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Application of fluorescence spectrum to precisely inverse paddy rice nitrogen content

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ABSTRACT

Paddy rice is important for Chinese agriculture and crop production, which largely depends on the leaf nitrogen (N) levels. The purpose of this study is to discuss the relationship between the fluorescence parameters and leaf N content of paddy rice and to test their performance in inversing N content of crops through back-propagation (B-P) neural network. In the correlative analysis of the fluorescence parameters and the N content, we found that the correlation between fluorescence ratios (F740/F685 and F685/F525 (F740, F685, F525 – intensity of fluorescence at 740, 685 and 525 nm, respectively)) and the N content (R^2 are 0.735 and 0.4342, respectively) is weaker than that between the intensity of fluorescence peaks (F685 and F740) and N content (R^2 are 0.9743 and 0.9686, respectively). Our studies show that the accuracy and precision of N content inversion which is acquired from the intensity of fluorescence peaks through the B-P neural network model are significantly improved (root mean square error (MSRE) = 0.1702, the residual changes between –0.1–0.1 mg/g) compared with the fluorescence ratio (MSRE = 0.3655, the residual changes from –0.3–0.3 mg/g). Results demonstrate that the intensity of fluorescence peaks can be as a characteristic parameter to estimate N content of crops leaf. The B-P neural network model will be serviceable approach in inversing N content of paddy leaf.

Keywords: remote sensing; N fertilization strategies; nutrient stress; chlorophyll

At present, most plant investigations mainly focus on studying crop tolerance for changing environmental conditions or the optimal growth conditions for improving crop production. It is can be estimated that crop response to nutrient stress which is induced by numerous factors. Nitrogen (N) is an indispensable nutrient for improving crop field and N content is also a significant nutrient diagnosis indicator that governs canopy carbon assimilation (Oppelt and Mauser 2004). Thus, it is significant to precisely estimate the N content of crops leaf in a large area scale, which can gain the distribution of N content that can be used by farmers in fertilization and to obtain the discipline of leaf energy exchange in agroecological system that can be utilized to manage the number of vegetation (Dobrowski et al. 2005).

In the literature, N levels can be conducted through passive remote sensing methods, active reflectance measurement, and passive fluorescence (Blackburn 1998). Gaining the relationship by remote sensing techniques is also often used to describe the correlation between crops leaf chlorophyll and N status in crop (Fox et al. 2001), and it has the potential to rapidly estimate N variation over large fields. However, it lacks sensitivity for detecting variation of N content at early stages of growth (Apostol et al. 2007). A decade ago, it

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was mentioned that the technique of laser-induced fluorescence (LIF) can be utilized to detect nutrient stress of crops (Chappelle et al. 1984, Subhash and Mohanan 1994). These studies analyzed the relationship between LIF spectral emission and the physiological condition of crops. In recent years using the fluorescence spectrum to estimate the canopy N status of paddy, triticale, soybean and cotton has been comprehensively studied (Nguyen and Lee 2006, Li et al. 2008, Janušauskaite and Feiziene 2012).

They emphasized to use the red and far-red chlorophyll fluorescence ratios as well as the blue-green fluorescence ratio to monitor variation of crop nutrient. The relevant research which would estimate the major nutrient content of crops through the intensity of fluorescence peaks is lacking (Živčák et al. 2014). The reason is that the shape of the plant leaves' fluorescence spectra (360–800 nm) is influenced by many factors (Stober and Lichtenthaler 1993). These traditional RF/FRF and BGF/FRF (the fluorescence ratios red to far-red and blue to far-red, respectively) parameters are insensitive to monitor the N variation in crops (Takeuchi et al. 2002), and relative studies are rare in precisely inversing N content of crops (Sayed 2003, Janušauskaitė et al. 2011).

As mentioned above, the purposes of our study are to analyze the variation of leaf N content influence on the intensity of the fluorescence spectrum, and to analyze the correlation between fluorescence intensity obtained by the LIF device and difference of N content, and to study the performance of the fluorescence parameters in estimating the variation of N content. Finally, we inversed the N content of paddy leaf through back-propagation (B-P) neural network, and compared the accuracy and precision of the result derived from fluorescence intensity and fluorescence ratio, respectively.

