Article

Quantifying Chlorophyll Fluorescence Parameters from Hyperspectral Reflectance at the Leaf Scale under Various Nitrogen Treatment Regimes in Winter Wheat

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Abstract: Chlorophyll fluorescence (ChlF) parameters, especially the quantum efficiency of photosystem II (PSII) in dark- and light-adapted conditions (F_v/F_m and F_v'/F_m'), have been used extensively to indicate photosynthetic activity, physiological function, as well as healthy and early stress conditions. Previous studies have demonstrated the potential of applying hyperspectral data for the detection of ChlF parameters in vegetation. However, the performance of spectral features that have been documented to estimate ChlF is not ideal and is poorly understood. In this study, ChlF parameters and leaf reflectance were collected in two field experiments involving various wheat cultivars, nitrogen (N) applications, and plant densities, during the growing seasons of 2014 to 2015 and 2015 to 2016. Three types of spectral features, including vegetation indices (VIs), red edge position (REP), and wavelet features, were used to quantify ChlF parameters F_v/F_m and F_v'/F_m' . The results indicated that traditional chlorophyll fluorescence vegetation indices (ChlF VIs), such as the curvature index (CUR) and D705/D722 were capable of detecting F_v/F_m and F_v'/F_m' under various scenarios. However, the wavelet-based REP (WREP-S4) and the wavelet feature (WF) (704 nm, scale 4) yielded higher accuracy than other spectral features in calibration and validation datasets. Moreover, the bands used to calculate WREP-S4 and WF (704 nm, scale 4) were all centered in the red edge region (680 to 760 nm), which highlighted the role of the red edge region in tracking the change of active ChlF signal. Our results are supported by previous studies, which have shown that the red edge region is vital for estimating the chlorophyll content, and also the ChlF parameters. These findings could help to improve our understanding of the relationships among active ChlF signal and reflectance spectra.

Keywords: chlorophyll fluorescence (ChlF) parameter; vegetation indices (VIs); red edge position (REP); continuous wavelet analysis (CWA); maximal photochemical efficiency of PSII (F_v/F_m), photochemical efficiency of PSII in the light (F_v'/F_m')

1. Introduction

Chlorophyll fluorescence (ChlF) is a reaction of the photosynthesis apparatus to cope with excess light energy, which is accompanied with photochemical reactions and heat dissipation [1]. When the maximum photosynthetic rate is impaired, ChlF increases under many non-optimal environments. Therefore, ChlF is a direct indicator of electron transport and photosynthetic activity [2]. The ChlF parameters have been measured using a pulse-amplitude modulation fluorometer with different active light sources under light-adapted or dark-adapted conditions. ChlF parameters which are widely used to express the energy transfer of plant photosynthesis are relevant ChlF variables that have been calculated based on standard methodologies as reported in the PAM-2000 manual [3].

In contrast to traditional remote sensing techniques, ChlF produced solely by plants, rapidly captures the specific and instantaneous change of plants' physiology status and response to various nutritional treatments, heat, and water conditions [4,5]. Among all the ChlF parameters, F_v/F_m , which is the ratio of variable to maximal fluorescence, is the initial maximal efficiency of photons captured by open photosystem II (PSII) reaction centers [6], and is a widely used parameter representing the health and growth of plants [7–9]. The F_v'/F_m' parameter, which is the efficiency of energy harvesting by oxidized (open) PSII reaction centers in light, serves as an indicator to assess plant stress, nutrient, and health status [10]. Both F_v/F_m and F_v'/F_m' are closely related to the actual activity of plant photosynthetic tissue, and have been applied to monitor the physiological status of plants and their reactions to the environment [11].

Given the small ChlF signal and low efficiency for monitoring, there has been limited progress in retrieving ChlF parameters at the leaf and canopy scales. Most studies have mainly focused on the vegetation indices (VIs) [12–16]. The essence of estimating ChlF parameters using VIs is based on the fact that ChlF emission is superimposed on the leaf reflectance in the red edge region (680 to 800 nm). The VIs used to monitor ChlF parameters is a reflectivity index reflecting fluorescence intensity, rather than a definite physical quantity. These ChlF VIs have been divided into two categories, the reflectance ratio VIs and the derivative VIs. For reflectance ratio VIs, the curvature index (CUR), R_{685}/R_{655} , and R_{680}/R_{630} have shown good relationships with ChlF parameters [12,13]. Meanwhile, derivative VIs, such as $(D_{688} \cdot D_{710})/D_{697}^2$ (DPi), D_{705}/D_{722} , and D_{730}/D_{706} , have been developed to detect the subtle ChlF signal, because it has been found that the double-peak feature at 690 to 710 nm in the derivative reflectance was related to the natural fluorescence in both short-term and long-term stress experiments [14,17]. Although the photochemical reflectance index (PRI) has been used as an interspecific index of photosynthetic radiation use efficiency for foliar and canopy leaves [18], there has been some predicted bias when estimating F_v/F_m using PRI [13].

The ChlF signal centers in the red edge region (680 to 800 nm) and the VIs used to estimate the ChlF parameters are mainly composed of bands in the red edge region, which can make it possible to monitor ChlF parameters using the red edge position (REP). The REP, defined as the wavelength of inflection point in the red edge region, has been widely used as an indicator of chlorophyll (Chl) content of various plants [19]. Many techniques have been developed to extract REP including linear interpolation (LI) [20], linear extrapolation (LE) [21], polynomial fitting (PF) [22], inverted Gaussian (IG) [23], and wavelet-based REP extraction technique (WREP) [19]. In the past few decades, although REP has been successfully applied to monitor Chl content, it has never been used to monitor plant physiological parameters, especially ChlF parameters directly related to photosynthesis and plant function. Moreover, the most suitable algorithm of REP extraction for ChlF parameters estimation is still unclear.

In general, wavelength selection of ChlF VIs is mainly based on existing research or experience, and therefore the bands relevant to ChlF characteristics have not been fully explored and utilized. However, continuous wavelet analysis (CWA) can capture the rich and subtle changes of the reflectance by simulating the similarity between the wavelet function at different scales within a continuous region [24]. This approach improves the use of spectral libraries and gives a better performance for estimating plant traits relative to that of VIs, including Chl content, leaf water content, and leaf mass

per area (LMA) [25,26]. However, to date, little is known regarding the selection of optimal spectral features using CWA to estimate plant ChlF parameters.

In this context, the objectives of this study included the following: (1) to identify the ChlF parameters in light- and dark-adapted conditions which are more related to plant physiological and biochemical status; (2) to investigate the potential of CWA and REP to exploit hyperspectral reflectance for ChlF parameter estimation under different treatments involving variable sowing densities, development stages, and N fertilization; and (3) to determine the sensitive features relevant to the representative ChlF parameters. It is projected that this research could provide an experimental basis and technical support for estimating ChlF parameters via hyperspectral reflectance.

