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Coupling pyrolysis with mid-infrared spectroscopy for the characterization of soil organic matter

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Dedicated to

Almighty God

&

Samkia, Bidbungha and Bisona

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Abbreviations

C: Carbon

DRIFTS: Diffuse reflectance Fourier transform spectroscopy

DSC: Differential scanning calorimetry

FTIR-EGA: Fourier transform infrared-evolved gas analysis

GHG: Greenhouse gas

MIRS: Mid-infrared spectroscopy

NMR: Nuclear magnetic resonance

NPA: Normalized peak area

OC: Organic carbon

PA: Peak area

PLSR: Partial least squares regression

Py-MIRS: Pyrolysis-mid-infrared spectroscopy

Py-GC/MS: Pyrolysis-gas chromatography/mass spectroscopy

SQA: Soil quality assessment

SOC: Soil organic carbon

SOM: Soil organic matter

TOC: Total organic carbon

TN: Total nitrogen

Chapter 1 General Introduction

1.1 Background

Over the past few decades, numerous studies have laid major emphasis on soil organic matter (SOM) because of its ability to sequester atmospheric carbon dioxide (CO_2) and its beneficial effects on soil fertility which ensures sustainable crop productivity of agro-ecosystems (Lal, 2004). It has been clearly established that agricultural management practices (e.g. fertilization) and land use lead to variations in the abundance and composition of SOM, subsequently affecting the main processes like microbial activities and nutrient release (Vázquez et al., 2016) occurring within the terrestrial ecosystem. In an effort to contribute to a deeper understanding of these impacts and the accompanying mechanisms which have profound effects on SOM dynamics and vice versa, there has been an increasing focus on developing and advancing methodological approaches which permit a better understanding of SOM composition and dynamics.

Due to a growing need to enhance the understanding and characterization of SOM, a lot of research has been conducted and a wide range of analytical techniques have been developed (Kögel-Knabner, 2000). In spite of the considerable advances made towards the establishment of improved routine techniques for SOM studies, important questions remain concerning the ability of these techniques to completely capture the SOM continuum (Plante et al., 2009).

This study sets out to address this knowledge gap caused by the limited ability of the existing techniques to contribute to unraveling the heterogeneous and complex structure of SOM to provide in-depth knowledge into the manifold constituents of SOM by developing new techniques, i.e. pyrolysis coupled with mid-infrared spectroscopy (Py-MIRS) together with diffuse reflectance Fourier transform mid-infrared spectroscopy (DRIFTS) to fingerprint the molecular composition and reveal changes in SOM chemistry in bulk soils from long-term experiments under different agricultural managements practices and land use.

1.1.1 SOM in agricultural soils

SOM is described as a complex, multi-structured, multicomponent pool of above and belowground decomposing plant and animal litter as well as microorganisms at different stages of decomposition and represents the largest global reservoir of terrestrial organic C (OC) (Paul et al., 2015; Tfaily et al., 2015). The soil organic C pool contains an estimated 1505 petagrams (Pg) C (Batjes, 2014) in the upper 100 cm of soil, while the deeper soil horizons from about 150 to 3000 cm contain an estimated 1778 to 3000 Pg C (Batjes, 2014; Lal, 2018) accounting for about 3.5 times the amount C of in vegetation (450-650 Pg C) and about 2.5 times the amount of atmospheric C (589-829 Pg C) respectively (Ciais et al., 2013). Variations in the SOC stocks in these pools as well as corresponding C fluxes are known to be significantly altered by anthropogenic activities such as land use conversion and agricultural management practices.

SOM composition, abundance, and global distribution affect the relevant processes within the ecosystem and also contain a great wealth of information on vegetation, climate, and parent materials (Batjes, 2014; Paul, 2016). SOM also controls major biological, physical, and chemical soil properties like aeration, nutrient retention, fertility and water infiltration, and major biochemical cycles, which have an impact on soil quality (Chenu et al., 2019).

About 1.4 billion hectares of the global land surface area is agricultural soils with arable land covering about 10% of this area (Jenkinson et al., 1991). Variations in the SOC stocks are to an extent largely influenced by agricultural management practices and land use. Decreased SOC stocks result from perturbation caused by tillage and the conversion of forests and grasslands to arable land while increased stocks are often brought about by the implementation of sustainable management practices such as the addition of organic amendments like manure and compost (Lal et al., 2015). The quantity and quality of organic amendments play a crucial role in the accumulation and dynamics of SOC (Jacobs et al., 2020). SOC dynamics play a pivotal role in the size of the SOC reservoir, soil functions, global C fluxes, and consequently the productivity and sustainability of the terrestrial ecosystem.

SOM has been allocated into a series of fractions or conceptual “pools” based on their decomposition rates or mean residence time (MRT) (Luo et al., 2014). These conceptual pools include the labile pool with a rapid turnover rate and high sensitivity to disturbance, and one to several recalcitrant pools with a slower turnover rate (Cambardella, 2005; Gregorich et al., 1994). One of the most promising approaches to clearly understand SOM dynamics is the use of long-term incubations that reveal SOM pools and their turnover rates. In addition, SOM models such as CENTURY, DIASY, and ROTH C have been frequently utilized to explore the dynamic changes of SOM and its effect on C fluxes and climate change.

SOM management has been proposed as a plausible climate change mitigation strategy due to its ability of the soil to sequester carbon and reduce atmospheric C concentrations. The amount of C stored in soil is highly dependent on the balance between input from plant and animal residues and C losses occurring through decomposition and erosion respectively (Ghimire et al., 2019; Vázquez et al., 2016). It has been documented that agricultural management practices like fertilization (organic and mineral), tillage as well as land use and related changes (e.g. grassland to arable land and vice versa) have profound effects on SOC stocks and inevitably on SOM quantity, quality, and soil productivity (Bai et al., 2018; Söderström et al., 2014). Land use change from grassland to arable land is known to deplete SOC stocks which are often exacerbated through mechanisms like erosion and management practices with little or no return of organic residues (Söderström et al., 2014). The increasing interest in SOM quality, therefore, underscores the need for the meaningful integration of management practices and land use that can restore or slow down the depletion of SOC stocks in agricultural soils and improve physical, chemical, and biological soil properties. Such practices include diverse crop rotations, tillage modifications, and enhanced organic amendments (Söderström et al., 2014).

Toosi et al. (2017) reported that fewer studies have been carried out to investigate the effects of management and land use on SOM composition with inconsistent findings in contrast to the widely studied effects on SOC stocks. These inconsistencies can be ascribed to the large size of the SOM pool and its functional and structural heterogeneity; hence the difficulty to detect changes in response to management and land use (Bünemann et al., 2018; Haynes, 2005). A plethora of analytical techniques are available and have been used to assess the SOM bulk chemistry (see section 1.2).

1.1.2 SOM stabilization mechanisms

The effectiveness of developing sustainable management practices and land use to preserve SOM quality in part depends on understanding SOM stabilization mechanisms which are key elements to reliably assess SOM dynamics. Stabilized SOM, also referred to as protected SOM or humus has a lower potential to be lost through respiration, leaching, or erosion compared to labile or less stable SOM. It has been proposed that SOM can be stabilized through the following mechanisms; (1) physical stabilization, (2) chemical (organo-mineral association) stabilization, and (3) inherent recalcitrance of SOM (biochemical protection) (Lützow et al., 2006; Six et al., 2002; Sollins et al., 1996). The occurrence and effectiveness of these mechanisms depend on factors like soil type and texture, mineralogy, and management practice.

Physical stabilization

SOM is physically protected from decomposition or made spatially inaccessible through the following processes: occlusion by aggregation, intercalation within phyllosilicates, hydrophobicity, and encapsulation in organic macromolecules (Lützow et al., 2006). Several lines of evidence suggest that the principal factor controlling the processes of physical SOM protection is its ability to associate with clay and silt particles (Hassink, 1997). Occlusion of SOM occurs either through the establishment of physical barriers that limit the accessibility of the decomposers and enzymes to organic substrates or the diffusion of oxygen (Plaza et al., 2013; Six et al., 2002). SOM can also be intercalated between layers of phyllosilicates only under acidic conditions (pH <5) with minimal dissociation. However, the characterization and quantification of intercalated OM are still unsatisfactory as there exist no specific routine techniques for its determination (Leifeld and Kögel-Knabner, 2001). While the labile OM can be protected from decomposition through encapsulation in organic macromolecules, there is limited evidence for the occurrence of this phenomenon in soils (Lützow et al., 2006). Physical protection of SOM is dependent on the level of aggregation which has been shown to be greater within micro-aggregates than macro-aggregates (Plaza et al., 2013).

Chemical stabilization

Chemical stabilization involves the intimate association of OM with minerals that inhibit degradation by decomposers and enzymes (Plaza et al., 2013; Six et al., 2002). The mechanisms usually involved with this association include ligand exchange, polyvalent cation bridges, and interactions like hydrophobic interactions (Lützow et al., 2006). It has been reported that metal ions like Ca^{2+}, Al^{3+}, and Fe^{3+} have the potential to bind with SOC (Baldock and Skjemstad, 2000), however, there is little information available on the effects of metal ion binding to SOC on its stability. It has been proposed that chemical protection is responsible for C associated with organo-mineral complexes and the amount of protection increases with increasing soil silt and clay content (Guggenberger et al., 1999; Hassink, 1997). Lützow et al. (2006) proposed three reasons why chemically stabilized OM is protected against decomposition and they include (i) decreased access for the decomposers and their enzymes, which control further food web interactions, (ii) reduced diffusion of enzymes into the intra-aggregate space; and (iii) restricted aerobic decomposition due to reduced diffusion of oxygen.

Biochemical stabilization

Stabilization of SOM by biochemical recalcitrance is achieved through the inherent chemical structure of the compounds (Krull et al., 2003). It has been proposed that chemical recalcitrance can either be a property of primary molecular structure, e.g. aromatic structures, or it can result from changes induced by decompositional processes like condensation reactions in the soil environment (Krull et al., 2003; Six et al., 2002). Chemical recalcitrance thus can be divided into primary recalcitrance (involving plant litter and rhizodeposits) and secondary recalcitrance (involving charcoal, microbial, fungal, faunal, and extracellular formations) (Lützow et al., 2006; Mutabaruka et al., 2007). Although chemical recalcitrance as suggested by Krull et al. (2003) is among the several mechanisms by which SOC can be protected for long periods, recalcitrance exhibited by some biomolecules does not necessarily convey inherent stability but may depend on whether a particular turnover time of compounds is considered as stable and if the recalcitrance of the same compounds vary in different environments (Torn et al., 2009).

Just as physical mechanisms, chemical, and biochemical mechanisms account for greater stability of SOM, while destabilization mechanisms render SOM susceptible to decomposition (Sollins et al., 1996). Destabilization is often thought to occur alongside stabilization as it is considered that destabilization may generate products that are more stable than the original compound or have the potential to be stabilized (Wolters, 2000). Destabilization mechanisms promote soil disturbances like tillage, erosion, and bioturbation that expose SOM to the elements and also foster a physical environment that promotes microbial and meso-faunal degradation of SOM (Torn et al., 2009). Substrate degradation from either biotic or abiotic sources like microbial degradation (Cairney and Burke, 1998) and photo-degradation (Zepp et al., 2003) respectively also serve as a mechanism for destabilization as its by-products are labile (Torn et al., 2009).

1.2 SOM characterization

SOM is dominantly composed (about 58%) of C and performs several physical, chemical, biological, and economic functions (e.g. energy and nutrient source, C sequestration, improving soil structure) (Ondrasek et al., 2019) in addition to providing a wide array of ecosystem services. SOM bulk chemistry is a very dynamic soil property and its dynamic and stabilization are strongly altered by physical, chemical, and/or biochemical protection mechanisms.

As previously mentioned, SOM has been widely acknowledged to have a tremendous impact on soil physical, chemical, biological, and chemical properties and as a result, exerting a strong influence on agricultural sustainability and climate change mitigation. As opposed to earlier studies on SOM which focused on investigating either the chemical structure or the biological activity of SOM, present-day research has focused on bridging this gap by jointly investigating both facets (Poirier et al., 2005). This is been fueled by the recognition that SOM chemical composition and to a great extent, microbial activity significantly influences the decomposition rates and shifts in SOM dynamics and stabilization. Hence, the in-depth characterization of SOM is essential to understand its dynamics, C cycling in agroecosystems, and determine the overall quality of soils. Soil fertility and quality are known to be inextricably linked to the chemical composition of SOM at a molecular level.

The individual components of SOM are often divided into separate pools on the basis of stability (see section 1.1.1). It is well known that agricultural management practices and land use greatly impact SOM characteristics. Determining the structure and composition of SOM such as the presence of individual biochemical compounds of SOM e.g labile C, recalcitrant C, etc., therefore, provide a means for assessing the impacts of agricultural management practices and land use on SOM quality (Ding et al., 2006; Wander and Traina, 1996).

A plethora of analytical techniques has been developed to characterize SOM bulk chemistry (composition). The technique(s) employed in the active research of SOM is largely dependent on the goal of the research pursued as well as the scale of the study; either at a molecular or global level. It was recently established by Dignac et al. (2017) that there is a dearth of analytical techniques that can be used to effectively characterize the SOM due to its molecular complexity.

1.2.1 Chemical characterization

Chemical characterization of SOM involves degradation techniques like acid hydrolysis, oxidation, and extraction that are selective in their attack on specific molecular structures. These techniques have been applied successfully to analyze changes in the composition of plant or microbial-derived biopolymers during microbial transformation processes (Kögel-Knabner, 1995). Hydrolysis and hot-water extraction techniques provide information on the SOM components and the labile SOM in soils like microbial biomass, proteins, fats, polysaccharides, lipids, and related compounds (Paul et al., 2006; Rovira and Vallejo, 2002). Chemical oxidation is a widely popular method used to separate and distinguish between resistant and active SOM fractions (Silveira et al., 2008), using hydrochloric acid (HCl), potassium permanganate (K_2MnO_4), hydrogen peroxide (H_2O_2), sodium persulfate ($Na_2S_2O_8$) and sodium hypochlorite (NaOCl). Mikutta et al. (2005) also reported that NaOCl and $Na_2S_2O_8$ are more effective in isolating organic C than H_2O_2. A review of different SOM separation methods by Mikutta et al. (2005) found that the separation of SOM is never complete and the efficiency of separation depends largely on the reaction conditions like pH and temperature as well as the sample properties like the presence of chemically stable organic compounds.

1.2.2 Physical fractionation

For decades, SOM has been studied by fractionating soil using physical, chemical, and/or biochemical approaches into fractions and characterizing the compounds associated with the different fractions, thereby providing information on the biological, physical, and chemical attributes of SOM and SOM dynamics (Paul et al., 2006). Powlson et al. (2013) noted that physical fractionation emphasizes the role of soil minerals and soil structure in SOC turnover and relates more discretely to *in situ* SOC dynamics. Physical fractionation involves the separation of SOM into size (sand: 2000 - 0.63 μm, silt: 0.63 - 0.2 μm, and clay: <0.2 μm) and density fractions (lighter fraction: <1.6–2 g cm^{-3}, and heavier fraction: >1.6–2 g cm^{-3}) (von Lützow et al., 2007), and is achieved by applying various degrees of dispersion to break the bonds between the elements of the soil structure (Christensen, 2001). With this approach, SOM is separated into fractions with different turnover rates differentiating the more decomposed materials from amorphous humus associated with soil minerals. This differentiation emphasizes the role of soil mineralogy and structure on SOM dynamics (Christensen, 1992). Notwithstanding the accuracy of the results obtained via fractionation, these approaches have been demonstrated to be very time-consuming, laborious, and subject to loss of SOM. Findings from different studies revealed that no single fractionation technique could be employed to achieve a completely satisfactory separation of SOM into different fractions based on their turnover times. This is because SOM is a continuum and not a series of discrete fractions.

1.2.3 Spectroscopic techniques

Over the last decades, the recent upsurge in finding new and reliable routine techniques to characterize SOM has led to considerable advances in spectroscopic techniques.

Infrared spectroscopy

Infrared (IR) spectroscopy is based on the interaction of molecules with electromagnetic energy in the infrared spectral range which is divided into three main regions: near- (14000 – 4000 cm^{-1}), mid- (4000 – 400 cm^{-1}), and far-infrared (400 – 10 cm^{-1}) (Raphael, 2011). IR radiation initiates vibrational movements in molecules inducing absorption in the IR region (Dufour, 2009). This molecular vibration is often triggered by a change in the dipole moment as the absorption of energy, E, matches the vibration frequency, υ (El-Azazy, 2018). This results in absorption bands whose identification and attribution to specific organic functional groups like the aliphatic chain, hydroxyl and carbonyl groups, etc. provide information on the molecular structure of the sample under investigation

Near-infrared (NIRS) and mid-infrared (MIRS) spectroscopy have tremendous potential for the rapid and inexpensive analysis providing substantial information on organic and inorganic C in agricultural products and soils (Cécillon et al., 2009; Demyan et al., 2012; McCarty and Reeves, 2006; Mirzaeitalarposhti et al., 2015; Raphael, 2011; Tinti et al., 2015 and references therein). Research has demonstrated that the NIR region is characterized by broad superimposed and weak vibrations resulting in few and broad absorption features of NIR soil spectra (Mouazen et al., 2016). Over the last decades, the utilization of MIRS in soil studies has evolved as it has been shown to be a more reliable technique for the qualitative and qualitative analyses of SOC. Different studies have reported with evidence that MIRS is superior to and outperformed NIRS when used to investigate soil C and other soil constituents (e.g. McCarty and Reeves, 2006; Viscarra Rossel et al., 2006 and references therein). This is because the mid-infrared region is dominated by intensive vibrational frequencies related to a variety of soil chemical attributes (Calderón et al., 2011), whereas the NIRS is dominated by weaker and broader signals from vibration overtones (Ludwig et al., 2008; Nguyen et al., 1991). A number of MIRS analyses utilize reflectance methods like the diffuse and attenuated total reflectance as well as photoacoustic methods. Photoacoustic spectroscopy (PAS) is based on the change in the pressure, PAS signal caused by the thermal expansion of the sample under investigation following the adsorption of electromagnetic radiation by its molecular structures resulting in local heating of the sample and subsequent non-radiative relaxation (Volkov et al., 2020). MIRS in the diffuse reflectance mode, Diffuse reflectance Fourier infrared spectroscopy (DRIFTS), has been used to characterize, quantify SOM and its constituents in bulk soil and fractions as well as predict soil physical and chemical properties (Demyan et al., 2012; Janik and Skjemstad, 1995; Leifeld, 2005; Ludwig et al., 2008; Matamala et al., 2017; McCarty and Reeves, 2006; Mirzaeitalarposhti et al., 2016, 2015; Parolo et al., 2017; Viscarra Rossel et al., 2006). DRIFTS involves the diffusion of radiation directly reflected on the soil sample which gives rise to a specular reflection that indicates a function of the refractive index and the absorptivity of the sample (Armaroli et al., 2004). However, MIRS has a limitation in that a vibrational frequency is often assigned to more than one compound and most times there is an overlap between vibrational frequencies of different compounds, i.e. organic compounds and minerals.

A combination of spectroscopic techniques like MIRS with Partial Least Squares (PLS), which was introduced to quantify chemical compounds (Haaland and Thomas, 1988) has also been used to estimate bulk soil characteristics from visible, near, and mid-infrared spectroscopic measurements (Janik and Skjemstad, 1995; Viscarra Rossel et al., 2006), quantify OC of particle-sized fractions (Cozzolino and Moron, 2006) and derive prediction models for specific SOM components in unknown samples using spectral data (Mirzaeitalarposhti et al., 2015; Viscarra Rossel et al., 2008). PLS is a chemometric method to derive prediction models for specific compounds from spectroscopic data (Zimmermann et al., 2007). PLS relates the spectra to the concentration of the soil component of interest, for instance, TOC using selected vibrational frequencies from the spectra.

Nuclear Magnetic Resonance (NMR) spectroscopy

NMR spectroscopy has been used to extensively investigate SOM structure for several decades. It measures chemical shifts in the frequency at which the atomic nucleus in a sample subjected to a magnetic field resonates relative to a reference compound (Bruun et al., 2010), yielding semi-quantitative information on SOM on a molecular level (Schnitzer, 2001). NMR has a large potential to analyze the different chemical species that are less sensitive or less abundant like ^{1}H and ^{13}C in SOM in liquid and solid-state respectively (Kögel-Knabner, 2000). The chemical composition of SOM has been characterized by solid-state ^{13}C NMR (Kögel-Knabner, 2002) resulting in a spectrum that is divided into four main regions: carbonyl, aromatic, O alkyl, and alkyl. The amount of each organic group in a sample is considered to be proportional to each region (Bruun et al., 2010). NMR is often proposed to have an upper hand over MIRS as the standard demineralization of soil samples by hydrofluoric (HF) acid for NMR removes minerals preventing mineral interference (Yeasmin et al., 2017). However, the treatment of soil samples with HF and the sequential washes lead to DOC losses and the disruption of organo-mineral interactions which alter the amount and composition of SOM (Sleutel et al., 2009). The utilization of NMR in soil studies has been known to be hampered by high concentrations of some paramagnetic metal ions like iron (Fe^{+}) (Schnitzer, 2001) thereby limiting the use of NMR in SOM studies. Earlier works by Arshad et al. (1988) and Skjemstad et al. (1994) suggested that the isolation of OM-rich fractions, particle-size separations, and the removal of metal ions could eliminate the interference caused by metal ions.

1.2.4 Thermal techniques

Several thermal analytic techniques in different approaches have been used to study the organic and mineral components of SOM. Conventionally, a soil sample is heated according to a heating program yielding information on the weight loss as a function of temperature using thermograms or detecting and analyzing evolved gases through spectroscopic techniques (Bruun et al., 2010; Demyan et al., 2013; Leinweber et al., 1992; Montecchio et al., 2006). These analyses can help to elucidate the nature and degree of association of organic matter with minerals (Plante et al., 2009). Thermal techniques like thermo-gravimetric analysis (TGA) and differential scanning calorimetry (DSC) have been used to characterize the thermal nature of SOM and its fractions as well as to measure soil organic and inorganic C (Leinweber et al., 1992; Siewert, 2004). Though rapid and sometimes inexpensive, these techniques have a drawback that the prevailing oxidative conditions under which they are carried out result in significant weight loss of interlayer water, and the decomposition of some inorganic soil constituents occur at similar temperatures as SOM degradation (Gaál et al., 1994). An improvement in the thermal analysis of SOM was achieved by coupling thermal techniques like evolved gas analysis (EGA) and pyrolysis (Py) with Fourier-transform infrared spectroscopy (FTIR), gas chromatography (GC), and/or mass spectrometry (MS) (Court and Sephton, 2009; De la Rosa et al., 2012, 2008; Demyan et al., 2013). These techniques permitted the quantitative and qualitative monitoring of thermal properties of SOM and the elucidation of chemical changes of SOM properties in bulk soil and its fractions. These techniques also revealed the chemical structure of SOM via the presence of peaks in mid-infrared and mass spectra assigned to specific organic compounds. This is in contrast to thermograms obtained via TGA which monitor the changes in the sample weight during measurements revealing no information about the chemical nature of SOM.

A review by Derenne & Quénéa, (2015) puts forth some potential limitations of analytical pyrolysis (coupled techniques) in SOM studies. They include; a large number of pyrolysis products due to the complexity of natural OM make identifications difficult (e.g with Py-GC/MS), the formation of artifacts through side reactions like decarboxylation resulting in pyrolysis products which are not building blocks of SOM, and the introduction of bias stemming from the release of thermally desorbed molecules at sub-pyrolytic (≤300 °C) and pyrolytic temperatures (≥700 °C).

1.2.5 Isotopic techniques

Isotopic techniques have proven to be a useful tool for investigating the influence of C_3 and C_4 vegetation on SOM dynamics (Bruun et al., 2004; Saiz et al., 2015). The ^{13}C natural abundance approach has been used to identify changes in vegetation patterns, determine human-induced impacts on SOC storage like land use and soil erosion, thereby providing information on SOM dynamics (Acton et al., 2013; Zacháry, 2019). This approach relies on the variation in the $^{13}C/^{12}C$ ratio mainly between C_3 and C_4 plants, for example, the distinct carbon isotopic ($\delta^{13}C$) values of grasslands that utilize the C_4 photosynthetic pathway compared to the $\delta^{13}C$ value of forests that utilize the C_3 photosynthetic pathway (Krull et al., 2007; Millard et al., 2010). Research has suggested that CO_2 enrichment of the atmosphere will cause global shifts in vegetation favoring C-rich C_3 forests over C_4 grasslands and savannahs, hence changes in SOM accumulation and dynamics (Acton et al., 2013). These approaches have gone a long way to provide insight into the proportion of respective residues in bulk soil and its size and density fractions as well as the residual amount from previous vegetation in the soil (Millard et al., 2010) and to detect changes in SOM and soil management systems like forest, pasture and plantation (Cadisch et al., 1996). Isotopic ratios also facilitate the interpretations and enhance the development of quality distribution models of SOM (Bruun et al., 2010).

Radiocarbon ^{14}C age measurements have been used to determine the mean residence time of SOM (Acton et al., 2013; Quideau et al., 2000 and references therein). While ^{14}C dating yields valuable information on SOM, it is possible that the true age of stabilized SOM measured in deeper soil layers is distorted as single analyses of bulk soil fail to consider fresh organic inputs from root decomposition and solubilized SOC (Acton et al., 2013).

1.2.6 Biological fractionation

Biological fractionation of SOM via soil incubation has shown promise in SOM studies as it identifies SOM that is easily decomposable or labile in intact or disturbed soil samples. Incubations are considered to be a direct measure of the microbial population and enzymes that mineralize SOM (Plante et al., 2011). Soil incubations under changing temperatures have been used to investigate how the temperature dependence of SOM decomposition is influenced by SOM composition, commonly referred to as temperature sensitivity (Q_{10}) (Fang et al., 2005). A study by Paul et al. (2006) showed that long-term incubation can be used to effectively identify labile and more resistant fractions of SOM. The biological mineralization of C is determined by measuring the CO_2 evolution; and CO_2 evolution rates have been used to identify management effects on SOM dynamics (Fortuna et al., 2003; Paul et al., 2003). Soil incubations are commonly considered ‘unnatural’ as the use of disturbed soil samples determines mineralization in the labile fraction of soils or the loosely protected organic matter accounts for the CO_2 flux. Another problem with soil incubation is the uncertainty associated with the temperature sensitivity of decomposition often caused by the instantaneous measurement of CO_2 rates at different times and the use of longer incubation times leading to the cropping up of artificial conditions (Kirschbaum, 1995). CO_2 fluxes emitted from the soil surface field-measured respiration have also been used to study SOM decomposition in agro-ecosystems (Ali et al., 2015). However, field measurements have often been criticized to be subject to significant error as the CO_2 emissions are confounded by root respiration and environmental factors like moisture (Yan et al., 2017).

1.2.6 Methodological Advances in SOM characterization

The concept of soil quality assessment (hereafter denoted as SQA) and soil management particularly in agricultural soils has recently been at the forefront of numerous research efforts. Agricultural management practices, as earlier mentioned, impact directly on SOM influencing soil fertility, nutrient availability, and C sequestration. The focus on the use of resource-intensive conventional laboratory techniques (plot, field, and site scale) like dry combustion and wet digestion to measurements at landscape (field, ecosystem, and regional) levels have been on the rise during the past few years (Nayak et al., 2019). This has resulted in recent developments of high-throughput methodologies with tremendous potential to improve SQA and management approaches at high spatial resolution.

Utilizing biological and biochemical indicators like pH, total OC, total OM, and water storage will greatly improve SQA as these indicators are essential in connecting abiotic soil properties to soil biochemical and biophysical transformations and potential plant growth (Lehman et al., 2015). The last decade has witnessed major methodological advances in molecular microbiology like genomics, proteomics, and transcriptomics which provide information on the abundance, diversity, activity, and functionality of indigenous microbial communities, subsequently yielding sensitive and robust indicators of soil quality (Bouchez et al., 2016) The implementation of *in situ* C measurement techniques like (field portable) Laser Induced Breakdown Spectroscopy (LIBS), Inelastic Neutron Scattering (INS), portable near-infrared spectroscopy (NIRS), and remote sensing have the potential to minimize soil disturbance, increases the ability to analyze large areas of soil, reduce sampling and errors, and are rapid, cost-effective and is promising for SQA (Gehl and Rice, 2007). These spectroscopic and remote sensing techniques are appealing to *in situ* C measurements due to their high throughput and sensitivity to soil chemical, physical and biological parameters. Combining *in situ* based analyses is hampered by soil properties like moisture content and particle size distribution, limited only to the first few millimeters of soil and the lack of calibration databases to relate spectral information to soil properties (Baveye and Laba, 2015; Bünemann et al., 2018; Gandariasbeitia et al., 2017). Combining *in situ* measurements with laboratory-based analyses is a promising alternative to improve SQA. X-ray computed tomography (CT), a non-destructive means to visualize and quantify soils in 3D, providing insights into soil functions (Helliwell et al., 2013) can be used as an alternative to avoid some drawbacks of *in situ* spectroscopy. However, this method is still far from being a routine technique for SQA (Bünemann et al., 2018).

Due to the importance of the molecular characterization of SOM in SQA, the existing traditional analytical techniques have been further developed to provide more insights regarding the formation, alterations, dynamics, and stability of SOM and its bulk chemistry. Recent developments in the molecular characterization of SOM include the introduction of high resolution techniques like magic angle spinning NMR and comprehensive multiphase (CMP) NMR for the detailed characterization of liquid, semisolid and solid components of SOM (Kögel-Knabner and Rumpel, 2018). Emerging microscale techniques like Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), near-edge X-ray spectroscopy and secondary ion mass spectrometry (isotopes (NanoSIMS) and molecular composition (TofSIMS)) have been applied to obtain information on SOM at microscales (e.g. below 50 nm (Solomon et al., 2005)) and advance the understanding of stabilization mechanisms (Kögel-Knabner and Rumpel, 2018).

While DRIFTS is a well-established technique in SOM studies (Demyan et al., 2012; Ellerbrock and Gerke, 2016; Margenot et al., 2015; Toosi et al., 2017; Veum et al., 2014, and references therein), inherent limitations of this technique such as interferences caused by vibration of O – H bonds greatly obscure the visibility of peaks assigned to organic compounds (e.g. aliphatic compounds). Such peaks are often invaluable in the assessment of SOM quality and dynamics as they are used as alternative proxies, hence the need to resolve such limitations. Additionally, results from several studies have revealed that an improved understanding and knowledge of the complex and heterogeneous nature of SOM can hence be achieved through a combination of analytical techniques (Albrecht et al., 2014; Antil et al., 2005; Demyan et al., 2013; Solomon et al., 2005). It is therefore of great importance to forge ahead with the development of SOM analytical techniques which can provide a more comprehensive picture of the SOM continuum in soils while minimizing the implications of methodological limitations of already existing techniques.

1.3 Objectives and hypotheses

The main objective of this thesis was to develop and use a coupled thermal and mid-infrared spectroscopic analytical technique to assess the molecular composition of SOM in soils from different agro-ecosystems. Additionally, this thesis also aimed to improve the sensitivity of mid-DRIFTS by enhancing the visibility of mid-infrared peaks of SOM compounds that act as alternative proxies for SOM quality

The general objectives of this study were:

(i) To develop an analytical technique, i.e. pyrolysis-mid-infrared spectroscopy (Py-MIRS), to fingerprint SOM bulk chemistry, providing information on the molecular composition of organic functional groups.

(ii) To apply the Py-MIRS technique developed to investigate SOM bulk chemistry as influenced by long-term fertilizer management practices and land use.

(iii) To investigate the effect of soil sample drying temperature on the mid-DRIFTS peaks and their potential as proxies for SOM quality.

In order to achieve the above-mentioned objectives, it was hypothesized that fingerprinting SOM bulk chemistry using Py-MIRS yields information on the composition of SOM as determined by the presence of different organic functional groups (e.g. aromatic, aliphatic). In addition, the composition of SOM as measured by Py-MIRS would be affected by long-term fertilizer management and land use (and change). Lastly, the relative amount of aliphatic compounds in SOM detected by mid-DRIFTS is strongly influenced by interfering vibrations of the O – H bonds and this phenomenon can be lessened by drying soils at higher temperatures prior to mid-DRIFTS analysis. It is further hypothesized that such higher sample drying temperatures do not strongly affect other organic functional groups (e.g. aromatic) determined via mid-DRIFTS but improve the ratio of aliphatic to aromatic groups which is a proxy for SOM stability.

