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Remotely assessing photosynthetic nitrogen use efficiency with *in situ* hyperspectral remote sensing in winter wheat

Hai-Yan Zhang[a], Xing-Xu Ren[a], Yi Zhou[a], Ya-Peng Wu[a], Li He[a], Ya-Rong Heng[a], Wei Feng[a,b,*], Chen-Yang Wang[a,b,*]

[a] *National Engineering Research Centre for Wheat, Henan Agricultural University, # Longzihu College District 15, Zhengzhou, Henan, 450046, PR China*
[b] *State Key Laboratory of Wheat and Maize Crop Science, Henan Agricultural University, # Longzihu College District 15, Zhengzhou, Henan, 450046, PR China*

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ABSTRACT

Non-destructive and rapid methods for estimating nitrogen efficiency are helpful in quantifying and communicating the N use efficiency (NUE) in agricultural production. To accurately evaluate NUE, we analysed the quantitative relationship between leaf photosynthetic nitrogen use efficiency (PNUE) and wheat NUE, and investigated the quantitative relationship between PNUE and hyperspectral indices in winter wheat. Our field experiments were carried out during 2013–2016 across different growth stages, cultivars, planting densities, nitrogen (N) rates, and water treatments, and a nitrogen efficiency index (NEI) was developed to evaluate PNUE. Three of eight previously developed vegetation indices (VIs) performed well when regressed against PNUE; however, they exhibited bias between vegetative and reproductive stages. The NEI was obtained by integrating the reflectance at the red band (680 nm) into simple ratio index (SR (760,850)), to become a modified simple ratio index (mSR (760,850,680)), then we added a floating coefficient [$1.8 + (R_{680}/R_{850})$] to mSR (760,850,680). Our novel index was better related with PNUE than previous VIs under different experimental conditions. There was no significant difference in the slope and intercept under the five experimental factors, suggesting that the new index is a superior tracker for PNUE changes under heterogeneous field conditions. These models accurately estimated PNUE in winter wheat, providing a theoretical basis and technical support for N fertilizer assessment and screening of N efficient wheat varieties.

1. Introduction

Wheat is the world's most widely cultivated food crops, with production becoming increasingly important with population growth. In developed countries, increased wheat production in recent years has led to continued growth in the use of N fertilizer (Ladha et al., 2005; Hirel et al., 2007; Beatty et al., 2010). Nevertheless, excessive use of N may lead to low efficiency in its use by crop and pollution problems. N use efficiency (NUE) is therefore an important parameter for N fertilizer assessment and grain production optimization.

NUE parameters can be defined using many methods (Barraclough et al., 2010) and, in general, NUE terminology is divided into three types: N agronomic efficiency, N recovery efficiency and N physiological efficiency (López-Bellido and López-Bellido, 2001). Most studies agree that NUE is the grain yield per unit of N supply (López-Bellido et al., 2005), and belong to the first category. NUE measures the ability to use nitrogen and the reasonable allocation of nitrogen, indicating the impact of nitrogen on plant growth and photosynthetic productivity. Physiological processes are limited by nitrogen, with plants able to effectively distribute nitrogen achieving optimal efficiency. The ratio between the rate of photosynthesis and the large quantity of N in the leaves, known as the photosynthetic N use efficiency (PNUE), can therefore be used to evaluate NUE (Ghannoum et al., 2005). A non-destructive, real-time and rapid method to determine PNUE would aid NUE estimation during growth. Leaf PNUE assessment using remote sensing could therefore help quantify and communicate the use efficiency of N in agriculture and food production.

