



Article Tracing Leaf Photosynthetic Parameters Using Hyperspectral Indices in an Alpine Deciduous Forest

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Abstract: Leaf photosynthetic parameters are important in understanding the role of photosynthesis in the carbon cycle. Conventional approaches to obtain information on the parameters usually involve long-term field work, even for one leaf sample, and are, thus, only applicable to a small area. The utilization of hyperspectral remote sensing especially of various vegetation indices is a promising approach that has been attracting increasing attention recently. However, most hyperspectral indices are only applicable to a specific area and specific forest stands, depending heavily on the conditions from which the indices are developed. In this study, we tried to develop new hyperspectral indices for tracing the two critical photosynthetic parameters (the maximum rate of carboxylation, V_{cmax} and the maximum rate of electron transport, J_{max}) that are at least generally applicable for alpine deciduous forests, based on original hyperspectral reflectance, first-order derivatives, and apparent absorption spectra. In total, ten types of hyperspectral indices were screened to identify the best indices, and their robustness was determined using the ratio of performance to deviation (RPD) and Akaike's Information Criterion corrected (AICc). The result revealed that the double differences (DDn) type of indices using the short-wave infrared (SWIR) region based on the first-order derivatives spectra performed best among all indices. The specific DDn type of indices obtained the RPD values of 1.43 ($R^2 = 0.51$) for V_{cmax} and 1.68 ($R^2 = 0.64$) for J_{max}, respectively. These indices have also been tested using the downscaled dataset to examine the possibilities of using hyperspectral data derived from satellite-based information. These findings highlight the possibilities of tracing photosynthetic capacity using hyperspectral indices.

Keywords: photosynthetic capacity; temperate forest; hyperspectral remote sensing; vegetation indices; ratio of performance to deviation (RPD)

1. Introduction

Global climate change has been projected to reduce global net primary production (NPP) and carbon stocks from soil [1], and has become a global threat to humans. To date, tremendous efforts have been made to reduce the impact of global climate change, for which the control of greenhouse gases (GHGs) emissions has become a popular solution [2]. Such an effort could be more effective if supported by increased reforestation, as tree species and all chlorophyll plants are important for mitigation purposes, since leaves capture CO_2 from the atmosphere, and combine it with water and energy from the sun to produce carbohydrates, a well-known process called photosynthesis that is critical for the carbon cycle [3,4].

To understand the role of photosynthesis in the carbon cycle, a comprehensive study of the dynamics of photosynthesis parameters is necessary. The proposed model by Farquhar et al. [5] has become a basic tool for assessing the photosynthetic capacity of each species [6]. Its two

critical parameters, the maximum rate of rubisco carboxylation (V_{cmax}), and the maximum rate of photosynthesis electron transport (J_{max}), are essential to the model, and are, thus, important to understand the exchange of carbon between the atmosphere and the terrestrial ecosystem [7].

Conventional approaches to obtain information on these two parameters usually involve long-term field work, even for one leaf sample [8,9], and can only be done on a small scale due to the time-consuming and highly labor-intensive work required. Alternatively, the approach of utilizing remote sensing information has increasingly attracted attention, and may potentially be used for estimating plant physiological, biochemical, and biophysical properties [10,11].

The utilization of remote sensing information generally involves two common approaches: empirical ways based on multispectral or hyperspectral information obtained from satellites, such as Hyperion, TianGong-1, EnMAP (Environmental Mapping and Analysis Program) and HyspIRI (Hyperspectral Infrared Imager) [12], or a radiative-transfer-model-based inversion like using SCOPE (Soil Canopy Observation, Photochemistry and Energy fluxes) [13], 4-Scale [14], or ProSAIL (Prospect + Scattering by Arbitrarily Inclined Leaves) [15]. However, most of the currently available radiative transfer models (RTMs) have not included photosynthesis parameters, especially V_{cmax} and J_{max}, as input variables, meaning that they are impossible to retrieve inversely. As an exception, the SCOPE model incorporated both radiative transfer and physiological processes, with V_{cmax} as one of the dozens (more than 30) inputting parameters [13,16]. Recent work by Camino et al. estimated V_{cmax} in wheat phenotyping trials using airborne hyperspectral-based solar-induced chlorophyll fluorescence (SIF) retrievals through SCOPE model inversions [17]. Their work involved the determination of numerous parameters, including the meteorological, leaf biophysical and structural parameters, leaf inclination angle distribution function (LIDF) parameters, and the broadband incoming shortwave radiation. As SCOPE is a very complex model, which has integrated different modules, the parameterization complexities can induce large errors in its application [16,18]. In addition, rather than obtained directly from reflectance, V_{cmax} used for the model was estimated from a previously established relationship with SIF. Therefore, directly remote sensing retrieval of physiological parameters to date has relied more on empirical approaches.

Since Rouse et al. proposed the popularly applied normalized difference vegetation index (NDVI) [19], the empirical approach has been widely followed and a number of indices have been developed for diverse purposes, including Gamon et al.'s photochemical reflectance index (PRI) [20] for physiological parameters [21–25]. To date, a large number of indices have been developed to fulfill the needs for monitoring and assessing plant structural and biochemical aspects [26–28], and most of the well-known indices reported were developed in multispectral information but with certain adjustments, such that these indices could potentially be used for hyperspectral reflectance. However, even though the use of vegetation indices for a quick assessment of photosynthesis or photosynthetic parameters has been attempted in several previous works [29–33], no consensus has yet been reached [34], and is dramatically behind the indices for structural or biochemical parameters. Furthermore, the few reported indices are generally only applicable to a specific area and specific forest stands depending on the condition for the index developed, or for specific leaf groups [35]. Our previous efforts to filter a universal index to trace photosynthetic parameters failed and no hyperspectral index has ever been applicable, especially for trees in alpine deciduous forests.

