The use of near-infrared reflectance spectroscopy in litter decomposition studies

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Summary — The biochemical nature of leaf litter is a key factor in regulation of its decomposition. Conventional wet chemical analysis of samples is destructive, time-consuming and expensive. The objective of this study was to evaluate the potentiality of near infrared reflectance spectroscopy (NIRS) for determining litter chemistry during the decomposition process using a wide range of species and decomposition stages. The litter of 8 species of evergreen and deciduous broad-leaved trees, conifers and shrubs were used in both laboratory and field experiments. Near-infrared reflectance measurements were made with an NIRS Systems 5000 spectrophotometer over the range 1100–2500 nm. Calibration samples were analysed for ash, carbon and nitrogen. Acid-detergent fibre (ADF) and acid-detergent lignin (ADL) were determined using Van Soest procedures. Stepwise regression (SR) calibrations and partial least squares (PLSR) calibrations were developed and compared as well as the effect of scatter correction. The PLS algorithm was used to create the predictive models using all the information in the spectrum to determine the chemical concentration. Using scatter correction always gave better results. Both regression methods provided acceptable validation statistics for C, N and ash. The PLSR had better prediction accuracy for ADF and ADL. For these two constituents, the improvement of SECV was 34 and 25% respectively. Our results showed that NIRS is an effective tool to predict nitrogen, ash and proximate carbon fractions in decomposition studies and that PSLR method improves calibration compared with SR method.

decomposition / leaf chemistry / litter / NIRS

Résumé — Utilisation de la spectroscopie proche infrarouge dans les études de décomposition de litières. La composition biochimique des litières est un des facteurs clés de la régulation de leur décomposition. Les méthodes d'analyse chimique par voie humide sont destructives, longues et coûteuses et ces contraintes sont rapidement limitantes dans les études en milieux hétérogènes et plurispécifiques, comme le sont les milieux forestiers spontanés méditerranéens. L'objectif de cette étude est d'évaluer les potentialités de la spectrométrie de réflexion dans le proche infrarouge (SPIR) pour l'étude et le suivi de la décomposition des litières forestières. Les échantillons utilisés proviennent d'expériences menées sur le terrain et en laboratoire sur 8 espèces méditerranéennes : feuillus caducifoliés et sempervirents, et résineux. Les spectres des litières, obtenues à différents stades de décomposition, ont été enregistrés entre 1100 et 2500 nm avec un spectrophotomètre NIRS 5000. Un tiers des échantillons a été analysé par voie humide : cendres totales, carbone, azote, ligno-cellulose et lignine (ADF et ADL méthode Van Soest). À partir de ces analyses, des modèles prédictifs de concentration de chaque composé chimique ont été établis, avec et sans correc-
tion de tendance, par deux méthodes de régression : i) régression multiple pas à pas (stepwise) et ii) au moyen d'un algorithme d'ajustement par la méthode des moindres carrés PLS (partial least squares). À la différence des méthodes de régression multiples basées sur un petit nombre de longueurs d'ondes, cette méthode utilise l'ensemble de l'information spectrale. La correction de tendance améliore toujours les résultats de calibration. Les deux méthodes de régression donnent des résultats comparables pour le carbone, l'azote et les cendres. Pour la ligno-cellulose et la lignine, les erreurs standards de validation obtenues par la méthode de calibration PLS sont inférieures de 25 et 34% à celles obtenues par la méthode de régression multiple. Ces résultats montrent que la SPIR peut être utilisée dans les études de décomposition et que la méthode de calibration basée sur l'ensemble du spectre (PLS) est plus performante pour la prédiction des fractions carbonées complexes. Par sa rapidité et sa fiabilité, cette méthode réduit les contraintes analytiques et permet d'aborder les études de décomposition en milieu hétérogène.

décomposition / litière / chimie du feuillage / SPIR

INTRODUCTION

Within a climatic area, the biochemical nature of leaf litter is certainly the most important factor in the regulation of its decomposition (O'Connell, 1988; Berg and McClaugherty, 1989; Taylor et al, 1989). The rate of decay varies with nitrogen and phosphorus concentration and also with carbon chemistry (Swift et al, 1979; McClaugherty and Berg, 1987). The carbon chemistry of the litter substrate is usually divided into 3 fractions: extractives (lipids, sugars, phenolics), polymer carbohydrates (cellulose, hemicellulose) and acid-insoluble compounds (AIC = lignins). In classical forage fiber analysis, the last 2 fractions constitute the ADF (acid detergent fiber) and the last fraction the ADL (acid detergent lignin). Each of these fractions represents a mixture of constituents extracted at the same time using the Van Soest analytical technique (1963); however, they are very useful to understand litter decay. Indeed, because lignins can operate both as a carbon and energy source and as a modifier of the activity of decaying organisms, they are as important as nutrient content for resource quality.