MATERIAL AND METHODS

Study areas and site description. These samples were collected during the summer of 2014 from the experimental rice field which is seated in Junchuan county, Suizhou, in the province of Hubei, China. The region is situated in the middle reaches of the Yangze river and is well-known as the Jianghan Plain (Song et al. 2011). The cultivar of the paddy rice was japonica rice, which was seeded on 27 April, 2014 and then transplanted on 1 June. The samples were gathered on 15 July and 1 August representing tillering stage and shooting stage, respectively.

LIF instrument. The LIF instrument is consisted of three main parts: the excitation source, optical receiver assembly and the data collection and treatment system. The laser source is an Nd:YAG that emitted pulses with the output power and the width per pulse being 1.5 mJ and 5 ns, respectively, and the omitted wavelength is 355 nm. A single-mode fiber optic with a diameter of 200 μm and 25° angular field of view is utilized to collect the fluorescence signal. The fluorescence emission spectral range of rice leaf is 360–800 nm with the sampling interval of 0.5 nm. The fluorescence induced by the ultraviolet laser through two convex lenses, and then entered the spectrometer after being through the cut-off filter of 355 nm. The relationship between the normalized fluorescence intensity and the wavelength is shown in Figure 1.

Methods. To compare the performance of intensity of fluorescence peak and fluorescence ratio

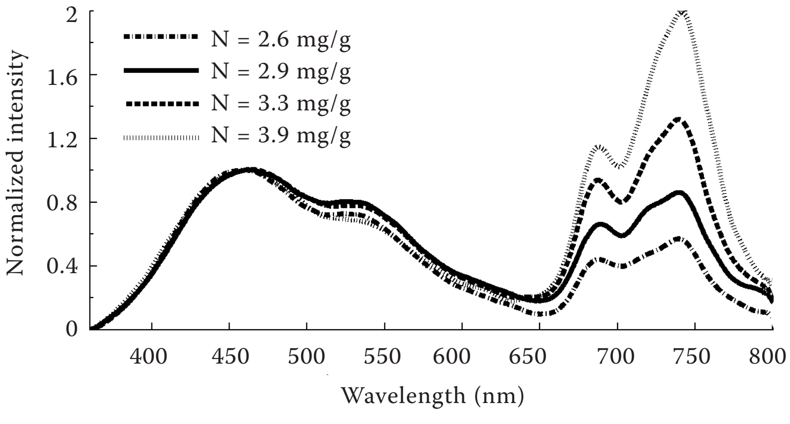

Figure 1. The relationship between the normalized fluorescence intensity (normalized to 1 at the 460 nm) and the wavelength under four different nitrogen (N) content of paddy leaf

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in inversing N content, the B-P neural network method was utilized in this thesis to predict the leaf N content. B-P neural network is a method that needs not to know the mapping relationship between a set of independent predictor variables and response variables. Each individual neuron in the nervous system is independent and essentially works alone (Goltsev et al. 2012). Moreover, it has the advantage of the input signals stimulating network capability to learn and recognize patterns (Samborska et al. 2014). All the experimental data which are composed of 276 measurements from all samples were randomly separated to two parts: 80% of the all data as the train set and 20% of the data as the validation set. The relationship between leaf fluorescence spectrum and leaf N content was modeled by utilizing the B-P neural network.

RESULTS AND DISCUSSION

Nitrogen content of paddy leaf. The rice leaves were destructively sampled by stochastically cutting six leaves with three replicates in each experimental field. A subsample was randomly chosen from the ground samples to test the N content (nitrate N) of paddy leaf by the plants nutrients tester TYS-3N (N resolution: ± 5%). To verify the N content measured by TYS-3N, the chemical approach is also used to determine the total N content of paddy leaf. The Kjeldahl method was utilized to analyze the content of total N and the result was shown in Figure 2. From Figure 1, we could know that there is a tightly linear correlation between the N content measured by TYS-3N and measured by the chemical approach, respectively (R^2 = 0.8942; root mean square error (MSRE) = 0.08349; sum of squares for error (SSE) = 0.3624).