The aim of this study, therefore, is to develop a robust index for tracing photosynthetic parameters using hyperspectral information for alpine deciduous forests. We used a dataset composed of six species that dominate in the habitat of the alpine temperate forest in Japan. The dataset includes synchronous measurements of photosynthetic parameters (V_{cmax} and J_{max}) and reflectance from two distinctive leaf groups, sunlit leaves, and shaded leaves of each species. A downscaled dataset is also attempted to investigate the possibilities of using satellite-borne hyperspectral data for future assessment.

2. Materials and Methods

2.1. Study Site

The study site is located in Nakakawane, one of the forestry research facilities of Shizuoka University ($35^{\circ}04'$ N and $138^{\circ}06'$ E, Shizuoka Prefecture, Japan). The land cover in this study site is a deciduous temperate forest, dominated by *Acer shirasawanum* Koidz. The elevations vary from 390 to 1560 m with an annual precipitation of approximately 2153 mm. Meanwhile, the annual mean temperature is around 17 °C [36]. The location of this site is shown in Figure 1.



Figure 1. Study site location: (a) north tower, and (b) south tower.

2.2. Measurements and the Dataset

A detached leaf sampling strategy has been followed in this study [37,38]. Leaf samples were collected in the years 2014, 2015, 2016, and 2018. All samples were taken pre-dawn by cutting the leaves from the branches and re-cutting underwater and were transported as soon as possible from the field to the laboratory under dark conditions. Measurements were taken with the dark-adapted leaves as soon as possible (no later than three days maximum) after the leaf samples were collected.

Gas exchange measurements were made for each sample following the standard method [8]. The LI-6400 (LI-COR Bioscience Inc., Lincoln, NE, USA) equipped with a leaf chamber was used. For dark-adapted samples, we took light response curves first in 2018, starting from a PAR (Photosynthetically Active Radiation) level of 0 μ mol·m⁻²·s⁻¹ and an ambient CO₂ (C_a) concentration of 400 μ mol mol⁻¹. After all the graphs became stable, we gradually tuned the PAR to 100, 200, 400, 700, 850, 950, and 1000 μ mol·m⁻²·s⁻¹ for about two minutes each to reach stably. The A/C_i curve (net CO₂ assimilation rate, A, versus calculated substomatal CO₂ concentration, C_i) measurements were made after having obtained the light saturation points from the light response curves. However, for the

years from 2014 to 2016 light response curves were not taken regularly, the A/C_i curve measurements were made with the PAR set to 1000 μ mol·m⁻²·s⁻¹, a light-saturated point as determined from previous light response curves. Then we decreased the C_a concentrations gradually from 400 μ mol·mol⁻¹ to 300, 200, 100 (but to 300, 250, 200, 150, 100 in 2018), and then finally to 50. Each step took about 2 minutes to stabilize. After that, we gradually increased the C_a back to 400 μ mol mol⁻¹, and then to 1200, 1500, 1800 in 2014-2016 while to 550, 650, 750, 850, 1000, 1200, 1500, 1700 in 2018, before finally reaching 2000 μ mol·mol⁻¹. The A/C_i curves were fitted using the plantecophys package [39] in R software [40] for V_{cmax} and J_{max}.

For each sample, once the gas exchange measurements finished, leaf scale reflectance was immediately taken using a field spectrometer (Analytical Spectral Devices Inc., Boulder, CO, USA) equipped with a leaf clip. Reflectance measurements were done with both white and dark backgrounds, repeated three times for each measurement. The average values of the three repeats were used for further analysis.

The dominant species in our study site is A. shirasawanum, which has unique leafy periods. This species comes into leaf earlier than other species but matures at the almost same time as the others and has a longer leaf senescence period. On the other hand, one of the target species in this study is *Fagus* crenata. This species is native to Japan and is categorized as an endemic Fagus species from Japan together with Fagus japonica. The species is usually widespread and even the dominant tree of Japanese deciduous forest. In total, six species were used for this study. The list of species is presented in Table 1. These species were all easy-to-access trees surrounding the towers. Shaded and sunlit leaves were taken from each species separately, and their positions were recorded. Only mature leaves were used for this study (from June to the middle of September in each year). This is also to eliminate outliers, since a typhoon hit the study site at the end of September 2018. An exception was only found for A. *shirasawanum* since the species has a longer leaf senescence period and suffered more serious stress from the typhoon than the other species. Therefore, we excluded all September data from each year of measurements for this species. However, for the year 2017 only F. crenata was measured. The dataset used in this study finally contained 170 leaf samples (Table 1). Besides F. crenata and A. shirasawanum, the other four species have a small number of leaf samples due to the small number of trees within our study site.

No.	Species Name	Number of Leaf Samples
1.	Acer shirasawanum Koidz.	46
2.	Betula grossa Siebold & Zucc.	14
3.	Carpinus tschonoskii Maxim.	11
4.	Fagus crenata Blume	84
5.	Stewartia monadelpha Siebold & Zucc.	6
6.	Stewartia pseudocamellia Maxim.	9

Table 1. List of species used in this study.

2.3. Reported Indices

We collected a number of reported indices to validate their applicability for estimating photosynthetic parameters. The results were also compared with the new indices developed in this study. We tested a series of well-known vegetation indices of hyperspectral remote sensing. The formulae of the reported indices are shown in Table 2.

