

Estimating tropical pasture quality at canopy level using band depth analysis with continuum removal in the visible domain

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Pasture quality, expressed as a percentage of total digestible nutrients (nitrogen, potassium, phosphorous, calcium and magnesium), is a major factor determining the grazing patterns of wildlife and livestock. Existing rangeland monitoring techniques seldom reflect the nutritive quality of the pastures and are consequently of limited value in explaining animal distribution. Techniques that can estimate pasture quality on a large scale are therefore critical in understanding and explaining wildlife and livestock distribution. We present the results of a greenhouse experiment designed to estimate the concentrations of nitrogen, potassium, phosphorous, calcium, magnesium and non-detergent fibre (NDF), using the reflectance of a tropical grass (*Cenchrus ciliaris*) canopy. Canopy spectral measurements were taken under controlled laboratory conditions using a GER 3700 spectroradiometer. We tested the utility of using the band depth analysis methodology in the visible region (where water absorption is less effective) to estimate foliar chemistry in fresh canopies. Continuum removal was applied to the visible absorption feature centred at 670 nm, and band depth ratios (BDRs) were calculated. Stepwise linear regression was used to select wavelengths from calculated BDRs that were highly correlated with foliar chemistry in a randomly selected training dataset. The resulting regression models were used to predict foliar chemistry in a test dataset. Results indicate that stepwise regression on bands calculated from continuum-removed reflectance spectra could predict foliar nutrient concentration with high accuracy. The correlations were highest for magnesium and nitrogen ($R^2=0.77$ and 0.73 respectively, using the normalized band depth index (NBDI)) between the measured and estimated biochemicals—a satisfactory result in estimating foliar chemistry in fresh standing pastures. With the advent of new sensors such as Hymap and MERIS, these results lay the basis for developing algorithms to rapidly estimate and ultimately map pasture quality in tropical rangelands.

1. Introduction

Pasture quality, expressed as a percentage of total digestible nutrients, is one of the major factors that determine the grazing patterns of wildlife and livestock (Bailey *et al.* 1996). Since pasture quality varies spatially, wildlife and livestock tend to exhibit preferences for certain sites, thereby affecting the grazing intensity of patches (Muya and Ogue 2000).

Existing rangeland monitoring techniques seldom reflect the nutritive quality of the pastures available. As such, these techniques are of limited value in explaining

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the distribution of wildlife and livestock. To understand and explain animal distribution, the development of techniques to estimate pasture quality is critical.

The traditional methods available for detecting pasture quality require detailed sampling and laboratory analysis. This usually results in the collection and analysis of inadequate data that are not representative of the population (Foley *et al.* 1998). By using narrow spectral channels of less than 10 nm, hyperspectral remote sensing offers the potential to detect and map variations in pasture quality. These narrow spectral channels allow the detection of detailed features, which could otherwise be masked by broadband satellites such as Landsat TM or NOAA AVHRR (Schmidt and Skidmore 2001).

Research has revealed a relationship between plant chemical constituents and the absorption of electromagnetic radiation (Curran 1989, Osborne *et al.* 1993, Johnson *et al.* 1994). The absorption mechanism originates from the energy transition of the molecular vibration (rotation, bending and stretching) of the C-H, N-H, O-H, C-N and C-C bonds, which are the primary constituents of the organic compounds of plant tissues (Elvidge 1990b). The chemical constituents of the plant tissue determine the nature and number of bonds present. Therefore, the wavelengths and the amount of energy reflected from the plant are partly a function of the chemical composition of that plant material (Foley *et al.* 1998). Curran (1989) lists 42 absorption features that have been related to particular foliar chemical concentrations.

Using spectroscopy to estimate plant biochemicals started with the laboratory analysis of dried ground samples (Norris *et al.* 1976). The United States Department of Agriculture (USDA) pioneered the laboratory-based near infrared reflectance spectroscopy (Williams and Norris 1987). The method developed rapidly in the 1990s, with emphasis on the estimation of canopy chemistry (Curran *et al.* 1992, Johnson *et al.* 1994, Wessman 1994, Fourty and Baret 1998). These studies used empirical multivariate approaches, such as stepwise linear regression, to select wavelengths correlated with the biochemical of interest from derivative spectra (Martin and Aber 1997). The stepwise regression method is robust; however, it suffers from problems of overfitting, especially when more wavebands than samples are used (Curran *et al.* 2001).

Recently, Kokaly and Clark (1999) developed a refined method that enhances and standardizes absorption features in order to minimize the effect of spectral variability that is independent of the biochemical concentration. Like the USDA methodology, this method uses stepwise regression. However, the effect of overfitting is minimized by concentrating on known absorption pits that are enhanced by continuum removal (Clark and Roush 1984). Kokaly and Clark (1999) applied continuum removal to broad absorption features of dry leaf spectra in the short-wave infrared region (1730 nm, 2100 nm and 2300 nm), and absorption band depths relative to the continuum were calculated. Bands in the short-wave infrared region were selected since it is a known region of chemical absorption (Curran 1989). The calculated band depths were normalized and stepwise linear regression was used to select wavelengths that are highly correlated with nitrogen, lignin and cellulose. The method showed strong correlation ($r^2=0.95$) between nitrogen concentration and the continuum-removed and band depth at five locations in the 2100 nm absorption feature.

