

# Estimation of canopy carotenoid content of winter wheat using multi-angle hyperspectral data

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## Abstract

Precise estimation of carotenoid (Car) content in crops, using remote sensing data, could be helpful for agricultural resources management. Conventional methods for Car content estimation were mostly based on reflectance data acquired from nadir direction. However, reflectance acquired at this direction is highly influenced by canopy structure and soil background reflectance. Off-nadir observation is less impacted, and multi-angle viewing data are proven to contain additional information rarely exploited for crop Car content estimation. The objective of this study was to explore the potential of multi-angle observation data for winter wheat canopy Car content estimation. Canopy spectral reflectance was measured from nadir as well as from a series of off-nadir directions during different growing stages of winter wheat, with concurrent canopy Car content measurements. Correlation analyses were performed between Car content and the original and continuum removed spectral reflectance. Spectral features and previously published indices were derived from data obtained at different viewing angles and were tested for Car content estimation. Results showed that spectral features and indices obtained from backscattering directions between 20° and 40° view zenith angle had a stronger correlation with Car content than that from the nadir direction, and the strongest correlation was observed from about 30° backscattering direction. Spectral absorption depth at 500 nm derived from spectral data obtained from 30° backscattering direction was found to reduce the difference induced by plant cultivars greatly. It was the most suitable for winter wheat canopy Car estimation, with a coefficient of determination 0.79 and a root mean square error of 19.03 mg/m<sup>2</sup>. This work indicates the importance of taking viewing geometry effect into account when using spectral features/indices and provides new insight in the application of multi-angle remote sensing for the estimation of crop physiology.

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**Keywords:** Multi-angle; Hyperspectral data; Vegetation index; Spectral feature; Canopy carotenoid content; Agriculture

## 1. Introduction

Life of vegetation is driven by photosynthesis, producing both oxygen and organic matter (Nelson and Yocum, 2006). Pigment content is an important indicator of crop growth condition that determines final crop yield (Haboudane et al., 2002). Chlorophyll (Chl) and some

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carotenoids (Car) are principal photosynthetic pigments. Chl absorb light energy and pass it to photosynthetic apparatus. Car, mainly composed of the xanthophyll cycle pigments and carotene, are responsible for accessory light harvesting and energy transfer (Gitelson et al., 2002; Ritz et al., 2000), as well as photo-protection thanks to the xanthophyll cycle. There is evidence that the conversion of the xanthophyll pigment violaxanthin to zeaxanthin via antheraxanthin protects the photosynthetic system from damage by excessive light (Demmig-Adams and Adams, 1996; Gitelson et al., 2006) and Car is persistently greater than Chl during leaf senescing (Biswal, 1995). Thus the changes of Car content can be an indicator of the response of crops to strong incidental light or temperature stress and crop phenology (Biswal, 1995; Tran and Raymundo, 1999). Due to these importance, knowledge on Car content provides complementary information on pigment composition, photosynthetic capacity, and crop growth condition.

Remote sensing provides a non-destructive, fast and effective way to obtain spatial and time-critical information on crop biophysical and biochemical parameters (Haboudane et al., 2004; Wu et al., 2008). In recent years, substantial efforts are expended in crop Car content estimation from remote sensing data, such as for cotton (Yi et al., 2014), winter wheat (Kong et al., 2016) and rice (Wang et al., 2009). However, estimation of Car content is more challenging, owing to the compound factors from other pigments, soil background reflectance or canopy structure (Sims and Gamon, 2002). Developing methods for accurate quantification of crop Car content is thus desirable.

Several approaches have been proposed for plant Car content estimation by exploiting spectral information, such as spectral indices or spectral transformations. Narrow-band spectral reflectance was considered to be able to discriminate spectral features from different pigments (Sykoti et al., 2011). Several Car indices have been developed in literatures (Blackburn, 1998a; Chappelle et al., 1992; Gitelson et al., 2002, 2006). The continuum removal transformation has been used as a spectral analysis tool to extract and enhance spectral features and to minimize extraneous factors, such as atmospheric absorption and background reflectance effects (Kokaly, 2001). A series of spectral features, e.g., the absorption depth and absorption area, were derived from continuum-removed spectra in some studies (Clark and Roush, 1984; Kokaly, 2001; Sanches et al., 2014).

Conventionally, Car content estimation was mostly based on spectral reflectance acquired from a near nadir direction (Fassnacht et al., 2015; Gitelson et al., 2002, 2006). However, studies showed that canopy spectral reflectance acquired from nadir observation was susceptible to influence from canopy structure and background soil reflectance, which might reduce the estimation accuracy of certain parameters, such as Car content (Kimes et al., 1985; Sims and Gamon, 2002). Reflectance anisotropy due to canopy structure, background and shadow may give rise to changes in remote sensing signals even with no change

in plant biophysical and biochemical parameters, which will limit the ability to accurately estimate the parameters from the nadir viewing spectral data (Chen et al., 2003). Complementary to the nadir observation, multi-angle observations (i.e., from different viewing angles) bring additional information on a target canopy (Stagakis et al., 2010). This provides a means to characterize reflectance anisotropy (Chen et al., 2003) and may lead to higher accuracy in estimation of certain canopy parameters (Kneubuehler et al., 2008). Many studies have focused on the assessment of canopy structural parameter estimation using multi-angle observation data (Asner et al., 1998; Gao et al., 2003), such as leaf area index (LAI) (Gu et al., 2015; Wu et al., 2010) and canopy gap fraction (Chen et al., 2005). Lately, multi-angle data have been found to be also promising for monitoring vegetation biochemical parameters (Kneubuehler et al., 2008). Huber et al. (2010) reported that the use of spectral indices derived from multi-angle observation data potentially improved the estimation of nitrogen and water content of forest. Stagakis et al. (2010) focused on investigating relationships between spectral indices and biochemical content (i.e. leaf pigment or water potential) in shrubs using multi-angle spectral data. Their results showed that spectral indices derived from off-nadir observations were more effective for biochemical variable estimation than that derived from nadir observation. Attempts have been made to develop new indices with angular information provided by multi-angle data. For instance, the Multi-angle vegetation index (MAVI) was introduced by He et al. (2016) to combine reflectance from two angles for better quantification of canopy nitrogen content.

So far, there have been fewer studies on assessing crop canopy Car content using multi-angle hyperspectral data. The objectives of this study were: (1) to investigate the capability of multi-angle viewing data in estimating crop canopy Car content; (2) to assess the performance of spectral features and indices derived from off-nadir directions respective to the nadir direction for Car content estimation, in order to find the best predictor of Car content.

## 2. Materials and methods

### 2.1. Study Site

The study was carried out at the Xiaotangshan Precision Agriculture Experimental Site (116°12'E, 40°13.2'N), in Changping district, Beijing, in 2004 and 2007. The soil is silty clay loam, with nutrient content about 14.2–14.8 g/kg of organic matter, 117.6–129.1 mg/kg of available potassium, and 20.1–55.4 mg/kg of available phosphorus in the topsoil layer (0–0.20 m depth). Six winter wheat cultivars were studied in 2007: three erectophile-type cultivars (Jing 411, Laizhou 3279 and I-93) and three horizontal-type cultivars (Linkang 2, 9428, 9507). In addition, four winter wheat cultivars were investigated in 2004: two erectophile-type cultivars (Jing 411, Laizhou 3279) and

two horizontal-type cultivars (Linkang 2, 9507). Each cultivar was planted in a plot with an area of  $45 \times 10.8 \text{ m}^2$ , and all plots were managed in the same way, including fertilization and irrigation treatments.

