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The temperature response of CO_2 production from bulk soils and soil fractions is related to soil organic matter quality

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Abstract. The projected increase in global mean temperature could accelerate the turnover of soil organic matter (SOM). Enhanced soil CO_2 emissions could feedback on the climate system, depending on the balance between the sensitivity to temperature of net carbon fixation by vegetation and SOM decomposition. Most of the SOM is stabilised by several physico-chemical mechanisms within the soil architecture, but the response of this quantitatively important fraction to increasing temperature is largely unknown. The aim of this study was to relate the temperature sensitivity of decomposition of physical and chemical soil fractions (size fractions, hydrolysis residues), and of bulk soil, to their quality and turnover time. Soil samples were taken from arable and grassland soils from the Swiss Central Plateau, and CO_2 production was measured under strictly controlled conditions at 5, 15, 25, and 35 °C by using sequential incubation. Physico-chemical properties of the samples were characterised by measuring elemental composition, surface area, ^{14}C age, and by using DRIFT spectroscopy. CO_2 production rates per unit (g) organic carbon (OC) strongly varied between samples, in relation to the difference in the biochemical quality of the substrates. The temperature response of all samples was exponential up to 25 °C, with the largest variability at lower temperatures. Q_{10} values were negatively related to CO_2 production over the whole temperature range, indicating higher temperature sensitivity of SOM of lower quality. In particular, hydrolysis residues, representing a more stabilised SOM pool containing older C, produced less CO_2 g^{-1} OC than non-hydrolysed fractions or bulk samples at lower temperatures, but similar rates at ≥25 °C, leading to higher Q_{10} values than in other samples. Based on these results and provided that they apply also to other soils it is suggested that because of the higher sensitivity of passive SOM the overall response of SOM to increasing temperatures might be higher than previously expected from SOM models. Finally, surface area measurements revealed that micro-aggregation rather than organo-mineral association mainly contributes to the longer turnover time of SOM isolated by acid hydrolysis.

Introduction

Soil organic matter (SOM) plays a key role in the global carbon (C) cycle, and it acts as an important C reservoir. Worldwide, C storage in organic matter (OM) of surface soils has been estimated at 2011 Gt C (Bolin and Sukumar 2000). Temperature is a major controlling factor for both net primary production (NPP) and storage and turnover of SOM, as shown by Jenny (1980)

across temperature and precipitation gradients in the U.S. and later by Burke et al. (1989). Temperature may also account for altitudinal gradients of SOM contents in mountain regions (Townsend et al. 1995; Leifeld et al. 2005), because it acts as a limiting factor for microbial activity at higher altitudes. Looking at the projected future change in global mean temperatures, the response of SOM to temperature is important for the assessments of possible atmospheric feedbacks from the SOM reservoir. Even a small decrease of the global SOC stock due to an increase in temperature would significantly add to the estimated current global C-flux caused by land-use change of around 1.7 Gt C (Sarmiento and Gruber 2002).

It is generally accepted that temperature is positively related to the rate of SOM decomposition (Kirschbaum 1995) and temperature sensitivity functions are used in common SOM models (Parton et al. 1988). Functions have been derived by means of incubation experiments of labelled plant materials (Sorensen 1981), or by short-term soil incubation (Hunt 1977), where most of the CO_2 derives from SOC with short turnover times. Based on these results, calculated temperature responses thus apply mainly to the readily available SOM. This SOM fraction is the major source of CO_2 from soil respiration, but accounts for only a minor portion of total SOM (e.g., Trumbore 2000). Also, older SOM is subject to decomposition when fresh material becomes rare, as observed in two forest stands in Germany (Dorr and Munnich 1986). For the estimation of the direction and magnitude of response of SOM to a raise in temperature, the behaviour of the older pool is crucial as it comprises most of the SOM, and thus any further change in pool size will significantly contribute to atmospheric CO_2.

In addition to external factors such as climate etc., residence times of SOM are controlled by its decomposability, which is mainly a function of its chemical nature, modified by several stabilisation mechanisms (Sollins et al. 1996). SOM partitioning into physico-chemical fractions has been shown to reflect these processes to some extent (e.g., Trumbore et al. 1990; Buyanovsky et al. 1994). From a conceptual point of view it is unlikely that the temperature response of different SOM fractions is consistent because SOM is chemically highly diverse, and several enzymes with specific temperature optima are involved in its decomposition. In a theoretical framework on the relationship between SOM quality and the temperature response of its decomposition Bosatta and Ågren (1999) argued that substrates of low quality should have a stronger temperature dependence than those of high quality because of a higher number of steps needed for decomposition of more complex substrates. Moreover, temperature-dependent processes other than enzymatic degradation may limit the rate of SOM turnover, including physico-chemical processes involved in SOM stabilisation, e.g. adsorption phenomena, which have been suggested to respond more to warming than enzyme-mediated processes (Thornley and Cannell 2001). These authors assumed that due to the faster stabilisation of SOM at higher temperatures less C will remain biochemically available. In turn, this could lead to an apparently lower temperature response

of older SOM. Evidence for this hypothesis comes from large-scale field observations, where the decomposition of SOM with higher ^{14}C age was argued to be more tolerant to temperature (Liski et al. 1999). Anderson (1991) reviewed the effects of climate change on decomposition processes, showing that under pine trees Q_{10} decreased during litter decomposition and with increasing depth from litter to humus horizons, i.e. the Q_{10} decreased with decreasing OM decomposability. In contrast to the studies of Liski et al. (1999) and Anderson (1991); Bol et al. (2003) hypothesised that recalcitrant SOM mineralises more efficiently at higher temperatures based on laboratory incubations and measurement of the ^{14}C age of the evolved CO_2, which is in accordance to the theory of Bosatta and Ågren (1999). In view of these findings it seems obvious that the role of temperature on SOM decomposition remains unclear, and that further work should attempt to more strictly separate effects of temperature from those of other factors influencing SOM decomposition and turnover.

The aim of this study was to relate the temperature sensitivity of physical and chemical soil fractions and of bulk soil, to their quality and ^{14}C age. It is not to relate the quality aspect to the turnover rate under native soil temperature in the field, because the variation in turnover rates in the field is determined by many factors other than temperature and quality (e.g., by input rates). We measured the temperature response of OM decomposition for plant residues, bulk soils and soil fractions, which represent SOM fractions with different turnover times. Because turnover time of SOM integrates the various stabilisation mechanisms, we refer to OM as being of different quality. In particular, acid hydrolysis residues were taken to represent a more stabilised SOM fraction. Hydrolysis residues are typically characterised by smaller decay rate constants/longer turnover times as compared to non-hydrolysable SOM (Leavitt et al. 1996; Falloon and Smith 2000). The temperature response may be related positively, negatively, or indifferently to quality. Here, we experimentally tested the hypothesis that the temperature response of decomposition is related to SOM quality such that materials of higher quality are characterised by a lower temperature sensitivity.

