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Studying the physical protection of soil carbon with quantitative infrared spectroscopy

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Abstract

Near and mid infrared reflectance spectroscopy (NIRS, MIRS) are time- and cost-effective tools for characterizing soil organic carbon (SOC). Here they were used for quantifying (i) carbon (C) dioxide (CO_2) emission from soil samples crushed to 2 and 0.2 mm, at 18 and 28°C; (ii) physical C protection, calculated as the difference between CO_2 emissions from 0.2- and 2-mm crushed soil at a given temperature; and (iii) the temperature vulnerability of this protection, calculated as the difference between C protection at 18 and 28°C. This was done for 97 topsoil samples from Tunisia, mostly calcareous, which were incubated during 21 days.

Soil CO_2 emission increased with temperature and fine crushing. However, C protection in 0.2–2 mm aggregates had little effect on the temperature vulnerability of CO_2 emission, possibly due to preferential SOC protection in smaller aggregates.

In general NIRS, and MIRS to a lesser extent, yielded accurate predictions of soil CO_2 emission ($0.60 \leq R^2 \leq 0.91$), and acceptable predictions of C protection at the beginning of incubation ($0.52 \leq R^2 \leq 0.81$) but not over the whole 21 day period ($R^2 \leq 0.59$). For CO_2 emission, prediction error was the same order of magnitude as, and sometimes similar to, the uncertainty of conventional determination, indicating that a noticeable proportion of the former could be attributed to the latter. The temperature vulnerability of C protection could not be modelled correctly ($R^2 \leq 0.11$), due to error propagation. The prediction of SOC was better with NIRS and that of soil inorganic C was very accurate ($R^2 \geq 0.94$), especially with MIRS. Soil CO_2 emission, C protection and its vulnerability were best predicted with NIR spectra, those of 0.2 mm samples especially. MIR spectra of 2 mm samples yielded the worst predictions in general.

NIRS prediction models suggested that CO_2 emission and C protection depended (i) on aliphatic compounds (i.e. labile substrates), dominantly at 18°C; (ii) on amides or proteins (i.e. microbial biomass), markedly at 28°C; and (iii) negatively, on organohalogens and aromatic amines (i.e. pesticides). MIRS models showed a negative influence of carbonates on CO_2 emission, suggesting they did not contribute to soil CO_2 emission and might form during incubation. They also suggested that CO_2 emission and C protection related to carboxylic acids, saturated aliphatic ones especially.

Keywords

Soil organic matter; soil respiration; carbon sequestration; carbonates; near infrared reflectance spectroscopy (NIRS); mid infrared reflectance spectroscopy (MIRS).

Introduction

Terrestrial ecosystems play a major role in regulating the atmospheric greenhouse gases concentrations, that of carbon (C) dioxide (CO_2) especially. The CO_2 flux balance between ecosystems and the atmosphere resulted in a global terrestrial sink of about 2.4 Pg C yr^{-1} over the period 2000–2009.[1] However, an increase in global ecosystem CO_2 emissions may shift the global terrestrial ecosystem from a sink into a source, accelerating the increase in atmospheric CO_2 concentration. The air temperature increase during recent years was positively and significantly correlated with soil CO_2 production, which increased by 0.1 Pg C yr^{-1} between 1989 and 2008.[2] Because half of CO_2-emitting soil respiration is estimated to be produced by microbial activity involved in organic matter decomposition (i.e. heterotrophic respiration),[3] the effect of increasing temperature on heterotrophic soil respiration is a major issue.[4,5]

The decomposition of soil organic matter (SOM) may be limited by three stabilization mechanisms, namely biochemical recalcitrance, adsorption on mineral surfaces, and physical protection in soil aggregates, the latter being the easiest to increase through appropriate soil management practices.[6,7] These stabilization mechanisms may be affected by temperature increase. The vulnerability to temperature of SOM stabilization by sorption on mineral surfaces or by biochemical recalcitrance has been studied;[8,9] but few studies have addressed the temperature vulnerability of physically protected SOM.[10] Physical protection of SOM in aggregates has usually been estimated by comparing SOM mineralization from aliquots crushed to 2 and 0.2 mm.[7,11] Additional CO_2 emission from 0.2-mm crushed aliquots is attributed to soil organic carbon (SOC) protected in 0.2–2 mm aggregates and deprotected through crushing to 0.2 mm. Thus addressing the temperature vulnerability of physically protected SOC requires incubating 2- and 0.2-mm crushed soil aliquots at different temperatures over several weeks, which is costly and time-consuming.

Near and mid infrared reflectance spectroscopies (NIRS and MIRS, respectively) are cost- and time-effective approaches that have been reported to provide accurate determinations of SOC concentration,[12,13] but also of different SOC fractions such as microbial biomass,[14,15] particle-size fraction SOC,[16,17] C-13 NMR (nuclear-magnetic resonance) fractions.[18,19]

The present work aimed to evaluate the applicability of NIRS and MIRS for studying soil CO_2 emissions, physical C protection in aggregates and its temperature vulnerability, as determined from laboratory incubations. This was done for a population of topsoil samples from Tunisia, mostly originating from calcareous soils (i.e. soils with carbonates).

Materials and methods

Sites and soils

The study analyzed 97 topsoil samples originating from 45 localities of the northern half of Tunisia. Sites possibly collected within a same locality were generally kilometres apart, under different land uses. The soils were sampled at 0–10 cm depth using a spade. They were mainly Calcaric Cambisols and Regosols, Kastanozems, and Chromic and Vertic Cambisols.[20] Most were calcareous, with soil inorganic carbon (SIC) ranging from 0 to 93 g C kg^{-1} soil and averaging 44 g C kg^{-1} soil (see the section on soil C determination below). Most samples were loamy; 23 were clayey and 14 sandy. The main land uses were forest, rangeland, orchards and crops. The SOC concentration ranged from 2 to 121 g C kg^{-1} soil and averaged 21 g C kg^{-1} soil (Table 1).

Soil preparation and soil organic and inorganic carbon determinations

The samples were air-dried, gently broken up along natural faults, homogenized, and sieved to 2 mm. Gravels greater than 2 mm were discarded. One part of the air-dried, 2 mm sieved soil samples was then ground and forced through a 0.2 mm sieve.

Soils were decarbonated prior to SOC determination: 10 mL of water were added to 1 g of soil and 0.5 M HCl solution was then dripped onto the sample until there was no more effervescence; then the samples were washed in water until pH reached 7. The SOC concentration was then determined on finely ground 25–30 mg aliquots by dry combustion using an elemental analyser (Thermo Fisher Scientific CHN NA2000, Waltham, MA, USA). The SIC content was calculated as 0.12 times the equivalent calcium carbonate content, assuming that most carbonates in the studied soils were calcium carbonates. The equivalent calcium carbonate content was determined on finely ground air-dried soil samples using a Bernard calcimeter, after calibration with a pure calcium carbonate standard, according to the French procedure NF ISO 10693.[21]

Mineralization assays

Twelve g (± 1 g) of each of the 2- and 0.2-mm crushed soil samples were incubated in triplicate at 18°C and 28°C for 21 days, with no pre-incubation, resulting in a total of ca. 1200 incubations. Deionized water was added to a water potential of -0.01 MPa. Each sample was then placed in a 1 L airtight jar with a vial containing 19 mL of aqueous sodium hydroxide solution (NaOH 0.5 M) to trap the CO_2 emitted. A vial containing 19 mL of deionized water was added to the jar to keep the relative air humidity constant in the headspace. The amount of CO_2 emitted was determined by back titration (HCl 0.5 M; pH 8.6) of the NaOH trap with an excess of barium chloride ($BaCl_2$ 1 M). At day 7, the jars were opened and the NaOH replaced; moreover the sample water content was checked, and deionized water was added to keep it constant. Thus trapped CO_2 was measured for two periods, from day 1 to day 7 and from day 7 to day 21, and was calculated for the total 21 day period. The difference between CO_2 emissions from 0.2- and 2-mm crushed samples accounts for C physically protected within 0.2–2 aggregates,[7,11] and is denoted "protection" in the present paper. It was calculated over 7 days and over 21 days. The difference between C protection at 18°C and 28°C was defined as the "vulnerability" of C protection to temperature elevation. It was also calculated over 7 and 21 days. The soil CO_2 emission, protection and vulnerability were generally expressed in mg C g^{-1} soil, but sometimes as proportions of SOC. The significance of differences between means was determined by paired t tests (e.g. between CO_2 emission at 18 and 28°C).