The wavelengths used for the Moderate Resolution Imaging Spectroradiometer (MODIS)-like indices were 858 nm for NIR (near infrared) and 645 nm for red. We also tried another combination of using the center Sentinel 2A wavelengths, in which the NIR was 833 nm and the red was 741 nm (vegetation red edge, Band 6). The reported indices were examined for their original reflectance form only.

No.	Name of Index	Formula of Index					
1.	Photochemical Reflectance Index, PRI [20]	$PRI = rac{R_{531} - R_{570}}{R_{531} + R_{570}}$					
2.	Red-edge Vegetation Stress Index, RVSI [27]	$RVSI = \left[\frac{(R_{714} + R_{752})}{2}\right] - R_{733}$					
3.	Structure-Insensitive Pigment Index, SIPI [41]	$SIPI = \frac{R_{800} - R_{445}}{R_{900} - R_{600}}$					
4.	Chlorophyll Absorption Ratio Index, CARI [26]	$CARI = (R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550})$					
5	modified Chlorophyll Absorption Ratio Index,	mCARI =					
5.	mCARI [42]	$\left[\left(R_{700} - R_{670} \right) - 0.2 * \left(R_{700} - R_{550} \right) \right] * \left(\frac{R_{700}}{R_{670}} \right)$					
6.	Normalized Difference Nitrogen Index, NDNI [28]	$NDNI = rac{\left[\log \left(rac{1}{R_{1510}} ight) - \log \left(rac{1}{R_{1680}} ight) ight]}{\left[\log \left(rac{1}{R_{1510}} ight) + \log \left(rac{1}{R_{1680}} ight) ight]}$					
7.	Normalized Difference Vegetation Index, NDVI [43] (Hyperspectral)	$NDVI = \frac{R_{860} - R_{690}}{R_{860} + R_{690}}$					
8.	Normalized Difference Vegetation Index, NDVI [19] (Moderate Resolution Imaging Spectroradiometer (MODIS)-like & Sentinel 2A-like)	$NDVI = \frac{NIR-Red}{NIR+Red}$					
9.	2-band Enhanced Vegetation Index, EVI 2 (Hyperspectral)	$EVI \ 2 = 2.5 * \frac{R_{860} - R_{690}}{R_{860} + (2.4 * R_{690}) + 1}$					
10.	2-band Enhanced Vegetation Index, EVI 2 [44] (MODIS-like & Sentinel 2A-like)	$EVI \ 2 = 2.5 * \frac{NIR-Red}{NIR+(2.4*Red)+1}$					

Table 2. Formulae of reported indices used in this study.

2.4. New Indices Development

Several reported index types were used in this study for developing new indices, including the given wavelength (R), simple ratio (SR), wavelength difference (D), a normalized difference (ND), and inverse differences (ID). Besides these five types, we also included the double differences (DDn), modified simple ratio 1 (mSR1), modified simple ratio 2 (mSR2), modified normalized difference (mND) and modified inverse differences (mID) to the list (Table 3). Furthermore, all types using original reflectance, first-order derivatives spectra and apparent absorption were screened to identify the best index and the best type of reflectance forms. The first-order derivatives and apparent absorption spectra were used to reduce background noise and the possibilities of overlapping spectral features [45,46]. The calculations of the first-order derivatives and the apparent absorption spectra are listed in Equation (1) and Equation (2), respectively.

$$1^{\text{st}}\text{Der}_{\lambda n} = (R_{\lambda n+1} - R_{\lambda n-1})/2 \tag{1}$$

$$Abs = \log\left(\frac{1}{R_{\lambda n}}\right)$$
(2)

The value of $R_{\lambda n}$ was the original reflectance value at wavelength λ_n .

In addition, we also examined the consistency of the developed indices with different spectral resolutions. This was done by downscaling the original spectral resolution to 5 nm, 10 nm, 20 nm, and 50 nm, with the purpose of investigating the possibilities of applying the developed indices using airborne or satellite-borne data. The screening was done using the MATLAB software (The MathWorks, Inc.).

Table 3. Index types used for developing new indices: Spectra at given wavelength (R), simple ratio (SR), wavelength difference (D), normalized difference (ND), inverse differences (ID), double differences (DDn), modified simple ratio 1 (mSR1), modified simple ratio 2 (mSR2), modified normalized difference (mND) and modified inverse differences (mID).

No.	Index Type	Formula of Index
1.	R(λ1)	$= R_{\lambda 1}$
2.	$SR(\lambda 1, \lambda 2)$	$=\frac{R_{\lambda 1}}{R_{\lambda 2}}$
3.	D(λ1, λ2)	$= R_{\lambda 1} - R_{\lambda 2}$
4.	ND($\lambda 1, \lambda 2$)	$=rac{(R_{\lambda 1}-R_{\lambda 2})}{(R_{\lambda 1}+R_{\lambda 2})}$
5.	$ID(\lambda 1, \lambda 2)$	$=\frac{1}{R_{\lambda 1}}-\frac{1}{R_{\lambda 2}}$
6.	$DDn(\lambda 1, \Delta \lambda)$	$= 2R_{\lambda 1} - R_{\lambda 1 - \Delta \lambda} - R_{\lambda 1 + \Delta \lambda}$
7.	mSR1($\lambda 1$, $\Delta \lambda$)	$=rac{({ m R}_{\lambda 1-\Delta \lambda}-{ m R}_{\lambda 1})}{{ m R}_{\lambda 1+\Delta \lambda}}$
8.	mSR2($\lambda 1$, $\Delta \lambda$)	$=\frac{(R_{\lambda 1-\Delta\lambda}-R_{\lambda 1})}{(R_{\lambda 1+\Delta\lambda}-R_{\lambda 1})}$
9.	mND($\lambda 1, \Delta \lambda$)	$=\frac{(R_{\lambda 1-\Delta \lambda}-R_{\lambda 1})}{(R_{\lambda 1-\Delta \lambda}+R_{\lambda 1}-2R_{\lambda 1+\Delta \lambda})}$
10.	mID($\lambda 1, \Delta \lambda$)	$=\mathrm{R}_{\lambda1-\Delta\lambda}ig(rac{1}{\mathrm{R}_{\lambda1}}-rac{1}{\mathrm{R}_{\lambda1+\Delta\lambda}}ig)$

