Published in final edited form as:
Soil Biol Biochem. 2008 July ; 40(7): 1923–1930. doi:10.1016/j.soilbio.2008.04.003.

Near infrared spectroscopy for determination of various physical, chemical and biochemical properties in Mediterranean soils

R. Zornoza[a,*], **C. Guerrero**[a], **J. Mataix-Solera**[a], **K.M. Scow**[b], **V. Arcenegui**[a], and **J. Mataix-Beneyto**[a]

[a]GEA (Grupo de Edafología Ambiental). Departamento de Agroquímica y Medio Ambiente, Universidad Miguel Hernández, Avda. de la Universidad s/n, 03202-Elche, Alicante, Spain.

[b] Department of Land, Air and Water Resources, University of California. One Shields Avenue, Davis, CA 95616, USA.

Abstract

The potential of near infrared (NIR) reflectance spectroscopy to predict various physical, chemical and biochemical properties in Mediterranean soils from SE Spain was evaluated. Soil samples (n=393) were obtained by sampling thirteen locations during three years (2003-2005 period). These samples had a wide range of soil characteristics due to variations in land use, vegetation cover and specific climatic conditions. Biochemical properties also included microbial biomarkers based on phospholipid fatty acids (PLFA). Partial least squares (PLS) regression with cross validation was used to establish relationships between the NIR spectra and the reference data from physical, chemical and biochemical analyses. Based on the values of coefficient of determination (r^2) and the ratio of standard deviation of validation set to root mean square error of cross validation (RPD), predicted results were evaluated as excellent (r^2>0.90 and RPD>3) for soil organic carbon, Kjeldahl nitrogen, soil moisture, cation exchange capacity, microbial biomass carbon, basal soil respiration, acid phosphatase activity, β-glucosidase activity and PLFA biomarkers for total bacteria, Gram positive bacteria, actinomycetes, vesicular-arbuscular mycorrhizal fungi and total PLFA biomass. Good predictions (0.81<r^2<0.90 and 2.5<RPD<3) were obtained for exchangeable calcium and magnesium, water soluble carbon, water holding capacity and urease activity. Resultant models for protozoa and fungi were not accurate enough to satisfactorily estimate these variables, only permitting approximate predictions (0.66<r^2<0.80 and 2.0<RPD<2.5). Electrical conductivity, pH, exchangeable phosphorus and sodium, metabolic quotient and Gram negative bacteria were poorly predicted (r^2<0.66 and RPD<2). Thus, the results obtained in this study reflect that NIR reflectance spectroscopy could be used as a rapid, inexpensive and non-destructive technique to predict some physical, chemical and biochemical soil properties for Mediterranean soils, including variables related to the composition of the soil microbial community composition.

*Corresponding author. Tel.: +34 966658336; Fax: +34 966658340. raul.zornoza@hotmail.com GEA (Grupo de Edafología Ambiental). Departamento de Agroquímica y Medio Ambiente, Edificio Alcudia. Universidad Miguel Hernández, Avda. de la Universidad s/n, 03202-Elche, Alicante, Spain..

Keywords

Near infrared reflectance; Mediterranean soils; organic matter; PLFA; soil enzymes

1. Introduction

The need for the development of more time- and cost- efficient methodologies for soil analysis is increasing. There is a great demand for rapid and predictive soil data to be used in environmental monitoring, soil quality assessment and precision agriculture and forestry (Cohen et al., 2005; Viscarra Rossel et al., 2006). For this reason near infrared (NIR) reflectance spectroscopy is considered as an alternative to complement (or even replace) conventional analytical methods.

NIR reflectance spectroscopy was initially developed in the early 1970's for rapid analysis of the moisture content of cereal grains (Chang et al., 2001). Over the past few decades, however, NIR reflectance spectroscopy has rapidly developed to become a fast and robust analytical method for many agricultural, pharmaceutical and food products (Blanco and Villarroya, 2002). In particular for soils, this technique permits the evaluation of different properties related to moisture and organic content matter, including carbon and nitrogen content or cation exchange capacity (Ben-Dor and Banin, 1995; Shepherd and Walsh, 2002; Islam et al., 2003; Chodak et al., 2004). Various authors have shown the effectiveness of NIR reflectance spectroscopy in estimating macro and micronutrients in soils (Dunn et al., 2002; Malley et al., 2002; Cozzolino and Morón, 2003; Islam et al., 2003), physical characteristics (Stenberg et al., 1995; Shepherd and Walsh, 2002; Cozzolino and Morón, 2003; Sorensen and Dalsgaard, 2005) and biochemical properties (Palmborg and Nordgren, 1993; Reeves et al., 2000; Chang et al., 2001; Coûteaux, et al., 2003; Cohen et al., 2005). In addition, this technique has been successfully used to predict maximum temperatures reached on burned soils (Guerrero et al., 2007). The conventional analytical methods exchangeable individually for most of the soil properties listed above are time consuming, destructive of samples and often use many chemical reagents. The advantages of using NIR reflectance spectroscopy include the simplicity of sample pre-treatment (sieving of soils), its lack of chemical reagents, its non-destructive nature, and the fact that it is rapid, inexpensive and accurate for analysis (Norris et al., 1976).

In the NIR region, the radiation is absorbed by the different chemical bonds, such as C–H, N–H, S–H, C=O and O–H of any chemical compounds present in the sample. Moreover, the radiation is absorbed in accordance with the concentration of these compounds. As a consequence, NIR reflectance spectra basically contain information about the organic composition of a soil sample. The NIR spectrum results from the overtones and combinations of fundamental vibrational bands for each of the chemical bonds, which are more strongly absorbed in the mid-infrared (MIR) region (Burns and Ciurczak, 2001). Nonetheless, as a consequence of overlapping bands, NIR information can not be directly interpreted from the obtained spectra. NIR reflectance spectroscopy is based on the use of calibrations, coupled with chemometrics techniques, which utilize absorbances at many wavelengths to predict particular properties of a sample (Batten, 1998). Normally, NIR spectra are used to establish a regression model in which the significant information contained in the spectra is concentrated into a few variables, optimized to produce the best correlation with the predicted property. Nevertheless, practically all published authors agree that to assure the reliability of this technique, it is necessary to include a great number of samples from zones with a wide range in the values of soil properties.

The objective of the present work is to explore the potential of models using NIR reflectance spectroscopy to estimate different physical, chemical and biochemical properties in soils from the Alicante Province of Spain, as rapid methods for soil analysis are essential to assess responses to perturbations, evaluate the effectiveness of restoration practices, or select the most sustainable management practices. Soils employed for this experiment were sampled from sites with different climatic conditions, vegetation cover and land uses, to guarantee a wide range in physical, chemical and biochemical characteristics, with the aim of creating models for extensive use in this region.

