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Predicting leaf and canopy ^{15}N compositions from reflectance spectra

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[1] We explored whether ^{15}N concentration could be predicted from reflectance spectra of fresh leaves, and, if so, whether the spectral features were related to the ^{15}N concentration on a canopy scale. Leaf scale reflectance (R) measurements were conducted in Ghanzi, Botswana using a spectrophotometer in March 2005 and canopy scale leaf R was measured in a series of successional fields in Northern Virginia, USA using the same instrument in September 2005. Results showed that there was a strong correlation between foliar ^{15}N concentration and spectral data in both visible and near-infrared wavelength regions. Stepwise regressions showed that the first-difference of the log1/R [(log 1/R)′] could explain 76 to 92% of the variation in foliar δ^{15}N, providing the most reliable correlations with foliar ^{15}N at bands near 600 and 700 nm. The present study indicates the possibility of estimating fresh leaf ^{15}N abundance from high-resolution reflectance at leaf and canopy levels. **Citation:** Wang, L., G. S. Okin, J. Wang, H. Epstein, and S. A. Macko (2007), Predicting leaf and canopy ^{15}N compositions from reflectance spectra, *Geophys. Res. Lett.*, *34*, L02401, doi:10.1029/2006GL028506.

1. Introduction

[2] In terrestrial ecosystems, the ratio of the rare, but naturally occurring, ^{15}N isotope to the highly abundant ^{14}N isotope is an index of many processes occurring in soils, plants, and the atmosphere [*Robinson*, 2001]. Methods for analyzing ^{15}N contents therefore are powerful tools that can be used to elucidate biogeochemical relationships. Traditionally, the concentration of foliar ^{15}N is measured in the laboratory using Isotope Ratio Mass Spectrometer (IRMS) with reproducibility better than 0.2‰. The purpose of this report is to comment on the feasibility of using high-resolution spectral data to estimate relative ^{15}N abundances in leaves.

[3] Analyses of foliar nitrogen concentration with reflectance spectrometry can be as accurate as traditional wet-chemistry procedures and in fact, in many laboratories, near-infrared spectrometry has replaced wet chemistry as the standard analytical procedure for plant biochemicals for dried and ground leaves [*Yoder and Pettigrew-Crosby*, 1995]. Furthermore, *Yoder and Pettigrew-Crosby* [1995] showed that nitrogen concentrations could be predicted from reflectance spectra of fresh leaves in the laboratory and possibly at canopy scales. *Martin and Aber* [1997] further demonstrated that foliar nitrogen concentrations could be estimated using satellite data. Because there is a close relationship between foliar ^{15}N concentrations and foliar N contents in many ecosystems [e.g., *Hobbie et al.*, 2000], there is a potential relationship between foliar ^{15}N and spectral reflectance. Here, we report on the relationship between foliar ^{15}N concentration and spectral reflectance (350–2500 nm) at both leaf and canopy scales for fresh leaves.

2. Materials and Methods

2.1. Study Sites and Field Measurements

[4] Leaf-level spectral reflectance was measured in Ghanzi, Botswana in March 2005 (21.65°S, 21.81°E). The vegetation in Ghanzi is open savanna dominated by *Acacia* species such as *Acacia luederizii* Engl. and *Acacia mellifera*. The mean annual precipitation is 370 mm and mean annual temperature is around 21°C [*Shugart et al.*, 2004]. Individual leaves from eight individual plants including both trees and grasses were chosen and 10–15 measurements were made for each individual. Leaf reflectance was measured with an ASD FieldSpec Pro FR portable spectroradiometer using a leaf contact probe with a halogen light source (ASD, Inc., Boulder, CO). This instrument has a spectral range of 350–2500 nm with 1 nm sampling intervals and a 10 Hz sampling rate. A one-second integration time was used for each measurement. Reflectance was calculated as the ratio of the reflected radiance of the leaf (minus dark-target radiance) to the reflected radiance of a Spectralon panel (minus dark-target radiance). Leaves were clipped from plants after spectral measurement, and placed in labeled paper bags to dry.

