The use of near infrared spectroscopy in carbon and nitrogen mineralisation studies

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Abstract

In order to achieve a significant reduction of environmental harmful nitrogen losses from agricultural soils or vegetable growing production systems, the calculation of N-fertilizer supply has to take into account not only the crops nitrogen demand and the soils mineral N content, but also the amount of easily decomposable organic nitrogen compounds. The turnover of these compounds can be calculated by the use of simulation models. But the quantification of the organic compounds in the soil is mostly done by time-consuming and labour-intensive incubation experiments. Their results often do not meet the needs of simulation models for parameterisation. Thus, they actually are rarely used for the prediction of nitrogen mineralisation in soils. A simple determination of these compounds is also necessary for the direct estimation of nitrogen release without the use of simulation models.

Nowadays near infrared spectroscopy (NIRS) is a widely used tool for the Analysis of organic materials, since it is a rapid method for the simultaneous quantification of several organic components. Basis for these analyses is the development of calibrations with samples with the content of the searched substance known from mostly chemical reference methods. The calibrations are calculated using multiple linear regression algorithms. The objective of this work was to investigate, if NIRS is a suitable method for the determination of soil components relevant for N-mineralisation.

For this purpose, NIR-spectra of soil samples from three different incubation experiments were taken. In the first study soils were examined, which had contents of organic matter varying in both amount and type due to the incorporation of different crop residues. Very different courses of mineralisation resulted in difficulties to develop accurate calibrations for net N-mineralisation rates. By restricting the number of samples to those, which were showing an approximately linear time-course of mineralisation, the estimation of the mineralisation rates determined for the evaluation dataset was improved

significantly. Although the number of regression parameters was reduced clearly, the fraction of explained variance was rising from 48% to 88%. The reason for this change in accuracy is the non-linear relationship between the organic compounds determining the NIR-spectrum and the measured mineralisation rates. This non-linearity is caused by the coupling of C and N cycles.

This problem can be reduced by the use of simulation models, since they are able to determine C and N pool sizes from the mineralisation course. The NIR-spectra can be assumed to depend upon these pool sizes linearly. This concept was chosen in a second investigation and a very close relationship between simulated cellulose-content and the content as estimated by NIRS was found (r^2=0.95). A comparison of the NIRS-equation determining the cellulose content in terms of important wavelengths and the spectrum of pure cellulose powder shows a good agreement of spectral features and thus underlines the usefulness of NIRS combined with simulation modelling.

Varying fractions of mineral soil compartments such as sand, silt and clay can lead to non-linear relationships between concentrations of organic soil components and their impact on the spectra. These non-linearities can be compensated for by the use of weight scaling factors, which account for the different transparency of the individual mineral soil compartments. This way the use of multiple linear regressions makes sense again. A last investigation shows the positive influence of these weight scaling factors on the precision of NIRS-equations, which are determining organic contents in the soil.

Keywords: Near infrared spectroscopy, N mineralisation, simulation models.

Kurzfassung

Um eine deutliche Reduktion von umweltschädlichen Stickstoffverlusten aus landwirtschaftlichen und gemüsebaulichen Produktionssystemen herbeizuführen, ist bei der Bemessung der Düngemengen nicht nur der Stickstoffbedarf der Kultur und der im Boden vorhandene mineralische Stickstoff zu berücksichtigen, sondern auch leicht umsetzbare organische Stickstoffverbindungen müssen Eingang in die Berechung finden. Die Umsetzung dieser Verbindungen kann durch den Einsatz von Simulationsmodellen abgebildet werden. Da allerdings die Quantifizierung der im Boden vorhandenen organischen Verbindungen bisher meist nur durch zeit- und arbeitsaufwendige Bebrütungsversuche erfolgt, deren Ergebnisse oft nicht für die Parametrisierung von Simulationsmodellen ausreichen, werden diese bisher kaum für die tatsächliche Prognose der zu erwartenden Stickstoffmineralisation eingesetzt. Auch für eine direkte Abschätzung der Stickstofffreisetzung ohne den Einsatz von Simulationsmodellen ist eine Quantifizierung dieser Verbindungen erforderlich.

Die Nah-Infrarot-Spektroskopie (NIRS) stellt heutzutage eine weitverbreitete Methode zur Analyse organischer Materialien dar. Ihre Vorzüge liegen in der schnellen simultanen Bestimmung mehrerer Inhaltsstoffe. Grundlage für solche Analysen ist die Entwicklung von geeigneten Kalibrationsgleichungen mit Proben, deren Gehalt an der zu bestimmenden Substanz durch Referenzmethoden bekannt ist. Diese Kalibration geschieht durch Anwendung multipler linearer Regressionsverfahren. Ziel dieser Arbeit war die Untersuchung, ob NIRS für die Bestimmung von mineralisations-relevanten Größen in Bodenproben geeignet ist.

Dazu wurden NIR-Spektren von Bodenproben aus drei verschiedenen Inkubationsversuchen aufgenommen. Zuerst wurden Proben untersucht, deren organischer Anteil nach Einarbeitung verschiedener Ernterückstände in Art und Menge sehr stark variierte. Sehr unterschiedliche Mineralisationsverläufe führten zu erheblichen Schwierigkeiten bei der Kalibration auf Netto-

Mineralisationsraten. Durch die Beschränkung auf Proben, in denen die Mineralisation zeitlich annähernd linear verlief, konnte trotz deutlicher Reduzierung der Regressionsparameter die Abschätzung der Mineralisationsrate durch NIRS erheblich präzisiert werden und zwar von 48% auf 88% erklärter Varianz im Validationsdatensatz. Der Grund für diese deutliche Änderung ist der nichtlineare Zusammenhang zwischen den das NIR-Spektrum bestimmenden Inhaltsstoffen und den ermittelten Mineralisationsraten aufgrund der Kopplung von C- und N-Kreislauf.

Dieses Problem kann durch die Anwendung von Simulationsmodellen reduziert werden, da sie aus dem Mineralisationsverlauf C- und N-Poolgrößen schätzen können, von denen die NIR-Spektren linear abhängen. Dieser Ansatz wurde in einer weiteren Untersuchung gewählt und eine sehr enge Beziehung zwischen simulierten und mittels NIRS geschätzten Cellulose-Gehalten wurde ermittelt (r^2=0.95). Ein Vergleich zwischen den in der NIR-Gleichung zur Cellulose-Bestimmung stark gewichteten Wellenlängen und dem Spektrum reiner Cellulose zeigt sehr deutliche Parallelen und untermauert somit die Anwendbarkeit der Kombination von NIRS mit mathematischen Simulationsrechnungen.

Veränderliche Anteile des mineralischen Hintergrundes aus Sand, Schluff und Ton können zu nichtlinearen Zusammenhängen zwischen organischen Inhaltsstoffkonzentrationen und deren spektralen Auswirkungen führen. Diese Nichtlinearitäten können kompensiert werden, indem multiplikative Gewichtungsfaktoren die unterschiedliche Transparenz der mineralischen Fraktionen widerspiegeln. Auf diese Weise ist die Anwendung von multipler linearer Regression zur Erstellung von Kalibrationen wieder sinnvoll. Eine abschließende Untersuchung zeigt deutlich die positiven Auswirkungen solcher Gewichtungsfaktoren auf die Präzision von NIRS-Gleichungen zur Bestimmung organischer Gehalte in Bodenproben.

Schlagworte: Nah-Infrarot-Spektroskopie, N-Mineralisation, Simulationsmodelle.

Contents

1 Introduction

Ecological aspects become more and more important in modern agriculture and horticulture. In addition to questions concerning biodiversity or pest management, high nitrate concentrations in soil and groundwater due to nitrate leaching have become a serious problem in this complex environment (Pang et al. 1998; Voss 1985). The reduction of harmful nitrogen flows to the environment without losses in crop yield and quality is a challenging task for the near future.

1.1 Fertilizer recommendations

For some greenhouse crops the goal of reducing nitrogen losses can be achieved by closed nutrient cycles. This can be realised by systems, in which all nutrient flows can be controlled, e.g. by growing plants in rockwool using drip irrigation with nutrient solution. But only a small fraction of crops can be cultivated under such controlled conditions. In field crops production, nitrogen supply and nitrogen demand have to be balanced carefully in order to avoid nutrient deficiency symptoms on one side and leaching of mineral nitrogen on the other. The N demand is known for the most important crops. If the N supply is supposed to match to these values as exactly as possible, fertilizer recommendations have to take into account not only the amount of mineral N in the soil at planting, but also the fraction of organic N, which can be mineralised until and during cultivation.

Especially the field production of vegetables is often associated with high amounts of residual mineral N in the soil (Navarro Pedreno et al. 1996). Many vegetables are harvested in a physiological state of growth and high N-demand. Since they are qualitatively and quantitatively very sensitive to the amount of available mineral N (Booij et al. 1996), considerable reserve factors are

calculated in the fertilizer management, leading to high residual mineral N values in the soil by the time of harvesting (Everaarts and Gysi 1993; Everaarts et al. 1996; Rahn et al. 1992). Additionally, high amounts of crop residues, which often contain many mineralisable N-compounds like proteins, stay on the field and their mineralisation is a valuable source of nitrogen for the next crops.

A prediction of this net N mineralisation has to consider several factors, namely weather, soil type, amount of mineralisable substances and the activity of the microbial biomass. Mathematical models can be used to calculate the mineralisation taking into account temperature, soil water content and other environmental factors. Many different models have been evaluated and were found to describe the course of mineralisation quite well (Jensen et al. 1996; Molina et al. 1997; Molina and Smith 1998). Most models use a number of different organic N pools in order to represent the differing decomposability of various N compounds. When predictions of the N-mineralisation are required, these models need to be initialised with starting values for the different nitrogen pools. Therefore the determination of the mineralisable N compounds in the soil is a prerequisite for any prediction of mineral N contents and in consequence for exact N fertilizer recommendations.

1.2 Characterisation of organic N compounds in soils

Biological, chemical or physical methods can be used for the measurement of mineralisable N (Olfs 1992). The most common method to determine a soil's mineralisation potential is the incubation of the soil at temperatures and water contents, which are close to optimum for the microbial biomass. This accelerated mineralisation is then determined by measuring the accumulation of mineral N after a defined incubation time (Keeney 1982; Stanford 1982). With this procedure one can only determine the net N mineralisation in a certain period of time, but it is not possible to differentiate between organic N pools with varying decomposability. Different pools can only be determined, when the

course of mineralisation is known from continuous monitoring of the mineral N content during incubation. Incubation experiments are always labour-intensive and extremely time-consuming. Hence they cannot be used economically for routine predictions.

The determination of organic soil compartments by various chemical extraction techniques (Bremner 1982) or by electro-ultra-filtration (EUF) (Nemeth 1985) is less time-consuming. Houba et al. (1986) compared extractions with $CaCl_2$ solution and EUF with standard extractions using KCl or NaCl solutions. High correlations were found between the results of EUF and these extraction methods. This means that subtraction of the results of two different extraction procedures does not deliver information about specific organic N fractions. There are no suggestions, how to retrieve information about the amount of different organic N compounds in the soil by EUF or extraction procedures.

Like incubation procedures, EUF and methods such as extractions with e.g. $CaCl_2$-solution are also labour-intensive due to the wet chemistry analyses, which have to be conducted. Another drawback of these methods is the fact, that extracts do not always appear to be useful N availability indices (Hossain et al. 1996; Köhler 1983), or that during incubation the amount of already mineralised N and the amount of extractable N are not always correlated clearly (Steffens et al. 1996).

The physical fractionation into size or density classes using silica suspensions is another possibility to characterise the soil organic matter (Christensen 1992; Meijboom et al. 1995). Hassink (1995) suggests to divide the organic matter into four size and density classes in order to parameterise mathematical mineralisation models. In this case different pools of N compounds can be determined, but the physical fractionation also comes along with a large labour requirement.

Apart from the dead organic matter, the microbial biomass is an important part of the soil organic matter. On one side microbial biomass is the driving force in the biochemical turnover in the soil, on the other side it is an easily decomposable fraction itself. Its determination is even more sophisticated than the characterisation of other pools of organic matter in the soil. Mainly two methods are used for the determination of the microbial biomass. In the fumigation-extraction method (FEM) (Brookes et al. 1985) the total microbial biomass is measured, while the substrate-induced respiration (SIR) (Anderson and Domsch 1978; Heinemeyer et al. 1989) only measures the active part of the microbial biomass.

A determination of different soil organic matter fractions such as easily decomposable and more recalcitrant compartments as well as the microbial biomass cannot be achieved economically by the methods listed above. Only special research interests might justify such an investment of time and work.

1.3 Near infrared spectroscopy

Near infrared reflectance spectroscopy (NIRS) is widely used for the determination of organic compounds in e.g. cereals, forages, dairy products and pharmaceuticals. The principle of NIRS is that chemical bonds like N-H or C-H absorb electromagnetic radiation at characteristic wavelengths, which means that the absorption at this wavelength is determined by the concentration of the absorbing compound (Colthup et al. 1990). The reflectance of a sample is scanned in the near infrared radiation (1100 nm - 2500 nm) in steps of 2 nm and the reflectance of the sample is divided by the reflectance of a standard material at each wavelength. The resulting relative reflectance R is then used to calculate the concentration c of the absorbing substance. According to Lambert-Beer's law electromagnetic radiation decreases exponentially when passing an absorbing material. The fraction of light absorbed by the sample (A) can then be written as

$$A = 1 - \exp(-b \cdot c \cdot x)$$

where β is the effective cross section, a coefficient with the dimension of an area, depending on the probability of an energy transfer to the chemical bond and x is the path length in the material passed by the radiation. Assuming that the transmittance through the sample can be neglected (R = 1 - A), the concentration is then proportional to the logarithm of the reciprocal reflectance.

$$b \cdot c \cdot x = \log\left(\frac{1}{R}\right)$$

The quantity $(-b \cdot c \cdot x)$ at wavelength i is referred to as absorbance a_i. A more detailed description of the theory of diffuse reflection in the NIR region is given by Olinger and Griffiths (1992).

Absorption peaks in the near infrared region are broad and overlapping (Workman and Burns 1992), which means that in mixtures of absorbing materials the left-hand term of the equation has to be replaced by a sum for all substances absorbing at this wavelength. On the other hand a certain chemical bond shows multiple absorption peaks representing the vibrational overtones, that can be excited in this bond (Ciurczak 1992).

Figure 1 shows spectra of three different crop residues. The differences between these spectra are quite evident. When incorporated into soil at a concentration of 3%, the spectral differences seem to vanish. The major difference between the soil spectra is a shift of the spectra to generally lower absorbance for finer soil textures. This effect, not helpful for the determination of organic soil constituents, can be reduced by the use of mathematical pre-treatments like scatter corrections or the use of first or second derivatives of the spectra (Barnes et al. 1989). The differences caused by the crop residues are small, but the resolution of commercial NIR-spectrometers is high enough to detect these differences.

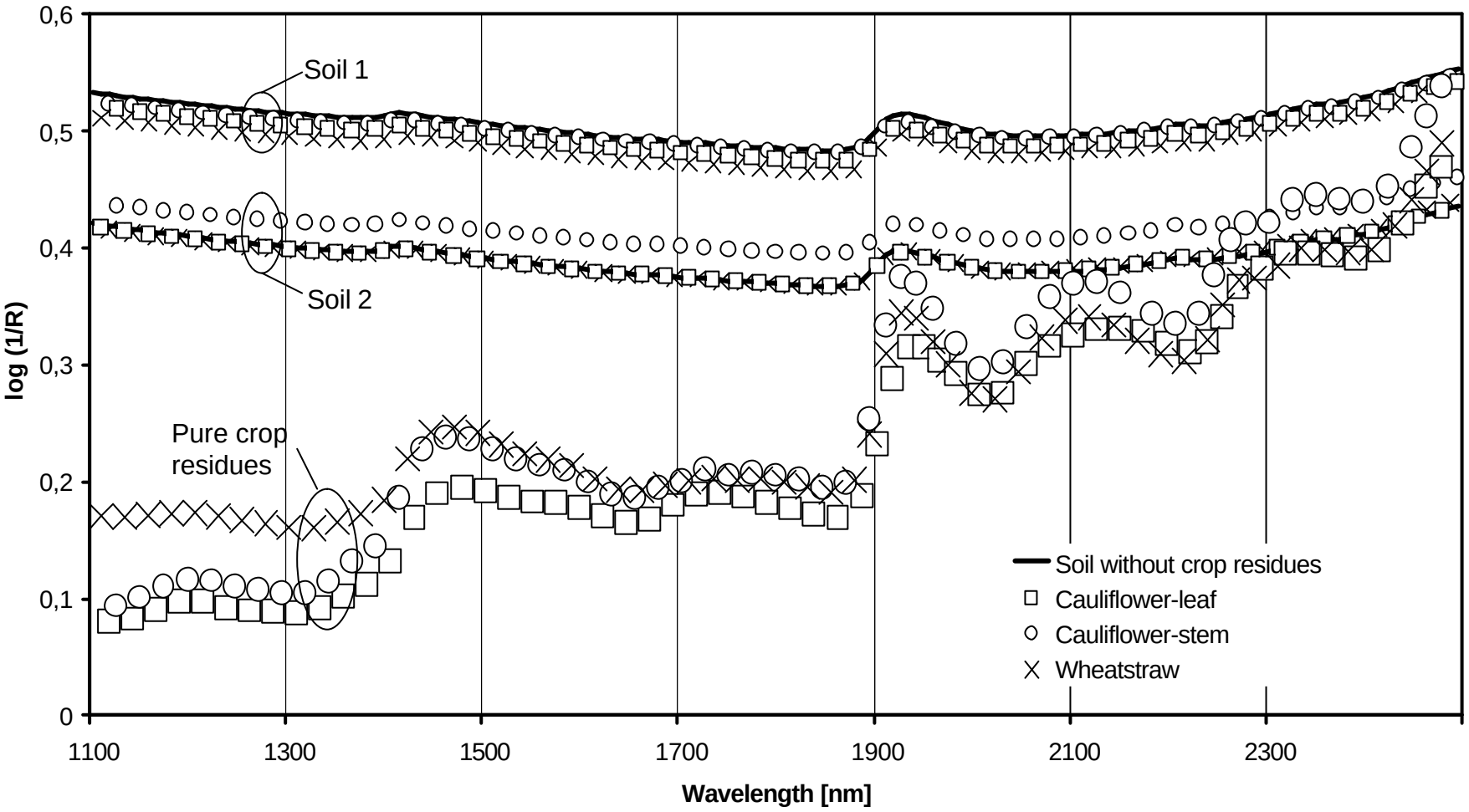

Figure 1 *NIR-spectra of pure crop residues (large symbols) and residues incorporated (small symbols) in two different soil types (concentration 3%)*

It is obvious, that the composition of a soil sample containing various organic compounds cannot be calculated directly from the spectrum. Calibrations have to be made using samples with a known concentration of the relevant compound.

There are several algorithms to solve the multivariate regression problem encountered when calibrating NIR-spectra with multi-collinear absorbance values. Due to the overlapping of absorption peaks and the collinearity of the absorbance at different wavelengths, methods using single wavelengths are usually less successful than methods like principal component regression (PCR) or partial least squares regression (PLS), which utilise the whole spectrum to extract the spectral fingerprint of a chemical compound. Both methods reduce the spectrum containing 700 absorbance values to a small number of spectral features (factors), which account for most of the variance in the spectra contained in the calibration dataset.

PCR only uses information of the spectra themselves to determine the factors (principal components). The covariance-matrix of the spectra is calculated containing the whole information of the variance among the spectra. The eigenvectors of this covariance matrix are the principal components. The first few principal components contain more than 99% of the variance, while the rest is negligible. A simple example is given in Figure 2. The absorbance at the wavelengths 1450nm and 1940nm, major absorption peaks of water, are highly correlated.

The introduction of principal components is nothing but a transformation of the coordinates to more useful directions. In the example the first principal component contains the whole information about the water content in the samples, while the second principal component is not used for the determination of the water content. Since they are eigenvectors of a matrix, the principal components are orthogonal (Mark 1992), which is an important prerequisite for their use as parameters in linear regressions. When changing

the coordinate system, the absorbances of a sample at the single wavelengths are replaced by the scores on the principal component axes.