1.4 Thesis Outline

This doctoral thesis is based on journal articles written during the Ph.D. studies. In Chapter 2, a novel technique is developed to fingerprint soil organic matter bulk chemistry in agricultural soils. Archive soil samples under different management practices from the Static Fertilization Experiment in Bad Lauchstädt, Germany were used to test the applicability of this technique to study soil SOM composition. Chapter 3 echoes the findings made in the previous chapter and following the application of the developed technique to soils from two long-term experiments: the Frame Experiment at Ultuna, Sweden, and the Observatory for Environmental Research near Lusignan, France differing agricultural management and land use. Chapter 4 explores the effect of drying temperatures

(32 °C, 55 °C, 65 °C, 75 °C, 85 °C, 95 °C, and 105 °C) on the quality indices of SOM as determined by mid-DRIFTS followed by a general discussion in chapter 5.

Chapter 2 Coupling pyrolysis with mid-infrared spectroscopy (Py-MIRS) to fingerprint soil organic matter bulk chemistry*

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2.1 Abstract

A novel method coupling pyrolysis with mid-infrared spectroscopy (Py-MIRS) was developed to characterize soil organic matter (SOM) chemistry in soils. The pyrolyzer was interfaced to the MIR spectrometer by means of a Brill cell^{TM} (CDS Analytica). The set-up generates pyrolysis fingerprint spectra from which individual pyrolysis products can be related to SOM bulk chemistry. Py-MIRS development involved the testing of experimental conditions like pyrolysis temperature (550, 700, 1000 °C), heating rate (20 °C s^{-1} and 20 °C ms^{-1}) and time (15, 30 and 60 s) using reference standard compounds ranging from carbohydrates to phenols varying in chemical and structural composition like levoglusogan, gluten, tannin, syringol, pectin and leucine falling within different compound categories (carbohydrates, amino acids, proteins, phenols, etc.) as well as soil samples. Pyrolysis yields of prominent specific functional groups, like aliphatics (C - H stretching at 2930 cm^{-1}) and C = C aromatics (1510 cm^{-1}), varied with pyrolysis temperature, heating rate and time. The preferred settings for high pyrolysis yield and minimized secondary reactions were obtained at a pyrolysis temperature of 700 °C, heating rate of 20 °C ms^{-1} and heating time of 30 s. The suitability of Py-MIRS to detect changes in SOM composition was evaluated by comparing Py-MIRS results to Diffuse Reflectance Fourier Transform mid-Infrared Spectroscopy (DRIFTS) results. Soil samples taken from the Static Fertilization Experiment, Bad Lauchstädt, Germany (Chernozem) revealed a major SOM contribution of the peak at 1750 cm^{-1} (C = O), followed by peaks at 950 (=C - H), 1510 (C = C), 1176 (C – H, O – H) cm^{-1}, with smaller contributions from the 2930 (C – H) and 3015 (CH_4) cm^{-1} peaks, apart from a dominant CO_2 peak. Using the preferred pyrolysis settings, Py-MIRS as well as DRIFTS results further indicated that soils receiving organic (e.g. farmyard manure) inputs were highly enriched in aliphatic groups, while their absence favored the accumulation of carboxyl and aromatic groups as well as polysaccharides. Py-MIRS allowed via semi-quantification of pyrolysis products a rapid monitoring of SOM bulk chemistry with a high degree of reproducibility. It was concluded that Py-MIRS represents a fast, effective and reproducible technique to characterize changes in the SOM bulk chemistry as a result of management practices. It also allows to minimize acknowledged constraints of other analytical techniques used to characterize SOM bulk chemistry such as mineral interferences and associated secondary reactions.

Keywords: pyrolysis, mid-infrared spectroscopy, soil organic matter, fertilization

2.2 Introduction

Soil organic matter (SOM) is a crucial indicator of soil quality as it governs soil properties like water-holding capacity, nutrient availability and soil structural stability (Lal, 2004). Hence, the understanding of changes in SOM characteristics resulting from management practices and land use change is essential to develop sustainable agricultural systems. Numerous analytical approaches have been developed to characterize SOM composition. These techniques include physical and/or chemical fractionation, spectroscopic techniques such as mid-infrared spectroscopy (MIRS) and nuclear magnetic resonance (NMR), thermal techniques like thermogravimetry (TG), differential scanning calorimetry (DSC), and differential thermal analysis (DTA) as well as analytic pyrolytic techniques e.g pyrolysis coupled with gas chromatography/mass spectrometry (Py-GC/MS) (Kögel-Knabner, 2000).

It has been recognized that physical and/or chemical fractionation may lead to unintended chemical changes of SOM (Kögel-Knabner, 2000). Alternatively, non-destructive, infrared spectroscopic techniques like Diffuse Reflectance mid-Infrared Spectroscopy (DRIFTS) provide efficient and reliable means for SOM characterization (Gerzabek et al., 2006;

Tatzber et al., 2009). However, the severe spectral band overlapping caused by broad spectral bands and interference from the soil mineral matrix (Nguyen et al., 1991) encountered when using DRIFTS may hinder the use of spectra for quantitative and qualitative analysis of SOM. However, heat and/or water loss and energy changes during thermal reactions in TG, DSC and DTA experiments cause overlaps between the organic and inorganic components of SOM thereby making it difficult to infer SOM bulk chemistry via thermal decomposition of SOM (Fernández et al., 2012). TG has been used in conjunction with Fourier transform infrared spectroscopy (FTIR) to investigate volatile compounds (Fu et al., 2009; Ibarra and Moliner, 1991). Yet, TG-FTIR is time consuming and employs slow heating rates (<100 °C min^{-1}) which can lead to charring and repolymerisation reactions (Fang et al., 2006). Alternatively, pyrolysis coupled with gas chromatography/mass spectrometry (Py-GC/MS) has also been used extensively to study SOM providing detailed insights into the molecular composition of SOM (De la Rosa et al., 2012; De la Rosa et al., 2008; Nierop et al., 2001; Rumpel et al., 2009; White et al., 2004). However, the drawbacks associated with Py-GC/MS include (i) the long analysis times (often >1 hour) which limits the analyses of large sample sets (Court and Sephton, 2009), (ii) the difficulty to interprete Py-GC/MS data due to the complex chromatographs with high information density (Wenig and Odermatt, 2010), and (iii) heat-induced arrangement of molecules influencing thermal degradation (Demyan et al., 2013).

Despite the considerable advances in the development of these thermal techniques for SOM studies, the lack of a well-established technique which reduces the limitations of the afore-mentioned methods has prompted the development of pyrolysis coupled to mid-infrared spectroscopy (Py-MIRS) as a promising, rapid and effective technique to characterize SOM. Pyrolysis uses thermal energy to cleave chemical bonds and yields molecular products that are detectable by MIRS via the identification of mid-infrared peaks corresponding to vibrations of organic and inorganic functional groups. Py-MIRS therefore provides information that can be used to assess the impact of various changes on the quality of SOM (Derenne and Quénéa, 2015). Thus, it is hypothesised that pyrolysis coupled with MIRS provides information on the composition of organic functional groups (e.g. aromatics, carbohydrates) of SOM. Py-MIRS has been employed to characterize biomass residues (Cardoso and Ataíde, 2013), coal (Ibarra and Moliner, 1991) and wood (Court and Sephton, 2009), while it has not been developed and validated yet for the characterization of SOM. The Py-MIRS fingerprint of samples can be used to identify SOM composition largely without interference from mineral vibrations, which limits the use of DRIFTS. However, the question that arises is if Py-MIRS is a suitable method to fingerprint soil organic matter bulk chemistry and to detect management related differences in SOM composition. The suitability of Py-MIRS to characterise changes in SOM was evaluated by comparing Py-MIRS results to those of DRIFTS.

The first study objective thus was to develop Py-MIRS as a technique to fingerprint SOM bulk chemistry using reference standard compounds varying in structural composition from simple sugars to complex polyphenols as well as bulk soil samples. The second objective was to apply this technique to verify changes in SOM composition resulting from different fertilizer management practices.

2.3 Materials and methods

2.3.1 Reference standard compounds for Py-MIRS method development

We used reference standard compounds as well as biological samples extracted from materials varying in chemical structure, ranging from simple sugars like glucose to complex polyphenols (Table 2.1) to develop the Py-MIRS technique. The standard samples provided initial information on the volatile products evolved during pyrolysis. These samples were selected as they covered a range of compounds commonly found in soil organic matter (Haddix et al., 2016) and have also been used in previous studies (Demyan et al., 2013; Haddix et al., 2016). The pyrolysis experimental conditions which were evaluated included pyrolysis temperature, heating rate and time in order to minimize associated secondary reactions and maximize pyrolysis yield efficiency. Pure substances were also diluted with preheated quartz sand (1:100) to test the effect of minerals on pyrolysis products. Standard samples were purchased from Sigma-Aldrich Chemie (Germany) with the exception of pectin and chitin (ICN Biomedicals, Inc., Germany), tannic acid (Carl Roth GmbH, Germany), levoglucosan (Merck KGaA, Germany) and syringol (ThermoFischer GmbH, Germany).

2.3.2 Soils for SOM characterization

Soil samples were taken from the Static Fertilization Experiment Bad Lauchstädt, Germany (51°24'N, 11°53'E). This field experiment was initiated in 1902 under a crop rotation of sugar beet, spring barley, potatoes and winter wheat while different fertilization schemes were introduced in 1978. The site is characterized by a mean annual temperature and precipitation of 8.8 °C and 484 mm respectively, and the soil classified as Haplic Chernozem. Archive soil samples from the years 1956, 1979, 1992, 2004 and 2008 taken from the plow layer (0-20 cm) were used for this study. The treatments selected for this study were: i) farmyard manure from cattle (FYM, 30 Mg ha^{-1} every second year), ii) mineral (NPK) fertilizer (123, 30 and 30 kg^{-1} ha^{-1} yr^{-1} N, P and K, respectively), iii) a combination of 30 Mg ha^{-1} FYM every second year and NPK fertilizer (FYM+NPK), and iv) a control (CON) without fertilizer inputs. The FYM+NPK and the FYM treatments had average (1956, 1992, 2004 and 2008) TOC contents of 22.2 mg g^{-1}, and 19.7 mg g^{-1}

respectively. TOC contents under the NPK and CON treatments were 16.7 mg g^{-1} and 14.7 mg g^{-1} respectively.

2.3.3 Py-MIRS

2.3.3.1 Py-MIRS instrumentation

The Py-MIRS unit consisted of three main components; a 5250 Trapping Pyrolyzer (CDS Analytica, Oxford, Pennsylvania, USA), a Type 8711 mass flow communicator (MFC) (Bürkert GmbH & Co. KG, Ingelfingen, Germany) and a Tensor-27 (Bruker Optik GmbH, Ettlingen, Germany) mid-infrared spectrometer (Figure 2.1). The pyroprobe was equipped with a resistively heated platinum filament. The pyrolyzer was interfaced to the Tensor-27 spectrometer by means of a Brill cell^{TM} (CDS Analytica). The Brill cell consists of a cylindrical stainless steel cell with potassium bromide (KBr) windows which was placed in the accessory chamber of the Tensor 27 spectrometer. The Brill cell was equipped with an inlet and outlet for the purge gas, a port for the pyroprobe insertion, and had a volume of approximately 20 ml. A Windows^{TM}-based CDS control software allowed the programming of different experimental settings like the initial and final pyrolysis temperature, time, heating rate and Brill cell temperature. The Brill cell temperature was set at 250 °C. The pyrolyzer was operated in the manual mode where the pyroprobe was inserted directly into the Brill cell through the probe inlet tube. With this procedure, the gases evolved during pyrolysis were instantaneously detected by the Tensor 27 spectrometer in the absorbance mode. The MFC connected to the flow controller of the pyrolyzer was used to monitor the helium (99.96 % purity) purge flow rates supplied to the Brill cell before and after each pyrolysis experiment. Helium was used to create an inert atmosphere during pyrolysis.

Table 2. 1: List of reference standard compounds and biological samples for Py-MIRS development.

Standard compounds/ Biological samples	**Compound category**	**Abbreviation**
Standards		
Gliadin	Protein	Gl
Glucose	Carbohydrate	Glu
Gluten	Protein	Glt
Leucine	Amino acid	Leu
Levoglucosan	Carbohydrate	Lev
Quebracho	Phenol	Que
Serine	Amino acid	Se
Syringic acid	Phenol	Syr A
Syringol	Aromatic	Sy
Tannin	Phenol	Tan
Vanillic acid	Phenol	Van A
Xylan	Carbohydrate	Xyl
Biological samples		
Chitin		Ch
Pectin		Pec

The flow rates were set by the MFC software A23 (Bürkert GmbH). A synchronized digital connection between pyrolyzer, MFC and spectrometer was established using the OPUS 7.0 software (Bruker Optik GmbH) enabling the operation of all three devices simultaneously during the pyrolysis experiment. The Tensor-27 spectrometer was equipped with a KBr beam splitter and mercury-cadmium-telluride (MCT) detector cooled by liquid nitrogen. The spectra were recorded using the OPUS software in the mid-infrared range (4000 cm^{-1} - 400 cm^{-1}). A scan was recorded every 0.3 s.

As the pattern of evolved volatiles varied between the different standard samples at different temperatures, coupled with their different chemical structures, the preferred pyrolysis temperature was evaluated in terms of the pyrolysis yield efficiency particularly the aliphatic C – H (2930 cm^{-1}) and the aromatic C = C (1510 cm^{-1}) groups which were detected in most standard compounds as well as soils while testing the different experimental conditions.

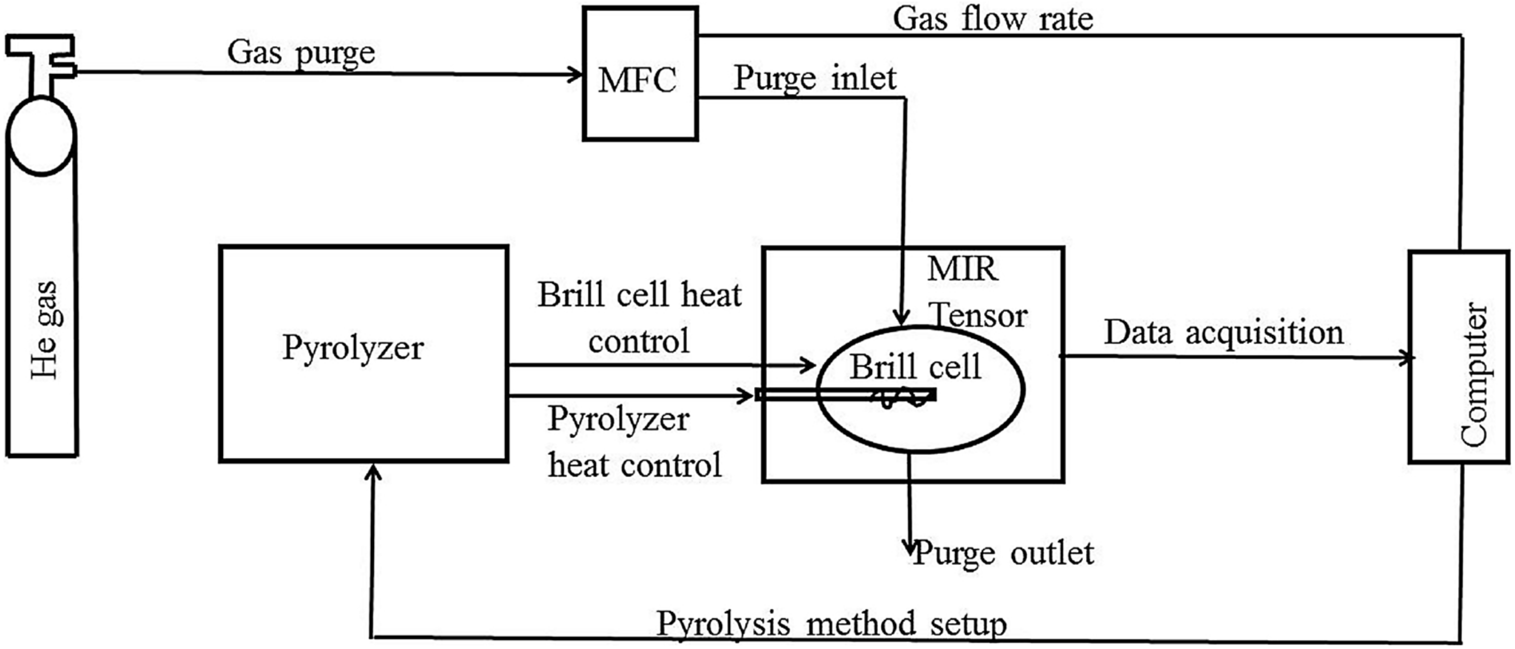

Figure 2. 1: Schematic of Py-MIRS experimental setup. MFC = Mass flow communicator

2.3.3.2 Py-MIRS method development

Approximately two milligrams of reference standard compounds and 35 mg bulk soil from the FYM treatment of the Bad Lauchstädt soils (sampling year 2004) were used in duplicate replicates. Samples were weighted into an open ended quartz tube (length = 25 mm, inner diameter = 1.9 mm) and fixed by loosely fitting quartz wool on both ends and placed in the filament coil of the pyroprobe. The sample weights were recorded before pyrolysis using a XS microbalance (Mettler-Toledo GmbH, Gießen, Germany) with a sensitivity of 1 µg. Each sample was analysed in triplicate.

Pyrolysis temperatures of 550, 700 °C and 1000 °C, heating rates of 20 °C s^{-1} and 20 °C ms^{-1} and heating times of 15 s, 30 s, and 60 s which have been frequently used in pyrolysis studies (Calderón et al., 2006; Court and Sephton, 2009; De la Rosa et al., 2012; Medina-Vera, 1996) were tested to obtain preferred experimental conditions. The purge rate of helium was set at 100 ml min^{-1} for 2 min to purge the cell before each pyrolysis run. The samples were pyrolysed using programmed temperature sequences of 100 °C for 2 s, then ramped at either 20 °C s^{-1} or 20 °C ms^{-1} to 550, 700 or 1000 °C and held either for 15, 30 or 60 s. Fifty-five repetitive scans were recorded during the evolution of volatile pyrolysis products from each sample and then averaged to give a single scan. The Py-MIRS spectra were further processed using OPUS 7.0 software (Bruker Optik GmbH). The recorded spectra were baseline corrected using the concave rubberband method with 64 baseline points and 10 iterations. As the pattern of evolved volatiles varied between the different standard samples at different temperatures, coupled with their different chemical structures, the preferred pyrolysis temperature was evaluated in terms of the pyrolysis yield efficiency particularly the aliphatic C – H (2930 cm^{-1}) and the aromatic C = C (1510 cm^{-1})

groups which were detected in most standard compounds as well as soils while testing the different experimental conditions.

After identification of suitable pyrolysis parameters (see Results and Discussion section), the following protocol was adopted for subsequent Py-MIRS measurements of soil samples to evaluate management effects. Following the insertion of the pyroprobe containing the sample into the Brill cell, the sample was heated at 100 °C for 2 s, then ramped at 20 °C ms^{-1} and held at 700 °C for 30 s. The wavenumber resolution and the aperture of the spectrometer were set at 4 cm^{-1}. The peak area was normalized by dividing the peak area by the sample weight or C content. The normalized peak area is also referred in this study as pyrolysis yield.

2.3.4 DRIFTS analysis of bulk soil

DRIFTS analysis of the soil samples used for Py-MIRS, as well as pyrolysis residue of soils and standard samples (glucose, xylan, tannic acid, and quebracho, Figure S1) was performed according to previously published routines (Demyan et al., 2012). The comparative analysis focused on three infrared bands which have the same functional group assignments like Py-MIRS. The double peak centred around 2930, 1530 cm^{-1} and 1159 cm^{-1} assigned to aliphatic C – H groups, aromatic C = C groups, and C – O bonds of poly-alcoholic and ether groups respectively (Demyan et al., 2012). Following an integration performed to obtain the corrected peak area (Figure S2), relative peak areas were calculated as % of the corrected peak area of each peak divided by the sum of the peak areas of the three peaks.

Prior to Py-MIRS and DRIFTS analyses, ball milled soil samples were dried overnight at 32 ˚C. Total organic carbon (TOC) of the soils samples as well as that of the pyrolysis residue of soils was determined by dry combustion with a Vario-EL III elemental analyser (Elementar, Hanau, Germany).

2.3.5 Statistical analysis

For analysis of data from the tests of preferred experimental conditions, mean comparisons of the peak areas at 2930 and 1510 cm^{-1} of xylan and quebracho as well as of soil samples (FYM treatment) were performed with a one-way analysis of variance (ANOVA) followed by a Tukey HSD test. Differences were considered to be significant at $P < 0.05$. In the absence of field replicates from the Bad Lauchstädt field experiment, descriptive statistical analyses were performed on the mean peak areas of the different peaks. All statistical analyses were performed in R (Software R.3.0.1, R foundation Statistical Computing, Vienna, Austria (https://www.r-project.org/).

2.4 Results

2.4.1 Interpretation of Py-MIRS spectra

Examples of Py-MIRS spectra from reference standard compounds, ranging from sugars, carbohydrates, amino acids, protein to phenols and from bulk soils are illustrated in Figures 2.2 and 2.3 respectively. The data revealed the variation in the pattern of evolved volatiles (peak pattern), e.g. the number of peaks and the absorbance intensity (peak area/yield) of the different peaks. These peaks were further assigned to different functional groups on the basis of their characteristic absorbance at the different frequencies.

The band at 4000-3400 cm^{-1} was assigned to O – H stretching bonds resulting from the release of water (Gu et al., 2013). The band at 3400-3200 cm^{-1} with a prominent peak at 3334 cm^{-1} was from N – H vibrations of amines (Fan et al., 2015). The absorbance in the wavenumber range 3200–3000 cm^{-1} was assigned to aromatic C – H stretching vibrations while the sharp peak at 3015 cm^{-1} indicated the presence of methane (CH_4) derived from the cracking of methoxy groups (Court and Sephton, 2009; Feng et al., 2006). The band at 3000-2800 cm^{-1} with a prominent peak at 2930 cm^{-1} was assigned to C – H vibrations of aliphatic compounds (Court and Sephton, 2009). The bands 3700-3600, 2400-2200 and 700-600 cm^{-1} were assigned to CO_2 (Court and Sephton, 2009; Gu et al., 2013). CO_2 is formed as a result of the cracking and reforming of carboxyl and carbonyl groups in organic compounds as well as decomposition of carbonates in inorganic compounds (Fan et al., 2015). The band at 2250-2000 cm^{-1} was assigned to CO, whose formation is attributed to the cleavage of C – O – C and C = O bonds (Fu et al., 2009). The bands at 1800-1600 cm^{-1}, with a prominent peak at 1750 cm^{-1}, were assigned to C = O stretching vibrations of aldehydes and ketones (Feng et al., 2006). Aromatic C = C stretching vibrations were identified by the band at 1585-1480 cm^{-1} with a dominant peak at 1510 cm^{-1} and at 1685-1550 cm^{-1} (Yang et al., 2007) in some standard samples like vanillic acid and tannic acid. The band at 1400-1100 cm^{-1} was assigned to O – H and C – O stretching vibrations of hydroxyl and alcoholic groups, polysaccharides and ethers, with the unique peak at 1176 cm^{-1} (Feng et al., 2006). The band at 1050-700 cm^{-1} with the double peak centred at 950 cm^{-1} was assigned to aromatic C – H vibrations of alkenes (Court and Sephton, 2009).

It was noted that the biological samples chitin and pectin had less complex spectra with fewer pyrolysis products (e.g. Figure. 2.2e) similar to those of soil (Figure 2.3). While for both protein and amino acids the band at 3400-3200 cm^{-1} assigned to N – H vibrations of amines was conspicuous (e.g. Figure 2.2g, j). Spectra from phenolic compounds, vanillic and syringic acids, revealed a prominent peak at 3582 cm^{-1} characteristic of O – H stretchings (e.g. Figure 2.2i).

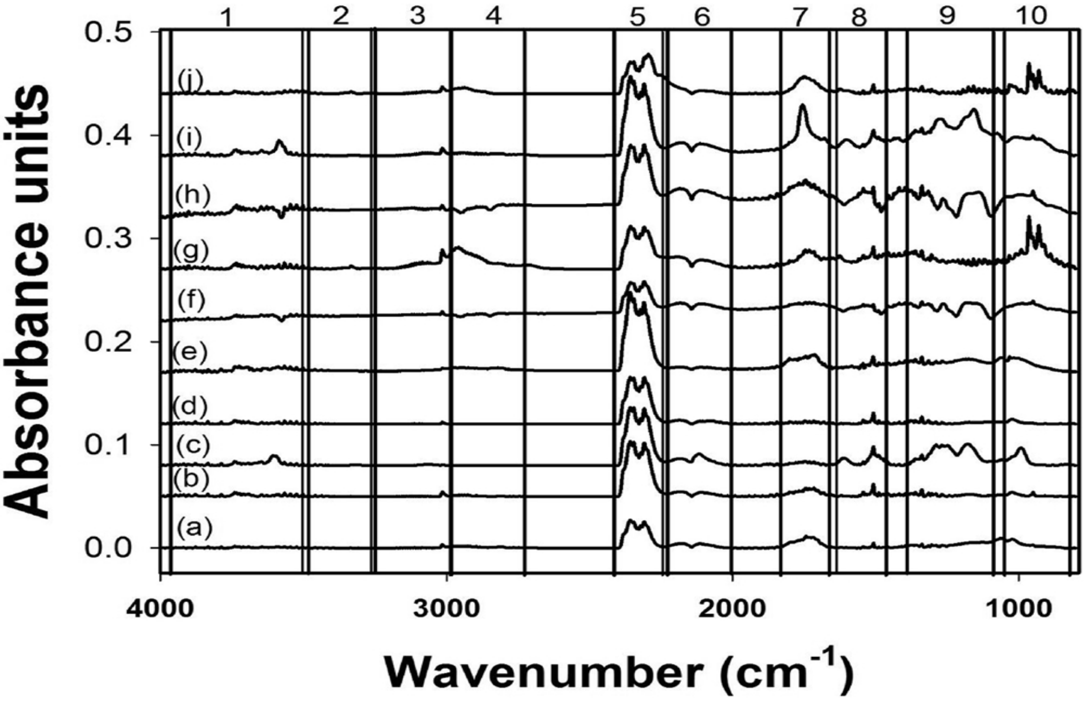

Figure 2. 2: Py-MIRS spectra of standard and biological compounds pyrolyzed from 100 to 700 °C at 20 °C ms^{-1} for 30 s (a) glucose, (b) xylan, (c) tannin, (d) quebracho, (e) pectine, (f) syringol, (g) leucine, (h) levoglucosan, (i) vanillic acid , and (j) gluten (spectra of further compounds tested see Figure S3). The indicated group frequencies were assigned to the following functional groups: 1: 4000-3400 cm^{-1} (O – H) stretch and CO_2); 2: 3400-3200cm^{-1} (N – H stretch); 3: 3200-3000 cm^{-1} (aromatic C – H stretch); 4: 3000-2800 cm^{-1} (aliphatic C – H stretch); 5: 2400-2200 cm^{-1} (CO_2); 6: 2250-2000 cm^{-1} (CO); 7: 1800-1600 cm^{-1} (C = O stretch); 8: 1650-1480 cm^{-1} (aromatic C = C stretch); 9: 1400-1100 cm^{-1} (O – H and C – O stretch) and 10: 1050-700 cm^{-1} (aromatic C – H stretch) (see Table 2.2). Normalized peak intensity in absorbance units (peak area) divided by sample weight.

For further investigation, wavenumber ranges associated with CO_2 and CO were avoided as the absorption at these peaks would be affected by mineral C in soils containing carbonates. In addition, the bands at 3400-3300 and 1685-1550 cm^{-1} were also omitted for further analysis as they were not discernable in Py-MIRS spectra of soils (Figure 2.3). Hence, only six absorption bands were used (Table 2.2) to further analyse the effect of fertilization on SOM composition using Py-MIRS. In addition, pyrolysis of blank quartz tubes with quartz wool analysis revealed no significant absorbance values in the selected wavenumber ranges (data not shown).

Table 2. 2: Investigated Py-MIRS peak areas and functional group assignments in soil samples

Wavenumber (cm^{-1})	**Functional group**
3015	CH_4[a]
2930	Aliphatic C-H vibrations[a]
1750	C=O stretching vibrations[b]
1510	Aromatic C=C stretching vibrations[c]
1176	C-O vibrations of polysaccharides, ethers, and alcoholic groups[a]
950	Aromatic =C-H vibrations (alkenes)[a]

[a](Court and Sephton, 2009), [b](Feng et al., 2006), [c](Yang et al., 2007)

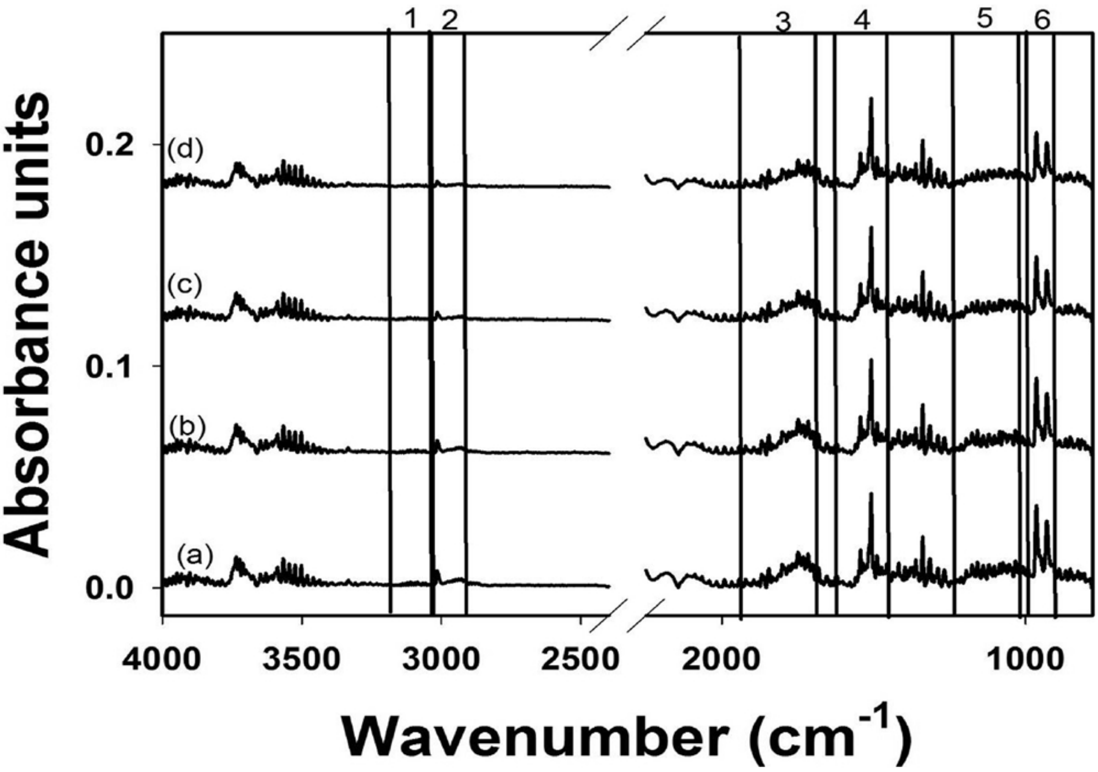

Figure 2. 3: Baseline corrected Py-MIRS spectra of Bad Lauchstädt soils from four treatments; (a) FYM + NPK, (b) FYM, (c) NPK and (d) CON. The indicated regions were assigned to the following peaks: 1: 3015 cm^{-1} (CH_4); 2: 2930 cm^{-1} (C – H); 3: 1750 cm^{-1} (C = O); 4. 1150 cm^{-1} (C = C); 5: 1176 cm^{-1} (C – O); 6: 950 cm^{-1} (=C – H) (see also Table 2.2). Note: the main CO_2 peak (2400-2200 cm^{-1}) was cut to make the other peaks more visible. Refer to Figure 2.2 for examples of uncut spectra.

2.4.2 Testing of pyrolysis experimental conditions

We utilized standard compounds that covered several compound categories present in soils to develop preferred Py-MIRS experimental conditions (pyrolysis temperature, heating rate and time) in order to maximize yield efficiency and minimize associated secondary reactions. As different standard compounds behave differently when subjected to different temperatures during pyrolysis and the nature of SOM which is highly heterogeneous and complex, the preferred pyrolysis temperature, heating rate and time were selected based on the differences in the peak area of the aliphatic C – H (2930 cm^{-1}) and aromatic C = C (1510 cm^{-1}) groups in xylan, quebracho (Figure 2.4) and soils (Figure 2.5). These two peaks were of particular interest as they were common in spectra of a majority of standard compounds as well as in soils at all pyrolysis temperatures tested.

Larger peak areas at 2930 and 1510 cm^{-1} for xylan were obtained with a faster heating rate of 20 °C ms^{-1} compared to 20 °C s^{-1} ($P < 0.05$) (Figure 2.4a). For quebracho, there was no difference in the peak area at both peaks between the heating rates. Hence, a heating rate of 20 °C ms^{-1} was used in subsequent pyrolysis experiments, thereby limiting the extent of secondary reactions.

