Savanna grass nitrogen to phosphorus ratio estimation using field spectroscopy and the potential for estimation with imaging spectroscopy

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Abstract

Determining the foliar N: P ratio provides a tool for understanding nutrient limitation on plant production and consequently for the feeding patterns of herbivores. In order to understand the nutrient limitation at landscape scale, remote sensing techniques offer that opportunity. The objective of this study is to investigate the utility of *in situ* hyperspectral remote sensing to estimate foliar N: P ratio. Field spectral measurements were undertaken, and grass samples were collected for foliar N and P extraction. The foliar N: P ratio prediction models were developed using partial least square regression (PLSR) with original spectra and transformed spectra. Spectral transformations included the continuum removal (CR), water removal (WR), first difference derivative (FD) and log transformation (Log(1/R)). The results showed that CR and WR spectra in combination with PLSR predicted foliar N: P ratio with higher accuracy as compared to FD and R spectra. The performance of CR and WR spectra were attributed to their ability to minimize sensor and water effects on the fresh leaf spectra, respectively. The study demonstrated a potential to predict foliar N: P ratio using field and HyMap simulated spectra and shortwave infrared (SWIR) found to be highly sensitive to foliar N: P ratio. The study recommends the prediction of foliar N: P ratio at landscape level using airborne hyperspectral data and could be used by the resource managers, park managers, farmers and ecologists to understand the feeding patterns, resource selection and distribution of herbivores (i.e. wild and livestock).

Keywords: nitrogen, phosphorus, N: P, water removed spectra, grass, estimation, savanna ecosystem, spectroscopy

1. Introduction

Estimation of foliar biochemical concentrations provide information for assessing ecosystem functioning, for example, nutrient cycling, gas exchange and plant productivity (Martin and Aber, 1997; Ollinger et al., 2002). Foliar biochemical concentrations such as nitrogen (N) and phosphorus (P) are primary indicators of physiological processes such as photosynthesis, leaf respiration and growth rates (Evans, 1989; Field and Mooney, 1986; Güsewell, 2004). Foliar N and P concentrations can also be used as an indicator for grass quality (McNaughton, 1988, 1990). The foliar N concentration is known to relate to the protein (Clifton et al., 1994) which is one of the main nutrient requirement for the herbivores (Prins and Beekman, 1989; Prins and van Langevelde, 2008). Prins and Beekman, (1987) reported that nutrient requirement of Buffalo (*Syncerus caffer*) is about 6.2% protein (i.e. 1 % of N), while Duncan, (1992) found out that lactating equids requires about 0.24% of P in their food for maintenance. The importance of N supply on dry matter production as well as protein content is well documented in agricultural literature (Marschner, 1995) while foliar P is one of the main nutrient requirements for lactating mammals (McNaughton, 1990). Therefore, grass quality information can be used to understand feeding patterns and distribution of wildlife and livestock (Drent and Prins, 1987; Duncan, 1992; McNaughton, 1990; Prins and Beekman, 1989).

To understand nutrient limitation in vegetation, foliar N: P ratio is a key indicator (Cech et al., 2008; Güsewell, 2004; Koerselman and Meuleman, 1996). The foliar N: P ratio reflects the balance of N and P supply which influence plants at all levels, i.e. the growth and reproduction of individual plants, plant species interactions, composition and diversity (Cech et al., 2008; Güsewell, 2004). Differences in foliar biochemical concentrations as captured by N: P ratio in plants provide information on which nutrient is limiting and could determine the plant production or biomass which could eventually affect the feeding activity of herbivores in a particular ecosystem (Daufresne and Loreau, 2001; Güsewell, 2004; Prins and van Langevelde, 2008). Smaller herbivores are highly N limited as compared to larger ones and not vice versa (Prins and van Langevelde, 2008), because they require more nutrients and energy for growth and to achieve their daily activities. This tool has been widely used in the limnetic related studies (Güsewell, 2004; Güsewell et al., 2003; Koerselman and Meuleman, 1996). A review of 40 fertilization studies reported the critical values to determine N or P limitation on plant production (Table 1) (Koerselman and Meuleman, 1996). Table 1 outlines the critical values for foliar N: P to determine nutrient limitation on plant production for various ecosystems. Koerselman and Meuleman's (1996) critical values cannot be applied in the savanna ecosystems, because wetland vegetation are mainly C3-type, while the savanna species are C4-type (Cech et al., 2008; Craine et al., 2008; Ludwig et al., 2001). Güsewell (2004) revealed that at a vegetation level, the critical N: P ratio is <10 (N-limiting) and >20 (P-limiting) based on short term fertilization, and argued that the values might be different at individual species. Ludwig *et al*. (2001) found that below the tree, N: P ratio value of 12 indicates that P is limiting, while on the open grassland the average of 6 indicates N limiting in the savanna ecosystems. Cech et al. (2008) also concluded on the critical values of N: P for determining the limitation of N and P for plant production in the savanna ecosystems (Table 1). There is no general consensus on which critical range of N: P values could be used, because there are limited experimental studies focusing on this, especially in the savanna ecosystems.

(Table 1)

Using remote sensing, there is a significant potential in estimating foliar biochemical concentration, especially using field spectroscopy. A simple technique is to correlate vegetation index and a biochemical concentration of interest, e.g. N (Abdel-Rahman et al., 2010; Ferwerda et al., 2005; Rivero et al., 2009). Challenges for using this approach include the influence of soil exposure and atmosphereas well as saturation especially during the peak productivity (Jackson and Huete, 1991; Mutanga and Skidmore, 2004; Tucker, 1977). Several attempts were made to address these challenges using the red edge region e.g. Mutanga and Skidmore (2004). The second approach is to use the specific regions of the spectra which are known to relate to the electron transition or physical bond vibrations of the specific foliar biochemical concentrations referred to as absorption features (Darvishzadeh et al., 2008; Knox et al., 2011). For example, bands centred at 430 nm, 640 nm, 910 nm, 1020 nm, 1420 nm, 1690 nm, 1940 nm, 2060, 2240 nm, 2300 nm, are the absorption features related to electron transition and physical bond vibrations of foliar N and protein (Curran, 1989; Knox et al., 2010; Kumar et al., 2001). The list of absorption features are well documented in Curran (1989) and Kumar et al. (2001). Absorption features were successfully used for estimating N, P and chlorophyll (Darvishzadeh et al., 2008; Knox et al., 2011). Thirdly, the use of whole or full spectrum became prominent following the application of multivariate techniques such as stepwise multiple linear regression (SMLR) and partial least square regression (PLSR). The use of full spectrum was mainly coupled with several spectral transformation techniques such as continuum removal, log transformed (Log(1/R)), derivatives as well as the water removal (Dawson and Curran, 1998; Kokaly and Clark, 1999; Ramoelo et al., 2011).

