



**HAL**  
open science

## Determination of total carbon and nitrogen contents in a range of tropical soils using NIRS: influence of replication and sample grinding and drying

Bernard G. Barthès, Didier Brunet, Henri Ferrer, Jean-Luc Chotte, Christian Feller

### ► To cite this version:

Bernard G. Barthès, Didier Brunet, Henri Ferrer, Jean-Luc Chotte, Christian Feller. Determination of total carbon and nitrogen contents in a range of tropical soils using NIRS: influence of replication and sample grinding and drying. *Journal of Near Infrared Spectroscopy*, 2006, 14 (5), pp.341-348. 10.1255/jnirs.686 . ird-04151205

**HAL Id: ird-04151205**

**<https://ird.hal.science/ird-04151205>**

Submitted on 4 Jul 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Determination of total carbon and nitrogen contents in a range of tropical soils using NIRS: influence of replication and sample grinding and drying

Bernard G. Barthès,<sup>a</sup> Didier Brunet,<sup>a</sup> Henri Ferrer,<sup>a</sup> Jean-Luc Chotte,<sup>a</sup> and Christian Feller<sup>b</sup>

<sup>a</sup>UR SeqBio (Séquestration du carbone et bio-fonctionnement des sols), IRD (Institut de recherche pour le développement), BP 64501, 34394 Montpellier cedex 5, France

<sup>b</sup>UR SeqBio (Séquestration du carbone et bio-fonctionnement des sols), IRD (Institut de recherche pour le développement), BP 434, 101 Antananarivo, Madagascar

## Abstract

Near infrared reflectance spectroscopy (NIRS) has been receiving increasing attention for the rapid and inexpensive determination of soil properties, total carbon ( $C_t$ ) and nitrogen contents ( $N_t$ ) especially. However, methodological aspects such as sample grinding and drying or replication have not been addressed extensively. The objectives of the paper were thus to assess how NIRS predictions of  $C_t$  and  $N_t$  were affected by sample grinding (2 mm sieving vs. 0.2 mm grinding), drying (air-drying vs. oven-drying at 40°C during 24 h), and replication (use of one to six subsamples to determine average spectra). This was performed on a range of tropical soils that differed widely in mineralogy (low and high activity clay soils, allophanic soils) and texture (sandy to clayey).

The accuracy of NIRS predictions of  $C_t$  and  $N_t$  was higher with oven-dried compared to air-dried samples, and more markedly, with 0.2 mm ground compared to 2 mm sieved samples. Replication had a positive effect on NIRS predictions when 2 mm sieved samples were used, especially for air-dried samples, but this effect was not clear with 0.2 mm ground samples. Thus the most accurate predictions of  $C_t$  and  $N_t$  were obtained with oven-dried finely ground samples, with limited response to sample replication. Accurate predictions were, however, also obtained with four replicates on oven-dried 2 mm sieved samples. Acceptable and less tedious results could thus be achieved when replacing fine grinding by replication. Even with this procedure, the  $r^2$  between predicted (NIRS) and measured (reference) values was 0.9 and the ratio of standard error of prediction to mean (CV%) was 20%, considered satisfactory for the heterogeneous sample set under study.

## Keywords

soil, carbon, nitrogen, near infrared reflectance spectroscopy (NIRS), sample preparation, replication

## Introduction

Studies on carbon and nitrogen cycles often require numerous determinations of soil carbon and nitrogen contents, which are rather time-consuming and expensive when standard analytical procedures are used. Therefore, much attention is given to possible alternatives such as near infrared reflectance spectroscopy (NIRS), which is a physical non-destructive, rapid and low-cost method. The NIRS analysis aims at developing calibration models to predict properties of materials (e.g. carbon content) according to their reflectance in the wavelength range between 800 and 2500 nm. Reflectance depends on vibrations in bonds between H and C, N, O, P, and S atoms. The models are developed from partial least square (PLS) calibration of spectral data carried out on samples that have also been characterized by reference methods. Calibration models are then used to characterize other samples from only their NIRS spectra.

NIRS was first used for the assessment of moisture content of seeds and other plant products,<sup>1</sup> and then for the evaluation of seed and forage quality.<sup>2</sup> Its first application to soils was reported during the sixties.<sup>3</sup> Accurate NIRS predictions of total soil carbon ( $C_t$ ) and nitrogen contents ( $N_t$ ) were reported during the seventies<sup>4</sup>, and to a larger extent, during the nineties.<sup>5-7</sup> Some methodological aspects have, however, not been addressed extensively. Some studies regarding NIRS determination of  $C_t$  and  $N_t$  reported that spectroscopic measurements of soil samples were performed in duplicate<sup>8-9</sup> or in triplicate<sup>10</sup> using different subsamples. However, the usefulness of replication has rarely been discussed. Moreover, several papers have discussed the usefulness of grinding samples for NIRS determination of  $C_t$ <sup>8,11-12</sup> whereas others have reported accurate predictions using un-ground samples.<sup>13-15</sup> Some authors even suggested that grinding the samples did not improve the prediction accuracy.<sup>16</sup> Additionally, the conventional determination of  $C_t$  and  $N_t$  by dry combustion (Elemental Analyzer) is generally carried out on an oven-dry basis (40°C). The question arises whether NIRS analyses should also be carried out on oven-dried samples. As far as soil samples are concerned, this point has not been addressed in the literature.