The standard deviation of the laboratory method (*SDL*) was calculated for CO_2 emission over 21 days, taking account of the three replicates per incubation, as proposed by Lindedam *et al.*:[22]

Equation (1)

$$SDL = \sqrt{\frac{\sum_{i=1}^{n} \sum_{j=1}^{m} \left(X_{ij} - \overline{X}_{j}\right)^{2}}{n \times m - 1}}$$

where X stands for CO_2 emission and $\overline{X}$ for its average, *i* is the individual laboratory replicate out of *n* replications (*n* = 3), and *j* the individual sample out of *m* samples (*m* = 97). It was calculated separately for 0.2- and 2-mm crushed samples at 18°C and 28°C. However, *SDL* could not be calculated for physical C protection, which was defined as the difference between mean CO_2 emission from 0.2 mm sample and mean CO_2 emission from 2 mm sample, each calculated over three replicates.

Spectral acquisition and pre-treatment

Before incubations, soil reflectance was measured on air-dried then overnight oven-dried (40°C) samples, on two aliquots of 2 mm samples and on one aliquot of 0.2 mm samples (because the latters were supposed to be more homogeneous).[23] On the one hand, aliquots of about 5 g were placed in a ring cup and scanned in the NIR region between 1100 and 2500 nm at 2 nm intervals (700 data points) using a Foss NIRSystems 5000 spectrophotometer (Laurel, MD, USA). On the other hand, aliquots of about 0.5 g were placed in a 17-well plate and scanned in the MIR region from 4000 to 400 cm^{-1} (i.e. 2500–25,000 nm) at 3.86 cm^{-1} resolution (934 data points), using a Nicolet 6700 diffuse reflectance Fourier transform spectrophotometer (Thermo Fisher Scientific Instruments, Madison, WI, USA). In both cases, the aliquot spectrum, automatically averaged from 32 elementary spectra, was recorded as apparent absorbance, which is the logarithm of the inverse of reflectance log(1/reflectance). For both NIRS and MIRS, more than 200 aliquots can be scanned daily. For 2 mm samples, the spectra of the two aliquots were averaged. Data analysis was conducted using the WinISI software (Infrasoft International, LCC, State College, PA, USA).

Several common spectrum mathematical pre-treatments were tested: no derivation (denoted 01) or first-order derivation with 4-point gap and smoothing (denoted 14), alone (denoted None) or in conjunction with standard normal variate transform (SNV), detrend (D), both SNV and detrend (SNVD), or standard multiplicative scatter correction (MSC), leading to a total of 10 pre-treatments (i.e. None01, None14, SNV01, SNV14, SNVD01, SNVD14, D01, D14, MSC01 and MSC14). It has often been observed that such procedures increase the signal-to-noise ratio, thus improve the prediction of sample properties using NIR spectra: derivation reduces baseline variation and enhances spectral features,[24] SNV transform reduces the particle-size effect,[25] the detrend transformation removes the linear or curvilinear trend of each spectrum,[25] and MSC transform removes additive and/or multiplicative signal effects.[26] Second-order derivative was not used because previous work had showed that it did not improve NIR prediction of soil properties.[27]

Multivariate analyses

For each of the four spectrum populations studied (NIR and MIR spectra of 0.2 and 2 mm samples), spectra were fitted to reference variables, namely soil C (total C, SOC and SIC), CO_2 emission, C protection and its vulnerability, using modified partial least square (mPLS) regression. The modification to PLS regression was to scale the reference data and reflectance data at each wavelength to have a standard deviation of 1.0 before each PLS term.[28] Cross-validation was performed to evaluate if the reference variables could be predicted from the spectra. For this purpose, each soil sample population was divided into six groups; five groups were used for fitting the model and one for testing it. The procedure was performed six times to use all samples for both model fitting and testing, then the residuals of the six predictions were pooled to calculate the standard error of cross-validation (*SECV*). The optimal number of mPLS terms to be used was that which resulted in the lowest *SECV*.

Before being divided into cross-validation groups, the sample population was ranked according to sample name, which reflected geographical proximity. Then the population was divided into six groups in a cyclical way, according to the venetian blinds method: the first, seventh, thirteenth samples, etc., were put in the first group; the second, eighth, fourteenth samples, etc., in the second group, and so on; until the sixth group, which included the sixth, twelfth, eighteenth samples, etc. Thus samples from a given region were in different cross-validation groups. Venetian blinds cross-validation is not relevant for blocked data with replicates, but there were no replicates in the present study: even though some samples originated from the same localities, they were under different land uses, and generally kilometres apart. Dividing the population into contiguous blocks can be considered more robust; but preliminary tests showed that some of such geographical blocks were poorly represented by the other blocks, which resulted in overly pessimistic predictions thus did not seem appropriate. Random group selection has often been used; but it results in variable prediction accuracy depending on the group selection, and requires a rather high number of iterations, and their averaging, to achieve more stable results.[29]

Whatever the spectrum population, no spectral outliers were removed, though some NIR spectra sometimes had very high Mahalanobis distance H,[30] up to 9. Indeed, preliminary tests showed that systematically removing such samples from further investigations did not improve prediction accuracy, thus was not useful. This might be due to limited robustness, or on the contrary, to the fact that the models applied fairly well to the samples considered though their spectra were distant from the rest of the population. For MIR spectra, H was always ≤ 3.5, and most generally < 3, which has been considered a threshold for spectral outlier removal.[28] Similarly, no calibration outliers were removed, though some authors recommended to remove samples with residues > 2.5 *SECV* and to perform another cross-validation.[28] Removing no outliers, either spectral or for calibration, was supposed to allow for model robustness, though external validation was not achieved.

The accuracy of the cross-validation was assessed using *SECV*, the part of variance explained (i.e. 1 – residual variance, or cross-validation determination coefficient, denoted R^2), and the ratio of standard deviation to *SECV* (commonly denoted *RPD*). After Chang *et al.* (2001)[14] and Dunn et al (2002),[31] NIRS models with $RPD \geq 2$ were considered accurate and those with $1.6 \leq RPD < 2.0$ acceptable for the prediction of soil properties. Similar criteria were applied for MIRS predictions.

Paired t tests were used to compare the averages of the best *RPD*s over different variables between different conditions (e.g. NIRS vs. MIRS prediction of CO_2 emission).

