Water removed spectra increase the retrieval accuracy when estimating savanna grass nitrogen and phosphorus concentrations

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Abstract

Information about the distribution of grass foliar nitrogen (N) and phosphorus (P) is important to understand rangeland vitality and to facilitate the effective management of wildlife and livestock. Water absorption effects in the near infrared (NIR) and shortwave infrared (SWIR) region pose a challenge for nutrient estimation using remote sensing. The aim of this study was to test the utility of water removed (WR) spectra in combination with partial least square regression (PLSR) and stepwise multiple linear regression (SMLR) to estimate foliar N and P, compared to spectral transformation techniques such as first derivative, continuum removal and log transformed spectra (Log(1/R)). The study was based on a greenhouse experiment with a savanna grass species (*Digitaria eriantha*). Spectral measurements were made using a spectrometer. *D. eriantha* was cut, dried and chemically analyzed for foliar N and P concentrations. A non-linear spectral matching technique was used to model the leaf water spectra, while

the WR spectra were determined by calculating the residual from the modelled leaf water spectra and observed leaf spectra. Results indicated that WR spectra yielded a higher N retrieval accuracy than a traditional first derivative transformation (R^2=0.84, RMSE=0.28 and R^2=0.87, RMSE=0.25, compared to R^2=0.59, RMSE=0.45 and R^2=0.59, RMSE=0.45 for PLSR and SMLR models, respectively). The highest P retrieval accuracy was derived from WR spectra using SMLR (R^2=0.64, RMSE=0.067), while the traditional first derivative and continuum removal resulted in R^2=0.47, RMSE=0.07 and R^2=0.40, RMSE=0.08, respectively. Only when using PLSR did the first derivative result in a higher P retrieval accuracy (R^2=0.47, RMSE=0.07) than the WR spectra (R^2=0.43, RMSE=0.070). It was concluded that the water removal technique could be a promising technique to minimize the perturbing effect of leaf water content when estimating grass nutrient concentrations.

Keywords: savanna ecosystem, nitrogen concentration, phosphorus concentration, water removal, continuum removal, bootstrapping

1. Introduction

Information about the distribution of grass nutrient concentration is crucial to understand rangeland health and facilitates effective management of wildlife and livestock. Several studies have established that grass nutrient concentration influences the feeding patterns and distribution of wildlife and livestock species in savanna rangelands (Drent and Prins 1987; Owen-Smith and Cooper 1987; McNaughton 1988; McNaughton

1990; McNaughton and Banyikwa 1995). Large herbivores are known to concentrate in nutrient rich sites in Southern Africa, e.g. termite mounds, sodic sites, sites beneath large trees (Owen-Smith and Danckwerts 1997; Grant and Scholes 2006; Treydte et al. 2007). Furthermore, studies showed that herbivore diversity increases with increasing soil fertility (Ollf et al. 2002) and that foliar nutrient concentration generally correlates positively with soil nutrient levels (Penning de Vries and Djiteye 1982).

Foliar nitrogen (N) and phosphorus (P) concentrations are important environmental factors for herbivores. The effect of increased N supply on dry matter production as well as protein content is well documented in agricultural literature (Marschner 1995), while P is one of the main requirements for lactating mammals (McNaughton 1990). Mapping both N and P would allow computing of the N:P ratio, which is a key indicator of nutrient limitation in vegetation (Koerselman and Meuleman 1996; Ludwig et al. 2001). Estimating N and P could therefore provide information on which nutrient is limiting for wildlife and livestock production in a particular landscape (Prins and van Langevelde 2008).

In order to identify where foliar N and P become important, hyperspectral remote sensing has been employed in various biomes, such as grasslands and savannas (Mutanga and Skidmore 2004; Mutanga et al. 2004a; Mutanga et al. 2004b; Bogrekci and Lee 2005; Ferwerda et al. 2005; Mutanga et al. 2005; Mutanga and Kumar 2007; Numata et al. 2009; Skidmore et al. 2010), forests (Martin and Aber 1997; Schlerf et al. 2010) and agricultural areas (LaCapra et al. 1996; Thenkabail et al. 2000; Hansen and Schjoerring 2003; Huang et al. 2004; Zarco-Tejada et al. 2004; Wang et al. 2009). Most of the

spectral absorption features that have been identified and used for N and P estimation are located in the near infrared (NIR) and shortwave infrared (SWIR). For example, N has absorption features centred at 430 nm, 460 nm, 640 nm, 660 nm, 910 nm, 1510 nm, 1940 nm, 2060 nm, 2180 nm, 2300 nm, 2350 nm, dominating in the SWIR region (Curran, 1989). The main leaf biochemicals absorbing in the SWIR region (1000-2500 nm) include lignin, cellulose, starch and proteins (Curran 1989; Kokaly and Clark 1999; Kumar et al. 2001). However, the accuracy of the estimation of N and P using NIR and SWIR absorption features is highly influenced by the reflectance of leaf water content, masking the subtle absorption features of other biochemicals (Gao and Goetz 1994; Gao and Goetz 1995; Fourthy and Baret 1998).