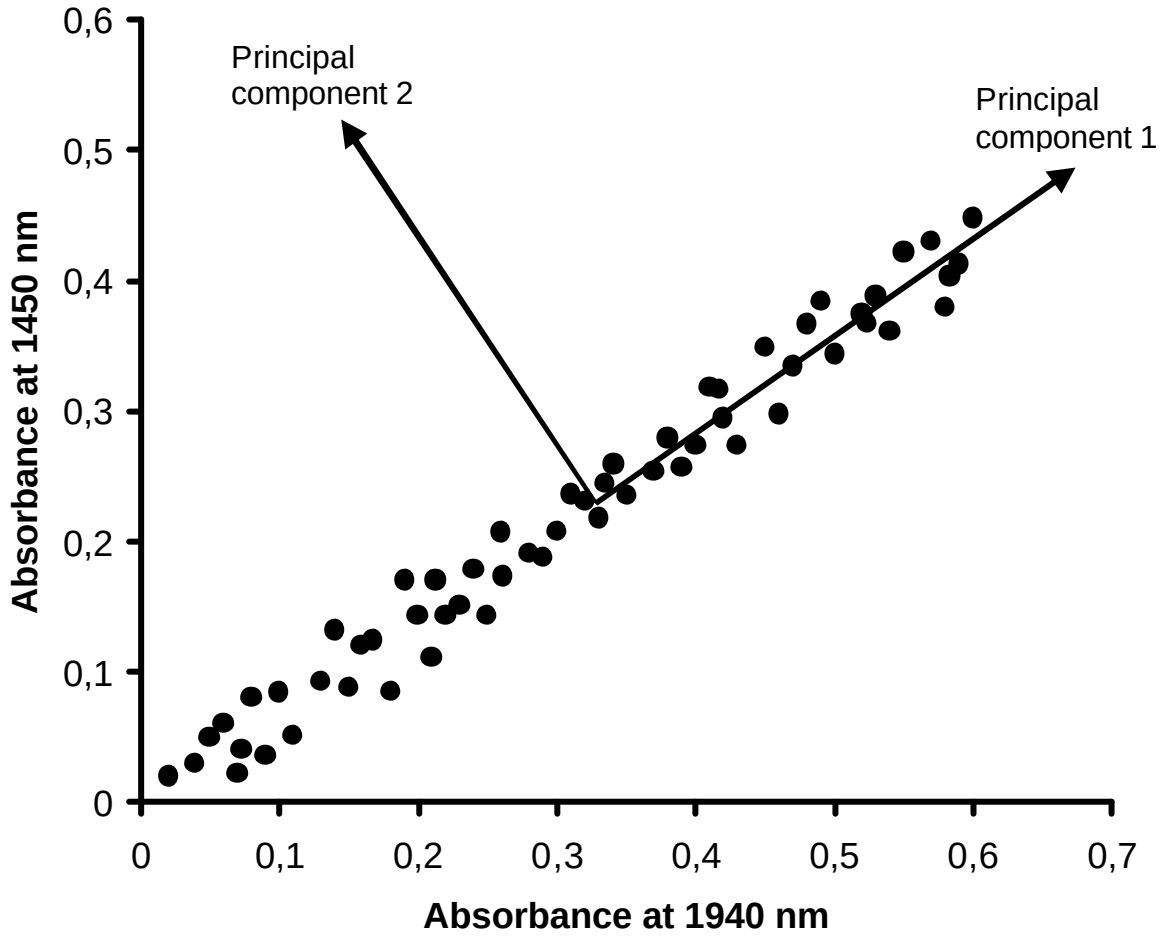

Figure 2 *Two-dimensional example for the generation of principal components (plot only for demonstration purposes, not actually measured values)*

In Figure 3, principal component representations of the NIR-spectra are shown for 6 replicates of the soil samples, which have been graphed in Figure 1. The first three principal components contain information characterizing the two soils, as seen in perspective a. Looking from a different angle (perspective b), it becomes obvious that also the variation in the spectra caused by the different crop residues is contained in the first three principal components.

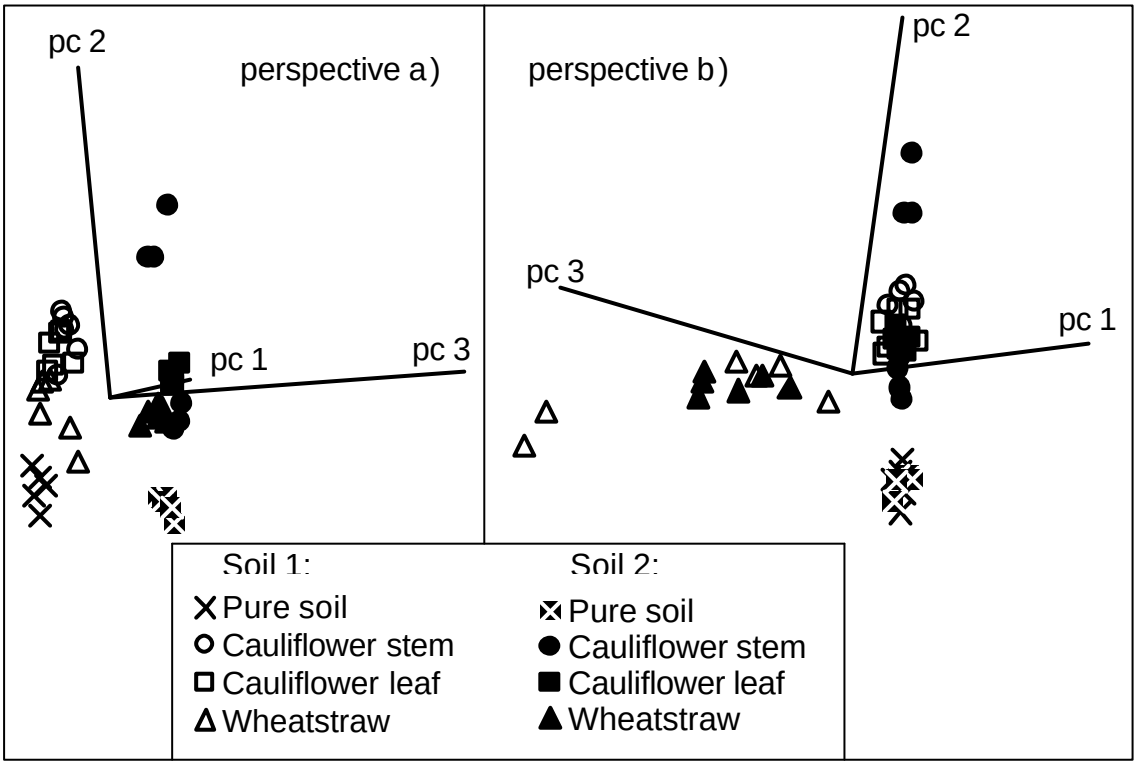

Figure 3 *Principle component (pc) presentation of the soil samples, which have been shown in Figure 1.*

PLS has similar properties as PCR for the introduction of PLS-factors is also a coordinate transformation to more useful axes than the absorbance at single wavelengths. The main difference is, that not only the NIR-spectra, but also the reference values, e.g. water content, of the calibration samples are used for the calculation of the PLS-factors (Bjørsvik and Martens 1992). PLS-factors are more difficult to interpret, but PLS-calibrations are faster and they usually need fewer factors for the explanation of the same variance than PCR-calibrations.

PLS-factors are also orthogonal due to the algorithm, which is used for their determination. A further improvement in the explanation of the variance can often be achieved with a modified PLS-algorithm (MPLS) as presented by Shenk and Westerhaus (1991). An illustrative comparison of principal components and PLS-factors is presented by Cowe et al. (1991).

The spectra can now be represented by a small number of scores without loosing important spectral information. The factors (PLS-factors or principal components) can then be used as parameters in a multivariate linear regression, the actual calibration. Afterwards, the spectral factors are inserted into the resulting regression equation. Thus, the calibration ends up with a NIRS-equation in the following form (Workman 1992):

$$c = b_0 + \sum_i a_i b_i$$

The concentration c of the searched ingredient in an unknown sample is then calculated from the measured absorbances a_i at each wavelength i, using the complementary coefficients b_i , which are characteristic for each NIRS-equation.

1.4 Cross validation and outlier detection

The number of factors used in the calibration is restricted by cross-validations (Snee 1976; Stone 1974) during the calibration process firstly to get a measure for the quality of the calibration and secondly to limit the number of parameters (principal components or PLS-factors) in the calibration. This increases the robustness of the NIRS-equation, since ‘over-fitting’ is avoided.

Also, outliers can be detected during the calibration process. There are spectral and regression outliers, which can be identified mathematically. If the spectrum of a sample cannot be described satisfactorily by the spectral factors, principal

components or PLS-factors, this sample is called a spectral outlier. Large residuals (> 3 · standard error of calibration) in the analytical data, which are not modelled by the NIRS-equation derived from the calibration, indicate regression outliers. More details about outlier detection are given by Martens and Næs (1989).

In the calibrations presented in this study, both spectral outliers and regression outliers were detected and eliminated from the calibration samples automatically in two successive outlier elimination stages. NIRS-calibrations were recalculated after each elimination stage. Regression statistics presented in this study therefore always belong to the final calibration after outlier elimination. In order to get a deeper understanding of their effects, spectral outliers are treated separately by manual elimination or outlier elimination is suppressed completely in some cases. This will be explained in the corresponding passages of the following chapters.

1.5 NIRS in soil analysis / Objective of this study

Recently, there is a growing interest in using NIRS for the analysis of soil (Malley 1998). The objective of this study was to evaluate the use of NIRS for the determination of soil organic matter, especially for the characterisation of easily decomposable fractions. A number of different aspects is associated with this scope. Several principal questions have to be answered in order to achieve this goal:

- Can easily mineralisable organic substances in the soil principally be detected and by NIRS distinguished from more recalcitrant fractions such as humic compounds, i.e. do they absorb electromagnetic radiation in the near infrared wavelength area, resulting in a specific spectrum?

- What is the detection limit, the lowest detectable concentration of the searched substances?

- Which mathematical pre-treatments and which regression algorithms are appropriate for the use of NIRS in soil analysis?

- Which quantities describing the amount of mineralisable organic matter can be used as reference values in the calibration process?

- How do the mineral soil fractions, especially silicates, influence the NIR-spectra of soil samples?

Other questions such as economic aspects of the NIRS-application in soil analysis or the accuracy, which can be reached, can be focused, when the questions listed above are answered. They will not be discussed intensively in this study.

An answer to the question about the principal chances to detect mineralisable substances in soil can be derived from other publications. Many chemical compounds, which are part of the decomposable soil fractions have been incorporated into the soil in form of crop residues. Their major constituents such as carbohydrates, lignin or proteins have been subject to a wide variety of studies, in which forages or silages are examined with NIRS (Batten 1998; Marten et al. 1984; 1989; Redshaw et al. 1986).

Also the successful use of NIRS as an analytical tool in litter decomposition studies has been reported (Couteaux et al. 1998; Gillon et al. 1999; McLellan et al. 1991). These investigations as well as NIRS-examinations of other materials used as soil amendments such as composts or cattle manure (Ben-Dor et al. 1997; Horst et al. 1996; Reeves and Van Kessel 2000) are closely related to the detection of mineralisable substances in soils. The samples examined in these studies did not contain considerable amounts of mineral compartments such as

sand or clay. Nevertheless, these publications are hints, that also in soils easily mineralisable organic substances might be detected by NIRS.

Detection limits are very different for individual chemical compounds due to varying extinction coefficients β. Hence, the detection limits cannot be discussed generally, but only in combination with the kind of reference data used in a calibration. Also the third question focusing on the appropriate mathematical pre-treatment and suitable regression algorithms can only be answered individually for each calibration. This study will therefore be concerned primarily with the last two questions, the utilisation of different kinds of data as reference values for NIRS-analyses of soil and the influence of mineral soil compartments on the NIR-spectra and on the accuracy of NIRS-calibrations.

The most direct determination of decomposition is the measurement of CO_2 or mineral N released from the soil in incubation experiments. Several authors used these quantities for calibrations (Nilsson et al. 1992; Palmborg and Nordgren 1993; Pietikainen and Fritze 1995; Reeves and Van Kessel 1999), but due to the coupling of C and N cycles, these net mineralisation values are not generally linearly correlated to the amounts of mineralisable compounds. Chapter 2 focuses on the use of mineralisation rates as reference values. Therefore other variation in the samples than different crop residues was kept small in the dataset used for this investigation.

In order to examine the capability of NIRS to detect easily decomposable soil fractions, Chapter 3 is concerned with the question, if cellulose, the most important organic component in the biosphere, can be detected in soil, where it makes up only a small part of the organic C. The detection of cellulose in purely organic samples has been reported to be quite successful (Czuchajowska et al. 1992; Langkilde and Svantesson 1995; Schultz and Burns 1990).

Silicates like sand, silt and clay are the major mineral constituents of soils. These are known not to absorb radiation in the near infrared wavelength area (Workman 1998). On the other hand the influence of mineral soil fractions on NIRS-calibrations for organic soil compartments has been pointed out in several publications (Krischenko et al. 1991; Morra et al. 1991; Zwanziger and Förster 1998). Couillard et al. (1996) have shown, that the predictive quality of calibrations for different soil compartments is improved, when the different amounts of sand, silt, clay and organic matter are modified by weight scaling factors. An improvement in predictive accuracy due to this modification has not yet been reported, when applied to natural soil samples.

In chapter 4 the influence of different soil textures on calibrations is focused. One soil type mixed with 20% quartz sand and 20% bentonite, respectively, was used in this investigation. Thus it was ensured, that the variance occurring in the two sample sets only derives from the mineral fraction of the soil. The modification developed by Couillard et al. (1996) is applied to the samples and its usefulness is discussed.

In chapter 5 NIRS is applied to a datasets containing a wide variety of soil textures. Again the application of weight scaling factors is tested. Another scope of this chapter is the question of suitable reference values for NIRS-calibrations. The samples of these datasets have been examined by different extraction procedures and by EUF (Appel 1998). Also sizes of different N-pools as simulated by NCSOIL (Molina et al. 1997) were available. These pool sizes were also used for calibrations.

2 Correlating near infrared spectra with N mineralisation parameters

Abstract

The prediction of nitrogen mineralisation in agricultural or horticultural soils is a major problem when fertilizer recommendations have to be given. Methods to estimate N-mineralisation like incubation of soil samples under optimum conditions are laborious and time-consuming. Other methods like different extraction procedures do not give reproducible results. Near infrared reflectance spectroscopy (NIRS) is a tool widely used in the determination of organic constituents of agricultural products. The aim of our study was to investigate, if NIRS can be applied to soil samples in order to determine the content of mineralisable nitrogen. We correlated results of an incubation experiment with near infrared spectra of soil samples which were scanned prior to incubation. 122 soil samples (sandy loam) from an experimental station and a nearby vegetable growing farm were collected. They contained crop residues varying in amount, time since incorporation and species of the residues. Total carbon and nitrogen in the samples were measured using an element analyser. The release of mineral nitrogen was measured by taking sub-samples 5 times during the 4 week incubation. Only a third of the samples showed an approximately linear N-mineralisation course. We used total contents of carbon and nitrogen as well as mineralisation rates at different stages of the incubation as reference values in the NIRS-calibration procedure. Half of the samples were used for calibration, the other half for validation purposes. For the total carbon and nitrogen content, the fraction of explained variance in the validation was r^2=0.93 and r^2=0.82, respectively. Applying the calibrations for mineralisation rates gave poor correlations (r^2<0.48), when all samples were used. Using only the samples with roughly linear mineralisation curves clearly improved the prediction for e.g. net N-mineralisation in four weeks to r^2=0.88. It can be concluded from these results, that only mineralisation rates in simple processes can be directly predicted by NIRS.

2.1 Introduction

Due to high levels of fertilizer application, nitrogen leaching has become a major problem in agriculture and horticulture. Most N fertilizer recommendations are still based solely on the nutrient requirements of the crop and the mineral N content present in the soil. However, if N-mineralisation from the organic matter is not considered, N fertilising recommendations will be too high. Thus, to reduce N overdosing, one has to find a good estimation of the N-mineralisation in the soil. Conversely, N fertilizer recommendations cannot be more exact than the prediction of N mineralisation or immobilisation in the soil. Traditionally, the mineralisation potential of a soil is estimated by in vitro incubations followed by wet chemical analyses. This method is very labour intensive, time-consuming and expensive.

In order to find methods suitable for monitoring purposes, attempts to avoid the incubation procedure have been made. Results of various extraction techniques like electro ultra filtration (Nemeth 1985) or $CaCl_2$-extraction (Appel et al. 1995) were correlated with incubation results. These methods are less time-consuming than incubation, but the wet chemistry is still labour-intensive. Additionally, it is shown, that the time courses of extractable organic N and mineralised N are not always well correlated (Köhler 1983, Steffens et al. 1996).

In the last decades, near infrared spectroscopy (NIRS) has replaced a major part of wet chemical analyses as it allows the fast and simultaneous determination of different organic compounds in e.g. grains, forages and dairy products. The principle of NIRS is to determine the absorbance of a sample in the near infrared wavelengths region, which is caused by chemical bonds in the sample (Ciurczak 1992).

Wavelength position and height of the spectral peaks cannot directly be used for determinations of chemical compositions, because spectra are too complex and peaks in the near infrared region are broad and overlapping (Workman and

Burns 1992). Instead, spectra have to be calibrated using samples in which the constituents of interest are known from reference methods. The multivariate regression problem, which is encountered when equations for one chemical substance have to be obtained from spectra with many collinear variables can be solved by e.g. partial least squares regression (PLS) or principal component regression (PCR) algorithms (Martens and Næs 1989; Shenk and Westerhaus 1991). Both methods reduce the original spectra consisting of 700 data points to a small number of spectral features (factors), which account for most of the variance of the spectra. These factors are then used as parameters in a normal linear regression procedure giving a NIRS-equation. Once calibrated, such a NIRS-equation can then be applied to other samples in order to determine the specific organic compound for which the equation had been developed before.

Recently there is a growing interest in using NIRS for soil analyses (Couillard et al. 1996; Malley 1998). Dalal and Henry (1986) measured organic C, total N and water content in soils. They divided the samples into two datasets, which were used for calibration and evaluation, respectively. They found fractions of explained variance between 0.85 and 0.94 for the separate evaluation dataset.

Also, correlations between NIR-spectra and the soils mineralisation potential were found. Palmborg and Nordgren (1993) were able to explain up to 95% of the basal respiration and substrate induced respiration (SIR) of forest soils from NIR-spectra. Meyer (1996) used NIRS to classify soils from South African sugarcane farms in four levels of N mineralisation potential. The probability of deriving the right mineralisation class from NIR-spectra in this practical application was above 90%.

Reeves and van Kessel (1999) correlated NIR-spectra with the accumulated soil inorganic nitrogen and the evolved CO_2. They used soils amended with different kinds of manure as well as unmanured controls. The coefficients of determination for the calibration (all soil samples included) were 0.63 and 0.56 for the evolved CO_2 and the released inorganic N, respectively.

All these results are encouraging as they are showing the potential of NIRS in rapid and effective detection of the soil organic matter. On the other hand, one has to keep in mind, that the application of NIRS to soils has some specific problems. Most agricultural or horticultural soils contain only a relatively small organic fraction, while the silicate minerals such as sand, silt and clay make up for the biggest part of the soil. These silicates are transparent to near infrared radiation (Workman 1998), but they still are subject for scattering processes, which cannot be described analytically. So, soils have properties contrasting to those materials for which NIRS has been developed for, which are mostly organic and only contain small mineral fractions.

Another difficulty rises from the fact, that the mineralisable fractions of the soil organic matter are usually very small and NIRS has not generally been found to perform very well for minor constituents in organic samples (Shenk and Westerhaus 1993).

Observing the decay of mineralising substances in incubation experiments, one has to remember, that most reference measurements give quantities of substances evolved from the soil like CO_2 or NO_3^- (Reeves and van Kessel 1999). By the time, when NIR-spectra are taken which is usually before incubation, the CO_2 or nitrate is not yet produced. Hence, the measured entity cannot directly contribute to the NIR-spectrum of a soil sample. Instead, the spectral differences are caused by different contents of organic compounds like cellulose or proteins which are subject to the mineralisation process. The correlation of spectra and produced CO_2 or nitrate is therefore indirect. This can lead to difficulties, if the organic sources for the mineralisation process in the unknown samples differ from those in the calibration samples. For instance, a calibration derived from samples containing different amounts of pig slurry will generally not be able to predict the nitrate release from samples containing vegetable residues.

The objective of this study was to investigate if mineralisation parameters of horticultural soils containing different amounts of crop residues can be directly correlated to their NIR-spectra and which conditions have to be fulfilled to get good prediction qualities. In addition it was investigated, if calibrations for total N and C can be developed even if very different amounts and kinds of organic substances are present in the soil samples.

2.2 Material and methods

Incubation

Soil samples of ca. 2 kg fresh mass were taken from an experimental station (73 samples) in southern Lower Saxony and from a nearby vegetable growing farm (49 samples). The soil was a silt loam derived from loess. Each of the 122 samples was taken from the top 20 cm of the soil collected from 3 different points of the same plot. The samples contained different kinds of crop residues The amounts of residues were not known. A detailed overview of the different residues is given in Table 1.

Table 1 Origin of the soil samples and type of plant residues incorporated

Number of samples	Type of plot	Residue type	Actual crop
3	long-term experiment	rye straw	rye
6	long-term experiment	winter barley	cauliflower
6	long-term experiment	winter wheat	spinach
6	long-term experiment	faba beans	none
6	long-term experiment	cauliflower	cauliflower
6	long-term experiment	winter wheat	mustard
16	experimental plot	cauliflower	none
6	experimental plot	white cabbage + clover	none
6	experimental plot	winter wheat	none
6	experimental plot	chicory leaves	none
6	experimental plot	lettuce	none
6	greenhouse	cucumber roots	cucumber
14	greenhouse	cucumber roots	tomato
6	field (11 days after ploughing)	white cabbage	none
9	field, freshly ploughed	white cabbage	none
9	field, freshly ploughed	tomato plants from greenhouse	none
5	field, freshly ploughed	pasture	none

The samples were hand-mixed thoroughly. Bigger parts of plant residues were thereby chopped to < 1 cm. Then the samples were divided into eight subsamples of 250 g each. These were used as follows:

1. measurement of mineral N (ammonium and nitrate),
2. NIRS-analysis after drying and milling,
3. drying, determination of soil dry matter, total N / total C content

4.- 8. Incubation, sampling after 3, 7, 14, 21, 29 days, measurement of mineral N (ammonium and nitrate) at each sampling date

For incubation the subsamples were filled in polyethylene pots and placed in climate chambers at 20°C under aerobic conditions. Controlling and irrigating regularly ensured that the water content was kept above a value that corresponded to 50% of the water holding capacity of the original soil.

