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Development of coupled mid-infrared spectroscopic and thermal analytical approaches for the characterization and modeling of soil organic matter dynamics of arable soils

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Abbreviations

DRIFT-MIRS: diffuse reflectance Fourier transform mid-infrared spectroscopy

FTIR-EGA: Fourier transform infrared (mid) spectroscopy-evolved gas analysis

In situ$_T$ DRIFTS: in situ thermal DRIFTS

MIRS: mid-infrared spectroscopy

Model pool: a conceptual soil organic matter pool as defined within a model that may or may not be linked to a measureable fraction.

PLSR: partial least squares regression as used in creating predictive models using mid-infrared data

POM: particulate organic matter. As separated first by size (wet sieving, >63 µm) and then heavy density separation (fraction <1.8 g cm^{-3})

rSOC: resistant soil organic carbon in the silt and clay fraction as resulting from oxidation with sodium hypochlorite at 95°C.

S+A: sand and stable aggregates fraction. Fraction after wet sieving of >63µm, and separation by heavy density liquid (fraction >1.8 g cm^{-3}).

Si+C: silt and clay fraction

SOC: soil organic carbon

SOM: soil organic matter

SOM fraction: part of the soil organic matter which has been separated via size, density, or chemical, or biological method or a combination of any of the four

SOM pool: considered as a more or less homogeneous group of soil organic matter as compared to the whole. Homogeneity can refer to either turnover time or to composition.

TOC: total organic carbon

Turnover: the length of time a particular fraction/group of SOM resides within a given pool before either moving to another pool or being mineralized.

1 Introduction

During the past 15 years or so, many researchers have focused on soil organic matter (SOM) not only due to its importance in agricultural ecosystems and nutrient cycling for crop production, but also due to the implications of SOM changes and feedbacks to the global carbon cycle. With this intense focus on better understanding of SOM, many advances have been made both in the methodological approach of studying soil organic matter and also in incorporating these measurements into modeling (Yadav and Malanson, 2007). Many of these advances have built on the previous early work done by some of the early soil scientists which recognized quite early on the complex nature of soil organic matter and its importance to the productivity of soils and long-term resilience (Schreiner and Shorey, 1911; Page, 1922; Sprengel, 1826).

This study sets forth to examine both changes in SOM composition under differing long term agricultural managements and also within season dynamics utilizing new methods of diffuse reflectance Fourier transform mid-infrared spectroscopy (DRIFT-MIRS), Fourier transform infrared evolved gas analysis (FTIR-EGA), in situ thermal DRIFTS (in situ$_T$ DRIFTS) in combination with physical, size and chemical fractionation in order to provide linkages to modeling of soil organic matter and soil organic matter dynamics.

In this dissertation an introduction and short review of the importance of SOM, SOM stabilization mechanisms, SOM pool dynamics, characterization methods, and modeling approaches is given (chapter 1). Next, the development of a specific mid-infrared peak method (DRIFT-MIRS) for studying SOM as applied in a Chernozem soil from the long-term Static Fertilization Experiment, Bad Lauchstädt, Germany will be explored (chapter 2). Further, the development of additional mid-infrared methods of Fourier transform infrared coupled evolved gas analysis system (FTIR-EGA) and in situ thermal DRIFTS (in situ$_T$ DRIFTS) to study SOM again of the Bad Lauchstädt site and also soils from the Kraichgau and Swabian Alb areas of southwest Germany is elucidated (chapter 3). These three approaches (DRIFT-MIRS, in situ$_T$ DRIFTS and FTIR-EGA) are then used in addition to classical size/density fractionation in an application for use as SOM pool parameterizations in a modeling exercise in the LUCIA-Century SOM model (chapter 4). Finally, this work ends

with an overall discussion of the findings in relation to current research and further research needs.

1.1 Background

1.1.1 The importance of soil organic matter (SOM)

Soil organic matter is derived from plant above ground and below ground residues, exudates, and microbial products. It is in various forms which will be relatively unstable or mobile in the soil system, lost from the system via leaching, erosion, or respiration. Losses from the local scale via leaching and erosion then can variously be decomposed within an aquatic system, or transported to a deep marine environment where it can be covered by more sediment and be "lost" to the global turnover cycle (Hedges et al., 1997). The major fluxes, though, concerning the global terrestrial carbon cycle are connected with inputs to the soil via litter fall and roots which are then the partially mineralized and remaining compounds stabilized via various mechanisms. The stocks of C contained in soil are estimated at 1500 to 2000 Pg in the top one meter depth and 2300 Pg to a depth of three meters (Jobbágy and Jackson, 2000). This amount is more than both terrestrial vegetation (550 ± 100 Pg) and also the atmosphere (800 Pg) contents of C (Houghton, 2007). Due to uncertainties, it is unclear at a global level the influence of changing climatic conditions on C cycling and also an accurate accounting of the amount and composition of C below 1 meter is lacking (Jobbágy and Jackson, 2000). The uptake or sink effect of land on the global C cycle has a high year to year variability due to errors in measurement, as it is calculated via several methods (Le Quéré et al., 2009). Thus land use changes or the rate at which C cycles in terrestrial systems may greatly influence the atmospheric and other stocks. Natural terrestrial systems are said to be at equilibrium or steady state with regard to their C balances, although in some instances this has shown not to be the case (Wardle et al., 2003). Even in "undisturbed" ecosystems there is variability or uncertainty from year to year whether the ecosystem is a net flux or sink of C as has been shown in the Amazon basin (Saleska et al., 2003) and also globally with estimates from -0.8 to 2.2 Pg yr^{-1} (Houghton, 2007). Additional global anthropogenic influences, such as atmospheric deposition of nitrogen, have the possibility to affect ecosystem functions (Boring et al., 1988) including C cycling.

Soil organic matter and soil productivity and quality are intimately linked. Sufficient SOM levels are essential for a healthy soil micro biome and likewise for efficient nutrient cycling.

Physically the soil is also improved by the added potential of aggregation and physical strength which increases aeration, water infiltration and retention, and decreases erosion. While a decrease in SOM likewise will reverse any of these benefits and could lead to a feedback for further soil degradation.

1.1.2 Stabilization mechanisms in SOM

Stabilization of SOM can be defined as the resistance of SOM from being lost via respiration, erosion, or leaching (Sollins et al., 1996). Stabilization mechanisms have been divided into three broad categories: a.) inherent chemical structure resistance against degradation, b.) spatial inaccessibility, and c.) interactions with surfaces and metal ions such as clay surfaces and oxides (Sollins et al., 1996; von Lützow et al., 2006; Six et al., 2002).

Firstly the chemical structure of compounds can lead to short-term to medium stability through complexity of substrate and can be divided into primary recalcitrance (structure of plant litter, rhizo-deposition, and organic fertilizers) and secondary recalcitrance (microbial and faunal products, extracellular, and charcoal formation) (von Lützow et al., 2006). Simple sugars and carbohydrates are quickly utilized by microorganisms, while aliphatics and finally lignin polymers are more resistant. Highly aromatic components (either biologically synthesized or through fire synthesis) generally have the highest stability due to the double C bonding and highly condensed structure. The polyphenol content of different plant residues has been found important in intermediate turnover of plant residues (Vityakon et al., 2000) and some have suggested that polyphenols inhibit microbial activity (Mandal et al., 2008). Alternatively it has been found that peat bog systems, structural polysaccharides are relatively resistant to decomposition in Sphagnum moss ecosystems (Hájek et al., 2011). Additionally lignin, another aromatic group containing chemical class, can be readily broken down by white rot fungus (Saiz-Jimenez and de Leeuw, 1984) and has be found to have a rapidly decomposable portion and a more stable portion in residues (Thevenot et al., 2010). An issue with following decomposition and breakdown of plant residues in soils is the issue with modifications made by microbial community which may only slightly modify the compound, but which changes its stability or nature dramatically. Most extraction methods are still crude forms, and are difficult to use for soils as any one compound class in soil may be a very small amount. It has been shown though that generally chemical recalcitrance in and of itself does not lead to long term stabilization of organic matter in the soil, with most of biogenic origin having a turnover time of <50 years (Marschner et al., 2008).

Secondly spatial inaccessibility is related to where the OM is located in relation to microbial communities, water, and oxygen. The spatial location can be related to horizon or depth from the surface with surface horizons receiving fresh inputs of OM and incorporation through plowing or bioturbation, while deeper horizons are more influenced by root inputs and dissolved OM from the surface horizons and their flow along macropores or preferential flow pathways (von Lützow et al., 2006). On the microscale, aggregation plays an important role in stabilization. Macroaggregates (>250 µm) are generally held together through fresh plant residues and roots, but are generally transitory and probably only provide stabilization for around a year. They are also sensitive to management and it has been postulated that tillage interrupts the formation of additional microaggregates through destruction of macroaggregates thereby short-circuiting the pathway of stable microaggregate formation (Six et al., 2000). Further the OM from the disrupted macroaggregates then either has the possibility to form organic-mineral complexes with available mineral surfaces or is available for utilization by microorganisms (Grandy and Neff, 2008). With decreasing aggregate size, stability generally increases and also the proportion of primary plant residues decrease, while microbial products increase (Grandy and Neff, 2008). An additional quality related to accessibility is hydrophobicity, which restricts the movement of water into a OM macromolecule, thus reducing the activity of microorganisms (von Lützow et al., 2006).

The third stabilization mechanism is related to the interactions with mineral surfaces. These interactions include Van der Wahl's forces, polyvalent cation bridges, ligand exchange, and hydrogen bonding among others (von Lützow et al., 2006). These tight interactions seem to protect SOM for longer time periods. Evidence for these interactions have been shown by the relationship between increasing C contents in $< 20 \mu m$ fraction and the weight of the $< 20 \mu m$ fraction (Hassink, 1997) and by increasing OC in the mineral fraction with increasing concentration of dithionite-citrate-bicarbonate extractable iron (Kögel-Knabner et al., 2008)

1.1.3 SOM dynamics-SOM pools

Dynamics of SOM vary at different temporal scales and these various temporal scales also relate to different theoretical SOM pools. Pools will be defined as a stock of SOM which is grouped either by its relative ease of decomposition or likewise its relative stabilization against decomposition or resistance to loss from the system. This will be followed by a discussion of environmental effects of soil organic matter dynamics.

The short-term turnover, as a working definition, would be changes within days to a growing season, up to a maximum of 1-2 years. These changes mostly involve easily decomposed compounds, but would also include compounds which will be incorporated into microbial bodies, and a portion of which will be respired as CO_2, but also another portion which will stay in the system as secondary metabolites which will generally increase in stability. As already mentioned previously, increases in respiration can be seen in the time frame of hours after the addition of glucose (Anderson and Domsch, 1978), after re-wetting dried soil, and after thawing of frozen soil. Root exudates can be rapidly utilized by microbes as has been shown by laboratory incubations with over 50% of applied root exudates being decomposed within 3 days at 20°C (Hütsch et al., 2002). While root exudates themselves are highly labile, inputs from root residues showed slower turnover during six months as also compared to shoot residues (Puget and Drinkwater, 2001). In temperate climates decomposition reaches a maximum in the early to mid growing season when temperatures and moisture are at near optimum levels, residues from the previous growing season are available, and new plant growth is adding root exudates to the soil. It has been shown on a global level atmospheric CO_2 concentrations increasing to a maximum during late spring or early summer depending on latitude and decreasing to a minimum at the end of the growing season showing a net uptake of C from the atmosphere at the height of the growing season and a release from the land surface during the winter and spring (D'Arrigo et al., 1987). In tropical climates, the growing season will correspond to a rainy season, while the rest of the season will have lower turnover due to lower moisture availability. Medium range turnover can be thought of in the time frame of decades and is more influenced by management or land use. It has been found that during a twenty year chronosequence after disturbance by fire, the first years after the SOM dynamics were driven mainly by the chemistry of the charcoal, but after 4 years mainly by fresh litter input (Alexis et al., 2012). Long term dynamics can be related more to climatic changes which have influenced not only temperature or precipitation but also vegetation type. Although, it is usually assumed that changes in management of a soil will result in an initial drastic change and reach equilibrium after several decades, it has been seen that even after 100 years or more of conversion of arable land to grassland, SOM is still increasing (Franko et al., 1997).

Generally microbial respiration will increase with increasing temperature, most likely up to some physiological limit where again activity will decline. Freezing greatly limits microbial activity, although microbial activity has been recorded down to -20°C in permafrost (Rivkina

et al., 2000). In temperate soils 24 to 30°C has been found as an optimum for C mineralization with a decrease thereafter (Bárcenas-Moreno et al., 2009; Wilhelmi and Rothe, 1990). An additional event especially in temperature and cold climates are freeze/thaw cycles that have been shown to result in an increase of organic substrates available for decomposition either through death of microorganisms or the physical changes in soil structure (Herrmann and Witter, 2002). The general assumption is that microbial activity doubles with every 10°C increase in temperature giving a Q_{10} value of 2, although general differences have been found depending on biome (Chen and Tian, 2005). The composition or quality of SOM may also have an influence on the temperature sensitivity of the bulk soil as some studies have shown older organic matter to be more sensitive to increases in temperature (Vanhala et al., 2007; Xu et al., 2012), less sensitive (Liski et al., 1999), but also no difference compared to younger C (Fang et al., 2005).

Moisture has been recognized for a relatively long time as an important factor influencing SOM dynamics (Birch, 1959). For microbial processes moisture impacts on physical, physiological, and biochemical processes and microbial processes are usually assumed to have a minimum at low and high matric potentials and a maximum somewhere in between (Moyano et al., 2013). Important moisture dynamics related to SOM dynamics are water holding capacity of the soil, wetting and drying events and duration of wetting and drying cycles. As with temperature, moisture may become the limiting factor for microbial turnover, when reaching dryness, there is no water left for diffusion of enzymes. It also seems that soil type or texture has an influence on the impact of moisture on C mineralization as sandy soils will have a lower proportion of micropores available for water films compared to clay soils (Jin et al., 2013). At the opposite extreme of the volumetric water content, in saturated conditions, aerobic respiration rapidly ceases and is taken over by anaerobic respiration which is much less efficient in decomposition. In these cases organic matter can increase due to suppressed decomposition. The cycles of wetting and drying have also been shown to have a pronounced effect on C mineralization and are important in C dynamics in semi-arid and arid environments (Borken and Matzner, 2009). Microbial community composition has been shown to change during the transition from pre-monsoon to post-monsoon periods (Cregger et al., 2012).

Soil texture and structure have several effects on SOM turnover. As already mentioned in the stabilization mechanisms section, fine texture soils provide a larger surface area, greater aggregation potential, and larger sorption capacity to protect organic matter from

decomposition. Specific clay minerals such as allophane and ferrihydrite, both with amorphous structures and high surface areas are especially good in reducing SOM turnover and provide resistance to land use change influences losses of SOM (Parfitt et al., 1997). A relationship between increasing clay content and decreased C turnover was seen in several studies (Van Veen et al., 1985; Ladd et al., 1985). Even though this relationship has generally been accepted it has been difficult to prove sometimes (Oades, 1988). It has been shown in short-term (7 days) that heavy textured soils provide a habitat for bacteria which makes it more difficult for protozoa predation, although the mineralization rates at the end of the experiment were similar lower clay content soils (Rutherford and Juma, 1992). Related to soil structure, well structured soils improve water movement and aeration which on the one hand will increase decomposition of non-protected SOM, but on the other hand will protect SOM inside of aggregates. Additionally improved soil structure can also effect above ground productivity which can lead to increased C inputs to the soil (Hamza and Anderson, 2003).

A factor that is less addressed in SOM cycling is the extent of the control of pH. Called the master variable in soil systems, pH and soil acidity are variable within natural systems depending on parent material, with acid parent materials such as in highly weathered oxic soils have been formed through millions of years of leaching of basic cations, leaving behind a high content of aluminum and iron oxides. There are instances of relatively recently formed soils, certain basalts and shales that have a naturally low pH value due to enrichment of alumina or iron containing minerals which upon weathering oxidize and form sulfuric acid. In human impacted systems, acid rain (Markewitz et al., 1998), long term fertilizer application (Barak et al., 1997), liming (Tang et al., 2003), and mining (Johnson and Skousen, 1995) can have short and long term effects on pH and soil acidity. While it has been shown in short-term experiments (100 day laboratory incubation) that microbial biomass and respiration increase due to increase of pH due to lime application (Curtin et al., 1998), the long-term effects have not been directly studied. It is hypothesized that a similar effect would be seen as from fertilizer application, increasing crop yields and increasing residue inputs to soil will result in increasing SOM (Haynes and Naidu, 1998). It has been found that generally at lower pH there is a greater occlusion of OM with mineral surfaces (Mayer and Xing, 2001)

Organic matter inputs are a driving force for SOM dynamics and are especially important in agroecosystems. This includes types of crops grown, cover crops, amount of residue returned to the field and also burning or not burning residues. The amount and nature of organic

matter inputs to the soil will affect SOM cycling. In the short term time, the C/N ratio will affect decomposition and N immobilization. Additionally the molecular composition of the litter (i.e. sugars, cellulose, and lignins) will affect the decomposition of litter (Wickings et al., 2012). Additionally the pH of the residues on the medium term could lead to decreases in soil pH which has been shown under Eucalyptus (Farley et al., 2009). Root residues are generally more enriched in recalcitrant compounds and generally are more preserved in the long term compared to above ground residues (Kätterer et al., 2011). In arable systems the amount of residue is very important to maintaining soil fertility and SOM levels. Whether the above biomass is removed for biofuel or forage usage, part returned, or the residue burned, will dramatically affect the annual C cycle of the system. In addition to the roots, it is usually required to return at least a portion of the above ground residues to the soil.

In agricultural systems soil tillage both the number of times, depth, and mixing of surface residues will influence the SOM turnover. Generally lower intensity tillage results in the build up and lower degradation of newly incorporated residues (Murage et al., 2007), while higher intensity tillage results in considerable mixing of the upper horizon of soil disrupting more of the macroaggregates leading to increased decomposition of SOM (Six et al., 2000). In addition to the mixing effect, the placement of residue has an important role at least in short-term SOM turnover, as was shown in a litter placement study which found only 30% of remaining residue after 14 months after incorporation in the soil, but 43 and 69% in litter bags and ^{14}C recovered straw, respectively surface applied after tillage (Holland and Coleman, 1987). Since the soil is mixed to a lower degree in conservation tillage, this also results in stratification in SOM very near the surface as compared to a more even distribution compared to conventional tillage (Dick et al., 1998).

1.1.4 SOM characterization methods

In studies of SOM, depending on the goal of the research, many methods have been used in order to characterize and study the composition and dynamics from a molecular scale to global. While measuring total soil carbon or total organic carbon gives an idea of the size of the total soil characterization of SOM which then may give more information on a functional property (e.g. respiration, nutrient availability) show influences of management, or other changes. The methods can be variable and depend on the soil type or the objective of the study.

Chemical

Chemical characterization is related to the total amount (C or IC), certain amount of organic compounds (cellulose, lignin, polyphenols). Additionally important elements such as oxide content (Al-, Fe-, Mn-) and the crystallinity of these oxides by the dithionite-bicarbonate extraction is used to study the importance of different binding mechanisms (Kaiser and Guggenberger, 2003). Specific surface area as measured by a multiple step absorption/desorption of N_2, has been utilized to study the microporosity and absorption of OM on mineral surfaces (Kaiser and Guggenberger, 2003). Additionally chemical oxidation has been used to separate an older SOM from younger, easily oxidizable SOM. Some oxidants are H_2O_2, $Na_2S_2O_8$, NaOCl and NaOCl with HF and HCl. In a review of the methods by (Helfrich et al., 2007), H2O2, $Na_2S_2O_8$ were generally found to isolate the oldest SOM based on ^{14}C dating.

Physical

Size or density separation of SOM is related to the hypothesis that SOM can be stabilized or intricately associated with the mineral fraction of the soil to different degrees depending on either the particle size or density. Size separation is usually based on the standard sand (2000-0.63 µm), silt (0.63 to 0.2 µm), and clay (<0.2 µm) separations (von Lützow et al., 2007) used for texture analysis. With decreasing particle size, there is an increasing surface area, thus more stabilizing effect for SOM. Density separation is also based on the same principle in that a heavy liquid is used to separate the soil into different densities (von Lützow et al., 2007). This in effect separates the particles into different mineralogies, as different minerals have different densities, with Fe- and Al- oxides having some of the highest densities of soil minerals (Rühlmann et al., 2006). The size and density approaches are often combined such that the sand or sand and silt portion are separated from the clay and then a density separation is done (Zimmermann et al., 2007a). These methods involve numerous steps which are time consuming and also can be prone to loss of SOM, which then factors into the distribution among the fractions.

Spectroscopic

Over the past two decades, with the development of more accurate, less expensive detectors, vibrational infrared spectroscopy (visible (VIRS), near infrared (NIRS) and mid infrared

(MIRS)) has expanded in the area of soil sciences. Vibrational Spectroscopy relies on the fact that compounds absorb or reflect differing amounts of energy at various wavelengths depending on their bond structure, configuration, and strength. In the field of soil science, some of the early applications were studying humic substances (Schnitzer and Hoffman, 1965), layer silicates (Farmer and Russell, 1964), lignin from different sources (Farmer and Morrison, 1964). These were investigations mainly into identifying which molecular vibrations could be found in certain substances, differences between the same substance from different locations or sources (Farmer and Morrison, 1964) or of assigning peaks to certain vibration groups related to the structure of the substance in the case of layer silicates (Farmer and Russell, 1964). MIRS has been used to identify litter types and decompositional status (Tatzber et al., 2011). It has also been used to study intact soil aggregate surface and the distribution of OM within preferential flow paths (Leue et al., 2010). A study by Reeves III et al. (2006) showed the application of MIRS and near infrared (NIRS) for the prediction of SOM fractions using partial least squares (PLS). Attempts have been made to make global or generic predictions for various soil properties include TOC but with less success then more site or region specific models (Terhoeven-Urselmans et al., 2010). Several drawbacks of these spectroscopic methods are that vibrational frequencies are generic, in that a certain vibrational group may be associated with multiple compounds and vibrational frequencies of different compounds can be overlapping (i.e. the C-O vibration of alcoholics and Si-O vibrations of minerals in the mid-infrared range). This is a reason that multivariate statistical procedures have become so popular for use in soil applications (Chen et al., 2002; Rossel et al., 2008) (i.e. which have very heterogeneous samples compared to chemical samples). Multivariate procedures such as partial least squared regression (PLSR) do not make any assumptions of the underlying molecular associations of the spectral intensities, but rather relate the measured spectra to the analyte concentration of interest (e.g. TOC) via a few principal components extracted from the spectra. If such constructed prediction models are accurate enough then they can be used to predict unknown samples, but if unknown samples are outside of the calibration range or are of another soil type, the prediction may not be successful. While it can be shown via correlation which spectral regions are more related or add more information to a certain principal component in PLSR (McCarty et al., 2002), the underlying molecular connection may not be clear.

Additional applications of spectroscopy include in situ DRIFTS (Toops et al., 2005) and evolved gas analysis (FTIR-EGA). These use the sample principals of regular solid powder

MIRS and apply them to a sample while heating (in situ DRIFTS) or to the gas of the soil sample while heating (FTIR-EGA). Both methods have been used for individually for different substances (i.e. coal (Murakami et al., 1997), clay minerals (Frost and Vassallo, 1996) they have not been used together so far for investigating SOM.

Thermal

Tradition thermal analyses have been used to study both SOM and properties related to SOM (such as clay type and clay content). Thermal gravimetric analysis (TGA) has been used to measure total organic and inorganic carbon in soils (Siewert, 2004). Additional methods such as differential scanning calorimetry (DSC) and differential thermal analysis have also used to characterize SOM and SOM fractions (Lopez-Capel et al., 2005). The methods are relatively rapid and do not require expensive columns, but do have the drawback that weight losses due to interlayer water and energy changes due to changes in the mineral structure also occur in the same temperature range of SOM degradation (Gaál et al., 1994).

Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) applies a magnetic field to the sample and depending on what element is being studied (usually ^1H and ^{13}C for SOM) provides a semi-quantitative assessment of the molecular groups in the SOM. The method has been used extensively to study bulk soils, fractions, differences between horizons, and also residues. A drawback of this method is usually in the solid-state there is strong influence from paramagnetic compounds which requires a destruction of the mineral phase through hydrofluoric acid (HF) dissolution (Randall et al., 1997) which may alter the nature of some component of SOM. If the HF dissolution is needed, then a large sample (100's of grams) may be needed to gain enough extracted humic acid for the analysis as the yield of humic acids especially for arable soils can be low.

Biological utilization

Soil incubations under controlled conditions have been used extensively to calculate Q_{10} values for temperature sensitivity (Hamdi et al., 2013) and to calculate labile and slow pools of SOM. Incubations often rely on sieved, homogenized samples which necessarily destroy the natural soil structure. Additionally many different incubation temperatures, durations, and

water contents have been used, making comparisons between experiments sometimes difficult. The utility of this method, though, is the better standard conditions to investigate the response of soil respiration to certain variables (e.g. moisture, temperature, substrate addition) as compared to the field. Measuring carbon gas fluxes (CO_2 and CH_4) from the soil is important to understanding short-term SOM dynamics. Gas fluxes vary greatly spatially and also with depth. Traditionally chamber methods with soda lime (Franzluebbers et al., 1995) were employed to measure the CO_2 flux over a certain period of time. Newer methods included infrared gas analyzers (both portable (Jensen et al., 1996) and automated (King and Harrison, 2002)), gradient flux method (Liang et al., 2004), eddy covariance stations (Janssens et al., 2001), and tunable diode laser absorption spectrophotometer (TDLAS) (Wingate et al., 2010) which enables measurements of isotopic ratios ($^{13}C/^{12}C$). Additionally gas samples can be taken from chambers and measured in the laboratory via gas chromatograph. Portable analyzers are able to take an instantaneous measurement within a few minutes, although chamber size my lead to high variation. For laboratory measurements devices which use infrared detectors (Heinemeyer et al., 1989) or conductometers (Palmborg and Nordgren, 1993)) can be used for continuous, automated measurements to measure soil respiration under controlled conditions.

Isotopic

With the advent of more accurate and less expensive mass spectrometry methods ^{13}C and ^{14}C methods have begun to be used in soil science to calculate a turnover or age of different fractions of SOM. The natural abundance method of ^{13}C uses the natural variations in $^{13}C/^{12}C$ ratios in mainly C3 and C4 plants to calculate the percentage of recent residues in the whole soil, amount in different fractions, and also the remaining amount of previous vegetation in the soil (Millard et al., 2010). This method, though, requires a C3 to C4 or vice a versa change in vegetation to have taken place and assumes that there is no discrimination in the decomposition process between C3 and C4 plant inputs. In ^{14}C measurements the radioactive isotope of ^{14}C is used to measure or date the age of SOM (Garnett et al., 2011). Labeling can also be done with pulse of ^{14}C or ^{13}C enriched air and then measured in the soil, roots, shoots, soil CO_2 flux and microbial biomass.

1.1.5 SOM models/modeling approaches

Soil organic matter modeling has been utilized for understanding the complex mechanisms and interactions of SOM already mentioned for exploratory and predictive purposes on many different scales (Van Keulen, 2001). Models range in complexity from single pool models to more complex models with multiple pools and many rate modifiers (e.g. temperature, moisture, clay content). A model can refer to a simple decay equation which follows the decomposition of a type of residue or litter at a constant rate (Jenny et al., 1949), multi pool models which have different pool turnover rates (Hansen et al., 1991; Jenny et al., 1949; Parton et al., 1987), or as a SOM sub-model imbedded in a larger model which includes other factors including hydrology, nitrogen uptake and plant growth (Hansen et al., 1991). Additionally the SOM model can be included in landscape spatially explicit models with erosion dynamics such as the Land Use Change Impact Assessment model (LUCIA) (Marohn et al., 2012) or global scale models coupled to general circulation models for studying climate forcings and feedbacks to the atmosphere from the land surface (Ostle et al., 2009).

One of the early attempts at OM decomposition modeling came from Jenny et al. (1949) with a single first order decomposition equation elaborated by Olson (1963):

$$C_t = C_o\, e^{-k*t} \qquad\qquad \text{Eq 1}$$

where C_t is carbon (litter) at time t, C_0 is carbon at time zero, and k is the turnover or decomposition rate. This equation assumes a constant decay rate for the litter input. As it has been found during incubation studies a general shape of rapid decrease of CO_2 evolution then reaching a more stable slowly decline period (Collins et al., 2000), a two pool model has often been utilized (Rovira and Rovira, 2010):

$$C_t = C_a\, e^{-k\_a*t} + C_s\, e^{-k\_s*t} \qquad \text{Eq 2}$$

where C_t is carbon (litter) at time t, C_a is carbon allocated to an active or labile pool, and k_a is the decay constant of the active pool while C_s and k_s are the size and turnover rate of the slow pool. This equation assumes that there is an easily decomposed fraction of OM which turns over rapidly (weeks to few years), while there is a more resistant portion which decays more slowly (decades). For litter decomposition dynamics, the one or two pools have been utilized extensively, while for soils these approaches and additionally a three pool model

have been used (Paustian et al., 1992). The passive pool has an even slower turnover time (400 to 2000 years) compared to the active and slow pools (Paustian et al., 1992). Additionally an approach of having an "inert" pool has been used (i.e. Roth-C) a portion of the SOM is isolated from the turnover cycle and is biologically inert (Coleman and Jenkinson, 1995; Falloon et al., 1998). Another way to think of the inert pool is a resistant or refractory SOM (RSOM) pool which is uncoupled from the other OM pools (Falloon and Smith, 2000). Alternatives to the many multi-pool models are the approaches of having a single pool but having a dynamic turnover rate which varies according to time (Rovira and Rovira, 2010; Ågren and Bosatta, 1996) or by a quality factor (Ågren and Bosatta, 1996). This approach is attractive as it dispenses with the problem of SOM allocation among the pools.

Different mechanisms (i.e. stabilization and microbial utilization) or controls (i.e. moisture, temperature, management) are dealt with either explicitly or implicitly in SOM models. How these factors are dealt with or implemented in a model is important for the user as this may affect the behavior of the model.

Substrate use efficiency

Substrate use efficiency is the portioning of C between growth and respiration and has been measured by various methods and therefore implemented into models in various forms (Manzoni et al., 2012). In most models microbial control on decomposition is indirectly modeled via changes in turnover rates via temperature, moisture, and tillage operators. The utilization is a set value, that is a certain percentage of C going from one pool to another via microbial utilization with the rest respired, as in the case of Century (Paustian et al., 1992). One of the early attempts modeling the direct control of microbial metabolism in decomposition was (Parnas, 1975). Daisy has allocated two different pools for soil microbial biomass (Hansen et al., 1991) while Century has a litter microbial biomass and soil microbial biomass (active pool) (Paustian et al., 1992). Another theoretical approach has been that there are two pools for the microbial biomass variously related to the rapid and slow growing or autochthonous and zymogenous (Stenström et al., 1998). In the approach of (Moorhead and Sinsabaugh, 2006), the microbial community is divided into three "guilds" related to the decomposition capacity of each groups; opportunists which rapidly colonize readily available litter, decomposers whom degrade cellulose and lignocelluloses, and finally miners whom utilize humified OM. The incorporation of such pools adds complexity to models, but may be

a needed addition for the understanding of the response of ecosystems to change (McGuire and Treseder, 2010).

Physical protection

Physical protection, which has been mentioned previously as an important mechanism in SOM stabilization, is usually dealt with via clay or silt plus clay content in SOM models (Krull et al., 2003). In two similar compartmental SOM models, Century and Roth-C, the effect of silt and clay contents on SOM levels is positively concave for Century, while negatively convex for Roth-C illustrating the same general trend but slightly different relationships (Paustian et al., 1997).

Dynamic controls on decomposition

Dynamic abiotic controls such as temperature, moisture, and tillage are usually dealt with as a modification of the *k* turnover rate, where the *k* rate is the turnover under optimal conditions, while the rate will be reduced when the conditions are different from optimum. In Century for example the temperature and moisture functions are equal across all pools. Additionally, intensity of tillage has been implemented as usually a continuum from no disturbance (no-till or low intensity land use) to high amount of disturbance (conventional plowing) in which all litter moves directly into soil pools or is respired in the case of Century (Paustian et al., 1992). A rate modifying effect for the CN-SIM model that seemed to take into account other influences of tillage (e.g. bulk density, porosity) was used by (Chatskikh et al., 2009). Conventional tillage was taken as a value of 1 while no-till was 0.48.

Pool initialization or parameterization

Initialization of pool sizes within compartmental models has been done via several approaches which can be divided up into four general methods; using a default setting from a long-term experiment, iteratively adjusting the pool sizes to fit the measured data of an experiment, a long-term equilibrium run, or measureable fractions used for pool initialization. Initialization of soil organic matter pool sizes depends again on the goals of the research and also the model type. Models which have a single pool will require the input of the TOC content of the soil and little else. More complex models require individual pool sizes and turnover rates (discussed above). Equilibrium can be assumed for a model start, so that the current pool distribution will be accurate or a pre-model simulation of the previous land use

can be done (Bruun and Jensen, 2002). This can also be done in a two step process, running the model for a very long time period (5000 years) for initializing the recalcitrant or passive pool and 100 years for the slow pool (Álvaro-Fuentes et al., 2012). In the presence of a change from C3 to C4 vegetation (or vice versa) one can estimate the proportion of new OM relative to old (Bernoux et al., 1998). Long term incubation (800 days) has been coupled with acid-hydrolysis to yield both rate constants and also pool sizes for the Century model (Paul et al., 2006). The incubation method (800 days) was used to determine the turnover rates for the active and slow pools and the size of the active pool, while acid hydrolysis was used to determine the size of the passive pool and by difference the slow pool (Collins et al., 2000). For the MiCNiT model the pools consist of soil microbial biomass and its activity as measured by substrate induced respiration, dissolved OC, and TOC which are all easily measured (Blagodatsky et al., 2011). Even in the case of using measureable fractions there still may be the need for iterative fitting of initial pool sizes from fractions as it may be difficult to transfer a measured fraction directly to a pool (Zimmermann et al., 2007b). The inert pool in Roth-C has been parameterized via radiocarbon ^{14}C measurements and if these are not available a transfer function has been developed based on TOC (Falloon et al., 1998). A review by (Falloon and Smith, 2000) showed a variety of methods, usually dealing with some type of acid hydrolysis or fractionation and acid hydrolysis to isolate a recalcitrant pool of SOM. Additionally sodium hypochlorite has also been used as an agent for recalcitrant pool measurements (Zimmermann et al., 2007b).

As a general observation in three compartment models, the slow pool is relatively poorly defined and as mentioned previously may be calculated as the difference between the active pool as measured by microbial biomass and the recalcitrant C. Due to the turnover time of a slow pool of decades, this can greatly influence even short-term modeling exercises. This leads to the importance of developing better methods for either pool parameterization, model pools, or both.

From a literature search using the search engine SCOPUS for the key words (http://www.scopus.com/home.url) "CENTURY", "soil organic matter", and "model." Papers were then used for the literature review if the CENTURY SOM model was used, SOM pool sizes and soil/site parameters were specified (clay content, mean annual precipitation (MAP), mean annual air temperature (MAT), model pool sizes either in percent or weight basis), and how SOC was allocated among the pools (mostly via a long-term equilibrium run). This resulted in 14 papers of 53 sites or soils (see Appendix 1 for specific sources). These sites

were from a large range of environments and soil types with clay contents from 2 to 63%, sand contents 10 to 94%, MAP 214 to 2648 mm, and MAT from -6.3 to 28.8°C. This shows the applicability of the CENTURY model but also shows the large range in pool size distribution especially between the slow and passive pools. There were no statistically significant trends between pool size and single abiotic variables (Figure 1). There were wide ranges in pool sizes. For example the slow pool ranged from 8 to 65% at around 20% clay content. Likewise around the same clay content, the passive pool ranged from 35 to 95%. The wide range of the pool allocations gives the indication that especially these slow and passive pools are generally not well defined.
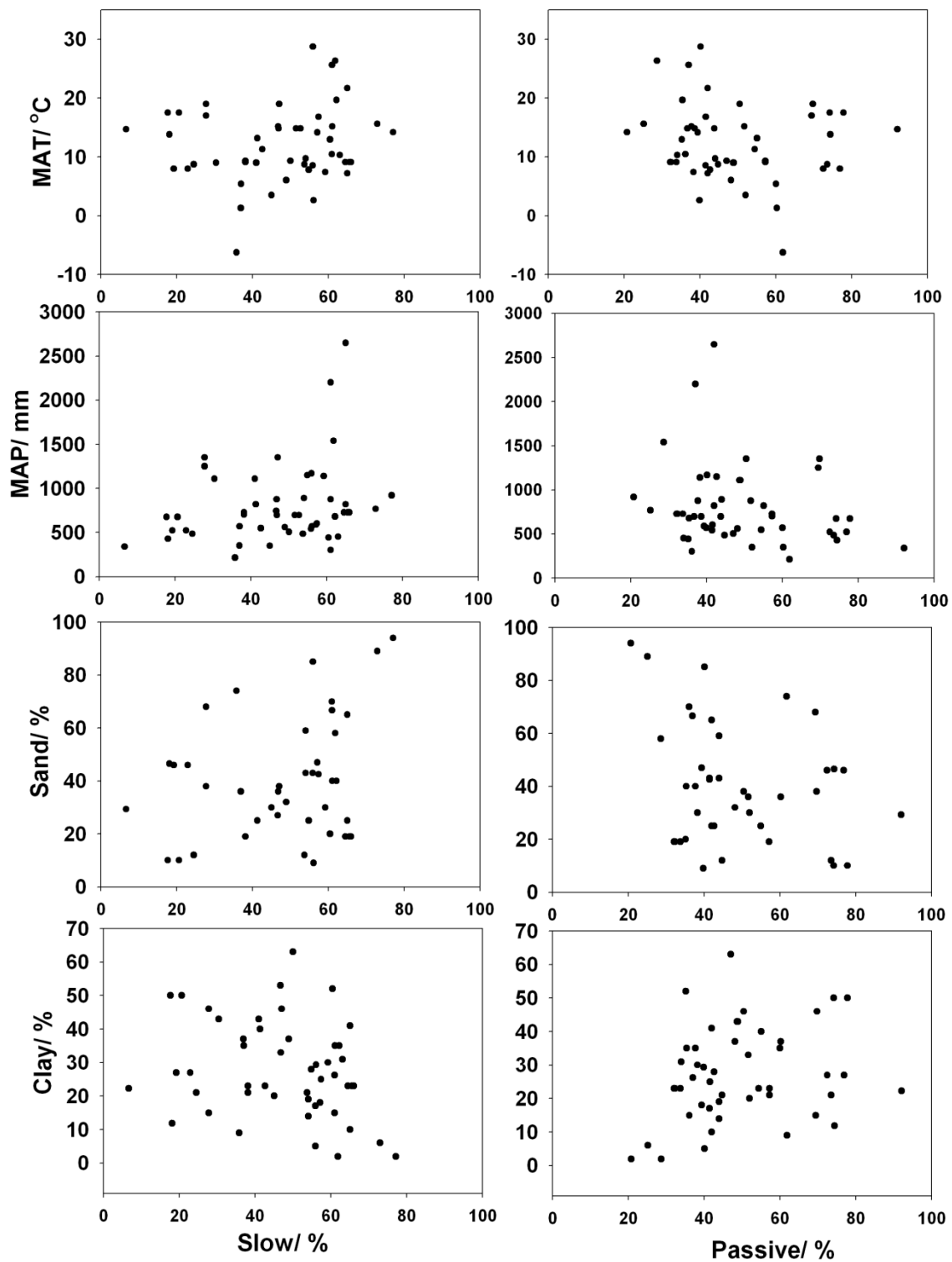
Figure 1. Review of Century slow and passive pools in relation to abiotic factors as taken

from literature (see Appendix 1). MAT mean annual temperature, MAP, mean annual

precipitation.

1.2 Objectives/hypotheses

The general objectives of this study were i.) to characterize SOM via vibrational spectroscopy linking specific organic functional groups to different stabilities of SOM as influenced by long-term fertilizer managements, ii.) investigate the utility of using a linked FTIR-EGA and in situ thermal DRIFTS system for additional characterization of SOM and iii.) apply these methods as a comparison to traditional methods (i.e. long-term equilibrium simulation, SOM size/density fractionation) of parameterizing SOM models for measuring short-term dynamics at the sites Kraichgau and Swabian Alb in SW Germany.

In order to advance our understanding of SOM composition and dynamics, it was determined that a multi-dimension approach was needed to address this issue. It was hypothesized that the relative composition or proportions of different organic functional groups (e.g. aliphatic, aromatic) as measured by mid-infrared spectroscopy would be affected as a result of long-term management and also absence of fresh organic inputs. Additional thermal stability as measured by FTIR-EGA of SOM and fractions would be related to its inherent stability and that different qualities of SOM would be evolved at different temperatures during heating. Finally, that these measures of different qualities of SOM would be able to be used as direct model inputs for SOM modeling.

1.3 Study outline

To explore the possibility of different mid-infrared active functional groups to reflect SOM quality changes, soil samples were taken from the Static Fertilization Experiment, Bad Lauchstädt (Figures 1 and 2) from years 1956, 1979, 1992, 2004, and 2008 and treatments of farmyard manure, NPK mineral fertilizer, combination of both, and control. DRIFTS measurements were taken of both the bulk soil and also soil organic matter fractions separated by size-density. This long-term experiment and also a short term experiment established in 2009 in the Kraichgau and Swabian Alb regions of south-west Germany (Figures 3 and 4) were used as examples to study the thermal stability of soils and how this relates to the inherent stability of SOM. In the Kraichgau and Swabian Alb sites bare fallow plots were established in order to follow the decomposition of soil organic matter without any

additional inputs and to gain SOM of different qualities. In parallel a long-term (490 days at 20°C) incubation was undertaken in order to study the decomposition under constant conditions. Lastly the experimental spectroscopic methods established were used in a modeling exercise using Century SOM model as implemented in LUCIA, land use change assessment tool.
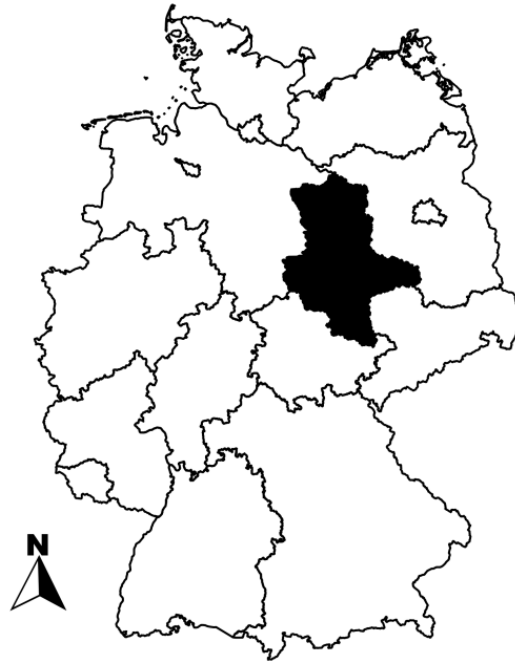
Figure 2. Map of Germany with the Federal State of Saxony-Anhalt in black.
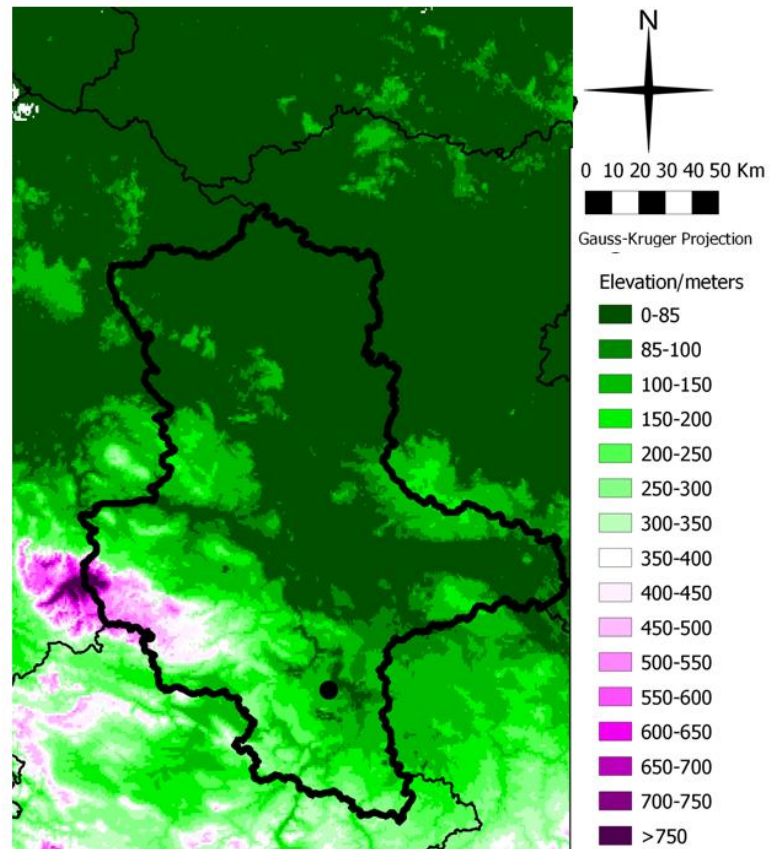
Figure 3. Elevation map of the Federal State of Saxony-Anhalt with the location of the Bad Lauchstädt Experiment site (●).
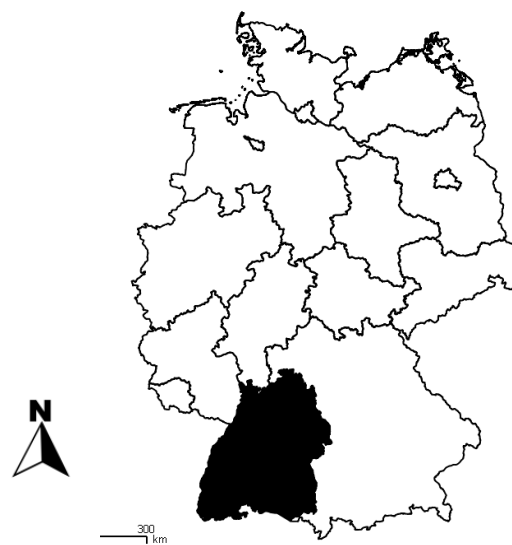
Figure 4. Map of Germany with the Federal State of Baden-Württemberg in black.
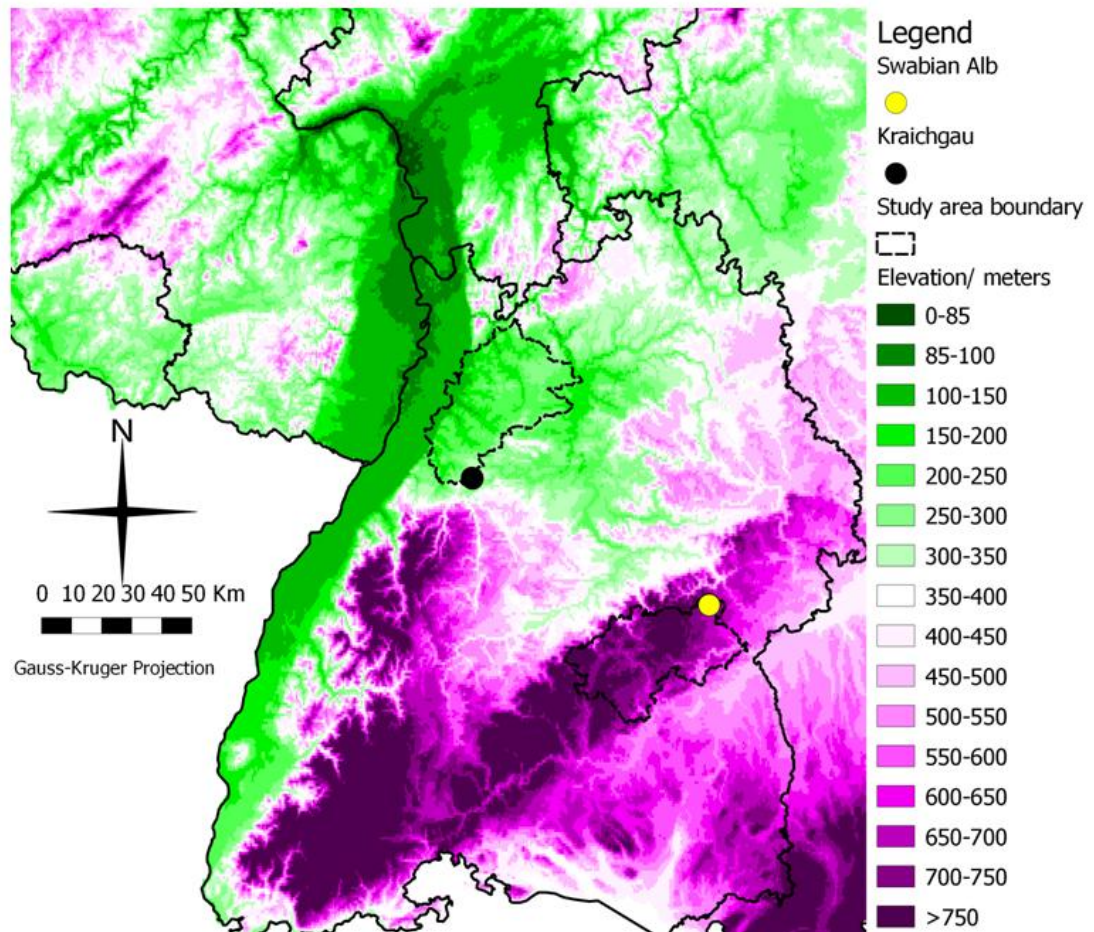
Figure 5. Elevation map of the Federal State of Baden-Württemberg with the locations of the study fields in the Kraichgau and Swabian Alb agro-ecological regions.

References

Ågren, G.I. & Bosatta, E. 1996. Quality: A bridge between theory and experiment in soil organic matter studies. *Oikos,* **76,** 522-528.

Alexis, M.A., Rasse, D.P., Knicker, H., Anquetil, C. & Rumpel, C. 2012. Evolution of soil organic matter after prescribed fire: A 20-year chronosequence. *Geoderma,* **189-190,** 98-107.

Álvaro-Fuentes, J., Morell, F.J., Plaza-Bonilla, D., Arrúe, J.L. & Cantero-Martínez, C. 2012. Modelling tillage and nitrogen fertilization effects on soil organic carbon dynamics. *Soil and Tillage Research,* **120,** 32-39.

Anderson, J.P.E. & Domsch, K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry,* **10,** 215-221.

Barak, P., Jobe, B.O., Krueger, A.R., Peterson, L.A. & Laird, D.A. 1997. Effects of long-term soil acidification due to nitrogen fertilizer inputs in Wisconsin. *Plant and Soil,* **197,** 61-69.

Bárcenas-Moreno, G., Brandón, M.G., Rousk, J. & Bååth, E. 2009. Adaptation of soil microbial communities to temperature: Comparison of fungi and bacteria in a laboratory experiment. *Global Change Biology,* **15,** 2950-2957.