2.5. Statistical Criteria

The premier criterion used in this study to evaluate the performance of an index is the ratio of performance to deviation (RPD) (Equation (3)) to observe the goodness of fit. It is expressed as a ratio of the standard error in prediction to the standard deviation of the samples. The RPD is calculated as follows:

$$RPD = \frac{SD}{SEP}$$
(3)

where SEP is the standard error prediction and SD is the standard deviation of photosynthetic capacity (V_{cmax} or J_{max}).

Based on the RPD results, the indices are then categorized into three different groups (A, B, or C). The category of each group refers to Chang et al. [47]. Category A means the indices can be used, while category B means the indices can be used with several improvements. Category C means the indices cannot be reliably applied.

However, the Akaike's information criterion corrected (AICc) (Equation (4)) served as the determinative criterion for the final selection of the candidates especially when several different indices give the same RPD value. The AICc is used due to its capability to address potential overfitting caused by a small sample size.

AICc =
$$(2k - 2\ln(\hat{L})) + \frac{2k^2 + 2k}{n - k - 1}$$
 (4)

where \hat{L} is the maximum value of the likelihood function, n is the sample size, and k is the number of parameters. In addition, the coefficient of determination (R²) and the root mean square error (RMSE) were also calculated.

3. Results

3.1. Properties of Leaf Photosynthetic Parameters and Hyperspectral Reflectance

Six species from Nakakawane were measured for photosynthesis activities from 2014 until 2018. The mean value of V_{cmax} was estimated to be 33.48 µmol m⁻² s⁻¹, ranging from 10.01 µmol m⁻² s⁻¹ to 61.21 µmol m⁻² s⁻¹, with a median of 31.95 µmol m⁻² s⁻¹. Meanwhile, the J_{max} data varied from 26.15 µmol m⁻² s⁻¹ to 160.80 µmol m⁻² s⁻¹, with the mean of 71.80 µmol m⁻² s⁻¹ and the median of 67.61 µmol m⁻² s⁻¹. Descriptive statistical results revealed that the V_{cmax} had a skewness of 0.284, a kurtosis of -0.851, and a standard deviation of 12.18 µmol m⁻² s⁻¹. On the other hand, the J_{max}

had 0.668 for skewness, -0.369 for kurtosis, and 29.98 μ mol m⁻² s⁻¹ for the standard deviation. The distributions of every photosynthetic parameter are shown in Figure 2.



Figure 2. Distribution of photosynthetic parameters: (**a**) the maximum rate of carboxylation (V_{cmax}), and (**b**) the maximum rate of electron transport (J_{max}).

Species-based descriptive statistics for photosynthetic parameters are shown in Figure 3. The highest values for V_{cmax} and J_{max} were both found in *Fagus crenata*, of 61.21 µmol m⁻² s⁻¹ and 160.80 µmol m⁻² s⁻¹, respectively. In contrast, the lowest value for V_{cmax} was observed in *Acer shirasawanum* (10.01 µmol m⁻² s⁻¹), while the lowest value for J_{max} was found in *Betula grossa* (26.15 µmo m⁻² s⁻¹). The highest variance of V_{cmax} was found in *B. grossa*, which reached 146.41 µmol m⁻² s⁻¹ (14 samples). This was followed by *Carpinus tschonoskii* (135.09 µmol m⁻² s⁻¹; 11 samples), and *F. crenata* (112.73 µmol m⁻² s⁻¹; 84 samples). However, different trends were noted for the J_{max}, for which *F. crenata* had the highest variance of 780.98 µmol m⁻² s⁻¹, followed by *C. tschonoskii* (713.10 µmol m⁻² s⁻¹) and *B. grossa* (668.18 µmol m⁻² s⁻¹).

On the other hand, the reflectance data also varied among different species (Figure 4). The *S. monadelpha* had the highest standard deviations of reflectance spectra at the range of red-edge to NIR. Nevertheless, *F. crenata* had the highest mean reflectance in most wavelengths, especially for the domain of short-wave infrared (SWIR). For different leaf groups, there is no significant variation of reflectance from 400 to 1400 nm. However, the standard deviations of sunlit leaves were higher at 700 nm (red edge) to 1100 nm (NIR area), while shaded leaves were higher at 1400 to 2500 nm (SWIR area). The result is presented in Figure 5.



Figure 3. Variation of photosynthetic capacity from each species. Boxes show 25% and 75% quartiles, while the thick center line represents the median. Dots represent outliers.



Figure 4. Variation of reflectance from each species. Lines represent mean of reflectance and the opaque zones represent standard deviations.



Figure 5. Variation of reflectance based on leaf categories. Lines represent mean of reflectance and the opaque zones represent standard deviations.

3.2. Performance of Reported Indices

The results from indices screening based on the original reflectance for both parameters using the reported indices are shown in Table 4. The reported indices used for this study gave poor performance, judged from their low RPD values and coefficients of determination (\mathbb{R}^2).

