Remote estimation of canopy nitrogen content in winter wheat using airborne hyperspectral reflectance measurements

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Abstract

Timely and accurate assessment of canopy nitrogen content (CNC) provides valuable insight into rapid and real-time nitrogen status monitoring in crops. A semi-empirical approach based on spectral index was extensively used for nitrogen content estimation. However, in many cases, due to specific vegetation types or local conditions, the applicability and robustness of established spectral indices for nitrogen retrieval were limited. The objective of this study was to investigate the optimal spectral index for winter wheat (\textit{Triticum aestivum} L.) CNC estimation using Pushbroom Hyperspectral Imager (PHI) airborne hyperspectral data. Data collected from two different field experiments that were conducted during the major growth stages of winter wheat in 2002 and 2003 were used. Our results showed that a significant linear relationship existed between nitrogen and chlorophyll content at the canopy level, and it was not affected by cultivars, growing conditions and nutritional status of winter wheat. Nevertheless, it varied with growth stages. Periods around heading stage mainly worsened the relationship and CNC estimation, and CNC assessment for growth stages before and after heading could improve CNC retrieval accuracy to some extent. CNC assessment with PHI airborne hyperspectra suggested that spectral indices based on red-edge band including narrowband and broadband CI\textsubscript{red-edge}, NDVI-like and ND\textsubscript{705} showed convincing results in CNC retrieval. NDVI-like and ND\textsubscript{705} were sensitive to detect CNC changes less than 5 g/m\textsuperscript{2}, narrowband and broadband CI\textsubscript{red-edge} were sensitive to a wide range of CNC variations. Further evaluation of CNC retrieval using field measured hyperspectra indicated that NDVI-like was robust and exhibited the highest accuracy in CNC assessment, and spectral indices (CI\textsubscript{red-edge} and CI\textsubscript{green}) that established on narrow or broad bands showed no obvious difference in CNC assessment. Overall, our study suggested that NDVI-like was the optimal indicator for winter wheat CNC retrieval.

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Keywords: Airborne hyperspectral; Spectral index; Canopy nitrogen content; Winter wheat

1. Introduction

Nitrogen (N) is the most important element that affects growing conditions and yield of crops (Ladha et al., 2005). A sufficient supply of nitrogen is crucial to the biochemistry of plants (Clevers and Kooistra, 2012): nitrogen deficiency significantly diminishes the photosynthetic yield of
crops (Feng et al., 2014), while excessive application of nitrogen fertilizer can cause environmental pollution (Ferguson et al., 2002; Hatfield et al., 2008; Inoue et al., 2012). Therefore, timely and accurate assessment of nitrogen status is critical in agricultural management so that the efficiency of nitrogen usage can be improved, thus ensuring a high grain yield while minimizing environmental damage (Tian et al., 2011). Traditional laboratory-based techniques are effective for diagnosing nitrogen status and making nitrogen fertilizer recommendations (Wu et al., 2007). However, these techniques are generally tedious, time-consuming and destructive, and thus cannot be repeated many times in order to make a more representative evaluation of canopy nitrogen status in a particular field or in the fields of a given area (Lemaire, 1997).

Remote sensing technologies have proved to be a potential source for estimates of variables related to biophysical, physiological or biochemical characteristics (Hansen and Schjoerring, 2003). Within the visible and near infrared wave range (400 nm–1000 nm), the absorption features of leaf spectral reflectance are mainly dominated by plant pigments and effects of the leaf cell structure. Research has shown that the absorption features in the blue and red spectral regions is strongly correlated with leaf chlorophyll (Chl) content and a close correlation between foliar nitrogen and chlorophyll content has been reported for various crops such as wheat, maize and potatoes, which provides the bridge for leaf nitrogen estimation using spectral features in visible and near infrared wave range (Clevers and Kooistra, 2012; Oppelt and Mauser, 2004; Yoder and Pettigrew-Crosby, 1995). Although the relationship between nitrogen and chlorophyll contents at the canopy level forms the basis for canopy nitrogen content (CNC) assessment, limited attention have been focused on the relationship among published studies on CNC retrieval. Whether this relationship is dependent on species type, phenological stage, growing conditions and nutritional status; different growing conditions and plant nutritional status; and linear estimator of canopy nitrogen content in both crops such as wheat, maize and potatoes, which provides the bridge for leaf nitrogen estimation using spectral features in visible and near infrared wave range (Clevers and Kooistra, 2012; Oppelt and Mauser, 2004; Yoder and Pettigrew-Crosby, 1995). Although the relationship between nitrogen and chlorophyll contents at the canopy level forms the basis for canopy nitrogen content (CNC) assessment, limited attention have been focused on the relationship among published studies on CNC retrieval. Whether this relationship is dependent on species type, phenological stage, growing conditions and nutritional status (Clevers and Kooistra, 2012), and how the relationship affects CNC estimation need to be intensively studied in order for better understanding of CNC retrieval.

