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Dehua Zhao, Jianlong Li, Jiaguo Qi

Agronomie 24 (2004) 463–471

DOI: 10.1051/agro:2004042

Original article

Hyperspectral characteristic analysis of a developing cotton canopy under different nitrogen treatments

Dehua ZHAO[a], Jianlong LI[a]*, Jiaguo QI[b]

[a] Department of Biological Science and Technology, Nanjing University, Nanjing 210093, China
[b] Center for Global Change and Earth Observations, Michigan State University, East Lansing, MI 48823, USA

(Received 21 August 2003; accepted 18 May 2004)

Abstract – The objective of this study was to analyze the differences of crop agronomy parameters, canopy spectral reflectance and spectral indices induced by different nitrogen application rates, and to assess the potential of discriminant analysis in discriminating cotton canopies at different nitrogen treatments. We conducted an experiment in cotton fields treated with four nitrogen application rates: 0%, 50%, 100% and 200% of the recommended rate. Results suggested that no single spectral index or canopy variable can fully characterize the canopies nitrogen status throughout the growing period. With a single vegetation index, discriminant analysis provided less than 45%, 48.8%, 61.6% accurate classifications of cotton canopies by nitrogen treatment during early, mid and late season, respectively. Nevertheless, using multi-vegetation-index model by stepwise procedure, 74.4%, 83.1% and 89.6% accuracies could be obtained during early, middle and late season, respectively.

cotton / hyperspectral remote sensing / spectral indices / nitrogen application rates / leaf area index / chlorophyll

1. INTRODUCTION

Today, various terms such as precision agriculture, precision farming and site-specific farming have been used in the remote sensing community. These are inter-changeable terms designed to help farmers apply variable levels of inputs to crop fields based on crop's production requirements. Sensors mounted on farm machines not only measure fertilizer input, but also they measure crop yields and their spatial distributions, making precision farming possible at very high spatial intervals. Farmers can apply different fertilizer rates to match the specific field requirements. However, the operational precision farming has been hampered by a lack of timely distributed information of crop and soil conditions, which can be compensated by remote sensing technology. Many studies have demonstrated the potential of remote sensing in precision agriculture that includes forecasting yields and productions, monitoring crop growing status and managing agricultural practices [33, 36, 44].

Nitrogen is one of the most important fertilizer elements for crop production. Research in precision farming has been more and more focused on nitrogen application rates during growing stages for high yield and quality, and for environmental pollution control [29, 38, 42]. Under normal conditions, nitrogen fertilizer influences nitrogen concentration and color in green leaf, which are related to photosynthetic pigment contents and photosynthesis rates. To monitor nitrogen status using remote sensing technology, previous studies primarily focused on how to estimate pigment or nitrogen contents in green vegetation at both leaf and canopy levels using a number of spectral indices [10, 18, 19, 23, 32, 41]. However, these previous studies had two limitations. The first is that nitrogen or pigment content does not always reflect canopy nitrogen status and the second is that nitrogen fertilizer affects not only canopy nitrogen and pigment content but also other biophysical and biochemical variables such as leaf area index and biomass. Spectral reflectance is the collective responses of these crop variables and ecological environmental properties [39].

The overall objective of this research was to analyze hyperspectral remote sensing capability to detect characteristic differences of cotton canopy under different nitrogen application rates and different growing stages. In order to address this overall objective, we must address the following three specific subobjectives: (1) to identify most representative agronomic cotton parameters capable of depicting characteristic difference under different nitrogen treatments, (2) to identify the optimal spectral wavelengths and appropriate vegetation indices charactering the difference of canopies induced by different nitrogen application rates, and (3) to test canonical discriminant analysis of detecting cotton nitrogen status and deficiencies under different nitrogen treatments.

* Corresponding author: jianlongli@hotmail.com

2. MATERIALS AND METHODS

2.1. Experimental design and treatments

An experiment was conducted in cotton fields located at Zhangjiagang, Jiangsu province, China (31° 50' N, 120º 49' E). The cotton cultivar was the local popular cotton (*Gossypium hirsutum* L.) cv. Sumian 3. Treatments included four nitrogen application rates: 0%, 50%, 100% and 200% of the recommended rate of 180 kg N ha^{-1} (termed N0, N90, N180 and N360, respectively). A completely randomized experiment was designed with four replicates. Each sample plot was two 1.2-m-big-small-rows (0.8 m for the big row and 0.4 m for the small row) wide and 14 m long ($2.4 \times 14 = 33.6\ m^2$), with a density of 45 000 plants/ha. Cotton was sown on 12 April in greenhouses and later transplanted on 28 May to fields of north/south row orientation. Phosphorus and potassium was supplied in adequate amounts according to the general nutrient status of the field as determined by soil samples: 180 kg ha^{-1} P_2O_5 and 240 kg ha^{-1} K_2O, respectively. Irrigation was not used due to the high rainfall (above 1200 mm) and high ground water table of the soil at the study site.

