doi: 10.17221/460/2017-PSE

Application of the chlorophyll fluorescence ratio in evaluation of paddy rice nitrogen status

Jian YANG[1,2,*], Lin DU[1], Wei GONG[2,3,*], Jia SUN[2], Shuo SHI[2,3], Biwu CHEN[2]

[1]Faculty of Information Engineering, China University of Geosciences, Wuhan, Hubei, P.R. China
[2]State Key Laboratory of Information Engineering in Surveying, Mapping and Remote Sensing, Wuhan University, Wuhan, Hubei, P.R. China
[3]Collaborative Innovation Centre of Geospatial Technology, Wuhan, Hubei, P.R. China
*Corresponding authors: wind_yang@whu.edu.cn; liesmars_lidar@foxmail.com

ABSTRACT

Yang J., Du L., Gong W., Sun J., Shi S., Chen B.W. (2017): Application of the chlorophyll fluorescence ratio in evaluation of paddy rice nitrogen status. Plant Soil Environ., 63: 396–401.

In this research, laser-induced fluorescence (LIF) technique combined with back-propagation neural network (BPNN) was employed to analyse different nitrogen (N) fertilization levels in paddy rice. Leaf fluorescence characteristics (FLCs) were measured by using the LIF system built in our laboratory and exhibited different FLCs with different nitrogen fertilization levels. The correlation between fluorescence intensity ratios (F685/F460, F735/F460 and F735/F685) and the dose of N fertilization was established and analysed. Then, the BPNN algorithm was utilized to validate that the different N fertilization levels can be classified based on the three FLCs. The overall identification accuracies of 2014 and 2015 were 90% and 92.5%, respectively. Experimental results demonstrated that the three FLCs with the help of multivariate analysis can be served as a helpful tool in the evaluation of paddy rice N fertilization levels. Besides, this study can also provide guidance for the selection of LIF Lidar channels in the following research.

Keywords: fluorescence characteristics; remote sensing; nutrient stress; *Oryza sativa*; machine learning

Paddy rice is one of the most important cereals and is cultivated worldwide (Huang et al. 2012). Currently, farmers tend to increase the dose of nitrogen (N) fertilization to improve paddy rice yield. Much N fertilizer is however wasted, resulting in severe environmental pollution. Passive remote sensing is the main technology for monitoring of N levels and has been studied in detail by investigators in the past decade (Wu et al. 2008, Tian et al. 2014). However, passive remote sensing cannot be operated all time. Therefore, active remote sensing was then proposed and have been widely utilized to monitor biochemical concentration of crops. The hyperspectral LiDAR was proposed by Gong et al. (2012). This active technology can measure both the spectral and spatial information of vegetation with high accuracy. Thus, it can be used to monitor the leaf biochemical content of crops by numerous researchers (Li et al. 2014).

In addition, Hoge and Swift (1981) found that chlorophyll in leaf can ray all or part of its absorbed energy at longer wavelengths after exposed to photons of a certain wavelength that was well known as fluorescence. Fluorescence technology has a potential for the pre-visual detection of nutrient stress (Pedrós et al. 2008). Then, Chappelle et al.

Supported by the Fundamental Research Funds for the Central Universities, China University of Geosciences (Wuhan), Grant No. CUG170661, and by the National Natural Science Foundation of China, Grant No. 41571370.

doi: 10.17221/460/2017-PSE

(1984b) investigated the remote monitoring of species differentiation and plant nutrient deficiency by using the laser-induced fluorescence (LIF) spectra of a green plant. They found that nutrient deficiency can affect the LIF spectra of corn and that different types of plant displayed different fluorescence spectral shapes (Chappelle et al. 1984a, 1985). Lichtenthaler and Buschmann (1987) analysed the fluorescence spectra of green leaves and pointed that red and far-red fluorescence peaks were closely related to the chlorophyll content in leaf.

