Final Response Letter to Referees' Comments

Referees' comments are black, while our responses are in blue. The mention of lines in our responses refers to the marked-up version of your revised manuscript

Referee 1

We thank the reviewer for the valuable and conscientious comments to our manuscript. Please see the replies to the comments in detail below and our revised version of the manuscript.

Language. Section structure should be streamlined and harmonized, as now paragraphs are sometimes indented and sometimes not, several paragraphs contain a single sentence, and use of spaces between paragraphs is inconsistent. This makes it difficult to follow the narrative within each section. Please check citation format, as now it does not always follow standard practice ("Author (year)" for in text citations and "(Author, year)" for citations in brackets), e.g., L38, 174, 185, 286. Several sentences are hard to understand because of missing commas. To give one example, please see L199-202, where I would suggest adding four commas as follows: "Initially, POM particles with a concentration of 0.48 gCcm-3 and a C/N ratio of 100, and necromass with a concentration of 0.32 gCcm-3 and C/N ratio of 10, amounting to a volume fraction of 5% of the solid area, were randomly added to the pore space, corresponding to 60 POM and 60 necromass particles, each with a size of 6-10 μm in diameter." In many instances word choice is not appropriate (though I am not a native speaker, so my impressions might be wrong), e.g., "Respired" instead of "Respirated" in Figure 1, L133 "outflows" instead of "sinks", L159 "Monod" instead of "Michaelis-Menten" (kinetics don't involve enzymes in this model), in some figure "amount" is actually a "content" (mass/mass). In other instances, the meaning of the chosen term is unclear, e.g., L44 "microbial C/N efficiency", L71 "experimental limitation", L401 "optimal" (what is optimized?), L558 "agitation", L570 "exclusive" (why are some pores exclusive?). These are just some examples. A thorough proof-reading is necessary, perhaps with help from a native speaker.

We thoroughly went through the manuscript and improved language as well as the arrangement of paragraphs and citations. We corrected the phrases according your remarks. However, we kept "amount" in the legends of the figures, as it refers to amount of C per g soil.

Methods structure. L74-93 present the model structure, so should be moved to the Methods. L260-270 do not present any result, but rather explain how model data is analyzed, so they belong to the Methods.

As suggested by the reviewer, we shifted the respective parts to the methods section.

Model implementation. It is not clear how the differential equations at the core of the model are solved in the hierarchical structure shown in Figure 4. Runge-Kutta method is used to solve the mass balance equations through time within a day, and then spatial fluxes are added, if I understand correctly. But if that is the case, is the solution converging numerically without successive iterations to feed back spatial flux information into the mass balance equations? Is the one-hour time step sufficiently short to ensure stability with an explicit method?

We use a non-iterative splitting approach (Line 585 in the submitted manuscript), where the solution of the diffusion equation is inserted without iteration into the mass balance equation. Specifically, Equations (1), (2), and (5–8) are solved explicitly for each voxel using a Runge–Kutta scheme. The resulting concentrations are then used as initial conditions to implicitly solve the diffusion equation. This is repeated 24 times within one day. To demonstrate that the chosen time step is adequate, we show the results of three additional simulations with doubled temporal resolution (48 equidistant steps per day) here. There are minor differences in absolute numbers but the same repartition of carbon among the different compartments. Note that due to the nature of the cellular automaton including some random movement of particles in the case of equal attractivity of several positions, there will always be (very small) differences in two simulations - even under the same initial conditions.

