Monitoring the nitrogen nutrition status of rice plants using spectral and image technologies

YE Chun[1,2], LIU Jizhong[1*], LIU Ying[1*], LI Yanda[2], CAO Zhongsheng[2], HUANG Huang[3]

1. School of Mechanical and Electrical Engineering, Nanchang University, Nanchang 330031, China;

2. Institute of Agricultural Engineering/Jiangxi Province Engineering Research Center of Intelligent Agricultural Machinery Equipment/Jiangxi Province Engineering Research Center of Information Technology in Agriculture, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, China;

3. Engineering College of Huazhong Agricultural University, Wuhan, 430070, China.

*Corresponding author: LIU Jizhong

Tel: 0791-83969629/13970070635

Email: jizhongl@163.com

Abstract

Background: We aimed to investigate methods to estimate the nitrogen (N) nutrition status of rice plants using data obtained using a digital camera and a spectroradiometer. The overall aim was to compare the advantages and potential of image technology and spectral technology to monitor rice N indexes accurately, inexpensively, and in real time to optimize fertilization strategies. Realizing the technical selection of definite spectrum or image diagnosis aiming at different rice nitrogen nutrition indexes. We conducted field trials of rice plants grown with different levels of N fertilizer in 2018 to 2019. Spectral information and images of the rice canopy were obtained, various image and spectral characteristic parameters were selected to construct models to estimate rice N status.

Results: The determination coefficients of the models constructed using the ratio vegetation index ($RVI_{[800,550]}$) and cover canopy (CC) as dependent variables were most significant. Among the models using spectral parameters, those constructed using $RVI_{[800,550]}$ to estimate rice N indexes had the obviously coefficient of determination (R^2) values, which were 0.69, 0.58, and 0.65 for the models to estimate leaf area index(LAI), aboveground biomass(AGB), and plant N accumulation(PNA). As for image parameter, those using CC to predict rice N indexes showed the highest R^2 values (0.76, 0.65, and 0.71 for the models to estimate LAI, AGB, and PNA, respectively) ($P < 0.01$). The model using the spectral parameter $RVI_{[800,550]}$ had a good fit and stability in estimating plant nitrogen accumulation ($R^2 = 0.65$, root mean square error (RMSE) = 1.35 $g \cdot m^{-2}$, relative RMSE (RRMSE) = 14.05%), and the model using the image parameter CC had a good fit in predicting leaf area index ($R^2 = 0.76$, RMSE = 0.28, RRMSE = 7.26%) and aboveground biomass ($R^2 = 0.65$, RMSE = 22.03 $g \cdot m^{-2}$, RRMSE = 7.52%). Different detection technology should be adopted for different rice varieties and rice N nutrition indexes.

Conclusions: Spectral and image parameters can be used as technical parameters to estimate rice N status. The spectral parameter $RVI_{[800,550]}$ can be used to accurately estimate plant nitrogen accumulation, and the image parameter CC can be used to accurately estimate leaf area index and aboveground biomass.

Key words: image; canopy coverage; spectral index; rice; nitrogen nutrition

Background

Rice (*Oryza sativa* L.) is one of the most important food crops both in China and around the world. It plays an important role in food security, and provides social and socioeconomic stability. Nitrogen (N) is one of the most important nutrients for the growth and development of rice plants. In China, the amount of N fertilizer applied to rice crops accounts for 37% of the N fertilizer used globally. However, the average utilization rate of N fertilizer is only 35% [1]. Increasing N applications can increase rice yield, but excessive N application causes a series of environmental problems, such as greenhouse gas emissions, soil acidification, and water pollution [2, 3]. In addition to nitrogen management, rice breeding also plays an important role in the process of increasing rice yield. GAO et al. [4]showed that hybrid rice had heterosis compared with conventional rice, the yield increase advantage mainly depends on the dry matter production advantage of aboveground plants. Therefore, the scientific and rational application of N fertilizer and study the difference of nitrogen nutrition between hybrid rice and conventional rice are of great significance for high-yielding rice.

Accurate N management is an essential part of the rice production management system. Accurate determination of the N nutrition status of rice is essential for accurate N management [5]. Leaf area index (LAI), aboveground biomass (AGB), and plant nitrogen accumulation (PNA) are important indicators that are used to characterize rice growth and N status [6]. They are usually determined by chemical analyses, which provide accurate results [7]. However, the disadvantages of chemical analyses are their high cost, lengthy and complex operation, and the need for expensive and potentially harmful chemical reagents. For these reasons, chemical analyses are insufficient to meet the needs of real-time monitoring of N nutrition in large-scale crops [8]. One alternative is to use near-ground hyperspectral equipment to monitor the N status of crops in a large area. Willkomm et al. used low-cost unmanned aerial vehicles to generate a high-resolution crop surface model (CSM) for rice. On the basis of comparisons of agronomic parameters (fresh and dry AGB, LAI, and plant nitrogen concentration) measured using hyperspectral methods and direct methods, it was concluded that the plant height of rice was significantly correlated with fresh AGB and LAI (coefficient of determination, R^2>0.8) [9]. He et al. accurately estimated N distribution in the vertical leaves of the rice canopy using a knapsack spectrometer, and the hyperspectral model was shown to have good predictability [10]. In addition, some special instruments for plant nutrition diagnosis have been developed, such as the SPAD chlorophyll meter [11] and the GreenSeeker spectrometer [12]. A portable spectrometer is easy to carry and use, but one of its disadvantages is that the diagnostic results are not reliable for crops with excess N absorption [13]. Consequently, this method cannot be used to evaluate crops growing with an excess of N.

Digital cameras are a common and inexpensive piece of equipment. They can collect image and spectral information with sufficient quality to use in predictions of crop nutrition status [13, 14] and yield [15] [16], and to monitor pests [17]. Li et al. extracted the dark green color index (DGCI) of image features, and concluded that DGCI was significantly correlated with the SPAD value of rice leaves. Thus, DGCI could be used to estimate the chlorophyll value of rice leaves and indirectly evaluate the growth and nutrition status of rice [18]. Jia et al. used a digital camera and a

Greenseeker hand-held sensor to monitor cotton growth and N status[19]. The results revealed an exponential relationship between the image parameter canopy cover (CC) and aboveground total N content. The R^2 value of the model was 0.926, and the root mean square error (RMSE) value was 1.631 g·m^{-2}. Lee et al. used image red-green-blue (RGB) parameters and CC to monitor rice nutrition status in real time [13, 20]. They established a stepwise multiple linear regression model based on a non-linear relationship between rice color indexes and CC. Using this model, information about the nutrient status of crops could be obtained quickly and non-destructively using image technology [21]. Models to predict leaf area index (LAI), biomass, and plant N accumulation (PNA) have been constructed using various methods. However, less attention has been paid to the advantages and disadvantages of spectral and image techniques in monitoring nitrogen nutrition in rice. Rice yield is affected by many factors, among which the succession of rice varieties and the improvement of fertilization measures play an important role in the formation of rice yield.

Hence, the objectives of this study were to: (1) assess the potential of rice canopy image parameters to monitor hybrid rice and conventional rice N status; (2) compare and analyze models based on image and spectral parameters to estimate rice N status; and (3) determine the accuracy, advantages, and disadvantages of the models constructed using different image and spectral parameters. The overall aim of our research was to provide a reference for the fast, inexpensive, and non-destructive monitoring of the N status of rice crops.

