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Estimation of vertical distribution of chlorophyll concentration by bi-directional canopy reflectance spectra in winter wheat

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Abstract An effective technique to measure foliage chlorophyll concentration (Chl) at a large scale and within a short time could be a powerful tool to determine fertilization amount for crop management. The objective of this study was to investigate the inversion of foliage Chl vertical-layer distribution by bi-directional reflectance difference function (BRDF) data, so as to provide a theoretical basis for monitoring the growth and development of winter wheat and for providing guidance on the application of fertilizer. Remote sensing could provide a powerful tool for large-area estimation of Chl. Because of the vertical distribution of leaves in a wheat stem, Chl vertical distribution characteristics show an obvious decreasing trend from the top of the canopy to the ground surface. The ratio of transformed chlorophyll absorption reflectance index (TCARI) to optimized soil adjusted

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vegetation index (OSAVI) was called the canopy chlorophyll inversion index (CCII) in this study. The value of CCII at nadir, ±20 and ±30°, at nadir, ±30 and ±40°, and at nadir, ±50 and ±60° view angles were selected and assembled as bottom-layer Chl inversion index (BLCI), middle-layer Chl inversion index (MLCI), and upper-layer Chl inversion index (ULCI), respectively, for the inversion of Chl at the vertical bottom layer, middle layer, and upper layer. The root mean squared error (RMSE) between BLCI-, MLCI-, and ULCI-derived and laboratory-measured Chl were 0.7841, 0.9426, and 1.7398, respectively. The vertical foliage Chl inversion could be used to monitor the crop growth status and to guide fertilizer and irrigation management. The results suggested that vegetation indices derived from bi-directional reflectance spectra (e.g., BLCI, ULCI, and MLCI) were satisfactory for inversion of the Chl vertical distribution.

Keywords Winter wheat · Bi-directional reflectance difference function · Chlorophyll concentration · Vertical distribution · Canopy chlorophyll inversion index

Abbreviations

Chl	Chlorophyll concentration
BRDF	Bi-directional reflectance difference function
TCARI	Transformed chlorophyll absorption reflectance index
CARI	Chlorophyll absorption in reflectance index
OSAVI	Optimized soil adjusted vegetation index
CCII	TCARI/OSAVI
BLCI	Bottom-layer Chl inversion index
MLCI	Middle-layer Chl inversion index
ULCI	Upper-layer Chl inversion index
RMSE	The root mean squared error
LOV	Leaf orientation value
LAD	Leaf angle distribution
VZA	View zenith angles

Introduction

The chlorophyll concentration (Chl) in leaves and canopies can be a key indicator of physiological stage, productivity, and stress (Shadchina et al. 1998; Filella et al. 1995). A precise estimation of plant Chl status is important for providing nitrogen (N) fertilization recommendation because the leaf Chl concentration is mainly determined by N availability (Filella et al. 1995). Reflectance in parts of visible region is mainly influenced by Chl (Thomas and Gausman 1977). Therefore, it is possible that reflectance spectra in this region could be used to estimate Chl and N status (Thomas and Oerther 1972). Using Chl reflectance, non-destructive technology has been developed for Chl detection. Linear relationships between portable leaf chlorophyll measurement (SPAD, a chlorophyll meter based on reflectance in the NIR (940 nm) and red (650 nm)) values and Chl were observed (e.g., Adamsen et al. 1999; Campbell et al. 1990), and the results suggested that the SPAD could be successfully applied in crop N management. The difficulty is how to effectively gather Chl information over a relatively wide area quickly from SPAD values. Remote sensing provides site-specific, large-area, promising, and non-destructive estimation of crop Chl status. It can be used to monitor N status because foliage Chl concentration is mainly determined by N availability (e.g., Filella et al. 1995). Numerous studies and experiments have been undertaken in the research for Chl estimation based on field measurements, model inversion or empirical and semi-empirical methods at the leaf or the canopy level (e.g., Curran et al. 1991; Miller et al. 1991; Daughtry et al. 2000; Demarez and Gastellu-Etchegorry 2000; Zarco-Tejada et al. 2001; Sims and Gamon 2002; Haboudane et al. 2002).

