Responses to Reviewer #3

We thank Reviewer #3 for their comments. Our responses are reported below in normal font, while the reviewer's comments are in *italic*.

The submitted manuscript aims at improving our understanding of the formation of mineral associated organic matter (MAOM), particularly the relative contributions of plant versus microbial derived carbon to MAOM formation. Therefore, they apply the innovative approach of fitting respective parameters of a simple soil microbial model in phase space. The datasets used are from decomposition experiments, where added labelled residues are traced from the particulate organic matter (POM) to the MAOM fraction. Instead of looking at temporal changes, the relative proportional changes of POM and MAOM along the decomposition continuum are mathematically formulated and solved, considering both C- and N- dynamics. The results showed that the in-vivo pathway (microbial derived) was more important than the ex-vivo pathway (plant-derived) MAOM-C and particularly MAOM-N formation. It was further possible to identify some controls on the relative importance of the two pathways. The authors conclude that the in vivo-pathway is particularly important in clay rich and carbon poor soils and that this path is accordingly stronger controlled by available mineral surfaces. The authors did a great job in writing and explaining each modelling step in the manuscript and I enjoyed reading it. I also believe that this could be an interesting new diagnostic, potentially applicable to the interpretation also of other incubation studies looking at the fate of labelled substrates in soils. Nevertheless, I still have some open questions as outlined below.

Thanks for these positive comments! we agree that the methodology can be adapted to other contexts where the goal is to study the pathway of transformation (stabilization as done here, or partitioning of carbon between microbial growth and respiration etc.) instead of the speed of the biogeochemical reactions.

Since not being a mathematician, I am wondering how sensitive the model output is towards violating some of the assumptions made when solving the equation. One is the assumption that microbial biomass is in quasi-steady state. This might apply for individual states of the model, but not when integrating across the decomposition continuum? Since there is no input to the model, microbial biomass is first building up after residue addition (growth > mortality) and then declining (growth < mortality) during the subsequent starvation phase. Both phases are required to reconcile the studied decomposition patterns.

Reviewer #2 had a similar comment regarding our assumption that microbes at quasi-equilibrium, so we respond to both comments in the same way.

The reviewer is correct that in these experiments microbial biomass likely increases first and then decreases when the substrate is consumed. We confirmed this trend by modelling (numerically) both substrates and microbial biomass in residues + POM and in MAOM (Eq. 1-2 and 5-6 in the main text), as shown in Figures R1 and R2 (denoted as 'full model'). For these model runs, we selected parameters reflecting the findings in the submitted manuscript and thus representative of the soil incubations we analyzed, but—different from the analytical model presented in the manuscript—we also had to specify kinetic parameters for residues + POM and microbial biomass. For the former we assumed residues + POM with a 6 month turnover time (reasonable for labile residues), whereas for the latter we considered two turnover rates (6 or 2 months, consistent with the range estimated for surface soils by Spohn et al., 2016). With the lower microbial mortality, there are some discrepancies between the full model and the minimal model presented in the manuscript—specifically, C accumulates in microbial biomass before being released and stabilized in MAOM in the full model, different from the fast transfer of C to MAOM in the minimal model (Figure R1B). This correspond to lower curves in the phase space—i.e., lower C in MAOM for a given C in residues + POM (Figure R1C). Thus, in the presence of microbial biomass with relatively slow turnover, we might be overestimating MAOM accumulation. However, this error decreases with faster microbial turnover (Figure R2A, B), or with slower decomposition rate of residues + POM (not shown). We can thus conclude that our approximation that microbes are in quasi-equilibrium is reasonable except when both microbial turnover is slow and residue turnover is fast.