With remote sensing techniques, much progress in nitrogen content assessment have been made in agricultural crops (Clevers and Gitelson, 2013; Clevers and Kooistra, 2012; Inoue et al., 2012; Schlemmer et al., 2013). Among these researches, a semi-empirical method based on spectral indices are commonly used for their high computation efficiency and universality. Sims and Gamon (2002) proposed two hyperspectral indices including normalized difference (ND705) and simple ratio (SR705), and found that ND705 and SR705 were good estimators of chlorophyll and nitrogen content. Work conducted by Clevers and Kooistra (2012) indicated that the red-edge chlorophyll index (CIred-edge) was linearly related to canopy chlorophyll content using PROSAIL simulations, and it was a good and linear estimator of canopy nitrogen content in both grassland and potato cropping systems. Based on the normalized difference vegetation index (NDVI) formula, Darvishzadeh et al. (2011) developed an inspiring NDVI-like index with hyperspectral data, and it showed remarkable performance in crop variables assessment, such as LAI. Its capability and applicability in other variables retrieval, such as nitrogen and chlorophyll, deserves investigation. To acquire information on agronomic variables at regional scale, the capability of spectral index method in retrieval of crop chlorophyll and nitrogen using multispectral satellite data has been investigated. Clevers and Gitelson (2013) found that the CIred-edge, the green chlorophyll index (CIgreen), and the MERIS terrestrial chlorophyll index (MTCI) were accurate and linear estimators of canopy chlorophyll and nitrogen contents by focusing on the potential of Sentinel-2 and Sentinel-3 satellites for crop parameters retrieval. Schlemmer et al. (2013) suggested that canopy chlorophyll and nitrogen content in maize could be accurately retrieved using CIgreen and CIred-edge based on near infrared, green and red-edge spectral bands of Sentinel-2 satellite. The above mentioned spectral indices established on hyperspectral (narrow) or multispectral (broad) bands indeed exhibited good performance in crop nitrogen and chlorophyll retrieval. Nevertheless, their universality and robustness, and whether these spectral indices established on broad or narrow bands affect their capability in CNC estimation need to be clear.

The development of airborne hyperspectral techniques offers valuable opportunities for agronomic variables retrieval. Airborne hyperspectral sensors could obtain abundant information related to canopy characteristics using numerous fine narrow bands within specific spectrum at regional scale, thus making it possible for rapid and real-time detection of crop variables. The aforementioned spectral indices have been proved to be good estimators for chlorophyll or nitrogen content assessment using field measured hyperspectral or satellite multispectral data. Nevertheless, their applicability in CNC assessment using airborne hyperspectral reflectance measurements is rarely reported. Comprehensive evaluation of these spectral indices in CNC retrieval based on airborne hyperspectral techniques could help to enhance the universality and robustness of these indices. Also, it could contribute to diagnosing nitrogen status in crops and provide basis for satellite remote sensing applications in agricultural production.

Therefore, the aim of the present study was to assess the estimation of CNC in winter wheat using spectral indices derived from airborne hyperspectral measurements. The specific objectives were to: (i) investigate the relationship between CNC and canopy chlorophyll content (CCC) and its influence on CNC estimation using multiple datasets obtained from field experiments conducted under different growing conditions and plant nutritional status; (ii) evaluate the predictive power of broadband and narrowband indices derived from airborne hyperspectral reflectance, that is, ND705, SR705, MTCI, NDVI-like narrowband CIred-edge and CIgreen, and broadband CIred-edge and CIgreen in winter wheat CNC retrieval. It
was anticipated that the results of this study would help to provide a reference for real-time monitoring of the CNC in cereal crops.

2. Materials and methods

2.1. Experimental design

To achieve the mentioned objectives, two different field experiments carried out during 2002 and 2003 growing periods were used, as described below.

Experiment 1 (Exp. 1): this experiment was conducted in 2002 at the National Experimental Station for Precision Agriculture (40°10′N, 116°27′E), Beijing, China. The field soil used was classified as silty clay loam. Three cultivars of winter wheat (9507, 9428 and Jingdong 8) were planted in 48 sampling plots that each in a size of 30 m × 32.4 m, where nitrogen fertilizer was applied at concentrations of 0, 150, 300 and 450 kg ha⁻¹, with one third being applied at pre-planting, one third at tillering stage (Zadoks scale 20, Z20) and the remaining at stem elongation stage (Z30). Water was also applied in amounts of 0, 225, 450 and 675 m³ ha⁻¹, with 50% being added at tillering stage (Z20) and 50% at elongation (Z30). The sampling dates included March 25 (tillering stage, Z25), April 2 (stem elongation, Z31), April 10 (stem elongation, Z34), April 18 (booting, Z41), May 5 (head emergence, Z54), May 17 (pollination, Z60), May 24 (pollination, Z68), and May 31 (milk development, Z73).