Results

Relationships Between Rice N Nutrition Indexes and Image/Spectral parameters

During the whole growth period of rice, the correlations between image or spectral parameters and N nutrition indexes of the whole growth period of rice were analyzed (Table 1). There were significant differences in the correlation coefficients between image and spectral parameters. The spectral parameters were all positively correlated with rice N indexes. RVI$_{[800,550]}$ was most correlated with PNA, DVI$_{[800,720]}$ was most correlated with LAI and AGB, and that the correlation coefficients ranged from 0.419 to 0.645. Different from spectral indexes, canopy coverage (CC), red normalized value (NRI) and hue (H) were significantly correlated with aboveground biomass, nitrogen accumulation and LAI of rice ($P < 0.01$), and the correlation coefficients ranged from 0.427 to 0.831. Among them， NRI was negatively correlated with rice N indexes, while Hue and CC were positively correlated with rice N indexes. The correlation coefficient between NRI and aboveground biomass, plant nitrogen accumulation and LAI was the highest, with an average of 0.74 ($P < 0.01$). Although there was a significant correlation between other parameters and N nutrition index of rice, the correlation coefficient was very low. Therefore, the image parameters CC, NRI, Hue and spectral index RVI$_{[800,550]}$ and DVI$_{[800,720]}$ were selected as sensitive parameters to construct rice N nutrition monitoring model furthermore.

Table 1

Correlations between rice N indexes (LAI, biomass, PNA) and image/spectral parameters

Image parameter	LAI	AGB	PNA	Spectral parameter	LAI	AGB	PNA
NRI	-0.685**	-0.828*	-0.698**	RVI$_{[800, 550]}$	0.572**	0.064**	0.574**
NGI	-0.071*	-0.311**	-0.316*	RVI$_{[800, 720]}$	0.504**	0.248*	0.512**
GDR	0.549	0.758	0.135	DVI$_{[800, 720]}$	0.645**	0.462**	0.419**
GMR	-0.109*	-0.493**	-0.088*	NDVI$_{[800, 680]}$	0.493**	0.158**	0.438**
Hue	0.707*	0.685*	0.427*	λrep	0.298**	0.069**	0.275**

CC	0.619**	0.831**	0.595**

Note: ** correlation significant at the 0.01 level, * correlation significant at the 0.05 level.

Construction of Rice N Nutrition Models Based on Image/Spectral Parameters

1) Models based on spectral parameters

Select the data of jointing period to build the model. The spectral parameters ($RVI_{[800,550]}$ and $DVI_{[800,720]}$) calculated in experiment 1 and experiment 2 were used as independent variables to predict N indexes of rice. The relationships between $RVI_{[800,550]}$,$DVI_{[800,720]}$and N nutrition indexes of rice were all polynomial functions. The R^2 values for models using $RVI_{[800,550]}$ to predict LAI, AGB, and PNA were 0.69, 0.58, and 0.65, respectively ($P < 0.01$). And for $DVI_{[800,720]}$ the coefficient were 0.54, 0.55, and 0.55, respectively (P < 0.01)(Fig.2).

Take $DVI_{[800,720]}$ as an example, there were significant differences between conventional rice and hybrid rice in the application of spectral parameters to predict rice nitrogen status. The relationships between $DVI_{[800,720]}$ and the N indexes of rice were all polynomial functions, the accuracy of monitoring rice N indexes by $DVI_{[800,720]}$ in Zhongjiazao 17 was higher than that in hybrid rice Changliangyou173(Fig.3).

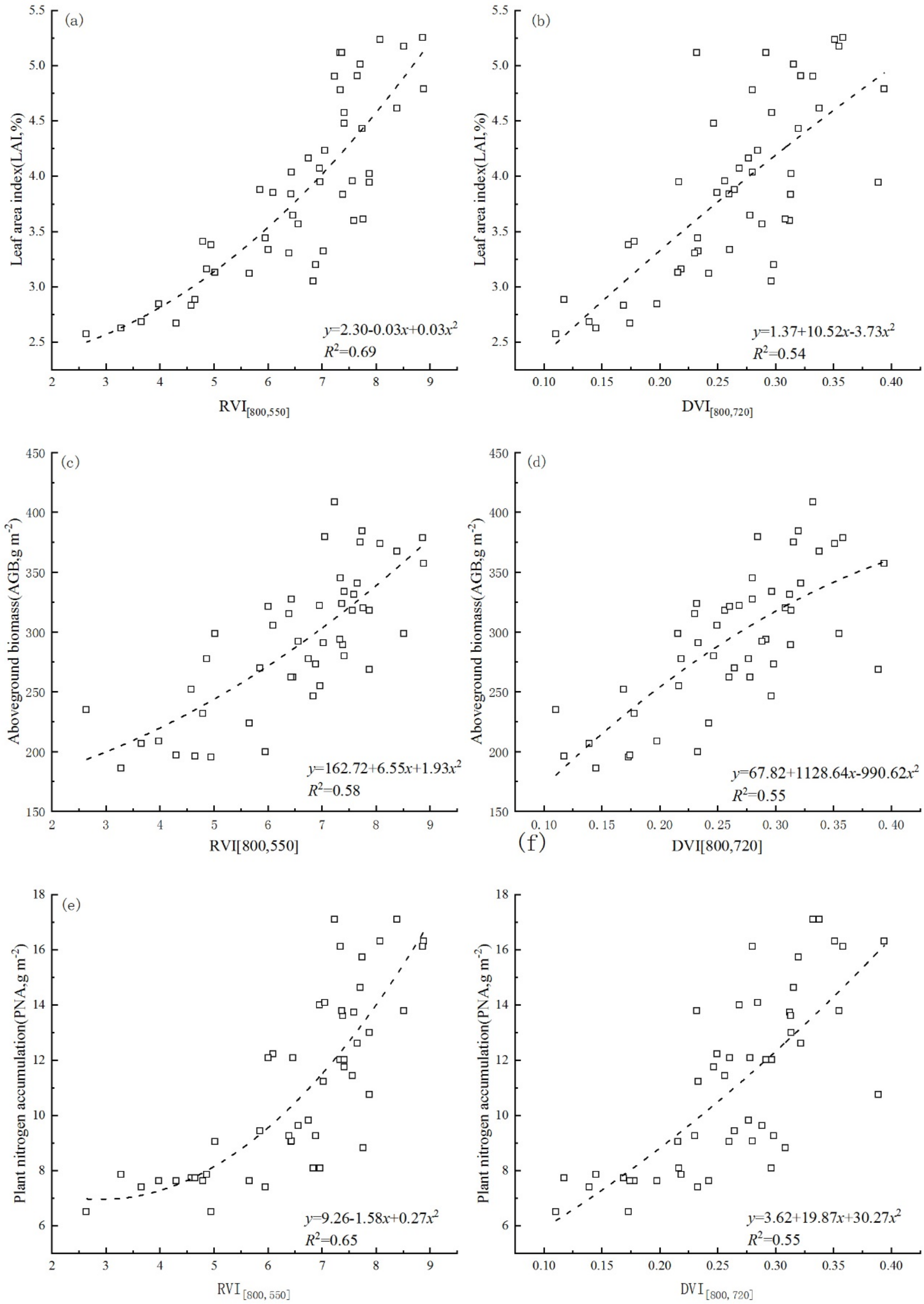

Fig. 2 Relationships between $RVI_{[800,550]}$,$DVI_{[800,720]}$ and rice N indexes