Reported Indices	Vc	nax	J _{max}			
Reported marces	RPD	R ²	RPD	R ²		
PRI	1.02	0.04	1.08	0.13		
RSVI	1.09	0.15	1.05	0.09		
SIPI	1.02	0.04	1.00	0.00		
CARI	1.05	0.09	1.02	0.03		
mCARI	1.05	0.08	1.02	0.03		
NDNI	1.08	0.14	1.12	0.19		
NDVI_Hyp	1.04	0.07	1.02	0.04		
NDVI_MOD	1.03	0.05	1.01	0.01		
NDVI_2A	1.13	0.22	1.11	0.19		
EVI2_Hyp	1.12	0.19	1.09	0.16		
EVI2_MOD	1.12	0.20	1.14	0.23		
EVI2_2A	1.15	0.23	1.17	0.26		

Table 4. Result of reported indices screening based on original reflectance spectra.

*bold type indicates the best ratio of performance to deviation (RPD), coefficient of determination (R²), and type of indices used.

3.3. Development of New Indices

3.3.1. All Data

We developed new indices based on the original reflectance, the first-order derivatives, and the apparent absorption for both parameters. The screening of all indices using the original spectra suggested that the double difference (DDn) type of indices gave the best performance for quantifying both V_{cmax} and J_{max} . The best index of this type used the first wavelength at 1652 nm and had an interval of 9 nm for V_{cmax} , which had an RPD of 1.42 and an R² of 0.50. It also had the minimum AICc (=5.34) among all index types. In comparison, the best index identified for J_{max} of this type used the nearby wavelength of 1644 nm with an interval of 16 nm and had an RPD of 1.75. Furthermore, its R² reached 0.67.

Similar analysis on the indices calculated from the first-order derivatives spectra again suggested that the DDn type index performed best for predicting V_{cmax} ($R^2 = 0.51$ and RPD = 1.43), with the central wavelength at 1831 nm and an interval of 419 nm. However, the wavelength difference (D) type index performed best for J_{max}, which used the wavelengths of 1629 nm and 1658 nm. This index also had an R^2 of 0.68 and its RPD was estimated as 1.78. The DDn type index with the central wavelength at 1600 nm and an interval of 65 nm was also effective to trace J_{max} with an R^2 of 0.64 and RPD of 1.68.

Furthermore, indices based on the apparent absorption spectra were also examined for their performance. The results suggested the mID type indices performed best for V_{cmax} while the DDn type for J_{max}. The R² between the mID index and V_{cmax} was higher than the SR, ND, and mSR1 indices (0.514 versus 0.513 for the other three indices, not shown in Table 5). The best mID index (R² = 0.51, RPD = 1.44) used the wavelength of 2199 nm with an interval of 9 nm, while the best DDn index (R² = 0.65, RPD=1.68) used 1651 nm with an interval of 11 nm for wavelengths before and after that.

Spectral Type		Index Type									
		R	SR	D	ND	ID	DDn	mSR1	mSR2	mND	mID
Maximum rate of rubisco carboxylation (V _{cmax})											
Original	RPD	1.17	1.35	1.41	1.35	1.18	1.42	1.35	1.30	1.30	1.35
reflectance	R ²	0.26	0.45	0.49	0.45	0.28	0.50	0.45	0.41	0.40	0.45
First-order	RPD	1.30	1.29	1.41	1.31	1.32	1.43	1.32	1.30	1.31	1.27
derivatives	\mathbb{R}^2	0.41	0.40	0.50	0.42	0.43	0.51	0.42	0.40	0.41	0.38
Apparent	RPD	1.17	1.44	1.35	1.44	1.34	1.39	1.44	1.29	1.29	1.44
absorption	\mathbb{R}^2	0.26	0.51	0.45	0.51	0.44	0.48	0.51	0.40	0.39	0.51
	Maximum rate of photosynthesis electron transport (I _{max})										
Original	RPD	1.20	1.41	1.44	1.41	1.29	1.75	1.41	1.48	1.48	1.41
reflectance	R ²	0.30	0.49	0.52	0.49	0.39	0.67	0.50	0.54	0.54	0.49
First-order	RPD	1.41	1.44	1.78	1.46	1.45	1.68	1.51	1.53	1.58	1.45
derivatives	\mathbb{R}^2	0.50	0.52	0.68	0.53	0.52	0.64	0.56	0.57	0.60	0.52
Apparent	RPD	1.19	1.51	1.41	1.51	1.41	1.68	1.51	1.47	1.47	1.51
absorption	R ²	0.29	0.56	0.49	0.56	0.49	0.65	0.56	0.53	0.53	0.56

Table 5. RPD and coefficient of determination based on different spectral types.

*bold type indicates the best RPD, coefficient of determination (R²), and type of wavelength used.

Further, for all indices based on the different types of spectral forms, the indices based on first-order derivatives spectra gave the best performance, which had the highest RPD values for first-order derivatives of both V_{cmax} and J_{max} .

3.3.2. Different leaf groups

We also explored the best indices for different leaf groups (sunlit and shaded) based on the original reflectance spectra, the first-order derivatives spectra and the apparent absorption spectra as well. The results for sunlit and shaded leaves are shown in Table 6. The bold type indicates the best RPD and coefficient of determination (R^2).