Experiment 2 (Exp. 2): several farmers’ fields located in the Shunyi (40°08′N, 116°39′E), Changping (40°13′N, 116°12′E) and Fangshan ((39°43′N, 115°59′E)) districts of Beijing were used for the field experiment carried out during 2003. Two major varieties of winter wheat (9507 and 9428) were grown in these fields, which were managed by local farmers. 21 sampling plots that had an area of 30 m × 30 m and in which a single cultivar had been planted were chosen for field surveys that were conducted on March 30 (tillering, Z27), April 7 (stem elongation, Z33), April 15 (stem elongation, Z37), April 23 (booting, Z45), May 1 (head emergence, Z50), May 9 (head emergence, Z56), May 17 (pollination, Z60), May 25 (pollination, Z68) and June 2 (milk development, Z73).

2.2. Data collection

2.2.1. Spectra measurements

The canopy reflectance was measured using an ASD FieldSpec Pro spectrometer (Analytical Spectral Devices, Boulder, CO, USA) under clear, blue-sky conditions between 10:00 h and 14:00 h (Beijing Local Time). In each experiment, a 1 m × 1 m area of crop was selected for measurement of the canopy reflectance and the measurements were obtained at a nadir position at approximately 1.3 m above the ground. The spectrometer was configured with a spectral range of 350 to 2500 nm and a field of view of 25°, and its spectral resolution (full width at half maximum, FWHM) was 1.4 nm for the region 350 to 1050 nm and 2 nm for the region 1050 to 2500 nm. The measured radiance was converted into reflectance by recording irradiance spectra from a 0.4 m × 0.4 m BaSO₄ calibration panel. All radiance measurements were recorded as an average of 20 individual measurements (minus dark current) using an optimized integration time.

2.2.2. Plant measurements

In both experiment, the 1 m × 1 m area of wheat samples were collected for biophysical and biochemical analysis after canopy spectra measurements. For each sample, all green leaves were separated from the stems. leaf area index (LAI) was determined with a dry weight method (Wang et al., 2005). Leaf segments of approximate area 0.06 m² were cut from the central part of about 30 leaves selected as reference leaves for LAI calculation. Both reference leaves and the remaining leaves were oven-dried at 70 °C to constant weight and weighed. LAI was calculated as Eq. (1):

\[
\text{LAI} = \frac{S_r W_t}{S_l W_r} \quad (1)
\]

where \( S_r \) (m²) is the area of the reference leaves, \( W_t \) (g) is the total dry weight of the sampled leaves, \( S_l \) is the sampled land area (m²) and \( W_r \) (g) is the dry weight of the reference leaves. Then specific leaf weight (SLW, g/m²) was calculated from the dried weight and the area of the reference leaves.

After LAI and SLW determination, leaf samples were ground to pass through a 40-mesh screen. Leaf nitrogen concentration (LNC, %) was determined by the Kjeldahl method (Bremner, 1960) with a B-339 distillation unit, and leaf chlorophyll concentration (LCC, mg/g) was obtained by the spectrophotometric method (Gao, 2006). Next, the CNC and CCC were calculated using the equations below (Zhao et al., 2012).

\[
\text{CNC} = \frac{(\text{LNC} \times \text{LAI} \times \text{SLW})}{100} \quad (2)
\]

\[
\text{CCC} = \frac{(\text{LCC} \times \text{LAI} \times \text{SLW})}{100} \quad (3)
\]

2.2.3. Airborne campaigns

Airborne hyperspectral images of the experimental fields were acquired using the Pushbroom Hyperspectral Imager (PHI) designed by the Shanghai Institute of Technical Physics of the Chinese Academy of Sciences and flown onboard a Yun-5 aircraft (Shijiazhuang Aircraft Manufacturing Company, China). The PHI sensor comprised a solid state, area array and silicon CCD device of 780 × 244 elements and had a spectral range of 400–850 nm, with a field of view of 21°. Its spectral resolution was 5 nm and the sensor was able to obtain images in a nadir-pointing direction with a 1 m × 1 m spatial resolution at an altitude of 1000 m above the ground. During the 2002 growing season, images were obtained at midday on April 18, May 17 and May 31. The images were radio-
metrically and geometrically corrected using an array of georeferenced light and dark targets (5 m × 5 m) located at the boundaries of the field site. The location of each target, as well as any field measurements were recorded using a differential global positioning system (Trimble Sunnyvale California, USA). In order to obtain the hyperspectral reflectance, radiometric correction was performed using the band by band moment matching method and the images were geometrically corrected using ground control points (Liu, 2002).

In addition, the PHI images were denoised with the Savitzky–Golay method to further improve the image quality (Xie et al., 2014). The denoised spectrum acquired with PHI sensor on April 18, May 17 and May 31 were presented in Fig. 1. In addition, the synchronous canopy reflectance, radiometric correction was performed using the PHI spectrum for biochemical retrievals. However, differences existed in the reflectance value in the near-infrared bands (780–850 nm). This might be largely related with the noise ratio of the PHI sensor in the near-infrared wavelengths.

