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Effect of C_3–C_4 vegetation change on $\delta^{13}C$ and $\delta^{15}N$ values of soil organic matter fractions separated by thermal stability

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Abstract

Carbon isotopic composition of soils subjected to C_3–C_4 vegetation change can be used to estimate C turnover in bulk soil and in soil organic matter (SOM) pools with fast and intermediate turnover rates. We hypothesized that the biological availability of SOM pools is inversely proportional to their thermal stability, so that thermogravimetry can be used to separate SOM pools with contrasting turnover rates. Soil samples from a field plot cultivated for 10.5 years with the perennial C_4 plant *Miscanthus×gigantheus* were analyzed by thermogravimetry coupled with differential scanning calorimetry (DSC). Three SOM fractions were distinguished according to the differential weight losses and exothermic or endothermic reactions measured by DSC. The $\delta^{13}C$ and $\delta^{15}N$ values of these three fractions obtained by gradual soil heating were measured by IRMS. The weight losses up to 190 °C mainly reflected water evaporation because no significant C and N losses were detected and $\delta^{13}C$ and $\delta^{15}N$ values of the residual SOM remained unchanged. The $\delta^{13}C$ values (−16.4‰) of SOM fraction decomposed between 190 and 390 °C (containing 79% of total soil C) were slightly closer to that of the *Miscanthus* plant tissues ($\delta^{13}C = -11.8$‰) compared to the $\delta^{13}C$ values (−16.8‰) of SOM fraction decomposed above 390 °C containing the residual 21% of SOM. Thus, the C turnover in the thermally labile fraction was faster than that in thermally stable fractions, but the differences were not very strong. Therefore, in this first study combining TG-DSC with isotopic analysis, we conclude that the thermal stability of SOM was not very strongly related to biological availability of SOM fractions. In contrast to $\delta^{13}C$, the $\delta^{15}N$ values strongly differed between SOM fractions, suggesting that N turnover in the soil was different from C turnover. More detailed fractionation of SOM by thermal analysis with subsequent isotopic analysis may improve the resolution for $\delta^{13}C$.

Introduction

The alteration in carbon (C) isotopic composition of soil organic matter (SOM) after C_3–C_4 vegetation change (and *vice versa*) has frequently been used to estimate C turnover rates and the incorporation of new C in SOM fractions (John et al., 2005; Kristiansen et al., 2005; Ludwig et al., 2003; 1987). Isotopic analyses are often performed on SOM fractions including density fractions (John et al., 2005; Magid et al., 2002) and particle size fractionation (Jolivet et al., 2003; Ludwig et al., 2003) as well as sequential

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extractions separated by various methods (Ellerbrock and Kaiser, 2005). Another method that is suitable for SOM fractionation is based on the different thermal stability of various C fractions in soil (Francioso et al., 2005; Lopez-Capel et al., 2005; Plante et al., 2005; Siewert, 2001, 2004). Thermal analysis coupled with differential scanning calorimetry (TG-DSC) is widely applied in coal, charcoal, peat, compost and lignite studies (see references in Lopez-Capel et al., 2005 and in Francioso et al., 2005). It has also been used to evaluate the humification state of SOM (Grisi et al., 1998; Siewert, 2001, 2004), the origin of humic acids (Francioso et al., 2005), and qualitative SOM alterations by various land uses (Plante et al., 2005).

TG-DSC involves a slow, continuous temperature increase leading to progressive decomposition (mainly oxidation) of organic compounds according to their thermal stability. Temperature increase coupled with weight loss measurements is called thermogravimetry (TG). Simultaneous with the weight loss measurements, energy released or consumed by the decomposition of organics or changes of mineral lattices is measured by differential scanning calorimetry (DSC). The thermal stability of organics in soil depends on the nature of the organic compound and binding on mineral particles. The initial weight losses below 190–200 °C mainly reflect the release of bound water. The exothermic decomposition of labile aliphatic and carboxyl groups occurs at around 300 °C, while the more refractory aromatic C is decomposed at higher temperatures (≈450 °C) (Leinweber and Schulten, 1999; Schulten and Leinweber, 1999).

The weight loss in these two phases can be used to compare the relative abundance of more and of less thermally labile C, while the position of DSC peaks reflects structure and chemical composition (Brown, 1988; Lopez-Capel et al., 2005; Plante et al., 2005). Calcium carbonate-containing minerals are usually decomposed at temperatures above 600 °C (Siewert, 2004; Lopez-Capel et al., 2005). Despite the consistent temperatures of various peaks, the results of TG-DSC remain qualitative and cannot be interpreted in terms of reaction mechanisms (Schultze, 1969; Yariv, 1991) because simultaneous reactions generate hidden peaks or cancel out peak intensities if the individual reactions are exothermic and endothermic (Plante et al., 2005). Additionally, adsorption of organics on clays may shift the temperatures and intensities of the peaks compared to model systems (Langier-Kuzniarowa, 2002).

