Estimation of winter wheat grain crude protein content from *in situ* reflectance and advanced spaceborne thermal emission and reflection radiometer image

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Abstract. The advanced technology in site-specific and spaceborne determination of grain crude protein content (CP) by remote sensing can help optimize the strategies for buyers in aiding purchasing decisions, and help farmers to maximize the grain output by adjusting field nitrogen (N) fertilizer inputs. We performed field experiments to study the relationship between grain quality indicators and foliar nitrogen concentration (FNC). FNC at anthesis stage was significantly correlated with CP, while spectral vegetation index was significantly correlated to FNC. Based on the relationships among nitrogen reflectance index (NRI), FNC and CP, a model for CP prediction was developed. NRI was able to evaluate FNC with a higher coefficient of determination of R^2= 0.7302 in Experiment A. The relationship between laboratory measured and remotely sensed FNC had a coefficient of determination of R^2=0.7279 in Experiment B. The method developed in this study could contribute towards developing optimal procedures for evaluating wheat grain quality by in situ canopy-reflected spectrum and ASTER image at anthesis stage. CP derived from both in situ spectrum and the ASTER image exhibited high accuracy and the precision in Experiment C. The RMSE were 0.893 % for in situ spectrum model and 1.654 % for ASTER image model, and the R^2 were 0.7661 and 0.7194 for both, respectively. It is thus feasible to forecast grain quality by NRI derived from in situ canopy-reflected spectrum and ASTER image. Our results indicated that the inversion of FNC and the evaluation of CP by NRI were surprisingly good.

Keywords: winter wheat (Triticum aestivum L), canopy reflectance, nitrogen reflectance index (NRI), grain crude protein content, ASTER image.

1 INTRODUCTION

Wheat (*Triticum aestivum* L) is one of the main grain crops in Northern China. Farmers, agricultural managers, and grain-processing enterprise managers are interested in estimating wheat grain quality at large scales before the wheat harvest. Grain of certain cultivars can often attract a price premium if it has higher crude protein content (CP) which makes it suitable for dough and bread making. CP above 12.5 % in wheat provides sufficient gluten to form good dough for bread making (Zhao et al, 2005) [1]. Therefore, cereal crops with higher CP are normally of better quality and have higher price premiums than those with lower CP. Advanced site-specific knowledge of CP determination before harvest would provide

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opportunities to adapt optimized strategies for grain harvesting, and to adjust inputs to optimize outputs (Zhao et al ,2005).

Plant canopy reflectance in visible-NIR wavelengths is predominantly influenced by chlorophyll concentration (Chl) related plant pigments (400-700 nm) and leaf cell structures (600-900 nm) (Bonham-Carter, 1987[2]; Gitelson and Merzlyak, 1994[3]). Ünsalan and Boyer (2004) [4] use the linearized vegetation indices with the blue and green bands to estimate vegetation density from satellite images. Saroj et al (2004) [5] developed an operational methodology for estimating soil moisture and crop biophysical parameters. Some spectral vegetation indices (VIs) had been used to estimate Chl,(Chappelle et al., 1992) [6]. Chl had been used to estimate crop nutrient status indirectly because much of N is incorporated in Chl (Filella et al., 1995[7]; Moran et al., 2000[8]). Arregui et al (2006) [9] used Chl meter SPAD (a handheld chlorophyll meter (Model SPAD 502, Minolta Camera Co., Ltd.) enables users to quickly and easily measure leaf greenness which is affected by leaf chlorophyll content) as a tool for N fertilization in winter wheat. Previous studies have proved that Chl concentration in plants was closely correlated with the foliar nitrogen concentration (FNC) (Shadchina, et al., 1998[10]; Serrano et al., 2000[11]). Ding et al (2005) [12] and McCullough (1994) [13] mentioned that there was a robust relationship between Chl concentration and soluble protein content. Xiao (2006) [14] studied the relationship between light absorption by leaf Chl and maximum light use efficiency. Debaeke et al (2006) [15] studied the relationship between the normalized SPAD index and the N nutrition index in wheat. Because of the stability of the Chl-N relation, the foliar nitrogen concentration (FNC) in winter wheat leaves could be determined by their spectral characteristics in the visual region. There are significant correlations between FNC and CP (Bhatia and Rabson, 1976[16]; Scheromm et al., 1992[17]; Boman et al., 1995[18]). Previous studies have observed that FNC affected the CP of winter wheat (Jamieson and Semenov, 2000[19]; Woodard and Bly, 1998[20]; Huang et al., 2004[21]). The interrelation among Chl concentration, FNC and CP made it possible to use reflectance pigment indices to predict grain protein content (Wang et al., 2004[22]).