As illustrated in Table 6, for sunlit leaves, the best performance indices based on the original reflectance form for V_{cmax} and J_{max} are both the DDn type. Similarly, the best indices based on the first-order derivatives spectra are also the DDn type of indices for J_{max} and for V_{cmax} . Further, the DDn type index using the apparent absorption spectra also performed best for J_{max} . Although the SR type index based on apparent absorption performed best for V_{cmax} (with maximum RPD value, not shown in Table 6), the R² values of SR, ND, DDn, mSR1, and mID were very similar (0.47 for SR, ND, mSR1 and mID indices, while 0.46 for DDn index). In addition, we also found that the RPD values generally increased from the indices based on original reflectance to indices based on first-order derivatives spectra.

For shaded leaves, the performance of indices based on the original reflectance mimicked that of sunlit leaves. The DDn type of indices were effective to trace both V_{cmax} and J_{max} of shaded leaves. Although the D type index based on the original reflectance performed best for V_{cmax} , the DDn type

Leaf	eaf Spectral Type		Index Type									
Groups			R	SR	D	ND	ID	DDn	mSR1	mSR2	mND	mID
	Maximum rate of rubisco carboxylation (V _{cmax})											
	Original	RPD	1.16	1.29	1.38	1.29	1.17	1.40	1.29	1.39	1.39	1.29
	reflectance	R ²	0.25	0.39	0.47	0.39	0.26	0.48	0.39	0.47	0.47	0.39
	First-order	RPD	1.32	1.37	1.40	1.35	1.41	1.45	1.37	1.37	1.37	1.36
	derivatives	R ²	0.42	0.46	0.48	0.45	0.49	0.51	0.46	0.46	0.46	0.45
	Apparent	RPD	1.17	1.39	1.29	1.39	1.32	1.37	1.39	1.33	1.33	1.39
Sunlit	absorption	R ²	0.25	0.47	0.39	0.47	0.42	0.46	0.47	0.43	0.43	0.47
	Maximum rate of photosynthesis electron transport (J _{max})											
	Original	RPD	1.24	1.42	1.57	1.42	1.33	1.75	1.42	1.60	1.60	1.42
	reflectance	R ²	0.34	0.50	0.59	0.50	0.42	0.67	0.50	0.60	0.60	0.50
	First-order	RPD	1.56	1.59	1.84	1.59	1.65	1.87	1.58	1.74	1.76	1.72
	derivatives	R ²	0.58	0.60	0.70	0.60	0.63	0.71	0.60	0.67	0.67	0.66
	Apparent	RPD	1.23	1.57	1.42	1.57	1.56	1.66	1.57	1.50	1.49	1.57
	absorption	R ²	0.33	0.59	0.50	0.59	0.58	0.63	0.59	0.55	0.54	0.59
			Ma	aximum	rate of ru	ibisco cai	rboxylati	on (V _{cma}	x)			
	Original	RPD	1.20	1.44	1.53	1.44	1.25	1.50	1.45	1.40	1.41	1.44
	reflectance	R ²	0.30	0.51	0.57	0.51	0.36	0.55	0.52	0.48	0.49	0.51
	First-order	RPD	1.41	1.43	1.51	1.49	1.43	1.62	1.46	1.50	1.55	1.43
	derivatives	R ²	0.49	0.51	0.55	0.55	0.51	0.62	0.53	0.55	0.58	0.50
	Apparent	RPD	1.19	1.48	1.44	1.50	1.47	1.52	1.48	1.39	1.40	1.52
Shaded	absorption	R ²	0.29	0.54	0.51	0.55	0.53	0.56	0.54	0.47	0.48	0.57
onadea			Maximu	um rate c	of photos	ynthesis	electron	transpor	t (J _{max})			
	Original	RPD	1.22	1.46	1.49	1.47	1.22	1.65	1.46	1.47	1.49	1.46
	reflectance	R ²	0.32	0.53	0.55	0.53	0.33	0.63	0.53	0.53	0.55	0.53
	First-order	RPD	1.43	1.53	1.70	1.60	1.37	1.62	1.42	1.60	1.46	1.41
	derivatives	R ²	0.51	0.57	0.65	0.61	0.46	0.62	0.50	0.61	0.53	0.49
	Apparent	RPD	1.22	1.50	1.47	1.50	1.38	1.66	1.50	1.46	1.48	1.50
	absorption	R ²	0.32	0.55	0.53	0.55	0.47	0.63	0.55	0.52	0.54	0.55

Table 6. RPD and R² from developed indices using different leaf group datasets.

*bold type indicates the best RPD and coefficient of determination (R²).

3.3.3. Different Species

We also investigated the best indices for each species in this study, to determine whether it is necessary to trace photosynthetic parameters for each species individually or for species groups instead. The results indicated that the indices based on the first-order derivative spectra performed better in quantifying the photosynthetic capacity parameters for all species. For most species, except *F. crenata* and *A. shirasawanum*, the indices based on the first-order derivative spectra have RPD values > 2.00 (irrespective of index type) for V_{cmax} and even better for J_{max}. For both *F. crenata* and *A. shirasawanum*, most index types have RPD values within 1.4 and 2.0 with V_{cmax} (except for R type for *A. shirasawanum*, RPD = 1.31). In comparison, all index types have RPD values within the range of 1.4 and 2.0 with J_{max} for the two species.

In general, the DDn type of indices based on the original reflectance had a respectable performance for all species when quantifying V_{cmax} . However, each species has its own specific best-fitting index. For example, for quantifying the V_{cmax} of *A. shirasawanum*, *F. crenata*, *S. monadelpha* and *S. pseudocamellia*, the DDn type of index should be chosen. For the other two species (*B. grossa* and *C. tschonoskii*), the best-performing indices type were the D and mSR2, respectively. In contrast, for J_{max}, the DDn type of index performed best with only two species (*A. shirasawanum* and *S. monadelpha*).

