

Using Canopy Hyperspectral Reflectance to Predict Root Biomass Carbon and Nitrogen Content

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Abstract

Full assessment of soil carbon (C) and nitrogen (N) pools is necessary for long-term sustainability of agricultural production and provides information on plant health and nutrient cycling. A major component of nutrient cycling is plant root C and N. Although root C and N contribute to nutrient cycling, determination of these quantities is laborious and tedious and is, therefore, not commonly done. In this study we attempt to determine the feasibility of using remotely sensed canopy reflectance as a proxy to determine root C and N data of live, standing forages. The study site was the United States Department of Agriculture-Grazinglands Research Laboratory located in El Reno, Oklahoma. Twelve plots in each of two sites (a native, tallgrass prairie and an improved, Old World Bluestem pasture) were used for collection and measurement of root C and root N and measurement of canopy reflectance using a field portable hyperspectral spectroradiometer. Root and soil samples were then taken from under the remote sensed area for total C and N analysis using the combustion method. The results of this study indicated that it is feasible to predict root C and N, but further study is required to improve model accuracy.

Keywords: Canopy reflectance, forage, remote sensing, root biomass carbon, root biomass nitrogen

1. Introduction

Van Ginkle et al. (1996) indicated that forage tissue nutrients in grassland aboveground biomass is shuttled to belowground biomass storage in the roots. This study reaffirmed previous research that 20-50% of all photosynthates, mainly carbon containing molecules, made in the aboveground biomass are transported to the belowground biomass (Merckx et al., 1986; VanVeen et al., 1989; Meharg & Killham, 1990; Swinnen et al., 1995). Additionally, root C and N is altered by climatic and abiotic factors, such as soil texture, pH, water holding capacity, and temperature (Avicé et al., 1996, Bouma et al., 1997, Gavito et al., 2001, Johnson et al., 2006, Scheffer and Aerts, 2000). Upon decomposition, C and N stored in the roots can return to the soil profile (Scheffer & Aerts, 2000).

Grasslands, which account for about 26% of the Earth's land area and about 70% of total agricultural area (Ramankutty et al., 2008; Ramankutty & Foley, 1999; FAO, 2010), are known to have root biomass equal to, or greater than, the mass of the aboveground forage (Yang et al., 2010). Robinson (2004) analyzed the allometric scaling equation proposed by West et al. (1997; 1999), and found that for relatively small plants, like grasses, the equation indicated that prediction of root mass was an isometric function of aboveground mass. Frank et al. (2010) also showed a strong (but non-linear) relationship between shoot biomass and root frequency of grasses growing under both dry and mesic conditions in Yellowstone National Park. Cheng et al. (2015) investigated the allometric partitioning theory on the above- and below-ground biomass in understory tropical plants (n = 1586), and noted a strong statistical relationship between the two variables. Moreover, this relationship was nearly isometric. Similarly, there is a strong relationship between leaf concentrations of N and that found in the roots. Kerkhoff et al. (2006) conducted an analysis on a large data set (n = 1287 plant species) containing N concentration of leaves, stems, roots, and reproductive structures of both woody and herbaceous species. They showed that a statistically significant relationship exists between the root N and that in the plant's leaves.

“Grasslands” is a general term and includes rangelands, native grasslands, and pastures, which are typically used to provide forage for grazing animals. Unlike rangelands and native grasslands, pastures are typically intensively managed (e.g., fertilized) and consist of forages selected and managed (e.g., by applying herbicides and fertilizers) to support animal-based agriculture. Agriculturalists that base their efforts on managed grasslands are, or will, face increasing pressure to maintain profitability in the face increasing environmental regulations. This is exemplified by Knoblauch et al. (2017) who noted that changes in the European milk market coupled with European environmental directives are forcing dairy farmers to improve pasture/forage production while at the same time reducing nutrient loads, primarily N, to the environment. To this end, Knoblauch et al. (2017) used vegetation indices determined from remotely sensed data collected from managed grasslands to investigate possible relationships between N fertilizer levels, plant biomass, and N dynamics. These researchers found that the Normalized Difference Vegetation Index (NDVI) and Simple Ratio (SR) were moderately related to plant biomass, but could not be used to link plant biomass to soil N processes.

