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Soil Biology & Biochemistry 37 (2005) 795–799

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Short Communication

Determination of water-soluble and total extractable polyphenolics in biomass, necromass and decomposing plant material using near-infrared reflectance spectroscopy (NIRS)

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Received 22 July 2003; accepted 23 August 2004

Abstract

Near infrared reflectance spectroscopy (NIRS) was used to predict the water-soluble and total extractable polyphenolics of plant material. Different life forms (forbs, grasses, shrubs, giant rosettes), organs (leaves, stems, roots) and decomposition stages (biomass, necromass and decomposing plant material) were studied. Prediction was good, with a R^2 in validation ranging from 0.91 to 0.93 and in prediction from 0.88 to 0.94. Various standard error ratios were used to assess the quality of the models, which are generally very good, being the model for predicting the water-soluble polyphenolics in the decomposing plant material the slightly less good. Because it is a cheap and rapid method, it would allow to perform a large screening for studies concerning (i) polyphenolics control on decomposition process and (ii) phenolics implication in herbivory.

Keywords: Near infrared reflectance spectroscopy; Polyplenols; Plant material quality; Decomposition

Near infrared reflectance spectroscopy (NIRS) is a rapid, cheap and non-destructive technique offering the potential for accurate and repeatable measurements of chemical constituents in organic materials (Norris et al., 1976; Williams, 1975). In litter decomposition studies, it has been used to determine neutral and acid detergent fibres (NDF and ADF) and acid detergent lignin (ADL) as extracted by the van Soest technique (Van Soest and Robertson, 1985; Joffre et al., 1992; Coûteaux et al., 1998; Mc Tiernan, 1998 unpublished PhD; Kurz-Besson, 2000 unpublished PhD; McTiernan et al., 2003); mass loss (Gillon et al., 1993; Kurz-Besson, 2000 unpublished PhD) or decomposability (Gillon et al., 1999). NIRS calibration of phenolic substances was carried out in some food materials, such as tea leaves (Schulz et al., 1999), or some forage species (Windham et al., 1988).

The NIRS determination is based in the use of regression models between the spectral information of a set of samples and their reference values. In this paper, we attempt to fit regression models to total and water-soluble extractable polyphenol content (TEP and WEP) of undecomposed (biomass and necromass) and decomposing plant material. These models would be an easy tool for rapid determination of phenolic compound content in plant material. These compounds are known to be a defence strategy of plants against herbivory (Hanley and Lamont, 2002; Kouki and Manetas, 2002) and a factor controlling plant residue decomposition rate (Northup et al., 1998; Loranger et al., 2002).

The plant material came from two studies: (i) a decomposition experiment where litter bags of 14 species were incubated for 1–2 years in two sites located in the high altitude Andes (Patacamaya in Bolivia at 3800 m asl and Gavidia in Venezuela at 3400 m asl) providing more than 3700 samples in different stages of decomposition (DECO set), and (ii) a production study at Gavidia providing 430 samples from 24 additional species (PROD set) (Table 1). Different life forms

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doi:10.1016/j.soilbio.2004.08.028

Table 1
Number of samples of the different kinds of plant material used for the conventional polyphenolics analyses