The winter wheat canopy reflectance spectrum is affected by wheat canopy (e.g., leaf area index and leaf angle distribution, leaf water content, mineral status, parasitic attacks), soil (soil properties, soil color), weeds (weed species, amounts), environment, and their interactions. In general, the estimation of Chl concentration is based on the relationships between upper-layer leaves/mixed leaves of the canopy and vegetation indices (Vis) (Pearcy and Seemann 1990; Poorter et al. 1995) whereas differences in Chl at different vertical layers are not taken into consideration. Due to the vertical distribution of leaves in a wheat stem, the Chl vertical distribution characteristics cause an obvious decreasing trend contribution from the canopy top to the ground surface (Wang et al. 2005a). The Chl vertical distribution showed it is necessary to improve the inversion precision of the middle and bottom layers Chl concentration for guiding fertilizer application.

Pearcy and Seemann (1990) pointed out that leaves near the bottom of the canopy had lower Chl a/b ratios as compared with upper leaves. Therefore, the accuracy of estimating Chl concentration by VIs can be strongly influenced by the non-uniformity of the plant canopy and the fact that the reflectance sensor utilized a tri-dimensional view (Lemaire et al. 1991).

Foliage Chl and N are usually transformed from lower- to upper-layer leaves during shortages of nitrogen supply (Hikosaka et al. 1994; Anten et al. 1995; Vouillot and Devienne-Barret 1999). Vertical gradients of leaf Chl (Pearcy and Seemann 1990; Lemaire et al. 1991) and leaf N are common features in canopies of crops (Shiraiwa and Sinclair 1993; Connor et al. 1995). Wang et al. (2004, 2005a, b) studied the foliage N vertical distribution characteristics along the canopy in winter wheat. The objective of this study was to investigate the relationships between bi-directional reflected VIs and the canopy Chl status, and to improve the estimation accuracy of foliage Chl vertical distribution. The vertical foliage Chl inversion can be used for monitoring the crop growth status and for guiding fertilizer application (Zhao et al. 2007).

Materials and methods

Experimental design

The field experiment was conducted at Beijing Xiaotangshan Precision Agriculture Experimental Base, in Changping district, Beijing (40°10.6′ N, 116°26.3′ E) from September 2002 to June 2003 (experiment A) and September 2003 to June 2004 (experiment B). Experimental data from experiment A were used to calibrate the Chl vertical distribution evaluation models, whereas the data from experiment B were used to validate the established Chl vertical distribution models. Each winter wheat variety was planted in a plot size of 32.4×30.0 m^2 under the same irrigation and nitrogen treatments.

The site is located in the warm temperate zone with a mean annual rainfall of 507.7 mm and a mean annual temperature of 13°C. The nutrient content of the soil (0–0.30 m) was as follows: 14.2–14.8 g kg^{-1} of organic matter; 0.81–1.00 g kg^{-1} of N–NO_3 nitrogen; 20.1–55.4 mg kg^{-1} of available phosphorus; and 117.6–129.1 mg kg^{-1} of available potassium.

Crop canopy geometry was classified by crop leaf orientation value (LOV). It was calculated from Eq. 1 (Pepper et al. 1977):

$$\mathrm{LOV} = \sum_{i=1}^{n} \left[a(h/L)_i/n \right], \quad (1)$$

where a is the leaf inclination angle ($a = 90° - \theta$); θ is the angle between leaf tangent and the stem); h is the distance from leaf base point to the zenith of the leaf; L is the leaf length; and n is the leaf number.

The wheat varieties with LOV $\geq$ 45° were treated as erectophile leaf angle distribution (LAD) varieties; those with 25° < LOV < 45° were treated as planophile LAD varieties, and those with LOV $\leq$ 25° were treated as horizontal LAD varieties. Eighteen winter wheat varieties were investigated in experiment A: six erectophile LAD varieties (Lumai21 (LM21), Jing411 (J411), P7, Laizhou3279 (LZ3279), Nongda3291 (ND3291), and I-93); six planophile LAD varieties (Jingwang10 (JW10), 6211, CA16, 95128, Jingdong8 (JD8), Chaoyou66 (CY66)); and six horizontal LAD varieties (Zhongyou 9507 (ZY9507), Jing 9428 (J9428), 4P3, Linkang2 (LK2), Nongda 3214 (ND3214), and Zhongmai 16 (ZM16)).

Twelve winter wheat varieties were investigated in experiment B at the same experimental base as in experiment A: six erectophile LAD varieties (I-93, J411, LZ3279, LM21, ND3291, and P7); and six horizontal LAD varieties (4P3, ZM16, J9428, ZY9507, LK2, and ND3214).