Conventional wet chemical analysis of samples is destructive, time-consuming and expensive when a large number of samples is required. Moreover, for some constituent, such as proximate carbon fractions, no standard method has been established (Ryan et al, 1990). Although near infrared reflectance spectroscopy (NIRS) has become widely used as a nondestructive method for quality analysis of grain (Williams, 1975) and forage (Norris et al, 1976), few ecological studies have used this technique. Dalal and Henry (1986), Krishnan et al (1980) and Morra et al (1991), used NIRS to predict C and N concentrations in soils. Card et al (1988), Wessman et al (1988) and McLellan et al (1991a, b) showed that NIRS may be useful for the determination of leaf chemistry.

Using a wide range of species and decomposition stages, our objectives were i) to determine the changes in spectra during decomposition process, ii) to evaluate the potential of NIRS for determining litter chemistry during decomposition, and iii) to compare the stepwise regression (SR) and partial least squares regression (PLSR) calibration methods.

MATERIALS AND METHODS

Litter decomposition experiment

Two data set collected from 2 experiments were used. The first experiment was conducted in the
laboratory and concerned 8 species (Quercus pubescens L, Quercus ilex L, Quercus coccifera L, Castanea sativa Miller, Pinus halepensis Miller, Fagus sylvatica L, Cistus monspeliensis L, Cistus albidus L). Leaf litter of these species were collected at the leaf-fall period near Montpellier. In the laboratory, a microcosm system, as described by Taylor and Parkinson (1988), was used. Air dried samples of 7.00 ± 0.01 g were remoistened in water for 24 h and were placed over a 2 mm nylon mesh on the soil surface of the microcosms. Microcosms were maintained at 22 °C and watered once a week maintaining soil moisture at 80% of field capacity. Five replicates of each litter were removed after 0.5, 1, 2, 4, 6, 10 and 14 months. The second experiment was conducted in the field, in a Q pubescens forest (50 km NE of Marseille, southern France), and concerned the 2 species Q pubescens and P halepensis. In this experiment, 5 mm mesh bags containing 10 g of air-dried litter, collected near this forest, were placed on the soil surface. Five replicates were removed after 5, 12, 19 and 26 months. All samples were dried in a ventilated oven at 60 °C until constant weight, weighed and then ground in a cyclone mill through a 1-mm mesh.

NIRS analysis

A total of 330 samples were scanned with a near-infrared reflectance spectrophotometer (NIRSystems 5000). Each sample was packed into a sample cell having a quartz-glass sample. Two reflectance measurements of monochromatic light were made from 1100 to 2500 nm to produce an average spectrum with 700 data points at 2 nm intervals over this range. The band-pass used 10 nm and the wavelength accuracy 0.5 nm. Reflectance (R) is converted to absorbance (A) using the following equation:

\[ A = \log \left( \frac{1}{R} \right) \]

Data analysis was conducted using ISI software system (Shenk and Westerhaus, 1991b).

Sample selection and chemical methods

Approximately one-third of the samples were selected for providing the calibration sample set analysed by wet chemical techniques. On the basis of the standardized H distances from the average spectrum in the space of the principal components, we first eliminated 4 samples (on the total population of 330 samples) with H > 3.0 (Shenk and Westerhaus, 1991a). The second algorithm used standardized H distance among pairs of samples to define neighbourhoods. The average distance between pairs of closest samples was 0.068, and using an H = 0.125, 91 samples were selected.

These samples were analysed for ash (550 °C for 3 h) and moisture (105 °C for 24 h). Carbon and nitrogen content were determined with a Perkin Elmer elemental analyser (PE 2400 CHN) and acid-detergent fiber (ADF) and acid-detergent lignin (ADL) were determined using Van Soest procedures (1963, 1965) adjusted for Fibertec (Van Soest and Robertson, 1985). Considering the important weight loss of litter after several months of incubation, analyses could not be achieved on all samples because of the lack of material.

