Spectroscopic Determination of Leaf Biochemistry: Use of Normalized Band-Depths and Laboratory Measurements and Possible Extension to Remote Sensing Measurements

Raymond F. Kokaly and Roger N. Clark

U.S. Geological Survey, MS 973
Box 25046, Federal Center
Denver, CO 80225-0046
raymond@speclab.cr.usgs.gov

1. INTRODUCTION

Ecosystems play an important role in the exchange of water, energy and greenhouse gases between soil, vegetation, and the atmosphere. The ability to detect changes in ecosystem processes such as carbon fixation, nutrient cycling, net primary production and litter decomposition is an important part in defining global biogeochemical cycles and identifying changes in climate. These processes have been linked in models of forest ecosystems to canopy biochemical content, specifically to the nitrogen, lignin and cellulose concentrations in vegetation (e.g. Aber and Federer 1992, and references therein). However, measurements of canopy chemistry by traditional field sampling methods are difficult to perform for large regional and global studies. Therefore, remote sensing measurement of canopy biochemistry is crucial to studying changes in ecosystem functioning.

Several studies have suggested that estimates of canopy chemistry based on remote spectroscopic measurements may be possible (e.g. Card et al. 1988, Curran 1989, Wessman et al. 1989, and Martin and Aber 1997). These studies used stepwise multiple linear regression to predict canopy chemistry from derivative reflectance spectra. This methodology is based on laboratory techniques developed in the agriculture industry for rapid estimation of forage quality parameters, for example, crude protein content and digestibility (Norris et al. 1976 and Marten et al. 1989). The original applications stressed the importance of controlled laboratory methods for reducing noise levels and the limited application of regression equations to samples of the same type used in calibration (Marten et al. 1989). Recently, Grossman et al. (1996) found the use of regression techniques with derivative reflectance spectra to give inconsistent results between dry leaf and needle data sets for forest vegetation. Under the NASA Accelerated Canopy Chemistry Program, analysis using derivatives had also given inconsistent results between test sites (ACCP 1994). Furthermore, although recent studies have correlated plant canopy chemistry to imaging spectrometer measurements (Johnson et al. 1994, LaCapra et al. 1996 and Martin and Aber 1997), the results at leaf and canopy scales are inconsistent and the derived regression equations are applicable to only the study area and are not reliable predictors for other remotely-sensed data.

Because of site-specific results in wavelength selection by derivative analysis and the sensitivity of the technique to noise, we employed a different approach. Before applying the stepwise multiple linear regression, we utilized continuum-removal and band ratios, traditional methods of spectral analysis used in remote sensing by the terrestrial geology and planetary science disciplines. Absorption band-depths were calculated from continuum-removed spectral features in reflectance data. Since all band-depths in an absorption feature were ratioed to the maximum depth at the center of the feature we termed the result "normalized band-depths." Subsequently, we used a multiple stepwise linear regression algorithm applied to normalized band-depths to select wavelengths highly correlated to laboratory measurements of chemical concentrations. Wavelength selection was based on only two of the seven data sets used in this paper. Subsequently, the selected wavelengths were tested for correlation with the chemical concentrations of the five other data sets. To test the robustness of this approach, regression equations developed from a subset of the data were used to predict the concentrations in the remaining samples. Finally, since a broadly applicable method for dry leaves serves only as a foundation for a remote sensing algorithm, the method was tested for its extension to remote sensing measurements of plant canopies. The influences of leaf water content, sensitivity to noise in the reflectance spectra, sensor bandwidths, incomplete vegetation coverage, and atmospheric influences were considered.

2. METHODS

2.1 Data Sets

Data used in this study were comprised of reflectance spectra and foliar chemistry measured from specimens of dried and ground leaves. These samples were gathered and analyzed for the NASA Accelerated Canopy Chemistry Program (ACCP 1994). We examined data from seven sites: three eastern U.S. forests (Blackhawk Island, Wisconsin, Harvard Forest, Massachusetts, and Howland, Maine), a slash pine plantation near Gainesville, Florida, rice fields in California, Douglas-fir seedlings grown in a greenhouse, and a data set consisting of a variety of tissue types from a diversity of plants collected from Long Term Ecological Research (LTER) sites. More than 30 deciduous and coniferous tree species were represented by the 840 samples. Overall, the foliar chemistry covered a wide range: 0.22 to 3.51% nitrogen, 7.60 to 44.62% lignin and 23.69 to 74.73% cellulose.