Several techniques have been used to minimize the effect of leaf water content on the remote sensing of foliar biochemicals, including spectral transformation such as vegetation indices, continuum-removed spectra, first derivative spectra and log-transformed spectra. Studies have estimated N using vegetation indices such as red edge position, which depends mainly on chlorophyll concentration (Clevers et al. 2002; Mutanga et al. 2004a; Cho and Skidmore 2006; Numata et al. 2009), assuming a positive correlation between leaf N and leaf chlorophyll concentration (Vos and Bom 1993; Yoder and Pettigrew-Crosby 1995). This approach is limited as it depends on the leaf or plant phenology, meaning the relationship will deteriorate as leaves senesce (Wang et al. 2009).

Derivatives, continuum-removal, and log transformed spectra (Log(1/R)) enhance absorption features of foliar biochemicals, while minimizing atmospheric, soil background,

and water absorption effects, as well as data redundancy (Yoder and Pettigrew-Crosby 1995; Dawson and Curran 1998; Cho and Skidmore 2006). For example, Log (1/R) is preferred to reflectance because it is linearly related to absorbing components (Hruschka 1987; Yoder and Pettigrew-Crosby 1995). Studies such as Yoder and Pettigrew-Crosby (1995) showed a strong relationship between Log (1/R), as well as the first derivative Log (1/R)', and N concentration. Fourty and Baret (1998) argued that transforming reflectance into their corresponding absorbance values improved the accuracy of estimates. Continuum removal has also been successfully applied to enhance absorption features for foliar N and P estimations (Kokaly and Clark 1999; Curran et al. 2001; Mutanga et al. 2005).

Water affects the absorption features for many foliar biochemicals when using fresh leaf spectra , and the removal of these effects has been recommended to increase the accuracy of foliar biochemical estimation in the SWIR (Gao and Goetz 1994; Gao and Goetz 1995; Dawson et al. 1998; Kokaly and Clark 1999; Mutanga and Skidmore 2004; Zhao et al. 2006) . Absorption by these chemicals, including lignin, starch, protein, and cellulose, is not very strong (weak absorbers) and so is generally masked by water absorption in fresh leaves (Kumar et al. 2001; Zhao et al. 2006). However, in dry leaf spectra, foliar biochemical absorption is generally highly differentiated and well correlated to the concentrations of foliar chemicals (Card et al. 1988; Elvidge 1990). Encouraging results have been attained using spectral transformations, e.g. normalized band depth (Kokaly and Clark 1999; Curran et al. 2001; Mutanga et al. 2004a). However, leaf water

still poses a challenge when using fresh leaf spectra to estimate biochemical concentrations (Fourthy and Baret 1998; Johnson 2001).

To overcome the masking effect of leaf water, Gao and Goetz (1994; 1995) successfully removed water absorption effects from fresh leaf spectra to estimate leaf components such as lignin and cellulose. They developed a non-linear least-squares spectral matching technique that calculates a fresh leaf spectrum as a non-linear combination of a leaf water spectrum and a dry matter spectrum. In a follow-up study, Schlerf et al. (2010) modified the technique and applied it successfully to estimate nitrogen concentrations in Norwegian spruce needles and named it the water removed approach (WR). To further adapt and apply this technique to the spectra of grass species common in savanna ecosystems the current study was undertaken.

Although stepwise multiple linear regression (SMLR) has been successfully used in foliar biochemical estimations, it has some limitations including multicollinearity, linear relationship assumptions, over-fitting (Curran 1989; Martens and Naes 2001) and difficulty in transferring the predictive models to other data sets (Grossman et al. 1996). To overcome these limitations many studies recommend the use of partial least square regression (PLSR) (William and Norris 1987; Hansen and Schjoerring 2003; Cho et al. 2007; Asner and Martin 2008). The advantage of PLSR is that the spectra are decomposed into latent factors using the response variable to reduce the data dimensionality problem in the model development process (reflectance spectra typically have a large number of bands constituting as many independent variables) (Geladi and Kowalski 1986; Geladi et al.

1999). The utility of PLSR in foliar biochemical estimation for N and P has been demonstrated, and both regression techniques (SMLR and PLSR) with various transformed spectra have been successfully applied (Huang et al. 2004; Asner and Martin 2008). However, the performance of PLSR, SMLR and WR spectra when estimating foliar P and N remains to be established.

The main aim of this study was to test the utility of water removed spectra (WR) in combination with PLSR and SMLR for estimating foliar N and P, and compare this to other existing spectral transformation techniques such as log (1/R), first derivative (FD), continuum removal (CR) and also the original reflectance (R). The study intended to quantify the retrieval accuracy of foliar N and P of a typical savanna grass grown under controlled conditions in a greenhouse. Reflectance spectra were collected using a visible – SWIR spectrometer. The hypothesis was that water removed spectra significantly increased the retrieval accuracy of nutrients, compared to first derivative of reflectance (or other spectral transformations).

2. Material and Methods

A grass species (*Digitaria eriantha)* was sown in pots and grown for four (4) months in a greenhouse. Soil water content as well as N and P fertilization were adjusted to produce high variation in foliar water, N and P. Reflectance measurements were acquired with a spectrometer. Foliar N and P concentrations were analyzed in the laboratory.