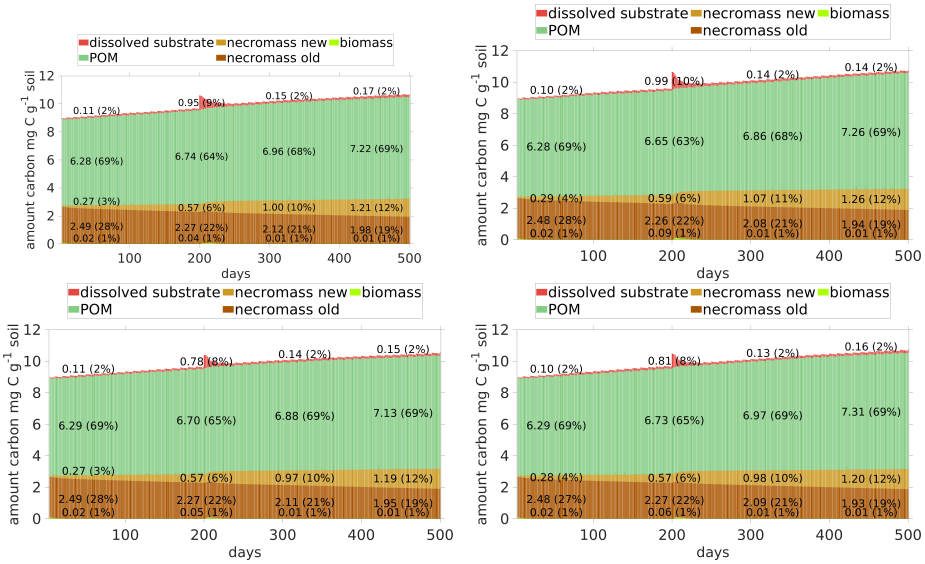

Figure 1: Compare C pool distribution of reference setting with 24 time steps per day (top left image) with different 3 simulations of the reference setting with 48 time steps per day (top right, and bottom row images). No significant difference can be observed.

Moreover, it is not clear how Eq. A2 is implemented. The inequalities compare partial derivatives on the left-hand side and contents on the right-hand side, which is not physically meaningful (units are different).

We define the concentration of nitrogen in soil solution within a voxel as N_s, which represents the maximum amount available for microbial assimilation during a given time step/moment t_k. In the subsequen timestep t_{k+1}, this nitrogen pool could be fully consumed (if the corresponding carbon pool demands this), such that $N_s^{t_{k+1}} = 0$. As the decision rule is implemented discretely, we reformulated the equation accordingly:

$$\left(\frac{N}{C}\right)_B \frac{C_B^{t_k} - C_B^{t_{k+1}}}{\Delta t} \geq \frac{N_S^{t_k} - N_S^{t_{k+1}}}{\Delta t} \quad \text{with } N_S^{t_{k+t}} = 0 \tag{1}$$

Model setup. Some important assumptions make the simulations hard to generalize. First, the soil is assumed saturated for the duration of the simulations, but in such conditions, over 500 days anoxic conditions would develop, leading to rather different processes and controlling factors for C and N dynamics. While perhaps more difficult computationally, considering partly saturated conditions would make simulations more realistic (then one could argue that oxygen is not a limiting factor), and also allow comparing the effects of particle arrangement and pore water content on C and N dynamics. Which one dominates?

In our setup, we consider a domain of 500x500x1 micrometers which corresponds to a system of microaggregates, primary particles and micropores in a range of 2-80 microns. This does not necessarily mean that the whole surrounding soil needs to be saturated as larger pores beyond our scale would drain first. In our study we want to focus on the interplay of C and N with the microbial community, not limited by oxygen or water. The model framework could be extended in future work to study, e.g., the changes under anoxic conditions. Please note that microbial carbon turnover with structure dynamics and different saturations and oxygen concentrations has been studied in Zech et al. (2024), JPNSS.

Second, POM is defined as particles with a size between 6 and 10 microns, while it is operationally defined as particles larger than about 50 microns. I agree that for this kind of numerical experiments, smaller POM particles are appropriate, but I would mention that they are smaller than according to the usual definitions.

We fully agree that POM of fresh litter may refer to particles up to 1 mm, while it also includes finer fractions with diameters less than 50 microns, e.g. Lavallee, Soong, Cotrufo (2020), GCB. Given the scale of the investigated domain, we took into account smaller POM particles (6–10 µm) to represent the fine particulate organic matter that is already partly decomposed and/or fragmented from larger POM particles. This choice allows us to capture microscale interactions within micro-aggregates between organic matter, microbes, and soil particles. We added a corresponding remark in lines 223 and 228.
Reference: Lavallee JM, Soong JL, and Cotrufo MF. "Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century". In: Global Change Biology 26.1 (2020), pp. 261–273.