2.3. Spectral indices

In the present study, published spectral indices that made accurate estimation of nitrogen in previous studies were chosen to assess CNC in winter wheat with PHI airborne hyperspectra. These indices included ND705, SR705, MTCI, narrowband CIred-edge and CIgreen. Meanwhile, the capability of broadband indices in CNC assessment were also investigated. Since the PHI sensor had a spectral range of 400–850 nm, with a 5 nm spectral resolution, we resampled the PHI hyperspectra with Sentinel-2 spectral response function to obtain broadband spectra, and broadband CIred-edge and CIgreen indices were then calculated to make a comparison with narrowband CIred-edge and CIgreen. In addition, the inspiring narrowband NDVI-like was also used for CNC assessment. It was calculated from the PHI reflectance spectra, using all possible 112 × 112 = 12544 wavelength combinations between 405 and 835 nm. The correlation coefficient ($R^2$) between the NDVI-like and the measured CNC were computed. Band combinations that formed the optimal index were determined for CNC estimation based on the highest $R^2$ values. Details about these indices were shown in Table 1.

2.4. Model evaluation

To evaluate the performance of spectral indices in CNC retrieval, best-fit functions between spectral indices and CNC were developed. Sensitivity of different spectral indices to detect changes in CNC was then tested through the use of the noise equivalent (NE) as the method reported by (Vina and Gitelson, 2005) and (Vina et al., 2011). Moreover, a $k$-fold cross-validation procedure was used to validate the behavior of spectral indices using PHI hyperspectra in CNC assessment. In our case, the hyperspectra dataset (144 samples, of which 4 samples with 0 LAI value were left out) was randomly split into ten sets ($k = 10$), and each set was estimated by the remaining samples. All the selected spectral indices were tested using the same $k$-fold partitions. This kind of validation was necessary because it reduced the dependence on a single random partition into calibration and validation datasets; also, it guaranteed that all samples were used for both training and validation with each sample used for validation exactly once. Then, the overall performance of these models were evaluated by statistics including coefficient of determination ($R^2$), root mean square error (RMSE) and relative RMSE (RRMSE). $R^2$ reflects the strength of linear statistical correlation between variables, while RMSE and MAE indicate absolute estimation errors and RRMSE express the error in relative terms, allowing the comparisons between different variables. The equations for these statistics are:

$$\text{NE} = \frac{\text{RMSE(VI vs. CNC)}}{\text{d(VI)/d(CNC)}}$$

$$\text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (V_{est}^i - V_{obs}^i)^2}$$

![Fig. 1. Comparison of spectrum acquired with ASD device and PHI sensor. (a), (b) and (c) are the spectrum of Jingdong 8 cultivar of winter wheat acquired on April 18, May 17 and May 31, respectively.](image-url)
The RMSE is the root mean square error of the best-fit function of the relationship “vegetation index (VI) vs. CNC” with respect to canopy nitrogen content, while, RRMSE = 100 × RMSE/\text{Mean}(\text{obs}) (6)

where \(d(VI)/d(CNC)\) is the first derivative of the best-fit function of the relationship “vegetation index (VI) vs. CNC” with respect to canopy nitrogen content, while, RMSE is the root mean square error of the best-fit function of this relationship. The NE has the advantage of allowing the direct comparison among different spectral indices in dynamic ranges. \(V_i^{\text{est}}\) and \(V_i^{\text{obs}}\) are the estimated, observed CNC values, respectively, \(n\) is the number of samples.

3. Results

3.1. Relationship between CNC and CCC

Relationship between chlorophyll and nitrogen was crucial, since crop nitrogen status could be accessed through chlorophyll estimates (Baret et al., 2007). Many studies suggested that crop nitrogen content was closely related with chlorophyll content, for instance, Ercoli et al. (1993) found a strong correlation between chlorophyll content and nitrogen content on a leaf-area basis. In the current study, linear relationship between CNC and CCC in winter wheat was found and this remarkable relationship was not affected by cultivars, growing conditions and nutritional status of winter wheat (Table 2). Nevertheless, the relationship did vary a little bit with growing stages. During the whole growth periods, the slope of the relationship between CNC and CCC shows an obvious decreasing tendency (Fig. 2a), and it remains constant from then until the milk development stage (Z73), when CNC and CCC stabilize. Reasons for this tendency could be explained that in early stages, such as tillering and stem elongation, nitrogen uptake exceeds the production of chlorophyll; with the advancement of growth periods, the consumption of a large amount of available nitrogen in biochemical reactions and the production of a large amount of chlorophyll cause the slope to decrease. It is worth noting that the heading stage (Z54) seems to be a boundary to separate the growth stages before and after heading, because slopes of the relationship exists great difference, which is more significant in stress condition (Exp. 1). This phenomenon might give a clue for CNC estimation separately for stages before and after heading to avoid the effect of heading stage.