However, it has been demonstrated that hyperspectral canopy reflectance data can be used to determine selected forage quality variables, including N (Wessman et al., 1988; Starks et al., 2004; Starks & Brown, 2010, Starks et al., 2016). Because of the connection between C and N in the canopy and that in the roots, it is hypothesized that hyperspectral canopy reflectance could serve as a proxy to estimate the concentrations of root C and N in standing, live forage. Such an approach would provide additional information concerning potential root contributions to the soil nutrient pool, thus allowing better management of chemical inputs and to reduce unintended inputs of N to stream and groundwater resources. Thus, our objective is to examine the feasibility of using hyperspectral canopy reflectance to predict root C and N.

2. Material and Methods

2.1 Site Description

This study was conducted at the Grazinglands Research Laboratory in El Reno, Oklahoma (35°34'4.19"N, 98° 3'36.22"W; 414 m above sea level) during May to August 2015 and May to August 2016. Precipitation in the study area is bimodal with rainfall occurring in May-June and October-September. The months of July and August are typically hot and dry. The 30-year normal (NCDC, 2017) monthly average precipitation is 65.8 mm, while the 30-year normal daily high temperature during May to August is 33.2°C, and the low is 21.2°C.

Two perennial forage systems were used in this study. One system was a large field consisting of an undisturbed, native tallgrass prairie (TGP) composed of a mixture of Big bluestem (*Andropogon gerardii* Vitman.), Little bluestem (*Schizachyrium scoparium* (Michx.) Nash), Indiangrass (*Sorghastrum nutans* (L) Nash), and Switchgrass (*Panicum vergatum* L.). This field is gently sloping ($\approx 1\%$) with an eastward aspect. The other forage system was located on a gently sloping ($\approx 2\%$) field with a westward aspect and consisted of a monoculture of Old World Bluestem (OWB; *Bothriochloa ischaemum* (L) Keng). Both the TGP and OWB fields were subjected to grazing by stocker cattle with animal numbers and grazing bouts based on visual inspection and measurements of biomass availability. The OWB site was established over ten years before the study began and is annually fertilized with 89.7 kg per hectare urea and treated with Grazon® (Dow AgroSciences, Midland, MI, USA) at 946.4 mL per acre. Fertilizer and herbicide treatments were not applied to the TGP field. The soil type for the research area was Bethany silt loam (a fine, mixed, superactive, thermic Pachic Paleustolls). Physical properties of the soil in the research area are presented in Table 1.

Table 1. Soil properties of the forage systems used in this study

Soil Property	Units	TGP	OWB
Texture	--	Silt Loam	Silt Loam
Bulk density	g cm ⁻³	1.4 (0.03)	1.4 (0.02)
Total Carbon	%	1.8 (0.1)	1.7 (0.1)
Total Nitrogen	%	0.2 (0.01)	0.2 (0.01)
pH	--	5.9 (0.4)	5.8 (0.2)
Organic matter	%	2.0 (0.32)	2.0 (0.28)
Electrical Conductivity	μS cm ⁻³	357 (28)	299 (54)

Note. TGP=Tallgrass prairie; OWB=Old World Bluestem. Values in parentheses are standard errors (n = 12 for each forage system).

2.2 Sampling Design

Four east-west transects, spaced about 100 m apart, were laid out in each field. Along each transect a 15 m² sampling area was established at the top, middle, and toe of the slope. Thus, each slope position was replicated four times in each field. For this study it was assumed that variations in topographic aspect and slope, forage type, fertilizer vs. no fertilizer treatment, and the effects of grazing (urine and fecal patches) would induce variations in root biomass C and N concentrations. Each replicate in each field was sampled on 6 and 25 June and 14 July in 2015 and on 6 and 22 June and 15 July in 2016; thus, n = 144 samples. For ease of reference below, we refer to the early June sampling dates of both years as “sampling date 1”, the late June sampling as “sampling date 2”, and the July sampling as “sampling date 3”.