Many researches were conducted on the effect of leaf N concentration on fluorescence spectra. They utilized the active LIF combined with passive reflectance measurements to analyse different N fertilization rates in field corn (McMurtrey et al. 1994, Yang et al. 2017). They found that leaf chlorophyll concentration was increased with increasing N fertilization rates, resulting in the change of the fluorescence spectra. Subhash and Mohanan (1994) analysed chlorophyll fluorescence characteristics (FLCs) that can be used as nutrient stress indicator in *Oryza sativa*, and suggested that far-red fluorescence peak had a great potential for remote sensing stress effects in plants. The differences in LIF characteristics of leaves between non-stressed and stressed plants were discussed in detail. Their experimental results showed that the fluorescence ratio between the red and far-red fluorescence peak was a sensitive and suitable stress indicator (Schweiger et al. 1996). In subsequent investigations, LIF technology was utilized to monitor the environment's (e.g., light stress, high temperature and drought (Živčák et al. 2008) influence on soybean, wheat and rice. In addition, Yang et al. (2015) analysed the correlation between fluorescence peaks and leaf N content in paddy rice. Thus, LIF is a helpful method in monitoring of the nutrient stress of plants. However, those studies focused only on the effect of leaf nutrient content on the FLCs in crops. The investigations of the correlation between the different N fertilization levels and FLCs, as well as the evaluation of different N fertilization levels using LIF technology combined with multivariate analysis have still been sparse.

Thus, the main objective of this research is to quantitatively analyse the correlation between different N fertilization levels and FLCs (F735, F685, F460 denote the fluorescence intensity at 735, 685 and 460 nm, respectively), as well as to evaluate different N fertilization levels in two-year experiments and with different rice cultivars based on the FLCs with the help of back-propagation neutral network (BPNN) in paddy rice.

MATERIAL AND METHODS

Treatments. The experiment was conducted in Wuhan, Hubei province, China. The experimental area has a typical subtropical monsoon climate with abundant rainfall; the area is sunny and hot during summer and cold during winter. Rainfall per year exceeds 1400 mm and sunshine duration per year surpasses 1500 h.

Yongyou 4949 of japonica and Yangliangyou 6 cultivar indica were planted on 27 April, 2014 and 27 May, 2015, respectively. During the entire growth period, six and four different doses of N fertilization of urea were utilized in 2014 and 2015, respectively (Table 1). The most optimal doses of N fertilization (urea) were 270 kg/ha and 180 kg/ha for 2014 and 2015 experimental areas according to the advice of the local farm extension service. N fertilization was divided into four splits: 30% at seeding, 20% at tillering, 25% at shooting and 25% at booting for 2014. N fertilization was divided into three splits: 60% at seeding, 20% at tillering and 20% at shooting for 2015. The experimental field has a block design with three replications per treatment with the same cultivation conditions. The leaves of paddy rice were gathered on 15 July, 2014 and 26 July, 2015.

Table 1. Field paddy rice dose of nitrogen (N) fertilization (urea) for each treatment in 2014 and 2015

	2014						2015			
	L0	L1	L2	L3	L4	L5	T0	T1	T2	T3
N fertilization (kg/ha)	0	189	229.5	270	310.5	351	0	120	180	240

L0 – no nitrogen; L1 – 189, L2 – 229.5, L3 – 270, L4 – 310.5, L5 – 351 kg N/ha; T0 – no nitrogen; T1 – 120, T2 – 180, T3 – 240 N/ha

doi: 10.17221/460/2017-PSE

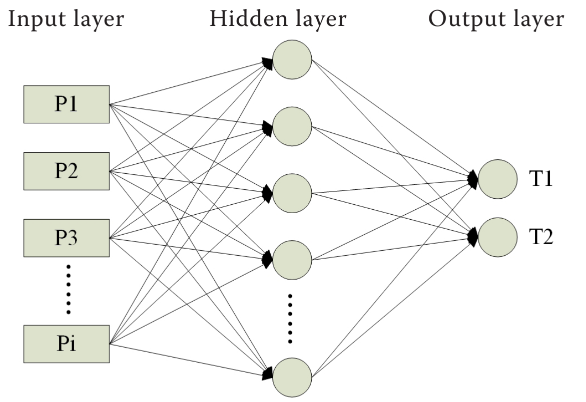

Figure 1. Scheme of a simple three-layer back-propagation neural network. Pi – input variables; Ti – target outputs

LIF system. The LIF measurement system consists of three main parts. The excitation source is 420 nm. Its output power is 1.6 mJ and the duration of pulse is 5 ns. The excited fluorescence signal was gathered by a telescope and then transmitted into a single-mode optical fiber with a diameter of 200 μm. The fluorescence signal entered the spectrometer and was recorded by an ICCD camera. A personal computer was used to store and process these spectral data. In addition, a 405 nm long-pass filter (LOPF-25C-405 with the edge of 421.5 nm) was positioned behind the telescope to eliminate reflected light from the laser.