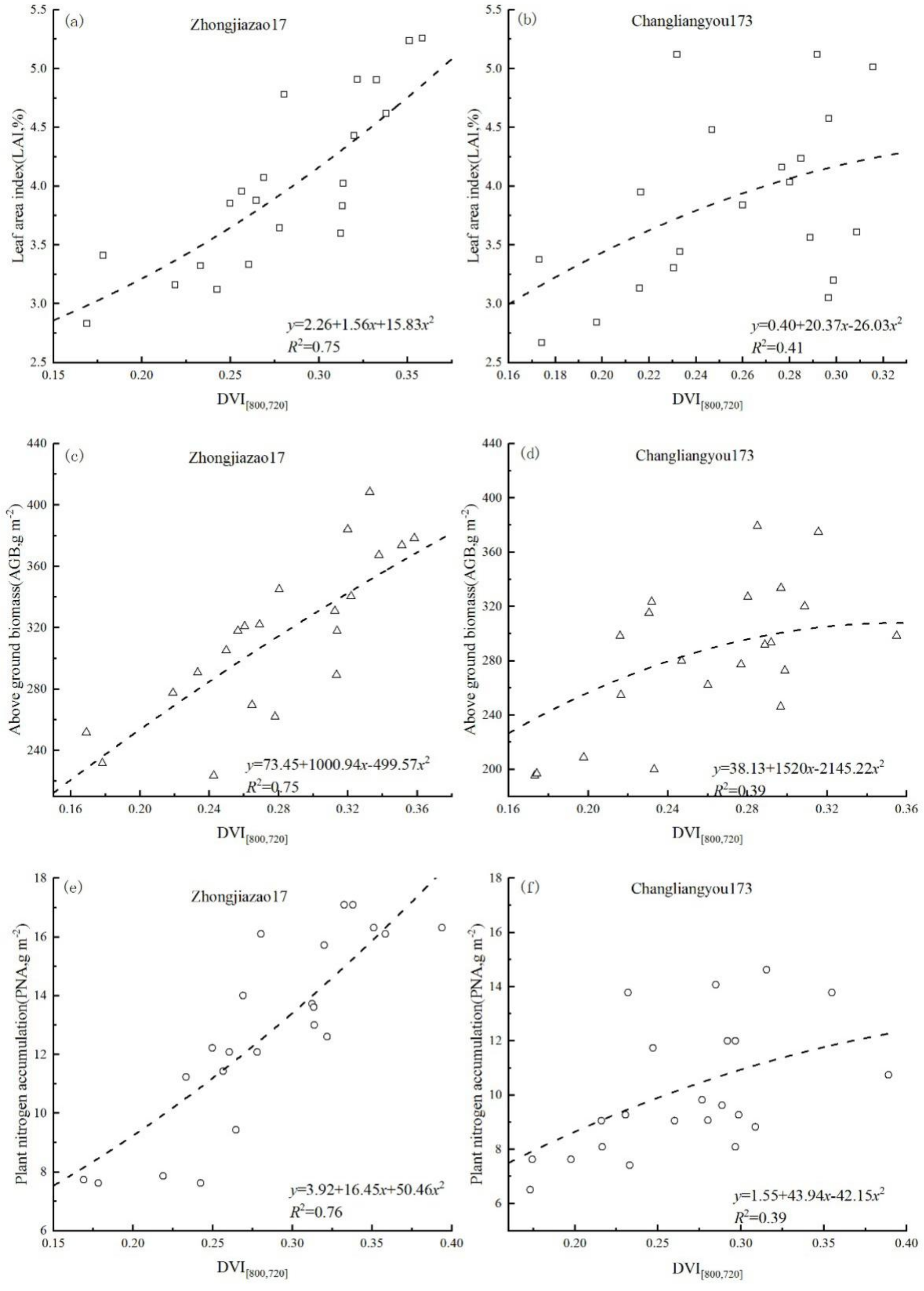

Fig. 3 Relationships between $DVI_{[800,720]}$ and rice N indexes

2) Models based on image parameters

The relationships between CC and N nutrition indexes of rice were all polynomial functions. The

R^2 values for models using CC to predict LAI, AGB, and PNA were 0.76, 0.65, and 0.71, respectively (P < 0.01). LAI, aboveground biomass and plant nitrogen accumulation of rice increased with the increase of CC, while the correlation coefficients between NRI, Hue and rice N indexes were not significant(R^2<0.5), the average correlation coefficient of NRI model was 0.16, and that of hue was 0.10. As for conventional rice and hybrid rice in the application of CC to predict rice nitrogen status, the average coefficient of models based on CC in hybrid rice Changliangyou173 was 0.74, which was higher than that in conventional rice Zhongjiazao17.

It can be seen from the above that the models based on $RVI_{[800,550]}$ and CC had good prediction effect for rice N indexes, and there were significant differences among different gene varieties.

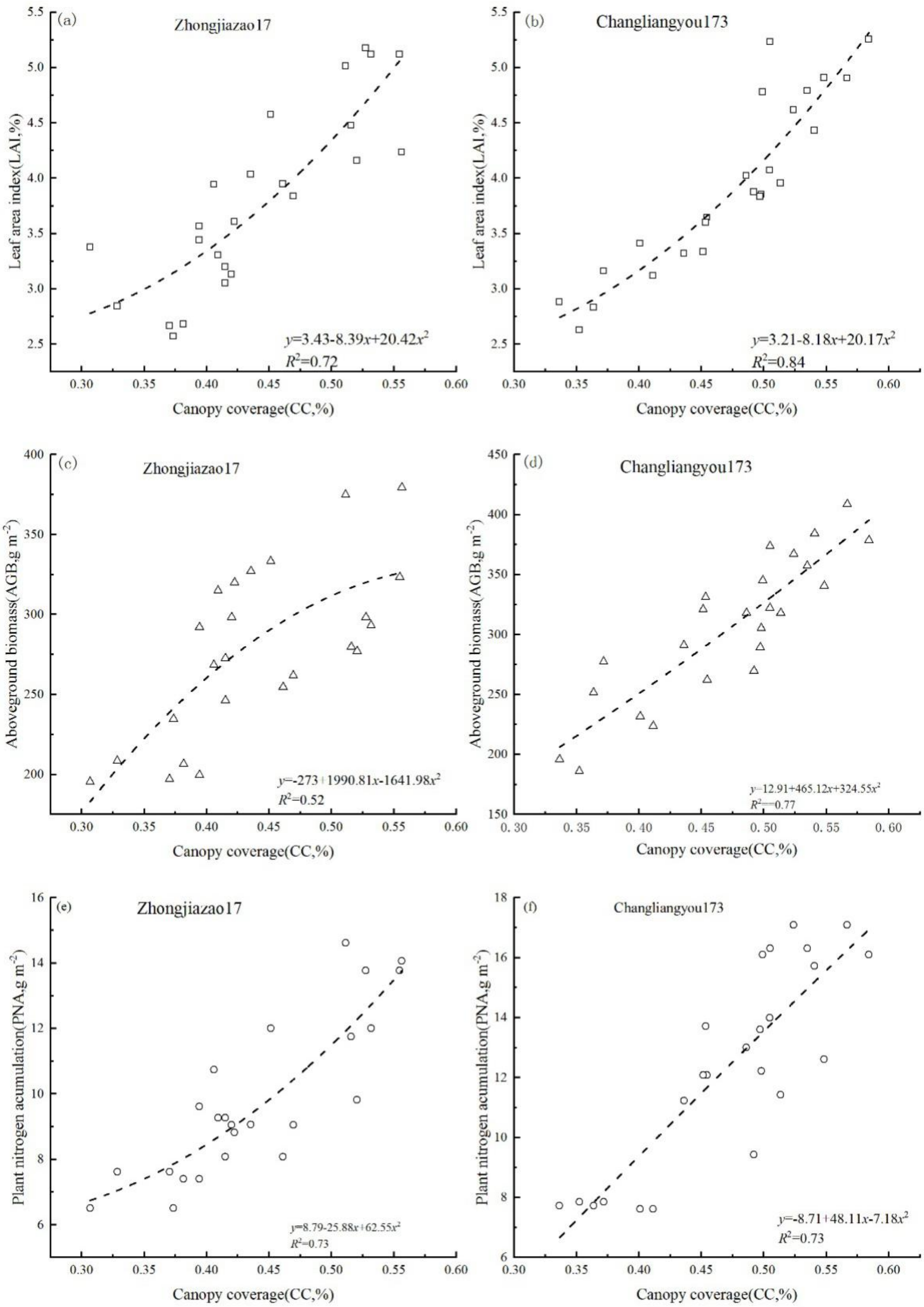

Fig. 4 Relationships between CC and rice N indexes.

