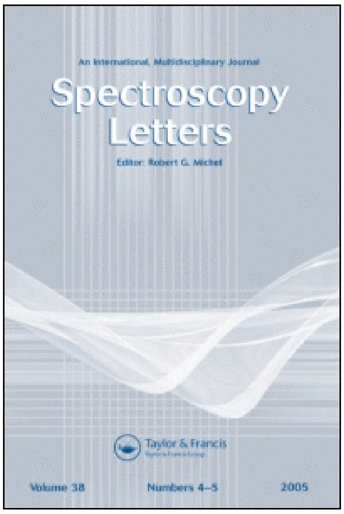

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Mid- and Near-Infrared Spectroscopic Determination of Carbon in a Diverse Set of Soils from the Brazilian National Soil Collection

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To cite this Article Madari, Beata E. , Reeves III, James B. , Coelho, Maurício R. , Machado, Pedro L. O. A. , De-Polli, Helvécio , Coelho, Ricardo M. , Benites, Vinícius M. , Souza, Lucas F. and McCarty, Gregory W.(2005) 'Mid- and Near-Infrared Spectroscopic Determination of Carbon in a Diverse Set of Soils from the Brazilian National Soil Collection', Spectroscopy Letters, 38: 6, 721 — 740

To link to this Article: DOI: 10.1080/00387010500315876

URL: http://dx.doi.org/10.1080/00387010500315876

Spectroscopy Letters, 38: 721–740, 2005
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ISSN 0038-7010 print/1532-2289 online
DOI: 10.1080/00387010500315876

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Received 27 November 2004, Accepted 16 May 2005

This paper was by special invitation as a contribution to a special issue of the journal entitled "Quantitative Vibrational Spectrometry in the 21st Century." This special issue was organized by Professor Miguel de la Guardia, Professor of Analytical Chemistry at Valencia University, Spain.

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Abstract: Calibrations for soil carbon content measured by combustion (total carbon, TC) and chromate oxidation by a modified Walkley-Black method (Walkley-Black carbon, WBC) from the Brazilian National Soil Collection were made using Fourier-transform near (1100 to 2500 nm; NIRS) and mid-infrared diffuse reflectance (2,500 to 25,000 nm; DRIFTS) spectroscopy combined with partial least squares (PLS). Calibration sets of sample populations of different carbon ranges, soil taxonomic classes, and soil textural groups were established. These are for TC ranges between 0.4 to 555.0, 0.4 to 99.1, and 0.4 to 39.9 $g\,kg^{-1}$: for WBC 0.2 to 401.0, 0.2 to 66.0, and 0.2 to 66.0, and 0.2 to 30.0 $g\,kg^{-1}$: for soil taxonomic classes Ferralsols and Acrisols; and for soil textural groups very clayey, clayey, and medium textures were examined. Calibrations obtained for the largest TC and WBC ranges were better compared to the lower ones, but lower root mean squared deviation (RMSD) and relative difference (RD = RMSD/mean value) were found for the lower carbon ranges. Taxonomic soil class was not an adequate criterium for calibration set formation. Soil texture had effect on calibrations, especially using NIR, because of the particle size effect to which NIR was more sensitive than mid-IR. In general, DRIFTS showed better performance than NIRS. NIRS only outperformed DRIFTS when used with calibration set fairly homogeneous in its particle size distribution. Results demonstrated that while calibrations can be developed using either DRIFTS or NIRS for even a very diverse set of soil samples, which will determine C over a wide range of concentrations inherent in such a diverse set, it is desirable to seperate sample populations by soil textural properties and choose the adequate spectral range (NIR or mid-IR) based on the textural group, for calibration development to achieve more accurate results.

Keywords: Carbon, chemometrics, DRIFTS, mid-infrared, near-infrared, NIRS, PLS, soil

INTRODUCTION

Over the past several decades, near-infrared (NIR) reflectance spectroscopy (NIRS) has become one of the dominant methods of analysis of agricultural products and samples where large numbers of samples are needed to be analyzed. This includes both commercial products such as the determination of protein levels in grain[1] for sale, and in research settings for the analysis of forages, silages etc.[2–4] More recently, in the past decade, the use of diffuse reflectance Fourier-transform mid-infrared (mid-IR) spectroscopy (DRIFTS) has also been found to be capable of performing quantitative analysis of similar products.[5–8] The relatively late arrival of DRIFTS as a method for analysis of agricultural products is due to the belief that, due to spectral distortions caused by the stronger absorbances in the mid-IR compared to the NIR, quantitative analysis on non-KBr diluted samples was not possible using mid-IR spectra of ground solids.[9] Although such distortions do occur in the DRIFTS spectra of agricultural products[9] including soils,[10] it appears that the use of multivariate calibration methods such as partial least squares (PLS), which base calibrations on the entire spectra as opposed to a few selected wavelengths, [3,4,11,12] are able to overcome problems caused by the distortions.[9]

Although the use of such spectroscopic techniques for analysis of forages, grains, and so forth, has been under study for several decades, only in the past decade or so has their application to soils become of increased interest.[13–20] Future implementation of the Kyoto Protocol[21] could be the driving force behind efforts to sequester carbon (C) in soils. Potential future policies for C sequestration in agriculture would require the measurement of soil C over time at many locations in order to determine whether, and if so, how much, C is being sequestered or lost from soils. Considering that assaying only one sample per hectare per year could still amount to tens of millions of samples, the need for a quick and inexpensive while still accurate analytical method for determining soil C is an absolute necessity. Standard methods such as combustion or chromate oxidation[22–25] are expensive, slow, or both. Although combustion is considered sufficiently accurate, chromate oxidation measures only organic C and is also known to be prone to biases, besides generating toxic wastes that must be disposed. Another method, loss-on-ignition, while relatively cheap and rapid, suffers from accuracy problems, because mineral fractions can also be decomposed by heating.[23–25] This is especially true for highly weathered tropical soils that contain high amounts of kaolinite and hydrated aluminosilicates (e.g., gibbsite, goethite) in their matrix that lose weight at lower temperatures than organic matter decomposition (600°C to 650°C).[26] Dehydroxylation of kaolinite causes weight loss of the sample at 450°C to 600°C, as well as dehydroxylation of gibbsite at 250°C to 350°C and of goethite at 300°C to 400°C.[27]

Finally, all of these methods require more than one determination to obtain information on both organic C and inorganic C (carbonates) and are not capable of determining other forms of C, such as soluble C, lignified C, charcoal, black carbon, and so forth, unless extractions of various types are performed and either the extractant or extracted sample reanalyzed for C. Contrasting these methods are spectroscopic techniques such as DRIFTS and NIRS, which, once calibrations are developed, can simultaneously determine any number of analytes from a single spectrum.[17]

With other agricultural materials such as grains and forages, it has often been found when using NIRS that the best results are obtained when calibrations are developed for specific products, for example a single species of forage, as opposed to using a single calibration for all forages.[3,4] Although research efforts on soils have demonstrated that both NIRS and DRIFTS can be used to determine soil C and even forms of soil C, none of these efforts has really addressed the question of whether all soils can be grouped together for calibration development or whether calibrations will be needed for different subgroups, based on, for example, C content. Few agricultural products have properties (analytes) which vary over the range found for C in soils. For the study reported here, total soil C (TC) ranged between 0.4 to 555 $\mathrm{g\,kg^{-1}}$, and the C that can be oxidized by the Walkley-Black method (chromate oxidation, WBC) between 0.2 to 402 $\mathrm{g\,kg^{-1}}$.