Although most biochemicals absorb electromagnetic energy in the short-wave infrared region, their detection using spectral bands in this region is largely masked

by the water of fresh plants (Elvidge 1990a). Kokaly and Clark (1999) report that to apply equations developed from dried leaves measured in the laboratory to remotely sensed canopy spectra, the influence of water should be computationally removed to an accuracy of within 10%. The solution to this is either to develop robust models that remove the effect of water or to focus on parts of the electromagnetic spectrum that are less sensitive to water. This study proposes a shift to the visible region, where leaf water is less sensitive.

Pigments determine most spectral features in the visible region between 400 and 780 nm (Penuelas *et al.* 1994). Since there is a strong correlation between pigments and biochemicals such as nitrogen (Katz *et al.* 1966, Penuelas *et al.* 1994, Yoder and Pettigrew-Crosby 1995), detecting foliar biochemicals by means of spectroscopy can be shifted to the visible. The question is whether the band depth analysis methodology can be applied to estimate foliar chemistry at canopy level by using the visible spectral bands.

In this study we aimed at improving and extending the band depth analysis methodology to estimate foliar chemistry (nitrogen, phosphorous, potassium, magnesium, calcium and non-detergent fibre (NDF)) in fresh tropical grass (*Cenchrus ciliaris*) canopies. This was done by shifting the focus of foliar analysis from the short-wave infrared region to the major band depth in the visible region (where leaf water is less sensitive), as well as by proposing new indices. The potential of univariate analysis in form of first derivative reflectance in foliar nutrient concentration estimation was also evaluated. We designed an experiment in which we varied the nitrogen content of *Cenchrus ciliaris* grass and measured canopy reflectance over four weeks.

2. Methods

2.1 Plant preparation

Blue Buffalo grass (*Cenchrus ciliaris*) was sown in a greenhouse for this experiment. *Cenchrus ciliaris* is a sweet perennial grass that grows naturally in Africa and is widely used as a pasture grass. The grass grows from 10 to 150 cm in height and is tufted and erect, branching and rooting at the nodes (Pooley 1998). The grass grows fast and is currently recommended for planting in dry land areas in order to provide pasture for ruminants.

A total of 96 pots (diameter 26 cm, height 24 cm, capacity 10 litres) were used for planting. Five seeds were sown in each pot in the greenhouse on 30 July 2001. The seedlings were grown under natural day length with a night temperature of 21°C and a day temperature of 25°C. The plants were supplied with an initial fertilization of 2.2 g of patent kali and 3.6 g of super phosphate per pot, and they were watered at least once every day.

The pots were randomly divided into three equal groups on 13 August 2001. To manipulate foliar concentration of nitrogen, the first group (called high nitrogen) was supplied with 3.4 ml (120 kg ha⁻¹) of ammonium nitrate per pot. The second group (called low nitrogen) was supplied with 1.14 ml (40 kg ha⁻¹) of ammonium nitrate per pot. This was supplied over several days until the end of harvesting. No nitrogen was added to the control group. The pots were arranged in blocks and rotated once a week to minimize any microclimate effect on the experiment.

2.2 Canopy spectral measurements

To manipulate a variation in chemical concentration, measurements were taken at the beginning of each week for four weeks from 3 September 2001. Measurements were taken from this date onwards because the grass had grown to an extent where it was largely covering the soil, thus minimizing the background effects. The plants were transferred in their pots from the greenhouse to a laboratory on each day of measurement. A total of eight pots from each group were measured every week. Measurements were conducted under laboratory conditions in order to control for sources of variation unrelated to plant vigour, such as change in illumination angle and atmospheric effects (Luther and Carroll 1999).

Reflectance measurements were taken using a GER 3700 spectroradiometer. The GER 3700 (Geophysical and Environmental Research Corporation) is a three dispersion grating spectroradiometer using Si and PbS detectors with a single field of view. The wavelength range is 350 nm to 2500 nm, with a resolution of 1.5 nm in the 350–1050 nm range, 6.2 nm in the 1050–1900 nm range, and 9.5 nm in the 1900–2500 nm range. The sensor, with a field of view of 3°, was mounted on a tripod and positioned 2 m above the ground at nadir position.

A halogen lamp fixed next to the sensor provided illumination. Each pot was placed on a fixed tray directly under the sensor for canopy spectral measurements.

The pots were rotated 45° after every fifth measurement in order to account for the bi-directional reflectance distribution function (BRDF) of each sample. Each spectrum was determined as an average of 20 spectral measurements per pot. The radiance was converted to reflectance, using scans of a spectralon reference panel. Four target measurements were made after measuring the reference (spectralon) panel. Figure 1 shows the mean and standard deviation of the measured spectra.