It is time-consuming and difficult at large scales for the inversion of FNC and the evaluation of CP by traditional field sampling methods because traditional field sampling methods are difficult to quantitatively evaluate the CP of crops before they are fully ripen. The current traditional laboratory methods of CP determination require the collection of matured grain samples, the preparation of samples, and extensive analytical procedures. They are very reliable, but only good for post-harvest quality evaluation. Therefore, it doesn't meet the requirements of farmers, agricultural managers, and grain-processing enterprise managers for estimating grain quality at large scales before wheat is harvested. However, hyperspectral remote sensing can play a vital role in providing time-specific and time-critical information due to its capability in assessing FNC variability. Some studies have focused on the relationship between canopy-reflected spectrum (vegetation index) and plant biochemical concentration. Driss *et al.* (2002) [23] divided the transformed Chl absorption reflectance index by the optimized soil-adjusted vegetation index (TCARI/OSAVI) to make accurate evaluations of crop Chl from airborne hyperspectral imagery. Zhou and Wang (2003) [24] studied leaf and spike reflectance of rice plants under contrasting nitrogen supplemental levels. However, there are only a few reports on forecasting CP via remote sensing data: Kokaly (2001) [25] estimated FNC over dry foliage by stepwise multiple linear regression technique using reflectance spectra, and it indicated that the 2054 nm and 2172 nm could be used for FNC and protein estimation. Hansen et al (2002) [26] predicted grain protein content in winter wheat and spring barley using repeated canopy reflectance measurements and partial least squares regression. Regression models had been constructed for CP prediction by FNC at anthesis and showed surprisingly good results. Wang et al (2004) [22] indicated that the plant pigment ratio (PPR, $(R_{550}-R_{450})/(R_{550}+R_{450})$) could be used to assess winter wheat grain quality based on the relationships among PPR, foliar Chl concentration, FNC, and CP. Liu et al (2006) [27] predicted winter wheat CP using multi-temporal EnviSat-ASAR and Landsat TM satellite images.

The objective of this study was to forecast winter wheat CP by selected spectral index extracted from in situ canopy reflectance and ASTER image around anthesis stage.

2 MATERIALS AND METHODS

2.1 Experimental design

***In situ* canopy reflectance experiment**

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, 2001 to June, 2002 (Experiment A, Zhao et al. (2005)) and September, 2002 to June, 2003 (Experiment B), respectively. Experimental data from Experiment A were used to establish the FNC evaluation models by selected vegetation index. Whereas the data from Experiment B were used to validate the FNC models established by the data from Experiment A.

The experimental site was located in the warm temperate zone with a mean annual rainfall of 507.7 mm and a mean annual temperature of 13℃. The nutrient content of the soil (0-0.30 m) was as follows: organic matter of 14.2–14.8 $g \cdot kg^{-1}$; N-NO3 nitrogen of 0.8–1.0 $g \cdot kg^{-1}$; available phosphorus of 20.1–55.4 $mg \cdot kg^{-1}$; and available potassium of 117.6–129.1 $mg \cdot kg^{-1}$ according to Olsen soil extraction methodology.