According to canopy structures and leaf functions of cotton plants, the whole cotton growing cycle was divided into three observing stages: (1) rapid growth period (early stage when the soil was partially covered by cotton and, therefore, its contribution to spectral signals was significant), (2) full green coverage period (middle stage when the canopy reached almost 100% cover), and (3) senescent period (late stage when cotton batting appeared and leaf became senescent). These growing stages were represented by the dates of July 15, August 14 and October 1, 2002, respectively, when agronomic and hyperspectral reflectance measurements were made in the experiment.

2.2. Hyperspectral reflectance measurements

A 512-channel spectroradiometer (from 300 nm to 1100 nm) by Analytical Spectral Devices™ (FieldSpec® FR) was used to acquire cotton canopy spectral data. The sensor's field of view was 15°. Due to noises in the both ends of the spectrum, only the data in the 400 nm to 1000 nm range was used in the hyperspectral characteristic analysis. Data were collected on cloudless days at solar elevation angles ranging between 50° and 55°, in order to minimize external effects from the atmosphere and changes in solar elevation. The radiometer was held at nadir orientation at 2.3 m above the canopy resulting in a footprint of 60 cm in diameter. Spectral reflectance was calculated as the ratio of measured radiance to radiance from a white standard reference panel. Immediately after the white standard radiance measurement, two spectra of cotton canopy were obtained – one made with the sensor located directly over the center of two rows on a ridge, the other one with the sensor located directly over the furrow. The two spectra were then averaged to represent a single mean field spectrum of a ridge (60 cm × 2 = 120 cm). Over each plot, this procedure was repeated ten times.

2.3. Agronomic variable measurements

On the same days when hyperspectral data were taken, six plants, which were believed to be sufficient to represent the canopy attributes at plot level, were harvested to determine the cotton agronomic characteristics. Every plant was separated into several parts and weighted for wet leaf and all aboveground biomass (g/m^2) calculations. Green leaf areas of two plants were measured with a leaf area meter to estimate specific leaf weights, and this variable was used to estimate plant leaf areas from leaf biomass values. Leaf area index of cotton was computed as the ratio of green leaf area per sampled area. A representative sample of leaf and stem was taken, the weight determined, then dried at 80 for at least 48 hours. Based on these measurements, aboveground dry biomass was determined. For chlorophyll content measurement, the leaf samples (0.15 g from the each plant taken according to its leaf weight) were grounded in 3 ml cold acetone/ Tris buffer solution (80:20Vol/Vol, pH = 7.8), centrifuged to remove particulates, and the supernatant diluted to a final volume of 15 ml with additional acetone/Tris buffer [24, 15]. The absorbance of the extract solutions was measured with the U-3000 spectrophotometer at 663 nm, 647 nm and 537 nm (Hitachi, Japan), and the equations derived were:

$$Chl_a = 0.01373\,A_{663} - 0.000897\,A_{537} - 0.003046\,A_{647} \quad (1)$$

$$Chl_b = 0.02405\,A_{647} - 0.004305\,A_{537} - 0.005507\,A_{663} \quad (2)$$

where A_x is the absorbance of the extract solution in a 1-cm path length cuvette at wavelength x. The units for all the equations are micromoles per milliliter (µmol ml^{-1}). The method used here is the same by Sims and Gamon [37]. Canopy chlorophyll density (g/m^2) was the chlorophyll content multiplied by leaf weights. Yield (seed cotton yield, kg/hm^2) was determined by hand harvesting 30 plants in each plot.

2.4. Data process and analysis

To enhance their sensitivities to green vegetation spectral signals and to reduce external effects such as those noises related to soil and atmospheric influences, many vegetation indices have been developed in past three decades. These vegetation indices can be divided into four groups as follows [27]: (1) Ratio-based vegetation indices which are calculated based on the ratio between red and near infrared reflectance. The normalized difference vegetation index (NDVI) [35] and the ratio vegetation index (RVI) [30] are the well-known and most commonly used ratio-based vegetation indices. (2) Soil-line based vegetation indices which are based on there being a line in spectral space along which bare soils will line up. The modified second soil-adjusted vegetation index (MSAVI2) is the example of this type vegetation index [34]. (3) Derivative vegetation indices, which was firstly introduced by Demetriades-Shaw [11] as the first and second-order derivative green vegetation indices. (4) Atmospheric corrected indices, such as the visible atmospherically resistant index (VARI) [16]. These vegetation indices had been shown to be well correlated with canopy parameters such as the leaf area index, aboveground biomass, leaf chlorophyll content, vegetation fractional cover. In this study, twenty four vegetation indices were selected (Tab. I) as a representative of each category.