The spectral transformation techniques are useful to enhance absorption features of foliar biochemical concentrations, while minimizing atmospheric, soil background, and water absorption effects, as well as data redundancy (Cho and Skidmore, 2006; Dawson and Curran, 1998; Yoder and Pettigrew-Crosby, 1995). Ramoelo et al. (2011) demonstrated that using WR and PLSR improves the estimation of foliar N and P in the controlled environment, because WR minimizes water absorption effect on fresh leaf spectra (Gao and Goetz, 1994, 1995). Continuum removal has also been applied to enhance absorption features for foliar biochemical concentrations (Curran et al., 2001; Kokaly and Clark, 1999; Mutanga et al., 2005). The Log (1/R) transformation is preferred to reflectance because it is linearly related to absorbing components (Hruschka, 1987; Yoder and Pettigrew-Crosby, 1995). Yoder and Pettigrew-Crosby (1995) showed a strong relationship between Log (1/R), as well as the first derivative of Log (1/R)', and foliar N concentration. Fourty and Baret (1998) argued that transforming reflectance into their corresponding absorbance values improved the accuracy of biochemical estimates. Continuum removal has also been applied to enhance absorption features for foliar biochemical estimations (Curran et al., 2001; Kokaly and Clark, 1999; Mutanga et al., 2005).

Foliar N concentrations has been estimated and mapped more often than P, especially using field and airborne hyperspectral data. This trend could be attributed to the following reasons;
    (i)   Low concentration of P in plants, normally 10 time lower than N (Knox et al., 2010; Meissner et al., 1999; Stark, 1970).
    (ii)   P has limited identified absorption features.

Nevertheless, foliar P in combination with N are crucialvariables for understanding the nutrient limitation (N: P ratio) for both plant and herbivores. Computation of N: P ratio depends on the

accurate estimation of foliar N and P, but P estimation is having challenges as noted above. Asner and Martin (2008) also argued that the retrieval of foliar P using hyperspectral remote sensing could be associated with the stoichiometry (i.e. indirect estimation based on the relationship between P and other foliar biochemicals) (Daufresne and Loreau, 2001; Elser et al., 1996). Predicting foliar N and P separately using field spectroscopy and later compute foliar N: P ratio could be faced by error propagation (combined errors from N and P prediction), because the retrieval accuracy of N and P is not consistently similar. To minimize these errors, direct estimation foliar N: P ratio using field spectroscopy is necessary. Nutrient limitation studies using foliar N: P ratios in the savanna ecosystem are rare, especially at landscape level using remote sensing. Using remote sensing has an advantage of the synoptic landscape view, which is impossible with conventional field measurement. In this study the field spectroscopy data will be tested, to demonstrate the possibility of using remote sensing to predict foliar N: P ratio. The objective of the study are twofold (1) to investigate the utility of field spectroscopy in combination with partial least square regression to predict foliar N: P ratio in the grass layer of the savanna ecosystem and (2).to evaluate a potential of predicting foliar N:P using airborne hyperspectral mapper (HyMap), through convolving field spectra into HyMap wavelength characteristics.

2. Data collection

2.1. Study area and sampling design

The study area is located (i.e. two corner co-ordinates; $24^00'0''$ S, $31^010'0''$ E and $25^00'$ S, $32^00''$E) in the Lowveld savanna at the north-eastern part of South Africa (Figure 1). The Lowveld landscape corresponds to the low lying area extending from the foot slopes of the Drakensberg Great Escarpment to the west and the Mozambique coastal plain to the east (Venter et al., 2003). The topography is gently undulating with flat patches in localized areas, and with an average height of 450m a.s.l. The study area covers a land use transect ranging from protected areas such as the private-owned Sabi Sands Game Reserve (SGR) and the state-owned Kruger National Park (KNP) to communal lands in the Bushbuckridge region. The western part of the transect (communal areas) receives higher mean annual rainfalls (800mm yr^{-1}.) as compared to the eastern side of the transect (580 mm yr^{-1}) (Venter et al., 2003). The annual mean temperature is about 22°C. The dominant geology includes granite and gneiss with local intrusions of gabbro (Venter et al., 2003). Consequently, these areas are characterized by gradients of soil moisture and nutrients. The soil fertility of gabbro areas are higher than the granitic ones (Ben-Shahar and Coe, 1992; Venter et al., 2003). The main vegetation communities include the "granitic lowveld" and the "gabbro grassy bushveld" (Mucina and Rutherford, 2006). In the gabbro patches, grass species such as *Setaria sphacelata* dominates the crest while species such as *Urochloa mosambicensis* dominates the valleys. Gabbro patches are dominated by grass species with high productive potential (e.g. *Urochloa mosambicensis*) compared to granite-derived soils (e.g. *Eragrostis rigidior and Pogonarthria squarrosa; cf.* Mutanga et al. (2004). The gabbro sites are dominated by fine leaves tree species such as *Acacia ssp* while the granite sites are dominated by broadleaves tree species such as *Combretum spp* and *Terminalia spp* (Ferwerda et al., 2006; Venter et al., 2003). Rangelands in the protected areas are grazed by wild herbivore such as impala (*Aepyceros melampus*), zebra (*Equus burchelli*), wildebeest (*Connochaetes taurinus*), buffalo (*Syncerus caffer*), etc., while the communal rangelands support grazing of cattle (*Bos*

taurus) and goats (*Capra hircus*) as well as sheep (*Ovis aries*), which determine various grazing intensities.