Based on correlations between the thermal stability of SOM fractions in various temperature ranges and the CO_2 production analyzed by soil incubations, Siewert (2001) suggested that the thermal stability of SOM fractions can be related to their biological degradability. Indirect confirmation of this hypothesis is the fact that organics decomposed by high temperatures are bound on mineral particles and composed on aromatic structures (Schulten and Leinweber, 1999) and, therefore, hardly accessible to soil microorganisms. This interpretation, however, that thermal stability and biological decomposability are closely related was based solely on correlations and was never demonstrated with direct methods.

We hypothesized that if thermal stability is closely coupled with biological decomposability, then in soils after a C_3–C_4 vegetation change, the thermally labile fractions will have $\delta^{13}C$ values closer to the new vegetation compared to thermally stabile SOM fractions. To test this hypothesis, we analyzed the thermal stability of organic matter of soil samples collected from a field planted with perennial C_4 crop (*Miscanthus* × *giganteus*) planted on a former C_3 grassland soil. We subjected the soil to TG-DSC, isolated individual SOM fractions, and measured $\delta^{13}C$ and $\delta^{15}N$ values of SOM fractions obtained after combustion at different temperatures.

Material and methods

Soil samples

Soil samples were taken from the long-term experimental field located in Stuttgart-Hohenheim, Baden-Württemberg, Germany (48°43′ N, 9°13′ E). The soil was a silty loamy Gleyic Cambisol (4.4% sand, 73% silt and 23% clay) without any significant textural change in soil profile. Mean annual temperature is 8.7 °C and average rainfall 680 mm a^{-1} (mean 1961–1990, meteorological station Stuttgart-Hohenheim). The soil properties were: pH 5.6 (no $CaCO_3$), bulk density

(0–10 cm) 1.1 mg m^3, C_{org} 28.0 mg g^{-1} and N_{tot} 2.03 mg g^{-1}, with a C:N ratio of 13.8.

Miscanthus × *giganteus* (Greef et Deu) was planted on 24 May 1994 on a former grassland plot and the stand was mulched annually in March. *Miscanthus* yields averaged 0.95 kg C m^{-2} a^{-1}. The *Miscanthus* cultivation period at the sampling time (25 October 2004) was 10.5 years. Soil samples were taken with a soil corer (inner diameter 6 cm) to a depth of 5 cm. Thus, only the upper 0–5 cm layer of the A_h horizon was investigated. The soil samples were air-dried at room temperature and sieved (2 mm mesh size). All visible root and plant remains were carefully removed with tweezers from a sub-sample of three grams, and the soil was ground in a ball mill (MM2, Fa Retsch) for one minute.

Thermogravimetry – Differential Scanning Calorimetry analysis

Thermogravimetric analysis and differential scanning calorimetry analysis were carried out simultaneously using a Netzsch STA 409EP instrument controlled by a TASC 414/3 controller (Fa Netzsch) (Plante et al., 2005). A soil sample (100.0 mg) was weighed on a platinum crucible and then heated from 20 to 1050 °C in an air atmosphere (1 L min^{-1}). The heating rate was 5 °C min^{-1}. The weight of the soil sample as well as energy (in μV mg^{-1}) released or consumed by substance oxidation or water evaporation were continuously scanned (every second). Calcined kaolinite previously heated at 1250 °C was used as the reference material for DSC measurements. As the replicate analyses for TG and DSC curves were nearly identical ($< \pm 2$ °C for peak temperatures, $< \pm 0.2\%$ for mass losses), only one replicate was reported. Based on the results of complete thermal analysis of samples from 20 to 1050 °C, replicate samples were thermally treated to either 190 or 390 °C (see below). These samples were left in the platinum crucibles after the combustion treatment to cool to room temperature, then prepared for isotopic analyses. Separate combustions were used for each isotopic analyses.

Peak fitting was performed with PeakFit (version 4.11, SYSSTAT Software Inc.) using the residuals method with Gaussian sum on area peaks, allowing for negative peaks (endothermic reactions) and varied peak shapes and widths (Plante et al., 2005). The PeakFit software automatically scans the trace and places peaks. No additional peaks were placed manually to improve the fit. Only the maximum of the peak corresponding to water losses was set as constant. All other peaks and their parameters were fitted iteratively until no changes in the fitting statistics were observed.

