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- Temperature sensitivity of mineral-associated SOM was not related to its age
- Temperature sensitivity of POM fraction was similar or slightly higher than MOM
- Within the POM fraction, old C was more sensitive to temperature than recent C

**Similar temperature sensitivity of soil mineral-associated organic carbon
regardless of age**

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Abstract

Most of the carbon (C) stored in temperate arable soils is present in organic matter (OM) intimately associated with soil minerals and with slow turnover rates. The sensitivity of mineral-associated OM to changes in temperature is crucial for reliable predictions of the response of soil C turnover to global warming and the associated flux of carbon dioxide (CO₂) from the soil to the atmosphere. We studied the temperature sensitivity of C in <63 µm fractions rich in mineral-associated organic matter (MOM) and of C in >63 µm fractions rich in particulate organic matter (POM). The fractions were isolated by physical separation of two light-textured arable soils where the C4-plant silage maize had replaced C3-crops 25 years ago. Differences in ¹³C abundance allowed for calculation of the age of C in the soil-size fractions (old C, C3-C > 25 years; recent C, C4-C < 25 years). We incubated bulk soils (< 2 mm) and size fractions sequentially at 6, 18, 26 and 34 °C (ramping up and down the temperature scale) and calculated the temperature sensitivity of old and recent C from ¹²CO₂ and ¹³CO₂ evolution rates. The temperature sensitivity was similar or slightly higher for POM than for MOM. Within the POM fraction, old C3-C was more sensitive to changes in temperature than recent C4-C. For the MOM fraction, the temperature sensitivity was unrelated to the age of C. Quantitative PCR analysis indicated that the proportions of bacteria, archaea and fungi did not change during incubation. Our results suggest that while OM stabilizing mechanisms affect the temperature sensitivity of soil C, temperature sensitivity appears unrelated to the age of mineral-associated OM.

Keywords: Soil organic matter, temperature sensitivity, decomposition, climate change, ¹³C natural abundance, Bayesian statistics

1. Introduction

Understanding the mechanisms that affect the turnover of organic matter (OM) in soil is crucial in order to predict the response of soil C storage to changes in climate and land use. The effect of changes in temperature on the decomposition of labile soil OM is well known, whereas less is known on the temperature effect on C in soil OM pools with turnover rates counted in decades and centuries (Trumbore, 2000). The soil OM with slow turnover rates is intimately associated with the soil mineral matrix and accounts for most of the C accumulated in the soil profile in temperate arable soils. Thus,

59 even small changes in turnover rate of stable OM may lead to substantial and long-lasting impacts on
60 the CO₂ flux from soil to atmosphere (Trumbore, 2000; Schmidt et al., 2011). This spawns a timely need
61 to link temperature sensitivity to the mechanisms that stabilize or destabilize C in mathematical models
62 that simulate long-term turnover of soil C.

63
64 Soil OM persists because either its chemical structure is resistant to enzymes released by decomposers
65 or it has become physically or chemically protected against decomposer activity. The decomposition rate
66 of particulate organic matter (POM) has been linked to its intrinsic chemical complexity (Melillo, 1982;
67 Tuomi et al., 2009), whereas the decomposition rate of stabilized OM relates to its incorporation into
68 organo-mineral complexes and reaction with the extensive surface areas of clay- and silt-sized particles
69 (Christensen, 2001; Kleber et al., 2007).

70
71 The decomposition of stable soil OM is often considered to be more sensitive to changes in temperature
72 than that of labile OM (Conant et al., 2008). This concept is based on the Arrhenius equation (Bosatta
73 and Agren, 1999) according to which the temperature sensitivity of chemical reactions increases with
74 increase in activation energy. Stable OM comprises chemically complex molecules for which
75 decomposition reactions require a high activation energy and thus are highly sensitive to temperature.
76 However, OM with long turnover time also includes chemically simple and labile proteins and
77 polysaccharides of microbial origin that have become stabilized in organo-mineral complexes (Kleber et
78 al., 2011). The Arrhenius equation does not necessarily apply when other mechanisms than molecular
79 complexity limit the decomposition process (Davidson et al., 2006). Stabilization by organo-mineral
80 interactions is known to reduce the temperature sensitivity of OM (Gillabel et al., 2010; Moinet et al.,
81 2018), but the temperature sensitivity of OM with different age residing within a given soil size fraction
82 remains unclear (Leifeld and Fuhrer, 2005; Plante et al., 2010; Poeplau et al., 2017).

83
84 The effect of OM association with minerals on the temperature sensitivity of recent (<25 years old) C
85 versus older C has not previously been studied. In this study it was determined by measuring the ¹²CO₂
86 and ¹³CO₂ evolved during incubation of OM fractions that were isolated by physical fractionation of soils
87 sampled in an experiment where C3-plants have been replaced by C4-plants 25 years ago.

88

89 The temperature sensitivity of C in soil OM with different mineral association was examined by
90 incubating two particle size fractions (<63 μm , mineral- associated OM (MOM) and >63 μm , particulate
91 OM (POM)). The soils were from an experiment where silage maize (a C4-plant) was grown for 25 years
92 on soil previously under C3-plants exclusively. The difference in $\delta^{13}\text{C}$ between plants with the C3 and the
93 C4 photosynthetic pathway is typically 15 ‰ (O'Leary, 1988). This allowed us to discriminate between
94 old and recent C (old, C3-C > 25 years; recent, C4-C < 25 years) present in a given fraction and
95 between CO_2 originating from old and recent soil C. The objectives were to quantify 1) the temperature
96 sensitivity of C residing in POM and MOM, and 2) the sensitivity of differently aged C associated within
97 each of these size fractions. We hypothesized: 1) that the temperature sensitivity of POM increases with
98 increasing age following Arrhenius equation because chemical recalcitrance controls the decomposition
99 of POM, and 2) that the temperature sensitivity of C in MOM is less affected by age than C in POM
100 because the mineral-association limits the decomposition of MOM. Thus, it follows 3) that the overall
101 decomposition of MOM is less sensitive to temperature than POM.

102

103 The composition of the soil microbial community may change with OM quality and incubation
104 temperature (Biasi et al., 2005). The balance between fungi, bacteria and archaea during the incubations
105 was determined using quantitative PCR, to reveal if respiration rate and changes in isotopic ratio relate
106 to changes in microbial community (Paterson et al., 2009).