In addition to the slope parameter, several interesting aspects were also observed in the coefficient of determination \(R^2\) and standard error (SE) of the relationships. The \(R^2\) for the relationship is the lowest during head emergence stage (Fig. 2b), and the SE value is the highest during the head emergence stage (Fig. 2c). Reasons for this might be that, apart from being consumed during the biochemical processes, nitrogen is also concentrated in the grains in stages such as head emergence and anthesis stages (Baret et al., 2007).

| Growth stage | N/Chl relationship | \(R^2\) | SE | N content |
|--------------|-------------------|--------|----|-----------|
| Z25          | \(N = 5.200 \times \text{Chl} + 0.124\) | 0.963  | 0.013 |            |
| Z31          | \(N = 3.080 \times \text{Chl} + 0.968\) | 0.884  | 0.065 |            |
| Z34          | \(N = 3.544 \times \text{Chl} + 1.352\) | 0.805  | 0.157 |            |
| Z41          | \(N = 3.107 \times \text{Chl} + 1.328\) | 0.739  | 0.193 |            |
| Z54          | \(N = 2.250 \times \text{Chl} + 1.688\) | 0.773  | 0.233 |            |
| Z60          | \(N = 2.479 \times \text{Chl} + 0.725\) | 0.862  | 0.215 |            |
| Z68          | \(N = 2.323 \times \text{Chl} + 0.801\) | 0.832  | 0.047 |            |
| Z73          | \(N = 2.429 \times \text{Chl} + 0.547\) | 0.776  | 0.083 |            |
| All          | \(N = 2.841 \times \text{Chl} + 0.984\) | 0.801  | 0.399 |            |

\(X. Zhou et al. / Advances in Space Research 58 (2016) 1627–1637\)
et al., 2007) and this may have disrupted the relationship between CNC and CCC. To further investigate the relationship between winter wheat CNC and growth stages, the average CNC, Standard deviation (SD) and Coefficient of variance (CV) of CNC for various growth stages are shown in Fig. 3. With the advance of growth stages, the Average CNC and SD value of CNC in winter wheat offer a trend of rise first then fall, the value comes to the maximum when heading stage occurs; while the situation for CV value is the opposite. The CV value of CNC represents the discrete degree of CNC value, the smaller the CV value, the more aggregated the CNC value, this could explain the reason that \( R^2 \) between CNC and CCC, and their relationship with CNC shows a high convergence of the points (Fig. 4). In contrast, relationships between CNC vs. SR\(_{705}\), MTCI, narrowband CI\(_{\text{red-edge}}\), narrowband CI\(_{\text{green}}\), broadband CI\(_{\text{red-edge}}\) and broadband CI\(_{\text{green}}\) all follow a power function. With the best-fit models, sensitivity of the eight spectral indices to CNC variations was tested through the use of NE\(\Delta\)CNC. Results in Fig. 5 show that indices behave differently to CNC variations. NDVI-like and ND\(_{705}\) have the smallest NE\(\Delta\)CNC values when CNC is less than 4 g/m\(^2\). Nevertheless, NE\(\Delta\)CNC values of these two indices are linearly related to CNC variations. For SR\(_{705}\) and MTCI, their NE\(\Delta\)CNC values are larger than that of NDVI-like and ND\(_{705}\) when CNC is less than 5 g/m\(^2\); however, the case is the contrary when CNC exceeds 5 g/m\(^2\). This indicates that normalized difference indices (NDVI-like and ND\(_{705}\)) might be sensitive to detect small CNC changes (<5 g/m\(^2\)), but vulnerable to saturate at high CNC values. CI\(_{\text{green}}\) and CI\(_{\text{red-edge}}\) show rather low NE\(\Delta\)CNC values responded to CNC changes, and the behavior of NE\(\Delta\)CNC for CI\(_{\text{red-edge}}\) is nearly invariant through the entire range of CNC variation, suggesting that CI\(_{\text{red-edge}}\) is less affected by saturation caused by CNC variations, this accords with the view of Clevers and Kooistra (2012).