Back propagation neural network. BPNN creates the relation between its units through a series of trials, with respect to multiple tasks, and is trained by repeatedly giving a series of input and output pattern sets to the neural network. When the mean squared error of the training set gets a minimum, the weights are fixed and the model is considered complete. A simple structure diagram of three-layer BPNN is shown in Figure 1. This simple network architecture included three parts: one output layer, one hidden layer and one input layer.

RESULTS AND DISCUSSIONS

N fertilization levels and fluorescence ratio. For each experimental field, the samples of paddy rice leaves were destructively sampled by randomly collecting six leaves at three different sites. All samples were the second fully expanded leaves from the top. These samples were sealed in freezer bags, kept in an ice tank and immediately transported to the laboratory for FLCs measurements (Yang et al. 2016). After that, all samples were immediately sent to the Wuhan Academy of Agricultural Science and Technology for measurement of leaf N content. The Kjeldahl method was utilized to determine the leaf total N content of paddy rice in this investigation (Yao et al. 2010). The mean values of leaf N content of each N fertilization level are shown in Figure 2; the black error bar denotes the standard deviation.

The leaf total N content increased with the increase of the dose of N fertilization in 2014 (Figure 2a) and 2015 (Figure 2b). The differences between no nitrogen (L0 and T0) and other different treatments in leaf N content were more

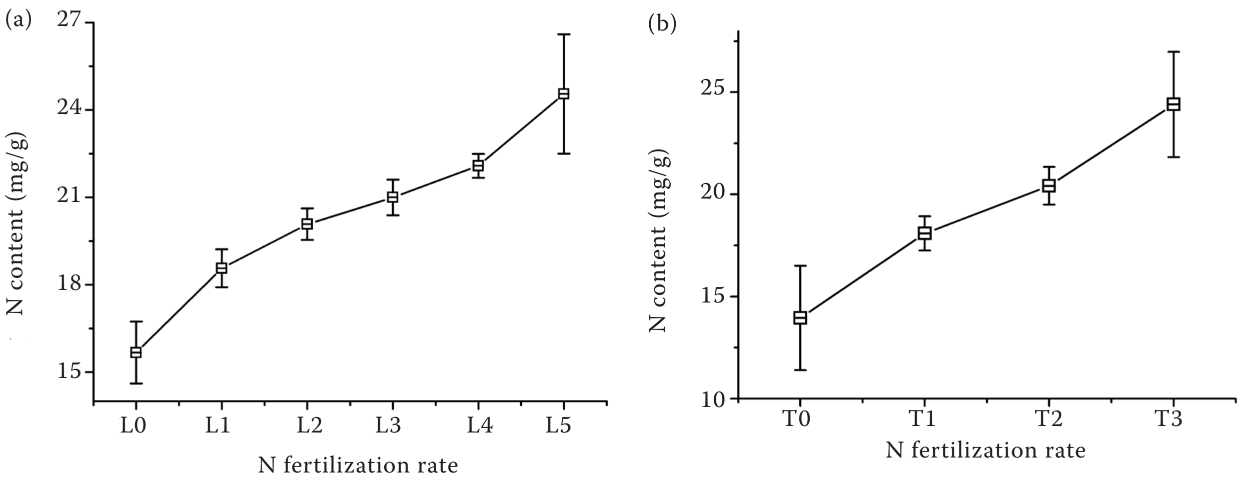

Figure 2. The nitrogen (N) content of rice leaf with the different N fertilization levels at different years: (a) 2014; (b) 2015. The black error bar represents the standard deviation of mean values. L0 – no nitrogen; L1 – 189, L2 – 229.5, L3 – 270, L4 – 310.5, L5 – 351 kg N/ha; T0 – no nitrogen; T1 – 120, T2 – 180, T3 – 240 N/ha

