# Determination of nitrogen, lignin, and cellulose content of decomposing leaf material by near infrared reflectance spectroscopy

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We report the results of a study of the near infrared reflectance spectra of decaying forest foliage. During the decay process, a broad absorbance feature develops in the 1100–2000 nm region of the near infrared spectrum. The magnitude of this feature is directly related to the age of the material (or to degree of decomposition) and may be useful in determining degree of decay in field samples. More specifically, multiple linear regression equations derived from second-derivative near infrared reflectance spectra are presented that predict the concentrations of nitrogen, lignin, and cellulose in decaying foliage. We conclude that near infrared reflectance spectroscopy is a very viable and attractive method for the simultaneous determination of these components in decaying foliage.

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Nous rapportons les résultats d'une étude du spectre de réflectance dans le proche infrarouge du feuillage forestier en décomposition. Lors du processus de décomposition, un large trait d'absorbance se développe dans la région de 1100–2000 nm du proche infrarouge. L'amplitude de ce trait est directement reliée à l'âge du matériel (ou au degré de décomposition) et peut être utile pour la détermination de la décomposition des échantillons au champ. Plus spécifiquement, des équations de régression linéaire multiple obtenues de la dérivée seconde du spectre de réflectance dans le proche infrarouge prédisant les concentrations d'azote, de lignine et de cellulose dans le feuillage en décomposition sont présentées. Nous concluons que la spectroscopie de réflectance dans le proche infrarouge est une méthode très viable et attrayante pour la détermination simultanée de ces composés dans le feuillage en décomposition.
[Traduit par la rédaction]

## Introduction

The carbon fraction (percent lignin, cellulose, and extractives) and nitrogen content of plant residues have been shown to be accurate predictors of litter decomposition dynamics (e.g., Melillo et al. 1982; Berg 1986; Meentemeyer and Berg 1986). Quantitative models of litter decomposition have been developed that use both initital litter chemistry and changes in litter chemistry through time (Aber et al. 1982, 1990; Parton et al. 1988). Such models are an important part of our cumulative understanding of the function of terrestrial ecosystems.

Standard wet chemistry analyses of the lignin and cellulose content of plant materials are both time-consuming and expensive. The multiple-extraction and digestion procedures generally used (e.g., Effland 1977; McClaugherty et al. 1985; Van Soest 1963) are also subject to changes in operator techniques or slight modifications in procedures, which introduce a bias to the analysis (McLellan et al. 1991).

Near infrared reflectance (NIR) spectroscopy offers the potential for rapid, repeatable, and accurate measurement of nitrogen and carbon-fraction chemistry of plant materials. This technique has been used for more than 20 years to measure protein, fiber, oil, moisture, and other constituents in agricultural products (Norris and Hart 1965; Norris et al. 1976; Shenk et al. 1981; Winch and Major 1981). In an earlier paper (Wessman et al. 1988*a*), we presented background information on the application of near infrared spectroscopy to foliar materials of native woody plants and a successful calibration for determination of nitrogen and lignin concentrations.

In this paper we present similar calibrations for foliage of woody plants that have been decayed in the field or laboratory for different lengths of time. The goals of this work were (*i*) to determine how decomposition affects reflectance spectra of foliage, (*ii*) to determine whether wavelengths used for the analysis of green and senescent foliage would also be appropriate for decaying foliage, and (*iii*) to develop a set of equations for converting NIR spectra into accurate estimates of nitrogen, lignin, and cellulose concentrations of decaying foliage.

## Methods

### *Litter materials*

Samples used in this study were drawn from several established, long-term decomposition studies. These included on-site decay studies in Wisconsin, Massachusetts, and Maine, as well as a laboratory study using several types of litter collected in Virginia. Altogether, 13 species were included in the sample set, representing material decayed for as little as 21 days and as much as 5 years (Tables 1 and 2). All litter samples were oven-dried (70°C, 48 h), ground to pass a 1-mm mesh, and stored in airtight containers in the dark prior to analysis.

