Comparing near and mid-infrared reflectance spectroscopy for determining properties of Malagasy soils, using global or LOCAL calibration

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Abstract

Nowadays near and mid-infrared reflectance (NIR, mid-IR) spectroscopy are recognized useful approaches for quantifying soil properties cost and time effectively. This work aimed at comparing predictions of soil carbon (C) and nitrogen (N) contents, C/N ratio, substrate induced respiration (SIR) and denitrifying enzyme activity (DEA) using NIR and mid-IR spectroscopy over a diverse set of 360 Malagasy topsoils. Partial least square regression was used for fitting NIR and mid-IR spectra to conventional data through procedures of calibration either global (one prediction model for all samples) or LOCAL (one prediction model per sample). Prediction accuracy was assessed according to validation r^2, standard error of prediction (SEP) in proportion of the mean, and ratio of standard deviation to SEP (RPD). Using both NIR and mid-IR spectroscopy, global calibration over the whole sample set yielded predictions that were excellent for C and N ($r^2 > 0.9$, SEP < 20%, RPD ≥ 3), good for C/N, acceptable for SIR, but poor for DEA. LOCAL calibration improved C/N and SIR predictions with both NIR and mid-IR spectroscopy, while DEA prediction became acceptable with NIR spectroscopy only. Additional improvement was achieved when LOCAL calibration was carried out over the fine-textured subset, especially for SIR ($r^2 > 0.9$, SEP < 20%, RPD > 3). By contrast, LOCAL calibration over the coarse-textured subset was not clearly useful for improving prediction accuracy. NIR outperformed mid-IR spectroscopy whatever the variable, the calibration procedure and the sample set (except for SIR over the coarse-textured subset, where both performed similarly), suggesting its possible superiority for tropical soils.

Keywords: Infrared spectroscopy; soil carbon; soil nitrogen; soil biological properties; LOCAL calibration

Introduction

Soil organic matter (SOM) is now well-recognized as a major factor controlling the capacity of soil resources to deliver agricultural and environmental services and sustain human societies.[1] Soil organic matter is an important component of soil fertility as it determines important soil functions such as nutrient mineralization or water retention. Recent concern about worldwide climate change has also increased interest for SOM and its role in the global carbon (C) and nitrogen (N) budgets through C sequestration and greenhouse gases (GHG) emissions. Thus, in agricultural systems, optimization of C and N cycling through SOM management can improve soil fertility and yields while reducing negative environmental impact.

Soil organic status as defined by the C and N concentrations has often been considered a reliable indicator for soil quality.[2] However, as soil microbiological properties are considered more sensitive than chemical and physical properties to changes in management and environmental conditions,[3] microbial-based indicators have often been used to directly account for the functional status of the soil.[4,5] Substrate-induced respiration (SIR) is a widely used physiological method for indirect measurement of soil microbial biomass.[6,7] When easily degradable substrate is added to soil an immediate increase in respiration rate (CO_2 emission) is obtained, the size of which is assumed to be proportional to the size of microbial biomass. Denitrifying enzyme activity (DEA, or potential denitrification) reflects the size of the pool of functionally active denitrifying enzymes in the soil. Denitrification is a major mechanism of loss of fertilizer N resulting in decreased efficiency of fertilizer use; it is also an important process of nitrous oxide (N_2O) emission from the soil to the atmosphere.[8] Measured in the laboratory under optimal conditions, the DEA assay reflects the enzymatic potential of the soil denitrifying bacteria to reduce nitrates (NO_3^-) to gaseous di-nitrogen (N_2) or to nitrous oxide (N_2O) when acetylene is added.[9] Beyond indications on soil functional status, SIR and DEA also provide information on the potential of soils to emit CO_2 and N_2O, respectively, which are important GHG linked to climate change.[10]

The usefulness of near infrared reflectance (NIR) spectroscopy for determining total C and N contents is well established now;[11-13] but its relevance has also been reported for characterizing soil biological properties such as microbial biomass,[11,14] respiration rates,[15,16]

potential nitrification,[14,17] and denitrification.[14,16] Indeed, NIR spectroscopy is a physical non-destructive, rapid, reproducible and low-cost method that characterizes materials according to their reflectance in the wavelength range between 800 and 2500 nm. It relies on calibration, which is a multivariate regression procedure that expresses a given property, determined using a conventional method, as a function of absorbance at all or selected wavelengths of the NIR region. The calibration equation can then be used to predict that property on new samples from their NIR spectra only, the acquisition of which is time- and cost-effective (< 1 min per sample, no consumables required).

Mid-infrared reflectance (mid-IR) spectroscopy is a similar approach but based on sample reflectance in the wavelength range between 2500 and 25,000 nm (i.e. 4000 and 400 cm^{-1}, respectively). In a review, Reeves[18] compared NIR and mid-IR technologies for soil C analysis and addressed their specificities as regards instrumentation, conditions of use (e.g. sample size and moisture) and even commercial support. Though less extensively and more recently used (for quantitative purposes) than NIR, mid-IR spectroscopy has similarly proven relevant for determining total soil C and N,[19,20] microbial biomass and mineralizable N.[20,21]

To date, most studies comparing both spectral domains have reported more accurate predictions of soil properties with mid-IR than with NIR spectroscopy (e.g. for soil C and N),[22-26] though the difference was sometimes slight. Superior performance of mid-IR spectroscopy has been reported to likely reflect higher information quality of on soil organic matter in the mid-IR spectral region.[27] Indeed, comparison between soil NIR and mid-IR spectra clearly shows that mid-IR spectra consist of many more defined peaks that NIR ones, the latter consisting of many overlapping combination and overtone peaks from the mid-IR region.[18,24] However, the published studies that compared predictions of soil properties using NIR and mid-IR spectroscopy often concerned rather homogeneous sample sets (e.g. collected in small areas or representing narrow textural ranges) and originated from temperate regions. It is worth noting that for Ferralsols originating from two distant Brazilian sites, Madari *et al.*[28] reported more accurate predictions using NIR than mid-IR spectroscopy for total C, while total N was slightly more accurately predicted by mid-IR spectroscopy. Considering soils from subtropical China, Shao and He[29] also reported that NIR outperformed mid-IR spectroscopy for predicting available N (but not available phosphorus and potassium; C was not studied).

Due to contrasted climatic conditions, Madagascar provides the major soil types used for food production in the tropics. The objective of the present study was to compare predictions using

NIR and mid-IR spectroscopy for total C and N, C/N ratio, SIR and DEA over a wide range of Malagasy soils.

Materials and methods

Soil collection

Soils were collected in order to obtain a sample set representative of the main soil types used for agriculture in Madagascar; the soil types under concern cover ca. 95% of the surface area of Madagascar.[30] The studied samples originated from eight sites from four regions with contrasted pedoclimatic conditions: the central Highlands (near the city of Antsirabe; sites 1, 3 and 5 in Table 1), which are under highland tropical climate; the eastern subequatorial coast (Manakara; site 2); the sub-arid Southwest (Tulear; sites 4 and 8); and the Northwest lowlands around the Alaotra lake under mid-altitude tropical climate (sites 6 and 7). The soil sample set also offered a wide range of texture, from clayey to sandy.