2.2 Continuum Removal

Working with reflectance data, broad absorption features in the dry leaf spectra centered near 1730, 2100, and 2300 nm, shown for a dry leaf spectrum in Figure 1a, were selected for continuum analysis. Previous studies have shown that many of the chemical bonds in foliar constituents have vibrational absorptions in these bands (see the review by Curran 1989). The continuum is an estimate of the other absorptions present in the spectrum, not including the one of interest. Once the continuum line was established, continuum-removed spectra for the absorption features were calculated by dividing the original reflectance spectrum by the corresponding reflectance of the continuum line (Figure 1b). Although imaging spectrometers such as AVIRIS fully cover the wavelength range from 350-2500 nm, the analysis here excludes wavelengths near strong atmospheric absorptions, around 1400 nm and 1900 nm, and regions where the signal-to-noise ratio (S/N) is low due to water absorption and decreasing solar flux (wavelengths greater than 2400 nm). From the continuum-removed reflectance, the band depth (D) for each channel in the absorption feature was computed by:

$$D = 1 - R' \tag{1}$$

where R' is the continuum-removed reflectance (Clark and Roush, 1984).

2.3 Band Depth Normalization

Reflectance spectra of vegetation canopies vary with changing leaf biochemistry but remote sensing measurements are also affected by atmospheric absorptions, the size of leaf cells, the abundance of other absorbers in the leaf (such as water), and the fractional areal coverage of leaves in heterogeneous landscapes. Therefore, analytical methods for estimation of plant biochemistry must overcome any sensitivity to these extraneous factors. Normalization of continuum-removed reflectance spectra minimizes these influences. The normalized band-depth (D_n) at all wavelengths within the continuum-removed absorption feature is calculated by dividing the band-depth of each channel by the band-depth at the band center (D_c):

$$D_n = D / D_c \tag{2}$$

where the band center is the minimum of the continuum-removed absorption feature. Variations of D_n with wavelength describe the shape of the absorption feature. Resulting differences in the shapes of absorption features between samples are correlated to foliar biochemistry.

2.4 Stepwise Multiple Linear Regression

Normalized band-depth, D_n, values for all wavelengths in the three continuum-removed absorption features were analyzed using a stepwise multiple linear regression routine to determine wavelengths correlated with chemistry. The stepwise regression was run separately for each of the three leaf constituents: nitrogen, lignin, and cellulose. A stepwise regression routine in IDL (Interactive Data Language), STEPWISE, was used. This routine is

based on an algorithm by Afifi and Azen (1971).

3. RESULTS & DISCUSSION

3.1 Wavelength Selection

Two of the eastern U.S. forest data sets, Blackhawk Island and Harvard Forest, were used to derive wavelengths correlated with nitrogen, lignin and cellulose concentrations. Stepwise multiple linear regression was applied to the normalized band-depths of these data. Table 1 lists the locations of selected wavelengths. Five wavelengths were selected in the nitrogen regressions, all in the 2100 nm absorption feature. Lignin required six wavelengths, two in the 1730 nm and four in the 2300 nm absorption features. Eight wavelengths, a few in each of the three broad absorption features, were selected for the cellulose regression.

3.2 Application to All Sites of Wavelengths Derived from Blackhawk Island & Harvard Forest

Following wavelength selection using Blackhawk Island and Harvard Forest sites, linear regression was used to establish regression equations at each of the sites. The strengths of regressions are assessed on high R^2 (where R is the correlation coefficient and R^2 indicates the proportion of variance accounted for by the correlation) and low standard error of calibration (SEC). The SEC is the root mean square error (RMSE) between the chemical concentrations calculated from the regression equation and the values obtained by wet chemistry laboratory methods. Nitrogen correlations were very high (R^2 from 0.90 to 0.97) and SEC were low (0.06 to 0.17% nitrogen by dry weight). Relative to the mean nitrogen concentrations the SEC were less than 10%. In general, cellulose correlations were fairly good (R^2 from 0.75 to 0.93). Correlations for lignin were all significant (R^2 from 0.65 to 0.83) except for the rice field data which had a low R^2 of 0.32. The rice data set had a much lower average lignin concentration relative to the other data sets (approximately 25% lower). In fact, many of the samples in the rice data set were below the range of lignin concentrations present in the forest data sets. However, the error of the SEC relative to the mean lignin concentration for the data set was low, only 6.2%.