107 2. Materials and Methods

108 2.1. Site and sampling

109 The study relied on soils sampled in a C3- to C4-vegetation conversion experiment at Askov
110 Experimental Station, Denmark (55°28N, 09°07E). Annual mean temperature and precipitation during
111 1981–2010 were 8.2 °C and 1079 mm, respectively. The experiment was established in 1987 when soil
112 (0-25 cm) was sampled from two agricultural sites. The Askov soil (ASK) has loamy sand texture with
113 14.1 % clay (< 2 μm), 21.2 % silt (2-20 μm), 30.6 % fine sand (20-200 μm), and 34 % coarse sand (200-
114 2000 μm). The Lundgaard soil (LUN) has coarse sand texture with 5.5 % clay, 7.7 % silt, 16% fine sand
115 and 70.8% coarse sand. The soils were sieved to <4 cm and placed outdoors in large open-ended
116 cylinders (0.76 m^2 ; 50 cm high) inserted 45 cm into the ground and resting on undisturbed subsoil. Until
117 1987, the ASK and LUN soils had been exclusively under C3-crops (mainly cereals). At the start of the

118 experiment (1988) both soils had a C/N ratio of 13, and ASK soil had pH of 6.4, and LUN soil had pH of
119 7.6 (H₂O). Soil pH is maintained by occasional addition of Ca(OH)₂. Soil C contents and $\delta^{13}\text{C}$ values are
120 presented in Table 1. Every year since May 1988, the soils have carried the C4-crop silage maize (*Zea*
121 *mays* L.). The maize receives NPK mineral fertilizers every spring and is whole-crop harvested for silage
122 in mid-October. Kristiansen et al. (2005) give further details. Soils sampled every two-to-four years since
123 1988 are archived in air-dry condition. The present study received soil that was sampled in 1988 before
124 the first maize crop (year = 0) and in 2013 after 25 years of maize cultivation (year = 25). The 1988
125 samples served as reference for the isotopic composition of C3-derived soil C. The soils were sieved to
126 < 2 mm.

127 **2.2. Soil fractionation and incubation**

128 The air-dry soil samples from 2013 were divided into four laboratory replicates for physical fractionation,
129 while the amount of soil archived in 1988 did not allow for replication. Samples were soaked overnight in
130 water and fractionated by stirring and wet sieving through a 63- μm mesh. Then the >63 μm (POM,
131 including sand) and <63 μm (MOM) size-fractions and bulk soil (< 2 mm) subsamples were dried at room
132 temperature to constant weight. Soil water holding capacity (WHC) was determined separately for each
133 soil fraction before incubation.

134

135 Samples of bulk soil, POM and MOM fractions from 2013 were rewetted to 60% WHC and pre-incubated
136 at 20 °C for two weeks to remove any labile C released during the fractionation process (De Nobili et al.,
137 2006). Four replicate samples were incubated in 120 mL glass bottles with a rubber septum. The amount
138 of sample incubated ranged between 3 and 31 g. The quantity of sample was determined in a pre-
139 experiment in order to allow for sufficient CO₂ for ^{13}C analysis when using the same incubation times for
140 all fractions. The amounts used for incubation were: 9 g of ASK bulk soil and 19 g of LUN bulk soil, 26 g
141 of ASK > 63 μm fraction and 31 g of LUN > 63 μm fraction, 9 g of ASK < 63 μm fraction and 3 g of LUN <
142 63 μm fraction. Three replicates were used for measurement of CO₂ evolution and one for quantitative
143 PCR (extraction of microbial DNA). After pre-incubation, bottles resided in a water bath for 24 hours to
144 adapt to the first incubation temperature (6 °C). After closing the septum, the bottle headspace was
145 flushed with moist CO₂-free air (80% N₂ and 20% O₂). The soil samples were incubated ramping up and
146 down the temperature steps: 6, 18, 26 and 34 °C (sequential method; Hamdi et al., 2013) to minimize

147 potential bias from any change in substrate quality during incubation (Leifeld, 2003). In the sequential
148 method, the CO₂-flux at a given temperature, and in our case also the $\delta^{13}\text{C}$ content of the respired CO₂,
149 is the average of the values measured at a given temperature when ramping up and down the
150 temperature scale. The incubation periods were as short as possible to avoid changes in OM quality
151 while ensuring measurable CO₂ concentrations. Soil samples equilibrated to the next incubation
152 temperature in a water bath for 24 hours before starting the CO₂ collection. Before starting the CO₂
153 collection, the bottle headspace was flushed with CO₂-free air. Then the bottles were incubated in the
154 water bath at each temperature level until the CO₂ concentration in the headspace reached about 1000
155 ppm. At that time point we measured the CO₂ concentration in the headspace and retrieved gas samples
156 for ¹³C analysis. Then the bottle was transferred to the next temperature level and the procedure
157 repeated. The incubation times for CO₂ collection at each temperature step were: Ramping up the
158 temperature range: 6 °C: 6 days, 18 °C: 2 days, 24 °C: 1 day, 34 °C: 12 hours, and ramping down the
159 temperature range: 24 °C: 1 day, 18 °C: 2 days, 6 °C: 8 days. The CO₂ concentrations were measured
160 using a Hewlett Packard 6890 gas chromatograph equipped with a TC detector and J&W Scientific
161 Megapore GS-Q column.

162 **2.3. Isotope analysis**

163

164 For isotope analysis, gas samples were retrieved in a He-flushed glass vial (12 ml Exetainer®, Labco
165 Limited, UK) using a syringe. The $\delta^{13}\text{C}$ of the CO₂ was determined using a DeltaPlusXL (Thermo
166 Finnigan) continuous flow isotope-ratio mass spectrometer and references NBS-19, NBS-18 and L-
167 SVEC to normalize raw isotope data. The analytical precision was $\pm 0.15\text{‰}$ (1 σ).