### *Wet chemistry analyses*

Different wet chemistry analytical techniques were applied to samples from the different studies. Nitrogen was measured either

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TABLE 1. Description of species of leaf litter, sites at which decomposition occurred, and length of decay sequence for samples used in this study

| Site | Reference | Species | Collections: Number | Collections: Duration |
|---|---|---|---|---|
| Blackhawk Island, WI | McClaugherty et al. 1985 | *Pinus strobus*<br>*Tsuga canadensis*<br>*Quercus alba*<br>*Populus tremuloides*<br>*Acer saccharum*<br>*Acer rubrum* | 11 | 2 years |
| Lead Mountain, ME | K.J. Nadelhoffer and J.D. Aber, unpublished | *Betula alleghaniensis*<br>*Prunus serotina*<br>*Picea rubens* | 4 | 5 months |
| Harvard Forest, MA | J.M. Melillo and J.D. Aber, unpublished | *Pinus resinosa*<br>*Betula alleghaniensis*<br>*Acer rubrum*<br>*Quercus rubra* | 2 | 1 year |
| | Melillo et al. 1989; Aber et al. 1990 | *Pinus resinosa*<br>*Betula papyrifera*<br>*Acer rubrum*<br>*Quercus rubra*<br>*Acer saccharum* | 17 | 5 years |
| Blacksburg, VA (laboratory) | A.E. Linkins and J.M. Melillo, unpublished | *Acer rubrum*<br>*Quercus alba*<br>*Cornus florida* | 8 | 6 months |
| Duke Forest, NC | Melillo and Aber 1984 | *Pinus resinosa* | 4 | 5 years |
| University of Wisconsin Arboretum, WI | Melillo and Aber 1984 | *Pinus resinosa* | 4 | 5 years |

TABLE 2. Number of samples used for each constituent in this study divided into broad-leaved deciduous and needle-leaved evergreen conifers, along with the range of each constituent within the complete sample set

| | Deciduous | | Coniferous | | Max concn. (%) | Min. concn. (%) |
|---|---|---|---|---|---|---|
| | No. samples | No. species | No. samples | No. species | | |
| Nitrogen | 328 | 9 | 123 | 4 | 3.16 | 0.44 |
| Lignin | 134 | 9 | 35 | 3 | 44.1 | 15.6 |
| Cellulose | 134 | 9 | 35 | 3 | 56.8 | 36.3 |

by the micro-Kjeldahl technique, with digestate analyzed by automated solution chemistry procedures (McClaugherty et al. 1985), or by CHN analysis (Cappo et al. 1987). Cross-laboratory comparisons have shown that these two techniques as performed by our different laboratories are equivalent (McLellan et al. 1991).

Lignin and cellulose concentrations were determined by either a wood-products chemistry procedure (Effland 1977; McClaugherty et al. 1985) or a nutrition–digestibility (forage fiber) procedure (Van Soest 1963; Goering and Van Soest 1970). All samples analyzed by the wood chemistry procedure were processed in the same laboratory and so do not contain interlaboratory bias (McLellan et al. 1991). Cross-laboratory comparisons show that results from these two procedures performed within the same laboratory are interconvertible (Ryan et al. 1990). For the analyses presented here, all lignin and cellulose data obtained by the forage fiber technique were converted to the wood chemistry technique using the equations in Ryan et al. (1990). Samples used in this study represent a very wide range nitrogen, lignin, and cellulose concentrations (Table 2).

*NIR spectroscopy*

Reflectance measurements were made with an NIR Systems model 6250 near infrared spectrophotometer. This device uses an oscillating concave holographic grating that covers a spectral range of 1100–2500 nm in 2-nm intervals with a bandwidth of 10 nm. Reflectance ($R$) is converted to absorbance ($A$) using the following equation:

$$A = \log(1/R)$$

Absorbance values are used for all data transformations and stratistical analyses.