More important, the C:N of POM is set to 100 g C/g N, which is reasonable for fresh conifer litter (or wood fragments), but much higher than most other plant residues. As a result, the simulated soil is almost always N limited, except at the time of root exudation, which provides inputs with lower C:N. I wonder if it should be the opposite—low organic matter C:N and high root exudate C:N?

We agree that natural litter spans a range of C/N ratios. In order to limit the number of scenarios we did not vary this parameter. As the reviewer noted, it is reasonable for fresh conifer or wood litter, but also for root litter. Poeplau et al. (2023) reported, e.g., C:N ratios from 50 - 124 there. We conducted an additional simulation using a lower C/N ratio for POM (C/N = 10). As expected the results indicate that nitrogen limitation did not occur under these conditions; therefore, a higher amount of microbial necromass was formed, while the overall biomass size remained unchanged. This lines up consistently with the findings in the manuscript. In order to limit the number of scenarios, we did not vary the C:N ratio of POM there. The C:N ratios of exudates have been varied in the scenario 'Pulse'. We added the reference in line 224 and described briefly the additional simulation in Appendix A5.
Reference: Poeplau C, Begill N, Liang Z, Schiedung M. Root litter quality drives the dynamic of native mineral-associated organic carbon in a temperate agricultural soil. Plant Soil. 2023;491(1-2):439-456. doi: 10.1007/s11104-023-06127-y.

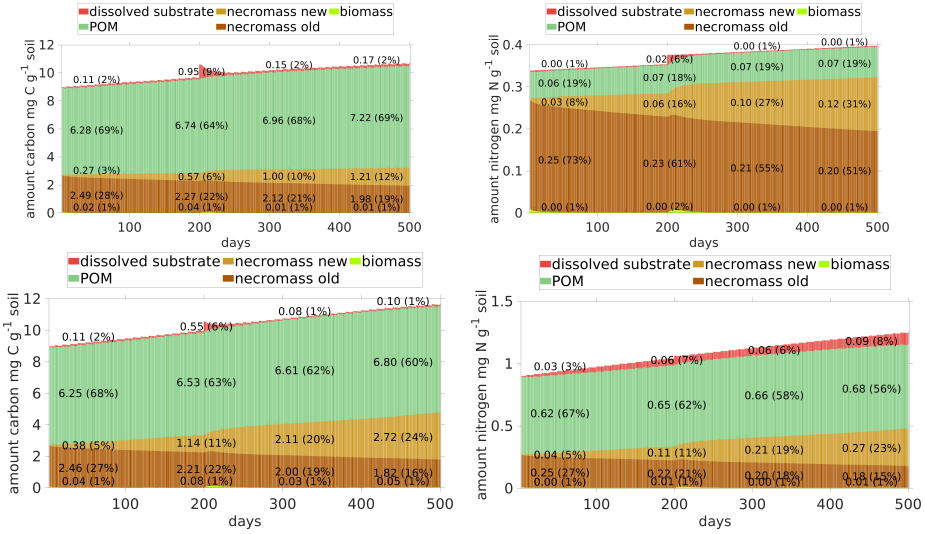

Figure 2: Comparison of C (left) and N (right) pools of two scenarios: top row: reference setting. bottom row: reference setting but with C/N ratio of POM = 10. In the bottom scenario the nitrogen limitation vanishes.

Results presentation. Model simulations provide numerical values for fluxes and contents, but the values themselves are in general not particularly important and can be read from the figures. Now the Results section reports several numerical values

that, in my view, distract from the main messages—what are the important trends and patterns we should look for in the figures? My suggestion is to streamline the Results section so that the answer to the research question (in this case a general aim rather than a question) emerges more clearly.

We thank you for that comment, removed several values in the text and streamlined the Results section.

Analysis of results. One of the most interesting results is briefly described in L373-383. There the interaction between spatial structure and organic matter stoichiometry emerges. I would expand this analysis beyond a qualitative statement based on visual inspection of a figure, making it quantitative. What can we say in general about these interactions across model runs within a scenario, or across scenarios?

