



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

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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 (CO₂) in order to relate evolved gas (i.e., CO₂) 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 was 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 CO₂ (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 min at 700 °C. Separately, the heating chamber was placed in a diffuse reflectance chamber (DRIFTS) for mea-

suring 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 CO₂ evolution (CO_{2,max}).

Results indicated that the FYM + NPK and FYM treatments of the Chernozem soils had a lower CO_{2,max} as compared to both NPK and CON treatments. On average, CO_{2,max} 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_{2,max} (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_{2,max} 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 CO₂ 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_2_{max} 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.

1 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 would evolve 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 Lützwow 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 Lützwow 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 stable 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 inter-layer water up to 250 °C and from 400 to 700 °C with crystallization and dehydroxylation reactions (Pansu and Gauthierou, 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. (2007, 2008) 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; (iii) very slow heating rates, as in the case of Dorodnikov et al. (2008, 2007) of 2 °C min⁻¹, may shift thermal reactions to significantly lower temperatures (Fernández et al., 2011); and (iv) these slow heating rates may possibly 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) were 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 (e.g., CO_2 at 2400 to 2200 cm⁻¹) and creating a calibration curve using a known standard (e.g., 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

Table 1. Study site characteristics.

Study site	Location	MAP*/mm	MAT*/°C	Soil*	Elevation/m a.s.l.*	pH	Clay/%
Bad Lauchstädt	51°24' N, 11°53' E	483	8.8	Haplic Chernozem	113	6.6	21
Kraichgau	48°55.7' N, 8°42.2' E	780	9.1	Cambisol/Regosol	322	6.7	18
Swabian Alb	48°31.7' N, 9°46.2' E	962	6.7	Cambisol	686	6.5	38

* MAP = mean annual precipitation, MAT = mean annual temperature, soil = FAO classification, m a.s.l. = meters above sea level.

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 CO₂ evolution (i.e., temperature of peak CO₂ evolution, changes in low temperature CO₂ evolution versus higher temperature CO₂ 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., 2007a). 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.

2 Materials and methods

2.1 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 CO₂ 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 CO₂ evolution to optimize combustion conditions.

2.2 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 %).

2.3 Fractionation

The soil fractionation method was modified from Zimmermann et al. (2007b). 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 × g. The clear supernatant was taken as extractable 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 × 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 × 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 frac-

tions 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).

2.4 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 CO₂ 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.

2.5 FTIR-EGA method

2.5.1 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) had 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) and 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

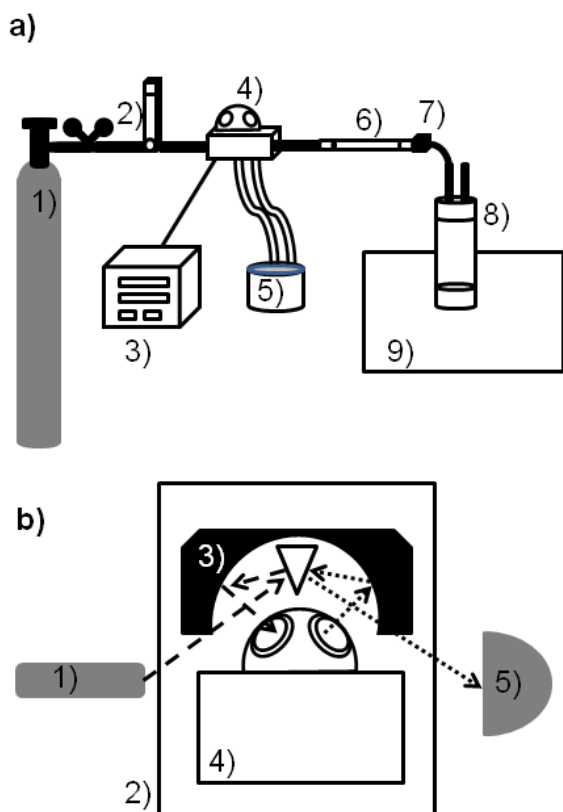


Fig. 1. Schematic of experimental setups. **(a)** FTIR-EGA system: (1) synthetic air purge/carrier gas, (2) pressure regulator and flow gauge, (3) automatic temperature controller, (4) high temperature reaction chamber with potassium bromide (KBr) windows and integrated sample cup, (5) recirculating cooling water, (6) glass tube with magnesium perchlorate ($\text{Mg}(\text{ClO}_4)_2$) 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.