Total C and N content were determined using an element analyser (vario EL, elementar Analysensysteme GmbH, Hanau, Germany).

NIRS-analysis and calibration

All subsamples dedicated for NIRS-analysis were dried at 105°C, milled to pass a 1mm-sieve and then spectrally analysed with a NIRSystems 5000 spectrometer (FOSS NIRSystems Inc., Silver Spring, USA) in the wavelength range from 1100 to 2500 nm in 2 nm-steps. Also, the subsamples of the end of the incubation process were measured in the same way.

The spectra were scatter corrected as described by Barnes et al. (1989). Calibrations were developed by using the first derivative of the original spectra. For the calibration process the PLS algorithm in the modified form presented by Shenk and Westerhaus (1991) was used.

In order to evaluate each calculated calibration, we used the following procedure: The set of soil samples was divided into two parts by picking every other sample and keeping these as an independent evaluation data set. The other samples were then used for calibration. Additionally, cross validations (Snee 1976; Stone 1974) were calculated firstly to get a measure for the quality of the calibration and secondly to limit the number of parameters (PLS-factors) in the calibration. This way we were able to avoid “over-fitting” in the calibration process.

Also, outliers are detected during the calibration process. There are spectral and regression outliers, which can be identified mathematically. If the spectrum of a sample cannot be described satisfactory by the spectral factors, principal

components or PLS-factors, this sample is called a spectral outlier. Large residuals in the analyte data, which are not modelled by the NIRS-equation derived from the calibration, indicate regression outliers. More details about outlier detection are given by Martens and Næs (1989).

In our calibrations, only regression outliers were detected and eliminated from the calibration samples automatically in two successive outlier elimination passes. NIRS-calibrations were recalculated after each elimination pass. Regression statistics given in our results therefore always belong to the final calibration after outlier elimination. In order to get a deeper understanding of their effects, spectral outliers were treated separately by manual elimination.

Apart from the NIRS-measurements of the unincubated samples, we also took spectra of the subsamples, which had been incubated for 29 days (last sampling date). Therefore these subsamples were divided in one half used for wet chemistry analyses and one half, which was dried and milled for NIRS-analysis. Thus we were able to calculate the spectral difference of the samples before and after incubation. We also calculated calibrations for these difference spectra on the observed mineralisation, which can be regarded to be the direct counterpart to these difference spectra.

2.3 Results

Incubation and sample selection

During incubation very different courses of mineral N content could be observed in the samples. Net immobilisation, linear and sigmoid mineralisation curves, exponential rise to maximum mineral N value or net mineralisation followed by a phase of net immobilisation were found. These curves indicated that different biochemical processes are prevailing in the different samples. The largest fraction (36 samples) were those with a linear course of mineralisation. A linear

approximation accounted for more than 90% of the variation in these samples. They will be referred to as linear samples.

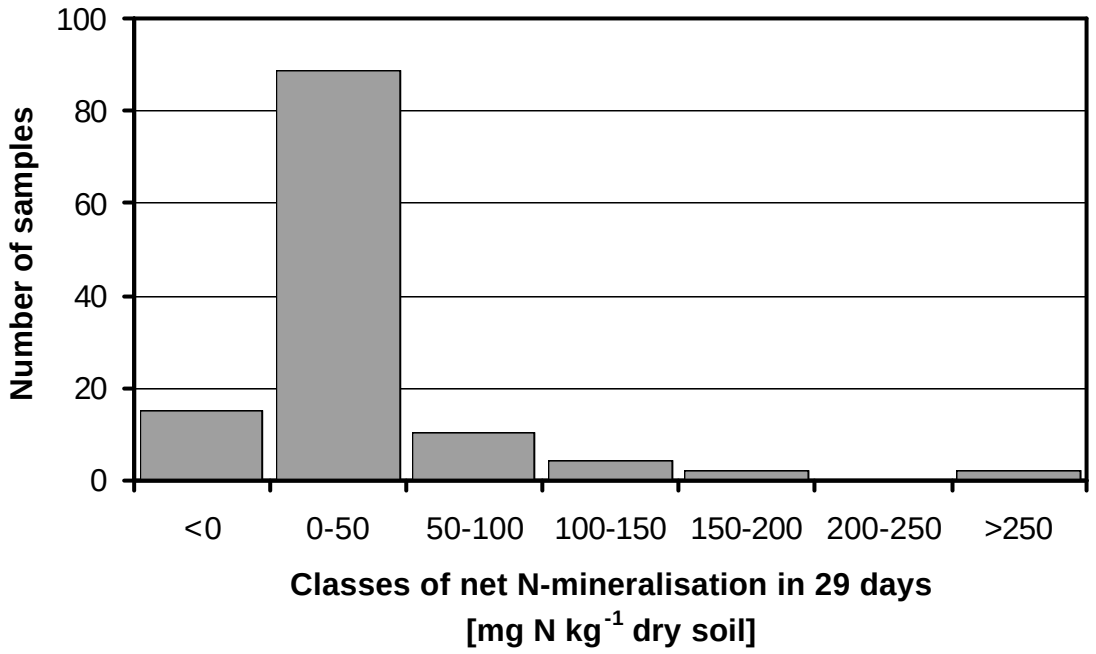

Figure 4 Distribution of soil samples in mineralisation classes

Over the whole incubation time of 29 days most samples (89 samples) showed a net mineralisation below 50 mg N kg^{-1} dry soil, while 15 samples had a net immobilisation (Figure 4). Two samples showed a very high net N-mineralisation. These samples were taken from ploughed plots containing residues from white cabbage. Large pieces of plant material in these two samples are probably the reason for the very high mineralisation rates. Also, their values for total carbon and nitrogen content were higher than in the other samples from these plots, which also is an indicator for high amounts of plant mass in these two samples.

Linear samples were found in all classes of samples showing net mineralisation. Especially all four samples with a net mineralisation higher than 150 mg N kg^{-1} soil showed an approximately linear rise in mineral nitrogen.

NIRS-calibrations using all samples

Irrespective of the mineralisation course, we correlated measured mineralisation properties to the spectra of all samples. Half of the samples (61 samples) were randomly picked for the calibration, the other half was used for evaluation. The best results, i.e. r^2=0.48 for the linear regression of predicted vs. observed mineralisation rates of the evaluation, were achieved with the net N-mineralisation in 29 days as reference value. The results achieved with this calibration as well as the results for calibrations on the mineralisation in the first seven days of incubation are presented in Table 2. Also, calibrations for the total C and N contents in the unincubated soil samples were calculated. These results are also shown in Table 2.

Table 2 Results of calibrations with 61 samples and evaluations with the remaining other 61 samples

Reference value	Number of regression outliers	Number of PLS-factors	r^2 calibration samples	r^2 cross validation	r^2 evaluation samples
Mineralisation in 29 days	2	3	0.50	0.60	0.48
Mineralisation in 7 days	2	5	0.62	0.59	0.10
Total C content	5	7	0.99	0.94	0.93
Total N content	4	8	0.96	0.93	0.87

Prior to calibration, spectral outliers were detected among the soil samples. These spectral outliers are commonly eliminated from the calibration set. Table 3 shows results of the calibration after elimination of the four outliers found in our study. The only improvement, that could be achieved, was a higher fraction of explained variance for the calibration samples and better results in the cross-validation in respect to the net N-mineralisation during the whole incubation time. Since there is no improvement in terms of explained variance in the

evaluation dataset, we decided to concentrate on the results of the calibrations without elimination of spectral outliers.

Table 3 *Results of calibrations (57 samples) and evaluations (61 samples) after elimination of four spectral outliers from the calibration dataset*

Reference value	Number of regression outliers	Number of PLS-factors	r^2 calibration samples	r^2 cross validation	r^2 evaluation samples
Mineralisation in 29 days	2	6	0.79	0.68	0.44
Mineralisation in 7 days	5	3	0.40	0.59	0.13
Total C content	7	5	0.96	0.93	0.90
Total N content	6	6	0.93	0.86	0.81

Figure 5 shows the calibration and evaluation results for total carbon content in the soils. The regression outliers, which were eliminated in the calibration process (not spectral outliers) are included in Figure 5. As a consequence, the slope of the linear regression predicted vs. observed of the calibration dataset can differ from unity. The evaluation shows a slope very similar to the slope of the calibration and as for the calibration data set, the NIRS-equation explains more than 90% of the variation in total C content in the samples.

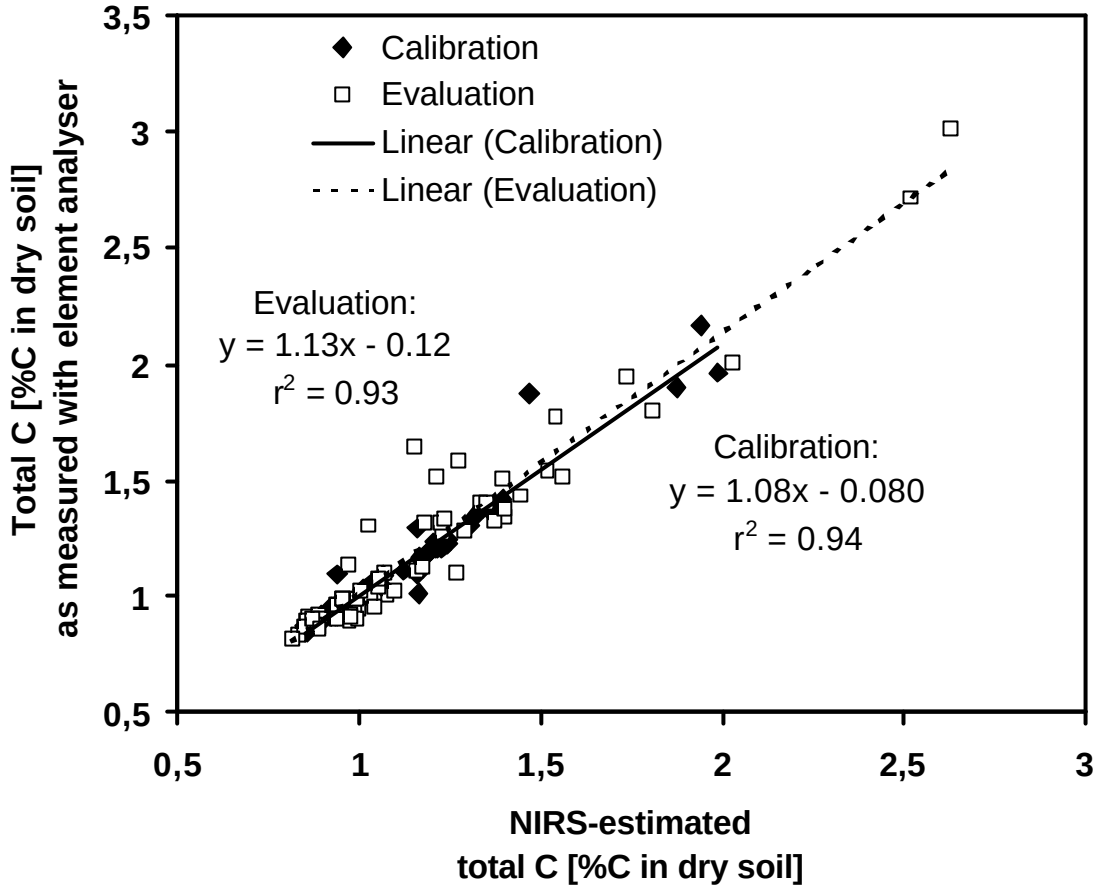

Figure 5 *Comparison between NIRS-estimated and reference total C content as measured with element analyser*

Very similar results were found for the total nitrogen content (Figure 6). In this case the fraction of variance explained by the NIRS-estimation is slightly below 90%. Again, there is only a very small difference between the slopes of the regression lines for calibration and evaluation samples. This indicates that both calibration and evaluation datasets are representative for the whole set of soil samples. Total C and total N content are explained by 7 and 8 PLS-factors, respectively.

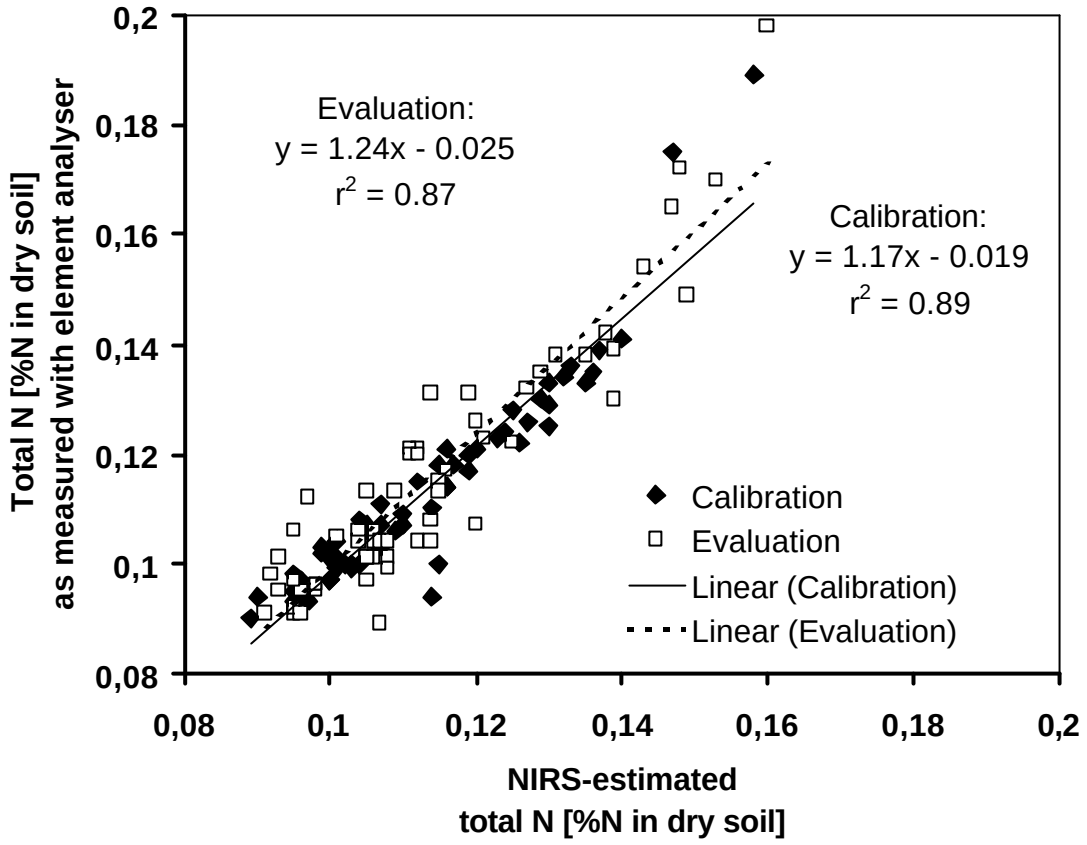

Figure 6 Comparison between NIRS-estimated and reference total N content as measured with element analyser

The results for the mineralisation rates are very poor compared to those presented above. The best results were achieved, when the net mineralisation during the total incubation time (29 days) was taken as reference value for the calibration. The mineralisation courses of several samples showed a development of mineral N content during the first days of incubation, which was not consistent to the net mineralisation during the rest of the time. This might be due to an extremely good aeration, when the samples were mixed. In order to find out whether such a “starting effect” was responsible for the poor results, the mineral N released on the first three days were subtracted from the 29 day values. However, the calibration derived from these new values showed only very small differences to the calibration for 29 day values (data not shown). There was also no improvement in calibration quality, when we used values of

the mineralisation in the first 14 or 21 days (data not shown). Therefore, we focused on the net mineralisation over the total time of 29 days as reference value. This calibration is presented in Figure 7.

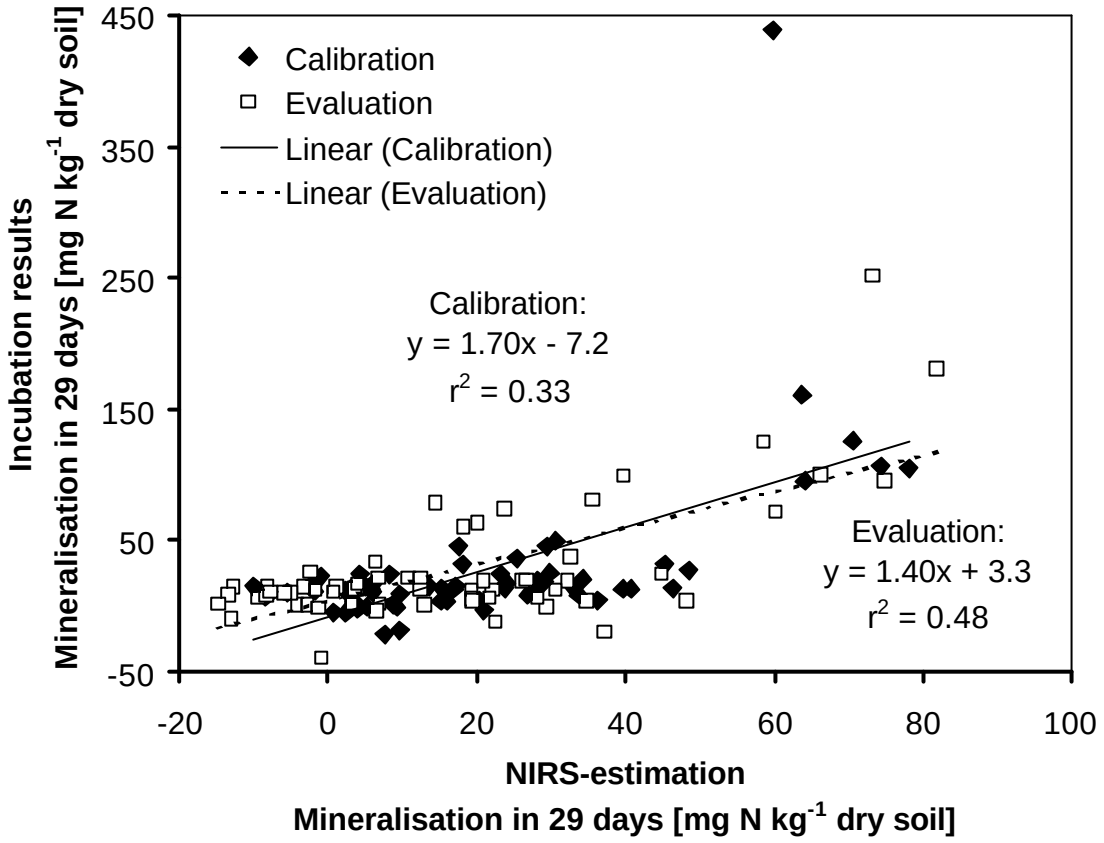

Figure 7 *Comparison of values for net N-mineralisation obtained from the incubation experiment and by NIRS-estimation*

NIRS-calibrations using only 'linear' samples

In addition to the calibrations with all soil samples we calculated NIRS-equations using only samples with an approximately linear course of mineralisation. The 36 linear samples were also divided in two subsets for calibration and evaluation containing 18 samples each. Elimination of regression outliers in the calibration process was suppressed due to the small number of samples.

The results in terms of fraction of explained variance of the total C and N content were as good as those of the calibrations with all samples (Table 4a). A major improvement could be achieved for the calibration on the net N-mineralisation. 58% of the variance in the evaluation samples could be explained by three PLS-factors.

As mentioned above, the two samples showing a very high net N-mineralisation of more than 250 mg N kg^{-1} dry soil (mineralisation outliers) were among the linear samples. We calculated new calibrations after eliminating these two samples from our set for they might have a predominant effect in a calibration process with such a small number of samples. Again there was a considerable improvement for the calibration on the net N-mineralisation (Table 4b). The new equation could explain 88% of the variance in the evaluation data set. For the linear samples we derived a mean daily mineralisation from the linear regression on the mineralisation data. This quantity was used as an additional reference value to calibrate on. For the calibration without elimination of the two samples showing the highest mineralisation, this calibration was somewhat better than the calibration on the total mineralisation, but after outlier elimination the calibration results were about the same (Table 4a,b).

Table 4 *Results of calibrations and evaluations with linear samples only: a) 18 samples each, including high mineralisation samples, b) 17 samples each, two high mineralisation samples eliminated*

a)

Reference value	Number of PLS-factors	r^2 calibration	r^2 cross validation	r^2 evaluation
Total C content	4	0.97	0.88	0.95
Total N content	4	0.94	0.80	0.90
Mineralisation in 29 d	3	0.81	0.68	0.58
Mean daily mineralisation	3	0.84	0.73	0.66

b)

Reference value	Number of PLS-factors	r^2 calibration	r^2 cross validation	r^2 evaluation
Total C content	3	0.91	0.81	0.90
Total N content	3	0.89	0.71	0.96
Mineralisation in 29 d	3	0.87	0.72	0.88
Mean daily mineralisation	3	0.87	0.72	0.86

From Table 4b) one can see, that the coefficient of determination as calculated from the cross validation, is not always a good predictor for how a calibration will perform on an independent evaluation dataset. Especially calibration datasets with a very low number of samples tend to give poor cross validation results, which may lead to an under-estimation of the predictive potential of the resulting NIRS-equation.