Because we have three separate specific sub-objectives in this study, we adapted three different analysis methods. To address first sub-objective, which is to identify cotton parameters

Table I. Spectral vegetation indices, formulations and references.

Indices	[b]Defined formulations and meanings	References
R_{680}	Reflectance at 680 nm	–
R_{800}	Reflectance at 800 nm	–
[a] D_{600}	Absorbed depth at 600 nm	–
[a] D_{720}	Absorbed depth at 720 nm	–
RVI	R_{680} / R_{800}	Pearson et al. 1972
NDVI	$(R_{800} - R_{680}) / (R_{800} + R_{680})$	Rouse et al. 1974
RE	Wavelength of red edge	Demetriades-Shah et al.1990
dRE	Value of the first derivative at the red edge	Demetriades-Shah et al. 1990
ddRE	Value of the second derivative at the red edge	Demetriades-Shah et al. 1990
PRI	$(R_{570} - R_{531}) / (R_{570} + R_{531})$	Gamon et al. 1992
MSAVI2	$\frac{1}{2}\left[(2R_{800}+1)-\sqrt{(2R_{800}+1)^2-8(R_{800}-R_{680})}\right]$	Qi et al. 1994
C_{420}	R_{420} / R_{695}	Carter, 1994
SIPI	$(R_{800} - R_{445}) / (R_{800} - R_{680})$	Peñuelas et al. 1995
AI	$\sum_{i=600}^{699} R_i \Big/ \sum_{i=500}^{599} R_I$	Gamon et al. 1999
WBI	R_{900} / R_{970}	Peñuelas et al. 1997
fWBI	$R_{900} / \min(R_{930\text{-}980})$	Peñuelas et al. 1997
PSRI	$(R_{680} - R_{500}) / R_{750}$	Merzlyak et al. 1999
GNDVI	$(R_{800} - R_{550})/(R_{800} + R_{550})$	Daughtry et al. 2000
OSAVI	$(1+0.16)\times(R_{800} - R_{670})/(R_{800} + R_{670} + 0.16)$	Daughtry et al. 2000
MCARI	$[(R_{700} - R_{670}) - 0.2(R_{700} - R_{550})\times(R_{700} / R_{670})]$	Daughtry et al. 2000
TCARI / OSAVI	$\frac{3[(R_{700} - R_{670}) - 0.2(R_{700} - R_{550})(R_{700} / R_{670})]}{(1+0.16)\times(R_{800} - R_{670})/(R_{800} + R_{670} + 0.16)}$	Haboudane et al. 2002
VARI	$(R_{555} - R_{680}) / (R_{555} + R_{680} - R_{480})$	Gitelson et al. 2002
mND_{705}	$(R_{750} - R_{705}) / (r_{750} + R_{705} - 2R_{445})$	Sims and Gamon, 2002
mSR_{705}	$(R_{750} - R_{445}) / (R_{705} - R_{445})$	Sims and Gamon, 2002

[a] Absorbed depths at 600 nm and 720 nm from the continuum-removed spectra of the red absorption feature ($R_{550–750\ nm}$) in the visible region.
[b] R_x is the reflectance at the wavelength of x nm.

capable of depicting difference among various nitrogen treatments, multiple comparison analysis was used. To address the second sub-objective, which is to identify the optimal spectral wavelengths and appropriate vegetation indices charactering the difference of canopies induced by different nitrogen application rates, one-way analysis of variance technique was used. To address the final sub-objective, which is to test the capability of combination vegetation indices for separating nitrogen treatments, canonical discriminant analysis was employed. The data were analyzed using statistical tool of the Statistical Product and Service Solutions (SPSS 11.0) and data was analyzed using these techniques as used by others [10, 15, 28]. There, multiple comparison analysis was used to test whether the differences in cotton canopy parameters were significant under different nitrogen treatments. One-way analysis of variance was used to show whether the means of reflectance at every wavelength and the 24 spectral indices were significantly different under different nitrogen treatments (see Tab. I). Canonical discriminant analysis was used to determine which combinations of spectral reflectance indices could accurately differentiate samples by nitrogen application rate.

3. RESULTS

3.1. Crop parameter differences between treatments

The most important factors that effect canopy spectral reflectance in the 400 nm to 1000 nm region are canopy structure

Table II. Multiple comparisons of mean values of four cotton variables observed on July 15, August 14 and October 1 and seed cotton yield under different nitrogen treatments (at 95% confidence level).