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 ($2\ \mu\text{m}$) 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.

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, sam-

ple size, heating rate, infrared spectrometer scan rate, and mid-infrared wave number integration range were optimized (see Supplement Figs. 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 s, with a resolution of $8\ \text{cm}^{-1}$. Synthetic air was used as the purge and carrier gas of the system with a flow rate of $15\ \text{L h}^{-1}$. The chamber was purged for 5 min 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 $68\ ^\circ\text{C min}^{-1}$ (see section below) and set-point temperature of $700\ ^\circ\text{C}$ were used. The set-point temperature was reached after 10 min and held for another 20 min, 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 min.

In the present experiment, lower heating rates were also tested (10 and $28\ ^\circ\text{C min}^{-1}$), 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 Fig. S3). Heating rates $> 68\ ^\circ\text{C min}^{-1}$ 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 $2200\ \text{cm}^{-1}$) 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 $15\ \text{L h}^{-1}$ 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 $8\ \text{cm}^{-1}$ 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 s 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 s 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.

2.5.2 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 Eq. (1):

$$\text{Area}_{\text{peak}} = \int_{2200\text{cm}^{-1}}^{2400\text{cm}^{-1}} A(\nu) \quad (1)$$

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

$$\text{Area}_{\text{cum}} = \sum_{j=1}^{300} j(\text{Area}_{\text{peak}}) \quad (2)$$

where j is the scan number, which was taken every 4 s for a total of 300 scans, and $j(\text{Area}_{\text{peak}})$ is the $\text{Area}_{\text{peak}}$ at scan j . The presence of a linear response of cumulative peak area found by Eq. (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 $\text{Area}_{\text{peak}}$ to analyzed sample weight:

$$\text{Area}_{\text{wt}} = \frac{\text{Area}_{\text{peak}}}{\text{sample weight (mg)}} \quad (3)$$

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

$$\text{Area}_{\text{C}} = \frac{\text{Area}_{\text{peak}}}{\text{sample weight} \times \text{C}_{\text{org}} \text{ content}} \quad (4)$$

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:

$$\text{CO}_{2_{\text{max}}} = \frac{700}{25} f(\text{Area}_{\text{peak}}), \quad (5)$$

during the heating from 25 to 700 $^{\circ}\text{C}$.

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

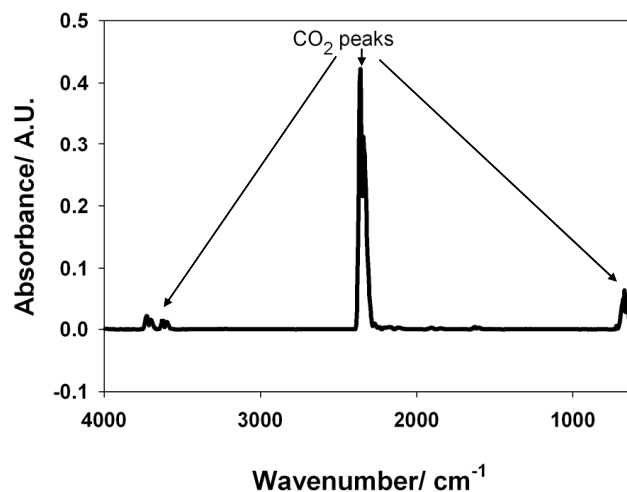


Fig. 2. Single scan of online evolved gas as measured by the FTIR-EGA system, with corresponding CO_2 peaks.

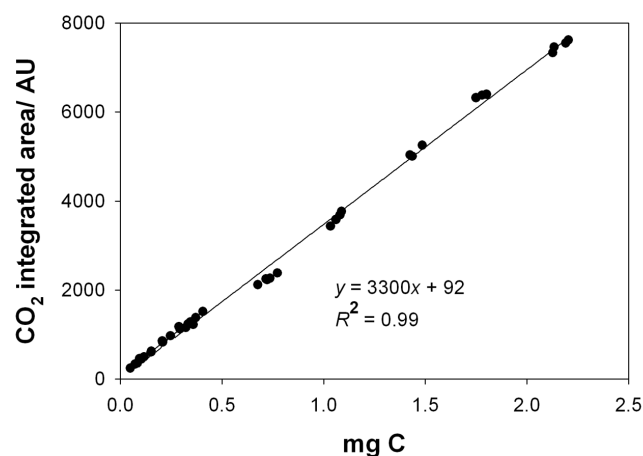


Fig. 3. CO_2 calibration curve of the FTIR-EGA with NaHCO_3 .