Sample analysis

Plant samples (shoots, roots, and rhizomes) were dried at 50 °C and ground. Ten to forty milligrams of ground soil samples and four to six milligrams of plant samples were weighed in tin capsules for $\delta^{13}C$ and $\delta^{15}N$ analyses. The $\delta^{13}C$ and $\delta^{15}N$ analyses of SOM fractions with different thermal stability were done on soil residues after heating steps. The C and N isotopic composition was measured with a Delta^{plus} XP isotope ratio mass spectrometer (Thermo Finnigan, Bremen) coupled to a Euro EA C/N analyzer (Eurovector Instruments and Software, Hekatech GmbH, Wegberg). N-phenylacetamide (acetanilid) was used as a standard for $\delta^{13}C$ and $\delta^{15}N$ analyses. The final isotopic composition was expressed as $\delta^{13}C$ and $\delta^{15}N$ units related to Pee Dee Belemnite ($^{13}C/^{12}C = 0.0112372$) and atmospheric N_2 ($^{15}N/^{14}N = 0.0036765$) as standards for C and N, respectively.

Calculations

The $\delta^{13}C$ and $\delta^{15}N$ values of individual SOM fractions were calculated based on the isotopic mass balance equation:

$$M_{Total} \times \delta_{Total} = M_{Fr1} \times \delta_{Fr1} + M_{Fr2} \times \delta_{Fr2} \quad (1)$$

where M_{Total} and δ_{Total} were total amount and $\delta^{13}C$ or $\delta^{15}N$ value of total SOM (not heated soil), M_{Fr1} and δ_{Fr1} were total amount and $\delta^{13}C$ or $\delta^{15}N$ value of the first SOM fraction (e.g. combusted between 190 and 390 °C) and M_{Fr2} and δ_{Fr2} were total amount and $\delta^{13}C$ or $\delta^{15}N$ value of the second SOM fraction (e.g. combusted between 390 and 1050 °C).

The study was conducted with four replications prepared separately by combustion of individual soil samples. The significance of differences

between different fractions for their $\delta^{13}C$ and $\delta^{15}N$ as well as C and N content was examined using one-way analysis of variance (ANOVA). Critical LSD values for 5% error probability were calculated.

Results

Thermal stability and differential scanning calorimetry analysis of the soil

Continuously increasing the temperature from 20 to 1050 °C led to weight losses from soil (Figure 1). The differential thermogravimetry (dTG) clearly showed 2 peaks: (1) between 20 and 190 °C and (2) between 190 and 390 °C. Additionally, the area above 390 °C may be separated into 2 subparts: (3a) between 390 and 570 °C and (3b) above 570 °C. The total weight losses amounted for 10.3% of the soil sample weight (Table 1). Assuming that the amount of SOM was two times higher than the C content in the soil (C_{org} = 2.80% of the soil weight), then only half of the total weight losses up to 1050 °C was associated with the thermal decomposition of SOM. No significant C and N losses were measured up to 190 °C, but the sample weight decreased by 2.25% (Table 1). Thus, the weight losses up to 190 °C were mainly associated with evaporation of free and chemically bound water. This was also clearly supported by negative DSC values, showing endothermic reactions by water evaporation (Figure 1). Starting at 190 °C, the weight losses simultaneously increased with strong DSC increases. This is clear evidence for energy release by thermal decomposition of organic substances. Accordingly, 87.6% of the weight losses between 190 and 390 °C were connected with SOM decomposition (Table 1). The weight losses during further temperature increase above 390 °C were much slower than below

