Determination of carbon and nitrogen contents in Alfisols, Oxisols and Ultisols from Africa and Brazil using NIRS analysis: Effects of sample grinding and set heterogeneity

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

Determination of carbon and nitrogen contents in Alfisols, Oxisols and Ultisols from Africa and Brazil using NIRS analysis: effects of sample grinding and set heterogeneity

Didier Brunet [a], Bernard G. Barthès [a], Jean-Luc Chotte [a], Christian Feller [b]

[a] *UR SeqBio (Séquestration du carbone et bio-fonctionnement des sols), IRD (Institut de recherche pour le développement), BP 64501, 34394 Montpellier cedex 5, France*
[b] *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) is a rapid and low-cost method that has been reported to provide accurate prediction of soil total carbon (Ct) and nitrogen (Nt) contents. However, methodological aspects such as sample preparation and set homogeneity have not been addressed extensively. The main objective of the paper was to assess how NIRS determination of Ct and Nt was affected by sample preparation (0.2 mm grinding vs. 2 mm sieving) and set heterogeneity (i.e. using the total set vs. texture or localised sets). The study was performed on a range of African and Brazilian Alfisols, Oxisols and Ultisols (297 samples, with 6-80% clay). The total set could be divided into a coarse-textured set, including samples from Burkina Faso and Benin mainly, and a clayey set, including samples from Brazil mainly. Calibration and validation procedures were carried out within the total set and within each of the two texture sets and the three localised sets (Burkina Faso, Benin, and Brazil).

Prediction of Ct using first derivatives with SNV (standard normal variate) scatter correction, which was the most appropriate spectrum preprocessing method, was in general more accurate for ground than for sieved samples. The difference was decisive when the determination was carried out on the localised clayey set, moderate on total and texture sets, and unclear on localised coarse-textured sets. Prediction of Nt was less clearly affected by sample preparation, though grinding was useful for the localised clayey set and harmful for localised coarse-textured ones. Thus the effect of sample preparation on prediction accuracy depended on soil texture, which may explain the contradictory results reported in the literature, often referring to narrow soil ranges. Using the same preprocessing method, the accuracy of Ct prediction generally increased with set homogeneity (i.e. from total to texture sets and from texture to localised sets), providing that clayey sets were ground. This agreed with deductions drawn from comparison between earlier studies carried out with either homogeneous or heterogeneous sets. The effect of set homogeneity on Nt prediction could

not, however, be clearly demonstrated by this study. In conclusion, the study confirmed the interest of NIRS for the rapid and accurate determination of Ct and Nt, especially when homogeneous sets including ground samples were used.

Keywords: Near infrared reflectance spectroscopy (NIRS); Soil; Carbon; Nitrogen; Texture

1. Introduction

Concerns about global climate change have renewed interest in carbon cycle studies, which often require numerous determinations of soil carbon content. Standard procedures for measuring soil carbon being time-consuming and expensive, much attention is given to possible alternatives such as near infrared reflectance spectroscopy (NIRS). NIRS is a physical non-destructive, rapid, reproducible and low-cost method that characterises materials according to their reflectance in the wavelength range between 800 and 2500 nm. Reflectance signals are produced by flexural and longitudinal vibrations in bonds between C, N, H, O, P, and S atoms, and provide information about the proportion of each element in the materials analysed. As organic materials are complex mixtures, their spectra result from the addition of elementary absorptions corresponding to each bond and are not directly informative. Calibration is a statistical process that relates spectral information to sample properties measured by conventional methods. Calibration equations are then used to predict these properties for other samples.

NIRS was first used for the assessment of moisture content of seeds and other plant products (Hart et al., 1962), then for the evaluation of seed and forage quality (Williams, 1975). Its application to soils has been mentioned from the 1960s (Bowers and Hanks, 1965), and studies reporting accurate prediction of soil carbon and nitrogen contents using NIRS were published in the 1970s (Al-Abbas et al., 1972) and have been on the increase since 1990 (Morra et al., 1991; Henderson et al., 1992; Sudduth and Hummel, 1993). However, some methodological aspects have not been addressed extensively. There are conflicting reports in the literature concerning the need for grinding the samples. Several papers have underlined the usefulness of grinding samples for NIRS determination of soil carbon content (Dalal and Henry, 1986; Reeves et al., 2002; Russell, 2003). These confirmed studies carried out on vegetative matter (Blosser et al., 1988). There are also reports in the literature of accurate NIRS prediction of soil carbon content using unground samples (Chang et al., 2001; Shepherd and Walsh, 2002; Morón and Cozzolino, 2004) and some have even suggested grinding the samples did not improve the prediction accuracy (Fystro, 2002). As soil grinding is often

tedious and time-consuming, it is clear that the usefulness of this process should be evaluated. Moreover, though several studies have been carried out on heterogeneous sets including wide ranges of soil types (Ben-Dor and Banin, 1995; Chang et al., 2001; Shepherd and Walsh, 2002), several authors consider the reliability of NIRS prediction questionable when studying heterogeneous sample sets (Sudduth and Hummel, 1996; Brown et al., 2005). More information concerning the effect of set heterogeneity on the accuracy of NIRS prediction is needed in order to apply NIRS more generally to soil characterisation. There also seems to be some confusion about the data preprocessing, such as derivation and scatter correction in order to optimise spectral features. For example, for NIRS prediction of soil carbon content, many authors used the first derivatives of the spectra (Chang et al., 2001; Reeves et al., 2002; Shepherd and Walsh, 2002; Coûteaux et al., 2003; Russell, 2003; Morón and Cozzolino, 2004) while others preferred the second derivatives (Salgó et al., 1998; Fystro, 2002).

This paper assesses the effect of sample preparation (0.2 mm grinding vs. 2 mm sieving) and set heterogeneity on the accuracy of NIRS determination of soil total carbon (Ct) and soil total nitrogen (Nt) contents for a range of Alfisols, Oxisols and Ultisols from Africa and Brazil. The effects of data preprocessing were also investigated (first vs. second derivatives of the spectra, and standard normal variate with or without detrending).

2. Materials and methods

2.1. Soil samples

The total set studied included 297 soil samples originating from Benin, Burkina Faso, Congo, Senegal, and Brazil, collected at depths ranging from 0-5 to 55-60 or 50-70 cm (Table 1). Samples were generally collected from pits, using cylinders, and were sometimes mixed to form composite samples (e.g. Congo and Brazil-SP). In nearby preparative laboratories, samples were spread out in trays and air dried at room temperature, before being stored in plastic bags or cardboard boxes, then transported to Montpellier, France. Then samples were gently crushed using a mortar and pestle and sieved through a 2 mm mesh. Aliquots were finely ground to pass a 0.2 mm mesh, also using a mortar and pestle.

The soils were all “low activity clay” soils, with clay minerals dominated by 1:1 phyllites, such as kaolinite, associated with more or less crystallised Fe, Al, Mn oxides and/or hydroxides. They belonged to three orders: Oxisols, Ultisols and Alfisols (Soil Survey Staff, 1999). In order to study the effect of set heterogeneity on NIRS prediction, the total set of samples was divided into two subsets according to soil texture: the first subset included 199 coarse-textured samples from Benin, Burkina Faso and Senegal (6% to 35% clay) and the

second subset, 98 clayey samples from Brazil and Congo (43% to 80% clay). The coarse-textured subset was denoted SA (for sandy), the clayey one CL, and the total set CLSA. Each subset was further divided according to the source country: Burkina Faso (BF, 79 samples), Benin (BE, 116 samples), and Brazil (BR, 87 samples); countries including a small number of samples (Congo, Senegal) were not considered individually. The sets including 2 mm sieved samples were denoted $CLSA_2$, SA_2, CL_2, BF_2, BE_2, and BR_2, and those including 0.2 mm ground samples, $CLSA_{0.2}$, $SA_{0.2}$, $CL_{0.2}$, $BF_{0.2}$, $BE_{0.2}$, and $BR_{0.2}$.

