



Comparison of different methods for estimating nitrogen concentration in flue-cured tobacco leaves based on hyperspectral reflectance

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ABSTRACT

Leaf nitrogen content (LNC) is an important indicator of tobacco quality and is used in the prediction of tobacco yield. Reflectance experiments for flue-cured tobacco were conducted over 2 consecutive years. Leaf hyperspectral reflectance and nitrogen content data were collected at 15-day intervals from 30 days after transplant until harvest. In this work, we identified the central band that sensitive to tobacco LNC and the optimum combination to establish new spectral indices (SR and NDVI), which were used in linear models of the specific ratio vegetation index (SR), normalized difference vegetation index (NDVI), stepwise multiple linear regression (SMLR), and back-propagation (BP) neural network models as independent variable or input factors. The central bands for the LNC were concentrated in the visible range (450–750 nm) in combination with the shortwave infrared range (1450–2500 nm) range. The optimum band combinations for SR and NDVI were (590 and 1980 nm) and (1970 and 650 nm), respectively. The BP neural network model was the most stable and accurate model ($R^2 = 0.91$, RMSE = 0.09, and $\bar{K} = 0.00$). The SR, NDVI, and SMLR models had R^2 values of 0.77, 0.76, and 0.86; RMSE values of 0.26, 0.51, and 0.60, and \bar{K} values of 0.05, 0.11, and 0.14, respectively. The results indicate the possibility of monitoring LNC by combining remote sensing with predictive models.

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1. Introduction

Nitrogen, the most limiting nutrients in plants, is crucial for plant growth, yield, and quality (Ju et al., 2008; Cox et al., 1993). It is the main component of proteins, nucleic acids, phospholipids, and chlorophyll, and is thus known as “the element of crop life” (Alvarado et al., 2000). Hence, precise management of plant nitrogen status is of great significance. However, the traditional methods of measuring plant nitrogen content are laborious, requiring destructive sampling, and producing hysteretic results (Wang et al., 2012). Hence, a new method for real-time, non-destructive, and wide-area estimation of plant nitrogen status is urgently needed. The nitrogen nutrition index (NNI) is a well-established diagnostic tool used in evaluate crop nitrogen status (Lemaire and Gastal, 1997). The NNI, developed by Lemaire and Salette (1984), is defined as the ratio of actual crop nitrogen concentration to the minimal nitrogen concentration required for maximum

biomass production (Lemaire et al., 1992; Greenwood et al., 1991). Misteale and Schmidhalter (2008) demonstrated a strong relationship between the red-edge inflection point (REIP) and NNI. In a later study, they also reported the ability to related in-season spectral measurements of the normalized difference vegetation index and REIP to canopy biomass and canopy nitrogen (Misteale and Schmidhalter, 2010). An integrated approach consists of calculating the NNI using remote sensing: the biomass is estimated using the leaf area index; while the nitrogen content is estimated based on the chlorophyll content at the canopy scale (Houlès et al., 2007; Lemaire et al., 2008; Chen et al., 2010a,b; Fitzgerald et al., 2010).

The key to nitrogen prediction quality of these approaches is a close relationship between chlorophyll and nitrogen (Gausman, 1977; Yoder and Pettigrew-Crosby, 1995). Currently, two approaches are available to establish the relationship between chlorophyll and nitrogen content. The first approach utilizes spectral reflectance measurements to determine the canopy chlorophyll content index (CCCI). CCCI was originally used in different nitrogen deficiency from water stress which separates out the confounding effects of canopy density lately (Barnes et al., 2000). The development and utility of the CCCI have been discussed by Fitzgerald

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et al. (2006, 2010). The second approach uses a SPAD 502[®] meter (Minolta Osaka Co., Ltd., Japan) to estimate nitrogen content based on leaf transmittance measurements at two wavelengths, centered at 650 and 940 nm (Chapman and Barreto, 1997; Duru, 2002; Pedro et al., 2012). Unfortunately, the relationship between chlorophyll pigments and nitrogen is not useful in every case, as it is highly dependent on the growing season (Evans, 1983) and cultivar (Spaner et al., 2005).

Kokaly (2001) presented an alternative method, which can directly evaluate crop nitrogen status from visible and infrared wavelengths. The method has been utilized by a number of research groups (Chappelle et al., 1992; Xue et al., 2004) and been adapted for wheat (Nathalie et al., 2011), rice (Chanseok et al., 2011), and maize (Weber et al., 2012). In fact, many previous studies utilized canopy reflectance to estimate the nitrogen concentration and other biophysical parameters (e.g., Liu et al., 2012; Takebe et al., 1990). However, Dorigo et al. (2007) and Asner and Martin (2008) suggested that factors such as non-photosynthetic canopy components, the background signal, and atmospheric conditions can influence the performance of canopy reflectance. In order to solve this problem, vegetation indices (Daughtry et al., 2000; Serrano et al., 2002; Chen et al., 2010a,b) and improved hyperspectral instruments (Hansen and Schjoerring, 2003) with numerous narrow wavebands and wide ranges have been employed to increase accuracy and to provide steadier and more comprehensive monitoring of plant growth. Leaf spectral reflectance provides accurate nitrogen status readings in leaves without the effects described above (Kokaly, 2001).