Winter wheat varieties were the main varieties in Northern China: Zhongyou 9507, Jing 411, and Jingdong 8. Zhongyou 9507 is a hard winter wheat with higher CP, Jing 411 is a soft winter wheat with lower CP, and Jingdong 8 is a semi-hard winter wheat with mid-range CP. Three winter wheat varieties were planted in 48 plots with a plot size of 32.4 m x 30.0 m under different irrigation and nitrogen treatments. The average LAI value of winter wheat at anthesis stage for the experiment sites was about 4.5. For each winter wheat variety, there were 4 irrigation treatments and 4 fertilization treatments. The 4 irrigation treatments were the following: no irrigation (W0); low irrigation (W1) = 0.023 m^3 m^{-2}; standard irrigation (W2) = 0.046 m^3 m^{-2}; and high irrigation (W3) = 0.069 m^3 m^{-2}. The 4 fertilization treatments were the following: no fertilization (N0); low fertilization (N1) = 5 g m^{-2}; standard fertilization (N2) = 20 g m^{-2}; and high fertilization (N3) = 35 g m^{-2} at reviving stage and erecting stage respectively.

ASTER image experiment

Research was conducted in Beijing suburbs of Tongzhou County (39°50’ 40°20’N, 117°10’E-117°30’E). Three winter wheat varieties of Zhongyou 9507, Jing 411, and Jingdong 8 were planted in the fields. In order to make the field crop information match the ASTER image, field experiments were conducted at the passing times of the Terra satellite during the growing season of 2004 (Experiment C). 48 plots with a plot size of 10 m x 10 m were used to validate the *in situ* spectrum measured CP, and another 24 plots with a plot size of 30 m x 30 m were used to validate the ASTER image derived CP.

2.2. Measuring methodology

***In situ* canopy reflectance**

All canopy-reflected spectrum measurements were taken from a height of 1.30 m above ground, under clear blue sky conditions between 11:00 and 14:00 Beijing Local Time, using an ASD FieldSpec Pro spectrometer (Analytical Spectral Devices, Boulder, CO, USA) fitted with 25° field of view fiber optics, functioning in the 350-2500 nm spectral region with a spectral resolution of 3 nm at 700 nm, 10 nm at 1400 nm and 2100 nm, and at a sampling interval of 1.4 nm between 350-1050 nm, and 2 mm between 1050-2500 nm. Reflected spectrum measurements from a 0.40 m × 0.40 m $BaSO_4$ calibration panel were used for the calculation of canopy-reflected spectrum. Twenty spectral samples were taken from one plot. The calibration panel radiance was measured for each plot.

ASTER image collection and processing

The ASTER image device recorded data in 14 spectral bands: 3 bands in visible near infrared (VNIR) with a 15 m spatial resolution; 6 bands in short wave infrared (SWIR) with a 30 m spatial resolution; and 5 bands in Thermal Infrared (TIR) with a 90 m spatial resolution. Geometric correction was carried through ground control point (GCP) registers. There were 30 GCPs used for the geometric correction with a total RMS Error of 0.4151.. The ASTER image was rectified with a projection of Universal Transverse Mercator. Atmospheric correction was carried out using the "Empirical Line Method"(ELM) method (Karpouzli and Malthus, 2003 [28]; G. M. Smith and E. J. Milton, 1999 [29]) based on ground measured spectrum data obtained by ASD field spectrometer, and the calibration targets chosen were bare flat concrete bleachery as the white object whose size was about 200 m * 250 m, and a reservoir with pure water as the dark object whose size was about 200 m * 150 m.

The 6 SWIR bands of the ASTER image device were re-sampled to 15 m plots by merging the results with band 3N by using bilinear method. All image processing was performed using the Environment for Visualizing Images (ENVI) version 4.1 (Research Systems, Inc., Boulder CO, USA) .

Foliar Chl and foliar nitrogen concentration (FNC)

After each reflectance measurement, wheat in the areas corresponding to the footprint of *in situ* spectrum was harvested to soil level, packed in cooled black plastic bags and transported to the laboratory for subsequent analysis. Chl was extracted with 80 % acetone and calculated with the formula described by Arnon (1949) [30]. The extracts of absorption features at wavelengths of 645 nm, and 663 nm were measured with a Heliosα spectrophotometer (Thermo Electron Company, Cambridge, United Kingdom). Leaf samples were dried separately in an oven at 70℃ and weighed to measure the FNC. Leaf samples were passed through a 40 mm mesh screen, and measured with the Kjeldahl technique (Bremner *et al.*, 1981) [31] before measuring the FNC. The apparatus was a B-339 Distillation Unit (Switzerland).