## 2.2. Field data acquisition

Field campaigns were carried out in 2007 during typical winter wheat growth stages: 28 April (booting), 8 May (heading), 19 May (milk-filling) and 29 May (milk-ripe). Field data in 2004 were collected in the same growth stages as in 2007 excluding the milk-ripe stage. Both canopy spectra and biophysical and biochemical parameters were measured. Detailed description of measurement protocols are given below.

### 2.2.1. Canopy multi-angle spectral measurements

An ASD FieldSpec 3 spectrometer (Analytical Spectral Devices, Boulder, CO), with a  $25^\circ$  field-of-view fiber optics, was used to measure canopy spectral reflectance under clear sky conditions between 11:00 a.m. and 13:00 p.m. (Beijing local time). It records spectral radiance with a sampling interval of 1.4 nm and a resolution of 3.0 nm between 350 and 1050 nm, and a sampling interval of 2.0 nm and a resolution of 10.0 nm between 1000 and 2500 nm. A rotating bracket was used to hold the instrument (Fig. 1), enabling spectral measurements of the same target from different angles in a short time. Table 1 provides solar zenith and azimuth angles during the measurements. The observation was made in the principle plane (constructed by the direction of incident direct sun light, and the direction of the normal to surface target) at different observation zenith angles. The nadir (i.e., observation zenith angle  $0^\circ$ ) spectral measurements were made at a height of 1.3 m above canopy top, generating an instantaneous field-of-view of about  $0.26 \text{ m}^2$ . The off-nadir spectral measurements were made at  $\pm 20^\circ$ ,  $\pm 30^\circ$ ,  $\pm 40^\circ$ ,  $\pm 50^\circ$  and  $\pm 60^\circ$  angles, where a positive angle corresponds to backscattering direction (measured back to the sun) and a negative angle corresponds to forward scattering direction (measured face to the sun). Each spectral measurement was preceded by an optimization measurement, and a white reference measurement was taken before and after canopy spectral measurement using a white Spectralon® (Lab-sphere, Inc. New Hampshire, USA) reference panel. More detailed information about the multi-angle spectral measurements can be found in previous studies (Huang et al., 2006; Wu et al., 2010). Twenty scans were performed and averaged to obtain spectral reflectance for each observing direction. Examples of canopy spectra between 400 and 1000 nm measured at  $\pm 20^\circ$ ,  $\pm 30^\circ$  and  $\pm 40^\circ$  angles are shown in Fig. 2.

### 2.2.2. Canopy Car content measurement

All samples of winter wheat in the plot ( $1 \times 1 \text{ m}^2$  area), within the footprint of canopy reflectance spectra acquisitions, were harvested by cutting off the aboveground por-

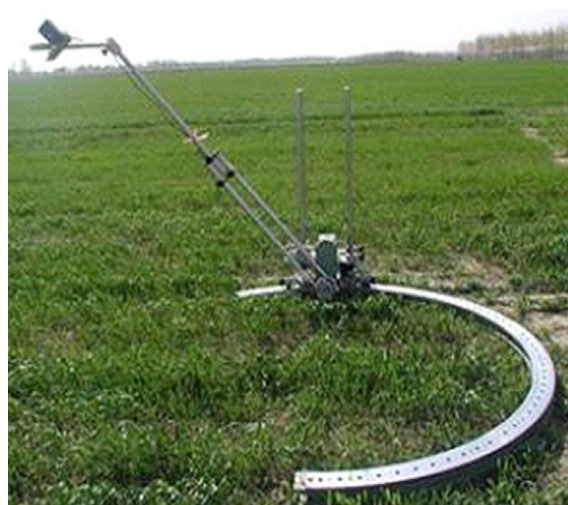


Fig. 1. The rotating bracket for canopy multi-angle spectral measurement. The semi-circular track controls the observation azimuth angle, the rotating boom controls the zenith angles, and the up-and-down arrow indicates that the observation height can be changed according to crop height.

tions, then put in cooled black plastic bags and transported to laboratory immediately to measure the biochemical parameters. Leaves that fully expanded and showed homogenous color as well as no visible sign of damage were sampled from the top to the bottom of the canopy. Circular pieces (about  $0.25 \text{ cm}^2$ ) were cut off from each leaf samples, mixed and weighted. Car was extracted by immersing and grinding the pieces in the aqueous acetone/distilled water buffer solution (80:20 vol:vol). Solutions were stored in darkness for more than 24 h till all Car had been extracted, as indicated by the white color of the leaf tissues. The extraction was then filtered and the absorbance was measured with a UV–VIS spectrophotometer (Perkin-Elmer, Lambda 5, Massachusetts, USA) at 440, 644 and 663 nm wavelength, and Car concentration (Car, mg/g) was determined using the equations given in the study by Lichtenthaler (1987). Canopy Car content ( $\text{mg/m}^2$ ) was calculated as (Gitelson et al., 2014; Yi et al., 2014):



Table 1  
Solar zenith and azimuth angles during the observations.

Date	Time	Solar zenith angle (°)	Solar azimuth angle (°)
2004.4.28	11:50–12:30	26.8–26.1	163.7–174.5
2004.5.8	11:50–12:30	23.9–23.2	162.1–173.9
2004.5.19	11:50–12:30	21.4–20.5	160.3–173.3
2007.4.28	11:45–12:40	27.6–26.6	158.7–169.1
2007.5.8	11:45–12:40	24.8–23.7	156.7–168.0
2007.5.19	11:45–12:40	22.3–21.0	154.4–166.8
2007.5.29	11:45–12:40	20.5–19.2	152.6–165.7

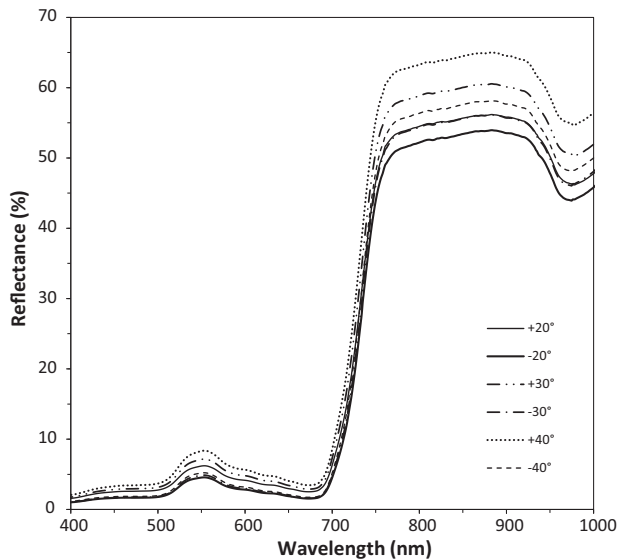


Fig. 2. Canopy spectra measured at  $\pm 20^\circ$ ,  $\pm 30^\circ$  and  $\pm 40^\circ$  angles.

$$\text{Canopy Car content (mg/m}^2\text{)} = \text{Car (mg/g)} \times \text{SLW (g/m}^2\text{)} \times \text{LAI} \quad (1)$$

where specific leaf weight (SLW) and LAI were determined in the lab. SLW ( $\text{g/m}^2$ ) was calculated as leaf dry weight per unit leaf area (Oren, 1984). The leaf area of a subsample of plant leaves was measured with a Li-Cor 3100 area meter (Lincoln, NB, USA) and the weight of leaves were recorded to scale up to the LAI of the  $1 \text{ m}^2$  sample area. LAI measured during multiple growth stages revealed green biomass variation across the season. Average LAI increased from about 1.97 at the booting stage to about 3.25 at the heading stage, and then decreased to 2.54 at the milk-ripe stage. More detailed information about LAI measurement can be found in Huang et al. (2006).