Various spectral transformation techniques were applied including WR spectra using PLSR and SMLR and a bootstrapping approach for validation.

2.1 Greenhouse experiment

2.1.1 Setup and sampling

The experiment was set up to produce high variation in foliar N and P concentrations, and foliar water content. A multiple factorial design was used (2x3x3) with 5 replications to ensure that each water treatment included at least 30 samples, generating a total of 90 samples (Morrison 2001). The selection of the grass species (*Digitaria eriantha*) was based on its wide occurrence in African savanna ecosystems and its importance as forage for livestock and wild herbivores. *D. eriantha* is a perennial grass species which grows either as a dense tussock, with or without extended stolons or as continuous stoloniferous sward. It can grow in a wide range of soil types from sands to heavy clays. Natural compost was mixed with fine red and sandy soils to form a basic stratum for sowing the grass seeds. Samples of this soil mix were taken to South Africa's Agricultural Research Council (ARC) for chemical analysis to determine its chemical composition. Then various levels of nutrients, based on Venter (1990) as well as Scholes and Walker (2004), were added. Limestone ammonia nitrate (N: 284 g/kg) and superphosphate (P: 83g/kg) were used for fertilization. Soil water levels were manipulated using different watering regimes, when the grass was fully grown and flowering. The various levels of water treatment applied were: (i) high level, where plants were watered twice a day, (ii) medium level, with plants watered once every two days and (iii) low level, with plants

watered once or twice a week. This was done for two weeks to ensure contrasting levels of water content, P and N concentrations in the soil, and hence in the grass leaves. The temperature was kept between 27 and 30^0C to mimic savanna ecosystems.

2.1.2 Spectral measurements

Canopy spectral measurements were taken for each pot using a FieldSpec ® 3 Portable Analytical Spectral Device (ASD®) spectrometer with a spectral range extending from 350 to 2500 nm, and a 1 nm bandwidth (www.asdi.com). Measurements were taken in a dark room to minimize wall reflections. A halogen lamp mounted on a tripod with a fixed illumination angle of 45 degrees was used as illumination source. Given the pot diameter of 15 cm, the canopy reflectance was measured by pointing the fibre optic with a field of view of 25 degrees in a nadir position, from about 33 cm above the grass canopy, to ensure that only spectral measurements of the grass canopy were taken. A white reference panel (spectralon) was used before each spectral measurement to convert spectral radiance into reflectance. Measuring followed the protocol used by e.g. Cho et al. (2007b), Mutanga et al. (2003) and Vaipasha et al. (2005). A single spectral measurement included an average of 10 scans. Each pot was rotated whilst 5 spectral measurements were taken and averaged to account for illumination differences and bi-directional reflectance effects (Wang et al. 2009).

2.1.3 Chemical analysis

For each pot all grasses were cut at the base and oven dried at 80^0 C for 24 hours. The dried grasses were sent to the Agricultural Research Council's Institute for Tropical and Subtropical Crops (ARC-ITSC) for chemical analysis. N and P were analyzed using the wet or acid digestion method, using perchloric and nitric acid for P and sulphuric acid for N (Giron 1973; Grasshoff et al. 1983).

2.2 Data Analysis

2.2.1 Spectral transformation techniques

The spectral reflectance data were pre-processed before transformation. Spectral smoothing was performed with the commonly used Savisky-Golay filter (Savitzky and Golay 1964), adding a second order polynomial least square function and 3-band window to remove signal noise. To compare the water removed (WR) spectra with the other spectral transformation techniques, taking some of the absorption features in the visible spectrum into account; the spectral region from 500 to 2450 nm was selected for data analysis.

Commonly used spectral transformation techniques such as log transformed spectra Log (1/R), first derivative, and continuum removal, were computed. Log (1/R) was determined by calculating a log function of the spectral reflectance's reciprocal (Hruschka

1987; Yoder and Pettigrew-Crosby 1995; Fourthy and Baret 1998). The first derivative of the spectral reflectance was derived using a first-difference approach. A first-difference transformation of the reflectance spectrum calculates differences in reflectance between adjacent wavebands. More details on this can be found in Dawson and Curran (1998). The continuum removed spectra were derived by applying a convex hull or a continuum line to the reflectance spectra connecting local spectral maxima (Kokaly and Clark 1999; Kokaly 2001; Mutanga et al. 2004a).

To reduce water absorption effects on weak biochemical absorption, the water removed spectra (WR) were derived from a non-linear least-squares spectral matching technique calculating a fresh leaf spectrum as a non-linear combination of a leaf water spectrum and a dry matter spectrum (Gao and Goetz 1994; Gao and Goetz 1995), modified by Scherlf et al. (2010), using the following equation:

$$R_{mod}(\lambda)=(A+B\,\lambda)exp\text{-}(C_wK_w(\lambda) + C_{dm}K_{dm}(\lambda)) \quad (1)$$

where $R_{mod}(\lambda)$ is the modelled reflectance for wavelength λ, C_w the water content, K_w the absorption coefficient of water, C_{dm} the dry matter content, K_{dm} the absorption coefficient of dry matter content, and A and B background model coefficients. A, B and C_w were unknowns determined using the mathematical optimization procedure called nelder-melder simplex method (Mathews and Fink 2004; Mathworks 2009). The absorption coefficient of water and the absorption coefficient of protein or dry matter were obtained from the PROSPECT leaf model (Jacquemoud et al. 1996). The leaf water

contribution to the total fresh leaf reflectance was modelled by filling in the three unknowns A, B, and C_w in equation (1), whilst setting C_{dm} at zero. Finally, the residual spectra between measured reflectance $R_{mes}(\lambda)$ and modelled reflectance $R_{mod}(\lambda)$ were computed as the water removed spectra $WR(\lambda)$:

$$WR(\lambda) = (R_{mes}(\lambda) - R_{mod}(\lambda)) / R_{mes}(\lambda) \quad (2)$$