All litter samples were redried overnight at 70°C, allowed to cool for 1 h, and then thoroughly mixed before analysis. Each sample was scanned once, rotated 90° within the sample cell holder, scanned a second time, thoroughly mixed outside the sample cell, scanned a third time, rotated 90° again, and scanned a fourth time. Spectra generated by each of the four scans actually represent the mean of 50 separate internal scans. Thus the final spectrum used in the result of 200 actual scans per sample. Each spectrum is

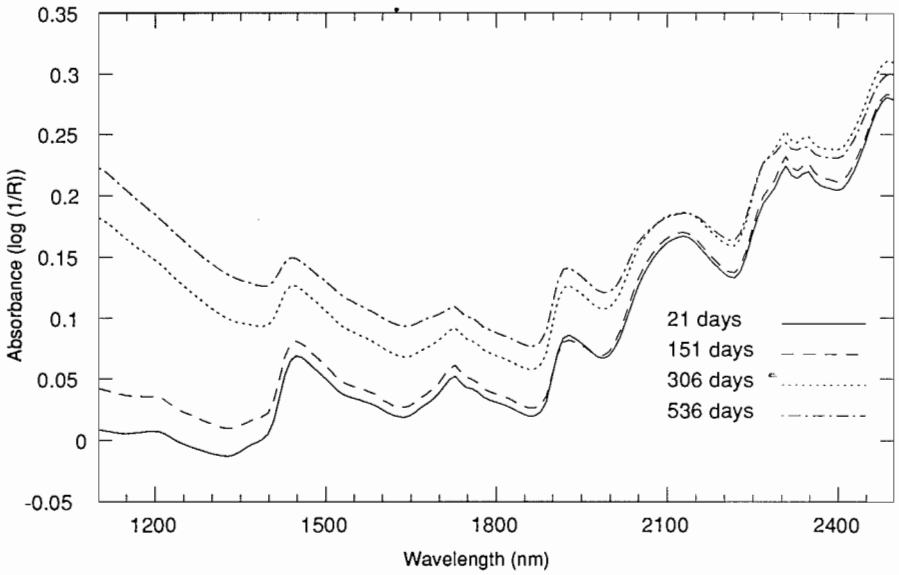


FIG. 1. Changes in the near infrared reflectance of decomposing litter material with time. These spectra are for *Quercus alba* foliage decomposing in the field on Blackhawk Island, Wisconsin. Note the large increase in absorbance with time, particularly between 1100 and 2000 nm.

TABLE 3. Wavelengths used in NIR regression equations

| Constants | Wavelength (nm) | Wavelength assignment |
|---|---|---|
| **Nitrogen** | | |
| K(0) = 2.148 | | |
| K(1) = −167.142 | 2174 | Protein |
| K(2) = −92.602 | 2378 | Oil |
| K(3) = 30.651 | 2090 | OH |
| K(4) = 357.657 | 1230 | CH |
| **Lignin** | | |
| K(0) = 33.034 | | |
| K(1) = 235.912 | 1438 | $CH_2$ |
| K(2) = −1564.618 | 2154 | $CONH_2$ |
| K(3) = 1265.537 | 1708 | $CH_3$ |
| K(4) = 991.175 | 2320 | Starch |
| **Cellulose** | | |
| K(0) = 41.277 | | |
| K(1) = −506.932 | 2252 | Cellulose |
| K(2) = −826.088 | 1898 | $CO_2H$ |
| K(3) = −549.229 | 2076 | Oil |
| K(4) = 624.905 | 1754 | $CH_2$ |

NOTE: Wavelength assignments are according to Osborne and Fearn (1986).

measured relative to measured reflectance from an internal ceramic chip. The average time required to complete the four scans was 4.5 min per sample.

For developing calibration equations, all spectra were transformed to second difference approximation of the second derivative (Wessman et al. 1988*a*; Casciero and DiGiovanni 1985). Derivative spectra were frequently used in equation development to reduce errors due to base-line shifts between samples and to accentuate fine-scale spectral features (Wessman et al. 1988*a*; Hruschka 1987).

Two approaches can be taken to the selection of wavelengths from which constituent concentrations will be predicted. The first is to use stepwise forward multiple linear regression, selecting wavelengths strictly on the basis of statistical relationships. This provides the best fit to the current data set, but unless the selected wavelengths have known biophysical meaning (i.e., represent overtones of bond types known to be important in the constituent of interest), the resulting equation may not be generalizable and may then be less accurate in predicting concentrations in additional tissues.