168

169 Subsamples of size fractions and bulk soil were ball-milled for isotope analysis. The C concentrations
170 were measured using CHN-analyser (CHN-1000, Leco) and ¹³C using NC2500 Carlo-Erba analyser
171 coupled with Delta^{plus} Advantage (Thermo Fisher Scientific) continuous flow isotope-ratio mass
172 spectrometer. The ¹³C results are given as $\delta^{13}\text{C}\text{‰}$ based on standard V-PDB and reference materials
173 (IAEA-CH3 and IAEA-CH7). The analytical precision was $\pm 0.15\text{‰}$ (1 σ). All soil isotope analyses were
174 run in duplicate and results averaged.

2.4. Quantitative PCR (qPCR)

Quantitative PCR (qPCR) was used for examining the proportions of fungi (ITS), bacteria (B16S) and archaea (A16S) in soil at each incubation temperature. Gene copy numbers were determined on the additional set of bottles incubated along with bottles used for CO₂-collection. Samples for DNA extraction were taken from the same bottle after incubation at 6, 18, 26 and 34°C, when ramping up the temperature scale. Microbial DNA was extracted from freeze-dried subsamples using the NucleoSpin Soil DNA extraction kit (Macherey-Nagel). Quantitative polymerase chain reaction (qPCR) assays were conducted in thin-walled PCR tubes (Qiagen) on a Rotor-Gene 6000 real time PCR machine (Corbett Life Science). We amplified qPCR products obtained with fungal ITS1F and ITS2 (Gardes and Bruns, 1993; White et al., 1990), bacterial 16S rRNA 1055F and 1392R (Lee et al., 1993; Olsen et al., 1986; Stahl et al., 1988) and archaeal 16S rRNA Arch967f and Arch1060R primer pairs (Amann et al., 1990; Reysenbach and Pace, 1995; Riley-Buckley, 2001). The assays were run with Maxima™ SYBR Green qPCR Master Mix (2X) (Thermo Scientific) in a 20 µl reaction volume containing 1µl template, 0.375 µM of each primer, and 1× qPCR master mix. For qPCR conditions see Peltoniemi et al. (2015). Fluorescence was measured at the end of each extension step. The standard curves were constructed with plasmids containing corresponding inserts, taking into account the concentration and molecular mass of the plasmid, including the insert. The samples and standards were run in duplicate. All samples were replicated and absence of PCR inhibition was verified through 1:10 dilution. The copy numbers in samples were calculated based on comparison with threshold cycle values of the standard curve; the numbers are given per gram of soil C.

2.5. Calculations

Soil C and respired CO₂ was divided into C3-C (pre-1988) and C4-C (post-1988) using the isotope mass-balance equation (Balesdent and Mariotti, 1996). The fraction of the total soil C originating from C4 plants, F_{C4-C} , was estimated as:

$$F_{C4-C} = \frac{\delta^{13}C_{t=25} - \delta^{13}C_{t=0}}{\delta^{13}C_{C4-C} - \delta^{13}C_{t=0}}, \quad (1)$$

where $\delta^{13}C_{t=25}$ is the value of soils retrieved in 2013 and $\delta^{13}C_{t=0}$ is the value of the corresponding samples from 1988. The calculation of $\delta^{13}C_{C4-C}$ accounts for the small changes in isotopic composition of maize

204 biomass recorded over the experimental period (Christensen et al., 2011). This was estimated for year
 205 2013 as follows:

$$207 \quad \delta^{13}C_{C4-C} = -0.043 \text{ year} + 74.15. \quad (2)$$

208
 209 The CO₂ respiration rates at a given temperature (up and down the temperature range) were averaged.
 210 The Gaussian temperature function is as follows:

$$212 \quad R(t) = R_0 e^{aT + bT^2}, \quad (3)$$

213
 214 where T is the temperature (°C) and the parameters are $R_0 > 0, a > 0, b < 0$ (Tuomi et al., 2008). These
 215 were fitted to measurements using Bayesian framework (Gelman et al., 2013). Measurement errors in
 216 the Gaussian mode were assumed to be independent and follow a log-normal distribution. This error
 217 distribution was a natural choice for exponential model to account heteroscedasticity. The prior
 218 information of the parameter R_0 was modelled with a log-normal distribution with mean 100 and
 219 variance 100^2 . Uninformative priors were used for parameters a and b , that is $p(a, b) \propto 1$. In this way,
 220 the following posterior was obtained:

$$222 \quad p(\log(R_0), a, b | y) \propto p(\log(R_0))p(a, b)p(y | \log(R_0), a, b) \propto \text{Normal}((V^{-1} + H^T H / \sigma^2)^{-1}(V^{-1}m + \\ 223 \quad HTy / \sigma^2), (V^{-1} + H^T H / \sigma^2)^{-1}), \quad (4)$$

224
 225 where each row in matrix H is $[1, T_i, T_i^2]$ with measurement temperatures T_i and data vector y contains
 226 the corresponding CO₂ measurements y_i . The matrix V and vector m relate to prior values. From the
 227 above posterior, analytical equations were derived for marginal densities.

228
 229 The temperature coefficient Q_{10} describes the change in respiration rate when temperature increases by
 230 10 °C and was calculated from a and b as follows:

$$232 \quad Q_{10}(T) = \frac{R(T+10)}{R(T)} = e^{10a + 20bT + 100b} \quad (5)$$

233

234 resulting in a log-normal posterior distribution for $Q_{10}(T)$.

235

236 We analysed the C age using the isotopic composition of soil converted from C3 to C4 vegetation 25
237 years ago. The soil C pool was previously observed to be in steady-state equilibrium (Thomsen and
238 Christensen, 2010). This allows a simple calculation of mean residence time (MRT) of C in bulk soil and
239 both POM and MOM fractions using the $\delta^{13}\text{C}$ values according to Amelung et al. (2008):

240

$$MRT = -\frac{t}{\ln(1 - F_{C4-C})}, \quad (6)$$

242

243 where F_{C4-C} is the C4-C fraction of the soil C pool (Eq. 1) and t is time in years since the vegetation
244 change ($t=25$). The significance of trends in temperature series was tested with a t -test for trend. The
245 calculations for the Eqs. 1-3. and 6 were done in MS Excel. Differences in the qPCR results (log-
246 transformed gene copy numbers) between different SOC fractions were tested with one-way ANOVA
247 combined with Tukey test (IBM SPSS Statistics 22). Test for data normality used the Shapiro-Wilk test,
248 and Levene test was used for testing of homogeneity of variances.