Variables	Treatments	Means[a]			Seed cotton Yield (kg/hm^2)
		Jul. 14	Aug. 15	Oct. 1	
Leaf area index (m^2/m^2)	N0	0.90 a	2.23 a	1.34 a	
	N90	0.97 ab	2.75 b	1.92 b	
	N180	1.06 bc	3.12 c	2.37 c	
	N360	1.12 c	3.25 c	2.33 c	
Chlorophyll content (%)	N0	1.25 a	0.85 a	0.53 a	
	N90	1.31 ab	1.23 b	1.07 b	
	N180	1.37ab	1.37 bc	1.26 c	
	N360	1.41 b	1.52 c	1.37 c	
Aboveground dry biomass (g/m^2)	N0	118.7 a	426.8 a	636.2 a	
	N90	130.2 ab	488.7 b	816.9 b	
	N180	139.4 bc	548.6 bc	911.7 c	
	N360	153.3 c	625.2 c	1032.3 d	
Canopy chlorophyll density (g/m^2)	N0	0.229 a	0.453 a	0.121 a	
	N90	0.275 b	0.863 b	0.464 b	
	N180	0.303 c	1.099 c	0.593 c	
	N360	0.332 d	1.259 d	0.633 c	
Seed cotton yield (kg/hm^2)	N0				2908.5 a
	N90				3914.9 b
	N180				4474.9 c
	N360				4592.3 c

[a] Means within columns followed by the same letter are not significantly different at 95% confidence level (Duncan's Multiple Range Test).

(generally expressed by many biophysical parameters such as leaf area index, biomass) and pigment status (such as chlorophyll, carotenoid and anthocyanins). In this study, leaf area index and aboveground dry biomass were used as the parameters to characterize canopy structure, leaf chlorophyll content were used to characterize vegetation pigment status, and canopy chlorophyll density was the combined variable characterizing both plant size and color properties. As expected, the nitrogen fertilizer treatments resulted in broad variations in the four crop variables. From Table II, on July 15, these agronomic variables increased with higher nitrogen fertilizer rates, and canopy structure variables (leaf area index and aboveground dry biomass) showed larger variations (24.4% and 29.2%) than canopy pigment variable (leaf chlorophyll content, 12.8%) among treatments of N0, N90, N180 kg N ha^{-1}. On August 14, the four variables also increased with nitrogen application rates. However, by contrary, the extent of variations in leaf chlorophyll content among different treatments (78.8%) was more than those of leaf area index and aboveground dry biomass (45.7% and 46.5%). On October 1, as superfluous nitrogen fertilizer easily resulted in quicker consenescence, leaf area index of N360 treatment was slightly lower than that of N180 treatment, but the other three variables continued to increase with nitrogen fertilizer rates. There were large differences in the four agronomic variables among different treatments with the biggest differences being 158.5%, 76.9% and 62.3% for leaf chlorophyll content, leaf area index and aboveground dry biomass, respectively. Results of multi-variable comparison analysis showed that only canopy chlorophyll density on July 15 and August 14 and aboveground dry biomass on October 1 showed significant difference between each two-treatments at 95% confidence level. The results also showed that the differences between nitrogen treatments became larger and larger with cotton growing. On July 15, canopy structure variable appeared to be a better indicator to separate the nitrogen treatments than canopy pigment variable. On August 14, the results were opposite. On October 1, the canopy variables between treatments existed very broad variations. No single crop canopy variable can fully characterize the status of the cotton canopy throughout the growing period. The canopy chlorophyll density variable containing both canopy structure and pigment information was a good indicator of crop nitrogen nutrient during all growth stages (Tab. II).

For cotton yield, nitrogen resulted in great increases in seed cotton yield. N90 treatment increased significantly compared with N0 treatment (34.6%, $p < 0.05$), and N180 treatment increased significantly compared with N90 treatment (14.3%, $p < 0.05$). But the difference between N180 and N360 treatments was not significant.