	Species	Family	Site	Undecomposed				Decomposing		
				Biomass	Necro-mass	Litter	Roots	Leaves	Stems	Roots
DECO study										
	Bitter potatoes	Solanaceae	Patacamaya		2			3	2	
	Sweet potatoes	Solanaceae	Patacamaya		1			3	1	
	Sheep dung		Patacamaya		1			7		
	Aristida asplundii	Poaceae	Patacamaya				1	2		
	Bouteloua simplex	Poaceae	Patacamaya		1			2		
	Erodium cicutarium	Geraniaceae	Patacamaya					4		
	Festuca orthophylla	Poaceae	Patacamaya				1	1		
	Oxalis bisfracta	Oxalidaceae	Patacamaya				1			
	Baccharis incarum	Asteraceae	Patacamaya				1	1	3	1
	Parastrephia lepidophylla	Asteraceae	Patacamaya		1				3	
	Stipa ichu	Poaceae	Patacamaya					2		1
	Triticum aestivum	Poaceae	Patacamaya					6		
DECO and PROD studies										
	Acaena elongata	Rosaceae	Gavidia	3		1	2	2		2
	Baccharis prunifolia	Asteraceae	Gavidia	2	1	1	1	2		3
	Espeletia schultzii	Asteraceae	Gavidia	2		1	1	3		2
	Hypericum laricifolium	Guttiferae	Gavidia	4	1		2	4		1
	Lupinus meridianus	Fabaceae	Gavidia	2		1		2	2	3
	Mixed roots	several	Gavidia							1
	Solanum tuberosum	Solanaceae	Gavidia				1	3	2	1
	Rumex acetosella	Polygonaceae	Gavidia	4		5		2		2
	Vulpia myurus	Poaceae	Gavidia	1				2		
PROD study										
	Agrostis jahnii	Poaceae	Gavidia	3		1				
	Agrostis trichodes	Poaceae	Gavidia	2		1				
	Arenaria venezuelana	Caryophylla-ceae	Gavidia	3		1				
	Baccharis tricuneata	Asteraceae	Gavidia	1		1				
	Bidens triplenervia	Asteraceae	Gavidia	3		1				
	Bromus carinatus	Poaceae	Gavidia	3	1	3				
	Calamagrostis pittieri	Poaceae	Gavidia	1						
	Cerastium racemosum	Caryophylla-ceae	Gavidia	1		1				
	Erodium cicutarium	Geraniaceae	Gavidia		1					
	Gamochaeta americana	Asteraceae	Gavidia			3				
	Geranium chamaense	Geraniaceae	Gavidia			1				
	Geranium multiceps	Geraniaceae	Gavidia	1		1				
	Gnaphalium meridanum	Asteraceae	Gavidia	1						
	Lachemilla moritziana	Rosaceae	Gavidia	1	1	2				
	Myrica pubescens	Myricaceae	Gavidia	3		1				
	Noticastrum marginatus	Asteraceae	Gavidia	1		1				
	Oenothera epilobifolia	Onagraceae	Gavidia	1						
	Orthosanthus chimboracensis	Iridaceae	Gavidia	2	1					
	Penisetum clandestinum	Poaceae	Gavidia	2						
	Sisyrinchium tinctorum	Iridaceae	Gavidia	3	1	2				
	Stevia elatior	Asteraceae	Gavidia	1		1				
	Trisetum irazuense	Poaceae	Gavidia	2	2					
	Root biomass + necromass	several	Gavidia	4						
	Root biomass	several	Gavidia	3						

(forbs, grasses, shrubs and giant rosettes) and organs (leaves, stems and roots) were studied, and comparisons were done between biomass, necromass and litter.

The samples were ground (1 mm) using a centrifugal mill (Cyclotec, Perstorp Analytical) and then packed into sample cells with a quartz (minimal reflectance) window. Infrared analysis was carried out using a NIR spectrophotometer (NIRSystems 6500, Perstorp Analytical) (Coûteaux et al., 1998; McTiernan et al., 1998; Kurz Besson 2000 unpublished PhD, McTiernan et al., 2003), which covers a spectral range of 400–2500 nm (i.e. visible and near infrared). Data were collected at 2 nm intervals giving a spectrum with 1050 data points. The sample cell was rotated during scanning and the energy reflected back from the sample was measured and

Table 2
Range of variation of water-soluble (WEP) and total extractable (TEP) polyphenols content (g kg^{-1} DW) of the reference values

	n	Mean	Median	Minimum	Maximum	SD
Decomposing plant material						
WEP	96	8.6	3.17	0.24	95.15	16.28
TEP	96	22.04	10.45	0.7	157.83	29.36
Undecomposed plant material						
WEP	101	36.85	16.29	2.09	233.28	51.91
TEP	101	64.56	32.43	5.97	321.17	72.16

recorded. Each spectrum comprised 64 averaged scans of the rotating sample. Reflectance was converted to absorbance (A) values via the following equation:

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

where R is the ratio of the reflectance of the sample to a reference standard.