2.2. Carbon and nitrogen analyses

The conventional determination of soil total carbon (Ct) and nitrogen (Nt) contents was carried out on finely ground (< 0.2 mm) and oven-dried (40°C during 24 h) aliquots by dry combustion using an Elemental Analyzer CHN Fisons / Carlo Erba NA 2000 (Milan, Italy). In the absence of carbonates, all carbon was assumed to be organic.

2.3. NIRS analysis

2.3.1. NIRS measurement

NIRS analysis uses empirical models to predict Ct and Nt from spectral data. The reflectance of the soil samples was determined in the near infrared region between 1100 and 2500 nm at 2 nm intervals using a Foss NIRSystems 5000 spectrophotometer (Silver Spring, MD, USA). The use of this spectral region resulted from the instrument specifications, and was relevant according to Chang et al. (2005), who achieved more accurate predictions of soil properties using the 1100-2500 nm than the 400-2500 nm region. Samples (ca. 5 g) were scanned using a ring cup, which is a 5 cm circular capsule with a quartz window, sealed at the back with cardboard. The ring cup was fixed within a rectangular transport cup.

Each sample spectrum, averaged from 15 spectra, was recorded as the logarithm of the inverse of the reflectance ($\log [1/R]$). Data analysis was conducted using the WinISI III-V1.50e software (Foss NIRSystems / Tecator Infrasoft International, LLC, Silver Spring, MD, USA). The data sets were reduced by keeping the first of every four consecutive spectral points (Sørensen and Dalsgaard, 2005), yielding 173 data points per spectra (Shenk and Westerhaus, 1991b, and Shepherd and Walsh, 2002, kept every fifth point).

2.3.2. Spectrum preprocessing

Derivatives were used to reduce baseline variation and enhance spectral features (Reeves et al., 2002). They were calculated over a 4 point gap with 4 point smoothing. Both first

derivatives (144) and second derivatives (244) were evaluated in conjunction with either a standard normal variate transform (SNV, mean centring and variance scaling), to reduce the particle size effect, or a standard normal variate transform with detrending (SNVD), which additionally removes the linear or curvilinear trend of each spectrum (Barnes et al., 1989). This gave a total of four preprocessing methods: SNV 144, SNVD 144, SNV 244, and SNVD 244.

2.3.3. Identifying spectral outliers

For each of the sample sets under study and each of the four preprocessing methods, a principal component analysis (PCA) was carried out on the complete set of sample spectra for the calculation of the Mahalanobis distance H (Mark and Tunnell, 1985). The H statistic provides quantitative information on how sample spectra deviate from the mean sample spectrum. Samples with $H > 3$ were considered spectral outliers and were eliminated from further investigations (Shenk and Westerhaus, 1991a). Depending on the sample set, preparation and preprocessing method, the number of spectral outliers ranged from 0 to 7, representing less than 5% of the set population (less than 3% in 41 out of the 48 set × preparation × preprocessing combinations).

2.3.4. Calibration

After eliminating the spectral outliers, modified partial least square regression (MPLS; Shenk and Westerhaus, 1991b) was used to relate the spectra with the measured Ct and Nt. The MPLS regression combines PCA and multiple regression in order to reduce a complex spectral matrix to a few orthogonal components and has often been considered an appropriate statistical method for studying soil organic properties (Fystro, 2002; Coûteaux et al., 2003). Each sample set was divided into a calibration subset and a remaining validation subset. The calibration subset of large sets (CLSA, SA) included 100 samples, whereas that of smaller sets (CL, BF, BE, BR) included 70% of the samples. The calibration subset was selected by the software to include the most representative samples of the set (i.e. closest neighbour samples, determined by Euclidian distances between spectra, were represented by one sample only). The literature has often reported random selection of calibration subsets (Ludwig et al., 2002; Shepherd and Walsh, 2002; Móron and Cozzolino, 2004) but it did not seem appropriate in this study as different random selections within the same set sometimes led to very different results. Cross validation was performed on the calibration set to determine the optimum number of terms to be included in the model (using too many factors may cause

overfitting, which reduces the validation performance). The calibration set was divided into six groups, five being used for developing the model and one for prediction. 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). The outliers for calibration (i.e. samples with $t > 2.5$) were removed and another cross validation was performed, the procedure being carried out twice, and 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 performance to deviation (RPD), which is the ratio of standard deviation to SECV. Though RPD > 3 has been recommended for NIRS applications in agriculture, forestry and food sciences (Williams, 1987; Baillères et al., 2002), the model accuracy has been considered acceptable in soil studies when RPD > 2 (Chang et al., 2001; Brown et al., 2005).

2.3.5. Validation

The prediction accuracy of the model was evaluated on the validation subset (which had not been used for model development), using the validation R^2 and standard error of prediction between predicted and measured values (SEP). According to Morra et al. (1991), the performance of prediction equations was also evaluated using the coefficient of variation (CV, in %), calculated as the ratio of SEP to the mean reference value. In addition to this validation CV, the calibration CV was defined as the ratio of SECV to the mean reference value.

3. Results and discussion

3.1. Reference data

Conventional determination results (CHN) of Ct and Nt are presented in Table 2 (the table does not consider individually the sets including a number of samples that was too small to allow modelling, i.e. Senegal and Congo). The samples under study represented a wide range of Ct and Nt, with an overall variation from 1.7 to 42.4 g C kg^{-1}, and from 0.01 to 2.30 g N kg^{-1}, respectively. Coefficient of determination (R^2) between Ct and Nt was 0.90 for CLSA, 0.70 for SA, 0.58 for CL, 0.88 for BF, 0.80 for BE, and 0.58 for BR (Nt was not determined on the samples from Congo). The data showed that Ct and Nt were much higher in clayey than in coarse-textured soils. The ratio of standard deviation to mean was higher

within the total set of samples (88%) than within texture or localised subsets (29% to 59%). It was also higher within coarse-textured subsets than within clayey ones (33% to 59% vs. 29% to 33%).

3.2. Effect of the spectrum preprocessing

Four preprocessing methods were applied to the spectra, in order to compare the scatter corrections SNV and SNVD, and the first and second derivatives. Considering each of the twelve sample sets (i.e. $CLSA_{0.2}$, $CLSA_2$, $SA_{0.2}$, SA_2, $CL_{0.2}$, CL_2, $BF_{0.2}$, BF_2, $BE_{0.2}$, BE_2, $BR_{0.2}$ and BR_2), SNVD 144, and to a lesser extent, SNV 144, often gave the most accurate calibration (i.e. low SECV, high calibration R^2 and RPD) and rarely gave poor calibrations, whereas SNV 244 and SNVD 244 often gave poor calibrations (Tables 3 and 4 for Ct, Tables 5 and 6 for Nt). Moreover, SNV 144 clearly gave the highest number of accurate predictions (lowest SEP and highest validation R^2 for eight and nine out of the twelve sets for Ct and Nt, respectively) and the lowest number of poor predictions, though it was sometimes outperformed (e.g. regarding Ct, by SNV 244 for BE_2 and by SNVD 144 for CL_2 and BR_2). Overall, SNV 144 proved to be the most appropriate preprocessing method for Ct and Nt prediction, especially when clayey sets were ground. Most of the data presented below was obtained using this preprocessing method.

While SNV transformation has been used by some authors for soil Ct (or organic carbon content, Corg) and Nt prediction (Reeves et al., 2002), SNVD seems to be more frequently used (Fystro, 2002; Ludwig et al., 2002; Coûteaux et al., 2003; Morón and Cozzolino, 2004). However, many authors tested several preprocessing methods and selected the most suitable for each property (Reeves and McCarty, 2001; McCarty et al., 2002; Russell, 2003; Sørensen and Dalsgaard, 2005). Moreover, some other authors have reported that normalisation did not improve the calibration (Shepherd and Walsh, 2002). First derivatives of the spectra have frequently been used for Ct, Corg and Nt prediction (Chang et al., 2001; Reeves et al., 2002; Coûteaux et al., 2003; Russell, 2003; Morón and Cozzolino, 2004) but second derivatives could sometimes result in more accurate results (Salgó et al., 1998; Fystro, 2002). Nevertheless, studying 2 mm sieved topsoil samples that were mostly coarse-textured, Sørensen and Dalsgaard (2005) found that the accuracy of Ct prediction was relatively insensitive to the preprocessing method. This does not agree with the results for SA_2, BF_2 and BE_2.