3.2. Reflectance differences between treatments

One-way analysis of variance was used to determine the wavelengths where differences of reflectance in the 450 individual

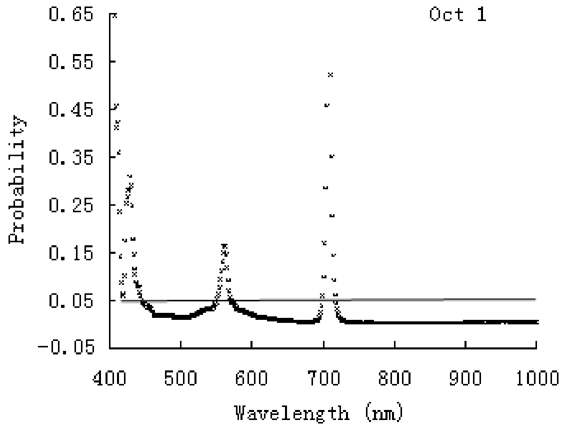

Figure 1. Results of one-way analysis of variance showing wavelengths where reflectance differences between the four nitrogen treatments (N0, N90, N180 and N360) for October 1.

narrow bands (400–1000 nm) between nitrogen treatments are significant. Because of the much significant differences in cotton canopies between N0 and other three treatments especially at late growth period (Tab. II), reflectance in most of the 450 individual narrow bands experienced significant difference among the four treatments on October 1 (Fig. 1). To select better bands sensitive to nitrogen status, only three treatments were used in this analysis (N90, N180 and N360). In general, our results showed that there was no significant difference under different nitrogen treatments in the visible region (380–700 nm), especially on August 14. However, there were a few channels that were significantly different between treatments on July 15 and October 1 at 90% confidence level. These bands are located at 540 nm and 680 nm. Nevertheless, for near-infrared region, there were always statistically significant differences between different treatments in all three growing periods (Fig. 2). These results are consistent with the results presented by Mutanga et al. [28].

3.3. Spectral index differences between treatments

The results in Figure 3 showed that the differences of the spectral indices were significant between nitrogen treatments (N0-N90, N90-N180 and N180-N360). On July 15, seven spectral indices (mSR705, mND705, SR680, D720, dRE, C420 and TCARI/OSAVI) showed significant differences between nitrogen treatments (N0-N90, N90-N180 and N180-N360) at 95% confidence level. On August 14, for the three combinations, eight spectral indices (mND705, mSR705, fWBI, ddRE, D720, AI, PRI and dRE) showed significant differences. On October 1, seventeen spectral indices performed significant differences between nitrogen treatments (mSR705, mND705, SR680, ND680, PSRI, ddRE, VARI, D720, D600, AI, R800, dRE, SIPI, MSAVI2, WBI, OSAVI and TCARI/OSAVI). As the cotton canopy developed, more spectral indices were able to discern the different between nitrogen treatments, because the effect of nitrogen on cotton canopy development became significant in the later growing season (Tab. II), and therefore, more and more indices experienced statistical differences between different nitrogen treatments. Four spectral indices (mSR705, mND705, D720 and dRE) always appeared to be able to discern nitrogen treatments at all growing stages (Fig. 3).

3.4. Canonical discriminant analysis of detecting nitrogen status

Firstly, canonical discriminant analysis was used to assess the power of each spectral index listed in Table I for discriminating canopies under different nitrogen application rates. The grouping variable in the model is the nitrogen application rate and independent variable is one of spectral indices alone (one-variable model). Then the accuracy rate values were determined: each of the spectral indices provided 30.6–45%, 28.8–48.8% and 28.8–61.6% accuracy classification of samples by nitrogen application rate during early, mid and late season conditions, respectively. This result indicates that only one spectral index alone is not enough to discern the nitrogen trends except by the end of the growing season where the indices of D720 and mND705 could provide more than 60% accuracy rates.

Secondly, canonical discriminant analysis was used to determine which combinations of spectral reflectance indices could

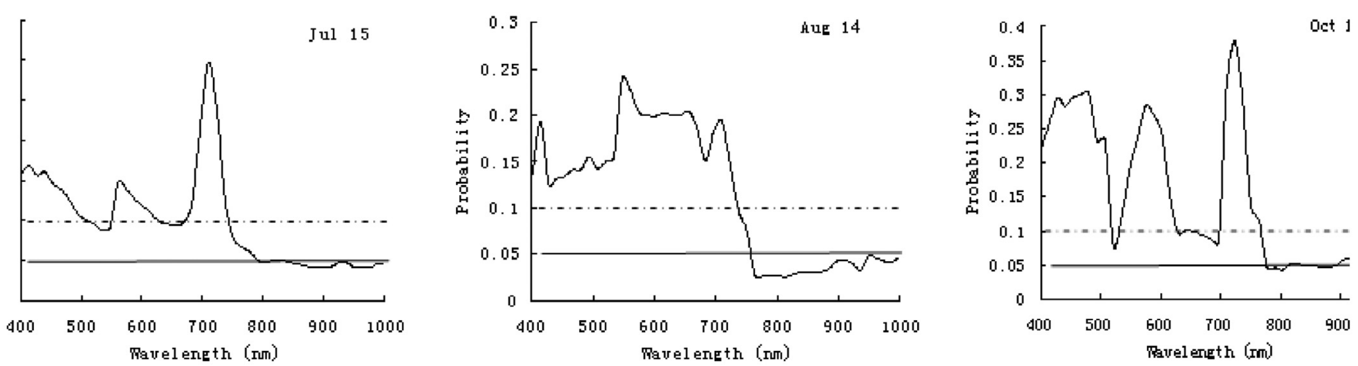

Figure 2. Results of one-way analysis of variance showing wavelengths where reflectance differences between the three nitrogen treatments (N90, N180 and N360) are significant observed on July 15, August 14 and October 1. Solid and dashed lines showed 95% and 90% confidence levels.

