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Thermal stability of soil organic matter pools and their $\delta^{13}C$ values after C_3–C_4 vegetation change

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Abstract

Carbon isotopic composition of soils subjected to C_3–C_4 vegetation change is a suitable tool for the estimation of C turnover in soil organic matter (SOM) pools. We hypothesized that the biological availability of SOM pools is inversely proportional to their thermal stability. Soil samples from a field plot with 10.5 years of cultivation of the C_4 plant *Miscanthus* × *gigantheus* and from a reference plot under C_3 grassland vegetation were analysed by thermogravimetry coupled with differential scanning calorimetry (TG-DSC). According to differential weight losses (dTG) and energy release or consumption (DSC), five SOM pools with increasing thermal stability were distinguished: (I) 20–190 °C, (II) 190–310 °C, (III) 310–390 °C, (IV) 390–480 °C, and (V) 480–1000 °C. Their $\delta^{13}C$ values were analysed by EA-IRMS. The weight losses in pool I were connected with water evaporation, since no significant C losses were measured and $\delta^{13}C$ values remained unchanged. The $\delta^{13}C$ of pools II and III in soil samples under *Miscanthus* were closer to the $\delta^{13}C$ of the *Miscanthus* plant tissues (−11.8‰) compared to the thermally stable SOM pool V (−19.5‰). The portion of the *Miscanthus*-derived C_4-C in total SOM in 0–5 cm reached 55.4% in the 10.5 years. The C_4-C contribution in pool II was 60% and decreased down to 6% in pool V. The mean residence times (MRT) of SOM pools II, III, and IV were similar (11.6, 12.2, and 15.4 years, respectively), while pool V had a MRT of 163 years. Therefore, we concluded that the biological availability of thermal labile SOM pools (<480 °C) was higher, than that of the thermal stable pool decomposed above 480 °C. However, the increase of SOM stability with rising temperature was not gradual. Therefore, the applicability of the TG-DSC for the separation of SOM pools with different biological availability is limited.

Keywords: C_3–C_4 vegetation change; *Miscanthus* × *gigantheus*; Differential scanning calorimetry; Thermogravimetry; TG-DSC; Thermal stability; $\delta^{13}C$; Soil organic matter

1. Introduction

Carbon (C) isotopic composition of soil organic matter (SOM) after C_3–C_4 vegetation change (and vice versa) has been frequently used in the last decade to estimate C turnover rates in soil and the incorporation of new C in various SOM pools (Balesdent and Mariotti, 1987; Volkoff and Cerri, 1987; Ludwig et al., 2003; John et al., 2003, 2005; Kristiansen et al., 2005). This approach is based on the different stable isotope composition (represented as $\delta^{13}C$ value) of residues from plants with C_3 and C_4 photosynthesis (Farquhar et al., 1989; Ehleringer and Cerling, 2002). After C_3–C_4 vegetation change the $\delta^{13}C$ value of SOM starts to change slowly from the original $\delta^{13}C$ value, which is closer to that of C_3 vegetation, to a new steady-state $\delta^{13}C$ value, which is closer to that of C_4 vegetation. When the new steady state is not reached (as in most studies) and the period after the C_3–C_4 vegetation change is known, the contribution of the new C_4-derived C to the total SOM can be calculated. Based on this C_4-C contribution and the period after vegetation change, the SOM turnover rates can be roughly estimated (Balesdent and Mariotti, 1996).

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Nearly all investigations using ^{13}C natural abundance have been conducted with maize (Balesdent and Balabane, 1996; Ludwig et al., 2003; Kristiansen et al., 2005). In Europe another C_4 plants—*Miscanthus* × *giganteus* (Greef et Deu.)—is successfully cultivated as a bio-energy crop (Beuch, 2000). This perennial C_4 plant can be cultivated from 15 up to 25 years without replanting and is harvested yearly, often in the following spring to reduce ash contents. The first results of C turnover for *Miscanthus*, based on ^{13}C natural abundance showed a higher contribution of *Miscanthus* C to the SOM than maize (Kao, 1997; Hansen et al., 2004).

