Dear Authors,

Both reviewers have kindly agreed to review your revised manuscript. They both acknowledge the improvements made and appreciate the effort you have invested. However, one of the reviewers remains critical of how you have used and interpreted the data on the proportion of EOM mineralization, particularly regarding the use of a non-linear model to fit this data. The reviewer presents a compelling argument in their review. In light of this, I have decided to request a major revision of this section of the manuscript, including reconsideration of the model used in the current Figure 2B and corresponding changes to the discussion section. Please also address the other comments provided by the reviewers throughout the manuscript and implement the requested changes. I will reconsider your manuscript after reviewing the revised version.

Looking forward reading a new version of your manuscript,

Kind regards.

We appreciate the editor's feedback for allowing us to revise our manuscript. In this rebuttal we provide a point-by-point response to the reviewers and refer to the line numbers of the document with visible changes. We understand the second reviewer's concern regarding the consideration of the origin in cumulative emissions, as no cumulative EOM emissions are expected when no EOM is added. The reviewer's comments on the lack of response of relative EOM mineralization to EOM dose have been addressed, and we have now applied a linear fit through the origin in the updated figure of relative EOM mineralization. The interpretation has been adjusted throughout the manuscript accordingly. We acknowledge that no evidence could be found for a lowered relative EOM mineralization at high dosage in the studied silt loam soil. Additionally, all other points raised by the reviewer have been addressed, as explained in our detailed responses below.

We hope that the changes made are satisfactory and meet the criteria for publication.

Best regards,

The authors

Reviewer #1:

The authors perfomed an extensive revision of the manuscript. The major comments have been adequately addressed, which made the manuscript better in my opinion and therefore I recommend the manuscript for publication.

I acknowledge that the authors have now included and discussed the overall C balance in the soils and in particular the effects on the net C balance.

The novelty of the study is now also better explained and the difference to the previous study is made clear.

Many aspects with respect to the SOM quality and soil structure are still not discussed. However, I understand that the data and experimental design do not really support an elaborate discussion on this.

Recommendations for the management of "real soils" are now made with more caution (comment on previous L.395). I still think that short-term lab incubation studies should not lead to management advice for real life, especially when they did not test at all the effect of repeated doses at all, but the effect of a one-time application of different doses. However, with the cautionary statement presented, I think this is fine.

We appreciate the reviewer's time in re-evaluating the revised manuscript and are grateful for the positive feedback and suggestion to publish our work after final revision.

One technical correction:

The abbreviation MBC should be explained once before it is used in the abstract.

Microbial biomass carbon was now spelled out in full, as this was only mentioned once in the abstract. (L.17)

Reviewer #2: Julia Schroeder

Mendoza et al. have addressed all reviewer's comments in their revision of the manuscript SOIL_2024-107. Now, the novelty of this study as compared to Mendoza et al. (2022b) is better introduced in the introduction. The new Figure 1 helps to provide a better overview on the hypotheses. However, I still highly disagree with the interpretation of Figure 2B and the following interpretations.