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 $^{\circ}\text{C}$ 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.

2.6 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 s 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.

2.7 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 CO₂ 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 CO₂ profile. The most rapid decreases of the peak intensities were found at the following temperatures: 1st peak at 320 °C of the C–O vibrations (2200–2000 cm⁻¹), 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 CO₂ 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

CO₂ 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_{2,max} among the treatments at the Bad Lauchstädt site. A mixed modeling approach was used to compare the CO_{2,max} 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 Results

3.1 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 extractable 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).

3.2 FTIR-EGA derived properties of organic substances

In order to quantify evolved CO₂ 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 NaHCO₃ decomposition can be found in Fig. 2, showing the three main vibrational modes of CO₂ (in order of decreasing intensity) at 2400–2200, 715–615, and 3760–3657 cm⁻¹. Absorbance values of > 2.5 of the main CO₂ peak at 2400–2200 cm⁻¹ resulted in a non-linear response of Area_{cum} and calculated C evolved from the NaHCO₃ (see Fig. S4). Thus, for the heating rate of 68 °C min⁻¹ and reaction chamber purge rate of 15 L h⁻¹, a C weight between 0.1–2.5 mg could be analyzed and resulted in a linear

Table 2. Mean values (\pm 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 ^b	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 ^c	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 and clay 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 μ m, rSOC < 63 μ m 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.

response ($R^2 = 0.99$) (Fig. 3). The three pure substances (i.e., glucose, xylan, tannic acid) had very different FTIR-EGA CO₂ thermograms, such as number of peaks, peak position and general shape of the curve of CO₂ evolution (Fig. 4). In the undiluted samples (Fig. 4a), xylan was found to have the first CO₂ evolution peak at 320 °C, one at 500 °C, and the highest CO₂ temperature peak at 680 °C. Undiluted glucose had a prominent peak at 442 °C and a shoulder at 537 °C. The first two CO₂ 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 CO₂ peak, but that was shifted to a higher temperature at 346 °C and a second peak at 518 °C. Glucose had only one CO₂ 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 min) from undiluted samples, while in the diluted samples, 90 % was recovered.

3.3 FTIR-EGA derived properties of soils and fractions

The CO_{2,max} 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) (Fig. 5). In the Bad Lauchstädt soils, the CO_{2,max} 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 signif-

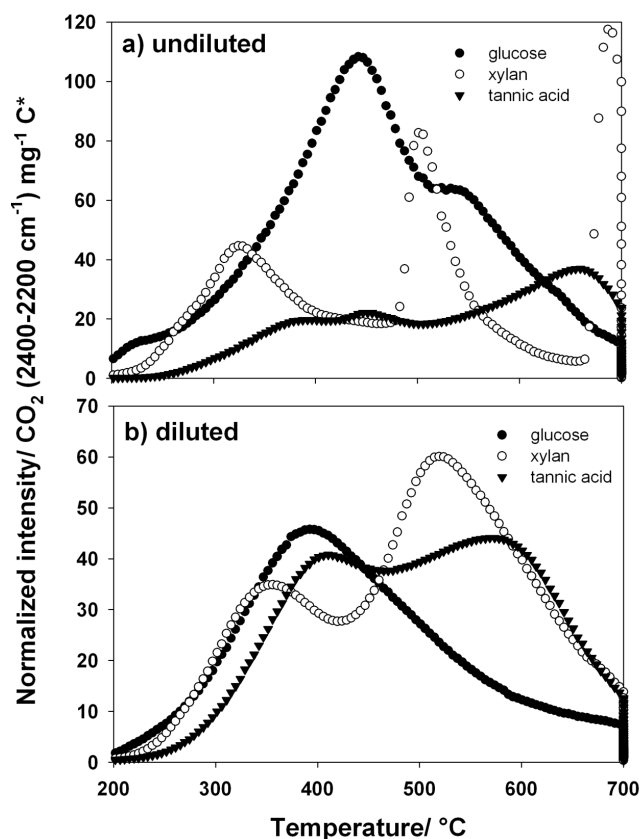


Fig. 4. FTIR-EGA CO₂ thermograms of three selected substances. (a) neat samples, (b) diluted 1 : 100 with preheated, ball-milled quartz sand. * Normalized intensity is the integrated area of the CO₂ peak from 2400 to 2200 cm⁻¹ normalized to C content of the sample.