Results

Reference data: soil C, CO_2 emission, physical C protection, and temperature vulnerability of this protection

The distributions of conventionally determined variables are presented in Table 1 (SOC, SIC, and 21-day incubation data), in mg C g^{-1} soil. Expressed as proportion of SOC, mean soil CO_2 emission over 21 days increased significantly with finer crushing ($p < 0.001$): from 2.9 to 5.0% SOC at 18°C, yielding a mean C protection of 2.1% SOC; and from 4.7 to 6.6% SOC at 28°C, resulting in a mean physical protection of 2.0% SOC. Thus the temperature vulnerability of C protection averaged +0.1% SOC over 21 days, indicating that protected C decreased when temperature increased (but not significantly; $p > 0.1$). The increase in CO_2 emission from 18 to 28°C was significant and averaged 1.7% SOC for soil < 2 mm and 1.6% SOC for soil < 0.2 mm ($p < 0.001$).

About 5% of the samples showed significant decrease in CO_2 emission after finer crushing (from 2 to 0.2 mm). No particular property could be associated with this result, especially regarding total C, SOC, SIC, or texture.

General considerations on NIRS and MIRS predictions

Soil CO_2 emission, C protection and its temperature vulnerability expressed as proportions of total C or SOC (mg C-CO_2 g^{-1} soil C or mg C-CO_2 g^{-1} SOC, respectively) were less accurately predicted than they were when expressed as proportions of total soil (mg C-CO_2 g^{-1} soil), thus are not presented here. It is worth noting that CO_2 emission from 0.2 mm samples was predicted using spectra of 0.2 and 2 mm samples; similarly CO_2 emission from 2 mm samples was predicted using spectra of 0.2 and 2 mm samples. Indeed, more accurate predictions are generally achieved using spectra of 0.2 mm soil samples;[27] but fine grinding is somewhat tedious, thus studying predictions using spectra acquired with both sample preparations is relevant.

For each studied variable, the results presented are those achieved using the pre-treatment that yielded the best prediction (i.e. maximum RPD). This best pre-treatment depended on the spectrum population and predicted variable; nevertheless, when using spectra of 2 mm samples, the best pre-treatment was often D14, with no clear trend otherwise.

NIRS and MIRS predictions

Predictions results are presented in Tables 2 (NIRS) and 3 (MIRS). The predictions of soil C were very accurate ($RPD \geq 3.9$) except for SOC using the MIR spectra of 2 mm samples ($RPD = 2.5$). The MIR spectra yielded particularly accurate predictions of SIC ($RPD \geq 5.8$), especially using 0.2 mm samples ($RPD = 7.8$).

Generally, CO_2 emission was accurately predicted using NIR spectra ($1.9 \leq RPD \leq 3.4$), and using MIR spectra to a lesser extent ($1.6 \leq RPD \leq 3.1$). On the whole, CO_2 emission was better predicted for 0.2- than for 2-mm incubated samples (mean $RPD = 2.4$ vs. 2.2, respectively; $p = 0.07$), at 28°C than at 18°C ($RPD = 2.5$ vs. 2.1; $p = 0.01$), over the first 7 days than over 21 days ($RPD = 2.4$ vs. 2.2; $p = 0.06$), with NIRS than with MIRS ($RPD = 2.5$ vs. 2.1; $p < 0.001$), and using scans from 0.2 than from 2 mm samples ($RPD = 2.4$ vs. 2.2; $p = 0.03$). Thus CO_2 emission was best predicted for 0.2 mm samples at 28°C over the first 7 days using the NIR spectra of 0.2 mm samples ($RPD = 3.4$). Considering CO_2 emission over 21 days, $SECV$ and SDL were the same order of magnitude (0.19-0.47 vs. 0.09-0.27 mg C g^{-1} soil, respectively; see SDL in Table 1), and were even similar for the incubation of 0.2 mm samples at 18°C (0.24-0.31 vs. 0.27 mg C g^{-1} soil, respectively).

The prediction of C protection was less accurate. It was nevertheless acceptable, and sometimes accurate over 7 days using spectra of 0.2 mm samples or using NIR spectra ($1.6 \leq RPD \leq 2.3$), but not over 21 days ($RPD \leq 1.6$), especially at 28°C ($RPD = 1.0$ using NIR or MIR spectra of 0.2 or 2 mm samples). MIRS did not allow accurate prediction of C protection ($RPD \leq 1.9$). On the whole, C protection was better predicted over 7 than over 21 days (mean $RPD = 1.8$ vs. 1.3, respectively; p = 0.01), using NIR than MIR spectra ($RPD = 1.6$ vs. 1.5; p = 0.03), and less clearly, using scans from 0.2 than from 2 mm samples ($RPD = 1.6$ vs. 1.5; p = 0.14), while temperature did not affect prediction accuracy ($RPD = 1.5$ vs. 1.6 at 18°C and 28°C, respectively; p = 0.78). Carbon protection was thus best predicted over 7 days at 28°C using NIR spectra of 0.2 mm samples ($RPD = 2.3$).

The vulnerability of C protection to temperature could not be modelled correctly using NIR or MIR spectra ($RPD \leq 1.1$).

As examples, the Figure 1 presents some comparisons between conventional determinations and predictions for soil CO_2 emission, C protection and its temperature vulnerability over 7 days (predictions made using the NIR spectra of 0.2 mm samples), and for SIC (predictions made using the MIR spectra of 0.2 mm samples).

General considerations on the determinants of NIRS and MIRS predictions

The predictions of CO_2 emission (or C protection) could not just be explained by NIRS or MIRS prediction of SOC and correlation between SOC and CO_2 emission (or C protection). Indeed, determination coefficient R^2 between SOC and CO_2 emission over 7 days was lower than prediction R^2 for CO_2 emission over 7 days using NIR spectra of 0.2 mm samples (0.75-0.85 vs. 0.80-0.91, respectively). Similarly, the loose correlation between SIC and CO_2 emission ($R^2 < 0.45$) could not explain the indirect prediction of CO_2 emission through the prediction of SIC. One might imagine indirect NIRS or MIRS prediction of CO_2 emission through the prediction of SOC, SIC, carbon-to-nitrogen ratio, clay content, other variables possibly, and multiple correlation between CO_2 emission and all these variables. However, the present work has not attempted to identify all the possible variables that would be involved in such multiple correlation. Moreover, measuring all these variables conventionally would be much more tedious and costly than acquiring NIR or MIR spectra.

Furthermore, examining the regression coefficients of prediction models provided information on the chemical compounds involved in the variables studied. Indeed, an mPLS prediction model expresses a variable of interest (e.g. CO_2 emission) as a linear combination of absorbances at all wavelengths. Spectral regions with large regression coefficients contribute more heavily to the prediction than those with small regression coefficients. Looking at chemical compounds assigned to heavily contributing regions helps identifying the determinants of the studied variables. Here this was done for the predictions of CO_2 emissions and C protection over the first 7 days using spectra of 0.2 mm samples, with no pre-pre-treatment (None01) because pre-treated spectra are often more difficult to interpret (though they often result in more accurate predictions).

Chemical determinants of NIRS and MIRS predictions

For NIRS, assignations were made according to Workman and Weyer,[32] in the absence of other citation. According to regression coefficients, the chemical compounds that seemed to contribute most to NIRS predictions were (Figure 2):

- for CO_2 emission: positively, aliphatic organic compounds (1710–1720 nm), and to a lesser extent but markedly at 28°C, amides or proteins (2110–2130 nm; less markedly, 2060–2065 nm; at 28°C, 1560–1590 nm) and hydroxyl (1425–1430 nm); negatively, chlorinated organic compounds (1860 nm), and to a lesser extent, at 28°C, aromatic amines (1500–1505 nm);
- for physical C protection: positively, aliphatic organic compounds (1710–1720 nm at 18°C, 1690–1720 nm à 28°C), amides or proteins especially at 28°C (2060–2070 and 2120 nm at 18°C; 1590 nm at 28°C), and to a lesser extent, hydroxyl (1425–1430 nm, more marked at 18°C); negatively, mainly halogenated organic compounds (1640–1650 nm, assigned to brominated compounds, more marked at 18°C; 1860–1870 nm, assigned to chlorinated compounds, more marked at 28°C) and at 28°C, lignin (1785–1790 nm),[33] and to a lesser extent, aromatic amines (1500–1510 nm) and amides or proteins (1520–1530 nm).