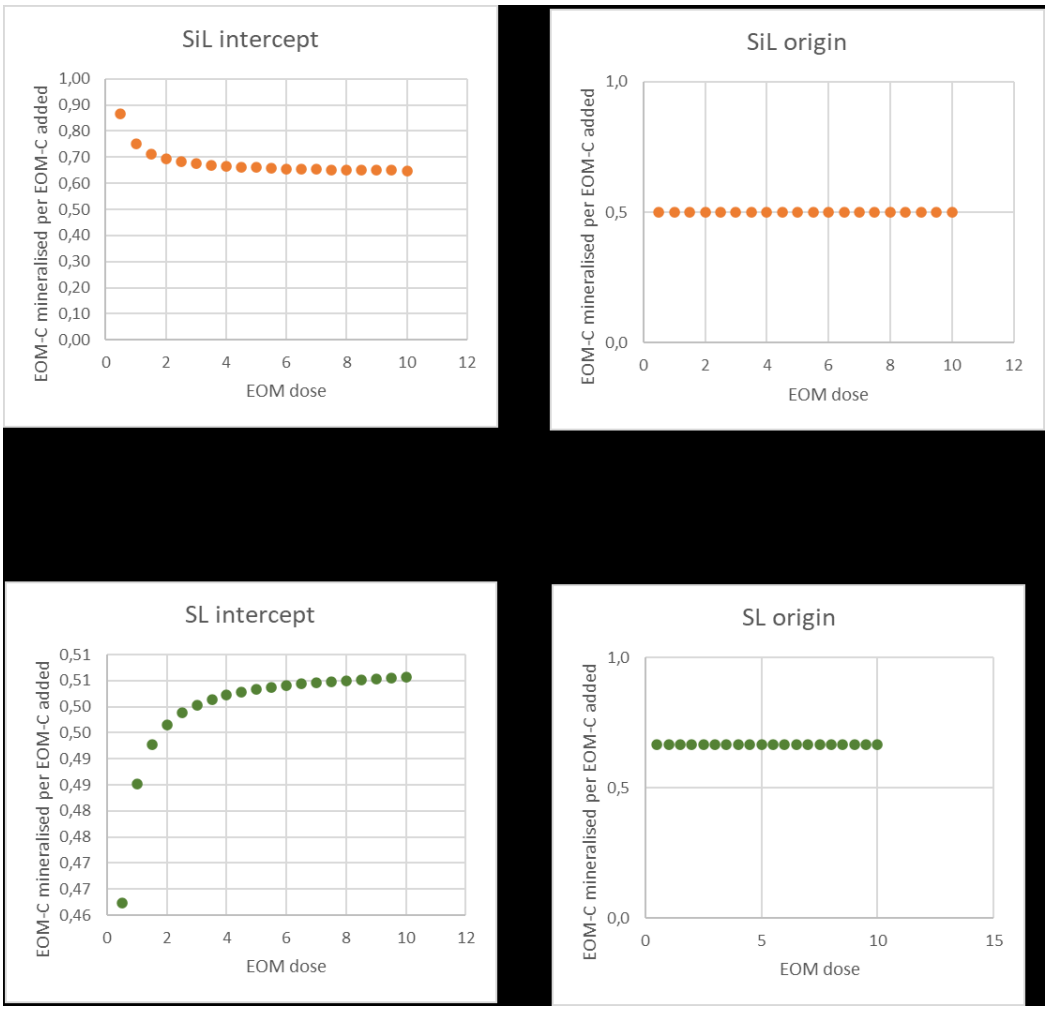
We are grateful to the reviewer for the considerable amount of time and energy invested in evaluating our manuscript and for the constructive comments, which we believe have helped to improve its quality. We believe that with these revisions, the main concern raised by the reviewer, i.e. no clear evidence for a dosage response on EOM mineralization in the silt loam soil, has now

been adequately considered (please see the addresses to the referee's comments below for further detail).

Relative EOM mineralisation (Figure 2).

I argued previously that the visualisation and interpretation of a non-linear decrease of the proportion of EOM mineralised at higher amounts of EOM added in SiL soils is misleading (Figure 1 in the original manuscript, i.e. Figure 2 in current version). This point is very crucial because this non-linear decline in EOM-C mineralised per EOM-C added is the fundament of further interpretation by the authors. In their reply, the authors have provided two examples to illustrate that the proportion of EOM does not necessarily need to be equal if cumulative EOM increases linearly with dose.

To further look into this problem, I extracted the data from their plots and fitted two linear regression lines. The first allowing for an intercept (*intercept*), the second one forcing the curve through the origin (*origin*). For SiL soils, this gave me the two functions y=279.59x + 50.195 (*intercept*) and y=292.36x (*origin*). Then I projected the cumulative EOM for 20 doses ranging from 0.5 to 10 g to visualise for myself, how the regression would affect the proportion of EOM mineralised per total EOM added. The first fit, i.e. *intercept*, did indeed result in two non-linear curves for SL and SiL, with opposing trends depending on whether the intercept was negative or positive. The second fit, i.e. *origin*, did not. In the *origin* scenario, the calculation of the proportion of EOM mineralised per EOM added (Figure 2B) gives the slope of the fit of EOM mineralised over EOM added (Figure 2A), which is then equal for all doses. As mentioned in my initial comment, Figure 2B is then the derivative of Figure 2A.