The pyrolysis yield for xylan and quebracho obtained with pyrolysis heating times of 15 s and 30 s was mostly higher compared to the yield at 60 s ($P < 0.05$) (Figure 2.4b). For xylan, the peak area at 2930 cm^{-1} decreased with increasing pyrolysis time, while the 1510 cm^{-1} peak area increased at 30 s and decreased at 60 s. For quebracho, the peak area at 2930 cm^{-1} was highest at 30 s while the peak area at 1510 cm^{-1} decreased with increasing pyrolysis time. It is worthy to note that the preferred pyrolysis time for soils may differ from that of the standard compounds used. We therefore tested different pyrolysis times using soil samples and obtained similar results to xylan and quebracho. Larger peak areas at the 2930 cm^{-1} peak for soils were obtained at 15 s and 30s compared to 60 s ($P < 0.05$) (Figure 2.5a). The pyrolysis yield for the peak at 1510 cm^{-1} obtained at 60 s was higher compared to the yield at 15 s and 30 s ($P < 0.05$).

For xylan, the peak areas at 2930 and 1510 cm^{-1} were lower at the pyrolysis temperature of 1000 °C compared to 550 and 700 °C ($P < 0.05$) (Figure 2.4c). For quebracho, the peak area for both functional groups tended to increase at 1000 °C. Similar trends were also observed for other reference compounds tested (see examples Figures S4, S5 and S6). Furthermore, in the spectra for compounds in the carbohydrate category, i.e. glucose, levoglucosan and xylan, we observed the appearance of peaks at 3015 and 950 cm^{-1} with increasing pyrolysis temperature (e.g. Figure S4a vs. Figure 2.2h). This could be partly due to impurities, e.g. xylan used in this study is extracted from beechwood and contains ≥ 90% xylose. Since beechwood contains about 24 % of lignin, it is therefore possible that there is some residual lignin in the extracted xylan and this is manifested during pyrolysis in several detectable bands. In the spectra from proteins (gliadin and gluten) and amino acids (leucine and serine) we noted a strong decline in the peaks at 2930 and 1710 cm^{-1} and the appearance or increase of the peaks at 3015, 1510 and 950 cm^{-1} in the spectra obtained at 700 °C compared to 500 °C (e.g. Figure S5). While syringol, tannin and quebracho, belonging to the aromatic compound category, showed generally an increase in pyrolysis yield with increasing pyrolysis temperature there was a decline in pyrolysis yield from 550 to 700 to 1000 °C with some bands like the 1800-1600 cm^{-1} (C = O group), while 1685-1550 cm^{-1} (C = C group) being completely absent in the spectra obtained at 1000 °C (e.g. Figure S6).

The same temperature range was also tested for soils (FYM), i.e. 550 vs 700 vs 1000 °C, revealing that the aliphatic and aromatic peak areas at 2930 and 1510 cm^{-1} respectively were higher at 700 °C compared to 550 and 1000 °C ($P < 0.05$) (Figure 2.5b).

2.4.3 Py-MIRS analysis of differently managed soils

Utilizing the wavenumber related peaks and their assigned functional groups, we were able to observe the influence of fertilization on the SOM composition of Bad Lauchstädt soils (Figure 2.6). The peak area normalized to the soil C content (g^{-1} soil C), which reflects the abundance of the different functional groups, revealed consistent changes in response to fertilization. The results revealed a major area contribution of the 1750 cm^{-1} peak, followed by the peaks at 950, 1510, 1176 cm^{-1}, with the least contributions from the 2930 and 3015 cm^{-1} peaks (apart from a dominant CO_2 peak, not shown). The prominent peak at 3015 cm^{-1} showed the largest peak area in the FYM+NPK treatment (0.08 peak area mg^{-1} C) and decreased in the FYM and NPK treatments being smallest in the CON treatment (0.06 peak area mg^{-1} C). The normalized peak area of the 2930 cm^{-1} peak assigned to aliphatic groups was largest in the FYM+NPK treatment (0.2 peak area mg^{-1} C), and again smallest in the CON treatment (0.1 peak area mg^{-1} C). At the 1750, 1176 and 950 cm^{-1} peaks, the normalized peak areas of the NPK and CON treatments were slightly higher than those the FYM+NPK and FYM treatments. The normalized peak areas at the 1510 cm^{-1} peak was largest in the NPK and CON treatments (0.8 and 0.9 peak area mg^{-1} C respectively).

The amount of C pyrolyzed in soil samples from the treatments was on average 60 % and was not statistically significant between the different treatments (FYM and non-FYM treatments).

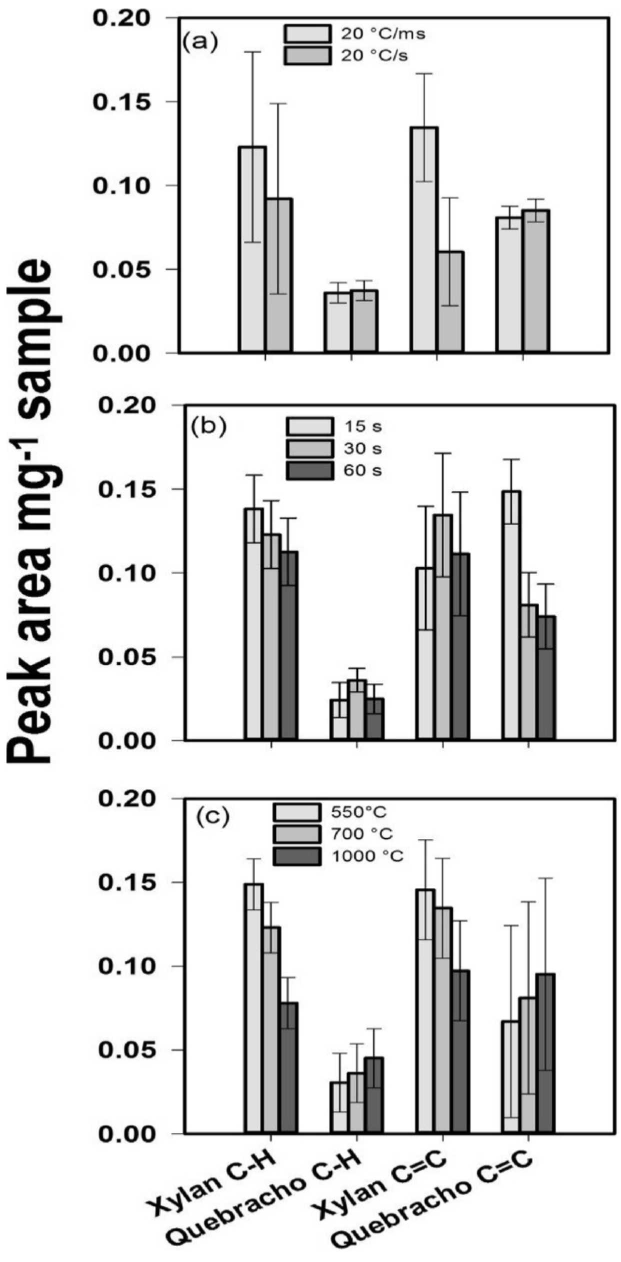

Figure 2. 4: Effect of (a) heating rate 20 °C ms^{-1} vs 20 °C s^{-1} at 700 °C; (b) time 15 vs 30 vs 60 s at 700 °C; (c) pyrolysis temperature 550 vs 700 vs 1000 °C and 20 °C ms^{-1} for xylan and quebracho and (d) pyrolysis temperature 550 vs 700 vs 1000 °C at 20 °C ms^{-1} for 30 s for soil (FYM) on the normalized peak area of the aliphatic C – H (2930 cm^{-1}) and aromatic C = C (1510 cm^{-1}) groups evolved during pyrolysis with standard errors (n=3).

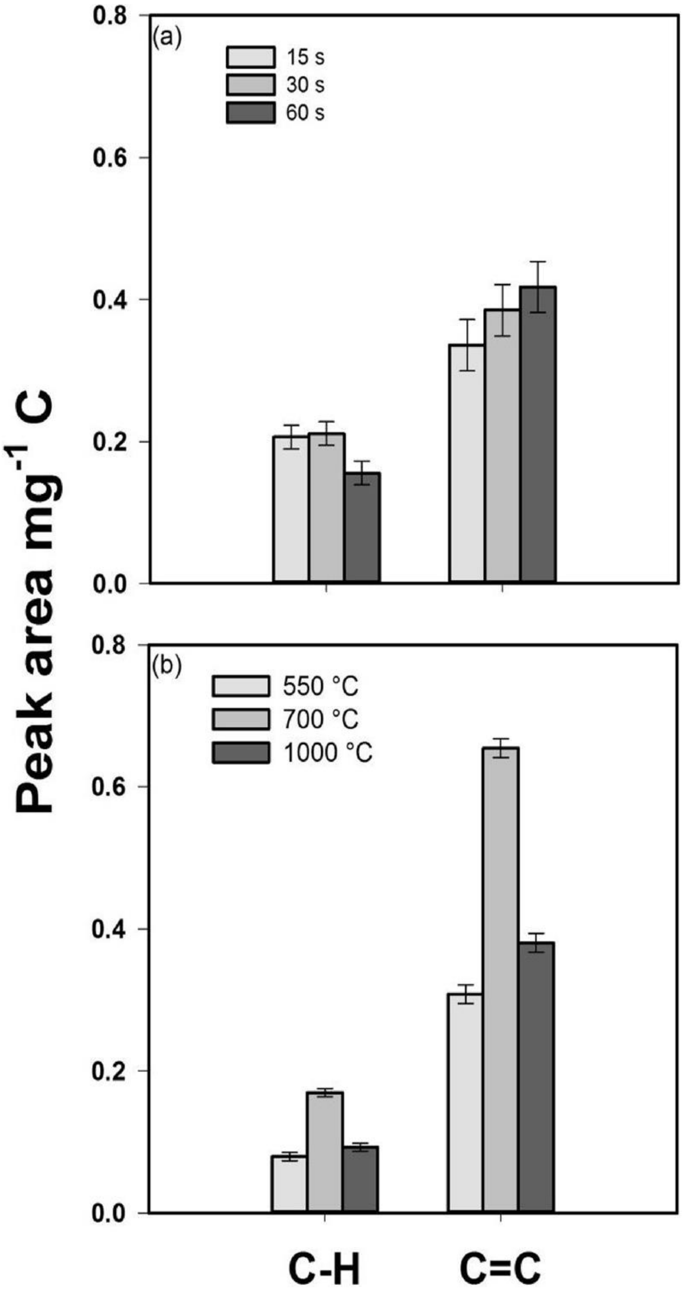

Figure 2. 5: Effect of (a) pyrolysis time 15 vs 30 vs 60 s at 700 °C and (b) pyrolysis temperature 550 vs 700 vs 1000 °C and 20 °C ms^{-1} for Bad Lauchstädt soils (FYM) on the normalized peak area of the aliphatic C – H (2930 cm^{-1}) and aromatic C = C (1510 cm^{-1}) groups evolved during pyrolysis with standard errors (n=3).

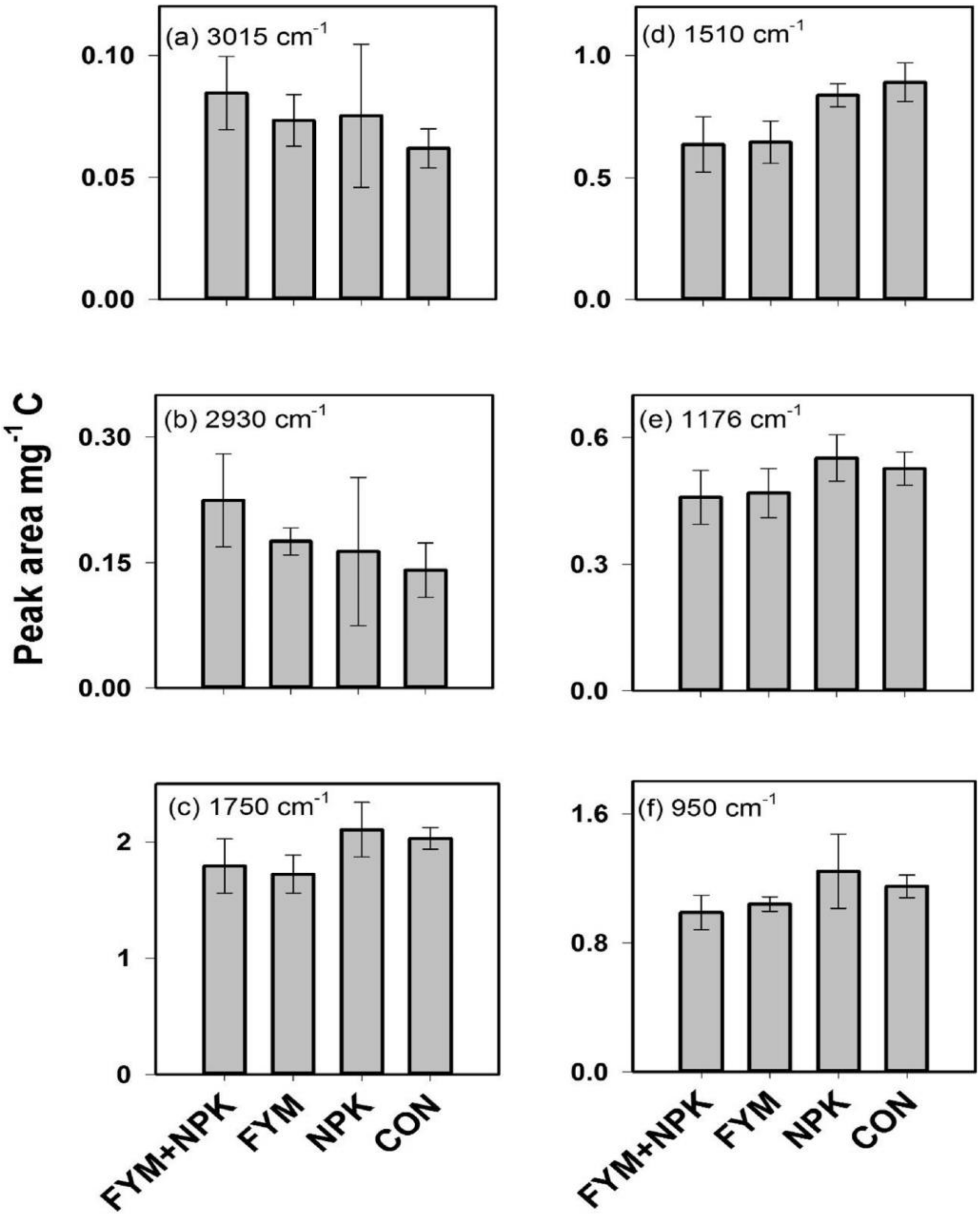

Figure 2. 6: **Py-MIRS peak area (normalized to soil C content) of bulk soil from Bad Lauchstädt at wavenumbers (a) 3015 cm^{-1} (CH_4); (b) 2930 cm^{-1} (C – H); (c) 1750 cm^{-1} (C = O); (d) 1510 cm^{-1} (C = C); (e) 1176 cm^{-1} (C – O); (f) 950 cm^{-1} (=C – H) along with standard deviations. Treatment averages of years 1956, 1979, 1992, 2004 and 2008. FYM + NPK = farmyard manure and mineral fertilizer; FYM = farmyard manure; NPK = mineral fertilizer; CON = control (no fertilizer/manure inputs).**

2.4.4 DRIFTS analysis of soils

The DRIFTS spectra of the Bad Lauchstädt soils revealed that the 2930 cm^{-1} peak contributed most to the total relative peak area, followed by the 1159 and 1530 cm^{-1} peaks, all being affected by fertilization (Figure 2.7). The relative peak area at 2930 cm^{-1} amounted up to 36.5% in the FYM+NPK treatment, being lowest in the NPK alone and CON treatments (13 and 19% respectively). The relative peak area at 1530 cm^{-1} also varied across all four treatments, being smallest in the FYM+NPK and FYM treatments (1.3 and 1.4% respectively) and largest in the NPK treatment (1.8%). The relative peak area at 1159 cm^{-1} peak was with 10.8% largest in CON.

The subsequent characterization of pyrolyzed soil with DRIFTS revealed that pyrolysis caused reductions in the intensity of bands at the 2930 and 1530 cm^{-1} peaks and an increase in band intensity at 1159 cm^{-1} (see Figure S7).

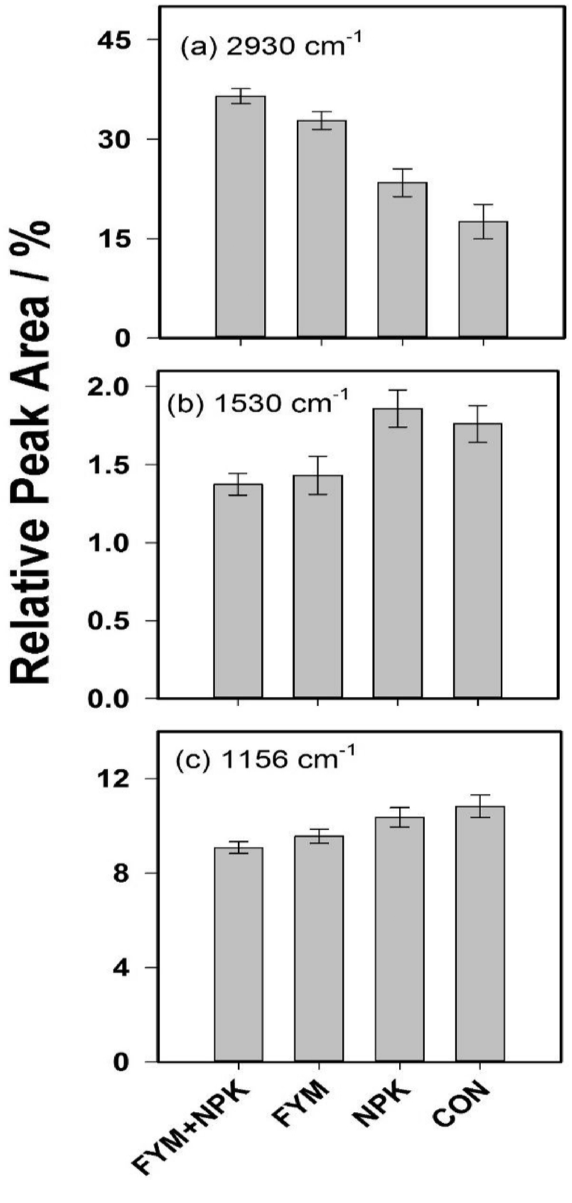

Figure 2. 7: DRIFTS relative peak area by fertilizer treatment and land use of bulk soil at wave-numbers: (a) 2930 cm^{-1} (C – H) (b) 1530 cm^{-1} (C = C) and (c) 1159 cm^{-1} (C – O – C) for Bad Lauchstädt with standard deviations (n=3). Treatment averages of years 1956, 1979, 1992, 2004 and 2008. FYM + NPK = farmyard manure and mineral fertilizer; FYM = farmyard manure; NPK = mineral fertilizer; CON = control (no fertilizer/manure inputs).

2.5 Discussion

2.5.1 Py-MIRS as a SOM fingerprinting technique

The acquisition of satisfactory Py-MIRS results was strongly dependent on the experimental conditions, i.e. temperature, heating rate and time. Thus the choice of preferred conditions was critical as these parameters influenced pyrolysis yield. The selection of the preferred experimental conditions was predominantly based on how these parameters influenced the product yield of the different standard compounds, as well as those of soil, while taking into consideration the chemical structures of the released compounds. Our tests showed that a faster heating rate led an increase in peak intensities. Additionally, while a heating time of 30 s seemed to favor the release of both aromatic and aliphatic groups, a longer heating time led to an increased production of aromatic groups and a simultaneous decrease in aliphatic groups in soils. A possible reason for the increased production of aromatic groups with a longer heating time is the occurrence of secondary reactions e.g. charring. However, care needs to be taken when determining the suitable heating time as the chemical structure of the compounds under investigation contributed significantly to the product yield as can be seen with our results of xylan and quebracho.

Furthermore, our results showed that pyrolysis temperature significantly influenced pyrolysis yield. While the intensity of peaks for some standard compounds at the lower temperature of 550 °C was higher, additional peaks appeared in the spectra of these compounds obtained at higher temperatures, an indication that evolution of some peaks was favored by pyrolysis temperature, e.g. the 3015 cm^{-1} peak in levoglucosan at 700 °C. We can surmise here that a pyrolysis temperature of 550 °C was not sufficient to fragment more resistant bonds. Considering the complex and heterogeneous nature of SOM, the objectives of our study and the influence of temperature on product yield, it was imperative that the choice of the preferred pyrolysis temperature be based on the results obtained for SOM. In contrast to the condensed tannin quebracho, the pyrolysis yields of aliphatics and aromatics of soils decreased with increasing temperature from 700 to 1000 °C, similar as with xylan. For soils, we thus identified a preferred pyrolysis temperature of 700 °C, heating time of 30 s and a heating rate of 20 °C ms^{-1}. A pyrolysis temperature of 700 °C has also been used in Py-GC/MS (Lu et al., 2011) and FTIR-EGA (Demyan et al., 2013) experiments. The heating time of 30 s is the same as that used in Py-GC/MS experiments by (Lu et al., 2011; Watanabe et al., 2005). Moreover, the sample amount subjected to pyrolysis affected the yield efficiency of pyrolysis products. During the pre-testing, a C content of 0.1 mg in the sample during each run resulted in unsatisfactory absorbance intensities (data not shown). The recommended sample weight was therefore set at ≥ 0.4 mg C (e.g. ~35 mg for soil samples) to achieve quantifiable Py-MIRS peaks.

Our study revealed little or no variation in the absorbance intensity of peaks at wavenumbers < 1000 cm^{-1}, where the strong influence of the soil mineral matrix is generally expected for DRIFTS (Nguyen et al., 1991). Py-MIRS analysis of clay minerals, e.g. sepiolite and allenite (Figure S8), also revealed no major interference from water peaks in the band at 1800-1300 cm^{-1} which may have affected the 1750 and 1510 cm^{-1} peaks. Hence, the Py-MIRS technique overcomes the limitations posed by mineralogical interferences, an important advantage over DRIFTS (Demyan et al., 2012). Py-MIRS is less time-consuming as runs take a few minutes to complete, unlike most Py-GC/MS runs which take longer (>1 hour). In addition, the analysis of Py-MIRS data is simplified and meaningful for the analyses of large sample numbers as a majority of retrieved peaks can be directly assigned to specific functional organic groups, and which were related to fertilizer management. Thus, Py-MIRS is particularly suitable for the analyses of large sample numbers, e.g. landscape SOM characterization or when extended time series are required. However, the Py-MIRS approach sacrifices a more detailed analyses of the molecular composition of SOM provided by Py-GC/MS, as it provides information on abundance of functional groups rather than individual molecular compounds.

A limitation associated with Py-MIRS is the detection limit of around 0.4 mg C in the sample during each run which might preclude its utilization in analysis of soils with low SOC contents using current setups. Another disadvantage of the Py-MIRS technique may be the efficiency of volatilization of some compounds within the sample, e.g. aromatic compounds, introducing bias when analyzing data, although similar limitations hold for Py-GC/MS.

2.5.2 Application of Py-MIRS to monitor SOM bulk chemistry

The developed Py-MIRS technique was successfully applied to bulk soils from Bad Lauchstädt and revealed striking differences in SOM bulk chemistry resulting from long-term fertilizer management. Comparing all four treatments, we observed that the SOM bulk chemistry was influenced by either presence or absence of organic and mineral fertilizer.

The larger peak area at 3015 cm^{-1} in the FYM treatments compared with NPK and CON treatments could partly be attributed to an enrichment of lignin and lignin-derived products from the straw supplied with FYM in SOM (De la Rosa et al., 2008; Lima et al., 2009). It was noted that the contribution of this peak was very low which is an indication these products do not constitute a major component of SOM (Kiem et al., 2000). Furthermore, our Py-MIRS results are in conformity with those obtained by DRIFTS and complement ^{13}C NMR spectroscopy results of Bad Lauchstädt soils by (Kiem et al., 2000) showing a decreased proportion in aliphatic groups (2930 cm^{-1}) in the CON treatment compared to the FYM+NPK treatment. However, aliphatic groups made a larger contribution to evaluated SOM compounds in DRIFTS (Figure 2.7) compared to Py-MIRS results (Figure 2.6), possibly due to mineral interferences associated with DRIFTS with overlapping organic/mineral bands at lower wavelengths. Nevertheless, relative fertilizer differences remained the same between the two methods.

The increasing normalized peak area at 1750 cm^{-1} in the NPK and CON treatments could be attributed to the enhanced decomposition of residual soil organic residues leading to the accumulation of more humified organic material in these treatments (Kiem et al., 2000). Our Py-MIRS results are complemented by ^{13}C NMR spectroscopy results of (Knicker and Lüdemann, 1995) of increased relative intensity of carboxyl-C with OM degradation of plant materials during incubation. Additionally, XANES spectroscopy results by (Gregorich et al., 2015) showed an increase in the carboxyl content found in soils under fallow treatment compared to pasture soils.

Py-MIRS revealed that the absence of organic inputs in led to the relative enrichment of aromatic groups in the peak at 1510 cm^{-1}. An enrichment of aromatic groups in unfertilized treatments compared with fertilized treatments has been found also using ^{13}C NMR spectroscopy by Kiem et al. (2000), likewise by our DRIFTS results, hence supporting our Py-MIRS results. Similarly, the absence of organic inputs led to an increase in the normalized peak area of the 1176 and 950 cm^{-1} peaks in the NPK and CON treatments compared to the FYM manure treatments as also shown by our DRIFT results. The decline in polysaccharides and ethers can be attributed to the enhanced biodegradation of residues in FYM treatments leading to a preferential loss of polysaccharides and the accompanying accumulation of aromatic groups as suggested by Dignac et al. (2005).

2.6 Conclusions

Py-MIRS has been developed to fingerprint SOM bulk chemistry in soils with different fertilizer management practices. The results showed that the yield efficiency of pyrolysis products was highly sensitive to and varied with the chemical structure of the sample (e.g. carbohydrates, proteins, soils, etc.) as well as experimental conditions like the pyrolysis temperature, heating rate and heating time. Our tests showed that a faster heating rate led an increase in peak intensities. Additionally, while a heating time of 30 s seemed to favor the release of both aromatic and aliphatic groups, a longer heating time led to an increased production of aromatic groups and a simultaneous decrease in aliphatic groups in soils. Pyrolysis temperate also had varied effects on pyrolysis yield for different compounds and functional groups. For soils the pyrolysis yields of aliphatics and aromatics decreased with increasing pyrolysis temperature from 700 to 1000 °C, similar as with xylan but in contrast to quebracho. Thus, the preferred conditions for the successful application of the Py-MIRS technique in soils included (i) a pyrolysis temperature of 700 °C, (ii) a rapid heating rate of 20 °C ms^{-1} and (iii) pyrolysis heating time of 30s.

Py-MIRS analysis of bulk soils permitted the detection of fertilizer management effects on SOM bulk chemistry in a similar order as DRIFTS, although peak area magnitude of some groups differed. Py-MIRS results provided evidence that soil mineral matrix interference was largely avoided thereby enhancing its sensitivity. However, further testing of the Py-MIRS approach on soils with different clay mineralogies and land use is required to verify the applicability of this technique to a broad range of ecosystems. While the results from our current study were based on the abundance of different C functional groups normalized to the C content of the samples in order to draw distinctions between the different treatments, the potential of this technique is not yet fully utilized. Hence, further research is needed to develop a more detailed quantification of compounds in the different functional groups identified. To achieve these goals and enable the analysis soil samples with low C contents, we suggest to prospectively develop a 2-step continuous pyrolysis process considering a sequence of ascending pyrolysis temperatures suitable to analyze such sample sets. In conclusion, Py-MIRS was introduced here as a semi-quantitative, rapid, effective and reproducible technique to characterize SOM composition. Thus, Py-MIRS is particularly suitable for the analyses of large sample numbers, e.g. landscape SOM characterization or when extended time series are required.

Acknowledgements

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2.7 Appendix 1: Supplementary information

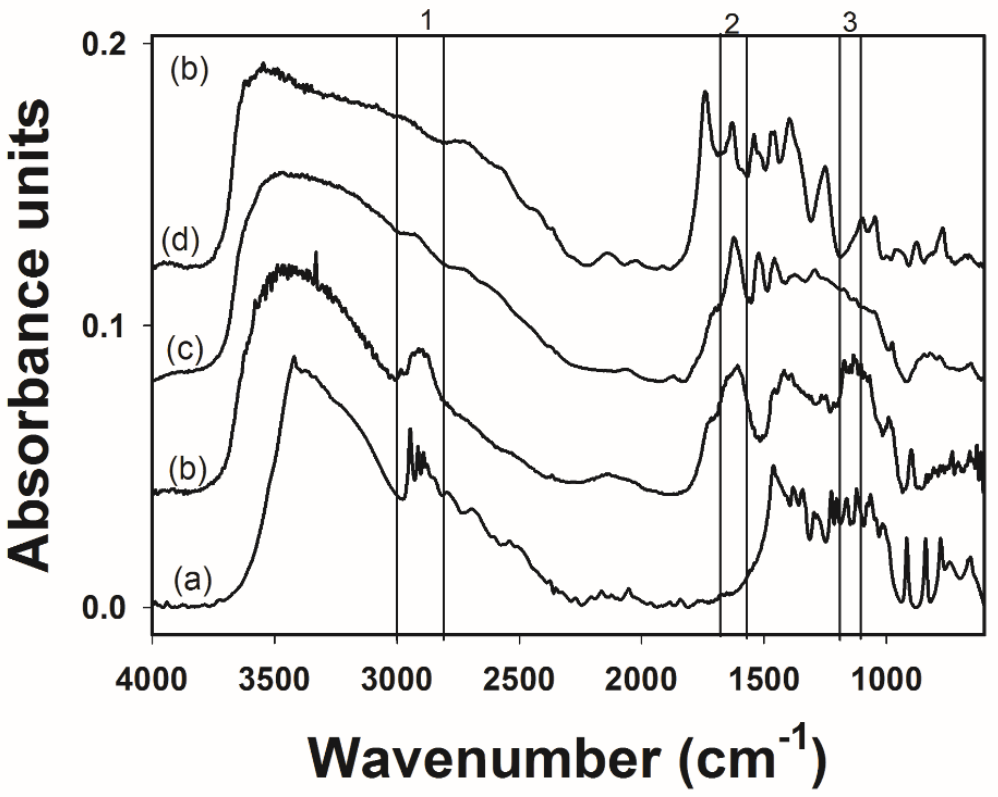

Figure S1: **DRIFTS spectra of organic substances: (a) glucose, (b) xylan, (c) tannic acid, and (d) quebracho. The indicated regions were assigned to the following molecular vibrations: 1: 2930 cm^{-1} (C – H); 2: 1530 cm^{-1} (C = C); 3: 1176 cm^{-1} (C – O).**

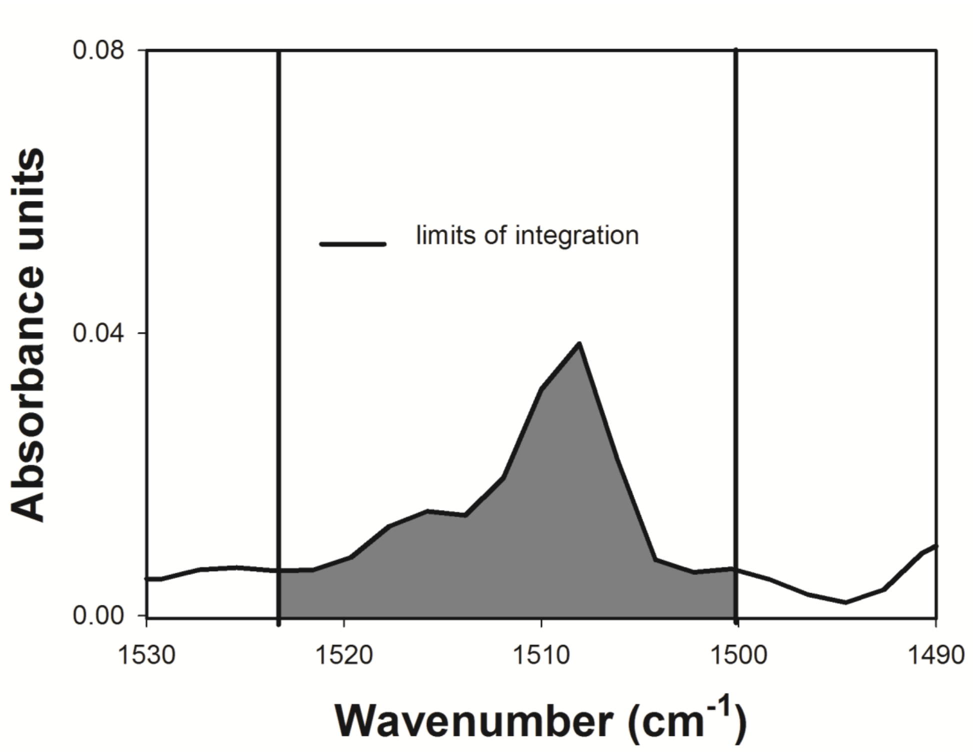

Figure S2: **Illustration of peak area integration performed between the left and right defined integration limits using zero absorbance as the baseline.**

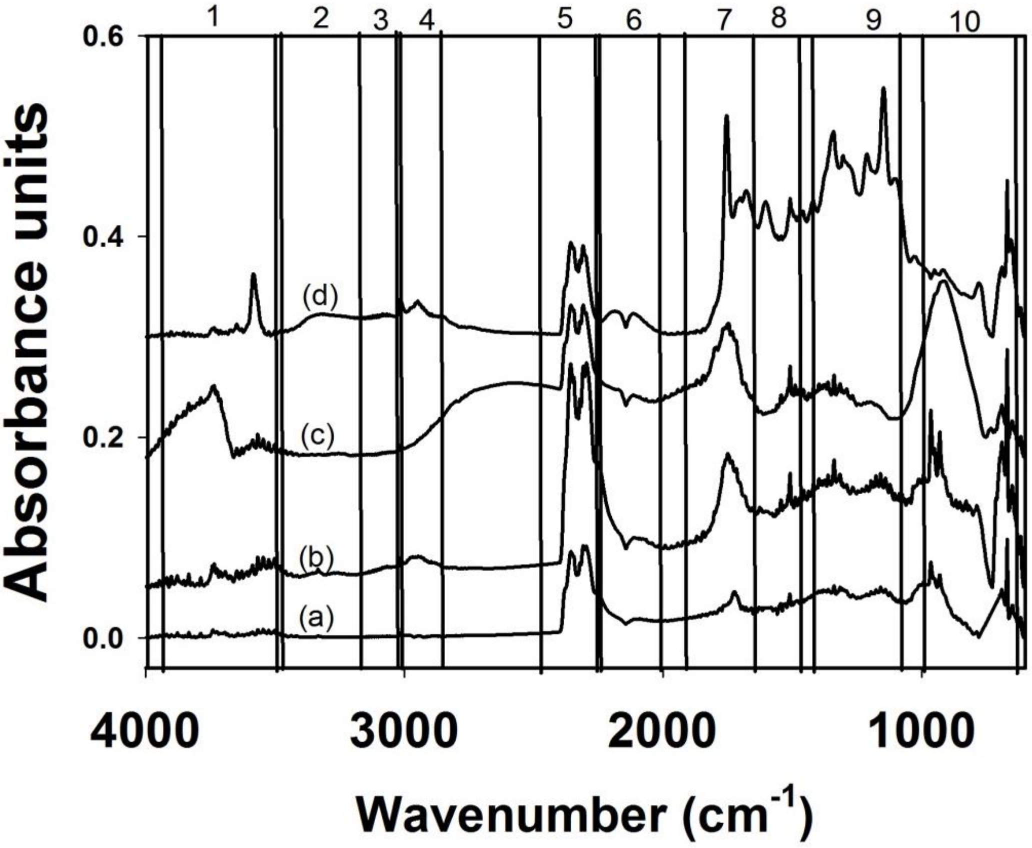

Figure S3: Py-MIRS spectra of organic substances pyrolyzed from 100 to 700°C at 20 °C ms^{-1} for 30 s (a) serine, (b) gliadin, (c) chitin, and (d) syringic acid. The indicated group frequencies were assigned to the following functional groups: 1: 4000-3400 cm^{-1} (O – H stretch and CO_2), 2: 3400-3200 cm^{-1} (N – H stretch), 3: 3200-3000 cm^{-1} (aromatic C – H stretch), 4: 3000-2800 cm^{-1} (aliphatic C – H stretch), 5: 2400-2200 cm^{-1} (CO_2), 6: 2250-2000 cm^{-1} (CO), 7: 1800-1600 cm^{-1} (C = O stretch), 8: 1650-1480 cm^{-1} (aromatic C = C stretch), 9: 1400-1100 cm^{-1} (O – H and C – O stretch) and 10: 1050-700 cm^{-1} (aromatic C – H stretch). Normalized peak intensity in absorbance units (peak area) divided by sample weight.