By comparison, the compounds that contributed heavily to NIRS prediction of SOC were (Figure 4, Supplementary Materials): positively, aliphatic (1690–1710 nm) and aromatic organic compounds (1670 nm) and amides or proteins (2120–2130 nm); negatively, aromatic amines (1500–1505 nm) and chlorinated compounds (1860 nm). The regions that contributed markedly to NIRS prediction of SIC were (Figure 4, Supplementary Materials): positively, hydroxyl (1430 nm), calcite (1760–1770 nm) and carbonates (2480 nm); negatively, silica (1395 nm), and possibly carbonates (1560–1580 and 2390 nm).[34,35]

For MIRS, assignations were made according to Socrates,[36] in the absence of other citation. The graphs that represented regression coefficients at every wavenumber displayed close zigzags between 1200 and 400 cm^{-1} (Figure 3), which in general rendered interpretation difficult in this region. The main chemical compounds that seemed to contribute heavily to MIRS predictions were:

- for CO_2 emissions: positively, aliphatic compounds at 18°C (2930–2920 cm^{-1}),[37,38] saturated aliphatic carboxylic acids (2780–2660 cm^{-1} at 18°C, 1740–1690 cm^{-1} at both temperatures), carboxylic acid salts (1620–1590 cm^{-1}, except for 2 mm samples at 28°C); negatively, carbonates mainly (2510–2490 cm^{-1} at 18°C; 1790–1800 and 865–860 cm^{-1} at both temperatures),[39,40,29] but also amides (1650–1635 cm^{-1});
- for physical C protection: positively, aliphatic compounds at 28°C (2925–2920 cm^{-1})[37,38], saturated aliphatic carboxylic acids (2750–2670 and 1705–1680 cm^{-1}), carboxylic acid salts (1600–1575 cm^{-1}), and to a lesser extent at 18°C, possibly nitriles (2420–2370 and 2300–2265 cm^{-1}); negatively, carbonates mainly (2540–2490 and 1805–1785 cm^{-1}, and at 18°C, 865–860 cm^{-1}),[39,40,29] but also amides (1650 cm^{-1}).

By comparison, the compounds that contributed most to MIRS prediction of SOC were (Figure 5, Supplementary Materials): positively, saturated aliphatic carboxylic acids mainly (1730 cm^{-1}), and negatively, carbonates (1800–1790 cm^{-1}). Moreover, carbonates were the main compounds that contributed to MIRS prediction of SIC, positively (2510, 1800–1790 and 820–815 cm^{-1}),[39,41,29] but also negatively (860 cm^{-1};[39] Figure 5, Supplementary Materials).

Discussion

CO_2 emissions, physical C protection and its temperature vulnerability

Incubation data confirmed previous results: finer crushing (from 2 to 0.2 mm) increased CO_2 emission significantly. This indicated that the compartmentalization of substrates and decomposers reduces the decomposability of otherwise labile material.[42,11] Temperature elevation also increased CO_2 emission significantly, which is a well established result.[43,5] Indeed, finer crushing, as well as temperature elevation, induces higher diffusion rates, higher C solubilisation (water soluble C content), higher cellular enzyme activity, and faster substrate uptakes.[11,44] This affects microorganism metabolism and growth rates, and leads to higher respiration rates.

Unlike C stabilization by biochemical recalcitrance[9] or sorption on mineral surfaces[8], C protection within 0.2 to 2 mm aggregates had little effect on the temperature vulnerability of CO_2 emissions (the temperature elevation caused 1.7% vs. 1.6% mean increase in CO_2 emissions of 2 vs. 0.2 mm samples, respectively). This weak influence of C protection on the temperature vulnerability of CO_2 emission, also reported by Plante *et al.*,[10] was probably because SOC protected against microbial mineralization is mainly located within microaggregates (< 0.2 mm) or associated with minerals.[6,45]

NIRS and MIRS predictions of soil C, CO_2 emission, C protection and its temperature vulnerability

Accurate NIRS and MIRS predictions of soil C concentration, either total or organic, as observed in the present study, have been reported and discussed extensively.[12] Excellent predictions of SIC have been reported but to a lesser extent, using either NIRS[46] or MIRS.[29] As already mentioned and discussed, most spectral regions that contributed heavily to SIC prediction have been assigned to carbonates, but some contributing regions have also been assigned to other constituents (e.g. silica).[35,29]

For the studied samples, CO_2 emission was accurately predicted in general by NIRS, and by MIRS to a lesser extent (the comparison NIRS vs. MIRS is discussed in the next section). The literature has also reported accurate prediction of basal soil respiration, using NIRS mainly, with *RPD* ranging from 2.1 to 2.6.[14,47,48] However, comparison between studies remains difficult because model performance depends on sample set diversity,[27] a fortiori when chemometric procedures differ. The important point is that NIR and MIR spectra of dry soil samples contain information on their CO_2 emission during incubation. Indeed, CO_2 emission results from the consumption of soil organic matter by microorganisms and thus reflects its chemical composition (e.g. labile materials are used first).

The predictions of physical C protection were less accurate than for CO_2 emission but were acceptable and sometimes accurate after 7 days, with NIRS and to a lesser extent with MIRS. The fact that MIR and even NIR spectra might be used for quantifying SOC fractions has been reported by many works,[14-19] and some even addressed the labile, stabilized or resistant nature of SOC fractions specifically.[16,49] So it is not surprising that the physically protected fraction of SOC could be quantified using infrared spectroscopy. In the present study, poor predictions of C protection were however achieved in general after 21 days, especially at 28°C. It is likely that some problems occurred at the end of the incubations at 28°C for two or three samples, which were very poorly predicted; but these problems have not been identified.

The prediction error (*SECV*) and the uncertainty of conventional determination (*SDL*) of CO_2 emission were the same order of magnitude, and even similar in some instances. Thus a noticeable proportion of the so-called "prediction error" could actually be attributed to the uncertainty of conventional determinations. Less accurate predictions for C protection than for CO_2 emissions might be attributed to error propagation, as the former was calculated by difference between CO_2 emissions from 0.2 mm and 2 mm samples. The temperature vulnerability of C protection was very poorly predicted, probably due to error propagation to a greater extent, as it was calculated by difference between C protection at 18 and 28°C.

Predictions using NIR vs. MIR spectra of 0.2 vs. 2 mm samples

Whatever the sample preparation, MIRS outperformed NIRS for SIC prediction; nevertheless NIRS prediction of SIC was very accurate. By contrast, the best prediction of SOC was always achieved using NIRS, while the best prediction of total C was either achieved using NIRS or MIRS depending on sample preparation. For the other variables, the best predictions were achieved using NIR spectra of 0.2 mm samples, then NIR spectra of 2 mm samples in general, and the worst predictions using MIR spectra of 2 mm samples. For the sample set and variables studied, NIRS could thus be considered preferable because it yielded better predictions except for total C and SIC, which it nevertheless predicted very accurately. Moreover, predictions were more accurate using 0.2 than 2 mm sample spectra, with the notable exceptions of SIC, and to a lesser extent SOC, using NIRS.