In summary, a set of wavelengths derived from an analysis of only the Blackhawk Island and Harvard Forest sites was found to be highly correlated with chemical concentrations for other data sets. These consistent results are significant considering that Grossman et al. (1996) found that wavelengths derived from any single data set were not able to reliably predict nitrogen concentrations in other data sets. Those tests were performed on log(1/R) and its first and second derivatives and only achieved low R^2 (.14-.49). In contrast, the results of this study, which used normalized band-depths describing the shape of absorption bands, show consistently high correlations and low errors across all data sets.

3.3 Predictive Ability of Regression Equations

Regression equations were tested for their ability to predict chemistry across data sets. Two-thirds of the samples from the eastern U.S. forest sites (Blackhawk Island, Harvard Forest and Howland, Maine) were used with the previously derived wavelengths to establish the coefficients in the regression equations. This calibration equation was then used to predict the chemical concentrations of the remaining validation data sets. The term Standard Error of Prediction (SEP) is commonly used to describe the prediction error. The SEP is the root mean square error between the chemical concentrations predicted from the regression equation and the values obtained by wet chemistry laboratory methods. As expected, correlations were highest and SEP lowest for the predictions of the remaining one-third of the eastern forest samples. The SEP in nitrogen estimates for slash pine and Douglas-fir data sets were slightly worse than the eastern forest validation set. The rice data set provided a test for the application of regression equations derived from forest foliage to non-forest vegetation. Nitrogen predictions were very good (R^2=0.83 and SEP=0.13%) The LTER data set also contained some sample tissues not present in the calibration data set: forest species different from the calibration set, grasses, bark, and roots. Nitrogen predictions were surprisingly good (R^2=0.93 and SEP=0.23%).

Similar to the nitrogen results, lignin and cellulose predictions for forest data were accurate. Cellulose estimates for the slash pine samples were good (SEP=2.60%, only a 7.2% error relative to the mean concentration).

The predictions of lignin and cellulose concentrations for the rice and LTER data were less accurate. Cellulose estimates for rice and LTER data have high errors of prediction (SEP of 7.49% and 6.88%, respectively) which might be influenced by structural or biochemical differences particular to these different vegetation types compared to all the other samples of tree foliage. Furthermore, mean concentrations for foliage constituents in the rice samples are significantly lower for nitrogen and lignin and much higher for cellulose than the other sites.

In order to be useful for remote sensing, an algorithm for predicting concentrations should be applicable over a wide variety of vegetation types. Nitrogen predictions were extremely robust for all new data sets. Rice and LTER predictions were good despite the fact that these tissues differ from the leaf and needle material of the calibration data. Lignin and cellulose predictions were good if the new samples were similar in type and concentration to those in the calibration data set. However, predictions for these biochemicals in non-forest tissues were not robust.

More generally applicable regression equations may be derived using a regression with all the samples. The results for regression constants and coefficients are given in Table 1. The results of regressions for nitrogen, lignin and cellulose gave R^2 values of 0.94, 0.64, and 0.83, respectively. The nitrogen results were excellent (SEC=0.17%) as shown in Figure 2. Given the wide range of concentrations and sample types in the 840 samples, these equations may be applicable to most dry leaf spectra obtained in future studies.

3.4 Remote Sensing Considerations

Schimel (1995) discusses the accuracy and precision required from remote sensing in order to map large scale variations in foliar nitrogen and lignin. Accuracy of ~0.5%(absolute) N is necessary to distinguish between ecosystems with differences in nitrogen large enough to affect photosynthesis. An accuracy of ~5.0% lignin concentration is needed to detect between-system gradients. The method used in this study has demonstrated errors below the required accuracy for nitrogen concentrations, even when extending predictions to different vegetation types (i.e., tree foliage to rice). However, accuracy will obviously degrade for extensions of this method to fresh, whole leaves or remotely sensed canopies. Additional complexities are encountered in vegetation spectra collected at the remote sensing scale, including: different instrument characteristics (S/N, sampling, and bandpass), atmospheric effects, leaf water, fractional vegetation coverage, and canopy architecture. This section of the paper addresses the effects of several, but not all, of these influences on this method.