doi: 10.17221/460/2017-PSE

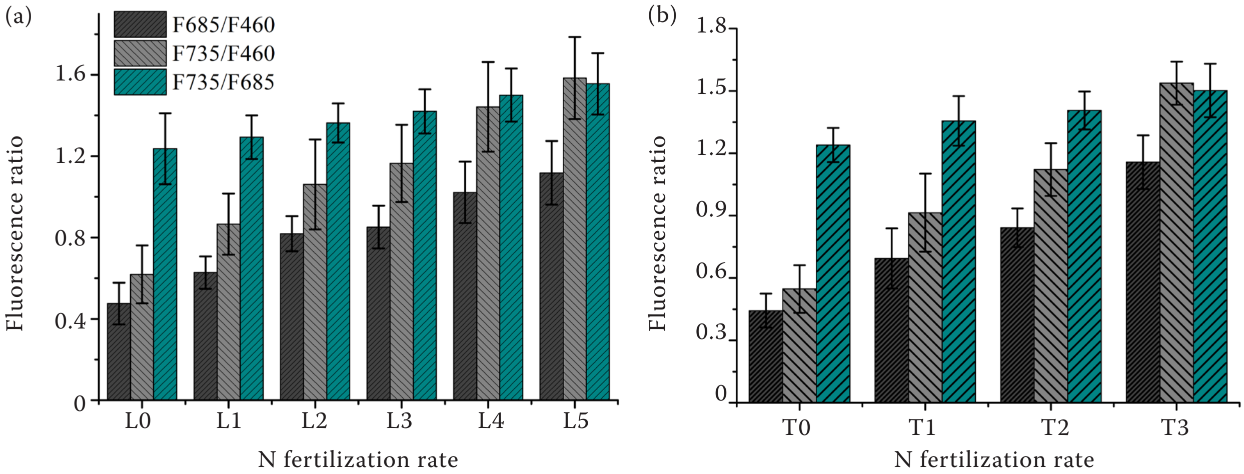

Figure 3. Mean values of normalized fluorescence intensity changing with different doses of nitrogen (N) fertilizer at different years: (a) 2014; (b) 2015. The black error bar denotes the standard deviation of the Leaf fluorescence characteristics. L0 – no nitrogen; L1 – 189, L2 – 229.5, L3 – 270, L4 – 310.5, L5 – 351 kg N/ha; T0 – no nitrogen; T1 – 120, T2 – 180, T3 – 240 N/ha

obvious compared with the differences among different N fertilization levels. In addition, Figure 2 showed a positive correlation between different N fertilization levels and leaf total N content. Based on previous researches (Hák et al. 1990), FLCs (F735/F460, F685/F460, and F735/F685) of samples of different N fertilization levels, which can be used to evaluate N status, were then measured by using the LIF system. The correlation between the mean values of these FLCs and the different N fertilization levels was achieved and shown in Figure 3. The black error bar denotes the standard deviation of mean values of the FLCs.

Figure 3 shows the changes of FLCs with N fertilization rate. The experimental results denoted that the FLCs increased with the increase of N fertilization rate. It can be found that the values of the FLCs (F735/F460, F685/F460) of the zero N fertilization levels (L0 and T0) were lower than other N fertilization levels. The reason is that its N content was lower than other treatments (Figure 1). In addition, compared with F735/F685, the fluorescence ratio of F735/F460 and F685/F460 both exhibited significant differences among different N fertilization levels in 2014 and 2015. McMurtrey et al. (1994) and Subhash and Mohanan (1994) achieved the same variation tendency of fluorescence characteristics for different dose of N fertilization. Therefore, these fluorescence ratios can be utilized to assess different N status in the further study.

Identification of N status. In this study, BPNN algorithm was used to verify the potential of the three FLCs for the identification of different doses of N fertilization. A simple three-layer BPNN was utilized. The input, hidden and output layers included three, five and one neuron, respectively. The Levenberg-Marquardt algorithm was used as a training function. About 63% of the fluorescence data were used to train BPNN model and the remaining 37% were applied to validate the model. The results of testing are listed in the confusion matrix for 2014 and 2015 (Tables 2 and 3).