2.3 Sampling Protocols

2.3.1 Remotely Sensed Data

Before root collection, canopy spectral reflectance data were collected in three replicates in each plot using an ASD field portable hyperspectral spectroradiometer (PANalytical, Inc., Westborough, MA). The spectroradiometer had a 25° field-of-view and the spectra were acquired 0.5 m above the canopy, producing a view diameter of about 22 cm. This view area typically represented the reflectance from a single plant or small group of identical plants. Prior to collecting the canopy spectra, the spectrum of a white reflectance panel (Labsphere Inc., New Hampshire, USA) was collected. Division of the canopy reflectance by the panel reflectance yielded a reflectance factor (RF) spectrum. The three RF spectra for each replicate were averaged and then converted into an absorbance spectrum via:

$$Absorbance = \log\left(\frac{1}{RF}\right).$$

2.3.2 Root Sampling and Preparation

Root samples were removed from the ground in a 15 x 15 x 15 cm block of soil (containing the plants measured by the spectroradiometer) from each of the 12 plots in the TGP and OWB fields. The soil block was placed in a bag, labeled and put on ice, transported to the laboratory, and stored at 4°C until processing.

Root processing consisted of removing large and fine roots from the soil block from each sampling point. Roots were picked out of the soil, washed, and dried at room temperature for one week. After the roots were dry, the sample was ground in a Wylie Mill to pass through 2 mm mesh sieve. Each sample was then weighed into approximately 2 g samples, wrapped in tin foil, and analyzed using a combustion analyzer (Carlo Erba Elementar, MAX, Milan, Italy) for C and N content.

2.4 Statistical Analysis

Statistical analysis of measured root C and N content was conducted using a mixed model procedure in R (R Core Team, 2017) to compare means (ANOVA) between forage systems and to evaluate possible impacts of sampling date, slope aspect, slope position, and their various interactions. Statistical significance of treatment effects was determined at $p = 0.05$. Contrasts were used to determine the effect of forage system and date on root C and N content.

The spectral absorbance data for a given replicate were paired with their respective measured root C and N values. The data pairs (n = 144) were entered into JMP Pro 13 (SAS Institute, Cary, NC) to develop calibration equations relating C or N to the spectral data using either recursive partitioning (RP) or artificial neural networks (ANNs). Recursive partitioning is a data mining technique in which the data are partitioned according to relationships between the predictors and a response variable; thus, creating a decision tree. The partitioning continues recursively until a desired level of fit is achieved. Artificial neural networks seek to associate a combination of all predictors with the response variable through a set of “hidden neurons” whose number is based upon the number of predictors and responses. The neurons learn through trial and error as the number of cases is presented to them and some level of learning is achieved. Because this was an exploratory study which produced a smaller than desired data set, we chose to use all of the data to construct and then evaluate the calibration equations using k-fold cross-validation. In k-fold validation, the calibration data set is randomly divided into 5 (in this case) somewhat equally-sized data sets. Four training data sets are used to construct a calibration (sometimes called prediction) equation, which is then used to predict the response variable in the test data set that was withheld. The error of cross-validation statistics are then determined and averaged as the algorithm cycles through the remaining data sets. The statistics used herein to evaluate the performance of the cross-validation are the root mean square error (RMSE) and/or the coefficient of determination (r^2).

3. Results

3.1 Weather Conditions

The average daily high and low temperatures from May to August in 2015 were 25.2 and 22.3°C, respectively, and the total precipitation during this time was 161 mm. In 2016 the average high and low temperatures over the same time period were 26.4 and 21.6°C, respectively, and the total precipitation was 126 mm.