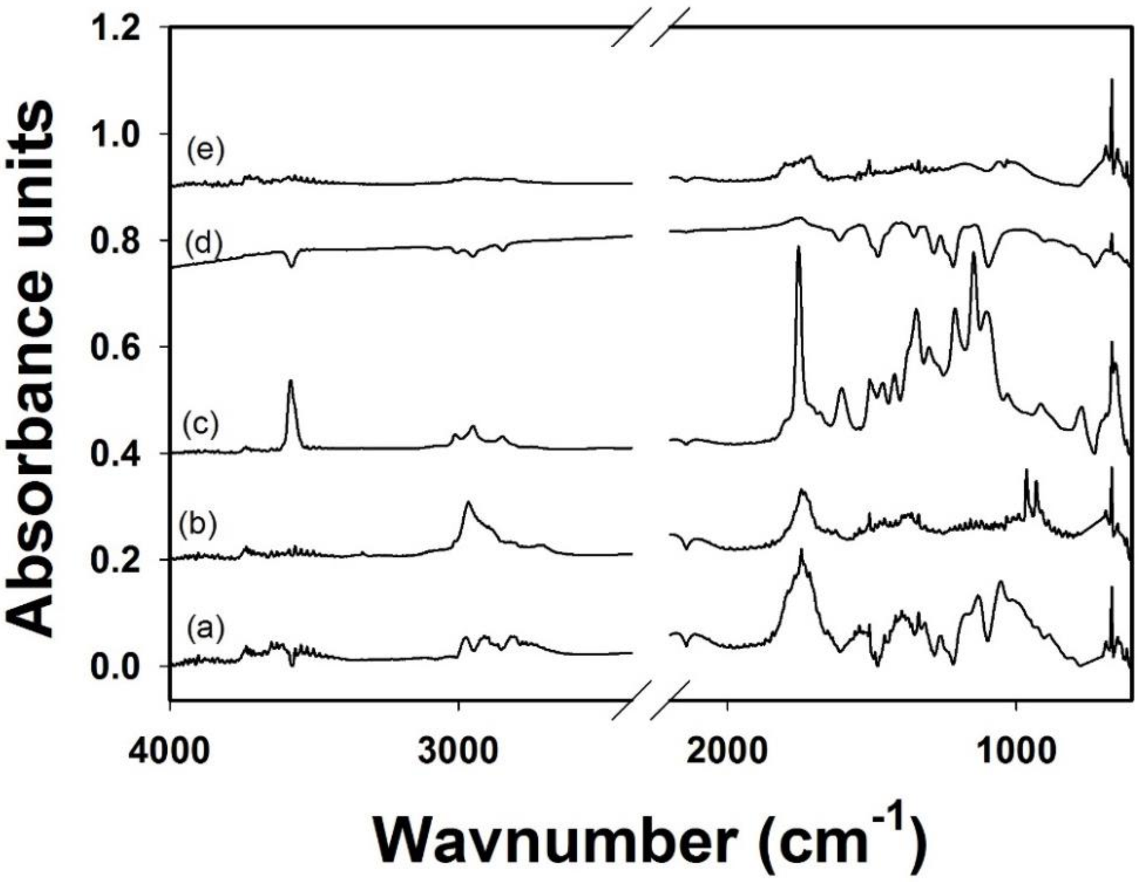

Figure S4: **Py-MIRS spectra of organic substances pyrolyzed from 100 to 550°C at 20 °C ms^{-1} for 30 s (a) levoglucosan, (b) leucine, (c) syringic acid, (d) syringol, (e) pectin. Note: The main CO_2 peak (2400-2200 cm^{-1}) was cut to make the other peaks more visible.**

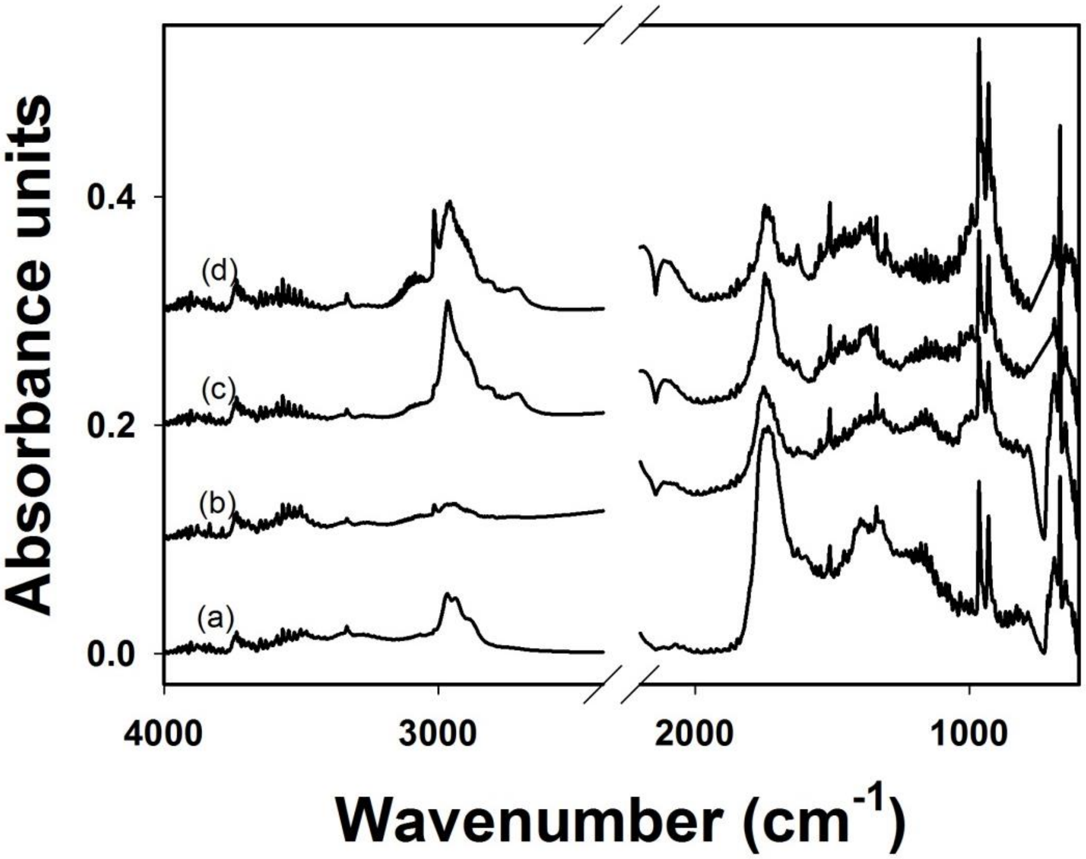

Figure S5: Py-MIRS spectra of organic substances pyrolyzed 20 °C ms^{-1} for 30 s; (a) gliadin at 550 °C, (b) gliadin at 700 °C, (c) leucine at 550 °C, (d) leucine at 700 °C. Note: The main CO_2 peak (2400-2200 cm^{-1}) was cut to make the other peaks more visible.

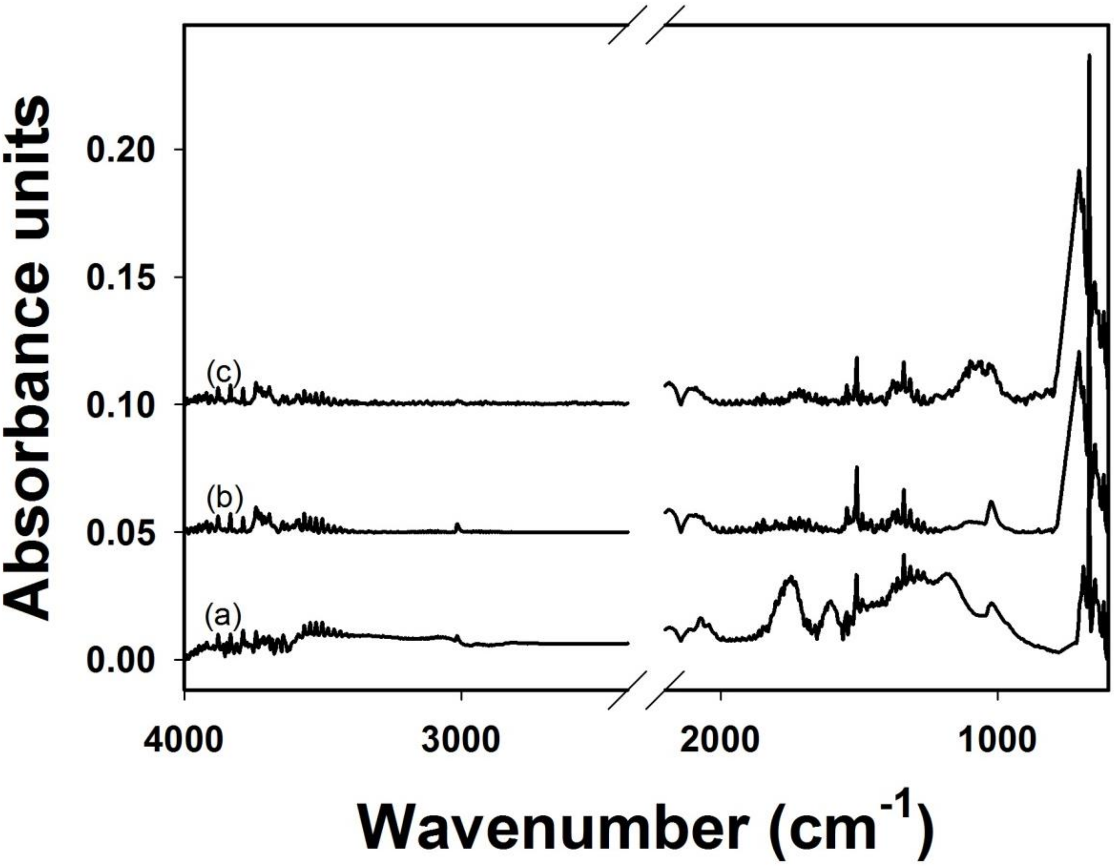

Figure S6: Py-MIRS spectra of quebracho 20 °C ms^{-1} for 30 s (a) 550 °C, (b) 700 °C, (c) 1000 °C. Note: The main CO_2 peak (2400-2200 cm^{-1}) was cut to make the other peaks more visible.

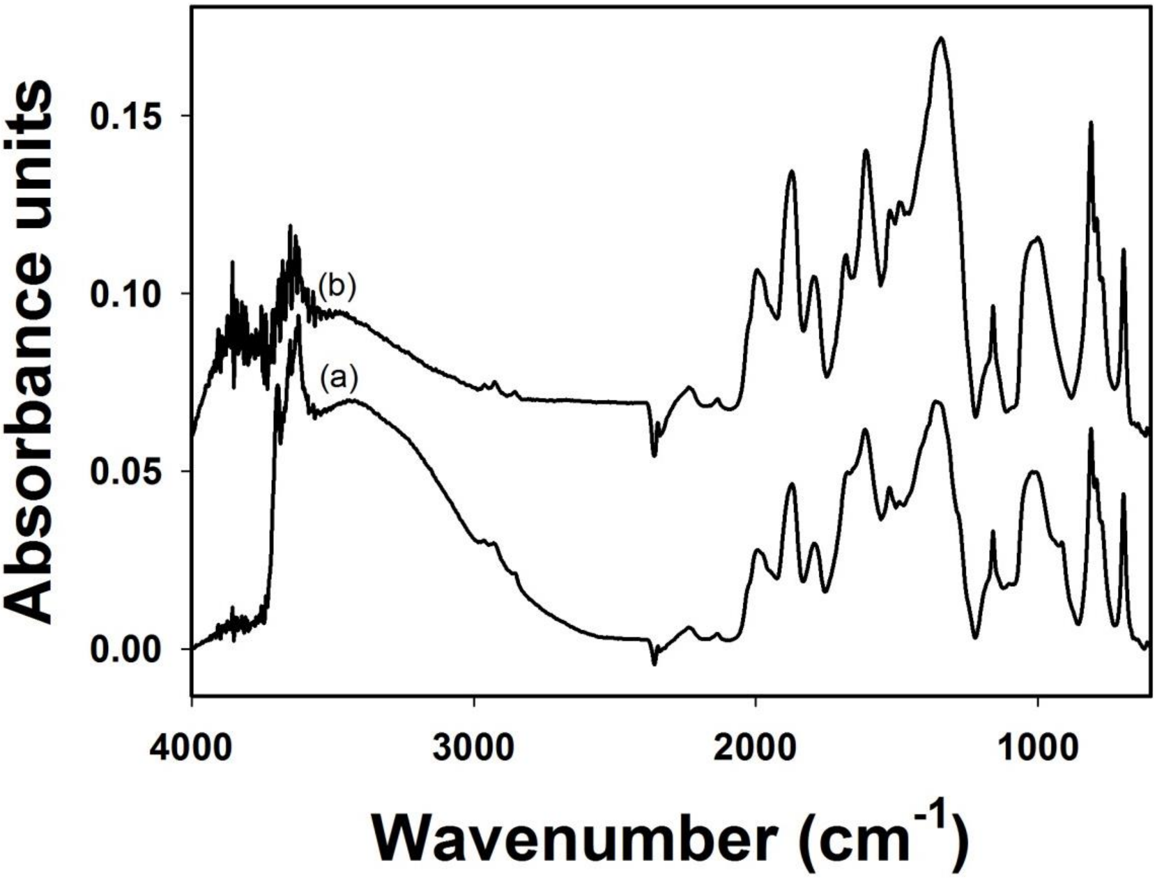

Figure S7: **DRIFTS spectra of (a) unpyrolyzed soil and (b) pyrolyzed soil from Bad Lauchstädt (FYM).**

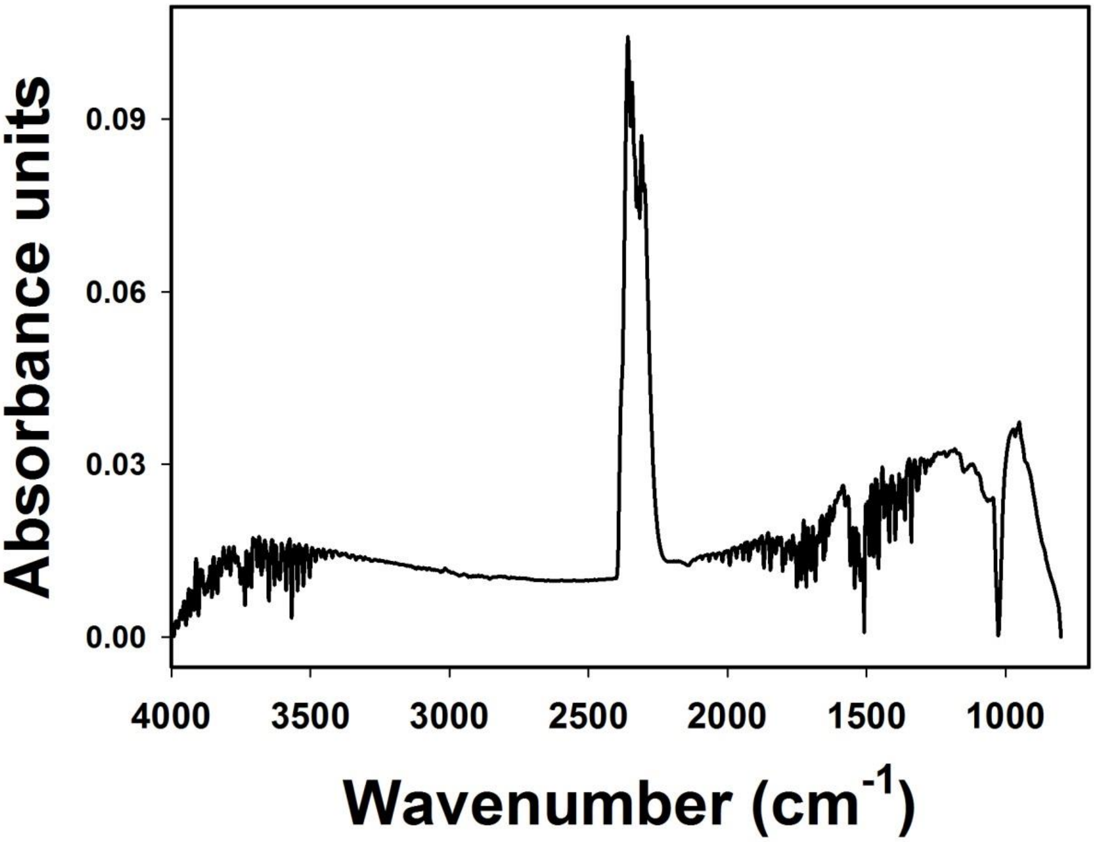

Figure S8: Py-MIRS spectra of sepiolite pyrolyzed from 100 to 550 °C at 20 °C ms^{-1} for 30 s.

Chapter 3 Pyrolysis-mid-infrared spectroscopy (Py-MIRS) reveals management and land use effects on soil organic matter molecular composition

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3.1 Abstract

This study aimed to evaluate the suitability of the newly developed pyrolysis-mid-infrared spectroscopy (Py-MIRS) technique to determine the effect of long-term management and land use practices on soil organic matter composition. Samples were taken from two long-term field experiments: (i) Ultuna, Sweden, from treatments farmyard manure (FYM), control without fertilizer inputs (CON) and continuous bare fallow (BF), and (ii) Lusignan, France, from arable land, grassland and grassland converted to arable land and analyzed by both Py-MIRS and DRIFTS. Results of both methods from the Ultuna soils showed an increase in the relative abundance of aliphatic C and methoxy groups in the FYM treatment, while in BF an increase in C = O, C = C, C – O, and =C – H groups occurred consistent with a lesser and increased stage of organic matter degradation respectively. Similarly, results of the Lusignan soils showed an increase in the carboxyl, aromatic, polysaccharide, and alkene content in the arable land compared to the grassland with larger inputs and less disturbance. Hence, Py-MIRS is, therefore, a suitable rapid semi-quantitative measure of the SOM compositional changes associated with agricultural management and land-use practices. Nevertheless, a lower sensitivity of the Py-MIRS-based ratios of C- to O-containing functional groups compared to the sensitivity of similar DRIFTS-based ratios indicates the need for further research to improve the functionality of Py-MIRS in terms of experimental settings. This will contribute to the increased understanding of SOM quality and dynamics in contrasting soil types subjected to different agricultural management practices and land use.

Keywords: Pyrolysis-MIRS, SOM composition, management, land-use, decomposition status

3. 2 Introduction

Soil organic matter (SOM) is a major component of the global carbon (C) and it is associated with soil fertility and quality. Its sustainable maintenance is vital as SOM mediates soil physical, chemical, and biological properties which in turn affect soil functions like providing a substrate for microbial activity, acting as a nutrient reservoir, and preserving the environment (Albrecht et al., 2014; Ding et al., 2002; Lal, 2004). Agricultural management practices such as fertilization, tillage as well as certain land use systems like grasslands are known to significantly influence SOM amount and quality (Antil et al., 2005; Ellerbrock and Gerke, 2016; Kaiser et al., 2007). Investigations on the influence of the aforementioned practices on SOM have mostly utilized long-term experiments since significant effects on SOM resulting from management are noticeable only after relatively long periods (Kaiser et al., 2007). The assessment of SOM composition as well as its alterations resulting from management practices and land use (and changes) should not only include the total OC stocks but should take into consideration the changes in the SOM molecular structure or bulk chemistry (Helfrich et al., 2006).

Several analytical techniques have been utilized to investigate differences in SOM like Fourier Transform Infrared (FTIR) spectroscopy (Demyan et al., 2012), pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) (Rumpel et al., 2009), nuclear magnetic resonance (NMR) spectroscopy (Kiem et al., 2000). However, only a few of these techniques are used routinely due to several methodological limitations frequently reported in literature. For example, the successful application of DRIFTS has over the years offered a means to investigate SOM quantity, composition, and turnover (Demyan et al., 2012; Tatzber et al., 2008; Toosi et al., 2017). Soil quality indicators derived using DRIFTS (e.g.

aliphatic and aromatic C contents) have elucidated the decomposition and transformation of SOM as influenced by agricultural management (Baldock et al., 1992; Demyan et al., 2012; Ellerbrock et al., 1999; Quideau et al., 2000; Veum et al., 2014). However, quantification of organic functional groups in DRIFTS spectra is often hampered by strong mineral signal interferences (Demyan et al., 2012) limiting its wider applicability.

This study focuses on the use of pyrolysis coupled with mid-infrared spectroscopy (Py-MIRS) to evaluate and elucidate SOM composition as influenced by management and land use. The Py-MIRS technique consists of a programmed heating employed to determine the organic components or functional groups contained in SOM (e.g. carboxylic groups) that are volatilized during the thermal degradation of OM in the samples under investigation. Nkwain et al. (2018) reported that the newly developed Py-MIRS technique can be used to effectively fingerprint bulk SOM chemistry by characterizing different organic compounds that are sensitive to management. Despite the advantages of Py-MIRS over other analytical techniques like DRIFTS, recommendations from this report stated that further investigation is required to verify the applicability of this technique to samples from different ecosystems and clay mineralogy.

In the present study, it was hypothesized that Py-MIRS and DRIFTS will yield complementary information in relation to the composition of SOM in response to management. The objectives were (i) to identify specific Py-MIRS peaks representing organic compounds that reflect changes in SOM composition, (ii) to investigate changing SOM composition under long-term agricultural management practices, land use systems, and alterations in land use. It has been suggested that since SOM analytical techniques are susceptible to limitations, like incomplete pyrolysis in the case of Py-MIRS (Nkwain et al., 2018), a combination of techniques that complement each other should be utilized

(Albrecht et al., 2014; Poirier et al., 2005). In this study, we used alternatively DRIFTS (Demyan et al., 2012) to evaluate SOM composition, establish the pertinence of Py-MIRS organic functional groups to reflect SOM composition, and finally to verify the applicability of Py-MIRS to evaluate SOM in soils from different ecosystems.

3.3 Materials and Methods

3.3.1 Soils

Soils were taken from two long-term experimental sites differing in agricultural management practices and land use (Table S1). The first study site was the Frame Experiment at Ultuna, Sweden (59°49’N, 17°38’E) established in 1956. Archive soil samples analyzed were from 1956, 1979, 1995, and 2005 and consisted of treatments of continuous bare fallow (BF), farmyard manure (FYM) (2 Mg C ha^{-1} yr^{-1}), and control (CON) which was cropped but did not receive any fertilizer (Kirchmann et al., 1996). The soil contained 36.5% clay and was classified as Eutric Cambisol. The second study site was the long-term Observatory for Environmental Research near Lusignan, France (46°25′N; 0°07′E) initiated in 2004 (Rumpel et al., 2009). The soil was classified as Dystric Cambisol (IUSS Working Group WRB 2007) with a loamy texture. The treatments used were arable land (AA), continuous grassland (GG), and grassland converted to arable land (GA). Soil samples were collected from a six-year-old grassland before it was converted to arable land and one year after the conversion of grassland to arable land.

3.3.2 Elemental analysis

CHN auto-analyser (CHN NA 1500, Carlo Erba) was used to determine the total organic carbon and nitrogen in soil samples by the dry combustion method.

3.3.3 Py-MIRS analysis

Py-MIRS analysis was performed according to the routine published by Nkwain et al. (2018). Py-MIRS spectra were recorded on a Tensor-27 Fourier transform mid-infrared spectrometer (Bruker Optik GmbH, Ettlingen, Germany) interfaced to a pyrolyzer (CDS Analytica, Oxford, Pennsylvania, USA) utilizing a Brill cell$^{\mathrm{TM}}$ (CDS Analytica). A mass flow communicator (MFC) connected to the flow controller of the pyrolyzer was used to monitor the flow rates of helium (99.96 % purity) used to provide inert conditions and to purge the Brill cell before and after each pyrolysis run. Prior to the analysis, ball-milled bulk soil samples were dried overnight at 32 °C. Approximately 35 mg bulk soil sample was weighted into an open-ended quartz tube fixed by loosely fitted quartz wool on both ends and placed in the filament coil of the pyroprobe. Following the insertion of the pyroprobe containing the sample into the Brill cell, the sample was heated to 700 °C for 30 s at 20 °C ms^{-1}. The spectra were recorded in absorbance units (AU) at a wavenumber resolution with a scan recorded every 0.3 s. Samples were analyzed in triplicate to ensure the reproducibility of Py-MIRS measurements. Spectral pre-processing included baseline correction, averaging, and peak area integration using the OPUS 7.5 processing software (Bruker Optik GmbH, Ettlingen, Germany).

The Py-MIRS soil spectra were assigned the following characteristics (Table 3.1, Figures 3.1, S1, and S2): The band at 4000 - 3400 cm^{-1} was assigned to O – H stretching vibrations (Gu et al., 2013). The absorbance in the wavenumber range 3200 - 3000 cm^{-1} was assigned to aromatic C – H stretching vibrations while the sharp peak at 3015 cm^{-1} indicated the presence of methane (CH_4) derived from methoxy groups of lignin and lignin-derived products (Court and Sephton, 2009; Feng et al., 2006). The prominent peak at 2930 cm^{-1} was assigned to C – H vibrations of aliphatic compounds (Court and Sephton, 2009). The bands 3700 - 3600, 2400 - 2200, and 700 - 600 cm^{-1} were assigned to carbon dioxide (CO_2) (Court and Sephton, 2009; Gu et al., 2013), while the band at 2250 - 2000 cm^{-1} was assigned to carbon monoxide (CO) (Fu et al., 2009). The peak at 1750 cm^{-1} was from the C = O stretching vibrations of carboxylic acid, aldehydes, and ketones (Feng et al., 2006). Aromatic C = C stretching vibrations were identified by the peak at 1510 cm^{-1} (Yang et al., 2007). The peak at 1176 cm^{-1} (Feng et al., 2006) was assigned to C – O stretching vibrations of polysaccharides, ethers, and alcoholic groups (Court and Sephton, 2009). The band at 1050 - 700 cm^{-1} with the double peak centered at 950 cm^{-1} was assigned to aromatic C – H vibrations of alkenes (Court and Sephton, 2009). Only the peaks recorded at 3012, 2930, 1750, 1510, 1159, and 950 cm^{-1} were used for further investigations in this study (Table 3.1).

Visual comparison of the Py-MIRS spectra between the fertilizer treatments and land use systems was found to be difficult as there were no major differences in the peak pattern (Figures 3.1, S1, and S2). We used the peak area integration approach (Figure S3) to permit quantitative comparisons of the Py-MIRS spectra among the different fertilizer treatments and land use systems. For the peak area integration, the upper and lower boundaries of the identified peaks were used to calculate the corrected peak area (PA_c). To permit the estimation of the relative C contents of each functional group, the PA_c of each functional group was related to soil C concentrations (mg^{-1} C) to obtain the normalized peak area (PA_n) following Eq. (1):

$$PA_n = {}^{PAc}/_{\text{Sample weight x C content}} \qquad (1)$$

Where C content is the relative OC content (mg g^{-1} soil) of the sample measured by dry combustion.