Materials and methods

Soil samples were taken at depths of 5–10 (A5, B5) and 25–30 cm (A30, B30) from two field sites located near Oensingen, Switzerland (47°17′ N, 07°44′ E, altitude 450 m above sea level, mean annual temperature 9 °C, average annual rainfall 1109 mm) under arable rotation (A) and under permanent grassland (B). The soil at the arable site is classified (WRB) as a stagnic Cambisol (pH 7.2, sand 2000–63 μm 0.25 kg kg^{-1}, silt 63–2 μm 0.33 kg kg^{-1}, clay <2 μm 0.42 kg kg^{-1} soil), and at the grassland site as a stagnic Gleysol (pH 5.6, sand 0.19 kg kg^{-1}, silt 0.38 kg kg^{-1}, clay 0.43 kg kg^{-1} soil).

Soil fractionation

Soils were sieved < 2 mm and air dried at 35 °C. Size fractions were obtained after shaking a 1:3 mixture (250 g soil plus 750 g distilled water) for 24 h on a horizontal shaker (frequency 60 min^{-1}), followed by sieving over a 63 μm mesh. The < 63 μm fraction was divided, and an aliquot of 100 g was hydrolysed in 400 ml 6 M HCl at 105 °C for 24 h. The hydrolysis residue was neutralized to pH 7 by addition of 12 M NaOH, and de-ionised by consecutive washing, centrifugation and decantation until the electrical conductivity adjusted to < 0.5 mS cm^{-1}. Residues were dried at 35 °C, gently mortared, mixed 1:1 with carbonate-free sand, and shaken in 200 ml of a nutrient solution (mg l^{-1}: NO3 0.68, NH4 0.52, P 0.34, K 1.16, B 0.002, Cu-EDTA 0.0004, Fe-DTPA 0.004, Mn-EDTA 0.002, Mo 0.0002, Zn-EDTA 0.0004) to restore the nutrient supply of the soil material. After drying, both the non-hydrolysed fraction < 63 μm and the HCl residues were inoculated with a fresh water extract from the same site (soil:water 1:3) containing 9 mg l^{-1} DOC, and allowed to equilibrate for 1 week before measuring respiration. Mixing with sand was performed in the same manner for the non-hydrolysed fraction < 63 μm to provide adequate aeration. Bulk densities of the physical fractions were at around 1.4 g cm^{-3}.

Respiration measurements

Measurements ($n = 6$) were carried out with intact soil cores (100 cm^{-3}), soil fractions obtained as described above, and a grass-clover sample composted for two month at 25 °C. Soil samples were adjusted to a water potential of 60 hPa before incubation and allowed to equilibrate for 1 week before the measurement. CO_2 emission was measured in an automatic static incubation chamber of 1500 cm^{-3} volume (Barometric Process Separation BaPS, UMS, Munich, Germany) with records every 10 min during several hours (25 and 35 °C) or up to 3 days (5 °C). The increase in gas concentration was calculated from the resulting 30 to about 220 measurements by linear regression.

To calculate total production, CO_2 concentrations in the headspace were corrected for solution and dissociation (CO_2) in soil water. Oxygen concentrations were not allowed to drop below 19% to avoid limitation in aerobic microbial activity. We choose 5, 15, 25, and 35 °C as incubation temperatures, with temperature during measurement kept constant at ± 0.1 °C. At the sampling site, temperatures up to 35 °C are typically reached in summer in the topsoil as evidenced by continuous soil temperature measurements since 2001. The order of incubation temperatures was the same for each sample, because we found that heating vs. cooling in a few cases resulted in different respiration rates at a particular temperature (hysteresis).

Together with fresh samples (hereafter referred to as bulk soils), the temperature response during a long-term incubation over 707 days was measured during six intervals for one sample subset ($n = 6$) taken from the arable site (hereafter referred to as long-term incubations). Temperature responses were characterised by calculating Q_{10} values for different temperature intervals:

$$Q_{10} = (k_2/k_1)^{[(10/T_2-T_1)]}$$

where T_2 and T_1 denote the temperatures during the measurement, and k_1 and k_2 the corresponding CO_2 production rate. The Q_{10} values are thus based on instantaneous rates rather than calculated by means of decay rate constants.

All temperature response measurements were done sequentially, i.e. the same sample was allowed to adjust to the particular temperature. This also applied to the long-term incubated samples, which were stored at 25 °C and where we measured instantaneous CO_2 production rates in the incubation chamber at the temperatures mentioned above at six dates over the total period of 707 days. With this approach, the possible development of different substrate qualities with time due to different decomposition rates and an adaptation of the microbial community to a particular temperature, as described by Leifeld (2003) was avoided. To experimentally test the applicability of parallel vs. sequential incubations, we conducted a preliminary incubation. For this purpose, we used samples from different sites during 159 days of incubation at 5, 15, 25 and 35 °C either in a parallel (replicates at different temperatures) or sequentially incubation (same samples rotated between different temperatures). Results of this preliminary study are reported in the results section under 'CO_2-production – comparing sequential vs. parallel incubation during 159 days'.

Chemical characterisation

Organic C and N was measured by dry combustion after removal of carbonates with 0.1 M HCl (CHN Na2000, ThermoQuest), and pH in the supernatant of a 0.1 M $CaCl_2$ solution after shaking (soil:solution 1:2.5). Chemical characterisation of soil samples was done by diffuse reflectance FT-IR spectroscopy (DRIFT). Samples were ground < 63 μm, mixed with KBr to obtain a 3% (weight) sample concentration, and measured with an Perkin Elmer Spectrum One B spectrometer with DRIFT equipment. Spectra were baseline corrected. The ^{14}C content of bulk soils and of hydrolysis residues was measured at the AMS facility of the Institute for Particle Physics of the Swiss Federal Institute of Technology, Zurich. After degassing the samples for 12 h at 40 °C, N_2 accessible surface area (SA) of fractions < 63 μm was measured with a NOVA

2200 surface analyser (Quantachrome) using 5 point isotherms and interpretation according to BET theory.