3.3. Measured vs. predicted values of Ct and Nt

For both $CLSA_{0.2}$ and $CLSA_2$ the comparison between measured and predicted values of Ct, using SNV 144, shows two distinct subpopulations (Figure 1): one represented low carbon, coarse-textured soils and the other, high carbon, clayey soils, among which numerous samples were poorly predicted. The difference in spatial pattern of measured vs. predicted Ct between the complete set (CLSA) and the two texture sets (SA and CL) indicates that the corresponding calibration models differed markedly. This probably related to the strong difference in soil properties between clayey and coarse-texture soils, beyond the difference in Ct. Figure 1 also shows the presence of a very poorly predicted sample for BE_2, and a trend to under-predict some of the highest Ct values for $CLSA_{0.2}$, $SA_{0.2}$, $BF_{0.2}$, BF_2, BE_2, and BR_2.

Figure 2 similarly presents measured vs. predicted values of Nt for the twelve sample sets, using SNV 144. Unlike the results for Ct, there was no clear division of CLSA into two subpopulations, and the tendency to under-predict relatively high values was less frequent. Figure 2 shows, however, that several values were markedly under-predicted for $BF_{0.2}$ and $BE_{0.2}$ (localised coarse-textured sets).

On the whole, the accuracy of NIRS prediction of Ct and Nt was satisfactory. Using SNV 144, validation R^2 ranged from 0.77 to 0.96 for Ct, except for BE_2 (0.55; but 0.76 with SNV 244), and from 0.63 to 0.90 for Nt. For texture sets (SA and CL) and localised sets (BF, BE, and BR), validation CV ranged from 7% to 18% for Ct, and from 8% to 25% for Nt, whereas it was 31% to 35% for the total set (CLSA). Prediction of Ct and Nt for the set collected in Benin was often less accurate than for the other sets. This could be explained by the relative heterogeneity of this set, which originated from a small area but included a wide range of textures due to the sharp increase in clay content with depth (< 10% in the topsoil but up to 40% at a 50 cm depth, the sand content always being more than 45%).

3.4. Effect of sample preparation

The effect of sample preparation on calibration accuracy was not clear for Ct (Tables 3 and 4). Compared to 2 mm sieving, 0.2 mm grinding resulted in a more accurate calibration for CLSA (lower SECV, higher R^2) but in a poorer calibration for BE, whereas similar calibrations with ground and sieved samples were recorded for the other sets. However, grinding generally gave more accurate Ct prediction than sieving: grinding always yielded higher validation R^2 than sieving (for localised sets especially), and yielded lower SEP for five out of the six sets (CL, BE and BR especially), but not for BF (homogeneous, sandy). Grinding, therefore, seems preferable to sieving for NIRS prediction of Ct.

In contrast, regarding Nt, grinding gave more accurate calibrations than sieving for five out of the six sets (BE and BF especially), but not for CL (Tables 5 and 6). Moreover, validation accuracy for Nt was not clearly affected by the sample preparation: it was greater with ground samples for two sets, greater with sieved samples for two sets and for two sets there was little difference between sieving and grinding. Nevertheless, grinding clearly improved the accuracy of Nt prediction for BR (homogeneous, clayey) and reduced it for BF (homogeneous, sandy).

Comparisons of NIRS prediction carried out on ground and sieved samples have been reported in several papers. For Australian Vertisols (0-60 cm soil layers, 40-75% clay), Dalal and Henry (1986) observed more accurate prediction of Corg and Nt with ground (< 0.25 mm) than with sieved (< 2 mm) samples: validation CV for Corg was ca. 15% and 25% for ground and sieved samples, respectively. Reeves et al. (2002) similarly reported more accurate calibration for Ct with ground than with sieved topsoil samples collected from a 20-ha field: calibration R^2 and CV were 0.93 vs. 0.78, and 7% vs. 13%, respectively. However, soil type, texture, and particle-size of ground and sieved samples were not specified. For a set of 0-10 cm samples collected on Sodosols, Chromosols and Vertisols within the same region, Russell (2003) also reported more accurate Ct and Nt prediction after grinding than after sieving: validation CV was 8% vs. 10% for Ct, and 11% vs. 15% for Nt, respectively. In contrast, Fystro (2002), studying sandy soils from three regions in Norway (from 59°N to 67°N), found better prediction of Corg and Nt for sieved (< 4 mm) than for ground (< 0.5 mm) samples. Gathering literature data would thus indicate that grinding samples results in more accurate NIRS prediction than sieving for clayey or localised sets, but in worse prediction for a sandy set originating from various locations. This is consistent with the results of this study, which showed that the effect of grinding on prediction accuracy was very positive for localised clayey sets, moderately positive for heterogeneous sets (for Ct), and unclear for localised coarse-textured sets. Considering a wide range of soils that could be divided in different subsets, the present study thus helped confirming deductions drawn from the gathering of studies carried out on narrow ranges of soils.

The effect of soil texture on the usefulness of sample grinding may be attributed to macro-aggregates and coarse sands. Chang et al. (2001) mentioned that size and arrangement of particles could affect the path of light transmission. Indeed, it is likely that the reflectance of clayey samples is influenced by their content in macro-aggregates and that this effect is reduced by grinding. Thus, grinding reduces aggregate influence on reflectance in clayey samples. However, sandy soils include few aggregates, thus 0.2 mm grinding mainly results

in the breaking up of coarse sands, and, owing to their crystalline nature (sands were mainly constituted of quartz in the soils under study), in the creation of new planes that can disturb light reflection. This may help explain why grinding was not useful for sandy samples. However, it seems preferable always to use the same sample preparation, especially when studying sets that include a wide diversity of soils, and so grinding might be recommended.

3.5. Effect of set heterogeneity

Regarding Ct, the heterogeneity of sample sets had a strong influence on calibration and validation statistics (Table 3 and 4, and Figure 1). This effect was assessed by studying changes in R^2, RPD and CV (ratio of SECV or SEP to mean) with set heterogeneity. Changes in SECV or SEP were not taken into consideration because these parameters depend on Ct range (e.g. comparing SECV values of 0.6 and 2.1 g C kg^{-1} is not relevant when the respective Ct ranges are 1.7-4.6 and 10.8-22.3 g C kg^{-1}). Calibration CV generally decreased (data not shown) and calibration R^2 and RPD increased as set homogeneity increased (i.e. from CLSA to SA and CL, and from SA to BF and BE; but not from CL to BR). The quality of calibration statistics for Ct thus increased with set homogeneity in general, except for the Brazilian clayey soils. Moreover, the accuracy of Ct prediction was clearly higher in the texture sets than in the total set: validation CV was markedly higher for SA and CL than for CLSA, while differences in validation R^2 were slight. For ground samples, prediction accuracy for Ct was also higher in the localised sets than in the texture sets: validation CV was slightly lower and validation R^2 generally higher for $BF_{0.2}$ and $BE_{0.2}$ than for $SA_{0.2}$ and for $BR_{0.2}$ than for $CL_{0.2}$. For sieved samples, the difference between localised sets and texture sets was less clear: the prediction accuracy was also higher for BF_2 than for SA_2, but it was lower for BE_2 than for SA_2 and lower for BR_2 than for CL_2. In conclusion, the accuracy of Ct prediction was clearly improved when the samples were separated into texture sets (i.e. clayey vs. coarse-textured soils). For ground samples the prediction was further improved by separation into localised sets. However, for sieved samples, separation into localised sets only improved the prediction for the sandy set.