Additionally to L373-383, in line 347-356 spatial results depicted in Figure 7 are discussed. There we describe the relation between the geometry of the pore space, the presence of microbial biomass and the availability of C using a normalized scale. We also included the final distribution of carbon among the various compartments for the scenario Static in Fig. 11 now, adding the bar entitled "Static" to allow a direct visual comparison to all other dynamic scenarios.

Specific comments

Thank you very much for your detailed comments. We revised the manuscript taking these comments into account. We only repeat them here if we'd like to answer more precisely.

L45: please define CUE

We added the reference to the defining equation in the text.

L73, 94: aims are repeated; I would suggest merging these sentences so all aims are in the same place

Figure 1: leaked nitrogen—is it including organic and inorganic N?

Indeed, as we do not distinguish between them. We added a note in line 155.

Figure 2: following the logic of the manuscript, Figure 2 might actually become Figure 1, as it illustrates the physical environment described by the model. Also, in current Figure 2, high C:N is associated to the rhizosphere, while in the model simulations POM has high C:N and root exudates have generally lower C:N; that is a bit confusing (note also that the labels "max" and "0" on the color bar for C:N are too small and can hardly be read)

We changed the order and improved the bars.

L105 (and elsewhere): I wonder if it is necessary to cite a long list of articles adopting a similar approach; perhaps it would be enough to cite the last one, or the one that sets the stage for this work

We removed two references.

L114: if POM is attached, it will become less bio-available, as it is associated to soil minerals

This is true and taken into account by the fully dynamic concept, where the degradation rate of every particle is depending ton the availabiltiy / access to pore space. We emphasized this in lines 55 and 198.

L189: just a curiosity—what happens if two soil particles end up overlapping when randomly placed in the domain?

The algorithm prevents two particles from occupying the same cell.

L224: are these rates expressed per unit root surface or per unit surface in the simulation domain?

The rate in Santangeli et al (2024) describes the release of C per root surface. Since we assume that the root passes by and releases the exudates into our domain centerline, we adjust the exudation rate to the size of the cell domain. We clarified this in the script in line 247ff.

Section 2.4: scenario names are not very intuitive, e.g., "3Pulse" refers to a scenario with 10 pulses. Maybe I would rename them "dynamic", "static", "CN", "10 pulse" without a number to keep it simple (the numbering matters less than the actual treatment you apply in each scenario)

We followed your suggestion to rename the scenarios

Figure 5 (and elsewhere): panel titles are generally placed on top of their panel, line styles are too similar to be able to distinguish the lines, colors for dissolved substrate and old necromass look the same in the bottom panels

To distinguish the lines, we replaced the dash-dotted line style with a small straight line with circles. In Figure 5, the old and new necromass have different line styles to improve readability despite their similar colors.

L326-329: I am not sure why this point regarding microbial biomass nearing equilibrium is presented here when showing results for N fluxes; the same argument holds for C fluxes

In this section we study the N fluxes in general. One observation is the relationship between assimilation and degradation fluxes. We would like to show how the fluxes influence biomass and how the dynamics of biomass can be reconstructed from them.

L373: not clear what "event" refers to

We clarified the reference to previous sentence (now line 410).

L437: parameters were not fitted but chosen to obtain reasonable results, which is not conceptually very different

We used measured parameters from experiments in our forward model without adjusting them to our simulation results. We added the word inverse, to make clear from what kind of parameter fitting we distinguish.

L464: plant residues in agricultural fields are likely much richer in N than assumed here

Please refer to the answer above containing Figure 2, especially the study by Poeplau et al. (2023), who reported root litter with C:N ratios ranging from 50 - 124.

L471: this sentence states what "equilibrium" means; I am not sure I understand where the argument is going

We removed this statement.

L475-482: this result is a direct consequence of the model assumptions, so it does not seem particularly insightful

We emphasize this as direct response from the model

L520: exudate "favorable C:N" does not translate to "exudate diversity"—you could have residues from a single species (no diversity) with very low C:N ratio (favorable C:N)

The term diversity is misleading in this context. We replaced it with composition.

L521: but structural changes of the magnitude experienced in agricultural fields are not included in this analysis...