As the estimation of the $\delta^{13}C$ values of bulk SOM is not very informative, various fractionation techniques including density (Magid et al., 2002; John et al., 2005) and particle size fractionation (Jolivet et al., 2003; Ludwig et al., 2003), as well as sequential extractions (Ellerbrock and Kaiser, 2005) were combined with the $\delta^{13}C$ analyses. One of the other methods suitable for SOM fractionation is based on thermal stability (Leinweber and Schulten, 1992; Siewert, 2001, 2004; Lopez-Capel et al., 2005; Plante et al., 2005). Thermal analysis by thermogravimetry coupled with differential scanning calorimetry (TG-DSC) involves a slow continuous temperature increase that leads to the progressive decomposition (mainly oxidation) of different organic compounds according to their thermal stability. Such a temperature increase coupled with the measuring of weight losses is termed thermogravimetry (TG). Energy released or consumed by the decomposition of organics is measured, simultaneous to the temperature increase, by differential scanning calorimetry (DSC).

Thermal analysis curves and heat fluxes provide important information on the structural composition of SOM (Provenzano and Senesi, 1999; Dell'Abate et al., 2002; Kuzyakov et al., 2006). The exothermic degradation of aliphatic and carboxyl groups at different temperatures was suggested to compare the proportions of labile and more stable components in SOM pools and whole soil (Brown, 1988; Siewert, 2001; Lopez-Capel et al., 2005).

The availability to microbial decomposition is an important characteristic of the SOM quality, which in turn allows to predict the transformation and turnover time of carbon in terrestrial ecosystems. Based on correlations between thermal stability of SOM pools in various temperature ranges and the CO_2 production by classical soil incubations, Siewert (2001) suggested that the thermal stability of SOM pools can be related to their biological degradability. It assumes that SOM pools decomposable at lower temperatures are more biologically accessible and utilizable compared to organics, which are decomposed at higher temperatures. However, the hypothesis of relation between the thermal stability of SOM pools and their availability to microbial mineralization was mainly supported by correlations between CO_2 evolution and thermogravimetrical weight losses (Siewert, 2001) and, to the best of our knowledge, has never been tested by direct methods.

Thus, the aim of our study was to test the hypothesis of close connection between thermal degradability and biological stability by comparing $\delta^{13}C$ values of SOM pools decomposed at increasing temperature in the soil after the vegetation changed from former C_3 grassland to C_4 plant *Miscanthus* × *giganteus*. If the hypothesis is true, then the thermally labile SOM pools would have $\delta^{13}C$ values closer to the new vegetation compared to thermally stabile SOM pools and the mean residence time (MRT) of labile pools would be shorter than those of stable ones.

The preliminary study testing this approach showed weak correlation between thermal and biological degradability when only three SOM pools were separated by TG-DSC (Kuzyakov et al., 2006). However, the preliminary study was insufficient for exact conclusions because of three reasons: (i) the absence of the reference soil developed solely under C_3 vegetation did not allow for estimation of the isotopic fractionation of C by thermal decomposition and by incorporation of C into pools with different thermal stability. So, in the absence of the reference soil the calculation of the MRT of the SOM pools was not possible; (ii) only three temperature ranges were separated; one of them was responsible for water evaporation and did not affected the $\delta^{13}C$ values; (iii) the fast rate of temperature increase ($5\,^{\circ}C\,min^{-1}$) during TG-DSC analysis in preliminary study led to some overlapping of pools with different thermal stability and therefore, did not allow their clear separation.

In the present study, the above-mentioned deficiencies were considered and therefore, the reference soil under grassland (solely C_3) was included into the experiment and the heating rate ($2\,^{\circ}C\,min^{-1}$) was greatly reduced. This allowed for the better separation of the pools, especially those decomposed under high temperature. The inclusion of the reference soil in the experiment design allowed for the calculation of MRT.

2. Materials and methods

2.1. Soil samples

Soil samples were taken from the long-term experimental field located in Stuttgart-Hohenheim, Baden-Wuerttemberg, Germany (48°43′ north latitude, 9°13′ east longitude). The soil on the plot with *Miscanthus* and reference plot was a loamy Gleyic Cambisol (WRB, 1998) without carbonates (no reaction with HCl). The soil properties under *Miscanthus* were: pH 5.6, bulk density $1.1\,g\,cm^{-3}$, C_{org} 2.13%, N_{tot} 0.18%, C-to-N ratio 12.0. The properties of the reference soil were: pH 6.3, bulk density $1.3\,g\,cm^{-3}$, C_{org} 1.62%, N_{tot} 0.18%, C-to-N ratio 9.1. Mean annual temperature is 8.7 °C and average rainfall 680 mm $year^{-1}$ (mean 1961–1990, meteorological station Stuttgart-Hohenheim).