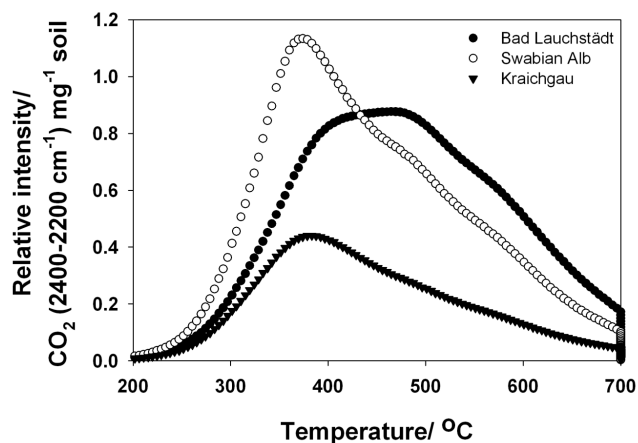


Fig. 5. FTIR-EGA average CO₂ thermograms of soils from three different agroecological sites (Bad Lauchstädt, Kraichgau, Swabian Alb). * Normalized intensity is the integrated area of the CO₂ peak from 2400 to 2200 cm⁻¹ normalized to weight of the sample.

icantly affected by FYM application, the FTIR-EGA method also indicated a difference between the FYM + NPK and FYM treatments. When comparing soils from the three sites (Fig. 5), the peak CO₂ 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_{2,max} at 384 °C, while that of the Kraichgau soils was at a significantly higher ($P < 0.05$) temperature (392 °C). The CO_{2,max} 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 CO₂ thermograms further revealed that while the Kraichgau and Swabian Alb soils had a similar CO₂ evolution pattern with a rapid increase in CO₂ evolution between 275 to 380 °C (Fig. 5), the Bad Lauchstädt soils had a much broader peak covering a range of approximately 80 °C (Fig. 5). Compared with the results from the elemental analyzer, total C contents as measured by FTIR-EGA were underestimated on average by 7 %.

Soils measured after incubation for 490 days showed a decrease in thermally evolved CO₂ (Fig. 7) and a shift in the CO_{2,max}. The difference CO₂ thermograms (Fig. 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 CO₂ was at 360 and 378 °C for the Kraichgau and Swabian Alb, respectively, with CO_{2,max} shifted to a slightly higher temperature (increase of 4 °C).

The temperature of the individual CO₂ 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) (Ta-

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*	419 (3)a
FYM*	428 (2)b
NPK*	473 (2)c
Control*	473 (2)c

* FYM + NPK (farmyard manure and mineral fertilizer), FYM (farmyard manure), NPK (mineral fertilizer), Control (no fertilizer inputs).

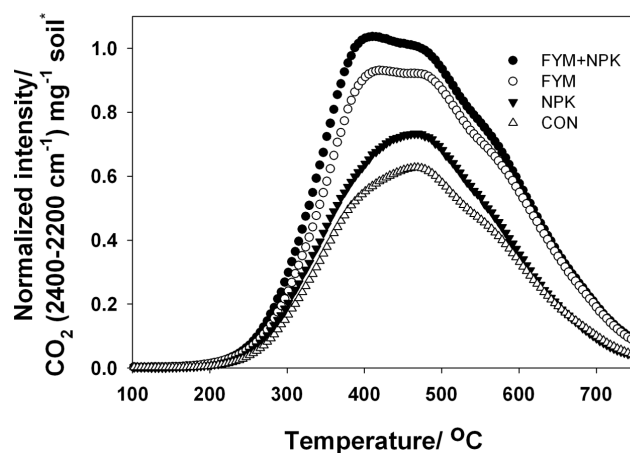


Fig. 6. FTIR-EGA CO₂ thermograms of bulk soil from the Static Fertilization Experiment, Bad Lauchstädt, heating from 25 to 700 °C at 68 °C min⁻¹ under synthetic air purge. Treatments: FYM + NPK (farmyard manure and mineral fertilizer), FYM (farmyard manure), NPK (mineral fertilizer), CON (no fertilizer inputs). * Normalized intensity is the integrated area of the CO₂ peak from 2400 to 2200 cm⁻¹ normalized to weight of the sample.

ble 4, Fig. 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 (Fig. 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.

