

Nitrogen influence on fresh-leaf NIR spectra

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Abstract

Simulations and measurements were used to derive information on the form and strength of the nitrogen (N) “signal” in near-infrared (NIR) spectra of fresh leaves. Simulations across multiple species indicated that in total, protein absorption decreased NIR reflectance by up to 1.8% absolute and transmittance by up to 3.7% absolute, all other inputs held equal. Associated changes in spectral slope were generally of $\pm 0.02\% \text{ nm}^{-1}$ absolute. Spectral effects were about an order of magnitude more subtle for a smaller, though potentially ecologically significant, change in N concentration of 0.5% absolute over measured. Nitrogen influence on spectral slope was fairly consistent across four empirical datasets as judged by wavelength dependence of N correlation, and there was reasonable agreement of observed and modeled slope perturbations with locations of known protein absorption features. Improved understanding of the form and strength of the N signal under differing conditions will support continued development of laboratory-based spectral measurement and analysis strategies for direct N estimation in individual fresh leaves. A pragmatic approach for canopy-level estimation by remote sensing, however, might additionally consider surrogate measures such as chlorophyll concentration or canopy biophysical properties. © 2001 Elsevier Science Inc. All rights reserved.

1. Introduction

Nitrogen provides crucial support for plant photosynthesis and ecosystem productivity and acts as a limiting resource in many systems (Field & Mooney, 1986; Lee, Harmer, & Ignaciuk, 1983). Productivity, in turn, is an index to global carbon cycling. Proteins are the primary nitrogenous compound in leaves, typically holding 70–80% of all N. An additional 5–10% of N is allocated to chlorophyll and lipoproteins (Chapin & Kendrowski, 1983), with the balance held as nucleic acids, amino acids, and inorganic N.

Several empirical studies have derived spectrally based estimates of N or protein concentration in fresh leaves (e.g., Curran, Dungan, Macler, Plummer, & Peterson, 1992; Johnson & Billow, 1996; Martin & Aber, 1994; Yoder & Pettigrew-Crosby, 1995). These analyses were based on the premise that organic molecular bonds comprising proteins absorb radiation at various peak locations in the 1100–2500 nm near infrared (NIR). Absorption in this region results from harmonics and overtones of fundamental absorption features centered at wavelengths greater than

2500 nm (Murray & Williams, 1987). Despite knowledge of these absorption feature centers, the broader spectral influence of nitrogen held in the fresh-leaf matrix has not been well described.

Indeed, there is no clear consensus that information on nitrogen (and other bioconstituents) can be robustly extracted from fresh-leaf spectra. Where stepwise regression is used to relate spectra to chemistry, as is common, it has been noted that wavelength selections tend to be inconsistent among studies and are not always clearly attributable to known harmonic and overtone absorptions. For example, Grossman et al. (1996) reported that regression responds to spectral overlaps with biochemicals other than those being examined, leaf anatomical characteristics, or instrument noise. Fourty and Baret (1998) reported low predictive performance of protein regression equations, cautioning that protein absorption is weak, nonspecific, and tends to be masked by water absorption. This has led to suspicion that regression methods, at least in the case of N, respond to factors other than absorption by the constituent of interest. From a more theoretical standpoint, Jacquemoud et al. (1996) found that inversion of a leaf reflectance model did a poor job of estimating leaf N. They reported that fresh-leaf spectra are “definitely insensitive” to protein, as protein constitutes only a small proportion of total leaf mass.

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Turning to canopy-level estimation, Schimel (1995) discussed the potential contribution of remote sensing to global-scale terrestrial biogeochemical cycling, suggesting that the ability to map foliar N changes on the order of 0.5% (dry weight basis) is required to “distinguish between ecosystems with differences in N large enough to influence photosynthesis.” Toward this general end, several studies have related remotely sensed data to canopy N and other biochemical constituents (e.g., Gastellu-Etchegorry et al., 1995; Johnson et al., 1994; LaCapra et al., 1996; Martin & Aber, 1997; Peterson et al., 1988; Wessman et al., 1988). Despite some apparent successes, however, remote estimation of canopy chemistry tends to be scene dependent (NASA, 1994) and therefore not robust.