The objective of this study was to examine the usefulness of DRIFTS and NIRS in the quantitative determination of total C as determined by combustion (TC) and C as determined by chromate oxidation using the Walkley-Black method (WBC) for a diverse set of tropical soils obtained from the Brazilian National Soil Collection. Calibrations were tested for the total number of samples and for subsample sets separated based on soil C content, taxonomic soil class, and soil texture.

EXPERIMENTAL

Samples

Three hundred sixty-seven soil profiles, representative of Brazilian territory, were selected from the National Soil Collection of Embrapa Soils, the Brazilian National Soil Research Center, Rio de Janeiro State, and from the soil collection of IAC–Campinas, the Agricultural Institute of Campinas, São Paulo State, Brazil. The distribution of the soil profiles over Brazil is shown in Fig. 1. Diagnostic soil horizons were selected, on average 3 horizons from each profile, resulting in 1135 soil samples for carbon measured by combustion (TC), and 1014 soil samples for carbon measured by dichromate oxidation (WBC).

Sample Preparation and Conventional Sample Analysis

All samples were bulk soils samples. Each sample was dried at 65°C and finely ground to pass an 80-mesh sieve.

The total carbon content (TC) of these samples was measured by combustion at 925°C[23] using a Perkin Elmer CHNS/O Series II 2400 Analyzer (Perkin Elmer Life and Analytical Sciences, Inc. Boston, MA, USA). Coefficient of variation of the method was 3%. Other methods that are very commonly used for soil carbon determination in soil in Brazilian laboratories are variations of the Walkley-Black procedure.[23] This is because under the

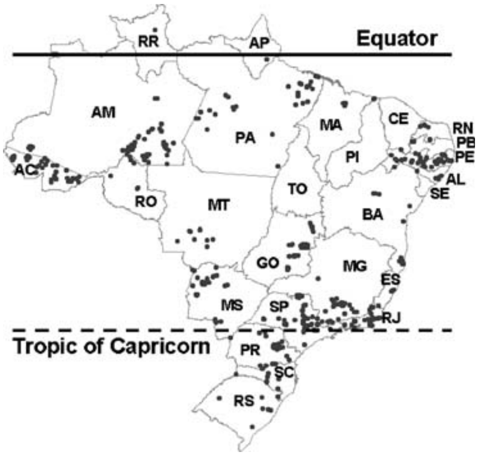

Figure 1. Distribution of the examined soil profiles in Brazil. Letters are abbreviations of names of Brazilian States. Northern Brazil: AC, Acre; AM, Amazonas; RR, Roraima; AP, Amapá; PA, Pará; RO, Rodônia; TO, Tocantins. Northeast Brazil: MA, Maranhão; PI, Piauí; CE, Ceará; RN, Rio Grande do Norte; PB, Paraíba; PE, Pernambuco; AL, Alagoas; SE, Sergipe; BA, Bahia. Central-west Brazil: MT, Mato Grosso; MS, Mato Grosso do Sul; GO, Goiás. Southeast Brazil; MG, Minas Gerais; ES, Espírito Santo; RJ, Rio de Janeiro; SP, São Paulo. Southern Brazil: PR, Paraná; SC, Santa Catarina; RS, Rio Grande do Sul.

acidic soil conditions and high leaching rates that predominate under tropical climate, most of the TC is in organic form (the highest level of inorganic carbon found in our samples was less than $50\,\mathrm{g\,kg^{-1}}$), and in many cases, the major part of the organic carbon can be measured by chromate oxidation. In this paper, we refer to the carbon that can be determined by chromate oxidation as Walkley-Black carbon (WBC). The standard method used at Embrapa Soils, which was used in this work, is also modified Walkley-Black procedure.[22] This involves oxidation of the soil organic matter by potassium dichromate solution ($K_2Cr_2O_7$, 0.4 N mixed with cc. H_2SO_4) combined with 5-min heating and boiling. After that, the solution is cooled down and distilled water is added to it, followed by titration with ammonium sulfate [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 0.1 N] in the presence of phosphoric acid (cc. H_3PO_4, 85%) using diphenylamine as indicator. These methods were used as reference for calibrations of the mid-IR and NIR techniques.

Fourier-Transform Diffuse Reflectance Mid-IR Spectroscopy

Samples were scanned in the mid-IR on a Digilab (Bio-Rad, Randolph, MA) FTS-7000 Fourier-transform spectrometer equipped with a KBr beam splitter and a DGTS (deuterated glycine trisulfate) detector. Samples were scanned from 4000 to 400 cm^{-1} (2500 to 25,000 nm) at 4 cm^{-1} resolution with 64 co-added scans per spectrum. Scanning was performed on ground, but non-KBr diluted,[9] samples using a Pike Autodiff autosampler/reflectance accessory (Pike Technologies, Madison, WI, USA) with KBr used as the blank reference standard.

Near-Infrared Spectroscopy

Samples were scanned in the near-infrared on a Digilab (Bio-Rad, Randolph, MA, USA) FTS-7000 Fourier-transform spectrometer equipped with a quartz beam splitter and an InSb liquid nitrogen cooled detector. Samples were scanned from 9091 to 4000 cm^{-1} (1100 to 2500 nm) at 4 cm^{-1} resolution with 64 co-added scans per spectrum. Scanning was performed using ground samples using the Pike Autodiff autosampler/reflectance accessory with S used as the reference standard.

Statistical Analysis

Summary statistics and correlation analysis were performed using SAS[28] PROC Means and FREQ version 8.