249 3. Results

250 3.1. Soil characteristics

251 The $<63 \mu\text{m}$ fraction (MOM) had a higher C concentration and contained more C than the $>63 \mu\text{m}$
252 fraction (POM) (Table 1). The MOM represented 67 to 69 % of the total soil C in ASK both in 1988 and
253 2013. For LUN, MOM accounted for 74 % in 1988 and 57 % in 2013, mainly because of a higher C
254 concentration in POM in 2013. The C concentration in MOM was about 5 % while that in POM ranged
255 from 0.3 to 1.3 %.

256

257 The MOM and POM isolated from LUN soil had very similar $\delta^{13}\text{C}$ values before maize growing started in
258 1988, whereas for ASK soil MOM was somewhat depleted in ^{13}C compared to POM (Table 1). Maize
259 cultivation increased the $\delta^{13}\text{C}$ values of each soil fraction, and the values of the POM increased most. In
260 2013, maize-derived C represented 37 % of the C in POM in ASK and 39 % in LUN, while maize-C

261 accounted for 14 % of the C in MOM at both sites (see Fig. 4). For LUN as well as ASK soil, the MRT of
262 C in POM was 50 years and about 170 years for C in MOM (Table 1).

263 **3.2. Temperature sensitivity of SOC fractions**

264 The ASK bulk soil, POM and MOM fractions all showed similar respiration rates during incubation (Fig.
265 1a). In LUN, the average respiration rate increased from the bulk soil to the MOM and POM. The
266 respiration rates were higher than deducted from the MRTs of the fractions (seen Table 1). For example,
267 the respiration rate measured at 18 °C equalled $80 \mu\text{g CO}_2 \text{ g}^{-1} \text{ C hour}^{-1}$, corresponding to about 0.2 g C
268 $\text{g}^{-1} \text{ C per year}$.

269
270 The Gaussian equation was used to describe the temperature dependence of the respiration. When
271 fitted to measured respiration from MOM and POM isolated from ASK soil, the 95 % probability
272 distributions of the two parameter values of this equation overlapped only little (Fig. 2a). This indicates a
273 different temperature dependence as illustrated by the Q_{10} value in Figs. 2c, e-g. For example, in the
274 temperature range 5 to 15 °C, the most probable Q_{10} value of the POM equalled 4.8 and that of the MOM
275 3.6. Because of the Gaussian equation, the Q_{10} values and the temperature sensitivity of the MOM and
276 POM fractions from ASK soil differed for different temperatures. The effect of temperature on the
277 respiration did not differ between size fractions from LUN soil (Fig. 2b, d-g).

278
279 The $\delta^{13}\text{C}$ value of respired CO_2 was some 4 ‰ higher than that of C in the corresponding soil fraction
280 (Fig. 3, Table 1). This indicates that decomposers favoured the younger maize-derived C. Maize-derived
281 C represented 63 % (LUN) and 70 % (ASK) of the C respired from POM, 52 % (ASK) and 60 % (LUN) of
282 the C respired from bulk soil, and 39 % (ASK and LUN) of the C respired from MOM (Fig. 4). The $\delta^{13}\text{C}$
283 values did not vary significantly with incubation temperature, except for C respired from POM isolated
284 from the LUN soil (Fig. 3). Here the $\delta^{13}\text{C}$ decreased with an increasing temperature indicating that an
285 increasing proportion of the older C3-C was respired at higher temperatures.

286 **3.3. Microbial community composition measured by quantitative PCR**

287 The ITS, B16S and A16S copy numbers did not change systematically with incubation temperature. In
288 the ASK soil, the POM fraction had lower ITS, A16S and B16S copy numbers per mg soil C than bulk
289 soil and MOM, but these differences were not statistically significant at all temperatures (Fig. 5). In the

290 LUN soil, the MOM fraction had higher ITS and B16S gene copy numbers compared to bulk soil and
291 POM fraction (Fig. 5).

292 **4. Discussion**

293 We studied the temperature dependence of C in bulk soil and their particle size fractions from two
294 differently textured arable soils converted from C3-plants to C4-plants 25 years ago. The >63 μm fraction
295 with a MRT of decades is dominated by particulate organic matter (POM), whereas the <63 μm fraction
296 is dominated by OM intimately associated with soil minerals (MOM) and with a MRT of more than a
297 century. Our estimates of old C3-derived C and recent C4-derived C depend on $\delta^{13}\text{C}_{y=0}$, determined for
298 the soils before maize cultivation was initiated in 1988. Accounting for the annual increase in $\delta^{13}\text{C}_{y=0}$ of
299 0.0083‰ (Christensen et al., 2011) changed the percentages of C3-C and C4-C in soil particle size
300 fractions by only 1%. Menichetti et al. (2015) showed that in experiments lasting for more than three to
301 four decades, isotopic fractionation could lead to larger changes in soil $\delta^{13}\text{C}$.

302

303 Our study relied on air-dried and sieved soil samples and a soil fractionation procedure that eliminates
304 soil structure. The potential effect of the rewetting on the observed temperature sensitivity of the soil OM
305 was minimized by pre-incubating samples for two weeks. The pre-incubation showed a flush of CO_2 for
306 bulk soil as well as POM from the LUN soil (Fig. S1), suggesting that this flush of CO_2 was not only due
307 to fractionation procedure. Based on the decreasing respiration rates during the pre-incubation it was
308 estimated that the C released from bulk soil accounts for less than 30 % of C in the >63 μm fraction and
309 more than 70% of C in the <63 μm fraction. If the respiration rate of the >63 μm fraction did not change
310 due to fractionation, then the unexplained increase in the respiration rate of <63 μm fraction after
311 fractionation corresponds to 11 % and 42 % of the total respiration in ASK and LUN, respectively. Thus,
312 part of the respiration from POM as well as MOM may originate from C released during re-wetting and
313 fractionation. For both soils, however, old C-3 derived C made up a larger proportion of the C respired
314 from MOM than from POM. For POM, a larger part of the soil C and respired C originated from recent C-
315 4 derived C (Fig. 4). This suggests that we did manage to separate two soil fractions with OM of different
316 age, allowing us to compare the temperature sensitivity of soil C older and younger than 25 years.