(Figure 1)

The study area comprised of eight experimental sites which were placed along the land use gradient: two sites in KNP (L1 gabbro, L2 granite), two sites in SGR (L3 granite, L4 gabbro), and four sites in the communal areas (L5-6 gabbro, L7-8 granite) (Fig. 1). The sites (totalling ca. 35000ha) were demarcated using 1:250, 000 geology maps and refined using 2008 SPOT 5 images (Wessels et al., 2011). The site selection process was designed to capture the nutrient contrast from low to high in granitic-derived soils to gabbro-derived soils, respectively. A line transect sampling design was used to collect field data (Fewster et al., 2005) in each site except L3 (because of access limitations). The topography influences the grass biomass in the savanna ecosystems with valley areas generally having higher grass biomass than crest areas. Along transects, a combination of purposive and systematic placement of sampling plot was undertaken. The distance between the plots was between 500m and 1000m depending on the accessibility and homogeneity of the area. The plot size was 30 m x 30 m. A total of 49 plots were surveyed and in each plot three to four subplots (0.5 m x 0.5m) were randomly selected to capture the plot variability. In each subplot, data on the sample location using the Leica®'s GS20 differential geographic positioning system (DGPS), dominant grass species and grass samples were collected. Grass samples were dried at 80^0C for 24 hours and the measurements were later averaged at plot level. The DGPS points were post-processed using Leica's GeoPro software and reference GPS data from Nelspruit station to produce less than 1 m positional accuracy. The fieldwork was undertaken in March/April 2009 towards the end of the wet season, when the grass biomass had achieved a maximum productivity to minimize the N/P and biomass interaction effects (Plummer, 1988; Skidmore et al., 2010).

2.2. Chemical analysis

The dried grass samples were taken to the South African Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC)-Nelspruit for chemical analysis. Firstly, the acid digestion technique was used, where perchloric and nitric acids were used for foliar P concentration retrieval and sulphuric acid was used for retrieving foliar N concentrations (Giron, 1973; Grasshoff et al., 1983; Mutanga et al., 2004a). Secondly, the colorimetric method by auto analyser was used to measure foliar N and P (Technicon Industrial Method 329-74 W; Technicon Industrial Systems, Farrytown, New York). These extraction methods were successfully used for grass foliar N and P by Mutanga et al. (2004) and Ramoelo et al. (2011).

2.3. Canopy Spectral measurements

The reflectance spectra were measured using an Analytical Spectral Device (ASD) spectroradiometer, Fieldspec 3®. The ASD spectral domain ranges from 350 to 2500 nm, with 1 nm band width. Within each plot, spectral measurements were made for each of the 3 to 4 randomly selected subplots. In each subplot, five spectral measurements were taken and later averaged to account for illumination and grass canopy structural differences as well as bidirectional effects (Mutanga et al., 2003; Wang et al., 2009). The measurements were taken between 10h30 and 15h00 on clear sunny days to minimize cloud effects and maximize illumination (Abdel-Rahman et al., 2010). A 25° field-of-view fibre optic was used. The fibre

optic pistol was held at 1m above the ground and at nadir to cover the entire subplot. A Spectralon reference panel was utilized before each measurement to calibrate the sensor and convert spectral radiance to reflectance.

3. Data analysis

3.1. Spectral pre-processing and transformation techniques

Spectral data were smoothed with using Savisky-Golay filter (Savitzky and Golay, 1964), adding a second order polynomial least square function and 3-band window to remove signal noise. Field spectral data were convolved to Hyperspectral Mapper (HyMap) wavelength characteristics to test the potential or applicability of airborne or imaging spectrometer in estimating foliar N:P. HyMap offers 128 spectral bands covering 400 to 2500 nm spectral regions, with a bandwith ranging from 15 to 20nm (http://www.hyvista.com/?page_id=440). HyMap is a commonly used data for mapping foliar biochemical at various ecosystems (Huang et al 2004; Skidmore et al. 2010).

Spectral transformation techniques such as log transformed spectra (Log (1/R)), first derivative, water removal and continuum removal were used. Log (1/R) transformation was determined by calculating a log function of reciprocal of the spectral reflectance (Fourty and Baret, 1998; Hruschka, 1987; Yoder and Pettigrew-Crosby, 1995). 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 (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, 2001; Kokaly and Clark, 1999; Mutanga et al., 2004b). The water removal 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, 1995), modified by Schlerf et al. (2010) and Ramoelo et al. (2011). The WR technique was implemented as per Ramoelo et al. (2011).

3.2. Regression analysis and bootstrapping

PLSR (Ehsani et al., 1999; Geladi and Kowalski, 1986; Viscarra Rossel, 2008) was used to predict the foliar N:P. PLSR was sought to be a robust multivariate technique, and proved to reduce the problem of over-fitting (Viscarra Rossel, 2008). PLSR has been successfully used for foliar biochemical estimations (Asner and Martin, 2008; Huang et al., 2004; Ramoelo et al., 2011). The performance of the transformed spectra and PLSR was measured using a bootstrapping approach (Efron, 1983). The advantage of bootstrapping is that it can be used efficiently when there is a limited sample size. Bootstrapping techniques iteratively resample the data set to be used for model development, making it an appropriate technique for assessing model accuracy (Verbyla and Litvaitis, 1989). To integrate PLSR and bootstrapping, bagging-PLSR was implemented using the ParLes 3.1 software (Viscarra Rossel, 2007, 2008). The bagging PLSR has advantage of improved prediction, and derives robust models insensitive to

over-fitting and provides uncertainty measure for prediction by computing the confidence interval (Viscarra Rossel, 2008).

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; Darvishzadeh et al., 2008; Viscarra Rossel, 2008). This Optimal number of factors was then used for model development and validation with 1000 bootstraps.