Due to the small sample sizes, the RPD values for *S. monadelpha* and *S. pseudocamellia* were higher than the other species and may, thus, provide misleading RPD results. The sample size was an important factor in ensuring accurate indices' predictions.

3.4. Evaluation of Developed Indices With Downscaled Resolutions

Indices based on downscaled spectra were also examined in this study, in order to investigate the possibility of expanding their applications from hyperspectral to multispectral data. We downscaled the original 1 nm spectra into several different resolutions, namely, 5 nm, 10 nm, 20 nm, and 50 nm. The screening results proved that the RPD values for indices based on original reflectance did not change significantly, and that the type of indices used to quantify the photosynthetic parameters remained consistent for all resolutions except for 50 nm.

The results indicated that the RPD values of each type of index were relatively constant even when the resolutions were changed. The DDn type of indices based on either original reflectance or apparent absorption spectra showed a non-significant decrease of the RPD values for tracing J_{max}. However, for the first-order derivatives spectra-based indices, the D type index performed better than the DDn type. Nevertheless, the RPD for the DDn index type was still respectable for different spectral resolutions. On the other hand, V_{cmax} can be traced better using the DDn type of indices based on the first-order derivatives and apparent absorption spectra. The RPD values of DDn type indices for V_{cmax} decreased with the spectral resolution of the original reflectance.

4. Discussion

Hyperspectral remote sensing, as a promising technique, has already been widely used for retrieving structural and biochemical parameters [11,48–50] Unfortunately, few studies have ever succeeded with physiological parameters [29,31,51,52]. Sensitivity analysis results of the newly developed model of soil-canopy spectral radiances, photosynthesis, fluorescence, temperature, and energy balance (SCOPE) [13] indicated that recognizable contributions of the physiological parameters (such as Ball–Berry stomatal conductance parameter, roughness length for the momentum of the canopy) were identified to reflectance [16]. What's more, V_{cmax} showed a great contribution to the full broadband sun-induced chlorophyll fluorescence (SIF) flux and calculating total fluorescence yield [53]. These results laid the possibility of using hyperspectral remote sensing to trace photosynthetic capacity parameters.

In this study, we tried to reveal the possibility of using hyperspectral information to detect and to quantify photosynthetic capacity parameters, namely, the maximum rate of rubisco carboxylation (Vcmax) and maximum rate of photosynthesis electron transport (Jmax). We investigated the possibilities of using hyperspectral vegetation indices, the most popular applied empirical approach in current remote sensing activities. We first investigated the feasibility of using the reported indices based on our specific dataset containing synchronous data of gas exchange and reflectance. In total, ten well-known indices were validated, including the PRI and RVSI that are usually used for physiological trait assessment, and some for predicting biochemicals such as pigments (SIPI, CARI, mCARI) and nitrogen content (NDNI), as well as several (e.g., NDVI and EVI2 for structural assessment, such as LAI (leaf area index) and green biomass). These ten reported indices are in widespread use and are related, more or less, to photosynthetic capacity [54–56].

None of the reported indices could be recommended, as clearly indicated by their RPD values (all below 1.4). Although NDVI and EVI2 performed slightly better using the range of the central wavelength of Sentinel 2A, their RPD values were still below 1.4. Similar results were also found for other indices, such as PRI and RVSI. Surprisingly, although both the PRI and RVSI indices have frequently been used to quantify physiological parameters, they performed poorly with both photosynthetic parameters. Furthermore, several indices that are usually used to quantify pigments also gave poor results.

Possibly, the main reason for the poor performance of the reported indices may be the range of wavelengths used for calculations. All of the reported indices were developed using specific wavelengths to fit their specific purposes. For example, PRI was developed using green wavelengths at 531 nm and 570 nm, both of which are useful to detect chlorophyll fluorescence [57,58]. As such, a number of previous researches have already proved the feasibility of PRI for tracing other plant physiological activities related to chlorophyll pigment [24,59,60], but apparently not for photosynthetic parameters. The other reported indices are largely based on the red and NIR wavelengths that are sensitive to trace the change of vegetation, except for NDNI using the SWIR range. However, the wavelengths used were not feasible to trace other physiological changes, especially photosynthetic capacity.

Clearly, new indices for quantifying photosynthetic capacity need to be developed. When using first-order derivatives spectra, we found DDn(1831,419) and D(1629,1658) to be the best indices for tracing V_{cmax} and J_{max} based on our dataset. The RPD values of both indices are 1.43 and 1.78 for V_{cmax} and J_{max} , respectively. Although this type of index is not the best on J_{max} , the performance of DDn(1600,65) is still acceptable (RPD = 1.68, $R^2 = 0.64$). We suggest that the DDn type of indices could be used as a general form for tracing photosynthetic parameters. We looked through the wavelengths and identified several negative coefficients of correlation (troughs) between the reflectance spectra and photosynthetic parameters. We found that our identified DDn index used a wavelength from the second trough and other troughs of first-order derivatives spectra, as shown in Figure 6. Meanwhile, the useful wavelengths for all of the indices based on original reflectance, first-order derivatives, and apparent spectra are presented in Figure 7. Most of the useful wavelengths for both parameters are from the peak regions or troughs region.



Figure 6. Correlation test between photosynthetic capacity and wavelength. The suffix 'Der' refers to the first-order derivative spectra, while the 'Ref' for the original reflectance, and the 'psA' for the apparent absorption spectra.