317

318 We estimate that on average 0.9% of the soil C was lost during the incubation. With this incubation set-
319 up, it was also possible to observe if the isotopic signature changed to the same direction when moving
320 up and down of the temperature scale (e.g. for POM in LUN, the $\delta^{13}\text{C}$ value exhibits slopes of -0.046 and
321 -0.060 with increasing temperature when measured up and down the temperature scale). Therefore, we
322 do not consider changes in substrate quality during the incubation to have affected our results.

323 **4.1. Temperature sensitivity and age of C**

324 In the LUN soil, the relative contribution of C3-derived C to respiration from POM increased with
325 temperature (Fig. 3), reflecting a higher temperature sensitivity of the old C3-C than the recent maize-
326 derived C4-C. This is ascribed to a chemically more complex structure of the part of POM that has
327 survived for > 25 years. The surviving old POM demands higher activation energy and thus shows higher
328 temperature sensitivity.

329

330 The high temperature sensitivity of old C in the POM fraction supports our first hypothesis that C release
331 from this fraction follows Arrhenius kinetics. We hypothesised that mineralisation of C in this fraction
332 reflects the intrinsic temperature sensitivity of C in POM as this fraction is freely available to microbes.
333 However, this effect of age was less pronounced in ASK loamy sand, and the trend was not statistically
334 significant. This may be due to less complete soil dispersion in the more clayey ASK soil. Even though
335 the fractionation procedure aimed at breaking all soil aggregates, some microaggregates (63-250 μm) in
336 the ASK soil may have survived dispersion. This may explain why the ASK size fractions and bulk soil
337 had rather similar respiration rates. For the coarser LUN soil with a more complete soil dispersion, POM
338 showed higher respiration rate. There was slightly more old C3-C respired from the POM fraction of the
339 LUN soil compared with that of the ASK soil, even though the proportions of C3-C and C4-C in POM
340 were almost identical for both soils. This supports the previous notion that physical protection of C within
341 microaggregates that survived dispersion may have affected POM availability in the ASK soil.

342

343 There was no change with temperature in the isotopic signal of CO_2 from the MOM fraction, suggesting
344 that mineral-associated recent C4-C and old C3-C in MOM exhibit similar temperature sensitivity. This
345 supports our second hypothesis stating that temperature sensitivity of MOM is less affected by age than
346 that of POM. Our results indicate that the most temperature sensitive OM pool in soil is the free, but
347 chemically recalcitrant C with intermediate turnover time, aligning with recent studies, which have also

348 found that C fractions with decadal-scale turnover times show the highest temperature sensitivity
349 (Gillabel et al., 2010; Hopkins et al., 2012).

350 **4.2. Temperature sensitivity of soil C fractions**

351 Our third hypothesis stated that organo-mineral (MOM) association of C reduces its intrinsic temperature
352 sensitivity compared to C in POM. This hypothesis was only partially supported by the lower temperature
353 sensitivity of MOM and higher sensitivity of POM in ASK soil (Fig. 2a) as illustrated by different Q_{10}
354 values, especially in the temperature range of 5–15 °C (Fig. 2e). This is in line with previous studies
355 showing that stabilization of C in organo-mineral complexes reduces its temperature sensitivity (Gillabel
356 et al., 2010; Moinet et al., 2018). However, differences in Q_{10} s of the incubated size fractions were less
357 clear at higher temperatures and not statistically significant for LUN soil. We speculate that the stabilising
358 effect of organo-mineral interaction was stronger in MOM from the finer textured ASK soil, whereby
359 stabilisation with minerals reduced the Q_{10} more compared to the Q_{10} of C in POM, which was similar for
360 both soils.

361

362 Studies using incubations of physically or chemically isolated fractions to study their temperature
363 sensitivity are scarce and results are partly contradictory. Plante et al. (2010) found the Q_{10} to increase in
364 the order: hydrolysis residue<POM<whole soil. In their study, POM was fractionated with wet sieving
365 size-separation method comparable to our study, whereas Q_{10} was determined from long-term (126-
366 days) parallel incubations. This could have decreased the Q_{10} of labile POM fraction more relative to bulk
367 soil Q_{10} values. Contrary to Plante et al. (2010), in our study there were no clear differences between the
368 Q_{10} values of POM and bulk soil. Benbi et al. (2014) found higher temperature sensitivity for POM
369 compared to MOM, which aligns with our results from the ASK soil. Their study relied on a size-
370 fractionation procedure similar to that of our study, but divided POM into two size classes (53-250 µm
371 and 250-2000 µm). Ding et al. (2014) reached opposite results, and found that the Q_{10} for the different
372 size-fractions increased in the order: sand<silt<clay. In their study, however, soil dispersion by
373 ultrasonication and the crushing of the size-fractions after drying prior to incubation could have increased
374 the availability of mineral associated OM. Flocculation of clay with CaCl_2 could have also affected C
375 availability from that fraction. Leifeld and Fuhrer (2005) found little difference between size fractions (<63
376 µm and >63 µm) whereas C in residues from acid hydrolysis of finer sized soil fractions showed a high
377 sensitivity to temperature. The residue obtained after acid hydrolysis is considered less suitable for

378 studying stable C in incubations studies. The isolated soil OM fractions are impacted by chemical
379 changes that may make mineral associated C either more available (Leifeld and Fuhrer, 2005) or less
380 available due to cation bridging with Ca^{2+} added to neutralise pH after hydrolysis (Plante et al., 2010).
381 Our fractionation procedure avoided these pitfalls. However, laboratory incubations with optimal
382 conditions for microbial decomposition always provide shorter turnover time estimates than methods
383 based on changes in the abundance of ^{13}C after C3- to C4-vegetation change (Feng et al., 2016). This
384 was also true in our study. Although decomposition rates of MOM in incubation studies may not refer
385 directly to field conditions, they are still valid when comparing the temperature sensitivity of older versus
386 younger C in a given fraction.