The objective of this paper was to assess the effects of sample preparation (2 mm sieving vs. 0.2 mm grinding, and air- vs. oven-drying) and replication (use of one to six subsamples to calculate average spectra) on the accuracy of NIRS determination of  $C_t$  and  $N_t$ . This was performed on a wide range of tropical soils, including low activity clay soils (e.g. Alfisols, Oxisols, Ultisols), high activity clay soils (Vertisols), and allophanic soils (Andosols).

## Materials and methods

### *Soil samples*

The set studied included 123 soil samples originating from tropical Africa and America, which represented a wide range of soil types, textures, and *Ct* and *Nt* (Table 1). The distribution of *Ct* was rather even over the sample set: 23, 28, 23, 21, 19 and 9 samples had *Ct* between 0 and 10, 10 and 20, 20 and 30, 30 and 40, 40 and 50, and greater than 50 g kg<sup>-1</sup>, respectively. The distribution of *Nt* was slightly less even: 22, 32, 19, 18, 22 and 10 samples had *Nt* between 0 and 0.8, 0.8 and 1.6, 1.6 and 2.4, 2.4 and 3.2, 3.2 and 4.0, and greater than 4.0 g kg<sup>-1</sup>, respectively. The composition of sets that have even distributions of studied properties is a current practice in NIRS applications.<sup>17</sup> In the present study, it was obtained by selecting 32 additional samples to complete the initial set of 91 samples. Considering the complete set, *Ct* ranged from 1.7 to 67.3 g kg<sup>-1</sup>, and *Nt* from 0.18 to 6.32 g kg<sup>-1</sup>. The samples were air-dried then gently crushed using a mortar and pestle, and sieved to pass a 2 mm mesh. Aliquots were finely ground to pass a 0.2 mm mesh, also using a mortar and pestle.

### *Reference analyses for carbon and nitrogen*

The reference determinations of *Ct* and *Nt* were carried out on finely ground (< 0.2 mm) and oven-dried (40°C) samples by dry combustion using an Elemental Analyzer (CHN Fisons / Carlo Erba NA 2000, Milan, Italy). Replication was not achieved for these conventional determinations. In the absence of carbonates, all carbon was assumed to be organic.

### *NIRS analyses*

Reflectance was measured between 1100 and 2500 nm at 2 nm interval with a Foss NIRSystems 5000 spectrophotometer (Silver Spring, MD, USA), using a static ring cup (5 cm diam.) containing ~ 5 g of soil. Each spectrum, automatically averaged from 15 scans, was recorded as the logarithm of the inverse of the reflectance (log [1/R], i.e. absorbance). Principal component analysis (PCA) and a modified partial least-square (MPLS) analysis were conducted using the WinISI III-v.1.50e software (Infrasoft International, LLC, State College, PA, USA). Absorbance bands being much wider than 2 nm in general and recorded spectra thus including redundant information,<sup>18</sup> spectral data sets were reduced to condense information. Following Sørensen and Dalsgaard,<sup>9</sup> this was achieved by keeping the first out of four adjacent spectral points, yielding 173 data points per spectra. Based on previous unpublished results, spectra were mean centered and variance scaled (standard normal variate

with 4 point gap and 4 point smoothing), then a first derivative was applied using the finite-difference method.<sup>19</sup>

A PCA was carried out on spectral data for the calculation of the Mahalanobis distance  $H$ .<sup>20</sup> Samples with  $H > 3$  were considered outliers and were eliminated from further investigations<sup>21</sup> ( $H > 3$ , probably due to particular sample properties, was observed for one Ultisol sample from Martinique with all four sample preparations [see below], and for two air-dried sieved Oxisol samples from Brazil). After eliminating spectral outliers, MPLS regressions were used to derive calibration models from spectra and reference values of  $Ct$  and  $Nt$ .<sup>22</sup> Each set was divided into a calibration subset, which included 90 samples, and a remaining validation subset, which included 32 or 30 samples. The calibration subset was selected by the software to include the most representative samples of the set.<sup>21</sup> Based on  $H$  distance between all pairs of spectra, an algorithm identified the spectrum that had the most neighbouring spectra closer than a minimal distance, retained that spectrum and discarded its neighbours. The process was continued until no samples remained with neighbours closer than the minimal distance, which was calculated by the software so that the calibration subset included 90 samples. Random selection of the calibration subset did not seem appropriate as different selections sometimes led to very different results, as reported by Brown *et al.*<sup>23</sup> Cross validation was performed on the calibration subset to determine the optimal number of terms to be included in the prediction model. For this purpose, the calibration subset was divided into six groups, five being used for developing the model and one for testing the calibration. The procedure was performed six times to use all samples for both model development and prediction. The residuals of the six predictions were pooled to calculate the standard error of cross validation between predicted and measured values ( $SECV$ ). Samples with residual greater than 2.5 times  $SECV$  (calibration outliers) were removed and another cross calibration was performed, the procedure being carried out twice. Then all remaining calibration samples were used to calculate the final model. The number of factors giving the lowest final  $SECV$  determined the optimal number of terms to be used for the calibration. The performance of the calibration model was assessed using the coefficient of determination ( $R^2$ ) and the ratio of standard deviation to  $SECV$  (denoted  $RPD$ ). The prediction accuracy of the model was evaluated on the validation subset, using validation  $r^2$  and standard error of prediction ( $SEP$ ) between predicted and measured values. According to Morra *et al.*,<sup>5</sup> the performance of the MPLS calibration model was also evaluated using the coefficient of variation ( $CV$ , in %), calculated as the ratio of  $SEP$  to the mean reference value. Additionally,

a paired *t*-test was carried out on the validation subset to assess whether measured and predicted values differed significantly.