3.4 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.

Table 4. Mean values (\pm standard error) for soil (Kraichgau and Swabian Alb) at the temperatures ($^{\circ}$ 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), uppercase letters indicate significant differences ($P < 0.05$) within a site among fractions.

Fraction	Site					
	Kraichgau			Swabian Alb		
	200–350 $^{\circ}$ C	350–450 $^{\circ}$ C	450–550 $^{\circ}$ C	200–350 $^{\circ}$ C	350–450 $^{\circ}$ C	450–550 $^{\circ}$ C
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		400(1)A			399(4)A	
rSOC ^a	265(1)C		482(17)Ba	265(5)C		392(15)Ab

^a Bulk soil < 2 mm, POM-sand fraction < 1.8 g cm⁻³, Sa + A sand fraction > 1.8 g cm⁻³, Si + C < 63 μ m, rSOC < 63 μ m treated with hot sodium hypochlorite.

^b 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.

Table 5. Temperature and relative contribution to total evolved CO₂ of fitted peaks/components (\pm standard errors) from an iterative least squares approach of thermally evolved CO₂ from 25 to 700 $^{\circ}$ C (68 $^{\circ}$ 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/ $^{\circ}$ C	% ^a	Temp/ $^{\circ}$ C	%	Temp/ $^{\circ}$ C	%	Temp/ $^{\circ}$ C	%
BL FYM + NPK ^a	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 CO₂.

^b BL – Bad Lauchstädt experiment; FYM + NPK – farmyard manure and NPK fertilizer, FYM – farmyard manure, NPK – NPK fertilizer, CON – control.

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 (Figs. 9–11). In the bulk soil samples, consistent changes were found for all three sites with regard to the C-H and $-\text{COO}^-/\text{C}=\text{C}$ vibrations. The C-H vibrations consistently decreased starting 220 to 250 $^{\circ}$ C and accelerating rapidly around 350 $^{\circ}$ C, while the $-\text{COO}^-/\text{C}=\text{C}$ vibrations began to decrease around 380 $^{\circ}$ C with a maximum decline around 440 $^{\circ}$ C. Between 300 to 370 $^{\circ}$ C, there was a drastic decrease in C-H vibrations while at the same temperature range there was a slight increase in $\text{C}=\text{C}/-\text{COO}$ vibrations and a stronger increase in $\text{C}=\text{C}$ vibrations. At approximately 400 $^{\circ}$ C, the $\text{C}=\text{C}/-\text{COO}$ vibrations decreased and after 430 $^{\circ}$ C the $\text{C}=\text{C}$ 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 $^{\circ}$ C.

Differences among the fractions via in situ_T DRIFTS is illustrated in Fig. 10 for the C-H and $-\text{COO}^-/\text{C}=\text{C}$ 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 $^{\circ}$ C, the decrease in intensity of the 2930 cm⁻¹ peak accelerated after 280 $^{\circ}$ C with the decrease in intensity ending around 450 $^{\circ}$ C. The POM fractions showed an increased in intensity after 550 $^{\circ}$ C, until 700 $^{\circ}$ C. The 1620 cm⁻¹ peak was found to be relatively stable from 200 and 220 $^{\circ}$ C and then increase in intensity from 280 $^{\circ}$ C to approximately 360 $^{\circ}$ C with some variation between fractions. The onset of decrease was around 380 $^{\circ}$ C, the same as for the bulk soils.