387 4.3. Microbial balance

388 The composition of the microbial community may affect mineralization rates and the isotopic composition
389 of respired CO_2 (Paterson et al., 2009). We did not directly analyse the microbial community, but
390 estimated the balance of the community targeting the three domains fungi, bacteria and archaea of the
391 microbial community by qPCR. No significant changes in the balance were observed with temperature
392 although the relative abundance of archaeal 16S rRNA copy numbers tended to increase with
393 temperature. Archaea are the least abundant microbial group in soil (Tamez-Hidalgo et al., 2016), and
394 their functional importance is less understood than that of bacteria and fungi. The small changes in this
395 microbial group are unlikely to explain observed changes in CO_2 production or isotope signatures of
396 respired CO_2 . The rather similar balance between the three domains in soil-size fractions and bulk soils
397 suggests that trends in isotopic composition of respired CO_2 were not derived from differences in the
398 composition of the microbial community. The qPCR analysis showed higher gene copy numbers in the
399 MOM than in the POM fraction isolated from LUN soil. Most of the decomposer community live attached
400 to the surfaces of clay and silt particles (Mills, 2003), whereby these particles may become enriched in
401 gene copies. Because qPCR records genes associated with live as well as dead microbial biomass, a
402 higher gene copy number in the MOM fraction may indicate a larger contribution of microbial necromass
403 to the stabilised C in this fraction (Liang et al., 2017).

404 5. Conclusions

405 The temperature sensitivity of C in the $<63\ \mu\text{m}$ particle size fraction (MOM), dominated by mineral
406 associated C, was smaller or similar to the sensitivity of C in the $>63\ \mu\text{m}$ size fraction (POM). The

407 temperature sensitivity of respiration increased with age of C in the POM fraction while the sensitivity
408 was unaffected by age of C in the MOM fraction where OM is stabilised by association with minerals. No
409 evidence was found for highly temperature sensitive C being a significant component of the MOM
410 fraction. Our results imply similar temperature sensitivity of older and younger C, when residing in
411 organo-mineral associations. This aligns with recent evidence suggesting that the most temperature
412 sensitive C is in soil OM pools cycling on intermediate time scales. Our results suggest that the
413 temperature sensitivity of most of the C residing in arable topsoils relates to the mechanisms that
414 stabilize C in soil rather than to the age of the OM stabilized in organo-mineral complexes.

415

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422

423 **Literature**

424 Amann, R.I., Krumholz, L., Stahl, D.A., 1990. Fluorescent-oligonucleotide probing of whole cells for
425 determinative, phylogenetic, and environmental-studies in microbiology. *Journal of Bacteriology* 172,
426 762-770.

427 Amelung, W., Brodowski, S., Sandhage-Hofmann, A., Bol, R., 2008. Combining biomarker with stable
428 isotope analyses for assessing the transformation and turnover of soil organic matter. *Advances in*
429 *Agronomy* 100, 155-250.

430 Balesdent, J., Mariotti, A., 1996. Measurement of soil organic matter turnover using ^{13}C natural
431 abundances. In: Boutton, T.W., Yamasaki, S.I. (Eds.), *Mass Spectrometry of Soils*. Marcel Dekker Inc.,
432 New York, pp. 83–111.

433 Biasi, C., Rusalimova, O., Meyer, H., Kaiser, C, Wanek, W., Barsukov, P, Junger, H., Richer, A. 2005.
 434 Temperature-dependent shift from labile to recalcitrant carbon sources of arctic heterotrophs. *Rapid*
 435 *Communications in Mass Spectrometry* 19:1401-1408.
 436 Benbi, D.K., Boparai, A.K., Brar, K., 2014. Decomposition of particulate organic matter is more sensitive
 437 to temperature than the mineral associated organic matter. *Soil Biology & Biochemistry* 70, 183-192.
 438 Bosatta, E., Agren, G.I., 1999. Soil organic matter quality interpreted thermodynamically. *Soil Biology &*
 439 *Biochemistry* 31, 1889-1891.
 440 Christensen, B.T., 2001. Physical fractionation of soil and structural and functional complexity in organic
 441 matter turnover. *European Journal of Soil Science* 52, 345-353.
 442 Christensen, B.T., Olesen, J.E., Hansen, E.M., Thomsen, I.K., 2011. Annual variation in $\delta(13)C$
 443 values of maize and wheat: Effect on estimates of decadal scale soil carbon turnover. *Soil Biology &*
 444 *Biochemistry* 43, 1961-1967.
 445 Conant, R.T., Drijber, R.A., Haddix, M.L., Parton, W.J., Paul, E.A., Plante, A.F., Six, J., Steinweg, J.M.,
 446 2008. Sensitivity of organic matter decomposition to warming varies with its quality. *Global Change*
 447 *Biology* 14, 868-877.
 448 Davidson, E.A., Janssens, I.A., Luo, Y.Q., 2006. On the variability of respiration in terrestrial
 449 ecosystems: moving beyond $Q(10)$. *Global Change Biology* 12, 154-164.
 450 De Nobili, M., Contin, M., Brookes, P.C., 2006. Microbial biomass dynamics in recently air-dried and
 451 rewetted soils compared to others stored air-dry for up to 103 years. *Soil Biology & Biochemistry* 38,
 452 2871-2881.
 453 Ding, F., Huang, Y., Sun, W.J., Jiang, G.F., Chen, Y., 2014. Decomposition of Organic Carbon in Fine
 454 Soil Particles Is Likely More Sensitive to Warming than in Coarse Particles: An Incubation Study with
 455 Temperate Grassland and Forest Soils in Northern China. *Plos One* 9, e95348.
 456 Feng, W., Shi, Z., Jiang, J., Xia, J., Liang, J., Zhou, J., Luo, Y., 2016. Methodological uncertainty in
 457 estimating carbon turnover times of soil fractions. *Soil Biology & Biochemistry* 100, 118-124.
 458 Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes - application to
 459 the identification of mycorrhizae and rusts. *Molecular Ecology* 2, 113-118.
 460 Gelman, A., Carlin, J.B., Stern, H.S., Dunson, D.B., Vehtari, A., Rubin, D.B., 2013. *Bayesian Data*
 461 *Analysis*, Third Edition. Taylor & Francis. Boca Raton.

462 Gillabel, J., Cebrian-Lopez, B., Six, J., Merckx, R., 2010. Experimental evidence for the attenuating
 463 effect of SOM protection on temperature sensitivity of SOM decomposition. *Global Change Biology* 16,
 464 2789-2798.

465 Hamdi, S., Moyano, F., Sall, S., Bernoux, M., Chevallier, T., 2013. Synthesis analysis of the temperature
 466 sensitivity of soil respiration from laboratory studies in relation to incubation methods and soil conditions.
 467 *Soil Biology & Biochemistry* 58, 115-126.