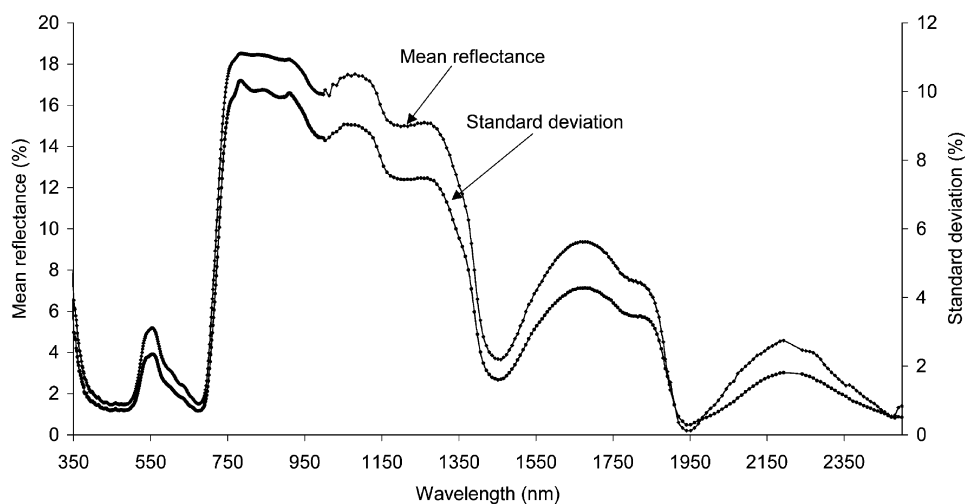


Figure 1. Mean and standard deviation of the canopy reflectance spectra of *Cenchrus ciliaris* grass ($n=96$) measured over four weeks.

2.3 Chemical analysis

Samples were oven dried at 70°C for 24 hours. The samples were ground through a 1 mm steel screen with a cyclone sample mill. Digestion of the samples was done before automated determinations. Organic matter was oxidized by applying hydrogen peroxide at relatively low temperature. Salicylic acid was added to prevent loss of nitrate. After decomposition of the excess H₂O₂, the digestion was completed by concentrated sulphuric acid at elevated temperature under the influence of selenium powder as a catalyst.

Nitrogen and phosphorous were measured with a segmented flow analyser at 660 nm wavelength. Potassium, calcium and magnesium were measured using a flame atomic emission spectrometer at 766.5 nm wavelength. NDF was determined using an Ankom 220 fibre analyser (Ankom Technology, Fairport, NY). Table 1 shows the resulting dataset.

2.4 Derivative reflectance

The first derivative reflectance (FDS) was calculated from each reflectance spectrum. A first difference transformation of the reflectance spectrum calculates the slope values from the reflectance and can be derived from the following equation (Dawson *et al.* 1998):

$$\text{FDS}_{\lambda(i)} = (R_{\lambda(j+1)} - R_{\lambda(j)}) / \Delta\lambda \quad (1)$$

where FDS is the first derivative reflectance at a wavelength i midpoint between wavebands j and $j+1$. $R_{\lambda(j)}$ is the reflectance at the j waveband, $R_{\lambda(j+1)}$ is the reflectance at the $j+1$ waveband and $\Delta\lambda$ is the difference in wavelengths between j and $j+1$. Correlations between the FDS and biochemicals were calculated at each wavelength, and correlograms were plotted.

2.5 Cross-spectrum (Fourier) analysis

Cross-spectrum analysis was executed between the correlograms. The purpose of cross-spectrum analysis is to uncover the correlations between two cyclical patterns at a certain frequency. This was presented in the form of squared coherency, which can be interpreted similarly to the squared correlation coefficient. The coherency value is the squared correlation between the cyclical components in the two series at the respective frequency.

Table 1. Descriptive statistics of the chemical variables measured in the laboratory ($n=96$).

Biochemical (mg g ⁻¹)	Mean	Minimum	Maximum	Confidence limit (95%)
N	22.5	9.27	44.9	2.1
P	3.39	1.95	4.97	0.15
K	3.8	2.6	5.28	0.13
Ca	4.3	2.37	7.9	0.28
Mg	3.14	1.43	14.69	0.37
NDF	46.98	1.58	57.68	1.53

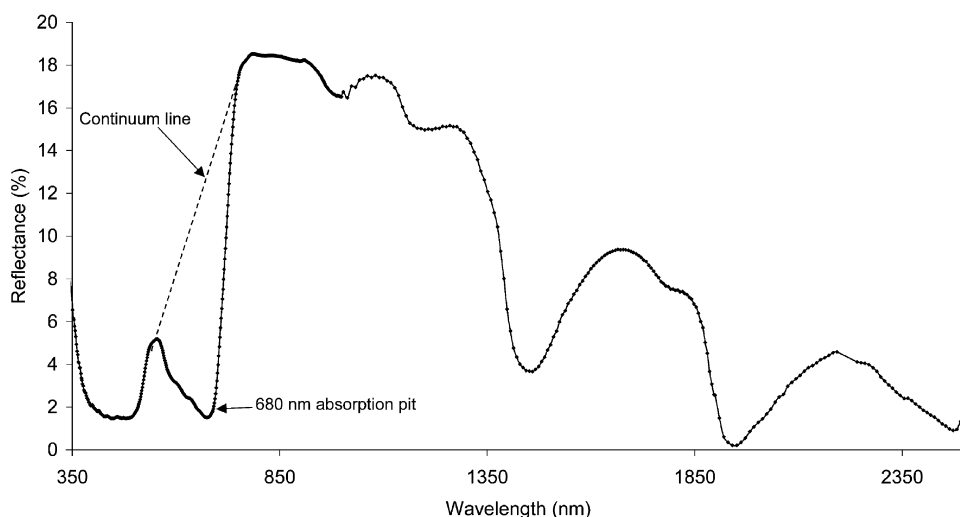


Figure 2. The 680 nm absorption feature illustrated on the mean reflectance spectra. The continuum is located between 550 nm and 750 nm.