[5] Canopy-level reflectance was measured at the Blandy Experimental Farm (BEF) in four successional fields (two early stages and two middle stages). The BEF study site is located in Clarke County, Virginia (39.15°N, 78.10°W). The BEF is a 283.5-ha experimental field with sites of distinct successional ages, which is owned and operated by the Department of Environmental Sciences at the University of Virginia. The BEF can be described as an agro-ecosystem consisting of agricultural fields, successional fields, deciduous woodlands and ephemeral wetlands. The average annual temperature (1971–2000) is 15°C and the mean annual precipitation is 100 cm (data obtained from the Martinsburg weather station, 40 km from BEF). This research utilized two sets of successional fields at the BEF, approximately 2 km apart. Four distinct successional fields have been maintained over the two sites: a 5-year-old field (early stage1), a 3-year-old field (early stage2), a 19-year-old field (middle stage1), and an 18-year-old field (middle stage2). The 5-year-old field and 19-year-old field are adjacent to each other, and the 3-year-old field and 18-year-old field are located at another site [*Bowers*,

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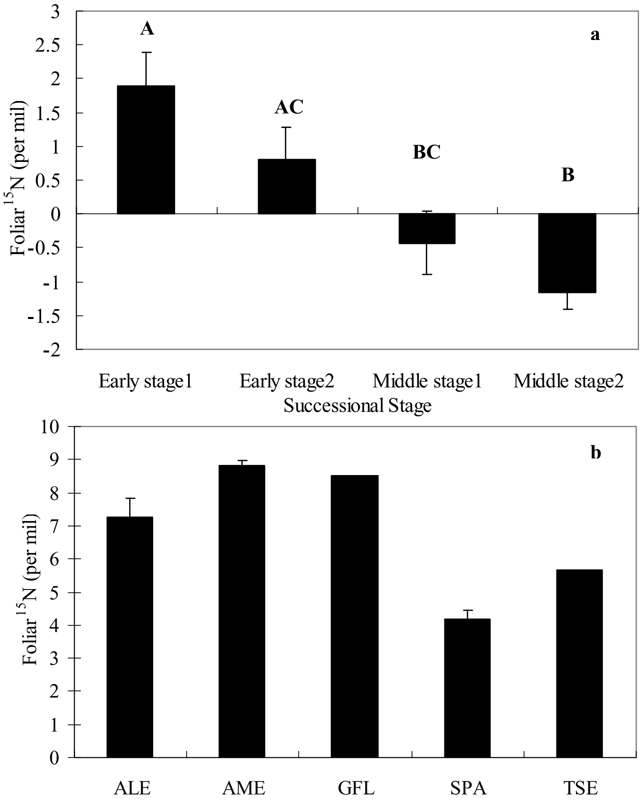

Figure 1. (a) Canopy level foliar δ^{15}N in four successional fields at the Blandy Experimental Farm, Virginia (Different capital letters indicate different mean values of foliar δ^{15}N). (b) Species sampling list and leaf level foliar δ^{15}N in Ghanzi, Botswana. ALE (*Acacia leuderitzii*), AME (*Acacia mellifera*), GFL (*Grewia flava*), SPA (*Schmidtia pappophoroides*), TSE (*Terminalia sericea*).

1993; *Riedel and Epstein*, 2005; *Emanuel et al.*, 2006]. The 3-year-old field and 5-year-old field are relatively homogeneous and dominated by *Solidago spp.*; there are other dicots such as *Asclepias syriaca* and *Carduus spp.*, and some shrub seedlings in the understory such as *Rhamnus cathartica* and *Celastrus orbiculatus*. The 18-year-old field is dominated by *Celastrus orbiculatus*, *Rhamnus cathartica*, *Maclura pomifera*, *Solidago spp*. and *Daucus carota*. The 19-year-old field is a mixture of woody species such as *Rhamnus cathartica*, *Celastrus orbiculatus* and *Ailanthus altissima*, and herbaceous dicots such as *Solidago spp.*, *Daucus carota*, *Asclepias syriaca*, *Carduus spp*.

[6] Canopy-level reflectance was measured with the ASD FieldSpec Pro FR spectroradiometer with a 20° field of view. The sun was used as the light source and a Spectralon panel was used to convert reflected radiance to reflectance. Around 20 nadir-viewing reflectance measurements were taken within five randomly located 1 × 1 m locations at each of the four sites. Different successional ages were used because there are distinct foliar ^{15}N signatures among successional ages (Figure 1a). After the spectral measurement, foliage was sampled from each 1×1 m^2 location, separated by species, and stored in paper bags to dry.