The retrieval accuracy of the PLSR model 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 RMSE. The scores or the variable of importance for prediction (VIP) (equation 1) was computed to determine which bands contributed more in the foliar N:P prediction model development (Viscarra Rossel, 2008). The VIP is calculated by:

$$\text{VIP}_k\,(a) = K \sum_a w^2{}_{ak}(\text{SSY}_a/\text{SSY}_t)\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(1)$$

where $\text{VIP}_k(a)$ is the importance of the kth predictor variable based on a model with a factors, w_{ak} corresponds to the loading weight of the kth variable in the ath PLSR factor, SSY_a is the explained sum of squares of y by a PLSR model with a factors, SSY_t is the total sum of squares of y, and K is the total number of predictor variables (Viscara Rossel 2008). The wavebands or predictors with high scores were associated with the known absorption features by Curran (1989) and Kumar et al. (2001) respectively. Because ASD has a 1 nm band width, the maximum difference of 20 nm between the bands with high scores and known absorption features were chosen for the consistent comparison.

4. Results

The full spectrum analysis results showed that foliar N: P could be successfully estimated using field spectra and partial least square regression. WR and CR spectra in combination with PLSR which produced higher estimation accuracy of foliar N: P compared to the log (1/R), R and FD spectra (Figure 2, Table 2). WR and CR spectra resulted to the similar root mean square error (RMSE) of 1.12, which equals to 14% of the mean (Table 2). CR spectra accounted for 85% of the variation whilst WR accounted for 81% (Figure 2). The Log (1/R), FD and R spectra achieved a foliar N: P estimation accuracy ranging between 16-19% of the mean and the foliar N: P variation explained range between 69-76%, which is relatively lower than the ones for WR and CR (Table 2). For the HyMap analysis, spectral data showed a potential to estimate foliar N: P accounting for about 50-64 % of the variation, with FD, CR and WR showing high performance (accounting for 61-64% of the variation). The accuracy of FD, CR and WR ranges between 1.40-1.47%, which is about 17-19% of the mean. The highest accuracy was attained by WR with the lowest RMSE (Table 2).

 (Table 2)
(Figure 2)
The results from the VIP (i.e. variable of importance for prediction) analysis derived for each spectral data and PLSR showed the importance of each bands for predicting foliar N: P (Figure

3). Figure 3 shows the top 20 bands most important for predicting foliar N: P, using various spectral data. In Figure 3, the dark bars indicate bands associated with the known absorption features (e.g. N, protein) (Kumar et al. 2001; Curran et al 1989) and grey is classified as one of the top 20 bands not associated with known absorption features. For example, 90% of the selected bands from WR are associated with known absorption features, with 40% related to protein and N. Sixty percent (60%) of the selected band from the R spectra is associated with the known absorption features, while FD, CR as well as Log (1/R) yielded the percentage between 70-85%. For HyMap spectral analysis, WR and CR selected bands associated with known absorption features by 45 and 70%, respectively. While the remaining spectral transformation techniques based on HyMap spectra were associated with known absorption features by < 30% (Figure 3).

Table 3 shows that most of the transformed spectra and PLSR models for estimating foliar N: P consistently select bands from the SWIR, for example; 1350-1360 nm, 1400-1450 nm, 1980-2000 nm, 2000-2060 nm and 2300-2360 nm. Similar results were achieved when spectral data were covolved to HyMap wavelength characteristics (Table 3). These substantiate the evidence in Figure 3, that most of the bands for estimating foliar N: P are located in the SWIR, and are mostly related to the known absorption features.

(Table 3)
(Figure 3)

The foliar N:P data values are normally distributed as confirmed by Shapiro-Wilk normality test (W=0.983, p=0.69) (Royston, 1982). The variance of foliar N: P is relatively high as indicated by 30% of the coefficient of variance (CV) and an average of 7.86 (Table 4). The descriptive statistics of N and P are also presented in Table 4, where N has an average of 0.71% and 0.10% for P. Figure 4 shows examples of the measured reflectance with their corresponding foliar N: P values. Reflectances of the contaminated regions, especially by water were removed. The relationship between N and P is not very high ($R^2 < 0.25$), a corresponding scatterplot in Figure 5.

(Table 4)
(Figure 4)
(Figure 5)

5. Discussion

The study demonstrated that foliar N: P ratio can be estimated using transformed spectra derived from field spectroscopy and HyMap simulated spectral datacombined with PLSR. WR and CR spectral data achieved higher foliar N: P ratio estimation accuracy for field spectroscopy, while for HyMap spectra, FD, CR and WR achived higher estimation accuracy. The performance of WR is because it minimizes the water absorption effects on the sensitive weak or subtle regions of the foliar biochemical concentrations (Gao and Goetz, 1994, 1995; Ramoelo et al., 2011; Schlerf et al., 2010). Coincidentally, the regions of the reflectance most affected by water absorption are very important for foliar biochemical estimation. The WR technique was developed by Gao and Goetz (1994, 1995) to address this problem, especially on fresh leaf

spectra. Gao and Goetz, (1994; 1995) tested the WR technique successfully for estimating foliar cellulose and lignin. Ramoelo et al. (2011) demonstrated that WR could be used to as one of the techniques for estimating foliar biochemical, as shown from the experimental study in the controlled environments. WR spectra are applied here for the first time to estimate foliar N: P and yielded the promising results.

CR spectra improve the absorption features of foliar biochemical concentrations through enhancing the differences in the absorption strength (Huang et al., 2004; Kokaly and Clark, 1999; Mutanga et al., 2005). CR spectra have been used for estimating foliar N and P, not N: P ratio but showed a good performance. Several studies demonstrated the applicability of CR spectra for foliar biochemicals, especially for foliar N and P (Huang et al., 2004; Ramoelo et al., 2011). Mutanga *et al.* (2004) estimated both foliar N and P with the coefficient of determination between 43 to 80% using continuum removal derived from the field spectrocopy data. Huang *et al.* (2004) achieved a coefficient of determination of 0.65 for estimating foliar N using airborne hyperspectral data. The latter studies showed consistent performance of CR on foliar biochemical estimations, similar trend observed in this study. The reflectance, Log (1/R) and FD spectra showed a potential to estimate foliar N: P ratio. In most cases, Log(1/R) and FD have higher foliar biochemical retrieval accuracy than using the reflectance spectra (Johnson, 2001; Yoder and Pettigrew-Crosby, 1995), and this differs with our results. It is possible that foliar N: P ratio interacts differently with spectra as compared to N or P, especially in terms of physical vibration bonds. Yoder and Pettigrew-Cosby (1995), Johnson (2001) and Fourty and Baret (1996) found that Log (1/R) improves estimation of foliar N than the reflectance, because there is a linear relationship between the foliar biochemical and its contribution to the Log(1/R) at the absorbed wavelength (Hruschka, 1987; Kumar et al., 2001).