2.6 Continuum removal

Continuum removal was applied to the major absorption pit in the visible region between 550 nm and 750 nm (figure 2). Although $R_{550-750}$ is strongly influenced by pigment absorption, several studies have established relationships between reflectance at specific wavelengths in this region and other foliar biochemicals (Ponzoni and Goncalves 1999, Curran *et al.* 2001, Gong 2002), largely emanating from the interrelationships between foliar biochemicals themselves. Continuum removal normalizes reflectance spectra in order to allow comparison of individual absorption features from a common baseline (Kokaly 2001). The continuum is a convex hull fitted over the top of a spectrum, utilizing straight-line segments that connect local spectra maxima.

The continuum is removed by dividing the reflectance value R for each point in the absorption pit by the reflectance level of the continuum line (convex hull) R_c at the corresponding wavelength (table 2). The first and last spectral data values are on the hull and therefore the first and last bands in the output continuum-removed data file are equal to 1. The output curves have values between 0 and 1, in which the absorption pits are enhanced and the absolute variance removed (Schmidt and Skidmore 2003). Figure 3 shows the band depths that were calculated from the continuum-removed spectra.

Table 2. Equations used for the calculation of spectral datasets.

Method	Equation
Continuum-removed spectra R'	$R' = R/R_c$
Band depth (BD)	$BD = 1 - R'$
Band depth ratio (BDR)	$BDR = D/D_c$
Normalized band depth index (NBDI)	$NBDI = (D - D_c)/(D + D_c)$

Where R = original reflectance values, R_c = reflectance values of the continuum line and D_c = band centre.

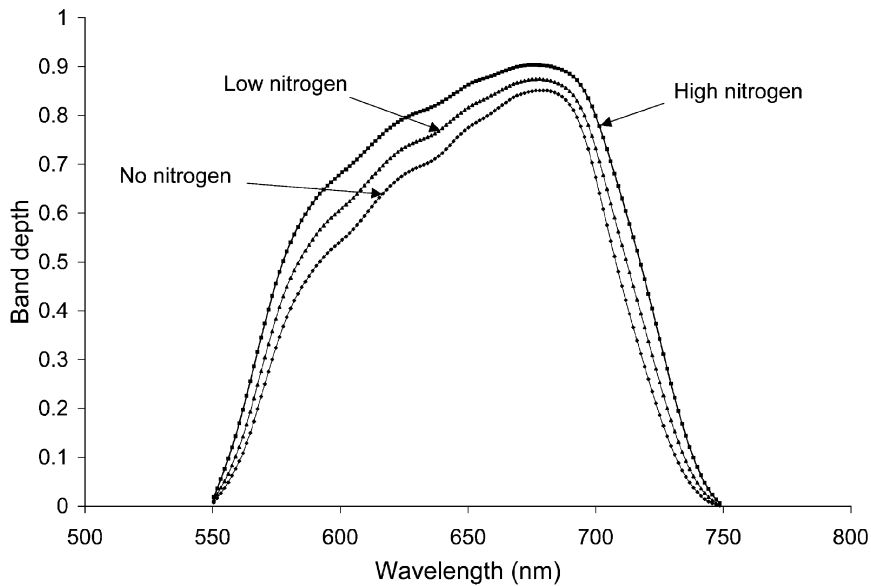


Figure 3. Band depth profiles for the 680 nm absorption feature in the no nitrogen, low nitrogen and high nitrogen supply treatments. The original reflectance spectra have been normalized by continuum removal.

This method has been found useful in mapping the distribution of minerals by comparing remotely sensed absorption band shapes with those in a reference library (Clark and Roush 1984). Efforts to apply the method in vegetation science have been made using dried plant material (Kokaly and Clark 1999). This method has not to our knowledge been extended to fresh canopies.

2.7 Band depth ratios

Factors such as atmospheric absorptions, water absorptions, soil background effects and BRDF affect the reflectance spectra. These can be minimized by normalizing the reflectance spectra (Kokaly and Clark 1999). This normalization procedure is usually executed on broadband satellite data such as Landsat TM or ASTER data.

The band depth ratio (BDR) was calculated by dividing the band depth of each channel by the band depth (BD) at the band centre (D_c), which is the maximum band depth. The new ratio, the normalized band depth index (NBDI), was calculated by subtracting the band depth (BD) from the band centre (D_c) and dividing it by their sum. In addition we proposed the use of continuum removed derivative reflectance (CRDR), which was calculated by applying equation (1) on the continuum-removed reflectance. Table 2 summarizes the equations used for calculating the indices.

2.8 Regression analysis

The dataset was randomly divided into a training dataset ($n=72$) and a test dataset ($n=24$), using the random generation function in S-PLUS. Stepwise linear regression was used to select spectral bands that were highly correlated with foliar chemistry in the training dataset. Stepwise regression fits a dependent

dataset (chemical concentration) using a linear combination of independent datasets (spectral indices). The wavelengths that were highly correlated with the biochemical of interest were selected. Regression models were computed from the training dataset.