In contrast, the effect of set heterogeneity on the calibration accuracy for Nt was unclear. Higher set homogeneity generally resulted in a lower calibration CV (i.e. from CLSA to SA then to BF, and from CLSA to CL then to BR; but not from SA to BE). However, greater homogeneity did not always result in higher calibration R^2 (e.g. R^2 was lower for BF than for SA and CLSA). The effect of set heterogeneity on the validation accuracy for Nt was also unclear. Higher set homogeneity resulted in a lower validation CV (i.e. from CLSA to SA and

CL, from SA to BF and BE, and from CL to BR) but also often in lower validation R^2 (e.g. from CLSA to SA and CL). Set homogeneity did not clearly affect Nt prediction on the whole, though prediction accuracy was greater for the localised clayey set (BR) and for the sieved localised sandy set (BF_2) than for the corresponding heterogeneous sets (CL and CLSA, and SA_2 and $CLSA_2$, respectively).

Higher validation CV and lower validation R^2 for heterogeneous than for homogeneous sets could be deduced from comparison between studies that included either homogeneous or heterogeneous sets (Figure 3; in this figure, set heterogeneity was expressed by the difference between maximum and minimum clay content, i.e. clay range). This was corroborated by studies including sets of variable heterogeneity, though heterogeneity rarely related to soil type: for Ct (or Corg) and Nt, validation CV was higher for whole Vertisol profiles than for the more homogeneous topsoil layers (30-40% vs. 15-25%; Dalal and Henry, 1986) and higher for silt plus clay fractions than for silt fractions alone (19% vs. 14-17%; Morra et al., 1991). On the whole, the literature suggests that prediction of Ct (or Corg) and Nt was less accurate for sets including a wide diversity of soils than for those including narrower ranges of soil types: validation CV and R^2 were 25%-45% vs. 6%-30%, and 0.55-0.82 vs. 0.85-0.99, respectively. For Ct this was confirmed by the results of the present study, specifically addressing the effect of soil range diversity. More accurate prediction for homogeneous than for heterogeneous sets implied that robust calibrations might be difficult to achieve, as reported by several authors (Sudduth and Hummel, 1996; Brown et al., 2005; Sørensen and Dalsgaard, 2005). Weak relationship between soil organic matter content and reflectance when soil samples are collected from large geographic areas has been attributed to probable parent-material influences on soil mineral reflectance (Henderson et al., 1992).

However, the results of this study suggest that Nt prediction was not clearly affected by set heterogeneity: increasing set homogeneity generally decreased both validation CV and R^2. Although this was not confirmed in the literature (cf. Figure 3), several papers suggested that Nt prediction is less clearly affected by set homogeneity than Ct prediction. Morra et al. (1991) observed that the increase in set homogeneity resulted in a greater decrease in validation CV for Ct than for Nt, and Dalal and Henry (1986) showed a similar result for sieved samples.

3.6. Comparison of prediction accuracy for Ct and Nt

Using SNV 144, the calibration accuracy was, in general, higher for Ct than for Nt except for $CLSA_2$ (similar accuracy for Ct and Nt) and BR_2 (higher R^2 and RPD for Nt). For the ground

sets, the validation accuracy was also higher in general for Ct than for Nt: Ct prediction was more accurate than Nt prediction for four out of the six ground sets, less accurate for $CLSA_{0.2}$ and there was no clear difference for $BR_{0.2}$. For sieved sets, however, the validation accuracy for Ct and Nt did not differ clearly: Ct prediction was more accurate for one set (SA_2), Nt prediction was more accurate for two sets ($CLSA_2$ and BE_2), and the accuracy of the Ct and Nt predictions was similar for the three other sets. To summarise: (i) of the sets where Ct prediction was more accurate than the Nt prediction, most were ground sets and (ii) prediction for the total set (CLSA) tended to be more accurate for Nt than for Ct. Also grinding and set homogeneity had a generally positive effect on Ct prediction, but no clear effect on Nt prediction.

Studying ground samples from Australian Vertisols (0-60 cm layers), Dalal and Henry (1986) found similar validation R^2 and CV for Corg and Nt (0.85-0.86, and ca. 15%, respectively). For a fairly homogeneous set of ground fine-textured samples (silty loam Ultisols), Reeves and McCarty (2001) also found similar calibration R^2 and CV for Corg and Nt (0.97 and 6%, respectively). For a more heterogeneous set of ground sandy samples originating from different regions of Norway (59°N to 67°N), data from Fystro (2002) showed that validation CV was similar for Corg and Nt but that R^2 was higher for Corg. For a fairly heterogeneous set of ground topsoil samples (three soil types within the same Australian region), data from Russell (2003) also showed a validation CV lower for Ct than for Nt (8% vs. 11%, respectively). Thus data from the literature confirmed that for ground samples, Ct and Nt prediction accuracy was similar for homogeneous fine-textured sets (cf. $BR_{0.2}$), but that it was more accurate for Ct than for Nt for moderately heterogeneous or for coarse-textured sets (cf. $SA_{0.2,}$ $CL_{0.2}$, $BF_{0.2}$ and $BE_{0.2}$).

For sieved samples, data from Dalal and Henry (1986) for Australian Vertisols showed similar validation CV for Corg and Nt for topsoil (0-60 cm) samples (ca. 25%), but higher validation CV for Corg than for Nt for whole profiles (i.e. less homogeneous: ca. 40% vs. 35%, respectively). However, data from Coûteaux et al. (2003) for a fairly homogeneous set indicated that prediction accuracy was greater for Ct than for Nt (validation R^2 and CV were 0.99 vs. 0.98 and 6% vs. 10%, respectively). Similarly, data from Salgó et al. (1998), Fystro (2002) and Russell (2003), for heterogeneous sample sets, showed more accurate prediction of Ct (or Corg) than Nt. Considering literature data as well as results from this study, it was thus difficult to find a clear trend when comparing prediction accuracy for Ct (or Corg) and Nt using sieved samples.

It has been reported that NIRS determination of Nt might be indirect, due to close correlation with Ct, rather than direct, due to specific N absorbers (Martin et al., 2002). In the present study, prediction accuracy was not always higher for Ct than for Nt (e.g. $CLSA_2$ and BR_2), and correlation between Nt and Ct was rather loose within several sets ($R^2 \leq 0.70$ for SA, CL and BR). Thus, from a general viewpoint, it could not be concluded that NIRS determination of Nt was indirect and resulted from NIRS determination of Ct and correlation between Ct and Nt. This confirmed that NIRS is able to determine Nt directly, as reported by Chang and Laird (2002). However, for BF_2, much lower calibration R^2 for Nt than for Ct (0.69 vs. 0.93) and close correlation between Nt and Ct ($R^2 = 0.88$) suggested that NIRS prediction of Nt might be indirect. The question also arose for $BF_{0.2}$, but to a lesser extent. Following Martin et al. (2002), it could be hypothesized that depending on the studied set, Nt was predicted best on its correlation with Ct when the correlation was high, but directly otherwise. In the present study, data strongly suggested that NIRS determination of Nt was direct for most sets, but that it might be indirect for some few other ones (i.e. BF, which was the most sandy set).

4. Conclusion

This study, carried out on a range of low activity clay soils, demonstrated the usefulness of NIRS to determine Ct and Nt in tropical soils. The most accurate prediction of Ct and Nt was generally achieved using scatter correction without detrending and first derivatives (preprocessing method SNV 144). Soil preparation had a clear effect on Ct prediction accuracy. This was, in general, higher for 0.2 mm ground samples than for 2 mm sieved samples, although grinding was less beneficial for sandy sets. The effect of soil preparation on Nt prediction was less clear in general, however grinding increased prediction accuracy for localised clayey sets and decreased it for localised sandy ones. On the whole, sample grinding could be recommended, as it yielded an overall improvement in the accuracy of Ct and Nt prediction. Moreover, set homogeneity affected the accuracy of Ct prediction, which was clearly higher when clayey and coarse-textured sets were analysed separately. For ground samples, the accuracy of Ct prediction further increased when localised sets were analysed. This was not true for sieved samples. Set homogeneity had less effect on Nt prediction, the accuracy was not improved by analysing texture sets separately, nevertheless it was often higher for homogeneous localised sets. On the whole, the study of homogeneous sample sets could be recommended for Ct and Nt prediction, suggesting that NIRS is particularly appropriate for localised studies. Finally, the present study confirmed deductions that could be drawn from comparisons between earlier studies (e.g. studies on clayey soils vs. studies on

sandy soils, studies on homogeneous sets vs. studies on heterogeneous sets), and helped optimize the conditions for NIRS determination of soil properties.