The second approach is to force wavelengths of known biophysical meaning into the equation. This yields a more generalizable equation with a strong theoretical basis, but may perhaps be less accurate for the current data set. In particular, this approach might fail to correct for interferences at particular wavelengths produced by other important constituents. The use of several wavelengths per calibration allows for the inclusion of several chemically distinct compounds within the broad, chemically defined categories "lignin" and "cellulose." A combination of user- and machine-selected wavelengths was used for all three constituents reported here.

When developing the calibration, approximately one-third of the samples were randomly selected and separated into a validation set. For nitrogen, 148 samples were used for validation and 303 for the initial calibration. For lignin, 56 samples were used for validation and 113 for the initial calibration. For cellulose, 55 samples were used for the validation and 114 for the initial calibration. In all three cases the two sets were combined to arrive at the final calibration equation.

After a preliminary equation had been developed for each constituent, concentrations of nitrogen, lignin, and cellulose were predicted for all four individual scans of each sample. If the predicted concentration of any constituent for any one scan differed by more than 10% from the mean of all four scans for that constituent, the sample was scanned again. This was done to identify and reanalyze those samples in which the packing of the material into the sample cell produced a nonuniform surface. For the final calibration set, all samples for which the estimated (NIR) value differed from the wet chemistry value by more than twice the standard error of calibration for that constituent were removed from the calibration set. Eighteen samples were removed from the nitrogen file by this procedure, seven from the lignin file, and seven from the cellulose file.

## Results and discussion

### *Differences between spectra of fresh and decomposing materials*

There is a general lack of information on the changes in spectral reflectance that occur during the decomposition

TABLE 4. Statistics for calibrations developed in this study

| | Initial calibration | | | Validation | | | Final calibration | | |
|---|---|---|---|---|---|---|---|---|---|
| | *n* | *r* | SEC | *n* | *r* | SEP | *n* | *r* | SEC |
| Nitrogen | 303 | 0.97 | 0.11 | 148 | 0.94 | 0.17 | 451 | 0.97 | 0.12 |
| Lignin | 112 | 0.91 | 1.73 | 57 | 0.87 | 3.03 | 169 | 0.91 | 2.03 |
| Cellulose | 114 | 0.90 | 2.12 | 55 | 0.84 | 2.90 | 169 | 0.90 | 2.20 |

NOTE: *r*, correlation coefficient of multiple linear regression; SEC, standard error of the calibration; SEP, standard error of prediction.



process. We have recorded both significant and cumulative changes in the basic spectral signature of leaf material as it decomposes (Fig. 1). In general, absorbance in the region between 1100 and 2000 nm increases continuously as decomposition proceeds. In all sample sequences analyzed (spectra from the same material through time), the generally low absorbance in this range for fresh foliage is converted to high absorbance through the development of a very broad scale feature. We do not know what causes this feature. It is interesting that absorbance increases continuously with time in this region, suggesting that measurement of this broad-scale feature may allow determination of the relative state of decay of a given tissue type. More work needs to be done in this area.

*Constituent calibrations*

All calibrations used wavelengths selected for biophysical meaning combined with additional wavelengths that provided good statistical fits to the data. We cannot at this time determine the chemical significance of all of the statistically fit wavelengths. This may be due either to the nonspecific nature of the lignin and cellulose wet chemistry analyses (i.e., several different types of compounds may not be dissolvable in sulfuric acid) or to imperfect knowledge of the pure-compound reflectance characteristics of specific compounds.

The wavelengths used in the nitrogen calibration were 2174, 2378, 2090, and 1230 nm (Table 3). The first wavelength (2174 nm) had the best statistical fit to the data and is in the region of a very strong protein absorption feature. An equation using this wavelength alone carries a correlation coefficient of 0.91 and a standard error of calibration (SEC) of 0.19. This wavelength also contains most of the information on nitrogen content in fresh and senescent foliage (McLellan et al. 1991). The third wavelength, 2090 nm, was selected because of good statistical fit and as representative of the O—H bond in cellulose, starch, and lignin. The second wavelength, 2378 nm, is associated with absorbtion by oil and may represent some of the condensed, hydrocarbonlike materials in both fresh and decomposed organic residues. The fourth wavelength, 1230 nm, is associated with absorption by C—H bonds.