((Table 1))

Soil samples were collected in the experimental designs of the Tany sy Fampandrosoana (TAFA) NGO under cultivated systems with the local fallow serving as a control. Some soils were also sampled in adjacent farmer fields, especially when no fallow was available close to the experimental design. The agricultural systems consisted of rainfed rice (*Oryza sativa* L.) as the main crop in all sites, except at Tulear, where rainfalls are not sufficient for upland rice cultivation, and where maize (*Zea mays* L.) was cultivated. These crops were cultivated traditionally by local farmers without fertilization and after hand tillage. Agricultural practices in the experimental design comprised conventional tillage (CT) and direct seeding mulch-based cropping systems (DMC). In DMC systems the main crop was usually associated with a legume cover crop except at Antsirabe-Andranomanelatra, where DMC systems were conducted with or without legume association. Agricultural systems had been established for at least 5 years and received annual application of cattle manure with or without mineral (NPK) fertilizers, depending on the sub-treatment. Moreover, depending on the site and main treatment (i.e. CT, DMC, farmer plot, and fallow), each sub-treatment was represented by one to four plots (replicates; Table 1). According to sub-treatment and plot size, three or six composite samples were collected per plot, yielding a total set of 360 samples. Each composite soil sample was made of ten soil cores collected at 0-5 cm depth, using a 5 cm diameter core sampler. On the whole, the sample set studied was thus heterogeneous but included eight subsets that were fairly homogeneous from mineralogical and textural

viewpoints. Soil samples were air dried, sieved at 2 mm, and an aliquot was finely ground to pass a 0.2 mm mesh sieve.

Conventional analyses

Conventional determinations of soil total carbon (C) and nitrogen (N) contents were carried out on finely ground (< 0.2 mm) and oven-dried (at 40°C during 24 h) aliquots by dry combustion using an Elemental Analyzer CHN Carlo Erba NA 2000 (Milan, Italy).

Substrate induced respiration (SIR) was assayed using an adaptation of the method proposed by Anderson and Domsch.[6] Twenty g of dry soil (< 2 mm) were placed into a 150 ml airtight vial. A solution containing 1.5 mg of C-glucose g^{-1} soil was added to ensure 60% of the soil water holding capacity. The flasks were then incubated at 25°C and the headspace volume was sampled after 2 and 4 hrs and analyzed for carbon dioxide (CO_2) using a gas micro chromatograph (Varian 3900-GC, Varian Chromatography Group, Walnut Creek, CA, USA). SIR was expressed in µg C-CO_2 h^{-1} g^{-1} dry soil. The CO_2 emissions in the first hours after the addition of readily available substrate correspond to the maximal initial respiratory response of the microbial populations without significant microbial growth, and serve as a physiological estimate of the initial microbial biomass in soil.[6,7]

Similarly, the denitrifying enzyme activity (DEA) test measures the initial activity of denitrifying enzymes from data on N_2O accumulated over a short duration just after optimal conditions for denitrification have been achieved, i.e. without growth of denitrifying populations, as described by Lensi *et al.*[34] Briefly, 30 g of dry soil (< 2 mm) were put in a 150 ml airtight vial. The atmosphere of the vial was evacuated and replaced by a 90:10 helium-acetylene mixture to provide anaerobic conditions and inhibition of the N_2O reductase activity. Before incubation at 28°C, the soil was humidified at 100% of its water holding capacity with a nutritive solution containing potassium nitrate (0.2 mg N g^{-1} soil), glucose (1 mg C g^{-1} soil) and glutamic acid (1 mg C g^{-1} soil). The N_2O efflux was measured in the headspace atmosphere of the vials using a gas chromatograph equipped with an electron capture detector (Varian Star 3400 CX, Varian Chromatography Group, Walnut Creek, CA, USA). DEA was expressed in µg N-N_2O h^{-1} g^{-1} dry soil.

External soil samples serving as standards were included in each analytical series to estimate the reproducibility and accuracy of the measurements. Mean coefficient of variation (i.e. ratio of standard deviation to mean) was 7.3% for SIR and 10.6% for DEA.

Spectral analyses

Sample reflectance in the NIR range was measured between 1100 and 2500 nm (i.e. 9091 and 4000 cm^{-1}, respectively) at 2 nm intervals with a Foss NIRSystems 5000 spectrophotometer (Laurel, MD, USA). The scan was performed on a 42 mm^2 area of a 5 g subsample (0.2 mm ground, oven-dried at 40°C) packed in a ring cup. Each spectrum, averaged from 32 co-added scans, was recorded as absorbance (log [1/reflectance]). Absorbance bands being much wider than 2 nm in general and recorded spectra thus including redundant information, spectral data sets were reduced to condense information and facilitate computational calculations[13]. This was done by keeping the first out of four adjacent spectral points, as proposed by the WinISI software, yielding 173 data points per spectrum.

Sample reflectance in the mid-IR range was measured between 4000 and 400 cm^{-1} (i.e. 2500 and 25,000 nm, respectively) at 3.86 cm^{-1} intervals with a Nicolet 6700 FT-IR spectrophotometer (Thermo Fischer Scientific, Madison, WI, USA). The scan was performed on a 12.6 mm^2 area of a 0.5 g subsample (0.2 mm ground, oven-dried at 40°C) packed in a well of an 18 well plate. As for NIRS, spectra resulted from the averaging of 32 co-added scans, were recorded as absorbance, and were condensed by keeping the first out of four adjacent spectral points. In addition, both spectrum ends were discarded due to noise, and the range 3961.14-439.70 cm^{-1} only was considered, yielding 229 data points per spectrum.

All spectral data analyses were conducted using the WinISI III-v.1.61 software (Infrasoft International, LCC, State College, PA, USA).

Several mathematical pretreatments were evaluated for spectrum pre-processing in order to reduce baseline variations, enhance spectral features, reduce particle-size effect, remove linear or curvilinear trends of each spectrum, or remove additive or multiplicative signal effects:[22,35,36,37] no derivation (denoted 01), first- or second-order derivation with 4, 5 or 10 point gap and smoothing (denoted 14, 15, 110, 24, 25, and 210, respectively), alone (denoted None) or in conjunction with standard normal variate transform (SNV), detrending (D), both SNV and detrending (SNVD), or multiplicative scatter correction (MSC).