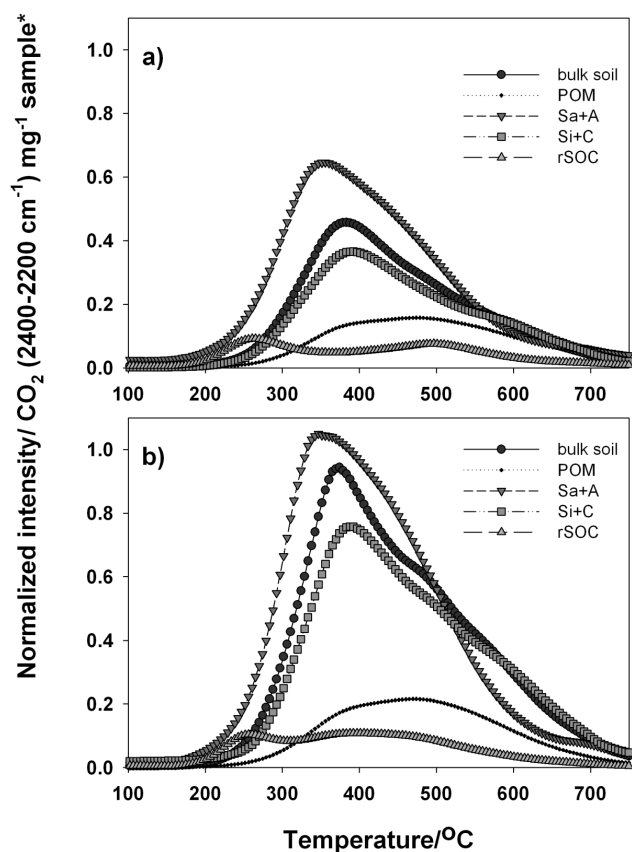


Fig. 7. FTIR-EGA CO₂ thermograms of bulk soil and fractions heating from 25 to 700 °C at 68 °C min⁻¹ under synthetic air purge from the (a) Kraichgau and (b) Swabian Alb sites. Bulk soil < 2 mm, POM-sand fraction < 1.8 g cm⁻³ fraction diluted 1 : 50 with preheated ball-milled quartz sand for thermal 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 CO₂ peak from 2400 to 2200 cm⁻¹ normalized to weight of the sample.

3.5 Peak fitting of FTIR-EGA CO₂ gas thermograms

With the temperatures of maximum change of different functional groups as measured by in situ 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 CO₂ thermogram, a peak fitting of the FTIR-EGA CO₂ gas profiles from bulk soils resulted in four different peaks or “fitted components” (Fig. 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

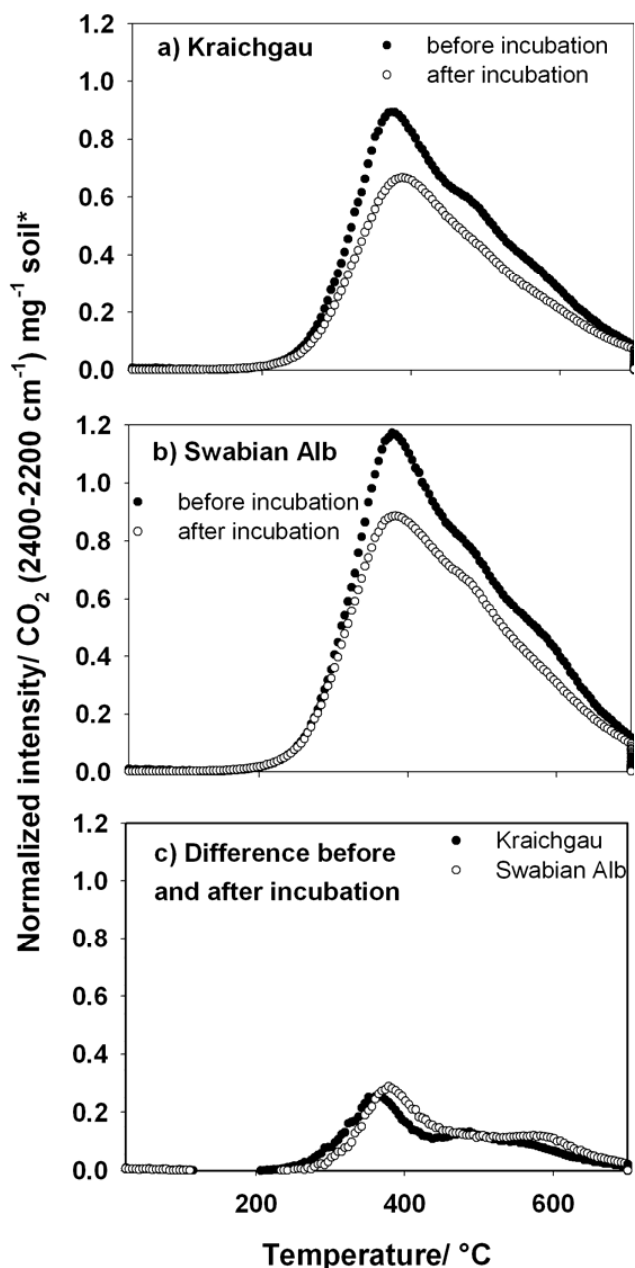


Fig. 8. Effects of 490 day incubation on thermally evolved CO₂ thermogram. (A) Kraichgau soils, (B) Swabian soils, (C) difference when subtracting thermogram of soil at the end of incubation from thermogram at the beginning of the incubation. * Normalized intensity is the integrated area of the CO₂ peak from 2400 to 2200 cm⁻¹ normalized to weight of the sample.