Selected bands in (Figure 3) were associated with the known absorption features which are listed in Curran (1989), Knox et al. (2010) and Kumar et al. (2001), which dominate the SWIR region (Table 2). This indicates that foliar N: P ratio estimation involves the bands associated with known absorption features with various vibration mechanisms for several foliar biochemicals. N, protein and starch or sugar were the main or dominant absorption features selected for predicting foliar N: P. For examples, N and protein features were centered around 910nm, 1420nm, 2060, 2180 and 2300nm, while for starch were centered around 970nm, 990nm, 1960nm, 2000nm, 2250nm and 2280nm. The bond vibration mechanisms associated with N and proteins were mainly C-H, N-H and C-O, while for starch ones were O-H, C-O and C-H. Despite the unavailability of P specific absorption features, starch absorption features are seen as promising in estimation of foliar N: P using field spectroscopy, and potentially using HyMap. Knox et al. (2012) used starch and sugar absorption features to estimate foliar P, because P is linked to the metabolic processes leading to starch development in the plant. The use of this approach is not as well established as the one for estimating foliar N using N and protein absorption features. Notwithstanding, other spectral data such as CR and FD (both field and HyMap) depict the importance of the chlorophyll absorption regions. The absorption process in the visible or chlorophyll absorption region is electron (e.g.), than physical bond vibrations. Generally, the bands which are not associated with known absorption features are associated with vegetation vigor, which can either influenced by chlorophyll and other pigments or nutrients such as lignin and cellulose. Selection of specific or known absorption features is not consistent over seasons (Knox et al., 2012). The developmental stages of a plant are coupled with changes in cell

structure, water content and functions, which in turn influence the reflectance which then impact on the selection of absorption features over a period of plant development (Knox et al. 2012). During the wet season (similar period to this study), absorption features especially for N are located in visible/ near infrared and SWIR (Ustin et al., 2009), while in dry season N features are active around SWIR region (Asner et al., 1998).

Using WR based on field spectra, 40% of the 90% of known absorption features were associated with N and protein. According to the range of values for nutrient limitation by Cech et al. (2008), our study area is limited by N and P because of foliar N: P ratio values are between 3 and 14. In this study, nutrient limitation could be associated with the contrast between low fertile soils derived from granite and high fertile soils derived from gabbro geological types (Grant and Scholes, 2006; Scholes, 1990; Venter et al., 2003). Similarly, Ludwig et al. (2001) reported that under the tree of the savanna ecosystems, 12 is already indicating P limitation, which means according to the range of values of foliar N: P ratio in the study, N or P could be limiting in some areas than the other. The low relationship between N and P (Figure 5) indicates that either N or P could be limiting and it is difficult to conclude that the accuracy of N: P ratio is dependent to N or P. In addition, Ludwig et al. (2001) mentioned that open grassland have an average foliar N: P ratio of 6 indicating that N is limiting, which similar results observed by this study (mean foliar N: P of 7.86). Craine et al. (2008) also revealed that unfertilized vegetation had a mean N: P ratio of 5.8, during their experiments in KNP, which is similar to the N: P values in the open grassland found by Ludwig et al. (2001), and by Cech et al. (2008) in the tropical savanna. The difference between Koerselman and Meuleman's (1996) in the European freshwater wetlands, as well as Ludwig et al. (2001) and Cech et al. (2008) in the savanna grasses (C4 type) could be attributed to the fact that savanna grasses have relatively lower N requirement as compared to wetlands plants (C3-type). Plants with C4 photosynthetic pathways have high nutrient efficiency than C3 plants (Craine et al., 2008; Wolfson and Tainton, 2000).

Phenology plays a crucial role in foliar biochemical estimation using spectroscopy. A combination of visible and SWIR is important to estimate foliar N: P during wet or peak productivity season, with minimum biochemical and biomass or leaf area index (LAI) interactions. During this period, a relationship between biomass or LAI with the visible region (even through vegetation indices) saturates or is asymptotic. Therefore, it is during this time when foliar biochemicals can be estimated without being compromised by the biomass or LAI effects (Skidmore et al. 2010; Plummer 1988).

6. Conclusions

The study demonstrated that foliar N: P ratio can be estimated using *in situ* hyperspectral remote sensing. Because this is first study to do this, there is a need to test the applicability of using airborne hyperspectral remote sensing data such as HyMap, to understand the landscape variability of foliar N: P. The challenge for estimating foliar N: P ratio at regional scale (up-scaling) is that satellite or multispectral remote sensing data have limited bands in the SWIR region, and this study indicated that most of the bands sensitive to foliar N:P are located in the SWIR. There is a need for several experimental studies for understanding how foliar N: P

influence grass productivity, and hence the feeding patterns of herbivores in the savanna ecosystems. Such experimental studies could play a crucial role in determining critical values of foliar N: P ratio for ascertaining nutrient limitation on grass productivity. The foliar N: P ratio as an indicator for nutrient limitation could be useful information to the ecologists, resource managers, farmers and park managers to understand which between N and P is limiting and how the limitation influence the resource selection (Manly et al., 1993), distribution, densities and population dynamics of herbivores (wild and livestock), at landscape level.

7. Acknowledgement

8. References

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Tables

Table 1: Critical values for determining nutrient limitation (N: P) for various ecosystems.