A principal component analysis was carried out on the spectral data of all samples for calculating the Mahalanobis distance H, and samples with $H > 3$ were considered spectral outliers and eliminated from further investigations.[38] The number of principal components used was that accounting for 99.9% of the total variance, and varied depending on the spectral range and the pretreatment (from 5 to 10 in the NIR, from 20 to 35 in the mid-IR). The sample set was then divided into a calibration subset, which included 150 samples, and a remaining validation subset. To optimize predictions on unknown samples originating from

the studied sites, the calibration subset was selected by the WinISI software to include the most representative samples of the set: 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 150 samples.[39], Validation was therefore external but not independent, which was not considered a problem because the objective was to compare the performances of NIR and mid-IR spectroscopy, not to maximize calibration robustness. Calibration models deriving reference values (e.g. soil C or N content) from absorbance spectra were built using partial least square (PLS) regression: PLS reduces the spectral data to a few orthogonal combinations of absorbance, called factors, that account for most spectral information and covary with reference values; cross validation being recommended for estimating the optimal number of factors in order to avoid overfitting.[39,40] Cross validation was performed by dividing the calibration subset into four groups, all but one being used for developing the model and one for prediction, the procedure being performed four times to use all samples for both model development and prediction. The residuals of all predictions were pooled to calculate the standard error of cross validation (SECV). Calibration outliers (i.e. with residual > 2.5 times SECV) were removed and another cross validation was performed, the procedure being carried twice, as recommended by WinISI. The number of factors after which final SECV no longer decreased meaningfully determined the optimal number of factors of the model. The model performance was evaluated on the validation subset (which had not been used for model development), according to standard error of prediction between predicted and measured values (SEP), corresponding coefficient of determination (r^2), and RPD (ratio of standard deviation to SEP); SEP was expressed in absolute value (e.g. $g\ kg^{-1}$ for soil C) and as proportion of mean reference value over the validation subset (in %). According to Chang *et al.*,[12] prediction models with RPD > 2 were considered accurate.

The above-described calibration procedure has been called “global” because a unique model is used to predict a given property for all samples of the validation subset.[41] In addition, LOCAL calibration was also carried out.[41] In that procedure, proposed in the WinISI software, a specific calibration equation is built for each sample of the validation subset, using samples selected from the calibration subset according to their similarity with that sample. Similarity is assessed by correlation coefficient between the spectrum of the sample to be characterized and those of the calibration subset samples. The number of calibration samples

was varied from 25 to 35 in steps of 5 in order to determine its optimum, which was assessed according to r^2, SEP and RPD. The optimal number of factors used in PLS regression was not determined through cross validation, which is not appropriate for LOCAL calibration.[41] Instead, each prediction was calculated as the weighed average of the predicted values generated with four to 15 PLS factors, each weight being calculated as the inverse of the product of root mean square (RMS) of X-residuals (i.e. difference between actual spectrum and spectrum approximated using the considered number of PLS factors) and RMS of regression coefficients. [41]

In order to assess the effect of set homogeneity on model performance, the total set was divided into a fine-textured set (192 samples with clay > 28%; i.e. sites 1 to 5) and a coarse-textured set (168 samples with clay < 28%; i.e. sites 6 to 8). Procedures for calibration (involving cross validation) and external validation were similar for the textural sets than for the total set, except that calibration subsets included 100 samples instead of 150.

Results

Conventional data

Table 2 presents statistics for the measured soil properties over the total set, fine-textured soils (sites 1 to 5), coarse-textured soils (sites 6 to 8), and for each site separately.

((Table 2))

Lowest C and N contents were observed in coarse-textured soils (sites 6 and 8), and highest values in the most clayey soil (site 1). The studied soil samples represented a quite wide range of C and N contents over the total set (from 6.4 to 59.1 g C kg^{-1} and from 0.75 to 4.67 g N kg^{-1}, respectively) but also within a site (see Table 2 for details). Even for site 3, which had the smallest sample size (n = 18), contrasted cultural practices resulted in a certain heterogeneity in soil properties (Table 1). When averaged per textural subset, the C and N contents were higher in fine- than in coarse-textured soils. The C-to-N ratio ranged from 6.9 to 14.8 with higher values in fine-textured soils. Substrate-induced respiration (SIR) varied from 0.3 to 13.6 µg $C\text{-}CO_2$ g^{-1} h^{-1}, and denitrifying enzyme activity (DEA) from values close to 0 to 11.2 µg $N\text{-}N_2O$ g^{-1} h^{-1}. The highest value of SIR was observed in a fine-textured soil (site 1) while DEA reached a maximum in a coarser-textured soil (site 6; Table 2). However, mean values for SIR or DEA did not differ significantly when samples were separated according to texture (Table 2). Over the total set, soil C and N contents were strongly correlated one to another ($R^2 = 0.96$; $p < 0.001$; n = 360), but they correlated poorly with SIR and DEA ($R^2 < 0.11$; see Table 3 for details). Over the fine-textured set, C and N contents correlated

more closely though still weakly with DEA (R^2 = 0.40 and 0.43 respectively; $p < 0.001$; n = 192), but not with SIR (R^2 = 0.03 and 0.05 respectively; $p < 0.05$; n = 192; Table 3). Over the coarse-textured set, most correlations between C or N and SIR or DEA were not significant ($R^2 < 0.10$; n = 168; Table 3).

((Table 3))

Predictions of soil properties on the total sample set using global calibration

Over the total set and using global calibration, the mathematical pretreatment that provided most accurate predictions with NIR spectroscopy was None 14, while most accurate predictions with mid-IR spectroscopy were achieved with SNV 01 for C, SIR and DEA, and with MSC for N and C/N (data not shown). With these pretreatments, the number of spectral outliers was 4 for NIR (i.e. ~1% of set size) and 0 or 1 for mid-IR (≤ 0.3%), depending on the variable considered. The number of calibration outliers ranged from 6 to 11 for NIR (i.e. 4-8% of calibration subset) and from 7 to 13 for mid-IR (5-9%), in the range reported by studies that have mentioned this information.[13,27]

As shown in Table 4, predictions of C and N using NIR and mid-IR spectroscopy were excellent using global calibration, and NIR outperformed mid-IR (r^2 = 0.93-0.97 vs. 0.92, SEP = 10-11% vs. 14-17%, RPD = 3.8-5.5 vs. 3.0-3.3, respectively). In addition, C was more accurately predicted than N using NIR spectroscopy (e.g. r^2 = 0.97 vs. 0.93), but this was less clear using mid-IR spectroscopy (e.g. r^2 = 0.92 vs. 0.92). The C/N ratio was accurately predicted too, though to a lesser extent, and NIR again outperformed mid-IR (e.g. r^2 = 0.83 vs. 0.78). The prediction of SIR was less satisfactory: it remained fairly accurate using NIR but not using mid-IR (r^2 = 0.79 vs. 0.66, SEP = 24% vs. 31%, RPD = 2.2 vs. 1.6). By contrast, DEA was poorly predicted, with mid-IR especially (e.g. r^2 = 0.28). Figure 1 compares measured vs. predicted values of SIR with NIR and mid-IR spectroscopy using global calibration over the total set.

((Table 4))

((Figure 1))

Predictions of soil properties on the total sample set using LOCAL calibration

Over the total set and using LOCAL calibration, best predictions using NIR spectroscopy were achieved with pretreatment None 14 for C, N, C/N and DEA, but with None 01 for SIR; best predictions using mid-IR spectroscopy were always achieved with SNV 01 (data not shown). With these pretreatments, the number of spectral outliers was 3 or 4 for NIR (i.e.