The indices based on the first-order derivatives spectra were found to perform better than those based on the original or apparent absorption spectra. The first-order derivatives spectra are known to be able to reduce noise between spectral data [45,46] and this is the probable reason. Another possible reason is that these identified indices all used wavelengths in the SWIR region (1300 nm to 2500 nm). The SWIR domain is known to be very informative for detecting physiological or chemical activity due to the wavelengths being absorbed or reflected by the object [61,62]. These SWIR wavelengths have properties that are more similar to visible light compared with the other infrared range. Based on Figure 7, we extracted six different wavelengths used by the DDn type of indices based on the first-order derivatives spectra. Wavelengths 1412 nm, 1831 nm, and 2250 nm were selected for V_{cmax}

and wavelengths 1535 nm, 1600 nm, and 1665 nm were selected for J_{max} . The wavelength 1412 nm used in the DDn index for V_{cmax} estimation was within the most sensitive domain of V_{cmax} . The first-order derivative spectra at 1412 nm were significantly related to V_{cmax} (the coefficient of correlation r = -0.62). Similarly, the wavelength 1600 nm used in the DDn index for J_{max} estimation was also within the most sensitive domain of J_{max} . The first-order derivative spectra at 1600 nm were significantly and positively related to J_{max} (the coefficient of correlation r = 0.68). According to Figure 8, there were no significant differences between species in this range of wavelengths. However, the value range of the wavelengths differed in each case. The value of each wavelength is presented in Figure 8.



Figure 7. Useful wavelengths from all index types for photosynthetic capacity. (**a**) Original reflectance, (**b**) first-order derivatives spectra, and (**c**) apparent absorption.

Furthermore, we investigated the robustness of the developed indices with different leaf groups. The difference in leaf groups (sunlit and shaded) used in this study is due to differences in their response to the photosynthesis process [63]. This leaf stratification also contributed to model accuracy when upscaling the leaf photosynthesis model to the canopy scale [64]. While in several vegetation indices that have already been developed, the sunlit leaves usually contain a higher ratio of chlorophyll A and are thicker than shaded leaves, the shaded leaves have more chlorophyll per dried leaf weight and emit the maximum chlorophyll fluorescence, higher than sunlit leaves [65].

We found that the DDn type of indices consistently traced J_{max} well, even when the wavelengths used were slightly different. For the shaded leaf group, the best DDn index used the 1664 nm for λ 1with a $\Delta\lambda$ of 54 nm, while for the sunlit leaf group the 1609 nm was used for λ 1 with the $\Delta\lambda$ of 45 nm. Meanwhile, the RPD values of the DDn type indices for tracing V_{cmax} decreased, especially in the sunlit leaves group. The sunlit leaves and shaded leaves have different physical and pigment conditions, where the shaded leaves usually emit higher chlorophyll fluorescence compared with sunlit leaves [65]. Furthermore, the sunlit leaves mostly represent the lowest quantum yield of electron transport and lowest pigment content [66] while the shaded leaves generally obtain only one-third of the canopy carbon gain [67].



Figure 8. Value range of first-order derivatives spectra for the DDn selected wavelengths.

In addition to leaf groups, the screening results for different species also confirmed that the DDn type of indices performed best for *B. grossa* and *F. crenata*. However, the RPD value of the DDn index for *A. shirasawanum* was also respectable. As mentioned before, *A. Shirasawanum* and *F. crenata* have a large number of samples, being the dominant and targeted species in our study, while for other species, the DDn performed respectably in comparison with the other types of index and when using different forms of spectra also performed better than the original reflectance spectra.

Further, we tested the indices with downscaled datasets to check the possibility of using airborne or satellite-borne data that generally have coarse spectral resolutions. The DDn type of indices kept their good performance for tracing J_{max} and even V_{cmax} , although the V_{cmax} results were slightly inferior to those of J_{max} . We also tried to use different types of spectral forms to test the consistency of the DDn index, and the performance for the DDn type of indices was found to be acceptable even for different spectral resolutions.

We concluded that the best type of index for quantifying photosynthetic parameters is the DDn type, which proved to be robust even for different species. The index also performed stably for downscaled resolution spectra, suggesting that it could also be used for airborne or satellite-borne coarse spectral data.

However, we realize that the indices have several shortcomings that must be resolved before they can be applied widely, such as that the number of species needs to be increased, as the sensitivity of hyperspectral indices is often considered to be species-dependent [68,69]. Furthermore, the sample sizes for several specific species were too small and could possibly therefore be overfitted, since it has been claimed that the sample size could influence the value of statistical indicators [70]. The first-order derivatives spectra are the best type of spectra for quantifying photosynthetic parameters, as hyperspectral indices based on the spectra show a certain balance between accuracy and robustness on retrieving plant properties [71]. Even though we are confident that this type of index could work for the alpine deciduous forest, much more study is still needed in order to provide generally applicable indices for tracing photosynthetic parameters. However, we foresee that such indices could be developed and we have taken an important step towards this objective.

5. Conclusions

New hyperspectral vegetation indices have been developed for tracing photosynthetic parameters in an alpine deciduous forest containing six species. The results demonstrated that the DDn type of indices performed best in tracing photosynthetic capacity. The DDn type of indices used the first-order derivatives spectra in the SWIR region wavelength at 1831 nm with $\Delta\lambda$ 419 nm for V_{cmax} and 1600 nm with $\Delta\lambda$ 65 nm for J_{max}. The result from this index type was relatively stable even for the downscaled resolution of 50 nm. The first-order derivatives spectra have been proven to be the best spectral form for tracing photosynthetic capacity. The DDn type of indices and first-order derivatives spectra have already demonstrated possibilities for tracing photosynthetic capacity using hyperspectral indices. The results obtained can contribute to the development of the vegetation index to assess photosynthetic capacity, especially for the alpine deciduous forest.

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