The control treatment (L0 and T0) can be completely identified (the identification rate can reach up to 100%) for producers and users, and the over-

Table 2. The confusion matrix of identification accuracy of the nitrogen (N) fertilization levels for 2014

		Predicted						
		L0	L1	L2	L3	L4	L5	producer (%)
True	L0	20	0	0	0	0	0	100
	L1	0	18	1	1	0	0	90
	L2	0	2	16	1	1	0	80
	L3	0	1	1	17	1	0	85
	L4	0	0	0	1	18	1	90
	L5	0	0	0	0	1	19	95
	user (%)	100	85.71	88.89	85	85.71	95	
Overall accuracy: 90%								

L0 – no nitrogen; L1 – 189, L2 – 229.5, L3 – 270, L4 – 310.5, L5 – 351 kg N/ha

doi: 10.17221/460/2017-PSE

Table 3. The confusion matrix of identification accuracy of the nitrogen (N) fertilization levels for 2015

		Predicted				
		T0	T1	T2	T3	producer (%)
True	T0	20	0	0	0	100
	T1	0	18	1	1	90
	T2	0	1	17	2	85
	T3	0	0	1	19	95
	user (%)	100	94.75	89.47	86.36	
Overall accuracy: 92.5%						

T0 – no nitrogen; T1 – 120, T2 – 180, T3–240 N/ha

fertilization or low-fertilization can be also well identified. Experimental and numerical results demonstrated that the total identification rates of 2014 (n = 120) and 2015 (n = 80) were 90% and 92.5%, respectively. Thus, fluorescence ratios (F685/F460, F735/F460, and F735/F685) with the help of BPNN can be effectively applied in the evaluation of N status and provide farmers with the direction of the decision-making of N fertilization strategies and guide them to rectify fast the lack of N fertilization. In addition, this study can also provide guidance for the selection of fluorescence Lidar channel in the following research.

In this study, the potential of the three FLCs combined with BPNN for the evaluation of different N fertilization levels was demonstrated. However, more investigations on the effect of different doses of N fertilization on FLCs are still needed to be further conducted. For the BPNN, the optimal network architecture and training approaches have not been discussed and established. The results obtained using the BPNN method were trained on the basis of our experiences in analysing restricted data combinations. Ideally, the best results should be compared by changing the numbers of hidden neurons and network architectures. Thus, further studies should be conducted on the effectiveness of the BPNN for identifying the dose of N fertilization-based LIF technology. Moreover, although different N treatments were set, different paddy rice growth seasons and other crops should be considered to obtain a more solid conclusion in future studies.

In summary, LIF combined with BPNN exhibited the potential for evaluating N fertilization levels in paddy rice. The numerical results demonstrated that the leaf total N content of paddy rice increased along with the dose of N fertilization, resulting in different FLCs. The mean values of the fluorescence ratios (F685/F460, F735/F460 and F735/F685) of different N fertilization levels displayed significant differences. The correlation between the fluorescence ratio and different doses of N fertilization was established for 2014 and 2015 fluorescence data. Then, BPNN was used to verify the ability of fluorescence ratio for identifying different leaf N status, and experimental results demonstrated that the overall identification accuracies of 2014 and 2015 were 90% and 92.5%, respectively. Therefore, LIF with the help of multivariate analysis can be used as a helpful tool for the estimation of N status and offer direction for the decision-making of farmers on N fertilizer strategies in the future. In addition, this research can also provide guidance for the selection of LIF Lidar channels in the following research. However, in order to obtain a solid conclusion, more investigations are still needed to be conducted on the effect of different N status on FLCs by using other cultivars of crops.

REFERENCES

Chappelle E.W., McMurtrey J.E., Wood F.M., Newcomb W.W. (1984a): Laser-induced fluorescence of green plants. 2: LIF caused by nutrient deficiencies in corn. Applied Optics, 23: 139–142.

Chappelle E.W., Wood F.M., McMurtrey J.E., Newcomb W.W. (1984b): Laser-induced fluorescence of green plants. 1: A technique for the remote detection of plant stress and species differentiation. Applied Optics, 23: 134–138.

Chappelle E.W., Wood F.M.Jr., Newcomb W.W., McMurtrey J.E. (1985): Laser-induced fluorescence of green plants. 3: LIF spectral signatures of five major plant types. Applied Optics, 24: 74–80.

Gong W., Song S.L., Zhu B., Shi S., Li F.Q., Cheng X.W. (2012): Multi-wavelength canopy LiDAR for remote sensing of vegetation: Design and system performance. ISPRS Journal of Photogrammetry and Remote Sensing, 69: 1–9.

