Postprint: Hyperspectral Remote Sensing Monitoring Models for Grain Protein Content of Winter Wheat at Different Growth Stages

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Abstract

To investigate the hyperspectral remote sensing monitoring model for winter wheat grain protein content under different nitrogen and phosphorus levels and improve model accuracy, this study examined winter wheat canopy spectral reflectance, plant nitrogen content, and grain protein content at maturity under different nitrogen-phosphorus coupling levels through a five-year continuous location experiment. Using correlation, regression, and other statistical analysis methods, grain protein content monitoring models based on plant nitrogen content at different growth stages were established. Subsequently, through grey relational analysis, the optimal vegetation indices for plant nitrogen content were selected, and plant nitrogen content monitoring models based on vegetation indices were established using partial least squares regression. Finally, using plant nitrogen content as a linking point, a winter wheat grain protein content monitoring model at maturity that integrates vegetation indices and plant nitrogen content was established according to the relationship "vegetation index—plant nitrogen content—grain protein content". The results showed that: the grain protein content monitoring models at maturity based on plant nitrogen content established at the jointing, booting, heading, filling, and maturity stages had good monitoring accuracy; at the jointing, booting, heading, filling, and maturity stages, plant nitrogen content monitoring models were established based on the Modified Chlorophyll Absorption Ratio Index (MCARI1), Normalized Difference Chlorophyll Index (NDCI), modified Normalized Difference Vegetation Index (mNDVI), MCARI1, and NDCI vegetation indices, respectively, with monitoring accuracies (R^2) of 0.826, 0.854, 0.867, 0.859, and 0.819; using plant nitrogen content as a linking point, grain protein content monitoring models based on vegetation indices at the jointing, booting, heading, filling, and maturity stages and integrating plant nitrogen content were established through the indirect relationship "vegetation index—plant nitrogen content—grain protein content" , with R^2 values of 0.935, 0.972, 0.990, 0.979, and 0.936; the models were validated using independent data, and the relative errors (RE) between predicted and measured values were 11.26%, 10.74%, 8.41%, 10.25%, and 11.36%, respectively, with root mean square errors (RMSE) of 2.221 g·kg ^{1}, 1.825 g·kg ^{1}, 1.214 g·kg ^{1}, 1.767 g·kg ^{1}, and 2.137 g·kg ^{1}. This demonstrates that linking plant nitrogen content through vegetation indices at different growth stages can effectively monitor grain protein content at maturity, and the models exhibit good inter-annual repeatability and inter-cultivar adaptability.

Full Text

Winter Wheat Grain Protein Content Monitoring Model Driven by Hyperspectral Remote Sensing Images at Different Growth Stages

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Abstract

Grain protein content (GPC) is a crucial indicator of wheat quality. Early detection of GPC using hyperspectral remote sensing data can enhance effective decision-making for acquiring and processing high-quality wheat. This study aimed to establish GPC estimation models based on winter wheat canopy hyperspectral reflectance at different growth stages under varying nitrogen and phosphorus application rates, with the overall goal of improving forecast precision. Experiments were conducted from 2009-2014 at Northwest A&F University in Shaanxi Province, using different winter wheat varieties with various drought resistances under five nitrogen fertilizer rates (0, 75, 150, 225, and 300 kg·hm ^{2} pure nitrogen) and four phosphorus rates (0, 60, 120, and 180 kg·hm ^{2} P O). Plant nitrogen content (PNC) and canopy hyperspectral reflectance were measured at jointing, booting, heading, filling, and maturity stages, and GPC was measured at maturity. The relationships among PNC, canopy hyperspectral reflectance, and GPC were explored using correlation analysis, regression analysis, grey relational analysis, and partial least squares regression.

The results showed that GPC monitoring models based on PNC at jointing, booting, heading, grain-filling, and maturity stages achieved high prediction accuracy. PNC monitoring models based on specific vegetation indices—modified chlorophyll absorption reflectance index (MCARI1) at jointing, normalized difference chlorophyll index (NDCI) at booting, modified normalized difference vegetation index (mNDVI) at heading, MCARI1 at filling, and NDCI at maturity—demonstrated strong performance with determination coefficients (R^2) of 0.826, 0.854, 0.867, 0.859, and 0.819, respectively. When linked with PNC using the "VI—PNC—GPC" approach, the integrated GPC monitoring models combining VI and PNC at jointing, booting, heading, filling, and maturity stages achieved R^2 values of 0.935, 0.972, 0.990, 0.979, and 0.936, respectively. Independent validation data showed relative errors (RE) between measured and predicted values of 11.26%, 10.74%, 8.41%, 10.25%, and 11.36%, with corresponding root mean square errors (RMSE) of 2.221 $g \cdot kg^{1}$, 1.825 $g \cdot kg^{1}$, 1.214 $g \cdot kg^{1}$, 1.767 $g \cdot kg^{1}$, and 2.137 $g \cdot kg^{1}$. These results demonstrate that monitoring PNC through vegetation indices at different growth stages can effectively predict GPC at maturity, with good inter-annual repeatability and inter-cultivar adaptability.