In Figure 8 the calibration and evaluation results for the mean daily mineralisation are pictured. The fraction of explained variance, the slope and the bias of the regression lines are almost identical. Three PLS-factors are used for this calibration.

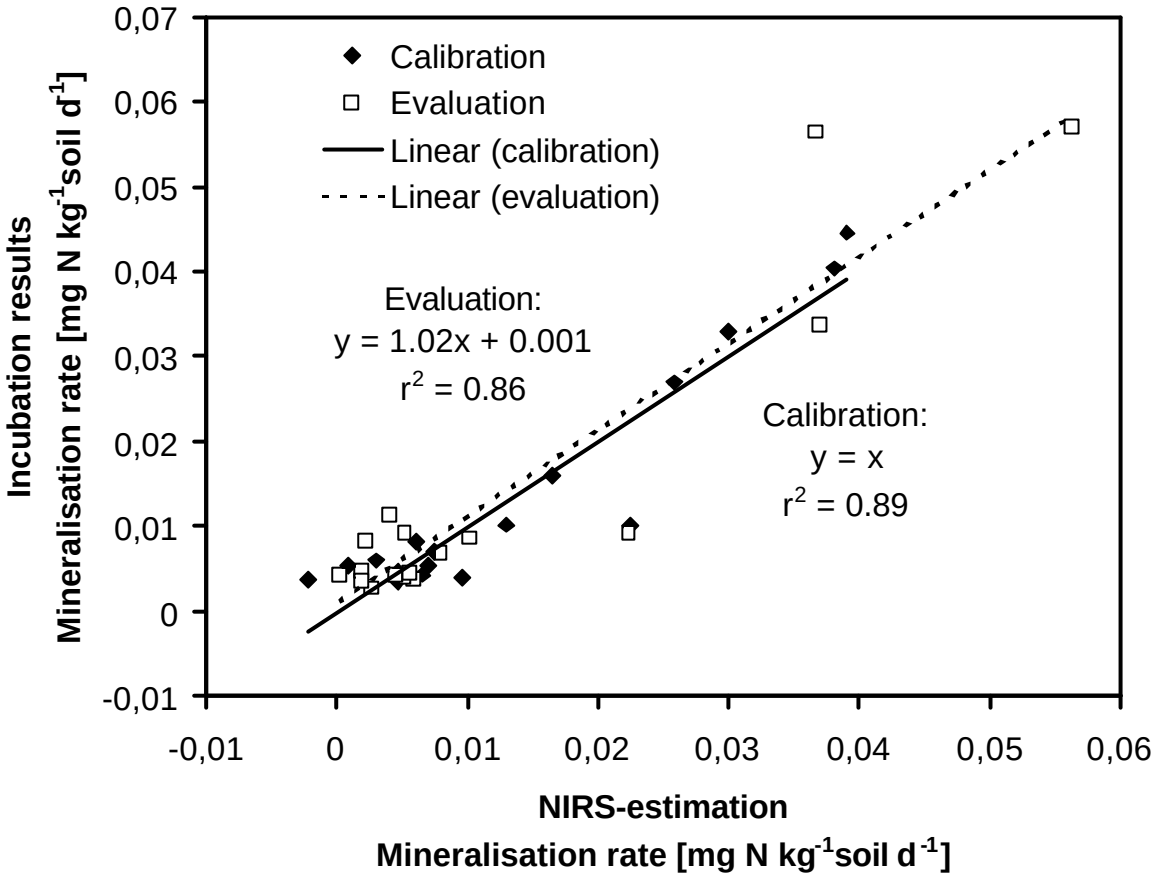

Figure 8 *Calibration and evaluation results for net N-mineralisation using only linear samples. The same dataset as in Table 4b was used.*

Difference spectra

We subtracted the spectra of the subsamples incubated for 29 days from the spectra of the unincubated subsamples. The resulting difference spectra were used for calibrations like the original spectra. Again we divided the whole

dataset in two halves for calibration and evaluation purposes, respectively. The only reference value, for which an acceptable calibration could be made, was the net N-mineralisation in 29 days. In this case 75% of the variance in the evaluation could be explained by the spectral difference. This value is significantly higher than the corresponding coefficient of determination obtained from calibrations with the original spectra (Table 2). The fraction of explained variance of other quantities (total C content, total N content) can be derived from their correlation to the net mineralisation. Therefore it can be concluded that those calibrations are indirect.

2.4 Discussion

With our investigation we wanted to get answers on two major questions. Firstly it was to be investigated, if mineralisation parameters of horticultural soils containing different amounts of crop residues can be predicted from their NIR-spectra and which conditions have to be fulfilled to get good prediction qualities. Secondly we wanted to see, if calibrations for total N and C can be developed even if very different amounts and kinds of are present in the soil samples.

Our results showed, that NIRS is a promising tool in predicting total C and N content in soils. Calibrations for these parameters can be derived even from soils with low content of organic substances from varying sources. The predictive accuracy is lower than from element analyses, but satisfying for fast determinations. For many applications, NIRS has the potential to substitute other more labour-intensive measurements of total C and N content.

The results for the C and N content imply that all kinds of organic compounds are detected by NIRS. From a theoretical point of view, there is no general restriction for the detection of organics in soils. A detection limit, i.e. a minimum detectable content of a specific substance can restrict the determination of specific compounds. As mentioned above the rapidly mineralisable organic

matter usually is only a small fraction of the total soil organic matter. Thus the differences in the NIR-spectra of soil samples are very small by nature, whereas noise in the spectra caused by the mineral soil compartments has an important influence. This signal/noise-ratio leads to a theoretical detection limit and makes calibrations difficult. Hence, results of mathematical algorithms like PLS or PCR (Martens and Næs 1989) have to be checked for plausibility especially when small fractions of the soil organic matter are subject to the calibrations.

Apart from these detection limits, we assume, that all organic substances in soils are in principal detected by NIRS. On the other hand it seems to be quite difficult to develop calibrations for the mineralisation potential of soils. The calibrations for mineralisation rates, which are built upon all soil samples in our experiment only provide a very low predictive quality. There are various reasons for these difficulties:

One problem has already been mentioned above. Each NIRS-equation is built up upon a certain calibration dataset containing variances in a special set of organic compounds. Only these substances can be taken into account correctly by the NIRS-equation, when it is applied to unknown samples. A solution for this problem might be a large database of samples, which vary in a large number of important organic substances. Then those samples can be used for calibration, which show spectra comparable to the spectrum of the unknown sample. This procedure has already been proposed by Shenk et al. (1997). These effects caused by different substances in calibration and evaluation datasets is probably not the origin of our problems to predict mineralisable N. We tried to get the same variance in both datasets, when samples for these two datasets had been picked.

The major problem related to the calibration of NIR-spectra with incubation results is the fact that the amount of mineralisable substances detected by NIRS and the actual mineralisation in an incubation experiment are different and their relation is not generally linear. But linearity between the reference

values on one side and the spectral variations on the other side is a prerequisite for the linear regressions to give useful results. All practical applications of NIRS, in which the amount of organic compounds are estimated by NIRS, are based on this linear relationship. The spectral variance can be assumed to be linearly correlated to the amount of the varying organic substance. The actual net N-mineralisation on the other hand is not always linearly correlated to the amounts of mineralisable substances in the sample.

Especially in horticultural soils with fresh vegetable residues like those used in our study there are physical factors like the size of the residue-particles, which have no effect on the NIR-spectra, but on the course of the decomposition. Two samples containing the same residues in different particle sizes thus have the same spectrum, when dried and milled like in our experiment. On the other hand they may give different values of net N-mineralisation after a specific time, since smaller particles can be accessed more easily by microbes. The variance in net N-mineralisation caused by these structural effects can neither be determined by NIRS, nor by any other chemical or spectroscopic type of measurement.

Interactions of different organic compounds can be another reason for non-linear relations between the amount of a certain substance and its effect on the actual mineralisation. For instance, easily accessible carbon pools like wheat straw can reduce the net N-mineralisation, but their impact is only linearly related to their amount, if enough nitrogen is available for immobilisation. If the accessible N-pools limit the immobilisation, the net N-mineralisation does not longer reflect the amount of these carbon sources. Their influence on the NIR-spectra is still linear.

Non-linearities can also arise from interactions of soil minerals with mineralisable organic compounds. Clay soils are known to reduce mineralisation rates compared to sandy soils. This effect can also not be considered by linear regression methods. The complex biochemical processes

in soils provide many more reasons for non-linearities. Growing or decreasing microbial populations are one of them.

The very different courses of net mineralisation in our experiment indicate, that different limitations dominate the turnover of N and C in the samples. Interactions of several organic compounds can be assumed. These different situations in the samples might be the reason for the poor predictive quality of the calibration for net N-mineralisation, when all samples were used for calibrations of mineralisation parameters.

Approximately linear courses of mineralisation can be found as start of a mineralisation following first order kinetics, when a large pool of mineralisable substance is available to microbes. The quantity, which then determines the rate of mineralisation, is simply a rate constant multiplied with the amount of available substrate. Hence, the two factors, which determine the mineralisation rate, amount and chemical composition of the substrate, are quantities, which can be expected to contribute to the NIR-spectrum of a soil sample. This influence of the single chemical components on the spectra is linear with respect to their influence on the mineralisation. This appears to be the reason for the good calibration, when only linear samples are used.

In principle, the amount of N mineralised in a certain period can also be estimated by NIRS, when the mineralisation in the samples is following other kinetics as long as the samples in the calibration dataset follow the same kinetic. We could apply this procedure only to linear samples, since there was not a sufficient number of other samples showing a uniform course of mineralisation. However, practically this procedure is not useful, since NIRS-estimations are not longer necessary, if the mineralisation course of a sample is already known.

The calibration built upon the spectral differences before and after incubation leads to significantly better results in the estimation of mineralised N than

calibrations with all unincubated subsamples. This is another indicator for the assumptions made above. The difference spectra do not contain the spectral information of the total amounts of mineralisable organic compounds, but the information of the differences of these amounts before and after incubation. This means, they represent the actual chemical turnover during the incubation process. Thus, linearity between spectral variations and net N-mineralisation is given again. Like calibrations of samples with uniform mineralisation course, these calibrations also are not useful for practical NIRS-application, since difference spectra of the unknown samples are needed. Thus the samples would have to be incubated in order to get NIRS-estimations.

Conclusion

NIRS can be used for the estimation of quantities, which are linearly related to amounts of organic compounds in the samples. The direct calibration of spectra on the amount of mineralised N in a certain time gives unsatisfying results, because non-linear relations between the actual mineralisation and the amount of mineralisable substances cause problems in the linear regression.

The potential of NIRS to estimate the amount of mineralisable organic compounds in soils can be utilized, when mineralisation courses of calibration samples are converted to amounts of mineralisable substances. This can be achieved by mathematical simulation of the mineralisation processes. More sampling dates and a longer incubation time are needed to determine the size of model pools with the necessary exactness. In the calibration process these model pools are then used as reference values. For unknown samples these pools can then be estimated by NIRS and the simulation model can predict the net N-mineralisation. Our further work will concentrate on this procedure.

3 Decomposition of plant residues as simulated by NCSOIL and measured by near infrared spectroscopy (NIRS)

Abstract

Plant residues are the major organic input to soils. Traditional methods monitoring the decay of plant residues in soils are time consuming and laborious. Once calibrated, the near infrared spectroscopy (NIRS) represents a rapid and inexpensive method to determine specific constituents in organic material. Cellulose is the most important organic component in the biosphere. Therefore, a method for monitoring the degradation of cellulose is expected to be a useful aid in studying the turnover of plant residues in soil. In order to prove whether the decay of cellulose in soil can be monitored by NIRS, we analysed soil samples from an incubation experiment using NIRS. A soil, one part amended with cellulose (2 g Cellulose kg^{-1} soil), the other without cellulose was incubated under aerobic conditions for 70 days in the dark at 15°C. Soil samples were taken at the beginning and twelve times within the incubation period. The decay of cellulose was simulated using the model NCSOIL. The soil samples were spectrally analysed with an NIR-spectrometer and the simulated cellulose content in the soil was used for the calibration of a NIRS-equation. Although the cellulose comprises only a very small part of the total organic carbon in the soil the decay of cellulose could be clearly monitored by NIRS. Ninety-five percent of the variation in the soil cellulose content as simulated by NCSOIL could be explained by the NIRS-equation (r^2 = 0,95).

We applied the NIRS-equation from the cellulose treatment to soil samples of another treatment (green manure using young leaves of endive salad mixed into the soil) which also had been scanned but not used for the calibration. The coefficient of determination for residual green manure in soil, predicted *versus* observed, was r^2 = 0.84 and 0.94 for the sandy and the clay soil, respectively.

We also analysed a sample of pure cellulose. For important wavelength regions, we found a parallel course of the NIRS-equation calibrated to describe the cellulose content in the soil and the NIR-spectrum of the pure cellulose powder. This result confirms that the NIRS-equation of the incubation experiment describes in fact the cellulose content in the soil and was not just the result of indirect correlations with other soil constituents. Hence, the NIRS-method provides an aid for keeping track of a specific and relatively small organic fraction among the background of the large amount of the total soil organic matter.

3.1 Introduction

Plant residues are the primary source of energy for the soil microbes and cellulose is the major organic constituent of the biosphere on earth (Raven et al. 2000). Generally, cellulose is rapidly decomposed in soils. Therefore, in a soil not recently amended with plant residues, cellulose contributes only negligibly to the total soil organic matter (designated in the following as “native soil organic matter”). Hence, keeping track of the decay of cellulose appears to be highly indicative for the decomposition of plant residues in soils.

Near infrared reflectance spectroscopy (NIRS) is widely used as a tool for fast determination of different organic compounds in forages, grains, dairy products or other organic materials. Several publications show, that cellulose can be detected by NIRS in different organic materials (Czuchajowska et al. 1992; Langkilde and Svantesson 1995; Schultz and Burns 1990). Also in decomposing substances like leaf material or pine needles the cellulose content can be determined by NIRS (Couteaux et al. 1998; Gillon et al. 1999; McLellan et al. 1991).

Recently, there is a growing interest in using NIRS for soil analyses (Couillard et al. 1996). The principle of NIRS is to determine the absorbance of a sample in the near infrared wavelengths region, which is caused by chemical bonds in the

sample. Before the reflectance spectra of a sample can be used to determine a specific organic compound, a calibration has to be developed for this compound. For this purpose a number of samples with different but known content of the specific organic compound need to be scanned by NIRS. The multivariate regression problem, which is encountered when equations for one chemical substance have to be obtained from spectra with many collinear variables can be solved by principle component regression (PCR) or partial least squares regression (PLS) algorithms (Burns and Ciurczak 1992; Martens and Næs 1989). Both methods reduce the original spectra consisting of 700 data points to a small number of spectral features (factors), which account for most of the variance of the spectra. These factors are then used as parameters in a normal linear regression procedure giving a NIRS-equation. Once calibrated, such a NIRS-equation can then be applied to other samples in order to determine the specific organic compound for which the equation had been developed before.

A major problem using NIRS in soil analysis is the low concentration of a single organic compound in the soil, since NIRS has not generally been found to perform well for minor organic components (Shenk and Westerhaus, 1993). Another problem is attributed to the disturbance caused by the mineral background of the soils.

Although these problems make the use of NIRS difficult, there is a growing interest in using NIRS for soil analysis. Once calibrated, NIRS is a rapid and inexpensive method and therefore it is predestinated for the routine soil analysis. In the last years numerous authors have reported encouraging results detecting various substances in soils by NIRS. Numerous studies show, that the total amount of C and N in soils can be estimated by NIRS quite well (Dalal and Henry 1986; Morra et al. 1991; Reeves et al. 1999). Successful determination of certain fractions of these total amounts has also been reported (Malley et al. 1999; Zwanziger and Förster 1998). But both publications deal with petrochemical pollutions in soils, which do not belong to the native soil organic

matter. Easily decomposable soil fractions have not yet been determined with NIRS. In most soils, the concentration of those fractions is much smaller than the fraction of recalcitrant soil organic matter. Palmborg and Nordgren (1993) were able to explain up to 95% of the basal respiration and substrate induced respiration (SIR) of forest soils from NIR-spectra.

Trying to find NIRS-equations which can be used to predict mineralisation parameters or to calculate the content of a certain organic substance with known mineralisation characteristics gives additional problems. The direct chemical measurement of the decaying organic substances in the soil is either impossible or very difficult.

The principle of the experiments cited above was to use a number of soils representing a wide range in total soil organic matter, measure a physical, chemical or biological soil parameter such as the mineralisation potential and then using such a soil parameter for the calibration of the NIRS-equation. A serious drawback of this procedure is that the soil parameters such as potentially mineralisable organic matter may be correlated with other spectral features than the spectra of the easily mineralisable compounds. Generally, soils with high organic matter content also have high mineralisation potential. For that reason not only the easily mineralisable fractions but also the total soil organic matter provides good correlations in most cases (Appel and Mengel, 1998). Hence, the spectral features of all kinds of organics (not just of the easily mineralisable fractions) might contribute to a NIRS-equation when soils with a wide range in the content of total soil organic matter were used for the calibration.

According to the problems described above, Appel and Mengel (1998) pointed out that a method providing an index for easily mineralisable soil organic matter must not only provide a good correlation when a wide range of different soils is used. The index must also be capable to indicate a change of the easily mineralisable substrate with time. The objective of our investigation was,

therefore, to test whether the change of a small and well defined fraction of the soil organic matter can be detected by NIRS during the period of its decay.

Observing such a decay in incubation experiments, one has to keep in mind that most measures give quantities of substances evolved from the soil such as CO_2. Once evolved from the soil, the CO_2 carbon is no longer a constituent of the soil sample. Hence, the measured CO_2 cannot directly contribute to a NIR-spectra of a soil sample. Instead, the spectral variation in soil samples with time are caused by indirect correlations, either due to decreasing contents of organic compounds which are mineralised such as cellulose or proteins or due to temporarily increasing organic fractions such as microbial biomass.

Reeves and van Kessel (1999) correlated NIR-spectra with the accumulated soil inorganic nitrogen and also with the evolved CO_2. They used soils amended with and without different kinds of manure. The coefficients of determination for the calibration (all soil samples included) were $r^2 = 0.63$ and 0.56 for the CO_2 and the inorganic N, respectively. However, NIRS-equations based on such indirect correlations might fail, particularly in cases when the mineralisation was caused by different organic sources. This may for example happen when soil samples manured with different amounts of pig slurry were used for the calibration and these calibrations were applied to recently green manured soils. Generally, in such a case a useful application of an NIRS-equation cannot be expected.

In order to avoid these problems we simulated the decomposition of the plant residues in soil using a validated model. By this means we calculated the remaining amount of added organic material in each sample and used these values for the development of the NIRS-equation.

3.2 Material and methods

Incubation

The soil samples were taken from an incubation experiment carried out 7 years earlier. The samples had been dried at 40 °C and stored in the laboratory for later use. Details of the experimental procedure were reported by Appel et al. (1995). For the present paper we also used samples of another treatment of this experiment which is not mentioned in this article. The soil used had been derived from alluvium and was sampled in spring from a farmer's field (0-20 cm) in Hessia, Germany (pH_{CaCl2}:5.2; total organic C: 8.09 g kg^{-1}; clay 11 %, sand 81 %). The soil was sieved (4 mm) immediately after sampling and then hand-mixed. 800 g portions were weighed and mixed either with 200 g bentonite (subsequently referred to as 'clay soil') or 200 g quartz sand ('sandy soil'). Thus we achieved two soils consisting of the same organic matter but of different mineral backgrounds. This gives us the opportunity to study the effect of the soil texture on NIRS being not confounded by any effects produced by a possibly different organic background in different soils. For the NIRS-analysis we used treatments receiving 110 mg N kg^{-1} soil as NH_4NO_3 (all amendments referred on soil dry weight base) and 908 mg C kg^{-1} soil as cellulose powder. The mineral N and the organic C were thoroughly mixed with each soil portion prior to incubation. We also analyzed the soil samples which had received green manure in form of endive leaves (C:N ratio 15.9) as described by Appel et al. (1995). The fresh plant matter provided 109 mg N kg^{-1} soil and was mixed with the soil prior to incubation. Thirty-nine pots were prepared for each treatment with the sandy soil and the clay soil. For control further 39 pots were filled with soil portions of each soil type not amended with cellulose nor with mineral fertiliser or with green manure. The soils were incubated under aerobic conditions at 15 °C in the dark for 70 days during which soil moisture was kept at 60 % of maximum water holding capacity. Triplicate pots (a-, b- and c-samples) from each treatment were sampled on day 0, 1, 2, 4, 7, 10, 14, 21, 28, 35, 42, 56, 70 of incubation. These samples were then extracted and analysed for extractable organic and inorganic N fractions by wet chemical analyses.