3.4.1 Leaf Water

The largest difference between reflectance spectrum of a ground, dry leaf and a spectrum of a vegetation canopy is due to leaf water. Leaves in a plant canopy can be composed of 40 to 80% water by weight (Elvidge 1990). Because water is highly absorbing in the near infrared, and because the water comprises so much of the leaf, the spectral signatures of the other chemical components are, to a large degree, masked by the water. To test the sensitivity of our method to water we added water absorptions to dry leaf spectra. The spectrum of a dry leaf plus water was computed using the Hapke (1981) radiative transfer theory. We measured spectra of liquid H_2O on a Nicolet Fourier Transform Spectrometer. Using these data and the known index of refraction of water (Irvine and Pollack 1968), we added water to dry leaf spectra. The absorption coefficients of the dry leaf component were derived by inverting the Hapke equations (e.g. Clark and Roush 1984), assuming the index of refraction of water, and deriving the absorption coefficients as a function of wavelength. Given the optical constants (index of refraction and absorption coefficients as a function of wavelength), reflectance spectra of water plus dry leaf were computed for 10%, 20%, 30%, 40%, 50%, 60%, 70%, and 80% water added by weight to the dry leaf.

The dry leaf plus water calculations were made for five different samples from the data set. These samples were selected to span a range of biochemical composition and include different species: red oak, white pine, hemlock, and red maple. The effect of increasing water concentration on calculations of leaf biochemistry was investigated by comparing chemistry estimates from the spectra with added water to the estimates from the original dry leaf spectra. The average error for all five samples was computed at each level of added water. Errors in nitrogen estimation remained small for 10% and 20% water contents (0.21% and 0.19%, respectively). Errors in calculation of lignin were also small until a water content reached 30% (SEP = 2.54%). Cellulose calculations were

most sensitive to increasing water content. To apply the equations developed from laboratory data to fresh leaf or remotely sensed canopy spectra in order to estimate concentrations of nitrogen lignin and cellulose, the spectra must be synthetically/computationally "dried" to an accuracy of at least 10%.

The impact of leaf water content on our normalized band-depth approach was compared to the effect on derivative methods. Derivative calculations were made according to Bolster et al 1996. Table 2 shows how a 10% water content affects the calculation of leaf chemistry relative to the dry leaf calculation. Our method has an SEP of 0.21% for nitrogen, however, the errors from 1st and 2nd derivative methods are much higher, 0.52% and 0.50%, respectively. The results show the long observed fact that water has a dominant influence on the reflectance from fresh leaves and canopies for wavelengths greater than 1400 nm. In our study, we found that with a 10% residual water content we could still accurately estimate leaf chemistry. If a method for removing the spectral signature of water from fresh leaf and remote sensing data can be developed to this accuracy then we may be able to apply our regression equations to these data to predict biochemical concentrations.

3.4.2 Fractional Coverage

A common difficulty in remotely sensing canopy chemistry is incomplete coverage of the surface by the canopy. When vegetation cover is not 100%, other components, such as rocks, soil, water, or man-made objects will contribute to the remotely sensed signal. We tested how the reflectance signature of an incomplete canopy would compare to the complete canopy by adding a soil spectrum to a dry leaf spectrum. We used a soil spectrum that had no strong absorption features. We combined the spectrum of a dry leaf with soil in a 75% to 25% mixture. Errors between the incomplete and complete vegetation cover are presented in Table 2 for our method using normalized band depths and the methods of 1st and 2nd derivatives. The calculations of chemical concentration from the soil contaminated spectrum are nearly identical to the original dry leaf results for the normalized band depth approach (RMSE=0.11% Nitrogen). Normalizing the band-depth alleviates the soil influences on the spectrum. The commonly employed derivative approaches are slightly more sensitive to fractional coverage.

3.4.3 Atmospheric Effects (Residual Atmosphere Absorptions)

Atmospheric influences on remotely sensed vegetation canopy spectra that must be considered include the incomplete removal of atmospheric absorptions. Residual atmosphere absorption features due to water vapor and carbon dioxide are commonly observed in AVIRIS data. Methods for estimating canopy chemistry must not be sensitive to these residuals. In our analysis we restricted the absorption features examined to avoid strong atmospheric absorption regions (see Figure 1a). We recommend that all analyses should similarly avoid these wavelength regions.