Grain quality indicators

CP was determined using the NIR Foss1241 (Foss Tecator, Sweden). The results were normalized on a 14% wet weight basis; Grain hardness was measured with a SKCS 4100 (Perten Instruments AB, Sweden); Wet and dried gluten content was measured with a 2200-Gluten Meter (Falling Number Co., Sweden); Hagberg falling number was measured using Germenic Brabender (AACC56-63 method; Dick , 1983 [32]).

3 RESULTS AND ANALYSIS

3.1. Relationship between FNC at anthesis stage and grain quality indicators

Correlation coefficients between FNC at anthesis stage and grain quality indicators, such as wet gluten, dry gluten, and Hagberg falling number were listed in Table 1(Experiment A). The correlations between FNC and foliar soluble sugar, FNC and stem soluble sugar, FNC and grain hardness were extremely negatively significant, where the correlation coefficients exceeded -0.492, -0.343 and -0.423, respectively. However, they were positively significant when compared with grain quality indicators such as CP, wet gluten, dry gluten, and Hagberg falling number, where the correlation coefficients exceeded 0.562, 0.529, 0.578, and 0.366, respectively. The FNC at the anthesis stage was thus highly correlated to CP. If the FNC at the anthesis stage could be monitored by remote sensing technology, the CP could be evaluated. It is thus feasible to estimate CP at the anthesis stage by FNC.

Table 1. Correlation coefficients of biochemical constituents and grain quality indicators.

	FNC	FSS	SSS	H	P	WG	DG	HFN
FNC	1.000							
FSS	-0.492**	1.000						
SSS	-0.343*	0.642**	1.000					
H	-0.423**	-0.02	0.167	1.000				
P	0.562**	-0.047	-0.036	-0.509**	1.000			
WG	0.529**	0.000	0.149	-0.520**	0.884**	1.000		
DG	0.578**	-0.051	0.035	-0.524**	0.961**	0.948**	1.000	
HFN	0.366**	-0.121	-0.381**	-0.643**	0.533**	0.254	0.447**	1.000

Note: 1. H—Hardness, P—Protein, WG—Wet gluten, DG—Dry gluten, HFN—Hagberg falling number

FN—Foliar nitrogen concentration, FSS—Foliar soluble sugar, SSS—Stem soluble sugar.

2. $r_{(0.05, 48)}$ =0.288; $r_{(0.01, 48)}$ =0.372

*: Significance level p=0.05; **: Significance level p=0.01.

3.2. Inversion of Chl and FNC by *in situ* canopy reflected spectrum

Grain quality of winter wheat is characterized by protein content, wet gluten, dry gluten content, and Hagberg falling number. Accumulation of FNC and its transfer to the grains is a physical process that forms grain proteins. All of 48 plots of three varieties under the 4 levels of water irrigation and 4 levels of nitrogen fertilization conditions were studied at 5 growth stages (erecting, elongation, heading, anthesis, and grain filling) in Experiment A and Experiment B, respectively. Data from Experiment A were used to develop the models which represented the relationship between Chl (FNC) and the selected vegetation indexes. Data from Experiment B were used to validate the established models. According to the statistical analysis of the relationship between FNC and canopy reflected spectrum or vegetation indices, the nitrogen reflectance index (NRI), derived from the spectral reflectance at 570nm and 670nm (green and red portions of visible spectra respectively), was defined by Schleicher *et al.* (2001) [33] as follows:

$$NRI = \frac{R_{570} - R_{670}}{R_{570} + R_{670}}, \quad (1)$$

The regression equation between the measured Chl and NRI of 3 varieties under different irrigation and N treatments was shown as bellow:

$$Y_1 (\%)=-6.792X_1 +5.154 \quad (0<X_1<0.4, n=48, R^2=0.790) , \quad (2)$$

Where Y_1 (%) is Chl in mg g^{-1}, and X_1 is NRI.