2. Materials and Methods

2.1 Soil samples

For this study, we used soil samples employed for previous researches. Soil samples were obtained from thirteen locations in the province of Alicante (SE Spain) during three years (2003-2005). The soil samples had a wide range in physical, chemical and biochemical characteristics (Table 1) due to variations in land use, vegetation cover and specific climatic conditions. Climate in all sites is Mediterranean, with mean annual temperature ranging from 13°C to 18°C, and mean annual precipitation from 270 mm to 600 mm, widely representative of the province of Alicante. The soil samples were collected from non-disturbed forest soils (259 samples), deforested forest soils (64 samples), arable soils (40 samples) and abandoned arable soils (40 samples). In year 2003, two land uses (non-disturbed forest and deforested forest) were selected in two locations (Sierra de Orihuela and Maigmó North slope). A plot of 200 m^2 was defined for each land use in each location. Samplings were carried out in winter, spring, summer and fall. In each of the four samplings, 16 soil samples per location were collected (8 from the non-degraded and 8 from the degraded zone). In year 2004, a single sampling was carried out in summer. A plot of 5 km^2 was defined in five non-distrubed forest sites (Sierra de Orihuela, Sierra de Crevillent, Maigmó South slope, Maigmó North slope and Puig Campana), where thirty soil samples were collected in each location. In year 2005, a single sampling was carried out in summer. We selected three land uses (undisturbed forest, arable and abandoned arable) in twelve locations (Sierra de Orihuela, Sierra de Crevillent, Maigmó South slope, Camara, Puig Campana, Peña de Sella, Puerto de Tudons, Puerto de Benifallim, Sierra del Reconco, Sierra de Salinas, Sierra de la Taja and Catí) A plot of 200 m^2 was defined for each land use in each location, where 3-5 soil samples were collected. See Zornoza et al. (2006, 2007, 2008) for more details about experimental design and soil sampling.

Regarding vegetation, non-disturbed forest soils have in common the presence of a tree stratum occupied by *Pinus halepensis* Miller, with understory vegetation dominated by some shrub species and *Brachipodium retusum* (Pers.) Beauv. as main herbaceous species. Deforested soils have suffered from a total elimination of the vegetation cover, although some spontaneous species have appeared, which consist of annual herbaceous species and some shrub, with vegetation cover <20%. Arable soils were cultivated with almond trees (*Prunus amygdalus* L.) Abandoned almond orchards presented some shrub species (dominated by *Rosmarinus officinalis* L., *Rhamnus lycioides* L., *Helichrysum stoechas* (L.) Moench, and *Cistus albidus* L.). Soils consisted of Calcixerolls, Haploxerolls, Xerorthents and Torriorhents (Soil Survey Staff, 1998), all of which had developed over calcaric bedrock.

In all samplings, individual soil samples (0-10 cm depth) were randomly collected from the mineral A horizon. Prior to analysis, the samples were air-dried for a week. After drying they were passed through a 2 mm mesh sieve. For all assays, two replicates per sample were used, and data are expressed on an oven dry weight basis. The number of soil samples used for the models construction was different depending on each soil property (Table 1).

2.2 Analytical Methods

Soil pH was measured in deionised water (1:2.5 w/v). Electrical conductivity (EC) was also measured in deionised water (1:5 w/v). Soil moisture was determined gravimetrically after heating the samples at 105°C for 24 h. Soil organic carbon (SOC) was determined by potassium dichromate oxidation (Nelson and Sommers, 1982). Total nitrogen (N_K) was determined by the Kjeldahl method (Bremmer and Mulvaney, 1982). Cation exchange capacity (CEC) was measured by the method described by Roig et al. (1980). Exchangeable phosphorus (P) was determined by the Burriel-Hernando method (Díez, 1982). Exchangeable Ca, Mg, K and Na were extracted with 1N ammonic acetate (Knudsen et al., 1982) and measured by atomic absorption and emission spectrophotometry. Water holding capacity (WHC) was assayed by the method expounded by Forster (1995). Microbial Biomass Carbon (MBC) was determined using the fumigation-extraction procedure (Vance et al., 1987), and the 0.5 M K_2SO_4 extracted carbon was measured in the same way as indicated for water soluble carbon (WSC). Basal soil respiration (BSR) was monitored for 4 days at 55% WHC and 25°C with a multiple sensor respirometer (Micro-Oxymax, Columbus, OH, USA). Metabolic quotient (qCO_2) was calculated by the relationship between BSR and MBC (BSR/MBC). Urease activity was measured according to the method of Nannipieri et al. (1980). Acid phosphatase activity was assayed by the method of Tabatabai and Bremmer (1969). The activity of β-glucosidase was determined according to Tabatabai (1982).

Phospholipid fatty acid (PLFA) analysis was carried out as described in Bossio et al. (1998). Briefly, fatty acids were extracted from 8 g soil samples using chloroform:methanol:phosphate buffer. PLFAs were separated from neutral and glycolipid fatty acids on a solid phase extraction column (0.58 Si; Supelco Inc., Bellafonte, PA). Afterwards mild alkaline methanolysis, samples were analysed using a Hewlett Packard 6890 Gas Chromatograph with 25 m Ultra 2 (5% phenyl)-methylpolysiloxane column (J & W Scientific, Folsom, CA). Fatty acids were quantified by comparison of the peak areas with those of an internal standard 19:0 peak. The peaks were named using bacterial standards and identification software from the Microbial Identification System (Microbial ID, Inc., Newark, DE). Fatty acid nomenclature used was that described by Frostegard et al. (1993). The fatty acids i 15:0, 15:0, a 15:0, i 16:0, 16:1ω7, i 17:0, a 17:0, cy 17:0, 17:0, 18:1ω7 and cy 19:0 were chosen to represent bacteria (Frostegard et al., 1993). The unsaturated PLFA 18:26ω was used as indicator of fungal biomass (Federle, 1986). PLFAs cy 17:0, 18:1ω7, cy 19:0, 17:1ω9c, 16:1ω9c, 18:1ω9c and 15:1ω4c were chosen to represent Gram-negative [G-] bacteria (Zelles et al., 1994). The branched, saturated i 14:0, i 15:0, a 15:0, i 16:0, i 17:0 and a 17:0 were chosen to represent Gram-positive [G+] bacteria (Zelles et al., 1994). The PLFAs 10 Me 16:0, 10 Me 17:0 and 10 Me 18:0 were selected as indicators of actinomycetes biomass (Zelles et al., 1994). The PLFA 16:1ω5 was used as representative of vesicular-arbuscular mycorrhizal [VAM] fungi (Olsson et al., 1995). The fatty acids 20:2ω6,9c, 20:3ω6,9,12c and 20:4ω6,9,12,15c were chosen to represent protozoa (White et al., 1996). The total biomass was estimated as the sum of all the extracted PLFAs [totPLFAs].

2.3 NIR spectra

Aliquots of around 50 g of soil samples were placed in glass Petri-dishes, and scanned on reflectance mode from 12000 to 3800 cm^{-1}. For these measurements we used a Fourier-Transform near infrared (FT-NIR) spectrophotometer (MPA, Bruker Optik GmbH, Germany), equipped with quartz beamsplitter and PbS detector. It was also equipped with an integrating macrosample sphere and rotating sample cup, allowing the scanning of large areas of the samples. In each of the reflectance measurements, 64 scans were averaged. Samples were measured in duplicate, increasing the surface of soil sample scanned. After

this, they were averaged again. The time employed for the spectral measurement was approximately 1 minute per sample. The resolution used for spectral analysis was 8 cm^{-1}. Background corrections were made before each sample scan. The x-scale of each NIR spectrum was transformed from wavenumber to wavelength, obtaining a 1000-absorbance point's spectrum between 830 and 2630 nm.