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

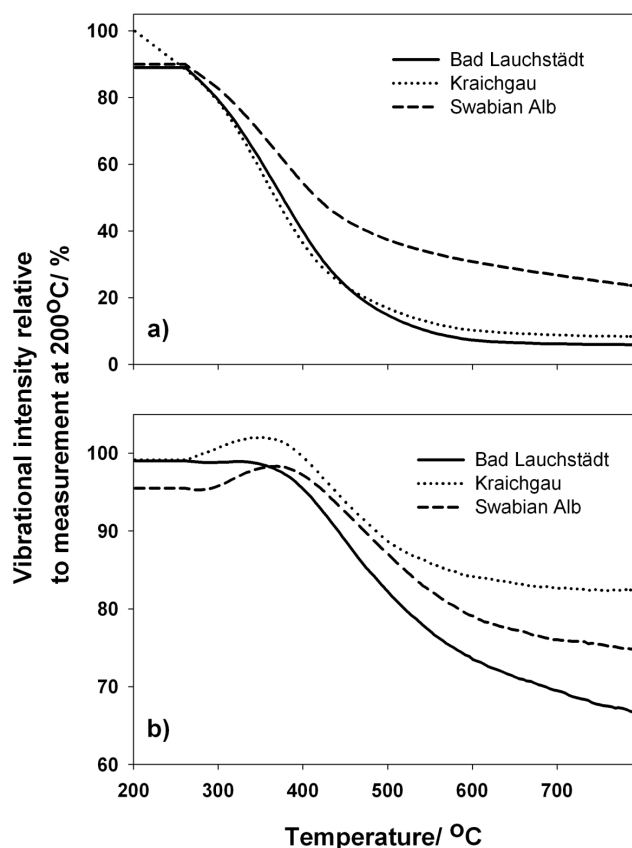


Fig. 9. In situ_T DRIFTS of bulk soil samples from Bad Lauchstädt, Kraichgau, and Swabian Alb of (a) C-H (2930 cm^{-1}) vibrations and (b) C=O/C=C (1620 cm^{-1}) vibrations.

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 treatments (49.1 and 49.2%).

As with the measured $\text{CO}_{2\text{max}}$, 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

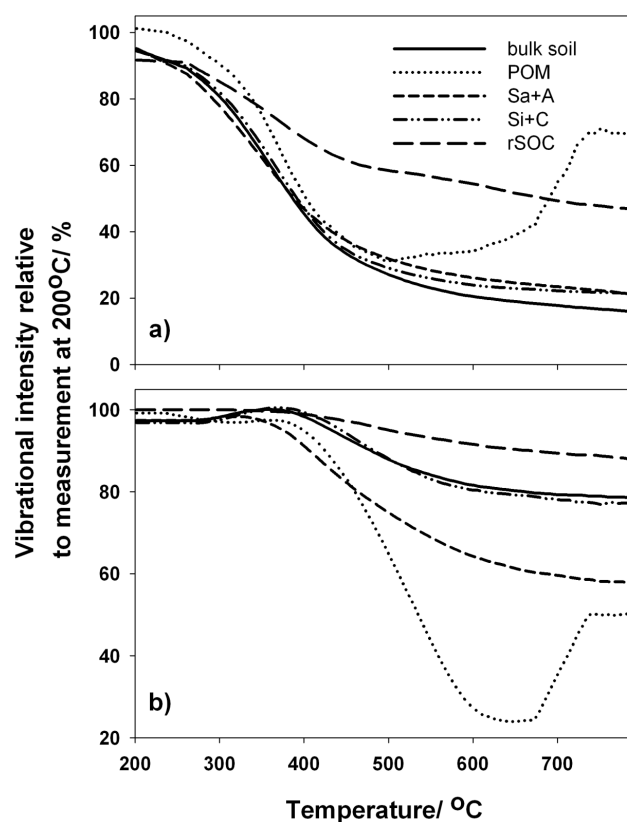


Fig. 10. In situ_T DRIFTS of the bulk soil and fractions of (a) C-H (2930 cm^{-1}) vibrations and (b) C=O/C=C (1620 cm^{-1}) vibrations. Average of Kraichgau and Swabian Alb sites.