Because the residual atmospheric absorptions partially overlap the absorptions due to leaf constituents, we tested our method for sensitivity to these residuals. We used MODTRAN (Berk et al, 1989) to calculate the transmittance spectra of a 30 m layer of atmosphere at an elevation of 8000 ft. A dry leaf spectrum was multiplied by the atmospheric transmittance of this layer. Calculations for chemistry were made using this "contaminated" spectrum and compared to the original dry leaf results. The average errors for the full data set are shown in Table 2 for our normalized band-depth approach and 1st and 2nd derivative methods. In general, all methods had small errors in nitrogen calculations. For the normalized band-depth approach, errors for all three estimates of concentrations were small. However, errors for 1st and 2nd derivative methods in the estimation of lignin and cellulose were larger.

3.4.4 Combined Effects of Atmosphere, Soil, and Leaf Water

In a final test for the sensitivity of these methods to influences encountered at the remote sensing level, we contaminated dry leaf spectra with 10% leaf water and added a soil background spectrum in a 75%/25% vegetation/soil combination. Next, we multiplied those spectra by the 30 m atmosphere residual. We performed these calculations for the five previously mentioned samples (see Section 3.4.1). The average errors for the estimates from the contaminated spectra are shown in Table 2. The advantage of the continuum removal and band normalization approach is evidenced in the much smaller errors as compared to the 1st and 2nd derivative methods. Nitrogen errors are doubled using the derivative approaches. Errors for lignin are extremely high for the 1st and 2nd

derivative methods, 15.52% and 28.72%, respectively.

4. CONCLUSIONS

Remote sensing algorithms are needed to measure canopy chemistry for large scale monitoring of ecosystem functioning. This paper examined the use of normalized band-depths calculated from continuum-removed reflectance spectra coupled with stepwise multiple linear regression to estimate leaf nitrogen, lignin, and cellulose concentrations. The method was designed with an awareness of the influences that will be encountered in remote sensing applications. A set of wavelengths highly correlated with leaf chemistry was determined. Independent applications of linear regression using normalized band-depths at these wavelengths to chemical concentrations of seven sites were accurately made. Furthermore, regression equations developed from a calibration set of data, including a variety of species from three eastern U.S. forests, were used to predict the chemical concentrations of slash pine, rice, and Douglas-fir samples. The method was consistent across independent data sets and a wide variety of species. The results of this study suggest that generally applicable equations can be developed to simply and rapidly estimate chemical concentrations in dry leaves from their reflectance spectra. These laboratory results are a necessary first step in establishing the validity of this empirical approach before analyzing remote sensing data. Although these results are encouraging, additional complexities must be considered for remote sensing data. Of all the influences on remotely sensed data that we considered, foremost is the effect of leaf water. In order for this method to work for fresh whole leaves or complete vegetation canopies, the influence of leaf water on spectral reflectance must be removed to within 10%. This presents a challenging problem and future research direction.

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Table 1. Wavelengths correlated to leaf chemistry by regression with Blackhawk Island and Harvard Forest data and coefficient values determined by regression with all data.

Estimated Biochemical	Wavelength (nm) (selected by regression with subset of data)	Coefficient value (determined by regression with all data)
Nitrogen		6.6059 (constant term)
	2036	-34.3577
	2050	24.3511
	2078	-13.8809
	2152	-3.0247
	2180	3.3388
Lignin		-10.2775 (constant term)
	1666	9.3277
	1762	77.9044
	2246	105.1030
	2266	9.9582
	2324	-62.2543
	2346	28.4725
Cellulose		-49.3231 (constant term)
	1660	-76.2645
	1766	46.9154
	2066	32.1212
	2186	202.1462
	2202	-315.4317
	2266	45.8069
	2288	-44.6616
	2322	47.1973

Table 2. Errors in estimates of leaf biochemistry as caused by changes in leaf water content, soil background, atmospheric residuals and all effects combined.