Results

Chemical characteristics of soils

Contents of SOC, N, and C/N ratios of the samples are listed in Table 1. SOC and N contents decreased with depth for bulk soils and size fractions. For both elements, concentrations were higher in the sand fraction as compared to the silt + clay fraction in the topsoil, and this ratio was reversed in the subsoil. With respect to land-use, the grassland site had a higher share of C in the sand fraction, as compared to the arable site. Carbon losses due to dissolution of SOC during physical fractionation, as calculated by difference between, on the one hand, the bulk soil and the combined sand + silt + clay fraction (not HCl-treated) on the other hand, were 10.2, 15.8, 2.1 and 7.9% for A5, A30, B5, and B30, respectively. C/N ratios were in a range of 5.0 to 9.5. Not all aggregates ≥63 μm were disrupted by shaking; therefore, silt + clay-associated C contributed also to the SOM in the sand-sized fraction.

Acid hydrolysis of the fraction < 63 μm removed 32, 32, 33 and 35% of the OC and 16, 19, 36 and 22% of the N (A5, A30, B5, B30), respectively. Acid hydrolysis residues of the silt + clay fraction were characterised by a

Table 1. Chemical composition of soils and soil fractions. A: arable rotation, B: permanent grassland.

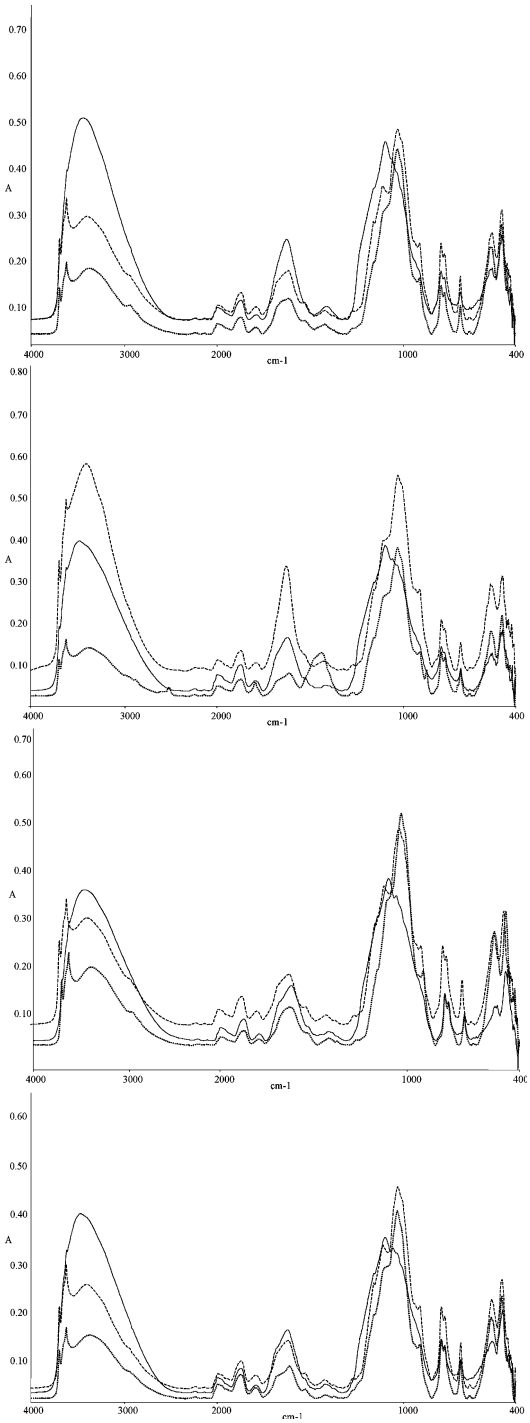
		OC g kg^{-1}	N g kg^{-1}	C/N g kg^{-1}	Share of fraction C to sample C (%)
Bulk soil	A 5–10 cm	34.0	3.6	9.5	
	A 25–30 cm	19.6	2.9	6.7	
	B 5–10 cm	32.1	4.2	7.7	
	B 25–30 cm	24.6	3.3	7.5	
Fraction ≥63 μm	A 5–10 cm	37.7	4.5	8.4	32.2
	A 25–30 cm	12.1	2.4	5.0	17.9
	B 5–10 cm	33.8	3.8	8.9	41.4
	B 25–30 cm	19.5	2.7	7.2	27.0
Fraction < 63 μm	A 5–10 cm	27.6	3.2	8.6	57.7
	A 25–30 cm	18.3	3.1	5.9	66.3
	B 5–10 cm	29.9	4.4	6.8	56.8
	B 25–30 cm	24.3	3.6	6.8	65.2
HCl residue < 63 μm	A 5–10 cm	18.9	2.7	7.0	39.5
	A 25–30 cm	12.4	2.5	5.0	44.9
	B 5–10 cm	20.1	2.8	7.2	38.2
	B 25–30 cm	15.7	2.8	5.6	42.1
Composted grass		385.9	31.4	12.3	

significantly higher ^{14}C age, as compared to the corresponding bulk soils (Table 2). Both, size fractionation and acid hydrolysis isolate carbon fractions of different ^{14}C age (Buyanovsky et al. 1994; Falloon et al. 1998). However, the HCl residues < 63 μm isolated here still had a wide range of ^{14}C ages, and thus could not be regarded as functionally identical with respect to SOM turnover across the four different samples. Percent modern C values in bulk soils indicated a high proportion of bomb ^{14}C in SOM. Thus, some of the SOM must have been accumulated during the period of anthropogenic $^{14}CO_2$ enrichment since the mid-1960's when atmospheric concentrations of $^{14}CO_2$ exceeded today's values. In terms of ^{14}C age, the values in Table 2 for the HCl residues translate to ages of 945–2515 years before present.