We removed this statement. We only consider structural changes on pore scale caused by Brownian motion, OM compounds or mechanical stresses due to, e.g., growing roots or earth worms.

L524-527: these last sentences distract from the main message of the manuscript by speculating on future work, while it would be more impactful to conclude with L523, where a key result is summarized (just my personal opinion)

We moved the sentences to the end of the discussion section.

L528: it would be nice if a version of the code to run a basic simulation could be provided

The code is available upon request from the authors, it is organized in a GitHub repository.

L543: at this point in the text the term "attractive" is not clear (it will be explained later)

The concept has been explained in Section 2.1.

Referee 2

We also thank reviewer 2 for the helpful comments to our manuscript. Please see the replies in detail below, we submit a revised version of the manuscript.

This paper presents a very interesting modeling exercise using an agent-based model that couples soil carbon dynamics driven by microbial activity with the dynamic formation of soil structure, resulting from the interplay between soil biological activity and physicochemical properties. Moreover, adding root exudates to the system increases the model's realism, enabling exploration of scenarios under varying conditions, including different root exudate qualities and quantities. The paper is well written, and I enjoyed reading it. And I feel it is a good concept that warrants further exploration. Although I really like the paper, I feel some conceptual aspects are not entirely clear and could have strong implications for the conclusions drawn from this modeling exercise. I will start pointing out the conceptual questions I have for the authors, and later, I will provide some minor comments on specific lines.

Conceptual questions:

Conceptually, the model describes two main external carbon inputs, root exudates and fresh POM, which are converted to DOC and later to necromass via microbial death. The idea of POM as one of the main "solid" forms of carbon could be a model assumption, but I wonder whether there could be a bit more discussion of what that means. I feel that litter will be the initial C input into the soil, and it will first be partitioned into POM, but it could also contribute to the MAOM (mineral-associated organic matter) as a more recalcitrant form of carbon, or even to the DOC pool. I understand the authors do not explicitly model MAOM,

but necromass could be assumed to be the more stable carbon form, whereas POM is the more readily degradable form. But as it is in the model, my understanding is that there is little difference in the degradation rates of necromass and POM. This is very evident from the results, where both pools increase over time, and I fail to see whether the system has a more stable or more easily degradable carbon pool. Also, in the model, POM and necromass are treated similarly (with the same degradation equations and the same first-order degradation rates), with the main difference in stoichiometry. It would be nice if the authors could reflect a bit on these model assumptions and not only describe their choices, but also whether this makes sense mechanistically. I would, for example, expect necromass to degrade far more slowly than POM. I can also imagine fractions of litter or root exudates being sorbed and quickly protected, thereby contributing to a more stable carbon fraction, not only to the POM or DOC pool. Root exudates also contribute to the POM fraction. However, these relationships are not part of the model. It probably isn't necessary to include them and re-run the model, but some explanation in the discussion and methods is important, especially as some model limitations. It could also be interesting to expand a bit on how carbon fractions are represented in different models. The introduction goes directly into modifications of the CAM framework, which is fair, but other frameworks for representing carbon dynamics in soil could at least be briefly mentioned.

Indeed POM does contribute to what is called MAOM in pool models as well as to DOC along its degradation. More precisely, in our spatially explicit model we do take into account the attachment of OM to mineral surfaces (as well as other surfaces). So there is no need to partition POM into less or more stable fractions, but the variation of the degradation rate is inherent in the dynamic model concept. The degradation rates are dependent on the degree of occlusion of the attached POM and necromass. As such our representation precisely allows to dynamically slow down the degradation of any occluded particle depending on the number of edges it shares with the pore space in this particular time step. As particles restructure and may break up again, faster degradation will be possible again. As we consider the exudates as immediately dissolved in water they directly contribute to the DOC concentration in water. This can be taken up by microbes. So we do not consider exudates as a separate phase here.
To simplify modeling we do not distinguish different turnover rates for the degradation of POM and necromass. They follow the same mechanism as opposed to the easily available exudates. Further complexity such as different degradation rates could of course simply be added, for the sake of more difficult interpretations of the results. The effect of different turnover rates for POM has been already studied in Zech et al. (2022, Global Change Biology). However, there is also experimental evidence which shows comparable turnover times, see, e.g. Joergensen and Wichern (2025, SBB). We will clarify these points in our revision and add more references to contextualize the model assumptions (see lines 30 and 54ff).
Reference: Joergensen RG, Wichern F (2025): Turnover of fungal glucosamine and bacterial muramic acid in comparison with soil organic carbon in two arable soils with distinct fungal communities, Soil Biology and Biochemistry 209, 109889.

https://doi.org/10.1016/j.soilbio.2025.109889.