3.2 Measured Root Carbon Content

Variation in root biomass C was not dependent upon slope position, sampling date or forage system ($p > 0.5$), specifically but did have significant interactions (Table 2). In fact, year was significant ($p \leq 0.5$) and interacted strongly with slope position and forage system, but not to the level of being statistically significant ($0.06 < p < 0.47$).

Table 2. Results of ANOVA for root biomass carbon (root C)

	Sum of Squares	Df	F-Value	p-Value
Y	436.68	1	54.16	≤ 0.05
SP	4.59	2	0.28	0.75
SD	10.97	2	0.68	0.51
FS	1.30	1	0.16	0.69
Y:SP	47.27	2	2.93	0.06
Y:SD	0.01	1	0.01	0.98
SP:SD	10.85	4	0.34	0.85
Y:FS	0.04	1	0.01	0.95
SP:FS	142.64	2	8.85	≤ 0.05
SD:FS	39.02	2	2.42	0.10
Y:SP:SD	12.44	2	0.77	0.47
Y:SP:FS	31.98	2	1.98	0.15
SP:SD:FS	65.11	4	2.02	0.10
Residuals	564.39	70		

Note. Y=year; SP=slope position; SD=sampling date; FS=forage system; DF=degrees of freedom. Table also has the effect of each interaction. n=144.

Sampling date did not significantly affect root c ($p=0.51$). Slope position was not significant to root c either ($p=0.75$). An interactions containing the variables slope position with forage system was significant ($p \leq 0.05$). Table 3 gives the mean root C values by slope position, forage system, and year. From Table 3 it is observed that the middle and toe slope positions in the TGP forage system have statistically similar mean root C values in 2015 and that these values are higher (statistically) than that observed at the top of the slope. In 2016, root C is about 3 to 7% higher (absolute) along the slope positions than observed in 2015.

Table 3. Least squares means of root biomass C as a function of slope position, forage system and year. Means in column within a year having differing superscript letters are significantly different at $p > 0.05$

	Mean Root Biomass C	
	2015	2016
<u>TGP</u>		
Top Slope	34.58 ^b	41.72 ^b
Middle Slope	37.91 ^a	42.80 ^a
Toe Slope	37.12 ^a	40.98 ^b
<u>OWB</u>		
Top Slope	38.35 ^a	42.38 ^a
Middle Slope	36.25 ^b	36.68 ^b
Toe Slope	36.93 ^b	41.64 ^a

Note. TGP=Tallgrass prairie; OWB=Old World Bluestem.

In 2016, however, the mid-slope position has the highest root C value and the top and toe slope positions are lower and statistically similar to each other in TGP. In the OWB forage system in 2015, the middle and toe slope positions had statistically similar root C values, which were lower than that observed at the top of the slope. In 2016, the top slope position again had the highest root C value followed closely by the toe slope positions. The mid slope position had both statistically and practically lower root C values.

Figure 1 is a box-and-whisker plot of root C as a function of forage system, slope, position, and sampling date. In Figure 1, sampling date represents the respective sampling time of both years, as explained earlier. From Figure 1 it is observed that root C varied considerably: from $\approx 24\%$ at the top of the slope in the TGP forage system during sampling date 1, to $> 40\%$ in both fields at various slope positions and sampling dates.