2. Materials and Methods

2.1. Experimental Design

Two experiments of winter wheat were conducted at Rugao (32°15'N, 120°38'E) in Jiangsu Province, China, during the growing seasons of 2014 to 2015 and 2015 to 2016. The data collected during the 2014 to 2015 growing season was only dedicated to the calibration of spectral computations, whereas that of the 2015 to 2016 dataset was used mainly as validation. As shown in Table 1, the experimental variables included various nitrogen (N) applications, planting densities, cultivars, and growing stages. A completely randomized block design was used with three replications in each experiment. Prior to seeding, 120 kg ha⁻¹ P₂O₅ and 135 kg ha⁻¹ KCl were applied for all treatments. For two winter wheat cultivars, the plant type of Shengxuan 6 is dispersed, and that of Yangmai 18 is erect. Then, 50% nitrogen (N) fertilizer was applied at the pre-planting stage and jointing period. Additional details about the experiments are provided in Table 1. The plot size was 6.5 × 5 m² for two experiments. The management of disease, pests, and weeds followed standard practices for chemical control in the local region.

Table 1. Basic information of two field experiments at Rugao.

Experiment (Exp.)	Season	Rowledge (cm)	Cultivar	N rate (kg/ha)	Samples (Fv/Fm, Fv'/Fm')	Sampling Date (Date/Phenological Stages/DAS) /Function
Exp. 1	2014 to 2015	25, 40	Shengxuan 6 (V1)	0 (N0),	36, 35	(9 th Apr/booting/164),
			Yangmai 18 (V2)	150 (N2), 300 (N4)	36, 36 36, 36 33, 33 12, 12	(16 th Apr/heading/172), (26 th Apr/anthesis/179), (1 st May/filling/187), (7 th May/7days after filling/193) (calibration)
Exp. 2	2015 to 2016	20, 30, 40	Yangmai 18 (V2)	0 (N0), 80 (N1), 150 (N2), 220 (N3)	62, 35 62, 30 62, 30	(9 th Apr/booting/160), (18 th Apr/heading/167), (5 th May/filling/187), (validation)

DAS: days after sowing.

2.2. Data Acquisition

2.2.1. Measurement of ChlF Parameters

The ChlF parameters (Table 2) of wheat leaves were measured using a portable modulated chlorophyll fluorometer PAM-2500 (Heinz Walz GmbH, Effeltrich, Germany) at several significant growth stages in two growing seasons. For each treatment, the top three fully expanded leaves of wheat were clamped at the center of PAM-2500 leaf clip holder. All light-adapted ChlF parameters were measured between 10:00 and 12:00 h in sunny conditions, whereas dark-adapted ChlF parameters (Fo, Fm, Fv/Fm), were obtained around 16:00 h after the leaves were subjected to dark adaptation for 30 minutes.

Table 2. Chlorophyll fluorescence (ChlF) parameters used in this study.

	Abbreviation	Description	Equation
dark-adapted condition	Fo	Minimal fluorescence yield	
	Fm	Maximal fluorescence yield	
	Fv/Fm	Maximal photochemical efficiency of PSII	$= (F_m - F_o) / F_m$
light-adapted condition	Fs	Steady-state fluorescence	
	Fo'	Initial fluorescence in the presence of NPQ	
	Fm'	Maximal fluorescence in the presence of NPQ	
	Fv'/Fm'	Photochemical efficiency of PSII in the light	$= (F_m' - F_s') / F_m'$
	Y(II)	Yield of quantum efficiency	
	NPQ	Non-photochemical quenching calculated with Fm	$= (F_m - F_m') / F_m'$
	ETR	Apparent photosynthetic electron transport rate	$= \text{Yield} \times \text{PFD} \times 0.5 \times 0.8$

2.2.2. Measurement of Leaf Physiological Parameters

After the ChlF parameters were obtained, measurements of leaf gas exchange, including net photosynthetic rate (A_n) and stomatal conductance (g_s), were taken on the same leaves under field conditions. These data were collected between 10:00 and 13:00 h on clear days using a LI-6400 portable photosynthesis system (LiCOR Inc., Lincoln, Nebraska, USA) during the 2014 to 2015 growing season. During the measurements, the photosynthetically active radiation (PAR) was set to $1200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, which corresponds to saturation in the field. The temperature and concentration of CO_2 in the chamber were maintained at 25°C and $380 \mu\text{mol}\cdot\text{mol}^{-1}$, respectively. The airflow rate through the leaf chamber was held constant at $500 \mu\text{mol}\cdot\text{s}^{-1}$. Air pressure and relative humidity were adjusted to near ambient levels. Finally, the plant leaves to be measured were transported to the laboratory for measuring the reflectance spectra.

2.2.3. Measurement of Leaf Reflectance

Leaf reflectance spectra were measured using an ASD FieldSpec Pro FR2500 spectrometer coupled with a leaf clip accessory (Analytical Spectral Devices, Boulder, CO, USA). The spectrometer, operating in the 350 to 2500 nm spectral range, provided a sampling interval of 1.4 nm and a spectral resolution of 3 nm between 350 and 1050 nm, and 2 nm and 10 nm between 1050 and 2500 nm, respectively. The leaf clip, used to collect leaf reflectance, was combined with a high-intensity reflectance probe attachment. It was equipped with an internal artificial light source that was independent of solar illumination. The clip also provided white and black background panels, of which the reflectance was approximately 100% and 0%, respectively. The field of view was limited to a diameter of 1.3 cm for the leaf clip, therefore measurements of leaf reflectance were not conducted before the jointing stage of winter wheat. After the dark current correction and optimization, the reflectance of the white panel was recorded as the reference. Individual spectral measurements, based on an average of three scans, were determined by standardizing a sample spectrum to that of the white reference panel.

2.2.4. Optical Measurements of Pigments in Laboratory

After the reflectance measurements, the surface area and fresh weight were determined immediately. The leaf area was measured with LI 3000 (LI-COR, Inc., Lincoln, NE, USA). Then, the leaves were cut into pieces and put into a volumetric flask (25 ml). The pigment content was extracted by ethanol (95%) and determined using a V1200 spectrophotometer (MAPADA Co., Shanghai, China) following standard wet chemistry procedures [27]. Then, the concentration ($\mu\text{g}/\text{ml}$) of Chl a,

Chl b, and carotenoid (Car) of samples were determined. Finally, the area based Chl_{ab} (Chl a + Chl b, ug/cm²) and Car (ug/cm²) were calculated.

2.3. Data Analysis and Utilization

2.3.1. Calculation of VIs

Five groups of VIs (Table 3) were calculated using the reflectance data. The first group was the ChlF VIs, including reflectance ratio VIs and derivative VIs, which were classified according to the previous study [28]. The second group was about status VIs, involving PRI and REPs extracted by five algorithms. These indices were selected because the variation of ChlF parameters is influenced comprehensively by biological activity and environmental conditions (fertilizer, solar radiation, temperature, and water) [29,30]. Thus, several VIs related to Chl content, structure properties, and water content were involved in this study for the detection of ChlF parameters.

