Nitrogen vertical distribution by canopy reflectance spectrum in winter wheat

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Abstract. Nitrogen is a key factor for plant photosynthesis, ecosystem productivity and leaf respiration. Under the condition of nitrogen deficiency, the crop shows the nitrogen deficiency symptoms in the bottom leaves, while excessive nitrogen will affect the upper layer leaves first. Thus, timely measurement of vertical distribution of foliage nitrogen content is critical for growth diagnosis, crop management and reducing environmental impact. This study presents a method using bi-directional reflectance difference function (BRDF) data to invert foliage nitrogen vertical distribution. We developed upper-layer nitrogen inversion index (ULNI), middle-layer nitrogen inversion index (MLNI) and bottom-layer nitrogen inversion index (BLNI) to reflect foliage nitrogen inversion at upper layer, middle layer and bottom layer, respectively. Both ULNI and MLNI were made by the value of the ratio of Modified Chlorophyll Absorption Ration Index to the second Modified Triangular Vegetation Index (MCARI/MTVI2) referred to as canopy nitrogen inversion index (CNII) in this study at ±40° and ±50°, and at ±30° and ±40° view angles, respectively. The BLNI was composed by the value of nitrogen reflectance index (NRI) at ±20° and ±30° view angles. These results suggest that it is feasible to measure foliage nitrogen vertical-layer distribution in a large scale by remote sensing.

Key words: winter wheat, vertical distribution, bi-directional reflectance difference function (BRDF), foliage nitrogen content

1. Introduction

Nitrogen is the key factor for plant photosynthesis, ecosystem productivity and leaf respiration [1-3], which is close related to crop yield and grain quality. Finding the optimal amount of nitrogen

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fertilization to match the demand of crop growth is critical for improving grain yield and reducing environmental impact \[4\]. However, nitrogen is also a limiting resource in many systems \[1\]. Excessive nitrogen fertilization on farmland can cause crop lodging, groundwater contamination, atmospheric pollution and other environmental problems \[5\]. Thus, fertilization should ideally be carried out according to crop growth conditions determined by the foliage nitrogen content. This can not only improve nitrogen use efficiency and crop’s yield and quality, but also minimize the environmental impact. Traditional methods of measuring foliage nitrogen, such as Kjeldahl method are time-consuming and labour intensive \[6\]. The values of SPAD was once used to measure foliage nitrogen based on the close relationship between foliage chlorophyll content and foliage nitrogen content \[7,8\], but the relationship between foliage chlorophyll content and foliage nitrogen content may not be linear at high nitrogen level \[9\], and the method cannot achieve foliage nitrogen content of large scale. The birth of remote sensing technology provides alternative for measuring the foliage nitrogen content at large scale timely.

Many spectral vegetation indices derived from canopy spectrum have been used to invert foliage and canopy nitrogen content by remote sensing, such as Normalized Difference Vegetation Index (NDVI) and Ratio Vegetation Index (RVI) of different bands as well as NDVI and RVI composed indices using first derivative or second derivative values of two bands \[10-12\], \[5\]. Bausch et al employed nitrogen reflectance index (NRI) to measure plant nitrogen \[13\]. Daughtry et al proposed a modified chlorophyll absorption ratio index (MCARI) and used it to measure chlorophyll concentration and nitrogen content \[9\]. To reduce the sensitivity to variation in LAI and soil background, a combined index (MCARI/MTVI2) was used to invert the nitrogen concentration and nitrogen content \[14,15\]. Chen et al proposed a new index named double-peak canopy nitrogen index (DCNI) to measure the nitrogen content \[15\]. Many indices such as normalized difference red edge index (NDRE) and red-edge chlorophyll index were also used to invert the plant nitrogen derived from the red-edge region \[8\]. These methods also utilized upper-layer nitrogen measurement, which normally cannot be a comprehensive response to the nitrogen content. In the case of nitrogen stress, the transmission of nitrogen is generally from the bottom-layer to upper-layer \[16\]. Nitrogen deficiencies usually exhibit in the bottom layer of foliage, while excess nitrogen application will first impact in the upper layer. Therefore, timely measurement of the vertical distribution of foliage nitrogen content is critical for growth diagnosis and reducing environmental impact. But there are few studies on vertical distribution of nitrogen content. As BRDF data can provide information about vegetation structures \[17\], more comprehensive than data observed at a single view angles, they have been used to detect foliage conditions and disturbance for forest ecosystem and inversion of chlorophyll vertical distribution in winter wheat \[18\], \[19\]. This paper presents a method to measure foliage nitrogen vertical-layer distribution by spectral vegetation indices of multiple viewing angles calculated from bi-directional reflectance difference function (BRDF) data.