Ecosystems	N-limitation	Co-limitation	P-limitation	Reference
Savanna (Tanzania)	< 9	9 – 10	> 10	Cech et al., (2008)
Freshwater Wetlands (Europe)	< 14	14 – 16	> 16	Koerselman and Meuleman (1998)
Savanna (Tanzania)	< 6	6 – 12	> 12	Ludwig et al., (2001)
Savanna (South Africa)	< 5.8	–	–	Craine et al., (2008)
Wetlands (Europe)	<10	–	> 20	Güsewell, (2004)

Table 2: The performance of each field and HyMap spectra combined with partial least square regression in estimating the foliar N: P and validated using bootstrapping

	R^2	RMSE	95% LCI	95% UCI	RRMSE (%)	No. of factors
Field Spectra						
CR	0.85	1.12	0.95	1.44	14.19	8
WR	0.81	1.12	0.93	1.40	14.19	9
R	0.76	1.22	1.01	1.52	15.46	13
Log (1/R)	0.73	1.30	1.07	1.62	16.47	12
FD	0.69	1.50	1.22	1.83	19.01	15
HyMap Spectra						
CR	0.61	1.47	1.22	1.84	18.62	9
WR	0.63	1.40	1.17	1.77	17.74	14
R	0.50	1.70	1.41	2.13	21.52	8
Log(1/R)	0.61	1.48	1.23	1.86	18.78	10
FD	0.64	1.43	1.19	1.80	18.16	10

CR=continuum removal, WR=water removal, R=original reflectance, FD=first-difference derivative, LCI=Lower bound confidence interval at 95% confidence level. UCI=Upper bound, RRMSE=relative root mean square error (% of mean)

Table 3: The selected regions by each spectral data and partial least square regression, and the consistency of each spectral data (field and HyMap Spectra) to estimate foliar N: P ratio.

Spectral Data	A	B	C	D	E	F	G	H	I
WR			x	X	X	X	Xx		Xx
CR	Xx	Xx			x		x	x	Xx
R		X	x	Xx		X	Xx	Xx	x
FD	x	x	Xx	Xx	Xx	X	X	Xx	X
Log (1/R)	Xx		x	X	X		Xx		X

WR=water removal, CR=continuum removal, R=reflectance, FD=first derivative, WR=water removal, CR=continuum removal, R=reflectance, FD=first derivative, A=400-470nm, B=530-560nm, C=1000-1130nm, D=1350-1360nm, E=1400-1450nm, F=1980-2000, G=2000-2060, H=2200-2300nm, I=2300-2360nm, **X**=Field Spectra, **x**=HyMap Spectra

Table 4: Descriptive statistics for the foliar N: P

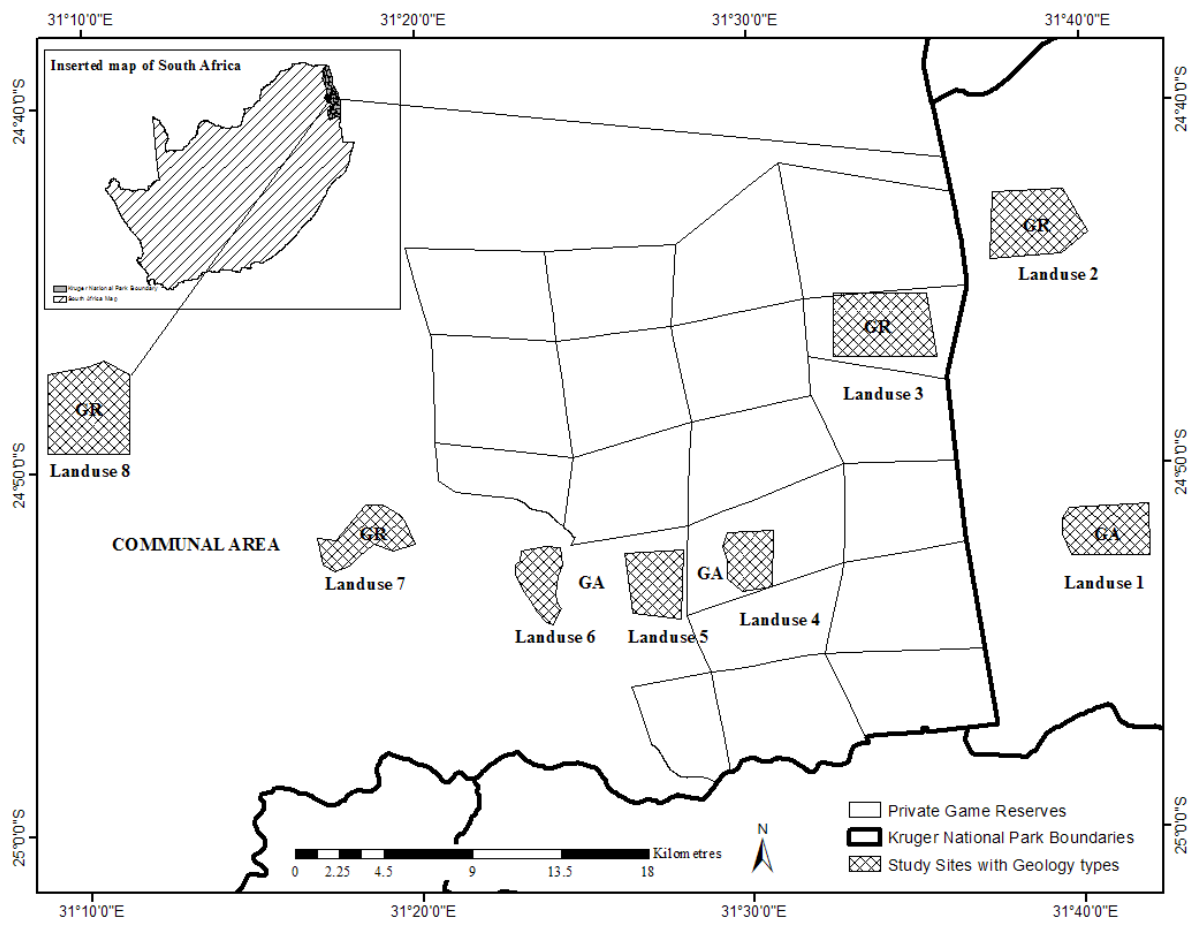| Biochemical	Min	Max	Mean	STDEV	Coefficient of Variation (CV)
N:P	2.63	14	7.89	2.35	0.30
N (%)	0.34	1.06	0.71	0.18	0.26
P (%)	0.04	0.29	0.10	0.05	0.49