#### *Treatments: sample preparation and replication*

NIRS analyses were carried out on 2 mm sieved and 0.2 mm ground air-dried samples, then on the same samples after oven-drying at 40°C during 24 h. Four sample preparations were thus considered: air-dried and sieved, air-dried and ground, oven-dried and sieved, and oven-dried and ground. For clayey samples and Andosols, oven-drying resulted in a decrease in weight that could reach 10% (data not shown). Moreover, spectroscopic measurements were carried out on six subsamples per sample, using independent refilling of the ring cup, and six final spectra were considered for each sample preparation: the spectrum obtained with the first subsample only, the average spectrum resulting from the two first subsamples, that resulting from the three first subsamples, etc., and the average spectrum resulting from all six subsamples. Thus 24 spectrum sets were studied, involving four sample preparations with one to six replicates. Three-way ANOVAs were carried out to assess whether *SEP* and validation  $r^2$  were affected by grinding, oven-drying, and replication level (three replication levels were considered: low, medium and high, corresponding to one or two, three or four, and five or six replicates, respectively).

## **Results**

### *Overall NIRS results*

Considering all treatments, *RPD* ranged from 2.88 to 3.93 for *Ct* and from 2.75 to 3.62 for *Nt* (Table 2). *SEP* ranged from 4.07 to 6.73 g kg<sup>-1</sup> for *Ct*, representing 16% to 26% of mean *Ct*, and from 0.30 to 0.52 g kg<sup>-1</sup> for *Nt*, representing 14% to 25% of mean *Nt*. Validation  $r^2$  ranged from 0.811 to 0.938 for *Ct* and from 0.866 to 0.944 for *Nt*. Whatever the sample preparation and the number of replicates, paired *t*-tests carried out on validation subsets indicated that predicted and measured values did not differ significantly at  $p < 0.05$ .

### *Influence of sample preparation*

On the whole, whatever the number of subsamples used to determine sample spectra, calibration accuracy for both *Ct* and *Nt* tended to increase (i.e. *SECV* tended to decrease and calibration  $R^2$  to increase) as follows: air-dried sieved < oven-dried sieved < oven-dried ground < air-dried ground (Table 2, Figures 1 and 2). However, sample preparations were not always ranked in this order when sample spectra were determined using one subsample only.

Validation accuracy for both  $Ct$  and  $Nt$  tended to increase (i.e.  $SEP$  tended to decrease and validation  $r^2$  to increase) as follows: air-dried sieved < oven-dried sieved < air-dried ground < oven-dried ground. Thus the accuracy of NIRS predictions of  $Ct$  and  $Nt$  was generally higher with ground than with sieved samples, and with oven- than with air-dried samples. Factorial ANOVAs indicated that the effects of grinding and oven-drying on  $SEP$  and validation  $r^2$  were significant for  $Ct$  and  $Nt$  ( $p < 0.05$ ), but that the interaction of grinding and oven-drying was not significant.

### *Influence of replication*

Regarding  $Ct$  or  $Nt$ , calibration accuracy was not clearly affected by the number of subsamples used to determine sample spectra (Table 2, Figures 1 and 2).

Replication had a positive effect of validation accuracy when 2 mm sieved samples were used:  $SEP$  tended to decrease and validation  $r^2$  to increase when the number of replicates increased, particularly for  $Ct$  prediction using air-dried sieved samples. By contrast, the effect of replication on validation accuracy was not clear when 0.2 mm ground samples were used (except for  $Ct$  prediction with oven-dried ground samples). Factorial ANOVAs indicated that  $SEP$  and validation  $r^2$  for  $Ct$  and  $Nt$  were not significantly affected by replication level, however they were significantly affected by the interactions grinding  $\times$  replication level, oven-drying  $\times$  replication level, and grinding  $\times$  oven-drying  $\times$  replication level ( $p < 0.05$ ). This confirmed that replication affected prediction accuracy with some sample preparations only (e.g. sieving).

## **Discussion**

### *Overall NIRS results*

For the soil samples under study,  $RPD$  was generally higher than 3 and always higher than 2.7, indicating that the ability of the models to predict  $Ct$  and  $Nt$  was satisfactory.<sup>24</sup> For sieved and ground samples, the ranges of validation  $r^2$  were 0.81-0.91 and 0.86-0.94, and the ranges of validation CV, 19-26% and 14-23%, respectively. These predictions of  $Ct$  and  $Nt$  were more accurate than most predictions carried out on sample sets that included wide ranges of soil types and texture: for heterogeneous sample sets, ranges of validation  $r^2$  and CV reported in the literature were 0.70-0.87 and 25-35%,<sup>9,13,14,25</sup> respectively. The relatively even distribution of samples across  $Ct$  and  $Nt$  classes might have contributed to this rather good prediction accuracy. The use of sets having even distributions of studied properties was

reported by Bertrand<sup>17</sup> as a current practice for NIRS applications in general, and was mentioned for soil studies by Sørensen and Dalsgaard.<sup>9</sup>

#### *Influence of sample preparation*

The accuracy of NIRS predictions of  $Ct$  and  $Nt$  was generally higher with oven- than with air-dried samples, and with 0.2 mm ground than with 2 mm sieved samples. As a consequence, the most accurate predictions were achieved using 0.2 mm ground oven-dried samples, and the less accurate with 2 mm sieved air-ground samples. Moreover, the accuracy of  $Ct$  and  $Nt$  predictions was more affected by grinding than by drying. Regarding  $Ct$  prediction, average decreases in  $SEP$  upon grinding and oven-drying were 1.17 and 0.89 g kg<sup>-1</sup>, and average increases in validation  $r^2$  were 0.051 and 0.039, respectively. Regarding  $Nt$ , average decreases in  $SEP$  upon grinding and oven-drying were 0.08 and 0.07 g kg<sup>-1</sup>, and average increases in validation  $r^2$ , 0.042 and 0.017, respectively.

