

Temperature sensitivity of decomposition: Discrepancy between field and laboratory estimates is not due to sieving the soil

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ABSTRACT

Is persistent soil organic matter (SOM), characterised by an old age and long-turnover time, more or less sensitive to changes in temperature than fast-cycling, recent SOM? Largely due to our limited understanding of the mechanisms of SOM formation, this question remains controversial. Laboratory incubation studies, through sieving the soil, may create conditions in which substrate accessibility is modified. The recent recognition of SOM accessibility as a defining factor of SOM persistency calls into question conclusions from these studies. Previously, in a study using root exclusion plots of increasing age, we showed in the field that the temperature sensitivity of SOM decomposition decreased with increasing persistence of SOM (Moinet et al., 2020), in opposition to many laboratory incubation studies. Here we sampled soils from the same root exclusion plots and conducted a laboratory incubation experiment to test the hypotheses that (i) the relationship between temperature sensitivity and SOM persistence is inverted as compared to the field, and (ii) the discrepancy is due to sieving the soil. We showed that, in the laboratory, the relationship was indeed inverted, with the temperature sensitivity being higher for the old root exclusion plots. However, sieving the soil at 2 mm did not affect estimates of the temperature sensitivity of SOM decomposition, suggesting that discrepancies between field and laboratory estimates are unlikely to stem from artificially modified substrate accessibility due to sieving.

The sensitivity of soil organic matter (SOM) decomposition to changes in temperature is a highly debated topic (Sulman et al., 2018). A major point of discussion concerns the relationship between SOM persistency and the relative temperature sensitivity of SOM decomposition (the change in decomposition rate as a proportion per unit change in temperature, of which Q_{10} is a commonly used indicator). Are persistent forms of SOM, characterised by an old age and slow turnover, more or less sensitive to changes in temperature than fast-cycling, recent SOM? The question has extensively been studied, but the literature collectively fails to provide a consensus (Conant et al., 2011). This can partly be attributed to our limited understanding of the complex set of mechanisms regulating the formation of SOM. The inherent chemical recalcitrance of organic molecules has historically been used to justify that SOM can resist microbial decomposition for centuries (Kleber and Johnson, 2010). However, much literature now advocates for uncoupling SOM persistency from its inherent chemical properties and proposing instead, or in addition, that properties of the soil matrix protect SOM, thereby reducing its accessibility to microbial decomposers (Schmidt et al., 2011; Dungait et al., 2012; Lehmann and Kleber, 2015).

Studies of the decomposition of substrates with different chemical structures have collectively revealed that recalcitrant substrates have higher temperature sensitivities than chemically labile substrates (Davidson and Janssens, 2006). These observations line up with soil laboratory incubation studies, which most often show positive correlations between temperature sensitivity and indicators of SOM persistence (Conant et al., 2011), and have led to the hypothesis that persistent SOM is more temperature sensitive than recent SOM because it is more chemically recalcitrant to decomposition, in line with enzymatic kinetic theory (Davidson and Janssens, 2006). Recently, we used root exclusion plots of varying age to show that the temperature sensitivity of SOM decomposition decreased with increasing SOM age (Moinet et al., 2020). This result from the field directly opposes kinetic theory and most laboratory incubation studies (Conant et al., 2011), suggesting that factors other than chemical recalcitrance must control the age and stability of SOM. Physically disrupting the soil in the laboratory can release substrates from protective mechanisms, therefore modifying both substrate accessibility to microbes and respiration rates (Curtin et al., 2014; Zakharova et al., 2014). Moreover, reducing substrate accessibility to microbes has been shown to constrain the temperature

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sensitivity of SOM decomposition (Gillabel et al., 2010; Moinet et al., 2018). In our previous study, we therefore speculated that our results departed from kinetic theory due to the rate limiting step for decomposition in the field being the release of substrates from physicochemical protection, and not the enzymatic degradation of the substrates themselves (Moinet et al., 2020). Most laboratory incubations would physically speed up this rate limiting step due to sieving, leading to conditions where substrate chemistry controls persistence and the temperature sensitivity of decomposition.