Acknowledgments

We thank Anastase Azontonde, Lydie Chapuis-Lardy, Edgar De Luca, Edmond Hien, and Aurélie Metay for sample collection. We also thank anonymous reviewers for their helpful recommendations.

References

Al-Abbas, A.H., Swain, P.H., Baumgardner, M.F., 1972. Relating organic matter and clay content to the multispectral radiance of soils. Soil Sci. 114, 477–485.

Baillères, H., Davrieux, F., Ham-Pichavant, F., 2002. Near infrared analysis as a tool for rapid screening of some major wood characteristics in a eucalyptus breeding program. Ann. Forest Sci. 59, 479–790.

Barnes, R.J., Dhanoa, M.S., Lister, S.J., 1989. Standard normal variate transformation and detrending of near-infrared diffuse reflectance spectra. Appl. Spectrosc. 43, 772–777.

Ben-Dor, E., Banin, A., 1995. Near-infrared analysis as a rapid method to simultaneously evaluate several soil properties. Soil Sci. Soc. Am. J. 59, 364–372.

Blosser, T.H., Reeves III, J.B., Bond, J., 1988. Factors affecting analysis of the chemical composition of tall fescue with near infrared reflectance spectroscopy. J. Dairy Sci. 71, 398–408.

Bowers, S.A., Hanks, R.J., 1965. Reflection of radiant energy from soils. Soil Sci. 100, 130–138.

Brown, D.J., Bricklemyer, R.S., Miller, P.R., 2005. Validation requirements for diffuse reflectance soil characterization models with a case study of VNIR soil C prediction in Montana. Geoderma 129, 251–267.

Chang, C.W., Laird, D.A., Mausbach, M.J., Hurburgh, C.R.J., 2001. Near-infrared reflectance spectroscopy-principal components regression analyses of soil properties. Soil Sci. Soc. Am. J. 65, 480–490.

Chang, C.W., Laird, D.A., 2002. Near-infrared reflectance spectroscopic analysis of soil C and N. Soil Sci. 167, 110–116.

Chang, C.W., Laird, D.A., Hurburgh, C.R.J., 2005. Influence of soil moisture on near-infrared reflectance spectroscopic measurements of soil properties. Soil Sci. 170, 244-255.

Coûteaux, M.M., Berg, B., Rovira, P., 2003. Near infrared reflectance spectroscopy for determination of organic matter fractions including microbial biomass in coniferous forest soils. Soil Biol. Biochem. 35, 1587–1600.

Dalal, R.C., Henry, R.J., 1986. Simultaneous determination of moisture, organic carbon and total nitrogen by near infrared reflectance spectrophotometry. Soil Sci. Soc. Am. J. 50, 120–123.

FAO-ISRIC-ISSS (Food and Agriculture Organization of the United Nations, International Soil Reference and Information Centre, International Society for Soil Science), 1998. World Reference Base for Soil Resources. FAO, Rome.

Fystro, G., 2002. The prediction of C and N content and their potential mineralisation in heterogeneous soil samples using Vis-NIR spectroscopy and comparative methods. Plant Soil 246, 139–149.

Hart, J.R., Norris, K.H., Golumbic, C., 1962. Determination of the moisture content of seeds by near-infrared spectrophotometry of their methanol extracts. Cereal Chem. 39, 94–99.

Henderson, T.L., Baumgardner, M.F., Franzmeier, D.P., Stott, D.E., Coster, D.C., 1992. High dimensional reflectance analysis of soil organic matter. Soil Sci. Soc. Am. J. 56, 865–872.

Ludwig, B., Khanna, P.K., Bauhus, J., Hopmans, P., 2002. Near infrared spectroscopy of forest soils to determine chemical and biological properties related to soil sustainability. Forest Ecol. Manage. 171, 121–132.

Mark, H.L., Tunnell, D., 1985. Qualitative near-infrared reflectance analysis. Anal. Chem. 58, 379–384.

Martin, P.D., Malley, D.F., Manning, G., Fuller, L., 2002. Determination of soil organic carbon and nitrogen at the field level using near-infrared spectroscopy. Can. J. Soil Sci. 82, 413-422.

McCarty, G.W., Reeves III, J.B., Reeves, V.B., Follett, R.F., Kimble, J.M., 2002. Mid-infrared and near-infrared diffuse reflectance spectroscopy for soil carbon measurement. Soil Sci. Soc. Am. J. 66, 640–646.

Morra, M.J., Hall, M.H., Freeborn, L.L., 1991. Carbon and nitrogen analysis of soil fractions using near-infrared reflectance spectroscopy. Soil Sci. Soc. Am. J. 55, 288–291.

Móron, A., Cozzolino, D., 2004. Determination of potentially mineralizable nitrogen and nitrogen in particulate organic matter fractions in soil by visible and near-infrared reflectance spectroscopy. J. Agric. Sci. 142, 335–343.

Reeves III, J.B., McCarty, G.W., 2001. Quantitative analysis of agricultural soils using near infrared reflectance spectroscopy and a fibre-optic probe. J. Near Infrared Spectrosc. 9, 25–34.

Reeves III, J.B., McCarty, G.W., Mimmo, T., 2002. The potential of diffuse reflectance spectroscopy for the determination of carbon inventories in soils. Environ. Pollut. 116, S277–S284.

Russell, C.A., 2003. Sample preparation and prediction of soil organic matter properties by near infrared reflectance spectroscopy. Commun. Soil Sci. Plant Anal. 34, 1557–1572.

Salgó, A., Nagy, J., Tarnóy, J., Marth, P., Pálmai, O., Szabó-Kele, G., 1998. Characterisation of soils by the near infrared technique. J. Near Infrared Spectrosc. 6, 199–203.

Shenk, J.S., Westerhaus, M.O., 1991a. Population definition, sample selection and calibration procedures for near infrared reflectance spectroscopy. Crop Sci. 31, 469–474.

Shenk, J.S., Westerhaus, M.O., 1991b. Population structuring of near infrared spectra and modified partial least square regression. Crop Sci. 31, 1548–1555.

Shepherd, K.D., Walsh, M.G., 2002. Development of reflectance spectral librairies for characterization of soil properties. Soil Sci. Soc. Am. J. 66, 988–998.

Soil Survey Staff, 1999. Soil Taxonomy. A Basic System of Soil Classification for Making and Interpreting Soil Surveys, 2nd Edn. United States Department of Agriculture, Natural Resources Conservation Service, Washington, DC.

Sørensen, L.K., Dalsgaard, S., 2005. Determination of clay and other soil properties by near infrared spectroscopy. Soil Sci. Soc. Am. J. 69, 159–167.

Sudduth, K.A., Hummel, J.W., 1993. Soil organic matter, CEC, and moisture sensing with a portable NIR spectrophotometer. Trans. ASAE, 36, 1571–1582.

Sudduth, K.A., Hummel, J.W., 1996. Geographic operating range evaluation of a NIR soil sensor. Trans. ASAE, 39, 1599–1604.

Williams, P.C. 1975. Applications of near-infrared reflectance spectroscopy to analysis of cereal grains and oilseeds. Cereal Chem. 52, 561–576.

Williams, P.C., 1987. Variables affecting near infrared reflectance spectroscopic analysis. In: Williams P.C., Norris K. (Eds.), Near Infrared Technology in the Agricultural and Food Industries. American Association of Cereal Chemists Inc., St. Paul, MN, pp. 143–167.

Table 1. Presentation of the studied samples: location, soil type, texture, climate, land use, and depth.

