

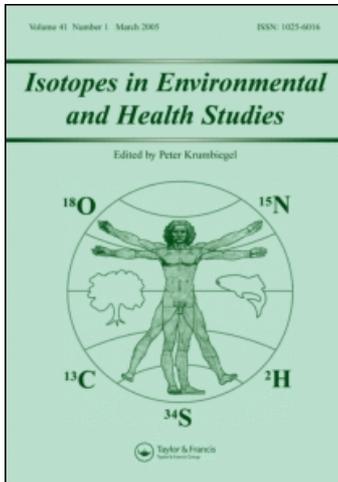
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Thermal stability of soil organic matter pools and their turnover times calculated by $\delta^{13}\text{C}$ under elevated CO_2 and two levels of N fertilisation†

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Soil from Free-Air Carbon dioxide Enrichment (FACE) plots (FAL, Braunschweig) under ambient air (375 ppm; $\delta^{13}\text{C}-\text{CO}_2 -9.8\text{‰}$) and elevated CO_2 (550 ppm; for six years; $\delta^{13}\text{C}-\text{CO}_2 -23\text{‰}$), either under 100% nitrogen (N) (180 kg ha^{-1}) or 50% N (90 kg ha^{-1}) fertilisation treatments, was analysed by thermogravimetry. Soil samples were heated up to the respective temperatures and the remaining soil was analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by Isotope Ratio Mass Spectrometry (IRMS). Based on differential weight losses, four temperature intervals were distinguished. Weight losses in the temperature range 20–200 °C were connected mostly with water volatilisation. The maximum weight losses and carbon (C) content were measured in the soil organic matter (SOM) pool decomposed at 200–360 °C. The largest amount of N was detected in SOM pools decomposed at 200–360 °C and 360–500 °C. In all temperature ranges, the $\delta^{13}\text{C}$ values of SOM pools were significantly more negative under elevated CO_2 versus ambient CO_2 . The incorporation of new C into SOM pools was not inversely proportional to its thermal stability. 50% N fertilisation treatment gained higher C exchange under elevated CO_2 in the thermally labile SOM pool (200–360 °C), whereas 100% N treatment induced higher C turnover in the thermally stable SOM pools (360–500 °C, 500–1000 °C). Mean Residence Time of SOM under 100% N and 50% N fertilisation showed no dependence between SOM pools isolated by increasing temperature of heating and the renovation of organic C in those SOM pools. Thus, the separation of SOM based on its thermal stability was not sufficient to reveal pools with contrasting turnover rates of C.

Keywords: carbon-13; IRMS; nitrogen-15; soil organic matter; thermogravimetry

1. Introduction

The atmospheric carbon dioxide (CO_2) concentrations are predicted to increase from the present 380 ppm to 470–560 ppm by 2050 [1]. This increase is a result of human activities such as

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combustion of fossil fuel, and land-use changes such as deforestation [2]. Research exploring ecosystem responses to elevated CO₂ has gained widespread attention in the last few decades [4,5,34]. Field experiments based on the Free-Air Carbon dioxide Enrichment (FACE) approach represent a great opportunity to investigate CO₂ effects on ecosystems under realistic natural conditions [3,6,7].

In most FACE experiments, stable-carbon isotopic tracers are used to study the carbon (C) transformations in plants and soils under CO₂ enrichment, since CO₂ used for enrichment is usually depleted in ¹³C relative to ambient atmospheric CO₂ [7–9]. Plants grown in elevated CO₂ atmosphere are therefore further depleted in ¹³C and, as the litter and rhizodeposits from these plants decompose and become incorporated into the soil organic matter (SOM), the soil δ¹³C will decrease. The contribution of the C metabolised under FACE conditions to total SOM can be calculated based on the δ¹³C of the SOM under ambient air and elevated CO₂ conditions. This new C contribution and the CO₂ treatment duration then allow the SOM turnover rates (TRs) to be estimated [10].

Beyond investigating the C pathways in the SOM under elevated atmospheric CO₂, the study of nitrogen (N) cycling provides insights into the interaction of these two elements in soil. Thus, the application of N fertilisers in FACE experiments was used to estimate the changes of N flows and plant uptake under elevated CO₂ [5,11].

Different techniques for the SOM fractionation that separate labile from recalcitrant soil C pools are useful to determine the dynamics of C and N in SOM [12]. One of the suitable approaches for the SOM partitioning is based on its thermal stability [13–18]. Thermogravimetry (TG) analysis involves a continuous, gradual temperature increase that decomposes (mainly oxidises) different organic compounds according to their thermal stability. TG could potentially provide a simple and rapid determination of gross changes in organic matter quality [17,18], but has only once been used to examine the quality of the SOM under altered environmental conditions such as elevated CO₂ concentration in the atmosphere [13]. In a previous experiment [13], the approach was tested on soil developed on loess. However, no correlation was detected between the thermal stability of the SOM pools and C and N turnover in those SOM pools, indicating the weakness of TG to separate SOM pools with different turnover times. In the current study, the soil developed on loamy sand probably has other interactions of organic and inorganic compounds, resulting in different (1) temperature intervals of decomposition for organic and inorganic compounds, (2) distribution of organic C and mineral N in SOM pools, and (3) rates of C renovation in SOM pools of different thermal stability. Hence, the aim of the present study was to characterise the SOM quality in soils on loamy sand from a FACE experiment (Braunschweig, Germany) conducted with crop rotation according to local farming practice, and two different N fertilisation levels under ambient and elevated concentrations of atmospheric CO₂ in terms of thermal properties.