I recognize that the authors' argumentation is valid from a mathematical point of view. An exponential fit of the EOM mineralised proportion over EOM dose is possible only if EOM mineralisation allows for an intercept.

I argue that it is not meaningful to assume that there is an intercept. Indeed, it seems much more logical to fit a model through the origin, assuming that the mineralization of EOM is zero, when no EOM is added. As mentioned before, the fit of the non-linear relationship in Figure 2B is weakly significant, and should not be overemphasised.

Furthermore, the question arises whether the observation of a non-linear fit in Figure 2B would really imply some underlying biological mechanisms or just point to uncertainties of the method, e.g. uncertainty of 13C-label at lower doses vs. higher doses? I would rather interpret the decline as caused by the intercept and the intercept being caused by methodological limitations.

The authors' assumed, that EOM mineralisation would slow down at high dose due to O2 depletion at the OM matter surrounding, limiting microbial activity. Assuming this to be the reason for slowed degradation, one would expect that the proportion of EOM mineralised per EOM added would further decline with dose, i.e. stronger limitation at higher O2 depletion and stop of

mineralisation. However, my little calculation exercise (i.e. *intercept*) implies that the proportion of EOM mineralised per EOM added will level off at a certain amount, reaching an asymptote at approximately 50% mineralisation. Furthermore, the authors' state that there they did not observe anaerobic conditions in L 380 "but still Eh remained at levels indicative of aerobic conditions".

Long story short, there is indeed little evidence that the proportion of mineralisation is slowed at high EOM dose in SiL and the authors need to revise Figure 2 and following interpretations.

We agree that a regression through the origin, rather than with an intercept, is more appropriate given the expectation of zero cumulative emissions when no EOM is added. Therefore, we have now fitted a linear model through the origin for cumulative emissions, and the interpretation of the resulting slopes has been updated in both the results section and the figure caption (L.257-263 and L.270-273). Even though there still appears to exist a trend of slowed EOM mineralization at high doses we no longer use this view in our interpretation and discussion. We acknowledge that more evidence (more doses) would have been required to conclude that the proportion of EOM mineralization would indeed decrease at high EOM doses in the silt loam soil. We have accordingly revised our interpretation. In line with this, we have removed the confidence intervals, model equation and determination coefficient from the lower panel of Fig. 2 bottom (L. 267) concerning the proportion of mineralized ryegrass C in silt loam soil and sandy loam soils. In the discussion we now also refrained from invoking a series of potential mechanisms to explain the previously misinterpreted trend in relative EOM mineralization.

Other points raised during the first review:

Novelty as compared to previous study published in Biology and Fertility of Soils is now better introduced.

Thanks, for the addition of the reasoning behind the selection of dose levels.

Relative SOC priming plot (Figure 3B). Revise changes in the paragraph added to L.446-453: "The slowed relative EOM mineralization" - see my argumentation above. Also revise L523-542: "…the slowed relative mineralization of EOM at increasing dose could be related to enhanced occurrence of local O2 limitation surrounding EOM litter, even though its addition in fact also stimulated macroporosity. …"

The mentioned lines 446-453 the previous manuscript version have now been revised (now L.461). We also omitted two sentences referring to the statistical significance of EOM dose on the C balance. A new sentence was added "Adding a low EOM dose was least favourable for the C balance at least in the sand loam soil (P <0.01), while no significant effect of EOM dose on the C balance was observed in silt loam soil." (L458-460).

The second sentence in the conclusion has been revised, – please see our response below in the Conclusion section.

MBC. I agree with the revisions. Figure 4 becomes now clearer.

Statistics. Thanks for clarification. I had confounded GLMMs and GLMs.

Figure 1 I highly appreciate the new figure, which illustrates the expected outcomes and hypotheses of the study. I recommend to add a short description of these to the figure caption to provide a quick overview on the hypotheses.

We thank the reviewer, and we agree to add such further description of the hypotheses - Please see our response below on 2 related remarks on the introduction (L.88-97 and L.107 of the previous manuscript version).