Simulation

The decay of the cellulose in the soil during the incubation period was calculated using the simulation model NCSOIL (Molina et al. 1997). The simulation with NCSOIL was conducted with the parameters in Table 5 as reported by Nicolardot and Molina (1994). The inorganic N accumulation in the control treatment (sandy soil) was used to adjust the initial values of pools 1 and 2 (autochthonous soil microbial biomass: 244 mg C kg^{-1} and easily mineralisable soil carbon: 2300 mg C kg^{-1}, respectively) as described by Appel (1999). These initial values from the control treatment were used for all other treatments. Another NCSOIL-parameter, however, needed to be adjusted differently for the treatments. This was the parameter describing the efficiency of the microbial population in using the plant residues for their growth.

Table 5 NCSOIL-Parameters

Parameter	Cellulose	Endive leaves	Pools (0 and 1)[a)]	Pool(2)[b)]	Pool(3)[c)]
C:N-ratio	1000	15.9	6.0	10.0	calculated[d)]
rate constants[e)] [d^{-1}]	0.2	0.3			$1.0 \cdot 10^{-5}$
for rapidly decomposable fraction [d^{-1}]			0.33	0.16	
for recalcitrant fraction [d^{-1}]			0.04	0.006	

a) refers to the zymogeneous and autochthonous microbial biomass, respectively

b) refers to the rapidly mineralisable soil organic fraction

c) refers to the recalcitrant soil organic fraction

d) calculated using the C-to-N-ratios of all other pools and the C-to-N-ratio measured for the total soil organic matter

e) rate constant for the decomposition at 28° C and optimum soil moisture

NIRS-calibration

Sixty-three of the samples (rest not enough soil left for analysis) with decreasing contents of cellulose were spectrally analyzed with a NIRSystems 5000 spectrometer (FOSS NIRSystems Inc., Silver Spring, USA) in the wavelength range from 1100 to 2500 nm. Each soil sample was milled to pass a 1 mm sieve.

We used both the PCR and the PLS algorithms for developing calibrations. The number of factors to explain the spectral variance in the samples was controlled by the cross-validation in order to avoid “over-fitting”. Calibrations with spectra of *n* wavelength end up in NIRS-equations of the form

$$c = b_0 + \sum_{i=1}^{n} b_i \cdot a_i$$

where a_i are the measured absorptions at wavelength *i* and b_i are the equations coefficients, which determine, to which extend a certain wavelength contributes to the calculation of the unknown concentration and *c* is this concentration, which has to be predicted from the spectra. The value b_0 is an additive constant originating from the regression procedure. This form does not depend on the regression method used in the calibration procedure.

All calibrations presented in this paper have been calculated with PCR and PLS as regression methods and with different mathematical pre-treatments of the raw spectra too, but the differences of the results were negligible. For the results presented in this article PLS using the first derivative of the spectra has been chosen as regression method, since it has become a very common method in NIRS analysis in the past years. The modified form described by Shenk and Westerhaus (1991) was used.

The NIRS-equation can be calibrated including all the samples. Alternatively, subsets of samples can be used for the calibration. This offers the opportunity to evaluate the NIRS-equation by applying it to the spectra of the other samples not used for the calibration. We used the following subsets:

a) One half of the soil samples were randomly picked for calibration, the rest taken for evaluation.
b) The a- and b-samples of the incubation were used for calibration, the c-samples for the evaluation.
c) The c-samples were used for the calibration, while the a- and b-samples were used for the evaluation.
d) The calibration was conducted with the sandy soil and the NIRS-equation was then applied to the clay soil.
e) The clay soil was used for calibration and the NIRS-equation was then applied to the sandy soil.

The last two subsets were supposed to show how the soil texture effects the calibration.

As a last step, pure cellulose powder (without soil) was scanned in order to examine, whether the NIRS-equations from the incubation experiment were based primarily on the main spectral features of cellulose. Thereby we wished to exclude the possibility, that the NIRS-equations were developed upon indirect correlations originated from other chemical substances just correlating with the cellulose content in our experiment.

3.3 Results and Discussion

The simulation model NCSOIL describes the mineralisation of cellulose using first order kinetics (Figure 9). Therefore, during the early period of incubation the cellulose content decreased rapidly followed by a period of less intense degradation. The simulated cellulose content ranged between 130 and 908 mg

cellulose-C kg^{-1} soil. Only small differences were calculated by the model between the sandy and the clay soil. The difference was caused by the slightly different initial mineral nitrogen content in the sandy and the clay soil. During the period of intense N-immobilization the decay rate of the cellulose was presumably limited by the nitrogen available for immobilization. This was considered by NCSOIL. The model does not consider any further effect of the soil texture such as a stabilizing of small organic metabolites on the clay mineral surfaces. This behavior of NCSOIL is in agreement with the results obtained with the green manured soil samples (Appel et al. 1995). The accumulation of inorganic N caused by the mineralisation of the green manure was only slightly affected by the different soil texture.

We used these simulated values as the reference for the calibration of the NIR-spectra. In other words, we calibrated the NIRS using the simulated data. On first view this may appear exceptionally. However, we think that the data obtained from a well validated simulation model such as NCSOIL may be more reliable than those of a chemical analysis of the soil samples, particularly when inhomogeneous distribution of the cellulose in the small subsamples used for analysis have to be feared as a source of variance. A loss of accuracy in chemical analyses may also be caused by the relatively low cellulose concentrations in the samples compared to the immense amount of soil mineral particles and in addition the existence of a huge background concentration of other organic constituents provided by the native soil organic matter.

Figure 9 shows that the cellulose content predicted by the NIRS-equation was very similar to those simulated by NCSOIL. Ninety-five percent of the variance in the cellulose content as simulated by NCSOIL was explained by the NIRS-equation ($r^2 = 0.953$). This close correlation between the simulated data and the NIR-spectra suggests twice, (1) the NIRS-method was capable to detect the cellulose in the soil matrix, and (2) the simulation by NCSOIL provides reliable data. The second suggestion comes out of the fact that associated to the simulated cellulose concentrations we found measurable counterparts

(NIR-spectra) in the soil samples. The course of the cellulose content as simulated by NCSOIL is not a simple exponential decay. Especially for the last sampling dates the decay is significantly slower. The calibration shown in Figure 9 was done with all soil samples. The measure r^2 in Figure 9 was calculated for the whole data set of 64 soil samples providing a NIRS-equation with 7 factors.

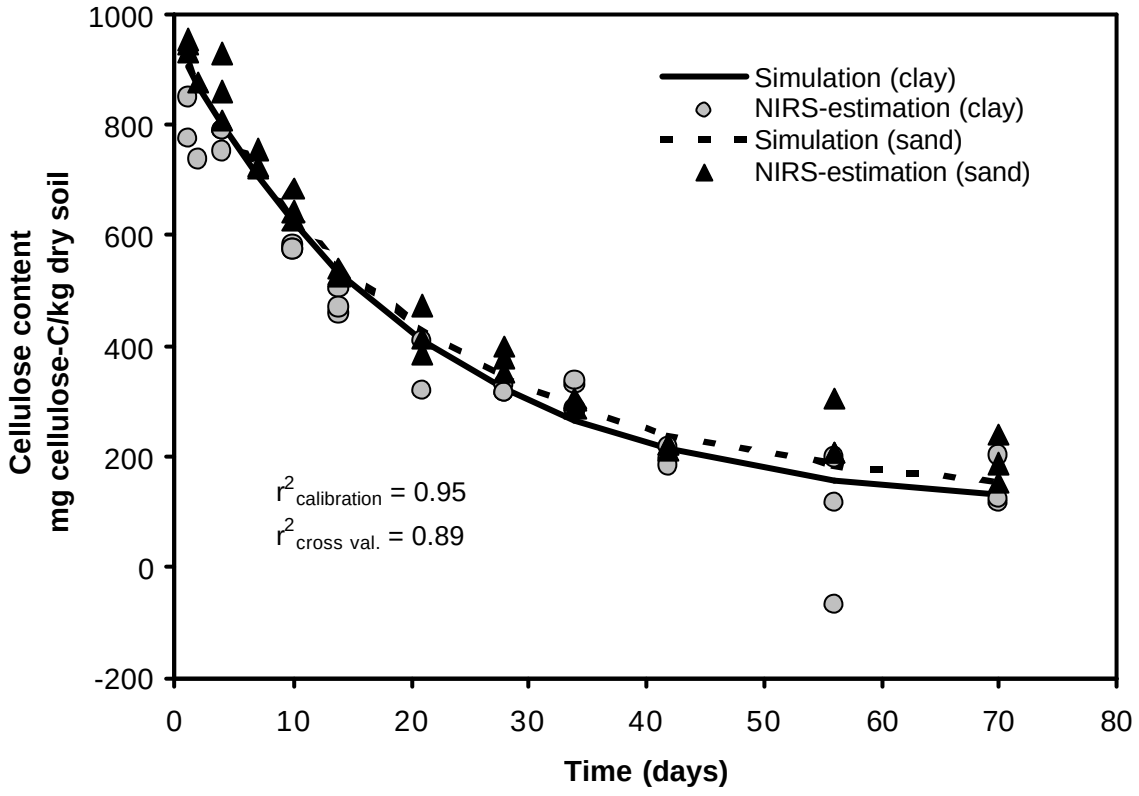

Figure 9 *Cellulose remaining in the soil during incubation as simulated by NCSOIL and estimated by a NIRS-equation*

Further calibrations were done using different subsets of the whole sample collection in order to evaluate the NIRS-equation. The NIRS-equations gained from these subsets were then applied to the samples not used for the calibration (= evaluation). The coefficients of determination provided by these evaluations ranged between r^2 = 0.80 and 0.86. The resulting slopes of the regression lines (predicted *versus* observed) did not significantly differ from 1.0 on a 5%-level (Table 6).

These results suggest that the NIRS-equations provide a reliable prediction of the cellulose content in the soil samples. However, one has to keep in mind that we used a set of soil samples without any variation in the constituents and in the content of the native organic matter. Such a variation in the background organic matter may reduce the explainable variance in similar experiments.

Table 6 Results for calibration and evaluation using different subsets of samples[1)]

Calibration subset	Evaluation subset	Number of PLS-factors	r^2 (cross val.)	r^2 (evaluation)	Slope (evaluation)
random2 34 samples	random1 33 samples	4	0.89	0.86	1.10
c-samples 22 samples	a + b samples 45 samples	3	0.88	0.83	1.00
a + b-samples 45 samples	c-samples 22 samples	7	0.91	0.80	0.87

[1)] For further explanation of subsets see material and methods section

The NIRS-equations gained from the calibration with the cellulose amended soils were applied to the NIR-spectra of the soil samples of the green manure treatment. The NIRS-equations from the sample subsets of the clay and sandy soil were applied each to the corresponding soil type of the green manure treatment. The comparison of the cellulose content in the soils as estimated by NIRS and the green manure carbon in the soils as simulated by NCSOIL is shown in Figure 10. The green manure carbon decreased from 1733 mg C kg^{-1} soil at the beginning of the experiment to 87 mg C kg^{-1} soil after 70 days of incubation. The coefficient of determination was $r^2 = 0.84$ and 0.94 for the sandy and the clay soil, respectively, when the NIRS-estimated cellulose was regressed on the green manure carbon. This confirms our suggestion that the

NIRS can be used as a tool to analyse the decomposition of plant residues in soils. Assuming a hundred percent sensitivity of the NIRS-estimation for cellulose content of the endive leaves, the slope of the regression line can be interpreted as the portion of cellulose on the total green manure carbon. The slope of the regression line was about 0.2 indicating a cellulose content of approximately 20 % of the total carbon in the endive leaves, which is a plausible value (Herrmann 2001). We cannot prove this estimation, since the cellulose content of the green manure had not been analysed.

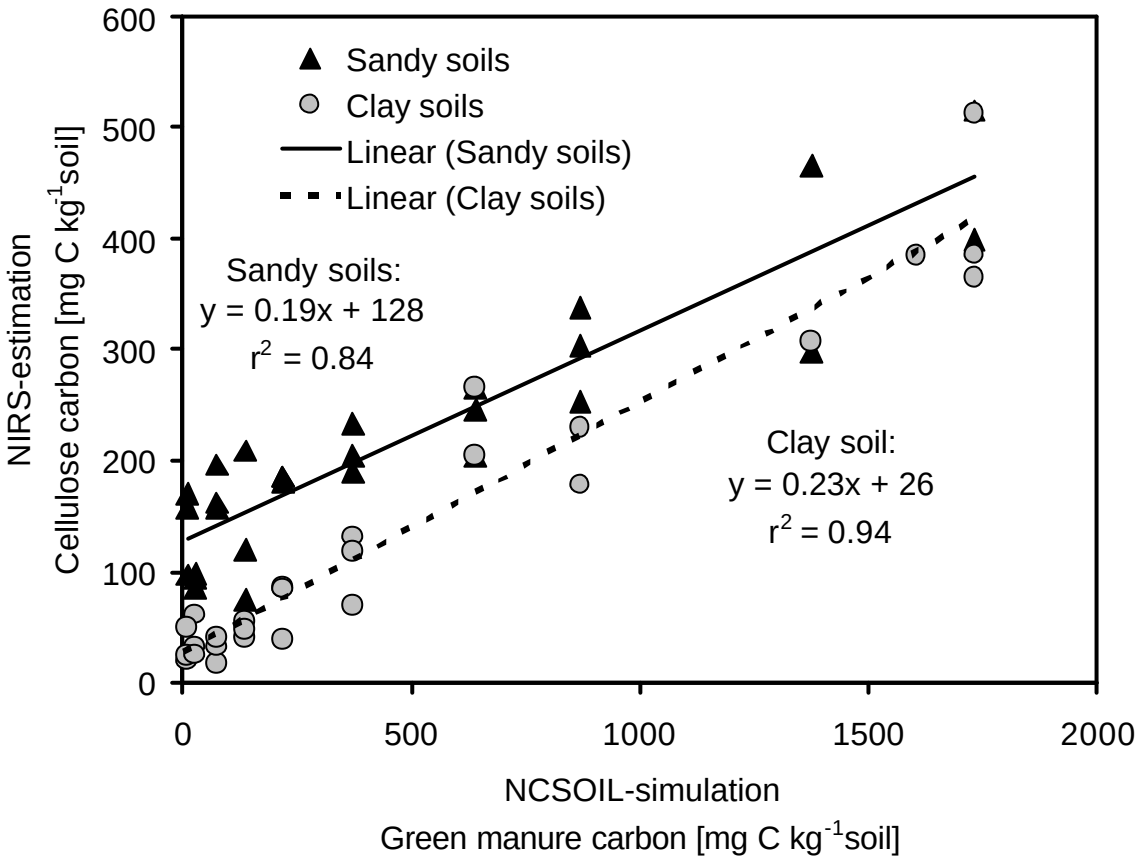

Figure 10 *Relationship between NIRS-estimated cellulose and NCSOIL calculated total green manure carbon in the clay and the sandy soils*

Pure cellulose powder was scanned by NIRS in order to examine whether the NIRS-equation reflects the cellulose or just another substance correlated with the cellulose content in the soils. For this purpose the coefficients b_i in the NIRS-equations can be plotted against the wavelengths l in the same way as the spectra themselves. These coefficients show peaks at those wavelengths, which are determined by PLS-regression to be positively correlated to the cellulose content. In Figure 11 this was done for the NIRS-equation gained from the calibration with the sandy soil samples (NIRS-equation) using only two spectral factors for the explanation of the cellulose variation. It has to be mentioned, that the first derivation of the spectra were used for the calculation of the NIRS-equation. One possibility to allow a comparison would be to show the first derivative of the spectrum of pure cellulose. But since the derivatives are very hard to interpret visually, not the actual b_i are shown in this figure, but the integrated values B_i as calculated from the following equation, where l_1 is the minimum wavelength of the NIR-spectrum (1100 nm):

$$B_i(l) = \int_{l_1}^{l} b_i \cdot dl'$$

This way, the slopes of the two plots given in figure 11 can be directly compared. The cellulose spectrum has passed the same scatter correction as the spectra used in the calibration. In this case, not the absolute values of the plots, but areas with a parallel course indicate, that the NIRS-equation actually represents the cellulose content. Wavelength areas, in which the B_i values do not show clear changes, are not important for the calculation of cellulose content with this NIRS-equation. Those wavelength areas, on the other side, where the B_i values show clear peaks, should correspond to peaks in the spectrum of pure cellulose.

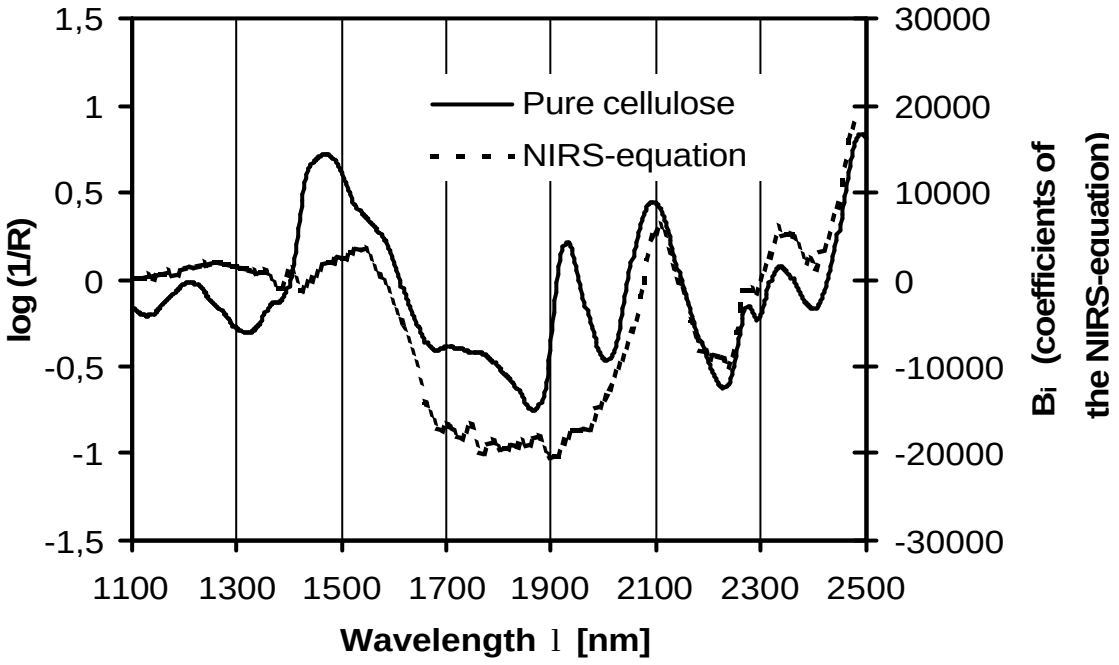

Figure 11 Comparison of NIRS-equation for cellulose from calibration with sandy samples (eqa-sand) and spectrum of pure cellulose powder

The major absorption bands of water are known to be around 1940 nm and 1450 nm (Sadtler 1981; Shenk et al. 1992). The residual water content in the cellulose powder affects the cellulose spectrum at these bands. The NIRS-equation does not show peaks in these areas. From 1100 to 1450 nm and from 1700 to 1900 nm the equation roughly gives horizontal lines. This means that the equation coefficients are around zero (integrated form is shown), so these wavelength areas have only little effect on the calculated cellulose content.

The parts of the spectrum, where absorption by water molecules is of importance, contribute only to a very low extend to the NIRS-equation for cellulose. The reason for this is, that peaks for water and cellulose are broad and overlapping in this part of the NIR-spectrum. Thus the absorption in the wavelength areas, which are influenced by both, water and cellulose, is not directly correlated to the cellulose content. Since regression algorithms as

simple mathematical instruments are not able to differentiate between water and cellulose effects, they only take into account wavelengths with clear correlations between the measured spectral variance and the variance of the searched chemicals. These clear correlations then lead to high coefficients b_i in the NIRS-equation.

In these other wavelength areas, particularly in those above 2000 nm, the cellulose spectrum and the NIRS-equation match quite well. Shenk et al. (1992) analysed the NIR-spectra of different organic substances. According to them, cellulose is characterized by a peak at 2336 nm. Scanning the pure cellulose we also found a characteristic peak at this wavelength (Figure 11). Calibrating the NIRS with the cellulose amended samples of the incubation experiment, the wavelengths of the coefficient peaks of the resulting NIRS-equation agree very well with those of the characteristic peaks from the pure cellulose (Table 7). The integrated NIRS-equation coefficients B_i do not show any major peaks at wavelengths other than those of cellulose (Figure 11, Table 8). This ensures that the NIRS-equation from the cellulose amended soil samples reflects principally the cellulose content in the soil and is not mainly caused by other soil constituents.

Table 7 *Comparison of absorption peaks from water and pure cellulose with those of the NIRS-equation gained from the cellulose amended incubated soil samples*

	Wavelengths of peaks [nm]								
Peaks of pure cellulose according to literature[1)]	1490	1780	1820	1930	2100	2270	**2336**	2352	2488
Peaks of pure water[1)]	1450	1790	1790	1940					
Peaks measured with pure cellulose in our study	1472	1762		1930	2094	2276	**2336**		2490
Peaks of the NIRS-equation gained from the incubated cellulose amended soil[2)]					2108	2268	**2332**	2348	2484

[1)] (Shenk et al. 1992)
[2)] Integrated coefficients B_i

The resolution of the NIRS-method for cellulose in soil is clearly below 0.1%. Total soil carbon was about 1 % in the incubation experiment and the fraction of cellulose-carbon among the total soil carbon ranged from about 1 to 10%, assuming that cellulose was negligible in the native soil organic matter. Hence, the cellulose concentration in the soil matrix was always below 0.1 %. To our knowledge, no other method than NIRS offers the possibility to analyse a specific organic compound in soils with a comparably low intensity of analytical work. Cellulose is by far the quantitatively most important constituent in the biosphere and therefore highly indicative for the decay of plant residues in soil. The NIRS-method appears, therefore, to become a useful tool to study the decay of organic residues in soil.