2.4 Models construction

Prediction models were constructed based on this following equation:

$$Y=b \bullet X \quad [1]$$

where Y is the target parameter (analyzed soil property in laboratory), b the calibration function and X the NIR spectra.

Two matrixes were constructed previously:

- the NIR-spectra matrix, composed of as many rows as soil samples used for calibration and 1000 columns (1000 absorbance values between 830 and 2630 nm). This matrix is the source of the X-term in the prediction models [1].

- the matrix of soil properties analyzed in laboratory, composed of as many rows as soil samples used for calibration and 1 column (with the value of the analyzed soil property in each soil sample). This matrix was the source of the Y-term in prediction model [1].

For the model construction (empirical calibration functions), we used partial least squares (PLS) regressions. PLS regression is widely employed as chemometrics in NIR analysis (Burns and Ciurczak, 2001; McCarty et al., 2002). Briefly, PLS reduces the NIR matrix to a few components, such as in a principal component analysis (PCA), but during the components extraction step in PLS, the data of the target parameter to be estimated is taken into account. The number of PLS components (so called PLS-vectors) used is the ‘rank’ of the PLS regression (the rank of the model). The first PLS-vectors are those which provide more information about the target parameter. In general terms, models with low ranks are preferred, because the higher the rank used, the higher the noise included.

Typical spectroscopic preprocessing of the spectra were tested: with no data preprocessing, first derivative, second derivative, linear offset subtraction, straight line subtraction, multiplicative scatter correction, vector normalization, min-max normalization, and combinations of them (Conzen, 2003). In fact, more than one thousand possibilities were tested using spectroscopic software (OPUS version 5.5, Bruker Optik GmbH, Germany) during each calibration. These procedures were made with the aim of reducing optical interference not related to the chemical composition of the sample such as, for example, those variations caused by different sample particle size (Blanco and Villarroya, 2002). Derivative treatment not only reduces scattering effects but also increases the resolution of spectral peaks (Burns and Ciurczak, 2001). In each case, we selected the preprocessing method so that the PLS algorithm can establish the best correlation between the spectral and the analytical data.

We used the cross validation method for the model construction. With this method, n-1 samples were used for calibration, while the excluded sample was estimated (and validated) with the others. This exclusion-step was repeated successively until all samples were validated with calibrations performed by the others. All of these procedures were conducted using the software OPUS version 5.5 (Bruker Optik GmbH, Germany).

2.5 Selection of models and statistics

The best models were defined as those which presented lower values of root mean square error in cross validation (RMSECV), low ranks and higher coefficient of determination (r^2). Furthermore, with the aim of comparing the accuracy of our models with others NIR-models cited in the literature, we calculated the RPD, being the ratio of the standard deviation of analysed data to RMSECV. The equations describing the statistics employed are:

$$r^2 = \left(1 - \frac{\sum (Differ_i)^2}{\sum (yi - ym)^2}\right) \quad [2]$$

$$\text{RMSECV} = \sqrt{\frac{1}{n} \cdot \sum_{i=1}^{n} (Differ_i)^2} \quad [3]$$

$$\text{where} \quad Differ_i = Y_i^{measured} - Y_i^{predicted} \quad [4]$$

$$\text{RPD} = \frac{\text{standard deviation of analysed data}}{\text{RMSECV}} \quad [5]$$

To evaluate the accuracy of models, the coefficient of determination and the RPD statistic were used. According to Saeys et al. (2005), a value for r^2 between 0.66 and 0.80 indicates approximate quantitative predictions, whereas a value for r^2 between 0.81 and 0.90 reveals good prediction. Calibration models having r^2 >0.90 are considered to be excellent. Regarding RPD statistic, an RPD<2 is considered insufficient for applications, whereas a value for RPD between 2 and 2.5 makes approximate quantitative predictions possible. For values between 2.5 and 3 predictions can be classified as good, and an RPD>3 indicates an excellent prediction.

When no accurate models were obtained, analytical data of soil properties were transformed using square roots or logarithms (Table 2). These transformations assure normal distribution of data, which favors a better fit by PLS regressions.

Apart from the PLS models, correlations were developed to study the relationships between physical, chemical and biochemical properties, and soil organic matter. This analysis was performed with the software SPSS for Windows, Version 13.0.

3. Results

3.1. Spectra

The NIR spectra of all soil samples were similar in appearance, with the lowest absorbance values in arable soils. Fig. 1 shows an example of spectra for an undisturbed forest soil, a deforested forest soil, an arable soil and an abandoned arable soil. All NIR spectra of the soil samples had the highest absorbance peaks at approx. 1400, 1900 and 2200 nm. The band at 1400 nm is usually associated with O-H and aliphatic C-H, whilst the absorbance band at 1900 nm is related with amide N-H and O-H. In the band of 2200 nm there are groups such as phenolic O-H, amide N-H, amine N-H and aliphatic C-H (Fidêncio et al., 2002; Cozzolino and Morón, 2003). Thus, owing to the overlap of bands, quantitative predictions are difficult by direct interpretations from the NIR spectra, and multivariate analyses are needed to discern the response of soil properties from spectral characteristics.

3.2. Calibrations of prediction models

The ability of NIR reflectance spectroscopy to predict the 27 soil properties is summarized in Table 2. The coefficient of determination (r^2), RMSECV, rank and RPD are given. Preprocessing methods were chosen as those which provided the best models for each soil property (lowest values in RMSECV and rank, and highest values in r^2). Scatter plots of predicted vs actual values for biochemical properties are shown in Fig. 2.

For physical and chemical properties, (Table 2) the best results were achieved for SOC (r^2=0.98; RPD=5.75), N_K(r^2=0.96; RPD=4.88), soil moisture (r^2=0.96; RPD=4.69) and CEC (r^2=0.92; RPD=3.46), considered as excellent predictions. Ca and Mg showed values of r^2>0.90, but RPD<2.5. Thus, these predictions, although good, cannot be considered excellent. Models for WSC and WHC were also accurate to predict these variables, with r^2>0.80 and RPD> 2.5. To the contrary, pH, EC, P and Na were poorly predicted, with RPD<2.

For the biochemical properties (Fig. 2), excellent predictions were achieved with MBC (r^2=0.91; RPD=3.26), BSR (r^2=0.92; RPD=3.59), acid phosphatase activity (r^2=0.93; RPD=3.66) and β-glucosidase activity (r^2=0.93; RPD=3.66). Urease activity was also predicted satisfactorily by NIR spectroscopy (r^2=0.80; RPD=2.66), whilst prediction for qCO_2 was very poor (r^2=0.60; RPD=1.92). NIR reflectance spectroscopy could satisfactory predict several variables related to soil microbial groups based on PLFAs biomarkers. Successful predictions were achieved for bacteria (r^2=0.93; RPD=3.74), G+ bacteria (r^2=0.91; RPD=3.24), actinomycetes (r^2=0.92; RPD=3.54), VAM fungi (r^2=0.91; RPD=3.04) and totPLFAs (r^2=0.91; RPD=3.14). The predictions of fungi and protozoa were not as good as those of the previous variables, and only approximate quantitative predictions are possible. The variable G- bacteria was poorly predicted (r^2=0.60; RPD=1.60).