2.3.2. Methods for REP Extractions

The explanation of five algorithms for REP extractions (listed in Table 3) are as follows:

(1) Linear Interpolation (LI)

REP is determined using a simple four-point LI method near the midpoint of the region 670 to 780 nm, where the reflectance can be simplified to a straight line [21]. The calculation procedure is shown as below:

$$R_{re} = (R_{670} + R_{780}) / 2 \quad (1)$$

$$REP = 700 + 40 \times \frac{R_{re} - R_{700}}{R_{740} - R_{700}} \quad (2)$$

where R_{re} is the reflectance average of 670 nm and 780 nm.

(2) Polynomial Fitting (PF)

A fifth-order PF function (shown below) was used to fit the reflectance curve over the red edge region 670 to 780 nm. The REP is defined as the wavelength corresponding to the maximum value of the first derivative spectra curve which is obtained by taking the first derivative of the fitted curve [23].

$$R(\lambda) = a_0 + \sum_{i=1}^5 a_i \lambda^i \quad (3)$$

where λ represents 111 bands of the reflectance from 670 nm to 780 nm.

(3) Linear Extrapolation (LE)

The first derivative spectra were obtained by a first difference transformation and then smoothing with the Savitzky–Golay method. Two points in the far-red region (680 to 700 nm) and two points in the near-infrared region (NIR) (725 to 760 nm) of the first derivative spectra were identified to construct two straight lines as follows [22].

$$FDR = m_1 \lambda + c_1 \quad (4)$$

$$FDR = m_2 \lambda + c_2 \quad (5)$$

where FDR is the first derivative spectra and m_1 , m_2 , c_1 , and c_2 represent the slopes and intercepts of the two respective straight lines. The REP is determined as the wavelength corresponding to the intersection of the straight lines as below:

$$REP = \frac{-(c_1 - c_2)}{m_1 - m_2} \quad (6)$$

(4) Wavelet-Based Red Edge Position (WREP)

The WREP is extracted in the red edge region of wavelet spectra as follows [20]:

$$\text{WREP} = \frac{w_1\lambda_2 - w_2\lambda_1}{w_1 - w_2} \quad (7)$$

where w_1 and w_2 are the wavelet coefficients of the two adjacent points below and above the horizontal zero line in the red edge region, respectively. λ_1 and λ_2 are the wavelengths corresponding to w_1 and w_2 , separately. The WREP has shown strong correlations with Chl content at the leaf level, especially at low scales [20]. Therefore, in this study, WREP was extracted at two low scales, such as scale 3 (WREP-S3) and scale 4 (WREP-S4).

2.3.3. Continuous Wavelet Analysis (CWA)

CWA is a powerful mathematical tool that processes spectroscopic signals and provides an effective approach for acquiring information about plant traits [31,32]. CWA is typically conducted in two steps, namely, continuous wavelet transform (CWT) and feature selection. In this study, CWT was analyzed using the "WV_CWT" function in IDL 8.3 (ITT Visual Information Solutions, Boulder, CO, USA).

A signal matrix ($1 \times n$) was generated by a wavelet function based on a given wavelet scale, a translational wavelet function, and a spectral signal. Thus, a $n \times m$ dimensional continuous wavelet transform coefficient matrix was produced after wavelet transformation at the m scale (where n represents the band and m is the scale). In this study, the mother wavelet function is the second derivative of the Gaussian function, because it has a similar shape to the leaf absorption features [33]. Each reflectance in the range 400–1000 nm where ChlF occurs was decomposed into continuous components at various scales. The CWT operations were conducted at a power of 2 ($2^1, 2^2, \dots, 2^8$) and are called by their respective power numbers (i.e., scale 1, scale 2, ... scale 8). Random noise due to reflectance measurement can be contained in the low scale components [32], and hence only the wavelet features at scales 3 to 8 were analyzed to produce a correlation scalogram (coefficient of determination, R^2). The regions with the highest 1% of R^2 for wavelet feature and ChlF parameters (F_v/F_m and F_v'/F_m') were highlighted in red, and the wavelet features with the highest R^2 in each region were extracted.

Table 3. Algorithms for different spectral features reported in literature.

Spectral Feature Type		Index Name (Abbreviation)	Index Formulation And Reference
VI	ChlF VI	Curvature index (CUR)	$(R_{675} \times R_{691})/R_{683}^2$ [12]
		Reflectance ratio VI	RVI (750,800) R750/R800 [12]
		RVI (685,655) R685/R655 [12]	
		RVI (680,630) R680/R630 [13]	
	Derivative VI	Double-peak index (DPi)	$(D_{688} \times D_{710})/D_{697}^2$ [14]
		D705/D722	D705/D722 [14]
		D730/D706	D730/D706 [14]
		DP22	$D\lambda\rho/D720$ [14]
		DPRI	$D\lambda\rho/D(\lambda\rho + 12 \text{ nm})$ [14]
	physiological VI	Photochemical reflectance index (PRI)	$(R_{531} - R_{570})/(R_{531} + R_{570})$ [33]
	Chl VI	MERIS terrestrial chlorophyll index (MTCI)	$(R_{754} - R_{709})/(R_{709} - R_{681})$ [34]
		Red edge chlorophyll index (CIred edge)	$(R_{800}/R_{720}) - 1$ [35,36]
	Structure VI	Normalized difference vegetation index (NDVI)	$(R_{810} - R_{690})/(R_{810} + R_{690})$ [37]
		Enhanced vegetation index (EVI)	$2.5 \times (R_{810} - R_{690})/(R_{810} + 2.4 \times R_{690} + 1)$ [38]
	Water VI	Normalized difference water index (NDWI)	$(R_{850} - R_{1240})/(R_{850} + R_{1240})$ [39]
Water index (WI)		R_{850}/R_{970} [40]	
REP	Linear interpolation (REP _{LI})	Explained as below, [41]	
	Polynomial fitting (REP _{PF})	Explained as below, [42]	
	Linear extrapolation (REP _{LE})	Explained as below, [21]	
	Wavelet-based REP extraction (WREP-S3)	Explained as below, [19]	
	Wavelet-based REP extraction (WREP-S4)	Explained as below, [17]	

2.4. Statistical Analysis

The VIs, REPs, and CWA were applied to build models to monitor the ChlF parameters of wheat leaf. The relationships among the spectral features and ChlF parameters were established from the data collected during the 2014 to 2015 growing season, which were validated using the data acquired during the 2015 to 2016 growing season. The 1:1 plot of the measured and predicted data was used to evaluate the model fitness. Calibration R^2 (R^2_C), see Equation (8), and the root mean square error of calibration (RMSE_C), see Equation (9), were statistical parameters used to evaluate the performances of all models in calibration. For validation, they are validation R^2 (R^2_V), see Equation (10); bias (Bias), see Equation (11); the root mean square error of prediction (RMSE_v), see Equation (12); and the relative root mean square error of prediction (RRMSE_v), see Equation (13). Higher values of R^2_C , R^2_V , and lower values of RMSE_C, Bias, RMSE_v, and RRMSE_v indicate higher accuracy of the model.

