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Evaluating chlorophyll density in winter oilseed rape (*Brassica napus* L.) using canopy hyperspectral red-edge parameters

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ABSTRACT

Accurate assessments of chlorophyll density (ChD) using hyperspectral techniques are important for effective evaluation of plant productivity and precise nitrogen (N) management in winter oilseed rape. To develop a quantitative estimation model for determining ChD in winter oilseed rape, field experiments with different N fertilizer levels were conducted over two successive years by measuring canopy hyperspectral reflectance and ChD at various developmental stages. The relationships between two types of parameters (existing red-edge spectral parameters and newly-developed red-edge area parameters) and ChD were investigated to determine the optimal red-edge spectral parameters (ORSPs) for ChD predictions. The Noise Equivalent (NE) model was adopted to evaluate the sensitivity and accuracy of the ORSPs for detecting changes in ChD across different growth stages. The results indicated that canopy hyperspectral reflectance and its first derivative spectra significantly varied with the levels of N fertilization. A strong correlation also existed between canopy reflectance data and ChD. Using a training dataset, the best results for assessing ChD status were observed when using the newly-developed red-edge area parameter, which indicated a difference between the double-peak areas based on the position of the main peak (DIDRmid). DIDRmid was the ORSP and exhibited a significant exponential relationship with ChD, with a coefficient of determination (R^2) of 0.88 and a standard error (SE) of 0.312. Tests conducted on the independent validation dataset showed that DIDRmid can be used to accurately predict ChD in oilseed rape, with a relative root mean square error (RRMSE) of 0.091 and a mean relative error (MRE) of 7.22%. Additionally, this ORSP also had relatively lower NE values and higher sensitivity and accuracy with respect to ChD estimation. Consequently, the ChD of winter oilseed rape can be stably estimated with the hyperspectral red-edge methods established in this study because the newly-developed red-edge area spectral parameter was effective and accurate in evaluating ChD.

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1. Introduction

Chlorophyll density (ChD, g m^{-2}) is the product of leaf fresh weight per unit ground area and leaf chlorophyll concentration. It not only provides crop pigment information but also indicates the properties of canopy coverage. It is a key factor in regulating the biophysical and physiological processes of many crops, including photosynthetic functioning (Barry et al., 2009), transformation of solar radiation into chemical energy (Gitelson et al., 2006), eco-environmental stress response and senescence (Merzlyak et al., 1999). Moreover, ChD is closely related to N nutritional status in crops because a large amount of N is incorporated in chlorophyll (Gitelson et al., 2003). Thus, accurate and timely estimation of ChD in crops is essential for site-specific N management, growth monitoring and yield prediction.

Traditional methods of measuring ChD through plant sampling, extraction and spectrophotometric determination in the laboratory (wet-chemistry technique) (Lichtenthaler, 1987), not only require destruction of the crop samples but are also labor-intensive and expensive (Feng et al., 2008; Kira et al., 2015). In recent years, several improved techniques have been proposed for rapidly monitoring the crop chlorophyll status of individual leaves. For example, the Soil and Plant Analyzer Development (SPAD), CCM-2000 and Dualex 4 methods have been successfully applied to measure foliage pigment in rice (Ali et al., 2014), paper birch (Richardson et al.,

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2002), corn (Tremblay et al., 2007) and grapevine (Cerovic et al., 2012). However, these instruments are limited to a small sampling area and are thus unable to assess ChD (or chlorophyll content) at regional scales (Huang et al., 2014). Moreover, leaf thickness, leaf position and the areas from which leaves are tested (e.g., top, middle and bottom) also influence the results (Daughtry et al., 2000). In contrast, hyperspectral techniques exhibit great potential for evaluating the nutritional status (Yang et al., 2010) and predicting values for crop communities instead of individual plants (Feng et al., 2014). These techniques have been proven to be reliable methods to monitor plant growth and nutritional status over large areas (Feng et al., 2015). The hyperspectral data of a canopy contain hundreds or thousands of contiguous spectral wavebands and complex information on crops (Gómez-Casero et al., 2010). Extraction of the most valid and effective information derived from canopy spectra is therefore a prerequisite for assessing crop chlorophyll status.

In recent years, two major approaches have been developed for remote estimation of chlorophyll status: (i) empirical relationships between chlorophyll and vegetation indices (VIs) (Kira et al., 2015) and (ii) physically-based retrieval methods such as canopy radiative transfer models (RTMs) (Baret et al., 2007; Darvishzadeh et al., 2008). These two methods are mutually complementary (Viña et al., 2011); it is difficult to obtain the optimal parameterized solutions for RTMs (Fang et al., 2003). Consequently, VIs have been proposed as better predictors of ChD and are used more extensively than RTMs due to their straightforward mechanisms and efficient computations (Viña et al., 2011; Delegido et al., 2013). VIs based on the red and near-infrared (NIR) wavebands, such as the ratio vegetation index (RVI), difference vegetation index (DVI) and normalized difference vegetation index (NDVI), have demonstrated close relationships with chlorophyll parameters (Hansen and Schjoerring, 2003; Broge and Mortensen, 2002). Moreover, canopy hyperspectral reflectance is always affected by the biophysical characteristics of vegetation, canopy shape and structure, atmospheric absorption and scattering, and soil backgrounds (Thenkabail et al., 2000; Ju et al., 2010). To capture more effective information from the canopy spectra, derivative spectra integrating two optimal bands were applied to minimize the influences of background interference and spectral noise (Li et al., 2013). Several studies showed that the first derivative reflectance (FDR) was sensitive to crop chlorophyll (Cho and Skidmore, 2006). Feng et al. (2008) reported that the ChD was highly related to the radio of the red-edge integral areas and the blue-edge integral areas (SDR/SDb). Liu et al. (2000) also demonstrated that the first derivative spectrum at 740–760 nm was highly correlated with ChD in rice.