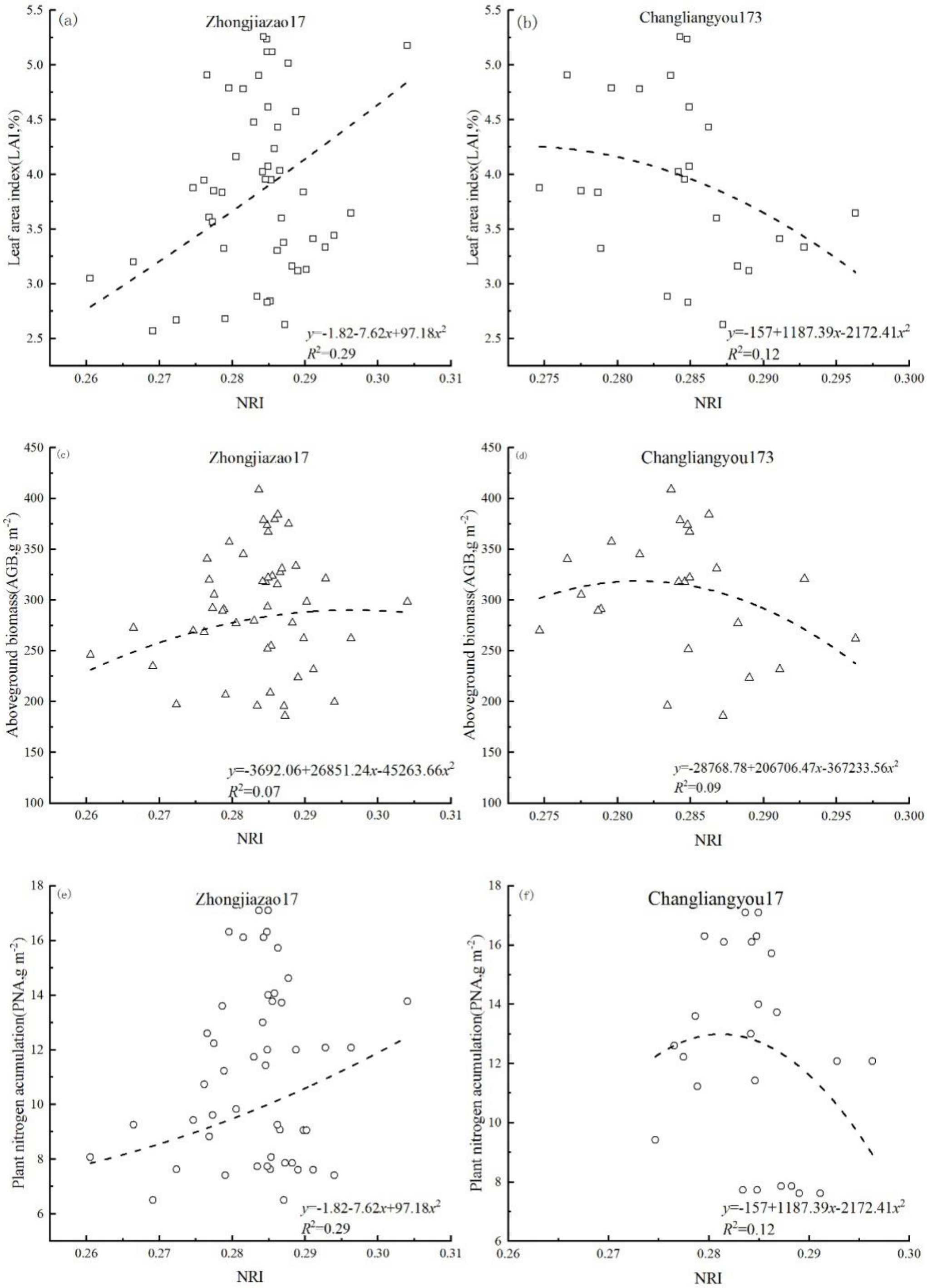

Fig. 5 Relationships between NRI and rice N indexes.

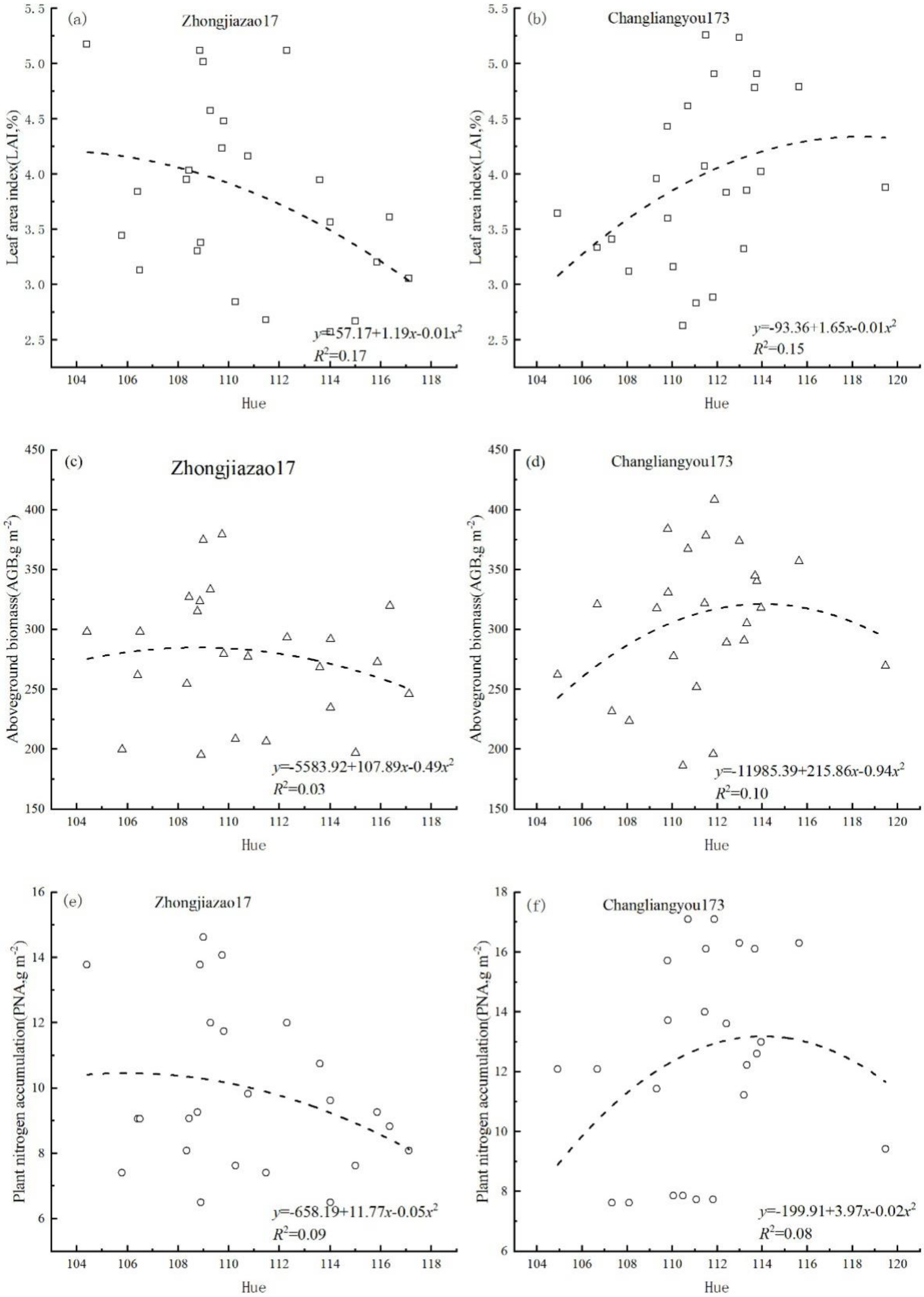

Fig. 6 Relationships between Hue and rice N indexes.