### 2.3. Spectral features and spectral indices

It should be noted that canopy spectra obtained at different viewing angles were processed and analyzed as an individual dataset in our study. Wavelengths shorter than 400 nm were not included in the analyses due to large noise. The continuum removal algorithm was used to process Car spectral features between 400 and 530 nm, with maxima around 500 nm (Chappelle et al., 1992). This was

accomplished by rationing the original spectral reflectance with a continuum line of a convex hull connecting local maxima of a spectrum, allowing for suppression of the impact from other pigments with absorption maxima at different wavelengths.

Two spectral features, the absorption depth (AD) and absorption area (AA), were derived from the continuum removed spectrum as shown in Fig. 3. In addition, four spectral indices proposed in the literature for Car content estimation were selected in this study (Table 2): 2 two-band indices (the pigment-specific simple ratio – PSSRc and the pigment-specific normalized difference – PSNDc) and two three-band indices (the carotenoid reflectance

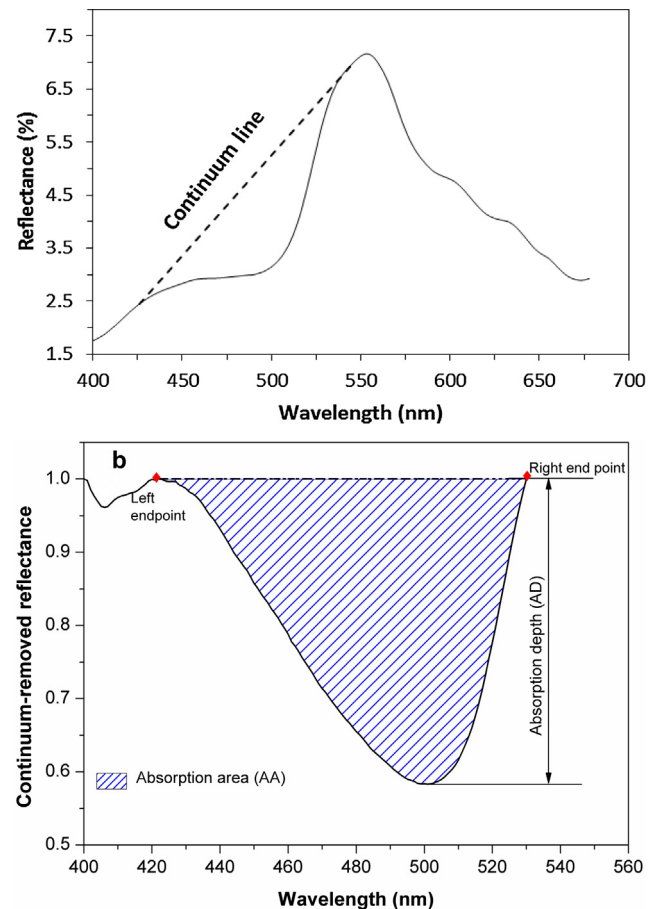


Fig. 3. (a) Example of a winter wheat canopy spectrum showing the continuum line over the Car absorption feature centered around 500 nm; (b) the absorption features, i.e., the absorption depth (AD) and absorption area (AA).

Table 2  
Spectral indices investigated in this study.

Spectral indices	Formula	References
PSSRc	$PSSRc = \frac{R_{800}}{R_{500}}$	Blackburn (1998b)
PSNDc	$PSNDc = \frac{R_{800} - R_{500}}{R_{800} + R_{500}}$	Blackburn (1998b)
Car <sub>green</sub>	$Car_{green} = (R_{510}^{-1} - R_{550}^{-1}) \times R_{770}$	Gitelson et al. (2006) and Yi et al. (2014)
Car <sub>red edge</sub>	$Car_{red\ edge} = (R_{510}^{-1} - R_{700}^{-1}) \times R_{770}$	Gitelson et al. (2006) and Yi et al. (2014)

index at the green or red<sup>1</sup> edge band, represented by Car<sub>green</sub> and Car<sub>red edge</sub>, respectively). The PSSRc and PSNDc indices were initially developed by incorporating the reflectance in the blue band at 500 nm and the reflectance in a near-infrared band at 800 nm, providing a measurement of the low reflectance in plant spectra relative to the highly reflective near infrared (NIR) plateau (Blackburn, 1998b). These two indices were found to correlate well with Car content. To reduce the influence of Chl on Car estimation, the Car<sub>green</sub> and Car<sub>red edge</sub> indices were proposed by Gitelson et al. (2006), who reported a new approach, i.e., a conceptual three-band model, to determine Car content. The two indices have been applied successfully for noninvasive estimation of Car content for different crop species.

#### 2.4. Assessment of the spectral features and indices

Performance of the spectral features and indices at different viewing angles was assessed by comparing the coefficient of determination ( $R^2$ ) and the root mean square error (RMSE) obtained from a linear regression analysis with canopy Car content. Since for both study years the number of samples was insufficient for creating independent calibration and validation datasets, the leave-one-out cross-validation approach was applied (Clevers and Kooistra, 2012). In addition, the relative sensitivity ( $S_r$ ) between a pair of spectral features or indices for Car content estimation was assessed using the following equation (Gitelson, 2004).

$$S_r = (dX/dY) \times (\Delta X/\Delta Y)^{-1} \quad (2)$$

where  $X$  and  $Y$  are two features or indices,  $dX$  and  $dY$  are the first derivatives with respect to Car content, and  $\Delta X$  and  $\Delta Y$  are the variation ranges of  $X$  and  $Y$ , respectively.  $S_r < 1$  indicates that  $Y$  is more sensitive to Car content than  $X$ ,  $S_r = 1$  indicates that their sensitivity are comparable, and  $S_r > 1$  indicates that  $X$  is more sensitive than  $Y$ .

### 3. Results

#### 3.1. Correlation between Car content and spectral reflectance

Correlation between canopy Car content and spectral reflectance at different viewing angles (i.e., nadir,  $\pm 20^\circ$ ,

$\pm 30^\circ$ ,  $\pm 40^\circ$ ,  $\pm 50^\circ$ ,  $\pm 60^\circ$ ) were inspected using linear regression analysis, and the results were shown in Fig. 4. In general, except for  $\pm 60^\circ$  view angles, spectral variation of correlation coefficients for all other observation angles revealed roughly similar patterns: two negative correlation peaks roughly in the blue (450–530 nm) and red (580–690 nm) regions and positive correlation in the NIR region (745–820 nm). It was worth noting that Car showed a strong correlation with red reflectance. This is because Chl has a strong absorption in the red region, and Car and Chl content covary to certain extent (Blackburn, 1998a). It was also observed that correlation coefficient changed from negative to positive at the red-edge region between 719 and 730 nm for all viewing angles except for  $-60^\circ$ . The insensitivity of reflectance to Car content in this region agreed with the findings by Stagakis et al. (2010).

As can be seen from Fig. 4, visible spectral reflectance at  $30^\circ$  backscattering angle had the strongest correlation with Car content among all viewing angles, with the strongest negative correlation at 648 nm in the red region ( $r = -0.71$ ) and at 507 nm in the blue region ( $r = -0.65$ ). The correlation at  $+40^\circ$  and  $+20^\circ$  viewing angles was also strong. The above results indicated that off-nadir spectral reflectance at backscattering directions could make a stronger contribution than the nadir reflectance to Car content estimation, which was consistent with the study by Huber et al. (2010). Contrary to the backscattering observations, the forward scattering observations did not show comparable results, although the best coefficients were gained at the  $-50^\circ$  viewing angle.