The *Miscanthus* × *giganteus* (Greef et Deu) was planted on May 24, 1994 on a former grassland plot. The above-ground standing biomass of the *Miscanthus* has been

harvested every year and the remaining foliage was mulched annually in March. The *Miscanthus* yields were 0.95 kg C m^{-2} $year^{-1}$ on average. Soil samples for total C and $\delta^{13}C$ analysis were taken on November 10, 2004. Soil samples from the reference plot under grassland were taken on October 27, 2004. The grassland on the reference plot with the domination of perennial ryegrass (*Lolium perenne* L.) has been cultivated for about 20 years with 2–3 annual cuttings. The grassland was not grazed and no manure was applied. The *Miscanthus* plot is located about 30 m from the reference plot. The samples were taken from individual points with the distant of 5–10 m from each other. The cultivation period at the time of sampling was 10 years and 6 months. Soil samples were taken with a soil corer (inner diameter 6 cm) to a depth of 5 cm. So, only the upper 0–5 cm layer of the Ah horizon was used. The soil samples were air-dried at room temperature and sieved (2 mm mesh size). After that, from a sub-sample of 5 g, all visible roots and plant remains were carefully removed with tweezers and the soil was ball milled (MM2, Fa Retsch) for 15 s.

2.2. Thermogravimetry–differential scanning calorimetry analysis

Thermogravimetric analysis (TG) and DSC analysis were carried out simultaneously using the Netzsch STA 409EP instrument, regulated by the TASC 414/3 controller (Fa Netzsch). About 100 mg of soil sample was weighed out on an alumina crucible and then heated from 20 to 1000 °C in air atmosphere (1 ml min^{-1}). The heating rate was 2 °C min^{-1}. The weight of the soil sample (in mg), as well as the heat (in μV mg^{-1}) released or consumed by substance oxidation or water evaporation was continuously scanned. Calcined kaolinite previously heated at 1250 °C was used as the reference material. The weight losses determined by dTG were used to set "threshold" temperature levels (190, 310, 390 and 480 °C) for subsequent $\delta^{13}C$ analysis (see below). Since the DSC curve of soil samples could not be exactly related to individual substances (as for the matrix-free organic substances), we used the DSC only to confirm the differential mass losses. After determination of "threshold" temperature levels by TG-DSC analysis, all soil samples were divided into four groups and combusted in a muffle oven (Heraeus MR-260) under the same conditions (sample weight 100 mg, heating rate 2 °C min^{-1}). Each group of soil samples was combusted to its particular "threshold" temperature. Then, the combustion samples were left to cool to room temperature and prepared for isotopic analyses.

2.3. Sample analyses

A total of 10–40 mg of soil samples before and after heating in the muffle oven were weighed in tin capsules for total C and $\delta^{13}C$ analyses. The amount of C and its isotopic composition was measured on the Thermo Finnigan isotope ratio mass spectrometer (IRMS) coupled to the Euro EA C/N analyser. Acetanilide was used as the internal standard for $\delta^{13}C$ analyses. The final isotopic composition is expressed as $\delta^{13}C$ units in relation to the international standard Pee Dee Belemnite.

2.4. Calculations

The $\delta^{13}C$ values of individual SOM pools were calculated based on the isotopic mass balance equation (Balesdent and Mariotti, 1996):

$$\delta^{13}C_{b.s.} \times C_{b.s.} = \delta^{13}C_{f.1} \times C_{f.1} + \delta^{13}C_{f.2} \times C_{f.2} + \cdots + \delta^{13}C_{f.n} \times C_{f.n}, \quad (1)$$

where $\delta^{13}C_{b.s.}$ is the $\delta^{13}C$ value of the bulk soil without combustion; $C_{b.s.}$ is the amount of C in the bulk soil without combustion; $\delta^{13}C_{f.1}$, $\delta^{13}C_{f.2}$,…,$\delta^{13}C_{f.n}$ are the $\delta^{13}C$ values of the SOM pools after combustion up to the subsequent "threshold" temperature levels; $C_{f.1}$, $C_{f.2}$,…, $C_{f.n}$ are the amounts of C in the SOM pools after combustion.

The portion of *Miscanthus*-derived C_4-C in SOM (% $C_{Miscanthus}$) was calculated according to Balesdent and Mariotti (1996) with the assumption of identical isotopic fractionation of the humification of C_3 and C_4 plant residues:

$$\%C_{Miscanthus} = \frac{\delta^{13}C_t - \delta^{13}C_3}{\delta^{13}C_4 - \delta^{13}C_3} \times 100, \quad (2)$$

where $\delta^{13}C_t$ is the $\delta^{13}C$ value of the soil with *Miscanthus*; $\delta^{13}C_3$ is the $\delta^{13}C$ value of the corresponding SOM fraction of the reference soil with continuous C_3 vegetation; $\delta^{13}C_4$ is the theoretical $\delta^{13}C$ value of a C_4 soil developed solely under *Miscanthus*, calculated based on the $\delta^{13}C$ of the *Miscanthus* plant and corrected for isotopic fractionation during humification by the subtraction of the differences between $\delta^{13}C$ of C_3 vegetation and $\delta^{13}C$ of the corresponding SOM fraction of the C_3 soil.