### 3.2. Assessment of CNC using airborne hyperspectral data

The best-fit models of the selected spectral indices vs. CNC are shown in Fig. 4. Results suggest that narrowband CI\(_{\text{red-edge}}\) index best captures the canopy nitrogen content, with the highest determination coefficient \((R^2 = 0.771)\), then followed by broadband CI\(_{\text{red-edge}}\) \((R^2 = 0.768)\) and NDVI-like \((R^2 = 0.759)\). ND\(_{705}\) and NDVI-like indices exhibit logarithmic relationship with CNC, and their relationship with CNC shows a high convergence of the points (Fig. 4). In contrast, relationships between CNC vs. SR\(_{705}\), MTCI, narrowband CI\(_{\text{red-edge}}\), narrowband CI\(_{\text{green}}\), broadband CI\(_{\text{red-edge}}\) and broadband CI\(_{\text{green}}\) all follow a power function. With the best-fit models, sensitivity of the eight spectral indices to CNC variations was tested through the use of NE\(\Delta\)CNC. Results in Fig. 5 show that indices behave differently to CNC variations. NDVI-like and ND\(_{705}\) have the smallest NE\(\Delta\)CNC values when CNC is less than 4 g/m\(^2\). Nevertheless, NE\(\Delta\)CNC values of these two indices are linearly related to CNC variations. For SR\(_{705}\) and MTCI, their NE\(\Delta\)CNC values are larger than that of NDVI-like and ND\(_{705}\) when CNC is less than 5 g/m\(^2\); however, the case is the contrary when CNC exceeds 5 g/m\(^2\). This indicates that normalized difference indices (NDVI-like and ND\(_{705}\)) might be sensitive to detect small CNC changes (<5 g/m\(^2\)), but vulnerable to saturate at high CNC values. CI\(_{\text{green}}\) and CI\(_{\text{red-edge}}\) show rather low NE\(\Delta\)CNC values responded to CNC changes, and the behavior of NE\(\Delta\)CNC for CI\(_{\text{red-edge}}\) is nearly invariant through the entire range of CNC variation, suggesting that CI\(_{\text{red-edge}}\) is less affected by saturation caused by CNC variations, this accords with the view of Clevers and Kooistra (2012).
Performance of spectral indices in CNC assessment were then validated with a 10-fold cross-validation strategy using best-fit functions between spectral indices and CNC. The final cross-validation results were determined by averaging the 10 sets’ validation results. The results (Fig. 6) suggest that behaviors of spectral indices in CNC assessment varies. Results established on narrowband CIred-edge show the highest accuracy ($R^2 = 0.627$, $RMSE = 1.400 \, \text{g/m}^2$, $RRMSE = 30.761\%$), then followed by broadband CI red-edge, ND 705, NDVI-like and others. Compared with ND 705, SR 705 is less accurate for CNC estimation. Broadband CI red-edge exhibits nearly the same results with that of narrowband CI red-edge, while, broadband CI green shows even better accuracy than that of narrowband CI green. For broadband CI green index, band 560 nm is the center wavelength and it has a 30 nm spectral width. This might help to add more spectral information than narrowband 560 nm, thus increasing the estimation accuracy of broadband CI green. On the whole, spectral indices that established on red-edge band, such as narrowband and broadband CI red-edge, NDVI-like, and ND 705, showed excellent results in CNC estimation. Generally, pure chlorophyll $a$ and $b$ have absorption peaks at blue
and red waveband regions, respectively (430 nm and 662 nm for Chl $a$; 425 nm and 644 nm for Chl $b$). Nevertheless, within flesh leaves, the absorption peaks might shift toward longer wavelengths due to interactions between chlorophyll molecules and the surrounding molecules such as protein, liquid and water (Nobel, 2009). Inoue et al. (2012) pointed out that the degree of the shift was about 10–50 nm as affected by the physiological status and the ratio between chlorophyll $a$ and $b$, and the absorption peak of chlorophyll at red band region might affect the red edge reflectance. Consequently, the red edge bands (700–750 nm) played a critical role in chlorophyll or nitrogen content estimation. Our current study results support this view as well.

The scatter plots of predicted CNC and measured CNC in Fig. 6 were further investigated to evaluate the CNC estimation with PHI airborne hyperspectra. Among the spectral indices, narrowband and broadband CI$_{\text{red-edge}}$, ND$_{705}$ and NDVI-like showed rather high convergence to the 1:1 line, which was consistent with their high accuracy in CNC retrieval. SR$_{705}$ and MTCI showed good estimate of CNC with $R^2$ higher than 0.530 and RMSE less than 1.600 g/m$^2$. Contrast to narrowband CI$_{\text{green}}$, performance of broadband CI$_{\text{green}}$ showed a slight improvement. Nevertheless, different from the scatters of other indices vs. CNC, one sample point showed obvious deviation from the scatters of both narrowband and broadband CI$_{\text{green}}$ vs. CNC, which indicates that CI$_{\text{green}}$ index might be less stable than other indices. Among all the indices, several samples that had CNC larger than 8 g/m$^2$ were severely underestimated. This underestimation suggests that all these spectral indices could be prone to saturate when CNC value gets higher than 8 g/m$^2$. To sum up, even though spectral indices behaved differently, satisfactory estimating results could be achieved by spectral indices methods in CNC retrieval, indicating that it is feasible and practicable to assess winter
wheat CNC with spectral indices derived from PHI airborne hyperspectral data.

3.3. Evaluation of spectral indices in CNC retrieval

Field measured hyperspectra and corresponding CNC of winter wheat that collected from the whole growth cycle in 2002 and 2003 were used to further evaluate the robustness of the selected spectral indices in CNC retrieval. The measured hyperspectra were resampled based on PHI spectral response function to keep consistent with the spectral resolution of PHI sensor. Meanwhile, we also resampled the hyperspectral dataset with Sentinel-2 spectral response function to obtain broad band spectral reflectance for broadband CI<sub>red-edge</sub> and CI<sub>green</sub> calculation. Then, sampling data from 2002 were used for model calibration through developing best-fit functions, while data from 2003 were used for model validation. In calibration results (Table 3), the best-fit models for spectral vs. CNC relationships are all power function. NDVI-like shows the best accuracy ($R^2 = 0.657$, RMSE = 1.309 g/m$^2$, RRMSE = 29.837%), then followed by ND<sub>705</sub> and others. Calibration model developed by MTCI shows the highest determination coefficient ($R^2 = 0.681$), nevertheless, it has the lowest accuracy (RMSE = 1.475 g/m$^2$, RRMSE = 33.620%). Narrowband and broadband CI<sub>red-edge</sub> show the same results, while broadband CI<sub>green</sub> exhibits better accuracy than narrowband CI<sub>green</sub> in calibration results.