2.2. Isotope and Elemental Analyses

[7] Leaf-level foliar δ^{15}N was estimated by averaging δ^{15}N for all leaves collected from individual plants. Canopy-level foliar δ^{15}N was estimated by averaging foliar δ^{15}N of all species (in general, the foliar δ^{15}N was similar across species for the same successional stage) for each sampling location. Prior to isotope and elemental analysis, foliar samples were dried at 60°C for 72 hours. After drying, they were ground and homogenized for isotope and elemental analysis. Stable nitrogen isotope analysis was performed using a Micromass Optima Isotope Ratio Mass Spectrometer (IRMS) coupled to an elemental analyzer (EA) (GV/Micromass, Manchester, UK). Nitrogen stable isotope compositions are reported in the conventional form (‰):

$$\delta^{15}\mathrm{N}(‰) = \left[\left({}^{15}\mathrm{N}/{}^{14}\mathrm{N}_{\mathrm{sample}}/{}^{15}\mathrm{N}/{}^{14}\mathrm{N}_{\mathrm{std}}\right) - 1\right] \times 1000$$

where $({}^{15}\mathrm{N}/{}^{14}\mathrm{N})_{\mathrm{sample}}$ is the nitrogen isotope composition of a sample, and $({}^{15}\mathrm{N}/{}^{14}\mathrm{N})_{\mathrm{std}}$ is the nitrogen isotope composition of the standard material. The standard material for stable nitrogen isotopes is atmospheric molecular nitrogen (AIR). Reproducibility of these measurements is approximately 0.2‰.

2.3. Data Analysis

[8] No pre-processing was applied to leaf-level reflectance spectra. To minimize the effect of variable shading in the canopy-level spectra, raw reflectance spectra were scaled so that the reflectance from wavelength 350 nm to 1780 nm spanned the interval [0,1]. Excessive noise due to low solar irradiance in the short-wave infrared (the region >1780 nm) prohibited the scaling and subsequent analysis. Leaf and scaled canopy reflectance spectra were smoothed with running averages, plus and minus four nanometers ($R_{\lambda(\mathrm{smooth})}$ = average ($R_{\lambda-4\ \mathrm{nm}}$, $R_{\lambda-3\ \mathrm{nm}}$, $R_{\lambda-2\ \mathrm{nm}}$, $R_{\lambda-1\ \mathrm{nm}}$, R_{λ}, $R_{\lambda+1\ \mathrm{nm}}$, $R_{\lambda+2\ \mathrm{nm}}$, $R_{\lambda+3\ \mathrm{nm}}$, $R_{\lambda+4\mathrm{nm}}$) following *Yoder and Pettigrew-Crosby* [1995]. First-difference spectra of log 1/R (log 1/R)$'$ was calculated from the difference between the values at each λ, plus and minus four bands, divided by the range of wavelength ($R' = (R_{\lambda+4} - R_{\lambda-4})/8$) [*Yoder and Pettigrew-Crosby*, 1995].

[9] Foliar δ^{15}N values between different succesional stages were compared using one-way ANOVA (SAS v. 9.1 PROC GLM), and a Tukey post hoc test was used to separate the means. The Pearson correlation coefficients were calculated between foliar δ^{15}N and R, between foliar δ^{15}N and (log 1/R)$'$ at all wavelengths using SAS (v. 9.1), and correlograms were created based on Pearson correlation coefficients to easily visualize the results.

[10] The method of *Sokal and Rohlf* [1995] was used to determine the significance of correlations for each band for R and (log 1/R)$'$. Stepwise regressions (SAS v. 9.1) were performed to find the best predictors (e.g. the best wavelengths) of foliar δ^{15}N at both leaf and canopy levels. Wavelength selection was based on the correlograms, and only the wavelengths associated with significant peaks ($p < 0.05$) were selected in stepwise regressions.

3. Results and Discussion

[11] Foliar δ^{15}N range in Virginia was between −1.5 to 3.4‰ (Figure 1a). In both sets of successional series, foliar