Bernoux, M., Cerri, C.C., Neill, C. & De Moraes, J.F.L. 1998. The use of stable carbon isotopes for estimating soil organic matter turnover rates. *Geoderma,* **82,** 43-58.

Birch, H.F. 1959. The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil,* **10,** 9-31.

Blagodatsky, S., Grote, R., Kiese, R., Werner, C. & Butterbach-Bahl, K. 2011. Modelling of microbial carbon and nitrogen turnover in soil with special emphasis on N-trace gases emission. *Plant and Soil,* **346,** 297-330.

Boring, L.R., Swank, W.T., Waide, J.B. & Henderson, G.S. 1988. Sources, fates, and impacts of nitrogen inputs to terrestrial ecosystems: review and synthesis. *Biogeochemistry,* **6,** 119-159.

Borken, W. & Matzner, E. 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Global Change Biology,* **15,** 808-824.

Bruun, S. & Jensen, L.S. 2002. Initialisation of the soil organic matter pools of the Daisy model. *Ecological Modelling,* **153,** 291-295.

Chatskikh, D., Hansen, S., Olesen, J.E. & Petersen, B.M. 2009. A simplified modelling approach for quantifying tillage effects on soil carbon stocks. *European Journal of Soil Science,* **60,** 924-934.

Chen, H. & Tian, H.-. 2005. Does a general temperature-dependent Q_{10} model of soil respiration exist at biome and global scale? *Journal of Integrative Plant Biology,* **47,** 1288-1302.

Chen, J., Gu, B., LeBoeuf, E.J., Pan, H. & Dai, S. 2002. Spectroscopic characterization of the structural and functional properties of natural organic matter fractions. *Chemosphere,* **48,** 59-68.

Coleman, K. & Jenkinson, D.S. 1995. *A model for the Turnover of Carbon in Soil. Model Description and User's Guide.* Lawes Agricultural Trust, Harpenden, UK.

Collins, H.P., Elliott, E.T., Paustian, K., Bundy, L.G., Dick, W.A., Huggins, D.R., Smucker, A.J.M. & Paul, E.A. 2000. Soil carbon pools and fluxes in long-term corn belt agroecosystems. *Soil Biology and Biochemistry,* **32,** 157-168.

Cregger, M.A., Schadt, C.W., McDowell, N.G., Pockman, W.T. & Classen, A.T. 2012. Response of the soil microbial community to changes in precipitation in a semiarid ecosystem. *Applied and Environmental Microbiology,* **78,** 8587-8594.

Curtin, D., Campbell, C.A. & Jalil, A. 1998. Effects of acidity on mineralization: pH-dependence of organic matter mineralization in weakly acidic soils. *Soil Biology and Biochemistry,* **30,** 57-64.

D'Arrigo, R., Jacoby, G.C. & Fung, I.Y. 1987. Boreal forests and atmosphere-biosphere exchange of carbon dioxide. *Nature,* **329,** 321-323.

Dick, W.A., Blevins, R.L., Frye, W.W., Peters, S.E., Christenson, D.R., Pierce, F.J. & Vitosh, M.L. 1998. Impacts of agricultural management practices on C sequestration in forest-derived soils of the eastern Corn Belt. *Soil and Tillage Research,* **47,** 235-244.

Falloon, P., Smith, P., Coleman, K. & Marshall, S. 1998. Estimating the size of the inert organic matter pool from total soil organic carbon content for use in the Rothamsted carbon model. *Soil Biology and Biochemistry,* **30,** 1207-1211.

Falloon, P.D. & Smith, P. 2000. Modelling refractory soil organic matter. *Biology and Fertility of Soils,* **30,** 388-398.

Fang, C., Smith, P., Moncrieff, J.B. & Smith, J.U. 2005. Similar response of labile and resistant soil organic matter pools to changes in temperature. *Nature,* **433,** 57-59.

Farley, K.A., Piñeiro, G., Palmer, S.M., Jobbágy, E.G. & Jackson, R.B. 2009. Stream acidification and base cation losses with grassland afforestation. *Water Resources Research,* **45**.

Farmer, V.C. & Russell, J.D. 1964. The infra-red spectra of layer silicates. *Spectrochimica Acta,* **20,** 1149-1173.

Farmer, V.C. & Morrison, R.I. 1964. Lignin in sphagnum and phragmites and in peats derived from these plants. *Geochimica et Cosmochimica Acta,* **28,** 1537-1546.

Franko, U., Crocker, G.J., Grace, P.R., Klír, J., Körschens, M., Poulton, P.R. & Richter, D.D. 1997. Simulating trends in soil organic carbon in long-term experiments using the CANDY model. *Geoderma,* **81,** 109-120.

Franzluebbers, A.J., Hons, F.M. & Zuberer, D.A. 1995. Tillage and crop effects on seasonal dynamics of soil CO_2 evolution, water content, temperature, and bulk density. *Applied Soil Ecology,* **2,** 95-109.

Frost, R.L. & Vassallo, A.M. 1996. The dehydroxylation of the kaolinite clay minerals using infrared emission spectroscopy. *Clays and Clay Minerals,* **44,** 635-651.

Gaál, F., Szöllosy, I., Arnold, M. & Paulik, F. 1994. Determination of the organic matter, metal carbonate and mobile water in soils simultaneous TG, DTG, DTA and EGA techniques. *Journal of Thermal Analysis,* **42,** 1007-1016.

Garnett, M.H., Bol, R., Bardgett, R.D., Wanek, W., Bäumler, R. & Richter, A. 2011. Natural abundance radiocarbon in soil microbial biomass: Results from a glacial foreland. *Soil Biology and Biochemistry,* **43,** 1356-1361.

Grandy, A.S. & Neff, J.C. 2008. Molecular C dynamics downstream: The biochemical decomposition sequence and its impact on soil organic matter structure and function. *Science of the Total Environment,* **404,** 297-307.

Hájek, T., Ballance, S., Limpens, J., Zijlstra, M. & Verhoeven, J.T.A. 2011. Cell-wall polysaccharides play an important role in decay resistance of Sphagnum and actively depressed decomposition in vitro. *Biogeochemistry,* **103,** 45-57.

Hamdi, S., Moyano, F., Sall, S., Bernoux, M. & Chevallier, T. 2013. Synthesis analysis of the temperature sensitivity of soil respiration from laboratory studies in relation to incubation methods and soil conditions. *Soil Biology and Biochemistry,* **58,** 115-126.

Hamza, M.A. & Anderson, W.K. 2003. Responses of soil properties and grain yields to deep ripping and gypsum application in a compacted loamy sand soil contrasted with a sandy clay loam soil in Western Australia. *Australian Journal of Agricultural Research,* **54,** 273-282.

Hansen, S., Jensen, H.E., Nielsen, N.E. & Svendsen, H. 1991. Simulation of nitrogen dynamics and biomass production in winter wheat using the Danish simulation model DAISY. *Fertilizer Research,* **27,** 245-259.

Hassink, J. 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. *Plant and Soil,* **191,** 77-87.

Haynes, R.J. & Naidu, R. 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: A review. *Nutrient Cycling in Agroecosystems,* **51,** 123-137.

Hedges, J.I., Keil, R.G. & Benner, R. 1997. What happens to terrestrial organic matter in the ocean? *Organic Geochemistry,* **27,** 195-212.

Heinemeyer, O., Insam, H., Kaiser, E.A. & Walenzik, G. 1989. Soil microbial biomass and respiration measurements: An automated technique based on infra-red gas analysis. *Plant and Soil,* **116,** 191-195.

Helfrich, M., Flessa, H., Mikutta, R., Dreves, A. & Ludwig, B. 2007. Comparison of chemical fractionation methods for isolating stable soil organic carbon pools. *European Journal of Soil Science,* **58,** 1316-1329.

Herrmann, A. & Witter, E. 2002. Sources of C and N contributing to the flush in mineralization upon freeze-thaw cycles in soils. *Soil Biology and Biochemistry,* **34,** 1495-1505.

Holland, E.A. & Coleman, D.C. 1987. Litter placement effects on microbial and organic matter dynamics in an agroecosystem. *Ecology,* **68,** 425-433.

Houghton, R.A. 2007. Balancing the global carbon budget. *Annual Review of Earth and Planetary Sciences,* **35,** 313-347.

Hütsch, B.W., Augustin, J. & Merbach, W. 2002. Plant rhizodeposition - An important source for carbon turnover in soils. *Journal of Plant Nutrition and Soil Science,* **165,** 397-407.

Janssens, I.A., Kowalski, A.S. & Ceulemans, R. 2001. Forest floor CO_2 fluxes estimated by eddy covariance and chamber-based model. *Agricultural and Forest Meteorology,* **106,** 61-69.

Jenny, H., Gessel, S.P. & Bingham, F.T. 1949. Comparative study of decomposition rates of organic matter in temperate and tropical regions. **68,** 419-432.

Jensen, L.S., Mueller, T., Tate, K.R., Ross, D.J., Magid, J. & Nielsen, N.E. 1996. Soil surface CO_2 flux as an index of soil respiration in situ: A comparison of two chamber methods. *Soil Biology and Biochemistry,* **28,** 1297-1306.

Jin, V.L., Haney, R.L., Fay, P.A. & Polley, H.W. 2013. Soil type and moisture regime control microbial C and N mineralization in grassland soils more than atmospheric CO_2-induced changes in litter quality. *Soil Biology and Biochemistry,* **58,** 172-180.

Jobbágy, E.G. & Jackson, R.B. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications,* **10,** 423-436.

Johnson, C.D. & Skousen, J.G. 1995. Minesoil properties of 15 abandoned mine land sites in West Virginia. *Journal of environmental quality,* **24,** 635-643.

Kaiser, K. & Guggenberger, G. 2003. Mineral surfaces and soil organic matter. *European Journal of Soil Science,* **54,** 219-236.

Kätterer, T., Bolinder, M.A., Andrén, O., Kirchmann, H. & Menichetti, L. 2011. Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. *Agriculture, Ecosystems & Environment,* **141,** 184-192.

King, J.A. & Harrison, R. 2002. Measuring soil respiration in the field: An automated closed chamber system compared with portable IRGA and alkali absorption methods. *Communications in Soil Science and Plant Analysis,* **33,** 403-423.

Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S., Eusterhues, K. & Leinweber, P. 2008. Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. *Journal of Plant Nutrition and Soil Science,* **171,** 61-82.

Krull, E.S., Baldock, J.A. & Skjemstad, J.O. 2003. Importance of mechanisms and processes of the stabilisation of soil organic matter for modelling carbon turnover. *Functional Plant Biology,* **30,** 207-222.

Ladd, J.N., Amato, M. & Oades, J.M. 1985. Decomposition of plant material in Australian soils. III. Residual organic and microbial biomass C and N from isotope- labelled legume material and soil organic matter, decomposing under field conditions. *Australian Journal of Soil Research,* **23,** 603-611.

Le Quéré, C., Raupach, M.R., Canadell, J.G., Marland, G., Bopp, L., Ciais, P., Conway, T.J., Doney, S.C., Feely, R.A., Foster, P., Friedlingstein, P., Gurney, K., Houghton, R.A., House, J.I., Huntingford, C., Levy, P.E., Lomas, M.R., Majkut, J., Metzl, N., Ometto, J.P., Peters, G.P., Prentice, I.C., Randerson, J.T., Running, S.W., Sarmiento, J.L., Schuster, U., Sitch, S., Takahashi, T., Viovy, N., Van Der Werf, G.R. & Woodward, F.I. 2009. Trends in the sources and sinks of carbon dioxide. *Nature Geoscience,* **2,** 831-836.

Leue, M., Ellerbrock, R.H. & Gerke, H.H. 2010. DRIFT mapping of organic matter composition at intact soil aggregate surfaces. *Vadose Zone Journal,* **9,** 317-324.

Liang, N., Nakadai, T., Hirano, T., Qu, L., Koike, T., Fujinuma, Y. & Inoue, G. 2004. In situ comparison of four approaches to estimating soil CO_2 efflux in a northern larch (*Larix kaempferi Sarg.*) forest. *Agricultural and Forest Meteorology,* **123,** 97-117.

Liski, J., Ilvesniemi, H., Mäkelä, A. & Westman, C.J. 1999. CO_2 Emissions from Soil in Response to Climatic Warming Are Overestimated: The Decomposition of Old Soil Organic Matter Is Tolerant of Temperature. *Ambio,* **28,** 171-174.

Lopez-Capel, E., Sohi, S.P., Gaunt, J.L. & Manning, D.A.C. 2005. Use of thermogravimetry-differential scanning calorimetry to characterize modelable soil organic matter fractions. *Soil Science Society of America Journal,* **69,** 136-140.

Mandal, B., Majumder, B., Adhya, T.K., Bandyopadhyay, P.K., Gangopadhyay, A., Sarkar, D., Kundu, M.C., Choudhury, S.G., Hazra, G.C., Kundu, S., Samantaray, R.N. & Misra, A.K. 2008. Potential of double-cropped rice ecology to conserve organic carbon under subtropical climate. *Global Change Biology,* **14,** 2139-2151.

Manzoni, S., Taylor, P., Richter, A., Porporato, A. & Ågren, G.I. 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist,* **196,** 79-91.

Markewitz, D., Richter, D.D., Allen, H.L. & Urrego, J.B. 1998. Three decades of observed soil acidification in the Calhoun experimental forest: Has acid rain made a difference? *Soil Science Society of America Journal,* **62,** 1428-1439.

Marohn, C., Schreinemachers, P., Quang, D.V., Berger, T., Siripalangkanont, P., Nguyen, T.T. & Cadisch, G. 2012. A software coupling approach to assess low-cost soil conservation strategies for highland agriculture in Vietnam. *Environmental Modelling and Software,.*

Marschner, B., Brodowski, S., Dreves, A., Gleixner, G., Gude, A., Grootes, P.M., Hamer, U., Heim, A., Jandl, G., Ji, R., Kaiser, K., Kalbitz, K., Kramer, C., Leinweber, P., Rethemeyer, J., Schäffer, A., Schmidt, M.W.I., Schwark, L. & Wiesenberg, G.L.B. 2008. How relevant is recalcitrance for the stabilization of organic matter in soils? *Journal of Plant Nutrition and Soil Science,* **171,** 91-110.

Mayer, L.M. & Xing, B. 2001. Division S-9 - Soil mineralogy: Organic matter-surface area relationships in acid soils. *Soil Science Society of America Journal,* **65,** 250-258.

McCarty, G.W., Reeves III, J.B., Reeves, V.B., Follett, R.F. & Kimble, J.M. 2002. Mid-infrared and near-infrared diffuse reflectance spectroscopy for soil carbon measurement. *Soil Science Society of America Journal,* **66,** 640-646.

McGuire, K.L. & Treseder, K.K. 2010. Microbial communities and their relevance for ecosystem models: Decomposition as a case study. *Soil Biology and Biochemistry,* **42,** 529-535.

Millard, P., Midwood, A.J., Hunt, J.E., Barbour, M.M. & Whitehead, D. 2010. Quantifying the contribution of soil organic matter turnover to forest soil respiration, using natural abundance d13C. *Soil Biology and Biochemistry,* **42,** 935-943.

Moorhead, D.L. & Sinsabaugh, R.L. 2006. A theoretical model of litter decay and microbial interaction. *Ecological Monographs,* **76,** 151-174.

Moyano, F.E., Manzoni, S. & Chenu, C. 2013. Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. *Soil Biology and Biochemistry,* **59,** 72-85.

Murage, E.W., Voroney, P.R., Kay, B.D., Deen, B. & Beyaert, R.P. 2007. Dynamics and turnover of soil organic matter as affected by tillage. *Soil Science Society of America Journal,* **71,** 1363-1370.

Murakami, K., Shirato, H. & Nishiyama, Y. 1997. In situ infrared spectroscopic study of the effects of exchanged cations on thermal decomposition of a brown coal. *Fuel,* **76,** 655-661.

Oades, J.M. 1988. The retention of organic matter in soils. *Biogeochemistry,* **5,** 35-70.

Olson, J.S. 1963. Energy storage and the balance of producers and decomposers in ecological systems. **44,** 322-331.

Ostle, N.J., Smith, P., Fisher, R., Ian Woodward, F., Fisher, J.B., Smith, J.U., Galbraith, D., Levy, P., Meir, P., McNamara, N.P. & Bardgett, R.D. 2009. Integrating plant-soil interactions into global carbon cycle models. *Journal of Ecology,* **97,** 851-863.

Page, H.J. 1922. Section III. - Papers and discussion on "organic constituents of the soil. The part played by organic matter in the soil system. *Transactions of the Faraday Society,* **17,** 272-287.

Palmborg, C. & Nordgren, A. 1993. Modelling microbial activity and biomass in forest soil with substrate quality measured using near infrared reflectance spectroscopy. *Soil Biology and Biochemistry,* **25,** 1713-1718.

Parfitt, R.L., Theng, B.K.G., Whitton, J.S. & Shepherd, T.G. 1997. Effects of clay minerals and land use on organic matter pools. *Geoderma,* **75,** 1-12.

Parnas, H. 1975. Model for decomposition of organic material by microorganisms. *Soil Biology and Biochemistry,* **7,** 161-169.

Parton, W.J., Schimel, D.S., Cole, C.V. & Ojima, D.S. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Science Society of America Journal,* **51,** 1173-1179.

Paul, E.A., Morris, S.J., Conant, R.T. & Plante, A.F. 2006. Does the acid hydrolysis-incubation method measure meaningful soil organic carbon pools? *Soil Science Society of America Journal,* **70,** 1023-1035.

Paustian, K., Parton, W.J. & Persson, J. 1992. Modeling soil organic matter in organic-amended and nitrogen- fertilized long-term plots. *Soil Science Society of America Journal,* **56,** 476-488.

Paustian, K., Levine, E., Post, W.M. & Ryzhova, I.M. 1997. The use of models to integrate information and understanding of soil C at the regional scale. *Geoderma,* **79,** 227-260.

Puget, P. & Drinkwater, L.E. 2001. Short-term dynamics of root- and shoot-derived carbon from a leguminous green manure. *Soil Science Society of America Journal,* **65,** 771-779.

Randall, E.W., Mahieu, N. & Ivanova, G.I. 1997. NMR studies of soil, soil organic matter and nutrients: spectroscopy and imaging. *Geoderma,* **80,** 307-325.

Reeves III, J.B., Follett, R.F., McCarty, G.W. & Kimble, J.M. 2006. Can near or mid-infrared diffuse reflectance spectroscopy be used to determine soil carbon pools? *Communications in Soil Science and Plant Analysis,* **37,** 2307-2325.

Rivkina, E.M., Friedmann, E.I., McKay, C.P. & Gilichinsky, D.A. 2000. Metbolic acitivity of permafrost bacteria below the freezing point. **66,** 3230-3233.

Rossel, R.A.V., Jeon, Y.S., Odeh, I.O.A. & McBratney, A.B. 2008. Using a legacy soil sample to develop a mid-IR spectral library. *Aust.J.Soil Res.,* **46,** 1-16.

Rovira, P. & Rovira, R. 2010. Fitting litter decomposition datasets to mathematical curves: Towards a generalised exponential approach. *Geoderma,* **155,** 329-343.

Rutherford, P.M. & Juma, N.G. 1992. Influence of texture on habitable pore space and bacterial-protozoan populations in soil. *Biology and Fertility of Soils,* **12,** 221-227.

Rühlmann, J., Körschens, M. & Graefe, J. 2006. A new approach to calculate the particle density of soils considering properties of the soil organic matter and the mineral matrix. *Geoderma,* **130,** 272-283.

Saiz-Jimenez, C. & de Leeuw, J.W. 1984. Pyrolysis-gas chromatography-mass spectrometry of isolated, synthetic and degraded lignins. *Organic Geochemistry,* **6,** 417-422.

Saleska, S.R., Miller, S.D., Matross, D.M., Goulden, M.L., Wofsy, S.C., Da Rocha, H.R., De Camargo, P.B., Crill, P., Daube, B.C., De Freitas, H.C., Hutyra, L., Keller, M., Kirchhoff, V., Menton, M., Munger, J.W., Pyle, E.H., Rice, A.H. & Silva, H. 2003. Carbon in Amazon Forests: Unexpected Seasonal Fluxes and Disturbance-Induced Losses. *Science,* **302,** 1554-1557.

Schnitzer, M. & Hoffman, I. 1965. Thermogravimetry of soil humic compounds. *Geochimica et Cosmochimica Acta,* **29,** 859-870.

Schreiner, O. & Shorey, E.C. 1911. Soil organic matter as material for biochemical investigation. *Journal of the Franklin Institute,* **171,** 295-300.

Siewert, C. 2004. Rapid screening of soil properties using thermogravimetry. *Soil Science Society of America Journal,* **68,** 1656-1661.

Six, J., Conant, R.T., Paul, E.A. & Paustian, K. 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant and Soil,* **241,** 155-176.

Six, J., Elliott, E.T. & Paustian, K. 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry,* **32,** 2099-2103.

Sollins, P., Homann, P. & Caldwell, B.A. 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma,* **74,** 65-105.

Sprengel, C. 1826. Ueber Pflanzenhumus, Humussäure und humussaure Salze (About plant humus, humic acids, and salts of humic acid). **8,** 145-220.

Stenström, J., Stenberg, B. & Johansson, M. 1998. Kinetics of Substrate-Induced Respiration (SIR): Theory. *Ambio,* **27,** pp. 35-39.

Tang, C., Rengel, Z., Diatloff, E. & Gazey, C. 2003. Responses of wheat and barley to liming on a sandy soil with subsoil acidity. *Field Crops Research,* **80,** 235-244.

Tatzber, M., Mutsch, F., Mentler, A., Leitgeb, E., Englisch, M., Zehetner, F., Djukic, I. & Gerzabek, M.H. 2011. Mid-infrared spectroscopy for topsoil layer identification according to litter type and decompositional stage demonstrated on a large sample set of Austrian forest soils. *Geoderma,* **166,** 162-170.

Terhoeven-Urselmans, T., Vagen, T.-., Spaargaren, O. & Shepherd, K.D. 2010. Prediction of soil fertility properties from a globally distributed soil mid-infrared spectral library. *Soil Science Society of America Journal,* **74,** 1792-1799.

Thevenot, M., Dignac, M. & Rumpel, C. 2010. Fate of lignins in soils: A review. *Soil Biology and Biochemistry,* **42,** 1200-1211.

Toops, T.J., Smith, D.B. & Partridge, W.P. 2005. Quantification of the in situ DRIFT spectra of $Pt/K/\gamma-A1_2O_3$ NO_x adsorber catalysts. *Applied Catalysis B: Environmental,* **58,** 245-254.

Van Keulen, H. 2001. (Tropical) soil organic matter modelling: Problems and prospects. *Nutrient Cycling in Agroecosystems,* **61,** 33-39.

Van Veen, J.A., Ladd, J.N. & Amato, M. 1985. Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with $[^{14}C(U)]$glucose and $[^{15}N](NH_4)_2SO_4$ under different moisture regimes. *Soil Biology and Biochemistry,* **17,** 747-756.

Vanhala, P., Karhu, K., Tuomi, M., Sonninen, E., Jungner, H., Fritze, H. & Liski, J. 2007. Old soil carbon is more temperature sensitive than the young in an agricultural field. *Soil Biology and Biochemistry,* **39,** 2967-2970.

Vityakon, P., Meepech, S., Cadisch, G. & Toomsan, B. 2000. Soil organic matter and nitrogen transformation mediated by plant residues of different qualities in sandy acid upland and paddy soils. *NJAS - Wageningen Journal of Life Sciences,* **48,** 75-90.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E. & Marschner, B. 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. *Soil Biology and Biochemistry,* **39,** 2183-2207.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B. & Flessa, H. 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions- a review. *European Journal of Soil Science,* **57,** 426-445.

Wardle, D.A., Hörnberg, G., Zackrisson, O., Kalela-Brundin, M. & Coomes, D.A. 2003. Long-term effects of wildfire on ecosystem properties across an island area gradient. *Science,* **300,** 972-975.

Wickings, K., Grandy, A.S., Reed, S.C. & Cleveland, C.C. 2012. The origin of litter chemical complexity during decomposition. *Ecology Letters,* **15,** 1180-1188.

Wilhelmi, V. & Rothe, G. 1990. The effect of acid rain, soil temperature and humidity on C-mineralization rates in organic soil layers under spruce. *Plant and Soil*, **121,** 197-202.

Wingate, L., Ogée, J., Burlett, R. & Bosc, A. 2010. Strong seasonal disequilibrium measured between the oxygen isotope signals of leaf and soil CO2 exchange. *Global Change Biology,* **16,** 3048-3064.

Xu, X., Luo, Y. & Zhou, J. 2012. Carbon quality and the temperature sensitivity of soil organic carbon decomposition in a tallgrass prairie. *Soil Biology and Biochemistry,* **50,** 142-148.

Yadav, V. & Malanson, G. 2007. Progress in soil organic matter research: Litter decomposition, modelling, monitoring and sequestration. *Progress in Physical Geography,* **31,** 131-154.

Zimmermann, M., Leifeld, J. & Fuhrer, J. 2007a. Quantifying soil organic carbon fractions by infrared-spectroscopy. *Soil Biology and Biochemistry,* **39,** 224-231.

Zimmermann, M., Leifeld, J., Schmidt, M.W.I., Smith, P. & Fuhrer, J. 2007b. Measured soil organic matter fractions can be related to pools in the RothC model. *European Journal of Soil Science,* **58,** 658-667.

2 Use of specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy to study the composition of organic matter in a Haplic Chernozem*

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2.1 Summary

This study assessed specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy (DRIFTS) for characterizing soil organic matter (SOM) composition of a Haplic Chernozem. Soils were collected from the Static Fertilization Experiment, Bad Lauchstädt, Germany, in five years from the farmyard manure (FYM), mineral fertilizer (NPK), combination (FYM+NPK), and no fertilizer (Control) treatments. Soils were extracted with hot water (HWE), and fractionated by size and density. Bulk soil and fractions were analyzed by DRIFTS. Peak areas at 2930, 1620, 1530, and 1159 cm^{-1} were selected as a range of organic functional groups (with limited mineral interference), integrated with a local baseline (corrected peak area) and each was divided by the summed area of the four peaks (relative peak area). Positive correlations between carbon (C) in fractions representing labile OM (<1.8 g cm^{-3}, 1.8-2.0 g cm^{-3}, C_{HWE}) and the corrected peak area at 2930 cm^{-1} (3010 to 2800 cm^{-1}) in the bulk soil indicated that this aliphatic peak corresponded to the more labile C compounds. Negative correlations between the same fractions and the corrected area of the predominantly aromatic peak at 1620 cm^{-1} (1660 to 1580 cm^{-1}) in the bulk soil suggested a relationship with more stable SOM compounds. All relative peaks areas were significantly affected by fertilizer treatment, with an increasing relative peak area at 2930 cm^{-1} in FYM compared with non-FYM. The ratio of the peaks at 1620 and 2930 cm^{-1} was positively correlated to the ratio of stable C (sum of C in >1.8 g cm^{-3} and clay fractions) to labile C (C content of <1.8 g cm^{-3} fraction) and thus taken as an indicator of SOM stability. The DRIFTS peak area method reflected changes in SOM quality and composition under long-term management as measured by size and density fractionation, indicating heterogeneous chemical composition of the latter. Further, the DRIFTS analysis of undiluted soil samples can be used to assess SOM composition in small sample sets if specular reflection and mineral interferences are considered.

2.2 Introduction

Soil organic matter (SOM) is not only an important indicator of soil quality, but is also a large component of the global carbon (C) budget. It has been suggested that alternative agricultural management, such as enhanced residue incorporation and organic amendments such as farm yard manure to soils increases soil fertility and C sequestration (Smith *et al.*, 2000). While the effects of agricultural management on bulk SOM have been documented

(Paul *et al.,* 2003), less is known about associated alterations of SOM composition and its stabilization in soil (Capriel, 1997).

The knowledge gap can be partly ascribed to the inherent complexity of SOM and its stabilization processes, leading to difficulties in examining changes in the composition of SOM (von Lützow *et al.*, 2006). Conventionally, SOM fractionation has been applied to study changes in SOM composition and to isolate components of similar chemical/physical composition or turnover rates (von Lützow *et al.*, 2007). Fractionations based on density and/or size separation techniques (Christensen, 2001) can be used to measure the quantity of SOM in fractions of differing quality to discriminate between labile and more stabilized C pools that are relevant to soil functions. The C content in hot-water extractions (C_{HWE}) has been correlated with microbial biomass C and described as a labile pool and a sensitive indicator of SOM quality (Sparling *et al.*, 1998; Hoffmann *et al.*, 2006). In contrast, longer turnover times have been assigned to clay-sized (Christensen, 2001) and heavy density fractions (>2 g cm^{-3}) (von Lützow *et al.*, 2007), which comprise more stabilized and protected SOM. Size and density fractionation, however, is time consuming, and influenced by the method of separation.

Diffuse reflectance Fourier transform mid-infrared spectroscopy (DRIFTS) is the diffuse reflectance of bending and stretching vibrations of different functional groups in the mid-infrared range from 4000 to 400 cm^{-1}. Organic and inorganic functional groups are characterized by vibrations at different wave-numbers and are visible as peaks on the mid-infrared spectrum. It has been suggested that identification of specific mid-infrared peaks, which correspond to vibrations of certain functional groups of organic compounds, may be suited to study SOM composition and dynamics. For example, Gerzabek *et al.* (2006) found that relative peak heights at 2920, 1630 and 1450 cm^{-1} of bulk soil and silt-sized fractions were correlated with the respective organic C contents of a Eutric Cambisol. Further, alkyl C in bulk soils and clay-sized fractions measured by ^{13}C nuclear magnetic resonance (NMR) could be clearly related to the integrated peak area of the C-H bending vibration between 3010–2800 cm^{-1} (Leifeld, 2006). A variety of methods for mid-infrared peak analyses have been used to investigate short and long-term changes in organic matter quality. Grube *et al.* (2006) used band intensity ratios 1034:1384, 1384:2925 and 2925:1034 cm^{-1} to follow organic matter changes in composts over the short-term (40 days). Spaccini *et al.* (2001) integrated the area between 3000–2800 cm^{-1} to investigate the decomposition of maize straw in a soil incubation experiment during one year. In another approach, a relative peak area

(area of individual peak divided by the sum of area of all investigated peaks) has been used to study longer-term changes in humic acids from soils under bare fallow over 36 years (Tatzber *et al.*, 2009), and also in bulk soils from a 140-year proglacial chronosequence (Egli *et al.*, 2010) which reflected changes in organic matter quality as measured by independent methods. Grube *et al.* (2006) and Tatzber *et al.* (2009) both used Fourier transform infrared (FT-IR) transmission measurements rather than the DRIFTS method, which is said to be the more rapid alternative to transmission spectroscopy (Nguyen *et al.*, 1991). Comparing single mid-infrared peaks with different SOM fractions using DRIFTS should provide further insights as to which peaks and integration method can be used as potential SOM quality or composition indicators under long-term agricultural management at a given site. Egli *et al.* (2010) used a DRIFTS relative peak area method to investigate SOM in young glacial moraine soils, but did not consider the influence of different soil minerals which may interfere with the 'visibility' of organic spectra. The large variability in mineralogy of different soils and the resulting potential overlap of the inorganic and organic vibrations of functional groups in the mid-infrared region suggests that the choice of individual peak selection to study changes in SOM composition may vary among soils. This effect makes comparisons between different soil classes or even within soil depths difficult but favours its use within experimental sites with uniform soil mineralogy.

Although OM in different size and/or density fractions has been successfully predicted using DRIFTS multivariate prediction models (Janik *et al.,* 2007; Zimmermann *et al.*, 2007), such models usually require a large number of samples (for calibration/validation) and are not well suited for less than 50 samples as often encountered when studying single experiments. Furthermore, a quantitative relationship between individual mid-infrared peaks and the composition of SOM as related to different fractions or qualities of SOM is not well-established and tested for undiluted bulk soils because most studies have used a dilution with KBr.

In this study, it was hypothesized that DRIFTS measurements of undiluted bulk soil can yield information on the quality and stability of SOM comparable with results of the size and density fractionation approach suitable for studying SOM composition and dynamics in agriculturally managed soils. Applying DRIFTS to SOM fractions directly might also corroborate their implied SOM composition and give insight into the variability in peak patterns. The objectives were (i) to identify specific DRIFTS peaks and corresponding peak integration method minimizing mineral interference, representing organic compounds of

varying C bond strengths which reflect different long-term fertilizer management effects in soil, (ii) to relate these peaks or their ratio to size and density fractions, which have different implied qualities/stabilities and (iii) and to apply this approach to verify changing SOM qualities under long-term agricultural management in the Static Fertilization Experiment at Bad Lauchstädt, Germany.

2.3 Materials and methods

Study area and experimental description

Soil samples were collected from the Static Fertilization Experiment at Bad Lauchstädt in Central Germany (Saxony-Anhalt) which was initiated in 1902 to study the effect of different fertilizer treatments on crop yields. Recently this experiment has also been used to study changes in SOM quality under different fertilizer treatments (Blair *et al.*, 2006; Leifeld *et al.*, 2006). The site is characterized by a continental climate with average annual precipitation of 484 mm and mean annual temperature of 8.8°C. Soils were formed from loess and classified as Haplic Chernozems (IUSS Working Group WRB, 2007). The investigated top-soil had an average of 2% organic C, 0.15% nitrogen (N), and 21% clay and 68% silt. The crop rotation of the long-term experiment was sugar beet, spring barley, potatoes, and winter wheat. Additional information about the field experiment and layout is given in Körschens *et al.* (1994).

The following four treatments were selected for the present study in individual treatment plots of 30 tons ha^{-1} farmyard manure every second year (FYM), yearly mineral fertilizer (NPK), 30 tons ha^{-1} farmyard manure every second year and NPK fertilizer (FYM+NPK) and control without fertilizer inputs (Control). Mineral fertilizer inputs (N was applied as calcium ammonium nitrate, phosphate (P) as triple superphosphate, and potassium (K) as potassium sulfate/magnesium sulfate monohydrate) averaged over a four-year rotation period were 103, 6, and 25 kg ha^{-1} N, P and K, respectively, in the FYM+NPK treatment, and 123, 30 and 30 kg ha^{-1} N, P, and K were applied in the NPK treatment. As archive samples were not available from every year and every treatment, samples from the years 1956, 1979, 1992, 2004, and 2008 were used for our study. Soil samples were taken after summer/autumn harvest, but before autumn ploughing and always following the winter wheat crop, except for 1979, when the crop was potatoes. In each treatment, 25 soil cores were taken with an auger (1-cm diameter and 20-cm length) and mixed to form a composite sample. Soil samples were

air-dried and passed through a 2-mm sieve with visible plant residues removed before analysis. The soil sampling protocol was the same in each sampling year.

2.3.1 Soil organic matter fractionations

Hot-water extraction (HWE) was performed according to Schulz & Körschens (1998). Briefly, 20 g <2 mm soil (n = 3) were boiled under reflux for one hour with distilled water (1:5, w:v). After rapidly cooling to room temperature, the mixture was centrifuged for ten minutes at 440 g and the supernatant filtered through a 0.45 µm membrane filter (Minisart RC 25 syringe membrane filters, Sartorius, Göttingen, Germany). The humification index (HIX), defined as the absorbances between 435 to 480 nm divided by 300 to 345 nm (Zsolnay *et al.*, 1999), was determined on diluted subsamples of the HWE using fluorescence spectroscopy. Dilutions of the HWEs were made to avoid inner filter or concentration effects. Size and density fractionations of selected samples from 1956, 1992 and 2004 were done according to Schulz (2004) modified from Shaymukhametov *et al.* (1984). The samples from these years were selected in order to gain insight in the dynamics of SOM fractions. First, plant residues from 20 g <2 mm air-dried soil samples (n = 2) were removed by flotation with deionized water. A 1:3 (w:v) soil-to-water suspension was then sonified (30.2 J s^{-1}) 15 times each for one minute followed by centrifugation at 110 g for three minutes. The supernatant containing the clay fraction was again centrifuged 440 g for ten minutes to separate a <1 µm clay fraction from a 1-2 µm fraction. Following clay separation, the remaining SOM fractions were isolated by heavy liquid density separation. Bromoform (96% stabilized with 1-3% ethanol) solutions of different densities were used to separate fractions of <1.8, 1.8-2.0 and >2.0 g cm^{-3}. The fractions were washed three times with ethanol (96%) to completely remove bromoform and dried at 40°C in a water bath to evaporate remaining ethanol.

Bulk soil and obtained fractions were analysed for total C (TC) by dry combustion according to DIN ISO 13878 (1998) with a Vario-EL III elemental analyser (Elementar-Hanau, Germany). Hydrochloric acid addition to bulk soil indicated that no carbonates were present in the samples; therefore TC was taken as total organic carbon (TOC) for the bulk soil and fractions. Hot water extracts were analysed for TOC (C_{HWE}) with a Multi N/C analyser (Analytik Jena, Germany). The reproducibility of C measurements was ± 0.4 %.

2.3.2 DRIFTS analysis

Samples of bulk soils and fractions for DRIFTS analyses were ball milled and dried overnight at 32º C before analysis. Mid-infrared spectra were recorded on a Tensor-27 (Bruker Optik GmbH, Ettlingen, Germany) Fourier transform spectrometer using a potassium bromide (KBr) beam splitter and a liquid nitrogen cooled mid-band mercury-cadmium-telluride detector. The spectrometer was mounted with a Praying Mantis diffuse reflectance chamber (Harrick Scientific Products, New York, USA) which was purged with dry air from a compressor (Jun-Air International, Nørresundby, Denmark) with a flow rate of 200 l hour^{-1}. The spectra were recorded in the mid-infrared range (4000 to 400 cm^{-1}) by combining 16 individual scans at a resolution of 4 cm^{-1}. The acquisition mode was double forward-backwards and the Blackman-Harris-3 apodization function was used. The spectra were recorded in absorbance units (A.U.) or -log(reflectance^{-1}). To gain knowledge on the reproducibility of the DRIFTS measurements, three sub-samples of each bulk soil sample were individually ball milled and each sub-sample was then scanned three times (16 co-added scans), giving a total of nine spectra per sample, which were later averaged. For each repetitive scan, the sample was returned to the sample container, mixed and then transferred to the measuring cup.

Spectral pre-processing included atmospheric correction for carbon dioxide (CO_2) and water, baseline correction and vector normalization in order to compensate for slight variations in air humidity, temperature and CO_2 concentration at the time of measurement. Peak area integration on the corrected spectra was performed using the spectral processing software OPUS version 6.5 (Bruker Optik GmbH). Results from the three separately processed sub-samples of each unique sample showed a relative standard deviation of less than 5% of the investigated peaks. For band interpretation, it must be considered that functional groups of both mineral and organic substances may have vibration frequencies in some of the same or overlapping wave-numbers. The two sharp peaks at 3695 and 3622 cm^{-1} were of O-H stretching of clay minerals (Nguyen *et al*., 1991) and the double peak centred at 2930 cm^{-1} of the C-H vibrations (Stevenson, 1982) which was superimposed on the broad O-H peak centred at 3400 cm^{-1} (Stevenson, 1982). Peaks at 1980 cm^{-1} and 1870 cm^{-1} were Si-O vibrations of quartz minerals (Nguyen *et al*., 1991). The peak at 1792 cm^{-1} was also due to Si-O vibrations of quartz minerals (Nguyen *et al*., 1991), but also stretching vibrations of carbonyl groups in alkyl and alkyl-aryl polyester bonds (Spaccini and Piccolo, 2007). The peak at 1620 cm^{-1} was assigned to predominately aromatic C=C stretching and/or asymmetric

–COO⁻ stretching (Baes and Bloom, 1989) but possibly also C=O vibrations (Stevenson, 1982). Aromatic C=C stretching vibrations (Baes and Bloom, 1989) were assigned to the 1525 cm^{-1} peak, while the peak at 1390 cm^{-1} was of OH deformation and C-O stretching (Stevenson, 1982). The peak at 1159 cm^{-1} was assigned to C-O bonds of both poly-alcoholic and ether functional groups (Spaccini and Piccolo, 2007), the 997 cm^{-1} peak to S=O, P-O-alkyl, or =C-O-C vibrations, and Si-O vibrations (Senesi et al., 2003) and the 975 cm^{-1} peak to aromatic CH out of plane bending (Baes and Bloom, 1989) and alumino-silicate lattice vibrations. The remaining peaks at 915 cm^{-1}, 810 cm^{-1}, 690 cm^{-1}, and 670 cm^{-1} were peaks from clay and quartz minerals (Nguyen et al., 1991). For peak area investigations, wave-numbers of functional groups associated with non-organic compounds such as silicates and alumino-iron oxides were avoided. These criteria removed the peaks <1000 cm^{-1} and the peaks at 1980, 1870, 1792 and 1390 cm^{-1}. The four remaining peaks (2930, 1620, 1530, 1159 cm^{-1}) were used for further investigation and were assigned to different organic functional groups and their potential stabilities based on current literature (Table 1). In our study different stabilities of functional groups of SOM, as represented by the identified DRIFTS peaks, were proposed on the basis of the energy state or strength of the chemical bonds of the functional groups such as aliphatic C-H being less stable than aromatic C=C bonds. At identified local peaks, upper and lower boundaries were established (see Figure 1a and Table 1); a local baseline was drawn between the boundaries, and an integration performed to calculate the corrected peak area. The corrected area from each peak was then divided by the sum of the area of the four peaks and multiplied by 100 to give the relative peak area. Relative peak areas were used to assess how the peak areas changed relative to each other as suggested by Niemeyer et al. (1992). Ratios of relative peak areas representing organic functional groups of different hypothesized stabilities (the ratio of the relative DRIFTS peaks at 1620 and 2930 cm^{-1}: 1620:2930) were also related to the distribution of C among the fractions (that is the ratio of the sum of C contained in clay and >1.8 g cm^{-3} fractions divided by the amount of C in <1.8 g cm^{-3} fraction). The ratio of aromatic to aliphatic compounds has been used previously to study the decomposition of composts (with ratios of 1655 to 2930 cm^{-1}) (Inbar et al., 1989) and organic surface horizons (ratios of 1630 to 2920 cm^{-1} (Haberhauer et al., 1998) with FT-IR transmission spectroscopy. Additionally, the ratio of band A (defined as peak intensity between 3020-2800 cm^{-1}) to band B (defined as the peak intensity between 1740-1710 and 1640 to 1620 cm^{-1}) has been used successfully to predict the wettability or hydrophobicity of soils (Ellerbrock et al., 2005). In the bulk soils used in

the current study, a distinct peak was not visible in the 1740–1710 cm^{-1} range, so only the peak at 1620 cm^{-1} was used for the relative peak area method and was assigned to aromatic C=C vibrations, as the ratio of aromatic to aliphatic peak areas changes with the relative degree of decomposition or humification of OM.

Table 1: Investigated DRIFTS peak areas and organic functional group assignments. Probable organic carbon functional group assignments were based on literature and hypothesized stabilities related to the bond strengths (i.e. C-H less stable compared to C=C) at the specific wavenumbers.

Peak Name	Integration Limits [cm^{-1}]	Probable Organic Carbon Assignment	Hypothesized Stability
2930	3010-2800	Aliphatic C-H stretching[a]	Labile
1620	1660-1580	Aromatic C=C and/or –COO$^-$ stretching[a]	Intermediate
1530	1546-1520	Aromatic C=C stretching[a]	Intermediate
1159	1170-1148	C-O bonds of poly-alcoholic and ether groups[b]	unknown

a - Baes and Bloom, 1989, b - Senesi et al., 2003.
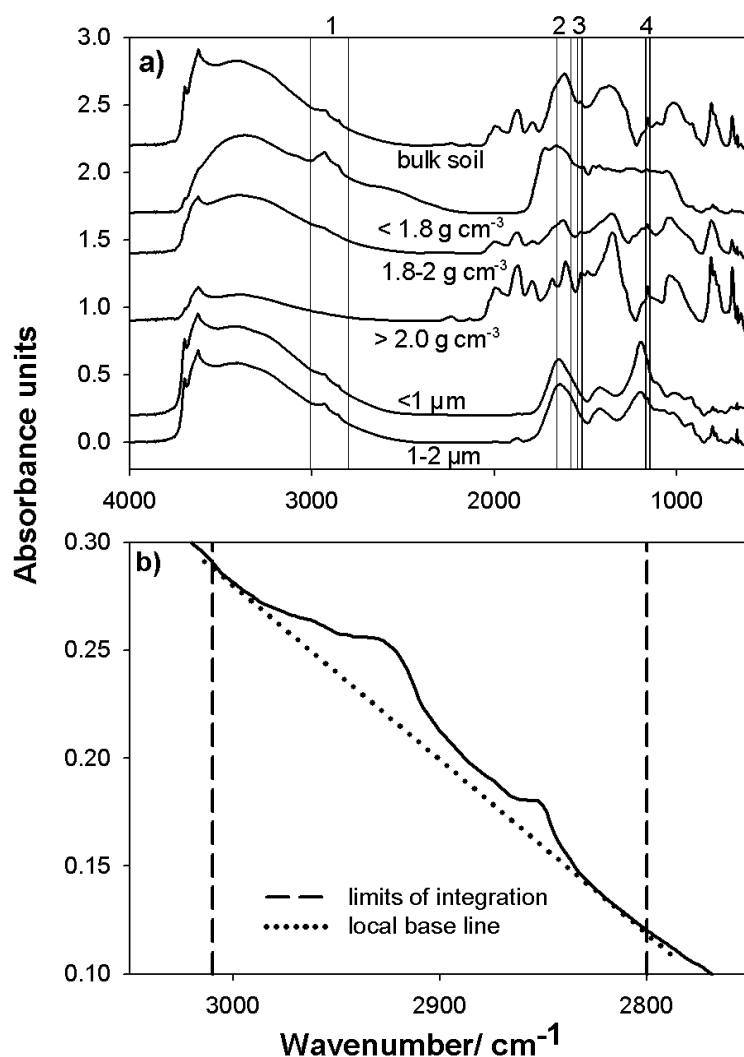
Figure 1 a) Baseline corrected DRIFTS spectra of undiluted (neat) bulk soil, <1.8 g cm^{-3}, 1.8-2.0 g cm^{-3}, >2.0 g cm^{-3}, <1 μm and 1-2 μm fractions from the Static Fertilization Experiment, Bad Lauchstädt, Germany. The indicated regions were assigned to the following molecular vibrations for peak area integration 1-2930 cm^{-1} (C-H), 2-1620 cm^{-1} (C=C and –COO-), 3-1530 cm^{-1} (C=C), and 4-1159 cm^{-1} (C-O) (see also Table 1), b) Depiction of the peak area integration performed with local baseline to gain the corrected peak area which was then used for calculating the relative peak areas.

It has been noted in other studies that soil samples must be diluted in order to reduce non-diffuse or Fresnel reflection and to avoid infrared peaks from appearing to be negative peaks as opposed to positive peaks also called band inversion (Bishop *et al.*, 1996). Band inversion can happen when there is a strongly absorbing compound in the sample. We performed tests

with two samples using 1:3 and 1:100 dilutions with KBr which showed no evidence of band inversion, even in the <1600 cm^{-1} region, where this usually occurs. There was, however, a disappearance of some peaks with a 1:100 dilution, indicating a loss of information with dilution (data not shown). Thus, with our soil samples, undiluted samples resulted in better scans for peak area integration because more sample was scanned leading to less heterogeneity than if mixed with a diluting agent as also noted by Reeves (2003).

2.3.3 Statistical analyses

Since there were no field replicates of the treatments, sample years were grouped for statistical testing of the fertilizer management effect. Statistical analyses were performed with SAS version 8.0 (SAS Institute Inc., Cary, NC, USA). Analysis of variance with Tukey-Kramer post-hoc testing was used to determine significant differences ($P <0.05$) between fertilizer treatments. Spearman rank correlations were used to examine correlations between the corrected peak areas and SOM fractions. Linear regressions were performed using Sigma Plot version 10.0 (Systat Software Inc., Chicago, IL, USA) on relative peak areas by year to study long-term SOM dynamics from 1956 to 2008. In the absence of field replicates for the sampled treatments and in view of their similar long-term trends, FYM+NPK and FYM (FYM treatments) were grouped together, while NPK and Control (non-FYM treatments) were grouped for linear regressions in order to follow the effect of farmyard manure application on DRIFTS peaks with time.

2.4 Results

2.4.1 Characteristics of soil organic matter

Average contents of TOC in the soils of the FYM+NPK and FYM treatments were significantly larger than in the Control ($P < 0.05$) (Table 2). Hot-water extractable carbon (C_{HWE}) contents were the largest in the soils of the FYM+NPK treatment, whereas C_{HWE} was nearly 50% smaller in the Control ($P < 0.05$). Although TOC was not significantly different between FYM and NPK treatments, C_{HWE} was 27% greater in the FYM treatment ($P >0.05$). The humification index (HIX) of C_{HWE} was not significantly affected by treatment ($P >0.05$), but was greatest in the Control. Carbon content of the <1.8 g cm^{-3} and 1.8-2.0 g cm^{-3} fractions was significantly larger in the FYM+NPK treatment compared with the Control ($P <0.05$).

Neither clay fractions nor the >2.0 g cm^{-3} fraction differed between FYM+NPK and Control (P >0.05).

Table 2: Soil organic carbon contents in bulk soil and fractions by treatment (sampling years 1956, 1992, and 2004). Treatments were: FYM+NPK (farmyard manure and mineral fertilizer, n=3), FYM (farmyard manure, n=1), NPK (mineral fertilizer, n=1), Control (CON, no fertilizer inputs, n=3). Different letters indicate significant differences within each row ($P < 0.05$) and standard errors are given in parentheses.

	Treatment				F[c]	LSD$_{0.05}$[e]
	FYM+NPK	FYM	NPK	CON		
TOC [g kg^{-1}][a]	23.4 (0.8)	19.7 (1.1)	18.3 (0.5)	16.5 (0.4)	18.1	0.5
C$_{HWE}$ [mg kg^{-1}][a]	657 (13)	598 (8)	470 (38)	332 (21)	27.2	21.9
HIX[a]	10.6 (0.7)	9.2 (0.5)	9.7 (0.7)	11.0 (0.6)	1.6	ns[f]
C$_{<1\mu m}$ [g kg^{-1}][a]	8.7 (0.4)	8.0[b]	7.6[b]	8.0 (0.1)	2.1[d]	ns
C$_{1-2\mu m}$ [g kg^{-1}][a]	5.5 (0.4)	4.9[b]	4.6[b]	4.9 (0.5)	4.5[d]	ns
C$_{<1.8 \text{ g cm}}$$^{-3}$ [g kg^{-1}][a]	5.8 (1.2)	5.3[b]	3.2[b]	2.5 (0.4)	13.3[d]	2.4
C$_{1.8-2.0 \text{ g cm}}$$^{-3}$ [g kg^{-1}][a]	0.5 (0.1)	0.4[b]	0.3[b]	0.2 (0.0)	27.5[d]	0.1
C$_{>2.0 \text{ g cm}}$$^{-3}$ [g kg^{-1}][a]	1.4 (0.3)	1.0[b]	0.7[b]	1.0 (0.2)	1.9[d]	ns

[a] TOC = total organic C; C$_{HWE}$ = hot water extractable C of bulk soil; HIX = humification index (435 to 480 nm divided by 300 to 345 nm) of C$_{HWE}$; C$_{<1\mu m}$ = C in <1 μm fraction; C$_{1-2\mu m}$ = C in 1-2 μm fraction; C$_{<1.8 \text{ g cm}}$$^{-3}$ = C in <1.8 g cm^{-3} fraction, C$_{1.8-2.0 \text{ g cm}}$$^{-3}$ = C in 1.8-2.0 g cm^{-3} fraction, C$_{>2.0 \text{ g cm}}$$^{-3}$ = C in >2.0 g cm^{-3} fraction.