Table 3. 1: Investigated Py-MIRS peak areas and functional group assignments in soil samples

Wavenumber (cm^{-1})	**Integration limits (cm^{-1})**	**Functional group assignment**
3015	3200 - 3000	CH_4[a]
2930	3000 - 2800	Aliphatic C – H vibrations[a]
1750	1850 - 1665	C = O stretching vibrations[b]
1510	1529 - 1495	Aromatic C = C stretching vibrations[c]
1176	1250 - 1130	C – O vibrations of polysaccharides, ethers, and alcoholic groups[a]
950	1050 - 700	Aromatic = C – H vibrations (alkenes)[a]

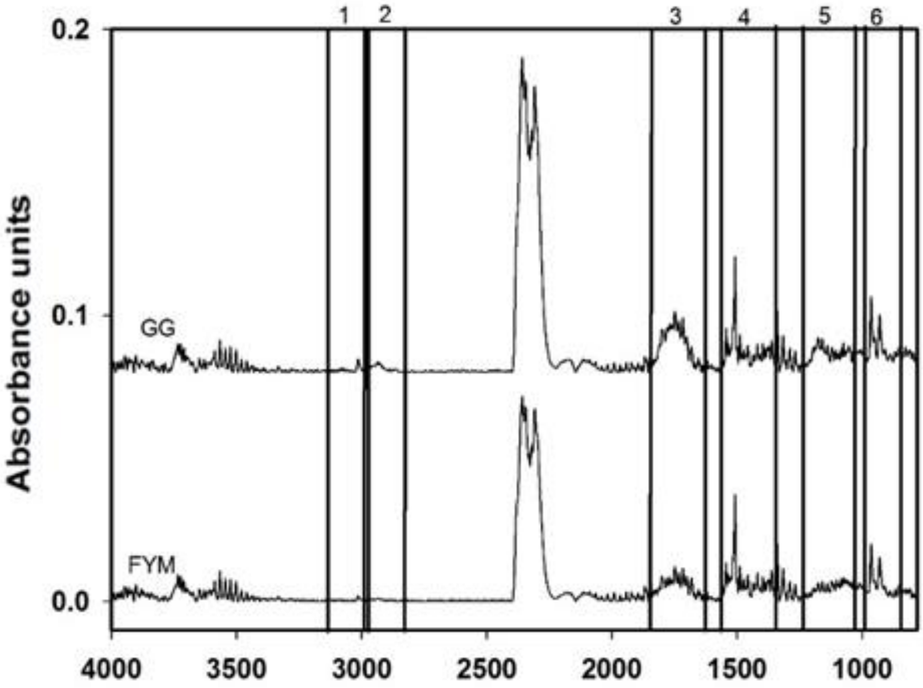

Figure 3. 1: Py-MIRS spectra of bulk soil under different fertilizer management and land use farm yard manure (FYM) from the Ultuna Frame experiment and grassland GG system from the Lusignan experiment. The indicated regions were assigned to the following functional groups: 1: 3015 cm^{-1} (CH_4); 2: 2930 cm^{-1} (C – H), 3: 1750 cm^{-1} (C = O); 4: 1510 cm^{-1} (C = C); 5: 1176 cm^{-1} (C – O); 6: 950 cm^{-1} (=C – H) (see also Table 3.1).

Peak area ratios that represent the decomposition status and recalcitrance of SOM commonly used in spectroscopic studies (Demyan et al., 2012; Ding et al., 2002; Gerzabek et al., 2006; Veum et al., 2014) were used in our Py-MIRS study to elucidate the impacts of fertilization and land use on the status of SOM. Three Py-MIRS peak area ratios were calculated using Eq. 2-4. Index 1, determines the ratio of aromatic to aliphatic functional groups or aromaticity and has been reported to increase with increasing decomposition and maturity SOM (Demyan et al., 2012; Margenot et al., 2015; Veum et al., 2014). Indices 2 and 3 determine the ratio of C (C = C and C – H, respectively) to O- functionality (C = O and C – O). Reports from previous studies show that the proportion of O-containing functional groups decrease relative to the total C content with a corresponding increase in recalcitrance and a decrease in biological activity (Toosi et al., 2017; Veum et al., 2014).

Index 1: $\frac{1510}{2930}$ (aromaticity) (2)

Index 2: $\frac{1510}{1750 + 1176}$ (aromatic C- to O-functionality) (3)

Index 3: $\frac{2930}{1750+1176}$ (aliphatic C- to O-functionality) (4)

3.3.4 DRIFTS analysis

Ball milled bulk soil samples (<2mm) and Py-MIRS sample residues were dried overnight at 32 °C before DRIFTS analysis. DRIFTS spectra were recorded on a Tensor-27 Fourier transform infrared spectrometer using a liquid nitrogen-cooled mercury-cadmium-telluride detector and a potassium bromide (KBr) beam splitter (Bruker Optik GmbH, Ettlingen, Germany). The spectrometer was mounted with a Praying Mantis diffuse reflectance chamber (Harrick Scientific Products, New York, NY, USA) and the spectra were recorded in the mid-infrared range from 4000 - 400 cm^{-1} in absorbance units (AU). Pre-processing of acquired spectra using the spectral processing software OPUS 7.5 (Bruker Optik GmbH, Ettlingen, Germany) included atmospheric compensation, baseline correction, and vector normalization and peak area integration to obtain the corrected peak area (PA_c). We used the peak area approach to permit quantitative comparison between samples from different agricultural management practices and land use (Demyan et al., 2012). Figure 3.2 shows an example of a DRFITS spectrum of bulk soil (see also Figure S4). The peaks used for investigations in this study focused on four infrared bands indicated in Figure 3.2, including peaks at 2929 cm^{-1} assigned to C – H vibrations, 1620 cm^{-1} for aromatic C = C and/or $-COO^-$ stretching vibrations, 1528 cm^{-1} for aromatic C = C stretching vibrations and 1159 cm^{-1} assigned to C – O bonds of both poly-alcoholic, and ether groups (Baes and Bloom, 1989; Demyan et al., 2012; Spaccini and Piccolo, 2007). The relative peak area (PA_r) was calculated by dividing the corrected peak area (PA_c) for each peak by the sum of the area of the four peaks investigated and multiplied by 100 (Eq. 5) The PA_r is used to assess how peak areas change relative to one another (Niemeyer et al., 1992)

$$PA_r = {PAc}/{\text{Sum of } PAc} * 100 \qquad (5)$$

Similar to Py-MIRS, the peak ratios of (1620+1528) / 2929 (aromaticity), (1620+1528) / 1159, and 2929 / 1159 (C- to O- functionality) which represent three widely used indices were calculated to explain the decomposition state of SOM and relate the differences in organic functional groups with soil quality (Demyan et al., 2012; Toosi et al., 2017).

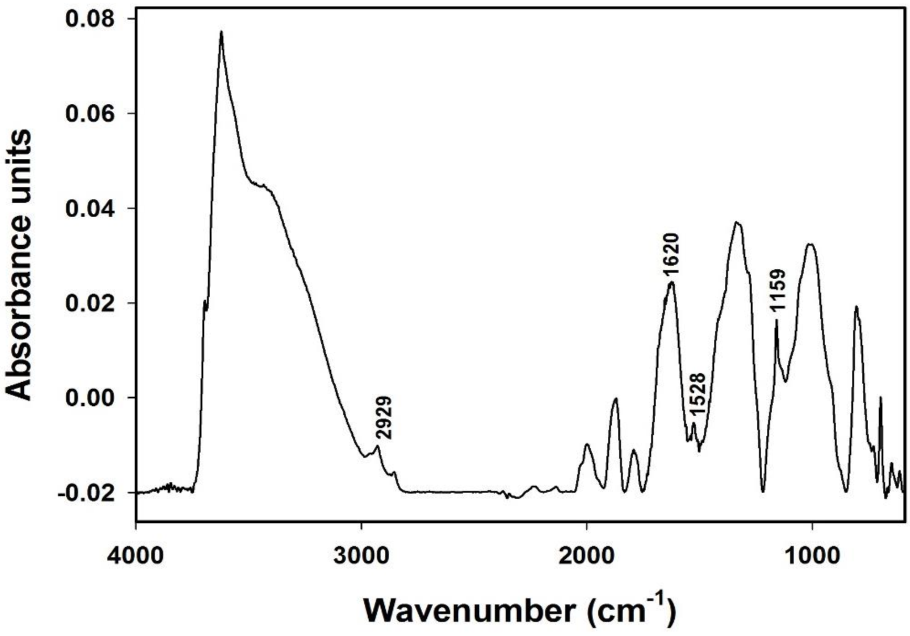

Figure 3. 2: Representative baseline corrected DRIFTS spectrum of bulk soil. The indicated peaks were assigned to the following molecular vibrations for peak area integration: 1: 2929 cm^{-1} (C – H), 2: 1620 cm^{-1} (COO- / C = C), 3: 1528 cm^{-1} (C = C) and 4: 1159 cm^{-1} (C – O).

3.3.5 Statistical analysis

The R software (R 4.0.3, R foundation Statistical Computing, Vienna, Austria, (https://www.r-project.org/) was used to determine significant differences due to the effects of fertilization as well as land use ($P < 0.05$). Mean comparisons of results were made by a one-way analysis of variance (ANOVA) using the Tukey range test. All data were checked for normality and no data transformations were performed. Principal component analysis (PCA) was also performed on the Py-MIR data to study in more detail the changes in Py-MIRS SOM composition resulting from fertilizer management and land use. The selected SOM functional groups and decomposition index ratios were included in the multivariate ordination order to provide information on key functional groups influencing ordination on PCA.

3.4 Results

3.4.1 Soil organic C and N

Table 3.2 shows the total organic C (TOC) and total nitrogen (TN) values of bulk soils from the Ultuna and Lusignan experiments. Among the fertilization practices in the Ultuna experiment, the soils receiving FYM inputs showed a significantly greater TOC ($P < 0.05$) relative to the CON and BF treatments. Compared with the TOC value of 1956, the CON and BF treatments lost over 28% and 38% respectively of their original OC, while the FYM treatment showed an increase in the OC levels (approximately 28%) after 49 years (Figure. S5).

Similarly, the TN values in the FYM treatment were significantly larger than in the CON and BF treatments respectively ($P < 0.05$). In Lusignan soils, AA soils had significantly smaller TOC and TN contents compared with the other systems, GG and GA. The conversion of grassland to arable land (GA) led to a slight but significant loss of OC (about 9.4%).

Table 3. 2: Average values of TOC, TN, and CN ratio (±standard errors) of bulk soil from Ultuna and Lusignan. Data for the Ultuna experiment are mean values from 1979, 1995, 2005 for the Ultuna experiment and 2008 for the Lusignan experiment

Site	Treatment	TOC/ mg g^{-1}	TN/ mg g^{-1}	CN
Ultuna[1]				
	FYM	19.8 (0.14)^{c}	2.1 (0.02)^{c}	9.6 (0.08)^{c}
	CON	11.7 (0.14)^{b}	1.2 (0.02)^{b}	9.4 (0.08)^{b}
	BF	10.3 (0.14)^{a}	1.0 (0.02)^{a}	9.1 (0.08)^{a}
Lusignan[1,2]				
	AA	11.2 (1.6)^{a}	1.2 (0.2)^{a}	9a
	GA	16.3 (2.0)^{b}	1.7 (0.2)^{b}	10^{b}
	GG	18.0 (1.4)^{b}	1.7 (0.1)^{b}	10^{b}

[1] letter within columns indicates significant differences at $P < 0.05$.
[2] Data from (Rumpel et al., 2009)
Treatment codes: FYM = farmyard manure; CON = control (no fertilizer/manure inputs); BF = bare fallow; AA = arable land; GG = grassland; GA = grassland to arable land.

3.4.2 Py-MIRS analysis

The changes in the normalized peak areas (PA_n) of the selected peaks representing SOM components varied significantly among the different fertilizer treatments and land use systems in the Ultuna and Lusignan experiments respectively (Fig. 3.3). It was observed that the PA_n of the C = O (1750 cm^{-1}) and =C – H (950 cm^{-1}) peaks contributed most to the SOM composition in both experiments, with moderate contributions from the CH_4 (3015 cm^{-1}) and aliphatic C – H (2930 cm^{-1}) peaks. The proportions of CH_4 and C – H peaks were significantly larger in the FYM treated soils system ($P < 0.05$) of the Ultuna experiment. The GG treatment of the Lusignan experiment showed a distinguishably (but not significant) a larger proportion of C – H peak area as well as a slightly larger proportion of the CH_4 peak. The non-FYM (CON and BF) treatments showed significantly higher PA_n of the C = O, C = C, C – O, and =C – H peaks compared to the FYM treatment ($P < 0.05$) in the Ultuna experiment, and the AA land use system in the Lusignan experiment although these differences were only significant for the C = C and =C – H peaks ($P < 0.05$). A significant positive relationship was found between the sum of peak areas of six Py-MIRS peaks investigated and the TOC content in the Ultuna ($R^2 = 0.73$, $P = <0.001$) and Lusignan ($R^2 = 0.75$, $P = 0.002$) (Figure S5). Temporal Py-MIRS data further revealed a decrease in the 3015 cm^{-1} PA_n from 1979 to 2005 in Ultuna soils (Figure S6). These results are consistent with the declining TOC in the CON and BF treatments (Figure S7) and with those of (Barré et al., 2016) who examined the energetic and chemical signatures of SOM in the BF treatment in Ultuna.

The Py-MIRS-based indices (Index 1-3) describing SOM decomposition and recalcitrance differed significantly between the different fertilizer treatments ($P < 0.05$) (Table 3.3). These indices showed a consistent pattern with fertilizer management and land use system; indices 1 and 2 followed the pattern of BF > CON > FYM and AA > GA > GG in the Ultuna and Lusignan experiments respectively. In contrast, index 3 showed the opposite pattern.

Table 3. 3: Py-MIRS based indices of decomposition and recalcitrance of SOM by fertilizer management and land use. Data are means (*n* = 3)

Site	**Treatment / Land use**	**Py-MIRS based indices** **Index 1**[2]	 **Index 2**[3]	 **Index 3**[4]
Ultuna				
	FYM	1.81a[1]	0.21a	0.12b
	CON	5.10b	0.30b	0.07a
	BF	5.82b	0.33c	0.06a
Lusignan				
	GG	1.80a	0.16a	0.09b
	GA	2.08a	0.17a	0.08ab
	AA	3.36b	0.27b	0.07a

[1] Different letters within columns indicate a significant difference at $P < 0.05$.
[2] Index 1:1510 / 2930
[3] Index 2: 1510 / (1750+1176)
[4] Index 3: 2930 / (1750+1176)

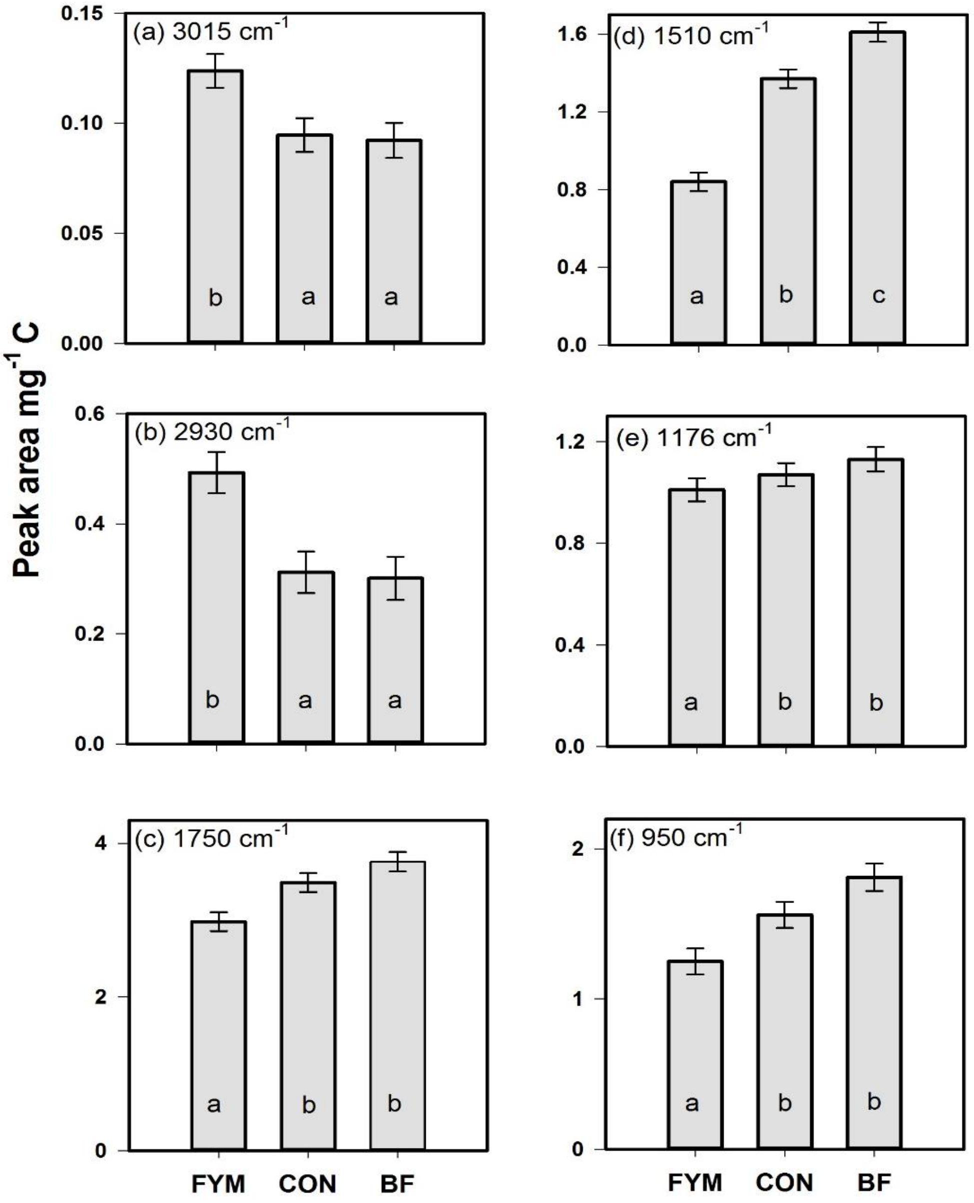

Figure 3. 3: Py-MIRS PA$_n$ by fertilizer management (Ultuna, Sweden) of bulk soil at peaks: (a) 3015 cm^{-1}, (b) 2930 cm^{-1}, (c) 1750 cm^{-1}, (d) 1510 cm^{-1}, (e) 1159 cm^{-1} and (f) 950 cm^{-1}. Treatment averages of years 1979, 1995 and 2005: FYM = farmyard manure; CON = control (no fertilizer inputs) and BF = continuous bare fallow. The letters indicate significant differences (*P* < 0.05) along with standard errors (*n* = 3).

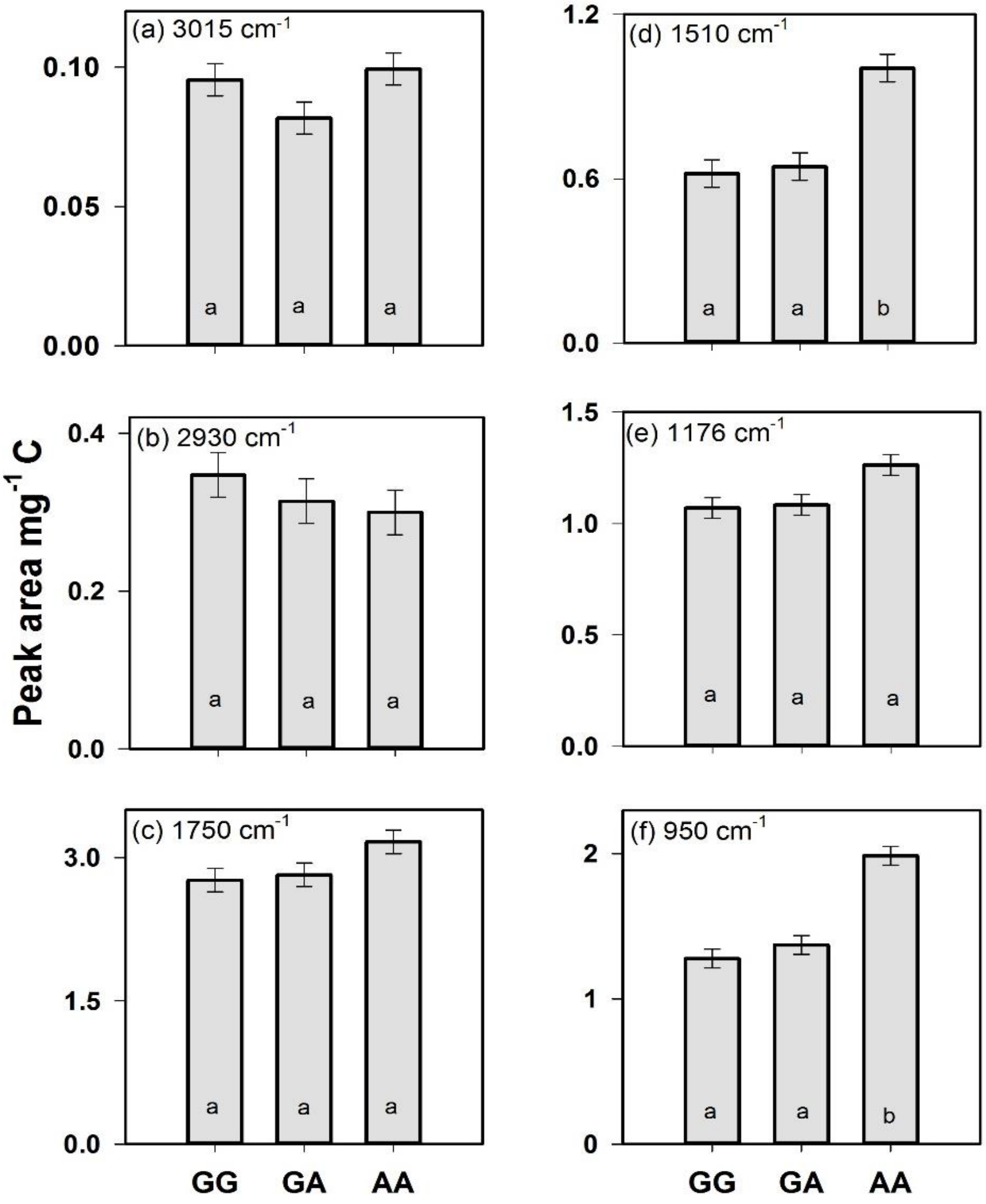

Figure 3. 4: Py-MIRS PA$_n$ by land use (Lusignan, France) of bulk soil at peaks: (a) 3015 cm^{-1}, (b) 2930 cm^{-1}, (c) 1750 cm^{-1}, (d) 1510 cm^{-1}, (e) 1159 cm^{-1} and (f) 950 cm^{-1}. GG = grassland; GA = grassland converted to arable land and AA = arable land. The letters indicate significant differences (P < 0.05) along with standard errors (n = 3).

3.4.3 DRIFTS analysis

In general, the DRIFTS spectra of bulk soils from the Ultuna and Lusignan experiments were similar, displaying the same peak patterns (Figure 3.1 and S2 a and b). However, the PA_r of the different organic functional groups revealed noticeable significant variations among the different fertilizer treatments and land use systems (Table 3.4). The C – H (2929 cm^{-1}) and C = C / COO^- (1620 cm^{-1}) functional groups were the most abundant functional groups in all soils. In the Ultuna experiment, the PA_r at 1620 cm^{-1} (COO^-/C = C), 1528 cm^{-1} (C = C) and 1156 cm^{-1} (C – O) peaks increased significantly in the non-FYM treatments (CON and BF), unlike the peak at 2929 cm^{-1} (C – H) which revealed a significant decline in both fertilizer treatments ($P < 0.05$). In the Lusignan experiment (Table 4), the PA_r at the 2929 cm^{-1} peak (C – H) was significantly greater in the GG than in the AA land use system. This is consistent with the Py-MIRS results reported in section 3.4.2. A comparison of the land use systems revealed that the AA land use had the highest PA_r for the peaks at 1620 (COO^-/C = C), 1528 (C = C), and 1156 (C – O) cm^{-1} compared to the GG and GA land use systems, although the differences were only significant for the peaks at 2930 and 1620 cm^{-1}. Similar to the Py-MIRS results, the DRIFTS-based indices differed significantly between the different fertilizer treatments and land use except for index 2 for Lusignan soils ($P < 0.05$) and also exhibited a consistent pattern with fertilizer management and land use (Table 3.4).

3.4.4 PCA analysis

PCA was performed on Py-MIRS data to analyze and compare Py-MIRS signals from different fertilizer treatments and land use systems. With the PCA, 84.4% and 98.8% of the total variation was explained by the first two principal components (PC1 and PC2) in the Ultuna and Lusignan experiments respectively (Figure 3.5). Three clusters were identified on each score plot by the fertilizer management practice and the land use system. Similar results of PCA analysis on DRIFTS data of both the Ultuna and Lusigan experiments are shown in Figure 3.6. On one hand, the distribution of samples in the Ultuna experiment was negatively linked with index 3 (left-hand side of Figure 3.5a) but positively linked with indices 1 and 2 (right-hand side of figure). On the other hand, in the Lusigan experiment, the distribution of samples was negatively linked with indices 1 and 2 (left-hand side of Figure 3.5b) but positively linked with index 3 (right-hand side of figure). This representation highlights the links between indices 1 and 2 and the advanced level of decomposition of OC in non-FYM (CON and BF) and AA soils as well as index 3 and easily decomposable OC in the FYM and GG and GA soils.

3.5 Discussion

3.5.1 Influence of agricultural management and land use on SOM composition

Long-term experiments are often required and used to investigate the effects of agricultural management practices on SOM composition. The long-term Frame Experiment in Ultuna and the Observatory for Environmental Research near Lusignan represent a series of management practices and land use systems respectively that have been shown to significantly influence, SOM composition, soil fertility, and quality. Results from our Py-MIRS study which are complemented by DRIFTS results were entirely consistent in revealing differences in SOM composition and decomposition status across the different fertilizer management practices and land use systems. These findings were consistent with previous research reporting the potential ability of Py-MIRS and DRIFTS to fingerprint SOM bulk chemistry to reveal consistent differences between the soils investigated (Nkwain et al., 2018). We can surmise that the greater proportion of the CH_4 peak indicated a higher lignin content derived from plant residues and organic inputs. The most predominant evidence of land use conversion in the Lusignan experiment, that is, from grassland which is biologically more active to arable land was seen in the decline in the proportion of this peak (up to 14%) one year after the conversion from GG to GA.

Table 3. 4: DRIFTS relative peak area (PA_r) and decomposition indices by fertilizer management and land use system of bulk soil. Data are means ($n = 3$).

	Treatment / Land use	DRIFTS relative peak area / %				DRIFTS-based indices		
		2929	**1620**	**1528**	**1159**	**Index 1[2]**	**Index 2[3]**	**Index 3[4]**
Ultuna								
	FYM	52.5c[1]	38.5a	0.9a	8.1a	0.8a	4.9a	6.6b
	CON	36.2b	52.6b	1.4ab	9.8b	1.6b	5.5ab	3.8a
	BF	29.6a	58.3c	1.8b	10.3b	2.2b	5.8b	2.9a
Lusignan								
	GG	39.3b	47.3a	3.4a	10.1a	1.3a	5.0a	3.9b
	GA	35.0ab	50.9ab	3.8a	10.3a	1.6b	5.3a	3.4b
	AA	26.0a	58.3b	4.0a	11.7a	2.5b	5.3a	3.2a

[1] Different letters within columns indicate significant difference at $P < 0.05$. [2] Index 1: (1620+1528) / 2929; [3] Index 2: (1620+1528) / (1159); [4] Index 3: 2929 / 1159

This decline has been suggested to be associated with the rapid decomposition of organic material, degradation of the aggregate structure, and accompanying changes in amount and type of vegetation and organic input following the conversion (Rumpel et al., 2009). Our results complement Py-GC/MS findings by Rumpel et al. (2009) which sustained similar observations of a decline in lignin compounds in arable land converted from grasslands. The aliphatic C – H group was prominent in the FYM and GG soils which is consistent with relative enrichment of aliphatic compounds derived from additional organic inputs of farmyard manure and aboveground and belowground biomass in the FYM and GG treatments respectively (Capriel, 1997; Demyan et al., 2012; Helfrich et al., 2006; Scotti et al., 2013). A comparable effect of fertilization on the aliphatic C – H group in SOM in the Bad Lauchstädt long-term experiment was found by Nkwain et al. (2018) using Py-MIRS.

The greater proportion of C = O, C = C, C – O, and =C – H peaks in the BF and AA soils of the Ultuna and Lusignan experiments respectively indicted higher contents of recalcitrant SOM. The absence of organic inputs in the BF soils in addition to soil disturbance (e.g. tillage) in arable land use system has been reported to enhance the rate of decomposition of SOM leading to the rapid degradation of organic material and the selective preservation of more recalcitrant organic compounds (Gregorich et al., 2015; Helfrich et al., 2006; Powlson et al., 1996; Toosi et al., 2017). Results of our Py-MIRS study of an increase in recalcitrant compounds in soils that do not receive any organic inputs are consistent with changes reported in a previous study by Nkwain *et al.* (2018) where Py-MIRS was used to fingerprint the bulk chemistry of SOM of a long-term experiment. Contrary to the results of our DRIFTS study, previous research at the Ultuna experiment using FTIR spectroscopy indicated that fallow soils had a greater and smaller proportion of the aliphatic

C – H and aromatic C = C groups respectively (Gerzabek et al., 2006). This discrepancy in results between this study and the aforementioned study can probably be due to the differences in methodological considerations utilized in the spectroscopic analysis i.e the (un)-dilution of samples with KBr. Additionally, the technique utilized to extract the proportion of the different organic functional groups varied between both studies. While our study utilized relative peak areas, the study by (Gerzabek et al., 2006) utilized relative peak heights. The same research revealed that grassland soils contained more aromatic components compared to the fallow soils and soils receiving organic and mineral fertilizers.

3.5.2 Py-MIRS-based indices

According to the model describing stages of the chemical changes associated with SOM decomposition proposed by Baldock et al. (1992), labile components of SOM like proteins and carbohydrates are preferentially degraded by the microbial community resulting in the temporary decrease in the amount of labile C components and the accumulation of aromatic C components. This stage is proceeded by the decomposition of and reduction in the content of aromatic C components. The final stage of decomposition involves the accumulation of more recalcitrant C components of SOM.

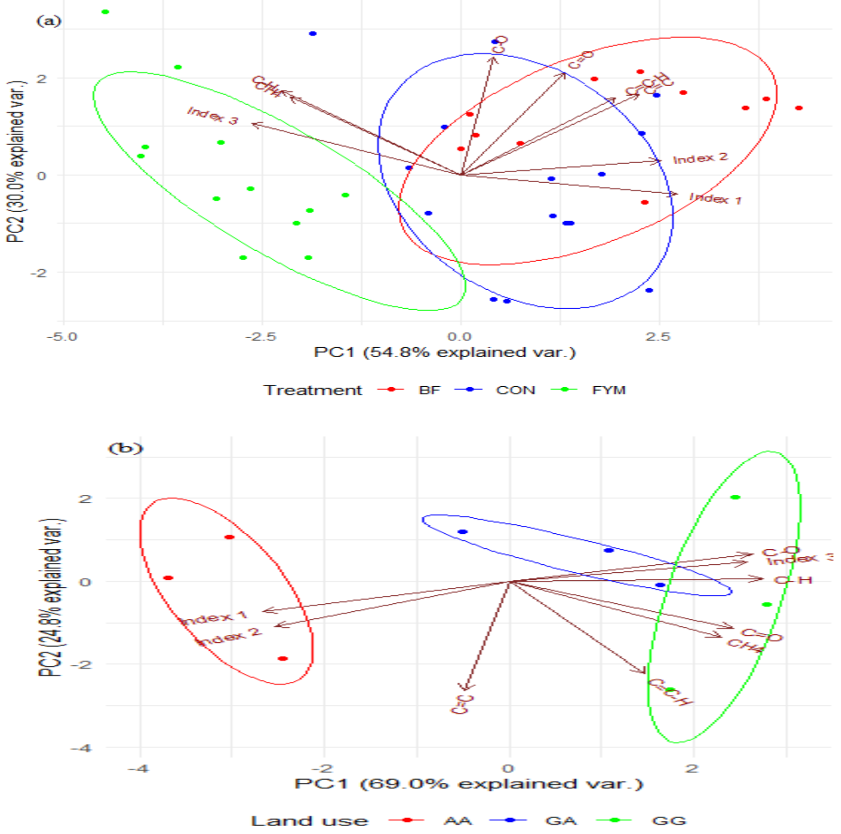

Figure 3. 5: Principal component analysis (PCA) score plot for the 1st and 2nd principal components for the Py-MIRS data for (a) fertilizer treatments in Ultuna soils and (b) land use systems in Lusignan soils. See section 3.3.3 for functional group assignments.

There was a clear influence of fertilization and land use on SOM composition and decomposition status and recalcitrance. Trends in the Py-MIRS and DRIFTS data between the different fertilizer treatments and land use systems suggest that SOM was more degraded in non-FYM (CON and BF) and AA soils, whereas SOM in soils with larger input of organic residues contained significant proportions of less decomposed material. Non-FYM soils showed significantly greater proportions of C = O, C = C, C – O groups, and lower proportions of aliphatic C – H. These were accompanied by higher ratios for indices 1 and 2 and a lower index 3 ratio suggesting a more advanced stage of decomposition for these soils (Baldock et al., 1992; Toosi et al., 2017). The larger proportion of the aliphatic C – H and overall lower ratios for indices 1, and 2 of the FYM treatment and the GG land use system suggest an average early stage of decomposition and probably a high level of microbial activity in these treatments (Margenot et al., 2015). It has also been previously suggested that less microbially processed SOM contains a greater proportion of O-rich functional groups like carboxyl (C = O) and polysaccharides (C – O) and a small proportion of aromatic (C = C) groups, hence lower aromaticity or index 1 ratio (Ding et al., 2002; Helfrich et al., 2006; Margenot et al., 2015; Veum et al., 2014). This is evident from the results of our Py-MIRS study which revealed greater proportions of C =O and C – O groups and a small proportion of the C – H group in the non-FYM treatments and the AA land use system.