2.9 Accuracy assessment

The regression models derived from the training dataset were applied to the CRDR, BD, BDR and NBDI from the test dataset in order to estimate foliar chemistry. The accuracy of the regression models in predicting foliar chemistry for the new datasets was tested by statistically comparing the predicted and measured biochemical concentrations. The accuracy of the estimation is reported in terms of R and R^2 between the estimated and measured biochemical concentrations. In order to provide confidence in the coefficient of determination between the estimated and the measured biochemicals, a modified bootstrap methodology was applied to test the predictive capability of multiple linear regression models developed between selected absorption feature variables and biochemicals. The standard bootstrapping procedure simulates the sampling distribution of any statistic by treating the observed data as if they were the entire statistical population under study. For each replication, a random sample of size N is selected, with replacement from the available data. The statistic of interest (in this case coefficient of determination R^2) is calculated for this bootstrapped subsample and recorded. The process is repeated a number of times in order to obtain the sampling distribution (Efron and Tibshirani 1994).

In this study, since we aimed at testing the predictive capability of regression models on an independent dataset, we modified the bootstrap procedure by first dividing the data into training and test sets and then replicate the test dataset. The regression model developed using the training dataset was tested on each bootstrapped subsample of the test dataset. This process was repeated 200 times and resulting R^2 values between the measured biochemicals and the predicted biochemicals were recorded. The mean and confidence intervals were calculated for the bootstrapped data. A routine, developed in IDL (Interactive Data Language) was used.

3. Results

3.1 Univariate correlation analysis

Correlations were calculated between the nutrient constituents (N, P, K, Ca, Mg, Ca, NDF) and the first derivative reflectance. The correlograms in figure 4 show the correlations between the FDS and biochemicals for 648 bands.

There are significant correlations between the FDS and most biochemicals. There are stronger correlations in the red edge region (748 nm) for nitrogen, calcium and magnesium. The concentration of NDF was correlated weakly with FDS along the whole spectrum.

Generally, all the correlograms show a similar shape. Table 3 shows squared coherency (squared correlation between the cyclical components in the two series at the respective frequency) values between the different correlograms.

There are strong cyclical components between nitrogen, calcium and magnesium ($p < 0.001$). The strong coherency between the correlograms is explained by the strong intercorrelation between the three biochemicals, as shown in table 4.

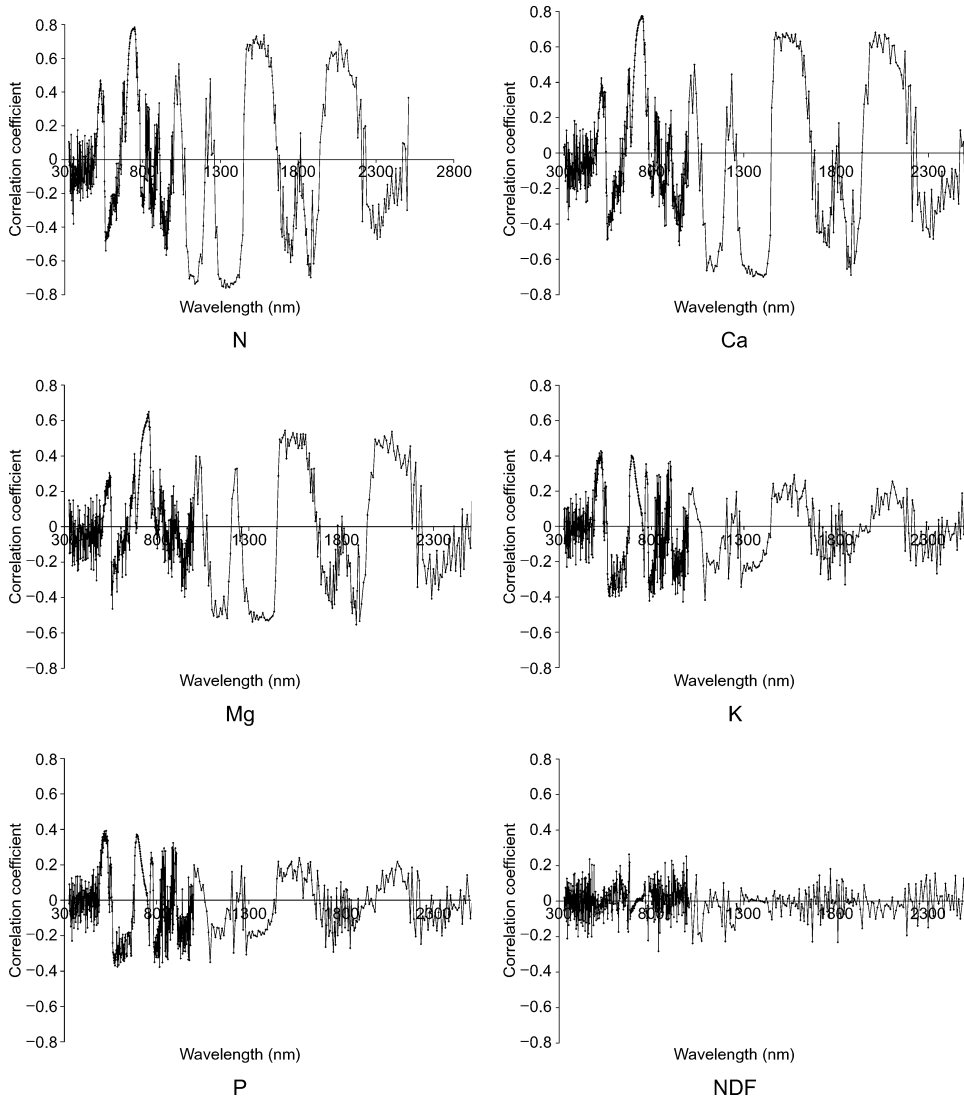


Figure 4. Relationship between first derivative reflectance (FDS) and biochemical concentration on the training dataset ($n=72$).