The modifications by Schlerf et al. (2010) included the use of known and published water and protein absorption coefficients and incorporating nelder-melder simplex methods to determine the unknowns as indicated above. This study adopted the same technique for the savanna grass species. $WR(\lambda)$ was used for analysis and compared with the other transformations of the spectra.

2.2.2 Regression analysis and bootstrapping

Two commonly used regression techniques were selected for data analysis, i.e. partial least square regression (PLSR) (Naes and Martens 1985; Geladi and Kowalski 1986; Naes et al. 1986; William and Norris 1987; Martens and Naes 1989; Ehsani et al. 1999; Geladi et al. 1999; Martens and Naes 2001; Viscarra Rossel 2008) and stepwise multiple linear regression (SMLR) (Grossman et al. 1996; Martin and Aber 1997; Kokaly and Clark 1999; Huang et al. 2004; Schlerf et al. 2010). To compare the retrieval accuracy of foliar N and P using the various spectral transformation techniques, a bootstrapping approach was used (Efron 1983). The advantage of bootstrapping is that it

can be used efficiently when only a limited number of samples are available. Bootstrapping was used as an alternative to the split method since it iteratively resample the data set to be used for model development, making it a good technique for assessing model accuracy (Verbyla and Litvaitis 1989). In this study, PLSR and SMLR were integrated with bootstrapping to derive calibrated and validated models. To integrate PLSR and bootstrapping, bagging-PLSR was implemented using the Parles 3.1 software (Viscarra Rossel 2007; Viscarra Rossel 2008). SMLR was integrated with bootstrapping using Mathworks (2009).

Using bagging-PLSR, independent or predictor variables were mean-centred to normalize them prior to further statistical analysis. The leave-one-out cross validation, as defined by the lowest root mean square error (RMSE), was used to determine the optimal number of factors or latent variables to be used for model development (Cho et al. 2007; Viscarra Rossel 2008; Darvishzadeh et al. 2008a). This Optimal number of factors was then used for model development and validation with the number of bootstraps equalling 1000. Bootstrapping with SLMR also used 1000 iterations and was implemented in Mathworks (2009). Only significant wavelengths were used in the model development using SMLR. Wavelengths were selected using the conventional rule for selecting independent variables in SMLR ("in" if $p<0.05$, and "out" if $p>0.01$).

For both models (PLSR and SMLR) the retrieval accuracy was defined by the bootstrapped mean of the coefficient of determination (R^2) and the RMSE. The confidence interval at a 95% confidence level was calculated for both R^2 and RMSE.

3 Results

3.1 Performance of WR spectra for foliar N estimation using PLSR and SMLR

Generally, the WR technique used in combination with PLSR yielded the highest N retrieval accuracy (R^2=0.84; RMSE=0.28, 17% of the mean), compared with other spectral transformation techniques (Table 1). The 95% confidence interval (CI) of the N retrieval accuracy (RMSE, 95% LCI=0.25 and UCI=0.33) confirmed the outperformance of the WR technique plus PLSR over other techniques. A similar trend with a slightly higher accuracy was obtained when the WR technique was combined with SMLR, producing the highest retrieval accuracy overall (R^2=0.87; RMSE=0.25, 15% of the mean; Table 1). The second most important spectral transformation for N estimation after the WR technique was continuum removal, producing a RMSE of 0.30 (18% of the mean), and a 95% confidence interval varying from 0.24 to 0.31, when using PLSR. Similar trends were obtained when the continuum removal was combined with SMLR (R^2=0.78; RMSE=0.34, 20% of the mean). The poorest performances were obtained with the first derivative, the Log(1/R), and the original reflectance spectra, with an R^2 of around 0.6 and a RMSE varying between 0.43 and 0.48 (about 26% of the mean; Table 1).

(Table 1)

N retrieval accuracies were not significantly (*t-value*=-0.18, *df*=8, *p*=0.859) different between PLSR and SMLR approaches. On average the PLSR accuracies were slightly

higher than those of the SMLR, with a higher R^2 and a lower RMSE. Generally, the results consistently demonstrated the high performance of the WR technique.

More bands were selected using SMLR and WR spectra (about 11 bands) to estimate N concentration, than for other spectral transformations (Table 2). Many of the important bands for all spectral techniques were located in the known absorption features for protein and N (Curran 1989; Kumar et al. 2001) (see Table 2 and Figure 1). For N estimation, Figure 1 depicts the PLSR weights plotted with the grass canopy spectra of the highest performing spectral transformations, namely WR and continuum removal. The original reflectance curve was added to Figure 1 for reference.