The bulk chemical composition of HCl-treated and non-treated size fractions, as revealed by means of DRIFT spectroscopy, is depicted in Figure 1. Dominant peaks assignable to SOM constituents occurred around 1620 cm^{-1} (asymmetric COO-/COOH-stretch and/or aromatic C = C stretch; Niemeyer et al. 1992) and at 1031 cm^{-1} (C–O–C from polysaccharides; (Ellerbrock et al. 1999). Aliphatic stretching bands in the region 2940–2900 cm^{-1} were almost absent in the samples. The main difference in composition before and after HCl treatment was visible in the region around 1000–1100 cm^{-1} where C–O–C from polysaccharides occur together with O–Si–O stretching of silicates. Due to HCl treatment, the contribution of the polysaccharide band was markedly reduced, most probably due to disruption of glycoside bonds between carbohydrates and/or the exchange with the supernatant of polyvalent cations between carbohydrates and mineral surfaces, and the concomitant release and removal of the carbohydrates. New absorption bands, indicative for the formation of artefacts due to the harsh HCl treatment, were not visible. A comparison of SA revealed a strong increase by a factor of around 5–9 in N_2-accessible SA due to the removal of hydrolysable SOM (Table 3). In contrast, the subsequent removal of the remaining SOM by hydrogen peroxide resulted in a small further increase in SA, or even a slight decrease (B5).

Table 2. Radiocarbon content and percent modern carbon from bulk soils and corresponding HCl residues < 63 μm. A: arable rotation, B: permanent grassland.

		$\Delta\ ^{14}C$ (‰)	% modern C
Bulk soil	A 5–10 cm	107	109.9
	A 25–30 cm	391	137.9
	B 5–10 cm	154	114.7
	B 25–30 cm	32	102.5
HCl residue < 63 μm	A 5–10 cm	−112	88.3
	A 25–30 cm	−269	72.7
	B 5–10 cm	−115	87.9
	B 25–30 cm	−135	85.9


0.70
0.60
0.50
0.40
A
0.30
0.20
0.10
4000
3000
2000
1000
400
cm-1
0.80
0.70
0.60
0.50
0.40
A
0.30
0.20
0.10
4000
3000
2000
1000
400
cm-1
0.70
0.60
0.50
0.40
A
0.30
0.20
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2000
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cm-1
0.60
0.50
0.40
A
0.30
0.20
0.10
4000
3000
2000
1000
400
cm-1

Figure 1. DRIFT spectra (top to bottom) A 5–10 cm, A 25–30 cm, B 5–10 cm, B 25–30 cm. A arable rotation, B permanent grassland. Dotted: Fraction ≥ 63 μm; dashed: Fraction < 63 μm; solid: Fraction < 63 μm HCl treated. Y-axes shows absorbance units.

CO_2 production – comparing sequential vs. parallel incubation during 159 days

For the preliminary experiment comparing sequential vs. parallel incubation of soils, calculated rate constants for CO_2 release from this experiment are summarised in Figure 2. Parallel incubation resulted in higher rate constants at 5 °C, and in smaller ones at 35 °C, compared to sequential incubation. Q_{10} values for the temperature range 5–35 °C were 1.21 and 1.62 for parallel and sequential incubation, respectively.

Table 3. N_2 surface area ($m^2\ g^{-1}$ soil) for the untreated fraction < 63 μm, corresponding hydrolysis residues, and hydrolysis residues after treatment with hydrogen peroxide. A: arable rotation, B: permanent grassland.

	< 63 μm	< 63 μm HCl	< 63 μm H_2O_2
A 5–10 cm	9.7	89.8	97.9
A 25–30 cm	16.2	76.4	99.1
B 5–10 cm	12.8	71.2	66.3
B 25–30 cm	19.1	91.7	93.8

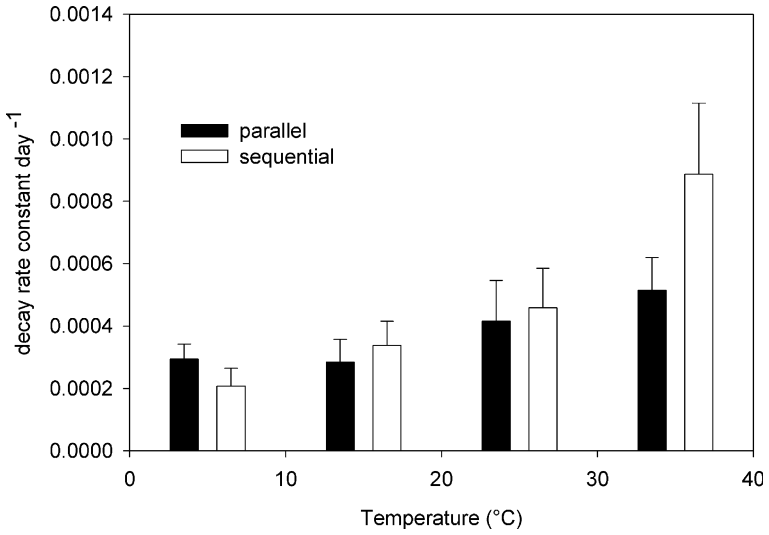

Figure 2. Mean SOC decay rate constants of long-tem measurements over 159 days for parallel (black) and sequential (white) incubations. Bars show 1 standard error ($n = 3$).

CO_2 production – results from bulk soils, soil fractions, and long-term incubated samples

Production rates (25 °C) for CO_2 per weight unit OC of bulk soils or of their size fractions varied between substrates (Figure 3). The sand fractions showed 4 (B5) to 11 (A30) times higher production compared to the corresponding bulk soil. CO_2 production of the composted grass sample (2067 mg C kg^{-1} C day^{-1}) was slightly below that of the SOC of the sand fractions. CO_2 production of the HCl residues < 63 μm was in the same order of magnitude as the non-hydrolysed < 63 μm fractions and bulk soils. From the CO_2 production and the time between inoculation of the HCl residues and CO_2 measurement, we calculated that for the sample with the smallest productivity the amount of CO_2-C produced exceeded the amount of DOC-C added by a factor of 5.8.