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.

4 Discussion

4.1 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).

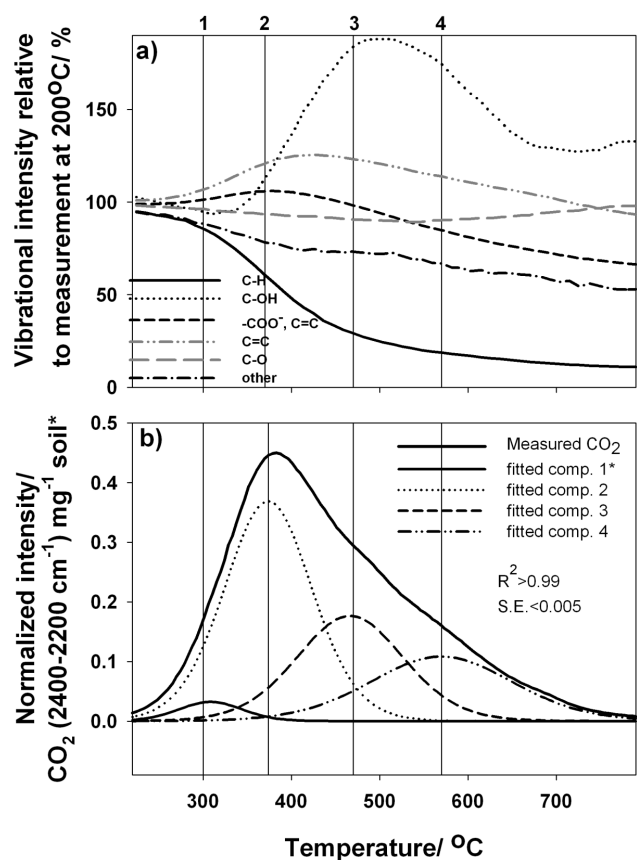


Fig. 11. Summary of qualitative changes in organic functional groups during heating as a guide to fitting components to quantitative evolved gas analysis: (a) in situ DRIFTS measurements of bulk soil from the Kraichgau site as a percentage of the vibrational intensity at 200 °C to identify temperature 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 Fig. 6b. * Normalized intensity is the integrated area of the CO₂ peak from 2400 to 2200 cm⁻¹ normalized to weight of the sample.

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_{2,max}. 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 CO₂ 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_{2,max}. 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_{2,max} 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. (2011) 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 CO₂ via FTIR-EGA, our results showed an increase from 433 to 442 °C after incubation. From these results, we could conclude that both CO_{2,max} 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_{2,max} 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 was 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 stable 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_{2,max} 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 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.

4.2 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 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 indi-

cated 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). 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 ¹³C 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 pyrolysis field ionization mass spectroscopy (Py-FIMS), Sleutel et al. (2011) showed 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 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 and 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 pyrolysis compounds after 98 yr of fertilizer deprivation compared to the full fertilized treatment, which complements 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 $> 540^{\circ}\text{C}$ 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.

4.3 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 $68^{\circ}\text{C min}^{-1}$ as the optimal heating rate for soils for our system to balance the need for reproducible heating rate and also minimize secondary reactions. This heating rate is similar to the $70^{\circ}\text{C min}^{-1}$ 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 ($68^{\circ}\text{C min}^{-1}$) compared to other studies (Siewert, 2004; Plante et al., 2011; Leinweber et al., 1992), which used a rate of $10\text{--}30^{\circ}\text{C min}^{-1}$ and therefore may have been 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 relative 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.

5 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 h^{-1} synthetic air purge with a scan every 4 s; (2) a rapid heating rate ($68^{\circ}\text{C min}^{-1}$) 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 in 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 clearly 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.

Supplementary material related to this article is available online at: <http://www.biogeosciences.net/10/2897/2013/bg-10-2897-2013-supplement.pdf>.

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