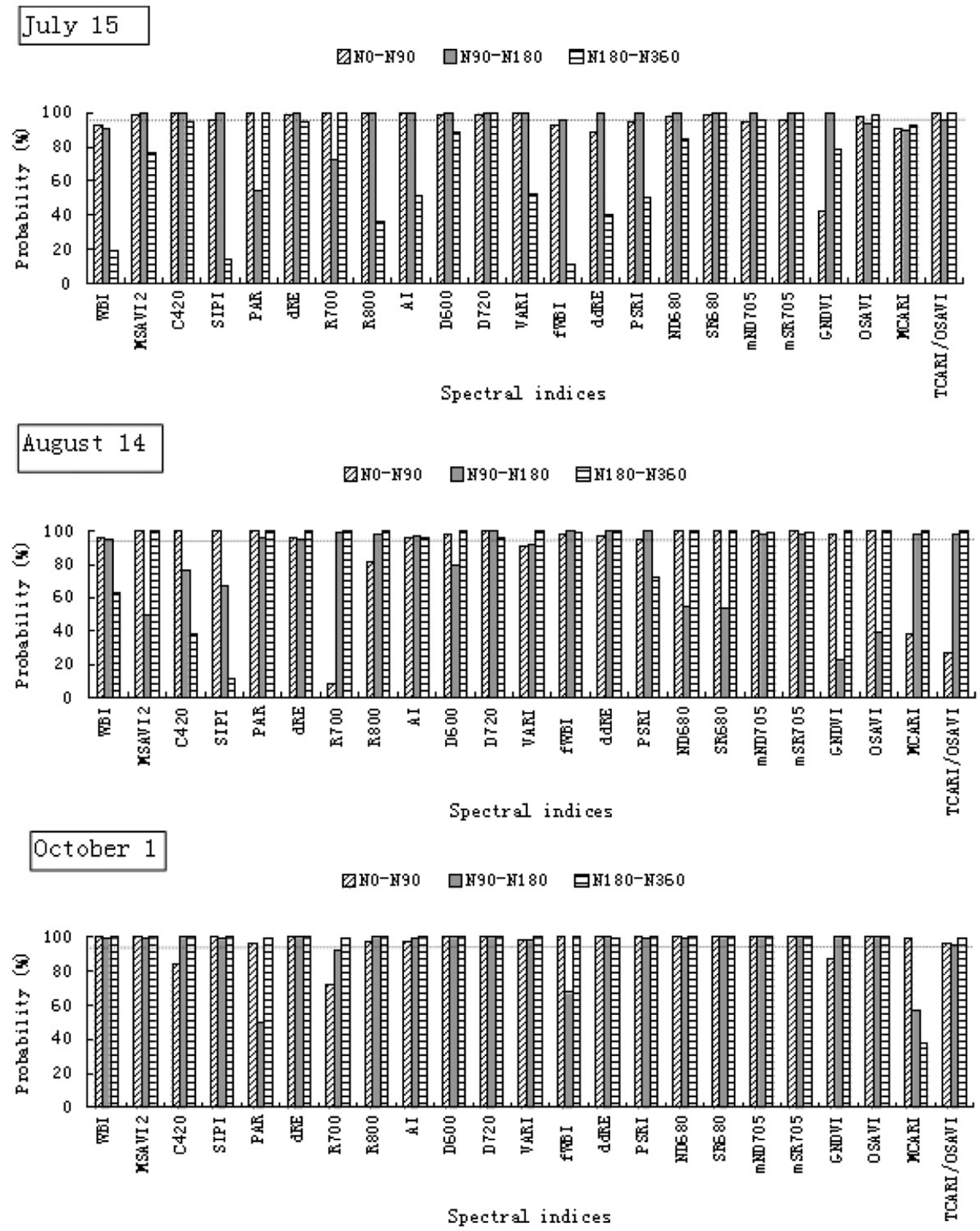

Figure 3. Results of one-way analysis of variance showing whether the differences of spectral indices between near two nitrogen treatments (N0 and N90, N90 and N180, N180 and N360) are significant observed on July 15, August 14 and October 1. Horizontal lines showed 95% confidence level.