Data acquisition

In situ canopy reflectance spectrum

In each plot, a 1 × 1 m^2 area of crop canopy was selected to measure canopy reflectance and to analyze biophysical and biochemical crop canopy features. Canopy reflectance was measured at a height of 1.3 m, under clear sky conditions between 10:00 and 14:00 local time, using an ASD FieldSpec Pro spectrometer (Analytical Spectral Devices, Boulder, CO, USA) fitted with a 25°-field-of-view fiber optic adaptor and operated in the 350–2500 nm spectral range. A 0.4 × 0.4 m^2 $BaSO_4$ calibration panel was used for calculating the baseline reflectance. Twenty scans were averaged into one spectral sample, representing the spectral observation at 1 × 1 m^2 plot. Calibration panel reflectance measurements were taken before and after the vegetation measurements.

Canopy BRDF reflectance spectrum

Canopy bi-directional reflectance difference function (BRDF) reflectance was measured using the same spectrum instrument as when measuring the in situ canopy reflectance. The instrument was fixed on a rotating bracket (Fig. 1), which enables BRDF observation of the same object in a short time duration. The observation planes were the principal plane and the cross-principal plane. The view zenith changes from 0 to 65° at intervals of 5°. The sun zenith changed during measurements from 32.8 to 41.2°. More detailed information about BRDF measurement can be found in the published papers by the author, and the experiments were done just in the same field as this paper mentioned (Huang et al. 2006; Wang et al. 2009).

Fig. 1 Rotating bracket for observing BRDF canopy reflectance

Foliar Chl vertical distribution sampling method

The plants in the 1-m^2 area were divided into three layers of equal vertical thickness (from top to bottom: layer 1 is the top, layer 3 is the bottom; see Fig. 2). The divided height was standardized as the actual height of the plant at the elongation stage (Zadok stage 3) and the average height from the top of the flag leaf to the ground at anthesis (Zadok stage 5) in experiment A (Zadoks et al. 1974). Four layers were divided at the anthesis stage in experiment B, with the layer 1, layer 2, and layer 3 almost divided as experiment A and the ear was regarded as one separate layer.

Foliar Chl concentration

After each reflectance measurement, wheat in the areas corresponding to the footprint of the in situ spectrum was harvested to soil level, packed in cooled black plastic bags and

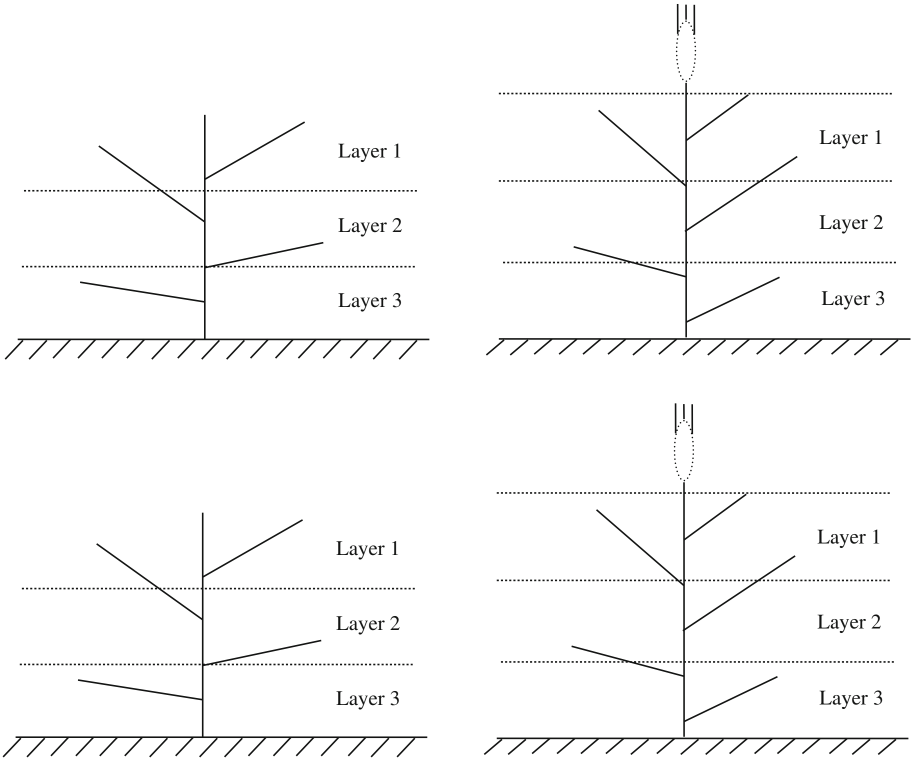

Fig. 2 The sketch map of different layers in the canopy at elongation (*left*) and anthesis (*right*) stages in the modeling experiment