The positive effect of fine grinding on the accuracy of NIRS predictions of  $Ct$  and  $Nt$  has been reported in the literature, especially for sets including clayey soils and for heterogeneous sets including several soil types.<sup>8,11,12</sup> Nevertheless, lower prediction accuracy with 0.5 mm ground than with 4 mm sieved samples was reported for coarse-textured soils.<sup>16</sup> These contradictory results might be explained by the size and arrangement of soil particles, which affect light transmission<sup>13</sup> and differ between clayey and coarse-textured soils. In clayey soils, aggregation is well developed and results in a wide range of particle sizes that affects reflectance adversely,<sup>11</sup> thus aggregate destruction upon grinding, which reduces the heterogeneity of particle-size distribution, might explain increasing prediction accuracy. By contrast, coarse-textured soils include few aggregates and their particle-size distribution is dominated by coarse quartz particles often coated with fine particles (except in very sandy soils). In that case, grinding requires much energy to break quartz particles up, which might alter sample composition, moreover it probably causes the peeling of coatings, which might result in a more heterogeneous particle-size distribution. The sample set under study was dominated by clayey soils (79 out of the 123 samples), thus higher prediction accuracy with ground than with sieved samples was consistent with the above-mentioned explanation and with literature data.

Comparisons between NIRS predictions using air-dried and oven-dried soil samples have not been reported in the literature yet. Nevertheless some studies compared NIRS predictions obtained with dried vs. field-moist or thawed samples. Regarding  $Ct$ , higher prediction accuracy has been reported with ground dried than with un-ground field-moist samples,<sup>26</sup> and



with thawed than with air-dried un-ground sandy samples,<sup>16</sup> however some studies reported similar accuracy with dried and field-moist un-ground samples.<sup>12,27</sup> Regarding *Nt*, higher prediction accuracy was generally observed with field-moist or thawed samples than with air-dried ones,<sup>16,26,27</sup> but no explanation was proposed. Chang *et al.*<sup>27</sup> mentioned that soil water might adversely affect the ability of NIRS to predict soil properties, because the strong H<sub>2</sub>O absorption bands often masked peaks associated with organic functional. Though oven-drying (40°C) has not been addressed in previous studies, its positive effect on prediction accuracy might thus be attributed to a reduction of this adverse influence of moisture on reflectance.

### *Influence of replication*

Replication (i.e. using several subsamples to determine sample spectra) had little influence on calibration results. Similarly, its effect on validation results was not clear in general when using 0.2 mm ground samples (except for *Ct* with oven-dried samples). By contrast, prediction accuracy tended to increase with the number of replicates for 2 mm sieved samples, air-dried ones especially, which yielded poor predictions. From a general viewpoint, replication helps characterizing heterogeneous samples, but is less useful for homogeneous samples. In clayey soils, grinding decreases the heterogeneity of sample-size distribution, due to the destruction of aggregates. As clayey soils dominated the studied set, samples were less heterogeneous in general in the ground than in the sieved set, which probably contributed to the smaller effect of replication on prediction accuracy for ground than for sieved samples.

As far as soils are concerned, determination of average spectra from several subsamples has been reported for 2 mm sieved samples,<sup>8-10,23</sup> and to a lesser extent, for finely ground samples.<sup>8</sup> However, though replication has been mentioned in the Materials and methods section of several papers, its interest has rarely been discussed. The question was addressed by Sørensen and Dalsgaard<sup>9</sup>, who noticed that the accuracy of NIRS predictions could not be improved significantly by performing double measurements instead of single ones. For 2 mm sieved soil samples, they found that the variability of independent single predictions of clay content was less than 0.8%. Similarly, in a study regarding the NIRS prediction of the composition of a ground pharmaceutical preparation, Blanco *et al.*<sup>28</sup> found that the variability of 12 independent results was lower than 0.9%. NIRS studies on plant materials (forages, litters, etc.), which have developed earlier than on soils, often involve the determination of average spectra using several subsamples, probably because plant samples are rather hard to grind and are not finely ground in general (e.g. 1 mm preparation<sup>29-31</sup>). Nevertheless, single NIRS measurement was reported with rather finely ground plant samples (0.5 mm

preparation<sup>32</sup>). It might thus be suggested that replication has sometimes been adopted in soil studies because it was widely used with plant materials, though it seems less necessary for soils than for plant materials. Indeed, fine grinding is easier for soil samples than for plant materials and yielded more accurate predictions than coarse grinding.