Keywords: Winter wheat; Hyperspectral remote sensing; Canopy spectral reflectance; Grain protein content; Plant nitrogen content; Vegetation index

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Introduction

Grain protein content (GPC) is a primary factor in evaluating wheat (*Triticum aestivum*) quality. Developing high-quality specialty wheat to ensure food security while improving grain quality represents an inevitable trend for enhancing agricultural production efficiency [1-5]. Traditional analytical methods for wheat grain quality cannot monitor GPC over large areas in real time and are time-consuming and labor-intensive. Therefore, there is an urgent need for a new technology that enables large-area, real-time, non-destructive monitoring of GPC before harvest.

Hyperspectral remote sensing, with its advantages of strong band continuity, high spectral resolution, and rich spectral information, provides an effective approach for real-time and rapid monitoring of crop nutritional status, population growth, yield, and quality [6-7].

Previous studies have demonstrated the feasibility of monitoring crop GPC using remote sensing technology. Hansen et al. [8] proposed predicting wheat GPC using canopy spectral reflectance and partial least squares regression. Basnet et al. [9] showed that monitoring cereal crop GPC based on satellite image spectral information yields good results. Reyniers et al. [10] achieved remote sensing monitoring of winter wheat GPC based on the normalized difference vegetation index (NDVI). Liu et al. [11] successfully predicted wheat GPC during the grain-filling stage by constructing the structure insensitive pigment index (SIPI) based on Landsat TM imagery. Wang et al. [12] reported good correlation between the plant pigment ratio index (PPR) at wheat flowering stage and GPC at maturity. Zhao et al. [13], based on nitrogen translocation principles, indicated good correlation between the water index (WI) at winter wheat flowering stage and GPC. Pettersson et al. [14] noted that the transition chlorophyll absorption reflection index (TCARI) could be used to predict crude protein content in barley grains.

Xue et al. [15] found that the correlation coefficient between rice (*Oryza sativa*) canopy spectral reflectance from jointing to grain-filling stage and GPC at maturity reached above 0.80 (P<0.01). Wang et al. [4] proposed the concept of indirectly predicting GPC by monitoring leaf nitrogen content through canopy spectral reflectance in the 820–1,140 nm band. Tian et al. [16] reported a highly significant exponential relationship between the ratio vegetation index (RVI /) after wheat heading and GPC. Xue et al. [17] achieved a prediction accuracy of 0.88 for wheat GPC by indirectly predicting it through canopy spectral reflectance monitoring of leaf nitrogen content. Li et al. [18] developed a wheat GPC monitoring model based on grain nitrogen translocation dynamics using TM remote sensing imagery, with RMSE ranging from 0.47% to 0.59%. Jin et al. [19] combined grey relational analysis (GRA) with partial least squares (PLS) to improve wheat GPC estimation methods through ratio and product indices. Li et al. [20] established a wheat GPC monitoring model by integrating GRA-PLS and coupling leaf nitrogen content monitoring models with grain nitrogen translocation models based on nitrogen translocation theory.

In summary, current GPC monitoring using hyperspectral remote sensing technology mostly relies on direct monitoring based on correlations between GPC and either a specific critical growth stage or vegetation indices. However, these studies have given limited consideration to the relationship between plant nitrogen status and vegetation indices across different growth stages, resulting in poor model stability and repeatability. Therefore, this study conducted five consecutive years of fixed-position research on winter wheat canopy spectral reflectance, plant nitrogen content (PNC), and GPC at maturity under different nitrogen and phosphorus levels. First, we established GPC monitoring models based on PNC at different growth stages. Then, we analyzed the grey correlation between vegetation indices and PNC at different growth stages to select optimal vegetation indices and established PNC monitoring models based on these indices using partial least squares regression. Finally, using PNC as a linking point, we established winter wheat GPC monitoring models at maturity that integrate vegetation indices and PNC across different growth stages following the "vegetation index–plant nitrogen content–grain protein content" framework. Independent data were used to validate these models, enhancing the agronomic explanatory mechanism and providing theoretical basis and technical support for monitoring wheat quality using hyperspectral remote sensing technology, thereby promoting dynamic regulation of winter wheat quality management.