In this study, we sampled soils from the new (1 month) and old (30 years) root exclusion plots used in Moinet et al. (2020) for a laboratory incubation experiment. The soils are described excessively drained silty stony soils and classified as Dystric Gleysol according to WRB classification (Moinet et al., 2020). We tested the hypothesis that, due to its effect on substrate accessibility, sieving the soil with a 2 mm mesh size would yield contrasting results to the field and show increasing temperature sensitivity with increasing persistence (increasing root exclusion plot age). We sampled eight intact soil cores (100 mm diameter and 200 mm depth) from each experimental plot (new and old) and placed them in PVC cylinders. The base of each cylinder was sealed with duct tape. Half of the cylinders (4 of each treatment) were emptied in a tray, passed through a 2 mm mesh size sieve and repacked in the same cylinder to the same volume to maintain a constant bulk density (both the material retained and passed through the sieve was repacked). The 16 soil cores were then brought back to the laboratory, weighed, and placed in a controlled environment chamber (Model HGC 1514, Weiss Gallenkamp, UK) in the dark at 20 °C and 80% humidity for 8 days. The water content of the soils was readjusted to the field value based on the mass lost after 7 days (which was less than 5% of the core's mass). After these 8 days equilibration period, the ambient temperature in the growth cabinet temperature was cycled through 5 °C step changes from 20 °C to 35 °C, then down to 5 °C and finally back up to 20 °C. Preliminary tests showed that the soil temperature in the soil cores was evenly distributed throughout the core and equal to ambient temperature after 10 h. Each temperature step was, therefore, maintained for 12 h. Measurements of soil CO₂ effluxes (R) were made on each soil core between 11 and 12 h after each temperature change using the same respiration chamber system (LI-8100, LI-COR Inc., Lincoln, NE, USA) as in the previous study (Moinet et al., 2020). There were no significant differences between R measured at 20 °C at the beginning, the middle and the end of the temperature cycle (ANOVA, $F_{1,34} = 2.79$, $p = 0.1$).

It was notable that standard errors of the means for R appeared lower for the sieved than for the non-sieved soil cores, suggesting lower variability in the sieved soil cores. However, mean R was nearly identical at each incubation temperature for sieved and non-sieved cores (Fig. 1). To test for the effect of the sieving on the temperature sensitivity of soil CO₂ efflux (R), the model from Lloyd and Taylor (1994) (equation (1)) was fitted on R for each root exclusion treatment separately. We used non-linear mixed-effect models (in the 'nlme' package (Pinheiro et al., 2018) using R version 3.4.2 (R Development Core Team, 2017)), including spatial replicates (core number) as a random effect and including a first order autocorrelation function to account for the non-independence of measurements made on the same soil core over time.

$$R = R_{10} e^{E_0 \left(\frac{1}{56.02} - \frac{1}{T_s - 227.1} \right)} \quad (1)$$

where R_{10} is a basal respiration rate at 10 °C, and E_0 is related to the relative temperature sensitivity. Neither parameter E_0 and R_{10} showed significant differences for sieved and non-sieved cores ($p > 0.5$ in all cases).

As a result, Q_{10} , calculated following Kirschbaum (2000) (equation (2)), was identical for sieved and non-sieved cores, showing the absence of an effect of sieving on the temperature sensitivity of SOM decomposition.