The regression equation between the measured Chl and FNC of 3 varieties under different irrigation and N treatments was shown as bellow:

$$Y_2 (\%)=0.331X_2 +1.619 \quad (0.55<X_2<5.6, R^2=0.924) , \quad (3)$$

where Y_2 (%)is FNC in percent, and X_2 is Chl in mg g^{-1}.

The relation between the measured FNC and the NRI of 3 varieties under different irrigation and soil nitrogen treatments was shown in Fig.1 (n=240).

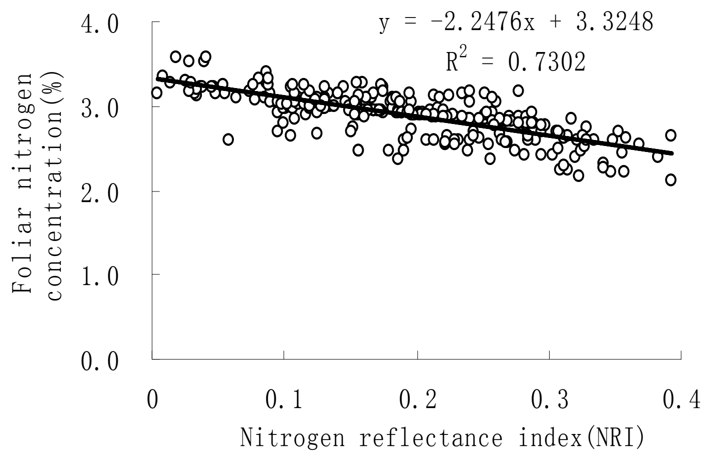

Fig. 1. Relationship between *in situ* spectrum derived NRI and FNC in Experiment A.

The 240 samples were measured in the laboratory from plot field sampling. The regression equation between FNC and NRI was determined to be the following

$$Y\ (\%) = -2.248\ X + 3.325\quad (0 < X < 0.4,\ R^2 = 0.7302)\ , \tag{4}$$

where Y（%）is FNC in percent, and X is the nitrogen reflectance index (NRI).

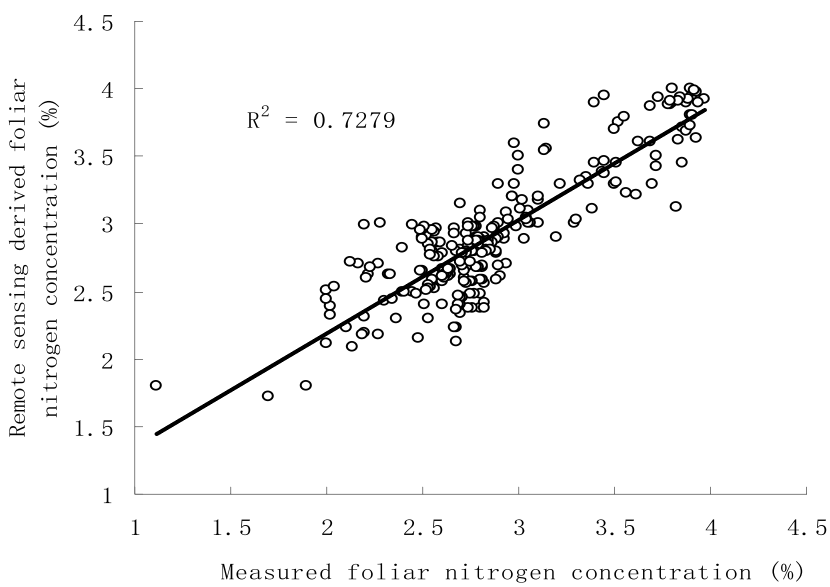

Fig. 2. Relationship between measured and *in situ* spectrum derived FNC in Experiment B.

We found a strong relationship between the measured FNC and NRI, with a coefficient of determination of R^2=0.7302 (n=240), which was very significantly positive ($r_{(0.01, 240)}$=0.181). The model of the relationship between FNC and NRI was validated using data in Experiment B. The coefficient of determination between remote sensing derived (Eq.(4)) and the measured FNC from Experiment B was 0.7279(n=240), which was extremely significant ($P < 0.01$).