Fluorescence spectra. The spectrum is normalized to 1 at 460 nm and we can find that chlorophyll fluorescence emission spectra are in the range between 650–800 nm, centering at 685 nm and 740 nm. Fluorescence emitted by antennae chlorophyll of photosystem I (740 nm) is more intensive than the emission from photosystem II (685 nm) (Günther et al. 1994). The dual waveband area that focused on the peak wavelength (685 nm and 740 nm) exhibited dramatic variation between the low and high N content. We can conclude that the intensity of fluorescence peaks (685 nm and 740 nm) is of significant difference with increasing of N content in paddy leaf. So the intensity of fluorescence peaks at 685 nm and 740 nm is feasible to estimate the variation of leaf N content. These results have similar varying tendency with the investigations of Janušauskaite et al. (2011) and Živčák et al. (2014).

Analysis of fluorescence parameters. Different N levels of paddy leaf result in different characteristics of fluorescence (Figure 2). Thus, the fluorescence spectrum techniques were frequently employed in physiological analysis of N variation in plants. Fluorescence ratio (F740/F685 and F685/F525 (F740, F685, F525 – intensity of fluorescence at 740, 685 and 525 nm, respectively)) and the maximum quantum yield ratio of photochemistry parameters were widely utilized in the estimation of the N status and in diagnosis of the crops growth. The reason is that it is simple to measure and interpret, but the fluorescence parameters are insensitive (Janušauskaite and Feiziene 2012, Živčák et al. 2014).

A comparison was made with the representatively spectral parameters that were published (Günther et al. 1994, Apostol et al. 2007), which is based on the correlation between the fluorescence spectral index and the N content of paddy leaf. The relationships between the N content of paddy leaf and fluorescence ratio (F740/F685, F685/F525), and the relationship between N content of paddy leaf and the intensity of fluorescence peaks (F685, F740) were established, as showed Figure 3.

Figures 3a,b show that the fluorescence ratio indices (F740/F685 and F685/F525) have a weak

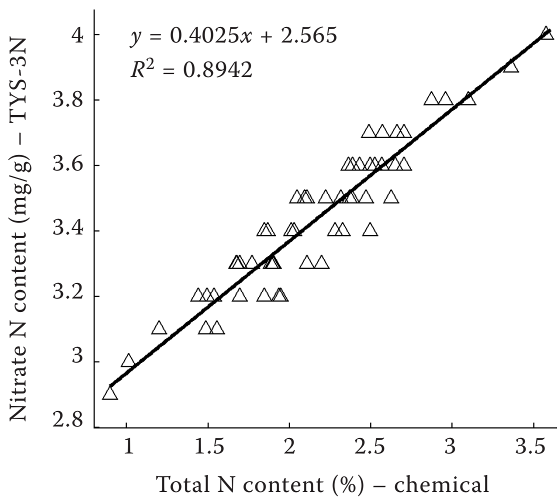

Figure 2. Relationship between nitrogen (N) content measured by TYS-3N and by chemical approach