In the present study, the highest prediction accuracy was generally obtained with six replicates on oven-dried ground samples (validation  $r^2$  and  $CV$  for  $C_t$  and  $N_t$  were 0.94 and 16-17%, respectively). However, using one single replicate on oven-dried ground samples did not decrease prediction accuracy markedly (0.91-0.94 and 14-17%, respectively). Moreover, replacing one single replicate on oven-dried ground samples by four replicates on oven-dried sieved samples also caused a limited decline in prediction accuracy (0.90-0.91 and 19-20%, respectively). Paired  $t$ -tests confirmed that the three procedures yielded  $C_t$  and  $N_t$  predictions that did not differ significantly at  $p < 0.05$ . As it is less tedious than fine grinding, replication on oven-dried sieved samples could thus be considered an acceptable procedure (Figure 3).

## **Conclusion**

The present study was carried out on a range of tropical soils that widely differed in mineralogy (low and high activity clay soils, allophanic soils), texture (sandy to clayey), and carbon content (1.7 to 67.3 g kg<sup>-1</sup>). It confirmed the interest of NIRS for the determination of soil carbon and nitrogen contents. Prediction accuracy was higher with oven-dried (40°C during 24 h) than with air-dried samples, and to a larger extent, with 0.2 mm ground than with 2 mm sieved samples. Moreover, replication (i.e. the use of several subsamples to calculate average NIRS spectra) increased prediction accuracy when 2 mm sieved samples were used, air-dried ones especially, but the effect of replication was less clear with ground samples.

The most accurate predictions of total soil carbon and nitrogen contents were generally obtained with oven-dried 0.2 mm ground samples and six replicates, but the decrease in prediction accuracy was limited when one single replicate only was used. Moreover, the decrease in prediction accuracy was also limited when the procedure involving one single replicate on oven-dried 0.2 mm ground samples was replaced by four replicates on oven-dried 2 mm sieved samples. As far as samples were oven-dried, acceptable results could thus be achieved when replacing tedious fine grinding by replication.

## Acknowledgments

We thank Anastase Azontonde, Edgar De Luca, Edmond Hien, Aurélie Metay, Tantely Razafimbelo, and Corinne Venkatapen for sample collection. We also thank anonymous referees for their helpful recommendations.

## References

1. J.R. Hart and K.H. Norris, *Cereal Chem.* **39**, 94 (1962).
2. P.C. Williams, *Cereal Chem.* **52**, 561 (1975).
3. S.A. Bowers and R.J. Hanks, *Soil Sci.* **100**, 130 (1965).
4. A.H. Al-Abbas, P.H. Swain and M.F. Baumgardner, *Soil Sci.* **114**, 477 (1972).
5. M.J. Morra, M.H. Hall and L.L. Freeborn, *Soil Sci. Soc. Am. J.* **55**, 288 (1991).
6. T.L. Henderson, M.F. Baumgardner, D.P. Franzmeier, D.E. Stott and D.C. Coster, *Soil Sci. Soc. Am. J.* **56**, 865 (1992).
7. K.A. Sudduth and J.W. Hummel, *Trans. ASAE* **36**, 1571 (1993).
8. C.A. Russell, *Commun. Soil Sci. Plant Anal.* **34**, 1557 (2003).
9. L.K. Sørensen and S. Dalsgaard, *Soil Sci. Soc. Am. J.* **69**, 159 (2005).
10. A. Salgó, J. Nagy, J. Tarnóy, P. Marth, O. Pálmai and G. Szabó-Kele, *J. Near Infrared Spectrosc.* **6**, 199 (1998).
11. R.C. Dalal and R.J. Henry, *Soil Sci. Soc. Am. J.* **50**, 120 (1986).
12. J.B. Reeves, III, G.W. McCarty and T. Mimmo, *Environ. Pollut.* **116**, S277 (2002).
13. C.W. Chang, D.A. Laird, M.J. Mausbach and C.R.J. Hurburgh, *Soil Sci. Soc. Am. J.* **65**, 480 (2001).
14. K.D. Shepherd and M.G. Walsh, *Soil Sci. Soc. Am. J.* **66**, 988 (2002).
15. A. Morón and D. Cozzolino, *J. Agr. Sci.* **142**, 335 (2004).
16. G. Fyströ, *Plant Soil* **246**, 139 (2002).
17. D. Bertrand, in *La Spectroscopie Infrarouge et ses Applications Analytiques*, Ed by D. Bertrand and E. Dufour. Dunod, Paris, p. 381 (2000).
18. D. Bertrand, in *La Spectroscopie Infrarouge et ses Applications Analytiques*, Ed by D. Bertrand and E. Dufour. Dunod, Paris, p. 351 (2000).
19. L. Collatz, *The Numerical Treatment of Differential Equations*. Springer-Verlag, Berlin (1966).
20. H.L. Mark and D. Tunnell, *Anal. Chem.* **58**, 379 (1985).
21. J.S. Shenk and M.O. Westerhaus, *Crop Sci.* **31**, 469 (1991).
22. J.S. Shenk and M.O. Westerhaus, *Crop Sci.* **31**, 1548 (1991).