Temperature response of CO_2 production

Most samples showed a single exponential relationship between CO_2 production and temperature with R^2 values between 0.999 and 0.923, thus indicating an Arrhenius-type of reaction with activation energies (E_a) between 142 to 244 (hydrolysis residues) and 71 to 123 kJ Mol^{-1} for all other samples. Despite the relative high coefficients of determination, residuals were high for all soil fractions except B5 < 63 μm, in particular for the estimated rates at 25 °C (Figure 4). As an example, the small image embedded in Figure 4 exemplifies

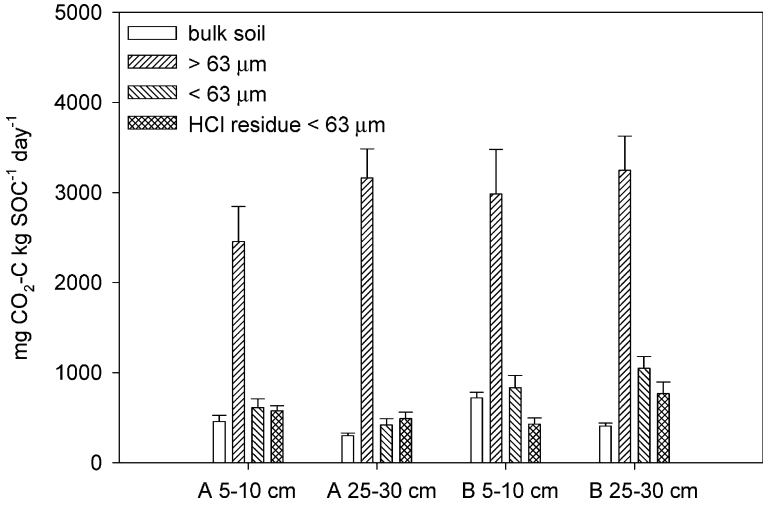

Figure 3. CO_2 production rates from bulk soils and corresponding soil fractions at 25 °C. A arable rotation, B permanent grassland. Bars show 1 standard error ($n = 6$).

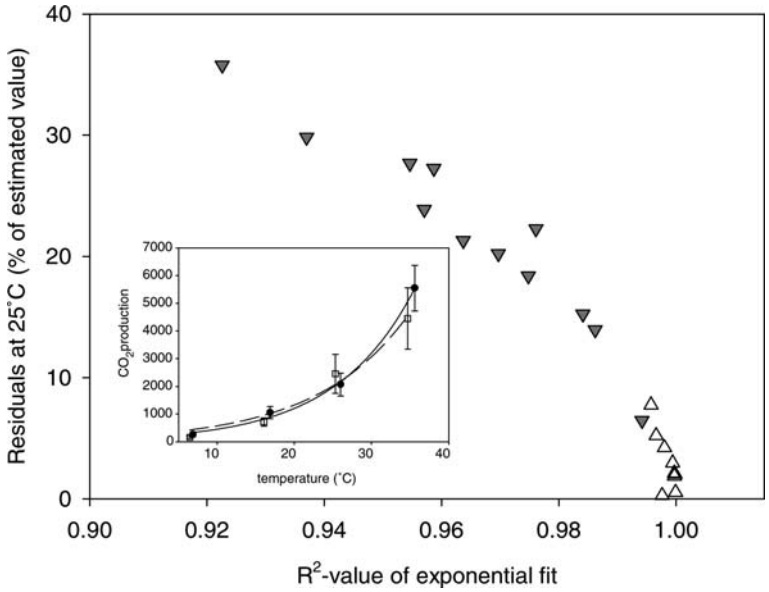

Figure 4. Scatter plot of *R*-squared values of exponential fitting vs. the residuals of the exponential model at 25 °C (% of predicted value). The open upside triangles represent bulk soils, long-term incubations, and the composted grass. The grey downward triangles represent soil fractions. Note that there is only one soil fraction where the residual is < 10% (B 5–10 cm < 63 μm) and thus similar to the bulk soils and long-term incubations. The small image illustrates the exponential relationship between temperature and CO_2 production for a bulk soil (closed circles) and a soil fraction (open squares). In the latter case, the model underestimates the measured production at 25 °C.

two samples with a different response to temperature. The data of the sample with the higher residual at 25 °C could better be described by a sigmoidal rather than an exponential function.

Long-term incubations over 707 days resulted in a typical double exponential decrease of CO_2 production rates (Figure 5). SOC lost during this period accounted for 5.7% of the total amount. As a measure of temperature sensitivity, Q_{10} values for the interval 15–35 °C are depicted in Figure 5. At the beginning of the long-term incubation, production rates were similar to those measured in bulk soils described above. Over time, production rates decreased by about 83%. Q_{10} values 15–35 °C showed no significant trend over time, similar to all other parameters describing temperature sensitivity (i.e., Q_{10} values for other temperature ranges, temperature quotients, E_a values).

Q_{10} values of short-term incubated bulk soil, and of the corresponding size fractions, were in the same range as those of long-term incubated bulk soil, but exceptionally higher for HCl residues < 63 μm (Figure 6). For most temperature intervals, the difference between HCl residues and the non-hydrolysed fractions ≥63 μm and < 63 μm was significant (Table 4). At 25 °C, produc-

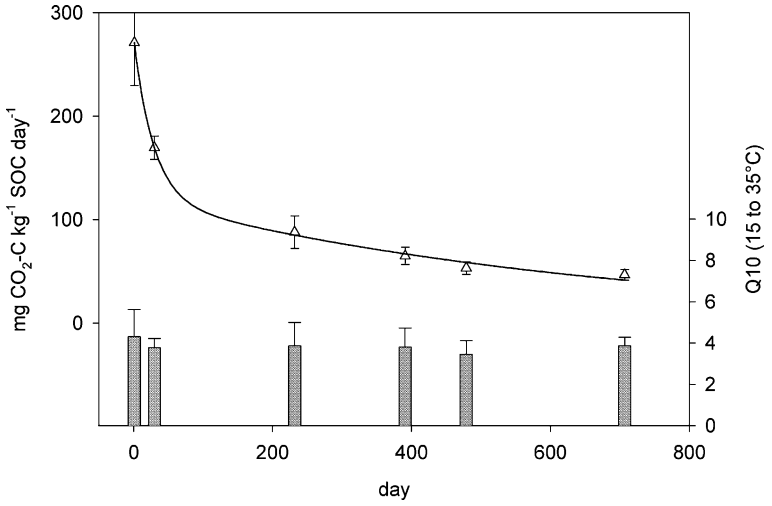

Figure 5. CO_2 production rates during a 707 days long-term incubation at 25 °C (curve), and temperature response at each particular measurement (bars) in Q_{10} values. Bars show 1 standard error ($n = 6$).