The goal of this study was to examine whether N absorption affects fresh-leaf spectra and, if so, assess the strength and form of the signal. Such information might subsequently support formulation of sensor requirements

Table 1
Samples used in modeling study, also comprising empirical dataset no. 4

1. oak (16.14); <i>Quercus pubescens</i>	2. black locust (25.22); <i>Robinia pseudoacacia</i>
3. chestnut (14.72); <i>Castanea sativa</i>	4. hazel (16.95); <i>Corylus avellana</i> L.
5. laurel (9.83); <i>Prunus laurocerasus</i>	6. maize (26.55); <i>Zea mays</i> L.
7. alfalfa (32.66); <i>Medicago sativa</i> L.	8. sorghum (23.69); <i>Sorghum halepense</i>
9. sunflower (34.89); <i>Helianthus annuus</i> L.	10. soy (31.64); <i>Soja hispida</i>
11. poplar (17.47); <i>Populus canadensis</i>	12. clover (31.35); <i>Trifolium pratense</i> L.
13. maple (18.09); <i>A. pseudoplatanus</i> L.	14. ash (19.92); <i>Fraxinus excelsior</i> L.
15. linden (21.78); <i>Tilia platyphyllos</i>	16. beech (17.17); <i>Fagus sylvatica</i> L.
17. potato (30.33); <i>Solanum tuberosum</i> L.	18. nettles (26.32); <i>Urtica dioica</i> L.
19. mulberry (20.56); <i>Morus nigra</i>	20. grape, wild (11.91); <i>V. silvestris</i>
21. walnut (20.08); <i>Juglans regia</i> L.	22. apricot (16.13); <i>Armeniaca vulgaris</i>
23. sage (19.16); <i>Salvia officinalis</i> L.	24. black cherry (17.87); <i>Prunus serotina</i>
25. red oak (13.56); <i>Quercus rubra</i>	26. birch (14.85); <i>Betula alba</i> L.
27. alder (22.21); <i>Alnus glutinosa</i>	28. willow (14.36); <i>Salix alba</i> L.
29. reeds (16.33); <i>Phragmites communis</i>	30. banana (20.76); <i>Musa ensete</i>
31. elm (16.55); <i>Ulmus glabra</i>	32. grape, red (18.69); <i>Vitis vinifera</i> L.
33. fig (19.67); <i>Ficus carica</i> L.	34. bamboo (15.72); <i>Bambusa acundinacea</i>
35. ivy (14.26); <i>Hedera helix</i> L.	36. palm (11.27); <i>Chamaerops humilis</i>
37. tomato (26.55); <i>Lycopersicum esculentum</i>	38. grape, white (19.12); <i>Vitis vinifera</i> L.

Protein concentration as % dry weight in parentheses, after measurements of the LOPEX (Hosgood et al., 1995).

Table 2

Range of biophysical and biochemical measurements used as LEAFMOD input

Thickness	86–583 μ
Specific leaf area	82–398 $\text{cm}^2 \text{g}^{-1}$
Equivalent water thickness	0.005–0.023 cm
Pigments	22.1–104.2 $\mu\text{g cm}^{-2}$ leaf area
Cellulose	9.1–37.2% dry weight
Lignin	1.1–27.5% dry weight
Nitrogen	1.7–5.9% dry weight
Protein	9.8–34.9% dry weight

38 fresh-leaf samples. Source: LOPEX archive (Hosgood et al., 1995).

and information extraction algorithms for improved spectral evaluation of nitrogenous compounds at leaf and canopy levels.

2. Methods

2.1. Simulation

A leaf-level radiative transfer model, LEAFMOD (Ganapol et al., 1998; Ganapol et al., 1999), was used to simulate percent reflectance (R_λ) and transmittance (T_λ) spectra of fresh, single leaves of 38 dicot and monocot samples (Table 1). The simulations were supported by data from the Leaf Optical Properties EXperiment (LOPEX; Hosgood et al., 1995). These measurements included R_λ and T_λ per sample, with the associated physical and chemical measurements of Table 2. R_λ and T_λ measurements were used to generate a scattering coefficient profile per sample and subsequently to evaluate simulation goodness of fit. Specific absorption coefficient profiles for protein (Fig. 1), lignin, cellulose, pigments, and water were adapted from the PROSPECT v2.01 leaf model (Jacquemoud et al., 1996).