2. Method

2.1. Experimental design
The experiment was conducted on winter wheat at Xiaotangshan Precision Agriculture Demonstration Base, which is situated in Changping district, the northeast of Beijing City (40°11’ N, 116°27’ E), China. The soil is classified as a silt clay loam with mean annual rainfall at 507.7mm and the mean annual temperature at 13°C. In years of 2004 and 2007, winter wheat cultivars with different canopy geometry structure were used in the experiment. The sown area of each winter wheat cultivar was 30.0×5.4 m² in 2004 and 45×10.8 m² in 2007. The experiment in 2004 was to establish the nitrogen vertical distribution model, while the experiment in 2007 was to validate the nitrogen vertical distribution model.

2.2. Data acquisition
2.2.1 In situ canopy reflectance spectrum

An ASD FieldSpec Pro spectrometer (Analytical Spectral Devices, Boulder, CO, USA) fitted with a 25 degree-field-of-view fiber optic adaptor was used to measure the canopy reflectance between 10:00 and 14:00 (Beijing local time) under clear sky condition. To obtain the information of various angles of plants, we used the same spectrum instrument measured in situ canopy reflectance spectrum to obtain canopy bi-directional reflectance function (BRDF) reflectance at the principle plane and the cross-principle plane. Introduced by Huang et al 2006 [20], a rotating bracket was used to fix the spectrum instrument. The solar zenith angle was 32.8° to 41.2° during the measuring period between 10:00 and 14:00 (Beijing local time), and the view zenith angle was from −65° to 65° with 5° interval. The negative angle represented face-to-the-sun, while the positive angle represented back-to-the-sun.

2.2.2 Foliar nitrogen vertical distribution sampling method and foliage nitrogen content

The foliar nitrogen vertical distribution sampling method used in this study was similar to the foliar chlorophyll vertical distribution sampling method used in the study by Huang et al 2010 [19]. Leaf nitrogen concentration was determined using Kjedahl (1883) methods. Then nitrogen content was calculated by the following formula:

\[
N \text{ content} = N \text{ concentration} \times \text{leaf dry weight} \div \text{sampling area}
\]

Statistical analysis and evaluation factor

To get the leaf nitrogen content of upper-layer, middle-layer and bottom-layer quickly and easily, we propose three new indices (ULNI, MLNI and BLNI) from BRDF data to invert the corresponding foliage nitrogen contents. The data of 2004 were used to establish the relationship between each index (ULNI, MLNI and BLNI) and its corresponding nitrogen content using regression analysis in Excel 2007, whereas the data of 2007 were utilized to verify the relationships. Coefficient of determination (R²) and root mean square error (RMSE) were used to test the strength of the relationship and accuracy of the estimation. A value of R² close to 1.0 indicates high precision and RMSE value close to 0 means high estimation accuracy.

\[
\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2}{n}}
\]

where \(\hat{y}_i\) and \(y_i\) were the predicted and measured foliage nitrogen contents, and \(n\) is the number of samples [21].

3. Results and analysis

The inversion of foliage nitrogen is also affected by leaf area index, non-photosynthetic surfaces and land surface. The ratio of Modified Chlorophyll Absorption Ration Index to the second Modified Triangular Vegetation Index (CNII) is well related to nitrogen content of wheat, and it have the properties that low sensitivity to variations in LAI, soil background and non-photosynthetic surfaces [14-15], therefore we used it to invert the foliage nitrogen inversion of upper and middle layer. NRI was used for the inversion of foliage nitrogen of bottom-layer, which has been used in measurement of the foliage nitrogen in irrigation corn and plant growth parameters even some plots with various water and nitrogen treatments [13,22]. The CNII and NRI are defined as follow [11,23]:

\[
\text{CNII} = \frac{MCARI}{MTVI2}
\]

\[
MCARI = (R_{700} - R_{670} - 0.2 \times (R_{700} - R_{550})) \times (R_{700} / R_{670})
\]

\[
MTVI2 = \frac{1.5 \times (1.2 \times (R_{600} - R_{550}) - 2.5 \times (R_{670} - R_{550})) \times (2 \times R_{500} + 1)^2 - (6 \times R_{600} - 5 \times \sqrt{R_{670}}) - 0.5}{(2 \times R_{500} + 1)^2 - (6 \times R_{600} - 5 \times \sqrt{R_{670}}) - 0.5}
\]
where \( MCARI \) was ameliorated from the Chlorophyll Absorption in Reflectance Index (CARI) for minimizing the combined effects of non-photosynthetic surfaces and soil reflectance \([9, 14]\). MTVI2 was improved from Modified Triangular Vegetation Index (MTVI1) by optimizing the constraint of the resistance to chlorophyll influence and keeping the sensitivity to LAI \([24]\), and it is a good index for predicting leaf area index. The index (CNII) has been proved to be sensitive to nitrogen and can invert foliage nitrogen of wheat effectively \([15]\).

As mentioned above, the foliage nitrogen information of bottom-layer can be expressed by the information at view angles of 20° and 30°. In this study, BLNI was made by the value of NRI at ±20°and ±30° view angles to invert the bottom-layer nitrogen. To represent the information of the full range of crop bottom-layer, BLNI was defined as:

\[
BLNI = 0.3 \times NRI_{20} + 0.2 \times NRI_{30} + 0.3 \times NRI_{-20} + 0.2 \times NRI_{-30}
\]

where \( NRI_{20}, \ NRI_{30}, \ NRI_{-20}, \text{ and } NRI_{-30} \) represent the NRI values of view zenith angles at 20°, 30°, −20°, and −30°. The negative angles represent face-to-the-sun and the positive angles represent back-to-the-sun.

From the data of 2004, the relationship between BLNI and foliage nitrogen content of bottom-layer can express by the following equation:

\[
y = kx + b \quad (R^2 = 0.6873, \text{RMSE} = 0.0549 \text{ g/m}^2)
\]

where \( x \) is BLNI, \( y \) is foliage nitrogen content of bottom-layer, \( k \) is slope, and \( b \) is intercept. It showed that BLNI was linearly related to the foliage nitrogen content of bottom layer. The values of \( k \) and \( b \) were 12.1 and -3.00 for the year of 2004 (Fig. 1a). The data of 2007 was used to validate the linear relationship between BLNI and foliage nitrogen content of bottom-layer. The result was consistent with the result of 2004, with the \( R^2 \) value of 0.7968 and RMSE value of 0.1913 (Fig. 1b).

**Figure 1.** Relationship between BLNI and measured foliage nitrogen content of bottom-layer in 2004 (a) and 2007 (b)

These linear relationships were statistically significant between BLNI and foliage nitrogen content of bottom-layer. As we mentioned above, the foliage nitrogen information of middle-layer can be expressed by the information at view angles of 30° and 40°. In this study, MLNI was made by the
value of CNII at ±30° and ±40° view angles to invert the middle-layer nitrogen. To represent the information of the full range of crop middle-layer, MLNI was defined as:

$$MLNI = 0.2 \times CNII_{v=30} + 0.3 \times CNII_{v=40} + 0.2 \times CNII_{v=-30} + 0.3 \times CNII_{v=-40}$$  \(8\)

where $CNII_{v=30}$, $CNII_{v=40}$, $CNII_{v=-30}$, and $CNII_{v=-40}$ represent the CNII values of view zenith angle at 30°, 40°, -30°, and -40°. The negative angles represent face-to-the-sun and the positive angles represent back-to-the-sun.

From the data of 2004, the relationship between MLNI and foliage nitrogen content of middle-layer could be expressed by the following equation:

$$y = k \times x + b \ (R^2=0.8132, \ RMSE=0.1891 \ g/m^2)$$

where $x$ is MLNI, $y$ is foliage nitrogen content of middle-layer, $k$ is slope, and $b$ is intercept. It showed that MLNI was linearly related to the foliage nitrogen content of middle-layer.