$$R^2_C = \left(\frac{\sum_{i=1}^n (X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum_{i=1}^n (X_i - \bar{X})^2} \sqrt{\sum_{i=1}^n (Y_i - \bar{Y})^2}} \right)^2 \quad (8)$$

$$\text{RMSE}_C = \sqrt{\sum_{i=1}^n (Y_i - M_i)^2 \frac{1}{n}} \quad (9)$$

$$R^2_V = \left(\frac{\sum_{i=1}^n (Y'_i - \bar{Y}'_i)(Y_i - \bar{Y})}{\sqrt{\sum_{i=1}^n (Y'_i - \bar{Y}'_i)^2} \sqrt{\sum_{i=1}^n (Y_i - \bar{Y})^2}} \right)^2 \quad (10)$$

$$\text{Bias} = \frac{\sum_i (Y_i - Y'_i)}{n} \quad (11)$$

$$\text{RMSE}_v = \sqrt{\sum_{i=1}^n (Y_i - Y'_i)^2 \frac{1}{n}} \quad (12)$$

$$\text{RRMSE}_v = \frac{\sqrt{\sum_{i=1}^n (M_i - Y_i)^2 \frac{1}{n}}}{\bar{Y}_i} \times 100\% \quad (13)$$

where Y_i is the measured value of samples, M_i is the predicted value of samples in the calibration dataset, $\bar{X}$ is the average of X_i , $\bar{Y}$ is the average of Y_i , Y'_i is the predicted values, $\bar{Y}'$ is the average of Y'_i for the validation dataset, and n is the number of samples.

3. Results

3.1. Relationships among Leaf Pigments, Physiological Indices, and ChlF Parameters

Table 4 displays the relationships among leaf pigments (Car and Chl_{ab}), physiological measurements (An and gs), dark-adapted ChlF parameters (Fo, Fm, and Fv/Fm), and steady-state ChlF parameters (Fo', Fs, Fm', Fv'/Fm', NPQ, and Y(II), ETR) acquired during the 2014 to 2015 growing season. The results showed that Fv/Fm and Fv'/Fm' have better relationships with the majority of items listed in Table 4 than other ChlF parameters. For instance, among the dark-adapted ChlF parameters, Fv/Fm was significantly related to Car, Chl_{ab}, An, gs, Fm, Fm', Fv'/Fm', and Y(II) with $R^2 > 0.5$ and p value < 0.001 . Other crop traits which showed moderately significant relationships with Fv/Fm (at $p < 0.01$) were Fs ($R^2 = 0.20$). Fo' and ETR had a remarkable relationship with Fv/Fm at the $p < 0.05$ level ($R^2 = 0.13$ and 0.15). For the steady-state ChlF parameters, Fv'/Fm' was significantly related with Car, Chl_{ab}, An, gs, Fv'/Fm', Fm', Y(II), and ETR ($R^2 = 0.34, 0.35, 0.47, 0.29, 0.58, 0.59, 0.45$, and 0.41 , respectively; $p < 0.001$). Fm and NPQ also had significant relationships with Fv'/Fm'. In general,

F_v/F_m and F_v'/F_m' were the representative ChlF parameters in dark- and light-adapted conditions. Due to their fundamental significance and practical utility, they are the subjects of our research, and discussed in later sections.

3.2. Dynamic Changes of F_v/F_m And F_v'/F_m' under Different N Treatments during the Growing Season

Figure 1A–D (Figure S1A–C, Figure S2A–C, Figure S3A–C, and Figure S4A–C) show the dynamic variation of F_v/F_m for winter wheat leaves in various treatments during the 2014 to 2015 growing season. The F_v/F_m of wheat leaves tended to increase with increasing N application, especially for the second and third leaves (Figure S1B,C). As shown in Figure 1A, F_v/F_m of wheat leaves in three N conditions increased from DAS 164 to DAS 172, and then decreased gradually from DAS 172 to DAS 187. The leaves in these three positions yielded a consistent tendency in the case of the F_v/F_m variation from DAS 164 to DAS 187 (Figure S1A–C, Figure S2A–C, Figure S3A–C and Figure S4A–C). However, the first expanded leaf under the N0 treatment, yielded a slightly different result (Figure S1A). The F_v/F_m kept increasing until DAS 179, and then decreased.

The temporal patterns of F_v'/F_m' measured during the 2014 to 2015 growing season were exhibited in Figure 1E–H, Figure S1D–F, Figure S2D–F, Figure S3D–F and Figure S4D–F). The F_v'/F_m' values under N4 applications were usually larger than that in N2 cases, but for the first leaves, it was slightly smaller than that in N2 cases from DAS 164 to DAS 179 (Figure S1D), but slightly larger from DAS 179 to DAS 187. The F_v'/F_m' values of the second and third leaves in the N2 and N4 cases were relatively close to that in the N2 treatment from DAS 164 to DAS 172 (Figure S1E,F), and higher than that in the N2 condition from DAS 172 to DAS 187. It was shown obviously that the F_v'/F_m' in the N2 and N4 treatments were higher than that in N0 case regardless of which leaf position. The F_v'/F_m' of the top three leaves increased gradually from DAS 164, and reached the maximum at DAS 172 or DAS 179. The F_v'/F_m' of the third leaves decreased more sharply than that of the first and second leaves.

Table 4. Relationships among leaf pigments, physiological indices, and ChlF parameters acquired during the 2015 growing season considering all data and all N treatments. The red color indicates significant correlations among Fv/Fm, Fv'/Fm', and other parameters listed in this table ($p < 0.05$).

	Leaf Pigment		Physiological Indices		Dark-adapted ChlF Parameters				Steady-state ChlF Parameters					
	Car	Chl _{ab}	An	gs	Fo	Fm	Fv/Fm	Fo'	Fs	Fm'	Fv'/Fm'	NPQ	Y(II)	ETR
Car	1													
Chl _{ab}	0.83***	1												
An	0.41***	0.32***	1											
gs	0.32***	0.26**	0.75	1										
Fo	0.0008	0.011	0.083	0.016	1									
Fm	0.20***	0.35***	0.12*	0.087*	0.54***	1								
Fv/Fm	0.51***	0.60***	0.55***	0.32***	0.017	0.54***	1							
Fo'	0.11**	0.11*	0.042	0.048	0.051	0.15*	0.13*	1						
Fs	0.13*	0.24***	0.0059	0.0097	0.34**	0.53***	0.20**	0.46***	1					
Fm'	0.32***	0.30***	0.30**	0.22*	0.0014	0.20**	0.52***	0.54***	0.31**	1				
Fv'/Fm'	0.34***	0.35***	0.47***	0.29***	0.017	0.17**	0.58***	0.0073	0.045	0.59***	1			
NPQ	0.035	0.0043	0.13*	0.078	0.22**	0.20**	0.085	0.12*	0.014	0.31**	0.25**	1		
Y(II)	0.063	0.016	0.39**	0.24**	0.0009	0.066	0.33***	0.0056	0.18**	0.25**	0.45***	0.53***	1	
ETR	0.063	0.016	0.40**	0.24**	0.37***	0.065	0.15*	0.0055	0.18**	0.25**	0.41***	0.53***	0.99***	1

* $p < 0.01$, ** $p < 0.05$, and *** $p < 0.001$.