3.2 Stepwise linear regression results

The four methodologies (CRDR, BD, BDR, NBDI) used stepwise linear regression to select bands that are correlated with biochemicals in the major absorption pit located in the visible region. The training dataset ($n=72$) was used in the regressions.

Table 5 shows the R and R^2 values for the correlations between foliar biochemicals and the four sets of spectral data.

Nitrogen, calcium and magnesium showed the strongest correlations ($> 60\%$) with all spectral datasets—a satisfactory result for estimating foliar biochemicals in fresh canopies. The lowest R^2 of 32% was recorded between potassium and NBDI. The regression models generated from the training data were tested to predict biochemicals in the test dataset.

Table 3. Cross-spectral (Fourier) analysis between correlograms. The correlations between two series of correlograms (cyclical patterns) are presented in the form of squared coherency.

Biochemical	N	P	K	Ca	Mg
N	1.000				
P	0.15	1.000			
K	0.05	0.96**	1.000		
Ca	0.99**	0.13	0.03	1.000	
Mg	0.97**	0.11	0.04	0.98**	1.000
NDF	0.51**	0.59**	0.56**	0.05	0.07

**Significance level: $p < 0.01$

3.3 Assessing the prediction capability of the regression equations

Regression equations developed from the training dataset ($n=72$) were applied to the test dataset ($n=24$) for all four (CRDR, BD, BDR, NBDI) spectral datasets. The result was a new dataset of predicted biochemical concentrations.

The accuracy of the models is presented in the form of R and R^2 . To install confidence in the predictive capability of the regression models, a modified bootstrap procedure was adopted. This involved randomly selecting a training subset ($n=72$) from the entire sample and then develop a regression model. The remaining test set ($n=24$) was iterated 200 times using bootstrapping. For each iteration, the regression model developed from the training dataset was used to predict biochemicals on the test subsample. Calculating R^2 values between the predicted and measured biochemicals on the test subsets assessed accuracy. The iteration process resulted in a total of 200 R^2 values and the mean R^2 as well as standard errors were calculated (table 6).

Figure 5 shows an example of the predicted versus measured biochemicals using a randomly selected test set on NBDI data.

In general, results in table 6 show that the R and R^2 values are lower than those from stepwise regression (presented in table 5). However, NBDI yielded the highest mean R^2 of 0.73, 0.67 and 0.77 for nitrogen, calcium and magnesium respectively. Correlation coefficients between NDF and all spectral datasets are not significant at $p < 0.05$. The regression models therefore statistically failed to predict NDF. The standard errors are low for all chemicals, implying that the bootstrap method estimated with a high precision.

Table 4. Intercorrelation coefficients (r) between biochemicals measured on *Cenchrus ciliaris* grass.

Biochemical	N	P	K	Ca	Mg
N	1.000				
P	0.254*	1.000			
K	0.204*	0.914**	1.000		
Ca	0.925**	0.153	0.118	1.000	
Mg	0.875**	-0.063	-0.109	0.882**	1.000
NDF	-0.211*	-0.423**	-0.414**	0.018	0.077

**Significance level: $p < 0.01$

*Significance level: $p < 0.05$

Table 5. Stepwise linear regression results of four sets of spectral data and foliar variables ($n=72$). R is the slope of the least squares regression line when x and y are measured in standardized units. R^2 is the fraction of the variance of one variable that is explained by the least squares regression on the other variable.

Variable	CRDR		BD		BDR		NBDI	
	R	R^2	R	R^2	R	R^2	R	R^2
N	0.85	0.73	0.85	0.73	0.83	0.70	0.87	0.76
P	0.65	0.42	0.56	0.31	0.57	0.33	0.68	0.46
K	0.65	0.43	0.60	0.37	0.61	0.38	0.62	0.38
Ca	0.84	0.72	0.86	0.75	0.87	0.76	0.87	0.77
Mg	0.78	0.62	0.64	0.41	0.73	0.53	0.94	0.89
NDF	0.65	0.43	0.59	0.35	0.66	0.44	0.57	0.32

4. Discussion

Pasture quality is one of the major factors that influence animal grazing distribution patterns. The usefulness of available ecological models in predicting the distribution of wildlife and livestock is being hampered by inadequate spatial data on pasture quality. Techniques that can estimate pasture quality on a large scale are therefore critical in explaining ecological patterns, as well as in managing livestock and wildlife populations. This study has shown that there is a relationship between spectral reflectance and some aspects of pasture quality measured at canopy level. The four methods applied produced different correlation coefficients in estimating foliar chemistry.

4.1 The first derivative correlograms

The correlograms (figure 4) show a strong relationship between first derivative reflectance and biochemical concentration. The relationship was very strong for nitrogen, calcium and magnesium, which yielded similar correlograms. The similarity of the correlograms was tested using bivariate (Fourier) spectral analysis. Table 3 shows that nitrogen, calcium and magnesium yielded very high squared coherencies ($p < 0.01$). Table 4 shows that there are strong intercorrelation coefficients between the measured biochemicals, which explains the similarity in the correlograms.

Table 6. Results of mean R^2 values for the test dataset. Data was randomly divided into training and test datasets. A regression model from the training dataset was used to predict biochemicals on 200 bootstrapped test datasets and R^2 values recorded. The standard error and confidence limits of the mean R^2 values for the test data are presented.

