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Assessing nutritional status of *Festuca arundinacea* by monitoring photosynthetic pigments from hyperspectral data

Feng Yang[a], Jianlong Li[a,*], Xiaoyu Gan[a], Yurong Qian[a], Xiaoling Wu[b], Qi Yang[a]

[a] *School of Life Science, Nanjing University, Nanjing 210093, PR China*
[b] *School of Agriculture, Nanjing Agriculture University, Nanjing 210095, PR China*

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ABSTRACT

The dynamics of photosynthetic pigment concentrations and composition provide important information about plant nutritional status. For this reason, hyperspectral remote sensing techniques for quantifying plant pigments on a large scale have received much attention. The spectral absorbance properties of photosynthetic pigments are manifested in the reflectance spectra of the canopy level. This offers the opportunity of using measurements of reflected radiation as a non-destructive method for analyzing nutritional status in grass. The objectives of this study were to analyze the relationships between photosynthetic pigments (chlorophyll, carotenoids, and chlorophyll/carotenoid ratio), canopy spectral characteristics, and vegetation indices (VIs) derived from broadband and narrowband analysis in tall fescue (*Festuca arundinacea*). The overall goal was to determine a sensitive indicator for assessing grass nutritional status using hyperspectral remote sensing techniques. Canopy spectral measurements from each treatment (Control, Low fertility and High fertility) were taken in the field using a FieldSpec® FR spectroradiometer. A large number (i.e. 22,500) of two-band combinations in the Normalized Difference Vegetation Index (NDVI) and the Ratio Vegetation Index (RVI) were then used for a linear regression analysis against photosynthetic pigments. Obvious differences in spectral reflectance existed between treatments within certain wavelength regions (400–1000 nm). In addition, the techniques of derivative analysis increased the separation of grasses with different fertility levels, providing the possibility of monitoring grass nutritional status. Unlike chlorophyll and carotenoid values individually, the ratio of chlorophyll/carotenoid was strongly correlated with canopy spectral reflectance (500–730 nm) ($P < 0.01$). Further investigation of the relationships between photosynthetic pigments and traditional broadband vegetation indices suggested that wavelengths of 610–690 and 752–1000 nm, the regions of the red and NIR channels of several multi-spectral sensors in place on the current generation of earth-orbiting satellites, were not the optimum wavelengths for NDVI and RVI analysis. However, since the NDVI was closely related to chlorophyll/carotenoid ratio, using the combinations of λ_1 at 540–560 nm and λ_2 at 750–950 nm produced a coefficient of determination $R^2 > 0.75$. Thus, it was possible to map variation in grass photosynthetic pigments using hyperspectral remote sensing. This provided an insight for building new models and vegetation indices to monitor grass nutritional status by following the dynamics of the chlorophyll/carotenoid ratio.

1. Introduction

Photosynthetic pigments (chlorophyll and carotenoids) that absorb solar radiation and transfer light energy to reaction centers in photosynthesis, are of tremendous significance in the biosphere. Low concentrations of photosynthetic pigments can directly limit photosynthetic potential and hence primary production (Filella et al., 1995). Chlorophyll therefore can be regarded as one of the Earth's most important organic molecules as it is essential for photosynthesis (Blackburm, 2007). Not only is it important in photosynthesis, but the molecular structure of chlorophyll incorporates a large proportion of total leaf nitrogen. This means that foliar chlorophyll concentration can also provide an accurate means of indirectly estimating plant nutritional status (Filella et al., 1995; Moran et al., 2000) and physiological condition (Zhao et al., 2007).

Carotenoids are the second major group of plant photosynthetic pigments. This class of pigments contributes to light-harvesting and also plays a photo-protective role, preventing damage to the photosynthetic system by energetic interconversions among the xanthophylls molecules (Ort, 2001; Merzlyak et al., 2003; Gitelson et al., 2006a,b). Carotenoids too can therefore provide a great deal

* Corresponding author at: School of Life Science, Nanjing University, Hankou Road 22, Nanjing 210093, PR China. Tel.: +86 25 86214644; fax: +86 25 83302728.
E-mail address: jlli2008@nju.edu.cn (J. Li).

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of complementary information regarding the physiological status of vegetation (Young and Britton, 1990).

Photosynthetic pigment levels can be directly related to stress physiology, as concentrations of carotenoids increase and chlorophyll generally decreases under stress conditions, such as water stress, heat stress or disease (Peñuelas and Filella, 1998; Dobrowski et al., 2005). Changes in the chlorophyll/carotenoid ratio therefore can be a good indicators of stress levels in plants (Netto et al., 2005). Consequently, the nutritional status and physiological stress level can be assessed by following the dynamics of photosynthetic pigment concentrations.

In a pasture setting, measurement of pigment ratios can be viewed as a means for understanding pasture nutritional status. Traditional methods of wet chemical pigment for analyzing pasture nutritional status are time consuming and expensive. They require detailed sampling and laboratory analysis, and result in the collection and analysis of inadequate data that are not representative of the plant population, if large areas are to be investigated (Foley et al., 1998). In contrast, remote sensing technology offers a noninvasive, rapid technique for predicting plant physiological and nutritional status, thereby reducing the tedious process of intensive sampling and laboratory analysis (Gitelson et al., 2006a,b).