2. Materials and methods

2.1. Study site

Soil samples were taken from the FACE facility, located in the Federal Agricultural Research Centre (FAL) in Braunschweig, Lower Saxony, Germany (10°26'E 52°18'N, 79 m a.s.l.). The experimental design consisted of four rings, each 20 m diameter, two of which were maintained under ambient air conditions (375 μmol mol⁻¹ CO₂). Two rings were conducted under elevated CO₂ conditions (550 μmol mol⁻¹ CO₂ during daylight hours). For further details see Ref. [19]. The CO₂ supplied to the FACE rings was strongly depleted in ¹³C. Mixed with ambient air, the

$\delta^{13}\text{C}$ value of the atmospheric CO_2 within the FACE rings was more negative (approximately -23‰) than the ambient air CO_2 within the control rings (-9.8‰) [20].

The research site has been used for agricultural purposes for the last 30 years, but only C_3 plants were cultivated [20]. All soil treatments, including irrigation and pesticide management, were carried out according to the local farming practices [19]. To study the interactions between C and N, N supply was restricted to 50% of adequate N in half of each of the plots [19]. The FACE experiment started in 1999 and was carried out in two crop rotation cycles, consisting of winter barley (*Hordeum vulgare* cv. Theresa) – cover crop: ryegrass (*Lolium multiflorum* cv. Lippstaedter Futtertrio) – sugar beet (*Beta vulgaris* cv. Wiebke) – winter wheat (*Triticum aestivum* cv. Batis) [19]. The soil for the experiment presented here was collected in 2005 when winter wheat was cultivated the second time. Average $\delta^{13}\text{C}$ values of crops growing on the experimental site were as follows: winter barley (leaves, stems, roots) -28.7‰ under ambient CO_2 , -39.0‰ under elevated CO_2 treatment; ryegrass (whole plants) -29.4 , -35.3‰ ; sugar beet (leaves, beet, roots) -28.1 , -41.9‰ ; winter wheat (leaves, stems, roots) -29.4 , -41.9‰ , respectively [20,21].

2.2. Soil sampling and samples processing

The soil at the site is cambisol/loamy sand, with a pH of 6.3–6.5 [19]. The concentrations of soil organic C and N (0–10 cm) are given in Table 1 (see bulk soil values).

Soil samples were taken at the end of July 2005 immediately after the final harvest of the crop six years after the FACE experiment started. Eight soil samples were collected randomly from the FACE rings under elevated CO_2 treatment and the control rings from both N treatments. Soil samples were air-dried at room temperature. All visible roots and plant remains were removed carefully and the soil was ball-milled into powder. Four of the eight prepared samples were chosen for TG analysis.

2.3. TG analysis

TG analysis was carried out using the Netzsch STA 409EP device, regulated by the TASC 414/3 controller (Fa Netzsch). 55 mg of soil sample was weighed out in an aluminium crucible and then heated from 20 °C to 1000 °C . During the heating, the air flowed into the chamber at a rate of 1.0 ml min^{-1} . The heating rate was 2.0 °C min^{-1} . Calcined kaolinite previously heated at 1250 °C was used as the reference material. The weight of the soil sample (in mg) was continuously scanned. The TG curves obtained for the soil samples from ambient and elevated CO_2 , 100% and 50% N fertilisation treatments were used to determine ‘threshold’ temperature levels. These levels were set as temperature values to heat soil samples in a muffle oven (Heraeus MR-260) for subsequent measurements of weight loss, C and N content, as well as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of SOM pools. Three groups of soil samples were heated up to 200, 360, and 500 °C . No heating (20 °C) corresponded to the bulk soil. Heating up to 200 °C removed mainly water, since no significant C and N contents were measured in residue. Heating up to 360 °C removed organic and inorganic substances already decomposed between 20 °C and 360 °C . The residual SOM pool remaining after heating to 360 °C was analysed for its C and N content, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The same approach was used to determine the SOM pool at the 500 °C temperature threshold. Then, considering the weight, C and N losses after heating to the threshold temperatures and the corresponding values (weight, C and N) were calculated for the intermediate SOM pools decomposed between 200 °C and 360 °C , or 360 °C and 500 °C (see below). The heating rate of the muffle furnace was the same as by TG-DSC analysis: 2.0 °C min^{-1} .

Table 1. Measured C, N amount and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ values in soil under ambient and elevated CO_2 treatments and under 100% and 50% N fertilisation in soil samples after heating up to threshold temperatures.