Figure 1. Variations of F_v/F_m (A–D) and F_v'/F_m' (E,F,H) for leaves in all treatments under different N levels during the 2014 to 2015 growing season. V1 and V2 represent the wheat cultivar Shengxuan 6, and Yangmai 18. D1 and D2 mean the plant density of 25 and 40 cm. N0, N2, and N4 indicate the N treatments of 0, 150, and 300 kg/ha, respectively. (A and E, the leaf of VID1; B and F, the leaf of VID2; C and G, the leaf of V2D1; D and H, the leaf of V2D2).

3.3. Semi-empirical Models for Estimating the Leaf F_v/F_m And F_v'/F_m' Using Spectral Features

Tables 5 and 6 show the performance of three types of spectral features (VIs, REP, and wavelet feature) for monitoring leaf F_v/F_m and F_v'/F_m' in calibration and validation datasets.

3.3.1. Performance of VIs-based F_v/F_m and F_v'/F_m' Models in Calibration and Validation Datasets

The best-fit functions for the relationships between the reflectance ratio VIs and wheat leaf F_v/F_m and F_v'/F_m' were mainly linear, with R_c^2 values ranging from 0.22 to 0.67 for F_v/F_m , and 0.35 to 0.56 for F_v'/F_m' . Only the R685/R655 showed logarithmic relationships with F_v/F_m . For both F_v/F_m and F_v'/F_m' , the CUR exhibited the best goodness of fit among all the reflectance ratio VIs in the calibration dataset (Figure 2A,F), superior to R680/R630, followed by R685/R655 and R750/R800. When applying the reflectance ratio VIs regression models to the validation dataset, the RRMSEv produced ranged from 1.95% to 3.43% and from 4.28% to 5.51% for predictive F_v/F_m and F_v'/F_m' , separately. The CUR yielded the best performance in validation for predicting F_v/F_m ($R_v^2 = 0.50$, Bias = 0.0056, RMSEv = 0.012, and RRMSEv = 1.95%) shown in Figure 3A and for F_v'/F_m' ($R_v^2 = 0.38$, Bias = 0.0072, RMSEv = 0.030, and RRMSEv = 4.28%) exhibited in Figure 3F.

For the derivative VIs listed in Table 3, the best fitness between F_v/F_m and D705/D722, $D\lambda\rho/D(\lambda\rho+12\text{ nm})$, and DPi were linear, except for D730/D706 and $D\lambda\rho/D720$ which were logarithmic. The R_c^2 values of the derivative VIs and leaf F_v/F_m ranged from 0.38 to 0.64. D705/D722, D730/D706, and $D\lambda\rho/D720$ gave similar performances in the calibration dataset ($R_c^2 = 0.63$) (Table 5). When detecting F_v'/F_m' using derivative VIs, all the best fitting lines were linear, with R_c^2 values from 0.22 to 0.60. D730/D706 and $D\lambda\rho/D720$ performed similar, which were inferior to D705/D722. When the regression models of the calibration dataset were applied to the datasets acquired during the 2015 to 2016 growing season, the RRMSEv ranged from 1.87% to 3.69% for estimating F_v/F_m , and 3.96% to 6.41% for F_v'/F_m' . The D705/D722 produced the best prediction accuracy for F_v/F_m ($R_v^2 = 0.50$, Bias = 0.0037, RMSEv = 0.015, and RRMSEv = 1.87%) shown in Table 5 and for F_v'/F_m' ($R_v^2 = 0.40$, Bias = 0.0031, RMSEv = 0.028, and RRMSEv = 3.96%) depicted in Table 6, significantly better than that of the other derivative VIs.

Table 5. Performance of models for estimating the Fv/Fm based on vegetation indices (VIs), red edge positions (REPs), and wavelet features in calibration and validation.

FvFm		Indices	Calibration			Validation			
			Equation	Rc ²	RMSEc	Rv ²	Bias	RMSEv	RRMSEv
VI	Reflectance ratio VI	CUR	$y = -0.3963x + 1.196$	0.66	0.017	0.50	0.0056	0.012	1.95
		R680/R630	$y = 0.2209x + 0.5797$	0.59	0.019	0.46	0.0047	0.016	1.96
		R685/R655	$y = 0.3209\ln(x) + 0.7388$	0.50	0.021	0.25	0.021	0.027	3.43
		R750/R800	$y = -2.008x + 2.761$	0.21	0.026	0.10	0.011	0.024	2.96
	ChlF VI	D705/D722	$y = -0.06905x + 0.8604$	0.64	0.018	0.50	0.0037	0.015	1.87
		D730/D706	$y = 0.07926\ln(x) + 0.8109$	0.62	0.018	0.42	0.0012	0.016	1.95
		Dλρ/D720	$y = -0.08262\ln(x) + 0.7919$	0.64	0.018	0.28	0.0045	0.021	2.65
		Dλρ/D(λρ+12 nm)	$y = -0.1255x + 0.9238$	0.43	0.022	0.03	0.012	0.028	3.49
		DPi	$y = -0.4849x + 0.9379$	0.38	0.023	0.27	0.023	0.029	3.69
	Physiological VI	PRI	$y = 1.009 + 0.7439$	0.50	0.021	0.23	0.015	0.022	2.80
	Chl VI	MTCI	$y = 0.04633 + 0.7071$	0.61	0.018	0.33	−0.014	0.019	2.41
		CIred edge	$y = 0.1664 + 0.7056$	0.60	0.018	0.39	−0.013	0.022	2.79
	Structure VI	NDVI	$y = 0.2546x + 0.5875$	0.32	0.024	0.28	0.025	0.031	3.85
		EVI	$y = 0.001713x + 0.7663$	0.021	0.029	0.10	0.025	0.031	3.92
	Water VI	NDWI	$y = -0.005260x + 0.7703$	0.0012	0.029	0.15	0.025	0.032	4.01
		WI	$y = 0.7209x + 0.02677$	0.032	0.029	0.16	0.027	0.033	4.12
REP	REP	REP _{LI}	$y = 0.005224x - 2.961$	0.62	0.018	0.45	0.034	0.037	4.62
		REP _{PF}	$y = 0.004448x - 2.383$	0.68	0.016	0.43	−0.027	0.030	3.78
		REP _{LE}	$y = 0.003633x - 1.786$	0.64	0.018	0.43	0.037	0.040	5.06
		WREP-S3	$y = 3.155\ln(x) - 19.94$	0.66	0.017	0.48	−0.013	0.019	2.33
		WREP-S4	$y = 0.005376x - 3.052$	0.67	0.017	0.51	0.0025	0.014	1.80
Wavelet feature	Wavelet feature	WF(702 nm, scale 3)	$y = -0.5217x + 0.7406$	0.70	0.016	0.48	0.0071	0.015	1.91
		WF(637 nm, scale 3)	$y = -3.514x + 0.8036$	0.69	0.016	0.37	0.0089	0.016	1.99
		WF(704 nm, scale 4)	$y = -0.2112x + 0.7305$	0.69	0.016	0.55	0.0050	0.014	1.81
		WF(592 nm, scale 5)	$y = -0.4664x + 0.7930$	0.69	0.016	0.40	0.0089	0.016	1.98
		WF(575 nm, scale 6)	$y = -0.1260x + 0.7571$	0.68	0.016	0.41	0.0047	0.016	1.99

Table 6. Performance of models for estimating the Fv'/Fm' based on VIs, REPs, and wavelet features in calibration and validation.