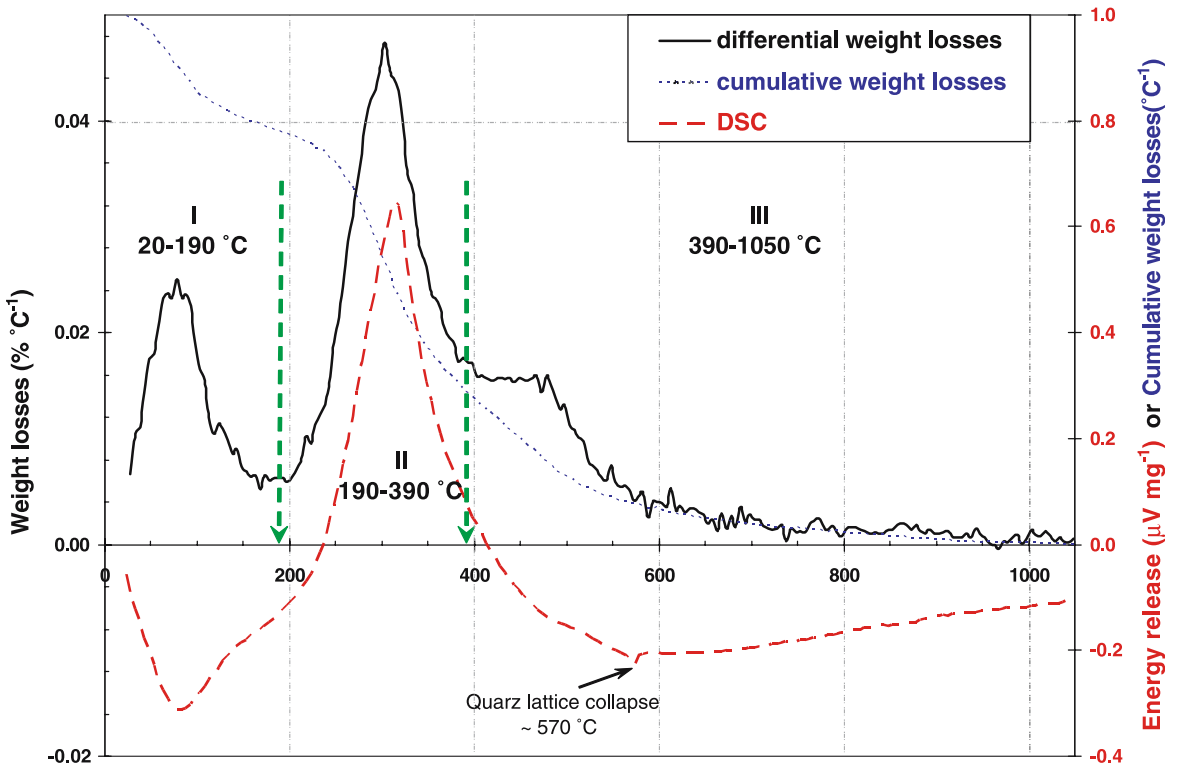

Figure 1. Differential thermogravimetry (dTG, left Y axis), cumulative weight losses (scaled to 1.0, right Y axis) and differential scanning calorimetry (DSC, right Y axis) of the soil under *Miscanthus*. Cumulative weight losses are scaled to 1.0 of the right Y axis and totaled 10.3% of the sample weight. Negative DSC values represent energy consumption (endothermic reactions), positive DSC values energy release (exothermic reactions). Two vertical dashed green arrows show three temperature ranges chosen for SOM fractionation.

Table 1. Losses of weight, C and N (±SE) in SOM fractions of different thermal stability and their $\delta^{13}C$ and $\delta^{15}N$ values measured and calculated according to isotopic mass balance. The values for 'Bulk SOM' and the fraction '390–1050' were measured by EA-IRMS and that for the fractions '20–190' and '190–390' were calculated according to isotopic mass balance (Eq. 1)

Fraction (°C)	Weight losses (%)	C content (%)	N content (%)	C/N	SOM[a] on losses (% of the losses)	$\delta^{13}C$ (‰ PDB)	$\delta^{15}N$ (‰ air N_2)
Bulk SOM	10.30	2.80 ± 0.01	0.203 ± 0.001	13.8	54.4	−16.42 ± 0.10	3.06 ± 0.29
20–190	2.25	0 ± 0.18	0 ± 0.011	–	0	–	–
190–390	5.07	2.22 ± 0.18	0.080 ± 0.011	27.9	87.6	−16.40 ± 0.10[b]	3.27 ± 0.29[b]
390–1050	2.95	0.60 ± 0.02	0.123 ± 0.005	4.6	40.7	−16.79 ± 0.06	5.14 ± 0.29
$LSD_{0.05}$	–	0.46	0.029	0.51	–	0.22	0.84

[a] Contribution of SOM losses to the total weight losses in the respective temperature range. A 50% C content in SOM is accepted in calculating the contribution of SOM to total weight losses (Francioso et al., 2005).
[b] Standard error of the $\delta^{13}C$ value of the intermediate SOM fraction (190–390 °C) is accepted as being equal to the highest error of one of the end members.

390 °C, and the DSC curve switched to negative values (Figure 1) showing energy consumption. Only 40.7% of the weight losses above 390 °C were explainable by decomposition of organic substances (Table 1). The remaining losses (59.3% of the total losses within this temperature range) mainly involved O, S, P and H losses by mineral lattice changes between 390 and 1050 °C. One such change was a small endothermic peak on the DSC curve at 573 °C; this indicates release of constitutional water, collapse of lattices of such clay minerals as kaolinite and halloysite, and structure modifications in silicates (Schultze, 1969, p.204). The weight losses above 573 °C were small (0.79% of the soil weight) and mainly reflected changes in the lattices of soil minerals and subsequent release of constitutional water residues, but not organic C decomposition.