Temperature threshold ($^{\circ}\text{C}$)	Treatments		C (mg g soil $^{-1}$)	N (mg g soil $^{-1}$)	$\delta^{13}\text{C}$ (‰ PDB)	$\delta^{15}\text{N}$ (‰ air N_2)
	CO_2	N (%)				
200	Ambient	100	8.86 \pm 0.58	1.01 \pm 0.07	-28.05 \pm 0.01	6.87 \pm 0.15
	Elevated	100	9.91 \pm 0.35	1.06 \pm 0.03	-29.47 \pm 0.07	7.07 \pm 0.06
	Ambient	50	10.13 \pm 0.72	0.95 \pm 0.06	-27.88 \pm 0.05	6.52 \pm 0.09
	Elevated	50	8.25 \pm 0.32	0.91 \pm 0.03	-29.72 \pm 0.05	6.40 \pm 0.07
<i>p</i> values ^a	CO_2		0.382	0.893	<0.01	0.678
	N		0.674	0.061	<0.01	<0.01
	$\text{CO}_2 \times \text{N}$		0.023	0.362	<0.01	0.140
360	Ambient	100	3.90 \pm 0.07	0.53 \pm 0.02	-26.55 \pm 0.15	9.81 \pm 0.13
	Elevated	100	4.24 \pm 0.12	0.58 \pm 0.02	-27.46 \pm 0.14	9.74 \pm 0.16
	Ambient	50	3.63 \pm 0.13	0.50 \pm 0.02	-26.68 \pm 0.04	9.35 \pm 0.11
	Elevated	50	4.47 \pm 0.14	0.57 \pm 0.02	-27.85 \pm 0.03	9.28 \pm 0.11
<i>p</i> values	CO_2		<0.01	<0.01	<0.01	0.595
	N		0.724	0.152	0.031	<0.01
	$\text{CO}_2 \times \text{N}$		0.064	0.467	0.239	0.980
500	Ambient	100	0.57 \pm 0.02	0.10 \pm 0.01	-22.59 \pm 0.72	6.01 \pm 0.13
	Elevated	100	0.62 \pm 0.07	0.11 \pm 0.01	-24.57 \pm 0.21	6.35 \pm 0.21
	Ambient	50	0.74 \pm 0.08	0.11 \pm 0.01	-21.61 \pm 0.20	6.16 \pm 0.28
	Elevated	50	0.58 \pm 0.05	0.10 \pm 0.01	-23.05 \pm 0.05	6.17 \pm 0.17
<i>p</i> values	CO_2		0.325	0.326	<0.01	0.440
	N		0.215	0.335	<0.01	0.939
	$\text{CO}_2 \times \text{N}$		0.072	0.416	0.417	0.476
Bulk soil ^b	Ambient	100	8.88 \pm 0.36	1.00 \pm 0.03	-27.97 \pm 0.04	7.11 \pm 0.16
	Elevated	100	9.94 \pm 0.71	1.04 \pm 0.05	-29.55 \pm 0.09	6.98 \pm 0.12
	Ambient	50	10.33 \pm 0.97	0.95 \pm 0.01	-27.80 \pm 0.03	6.53 \pm 0.10
	Elevated	50	8.68 \pm 0.65	0.94 \pm 0.05	-29.73 \pm 0.07	6.70 \pm 0.15
<i>p</i> values	CO_2		0.482	0.640	<0.01	0.878
	N		0.281	0.099	0.900	<0.01
	$\text{CO}_2 \times \text{N}$		0.136	0.579	0.016	0.290

Values are means of eight replicates \pm SE.

^aEffects were not significant ($p > 0.05$) or significant at reported *p* values.

^bBulk soil represented not-heated soil samples.

2.4. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses

Isotope C and N ratios, as well as C and N contents, were measured with an elemental analyser in a dual-element analysis mode (Carlo Erba 1108, Milano, Italy) for Dumas combustion. This was followed by gas chromatographic separation of the gaseous combustion products, which were then fed into a gas-isotope ratio mass spectrometer (delta S Finnigan MAT, Bremen, Germany) through a ConFlo III open-split interface (Finnigan MAT). Relative isotope abundances, denoted as δ -values, were calculated according to the following equation:

$$\delta\text{-value} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \cdot 1000\text{‰}, \quad (1)$$

where R_{sample} and R_{standard} are the ratios of heavy isotope to light isotope of the samples and the respective standards. Standard gases were calibrated with respect to international standards (N_2 in the air and CO_2 in PeeDee belemnite) by the use of the reference substances N_1 and N_2 for nitrogen isotope ratios and Australian National University (ANU) sucrose and NBS 19 for the carbon isotopes [22].

2.5. Calculations and statistics

The calculation of weight losses, C and N content as well as the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of SOM pools decomposed in different temperature intervals, is described in details in a previous study [13].

The portion of FACE-derived C (P_{FACE}) in SOM was calculated according to Balesdent [23] and adapted for FACE conditions:

$$P_{\text{FACE}} = \frac{(\delta^{13}\text{C}_{\text{elev.}} - \delta^{13}\text{C}_{\text{amb}})}{(\delta^{13}\text{C}_{\text{elev.theor.}} - \delta^{13}\text{C}_{\text{amb}})} 100, \quad (2)$$

where $\delta^{13}\text{C}_{\text{elev.}}$ is the $\delta^{13}\text{C}$ value of the SOM under elevated CO_2 treatment, $\delta^{13}\text{C}_{\text{amb}}$ is the $\delta^{13}\text{C}$ value of the SOM under ambient air treatment, and $\delta^{13}\text{C}_{\text{elev.theor.}}$ is the theoretical $\delta^{13}\text{C}$ value of the SOM developed under vegetation grown under continuously elevated CO_2 conditions. The isotopic signatures of the theoretical SOM were calculated based on the difference between the $\delta^{13}\text{C}$ of the plants growing under elevated CO_2 and the $\delta^{13}\text{C}$ of bulk soil and SOM pools decomposed at 200–360 °C, 360–500 °C, 500–1000 °C under ambient CO_2 . The values were corrected for isotopic fractionation during humification by subtracting the differences between $\delta^{13}\text{C}$ of vegetation growing under ambient conditions and $\delta^{13}\text{C}$ of the corresponding SOM pool of the soil developed under ambient CO_2 treatment:

$$\delta^{13}\text{C}_{\text{elev.theor.}} = \delta^{13}\text{C}_{\text{elev.plant}} - (\delta^{13}\text{C}_{\text{amb.plant}} - \delta^{13}\text{C}_{\text{amb}}), \quad (3)$$

where $\delta^{13}\text{C}_{\text{elev.plant}}$ is the $\delta^{13}\text{C}$ of the plants under elevated CO_2 treatment, $\delta^{13}\text{C}_{\text{amb.plant}}$ is the $\delta^{13}\text{C}$ value of the plants under ambient CO_2 treatment, and $\delta^{13}\text{C}_{\text{amb}}$ is the $\delta^{13}\text{C}$ of the bulk soil and the corresponding SOM pool of the soil developed under ambient CO_2 treatment.

To calculate the contribution of new FACE-derived C in SOM for a particular period of the FACE experiment and annual TRs of SOM, a simple exponential approach was selected [23]:

$$\text{TR} = -\ln(1 - M/100)/t, \quad (4)$$

where M is the portion of C_{FACE} (Equation (2)) in SOM and t is the time of soil exposition to elevated CO_2 concentrations.

The mean residence time of FACE-derived C in bulk soil and in SOM pools was calculated as a reciprocal to the TRs [24]:

$$\text{MRT} = 1/\text{TR}. \quad (5)$$

The study was conducted with four replicates originating from elevated and ambient CO_2 , full- and half-N fertilisation treatments for TG and with eight replicates for muffle combustion, C, N measurements and isotopic analysis. The significance of differences between $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, as well as the C and N content of different pools under ambient and elevated CO_2 treatments was examined using two-way analysis of variance (ANOVA). The standard errors of means are presented in the figures and tables as the variability parameter.

3. Results

3.1. TG, weight losses, C and N content of SOM pools

Data for TG analysis were represented by cumulative weight losses (TG, percentage from initial weight of a soil sample) and differential weight losses (differential thermogravimetry (dTG), percentage loss of total weight per degree Celsius heating) (Figure 1). The dTG curves showed

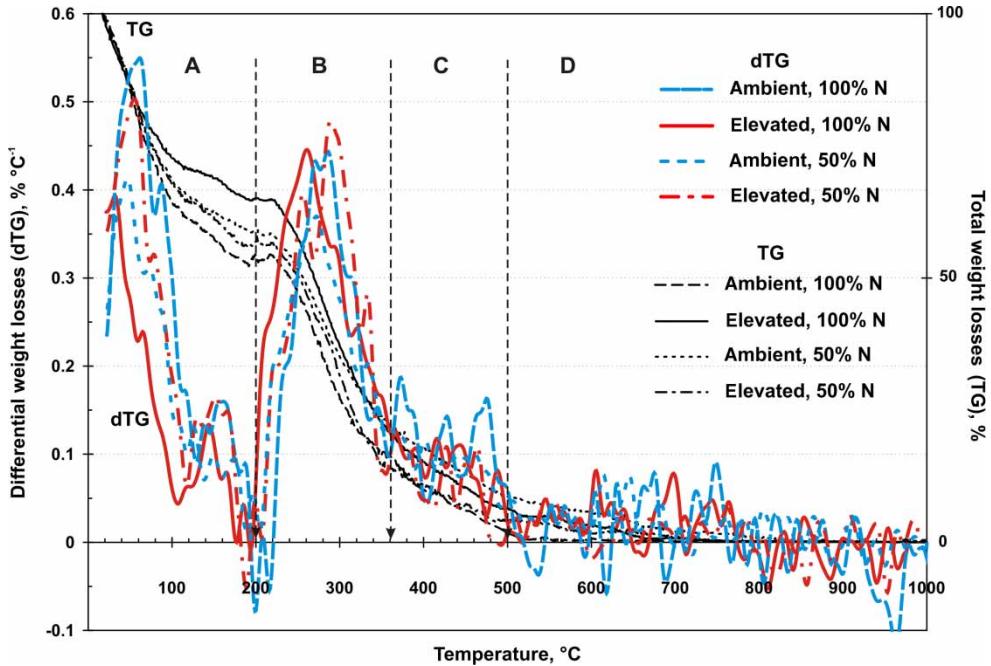


Figure 1. dTG of soil under ambient/elevated CO_2 and 100/50% N fertilisation treatments. Cumulative losses (TG) are scaled to 100% of the right Y axis. Arrows with dotted lines show the 'threshold' temperature chosen for SOM fractionation.

two well-defined peaks of differential weight losses from soil samples under elevated and ambient CO_2 treatments for both 100% and 50% N fertilisation during heating from 20 °C to 1000 °C: (1) between 20 °C and 200 °C (temperature range A) and (2) between 200 °C and 360 °C (temperature range B). Less intensive weight losses occurred in temperature ranges C and D.