transported to the laboratory for subsequent analysis. Chl was extracted with 80% acetone and calculated with the formula described by Arnon (1949). The absorption features at wavelengths of 645 and 663 nm were measured with a Helios α spectrophotometer (Thermo Electron Company, Cambridge, UK).

Results and analysis

Wheat canopy characteristics at different view zenith angles (VZA)

This study focused on the inversion of foliage Chl vertical layers distribution by BRDF data, so as to provide a theoretical basis for monitoring the growth and development status of winter wheat and for providing guidance on the application of nitrogen fertilizer.

The digital camera pictures of different LAD varieties under different VZA are shown in Fig. 3 (the VZA was 0, 20, 30, 40, 50, and 60°). The pictures indicated the different upper-, middle-, and bottom-layer crop characteristics for different LAD varieties (Fig. 3a is the observation pictures of erectophile LAD variety Laizhou 3279, Fig. 3b is the observation pictures of planophile LAD variety Jingwang 10 and Fig. 3c is the observation pictures of horizontal LAD variety Linkang 2).

The digital camera pictures of different LAD varieties for 0° (nadir VZA) contained more upper-layer foliage and background soil information than those of the other bi-

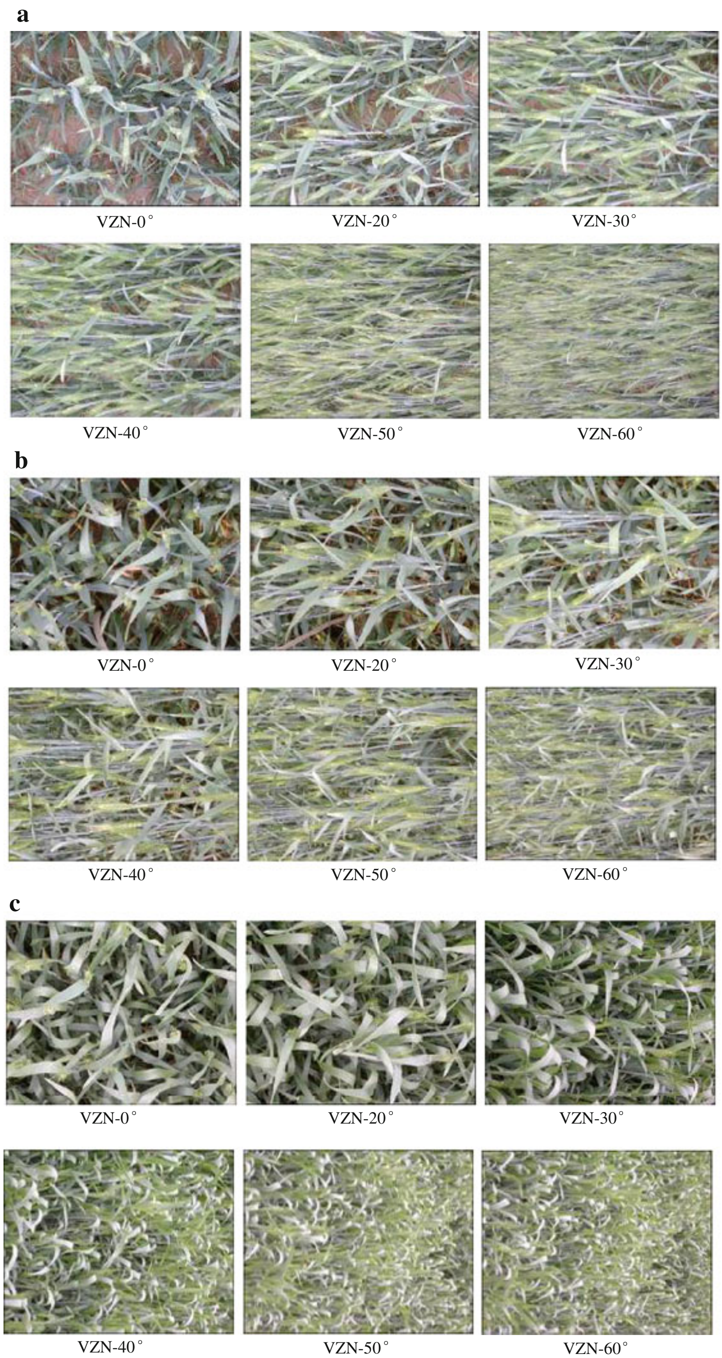

Fig. 3 Bi-directional view zenith angle (VZA) observation pictures of different LAD varieties at jointing stage. *Note*: **a** Erectophile LAD variety Laizhou 3279. **b** Planophile LAD variety Jingwang 10. **c** Horizontal LAD variety Linkang 2

directional VZA. The digital camera pictures of different LAD varieties for 20 and 30° VZA contained more bottom-layer foliage information than those of the other bi-directional VZA. The digital camera pictures of different LAD varieties for 40° VZA contained more middle-layer foliage information than that of the other bi-directional VZA. The digital camera pictures of different LAD varieties for 50 and 60° VZA contained more upper-layer foliage information than those of the other bi-directional VZA. This indicated that the crop upper layer, middle layer, and bottom layer information could be expressed by VZA at '50 and 60°', '30 and 40°', and '20 and 30°', respectively.