1. Materials and Methods

1.1 Experimental Site The experiment was conducted at the North Campus of Northwest A&F University (108°10 E, 34°10 N, altitude 454.8 m) in a temperate continental monsoon climate zone, with an average annual temperature of 12-14°C, frost-free period of 220 days, annual evaporation of 1,400 mm, and average annual precipitation of 621.6 mm. The experimental soil was silty clay loam. Nutrient content in the 0–20 cm soil layer was: organic matter 14.26 g · kg ^{1}, total nitrogen 0.90 g · kg ^{1}, alkaline hydrolyzable nitrogen 36.00 mg · kg ^{1}, and available phosphorus 17.64 mg · kg ^{1}. In the 20–40 cm layer, nutrient content was: organic matter 10.04 g · kg ^{1}, total nitrogen 0.62 g · kg ^{1}, alkaline hydrolyzable nitrogen 25.29 mg · kg ^{1}, and available phosphorus 23.85 mg · kg ^{1}.

1.2 Experimental Design The experiment was implemented from 2009 to 2014 using a randomized block design. Winter wheat varieties with large planting areas in the region were selected, and two varieties with different drought resistance levels were planted each year based on their relative drought resistance indices. According to local wheat production practices, five nitrogen levels and four phosphorus levels were established with three replicates. Each plot measured 3 m × 10 m. The five nitrogen (urea, 46% N) levels were: N0 (no fertilizer), N1 (75 kg · hm ^{2} pure nitrogen), N2 (150 kg · hm ^{2} pure nitrogen), N3 (225 kg · hm ^{2} pure nitrogen), and N4 (300 kg · hm ^{2} pure nitrogen). Total nitrogen fertilizer was applied as 60% basal fertilizer and 40% topdressing, applied after regreening and before jointing. The four phosphorus (16% CaP H O) levels were: P0 (no fertilizer), P1 (60 kg · hm ^{2} P O), P2 (120 kg · hm ^{2} P O), and P3 (180 kg · hm ^{2} P O), applied as basal fertilizer in one application. No potassium fertilizer was applied, and other field management followed high-yield practices in the Loess Plateau. The seeding rate was 187.50 kg · hm ^{2} (approximately 0.56 kg per plot). Details of varieties, sowing, harvest, and data collection dates for different years are shown in Table 1 .

1.3 Measurements

1.3.1 Canopy Spectral Reflectance Measurement Canopy spectral reflectance of winter wheat was measured using an ASD FieldSpec Pro FR-2500 backpack field spectroradiometer (Analytical Spectral Devices, USA) with a wavelength range of 350–2,500 nm. The spectral sampling interval was 1.4 nm with 3 nm resolution for 350–1,000 nm, and 2 nm interval with 10 nm resolution for 1,000–2,500 nm. Measurements were taken at jointing, booting, heading, grain-filling, and maturity stages under clear, windless conditions between 10:00–14:00. The sensor probe was positioned vertically downward with a 7.5° field of view, approximately 25–30 cm above the canopy top, covering a ground diameter of 0.5 m. Ten spectral samples were recorded at each observation point, with three replicates per plot, and the arithmetic mean was used as the canopy spectral reflectance. Standard whiteboard calibration (reflectance = 1) was performed after each treatment measurement to ensure accurate subsequent measurements.

1.3.2 Plant Nitrogen Content (PNC) Determination PNC determination was synchronized with spectral reflectance measurements. After spectral measurement, 20 representative plants with consistent growth were selected within the same sampling range. Different plant organs were separated, killed at 105°C for 30 minutes, dried at 85°C to constant weight, weighed separately, ground, and digested with H SO -H O . PNC was then determined using a Swiss Buchi automatic B-339 Kjeldahl nitrogen analyzer.

1.3.3 Grain Protein Content (GPC) Determination At maturity, wheat from yield measurement plots was air-dried, threshed, ground, digested with H SO -H O , and analyzed using a Swiss Buchi automatic B-339 Kjeldahl nitrogen analyzer. Wheat GPC (grain protein content, g · kg ^{1}) was calculated as grain total nitrogen content multiplied by 5.823 [21].

1.4 Vegetation Index Selection Based on comprehensive review of previous research on vegetation indices, plant nitrogen content, and grain protein content, we categorized and selected nitrogen-related vegetation indices for application in GPC monitoring models at different growth stages. The main vegetation indices selected and their calculation methods are shown in Table 2 .