While conducting this analysis, we also compared the analytical solution of the model in phase space to the numerical solution obtained by plotting the numerically simulated time trajectory of C in MAOM as a function of C in residues + POM. As expected, the two results are undistinguishable (black and gray dashed curves in Figure R1C and R2C). Adding these figures in a revised manuscript does not seem necessary, but we would include this discussion on the quasi-equilibrium approximation in a new section of the Discussion "4.2. Model limitations." In this new section, we would explain: "The model was constructed with five compartments (including POM and MAOM substrates and microbial biomass, as well as DOM), but assuming that microbial biomass and DOM are at quasi-equilibrium allows reducing the model to two compartments. This simplification has minor consequences on the POM and MAOM dynamics as long as both microbial biomass and DOM turn over faster than the POM and MAOM substrates. Microbial biomass has a turnover time in the order of a few months (Spohn et al., 2016) and DOM dynamics are even faster—shorter than the turnover of POM and MAOM. Therefore, our quasi-equilibrium assumption appears to be reasonable."
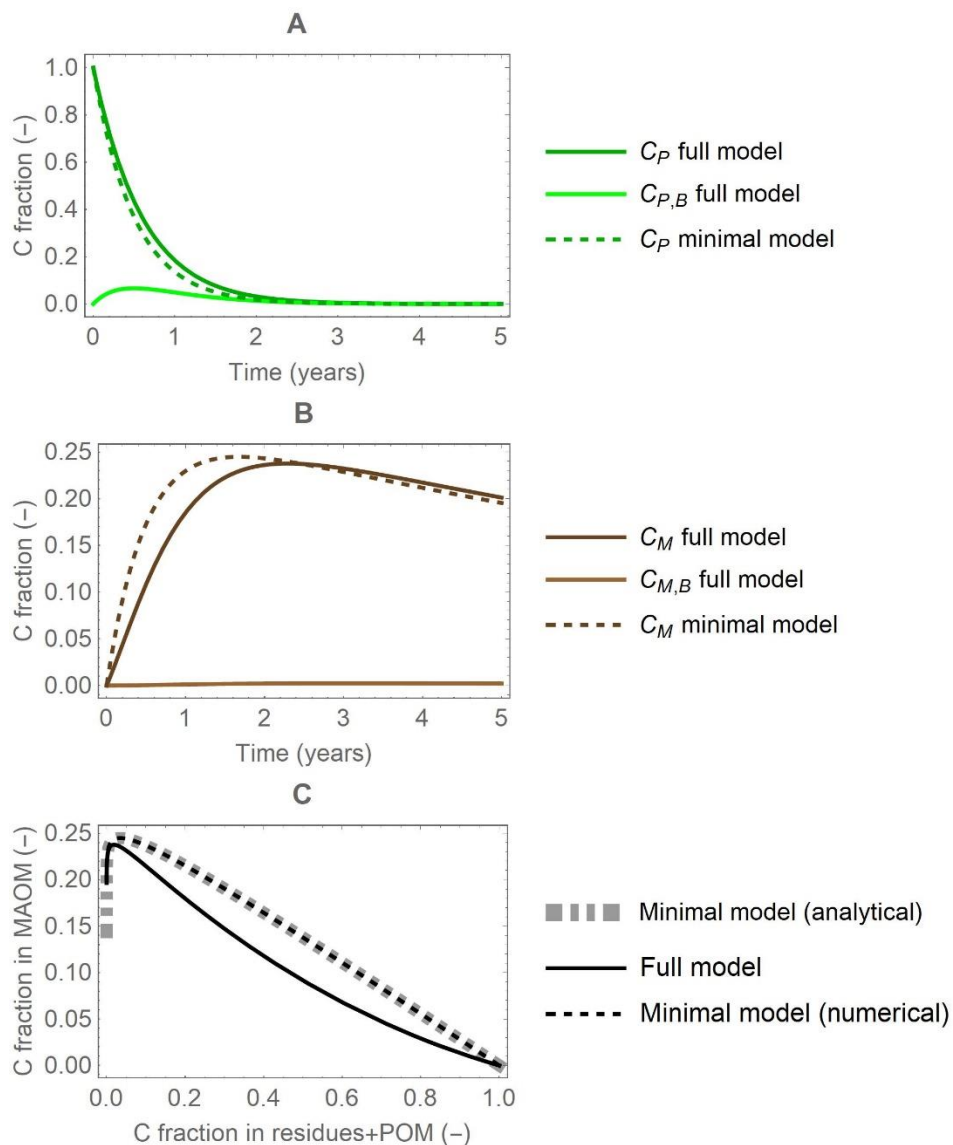
Figure R1. Temporal trends in the fractions of added C in: A) residues + POM (microbial biomass, c_{PB}, and total, c_P) and B) MAOM (microbial biomass, c_{MB}, and total, c_M). C) Phase space representation of the same time trajectories—i.e., c_M is plotted as a function of c_P. In all panels, curves refer to the 'full model' where microbial biomass dynamics are included (solid lines), and the 'minimal model' where microbial biomass is assumed in quasi-equilibrium (dashed lines). In panel C, the minimal model is solved both numerically (black dashed line) and analytically as in the submitted manuscript (gray thick dashed line). Parameters: b=1, m=1, l=0.1, e=0.2, κ=0.05; we also assumed first order kinetics for residues + POM decomposition, with decay constant of 2 y^{-1}, and for microbial mortality, with rate constant of 2 y^{-1}.
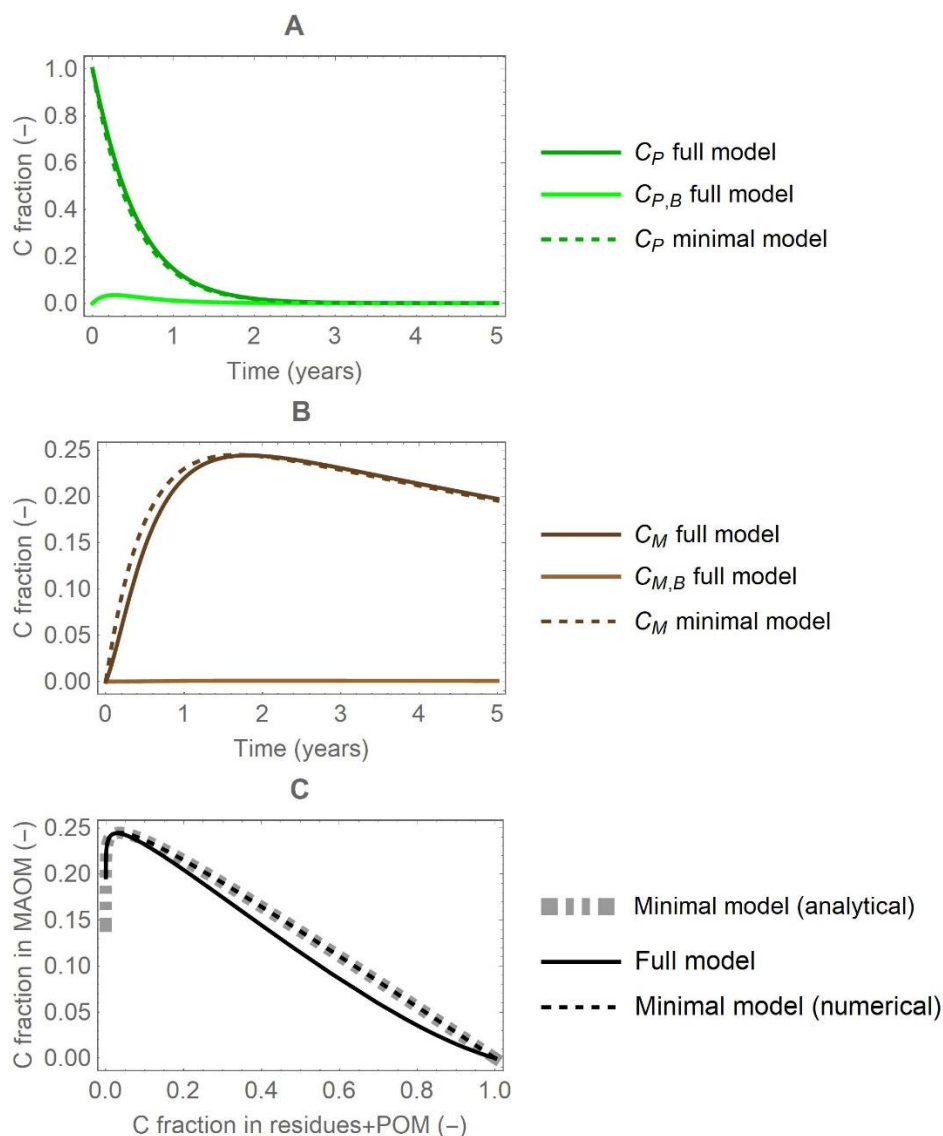
Figure R2. Same as Figure R1, but with a microbial mortality rate constant of 6 y⁻¹.