#### 3.2. Correlation between Car content and continuum-removed spectral reflectance

We analyzed the linear correlation between Car content and continuum-removed spectral reflectance at different viewing angles and the results were shown in Fig. 5. Compared with that of the original spectral reflectance, the correlation appeared to be stronger in the blue region (400–530 nm) except for the  $-60^\circ$  angle, indicating that the continuum-removed spectral reflectance seemed to be more effective than the original reflectance for Car estimation. This can be attributed to the suppression of external influences on Car estimation through continuum removal (Kokaly, 2001).

As shown in Fig. 5, Car correlated well with the continuum-removed spectra in the 450–520 nm wavelengths for most of the viewing angles, coinciding with the spectral region of Car absorption and resembling the

<sup>1</sup> For interpretation of color in Figs. 3–5, the reader is referred to the web version of this article.

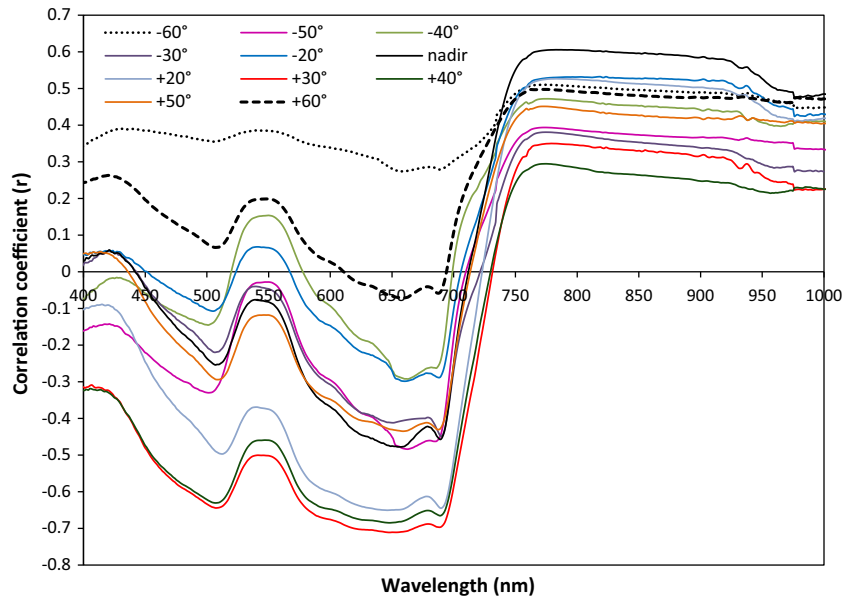


Fig. 4. Correlation between canopy Car content and spectral reflectance at different viewing angles ( $n = 36$ ).

blue peak in the original spectra analysis (Fig. 4). It should be noted that the strongest correlation at around 500 nm in the blue region was observed for all viewing angles except for  $-60^\circ$ . This was in consistent with Chappelle et al. (1992) who found that Car absorption reached maximum at around 500 nm.

The continuum-removed spectral data from backscattering directions at  $20^\circ$ ,  $30^\circ$  and  $40^\circ$  were better correlated with Car content than at the nadir, with the strongest correlation ( $r = -0.76$ ) achieved at  $+30^\circ$  (Fig. 5a). All forward scattering observations showed a weaker correlation in the blue region compared to the nadir observation (Fig. 5b). The observation from  $-50^\circ$  had a higher  $r$  than the rest of the forward viewing angles, which was in consistence with analysis results of the original spectral reflectance (Fig. 4).

### 3.3. Relationship between Car content and spectral features and indices

The  $R^2$  and RMSEs derived from linear regression between Car content and the selected spectral features and indices from all viewing directions are shown in Fig. 6. For all the selected spectral features and indices, the strongest correlation with Car content was obtained at  $+30^\circ$  angle, while the weakest correlation was found at  $\pm 60^\circ$ . In particular, the highest coefficient ( $R^2 = 0.79$ ,  $p < 0.001$ ) and the lowest RMSE ( $18.15 \text{ mg/m}^2$ ) for Car content estimation was achieved using a model using AD at  $+30^\circ$ , followed by models using AD at  $+40^\circ$  and AA at  $+30^\circ$  ( $R^2 = 0.77$  and  $0.68$ , respectively). Significant correlations ( $R^2 > 0.6$ ,  $p < 0.001$ ) were also observed for AD and AA models at  $+20^\circ$ , AA and  $\text{Car}_{\text{green}}$  models at  $+40^\circ$  and  $+30^\circ$  respectively, and AD model from the nadir. These results indicated that a model based on backscattering observation data (i.e.,  $+20^\circ$ ,  $+30^\circ$ ,  $+40^\circ$ ) will achieve a

better accuracy in canopy Car estimation than that achieved from nadir observation data. The forward scattering directions showed a lower  $R^2$  for all spectral parameters compared to the corresponding nadir and back scattering observing angles. Maximum  $R^2$  value in the forward scattering observations was found in the AD model based on  $-50^\circ$  angle data ( $R^2 = 0.38$ ,  $p < 0.001$ ).

For a further investigation, comparisons of the relationships between canopy Car content and the spectral parameters obtained at  $+30^\circ$  and at nadir were made, and the results were shown in Figs. 7 and 8. All six models achieved a higher accuracy using  $+30^\circ$  viewing data than the corresponding nadir viewing data.  $R^2$  of AD, AA and  $\text{Car}_{\text{green}}$  models were 0.79, 0.68 and 0.60, improved by about 27.4%, 17.2% and 15.4%, respectively, over their respective nadir models ( $R^2 = 0.62$ , 0.58, 0.52, respectively). This manifested that  $+30^\circ$  back scattering viewing angle was more sensitive to Car content variation. AD outperformed the other spectral features and indices. Although less powerful than the two spectral features (i.e., AD and AA),  $\text{Car}_{\text{green}}$  and  $\text{Car}_{\text{red edge}}$  were also capable for winter wheat Car content estimation, with  $R^2$  of 0.60 and 0.59 respectively. The two two-band indices (PSSRc and PSNDc) showed almost the same pattern for both  $+30^\circ$  and the nadir direction, owing to the similarity of their mathematical forms. The accuracy for Car content estimation using these two indices was lower ( $R^2$  ranging from 0.31 to 0.40) than that using the three-band indices, consistent with the findings by Gitelson et al. (2006).

We analyzed the relative sensitivity (Sr) to canopy Car content between pairs of spectral features/indices at  $30^\circ$  backscattering observation (Table 3). Sr of AD versus PSSRc, PSNDc,  $\text{Car}_{\text{green}}$  and  $\text{Car}_{\text{red edge}}$  were higher than 1, indicating that AD was more sensitive than these spectral indices. And the sensitivities of AD and AA were