The PeakFit software revealed five peaks composing each of the DSC and dTG curves (Figure 2). The first three fitted peaks in each of the DSC and dTG were clear and not subject to significant overlap. The water evaporation maxima occurred at about 80 °C according to both dTG and DSC. The exact position of the second peak responsible for most of the SOM decomposition was slightly lower according to dTG (299 °C) than to DSC (312 °C) (Figure 2). This shift between dTG and DSC was more pronounced for subsequent temperature fractions. The location and shape of the last two peaks could not be exactly fitted (Figure 2) because the dTG and DSC curves were very flat (Figure 1) and must therefore be interpreted with caution.

According to the thermal stability of SOM as evaluated by dTG and DSC curves as well as by peak fitting, three temperature ranges were chosen for subsequent $\delta^{13}C$ and $\delta^{15}N$ analyses: (1) 20–190 °C, (2) 190–390 °C, and (3) above 390 °C (Figures 1 and 2). Because the C content in the samples above 570 °C was very small and insufficient for $\delta^{13}C$ analysis, we combined both high temperature fractions (3a and 3b) into one, which was referred to as 390–1050 °C.

C and N isotopic composition of SOM fractions with different thermal stability

The $\delta^{13}C$ signature of the bulk soil (thermally untreated) was -16.4 ± 0.10‰. Two thermal SOM fractions were analyzed after heating up to 190 °C: the fraction between 190 and 390 °C and all SOM remaining after 390 °C. Heating to 390 °C removed the SOM fraction between 190 and 390 °C, and only the SOM remaining after 390 °C was analyzed. The C and N isotopic composition showed that heating the soil to 190 °C did not change $\delta^{13}C$ and $\delta^{15}N$ values compared to the bulk soil (Figure 3). This was consistent with the absence of C and N losses up to 190 °C (Table 1). Further heating to 390 °C, however, changed both $\delta^{13}C$ and $\delta^{15}N$ values statistically significant (Figure 3). The $\delta^{13}C$ decrease of 0.4‰ implied that the C in the thermally stable SOM fractions remained closer to the previous C_3 vegetation ($\delta^{13}C = -28.0$‰) compared to the less thermally stable SOM fractions. In contrast to $\delta^{13}C$, $\delta^{15}N$ values increased by 2.1‰ after heating to 390 °C (Figure 3).