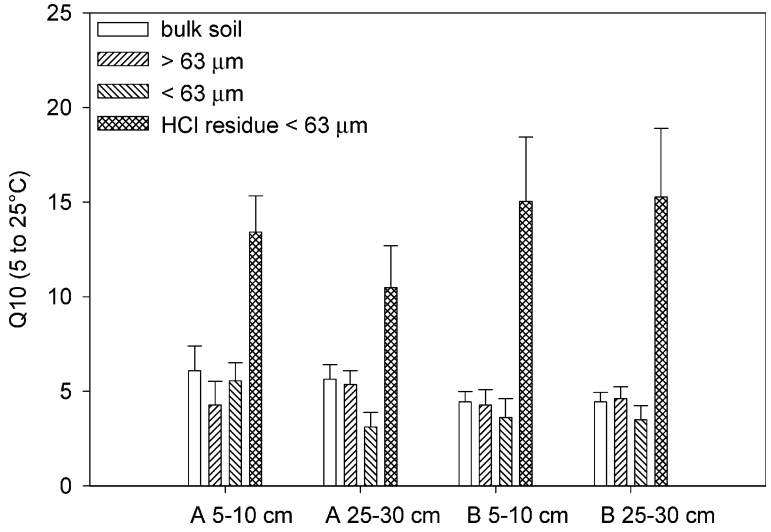

Figure 6. Q_{10} values of CO_2 production 5–25 °C for bulk soils and corresponding physico-chemical fractions. A arable rotation, B permanent grassland. Bars show 1 standard error ($n = 6$).

tivity of HCl residues was not much different from bulk soils and from the non-hydrolysed fraction < 63 µm (Figure 2). Hence, this difference was attributed to the much lower production rates of the HCl residues at 5 °C (2.2–4.3), as compared to bulk soils and size fractions < 63 µm (3–90) or size fractions

Table 4. Q_{10} values of CO_2 production from physico-chemical soil fractions and bulk soils for six different temperature ranges. Different letters in rows indicate significant differences among sample type ($P < 0.05$; Tukey-Kramer).

Q_{10} range (°C)	≥63 μm	< 63 μm	< 63 μm HCl	Bulk soils and long-term incubated samples
5–15	5.3 a	6.1 a	15.0 b	6.6 a
5–25	4.0 a	4.6 a	13.6 b	4.9 a
5–35	3.4 a	3.5 a	7.2 b	4.6 a
15–25	3.5 a	4.1 a	12.1 b	3.6 a
15–35	2.5 a	2.7 a	5.3 b	3.9 ab
25–35	1.8 a	2.0 a	2.0 a	4.0 b

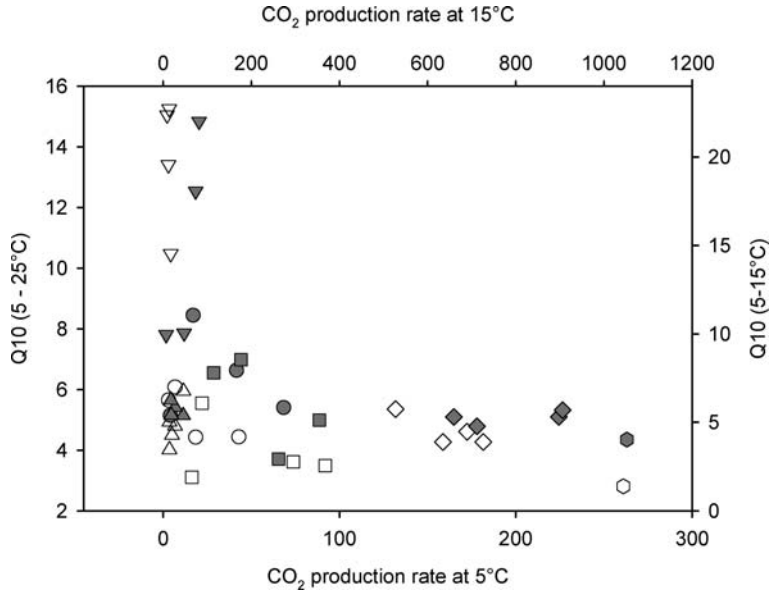

Figure 7. CO_2 production rates (mg CO_2–C kg^{-1} SOC d^{-1}) at 5 °C plotted against Q_{10} values calculated for the temperature range 5–25 °C (open symbols) and rates at 15 °C plotted against Q_{10} values for the temperature range 5 to 15 ° C (grey symbols). ○ bulk soils, □ fraction < 63 μm, ▽ HCl residues, ◇ fraction ≥63 μm, △ long-term incubations, hexagons composted grass.

≥63 μm (132–182 mg CO_2-C kg^{-1} SOC day^{-1}), respectively. Q_{10} values of the non-hydrolysed fractions ≥63 μm and < 63 μm were not significantly different, but consistently higher for the latter.

To further evaluate possible systematic interactions between temperature response and substrate quality, Q_{10} for two different temperature intervals (5–25 °C and 5–15 °C) were compared with corresponding CO_2 production rates (k) at 5 and 15 °C, respectively (Figure 7). Mean Q_{10} values were 6.2 for 5–25 °C and 7.8 for 5–15 °C (2.8 and 5.2 for 25–35 °C and 15–25 °C, respec-

tively), but varied strongly between samples. For both temperature ranges, large Q_{10} values corresponded to small CO_2 production rates at the respective temperature, and vice versa. HCl residues showed the largest temperature response. The negative relationship between Q_{10} and k was significant (ln-transformed data) for the two temperature ranges shown in Figure 7 ($p < 0.001$ for Q_{10} 5–25 °C vs. k_5 and $P < 0.05$ for Q_{10} 5–15 °C vs. k_{15}) and for the majority of other combinations of Q_{10} vs. k. In contrast to the pattern in Figure 7, bulk soils and long-term incubated samples rather than HCl-residues showed the highest Q_{10} values in combination with a small k above 25 °C.

Discussion

Parallel incubations have been used previously to characterise the temperature response of SOM mineralisation (Dalias et al. 2001; Bol et al. 2003). In our investigation, parallel and sequential incubations showed pronounced differences in rate constants and, more importantly, Q_{10} values. We believe this to be an effect of different amounts of available substrate remaining at each temperature in the samples over time in the parallel approach. The temperature response calculated after a given period strongly relies on the remaining decomposable substrate at a given temperature, and thus is systematically wrong in parallel incubations (see detailed discussion in Reichstein et al. 2000; Leifeld 2003). Consequently, parallel incubations should be avoided when temperature effects are to be assessed in laboratory experiments. The approach used here is yet another alternative to circumvent sources of variability in Q_{10} values, in addition to methods discussed by Reichstein et al. (2000).

Different carbon sources and their relation to CO_2 production

We hypothesised that samples with varying physico-chemical properties are of different SOM quality, and that incubation of these experimentally separated materials will show differences in temperature response. The differences in CO_2 production, chemical composition, age, and allocation in the soil matrix observed in the different samples confirmed a wide range of SOM properties and provided a good basis for the detection of potential temperature effects related to quality.