Line-to-line comments

Abstract

L13: Consider to mention your hypotheses with regards to differences to soil texture already in the abstract.

We agree and added the following sentence: *"The percentage of mineralized EOM was expected to increase linearly with EOM dose in sandy loam soil and level off in silt loam soil due to limited O_2 supply to maintain aerobic microbial activity."* in L.13-15

L 15: Delete "economic". Unclear what you mean by that. Do you mean "no increased microbial growth" or "no increased microbial efficiency" or "no changes in microbial growth efficiency"?

We can see that this term was unclear, and the sentence has now been adjusted to: *"Likewise, formation of microbial biomass carbon was proportional to the EOM dose, suggesting no reduction in microbial growth efficiency at higher C concentrations"* in L.17-18.

L 17-19: I do not agree with the interpretation that the percentage of mineralised EOM decreased with dose and the following hypothesised mechanisms. See detailed comment. Consider revision.

We acknowledge the referee's points – see our overall response above and in-depth responses to comments raised in the discussion section. We also adjusted the formulation in the abstract and the sentence reads now: *"In the silt loam soil, a decreasing tendency in the percentage of mineralized EOM was apparent but could not be confirmed statistically. We therefore conclude that as in the sandy loam soil the proportion of EOM mineralization was not affected with increasing dose…"* in L.18-21. We have also added a sentence "Consistent with this lack of response in the proportion of EOM mineralization to EOM dose, *soil Eh did not decrease with increasing EOM dose, indicating no O_2 limitations.*" in L.23-24.

L 20: Delete "textured". "In both soils" is sufficient.

Done

L 21-25: With regards to the high uncertainty of slowed EOM mineralisation in SiL at high dose, I recommend to revise this part. It seems very speculative.

We agree that the sentence *"At the same time the higher microbial activity might have sufficiently lowered soil Eh close to the large added EOM particles, limiting its relative degradability at high dose, suggesting a potential new mechanism for understanding SOC cycling."* was speculative and therefore removed it.

We also further slightly rephrased the preceding sentence more conditionally: *"The observed stimulation of soil macroporosity at higher EOM doses in the silt loam soil might have contributed to sustaining aerobic conditions required for SOC mineralization."* in L.28

Introduction

L 88-97: I really like this new passage. The novelty of this study is now better introduced.

L 107: Helpful figure! Doesn't the green line show mineralised EOM instead of % mineralised EOM (curve would start high and become lower at high dose in the latter case)? I recommend to add a short explanation on the hypotheses with regards to differences to soil texture to the caption of Figure 1.

The plotted lines are showing the % mineralized EOM, not cumulative EOM mineralization. We expected the proportion of EOM mineralized to increase with dose in the sandy loam soil and flatten at high doses in the silt loam soil. To clarify further this, we slightly adjusted L.100. *"We hypothesized that the mineralized percentage of added EOM (further referred to as relative EOM mineralization) would increase with increasing application dose, due to closer contact of EOM and decomposers."* . +

We also added a description of the hypotheses to the caption and clarified the expected mechanisms as follows: ***"Figure 1.*** *Expected outcomes and hypotheses regarding the effect of EOM application dose on EOM and native SOC mineralization in sandy loam and silt loam soils. Overall, we expect the proportion of EOM mineralization to increase with higher EOM doses in sandy loam soil due to closer contact between EOM and microbes at higher doses. In silt loam soil, the proportion of EOM mineralization is expected to level off at higher doses, due to a higher chance of limited O_2 supply, which may restrict aerobic microbial activity compared to sandy loam soil. Priming of SOC mineralization is expected to increase with higher EOM doses because of enhanced co-metabolism and formation of macroporosity."* in L.109-114.

Materials and methods

L 161: Do you mean every 1-2 hours?

We meant every 1-2 seconds with the temporal resolution of measurement of CO_2 emitted and recorded for about 10 minutes per each soil core. This was already stated in L.166-167.

L 196: CFE-extraction was done in a 1:2 v/w ratio of soil-to-K2SO4. Why didn't you stick to a 1:4 ratio (Joergensen, 1996)? The ratio may affect the kEC. Can you add a reference?