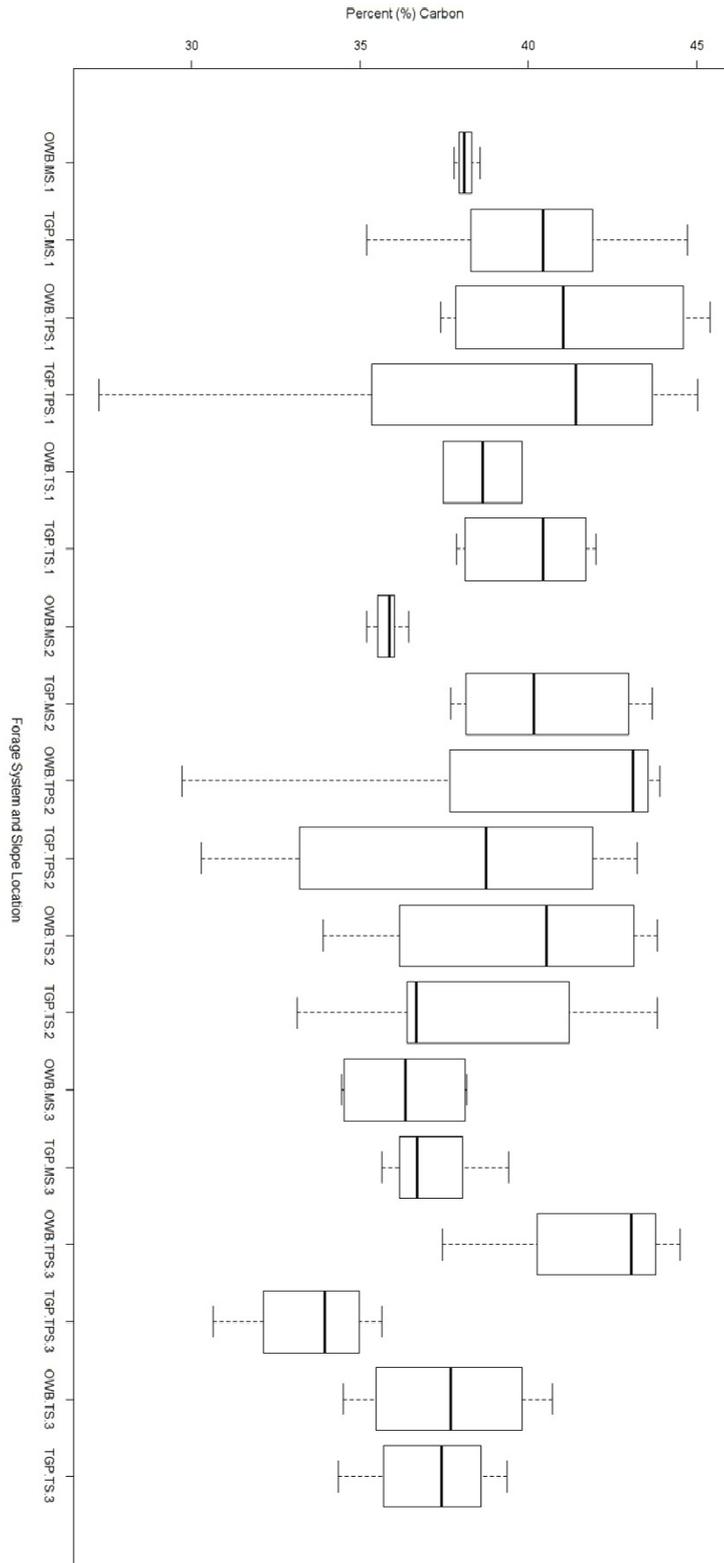


Figure 1. Root biomass percent C as a function of forage system (TGP = tallgrass prairie, OWB = Old World Bluestem), slope position (TPS = top of slope, MS = middle of slope, TS = Toe of slope), and sampling period (1, 2, or 3). (Note: sampling period 1 = the early June sampling in both 2015 and 2016, sampling period 2 = the late June sampling in both 2015 and 2016, and sampling period 3 = the July sampling in both 2015 and 2016)

3.3 Measured Root Nitrogen Content

Root N did not vary as a function of forage system ($p = 0.65$) or sample date ($p = 0.25$) (Table 4). However, root N did vary significantly as a function of year ($p < 0.05$), slope position ($p \leq 0.05$) and had a tendency to interact with forage system ($p = 0.07$), slope aspect and sampling date ($p \leq 0.05$), sampling date and forage system ($p \leq 0.05$), year, slope position and sampling date ($p \leq 0.05$) and slope position, sampling date and forage system ($p \leq 0.05$). Root N content in 2016 was statistically greater than in 2015 in both TGP and OWB. The first sampling showed statistically similar root N content in both forage systems (0.85%).