Ground remote sensing technologies have been widely used for precise management of crops, however, its potential application for high throughput phenotyping has gathered interest in recent years (Araus and Cairns, 2014; Liebisch et al., 2015). This common method has involved the development of a large number of vegetation indices associated with protein concentration, chlorophyll fluorescence, nutrient status and grain yield (Xing et al., 2007; Xue et al., 2007; Feng et al., 2016). A wide range of hyperspectral indices have been investigated to assess chlorophyll content using leaf reflectance

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* Corresponding authors. Present address: National Engineering Research Centre for Wheat, # Longzihu College District 15, Zhengzhou, Henan, 450046, PR China.
E-mail addresses: fengwei78@126.com (W. Feng), xmzxwang@163.com (C.-Y. Wang).

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(Daughtry et al., 2000; Haboudane et al., 2002; Dash and Curran, 2004; Maire et al., 2004; Gitelson et al., 2005). A number of general parameters and angular insensitivity vegetation indices have been built to estimate nitrogen content (Feng et al., 2014, 2016; He et al., 2016). In addition, a large number of studies have shown significant correlation between leaf area index (LAI) and hyperspectral remote reflectance in the red and near-infrared (NIR) wavebands (Xavier and Vettorazzi, 2004; Pocewicz et al., 2007). Furthermore, the estimation of water content using remote sensing has been widely researched (Sims and Gamon, 2003). Numerous studies have revealed the potential of methods based on remote sensing data to estimate grain production over a wide range of conditions (Pinter et al., 2003; Hadria et al., 2010; Vergara-Díaz et al., 2016). Protein concentration is known to influence the quality of wheat products, hence methods to estimate protein concentration have been studied using canopy spectral reflectance (Hansen et al., 2002; Xue et al., 2007). These parameters can accurately predict plant physiological indices.

In recent years, research has mainly focused on quantifying relationships between functional indicators and field crop remote sensing reflectance information. For example, the relationship between the net photosynthetic rate (P_N) and vegetation indices (VIs) is thought to be curvilinear (Choudhury, 2001). Recently, research has identified the linear relationship between leaf P_N characteristics and the spectral reflectance of field crops, with the ratio index R_{810}/R_{680} identified as the optimum vegetation index for estimating rice leaf photosynthetic characteristics (Tian et al., 2005). Furthermore, chlorophyll fluorescence performance indices are important functional indicators in crops, therefore the estimation of chlorophyll fluorescence indices has been researched using remote sensing (Xing et al., 2007). In addition, light-use efficiency (LUE), which is defined as the ratio of the P_N to the photosynthetically active radiation (PAR) (Nakaji et al., 2007), is a significant functional index of crops. The physiological reflectance index (PRI) produced from narrow waveband spectral measurements of a sunflower canopy was previously used to estimate LUE (Gamon et al., 1992). Moreover, the PRI can be used to monitor the xanthophyll cycle, water, and the disease index of yellow rust (Huang et al., 2007; Rossini et al., 2013). Yet, further information is required on quantitative and effective methods for monitoring NUE in crops such as wheat under different experiment conditions using remote sensing techniques.

This study aims to create a model for estimating NUE by (i) investigating the relationship between PNUE and NUE under different experimental conditions; (ii) developing a novel index for evaluating PNUE; (iii) comparing the reliability of the common indices and novel combined index for assessing PNUE, and (iv) quantify the relationships between PNUE and the new index, and determine the united equation for monitoring PNUE. All this will be useful to provide a technical analysis method for fast, non-destructive and real-time monitoring of PNUE in winter wheat.