Chemometrics

Calibration development was carried out using SAS PLS with a custom made program.[29,30] All spectra were mean centered and variance scaled. In addition, spectral pretreatments using first and second gap derivatives with gaps of 4 to 64 data points with and without multiplicative scatter correction (MSC) were tested. In all, 22 different spectral pretreatments were tested for all analytes. Initial testing was performed using different spectral ranges and data point averaging to determine the optimal spectral range to use in the final PLS calibration development. Final calibrations were done using the entire spectral range and averaging every 4 data points, for both mid-IR and NIR spectra. Calibrations were developed using the one-out cross-validation procedure with and without an independent test set. Calibrations were also examined using GRAMS PLSPlus V2.1G.[31]

Calibration Data Sets

A total of 1135 samples for TC and 1014 samples for WBC were available for NIRS and DRIFTS. Samples were also divided into calibration sets based on C content, taxonomic soil class, and soil texture. In total, 17 data sets were created for calibration development for each spectral range. The sample numbers and carbon (TC and WBC) values for each data set are presented in Table 1.

The first calibration set (CARBON1) included all 1135 soil samples. The second set (CARBON2) consisted of the samples that contained less than 100 g kg^{-1} TC. The third set (CARBON3) included the samples that contained less than 40 g kg^{-1} TC, which is the TC range that was most common among the examined soil samples (85%), and is most representative of tropical soils. The fourth set (CARBON4) included the soil samples that belong to two specific soil types, Histosols and Spodosols, that have higher TC levels and unique soil organic matter characteristics compared to most

Table 1. Basic descriptive statistics of the sample data sets

No.	Sample data sets	N[a]	Mean[b] (g kg^{-1})	SD[c] (g kg^{-1})	Minimum[b] (g kg^{-1})	Maximum[b] (g kg^{-1})
Sample data sets based on carbon content						
1	CARBON1	1135	30.52	67.04	0.40	555.00
2	CARBON2	1045	16.69	15.46	0.40	99.10
3	CARBON3	970	13.48	9.62	0.40	39.90
4	CARBON4	88	191.92	158.86	0.80	555.00
5	CARBON5	1014	19.16	42.46	0.20	401.90
6	CARBON6	963	10.61	9.76	0.20	66.00
7	CARBON7	911	8.91	6.55	0.20	30.00
Sample data sets based on taxonomic soil class						
8	SOILCLASS1	367	17.65	14.65	2.00	85.10
9	SOILCLASS2	189	14.92	15.00	1.70	91.60
10	SOILCLASS3	355	17.48	14.72	2.00	85.10
11	SOILCLASS4	184	14.36	14.56	1.70	91.60
Sample data sets based on soil textural group						
12	TEXTURE1	153	21.90	32.65	1.70	254.80
13	TEXTURE2	487	20.23	27.42	0.40	308.50
14	TEXTURE3	351	18.50	17.50	0.40	115.50
15	TEXTURE4	136	17.27	29.29	0.90	222.00
16	TEXTURE5	445	14.38	24.40	0.20	287.00
17	TEXTURE6	330	11.22	9.76	0.50	99.70

[a] Number of samples.

[b] Mean, minimum, and maximum values refer to TC or WBC respective to the sample set.

[c] Standard deviation; Calibration sets CARBON1 to 4 are based on soil total carbon (TC) content, CARBON5 to 7 are based on Walkley-Black carbon (WBC) content; SOILCLASS1 and 2, and 3 and 4 are based on soil taxonomy class for TC and WBC calibration, respectively, SOILCLASS1 and 3 are ferralsols and SOILCLASS2 and 4 are acrisols; TEXTURE1 to 3 and 4 to 6 are based on soil textural grouping for TC and WBC calibration, respectively, TEXTURE1 and 4 are very clayey, TEXTURE2 and 5 are clayey, and TEXTURE3 and 6 are medium textural groups.

of the other soil types, and also most of the samples with more than 100 g kg^{-1} TC belonged to these two soil classes. Sets CARBON5, 6, and 7 are the calibration sets for WBC including all 1014 samples, those containing WBC less than 70 g kg^{-1}, and less than 30 g kg^{-1}, respectively. The majority of the soil samples (89%) fell into the lowest WBC range set.

To examine taxonomic soil class effect on calibration for TC and WBC, we separated samples belonging to the two most common soil classes, Latossolos and Argissolos, according to the Brazilian Soil Classification System.[32] According to the World Reference Base,[33] these soils would be classified as Ferralsols and Acrisols, respectively. The data sets SOILCLASS1 and 2 consists of soil samples of Ferralsols and Acrisols, respectively, for TC calibration. The data sets SOILCLASS3 and 4 consist of soil samples of Ferralsols and Acrisols, respectively, for WBC calibration.

The sample sets TEXTURE1 to 3 include soil samples with very clayey, clayey, and medium texture for TC calibration, and sets TEXTURE4 to 6 include soil samples belonging to the same textural groups for WBC calibration. The set of soil classified as very clayey texture contained more than 60% clay fraction (<0.002 mm diameter). The set of soils classified as clayey texture contained 35% to 60% clay fraction. Medium textured soils are those that contained less than 35% clay fraction and more than 15% sand fraction (particles between 2.000 and 0.053 mm in diameter). For the determination of the textural classes used in this work, the criteria of the Brazilian Soil Classification System[32] was used. No sample set for sandy textural group was separated because of insufficient number of soil samples belonging to this textural group in our database.

RESULTS AND DISCUSSION

Sample Composition and Calibration Sets

The carbon content (TC and WBC) for the samples is presented in Table 1. All sample sets consisted of soils with a wide range of C values; minimum range of low to high values varied by about 100-fold for the CARBON3 sample set. It is worthwhile noting that both the minimum and maximum values found for WBC (in sets CARBON5–7, SOILCLASS3–4, and TEXTURE4–6) were lower than the values found for TC. This may be due to the determination of nonorganic and recalcitrant organic forms of C by combustion, which are not determined by chromate oxidation. With the exception of the CARBON4 set, all sample sets contained more than sufficient number of samples (minimum of 136 samples) to permit excellent chemometric evaluation of the data. Because use of 88 samples (CARBON4) is borderline for calibration development, such calibrations may be subject to over-fitting, and these results should be evaluated with caution.

Mid-IR and NIR Spectra

Shown in Figs 2 and 3 are the mid-IR and NIR spectra of the samples in the CARBON1 calibration set with the highest and lowest TC contents. As demonstrated, the mid-IR spectra (Fig. 2) contain considerably more visually apparent information than the corresponding NIR spectra (Fig. 3). It is worth noting the extremely low absorbances seen across the entire spectral range for the NIR spectrum of the low C soil. This is due to the fact that inorganic components of soil such as silica absorb very little in the NIR.[10] In contrast, the mid-IR spectrum of even the low C sample (Fig. 2, bottom line) contains strongly absorbing bands and bands not prominent in the high C sample (Fig. 2, top line), for example, the three bands between 2000 and 1800 cm^{-1} are due to silica.[10]

The high C sample is from an organic soil (Histosol by the soil classification of the World Reference Base [WRB][33]) containing 555.0 $\mathrm{g\,kg}^{-1}$ TC and 207.0 $\mathrm{g\,kg}^{-1}$ WBC. In this soil, as often in organic soils, the mineral phase contributes to less than 50% of the soil matrix, and consequently, the contribution of the silica is less to the mid-IR spectrum. The spectrum of this soil well reflects the high organic matter content, principally in the 2900 to 2940 cm^{-1} region that is characteristic for aliphatic C–H stretching.[34] These aliphatic components are relevant to recalcitrant humified material, and a strong aliphatic signature matches the known chemical composition of the humic substances fraction of this soil, which consists of 51% humin and 36% humic acids, which are the more stable humic fractions.[35] The low carbon sample is from a sandy soil (Haplic Arenosol by WRB) from the BA horizon at 30–45 cm depth and has 0.4 $\mathrm{g\,kg}^{-1}$ total C content. This sample has 581, 95, and 324 $\mathrm{g\,kg}^{-1}$ sand, silt, and clay content, respectively, that results in a very strong contribution of silica to the mid-IR spectrum.