Weight losses occurred in similar temperature intervals with all samples, allowing similar temperature threshold levels to be chosen for thermal fractionation of SOM. To fractionate the SOM into individual pools with different thermal stability, three threshold temperature levels were chosen: 200 °C, 360 °C, and 500 °C (Figure 1). Three threshold temperatures allowed four SOM pools to be isolated according to the procedure described above (Section 2). The total weight loss, as well as weight loss of the four individual SOM pools, did not differ significantly between the ambient air and CO_2 enrichment samples. However, N fertilisation significantly influenced total weight loss from bulk soil and soil samples decomposed between 20–200 °C, 200–360 °C, and 500–1000 °C (Figure 2). Peak weight losses were found in the SOM pool decomposed between 200 °C and 360 °C ranging from $2.23 \pm 0.12\%$ of the initial weight of soil under elevated CO_2 treatment and 100% N fertilisation, to $1.65 \pm 0.02\%$ of soil under elevated CO_2 treatment and 50% N fertilisation. The lowest weight losses were observed at 20–200 °C and 500–1000 °C, where the soil samples of all treatments lost on average 0.2% of total weight losses (Figure 2).

For all treatments except one, the maximum C losses corresponded to maximal weight losses in SOM pools decomposed at 200–360 °C. The exception was in the treatment with elevated CO_2 and 50% N, where maximum C losses were detected in SOM pools decomposed at 360–500 °C (Figure 2, Table 2). Maximum N losses, in contrast, were measured in SOM pools decomposed in two temperature ranges: 200–360 °C and 360–500 °C. The lowest amounts of C and N were measured in SOM decomposed above 500 °C in soils under all treatments. Minor insignificant C and N losses were detected by decomposition between 20 °C and 200 °C because the main weight losses in this temperature interval are connected with water evaporation (Table 2).

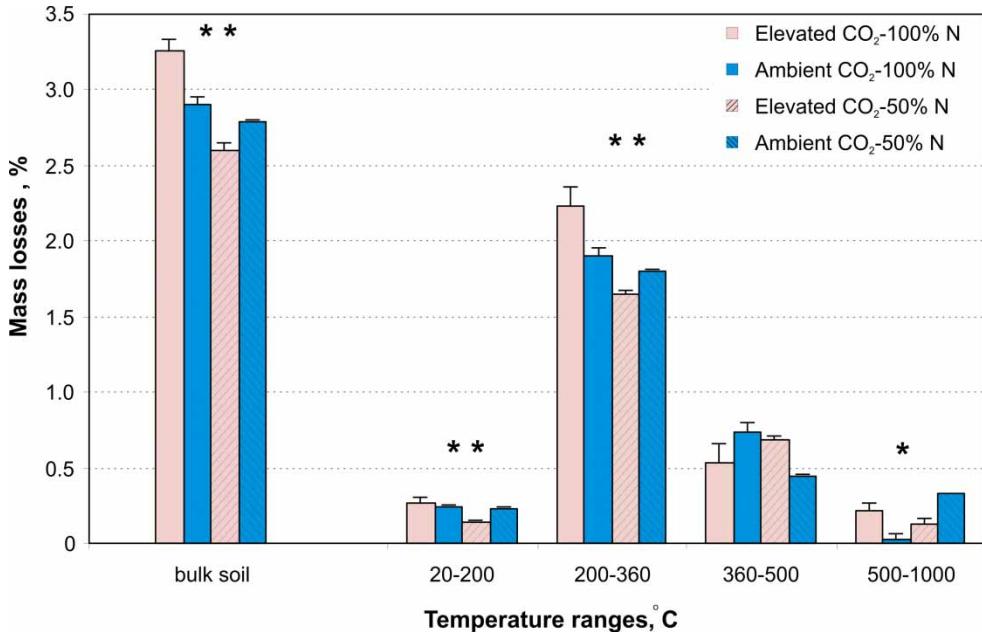


Figure 2. Weight losses of bulk soil and SOM pools of different thermal stability under ambient/elevated CO₂ and 100/50% N fertilisation treatments after combustion in a muffle oven. Bulk soil represents samples combusted from 20 °C to 1000 °C. SOM pools showed the distribution of weight losses from bulk soil within four temperature ranges. Whiskers present standard error (\pm SE). * $p < 0.05$ and ** $p < 0.01$ represent the significance of differences between 100% and 50% N fertilisation treatments. The effect of CO₂ treatment was insignificant.

3.2. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and amount of FACE-derived C in SOM pools of different thermal stability

A significant shift in $\delta^{13}\text{C}$ values of bulk soil and SOM pools decomposed in temperature ranges was observed after approx. six years of exposition of crops in an agroecosystem to isotopically labelled CO₂ (Tables 1 and 2). The observed average increase of 5.8‰ in $\delta^{13}\text{C}$ values between treatments with increasing combustion temperature (Tables 1 and 2) is probably due to the slow combustion process, when the lighter ¹²C isotope decomposes faster than the heavier ¹³C, leading to an accumulation of the latter in the residual SOM pools [16].

Statistical analysis showed significant difference in $\delta^{15}\text{N}$ values of bulk soil and SOM pools with different thermal stability between ambient and elevated CO₂ treatments by application of 100 and 50% N fertilisers (Tables 1 and 2). $\delta^{15}\text{N}$ values of the only SOM pool decomposed >500 °C were affected neither by N nor by CO₂ treatments.