The higher ^{14}C age of the HCl residues suggests that this fraction contributes to a biochemically more stable SOM pool. The HCl residues also confirmed the expected differences in chemical composition (DRIFT) as compared to the other fractions or bulk soils. Chemically labile carbohydrates and organic C with young ^{14}C age significantly contributed to the overall composition of SOM in samples from each site, and HCl treatment resulted in a relative enrichment of older SOM with smaller carbohydrate contents, as compared to non-hydrolysed soils. A question to be addressed is the possible formation of

melanoidin-like artefacts during the HCl treatment as described for the acid treatment of mycobacteria by Allard et al. (1997), which might effect the chemical structure and thus the decomposability of the remaining material. The use of FT-IR spectroscopy has been found to be an efficient tool for the detection of such melanoidin-type structures (Poirier et al. 2000). To control whether the HCl treatment resulted in the formation of new structures, we compared the HCl-treated size fraction with its non-treated precursor, and also compared the DRIFT spectra of the HCl residues with that of a custom-made melanoidin in our lab. Any occurrence of newly formed covalent bonds along with the HCl treatment, in particular of a melanoidin-like compounds, could not be observed (see Figure 1). The spectra of the pure melanoidin (not shown) comprised pronounced bands at around 1676 cm^{-1} (C = N of imines), 1198 cm^{-1} (most probably C–N of amines), and at around 2940 cm^{-1} (C–H aliphatic stretching). These bands were not visible in the HCl treatments. Together, these findings indicate that the formation of new compounds during HCl treatment in our samples is very unlikely.

Sorption of SOM onto mineral surfaces is supposed to decrease SA of mineral soils, which is mainly due to clogging of micropores by the absorbed material (Kaiser and Guggenberger 2003). The pronounced increase in N_2 SA after HCl treatment implies that the younger, hydrolysed SOM was intimately associated with the mineral matrix in the <63 μm fraction. The SOM remaining after this treatment was chemically less protected, as indicated by small changes in SA after complete removal of SOM. Therefore, we expect mechanisms other than chemical protection via organo-mineral association to contribute to the longer turnover times of HCl residues in soil. Surprisingly, HCl residues were as active as their non-hydrolysed counterparts <63 μm at higher temperatures (25 and 35 °C), whereas CO_2 production was significantly smaller in the lower temperature range. From the comparison of the fine fractions, we conclude that despite its higher age and its less favourable chemical composition, SOM remaining after acid hydrolysis is biochemically not stable. Possibly, physical protection in microaggregates rather than surface reactions contributes to the stability of HCl residues in the field, and removal of binding agents during hydrolysis releases some of this material, which then becomes available for microorganisms. Differences in the chemical composition between hydrolysed and non-hydrolysed fractions are expected to contribute to the smaller CO_2 production at lower temperatures, thus indicating the influence of SOM quality.

The macro-OM in the sand fraction had a much higher activity similar to that of the 4-week-old composted grass. In the first place, the enhancement of the activity of SOM in the sand fraction is most probably due to the disruption of the physical integrity of soil aggregates, resulting in the removal of physical protection. This is in agreement with previous findings on density (Hassink 1995) and size (Leinweber 1995) fractions showing light and coarse SOM to be more easily mineralisable than mineral associated SOM, as it was present here in the size fractions <63 μm. Secondly, sand-sized SOM is typically much less

transformed than mineral-associated SOM (e.g. Leifeld and Kögel-Knabner, 2005) and thus forms favourable microbial food. The breakdown of aggregates and the subsequent amplified release of CO_2 also explains, why the cumulated CO_2 from the size fractions ≥63 μm and <63 μm of a particular sample exceeds that of the undisturbed bulk soil.

Temperature response

Generally, our data confirm that Q_{10} values decrease with increasing temperature, as reported elsewhere (Lloyd and Taylor 1994; Kirschbaum 1995). In a first attempt, we tried to separate temperature effects induced by different SOM qualities by means of long-term experiments with intact soil cores. During sequential incubation, CO_2 production rates declined which is typical for the decomposition of a substrate composed of two different pools with individual rate constants. The temperature response of CO_2 production remained almost unchanged over time, but only 5.7% of the SOM in the sample was mineralised during that period, corresponding to 'active' SOM. In other words, this approach gives no information regarding the temperature sensitivity of the remaining 94.3% of more stable SOM, but it shows that the temperature response of so called 'active' SOM seems to be relatively consistent, as compared to the higher variability of Q_{10} values in physico-chemical soil fractions. A similar result has been recently reported for the sequential incubation of soil samples from a Scottish spruce site during 108 days (Fang et al. 2005). In their study, the authors conclude that the 'resistant' SOM pool has a similar response to temperature as the 'labile' one. However, because their pools were arbitrarily defined rather than physically separated, it is probable, that the contribution of older SOM to the total CO_2 flux has been significantly overestimated. The long-term incubation in our study indicates that the temperature response is not related to CO_2 production as long as the emitted CO_2 derives from a relatively homogeneous SOM pool and as long as the flux depends only on pool size and not on SOM quality.

The temperature response of incubated bulk soils and long-term incubated samples was above average between 25 and 35 °C (mean $Q_{10} = 2.8$), and these samples produced less CO_2 at 25 °C than the others. In Figure 4, we illustrated that the soil fractions did not obey the Arrhenius equation above 25 °C, in contrast to bulk soils and long-term incubated samples. These data imply convergence to the biochemical temperature optima between 25 and 35 °C for the soil fractions, leading to a flattened slope. Because the shape of the curve above 25 °C is dominated by approaching the temperature optima of microbial activity for the soil fractions, any further comparative interpretation of their temperature response relative to that of the bulk soils seems inappropriate for higher temperatures.

Q_{10} values of highly labile fractions ≥63 μm and of the composted grass were smaller than the mean of all samples at any temperature interval, and close to

published values from other studies. Because sand-sized SOM and composted grass showed typical Q_{10} values and concurrent high CO_2 production rates, it seems likely that it is material of this origin which accounts for most of measured temperature effects in incubation studies and for most of the heterotrophic CO_2 efflux from soils in the field. Some of these literature values are used for the parameterisation of SOM models. For example, Sorensen (1981) reported Q_{10} values over the temperature range $+10$ °C to $+30$ °C of 1.40–1.80 for incubated, labelled cellulose, and Kirschbaum (1995) reported a mean Q_{10} of 2.5 at 20 °C, reviewing 20 different data sources. During parallel incubation of subalpine soil samples at 5, 15, and 25 °C, Reichstein et al. (2000) found Q_{10} values of between 2.5 and 2.8.