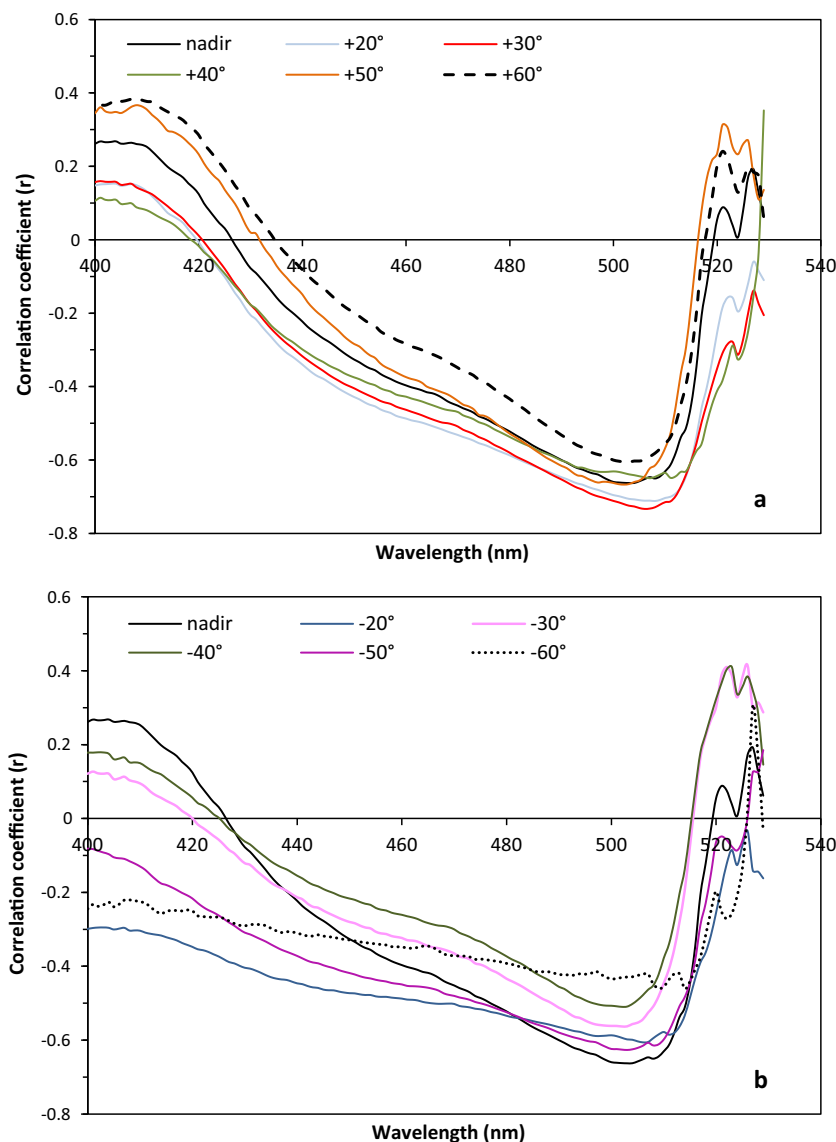


Fig. 5. Correlation between Car content and continuum-removed spectral reflectance at different viewing angles: (a) nadir and backscattering and (b) nadir and forward scattering ( $n = 36$ ).

comparable as  $S_r$  was relatively close to 1 ( $S_r = 1.03$ ). Similarly, the two three-band spectral indices presented advantage in sensitivity to Car content, compared to the two-band indices.

### 3.4. Comparison of spectral features and indices for different plant cultivars

Plant cultivars with different canopy structure may impact spectral measurements differently. To assess the impact of different cultivar on canopy Car content estimation, we compared the correlation coefficients ( $r$ ) between Car content and spectral features and indices for the two winter wheat cultivar types (Table 4). For both the erectophile-type and the horizontal-type cultivars, all spectral features and indices derived from  $+30^\circ$  observation data exhibited the strongest linear correlation with Car

content. Except for AA, the differences of  $r$  between these two types of cultivars were much smaller at  $+20^\circ$ ,  $+30^\circ$ ,  $+40^\circ$  viewing angles than that at the nadir direction, indicating that spectral data obtained from the backscattering direction reduced the effects of canopy structure on quantification of canopy Car content. The higher sensitivity of the spectral indices to plant cultivars at the nadir direction was in conformity with the study by Sims and Gamon (2002). Thus indices based on nadir observation data were susceptible to impact from plant types and are inferior for Car content estimation at the regional or global scales. For the forward scattering observations (viewing angles from  $-20^\circ$  to  $-40^\circ$ ), all spectral features and indices performed better for erectophile-type than horizontal-type cultivars, possibly due to a smaller proportion of shadow produced by the leaves of the erectophile-type, hence less impact on Car estimation from shadowing effect.



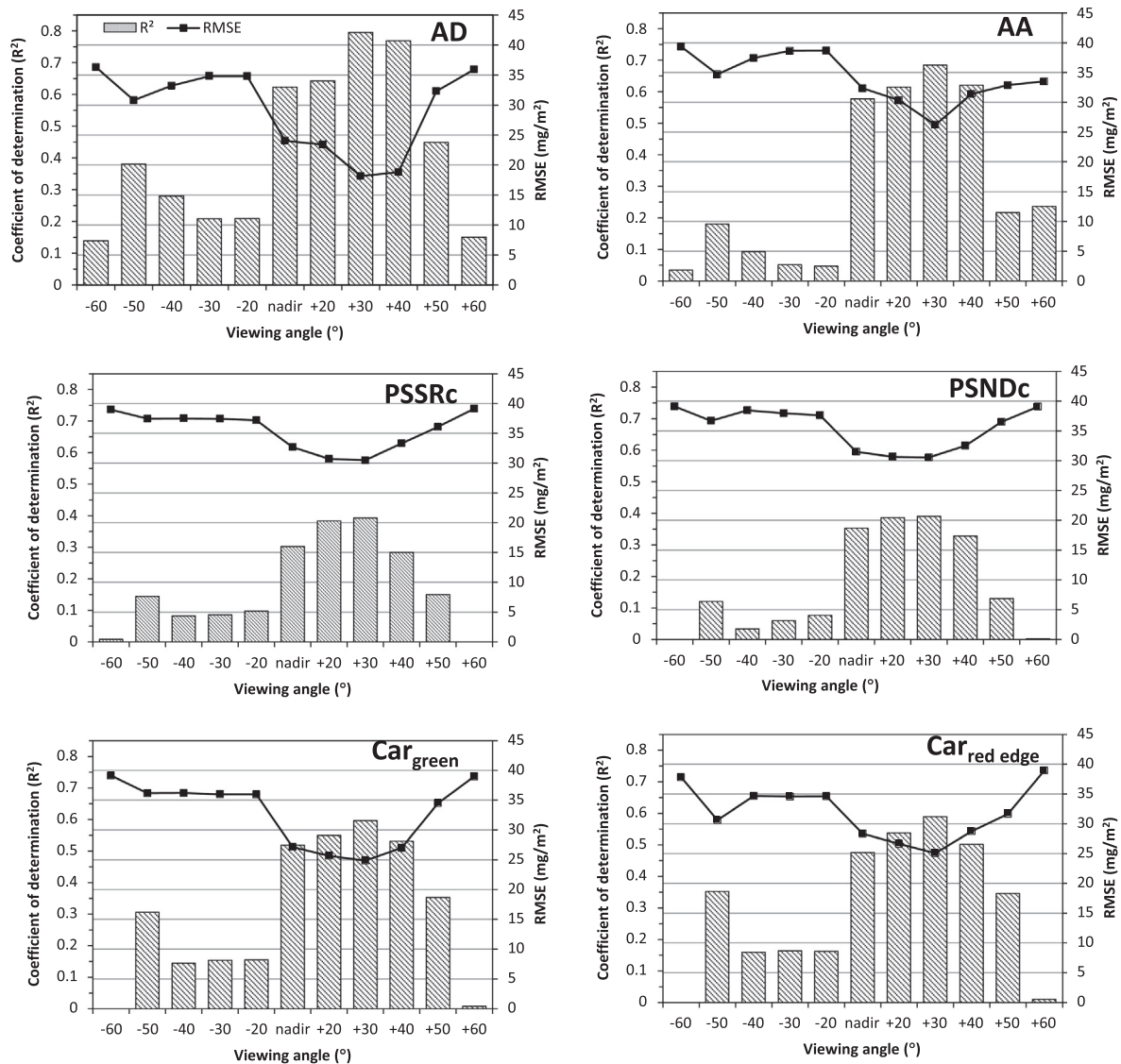


Fig. 6. Coefficients of determination ( $R^2$ ) and root mean square error (RMSE) of linear regression between canopy Car content and the spectral features/indices at different viewing angles ( $n = 36$ ).

