



## Research paper

## Using remote sensing to estimate forage biomass and nutrient contents at different growth stages

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## ABSTRACT

Lignocellulosic biomass is an important feedstock for the second generation bioenergy production. A sustainable supply of biomass feedstock having consistent composition is critical for a biorefinery. This requires a timely monitoring and estimation of the biomass yield and composition in the field. It is not clear if one can use the near infrared (NIR) vegetation canopy reflectance measured in the field and build a calibrate model to estimate biomass yield and nutrient contents (compositions) for the vegetation from vegetative growth through dormancy stages. In this study, the NIR canopy reflectance of a grass/legume mixture was measured in a field with a spectroradiometer with wavelength ranging from 400–2500 nm. The plants were then clipped, dried, and ground to measure the biomass yield, neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP). The measurements were conducted at the boot, peak growth, and at dormancy stages. Partial least significant regression (PLSR) models were built using data from each individual growth stage as well as all stages combined. Except for the yield model at peak growth stage and the NDF and ADF models at dormancy stage, models developed from each of the individual stages generally estimated the yield, CP, NDF, and ADF poorly, with  $R^2_{cv}$  ranging between -0.31 and 0.42. When data from all three growth stages were included, the accuracy of all models was greatly improved, with  $R^2_{cv}$  ranging between 0.77 and 0.80. Furthermore, multiple linear regression (MLR) models developed with 7–9 most significant wavelengths selected from 400–2500 nm estimated the yield, ADF, NDF, and CP equally well compared with the PLSR models. The estimates from MLR model showed strong correlations between the measured and estimated values, with  $R^2$  of 0.72, 0.67, 0.78, and 0.66 for the yield, ADF, NDF, and CP, respectively. These results indicate that biomass feedstock yield and composition can be estimated by the in-situ NIR canopy reflectance using a multiple linear regression model with 7–9 wavelengths. Further calibration is needed to use the model to other geological locations.

## 1. Introduction

The second generation of biofuels, such as bioethanol and hydrocarbons, are derived from lignocellulosic biomass instead of grain-based starch [1], and a consistent supply of lignocellulosic biofuel feedstock with uniform quality is essential for a bio-refinery. The concentration of cellulose, hemicellulose, and lignin in the biomass feedstock will determine the conversion rate and ethanol yield [2]. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude protein (CP) have long been used as parameters to assess the available energy values and digestibility of forage for ruminant animal feed [3–5]. Because the energy conversion from the lignocellulosic feedstock to biofuels by yeast fermentation is dependent on the degradability of the

feedstock, researchers have been using ADF and NDF as the criteria to evaluate the digestibility of biofuel feedstocks and to estimate the cellulose and hemicellulose concentrations, and bioethanol yield from the feedstock [6,7].

Traditional methods are time consuming or costly for the NDF and ADF measurements, which takes several steps including sample collecting, drying, grinding, and wet chemical assay [5,8,9]. Recently, near infrared reflectance spectroscopy (NIRS) technology has been developed as a rapid and low-cost method for analysis of ADF and NDF [10–12] or biochemical compositions [13–15] of the biomass feedstock. The NIRS technology is based on partial least squares regression model built on the near infrared reflectance of biomass and the actual wet chemical analysis of biomass compositions [16], and many good models

*Abbreviations:* ADF, Acid detergent fiber; NDF, Neutral detergent fiber; CP, Crude protein; NIRS, Near infrared spectroscopy; PLSR, Partial least significant regression; MLR, multiple linear regression; NDVI, normalized differences vegetation index

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or calibration curves have been developed for benchtop NIRS analysis of biomass compositions.

Although the benchtop NIRS skips the wet chemical assay, it still requires collection, drying, and grinding of the biomass samples for NIR scan. There is a need to develop a quick and non-destructive method to estimate biomass feedstock yield and compositions in the field. Because plant biomass yield and composition change during the growing season [17–19], and the biomass feedstock producers and the bio-refinery managers need to have a simple, quick, and non-destructive technology to monitor the biomass yield and composition (quality) throughout the plant growing season and determine the good harvest timing for optimum yield and quality. Field remote sensing technology may provide a real-time non-destructive estimate of forage biomass and quality efficiently in the field. Several studies have documented the use of remote sensing technology to estimate forage productivity and quality of the pastures and range lands for livestock or wildlife grazing [20–23] as well as for estimate of forest aboveground biomass [24].

Plant vegetative indices, such as the normalized difference vegetation index (NDVI), calculated from the leaf or canopy spectral reflectance data have proved to be useful in estimating aboveground vegetative biomass and quality [24–26]. Albayrak et al. [25], for example, used a portable spectroradiometer to collect the vetch (*Vicia* spp.) canopy reflectance data from 325 to 1150 nm wavelength at 1 nm intervals. The spectral reflectance data were combined into four broad wavebands, i.e. blue (450–520 nm), green (520–600 nm), red (630–690 nm) and near infrared (NIR; 760–900 nm). Two reflectance indices were then calculated: ratio of  $R_{\text{NIR}}/R_{\text{Red}}$  and the NDVI  $(R_{\text{NIR}} - R_{\text{Red}}) / (R_{\text{NIR}} + R_{\text{Red}})$ . Results showed that both  $R_{\text{NIR}}/R_{\text{Red}}$  and NDVI could adequately predict ADF and NDF concentrations of the vetch species at the beginning of the flowering stage and throughout the seed-filling stage. Hansen and Schjoerring [26] used a spectrometer with wavelength range from 400 to 900 nm with 1 nm intervals to measure wheat canopy reflectance during the vegetative growth stage from early stem elongation until heading. Green wheat samples were also cut and brought to laboratory to determine biomass yield and nitrogen content. The authors concluded that selection of correct wavelength and bandwidths is important; short bands performed better than broadbands, and selection of the optimal waveband combination in NDVI improve the prediction. Lawrence and Ripple [27] found that modeling biomass with bandwise regression explained more variability than models based solely on NDVI.