Table 2. Hyperspectral vegetation indices used in this study

Vegetation index	Formula	Reference
Modified normalized difference vegetation index (mNDVI)	mNDVI=(R −R)/(R +R −2R)	[22]
Normalized difference chlorophyll index (NDCI)	NDCI=(R −R)/(R +R)	[23]
Normalized difference index (NDI)	NDI=(R −R)/(R +R)	[24]
Modified triangle vegetation index (TVIBL)	TVIBL=0.5[120(R −R)−200(R −R)]	[25]
Modified chlorophyll absorption reflectance index (MCARI1)	MCARI1=1.2[2.5(R −R)−1.3(R −R)]	[26]
Red edge normalized difference vegetation index (NDVI)	NDVI =(R −R)/(R +R)	[27]
Green normalized difference vegetation index (GREEN-NDVI)	GREEN-NDVI=(R −R)/(R +R)	[28]
Red edge position index (REP)	REP=700+40[(R +R)/2−R]/(R −R)	[29]
Modified simple ratio index (mSRI)	mSRI=(R −R)/(R +R)	[30]

Vegetation index	Formula	Reference
Structure insensitive pigment index (SIPI)	SIPI=(R −R)/(R+R)	[28]
Plant pigment ratio (PPR)	PPR=(R −R)/(R+R)	[12]

Note: R represents spectral reflectance, and the number following R is wavelength.

1.5 Grey Correlation Analysis Correlation degree measures the association between different factors. Higher correlation indicates more consistent changing trends. Grey correlation analysis is a systematic method that measures the degree of association between different factors within a system based on the similarity or dissimilarity of their development trends. It identifies correlations between characteristic data sequences reflecting system behavior and related factor sequences affecting system behavior by processing these data to quantitatively describe the influence of different factors on the system [30]. If correlation degree is considered as the distance between factors, it can only be positive [31]. This study used the correlation degree between PNC and vegetation indices to evaluate their synchronous change degree. First, correlation analysis was performed, and vegetation indices positively correlated with PNC were selected for grey correlation analysis.

1.6 Data Processing and Analysis Experimental data were compiled according to different years, nitrogen-phosphorus coupling levels, growth stages, and varieties. Canopy spectral reflectance data were preprocessed using ViewSpec software. Data from 2012–2013 were used to analyze the effects of different nitrogen and phosphorus levels on GPC at maturity. Comprehensive field data from 2010–2013 were used for correlation analysis between PNC at different growth stages and GPC at maturity to establish PNC-based GPC monitoring models. Simultaneously, correlation and grey correlation analyses were conducted between PNC and vegetation indices at different growth stages to select optimal vegetation indices. Partial least squares regression was used to establish vegetation index-based PNC monitoring models, validated with independent data from 2009–2010. Finally, using PNC as a linking point, PNC-based GPC models and vegetation index-based PNC models were combined following the "vegetation index—plant nitrogen content—grain protein content" framework to establish integrated GPC monitoring models at maturity. These models were validated using independent data from 2013–2014.

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2. Results

2.1 Effects of Nitrogen and Phosphorus Levels on Winter Wheat GPC Using GPC at maturity for 'Zhoumai 18' and 'Xiaoyan 22' in 2012–2013 as examples, we analyzed the effects of different nitrogen and phosphorus levels on winter wheat GPC (Figure 1 [Figure 1: see original paper]). Results showed that under the same nitrogen level, GPC increased significantly with increasing phosphorus application (N2P0, N2P1, N2P2, N2P3) ($P<0.05$). For 'Zhoumai 18' , N2P3 (139.22 g · kg ^{1}) increased by 24.88 g · kg ^{1}, 15.40 g · kg ^{1}, and 10.13 g · kg ^{1} compared to N2P0 (114.33 g · kg ^{1}), N2P1 (123.81 g · kg ^{1}), and N2P2 (129.09 g · kg ^{1}), representing increases of 17.87%, 11.06%, and 7.28%, respectively. Under the same phosphorus level, GPC also increased significantly with increasing nitrogen supply (N0P2, N1P2, N2P2, N3P2, N4P2) ($P<0.05$). For 'Zhoumai 18' , N4P2 (173.74 g · kg ^{1}) increased by 81.33 g · kg ^{1}, 58.64 g · kg ^{1}, 44.65 g · kg ^{1}, and 9.63 g · kg ^{1} compared to N0P2 (92.41 g · kg ^{1}), N1P2 (115.10 g · kg ^{1}), N2P2 (129.09 g · kg ^{1}), and N3P2 (164.11 g · kg ^{1}), representing increases of 46.81%, 33.75%, 25.70%, and 5.54%, respectively. 'Xiaoyan 22' showed similar trends. These results indicate that GPC increased gradually with nitrogen and phosphorus supply, but the rate of increase diminished. Nitrogen application showed more pronounced effects on GPC improvement than phosphorus application. Similar trends were observed across different years and varieties, though some inter-varietal differences existed, likely related to differences in nitrogen and phosphorus absorption efficiency and genetic characteristics.