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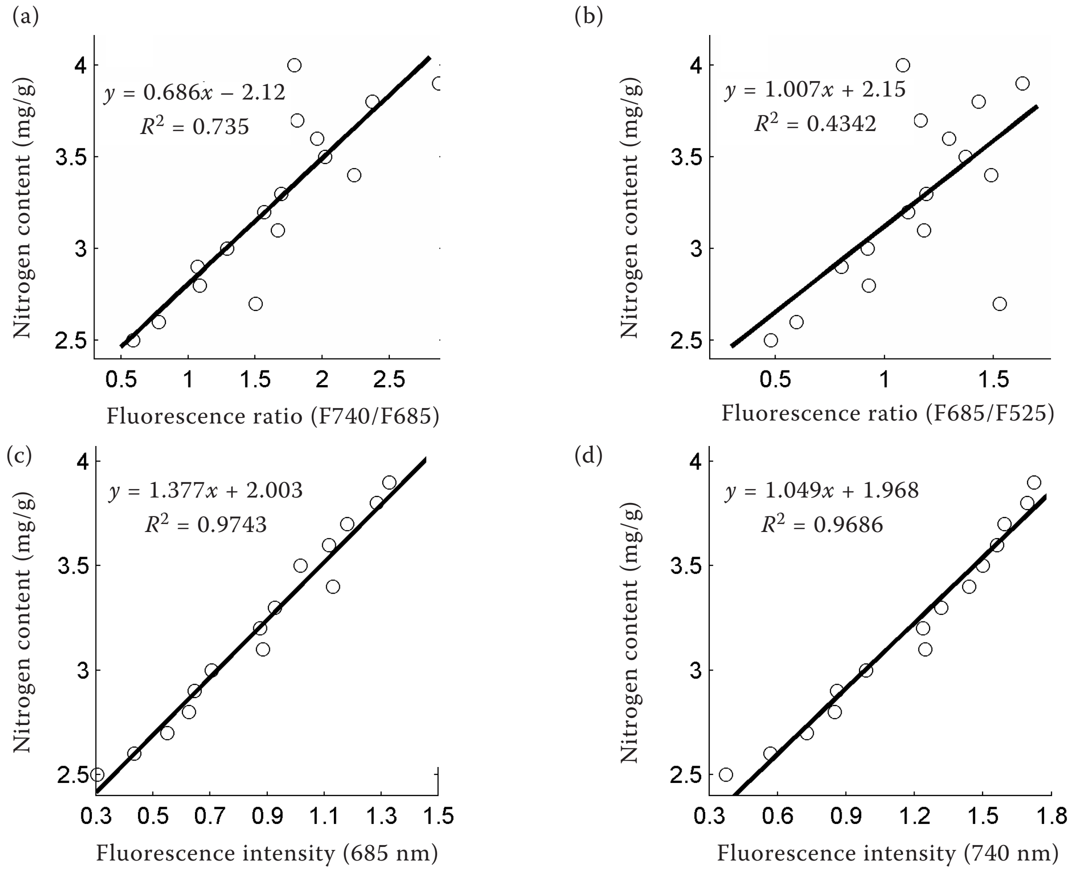

Figure 3. Relationship between the nitrogen (N) contents of paddy leaf and different fluorescence spectrum parameters (a) F740/F685; (b) F685/F525; (c) F685; (d) F740. F740, F685, F525 – intensity of fluorescence at 740, 685 and 525 nm

correlation with the N content of rice leaf (R^2 = 0.735 and R^2 = 0.4342, respectively). On the contrary, there is significant correlation between the intensity of fluorescence peaks (F685 and F740) and the increase of N content from 2.5–4.0 mg/g. The fluorescence intensity exhibited a direct linear relationship with the N content with R^2 = 0.9743, RMSE = 0.1421 and SSE = 0.09954 at 685 nm (Figure 3c) and R^2 = 0.9684, RMSE = 0.1118 and SSE = 0.08141 at 740 nm (Figure 3d). So we can directly use the information of fluorescence intensity to inverse the N content.

Nitrogen content inversion. The train set of experimental data was utilized to train the B-P neural network model with the same number of latent variables and other parameter settings. The results of inversion N content, which were acquired from the fluorescence ratio (F740/F685 and F685/F525) and intensity of fluorescence peaks (F685 and F740) respectively, are shown in Figure 4.

The robustness of the B-P neural network models significantly depends on the correlation between N levels and the fluorescence parameters. The accuracy and precision of the inversion were greatly reduced and the results of inversion deviated sufficiently from the line of 1:1 (Figures 4a,b). On the contrary, there is a significant linear correlation between the intensity of fluorescence peak and the variation of N content (Figures 3c,d). The ability of inversion was greatly improved and the results of prediction were nearly in accordance with the line of 1:1 (Figures 4c,d). Compared with the results of inversion acquired from the fluorescence ratio indices, the intensity of fluorescence peaks parameters has higher sensitivity and effectiveness in estimating the variation of N content. So the intensity of fluorescence peak can be as a characteristic index and directly used to monitor the N status of crops.