Three simulation cases were considered: (1) Case 1x with measured or nominal protein (Table 1) to establish the capability of the model to “reconstruct” spectral measurements using all available information; (2) Case 0x with protein omitted, as a boundary condition; and (3) Case 1+x with protein elevated over nominal to mimic an N increase of 0.5% dry weight (in keeping with Schimel, 1995). Set

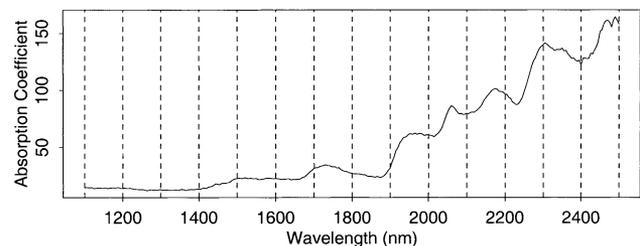


Fig. 1. Protein-specific absorptivity profile ($\text{cm}^2 \text{g}^{-1}$), adapted from PROSPECT model v2.01 (Jacquemoud et al., 1996) as described by Ganapol et al. (1999).

1⁺ x protein concentrations were specified by assuming a nitrogen/protein conversion factor of 6 (after Williams, 1987). All input parameters other than protein were held constant across cases. R_λ and T_λ simulations were of range 400–2500 nm and interval (step) 10 nm for a total of 211 wavelengths (λ).

Several quantities were calculated to evaluate the simulations. Root-mean-square (RMS) error was used to express goodness of fit vs. LOPEX measurement per sample:

$$RMS_R = \sqrt{\sum_{\lambda} (R_\lambda - R_{\lambda,m})^2 / n}, \tag{1}$$

$$RMS_T = \sqrt{\sum_{\lambda} (T_\lambda - T_{\lambda,m})^2 / n}, \tag{2}$$

where R_λ (T_λ) was the predicted spectral response at wavelength λ , $R_{\lambda,m}$ ($T_{\lambda,m}$) was measured response at wavelength λ , and n was the number of wavelengths (211; Eqs. (1) and (2)).

Reflectance and transmittance differences were calculated per wavelength as:

$$\Delta R_\lambda = R_{\lambda,p1} - R_{\lambda,p2}, \tag{3}$$

$$\Delta T_\lambda = T_{\lambda,p1} - T_{\lambda,p2} \tag{4}$$

where p1 and p2 were different protein values. ΔR_λ (ΔT_λ) was the amplitude difference between two simulations of differing protein. Table 3 shows an example calculation of Eq. (3).

It is known that spectral conversion to first derivative (slope), frequently approximated by differencing and smoothing, reduces the effect of wavelength-independent baseline shifts (Demetriades-Shah, Steven, & Clark, 1990, Hrushka, 1987). Here, differences in spectral slope were calculated per step as:

$$\Delta R'_\lambda = \Delta R_{\lambda+} - \Delta R_{\lambda-}, \tag{5}$$

$$\Delta T'_\lambda = \Delta T_{\lambda+} - \Delta T_{\lambda-} \tag{6}$$

where $\lambda+$ and $\lambda-$ were the long and short wavelengths of each step, respectively. Output wavelengths were assigned as the center points of each original step (i.e., 405, 415, . . . , 2495 nm), and differences were expressed on a nm⁻¹ basis

Table 3
Example calculations extracted from predicted reflectance spectra (R_λ) of Sample 1 (oak), Case 1x minus Case 0x protein

Case	Variable	λ	
		2140 nm	2150 nm
1x	R	15.73%	16.03%
0x	R	16.99%	17.44%
	ΔR	-1.26%	-1.41%
	$\Delta R'_{2145}$	-0.15% over 10 nm = -0.015% nm ⁻¹	

Table 4
Fresh-leaf datasets used for correlation analysis

Set	Species	No. of samples	Instrument	N range (% dry weight)
1	Douglas fir ^a	87	NIRS6500	0.7–3.3
2	grape ^b	82	NIRS6500	2.0–3.9
3	bigleaf maple ^c	83	NIRS6500	1.0–4.5
4	various species ^d	38	Perkin-Elmer	1.7–5.9

^a Johnson and Billow (1996).
^b Johnson (1999).
^c Yoder and Pettigrew-Crosby (1995).
^d Hosgood et al. (1995).