Achieving better NIRS predictions using spectra acquired on more finely crushed soil samples (i.e. 0.2 vs. 2 mm) is an overall trend that has already been reported and discussed, as well as the attenuation of this trend for coarse-textured samples.[23,27] Better MIRS prediction of soil properties using finely than coarsely crushed samples has also been reported.[50] Indeed, whatever the spectral range, fine crushing homogenizes the sample and reduces light diffusion, which both allow better predictions. This is particularly true for MIRS because the spectrometer's beam aperture is often around 1-2 mm in diameter.[50]

Comparisons between NIRS and MIRS predictions of soil properties have led to somewhat contradictory reports in the literature. As MIR spectra seem much more informative a priori than NIR spectra due to their numerous peaks relating to fundamental bond vibrations, it has been considered that they should logically yield more accurate predictions, and a number of papers have supported this view.[e.g. 12,24,51] However, other studies have been much less conclusive, especially when samples were prepared similarly (e.g. similar crushing), and either reported better MIRS or NIRS predictions depending on the soil properties and sample sets considered.[18,52-57] For instance, for litters and topsoils from north-western Europe, Ludwig *et al.*[18] achieved more accurate NIRS predictions of SOC, nitrogen (N) and lignin contents but more accurate MIRS predictions of microbial biomass and SOC/N ratio. For Maryland soils, Igne *et al.*[54] reported better SOC and texture predictions with MIRS but better N predictions with NIRS, when using comparable bench-top devices. For Canadian soils, Yang *et al.*[57] achieved better MIRS predictions of fine-fraction SOC and N but better NIRS predictions of coarse-fraction SOC and N. The absence of superiority of MIRS seems noticeable for tropical soils. For example, on Brazilian Ferralsols, Madari *et al.*[53] obtained slightly better N predictions with MIRS but clearly better SOC predictions with NIRS. In soils from sub-tropical China, Shao and He[56] observed better predictions of available N with NIRS but better predictions of available phosphorus and potassium with MIRS (SOC was not studied). Rabenarivo *et al.* [13] even reported systematically better NIRS than MIRS predictions for Malagasy Ferralsols. They suggested that the overlap of absorption regions relating to metal sesquioxides and organic compounds could represent an obstacle for MIRS prediction of soil organic properties: for instance, metal oxides may absorb in the 1020–970 cm^{-1} region (when more than one oxygen atom is bound to a single metal atom) and in the 1100–825 cm^{-1} region (when containing a metal-to-oxygen double bound);[36] while carbohydrates absorb in the 1080–1030 and 960–730 cm^{-1} regions and polysaccharides in the 1170–950 cm^{-1} region.[58] Information useful for SOC prediction could thus be masked in MIR spectra, at least partially, due to the abundance of minerals such as iron and aluminum sesquioxides. Sesquioxides have not been studied in the Tunisian soils considered in the present work; but it is well established that dry and warm conditions are favourable to the formation of haematite, which, in calcareous soils, is not leached from the topsoil.[59] The worse overall performance of MIRS, compared to NIRS, in the prediction of some properties of soils such as Mediterranean carbonated soils might thus be attributed to mineralogy. Nevertheless, SIC was better predicted and the influence of carbonates on CO_2 emission was clearer using MIRS than NIRS (see the next section). Though the review of Reeves emphasizing soil C analysis underlined that MIRS is often more accurate and produces more robust calibrations than NIRS when analyzing dried ground samples,[12] this can hardly be generalized for all soils and soil properties.

Chemical determinants of NIRS and MIRS predictions

The regression coefficients of NIRS prediction models indicated that CO_2 emission over 7 days related positively to the amount of aliphatic organic compounds, and to a lesser extent but markedly at 28°C, to amides or proteins. Aliphatic organic compounds are easily degradable substrates, preferentially used by soil microorganisms.[43] Their influence on NIRS prediction of CO_2 emission over 7 days reflects the importance of labile substrate availability during the first incubation days. Amides or proteins might be considered a proxy for soil microbial biomass, because microorganisms concentrate soil organic N. The influence of amides or proteins on CO_2 emission would thus reflect that of microbial biomass on respiration. This influence was relatively more marked at 28 than at 18°C, possibly because substrate availability was less limiting at 28°C due to better substrate diffusion and use by microorganisms.[44,9] Furthermore, regression coefficients of NIRS models suggested that CO_2 emission was affected negatively by organochlorines and aromatic amines. This might relate to the toxicity of several of these molecules, which are used as pesticides or are pesticide metabolites.[60] According to regression coefficients of NIRS predictions, physical C protection, defined as additional CO_2 emission from 0.2 mm samples (compared to 2 mm samples), related positively to aliphatic organic compounds, dominantly at 18°C, and to amides or proteins, markedly at 28°C. This suggested that the mineralization of C deprotected by fine grinding depended firstly on substrate availability at 18°C and on microbial biomass at 28°C, as was the case for CO_2 emission. The negative contribution of halogenated compounds and aromatic amines to physical C protection indicated that they would cause higher reduction in CO_2 emission from 0.2 that from 2 mm samples, possibly because 0.2–2 mm aggregates would contain microhabitats not affected by these compounds.

Interestingly and surprisingly, regression coefficients of MIRS predictions showed a negative influence of carbonates on soil CO_2 emissions. This strongly suggested that carbonates did not contribute to CO_2 emissions, and even, that emissions could be reduced as a consequence of carbonate formation.[61,62] Thus MIRS could help evaluating the carbonate impact on soil CO_2 emissions, which is a challenging issue in calcareous soils.[63,64] Regression coefficients in the MIR also indicated that CO_2 emissions and C protection related to carboxylic acids, saturated aliphatic ones especially, which is consistent with the fact that fulvic acids represent the most labile form of humus.[58] On the whole, except regarding carbonates, the regression coefficients were more difficult to interpret, and tended to be less informative on the compounds involved, in the MIR than in the NIR. This was unexpected because MIR spectra are considered more informative a priori;[51,12] but the higher informativeness of MIR spectra is still debated.[18,13]

Conclusion

Incubation data confirmed that soil respiration (CO_2 emission, strictly speaking) increases with temperature elevation and with finer crushing. They also indicated that physical C protection in 0.2–2 mm aggregates had little effect on the temperature vulnerability of soil respiration, possibly due to preferential SOC protection in aggregates < 0.2 mm.

In general NIRS and MIRS predictions of soil CO_2 emission were accurate and those of physical C protection acceptable, which confirmed that NIR and MIR spectra contain useful information on SOC quality, even in terms of physical protection. However, the temperature vulnerability of C protection could not be modelled correctly by NIRS or MIRS, which was attributed to error propagation when calculating this vulnerability conventionally.

The prediction of SOC was better using NIRS than MIRS. The prediction of SIC was better using MIRS, but was very accurate even with NIRS. The best predictions of CO_2 emission and C protection were generally achieved with NIR spectra of 0.2 mm samples, then with NIR spectra of 2 mm samples, and the worst with MIR spectra of 2 mm samples. For the variables considered (except SIC), NIRS thus seemed more appropriate for studying Mediterranean carbonated soils.