Validation results in Table 4 show that NDVI-like index exhibits the highest accuracy ($R^2 = 0.659$, RMSE = 1.221 g/m$^2$, RRMSE = 27.824%), then followed by ND<sub>705</sub> and broadband CI<sub>green</sub>. In CNC assessment with PHI hyperspectra, NDVI-like and ND<sub>705</sub> also showed good accuracy. Their excellent performance in CNC estimation with field measured hyperspectra (Rank 1 and Rank 2 in both calibration and validation results) suggests that they are robust and accurate in CNC estimation using various hyperspectral datasets. Comparing the behavior of ND<sub>705</sub> and SR<sub>705</sub> in CNC estimation with both PHI airborne hyperspectra (Fig. 6) and field measured hyperspectra (Tables 3 and 4), we could find that ND<sub>705</sub> showed better estimation accuracy than SR<sub>705</sub>. These two indices were composed by 750 and 705 nm, but with different formations. Their varied performance suggests that indices of normalized difference formation (ND<sub>705</sub>) might be more accurate than that of simple ratio form (SR<sub>705</sub>) for CNC retrieval. In validation results, broadband CI<sub>red-edge</sub> and narrowband CI<sub>red-edge</sub> showed nearly the same results, while broadband CI<sub>green</sub> behaved even better than narrowband CI<sub>green</sub>. The performance of narrowband and broadband indices in CNC estimation with field measured hyperspectra indicates that spectral indices (CI<sub>red-edge</sub> and CI<sub>green</sub>) that established on narrow or broad bands show no obvious difference in CNC retrieval.

4. Discussion

In the present study, we confirmed a significant linear relationship between CNC and CCC in winter wheat, which made it possible for CNC estimation using spectral features in visible and near-infrared wave range. Moreover, we found that the relationship was unacted on the cultivars, growing conditions and nutritional status of winter wheat. Nevertheless, the relationship did vary a little bit with growing stages, and the periods around heading stage severely affected the relationship: $R^2$ for the relationship was low and SE value was high when heading stage occurred (Table 2 and Fig. 2). Changes of winter wheat CNC demonstrated that the periods around heading stage influenced the variation of CNC: the average CNC was the highest and CV of CNC was the lowest when heading stage approached (Fig. 3). Correlation between CNC with NDVI-like index indicated that the periods around heading stage severely affected the CNC retrieval: $R^2$ for the correlation is the lowest when heading stage occurred (Fig. 3). All of these analysis indicated that the CNC/CCC relationship and CNC retrieval were influenced by growth stages, especially by the periods around heading stage. Given that the slopes of CNC/CCC relationship varied obviously for stages before and after heading (Table 2), we conducted CNC estimation for growth stages before and after heading stage using NDVI-like index (Table 5). Compared with the performance of NDVI-like in calibration (Table 3) and validation (Table 4), estimating results of CNC for growth stages before heading showed great improvement (Table 5); while results of CNC for growth stages after heading showed satisfactory results in accord with that in Tables

| Index        | Rank | Model equation | $R^2$ | RMSE (g/m$^2$) | RRMSE (%) |
|--------------|------|----------------|-------|----------------|-----------|
| ND<sub>705</sub> | 2    | $y = 10.827x^{1.539}$ | 0.641 | 1.323          | 30.168    |
| SR<sub>705</sub> | 7    | $y = 0.875x^{1.439}$   | 0.620 | 1.446          | 32.965    |
| MTCI         | 8    | $y = 0.557x^{1.462}$   | 0.681 | 1.475          | 33.620    |
| NDVI-like    | 1    | $y = 18.403x^{1.407}$  | 0.657 | 1.309          | 29.837    |
| Narrowband CI<sub>red-edge</sub> | 3    | $y = 1.813x^{0.784}$   | 0.618 | 1.407          | 32.078    |
| Narrowband CI<sub>green</sub>    | 6    | $y = 0.974x^{0.942}$   | 0.581 | 1.433          | 32.676    |
| Broadband CI<sub>red-edge</sub>  | 4    | $y = 1.815x^{0.791}$   | 0.617 | 1.407          | 32.084    |
| Broadband CI<sub>green</sub>     | 5    | $y = 0.964x^{0.926}$   | 0.584 | 1.428          | 32.548    |

Note: the Rank was a comprehensive evaluation based on $R^2$, RMSE and RRMSE, the same below.
3 and 4. This implies that CNC estimating for growth stages before and after heading could relieve the effect of heading stage on CNC retrieval to some extent.