(Eqs. (5) and (6)). See Table 3 for example calculation of Eq. 5.

2.2. Observation

In addition to the simulations, four intact fresh-leaf datasets were analyzed (Table 4). The sets are fully described in Hosgood et al. (1995), Johnson (1999), Johnson and Billow (1996), and Yoder and Pettigrew-Crosby (1995). Briefly, however, each set included laboratory spectrophotometric measurements and associated data on N concentration, commonly regarded as a surrogate measure for protein (Williams, 1987). Sets 1–3 were monospecific: Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco.], grape (*Vitis vinifera* L.), and bigleaf maple (*Acer macrophyllum* P.). Set 4 included the 38 species of Table 1. The cultivation regime of Sets 1 and 3 specifically attempted to control for differences other than N concentration.

Sets 1–3 were measured with an NIRSystems Model 6500 scanning monochromator (Perstorp Analytical, Silver Spring, MD). The instrument made bidirectional reflectance measurements in the 400–2500 nm region with 2 nm step, 10 nm spectral resolution with 1050 channels in total. Set 4 was developed with a Perkin-Elmer Lambda 19 double-beam spectrophotometer (Perkin-Elmer, Norwalk, CT) equipped with an integrating sphere. Hemispherical reflectance measurements were made in the 400–2500 nm region with 1 nm step and spectral resolution 1–5 nm depending on wavelength. For this study, the Perkin-Elmer scans were resampled without interpolation to a 10 nm step.

All reflectance measurements were converted to pseudoabsorbance ($A_\lambda = \log_{10} 1/R_\lambda$) and then to absorbance first difference (A'_λ). First difference conversion and subsequent smoothing of Sets 1–3 used the “1-4-4 Math Treatment” of the Infrasoft International NIRS2 software package (Perstorp Analytical). The procedure for Set 4 invoked the “diff” and “smooth” functions of the Splus software package (MathSoft, Seattle, WA). Both approaches computed differences as

$$A'_\lambda = A_{\lambda+} - A_{\lambda-}, \tag{7}$$

with 8 nm intervals used for Sets 1–3 and 10 nm for Set 4 (Eq. (7)). All results were smoothed by running means. Finally, correlation (r) between A'_λ and N was calculated per dataset for the 1100–2500 nm region.

3. Results

3.1. Simulation

Mean RMS errors for Case 1x (reconstruction) were low ($RMS_R=1.1\%$, $RMS_T=1.7\%$), indicating that the model produced an excellent fit to measurements throughout the 400–2500 nm region, for the 38 samples. For Case 0x, mean errors increased to 1.7% and 2.4%, respectively, still good but higher than for 1x. Protein omission served to increase RMS_R in 36 samples and increase RMS_T in 33 samples.

Trends in ΔR_λ and ΔT_λ were similar to each other (Figs. 2 and 3). Both suggested that protein exerts an absorbing influence throughout the NIR, with greatest effect in the 2150–2350 nm region and least sensitivity near water absorption peaks (~ 1450 and ~ 1940 nm). Absolute amplitude of ΔT_λ generally exceeded ΔR_λ for a given protein difference. The boundary condition