(Table 2)

(Figure 1)

3.2 Performance of WR spectra for foliar P estimation using PLSR and SMLR

Generally, the WR technique yielded the highest P retrieval accuracy compared to other spectral transformation techniques. Contrary to what was observed with WR-PLSR, the WR-SMLR technique yielded a higher coefficient of determination (R^2=0.64) with a slightly higher P retrieval accuracy (RMSE=0.06, 18% of the mean; Table 1). This suggests that overall the WR technique minimized water masking effects on features sensitive to P. The WR technique had a higher accuracy compared to the continuum removal technique (RMSE=0.07, 20% of the mean), which again was the second performer with an about 27% lower R^2 value (Table 1). Using PLSR, the first-derivative

transformation produced the highest estimates of P with a RMSE of 0.068 (20% of the mean), slightly higher than the WR technique with its RMSE of 0.070 (20.6% of the mean). Continuum removal and PLSR yielded a RMSE of 0.08 (22% of the mean). The lowest performing spectral transformation techniques for estimating P with PLSR were Log (1/R) and original reflectance, with a RMSE of 0.08 (24% of the mean) and of 0.07 (20% of the mean), respectively. Again P retrieval accuracies using SMLR were not significantly different (*t-value*=-1.1095, *df*=8, *p*=0.299) from those using PLSR. Log (1/R) and original reflectance generally obtained poor results with both regression techniques.

Using the WR technique, the stepwise regression technique yielded the highest number of bands for estimating P concentrations compared to the other transformation approaches. Some of the selected bands corresponded with known absorption features of starch, as cited in Curran (1989) and Kumar et al. (2001) (Table 2), as was also observed in the PLSR weights noted in Figure 2. For P estimation, Figure 2 depicts the PLSR weights plotted with the grass canopy spectra of the highest performing spectral transformations, namely WR and the first derivative spectra. The original reflectance curve has been added to Figure 2 for reference.

(Table 2)

(Figure 2)

3.3 Foliar P and N concentrations in grass canopies

A high variability of foliar N and P was observed in the samples of *D. eriantha* cultivated in the greenhouse and treated with various nutrient and water levels. Descriptive statistics for foliar N and P are detailed in Table 3; the mean foliar N and P concentrations were 1.68% and 0.34% of dry matter respectively. The distribution of both N and P foliar concentrations across all treatments were normal, as tested by the Kalmogorov-Smirnov normality test (p>0.05, 4.39 and 2.16 chi square test, respectively).

(Table 3)

4 Discussion

4.1 WR spectra for estimating foliar N

This study demonstrated the potential of the WR approach as one of the spectral transformation techniques that could be used to increase the accuracy of foliar N estimation. By reducing the water effect across the fresh leaf spectra this technique enhances weak or subtle absorption features. The regions of the electromagnetic spectrum most affected by water are the NIR and SWIR, important regions for distinguishing various biochemical concentrations. This was illustrated by the selection of more bands in the SWIR region being related to foliar N concentration when using WR instead of reflectance (Table 2, Figure 1). Some of these bands correspond to known absorption

features cited by Curran (1989) and Kumar et al. (2001) due to several absorption mechanisms including electron transition in the visible region, C-H stretch of the 2nd overtone mainly in the 1100-1300 nm region, and C=O, O-H, N-H, C-O, C-H as well as C-C for the region within 1300-2380 nm (Kumar et al. 2001). Generally this study showed the applicability of the WR technique for savanna grass species, with a higher R^2 of 0.87 for N estimation compared to the results (R^2=0.52) attained by Schlerf et al. (2010). Gao and Goetz (1994; 1995) successfully implemented the WR technique for lignin and cellulose estimations, highlighting the importance of minimizing water effects on the SWIR. The WR technique is easy to implement but requires a careful and proper parameterization of the least square spectral mixture analysis model to provide reliable results (Gao and Goetz 1994).

The continuum removal technique yielded the second highest accuracy for estimating foliar N concentrations with both PLSR and SMLR. Continuum removal enhanced the differences in absorption strength (Clark and Roush 1984; Schmidt and Skidmore 2001; Schmidt et al. 2004). The highest R^2 obtained in this study using continuum removal was 0.81. This is consistent with the N retrieval accuracy of forest sites reported by Kokaly and Clark (1999), where an R^2 of 0.75 to 0.94 was attained using continuum removal and continuum removal-derived indices. This study attained higher accuracy results for N based on continuum removal than a study by Mutanga et al. (2005) on *Cenchrus ciliaris* grown in the greenhouse. Estimating N with Log (1/R) yielded a higher retrieval accuracy than with reflectance, but not as high as with the WR and continuum removal techniques. Yoder and Pettigrew-Crosby (1995) showed Log

(1/R) performed accurately estimating N concentrations, compared to reflectance. Similar results were also attained by Fourty and Baret (2001). They argued that by transforming reflectance to absorbance Log (1/R) values the accuracy of biochemical estimates was improved. Log (1/R) is likely to be used instead of the original reflectance because of the linear relation between the absorbing components and its contribution to the Log (1/R) value at the wavelength absorbed (Hruschka 1987). However, the present study shows the performance of WR to be higher to that of Log (1/R).