Inversion of foliage Chl vertical distribution by bi-directional canopy reflectance spectrum

Leaf area index (LAI) plays an important role in the canopy reflectance spectrum; specifically, the LAI dominated the near-infrared spectrum characteristics of the canopy reflectance spectrum. When we attempt to invert crop Chl, the effect of LAI on canopy reflectance spectrum should be eliminated or minimized as much as possible. Huang et al. (2004a) used the canopy chlorophyll inversion index (CCII) for the inversion of Chl among different LAD varieties by canopy reflected spectra. In addition, Huang et al. (2004b) have also conducted a thorough study involving three cultivars under four levels of water irrigated and four levels of nitrogen fertilized conditions, and revealed that with LAI values ranging from 1 to 5, a very good agreement existed between laboratory measured chlorophyll concentration and CCII, which confirmed that CCII can be used to minimize the effects of LAI and non-photosynthetic materials on retrieval of canopy chlorophyll concentration. The CCII was defined as follows:

$$\text{CCII} = \text{TCARI} / \text{OSAVI}, \qquad (2)$$

$$\text{TCARI} = 3[(R_{700} - R_{670}) - 0.2(R_{700} - R_{550})(R_{700}/R_{670})]$$

$$\text{OSAVI} = (1 + 0.16)(R_{800} - R_{670})/(R_{800} + R_{670} + 0.16),$$

where TCARI was ameliorated from the Modified Chlorophyll Absorption in Reflectance Index (MCARI), which was proposed by Daughtry et al. (2000) as the Chlorophyll Absorption in Reflectance Index (CARI) developed by Kim et al. (1994). TCARI is resistant to non-green biomass effects, but it is still sensitive to the underlying soil reflectance properties, particularly for low LAI (Rondeaux et al. 1996). To overcome this problem, Daughtry et al. (2000) proposed that the MCARI be combined with a soil-line vegetation index such as the Optimized Soil-Adjusted Vegetation Index (OSAVI; Rondeaux et al. 1996). OSAVI belongs to the Soil-Adjusted Vegetation Index (SAVI; Huete 1988). This index (CCII) proved to be sensitive to Chl and very resistant to LAI (Huang et al. 2004a, b; Haboudane et al. 2002). This paper introduced the application of CCII at different VZA angles to invert the Chl vertical distribution (upper-layer, middle-layer, and bottom-layer Chl) among different LAD varieties.