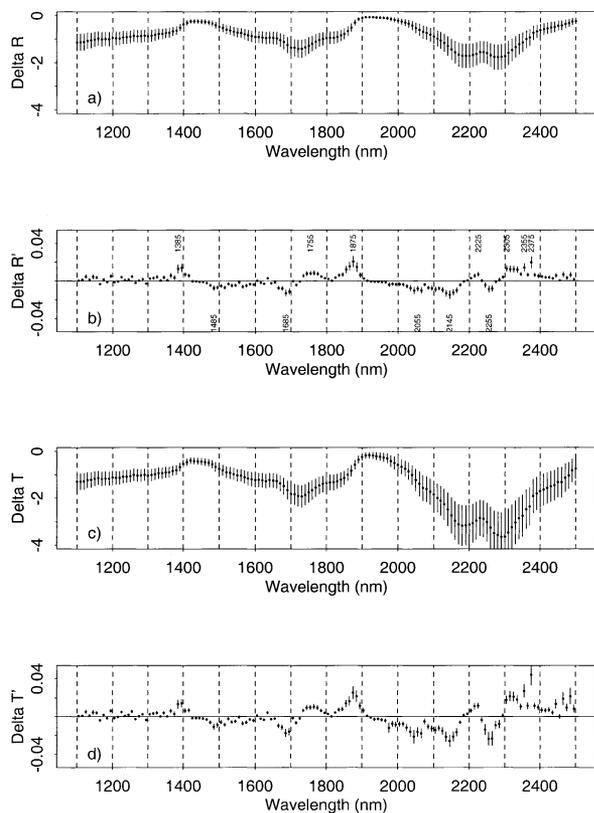


Fig. 2. Simulation results for Case 1x minus Case 0x, mean ± 1 S.D. for 38 samples of Table 1. (a) ΔR_λ (%), (b) slope difference $\Delta R'_\lambda$ (%), (c) ΔT_λ ($\% \text{ nm}^{-1}$), (d) slope difference $\Delta T'_\lambda$ ($\% \text{ nm}^{-1}$).

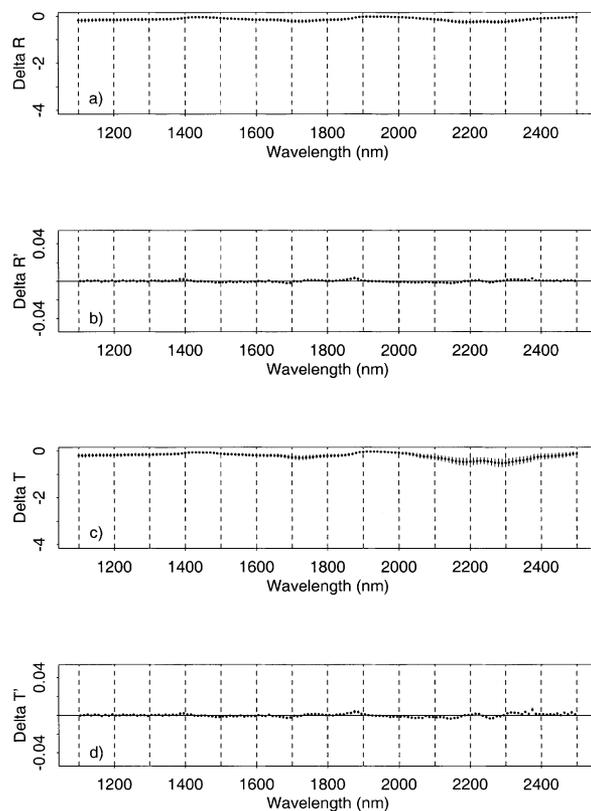


Fig. 3. Simulation results for Case 1^+x minus Case 1x, mean ± 1 S.D. for 38 samples of Table 1. (a) ΔR_λ (%), (b) $\Delta R'_\lambda$ (%), (c) ΔT_λ ($\% \text{ nm}^{-1}$), (d) $\Delta T'_\lambda$ ($\% \text{ nm}^{-1}$).

protein differences expressed between Cases 1x and 0x produced ΔR_λ and ΔT_λ maxima of about 1.8% and 3.7%, respectively. For the more subtle protein difference between Cases 1^+x and 1x, maxima were about 0.2% and 0.5%, respectively.

Trends in $\Delta R'_\lambda$ and $\Delta T'_\lambda$ were also similar, with $\Delta T'_\lambda$ generally exceeding $\Delta R'_\lambda$ (Figs. 2 and 3). Both variables were of range $\pm 0.02\% \text{ nm}^{-1}$ for Case 1x minus Case 0x. Maxima (arbitrarily defined as $\Delta R'_\lambda > |0.005\% \text{ nm}^{-1}|$) occurred at 1385, 1485, 1685, 1755, 1875, 2055, 2145, 2225, 2255, 2305, 2355, and 2375 nm. There was some indication (more evident for $\Delta T'_\lambda$) that the strongest region of slope difference occurs >2000 nm. For Case 1^+x minus Case 1x, $\Delta R'_\lambda$ and $\Delta T'_\lambda$ were generally of range $\pm 0.002\% \text{ nm}^{-1}$.