Analysis of accuracy and precision. In order to assess the accuracy and precision of the inversion

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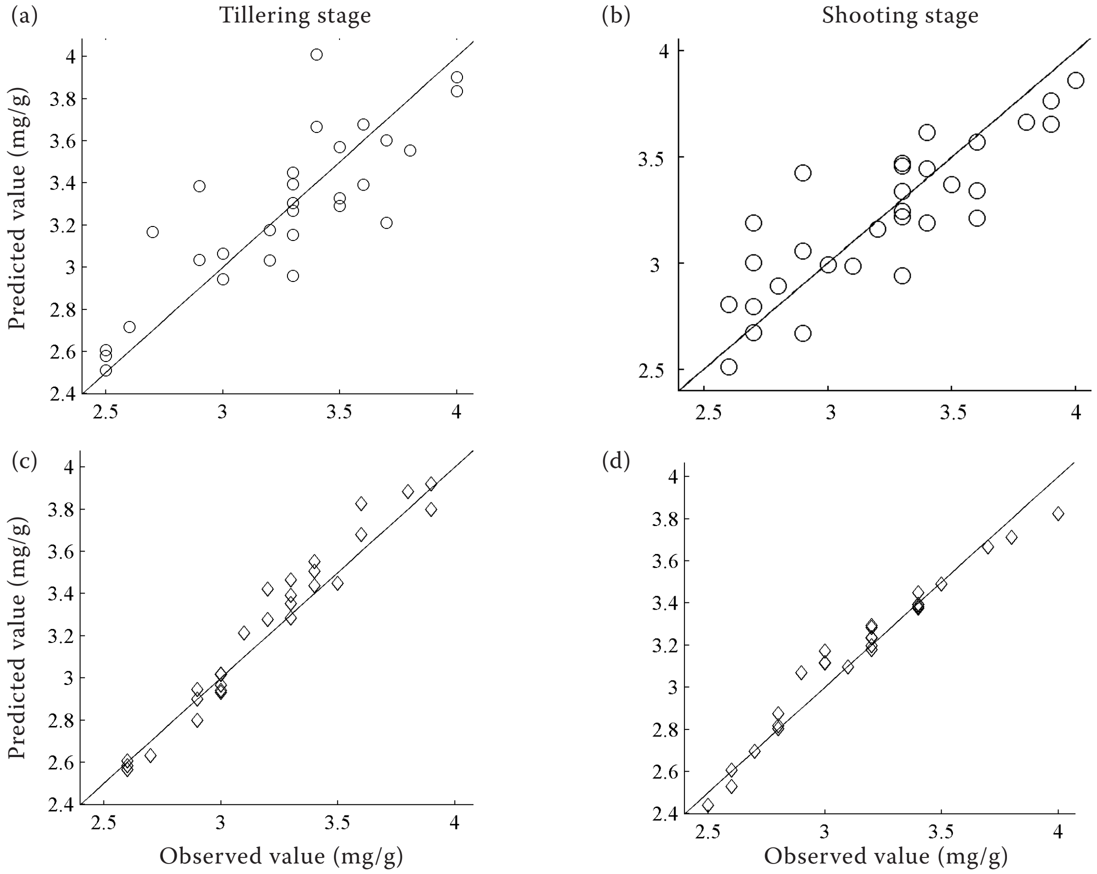

Figure 4. The relationship between the observed and predicted leaf nitrogen (N) content based on different fluorescence parameters at different stages. (a) and (b) present the predicted value inversed by the fluorescence ratio (F740/F685 and F685/F525) at tillering stage and shooting stage, respectively; (c), (d) present the predicted value inversed by the intensity of fluorescence peaks (F685 and F740) at tillering stage and shooting stage, respectively. F740, F685, F525 – intensity of fluorescence at 740, 685 and 525 nm

results, the RMSE and the residual are used. The RMSE of predictive N content of paddy leaf, which is based on the fluorescence ratios (the F740/F685 and F685/F525 as the variables), are 0.3295 and 0.3655 as response to tillering stage (Figure 5a) and shooting stage (Figure 5b), respectively. Those based on the intensity of fluorescence peaks (F685 and F740 as the variables) are 0.1702 and 0.1445 as response to tillering stage (Figure 5c) and shooting stage (Figure 5d), respectively. The residual of predictive value acquired from the intensity of fluorescence peaks is smaller (vary from –0.1–0.1 mg/g) than that acquired from the fluorescence ratio (vary from –0.3–0.3 mg/g). Results (Figures 4 and 5) reveal that the fluorescence intensity can be a characteristic parameter to estimate the N stress of crops and the B-P neural network method is a potentially useful method to precisely inverse the N content.