3.3. Evaluation of CP by *in situ* canopy-reflected spectrum and ASTER image at anthesis stage

The characteristics of the 9 ASTER spectral bands in V-NIR, SWIR region were shown in Table 2.

Table 2. Spectral and spatial characteristics of the ASTER image sensor in V-NIR and SWIR region.

Bands	Band N^o.	Spectral (nm)	Spatial Resolution (m)	Radiometric Accuracy	Radiometric Resolution
VNIR	1 2 3N 3B	520-600 630-690 780-860 780-860	15	±4% ±4% ±4% ±4%	≤0.5% ≤0.5% ≤0.5% ≤0.5%
SWIR	4 5 6 7 8 9	1600-1700 2145-2185 2185-2225 2235-2285 2295-2365 2360-2430	30	±4% ±4% ±4% ±4% ±4% ±4%	≤0.5% ≤1.3% ≤1.3% ≤1.3% ≤1.0% ≤1.3%

The authors have studied the correlation coefficient between the V- NIR region from ASTER and CP.

Table 3. Correlation coefficients of CP and different bands in V-NIR region derived from ASTER image sensor system.

	B1	**B2**	**B3**	**NRI**	**NDVI**
CP	0.377	**0.594**	**0.539**	**-0.417**	0.0181

Note:
1) CP =Crude protein content;
2) NDVI=(B3-B2)/(B3+B2); NRI=(B1-B2)/(B1+B2);

As shown in Table 3, the correlation coefficient between V-NIR vegetation index and CP was -0.417, and it reached the significant level (significant level ($r_{0.05,\ 240}$=0.388). So NRI vegetation index derived from ASTER was used for the predicting of CP.

The NRI in this paper for ASTER image was as follows

$$NRI_{ASTER} = \frac{R_{B1} - R_{B2}}{R_{B1} + R_{B2}}, \quad (5)$$

The ASTER image at the winter wheat anthesis stage (May 17, 2004）in Experiment C was chosen for this study. Using the models of the relationship between FNC and *in situ* field canopy-reflected spectrum, we derived the NRI from the data in Experiment A. NRI spectral index at the anthesis stage was used to estimate CP.

CP was forecasted from NRI at anthesis stage using the following equation:

$$P\ (\%) = -11.7594\ NRI + 17.3953 \quad 0 < NRI < 0.4,\ (R^2 = 0.7432), \quad (6)$$

where *P(%)* is percent CP.

Grain flour dried gluten was forecasted from NRI at anthesis stage using the following equation:

$$DG\ (\%) = -15.2432\ NRI + 22.5488 \quad (0 < NRI < 0.4,\ R^2 = 0.7259), \quad (7)$$

where *DG (%)* is percent dry gluten .

According to equation (6), we mapped ASTER image based CP at anthesis stage by ASTER NRI. The mapped result was shown in Fig. 3

It showed the forecasted CP in Beijing suburbs of Tongzhou. The site-specific knowledge of CP provides valuable opportunities to manage grain harvest differently. From this mapping result, we could find that the CP of the winter wheat was geographically different. In the east of Tongzhou county, such as most of Xiji town, it was generally higher than 15.1 % and up to >17.1 %.In the south of Tongzhou county, such as most of Yujiawu town and Taihu town, it was generally lower than 15.0 % and down to <12.5 %. In the western Tongzhou county, most of Taihu town, it ranged from 15.1% to17.0 %.