Statistical methods

Stepwise regression (SR) calibrations and partial least squares (PLSR) calibrations were developed and compared for C, N, ADF, ADL, and ash with each calibration using 6 math treatments corresponding to first and second derivative and a gap of 5, 10, and 15 data points or 10, 20, and 30 nm. For all these previous math treatments, results obtained with and without the detrending method (Barnes et al, 1989) were compared.

Stepwise is performed by selecting the wavelength that is the most highly correlated with the reference values and adding it to the equation. The second wavelength is added by calculating all partial correlations with all other wavelengths and selecting the wavelength with the highest partial correlation. The process continues until the addition of a wavelength makes no additional improvement in explaining the variation in the reference value (F value significant at 0.01). After each wavelength is added to the equation, the program re-evaluates all wavelength in the equation before continuing (Windham et al, 1989; Shenk and Westerhaus, 1991b).

Partial least squares (PLS) algorithm was used to create predictive models (Martens and Jensen, 1982). PLS differs from wavelength searches in that it uses all the information in the
spectrum to determine the analyte concentration, a fundamental advantage over single wavelength applications. Because the entire spectrum is used, each wavelength is averaged into the answer. PLS is the marriage of principal component analysis (PCA) and multiple linear regression (MLR). PCA reduces the spectral data to a few combinations of the absorptions that account for most of the spectral information but also relates to the sample reference values (Shenk and Westerhaus, 1991b). The first vector (called a loading) used by the PLS algorithm is the result of the cross multiplication of the spectral variance of the data and the correlation spectrum. The first loading is used to fit the training spectra based on a least square is then correlated with the chemical value. This results in an overall correlation coefficient and a preliminary estimate of the chemical values. The residual errors between the actual and predicted chemical values are calculated, as are the residual spectra from the curve fitting process. Both of these residuals are plugged back into the start of the program. The same calculations are performed on the residuals to obtain the second loading and scores. This stepwise addition of loadings continues until sufficient terms have been added to explain the chemical data. Cross validation is used to estimate the optimal number of terms in the calibration to avoid overfitting. It consists of selecting, for instance, 1 quarter of the samples for the prediction and 3 quarters to develop the model. The algorithm is repeated 4 times and all the residuals of the 4 predictions are pooled to provide a standard error of cross validation (SECV) on independent samples. The minimum SECV determines the number of terms to be used. The final model is then recalculated with all the samples to obtain the standard error of calibration (SEC).

In order to compare the 2 calibration methods, only the math treatment that provided the most accurate prediction of each constituent has been taken into account.

RESULTS AND DISCUSSION

Changes in spectra during decomposition process

The modification of the litter chemical components during decomposition was related to a progressive and important distortion of the spectra. The example of Quercus pubescens litter shows that this alteration is far more rapid in the laboratory than in the field (fig 1a, b). As decomposition progresses, absorbance in the region between 1100 and 1400 nm increases as emphasized by Mc Lellan et al (1991a). This baseline shift can be related to the modification of the mineral matter/organic matter ratio of the samples as decomposition progresses. Ash concentration increases with time and decay state: from 82, 98, 128, 180 to 216 g kg⁻¹ dry matter at 0, 5, 12, 19 and 26 months of decomposition in field experiments whilst this concentration varies from 55, 78, 180, 248 to 441 at 0, 0.5, 2, 4 and 6 months in laboratory experiments. The increased reflectance in the 1100-1400 region caused by

![Fig 1. a Spectra of litter samples of Quercus pubescens at the beginning and after 5, 12, 19 and 26 months during the field decomposition experiment. b Spectra of litter samples of Quercus pubescens at the beginning and after 0.5, 2, 4 and 6 months during the laboratory decomposition experiment.](image-url)
the increase of mineral component agrees with data from Paul (1988) for soil contamination in silage and Windham et al (1991) for increasing ash concentration in forage, esophageal and fecal samples.

**Calibration equations**

The calibration equations were carried out on samples characterized by a wide range of chemical components concentration (table I). Furthermore, as emphasized by McLellan et al (1991a), the chemical nature of decomposing plant materials was more heterogeneous than that of green foliage. This sample heterogeneity could have led to low determination coefficients and high SEC and SECV. Yet, on the whole, SEC and SECV were weak except for ADF (tables II, III). The use of scatter correction gave similar or better result in all cases. Determination coefficients ranged from 0.87–0.99 (except 0.82 and 0.78 for ADF by SR with and without scatter correction). The 2 methods of regression gave similar good prediction results for C, N and Ash. The PLSR had better prediction accuracy for ADF and ADL. For these 2 constituents, the improvement of SECV was 34 and 25% respectively.