Table 4. Descriptive statistics for root nitrogen in studied forage systems in 2015 and 2016.

	Sum of Squares	Df	F-Value	p-Value
Y	0.36	1	14.66	≤ 0.05
SP	0.15	2	3.10	≤ 0.05
SD	0.07	2	1.399	0.25
FS	0.01	1	0.20	0.65
Y:SP	0.01	2	0.29	0.74
Y:SD	0.01	1	0.42	0.52
SP:SD	0.28	4	2.76	≤ 0.05
Y:FS	0.12	1	4.91	≤ 0.05
SP:FS	0.14	2	2.82	0.07
SD:FS	0.36	2	7.32	≤ 0.05
Y:SP:SD	0.06	2	1.18	0.31
Y:SP:FS	0.16	2	3.16	≤ 0.05
SP:SD:FS	0.43	4	4.31	≤ 0.05
Residuals	1.97	79		

Note. Y=year; SP=slope position; SD=sampling date; FS=forage system; DF=degrees of freedom. Table also has the effect of each interaction. n=144.

Table 5 gives the results of the means comparison test for root N as function of year, slope position, sampling date, and forage system. From Table 5 it is observed that in both 2015 and 2016, the top slope position in the TGP forage system had the highest root N content, and that root N decreased with lower slope position in both years. In 2015, root N tended to be similar among slope positions in the OWB forage system, but the top slope position had higher root N in 2016 as compared to the other slope positions.

Table 5. Least squares means of root biomass N as a function of slope position, forage system and year. Means in column within a year having differing superscript letters are significantly different at $p > 0.05$

	Mean Root Biomass N	
	2015	2016
<u>TGP</u>		
Top Slope	0.73 ^a	0.88 ^a
Middle Slope	0.63 ^b	0.80 ^a
Toe Slope	0.57 ^c	0.69 ^b
<u>OWB</u>		
Top Slope	0.51 ^b	0.91 ^a
Middle Slope	0.58 ^a	0.80 ^a
Toe Slope	0.60 ^a	0.81 ^b

Note. TGP=Tallgrass prairie; OWB=Old World Bluestem.

Figure 2 is a box-and-whisker plot of root N as a function of forage system, slope, position, and sampling date. From Figure 3 it is observed that root N varied from below 0.4% at the mid-slope positions in both the OWB and TGP forage systems during sampling period 2 and 3, respectively, to > 1.2% in OWB during sampling period 1 in both the middle and top slope positions.