Location	Soil type [a]	Soil texture and clay content	Mean annual rainfall and temperature	Management	Number of samples and depth
Sonkorong, Senegal 13°45'N, 15°40'W	Lixisol	Sandy ca. 12%	700 mm yr^{-1} 30°C	Fallow (shrub savanna)	4 samples 0-10 cm
Saria, Burkina Faso 12°16'N, 02°09'W	Kanhaplic Haplustalf, Ferric Acrisol	Sandy to sandy clay loam 9-32%	800 mm yr^{-1} 28°C	Sorghum	79 samples from 0-10 to 30-40 cm
Agonkanmey, Benin 06°24'N, 02°20'E	Typic Kandiustult, Dystric Nitisol	Sandy to clay loam 6-40%	1200 mm yr^{-1} 27°C	Maize-based cropping systems	116 samples from 0-5 to 55-60 cm
Loudima, Congo 04°00'S, 13°30'E	Typic Haplorthox, Orthic Ferralsol	Clayey 52-76%	1100 mm yr^{-1} 25°C	Savanna and cassava-based cropping systems	11 samples 0-10 and 10-20 cm
Brasilia, Brazil (DF) 15°50'S, 47°50'W	Typic Haplustox, Orthic Ferralsol	Clayey 44-79%	1400 mm yr^{-1} 22°C	Pastures, savanna and forest	39 samples from 0-10 to 50-70 cm
Goiânia, Brazil (GO) 16°29'S, 49°19'W	Typic Haplustox, Orthic Ferralsol	Clayey 43-46%	1500 mm yr^{-1} 23°C	Rice-soybean rotation with possible cover crop	8 samples 0-10 and 10-30 cm
Pradópolis, Brazil (SP) 21°22'S, 48°03'W	Typic Hapludox, Orthic Ferralsol	Clayey 66-80%	1600 mm yr^{-1} 23°C	Sugarcane and forest	40 samples 0-5 and 5-10 cm

[a] Soil Survey Staff (1999) and/or FAO-ISRIC-ISSS (1998).

Table 2. Conventional determination (CHN) of Ct and Nt: minimum, maximum, mean, and standard deviation (SD) for the total set, the texture subsets, and the localised subsets; n is the number of samples.

Set	Ct (g C kg^{-1})					Nt (g N kg^{-1})				
	n	Min	Max	Mean	SD	n	Min	Max	Mean	SD
Total set (CLSA)	297	1.67	42.40	10.45	9.21	286	0.01	2.30	0.64	0.56
Coarse-textured subset (SA)	199	1.67	9.85	4.62	1.82	199	0.01	0.76	0.32	0.15
Clayey subset (CL)	98	10.80	42.40	22.28	6.40	87	0.40	2.30	1.38	0.45
Burkina Faso (BF; coarse-textured)	79	1.67	9.85	4.26	1.73	79	0.16	0.73	0.36	0.12
Benin (BE; coarse-textured)	116	1.72	9.72	4.83	1.87	116	0.01	0.76	0.29	0.17
Brazil (BR; clayey)	87	10.80	42.40	22.19	6.35	87	0.40	2.30	1.38	0.45

Table 3. Calibration and validation results for Ct depending on sample preparation (0.2 vs. 2 mm), set homogeneity (total set vs. texture subsets), and preprocessing method (first vs. second derivatives, i.e. 144 vs. 244, and with vs. without detrending, i.e. SNVD vs. SNV, respectively).

Set	Preprocessing method	Calibration set				Validation set			
		n_1	SECV (g C kg^{-1})	R^2	RPD	n_2	SEP (g C kg^{-1})	R^2	CV (%)
$CLSA_{0.2}$	SNV 144	91	2.73	0.89	3.0	191	3.54	0.84	33.9
total set	SNVD 144	88	2.18	0.94	3.9	193	5.12	0.67	49.0
	SNV 244	91	2.87	0.86	2.7	191	3.48	0.85	33.3
	SNVD 244	89	2.77	0.86	2.7	191	3.65	0.84	34.9
$CLSA_2$	SNV 144	92	3.33	0.87	2.8	193	3.69	0.81	35.3
total set	SNVD 144	90	3.94	0.83	2.4	195	4.04	0.78	38.7
	SNV 244	87	3.36	0.84	2.5	197	5.38	0.62	51.5
	SNVD 244	88	3.41	0.83	2.5	197	5.52	0.61	52.8
$SA_{0.2}$	SNV 144	95	0.64	0.89	3.0	92	0.61	0.86	13.2
total coarse-	SNVD 144	90	0.66	0.88	2.9	96	0.83	0.71	18.0
textured	SNV 244	95	0.72	0.86	2.7	96	0.84	0.66	18.2
subset	SNVD 244	95	0.72	0.86	2.7	96	0.84	0.66	18.2
SA_2	SNV 144	93	0.65	0.91	3.3	93	0.66	0.81	14.3
total coarse-	SNVD 144	92	0.61	0.92	3.5	94	0.78	0.73	16.9
textured	SNV 244	94	0.56	0.93	3.8	96	0.73	0.73	15.8
subset	SNVD 244	94	0.57	0.93	3.7	96	0.73	0.73	15.8
$CL_{0.2}$	SNV 144	64	2.09	0.91	3.4	28	1.68	0.84	7.5
total clayey	SNVD 144	59	2.16	0.85	2.6	25	3.14	0.75	14.1
subset	SNV 244	66	2.84	0.74	2.0	26	2.30	0.86	10.3
	SNVD 244	65	2.59	0.85	2.6	26	2.71	0.79	12.2
CL_2	SNV 144	63	2.06	0.88	2.9	26	2.11	0.80	9.5
total clayey	SNVD 144	64	2.04	0.89	3.0	28	1.86	0.89	8.3
subset	SNV 244	67	2.57	0.78	2.1	26	3.00	0.64	13.5
	SNVD 244	68	2.86	0.77	2.1	26	3.01	0.65	13.5

n_1 is the number of samples after the elimination of calibration outliers.
n_2 is the number of samples used for the validation.
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.
CV is the coefficient of variation, defined as the ratio of SEP to the mean.

Table 4. Calibration and validation results for Ct depending on sample preparation (0.2 vs. 2 mm), localisation (Burkina Faso BF, Benin BE, and Brazil BR), and preprocessing method (first vs. second derivatives, i.e. 144 vs. 244, and with vs. without detrending, i.e. SNVD vs. SNV, respectively).

Set	Preprocessing method	Calibration set				Validation set			
		n_1	SECV (g C kg^{-1})	R^2	RPD	n_2	SEP (g C kg^{-1})	R^2	CV (%)
$BF_{0.2}$	SNV 144	48	0.38	0.92	3.6	22	0.44	0.96	10.3
coarse-textured	SNVD 144	48	0.42	0.89	3.0	22	0.44	0.94	10.3
(included in SA)	SNV 244	52	0.46	0.91	3.4	22	0.60	0.86	14.1
	SNVD 244	50	0.46	0.91	3.3	22	0.60	0.87	14.1
BF_2	SNV 144	51	0.41	0.93	3.8	20	0.38	0.87	8.9
coarse-textured	SNVD 144	49	0.41	0.91	3.3	21	0.42	0.83	9.9
(included in SA)	SNV 244	49	0.37	0.94	4.2	22	0.43	0.84	10.1
	SNVD 244	49	0.41	0.93	3.9	22	0.46	0.84	10.8
$BE_{0.2}$	SNV 144	78	0.63	0.91	3.4	33	0.50	0.78	10.4
coarse-textured	SNVD 144	74	0.54	0.93	3.9	34	0.78	0.59	16.1
(included in SA)	SNV 244	78	0.71	0.88	2.9	30	0.74	0.58	15.3
	SNVD 244	77	0.72	0.87	2.8	30	0.64	0.65	13.3
BE_2	SNV 144	75	0.57	0.93	3.7	35	0.86	0.55	17.8
coarse-textured	SNVD 144	75	0.56	0.93	3.8	35	0.67	0.72	13.9
(included in SA)	SNV 244	74	0.63	0.91	3.3	35	0.62	0.76	12.8
	SNVD 244	73	0.58	0.93	3.6	35	0.63	0.75	13.0
$BR_{0.2}$	SNV 144	53	2.49	0.86	2.6	25	1.50	0.89	6.8
clayey	SNVD 144	52	2.24	0.89	3.0	25	2.84	0.59	12.8
(included in CL)	SNV 244	55	2.32	0.86	2.7	27	5.54	0.39	25.0
	SNVD 244	55	2.33	0.86	2.7	27	5.51	0.40	24.8
BR_2	SNV 144	51	2.26	0.78	2.1	25	2.18	0.77	9.8
clayey	SNVD 144	56	2.36	0.86	2.6	26	1.97	0.84	8.9
(included in CL)	SNV 244	58	2.35	0.85	2.6	27	2.70	0.69	12.2
	SNVD 244	58	2.54	0.85	2.6	27	2.06	0.81	9.3

n_1 is the number of samples after the elimination of calibration outliers.
n_2 is the number of samples used for the validation.
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.
CV is the coefficient of variation, i.e. the ratio of SEP to the mean.
SA and CL are the coarse-textured and the clayey subset, respectively.