Regression coefficients of NIRS prediction models suggested that CO_2 emission and physical C protection depended on aliphatic compounds (i.e. labile substrates), dominantly at 18°C, and on amides or proteins (i.e. microbial biomass), markedly at 28°C. Moreover, NIRS models suggested that CO_2 emission and physical C protection were affected negatively by organohalogens and aromatic amines (i.e. pesticides). Regression coefficients of MIRS prediction models showed a systematic and negative influence of carbonates on CO_2 emission. This suggested that carbonates did not contribute to CO_2 emission, which could even be reduced as a consequence of carbonate formation. This indicates that MIRS could help addressing challenging issues such as that of carbonate impact on soil CO_2 emissions. Regression coefficients of MIRS models also suggested that CO_2 emission and C protection related to carboxylic acids, saturated aliphatic ones especially. However, except regarding carbonates, MIRS regression coefficients seemed, on the whole, more difficult to interpret and less informative than their NIR counterparts, which was unexpected.

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Table 1. Distribution of conventionally determined variables: soil organic and inorganic carbon (SOC, SIC), CO_2 emission, physical C protection, and temperature vulnerability of this protection over 21 days (the physical C protection is the difference between CO_2 emission from 0.2 and 2 mm samples; the temperature vulnerability of this protection is the difference between C protection at 18 and 28°C).

	SOC	SIC	CO_2 emission				C protection		Vulnera-bility
			0.2 mm soil		2 mm soil				
			at 18°C	at 28°C	at 18°C	at 28°C	at 18°C	at 28°C	
	mg C g^{-1} soil		mg C g^{-1} soil				mg C g^{-1} soil		mg C g^{-1} soil
Min	2.0	0.0	0.04	0.16	0.03	0.09	-0.31	-0.27	-0.84
Max	121.0	92.9	3.55	4.50	1.84	3.64	1.77	1.14	1.24
Mean	21.1	43.6	0.84	1.20	0.48	0.84	0.35	0.36	-0.01
Median	14.7	49.2	0.63	0.84	0.37	0.60	0.27	0.29	-0.01
SD	23.1	25.6	0.65	0.90	0.37	0.73	0.33	0.33	0.40
SDL	ND	ND	0.27	0.24	0.09	0.18	ND	ND	ND

SD: standard deviation of the means over three replicates per sample (97 samples). *SDL*: standard deviation over all incubations, including three replicates per sample, cf. equation (1).[22] ND: not determined.

Table 2. NIRS cross-validation results (total C, SOC and SIC are in mg C g^{-1} soil; CO_2 stands for CO_2 emission, over 7 or 21 days, at 18 or 28°C, from 0.2 or 2 mm samples, and is in µg C g^{-1} soil; C protection is the difference between CO_2 emission from 0.2 and 2 mm samples, in the same unit; vulnerability is the difference between C protection at 18 and 28°C, in the same unit).

Variable	Best pre-treatment	*N*	Mean	*SD*	*SECV*	*R²*	*RPD*	Terms
*Predictions using NIR spectra of **0.2 mm** samples*								
Total C	MSC14	97	64.7	38.2	8.0	0.96	4.8	11
SOC	MSC14	97	21.1	23.1	4.9	0.96	4.7	13
SIC	None14	97	43.6	25.6	6.3	0.94	4.0	9
CO_2 7d 0.2mm 18°	SNVD01	97	480	381	154	0.83	2.5	6
CO_2 7d 0.2mm 28°	D01	97	683	524	154	0.91	3.4	7
CO_2 7d 2mm 18°	D01	97	249	169	76	0.80	2.2	7
CO_2 7d 2mm 28°	None01	97	418	322	119	0.86	2.7	7
CO_2 21d 0.2mm 18°	D01	97	839	624	235	0.86	2.7	7
CO_2 21d 0.2mm 28°	D14	97	1204	882	380	0.81	2.3	4
CO_2 21d 2mm 18°	None01	97	484	369	192	0.73	1.9	7
CO_2 21d 2mm 28°	SNVD14	97	844	727	245	0.89	3.0	15
C protection 7d 18°	SNVD14	97	232	247	149	0.63	1.7	2
C protection 7d 28°	SNV01	97	266	226	98	0.81	2.3	7
C protection 21d 18°	None14	97	355	330	211	0.59	1.6	3
C protection 21d 28°	None14	97	360	325	318	0.04	1.0	1
Vulnerability 7d	None01	97	-34	168	169	-0.03	1.0	1
Vulnerability 21d	SNVD14	97	-5	398	377	0.09	1.1	1
*Predictions using NIR spectra of **2 mm** samples*								
Total C	SNVD14	97	64.7	38.2	8.4	0.95	4.5	9
SOC	SNV14	97	21.1	23.1	4.7	0.96	4.9	12
SIC	None01	97	43.6	25.6	5.7	0.95	4.5	14
CO_2 7d 0.2mm 18°	SNV01	97	480	381	136	0.87	2.8	14
CO_2 7d 0.2mm 28°	MSC01	97	683	524	197	0.86	2.7	10
CO_2 7d 2mm 18°	D14	97	249	169	83	0.76	2.0	11
CO_2 7d 2mm 28°	D14	97	418	322	130	0.84	2.5	10
CO_2 21d 0.2mm 18°	MSC01	97	839	624	235	0.86	2.7	15
CO_2 21d 0.2mm 28°	D14	97	1204	882	449	0.74	2.0	9
CO_2 21d 2mm 18°	D14	97	484	369	190	0.73	1.9	10
CO_2 21d 2mm 28°	D14	97	844	727	278	0.85	2.6	10
C protection 7d 18°	SNVD01	97	232	247	139	0.68	1.8	13
C protection 7d 28°	D01	97	266	226	113	0.75	2.0	7
C protection 21d 18°	SNVD01	97	355	330	224	0.54	1.5	9
C protection 21d 28°	SNV01	97	360	325	313	0.06	1.0	1
Vulnerability 7d	D14	97	-34	168	169	-0.02	1.0	1
Vulnerability 21d	SNVD14	97	-5	398	373	0.11	1.1	1

N is the number of samples used for cross validation. *SD* is the standard deviation of the means over three replicates per sample. *SECV* is the standard error of cross validation. Mean, *SD* and *SECV* are in the unit of the variable. *R²* is the determination coefficient. *RPD* is the ratio of *SD* to *SECV*.

Table 3. MIRS cross-validation results (total C, SOC and SIC are in mg C g^{-1} soil; CO_2 stands for CO_2 emission, over 7 or 21 days, at 18 or 28°C, from 0.2 or 2 mm samples, and is in µg C g^{-1} soil; C protection is the difference between CO_2 emission from 0.2 and 2 mm samples, in the same unit; vulnerability is the difference between C protection at 18 and 28°C, in the same unit).