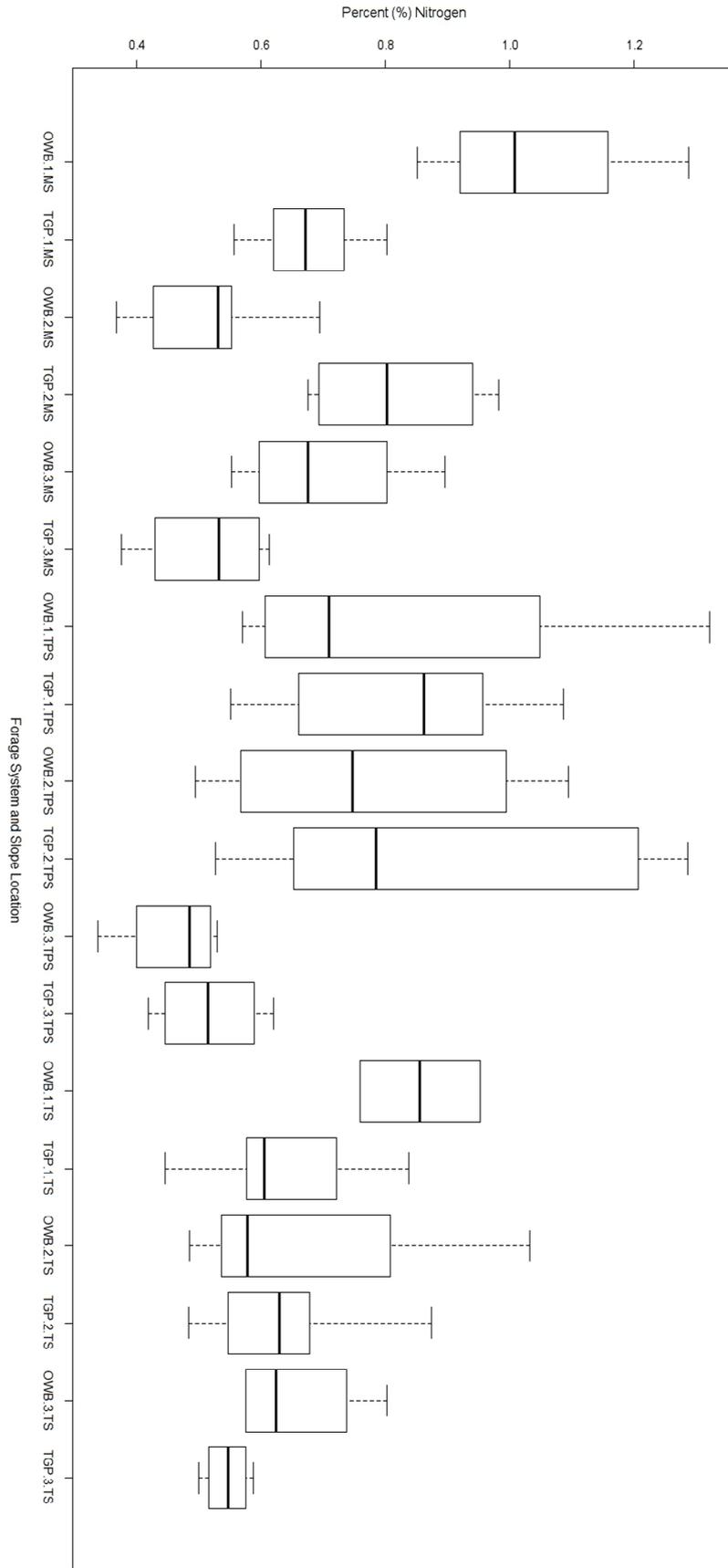


Figure 2. Root biomass percent N as a function of forage system (TGP = tallgrass prairie, OWB = Old World Bluestem), slope position (TPS = top of slope, MS = middle of slope, TS = Toe of slope), and sampling period (1, 2, or 3). (Note: sampling period 1 = the early June sampling in both 2015 and 2016, sampling period 2 = the late June sampling in both 2015 and 2016, and sampling period 3 = the July sampling in both 2015 and 2016)

3.4 Remotely Sensed Root C and N

In the calibration phase, the RP technique indicated that about 76% of the variation in measured root C was accounted for by the canopy reflectance spectra. However, during calibration, the ANN only accounted for about 23% of the variability in measured root C. In the cross-validation phase, both the RP and ANN procedures indicated that about 56% (RP) and 62% (ANN) of the variability in root C could be explained by the canopy reflectance spectra (Table 6). (In the application of ANNs it is better to have a network that tests well despite low training statistics than to have a network that trains well but does not predict well (Lawrence, 1994).) The RMSEs were from 2.2% (RP) to 2.9% (ANN) in the cross-validation.

Table 6. Calibration and cross-validation of relationships developed between the canopy hyperspectral reflectance data and other

RV	RP				ANN			
	Calibration		Validation		Calibration		Validation	
	RMSE	r^2	RMSE	r^2	RMSE	r^2	RMSE	r^2
Root C	--	0.76	2.23	0.56	3.92	0.23	2.92	0.62
Root N	--	0.78	0.09	0.67	0.15	0.47	0.09	0.80

Note. RMSE=root mean square error; RV=response variables; root C=root carbon; root N=root nitrogen; RP=recursive partitioning; ANN=artificial neural network.