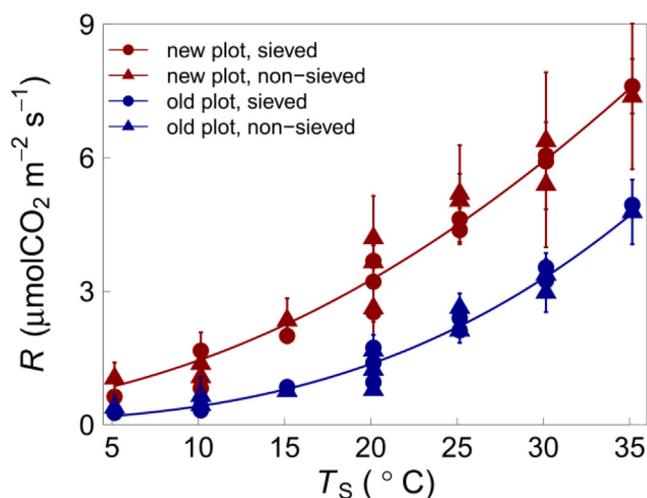


Fig. 1. Soil CO₂ efflux (R) for the sieved and non-sieved cores from new and old root exclusion treatments. The lines are fitted using estimated parameters with equation (1) with both parameters R_{10} (the basal respiration at 10 °C) and E_0 (related to the relative temperature sensitivity) varying for the root exclusion treatments. Vertical bars represent one standard error of the mean ($n = 4$).

$$Q_{10} = e^{10 \times E_0 / (T_s - 227.1)^2} \quad (2)$$

For testing the effect of the root exclusion treatments on the temperature sensitivity of SOM decomposition, values of R from sieved and non-sieved cores were used without distinction. Parameter R_{10} was significantly lower ($F_{1,129} = 96.49$, $p < 0.0001$) for the old ($0.4 \pm 0.1 \mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$) than for the new ($1.5 \pm 0.1 \mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$) root exclusion treatment. Contrastingly, parameter E_0 was significantly higher ($F_{1,129} = 22.09$, $p < 0.0001$) for the old ($159 \pm 30 \text{ K}$) than new ($299 \pm 17 \text{ K}$) root exclusion treatment. As a result, Q_{10} was higher for the old than for the new root exclusion treatment and was consistently greater than 2 for both treatments (Fig. 2). The results only partly supported our hypothesis: laboratory incubations did indeed yield contrasting results to those obtained in the field (Fig. 2), but this contradiction could not be attributed to an effect of sieving the soil.

There is evidence that modifying substrate accessibility to microbial decomposers results in modified temperature sensitivity of decomposition (Gillabel et al., 2010; Moinet et al., 2018). There is also evidence that disturbance of the soil, such as sieving, may result in modified substrate availability (Curtin et al., 2014; Zakharova et al., 2014). However, Meyer et al. (2019) found no effect of sieving on the Q_{10} of soil respiration and Curtin et al. (2014) suggested that large compressive forces were necessary to modify the soil respiration rates, and that sieving was insufficient to modify those rates. Although Curtin et al. (2014) used a 4 mm sieve to reach these conclusions, their result would support our observations that R remained unchanged as a result of sieving, suggesting that the sieving treatment did not modify substrate accessibility to decomposers. Moreover, every soil core was carefully extracted to minimize disturbance within. We assume that substrate accessibility in the soil cores, both sieved and non-sieved, remained unmodified as compared to that in the field. Some alternative mechanism, therefore, must explain the discrepancy between the field and the laboratory results.

Another notable difference characterizing the field and laboratory approaches are the soil layers considered in the analysis and interpretation. Indeed, only the top 200 mm of the soil was sampled for laboratory incubations, while soil CO₂ collected in the field arose from SOM decomposition occurring through the whole soil profile. Root distribution of grass species has long been known to decrease with depth, and typically concentrate in the top 150–200 mm for ryegrass (McNally et al., 2015), the dominating species (*Lolium perenne* L.) at our