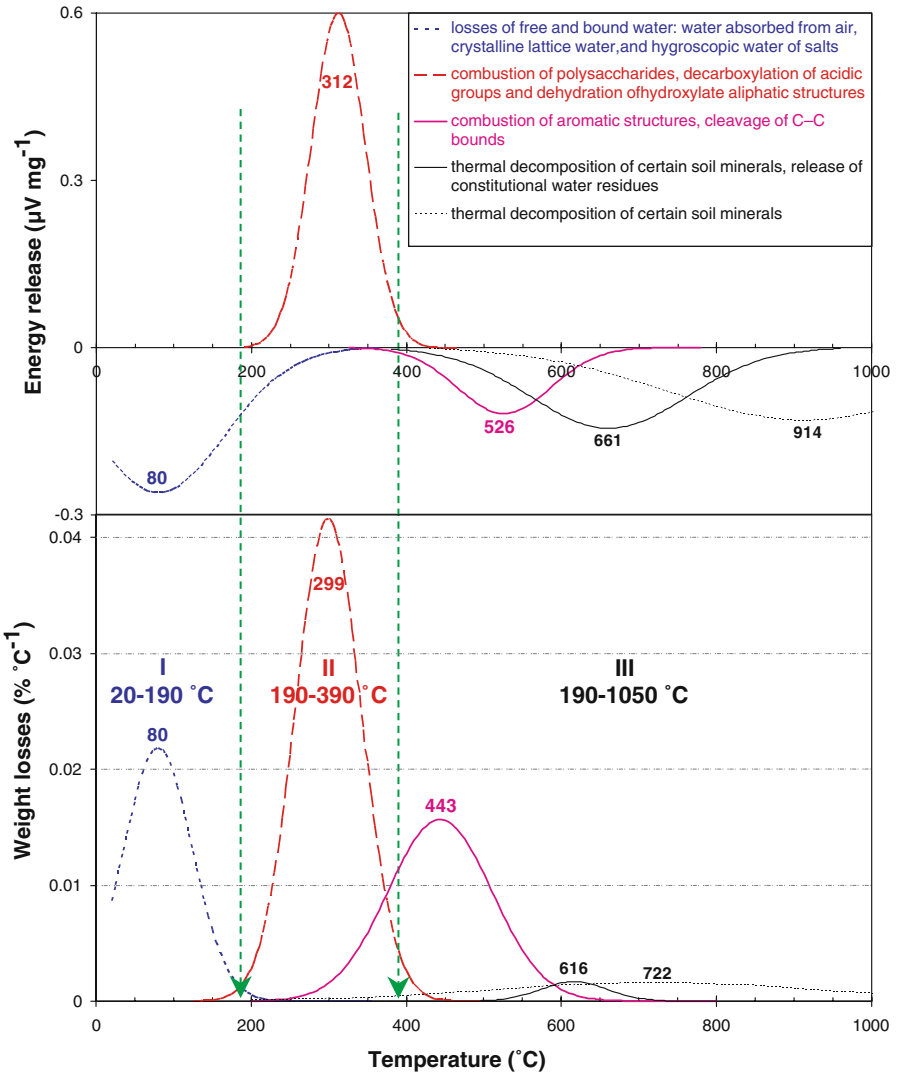

Figure 2. Peak fitting analysis of the differential scanning calorimetry (top) and differential thermogravimetry (bottom) of the loamy soil under *Miscanthus*. Negative DSC values represent energy consumption (endothermic reactions), positive DSC values energy release (exothermic reactions). Dashed arrows show the temperature ranges chosen for SOM fractionation. Values show the temperature maxima of the peaks.

Considering the C and N losses after heating to 190 and 390 °C and based on the isotopic mass balance (Eq. 1), we calculated the $\delta^{13}C$ and $\delta^{15}N$ values of the intermediate fraction (Table 1). The intermediate fraction combusted between 190 and 390 °C contained 79% of C of SOM and had a significantly higher $\delta^{13}C$ value than bulk soil or the fraction combusted above 390 °C. Compared to $\delta^{13}C$, the $\delta^{15}N$ value of SOM fraction between 190 and 390 °C did not significantly differ from the bulk soil, but differed from the fraction above 390 °C (Table 1).

Discussion

Thermal stability

Gradual heating of soil samples yielded unequal weight losses at different temperatures and allowed the separation of at least three temperature ranges (Figures 1, 2). The first interval, up to 190 °C, mainly reflected losses of bound water because no C and N were lost and no significant changes of isotopic composition were observed. This water mainly consists of water absorbed

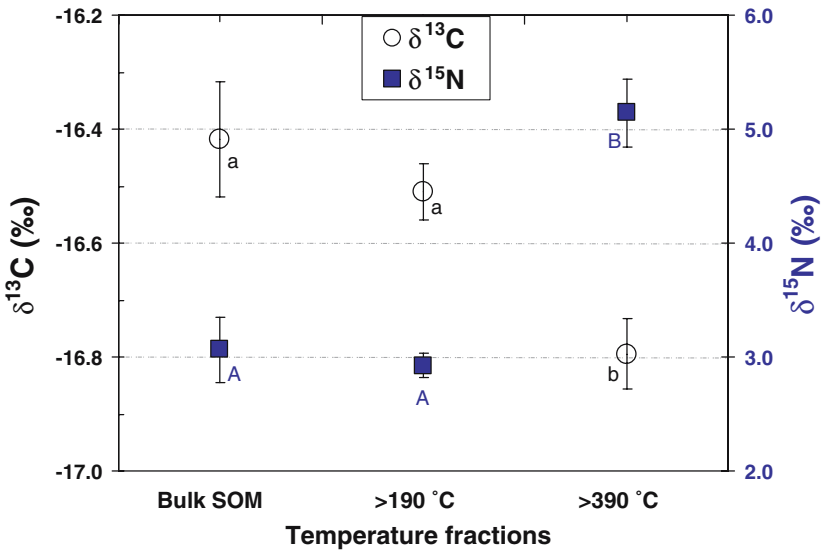

Figure 3. Measured $\delta^{13}C$ (left Y axis) and $\delta^{15}N$ (right Y axis) values of bulk soil (>20 °C) under *Miscanthus* and two SOM fractions: remaining after heating up to 190 °C (>190 °C) and remaining after heating up to 390 °C (>390 °C). Significant differences (LSD, $P=0.05$) are denoted with different letters: small letters for $\delta^{13}C$, capital letters for $\delta^{15}N$. Whiskers present standard error (±SE).

from air, crystalline lattice water, and hygroscopic water of salts (Gaál et al., 1994). A similar conclusion was drawn by Siewert (2001), who compared weight losses up to 190 °C of various soils preconditioned by increasing air moisture. Depending on preconditioning, the weight losses in his soils were between 1.3% (after drying over silica gel) and 5.5% (after preconditioning in air with 100% humidity). Weight losses in our study were 2.25% (Table 1). Part of these losses involved the decomposition of certain organic substances such as free amino and carboxylic acids as well as the volatilization of other organic compounds from the soil. However, the portion of these compounds was very small and had no effect on the C and N composition of the remaining fraction (Table 1).