4 Influence of soil texture on the use of near infrared spectroscopy (NIRS) in C- and N-mineralisation studies

Abstract

In the last years there is a growing interest in using NIRS in carbon- and nitrogen mineralisation studies. Here, a major problem is the unknown influence of soil texture on the spectra and thus on the prediction results. In our study we used soil samples from a 70-day incubation experiment with two different soil textures for the spectral analysis. These two soil textures were achieved by mixing the soil with 20% bentonite and 20% quartz sand, respectively. Four different fertilizer treatments with three repetitions had been prepared with each soil type. For our NIRS-investigation we used the treatments: a) NH_4NO_3 + Cellulose and b) chopped endive leaves. Values simulated with NCSOIL were used as reference values in the calibration process. Calibrations derived from soils mixed with clay give different results for organic substances than those derived from sandy soils, which can lead to misinterpretations when calibrations are transferred to another soil type. Scaling of the reference values according to the soil mineral components can avoid this problem to some degree.

4.1 Introduction

Near infrared reflectance spectroscopy (NIRS) is widely used as a tool for fast determination of different organic compounds in forages, grains, dairy products or other organic materials. The principle of NIRS is to determine the absorbance of a sample in the near infrared wavelengths region, which is caused by chemical, especially organic bonds in the sample. A calibration for determining contents of various substances from the spectra is created using different multivariate regression methods like principle component regression (PCR) or

partial least squares regression (PLS) (Burns and Ciurczak, 1992; Martens and Næs 1989). Recently, there is a growing interest in using NIRS for soil analyses (Couillard et al. 1996). In the last years numerous authors have reported encouraging results detecting various substances in soils using NIRS. Dalal and Henry (1986) measured organic C, total N and water content in soils. They divided the samples into two datasets, which were used for calibration and evaluation, respectively. They found fractions of explained variance between 0.85 and 0.94 for the separate evaluation dataset. Reeves and van Kessel (1999) correlated NIR-spectra with the accumulated soil inorganic nitrogen and the evolved CO_2. They used soils amended with different kinds of manure as well as unmanured controls. The coefficients of determination for the calibration (all soil samples included) were 0.63 and 0.56 for the evolved CO_2 and the released inorganic N, respectively. Quantities related to the soil texture like clay content, cation exchange capacity (CEC) and specific surface area (SSA) have been successfully determined by NIRS (Ben-Dor et al. 1991; Matsunaga and Uwasawa 1992; Sudduth and Hummel 1993).

All these publications indicate that there are chances to detect mineralisable organic substances in soils by NIRS. However, one problem appears to be obviously associated with the disturbance caused by the soils mineral background. Different soil textures and particle sizes cause different scattering conditions among the mineral particles. This could be a serious restriction in the use of NIRS for soil analysis. The influence of mineral soil fractions on NIRS-calibrations for organic soil compartments has been pointed out in several publications (Krischenko et al. 1991; Morra et al. 1991; Zwanziger and Förster 1998). In these articles it is recommended to use calibrations derived from samples with the same soil type as the examined samples for the determination of organic substances. This would mean, that calibrations cannot be transferred to other sites and the usefulness of NIRS for soil analyses would be economically ambiguous due to an enormous labour requirement for a huge number of calibrations.

Artificial mixtures with different proportions of sand, silt and clay have been examined by Couillard et al. (1996). They found different influences of the different soil minerals on the NIR-spectra, e.g. a coating effect of clay, which could be compensated by a model using scaling factors for the different soil separates. Attempts to apply this model to natural samples failed, showing no improvement in prediction accuracy. Therefore, our interest was focused on how the soil texture affects NIRS-measurements in more natural samples. In order to get a deeper understanding of the mechanisms by which the soil texture may influence the NIRS-measurement, we attempted to restrict the variance in the samples to a very small set of parameters. This was possible by using soil samples from an incubation experiment where two different soil textures were prepared by mixing the soil with quartz sand or bentonite, respectively. Thus we got a dataset with a defined source of variance, offering the chance of interpretable results.

4.2 Material and methods

Incubation

The soil samples analysed by NIRS were part of an incubation experiment carried out about 7 years earlier and stored after drying (40 °C) in the laboratory for later use. Details of the experimental procedure were reported by Appel et al. (1995) for the soils treated with and without green manure. The soil used was derived from alluvium and was sampled in spring from a farmer's field (0-20 cm) in Hessia, Germany (pH_{CaCl2}:5.2; total organic C: 8.09 g kg^{-1}; clay 11 %, sand 81 %). The soil was sieved (4 mm) immediately after sampling and then hand-mixed. 800 g portions were weighed and mixed either with 200 g bentonite (subsequently referred to as 'clay soil') or 200 g quartz sand ('sandy soil'). This provided two soils consisting of the same organic but different mineral backgrounds. For the NIRS-analysis we used treatments receiving

a) 110 mg N kg^{-1} soil as NH_4NO_3 and 908 mg C kg^{-1} soil as cellulose powder (all amendments referred on soil dry weight base),
b) green manure (chopped endive leaves offering 1730 mg C kg^{-1} soil and 109 mg N kg^{-1} soil)

Thirty-nine pots were prepared for each treatment with the sandy soil and the clay soil.

The soils were incubated under aerobic conditions at 15 °C in the dark for 70 days during which soil moisture was kept at 60 % of maximum water holding capacity. Triplicate pots from each treatment were sampled on day 0, 1, 2, 4, 7, 10, 14, 21, 28, 35, 42, 56, 70 of incubation. Aliquots of the soils were extracted and analysed for extractable organic and inorganic N fractions. The subsequent reference values for the cellulose content and the remaining content of green manure during the incubation were obtained from simulations with NCSOIL (Molina et al. 1997).

NIRS-calibration

128 samples were spectrally analysed with a NIRSystems model 5000 spectrometer (FOSS NIRSystems Inc., Silver Spring, USA) in the wavelength range from 1100 to 2500 nm. The rest of the samples could not be examined, since the amount was not sufficient for NIRS-analysis. Each soil sample was dried and milled to pass a 1 mm sieve. Then three ring cups were filled with each soil sample. The spectra of these cups were averaged after a first outlier examination. Thus it was ensured that no effects like dirty cups or non-representative sub-samples had disturbing influence on the spectra.

There are several algorithms which can be used for solving the multivariate regression problem, which is encountered when equations for one chemical substance have to be obtained from spectra with many collinear variables (Burns and Ciurczak 1992). The most common methods are principle component regression (PCR) and partial least squares regression (PLS). Both

methods reduce the original spectra consisting of 700 data points to a small number of spectral features (factors), which account for most of the variance of the spectra. The number of factors to explain the spectral variance in the samples is controlled by the cross-validation during the calibration procedure in order to avoid "over-fitting". In this study PLS in the modified form (MPLS) as described by Shenk and Westerhaus (1991) using the first derivative of the spectra was chosen as regression algorithm after comparing different regression methods. This comparison, which was conducted using all 63 cellulose amended samples in the calibration, is summarized in Table 8. In addition to the number of factors used to explain the variance, Table 8 shows the coefficient of determination (r^2) for the calibration and the cross-validation and the number of regression outliers.

Table 8 *Comparison of different regression methods and mathematical treatments with respect to the coefficent of determination and the number of regression outliers*

Regression method	Derivative (0=original spectra)	Number of PLS-factors	r^2 calibration	r^2 cross val.	Number of regression outliers
MPLS	1	7	0.96	0.89	1
MPLS	0	7	0.95	0.94	1
PLS	1	6	0.93	0.88	0
PLS	0	7	0.95	0.94	4
PCR	1	4	0.89	0.90	3
PCR	0	8	0.95	0.94	4

Calibrations with the raw spectra give higher coefficients of determination in the cross validation, but need more factors to explain the variance and more regression outliers have to be removed from the calibration dataset. MPLS is

giving the best results when no mathematical pre-treatment is applied to the spectra. Using a scatter correction (Barnes et al. 1989) and the first derivative of the spectra as pre-treatment, every regression method has got its own advantage: MPLS gives the best result in r^2, normal PLS needs no outliers elimination and PCR needs only four factors to explain the variance. No regression method can be said to perform best on this dataset. We decided to use MPLS and the first derivative of the spectra for our further calculations.

It has to be pointed out, that the NIRS-equations found in these calibrations can be shown to really predict the cellulose content in the soil samples. This cannot be concluded directly from the correlations of NIR-spectra and simulated cellulose contents, but from comparisons of NIRS-equations for cellulose-content and the NIR-spectrum of pure cellulose-powder. A more detailed publication on this aspect is presented in chapter 3.

Except building up a NIRS-equation by making use of all samples in the calibration procedure, different subsets of samples were used for calibration and evaluation purposes: a) One half of the samples were randomly picked for calibration, the rest taken for evaluation. b) samples of two repetitions of the incubation process were used for calibration, the third repetition for evaluation. c) calibration was conducted using the soil samples with sand addition, the equation gained with sand samples was evaluated using the soils with clay addition and vice versa. This last separation was supposed to show, to which extend a calibration can be used for soils with differing mineral background.

4.3 Results

Impact of soil texture on calibration for cellulose

Five PLS-factors were necessary to explain more than 90 % of the variance in the cellulose content when both soils were used for the calibration of the NIRS-equation (Figure 12).

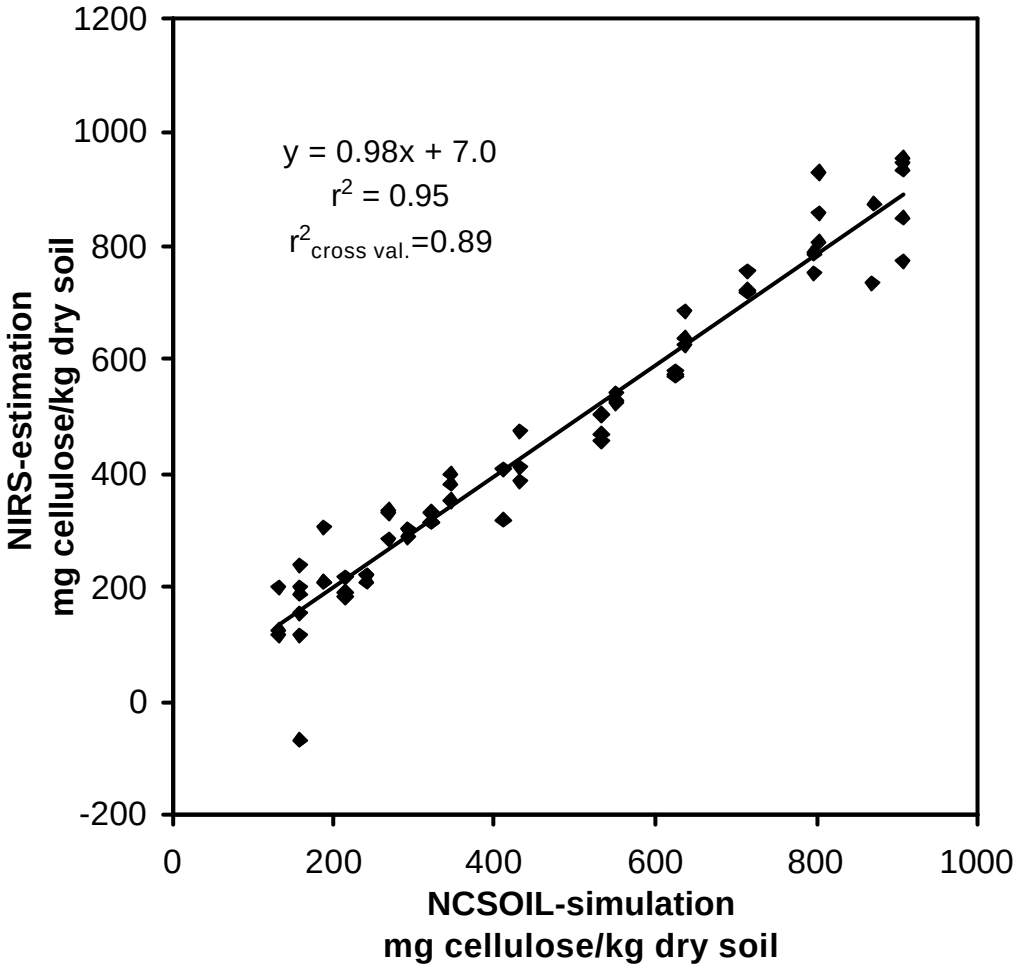

Figure 12 Calibration using all 63 cellulose-amended soil samples and using 7 spectral factors to explain the variance

Using only a subset of the samples consisting only on the sandy soil or the clay soil, respectively, clearly reduced the number of factors needed for the explanation of cellulose variance. One obvious difference between the calibration with all samples and the calibrations with only one soil type can be seen from the proportion of variance described by the first PLS-factor (Figure 13). In calibrations with all soil samples the first factor explains less than 20% of the variance, regardless which kind of mathematical treatment or which regression method is used.

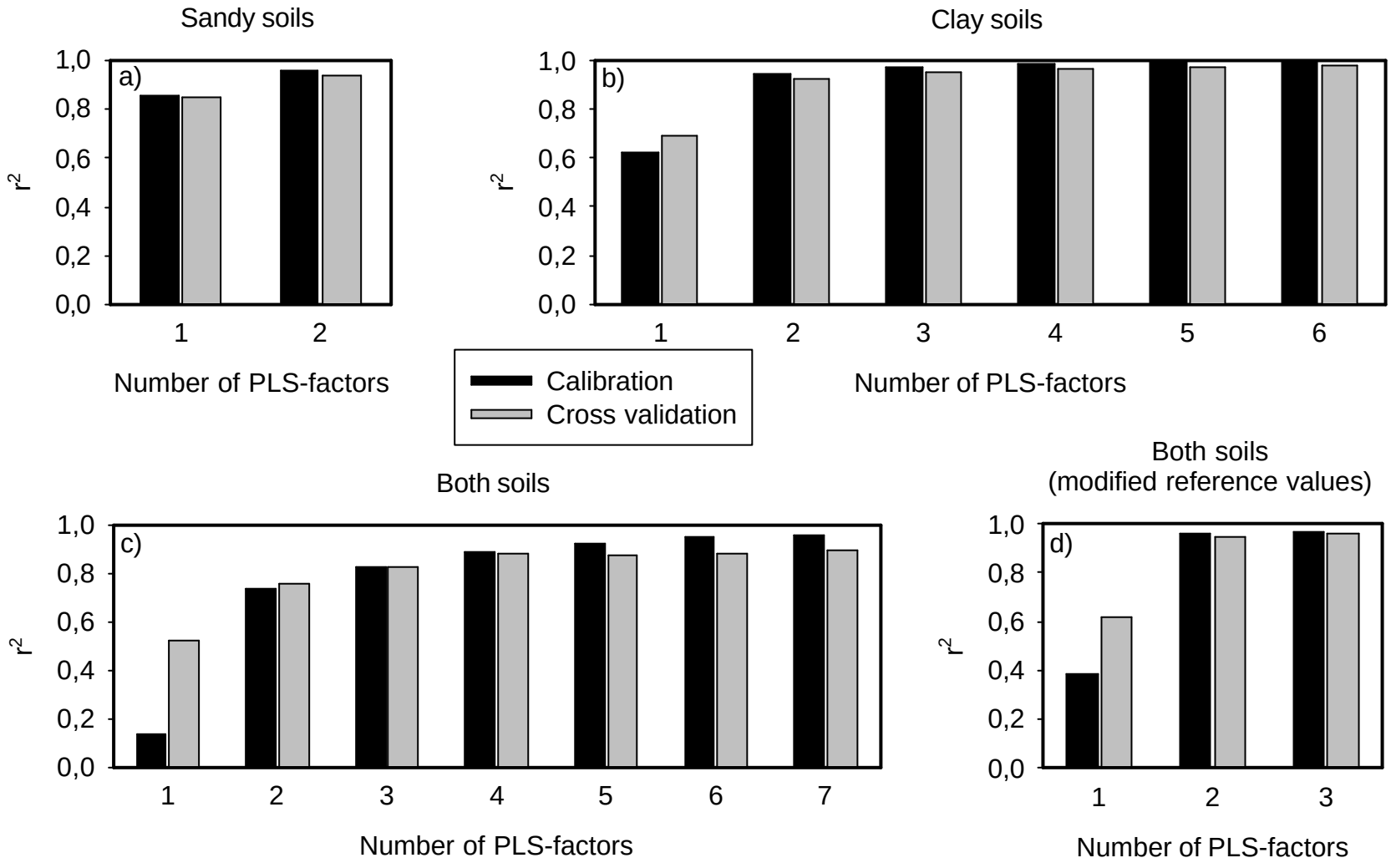

Figure 13 Quality of the calibration in terms of r² and 1-VR as related to the number of PLS-factors

This clearly indicates, that the main spectral variance is related to the difference of the mineral additions, clay and quartz sand. In the sandy soil, more than 80% of the cellulose variance can be explained with the 1st factor since there is no spectrally dominating variance by mineral additions. Using the clay soils in the calibration leads to more than 60% of the variance explained by the first factor (Figure 13).

The NIRS-equation calibrated using the sandy soil samples was applied for predicting the cellulose clay soils and *vice versa.* By this cross evaluation about 80 % of the variance in cellulose content was explained (r^2 = 0.79 and 0.94, Table 9). However, the slope of the linear regression predicted versus observed was substantially different from one (Table 9). This difference is significant on a level of α = 1%.

Table 9 *Evaluation results for different subsets of samples used for calibration and evaluation*

Calibration subset	Evaluation subset	Number of PLS-factors	r^2 (cross val.)	r^2 (evaluation)	Slope (evaluation)
sand	clay	2	0.93	0.79	0.41
clay	sand	6	0.98	0.94	2.03
random2	random1	4	0.89	0.86	1.10
repl. c	repl. a+b	3	0.88	0.83	1.00
repl. a+b	repl. c	7	0.91	0.80	0.87

This varying slope in the evaluation indicates a different “sensitivity” of the NIRS-equations for the searched substance caused by the different soil textures used in the calibration. Dividing the whole number of samples in either two fractions of randomly chosen samples or using the different replications of the incubation as data sets for calibration and evaluation, results in r^2-values above

0.80 and slopes between 0.87 and 1.10. In these cases the calibration and evaluation data sets roughly show the same spectral variation, which leads to better evaluation results, i. e. slopes do not significantly differ from 1.0 (α = 5%).

Impact of soil texture on calibration for green manure

Very similar results like those obtained from cellulose calibrations are found when calibrations are done for the residual amount of a green manure. Again we divided the samples amended with green manure in sandy and clay-soils for calibration and validation. As above the calibration made up with the sandy soils underestimated the content in the clay soils and *vice versa*. The slopes of the regression lines in the validations were 0.40 and 1.60, respectively.

The calibration and evaluation results are shown in Figure 13. The coefficient of determination is satisfactory (r^2=0.88 and r^2=0.69), but the slope and the intercept of the regression lines are affected when applying a NIRS-equation to another soil type. The slopes of the evaluation as presented in Figure 14 differ significantly from 1.0 on a 5%-level. The equation derived from the clay-soils spectra not only over-estimates the slope but also the average content of the green manure in the sandy soils and *vice versa*.

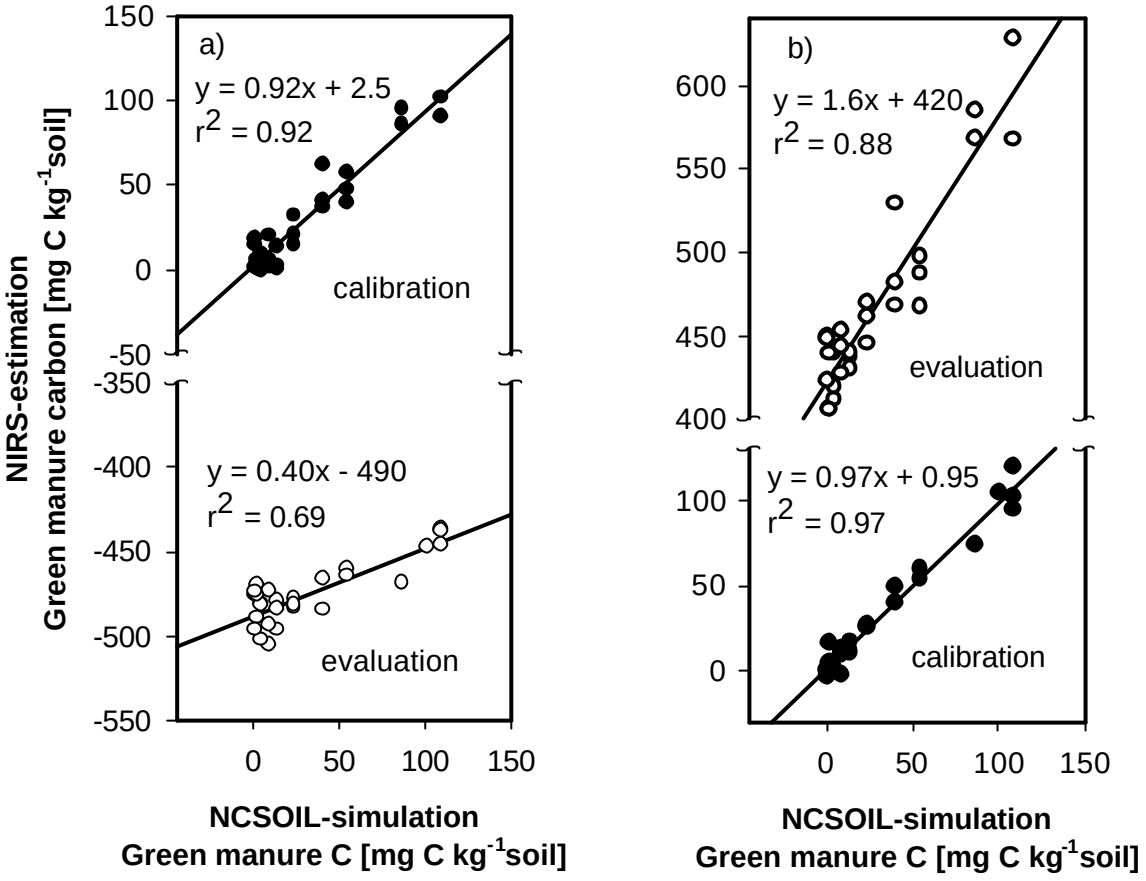

Figure 13. a) Calibration with sandy soils, evaluation with clay soils, b) calibration with clay soils, evaluation with sandy soils.