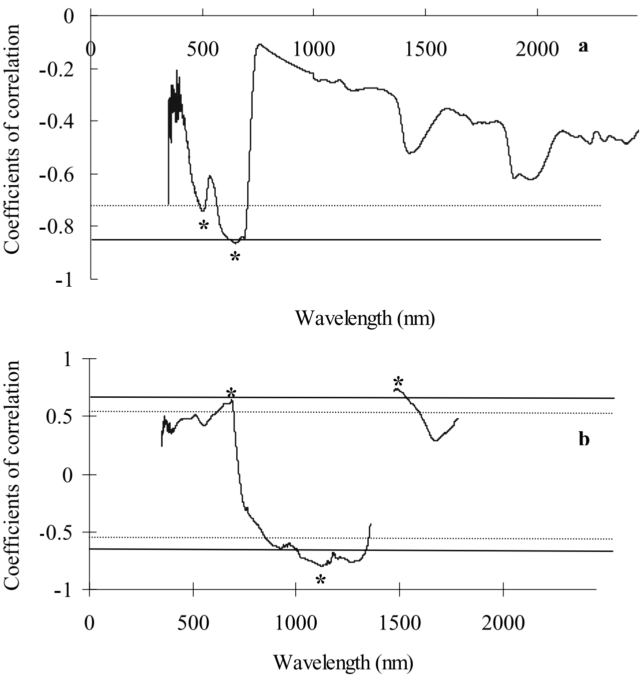

Figure 2. (a) Coefficients of correlation between foliar δ^{15}N and leaf-level reflectance (R) (dash horizontal bars indicate the area of significance, $p < 0.05$; solid horizontal bars indicate the area of significance, $p < 0.01$, the regions marked with an asterisk indicate regions that may be useful for predicting foliar δ^{15}N, the ranges of wavelength for asterisk regions from left to right are $\lambda = 483-517$ and $\lambda = 617-703$ nm). (b) Coefficients of correlation between foliar δ^{15}N and canopy-level reflectance (R) (dash horizontal bars indicate the area of significance, $p < 0.05$; solid horizontal bars indicate the area of significance, $p < 0.01$, the regions marked with an asterisk indicate regions that may be useful for predicting foliar δ^{15}N, the ranges of wavelength for asterisk regions from left to right are $\lambda = 670-694$, $\lambda = 1098-1319$ and $\lambda = 1480-1522$ nm).

δ^{15}N at the canopy level was significantly higher in the early successional field than that in the middle successional field (Figure 1a) although the difference between middle stage1 and early stage2 was not significant. Foliar δ^{15}N range in Ghanzi was between 4.2 to 8.8‰ (Figure 1b).

[12] Correlograms provide a clear picture of the relationships between spectral reflectance and foliar δ^{15}N. Horizontal bars in the correlograms of Figures 2 and 3 are the boundary of correlation coefficients beyond which the results are significant (dash line for $p < 0.05$ and solid line for $p < 0.01$). The regions marked with an asterisk indicate regions that may be useful for predicting foliar δ^{15}N. These regions were chosen based on three criteria: 1) correlation coefficients are statistically significant ($p < 0.05$), 2) the region is wide enough to be used in normal field and laboratory conditions (>15 nm) and 3) the regions do not fall in wavelength ranges where atmospheric water vapor or other factors might interfere with field or remote sensing-based spectroscopy. For instance, water vapor absorptions at ~940 nm, ~1140 nm, 1360–1470 nm and 1800–2000 nm preclude the use of these regions in practical applications. Significant noise >1780 nm due to weak solar irradiance in this region also eliminates these regions from practical use for the spectroscopic estimation of ^{15}N.

[13] At the leaf scale, two broad regions were observed in which the correlation between R and foliar δ^{15}N were significant ($\lambda = 483-517$ and $617-703$ nm, $|r| = 0.71$ to 0.86) (Figure 2a). At the canopy scale, the strong relationship between R and foliar δ^{15}N appears at several ranges including both visible and near-infrared (VNIR) regions ($|r| = 0.60-0.80$) (Figure 2b). Although VNIR wavelength regions exhibit very high correlations, the use of R to estimate foliar ^{15}N may not be practical for remote sensing applications. This is due to the fact that variable lighting conditions can cause significant changes in the overall reflected brightness. The use of R to estimate ^{15}N should be limited to cases where lighting conditions and geometry can be controlled (e.g., a leaf-contact probe or laboratory conditions) or R empirically showing the advantages.

[14] With the first-difference transformation [(log 1/R)′], there are several narrow regions in the VNIR that appear to be useful for the estimation of ^{15}N (Leaf level: Figure 3a; Canopy-level: Figure 3b). First-difference spectra highlight regions where the spectral reflectance changes due to variations in absorption or scattering in the foliage. The