Variable	Best pre-treatment	*N*	Mean	*SD*	*SECV*	R^2	*RPD*	Terms
*Predictions using MIR spectra of **0.2 mm** samples*								
Total C	SNV01	97	64.7	38.2	5.7	0.98	6.7	16
SOC	None01	97	21.1	23.1	5.4	0.95	4.3	13
SIC	D01	97	43.6	25.6	3.3	0.98	7.8	12
CO_2 7d 0.2mm 18°	None14	97	480	381	172	0.79	2.2	5
CO_2 7d 0.2mm 28°	D01	97	683	524	172	0.89	3.1	15
CO_2 7d 2mm 18°	SNVD01	97	249	169	93	0.70	1.8	9
CO_2 7d 2mm 28°	SNVD01	97	418	322	146	0.79	2.2	9
CO_2 21d 0.2mm 18°	MSC01	97	839	624	290	0.78	2.1	8
CO_2 21d 0.2mm 28°	SNV01	97	1204	882	471	0.71	1.9	12
CO_2 21d 2mm 18°	MSC14	97	484	369	233	0.60	1.6	3
CO_2 21d 2mm 28°	SNVD14	97	844	727	305	0.82	2.4	12
C protection 7d 18°	None14	97	232	247	154	0.61	1.6	5
C protection 7d 28°	None14	97	266	226	117	0.73	1.9	3
C protection 21d 18°	MSC14	97	355	330	226	0.53	1.5	3
C protection 21d 28°	MSC14	97	360	325	310	0.08	1.0	2
Vulnerability 7d	D01	97	-34	168	168	-0.01	1.0	1
Vulnerability 21d	SNV01	97	-5	398	394	0.01	1.0	2
*Predictions using MIR spectra of **2 mm** samples*								
Total C	MSC14	97	64.7	38.2	9.8	0.93	3.9	8
SOC	D14	97	21.1	23.1	9.1	0.84	2.5	6
SIC	SNVD01	97	43.6	25.6	4.4	0.97	5.8	7
CO_2 7d 0.2mm 18°	D14	97	480	381	189	0.75	2.0	3
CO_2 7d 0.2mm 28°	D14	97	683	524	207	0.84	2.5	4
CO_2 7d 2mm 18°	None14	97	249	169	97	0.67	1.8	3
CO_2 7d 2mm 28°	None14	97	418	322	146	0.79	2.2	3
CO_2 21d 0.2mm 18°	D14	97	839	624	312	0.75	2.0	3
CO_2 21d 0.2mm 28°	D14	97	1204	882	468	0.72	1.9	4
CO_2 21d 2mm 18°	MSC14	97	484	369	219	0.64	1.7	4
CO_2 21d 2mm 28°	D14	97	844	727	332	0.79	2.2	4
C protection 7d 18°	D14	97	232	247	171	0.52	1.4	2
C protection 7d 28°	D14	97	266	226	121	0.71	1.9	3
C protection 21d 18°	D14	97	355	330	238	0.47	1.4	3
C protection 21d 28°	MSC14	97	360	325	320	0.02	1.0	2
Vulnerability 7d	SNV01	97	-34	168	168	0.00	1.0	1
Vulnerability 21d	SNVD01	97	-5	398	389	0.03	1.0	1

N is the number of samples used for cross validation. *SD* is the standard deviation of the means over three replicates per sample. *SECV* is the standard error of cross validation. Mean, *SD* and *SECV* are in the unit of the variable. R^2 is the determination coefficient. *RPD* is the ratio of *SD* to *SECV*.

Figure 1. Comparisons between NIRS predictions and conventional determinations of soil CO_2 emission, C protection and its temperature vulnerability over 7 days (in µg C-CO_2 g^{-1} soil), and between MIRS predictions and conventional determinations of SIC (in g kg^{-1} soil). All predictions were made using the spectra of 0.2 mm samples.

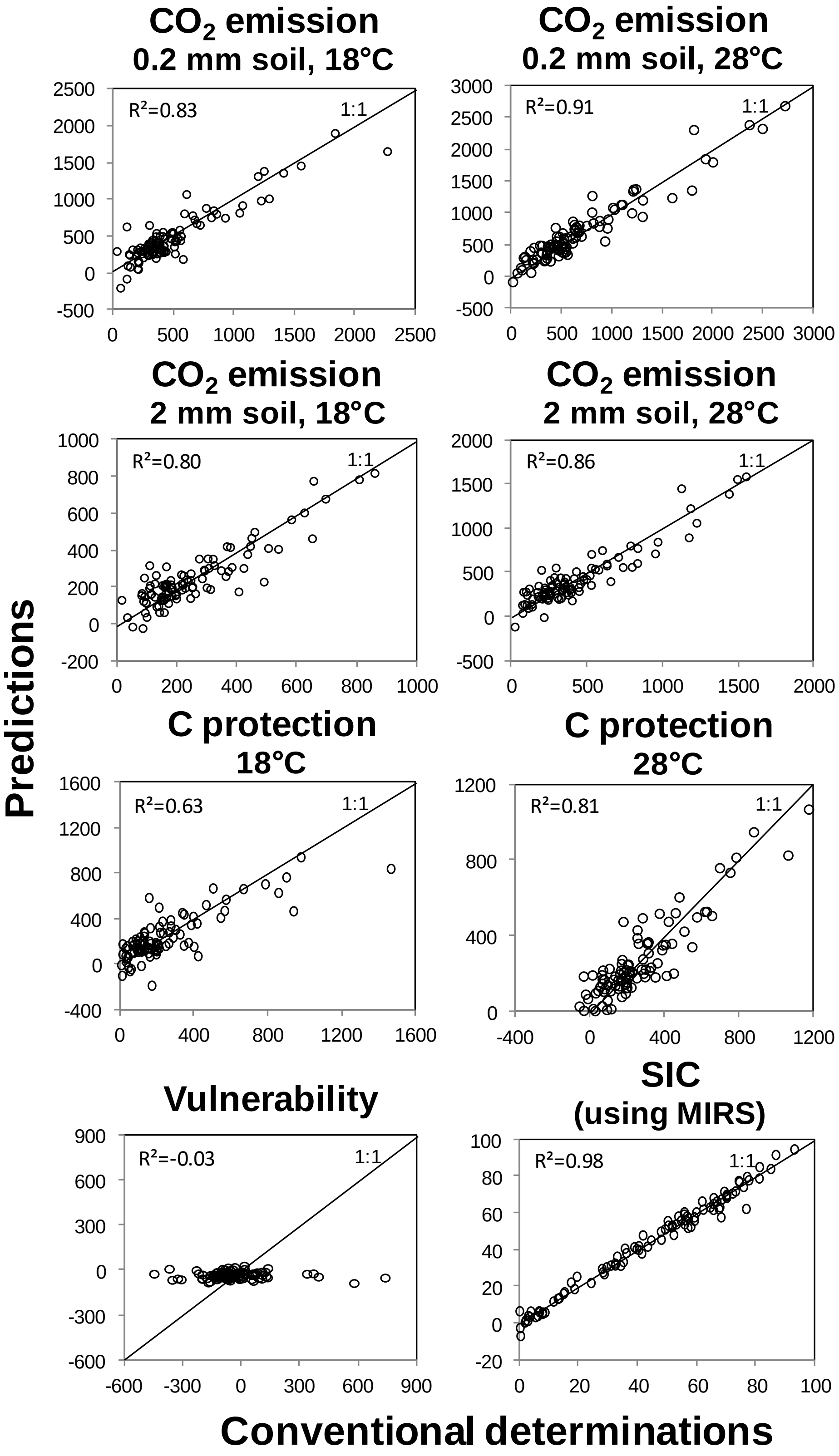


Figure 2. Regression coefficients of NIRS prediction models of CO_2 emission from 0.2 and 2 mm samples, and of soil C protection, at 18°C and 28°C, over the first 7 days of incubation (predictions made using NIR spectra of 0.2 mm samples, with no pre-treatment).