The calculated amount of new C in bulk soil calculated on the basis of $\delta^{13}\text{C}$ values of SOM under ambient and elevated CO₂ treatments reached $12.6 \pm 0.23\%$ of total C under 100% N fertilisation and $15.4 \pm 0.16\%$ under 50% N fertilisation (Figure 3). The amount of FACE-derived C was different in the SOM pools. With 100% N fertilisation, it amounted to 14.2% at 200–360 °C, 5.8% at 360–500 °C, and 15.8% at 500–1000 °C. In the 50% N fertilisation samples, the amount of FACE-derived C was 27.4, 4.9, and 11.5% at the respective temperature ranges (Figure 3).

Based on the amount of FACE-derived C, the calculated TRs and MRTs showed a TR of 42 years under 50% N treatment compared with 52 years under 100% N treatment for bulk soil (Table 3). The fastest SOM TRs corresponding to an MRT of 22 years were detected in the SOM pool from the 50% N treatment, which decomposed from 200 °C to 360 °C. The MRT calculated for the same SOM from the 100% N treatment amounted to 46 years, and hence was two times

Table 2. Calculated amounts of C, N and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ values in soil under ambient and elevated CO_2 treatments and under 100% and 50% N fertilisation in SOM decomposed in different temperature ranges.

Temperature threshold ($^{\circ}\text{C}$)	Treatments		C (mg g soil $^{-1}$)	N (mg g soil $^{-1}$)	$\delta^{13}\text{C}$ (‰ PDB)	$\delta^{15}\text{N}$ (‰ air N_2)
	CO_2	N (%)				
20–200	Ambient	100	0.02 ± 0.69	N.D.	N.D.	N.D.
	Elevated	100	0.03 ± 0.79	N.D.	N.D.	N.D.
<i>p</i> values ^a	Ambient	50	0.2 ± 0.72	N.D.	N.D.	N.D.
	Elevated	50	0.43 ± 0.64	0.03 ± 0.06	N.D.	N.D.
	CO_2		0.972	N.D.	N.D.	N.D.
	N		0.861	N.D.	N.D.	N.D.
	$\text{CO}_2 \times \text{N}$		0.489	N.D.	N.D.	N.D.
200–360	Ambient	100	4.96 ± 0.59	0.47 ± 0.07	-29.24 ± 0.57	3.43 ± 0.18
	Elevated	100	5.67 ± 0.37	0.48 ± 0.03	-31.03 ± 0.33	3.70 ± 0.09
<i>p</i> values	Ambient	50	6.50 ± 0.73	0.45 ± 0.06	-28.57 ± 0.53	3.28 ± 0.14
	Elevated	50	3.78 ± 0.35	0.34 ± 0.04	-32.01 ± 0.45	1.30 ± 0.11
	CO_2		0.052	0.473	0.048	<0.01
	N		0.400	0.253	0.082	<0.01
	$\text{CO}_2 \times \text{N}$		0.126	0.083	0.763	<0.01
360–500	Ambient	100	3.33 ± 0.08	0.44 ± 0.02	-27.21 ± 0.10	10.62 ± 0.09
	Elevated	100	3.62 ± 0.14	0.47 ± 0.02	-27.94 ± 0.16	10.53 ± 0.12
<i>p</i> values	Ambient	50	2.90 ± 0.15	0.39 ± 0.02	-27.94 ± 0.27	10.23 ± 0.12
	Elevated	50	3.89 ± 0.15	0.47 ± 0.02	-28.55 ± 0.32	9.93 ± 0.12
	CO_2		0.028	0.076	<0.01	0.095
	N		0.109	0.529	<0.01	<0.01
	$\text{CO}_2 \times \text{N}$		0.046	0.182	0.374	0.231
500–1000	Ambient	100	0.56 ± 0.02	0.096 ± 0.001	-22.59 ± 0.72	6.01 ± 0.13
	Elevated	100	0.62 ± 0.07	0.112 ± 0.003	-24.57 ± 0.21	6.35 ± 0.21
<i>p</i> values	Ambient	50	0.74 ± 0.08	0.110 ± 0.004	-21.61 ± 0.20	6.16 ± 0.28
	Elevated	50	0.58 ± 0.05	0.102 ± 0.002	-23.05 ± 0.05	6.17 ± 0.17
	CO_2		0.343	0.177	<0.01	0.440
	N		0.277	0.436	<0.01	0.939
	$\text{CO}_2 \times \text{N}$		0.097	0.003	0.417	0.476

Values are means of eight replicates \pm SE. ^aEffects were not significant ($p > 0.05$) or significant at reported p values.

higher. The longest MRTs of 116 and 140 years corresponded to SOM pools decomposed at 360–500 $^{\circ}\text{C}$ under 100% and 50% N treatments, respectively (Table 3).

4. Discussion

4.1. TG of SOM pools

Weight losses during gradual heating allowed a separation into four conditional temperature intervals (Figure 1). The first interval A (up to 200 $^{\circ}\text{C}$) reflected the losses of free and bound water [25]. Since the losses of C and N were minor, no change of isotopic composition was observed (Table 2). The temperature ranges B (200–360 $^{\circ}\text{C}$) and C (360–500 $^{\circ}\text{C}$) showed the largest losses of C and N. As observed by Kristensen [26], the organic substances decomposed up to 310 $^{\circ}\text{C}$ comprised plant material such as cellulose. Herrera *et al.* [27] used dTG to determine that the major percentage weight loss of hemicelluloses and celluloses occurs at the temperature range between 200 $^{\circ}\text{C}$ and 350 $^{\circ}\text{C}$. At higher temperatures (360–500 $^{\circ}\text{C}$), the decomposed organics comprise stable constituents with aromatic compounds such as lignin, humic substances, and kerogens [16,18,26]. In the last temperature range D (500–1000 $^{\circ}\text{C}$), the losses of organic matter were the