Figures

Figure 1: Study area map showing a location of the north-eastern part of South Africa.
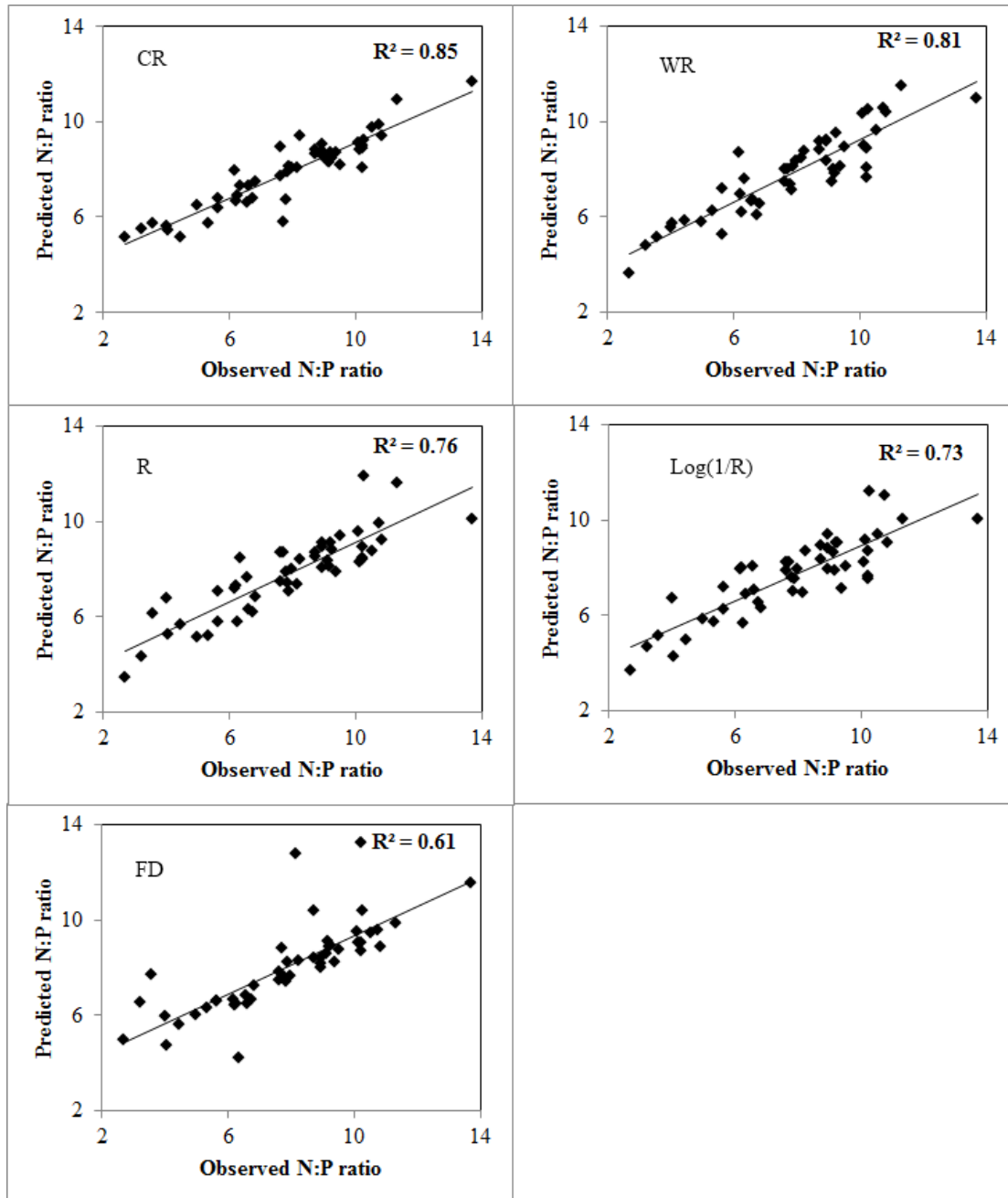GA=Gabbro, GR=Granite.