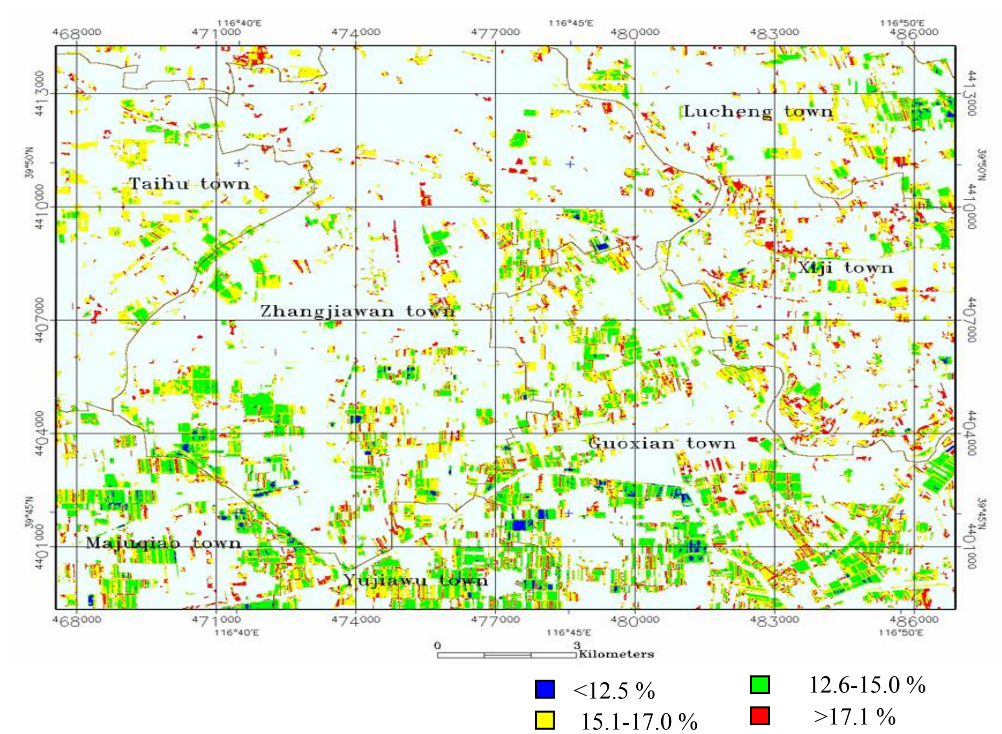

Fig. 3. Mapping ASTER image based CP (%) at anthesis stage in Experiment C.

In order to test whether the model was reliable and applicable for CP prediction, the data collected in the validation treatment were used for testing the models. Root mean square error (RMSE) was employed to test the reliability and estimation accuracy between measured CP and estimated CP.

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\theta_i - \hat{\theta}\right)^2} \tag{8}$$

In equation (8), θ_i and $\hat{\theta}$ are CP observed and remote sensing derived respectively, and n is the number of samples. Massart et al. (1988) [34] 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.

As shown in Fig. 4, the *in situ* spectrum derived CP exhibited high accuracy (its slope was 1.0329 and the intercepts was -0.2013), and precision (R^2= 0.7661, n=48) and the RMSE was 0.893. The ASTER image derived CP did not exhibit very high accuracy (its slope was 0.9254, and the intercepts was 1.125) or precision (R^2= 0.719, n=24), and the RMSE was 1.654. It was thus feasible to forecast grain quality by NRI spectral index derived from *in situ* canopy-reflected spectrum and ASTER image. The result indicated that winter wheat CP in a large scale could be forecasted by *in situ* spectrum and ASTER image at anthesis stage.