3.2. Observation

Empirical sets 1–3 had similar patterns of correlation between N and absorbance first difference (Fig. 4). In Set 4, the pattern was less pronounced and $r(A'_\lambda, N)$ was generally lower. Fig. 5 shows the spectral regions where $r(A'_\lambda, N) > |0.5|$. Several such regions, centered at about 1190, 1265, 1675, 2075, and 2160 nm, were common to Sets 1–3, with Set 3 including additional, unique regions.

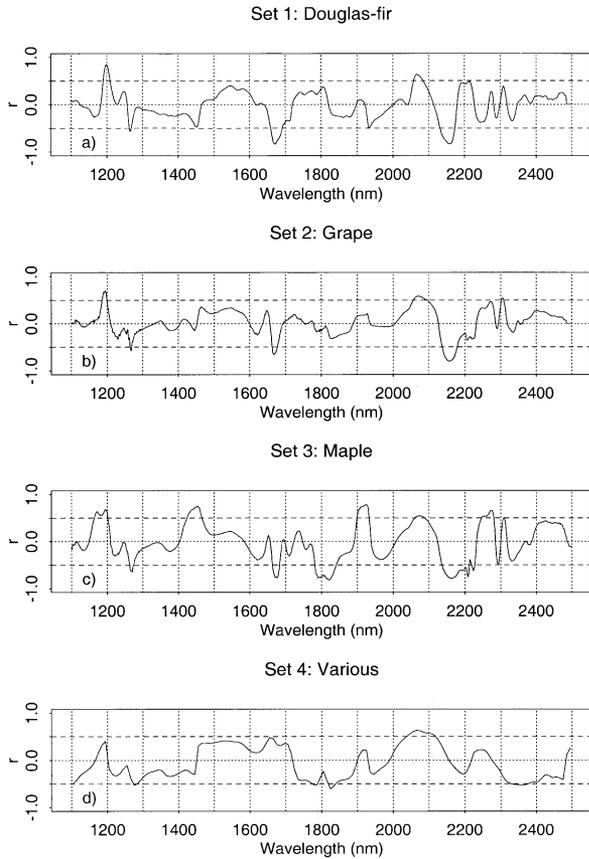


Fig. 4. Correlation coefficients (r), absorbance first difference (A'_λ) vs. foliar nitrogen (N), observed in four fresh-leaf sample sets. Horizontal dashed lines shown for reference at $r(A'_\lambda, N) = \pm .5$.

Set 4 had two regions in common with the other sets: ~ 1265 and ~ 2075 nm.

Fig. 5 includes additional information: (1) 12 LEAFMOD-predicted locations of slope perturbation associated with protein after Fig. 2b, and (2) locations of “major” protein absorption features reported in the literature (Peterson & Hubbard, 1992; Williams & Norris, 1987). Of these eight features, three occur within 5 nm of a prediction and

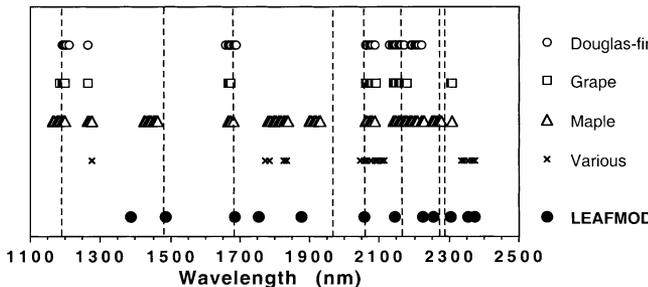


Fig. 5. Spectral regions where $r(A'_\lambda, N) > .5$ per dataset, after Fig. 4. Also shown as “LEAFMOD” are predicted $\Delta R'_\lambda$ maxima and minima of Fig. 2b. For additional reference, locations of major protein features reported by Peterson and Hubbard (1992) and Williams and Norris (1987) are shown as vertical dashed lines. Datasets offset vertically for legibility.