~1% of set size), and was 1 for mid-IR (0.3%). No calibration outliers are identified in the calibration subset through LOCAL calibration as properties are predicted for each sample separately, using samples selected from the calibration subset according to their spectral proximity with that particular sample.

Whatever the variable considered, LOCAL calibration improved predictions when compared with global calibration (Table 4): predictions of C and N using NIR and mid-IR spectroscopy, which were very accurate using global calibration, became even better (e.g. for NIR prediction of C yielded $r^2 = 0.99$, SEP = 6%, and RPD > 8); the prediction of C/N became excellent with NIR ($r^2 = 0.92$) and fairly accurate with mid-IR ($r^2 = 0.84$), as became also predictions of SIR with NIR and mid-IR ($r^2 = 0.86$ and 0.76, respectively); the prediction of DEA became acceptable with NIR ($r^2 = 0.61$, RPD = 1.5) but not with mid-IR ($r^2 = 0.35$, RPD = 1.3). The accuracy of predictions with both NIR and mid-IR decreased as follows: C > N > C/N > SIR > DEA. In addition, using LOCAL calibration over the total set still resulted in more accurate predictions using NIR than mid-IR. Figure 2 compares measured vs. predicted values of SIR with NIR and mid-IR spectroscopy using LOCAL calibration over the total set.

((Figure 2))

Improvement upon LOCAL calibration was clearer with mid-IR than with NIR spectroscopy as regarded C (e.g. r^2 increased from 0.92 to 0.97 for mid-IR and from 0.97 to 0.99 for NIR); but the trend was opposite for N (e.g. r^2 increased from 0.92 to 0.94 for mid-IR and from 0.93 to 0.97 for NIR), C/N and DEA (e.g. r^2 increased from 0.28 to 0.35 for mid-IR and from 0.41 to 0.61 for NIR), while no clear trend could be seen for SIR.

Predictions of soil biological properties on textural subsets using LOCAL calibration

Attempts were made to improve prediction accuracy for SIR and DEA by increasing set homogeneity through textural distinction (< 28% vs. > 28% clay), again using LOCAL calibration (Table 5). This was not done for C, N and C/N, which were already predicted accurately using LOCAL calibration without this distinction. For NIR, most useful pretreatments for coarse- and fine-textured subsets were SNV 01 and None 01 for SIR, and SNV 14 and SNV 01 for DEA, respectively; for mid-IR, None 01 was the most useful pretreatment for SIR and DEA in both textural subsets (data not shown). With these pretreatments, the number of spectral outliers in the coarse-textured subset was 5 for NIR (i.e. 3% of subset size) and 0 or 1 for mid-IR (≤ 0.6%); in the fine-textured subset, it ranged from

0 to 2 for NIR and mid-IR (≤ 1%). There are no calibration outliers in LOCAL calibration (see previous section).

((Table 5))

Both variables were more accurately predicted over the fine-textured subset than over the total set: predictions of SIR using NIR and mid-IR spectroscopy became excellent (r^2 = 0.93-0.95 vs. 0.76-0.86, SEP = 15-18% vs. 20-24% of the mean, RPD = 3.4-4.3 vs. 2.0-2.5, respectively); DEA prediction became fairly accurate with NIR (r^2 = 0.74 vs. 0.61, RPD = 1.9 vs. 1.5) but not with mid-IR (r^2 = 0.45, RPD = 1.3).

By contrast, predictions were not improved over the coarse-textured subset using NIR spectroscopy: for SIR they were worse than over the total set (e.g. r^2 = 0.77 vs. 0.86), and for DEA they were similar than over the total set. Regarding mid-IR, SIR prediction tended to be more accurate over the coarse-textured subset than over the total set (e.g. r^2 = 0.80 vs. 0.76), but this was less clear for DEA. As a consequence, predictions of SIR using NIR and mid-IR had similar accuracy over the coarse-textured subset.

Predictions were more accurate over the fine- than over the coarse-textured subset, especially for SIR and/or using NIR. Moreover, in both textural subsets SIR remained more accurately predicted than DEA using a given approach (NIR or mid-IR spectroscopy), and on the whole, NIR again outperformed mid-IR (except for SIR on the coarse-textured set as mentioned above).

Figure 3 compares measured vs. predicted values of SIR with NIR and mid-IR spectroscopy using LOCAL calibration over each textural subset.

((Figure 3))

Discussion

Accuracy of predictions of soil properties with NIR and mid-IR spectroscopy using global calibration

Using global calibration and PLS regression, predictions of soil C and N with NIR and mid-IR spectroscopy were excellent, as reported in reviews by Malley *et al.*[42] for NIR and by Viscarra Rossel *et al.*[24] for NIR and mid-IR. It is worth noting that the set studied here was heterogeneous geographically and texturally while accurate predictions reported in the literature have often concerned more homogeneous sample sets (e.g. originating from one site); knowing that the accuracy of prediction models tends to increase with set homogeneity[43] even though minimum variability is required for calibration.[44] Using global calibration, soil C/N was fairly accurately predicted, and this is consistent with literature data, which are much

less numerous than regarding C and N contents: for example, with NIR, validation r^2, SEP and RPD of 0.88, 12% and 3.1, respectively, were reported by Chang and Laird[45] for a set including mixtures of five Midwest USA topsoils with limestone and organic materials, and values of 0.88, 11% and 3.5, respectively, were reported by Barthès *et al.*[46] for a range of sandy tropical topsoils (vs. 0.83, 7% and 2.4 in the present study, respectively); with mid-IR, cross-validation R^2, SECV and RPD of 0.70, 6% and 1.8 respectively (vs. 0.78, 8% and 2.1 for validation in the present study), were reported by McCarty and Reeves[23] for a sample set originating from a 20 ha field, while cross-validation R^2 of 0.96 was reported by Ludwig *et al.*[21] for a set of topsoil and litter samples originating from 16 sites in north-western Europe.