2.2 GPC Monitoring Models Based on PNC at Different Growth Stages Using comprehensive field data from 2010–2013 (n=120), correlation analysis was performed between PNC at different growth stages and GPC at maturity (Table 3). PNC at jointing, booting, heading, grain-filling, and maturity stages showed extremely significant correlations with GPC at maturity ($P<0.01$), with correlation coefficients (r) of 0.781, 0.810, 0.851, 0.876, and 0.849, respectively, indicating that GPC could be predicted based on PNC at different growth stages. Regression analysis revealed that quadratic functions ($y = ax + bx^2 + c$) provided the best fit for all growth stages, with determination coefficients (R^2) of 0.636, 0.661, 0.767, 0.811, and 0.770 for jointing, booting, heading, grain-filling, and maturity stages, respectively. Lower fitting accuracy at jointing and booting stages may be attributed to nitrogen accumulation being primarily used for vegetative growth during these stages, while from heading to grain-filling, the transition from vegetative to reproductive growth facilitates nutrient transport and accumulation in grains, resulting in higher fitting accuracy between PNC and GPC during grain-filling.

Table 3. Monitoring models of grain protein content (y) of winter wheat based on plant nitrogen content (x) at different growth stages (n=120)

Growth stage	Correlation coefficient (r)	Equation	Determination coefficient (R^2)
Jointing	0.781**	$y = -0.748x^2 + 7.561x - 2.315$	0.636
Booting	0.810**	$y = -0.297x^2 + 4.381x + 4.658$	0.661
Heading	0.851**	$y = -0.922x^2 + 8.688x - 3.459$	0.767
Filling	0.876**	$y = -0.844x^2 + 9.595x - 10.014$	0.811
Maturity	0.849**	$y = -1.027x^2 + 7.794x + 2.167$	0.770

** represents significant correlation at the 0.01 level.*

2.3 Grey Correlation Analysis Between Vegetation Indices and PNC at Different Growth Stages Using field data from 2010-2013 (n=120), correlation analysis was conducted between PNC and vegetation indices at different growth stages (Table 4). Except for PPR, which showed no significant correlation with PNC (P>0.05), and SIPI, which showed extremely significant negative correlation (P<0.01), all other vegetation indices (mNDVI, NDCI, NDI, TVIBL, MCARI1, NDVI , GREEN-NDVI, mSRI, REP) showed extremely significant positive correlations with PNC (P<0.01). Therefore, based on correlation theory, vegetation indices positively correlated with PNC were selected for grey correlation analysis.

Table 5 presents the grey correlation analysis between vegetation indices and PNC at different growth stages (n=120). The ranking of grey correlation degrees was: jointing stage: MCARI1 > NDCI > mNDVI > NDI > TVIBL > NDVI > GREEN-NDVI > REP > mSRI; booting stage: NDCI > MCARI1 > mNDVI > NDI > TVIBL > NDVI > REP > GREEN-NDVI > mSRI; heading stage: mNDVI > NDCI > NDI > MCARI1 > TVIBL > NDVI > GREEN-NDVI > REP > mSRI; grain-filling stage: MCARI1 > mNDVI > REP > NDI > TVIBL > NDCI > NDVI > GREEN-NDVI > mSRI; maturity stage: NDCI > mNDVI > NDI > TVIBL > MCARI1 > NDVI > REP > GREEN-NDVI

> mSRI. The vegetation indices with highest grey correlation with PNC at each growth stage were selected for PNC monitoring.