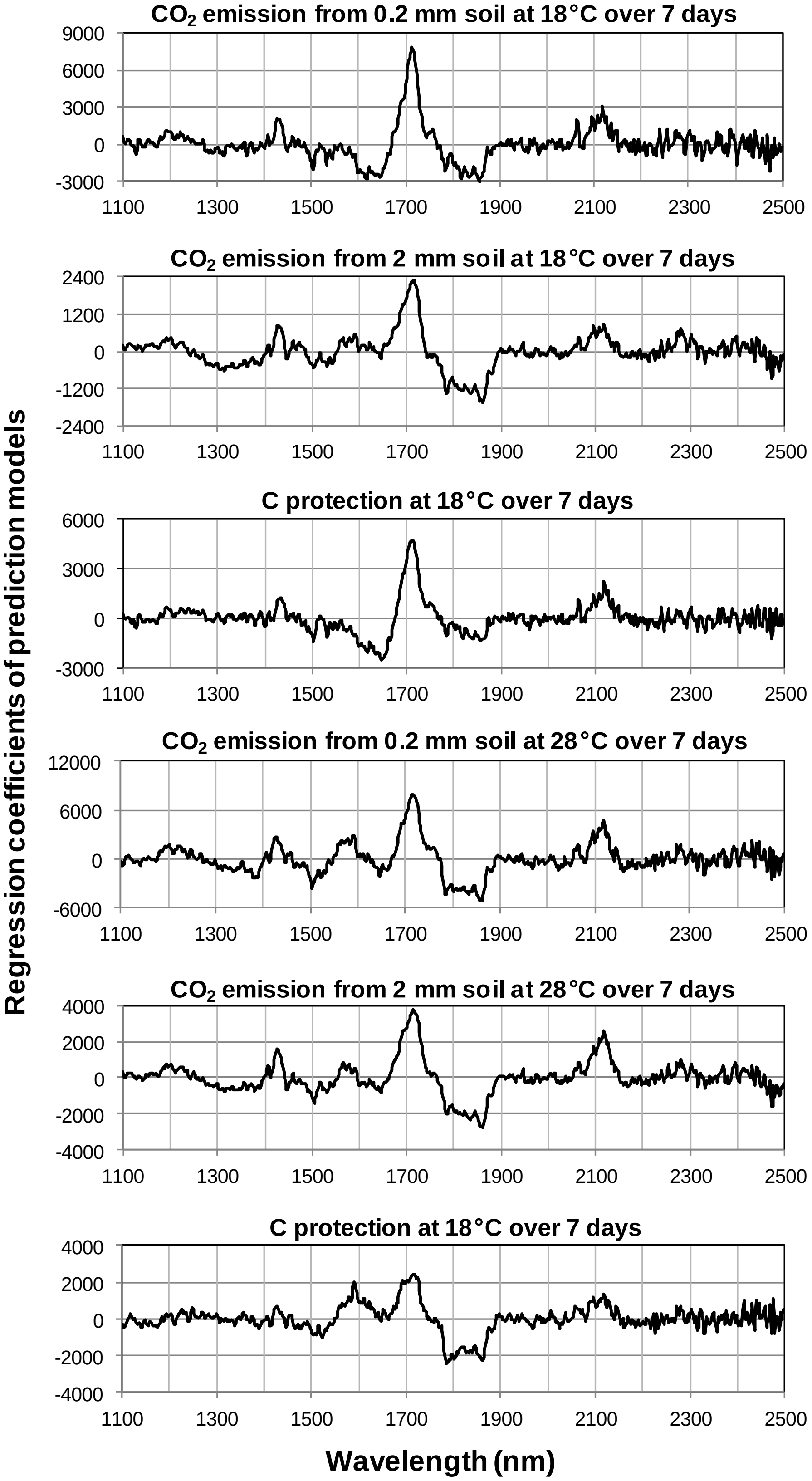
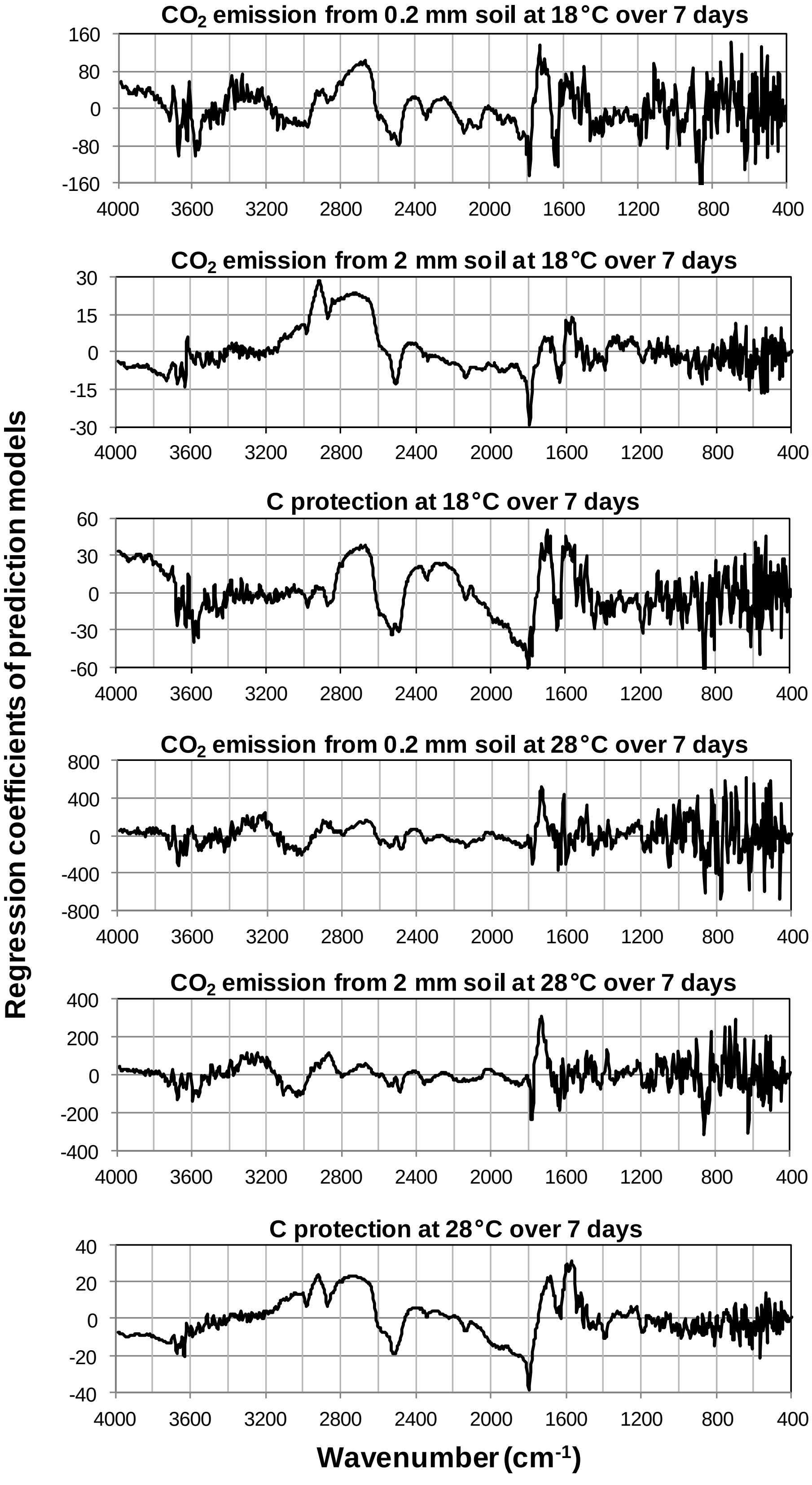


Figure 3. Regression coefficients of MIRS prediction models of CO_2 emission from 0.2 and 2 mm samples, and of soil C protection, at 18°C and 28°C, over the first 7 days of incubation (predictions made using MIR spectra of 0.2 mm samples, with no pre-treatment).



CO_2 emission from 0.2 mm soil at 18°C over 7 days

CO_2 emission from 2 mm soil at 18°C over 7 days

C protection at 18°C over 7 days

CO_2 emission from 0.2 mm soil at 28°C over 7 days

CO_2 emission from 2 mm soil at 28°C over 7 days

C protection at 28°C over 7 days