Fv'/Fm'		Indices	Calibration			Validation			
			Equation	Rc^2	RMSEc	Rv^2	Bias	RMSEv	RRMSEv
VI	Reflectance ratio VI	CUR	$y = -0.4089x + 1.119$	0.55	0.022	0.38	0.0072	0.030	4.28
		R680/R630	$y = 0.2097x + 0.4987$	0.42	0.025	0.35	0.0074	0.030	4.28
		R685/R655	$y = 0.2811x + 0.3689$	0.37	0.026	0.18	0.022	0.039	5.51
		R750/R800	$y = -2.928x + 3.583$	0.36	0.026	0.24	0.0050	0.031	4.40
	ChlF VI	D705/D722	$y = -0.07581x + 0.7785$	0.60	0.021	0.40	0.0031	0.028	3.96
		D730/D706	$y = 0.1405x + 0.5920$	0.54	0.022	0.36	0.0034	0.030	3.96
		$D\lambda\rho/D720$	$y = -0.04609x + 0.7422$	0.52	0.023	0.26	0.0087	0.033	4.61
		$D\lambda\rho/D(\lambda\rho+12\text{ nm})$	$y = -0.1332x + 0.8426$	0.38	0.026	0.09	0.019	0.045	6.41
		DPi	$y = -0.4247x + 0.8264$	0.22	0.029	0.02	0.026	0.044	6.22
	Physiological VI	PRI	$y = 1.084x + 0.6512$	0.45	0.024	0.08	0.010	0.037	5.21
	Chl VI	MTCI	$y = 0.04936x + 0.6122$	0.55	0.022	0.3	-0.014	0.029	4.17
		CIred edge	$y = 0.1821x + 0.6088$	0.57	0.022	0.32	-0.011	0.030	4.07
	Structure VI	NDVI	$y = 0.2999x + 0.4644$	0.35	0.026	0.11	0.0025	0.041	5.88
		EVI	$y = -0.000004619x + 0.6792$	0.0000012	0.033	0.05	0.022	0.042	5.92
	Water VI	NDWI	$y = 0.008163x + 0.6797$	0.0022	0.033	0.06	0.022	0.041	5.82
		WI	$y = 0.8144x - 0.1604$	0.032	0.032	0.03	0.025	0.042	6.00
REP	REP	REP _{LI}	$y = 0.005767x - 3.439$	0.60	0.021	0.38	0.0069	0.029	4.15
		REP _{PF}	$y = 0.004753x - 2.690$	0.61	0.021	0.40	0.0050	0.028	3.94
		REP _{LE}	$y = 0.003943x - 2.095$	0.59	0.021	0.41	0.0035	0.027	3.84
		WREP-S3	$y = 0.004762x - 2.693$	0.59	0.021	0.43	0.0016	0.027	3.76
		WREP-S4	$y = 0.005792x - 3.438$	0.61	0.021	0.43	0.0028	0.026	3.74
Wavelet feature	Wavelet feature	WF(630 nm, scale 3)	$y = -5.381x + 0.7119$	0.59	0.021	0.24	-0.0034	0.031	4.41
		WF(702 nm, scale 3)	$y = -0.5553x + 0.6480$	0.62	0.020	0.37	-0.0009	0.028	4.03
		WF(465 nm, scale 4)	$y = 4.335x + 0.7136$	0.58	0.021	0.31	-0.0112	0.031	4.46
		WF(704 nm, scale 4)	$y = -0.2197x + 0.6440$	0.62	0.020	0.43	-0.0028	0.027	3.79
		WF(707 nm, scale 7)	$y = -0.1804x + 0.7008$	0.61	0.020	0.37	-0.0012	0.028	3.94

Figure 2. Leaf F_v/F_m and F_v'/F_m' values plotted against five spectral features which performed best in each group: Curvature index (CUR) (A,F), D705/D722 (B,G), wavelet-based REP (WREP-S4) (C,H), Clred edge (D,I), and wavelet feature (WF) (704 nm, scale 4) (E,J). The black solid line is for all data in the growing season. All regressions are statistically significant ($p < 0.01$). Data points from the booting, heading, anthesis, and filling stages are shown in red (circle), magenta (triangle), green (square), and blue (cross), respectively.

Figure 3. Plots of measured and predicted leaf F_v/F_m and F_v'/F_m' developed from the regression models using CUR (A,F), D705/D722 (B,G), WREP-S4 (C,H), Clred edge (D,I), and wavelet feature (WF) (704 nm, scale 4) (E,J). Data points from the booting, heading, and filling stages are shown in red (circle), green (triangle), and blue (cross), respectively.

The PRI, as a physiological VI, showed a little worse calibration performance than some ChlF VIs, and it did not yield a positive result in validation. The sensitivities of structure VIs and water VIs to F_v/F_m and F_v'/F_m' were much poorer than that of Chl VIs. The MTCI and Clred edge had similar performances for predicting F_v/F_m and F_v'/F_m' in the calibration dataset, with R^2 around 0.61 and 0.55, respectively. But with regards to the predictive accuracy of leaf F_v/F_m inversion, the best validation results were obtained by Clred edge ($R_v^2 = 0.39$, Bias = -0.013 , $RMSE_v = 0.022$, and $RRMSE_v = 2.79\%$), which also was the most accurate Chl VIs to derive leaf F_v'/F_m' ($R_v^2 = 0.32$, Bias = -0.011 , $RMSE_v = 0.030$, and $RRMSE_v = 4.07\%$). In addition, the VIs related to structure properties and water content were proven to be invalid to estimate F_v/F_m and F_v'/F_m' in the validation dataset.

3.3.2. Performance of REP for Predicting Leaf Fv/Fm and Fv'/Fm' in Calibration and Validation

The best relationships between the REPs and Fv/Fm all were linear except WREP-S3, and all linear functions for Fv'/Fm'. There are not much difference between the five REPs monitoring A Fv/Fm or Fv'/Fm'. However, with regards to the validation performance, WREP-S4 showed better performance in estimating leaf Fv/Fm and Fv'/Fm' than the other four REPs, with the highest predictive accuracy ($R_v^2 = 0.51$ for Fv/Fm and $R_v^2 = 0.43$ for Fv'/Fm') and the lowest error (RRMSEv = 1.80% for Fv'/Fm' and RRMSEv = 3.74% for Fv'/Fm') as shown in Figure 2C,H, followed by WREP-S3.