In this study, the values of CCII at nadir, ± 20° (positive VZA is observing back to the sun and negative VZA is observing facing the sun in the same principle plane, the same as the following), and ± 30° view angles were assembled as bottom-layer Chl inversion index (BLCI) for inversion of bottom-layer Chl; the CCII at nadir VZA contained more background soil information, and the CCII at 20° and 30° VZA contained more bottom-layer foliage information. The BLCI was defined as:

$$\mathrm{BLCI} = \mathrm{BLCI_b} / \mathrm{BLCI_f}, \quad (3)$$

$$\mathrm{BLCI_b} = (0.4 * \mathrm{CCII_{A20}} + 0.6 * \mathrm{CCII_{A30}})/\mathrm{CCII_{A0}},$$

$$\mathrm{BLCI_f} = (0.4 * \mathrm{CCII_{A-20}} + 0.6 * \mathrm{CCII_{A-30}})/\mathrm{CCII_{A0}},$$

where $\mathrm{BLCI_b}$ and $\mathrm{BLCI_f}$ are back-to-the-sun and facing-the-sun VZA, respectively; $\mathrm{CCII_{A0}}$, $\mathrm{CCII_{A20}}$, $\mathrm{CCII_{A30}}$, $\mathrm{CCII_{A-20}}$, and $\mathrm{CCII_{A-30}}$ are the CCII values for the VZA at nadir, 20° back-to-the-sun, 30° back-to-the-sun, 20° facing-the-sun, and 30° facing-the-sun VZA.

The BLCI is used to establish a predictive linear regression equation to estimate bottom-layer foliage Chl. The prediction regression equation of bottom-layer foliage Chl by BLCI has the form ($n = 36$):

$$y = 4.3628x - 1.9221\left(R^2 = 0.7738\right), \quad (4)$$

where x is BLCI, and y is bottom-layer foliage Chl.

The reliability of the regression equation can be seen in Fig. 4. Canopy bi-directional reflectance spectrum and Chl were acquired among different LAD varieties. They reveal a good agreement between bottom-layer foliage Chl and CCII, with a Pearson $R^2 = 0.7738$ ($n = 36$). The regression was processed in the software of SPSS 13.0, and so as the following regressions.

In this study, the values of CCII at nadir, ±30 and ±40° view angles were assembled as the middle-layer Chl inversion index (MLCI) for inversion of middle-layer Chl. The CCII at nadir VZA contained more background soil information, and the CCII at 30 and 40° VZA contained more middle-layer foliage information. The MLCI was defined as:

$$\mathrm{MLCI} = \mathrm{MLCI_b} / \mathrm{MLCI_f}, \quad (5)$$

$$\mathrm{MLCI_b} = (0.4 * \mathrm{CCII_{A30}} + 0.6 * \mathrm{CCII_{A40}})/\mathrm{CCII_{A0}},$$

$$\mathrm{MLCI_f} = (0.4 * \mathrm{CCII_{A-30}} + 0.6 * \mathrm{CCII_{A-40}})/\mathrm{CCII_{A0}},$$

where $\mathrm{MLCI_b}$ and $\mathrm{MLCI_f}$ are back-to-the-sun and facing-the-sun VZA, respectively; $\mathrm{CCII_{A0}}$, $\mathrm{CCII_{A30}}$, $\mathrm{CCII_{A40}}$, $\mathrm{CCII_{A-30}}$, and $\mathrm{CCII_{A-40}}$ are the CCII value for the VZA at nadir, 30° back-to-the-sun, 40° back-to-the-sun, 30° facing-the-sun, and 40° facing-the-sun VZA.

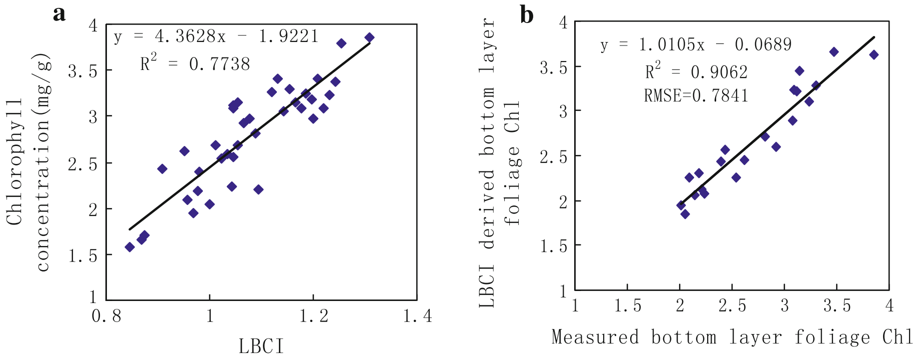

Fig. 4 BLCI derived bottom-layer foliar Chl. *Note*: **a** Relationship between measured bottom-layer foliar Chl and BLCI. **b** Relationship between laboratory measured and BLCI derived bottom-layer foliar Chl

The MLCI was used to establish a predictive regression equation to estimate middle-layer foliage Chl. The prediction regression equation of middle-layer foliage Chl by MLCI has the form ($n = 36$):

$$y = 1.9019x + 1.223\left(R^2 = 0.6713\right), \quad (6)$$

where x is MLCI, and y is middle-layer foliage Chl.