The red-edge (680–760 nm), a special transition region where there is an abrupt increase from low red spectral reflectance to high infrared spectral reflectance, is caused by strong pigment absorption near 680 nm and high multiple canopy scattering at 760 nm (Zhu et al., 2014; Smith et al., 2004). Researchers have indicated that the red-edge region is less sensitive to the impact of the atmospheric environment and soil background (Clevers, 1999; Mutanga and Skidmore, 2007) and provides more accurate information for assessing nutritional status and developmental stages (Zhang et al., 2015). Numerous spectral parameters have been developed using red-edge reflectance to quantify the variation of chlorophyll concentration. The most widely applied chlorophyll indices in this region are (i) spectral variables based on spectral position and area, such as the REP (red-edge position) (Pinar and Curran, 1996) and REA (red-edge amplitude) (Ju et al., 2010) and (ii) certain classical pigment spectral indices, e.g., MCARI (Modified Chlorophyll Absorption in Reflectance Index) (Daughtry et al., 2000), CARI (Chlorophyll Absorption Ratio Index) (Kim et al., 1994), SIPI (Structured Independent Pigment Index) (Peñuelas et al., 1994) and three-band VIs (Sims and Gamon, 2002; Dash and Curran, 2007). Many investigators have shown that REP can be used to diagnose the chlorophyll content (Pinar and Curran, 1996; Mutanga and Skidmore, 2007) because an increasing chlorophyll concentration broadens the absorption feature centered around 680 nm, resulting in shifts in REP to longer wavebands (Dawson and Curran, 1998). However, most of the published literature primarily refers to the classical VIs for estimating the chlorophyll content, and few studies have used area-based parameters for monitoring the ChD status, such as a population-index. Area-based VIs contain a large amount of spectral information and have a simple structure. In addition, red-edge area parameters based on the first derivative spectral reflectance could potentially minimize background interference, resolving overlapped spectra and enhancing the signal-to-noise ratio (Demetriades-Shah et al., 1990; Cloutis, 1996). It is important to develop new red-edge area spectral parameters to further improve the robustness and accuracy of assessing crop ChD using hyperspectral data.

Oilseed rape, the second largest oil crop produced around the world, not only provides edible oil but is also an important source of biodiesel (Kim et al., 2013). As a major indicator of nutrients, ChD is involved in various biochemical and physiological processes that are vital for oilseed rape production (Avice and Etienne, 2014). Real-time and non-destructive assessment of oilseed rape ChD is important for evaluating crop productivity and improving the precise management of N. The objectives of our study are as follows: (1) to develop new spectral parameters using the red-edge of canopy spectral reflectance that strengthens the robustness of ChD measurements in winter oilseed rape; (2) to compare the suitability and general applicability of the existing spectral parameters and newly-developed red-edge area parameters; and (3) to establish quantitative monitoring models for estimating ChD.

2. Materials and methods

2.1. Experimental site

Field experiments were conducted in Wuxue city (30°06′ N, 115°35′ E), Hubei province, central China (Fig. 1a) from September 2013 to May 2014 and September 2014 to May 2015. The experimental site is situated in a subtropical monsoon climate zone with a mean annual precipitation of 1358 mm and a mean annual temperature of 16.4 °C. The soil chemical characteristics (0–20 cm soil layer) of the experimental site are shown in Table 1.

2.2. Experimental design

The treatments were set up in a randomized complete block design with 3 replications. The individual plot area was 20 m^2 (10.0 m × 2.0 m). The detailed N fertilization treatments for winter oilseed rape in 2013–2014 and 2014–2015 were as follows: (i) no N application (N0); (ii) 90 kg N ha^{-1}, applied as urea (N90); (iii) 180 kg N ha^{-1} (N180); (iv) 270 kg N ha^{-1} (N270); and (v) 360 kg N ha^{-1} (N360). During the 2014–2015 growing season, three additional treatments, i.e., 45 kg N ha^{-1} (N45), 135 kg N ha^{-1} (N135) and 225 kg N ha^{-1} (N225), were applied to thoroughly study the effects of N nutrition on the plant ChD and hyperspectral reflectance properties of winter oilseed rape.

Except for N, other nutrients such as phosphorus (P), potassium (K) and boron (B) were applied in the same amounts to all the treatments. The recommended P, K and B fertilizer rates were 90 kg P_2O_5 ha^{-1}, 120 kg K_2O ha^{-1}, and 1.6 kg B ha^{-1}, consisting of superphosphate (12% P_2O_5), potassium chloride (60% K_2O), and borax (10.8%), respectively. All nutrient sources were applied as basal fertilizer one day before transplantation.

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