2. Methods

2.1. Experimental design

Five experiments were conducted across three growing seasons at two different locations in China. Various water management, N rates, planting densities, and cultivars of hexaploid winter wheat (*Triticum aestivum* L.) were studied (Table 1). The first four experiments were conducted at the experimental station of Henan Agricultural University located in Zhengzhou City (34°51′N, 113°35′E) with fluvo-aquic soil (classified as Aquic Inceptisol, Soil Survey Staff, 2010). Experiments 1–2 and 3–4 were carried out in 2014–2015 and 2015–2016, respectively. Experiment 5 was completed in 2013–2014 at Shangshui experimental station in Zhoukou City (33°33′N, 114°37′E), with lime concretion black soil (classified as Aquic Vertisols, Soil Survey Staff, 2010). Two cultivation treatments for N rates and planting density were conducted in two consecutive seasons from November 2014 to June 2016, with a low rainfall season in 2015–2016 and a normal rainfall season in 2014–2015 at the Zhengzhou. Two wheat varieties were used, an erect type cultivar, Yumai 49–198, and a horizontal cultivar, Zhengmai 9694. Rows were planted in a north-south direction at a distance of 18 cm apart in a plot 7 × 2.9 m in Exp. 1–4 and 9 × 6 m in Exp.5. The experiment used a randomized complete block design and a factorial arrangement of treatments was used with three replications. Management procedures were as per the local standard practice for winter wheat production.

2.2. Measurement of canopy reflectance

The spectral reflectance of canopy measurements were obtained from a height of 1 m above the canopy (height of wheat was 70–80 cm at maturity). The measurements were carried out on cloudless or near cloudless days between 10am and 2 pm (Beijing local time), using an ASD Field Spec Pro spectrometer (Analytical Spectral Devices, USA). The sensor was fitted with 25° field-of-view fibre optics operating over the wavelength 325–1075 nm with a sampling interval of 1.6 nm A 40 cm × 40 cm $BaSO_4$ calibration panel was used to calculate black and baseline reflectance. To minimize environmental effects, spectral reflectance measurements were carried out at 10 sample sites in each plot, for each measurement, 20 scans were processed internally by the ASD spectrometer with an interval of 8 s. For each experiment, data were obtained at five developmental stages: jointing, booting, heading, anthesis and grain filling of wheat.

2.3. Measurement of agronomic parameters

The LAI value was obtained using the LAI-2000 Plant Canopy Analyzer (Li-Cor, Inc.) before sunrise or at sunset, using two separate inter-calibrated sensor units. The LAI-2000 field of view was limited with a 90° view-cap, to minimize the influence of the operator and of adjacent plots (Weiss et al., 2004).

P_N of the first fully-expanded top leaves were measured with the canopy spectral reflectance at the same time using an LI-6400 photosynthesis system (Li-Cor Inc., Lincoln, USA). The measurements were carried out in an open system with a CO_2 concentration of 385 umol l^{-1} (Mu et al., 2010).

After the photosynthetic measurements, samples were collected for measuring leaf nitrogen concentration (LNC). In the laboratory, green leaves were separated from the stems then placed in an oven for 30 min at 105 °C to deactivate enzymes, then dried at 70 °C to a constant weight, and dry weight (DW) recorded. The dry samples were then ground and N concentrations determined using a dry combustion method with a Dumas Elementary Analyser (Macro-N, Foss Heraeus, Hanau, Germany) (Zheng et al., 2016). Plant N uptake was determined by multiplying the N content by the dry biomass, and PNUE was determined using the following formula (Ghannoum et al., 2005):

$$\mathrm{PNUE}(\,100\mu\mathrm{mol\,m^{-2}\,s^{-1}}) = \frac{P_N\ (\mu\mathrm{mol\,m^{-2}\,s^{-1}})}{\mathrm{concentration\ N}} \tag{1}$$

At harvest, samples were taken from a 2.0 m × 6 row (1.5 m) quadrat (with one border row) at e centre of each wheat plot. From this sample, thousand seed weight was measured. Seed weight was determined by drying the sampled plants at 70 °C to constant weight. The NUE was determined using the following formula (López-Bellido and López-Bellido, 2001):

$$\mathrm{NUE}(\,\mathrm{kg\,kg^{-1}}) = \frac{\mathrm{grain\,yield(kg\,ha^{-1})}}{\mathrm{plant\,N\,uptake(kg\,ha^{-1})}} \tag{2}$$

2.4. Data analysis

2.4.1. Construction of the new parameter

The Savitaky-Golay smoothing method was used to pre-process the