4.2 WR spectra for estimating foliar P

The performance of the first derivative and WR techniques in terms of P retrieval accuracy highlights the importance of reducing the influence of water on the fresh leaf spectra. As shown in Table 2 and Figure 2, many bands highlighted by the models as sensitive to P correspond to known absorption features of starch. The relationship between P and starch is understood to be based on an energy molecule rich in P called *adenosine triphosphate* (ATP), which is used in starch formation (Heldt et al. 1977; Sava Stankovic 1978; Larcher 1980; Okita 1992). The energy from the sun is converted into chemical energy stored as a form of ATP and then used to bond with carbon dioxide (CO_2) and hydrogen to form starch (Larcher 1980). To date, few studies have focused on the estimation of P using remote sensing. Bogrekci and Lee (2005) used wavelengths from 225 to 2550 nm, while Mutanga and Kumar (2007) showed that bands in the SWIR were more sensitive to P, which is similar to the findings in this study. The high precision of N and P estimation using the WR technique is evident from the lower confidence

limits of the correlation coefficient and the RMSE derived from the bootstrapping technique (Table 1).

4.3 Comparing PLSR and SMLR for foliar N and P estimation

PLSR consistently performs well in estimating N. Comparative studies using both PLSR and SMLR indicated the predictive power of PLSR. For example, Bogrekci and Lee (2005) showed that PLSR had a higher P accuracy than SMLR on grass leaves. This study showed different results, with the SMLR-WR technique yielding a higher accuracy for P than the PLSR-WR did. SMLR is normally confounded with difficulties transferring the predictive models to the other data sets or other areas (Grossman et al. 1996). On average, including all other spectral transformation techniques, PSLR still showed its high predictive power. Hansen and Schjoerring (2003) concluded that PLSR can be used as an alternative univariate statistical technique. This is mainly because PLSR minimizes the multicollinearity effects by decomposing the spectral data into non-collinear latent variables. Over-fitting may be minimized using PLSR by selecting an optimal number of latent variables rather than having more redundant explanatory variables (Viscarra Rossel 2007; Viscarra Rossel 2008).

5 Conclusion

The estimation of N, P, and N:P at landscape level forms an important objective to facilitate investigation into the feeding patterns and distribution of wildlife and livestock in African savannas. The study tested the applicability and performance of the water removal technique in estimating P and N concentrations on grass canopies and compared this technique with other spectral transformation techniques such as first derivative, Log (1/R) and continuum removal, as well as the original reflectance. This study suggests that the water removed approach is a useful technique to retrieve foliar N and P concentrations from grass in savannas, especially N which was consistently estimated with high accuracy by both SMLR and PLSR. This study focused on a single species; future studies should consider multiple species at field, airborne or satellite level to test the utility of the water removed technique at landscape level in savanna ecosystems.

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Tables and Figure captions

Tables

Table 1: The performance of various spectral transformations for estimating N and P using PLSR and SMLR combined with the bootstrapping technique

Spectra		r^2	LCI 95%	UCI 95%	RMSE	LCI 95%	UCL 95%	*no. of factors or bands
CR	N *vs.* PLSR	0.81	0.68	0.94	0.30	0.26	0.35	8
	N *vs.* SMLR	0.78	0.69	0.78	0.34	0.33	0.38	4
	P *vs.* PLSR	0.40	0.38	0.41	0.08	0.06	0.08	6
	P *vs.* SMLR	0.37	0.18	0.38	0.07	0.07	0.09	2
FD	N *vs.* PLSR	0.59	0.48	0.70	0.45	0.39	0.53	3
	N *vs.* SMLR	0.59	0.48	0.70	0.45	0.39	0.53	9
	P *vs.* PLSR	0.47	0.46	0.48	0.07	0.06	0.08	7
	P *vs.* SMLR	0.25	0.12	0.27	0.08	0.08	0.09	4
Log(1/R)	N *vs.* PLSR	0.62	0.50	0.74	0.43	0.38	0.50	6
	N *vs.* SMLR	0.60	0.54	0.62	0.45	0.43	0.50	4
	P *vs.* PLSR	0.17	0.16	0.18	0.08	0.07	0.10	4
	P *vs.* SMLR	0.00	0.01	0.10	0.09	0.92	0.10	1
R	N *vs.* PLSR	0.60	0.49	0.71	0.44	0.38	0.52	4
	N *vs.* SMLR	0.55	0.48	0.56	0.48	0.46	0.57	4
	P *vs.* PLSR	0.18	0.17	0.19	0.08	0.07	0.10	4
	P *vs.* SMLR	0.10	0.01	0.11	0.06	0.09	0.10	3
WR	N *vs.* PLSR	0.84	0.71	0.97	0.28	0.25	0.33	6
	N *vs.* SMLR	0.87	0.82	0.88	0.25	0.24	0.31	11
	P *vs.* PLSR	0.43	0.42	0.44	0.07	0.06	0.08	5
	P *vs.* SMLR	0.64	0.48	0.64	0.06	0.05	0.07	9

N=Nitrogen, P=Phosphorus, CR=continuum removal, FD=first derivative, R=original reflectance, WR=water removed spectra. ***** *factors for partial least square regression (PLSR) and bands for stepwise multiple linear regression (SMLR)*

Table 2: Wavelengths selected using SMLR, marking wavelengths corresponding to known absorption features.