3) Regression validation

To test the accuracy of the models, those based on the spectral parameter $RVI_{[800,550]}$ and the image parameter CC were tested and evaluated using data from experiment 3 obtained at the jointing stage (Fig. 7, Fig.8). The RMSE, RRMSE, and r^2 values were calculated to evaluate the accuracy and stability of the models. The result showed that the r^2 from $RVI_{[800,550]}$ regression equations were 0.51,0.47 and 0.86 respectively, and that the RMSE values were 0.77, 42.18 and 1.35, respectively (Fig. 7a, 7b, 7c). As shown in Fig. 8, there was good consistency between the observed value and the value predicted by the model constructed using the image parameter CC as the independent variable except for predict PNA (r^2 values of 0.86, 0.77, and 0.52 for LAI, AGB, and PNA, respectively; $P < 0.05$). The RMSE values from CC regression equations were 0.28, 22.03 and 2.38, respectively (Fig. 8a, 8b, 8c). Among all the models, the PNA model based on $RVI_{[800,550]}$ showed ideal test result, with a higher r^2 and smaller RMSE , RRMSE values than that from CC regression equations, while the test result of LAI and AGB equations based on CC showed better result with higher r^2 and smaller RMSE , RRMSE values than that from $RVI_{[800,550]}$ regression equation.

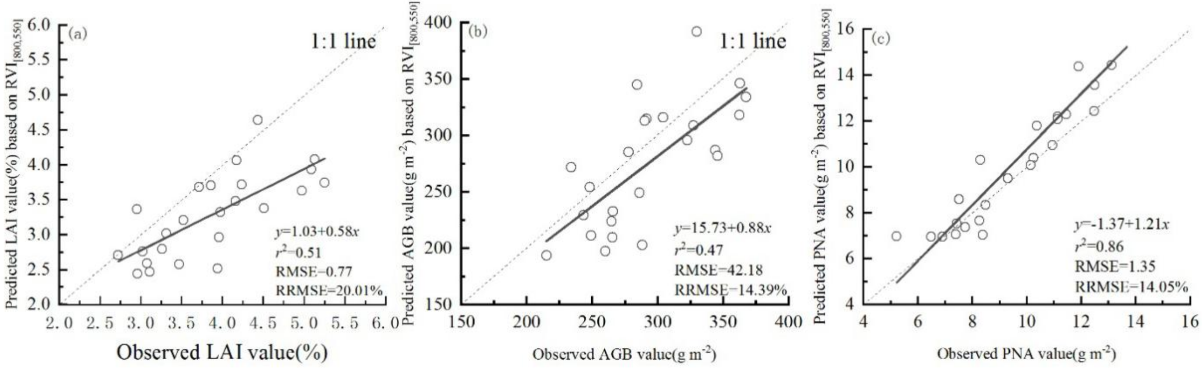

Fig. 7. Relationship between observed values in rice plants and predicted values from models based on $RVI_{[800,550]}$.

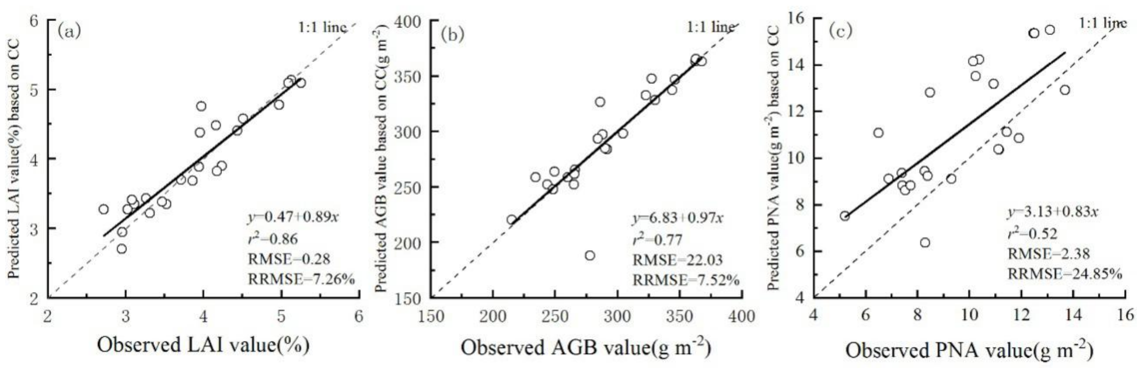

Fig. 8 Relationship between observed values in rice plants and predicted values from models based on CC.

Discussion

Comparison of Methods to Estimate Rice N Status

In recent years, accurate and non-destructive spectral and image techniques have been developed for the real-time monitoring of crop growth and N nutrition[21, 22]. However, few studies have compared and contrasted models constructed using data obtained using these two techniques. Hyperspectrometry has many advantages, including precise measurements and abundant spectral data [23, 24]. Single bands readily become saturated, it is better to use data from two or more bands as spectral parameters to create models to estimate the biochemical parameters of vegetation [25]. In the present study, $RVI_{[800,550]}$, a dual-band vegetation index at the jointing stage, was used as an independent variable in models to estimate the N status of rice. The R^2 values of models using $RVI_{[800,550]}$ to estimate LAI, AGB, and PNA were 0.69, 0.58, and 0.65, respectively. While the models using $DVI_{[800,720]}$ had poor fitting abilities. Different spectral parameters have different effects in different application environments. Zhao et.al. [26]constructed a regression model of a maize N nutrition index using a dual-band spectral index (R_{710}, R_{512}), and it was proven to be a very good predictor. Sun et al . [27] used hyperspectral technology and BP neural network to establish the estimation model of nitrogen concentration in rice leaves , which was better than the traditional multiple linear regression model. The results showed that the dual-band vegetation index model and BP neural network model were better than the traditional multiple linear regression model, but the cost of hyperspectral technology was high and the operation was complicated.

Compared with spectral technology, image technology does not need special equipment to diagnose crop N status. This greatly reduces the cost of detection and provides a reliable basis for precision agriculture. The intensity of R, G, and B colors in the canopy image provides information about most plant organs. The quantification of the intensity values of these visible colors (R and G) can describe plant color [28], which can reflect its nutrient status, especially N content and absorption. Several studies have shown that RGB color space parameters extracted from vegetation canopy images can be used to predict vegetation yield and nutrient status [15, 19, 20]. Among the models constructed with image parameters in this study, those constructed using NRI were unstable, possibly because the parameters of NRI were obtained by extracting RGB values from images. These values can be affected by the time and the weather when the image was acquired. The model constructed using CC had a good fitting effect. The R^2 values of the models using CC to estimate LAI, AGB, and PNA were 0.76, 0.66, and 0.71, respectively, consistent with the conclusion that CC is a reliable parameter to estimate vegetation N content [29]. The CC value is obtained by removing the influence of soil and water in the image. Compared with other image parameters, CC is obtained more easily and is not affected by weather or light intensity.