Figure 2: Scatterplots indicating the performance of each spectra (Field based) and partial least square regression in estimating the foliar N: P validated using the bootstrapping. CR=continuum removal, WR=water removal, R=original reflectance, FD=first-difference derivative.
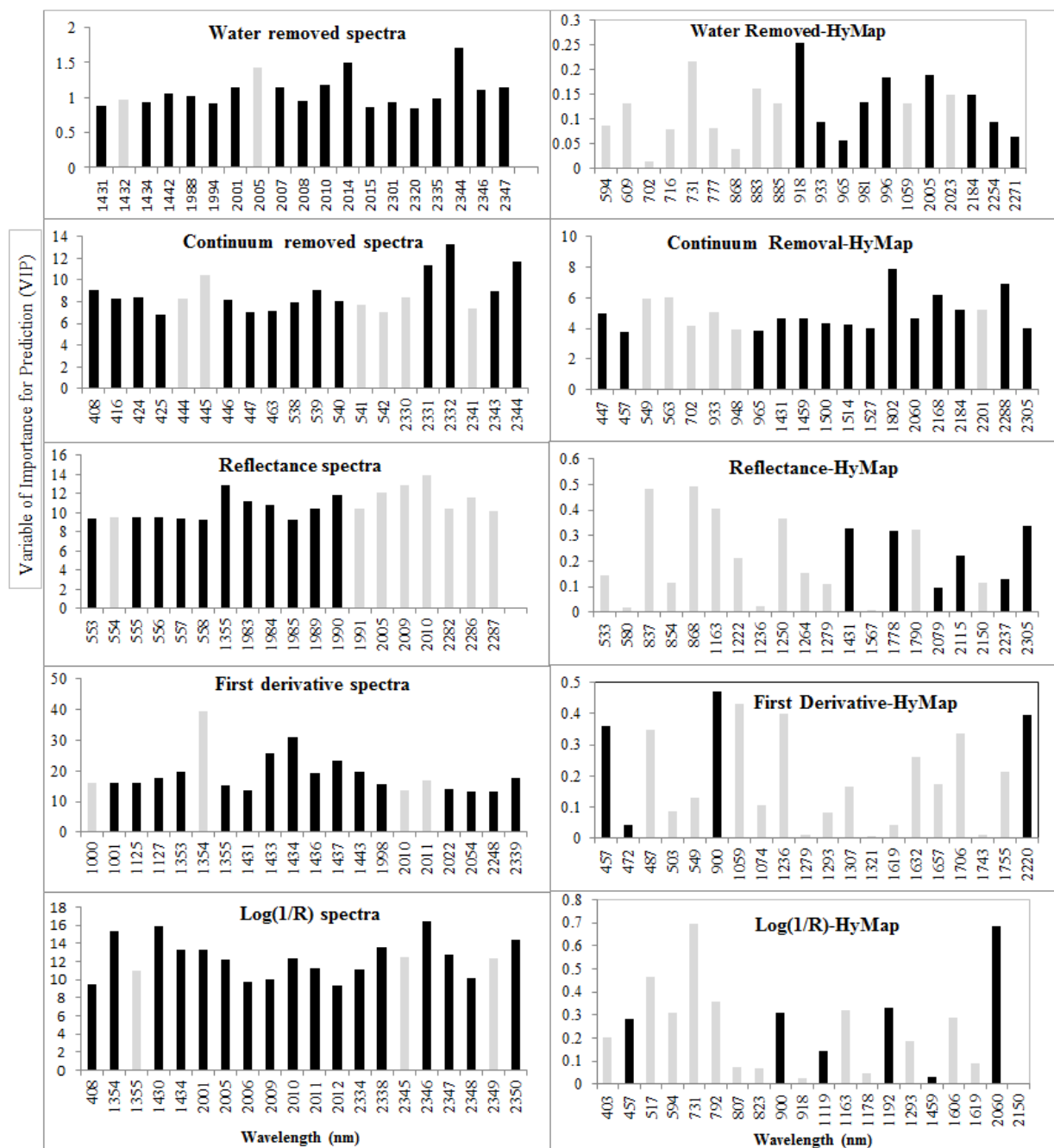
Figure 3: The variable of importance for prediction (VIP) i.e. important wavebands for predicting foliar N: P ratio derived from each spectral data and partial least square regression (PLSR). The displayed bars are the top 20 bands selected from the performance of each spectral data. The dark or **bold bars** indicate that selected bands are associated with known absorption features listed by Curran, (1989) and Kumar et al., (2001) and grey bars are bands belonging to the top 20, but not associated with the known absorption features.
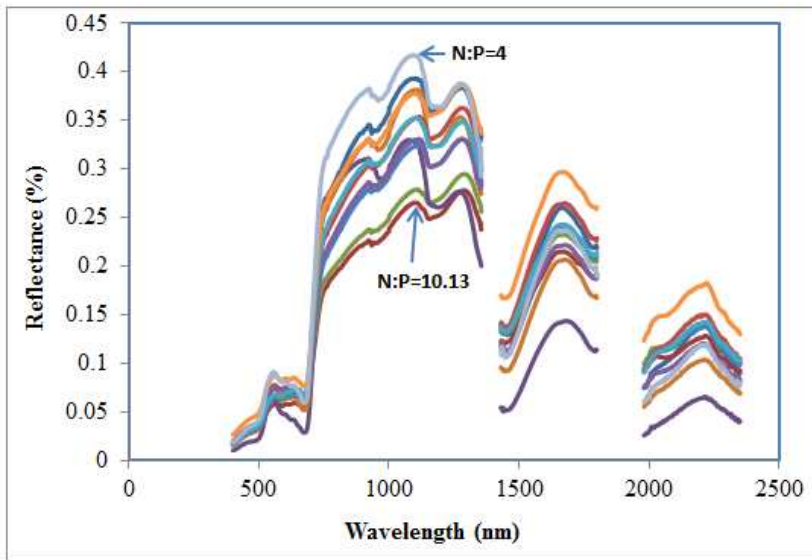
Figure 4: Spectra with the corresponding low and high foliar N:P values. Due to atmospheric or water contaminations of the spectra, regions around no-value areas above were regarded as noise and removed.
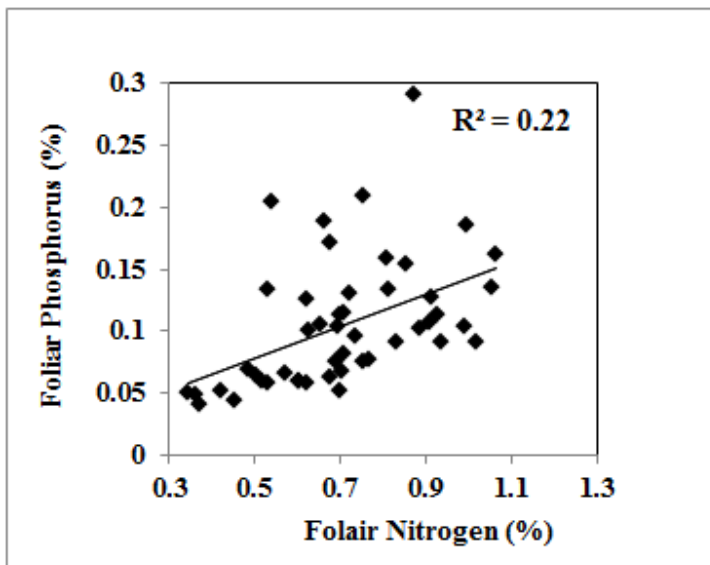

Figure 5: Shows a relationship between foliar N and P concentrations (%)