468 Hopkins, F.M., Torn, M.S., Trumbore, S.E., 2012. Warming accelerates decomposition of decades-old
 469 carbon in forest soils. *Proceedings of the National Academy of Sciences of the United States of America*
 470 109, E1753-E1761.

471 Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: self-
 472 assembly of organic molecular fragments into zonal structures on mineral surfaces. *Biogeochemistry* 85,
 473 9-24.

474 Kleber, M., Nico, P.S., Plante, A., Filley, T., Kramer, M., Swanston, C., Sollins, P., 2011. Old and stable
 475 soil organic matter is not necessarily chemically recalcitrant: implications for modelling concepts and
 476 temperature sensitivity. *Global Change Biology* 17, 1097–1107,

477 Kristiansen, S.M., Hansen, E.M., Jensen, L.S., Christensen, B.T., 2005. Natural C-13 abundance and
 478 carbon storage in Danish soils under continuous silage maize. *European Journal of Agronomy* 22, 107-
 479 117.

480 Lee, S.H., Malone, C., Kemp, P.F., 1993. Use of multiple 16S ribosomal-RNA-targeted fluorescent-
 481 probes to increase signal strength and measure cellular RNA from natural planktonic bacteria. *Marine*
 482 *Ecology Progress Series* 101, 193-201.

483 Leifeld, J., 2003. Comments on "Recalcitrant soil organic materials mineralize more efficiently at higher
 484 temperatures" by R. Bol, T. Bolger, R. Cully, and D. Little; *J. Plant Nutr. Soil Sci* 166, 300-307 (2003).
 485 *Journal of Plant Nutrition and Soil Science* 166, 777-778.

486 Leifeld, J., Fuhrer, J., 2005. The temperature response of CO₂ production from bulk soils and soil
 487 fractions is related to soil organic matter quality. *Biogeochemistry* 75, 433-453.

488 Liang, C., Schimel, J.P., Jastrow, J.D. 2017. The importance of anabolism in microbial carbon over soil
 489 carbon storage. *Nature Microbiology* 2, 17105.

490 Melillo, J.M., Aber, J.D., Muratore, J.F., 1982. Nitrogen and lignin control of hardwood leaf litter
 491 decomposition dynamics. *Ecology* 63, 621-626.

492 Menichetti, L., Houot, S., van Oort, F., Katterer, T., Christensen, B.T., Chenu, C., Barre, P., Vasilyeva,
 493 N.A., Ekblad, A., 2015. Increase in soil stable carbon isotope ratio relates to loss of organic carbon:
 494 results from five long-term bare fallow experiments. *Oecologia* 177, 811-821.
 495 Mills, A.L., 2003. Keeping in touch: microbial life on soil particle surfaces. *Advances in Agronomy* 78, 1-
 496 43.
 497 Moinet, G.Y.K., Hunt, J.E., Kirschbaum, M.U.F., Morcom, C.P., Midwood, A.J., Millard, P., 2018. The
 498 temperature sensitivity of soil organic matter decomposition is constrained by microbial access to
 499 substrates. *Soil Biology & Biochemistry* 116, 333-339.
 500 O'Leary, M.H., 1988. Carbon isotopes in photosynthesis. *Bioscience* 38, 328-336.
 501 Olsen, G.J., Lane, D.J., Giovannoni, S.J., Pace, N.R., Stahl, D.A., 1986. Microbial ecology and evolution
 502 - a ribosomal-RNA approach. *Annual Review of Microbiology* 40, 337-365.
 503 Paterson, E., Midwood, A.J., Millard, P., 2009. Through the eye of the needle: a review of isotope
 504 approaches to quantify microbial processes mediating soil carbon balance. *New Phytologist* 184, 19-33.
 505 Peltoniemi, K., Laiho, R., Juottonen, H., Kiikkilä, O., Makiranta, P., Minkkinen, K., Pennanen, T., Penttilä,
 506 T., Sarjala, T., Tuittila, E.-S., Tuomivirta, T., Fritze, H., 2015. Microbial ecology in a future climate: effects
 507 of temperature and moisture on microbial communities of two boreal fens. *FEMS Microbiology Ecology*
 508 91, fiv062, <https://doi.org/10.1093/femsec/fiv062>.
 509 Plante, A.F., Conant, R.T., Carlson, J., Greenwood, R., Shulman, J.M., Haddix, M.L., Paul, E.A., 2010.
 510 Decomposition temperature sensitivity of isolated soil organic matter fractions. *Soil Biology &*
 511 *Biochemistry* 42, 1991-1996.
 512 Poeplau, C., Kätterer, T., Leblans, N.I.W., Sigurdsson, B., 2017. Sensitivity of soil carbon fractions and
 513 their specific stabilization mechanisms to extreme soil warming in a subarctic grassland. *Global Change*
 514 *Biology* 23, 1316-1327.
 515 Reysenbach, A.-L., Pace, N.R., 1995. Reliable amplification of hyperthermophilic Archaeal 16S rRNA
 516 genes by the polymerase chain reaction, In: Robb, F.T., Place, A.R., Sowers, K.R., Schreier, A.R.,
 517 DasSarna, S., Fleischman, E.M. (Eds.), *Archaea: A Laboratory Manual*. Cold Spring Harbor Laboratory
 518 Press, New York, USA, pp. 101-105.
 519 Riley-Buckley, S.M., 2001. Microbial communities in pristine and tetrachlorine-contaminated aquifer
 520 sediments. Ph.D. Thesis. Michigan State University, Michigan, USA.