4. Discussion

The soil properties which were most strongly correlated with soil reflectance properties were SOC, N_K and soil moisture, as often reported in literature (Chang et al., 2001; Fystro, 2002; Fidêncio et al., 2002; Ludwig et al., 2002; Shepherd and Walsh, 2002; Islam et al., 2003; Chodak et al., 2004). This makes sense because NIR reflectance spectroscopy provides information about the relative proportions of bonds such as C-H, N-H, S-H and O-H, present in the organic compounds (O-H also included in the water molecule). Although moisture was determined in air-dried soil samples, there is still remaining water adsorbed on the surface areas of clay minerals and organic matter, in equilibrium with atmospheric water vapor.

Similar results to those achieved in our study with CEC have been reported in other studies (Chang et al., 2001; Dunn et al., 2002; Chodak et al., 2004). However, no articles have been found in literature describing models relating to WHC, despite the fact that this parameter reflects the capacity of a soil to retain water, an important attribute for soil quality assessment or agriculture management. Exchangeable Ca and Mg were also satisfactorily predicted, as has been observed by other researchers (Malley et al., 2002; Shepherd and Walsh, 2002; Dunn et al., 2002; Cozzolino and Morón, 2003; Chodack et al., 2004).

Nevertheless, CEC, WHC and exchangeable Ca and Mg do not have a primary response in the NIR region, and so, they are not directly predicted by NIR reflectance spectroscopy. Thus, correlation of these properties with soil organic matter (r>0.70; P<0.001) may explain some of this effect. Chang et al. (2001) carried out an experiment to verify if predictions of variables that do not have primary response in the NIR region were achieved by correlations with properties exhibiting a primary response. These authors compared the predictions of

secondary properties (such as CEC) by multiple linear regression using primary properties as explanatory variables, with predictions using the NIR spectroscopy. They found that while high regression coefficients were obtained by multiple linear regressions, high regression coefficients were also obtained using the NIR spectroscopy, which confirmed their hypothesis. Thus, the ability of NIR-PLS to predict CEC, WHC and exchangeable Ca and Mg, may be due to the strong dependence of these properties on organic matter and clays, which have a primary response in the NIR region. These properties are principally controlled by clay and organic matter type and content, which have functional groups with variable charges responsible for the adsorption of the different cations and water. In addition, exchangeable Ca and Mg dominate the exchange complex in the studied soils. Thus, their concentrations highly depend on the functional groups of organic matter and clays, and, as a result, they are well predicted by NIR spectroscopy.

Poor predictions have been obtained for exchangeable K, Na, and P, as observed in previous studies (Chang et al., 2001; Malley et al., 2002; Shepherd and Walsh, 2002; Dunn et al., 2002; Cozzolino and Morón, 2003; Chodak et al., 2004). In addition, pH and EC were also unsatisfactorily predicted, confirming similar findings by Chang et al. (2001), Dunn et al. (2002), Islam et al. (2003) or Pirie et al. (2005). These properties do not have a primary response in the NIR region. Thus, the ability of NIR-PLS to predict these properties would depend on their relationships with organic matter and clay content, as observed for CEC, WHC and exchangeable Ca and Mg. However, exchangeable K, Na and P, pH and EC were not correlated with organic matter, and this may be the reason why no satisfactory predictions were achieved for these variables, suggesting that organic matter type and content and clay content do not directly govern these properties in the studied soils.

We have demonstrated the premise of using NIR reflectance spectroscopy to predict various biochemical properties. Contrary to the latter parameters, these properties are based on organic compounds, and, as a consequence, have functional groups able to absorb radiation in the NIR region and provoke direct changes in the reflectance characteristics of the samples. This technique has not been as widely used for biochemical as for traditional soil chemical properties. Palmborg and Nordgren (1993) reported values of r^2=0.70 and r^2>0.90 in prediction models for MBC and BSR respectively. Cheng et al. (2001) obtained approximate predictions for MBC (r^2=0.60; RPD=1.10) and BSR (r^2=0.82; RPD=2.31). Excellent predictions were achieved by Coûteaux et al. (2003) for MBC (r^2=0.95; RPD=4.40). It is interesting to point out that although MBC and BSR were well predicted by NIR spectroscopy, the qCO_2, a ratio of BSR to MBC, was poorly predicted. One possible explanation is that the efficiency in C use, represented by this quotient, strongly depends on the physiological status of the microbial community, a characteristic unlikely to influence the spectra to register a measurable effect. Certain enzyme activities were also satisfactorily predicted by NIR reflectance spectroscopy, as reported by Cohen et al. (2005) for acid phosphatase (r^2=0.94; RPD=2.62), β-glucosidase (r^2=0.96; RPD=2.64), dehydrogenase (r^2=0.96; RPD=1.89) and peptidase (r^2=0.94; RPD=2.37). Nevertheless, Reeves et al. (2000) achieved poor predictions for acid phosphatase, urease, arylsulfatase and dehydrogensase, with r^2<0.75.

There have been few attempts to predict variables related to the soil microbial community composition by NIR reflectance spectroscopy. The first study we know that measured relationships between microbial community data and soil reflectance was that expounded by Johnson et al. (2003). The authors did not develop quantitative prediction models, but observed that the grouping of soil samples based on their soil reflectance properties was similar to the grouping based on DNA fingerprinting. Rinnan and Rinnan (2007) applied NIR reflectance spectroscopy to predict, as in our research, groups of microorganisms based on PLFAs. These authors could satisfactorily predict totPLFAs (r^2=0.78), fungi (r^2=0.78)

and the ratio fungi to bacteria (r^2=0.80), the predictions being similar to those shown in our study for fungi, but poorer for totPLFAs. The ability of NIR to predict these variables based on PLFAs, if it holds true for a larger set of samples under different conditions, is particularly promising because PLFA analyses are expensive, laborious and require great amounts of chemical reagents, while NIR is much faster, inexpensive and needs no chemical reagents. Since soil microbial properties have proved to be sensitive and reliable indicators for soil quality (Nannipieri et al., 1990; Dick et al., 1996), there has been a great expansion of research into the possibilities of using variables related to soil microbial community structure to assess degradation processes, restoration strategies or management practices. As a consequence, it is worthwhile to direct efforts on the applicability of NIR to create accurate prediction models for PLFAs biomarkers in soil. Cohen et al. (2005) and Rinnan and Rinnan (2007), however, speculated that the low concentrations of microbiological variables in the soil matrix make it unlikely that it will be possible to obtain direct measurable effects by changes in the reflectance characteristics, and good predictions could be the consequence of high correlations with total soil organic matter quantity and quality. Rinnan and Rinnan (2007) observed that the regression coefficients of the microbiological variables were rather similar to those of organic matter, concluding that NIR detected a combination of soil constituents containing organic functional groups, which are related to the studied microbiological variables. Nonetheless, in our study we have not found high similarities between the regression coefficients of the biochemical properties and SOC. Furthermore, the structure of the PLS vectors are also different for all these properties, suggesting that calibrations have been carried out independently, with different spectral regions implied in each property. As it is shown in Table 3, correlations between SOC concentration and NIR absorbance data have been developed in the ranges 1732-1914 and 2092-2630 nm. Nonetheless, for the calibration of the biochemical properties, other ranges of the spectrum have been used. Concretely, there are regions under 1732 nm used in most calibrations, indicating that this region has variations in the spectral data which have their origin in variations in the biochemical properties concentrations. That is to say, this region must contain information about functional groups bound to the biochemical properties, as it is present in practically all cases. As a consequence, calibrations have been developed by means of the best correlations between the concentration of the biochemical properties and the variations in the spectra. In Table 4 we show the values of r^2 after simple linear regressions of biochemical properties with SOC, and the values of r^2 achieved after PLS regressions with NIR spectra. The values of r^2 in the linear regressions are always lower than r^2 obtained with NIR spectra. Hence, PLS regressions extract information from the NIR spectra directly related to the biochemical properties, as this accuracy can not be explained by direct correlations with SOC.