Table 5. Calibration and validation results for Nt depending on sample preparation (0.2 vs. 2 mm), set homogeneity (total set vs. texture subsets), and preprocessing method (first vs. second derivatives, i.e. 144 vs. 244, and with vs. without detrending, i.e. SNVD vs. SNV, respectively).

Set	Preprocessing method	Calibration set				Validation set			
		n_1	SECV (g N kg^{-1})	R^2	RPD	n_2	SEP (g N kg^{-1})	R^2	CV (%)
$CLSA_{0.2}$	SNV 144	85	0.17	0.87	2.8	184	0.21	0.86	32.8
total set	SNVD 144	85	0.20	0.85	2.5	188	0.33	0.67	51.6
	SNV 244	90	0.19	0.83	2.5	188	0.23	0.84	35.9
	SNVD 244	91	0.20	0.83	2.5	188	0.23	0.84	35.9
$CLSA_2$	SNV 144	87	0.20	0.87	2.8	186	0.20	0.86	31.3
total set	SNVD 144	88	0.26	0.81	2.3	187	0.24	0.79	37.5
	SNV 244	88	0.22	0.81	2.3	189	0.22	0.83	34.4
	SNVD 244	88	0.22	0.82	2.3	189	0.22	0.83	34.4
$SA_{0.2}$	SNV 144	97	0.06	0.88	2.8	92	0.07	0.72	21.9
total coarse-	SNVD 144	94	0.05	0.89	2.4	96	0.07	0.70	21.9
textured	SNV 244	95	0.07	0.84	2.4	96	0.08	0.59	25.0
subset	SNVD 244	94	0.07	0.84	2.4	96	0.08	0.60	25.0
SA_2	SNV 144	94	0.06	0.87	2.8	93	0.08	0.65	25.0
total coarse-	SNVD 144	95	0.07	0.84	2.6	94	0.07	0.67	21.9
textured	SNV 244	97	0.07	0.83	2.6	96	0.07	0.68	21.9
subset	SNVD 244	97	0.07	0.83	2.6	96	0.06	0.68	18.8
$CL_{0.2}$	SNV 144	54	0.22	0.78	2.1	25	0.18	0.81	13.0
total clayey	SNVD 144	53	0.18	0.85	2.5	23	0.35	0.67	25.4
subset	SNV 244	57	0.19	0.83	1.7	24	0.41	0.57	29.7
	SNVD 244	57	0.19	0.83	2.4	24	0.40	0.58	29.0
CL_2	SNV 144	60	0.18	0.84	2.5	23	0.21	0.82	15.2
total clayey	SNVD 144	59	0.16	0.85	2.7	23	0.20	0.84	14.5
Subset	SNV 244	60	0.16	0.88	2.9	23	0.23	0.73	16.7
	SNVD 244	60	0.16	0.88	2.9	23	0.23	0.72	16.7

n_1 is the number of samples after the elimination of calibration outliers.
n_2 is the number of samples used for the validation.
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.
CV is the coefficient of variation, i.e. the ratio of SEP to the mean.

Table 6. Calibration and validation results for Nt depending on sample preparation (0.2 vs. 2 mm), localisation (Burkina Faso BF, Benin BE, and Brazil BR), and preprocessing method (first vs. second derivatives, i.e. 144 vs. 244, and with vs. without detrending, i.e. SNVD vs. SNV, respectively).

Set	Preprocessing method	Calibration set				Validation set			
		n_1	SECV (g N kg^{-1})	R^2	RPD	n_2	SEP (g N kg^{-1})	R^2	CV (%)
$BF_{0.2}$	SNV 144	49	0.04	0.84	2.5	22	0.06	0.63	16.7
coarse-textured	SNVD 144	49	0.03	0.86	3.0	22	0.06	0.69	16.7
(included in SA)	SNV 244	50	0.04	0.85	2.5	22	0.05	0.76	13.9
	SNVD 244	50	0.04	0.86	2.5	22	0.05	0.75	13.9
BF_2	SNV 144	48	0.05	0.69	1.8	20	0.03	0.87	8.3
coarse-textured	SNVD 144	49	0.04	0.89	3.0	21	0.05	0.62	13.9
(included in SA)	SNV 244	49	0.05	0.75	2.0	22	0.05	0.74	13.9
	SNVD 244	49	0.05	0.75	2.0	22	0.05	0.74	13.9
$BE_{0.2}$	SNV 144	78	0.06	0.90	3.2	33	0.06	0.76	20.7
coarse-textured	SNVD 144	80	0.06	0.91	3.2	34	0.08	0.46	27.6
(included in SA)	SNV 244	78	0.07	0.87	2.7	30	0.08	0.28	27.6
	SNVD 244	78	0.07	0.87	2.7	30	0.08	0.29	27.6
BE_2	SNV 144	77	0.07	0.87	2.7	35	0.05	0.66	17.2
coarse-textured	SNVD 144	78	0.07	0.87	2.7	35	0.07	0.63	24.1
(included in SA)	SNV 244	77	0.07	0.88	2.7	35	0.07	0.57	24.1
	SNVD 244	77	0.07	0.87	2.7	35	0.07	0.58	24.1
$BR_{0.2}$	SNV 144	55	0.17	0.85	2.6	25	0.14	0.90	10.1
clayey	SNVD 144	53	0.17	0.86	2.7	25	0.17	0.87	12.3
(included in CL)	SNV 244	54	0.19	0.82	2.4	27	0.39	0.60	28.3
	SNVD 244	54	0.19	0.82	2.4	27	0.38	0.61	27.5
BR_2	SNV 144	55	0.18	0.84	2.5	25	0.18	0.85	13.0
clayey	SNVD 144	58	0.18	0.84	2.5	26	0.21	0.79	15.2
(included in CL)	SNV 244	59	0.21	0.78	2.1	27	0.18	0.84	13.0
	SNVD 244	57	0.22	0.77	2.1	27	0.22	0.76	15.9

n_1 is the number of samples after the elimination of calibration outliers.
n_2 is the number of samples used for the validation.
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.
CV is the coefficient of variation, i.e. the ratio of SEP to the mean.
SA and CL are the coarse-textured and the clayey subset, respectively.

Figure 1. Comparison of measured and predicted Ct values for validation subsets from the total set (CLSA), the coarse-textured set (SA), the clayey set (CL), Burkina Faso (BF), Benin (BE), and Brazil (BR), using 0.2 mm ground or 2 mm sieved soil samples (with SNV 144).