521 Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M.,
 522 Kögel-Knabner, I., Lehmann, J., Manning, D.A.C, Nannipieri, P, Rasse, D.P., Weiner, S., Trumbore,
 523 S.E., 2011. Persistence of soil organic matter as an ecosystem property. *Nature* 478, 50-56.
 524 Stahl, D.A., Flesher, B., Mansfield, H.R., Montgomery, L., 1988. Use of phylogenetically based
 525 hybridization probes for studies of ruminal microbial ecology. *Applied and Environmental Microbiology*
 526 54, 1079-1084.
 527 Tamez-Hidalgo, P., Christensen, B.T., Lever, M.A., Elsgaard, L., Lomstein, B.Aa., 2016. Endospores,
 528 prokaryotes, and microbial indicators in arable soils from three long-term experiments. *Biology and*
 529 *Fertility of Soils* 52, 101-112.
 530 Thomsen, I.K., Christensen, B.T., 2010. Carbon sequestration in soils with annual inputs of maize
 531 biomass and maize-derived animal manure: Evidence from ¹³C abundance. *Soil Biology & Biochemistry*
 532 42, 1643-1646.
 533 Trumbore, S., 2000. Age of soil organic matter and soil respiration: Radiocarbon constraints on
 534 belowground C dynamics. *Ecological Applications* 10, 399-411.
 535 Tuomi, M., Vanhala, P., Karhu, K., Fritze, H., Liski, J., 2008. Heterotrophic soil respiration - Comparison
 536 of different models describing its temperature dependence. *Ecological Modelling* 211, 182-190.
 537 Tuomi, M., Thum, T., Järvinen, H., Fronzek, S., Berg, B., Harmon, M., Trofymow, J.A., Sevanto, S. &
 538 Liski, J. 2009. Leaf litter decomposition - Estimates of global variability based on Yasso07 model.
 539 *Ecological Modelling* 220:3362-3371. [doi:10.1016/j.ecolmodel.2009.05.016](https://doi.org/10.1016/j.ecolmodel.2009.05.016)
 540 White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Analysis of phylogenetic relationships by amplification
 541 and direct sequencing of ribosomal RNA genes, In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J.
 542 (Eds.), *PCR Protocols: a Guide to Methods and Applications* Academic Press, New York, USA, pp. 315–
 543 322.

544 **Figure captions**

545 Figure 1. Measured respiration rates at different temperatures and the modelled Gaussian temperature
 546 curve for soil particle size fractions and bulk soil: 1 a) ASK and 1 b) LUN.

547

548 Figure 2. 95% probability contours of parameter values *a* and *b* in the Gaussian model for the bulk soil
 549 and particle size fractions in ASK (a) and in LUN (b), *Q*₁₀ as a function of temperature in ASK (c) and in

550 LUN (d). Dashed lines represents 95% credible intervals (Bayesian confidence intervals). Posterior
551 distributions of Q_{10} calculated for temperature range of 5-15 °C (e), 15-25 °C (f) and 25-35 °C (g).

552

553 Figure 3. Mean $\pm$ standard deviation (N=6) of isotopic composition of CO₂ respired from the bulk soils
554 and soil size fractions at different temperatures in ASK (a) and in LUN (b). Lines represent linear
555 regressions fitted to the means: a) ASK: MOM ($y = 0.03x - 22.44$, $R^2=0.70$, $p=0.16$), POM ($y = -0.03x -$
556 16.59 , $R^2=0.63$, $p=0.21$), Bulk soil ($y = 0.01x - 19.94$, $R^2=0.08$, $p=0.72$) b) LUN: MOM ($y = -0.00x - 21.24$,
557 $R^2=0.01$, $p=0.89$), POM ($y = -0.05x - 16.80$, $R^2=0.92$, $p=0.04$), bulk soil ($y = -0.05x - 17.49$, $R^2=0.68$,
558 $p=0.18$). The trend in POM fraction ($>63 \mu\text{m}$) in LUN is statistically significant.

559

560 Figure 4. The proportions of C4-C and C3-C in soil and in respired CO₂ calculated from their isotopic
561 composition in ASK (a) and LUN (b). The $\delta^{13}\text{C}$ values for CO₂ are averages four replicates and 4
562 temperatures measured twice.

563

564 Figure 5. Mean $\pm$ standard deviation of the gene copy numbers/ mg soil C for ITS gene in a) ASK and b)
565 LUN, for B16S in c) ASK and d) LUN, and A16S in e) ASK and f) LUN. The Anova and Tukey's test for
566 differences in gene copy numbers between different SOC fractions (bulk, MOM $<63 \mu\text{m}$ and POM <63
567 μm) were tested separately in each temperature. Different letters indicate statistically significant
568 differences between fractions. The figure presents non-transformed data, but the y-axis in the figure is in
569 log-scale. For the statistical analysis log-transformed data was used.

570

571 **Tables**

572 Table 1. Mean $\pm$ standard deviation of C contents, mass proportions and isotopic composition of ASK
573 and LUN bulk soils, POM and MOM fractions in 1988 and 2013 as well as the mean residence time
574 (MRT) of C. In 2013, N=2 for $\delta^{13}\text{C}$ except for the POM fraction where N=8.

	N	1988 (t=0)			2013 (t=25)			MRT years
		C (%)	Mass (%)	$\delta^{13}\text{C}$ (‰)	C (%)	Mass (%)	$\delta^{13}\text{C}$ (‰)	
		2	1	4	2	4	2/8	
ASK								
Bulk soil		2.5 $\pm$ 0.1	100	-27.9 $\pm$ 0.2	2.4 $\pm$ 0.1	100	-24.5 $\pm$ 0	100 $\pm$ 4
MOM		5.4 $\pm$ 0.3	33	-28.2 $\pm$ 0.1	5.7 $\pm$ 0.3	32 $\pm$ 3.9	-26.1 $\pm$ 0.1	170 $\pm$ 8
POM		1.3 $\pm$ 0.3	67	-27.4 $\pm$ 0.4	1.2 $\pm$ 0.1	68 $\pm$ 3.9	-22.1 $\pm$ 0.8	54 $\pm$ 9
LUN								
Bulk soil		1 $\pm$ 0.1	100	-26.9 $\pm$ 0	0.9 $\pm$ 0	100	-22.6 $\pm$ 0.3	71 $\pm$ 7
MOM		4.8 $\pm$ 0.1	15	-26.7 $\pm$ 0.2	4.9 $\pm$ 0.1	12 $\pm$ 1.7	-24.8 $\pm$ 0.2	170 $\pm$ 15
POM		0.3 $\pm$ 0.2	85	-26.7 $\pm$ 0.5	0.5 $\pm$ 0.2	88 $\pm$ 1.7	-21.4 $\pm$ 1	51 $\pm$ 12

575

Figure2

Figure3

Figure4

Figure5

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