To conclude, this study confirms the usefulness of NIR spectroscopy for the prediction of various properties in Mediterranean soils. NIR spectroscopy offers a number of important advantages over other methods. It is a rapid, non-destructive method, requires minimal pre-treatment of samples (only air-drying and sieving), it is highly accurate and free of chemical reagents and harmful waste production. NIR spectroscopy used in association with sophisticated chemometrics tools permits the construction of accurate and reliable prediction models for several physical, chemical and biochemical properties, including variables related to the microbial community composition of the soil. Therefore, NIR spectroscopy could be used as a rapid analytical tool for soil quality assessment and soil management. Furthermore, low costs of sample evaluation would allow high spatial and temporal resolution for routine monitoring across large areas, which may greatly reduce management uncertainty (Cohen et al., 2005). However, further work is needed to develop more reliable models including a larger number of samples from different soil types and zones with a wide range of soil characteristics. Nonetheless, the excellent prediction models that were

developed here, with a large number of soil samples (393) from different land uses and soil types in SE Spain, could be used to characterize other soils collected in this general region.

Acknowledgments

This research was supported by the Generalitat Valenciana co-financed FEDER program. R. Zornoza and V. Arcenegui acknowledge a grant from the Ministry of Education and Science of the Government of Spain and Caja de Ahorros del Mediterráneo, respectively. Support for PLFA analysis was supported by the National Institute of Environmental Health Sciences (NIEHS), NIH. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of these agencies.

References

Batten GD. Plant analysis using near infrared reflectance spectroscopy: the potential and the limitations. Australian Journal of Experimental Agriculture. 1998; 38:697–706.

Ben-Dor E, Banin A. Near infrared analysis as a rapid method to simultaneously evaluate several soil properties. Soil Science Society of America Journal. 1995; 59:364–372.

Blanco M, Villarroya I. NIR spectroscopy: a rapid-response analytical tool. Trac-Trends in Analitical Chemistry. 2002; 21:240–250.

Bossio DA, Scow KM, Gunapala N, Graham KJ. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial Ecology. 1998; 36:1–12. [PubMed: 9622559]

Bremmer, JM.; Mulvaney, CS. Nitrogen total.. In: Page, AL.; Miller, RH.; Keeney, DR., editors. Methods of soil analysis, Part 2, Chemical and microbiological properties. American Society of Agronomy; Madison: 1982. p. 595-624.

Burns, DA.; Ciurczak, EW. Handbook of near-infrared analysis. Second edition. Marcel Dekker; New York: 2001. revised and expanded

Chang C, Laird DA. Near-Infrared reflectance spectroscopic analysis of soil C and N. Soil Science. 2002; 167:110–116.Soil Science Society of America Journal. 65:480–490.

Chang C, Laird DA, Mausbach MJ, Hurburgh CR Jr. Near-Infrared Reflectance Spectroscopy-Principal Components Regression Analyses of soil properties. Soil Science Society of America Journal. 2001; 65:480–490.

Chodak M, Khanna P, Horvarth B, Beese F. Near infrared spectroscopy for determination of total and exchangeable cations in geologically heterogeneous forest soils. Journal of Near Infrared Spectroscopy. 2004; 12:315–324.

Cohen MJ, Prenger JP, DeBusk WF. Visible-Near infrared reflectance spectroscopy for rapid, non-destructive assessment of wetland soil quality. Journal of Environmental Quality. 2005; 34:1422–1434. [PubMed: 15998865]

Conzen, JP. A practical guide for developing methods in the quantitative analytical chemistry. Bruker Optik GmbH; Alemania: 2003. Multivariate calibration..

Coûteaux M-M, Berg B, Rovira P. Near infrared reflectance spectroscopy for determination of organic matter fractions including microbial biomass in coniferous forest soils. Soil Biology & Biochemisty. 2003; 35:1587–1600.

Cozzolino D, Morón A. The potencial of near-infrared reflectance spectroscopy to analyse soil chemical and physical characteristics. Journal of Agricultural Science. 2003; 140:65–71.

Dick, RP.; Breakwell, DP.; Turco, RF. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators.. In: Doran, JW.; Jones, AJ., editors. Methods for Assessing Soil Quality. Soil Science Society of America, Special Publication 49; Madison, WI: 1996. p. 247-271.

Díez JA. Consideraciones sobre la utilización de la técnica extractiva de Burriel-Hernando para la evaluación de fósforo asimilable en suelos. Anales de Edafología y Agrobiología. 1982; 41:1345–1353.

Dunn BW, Beecher HG, Batten GD, Ciavarella S. The potential of near-infrared reflectance spectroscopy for soil analysis – a case study from the Riverine Plain of south-eastern Australia. Australian Journal of Experimental Agriculture. 2002; 42:607–614.

Federle, TW. Microbial distribution in soil.. In: Megusar, F.; Gantar, M., editors. Perspectives in microbial ecology. Slovene Society for Microbiology; Ljubljana: 1986. p. 493-498.

Fidêncio PH, Poppi RJ, Andrade JC, Cantarella H. Determination of organic matter in soil using near-infrared spectroscopy and partial least squares regression. Communications in Soil Science and Plant Analysis. 2002; 33:1607–1615.

Forster, JC. Soil physical analysis.. In: Alef, K.; Nannipieri, P., editors. Methods in Applied Soil Microbiology and Biochemistry. Academic Press Inc.; San Diego, CA: 1995. p. 106-111.

Frostegard A, Bååth E, Tunlid A. Shifts in the composition of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology & Biochemistry. 1993; 25:723–730.

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

Guerrero C, Mataix-Solera J, Arcenegui V, Mataix-Beneyto J, Gómez I. Near-Infrared Spectroscopy to estimate the Maximum Temperatures reached on burned soils. Soil Society of America Journal. 2007; 71:1029–1037.

Islam K, Stingh B, McBratney. Simultaneous estimation of several soil properties by ultra-violet, visible, and near-infrared reflectance spectroscopy. Australian Journal of Soil Research. 2003; 41:1101–1114.

Johnson MJ, Lee KY, Scow KM. DNA fingerprinting reveals links among agricultural crops, soil properties, and the composition of soil microbial communities. Geoderma. 2003; 114:279–303.