23. D.J. Brown, R.S. Brickleyer and P.R. Miller, *Geoderma* **129**, 251 (2005).
24. P.C. Williams, in *Near Infrared Technology in the Agricultural and Food Industries*, Ed by P.C. Williams and K. Norris. American Association of Cereal Chemists Inc., St. Paul, MN, p. 143 (1987).
25. G.W. McCarty, J.B. Reeves, III, V.B. Reeves, R.F. Follett and J.M. Kimble, *Soil Sci. Soc. Am. J.* **66**, 640 (2002).
26. D.F. Malley, L. Yesmin and R.G. Eilers, *Soil Sci. Soc. Am. J.* **66**, 1677 (2002).
27. C.W. Chang, D.A. Laird and C.R.J. Hurburgh, *Soil Sci.* **170**, 244 (2005).
28. M. Blanco, J. Coello, H. Iturriaga, S. MasPOCH and N. Pou, *Fresen. J. Anal. Chem.* **368**, 534 (2000).
29. R. Joffre, D. Gillon, P. Dardenne, R. Agneessens and R. Biston, *Ann. Sci. For.* **49**, 481 (1992).
30. M.M. Coûteaux, K.B. McTiernan, B. Berg, D. Szuberla, P. Dardenne and P. Bottner, *Soil Biol. Biochem.* **30**, 583 (1998).
31. L. Thuriès, D. Bastianelli, F. Davrieux, L. Bonnal, R. Oliver, M. Pansu and C. Feller, *J. Near Infrared Spectrosc.* **13**, 187 (2005).
32. V. Bouchard, D. Gillon, R. Joffre and J.C. Lefeuvre, *J. Exp. Mar. Biol. Ecol.* **290**, 149 (2003).
33. Soil Survey Staff, *Soil Taxonomy. A Basic System of Soil Classification for Making and Interpreting Soil Surveys, 2nd Edn. Agriculture Handbook, 436*. United States Department of Agriculture, Natural Resources Conservation Service, Washington, DC (1999).

**Table 1.** Presentation of the soil samples.

Location	Texture and soil type <sup>33</sup>	Number of samples	Sample depth (cm)	$Ct^a$ (g kg <sup>-1</sup> )	$Nt^a$ (g kg <sup>-1</sup> )
Benin	Sandy loam Ultisol	11	0-5 to 25-30	5.7 ± 4.0	0.48 ± 0.36
Brazil	Clayey Oxisol	13	0-5 and 5-10	19.2 ± 6.4	1.34 ± 0.49
Burkina Faso	Sandy Alfisol	11	0-10 to 20-40	6.1 ± 4.0	0.48 ± 0.26
Congo <sup>b</sup>	Clayey Oxisol	6	0-10 and 10-20	21.8 ± 7.2	1.30 ± 0.34
Madagascar	Clayey Inceptisol	15	0-5 and 5-10	45.2 ± 4.8	3.42 ± 0.38
Martinique <sup>c</sup>	Clayey Inceptisol	15	0-10	25.3 ± 13.8	2.24 ± 1.07
Martinique <sup>c</sup>	Clayey Ultisol	10	0-10 to 20-30	24.1 ± 6.6	2.00 ± 0.49
Martinique <sup>c</sup>	Clayey Vertisol	20	0-10 to 20-30	28.6 ± 15.5	2.50 ± 1.37
Martinique <sup>c</sup>	Andosol	21	0-10 to 60-70	36.8 ± 13.6	3.26 ± 1.32
Senegal	Sandy Alfisol	1	0-10	5.5	0.39
Total set	-	123	0-5 to 60-70	25.7 ± 15.9	2.11 ± 1.36

<sup>a</sup> mean ± standard deviation.

<sup>b</sup> Congo-Brazzaville.

<sup>c</sup> French West Indies.

**Table 2.** Calibration and validation results for  $Ct$  and  $Nt$  using 2 mm sieved or 0.2 mm ground air-dried or oven-dried soil samples with one to six replicates (i.e. spectra averaged over one to six subsamples).

Number of repli- cates	$Ct$				$Nt$										
	Calibration set		Validation set		Calibration set		Validation set								
	$n_1$	$SECV$ g kg <sup>-1</sup>	$R^2$	$RPD$	$n_2$	$SEP$ g kg <sup>-1</sup>	$r^2$		$n_1$	$SECV$ g kg <sup>-1</sup>	$R^2$	$RPD$	$n_2$	$SEP$ g kg <sup>-1</sup>	$r^2$
2 mm sieved air-dried samples															
1	83	5.03	0.907	3.29	30	6.73	0.811		82	0.42	0.902	3.21	30	0.49	0.867
2	85	5.66	0.880	2.88	30	6.63	0.829		85	0.46	0.875	2.84	30	0.52	0.870
3	84	5.53	0.883	2.93	30	6.55	0.836		84	0.44	0.881	2.90	30	0.51	0.880
4	82	4.97	0.906	3.28	30	6.47	0.838		82	0.42	0.898	3.15	30	0.51	0.866
5	85	5.64	0.881	2.90	30	5.85	0.859		82	0.47	0.867	2.75	30	0.43	0.907
6	85	5.65	0.880	2.89	30	5.87	0.858		83	0.45	0.878	2.87	30	0.43	0.908
0.2 mm ground air-dried samples															
1	83	4.91	0.907	3.28	32	5.06	0.905		81	0.35	0.922	3.59	32	0.41	0.918
2	83	4.80	0.912	3.36	32	5.02	0.907		81	0.35	0.924	3.62	32	0.41	0.920
3	81	4.39	0.926	3.67	32	5.41	0.892		82	0.37	0.910	3.36	32	0.40	0.923
4	81	4.27	0.930	3.77	32	5.44	0.892		83	0.36	0.916	3.47	32	0.40	0.927
5	81	4.36	0.930	3.74	32	5.78	0.864		82	0.35	0.922	3.56	32	0.37	0.927
6	84	4.91	0.911	3.35	32	5.34	0.885		81	0.35	0.923	3.60	32	0.42	0.910
2 mm sieved oven-dried samples															
1	83	5.36	0.894	3.07	32	6.18	0.848		82	0.40	0.922	3.58	32	0.45	0.874
2	80	4.22	0.936	3.93	32	5.62	0.871		83	0.40	0.923	3.59	32	0.42	0.883
3	84	5.46	0.889	3.00	32	5.48	0.884		83	0.45	0.897	3.11	32	0.40	0.910
4	83	5.23	0.902	3.17	32	5.05	0.898		81	0.40	0.911	3.33	32	0.39	0.909
5	81	4.93	0.909	3.32	32	5.91	0.867		83	0.43	0.907	3.28	32	0.42	0.898
6	81	4.74	0.917	3.46	32	5.50	0.884		82	0.43	0.910	3.32	32	0.42	0.898
0.2 mm ground oven-dried samples															
1	82	4.88	0.914	3.37	32	4.43	0.913		84	0.44	0.898	3.13	32	0.30	0.941
2	82	4.65	0.920	3.53	32	4.62	0.907		85	0.42	0.902	3.20	32	0.32	0.936
3	83	4.75	0.918	3.47	32	4.20	0.926		83	0.44	0.894	3.05	32	0.31	0.943
4	82	4.62	0.918	3.49	32	4.21	0.934		81	0.38	0.914	3.39	32	0.35	0.943
5	82	4.64	0.918	3.48	32	4.18	0.935		81	0.38	0.914	3.39	32	0.35	0.943
6	83	4.67	0.917	3.46	32	4.07	0.938		81	0.37	0.914	3.40	32	0.35	0.944