The proportion of C = O and C – O groups decreased in the order of BF > CON > FYM and AA > GA > GG whereas the proportion of the C – H group increased in this order. This pattern and the resulting increase in the index 3 ratio is indicative of an increasing degree of decomposition in the order BF < CON < FYM and AA < GA < GG. The significantly higher ratio for index 3 in FYM and GG soils is consistent with the decrease in O-rich functional groups (C = O, C – O) suggested being linked to decreased biological activity and recalcitrance of SOM as well as the accumulation of more processed C in these samples (Ding et al., 2002, Margenot et al., 2015). The high index 3 ratio is also indicative that these soils consisted predominantly of fresh or slight decomposed plant residues (Helfrich et al., 2006). While the DRIFTS results revealed a quite close relationship between the aliphatic C – H group to Index 3 (aliphatic C- to O-functionality), and C

= C / COO$^-$ group to Index 1 (aromaticity), this relationship was less predominant with the Py-MIRS results. This difference could be explained by the temperature-dependence of pyrolysis yield of the different organic functional groups (e.g. underestimation of aliphatic groups) as well as the possible formation of artifacts (e.g. charring) during secondary reactions with current settings.

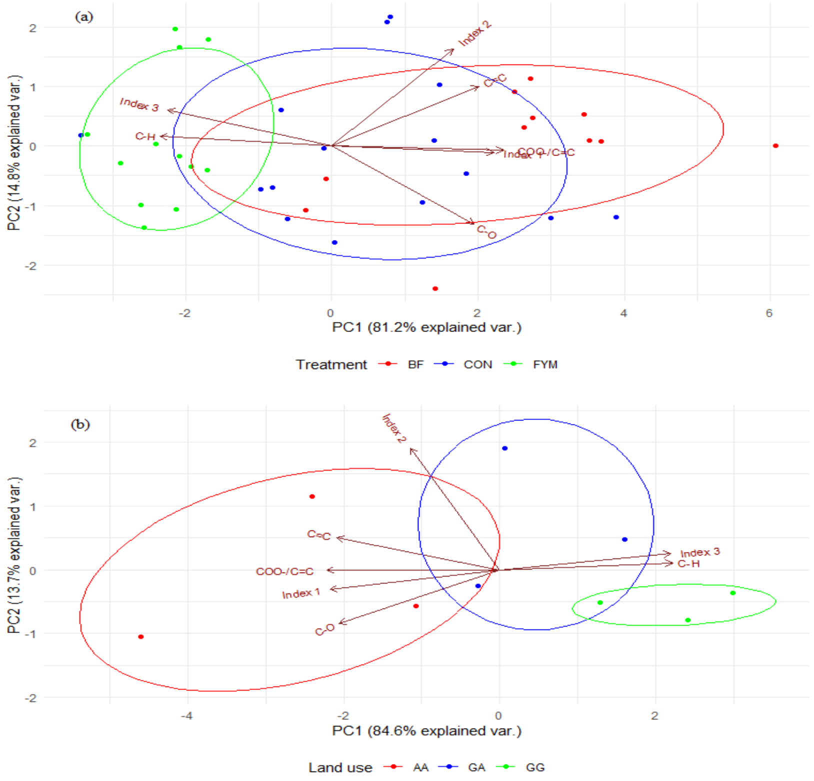

Figure 3. 6: Principal component analysis (PCA) score plot for the 1st and 2nd principal components for the DRIFTS data for (a) fertilizer treatments in Ultuna soils and (b) land use systems in Lusignan soils. See section 3.3.4 for functional group assignments.

Reports from previous studies also show that the proportion of O-containing functional groups decrease relative to the total C content with a corresponding increase in recalcitrance and decrease in biological activity (Toosi et al., 2017; Veum et al., 2014). On the other hand, reduced Py-MIRS and DRIFTS ratios of aromaticity in FYM practice and GG land use system is consistent with increased inputs of labile C and a more rapid turnover of SOM (Veum et al., 2014). We can surmise that interactions between organic inputs (farmyard manure, litter, plant residues), the microbial community, and degree of tillage or soil disturbance can be responsible for the alterations in SOM composition under different management and land use systems.

3.6 Conclusions

The results of our study show that agricultural management practices like fertilization and land use (and changes) influenced not only the total organic C and N contents which represent traditional indicators of SOM quality, but also the composition of SOM. Py-MIRS gives a broad overview of the different organic functional groups in SOM, thereby permitting the monitoring of changes in SOM composition based on the proportion of each group. This reflects the properties of different organic inputs (manure or plant residues/biomass) or their absence under different fertilization practices and land use systems as well as the extent of SOM decomposition which results in the production and accumulation of microbially-derived substances (Helfrich et al., 2006). Biologically more active grasslands and soils fertilized with farmyard manure enhances biochemical properties like total OC and N and increased labile (C – H) compounds, whereas fallow soils are enriched with more recalcitrant compounds and are more degraded thereby exhibiting greater stability of SOM in these soils. The decomposition indices provide useful indicators to assess the extent of SOM decomposition. The results showed that the overall decomposition of soils that have been under contrasting fertilizer management for 49 years followed the order continuous bare fallow > control > farmyard manure. Also, the decomposition of soils under contrasting land use systems was in the order arable land > converted grassland to arable land > grassland. These results were confirmed by data from the DRIFTS analysis, a possible indication of this technique to detect changes in SOM composition following land use change. The ability of DRITFS to assess SOM composition as affected by agricultural management practices and land use has been reported by several studies. However, Py-MIRS and DRIFTS did not show any significant distinguishable changes in SOM composition associated with short-term effects land use change, that is, GA land use system, although the trends towards degradation are evident.

The application of Py-MIRS to assess SOM composition is novel and still open to fine-tuning to improve its robustness. Utilizing the Py-MIRS technique to study compositional changes in SOM overcomes the limitations associated with DRIFTS (Nkwain et al., 2018) due to the overlap of peaks associated with both organic and mineral vibrations. Py-MIRS thus allows a rapid and meaningful characterization of land use and management induced changes in important molecular functional groups and hence changes in SOM quality also from a range of different soils. To put in other words, our results confirm the pertinence of Py-MIRS to elucidate SOM composition and the degree of decomposition as influenced by fertilizer management and land use. While the PCA data revealed an overall consistency in the results obtained using both techniques, some differences in the relationship between the decomposition indices and organic functional groups were observed. It can be inferred that, based on the current Py-MIRS settings, the decomposition indices utilized in this study were more fitting for the DRIFTS results. This, therefore, opens up an avenue for further research on improving the Py-MIRS technique that will involve using specific pyrolysis temperatures to target specific organic functional groups based on their hypothesized stabilities to optimize the pyrolysis yield. Additionally, due to the semi-quantitative nature of Py-MIRS results, (on average about 60% of SOC is pyrolyzed (Nkwain et al., 2018)), further research is recommended to improve the understanding of structural changes occurring within a sample during pyrolysis and its applicability to a wider range of soils. We, therefore, recommend the combination of both Py-MIRS and DRIFTS techniques in assessing SOM as they complement each other and can provide information on specific building blocks of SOM and further elucidate the indiscernible changes in SOM composition resulting from external factors.

Acknowledgments

We acknowledge the financial support of the German Research Foundation (DFG) under the framework of The FOR1695 “Agricultural Landscapes under Global Climate Change – Processes and Feedbacks on a Regional Scale. We would also like to thank Carolin Stahl for the technical support.

3.7 Appendix 2: Supplementary information

Table S1: Study site characteristics

Study site	**Location**	**Soil**	**MAP* [mm]**	**MAT* [°C]**	**Elevation [m.a.s.l*]**	**pH [H_2O]**	**Clay [%]**
Ultuna	60°N, 17°E	Eutric Cambisol	542	5.8	14	6.6	36.5
Lusignan	46°25N, 0°07’E	Dystric Cambisol	900	10.5	119	6.3	17

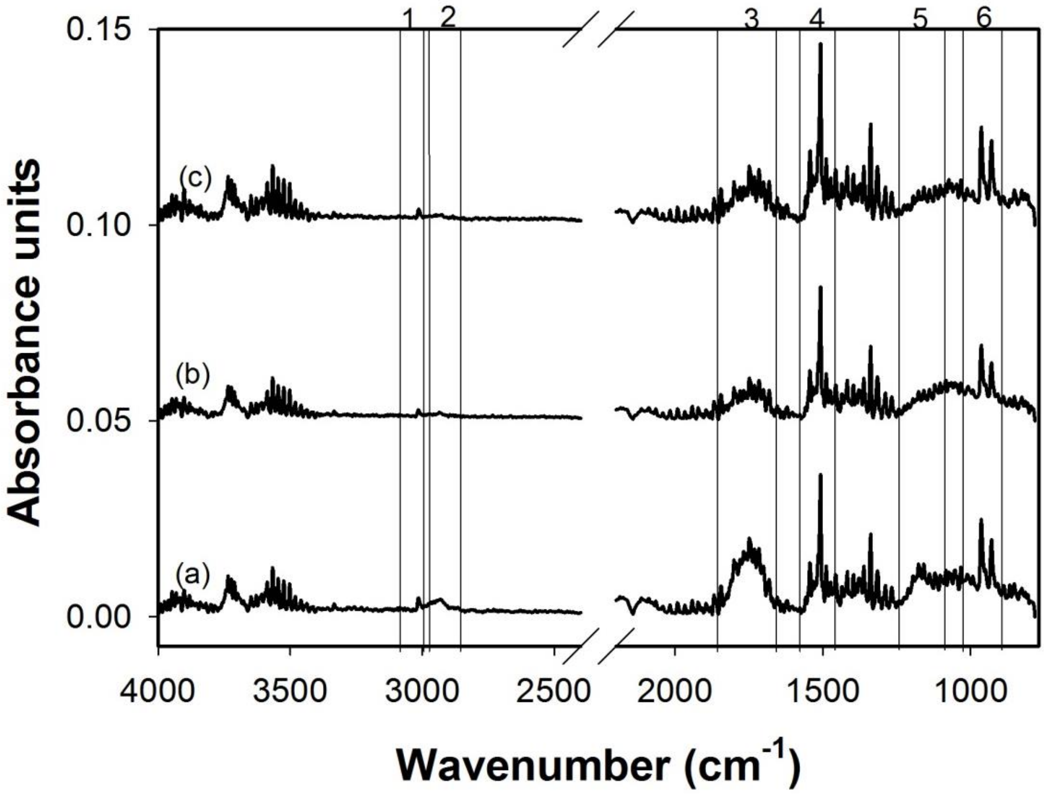

Figure S1: **Baseline corrected Py-MIRS spectra of Ultuna soils from three treatments; (a) FYM, (b) CON, and (c) BF sampled in 2005. The indicated regions were assigned to the following peaks: 1: 3015 cm^{-1} (CH_4); 2: 2930 cm^{-1} (C – H); 3: 1750 cm^{-1} (C = O); 4: 1510 cm^{-1} (C = C); 5: 1176 cm^{-1} (C – O); 6: 950 cm^{-1} (=C – H). Note: The peak at 2400–2200 cm^{-1} was cut to ensure the other peaks are more visible.**

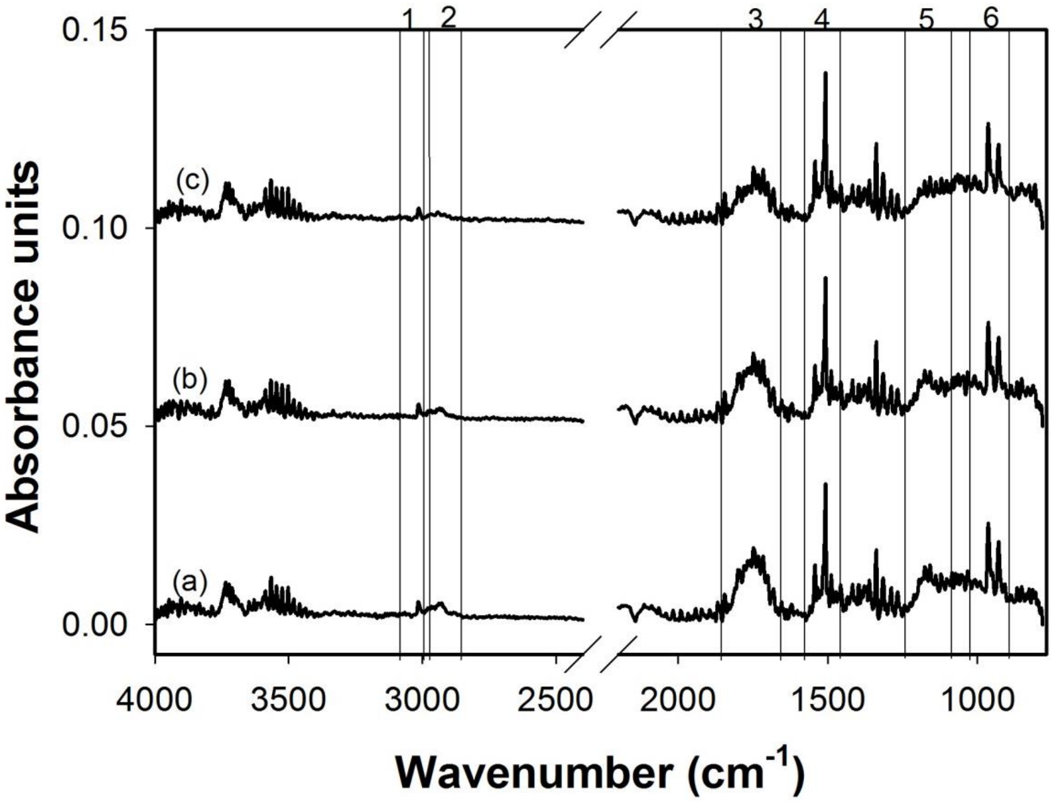

Figure S2: **Baseline corrected Py-MIRS spectra of Lusignan soils from three treatments; (a) GG, (b) GA, and (c) AA. The indicated regions were assigned to the following peaks: 1: 3015 cm^{-1} (CH_4); 2: 2930 cm^{-1}(C – H); 3: 1750 cm^{-1} (C = O); 4: 1510 cm^{-1} (C = C); 5: 1176 cm^{-1} (C – O); 6: 950 cm^{-1} (=C – H) Note: The peak at 2400–2200 cm^{-1} was cut to ensure the other peaks are more visible.**

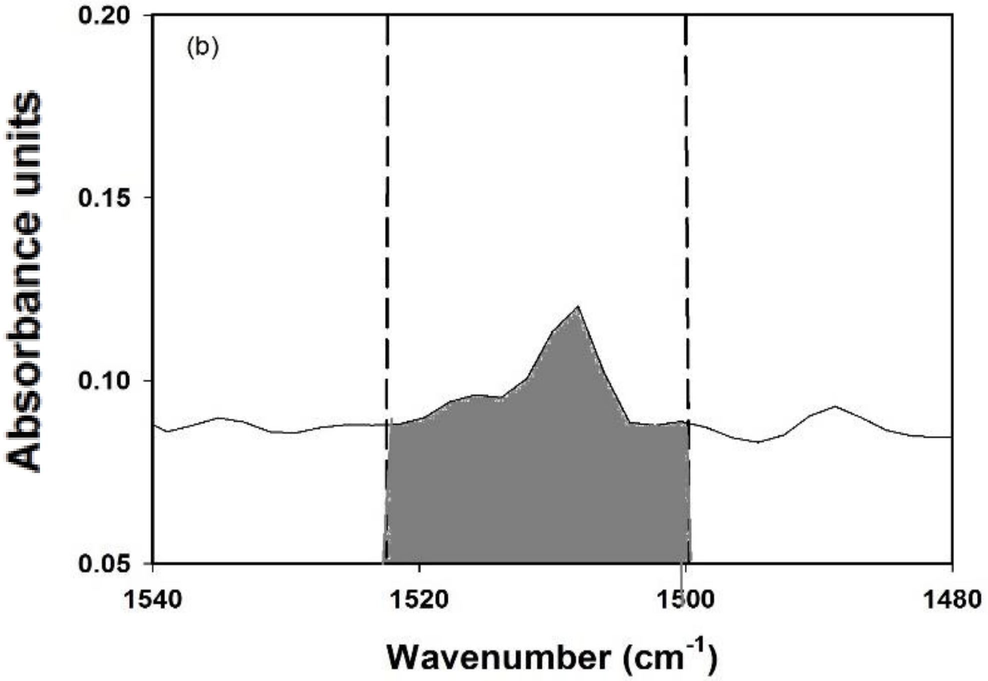

Figure S3: **Illustration of peak area integration of the 1510 cm^{-1} peak performed between the left and right defined integration limits using zero absorbance**

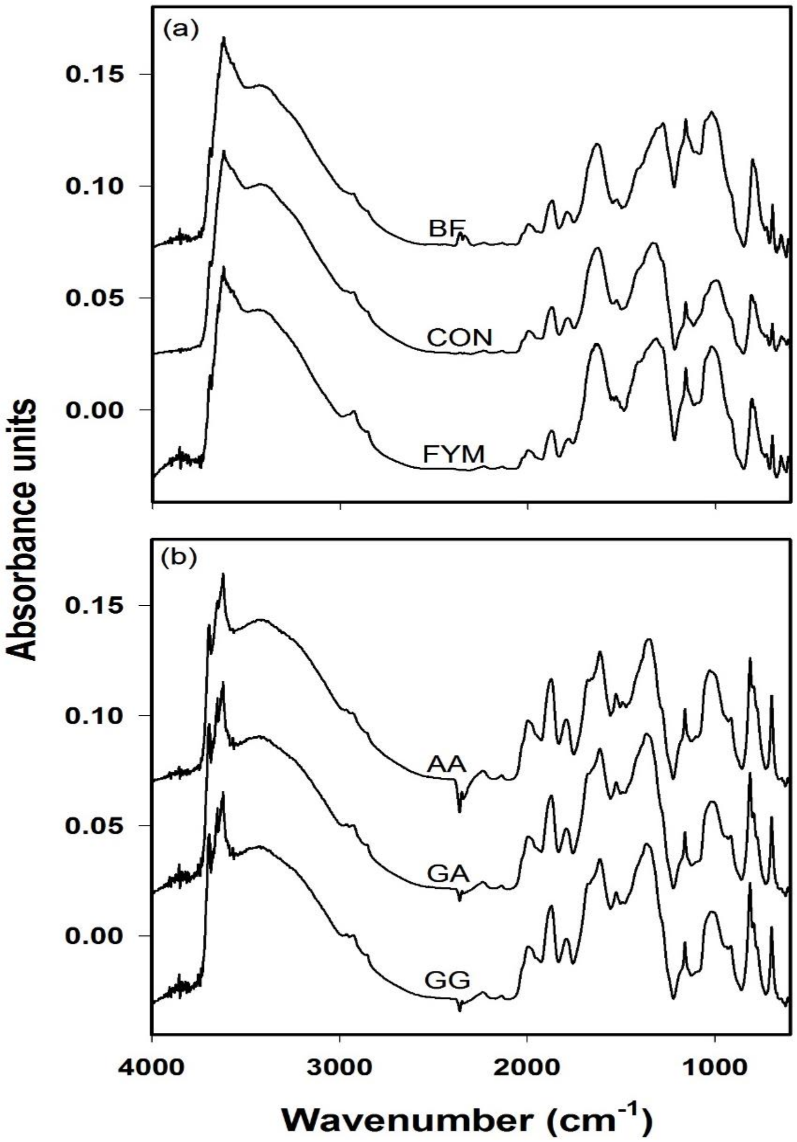

Figure S4: Baseline corrected DRIFTS spectra of (a) Ultuna soils from three treatments; FYM, CON, and BF sampled in 2005 and (b) Lusignan soils from three land use systems; GG, GA, and AA.

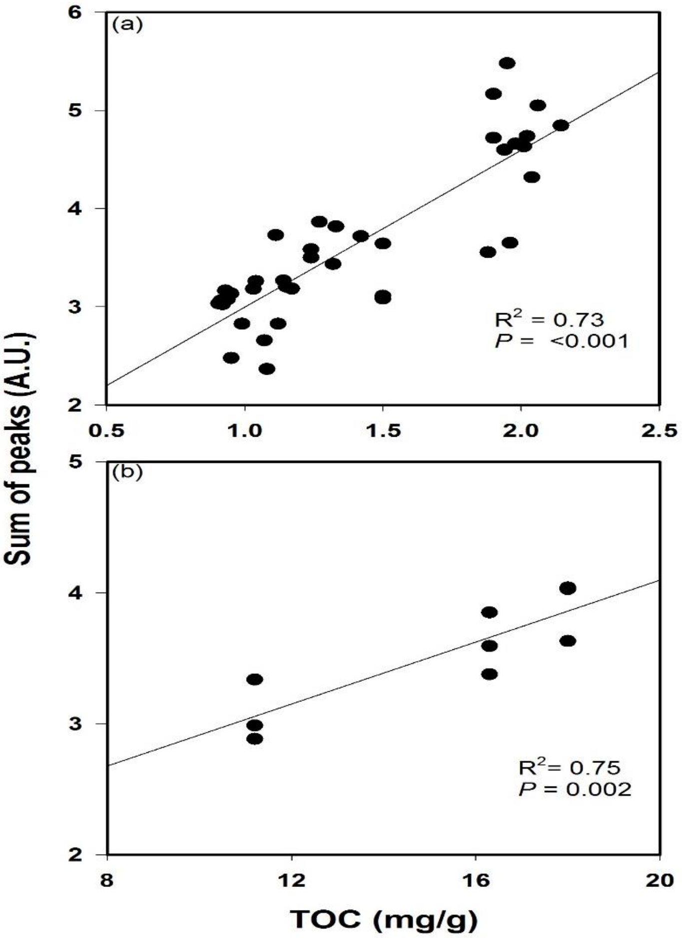

Figure S5: **Relationship between the sum of six investigated Py-MIRS peak areas and the TOC content in (a) Ultuna and (b) Lusignan soils.**

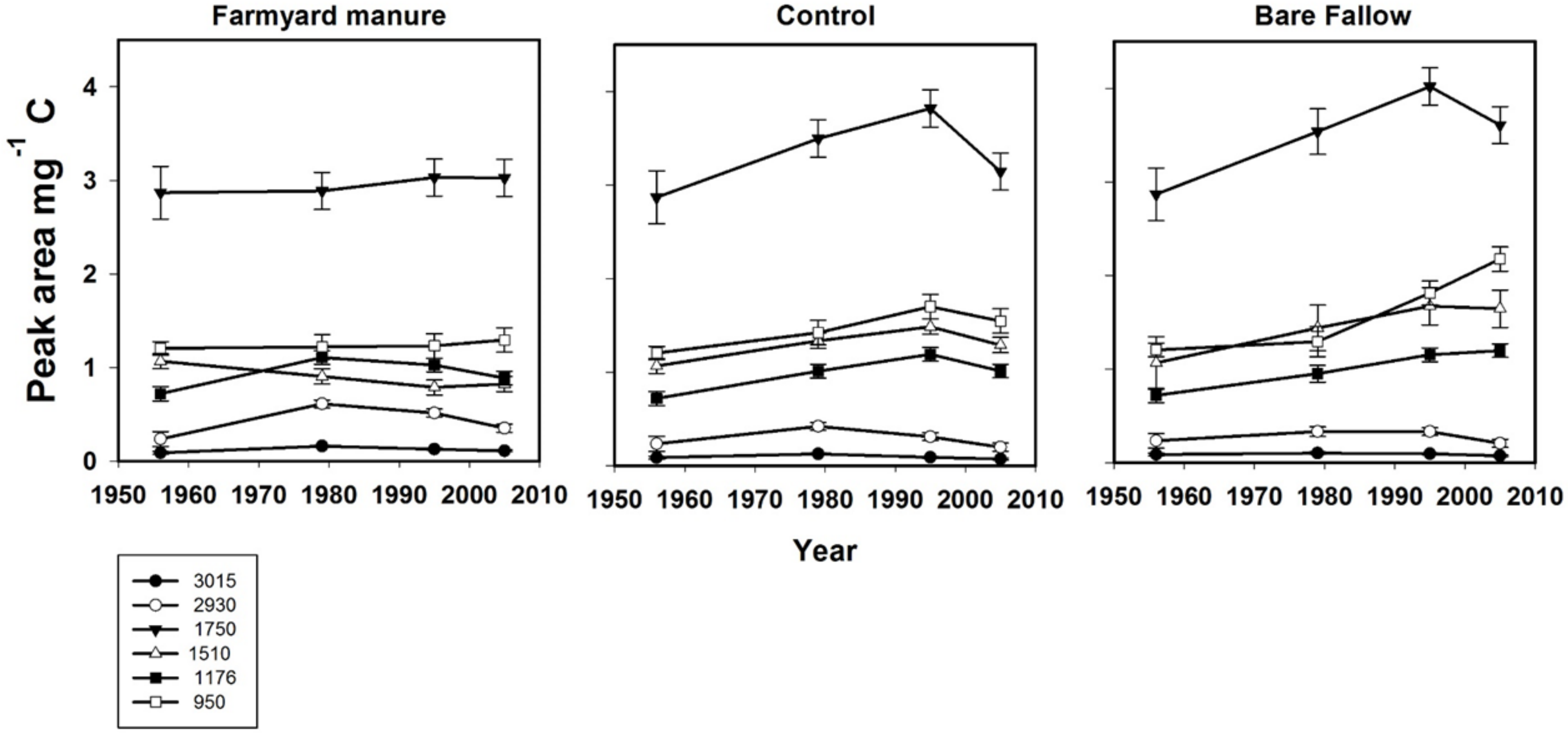

Figure S6 Dynamics of Py-MIRS of peak areas normalized to the organic C content by sampling year of FYM, CON, and BF treatments for the Ultuna soils. Bars indicate standard error (*n* = 4).

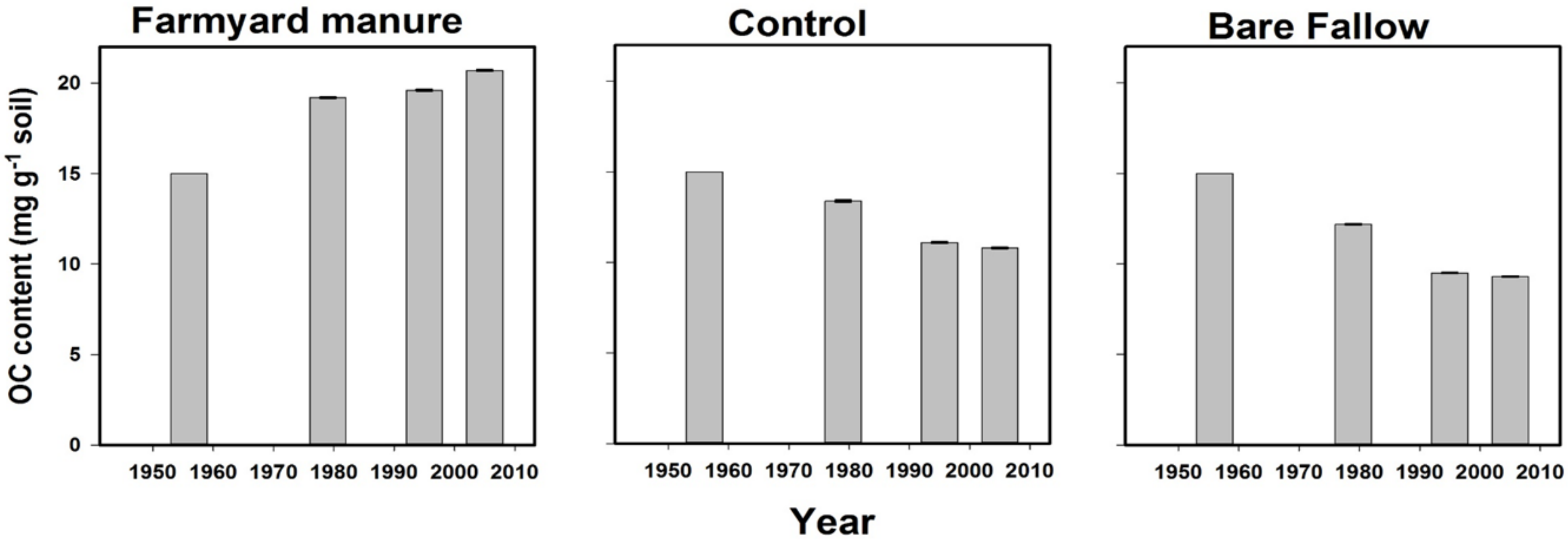

Figure S7. Dynamics of TOC content by sampling year of FYM, CON, and BF treatments for the Ultuna soils. Bars indicate standard error (*n* = 4).

Chapter 4 Soil sample drying temperature affects specific organic mid-DRIFTS peaks and quality indices*

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4.1 Abstract

Peak area (PA) integration of diffuse reflectance mid infrared Fourier transform spectroscopy (DRIFTS) spectra is useful to study soil organic matter (SOM) quality and as a potential modelling proxy. Residual water of soil samples after drying affects DRIFTS spectra, mainly in the region >2500 cm^{-1}. Therefore, SOM quality related PA at different wavenumbers should be influenced to varying degrees. We studied how absolute, normalized and relative PA related to SOM functional groups (at 2930, 1620, 1530 and 1159 cm^{-1}), as well as their ratios were influenced by oven drying of soil samples at increasing temperatures (from 32 °C to 105 °C).

All organic and even mineral associated PA significantly increased with drying temperature. The PA of aliphatic C – H stretching (2930 cm^{-1}), located on the shoulder of the broad O – H stretching PA, was influenced most strongly. Our results indicate that using 105 °C as drying temperature and storing samples in a desiccator is the best way to minimize water interference. These findings apply to relative PA and ratios of organic compounds, while no effect of drying temperatures on PA correlations to C or N were found.

All organic and even mineral associated PA significantly increased with drying temperature. The PA of aliphatic C – H stretching (2930 cm^{-1}), located on the shoulder of the broad O – H stretching PA, was influenced most strongly. Our results indicate that using 105 °C as drying temperature and storing samples in a desiccator is the best way to minimize water interference. These findings apply to relative PA and ratios of organic compounds, while no effect of drying temperatures on PA correlations to C or N were found.

Keywords: Soil; DRIFTS; peak area analysis; Mineral interference; Sample preparation; Drying temperature

4.2 Soil sample drying temperature affects specific organic mid-DRIFTS peaks and quality indices

Ratios of peak areas (PA) from diffuse reflectance mid infrared Fourier transform spectroscopy (DRIFTS), which is typically used for soil properties prediction like soil texture, C and N content, inform about soil organic matter (SOM) quality (Demyan et al., 2012). One example is the ratio of PA 2930 cm^{-1} (C – H stretching, hypothesized proxy for labile SOM) to 1620 cm^{-1} (aromatic C = C and/or COO^{-} stretching, hypothesized proxy of recalcitrant SOM), as measure of SOM hydrophobicity (Ellerbrock and Gerke, 2004) or SOM stability (Demyan et al., 2012; Margenot et al., 2015). The SOM related peaks of bulk soil DRIFTS spectra have interference by minerals and water, for example the 2930 cm^{-1} PA is superimposed on the broad O – H peak (Demyan et al., 2012). Since regions of DRIFTS spectra > 2500 cm^{-1} are most strongly influenced by O – H bonds, it is likely that residual water and hence drying temperature has an unwanted impact. Nevertheless, no consensus on optimal drying temperature exists: different authors applied a wide range of drying temperatures from 32 °C (Demyan et al., 2012), 65 °C (Matamala et al., 2017), up to 105 °C (Parolo et al., 2017). To our knowledge, the influence on PA has not been comprehensively studied, so this was our study’s objective. We hypothesized, that intensities of organic matter functional peaks with O – H interference are more affected by drying temperatures than others, and that an optimal drying temperature exist where the effect of temperatures on PA and their ratios plateaus.