Units are g C contained in the fraction per kg of whole soil calculated from the mass balance of the weight of the recovered fraction.

[b] Only one sample from treatments FYM and NPK was fractionated, therefore standard errors were not calculated.

[c] F-statistic.

[d] Statistical comparison only between FYM+NPK and Control treatments.

[e] Least significant difference (P <0.05).

[f] not significantly different (P <0.05).

2.4.2 DRIFTS analysis of bulk soil

In the spectra of the studied bulk soil samples, the 1620 cm^{-1} peak contributed most to the total relative area in bulk soil scans, followed by peaks at 2930 cm^{-1}, 1159 cm^{-1} and 1530 cm^{-1} (Figure 1 and 2). The DRIFTS peaks of the four investigated bands were affected significantly by fertilizer treatment ($P <0.05$) (Figure 2). The average relative area of the 2930 cm^{-1} peak was 36.5% in the FYM+NPK treatment, decreasing in the FYM treatment (4% smaller), and was 13% and 19% smaller in NPK and Control, respectively. The relative peak areas at 1620 cm^{-1} also varied significantly across all four treatments with the smallest peak area in the FYM+NPK, and the largest in the Control ($P < 0.05$). The peaks at 1530 cm^{-1} and 1159 cm^{-1} only showed a difference between the FYM treatments and non-FYM treatments ($P <0.05$), with larger relative peak areas in the NPK and Control. A positive relationship was found between the ratio of the peaks at 1620 and 2930 cm^{-1} (1620:2930) and the ratio of stable C (sum of C contained in clay and >1.8 g cm^{-3} fractions) to labile C (amount of C in the <1.8 g cm^{-3} fraction) ($R^2=0.62$, $P = 0.012$) (Figure 3).
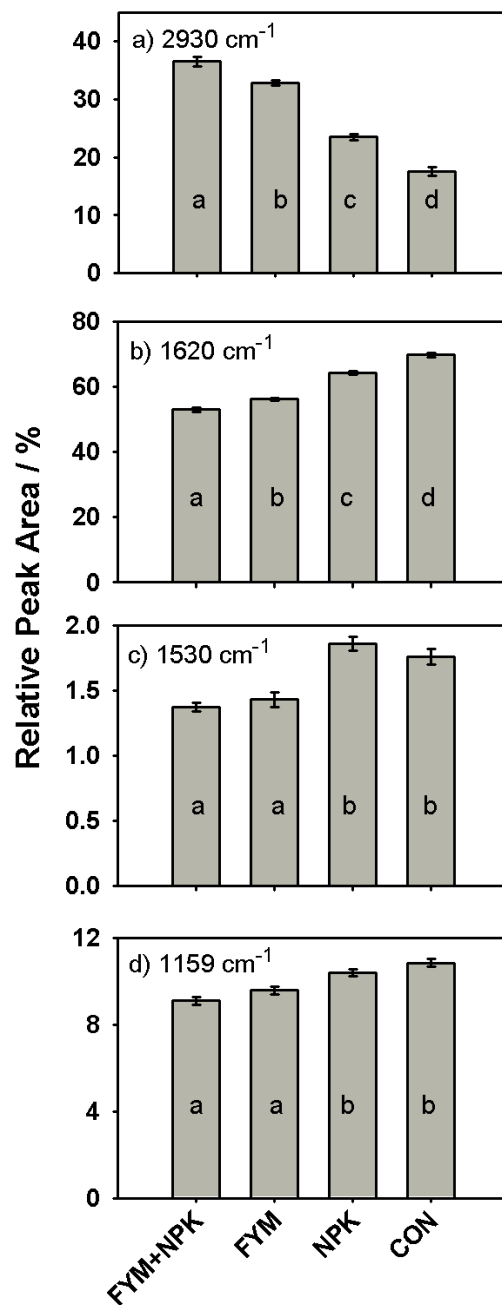
Figure 2. DRIFTS relative peak area by fertilizer treatment of bulk soil at wavenumbers: a) 2930 cm-1 b) 1620 cm-1, c) 1530 cm-1, and d) 1159 cm-1. Treatment averages of years 1956, 1979, 1992, 2004, and 2008: FYM+NPK = farmyard manure and mineral fertilizer; FYM = farmyard manure; NPK = mineral fertilizer; CON = control (no fertilizer/manure inputs). Letters indicate significant differences (P <0.05) along with standard errors.
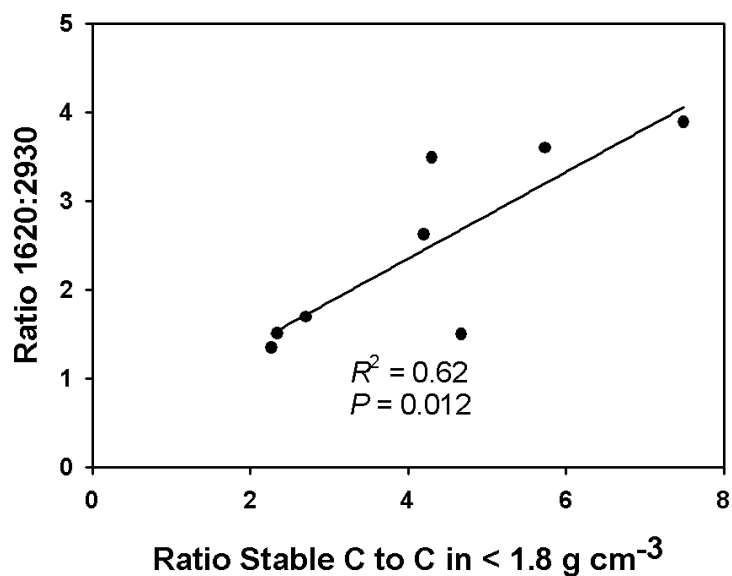
Figure 3. Relationship between the ratio of the DRIFTS peak areas at 1620 and 2930 cm^{-1} and the ratio of stable C (sum of C contained in clay and >1.8 g cm^{-3} fractions) to labile C (amount of C in the <1.8 g cm^{-3} fraction) in soils collected in 1956, 1992, and 2004.

The relative peak areas of the bulk soil by sampling year increased slightly in the 2930 cm^{-1} in the FYM managements ($R^2 = 0.75$, $P = 0.057$), but there was no significant change in the non-FYM managements (Figure 4). Likewise, the 1620 cm^{-1} relative peak area decreased in the FYM ($R^2 = 0.77$, $P = 0.05$), but no trend was observed in the non-FYM treatments. There was no statistically discernible linear trend in the relative area of the other two peaks (1530 and 1159 cm^{-1}) among the sample years.
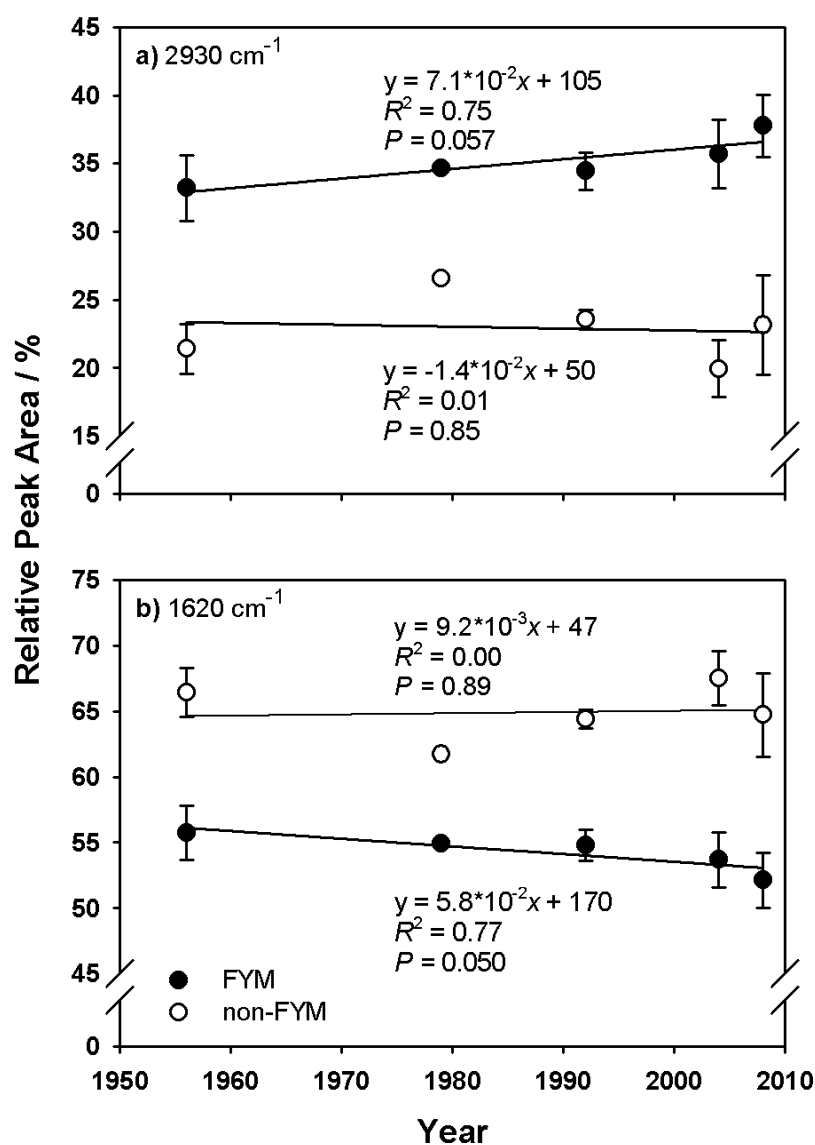
Figure 4. Dynamics of DRIFTS relative peak areas by sampling year of farmyard manure (FYM) and non-farmyard manure treatments (non-FYM): a) 2930cm^{-1} relative peak area, b) 1620cm^{-1} relative peak area. Symbols: ● = FYM, average of both farmyard manure treatments (FYM+NPK and FYM); ○ = non-FYM, average of NPK (mineral fertilizer) and CON (control no fertilizer input) treatments. Bars indicate standard errors.

2.4.3 Spectra of size and density fractions

In the spectra from the size and density fractions, the 2930 cm^{-1} relative peak area of the <1.8 g cm^{-3} fraction was the largest among the size and density fractions with a 88.7% relative peak area (Figure 5), while there was no detectable organic peak present at this wavelength for the >2.0 g cm^{-3} fraction (data not shown). The relative 2930 cm^{-1} peak areas of the <1 μm

and 1-2 μm clay fractions (50.7 and 62.9% respectively) were larger than that of the 1.8-2.0 g cm^{-3} fraction (20.9%), which had the largest variation. Relative peak areas at 1620 cm^{-1} were largest in the >2.0 g cm^{-3} fraction (79.5%) (data not shown) declining with decreasing density to 10.9% in the <1.8 g cm^{-3} fraction, whereas the clay fractions were intermediate between the <1.8 g cm^{-3} fraction and the fractions 1.8-2.0 and >2.0 g cm^{-3}. The ratio of the relative peak areas at 1620 and 2930 cm^{-1} (1620:2930) was greatest in the fraction 1.8-2.0 g cm^{-3} (median = 2.17) and smallest in the <1.8 g cm^{-3} fraction (median = 0.12). The peaks at 1530 and 1159 cm^{-1} were not discernible as distinct peaks in all the fractions, possibly because of a lack of a significant contribution of the respective organic compounds, and were not quantified by peak area integration.
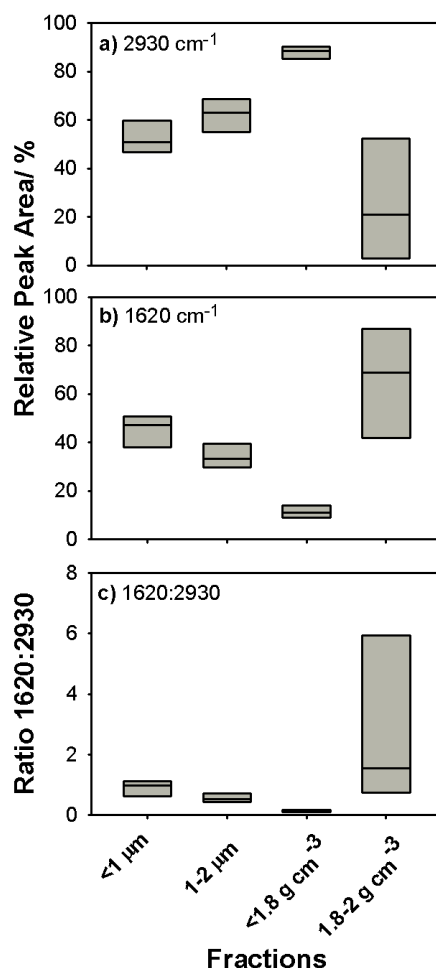
Figure 5. DRIFTS spectra of soil organic matter fractions: a) relative peak area of 2930 cm^{-1}, b) relative peak area of 1620 cm^{-1}, c) Ratio of the peak area at 1620 cm^{-1}-to-2930 cm^{-1}). Values are from the years 1956, 1992, and 2004. The center line indicates the median and the end lines the 25th and 75th percentiles. Legend: <1μm and 1-2μm = clay fractions; <1.8 g cm-3 and 1.8-2.0 g cm-3

2.4.4 Bulk soil peak area correlations with SOM fractions

Correlations (results presented are the correlation coefficient r) of the 2930 cm^{-1} and 1620 cm^{-1} corrected peaks areas of the bulk soil samples with SOM characteristics were correlated significantly to measured SOM contents and fractions while correlations with 1530 cm^{-1} and 1159 cm^{-1} were mostly smaller (Table 3). The corrected 2930 cm^{-1} peak area was positively correlated with TOC ($r = 0.88$, $P < 0.0001$), C_{HWE} ($r = 0.93$, $P < 0.0001$), <1.8 g cm^{-3} fraction C ($r = 0.86$, $P < 0.01$), and 1.8-2.0 g cm^{-3} fraction C ($r = 0.90$, $P < 0.05$). The corrected peak area at 1620 cm^{-1} was negatively correlated to TOC ($r = -0.68$, $P < 0.01$), clay fraction <1 µm C ($r = -0.79$, $P < 0.01$), and <1.8 g cm^{-3} fraction C ($r = -0.95$, $P < 0.01$).

Table 3: Spearman correlation (H1:$\rho \neq 0$) coefficients between DRIFTS corrected peak areas of bulk soil and soil organic carbon parameters.

		Correlation coefficient by peak			
	N	2930 cm^{-1}	1620 cm^{-1}	1530 cm^{-1}	1159 cm^{-1}
TOC	15	0.88***	-0.68**	-0.49*	-0.08
C_{HWE}	15	0.93***	-0.79**	-0.57*	0.03
HIX_{HWE}	15	-0.13	-0.13	-0.13	-0.65**
$C_{<1µm}$	8	0.69	-0.79*	-0.67	-0.07
$C_{1-2µm}$	8	0.40	-0.33	-0.17	0.00
$C_{<1.8\ g\ cm^{-3}}$	8	0.86**	-0.95**	-0.69*	-0.17
$C_{1.8-2.0\ g\ cm^{-3}}$	8	0.90*	-0.81	-0.48	0.12
$C_{>2.0\ g\ cm^{-3}}$	8	0.24	-0.55	-0.43	-0.67*

Significance levels: *** = $P < 0.0001$, ** = $P < 0.01$, * = $P < 0.05$.

TOC = total organic C; C_{HWE} = hot water extractable C; HIX = humification index (435 to 480 nm divided by 300 to 345 nm) of C_{HWE}; $C_{<1µm}$ = C of <1 µm fraction; $C_{1-2µm}$ = C of 1-2 µm fraction; $C_{<1.8\ g\ cm^{-3}}$ = C of <1.8 g cm^{-3} fraction, $C_{1.8-2.0\ g\ cm^{-3}}$ = C of 1.8-2.0 g cm^{-3} fraction, $C_{>2.0\ g\ cm^{-3}}$, C of >2.0 g cm^{-3} fraction.

2.5 Discussion

2.5.1 Comparing DRIFTS of bulk soil with size and density fraction characteristics to derive information on SOM composition and quality

DRIFTS peaks of the bulk soil with the C contents of the size and density fractions and hot-water extractable C (C_{HWE}) were used to study the relationship between different SOM compounds represented by the four peak areas 2930, 1620, 1530 and 1159 cm^{-1} and with the C contents of different fractions. The positive correlation of the corrected 2930 cm^{-1} peak area with C_{HWE} indicated the hypothesized labile nature of the compounds related to this peak; C_{HWE} has been used previously as a measure of a relatively labile pool of SOM (Hoffmann, *et al.*, 2006). The link between the corrected 2930 cm^{-1} peak area to labile organic compounds was further corroborated with the highly significant correlation with the light fraction C content (<1.8 g cm^{-3}), which represents an active SOM pool (von Lützow *et al.*, 2007). Additionally, the peak around 2930 cm^{-1} reflects mainly aliphatic C-H stretching (Baes and Bloom, 1989) which are less stable than aromatic bonds. Both hot-water extracts and light fractions contain heterogeneous materials, though they are dominated by labile organic compounds. Thus, positive relationships with these indicators with DRIFTS results do not necessarily provide proof of a casual relationship. However, both indicators support the theoretical framework based on the energy state of the predominant C-H stretching at 2930 cm^{-1}. The corrected peak area of 3010 to 2800 cm^{-1} has also been correlated with humic substances in sediments of different composition and supposed stabilities (Tremblay & Gagné, 2002). In contrast to Tremblay & Gagné's approach and their contrasting environment, we observed changes i) in the relative abundance of peaks which takes into account shifts between more labile and stable C compounds and ii) between samples obtained from the same soil where the mineralogy is constant and any differences in the studied peaks must have occurred as the result of changes in SOM. Since spectral regions around 3050 cm^{-1} are also known vibrations of C-H functional groups associated to aromatic compounds (Baes and Bloom, 1989), careful interpretation of the spectral assignments for each case is necessary.

Significant negative correlations of the corrected peak areas at 1620 cm^{-1} with C_{HWE} and $C_{<1.8 \ g \ cm^{-3}}$ indicated that the 1620 cm^{-1} peak may be related more to recalcitrant organic

compounds. This was also supported by the fact that the corrected peak areas at 1620 cm^{-1} in the Control, which had received the smallest amount of organic inputs (crop stubble and roots), was largest and slowly increased over time through the continuous decomposition of more labile compounds. The negative correlations with $C_{<1\mu m}$ and $C_{1.8\text{-}2.0 \text{ g cm}}^{-3}$, however, did not directly support this conclusion, as these fractions should have contained relatively stabilized SOM (Schulz et al., 2011). This provides evidence for the heterogeneity of the isolated SOM fractions: that although they are composed of a continuum of organic compounds, they may be more enriched or depleted in certain compounds. Additionally, it has been suggested that because there is usually more than one stabilization mechanism present, this may result in the non-homogeneous turnover rate of organic compounds within most of the fractions (von Lützow et al., 2007). The negative correlations of the peak at 1530 cm^{-1} and the SOM fractions followed the same trend as 1620 cm^{-1}, but with generally poorer correlation coefficients. Both of these peaks were assigned to aromatic C=C vibrations, while the 1620 cm^{-1} peak was also assigned to the –COO^{-} stretching vibrations. The absence of the –COO^{-} vibrations at 1530 cm^{-1} may partly explain the poorer correlations of the peak with the fractions' C contents. Unexpectedly, the corrected peak 1159 cm^{-1} peak area did not correlate with any of the fractions except for the humification index (HIX) and the fraction >2.0 g cm^{-3}. The negative correlation with the HIX, which increases with increased humification of the hot-water extract, may indicate that this peak represents labile compounds and results in the negative correlation with the >2.0 g cm^{-3} fraction. However, the relative contribution of this peak area was greater for the Control than for FYM+NPK treatments which would not support the contention that the 1159 cm^{-1} peak represents compounds of greater lability.

The increase in the 1620:2930 ratio with increasing stabilized C in the SOM fractions also confirmed other studies which have found a positive relationship with a aromatic to aliphatic ratio and the amount of humic substances (Inbar *et al.*, 1989) and increasing degree of decomposition in forest soil organic horizons (Haberhauer *et al.*, 1998). Through correlations with SOM fractions, distribution of C among the fractions, and their implied stabilities from literature, the hypothesis was confirmed that the aliphatic stretching peak at 2930 cm^{-1} was dominated by SOM components of greater lability, and the peaks at 1620 and 1530 cm^{-1} represented more stable components in our soils. The evidence for attributing SOM quality characteristics for 1159 and 1530 cm^{-1} peaks was less clear. These findings might not be relevant to other soils, as mineralogical interferences might obscure or alter visibility and extent of peaks in different soils and complicate comparisons across sites. On the other hand,

the preferential pre-selection of peaks took this aspect partly into consideration by selecting those peaks which do not have strong mineral interferences. Additionally, the choice of integration to consider overlapping vibrations of different functional groups at a certain peak, and using a peak analysis approach with corrected or absolute areas may also help to extend the applicability of the method.

2.5.2 Confirming quality findings with DRIFTS analysis of SOM fractions

The selected peak areas as applied to the SOM fractions themselves corroborated our conclusions from the correlation analyses of different stabilities of the fractions determined by DRIFTS. The largest relative value for the 2930 cm^{-1} peak was found in the <1.8 g cm^{-3} fraction, again indicating that this peak represented labile organic compounds. Organic C in the <1.8 g cm^{-3} was shown by (Schulz et al., 2011) to be only loosely associated with minerals and consisted of relatively un-decomposed plant residues with weak physical protection: this was also indicated by the small value for the 1620:2930 ratio of the <1.8 g cm^{-3} fraction of our soil. The larger relative value for the 1620 cm^{-1} peak area of the two clay fractions compared with the relatively less decomposed <1.8 g cm^{-3} fraction indicates their greater degradation. Leifeld & Kögel-Knabner (2005) suggested that clay fractions may have both old, relatively inert C which does not respond to land use and another more active component which does respond to changes. This could explain why the 2930 cm^{-1} relative peak areas for the clay fractions were greater than that of the density fraction of 1.8-2.0 g cm^{-3}: this supports the idea of an interaction of stabilization mechanisms and heterogeneity within these fractions. The greater 1620:2930 ratio of the clay fractions compared with the <1.8 g cm^{-3} fraction is also compatible with their slower turnover rate compared with fresh material (von Lützow *et al.*, 2007). Using relative peak areas of the SOM fractions, it was found that not only the quantity of OM contained in each fraction was different, but also the composition. This has also been observed by using ^{13}C NMR (Kögel-Knabner *et al*., 2008); Gerzabek *et al.*, 2006); however, DRIFTS is more rapid, less costly, and is also able to show the inherent heterogeneity of the SOM fractions.

2.5.3 SOM dynamics in the Static Fertilization experiment as demonstrated by bulk soil DRIFTS

As the corrected peak areas of the bulk soil were significantly correlated to different stabilities and qualities of SOM (also reflected in the relative peak areas of the SOM fractions themselves), the differences in relative peak area in different fertilizer treatment indicated the changing quality of SOM under the long-term management. We can surmise that the peak at 2930 cm^{-1} increased mainly by additional residue inputs, both through FYM and also increased plant residues in the NPK treatment: this also followed the same trend as TOC and C_{HWE}. A similar influence of organic and mineral inputs on the corrected peak area between 3000 and 2800 cm^{-1} normalized to TOC was found by Capriel (1997) . This peak changed in medium textured soils during a one-year incubation experiment (Spaccini *et al.*, 2001), but also seemed changed over the longer term with farmyard manure application as we have also shown. . An increase in labile SOM of FYM+NPK compared with the Control has been found by using other methods including $KMnO_4$ oxidation (Blair *et al.,* 2006) and differential scanning calorimetry (Leifeld *et al.*, 2006) and supports what was found with the specific DRIFTS peak method. The increasing relative peak area at 1620 cm^{-1} in the NPK and Control treatments compared with the FYM treatments showed an enrichment of more stable organic compounds. This is consistent with smaller TOC, C_{HWE} and <1.8 g cm^{-3} contents of the Control compared to the FYM+NPK and FYM treatments. Our DRIFTS results complement the ^{13}C NMR spectroscopy results of Kiem *et al*. (2000) of increased aromaticity in the Control treatment compared with the FYM+NPK treatment in the Bad Lauchstädt long-term experiment. Additionally, an increase in the aromatic nature (1620 cm^{-1} peak) of SOM has been found in increasing maturities of manures (Hsu & Lo, 1999), in soil under treatments of fallow and no organic inputs (Gerzabek *et al.*, 2006), and in increasingly decomposed forest litter layers and Ah horizons (Haberhauer *et al.*, 1998; Haberhauer & Gerzabek, 1999). Furthermore, with increasing soil depth from the surface to 30 cm, the aliphatic peak at 2920 cm^{-1} in the humin fraction declined and the aromatic peak at 3050 cm^{-1} increased (Tatzber *et al.,* 2007). Likewise, in our study, according to the bulk soil DRIFTS results, the SOM in the Control treatment has become relatively more aromatic (increased 1620 cm^{-1} peak) compared with the FYM+NPK treatment (decreased 1620 cm^{-1} peak). It seems that the compounds represented by the peaks at 1530 and 1159 cm^{-1} were mainly influenced by either the presence or absence of FYM and not by mineral fertilizer. It was not immediately clear why this was the case, as these peaks represent very different C compounds (1530 cm^{-1}: C=C

aromatics (Baes & Bloom, 1989), and 1159 cm^{-1}: C-O poly-alcohol and ether group vibrations (Senesi *et al.*, 2003). Additional sampling years and greater SOM characterization would be needed in order to detect more precisely the temporal trends in these two peaks. Conversely, Spaccini *et al.* (2001) did not detect any significant peak changes in sandy and clayey soils of a one-year incubation experiment, possibly indicating that the method may have interferences in certain soil textures.

2.6 Conclusions

This study substantiated the hypothesis that specific peaks obtained by DRIFTS of bulk soils can be used to study the changing composition of organic matter in a Haplic Chernozem. It was shown that long-term application of organic and mineral fertilizers affected not only the quantity, but also the composition of SOM as compared with an unfertilized control treatment. These changes were strongly reflected in DRIFTS spectra, especially in some of the selected predominant aliphatic and aromatic peak areas at 2930 and 1620 cm^{-1}, respectively. For the Haplic Chernozem the ratio of the relative peak areas at 1620 and 2930 cm^{-1} (1620:2930) was particularly shown to be a useful indicator of changes in stable and labile C in bulk soil samples. Here we demonstrated that this approach applied to our size and density fractions also reflected the interaction of organic matter stabilization mechanisms and heterogeneity.

While the current study confirms similar results from other investigations, other studies have found relationships of other peak ratios and OM quality in different environments, suggesting that the findings might not be fully transferable to all soils as mineralogical interferences might obscure or alter visibility and the quantification of peaks in different soils. This would complicate comparisons across sites. However, as we show, a careful pre-selection of peaks avoiding strong mineral interferences is needed. Additionally, the choice of integration method considering possible overlapping vibrations of different functional groups at a certain peak, and analysis using corrected peak or relative peak areas are important. The demonstrated relationship between specific peaks and SOM stability and use of undiluted soil samples should enhance the applicability of DRIFTS, if specular reflection is avoided, as an improvement over FT-IR transmission spectroscopy. The peak area method is probably best suited for investigations within the same soil type and with small numbers of samples which are not suitable for multivariate prediction models. The demonstrated relationships of relative

peak areas with the suggested composition of SOM need to be tested when applying the method to soils with different mineralogies. Further research is needed to quantify organic C in the different peak regions regardless of possible mineral interferences in order to make a better link between the peak areas and functional SOM pools. This may be possible through the use of coupling technologies such as thermal analysis, paralysis, or fluorescence spectroscopy.

References

Baes, A.U. & Bloom, P.R. 1989. Diffuse reflectance and transmission Fourier transform infrared (DRIFT) spectroscopy of humic and fulvic acids. *Soil Science Society of America Journal,* **53,** 695-700.

Batjes, N.H. 1996. Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science,* **47,** 151-163.

Bishop, J.L., Koeberl, C., Kralik, C., Fröschl, H., Englert, P.A.J., Andersen, D.W., Pieters, C.M. & Wharton Jr., R.A. 1996. Reflectance spectroscopy and geochemical analyses of Lake Hoare sediments, Antarctica: Implications for remote sensing of the Earth and Mars. *Geochimica et Cosmochimica Acta,* **60,** 765-785.

Blair, N., Faulkner, R.D., Till, A.R., Körschens, M. & Schulz, E. 2006a. Long-term management impacts on soil C, N and physical fertility. Part II: Bad Lauchstädt static and extreme FYM experiments. *Soil and Tillage Research,* **91,** 39-47.

Capriel, P. 1997. Hydrophobicity of organic matter in arable soils: influence of management. *European Journal of Soil Science,* **48,** 457-462.

Christensen, B.T. 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. *European Journal of Soil Science,* **52,** 345-353.

DIN ISO 13878. 1998. Bodenbeschaffenheit - Bestimmung des Gesamt-Stickstoffs durch trockene Verbrennung (Elementaranalyse). Beuth, Berlin, Wien, Zürich.

Egli, M., Mavris, C., Mirabella, A. & Giaccai, D. 2010. Soil organic matter formation along a chronosequence in the Morteratsch proglacial area (Upper Engadine, Switzerland). *Catena,* **82,** 61-69.

Ellerbrock, R.H., Gerke, H.H., Bachmann, J. & Goebel, M.-O. 2005. Composition of Organic Matter Fractions for Explaining Wettability of Three Forest Soils. *Soil Science Society of America Journal,* **69,** 57-66.

Gerzabek, M.H., Antil, R.S., Kögel-Knabner, I., Knicker, H., Kirchmann, H. & Haberhauer, G. 2006. How are soil use and management reflected by soil organic matter characteristics: a spectroscopic approach. *European Journal of Soil Science,* **57,** 485-494.

Grube, M., Lin, J.G., Lee, P.H. & Kokorevicha, S. 2006. Evaluation of sewage sludge-based compost by FT-IR spectroscopy. *Geoderma,* **130,** 324-333.

Haberhauer, G. & Gerzabek, M.H. 1999. Drift and transmission FT-IR spectroscopy of forest soils: an approach to determine decomposition processes of forest litter. *Vibrational Spectroscopy,* **19,** 413-417.

Haberhauer, G., Rafferty, B., Strebl, F. & Gerzabek, M.H. 1998. Comparison of the composition of forest soil litter derived from three different sites at various decompositional stages using FTIR spectroscopy. *Geoderma,* **83,** 331-342.

Hoffmann, S., Schulz, E., Csitári, G. & Bankó, L. 2006. Influence of mineral and organic fertilizers on soil organic carbon pools. *Archives of Agronomy and Soil Science,* **52,** 627-635.

Hsu, J.-H. & Lo, S.-L. 1999. Chemical and spectroscopic analysis of organic matter transformations during composting of pig manure. *Environmental Pollution,* **104,** 189-196.

Inbar, Y., Chen, Y. & Hadar, Y. 1989. Solid-state carbon-13 nuclear magnetic resonance and infrared spectroscopy of composted organic matter. *Soil Science Society of America,* **53**, 1695-1701.

IUSS Working Group WRB. 2007. *World Reference Base for Soil Resources 2006, first update 2007. World Soil Resources Reports.* Rep. 103. FAO, Rome.

Janik, L.J., Skjemstad, J.O., Shepherd, K.D. & Spouncer, L.R. 2007. The prediction of soil carbon fractions using mid-infrared-partial least square analysis. *Australian Journal of Soil Research.,* **45,** 73-81.

Kiem, R., Knicker, H., Körschens, M. & Kögel-Knabner, I. 2000. Refractory organic carbon in C-depleted arable soils, as studied by ^{13}C NMR spectroscopy and carbohydrate analysis. *Organic Geochemistry,* **31,** 655-668.

Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S., Eusterhues, K. & Leinweber, P. 2008. Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. *Journal of Plant Nutrition and Soil Science,* **171,** 61-82.

Körschens, M., Stegemann, K., Pfefferkorn, A., Weise, V. & Müller, A. 1994. *Der Statische Düngungsversuch Bad Lauchstädt nach 90 Jahren. Einfluß der Düngung auf Boden, Pflanze und Umwelt.* B.G. Teubner Verlagsgesellschaft, Stuttgart.

Leifeld, J. 2006. Application of diffuse reflectance FT-IR spectroscopy and partial least-squares regression to predict NMR properties of soil organic matter. *European Journal of Soil Science,* **57,** 846-857.

Leifeld, J., Franko, U. & Schulz, E. 2006. Thermal stability responses of soil organic matter to long-term fertilization practices. *Biogeosciences,* **3,** 371-374.

Leifeld, J. & Kögel-Knabner, I. 2005. Soil organic matter fractions as early indicators for carbon stock changes under different land-use? *Geoderma,* **124,** 143-155.

Nguyen, T.T., Janik, L.J. & Raupach, M. 1991. Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy in soil studies. *Australian Journal of Soil Research,* **29,** 49-67.

Niemeyer, J., Chen, Y. & Bollag, J.-M. 1992. Characterization of humic acids, composts, and peat by diffuse reflectance Fourier-transform infrared spectroscopy. *Soil Science Society of America Journal,* **56,** 135-140.

Paul, E.A., Morris, S.J., Six, J., Paustian, K. & Gregorich, E.G. 2003. Interpretation of soil carbon and nitrogen dynamics in agricultural and afforested soils. *Soil Science Society of America Journal,* **67,** 1620-1628.

Reeves III, J.B. 2003. Mid-Infrared diffuse reflectance spectroscopy: Is sample dilution with KBr necessary, and if so, when? *American Laboratory,* **35,** 24-28.

Schulz, E. & Körschens, M. 1998. Characterization of the Decomposable Part of Soil Organic Matter (SOM) and Transformation Processes by Hot Water Extraction. *Eurasian Soil Science,* **31,** 809-813.

Schulz, E., Breulmann, M., Boettger, T., Wang, K.-R. & Neue, H.-U. 2011. Effect of organic matter input on functional pools of soil organic carbon in a long-term double rice crop experiment in China. *European Journal of Soil Science,* **62,** 134-143.

Senesi, N., D'Orazio, V. & Ricca, G. 2003. Humic acids in the first generation of EUROSOILS. *Geoderma,* **116,** 325-344.

Shaymukhametov, M.S., Titova, N.A., Travnikova, L.S. & Labenets, Y.M. 1984. Use of physical fractionation methods to characterize soil organic matter. *Soviet Soil Science,* **16,** 117-128.

Smith, P., Powlson, D.S., Smith, J.U., Falloon, P. & Coleman, K. 2000. Meeting Europe's climate change commitments: quantitative estimates of the potential for carbon mitigation by agriculture. *Global Change Biology,* **6,** 525-539.

Spaccini, R. & Piccolo, A. 2007. Molecular characterization of compost at increasing stages of maturity. 1. Chemical fractionation and infrared spectroscopy. *Journal of Agricultural and Food Chemistry,* **55,** 2293-2302.

Spaccini, R., Piccolo, A., Haberhauer, G., Stemmer, M. & Gerzabek, M.H. 2001. Decomposition of maize straw in three European soils as revealed by DRIFT spectra of soil particle fractions. *Geoderma,* **99,** 245-260.

Sparling, G., Vojvodić-Vuković, M. & Schipper, L.A. 1998. Hot-water-soluble C as a simple measure of labile soil organic matter: The relationship with microbial biomass C. *Soil Biology and Biochemistry,* **30,** 1469-1472.

Stevenson, F.J. 1982. *Humus chemistry. Genesis, composition, reactions.* Wiley and Sons, New York.

Tatzber, M., Stemmer, M., Spiegel, H., Katzlberger, C., Haberhauer, G., Mentler, A. & Gerzabek, M.H. 2007. FTIR-spectroscopic characterization of humic acids and humin fractions obtained by advanced NaOH, $Na_4P_2O_7$, and Na_2CO_3 extraction procedures. *Journal of Plant Nutrition and Soil Science,* **170,** 522-529.

Tatzber, M., Stemmer, M., Spiegel, H., Katzlberger, C., Zehetner, F., Haberhauer, G., Garcia-Garcia, E. & Gerzabek, M.H. 2009. Spectroscopic behaviour of ^{14}C-labeled humic acids in a long-term field experiment with three cropping systems. *Australian Journal of Soil Research,* **47,** 459-469.

Tremblay, L. & Gagné, J.-P. 2002. Fast quantification of humic substances and organic matter by direct analysis of sediments using DRIFT spectroscopy. *Analytical Chemistry,* **74,** 2985-2993.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E. & Marschner, B. 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. *Soil Biology and Biochemistry,* **39,** 2183-2207.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B. & Flessa, H. 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions- a review. *European Journal of Soil Science,* **57,** 426-445.

Zimmermann, M., Leifeld, J. & Fuhrer, J. 2007. Quantifying soil organic carbon fractions by infrared-spectroscopy. *Soil Biology and Biochemistry,* **39,** 224-231.

Zsolnay, A., Baigar, E., Jimenez, M., Steinweg, B. & Saccomandi, F. 1999. Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. *Chemosphere,* **38,** 45-50.

3 Combining a coupled FTIR-EGA system and in situ DRIFTS for studying soil organic matter in arable soils[*]

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3.1 Abstract

An optimized spectroscopic method combining quantitative evolved gas analysis via Fourier transform infrared spectroscopy (FTIR-EGA) in combination with a qualitative in situ thermal reaction monitoring via diffuse reflectance Fourier transform infrared spectroscopy (in situ$_T$ DRIFTS) is being proposed to rapidly characterize soil organic matter (SOM) to study its dynamics and stability. A thermal reaction chamber coupled with an infrared gas cell was used to study the pattern of thermal evolution of carbon dioxide (CO_2) in order to relate evolved gas (i.e., CO_2) to different qualities of SOM. Soil samples were taken from three different arable sites in Germany: i) the Static Fertilization Experiment, Bad Lauchstädt (Chernozem), from treatments of farmyard manure (FYM), mineral fertilizer (NPK), their combination (FYM+NPK) and control without fertilizer inputs; ii) Kraichgau; and iii) Swabian Alb (Cambisols) areas, Southwest Germany. The two latter soils were further fractionated into particulate organic matter (POM), sand and stable aggregates (Sa+A), silt and clay (Si+C), and NaOCl oxidized Si+C (rSOC) to gain OM of different inferred stabilities; respiration measured from fresh soil samples incubated at 20°C and 50% water holding capacity for 490 days. A variable long path length gas cell was used to record the mid-infrared absorbance intensity of CO_2 (2400 to 2200 cm^{-1}) being evolved during soil heating from 25 to 700°C with a heating rate of 68°C min^{-1} and holding time of 10 minutes at 700°C. Separately, the heating chamber was placed in a diffuse reflectance chamber (DRIFTS) for measuring the mid-infrared absorbance of the soil sample during heating. Thermal stability of the bulk soils and fractions was measured via the temperature of maximum CO_2 evolution (CO_{2max}). Results indicated that the FYM+NPK and FYM treatments of the Chernozem soils had a lower CO_{2max} as compared to both NPK and CON treatments. On average, CO_{2max} of the Chernozem was much higher (447°C) as compared to the Cambisol sites (Kraichgau 392°C; Swabian Alb 384°C). The POM fraction had the highest CO_{2max} (477°C), while rSOC had a first peak at 265°C at both sites and a second peak at 392°C for the Swabian Alb and 482°C for the Kraichgau. The CO_{2max} increased after 490 day incubation, while the C lost during incubation was derived from the whole temperature range but a relatively higher proportion from 200 to 350°C. In situ$_T$ DRIFTS measurements indicated decreases in vibrational intensities in the order of C-OH = unknown C vibration < C-H < -COO/ C=C < C=C with increasing temperature, but interpretation of vibrational

changes was complicated by changes in the spectra (i.e., overall vibrational intensity increased with temperature increase) of the sample during heating. The relative quality changes and corresponding temperatures shown by the in situ$_T$ DRIFTS measurements enabled the fitting of four components or peaks to the evolved CO_2 thermogram from the FTIR-EGA measurements. This gave a semi-quantitative measure of the quality of evolved C during the heating experiment, lending more evidence that different qualities of SOM are being evolved at different temperatures from 200 to 700°C. The CO_{2max} was influenced by long-term FYM input and also after 490 days of laboratory incubation, indicating that this measurement is an indicator for the relative overall SOM stability. The combination of FTIR-EGA and in situ$_T$ DRIFTS allows for a quantitative and qualitative monitoring of thermal reactions of SOM, revealing its relative stability, and provides a sound basis for a peak fitting procedure for assigning proportions of evolved CO_2 to different thermal stability components.

3.2 Introduction

Various thermal analytical methods have been explored to study the quantity of soil organic matter (SOM) (Gaál et al., 1994) and its quality (Lopez-Capel et al., 2005), the basic hypothesis being that more labile OM such as carbon (C) associated with microbial biomass or light fraction C would also be thermally degraded at lower temperatures due to the lower activation energy needed to break the associated bonds, and that more stable compounds being evolved at higher temperatures. Thermal degradation of organic molecules is affected by the arrangement of the molecules in the larger macromolecule and likewise influenced by interactions with mineral surfaces (Blumstein, 1965). These biochemical characteristics are also the basic factors which influence the stabilization of OM in soils (von Lützow et al., 2007; von Lützow et al., 2008). Further, organo--mineral interactions such as ligand exchange, cation ion bridging, and complexation are influenced by clay as well as Fe- and Al-oxide content and type of soil (von Lützow et al., 2008).

Traditional thermal analytical techniques relied on weight loss (i.e., thermal gravimetric analysis (TGA)), energy changes (differential scanning calorimetry (DSC)), or differential thermal weight loss (DTA) between a sample and a reference material (Siewert, 2004); (Leinweber et al., 1992). Different land uses (e.g., grassland, arable, fallow) and different SOM fractions (e.g., light free and inter-aggregate OM) have been shown to affect the distribution of weight losses during heating treatments (Lopez-Capel et al., 2005), where it

was inferred that weight losses at lower temperatures were related to labile SOM and at higher temperature more stabile SOM was degraded. The TGA/DSC/DTA methods have the drawback that the thermal decomposition of SOM may be difficult to infer due to additional reactions in the same temperature range (Gaál et al., 1994), such as loss of absorbed or interlayer water up to 250°C and from 400 to 700°C with crystallization and dehydroxylation reactions (Pansu and Gautheyrou, 2006). Isotope measurements *ex post facto* of different thermal treatments have been used to determine the age or contribution of maize residues to SOM after conversion from winter wheat. (Dorodnikov et al., 2008; Dorodnikov et al., 2007) found that SOM turnover times, as calculated via ^{13}C enrichment from free air carbon enrichment (FACE) experiments, did not increase with increasing temperature treatment, although C evolved at temperatures >480°C was slightly less bio-available compared to C evolved at temperatures <480°C. Likewise, Helfrich et al. (2010) did not find a significant increase in recent maize-derived SOM in a >1.8 g cm^{-3} fraction as measured by ^{13}C dating after a C3 to C4 vegetation conversion with increasing heating treatments up to 500°C.

Although these studies cast doubt on the use of thermal analytical approaches as an indicator of SOM stability, several factors may have affected the results: i) it was hypothesized that younger SOM would be thermally decomposed at lower temperatures, which may not be the case anymore if the light fraction has been removed and the remaining sample had undergone microbial mediated transformations; ii) it is not well understood what changes a soil sample undergoes after being exposed to a thermal treatment and then cooled to room temperature to be analyzed for isotope composition; and iii) very slow heating rates, as in the case of (Dorodnikov et al., 2008; Dorodnikov et al., 2007)) of 2°C min^{-1}, may shift thermal reactions to significantly lower temperatures (Fernández et al., 2011); and may possibly v) lead to increased char formation, as has been shown during combustion of cellulose (Baldry et al., 1988). Hence, an improved method to directly quantify thermal decomposition products and their quality would greatly advance the investigation of SOM via thermal analysis.

To avoid the aforementioned drawbacks of TGA/DTG and *ex post facto* analysis, on-line analyses such as FTIR-EGA (Fourier transform infrared-evolved gas analysis) (Plante et al., 2009) and in situ$_T$ DRIFTS (in situ monitoring of thermal reactions via diffuse reflectance Fourier transform infrared spectroscopy) (Murakami et al., 1997) could be developed for monitoring thermal reactions of SOM. An online method provides the direct measurement of the combustion/decomposition products of thermal degradation of OM and has been used in conjunction with TG/DSC for studying SOM (Fernández et al., 2012). FTIR-EGA has been

used extensively for the rapid quantification of gaseous reaction products such as CO_2, carbon monoxide, water, aliphatics and others for the analysis of lignite, chars of coal and spruce wood (Haselsteiner et al., 2011), and inorganic compounds (e.g., calcium carbonate, barium chloride, etc.) (Mittleman, 1990). This method relies on the vibrational frequencies of polar gas molecules in the mid-infrared range from 4000 to 400 cm^{-1} and it is usually the case that combustion products can be quantitatively determined by integrating the wavenumbers of the gas of interest (i.e., CO_2 at 2400 to 2200 cm^{-1}) and creating a calibration curve using a known standard (i.e., sodium bicarbonate) (Court and Sephton, 2009); (Toops et al., 2005). The in $situ_T$ DRIFTS method, on the other hand, allows observations of the changes in vibrational intensities at wavenumbers of interest of a sample during heating (Toops et al., 2005). Hence, combining FTIR-EGA and in $situ_T$ DRIFTS has the potential for the rapid characterization of SOM based on the properties of the evolved gas profile (i.e., peak temperature, distribution of evolved gas) and the changes in vibrational intensity of mid-infrared wavenumbers during heating. In addition to quantifying the total C amount in a soil sample, by optimizing the combustion conditions the distribution and peak temperature may be related to different chemical and or physical traits. This could then be linked to changes in vibrational frequencies of the soil at different temperatures during in $situ_T$ DRIFTS measurements to unravel qualitative changes in the types of compounds undergoing thermal degradation.

The objectives of this study were to i) develop improved experimental techniques of FTIR-EGA and in $situ_T$ DRIFTS systems for analyzing SOM across the range of C contents and stability found in bulk soil and SOM fractions; and ii) to use these techniques to characterize SOM of different stabilities, treatments, and microbial decomposition status. The hypothesis was that the characteristics of thermal CO_2 evolution (i.e., temperature of peak CO_2 evolution, changes in low temperature CO_2 evolution versus higher temperature CO_2 evolution) are affected by different qualities of SOM. Soils or fractions which are more enriched in labile compounds (e.g., farmyard manure treated soil, POM) will have lower thermal stability as compared with soils or fractions which are more enriched in stable compounds (e.g., soil with long history of low residue return after harvest, silt+clay fraction (von Lützow et al., 2007), or a chemically resistant fraction (Zimmermann et al., 2007b). The third objective was to develop an innovative approach to combine the use of FTIR-EGA and in $situ_T$ DRIFTS to provide a theoretical molecular based framework for deconvolution of different components of the evolved gas thermogram.

3.3 Materials and methods

3.3.1 Organic compounds for experimental testing

Pure, reagent grade glucose (anhydrous, BDH Chemicals Ltd, Poole, UK), xylan (Sigma-Aldrich Chemie, Munich, Germany), and tannic acid (Carl Roth GmbH, Karlsruhe, Germany) were compared to gain initial information on thermally evolved CO_2 of different organic substances in the absence of any mineral influence. These substances were chosen as they vary in their chemical structure, ranging from a simple sugar in glucose to the more complex polyphenol type structure of tannic acid, and to test the effect of a dilution with preheated quartz sand (1:100) on CO_2 evolution to optimize combustion conditions.

3.3.2 Soils

Soil samples were from three different sites in Germany. The first site was the Static Fertilization Experiment, Bad Lauchstädt, Germany (51°24' N, 11°53' E). Archive soil samples were taken from the years 1956, 1992, and 2004 of the treatments of farmyard manure (FYM, 30 t ha^{-1} every second year), and NPK (mineral) fertilizer (123, 30 and 30 kg ha^{-1}yr^{-1} N, P and K, respectively), combination of both (FYM+NPK, 30 t ha^{-1} farmyard manure every second year and 103, 6 and 25 kg ha^{-1}yr^{-1} N, P and K, respectively as mineral fertilizers), and a control (CON) to examine how long-term fertilizer treatments affect SOM properties and thermal characteristics of the bulk soil. The experiment has been running since 1902 under a rotation of winter wheat, potatoes, spring barley, and sugar beets. Soil samples were taken in the fall following harvest of winter wheat from a depth of 0 to 30 cm. The soils were classified as Chernozems (IUSS Working Group WRB, 2007). Bulk mineralogy (<2 mm) is predominantly quartz with lesser amounts of feldspars and micas (Dreibrodt et al., 2002). More information about the experiment can be found in (Körschens et al., 1994) and Table 1. The second study site (48°55.7' N, 8°42.2' E) was in the vicinity of Pforzheim, Germany, in the Kraichgau region, which is characterized by loess parent material and intensive agriculture. Soils sampled were classified as Cambisols (IUSS Working Group WRB, 2007) with bulk mineralogy a mixture of quartz, feldspars, and micas. The third site (48°31.7' N, 9°46.2' E) was near the village of Nellingen, Germany, in the Swabian Alb. The Swabian Alb soils were formed from limestone residuum and also classified as Cambisols (IUSS Working Group WRB, 2007) with a similar mineralogy as the Kraichgau soil. The crop rotation in the fields of both areas typically consists of winter wheat, maize, and oilseed rape. In both the Kraichgau and Swabian Alb sites, two fields were selected with three plots

each with a subplot sampled in the vegetation (winter wheat and maize in the Kraichgau, winter wheat and oil seed rape in the Swabian Alb) and a bare fallow subplot which was kept clear of vegetation during the experiment by hand pulling and periodic spot spraying of glyphosate. The fallow plots were used to investigate the turnover of SOM without fresh organic inputs such as roots, aboveground crop residues, and manure/slurry. Soil samples were taken one month after the establishment of the bare fallow subplots and in adjacent vegetated subplots at the end of May to beginning of June 2009. Soil samples for fractionation (Kraichgau and Swabian Alb) and thermal analysis (all three sites) were air-dried and made to pass a 2 mm sieve. Visible roots and plant residues were removed prior to analysis. The mean annual precipitation and elevation gradients, from lowest to highest, are Bad Lauchstädt (483 mm), Kraichgau (780 mm), and Swabian Alb (962 mm) (Table 1). Mean annual temperature is nearly the same in the Kraichgau and Bad Lauchstädt sites (9.1 and 8.8°C, respectively), but more than two degrees lower in the Swabian Alb (6.7°C). The clay percentage was highest in soils of the Swabian Alb site (38%) and lowest in those of the Kraichgau site (18%).