More than 4000 spectra were collected. Data analyses were performed using WinISI II-version 1.02a software (Foss NIRSystems/Tecator, Infrasoft International, LLC). A sub-set of 96 samples was selected from the DECO set by elimination of samples with close spectra using a principal component analysis (PCA) performed on the global spectral information (DECO selection) after checking on their normal distribution. On this selected set, conventional analyses were performed in order to build up calibration models. Then the spectra of the PROD set were compared to the average spectrum of the DECO selection, using the Mahalanobis distance H (Mahalanobis, 1936) in order to test if the calibration models based on the DECO selection may be used to predict their chemical composition. For 140 spectra of the PROD set, the H value was higher than 3, which is the threshold used for eliminating outliers, indicating that the regression model for the DECO set was not appropriate for the PROD set. Therefore, calibration models for the PROD set were built up on a selection of 101 samples (PROD selection) on which conventional analyses were performed.

Conventional analyses were performed according to the TSBF method (Anderson and Ingram, 1993): water-soluble compounds (WEP) were extracted by mixing 1 g of plant material with 60 ml of cold water during 2 h and centrifuged for 8 min at 1500 *g*; methanol-soluble compounds (TEP) were extracted by heating the residue of water extraction at 80 °C in 50 ml of 50% (v/v) methanol in water for one hour and centrifuged for 8 min at 1500 *g*. The phenolic compounds were determined with a colorimeter (DR/890—HACH company, Colorado, USA) in both supernatants with the HACH™ method no 8193 (Anonymous, 1999) using Tanniver® 3 Tannin–Lignin reagent as tyrosine reagent according to the Folin Ciocalteu principle which takes into account all hydroxyl aromatic compounds (Chapuis-Lardy et al., 2002). Phenolics were expressed as tannic acid equivalent. In an evaluation of the methods for measuring phenolics, Yu and Dahlgren (2000) suggested that the Folin Ciocalteu method provides a rapid test for the characterization of extractable phenolics which may have a great physiological and/or ecological significance.

The ranges of variation of the reference values are given in Table 2. The mean values of the DECO selection were lower than those of the PROD selection probably due to the leaching of the polyphenolics during the decomposition process or the decrease of extractability (Maie et al., 2003). The medians were lower than the means because of the low number of samples with high concentrations. In the DECO selection, the samples with a high concentration were generally the initial material (senescent material) and in the PROD selection generally the green leaves. In both sub-sets the range of variation was large.

The calibration models were built up on both DECO and PROD selections using least square mean multiple regressions between the measured concentrations and the wavelengths of the spectra (256 variables). The characteristics of the calibration equations are given in Table 3.

Cross validation was used to determine the optimal number of terms for the calibration. The calibration set was arbitrarily divided into four groups. Three groups were selected for developing the model and the fourth for prediction. This validation procedure was performed four times, to use all samples for both model development and prediction. The residuals of the four predictions were pooled to provide a standard error of cross validation (SECV). All the samples were used to calculate the final model and the residuals give the standard error of calibration (SEC).

Different criteria are generally used to assess the quality of the models. The coefficient of determination R^2, the most commonly used, should be higher than 0.8 for quantitative predictions. For excellent models, the SEC-to-SD ratio should be ≤ 0.2, where SD is the standard deviation of the reference values. If $0.2 < \text{SEC-to-SD ratio} \leq 0.5$, quantitative predictions is possible (Coûteaux et al., 2003). The SD-to-SECV ratio should be ≥ 2 (Chang et al., 2001; Chang and Laird, 2002), SEP-to-SEC ≤ 1.2 and the SD-to-SEP ratio should be ≥ 2.5 (Mathison et al., 1999). In this study the R^2 value ranged from 0.90 to 0.93, the SEC-to-SD ratio from 0.27 to 0.32, SD-to-SECV ratio from 1.3 to 2.2, the SEP-to-SEC ratio from 0.97 to 1.25 and the SD-to-SEP from 2.69 to

Table 3
NIRS calibration and validation statistics

Parameters	Calibration								Validation				Quality parameters			
	N	Term numbers	X-outliers	Math treatment	SEC[a] (g kg^{-1} DW)	SD[a]	SECV	R^2	*n*	X-outliers	SEP[a] (g kg^{-1} DW)	R^2	SEC/SD	SD/SECV	SD/SEP	SEP/SEC
Decomposing plant material																
WEP[a]	84	8	9	2,4,4	1.92	6.427	5.06	0.911	90	6	2.39	0.919	0.30	1.3	2.69	1.25
TEP[a]	86	8	7	2,8,4	6.10	20.24	10.28	0.910	90	6	7.11	0.887	0.30	2.0	2.85	1.17
Undecomposed plant material																
WEP[a]	94	8	7	2,6,4	11.39	42.58	18.98	0.928	95	6	11.1	0.938	0.27	2.2	3.85	0.97
TEP[a]	94	6	7	2,4,4	20.13	63.53	28.87	0.900	95	6	22.0	0.883	0.32	2.2	2.89	1.09

[a] Abbreviations are explained in the text

3.85. The best models were obtained for WEP and TEP using the PROD selection and TEP with the DECO selection. The model for WEP using the DECO selection was less good with nevertheless an R^2 of 0.91 that allows a rough quantitative evaluation.