Knudsen, D.; Peterson, GA.; Pratt, PF. Lithium, Sodium and Potassium.. In: American Society of Agronomy and Soil Science Society of America Journal. , editor. Methods of soil analysis. Vol. 2. Madison, WI: 1982. p. 225-246.

Ludwig B, Khanna PK, Bauhus J, Hopmans P. Near infrared spectroscopy of forest soils to determine chemical and biological properties related to soil sustainability. Forest Ecology and Management. 2002; 171:121–132.

Malley DF, Yesmin L, Eilers RG. Rapid analysis of hog manure and manure-amended soils using near-infrared spectroscopy. Soil Science Society of America Journal. 2002; 66:1677–1686.

McCarty GW, Reeves JB III, Reeves VB, Follett RF, Kimble JM. Mid-infrared and near-infrared diffuse reflectance spectroscopy for soil carbon measurement. Soil Science Society of America Journal. 2002; 66:640–646.

Nannipieri P, Ceccanti B, Cervelli S, Matarese E. Extraction of phosphatase, urease, protease, organic carbon and nitrogen from soil. Soil Science Society of America Journal. 1980; 44:1011–1016.

Nannipieri, P.; Greco, S.; Ceccanti, B. Ecological significance of the biological activity in soil.. In: Bollag, JM.; Stotzky, G., editors. Soil Biochemistry. Vol. 6. Marcel Dekker Inc.; 1990. p. 293-355.

Nelson, DV.; Sommers, LE. Total carbon, organic carbon, and organic matter.. In: Page, AL.; Miller, RH.; Keeney, DR., editors. Methods of soil analysis. Part 2. Chemical and biological methods. American Society of Agronomy and Soil Science of America; Madison, WI: 1982. p. 539-579.

Norris KH, Barnes RF, Moore JE, Shenk JS. Predicting forage quality by infrared reflectance spectroscopy. Journal of Animal Science. 1976; 43:889–897.

Olsson PA, Bååth E, Jakobsen I, Söderström B. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycological Research. 1995; 99:623–629.

Palmborg C, Nordgren A. Partitioning the variation of microbial measurements in forest soil into heavy metal and substrate quality dependent parts by use of near infrared spectroscopy and multivariate statistics. Soil Biology & Biochemistry. 1995; 28:711–720.

Pirie A, Singh B, Islam K. Ultra-violet, visible and near-infrared diffuse reflectance spectroscopic techniques to predict several soil properties. Australian Journal of Soil Research. 2005; 43:713–721.

Reeves JB III, McCarty GW, Meisinger JJ. Near infrared reflectance the analysis of agricultural soils. Journal of Near Infrared Spectroscopy. 1999; 7:179–193.

Reeves JB III, McCarty GW, Meisinger JJ. Near infrared reflectance spectroscopy for the determination of biological activity in agricultural soils. Journal of Near Infrared Spectroscopy. 2000; 8:161–170.

Rinnan R, Rinnan A. Application of near infrared reflectance (NIR) and fluorescence spectroscopy to analysis of microbiological and chemical properties of artic soil. Soil Biology & Biochemistry. 2007; 39:1664–1673.

Roig A, Romero M, Lax A, Fernández FG. Estudio comparativo de métodos de determinación de capacidad de cambio catiónica en suelos calizos. Anales de Edafología y Agrobiología. 1980; 39:2021–2032.

Saeys W, Mouazen AM, Ramon H. Potential for onsite and online analysis of pig manure using visible and near infrared reflectance spectroscopy. Biosystems Engineering. 2005; 91:393–402.

Shepherd KD, Walsh MG. Development of reflectance spectral libraries for characterization of soil properties. Soil Science Society of America Journal. 2002; 66:988–998.

Soil Survey Staff. Keys to soil taxonomy. 8th ed.. USDA-NRCS; Washington DC: 1998. p. 326

Sorensen LK, Dalsgaard S. Determination of clay and other soil properties by near infrared spectroscopy. Soil Science Society of America Journal. 2003; 69:159–167.

Stenberg B, Nordkvist E, Salomonsson L. Use of near infrared reflectance spectra of soils for objective selection of samples. Soil Science. 1995; 159:109–114.

Tabatabai, MA. Soil enzimes.. In: Page, AL.; Miller, RH.; Keeney, DR., editors. Methods of Soil Analysis, Part 2. 2nd ed.. American Society of Agronomy and Soil Science of America; Madison, WI: 1982. p. 501-538.Agronomical Monograph No. 9

Tabatabai MA, Bremner JM. Use of *p*-nitrophenyl phosphate in assay of soil phosphatase activity. Soil Biology & Biochemistry. 1969; 1:301–307.

Vance ED, Brookes PC, Jenkinson DS. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry. 1987; 19:703–707.

Viscarra Rossel RA, Walvoort DJJ, McBratney AB, Janik LJ, Skjemstad JO. Visible, near infrared, mid infrared or combined diffuse reflectance spectroscopy for simultaneous assessment of various soil properties. Geoderma. 2006; 131:59–75.

White D, Stair J, Ringelberg D. Quantitative comparisons of in situ microbial diversity by signature biomarker analysis. Journal of Industrial Microbiology and Biotechnology. 1996; 17:185–196.

Zelles L, Bai QY, Ma RX, Rackwitz R, Winter K, Beese F. Microbial biomass, metabolic quotient and nutritional status determined from fatty acid patterns and polyhydroxybutyrate in agriculturally-managed soils. Soil Biology & Biochemistry. 1994; 26:439–446.

Zornoza R, Guerrero C, Mataix-Solera J, Arcenegui V, García-Orenes F, Mataix-Beneyto J. Assessing air-drying and rewetting pretreatment effect on some soil enzyme activities under semiarid Mediterranean conditions. Soil Biology & Biochemistry. 2006; 38:2125–2134.

Zornoza R, Mataix-Solera J, Guerrero C, Arcenegui V, Mayoral AM, Morales J, Mataix-Beneyto J. Soil properties under natural forest in the Alicante Province of Spain. Geoderma. 2007; 142:334–341.

Zornoza R, Mataix-Solera J, Guerrero C, Arcenegui V, Mataix-Beneyto J, Gomez I. Validating the effectivity and sensisitivity of soil quality indices based on natural soils under Mediterranean conditions. Soil Biology & Biochemistry. 2008 in press.