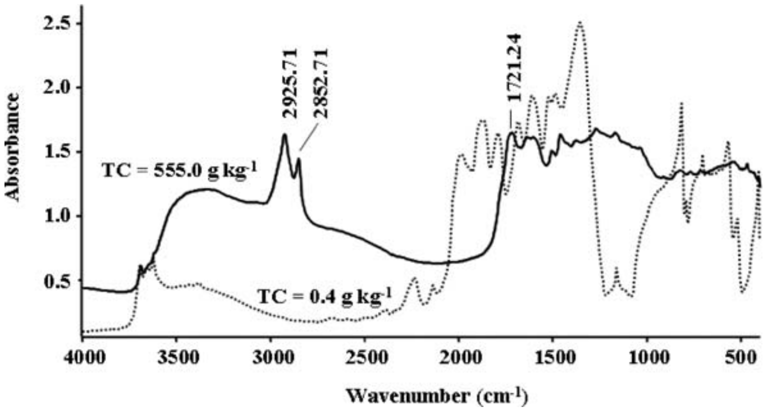

Figure 2. Mid-infrared spectrum of soil samples with lowest (0.4 $\mathrm{g\,kg}^{-1}$) and highest (555.0 $\mathrm{g\,kg}^{-1}$) TC.

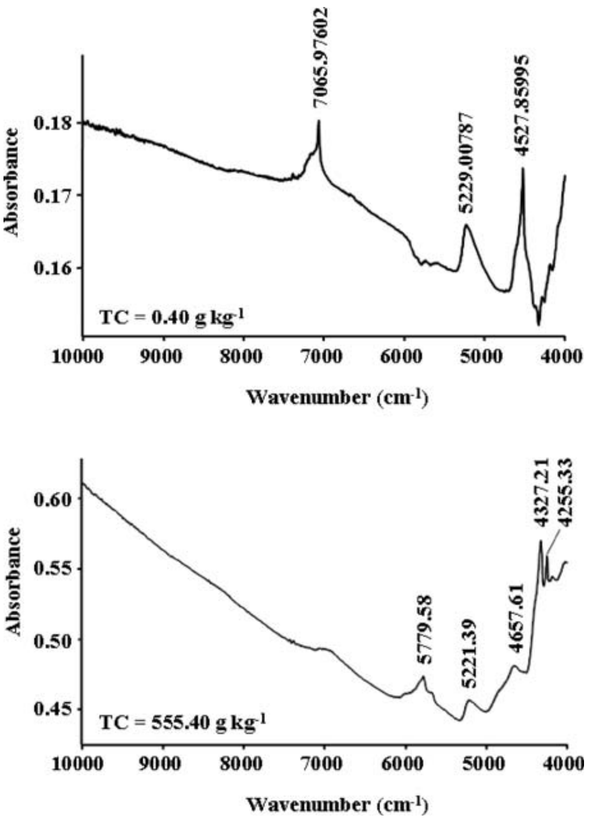

Figure 3. Near-infrared spectrum of soil samples with lowest ($0.4\,g\,kg^{-1}$) and highest ($555.0\,g\,kg^{-1}$) TC.

Calibrations for Carbon as Determined by Combustion (TC)

Complete Sample Set

Table 2 contains calibration results for the various carbon range datasets (CARBON1–7) testing based on NIRS and DRIFTS. As shown, with the exception of the calibration containing only Histosols and Spodosols (CARBON4), calibrations based on mid-IR spectra always outperformed those based on NIR spectra. In both cases, the same analyte values were used and the same sample scanned eliminating all but the basic spectral information content as the basis for the differences. These comparative results are similar to those seen in less diverse sample sets including those containing only a single soil type.[16–19] Examination of the mid-IR and NIR calibrations for TC (CARBON1) indicates that the biggest difference is slightly lower accuracy in the NIR determinations of samples with values less than

Table 2. Mid-infrared (DRIFTS) and near-infrared (NIRS) calibration results

Sample data sets	MSC[a]	Derivative	GAP[b]	No. factors[c]	R^2	RMSD[d]	RD[e]
DRIFTS, total carbon (TC)							
CARBON1	Yes	First	4	13	0.947	15.38	0.504
CARBON2	Yes	First	8	12	0.856	5.87	0.352
CARBON3	Yes	First	16	11	0.811	4.18	0.312
CARBON4	No	Second	4	4	0.948	36.14	0.188
DRIFTS, Walkley-Black carbon (WBC)							
CARBON5	Yes	First	8	13	0.934	10.88	0.568
CARBON6	Yes	First	4	12	0.810	4.24	0.400
CARBON7	No	First	6	11	0.840	2.62	0.294
NIRS, total carbon (TC)							
CARBON1	No	Second	8	15	0.931	17.66	0.574
CARBON2	No	First	16	13	0.739	7.90	0.473
CARBON3	No	First	8	12	0.750	4.80	0.356
CARBON4	No	First	4	4	0.952	34.56	0.180
NIRS, Walkley-Black carbon (WBC)							
CARBON5	No	First	16	13	0.809	18.55	0.968
CARBON6	No	Second	8	10	0.712	5.23	0.478
CARBON7	No	Second	8	9	0.726	3.43	0.385
DRIFTS, taxonomic soil class, total carbon (TC)							
SOILCLASS1	No	First	4	10	0.861	5.44	0.308
SOILCLASS2	Yes	First	8	11	0.914	4.40	0.295
DRIFTS, taxonomic soil class, Walkley-Black carbon (WBC)							
SOILCLASS3	No	First	4	10	0.862	5.45	0.312
SOILCLASS4	Yes	Second	16	10	0.905	4.49	0.313
NIRS, taxonomic soil class, total carbon (TC)							
SOILCLASS1	No	First	16	9	0.725	7.65	0.433
SOILCLASS2	No	Second	4	5	0.854	5.72	0.383
NIRS, taxonomic soil class, Walkley-Black carbon (WBC)							
SOILCLASS3	No	First	16	9	0.725	7.70	0.440
SOILCLASS2	No	Second	4	4	0.784	6.75	0.470
DRIFTS, soil textural group, total carbon (TC)							
TEXTURE1	Yes	First	16	5	0.953	7.05	0.322
TEXTURE2	Yes	First	4	12	0.954	5.89	0.291
TEXTURE3	Yes	First	8	12	0.905	5.39	0.291
DRIFTS, soil textural group, Walkley-Black carbon (WBC)							
TEXTURE4	Yes	Second	32	7	0.967	6.19	0.358
TEXTURE5	Yes	First	4	12	0.962	5.52	0.384
TEXTURE6	Yes	First	8	13	0.917	5.11	0.455