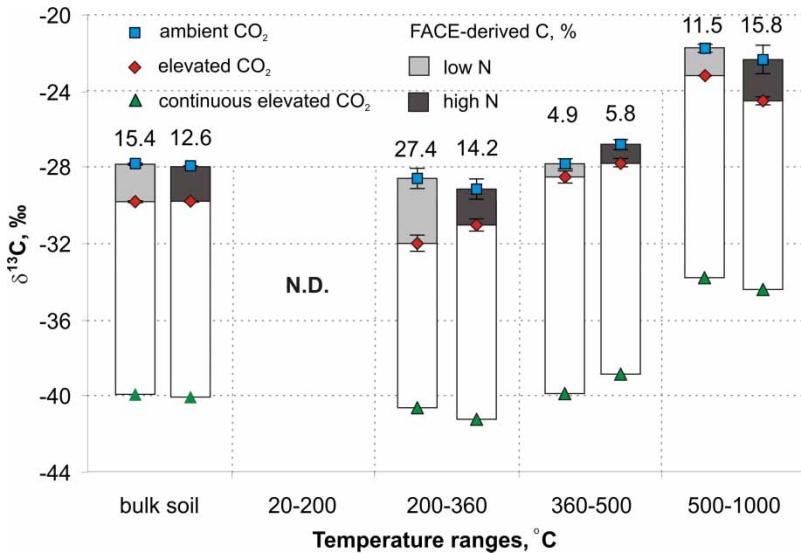


Figure 3. The $\delta^{13}\text{C}$ values of bulk soil and three SOM pools with different thermal stability under ambient/elevated CO_2 and 100/50% N fertilisation treatments. The calculated theoretical $\delta^{13}\text{C}$ values (Equation (3)) of soil developed under continuous elevated CO_2 conditions were used to estimate the portion of FACE-derived C in the SOM under two N treatments. The portion of FACE-derived C is shown above the columns as a percentage of the total amount of C in bulk soil and SOM pools. Whiskers present standard error ($\pm\text{SE}$).

smallest compared with other SOM pools (Table 2) as most weight losses here reflect clay mineral decomposition [28].

4.2. Isotopic composition and the portion of FACE-derived C in SOM pools under 100% and 50% N treatments

The results of our experiment showed that neither elevated CO_2 treatment nor different N fertilisation regimes significantly changed the total amount of C or N in the soil. However, an input of

Table 3. Amount of FACE-derived C, turnover rates (TR) and mean residence time (MRT) of carbon in bulk soil and SOM pools isolated in three temperature ranges for soils under 100% and 50% N fertilisation.

Temperature ranges (°C)	Treatment		TR (year ⁻¹)	MRT (years)
	N (%)	FACE derived C ^a (%)		
20–200		N.D.	N.D.	N.D.
200–360	100	14.2 ± 0.5	0.0219	46
	50	27.4 ± 0.3**	0.0458	22
360–500	100	5.8 ± 0.7	0.0086	116
	50	4.9 ± 1.3	0.0071	140
500–1000	100	15.8 ± 0.5	0.0246	41
	50	11.5 ± 0.4*	0.0174	57
Bulk soil	100	12.6 ± 0.3	0.0193	52
	50	15.4 ± 0.2*	0.0239	42

The calculations were made using Equations (2) and (3). Values are means of eight replicates $\pm\text{SE}$. ^aFACE-derived C, TR and MRT could only be assessed at elevated CO_2 treatment. Therefore, the effect of CO_2 was not included for these variables. * $p < 0.05$ and ** $p < 0.01$ represent the significance of differences between 100% and 50% N treatment.

newly synthesised C into the SOM is documented as the $\delta^{13}\text{C}$ values of both bulk SOM, and most of the SOM pools shifted, indicating a significant impact of C from the labelled atmospheric CO_2 (Tables 1 and 2).

The lack of changes in total C [7] and N [29] under elevated CO_2 conditions were expected because SOM formation and decomposition processes are not strongly affected by elevated CO_2 . N fertilisation (both 100% and 50% N) under an elevated CO_2 environment had no effect on total C and N content in bulk soil. These results go along with the results of some other researchers [5,12,29,30]. However, a number of experiments gave contrary results suggesting an increase of total C and N due to elevated CO_2 and high N fertilisation rates of $>30 \text{ kg N ha}^{-1}$. According to de Graaff *et al.* [29] such observations are so far restricted to soils under herbaceous plant species. In our study, significant effects of CO_2 and fertilisation treatments on C and N content were observed in SOM pools decomposed under intermediate (C) and high (N) temperature ranges of 360–500 °C and 500–1000 °C (Table 2).

The isotopic signatures of the carbon in bulk soils, and SOM pools under ambient and elevated CO_2 , allowed the calculation of the amount of new FACE-derived C incorporated into SOM pools at both levels of N fertilisation. After six years of CO_2 fumigation, the portion of new FACE-derived C in bulk soil under the 100% N reached 12.6%, and under 50% N treatment, 15.4% of total C (Figure 3).