It is well known that the vegetation at different growth (or phenological) stages differs greatly in morphology and in the biochemical content and structure. At the maturity stage, for example, the stem to leaf ratio and the amount of lignin, cellulose, and hemicellulose increase, and the amount of chlorophyll decreases at the same time [28,29]. Therefore, the spectral responses of plants are likely changed during different growth stages [30–32]. In addition, environmental factors also have great impact on remote sensing measurements [33]. Knox et al. [23] used a Carnegie Airborne Observatory Alpha sensor with a spectral range from 350 to 1058 nm to map the forage nutrients in the dry season in an African Savanna. They found that in order to improve the accuracy of the mapping of forage quality in the dry season (matured vegetation), the spectra within the shortwave infrared region should be included. Similarly, Porter et al. [34] used a hand-held Crop Circle sensor and Landsat satellite images to model pasture biomass and found that the accuracy of biomass estimation was correlated with certain spectral regions at different phenological growth stages. The red, red edge and the near-infrared regions were more responsive at the boot and peak growth stages, while shortwave infrared region was more responsive at the dormancy stage. We expected that a multiple linear regression (MLR) model with several wavelengths is likely estimating the biomass yield and quality better than a single vegetative index, such as NDVI.

To date, many researchers have estimated forage biomass and quality or nutrient status with different remote sensing methods.

However, most of the studies only measured the canopy reflectance on a short growing period of the forage, such as in July or August [35,36], or on several measurement dates during the vegetative growth stages [25,36–39]. Up to now, applying a model from one stage to another stage has not been well documented, especially for the dormancy stage. Knox et al. [33] combined ecological and spectral absorption data to model forage nutrients of a grassland-savanna ecosystem in wet and dry seasons. They found difficult to create a temporally universal prediction model and suggested that additional variables are needed for the model to apply to different seasons.

The objective of this study was to investigate the feasibility of conducting a non-destructive remote sensing with NIR canopy reflectance and employing a universal regression model to all growth stages to estimate the biomass yield. CP, ADF and NDF contents in the field. A simple, accurate method for quick, nondestructive, and real-time estimation of biomass yield and forage quality across growth stages would be very beneficial to feedstock producers, farm managers, and biofuel refineries.

## 2. Methods

### 2.1. Site description

This study was conducted during the 2012 growing season on a conservation reservation program (CRP) pastureland at Benchland, Montana, near Montana State University Central Agricultural Research Center (47°05'21" N, 110°00'44" W, elevation 1400 m). The site has an annual precipitation of 400 mm and annual mean temperature of 7.1 °C. The pastureland has a fine clay loam soil [17], and the vegetation is a uniform mixture of grass and alfalfa (*Medicago sativa*) (70% grass: 30% alfalfa). The pasture was planted in 2003 and the grass community is dominated by intermediate wheatgrass (*Thinopyrum intermedium*) and pubescent wheatgrass (*Thinopyrum trichophorum*), with small amount of tall wheatgrass (*Thinopyrum ponticum*). The experiment was a split-plot design with three replications. The main plots were two harvest timings (peak growth (flowering) stage and dormancy stage) with three replications; the subplots were three nitrogen (N) rates (0, 56, and 112 kg ha<sup>-1</sup> nitrogen) in the form of urea. The urea was applied by broadcast in the spring of 2012, which created different biomass growth and nutrient concentrations in the pasture. The plot dimensions were 91 m × 20 m. Within each plot, three 1-m<sup>2</sup> representative (with mixture of alfalfa and grass) quadrats were randomly selected for canopy reflectance measurement and biomass sampling.

### 2.2. Measurements

Canopy reflectance measurements were performed on a clear-sky day at the growth stages of boot stage (mid May to early June), peak growth (late June to early July), and dormancy (Mid to late October), respectively, using a portable ASD FieldSpec FR spectroradiometer (Analytical Spectral Devices, Boulder, CO, USA). The instrument was equipped with a fiber optic cable and accompanied with a fore optic accessory. The instrument takes spectral measurements from 350–2500 nm wavelength range at 1-nm sampling intervals. The spectral measurement was performed from a height of approximately 1 m above the grass canopy with a 25° field-of-view fore-optic. Prior to spectral measurement of each plot, calibration was done to reduce solar variation by using a spectralon (Labsphere, Sutton, NH, USA) reference panel (white reference). Each plot was measured three times and each measurement consisted of the internal averages of 10 scans through the RS<sup>3</sup> Spectral Acquisition Software.

After three measurements were taken in each plot, the vegetation of the 1 m<sup>2</sup> quadrat within the ASD field-of-view was immediately hand clipped to 2.5 cm above the ground level. The clipped vegetation was dried at 40.6 °C for 72 h in an air forced oven. Total dried vegetation was then weighed to calculate the biomass yield in kg ha<sup>-1</sup>. The dry

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