correctly classify samples by nitrogen application rate (Tabs. III–IV). The grouping variable in the model is the nitrogen application rate and independent variables are the spectral indices listed in Table I. On July 15, D720, PRI, fWBI, mND705, OSAVI and MSAVI2 were selected and the results provided 74.4% classification accuracy of cotton canopies. For August 14 data, seven indices were retained in the model in the order of mND705, dRE, C420, MCARI, PRI, R800 and WBI, with classification accuracy of 83.1%. On October 1, five indices (D720, mND705, TCARI/OSAVI, PRI, fWBI) were retained, and the results provided 89.4% accuracy. Results also showed that more and more cotton canopies could be correctly classified by nitrogen application rate from early to late stages (accuracies were 74.4%, 83.1% and 89.4%, respectively). Only two indices (PRI and mND705) were always selected by stepwise procedure for the three growth periods. Cotton crops with extreme nitrogen application rates (N0 and N360) could be well discriminated.

Table III. Optimal spectral indices combinations of detecting cotton nitrogen status identified by canonical discriminant analysis.

Jul. 15	Aug. 14	Oct. 1
D_{720}	mND_{705}	D_{720}
PRI	dRE	mSR_{705}
fWBI	C_{420}	TCARI/OSAVI
mND_{705}	MCARI	PRI
OSAVI	PRI	fWBI
MSAVI2	R_{800}	
	WBI	

4. DISCUSSION AND CONCLUSIONS

It is known that nitrogen nutrition can influence many biophysical/biochemical variables of crop canopy, including the four variables used in this paper (Tab. II). In spite of the close relationships between the different variables under different nitrogen treatments, no single variable, including nitrogen content in crops, is able to fully describe the state of crops nitrogen status throughout the growing period. The leaf-level variable that is more directly related with nitrogen deficiency is chlorophyll, while other effects (canopy structure) are indirectly related with nitrogen concentration. The relationship between nitrogen and chlorophyll is positive and linear [45]. In good agreement with other results from the literature [39], our results suggested that cotton in higher nitrogen application rate always had higher chlorophyll content in leaf than their counterparts in lower nitrogen application rate throughout the season. But the leaf-level variable, chlorophyll content (%), did not always perform better than canopy structure variables such as leaf area index and aboveground dry biomass in describing canopies under different nitrogen rates throughout the season (Tab. II). In the early season, plants in higher nitrogen application rate had faster leaf expansion and larger biomass accumulation than those of lower nitrogen application rate. Indeed, the largest different of leaf area index and aboveground dry biomass between nitrogen treatments (24.4% and 29.2%) was even larger than that of chlorophyll content (12.8%). But this pattern changed during mid-season, and the chlorophyll content had larger variation than leaf area index or aboveground dry biomass between nitrogen treatments. The canopy-level variable of chlorophyll, canopy chlorophyll density, performed well in describing cotton canopies under different nitrogen treatments in all the three stages. This result can largely be explained by the fact that the canopy chlorophyll density contains both canopy-level (structure) and leaf-level (pigment) information of crops [43]. Moreover, the variable of canopy chlorophyll density also can be estimated more accurately by remotely sensed data than some other popular biophysical variables such as leaf area index, biomass and green crop area index [5, 25].

Spectral reflectance of cotton canopy in the 400–700 nm region is primarily governed by the abundance of chlorophyll and other pigments absorbing most of the incident radiation [6, 40]. In consistent with other results, plants reflect more in visible region due to lower chlorophyll concentration as induced by nitrogen stress [10, 21, 36]. Our results also showed that in the visible region, from 620 and 700 nm, especially during the early-season and late-season, statistical differences between nitrogen treatments (N90, N180 and N360) at 90% level were significant. But in the mid-season, differences in reflectance in visible region between the three nitrogen treatments were not significant at 90% level, which can largely be explained by the insignificant difference of cotton canopy ground cover among treatments (near full closure for each treatment). Nevertheless, reflectance in the near infrared region showed significant differences under different treatments in all of the three measurement periods, which largely depend on the differences in the canopy structure variables such as leaf area index and biomass, even during mid-season when leaf area index is large than 2.5

Table IV. The confusion matrix results observed on July 15, August 14, and October 1 using canonical discriminant analysis.