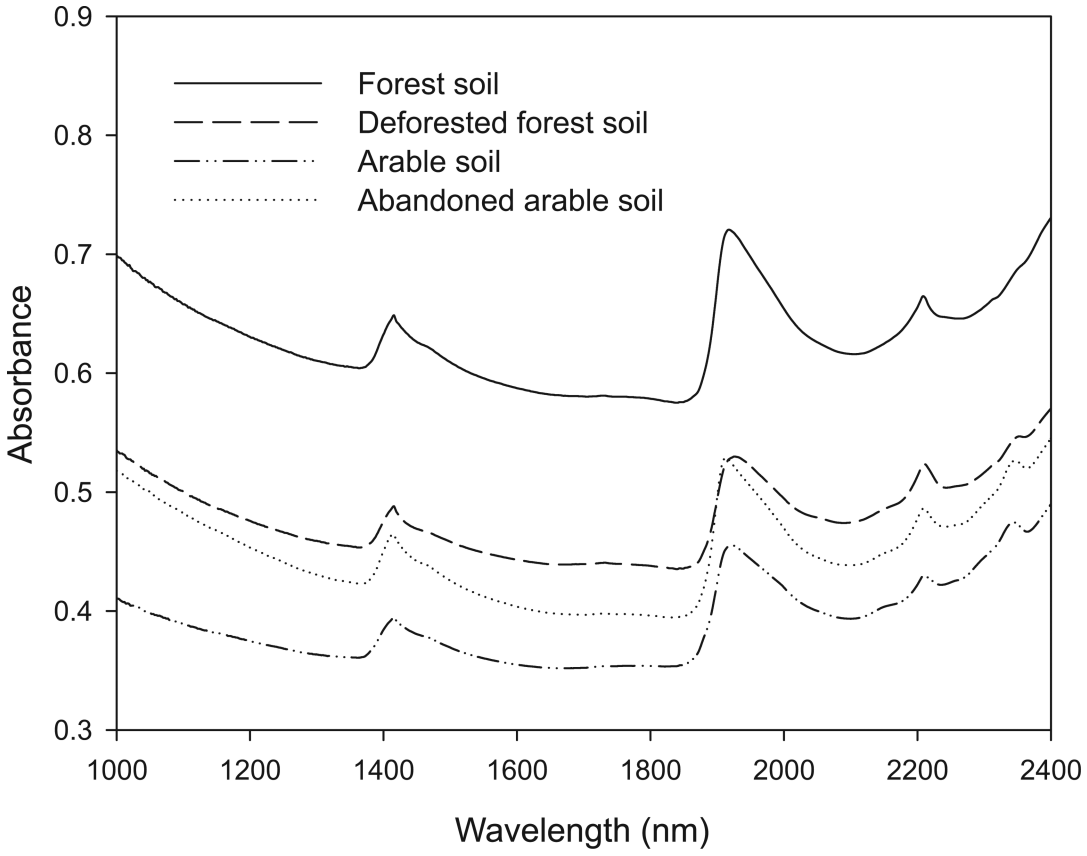

Figure 1.
Representative NIR spectra of 4 soil samples chosen to illustrate the variation in absorbance across the different land uses employed in this study.

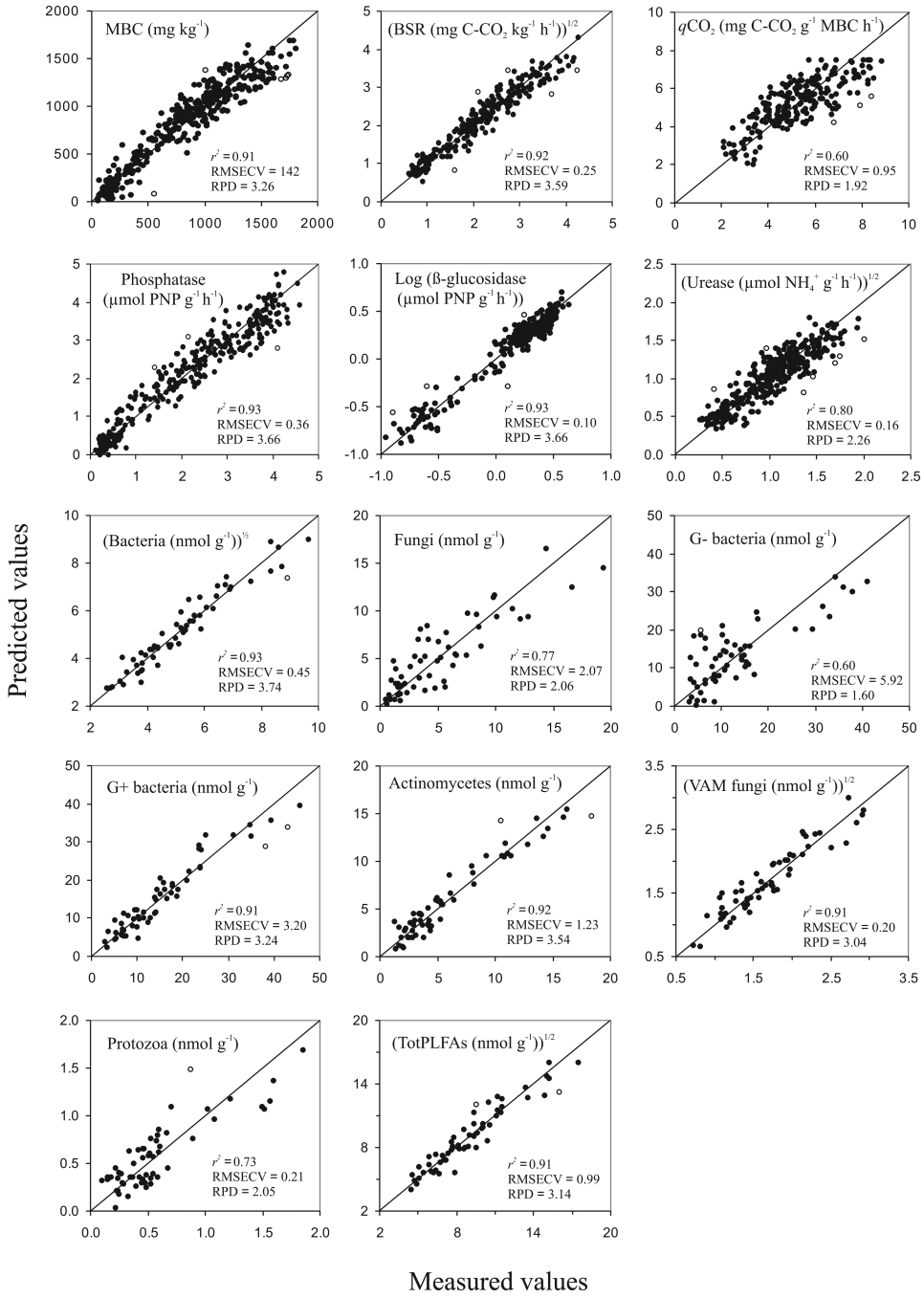

Figure 2.

Relationships between biochemical properties measured by standard laboratory procedures and predicted by NIR reflectance spectroscopy. MBC: microbial biomass carbon; BSR: basal soil respiration; VAM fungi: vesicular-arbuscular mycorrhizal fungi; totPLFAs: total biomass PLFAs. The 1:1 line is indicated in each figure. Blank symbols denote outliers (not removed).

Soil Biol Biochem. Author manuscript; available in PMC 2012 December 07.