Application of modified reference values

Couillard et al. (1996) suggest to modify the reference values when samples contain different soil mineral backgrounds. This modification is derived from results of investigations with artificial mixtures of sand, silt, clay and organic matter. Laboratory weight scaling factors are introduced and the different fractions of soils are supposed to be multiplied by these factors (Couillard et al. 1996). Table 10 shows our unmodified values for the different soil compartments in the cellulose samples, the scaling factors according to Couillard et al. and our new values after the modification. Couillard et al. (1996) give scaling factors of 1.41 and 1.63 for kaolinite and montmorillonite clay, respectively. We used an average value of 1.52 for clay in our calculations.

After multiplying the contents with the weight scaling factors, we had to normalize the values to sum up to 100 % again.

Table 10 *Contents of soil separates as before and after the modification as suggested by* Couillard et al. (1996)

Soil compartment	Original content [%] in		Scaling factor	Modified content [%] in	
	sandy soil	clay soil		sandy soil	clay soil
Sand	83.7	63.7	0.15	41.8	16.6
Silt	6.3	6.3	0.39	8.2	4.3
Clay	8.7	28.7	1.52	43.8	75.8
Organic matter	1.1	1.1	1.41	5.2	2.7
cellulose	0.2	0.2	1.41	1.0	0.5

After the modification the new reference values for cellulose as used for NIRS-calibrations and evaluations differ clearly due to the different contents of sand and clay. Depending on the exact type of clay, the modified cellulose contents in the sandy soils are 1.87 to 1.96 times higher than in the clay soil samples. The same factor can be used, if the modification is applied to the samples, which received green manure.

If calibrations are calculated with these new reference values, some improvements can be achieved. If soils of both types are used in the calibration, fewer PLS-factors are needed to explain the variance of the cellulose content. This fact is shown in Figure 13 part d). With three factors, both r^2 values for calibration and cross validation already are above 0.95 and even with only two factors almost the same results can be achieved.

When calibrations for cellulose are made using only samples from one soil type, calibration statistics stay the same as those without modification, since scaling

factors are uniform for each soil type. Applying the calibrations to the other soil type, mainly the slope of the regression line predicted vs. observed is changed. Contrary to the unmodified reference values, it does no longer differ significantly from 1.0 (α = 5%) in both cases as shown in Table 11. When other subsets of samples were used for calibration and evaluation (randomly chosen or different replicates), the proportion of explained variance is larger than when unmodified reference values are used.

Table 11 *Evaluation results for different subsets of samples used for calibration and evaluation using modified values for the cellulose content*

Calibration subset	Evaluation subset	Number of factors	r^2 (cross val.)	r^2 (evaluation)	Slope (evaluation)
Sand	Clay	3	0.95	0.78	0.87
Clay	Sand	6	0.98	0.93	0.89
Random1	Random2	6	0.95	0.97	0.89
Rep a+b	Rep c	3	0.95	0.96	1.18
Rep c	Rep a+b	4	0.97	0.96	0.91

Similar results are achieved, when the modification is applied to samples containing green manure. Again, the slopes of the regression line predicted vs. observed does not significantly differ from 1.0 for the evaluation subsets (α = 5%). In both cases, cellulose and green manure treatments, the intercept for the evaluation as seen in Figure 13 cannot be completely removed by the modification. However, it is smaller after the modification in all cases.

4.4 Discussion

Spectral difference of sandy and clay soils

Regarding the ability of the 1st factor to explain cellulose variance, it is obvious, that the spectral difference of sandy and clay soils dominates in the calibration with spectra from both soils.

This spectral difference of the sandy and the clay soils cannot be simply explained by spectral features of sand or clay itself. Both are silicates, which have no absorption bands in the near infrared region (Workman 1998). So these mineral additions are “transparent” for NIR-radiation. Several other differences might contribute to different spectra. The larger mean particle size of sandy soils compared to soils with clay addition mainly shifts the spectra to generally higher absorptions. These differences are almost completely removed by scatter correction and application of the 1st derivative (Barnes et al. 1989). So the difference seen in the calibrations with sand and clay cannot be explained this way, since the calibrations use these mathematical treatments. Different water contents due to higher water adsorption at clay mineral surfaces can explain the spectral difference making the 1st factor in the calibration inefficient for explaining cellulose variance when samples from both soil types were considered.

Different “sensitivities” of NIRS-equations

The different “sensitivity” of the NIRS-equations from the sandy and the clay soil became obvious when they were applied to the other soil type and vice versa. This cannot be explained simply by the hygroscopicity of the clay. However, using the following physical analogy the effect becomes plausible. Both sand and clay are transparent to NIR-radiation, but like glass surfaces both reflect a part of the visible light, all surfaces of silicates, sand as well as clay scatter a fraction of the NIR-radiation. Smaller mean particle size is associated with a higher density of surfaces per unit volume which results in a lower penetration

depth for the NIR-radiation. This effect is similar to the higher transparency of big ice-cubes compared to “white” snow. Thus having two soils with the same concentration of organic material per kg dry soil, less information of this organic compounds will reach the detector in the soil with lower mean particle size. So if the NIR-spectrometer gets less spectral information from the cellulose in the clay soil, the coefficients b_i in the NIRS-equation must be higher to explain the same cellulose variance as in the sandy soil. The NIRS-equation has to compensate the lower amount of spectral information from the organic compounds, it has to “react more sensitive”. This way the content of the predicted substance is over-estimated and the slope of the linear regression in the evaluation plot is too high in sandy soils when applying the equation gained from clay soils and *vice versa*.

The size of clay particles has the same order of magnitude as the wavelength of the near infrared radiation. Thus other physical effects than reflection occur, when this radiation interacts with clay particles. So the analogy described above cannot be used for quantitative predictions. However, the principle effects can be represented qualitatively by this consideration.

The intercept of the regression lines, which is caused by the transfer of a NIRS-equation to a soil with another texture has mainly the same reason, but it cannot be interpreted that simply. Its absolute value is strongly influenced by the regression algorithm and the mathematical treatment of the spectra prior to calibration. Thus the bias cannot be corrected by addition of a soil texture depending value.

One conclusion from this consideration is, that the detection limit for organic soil constituents is lower in sandy soils than in soils with a high content of clay, i.e. lower concentrations of organic material can be detected in sandy soils.

Scaling of reference values

Modification of the reference values by scaling of the different soil separates according to the scheme presented by Couillard et al. (1996) is a possibility to compensate the different visibility of the mineral soil size fractions. In this case the different soil components are weighted by their transparency in the sample. Thus spectral features of the soil samples and their reference values match better after the modification. This leads to several improvements in the calibrations: This compensation explains, why calibrations with both sand and clay samples only need fewer PLS-factors to explain the cellulose variance, than without the modification. Another effect is, that higher values of r^2 can be reached for the evaluations, when calibration and evaluation datasets both consist of samples with different soil types. When the samples are divided in sandy and clay soils for calibration and evaluation, respectively, the slopes of the regression lines predicted vs. observed, which are related to the sensitivities of the NIRS-equations from different soil types, are shifted to be close to one by the modification. The bias, which is observed for the evaluation in this case, cannot be compensated by the modification.

Conclusion

Although silicates like sand and clay are transparent in the near infrared wavelength region, different soil textures have a considerable impact on the measured NIR-spectra. One possibility, how correct predictions can be made, is the use of a calibration dataset, in which the same soil textures are represented like in the samples with unknown organic content. The other possibility is the use of scaling factors depending on the transparency of the soil samples, which compensate the soil texture effect, when predicting organic contents.

Experiments with a wider variety of soil textures may be helpful to determine, whether it is possible to calculate clay, silt and sand contents simultaneously with the organic material. In this case the scaling factors needed to correct the organic contents could be calculated from the same measurement and accurate predictions would be possible solely from NIR-spectra.

5 Near infrared spectroscopy (NIRS) for the characterisation of organic matter in soils with nonuniform texture

Abstract

The objective of this study was to investigate the influence of different soil texture on near infrared spectra and the development and application of calibrations on various soil constituents. The usefulness of weight scaling factors multiplied to the amount of mineral soil compartments in order to improve the accuracy of NIRS-estimations was tested. Another scope of this investigation was to evaluate the application of NIRS for the determination of different organic soil fractions.

23 soils of an incubation experiment carried out earlier were scanned with a NIR-spectrometer. PLS-calibrations were conducted to develop NIRS-equations for the estimation of the analytical data from NIR-spectra. The results show that weight scaling factors are a simple method to achieve a significant improvement in the accuracy of NIRS-based determinations of organic matter in soils. These factors compensate for the effect of different soil textures on NIR-spectra caused by the different transparency of sand, silt and clay. Due to the small number of samples and partly high correlations among the reference data, interpretations of the calibration results for smaller organic soil fractions are nearly impossible. Indirect correlations between NIR-spectra and these organic matter fractions have to be considered.

5.1 Introduction

The characterisation of decomposable organic soil compartments is important for the determination of soil fertility. Apart from the determination of organic matter content, incubation of soil samples combined with respiration measurements (Magliulo and Renella 1997; Nordgren 1988) or subsequent determination of released mineral N are common methods to gain information about organic compounds in the soil. Also various extraction methods (Dou et al. 2000; Houba et al. 1986; Keeney 1982) and electro ultra filtration (EUF) (Nemeth 1985) are used for this purpose. A further method is the physical fractionation into size and density classes (Christensen 1992; Meijboom et al. 1995). All these methods are time-consuming and require a lot of work, which reduces their potential for monitoring purposes. Frequent determinations of soil characteristics would be expensive and economically ambiguous.

In the last decades near infrared reflectance spectroscopy (NIRS) has been widely used for the determination of organic compounds of e.g. grains, dairy products, forages and pharmaceuticals. Once calibrated, NIRS is a rapid, non-chemical, non-destructive technique for the simultaneous determination of various, mainly organic constituents in a wide variety of samples.

There are only very few applications of NIRS in the routine analysis of soils (Meyer 1989), but its potential for soil analysis has been lined out in many publications (Couillard et al. 1997; Fox et al. 1993; Krischenko et al. 1991; Salgó et al. 1998). A serious difficulty is the influence of soil minerals on the NIR-spectra. These minerals, which are mainly silicates, do not absorb NIR-radiation (Workman 1998), but they affect the scattering conditions in the sample. Several authors have pointed out, that the accuracy of NIRS-calibrations for soil analysis is severely reduced, when applied to datasets containing soils with different textures, i.e. different contents of the compartments clay, silt and sand (Krischenko et al. 1991; Morra et al. 1991; Zwanziger and Förster 1998).

For artificial mixtures of soil separates Couillard et al. (1996) have shown, that this negative effect caused by different soil textures can be compensated to some degree by the introduction of weight scaling factors for the reference values of analyte contents. In chapter four the application of these weight scaling factors to the sand and clay content of soils with two different textures gave an improvement in predictive accuracy for the cellulose content in these samples.

The aim of this study was to show, if the use of weight scaling factors has a generally positive effect on the calibration on various soil constituents in a dataset with a high natural variation in clay, silt and sand content. Another goal was to investigate, if the results of $CaCl_2$-extractions and EUF-results can be estimated by NIRS. In addition to these values, pool sizes in these samples as estimated by NCSOIL (Molina et al. 1997) from results of incubation experiments were tested as reference values for NIRS.

5.2 Material and methods

Soil samples from an earlier incubation experiment were the basis for our investigations. This incubation experiment is described in detail elsewhere (Appel 1998, 1999). 23 soils from different locations had been prepared for incubation in six replicates. After drying and rewetting three replicates of each soil were prepared. Six polyethylene pots per replicate (one per sampling date) were filled with 200g soil and incubated at 25°C under aerobic conditions.

Subsamples of all replicates were used for NIRS-analysis. All 138 samples, dedicated for NIRS-analysis were dried at 40°C, milled to pass a 1mm-sieve and then scanned with a NIRSystems 5000 spectrometer (FOSS NIRSystems Inc., Silver Spring, USA) in the wavelength range from 1100 to 2500 nm in 2 nm-steps. Also, subsamples of the end of the incubation process were measured in the same way. Spectra of the three replicates were averaged for

further calculations. At the beginning of incubation, dried and rewetted soils and those without this treatment are equal when prepared for NIRS. Also the reference values are still the same. Hence, we averaged all the six replicates from the beginning. This gives a total number of 23 spectra for the start and 46 spectra for the end of the incubation.

The spectra were scatter corrected as described by Barnes et al. (1989). Calibrations were developed by using the first derivative of the original spectra. For the calibration process the partial least squares (PLS) (Høskuldsson 1988; Wold et al. 1983), algorithm in the modified form presented by Shenk and Westerhaus (1991) was used. When developing a calibration, cross validations (Snee 1976; Stone 1974) were calculated firstly to get a measure for the quality of the calibration and secondly to limit the number of parameters (PLS-factors) in the calibration. This way we were able to avoid “over-fitting” in the calibration process.

Using results from sampling dates 1 to 5, the mineralisation process was simulated with NCSOIL. Simulation parameters as reported by Nicolardot and Molina (1994) were used. The sizes of the two major organic pools in NCSOIL, the more rapidly decomposable pool 2 and the recalcitrant pool 3, were calculated for the starting date. Further details of these calculations have been described by Appel (1999) These pool sizes were then used for calibration purposes. Also, the mean mineralisation rate for the whole incubation period was used as a parameter to calibrate on.

Prior to incubation $CaCl_2$-extractions and EUF measurements were made with subsamples of the 23 soils (Appel et al. 1995). Both methods were conducted at two temperature levels, namely 20°C and 80°C. The N-fractions in mg N kg^{-1} dry soil determined by these methods also were used as reference values for NIRS-calibrations. Other reference values were the soil pH and the soil content of organic C and organic and mineral N.

The amounts of sand, silt and clay in the soil were mainly used for the determination of the weight scaling factors, but also calibrations for these contents were calculated. The proportions of sand, silt and clay in the 23 soils are given in Table 12. Also the contents of organic C and N as well as the mean mineralisation rate are shown.

The last column of Table 13 contains the values for optical density. This value is calculated from the contents of sand, silt and clay. These contents are multiplied by the weight scaling factors (sand: 0.15; silt: 0.39; clay: 1.52) as given by Couillard et al. (1996). The three products are then summed up to the optical density. Since the content of organic matter was rather small compared to the mineral soil compartments, we did not take it into account for the calculation of the optical density.

All reference values were divided by the optical densities of the samples in order to represent the effect of different transparency of the soils. Calibrations were calculated for the modified as well as for the unmodified reference values. In practical applications, the analytical data as estimated by NIRS-equations based on such modified values have to be multiplied by the optical densities of the samples.

Table 12 *Features of the 23 different soils used in the incubation experiment described by Appel (1998). 'Optical density' means the sum of the mineral soil separates each multiplied by the factor given by Couillard et al. (1996).*

Sample Number	Organic N [%]	Organic C [%]	Clay [%]	Silt [%]	Sand [%]	Min. rate [mg N kg^{-1} d^{-1}]	Optical density
1	0.120	1.24	18.6	50.6	30.8	0.91	52.6
2	0.167	1.57	17.6	69.0	13.4	0.76	55.7
3	0.159	1.66	13.4	64.8	21.8	0.98	48.9
4	0.167	1.91	5.1	24.8	70.1	0.96	27.9
5	0.146	1.52	4.5	24.2	71.3	0.89	27.0
6	0.152	1.44	23.7	72.1	4.5	0.69	64.8
7	0.153	1.30	24.6	68.6	6.8	1.01	65.2
8	0.157	1.40	22.4	73.1	4.5	0.93	63.2
9	0.156	1.30	21.2	74.6	4.2	0.94	61.9
10	0.246	2.42	24.1	71.4	4.5	0.94	65.2
11	0.111	1.16	13.5	32.1	54.4	0.46	41.2
12	0.209	1.80	33.0	53.6	13.4	0.41	73.1
13	0.087	0.87	16.8	70.7	12.5	0.20	55.0
14	0.196	1.95	26.2	52.4	21.4	0.54	63.5
15	0.233	2.13	22.9	68.1	9.0	0.74	62.7
16	0.133	1.24	18.8	76.3	4.9	0.56	59.1
17	0.096	1.03	14.0	77.6	8.4	0.55	52.8
18	0.055	0.80	8.6	15.5	75.9	0.41	30.5
19	0.095	1.05	14.2	73.4	12.4	0.55	52.1
20	0.114	1.17	23.1	47.9	29.0	0.46	58.1
21	0.122	1.26	13.6	50.6	35.8	0.56	45.8
22	0.135	1.17	22.4	71.5	6.1	0.87	62.8
23	0.108	1.05	15.5	79.5	5.0	0.63	55.3

5.3 Results

First, we concentrated on the 23 spectra at the beginning of the incubation in order to test whether these spectra allow predictions of the reference values The major problem about the dataset in this investigation is the small number of samples.. So for the development of a first set of NIRS-equations we used all 23 soils for calibrations. The results of these calibrations in terms of coefficient of determination for calibration and cross validation are presented in Figure 14 and Figure 15, respectively. Up to five PLS-factors were used for the explanation of the variation in analytical data.