The reliability of the regression equation can be seen in Fig. 5. As shown in Fig. 5a, canopy bi-directional reflectance spectrum and Chl were acquired among different LAD varieties. It reveals a good agreement between middle-layer foliage Chl and CCII, with a coefficient of determination $R^2 = 0.6713$ ($n = 36$); it reached a very significant positive level ($r_{(0.01,36)} = 0.418$).

In this study, the value of CCII at nadir, ±50 and ±60° view angles was assembled as the upper-layer Chl inversion index (ULCI) for inversion of upper-layer Chl. The CCII at nadir VZA contained more background soil information, and the CCII at 50 and 60° VZA contained more upper-layer foliage information. The ULCI was defined as:

$$\mathrm{ULCI} = \mathrm{ULCI_b}/\mathrm{ULCI_f}, \quad (7)$$

$$\mathrm{ULCI_b} = (0.4 * \mathrm{CCII_{A50}} + 0.6 * \mathrm{CCII_{A60}})/\mathrm{CCII_{A0}},$$

$$\mathrm{ULCI_f} = (0.4 * \mathrm{CCII_{A-50}} + 0.6 * \mathrm{CCII_{A-60}})/\mathrm{CCII_{A0}},$$

where $\mathrm{ULCI_b}$ and $\mathrm{ULCI_f}$ are back-to-the-sun and facing-the-sun VZA, respectively; $\mathrm{CCII_{A0}}$, $\mathrm{CCII_{A50}}$, $\mathrm{CCII_{A60}}$, $\mathrm{CCII_{A-50}}$, and $\mathrm{CCII_{A-60}}$ are the CCII value for the VZA at nadir, 50° back-to-the-sun, 60° back-to-the-sun, 50° facing-the-sun and 60° facing-the-sun VZA.

The MLCI is used to establish a predictive regression equation to estimate upper-layer foliage Chl. The prediction regression equation of upper-layer foliage Chl by MLCI has the form ($n = 36$):

$$y = 4.3199x - 1.2487\left(R^2 = 0.6435\right), \quad (8)$$

where x is MLCI, and y is upper-layer foliage Chl. The reliability of the regression equation can be seen in Fig. 6a. Canopy bi-directional reflectance spectrum and Chl were acquired among different LAD varieties. It revealed a good agreement between upper-layer

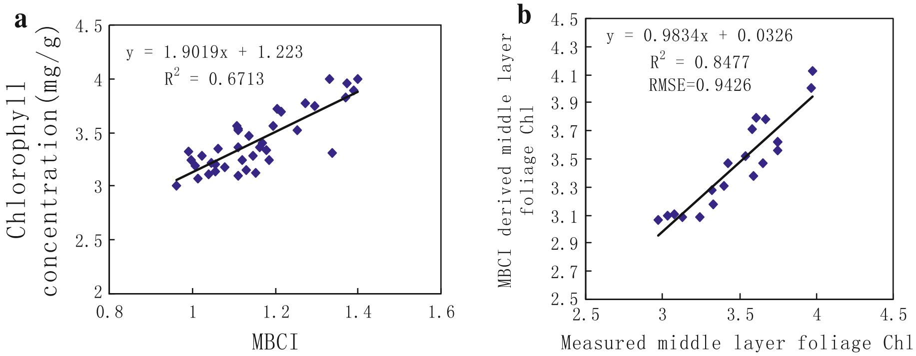

Fig. 5 MLCI derived middle-layer foliar Chl. *Note*: **a** Relationship between measured middle-layer foliar Chl and MLCI. **b** Relationship between laboratory measured and MLCI derived middle-layer foliar Chl

foliage Chl and CCII, with a coefficient of determination $R^2 = 0.6435$ ($n = 36$); it reached a significant positive level ($r_{(0.01,36)} = 0.418$).