(*continued*)

Table 2. Continued

Sample data sets	MSC[a]	Derivative	GAP[b]	No. factors[c]	R^2	RMSD[d]	RD[e]
NIRS, soil textural group, total carbon (TC)							
TEXTURE1	No	First	8	10	0.961	6.42	0.293
TEXTURE2	No	First	8	12	0.930	7.26	0.359
TEXTURE3	No	Second	32	8	0.866	6.39	0.345
NIRS, soil textural group, Walkley-Black carbon (WBC)							
TEXTURE4	No	Second	4	8	0.975	5.33	0.309
TEXTURE5	No	First	8	12	0.938	7.00	0.487
TEXTURE6	No	Second	32	8	0.871	6.37	0.568

[a] Multiplicative scatter correction.

[b] Number of data points skipped for derivatives.

[c] Number of partial least squares factors used in calibration.

[d] Root mean squared deviation.

[e] Relative difference; Calibration sets CARBON1 to 4 are based on soil total carbon (TC) content ($0.4 \leq C \leq 555.0$, $0.4 \leq C \leq 99.1$, $0.4 \leq C \leq 39.9$, and $0.8 \leq C \leq 555.0\,g\,kg^{-1}$, respectively); CARBON5 to 7 are based on Walkley-Black carbon (WBC) content ($0.2 \leq C \leq 401.9$, $0.2 \leq C \leq 66.0$, and $0.2 \leq C \leq 30.0\,g\,kg^{-1}$, respectively); SOILCLASS1 and 3 and 2 and 4 are based on soil taxonomy class (Ferralsols and Acrisols, respectively) for TC and WBC calibration; TEXTURE1 and 4, 2 and 5, and 3 and 6 are based on soil textural grouping (very clayey, clayey, and medium texture, respectively) for TC and WBC calibration.

$100\,g\,kg^{-1}$ C, although a few of the samples with higher TC values (~300 to $400\,g\,kg^{-1}$) are more poorly determined by the mid-IR calibration (Fig. 4). This may be a consequence of the mid-IR calibration determining the more numerous lower C samples better than the corresponding NIR calibration (Fig. 5).

There is uncertainty concerning the degree to which a single calibration can fit a wide range of soil C values. For many products, such as forages and grains, the best results are obtained when calibrations are developed using only samples for a specific product, for example, single forage or even type of grain[2,4]. Although work using a set of soils representing the Great Plains region of the United States indicated that calibrations could be developed using a diverse set of samples, insufficient number of samples were available to determine whether using more limited ranges of C values would result in better calibrations.[16]

Samples with Less Than $100\,g\,kg^{-1}$ and Less Than $40\,g\,kg^{-1}$ Carbon (TC)

Results based on samples with a total C content less than $100\,g\,kg^{-1}$ are shown in Table 2. This sample group ($TC < 100\,g\,kg^{-1}$) was created by taking out

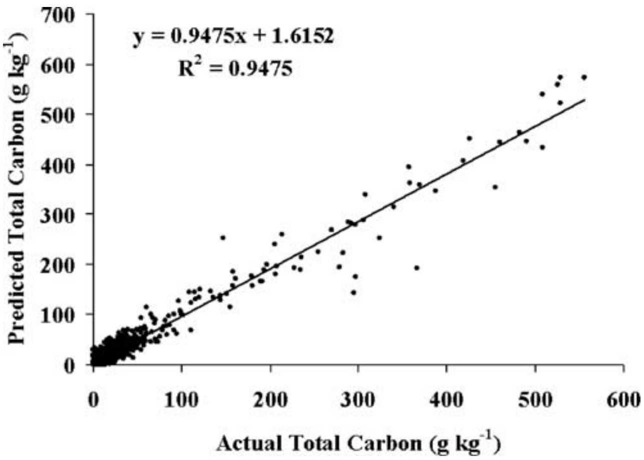

Figure 4. Final calibration results for C by combustion (TC) for all 1135 samples using mid-infrared spectra.

samples that contain more than 100 g kg^{-1} TC plus every sample that belonged to the Spodosol and Histosol soil type, even if the TC content was under 100 g kg^{-1}. This was done because the organic matter of Histosols and Spodosols has unique characteristics compared to most of the other soil types, and also, most of the samples with more than 100 g kg^{-1} TC belonged to these two soil classes. Although the results for samples appear, based on RMSD values, to be somewhat better than when all the samples are present, the error (RMSD or RMSD/Mean value = RD) is higher than what was seen in previous work where RD values half or less than these values were obtained.[16] Similar results were obtained when only samples

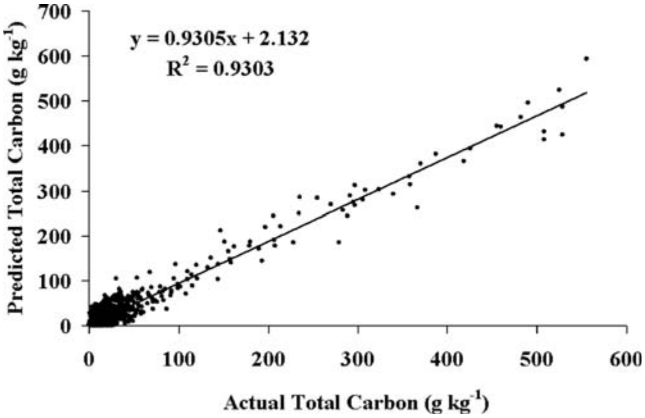

Figure 5. Final calibration results for C by combustion (TC) for all 1135 samples using near-infrared spectra.

with less than 40 g kg^{-1} TC were examined. These results would seem to indicate that factors other than TC content may need to be considered in determining the basis for calibration sets for TC in soils. It also has to be noted that the NIRS calibrations in both cases performed considerably poorer than the DRIFTS calibrations indicating less robustness, as noted in previous work.[16]