As shown in Table 4, the models with AD at +30° achieved stronger correlations to Car content than all the others for both types of plant cultivars ( $r = 0.89$ ). The two spectral features had a strong linear correlation in +30° and nadir directions ( $r$  ranged from 0.75 to 0.89), demonstrating that they were the most suitable for canopy Car content estimation of different types of winter wheat cultivars. Car<sub>green</sub> and Car<sub>red edge</sub> yielded relatively weaker correlations with Car content for the horizontal-type than for the erectophile-type at the nadir and +30° viewing angle; the  $r$  values of both indices varied from 0.77 to 0.85 for the latter type, whereas they varied from 0.56 to 0.79 for the former type, with the lowest  $r$  appearing in Car<sub>red edge</sub> from the nadir direction. Besides, PSSRc and PSNDc at +30° yielded moderate correlations for the erectophile-type ( $r = 0.74$  and  $0.71$ , respectively); however, neither showed a strong correlation for nadir observation

for the horizontal-type cultivars ( $r = 0.46$  and  $0.47$ , respectively), indicating that they were less sensitive to changes of Car content in this type of cultivar.

### 3.5. Model validation

Based on the results presented above, models based on the nadir, +20°, +30° and +40° observations were evaluated only. The  $R^2$  and RMSE between the measured and estimated Car content derived using the leave-one-out cross-validation approach are shown in Fig. 9. All the spectral parameters derived from +30° performed better to predict Car content than that from nadir and the other two backscattering angles, with a relatively higher  $R^2$  and lower RMSEs. To identify the best spectral feature/index for canopy Car content estimation, scatter-plots of the estimated and measured canopy Car content for the six



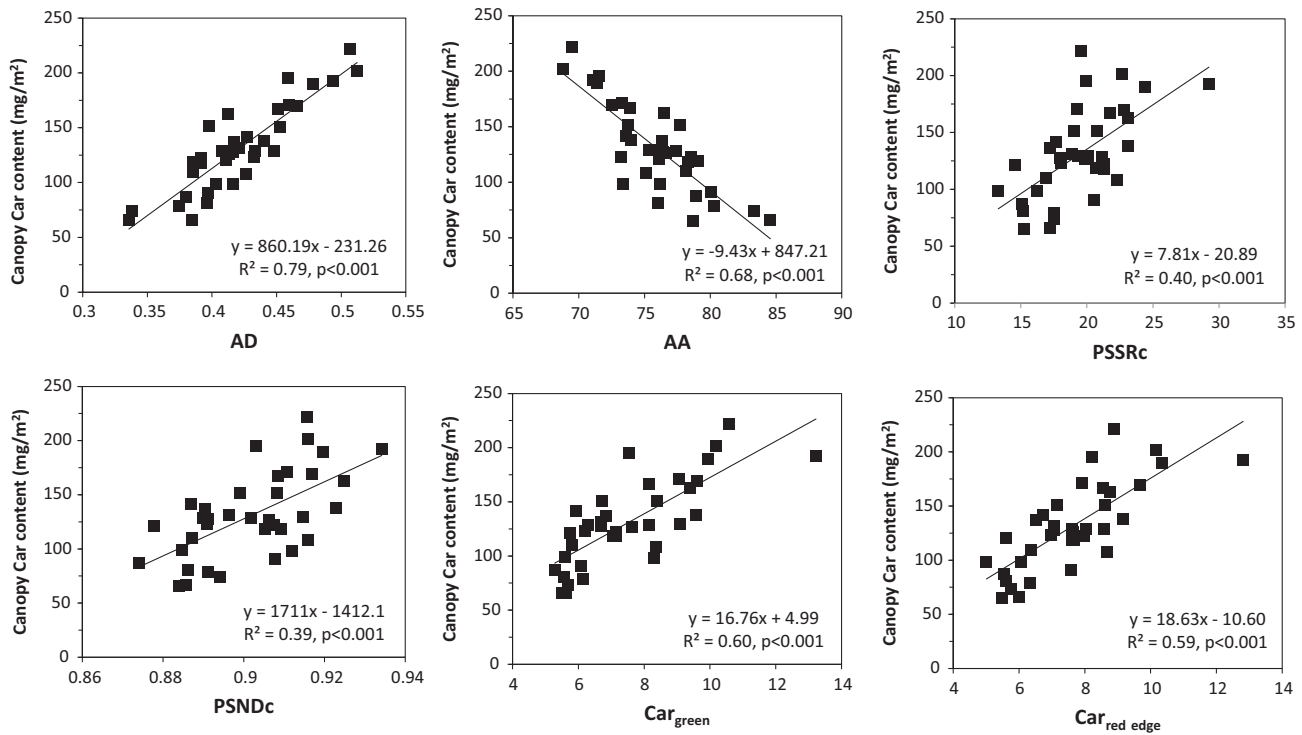


Fig. 7. Relationship between canopy Car content and spectral features/indices at +30° viewing angle (n = 36).

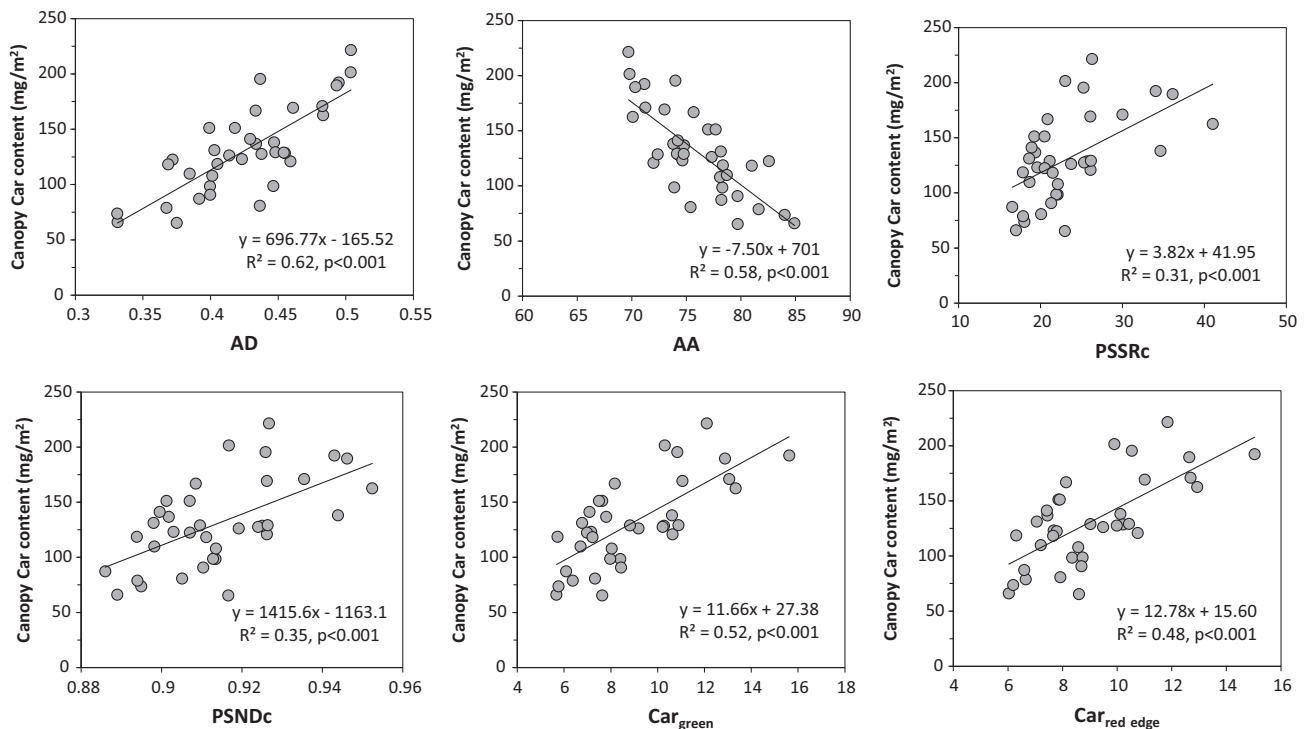


Fig. 8. Relationship between canopy Car content and spectral features/indices in the nadir direction (n = 36).