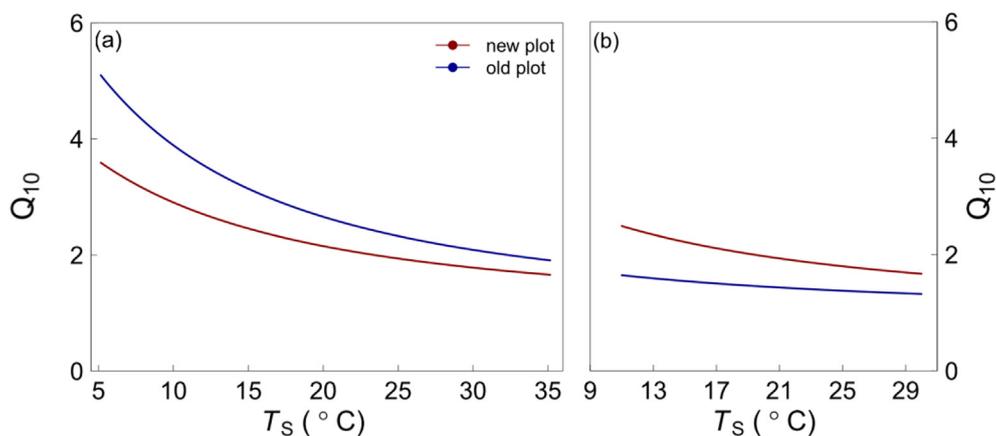


Fig. 2. Relative temperature sensitivity (Q_{10}) in new and old root exclusion plots (a) calculated from the laboratory measurements of soil CO_2 efflux from sieved and non-sieved cores without distinction and (b) from field data published in from Moinet et al. (2020) (redrawn to fit the same y axis).

experimental site. Because of the presence of dead root litter, the contribution of the top 200 mm of soil to the total CO_2 efflux in the field was likely greater in the new compared to the old root exclusion plots. Consequently, more of the CO_2 efflux in the old plots came from deeper soil layers where temperature likely did not vary as much than at 10 cm depth (where the temperature measurements were made). Therefore, in the field, part of the measured CO_2 efflux was likely unaffected by the measured temperature and this part was proportionally greater for old than new plots. This was not the case in the laboratory where the measured temperature was representative of the whole 200 mm of soil.

Radiocarbon dating has shown that the age of SOM increases with depth (Rumpel and Kögel-Knabner, 2011). If older SOM (in the subsoil and in the old root exclusion plots) had higher temperature sensitivity than younger SOM (in the topsoil and in the new root exclusion plots), as suggested by the present study, we would have expected that underestimating the contribution of subsoil to Q_{10} estimates in the field lead to smaller differences between new and old plots as compared to laboratory estimates, but not an inverted relationship. For such a depth effect to explain the discrepancy between laboratory and field results, topsoil and subsoil would have to display contrasting relationship between SOM persistence and temperature sensitivity. Gillabel et al. (2010) showed diverging persistence-temperature sensitivity relationships between topsoil and subsoil, partly supporting this speculative explanation. They used time-induced substrate depletion to show that SOM decomposition in subsoil samples (95–105 cm depth) was nearly insensitive to temperature (Q_{10} close to 1) irrespective of the substrates age (from 0 to 6 months), while the Q_{10} for topsoil (5–15 cm depth) increased from approximately 2 to 4 with increasing substrate depletion (e.g. increasing age of the remaining substrate).

Part of the controversy around the relationship between temperature sensitivity and SOM persistence has previously been attributed to laboratory incubations providing conditions supporting wrongly recalcitrance as a mechanism explaining SOM stability (Kleber et al., 2011). Indeed, there is a general trend for laboratory incubation studies and field or cross-site studies to suggest contrasting conclusions. Our study showed that sieving at 2 mm did not affect estimates of the temperature sensitivity and cannot account for those discrepancies. This short study provides one answer but opens many questions. We speculate that the mechanisms regulating SOM persistence may change with depth, suggesting that many laboratory studies, by focusing on topsoil, and many field studies, by disregarding this potential effect in interpreting the data, have to date provided incomplete interpretations of their data.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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