Table 1. Study site characteristics.

Study Site	Location	MAP [1] / mm	MAT / °C	Soil	Elevation / MASL	pH	Clay / %
Bad Lauchstädt	N 51° 24', E 11° 53'	483	8.8	Haplic Chernozem	113	6.6	21
Kraichgau	N 48° 55.7', E 8° 42.2'	780	9.1	Cambisol/Regosol	232	6.7	18
Swabian Alb	N 48° 30', E 9° 42'	962	6.7	Cambisol	686	6.5	38

[1] MAP-mean annual precipitation, MAT-mean annual temperature, soil-FAO classification, MASL-meters above sea level.

3.3.3 Fractionation

The soil fractionation method was modified from (Zimmermann et al., 2007a). Briefly, 30 g of <2 mm soil was made up to 150 ml with deionized water and sonified for two minutes at

30 J s^{-1}. The mixture was poured onto a 63 μm sieve and gently sieved until water passing through the sieve was clear. The <63 μm portion was collected and dried at 40°C overnight to yield the silt and clay (Si+C) fraction. Before drying the bulk, moist Si+C fraction, 1 ml of 0.01 M calcium chloride solution for flocculation was added to a 20 ml subsample of the suspension and centrifuged for 15 min at 3000 x g. The clear supernatant was taken as extractible OC. The fraction remaining on the 63 μm sieve was transferred to a 10 ml test tube and 5 ml of 1.8 g cm^{-3} density sodium polytungstate (SPT) solution was added, mixed, and centrifuged for 15 min at 3000 x g. The fraction floating on top of the SPT was poured off and 4 ml SPT was added to the remaining fraction, mixed, and centrifuged again to remove any remaining light fraction material. The floating material yielded the particulate organic matter (POM) and the heavy material yielded the sand and >63 μm stable aggregates (Sa+A) fraction. The separated fractions were rinsed with deionized water and dried at 40°C. A sub-sample of the silt and clay fraction was combined with 10 ml sodium hypochlorite and placed in a water bath at 95°C for 15 min (Anderson, 1963) to speed up the partial oxidation of the labile organic matter. The mixture was centrifuged for 8 min at 200 x g, decanted, and the oxidation, centrifugation, and decanting steps repeated twice. Finally, the residue was rinsed with deionized water and dried at 40°C to yield a resistant organic carbon (rSOC) fraction. Two aliquots of each soil sample were fractionated separately.

Total carbon (TC) of bulk soil and soil fractions were measured by dry combustion. Carbonate content of bulk soils was measured by the HCl gasometric method (Scheibler method) (Schlichting et al., 1995), while carbonates in the SOM fractions were destroyed by fumigation with hydrochloric acid (Harris et al., 2001). Bulk soils and fractions were then measured for TC by dry combustion with a Vario-EL III elemental analyzer (Elementar, Hanau, Germany).

3.3.4 Incubation experiment

Field moist samples from the Kraichgau and Swabian Alb sites' vegetated plots were sieved to <4 mm and stored at 4°C. One kg moist soil was transferred to 2.5 l glass jars and incubated in a dark, climate controlled room at 20°C for 490 days. The lids to the jars were left slightly open to enable gas exchange, but to prevent rapid drying. Additionally, a 100 ml beaker of deionized water was placed in the jars for better moisture retention. Periodically, deionized was added to the soils to keep the soil moisture content at 50% of field capacity.

Additionally, at the beginning of the experiment, two 20 g moist weight replicates were taken from each field sample, sieved to 2 mm, adjusted to 50% of field capacity and placed in a Respicond IV automated respirometer (Nordgren Instruments, Bygdeå, Sweden) for the continuous measurement of CO_2 evolution during 44 days at 20°C (the same temperature as the climate controlled room). At the end of the 44 day period, the incubation temperature was increased to 25°C (standard temperature for microbial biomass determination) and, after stabilization of respiration, a 1:3 (w:w) glucose and talc mixture was added to measure (substrate-induced) microbial biomass (Anderson and Domsch, 1978). A pre-test was done with varying amounts of glucose mixture, with the maximum respiration response being ~0.16 g for Kraichgau and ~0.32 g for Swabian Alb, respectively.

3.3.5 FTIR-EGA method

Setup of FTIR-EGA thermal chamber

For measuring thermally evolved gases, a high temperature heating system was utilized in conjunction with a Bruker Tensor 27 (Bruker Optik GmbH, Ettlingen, Germany) infrared spectrometer. The high temperature reaction chamber (HTC) (Harrick Scientific Products, Pleasantville, NY, USA) and with an integrated sample holder which could hold approximately 50-70 mg of soil sample and was equipped with a cartridge type heating element and a K-type thermocouple. The HTC was interfaced with an automatic temperature controller (Harrick Scientific Products, Pleasantville, NY, USA) with an integrated temperature/process controller (Watlow Winona, Minnesota, USA) for programmed heating rates and set-point temperatures. The HTC was closed by a gas tight dome with a high temperature O-ring. The dome had three windows, two made from potassium bromide to enable diffuse reflectance measurements (DRIFTS) of the soil, while the third window was made from quartz glass. The approximate internal volume of the heating chamber with installed dome was 20 ml. In the gas measurement mode (FTIR-EGA), the heating chamber was linked via a stainless steel Swagelok system (Swagelok-Stuttgart GmbH, Reutlingen, Germany) to a variable long path gas cell (Bruker Optik GmbH) which was placed in the accessory chamber of the Tensor 27 spectrometer (Fig. 1a). In the gas transfer line, immediately after the heating chamber, there was a water trap of magnesium perchlorate and a sintered metal filter (2 μm) to prevent any particulate matter from reaching the gas cell and to act as a heat sink. The gas cell had a volume of 136 ml and a path length of 1 m.
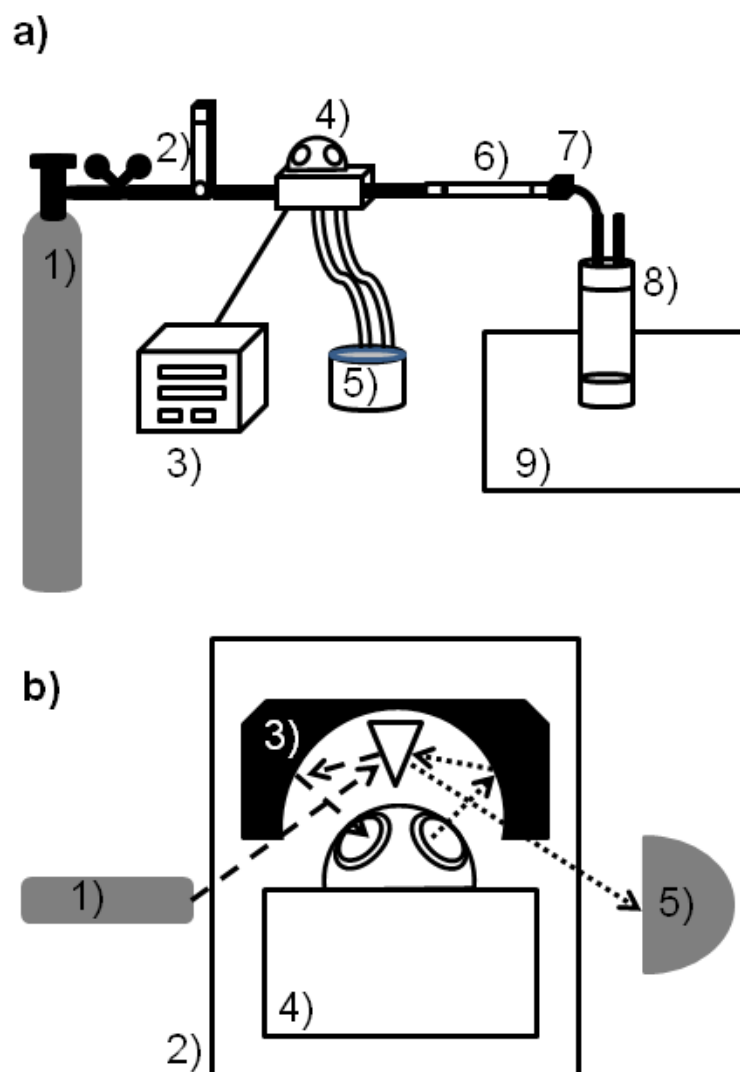
Figure 1. Schematic of experimental setups. (a) FTIR-EGA system: 1) synthetic air purge/carrier gas, 2) pressure regulator and flow gauge, 3) automatic temperature controller, 4) high temperature reaction chamber with potassium bromide (KBr) windows and integrated sample cup, 5) recirculating cooling water, 6) glass tube with magnesium perchlorate $(Mg(ClO_4)_2)$ water trap, 7) sintered metal particulate trap, 8) variable path length gas cell, 9) FTIR spectrometer). **(b)** In situ$_T$ DRIFTS system: (1) infrared source, 2) diffuse reflectance chamber, 3) globular mirror, 4) high temperature reaction chamber with potassium bromide (KBr) windows and integrated sample cup, 5) detector. Dashed lines indicate source infrared beam; dotted lines indicate diffusely reflected infrared beam.

Taking into consideration the range of C contents of the samples to be analyzed (0.13% being the lowest in the NaOCl oxidized silt+clay fraction to 48% in tannic acid) for this study, the state conditions of purge flow rate, sample size, heating rate, infrared spectrometer scan rate, and mid-infrared wave number integration range were optimized (see supplement figures S1-S4). This optimization was necessary to quantitatively determine the mid-infrared spectrometer signal response to total C amount, minimize secondary reactions, and to ensure similar heat transfer rates from the heating element to the sample, as the shape and distribution of the evolved gas were to be used as additional parameters to link to SOM properties.

After the pre-test the following procedure was adopted for the FTIR-EGA measurements. A scan was recorded every 4 seconds, with a resolution of 8 cm^{-1}. Synthetic air was used as the purge and carrier gas of the system with a flow rate of 15 L hr^{-1}. The chamber was purged for 5 minutes after introducing the sample. Additionally, a baseline was taken before the beginning of each analysis in order to zero the signal in the gas cell. An optimized heating rate of 68°C min^{-1} (see section below) and set-point temperature of 700°C were used. The set-point temperature was reached after 10 minutes and held for another 20 minutes, which resulted in a total of 400 individual FTIR scans. After pretesting, in which it was found that CO_2 evolution ended after a shorter time duration, the final number of scans taken was reduced to 300 for a total analysis time of 20 minutes.

In the present experiment, lower heating rates were also tested (10 and 28°C min^{-1}), but were found to result in an increase in the formation of secondary reaction products during heating, as shown by in situ$_T$ DRIFTS (see Figure S3). Heating rates >68°C min^{-1} resulted, however, in non-linear increases in temperature and an overshoot of the final set point temperature. After the heating rate was set, the purge gas flow rate was adjusted in order that the maximum absorbance of the CO_2 peak (2400 to 2200 cm^{-1}) evolution was <2.5 absorbance units (A.U.). If the absorbance is >2.5 A.U., then there may not be a linear relationship between absorbance and the content of the compound of interest (Conzen, 2003). A relatively high purge rate of 15 L hr^{-1} was needed in order to minimize retention time in the gas cell due to the large volume (136 mL). The scanning or wavenumber resolution of 8 cm^{-1} was found adequate, as the main gas of interest, CO_2, has a relatively broad spectral absorbance range (100 wavenumbers) and does not require a very high resolution. The time in between each scan was first set at 40 seconds as this was the calculated time to purge the entire system, but was found to be too coarse a measurement interval, especially for rapid increases in CO_2

evolution. A scan every 4 seconds gave a better time resolution of the rate of change with respect to time of CO_2 evolution. It was found that if any of these state variables were changed (i.e., purge rate, scan time, resolution, or heating rate), then a new calibration curve would need to be constructed to quantify CO_2 evolution.

3.3.6 Deriving FTIR-EGA thermal characteristics

The individual scans from a single FTIR-EGA analysis were then assembled into a single file or thermogram for further processing using the spectral processing software OPUS v 6.5 (Bruker Optik GmbH). The spectra were baseline corrected using the concave rubberband method with 64 baseline points and 10 iterations. Integration was performed on the baseline corrected spectra between 2400 to 2200 cm^{-1}, following equation 1:

$$Area_{peak} = \int_{2200cm^{-1}}^{2400cm^{-1}} A(v) \tag{1}$$

where $A(v)$ is the absorbance value of the investigated wavenumbers. The cumulative peak area was calculated by equation 2:

$$Area_{cum} = \sum_{j=1}^{300} j(Area_{peak}) \tag{2}$$

where j is the scan number, which was taken every 4 seconds for a total of 300 scans, and $j(Area_{peak})$ is the $Area_{peak}$ at scan j. The presence of a linear response of cumulative peak area found by equation 2 to the theoretical CO_2 yield of the thermal decomposition of a sodium bicarbonate standard was checked to establish a quantitative relationship.

Each CO_2 evolution thermogram was exported from OPUS and further processed in Excel (Microsoft) to normalize $Area_{peak}$ to analyzed sample weight:

$$Area_{wt} = \frac{Area_{peak}}{sampleweight(mg)}. \tag{3}$$

Furthermore, different characteristics of the nature of CO_2 evolution were calculated including normalizing $Area_{peak}$ to C content:

$$Area_C = \frac{Area_{peak}}{sampleweight \; x \; C_{org} \; content} \tag{4}$$

where C_{org} *content* is the total organic C content of the sample as measured by elemental analyzer minus any carbonates.

The temperature of maximum CO_2 evolution was identified with the following equation:

$$CO_{2\,max} = \max_{25}^{700} f(Area_{peak}),$$ (5)

during the heating from 25 to 700°C.

Due to the large range in C contents of samples analyzed (0.1 to 48% OC), the sample weight was adjusted to fit into a range from 0.1 to 2.5 mg OC content in order to stay within the linear range of the calibration curve (Fig. 3). In the case of POM, which had between 26-32% C by weight, the above C content would only result in a maximum sample weight of 6 mg as compared to 30 to 50 mg of the other fractions and bulk soil. Thus, due to the small sample weight and the small volume occupied within the heating chamber, a 1:50 dilution was made with ball-milled quartz sand, which had previously been heated to 600°C overnight to remove any C impurities. This dilution ratio was used to gain roughly the same volume of material as was analyzed with the bulk soil and lower C content fractions and to result in C amounts between the 0.1 to 2.5 mg range.
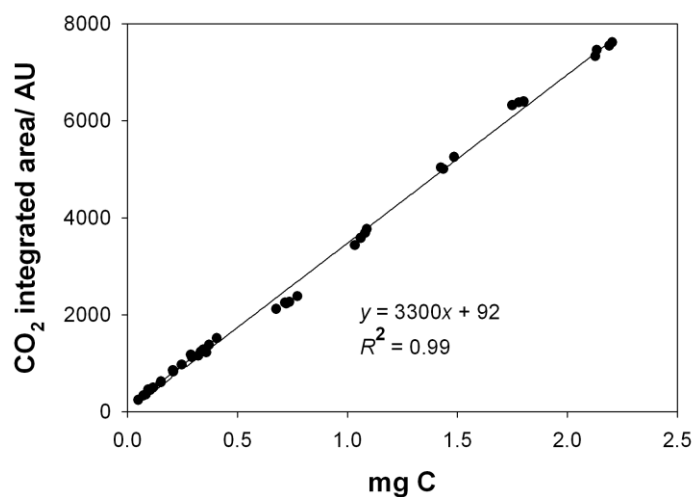
Figure 3. CO_2 calibration curve of the FTIR-EGA with $NaHCO_3$.

3.3.7. In situ$_T$ DRIFTS method

To follow the molecular vibrational changes of the soil sample during heating, a Praying Mantis diffuse reflectance (DRIFTS) chamber (Harrick Scientific) was fitted in the Tensor 27

(Bruker Optik GmbH) and the HTC (Harrick Scientific) placed inside the DRIFTS chamber to record absorbance measurements from 4000 to 400 cm^{-1} of the bulk soil or SOM fraction surfaces during heating, thus referred to in this paper as in situ$_T$ DRIFTS (Fig. 1b). The heating conditions and purge rate remained the same as in the FTIR-EGA mode. A scan was taken every 4 seconds at a resolution of 4 cm^{-1}. Potassium bromide (KBr) was used to take a background scan before measuring the sample. Additionally, the POM fraction was diluted 1:50 with KBr for analysis. Identification of important wavenumbers corresponding to OM functional groups was done based on the assignments by Demyan et al. (2012) and also by inspecting which wavenumbers were changing in intensity during the heating experiment. As previously mentioned, not only is OM thermal degradation ongoing during this temperature interval (200-700°C) but also water loss and mineral changes (silica structure) occur. The known OH stretching area at 3400 to 3000 cm^{-1} was used to monitor changes in water loss and was compared to behavior of other bands to determine if there was also an influence of water loss on the decrease of a peak instead of OM combustion. Collected spectra were baseline corrected as described above and smoothed using a running average with a 15 point window.

3.3.8 Data combination of FTIR-EGA and in situ$_T$ DRIFTS for curve fitting

The *Area$_{wt}$* outputs from a single FTIR-EGA analysis (300 readings) (Eq. 3) of bulk soil were then subjected to a curve fitting procedure. First, changes in molecular vibration intensities related to specific OM compounds were followed during the heating process between 200 and 700°C using in situ$_T$ DRIFTS. A decrease in the identified molecular vibrational peaks during this period were assumed to coincide with the combustion of OM. Points of maximum decrease were identified by using 100% of the value at 200°C (start of thermal degradation of OM) and calculating the rate of change between each measurement point. These points of largest decrease were then taken as center points for the fitted components of the peak fitting procedure of the FTIR-EGA evolved CO_2 of the bulk soil. A series of constraints were made for the curve deconvolution routine based on the results of the in situ$_T$ DRIFTS results. Temperatures of maximum decrease in intensity of a vibrational organic functional group above 200°C were taken to be the peak of an individual "component" of the FTIR-EGA CO_2 profile. The most rapid decreases of the peak intensities were found at the following temperatures: C-O vibrations (2200-2000 cm^{-1}) at 320°C and taken as 1st peak, 2nd peak at 380°C of the C-H vibrations (3000-2800 cm^{-1}), 3rd peak COO-/C=C (1620 cm^{-1}), and 4th peak

at 1525 cm^{-1} (C=C). An iterative least squares approach was used for the curve fitting of the CO_2 evolved gas profiles using the software PeakFit version 4.12 (Systat Software, San Jose, California, USA). Additionally, the following rules were followed during FTIR-EGA peak fitting: peaks were placed first where there was a visible peak or a change in the rate of increase or decrease of CO_2 evolution as determined by 2nd derivative, peaks were then added at the local maximum of the residuals to improve the R^2 and decrease the standard error (SE). The temperatures of these peak positions were again verified with the results from the in situ$_T$ DRIFTS given above to justify the presence of a specific component at a certain temperature. The residuals of the final fit were also inspected for normality and heteroscedasticity. A fitting procedure was considered completed when $R^2 > 0.999$, SE < 0.005 and there was a homoscedasticity of residuals. The peak fitting was repeated three times on a single analytical replicate to ensure stability or robustness of the fit and to determine if the fit was the most likely combination of solutions based on the data.

Analysis of variance (significance at $P < 0.05$) was used to compare CO_{2max} among the treatments at the Bad Lauchstädt site. A mixed modeling approached was used to compare the CO_{2max} of the bulk soils and fractions in Kraichgau and Swabian Alb areas. The factors were site and fraction, with plot location as the random error.

3.4 Results

3.4.1 Soil characteristics

Results of total C elemental analysis revealed that the soils of Swabian Alb had nearly double the TOC content (18.33 mg C g^{-1} soil) compared to the Kraichgau site (9.17 mg C g^{-1} soil) (Table 2). The TOC contents ranged from a low 14.8 in the CON to 21.2 mg C g^{-1} soil in the FYM+NPK treatment. Carbon contents of the fractions were generally twice the amount in the Swabian Alb as compared to the Kraichgau with the exception of extractible SOM, which was only 0.04 mg C g^{-1} soil in the Kraichgau compared to 0.36 mg C g^{-1} soil in the Swabian Alb. Additionally, microbial biomass C as measured by the SIR method of incubated soils was more than twice as much in the Swabian Alb site (0.59 mg C g^{-1} soil) as compared to the Kraichgau site (0.24 mg C g^{-1} soil).

Table 2. Mean values (± standard errors) of organic carbon (OC) of bulk soil and fractions from the sites of Bad Lauchstädt, Kraichgau, and Swabian Alb based on oven dried (105°C) basis. All values are mg C g^{-1} bulk soil.

Soil parameter[a]	Site					
	Kraichgau	Swabian Alb		Bad Lauchstädt		
			FYM+NPK	FYM	NPK	CON
Bulk soil	9.17 (0.24)	18.33 (1.16)	22.1(1.1)	20.4(0.8)	16.5(0.3)	14.8(0.3)
Extractible SOM	0.04 (0.00)	0.36 (0.02)	nd[b]	nd	nd	nd
POM	0.84 (0.07)	1.34 (0.14)	nd	nd	nd	nd
Sa+A	0.42 (0.02)	0.63 (0.09)	nd	nd	nd	nd
Si+C	7.92 (0.14)	13.31 (0.59)	nd	nd	nd	nd
rSOC	1.38 (0.05)	2.94 (0.24)	nd	nd	nd	nd
C$_{mic}$	0.24 (0.04)	0.59 (0.07)	nd	nd	nd	nd
C lost[4]	0.9 (0.01)	1.9 (0.07)	nd	nd	nd	nd

a Bulk soil <2 mm, Extractible SOM-dissolved SOM captured during wet sieving of silt andclay from sand and stable aggregates, POM-sand fraction < 1.8 g cm^{-3}, Sa+A sand fraction > 1.8 g cm^{-3}, Si+C < 63 μm, rSOC < 63 μm treated with hot sodium hypochlorite, C$_{mic}$-microbial biomass as measured by substrate induced respiration method (Andersen and Domsch, 1993), [b] Not determined. c Carbon lost over 490 day incubation at 20°C and 50% water holding capacity.

3.4.2 FTIR-EGA derived properties of organic substances

In order to quantify evolved CO_2 from the thermal reactions, sodium bicarbonate was used as a standard due to its known stoichiometric thermal decomposition (Janković, 2009). An example of a single mid-infrared gas cell scan of evolved gas from $NaHCO_3$ decomposition can be found in Fig. 2, showing the three main vibrational modes of CO_2 (in order of decreasing intensity) at 2400-2200, 715-615, and 3760-3657 cm^{-1}. Absorbance values of >2.5 of the main CO_2 peak at 2400-2200 cm^{-1} resulted in a non-linear response of $Area_{cum}$ and calculated C evolved from the $NaHCO_3$ (see Figure S4). Thus, for the heating rate of 68 °C min^{-1} and reaction chamber purge rate of 15 L hr^{-1}, a C weight between 0.1-2.5 mg could be analyzed and resulted in a linear response ($R^2 = 0.99$) (Fig. 3). The three pure substances (i.e., glucose, xylan, tannic acid) had very different FTIR-EGA CO_2 thermograms, such as number

of peaks, peak position and general shape of the curve of CO_2 evolution (Fig. 4). In the undiluted samples (Fig. 4a), xylan was found to have the first CO_2 evolution peak at 320°C, one at 500°C, and the highest CO_2 temperature peak at 680°C. Undiluted glucose had a prominent peak at 442°C and a shoulder at 537°C. The first two CO_2 peaks of undiluted tannic acid were present at 380 and 450°C, and a final peak at 660°C. To test if there was any effect on sample volume to FTIR-EGA derived thermal characteristics, a dilution of 1:100 (w/w) with preheated quartz sand was done. This dilution resulted in relatively the same volume of heating chamber occupied compared with the bulk soils for the same quantity of OC. The dilution resulted in shifts of the peak temperatures and also the peak heights relative to C content. Xylan still had the earliest CO_2 peak, but that was shifted to a higher temperature at 346°C and a second peak at 518°C. Glucose had only one CO_2 peak at 380°C. Tannic acid had two poorly resolved peaks, one at 410°C and a final peak around 577°C. As a percentage of total evolved C, 80% was recovered upon reaching 700°C (10 minutes) from undiluted samples, while in the diluted samples, 90% was recovered.
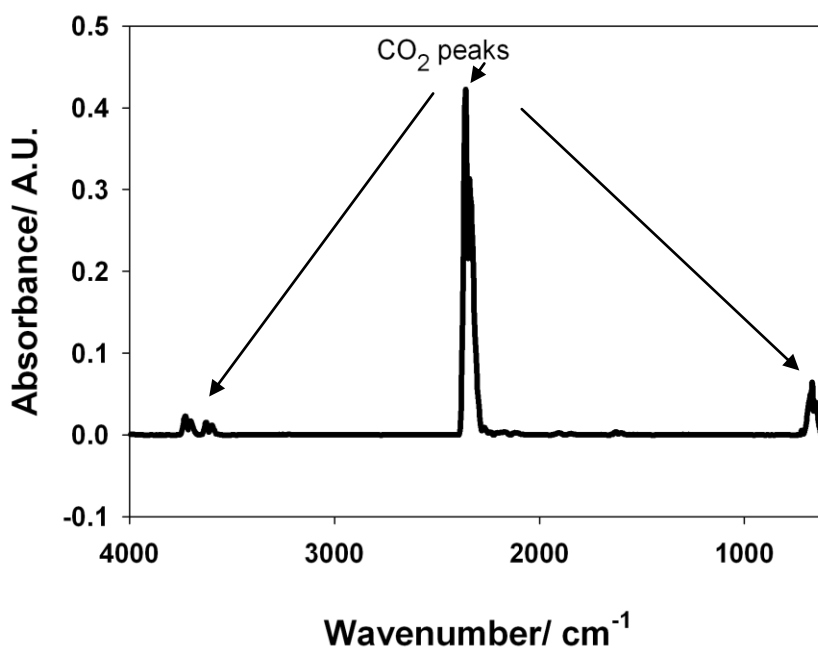
Figure 2. Single scan of online evolved gas as measured by the FTIR-EGA system, with corresponding CO_2 peaks.
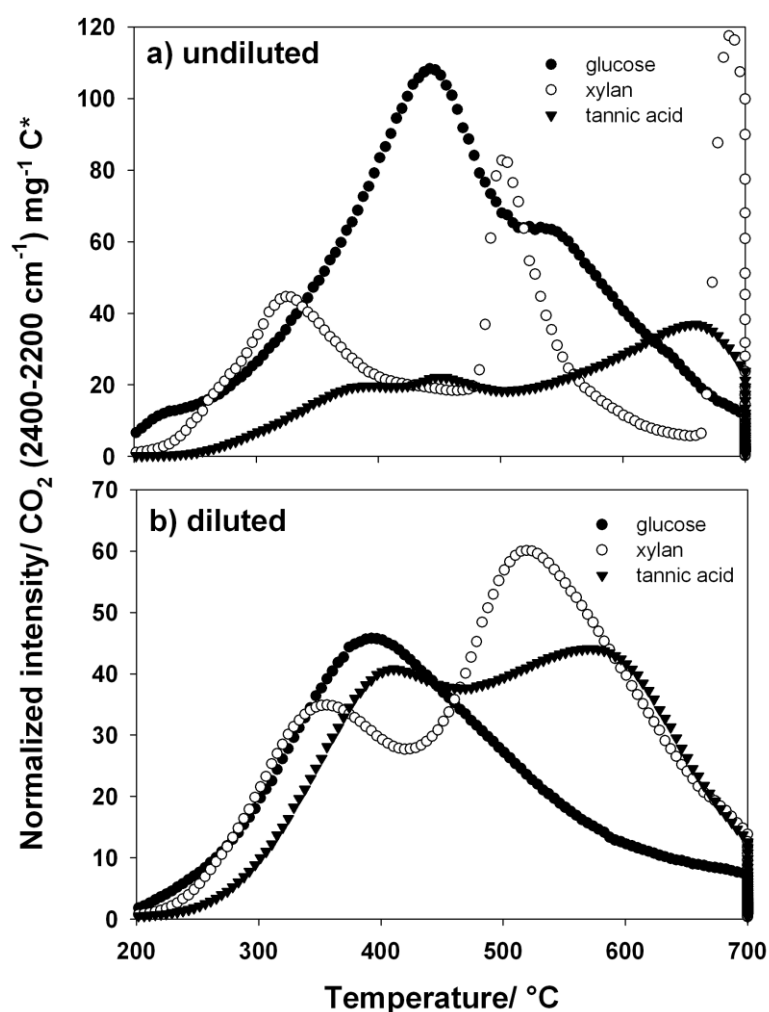
Figure 4. FTIR-EGA CO2 thermograms of three selected substances. A) neat samples, B) diluted 1:100 with preheated, ball-milled quartz sand. * Normalized intensity is the integrated area of the CO_2 peak from 2400 to 2200 cm^{-1} normalized to C content of the sample.

3.4.3 FTIR-EGA derived properties of soils and fractions

The CO_{2max} varied both by long-term fertilizer application in the case of the Bad Lauchstädt soils (Table 3) and also when comparing by site (Bad Lauchstädt, Kraichgau, Swabian Alb) (Figure 5). In the Bad Lauchstädt soils, the CO_{2max} increased from 419°C in the FYM+NPK treatment to 428°C in FYM and 473°C in the NPK and control soils. Compared to the TOC contents (Table 2), which were only significantly affected by FYM application, the FTIR-EGA method also indicated a difference between the FYM+NPK and FYM treatments (Fig 6). When comparing soils from the three sites (Fig. 5), the peak CO_2 evolution not only had

different maximum heights for the three soils, but also different temperatures. The average for the Swabian Alb site soils had the lowest CO_{2max} at 384°C, while that of the Kraichgau soils was at a significantly higher (P <0.05) temperature (392°C). The CO_{2max} average of all Bad Lauchstädt samples was much later at 447°C (P <0.05), even factoring in the effect of long-term manure treatment. The CO_2 thermograms further revealed that while the Kraichgau and Swabian Alb soils had a similar CO_2 evolution pattern with a rapid increase in CO_2 evolution between 275 to 380°C (Figure 5), the Bad Lauchstädt soils had a much broader peak covering a range of approximately 80°C (Figure 5). Compared with the results from the elemental analyzer, total C contents as measured by FTIR-EGA were underestimated on average by 7%.

Table 3. FTIR-EGA measured peak temperature of CO2 evolution during heating from 25 to 700°C of soils from the Static Fertilization Experiment, Bad Lauchstädt (average years 1956, 1992, 2004). Values in parentheses are standard errors. Different letters within each column indicate a significant difference (P < 0.05) among the four treatments.

Treatment	Peak Temperature/ °C
FYM+NPK[1]	419 (3)a
FYM	428 (2)b
NPK	473 (2)c
Control	473 (2)c

[1] FYM+NPK (farmyard manure and mineral fertilizer), FYM (farmyard manure), NPK (mineral fertilizer), Control (no fertilizer inputs).
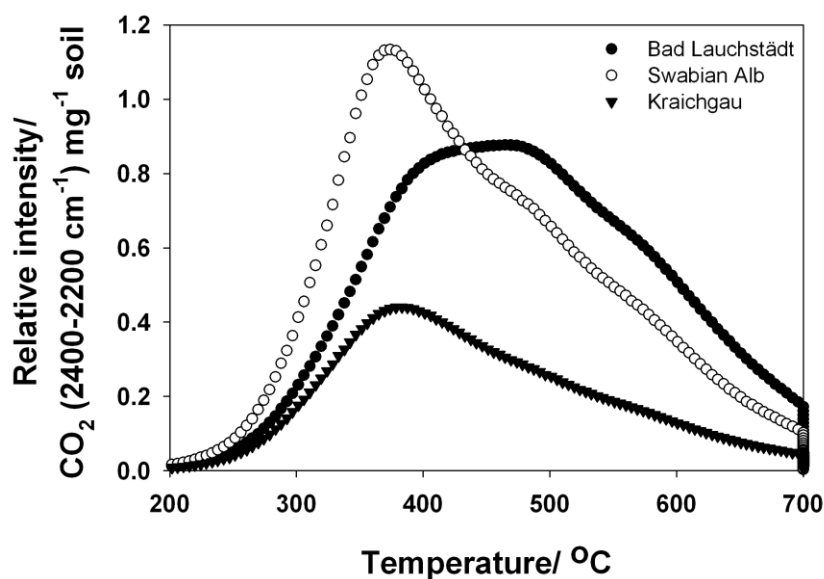
Figure 5. FTIR-EGA average CO2 thermograms of soils from three different agroecological sites (Bad Lauchstädt, Kraichgau, Swabian Alb). * Normalized intensity is the integrated area of the CO_2 peak from 2400 to 2200 cm^{-1} normalized to weight of the sample.
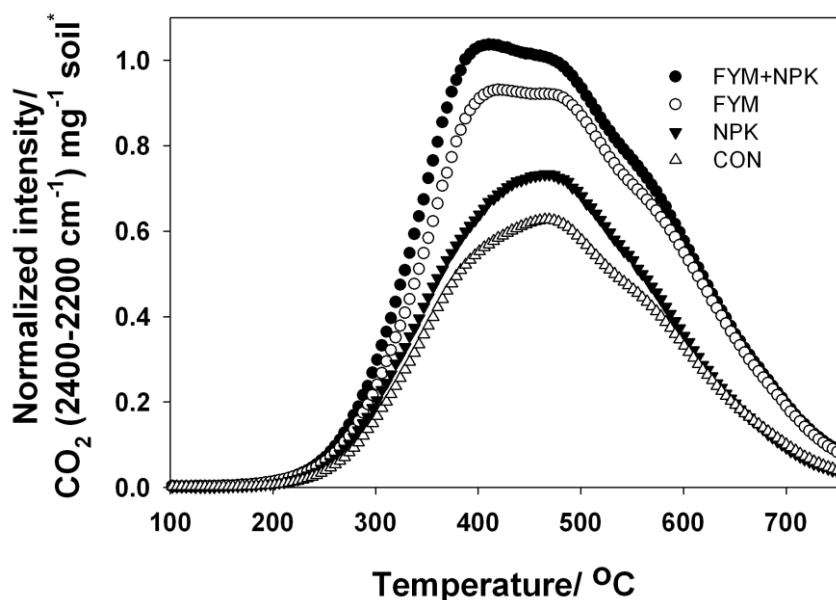
Figure 6. FTIR-EGA CO2 thermograms of bulk soil from the Static Fertilization Experiment, Bad Lauchstädt heating from 25 to 700°C at 68°C min-1 under synthetic air purge. Treatments: FYM+NPK (farmyard manure and mineral fertilizer), FYM (farmyard manure), NPK (mineral fertilizer), Control (no fertilizer inputs). * Normalized intensity is the integrated area of the CO_2 peak from 2400 to 2200 cm^{-1} normalized to weight of the sample.

Soils measured after incubation for 490 days showed a decrease in thermally evolved CO_2 (Figure 7) and a shift in the CO_{2max}. The difference CO_2 thermograms (Figure 7C) taken by subtracting the two thermograms show from which temperature regions C has been released during the long-term incubation. The maximum difference between pre- and post-incubation evolved CO_2 was at 360 and 378°C for the Kraichgau and Swabian Alb, respectively, with CO_{2max} shifted to a slightly higher temperature (increase of 4°C).
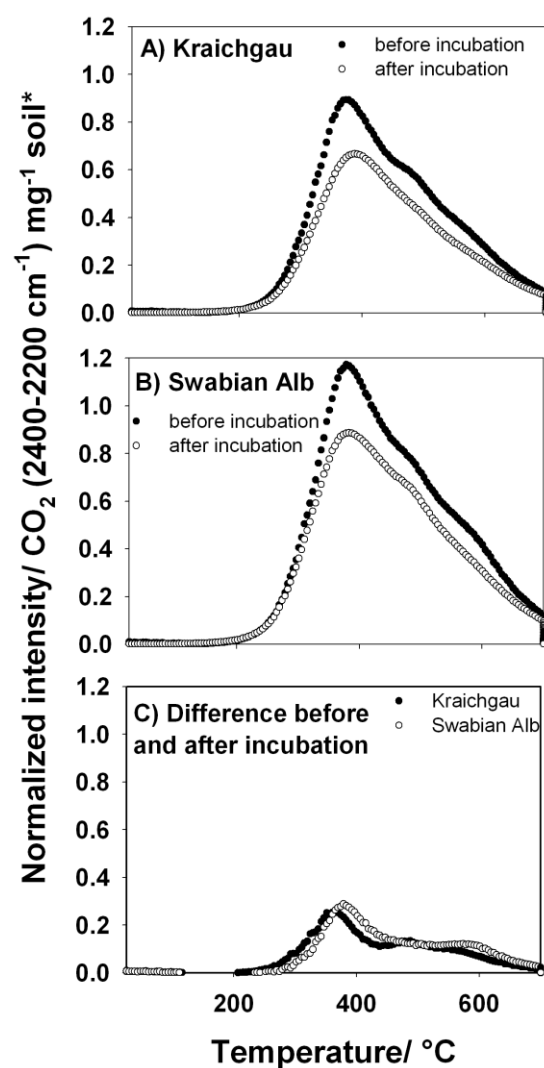
Figure 7. Effects of 490 day incubation on thermally evolved CO2 thermogram. A) Kraichgau soils, B) Swabian soils, C) difference when subtracting thermogram of soil at beginning of incubation from thermogram at the end of the incubation. * Normalized intensity is the integrated area of the CO_2 peak from 2400 to 2200 cm^{-1} normalized to weight of the sample.

The temperature of the individual CO_2 evolution peaks of SOM fractions separated from the bulk soil of the Kraichgau and Swabian Alb sites were generally found to be the same among the sites, with the exception of the second peak of rSOC fraction, which was much higher at the Kraichgau site (482°C) compared to the Swabian Alb site (392°C) (Table 4, Figure 8). The largest differences in relation to the bulk soil were the rSOC fractions, which had two well defined peaks and the POM, which had a first poorly defined peak or "shoulder" around 380°C, the same temperature as the bulk soil, but then a main peak much later compared to the bulk soil at 477°C (Figure 8). C contents of the fractions as estimated by FTIR-EGA compared with the elemental analyzer were 7% overestimated in the case of Sa+A and rSOC, 8% underestimated in Si+C and 20% underestimated in the case of POM.

Table 4. Mean values (± standard error) for soil (Kraichgau and Swabian Alb) at the temperatures (°C) of peaks or "shoulders" of thermally evolved CO2 of bulk soil and SOM fractions as measured by FTIR-EGA. Lowercase letters indicate significant differences ($P < 0.05$) between peak temperatures in a row (among the sites), different letters indicate significant differences ($P < 0.05$) within a site, among fractions.

	Site					
	Kraichgau			Swabian Alb		
	200-350°C	350-450°C	450-550°C	200-350°C	350-450°C	450-550°C
fraction						
Bulk soil[a,b]		392(2)Aa			384(2)Ab	
POM[a] *			477(5)B			476(6)B
Sa+A[a]		362(1)A			369(7)A	
Si+C[a]		400(1)A			399(4)A	
rSOC[a]	265(1)C		482(17)Ba	265(5)C		392(15)Ab

[1] Bulk soil <2 mm, POM-sand fraction <1.8 g cm^{-3}, Sa+A sand fraction >1.8 g cm^{-3}, Si+C <63 µm, rSOC <63 µm treated with hot sodium hypochlorite. [2] No value indicates that a peak was not present in these temperature regions.

* POM fraction was diluted 1:50 with preheated ball-milled quartz sand for thermal analysis
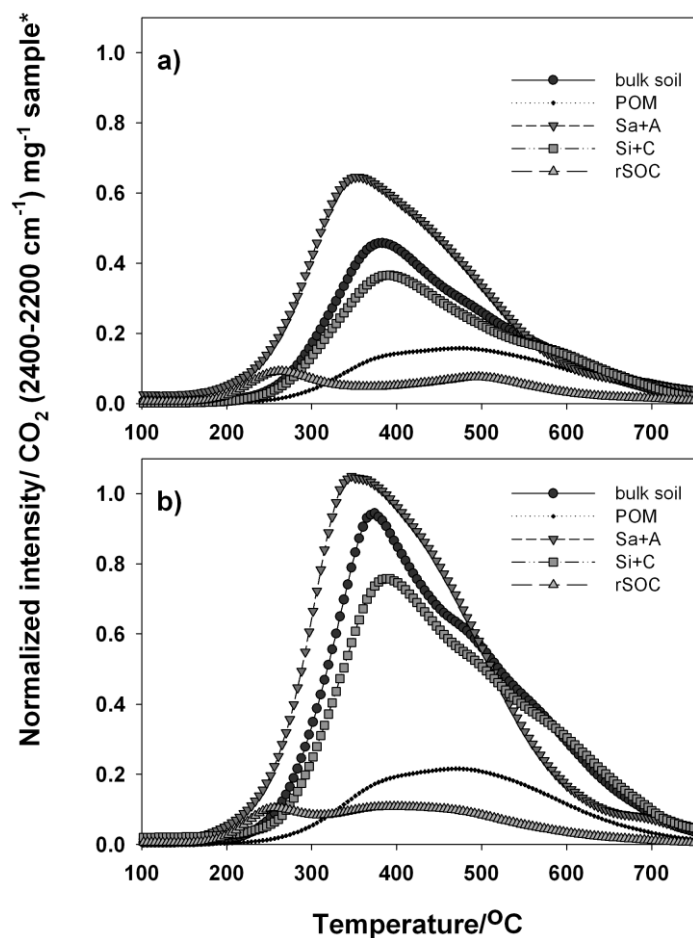
Figure 8. FTIR-EGA CO2 thermograms of bulk soil and fractions heating from 25 to 700°C at 68°C min-1 under synthetic air purge from the (a) Kraichgau and (b) Swabian Alb sites. Bulk soil <2 mm, POM-sand fraction < 1.8 g cm^{-3}: fraction diluted 1:50 with preheated ball-milled quartz sand for thermal analysis, Sa+A sand fraction > 1.8 g cm^{-3}, silt+clay < 63 μm, rSOC < 63 μm treated with hot sodium hypochlorite. * Normalized intensity is the integrated area of the CO_2 peak from 2400 to 2200 cm^{-1} normalized to weight of the sample.

3.4.4 In situ$_T$ DRIFTS

In situ$_T$ thermal DRIFTS measurements showed changing vibrational intensities which depended on the heating chamber temperature and the functional groups being studied. Certain organic functional group associated vibrations (i.e., aliphatic, carboxylate, aromatic) differed in their temperature sensitivity shown by specific temperature ranges of changing vibrational intensity (Figures 9-11). In the bulk soil samples, consistent changes were found for all three sites with regard to the C-H and -COO$^-$/C=C vibrations. The C-H vibrations

consistently decreased starting 220 to 250°C and accelerating rapidly around 350°C, while the -COO⁻/C=C vibrations began to decrease around 380°C with a maximum decline around 440°C. Between 300 to 370°C, there was a drastic decrease in C-H vibrations while at the same temperature range there was a slight increase in C=C/-COO vibrations and a stronger increase in C=C vibrations. At approximately 400°C, the C=C/-COO vibrations decreased and after 430°C the C=C vibrations decreased. Vibration intensities of wavenumbers below 1000 cm^{-1} were rather difficult to interpret due to the lack of clear trends, high variation from one scan to the next, and absorbance values of >2.5 A.U., especially at temperatures >500°C.
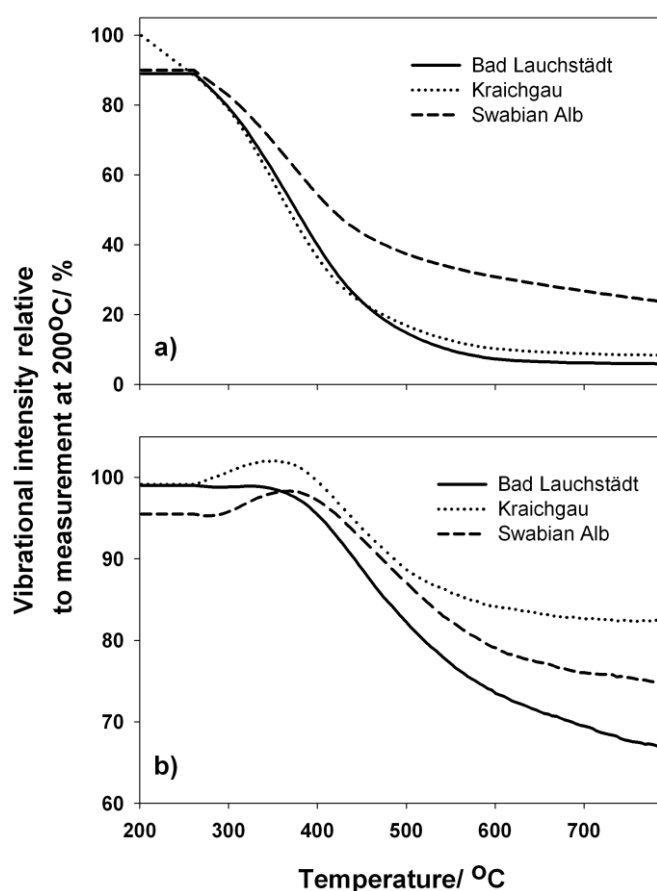
Figure 9. In situ$_T$ DRIFTS of bulk soil samples from Bad Lauchstädt, Kraichgau, and Swabian Alb of a) C-H (2930 cm^{-1}) vibrations and b) C=O/C=C (1620 cm^{-1}) vibrations.

Differences among the fractions via in situ$_T$ DRIFTS is illustrated in Figure 10 for the C-H and -COO⁻/C=C vibrational groups as an average of the Kraichgau and Swabian Alb samples. The behavior of the 2930 cm^{-1} peak is consistent among the fractions with the exception of

the rSOC fraction from the Kraichgau site. After an initial slight decrease between 200 to 250°C, the decrease in intensity of the 2930 cm^{-1} peak accelerated after 280°C with the decrease in intensity ending around 450°C. The POM fractions showed an increased in intensity after 550°C, until 700°C. The 1620 cm^{-1} peak was found to be relatively stable from 200 and 220°C and then increase in intensity from 280°C to approximately 360°C with some variation between fractions. The onset of decrease was around 380°C, the same as for the bulk soils.
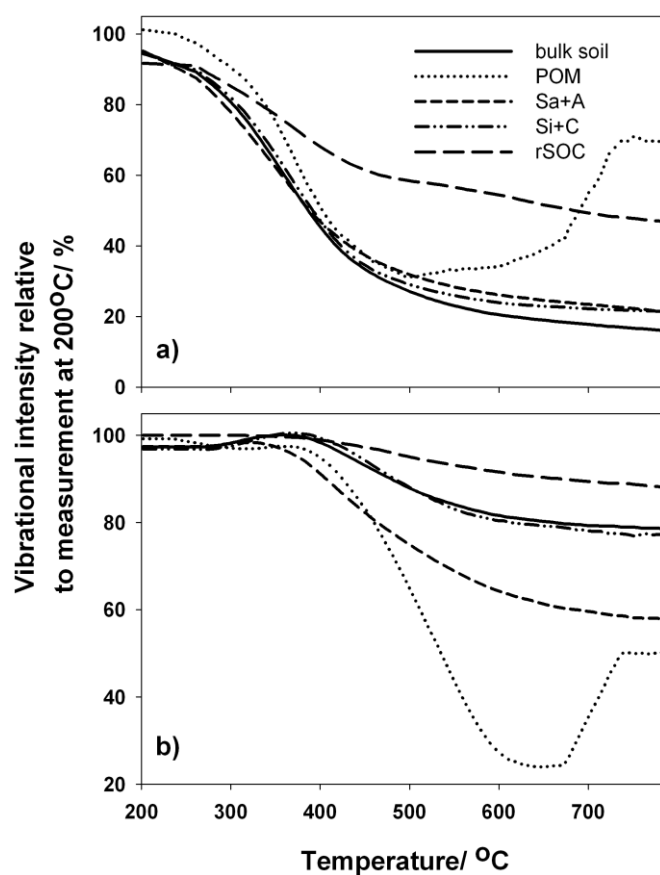
Figure 10. In situ$_T$ DRIFTS the bulk soil and fractions of a) C-H (2930 cm^{-1}) vibrations and b) C=O/C=C (1620 cm^{-1}) vibrations. Average of Kraichgau and Swabian Alb sites.

3.4.5 Peak fitting of FTIR-EGA CO_2 gas thermograms

With the temperatures of maximum change of different functional groups as measured by in situ$_T$ DRIFTS analyses as the first step for peak placement and determining the number of peaks followed by an examination of the shape of the evolved CO_2 thermogram, a peak fitting of the FTIR-EGA CO_2 gas profiles from bulk soils resulted in four different peaks or

"fitted components" (Figure 11). Component 1 corresponded to the decrease of the C-OH vibration and the C-H vibration. Component 2 was mainly the C-H vibration, while component 3 was –COO/C=C vibrations. Finally, component 4 corresponded to C=C vibration and also again C-OH vibrations. The main effect in the Bad Lauchstädt experiment was seen between the two FYM treatments compared to the two without FYM (Table 5). The temperature of the center of the first component was at 298 and 301°C for FYM+NPK and FYM, respectively, while at 293 and 292°C for NPK and CON, respectively. The relative contribution of fitted component 1 was similar between the treatments (3.3 to 4.2%). Larger differences were found with components 2 and 4 between the treatments. For example, component 2 was representative of 25 and 23% of the evolved CO_2 for the FYM+NPK and FYM treatments compared to 18.8 and 18.6% in the non-FYM treatments. The opposite trend was seen with component 4, and which was the largest pool, where the FYM treatments had a lower share (43.3 and 45.1%) compared to the non-FYM treatements (49.1 and 49.2%).

Table 5. Temperature and relative contribution to total evolved CO_2 of fitted peaks/components (± standard errors) from an iterative least squares approach of thermally evolved CO_2 from 25 to 700°C (68°C min^{-1} heating rate) as measured by FTIR-EGA. Peak temperatures of the components were derived from relative changes of vibrational intensity of organic functional groups by in situ$_T$ DRIFTS.