3.3.3. Models for Estimating the Leaf Fv/Fm and Fv'/Fm' Based on Wavelet Features

Figure 4A shows the top 1% wavelet feature regions relevant to the ChlF parameter Fv/Fm for the dataset acquired during the 2014 to 2015 growing season. These wavelet features were formed in five feature regions in the visible and near-infrared regions. All of them occurred from scale 3 to scale 6. These five wavelet features were identified as significantly related to Fv/Fm ($P < 0.0001$) with R_c^2 values for the linear regressions ranging from 0.68 to 0.70 (Table 5). According to the R_c^2 values, the wavelet features sensitive to Fv/Fm were WF (702 nm, scale 3), WF (637 nm, scale 3), WF (704 nm, scale 4), WF (592 nm, scale 5), and WF (575 nm, scale 6) in sequence, which gave similar performances for the estimation of the Fv/Fm of winter wheat. When the regression models of the calibration dataset were used as the validation dataset, WF (704 nm, scale 4) showed the best transferability yielding the highest precision ($R_v^2 = 0.55$) and lowest predicted error (Bias = 0.0050, RMSEv = 0.014, and RRMSEv = 1.81%; Figure 3E and Table 5).

Figure 4. The wavelet feature regions with the top 1% coefficients of determination between wavelet power and wheat leaf Fv/Fm and Fv'/Fm' for the dataset acquired during the 2014 to 2015 growing season.

Six feature regions relevant to variation of leaf ChlF parameter Fv'/Fm' were determined according to their relationships with extracted wavelet power, which covered the visible region between 460 and 710 nm (Figure 4B), distributing on scale 3, 4, 7, and 8. These feature regions are WF (465 nm, scale 4), WF (630 nm, scale 3), WF (702 nm, scale 3), WF (704 nm, scale 4), WF (707 nm, scale 7), and WF (688 nm, scale 8). The wavelength of the first feature was closed to the blue band, in which the N-carbon compounds have absorption characteristics. Other features were located in the red region and red edge area, including the 630 nm, 688 nm, and 702 to 707 nm, in which the reflectance is strongly absorbed by Chl. Among these six wavelet features, the top five features were selected to estimate the leaf Fv'/Fm'. All of the best-fit functions for the relationships between wavelet features and leaf Fv'/Fm' were linear, with R_c^2 values from 0.58 to 0.62. The WF (465 nm, scale 4) located in the blue region produced the worst agreement in the calibration dataset and the independent validation dataset as compared with the other four wavelet features. And regardless of which dataset, the best predictive

accuracy and the lowest predictive error were found when using the WF (704 nm, scale 4), followed by WF (702 nm, scale 3), and WF (707 nm, scale 7).

4. Discussion

4.1. Signature Components of Leaf ChlF Parameters and Their Variation with N Regimes

Compared with other ChlF parameters in dark-adapted and steady-state condition, F_v/F_m and F_v'/F_m' , two ChlF parameters related to the photochemical efficiency of PSII, were more significantly correlated with leaf pigments, physiological indices, and other ChlF parameters (Table 4). F_v/F_m and F_v'/F_m' , relevant to the quantum function of the PSII reaction center, have been used to study plant response to environmental factors [43–45], analyze the correlation to net photosynthesis and quantum yield, indicate leaf N and sulfur content, Chl concentration [46], and detect plant stress and evaluate the nutritional and healthy status of plants [47]. Therefore, F_v/F_m and F_v'/F_m' are seen as the subjects of this study due to their fundamental significance and practical utility.

Compared with the variation of L1, L2, and L3 under N0, N2, and N4 treatments (Figure S1) for wheat leaf in V1D1, those in V1D2, V2D1, and V2D2 were similar overall. This seasonal trend is common, existing in multiple genotypes of wheat sown with different plant densities. In general, the changes of F_v/F_m and F_v'/F_m' for wheat leaves at different leaf positions and different N treatments were basically the same as the findings of other studies [4]. At the heading or anthesis stages, F_v/F_m and F_v'/F_m' reached a peak, and then decreased, which is mainly due to the exuberant growth and strong physiological activity of plants. Within a certain range of fertilizer applications (i.e., N0 to N2), F_v/F_m increased with an increase of N application [4]. It shows that the efficiency of light energy conversion and potential activity of photosynthetic reaction center were increased, with decreased damage on the photosynthetic apparatus. However, there was a slight decrease in F_v'/F_m' in the N4 treatment as compared with the N2 application (Figure 1D), and similar F_v/F_m in the N4 and N2 cases (Figure 1A) [48]. Photosynthetic capacity was not improved under excess N, which indicates that excessive N is not effective in photosynthetic performance. The reason why N can cause a change of ChlF parameters is because different N applications affect the photochemistry of PSII by altering the quantum yield of PSII electron transport [49].

4.2. Comparing VIs and REPs for Quantitatively Retrieving F_v/F_m and F_v'/F_m'

Many ChlF VIs have been proposed to exploit the effect of ChlF on the apparent reflectance spectrum in the red edge region (from 650 to 800 nm) [13]. By dividing the sample into different growth periods, it was concluded that phenological stages did not cause a significant impact on the performance of these spectral features for monitoring F_v/F_m and F_v'/F_m' (S5). Among the reflectance ratio VIs, the CUR yielded the best relationship with leaf F_v/F_m and F_v'/F_m' (Figure 3A), followed by R680/R630, which was also found in the case of tracking F_v/F_m variations under various Chl contents [12]. Both R680/R630 and CUR can well capture the reflectance changes centered around 680 nm, therefore, they can track the curvature of reflectance caused by ChlF emission (in the red edge region from 670 nm to 770 nm, with the central emission at 690 nm and 730 nm) as reported earlier [14]. Derivative VIs minimizing other confounding effects, can detect subtle changes due to the ChlF emission in the red edge region. Among all the derivative VIs listed in this study, the best predictive accuracy was found when using D705/D722 to estimate F_v/F_m and F_v'/F_m' in various N treatments, which is consistent with an independent study [13]. Our study provided confirmatory evidence that the inference of ChlF using reflectance ratio VIs and derivative VIs is feasible with acceptable accuracy.