Among all the analysed constituents, ADF is the most complex, as it is made up of all lignins and cellulloses which probably have different decomposition rates. ADF thus results from several different components in variable proportions, registered by chemical analysis as a single entity, but probably related to different spectra. The SEC value indicates that different chemical components are not expressed by the ADF global value measured here.

Graphic comparisons between values predicted with NIR calibration equations and those obtained by chemical analyses are displayed in figure 2. The prediction equation is all the more effective as the

### Table I. Wet chemical range (%) for each constituent within the calibration sample set.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>51</td>
<td>45.3</td>
<td>27.3 – 58.6</td>
</tr>
<tr>
<td>N</td>
<td>47</td>
<td>1.09</td>
<td>0.45 – 1.94</td>
</tr>
<tr>
<td>ADF</td>
<td>73</td>
<td>53.4</td>
<td>33.4 – 70.4</td>
</tr>
<tr>
<td>ADL</td>
<td>70</td>
<td>33.1</td>
<td>17.7 – 51.6</td>
</tr>
<tr>
<td>Ash</td>
<td>68</td>
<td>15.9</td>
<td>3.4 – 44.1</td>
</tr>
</tbody>
</table>

### Table II. Modified partial least squares regression. Equation calibration statistics for C, N, ADF, ADL and Ash.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>With scatter correction</th>
<th>Without scatter correction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEC</td>
<td>R²</td>
</tr>
<tr>
<td>C</td>
<td>51</td>
<td>0.68</td>
<td>0.98</td>
</tr>
<tr>
<td>N</td>
<td>47</td>
<td>0.04</td>
<td>0.99</td>
</tr>
<tr>
<td>ADF</td>
<td>73</td>
<td>2.64</td>
<td>0.90</td>
</tr>
<tr>
<td>ADL</td>
<td>70</td>
<td>1.93</td>
<td>0.96</td>
</tr>
<tr>
<td>Ash</td>
<td>68</td>
<td>0.87</td>
<td>0.99</td>
</tr>
</tbody>
</table>

* Math treatment indicates the mathematical transformation of spectral data: the first number is the order of the derivative function, the second is the segment length in data points over which the derivative was taken, and the third the segment length over which the function was smoothed.
Table III. Stepwise regression. Equation calibration statistics for C, N, ADF, ADL and Ash.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>With scatter correction</th>
<th>Without scatter correction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SEC</td>
<td>$R^2$</td>
</tr>
<tr>
<td>C</td>
<td>51</td>
<td>0.76</td>
<td>0.97</td>
</tr>
<tr>
<td>N</td>
<td>47</td>
<td>0.06</td>
<td>0.96</td>
</tr>
<tr>
<td>ADF</td>
<td>73</td>
<td>3.61</td>
<td>0.82</td>
</tr>
<tr>
<td>ADL</td>
<td>70</td>
<td>3.30</td>
<td>0.91</td>
</tr>
<tr>
<td>Ash</td>
<td>68</td>
<td>1.58</td>
<td>0.98</td>
</tr>
</tbody>
</table>

* Math treatment indicates the mathematical transformation of spectral data: the first number is the order of the derivative function, the second is the segment length in data points over which the derivative was taken, and the third the segment length over which the function was smoothed.

Fig 2. Relationship between NIR predicted values and wet chemistry values (% of dry matter).
points are near the theoretical correspondence 1:1 (diagonal line).

The results obtained show that NIRS is an effective tool to predict nitrogen, ash, and proximate carbon fractions from forage fiber techniques in the study of decomposition of leaf litter from a variety of evergreen and deciduous broad-leaved species, conifers and shrubs. However, the interpretation of forage fiber analysis in decomposing leaf material remains difficult. Ryan et al (1990) emphasized that "forage fiber lignin analysis may be less sensitive than the forest products lignin analysis to changes that occur during decomposition". Complementary studies are now in progress using the same plant material to test NIRS efficiency in order to determine carbon chemistry according to forest product techniques.

ACKNOWLEDGMENTS

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