We have updated a reference for the used method, we now cited Carter and Gregorich 2008 instead of Vance et al., 1987 in L.199. The method proposed by Carter and Gregorich 2008 applies to extraction ratios from (oven dry) 1:2 to 1:5 with no further impact on the extraction efficiency mentioned. Our 1:2 ratio - moist soil:K_2SO_4 would in fact result in about a 1:2,5 dry soil : K_2SO_4 ratio, i.e. within the proposed range proposed. Tate (1988; Soil Biol. Biochem.1988 20 329335) compared 1:2.5 and 1:5 ratios and found no effect onto extraction efficiency for both statistically significant difference using two extractant-to-soil ratios.

Results

L 254-256: Revise result that relative EOM mineralisation in silt loam soil slowed down at high EOM dose.

This part has now been modified to *"The relative fraction of added EOM mineralized after 90 days was independent of soil texture. However, in the silt loam soil, the relative fraction of mineralized EOM tended to decrease with increasing EOM dose (Fig. 2 bottom). Given the limited number of EOM doses included and close linear response of the cumulative EOM mineralization to EOM dose (with an intercept of zero due to the absence of cumulative EOM mineralization when no EOM is added), this trend should be interpreted with caution."* in L.256-262.

L 279: "the extra amount of SOC mineralized vs. the unamended controls relative to the unamended controls" - delete and in caption of Figure 3.

The sentence is now deleted as suggested. The remaining text is slightly adjusted accordingly, and now reads: *"The lower graph compares the relative priming of native SOC mineralization in both soil textures."* (L.284-285).

Discussion

The discussion needs a major revision, given that there is no evidence for a slowed EOM mineralisation with EOM dose in the silt loam soil.

As explained in our overall response above, we agree and revised the discussion in the following points:

1° We acknowledge that there is no proof to assume a dosage response in the reformulated text.

2° We reorganized the second paragraph of the discussion in which we previously discussed several potential explanations for an EOM mineralization dosage response in silt loam soil. The first part of that discussion on soil N availability has been removed (L.371-375), and now it is just briefly mentioned under section 4.2 to demark the differences in methodological approach between our study and other published work on this topic (L.422-423).

3° We now only concentrate on our actual observation of a lacking response of Eh to EOM dose and refrain now from making any further reference to other mechanisms not under investigation like occlusion of the added EOM into soil aggregates (L.387-394)

Specifically, the following text was changed

"In the sandy loam soil and silt loam soils, EOM-derived C mineralization was overall independent of its application dose. A decreasing tendency did emerge with increasing EOM dose in the silt loam soil (Fig. 2), did not provide sufficient evidence to support our hypothesis that the relative EOM mineralization would increase with increasing EOM dose" in L.347-350.

"The unresponsiveness of relative EOM mineralization to EOM application dose in the sandy loam soil and silt loam soils was consistent with…" in L.357.

"Drawing conclusions for EOM management in the field based on this 90-day lab incubation experiment at 20°C is to be made with care. Nevertheless, the ordination of relative EOM mineralization patterns remained consistent among the established dose treatments over time and are projected to remain likewise for at least some time (Fig. A1). For instance, mineralization over 137 days at the established 20°C in the lab experiment equates to about one year in the field in Belgium (9.7°C on average) (De Neve et al., 1996). Thus, our results suggest no, or at most, a limited negative effect of adding EOM at increasing doses on its annual mineralization, a traditionally used metric in C-balance calculations (the so-termed humification coefficient). However, empirical evidence from field experiments is now needed to confirm these findings." in L.395-403.

L 364: The revised sentence makes no sense anymore. Check.

The EOM-mediated MBC increase was NOT proportional to EOM dose, we have corrected that: *"In our experiment, the EOM-mediated MBC increase was not proportional to EOM dose in both soil textures (Fig. 4; bottom), suggesting that further growth of MBC was energetically equal for the different included substrate concentrations."* in L.364.