Site	Component 1		Component 2		Component 3		Component 4	
	Temp/°C	%[a]	Temp/ °C	%	Temp/°C	%	Temp/°C	%
BL FYM+NPK[2]	298 (2)	4.2 (0.1)	361 (2)	25.0 (1.8)	442 (2)	27.3 (1.1)	540 (3)	43.4 (0.7)
BL FYM	301 (3)	4.4 (0.3)	367 (3)	23.0 (0.7)	446 (3)	26.9 (0.8)	547 (6)	45.1 (1.1)
BL NPK	293 (1)	3.3 (0.0)	360 (0)	18.8 (0.4)	439 (1)	28.1 (0.7)	528 (5)	49.1 (1.0)
BL CON	292 (2)	3.7 (0.2)	357 (1)	18.6 (1.3)	435 (1)	28.6 (2.5)	527 (5)	49.2 (1.6)
Kraichgau	290 (2)	3.4 (0.2)	353 (1)	46.1 (0.6)	445 (1)	27.3 (0.4)	549 (2)	23.3 (0.6)
Swabian Alb	289 (2)	4.7 (0.3)	344 (1)	35.3 (1.0)	432 (2)	33.9 (0.7)	541 (2)	26.1 (0.9)

[a] Percent contribution of component to total evolved CO_2. [b] BL – Bad Lauchstädt experiment; FYM+NPK – farmyard manure and NPK fertilizer, FYM – farmyard manure, NPK – NPK fertilizer, CON-control.
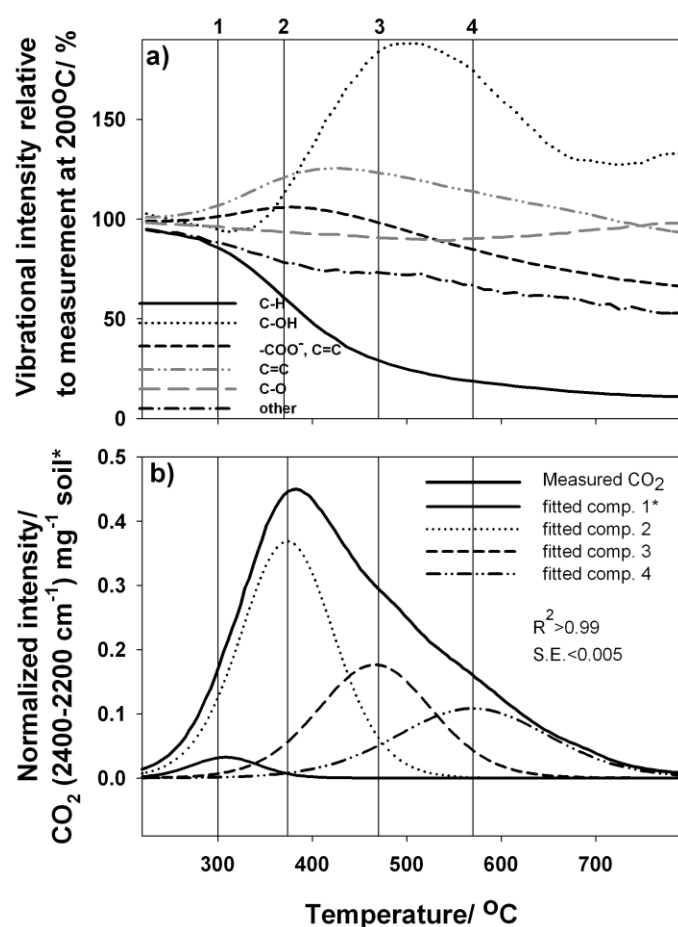
Figure 11. Summary of qualitative changes in organic functional groups during heating as a guide to fitting components to quantitative evolved gas anlaysis: (a) In situ$_T$ DRIFTS measurements of bulk soil from the Kraichgau site as a percentage of the vibrational intensity at 200°C to identify temperature of maximum change of functional groups. (**b**) Peak fitting with four components of evolved gas profile as measured by FTIR-EGA of bulk soil from Kraichgau site using previously identified temperatures of maximum change of functional groups. Statistics are from the goodness of fit of the four fitted components to the measured data. Reference lines 1 to 4 indicate the peak of the fitted components of the FTIR-EGA gas profile in **6b**. * Normalized intensity is the integrated area of the CO_2 peak from 2400 to 2200 cm^{-1} normalized to weight of the sample.

As with the measured CO_{2max}, the center of each fitted component was at a slightly lower temperature in the Swabian Alb compared to the Kraichgau soils. Component 1 had a peak center at almost the same temperature in both soils (i.e., Kraichgau, 290°C; Swabian Alb, 289°C), but there was a nearly 10°C difference between the two sites regarding the second

and third components. Component 2 in the Kraichgau had a peak center at 353°C compared to 344°C in the Swabian Alb, while that of component 3 was at 445°C in the Kraichgau compared to 432°C in the Swabian Alb. Again, a similar but smaller difference was found for component 4 with its center at 549°C in the Kraichgau soils compared to 541°C in the Swabian Alb soils. The largest contribution to the total CO_2 evolution was provided by component 2 (35-46%) for both soils. Component 1 was the smallest fraction, being slightly higher in Swabian Alb soils (4.7%) compared to Kraichgau (3.4%). Larger differences between sites were found for components 2 and 3 with 46 and 27.3% for the Kraichgau and 35 and 34% for the Swabian Alb. Lastly, component 4 was larger in the Swabian Alb at 26% compared to 23% in the Kraichgau.

3.5 Discussion

3.5.1 Thermal stability of organic matter of soils and fractions

Using the temperature of peak CO_2 evolution as one indicator of thermal stability, the results from the three contrasting soils indicated that there may be a large portion of stable OM present in the Bad Lauchstädt soils given the large proportion of a high temperature component and its associative thermal behavior with aromatic-like structures. These findings are in agreement with what others have found during previous analyses of soils from Bad Lauchstädt by DTA and TGA (Leifeld et al., 2006), as well as pyrolysis-field ionization mass spectrometry methods (Leinweber and Schulten, 1993). This thermal stability may be due to the presence of very stable humus compounds formed within a Chernozem or the presence of black C (Eckmeier et al., 2007). The effect of long-term manure application was evident in the FYM+NPK and FYM treatments of the Bad Lauchstädt soils with a relative enrichment of thermally labile substances as indicated by a lower temperature of CO_{2max}. It has been shown previously that in the control treatment relative to the FYM+NPK treatment there is a relative enrichment of C in the clay, heavy density fractions, and of aromatic functional groups in the bulk soil via specific DRIFTS peak areas at the expense of the more labile fractions (Demyan, et al., 2012).

Further evidence of a link between FTIR-EGA results and dynamic changes of SOM quality was shown by the changes in thermal stability after 490 day incubation, which confirmed that ongoing microbial decomposition altered the thermal CO_2 thermogram of the remaining SOM. Although, after a 490 day laboratory incubation experiment, the quality of lost C spanned the entire range of thermal stabilities, the maximum peak of C lost during incubation

was slightly lower than the non-incubated soil CO_{2max}. This indicated that the possible source of a large part of the respired C during incubation came from the Si+C fraction, which was also the largest fraction by weight in the bulk soil, and also had a similar peak temperature as compared with the bulk soil. While the effect of the 490 day incubation did shift the CO_{2max} to a slightly higher temperature, the C loss was not confined to lower temperatures (below 350°C), but was also evolved at higher temperatures indicating, some apparently labile compounds easily decomposable to microorganisms are quite thermally resistant, as shown for the pure substances. (Plante et al., 2009)1) also found that soils incubated for 588 days at 35°C have a higher thermal stability, as indicated by the temperature at which 50% of the mass loss or energy loss occurred measured via TG/DSC. Using the same 50% as threshold, but with evolved CO_2 via FTIR-EGA, our results showed an increase from 433 to 442°C after incubation. From these results, we could conclude that both CO_{2max} and 50% evolved C loss as measured by FTIR-EGA were indicators of the relative stability of SOM in these soils.

The thermal stability gradient of the SOM fractions did only partially follow their implied stabilities. There was a gradient in the mineral associated fractions tending from lower to higher thermal stability as shown by CO_{2max} in the order Sa+A < bulk soil < Si+C but was not statistically different. The non-mineral associated POM did not fit into this relationship, as it had a higher peak temperature compared to the Sa+A, Si+C and bulk soil. As was shown with the pure substances, a high thermal stability was also found for components within xylan and tannic acid. These components are present in the form of hemicelluloses and polyphenols/lignins in relatively undecomposed plant residues having lost their labile compounds prior to sampling, as being the case with the analysed POM having been sampled seven months after last residue inputs. Dell'Abate et al. (2000) confirmed such changes in thermal stability during decomposition via DSC and TG measurements of compost in a 56 day incubation observing an increase in the proportion of the higher temperature (450 °C) peak relative to the lower temperature peak (300 °C), indicating an enrichment in more thermally stabile humic substances. Others have also shown that there is a resistant POM fraction based on the size separation of >100 µm of <1.8 g cm^{-3} being more labile and 100 to 53 µm of <1.8 g cm^{-3} being more stable (Cadisch et al., 1996). However, in our case this comparison may not be straightforward, as it was shown in this study that dilution with quartz sand changed the CO_{2max} of POM. A similar observation was made by Rovira et al. (2000) who observed that mineral interferences in the change of thermal stability of pure residues

compared with residues mixed with red earth, even after subtracting the DSC curves of the unamended red earth from the DSC curves of the red earth mixed with residues.

Sodium hypochlorite (NaOCl) is normally used to efficiently remove OM from soils and clays without greatly altering the clay mineral structure (Mikutta et al., 2005). Our FTIR-EGA results indicated that the NaOCl treatment has greatly altered the nature of the Si+C fraction, as measured on the remaining residue (rSOC), so that a much less thermally stable peak evolved at relatively low temperature. This may have been the effect of the complete dispersion of any microaggregates and also exchange of organic substances from the clay minerals. It was surprising that this relatively thermally labile pool had survived oxidation by the NaOCl. That may have been due to the fact that NaOCl does not completely attack aliphatic C compounds (Sleutel et al., 2009), which has been shown to have a lower thermal stability as compared to aromatic C (Schulten and Leinweber, 1999) and seems to lend evidence that physiochemical protection is playing a role in influencing the thermal stability of the Si+C fraction. This was also shown qualitatively in our in $situ_T$ DRIFTS measurements of the bulk soils in which C-H vibrations decreased rather rapidly and at an earlier temperature compared to C=C vibrations. Due to the evident chemical alteration of the rSOC fraction by the NaOCl oxidation that has been used to isolate a supposedly very stable fraction, the thermal properties are greatly altered and this fraction may not be suitable for analysis by FTIR-EGA to infer biological stability.

3.5.2 Allocation of thermally evolved carbon

Using a novel approach of a peak fitting procedure, we were able to identify different "pools" of SOM with characteristic thermal signatures. This might lend evidence to link with functional properties such as used in SOM decomposition models. Through the peak fitting procedure, evolved C, as measured by the FTIR-EGA, was allocated into several peaks or fitted components. Additional support for obtained pool allocation results were that the centroid or maximum of each fitted peak was consistent among the three sites and that these peaks coincided with different maximum or near maximum decreases in vibrational absorbances. As measured by in $situ_T$ DRIFTS, there was an initial slight decline in C-O, C-OH, and C-H vibrations, then a strong decrease in C-H and continued decline in C-O vibrations, and finally a decline in C=C, lending evidence to different organic compounds being decomposed at different temperatures. Three main trends were seen in the changing

vibrational intensities over the heating experiment. First, at relatively low temperatures (200 to 300°C) a decrease in intensity was found for the C-O and C-H vibrations. This was evidence for the breakdown of aliphatic groups and also carbohydrates. Secondly, at slightly higher temperatures the rapid decrease of C-H groups indicated the cracking of longer chained aliphatic compounds with an almost simultaneous increase in both C=O/C=C and C=C intensities. This clearly indicates the formation of secondary products such as more condensed aromatic structures, which was previously suggested during the heating under an inert atmosphere of brown coal (Murakami et al., 1997)(Murakami et al., 1997). Thirdly, there was a final decrease of both the C=O/C=C and C=C peaks at >400°C. The lower temperature at which the C=O/C=C peak decreased relative to the C=C peak showed decarboxylation happening before the breakdown of the aromatic structures, which was also found by (Lu et al., 1997) via FTIR emission spectroscopy of humic substances. (Kiem et al., 2000)) found a similar stability or depletion sequence in comparisons of long-term managed fallow treatments compared with fertilized treatments using ^{13}C nuclear magnetic resonance and carbohydrate analysis. In the case of the increase in the "C-O" group at 2226 cm^{-1}, this suggested the formation of nitriles as was earlier found in humic acid salts (Woelki and Salzer, 1995)as C≡N vibrations can also be present in this mid-infrared range. Additionally, via pyrolyis field ionization mass spectroscopy (Py-FIMS), (Sleutel et al., 2011)) shown that at <400°C combustion products are relatively enriched in lipids, carbohydrates, and peptides compared to >400°C where there was a greater proportion of alkyl-aromatics. Other previous work on Py-FIMS has led to the general molecular framework of three thermal classes: i) unbound undecomposed plant fragments relatively rich in aliphatics; ii) a thermally labile fraction containing N-containing compounds and carbohydrates associated with humified OM; and iii) thermally stable mineral bound OM (Schulten & Leinweber 1999). In the soils of the Bad Lauchstädt experiment, Mertz et al. (2005) found in the coarse clay fractions a general increase of thermal stability of pyrolyis compounds after 98 years of fertilizer deprivation compared to the full fertilized treatment, which would complement our results of increased thermal stability of the bulk soil in the CON treatment relative to the FYM+NPK treatment. In our experiment, the peak fitting results also showed an enrichment in the most thermally stable compounds in the non-FYM treatments as compared to the FYM-treatments.

In the current study, the larger percentage of fitted component 1 in the Swabian Alb compared to the Kraichgau may be related to the slightly larger portion of labile C in the Swabian Alb site as measured by extractible C and microbial biomass. Additionally, the

larger percentage in the most thermally stable component, the 4th component, in the Swabian Alb may have been a result of the higher clay content of the Swabian Alb compared to the Kraichgau soils. Further vibrational functional groups may be of interest and related to OM, but in our study it was found that the absorbance values of the wavenumbers <1000 cm^{-1}, where there is a strong influence of mineral vibrations (Nguyen et al., 1991) increased during the entire heating procedure or were highly variable from one scan to the next, complicating interpretation. As the studied vibrational frequencies were selected based on the fact that they represent mostly organic and not mineral functional groups (Demyan et al., 2012), any changes in absorbance intensity due to mineralogical changes especially >540°C should have been avoided. This combined use of both in situ$_T$ DRIFTS and FTIR-EGA information to assign evolved CO_2 of different temperature intervals to different qualities of organic matter is a promising and new step towards application of this method.

3.5.3 Specific considerations using FTIR-EGA thermal approaches

Several conditions must be met to obtain satisfactory FTIR-EGA results. First, a major drawback in oxidative thermal combustion reactions is the potential of formation of secondary reactions, e.g., charring, during the heating process. It is thus important to set up experimental conditions with minimal interference from such secondary reactions. Our tests showed that low heating rates favored formation of secondary reactions. We therefore identified 68 °C min^{-1} as the optimal heating rate for soils for our system to balance the need for reproducible heating rate and also minimizing secondary reactions. This heating rate is similar to the 70°C min^{-1} used in pyrolysis-field ionization mass spectrometry (Py-FIMS) experiments by (Schulten and Leinweber, 1993). This may also have led to the occurrence of only one CO_2 peak for the bulk soils as opposed to traditional TGA or DSC profiles which even for bulk soils usually show two distinct peaks of OM associated weight loss (Dell'Abate et al., 2003). A possible reason for the lack of additional peaks is that we used a much higher heating rate (68°C min^{-1}) compared to other studies (Siewert, 2004); (Plante et al., 2009)1; (Leinweber et al., 1992), which used a rate of 10-30 °C min^{-1} and therefore may be subject to more charring effects.

Secondly, the amount of potential CO_2 evolution will determine the experimental conditions used during analysis. In our case a C content of >2.5 mg in the sample cup resulted in absorbances of >2.5 A.U. which were no longer linear in respect to C concentration.

Thirdly, the combustion conditions should be similar among the tested materials. This is particularly true for material with high OC content. In the case of POM and the organic standards (e.g., glucose, xylan, tannic acid), the CO_2 evolution profiles were changed by diluting a sample with pre-heated quartz sand. This may have been the consequence of increasing the aeration within the sample and also changing its heat conductivity compared to a pure sample. It has been suggested that secondary reactions such as charring are present during combustion of cellulose, hemicellulose, and tannic acid (Court et al., 2009). As these secondary reactions do not necessarily reflect the thermal stability of the compound but are rather a result of the heating procedure, care must be taken in interpreting the thermal characteristics of high OC content substances. For the current experimental setup, we propose that for non-mineral samples (POM, organic soils, plant samples) a dilution with inert material (quartz sand) is beneficial in replicating the thermal conditions of mineral soils and fractions. This will reduce charring and standardize the aeration and thermal conductivity of the sample, which is important at high heating rates.

3.6 Conclusion

Linking a high temperature thermal reaction chamber with a FTIR gas cell was shown to be able to quantify evolved gas (CO_2) in order to characterize SOM of different soils and SOM fractions. The conditions for an optimized FTIR-EGA procedure were 1) the use of a standard with well known thermal decomposition reaction (i.e., $NaHCO_3$) to quantify mid-infrared absorbance units to CO_2 production and to identify the optimal purge and scan rates, which for our 136 mL gas cell were 15 L hr^{-1} synthetic air purge with a scan every 4 s; 2) a rapid heating rate (68°C min^{-1}) to minimize char formation, and 3) a dilution of high C content samples, especially POM and plant samples, for similar heating conditions in the heating chamber as compared to mineral soils. The amount and shape of evolved CO_2 under an oxidizing atmosphere evolved from 25 to 700°C depending on the chemical composition (e.g., simple C-C bonding of glucose compared to complex ring structure of tannic acid), type of soil (e.g., Chernozem versus Cambisol) and also fraction of soil investigated (e.g., silt and clay fraction versus bulk soil versus POM). Temperature-dependent changes in the intensity of mid-infrared molecular vibrations also indicated different OM compounds decomposing at different temperatures.

In a novel approach, a peak fitting procedure based on changes of molecular vibrations derived from situ$_T$ DRIFTS measurements was used to allocate C evolved at different

temperature into separate C pools of inferred stability. The in $situ_T$ DRIFTS results gave evidence of different C components (i.e., C-OH = unknown C vibration < C-H < -COO and C=C < C=C) being evolved at different times during the 220 to 700°C, which justified and lent a quality aspect to the curve fitting procedure for the FTIR-EGA evolved CO_2. A combination of the FTIR-EGA and in $situ_T$ DRIFTS methods will provide an improved approach for SOM characterization. Even though analysis of soils with different clay mineralogies and land use types is needed to test the applicability of this method to different soils and land uses, the current results prove that the combined use of FTIR-EGA and in $situ_T$ DRIFTS provides a good theoretical molecular basis and a major step forward for the fitting of different components to the evolved gas thermogram.

Acknowledgements

References

Anderson, J.U. 1963. An improved pretreatment for mineralogical analyses of samples containing organic matter. *Clay and Clay Minerals,* **10,** 380-388.

Anderson, J.P.E. & Domsch, K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry,* **10,** 215-221.

Blumstein, A. 1965. Polymerization of adsorbed monolayers. II. Thermal degradation of the inserted polymer. *Journal of Polymer Science Part A: General Papers,* **3,** 2665-2672.

Cadisch, G., Imhof, H., Urquiaga, S., Boddey, R.M. & Giller, K.E. 1996. Carbon turnover (δ13C) and nitrogen mineralization potential of particulate light soil organic matter after rainforest clearing. *Soil Biology and Biochemistry,* **28,** 1555-1567.

Conzen, J. 2003. *Multivariate Calibration. A practical guide for developing methods in the quantitative analytical chemistry.* Bruker Optik GmbH, Germany.

Court, R.W. & Sephton, M.A. 2009. Quantitative flash pyrolysis Fourier transform infrared spectroscopy of organic materials. *Analytica Chimica Acta,* **639,** 62-66.

Dell'Abate, M.T., Benedetti, A. & Brookes, P.C. 2003. Hyphenated techniques of thermal analysis for characterisation of soil humic substances. *Journal of Separation Science,* **26,** 433-440.

Dorodnikov, M., Fangmeier, A., Giesemann, A., Weigel, H.-., Stahr, K. & Kuzyakov, Y. 2008. Thermal stability of soil organic matter pools and their turnover times calculated by δ13C under elevated CO2 and two levels of N fertilisation. *Isotopes in environmental and health studies,* **44,** 365-376.

Dorodnikov, M., Fangmeier, A. & Kuzyakov, Y. 2007. Thermal stability of soil organic matter pools and their δ13C values after C3–C4 vegetation change. *Soil Biology and Biochemistry,* **39,** 1173-1180.

Dreibrodt, S., Kleber, M. & Jahn, R. 2002. Das mineralinventar der versuchsfla¨che "statischer dauerdu¨ngungsversuch v120, bad lauchsta¨dt". *Archives of Agronomy and Soil Science,* **48,** 227-240.

Eckmeier, E., Gerlach, R., Gehrt, E. & Schmidt, M.W.I. 2007. Pedogenesis of Chernozems in Central Europe — A review. *Geoderma,* **139,** 288-299.

Fernández, J.M., Peltre, C., Craine, J.M. & Plante, A.F. 2012. Improved characterization of soil organic matter by thermal analysis using CO 2/H 2O evolved gas analysis. *Environmental Science and Technology,* **46,** 8921-8927.

Fernández, J., Plante, A., Leifeld, J. & Rasmussen, C. 2011. Methodological considerations for using thermal analysis in the characterization of soil organic matter. *Journal of Thermal Analysis and Calorimetry,* **104,** 389-398.

Gaál, F., Szöllosy, I., Arnold, M. & Paulik, F. 1994. Determination of the organic matter, metal carbonate and mobile water in soils simultaneous TG, DTG, DTA and EGA techniques. *Journal of Thermal Analysis,* **42,** 1007-1016.

Harris, D., Horwáth, W.R. & Van Kessel, C. 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. *Soil Science Society of America Journal,* **65,** 1853-1856.

Haselsteiner, T., Erbel, C., Kunze, C. & Spliethoff, H. 2011. An in situ optical gas phase analysis approach for TGA: Its assessment and application. *Applied Energy,* **88,** 5200-5207.

IUSS Working Group WRB. 2007. *World Reference Base for Soil Resources 2006, first update 2007. World Soil Resources Reports.* Rep. 103. FAO, Rome.

Janković, B. 2009. Kinetic analysis of isothermal decomposition process of sodium bicarbonate using the weibull probability function-estimation of density distribution functions of the apparent activation energies. *Metallurgical and Materials Transactions B: Process Metallurgy and Materials Processing Science,* **40,** 712-726.

Kiem, R., Knicker, H., Körschens, M. & Kögel-Knabner, I. 2000. Refractory organic carbon in C-depleted arable soils, as studied by ^{13}C NMR spectroscopy and carbohydrate analysis. *Organic Geochemistry,* **31,** 655-668.

Körschens, M., Stegemann, K., Pfefferkorn, A., Weise, V. & Müller, A. 1994. *Der Statische Düngungsversuch Bad Lauchstädt nach 90 Jahren. Einfluß der Düngung auf Boden, Pflanze und Umwelt.* B.G. Teubner Verlagsgesellschaft, Stuttgart.

Leifeld, J., Franko, U. & Schulz, E. 2006. Thermal stability responses of soil organic matter to long-term fertilization practices. *Biogeosciences,* **3,** 371-374.

Leinweber, P. & Schulten, H.-. 1993. Dynamics of soil organic matter studied by pyrolysis—field ionization mass spectrometry. *Journal of Analytical and Applied Pyrolysis,* **25,** 123-136.

Leinweber, P., Schulten, H.-. & Horte, C. 1992. Differential thermal analysis, thermogravimetry and pyrolysis-field ionisation mass spectrometry of soil organic matter in particle-size fractions and bulk soil samples. *Thermochimica Acta,* **194,** 175-187.

Lopez-Capel, E., Sohi, S.P., Gaunt, J.L. & Manning, D.A.C. 2005. Use of thermogravimetry-differential scanning calorimetry to characterize modelable soil organic matter fractions. *Soil Science Society of America Journal,* **69,** 136-140.

Lu, X.Q., Vassallo, A.M. & Johnson, W.D. 1997. Thermal stability of humic substances and their metal forms: An investigation using FTIR emission spectroscopy. *Journal of Analytical and Applied Pyrolysis,* **43,** 103-114.

Mikutta, R., Kleber, M., Kaiser, K. & Jahn, R. 2005. Review: Organic Matter Removal from Soils using Hydrogen Peroxide, Sodium Hypochlorite, and Disodium Peroxodisulfate. *Soil Science Society of America Journal,* **69,** 120-135.

Mittleman, M. 1990. Quantitative TG/IR. *Thermochimica Acta,* **166,** 301-308.

Murakami, K., Shirato, H. & Nishiyama, Y. 1997. In situ infrared spectroscopic study of the effects of exchanged cations on thermal decomposition of a brown coal. *Fuel,* **76,** 655-661.

Nguyen, T.T., Janik, L.J. & Raupach, M. 1991. Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy in soil studies. *Australian Journal of Soil Research,* **29,** 49-67.

Pansu, M. and J. Gautheyrou. 2006. *Handbook of Soil Analysis. Mineralogical, Organic and Inorganic Methods.* Springer Verlag Berlin Heidelberg, The Netherlands.

Plante, A.F., Fernández, J.M. & Leifeld, J. 2009. Application of thermal analysis techniques in soil science. *Geoderma,* **153,** 1-10.

Schlichting, E., Blume, H.P. & Stahr, K. 1995. *Bodenkundliches Praktikum.* Blackwell Wissenschafts-Verlag, Berlin, Vienna.

Schulten, H.-. & Leinweber, P. 1993. Pyrolysis-field ionization mass spectrometry of agricultural soils and humic substances: Effect of cropping systems and influence of the mineral matrix. *Plant and Soil,* **151,** 77-90.

Siewert, C. 2004. Rapid screening of soil properties using thermogravimetry. *Soil Science Society of America Journal,* **68,** 1656-1661.

Sleutel, S., Leinweber, P., Van Ranst, E., Kader, M.A. & Jegajeevagan, K. 2011. Organic matter in clay density fractions from sandy cropland soils with differing land-use history. *Soil Science Society of America Journal,* **75,** 521-532.

Sleutel, S., Leinweber, P., Ara Begum, S., Kader, M.A. & De Neve, S. 2009. Shifts in soil organic matter composition following treatment with sodium hypochlorite and hydrofluoric acid. *Geoderma,* **149,** 257-266.

Toops, T.J., Smith, D.B. & Partridge, W.P. 2005. Quantification of the in situ DRIFT spectra of Pt/K/γ-A12O3 NOx adsorber catalysts. *Applied Catalysis B: Environmental,* **58,** 245-254.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E. & Marschner, B. 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. *Soil Biology and Biochemistry,* **39,** 2183-2207.

von Lützow, M., Kögel-Knabner, I., Ludwig, B., Matzner, E., Flessa, H., Ekschmitt, K., Guggenberger, G., Marschner, B. & Kalbitz, K. 2008. Stabilization mechanisms of organic matter in four temperate soils: Development and application of a conceptual model. *Journal of Plant Nutrition and Soil Science,* **171,** 111-124.

Woelki, G. & Salzer, R. 1995. Thermal investigations of the structure of two humic acid salts by in situ FTIR spectroscopy. *Fresenius' journal of analytical chemistry,* **352,** 529-531.

Zimmermann, M., Leifeld, J. & Fuhrer, J. 2007a. Quantifying soil organic carbon fractions by infrared-spectroscopy. *Soil Biology and Biochemistry,* **39,** 224-231.

Zimmermann, M., Leifeld, J., Abiven, S., Schmidt, M.W.I. & Fuhrer, J. 2007b. Sodium hypochlorite separates an older soil organic matter fraction than acid hydrolysis. *Geoderma,* **139,** 171-179.

4 Implications of spectroscopic and thermo-spectroscopic approaches for pool parameterization of a SOM model

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4.1 Abstract

New methods are needed to parameterize various pools (particularly intermediate turnover pools which, thus far, are often conceptual only) of multi-compartment soil organic matter (SOM) models. The objectives of this study were to examine different SOM pool initialization approaches to model short and medium-term (decadal) carbon dynamics at two study sites in Southwest Germany (Kraichgau and Swabian Alb) using the Land Use Change Impact Assessment tool with a SOM sub-model based on Century (LUCIA-Century). Measurements were done in two representative cropped fields within each study site. Three plots were allocated in each field with subplot measurements done under the crop and in an adjacent bare fallow subplot to follow the decomposition of SOM without any input of fresh plant material or manure application. Total organic carbon (TOC) and soil surface carbon dioxide (CO_2) fluxes were used to test the short-term accuracy of the different pool initialization methods. Additionally, a 20 year simulation was used to explore the sensitivity on SOC of the different initializations methods. Besides a *Default* setup (1), model parameterization was based on (2) a fitting to a long-term equilibrium (long-term 140 year model run; *Equilibrium*), and measured fractions derived from (3) diffuse reflectance Fourier transform mid-infrared spectroscopy (*DRIFTS*), (4) Fourier transform infrared-evolved gas analysis coupled to in situ thermal DRIFTS (*EGA-1* and *EGA-2* initializations) and (5) classical SOM fractionation (*Fractions*). Model parameterization included the allocation of initial sizes of the active, slow, and passive pools for the Century SOM module. Results showed that short-term accuracy of both soil organic carbon (SOC) contents and soil CO_2 fluxes were only slightly affected by initial parameterization. Generally, measured CO_2 fluxes were underestimated earlier in the season (May-June) and better modeled later in the season (July-November). Model efficiency ranged from -4.28 to 0.35 for CO_2 fluxes. The *Fractions*, *DRIFTS*, and *EGA-1* methods were the most accurate methods (shown by root mean square error, modeling efficiency, and model bias) for predicting soil CO_2 fluxes during the short-term simulation (2009-2010). Modeled results of SOC were within ± one standard error of the measured values at the end of the measurement period at both sites for the bare fallow plots and the vegetated Swabian Alb plots, but not for the vegetated Kraichgau plots. Over a 20-year simulation, however, there were large predicted ranges in SOC (6 Mg ha^{-1} and 14 Mg ha^{-1} in the Kraichgau and Swabian Alb sites, respectively) between the different parameterizations. This showed that correct initialization of SOM pools, while not affecting

SOC levels much over one or two growing seasons, had large impacts for the medium-term simulations. This was controlled by the percentage allocated to the intermediate turnover pool which varied from 35 to 75%. *DRIFTS* and *EGA-1* were able to give a reasonable estimate of the intermediate SOM pool with relatively little sample preparation and analysis time.

4.2 Introduction

Soil organic matter (SOM) modeling has increasingly become an important tool to study SOM dynamics as responding to different agricultural management (Grant, 1997), land use change (Cerri et al., 2004), changing climate (Heinemeyer et al., 2010), and for calculating carbon (C) credits (Yadav et al., 2009). Agricultural models such as Roth-C (Jenkinson and Coleman, 1994; Coleman et al., 1997), Daisy (Hansen et al., 1991) and Century (Parton et al., 1987) include a compartmental SOM sub model with two or more pools of varying size, turnover rates, and C to nitrogen (N) ratios. As most SOM models are primarily C driven, emphasis has been given to the parameterization of soil organic carbon (SOC) pools with mostly fixed C:N ratios. Several approaches have been used to initialize or parameterize these SOC pools. This is particularly challenging for purely conceptual pools of intermediate turnover which do not have any measureable counterpart. Initial approaches included changing SOC pool sizes iteratively and then running a 10-30 year simulation until the pool sizes better fitted measured SOC data for the start of the experiment (Kelly et al., 1997). Alternatively, long-term incubations have been used for determining the size of both the active and intermediate pools (Collins et al., 2000).

A commonly used approach to initialize SOM pools is to simulate past climate conditions, management and residue input to the soil (Bruun and Jensen, 2002) often seeking equilibrium conditions under a set environment. Further approaches try to identify methods which allow the division of SOM into several isolated fractions or pools that have a certain implied stability, e.g. soil fractionation based on particle size, density, chemical methods or a combination of them (Skjemstad et al., 2004; Zimmermann et al., 2007c). Isotopic tracers have also been applied to identify fluxes of organic C to identify SOM pools and their turnover, e.g. manure labeled with [14]C (Tatzber et al., 2009) or "natural" tracers such as [13]C:[12]C ratios associated with the changing from a C3 to a C4 crop (Ludwig et al., 2003) or the flux of ^{14}C from thermonuclear bomb testing during the middle part of the 20th Century (O'brien and Stout, 1978). Transfer functions including SOC and clay content have been used

to initialize pools in Roth-C (Weihermüller et al., 2013). Finally, different statistical fitting or optimization approaches have been found useful in initializing SOM pools. These aim to fit the model outputs to one or more measured outputs (e.g. SOC) while adjusting several model parameters to optimize the fit and have included the Bayesian (Yeluripati et al., 2009), or Gauss-Marquardt-Levenberg estimation technique (Liu et al., 2008), and Monte Carlo simulations (Stamati et al., 2013).

Drawbacks of the previously mentioned methods range from improper assumptions to difficulty in implementing analytical methods in all situations. The actual lack of equilibrium conditions under changing agricultural land use has been cited (Wutzler and Reichstein, 2007), which has been shown under long-term experiments in the case of the Askov experiment in Denmark with still declining SOM (Bruun et al., 2003) or when prior land use is not well known. The influence in the slow SOM pool may still be responding theoretically up to 100 years after management change (Bruun and Jensen, 2002), while it has been pointed out that the uncertainty derived from not knowing the initial starting conditions has rarely been accounted for (Juston et al., 2010; Lardy et al., 2011; Stamati et al., 2013). Additionally, individual soil fractions may not be related to a single homogenous pool (von Lützow et al., 2007), a precondition for most common SOC models, or that C3 to C4 vegetation changes are not always present at a site. As the effects from thermonuclear testing are decreasing in the atmosphere, the impact of this anthropogenic ^{14}C tracer source is lessening and thus plant inputs to the soil will have a ^{14}C value closer to pre-bomb SOM (Rutberg et al., 1996). Incubation methods can be influenced by the method used and also it is not always clear how long the experiment must be run in order to make a separation between the active and slow pools. Lastly isotopic labeling of plants or plant residues may not be applicable for field experiments, as too costly or difficult to implement on a field scale basis. The statistical methods (e.g. Bayesian, Monte Carlo) are adequate at modifying parameters for optimal model fit, but may ignore the underlying model architecture or mechanisms while arriving at a best fit. Alternatively, SOM models can be designed around measurable pools but as pools in reality are not uniform this still needs further investigation.

The fore mentioned drawbacks make the search for new methods or approaches better suited for SOM pool identification enticing. Different spectroscopic and thermal analytical methods have previously shown promising results. For example, several C-compound specific mid-infrared peaks have been shown to be indicative of SOM quality, as well as allowing

monitoring of the depletion of labile substrates in different soil fractions over time (Demyan et al., 2012) and thus may have the potential to be used as a model pool initialization approach. Mid-infrared spectroscopy has been used both qualitatively and quantitatively to estimate or measure different SOM fractions (Zimmermann et al., 2007b). Mid-infrared peak intensity has been shown to change over time in response to decomposition (Spaccini et al., 2001), different agricultural management (Capriel, 1997), and soil formation (Egli et al., 2010). Additionally, thermal analyses have been used to isolate SOM fractions of different implied stability (Manning et al., 2005). If these methods can be further developed they could represent a rapid and cost effective method for parameterizing SOM models. Demyan et al. (2013) developed a novel approach to deconvolute thermal oxidation spectra into different meaningful fractions by combining thermal evolved gas analysis (FTIR-EGA) with in situ monitoring of diffuse reflectance data (in situ$_T$ DRIFTS). To our knowledge, no work has focused on using either SOM molecular vibrational characteristics (DRIFTS) or coupled FTIR-EGA with in situ$_T$ DRIFTS for direct SOM model pool parameterization.

To date, no universal method exists in parameterizing conceptual SOM models and few efforts have been made to compare the effectiveness or impact on the long-term (20 or more years) of different SOM parameterization approaches. Thus, the goal of this research was to investigate the impact of different analytical approaches to parameterize SOM models with defined pools with constant C:N ratios. The approach included the use of different methods to obtain measureable SOM fractions (i.e. size/density fractionation, spectroscopic, thermally derived) as model pools and to better understand the potentials and implications of using these different measureable SOM qualities for parameterization of a widely used SOM model (Century). The specific objectives of this study were 1.) to compare different physical/chemical soil fractionation, spectroscopic and thermal analytical approaches for pool parameterization to model short-term C dynamics at two arable sites in Southwest Germany on contrasting soils by the Century SOM model implemented in the Land Use Change Impact Assessment (Century-LUCIA, (Marohn and Cadisch, 2011)) tool and 2.) identify the specific effects of different model pool distributions on the sensitivity of medium-term (20 year) simulations.

4.3 Materials and methods

4.3.1 Site characteristics

The first site (48° 55.7' N, 8° 42.2' E) was in the Kraichgau area (Southwest Germany) which is characterized by loess derived soils. The second site (48° 31.7' N, 9° 46.2' E) was in the Swabian Alb region. The Swabian Alb soils were formed from upper Jurassic limestone. The Kraichgau site has an annual precipitation of 780 mm yr^{-1} and annual average temperature of 9.1°C (Pforzheim-Eutingen Station (DWD, 2013)) and the Swabian Alb 962 mm yr^{-1} of precipitation and average temperature of 6.7°C (Merklingen Station (DWD, 2013)). Both sites have a temperate climate (Cfb, Kottek et al., 2006). A more detailed description of the study sites can be found in Wizemann et al. (submitted). Soils at the two field sites have been under arable land use since at least the late Middle Ages (farmers' personal communication). Typical crops in rotation have consisted of cereals (e.g. winter wheat, oats, barley), and more recently silage maize, and oilseed rape.

In both the Kraichgau and Swabian Alb sites two farmers' fields were selected as being representative of the respective study areas and three plots were designated in each field with a subplot sampled in the vegetation, while adjacent to these vegetated plots, bare fallow plots were established during spring of 2009 in order to study SOM dynamics without residue inputs and the relationship of soil carbon dioxide (CO_2) fluxes between root free soil and soil with plants. Plots were located in representative locations in each field, with each subplot measuring 25 m^2. Planting, harvesting, and fertilizer application dates are listed in Table 1.

4.3.2 Soil sampling

Soils were sampled in May, 2009 and November, 2010. Soil samples were taken with a soil corer at three different depths, 0-30, 30-60, and 60-90 cm. Each sample was a composite of 6-8 sampling points within each subplot. Visible plant residues and roots were removed, and the soil gently crushed and mixed by hand in the field and transported to the laboratory in coolers and kept at 4°C. Soil samples for mineral nitrogen (N_{min}) and microbial biomass were extracted the next day, while the remaining soil was dried for further analysis. Samples from May 2009 were used for physical and chemical characterization, and were used for model initialization.

Table 1. Management and crop rotations during 2008-2010.

Crop[a]	Planting	Harvest	Fertilizer date, type[b], amount/ kg N ha^{-1}	Manure/ kg ha^{-1}
			Kraichgau	
ww	01.Nov.08	06.Aug.09	19.Mar.09, KA 61; 7.Apr.09, Urea 122; 6.Jun.09, KA 49	none
mustard silage maize	28.Aug.09 17 Apr.10	14.Oct.10	15.Apr.10, Urea 164; 17.Apr.10, DAP 29	16.Apr.09 1600 Biogas slurry
			Swabian Alb	
ww	13.Oct.08	20.Aug.09	19.Mar.09 & 07.Apr.09, Urea 115; 5.Jun.09, KAS 24	none
wr	30.Sep.09	05.Sep.10	24.Aug.10, UAN 46; 19.Sept.10, 13/10/18 26; 9.Apr.10, Piamon 66	none

[a]-catch crop, ww-winter wheat, wr-winter oilseed rape. Crop varieties: Kraichgau ww- *Cubus*, silage maize- *Agro-Gas*, Swabian Alb: ww-*Skalmeje*, wr-*Visby*. [b]- DAP-diammonium phosphate, KA-calcium ammonium nitrate, KAS-calcium ammonium sulfate, UAN-urea and ammonium nitrate, Piamon-32% nitrogen and 12% Sulfur.

4.3.3 Soil physical and chemical parameters

Soil bulk density was determined by the intact core method, and pH in a 1:2 (w/v) 0.01 M CaCl$_2$ solution. Soil texture was determined by laser defractometer (LS 200 Series, Beckman Coulter GmbH, Krefeld, Germany). Exchangeable potassium was determined by the BaCl$_2$-TEA replacement method (Gillman and Sumpter, 1986) and available phosphorous by the calcium lactate/acetate method (CAL-P) (Schüller, 1969).

4.3.4 Physical/chemical SOM fractionation

Five different SOM fractions were isolated using the SOM fractionation method of (Zimmermann et al., 2007b). Briefly, 30 grams of <2 mm air dried soil was made up to 100 ml with deionized water and macroaggregates dispersed by sonfication (30 J s^{-1}). The water and soil passing a 63 µm sieve, was then centrifuged at 3000 x g for 15 min and the pellet dried at 40°C representing silt and clay (Si+C), while a subsample of the clear supernatant was taken for dissolved organic carbon (DOC) analysis (Multi N/C analyser, Analytik Jena, Jena, Germany). The >63 µm fraction remaining on the sieve was subject to density separation by sodium polytungstate (SPT) solution of 1.8 g cm^{-3}. The <1.8 g cm^{-3} was the particulate organic matter (POM), while the >1.8 g cm^{-3} was the sand and stable aggregate fraction (S+A). A subsample of the Si+C fraction was oxidized with 6% sodium hypochlorite (NaOCl) for 15 min at 95°C, centrifuged at 200 x g for 8 min and repeated twice to isolate a chemically resistant organic matter fraction (rSOC).

Carbonate content of bulk soils was measured by the gasometric Scheibler method (Schlichting et al., 1995), while carbonates in the SOM fractions were destroyed by fumigation with hydrochloric acid (Harris et al., 2001) prior to total carbon (TC) determination. Dry combustion (Vario-EL III elemental analyser, Elementar, Hanau, Germany) was used to measure TC of the bulk soil and total organic carbon (TOC) of the SOM fractions.

4.3.5 MIRS-DRIFTS method

Mid-infrared spectroscopy measurements via the diffuse reflectance method (DRIFT-MIRS) were performed on dried, ball milled soil samples with a Bruker Tensor 27 (Bruker Optik GmbH, Ettlingen, Germany) mid-infrared spectrometer equipped with a Praying Mantis diffuse reflectance chamber (Harrick Scientific Products, Pleasantville, NY, USA). Wavenumbers from 4000 to 600 cm^{-1} with a 4 cm^{-1} resolution were scanned with 16 co-

added scans combined for one analysis with three analytical replicates averaged per sample. Specific peaks were analyzed according to Demyan et al. (2012) with integrations with a local base line performed from 3010 to 2800 cm^{-1} (2930 cm^{-1} peak assigned to aliphatic C-H vibrations (Baes and Bloom, 1989)) and 1660 to 1580 cm^{-1} (1620 cm^{-1} peak assigned to COO$^-$ and C=C vibrations (Baes and Bloom, 1989)). Then these relative peak areas at 2930 cm^{-1} and 1620 cm^{-1} were taken as the size of active (C_a) and slow (C_s) pools of the Century model, respectively, while the passive pool (C_p) was calculated as difference ($C_p = 100 - C_a - C_s$). Further explanation is given below in the SOM pool parameterization section.

4.3.6 FTIR-EGA and in situ$_T$ DRIFTS methods

For measuring thermally evolved gases during oxidative combustion of SOM, a high temperature heating system was utilized in conjunction with the Bruker Tensor 27 infrared spectrometer (Demyan et al., 2013). The high temperature reaction chamber (HTC, Harrick Scientific Products, Pleasantville, NY, USA) was equipped with a cartridge type heating element and a K-type thermocouple and interfaced with an automatic temperature controller (Harrick Scientific Products, Pleasantville, NY, USA) with an integrated temperature/process controller (Watlow Winona, Minnesota, USA). The dome had three windows, two made from potassium bromide (KBr) to enable diffuse reflectance measurements of the soil during the heating period (in situ$_T$ DRIFTS). Furthermore, the heating chamber was linked via a stainless steel Swagelok system (Swagelok-Stuttgart GmbH, Reutlingen, Germany) to a variable long path gas cell (Bruker Optik GmbH, Ettlingen, Germany) which was placed inside the Tensor 27 spectrometer to monitor CO_2 evolution (Fourier transform infrared-evolved gas analysis, FTIR-EGA) during the heating period. The gas cell had a volume of 136 ml and a path length of 1 m.

For the FTIR-EGA measurements, a scan was recorded every 4 sec, with a resolution of 8 cm^{-1} in the gas cell. Synthetic air was used as the purge and carrier gas of the system with a flow rate of 15 L hr^{-1}. The chamber was purged after introducing the sample for 5 min. Additionally, a baseline was taken before the beginning of each analysis in order to zero the signal in the gas cell. A heating rate of 68°C min^{-1} and set-point temperature of 700°C were used. The set-point temperature was reached after 10 min and held for another 20 min, which resulted in a total of 300 individual FTIR scans.

Alternatively, the Praying Mantis diffuse reflectance chamber was fitted in the Tensor 27 and the thermal reaction chamber placed inside in order to take DRIFTS measurements of the soil

or SOM fraction surface during heating (i.e., in situ$_T$ DRIFTS). The heating conditions and purge rate remained the same. A scan was taken every 4 sec, at a resolution of 4 cm^{-1}. Potassium bromide was used to take a background scan before measuring the sample.

4.3.7 Fresh soil analysis

Microbial biomass was measured by the chloroform fumigation extraction method (Vance et al., 1987) on field moist, homogenized, unsieved soil samples. Extraction was performed using 20 g soil and 80 ml 0.5 M K$_2$SO$_4$ solution for 30 min on an oscillating shaker at 200 revolutions min^{-1}, while in parallel another 20 g subsample was placed in a desiccator with a 50 ml beaker of ethanol free chloroform at 25°C under vacuum and kept overnight. The fumigated subsample was then extracted identically as the non-fumigated subsample. Extracts were then analyzed using a Multi C/N analyser (Analytik Jena) for both OC and N contents. Microbial biomass was taken as the difference between the C or N contents in the fumigated and non-fumigated samples multiplied by 0.45 for microbial biomass C (C$_{mic}$) (Joergensen, 1996) and 0.54 for microbial biomass N (N$_{mic}$) (Joergensen and Mueller, 1996). Non-fumigated extracts were taken as extractible C and N and additionally analyzed for ammonium (NH$_4^+$) and nitrate (NO$_3^-$) contents via a continuous flow analyzer (Alliance Instruments GmbH, Ainring, Germany).

4.3.8 Soil surface CO$_2$ fluxes

Field soil surface daytime CO$_2$ fluxes were measured via EGM-2 and EGM-4 non-dispersive infrared field Environmental Gas Monitor instruments equipped with a soil respiration chamber and a soil temperature probe (PP Systems Amesbury, Massachusetts, USA). Three measurement campaigns were done during the year (end of May beginning of June, middle of July, end of October) to coincide with soil sampling times. During the week of soil sampling, soil CO$_2$ fluxes (I$_{CO2}$) were measured during the daytime (usually between 08:00 and 18:00) for 5 days. Due to weather (heavy rain) or farmer field operations (herbicide spraying), the measurement days were sometimes not consecutive. The measurement order of the plots was rotated each day. Six measurements were taken in each subplot i.e., vegetated and bare fallow treatments. Data of the EGM-2 were calibrated against the EGM-4 to ensure compatible data. To compare with model results, raw data from the closed chambers as measured in g CO$_2$ m^{-2} hr^{-1} were converted to Mg C ha^{-1} day^{-1}. Results are presented in Mg C ha^{-1} day^{-1}. Soil temperature at 10 cm and I$_{CO2}$ were related via the following relationship

$$I_{CO2} = a \; e^{bT} \; ,$$
Eq 1

where *a* and *b* are fitted constants and *T* is soil temperature measured at 10 cm depth.

4.3.9 Plant phenology and crop management

In adjacent plots to where soil sampling was conducted, crop leaf area index (LAI; LAI-2000 Plant Canopy Analyzer, LI-COR Biosciences, Bad Homburg, Germany), final harvest of above- and belowground biomass (belowground measured 2009/estimated from harvest index in 2010) were measured during the 2009 and 2010 growing seasons. Table 1 shows crop planting and harvesting dates, as well as fertilizer and manure inputs. Plant phenology (BBCH growth stages according to Meier (2001)) and management information were used to parameterize the LUCIA-Century model and to assure that model C residue inputs were of corresponding values.

4.3.10 Abiotic variables

At a central location in each field, a weather station was installed in spring of 2009 to measure rainfall, air temperature, humidity, wind speed, solar radiation, and soil temperature at 6 and 15 cm. Interpolation was used to fill small data gaps (<1 day), while larger data gaps (>1 day) were filled via a transfer function developed from data of a weather station in an adjacent field. Simple linear regressions were performed for the variable of interest (e.g. soil temperature) with the variable of the adjacent field acting as the predictor and the field with missing data as the predicted.

Long-term weather data from the German weather service (Stötten for Swabian Alb and Öhringen for Kraichgau (DWD, 2013)) were used for the long-term equilibrium model run and 20 year simulation (see below). Reference evapotranspiration (*ETo*, [mm d^{-1}]) was estimated via the FAO Pennman-Monteith equation (Allen et al., 1998)) using average daily wind speed [m s^{-1}], average daily air temperature, minimum and maximum daily air temperature [°C], and average daily relative humidity (*RH* %). Soil temperature for the equilibrium run and 20 year simulation was first estimated via a Fourier expansion function from air temperature and day of the year (Roodenburg, 1985). The LUCIA-Century model was run, using this soil temperature estimate as an input. The generated LAI from the model run was then used in the approach of (Kätterer and Andrén, 2009) to estimate a soil temperature at 10 cm using air temperature, LAI, and snow cover which were then used as the final soil temperature input for the 20 year simulation.

4.4 Model conditions

4.4.1 Model structure

The Land Use Change Impact Assessment tool LUCIA (Marohn and Cadisch, 2011) is a dynamic spatially explicit landscape scale model, which runs at a daily time step. LUCIA is typically used for watershed simulations of land cover change over decades (Marohn et al., 2012), while for this study version 1.2 was used at the single plot level. The model integrates established concepts of hydrology (KINEROS 2; (Semmens et al., 2008)), erosion (Rose et al., 1983), plant growth (WOFOST-CGMS; (Supit, 2003)), litter decomposition and SOM transformations (Century; Parton et al., 1987) and combines them with routing functions provided by the PCRaster programming language (van Deursen, 1995) and farmer management options.

Daily input data required includes average air and soil temperature, rainfall, reference evapotranspiration (*ETo*) and solar radiation. Soils consist of two user-defined horizons, which require initial input data of SOC, texture, bulk density and coarse fragment contents (>2mm) to calculate hydraulic variables such as porosity, field capacity, and hydraulic conductivity, based on pedotransfer functions by (Saxton and Rawls, 2006). The stand level plant module builds on WOFOST routines and leaves, stems and roots have specific N and lignin contents, which determine their decomposition characteristics once they become litter. Litter decomposition into SOM as well as SOM dynamics follow the Century concept of active, slow and passive C pools, as implemented in LUCIA and will be referred to as the LUCIA-Century model version 1.2 (Marohn and Cadisch, 2011). The LUCIA-Century model was used in the current study to test five different methods for parameterization of SOM pool sizes (see section 4.4.6). The SOM pool turnover rates were left as defaults ($k_a = 0.02$ d^{-1}, $k_s = 0.00543$ d^{-1}, $k_p = 1.86 \times 10^{-5}$ d^{-1} for active, slow, and passive pools, respectively) (Parton et al., 1987) (Appendix 3, Table A.1) and all pool C:N ratios were left as Century defaults. A pre-modeling step involved updating the temperature function affecting SOM turnover. The original Century function was an exponential temperature response function (Parton et al., 1987), while in the DayCent daily time step model, this has been changed to a tangential function (Del Grosso et al., 2005), which results in higher respiration at lower temperatures and better reflected conditions in the study region.