In previous studies, estimations of nitrogen or other physiological parameters from hyperspectral monitoring mainly relied on empirical physiological indices (Haboudane et al., 2002; Dash and Curran, 2004; Jin et al., 2012). However, a limitation of prior results is that the distribution of nitrogen varies among plant species. Thus, the spectral index for nitrogen concentration can also be expected to be various.

Few studies of tobacco have used remote sensing, although tobacco is cultivated in several Chinese provinces as a significant economic crop with large yield and planting area. Tobacco is a broadleaf crop with a leaf harvest and a low planting density. There are many differences between tobacco and narrow-leaf and closely planted crops such as rice, wheat, and cotton from which the flower or fruit is harvested. Thus, further work is needed to evaluate the usage of hyperspectral remote sensing to measure nitrogen status in tobacco.

In this study, to analyze the nitrogen content of tobacco leaves, hyperspectral remote sensing and two years of data from the same experimental site was applied. The aim of this work was to (1) identify the central bands for tobacco LNC, (2) develop vegetation indices for accurate and stable tobacco LNC monitoring, and (3) evaluate optimal models for monitoring tobacco LNC.

2. Materials and methods

2.1. Experimental design

An open field experiment was conducted in Fangcheng City (33°15'N, 112°54'E), Henan Province, China, in 2011 and 2012, with flue-cured tobacco (*Nicotiana tabacum* L.) cv. Yunyan 87 as the experimental tobacco. The field soil was classified as a yellow loam (Alfisol in U.S. Taxonomy) with 11.45 g kg⁻¹ organic matter, 0.72 g kg⁻¹ total N, 55.01 mg kg⁻¹ alkali-hydrolyzable nitrogen, 18.0 mg kg⁻¹ available phosphate, and 135.21 mg kg⁻¹ available potassium (0–0.25 m soil depth). Six light quality treatments were employed, with natural light as the control. Experiments 1–5 were treated with red (R), yellow (Y), green (G), blue (B), and white

(W) light filters, respectively. The experiment was a randomized complete block design using a factorial arrangement of treatments with three replicates. For every treatment, 48 tobacco plants were planted, with spacing of 1.20 m × 0.50 m, covering an area of 34.56 m². Each light filter was placed on a vaulted iron bracket 2.8 m tall, with a bottom width of 6 m and length of 6 m, covering about 36 m², respectively. The brackets were positioned in a north–south direction in random order, while the south and north openings were uncovered to allow for ventilation. Light intensity was improved by using a colorless filter and adjusting the height of the bracket. Tobacco plants were transplanted on April 26, 2011 and April 25, 2012. Other cultivation and management methods were the same as those typically used in a local field producing high-quality tobacco.

2.2. Measurement of leaf reflectance

During the experimental periods in 2011 and 2012, ground-based hyperspectral leaf reflectance was measured every 15 days beginning 30 days after transplant until harvest using a Field Spec Pro FR spectroradiometer (Analytical Spectral Devices (ASD), Boulder, CO, USA) equipped with a leaf clip. The instrument recorded reflectance between 350 and 1000 nm with a sampling interval of 1.40 nm and a resolution of 3 nm, and reflectance between 1000 and 2500 nm with a sampling interval of 2 nm and a resolution of 10 nm. Measurements were taken under clear-sky conditions between 10:00 and 14:00 Beijing local time. Using the ASD leaf clips, leaves were measured in a confined environment with stable operation of the simulated light source to reduce spectral data error. For each treatment, three healthy tobacco plants were typically measured with three upper, middle, and lower leaves tested for each plant. For each leaf sample, reflectance was measured at five points: the leaf tip, the upper left, upper right, lower left, and lower right of each leaf. For each point, five reflectance curves were made. The average reflectance of the 25 reflectance measurements was used as the value of the leaf's spectral reflectance. A white Spectralon reference panel was used under the same conditions to convert the spectral radiance measurements before each test, and the scan time was 0.2 s.

2.3. Plant measurements

After leaf reflectance was measured in the field, the same plants for each treatment were destructively sampled and transferred to the laboratory to measure LNC. For each sample, all green leaves were separated and dried at 105 °C for 30 min, and then at 60 °C until they reached a constant weight. Dried leaf samples were then ground to pass through a 0.3-mm screen, and finally, LNC was determined by the micro-Kjeldahl method.

2.4. Methods

By constructing the spectral vegetation index, vegetation reflectance and external factors (such as non-photosynthetic canopy components and atmospheric conditions) can be maximized and minimized, respectively. In this work, the specific ratio vegetation index (SR) and normalized difference vegetation index (NDVI) were calculated using any two-band combination in the 400–2450 nm wavelength range.

$$SR = \frac{R_{\lambda 1}}{R_{\lambda 2}} \quad (1)$$

$$NDVI = \frac{R_{\lambda 1} - R_{\lambda 2}}{R_{\lambda 1} + R_{\lambda 2}} \quad (2)$$

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