Very few studies exist on the effect of elevated CO_2 and N fertilisation on the SOM turnover under agricultural crops lasting for a period longer than five years. Most of the results available are from two- to three-year experiments. Thus, Leavitt *et al.* [7] reported C inputs by spring wheat in a two-year CO_2 enrichment experiment to be 6.3% in the upper 15 cm of soil. In another experiment with spring wheat exposed for three years to elevated CO_2 (FACE-Hohenheim) and N fertilisation ($140 \text{ kg ha}^{-1} \text{ a}^{-1}$), the amount of newly metabolised C reached 7.3% in upper 0–10 cm bulk soil [13]. Studies with cotton showed that the average amount of new C was 12–13% after three years of elevated CO_2 [31]. However, Van Kessel *et al.* [5] reported higher C inputs of 23.5% under a high N fertilisation rate ($540 \text{ kg ha}^{-1} \text{ a}^{-1}$) and 25.5% under a lower rate ($140 \text{ kg ha}^{-1} \text{ a}^{-1}$) in an experiment with grassland consisting of *Lolium perenne* and *Trifolium repens* under FACE CO_2 enrichment for 10 years. These larger new C inputs mainly reflected grassland plant species, a longer CO_2 fumigation time, higher N fertilisation rate and the absence of tillage [5]. Giesemann [20] reported the amount of newly metabolised FACE-derived C in the same soil used in current study to be as much as 16.6% of C in the soil C pool under the 100% N fertilisation plot down to 10 cm depth at the end of the first crop rotation period.

The incorporation of FACE-derived C in the temperature labile SOM pool, decomposing at 200–360 °C under 50% N fertilisation, was two times faster (27.4%) than under 100% N treatment (14.2%) (Figure 3). However, the C turnover in the more temperature-stable SOM pools decomposed at 360–500 °C and 500–1000 °C, respectively, were higher under the 100% N treatment than under the 50% N treatment (Table 3). MRTs of C in SOM pools were not dependent on increasing thermal stability of those SOM pools (Table 3). The MRT of the SOM under 100% N increased in temperature intervals 500–1000 °C (41 years) < 200–360 (46) < bulk soil (52) < 360–500 (140). Under 50% N treatment, the values were 200–360 °C (22 years) < bulk soil (42) < 500–1000 (57) < 360–500 (116). Thus, MRTs of the SOM under 100% N fertilisation were similar at 200–360 °C and 500–1000 °C (46 and 41 years, respectively). Under 50% N treatment, the MRT of the SOM pool decomposed at 200–360 °C was 2.5 times shorter (22 years) than at 500–1000 °C (57 years), whereas the SOM decomposed at 360–500 °C under both N fertilisation treatments had the longest MRT of 116–140 years (Table 3).

The data obtained in the current investigation suggest that TG is not sufficient as an approach in isolating SOM pools with different turnover times. This conclusion is supported by our previous findings in an experiment with *Miscanthus x giganteus* [14,15], where, after 10.5 years of cultivation of C_4 plant *Miscanthus* on C_3 grassland, the C exchange reached on average 50% in

bulk (not heated) soil and SOM pools decomposed up to 190 °C, 310 °C, 390 °C, respectively. The MRT of the SOM decomposed up to 190 °C, 310 °C, and 390 °C was 13, 12, and 15 years, respectively. Only the most thermally stable SOM pool, which decomposed >480 °C and had the lowest amount of *Miscanthus*-derived C (9%) showed an MRT of 163 years [14]. Another attempt at the thermal isolation of SOM pools was made on soil from the FACE experiment (Hohenheim, Stuttgart) [13]. This soil had been subjected to elevated CO₂ (+160 ppm) compared with ambient air (380 ppm) and an annual N fertilisation (140 kg ha⁻¹) for three years. In contrast to the soil used in the Braunschweig FACE experiment, which developed on loamy sand, the soil from the FACE-Hohenheim developed on loess and hence had higher organic C and mineral N contents. However, five separated SOM pools decomposed at 20–200 °C, 200–310 °C, 310–400 °C, 400–480 °C, and 480–1000 °C did not show contrasting TRs. Thus, the MRTs of C in SOM pools increased as follows: 12 years (310–400 °C) <28 (480–1000) <35 (400–480) <92 (200–310) <149 (20–200). Furthermore, there was no dependence between the portion of FACE-derived C in a SOM pool and increasing thermal stability of a SOM pool.

Helfrich *et al.* [33] supported our impression that TG is weak in separating SOM pools with different turnover times. Thus, in their study, the efficiency of thermal oxidation at different temperatures (from 200 to 500 °C) was tested for different soils (loamy sand and silty loam) under different land use regimes (field, grassland, forest). The results showed that the thermal oxidation at different temperatures did not yield residual SOM pools of contrasting MRTs in the silty loam (58 years in non-heated soil and 77 years in soil samples decomposed up to 300 °C). Only the silty loam with large amounts of black carbon, which is relatively stable with regards to thermal decomposition [32], showed an increase of the C-turnover time (940 years compared with the non-heated soil with 220 years) [33].

A possible explanation for the inability of thermal oxidation for isolating SOM pools of contrasting turnover times is that the fractionation of SOM pools, according to their thermal stability, is rather close to chemical separation. In turn, Balesdent [23] found that chemical separations of SOM failed to isolate the SOM pools of different turnover time because different biochemical plant components (cellulose, lignin) are decomposed in a wide temperature range [16]. Individual components of plant residues may be directly incorporated into, or even mixed with, the thermal stable SOM pools and will so mask low TRs of these pools.

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