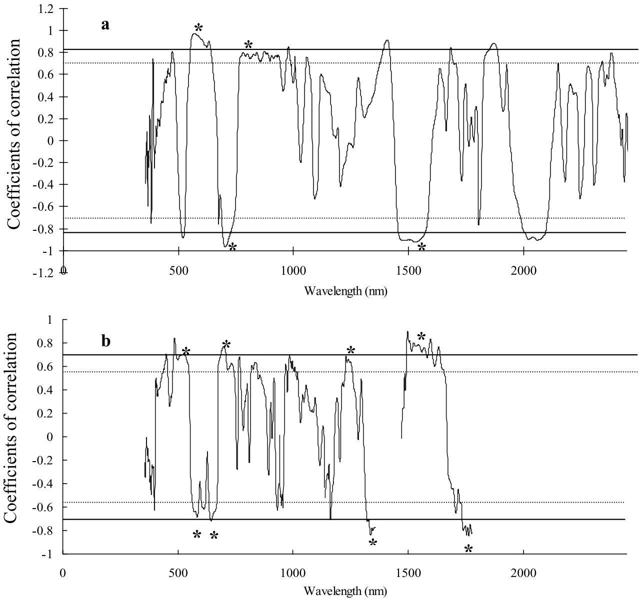

Figure 3. (a) Coefficients of correlation between foliar δ^{15}N and leaf-level first difference of log 1/R (log 1/R)′ (dash horizontal bars indicate the area of significance, $p < 0.05$; solid horizontal bars indicate the area of significance, $p < 0.01$, the regions marked with an asterisk indicate regions that may be useful for predicting foliar δ^{15}N, the ranges of wavelength for asterisk regions from left to right are $\lambda = 587-637$, $690-745$, $798-940$ and $1504-1573$ nm). (b) Coefficients of correlation between foliar δ^{15}N and canopy-level first difference of log 1/R (log 1/R)′ (dash horizontal bars indicate the area of significance, $p < 0.05$; solid horizontal bars indicate the area of significance, $p < 0.01$, the regions marked with an asterisk indicate regions that may be useful for predicting foliar δ^{15}N, the ranges of wavelength for asterisk regions from left to right are $\lambda = 517-535$, $580-590$, $639-661$, $693-708$, $1249-1254$, $1345-1358$, $1509-1604$ and $1760-1778$ nm).

Table 1. Regressions Predicting Foliar δ^{15}N at Both Leaf Level and Canopy Level[a]

	λ, nm	Partial R^2	Cumulative R^2	p
Leaf level				
R	619	0.46		0.06
	695	0.36	0.82	0.02
Log (1/R)′	603	0.46		0.07
	704	0.46	0.92	0.004
Canopy level				
R	1135	0.63	0.63	0.004
Log (1/R)′	702	0.67		0.0006
	639	0.09	0.76	0.08

[a] See text for an explanation of the choice of bands. At the leaf level, regressions for R, were performed with λ = 483–517 nm, 617–703 nm; regressions for Log (1/R)′, were performed with λ = 587–637 nm, 690–745 nm, 798–846 nm, 904–940 nm and 1504–1573 nm. At the canopy level, regressions for R, were performed with λ = 670–694 nm, 1098–1136 nm, 1261–1319 nm and 1480–1522 nm; regressions for Log (1/R)′, were performed with λ = 517–535 nm, 639–661 nm, 693–708 nm, 932–934 nm, and 1249–1254 nm.

(log 1/R)′ spectra therefore tend to highlight the edges of absorption features, such as those due to chlorophyll. At both the leaf and canopy levels, significant correlations between foliar δ^{15}N and (log 1/R)′ at ~600 nm and ~700 nm were found. These wavelengths occur at the edge of chlorophyll absorptions and most likely arise from variable widths of chlorophyll absorption bands in samples with different ^{15}N abundances. Because first-difference spectra are less sensitive to lighting conditions, we consider (log 1/R)′ to be more practical for remote sensing-based determination of ^{15}N than R. In this way, our finding concerning ^{15}N abundance follows those of *Martin and Aber* [1997] for N concentration. Based on (log 1/R)′ stepwise regressions results, at leaf scale, the best predictors for foliar δ^{15}N are λ = 603 nm and λ = 704 nm; at the canopy scale, the best predictor for foliar δ^{15}N is λ = 702 nm (Table 1).