Table 4. Correlation between vegetation indices and plant nitrogen content (PNC) of winter wheat at different growth stages (n=120)

Vegetation index	Jointing	Booting	Heading	Filling	Maturity
mNDVI	0.891**	0.875**	0.931**	0.884**	0.892**
NDCI	0.897**	0.924**	0.904**	0.831**	0.905**
NDI	0.868**	0.842**	0.892**	0.851**	0.856**
TVIBL	0.862**	0.835**	0.841**	0.847**	0.854**
MCARI1	0.909**	0.927**	0.841**	0.853**	0.829**
NDVI	0.881**	0.841**	0.853**	0.844**	0.821**
GREEN-NDVI	0.889**	0.853**	0.797**	0.833**	0.781**
SIPI	-0.681**	-0.673**	-0.554**	-0.467**	-0.616**
mSRI	0.815**	0.715**	0.832**	0.793**	0.714**
REP	0.826**	0.832**	0.801**	0.627**	0.751**
PPR	-0.383NS	-0.404NS	-0.363NS	-0.285NS	-0.154NS

- and ** represent significant correlation at 5% and 1% levels, respectively. NS represents no significant correlation.*

Table 5. Grey correlation degrees and rank of vegetation indexes for plant nitrogen content (PNC) of winter wheat at different growth stages (n=120)

Vegetation index	Jointing	Booting	Heading	Filling	Maturity
	=0.5	Order	=0.5	Order	=0.5
mNDVI	0.891	3	0.875	3	0.931
NDCI	0.897	2	0.924	1	0.904
NDI	0.868	4	0.842	4	0.892
TVIBL	0.862	5	0.835	5	0.841
MCARI1	0.909	1	0.927	2	0.841
NDVI	0.881	6	0.841	6	0.853
GREEN-NDVI	0.889	7	0.853	8	0.797
mSRI	0.815	9	0.715	9	0.832
REP	0.826	8	0.832	7	0.801

Meanings of vegetation index codes are shown in Table 4.

2.4 Establishment and Validation of PNC Hyperspectral Monitoring Models at Different Growth Stages Based on grey correlation analysis (Table 5), optimal vegetation indices stably correlated with PNC were identified for each growth stage. Using the correlations between PNC and GPC at different growth stages (Table 3), partial least squares regression was employed to establish PNC monitoring models based on optimal vegetation indices (n=120). Model accuracy was validated using independent data from 2009-2010 (n=40) (Table 6).

The fitted models showed that PNC models based on MCARI1 at jointing, NDCI at booting, mNDVI at heading, MCARI1 at grain-filling, and NDCI at maturity achieved fitting accuracies (R^2) of 0.826, 0.854, 0.867, 0.859, and 0.819, respectively, with standard errors (SE) of 0.213, 0.191, 0.136, 0.177, and 0.243. Independent validation showed relative errors (RE) between predicted and measured values of 14.08%, 13.63%, 10.31%, 12.17%, and 15.16%, with RMSE values of 0.324 $g \cdot kg^{1}$, 0.317 $g \cdot kg^{1}$, 0.125 $g \cdot kg^{1}$, 0.230 $g \cdot kg^{1}$, and 0.421 $g \cdot kg^{1}$ for jointing, booting, heading, grain-filling, and maturity stages, respectively. These results indicate that PNC monitoring models based on different vegetation indices at different growth stages have good fitting and validation performance, with linear equations of the form PNC = $d \cdot VI + e$ providing optimal fitting across all growth stages.

Table 6. Simulating (n=120) and performance (n=40) of monitoring models between plant nitrogen content (y) and the best vegetation index (x) of winter wheat at different growth stages

Growth stage	Vegetation index	Fitting model	Performance model
		Equation	R^2
Jointing	MCARI1	$y = 2.215x + 4.233$	0.826
Booting	NDCI	$y = 1.471x + 6.483$	0.854
Heading	mNDVI	$y = 0.738x + 9.145$	0.867
Filling	MCARI1	$y = 0.927x + 5.314$	0.859
Maturity	NDCI	$y = 0.898x + 6.164$	0.819

Meanings of vegetation index codes are shown in Table 4.

2.5 Establishment and Validation of GPC Hyperspectral Remote Sensing Monitoring Models Since PNC at different growth stages showed extremely significant correlations with GPC at maturity ($P<0.01$), GPC prediction models could be established based on PNC. Quadratic functions ($y = ax + bx^2 + c$) provided the best fit for PNC-GPC relationships, while vegetation index-based PNC models showed high fitting accuracy and validation performance with linear equations (PNC = $d \times VI + e$) being optimal. Therefore, vegetation indices and GPC could be linked through PNC at different growth stages. Following the "VI—PNC—GPC" framework and using PNC as the linking point, vegetation index-based PNC monitoring models and PNC-based GPC monitoring models were combined to establish integrated GPC remote sensing monitoring models at maturity:

$$\text{GPC} = a \times (d \times \text{VI} + e) + b \times (d \times \text{VI} + e)^2 + c \quad (1)$$

where GPC is grain protein content, VI is vegetation index, and a, b, c, d, e are constants.