In conclusion, the N content of paddy leaf has a stronger linear correlation with the fluorescence intensity (R^2 = 0.9743 and R^2 = 0.9684) than the fluorescence ratio (R^2 = 0.735 and R^2 = 0.4342) in our trail. Thus the intensity index of the fluorescence spectrum can also be a characteristic index and directly used to inverse the N content of paddy leaf and to monitor the variation of N status of crops. The results of inversion show that the B-P neural network method is a potentially useful approach in evaluating the N content of paddy leaf in the field and satisfactory results (the residual vary from –0.1–0.1 mg/g) can be obtained through fluorescence intensity. If more spectral and field measurements are included, the model can be suggested and may be extended to on-time monitoring N levels of paddy rice globally, and it can be used to provide the support for decision-making of farmers in their N fertilization strategies.

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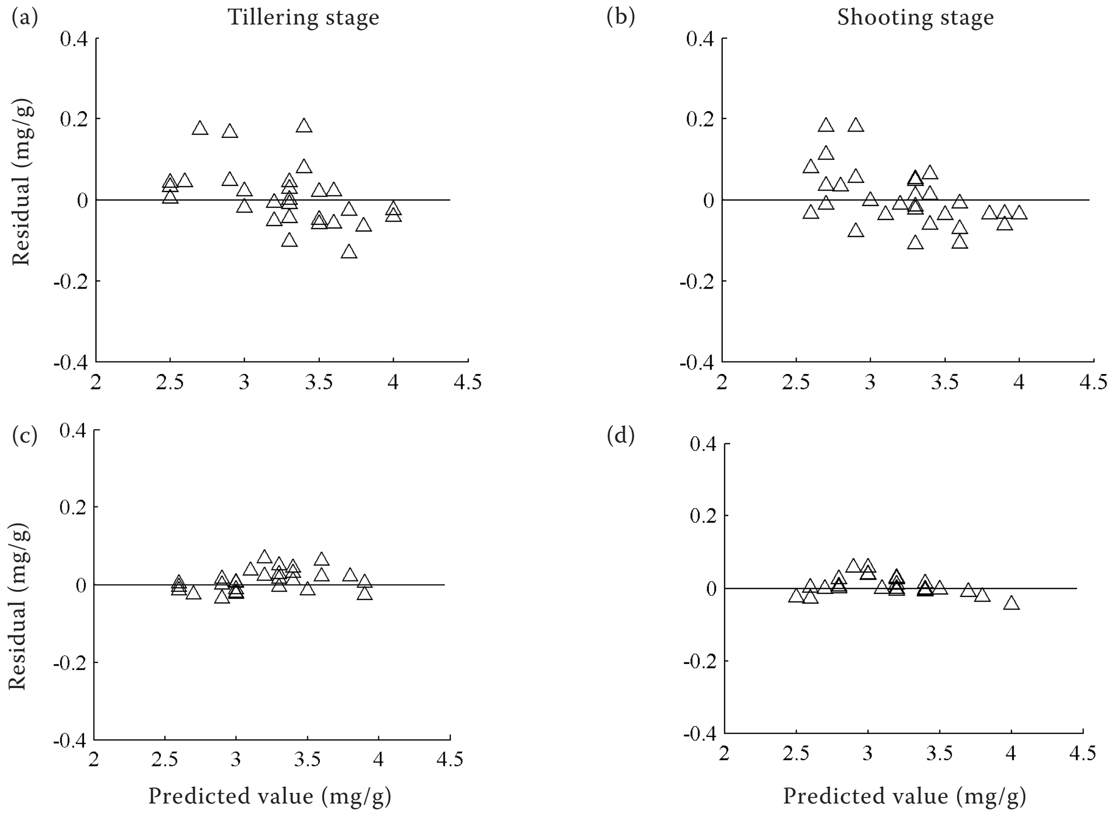

Figure 5. The residual of predictive paddy leaf nitrogen (N) content based on: (a, b) fluorescence ratios; (c, d) intensity of fluorescence peaks

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