Hák R., Lichtenthaler H.K., Rinderle U. (1990): Decrease of the chlorophyll fluorescence ratio F690/F730 during greening and development of leaves. Radiation and Environmental Biophysics, 29: 329–336.

Hoge F.E., Swift R.N. (1981): Airborne simultaneous spectroscopic detection of laser-induced water Raman backscatter and fluorescence from chlorophyll *a* and other naturally occurring pigments. Applied Optics, 20: 3197–3205.

Huang X.H., Kurata N., Wei X.H., Wang Z.-X., Wang A., Zhao Q., Zhao Y., Liu K.Y., Lu H.Y., Li W.J., Guo Y.L., Lu Y.Q., Zhou

doi: 10.17221/460/2017-PSE

C.C., Fan D.L., Weng Q.J., Zhu C.R., Huang T., Zhang L., Wang Y.C., Feng L., Furuumi H., Kubo T., Miyabayashi T., Yuan X.P., Xu Q., Dong G.J., Zhan Q.L., Li C.Y., Fujiyama A., Toyoda A., Lu T.T., Feng Q., Qian Q., Li J.Y., Han B. (2012): A map of rice genome variation reveals the origin of cultivated rice. Nature, 490: 497–501.

Li W., Sun G., Niu Z., Gao S., Qiao H.L. (2014): Estimation of leaf biochemical content using a novel hyperspectral full-waveform LiDAR system. Remote Sensing Letters, 5: 693–702.

Lichtenthaler H.K., Buschmann C. (1987): Chlorophyll fluorescence spectra of green bean leaves. Journal of Plant Physiology, 129: 137–147.

McMurtrey J.E., Chappelle E.W., Kim M.S., Meisinger J.J., Corp L.A. (1994): Distinguishing nitrogen fertilization levels in field corn (*Zea mays* L.) with actively induced fluorescence and passive reflectance measurements. Remote Sensing of Environment, 47: 36–44.

Pedrós R., Moya I., Goulas Y., Jacquemoud S. (2008): Chlorophyll fluorescence emission spectrum inside a leaf. Photochemical and Photobiological Sciences, 7: 498–502.

Schweiger J., Lang M., Lichtenthaler H.K. (1996): Differences in fluorescence excitation spectra of leaves between stressed and non-stressed plants. Journal of Plant Physiology, 148: 536–547.

Subhash N., Mohanan C.N. (1994): Laser-induced red chlorophyll fluorescence signatures as nutrient stress indicator in rice plants. Remote Sensing of Environment, 47: 45–50.

Tian Y.-C., Gu K.-J., Chu X., Yao X., Cao W.-X., Zhu Y. (2014): Comparison of different hyperspectral vegetation indices for canopy leaf nitrogen concentration estimation in rice. Plant and Soil, 376: 193–209.

Wu C.Y., Niu Z., Tang Q., Huang W.J. (2008): Estimating chlorophyll content from hyperspectral vegetation indices: Modeling and validation. Agricultural and Forest Meteorology, 148: 1230–1241.

Yang J., Shi S., Gong W., Du L., Ma Y.Y., Zhu B., Song S.L. (2015): Application of fluorescence spectrum to precisely inverse paddy rice nitrogen content. Plant, Soil and Environment, 61: 182–188.

Yang J., Du L., Sun J., Zhang Z., Chen B., Shi S., Gong W., Song S. (2016): Estimating the leaf nitrogen content of paddy rice by using the combined reflectance and laser-induced fluorescence spectra. Optics Express, 24: 19354–19365.

Yang J., Sun J., Du L., Chen B., Zhang Z., Shi S., Gong W. (2017): Effect of fluorescence characteristics and different algorithms on the estimation of leaf nitrogen content based on laser-induced fluorescence lidar in paddy rice. Optics Express, 25: 3743–3755.

Yao X., Zhu Y., Tian Y.C., Feng W., Cao W.X. (2010): Exploring hyperspectral bands and estimation indices for leaf nitrogen accumulation in wheat. International Journal of Applied Earth Observation and Geoinformation, 12: 89–100.

Živčák M., Brestič M., Olšovská K. (2008): Assessment of physiological parameters useful in screening for tolerance to soil drought in winter wheat (*Triticum aestivum* L.) genotypes. Cereal Research Communications, 36: 1943–1946.

Received on July 27, 2017
Accepted on September 13, 2017
Published online on September 18, 2017