For lignin, the first and third wavelengths (1438 and 1708 nm) were operator selected, while the second and fourth (2154 and 2320 nm) were selected statistically (Table 3). The region around 1430 nm has been identified as an absorption feature for lignin (Osborne and Fearn 1986). However, this wavelength is not as effective in predicting lignin concentration alone as 2174 nm is for nitrogen ($r = 0.63$, SEC = 3.83).

Two problems prevent a more accurate determination of lignin concentrations by a single wavelength in the near infrared. First, lignin is operationally defined as that material which cannot be digested in 72% sulfuric acid under the conditions of the digestion. This is acknowledged to be a useful but only proximate separation. Thus the wet chemistry lignin fraction is likely to contain a mixture of relatively stable organic compounds. To restate this, the chemical analysis for lignin is less specific than is the Kjeldahl procedure for nitrogen. Second, even if lignin could be chemically isolated, it still consists of several different types of molecules combined in many ways. Thus the chemistry of lignin is quite variable from tissue to tissue and species to species. Still, lignin content of foliage is a very good predictor of decay rate in both litter and soils (e.g., Melillo et al. 1982; Wessman et al. 1988*b*). NIR offers the potential to both standardize the measurement of lignin (McLellan et al. 1991) and greatly decrease the cost of analyses. The second to fourth wavelengths, 2154, 1708, and 2320 nm (associated with $CONH_2$, $CH_3$, and starch, respectively), may represent important bond types present in lignin, but we cannot at this time explain why they offer a better fit to the data than other wavelengths representing the same bond types.

For cellulose, the first and fourth wavelengths (2252 and 1754 nm) were selected statistically. Wavelengths two and three (1898 and 2076 nm) were operator selected (Table 3). The wavelength 2252 nm has been identified as a cellulose wavelength and also had good correlation with cellulose measurements within this data set ($r = 0.90$ SEC = 2.20). The second wavelength (1898 nm) is associated with $CO_2H$ bonds, the third wavelength (2076 nm) with oil, and the fourth wavelength (1754 nm) with $CH_2$ bonds.

*Statistical analyses of calibrations*

For all constituents, the SEC was lower than the standard error of prediction (SEP) (Table 4). This suggests that additional information about relationships between wavelengths and constituent concentrations was contained in the validation set. As the division between calibration and validation sets was random, we did not attempt to adjust this division to achieve better results.

Wessman et al. (1988*a*) did not see the same pattern in their analysis of fresh and senescent foliage, even though their sample size was smaller. Their SEC and SEP for nitrogen were both 0.11, and for lignin were 3.02 and 3.14, respectively. We suggest that this difference arises from the more heterogeneous nature of decomposing plant materials relative to green or senescent tissues. This greater heterogeneity would require a larger number of samples to characterize accurately wavelength–concentration relationships. However, the larger sample size in this study results in lower SEC values for the final calibrations for lignin and cellulose than reported in either of two studies of fresh and senescent foliage (Wessman et al. 1988*a*; McLellan et al. 1991).

Further validation of these calibrations should be attempted on additional sets of partially decomposed foliage that have been analyzed by standard wet chemistry procedures.

## Conclusion

Even though a large and broad absorption feature develops in the NIR spectra of decaying foliage, our analyses suggest that NIR spectroscopy is a valid method for measuring the nitrogen, lignin, and cellulose concentrations within decomposing foliar litter material over a wide range of litter ages. Because this method is more rapid, less expensive, and more easily standardized than traditional wet chemistry procedures, we feel that it has the potential to become the standard method for measuring these constituents in both fresh and decomposed plant materials.

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ABER, J.D., MELILLO, J.M., and FEDERER, C.A. 1982. Predicting the effects of rotation length, harvest intensity and fertilization on fiber yield from northern hardwood forests in New England. For. Sci. **28**: 31–45.

ABER, J.D., MELILLO, J.M., and MCCLAUGHERTY, C.A. 1990. Predicting long-term patterns of mass loss, nitrogen dynamics, and soil organic matter formation from initial fine litter chemistry in temperate forest ecosystems. Can. J. Bot. **68**: 2201–2208.

BERG, B. 1986. Nutrient release from litter and humus in coniferous soils—a mini review. Scand. J. For. Res. **1**: 359–369.