4.4.2 Modeling

A four step modeling procedure was followed (Fig. 1) in which the approach was divided into several sub-steps.

4.4.2.1 Iterative calibration of the crop growth model

At the beginning of all model runs, soil parameters (i.e. TOC, TN, N_{min}) were set to the measured values at the start of the experiment in May 2009 (Table 2). Table 2 gives the site soil characteristics of the two study sites. The Swabian Alb topsoils had nearly two times SOC of the Kraichgau soils (0.92 and 1.83% in the Kraichgau and Swabian Alb, respectively). Sand contents were relatively similar (between 3 to 8%), but clay contents differed strongly with 18% in the Kraichgau soils and 38% in the Swabian Alb. Total N contents were again nearly double in the Swabian Alb compared to the Kraichgau (0.78 to 1.30 g kg^{-1} in the Kraichgau and Swabian Alb, respectively). At time of sampling the topsoil, N_{min} concentration was much higher in the Swabian Alb (65.0 mg kg^{-1}) compared to the Kraichgau (15.3 mg kg^{-1}). The pH of both sites was circum neutral (6.8 for the Kraichgau and 6.6 for the Swabian Alb). The 30-90 cm horizon was relatively similar to the 0-30 cm horizon in texture and pH, while for TOC and TN, values were nearly 2:3 less.

An adjacent field at each study site was used for calibration of the crop growth module. The crop rotation was silage maize and winter wheat in the Kraichgau in 2009 and 2010 respectively, and winter rape (2009) and winter wheat (2010) in the Swabian Alb. Meteorological data (Fig. 2) from the weather stations of the study sites were used from April 2009 until November 2010. Measured soil data was used for soil input parameters, while the default SOM pool distribution was used, as the SOM pool parameterization was found to not have an effect on the final crop biomass. Plant growth parameters (degree days to flowering and maturity, assimilation rate, and partitioning) of the WOFOST maize, winter wheat, oilseed rape (also used for winter mustard), summer barley routines (Supit, 2003) were used as a starting point and then iteratively modified during the manual calibration phase in order to achieve the correct growth pattern using LAI, plant biomass, planting and harvesting times, and harvest biomass for the used cultivars in LUCIA-Century.

Table 2. Initial site soil characteristics used for model parameterization.

	Horizon depth/cm	CF /%[a]	Db/g m^{-3}	Sand/%	Clay/%	SOC/%	TN/g kg^{-1}	N$_{min}$/ mg kg^{-1}	P$_{aval}$/ mg kg^{-1}	K$_{exch}$/ mg kg^{-1}	pH
Kraichgau	0-30	0	1.3	3	18	0.92	0.78	15.33	40	210	6.8
	30-90	0	1.5	2	18	0.29	0.22	4.0	8	90	6.4
Swabian Alb	0-30	7	1.32	6	38	1.83	1.30	65.0	55	240	6.6
	30-90	17	1.4	8	40	0.70	0.80	8.0	6	70	6.6

[a]CF-coarse fragments (>2 mm), Db-bulk density, SOC-soil organic carbon, TN-total nitrogen, N$_{min}$-soil mineral nitrogen, P$_{aval}$-available phosphorous taken from representative soil profile data (calcium acetate lactate method), K$_{exch}$-exchangeable soil potassium (BaCl$_2$-TEA method).
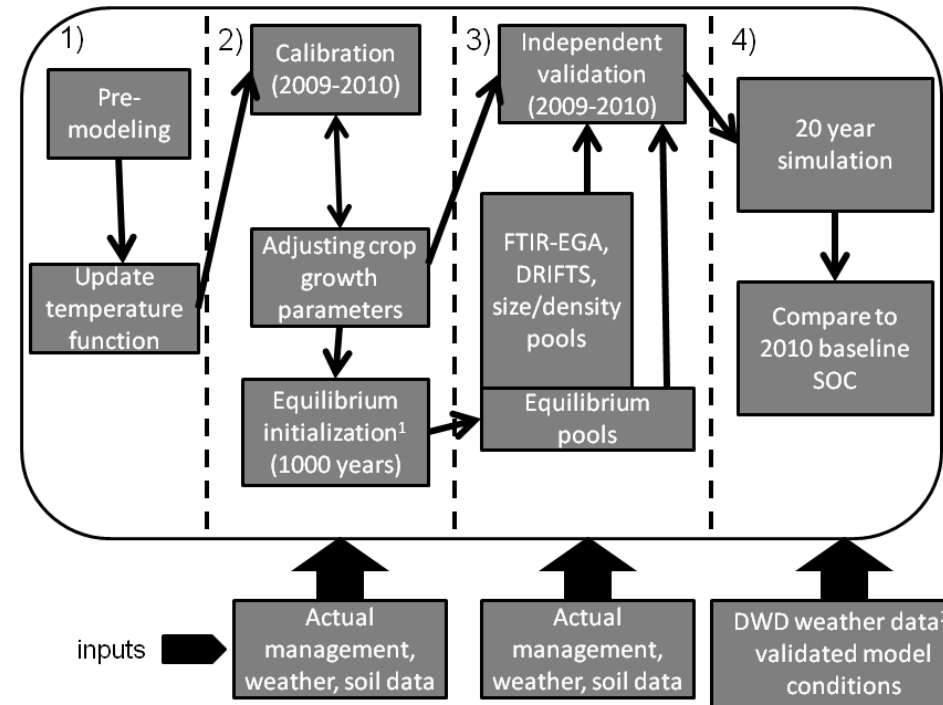
Figure 1. Modeling outline: 1) the first step was an iterative calibration of the plant growth module using measured values for both the Kraichgau and Swabian Alb sites, using measured weather and soil data from April 2009 until November 2010. 2) the second step was a validation of the crop growth parameters from the calibration step applied to an independent field for both sites, 3) while the third step utilized the different SOM pool initialization methods of FTIR-EGA, DRIFTS, size/density fractionation, and equilibrium run for the April 2009 to November 2010 period for measured short-term pool dynamics, and then 4) for a 20-year simulation to examine the medium-term impact of different pool parameterization compared to a 2010 baseline measured SOC level. [1]- The calibrated plant growth module was used for a 140-year simulation to gain the equilibrium SOM distribution between active (C_a), slow (C_s), and passive (C_p) pools, equilibrium run was done with German Weather Service (DWD) data as inputs from the years 1952-2007 looped for the long-term simulation. [2]-DWD- German Weather Service data from the years 1989 to 2008 was used for the 20-year simulation.
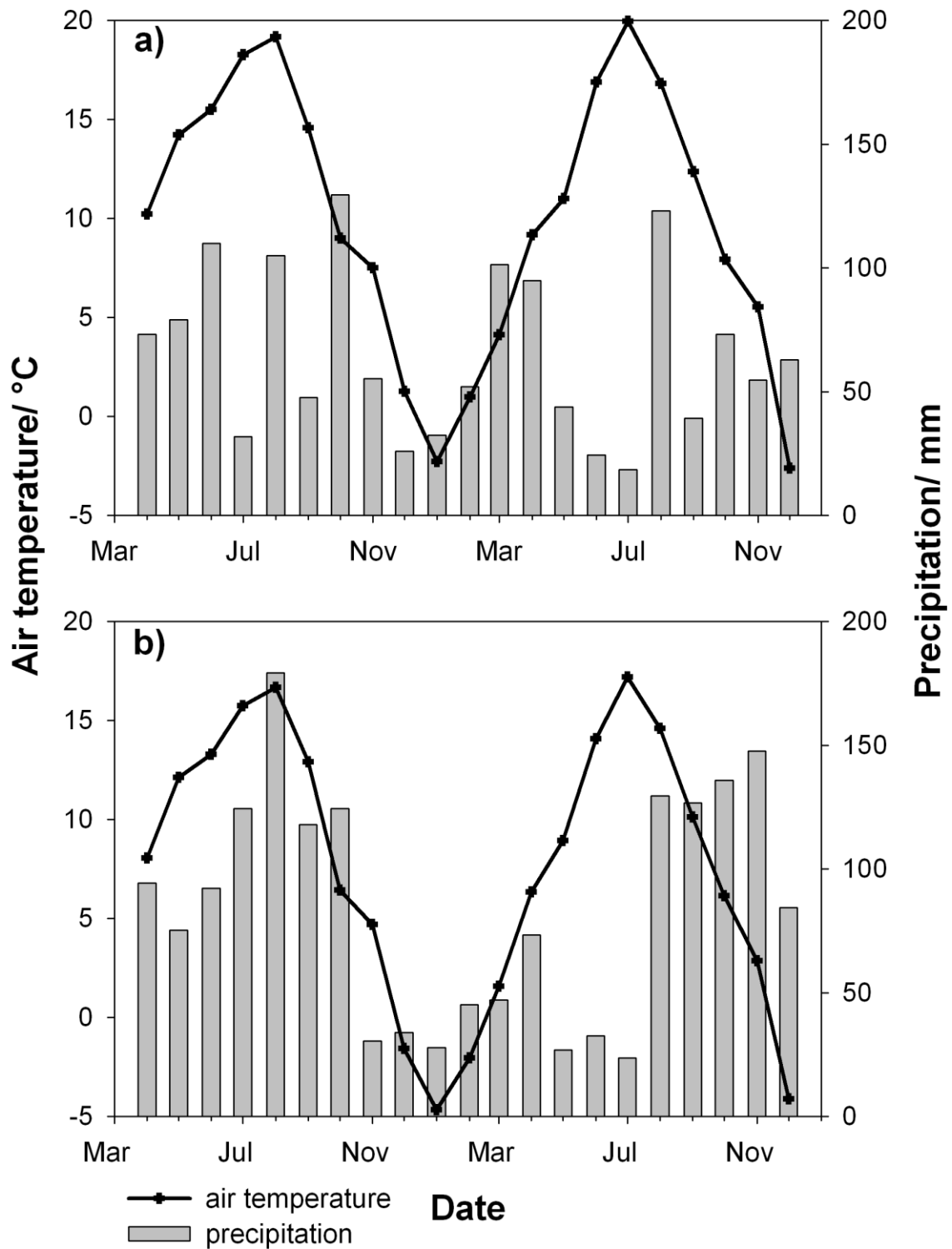
Figure 2. Measured monthly air temperature and precipitation from April 2009 until December 2010 of a) Kraichgau and b) Swabian Alb sites.

4.4.2.2 Crop module validation

After calibration of crop growth parameters with measured values from the 2009 and 2010 growing seasons, these parameters were applied to an adjacent field at both sites (step 2 in the modeling simulation) for a validation of the crop growth module. Appendix 3, Fig. A.1 shows the comparison between measured LAI and modeled LAI during the validation phase, using the results obtained during the calibration phase of the plant growth module. Measured and modeled final aboveground biomass, root biomass, and aboveground litter inputs (2009 and 2010, respectively) are given in Table 3.

4.4.2.3 SOM pool size parameterization

The pools size parameterization methods were:

1) Values from Parton (1987) were used as a *Default* parameterization which is usually used as a starting point for pool parameterization.

2) A parameterization was done running a long-term simulation (140 years) to arrive at pool equilibrium values, which was called the *Equilibrium* method. Historical daily weather data (e.g. air temperature and precipitation) from 1952 to 2007 from the Stötten and Öhringen weather stations were used as model inputs. The crop parameters derived in the calibration step were used for the crop growth module. Planting times, rotation, fertilizer application details can be found in Appendix 3, Table A.3.

3) A DRIFT-MIRS relative peak area method was used as defined in Demyan et al. (2012) and detailed above (Section 3.5) and called *DRIFTS*. Previously, the peak (2930 cm^{-1}) assigned to C-H groups correlated well with hot water extractable C, and was taken as the active pool, while the peak at 1620 cm^{-1} (–COO/C=C groups) was taken as the slow pool, as this peak has been shown to be negatively correlated to labile SOC and was assigned to more stable aromatic groups (Demyan et al., 2012). The % sum of these two pools was then subtracted from 100 and was taken as the passive pool.

4) A FTIR-EGA method based on thermal characteristics of evolved C during SOM oxidation under certain temperature intervals (Demyan et al., 2013) was used for a FTIR-EGA/in situ$_T$ DRIFTS method called *EGA-1*. An example is shown in Fig. 3. For this parameterization, fitted component 1 was taken as the active pool (peak temperature around 290°C corresponding to decreases in vibrational intensity of C-O and C-H groups), the slow pool was the component 2 (peak temperature around 360°C and corresponding to a decrease in C-H vibrations), while the passive pool was a sum of

Table 3. Observed (average and standard error) and modeled above- and belowground biomass and residues carbon inputs to the soil for model validation.

Site	AGB[a]/ Mg DW ha^{-1}		BGB/ Mg DW ha^{-1}		AGR-C/ Mg C ha^{-1}		BGR-C/ Mg C ha^{-1} (0-30 cm)		BGR-C/ Mg C ha^{-1}
	obs	mod	obs	mod	obs	mod	obs	mod	mod
Kraichgau									
winter wheat	17.4 (0.2)	20.6	1.7 (0.3)	1.2	0.34 (0.02)	0.39	0.36 (0.01)	0.5	1.6
mustard-maize	20.1 (1.1)	21.5	1.2 (0.1)	1	0.28 (0.01)	0.3	0.48 (0.08)	0.27	2.5
Swabian Alb									
winter wheat	17.7 (0.8)	18.5	1.4 (0.4)	1.2	0.40 (0.1)	0.26	0.5 (0.1)	0.51	1.7
winter rape	9.3 (1.1)	10.6	0.8 (0.1)	0.7	1.90 (0.3)	3.43	0.3 (0.0)	0.29	0.9

[a]AGB-aboveground biomass (dry weight), BGB-belowground biomass (dry weight), AGR-aboveground residue at harvest, BGR-belowground residue at harvest, BRG crop-belowground residue including final harvest and cumulative dead root inputs during crop cycle.

components 3 and 4 which corresponded to decreases in –COO/C=C and C=C vibrations at higher temperatures.

5) The second FTIR-EGA/in situ$_T$ method (*EGA-2*) was a derivation of EGA-1 in that the active pool size was identical (component 1) but the slow pool was the sum of fitted components 2 and 3, while the slow pool was fitted component 4 (Fig. 3).

6) Using the fractions isolated by the method of (Zimmermann et al., 2007b) the pool sizes were initialized as follows, the extractable and POM amounts were taken as the active pool, the sum of Sa+A fraction and Si+C minus rSOC was taken as the slow pool, and the rSOC fraction was taken as the passive pool. The method was defined as *Fractions*.

All other values used in the modeling exercises were identical between the different pool parameterization methods.

4.4.2.4 Short-term model performance

As this paper was focused on the SOM dynamics, several performance indicators were used to compare observed values with modeled values of soil CO_2 flux and SOC: The coefficient of determination (r^2) (Nash and Sutcliffe, 1970):

$$r^2 = \left(\frac{\sum_{i=1}^{n}(O_i - \bar{O})(P - \bar{P})}{\sqrt{\sum_{i=1}^{n}(O_i - \bar{O})^2}\sqrt{\sum_{i=1}^{n}(P_i - \bar{P})^2}} \right)^2 \qquad \text{Eq 2}$$

where n is the number of observations, O_i are the observed values, $\bar{O}$ is the average of the observed values, P_i are the predicted values with a perfect model fit to observed values $r^2 = 1$. The relative root mean error square (RMSE$_\%$) (Loague and Green, 1991) was calculated as:

$$RMSE_\% = \frac{100}{\bar{O}} \cdot \sqrt{\frac{\sum_{i=1}^{n}(P_i - O_i)^2}{n}} \qquad \text{Eq 3}$$

with an optimum value RMSE = 0. Furthermore, we computed the model efficiency (Loague and Green, 1991):

$$EF = \frac{(\sum_{i=1}^{n}(O_i - \bar{O})^2 - \sum_{i=1}^{n}(O_i - P_i)^2)}{\sum_{i=1}^{n}(O_i - \bar{O})^2} \qquad \text{Eq 4}$$

An optimum value is EF = 1, which will indicate a perfect fit of the model, while values <0 indicate that predicted values are worse than using the observed mean value. Model bias (MBE) (Bennett et al., 2013) was defined as:

$$MBE = \frac{\sum_{i=1}^{n} P_i - O_i}{n} \qquad \text{Eq 5}$$

where an optimum value is MBE = 0.
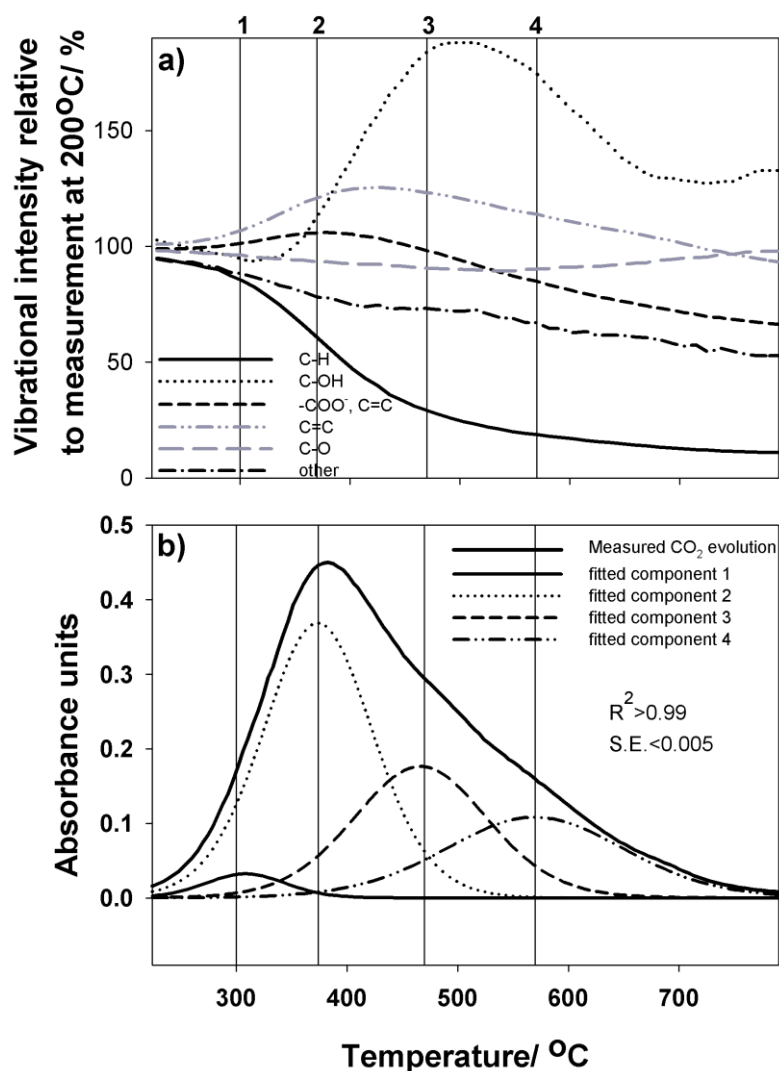
Figure 3. Derivation of the SOM pools from the combined in situ$_T$ DRIFTS and FTIR-EGA:
a-In situ$_T$ DRIFTS measurements of bulk soil from the Kraichgau site as a percentage of the vibrational intensity at 200°C to identify temperature of maximum change of functional groups. **b**- Peak fitting with four components of evolved gas profile as measured by FTIR-EGA of bulk soil from the Kraichgau site using previously identified temperatures of maximum change of functional groups. Statistics are from the goodness of fit of the four fitted components to the measured data. Figure is taken from Demyan et al. (2013). *- Normalized intensity is the integrated area of the CO_2 peak from 2400 to 2200 cm^{-1} normalized to weight of the sample. **-fitted component.

4.4.2.5 Medium-term simulations

The Century model was then run for an additional 20 years to examine the effect of the different pool parameterizations on the medium-term effect of SOC with weather data of 1989-2008 from the weather stations of Öhringen for the Kraichgau and Stötten for the Swabian Alb (DWD, 2013) (Appendix 3, Table A.2) and typical crop rotations of the areas (Appendix 3, Table A.3) used as inputs. Twenty years were used as time duration to show differences brought about by the proportion of the intermediate pool, which on a scale of 20 years potentially 10% of the pool would turn-over based on the k_s value of $5.43*10^{-4}$ d^{-1}. Since the cropping systems at both sites have already been running for decades, it was assumed for this SOM parameterization method that SOM would be in equilibrium without considerable changes in total SOM contents if continuing these cropping systems under the same conditions. With this in mind, the SOC content result at the end of 20 years of different parameterization methods were compared to a baseline of ±1 standard error of the measured SOC value at the end of 2010, assuming under continuing current agricultural practices that SOC would be maintained at current levels.

4.5 Results

4.5.1 Site CO_2 flux characteristics

The general pattern of soil CO_2 flux was found to increase from the early growing period, reaching a maximum during the summer months, and decreasing again in the fall (Fig. 4) under the vegetation. There was a large variation in the measurements in the Kraichgau in May of 2009 under winter wheat, ranging from 0.03 to 0.07 Mg C $ha^{-1}d^{-1}$. In the Kraichgau under maize in 2010, measured CO_2 fluxes were lower, especially late in the season in November. The Swabian Alb followed the same general pattern, although there was strong variation during the end of the winter oilseed rape growing period. Similarly in the bare fallow plots (Fig. 5), a maximum CO_2 flux was reached during the summer growing months and declining to the end of the growing period. At both sites measured CO_2 fluxes were higher during the first year compared to the second, though the difference was less in the Swabian Alb soils compared to the Kraichgau. Looking at the overall average CO_2 flux (Table 4), the flux was similar under vegetation between the two sites ($3.12*10^{-2}$ and $3.08*10^{-2}$ Mg C $ha^{-1}d^{-1}$ in the Kraichgau and Swabian Alb, respectively). The CO_2 flux in the bare
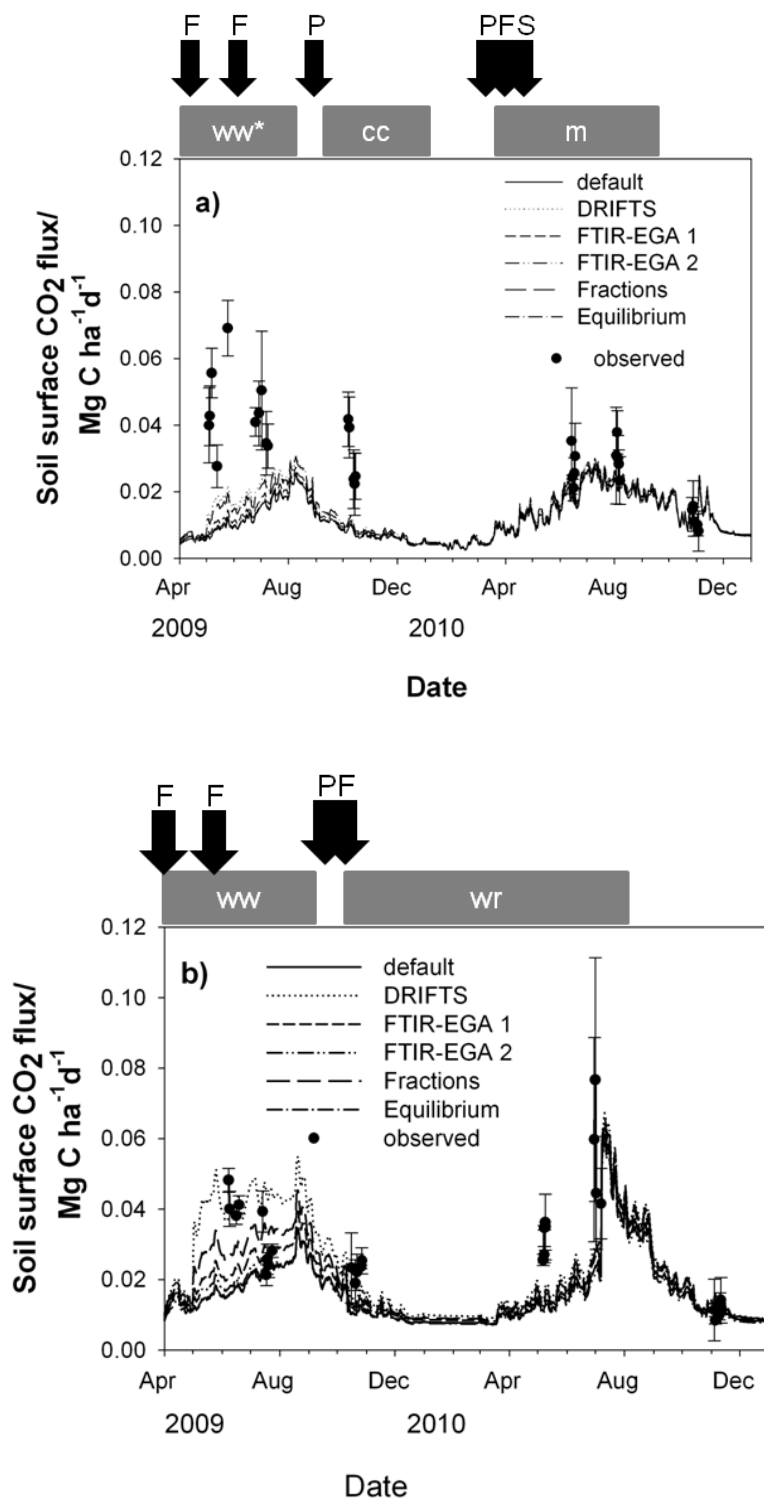
Figures 4a & 4b. Measured and modeled soil surface CO_2 flux showing the results of different parameterization methods for the Kraichgau site (4a): ww-winter wheat, cc cover crop, m-maize, F-fertilizer application, P-plowing, S-slurry application; and Swabian Alb site (4b): ww-winter wheat, wr-winter oilseed rape, F-fertilizer application, P-plowing, S-slurry application.
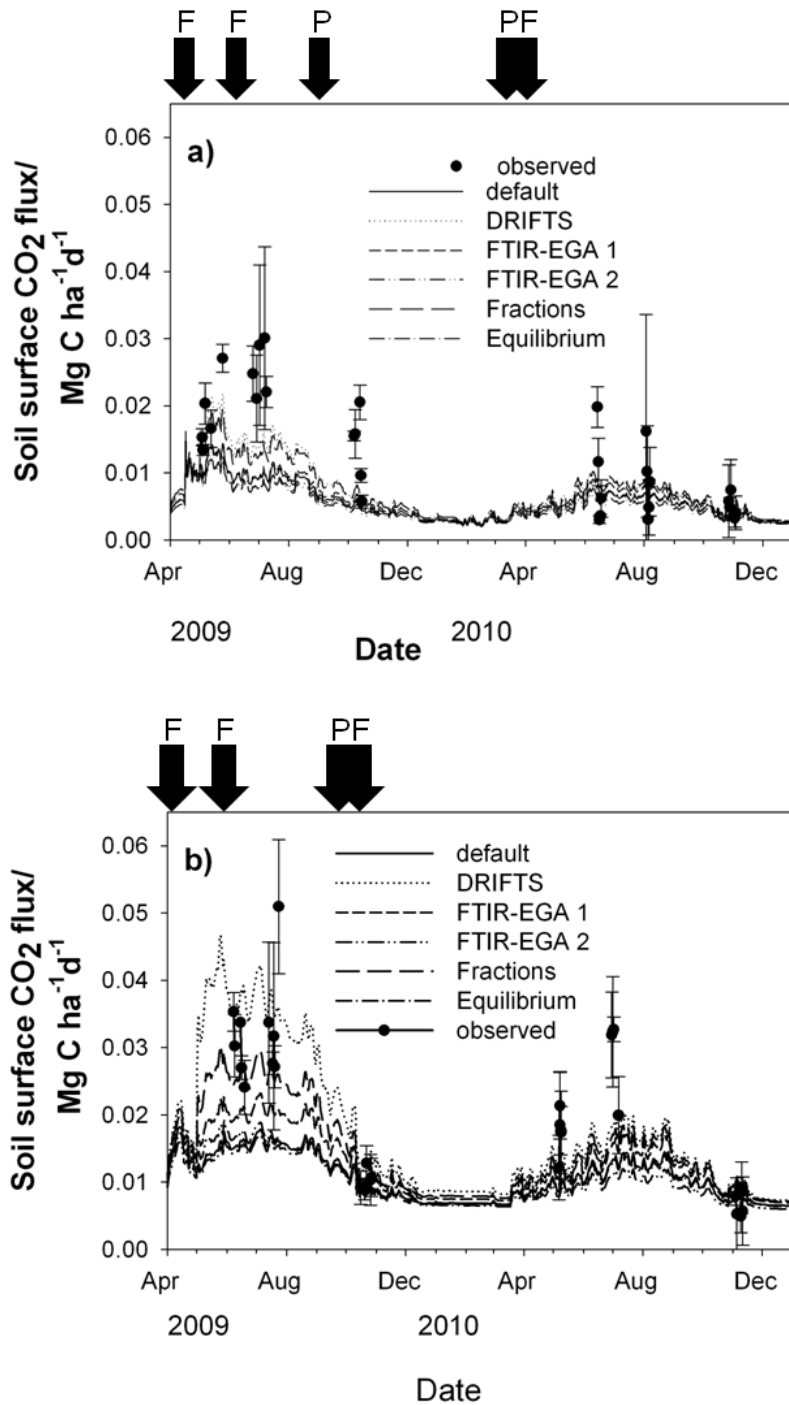
Figures 5a & b. Measured and modeled soil surface CO_2 flux showing the results of different parameterization methods under bare fallow for the Kraichgau site (5a) F-fertilizer application, P-plowing, and Swabian Alb site (5b) F-fertilizer application, P-plowing.

Table 4. Average of measured CO_2 fluxes by site during 2009-2010. Values in parentheses are the standard error.

Site	Bare fallow $Mg\ C\ ha^{-1}\ d^{-1}$		Vegetated[a] $Mg\ C\ ha^{-1}\ d^{-1}$		ratio bare:vegetated
Kraichgau	$1.35*10^{-2}$	$(1.63*10^{-3})$	$3.12*10^{-2}$	$(1.63*10^{-3})$	0.43
Swabian Alb	$2.08*10^{-2}$	$(1.39*10^{-3})$	$3.08*10^{-2}$	$(1.94*10^{-3})$	0.67

[a]-crops for the Kraichgau site were winter wheat and silage maize, and the Swabian Alb were winter wheat and oil seed rape.

fallow was lower at both sites compared to that under vegetation ($1.35*10^{-2}$ and $2.08*10^{-2}$ Mg C ha^{-1}d^{-1} in the Kraichgau and Swabian Alb, respectively). The ratio of bare to vegetated CO_2 flux was 0.43 in the Kraichgau and 0.67 in the Swabian Alb.

The temperature response of soil respiration at each site was similar as shown in Fig. 6. Since certain dates (e.g. 25 May 2009) were found to be greatly underestimated by the modeling, the time of CO_2 measurement was investigated relative to the diurnal pattern of soil temperature (Fig. 7). It was found that on these days the CO_2 measurements represent an average for the daily flux as they were taken nearly at the point of the average soil daily temperature.

4.5.2 SOM pool parameterization by different methods

SOM pool parameterization results for the model initialization for the 2009-2010 simulations are given in Table 5. The *Default* method was 5, 55, and 40% for C_a, C_s, and C_p, respectively, for both sites. In the Kraichgau, the *Equilibrium* method allocated 0.88% to the C_a pool which was the lowest amount out of any of the methods. The *DRIFTS* method by contrast allocated 14% to the C_a pool. The *Fractions* method allocated the lowest amount of any methods to the passive pool (15%). The *EGA-1* and *EGA-2* methods both allocated 3.4% to the C_a pool, but 73.4% was allocated to C_s from *EGA-1* while 46.1% from *EGA-2*. For Swabian Alb, besides the default method, which was the same for both sites, pool allocation among the methods followed the same trend as in Kraichgau. The lowest C_a resulted from the equilibrium pool method while the largest derived from the *DRIFTS* method. Contrasting the
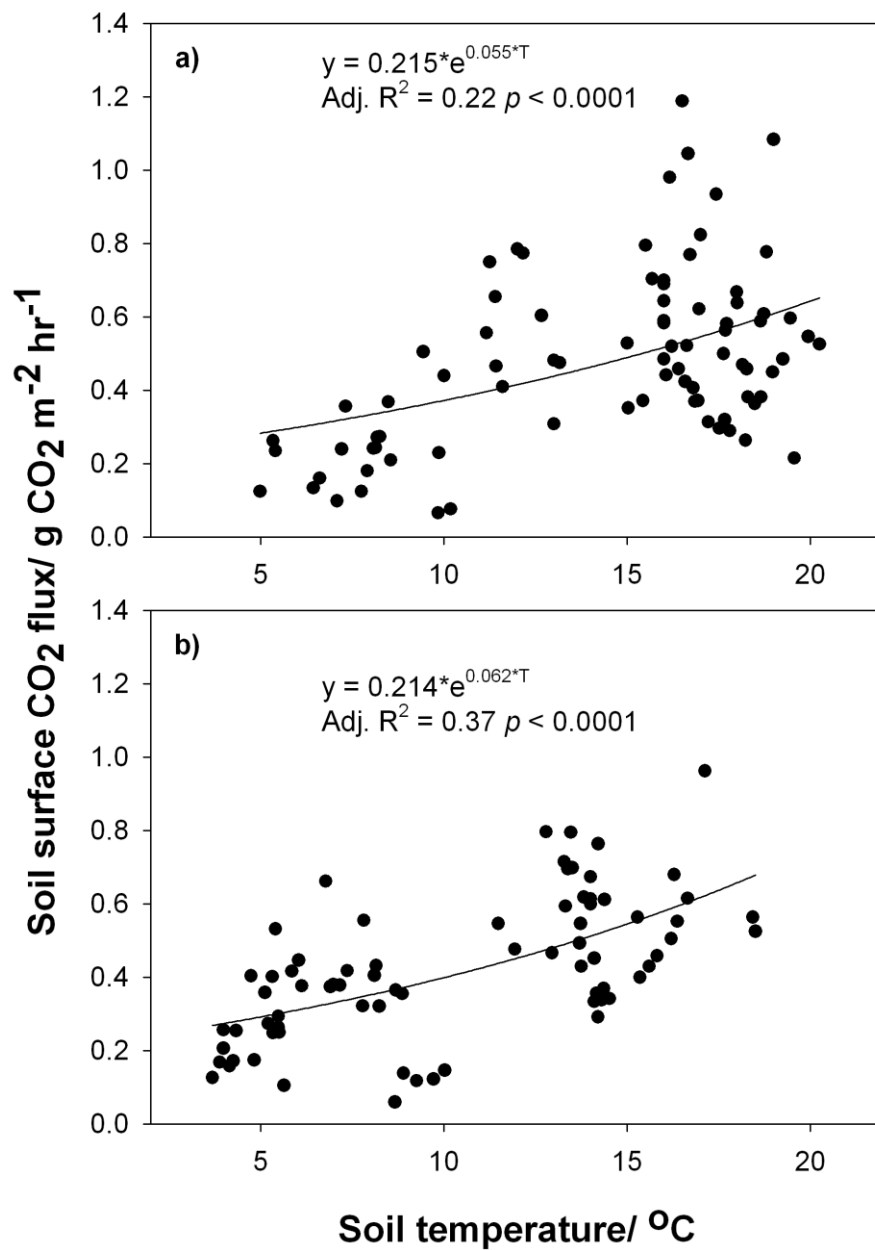
Figures 6a & b. Relationship between temperature and measured soil surface CO_2 flux at the a) Kraichgau and b) Swabian Alb sites during measurements taken during 2009 and 2010 growing seasons.
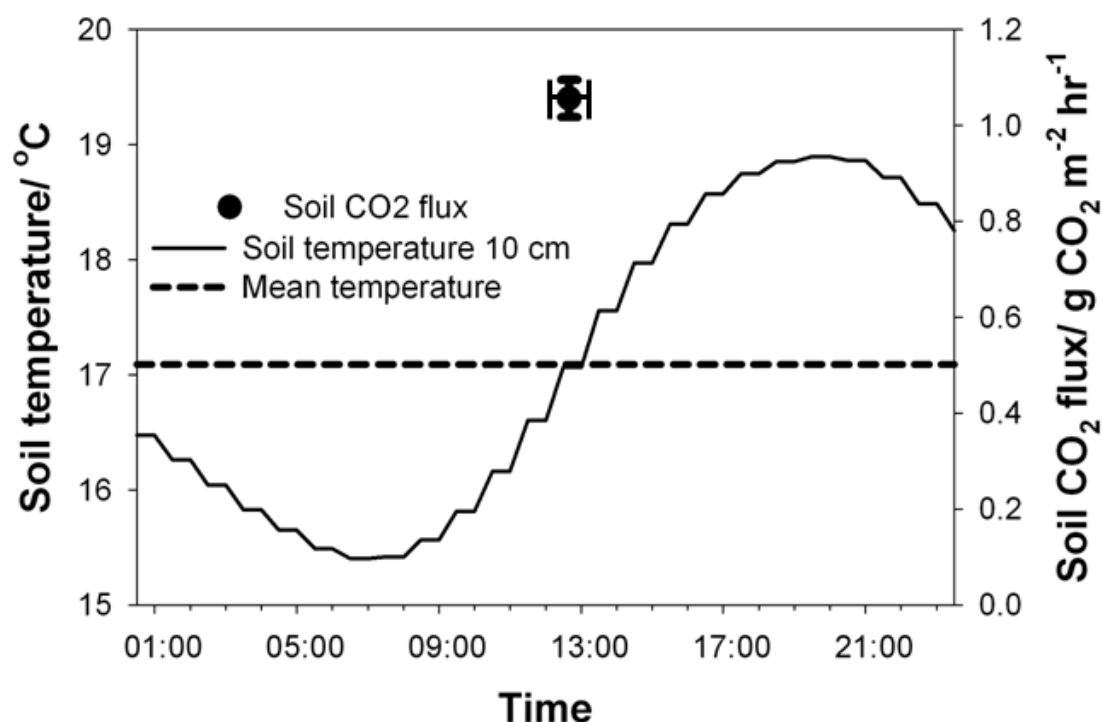
Figure 7. Measured soil surface CO_2 flux in relationship to soil temperature at 10 cm for 25 May 2009.

Table 5. SOM pool parameterization estimates by different methods.

Method[a]	Kraichgau			Swabian Alb		
	C_a/%[b]	C_s/%	C_p/%	C_a/%	C_s/%	C_p/%
Default	5	55	40	5	55	40
Equilibrium	0.9	62.3	36.8	1.3	53.3	45.4
DRIFTS	14.0	67.6	18.3	23.6	62.1	14.3
Fractions	9.5	75.5	15.0	10.9	70.3	18.8
EGA-1	3.4	73.4	23.3	4.7	69.2	26.1
EGA-2	3.4	46.1	50.6	4.7	35.3	60.0

[a]-methods are as follows: *Default*-from Parton et al. (1987), *Equilibrium*-model run for 140 years using crop rotations (see Table 2 and daily weather values from 1952-2008), *DRIFTS*-diffuse reflectance Fourier transform infrared spectroscopy derived pools, *Fractions*-SOM fractionation method according to Zimmermann et al. (2007a), *EGA* in situ$_T$ DRIFTS-evolved gas analysis and in situ thermal DRIFTS (Demyan et al., 2012).[b]-C_a-percent of SOC allocated to Century active pool, C_s-percent of SOC allocated to slow pool, C_p- percent of SOC allocated to passive pool.

two sites, all methods, except for the *Default* method, allocated a larger C_a in the Swabian Alb site compared to that of the Kraichgau. Conversely, all methods except *DRIFTS* found a higher C_p in the Swabian Alb compared to the Kraichgau site.

4.5.3 SOM dynamics of short-term simulation

Modeled day time soil CO_2 fluxes are shown in Figs. 4 and 5 along with model statistics in Table 6. The general behavior of all model parameterizations was similar in that during the middle of the growing season CO_2 fluxes were underestimated, while the model approximated late season CO_2 fluxes more accurately. In general, respiration in the Swabian Alb was more accurately modeled compared to the Kraichgau as measured by RMSE and EF statistics (Table 6) and the bare fallow treatment in the Swabian Alb was most successfully modeled.

During the first year of simulation, SOM parameterization methods varied greatly as compared to the second year, particularly in the vegetated plots. In the Kraichgau a maximum flux difference of $9.9*10^{-3}$ Mg C ha^{-1} d^{-1} was found between the *DRIFTS* ($2.2*10^{-2}$ Mg C ha^{-1} d^{-1}; Fig. 4a) and *Default* ($1.2*10^{-2}$ Mg C ha^{-1} d^{-1}) method on 25.05.2009, while in 2010 the maximum difference of $2.5*10^{-3}$ Mg C ha^{-1} d^{-1} was between *Fractions* ($2.3*10^{-2}$ Mg C ha^{-1} d^{-1}) and *EGA-2* ($2.0*10^{-2}$ Mg C ha^{-1} d^{-1}) on 06.06.2010. The maximum modeled flux difference at the Swabian Alb site of $3.3*10^{-2}$ Mg C ha^{-1} d^{-1} (Fig. 4b) occurred on 26.05.2009 between *DRIFTS* ($5.1*10^{-2}$ Mg C ha^{-1} d^{-1}) and *Equilibrium* ($1.7*10^{-2}$ Mg C ha^{-1} d^{-1}). Likewise the maximum difference of modeled values in 2010 at the Swabian Alb was much lower $6.8*10^{-3}$ Mg C ha^{-1} d^{-1} between *DRIFTS* ($3.2*10^{-2}$ Mg C ha^{-1} d^{-1}) and *EGA-2* ($2.3*10^{-2}$ Mg C ha^{-1} d^{-1}) on 04.07.2010.

The SOC did not show much change over the measurement period and even after the bare fallow period from May 2009 until November 2010 there was no significant measurable change, even though there was a general trend of decreasing SOC compared to the first sampling (Figs. 8a & 8b). Modeled SOC contents of the 0-30 cm horizon were predicted within ±1 standard error, except for the Kraichgau vegetated plots, where SOC was modeled higher than the measured values (measured 35.6 Mg C ha^{-1}, modeled 38.2 Mg C ha^{-1}).

4.5.4 SOM dynamics of long-term simulation

Modeled long-term (20 years) SOC contents to 30 cm continued the inter-annual dynamics of an increase of SOC upon crop residue incorporation and manure application and subsequent

Table 6. Indicators of modeling accuracy of two year (2009-2010) simulations of soil CO_2 flux by different SOM parameterization methods.

| | Kraichgau | | | | | | | |
| | vegetated | | | | bare fallow | | | |
Method[a]	RMSE% [b]	EF	r^2	MBE	RMSE%	EF	r^2	MBE
Default	55	-4.28	0.42	0.016	76	-0.15	0.38	0.006
Equilibrium	76	-1.46	0.01	0.016	80	-0.29	0.34	0.006
DRIFTS	65	-0.79	0.07	0.013	60	0.26	0.42	0.003
Fractions	67	-0.90	0.03	0.014	64	0.18	0.41	0.004
EGA-1	73	-1.27	0.00	0.015	74	-0.11	0.36	0.005
EGA-2	77	-1.48	0.01	0.017	81	-0.32	0.36	0.007

| | Swabian Alb | | | | | | | |
| | vegetated | | | | bare fallow | | | |
Method	RMSE%	EF	r^2	MBE	RMSE%	EF	r^2	MBE
Default	69	-0.20	0.25	0.012	66	-0.03	0.44	0.008
Equilibrium	69	-0.22	0.25	0.013	68	-0.08	0.43	0.008
DRIFTS	64	-0.05	0.10	0.003	57	0.25	0.36	0.001
Fractions	62	0.03	0.17	0.007	53	0.35	0.40	0.003
EGA-1	64	-0.04	0.22	0.010	58	0.21	0.43	0.005
EGA- 2	70	-0.24	0.20	0.013	67	-0.05	0.39	0.008

[a]-methods are as follows: *Default*-from Parton et al. (1989), *Equilibrium*-model run for 56 years using crop rotations (see Appendix 3 and daily weather values from 1952-2008), *DRIFTS*-diffuse reflectance Fourier transform infrared spectroscopy derived pools, *Fractions*-fractionation method according to Zimmermann et al. (2007), *EGA-1* and *EGA-2*-evolved gas analysis and in situ thermal DRIFTS (Demyan et al., 2012). [b]-RMSE%-root mean square error of prediction, EF-modeling efficiency, r^2-coefficient of determination, MBE-model bias. Optimum values are RMSE-0, EF-1, CD-1, MBE-0.

decrease of SOC during decomposition for all parameterization methods (Figs. 9a & 9b). At the end of the 20 year period the difference in SOC estimates among the different SOM parameterization methods was 6 Mg C ha^{-1} for the Kraichgau and 14 Mg C ha^{-1} for the Swabian Alb. This represents 17 and 19% of the 2010 measured SOC values of the Kraichgau and Swabian Alb soils, respectively. For the Kraichgau site, the *EGA-2* resulted in the highest SOC, followed by the *Default* and *Equilibrium* methods, with the *EGA-1*, *DRIFTS*, and *Fractions* methods grouped together with the lowest modeled SOC amounts (37 Mg C ha^{-1}). In the Swabian Alb the *EGA-2* again estimated the highest SOC amount (79 Mg

C ha^{-1}), followed by the *Equilibrium* and *Default* methods (75 Mg C ha^{-1}). *EGA-1*, *Fractions*, and *DRIFTS* were again grouped closely together (68, 66, and 65 Mg C ha^{-1}, respectively).
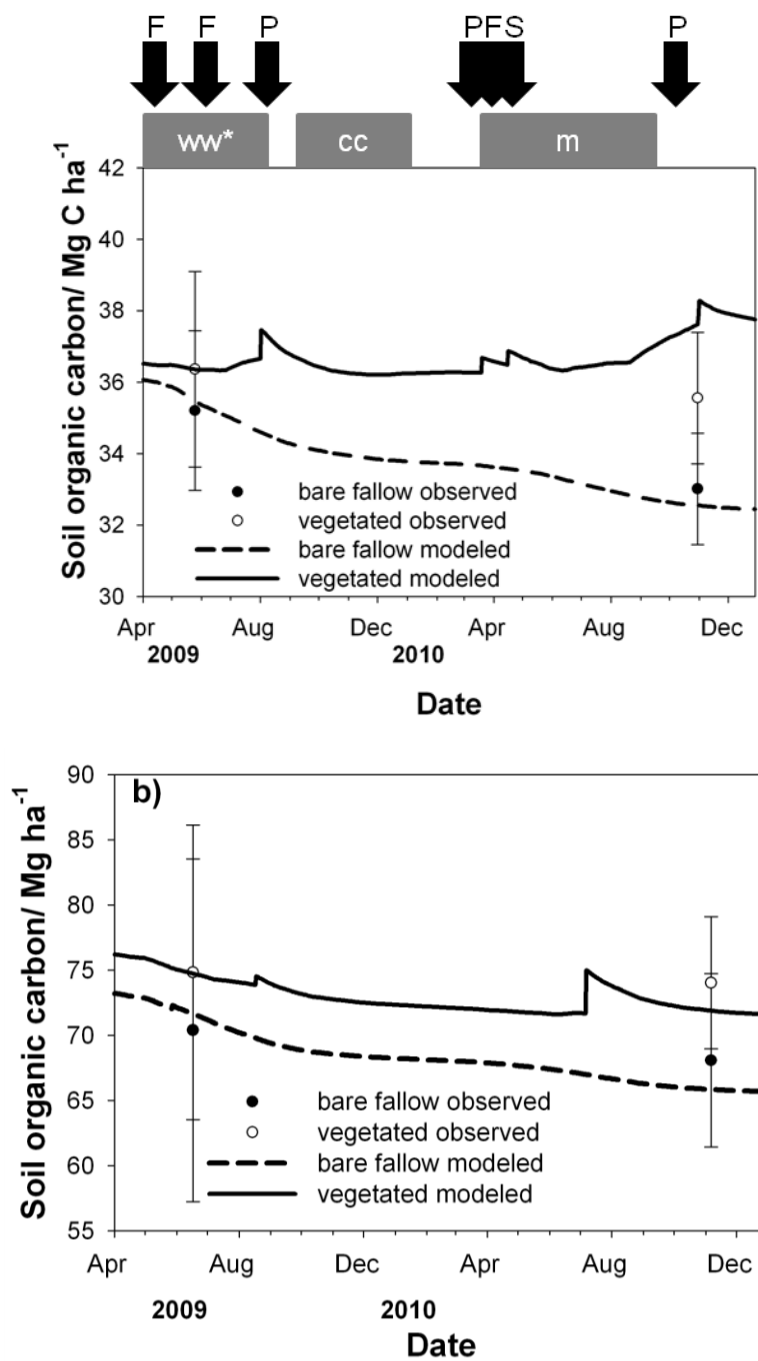
Figure 8. Observed and modeled (*DRIFTS* parameterization) SOC of bare fallow and vegetated plots at the a) Kraichgau sites during the 2009 and 2010 growing seasons.*ww-winter wheat, cc-cover crop, m-maize, F-fertilizer application, P=plowing, S-biogas slurry application and b) Swabian Alb site during the 2009 and 2010 growing seasons. *ww-winter wheat, wr-winter oilseed rape, F-fertilizer application, P-plowing.
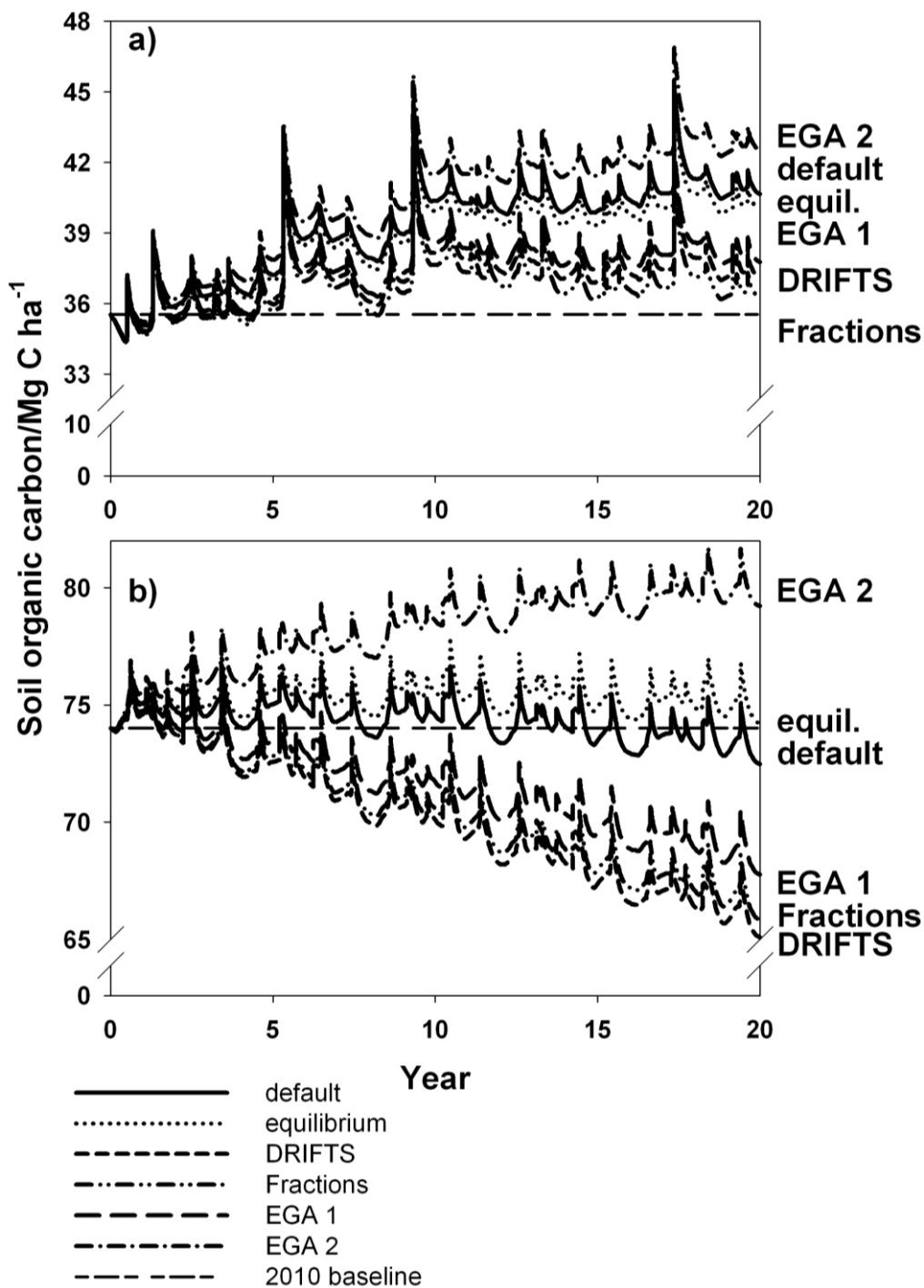
Figure 9. Medium-term simulation (20 years) of SOC contents in 0-30 cm depth using different SOM pool size parameterization methods[1] for a) Kraichgau and b) Swabian Alb sites. [1] *Default*-from Parton et al. (1987), *equilibrium*-model run for 140 years, *DRIFTS*-diffuse reflectance Fourier transform infrared spectroscopy derived pools, *Fractions*- SOM fractionation method according to Zimmermann et al. (2007a), *EGA-1* and *EGA-2*-in situ$_T$ DRIFTS-evolved gas analysis and in situ thermal DRIFTS method 1 and method 2. **Note**: y-axis scale break. Different scales were used for the y-axis in order to make the differences between methods visible.