To calculate the annual contribution of new *Miscanthus*-derived C_4-C in SOM and annual turnover rates (TR) of SOM, a simple exponential approach was selected (Balesdent and Mariotti, 1996):

$$TR = \frac{-\ln\left(1 - (\%C_{Miscanthus}/100)\right)}{\text{time}}, \quad (3)$$

where time (years) is the cultivation period of *Miscanthus* and $\%C_{Miscanthus}$ is the portion of C_3-C that was replaced by C_4-C derived from *Miscanthus* (Eq. (2)).

The MRT of *Miscanthus*-derived C_4-C in bulk soil and in SOM pools was calculated as a reciprocal to the turnover rates (Gregorich et al., 1995).

The study was conducted with seven replications for *Miscanthus* soil samples and three replications for the reference soil. The significance of differences between $\delta^{13}C$, as well as the C content of different pools was examined using the two-way analysis of variance (ANOVA). The standard errors of means were presented on the figures and in the table as variability parameter.

3. Results

3.1. Thermal stability and differential scanning calorimetry analysis of the soil

The dTG clearly showed 3 maximums of weight losses at temperature increases (Fig. 1): (1) between 20 and 190 °C, (2) between 190 and 310 °C and (3) between 390 and 480 °C. The DSC traces were characterised by three temperature ranges, two with endothermic reactions: (1) from ambient temperature to 210 °C in soil under grassland and to 220 °C in soil under *Miscanthus*; (2) from 335 to 1000 °C in both reference soil and soil under *Miscanthus*; and one range with exothermic reactions between 210 or 220 and 335 °C (Fig. 1). The total weight losses of the soil under *Miscanthus* and the reference plot were 8.45% and 8.27%, respectively (Table 1). Assuming that the amount of SOM is 1.724 times higher than the C content (Post et al., 2001), then only 34.0% and 43.5% of the total weight losses at up to 1000 °C could be connected with the decomposition of SOM in the reference soil and soil under *Miscanthus*, respectively (Table 1).

No significant C losses were measured in both soils at the temperature of up to 190 °C, but samples weight decreased by about 0.71% (Table 1). It means that the weight losses in the temperature area up to 190 °C were mainly connected with water evaporation. This was also clearly proven by the negative values of DSC that showed endothermic reactions by water evaporation (Fig. 1). Starting from 190 °C, the weight losses increased simultaneously with strong DSC increases. This was a clear evidence of energy release by the thermal decomposition of organic substances. The maximum weight losses and energy release occurred between 190 and 310 °C,

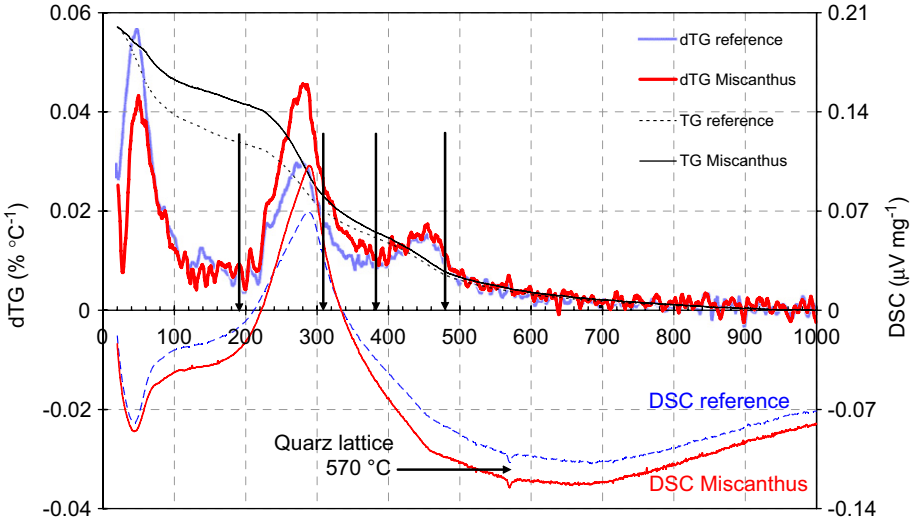

Fig. 1. Differential thermogravimetry (dTG, left *Y*-axis) and Differential Scanning Calorimetry (DSC, right *Y*-axis) of reference soil under grassland and soil under *Miscanthus*. Cumulative losses (TG) are scaled to 100% of the left *Y*-axis and total cumulative losses amounted to 8.27% and 8.45% for reference soil and soil under *Miscanthus*, respectively. Negative DSC values represent energy consumption (endothermic reactions) and positive DSC values represent energy release (exothermic reactions). Arrows show the "threshold" temperature chosen for SOM fractionation.