Table 5

Locations of eight major protein features (after Peterson & Hubbard, 1992; Williams & Norris, 1987), nearest predicted slope perturbation after Fig. 2b, and difference (Δ)

Protein feature (nm)	Nearest prediction (nm)	$ \Delta $
1187	–	–
1485	1485	0
1690	1685	5
1972	–	–
2055	2055	0
2168	2145	23
2274	2255	19
2294	2305	11

“–” if $\Delta > 25$ nm.

three others occur within 25 nm (Table 5). Best agreement among observed correlation peaks, model predictions, and published absorption features was observed in the following regions: ~ 2075 nm (all sets), ~ 2160 nm (Sets 1–3) and ~ 1675 nm (Sets 1–3).

4. Discussion

Simulations indicated that absorption by N (held as protein) affects both the amplitude and slope of fresh-leaf NIR spectral profiles, all other things held equal. However, amplitude and slope effects associated with a change of 0.5% N were subtle and might be considered a challenging basis for information extraction.

Some discrepancies were noted between locations of predicted slope perturbations and major protein absorption features (Table 5). The overall greater number of predictions (12) vs. major features (8) may result from model sensitivity to “lesser” protein features embodied in the specific absorptivity profile and should not necessarily be regarded as an artifact. The converse situation, or lack of prediction at or near a major feature, occurs at 1187 and 1972 nm. Prediction failure near 1187 nm probably indicates an anomaly in the protein-specific absorptivity profile used here (Fig. 1), which contains no obvious peak in this spectral region. The profile, which was determined with dry leaf spectra, does contain a broad peak near 1970 nm. Prediction failure in this region on fresh leaves possibly indicates confusion introduced by water, which has a strong absorption peak centered at 1940 nm (Curcio & Petty, 1951).

Comparison of empirical datasets revealed consistent trends in $r(A'_\lambda, N)$, particularly for Sets 1–3. However, the consistency was not such that one would expect an identical wavelength set, in the case of stepwise regression for instance, to be objectively selected across sets. Less agreement was seen with Set 4, possibly due its composition of multiple species with different scattering characteristics. All sets considered, good agreement was seen between predicted and observed slope perturbations.

5. Conclusions

Predicted and empirical data were used to derive information on the form and strength of nitrogen influence on fresh-leaf spectra. Boundary condition simulations showed that in total, protein absorption decreased NIR reflectance by as much as 1.8% absolute and transmittance by up to 3.7% absolute, with slope changes generally of range $\pm 0.02\% \text{ nm}^{-1}$. Notably, spectral sensitivity was approximately an order of magnitude smaller for a change of 0.5% N, a value that has been previously suggested as a remote-sensing requirement for regional to global ecosystem monitoring.

Protein influence on spectral slope was fairly consistent across empirical datasets as judged by wavelength dependence of N correlation. Generally, good alignment was seen among observed and predicted locations of slope perturbation and major protein absorption features. Key areas of spectral sensitivity were centered at approximately 2075, 2160, and 1675 nm.

Most laboratory studies to date have examined leaf sets of relatively wide N range. Future studies might further address (1) the extent to which laboratory spectrophotometers can exploit spectral effects of subtle N changes expected in natural ecosystems as early response indicators of environmental change, and (2) impacts of species and leaf/plant condition on the form and strength of the N signal.

For canopy-level remote sensing, the situation is perhaps more challenging due to uncertainty about the composition of scene components (e.g., “mixed” pixels), greater presence of confusion factors, and data quality considerations. Even allowing for possible leaf-to-canopy amplification of absorption features (NASA, 1994), “direct” estimation of canopy N, based strictly on N-related absorption features, might be regarded as problematic given the subtlety of the signal. A pragmatic approach might in addition, or instead, consider surrogate measures such as chlorophyll concentration for monitoring short-term physiological response to environmental change. Longer-term responses might be monitored through canopy architecture changes related to leaf area index, leaf angle distribution, or eventually altered species composition.

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by the Advanced Techniques Unit of the Institute for Remote Sensing Applications/Joint Research Centre of the European Commission.

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