The REP, the wavelength of the inflexion point in the red edge region of reflectance, has received much attention over the years for understanding the properties of plants, such as the Chl content, N content of the leaf, and the leaf area index [50,51]. Among five methods widely used to extract REP, WREP-S4 yielded more stable results than VIs relevant to ChlF, physiological status, Chl content,

water content, and structure properties. In comparison with VIs related to physiological status, those for Chl content, water content, and structure properties were not effective for monitoring ChlF parameters. With respect to NDVI and PRI, they varied with the development of vegetation, but NDVI can be saturated before PRI [52]. And the bands sensitive to the photosynthesis and water content are definitely different. Although Clred edge showed acceptable performance in calibration, the transferability of Clred edge-based model was not ideal. However, REP can characterize signals in the red edge region, where ChlF exists. Therefore, according to the good agreement between REP and Fv/Fm , Fv'/Fm' , it implies that REP can be used as the functions of ChlF emission bands in the red edge region, which is consistent with previous theoretical descriptions of ChlF effects on reflectance or the derivative spectra [14].

4.3. Comparison between CWA and Other Spectral Features for Estimating Fv/Fm and Fv'/Fm'

CWA, as an effective mathematical method, has been extensively used to retrieve useful information from hyperspectral data, such as leaf biochemical parameters [25]. Comparing the wavelet features associated with Fv/Fm and Fv'/Fm' , we found that WF (702 nm, scale 3), WF (637 nm, scale 3), and WF (704 nm, scale 4) were their common wavelet features. Among these three wavelet features, WF (704 nm, scale 4) outperformed and exhibited the best stability, not only for Fv/Fm estimation but also for Fv'/Fm' . Therefore, WF (704 nm, scale 4) is considered to be a robust spectral feature for tracking Fv/Fm and Fv'/Fm' collected throughout various significant growth stages and different N treatment regimes.

According to a comprehensive evaluation of the performances in calibration and validation, the reflectance ratio VI CUR, the derivative VI D705/D722, the REP extracted by WREP-S4 technique, Clred edge related to Chl content, and WF (704 nm, scale 4) derived by CWA performed best and exhibited good reliability in their respective groups. The best wavelet feature WF (704 nm, scale 4) was superior to WREP-S4, but not significantly. Both of them were extracted at the fourth scale and centered around 704 nm. WF (704 nm, scale 4) has been used to retrieve the leaf Chl contents of cereal crops [19], and therefore it confirms that this wavelet feature is not only sensitive to Chl content but also ChlF signal. In summary, WREP-S4 and wavelet feature WF (704 nm, scale 4) permitted operational quantification of Fv/Fm and Fv'/Fm' using hyperspectral data.

On the basis of a comparison of the performance of these spectral features for detecting Fv/Fm and Fv'/Fm' , we concluded that Fv/Fm could be estimated more accurately than Fv'/Fm' . This may be because the change of Fv/Fm is very small under non-stress conditions and it is not affected by species and growth conditions. The performance of WREP and wavelet feature for detecting ChlF parameters should be tested for more vegetation types. Moreover, at the leaf scale, ChlF signal is to a certain extent influenced by environmental factors, however, when these findings are extended to the canopy and regional scales, additional complexity should be considered, such as canopy structure, canopy heterogeneity, and the interaction of ChlF signal with atmosphere.

5. Conclusions

In this study, three reflectance-based approaches (VIs, REPs, and wavelet features) were used to extract the optimal spectra features from apparent reflectance for estimation of two significant ChlF parameters, Fv/Fm and Fv'/Fm' . They were related to photosynthetic apparatus, especially PSII activity, and were more relevant to pigment content and physiological parameters than other ChlF parameters. In each group, the spectral feature most relevant to Fv/Fm and Fv'/Fm' were the same, and all of them were calculated based on bands in the red edge region, where the ChlF signal occurs. The results also showed that the ChlF VIs (CUR and D705/D722) are effective indicators for the estimation of Fv/Fm and Fv'/Fm' under various treatment regimes. Meanwhile, the performance of REPs and wavelet feature for the estimation of Fv/Fm and Fv'/Fm' were evaluated and compared with that of traditional VIs related to ChlF, physiological status, Chl content, water content, and structure properties. The accuracy of WREP-S4 in both calibration and validation was better than REPs extracted by other

algorithms. Among all wavelet features, WF (704 nm, scale 4) yielded the best predictive accuracy for Fv/Fm and Fv'/Fm' estimation. Additionally, both WREP-S4 and WF (704 nm, scale 4) were all centered around 704 nm. This work demonstrates that the use of WREP-S4 and WF (704 nm, scale 4) provide more reliable assessments of the ChlF parameters Fv/Fm and Fv'/Fm' than empirical methods based on traditional VIs. Therefore, the red edge region can be exploited for estimation of Fv/Fm and Fv'/Fm'. These findings provide new insight for quantifying ChlF signal more accurately using reflectance-based approaches.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2072-4292/11/23/2838/s1>, Figure S1: The variations of Fv/Fm (A, B, C) and Fv'/Fm' (D, E, F) for leaves in V1D1 treatments under different N levels in the 2014–2015 growing season. V1 represents the wheat cultivar Shengxuan 6, D1 means the rowledge 25 cm. (A, D: the top first expanded leaf (L1); B, E: the top second expanded leaf (L2); C, F: the top third expanded leaf (L3)). N0, N2 and N4 indicate the N treatments of 0, 150 and 300 kg/ha, respectively., Figure S2. The variations of Fv/Fm (A, B, C) and Fv'/Fm' (D, E, F) for leaves in V1D2 treatments under different N levels in the 2014–2015 growing season. V1 represents the wheat cultivar Shengxuan 6, D2 means the rowledge 40 cm. (A, D: the top first expanded leaf (L1); B, E: the top second expanded leaf (L2); C, F: the top third expanded leaf (L3)). N0, N2 and N4 indicate the N treatments of 0, 150 and 300 kg/ha, respectively. Figure S3. The variations of Fv/Fm (A, B, C) and Fv'/Fm' (D, E, F) for leaves in V2D1 treatments under different N levels in the 2014–2015 growing season. V2 represents the wheat cultivar Yangmai 18, D1 means the rowledge 25 cm. (A, D: the top first expanded leaf (L1); B, E: the top second expanded leaf (L2); C, F: the top third expanded leaf (L3)). N0, N2 and N4 indicate the N treatments of 0, 150 and 300 kg/ha, respectively. Figure S4. The variations of Fv/Fm (A, B, C) and Fv'/Fm' (D, E, F) for leaves in V2D2 treatments under different N levels in the 2014–2015 growing season. V2 represents the wheat cultivar Yangmai 18, D2 means the rowledge 40 cm. (A, D: the top first expanded leaf (L1); B, E: the top second expanded leaf (L2); C, F: the top third expanded leaf (L3)). N0, N2 and N4 indicate the N treatments of 0, 150 and 300 kg/ha, respectively.

Author Contributions: M.J. and X.Y. designed the experiment; M.J., D.L., Y.W., and X.W. contributed to the field experiments and data collection; M.J., D.L., R.C., T.C., Y.Z., and X.Y. performed the overall data analysis; T.C., Y.Z., X.Y., C.X., G.O., H.L., and C.Z. contributed to data collection; M.J., D.L., R.C., and X.Y. drafted the manuscript; all authors read and approved the final manuscript.

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