Variable	CRDR			BD			BDR			NBDI		
	Mean R^2	SE	95% CL	Mean R^2	SE	95% CL	Mean R^2	SE	95% CL	Mean R^2	SE	95% CL
N	0.52	0.00	0.01	0.67	0.00	0.00	0.70	0.01	0.00	0.73	0.01	0.00
P	0.02	0.01	0.01	0.16	0.01	0.01	0.33	0.01	0.01	0.17	0.00	0.02
K	0.02	0.01	0.01	0.32	0.01	0.02	0.01	0.01	0.01	0.33	0.01	0.01
Ca	0.61	0.01	0.01	0.42	0.01	0.01	0.61	0.00	0.00	0.67	0.00	0.00
Mg	0.58	0.00	0.00	0.75	0.01	0.00	0.67	0.00	0.00	0.77	0.00	0.00
NDF	0.02	0.01	0.01	0.03	0.01	0.01	0.02	0.01	0.01	0.01	0.00	0.00

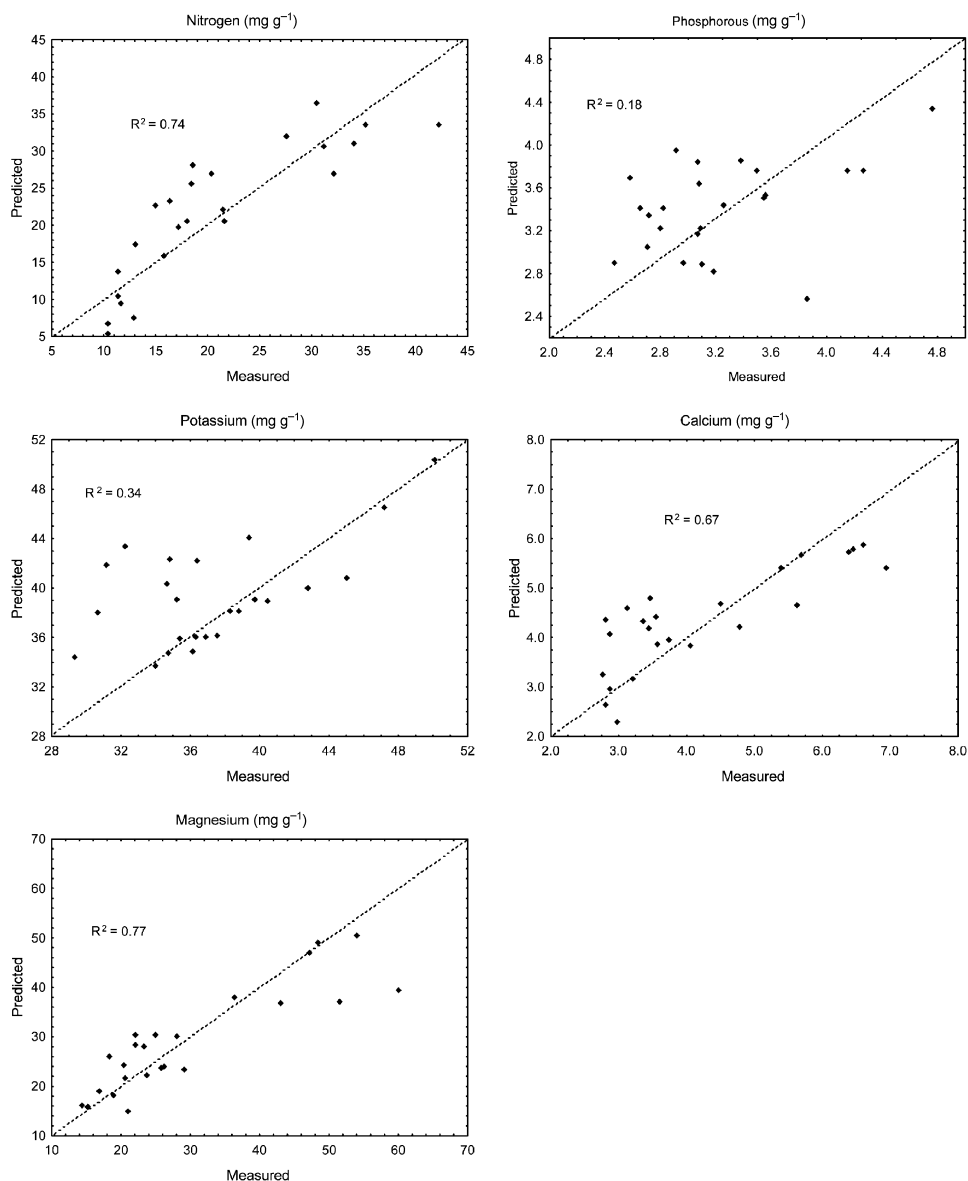


Figure 5. Results of regressions between foliar variables (variables that are significant at $p < 0.05$ are shown) for a test dataset and foliar variables estimated using stepwise regression equations developed from a training dataset ($n=72$) on NBDI.

The red edge yielded high correlations between the first derivative reflectance and foliar biochemicals. This confirms the hypothesis that the red edge slope is sensitive to a variation in foliar chemistry (Yoder and Pettigrew-Crosby 1995). NDF was poorly correlated with the whole spectrum although it comprises a more substantial portion of the plant material. NDF is not a well-defined compound since it is a mixture of many other compounds, such as lignin and cellulose (Robbins 1983, Belovsky and Schmitz 1994), and therefore does not have clearly defined absorption bands in the spectrum.