Fig. 1 shows the predicted values plotted against the measured values for WEP and TEP concentrations of the DECO and the PROD selections. The R^2 of the linear regression ranged from 0.88 to 0.94, the slope from 0.86 to 0.92, which confirm the good quality of the models.

It can be concluded that NIRS can accurately determine the content of water-soluble and total extractable polyphenolics for a large range of concentrations and kinds of plant materials. Because it is a cheap, rapid and non-destructive method, it would allow performing a large screening for studies concerning (i) polyphenolic control on

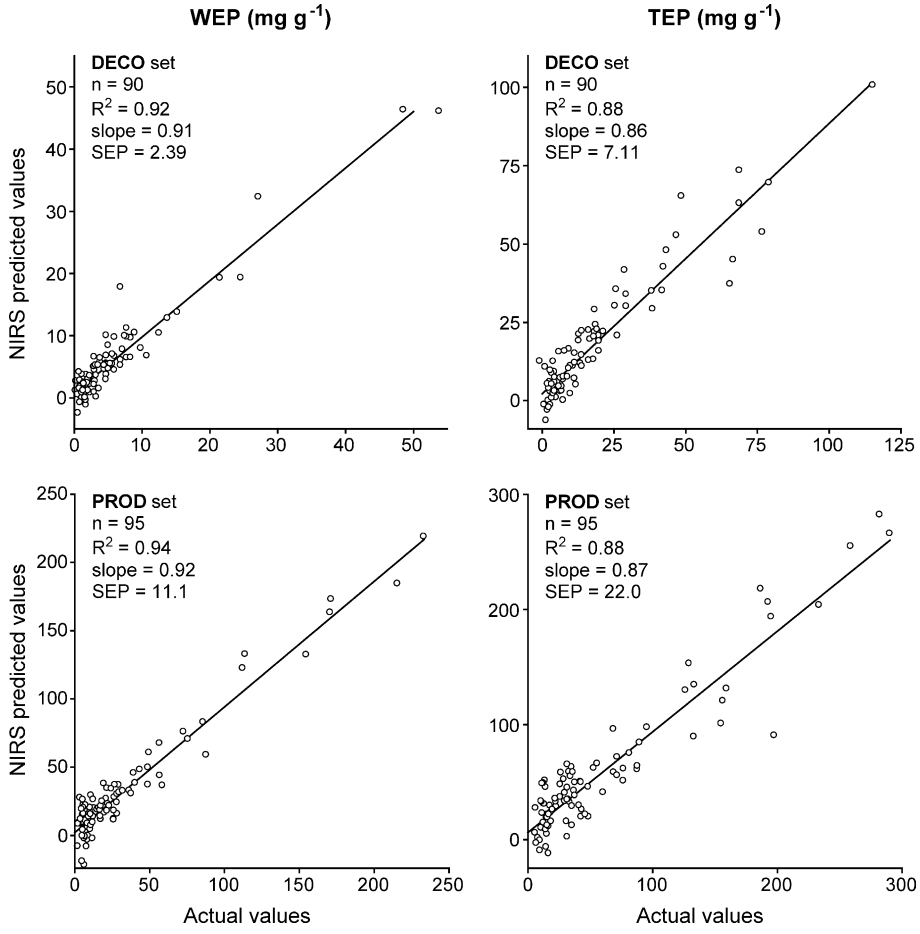

Fig. 1. Relationship between NIRS-predicted and measured water-soluble extractable polyphenolics (WEP) and total extractable polyphenolics (TEP) concentration in the DECO and the PROD selected samples.

decomposition process and (ii) polyphenolic implication in herbivory and adaptive mechanisms of plants.

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