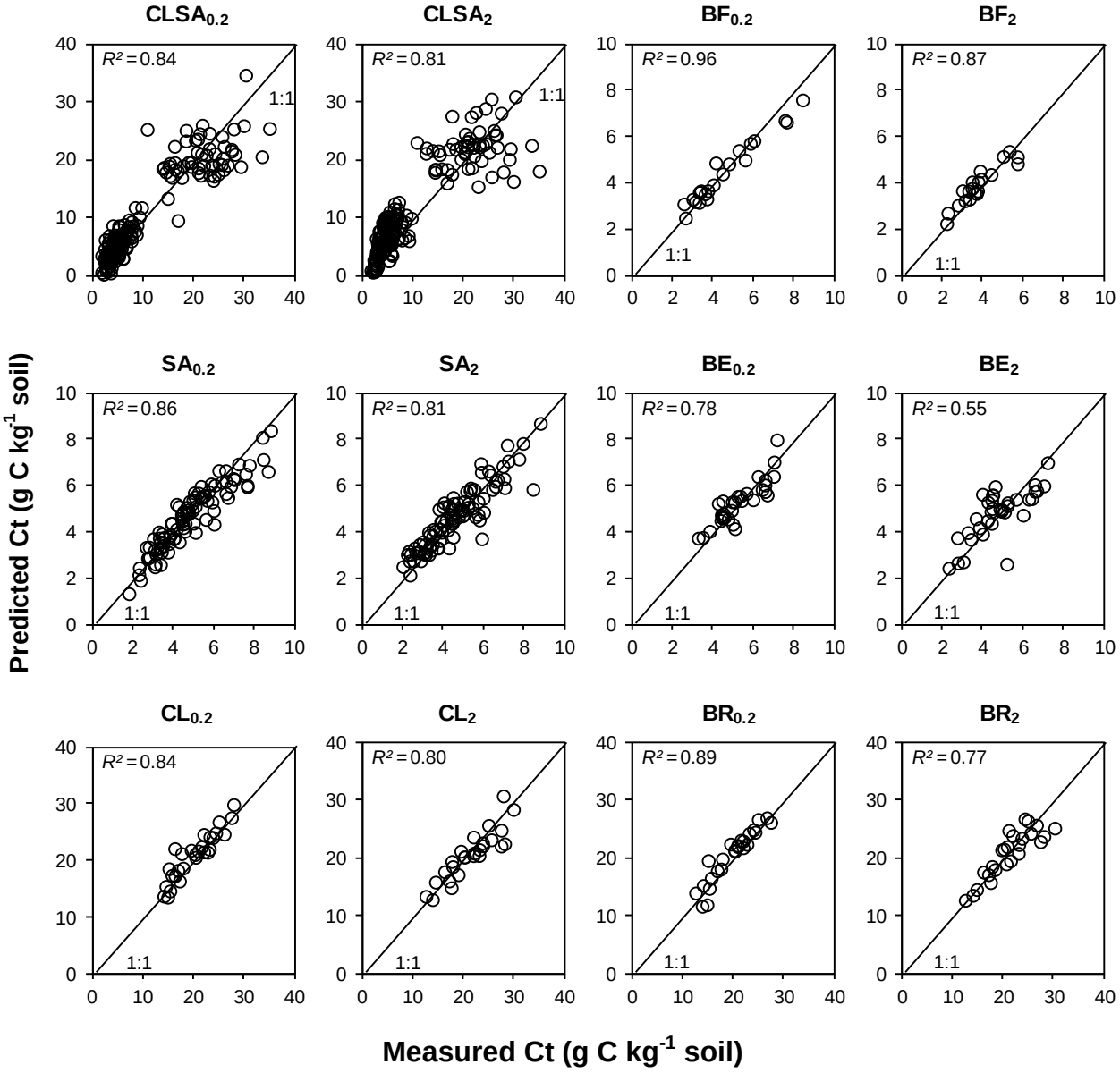

Figure 2. Comparison of measured and predicted Nt values for validation subsets from the total set (CLSA), the coarse-textured set (SA), the clayey set (CL), Burkina Faso (BF), Benin (BE), and Brazil (BR), using 0.2 mm ground or 2 mm sieved soil samples (with SNV 144).

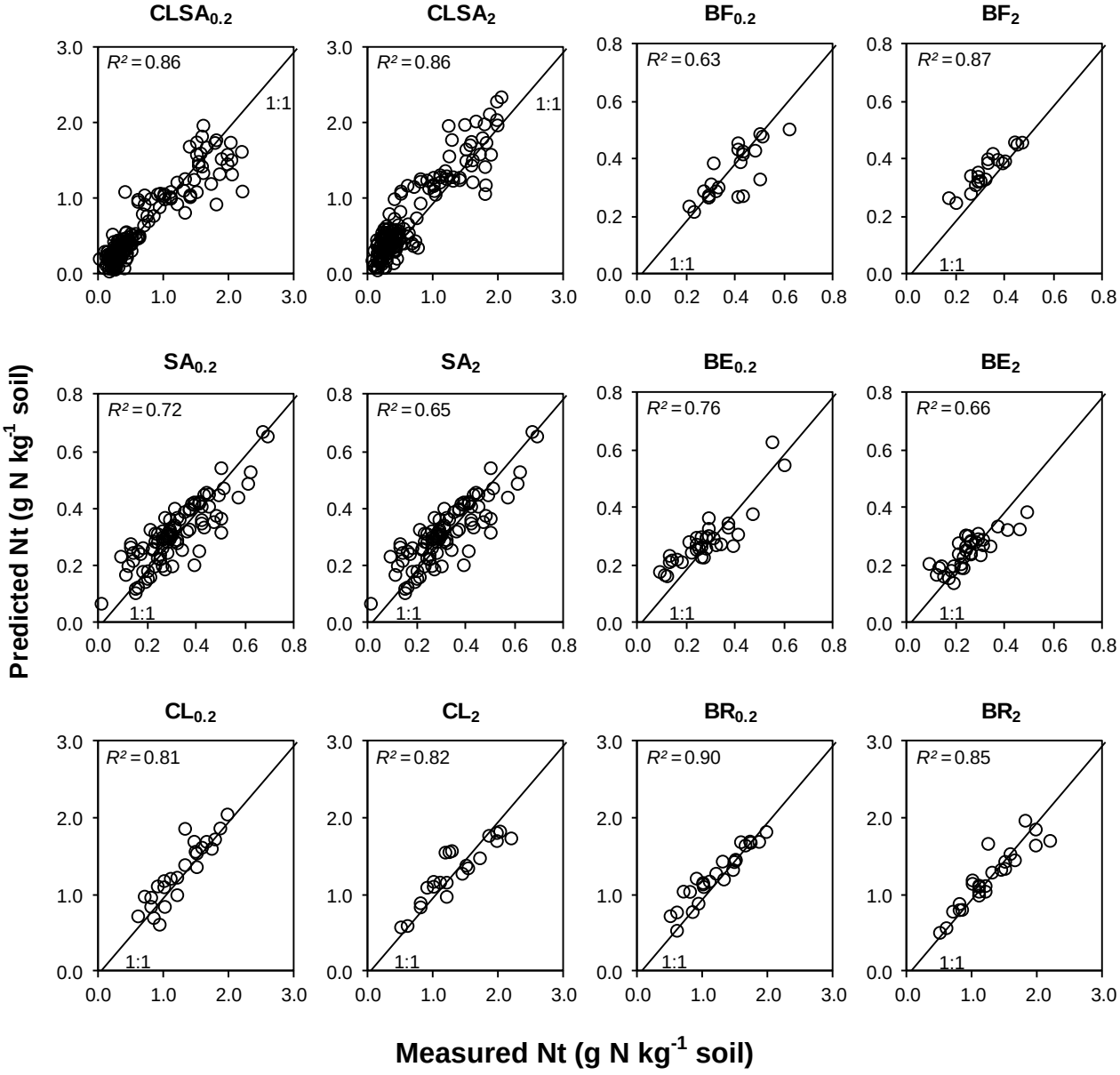

Figure 3. Effect of set heterogeneity, expressed by the difference between maximum and minimum clay content (denoted clay range), on validation CV and R^2 for Ct (or Corg) and Nt. Comparison of data from this study and from the literature (References: Dalal and Henry (1986), Ben-Dor and Banin (1995), Chang et al. (2001), Reeves and McCarty (2001), Chang and Laird (2002), Ludwig et al. (2002), McCarty et al. (2002), Shepherd and Walsh (2002), Coûteaux et al. (2003), Russell (2003), and Sørensen and Dalsgaard (2005); for most of these studies, clay range was estimated).