Due to its narrow and continuous spectral bands, hyperspectral remote sensing is now becoming a key tool for detecting important information about the spatial and temporal dynamics of nutritional status in vegetation. In terms of pasture land and forage grasses, it is providing important contributions to scientific investigations and particularly for applied pasture management (Numata et al., 2007). In order to map pasture nutritional status and quality, the underlying principle is that pastures with varying nutrient levels will differentially reflect specific wavelengths due to the dynamic changes in the photosynthetic pigments of the plants comprising the pasture.

Most of the existing research has concentrated on pigment indices such as green and red-edge chlorophyll indices, which have been proposed as a means of solving the problems of the overlapping absorption spectra of different pigments (Chappelle et al., 1992; Gitelson et al., 2005). In addition, Datt (1998) and Gitelson et al. (2003) have analyzed the linear relationship between plant pigment contents and reflectance. Chlorophyll content and reciprocal reflectances have a high linear correlation in the green and red-edge spectral regions (Gitelson et al., 2003). These studies were primarily focused on chlorophyll, with less attention paid to carotenoids and chlorophyll/carotenoid ratios (Peñuelas et al., 1995; Gitelson et al., 1996, 2006a,b; Datt, 1998; Merzlyak et al., 1999; Zarco-Tejada et al., 2005). Moreover, these studies also did not address grass nutritional status through changes in photosynthetic pigments. Relatively little research has been focused on predicting nitrogen concentration or macronutrients levels (nitrogen, phosphorous, potassium, calcium and magnesium) derived form hyperspectral remote sensing as a means of mapping variations in pasture quality (Mutanga et al., 2003, 2004).

The C:N ratio is also considered to be a measure of canopy pasture quality and this also can be assessed using aerial hyperspectral imagery (Phillips et al., 2006; Beeri et al., 2007). However, at this point in time, no studies have attempted to discriminate between cool-season grasses with different nutrient levels using their reflectance spectra at canopy scale, or to estimate grass nutritional status by photosynthetic pigments according to specific spectral channels that are sensitive to variation in photosynthetic pigments in grass canopies.

Tall fescue (*Festuca arundinacea*) is a predominant cool-season perennial grass grown on football fields and golf courses, and is also an important forage grass species. The temperature ranges for tall fescue cultivation are between 18 and 36 °C (Lu et al., 2008). Because of its excellent agronomic characteristics (Bacon, 1995; Al-Faraj et al., 2001), tall fescue is the most widely planted grass in transition zone pastures. In recent years, it has been extensively planted in central eastern China and temperate regions for both turf and forage (Yu and Lin, 2006).

In this study, the aims were: (1) to investigate the changes in tall fescue photosynthetic pigments (chlorophyll, carotenoids and chlorophyll/carotenoid ratios) and canopy spectral reflectance under different fertility levels; (2) to analyze the relationships between grass photosynthetic pigments and spectral reflectance data to determine the optimal region of spectrum and spectral characteristics; (3) to compare VIs derived from narrow and broadband red-NIR spectral data for photosynthetic pigments analysis, and to analyze the consistency of the optimal regions of the spectrum. Lastly, grass nutritional status was assessed using the optimal spectral region, vegetation index, and photosynthetic pigment measurements. To address these aims, the response of the spectrum at canopy level to a variation in the fertility supply and the concentrations of photosynthetic pigments (chlorophyll and carotenoid) were measured.

2. Materials and methods

2.1. Plant preparation and experimental design

Seeds of tall fescue (*F. arundinacea*) were sown and grown in mixture of soil, sand and organic matter (3:1:1, v/v/v) in polyethylene pots (20 cm in diameter and 15 cm in height, with 1 g of seeds in each pot), and kept in natural conditions with a night temperature of 20 °C and a day temperature of 30 °C. The plants were watered at least once every day, and kept at height of 14 cm by clipping weekly.

After four weeks, the pots were randomly divided into three equal groups and arranged in a randomized block design on 7 May 2008. To create a variation in foliar concentrations of photosynthetic pigments, the first group (designated as low fertilizer) was supplied with 50 kg/ha of compound fertilizer (N:P:K, 10:9:9) per pot. The second group (designated as high fertilizer) was supplied with 150 kg/ha of compound fertilizer per pot. Fertilizer was supplied once a week. No fertilizer was added to the control group. The pots were rotated once a week to minimize any effects of microclimate on the experiment.

2.2. Canopy spectral measurements

A 512-channel spectroradiometer (from 350 to 1050 nm) manufactured by Analytical Spectral Devices™ (ASD, FieldSpec® FR) was used to collect tall fescue canopy spectral data. The sensor's field of view was 15°. Noise at both ends of the spectrum limited the useful data range to between 400 and 1000 nm in this study. Data were collected on cloudless days with solar elevation angles ranging from 50° to 55°, in order to minimize external effects from the atmospheric conditions and changes in solar position.

Spectral measurements were taken at the beginning of every week for four weeks from May 15 to June 20, 2008. Measurements were taken from this date and the grass had grown to an extent that it largely covered the soil, thus minimizing the background effects. The spectroradiometer was held at nadir angle at 60 cm above the canopy, resulting in a 15 cm diameter footprint on the ground. The pots were rotated by 90° after every third measurement. Each spectrum was determined as the mean of 12 spectral measurements per pot. Spectral reflectance was calculated as the ratio of measured radiance to the radiance from a white standard reference panel (Labsphere spectralon panel). Immediately after the white standard radiance measurement, spectral of grass canopy per pot were obtained.