Advantages and Disadvantages of Models using Spectral and Image Parameters

The results of previous studies indicated that the booting stage is the peak period of rice plant growth, when the LAI is the highest. The booting stage is considered as the best time and cut-off point for estimating rice yield using remote sensing. However, some other studies have found that the early heading stage is the best time to use the spectral index RVI and color indexes to estimate rice LAI [30, 31]. In our study, through the correlation analysis of spectral parameters and image parameters with the nitrogen nutrition index of the whole growth period of rice, the parameters with larger correlation value were selected for modeling. According to the practice of fertilization in the double cropping rice region of southern China, the last fertilizer, panicle fertilizer, must be applied before booting stage to supply the nutrition needed after booting. Therefore, in order to achieve accurate fertilization before booting, the data of jointing stage were used for modeling. The results of the comparative analysis of the constructed models (Table 2) showed that the stability (RMSE value) of the model using CC to predict PNA was lower than that using $RVI_{[800,550]}$. While to predict LAI and AGB, they were higher than that using $RVI_{[800,550]}$. Among all the constructed models, the model to estimate LAI using CC had a high fitting ability and good stability (higher R^2 value, small RMSE value). The fitting ability of the model to predict AGB using CC was also high(higher R^2 value, small RMSE value). In general, the model using the spectral parameter $RVI_{[800,550]}$ to predict PNA had a good fitting ability and good stability, while the model using the image parameter CC to predict LAI and AGB had a good fitting ability and stability. From the viewpoint of LAI, AGB prediction, CC can be used as alternative technical parameters for estimating, and $RVI_{[800,550]}$ can be used as alternative technical parameters for estimating PNA.

Table 2 Comparison of model test results

Dependent variable	Independent variable	Estimation model	R^2	RMSE	RRMSE(%)	r^2
$RVI_{[800,550]}$	LAI	$y= 2.31-0.03x+0.03x^2$	0.69	0.77	20.01	0.51
	AGB	$y = 162.72+6.55x+1.93x^2$	0.58	42.18	14.37	0.47
	PNA	$y= 9.26-1.58x+0.27x^2$	0.65	1.35	14.05	0.86
CC	LAI	$y = 3.34-8.08x+19.75x^2$	0.76	0.28	7.26	0.86
	AGB	$y =-88.71+1022.24x-403.05x^2$	0.65	22.03	7.52	0.77
	PNA	$y = 3.11-4.40x+46.88x^2$	0.71	2.38	24.85	0.52

In addition, different rice varieties also had influence on model construction. The difference in nitrogen nutrition diagnosis between hybrid rice and conventional rice may also be related to nitrogen use efficiency. Previous studies have shown that the nitrogen accumulation in hybrid rice is significantly higher than that in conventional rice as the nitrogen supply level increases[4]. Peng et al. [32]indicated that the application ratio of panicle fertilizer should be increased to promote nutrient absorption and accumulation in the middle and late growth stage of hybrid rice. There was a significant correlation between vegetation reflection and nitrogen accumulation, which could be analyzed using multi-term linear regression method[33], consistent with this study. Moreover, the correlation between crop population reflection spectrum and nitrogen accumulation was better than that between digital image and nitrogen accumulation. The two-band combination has advantages in the inversion of nitrogen accumulation.

An effective strategy to optimize N use for rice should be suitable for the methods used by farmers, while taking account of factors such as cultivars that affect the N requirements of rice and the efficiency of its use. There are still many uncertain factors in remote sensing of crop N status. In this study, we did not consider the effects of several imaging factors (shooting angle, storage format, shooting time, and camera resolution). To obtain a reliable and universal model, it is necessary to further standardize imaging factors, test varieties, growth period, and test points, and to integrate soil and climate data. This will improve the accuracy of models so that they can be used to quickly diagnose the nutrient status of field crops and establish a tailored fertilization system.

Conclusion

In this study, we constructed models to estimate rice N indexes with the image parameter and the spectral parameter. We analyzed the accuracy and stability of the models to predict LAI, AGB, and PNA. The results showed that the R^2 values of the models constructed with the image parameter

CC and the spectral parameter $RVI_{[800,720]}$ were very significant. Compared with other models, the polynomial model constructed using CC to predict LAI ,AGB and the model constructed using $RVI_{[800,550]}$ to predict PNA during the jointing stage had better prediction and test results. Our results showed that image parameters can be used to estimate rice N status (especially LAI and AGB). We conclude that image technology can be used as a low-cost, non-destructive, and rapid method to monitor rice N status instead of spectral technology, which could be suitable for the methods used by farmers.

Materials and Methods

Study Area and Experimental Details

Three independent experiments were performed in this study.

Experiment 1 (Exp. 1): This experiment was carried out at the Gao'an base of Jiangxi Academy of Agricultural Sciences (28°25′27″ N， 115°12′15″ E), Jiangxi Province, China, in 2018. This area is in a mid-subtropical monsoon climate zone, with an annual average temperature of 17.6 °C, annual average sunshine of 1668.2 h, and annual precipitation of 1718.4 mm. The soil properties were as follows: 38.80 $g{\cdot}kg^{-1}$ organic matter, 2.53 $g{\cdot}kg^{-1}$ total N, 42.4 $mg{\cdot}kg^{-1}$ ammonium N, 1.04 $mg{\cdot}kg^{-1}$ nitrate N, 16.78 $mg{\cdot}kg^{-1}$ rapidly available phosphorus (P), 120.1 $mg{\cdot}kg^{-1}$ rapidly available potassium (K), and pH 5.5. A split-plot design was used with cultivar as the main plot and N treatment as the sub-plot with three replications. The experiment included two rice cultivars (Conventional rice: Zhonjiazao17; Hybrid rice: Changliangyou173) and four N application levels (0, 75, 150, 225 $kg{\cdot}hm^{-2}$). The row and plant spacing was 24 cm × 14 cm. Three seedlings were planted in each hole in the north-south direction. The plots were separated by ridges and were irrigated independently. The plot area was 30 m^2. Seeds were sown on 23 March and seedlings were transplanted on 23 April at three planting densities. All experimental plots were also supplemented with 75 kg·hm^{-2} P_2O_5 as P fertilizer and 150 kg·hm^{-2} K_2O as K fertilizer. The P fertilizer was added as base fertilizer, and N and K fertilizers were applied at three stages: 40% as base fertilizer, 30% at the tillering stage, and 30% at the ear-filling stage. Other cultivation measures were consistent with local high-yielding cultivation practices.

Experiment 2 (Exp. 2): This experiment was carried out at the Gao'an base of Jiangxi Academy of Agricultural Sciences in 2019. The soil properties were as follows: 38.60 g·kg^{-1} organic matter, 2.51 g·kg^{-1} total N, 42.0 mg·kg^{-1} ammonium N, 1.09 mg·kg^{-1} nitrate N, 16.88 mg·kg^{-1} rapidly available P, 120.3 mg·kg^{-1} rapidly available K, and pH 5.5. A split-plot design was used with cultivar as the main plot and N treatment as the sub-plot with three replications. Seeds were sown on 25 March and seedlings were transplanted on 24 April at three planting densities. The four N application levels, row spacing, row direction, plot area, and types and amounts of NPK fertilizers were the same as those in Exp. 1.