Soil microbial activities were less accurately predicted that C, N and C/N. Nevertheless, the prediction of SIR was satisfactory with NIR (validation $r^2 = 0.79$, SEP = 24%, RPD = 2.2). This agrees with literature results regarding the prediction of soil respiration, which mostly involved VisNIR (visible and NIR, i.e. 400-2500 nm): Chodak *et al.*[47] and Schimann *et al.*[16] achieved acceptably accurate predictions of SIR on smaller sets (calibration $R^2 = 0.86$ and 0.73, SECV = 39-40% and RPD = 1.6-1.7, for 80-100 forest topsoils from different sites in Poland, and for two reforestation chronosequences in French Guiana, respectively); Palmborg and Nordgren[15] obtained better prediction of SIR but on a small homogeneous set (cross-validation $R^2 = 0.88$ for 30 forest topsoil samples at one site). By contrast, DEA was poorly predicted by NIR spectroscopy in the present study (validation $r^2 = 0.41$, SEP = 114%, RPD = 1.3). Using global calibration for soil DEA prediction, Cécillon *et al.*[14] also obtained poor results on a smaller set (cross-validation $R^2 = 0.38$, SECV = 67% and RPD = 1.3 for 50 topsoil and earthworm cast samples from forest plots affected by wildfire), while Schimann *et al.*[16] reported good results on reforestation chronosequences (cf. above; calibration $R^2 = 0.85$, SECV = 59% and RPD = 2.2). As regarded predictions using mid-IR spectra and global calibration, results were barely acceptable for SIR and disappointing for DEA. No comparable studies were found in the literature. It is worth noting that part of prediction imprecision attributed to NIR or mid-IR spectroscopy actually resulted from the variability of conventional data: indeed, laboratory replication carried out on external samples yielded coefficients of variation of ca. 7% for SIR and 11% for DEA.

Prediction improvement through LOCAL calibration and sample stratification

LOCAL calibration improved predictions of C, N, C/N, SIR and DEA for both NIR and mid-IR spectroscopy. For a given variable, LOCAL calibration builds a prediction model for each sample separately using spectral neighbours selected in the calibration subset, while global

calibration uses the whole calibration subset to build a unique prediction model for all samples. Several authors have reported more accurate prediction of soil properties with LOCAL than with global PLS calibration using NIR spectra: for organic C, N, clay, silt and sand over a large and diverse set from Italy;[48] and for organic C, clay and cation exchange capacity (CEC) over a large and diverse set from Belgium.[49] LOCAL PLS regression even outperformed least squares support vector machine regression (based on supervised learning) for predicting C and CEC, but not N, for the Belgium set.[50] Igne *et al.*[25] did not report clearly better predictions of C, N and texture with a kind of LOCAL PLS calibration than with global PLS calibration, but the set they studied was rather homogeneous (315 topsoils from five fields in Maryland), which does not highlight the advantages of LOCAL-like methods.

Sample stratification on the basis of soil texture was partially satisfactory. For the fine-textured subset, predictions of SIR and DEA were more accurate than for the total set. Stratification effect was less clear for the coarse-textured subset, as change in prediction accuracy depended on the soil property and spectral range: for SIR, improvement with mid-IR but degradation with NIR; for DEA, little change with either NIR or mid-IR. Schimann *et al.*[16] used VisNIR spectroscopy to predict SIR and DEA on soils from two reforestation chronosequences and also performed textural stratification. Regarding SIR, they reported that cross validation was better in the clayey subset ($R^2 = 0.89$) than in the total set (RPD = 0.73), but that it was worse in the sandy subset ($R^2 = 0.31$). Regarding DEA, cross validation were similar in the clayey subset ($R^2 = 0.84$) and the total set ($R^2 = 0.85$), and slightly worse than in the sandy subset ($R^2 = 0.87$). According to Schimann *et al.*[16], textural stratification had thus variable effects on SIR prediction depending on the texture, and little effect on DEA prediction. Along with the results of the present study, this suggests that textural stratification is useful for fine-textured samples but not for coarse-textured ones. Moreover, less accurate predictions of biological properties in coarse-textured samples could be caused by their lower content in organic matter and the greater heterogeneity of its spatial distribution, which might lead to discrepancies between subsamples used for conventional and spectral analyses.[46] Studying organic C in a set of 1626 soil samples from northern Belgium grasslands, Van Waes *et al.*[51] also observed that the accuracy of prediction using NIR spectroscopy decreased as follows: clayey set > total set > sandy set.

It is worth noting that whatever the sample set, R^2 for the relationship between SIR or DEA and C or N (cf. Table 3) was much lower than corresponding validation r^2 for prediction of SIR or DEA using NIR spectroscopy (cf. Tables 4 and 5; e.g. in the fine-textured set: $R^2 = 0.40$ between C and DEA vs. validation $r^2 = 0.74$ for the prediction of DEA using NIR

spectroscopy). Thus predictions of SIR and DEA using NIR spectroscopy could not be considered as resulting from the prediction of C or N and correlation between C or N and SIR or DEA. Similarly, correlations between SIR or DEA and C or N were not close enough for explaining indirect prediction of SIR and DEA as a result of C or N prediction.

Comparison between predictions of soil properties using NIR and mid-IR spectroscopy

Whatever the variable, the calibration procedure and the set considered, NIR provided more accurate predictions than mid-IR for the studied soil samples (except for SIR in the coarse-textured subset, where both domains yielded similarly accurate predictions). This contrasts with most published comparisons between spectral domains, which have concluded that mid-IR produced more accurate predictions than NIR for soil C and N contents in general.[20,22-28] It is worth noting that most of these comparisons concerned soils from temperate regions such as Maryland[20,22,23,25] or west central North America[26,27], and that results regarding soils from tropical areas were not very conclusive. For example, in a representative range of Brazilian soils, Madari *et al.*[52] reported that mid-IR generally outperformed NIR for C prediction except when prediction was carried out on fairly homogeneous textural sets. On a sample set originating from two Brazilian Ferralsols, Madari *et al.*[28] obtained slightly more accurate N predictions with mid-IR (calibration $R^2 = 0.99$ vs. 0.97) but clearly more accurate C predictions with NIR (0.99 vs. 0.93). For a range of topsoils from subtropical China, Shao and He[29] observed better predictions of available N with NIR than with mid-IR, but they observed the opposite for available phosphorus and potassium (C was not studied). Moreover, for Maryland soils, Reeves *et al.*[20] reported that mid-IR outperformed NIR for soil C and N predictions but that both provided similarly accurate predictions for soil mineralizable N and microbial biomass N. Ludwig *et al.*[21], who studied topsoil and litter samples from 16 sites in north-western Europe, concluded that mid-IR was not superior to NIR for predicting soil and litter properties: indeed, when compared with mid-IR, NIR yielded more accurate predictions for C, N, lignin content and N mineralization, but less accurate for microbial biomass and C/N. Also studying Maryland topsoils, Igne *et al.*[25] reported better C and texture predictions with mid-IR but better N predictions with NIR, when using comparable bench-top devices. For a range of topsoils from temperate China, Dong *et al.*[53] similarly reported that some variables were better predicted with mid-IR, such as pH in water and concentrations in organic matter, arsenic, copper, while others were better predicted with NIR, such as zinc, lead and chromium concentrations. In a comparable way, Yang *et al.*[54], who studied particle-size fraction C and N in a Canadian clay loam gleysol, concluded that fine-fraction C and N

were better predicted with mid-IR but coarse-fraction C and N with NIR. Though the review of Reeves[18] emphasizing soil C analysis mentioned that mid-IR is often more accurate and produces more robust calibrations than NIR when analyzing dried ground samples, this does not seem generalizable for all soils and soil properties.