The second temperature range (190–390 °C) yielded the greatest weight losses, 87.6% of which were connected with thermal decomposition of organic substances (Table 1). Seventy-nine percent of total SOM was decomposed and 49% of the overall weight losses were observed in this temperature range. The location of this peak (~300 °C) exactly coincided with the first exothermic peaks observed by decomposition of humic acids extracted from different peats, lignites and leonardites (Francioso et al., 2005). This exothermic peak probably reflected thermal combustion of polysaccharides, decarboxylation of acidic groups and dehydration of hydroxylate aliphatic structures (Dell'Abate et al., 2002). The average weight losses in the second temperature range from soils on different geological substrates were about 39% (12–62% depending on the origin) (Siewert, 2001). Siewert (2001), however, used a temperature range between 200 and 450 °C, which was 50 °C broader than ours. Therefore, the thermal decomposition of organic matter in our soil in the second temperature range was less than in Siewert's (2001) study.

In the last temperature range (>390 °C), 2.95% of soil mass was lost, whereby only 41% of these losses were connected with SOM decomposition. The location of the third fitted peak (Figure 2) coincided with the second peaks observed by decomposition of humic acids extracted from different peats, lignites and leonardites (Francioso et al., 2005). This decomposition mainly reflected the combustion of aromatic structures and cleavage of C–C bonds (Peuravuori et al., 1999), and the remaining 59% of the weight losses above 390 °C involved the thermal decomposition of certain soil minerals and the release of constitutional water residues from different minerals of the kaolinite

group occurring above 570 °C (Schultze, 1969, p. 204).

Separation of peaks obtained by fitting showed some overlapping (Figure 2). Despite the overlapping, the second and third peaks coincided exactly with peaks from extracted humic acids (Francioso et al., 2005). The extent of this overlap and therefore the quality of fitting depends on the properties of the organic compounds making up the SOM as well as on the heating conditions. We used the heating rate of 5 °C min^{-1} applied by Siewert (2004) and which was two times lower than those used in some other studies (e.g. Lopez-Capel et al., 2005; Plante et al., 2005). As organic decomposition is not instant, we expect slower heating rate to increase the resolution of individual peaks on both dTG and DSC curves.

Carbon isotopic composition

The C isotopic composition of the bulk soil of $-16.4‰ \pm 0.1‰$ showed a very high contribution of *Miscanthus*-derived C to the SOM during the last 10.5 years. The average shoot- and root-weighted $\delta^{13}C$ values of *Miscanthus* growing on the soil were $-11.8 \pm 0.2‰$ and those of grassland plants previously grown on this plot were $-28.0 \pm 0.3‰$. The isotopic discrimination by humification of C_3 plant residues measured for the reference soil originated solely under C_3 vegetation was equal to 1.0‰ (Schneckenberger and Kuzyakov, 2006). We therefore calculated theoretical $\delta^{13}C$ value of the SOM originated solely under *Miscanthus* ($\delta^{13}C = -10.8‰$). This value was used to calculate the portion of *Miscanthus*-derived C in the SOM according to Balesdent and Mariotti (1996). The amount of C exchanged in the total SOM during the last 10.5 years was 64.6%. This is much higher than the contribution of new C observed in other studies with maize for varying cultivation periods (Flessa et al., 2000; Jolivet et al., 2003). This higher contribution of *Miscanthus*-derived C reflects much larger above- and below-ground biomass compared to maize and the annual mulching of some of the above-ground plant residue in the upper 0–5 cm. Note also that we investigated here only the upper 0–5 cm, whose C turnover is generally faster than the 0–20 or 0–30 cm soil depth used in other studies (e.g. Kristiansen et al., 2005). However, the SOM proportions of 19–31% after 5 years of cultivating the perennial C_4 grass *Panicum virgatum L.* (Switchgrass) after just 5 years were similar to the high contributions of C_4-C to SOM by *Miscanthus* (Garten and Wullschleger, 2000). In comparison, C_4-C in Ap horizons (0–20 to 0–30 cm) in various studies ranged from 22 to 40% of the SOC after 8–35 years of maize cultivation (Ludwig et al., 2003). The mean annual increase of about 0.33 g C_4-C kg^{-1} soil a^{-1} in the upper 40 cm in the soils under Switchgrass was twice as high as summarized for upper 30 cm of maize soils (Schneckenberger and Kuzyakov, 2006).