To evaluate model stability and applicability, the optimized models were validated using measured data from different varieties in 2013-2014 (n=40). GPC model predictions at different growth stages are shown in Table 7 .

Results showed good validation performance for GPC models fitted through MCARI1–PNC–GPC at jointing, NDCI–PNC–GPC at booting, mNDVI–PNC–GPC at heading, MCARI1–PNC–GPC at grain-filling, and NDCI–PNC –GPC at maturity. The optimal monitoring models for different growth stages were:

Jointing stage:

$$\text{GPC} = 0.451\times(0.572\times\text{MCARI1}+0.244)+0.339\times(0.572\times\text{MCARI1}+0.244)^2+0.473 \quad (R^2 = 0.935) \quad (2)$$

Booting stage:

$$\text{GPC} = 0.486\times(0.519\times\text{NDCI}+0.327)+0.611\times(0.519\times\text{NDCI}+0.327)^2+0.526 \quad (R^2 = 0.972) \quad (3)$$

Heading stage:

$$\text{GPC} = 0.486\times(0.725\times\text{mNDVI}+0.424)+0.611\times(0.725\times\text{mNDVI}+0.424)^2+0.418 \quad (R^2 = 0.990) \quad (4)$$

Grain-filling stage:

$$\text{GPC} = 0.577\times(0.691\times\text{MCARI1}+0.517)+0.838\times(0.691\times\text{MCARI1}+0.517)^2+0.733 \quad (R^2 = 0.979) \quad (5)$$

Maturity stage:

$$\text{GPC} = 0.591\times(0.486\times\text{NDCI}+0.828)+0.656\times(0.486\times\text{NDCI}+0.828)^2+0.733 \quad (R^2 = 0.936) \quad (6)$$

Independent validation (n=40) showed relative errors (RE) between predicted and measured values of 11.26%, 10.74%, 8.41%, 10.25%, and 11.36%, with RMSE values of 2.221 g·kg ^{1}, 1.825 g·kg ^{1}, 1.214 g·kg ^{1}, 1.767 g·kg ^{1}, and 2.137 g·kg ^{1} for jointing, booting, heading, grain-filling, and maturity stages, respectively. These results demonstrate that GPC of different winter wheat varieties under different nitrogen and phosphorus levels can be predicted using key vegetation indices at different growth stages, with better monitoring performance at heading and grain-filling stages. Figure 2 [Figure 2: see original paper] compares measured and predicted GPC values at different growth stages.

Table 7. Performance of monitoring model of grain protein content of winter wheat at different growth stages (2013-2014)

Growth stage	Method	Fitting precision (R^2)	Relative error (RE) (%)	Root mean square error (RMSE) (g · kg ¹)
Jointing	MCARI1—PNC—GPC	0.935	11.26	2.221
Booting	NDCI—PNC—GPC	0.972	10.74	1.825
Heading	mNDVI—PNC—GPC	0.990	8.41	1.214
Filling	MCARI1—PNC—GPC	0.979	10.25	1.767
Maturity	NDCI—PNC—GPC	0.936	11.36	2.137

PNC: plant nitrogen content; GPC: grain protein content; other codes in the method column are shown in Table 4.

3. Discussion

3.1 PNC-Based GPC Monitoring Models PNC at different growth stages showed extremely significant correlations with GPC at maturity ($P<0.01$), indicating that GPC can be predicted based on PNC at different growth stages, consistent with previous research [16,18,21,37]. Although GPC could be predicted from PNC from jointing to maturity, higher monitoring accuracy was achieved during late grain-filling stages. This is because wheat grain protein formation and accumulation is a dynamic process with different growth priorities at different stages. Before grain-filling, vegetative growth dominates and nitrogen assimilation accumulates slowly, while after grain-filling, the transition from vegetative to reproductive growth facilitates rapid nitrogen assimilation and accumulation in grains, resulting in differences in monitoring accuracy across growth stages.

3.2 Vegetation Index-Based PNC Monitoring Models Based on correlation analysis between vegetation indices and PNC at different growth stages, we analyzed grey correlation degrees and ranked vegetation indices to select optimal ones. These indices, constructed using sensitive bands closely related to plant nitrogen, enhanced correlation with PNC through multi-band combinations, better reflecting PNC status at different growth stages. Partial least squares regression was then used to establish vegetation index-based PNC monitoring models at different growth stages. Models based on MCARI1, NDCI, mNDVI, MCARI1, and NDCI at jointing, booting, heading, grain-filling, and maturity stages showed good monitoring accuracy (R^2) and standard error (SE). Independent validation using data from different years and variety types demonstrated good performance, indicating model stability and reliability.