The higher accuracy of mid-IR compared to NIR spectroscopy predictions of soil properties has been attributed to the considerably more details that exist in mid-IR spectra allowing more robust calibrations.[22,27] Furthermore, as stated by Ludwig *et al.*,[21] it is generally assumed that the mid-IR region is more useful than the NIR region because the former is dominated by intensive vibration fundamentals and the latter by weaker and broader signals from vibration overtones and combination bands. The superiority of mid-IR spectroscopy has mainly been inferred from studies on soils from temperate regions but it may possibly not hold for soils from tropical regions. Mineralogy often represents an important difference between soils from both regions, and minerals such as iron (Fe) and/or aluminium (Al) sesquioxides (mainly hematite, goethite and/or gibbsite) are abundant in tropical regions, especially in Madagascar but much less frequently in temperate regions. Moreover, it is well established that minerals have a much greater signature in the mid-IR region than in the NIR one, as the latter contains primarily information from the organics and from hydroxyls.[18,20,24] In addition, minerals and organics may absorb in similar mid-IR regions. For instance, metal oxides may absorb in the regions 1020-970 cm^{-1} (when more than one oxygen atom is bound to a single metal atom) and 1100-825 cm^{-1} (when containing a metal-to-oxygen double bound).[55] Carbohydrates absorb in the regions 1080-1030 and 960-730 cm^{-1}, and polysaccharides in the region 1170-950 cm^{-1}.[56] Silica also absorbs at 1130-1110 cm^{-1}, but is not specific to soils from tropical regions.[55] Such proximity or overlap of absorption regions relating to mineral and organic components may represent obstacles for prediction of soil organic properties with mid-IR. We suggest the hypothesis that information useful for the prediction of soil organic and biological properties could be partially masked in mid-IR spectra, due to the abundance of minerals such as Fe and Al sesquioxides. This might explain why NIR outperformed mid-IR in the present study.

It is worth noting that some cited studies[24,29] compared predictions using mid-IR spectra of finely ground samples and NIR spectra of more coarsely prepared samples, while others did not clearly specify sample preparation.[20,25,27] Difference in sample preparation might be an artifactual reason for more accurate predictions with mid-IR than with NIR. Indeed, as often reported for soil C and N contents, fine grinding increases the accuracy of predictions in general.[13,22,43] Most comparisons between predictions of soil properties using mid-IR and NIR

spectra of similarly ground samples, as in the present study, did not demonstrate clearly the superiority of mid-IR spectroscopy.[21,28,52,53,54] In the present study, another possible artifactual reason for more accurate predictions with NIR than mid-IR could be the larger area scanned for the former (42 mm²) than for the latter (12.6 mm²). However, as scanned samples were finely ground (< 0.2 mm) thus homogenised, the influence of scanned area on prediction accuracy was probably limited; but it could not be excluded completely. This possible artefact was hardly avoidable because scanning similar area with both types of spectrometers is not easy. By contrast, preparing samples similarly (e.g. < 0.2 mm) is easily achievable and should always be the rule when comparing predictions of soil properties using NIR and mid-IR spectra.

Conclusions

The studied sample set was representative of most agricultural soils from Madagascar and covered wide ranges of SOM content and texture. Over this large and heterogeneous set, global calibration of NIR and mid-IR spectra using PLS regression resulted in predictions that were excellent for C and N contents, good for C/N ratio, acceptable for SIR, but poor for DEA. LOCAL calibration over the whole set improved prediction accuracy for all variables: predictions became excellent or good for C/N and SIR; they became acceptable for DEA with NIR but not with mid-IR. Considering two textural subsets separately had variable effects. Predictions were more accurate for the fine-textured subset compared to the total set, being excellent for SIR with both NIR and mid-IR, and acceptable for DEA with NIR (but not with mid-IR). By contrast, predictions were not clearly better for the coarse-textured subset compared to the total set. Improvement of prediction accuracy using LOCAL calibration and/or for fine-textured soils only is consistent with the few papers that have addressed comparable questions.

This work shows that NIR provided more accurate predictions than mid-IR whatever the variable, the calibration procedure, and the sample set considered (except for SIR in the coarse-textured subset, where both domains yielded similarly accurate predictions). A priori, this contrasts with a number of published studies which have often reported more accurate predictions of soil properties with mid-IR than with NIR. However, as far as it does not depend on artefacts such as finer grinding of subsamples scanned in the mid-IR range, the reported superiority of mid-IR may be limited to soils from temperate areas; indeed, the few studies on tropical soils have not observed it clearly. We suggest that the possible superiority of NIR for predicting the organic and biological properties of tropical soils could result from

the abundance of some minerals, and as a consequence, the possible masking of organic matter peaks by the mineral component peaks in the mid-IR spectra. Further investigations are necessary for addressing the influence of mineralogy on the predictions of soil organo-biological properties using NIR and mid-IR spectroscopy. In addition, further work should address the accuracy of predictions for samples originating from new Malagasy sites, as proposed by Brown *et al.*[57], in order to test the robustness of predictions using the present sample set for calibration.

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Table 1. Presentation of the study sites and soil samples (0-5 cm depth).

Site No.	Site location	Elevation (m)	Mean rainfall (mm yr^{-1}) / air temperature (°C)	Soil type[a] (CPCS / FAO)	Average clay-silt-sand (%)[b]	Land use[c]
1	Antsirabe - Andranomanelatra 19°46' S, 47°06' E N = 48	1650	1600 / 16	Ferrallitic soil / Ferralsol	60-20-20	CT (1×2×6), DMC (1×4×6), fallow (1×2×3), farmer plot (1×2×3)
2	Manakara - Andasy 22°12' S, 47°50' E N= 60	50	2500 / 23	Ferrallitic soil / Ferralsol	45-40-15	DMC (2×4×6), fallow (1×2×3), farmer plot (1×2×3)
3	Antsirabe - Antsapanimahazo 19°40' S, 47°09' E N= 18	1700	1600 / 16	Ferrallitic soil / Ferralsol	35-40-25	CT (1×1×6), DMC (1×1×6), fallow (1×2×3)
4	Tulear - Andranovory 23°07' S, 44°13' E N = 36	440	700 / 28	Fersiallitic soil / Cambisol	30-25-45	DMC (2×3×3), fallow (1×2×3), farmer plot (1×2×3)
5	Antsirabe – Ivory 19°33' S, 46°24' E N= 30	940	1200 / 16	Ferrallitic soil / Ferralsol	30-35-35	CT (1×1×6), DMC (1×2×6), fallow (1×2×3), farmer plot (1×2×3)
6	Alaotra Lake - Marololo Baiboho 17°32' S, 48°31' E N = 78	770	1200 / 20	Alluvial soil / Fluvisol	20-20-60	CT (2×3×6), DMC (2×3×6), farmer plot (1×2×3)
7	Alaotra Lake - Marololo Tanety 17°32' S, 48°32'E N = 54	800	1200 / 20	Ferrallitic soil / Ferralsol	20-35-45	CT (2×1×6), DMC (2×3×6), fallow (1×2×3)
8	Tulear - Sakaraha 22°54' S, 44°37' E N = 36	640	800 / 28	Ferruginous soil / Arenosol	10-10-80	DMC (2×4×3), fallow (1×2×3), farmer plot (1×2×3)

[a] according to the CPCS[30] and FAO[31] classifications.
[b] from Razafimbelo[32].
[c] conventional tillage (CT), direct seeding mulch-based cropping systems (DMC); the figures refer to: (i) the number of "input" sub-treatments per treatment × (ii) the number of plot replicates per sub-treatment × (iii) the number of composite samples per plot, respectively.