4.6 Discussion

4.6.1 Different measured fractions as model pool inputs

It was evident that some of the pool size parameterizations (i.e. *DRIFTS* large active pool size) would be very different from pool distributions used in previous studies. The decision of how to split the different measured fractions into the different modeled pools is always a difficult operation. In the current study this can be seen in the *EGA-1* and *EGA-2* methods, where fitted component 3 was allocated either to the slow or to the passive pool. As was shown in the results, this had major consequences for the 20 year simulation as this 3rd component was a large portion of the TOC (27.3 and 32.9% in the Kraichgau and Swabian Alb, respectively). As the functional groups that the 3rd and 4th fitted components represent were different (-COO and C=C in the 3rd component and solely C=C in the 4th component), it may make sense to allocate them to the slow and passive pools, respectively. For pool assignments for the fraction method, we did not split any of the fractions between more than one model pool. While it may be the case that the SOM fractions are not homogeneous, especially the POM fraction, it is not an easy matter to divide them among model pools. While the *EGA-1* and *EGA-2* methods have not been used previously for SOM pool parameterization, and therefore are difficult to compare to other studies, there are examples of splitting and allocating size/density/chemical fractions to model pools (Zimmermann et al., 2007c; Leifeld et al., 2009; Xu et al., 2011). For the Roth-C model and using the same physical/chemical fractionation method as in the current study (Zimmermann et al., 2007c), the measured fractions were split via running the Roth-C to equilibrium with an assumed annual C residue input and then used the model pool ratios between the decomposable and resistant plant residues and biomass and humus pools as the ratios to allocate the measured fractions to the model pools, so it was not a simple one to one relationship between a fraction and a model pool. In the current study, we wanted to independently investigate the strengths and weaknesses of the new approaches (i.e. *DRIFTS* and *EGA-1* and *EGA-2*) in order to test how they behaved in the Century model framework. Previously, SOM fractions separated based on physical (e.g. size, density) (Zimmermann et al., 2007c), chemical (Zimmermann et al., 2007a), and chemical combined with biological methods (Paul et al., 2006) have been used as measureable model inputs. Additionally, near infrared spectroscopy (NIRS) has been used through a partial least squares cross validation approach to predict Roth-C generated SOM pools (Michel and Ludwig, 2010).

The parameterization methods utilized various principles relating to the recalcitrance of SOM to allocate C to the different pools. The *DRIFTS* method relied on the hypothesis that the integrated area of the C-H groups (around 2930 cm^{-1}) was influenced more by labile compounds and has previously been related to hot water extractible C (Demyan et al., 2012). The *EGA* methods were an extension of the *DRIFTS* application with the added analysis of the evolved gas while heating and also monitoring of the DRIFT spectrum of the soil sample while heating. This allowed both a quantification of the evolved C during a controlled increase of temperature, but also the changes in DRIFT peak intensity giving a quality aspect to the evolved C, with different stabilities of C evolved at higher temperatures. The *Fractions* method was based on classic SOM size-density fractionation, which aimed to isolate SOM with less to more association with the mineral fraction and thereby lower to higher recalcitrance (Zimmermann et al., 2007a). The *Equilibrium* method was the only model based parameterization method which used assumed long-term management and inputs for both sites and relied on the structure of the model to derive the pool allocations. The analytical methods, although somewhat differing in principle, had linkages. It has been shown that light fraction OM was enriched in C-H groups as measured by DRIFTS in contrast to heavy mineral fractions (Demyan et al., 2012). Likewise a chemically resistant fraction was found to have a second peak of CO_2 evolution not found in other fractions as measured by EGA (Demyan et al., 2013) lending evidence that the fraction contained very recalcitrant OM. This at least theoretically implied that through the different methods, they were measuring partly similar portions of the same labile to recalcitrant SOM pools.

Another promising result of the SOM pool initializations was that in the tested methods of *DRIFTS*, *Fractions*, *EGA-1*, and *EGA-2*, the general trend of a larger active pool in the Swabian Alb soils compared to the Kraichgau soils was consistently found, indicating that the methods, in part, are measuring some part of the same active pool even though they are relying on different properties, such as solely molecular vibrations for *DRIFTS*, size/density/chemical separation in SOM fractionation, and a combination thermal decomposition and molecular characterization for the *EGA-1* and *EGA-2* methods.

4.6.2 Importance of pool parameterization to simulate short-term soil C dynamics

In the current study, modeled SOC contents were within the standard error of measured values at the end of the short-term simulation, except for the Kraichgau vegetated plots. This

seems to indicate that generally residue inputs to the soil were more or less correct and the turnover times were relatively accurate, with the exception of the Kraichgau vegetated plots. The general inter-annual SOC dynamics were represented by the model which has also been found for applications of Century to arable soils of long-term experiments. The fact that the SOC levels of the bare plots were modeled correctly leads one to believe that the turnover rates for the different SOM pools are reasonable.

The difference in measured and modeled SOC for the Kraichgau vegetated plots could indicate an influence of several factors such as too much litter additions to the soil either through aboveground or root inputs. The modeled root biomass at harvest was nearly always within ±1 standard error of the measured values while the aboveground residue inputs were also well modeled. What is not taken into account in the root measurements are the dead roots and exudates deposited during the season (rhizo-deposition), so that the final root biomass measurement is under estimating the amount of root residue input to the soil.

The more dynamic soil CO_2 flux values were less well modeled, although the general tendency of increasing respiration in the spring to a maximum during the summer and declining toward the end of the growing season was reflected in the modeled results, but soil CO_2 fluxes were generally underestimated. (Jensen et al., 1996) also found a similar decline in soil surface CO_2 fluxes after incorporation of crop residues during late August until the beginning of October using an EGM-1 CO_2 analyzer. It was shown that initial flux values were much higher under residue incorporated plots compared to non-amended plots, but that this difference nearly disappeared as the soil temperature decreased below 10°C.

The effects of the different parameterization methods were more evident in the 1st year (2009) as compared to the 2nd year with the 1st year difference being controlled by the allocation to the C_a pool as *DRIFTS* was found to have the maximum single day flux at both sites and this method allocated the largest amount to C_a. While alternatively *Equilibrium* had the lowest flux on the same day and had the smallest allocation to the active pool. In the Kraichgau, then, during the second year, the intermediate pool was found to have more influence as the *Fractions* method had the maximum daily flux and also the largest allocation to the C_s pool and *Fractions*, with the smallest C_s allocation, had the lowest flux on the same day. In the Swabian Alb, though, *DRIFTS* still had the maximum flux, which was due to the initial large allocation to the C_a pool (23.6%) and general slower turnover due to lower annual temperature.

Others also have had varying success in modeling soil surface CO_2 fluxes. Chirinda et al. (2011) found model efficiencies ranging from -0.95 to 0.54 when modeling fluxes from winter wheat using FASSET and MoBiLE-DNDC and yearly CO_2 fluxes were underestimated by 10 to 30%. As evidenced by the wide range in the initial active pool percentages (1 to 14%), the response of soil CO_2 flux was greater during the initial year (2009) as compared to 2010 when all methods resulted in similar CO_2 fluxes.

In general, it is difficult to compare point measurements of temporally highly variable values such as soil respiration to daily model outputs. (Jensen et al., 1996) modeled CO_2 flux with the SOILCO$_2$ model and found a strong decline after rainfall events when the soil moisture was at or above field capacity, owing to the fact of the low diffusivity of CO_2 through water filled pore space. In general, our model results between the plots showed a very low variation while the measured values were much higher. A point of error in the CO_2 flux measurements would be temporally up-scaling point measurements taken over several hours to a 24 hour period. This would most likely lead to the most error during days of large diurnal temperature changes and measuring at either the low or high extreme of this range. In the current study, this did not seem the case as during days that were greatly underestimated by the model (e.g. Fig. 7), the CO_2 flux measurements were taken at soil temperatures close to the average daily value.

In regards to plant growth and residue inputs, root exudates, which are assumed to be around 30% for cereal crops of C allocated belowground (Kuzyakov and Domanski, 2000) are not explicitly included in the model. Especially during times of rapid root growth and exudate production, this can have a significant effect on soil respiration as the exudates provide an easily utilizable source of C for soil microbes. Possibly this is one reason why there is an underestimation of soil CO_2 flux by the model in the early growing period. As is shown both by the estimates for root biomass at harvest compared to the measured (low) and by the simulation over two growing seasons for the Kraichgau, there is an abundance of root litter entering the soil that is not decomposing quickly and is leading to an increase in SOM.

4.6.3 Medium-term implications

During a medium-term model simulation, the slow and passive pool sizes start to affect the SOC contents more strongly, which was seen by the divergence after 20 years of the modeled SOC contents among the different model parameterization methods. As we did not test the

pool parameterizations with a long-term experiment, we tested the methods against an assumed steady state as measured by the SOC contents at the end of 2010. If the assumption of a current steady state SOC is correct, then for the Kraichgau the *Fractions* method, followed by the *DRIFTS* and *EGA-1* methods were within one standard error of the baseline, while in the Swabian Alb, the *Equilibrium* method was the closest to the baseline value. There is some evidence of mostly relatively stable long-term SOC contents in the region. In the neighboring state of Bavaria 54% of SOC contents in cropland plots were unchanged, 25% significantly decreased, while 21% significantly increased during the period 1986 to 2007 (Capriel, 2013). In some long-term experiments in Western Europe, varying results have been given for the dynamic level of SOC under different fertilizer and crop management regimes (Kelly et al., 1997). Werth et al. (2005) studied long-term SOM in the Black Forest and Swabian Alb areas in differently managed grasslands and found some treatments declining in SOC. Additionally, in a 13-year liming experiment in Norway Spruce forests it was found that SOC was declining in organic horizons (Lorenz et al., 2001). In Central Germany, it was shown in a two-year study using eddy covariance measurements and modeling, that one year the soil was a net sink of C, while the next year a source and modeling of pre-experiment site conditions showed that SOC levels have decreased around 7% during the last 30 years under typical management (Anthoni et al., 2004).

The amount of litter returns to soil is a major driver of SOC dynamics and hence for the equilibrium run, historical litter returns to the soil are rough estimates at best. As has been shown (Prince et al., 2001) as crop yield per hectare has increased, the harvest index has also changed, making it difficult to estimate returns of residue to soil from yields. Additionally, farmers' practices over the years may also have changed such as removing all residues in some years, while retaining in others. This would additionally add to uncertainties in the long-term equilibrium run and additionally to the influence on SOM pools in the past. This would be a major reason to use measured fractions instead of assuming equilibrium at a site of unknown historical land use.

Correct model initialization is important as was shown by our long-term simulation that already after 20 years a more then 14 Mg ha^{-1} divergence could be found among the model initialization methods. In general the *EGA-2* method allocates too much to the passive pool and results in too slow of a turnover and accumulation of SOC. It seems that the allocation in *EGA-1* is a more acceptable approach. As mentioned previously about the importance and

difficulty of splitting measured fractions into model pools, this would also be affected by the constant C:N ratio and turnover time.

4.6.4 Better matching model pools with measured pools

Century and other compartmental SOM models are based on the assumption that SOM within a certain pool is homogeneous and has a single turnover rate which responds equally to soil moisture, temperature, and other external variables. As we know that this is not necessarily the case, it may be advantageous to design model pools that more mimic natural pools which are easily measureable and more compositionally (e.g. chemically, physically) homogenous and react similarly in relation to temperature, moisture, or other factors. The approaches tested in this study cover a wide range of properties measured via different analytical methods (chemical, size, density, vibrational spectroscopy, thermal analytical coupled to vibrational spectroscopy), and while the approaches allocated different amounts to the active, slow, and passive pools, found the same general distribution for the two soils studied. The active and passive pools were large in the Swabian Alb compared to the active and passive pools in the Kraichgau site.

An alternative modeling approach instead of the compartmental SOM models is the continuous quality SOM approach (Ågren and Bosatta, 1996). The continuous quality approach (Q-Soil), while not easily measureable, has a gradient of SOM quality which can vary simultaneously in temperature, moisture, and other parameters along the entire range, more closely approximating the "natural" response of SOM. The EGA analytical methods used in the current study offer a continuous measurement of the thermally evolved CO_2 from a soil sample and a "quality" attribute from the corresponding in $situ_T$ DRIFTS data. It may be possible to adopt this analytical method for use in deriving a continuous quality attribute for the Q-Soil model, but this would require additional testing with the EGA model inputs on long-term experimental data to follow longer term dynamics.

4.7 Conclusions

Six different SOM pool parameterization methods were explored for use in the Century module implemented in LUCIA. It was found that there were differences in pool parameterization effects during the first year (2009), but decreasing in the second year. As compared to measured values of soil respiration and TOC, generally predictions were more successful for the Swabian Alb site compared to the Kraichgau. Possibly this was due either

to too much residue input in the way of roots in the Kraichgau, which decompose more slowly compared to aboveground residues, or also that the pool turnover times were too slow for the Kraichgau. This does not seem to be the case as the bare plot SOC dynamics were modeled relatively well. The methods of *DRIFTS*, *Fractions*, and *EGA-1* parameterizations were similar in the results, as the allocation to the slow pool were all in the same range, while the default and *EGA-2* method were found to be similar. For the short-term dynamics of CO_2, *Fractions* and *DRIFTS* methods had the highest indicators of model success, but in most cases had a modeling efficiency of <0, meaning that the model result was worse than using a simple average for predicting the measured values. Overall, in the medium-term simulation (20 years) there was a wide divergence between the pool parameterizations at the end of the simulation period. In the Kraichgau site, the *Fractions*, *DRIFTS*, and *EGA-1* were the closest to the 2010 baseline for SOC, while in the Swabian Alb the *Equilibrium* method was the closest. The different simulation behaviors between the sites may indicate either too high of a residue input at the Kraichgau or too slow of a turnover, as there was a general tendency for increasing SOC. Overall the measured SOM pools performed better as compared to the default and equilibrium derived SOM pools. This study has shown that the wide variation in initial C_a affected the first year results, but not the following year in regards to short-term variation in SOC dynamics, while the C_s parameterization method influenced medium-term results, over a 20 year time period.

Acknowledgements

References

Ågren, G.I., Bosatta, E., 1996. Quality: A bridge between theory and experiment in soil organic matter studies. *Oikos* **76**, 522-528.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotranspiration: Guidelines for computing crop water requirements. FAO irrigation and drainage paper no. 56.

Anthoni, P.M., Freibauer, A., Kolle, O., Schulze, E., 2004. Winter wheat carbon exchange in Thuringia, Germany. *Agricultural and Forest Meteorology* **121**, 55-67.

Baes, A.U., Bloom, P.R., 1989. Diffuse reflectance and transmission fourier transform infrared (DRIFT) spectroscopy of humic and fulvic acids. *Soil Science Society of America Journal* **53**, 695-700.

Bennett, N.D., Croke, B.F.W., Guariso, G., Guillaume, J.H.A., Hamilton, S.H., Jakeman, A.J., Marsili-Libelli, S., Newham, L.T.H., Norton, J.P., Perrin, C., Pierce, S.A., Robson, B., Seppelt, R., Voinov, A.A., Fath, B.D., Andreassian, V., 2013. Characterising performance of environmental models. *Environmental Modelling and Software* **40**, 1-20.

Bruun, S., Christensen, B.T., Hansen, E.M., Magid, J., Jensen, L.S., 2003. Calibration and validation of the soil organic matter dynamics of the daisy model with data from the askov long-term experiments. *Soil Biology and Biochemistry* **35**, 67-76.

Bruun, S., Jensen, L.S., 2002. Initialisation of the soil organic matter pools of the Daisy model. *Ecological Modelling* **153**, 291-295.

Capriel, P., 2013. Trends in organic carbon and nitrogen contents in agricultural soils in Bavaria (south Germany) between 1986 and 2007. *European Journal of Soil Science* **64**, 445-454.

Capriel, P., 1997. Hydrophobicity of organic matter in arable soils: Influence of management. *European Journal of Soil Science* **48**, 457-462.

Cerri, C.E.P., Paustian, K., Bernoux, M., Victoria, R.L., Melillo, J.M., Cerri, C.C., 2004. Modeling changes in soil organic matter in Amazon forest to pasture conversion with the Century model. *Global Change Biology* **10**, 815-832.

Chirinda, N., Kracher, D., Lægdsmand, M., Porter, J.R., Olesen, J.E., Petersen, B.M., Doltra, J., Kiese, R., Butterbach-Bahl, K., 2011. Simulating soil N_2O emissions and heterotrophic CO2 respiration in arable systems using FASSET and MoBiLE-DNDC. *Plant and Soil* **343**, 139-160.

Coleman, K., Jenkinson, D.S., Crocker, G.J., Grace, P.R., Klír, J., Körschens, M., Poulton, P.R., Richter, D.D., 1997. Simulating trends in soil organic carbon in long-term experiments using RothC-26.3. *Geoderma* **81**, 29-44.

Collins, H.P., Elliott, E.T., Paustian, K., Bundy, L.G., Dick, W.A., Huggins, D.R., Smucker, A.J.M., Paul, E.A., 2000. Soil carbon pools and fluxes in long-term corn belt agroecosystems. *Soil Biology and Biochemistry* **32**, 157-168.

Del Grosso, S.J., Parton, W.J., Mosier, A.R., Holland, E.A., Pendall, E., Schimel, D.S., Ojima, D.S., 2005. Modeling soil CO2 emissions from ecosystems. *Biogeochemistry* **73**, 71-91.

Demyan, M.S., Rasche, F., Schütt, M., Smirnova, N., Schulz, E., Cadisch, G., 2013. Combining a coupled FTIR-EGA system and in situ DRIFTS for studying soil organic matter in arable soils. *Biogeosciences* **10**, 2897-2913.

Demyan, M.S., Rasche, F., Schulz, E., Breulmann, M., Müller, T., Cadisch, G., 2012. Use of specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy to study the composition of organic matter in a haplic chernozem. *European Journal of Soil Science* **63**, 189-199.

DWD, 2013. German weather service. 2013.

Egli, M., Mavris, C., Mirabella, A., Giaccai, D., 2010. Soil organic matter formation along a chronosequence in the morteratsch proglacial area (Upper Engadine, Switzerland). *Catena* **82**, 61-69.

Gillman, G.P., Sumpter, E.A., 1986. Modification to the compulsive exchange method for measuring exchange characteristics of soils. *Australian Journal of Soil Research* **24**, 61-66.

Grant, R.F., 1997. Changes in soil organic matter under different tillage and rotation: Mathematical modeling in ecosys. *Soil Science Society of America Journal* **61**, 1159-1175.

Hansen, S., Jensen, H.E., Nielsen, N.E., Svendsen, H., 1991. Simulation of nitrogen dynamics and biomass production in winter wheat using the Danish simulation model DAISY. *Fertilizer Research* **27**, 245-259.

Harris, D., Horwáth, W.R., Van Kessel, C., 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. *Soil Science Society of America Journal* **65**, 1853-1856.

Heinemeyer, A., Croft, S., Garnett, M.H., Gloor, E., Holden, J., Lomas, M.R., Ineson, P., 2010. The MILLENNIA peat cohort model: Predicting past, present and future soil carbon budgets and fluxes under changing climates in peatlands. *Climate Research* **45**, 207-226.

Jenkinson, D.S., Coleman, K., 1994. Calculating the annual input of organic matter to soil from measurements of total organic carbon and radiocarbon. *European Journal of Soil Science* **45**, 167-174.

Jensen, L.S., Mueller, T., Tate, K.R., Ross, D.J., Magid, J., Nielsen, N.E., 1996. Soil surface CO2 flux as an index of soil respiration in situ: A comparison of two chamber methods. *Soil Biology and Biochemistry* **28**, 1297-1306.

Joergensen, R.G., Mueller, T., 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the kEN value. *Soil Biology and Biochemistry* **28**, 33-37.

Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the kEC value. *Soil Biology and Biochemistry* **28**, 25-31.

Juston, J., Andrén, O., Kätterer, T., Jansson, P., 2010. Uncertainty analyses for calibrating a soil carbon balance model to agricultural field trial data in Sweden and Kenya. *Ecological Modelling* **221**, 1880-1888.

Kätterer, T., Andrén, O., 2009. Predicting daily soil temperature profiles in arable soils in cold temperate regions from air temperature and leaf area index. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science* **59**, 77-86.

Kelly, R.H., Parton, W.J., Crocker, G.J., Graced, P.R., Klír, J., Körschens, M., Poulton, P.R., Richter, D.D., 1997. Simulating trends in soil organic carbon in long-term experiments using the Century model. *Geoderma* **81**, 75-90.

Kottek, M., Grieser, J., Beck, C., Rudolf, B., Rubel, F., 2006. World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift* **15**, 259-263.

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. review. *Journal of Plant Nutrition and Soil Science* **163**, 421-431.

Lardy, R., Bellocchi, G., Soussana, J., 2011. A new method to determine soil organic carbon equilibrium. *Environmental Modelling and Software* **26**, 1759-1763.

Leifeld, J., Reiser, R., Oberholzer, H., 2009. Consequences of conventional versus organic farming on soil carbon: Results from a 27-year field experiment. *Agronomy Journal* **101**, 1204-1218.

Liu, S., Anderson, P., Zhou, G., Kauffman, B., Hughes, F., Schimel, D., Watson, V., Tosi, J., 2008. Resolving model parameter values from carbon and nitrogen stock measurements in a wide range of tropical mature forests using nonlinear inversion and regression trees. *Ecological Modelling* **219**, 327-341.

Loague, K., Green, R.E., 1991. Statistical and graphical methods for evaluating solute transport models: Overview and application. *Journal of Contaminant Hydrology* **7**, 51-73.

Lorenz, K., Preston, C.M., Feger, K., 2001. Long-term effects of liming on microbial biomass and activity and soil organic matter quality (^{13}C CPMAS NMR) in organic horizons of Norway spruce forests in southern Germany. *Journal of Plant Nutrition and Soil Science* **164**, 555-560.

Ludwig, B., John, B., Ellerbrock, R., Kaiser, M., Flessa, H., 2003. Stabilization of carbon from maize in a sandy soil in a long-term experiment. *European Journal of Soil Science* **54**, 117-126.

Manning, D.A.C., Lopez-Capel, E., Barker, S., 2005. Seeing soil carbon: Use of thermal analysis in the characterization of soil C reservoirs of differing stability. *Mineralogical Magazine* **69**, 425-435.

Marohn, C., Cadisch, G., 2011. Documentation of the LUCIA model. Department of Plant Production and Agroecology in the Tropics and Subtropics, Univ. of Hohenheim, Stuttgart. v 1.2, Available from: www.lucia.uni-hohenheim.de (2011). Accessed 25 September, 2013.

Marohn, C., Schreinemachers, P., Quang, D.V., Berger, T., Siripalangkanont, P., Nguyen, T.T., Cadisch, G., 2012. A software coupling approach to assess low-cost soil conservation strategies for highland agriculture in Vietnam. Environmental *Modelling and Software* **45**, 116-128.

Michel, K., Ludwig, B., 2010. Prediction of model pools for a long-term experiment using near-infrared spectroscopy. *Journal of Plant Nutrition and Soil Science* **173**, 55-60.

Nash, J.E., Sutcliffe, J.V., 1970. River flow forecasting through conceptual models part I - A discussion of principles. *Journal of Hydrology* **10**, 282-290.

O'brien, B.J., Stout, J.D., 1978. Movement and turnover of soil organic matter as indicated by carbon isotope measurements. *Soil Biology and Biochemistry* **10**, 309-317.

Parton, W.J., Schimel, D.S., Cole, C.V., Ojima, D.S., 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Science Society of America Journal* **51**, 1173-1179.

Paul, E.A., Morris, S.J., Conant, R.T., Plante, A.F., 2006. Does the acid hydrolysis-incubation method measure meaningful soil organic carbon pools? *Soil Science Society of America Journal* **70**, 1023-1035.

Prince, S.D., Haskett, J., Steininger, M., Strand, H., Wright, R., 2001. Net primary production of U.S. Midwest croplands from agricultural harvest yield data. *Ecological Applications* **11**, 1194-1205.

Roodenburg, J., 1985. Estimating 10-cm soil temperatures under grass. *Agricultural and Forest Meteorology* **34**, 41-52.

Rose, C.W., Williams, J.R., Sander, G.C., Barry, D.A., 1983. A mathematical model of soil erosion and deposition processes: I. theory for a plane land element. *Soil Science Society of America Journal* **47**, 991-995.

Rutberg, R.L., Schimel, D.S., Hajdas, I., Broecker, W.S., 1996. The effect of tillage on soil organic matter using [14]C: A case study. *Radiocarbon* **38**, 209-217.

Saxton, K.E., Rawls, W.J., 2006. Soil water characteristic estimates by texture and organic matter for hydrologic solutions. *Soil Science Society of America Journal* **70**, 1569-1578.

Schlichting, E., Blume, H.P., Stahr, K., 1995. Bodenkundliches Praktikum.

Schüller, H., 1969. Die CAL-methode, eine neue Methode zur bestimmung des pflanzenverfügbaren Phosphors in Böden. *Zeitschrift für Pflanzenernährung und Bodenkunde* **123**, 48-63.

Semmens, D.J., Goodrich, D.C., Unkrich, C.L., Smith, R.E., Woolhiser, D.A., Miller, S.N., 2008. KINEROS2 and the AGWA Modeling Framework. In: Wheater, H., Sorooshian, S., Sharma, K.D. (Eds.), Hydrological Modelling in Arid and Semi-Arid Areas. Cambridge University Press, London, pp. 49-69.

Skjemstad, J.O., Spouncer, L.R., Cowie, B., Swift, R.S., 2004. Calibration of the Rothamsted organic carbon turnover model (RothC ver. 26.3), using measurable soil organic carbon pools. *Australian Journal of Soil Research* **42**, 79-88.

Spaccini, R., Piccolo, A., Haberhauer, G., Stemmer, M., Gerzabek, M.H., 2001. Decomposition of maize straw in three European soils as revealed by DRIFT spectra of soil particle fractions. *Geoderma* **99**, 245-260.

Stamati, F.E., Nikolaidis, N.P., Schnoor, J.L., 2013. Modeling topsoil carbon sequestration in two contrasting crop production to set-aside conversions with RothC - calibration issues and uncertainty analysis. *Agriculture, Ecosystems and Environment* **165**, 190-200.

Supit, L., 2003. *Updated system description of the WOFOST crop growth simulation model as implemented in the crop growth monitoring system applied by the European Commission*. Treemail Publishers, Heelsum, The Netherlands.

Tatzber, M., Stemmer, M., Spiegel, H., Katzlberger, C., Zehetner, F., Haberhauer, G., Roth, K., Garcia-Garcia, E., Gerzabek, M.H., 2009. Decomposition of carbon-14-labeled organic amendments and humic acids in a long-term field experiment. *Soil Science Society of America Journal* **73**, 744-750.

van Deursen, W.P.A., 1995. Geographical information systems and dynamic models: Development and application of a prototype spatial modelling language. PhD Thesis. University of Utrecht, The Netherlands. pp. 190.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* **19**, 703-707.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. *Soil Biology and Biochemistry* **39**, 2183-2207.

Weihermüller, L., Graf, A., Herbst, M., Vereecken, H., 2013. Simple pedotransfer functions to initialize reactive carbon pools of the RothC model. *European Journal of Soil Science* .**64,** 567-575.

Werth, M., Brauckmann, H., Broll, G., Schreiber, K., 2005. Analysis and simulation of soil organic-carbon stocks in grassland ecosystems in SW Germany. *Journal of Plant Nutrition and Soil Science* 1**68**, 472-482.

Wizemann, H.-D., Ingwersen, J., Högy, P., Warrach-Sagi, K., Streck, T., Wulfmeyer, V., submitted. Long-term dynamics of water vapor, carbon dioxide and sensible heat fluxes over agricultural crops in two regional climates of south-west Germany. *Agriculture, Ecosystems, and Environment*. submitted.

Wutzler, T., Reichstein, M., 2007. Soils apart from equilibrium - consequences for soil carbon balance modelling. *Biogeosciences* **4**, 125-136.

Xu, X., Liu, W., Kiely, G., 2011. Modeling the change in soil organic carbon of grassland in response to climate change: Effects of measured versus modelled carbon pools for initializing the Rothamsted carbon model. *Agriculture, Ecosystems & Environment* **140**, 372-381.

Yadav, V., Malanson, G.P., Bekele, E., Lant, C., 2009. Modeling watershed-scale sequestration of soil organic carbon for carbon credit programs. *Applied Geography* **29**, 488-500.

Yeluripati, J.B., van Oijen, M., Wattenbach, M., Neftel, A., Ammann, A., Parton, W.J., Smith, P., 2009. Bayesian calibration as a tool for initialising the carbon pools of dynamic soil models. *Soil Biology and Biochemistry* **41**, 2579-2583.

Zimmermann, M., Leifeld, J., Abiven, S., Schmidt, M.W.I., Fuhrer, J., 2007a. Sodium hypochlorite separates an older soil organic matter fraction than acid hydrolysis. *Geoderma* **139**, 171-179.

Zimmermann, M., Leifeld, J., Fuhrer, J., 2007b. Quantifying soil organic carbon fractions by infrared-spectroscopy. *Soil Biology and Biochemistry* **39**, 224-231.

Zimmermann, M., Leifeld, J., Schmidt, M.W.I., Smith, P., Fuhrer, J., 2007c. Measured soil organic matter fractions can be related to pools in the RothC model. *European Journal of Soil Science* **58**, 658-667.

5 General Discussion

5.1 Molecular characterization of bulk SOM and distribution within SOM fractions

Mid-infrared spectroscopy, utilizing the diffuse reflectance method, has shown itself to be very valuable in soil analysis for the main reasons of ease of sample preparation (dried, ball milled), rapid analysis of a sample, and wide ranging applicability to different soils. The development of chemometric models (soft-modeling), specifically partial least squares and principal component analysis has allowed the successful prediction of soil physical/chemical (Cobo et al., 2010) and biological (Rasche et al., 2013) soil properties. With these soft-modeling approaches, there are no assumptions to the underlying data structure, e.g., that a specific vibrational peak represents a certain parameter of interest. If more of a link of a peak or certain peaks to different SOM properties can be found, this would add to the functionality of the mid-infrared technique in that soil properties could be deduced based strictly on a peak intensity or combination of peaks, without the need for development of chemometric models.

While mid-infrared spectra offer additional information as compared to near-infrared spectra (Soriano-Disla et al., 2014), there is the issue with vibrational functional groups being of overlapping ranges or the same range as another functional group. Special care needs to be taken for a direct interpretation or assignment of the spectral peaks to a molecular functional group or groups. This is why studying a single soil but with differing treatments is valuable in discerning changes which are the result of management and not of soil type or mineralogy. In the current study (chapter 2), it was shown that DRIFT-MIRS specific peaks can be used semi-quantitatively to study the changing composition of SOM in bulk soil in response to different long-term fertilizer applications of either farmyard manure or mineral fertilizer. When certain assumptions hold (i.e. minimal specular reflection and similar mineralogy), this method offers a rapid assessment on the relative contribution of certain functional groups (e.g. C-H, -COO$^-$, and C=C) in SOM. Additionally the ratio of the aromatic to the aliphatic peaks (1620 cm^{-1} to 2930 cm^{-1}) in the samples from the Haplic Chernozem were positively correlated to the ratio of stable C (that within the >1.8 g cm^{-3} and clay fractions) to labile C (that contained within the light density fraction of <1.8 g cm^{-3}). It offers a quicker, non-destructive alternative to expensive and time consuming analyses (e.g. nuclear magnetic resonance). Previous studies have investigated the use of other peaks for studying SOM (Egli

et al., 2010; Grube et al., 2006) which either were not found in the current study or were not found to be affected by the long-term treatments. It could be the case that certain peak areas may reflect the fact of different mineralogies or textures of the soils or parent materials. Another promising approach in linking peaks with SOM quality is the use of 2D correlation and combining MIRS and NMR data (Forouzangohar et al., 2013). A possible way forward would be to use different statistical methods in order to discern mineral contribution as a first step and then as a second step, to distinguish spectral differences based on SOM differences. This would require some *a priori* information about the soils and a hypothesis about which peak(s) should represent either solely mineral, mineral and SOM, and solely (or nearly solely) SOM. Once the three groups of peaks are formed, this could be used as constraints or data inputs for a cluster or principle components analysis using a decision tree of where to divide the sample set. Principal component analysis has been used to distinguish different soil types which were then used as different classes to form predictions for soil nitrate (Linker et al., 2005) and decision tree regression has been used for predicting soil parameters as an alternative to PLSR ((Minasny and McBratney, 2008).

5.2 Thermal stability and SOM quality

The current study used the working hypothesis that under thermal decomposition, less stable SOM component will be evolved at lower temperatures, while more stable compounds will be evolved at higher temperatures, though this premise has also been cast into doubt by previous studies (as already mentioned). Intuitively, the hypothesis makes sense, as the strength of a chemical bond should be proportional to the energy that is required to break it, either through microbial utilization or through thermal decomposition, which is why previously thermal stability was related SOM stability. If we go back to the development and first utilization of the main thermo-analytical methods such as thermogravimetry or scanning colorimetery, these were mainly used in material sciences and on relatively simple compounds (e.g. clay minerals, chemical catalysts). The reaction pathways were relativity simple, usually a loss of water or dehydration reactions, changes in crystal structure, and evolvement of different volatiles if present. It was recognized that applying this methods to SOM, that there were overlapping reactions and that SOM was thermally decomposed over a large temperature range (ca. 200-700°C) (Gaál et al., 1994). Additionally it is known that SOM is made up of a multiple of organic and inorganic functional groups which can be

associated with the mineral portion of the soil. There is also the production of reaction intermediates during analysis especially when using a non-oxidizing atmosphere. These factors could be the reasons why sometimes there is not a clear connection between SOM thermal stability and age. In the current study, through the use of in situ$_T$ DRIFTS in combination with FTIR-EGA and a peaking fitting routine, it was possible to follow both the thermal degradation of SOM while heating and also create a quality distribution of the evolved C based on changes in molecular vibration intensities (chapter 3). While evolved gas analysis under an oxidizing atmosphere does not give much information by itself other than the total amount of C evolved, when coupling with monitoring of the mid-infrared spectra of the surface of the soil sample itself, it was shown that different functional groups were being thermal degraded at different temperatures in the order of C-O, C-H, -COO$^-$/C=C, and C=C during the interval from 200 to 700°C. This then enabled a least squares peak fitting procedure to be done on the evolved gas thermograms giving a distribution of different fractions or stabilities of the SOM. Typically peak or curve fitting is used in vibrational spectroscopy to unravel overlapping peaks for further quantification (Meier, 2005), while in the current study, peak fitting was used on the EGA-FTIR thermogram. In cases of severely overlapped peaks, further independent information should be obtained regarding peak position (Meier, 2005), which in our case has been supplied by the maximum or near maximum decreases in vibrational intensity as measured by in situ$_T$ DRIFTS. Without this independent biochemical information, the curve fitting of the thermogram would just be a statistical exercise, without any biochemical basis. This seems to show that indeed, there are overlapping reactions during thermal decomposition.

Other studies have investigated the use of in situ$_T$ DRIFTS mainly for the study of humic acids (Lu et al., 1997), coal samples (Murakami et al., 1997), or clay minerals (Frost and Vassallo, 1996) in which thermal reactions were monitored. While this new method is not an inline method, that is the in situ$_T$ DRIFTS and FTIR-EGA procedures must be done one at a time, unless there is a second mid-infrared spectrometer available, the instrumentation does not have expensive columns to replace such as in a mass spectrometer or gas chromatograph, and sample preparation only requires a finely ball milled sample. This method joins the other established methods of coupling different types of analyses such as thermogravimetry and EGA (Ferrasse et al., 2003), gas chromatography (GC) and mass spectroscopy (MS) (De la Rosa et al., 2008), and differential thermal analysis/thermogravimetry with a field ionization mass spectrometer (FIMS) (Leinweber et al., 1992) for studying soils or SOM fractions.

Interpretation of FTIR-EGA data is relatively straightforward for polar gases (e.g. CO_2, H_2O, NH_3) and can be calibrated with known standards (Eigenmann et al., 2006), and when coupled with in situ$_T$ DRIFTS measurements, gives a complimentary quality indicator during the reaction sequence. This is in contrast to the large numbers of peaks in the data output of FIMS, the problems with overlapping reactions in thermogravimetery, and already mentioned expensive columns for GC and MS.

5.3 Linkage of measureable fractions to model pools

It has been previously pointed out, that most conceptual SOM model pools do not very well correspond to fractions that are easily measured (Christensen, 1996). Smith et al. (2002) argued that a measured SOM fraction is only equivalent to a model pool if it is both unique (related to the inputs, decomposition products, and turnover) and non-composite (do the proportions of decomposition products, order and rate of decomposition change with different soil conditions). At present most models assume an equal response of all pools to abiotic factors which is being cast into doubt in the case of temperature response of different ages of SOM (Vanhala et al., 2007; Conen et al., 2008). While it would not necessarily be better to have additional SOM model pools compared to the 3 to 5 that are common in most models, it may also cause difficulties in forcing different measureable fractions into a defined model pool. As was mentioned previously, Zimmermann et al. (2007) used a model run (Roth-C) to derive the partitioning of different fractions into model pools. In the case of Sitompul et al. (2000), a modification of the slow Century pool was made in order to better fit decomposition as measured by different density fractions in a land-use change sequence. In the current work experience of using different measureable fractions as model pool inputs, it was seen that with the current model construction and turnover rates of pools, that certain pool allocations (i.e. EGA-2, Chapter 4) were not suited to the model structure to simulation SOM dynamics. Perhaps as in the approach of Sitompul et al. (2000), a splitting of the slow model pool could facilitate better results, but this would also raise the question of whether this model structure could be applied at other sites.

A positive result from the current work, was that in all SOM pool parameterization methods except for the default pool parameterization, the active and passive pools were smaller in the Kraichgau, while the passive pool was larger in the Swabian Alb in the model initialization.

This consistent distribution would indicate that the different methods are in some way measuring the same active, slow, and passive pools.

Alternatively, the mechanisms behind substrate availability and microbial use efficiency of a particular residue may be more important driving factors in SOM turnover and mineralization then previously though, which have implications for long-term stabilization (Sierra et al., 2011). Since these factors are often confounding in that one factor may increase turnover, while another factor may inhibit it, making it difficult to study under field conditions. A first step could be to focus on the mechanisms as affected by different forcing variables (temperature, moisture, substrate quality, soil type) in the laboratory where conditions are easier to control and then upscale to the plot level through the use of bare fallow plots to study native SOM and microbial interactions, or to apply residues of different qualities. Another method could be to use root exclusion via fine mesh (i.e. Gavrichkova et al., 2010) to exclude root growth into inter-row areas but still have the shading effect and water and nutrient uptake from the crop that would better mimic "natural" conditions compared to fallow plots and be easier to implement compared to pulse isotopic labeling. Such an approach could attempt to reconcile the differences in short-term (within season) dynamics, to be combined with the characterization of the slow and passive pools via FTIR-EGA and DRIFTS for medium and long-term SOM dynamics.

5.4 Future work

It is evident not only from the work in this dissertation, but in the wealth of SOM work, that there is not a "one size fits all" approach for studying SOM, nor is there one single method which can accomplish all research objectives in the realm of SOM characterization. A strength of any one method is if it can be applied across many different conditions, soil types, management types, and ecosystems and compliments results found via other methods. While in the current study (chapters 2-4) we have applied the various methods to a wide variety of compounds (e.g. chemical standards, SOM fractions, 3 different soils of different managements), it would be a further test of the methods to apply to different land-use types (e.g. forest, grasslands) and also to a variety of soil types. As already seen in the current study, there was a different behavior of the different pool parameterization methods between the two sites, suggesting additional influences on the SOM dynamics which was not well modeled. Inputs of residues, especially below ground root and root exudates are very

important to the C dynamics and must be correctly accounted for. With model integration, future work should focus on the further incorporation of measureable compounds/pools to use as model inputs. While conceptual pools that work on a mechanistic basic and do provide for the relative accurate modeling of SOC dynamics, nearly always the models must be adapted for use in different soils/sites/ecosystems. The interaction of environmental factors (e.g. temperature, moisture, SOM quality) which influence the turnover and cycling of SOM need to be further investigated in order to be able to prediction future behavior of the soil system under climate change. Further development of methods to measure microbial community dynamics and soil structure and SOM distribution in situ with new sensor technology (e.g. Allen et al., 2007) and incorporation of this additional information may be a promising way forward to further SOM modeling in addition to the physiological status and changes of the microbial biomass during the year (Kramer et al., 2013). If the goal of a model is merely to predict changes in SOM in response to different variables up to the present, then the models thus far have done a fairly good job at this task but usually need to be adjusted for new sites. If we want to deepen our understanding of the complex mechanisms and interrelationships between different components of the system and arrive at reasonably accurate forecasts for the future, we need to develop both better methods for SOM characterization of not just the quantity but also the quality and measuring both short term and long term dynamics. Additionally, it is essential to develop long-term experiments in land use or geographical areas which are underrepresented in the current suite of long-term experiments (tropics, semi-arid, both extensive and intensive land uses) for the study of SOM dynamics and to maintain existing networks and experiments. In the current study and in many others, the Static Fertilization Experiment at Bad Lauchstädt (Körschens et al., 1994) has proven invaluable as it offers a long-term record of differing fertilizer management schemes and crop and soil data that can be studied using various methods.

SOM modeling approaches such as the Q-soil model (Ågren and Bosatta, 1996) cognitively provide a better conceptual fit to what hypothesized SOM pools are like, as compared to the classical discrete or compartmental models (e.g. Century, Daisy). The model has been used previously, but in the application of following the decomposition of a single litter source such as pine and spruce forests (Ågren and Hyvönen, 2003), or of studying the impact of different residues applied over decades where starting native SOM was assumed to have the same quality distribution (Hyvönen et al., 1996). Most likely an issue in the limited amount of

applications of this model has been the ability of current SOM characterization methods to measure the quality distribution of SOM that is related to its turnover and defining what exactly is "quality." While several methodological approaches have been proposed (Bruun et al., 2010), these methods have not been applied to the quality distribution question for model incorporation. It seems as though a combination of methods will probably be needed in order to characterize SOM in multiple facets relevant for both short and long-term turnover such as substrate quality and association with mineral surfaces. Such an approach as suggested in (Bruun et al., 2010) could combine a continuous density separation via a heavy liquid (Dyrkacz et al., 1996) and then chemical characterization (DRIFTS, FTIR-EGA, Fe/Al oxide content, stable isotopes) of discrete fractions taken from the continuous separation. In this way not only is SOM separated on an increasing association with mineral particles, but also the chemical composition is studied.

We have seen that even though there has been much done in the way of SOM research over the last 15-20 years, we are still struggling to understand not only the complex interplay of factors operating on a mechanistic level, but also at large spatial scales (e.g. continental/global) or over time. Understanding the feedbacks between the different cycles on different scales will require the development of additional new techniques and integration of different earth science (geology, meteorology, paleoclimatology, and hydrology) and social sciences with the agricultural/soil sciences. The general topics that we are studying today have already been realized for nearly a century and are building on the early work of some of the first soil and agricultural scientists, whom even a century ago realized the complex web of factors influencing the soil-sphere. In the intervening century, our methods have advanced exponentially, but as the saying goes, "the more we know, the more we know we don't know." Hopefully this dissertation can serve as a small advancement in development of methods in the quest that McBratney et al. (2006) has called "solving the soil data crisis."

References:

Ågren, G.I. & Hyvönen, R. 2003. Changes in carbon stores in Swedish forest soils due to increased biomass harvest and increased temperatures analysed with a semi-empirical model. *Forest Ecology and Management,* **174,** 25-37.

Ågren, G.I. & Bosatta, E. 1996. Quality: A bridge between theory and experiment in soil organic matter studies. *Oikos,* **76,** 522-528.

Allen, M.F., Vargas, R., Graham, E.A., Swenson, W., Hamilton, M., Taggart, M., Harmon, T.C., Rat'ko, A., Rundel, P., Fulkerson, B. & Estrin, D. 2007. Soil sensor technology: Life within a pixel. *Bioscience,* **57,** 859-867.

Bruun, S., Ågren, G.I., Christensen, B.T. & Jensen, L.S. 2010. Measuring and modeling continuous quality distributions of soil organic matter. *Biogeosciences,* **7,** 27-41.

Christensen, B.T. 1996. Matching measurable soil organic matter fractions with conceptual pools in SimulationModels of carbon turnover: Revision of model structure. p. 143-159. *In* D.S. Powlson, P. Smith and J.U. Smith (eds.) Evaluation of soil organic matter models. Springer, Berlin.

Cobo, J.G., Dercon, G., Yekeye, T., Chapungu, L., Kadzere, C., Murwira, A., Delve, R. & Cadisch, G. 2010. Integration of mid-infrared spectroscopy and geostatistics in the assessment of soil spatial variability at landscape level. *Geoderma,* **158,** 398-411.

Conen, F., Karhu, K., Leifeld, J., Seth, B., Vanhala, P., Liski, J. & Alewell, C. 2008. Temperature sensitivity of young and old soil carbon – Same soil, slight differences in 13C natural abundance method, inconsistent results. *Soil Biology and Biochemistry,* **40,** 2703-2705.

De la Rosa, J.M., González-Pérez, J.A., González-Vázquez, R., Knicker, H., López-Capel, E., Manning, D.A.C. & González-Vila, F.J. 2008. Use of pyrolysis/GC-MS combined with thermal analysis to monitor C and N changes in soil organic matter from a Mediterranean fire affected forest. *Catena,* **74,** 296-303.

Dyrkacz, G.R., Ruscic, L., Marshall, C.L. & Reagan, W. 1996. Separation and characterization of FCC catalysts using density gradient separation. *Energy and Fuels,* **10,** 849-854.

Egli, M., Mavris, C., Mirabella, A. & Giaccai, D. 2010. Soil organic matter formation along a chronosequence in the Morteratsch proglacial area (Upper Engadine, Switzerland). *Catena,* **82,** 61-69.

Ferrasse, J.H., Chavez, S., Arlabosse, P. & Dupuy, N. 2003. Chemometrics as a tool for the analysis of evolved gas during the thermal treatment of sewage sludge using coupled TG–FTIR. *Thermochimica Acta,* **404,** 97-108.

Forouzangohar, M., Cozzolino, D., Smernik, R.J., Baldock, J.A., Forrester, S.T., Chittleborough, D.J. & Kookana, R.S. 2013. Using the power of C-13 NMR to interpret infrared spectra of soil organic matter: A two-dimensional correlation spectroscopy approach. *Vibrational Spectroscopy,* **66,** 76-82.

Frost, R.L. & Vassallo, A.M. 1996. The dehydroxylation of the kaolinite clay minerals using infrared emission spectroscopy. *Clays and Clay Minerals,* **44,** 635-651.

Gaál, F., Szöllosy, I., Arnold, M. & Paulik, F. 1994. Determination of the organic matter, metal carbonate and mobile water in soils simultaneous TG, DTG, DTA and EGA techniques. *Journal of Thermal Analysis,* **42,** 1007-1016.

Gavrichkova, O., Moscatelli, M.C., Kuzyakov, Y., Grego, S. & Valentini, R. 2010. Influence of defoliation on CO2 efflux from soil and microbial activity in a Mediterranean grassland. *Agriculture, Ecosystems and Environment,* **136,** 87-96.

Grube, M., Lin, J.G., Lee, P.H. & Kokorevicha, S. 2006. Evaluation of sewage sludge-based compost by FT-IR spectroscopy. *Geoderma,* **130,** 324-333.

Hyvönen, R., Ågren, G.I. & Andrén, O. 1996. Modelling long-term carbon and nitrogen dynamics in an arable soil receiving organic matter. *Ecological Applications,* **6,** 1345-1354.

Körschens, M., Stegemann, K., Pfefferkorn, A., Weise, V. & Müller, A. 1994. *Der Statische Düngungsversuch Bad Lauchstädt nach 90 Jahren. Einfluß der Düngung auf Boden, Pflanze und Umwelt.* B.G. Teubner Verlagsgesellschaft, Stuttgart.

Kramer, S., Marhan, S., Haslwimmer, H., Ruess, L. & Kandeler, E. 2013. Temporal variation in surface and subsoil abundance and function of the soil microbial community in an arable soil. *Soil Biology and Biochemistry,* **61,** 76-85.