The SOM fraction decomposed in the low temperature range (190–390 °C) had higher $\delta^{13}C$ values that the fraction above 390 °C (Table 1). Accordingly, the contribution of new C_4-C to this fraction was higher than to the second fraction. The differences between both SOM fractions, however, were small and the portion of new C was therefore very similar. This led us to conclude that the proportion of C with short-term turnover (years and decades) was nearly similar in thermally labile and thermally stabile fractions. This initially appears to contradict Siewert's (2001) hypothesis of close correlation between thermal stability and biological decomposability. Reviewing literature, we found that Plante et al. (2005) also observed statistically significant but small differences in the alterations of the total mass of SOM fractions separated by TG-DSC after land use changes. This is consistent with our results that the turnover rates of SOM fractions with different thermal stability were similar.

Nitrogen isotopic composition

Surprisingly, the $\delta^{15}N$ values of SOM fractions differed strongly ($\sim 1.9‰$) between the fractions, and the difference was about 6 times higher than the standard error of means. The field was not fertilized with N or any other nutrients, at least in the last 10 years. Unfortunately, no data were available about the $\delta^{15}N$ values of fertilizers applied before *Miscanthus* was planted on this field. This prevents drawing definitive conclusions about the reasons for $\delta^{15}N$ differences in SOM fractions. Nonetheless, nearly all mineral N fertilizers applied at least over the last 30 years in

Germany are known to have been produced by the Haber-Bosch technique, converting air N_2 to ammonia (Jenkinson, 2001). The $\delta^{15}N$ values of N fertilizers produced by Haber-Bosch conversion are the same as those of the air N_2 and are close to 0‰. The mean total N deposition in Germany is between 25 and 50 kg ha^{-1} $year^{-1}$, depending on the determination method and definition used (References in Russow and Böhme, 2005), whereby higher values are common for highly industrial areas such as Stuttgart. This is significant annual N input, especially for crops having a low N demand like *Miscanthus*. The $\delta^{15}N$ value of atmospheric gaseous NO_x deposition varies from +1.3‰ to +6.4‰ (Durka et al., 1994; Mayer et al., 2001; Saurer et al., 2004) more positive values are common for higher pollution. We speculate that the N remaining in soil after nitrate leaching should be enriched in ^{15}N, if the nitrate leached was depleted in ^{15}N compared to the deposition and soil N (Burns and Kendall, 2002). If the N turnover of the low-temperature fraction is faster than that of the high-temperature fraction, then the microbial availability of N from the latter would be lower. This can lead to accumulation of heavier N in the high-temperature fraction. Direct measurements on soils without land use changes and with known $\delta^{15}N$ values of fertilizer and of atmospheric deposition are required before drawing definite conclusions about different $\delta^{15}N$ values of both SOM fractions.

The $\delta^{15}N$ results showed strong differences between fractions and contrasted with the $\delta^{13}C$ results that showed statistically significant but minor differences between fractions. Therefore, we expect differences in C and N turnover in the soil. Similar conclusions involving different turnover and pathways of C and N were drawn based on incorporation of ^{14}C- and ^{15}N-labeled amino acids and nucleic bases into SOM fractions separated by sequential extraction (Kuzyakov, 1997). Differences in C and N turnovers partly reflect the release of C into the atmosphere, whereas N remains in the soil and is subsequently reutilized by plants and microorganisms. These differences are connected also with the fact that most N-containing organic substances are utilized much faster by microorganisms than most-N free organic substances. Therefore, the N turnover in soils should be faster than the C turnover (Kuzyakov, 1997).

Conclusions and outlook

TG and DSC can be used to separate SOM fractions with different thermal stabilities. The current study is the first application combining the advantages of thermal techniques such as TG-DSC with stable isotope analysis after C_3–C_4 vegetation changes. This combination showed that the thermally labile fraction had slightly faster turnover rates than the fraction with high thermal stability. However, the connection between thermal and biological degradability was weak because the difference in $\delta^{13}C$ values between SOM fractions after C_3–C_4 vegetation changes was only 0.4‰. Contrasting to $\delta^{13}C$, $\delta^{15}N$ values differed much more strongly between the fractions decomposed at increasing temperatures. We therefore assume that the N turnover in SOM differs from that of C.

Future improvement of the suggested method, including more temperature gradations and a slower temperature increase, will enhance the resolution of the C isotopic composition of the SOM fractions. Isotopic analyses of the reference soil without vegetation changes (developed solely under C_3 vegetation) will allow estimation of ^{13}C fractionation by incorporation of organic residues into fractions of different thermal stability. Subsequently, the contribution of C_4-C and C_3-C to the SOM fractions can be calculated and C mean residence time and turnover rates in individual fractions can be estimated. The suggested coupling of TG-DSC and isotopic analyses of SOM could also be tested on FACE soil samples and in experiments with application of ^{13}C-, ^{14}C- and ^{15}N-labeled substances.

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