Table 2. Total carbon (C, g kg^{-1}) and nitrogen (N, g kg^{-1}), C-to-N ratio, substrate induced respiration (SIR, µg $C\text{-}CO_2$ g^{-1} h^{-1}), and denitrifying enzyme activity (DEA, µg $N\text{-}N_2O$ g^{-1} h^{-1}) in the studied samples, as determined by conventional methods (n: number of samples per set; Min: minimum; Max: maximum; SD: standard deviation)

Variable		Total	Site 1	Site 2	Site 3	Site 4	Site 5	Fine-textured	Site 6	Site 7	Site 8	Coarse-textured
	n	360	48	60	18	36	30	192	78	54	36	168
C	Min	6.4	24.5	32.2	28.7	11.0	9.4	9.4	6.4	12.2	6.6	6.4
	Max	59.1	59.1	47.4	47.0	23.6	21.8	59.1	19.6	24.6	29.1	29.2
	Mean	24.0	41.7	39.8	36.7	17.8	16.0	32.2	12.1	17.0	15.6	14.5
	SD	13.0	8.9	3.6	5.1	2.7	4.3	12.4	3.6	2.9	5.1	4.3
N	Min	0.75	1.95	2.20	2.08	1.21	1.09	1.09	0.74	1.38	0.75	0.75
	Max	4.67	4.67	3.58	3.63	1.86	1.82	4.67	2.03	2.51	2.36	2.51
	Mean	2.04	3.20	3.04	2.81	1.56	1.37	2.52	1.28	1.81	1.45	1.49
	SD	0.86	0.71	0.32	0.42	0.18	0.22	0.88	0.29	0.29	0.37	0.39
C/N	Min	6.9	12.3	12.2	12.5	9.1	8.5	8.4	6.9	8.3	8.7	6.9
	Max	14.8	14.8	14.6	13.8	13.6	14.4	14.8	11.6	10.8	12.3	12.4
	Mean	11.2	13.1	13.1	13.1	11.4	11.5	12.5	9.3	9.4	10.6	9.6
	SD	1.9	0.6	0.5	0.4	1.1	1.8	1.2	1.2	0.4	1.0	1.1
SIR	Min	0.3	2.6	0.8	3.0	5.7	1.6	0.8	2.5	2.2	0.4	0.4
	Max	13.6	11.3	4.2	8.7	13.6	7.1	13.6	11.5	6.3	9.5	12.5
	Mean	5.2	5.9	2.1	4.9	9.2	4.0	4.9	6.7	4.4	4.6	5.5
	SD	2.7	2.1	0.9	1.7	1.8	1.4	3.0	2.1	1.1	2.4	2.3
DEA	Min	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0
	Max	11.2	7.8	6.1	4.1	0.3	0.1	7.8	11.2	1.0	5.2	11.2
	Mean	1.2	1.6	2.0	1.1	0.1	0.0	1.2	2.0	0.2	0.8	1.2
	SD	1.7	1.7	1.7	1.3	0.1	0.0	1.7	2.2	0.2	1.0	1.7

Table 3. Coefficient of determination (R^2) between some soil properties (total content in carbon, C, and nitrogen, N; substrate induced respiration, SIR; and denitrifying enzyme activity, DEA) for total, coarse- and fine-textured sample sets.

Sample set	C vs. N	C vs. SIR	C vs. DEA	N vs. SIR	N vs. DEA
Total (n = 360)	0.96***	0.02*	0.10***	NS	0.11***
Coarse-textured (n = 168)	0.94***	0.09***	NS	NS	NS
Fine-textured (n = 192)	0.98***	0.03*	0.40***	0.05**	0.43***

Asterisks indicate the statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; NS: not significant).

Table 4. Validation results regarding the predictions of total soil carbon (C) and nitrogen (N), substrate induced respiration (SIR) and denitrifying enzyme activity (DEA) with NIR and mid-IR spectroscopy using global or LOCAL calibration over the total set.

Variable	Parameter	Global calibration		LOCAL calibration	
		NIR	mid-IR	NIR	mid-IR
C	n[a]	206	209	206	209
	SEP[b] (g kg^{-1})	2.1	3.1	1.4	1.7
	SEP[b] (%)	10	17	6	9
	bias (g kg^{-1})	0.2	-0.5	-0.4	0.3
	r^2	0.97	0.92	0.99	0.97
	RPD[c]	5.5	3.3	8.6	6.1
N	n	206	210	206	209
	SEP (g kg^{-1})	0.21	0.25	0.14	0.18
	SEP (%)	11	14	7	10
	bias (g kg^{-1})	-0.01	0.11	-0.05	0.02
	r^2	0.93	0.92	0.97	0.94
	RPD	3.8	3.0	5.6	4.0
C/N	n	206	210	206	208
	SEP	0.8	0.8	0.5	0.7
	SEP (%)	7	8	5	6
	bias	-0.0	-0.4	0.0	-0.0
	r^2	0.83	0.78	0.92	0.84
	RPD	2.4	2.1	3.4	2.5
SIR	n	194	196	194	196
	SEP (µg C-CO_2 g^{-1} h^{-1})	1.2	1.7	1.0	1.3
	SEP (%)	24	31	20	24
	bias (µg C-CO_2 g^{-1} h^{-1})	0.1	0.6	0.1	0.2
	r^2	0.79	0.66	0.86	0.76
	RPD	2.2	1.6	2.5	2.0
DEA	N	185	187	185	179
	SEP (µg N-N_2O g^{-1} h^{-1})	1.3	1.6	1.1	1.4
	SEP (%)	114	130	105	121
	bias (µg N-N_2O g^{-1} h^{-1})	0.3	0.4	0.1	0.4
	r^2	0.41	0.28	0.61	0.35
	RPD	1.3	1.2	1.5	1.3

[a] n is the number of samples of the validation subset.
[b] SEP is the standard error of prediction, either expressed in the variable unit or in proportion of the validation subset mean (conventional determinations).
[c] RPD is the ratio of standard deviation (of the validation subset) to SEP.

Table 5. Validation results regarding the predictions of substrate induced respiration (SIR) and denitrifying enzyme activity (DEA) with NIR and mid-IR spectroscopy using LOCAL calibration in the coarse- and fine-textured subsets.