Table 1
Losses of weight and amount of C (±SE) in SOM pools with different thermal stability for reference soil under grassland (ref) and soil under *Miscanthus* (Misc), and their $\delta^{13}C$ values measured or calculated according to isotopic mass balance

Temperature fraction (°C)	Weight losses (%)		C content (%)		SOM[a] on total losses (%)		$\delta^{13}C$ (‰ PDB)	
	Ref	Misc	Ref	Misc	Ref	Misc	Ref	Misc
20–1000[b]	8.27	8.45	1.62±0.13	2.13±0.09	33.7	43.5	–26.47±0.26	–17.65±0.22
20–190[c]	0.70	0.72	0±0.05	0±0.09	0	0	ND	ND
190–310[c]	2.62	2.68	0.49±0.04	0.81±0.05	31.9	51.9	–28.91±0.67	–18.80±0.33
310–390[c]	2.26	2.31	0.90±0.03	1.14±0.06	68.8	85.3	–25.91±0.25	–16.92±0.24
390–480[c]	1.41	1.44	0.22±0.02	0.21±0.01	27.4	25.7	–24.95±0.29	–17.10±0.17
480–1000[b]	1.12	1.30	0.03±0.002	0.02±0.001	4.7	3.3	–20.92±0.66	–19.54±0.48
$LSD_{0.05}$[d]	0.42		0.17				1.14	

[a]SOM (%) was determined as multiplication of the amount of C (%) by 1.724.
[b]Measured.
[c]Calculated by isotopic mass balance equation.
[d]The least significant differences ($P<0.05$) were calculated for two-way ANOVA: soil × temperature.

amounting to 30% of total weight losses (8.27% and 8.45%) for both reference soil under grassland and soil under *Miscanthus*. The maximal DSC values of 0.07 and 0.10 $\mu V\,mg^{-1}$ were measured at 290 °C in reference soil and in soil under *Miscanthus*, respectively (Fig. 1). However, the maximum weight losses did not correspond to the maximum SOM losses. According to the C losses at individual temperature ranges, the maximum SOM decomposition was observed in the temperature range between 310 and 390 °C, where the soil under *Miscanthus* lost 85.3% of SOM from mass losses of fraction and the losses of organic matter from the reference soil amounted to 68.8% (Table 1). The third maximum of mass losses was related to the temperature fraction from 390 to 480 °C (Fig. 1). About 27.4% and 25.7% of total weight losses in this temperature range corresponded to SOM decomposition in soil under grassland and soil under *Miscanthus*, respectively (Table 1). The weight and SOM losses by further temperature increase to above 480 °C were much slower than at below this temperature. At up to 1000 °C, the reference soil and soil under *Miscanthus* lost only 1.12% and 1.30% of weight. SOM losses amounted to 4.7% and 3.3% of the weight losses within the last temperature range for soils under grassland and *Miscanthus*, respectively (Table 1). The DSC curve switched into negative values after the temperature increased to 340 °C and above showing energy consumption.

3.2. C isotopic composition of SOM pools with different thermal stability

According to the thermal stability of the SOM, five temperature ranges were chosen for soil heating in the muffle oven for the subsequent isotopic analyses: (1) no heating (20 °C) corresponds to the bulk soil, (2) heating up to 190 °C, (3) heating up to 310 °C, (4) heating up to 390 °C, and (5) heating up to 480 °C (Fig. 1). Four SOM pools with increasing thermal stability were obtained after the combustion up to the "threshold" temperatures: pools with peaks between 190 and 310 °C, and between 390 and 480 °C; intermediate pool between 310 and 390 °C; all SOM remaining after 480 °C. The heating up to 310 °C removed the SOM pool with the "threshold" temperatures of between 190 and 310 °C, so only the SOM remaining after 310 °C was analysed. The same approach was used to determine other SOM pools. The temperature range between 310 and 390 °C (intermediate fraction) was selected for better separation of the fractions decomposed below 310 °C and above 390 °C.