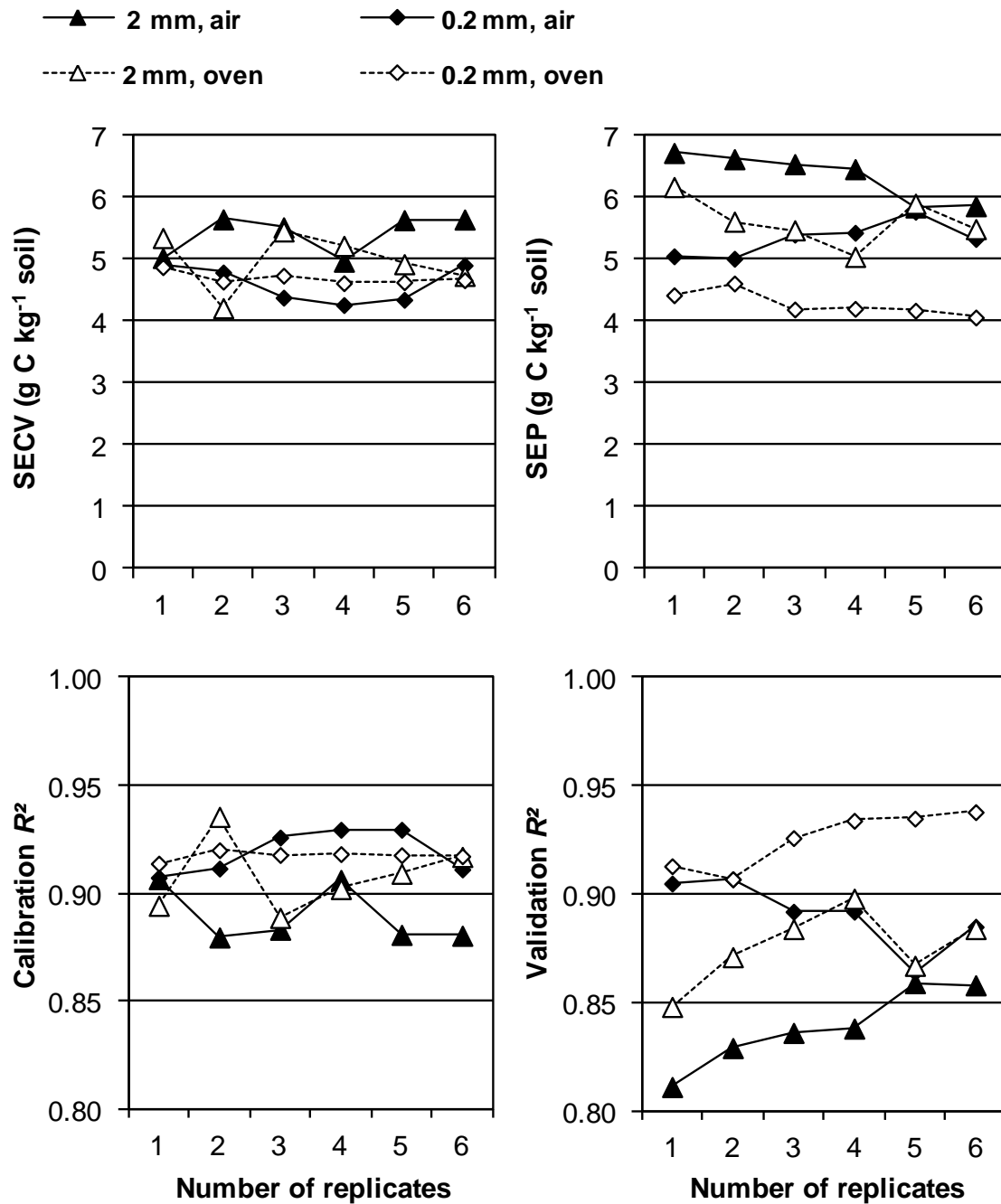
$n_1$  is the number of samples after the elimination of calibration outliers.