spectral features and indices at +30° were shown in Fig. 10. All models reached the 0.001 significance level. AD and AA had a higher  $R^2$  and a lower RMSE ( $R^2$  and RMSE of 0.76 and 19.03 mg/m<sup>2</sup> for AD, and 0.65 and 23.16 mg/m<sup>2</sup> for

AA), demonstrating that they were the best model for canopy Car content estimation. Prediction using the Car<sub>green</sub> also had a relatively low RMSE (26.73 mg/m<sup>2</sup>) and a high  $R^2$  (0.54); however there was a larger bias

Table 3

Relative sensitivity (Sr) of spectral features/indices with respect to canopy Car content at +30° viewing angle.

X	Y					
	AD	AA	PSSRc	PSNDc	Car <sub>green</sub>	Car <sub>red edge</sub>
AD	1	−1.03	1.61	1.53	1.14	1.26
AA		1	−1.45	−1.38	−1.08	−1.14
PSSRc			1	0.95	0.70	0.78
PSNDc				1	0.74	0.82
Car <sub>green</sub>					1	1.11
Car <sub>red edge</sub>						1

Table 4

The linear correlation coefficient (r) between canopy Car content and spectral features and indices for the two types of cultivars.

Plant cultivars	Spectral features/ indices	−60°	−50°	−40°	−30°	−20°	nadir	+20°	+30°	+40°	+50°	+60°
Erectophile-type cultivars	AD	0.46	0.77***	0.81***	0.80***	0.79***	0.85***	0.83***	0.89***	0.88***	0.76***	0.45
	AA	−0.43	−0.67**	−0.75***	−0.74***	−0.69**	−0.81***	−0.75***	−0.83***	−0.75***	−0.49*	−0.40
	PSSRc	0.28	0.61**	0.42	0.51*	0.55*	0.71***	0.73***	0.74***	0.51*	0.50*	0.35
	PSNDc	0.33	0.59*	0.51*	0.55*	0.51*	0.62**	0.68**	0.71***	0.47	0.43	0.36
	Car <sub>green</sub>	0.32	0.67**	0.74***	0.75***	0.76***	0.81***	0.79***	0.85***	0.68**	0.62**	0.47
	Car <sub>red edge</sub>	0.31	0.67**	0.74***	0.75***	0.77***	0.77***	0.80***	0.83***	0.66**	0.62**	0.46
Horizontal-type cultivars	AD	0.29	0.51*	0.31	0.07	0.13	0.76***	0.79***	0.89***	0.87***	0.60*	0.37
	AA	−0.09	−0.32	−0.05	0.13	0.12	−0.75***	−0.68**	−0.83***	−0.63**	−0.50*	−0.42
	PSSRc	0.39	0.27	0.31	0.27	0.28	0.46	0.62**	0.65**	0.56*	0.35	0.19
	PSNDc	0.32	0.22	0.28	0.22	0.22	0.47	0.63**	0.66**	0.58*	0.34	0.15
	Car <sub>green</sub>	0.33	0.21	0.09	0.08	0.10	0.70**	0.76***	0.79***	0.76***	0.66**	0.09
	Car <sub>red edge</sub>	0.32	0.30	0.15	0.12	0.17	0.56*	0.74***	0.78***	0.73***	0.64**	0.08

\* Significance at 0.05 level.

\*\* Significance at 0.01 level.

\*\*\* Significance at 0.001 level.

between field measurements and predictions, as shown with a larger overestimation at low Car content and underestimation at high Car content.

#### 4. Discussion

In this study, we investigated the correlation between canopy Car content and the original and the continuum-removed spectral reflectance at the nadir and the off-nadir directions. The spectral features derived from the continuum-removed spectra and the four selected spectral indices at different viewing zenith angles in the principal plane were evaluated for winter wheat canopy Car content estimation, in order to investigate the capability of multi-angle data. Based on the results presented above, the spectral features and indices derived from +20°, +30° and +40° viewing angle achieved stronger correlation than that from nadir direction, demonstrating that an improved Car estimation model could be developed from data obtained from a backscattering direction. Literature studies show that nadir observation is highly influenced by canopy gap and soil background reflectance (Sandmeier et al., 1998; Stagakis et al., 2010). The contribution to spectral reflectance from the gaps and background soil within the field of view is larger at the nadir direction, leading to a

decreased contribution from plant tissues. However, for off-nadir observations from backscattering directions, the field of view is dominated by plant tissues, hence, impact from canopy gap and background soil is minimized. This partly explained our results in this study that, a better performance for Car content estimation was achieved using observations from the backscattering directions. Our previous study showed that spectral reflectance obtained from a backscattering direction at 20°, 30° or 40° can be reliably applied to monitor pigment content in the middle and bottom layers of winter wheat canopy (Huang et al., 2011). Since leaves at the middle and lower layers of canopy were also used to measure Car content in the laboratory experiment and they have a contribution to canopy pigment content, estimation of canopy Car content may benefit from data contained in the off-nadir directions.

However, in the forward scattering directions at 20°, 30° and 40°, the correlation between Car content and the spectral features and indices were not as strong as that in the backscattering directions. This was mainly due to the shadowing effects resulted from viewing and illumination geometry (Galvao et al., 2009; Gu et al., 2015; Kneubuehler et al., 2008), inducing a decreased spectral signature of plant tissues (Fig. 2). Canopy spectral reflectance measured from these forward scattering directions between 400 and

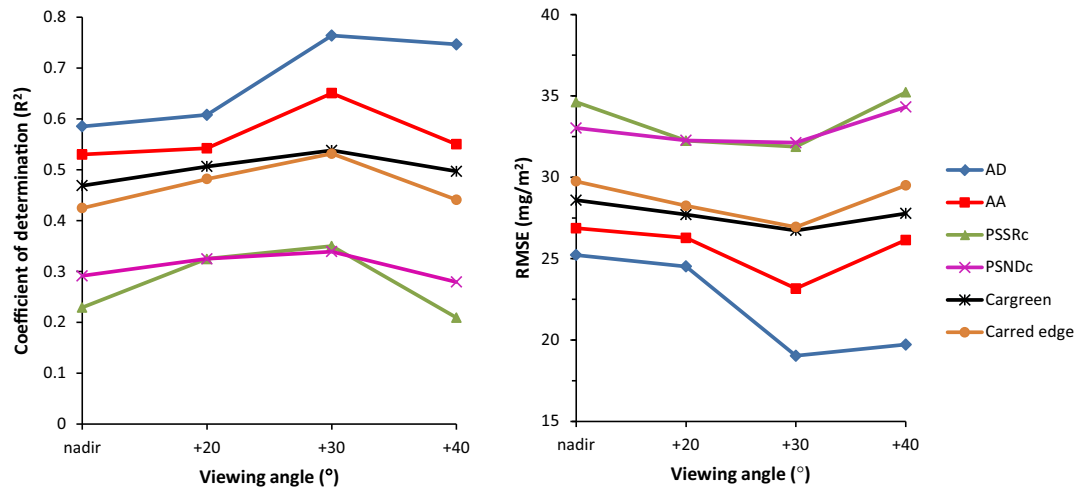


Fig. 9. Validation of the relationships between measured and estimated canopy Car content at the nadir, +20°, +30° and +40° viewing angles ( $n = 36$ ).