CAPPO, K.A., BLUME, L.J., RAAB, G.A., BARTZ, J.K., and ENGELS, J.L. 1987. Total carbon and nitrogen. Sect. 14. *In* Analytical methods manual for the Direct/Delayed Response Project Soil Survey. U.S. Environ. Prot. Agency Tech. Rep. 600/8-87/020.

CASCIERO, J.L., and DIGIOVANNI, E.J. 1985. Manual for the Compscan System. Pacific Scientific Co., Silver Springs, MD.

EFFLAND, M.J. 1977. Modified procedure to determine acid insoluble lignin in wood and pulp. Tappi, **60**(10): 143–144.

GOERING, H.K., and VAN SOEST, P.J. 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). U.S. Dep. Agric. Agric. Handb. 379.

HRUSCHKA, W.R. 1987. Data analysis: wavelength selection methods. *In* Near infrared technology in the agricultural and food industries. *Edited by* P. Williams and K. Norris. American Association of Cereal Chemists, St. Paul, MN.

MCCLAUGHERTY, C.A., PASTOR, J., ABER, J.D., and MELILLO, J.M. 1985. Forest litter decomposition in relation to soil nitrogen dynamics and litter quality. Ecology, **66**: 266–275.

MCLELLAN, T., MARTIN, M.E., ABER, J.D., MELILLO, J.M., NADELHOFFER, K.J., and DEWEY, B. 1991. Comparison of wet chemistry and near infrared reflectance measurements of carbon-fraction chemistry and nitrogen concentration of forest foliage. Can. J. For. Res. **21**: 1689–1693.

MEENTEMEYER, V., and BERG, B. 1986. Regional variation in rate of mass loss of *Pinus sylvestris* needle litter in Swedish pine forests as influenced by climate and litter quality. Scand. J. For. Res. **1**: 167–180.

MELILLO, J.M., and ABER, J.D. 1984. Nutrient immobilization in decaying litter. Nato Conf. Ser. 1, **6**: 193–215.

MELILLO, J.M., ABER, J.D., and MURATORE, J.M. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology, **63**: 621–626.

MELILLO, J.M., ABER, J.D., LINKINS, A.E., RICCA, A., FRY, B., and NADELHOFFER, K.J. 1989. Carbon and nitrogen dynamics along the decay continuum: plant litter to soil organic matter. Plant Soil, **115**: 189–198.

NORRIS, K.H., and HART, J.R. 1965. Direct spectrophotometric determination of moisture content of grain and seeds. Humidity Moisture Meas. Control Sci. Ind. Pap. Int. Symp. **4**: 19–25.

NORRIS, K.H., BARNES, R.F., MOORE, J.E., and SHENK, J.S. 1976. Predicting forage quality by infrared reflectance spectroscopy. J. Anim. Sci. **43**: 889–897.

OSBORNE, B.G., and FEARN, T. 1986. Near infrared spectroscopy in food analysis. Longman Scientific and Technical Publishing Co.

PARTON, W.J., STEWART, J.W.B., and COLE, C.V. 1988. Dynamics of C, N, P, and S in grassland soils: a model. Biogeochemistry, **5**: 109–132.

RYAN, M.G., MELILLO, J.M., and RICCA, A. 1990. A comparison of methods for determining proximate carbon fractions of forest litter. Can. J. For. Res. **20**: 166–171.

SHENK, J.S., LANDA, I., HOOVER, M.R., and WESTERHAUS, M.O. 1981. Description and evaluation of near infrared reflectance spectro-computer for forage and grain analysis. Crop Sci. **21**: 355–358.

VAN SOEST, P.J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Off. Anal. Chem. **46**: 829–835.

WESSMAN, C.A., ABER, J.D., PETERSON, D.L., and MELILLO, J.M. 1988*a*. Foliar analysis using near infrared reflectance spectroscopy. Can. J. For. Res. **18**: 6–11.

——— 1988*b*. Remote sensing of canopy chemistry and nitrogen cycling in temperate forest ecosystems. Nature (London), **335**: 154–156.

WINCH, J.E., and MAJOR, H. 1981. Predicting nitrogen and digestibility of forages using near infrared reflectance photometry. Can. J. Plant Sci. **61**: 45–51.