Table 1

Composition of soils used in this study

Soil property[a]	n	Mean	SD	Minimum	Maximum
SOC (g kg^{-1})	393	50	36	3	145
N_K (g kg^{-1})	393	3.26	1.94	0.34	8.94
Moisture (%)	393	5.12	2.95	0.57	13.18
WSC (mg kg^{-1})	393	232	127	34	598
CEC ($cmol^+$ kg^{-1})	265	17.5	7.8	3.7	42.2
pH	393	8.13	0.35	7.44	9.34
EC (μS cm^{-1})	393	238	94	54	601
WHC (%)	393	86	26	43	162
Exchangeable P (mg kg^{-1})	393	4.97	2.78	0.39	22.37
Exchangeable Ca (g kg^{-1})	60	11.9	2.5	8.6	18.7
Exchangeable Mg (g kg^{-1})	60	0.72	0.56	0.21	0.69
Exchangeable Na (g kg^{-1})	60	0.59	0.22	0.13	1.07
Exchangeable K (g kg^{-1})	60	0.60	0.23	0.14	1.06
MBC (mg kg^{-1})	393	828	463	29	1873
BSR (mg C-CO_2 kg^{-1} h^{-1})	393	5.46	4.11	0.35	18.18
qCO_2 (mg C-CO_2 g^{-1} MBC h^{-1})	393	5.2	1.5	2.1	8.8
Phosphatase (μmol PNP g^{-1} h^{-1})	393	2.08	1.33	0.12	4.58
β-glucosidase (μmol PNP g^{-1} h^{-1})	393	1.69	0.93	0.08	4.29
Urease (μmol NH_4^+ g^{-1} h^{-1})	393	1.23	0.77	0.07	4.04
Bacteria (nmol g^{-1})	60	29.8	20.0	6.8	93.1
Fungi (nmol g^{-1})	60	4.99	4.25	0.50	19.30
G- bacteria (nmol g^{-1})	60	12.88	9.49	3.06	40.96
G+ bacteria (nmol g^{-1})	60	14.82	9.35	2.99	45.59
Actinomycetes (nmol g^{-1})	60	6.05	4.35	1.26	18.38
VAM fungi (nmol g^{-1})	60	3.14	2.01	0.52	8.52
Protozoa (nmol g^{-1})	60	0.56	0.40	0.14	1.86
Total biomass PLFAs (nmol g^{-1})	60	88.9	61.8	20.1	304.5

[a] SOC: soil organic carbon; NK: Total nitrogen; WSC: water soluble carbon; CEC: cation exchange capacity; EC: electrical conductivity; WHC: water holding capacity; MBC: microbial biomass carbon; BSR: basal soil respiration; qCO_2: BSR/MBC; VAM fungi: vesicular-arbuscular mycorrhizal fungi. PNP: *p*-nitrophenol.

Table 2

Results of the cross-validated predictions for various soil properties using partial least-squares (PLS) regression.

Soil property[a]	Data transformation	preprocessing[b]	r^2	RMSECV	Rank	RPD
SOC	-	1D + MSC	0.98	6.25	18	5.75
N_K	-	-	0.95	0.41	9	4.69
Moisture	-	1D + MSC	0.96	0.61	11	4.88
WSC	Log	VN	0.86	0.12	15	2.67
CEC	Log	VN	0.92	0.06	15	3.46
pH	-	MSC	0.72	0.14	9	1.90
EC	-	VN	0.57	62	17	1.73
WHC	-	2D	0.86	9.89	6	2.69
Exchangeable P	-	MMN	0.46	2.02	11	1.36
Exchangeable Ca	-	MSC	0.95	0.54	13	2.46
Exchangeable Mg	-	1D + MSC	0.91	0.17	13	2.20
Exchangeable Na	-	SLS	0.13	0.21	9	1.08
Exchangeable K	-	2D	0.79	0.11	7	2.19
MBC	-	MSC	0.91	142	13	3.26
BSR	$\sqrt{}$	VN	0.92	0.25	13	3.59
qCO_2	-	MMN	0.60	0.95	13	1.92
Phosphatase	-	VN	0.93	0.36	13	3.66
β-glucosidase	Log	MMN	0.93	0.10	16	3.66
Urease	$\sqrt{}$	VN	0.80	0.16	12	2.26
Bacteria	$\sqrt{}$	1D + MSC	0.93	0.45	24	3.74
Fungi	-	SLS	0.77	2.07	11	2.06

Soil property[a]	Data transformation	preprocessing[b]	r^2	RMSECV	Rank	RPD
G- bacteria	-	SLS	0.60	5.92	15	1.60
G+ bacteria	-	1D + SLS	0.91	3.20	17	3.24
Actinomycetes	-	1D	0.92	1.23	16	3.54
VAM fungi	$\sqrt{}$	1D + SLS	0.91	0.20	13	3.04
Protozoa	-	1D + VN	0.73	0.21	16	2.05
TotPLFAs	$\sqrt{}$	1D + MSC	0.91	0.99	11	3.14

[a] SOC: soil organic carbon; N_K: Total nitrogen; WSC: water soluble carbon; CEC: cation exchange capacity; EC: electrical conductivity; WHC: water holding capacity; MBC: microbial biomass carbon; BSR: basal soil respiration; qCO_2: BSR/MBC; VAM fungi: vesicular-arbuscular mycorrhizal fungi; TotPLFAs: total biomass PLFAs.

[b] 1D: first derivative; 2D: second derivative; SLS: straight line subtraction; MSC: Multiplicative scatter correction; VN: Vector normalization; MMN: Min-max normalization.

Table 3

Spectral ranges used for calibrations of soil organic carbon and the biochemical properties.

Soil property[a]	Spectral ranges (nm)
SOC	1732-1914, 2092-2630
MBC	1374-2092, 2270-2630
BSR	1372-2092, 2270-2452
Phosphatase	1014-1195, 1732-2092, 2270-2630
β-glucosidase	1372-2272
Urease	1195-1734, 1912-2094
Bacteria	1195-1913, 2092-2274, 2452-2632
Fungi	1193-1555, 1912-2453
G- bacteria	1737-1914
G+ bacteria	1014-1195, 1373-1724, 1912- 2274, 2452-2632
Actinomycetes	1014-1195, 2092-2274, 2452-2632
VAM fungi	1014-1195, 1374-1555, 2092-2274, 2452-2632
Protozoa	1014-1195, 2092-2274, 2452-2632
TotPLFAs	1374-1734, 1912-2094, 2452-2632

[a]SOC: soil organic carbon; MBC: microbial biomass carbon; BSR: basal soil respiration; qCO_2: BSR/MBC; VAM fungi: vesicular-arbuscular mycorrhizal fungi; TotPLFAs: total biomass PLFAs.

Table 4

Determination coefficients (r^2) for simple linear regressions of the biochemical properties with soil organic carbon, and PLS regressions.

Soil property (X)	Simple linear regression (Y=SOC)	PLS regression (Y=NIR spectra)
	r^2	r^2
MBC	0.76	0.91
BSR	0.68	0.92
qCO_2	0.10	0.60
Phosphatase	0.88	0.93
β-glucosidase	0.55	0.93
Urease	0.46	0.80
Bacteria	0.81	0.93
Fungi	0.49	0.77
G- bacteria	0.49	0.60
G+ bacteria	0.81	0.91
Actinomycetes	0.82	0.92
VAM fungi	0.73	0.91
Protozoa	0.68	0.73
TotPLFAs	0.79	0.91

SOC: soil organic carbon; MBC: microbial biomass carbon; BSR: basal soil respiration; qCO_2: BSR/MBC; VAM fungi: vesicular-arbuscular mycorrhizal fungi; TotPLFAs: total biomass PLFAs.