Spectra *vs.* N/P	**Selected wavelengths for N and P estimation using SMLR**										
CR *vs.* N	732	1057	1836	**2129***							
CR *vs.* P	744	785									
FD *vs.* N	549	**673***	734	**1666***	1807	1908	2260	2290	**2374***		
FD *vs.* P	839	**1204***	**1667***	2450							
Log(1/R) *vs.* N	504	605	698	1396							
Log(1/R) *vs.* P	**638***	691	878								
R *vs.* N	511	604	696	1394							
R *vs.* P	640										
WR *vs.* N	522	**675***	1087	1159	1299	1360	2014	2038	**2056***	**2141***	2342
WR *vs.* P	935	1036	**1209***	**1974***	**2061***	2296	**2320***	2364	2379		

CR=continuum removal, FD=first derivative, R=original reflectance, WR=water removed spectra.
**Known absorption features protein / N for nitrogen (N) and starch for phosphorus (P) (Curran 1989).*

Table 3: Descriptive statistics of foliar N and P for *Digitaia eriantha*

Measured Variables	No. of Obs.	Min	Max	Mean	StDev
Nitrogen (N%)	90	0.65	3.73	1.68	0.25
Phosphorus (P%)	90	0.17	0.63	0.34	0.07

Figures

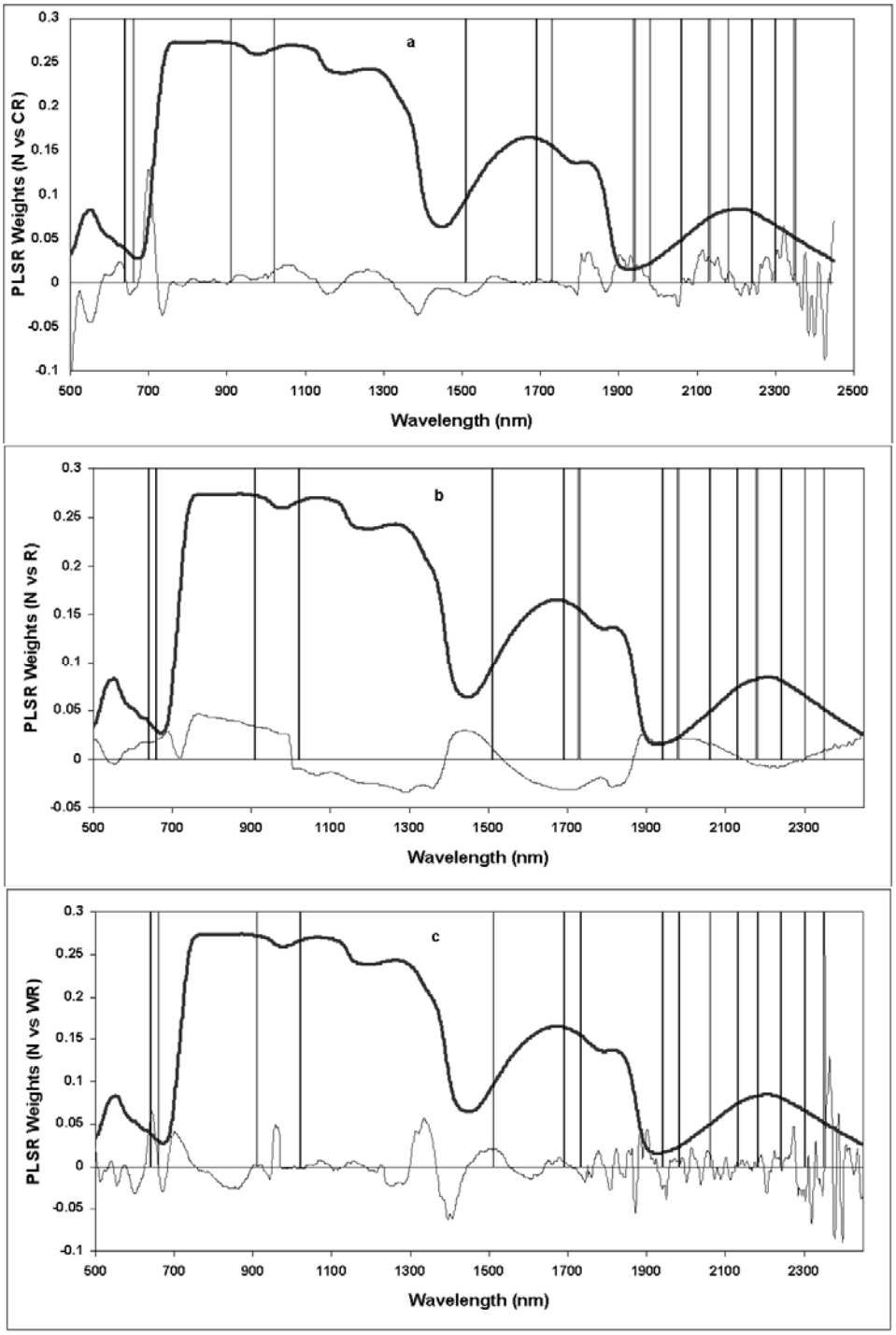