L 380: "but still Eh remained at levels indicative of aerobic conditions" - this is another argument against the slowed EOM mineralisation with increased EOM dose. + L 385 "In conclusion, we could not identify the cause of these phenomenon, and further research is required to explore the potential mechanisms leading to a relative temporal stabilization of EOM when added at larger doses." - This further suggests, that the authors' may have misinterpreted their results.

The discussion has been revised and several sentences were adjusted. L.385-387 now reads: *"Hence, from the Eh readings, we observed no indication that O_2 limitations would have restricted the relative EOM mineralization in the silt loam soil at higher EOM doses as we initially hypothesized."*

L 444: Add a reference for the 1-3% SOC mineralisation in the field.

We now refer to Vleeshouwers and Verhagen (2002) wherein annual SOC mineralization for several estimates for Western European countries are listed. Based on that study we also modified the previously given 1-3% into 2-3% in L.455 (Vleeshouwers & Verhagen, 2002; Global Change Biology 8, 519-530).

L 451-453: Unclear whether this conclusion is backed up statistically? There is no statistical analysis mentioned.

It is, and we now reformulated the sentence and added a p value: *"Adding a low EOM dose had the least favourable effect on the C balance at least in the sandy loam soil ($P < 0.01$), while no significant effect of EOM dose on the C balance was observed in the silt loam soil"* in L.458-460.

L472-474: Only one regression line was fitted for the relative SOC priming over EOM dose in Figure 3 and only one slope is provided in the Results section. Why are different slopes given here? You can not base your discussion on results you did not present and which are furthermore not significant. Please revise the discussion around this point.

We acknowledge that the slopes for each texture should not be reported here, as they were not significantly different. Therefore, we have removed the corresponding sentence (L.480-482) and revised the text accordingly as follows: *"We furthermore found positive linear relationships (via linear regressions) between the silt loam soil volume fraction of pore neck size classes 60–100 and >300 µm and relative SOC priming (R^2 = 0.34 and 0.36; and, P = 0.09 and 0.08, respectively), and a negative relation with the 3-9 µm class that also depended on EOM dose. We therefore hypothesize that the development of macroporosity might have contributed to the promotion of relative SOC priming in the silt loam soil. In contrast, no such relationships existed for the sandy loam soil and the observed increase in relative SOC priming with EOM dose must have been mediated by other mechanisms."* in L.483-489.

Figure 8: Please revise the trend in % min EOC in silt loam. Unclear what primed SOC revers to (e.g. relative SOC priming, absolute, relative SOC priming per EOM added). Please clarify.

The picture depicts relative SOC priming and that has now been clarified in the caption (in L.519). Moreover, the % EOM mineralized has been adjusted with the new interpretation of no EOM dose effect on relative EOM mineralization (L.525-526).

Conclusion

Needs revision with regards to slowed EOM mineralisation with EOM dose in the silt loam soil.

The conclusion is now revised, and the main adjusted parts read:

"Overall, our results showed no response of relative EOM mineralization to EOM dose in heavy- nor in light-textured soil, in line with a null response of MBC formation to EOM dose. A large range of doses and soil textures including clay and sandy soils would help clarify if such a dosage independency is consistently observed. Our experiment revealed a lower bulk soil Eh in the silt loam soil than in the sandy loam soil, as expected. However, since Eh remained within the aerobic

range even at a high EOM dose, it suggests that O_2 supply was sufficient to sustain the proportionally higher absolute EOM mineralization. We hypothesize that the enhanced macroporosity at the established higher EOM doses may have improved soil aereation, preventing the onset of O_2 limitations." in L.531-538.

Tentative C balance calculations finally indicated that adding EOM at a low dose (around 0.5 g kg-1), had the least favourable effect on SOC at least for the sandy loam soil and no effect in the silt loam soil." in L.545-547.

The following sentences were removed:

"The formation of MBC was independent of EOM dose; thus, we found no evidence suggesting a more economical growth of heterotrophs at higher substrate doses." in L.538-539.

"We expect that with the generally observed lower bulk soil Eh in the silt loam soil, the slowed relative mineralization of EOM at increasing dose could be related to enhanced occurrence of local O_2 limitation surrounding EOM litter, even though its addition in fact also stimulated macroporosity." in L.539-542.