Leinweber, P., Schulten, H.-. & Horte, C. 1992. Differential thermal analysis, thermogravimetry and pyrolysis-field ionisation mass spectrometry of soil organic matter in particle-size fractions and bulk soil samples. *Thermochimica Acta,* **194,** 175-187.

Linker, R., Shmulevich, I, Kenny, A. & Shaviv, A. 2005. Soil identification and chemometrics for direct determination of nitrate in soils using FTIR-ATR mid-infrared spectroscopy. *Chemosphere,* **61,** 652-658.

Lu, X.Q., Vassallo, A.M. & Johnson, W.D. 1997. Thermal stability of humic substances and their metal forms: An investigation using FTIR emission spectroscopy. *Journal of Analytical and Applied Pyrolysis,* **43,** 103-114.

McBratney, A.B., Minasny, B. & Viscarra Rossel, R. 2006. Spectral soil analysis and inference systems: A powerful combination for solving the soil data crisis. *Geoderma,* **136,** 272-278.

Meier, R.J. 2005. On art and science in curve-fitting vibrational spectra. *Vibrational Spectroscopy,* **39,** 266-269.

Minasny, B. & McBratney, A.B. 2008. Regression rules as a tool for predicting soil properties from infrared reflectance spectroscopy. *Chemometrics and Intelligent Laboratory Systems,* **94,** 72-79.

Murakami, K., Shirato, H. & Nishiyama, Y. 1997. In situ infrared spectroscopic study of the effects of exchanged cations on thermal decomposition of a brown coal. *Fuel,* **76,** 655-661.

Rasche, F., Marhan, S., Berner, D., Keil, D., Kandeler, E. & Cadisch, G. 2013. MidDRIFTS-based partial least square regression analysis allows predicting microbial biomass, enzyme activities and 16S rRNA gene abundance in soils of temperate grasslands. *Soil Biology and Biochemistry,* **57,** 504-512.

Sierra, C.A., Harmon, M.E. & Perakis, S.S. 2011. Decomposition of heterogeneous organic matter and its long-term stabilization in soils. *Ecological Monographs,* **81,** 619-634. Sitompul, S.M., Hairiah, K., Cadisch, G. & Van Noordwijk, M. 2000. Dynamics of density fractions of macro-organic matter after forest conversion to sugarcane and woodlots, accounted for in a modified Century model. *NJAS - Wageningen Journal of Life Sciences,* **48,** 61-73.

Smith, J.U., Smith, P., Monaghan, R. & MacDonald, A.J. 2002. When is a measured soil organic matter fraction equivalent to a model pool? *European Journal of Soil Science,* **53,** 405-416.

Soriano-Disla, J.M., Janik, L.J., Viscarra Rossel, R.A., MacDonald, L.M. & McLaughlin, M.J. 2014. The performance of visible, near-, and mid-infrared reflectance spectroscopy for prediction of soil physical, chemical, and biological properties. *Applied Spectroscopy Reviews,* **49,** 139-186.

Vanhala, P., Karhu, K., Tuomi, M., Sonninen, E., Jungner, H., Fritze, H. & Liski, J. 2007. Old soil carbon is more temperature sensitive than the young in an agricultural field. *Soil Biology and Biochemistry,* **39,** 2967-2970.

Zimmermann, M., Leifeld, J., Schmidt, M.W.I., Smith, P. & Fuhrer, J. 2007b. Measured soil organic matter fractions can be related to pools in the RothC model. *European Journal of Soil Science,* **58,** 658-667.

6 Summary

Soil organic matter (SOM) is a large part of the global carbon cycle both as a stock, as a source of fluxes (gaseous, dissolved, or sediments) to other stocks, and is also an important component of soil fertility and likewise plant productivity. Due to the growing need for additional data for both global studies related to climate change and soil fertility, additional information is needed not only on the total quantity of SOM, but its distribution within time and space and also its quality. In this study the use of mid-infrared spectroscopy in different applications was explored as an indicator of soil quality or composition, to measure the distribution of quality in different soils and fractions, and how these new methods could be used for SOM model parameterizations compared to other methods for both short and medium term model simulations. Firstly, certain mid-infrared active functional groups as measured with diffuse reflectance spectroscopy (DRIFTS) were studied in a long term fertilization experiment (Bad Lauchstädt) to ascertain the suitability of these different functional groups as indicators of the long term impacts of different fertilizer applications and also in various SOM fractions as separated by size-density approaches. Secondly, a coupled mid-infrared thermally evolved gas analysis was combined with in-situ monitoring of changes in vibrational functional groups to assign different qualities to different temperature ranges during a thermal oxidation experiment to 700 °C. Lastly, these two approaches were compared to traditional SOM fractionation as more rapid alternatives to parameterizing SOM pool sizes in the Century multi-compartment SOM model applied to arable soils at sites in the Kraichgau and Swabian Alb areas in Southwest Germany.

In the long-term experiment (Bad Lauchstädt) it was found that certain vibrational functional groups (i.e. aliphatic (2930 cm^{-1}) and aromatic (1620 cm^{-1})) in bulk soil varied ($P < 0.05$) according to long-term farmyard manure (FYM) and/or mineral fertilizer application. The application of 30 Mg ha^{-1} every second year of FYM increased the proportion of aliphatics as compared to aromatics, while the opposite was true for the control treatment (without any mineral and FYM fertilizer). The ratio of the aromatic to aliphatic relative peak areas were found to be positively related to the ratio of stabilized (SOC in heavy density fractions and clay size fraction) to labile SOC (light density fraction). This indicated that this peak area ratio (aromatic to aliphatic) is an indicator for the relative contribution of stable to labile SOM as a stability index.

In the next phase of the methodological development, evolved gas analysis (EGA) was used during a programmed heating of soil samples to 700°C to link EGA characteristics with SOM. An additional methodological step was the utilization of in-situ diffuse reflectance (in situ$_T$ DRIFTS) measurements during heating as an indicator of the nature of SOM being decomposed at different temperatures. Thermal stability was found to be affected by experimental conditions and also sample type. The heating rate, amount of C in the sample, and volume of the sample in the heating chamber changed the rate and overall shape of the CO_2 evolution curve and needed to be optimized when comparing different SOM fractions. In the long term experiment of Bad Lauchstädt, a decreasing thermal stability as measured by temperature of maximum CO_2 evolution was found in the order from control > mineral fertilizer > manure > manure and mineral fertilizer. Furthermore, after a 490 day soil incubation at 20°C the thermal stability of SOC increased, but only slightly. In the in-situ$_T$ DRIFTS method, the intensity of previously identified vibrational functional groups decreased (degraded) at different temperatures. The functional groups decreased in the order of aliphatic, alcoholic, and carboxylates, and at higher temperatures, also aromatic groups decreased. These findings were used as rules for fitting multiple peaks to the total evolved CO_2 curve to derive SOM pools of different reactivity.

Pools derived from the measured fractions of mid-infrared functional groups (aliphatic, carboxylate/aromatic, aromatic), evolved gas analysis (CO_2) fitted peaks (centered at 320, 380, 540°C), and size-density fractionation (particulate organic matter, heavy density fraction, silt and clay fraction) in addition to a long-term equilibrium model run, were used to parameterize the SOM pools of the Century model as implemented in the Land Use Change Assessment tool (LUCIA) and compared to measured soil surface CO_2 fluxes and soil organic carbon (SOC) contents after 2 years. The best fits for the short term study were found to be the SOM fractionation DRIFTS and EGA pool initialization methods, but the differences over two years were very small for the three different parameterization methods and generally CO_2 fluxes were underestimated. A 20 year simulation, keeping all rate constants the same, on the other hand, showed large changes in both the SOC (14 Mg ha^{-1} to 30 cm) and the distribution in the pools. As compared to the 2010 baseline SOC, the DRIFTS, EGA-1, and SOM fractionation methods were closest in the Kraichgau site, while the equilibrium method was closest in the Swabian Alb.

Overall, DRIFT mid-infrared spectroscopy showed its utility as a rapid assessment of the general distribution of stable to labile SOM in bulk soil. Additionally, when coupled with

EGA and in-situ DRIFTS measurements, the integrated method can provide additional information during the thermal degradation of SOM during heating. All methods investigated found changes as a result of soil fertilization management, and between SOM fractions. Lastly, it was shown that such information can be used for direct SOM model inputs, although the methods should be tested on further land uses and soil types. These mid-infrared thermally coupled spectroscopic techniques represent an advance in the use of mid-infrared spectroscopy in the field of detailed SOM characterization for modeling SOM dynamics.

7 Zusammenfassung

Organische Bodensubstanz (OBS) hat als Kohlenstoffspeicher, sowie als Ursprung von Stoffflüssen (gasförmig, gelöst, oder als Sediment) zu anderen Speichern oder Prozessen, einen großen Anteil am globalen Kohlenstoffkreislauf und ist ein wichtiger Faktor der Bodenfruchtbarkeit und somit der pflanzlichen Produktivität . Auf Grund des steigenden Bedarfs an genaueren Daten für globale Studien zu Klimawandel und Bodenfruchtbarkeit werden zusätzliche Informationen, nicht nur für die Ermittlung der Gesamtmenge an OBS benötigt, sondern auch für dessen Verteilung in Raum und Zeit und zu dessen Qualität. In dieser Studie wurde die Verwendung der Spektroskopie im mittleren Infrarot-Bereich (MIR-Spektroskopie) als Indikator für Bodenqualität oder Bodenzusammensetzung für verschiedene Anwendungen untersucht, um somit die Verteilung der Beschaffenheit verschiedener Böden und Fraktionen zu messen und um zu erörtern wie diese neuen Methoden im Vergleich zu traditionellen Methoden für Kurz- und Langzeitsimulationen bei einer OBS-Modellparametisierung verwendet werden können. Als erstes wurden durch Diffus-Reflexions-Infrarot-Fourier-Transformations-*Spektroskopie (DRIFTS)* bestimmte, im mittleren Infrarot aktive, funktionelle organische Gruppen in Bodenproben von einem Langzeitdüngungsexperiment (Bad Lauchstädt) untersucht, um die Eignung der verschiedenen schwingungsfähigen funktionellen Gruppen als Indikatoren für die langfristigen Auswirkungen verschiedener Düngebehandlungen, als auch deren Vorkommen in verschiedenen, mit Dichtefraktionierungsmethoden getrennten, OBS-Fraktionen festzustellen. Als zweites wurde eine gekoppelte MIR-Emissionsgasanalyse mit einem In-Situ-Monitoring kombiniert um bei einer thermischen Oxidation bis 700°C den in unterschiedlichen Temperaturbereichen auftretenden Veränderungen der schwingungsfähigen, funktionellen Gruppen bestimmte Eigenschaften zuordnen zu können. Schließlich wurden anhand von Ackerböden von Standorten in der Kraichgau und Schwäbischen Alb in Südwestdeutschland beide Ansätze als effizientere Alternativen zu traditionellen OBS-Fraktionierungen zur Parametrierung von OBS-Poolgrößen im Century-OBS-Modell verglichen.

Das Langzeitexperiment (Bad Lauchstädt) hat gezeigt, dass bestimmter schwingungsfähige, funktionelle Gruppen (z.B. aliphatisch (2930 cm^{-1}) und aromatisch (1620 cm^{-1})) sich je nach Langzeitapplikation von Stalldünger und/oder Mineraldünger im Boden unterscheiden ($P <$ 0.05). Die Applikation von 30 Mg ha^{-1} Stalldünger alle zwei Jahre erhöhte den Anteil an

Aliphaten gegenüber Aromaten, während das Gegenteil für die Kontrollbehandlung (ohne Mineraldünger oder Stallmist) gilt. Das Verhältnis der relativen Peakflächen der Aromaten zu dem der Aliphaten steht in positivem Zusammenhang zum Verhältnis von stabilem (organischer Bodenkohlenstoff (SOC) in schweren Dichtefraktionen und Tonfraktion) zu labilem SOC (leichte Dichtefraktion). Das ließ darauf schließen, dass das Peakflächenverhältnis (aromatisch zu aliphatisch) ein Indikator für den relativen Beitrag von stabilem zu unbeständigem OBS ist und somit als Stabilitätsindex dient.

In der nächsten Phase der methodischen Entwicklung, wurde die Emissionsgasanalyse (EGA) während einem programmierten Aufheizen von Bodenproben bis 700°C eingesetzt, um die Verbindung von EGA-Eigenschaften mit OBS zu untersuchen. Ein zusätzlicher Verfahrensschritt war die Verwendung der In-Situ Diffus-Reflexions (in situ$_T$ DRIFTS) Messungen, als ein Indikator des Zersetzung der OBS s bei verschiedenen Temperaturen. Es wurde festgestellt, dass die thermische Stabilität sowohl durch die experimentellen Bedingungen als auch durch den Probentyp beeinflusst wurde. Die Aufheizgeschwindigkeit, die Menge C in der Probe und das Volumen der Probe in der Heizkammer veränderten die Geschwindigkeit und die Gesamtform der CO_2-Emissionskurve und mussten für einen Vergleich der verschiedenen OBS Fraktionen optimiert werden. Auch bei dem Langzeitexperiment wurde anhand der gemessenen Temperatur, der maximalen CO_2^- Entwicklung, eine abnehmende thermische Stabilität in folgender Reihenfolge gefunden: Kontrolle > Mineraldünger > Stallmist > Stallmist und Mineraldünger. Es wurde auch festgestellt, dass sich nach einer 490 Tage dauernden Inkubation die thermische Stabilität geringfügig erhöhte. Bei der In-Situ$_T$ DRIFTS Methode nahm die Intensität der zuvor identifizierten schwingungsfähigen funktionellen Gruppen bei verschiedenen Temperaturen ab. Zuerst verringerte sich die Intensität der aliphatischen, alkoholischen und Carboxylatgruppen, bei höheren Temperaturen auch bei aromatischen Gruppen. Diese temperaturabhängigen Veränderungen dienten der Identifizierung von mehreren Peaks unterschiedlicher Reaktivität auf der CO_2-Kurve, aus denen sich verschiedene OBS-Modell-Pools ableiteten lassen.

Die von den gemessenen Fraktionen der funktionellen Gruppen im mittleren Infrarotbereich (aliphatisch, carboxyl/aromatisch, aromatisch), den angepassten Peaks der CO_2-Emissionsgasanalyse (320°C, 380°C, 540°C), und von den Größen- und Dichtefraktionierung (partikuläre organischen Substanz, schwere Dichtefraktion, Schluff- und Tonfraktion) abgeleiteten Pools, wurden zusammen mit dem Langzeitlauf eines Gleichgewichtsmodells

verwendet um die OBS-Pools des Century-Modells, welches Bestandteil des Modells zur Bewertung von Landnutzungsänderung (LUCIA) ist, zu parametrisieren und mit den an der Bodenoberfläche in zwei Jahren gemessenen CO_2-Flüssen und der organischem Bodenkohlenstoffmenge verglichen. Der erfolgreichsten OBS-Modellpool-Parameterisierungsansätze für die Kurzzeitstudie waren die OBS-Fraktionierung zusammen mit den DRIFTS und EGA Pool-Initialisierungsmethoden. Die Unterschiede über zwei Jahre Laufzeit für die drei verschiedenen Parametrisierungsmethoden waren sehr klein und für gewöhnlich wurden die CO_2-Flüsse unterschätzt. Andererseits hat eine Simulation über einen Zeitraum von 20 Jahren, bei Beibehaltung aller anderen Konstanten, große Veränderungen sowohl bei der Menge an SOC (14 Mg ha^{-1} bis 30 cm) als auch bei der Verteilung in den Pools gezeigt. Im Vergleich zur SOC-Basislinie von 2010, zeigten die DRIFTS, EGA-1 und OBS-Fraktionierungsmethoden für den Standort Kraichgau die größte Annäherung, während die Equilibrium-Methode für den Standort Schwäbische Alb am nächsten war.

Insgesamt zeigte die DRIFT MIR-Spektroskopie ihre Nützlichkeit als schnelle Bewertungsmethode für die allgemeinen Verteilung von stabiler zu unbeständiger organischen Substanz im Boden. Ergänzend kann diese Technik, zusätzliche Informationen zur thermischen Zersetzung von OBS beim Erhitzen liefern, wenn sie mit EGA und In-Situ$_T$ DRIFTS Messungen gekoppelt wird. Alle untersuchten Methoden zeigten Veränderungen zwischen den OBS-Fraktionen als Folge des Bodendüngemanagements. Schließlich wurde gezeigt, dass diese Informationen für direkte OBS-Modelleingaben verwendet werden können, wobei die Methode aber auch noch für andere Landnutzungen und Bodenarten getestet werden sollte. Diese thermisch gekoppelten spektroskopischen Techniken im mittleren Infrarot stellen einen Fortschritt bei der Verwendung der MIR-Spektroskopie auf dem Gebiet der detaillierten OBS-Charakterisierung zur Modellierung von OBS-Dynamiken dar.

Appendix 1: Sources for CENTURY Soil organic matter modelling pool size literature search

Grassland land use

location	SOM pool equilibriza-tion/initalization method	equilibrium landuse	Century version	depth/ cm	sand/ %	clay/ %	MAP/ mm	MAT/ °C	TOC/ Mg ha^{-1}	soil type	A/ %	S/ %	P/ %	source
Nairobi National Park, Kenya	5000 yr. eq.	grassland	3	30	40	35	680	19.7	23.5	vertisol	2.5	62.2	35.3	Parton, 1993
Klong HK Hat Yai, Thailand	5000 yr. eq.	grassland	3	30	58	2	1540	26.4	19.9	humic gley	9.5	61.8	28.6	Parton, 1993
Montecillos Chapingo, Mexico	5000 yr. eq.	grassland	3	30	47	18	590	14.2	53.4	solonet	3.6	57.1	39.3	Parton, 1993
Lamto, Ivory Coast	5000 yr. eq.	grassland	3	30	85	5	1170	28.8	15.2	sandy	3.9	55.9	40.1	Parton, 1993
CPER Pawnee, Colorado, USA	5000 yr. eq.	grassland	3	20	70	15	300	10.5	20.5	sandy loam	2.9	61.0	36.1	Parton, 1993
Konza Prairie, Kansas, USA	5000 yr. eq.	grassland	3	30	25	40	818	13.2	50.9	clay loam	3.7	41.3	55.0	Parton, 1993
Khomutov, Ukraine	5000 yr. eq.	grassland	3	30	20	52	441	13	61.2	vermiboroll	4.4	60.5	35.1	Parton, 1993
Kursk, Russia	5000 yr. eq.	grassland	3	30	32	37	560	6.05	112.5	haplaboroll	3.0	48.9	48.1	Parton, 1993
Otradnoye, Russia	5000 yr. eq.	grassland	3	30	43	17	543	8.55	55.5	soddy podzolic	2.7	55.9	41.4	Parton, 1993
Shortandy, Kazahkstan	5000 yr. eq.	grassland	3	30	36	37	351	1.3	59.6	calciboroll	2.9	36.9	60.2	Parton, 1993
Tuva, Russia	5000 yr. eq.	grassland	3	30	74	9	214	-6.3	41.9	Haplustosol	2.4	35.8	61.8	Parton, 1993
Oensingen,Switz.	Monte Carlo	unknown	DayCent	20	na	43	1109	9	68.4	Eutri-Stagnic Cambisol	10.3	41.0	48.7	Yeluripati, 2009
Oensingen,Switz.	Monte Carlo	unknown	DayCent	20	na	43	1109	9	68.4	Eutri-Stagnic Cambisol	20.7	30.4	49.0	Yeluripati, 2009
Park Grass-control, UK	10-30 yr. eq.	managed grassland	4	20	19	23	728	9.1	68.2	Aquic Palendalf	1.8	64.5	33.7	Kelley et al., 1997
Park Grass-organic, UK	10-30 yr. eq.	grassland+FYM (post-1905)	4	20	19	23	728	9.1	71.7	Aquic Palendalf	2.4	65.6	32.1	Kelley et al., 1997
Park Grass-inorganic, UK	10-30 yr. eq.	grassland+NPK (post-1905)	4	20	19	23	728	9.1	71.2	Aquic Palendalf	1.7	66.0	32.3	Kelley et al., 1997

Forest land use

location	SOM pool equilibriza-tion/initalization method	equilibrium landuse	Century version	depth/ cm	sand/ %	clay/ %	MAP/ mm	MAT/ °C	TOC/ Mg ha^{-1}	soil type	A/ %	S/ %	P/ %	source
Lavarone, Italy	5000 yr. eq.	managed forest	4.5	20	25	28	1150	7.8	84.4	humic umbrisol	2.5	54.9	42.6	Chiti et al., 2010
San Rossore, Italy	5000 yr. eq.	managed forest	4.5	20	94	2	920	14.2	50.9	albic arenosol	2.2	77.1	20.7	Chiti et al., 2010
Roca 1, Italy	5000 yr. eq.	managed forest	4.5	20	36	33	876	15.2	55.1	chromic luvisol	1.6	46.8	51.7	Chiti et al., 2010
Roca 2, Italy	5000 yr. eq.	managed forest	4.5	20	40	35	876	15.2	55.7	chromic luvisol	1.3	61.0	37.7	Chiti et al., 2010
Collelogo, Italy	5000 yr. eq.	managed forest	4.5	20	30	30	1140	7.4	92.2	humic alisol	2.6	59.2	38.2	Chiti et al., 2010
Castelporziano, Italy	5000 yr. eq.	managed forest	4.5	20	89	6	767	15.6	29.1	haplic arenosol	2.0	72.9	25.1	Chiti et al., 2010
Secano Interior, Chile,	2000 yr. eq.	open woodland to Espino	4.5	20	na	28-^{3}40	695	14.8	44.7	Ultic Palexeralfs	2.6	51.5	38.6	Stople et al., 2008
Secano Interior, Chile,	2000 yr. eq.	open woodland to Espino	4.5	20	na	28-40	695	14.8	40.2	Ultic Palexeralfs	2.4	46.8	43.8	Stople et al., 2008
Secano Interior, Chile,	2000 yr. eq.	open woodland to Espino	4.5	20	na	28-40	695	14.8	24.7	Ultic Palexeralfs	2.2	52.6	36.7	Stople et al., 2008
Geescroft wilderness, UK		hardwood forest	Parton, 87	20	na	21	704	9.3	24.9	Aquic/Typic Paleu-dalf	4.7	38.1	57.2	Falloon and Smith, 2002
Calhoun Forest, SC, USA	10-30 yr. eq.	loblolly pine forest	4	20	68	15	1250	17	14.4	Typic Kanhapludult	2.8	27.8	69.4	Kelley et al., 1997
Geescroft, UK	10-30 yr. eq.	hardwood	4	20	19	23	728	9.1	26.2	Aquic Palendalf	4.7	38.1	57.2	Kelley et al., 1997
Yarraman State For., Australia	3000 yr. eq.	tropical rainforest	4.5.1	30	27	53	744	4.4 to 29.7	138.0	Ferrasol	na	46.7	na	Richards et al., 2007
Nova Vida Ranch, Brazil	10000 yr. eq.	open humid tropical forest	4	20	67	26.2	2200	25.6	25.0	Kandiuldults, Paleudults	2.0	61.0	37.0	Cerri et al., 2004

Arable land use

location	SOM pool equilibriza-tion/initalization method	equilibrium landuse	Century version	depth/ cm	sand/ %	clay/ %	MAP/ mm	MAT/ °C	TOC/ Mg ha^{-1}	soil type	A/ %	S/ %	P/ %	source
Agramunt, Spain	5100 yr. eq.	open forest to barley-fallow	4	30	47	11.8	430	13.8	29.8	Typic Xerofluvent	7.5	18.1	74.4	Álvaro-Fuentes, et al., 2012
Prague-organic+NPK, Czech Republic	10-30 yr. eq.	rotation-1	4	20	46	27	523	8	26.2	Medudalf	4.7	22.9	72.5	Kelley et al., 1997
Prague-control, Czech Republic	10-30 yr. eq.	sugar beet, spring wheat	4	20	46	27	523	8	31.2	Medudalf	3.9	19.2	76.9	Kelley et al., 1997
Tammworth, Australia	10-30 yr. eq.	lucerne/clover+cereal	4	20	10	50	676	17.5	24.3	Pellic Vertisol	5.2	20.6	74.2	Kelley et al., 1997
	10-30 yr. eq.	fallow, cereal	4	20	10	50	676	17.5	28.3	Pellic Vertisol	4.5	17.7	77.8	Kelley et al., 1997
Waite, Australia	10-30 yr. eq.	wheat, fallow	4	20	43	25	604	16.8	113.3	Rhodoxeralf	1.1	57.4	41.5	Kelley et al., 1997
	10-30 yr. eq.	wheat, oat, pasture, fallow	4	20	43	25	604	16.8	113.3	Rhodoxeralf	1.1	57.4	41.5	Kelley et al., 1997
Ultuna, Sweden	other 1	na	na	20	na	35	570	5.4	na	Typic Eutrochrept	3.0	37.0	60.0	Paustian et al., 1992
La Montan̄a Research Station, Costa Rica	na.	na	4	20	25	41	2648	21.7	51.7	Eutric Cambisol	3.0	65.0	42.0	Oelbermann & Voroney, 2011
Uni. Guelph Agrofor. Res. St., Canada	na	temperature forest	4	20	65	10	820	7.2	45.0	luvisol	3.0	65.0	42.0	Oelbermann & Voroney, 2011
Kellogg Biolog. St., MI, US	other 2	na	DSSAT-CEN-TURY	20	43	19	890	9.7	41.1	Typic Hapludalf	2.0	54.0	44.0	Basso et al., 2011
Kellogg Biolog. St., MI, US	other 2	na	DSSAT-CEN-TURY	20	59	14	890	9.7	30.9	Typic Hapludalf	2.0	54.0	44.0	Basso et al., 2011
Zaragoza province, Spain	5100 yr. eq.	tree/grass system	Parton 87, 94	30	29	22.3	340	14.7	32.1	Xerollic Calciorthid	1.3	6.7	92.0	Álvaro-Fuentes et al., 2009
Zaragoza province, Spain	5100 yr. eq.	tree/grass system	Parton 87, 94	30	29	22.3	340	14.7	32.1	Xerollic Calciorthid	1.3	6.7	92.0	Álvaro-Fuentes et al., 2009
Coimbra, Brazil	6100 yr. eq.	forest to maize/beans	4	20	38	46	1350	19	64.0	Typic Kandiudult	2.5	27.8	69.7	Carvalho Leite et al., 2004
Coimbra, Brazil	6100 yr. eq.	forest to maize/beans	4	20	38	46	1350	19	64.0	Typic Kandiudult	2.5	47.0	50.5	Carvalho Leite et al., 2004
Saskatchewan, Canada	Monreal et al., 1997	no vegetation	Parton 87, 94	10	30	20	350	3.5	1.5	Typic Haploboroll	3.0	45.0	52.0	Wang et al., 2002
Martonvasar, Hungary	fit	forest arable with FYM	Parton, 87	20	na	31	453	10.3	73.4	Calcic Chernozem	2.6	63.0	34.0	Falloon and Smith, 2002

Arable land use (cont.)

location	SOM pool equilibriza-tion/initalization method	equilibrium landuse	Century version	depth/ cm	sand/ %	clay/ %	MAP/ mm	MAT/ °C	TOC/ Mg ha^{-1}	soil type	A/ %	S/ %	P/ %	source
Woburn-ley arable, UK	fit	fallow, cereal	Parton, 87	20		63	506	9.3	32.6	Cambic Arenosols	3.0	50.0	47.0	Falloon and Smith, 2002
Bad Lauchstadt-FYM+NPK, Germany	10-30 yr. eq.	Rotation-2	4	20	12	21	484	8.7	55.9	Haplic chernozem	1.6	53.7	44.7	Kelley et al., 1997
Bad Lauchstadt-nil, Germany	10-30 yr. eq.	Rotation-2	4	20	12	21	484	8.7	44.9	Haplic chernozem	2.0	24.5	73.5	Kelley et al., 1997

1-   sugar beet, spring wheat+organic+NPK

2-   sugar beet, winter wheat, spring barley, potatoes

3-   clay loam texture, not exactly specified

na-not available or not specified in text.

Additional initialization methods. Fit-pools adjusted to fit measured TOC values, 1-slow pool varied between 37 to 47% to fit measured values, 2-iterative method based on site conditions.

Equilibrium land use-land use used for long-term equilibrium run, MAP-mean annual precipitation, MAT-mean annual temperature, TOC-total organic carbon, soil type-either by FAO-WRB classification (IUSS Working Group WRB, 2007) or by Soil Taxonomy (Soil Survey Staff, 1998), A-active pool, S-slow pool, P-passive pool size.

References

Álvaro-Fuentes, J., Morell, F.J., Plaza-Bonilla, D., Arrúe, J.L. & Cantero-Martínez, C. 2012. Modelling tillage and nitrogen fertilization effects on soil organic carbon dynamics. *Soil and Tillage Research,* **120,** 32-39.

Basso, B., Gargiulo, O., Paustian, K., Philip Robertson, G., Porter, C., Grace, P.R. & Jones, J.W. 2011. Procedures for initializing soil organic carbon pools in the DSSAT-CENTURY model for agricultural systems. *Soil Science Society of America Journal,* **75,** 69-78.

Carvalho Leite, L.F., De Sá Mendonça, E., Oliveirade De Almeida MacHado, P.L., Inácio Fernandes Filho, E. & Lima Neves, J.C. 2004. Simulating trends in soil organic carbon of an Acrisol under no-tillage and disc-plow systems using the Century model. *Geoderma,* **120,** 283-295.

Cerri, C.E.P., Paustian, K., Bernoux, M., Victoria, R.L., Melillo, J.M. & Cerri, C.C. 2004. Modeling changes in soil organic matter in Amazon forest to pasture conversion with the Century model. *Global Change Biology,* **10,** 815-832.

Chiti, T., Papale, D., Smith, P., Dalmonech, D., Matteucci, G., Yeluripati, J., Rodeghiero, M. & Valentini, R. 2010. Predicting changes in soil organic carbon in mediterranean and alpine forests during the Kyoto Protocol commitment periods using the CENTURY model. *Soil Use and Management,* **26,** 475-484.

Falloon, P. & Smith, P. 2002. Simulating SOC changes in long-term experiments with rothC and CENTURY: Model evaluation for a regional scale application. *Soil Use and Management,* **18,** 101-111.

IUSS Working Group WRB 2007. World Reference Base for Soil Resources 2006, first up date 2007. World Soil Resources Report No. 103, FAO, Rome.

Kelly, R.H., Parton, W.J., Crocker, G.J., Graced, P.R., Klír, J., Körschens, M., Poulton, P.R. & Richter, D.D. 1997. Simulating trends in soil organic carbon in long-term experiments using the century model. *Geoderma,* **81,** 75-90.

Monreal, C.M., Zentner, R.P. & Robertson, J.A. 1997. An analysis of soil organic matter dynamics in relation to management, erosion and yield of wheat in long-term crop rotation plots. *Canadian Journal of Soil Science,* **77,** 553-563.

Oelbermann, M. & Voroney, R.P. 2011. An evaluation of the century model to predict soil organic carbon: Examples from Costa Rica and Canada. *Agroforestry Systems,* **82,** 37-50.

Parton, W.J. 1993. Observations and modeling of biomass and soil organic matter dynamics for the grassland biome worldwide. *Global Biogeochemical Cycles,* **7,** 785-809.

Parton, W.J., Schimel, D.S., Cole, C.V. & Ojima, D.S. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Science Society of America Journal,* **51,** 1173-1179.

Paustian, K., Parton, W.J. & Persson, J. 1992. Modeling soil organic matter in organic-amended and nitrogen- fertilized long-term plots. *Soil Science Society of America Journal,* **56,** 476-488.

Richards, A.E., Dalal, R.C. & Schmidt, S. 2007. Soil carbon turnover and sequestration in native subtropical tree plantations. *Soil Biology and Biochemistry,* **39,** 2078-2090.

Soil Survey Staff. 1998. Keys to Soil Taxonomy Eighth Edition. U.S. Department of Agriculture. Natural Resources Conservation Service.

Stolpe, N., Muñoz, C., Zagal, E. & Ovalle, C. 2008. Modeling soil carbon storage in the "Espinal" agroecosystem of central Chile. *Arid Land Research and Management,* **22,** 148-158.

Wang, H., Curtinb, D., Jamea, Y.W., McConkeya, B.G. & Zhouc, H.F. 2002. Simulation of soil carbon dioxide flux during plant residue decomposition. *Soil Science Society of America Journal,* **66,** 1304-1310.

Yeluripati, J.B., van Oijen, M., Wattenbach, M., Neftel, A., Ammann, A., Parton, W.J. & Smith, P. 2009. Bayesian calibration as a tool for initialising the carbon pools of dynamic soil models. *Soil Biology and Biochemistry,* **41,** 2579-2583.

Appendix 2: Supplementary information for chapter 3.

For the optimization of the FTIR-EGA and in $situ_T$ DRIFTS systems, purge rate, heating rate, and calibration range were investigated in order to obtain the optimal operating conditions. Figure A.1 shows the effect of purge rate of the heating chamber and gas cell. At low purge rate the CO_2 concentration is more than twice as high and the residence time of the gas in the system is much longer, as seen by the lack of a decrease of the curve upon reaching the end of the programmed heating at 700°C. The change in heating rate from 10 to 68°C also produced a marked change in the evolved gas profile (Fig. A.2). An increase in both the peak temperature of maximum CO_2 evolution was found and also an increase in the absorbance values. Figure A.3 shows the effect of the two extreme heating rates on the change in relative intensity of the mid-infrared peak at 1620 cm^{-1} (COO=/C=C). The lower heating rate seems to produce a greater overall increase in this peak area and likewise at the end of the programmed heating increase, the intensity of the peak has not declined as much as compared to 68°C min^{-1} possibly indicating the formation and retention of more thermal recalcitrant C which justified our use of the higher heating rate. Figure A.4 shows the linear range of the FTIR-EGA gas cell calibration with $NaHCO_3$ and also shows the "overflow" or non-linear area where the absorbance values no-longer respond linearly to an increase in C content of the sample. Additionally if any of the variables are changed in the experimental setup (heating rate, purge rate, purge gas), then a new calibration must be developed.
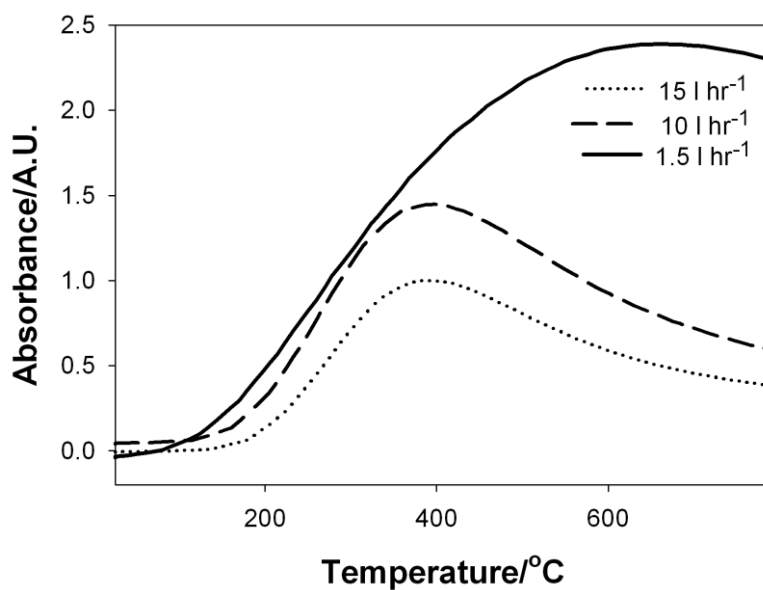
Figure A.1. Effect of flow rate (synthetic air) on evolved gas profile of CO_2 as measured by FTIR-EGA of bulk soil from Kraichgau site. Sample was heated from 25 to 700°C at 68°C min^{-1} under synthetic air purge.
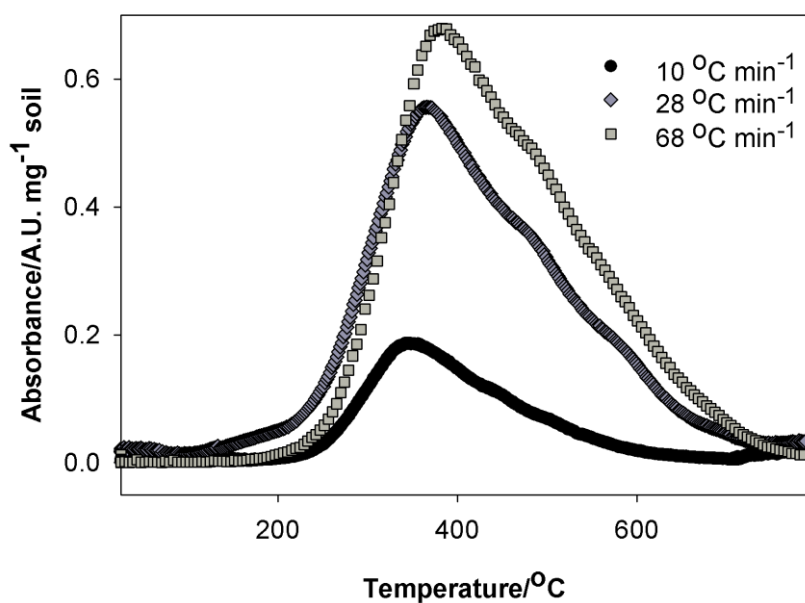
Figure A.2. Effect of heating rate on evolved gas profile of CO_2 as measured by FTIR-EGA of bulk soil from Kraichgau site heated from 25 to 700 °C under synthetic air purge.

Figure A.3. In situ$_T$ DRIFTS of effect of heating rate on vibrational intensity change of peak at 1620 cm^{-1} (COO-/C=C) of bulk soil samples heated from 25 to 700°C under synthetic air purge.
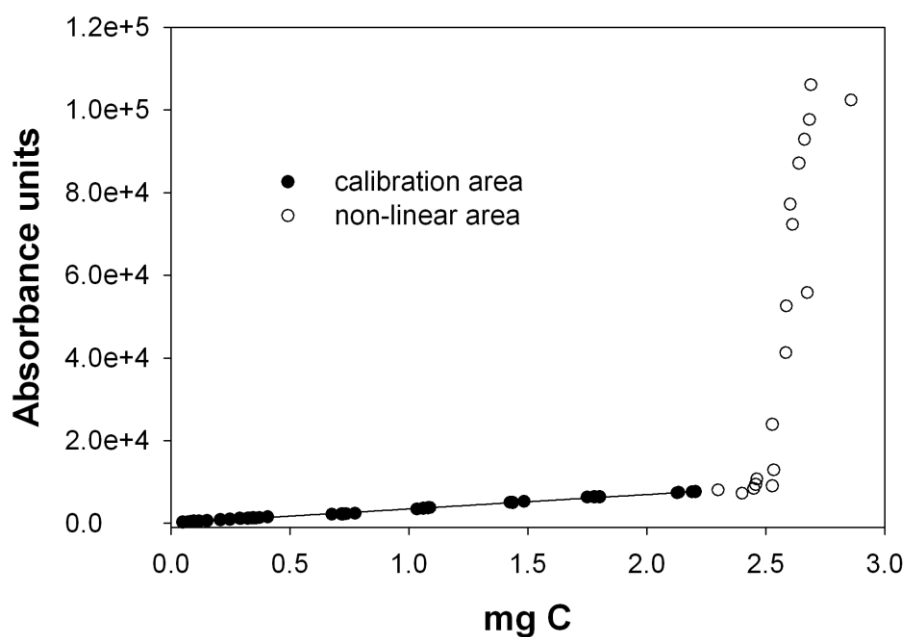
Figure A.4. Extended range of NaHCO$_3$ standard showing no-linear response with increasing C content under synthetic air purge.

Appendix 3: Supplementary information for chapter 4

Table A.2. Rate constants.

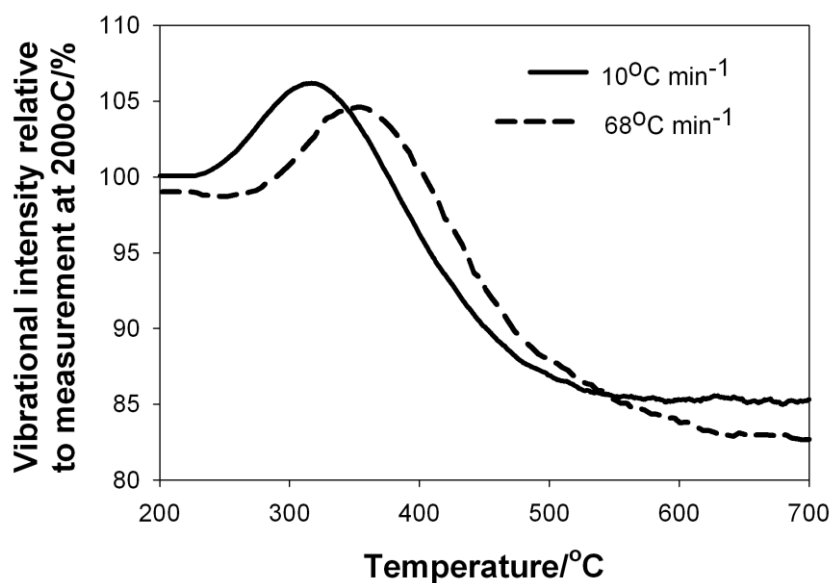| pool	rate constant (K)
surface litter metabolic	**$4*10^{-2}$**
surface litter structural	**$1.08*10^{-2}$**
soil litter metabolic topsoil	**$5*10^{-2}$**
soil litter metabolic subsoil	$5*10^{-3}$
soil litter structural topsoil	**$1.34*10^{-2}$**
soil litter structural subsoil	$1.34*10^{-3}$
active topsoil	**$2*10^{-2}$**
slow topsoil	**$5.43*10^{-4}$**
passive topsoil	**$1.86*10^{-5}$**
active subsoil	$2*10^{-3}$
slow subsoil	$4.43*10^{-5}$
passive subsoil	$1.86*10^{-6}$
CN ratios	
target metabolic	**25**
structural	**150**
active	**8**
slow	**11**
passive	**11**

bold=Century default (Parton et al., 1987)

Table A.3. Monthly weather data (DWD, 2013) (average of 1989-2008) used for 20 year simulation.

Site	Location	Elevation	parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Annual
Kraichgau	N 49°12', E 09°31'	276 m	ppt (mm)	63	57	63	64	85	94	75	76	59	63	68	74	841
(Öhringen)			solar radiation (W m^{-2})	39	69	112	166	211	228	230	196	145	88	46	33	130
			air temperature (°C)	0.1	1.4	4.7	8.6	13.1	16.2	18.0	17.4	14.1	9.5	4.5	1.3	9.1
			Eto (mm day^{-1})	0.39	0.48	1.21	1.89	2.91	3.43	3.71	3.08	2.01	1.03	0.52	0.36	1.8
			soil temp 10 cm (°C)	-0.9	0.2	3.3	6.8	11.0	14.1	15.7	15.2	12.2	8.0	3.3	0.2	7.4
Swabian Alb (Stötten)	N 48°40' E 09°51'	734 m	ppt (mm)	78	68	71	88	109	135	101	107	75	72	83	82	1068
			solar radiation (W m^{-2})	40	69	111	161	206	227	231	194	145	89	48	33	130
			air temperature (°C)	-2.1	-1.1	2.2	6.0	10.4	13.5	15.6	15.3	12.5	7.9	2.3	-1.0	6.8
			Eto (mm day^{-1})	0.38	0.61	1.20	1.97	2.77	3.25	3.60	3.09	2.11	1.15	0.58	0.40	2
			soil temp 10 cm (°C)	-2.5	-1.5	1.6	5.3	9.6	12.7	14.5	14.1	11.3	7.0	1.9	-1.4	6.0

*-ppt-precipitation, SR-solar radiation, AT-air temperature 2 meters above ground, ETo-reference evapotranspiration (estimated by Penman equation), ST-soil temperature at 10 cm depth (estimated by Kätterer a & O. Andrén, 2008)

Table A.4. Management parameters during 20 year simulation.

crop	planting date	harvest date	N	P	K	manure application	remaining residue/ %
Kraichgau							
winter wheat	01 Nov	06 Aug	149	46	0		10
mustard-maize	28 Aug, 17 Apr	14 Oct	192	75	0	Apr 16 1600 Biogas slurry	10
winter wheat	07 Nov	09 Aug	232	0	0		10
winter rape	20 Aug	20 Jul	194	37	66	Aug 20, 32 biogas slurry	100
Swabian Alb							
winter wheat	13 Oct	20 Aug	255	0	0		10
winter rape	30 Sep	05 Sep	153	44	36		100
winter wheat	22 Sep	20 Aug	235	20	36		10
mustard-maize	01 Sept, 25 Apr	19 Sep	92	0	0	biogas slurry Apr 20 1400 kg	10
mustard-summer barley	30 Oct, 01 Apr	15 Jul	92	0	0		10

8 Curriculum vitae

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Born 3 June, 1981 Columbus, Ohio, USA

Education:

Sept 2008-present	PhD candidate. Faculty of Agricultural Science, University of Hohenheim, Stuttgart, Germany. Dissertation: Development of coupled mid-infrared spectroscopic and thermal analytical approaches for the characterization and modeling of soil organic matter dynamics of arable soils
Jan 2004-June 2006	MSc., Soil Science at Ohio State University, Columbus, Ohio, USA. 2006. Thesis: Chemical and physical changes in twenty five year-old minesoils in Southeast Ohio
Oct 1999-Dec 2003	BSc., Environmental Science at Ohio State University, Columbus, Ohio, USA. 2003.
June 1999	High School Diploma, Central Catholic High School, Canton, Ohio, USA.

Work experience

Apr 2013-present	Scientific worker at the University of Hohenheim Institute of Crop Science within the Research Unit "Agricultural Landscapes under Global Climate Change – Processes and Feedbacks on a Regional Scale (FOR 1695)"
Sept 2008-Mar 2012	Scientific worker- at the University of Hohenheim, 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"
Jun 2006-Aug 2008	U.S. Peace Corps environmental education volunteer- Gyumri, Armenia. Soil and water quality research project development and implementation, informal environmental education activities, and local NGO capacity building.
Jan 2004-Jun 2006	Graduate teaching/research associate in the School of Natural Resources, The Ohio State University, Columbus, Ohio, USA. Teaching associate for introductory soil science laboratory and pedology courses.

Nov 1999-Nov 2005	Ohio Air National Guard-Services Journeyman November 1999-November 2005. Food preparation for large scale bases in austere conditions.
Sept 2001-Dec 2003	Laboratory (part time), Soil Characterization Lab with the Cooperative Soil Survey, The Ohio State University, Columbus, Ohio, USA. Job included all tasks pertaining to handling of samples received for the Cooperative Soil Survey including soil sample processing and performing analysis on total carbon, pH, particle size, extractable bases, X-ray diffraction, and surface area.
Oct 2000-Sept 2001	Laboratory technician (part time), Plant Biotechnology Center, The Ohio State University, Columbus, Ohio, USA. Job included general lab duties such as dish washing, making of solutions and growth media, and care of corn and Arabidopsis plants.

Publications

Demyan, M.S., Rasche, F., Schütt, M., Smirnova, N., Schulz, E. & Cadisch, G. 2013. Combining a coupled FTIR-EGA system and in situ DRIFTS for studying soil organic matter in arable soils. *Biogeosciences*, **10,** 2897-2913.

Demyan, M.S., Rasche, F., Schulz, E., Breulmann, M., Müller, T. & Cadisch, G. 2012. Use of specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy to study the composition of organic matter in a Haplic Chernozem. *European Journal of Soil Science,* **63,** 189-199.

Other publications not included in dissertation

Giacometti, C., Demyan, M.S., Cavani, L., Marzadori, C., Ciavatta, C. & Kandeler, E. 2013. Chemical and microbiological soil quality indicators and their potential to differentiate fertilization regimes in temperate agroecosystems. *Applied Soil Ecology,* **64,** 32-48.

Rhoades, J.L., Demyan, M.S. & Orr, B. 2011. Impacts of Deforestation and Land Cover Change on Mountain Soils in Hrazdan, Armenia. *Journal of Sustainable Forestry,* **30,** 677-696.

Siewert, C., Demyan, M.S. & Kučerík, J. 2012. Interrelations between soil respiration and its thermal stability. *Journal of Thermal Analysis and Calorimetry,* **110,** 413-419.

Conference contributions

Demyan, S., F. Rasche, S. Becker-Fazekas, M. Schütt, A. R. Zinkeng, E. Schulz, T. Müller, G. Cadisch. Coupled mid-infrared spectroscopy (MIRS) and thermally evolved gas analysis to study SOM dynamics in arable soils. 3rd International Symposium on Soil Organic Matter: Organic matter dynamics-from soils to oceans. 11th-14th July, 2011. Leuven, Belgium. Oral presentation.

Demyan. S., F. Rasche, E. Schulz, S. Becker-Fazekas, M. Breulmann, T. Müller, and G. Cadisch. 2010. Soil organic matter (SOM) characterization by coupled mid-infrared spectroscopy and thermal analyses to compliment SOM fractionation. 4th International Symposium on Organic Matter Stabilization: Organic matter stabilization and ecosystem functions. 19th-23rd September 2010. Presqu'île de Giens, France. Poster

Demyan, S., N. Smirnova, F. Rasche, T. Müller, G. Cadisch. 2010. Characterization of Soil Organic Matter by Mid-Infrared Spectroscopy/Evolved Gas Analysis, Long Term Dynamics. Workshop: Regulation of soil organic matter and nutrient turnover in agriculture. University of Kassel, 12th-13th November 2009. Oral presentation

Demyan, S., F. Rasche, S. Becker-Fazekas, E. Schulz, T. Müller, and G. Cadisch. Soil organic matter characterization utilizing mid-infrared spectroscopy and thermal analyses. Euroleague for Life Sciences (ELLS) Science Student Conference on Climate Change, 4th-5th November, 2009 Hohenheim 2009. Poster

Demyan, S. and N. Smeck. 2005. Rapid pedogenesis in minesoils, changes in minesoils over 25 years. Soil Science Society of America Conference, Salt Lake City. Oral presentation.

Other experiences

Aug 2012	expert for short-term IAEA-FAO training mission on mid-infrared spectroscopy for research uses to Lima, Peru
Jul 2004	Soil Science-Ecological Field Excursion to Western Siberia, Russia (July 4th-28th, 2004)
2002-2004	Soil Judging at the American Society of Agronomy Regional Contests (2002, 2003) and National Contest (2004)
2002-2003	deployed in support of Operation Enduring Freedom in 2002 and 2003 as a member of the Ohio Air National Guard
Dec 2002	American Society of Agronomy Cross Cultural Exchange Scholarship
Mar 1998	Eagle Scout, Boy Scouts of America

Activities and Organizations

-Association of Ohio Pedologists –associate member
-Soil Science Society of America –graduate student member
-Associate Professional Soil Scientist (since 2007)
-Ohio State Soil Judging Team (2002-2004)