Finally, calibrations based on only the Histosols and Spodosols (CARBON4, Table 2) were quite good ($R^2 = 0.95$) from low TC to high TC in both spectral ranges. The RMSD (the dispersion of the data from the medium value) (36.14 and 34.56 for DRIFTS and NIRS, respectively), however, was much higher than in the case of the calibration for the entire C range (15.38 and 17.66 for DRIFTS and NIRS, respectively) or for the lower C ranges (values between 7.90 and 4.18), but the RD (0.188 and 0.180 for DRIFTS and NIRS, respectively) was lower than for any other calibrations (minimum value 0.312 for CAR3 calibration set). This indicates that soil type may be an important factor in selecting samples for calibrations for TC rather than C content alone. Carbon is present in the soil in different forms. These forms (fractions), most of the time, are separated operationally, and the fractions represent different chemical or physical characteristics.[34,36,37,38] Most mineral soils in the tropics have low total carbon levels (between ~0 and 40 g kg^{-1}). Typically, 80% of the organic matter is in forms of humic substances, and within these as humin, and fulvic acids.[37] By contrast, soil types such as Histosols and Spodosols in certain soil horizons have higher organic matter content (>200 g kg^{-1}) and represent different distribution of C between the fractions. Histosols for example may contain high levels of undecomposed organic matter and within the humic fractions have higher proportions of the humic acid fraction compared to mineral soils.[35] Spodosols may also have high levels of carbon (>100 g kg^{-1}), however not as a rule. In Spodosols, lower molecular weight organic acids percolate down through the soil profile chelating and complexing hydrolysation products of clay minerals and accumulate in lower soil horizons in close association with the mineral phase of the soil.[39,40] Higher carbon content and the carbon being in different forms or in different distribution between the carbon forms may influence calibrations and thus justify the separation of these soil groups for calibration purposes.

Calibrations for Carbon as Determined by Chromate Oxidation (WBC)

Results for the total range of C (calibration set CARBON6) as determined by chromate oxidation (WBC) were similar to those for the total range of C (calibration set CARBONI) by combustion (TC) using mid-IR spectra (Table 2). The RMSD (10.88) was lower for WBC, but the RD (0.568) was higher than for TC (RMSD = 15.38, RD = 0.504). Using NIR spectra (CARBON6 set), the calibrations obtained for WBC were much poorer (Table 2) than the corresponding calibration based on combustion (TC). It is still unknown why NIR spectroscopy is outperformed by mid-IR, especially for WBC. However, these results indicate that the great information content present in the mid-IR spectra allows the mid-IR calibration to better differentiate the forms of C determined by chromate oxidation from those not so determined. In the case mid-IR, the lower R^2 obtained for WBC (0.93) compared to TC (0.95) is likely due to the inability of the reference methods to adequately measure the type of carbon they are designed to. The performance of dry combustion to measure TC in soil seems to be better than the performance of the modified Walkley-Black procedure to measure organic carbon (WBC). It is known that some of the samples contain high levels of black carbon ($>300\,g\,kg^{-1}$) that is detected by infrared spectroscopy as organic carbon.[41] However, the Walkley-Black procedure is to a large degree not capable of measuring this highly stable form of carbon.[42–44]

Developing calibrations using samples with less than 70 and $30\,g\,kg^{-1}$ WBC resulted in similar calibrations when compared to those based on TC. The calibrations for these sets (CARBON5–7) were always, however, only slightly lower than for the calibrations for TC, except for the calibration using the CARBON7 set (DRIFTS, WBC $<30\,g\,kg^{-1}$) that gave better calibration than CARBON3 (TC $<40\,g\,kg^{-1}$, and even the CARBON6 (WBC $<70\,g\,kg^{-1}$) sets. This may be due to the higher accuracy of the chromate oxidation method in measuring WBC in low carbon samples that have, consequently and proportionally, also lower concentration of the recalcitrant organic carbon that is not determined by the method.

Calibrations for Taxonomic Soil Classes

In general, calibrations for soil carbon based on separation into taxonomic soil classes did not perform better than calibrations for data sets based on soil carbon content ranges (Table 2). In all case, better R^2 was obtained using the mid-IR spectral region. Comparing between the two examined soil classes (Ferralsols and Acrisols), always better calibration was obtained for the Acrisols class, regardless the method of C determination (TC or WBC), and the spectral region. The best calibration was achieved for TC for the Acrisols class ($R^2 = 0.91$, RMSD = 4.40, RD = 0.295, using DRIFTS). This was lower than the best calibration obtained for the sample sets containing the largest TC range (CARBON1, $R^2 = 0.947$, RMSD = 15.38, RD = 0.504, using DRIFTS).

Calibrations for Soil Textural Groups

Particle size distribution affects infrared spectra, especially in the near-IR region. For this reason, calibrations for TC and WBC were done using sample sets of different soil textural classes: very clayey, clayey, and medium texture. It is shown in Table 2 that when using DRIFTS, an $R^2 > 0.900$ (0.905–0.967) was obtained for all examined textural groups. When using NIRS, R^2 between 0.866 and 0.975 were obtained. This suggests that NIRS is likely more sensitive for particle size distribution than DRIFTS. Near-infrared spectroscopy has an excellent performance for the textural class that has a more homogeneous particle size distribution (very clayey: TEXTURE1 and 4; $R^2 = 0.961$ and 0.975 for TC and WBC, respectively), and its performance declines as the heterogeneity of the particle size distribution of the soils in the textural groups increases (clayey and medium textural groups: TEXTURE2 and 5, and TEXTURE3 and 6; $R^2 = 0.930$ and 0.938, and $R^2 = 0.866$ and 0.871, for TC and WBC, for the clayey and medium texture groups, respectively). Moreover, using NIRS for calibration resulted in better R^2 for the very clayey textural group both for TC and WBC, whereas in the other two textural groups DRIFTS outperformed NIRS. The very clayey textural group is more homogeneous than the other two, because it consisted of, mostly (60%), particles of the same particle-size class (diameter <0.002 mm). The clayey and medium textural groups contain less of this particle-size class and more of particles of different sizes. DRIFTS proved to be less sensitive for the textural effect. This is supported by the finding that for the DRIFTS calibrations, there was less difference between the R^2 of the very clayey (TEXTURE1, 0.953; and TEXTURE4, 0.967) and clayey (TEXTURE2, 0.954; and TEXTURE5, 0.962) textural groups both for TC and WBC. The R^2 declined in case of the most heterogeneous textural group examined, the medium (TEXTURE3, 0.905; and TEXTURE6, 0.917). The textural effect is also the most probable reason for getting better calibration results with DRIFTS compared to NIRS for the calibration sets created based on carbon content and taxonomic soil class, as all of those sets contained soil samples belonging to different textural groups.