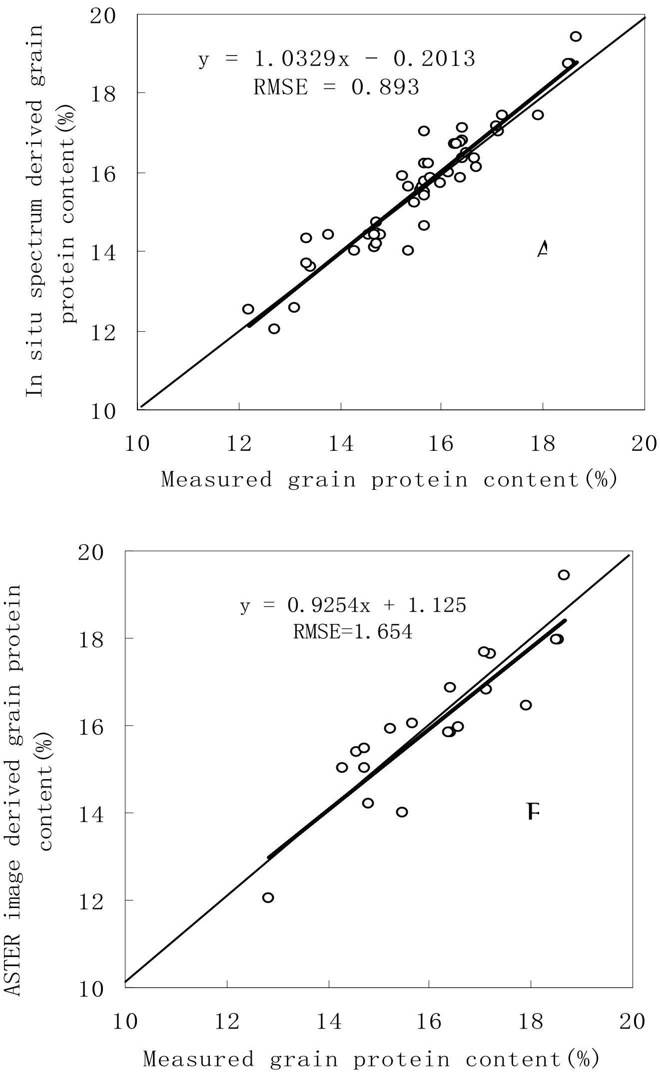

Fig. 4. Relationship between the measured and *in situ* spectrum derived CP in Experiment B, and ASTER image derived CP in Experiment C.

4 DISCUSSION

It is important to evaluate wheat grain quality before the harvest. However, it is difficult to evaluate grain quality before it is ripe using current methods. Remote sensing can potentially rapidly determine the grain quality condition of crops over large areas (Walburg *et al.*, 1982[35]; Blackmer *et al.*, 1994[36]). Hansen *et al.* (2002) [26] successfully evaluated winter wheat CP by using early, repeated, remotely sensed multi-spectral data.

Since this study was carried under almost the same ecological conditions, it cannot be applied directly to different ecological conditions. There should then be further studies on how environmental conditions influence on grain quality. Our study proposed optimal procedures for predicting large scale wheat CP through analysis of canopy reflectance spectra before harvest. In addition, these models are reliable by testing with the separate data set from a different location.

With the experimental results, we are developing some simple instruments with selected sensitive bands. For example, we are developing optical camera lens and sensors that focus on 570nm and 670nm and could be placed on agricultural machines. This would allow for on-site and non-sampling modes of winter wheat grain CP forecasting, fertilizing, and water guidance without a priori knowledge. Such portable instruments can also estimate grain quality at anthesis stage by the regression models of this paper. Moreover, a careful analysis should be carried out to investigate the effects of band width and sensor height above the ground.

5 CONCLUSION

The objective of this paper was to develop a method to evaluate winter wheat FNC and CP. Accumulation of FNC and its transfer to the grain is the physical link process that produces grain proteins. This current study showed robust correlations between NRI and FNC, suggesting that NRI is a promising indictor to predict winter wheat CP. The NRI proved to be able to evaluate FNC with a coefficient of determination of R^2= 0.730 using the data in Experiment A. The relationship between laboratory measured and remote sensing derived FNC had a coefficient of determination of R^2= 0.728 in Experiment B.

In situ spectrum derived from grain crude protein content exhibited high accuracy, the RMSE was 0.893 %. The ASTER image derived from grain crude protein content exhibited not very high accuracy and precision, and the RMSE was 1.654 %. According to the results of this paper, the relationships among NRI, leaf nitrogen concentration, and CP were significant. A model for evaluating CP was established based on the transfer principle of FNC, which made it possible to optimize crop nitrogen management for grain quality.

In situ spectrum derived CP exhibited high accuracy, and precision, the lower RMSE. However, the ASTER image derived CP did not exhibit very high accuracy and precision. According to the results, the relationships among NRI, FNC, and CP were significant. A model for evaluating CP was established based on the transfer principle of FNC, which made it possible to optimize crop N management for wheat grain quality.

Acknowledgments

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