Observed dates	Different nitrogen treatments	Number of cases	Predicted group membership			
			N0	N90	N180	N360
Jul. 15	N0	40	33	2	5	0
	N90	40	2	31	4	3
	N180	40	4	6	22	8
	N360	40	0	4	3	33
	Accuracy (%)	74.4				
Aug. 14	N0	40	35	2	3	0
	90	40	4	32	2	2
	180	40	2	5	29	4
	360	40	0	2	1	37
	Accuracy (%)	83.1				
Oct. 1	N0	40	39	1	0	0
	90	40	1	35	3	1
	180	40	0	1	34	5
	360	40	0	0	5	35
	Accuracy (%)	89.4				

for each of the three treatments due to the multiple scattering of radiation in near infrared. Our results are consistent with other results from the literature [28].

It is known that nitrogen stress affects both (i) chlorophyll concentration and, (ii) plant development and growth. Most of the vegetation indices (Tab. I) were established for the purpose of estimating plant biophysical/biochemical variables, and have been related to crop variables such biomass, leaf area index and chlorophyll [4, 5, 17, 22, 34, 36, 37]. In general, since nitrogen conditions result in a significant variation in these agricultural variables, which have been approved by this study, it is also possible to discriminate canopies grown under different nitrogen treatments using these vegetation indices [28, 39]. Our results suggested that four spectral indices (mSR705, mND705, D720 and dRE), which could be well used to estimate chlorophyll status [1, 9, 20, 37], always experienced significant difference between each two-treatment (N0-N90, N90-N180 and N180-N360) during the three measurement periods. Therefore, the difference in chlorophyll content induced by different nitrogen application rate is always one of the most important factors for nitrogen stress detection by remotely sensed data. Nevertheless, since chlorophyll status (especially when expressed as canopy chlorophyll density) also significantly correlates with canopy structure variables such as leaf area index and biomass (Tab. II), the difference of canopy structure may also be important for reflectance measurement of cotton nitrogen stress. In fact, these vegetation indices also significantly correlated with canopy structure variables such as leaf area index and biomass [1, 20]. In most cases, the difference in canopy structure as well as in chlorophyll concentration induced by nitrogen are the important factors in determining whether the canopies can be accurately differentiated, despite of the direct and linear relationship between nitrogen and chlorophyll. The newer spectral indices, such as MCARI, OSAVI and TCARI/OSAVI have been demonstrated to be highly correlated with chlorophyll with minimum leaf area index effects [19], did not necessarily perform better than the indices significantly correlating with both chlorophyll and canopy structure variables in separating the canopies under different nitrogen rates.

However, spectral reflectance is the collective responses of these crop variables and ecological environmental properties [39]. A single variable alone is not able to fully describe the state of crops nitrogen throughout the growing periods. As a result, a single vegetation index, including the two-band and three-band vegetation indices developed for a certain crop variable estimation, is not enough for nitrogen stress detection. With a single vegetation index as the indicator, 30.6–45%, 28.8–48.8% and 28.8–61.6% overall success rates could be obtained during early, mid and late season, respectively. Canonical discriminant analysis with a stepwise procedure offers great potential for identifying the canopies under ecophysiological stress [13, 39]. Our results showed that cotton canopies could be successfully differentiated according to their nitrogen application rate, even at early growth stage. Using the 24 spectral indices as independent variables, a canonical discriminant analysis resulted in an accurate classifications and estimations of developing cotton under different nitrogen treatments during early, middle and late of growing periods with accuracies of 74.4%, 83.1% and 89.6%, respectively. When remotely sensed information combined with other characteristics of field will be of interest in precision agriculture. In early or middle growth stages, it is critical to detect nitrogen deficiencies to allow farmers to apply appropriate fertilizers. Even in late growing stages, it is important to detect nitrogen deficiencies for accurate cotton yield prediction [19]. If the major limiting factors affecting crop growth have been identified, it may be feasible for farmers and agricultural managers to divide crop-fields into management units using remote sensing data, and then make the proper time-specific and time-critical management decisions [2, 12, 19].

However, an important issue in crop stress detection should be noted. As we had mentioned earlier, canopy structure and pigment status of cotton were the most important factors affecting canopy spectral reflectance. Because of the interaction between different crop growth conditions such as fertilizers (e.g. nitrogen, phosphorus and potassium), environment conditions (e.g. light, temperature, soil and water) and management practices (plant density, planting dates and cultivars), the change in spectral reflectance of plants is mainly resulting from the different canopy structure and pigment which can be influenced by each of the former factors. It would be difficult to determine the type of nutrient stress over large fields at canopy levels based only on spectral response [3, 8, 39]. More efforts and sophisticated approaches are needed to overcome this difficulty.

Acknowledgement: We would very happy to thank two referees and Prof. Alexandra N. Kravchenko of Michigan State University for helpful revisions.

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