The analysis of C isotopic composition showed that the heating up to 190 °C does not change $\delta^{13}C$ values of reference soil or soil under *Miscanthus* (Fig. 2). This is in agreement with the absence of total C losses for temperatures up to 190 °C (Table 1). Further heating up to 310 °C increased $\delta^{13}C$ values by 0.99‰ in reference soil, and by 0.64‰ in soil under *Miscanthus* (Fig. 2). The $\delta^{13}C$ value of SOM pool remaining after combustion of up to 390 °C showed a significant difference only in the reference soil, where it increased by 1.12‰, compared to $\delta^{13}C$ in the SOM decomposed up to 310 °C. The largest shift in $\delta^{13}C$ value occurred in the SOM remaining after heating up to 480 °C: the $\delta^{13}C$ in the soil under *Miscanthus* decreased by 2.2‰ which implied, that C in the thermal stable SOM pools remained closer to the previous C_3 vegetation ($\delta^{13}C = -28.0$‰) compared to the C of less stable SOM pools, that decomposed at temperatures of up to 190, 310, and 390 °C. In contrast to the soil under *Miscanthus*, the value of $\delta^{13}C$ in the reference soil in the same fraction (>480 °C) showed an increase of up to 3.55‰ in comparison with the $\delta^{13}C$ value in the SOM fraction decomposed up to 390 °C. The observed ^{13}C enrichment in the SOM pools with increasing thermal stability of the reference soil is mainly connected with isotopic discrimination of ^{13}C by decomposition during slow temperature increase.

3.3. Portion of Miscanthus-derived C_4-C and mean residence time of the SOM pools

The portion of *Miscanthus*-derived C_4-C in the SOM was calculated according to the approach by Balesdent and Mariotti (1996) (Fig. 2). During 10.5 years of *Miscanthus* cultivation, the amount of new C_4-C in the SOM of bulk soil reached 55.4% (Table 2). However, the C_4-C contribution in the 190–310 °C pool was 60.4% and decreased down to 6.4% in the pool decomposed above 480 °C. Based on this C_4-C contribution and the period after vegetation change (10.5 years), the SOM turnover rates were calculated (Table 2). The C turnover in the pools decomposed at lower temperatures was much faster than that in the pools decomposed at higher temperatures. The MRT, calculated as the reciprocal to the turnover rates (Gregorich et al., 1995) of SOM pools 190–310, 310–390, and 390–480 °C were similar: 11.6, 12.2, and 15.4 years, respectively. The pool above 480 °C had a much longer MRT of 162.6 years (Table 2). However, the amount of C_4-C incorporated in the last pool as well as the total amount of C_{org} in this pool was much lower than in the pools decomposed below 480 °C. Therefore, the pool decomposed above 480 °C is of minor ecological importance.

4. Discussion

4.1. Thermal stability

In contrast to our previous study (Kuzyakov et al., 2006), the temperature increase in this study was 2.5 times slower and TG-DSC was used to separate the SOM pools with different thermal stability. Slower temperature increases allowed for much better resolution of individual maximums of mass losses, especially of the maximum above 390 °C. According to the dTG curve, five temperature ranges were grouped (Fig. 1). The first interval up to