I wonder if the authors could explain a little bit about the practical relevance of your scenarios. This would be very important because in some cases, parameters are taken from different studies, and I do not know if they all match the system the authors are trying to simulate. I fully understand that it is not possible to find everything in one study, but an initial explanation of which system (conventional arable land, organic, grassland, etc.) the authors are simulating would help us better understand whether the parameters are realistic and which ones might be a bit of a stretch. This is also important during the discussion. There is mention that the simulations match this and that study, but I am not sure whether the study's conditions match the scenarios.

As we presented in Section 2.3, the parametrization of the model is oriented at the experimental platforms explained in Vetterlein et al., 2021, where a multitude of studies has been conducted at the same sites and with the same soils to study self-organization of the rhizosphere shaped by maize plants. We create a 2D representation of the loam of the soil plot experiment (SPE), following measurements of the particle size distributions there (Table 3). It stems from a haplic Phaeozem soil under agricultural use in central Germany and has been excavated and sieved before the experiments to provide a consistent, comparable basis for the various experiments. We added this information in lines 205ff. If possible we used parameters from those related studies, e.g. from Vetterlein et al. (2021), Brax et al. (2020), Niedeggen et al. (2024) and Santangeli et al. (2024). Other microbial model parameters, such as the maximum microbial concentration in a cell of the CAM, go back to previous work of Zech et al. (2022b), who built upon Portell et al. (2018). Most of the initial values (e.g., initial DOC concentration or microbial biomass) are not very sensitive as the system equilibrates in the first days (other values tested). We note that our modeling approach does not use any fitted parameter, but is a forward simulation based on the given assumptions. Notably the results of this assumptions are consistent within the experimentally observed ranges (e.g. total N and C masses as in Vetterlein et al. (2021), lines 234ff, or the total respiration, which aligns with measurements of Niedeggen et al. (2024), as noted in lines 340ff.)

Along the same lines as the model scenarios, I am really hesitant about how the root exudation process is represented. I understand you will not include different types of exudates, but to me, exudation is a continuous process, not really occurring in single pulses here and there. It is clear that there will be moments when exudation is higher, triggered by environmental conditions or developmental stages, but in the simulations, it seems random—or at least I did not see a justification. I wonder if the authors could add something about this in the methods, such as why this root exudation mode was selected, and maybe discuss a bit more about a more realistic root exudation process and its potential consequences.

In a previous study we modeled in detail the exudation as dependent on the age of the root by simulating a growing fine root tip intersecting the model domain and the subsequent shrinkage (Rötzer et al, 2023). Here, we do not explicitly model the root itself. As the focus was on the effect of different C sources and N

availability, we decided to take a more simplified representation of exudates. We agree that exudate composition varies with plant age, as has been also shown by Santangeli (2024) in the rhizosphere project. In the scenario 2CN we do investigate the influence of different types of exudates with C:N ratios of 10, 22, 40, and 100. We agree that the design of the ten pulses in the scenario 'Pulse' is somewhat artificial, however day-night cycles are evident for exudation processes and the main aim was to investigate the impact of an increased amount of exudates. In general, the overall goal of the simulations is not the reproduction of the most realistic setting, but to study the effect of the interplay of certain processes and the effects of precisely defined changes. Nevertheless as many parameters as possible were taken from a coherent experimental setting.

In several parts of the paper, there is mention of "gluing spots", but the authors briefly describe them, I believe, in the discussion as EPS substances, for example. The model, however, does not explicitly represent the production of these substances by microbes or the costs associated with this investment, which will be reflected in the CUE, so I wonder what the authors mean by this conceptually and in the model.