The method applied in this study that combining measured CNC with spectral indices derived from PHI hyperspectra has further demonstrated the feasibility for extracting CNC in winter wheat using airborne hyperspectral data. Among the selected spectral indices, spectral indices established on red-edge band that including narrowband and broadband CI<sub>red-edge</sub>, NDVI-like and ND<sub>705</sub> showed convincing results in CNC retrieval using PHI airborne hyperspectra (Fig. 6). NDVI-like and ND<sub>705</sub> were employed for CNC assessment using semi-empirical methods based on spectral indices including ND<sub>705</sub>, SR<sub>705</sub>, MTCI, NDVI-like, narrowband and broadband CI<sub>red-edge</sub>, and narrowband and broadband CI<sub>green</sub>. Then the whole 2002 and 2003 field data were used to further investigate the robustness and applicability of these indices in CNC retrieval.

Our main findings were that a significant linear correlation existed between CNC and CCC, which provided a bridge for CNC assessment using spectral features in the visible and near-infrared wave range. Moreover, this relationship was not affected by cultivars, growing conditions and nutritional status of winter wheat. Nevertheless, it varied with growth periods, and the periods around heading stage mainly affected the relationship and CNC estimation. Sensitivity to CNC variations suggested that NDVI-like and ND<sub>705</sub> might be sensitive to detect small CNC changes (<5 g/m<sup>2</sup>), but vulnerable to saturate at high CNC values, while narrowband and broadband CI<sub>red-edge</sub> were sensitive to a wide range of CNC changes. CNC assessment using PHI hyperspectra suggested that spectral indices based on red-edge band including narrowband and broadband CI<sub>red-edge</sub>, NDVI-like and ND<sub>705</sub> showed convincing results in CNC retrieval. Evaluation of spectral indices in CNC assessment with field measured hyperspectra indicated that NDVI-like was robust and accurate for CNC estimation using various hyperspectral datasets. Spectral indices (CI<sub>red-edge</sub> and CI<sub>green</sub>) that established on narrow or broadband bands showed no obvious difference in CNC retrieval, broadband indices were suitable for CNC assessment as well. Overall, our study suggests that NDVI-like index was the optimal indicator for winter wheat CNC retrieval.

### Table 4
Validation results of CNC (g/m<sup>2</sup>) using the whole dataset acquired in 2003 (n = 189).

| Index         | Rank | Model equation | $R^2$ | RMSE (g/m<sup>2</sup>) | RRMESE (%) |
|---------------|------|----------------|-------|------------------------|------------|
| ND<sub>705</sub> | 2    | $y = 0.659x + 1.614$ | 0.644 | 1.236                  | 28.179     |
| SR<sub>705</sub> | 7    | $y = 0.781x + 1.327$ | 0.631 | 1.362                  | 31.046     |
| MTCI          | 8    | $y = 0.733x + 1.343$ | 0.511 | 1.587                  | 36.180     |
| NDVI-like     | 1    | $y = 0.682x + 1.587$ | 0.659 | 1.221                  | 27.824     |
| Narrowband CI<sub>red-edge</sub> | 6    | $y = 0.733x + 1.350$ | 0.631 | 1.293                  | 29.465     |
| Narrowband CI<sub>green</sub>     | 4    | $y = 0.733x + 1.215$ | 0.636 | 1.269                  | 28.934     |
| Broadband CI<sub>red-edge</sub>   | 5    | $y = 0.732x + 1.355$ | 0.632 | 1.291                  | 29.423     |
| Broadband CI<sub>green</sub>      | 3    | $y = 0.736x + 1.215$ | 0.642 | 1.259                  | 28.700     |

### Table 5
CNC estimating results for stages before and after heading stage with NDVI-like index (n = 569).

| Growth stages          | n    | Model equation | $R^2$ | RMSE (g/m<sup>2</sup>) | RRMESE (%) |
|------------------------|------|----------------|-------|------------------------|------------|
| Stages before heading  | 192  | $y = 18.282x + 1.252$ | 0.784 | 1.041                  | 21.357     |
|                        | 84   | $y = 0.886x + 0.966$  | 0.804 | 1.022                  | 25.761     |
| Stages after heading   | 188  | $y = 20.419x + 1.662$ | 0.730 | 1.259                  | 32.393     |
|                        | 105  | $y = 0.512x + 2.193$  | 0.580 | 1.371                  | 28.991     |

5. Conclusions

This study aimed to evaluate the estimation of CNC in winter wheat using spectral indices derived from airborne hyperspectral measurements. Two field survey datasets acquired in 2002 and 2003 during the major growth stages of winter wheat were used to investigate the relationship between CNC and CCC. The PHI airborne hyperspectral imagery obtained corresponding to experiment in 2002 were employed for CNC assessment using semi-empirical methods based on spectral indices including ND<sub>705</sub>, SR<sub>705</sub>, MTCI, NDVI-like, narrowband and broadband CI<sub>red-edge</sub>, and narrowband and broadband CI<sub>green</sub>. Then the whole 2002 and 2003 field data were used to further investigate the robustness and applicability of these indices in CNC retrieval.
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