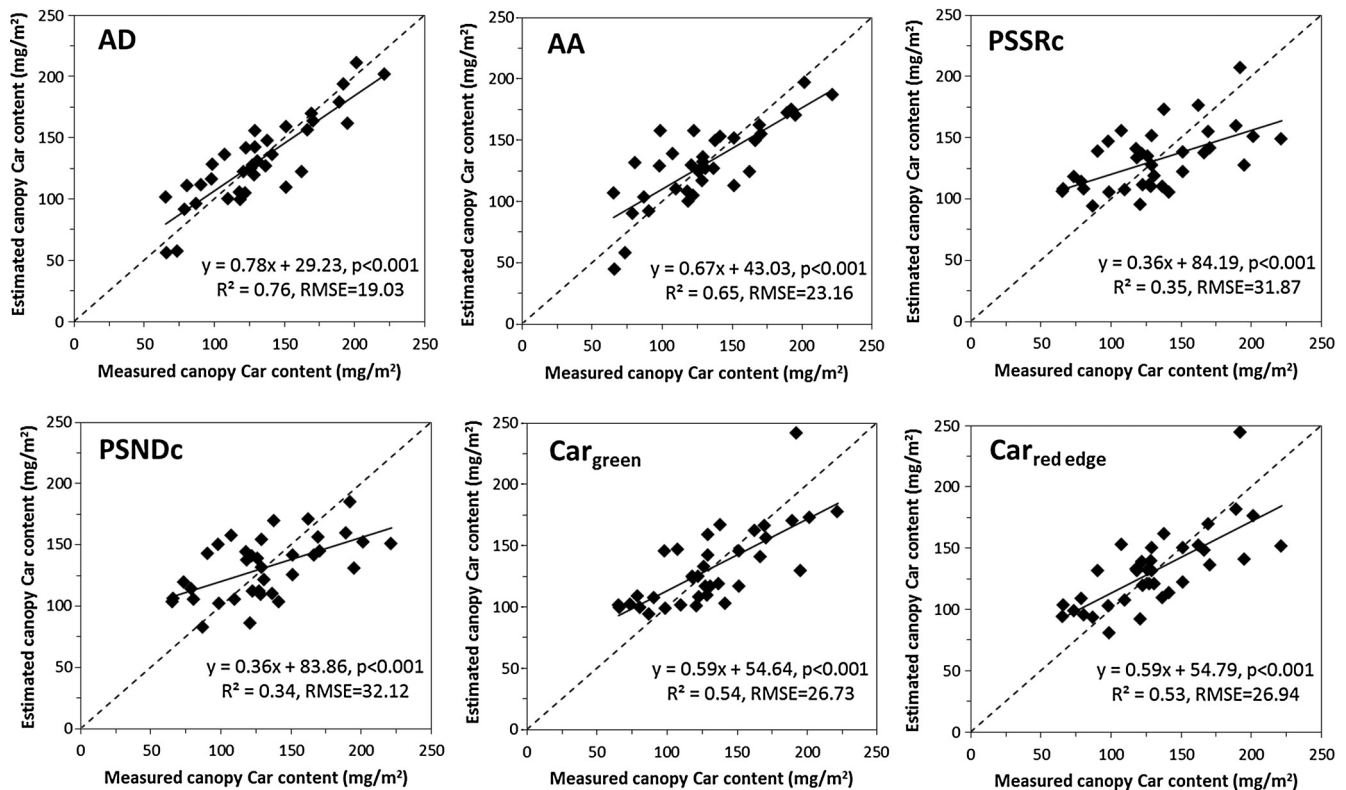


Fig. 10. Scatterplots between measured and estimated Car content using spectral features and indices at +30° angle ( $n = 36$ ).

1000 nm was lower than that measured from the corresponding backscattering directions. The performance of data obtained from the  $-50^\circ$  direction was comparable with that obtained from the  $+50^\circ$  direction, and slightly better than that obtained from other forward scattering directions. This was because shadowing effects on spectral reflectance from this direction was reduced (Ni et al., 1999).

It is acknowledged that the larger the view zenith angle, the less the fraction of gaps and background soil in the field of view (Stagakis et al., 2010). However, our study showed

that at  $\pm 60^\circ$  angles, the spectral features and indices exhibited the weakest correlation with Car content. This was because the penetration depth of light was small at a larger zenith angle, so that information from the upper-layers of a crop canopy was captured only (Huang et al., 2011). As part of the multi-angle spectral dataset used in this study was collected during late growth period of wheat (heading to milk-ripe stages), the upper-layer materials were almost dominated by the heads of winter wheat, which affected the canopy spectral reflectance (Haboudane et al., 2004). Thus,

spectral reflectance measured at larger zenith angles (e.g., 60° or beyond) is mainly determined by the heads rather than pigments containing leaves, therefore the performance for pigment content estimation at these angles is poor.

Among all spectral parameters tested, the two spectral features, i.e., AD and AA, were proven to be the most suitable for Car estimation using multi-angle spectral data in the study. This was because the continuum removal transformation reduced the influences from factors not related to Car and changes in shapes of spectral absorption features best captured the variation of Car content (Clark and Roush, 1984; Sanches et al., 2014). A variety of spectral features have been derived from continuum-removed spectra for the determination of chlorophyll (Sanches et al., 2014), nitrogen (Kokaly, 2001) and water content (de Jong et al., 2014) in plants. Our results indicated that spectral features also hold a promising potential for crop Car estimation.

The three-dimensional information of the Earth's surface can be directly acquired by multi-angle remote sensing. This will open an opportunity to provide quick and abundant information on vegetation structural properties. For example, several studies have been devoted to estimation of LAI using multi-angle spaceborne remote sensing data (e.g., Multi-Angle Imaging Spectroradiometer (MISR), Compact High-Resolution Imaging Spectrometer (CHRIS)) (Chen et al., 2003; Gu et al., 2015). Huber et al. (2010) stated that accurate mapping of forest biochemical parameters is impacted by vegetation structure, so biochemical parameter estimation might be improved with additional information contained in multi-angular data. According to our study, multi-angle spaceborne sensors could be developed with viewing angles between 20° and 40° at the visible and NIR wavebands, so as to improve the estimation of Car content or other pigments in crops. However, biochemical parameter estimation from multi-angle spaceborne data will be more complicated, owing to the greater uncertainty (e.g., mixed pixels), especially for data observed from different directions. Besides, the application of multi-angle observations may bring additional uncertainties to vegetation indices (Pocewicz et al., 2007; Verrelst et al., 2008), which would be a challenge and requires further studied in the future.

## 5. Conclusions

This study showed that multi-angle viewing hyperspectral data have strong capability for crop canopy carotenoid (Car) content estimation. Spectral features and indices derived from spectral reflectance at backscattering viewing angles from 20° to 40° generally provides better estimation accuracy than that from conventional nadir direction, and 30° backscattering spectral data showed the strongest correlation. The best spectral feature that can track dynamic changes of Car content was the absorption depth (AD) at around 500 nm derived from observation data at 30° view

zenith angle from backscattering direction, as it can reduce the effects from soil background and canopy structures associated to plant cultivars. These results indicated the importance of accounting for viewing geometry effect when analyzing spectral features and indices. More importantly, the application of multi-angle observation would offer great possibilities to enhance the accuracy of Car content estimation, to facilitate detection of variability and variation in pigment composition and physiological status, for water and nutrient management in precise agriculture at a large scale.

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