3.3 GPC Hyperspectral Remote Sensing Monitoring Models Winter wheat grain protein formation and accumulation is a dynamic process [38-39]. This study used PNC at different growth stages as the entry point to link vegetation indices with GPC at maturity. Following the "VI—PNC—GPC" framework, VI, PNC, and GPC were coupled, introducing plant nitrogen status and VI into GPC monitoring models. Independent data validation demonstrated high model performance, enabling GPC prediction for different drought-resistant wheat varieties under different fertility levels in the study region. Using PNC, an agronomic parameter closely related to GPC, as an intermediate variable to establish vegetation index-based GPC monitoring models enhances the agronomic explanatory mechanism, enabling GPC monitoring at maturity through hyperspectral remote sensing at different growth stages with good validation performance.

3.4 Future Prospects for GPC Monitoring Models This study established maturity GPC monitoring models based on the "VI—PNC—GPC" framework through five years of continuous research on PNC, canopy spectral reflectance, and GPC at maturity for different drought-resistant wheat varieties under varying nitrogen and phosphorus levels, achieving high validation accuracy. The modeling process incorporated canopy spectral characteristics and plant nitrogen status, improving prediction and validation accuracy. However, due to experimental constraints, model development and validation were conducted within the same experimental site with consistent soil, water, and light conditions. Future research should expand modeling factors across different ecological regions to enhance model extrapolation and stability. Additionally, this study established GPC monitoring models based solely on PNC, without considering regional ecological factors such as accumulated temperature, rainfall, and soil conditions. Therefore, it is necessary to combine physiological parameters with regional ecological factors to establish models with clear physical meaning, promoting the application of hyperspectral remote sensing technology in precision agriculture.

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4. Conclusions

1) Under the same nitrogen level, GPC increased significantly with increasing phosphorus application ($P<0.05$) by 7.28%-17.87%. Under the same phosphorus level, GPC increased significantly with increasing nitrogen supply ($P<0.05$) by 5.54%-46.81%. GPC at maturity was extremely significantly correlated with PNC at different growth stages ($P<0.01$), with correlation coefficients (r) of 0.781-0.876. GPC monitoring models could be established based on PNC at different growth stages, with quadratic functions ($y = ax + bx^2 + c$) providing the best fit. Determination coefficients (R^2) were 0.636, 0.661, 0.767, 0.811, and 0.770 for jointing, booting, heading, grain-filling, and maturity stages, respectively.

2) Through correlation analysis and grey correlation analysis, optimal vegetation indices were selected for different growth stages. Using partial least squares regression, PNC monitoring models were established based on MCARI1, NDCI, mNDVI, MCARI1, and NDCI for jointing, booting, heading, grain-filling, and maturity stages, respectively. Model fitting accuracies (R^2) were 0.826, 0.854, 0.867, 0.859, and 0.819, with standard errors (SE) of 0.213, 0.191, 0.136, 0.177, and 0.243. Independent validation showed relative errors (RE) of 14.08%, 13.63%, 10.31%, 12.17%, and 15.16%, with RMSE values of 0.324 g · kg ^{1}, 0.317 g · kg ^{1}, 0.125 g · kg ^{1}, 0.230 g · kg ^{1}, and 0.421 g · kg ^{1}, respectively, demonstrating good monitoring and validation performance of PNC models based on different vegetation indices at different growth stages.

3) Using PNC as a linking point and following the "VI—PNC—GPC" framework, vegetation index-based PNC monitoring models and PNC-based GPC monitoring models were combined to establish integrated GPC monitoring models that incorporate both vegetation indices and dynamic PNC at different growth stages, enhancing agronomic explanatory mechanisms. Independent validation of GPC monitoring models at jointing, booting, heading, grain-filling, and maturity stages showed relative errors (RE) of 11.26%, 10.74%, 8.41%, 10.25%, and 11.36%, and RMSE values of 2.221 g · kg ^{1}, 1.825 g · kg ^{1}, 1.214 g · kg ^{1}, 1.767 g · kg ^{1}, and 2.137 g · kg ^{1}, respectively. These results demonstrate that monitoring PNC through vegetation indices at different growth stages can effectively predict GPC at maturity, with good model stability and applicability.

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Note: Figure translations are in progress. See original paper for figures.