Simulated Effect on Remote Sensing Data	Chemical Estimated	RMSE Error for "contaminated" data		
		Normalized Band-depth	1st Derivative	2nd Derivative
10% Leaf Water	Nitrogen	0.21	0.52	0.50
	Lignin	0.47	29.70	2.43
	Cellulose	3.20	26.86	3.23
25% Soil Background	Nitrogen	0.11	0.23	0.11
	Lignin	1.02	0.77	1.36
	Cellulose	3.56	4.27	4.14
Atmosphere Residual (30m)	Nitrogen	0.08	0.14	0.04
	Lignin	1.70	8.60	14.87
	Cellulose	0.77	6.38	3.02
Combined Effects	Nitrogen	0.21	0.43	0.43
- 10% Leaf Water	Lignin	1.70	15.52	14.36
- 25% Soil Background	Cellulose	2.68	28.72	3.19
- Atmosphere Residual (30m)				

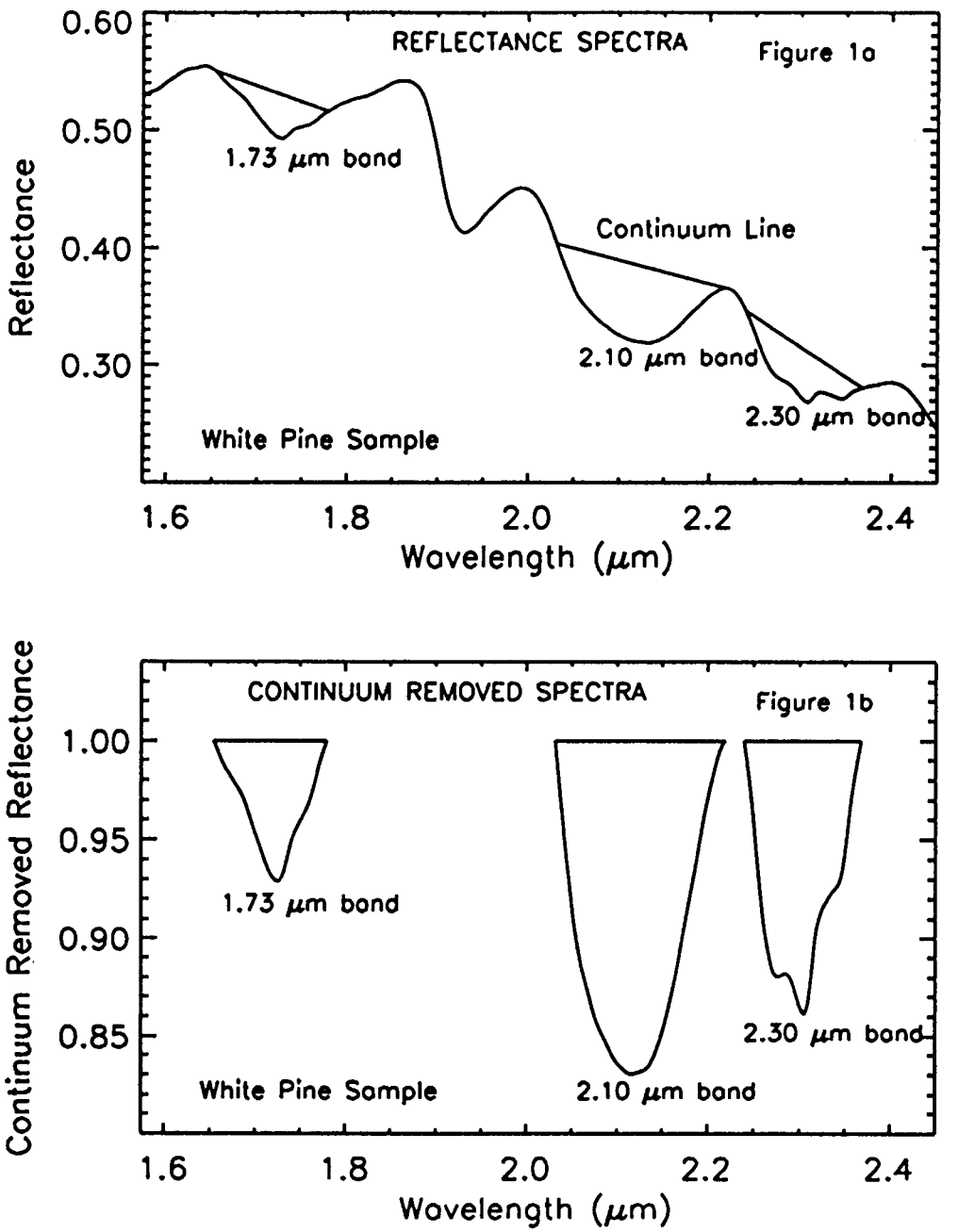

Figure 1. Example spectrum of white pine:
(a) reflectance spectrum with continuum lines
(b) continuum removed reflectance spectrum.