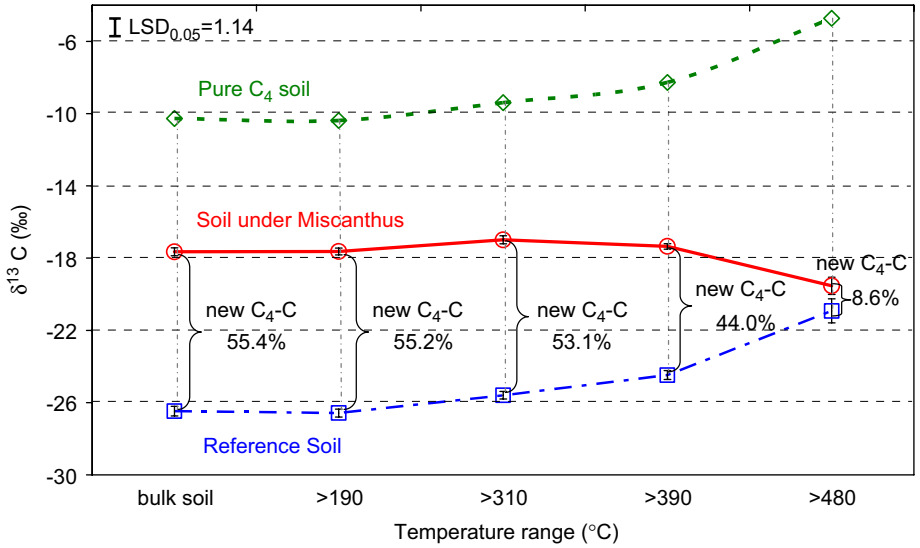

Fig. 2. δ^{13}C values of bulk reference soil under grassland and soil under *Miscanthus* and four SOM pools remaining after heating up to 190 °C (>190 °C), up to 310 °C (>310 °C), up to 390 °C (>390 °C), and up to 480 °C (>480 °C). The calculated theoretical δ^{13}C values of C_4 soil developed solely under C_4 vegetation (pure C_4 soil) were used to estimate the portion of C_4-C in the SOM. Whiskers present standard error (±SE). LSD $_{0.05}$ of means amounting for 1.14‰ shows significant differences between the two soils and temperature ranges.

Table 2
The portion of C_4-C, turnover rates, and mean residence time (MRT) of SOM (±SD) accumulated in 10.5 years of *Miscanthus* cultivation

Temperature fraction (°C)	*Miscanthus*-derived C_4-C (%)	Turnover rates (year^{-1})	MRT (years)
20–1000	55.4±2.3	0.077±0.005	13.1±0.8
20–190	ND	ND	ND
190–310	60.4±5.2	0.090±0.013	11.6±1.6
310–390	57.9±3.1	0.083±0.007	12.2±1.0
390–480	49.6±2.1	0.065±0.004	15.4±0.9
480–1000	6.4±0.7	0.006±0.001	163±18

190 °C was connected with the losses of hygroscopic water, since no C was lost and no changes of isotopic composition were observed. The consumption of energy by water volatilization was clearly confirmed by negative DSC values up to 210 or 220 °C in reference soil and soil under *Miscanthus*, respectively. The endothermic reactions measured by DSC up to 120–150 °C by TG-DSC studies of SOM were observed by many authors (Dell'Abate et al., 2003; Francioso et al., 2005; Plante et al., 2005). This bound water mainly consisted of water absorbed from air moisture and hygroscopic water of salts (Gaál et al., 1994).

The temperature ranges between 190–310 and 310–390 °C were characterized by the most intensive weight and SOM losses (Table 1). Moreover, the portion of SOM contributing to weight losses in the temperature range 310–390 °C amounted to 85% in soil under *Miscanthus*. The decomposition of organic compounds in these temperature ranges led to strong energy release with maximum at 290 °C. Nearly the same temperature (~300 °C) corresponded to the exothermic maximum observed by decomposition of humic acids extracted from different peats, lignites and leonardites (Francioso et al., 2005). This exothermic reactions reflected thermal decomposition of polysaccharides, decarboxylation of acidic groups and dehydration of hydroxylate aliphatic structures (Dell'Abate et al., 2002).

The third maximum of mass losses was related to the temperature fraction of 390 to 480 °C (Fig. 1). About 26% of the mass losses that occurred in this fraction were from SOM decomposition for both reference soil under grassland and soil under *Miscanthus* (Table 1). The decomposed organics could be referred to as stable constituents with aromatic compounds, such as lignin dimers (Leinweber and Schulten, 1992; Siewert, 2004; Lopez-Capel et al., 2005), although the mass losses were not supported by the DSC curve, which showed endothermic reactions from 317 and up to 1000 °C. As suggested by Plante et al. (2005), the energy consumption within this temperature range occurred due to the dehydroxylation of the clay minerals, prevailing the decomposition of organics.

In the last temperature range (>480 °C), 1.12% and 1.30% of mass were lost in the reference soil and soil under *Miscanthus*, respectively, whereas only 5.4% and 4.2% of these losses were connected with SOM decomposition in both soils, respectively. The other 95% were referred to the O, S, P, and H losses through mineral changes at temperatures between 480 and 1000 °C (Schultze, 1969). One of such changes could be seen as a small endothermic peak on the DSC curve at 570 °C that indicated the release of constitutional water and the collapse of the lattice of such clay minerals as kaolinite and halloysite (Schultze 1969, p. 204).