Temperature sensitivities for fractions < 63 μm were slightly, but not significant different to those of the sand fractions and bulk soils, whereas HCl residues < 63 μm were less active in the temperature range 5–15 °C than any other sample type, and had significantly higher Q_{10} values at temperatures ≤ 25 °C. Below 25 °C, we found a significant negative relationship between CO_2 production rates and the corresponding temperature response across the different samples, which was mainly due to the temperature response of HCl residues. From the experimental design it is difficult to formulate an unbiased and clear hypothesis for the difference in temperature response, because the incubation conditions differed from field conditions. In particular, we expect different microbial communities to exist across the physical and chemical fractions and the bulk soils due to disturbances during pre-treatment, although the inoculum was extracted from the same site. However, because the microbial activity of the HCl residues at higher temperatures was not different from that of the non-hydrolysed fraction < 63 μm, a general physiological or nutritional limitation for the microorganisms at lower temperatures is unlikely. It seems more probable that the chemical composition of the substrate remaining after HCl treatment affects the decomposition rate, particularly at lower temperatures. SOM remaining after acid hydrolysis is relatively depleted in carbohydrates, as shown by our results, and in general is enriched in more stable and energy-rich compounds like alkyl and aromatic C (Kögel-Knabner 1997). Oxidation of these compounds is hampered by the need for higher activation energies. In general, these experimental findings agree with the theory developed by Bosatta and Ågren (1999) who proposed a higher temperature sensitivity of decomposition for energy-rich compounds. Our findings also support results of a recent modelling study by Knorr et al. (2005), who presented evidence that decomposition of non-labile SOC is more sensitive to temperature than labile SOC by fitting a flexible activation energy of two- and three-pool models to experimental data. However, in contrast to these more theoretical indications, our study directly demonstrates that differences in temperature sensitivity are coupled to SOM quality. In consequence of these complementary findings, biochemically unfavourable substrate accumulated in the soil at lower temperature regimes would be relatively more temperature sensitive than easily degradable material.

What is the implication of these findings for the interpretation of CO_2 data and SOM turnover times in the field? Because of the small contribution of the stable SOM to the CO_2 efflux from heterotrophic soil respiration, its increased contribution at elevated temperatures relative to the 'active' SOM may be not detectable in the short-term, but may deplete SOM stocks in the long-term. However, there are some findings cited as evidence that temperature may effect CO_2 production from soil less than found in this study, or in studies reviewed by Kirschbaum (1995) or Kätterer et al. (1998), for example. In a soil warming experiment in a mid-latitude hardwood forest, Melillo et al. (2002) concluded that the decay rate of the first SOM pool (labile) was highly temperature-sensitive, whereas the decay rate of the second pool (more stable) was not. Giardina and Ryan (2000) even argued that decomposition rates of organic carbon in mineral soil do not vary with temperature, based on decomposition data from 82 sites worldwide. To understand this apparent contradiction among studies it is crucial to recognize what exactly is being measured in a particular experiment. For example, Melillo et al. (2002) measured CO_2 efflux from forest soil with static chambers of heated vs. non-heated plots and found that over the last four years of their 10-year study, the stimulatory effect of warming on soil respiration markedly decreased. This finding is not in opposite to the results reported here, because the study of Melillo et al. (2002) monitors primarily the decay of a labile SOM pool due to elevated temperature. In such an experimental set-up, the effect of elevated temperature on older SOM, which accounts for only a minor portion of the CO_2 efflux, might not be detectable. Effects of pool size probably apply also for the results presented by Giardina and Ryan (2000). They showed that the SOC turnover time calculated by means of cumulative mass loss of soil samples incubated in the lab at constant temperatures representative of field conditions was not related to the incubation temperature. Clearly, an effect of the size of the easily decomposable SOM pool will have a major effect on the cumulative CO_2 efflux, and the turnover time derived from it, a topic which has also been addressed by Davidson et al. (2000) and Kirschbaum (2004). Similarly, the finding of a temperature tolerant old SOM pool by Liski et al. (1999) has been questioned by Ågren and Bosatta (2002) who argued that the apparent temperature insensitivity was caused by a difference in the SOM quality distribution (which is the distribution of SOM among different pools) rather than a difference in temperature sensitivity. With respect to the use of field measurements to derive Q_{10} values for soil respiration, Curiel Yuste et al. (2004) recently discussed seasonal effects in a mixed temperate forest. Their study showed that measured Q_{10} values were strongly influenced by the deciduousness of the vegetation, and the authors concluded that differences in Q_{10} values were not only due to different temperature sensitivities, but also to different seasonal patterns of plant activity, which are also related to pool sizes through the seasonal variation of root respiration contributing to the measured flux. The effect of varying pool sizes and temperatures over time on calculated Q_{10} values have also been discussed by Gu et al (2004). By using a modelling approach, they explained an underestimation of

Q_{10} values in field experiments by a displacement of phase between the variation in pool size and temperature (and likewise, its overestimation as being in phase). Their finding of systematic errors is similar to those described here for the calculation of Q_{10} values by means of CO_2 production rates in parallel incubations. For the interpretation of temperature effects it is therefore necessary to clearly distinguish the variation in CO_2 efflux rates or in calculated SOM turnover rates with temperature, which both are largely effected by varying pool sizes, from the actual temperature response of biochemical reaction kinetics in both, field experiments and laboratory incubations. The temperature response of biochemical reaction kinetics has been addressed in our study and is an important factor within a complex of factors modulating SOM turnover, but it is by far not the only one.

Conclusions

The results show systematic differences in Q_{10} values and indicate that for the investigated soils the temperature sensitivity of SOM decomposition is negatively related to SOM quality, the latter characterised by means of physicochemical properties. We conclude that SOM fractions with longer turnover times ('passive SOM') respond more sensitive to an increase in temperature than so-called 'active SOM' and further experiments using experimental pool separation from different sites with respect to soil properties, land-use and land-use history are needed. Although the contribution of the older fraction to the total CO_2 efflux under field conditions cannot be extrapolated from our laboratory incubations and is considered to be small, the results suggest that under constant OM input an increase in temperature will lead to a depletion of passive SOM in the long term, in particular in cooler regions.

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