To test our hypothesis, we inspected four SOM quality related DRIFTS PA after increasing drying temperatures. We used 21 archive soil samples, representing a broad range of soil types, textures and SOM contents from topsoil and subsoil. They originated from long-term experiments in Bad Lauchstädt in Germany, the Frame Experiment at Ultuna, Sweden, field sites of University of Hohenheim and the SURUMER project in subtropical China (Table S1). These ball-milled, air dried soil samples had been stored since collection. Prior DRIFTS measurements they were dried at increasing temperatures, i.e. 32 °C, 55 °C, 65 °C, 75 C, 85 °C, 95 °C and 105 °C for 24 h. After each 24 h drying, samples were cooled in a desiccator. Four sub-samples of each soil were then measured in an HTS-XT microplate extension, mounted to a Tensor-27 spectrometer (Bruker Optik GmbH, Ettlingen, Germany) with the settings proposed by Demyan et al. (2012), but using gold instead of KBr as background. Due to limited amounts, the same samples were used for all temperatures (only with increasing temperature), as a previous test indicated no difference to using new sample material each time. After baseline correction and vector normalization of spectra, four PA of interest related to SOM functional groups at 2930 cm^{-1} (mainly C – H, limits 3010-2800 cm^{-1}), 1620 cm^{-1} (mainly aromatic C = C/COO^{-}, 1660-1580 cm^{-1}), 1530 cm^{-1} (mainly aromatic C = C, 1546-1520 cm^{-1}) and 1159 cm^{-1} (mainly C – O, 1170-1148 cm^{-1}) were extracted as the integral on top of a local baseline constructed between the intersect of the spectra and a vertical line at the integration limits (Demyan et al., 2012) as shown in Figure 4.1b. Other potential assignments are in Table S2**.** The average PA of four sub-samples were taken for further calculations. All PA, including inorganic peaks increased with drying temperatures. Therefore, PA of interest were normalized by dividing them by the 1880 cm^{-1} quartz/silicates PA (1925cm^{-1} – 1835 cm^{-1}) (Parolo et al., 2017), designated hereafter with subscript ($_{n}$). Finally, relative ($_{r}$) PA (each peak divided by the sum of all four PA (Demyan et al., 2012)) were computed. A mixed model (*lmer* of *lme4* package in R Software 3.4.3) was used to test for significant drying temperature effects on PA, ratios of aliphatic to aromatic PA and the integrated full spectra (4000 – 400 cm^{-1}). A random slope and intercept of the “drying temperature” effect was allowed for each sample, to account for autocorrelation of data due to repeated measurements. Least square means of the temperature effect and PA were calculated with the *emmeans* R package. Additionally, Pearson’s correlations of PA at different drying temperatures and organic C and N were computed.

The broad O – H peak centered at 3400 cm^{-1} significantly decreased with increasing drying temperature (~2% reduction from 32 °C to 105 °C, Figure S1), while the absorbance of the entire DRIFTS spectra increased (Figure 4.1 and Figure S1). All PA (including inorganic vibrational peaks) increased significantly with increased drying temperatures. Samples with low organic C content (0.8 %) with initially small to negative 2930 cm^{-1} PA at 32 °C (Figure 4.1 a, c) were increasingly visible at higher drying temperatures. The normalized PA $_{n}$2930 cm^{-1} and $_{n}$1530 cm^{-1} increased significantly with drying temperature, while $_{n}$1159 cm^{-1} decreased (all $P < 0.0001$) but $_{n}$1620 cm^{-1} had no trend (Figure 4.2). Also relative PA were significantly affected by drying temperature with the $_{r}$2930 cm^{-1} increasing, while all others decreased. An effect also existed for both ratios of peaks (2930 cm^{-1}/1620 cm^{-1} and 2930 cm^{-1}/1530 cm^{-1}), all strongly and significantly increasing with higher temperatures (Figure 4.2). Strong correlations of all PA with C or N content existed, but were not influenced by drying temperatures (Table S3). The correlations of $_{n}$2930 cm^{-1} and $_{n}$1530 cm^{-1} with C and N were stronger than for the original PA at 2930 cm^{-1} and 1530 cm^{-1}, while normalizing changed correlations of $_{n}$1620 cm^{-1} and $_{n}$1159 cm^{-1} from significant and negative to non-significant and positive.

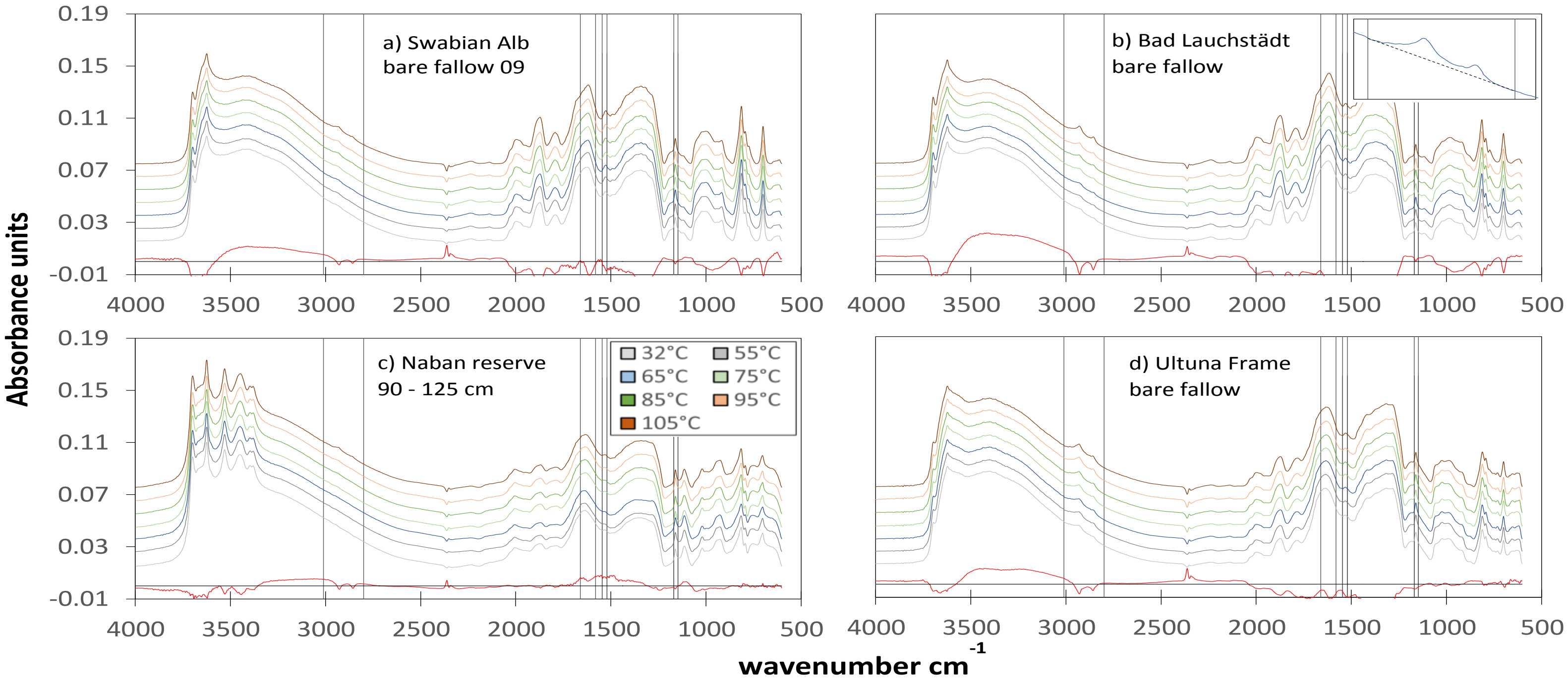

Figure 4. 1: Baseline corrected example DRIFTS Spectra of four different bulk soils (Table S1), dried at different temperatures. The red line indicates the difference of the 32 °C – 105 °C absorbance (3X scaled for better illustration). The integration limits of the four peaks of interest are shown by vertical lines. An example of an integrated 2930 cm^{-1} peak is given in b) top right. For integration, a local baseline is drawn between the intersects of the vertical lines at the integration limits (3010 and 2800 cm^{-1}) with the spectra, which is the lower boundary on the integral. More details on the integration can be found in (Demyan et al., 2012). (For interpretation of the references to the colour in this figure legend, the reader is referred to the web version of this article).

While all peak areas and the absorbance of the total spectra increased with drying temperature, the visibility of the 2930 cm^{-1} PA increased the most. This phenomenon led to a strong positive effect of drying temperature on the PA ratios of aliphatic to aromatic SOM functional groups. For example, the means of the 2930 cm^{-1}/1620 cm^{-1} ratio between 32 °C and 105 °C changed from 0.2 to 0.6 and the 2930 cm^{-1}/1530 cm^{-1} ratio means from 2 to 4. In fact, it seems as if the broad O – H can strongly over shade the 2930 cm^{-1} PA at low drying temperatures (Figure 4.1), which is reduced with drying. This challenge with a small 2930 cm^{-1} PA might also be the reason why several authors (Demyan et al., 2012; Margenot and Hodson, 2016) used rank correlations instead of Pearson correlations to relate relative PA/ratios to SOM fractions.

Several research groups have directly interpreted 2930 cm^{-1}/1620 cm^{-1} ratios as a measure of hydrophobicity, applying different drying temperatures but the results of this study suggest, that temperature should be standardized. At first, it looks like our results for DRIFTS spectroscopy contradict the results of (Duboc et al., 2016), who found no significant effect of drying temperature for ATR spectroscopy. However, they assumed that samples equilibrated moisture with ambient air as they did not store samples in a desiccator. In fact, our samples also regained ambient air water outside the desiccator, which also spectra showed within just a few hours. Combining the two studies results suggest that 1) organic matter functional groups are not changed by temperatures up to 105 °C, so 2) the change in DRIFTS spectra relates to loss of the residual water, which over shaded peaks in the broad O-H region, and which the soil can regain from ambient air.

Contrary to the initial hypothesis, no plateau of PA was detected in this study. As temperatures above 105 °C might start altering SOM, and it is standard for measuring water content, higher temperatures likely have a negligible effect on water evaporation compared to possible SOM alterations. To make future studies comparable, we therefore suggest using 105 °C as standard drying temperature for DRIFTS ratios or relative PA and to keep samples in desiccators.

As correlations were not affected by drying temperatures, the water was mainly interfering with relative PA and ratios, especially for samples with small SOM contents (very small or negative 2930 cm^{-1} peaks compared to baseline). Comparison across different temperatures might still be possible, using the regression slopes between temperature and PA (Table S4) gained from the mixed model, for example:

$$2930\ cm^{-1}/1620\ cm^{-1} = 0.07888 + 0.00492\ T\ (\text{in °C}) \qquad (1)$$

It would be interesting to see, whether the regressions are universally applicable, as we used a range of soil samples from around the world. We would advise further test for different soils (e.g. with high sand contents) and different DRIFTS equipment. We invite others to share their datasets, if they measured the same samples at different drying temperatures. While the PA approach is promising to study SOM quality, this study showed that the drying temperature considerably affects relative PA and ratios, but not correlations. The ratios could then give valuable information on SOM quality for large scale datasets or for SOM modelling.

Acknowledgement

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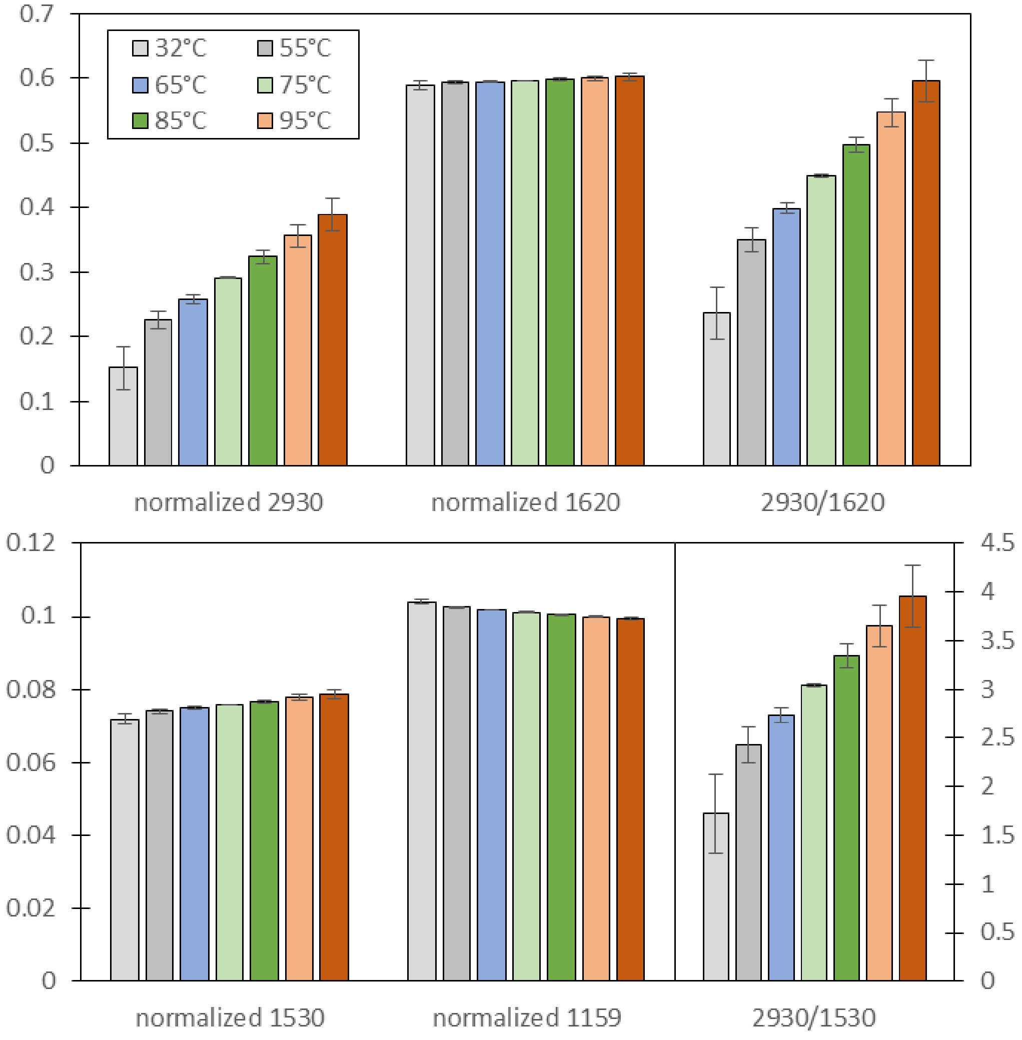

Figure 4. 2: **Least square means estimations for quartz/silicates normalized peak areas of interest and two stability indices ratios across all samples. Significant differences between all temperatures existed for all peaks and ratios (excluding the 1620 cm^{-1} peak). Error bars indicate 95 % confidence intervals of the drying temperature effect.**

4.3 Appendix 3: Supplementary information

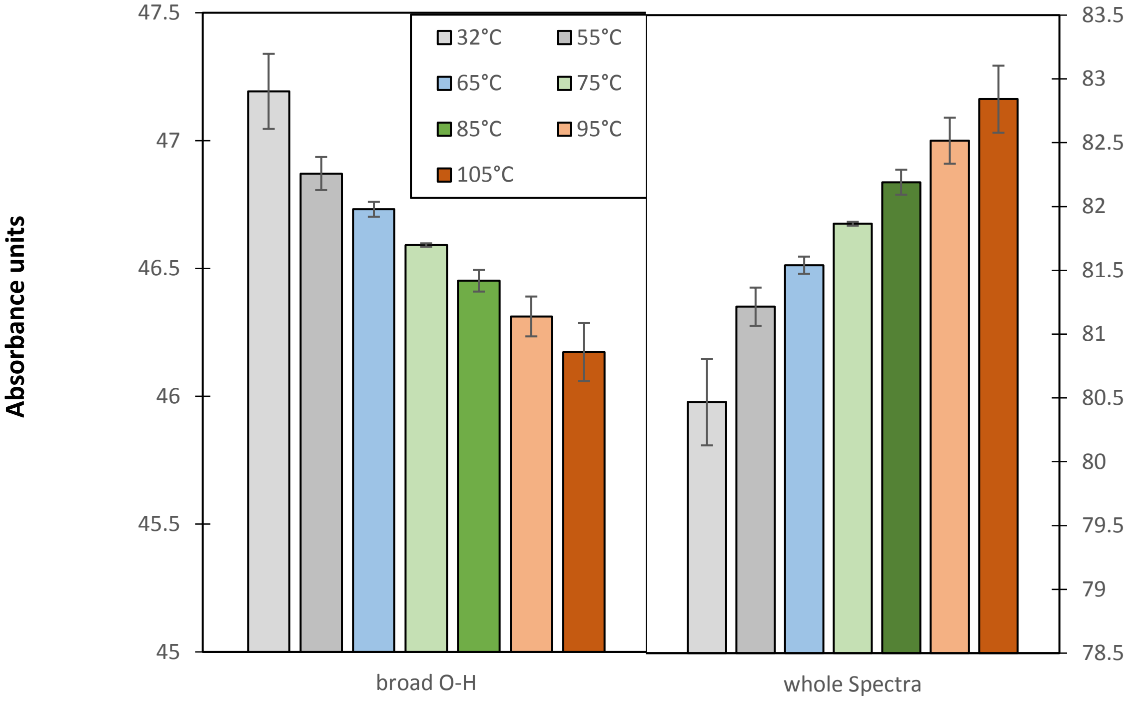

Figure S1. Least square means estimations of the broad O – H peak area (3800 to 2400 cm^{-1}) and the peak area of the whole spectra (4000 to 400 cm^{-1}) across all samples. Significant differences between all temperatures existed. Error bars indicate 95 % confidence intervals of the drying temperature effect.

Table S1. Origin and selected soil properties of the samples, used in this study. The contents of TOC and TN were analyzed by dry combustion in a Variomax CN analyzer (Hanau, Germany). Texture was measured for the samples from Swabian Alb, Kraichgau and SURUMER, and from the literature for the rest.

Site	**Plot Location**		**Treatment**	**Year of sampling**	**Depth (cm)**	**TOC (%)**	**TN (%)**	**Sand (%)**	**Clay (%)**	**Bulk density (g/cm^3)**
	UTM Degrees Lat	**UTM Degrees Long**								
Kraichgau	48.925723°	008.716103°	bare fallow since 09	2012	0 – 30	0.81	0.09	1.8	17.1	1.37
Swabian Alb	48.528354°	009.770306°	bare fallow since 09	2015	0 – 30	1.27	0.15	6.2	37.8	1.31
Swabian Alb	48.526689°	009.769955°	under cropping	2015	0 – 30	2.44	0.28	6.2	37.8	1.31
Kraichgau	48.927616°	008.716771°	bare fallow since 09	2015	0 – 30	0.89	0.11	1.8	17.1	1.37
Kraichgau	48.927616°	008.716771°	under cropping	2009	0 – 30	1.05	0.10	1.8	17.1	1.37
Kraichgau	48.927616°	008.716771°	under cropping	2010	60 – 90	0.20	0.02	0.8	16.1	1.51
Swabian Alb	48.526689°	009.769955°	under cropping	2010	30 – 60	0.78	0.06	8.4	48.4	1.32
Swabian Alb	48.527877°	009.768333°	bare fallow since 09	2010	0 – 30	1.53	0.24	6.2	37.8	1.31

Table S1 cont'd

Bad Lauchstädt	51.391605°	011.877028°	no FYM, NPK biannual	2008	0 – 20	1.61	0.13	11.0	21.0	NA
Bad Lauchstädt	51.391605°	011.877028°	50t/ha year FYM	2008	0 – 20	1.98	0.17	11.0	21.0	NA
Bad Lauchstädt	51.391605°	011.877028°	100t/ha year FYM	2008	0 – 20	2.55	0.22	11.0	21.0	NA
Bad Lauchstädt	51.391605°	011.877028°	200t/ha year FYM	2008	0 – 20	4.03	0.37	11.0	21.0	NA
Bad Lauchstädt	51.3901520°	011.8797610°	30 t/ha year FYM + NPK	2008	0 – 30	2.34	NA	11.0	21.0	NA
Bad Lauchstädt	51.3901520°	011.8797610°	control, no additions	2008	0 – 30	1.65	NA	11.0	21.0	NA
SURUMER	22.1703863°	100.6605682°	field survey	2014	0 – 15	1.16	0.14	19.4	35.8	1.25
SURUMER	22.1844893°	100.6627704°	field survey	2014	90 – 130	0.62	0.10	9.4	49.5	1.40
SURUMER	22.1763047°	100.5625986°	field survey	2014	0 – 30	5.05	0.45	32.7	34.5	0.56
SURUMER	22.2059529°	100.6184263°	field survey	2014	90 – 125	0.93	0.06	40.4	36.0	1.17
Ultuna Frame	59.8218790°	017.6563480°	farmyard_manure	2005	0 - 20	2.04	0.20	23.0	36.0	1.24
Ultuna Frame	59.8218790°	017.6563480°	sewage sludge	2005	0 - 20	2.47	0.26	23.0	36.0	1.02
Ultuna Frame	59.8218790°	017.6563480°	bare fallow since 56	2005	0 - 20	0.95	0.10	23.0	36.0	1.43

Table S2. DRIFTS peaks used in this study together with their integration limits

Peak name in article	**Integration limits (cm^{-1})**	**Main assignment (Demyan et al., 2012)**	**Potential other contributors**
2930 cm^{-1}	3010 – 2800	Aliphatic C-H stretching	Superimposed on broad O-H peak (Stevenson, 1994)
1620 cm^{-1}	1660 – 1580	Aromatic C=C and/or COO− stretching	C=C of alkenes (Coates, 2006), N-H of primary amides (Socrates, 2004), C=O (Stevenson, 1994)
1530 cm^{-1}	1546 – 1520	Aromatic C=C stretching	N-O (Coates, 2006), C=N , N-H (Socrates, 2004)
1159 cm^{-1}	1170 - 1148	C–O bonds of poly-alcoholic and ether groups	C-H, SO_2 (Socrates, 2004)
broad O-H peak area	3800 - 2400	O-H from water as well as clay minerals	

Table S3. Correlations between peak areas (and normalized peak areas) and total organic carbon (TOC), and total organic nitrogen (TN). Correlations > ABSOLUTE (0.444) are significant (n = 19; DF = 18). The strength of color indicates a significant positive (green) or negative (red) correlation

Correlation of TOC with								
Temperature (°C)	2930 cm^{-1}	n2930 cm^{-1}	1620 cm^{-1}	n1620 cm^{-1}	1530 cm^{-1}	n1530 cm^{-1}	1159 cm^{-1}	n1159 cm^{-1}
32	0.77	0.85	-0.69	0.14	-0.70	-0.85	-0.60	0.18
55	0.73	0.77	-0.64	-0.01	-0.66	-0.83	-0.56	0.08
65	0.75	0.81	-0.63	0.06	-0.68	-0.84	-0.55	0.11
75	0.76	0.82	-0.62	0.12	-0.68	-0.80	-0.56	0.18
85	0.74	0.79	-0.63	0.09	-0.67	-0.81	-0.56	0.15
95	0.72	0.80	-0.63	0.19	-0.68	-0.84	-0.57	0.24
105	0.76	0.83	-0.62	0.19	-0.68	-0.81	-0.58	0.20

Correlation of TN with								
Temperature (°C)	2930 cm^{-1}	n2930 cm^{-1}	1620 cm^{-1}	n1620 cm^{-1}	1530 cm^{-1}	n1530 cm^{-1}	1159 cm^{-1}	n1159cm^{-1}
32	0.76	0.83	-0.62	0.06	-0.62	-0.78	-0.50	0.11
55	0.72	0.74	-0.58	-0.09	-0.58	-0.75	-0.45	0.01
65	0.74	0.77	-0.57	0.03	-0.59	-0.76	-0.43	0.04
75	0.74	0.79	-0.57	0.04	-0.60	-0.73	-0.45	0.12
85	0.71	0.75	-0.58	0.00	-0.59	-0.73	-0.46	0.08
95	0.70	0.76	-0.58	0.11	-0.60	-0.76	-0.46	0.17
105	0.71	0.77	-0.58	0.07	-0.60	-0.73	-0.47	0.13

Table S4. Summary of Statistic models for different peak areas. Number of degrees of freedom was 125, with exception of the SI2 model (DF = 95).

Variable	**Intercept**	**standard error (Intercept)**	**Slope of temperature**	**standard error (Slope of temperature)**
2930 cm^{-1}	8.04E-03 $^{n.s.}$	2.58E-02	2.83E-03 ***	2.27E-04
1620 cm^{-1}	4.58E-01 ***	2.46E-02	1.02E-03 ***	1.02E-04
1530 cm^{-1}	5.88E-02 ***	7.67E-03	1.84E-04 ***	1.39E-05
1159 cm^{-1}	8.45E-02 ***	3.20E-03	9.76E-05 ***	7.70E-06
normalized 2930 cm^{-1}	4.82E-02 $^{n.s.}$	4.51E-02	3.24E-03 ***	3.78E-04
1/normalized 1620 cm^{-1}	n.s.	n.s.	n.s.	n.s.
normalized 1530 cm^{-1}	6.90E-02 ***	5.64E-03	9.17E-05 ***	1.65E-05
1/normalized 1159 cm^{-1}	9.42E+00 ***	5.29E-01	6.10E-03 ***	7.61E-04
SI = 2930 cm^{-1}/ 1620 cm^{-1}	7.89E-02 $^{n.s.}$	6.33E-02	4.92E-03 ***	4.75E-04

For SI2 the samples "P1a(0-30)","UL05-15","BL791","BL779","P3(90-125)" had to be removed (outliers)

SI2 = 2930 cm^{-1}/ 1530 cm^{-1}	7.47E-01 $^{n.s.}$	6.63E-01	3.05E-02 ***	4.33E-03
relative 2930 cm^{-1}	5.02E-02 $^{n.s.}$	4.50E-02	2.12E-03 ***	1.51E-04
relative 1620 cm^{-1}	7.26E-01 ***	3.38E-02	-1.62E-03 ***	1.20E-04
relative 1530 cm^{-1}	8.93E-02 ***	9.39E-03	-1.25E-04 ***	2.50E-05
relative 1159 cm^{-1}	1.35E-01 ***	6.74E-03	-3.77E-04 ***	2.50E-05
whole spectra	7.94E+01 ***	7.90E-01	3.25E-02 ***	4.18E-03
broad O – H	4.76E+01 ***	6.55E-01	-1.40E-02 ***	1.81E-03

Chapter 5 General Discussion

5.1 Overview

Numerous studies have been performed to provide in-depth knowledge on SOM, its composition, and dynamics. However, the methods used in the past to investigate SOM presented only a partial view of SOM composition as important questions remain open, e.g. the molecular fingerprinting, possible quantification of some of its components, alterations in bulk chemistry, and SOM-mineral interactions. Worth noting is the fact that a more comprehensive characterization of SOM is crucial with regard to its stability and dynamics, and eventual recommendations on sustainable management practices of agro-ecosystems that are directly influenced by anthropogenic inputs. It is therefore of primary importance to have a routine technique that will not only provide a generic but also a more comprehensive molecular characterization of SOM.

This thesis has focused on the development of the Py-MIRS technique to investigate the molecular composition of SOM in bulk soil and to assess how the SOM fingerprint is affected by the implementation of different agricultural management practices and human-induced land-use changes. The methodological implications of Py-MIRS were also investigated to generate information as to how they influence the assessment of SOM composition.

In this chapter, the development and application of the developed Py-MIRS technique from Chapter 2 is discussed as well as the anthropogenic influences on SOM as shown in Chapters 2 and 3. The effects of sample preparation and other limitations of the techniques utilized in this study are further assessed as already indicated in Chapters 2 and 4. Finally, recommendations regarding the next possible steps to improve SOM studies are proposed.

5.2 Py-MIRS as a SOM fingerprinting tool

This study focused on the development of an analytical technique, i.e. Py-MIRS to assess SOM bulk chemistry in soils as influenced by agricultural management practices and land use by producing a fingerprint that provides information on the molecular composition of SOM (Chapters 2 and 3). The potential limitations (Table 5.1) of some of the already existing pyrolytic techniques in addition to chemical, physical and spectroscopic techniques have prompted the need for the development of powerful and versatile techniques to resolve these inadequacies. Analytical pyrolysis, which utilizes thermal energy to physically cleave chemical bonds, provides a wealth of information on SOM on a molecular level (Derenne and Quénéa, 2015). The importance of analytical pyrolysis as a routine technique widely applied to soil studies emerged more than three decades ago and has exhibited tremendous potential as a tool to characterize SOM, particularly in bulk soil.

A number of steps were taken during the development of Py-MIRS to ensure that it is a suitable molecular fingerprinting and semi-quantitative tool for the investigation of SOM. The current study demonstrates that crucial experimental conditions like pyrolysis temperature, heating rate, and pyrolysis time significantly influence the molecular fingerprint of the sample under investigation. The different peaks assigned to C compounds or organic functional groups of SOM shown in the fingerprint spectra have been reported by several other studies on organic materials like wood and cellulose (Court and Sephton, 2009; Lu et al., 2011; Yang et al., 2007) and are known to be related to and derived from specific components of SOM (White et al., 2004). The differences in the fingerprint of bulk soils and standard compounds, varying in chemical structure ranging from simple sugars to complex polyphenols, clearly demonstrate that the experimental conditions, most importantly pyrolysis temperature greatly determine the type and amount of organic compounds pyrolyzed. Our analysis confirms earlier suggestions of other authors (Fernández et al., 2010; Haddix et al., 2016; Plante et al., 2009) that it is important to be cautious when comparing pyrolysis products and amounts from other studies that utilize different experimental conditions like pyrolysis temperatures, heating rates and/or times.

The developed technique utilizes the normalized (to sample weight or OC content) peak area to describe the proportion of each C compound present in the sample. It was imperative to normalize the spectra in order to surmount the variations in spectral patterns due to confounding factors like inconsistencies in sample weight, radiation source fluctuations, and scattering of the IR beam (Gautam et al., 2015), and most importantly TOC. The normalized peak areas, therefore, permit the comparison and establishment of relations between samples with varying TOC content and allow the relative comparison of SOM compounds among and between different samples across a wide range of soil mineralogies. Included in the development of the technique was the use of reference standard compounds diluted with preheated quartz sand and clay minerals like sepiolite and allenite to disclose the effect of interferences caused by the soil's mineral matrix and water. It was established that there is minimal or no interference from the mineral matrix and/or water on the Py-MIRS spectra when it was compared with the Py-MIRS spectra of the reference compounds only (Chapter 1, Appendix 1). While one could argue that clay minerals can act as a catalyst during pyrolysis leading to the formation of secondary artifacts, our results show that most newly formed polycyclic aromatic hydrocarbons (PAHs) and a simultaneous enrichment of aromatic compounds that subsequently can lead to significant alterations in the fingerprint (Faure et al., 2006), hence the Py-MIRS fingerprint spectra of bulk soils did not corroborate this argument. Py-MIRS fingerprint spectra obtained from the soil samples varying in clay content between 16 – 37 % from the long-term experiments together with DRIFTS spectra of pyrolyzed soil samples showed no evidence of alterations in the fingerprint spectra as the general pattern of the spectra obtained, using both techniques, remained unchanged for all samples.

The interpretation of the spectra was relatively straightforward and less complicated, compared to existing techniques like DRIFTS, as vibrations from minerals that might interfere or overlap with and/or obscure the visibility of distinct peaks assigned to organic functional groups were not detected. This allowed the rapid assessment of the contribution of some functional groups in SOM (e.g. C – H, C = O, C = C, C – O and =C – H), revealing changes in SOM molecular composition due to anthropogenic impact including but not limited to management practices. Previous studies have utilized pyrolysis coupled to Fourier transform infrared spectroscopy (Py-FTIR) mainly for the characterization of samples like coal (Ibarra & Moliner, 1991), rice straw (Fu et al., 2009), and wood (Court and Sephton, 2009). While the fingerprint spectra of the aforementioned materials revealed some similarities with SOM fingerprint spectra, some peaks e.g 1510 cm^{-1} (C = C) were found in our study which were not present in these other studies and vice versa.

Among the benefits of this technique is the fact that it involves relatively little sample preparation, avoids the undesirable interference of the soil mineral matrix often encountered with the DRIFTS technique, enables rapid analysis of large soil sample sets across different agro-ecosystems, and permits a more simplified analysis of spectra compared to other established routine techniques like Py-GC/MS. A major problem with this technique is the low detection limit of 0.4 mg C which currently prevents the investigation of soils with low SOC contents as they reveal very low units of absorbance in the fingerprint spectra. A possible way forward to overcome the limitation of low detection limits is to investigate the utilization of different mid-IR detectors and sensors e.g. gas sensors using optical and photoacoustic detectors (Popa and Udrea, 2019) with higher sensitivity to gases that can enhance absorption and the signal-to-noise ratio. The feasibility of coupling pyrolyzers to spectrometers equipped with these detectors, however, needs to, first of all, be explored. Further limitations of Py-MIRS include incomplete pyrolysis (see Chapter 2 and Table 5.1) and the occurrence of secondary reactions leading to the formation of artifacts. It is also difficult to establish a uniform protocol as the Py-MIRS results vary with the different Py-MIRS experimental conditions, particularly pyrolysis temperature. As a result, it is important to establish the fact that Py-MIRS experimental conditions will vary based on the study objectives as well as the type of samples under investigation.

5.3 Use of Py-MIRS to reveal anthropogenic impacts on SOM molecular composition

In this current study, it was hypothesized that the molecular composition of SOM measured by Py-MIRS would be affected by long-term management and land use. Several studies have over the past few decades applied different analytical techniques ranging from fractionations, spectroscopic, isotopic, biological, and thermal approaches (Albrecht et al., 2014; Calderón et al., 2011; Demyan et al., 2012; Panettieri et al., 2013; Rumpel et al., 2009; Zimmermann et al., 2007) to interpret changes in SOM molecular composition induced by management practices and land use (and change). However, most of these findings have been inconsistent due to the absence of a standard set of experimental conditions, in addition to the limitations exhibited by these techniques which influence and limit the effective interpretation of findings and the comparison of results obtained between studies (Toosi et al., 2017).