$n_2$  is the number of samples in the validation set.

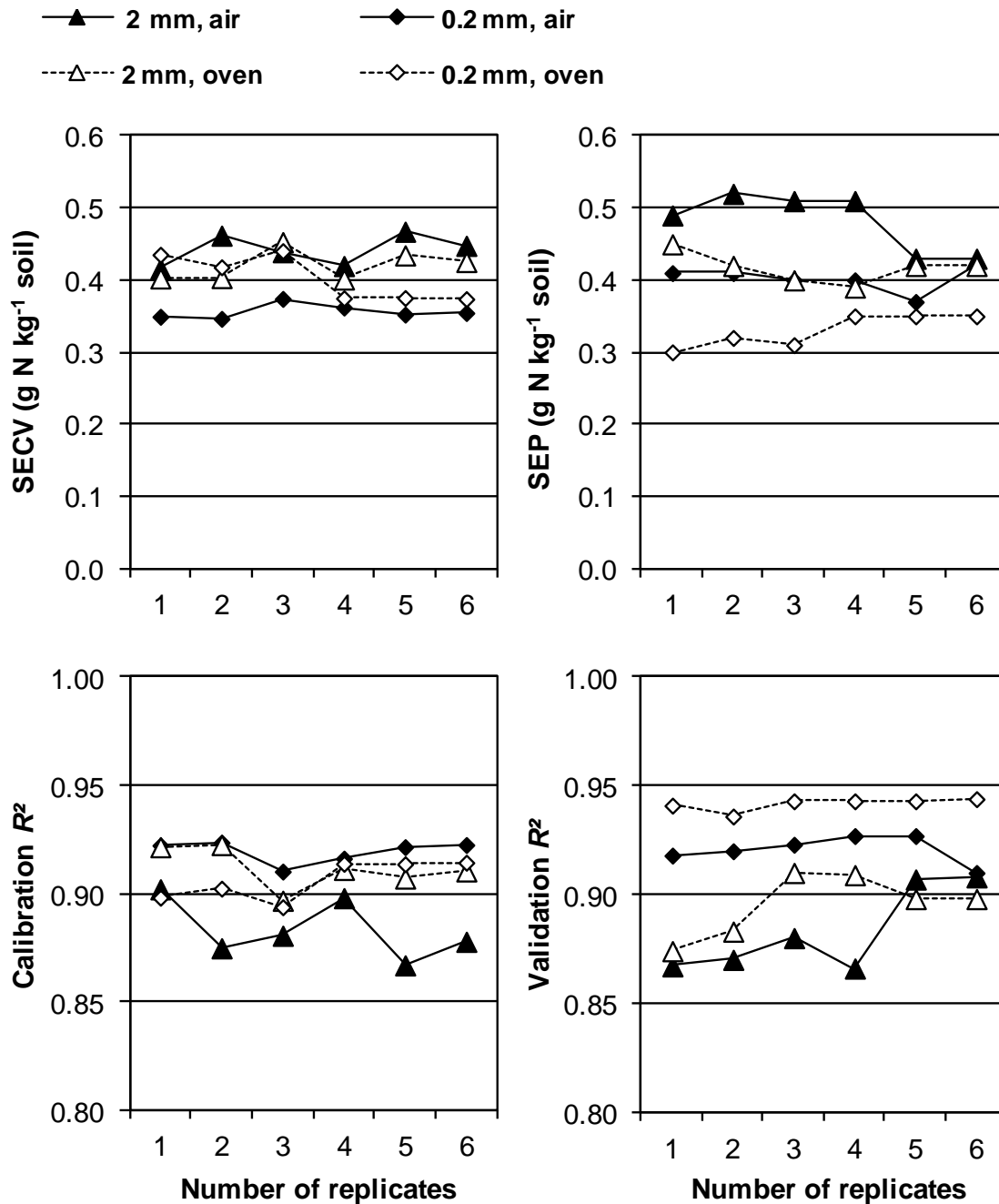
$SECV$  and  $SEP$  are standard error of cross validation and of prediction, respectively.

$RPD$  is the ratio of performance to deviation, i.e. the ratio of standard deviation to  $SECV$ .

**Figure 1.** Effect of replication (use of several subsamples to calculate average sample spectra), 2 mm sieving vs. 0.2 mm grinding, and air- vs. oven-drying of soil samples on calibration and validation results for *Ct* (air-dried sieved ▲; air-dried ground ■; oven-dried sieved △; oven-dried ground □).



**Figure 2.** Effect of replication (use of several subsamples to calculate average sample spectra), 2 mm sieving vs. 0.2 mm grinding, and air- vs. oven-drying of soil samples on calibration and validation results for *Nt* (air-dried sieved ▲; air-dried ground ■; oven-dried sieved △; oven-dried ground □).





**Figure 3.** Comparison of measured (reference) and predicted (NIRS) determinations of  $C_t$  ( $\text{g kg}^{-1}$ ) carried out on (a) 0.2 mm ground oven-dried samples with six replicates, (b) 0.2 mm ground oven-dried samples with one single replicate, and (c) 2 mm sieved oven-dried samples with four replicates.