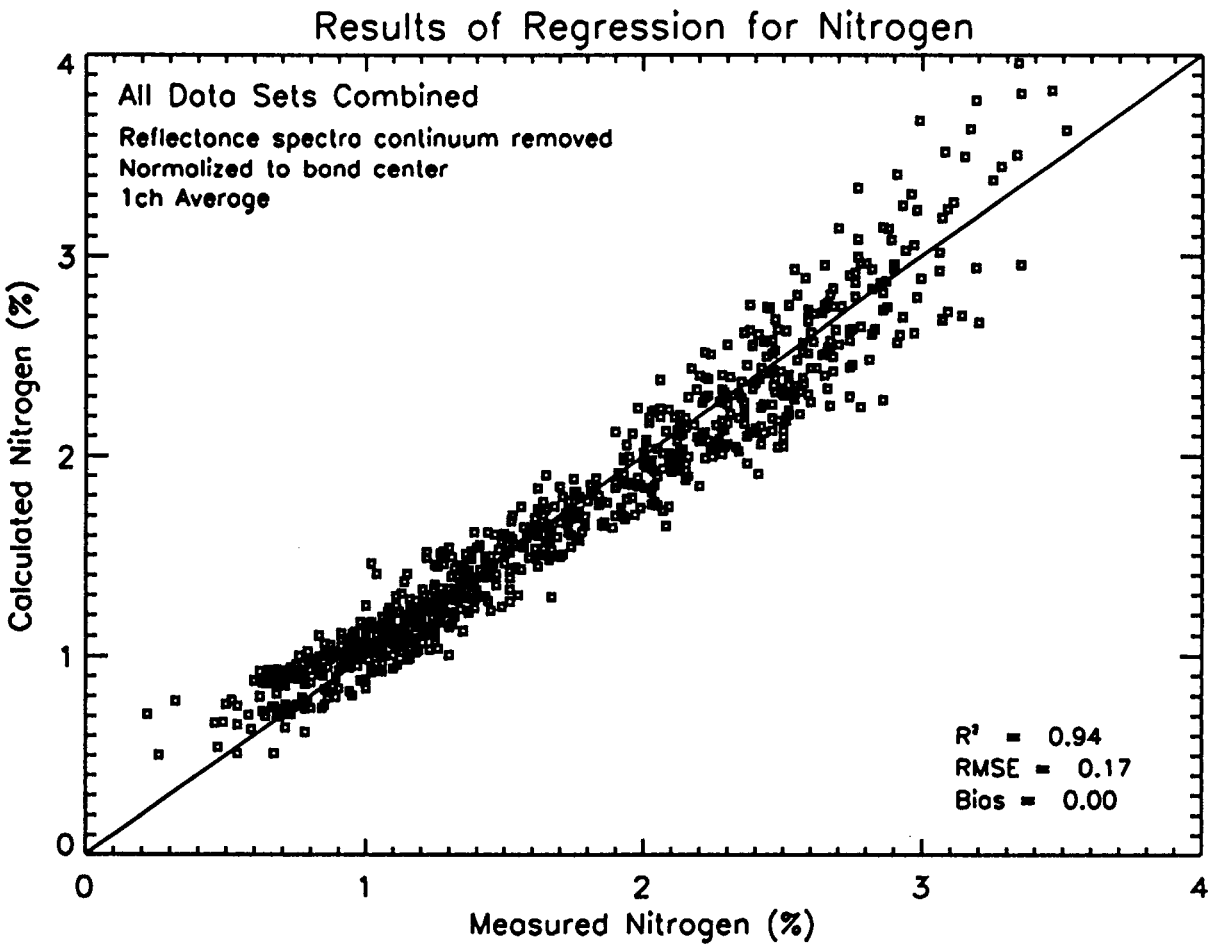

Figure 2. Regression results for nitrogen concentration using normalized band depths and all data sets combined (840 samples).