The values of $k$ and $b$ were -20.2 and 3.14 for the year of 2004 (Fig. 2a). The validation using data of 2007 showed a consistent linear relationship between MLNI and foliage nitrogen content of middle-layer with $R^2$ value of 0.6377 and RMSE value of 0.4589 g/m2. The values of $k$ and $b$ were -21.9 and 6.63 (Fig. 2b).

The linear relationship between MLNI and foliage nitrogen content of middle-layer was statistically significant. The foliage nitrogen information of upper-layer can be expressed by the information at view angles of 40° and 50°. In this study, ULNI was made by the value of CNII at ±40° and ±50° view angles to invert the upper-layer nitrogen. To represent the information of the full range of crop upper-layer, ULNI was defined as:

$$ULNI = 0.2 \times CNII_{v=40} + 0.3 \times CNII_{v=50} + 0.2 \times CNII_{v=-40} + 0.3 \times CNII_{v=-50}$$  \(9\)

where $CNII_{v=40}$, $CNII_{v=50}$, $CNII_{v=-40}$, and $CNII_{v=-50}$ represent the CNII values of view zenith angle at 40°, 50°, -40° and -50°.

From the data of 2004, the relationship between ULNI and layer foliage nitrogen content of upper-layer could be expressed by the following equation:

$$y = k \times x + b \ (R^2=0.7237, \ RMSE=0.2187 \ g/m^2)$$
where x is ULNI, y is foliage nitrogen content of upper-layer, k is slope, and b is intercept. It showed that ULNI was linearly related to the foliage nitrogen content of upper layer. The values of k and b were -15.9 and 3.69 for the year of 2004 (Fig. 3a).

The data of 2007 was used for the validation of the linear relationship between ULNI and upper-layer foliage nitrogen, with $R^2$ value of 0.7041 and RMSE value of 0.5313 g/m$^2$. The values of k and b were -23.4 and 7.21 (Fig. 3b). The relationship was statistically significant.

These results indicate that foliage nitrogen vertical distribution of winter wheat can be effectively measured in real time over a large area by three spectral vegetation indices calculated from bi-directional reflectance difference function (BRDF) data. However, calibration must be conducted every year to obtain the specific values of k and b for the inversion model.

![Figure 3](image)

**Figure 3.** Relationship between ULNI and measured foliage nitrogen content of upper-layer in 2004 (a) and 2007(b)

4. **Discussion**

The spectrum is affected differently by various confounding factors. The combined index (MCARI/MTVI2) appears to be suitable for the inversion of foliage nitrogen of upper-layer and middle-layer, because it is not sensitive enough to LAI variations, soil background effects and non-photosynthetic surface\textsuperscript{[14]}. NRI was selected for the inversion of foliage nitrogen of bottom-layer, because it has been used in measurement of the foliage nitrogen in irrigation corn and plant growth parameters even some plots with various water and nitrogen treatments\textsuperscript{[13, 22]}. The objective of this study is to more accurately nitrogen vertical distribution by BRDF data.

In the future, experiment should be carried out on different crops with different environment factors to further generalize and improve the accuracy of the models. Since this study only used the data of observed cross-principle plane, future studies should consider the application of more observation planes such as the combination of cross-principle plane and principal plane for obtaining more comprehensive information about crop.

5. **Conclusion**

This paper shows that foliage nitrogen vertical-layer can be measured from bi-directional reflectance difference function (BRDF) data with three simple empirical regression models. ULNI and MLNI were made by the value of CNII at ±40° and ±50°, and at ±30° and ±40° view angles for upper-layer
and middle-layer nitrogen inversion, respectively. BLNI was composed by the value of nitrogen reflectance index (NRI) at ±20° and ±30° view angles for bottom-layer nitrogen inversion. The result indicates that foliage nitrogen vertical distribution of winter wheat can be effectively measured in real time over a large area by three spectral vegetation indices calculated from bi-directional reflectance difference function (BRDF) data.

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