To test whether the model was reliable and applicable for the upper-layer, middle-layer, and bottom-layer Chl prediction, the data collected in the validation treatment (experiment B) were tested in the models. Root-mean-square error (RMSE) was employed to test the reliability and estimation accuracy between measured and estimated different-layer Chl:

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\theta_i - \hat{\theta}\right)^2}, \tag{9}$$

where θ_i and $\hat{\theta}$ are observed and remote-sensing-derived Chl, respectively; n is the number of samples. Massart et al. (1988) suggested a correlation value close to 1.0 would indicate high precision and a slope close to 1.0 when the intercept is zero would indicate high accuracy. The BLCI derived bottom-layer Chl exhibited high accuracy (its slope was 1.0105 and the intercept was −0.0689), and precision ($R^2 = 0.9062$, RMSE = 0.7841) (Fig. 4b). The MLCI-derived middle-layer Chl exhibited high accuracy (its slope was 0.9873 and the intercept was 0.0326) and high precision ($R^2 = 0.8477$, RMSE = 0.9426) (Fig. 5b). The ULCI-derived upper-layer Chl exhibited medium accuracy (its slope was 1.0500 and the intercept was 0.197) and medium precision ($R^2 = 0.8174$, RMSE = 1.7398) (Fig. 6b).

The result indicated that winter wheat Chl at different layers on a large scale could be forecast by bi-directional canopy-reflected spectra. The vertical foliage Chl inversion can be used for monitoring the crop growth status and for guiding the nitrogen fertilization management. The results suggested that vegetation indices derived from bi-directional reflected spectra (e.g., BLCI, ULCI, and MLCI) are satisfactory for inversion of Chl vertical distribution.

Discussion

Because this study was carried out under the selected varieties and the same ecological conditions, the influence of the sun angle as well as the range validity of chlorophyll concentration and LAI was not carefully studied. The results could not be applied directly

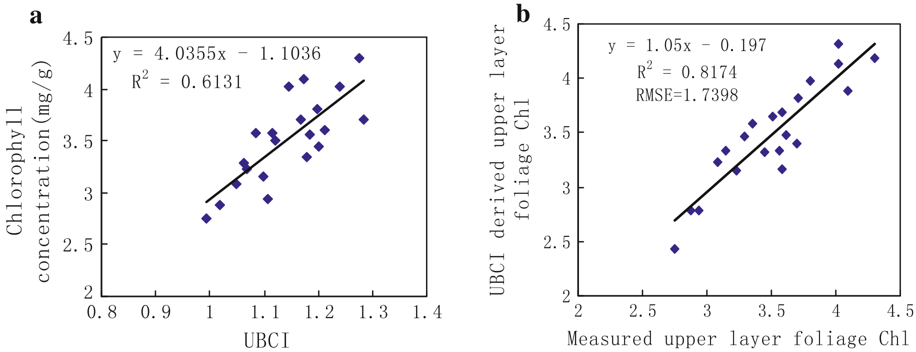

Fig. 6 ULCI derived upper-layer foliar Chl. *Note*: **a** Relationship between measured upper-layer foliar Chl and ULCI. **b** Relationship between laboratory measured and ULCI derived upper-layer foliar Chl

to different ecological conditions and other varieties. Thomas et al. (2009) mentioned that multi-angular high spectral resolution remote sensing can help guiding the application of spectral vegetation indices for determining canopy disturbance at different stages and will also have possible implications for the design of future airborne observations or satellite missions.

Haboudane et al. (2002) presented a combined index for predicting Chl from canopy reflectance spectra while minimizing LAI influence and underlying soil background effects. The method in this paper contributes to the development of optimal procedures for Chl vertical distribution inversion through the analysis of canopy bi-directional reflected spectra. Moreover, a careful analysis should be carried out to investigate the effects of these bi-directional observation angles and crop growth stages, including some other vegetation indices and band-widths. Therefore, further studies are needed to confirm and to improve the results of this study.

Conclusion

The objective of this paper was to develop a method to evaluate winter wheat foliar Chl vertical distribution. The Chl vertical distribution characteristics along the canopy showed an obvious decreasing trend from the canopy top to the ground surface. The BLCI with VZA at nadir, ±20 and ±30° was selected for bottom-layer Chl inversion; the MLCI with VZA at nadir, ±30 and ±40° was selected for middle-layer Chl inversion; the ULCI with VZA at nadir, ±50 and ±60° was selected for upper-layer Chl inversion. The results suggested that vegetation indices derived from bi-directional reflectance spectra (e.g., BLCI, ULCI, and MLCI) were satisfactory for inversion of Chl vertical distribution.

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