4.2. Carbon isotopic composition and the portion of the Miscanthus-derived C_4-C in SOM

The average shoot and root $\delta^{13}C$ values of *Miscanthus* growing on the soils were –11.8‰ and of grassland plants previously grown on this plot were –28.0‰. Considering the increase of the $\delta^{13}C$ value of the soil under *Miscanthus* up to 8.82‰ comparing to the $\delta^{13}C$ value of bulk soil under continuous C_3 vegetation (–26.47‰) and the isotopic discrimination of 1.0‰ by humification (Agren et al., 1996; Ehleringer and Cerling, 2002), the amount of C exchanged in the total SOM during the last 10.5 years was 55.4% (Table 2). This is much higher than the contribution of new C within 10 years, observed in most studies done on maize (Flessa et al., 2000; Ludwig et al., 2003). This higher contribution of *Miscanthus*-derived C was reflected in its much greater above and below-ground biomass and annual mulching of part of the above-ground plant residues in comparison to maize. The large amount of *Miscanthus*-derived C in our study contradicted the findings of the study by Foereid et al. (2004), which was conducted with *Miscanthus*, where the portion of new C_4-C reached only 18% after 11 years. The difference is probably connected to the fact that various soil layers had been investigated in their study. In our study, only the upper 0–5 cm was used, where the C turnover is faster compared to the soil depths of 0–10, 0–20 or even 0–30 cm, which were used in other studies (Gregorich et al., 1995; Flessa et al., 2000; Foereid et al., 2004).

The turnover rates (TR) corresponding to the MRT of 13.1 years for bulk soil and of 11.6, 12.2, and 15.4 years for pools decomposed between 190–310, 310–390 and 390–480 °C, respectively, were faster than the MRTs in the most of other studies with maize (Huggins et al., 1998; Collins et al., 1999). However, Gregorich et al. (1995) obtained a similar MRT of 15 years for soil under maize. These fast turnover rates and short MRT were connected to the very high input of *Miscanthus* C in the upper 0–5 cm soil layer and the labile compounds that were accumulated in the SOM during the period of *Miscanthus* cultivation (10.5 years). Foereid et al. (2004) showed that the stability of *Miscanthus*-derived SOM was correlated to the time of *Miscanthus* cultivation. The *Miscanthus*-derived C in the 11-year old *Miscanthus* field had a MRT not much longer than the fresh residues (1 year), while the MRT of the older field (18-year old) was longer (3.5 years).

The MRT of C_4-C in SOM pool decomposed above 480 °C was more than 160 years (Table 2). So due to very low incorporation of *Miscanthus*-derived C into this SOM pool (6.4%), this pool could be considered as an inert or recalcitrant one. Many other studies had looked for appropriate methods to separate the recalcitrant fraction of the SOM and considered this pool by modelling (reviewed by Ludwig et al., 2003). Most methods failed in the experimental estimation of the inert fraction because the recalcitrance may be connected with various chemical, physical, and biological properties (Six et al., 2001; Ludwig et al., 2003; Plante et al., 2005). Foereid et al. (2004) hypothesized that the recalcitrance of SOM under *Miscanthus* cropping was connected to the formation of insoluble carbon in the SOM, which increased its stability upon microbial attack.

The slow turnover rate of the pool with the highest thermal stability suggested its recalcitrance. However, the amount of this fraction (14% of SOM) in our study was lower than the results of other studies, where the inert SOM amounted to 30–40% of the total C (John et al., 2005; Kristiansen et al., 2005).

The experimental data showed that the differences in $\delta^{13}C$ values between SOM pools 190–310, 310–390, 390–480 °C were small and therefore, the portion of new Miscanthus-derived C was very similar (Tables 1 and 2). Consequently, the portion of C subjected to a short-time turnover with rates of years and decades was nearly similar in all SOM pools decomposed up to 480 °C. Only small amounts of C (<1% of Corg) in the SOM pool decomposed above 480 °C could be referred to as a stable C with MRT of about 160 years.

5. Conclusions

Thermogravimetry coupled with differential scanning calorimetry (TG-DSC) was suitable to separate soil organic matter (SOM) pools based on their stability to thermal decomposition. Despite clear separation of SOM pools of different thermal stability by TG-DSC, the isotopic analyses showed that the accordance between thermal and biological degradability was not gradual. All SOM pools with low and medium thermal stability decomposed below 480 °C had faster turnover rates than the pool, decomposed above 480 °C. Microbial availability of all pools decomposed below 480 °C was similar (MRT of 12–15 years) suggesting no clear correlation between thermal and microbial decomposability. A long MRT of 160 years was measured for the thermally stable pool decomposed above 480 °C. However, the ecological significance of this pool is of minor importance because of very low C amounts in the pool. Due to the very poor correlation between $\delta^{13}C$ values and thermal stability of SOM pools we conclude that the applicability of the TG-DSC for the separation of SOM pools with different biological availability is limited.

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