The current study used the working hypothesis that SOM bulk chemistry as revealed by Py-MIRS spectral fingerprint would be affected by long-term fertilizer management and land use. Additionally, it was hypothesized that the fingerprint of DRIFTS peaks obtained confirmed the pertinence of Py-MIRS as an analytical technique that can be adopted for routine SOM studies. The normalized peak area enabled the derivation of semi-quantitative estimates of the Py-MIRS peaks namely, CH_4, aliphatic C – H, C = O, C = O, C – O, and aromatic C – H functional groups to draw distinctions between the different treatments and land use. The normalized peak areas derived from the different treatments in the soils under investigation suggested distinguishable alterations in the SOM molecular composition resulting from anthropogenic inputs of organic and/or mineral fertilizer as well as land use. Py-MIRS corrected peak areas (not normalized by C content) and the DRIFTS relative peaks areas do not give much information by themselves other than insight into the composition of the soil sample and the possible contribution of each organic C compound to the SOM composition. Therefore, normalizing the corrected peak areas by C content transformed the data obtained to a specific range permitting relationships between the different treatments on SOM quality to be sought and established. In addition, a relatively straightforward interpretation of the Py-MIRS fingerprint spectrum was made possible by the absence of overlapping wavenumber bands and interferences caused by vibrations of silicate and quartz minerals. This phenomenon is commonly experienced with spectroscopic techniques like DRIFTS and NMR, thereby giving this technique an advantage over DRIFTS and other SOM analytical techniques. This technique is therefore an addition to the library of already established techniques, like FTIR-EGA (Demyan et al., 2013), Py-GC/MS (Rumpel et al., 2009), and Py-FIMS (Leinweber et al., 1992) to study bulk SOM and SOM fractions. While it was observed that DRIFTS provides valuable complementary information to Py-MIRS results and also confirms its pertinence, the daunting task of carefully assigning organic peaks to certain functional groups without interference from minerals introduces bias in the use of organic peaks when using DRIFTS as proxies for SOM quality and its applicability to soil samples with different mineralogies and textures (Demyan et al., 2012; Laub et al., 2019; Reeves, 2012).

We also attempted to derive indices which are ratios of representative Py-MIRS or DRIFTS peaks to describe the decomposition status and recalcitrance of SOM in these samples (Chapter 3). The results revealed a significant influence of management and land use on SOM decomposition status as indicated by the indices. A similar approach of using the relative contribution of peak areas and their ratios obtained via other techniques, e.g ^{13}C NMR and FTIR spectroscopy, rock eval pyrolysis, has been extensively applied to assess changes in SOM quality with management and land use (Albrecht et al., 2014; Demyan et al., 2012; Ellerbrock et al., 1999; Margenot et al., 2015; Rumpel et al., 2009; Toosi et al., 2017; Veum et al., 2014).

Taking a step further to test the usefulness of Py-MIRS in SOM quality assessment, principal component analysis (PCA) (Chapter 3) on Py-MIRS results revealed key variables that could explain the overall variability and potential overlaps between samples of the different treatments and agro-ecosystems in terms of SOM composition and decomposition status. The Py-MIRS indices together with the PCA result provide some insight into the dynamics of SOM in these samples thereby highlighting the robustness of this technique. While the DRIFTS PCA results demonstrate a good link for instance between the aliphatic C – H group to index 3 and COO/C = C group to index 1, this link was less predominant with the Py-MIRS PCA results. This, therefore, suggests that based on the current Py-MIRS protocol, the use of indices to describe the decomposition status of SOM is more fitting for DRIFTS spectra. Based on this, it is important to keep in mind the fact that Py-MIRS results vary with experimental conditions particularly pyrolysis temperature. Therefore, to fully determine and grasp the decomposition status of SOM and the effect on SOM quality, we recommend further research on fine-tuning Py-MIRS experimental settings and how it affects the representative Py-MIRS peak ratios used to explain the decomposition status.

As earlier established, SOM is a key indicator of soil quality as it influences chemical, biological and chemical properties (Campbell and Paustian, 2015; Gholizadeh et al., 2013). SOM also plays an important role in many ecosystem functions like C storage and is known to be sensitive to direct and indirect anthropogenic changes including but not limited to agricultural management practices and land use as elaborated in this study (Chapters 2 and 3). According to Janzen et al. (1997), optimum levels of SOM content for the sole purpose of maximizing sustainable productivity are influenced by SOM composition, net primary production, supplementary energy inputs (e.g. fertilization), and other soil and climatic conditions (e.g. pH, texture, temperature). Agricultural management practices and land use are also known to have a profound influence on SOM dynamics. Since SOM is an important indicator of soil quality, it is important to note that the changes in management practices and land use will influence SOM, soil quality, and consequently soil productivity. As such, the assessment of SOM quality is paramount to understanding SOM and facilitating the determination of management practices for sustainable production and/or maintenance or increase of SOM (Baldock et al., 2014; St. Luce et al., 2014) in the light of the current climate change crisis. An increase in the labile SOM fraction is interpreted as an improvement in SOM quality and consequently soil productivity. It has been established that much of the changes in SOM resulting from management practices and land use as well as climate change occur in the labile fraction (Gregorich et al., 1994; Janzen et al., 1997). This can be explained by the increased rates of decomposition of the labile SOM fraction due to increased temperature and soil disturbance from tillage practices, or alterations in organic inputs.

The understanding of how SOM responds to different agricultural management and land use determines the extent to which the efforts made to maintain soil quality can be accomplished. Additionally, the assessment of SOM quality with respect to specific functions of SOM, for instance, improving soil productivity or its role as an environmental filter is required to determine which sustainable management practices can/should be adopted. The importance of adopting sustainable management practices to maintain and/or to improve SOM quality as well as soil productivity cannot be disputed due to their impact on SOM pools and the sequestration potential of agro-ecosystems. Fageria (2012) reported a couple of management practices that can improve SOM quality and some of these include conservation tillage, the application of organic and/or mineral fertilizers, maintaining pasture land, etc. In this work, we have demonstrated that Py-MIRS can be used to fingerprint SOM chemistry illustrating the relative contribution of the different components of SOM together with the resulting quality and dynamics of SOM arising from the implementation of different agricultural management practices and land use. Based on our Py-MIRS results reported in chapters 2 and 3, we could identify that the application of organic (FYM) and mineral fertilizers and grassland increases the easily decomposable fraction of SOM (the peak at 2930 cm^{-1}) and the absence of fertilizers or crop residues leads to the accumulation of recalcitrant fractions (e.g. the peak at 1510 cm^{-1}) of SOM. Hence Py-MIRS results provide more in-depth insights into which management practice or land use can improve/maintain SOM quality. To further understand this phenomenon, we recommend that future research includes comparisons of Py-MIRS results with soil productivity (yield) and relevant soil property data e.g. cation exchange capacity, aggregate stability, nutrient cycling (nitrogen, phosphorous, etc).

5.4 Methodological considerations for using DRIFTS to investigate SOM quality

The inconsistencies associated with the analytical techniques used in SOM assessments have shown profound effects on the outputs and the subsequent interpretations and implementation of findings. DRIFTS analysis has for long been used to characterize SOM in bulk soils and fractions (Demyan et al., 2012; Gerzabek et al., 2006). The practical assessment of SOM using DRIFTS involves the identification of specific mid-infrared peaks at different wavenumbers which correspond to vibrations of certain organic functional groups. The interpretation of these peaks is often hampered by the overlapping of mineral and organic functional groups as well as O – H vibrations at the same wavenumber and this is often made more difficult by the variability in the mineralogy of different soils. This explains the large variation in the choice of individual peaks between studies (Demyan et al., 2013; Ellerbrock and Gerke, 2016; Gerzabek et al., 2006; Mirzaeitalarposhti et al., 2015; Toosi et al., 2017).

Table 5. 1: Advantages and disadvantages of some SOM analytical techniques

Technique	Advantage	Disadvantage	References
FTIR/DRIFTS	- Requires small sample volumes with minimum preparation - Short acquisition and analysis times - Non-destructive analysis of samples - No generation of chemical residues	- Spectral distortion due to band inversion at wavenumbers >1150 cm^{-1} - Overlap between organic and inorganic mid-IR peaks - Occurrence of spectral band overlap at the same wavenumber bands - Interference of water on organic mid-IR peaks	- Reeves (2010) - Nguyen et al. (1991)
Py-GC/MS	- No sample pretreatment is required - Requires small sample volumes	- Long analyses times - Complex pyrograms and mass spectra - No quantitative analysis - Lack of detection of non-GC amendable compounds - Generates chemical residues in the presence of tetramethylammonium hydroxide (TMAH)	- Derenne and Quénéa (2015) - Committee and No. 85 (2018)

Table 5: Advantages and disadvantages of some SOM analytical techniques (cont'd)

NMR	- Pretreatment eliminates the influence of mineral matrix - High sensitivity - Suitable for samples in liquid and solid-state	- Reduced cross-polarization efficiency of unprotonated carbons or mobile components - Reveals broad, poorly resolved peaks assigned to major functional groups - Baseline distortion due to the dead-time at the start of detection - Loss of structural information and poor quantification	- Mao et al. (2000) - Zhang et al. (2017)
Physical-chemical fractionation	- Easy performance - No uniform procedure	- Long analysis times - Describes only a narrow spectrum of SOM - The use of chemicals alters the nature of SOM - Generates chemical residues	- Morona et al. (2017)
TGA, DSC, EGA	- No generation of chemical residue - No sample pretreatment required	- Long analysis times - Interfering reactions at the same temperature range - The use of slow heating rates result in secondary reactions	- Mao et al. (2000) - Zhang et al. (2017)

Table 5: Advantages and disadvantages of some SOM analytical techniques (cont'd)

Py-MIRS	- Short acquisition and analysis times - No sample pretreatment required - Easy identification of individual compounds - Requires small sample volumes - No influence of mineral matrix - Provides semi-quantitative analysis of SOM components	- Generates pyrolysis residue - Low detection limit for samples of low SOC - The possible occurrence of incomplete pyrolysis - Absence of spectral library to facilitate detection of unknown samples	- Nkwain et al. (2018)

The methodological conditions like sample drying temperature and sample dilution on the organic peaks and the impact on the indices derived to describe SOM have not received much attention. It is therefore important to illustrate the importance of methodological effects in order to provide more insight into the DRIFTS results and that would be a valuable step towards comparability between studies. Strong water absorptions in the mid-infrared range particularly at wavenumbers >2500 cm^{-1} degrade the spectra and subsequently influence the interpretation of peaks. To shed more light on this, our study set out to investigate the effect of sample drying temperature ranging from 32 to 105 °C on the peak area of the peaks at 2930, 1620, 1530, and 1159 cm^{-1} and the ratio of 2930:1620 cm^{-1} (Chapter 4). It was demonstrated that increasing sample drying temperatures led to an overall increase in the absorbance units of the DRIFTS spectra, a decline in the O – H peak that significantly increased the peak area at 2930 $cm^{-1,}$ and a corresponding increase in the 2930:1620 cm^{-1} ratio. A similar study by Fernández et al. (2010), in an attempt to ensure reliable comparison of results between studies, revealed results obtained from the thermal analysis of SOM fractions were highly affected by experimental conditions like heating rate. It is therefore indispensable to consider that methodological conditions can strongly influence the results obtained and the comparability of results from different studies which is often overlooked. A recent study by Laub et al. (2020) also reported that the DAISY model initializations using DRIFTS stability indices of samples from experimental field sites in different agro-ecosystems with a drying temperature of 105 °C resulted in a lower model error compared to samples with drying temperatures of 30 and 65 °C. While it was beyond the scope of our study to provide an optimal drying temperature for samples before DRIFTS analyses, our findings serve as an important step in the right direction towards the establishment of recommendations to ensure the acquisition of comparable data for the analyses of SOM. While this study was not transferred to the Py-MIRS, it is worthwhile to investigate if sample drying temperatures affect the Py-MIRS peak intensities and their resulting peak areas.

5.5 Future work and recommendations

The widespread applicability of different analytical techniques to study SOM has generated a wealth of information on the chemical components and structures of SOM. It has been demonstrated by several authors that although several techniques are utilized in SOM studies, understanding the relationships between SOM in different soils analyzed using different analytical methods remains a challenging task (e.g. Albrecht et al., 2014; Roper et al., 2019). In other words, broader interpretations of SOM are limited as results are often not reliably comparable. It is therefore fundamentally important that there exists a technique that can be applied to different soils with mineralogical and textural differences subjected to different management practices and land uses and can satisfactorily complement results derived via other techniques. While in this current study we developed and applied Py-MIRS to different soils subjected to different management practices and land uses and reference standard compounds (Chapters 2 and 3), its robustness can be tested further by applying it to different land uses (e.g forest, pasture) as well as an array of soil types with varying TOC contents. As seen in this study, the technique is suited for samples with organic C contents of ≥1%. To sufficiently explore the potential of this technique, it would be crucial to further develop the technique so that it can be applied to soils with low organic C contents like highly degraded and eroded soils and also to a broad range of ecosystems. This will include the development and implementation of the thermal desorption process in pyrolysis which involves the trapping of volatile pyrolysis products with adsorbents, and which after release are later transferred to the detector and further testing of different pyrolysis temperatures. Additionally, the option of comparing Py-MIRS results of SOM characterization to those obtained from the use of high resolution techniques like magic angle spinning NMR, comprehensive multiphase (CMP) NMR, and Near-edge X-ray spectroscopy and secondary ion mass spectrometry (see Chapter 1.2.7) to fully apply and understand the pertinence of Py-MIRS over a broad range of SOCs and mineralogies.

The results from this study showed that the pattern of the fingerprint spectra varied between the different reference standard compounds and bulk soil and was contingent on the different experimental conditions. Given that Py-MIRS has been shown to be a suitable molecular fingerprinting tool, a promising approach to improve the precision and efficiency of Py-MIRS would be to develop a searchable spectral library. The observed Py-MIRS spectra and identified diagnostic peaks from reference standard compounds, bulk soils, and fractions from different agro-ecosystems and treatments at different pyrolysis temperatures can be cataloged to make new identifications of unknown compounds or samples and also provide a training set for spectral pattern recognition. For instance, the Py-MIRS spectra of several reference compounds analyzed (Chapter 2) varied with the chemical structure of the compound and the temperature at which the compound is pyrolyzed. This information can and will provide a starting point in recognizing different spectral patterns from a variety of samples and subsequently permit the identification of unknown samples.

As already seen in this current study, there were (significant) differences in the SOM molecular composition between the different soils, a strong indication that organic inputs from fertilization and land use played a vital role. If we want to deepen our understanding of the essential role of SOM on microbial processes and nutrient release and permit the detailed quantification of SOM components via Py-MIRS, we need to incorporate the possibility of quantitatively determining soil components like pH, bulk density, total C (TOC) and total nitrogen (TN) into this technique via the use of multivariate analysis like Partial Least Squares Regression (PLSR). This would require a priori assumption that the utilization of faster heating rates, like 20 °C ms^{-1}, greatly reduces the formation of secondary artifacts and a comprehensive understanding of pyrolytic responses for different samples at the preferred pyrolysis temperature of 700 °C.

An average of 60 % of pyrolyzed C (Chapter 2) in the soils from the different treatments suggests the occurrence of incomplete pyrolysis. While ensuring complete pyrolysis of samples, it is important to note that achieving complete pyrolysis will require longer heating times which lead to the formation of secondary artifacts in samples, particularly as different samples have different pyrolytic responses and consequently introduce bias in the interpretation of results (Haddix et al., 2016). We, therefore, advise users of the need to balance the needs of their specific research objectives and with the need for comparability between existing studies and an already established routine technique.

The use of specific peaks and peak area ratios (e.g. 2930:1620 cm^{-1}) have shown to be useful indicators of changes in SOM quality of soils as is the case with many studies using DRIFTS (Demyan et al., 2013, 2012; Laub et al., 2019; Toosi et al., 2017) could provide valuable information on the SOM quality and dynamics for SOM organic modeling. Due to the increasingly important consideration to integrate modeling approaches in SOM studies, these peaks and ratios have been used to initialize and parameterize SOM models like the Q-soil (Ågren and Bosatta, 1998) and DAISY (Laub et al., 2020). Q-soil has been used to model the continuous distribution of the different qualities of SOM and this has shown to be a promising concept which reflects the actual continuum of SOM. We attempted to compare different analytical (fractionation, DRIFTS, FTIR-EGA, Py-MIRS) measures of a continuous distribution of SOM quality (q_{soil}) of different long-term experiments using the Q-soil model. Preliminary results from this unpublished research revealed that measured and modeled q_{soil} indicated changes over time as a result of different organic amendments and Py-MIRS was generally the most sensitive to reflect quality changes over time.

The implementation of the peaks derived with Py-MIRS and DRIFTS into SOC models would not only provide information on the molecular composition of SOM but also show that soil quality is reflected by the different proportions of organic compounds which are considered to be either labile or recalcitrant. Additionally, we recommend the development of a multi-step continuous pyrolysis process with a sequence of ascending pyrolysis temperatures that target the specific organic compounds based on their hypothesized thermal stabilities revealing their origin(s) that will also provide more sensitive resolution of labile and more stable pyrolysis products and a further improvement in the initialization and parametrization of SOM models.

Our results also revealed that Py-MIRS has tremendous potential as a rapid and effective technique to detect changes in SOM molecular composition arising from management practices and land use in a large number of samples. Despite the impressive advances made in research on SOM composition and dynamics and the understanding of different analytical techniques utilized, it is worthwhile to note that the existence of limitations among the different techniques as well as their sensitivities to detect changes in SOM composition and dynamics vary. Fluctuating equilibrium levels of SOM which are dependent on organic material inputs, mineralization, and decomposition rate among other factors impact the SOC budget which in turn influences SOC sequestration, soil quality, and food security. The implementation of an integrated approach that involves the development of new analytical techniques to investigate changes in SOM molecular composition, social sciences involving governance and policy, and earth science like geology and meteorology which study physical processes and the factors controlling them is required. Although the development of our technique highlights a great advancement in SOM studies and its potential to be applied to soils with mineralogical differences, much remains to be done to enhance the reliable comparison of results from different studies utilizing different techniques. Until then, great caution should be exercised if any of such comparisons need or has to be made. We expect that this dissertation contributes to the quest for a rapid, efficient, and routine analytical technique to enhance the understanding of SOM dynamics.

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Summary

Soil organic matter (SOM) is known to play an important role in the global carbon cycle due to its ability to sequester atmospheric carbon dioxide (CO_2) and maintenance of soil physical, chemical, and biological properties. Due to the growing need to enhance the understanding of SOM composition and dynamics as influenced by natural and anthropogenic factors, in addition to the limited ability to exist analytical techniques to provide in-depth knowledge into the constituents of SOM, a lot of research is currently focused on the development of new techniques to address the aforementioned concerns.

In this study, a novel analytical technique, pyrolysis coupled with mid-infrared spectroscopy (Py-MIRS) was developed and applied to study SOM bulk chemistry in soils by measuring certain mid-infrared organic functional groups. Secondly, the developed Py-MIRS technique was applied to soil samples from different long term experiments to investigate the effects of agricultural management practices and land uses by monitoring the different functional groups. Lastly, the implications of methodological considerations of diffuse reflectance Fourier transform mid-infrared spectroscopy (DRIFTS) on specific mid-infrared functional groups and quality indices were investigated on soils from a number of long-term field experiments. Py-MIRS was developed by testing critical experimental conditions like pyrolysis temperature, heating rate, and time using a range of reference standard compounds varying in chemical and structural composition and bulk soils. Pyrolysis yield of aliphatic (C – H) and aromatic (C = C) mid-infrared functional groups were found to vary with experimental conditions. Preferred experimental conditions identified were: pyrolysis temperature of 700 C, a heating rate of 20 C ms^{-1} and hold time of 30 s were chosen based on the pyrolysis yield of specific mid-infrared function groups. Bulk soil samples taken from the long-term field experiment in Bad

Lauchstädt, Germany were used to test the applicability of Py-MIRS to study SOM bulk chemistry. It was found that certain organic functional groups varied with the presence or absence of farmyard manure (FYM) and mineral fertilizer inputs. The application of FYM led to an enrichment of the aliphatic (2930 cm^{-1}) functional group, while the absence of FYM or fertilizer inputs led to an accumulation of carboxyl (1750 cm^{-1}) and aromatic (1510 cm^{-1}) and poly-alcoholic (1159 cm^{-1}) functional groups. The suitability of Py-MIRS to study SOM was tested by comparing DRIFTS results to Py-MIRS which revealed management effects in similar orders but with differences in peak area magnitudes in certain functional groups with similar assignments as Py-MIRS (i.e aliphatic (2930 cm^{-1}), aromatic (1530 cm^{-1}) and poly-alcoholic and ether (1159 cm^{-1})) functional groups.

As a next step in the methodological development, the suitability of the newly developed Py-MIRS was further evaluated by testing the effect of long-term management and land use on the molecular composition of SOM in bulk soils taken from long-term field experiments in Ultuna, Sweden, and Lusignan, France. Results from the Ultuna experiment revealed that the absence of fertilizer application in the bare fallow (BF) and control (CON) treatments increased the amount of certain functional groups (i.e. carboxyl (1750 cm^{-1}), aromatic (1510 cm^{-1}), and alkenes (950 cm^{-1}) compared to the farmyard manure (FYM) treatment receiving 2 Mg C ha^{-1} yr^{-1} ($P < 0.05$). Additionally, the influence of land use (grassland and arable land) and land use change (grassland converted to arable land) on SOM molecular composition investigated on samples from Lusignan revealed that the proportion of the carboxylic, aromatic, and poly-alcoholic functional groups and alkenes increased in the arable land compared to the grassland. The conversion of grassland to arable land also led to a slight decline in the aliphatic group and a slight increase in the aromatic group.

In the long-term experiments of Bad Lauchstädt, Ultuna, Hohenheim, and China it was found that increasing drying temperatures from 32 °C to 105 °C increased the absorbance of the spectra and the peak area of certain organic functional groups (i.e. aliphatic (2930 cm^{-1}), aromatic (1620 cm^{-1} and 1530 cm^{-1}) and poly-alcoholic (1159 cm^{-1})) in bulk soil using DRIFTS. The ratio of the aliphatic to aromatic (2930 / 1620 cm^{-1} and 2930 / 1530 cm^{-1}) peak areas were found to significantly increase with increasing drying temperatures ($P < 0.05$). This implies that methodological considerations such as drying temperature are crucial for the understanding of SOM composition and in modeling SOM dynamics

The newly developed Py-MIRS technique and the evaluation of the effect of drying temperatures on peak areas obtained with DRIFTS demonstrate progress in the use of pyrolytic and spectroscopic techniques in the domain of SOM characterization. Py-MIRS revealed its potential as a rapid, reproducible, and effective technique to yield information on SOM molecular composition with minimal constraints due to mineral interferences and secondary thermal reactions. Py-MIRS also provided some insights into sustainable practices that improve SOM quality. However, the technique requires further development and testing on different clay mineralogies and land uses.

Zusammenfassung

Die organische Bodensubstanz (OBS) spielt aufgrund der Möglichkeit atmosphärisches CO_2 zu speichern, sowie ihrem Beitrag zum Erhalt der physikalischen, chemischen und biologischen Bodeneigenschaften, eine wichtige Rolle im globalen Kohlenstoffkreislauf. Es besteht ein wachsender Bedarf die Zusammensetzung und Dynamiken von OBS zu verstehen, insbesondere wie diese durch natürliche und anthropogene Faktoren beeinflusst werden. Zudem sind die derzeit vorhandenen Analysetechniken zur Bestimmung der Zusammensetzung von OBS in ihrer Eignung begrenzt. Die Entwicklung neuer Techniken zur Untersuchung der OBS Zusammensetzung ist daher ein wichtiges Anliegen der Wissenschaft.

In der vorliegenden Arbeit wurde eine neue Analysetechnik, Pyrolyse gekoppelt mit Spektroskopie im mittleren Infrarot-Bereich (Py-MIRS), entwickelt und angewandt, mit dem Ziel die molekulare Zusammensetzung von OBS in Böden, durch die Messung bestimmter funktionaler organischer Verbindungen, zu bestimmen. In einem zweiten Schritt wurde die entwickelte Py-MIRS Technik zur Messung von Bodenproben verschiedener Langzeitversuche angewandt. Hierbei wurde der Effekt unterschiedlicher landwirtschaftlicher Praktiken und Landnutzungen, durch Monitoring funktionaler organischer Verbindungen, untersucht. Zuletzt wurden die Implikationen methodologischer Abschätzungen von Diffus-Reflexions-Infrarot-Fourier-Transformations-Spektroskopie (DRIFTS) auf bestimmte funktionale organische Verbindungen und Qualitätsindexe auf Böden einer Reihe von Langzeitfeldversuchen erforscht. Py-MIRS wurde durch das Testen kritischer Versuchsspezifikationen wie Pyrolysetemperatur, Erhitzungsrate und -zeit, unter Berücksichtigung einer Reihe von Referenzverbindungen unterschiedlicher chemisch-struktureller Zusammensetzung sowie

Bodenproben, entwickelt. Die Ausbeute der Pyrolyse bezogen auf aliphatische (C – H) und aromatische (C = C) funktionelle Gruppen im mittleren Infrarotbereich variierte mit den Versuchsbedingungen. Die präferierten Versuchsbedingungen von 700 °C Pyrolysetemperatur, 20 °C ms^{-1} Erhitzungsrate und 30 s Erhitzungszeit wurden basierend auf der Pyrolyseausbeute spezifischer funktionelle Gruppen im mittleren Infrarotbereich ausgewählt. Die Anwendungsmöglichkeiten von Py-MIRS zur Erforschung der chemischen Zusammensetzung von OBS wurden basierend auf Bodenproben von Langzeitfeldversuchen aus Bad Lauchstädt in Deutschland erforscht. Es zeigte sich, das bestimmte organische funktionale Gruppen je nach An- oder Abwesenheit von Stallmist- und Mineraldüngung variierten. Die Düngung mit Stallmist führte zu Anreicherung von aliphatischen (2930 cm^{-1}) funktionellen Gruppen, wohingegen die Abwesenheit von Stallmist- oder Mineraldüngung zu einer Anreicherung von carboxylischen (1750 cm^{-1}), aromatischen (1510 cm^{-1}) and polyalkoholischen (1159 cm^{-1}) funktionellen Gruppen führte. Die Eignung von Py-MIRS zur genaueren Erforschung von OBS wurde durch den Vergleich von Py-MIRS und DRIFTS Ergebnissen getestet. Hierbei zeigten sich Effekte des unterschiedlichen Managements mit ähnlichen Umfang jedoch in unterschiedlichen Größenordnungen der Peakflächen bestimmter funktionellen Gruppen, welche die gleiche Zuweisung wie Py-MIRS (aliphatische (2930 cm^{-1}), aromatische (1530 cm^{-1}) and polyalkoholischen (1159 cm^{-1}) funktionelle Gruppen) hatten.

Als nächster Schritt der Methodenentwicklung wurde die weitergehende Eignung der entwickelten Py-MIRS Technik, anhand der Effekte von Langzeitmanagement sowie Landnutzung auf die molekulare Zusammensetzung von OBS in Bodenproben aus Ultuna in Schweden und Lusignan in Frankreich, evaluiert. In den Langzeitversuchen in Ultuna zeigte sich, das die Abwesenheit von Düngung in Brache- und Kontrollbehandlungen den

Menge von bestimmten funktionalen Gruppen (Carboxyl- (1750 cm^{-1}), Aromaten- (1510 cm^{-1}) und Alkenverbindungen (950 cm^{-1})) im Vergleich zur Stallmistdüngung mit 2 Mg C ha^{-1} yr^{-1} erhöhte ($P < 0.05$). Zusätzlich wurde der Einfluss von Landnutzung (Grasland und Ackerland) und Landnutzungsänderung (Umbruch von Grasland zu Ackerland) auf die molekulare Zusammensetzung von OBS anhand von Proben aus Lusignan erforscht. Dies ergab, dass sich die relative Menge an carboxylischen, aromatischen und polyalkoholischen funktionellen Gruppen sowie Alkenen, unter Ackerland im Vergleich zu Grasland, erhöhte. Der Umbruch von Grasland zu Ackerland führte zu einem leichten Rückgang der aliphatischen funktionellen Gruppen und einer leichten Zunahme der aromatischen funktionellen Gruppen.

Anhand von Proben aus Langzeitversuchen (Bad Lauchstädt, Ultuna, Hohenheim und China) wurde weiterhin herausgefunden, dass die Erhöhung der Trocknungstemperaturen von 32 °C auf 105 °C für die Analyse von Bodenproben mit DRIFTS die spektrale Absorption sowie die Peakflächen für bestimmte funktionelle organische Gruppen (Aliphaten (2930 cm^{-1}), Aromaten (1620 cm^{-1} und 1530 cm^{-1}) und Polyalkohole (1159 cm^{-1})) erhöhte. Das Verhältnis von aliphatischen zu aromatischen (2930 / 1620 cm^{-1} und 2930 / 1530 cm^{-1}) Peakflächen erhöhte sich signifikant mit höheren Trocknungstemperaturen ($P < 0.05$). Dies bedeutet, dass methodologische Berücksichtigungen wie die der Trocknungstemperatur entscheidend sind, um die OBS Zusammensetzung, besser zu verstehen und um die OBS Dynamiken zu modellieren.

Die entwickelte Py-MIRS Technik sowie die Erforschung des Effekts der Trockentemperatur auf DRIFTS Peakflächen markieren einen bedeutenden Fortschritt in der Nutzung pyrolytischer und spektraler Techniken im Bereich der OBS Klassifizierung. Py-MIRS zeigte Potential als schnelle, reproduzierbare und effektive Technik um

Informationen über die molekulare Zusammensetzung von OBS, mit minimalen Einschränkungen aufgrund von Mineralinterferenz und sekundären thermischen Reaktionen, zu gewinnen. Zudem bot Py-MIRS Einblicke in nachhaltige Praktiken zur Verbesserung der OBS Qualität. Weitere Tests und eine Weiterentwicklung der Technik sind jedoch für Böden anderer Tonmineralogie sowie für andere Landnutzungen nötig.

Curriculum Vitae

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Work Experience

06/2017-Present	Research assistant, Institute of Agronomy in the Tropics and Subtropics, University of Hohenheim, Stuttgart, Germany.
06/2012-03/2017	Scientific worker, University of Hohenheim, Stuttgart, Germany Institute of Plant Production and Agroecology in the Tropics and Subtropics, Stuttgart, Germany within PAK Project 346, “Structure and Functions of Agricultural Landscapes under Global Climate Change-Processes and Projections on a Regional Scale”
10/2008 – 08/2009	Project Assistant, University of Buea, Cameroon, IDRC-UB Women and Land Rights Research Project.

Educational Background

06/2016 – Present	Ph.D. candidate. Faculty of Agricultural Science, University of Hohenheim, Stuttgart, Germany. Dissertation: Coupling pyrolysis with mid-infrared spectroscopy for the characterization of soil organic matter.
09/2009 – 09/2011	M.Sc. Physical Land Resources (Soil Science), University of Ghent Belgium. Thesis: Greenhouse gas emission from biochar amended soils.
10/2005 – 07/2008	B.Sc. in Geography, University of Buea, Cameroon
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List of scientific publications included in the dissertation

Laub, M., Blagodatsky, S., **Nkwain, Y.F.** & Cadisch, G. 2019. Soil sample drying temperature affects specific organic mid-DRIFTS peaks and quality indices. *Geoderma*, **355**, 113897.

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Conference contributions

Demyan, M.S., Mirzaeitalarposhti, R., Rasche, F., **Funkuin**, **Y.N**., Ali, R.S., Müller, T. and Cadisch, G. 20 April, 2016. General Assembly of the European Geosciences Union. Vienna, Austria. Oral presentation.

Funkuin, Y.N., Demyan, M.S., Rasche, F. and Cadisch, G. Coupling pyrolysis with mid-infrared spectroscopy (Pyro-MIRS) to study soil organic matter in arable soils. 5th International Symposium on Soil Organic Matter: Structure, Origin, Mechanisms. 20th - 24th September 2015. Göttingen, Germany. Poster presentation.

Demyan, M.S., Marohn, C., Rasche, F., Mirzaeitalarposhti, R., **Funkuin, Y. N**., Ali, R.S., Högy, P. Ingwersen, J., Wizemann, H.-D., Müller, T., Cadisch, G. Implications of spectroscopic and thermo- spectroscopic approaches for pool parameterization of soil organic matter models. 29 April 2014. General Assembly of the European Geosciences Union. Vienna, Austria. Oral presentation.

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