Experiment 3 (Exp. 3): This experiment was carried out at Jiebu, Xingan County (28°25′27″ N, 115°12′15″ E), Jiangxi Province, China in 2019. This area is in a humid subtropical monsoon climate zone, with an annual average temperature of 20.4 °C, annual average sunshine of 1684.8 h, and annual precipitation of 1520 mm. The soil properties were as follows: 28.20 g·kg^{-1} organic matter, 127.1 mg·kg^{-1} available N, 29 mg·kg^{-1} rapidly available P, and 120.0 mg·kg^{-1} rapidly available K. This experiment included two rice cultivars (Conventional rice: Zaoxian618; Hybrid rice: Xiangzaoxian45) and four N application levels. The four N application levels, row spacing, row direction, plot area, and types and amounts of NPK fertilizers were the same as those in Exp. 1.

Field Data Collection

Repeated destructive sampling was carried out in each plot for Exp. 1 and Exp. 2 Three rice plants from each experimental plot were randomly selected to determine LAI. For each sample, the green leaves were separated from the stems, and the leaf area (LA) was immediately determined by multiplying length by width. The LAI for each plot was calculated based on the planting densities. After bagging, the plant samples were heated in an oven at 105 °C for 30 min, dried to constant weight at 80 °C, and then weighed to determine the dry weight per unit area. Samples were crushed before determining N content using the Kjeldahl method. The PNA value was calculated as follows:

$$\text{PNA (g N}\cdot\text{m}^{-2}) = \text{LNC (\%)} \times \text{LDW (g DW}\cdot\text{m}^{-2}) + \text{SNC (\%)} \times \text{SDW (g DW}\cdot\text{m}^{-2}) + \text{PNC (\%)} \times \text{PDW (g DW}\cdot\text{m}^{-2}), \quad (1)$$

Where LNC is leaf N content, LDW is leaf dry weight, SNC is stem N content, SDW is stem dry weight, PNC is plant N content, and PDW is plant dry weight. Before sampling, images of the rice canopy were obtained using a Canon EOS 100D digital camera (resolution, 72 DPI) (Canon, Tokyo, Japan) . The camera lens was about 1.0 m away from the rice canopy at an angle of 60° relative to the ground. The camera was set to auto mode to control the color balance automatically. The images were stored in JPEG format with a resolution of 5184 × 3456 pixels.

A FieldSpec Handheld 2 spectroradiometer (Analytical Spectral Devices, Boulder, CO, USA) was used to measure the spectra of the rice plant population. The band range was 325~1075 nm. Spectral data were obtained at the same time as agronomic sampling and image sampling. The vertical height between the probe and the canopy was 1 m. The field of view angle was 25° and reference plate correction was carried out before and after acquiring each target spectrum. The average value was calculated from 10 repeated measurements within the field of view. Five fields of view were analyzed for each plot.

Data Processing and Analysis

1) Image data processing

In the periods of rice growth, the canopy does not completely obscure the ground, so images contain soil, water, and other non-canopy items. Consequently, it is necessary to segment and extract the canopy part from the image. Image segmentation eliminates interference from non-canopy items so that data for the crop canopy can be extracted and analyzed. We used the Otsu threshold segmentation algorithm to segment images. This image segmentation method is based on the difference of reflectance spectra between green vegetation and soil in the visible light region. Figures 9a and 9b show the original and segmented images of the rice canopy, respectively (Fig. 9b shows the rice canopy area in white).

(a) (b)

Fig. 9. Canopy images of rice before (a) and after (b) applying Otsu threshold segmentation algorithm.

At the same time, the histogram program in Adobe Photoshop 7.0 software was used to obtain the red, green, and blue intensity values of the image. Using combinations of these three color parameters, a variety of color parameters can be obtained. Table 3 showed that the references of image parameters previous researchers used to indirectly characterize crop nitrogen nutrition. In this study, eight color parameters including image R-G-B were selected.

Table 3 Image characteristic values and calculation methods

Parameter	Abbreviation	Algorithm formula	Reference
Normalized value of red band	NRI	NRI=$R/R+G+B$	[21]
Normalized value of green band	NGI	NGI=$G/R+G+B$	
Green blue band ratio index	GDR	GDR=G/R	
Green blue band difference index	GMR	GMR=$G-R$	
Hue	Hue	if R=max, $H=(G-B)/(\max-\min)*60$ if G=max, $H=120+(B-R)/(\max-\min)*60$if B=max, $H=240+(R-B)/(\max-\min)*60$ if $H<0$, $H=H+360$	

2) Spectral data processing

Table 4 showed that the references of spectral reflectance parameters previous researchers used to indirectly characterize crop nitrogen nutrition.

Table 4 Algorithms for different spectral parameters

Spectral parameter	Abbreviation	Algorithm formula	Reference
Reflectance	R_λ		
Ratio vegetation index	RVI(λ_1, λ_2)	$R_{\lambda 1}/ R_{\lambda 2}$	[34]

Differential vegetation index	DVI(λ_1, λ_2)	$R_{\lambda 1} - R_{\lambda 2}$	[34]
Normalized difference vegetation index	NDVI (λ_1, λ_2)	$\frac{\lvert R_{\lambda 1} - R_{\lambda 2} \rvert}{R_{\lambda 1} + R_{\lambda 2}}$	[35]
Red edge position wavelength	λ_{rep}	$710 + 50 \times \left(\frac{1/2(R_{870} + R_{660}) - R_{710}}{R_{760} - R_{710}} \right)$	[36]

3) Data analysis

In the models, the rice N nutrition index was set as the dependent variable, and image parameters and spectral parameters were set as independent variables. The quantitative relationships between rice N nutrition indexes and parameters in Exp. 1 and Exp. 2 were fitted and analyzed using Microsoft Excel 2010 software. We tested various relationships between them (linear function, exponential function, logarithmic function, polynomial function, and power function), and the function with the highest R^2 value was selected as the estimation model. Data from Exp. 3 were used to test the predictive ability of the models. The reliability of each model was evaluated by calculating the RMSE, relative root mean square error (RRMSE), and R^2 values. A 1:1 relationship between observed and simulated values was drawn to show the fitting degree and the predictive effect of the model. The following formulae were used to calculate RMSE and RRMSE:

$$\mathrm{RMSE} = \sqrt{\frac{1}{n} \times \sum_{i=1}^{n} \left(P_i - O_i \right)^2} \quad , \tag{2}$$

$$\mathrm{RRMSE} = \mathrm{RMSE} / \overline{O_i} \times 100\% \; . \tag{3}$$

In the above formulae, n is the number of samples tested for model test; P_i is the predicted value of the model, $\overline{p_i}$ is the average value of the predicted value; O_i is the measured value; and $\overline{O_i}$ is the average value of the measured values.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YC, LJZ and LYD designed the study; YC and CZS measured rice canopy. YC and CZS managed the field experiment. YC, LY and HH conducted statistical analyses and drafted the manuscript. All authors contributed to the interpretation of results and/or drafting the manuscript. All authors read and approved the final manuscript.