In the calibration phase, the RP technique indicated that about 78% of the variation in measured root N was accounted for by the canopy reflectance spectra, whereas when the spectral were used in the ANN technique, only about 47% of the variability could be explained (Table 6). The results for root N cross-validation were stronger than those observed for root C where 67% (RP) and 80% (ANN) of the variation in measured root N was accounted for by the canopy reflectance spectra. The RMSEs were 0.67% (RP) and 0.09% (ANN). It is also observed that the calibration r^2 in the ANN was lower than that observed for the cross-validation (Table 6).

4. Discussion and Conclusions

Although the systems we included in our study represented unmanaged and managed forages (i.e., the managed system included N applications), our results showed that forage system was not a factor in inducing variation in root biomass C or N. However, large variations in these quantities were observed and were a function of both year and slope position. We deemed the variation large enough to investigate the feasibility of using hyperspectral canopy reflectance to predict root biomass C and N.

Both the recursive partitioning (RP) and artificial neural networks (ANN) indicated that, during cross-validation, from 56% to 80% of the variation in root C and N could be explained by the hyperspectral canopy reflectance data. Although the RP technique showed higher r^2 values during equation calibration, the ANN approach yielded higher r^2 (0.62 and 0.80 for root C and N, respectively) during equation cross-validation; that is, the ANN equations tested better than the decision trees developed in the RP approach. It should be noted that we did not evaluate the use of partial least squares (PLS) regression, a linear regression technique, in developing predictive equations for root C and N. This was not discussed in the main body of paper since the PLS results indicated that the r^2 s from the cross-validation were < 0.06 . This finding from the PLS, coupled with RP and ANN results, suggests that non-linear approaches like RP and ANN may be more appropriate for quantifying root C and N from canopy reflectance. Identifying the source of the non-linearity between root C and N and canopy reflectance was beyond the objectives of this study.

The finding from our preliminary study suggests that it is feasible to predict root biomass C and N via remote sensing of live forage canopies using hyperspectral canopy reflectance data. However, a more robust examination is needed to test the applicability of this approach over a wider range of root C and N concentrations, and preferably over a range of forage and agronomic crop types. Our study was performed on live standing grasses with an erectophile morphology from which canopy reflectance was measured from the nadir position. Although our measurements were made on closed canopies to minimize reflectance from extraneous sources, it is possible that reflected radiation from exposed leaf litter or soil could have contributed to the "canopy reflectance" in some cases; thus, reducing the accuracy of our calibration equations. The possible impact of litter and soil reflectance was not evaluated in our study.

In our study we used reflected radiation from plant canopies over the 400-2200 nm spectrum. We suggest that the use of hyperspectral reflectance data to assess root C and N is likely to be more robust than approaches using

current vegetation indexes typically calculated from wide-band multi-spectral radiometers on satellite-, aircraft-, and handheld- platforms. The hyperspectral data provides narrow-band data that is better related to plant chemistry. However, from an agricultural producer's point of view, hyperspectral spectroradiometers that collect data from the region noted above are often costly and not practical for use. Thus, further research is needed to isolate and minimize the specific wavebands needed to quantify the variable(s) of interest in order to reduce cost and increase likelihood of use.

Full assessment of soil C and N pools is necessary to develop methods to ensure long-term sustainability of agricultural production and to provide information on plant health and nutrient cycling. Should remote sensing of root C and N be further corroborated, it may be possible to provide a tool to agricultural producers to assist in better managing fertilizer inputs for forages and crops, thereby increasing profitability and enhancing environmental outcomes. At a larger scale, remote sensing may provide useful information regarding the state of root C and N, which could be coupled with allometric equations to assess landscape and ecosystem level nutrient cycling.

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