4. Conclusions

[15] Both visible and NIR wavelengths have been used to estimate foliar nitrogen [*Yoder and Pettigrew-Crosby*, 1995; *Martin and Aber*, 1997; *Read et al.*, 2002]. Because of the close relationship between foliar N concentration and foliar ^{15}N abundance, it is not surprising that there are strong correlations between foliar ^{15}N concentration and spectral data in the VNIR at the leaf scale and canopy scales. The R exhibits correlograms with wider regions of significance than those of (log 1/R)′. However, the first-difference (derivative) spectra are probably better predictors than the simple reflectance spectra. Environmental factors such as brightness and shading can influence total reflectance, whereas the shape of the reflectance curve, as characterized by the first-difference curve is more conservative (that is, is less impacted by environmental or analytical conditions). This is why traditionally first-difference spectra have been used to resolve fine-scale spectra from background noise [*Wessman et al.*, 1988; *Martin and Aber*, 1993; *Yoder and Pettigrew-Crosby*, 1995]. In the current study, (log 1/R)′ shows stronger correlations with foliar δ^{15}N than R, and the correlated bands occur over narrower wavelength regions. In stepwise regressions, (log 1/R)′ predictors generated higher R^2 values (R^2 = 0.76 and 0.92 respectively) than R predictors at both leaf scale and canopy scale (Table 1). Based on (log 1/R)′ stepwise regressions results, at leaf scale, the best predictors for foliar δ^{15}N are within visible ranges (λ = 603 nm and λ = 704 nm); at the canopy scale, the best predictor for foliar δ^{15}N is λ = 702 nm.

[16] Although there are factors such as waxy cuticles [*Vanderbilt et al.*, 1985] and trichome density [*Levizou et al.*, 2005] that may constrain the application of remote sensing on foliar chemical analysis, as found in this study, there is a strong relationship between foliar δ^{15}N and leaf reflectance at both leaf scale and canopy scale. Spectral analysis of foliar δ^{15}N, by its non-destructive and continuous nature, is a promising tool to detect terrestrial foliar δ^{15}N spatial patterns and dynamics using airborne or satellite-borne sensors through ground validation processes.

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References

Bowers, M. A. (1993), Influence of herbivorous mammals on an old-field plant community years 1–4 after disturbance, *Oikos*, *67*, 129–141.

Emanuel, R. E., J. D. Albertson, H. E. Epstein, and C. A. Williams (2006), Carbon dioxide exchange and early old-field succession, *J. Geophys. Res.*, *111*, G01011, doi:10.1029/2005JG000069.

Hobbie, E. A., S. A. Macko, and M. Williams (2000), Correlations between foliar δ^{15}N and nitrogen concentrations may indicate plant-mycorrhizal interactions, *Oecologia*, *122*, 273–283.

Levizou, E., P. Drilias, G. K. Psaras, and Y. Maneras (2005), Nondestructive assessment of leaf chemistry and physiology through spectral reflectance measurements may be misleading hen changes in trichome density co-occur, *New Phytol.*, *165*, 463–472.

Martin, M. E., and J. D. Aber (1993), Measurements of canopy chemistry with 1992 AVIRIS data at Blackhawk Island and Harvard Forest, in *Summaries of the 4th Annual JPL Airborne Geoscience Workshop*, vol. I, *AVIRIS Workshop*, edited by R. O. Green, pp. 113–116, NASA Jet Propul. Lab., Pasadena, Calif.

Martin, M. E., and J. D. Aber (1997), High spectral resolution remote sensing of forest canopy ligin, nitrogen and ecosystem process, *Ecol. Appl.*, *7*(2), 431–443.

Read, J. J., L. Tarpley, J. M. McKinion, and K. R. Reddy (2002), Narrow-waveband reflectance ratios for remote estimation of nitrogen status in cotton, *J. Environ. Qual.*, *31*, 1442–1452.

Riedel, S. M., and H. E. Epstein (2005), Edge effects on vegetation and soils in a Virginia old-field, *Plant Soil*, *270*, 13–22.

Robinson, D. (2001), δ^{15}N as an integrator of the nitrogen cycle, *Trends Ecol. Evol.*, *16*, 153–162.

Shugart, H. H., S. A. Macko, P. Lesolle, T. A. Szuba, M. M. Mukelabai, P. Dowty, and R. J. Swap (2004), Kalahari transect wet season campaign of year 2000, *Global Change Biol.*, *10*, 273–280.

Sokal, R. R., and F. J. Rohlf (1995), *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd ed., W. H. Freeman, New York.

Vanderbilt, V. C., L. Grant, L. L. Biehl, and B. F. Robinson (1985), Specular, diffuse, and polarized light scattered by two wheat canopies, *Appl. Opt.*, *24*, 2408–2418.

Wessman, C. A., J. D. Aber, D. L. Peterson, and J. M. Melillo (1988), Foliar analysis using near infrared reflectance spectroscopy, *Can. J. For. Res.*, *18*, 6–11.

Yoder, B., and R. Pettigrew-Crosby (1995), Predicting nitrogen and chlorophyll content and concentrations from reflectance spectra (400–2500 nm) at leaf and canopy scales, *Remote Sens. Environ.*, *53*, 199–211.

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