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References

1. Mohammend Y A, J.K., Chim B K, Emily R, Kevin W, Jeremiah M, Guilherme T, Kefyalew G D, William R, *Nitrogen fertilizer management for improved grain quality and yield in winter wheat in Oklahoma.* Journal of Plant Nutrition, 2013. **36**: 749-761.
2. Han, H., et al., *Study on nitrogen removal from rice paddy field drainage by interaction of plant species and hydraulic conditions in eco-ditches.* Environ Sci Pollut Res Int, 2019. **26**(7): 6492-6502.
3. Zhao, X., et al., *Nitrogen runoff dominates water nitrogen pollution from rice-wheat rotation in the Taihu Lake region of China.* Agriculture, Ecosystems & Environment, 2012. **156**: p. 1-11.
4. GAO Shuai, P.Y., SUN Yuming,GUO Junjie, WANG Chengzi, LING NIng, ZHANG Yan,GUO Shiwei, *Effects of different nitrogen supply on yield and nitrogen utilization of conventional rice and hybrid rice.* Journal of Arid Nanjing Agricultural University, 2018. **6**(41): 1061-1069.
5. E. Raymond Hunt, M.C., Craig S. T. Daughtry, James E. Mcmurtrey, Charles L. Walthall, *Evaluation of Digital Photography from Model Aircraft for Remote Sensing of Crop Biomass and Nitrogen Status.* 2005.
6. Inoue, Y., Moran, M.S., Horie, T., *Analysis of spectral measurements in paddy field for predicting rice growth and yield based on a simple crop simulation model.* Plant Production Science 1998. **1**: 269–279.
7. Jin J Y, B.Y.L., Yang L P, *Technology and Equipment of Efficient Soil Testing*, in *China Agriculture Press*. 2006: Beijing.
8. Gang Pan, F.-m.L., and Guo-jun Sun, *Digital Camera Based Measurement of Crop Cover for Wheat Yield Prediction.* IEEE, 2007. **47**(2): 135-146.
9. Congress, X.I., *Non-destructive monitoring of rice by hyperspectral in-field spectrometry and UAV-band remote sensing :case study of filed-grown rice in north rhine-westphalia,Germany*, in *Remote Sensing and Spatial Information Sciences*, A.B. M. Willkomm, G. Bareth, Editor. 2016: Czech Republic. 12-19.
10. Jiaoyang He, X.Z., Wanting Guo,Yuanyuan Pan,Xia Yao,Tao Cheng,Yan Zhu,Weixing Cao,Yongchao Tian, *Estimation of Vertical Leaf Nitrogen Distribution Within a Rice Canopy Based on Hyperspectral Data.* Frontiers in Plant Science 2020.
11. Jie Li, Y.F., Xiaoke Wang,Jinfeng Peng,Dinghua Yang,Guiling Xu,Qiangxin Luo,Lingli Wang,Da Ou,Wei Su *Stability and applicability of the leaf value model for variable nitrogen application based on SPAD value in rice.* PLOS ONE 2020.
12. A.M. Ali, H.S.T., S. Sharma, Varinderpal-Singh, *Prediction of dry direct-seeded rice yields using chlorophyll meter, leaf color chart and GreenSeeker optical sensor in northwestern India*

Field Crops Research 2014.
13. Lee, K.-J. and B.-W. Lee, *Estimation of rice growth and nitrogen nutrition status using color digital camera image analysis.* European Journal of Agronomy, 2013. **48**: 57-65.
14. Liu, X.-j., et al., *Leaf area index based nitrogen diagnosis in irrigated lowland rice.* Journal of Integrative Agriculture, 2018. **17**(1): 111-121.
15. Liu, K., et al., *Evaluation of grain yield based on digital images of rice canopy.* Plant Methods, 2019. **15**: 28.
16. Wang, Y., et al., *Estimation of Rice Growth Parameters Based on Linear Mixed-Effect Model Using Multispectral Images from Fixed-Wing Unmanned Aerial Vehicles.* Remote Sensing, 2019. **11**(11).
17. Dongyan Zhang, X.Z., Jian Zhang,Yubin Lan,Chao Xu,Dong Liang, *Detection of rice sheath blight using an unmanned aerial system with high-resolution color and multispectral imaging.* PLOS ONE 2018.
18. Jinwen, L., *Determination of Canopys Average SPAD Readings Based on the Analysis of Digital Images.* Agrotechnology, 2014. **03**(01).
19. Jia, B., et al., *Use of a digital camera to monitor the growth and nitrogen status of cotton.* ScientificWorldJournal, 2014. **2014**: 602647.
20. Jia, L., et al., *Use of Digital Camera to Assess Nitrogen Status of Winter Wheat in the Northern China Plain.* Journal of Plant Nutrition, 2004. **27**(3): 441-450.
21. Wang, Y., et al., *Estimating nitrogen status of rice using the image segmentation of G-R thresholding method.* Field Crops Research, 2013. **149**: 33-39.
22. Zhou ZJ, P.F., Thomsen AG, Andersen MN. , *A RVI/LAI-reference curve to detect N stress and guide N fertigation using combined information from spectral reflectance and leaf area measurements in potato.* European Journal of Agronomy, 2017. **87**: 1-7.
23. MILLER, J.R., HARE, E. W., WU, J., <*Quantitative characterization of the vegetation red edge reflectance 1 An inverted Gaussian reflectance model.pdf*>. International Journal of Remote Sensing, 1990. **11**: 10.
24. Mistele, B. and U. Schmidhalter, *Estimating the nitrogen nutrition index using spectral canopy reflectance measurements.* European Journal of Agronomy, 2008. **29**(4): 184-190.
25. Tian Y C, Y.J., Yao X, Cao W X,Zhu Y, *Monitoring canopy leaf nitrogen concentration based on leaf hyperspectral indices in rice[J].* .Acta Agronomica Sinica, 2010. **36**(9): 1529-1537.
26. Ben Zhao, A.D., Syed Tahir Ata-Ul-Karim, Zhandong Liu, Zhifang Chen, Zhihong Gong, Jiyang Zhang, Junfu Xiao, Zugui Liu, Anzhen Qin, Dongfeng Ning, *Exploring new spectral bands and vegetation indices for estimating nitrogen nutrition index of summer maize.* European Journal of Agronomy 2018. **93**: 113-125.
27. SUN Xiaoxiangm WANG Fangdong, Z.X., XIE Wen,GUO Xi, ***The estimation models of rice leaf nitrogen concentration based on canopy spectrum and BP neural network***. Chinese Journal of Agricultural Resources and Regional Planning, 2019. **40**(35-44).
28. Mulla, D.J., *Twenty five years of remote sensing in precision agriculture: Key advances and remaining knowledge gaps.* Biosystems Engineering, 2013. **114**(4): 358-371.
29. Hadjimitsis, D.G., C.R.I. Clayton, and A. Retalis, *The use of selected pseudo-invariant targets for the application of atmospheric correction in multi-temporal studies using satellite remotely sensed imagery.* International Journal of Applied Earth Observation and Geoinformation, 2009. **11**(3): 192-200.

30. Zhou, X., et al., *Predicting grain yield in rice using multi-temporal vegetation indices from UAV-based multispectral and digital imagery.* ISPRS Journal of Photogrammetry and Remote Sensing, 2017. **130**: 246-255.
31. Shibayama M C, S.T.H., Takada E J, Inoue A H, Morita K H, Yamaguchi T K Y, Takahashi W T R, Kimura A H, *Estimating rice leaf greenness (SPAD) using fixed-point continuous observations of visible red and near infrared narrow-band digital images.* Plant Production Science, 2012. **15**(4): 293-309.
32. J, P.S.C.K.G.K.M., *Relationship between leaf photosynthesis and nitrogen content of field-grown rice in tropics.* Crop Science, 1995. **6**(35): 1627-1630.
33. S. G. Bajwa, A.R.M., R. J. Norman, *Canopy reflectance response to plant nitrogen accumulation in rice.* Precision Agriculture, 2010. **11**: 488-506.
34. Jordan, C.F., *Derivation of Leaf-Area Index from Quality of Light on the Forest Floor.* Ecology, 1969. **50**.
35. Rouse, J.W.H., R.H.; Schell, J.A.; Deering, D.W. . *Monitoring vegetation systems in the great plains with ERTS*. in *In Third Earth Resources Technology Satellite-1 Symposium-Volume I: Technical Presentation*. 1974. Washington, DC, USA, : NASA.
36. Liu, M., et al., *Monitoring stress levels on rice with heavy metal pollution from hyperspectral reflectance data using wavelet-fractal analysis.* International Journal of Applied Earth Observation and Geoinformation, 2011. **13**(2): 246-255.