4.2 Band selection by stepwise linear regression

The bands selected by stepwise linear regression are close to chemical bonds in the leaf material. The highest frequency of the selected bands is around 680 nm, a region of known pigment absorption (Gates *et al.* 1965, Lichtenthaler *et al.* 1996). Studies have shown a strong nitrogen-pigment relationship as the porphyrin ring of the chlorophyll molecule contains nitrogen atoms (Katz *et al.* 1966, Penuelas *et al.* 1994, Yoder and Pettigrew-Crosby 1995). Pigment absorption bands could estimate several plant biochemical compounds since they contain similar atomic bonds.

Many wavelengths selected in the red edge region also confirm the region's relationship with biochemical compounds. Lichtenthaler *et al.* (1996) showed that reflectance ratios in the red edge (R_{750}/R_{700}) were positively correlated with chlorophyll and nitrogen concentrations. Wavelengths in the green reflectance region (550–580 nm) were also selected by stepwise linear regression. This confirms the results of many studies that computed ratios by using spectral bands in this region (Buschmann and Nagel 1993, Yoder and Pettigrew-Crosby 1995, Lichtenthaler *et al.* 1996) and correlated them with nitrogen and chlorophyll concentrations.

4.3 Regression results

The use of stepwise regression to estimate foliar biochemicals using the four methods resulted in high R^2 values between the measured and predicted biochemicals. These results are similar in pattern to those obtained by Curran *et al.* (2001) for dried ground plant materials. The study by Curran *et al.*, however, yielded higher correlations between the BDR and biochemicals ($R^2=0.99$ for nitrogen) than those obtained in this study. This difference was to be expected since this study applied the methodology to fresh standing canopies, which are significantly affected by canopy architecture, background effects and BRDF.

However, the satisfactory results obtained in this study benefited from using the visible bands, where the water effect is minimal, as well as from using the normalized band indices, which models out the effect of other factors that are independent of the biochemicals of interest (Kumar *et al.* 2001). Leaf water has the greatest influence on spectral absorption in the short-wave infrared region. Kokaly and Clark (1999) showed that the influence of water should be removed to an accuracy of within 10% if mid infrared absorption bands are to be used for estimating foliar chemistry. This difficult process has been avoided in this study by shifting the focus to visible bands, which are largely dominated by pigment absorption. The selection of red edge bands by stepwise regression was also advantageous as many red edge indices are insensitive to soil background effects (Clevers *et al.* 2000, Kumar *et al.* 2001).

NBDI resulted in the highest R^2 values: 0.76, 0.77 and 0.89 with nitrogen, calcium and magnesium concentrations, respectively. A similar pattern was also observed with the other spectral dataset, with lower but still significant coefficients of determination. The results shown in this study indicate that foliar biochemicals can be estimated at canopy level. This suggests that the absorption feature analysis with continuum-removal methodology (assuming an accurate atmospheric correction of the data) may be applied in the remote sensing of pasture quality and ultimately in mapping its spatial distribution.

By applying models derived from stepwise linear regression, it was possible to successfully estimate biochemical concentrations in an independent test dataset. (table 6 and figure 5). The results for magnesium regressions using the NBDI spectral dataset had the highest correlations ($R^2=0.77$). Success in extending the regression equations using the spectral datasets may have been because of the normalization procedure using continuum removal. Continuum removal enhances differences in absorption strength (Mutanga *et al.* 2003). In a study on spectral discrimination of vegetation types in a coastal wetland (Schmidt and Skidmore 2003) found out that continuum removal increases the separability of vegetation types in the visible region, thereby confirming our result. However, the lowest correlations were obtained for NDF data using all spectral datasets, which can be attributed to the unrefined state of NDF.

Overall, the result presented in this study is encouraging in the remote sensing of foliar chemistry when considering that the measurements were taken at canopy scale. This lays the basis for up-scaling the methodology to estimate pasture quality by using airborne remote sensing.

5. Conclusions

This study has applied an empirical method to estimate pasture quality at canopy level. The method was tested under controlled laboratory conditions to minimize atmospheric and geometric effects. The following conclusions can be drawn from this study:

- The major absorption pit in the visible domain can be used as an alternative in estimating pasture quality in fresh standing canopies, thereby avoiding the short-wave infrared region where foliar absorption features are largely masked by water. However, more experimental confirmation is required to ascertain the effect of biomass.
- The new index, NBDI, performed better than any other method tested in estimating pasture quality (bootstrapped mean R^2 of 0.73, 0.67, 0.77, 0.17 and 0.33 for nitrogen, calcium, magnesium, phosphorous and potassium respectively between estimated and observed foliar biochemical concentrations). Like any other method used in this study, NBDI could not predict NDF.
- Continuum removal (a widely used method in geological hyperspectral applications) is a useful normalization tool that enhances band depth differences, thereby improving the spectroscopic estimation of vegetation quality.

Overall, it is concluded that a lot of information is contained in hyperspectral absorption features. It is shown that a number of foliar biochemicals, which have hitherto been overlooked in vegetation science, can be estimated using high resolution reflectance spectra. With high quality field spectral collection and calibration, it is anticipated that the methods may be used to estimate *in situ* pasture quality in tropical grasslands.

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