CONCLUSIONS

Results demonstrate that NIR, and particularly Mid-IR spectroscopy (NIRS and DRIFTS, respectively), are promising techniques for the development of calibrations for quantitative soil carbon analysis. Examination of calibrations based on 1135 and 1014 samples (carbon determined by combustion [TC] and chromate oxidation [WBC] respectively), from the Brazilian National Soil Collection have demonstrated that calibrations can be developed using either DRIFTS or NIRS for even a very diverse set of soil samples, which will determine C over a wide range of concentrations inherent in such a diverse set. However, to obtain more reliable predictions for soil C content using a very diverse set as calibration set does not appear to be the most useful approach. Developing calibrations for ranges of soil C content decreased the error of the calibrations (RMSD and RD), however resulted in lower accuracy (R^2). Calibrations based on soil textural classes alone also do not seem to be the right approach for soil carbon content prediction. The reason for this might be that calibrations using a set of samples of great variance in textural composition suffers from the sensitivity of infrared spectroscopy on particle size distribution of the samples, especially in the case of NIRS. Near-infrared spectroscopy had excellent performance ($R^2 = 0.961$ to 0.975) when applied for a calibration set that contained samples that had very similar particle size distribution; however, its performance declined for more heterogeneous sample populations, regarding particle size. Diffuse reflectance infrared spectroscopy, by being less influenced by particle size distribution, gave reasonable performance ($R^2 > 0.95$) for sample sets containing soils of a wider range of particle size distribution. For calibration purposes for soil carbon content (both TC and WBC) prediction, therefore, it seems to be desirable to create calibration sets based on soil textural properties, and then to use the most adequate spectral region (NIR for the more homogeneous sets, or mid-IR for the less homogeneous ones) for the calibration.

Finally, it is relevant to note that infrared spectroscopy is not an independent technique for soil carbon analysis. Its accuracy is dependent on the accuracy of the standard method that is used to provide data for calibration development.

ACKNOWLEDGMENT

This project was supported by the National Soil Research Center (Embrapa Soils, Rio de Janeiro, Brazil, Project Nr. 03.02.5.40.03) and the Animal Manure and By-Products and Environmental Quality Laboratories (AMBL and EQL, ARS, USDA at Beltsville, MD, USA). The authors are grateful for the Labex-USA Program of the Brazilian Agricultural Research Corporation (Embrapa Labex-USA) for providing institutional background for collaboration between the above-mentioned institutions. Thanks are extended to Ronaldo Pereira de Oliveira (Embrapa Soils) for his dedication to build up the database of this project, as well as to the Brazilian National Research Council (CNPq/PIBIC) for the grant provided for L.F. Souza. All the hours of work to maintain the analyzers by Barry Francis (AMBL, BARC East, USDA, Beltsville, MD, USA), Felipe S. dos Santos, and Paula Silva Pinto (undergraduate students of the Pontificate Catholic University, Rio de Janeiro, and of the Federal Fluminense University, Niterói, Brazil) are greatly appreciated.

REFERENCES

1. Tkachuk, R. Analysis of whole grains by near-infrared reflectance. In *Near Infrared Technology in the Agricultural and Food Industries*; Williams, P. C.,

Norris, K., Eds.; American Association of Cereal Chemists, Inc.: St. Paul, MN, 1987; pp. 233–240.

2. Williams, P. C.; Norris, K. *Near-Infrared Technology in the Agricultural and Food Industries*; American Association of Cereal Chemists, Inc.: St. Paul, MN, 1987.
3. Williams, P.; Norris, K. *Near-infrared Technology in the Agricultural and Food Industries*, 2nd ed.; American Association of Cereal Chemists, Inc.: St. Paul, MN, 2001.
4. Roberts, C.; Workman, J. J.; Reeves, J. B., III. *NIR in Agriculture*; ASA-CSSA-SSSA: Madison, WI, 2004.
5. Reeves, J. B., III. Near- versus mid-infrared diffuse reflectance spectroscopy for the quantitative determination of the composition of forages and by-products. *J. Near Infrared Spectrosc.* **1994**, *2*, 49–57.
6. Reeves, J. B., III. Improvement in Fourier near- and mid-infrared diffuse reflectance spectroscopic calibrations through the use of a sample transport device. *Appl. Spectrosc.* **1996**, *50*, 965–969.
7. Reeves, J. B., III.; Delwiche, S. L. Determination of protein in ground wheat samples by mid-infrared diffuse reflectance spectroscopy. *Appl. Spectrosc.* **1997**, *51*, 1200–1204.
8. Reeves, J. B., III.; Zapf, C. M. J. Mid-infrared diffuse reflectance spectroscopy for discriminant analysis of food ingredients. *Agric. Food Chem.* **1998**, *46*, 3614–3622.
9. Reeves, J. B., III. Mid-infrared diffuse reflectance spectroscopy: Is sample dilution with KBr neccesary, and if so, when. *American Laboratory* **2003**, *35* (8), 24–28.
10. Reeves, J. B., III.; Francis, B. A.; Hamilton, S. K. Specular reflection and diffuse reflectance spectroscopy of soils. *Appl. Spectrosc.* **2005**, *59*, 39–46.
11. Beebe, K. R.; Pell, R. J.; Seasholtz, M. B. *Chemometrics: A practical guide*; John Wiley & Sons: New York, 1998; pp. 46–47.
12. Naes, T.; Isaksson, T.; Fearn, T.; Davies, T. *A User Friendly Guide to Multivariate Calibration and Classification*; NIR Publications: Chichester, West Sussex, UK, 2002.
13. Janik, L. J.; Merry, R. H.; Skjemstad, J. O. Can mid-infrared diffuse reflectance analysis replace soil extractions? *Aust. J. Exp. Agric.* **1998**, *38*, 681–696.
14. Malley, D. P.; Martin, P. D.; Ben-Dor, E. Analysis of soils. In *NIR in Agriculture*; Roberts, C., Workman, J. J., Reeves, J. B., III, Eds.; ASA-CSSA-SSSA: Madison, WI, 2004; pp. 729–784.
15. McCarty, G. W.; Reeves, J. B., III. Development of rapid instrumental methods for measuring soil organic carbon. In *Assessment Methods for Soil Carbon*; Lal, R., Kimble, J. M., Follett, R. F., Stewart, B. S., Eds.; CRC Press: Boca Raton, FL, 2001; pp. 371–380.
16. McCarty, G. W.; Reeves, J. B., III.; Reeves, V. B.; Follett, R. F.; Kimble, J. M. Mid-infrared and near-infrared diffuse reflectance spectroscopy for soil carbon measurement. *Soil Science Soc. Am. J.* **2002**, *66*, 640–646.
17. Reeves, J. B., III.; McCarty, G. V.; Meisenger, J. J. Near infrared reflectance spectroscopy for the analysis of agricultural soils. *J. Near Infrared Spectrosc.* **1999**, *7*, 179–193.
18. Reeves, J. B., III.; McCarty, G. V.; Reeves, V. B. Mid-infrared diffuse reflectance spectroscopy for the quantitative analysis of agricultural soils. *J. Agric. Food Chem.* **2001**, *49*, 766–772.
19. Reeves, J. B., III.; McCarty, G. V.; Mimmo, T. V.; Reeves, V. B.; Follett, R. F.; Kirnble, J. M.; Galletti, G. C. Spectroscopic calibrations for the determination

of carbon in soils, 17th World Congress of Soil Science, Thialand, 2002, Paper no. 398, 398-1 to 9.