The concept of the gluing spots describes in particular a change of surface properties. It has been introduced in detail in Zech et al. (2022), GCB:
"The microbial turnover of POM produces microbial remnants that may interact with the mineral particles serving as gluing agent for particle adhesion within the soil matrix (Hattori, 1988; Watteau et al., 2012). Such organo-mineral interfaces can temporarily cover mineral surfaces and probably enhance their surface reactivity as a gluing agent in the vicinity of the decomposing POM particle (Chenu & Stotzky, 2002; Huang et al., 2015; McCarthy et al., 2008). Temporary OM-covered mineral edges are assumed to provide gluing spots on mineral surfaces and enhance the formation of aggregates (Bucka et al., 2019). Based on the mineralization of OM, the gluing effect is temporarily limited. In accordance with adsorption studies (Gao et al., 2017, 2020), the surface conditioning effect of organic compounds on mineral surfaces (Dufrêne et al., 1999; Kleber et al., 2007) may be locally retained even after degradation of the OM coating."
We added a brief explanation together with the reference to Zech et al. (2022) in lines 76ff.
In this study we do not explicitly account for a concentration of a gluing agent. EPS is just one example substance mentioned in the introduction which changes these properties. The gluing spots along the mineral surfaces in our study develop as POM is attached to them or biomass. The cost of the production of EPS (which is not explicitly considered here) is implicitly taken into account in the basal respiration of the biomass, and the calculation of the CUE.

The conclusions are a bit weak, as new ideas are included that, to me, do not necessarily represent the paper's main point. For example, I'm not really sure whether you have explicitly slow-cycling or fast-turnover carbon pools in the model, since they are treated similarly. It could be that I am wrong, so if you can provide a supplementary figure of the decay of both fractions, POM and necromass, that could be interesting. The scale at which you present the two of them in the current figures does not allow for any distinction. Also, the conclusions about management

practices in lines around line 520 are correct ("Conversely, in soils that are subject to frequent structural changes—such as agricultural croplands—microbial activity is notably higher due to the regular exposure and decomposition of previously inaccessible organic matter. This increased accessibility enhances microbial biomass formation, leading to higher elevated CO2 emissions"), but I do not see how you can derive them from this study. Unless I am totally wrong, I see your dynamic model as a natural progression of the soil structure rather than a man-made intervention. Is this accurate? This means that, with or without human intervention, the dynamic nature of soil structure leads to greater emissions, so it needs to be accounted for in models. I think this is the main conclusion. I could derive these conclusions if you had a well-mixed model that could simulate the field after tillage, for example.

Thank you for highlighting these aspects. Firstly, we refer to slow cycling for the turnover of POM and necromass, and to fast cycling for the easily available carbon stemming from exudates. Although we assume the same underlying degradation rate for POM and necromass, their creation in the model is very different. POM is an external source while the growth of necromass is highly dynamic and dependent on the growth of biomass, which in turn needs the appropriate C:N relations. The result of this interplay of growth and decay is given in Fig. 11. Note that the constant recycling of microbial biomass during growth is a process respected by our approach. The statement on the enhanced decay is just a consequence of the fact that our model takes into account the degree of occlusion of the particles in the degradation rate, and thus less occlusion (given by intensified break-up of structures by external forces, e.g.) leads to higher turnover (and thus higher CO2 production). This has been highlighted already in a previous paper comparing different soils and degradation rates (Zech et al., 2022, Global Change Biology). We do not distinguish different processes for the break-up, but just have a random probability for each junction to break up again. The reason could be earthworms, tillage or disturbances. However, it is correct that our focus lies on the natural re-aggregation processes. We deleted the statement referring to a larger scale.

Minor comments:

Line 10: What does µCT data mean?

Indeed µCT data are not explicitly used here so we delete this from the introduction. The particle shapes stem from dynamic image analyses.

Line 354: What do you mean by "favorable conditions"? I assume this is based solely on C concentration because the model has no temperature or moisture dependencies.

Yes indeed we refer to the C:N ratio here. We reformulated the sentence.

Line 524: Maybe delete "elevated"

We removed the word.