Variable	Parameter	Coarse-textured subset		Fine-textured subset	
		NIR	mid-IR	NIR	mid-IR
SIR	n[a]	54	58	88	88
	SEP[b] (µg C-CO_2 g^{-1} h^{-1})	1.1	1.0	0.7	0.9
	SEP[b] (%)	18	19	15	18
	bias (µg C-CO_2 g^{-1} h^{-1})	-0.0	0.2	-0.1	-0.1
	r^2	0.77	0.80	0.95	0.93
	RPD[c]	2.1	2.2	4.3	3.4
DEA	n	57	61	74	75
	SEP (µg N-N_2O g^{-1} h^{-1})	1.2	1.2	0.7	1.2
	SEP (%)	97	114	90	102
	bias (µg N-N_2O g^{-1} h^{-1})	-0.2	0.2	0.1	0.31
	r^2	0.60	0.37	0.74	0.45
	RPD	1.5	1.3	1.9	1.3

[a] n is the number of samples of the validation subset.
[b] SEP is the standard error of prediction, either expressed in the variable unit or in proportion of the validation subset mean (conventional determinations).
[c] RPD is the ratio of standard deviation (of the validation subset) to SEP.

Figure 1. Comparisons between measured and predicted SIR values for the validation subset of the total set, predictions resulting from global calibration using NIR (A) or mid-IR (B) spectra.

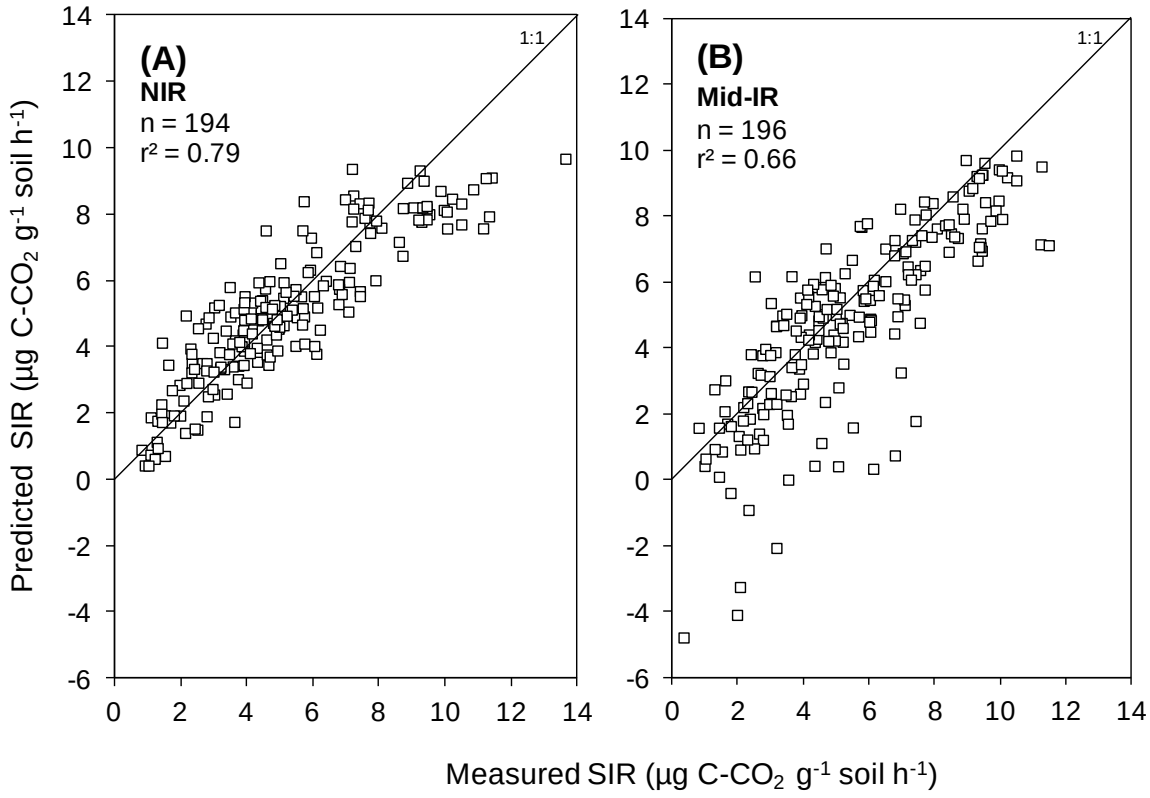

Figure 2. Comparisons between measured and predicted SIR values for the validation subset of the total set, predictions resulting from LOCAL calibration using NIR (A) or mid-IR (B) spectra.

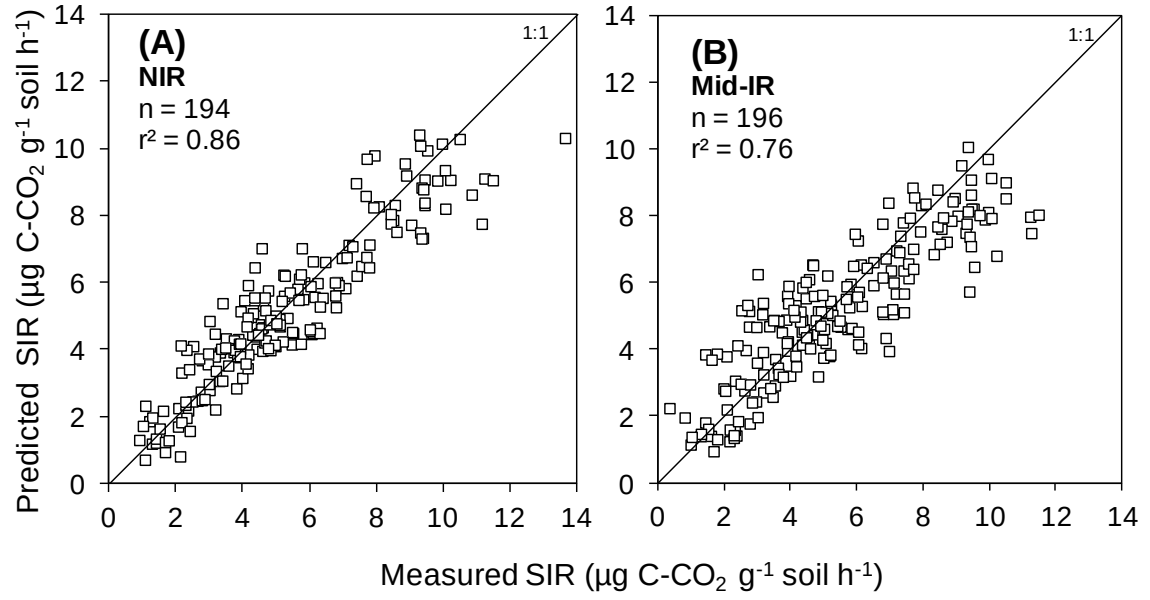

Figure 3. Comparisons between measured and predicted SIR values for the validation subset of the textural subsets, predictions resulting from LOCAL calibration using NIR in the fine- (A1) and coarse-textured (A2) subsets or from LOCAL calibration using mid-IR in the fine- (B1) and coarse-textured (B2) subsets.