20. Reeves, J. B., III.; McCarty, G. W. Quantitative analysis of agricultural soils using near infrared reflectance spectroscopy and a fiber-optic probe. *J. Near Infrared Spectrosc.* **2001**, *9*, 25–43.
21. Kyoto Protocol. Kyoto Protocol to the United Nations Framework Convention on Climate Change. 1997. Available at http://www.un.org/millennium/law/xxvii-23.htm.
22. Empresa Brasileira de Pesquisa Agropcuária–Centro Nacional de Pesquisa de Solos. In *Manual de métodos de análise de solo*, 2nd ed.; Centro Nacional de Pesquisa de Solos: Rio de Janeiro, Brazil, 1997; pp. 47–49.
23. Nelson, D. W.; Sommers, L. E. Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis, Part 3*; Sparks, D. L., Page, A. L., Helmike, P. A., Loeppert, R. H., Softanpour, P. N., Tabatabai, M. A., Johnston, C. T., Summer, M. E., Eds.; SSSA-ASA: Madison, WI, 1996; pp. 997–983.
24. Watson, R. T.; Noble, I. R.; Bolin, B.; Ravindranath, N. H.; Verardo, D. J.; Dokken, D. J. *Land Use, Land-Use Change, and Forestry, A Special Report of the IPCC*; Cambridge University Press: Cambridge, 2000; pp. 53–126.
25. Lal, R.; Kimble, J. M.; Follett, R. F.; Stewart, B. S. *Methods of Assessment of Soil Carbon*; CRC Press: Boca Raton, FL, 2001.
26. Tabatabai, M. A. Soil organic matter testing: An overview. In *Soil Organic Matter: Analysis and Interpretation*; Tabatabai, M. A., Ed.; SSSA Special Publication No. 46; SSSA: Madison, WI, 1996; pp. 1–9.
27. Tan, K. H.; Hajek, B. F.; Barshad, I. Thermal analysis techniques. In *Methods of Soil Analysis, Part 1*; Klute, A., Ed.; SSSA-ASA: Madison, WI, 1986; pp. 151–183.
28. SAS Institute. *SAS̈ User's Guide, Statistics*, Release 6.03, Ed.; SAS Institute: Cary, NC, 1988.
29. Reeves, J. B., III.; Delwiche, S. R. SAS partial least squares regression for analysis of spectroscopic data. *J. Near Infrared Spectrosc.* **2003**, *11*, 415–431.
30. Reeves, J. B., III.; Delwiche, S. R. Using SAS for PLS calibrations of spectroscopic data. *NIRS News* **2004**, *15* (3), 10–15.
31. PLSplus V2.1G. Galactic Industries Corporation: Salem, NH, 2004.
32. Empresa Brasileira de Pesquisa Agropcuária–Centro Nacional de Pesquisa de Solos. In *Sistema Brasileiro de Classificação de Solos*, 1st ed.; Centro Nacional de Pesquisa de Solos: Rio de Janeiro, Brazil, 1999.
33. FAO; ISRIC; ISSS. In *World Reference Base for Soil Resources*; Wageningen/Rome: FAO, ISRIC, ISSS, 1994.
34. Stevenson, F. J. *Humus chemistry. Genesis, Composition, Reactions*, 2nd ed.; Wiley & Sons Inc.: Toronto, 1994.
35. Valladares, G. S.; Benites, V. M.; Pereira, M. G.; Anjos, L. H.C.; Ebeling, A. G. *Proposta para Classificação de Organossolos em Níveis Inferiores com Base nas Frações Húmicas*; Bol. de Pesquisa e Desenvolvimento, No. 2: Embrapa Solos: Rio de Janeiro, RJ, Brazil, 2003.
36. Swift, R. S. 1996. Organic matter characterization. In *Methods of Soil Analysis, Part 3*; Sparks, D. L., Page, A. L., Helmike, P. A., Loeppert, R. H., Softanpour, P. N., Tabatabai, M. A., Johnston, C. T., Summer, M. E., Eds.; SSSA-ASA: Madison, WI, 1996; pp. 1011–1020.
37. Schnitzer, M.; Khan, S. U. *Soil Organic Matter; Developments in Soil Science 8*; Elsevier Scientific Publishing Company: Amsterdam, 1978.

38. Theng, B. K. G.; Tate, K. R.; Sollins, P. Constituents of organic matter in temperate and tropical soils. In *Dynamics of Soil Organic Matter in Tropical Ecosystems*; Coleman, D. C., Oades, J. M., Uehara, G., Eds.; University of Hawaii Press: Honolulu, 1989; pp. 5–32.
39. Qualls, R. G.; Takiyama, A.; Wershaw, R. L. Formation and loss of humic substances during decomposition in a pine forest floor. *Soil Sci. Soc. Am. J.* **2003**, *67*, 899–909.
40. Ussiri, D. A. N.; Johnson, C. E. Sorption of organic carbon fractions by Spodosol mineral horizons. *Soil Sci. Soc. Am. J.* **2004**, *68*, 253–262.
41. Cunha, T. J. F. *Ácidos húmicos de solos antropogênicos: estudo químico e espectroscópico*; Ph.D. Thesis; Federal Rural University of Rio de Janeiro, Institute of Agronomy: Seropédica, RJ, Brazil, 2005.
42. Golchin, A.; Clarke, P.; Baldock, J. A.; Higashi, T.; Skjemstad, J. O.; Oades, J. M. The effects of vegetation and burning on the chemical composition of soil organic matter in a volcanic ash soil as shown by ^{13}C NMR spectroscopy. I. Whole soil and humic acid fraction. *Geoderma* **1997**, *76*, 155–174.
43. Glaser, B.; Haumaier, L.; Guggenberger, G.; Zech, W. Black carbon in soils: the use of benzenecarboxylic acids as specific markers. *Org. Geochem.* **1988**, *29*, 811–819.
44. Poirier, N.; Derenne, S.; Rouzaud, J-N.; Largeau, C.; Mariotti, A.; Balesdent, J.; Maquet, J. Chemical structure and sources of the macromolecular, resistant, organic fraction isolated from a forest soil (Lacadée, south-west France). *Org. Geochem.* **2000**, *31*, 813–827.