Figure 1: Partial least square regression (PLSR) weights showing the contribution of each wavelength in the development of models for nitrogen (N) estimation using a) continuum removal (CR) spectra (N *vs.* CR), b) reflectance (R) spectra (N *vs.* R), c) WR (water removed) spectra (N *vs.* WR), plotted with the grass canopy reflectance for reference. The more positive the weight is on a particular wavelength, the more contribution it has towards the model development (and *vice versa*). Vertical lines indicate known N absorption features as listed in Curran (1989)

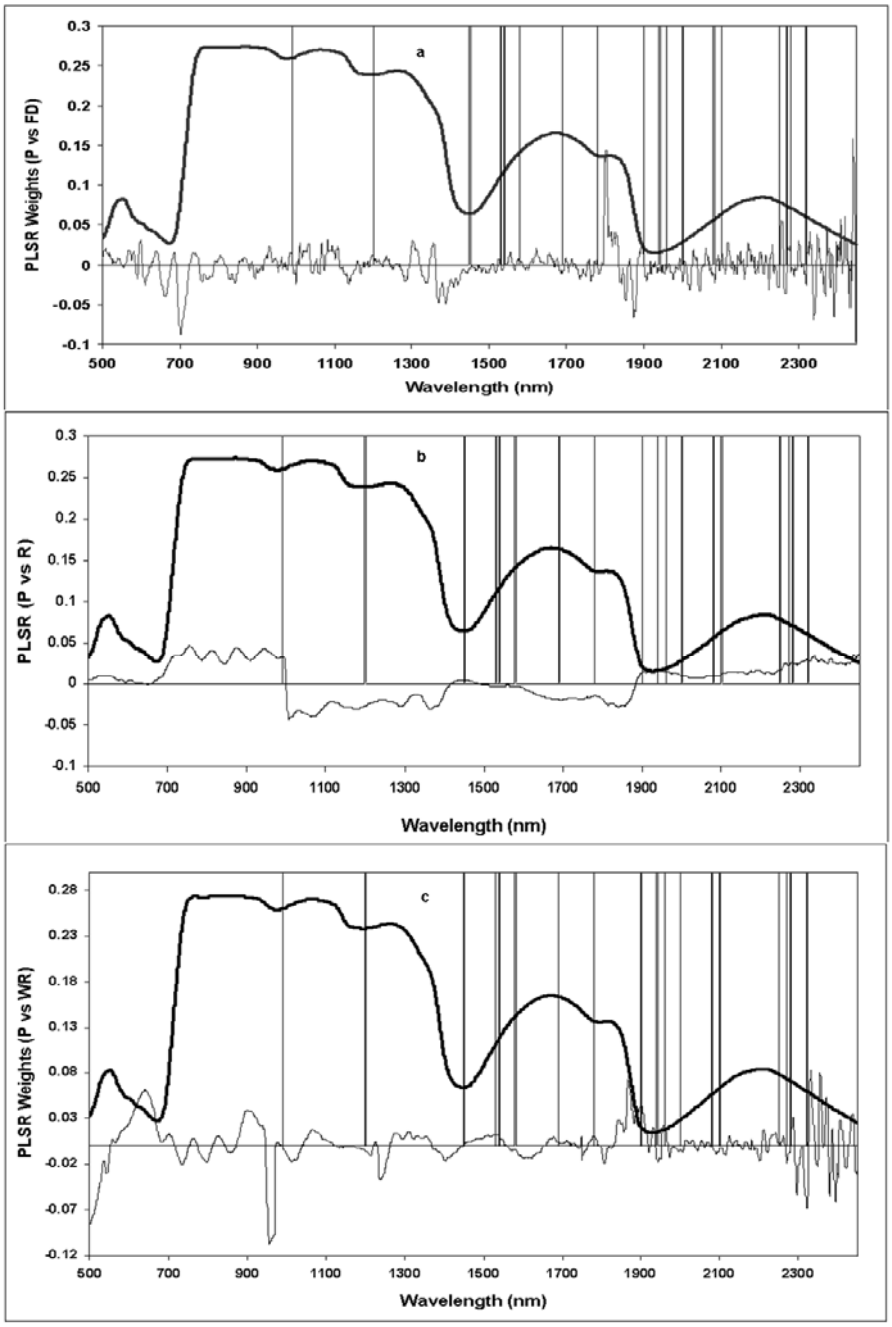

Figure 2: Partial least square regression (PLSR) weights showing the contribution of each wavelength in the development of models for estimating P using a) first derivative (FD) spectra (phosphorus (P) *vs.* FD), b) reflectance (R) spectra (P *vs.* R), c) water removed (WR) spectral data (P *vs.* WR), plotted with the grass canopy reflectance for reference. The more positive the weight is on a particular wavelength, the more contribution it has towards the model development (and *vice versa*). Vertical lines indicate known of starch absorption features as listed in Curran (1989).