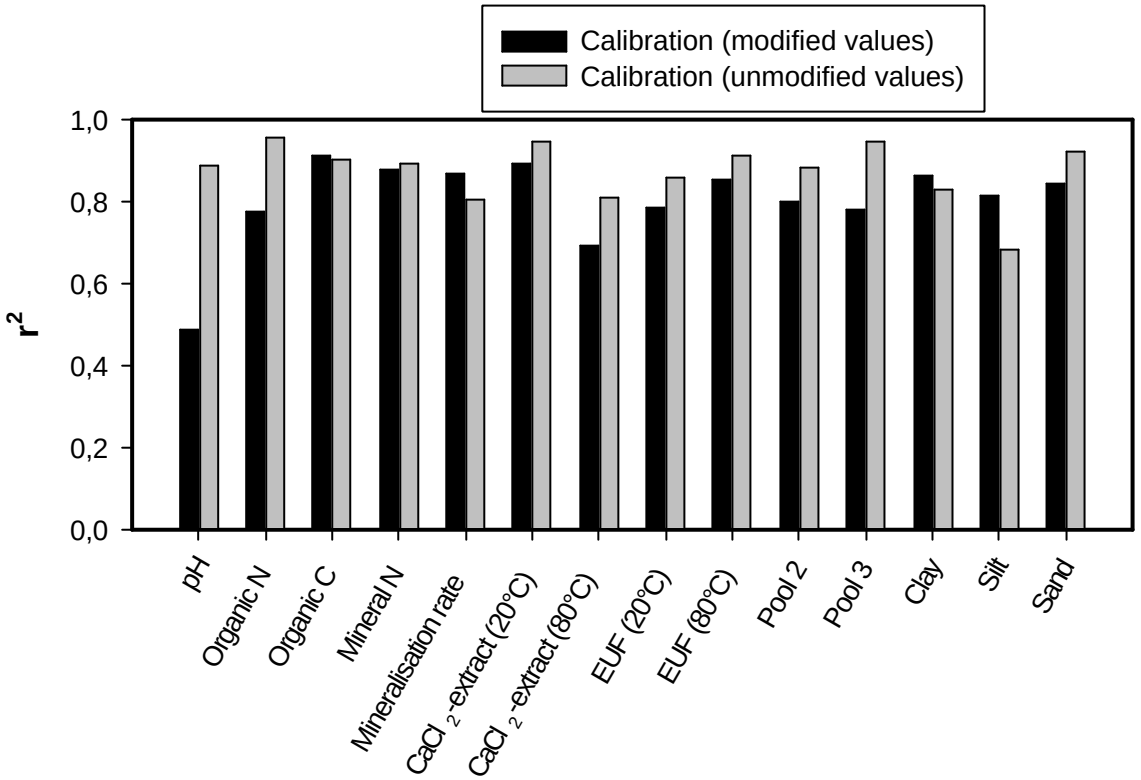

Figure 14 Coefficients of determination for calibrations upon unmodified and modified reference values using all 23 soils for the calibration

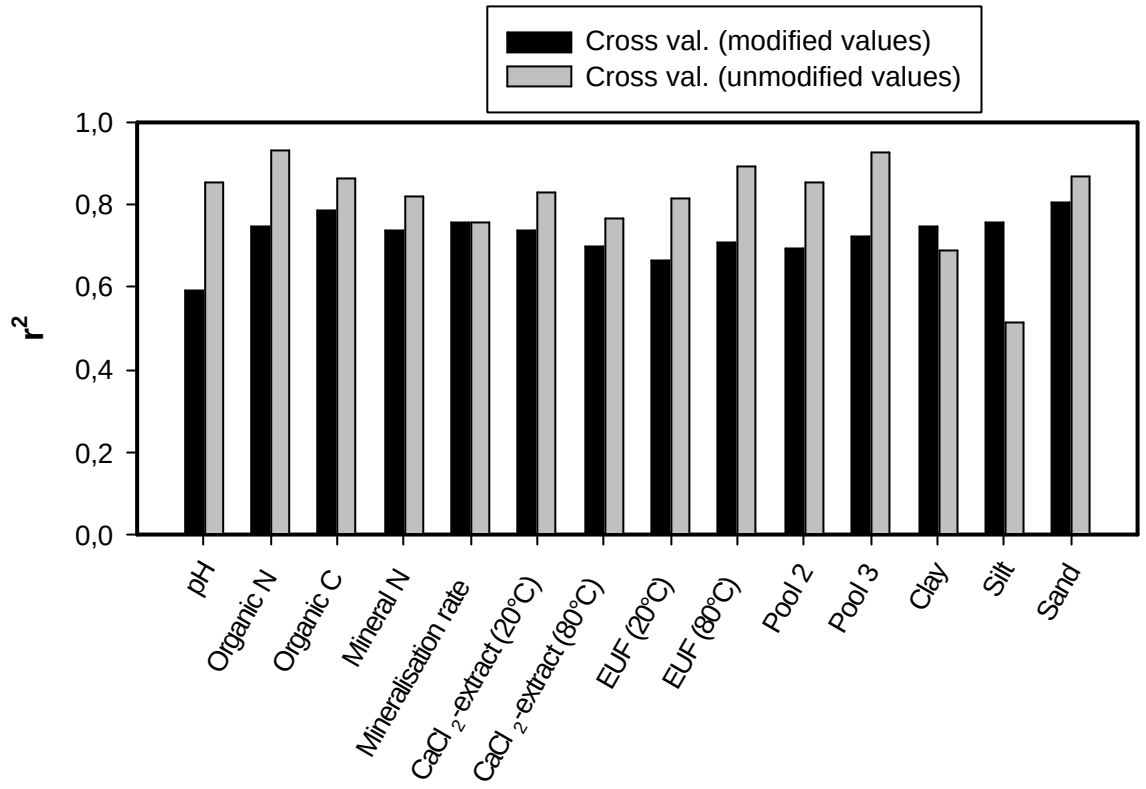

Figure 15 *Cross-validation results in terms of r^2 for calibrations upon unmodified and modified reference values using all 23 soils for the calibration*

The improvement achieved by the use of weight scaling factors to modify the reference values is obvious for most of the data depending on organic matter contents. There was no improvement in the determination of mineral soil compartments. These effects can already be seen from the coefficients of determination (Figure 14), but they are still more apparent in respect to the cross-validation results (Figure 15). Especially calibrations for silt content were much better without modifying the reference data.

These results are even more obvious, when only 12 soils were used for the calibration and the remaining 11 soils for evaluation. In this case only up to three PLS-factors could be used in the regression. The coefficients of determination of the evaluation dataset is presented in Figure 16. The values

are very low for the unmodified reference values, whereas the differences to the calibration with modified values is bigger in this case. Apart from organic C and N contents those parameters gave good evaluation results, which can be associated with the mineralisable organic matter, not only the net N mineralisation rate, but also values from $CaCl_2$-extraction, EUF and NCSOIL pool sizes. Again, the calibrations for mineral soil compartments are an exception.

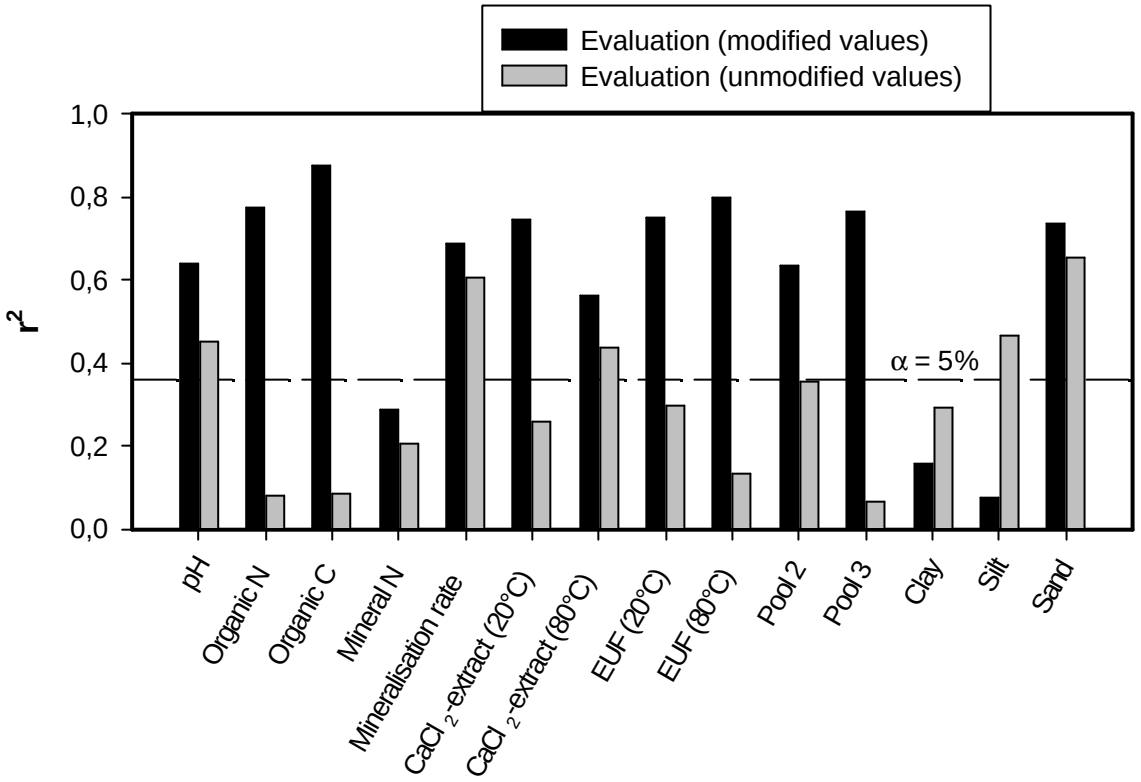

Figure 16 *Coefficients of determination for the evaluation of calibrations upon unmodified and modified reference values using all 12 soils for the calibration and 11 soils for evaluation. Coefficients of determination above 0.36 and 0.72 were significant on a level of $\alpha = 5\%$ and $\alpha = 0.1\%$, respectively.*

The results for the organic compartments have to be regarded critically due to the possibility of correlation to the total contents of C and N. The higher the correlation to one of these two values, the higher is the probability for indirect correlations as a basis for the calibration. The coefficients of correlation among the reference data except for sand, silt and clay are given in Table 13.

The spectra of the incubated samples were used to calculate spectral differences before – after incubation (see also chapter 2). The difference spectra of the dried-rewetted samples on one side and the samples without this treatment on the other did not show significant differences (data not shown). The difference spectra were also used for the calibration of the mineralisation rate for they are the direct spectral counterparts to the chemical turnover during incubation. These calibrations did not provide any new information (data not shown).

Table 13 *Coefficients of correlation between reference values of the 23 soils (bold numbers: significant correlation on the 0.1%-level)*

Reference value	Organic C	Organic N	Mineral N	pH	Miner. rate	$CaCl_2$ 20°C	$CaCl_2$ 80°C	EUF 20°C	EUF 80°C	Pool2	Pool3
Organic C	—	**0.953**	0.445	0.248	0.437	**0.703**	**0.725**	**0.687**	**0.774**	**0.785**	**0.941**
Organic N	**0.953**	—	0.447	0.463	0.45	**0.693**	**0.811**	**0.822**	**0.84**	**0.81**	**0.994**
Mineral N	0.445	0.447	—	0.219	**0.856**	**0.821**	0.418	0.405	0.488	**0.755**	0.369
pH	0.248	0.463	0.219	—	0.417	0.301	**0.694**	**0.673**	**0.65**	0.503	0.443
Miner. rate	0.437	0.45	**0.856**	0.417	—	**0.794**	0.61	0.441	0.598	**0.849**	0.355
$CaCl_2$ 20°C	**0.703**	**0.693**	**0.821**	0.301	**0.794**	—	0.617	**0.696**	0.626	**0.849**	0.632
$CaCl_2$ 80°C	**0.725**	**0.811**	0.418	**0.694**	0.61	0.617	—	**0.772**	**0.908**	**0.835**	**0.775**
EUF 20°C	**0.687**	**0.822**	0.405	**0.673**	0.441	**0.696**	**0.772**	—	**0.785**	**0.692**	**0.814**
EUF 80°C	**0.774**	**0.84**	0.488	**0.65**	0.598	0.626	**0.908**	**0.785**	—	**0.844**	**0.804**
Pool2	**0.785**	**0.81**	**0.755**	0.503	**0.849**	**0.849**	**0.835**	**0.692**	**0.844**	—	**0.739**
Pool3	**0.941**	**0.994**	0.369	0.443	0.355	0.632	**0.775**	**0.814**	**0.804**	**0.739**	—

5.4 Discussion

As an answer to one of the major goals of this investigation, the results show a considerable improvement achieved by the use of modified reference values according to Couillard et al. (1996). This can be seen from calibrations with all 23 soils as well as from the evaluation results, when the dataset is divided for calibration and evaluation. This indicates, that the weight scaling factors are compensating to some degree the effects of different soil textures on the spectral information of soil organic matter.

Contrary to the improvement achieved for the calibration on organic fractions in the soil, the results for the mineral soil compartments clay and especially silt are better without modification of the reference data. This does not contradict to the physical model presented in chapter 4. Assuming that scattering at the surface of silicate particle is the major reason for the effect of soil mineral composition on the NIR-spectra, it can be concluded, that the specific surface area (SSA), i.e. the silicate surface area per unit volume in the soil, regulates the transparency of the sample. Sandy samples with lower SSA are more transparent and thus more information about the organic matter can be detected by NIRS than in soils high in clay content. According to this model, information about the distribution of the silicate surfaces cannot be gained from NIRS. This means that NIRS will not differentiate between samples with the same SSA, i.e. silt can have the same effect on the NIR-spectrum as a mixture of clay and sand. Hence only sandy soils with a low SSA, which provide much information about their organic contents to NIRS, are not likely to be mixed up with other soil textures. Better calibration and evaluation accuracy for sand content than for silt and clay are consequence in this model.

Another scope of this study was to investigate the usefulness of NIRS to estimate the size of organic N fractions in the soil determined by $CaCl_2$-extraction, EUF and NCSOIL-simulation. Due to the small number of samples in this investigation and the correlation of these fractions to the total contents of

organic C and N, which can be assumed to have a higher influence on the NIR-spectra, clear answers on these questions would be very ambiguous, but the results of our calibrations do not contradict to the possibility to estimate these fractions by NIRS. The good calibration and evaluation results for the NIRS-estimation of the net N-mineralisation rate cannot be explained simply by their correlation to total organic C and N contents. In this case the fraction of variance explained by NIRS is significantly higher than the correlation coefficients between N-mineralisation rate and organic C and N content (Figure 17, Table 14).

Conclusion

This investigation clearly shows, that the negative influence of different soil textures on the NIRS-estimation of organic soil fractions can be significantly reduced by mathematical pre-treatment of the reference data. Weight scaling factors multiplied with the amount of sand, silt and clay in the soil samples are a simple possibility to improve the accuracy of NIRS-estimations. Investigations like this one, but with a larger number of soil samples would help to get more reliable information about the usefulness of NIRS to estimate the size of different fractions of the soil organic matter.

6 Final discussion

Most N fertilizer recommendations take into account only the crop's nitrogen demand and the mineral nitrogen content in the soil. Mineralisation from decomposing organic matter is often not considered and leads to a mismatch between supply and demand and in the consequence to environmental harmful N-leaching. This problem can only be reduced by quantitative estimations of the net N-mineralisation rates in the soil. One of the two main components for accurate estimations is a mathematical model of the mineralisation processes in the soil, which has to take into account the influence of environmental factors as temperature and water content as well as the decomposability of different organic compounds and interactions caused by the coupling of carbon and nitrogen cycles. The other major prerequisite is an analytical method for the fast and effective characterisation of the soil organic matter with a special focus on rapidly mineralisable fractions. This study was conducted to investigate the suitability of NIRS for these soil analyses. Therefore, soil samples with different textures and varying content and composition of organic material were examined by NIRS and by reference methods.

NIRS-equations, by which constituents of samples can be determined from their NIR-spectra, are derived by calculating calibrations using samples with analyte data known from a reference method. Since multiple linear regression methods are used for such calibrations, linearity between the reference values and the spectral features is an important condition for accurate NIRS-equations.

Two principle sources for non-linearities exist in the relationship between NIR-spectral features and reference data of a sample: On one side there can be nonlinear relations between chemical concentrations and functional reference data, and on the other side non-linearities may be found in the relationship of NIR-spectra and the chemical concentrations, which are responsible for the spectra. In this study it was discovered that both kinds of non-linearities are likely to appear, when NIR-spectra are used for estimations in carbon or nitrogen mineralisation studies.

The first kind of non-linear relationships appears, when e.g. net N mineralisation rates are used as reference data in NIRS-calibrations, as shown in chapter 2. Causes for these non-linear effects are found in the coupling of carbon and nitrogen turnover in the soil. When net N mineralisation depends on both organic carbon compounds as energy sources and organic nitrogen sources, which both show individual effects on the NIR-spectra, linear correlations between spectral changes and changes in net N mineralisation rates can only occur in one of the following cases: Either only one source, carbon or nitrogen, limits the decomposition process in the soil or there is a linear relationship between the changes in both sources. These preconditions can usually not be assumed to be met in natural soils. NIRS-calibrations built up with a dataset, which fulfils these conditions, will fail, when the resulting NIRS-equations are applied to soils with other compositions of mineralisable compounds.

In order to avoid the problems associated with the use of net N mineralisation rates as reference data in NIRS-calibrations, these values have to be converted to quantities, which are linearly correlated to the concentration of decomposable organic compounds in the soil. Therefore, mathematical simulation models are needed for the determination of organic pool sizes in the calibration samples. If interactions between carbon and nitrogen pools affect the course of net mineral nitrogen content in the soil, these effects have to be modelled from data such as CO_2-respiration or released mineral nitrogen measured regularly during incubation experiments. There are several well-evaluated models, which seem to be suitable for this purpose (Powlson et al. 1996). In this case the calibration process is connected with time-consuming and labour-intensive incubation experiments followed by model calculations, but once calibrated, NIRS provides the information about the pool sizes in a fast scan without the need of chemical pre-treatment of the examined sample. Thus, NIRS might be a valuable tool for the prediction of N-mineralisation in soils, which contain large amounts of added organic material rich in nitrogen.

Especially vegetable growing is associated with high amounts of N-containing crop residues. Sequences of several crops each with a short time of cultivation on one hand require fast estimations of the forthcoming development of mineral N content in the soil, on the other hand residues of several succeeding crops can be present in the soil, which makes estimations of the decomposable organic material quite difficult. Hence, these horticultural soils appear to be an area, in which the use of NIRS for the estimation of net N-mineralisation might be very useful.

The procedure of combining incubation experiments with mathematical modelling of decomposition processes has been used in the investigation described in chapter 3. It is shown that the content of cellulose remaining in the soil as simulated by NCSOIL could be estimated from NIR-spectra quite accurately. The spectral peaks of pure cellulose were compared with the spectral features, which have a very high importance in the determination of cellulose content according to the developed NIRS-equation. This comparison shows, that not indirect correlations depending on e.g. the mineral N content in soil, but the spectral information of actual cellulose content is responsible for the good calibration and evaluation results. This indicates, that the combination of NIRS with mathematical modelling of the mineralisation process can be a suitable tool for the description of the chemical turnover in soils. The mathematical modelling is needed for the conversion of non-linear relationships between spectra and functional reference data into linear relations between spectra and chemical concentrations in the soil. Cellulose as one of the most important organic compounds in the biosphere is a major energy source for the microbial community in all kinds of soils and thus deserves special attention when mineralisation processes in the soil have to be described. Hence, the positive results achieved in this investigation pronounce the possibility to determine organic fractions in the soil, which are quite important for decomposition processes.

The other source of non-linearities is the relationship between spectral features and chemical concentrations. Linearity between the spectra and the concentration of the absorbing chemical compound is the basis of NIRS-applications and assumed by the use of Lambert-Beer's law of the absorption of electromagnetic radiation. This law is an approximation of the scattering processes in absorbing media and can also be used for the NIRS-analysis of soil samples as long as the effect of soil minerals, mainly silicates, on the scattering conditions is equal for all samples. In chapter 4 and 5 it is shown, that this prerequisite for linearity is hurt by varying soil textures, i.e. varying amounts of sand, silt and clay.

Due to the large amount of work and time, which has to be invested into the development of NIRS-equations, economic considerations do not allow individual calibrations for a wide variety of soil types. So the effect of different textures has to be reduced by other, simpler methods. A physical model presented in chapter 4 is able to explain the textural effect qualitatively. A quantitative estimation of this effect is given by Couillard et al. (1996). The application of weight scaling factors, derived from artificial mixtures of soil separates in their study, is examined for natural soils in chapter 5. It is shown, that this method is able to compensate for the textural effect to a considerable degree.

These weight scaling factors have to be multiplied to the amount of sand, silt and clay in order to represent the different transparency of these minerals. Therefore, the amount of these minerals have to be determined, when this mathematical pre-treatment is used. Since soil types do not change on the time-scale, which is important for biological processes in the soil, and their spatial variation is mostly far smaller than the variation of land use and organic composition of the soil, the additional determination of the soil mineral compartments is acceptable from the economic point of view.

A further simplification might be possible if the textural features of the soil, which have an influence on its NIR-spectrum can be derived from the spectrum itself. From the results presented in chapter five it can be seen that there exist difficulties in the determination of sand, silt and clay content from NIR-spectra, but according to the physical model described in chapter four, only information about the specific surface area (SSA) of the soil is needed to assess the influence on the NIR-spectra. Hints for the suitability of NIRS to determine the SSA already exist in the literature (Ben Dor et al. 1991). Assuming the suitability of NIRS to determine the SSA as well as the soil organic components, the additional input of soil texture information would not be necessary any more. Further work is needed to investigate the feasibility of this method.

Since many mathematical models exist, which describe the chemical turnover in the soil (Plentinger and Penning de Vries 1996), further studies might also be concerned with the search for models, which can be combined with NIRS more efficiently. Most models divide the soil organic matter in more rapidly decomposable or recalcitrant pools, but the fractionation is quite different in the individual models (Molina and Smith 1998). NIRS-calibrations are less difficult, if reference data can directly be related to concentrations of individual compounds or chemical groups like carbohydrates or proteins, since important spectral features of such groups are likely to be very similar. Thus, very complex models might seem to be appropriate to meet the demands of NIRS. Some models use a large number of pools with sophisticated interrelations among these pools (Grant et al. 1993), but many of these pools such as several pools of microbial biomass are very small and concentrations of organic compounds characteristic for these pools are extremely low in soil samples. Thus, chances for a NIRS-based determination of these pool sizes are questionable.

An even more serious drawback of models using a lot of different pools is the extreme difficulty to extract reliable, reproducible pool sizes from e.g. incubation experiments for such a model. Hence, NIRS-calibrations for these pool sizes are very problematic.

Very simple models on the other hand are easier to parameterise using incubation results, but if they do not account for the major interactions of easily accessible and recalcitrant pools of carbon and nitrogen, they are not able to convert mineralisation data to quantities, which are linearly correlated with chemical concentrations in the soil. So the models suitable for a combination with NIRS have to be a compromise having regard to both kinds of arguments.

Apart from focusing on sizes of model pools as reference data in NIRS-calibrations, the determination of some important organic compounds such as proteins, cellulose or lignin in soil samples might help to estimate the chemical turnover during decomposition processes in the soil. Several studies show the suitability of NIRS to keep track of such decomposition processes in pure litter samples (Couteaux et al. 1998; Gillon et al. 1999; McLellan et al. 1991). The usefulness of NIRS for the determination of cellulose has been pointed out in chapter 3. The accuracy of this kind of calibrations can be improved, if the NIR-spectrum of the pure analyte is known. Algorithms utilising this spectral information of pure analyte material in the calibration process have been developed (Schönkopf et al. 1991).

Since chemical analyses of decomposing plant material as a small fraction in soil samples are very difficult, further experiments with a well-known variation of different organic soil amendments appear to be useful. This might be a promising way for a more detailed evaluation of the potential of NIRS for the estimation of carbon and nitrogen mineralisation in soils.

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