Also, equation 8 suggests that the decomposition of the POM and the MAOM pool are proportional along the decomposition process (right?). Is that the case in the real world, or would it not change e.g. with increasing depletion of easily degradable compounds with increasing decomposition in the POM fraction, which is not necessarily paralleled in the MAOM fraction when this is indeed built up from microbial residues?

Equation (8) (i.e., $D_M \approx \kappa D_P \frac{c_M}{c_P}$,) states that there is a proportionality between MAOM decomposition rate constant and residues + POM decomposition rate constant. This does not mean that the rates themselves are proportional. In fact, we assume that both decomposition rates (for MAOM, D_M, and for residues + POM, D_P) are linear functions of the carbon contents in those two compartments (for MAOM, c_M, and for residues + POM, c_P). By definition, the decomposition rate constants for these two rates are given by the rates divided by the carbon contents in the respective compartment (for MAOM, $\frac{D_M}{c_M}$, and for residues + POM, $\frac{D_P}{c_P}$). The only assumption we make is that these rate constants are proportional. Thus, it is still possible for the decomposition rate of residues + POM to be at first larger than that of MAOM, and then lower when most of the residue carbon is already in the MAOM compartment. We can clarify this in the Methods (after Eq. (8)): "This assumption only implies a proportionality between the decay constants, while the actual rates will still be different depending on the relative abundance of C in residues + POM and MAOM."

In the second part of this comment, the reviewer correctly points out that the chemical characteristics of the carbon compartments differ through time. Indeed, we have not accounted for such a change. It is possible that the decay constant for the residues + POM decomposition decreases through time due to consumption of labile compounds, but to capture this effect we would need a model with more compartments and thus more parameters. In most datasets we used, POM and MAOM were regarded as lumped

compartments without chemical differentiation. Therefore, it would be difficult to parameterize a more complex model with these datasets. We can comment on this model limitation in a new Discussion section "4.2. Model limitations."

In this new section we would explain: "POM and MAOM contain compounds with contrasting chemical characteristics (depending on residue chemistry in POM and on the stabilization pathway in MAOM), but we neglected these chemical differences both to keep the model simple and because of limited data to parameterize more than one compartment for POM and one for MAOM. As a consequence, we also neglected the decreasing rates of decomposition through time as a result of accumulating recalcitrant compounds. However, we can expect that less decomposable compounds remain in both POM and MAOM, so that the ratio of the decay constants for these compartments (i.e., parameter κ) should remain relatively stable, which is the only assumption we need to make in our derivation. Therefore, neglecting chemical heterogeneity would have major consequences when predicting decomposition rates, but is likely less problematic when modelling POM and MAOM in phase space."

Another assumption of the modelling approach is that ex-vivo and in-vivo formed MAOM have the same decomposition. Since they will differ in chemical composition and CN ratio, this might not necessarily be the case as also discussed by the authors in lines 545ff and could also have biased the results.

This is also a good point, but as explained above, with the current data we could not test this very reasonable hypothesis. We would explain our rationale for chemically lumped compartments in the new section "4.2. Model limitations"—please see our response above.

If I understand it correctly, parameter fitting was not done based on total carbon and nitrogen in the two pools (POM and MAOM) of the studied soils, but just the fate of labelled residues added and their transfer to the MAOM fraction was considered, right? Correlations to bulk soil carbon concentrations of the original samples were done afterwards to see how the fitted parameters were affected by background SOC?

Yes, the reviewer is correct. Total SOC is used to characterize the soil environment, based on the idea that different SOC contents might affect the contributions of different stabilization pathways via e.g., saturation of mineral surfaces or maintenance of a more or less active microbial community. We can clarify this point in the Methods section 2.2.2: "The model was fitted to residue-derived C and N contents in both residues + POM and MAOM fractions." Moreover, we can specify in section 2.2.3 that SOC is regarded as an index of overall C availability.

Nitrogen will probably play an important role for model parameterization, as the in vivo pathway leads to higher MAOM-N contents than the ex vivo path. Some of the datasets seem to have contained information on both, nitrogen and carbon, others not (table S1). How were the different data-streams then used for model fitting?

Too few datasets contained time series of N contents in POM and MAOM, so we could not use individual N datasets for parameter estimation, as we did for the C data. However, we used N data in Figure 3 to provide a general constraint on the model parameters. We also provide some examples of model fitting in Figure 4, but without using the estimated parameters in further analyses. This can be clarified in the Methods section 2.2.2: "Too few datasets included residue-derived N contents in soil fractions for a systematic analysis, so model parameters were fitted only to the C data, except for a few examples."

I was surprised that Figure 3 shows a proportional decline of C and N in POM with decomposition, since my understanding was so far, based on litter decomposition experiments (which should be equivalent to POM decomposition), that C is lost in excess to N, leading to a relative enrichment of N versus C particularly during the early stages of litter decomposition (e.g. https://doi.org/10.1016/j.ejsobi.2018.02.003, https://doi.org/10.1007/s10021-004-0026-x). The concept of the present study and outcome of the model-data integration is now, that if litter or POM is decomposed next to minerals instead of in litter bags, nitrogen is transferred to the mineral phase as microbial necromass and not accumulating in the POM fraction. It would be nice, if the authors could elaborate on these different observations a bit more.

We agree that this pattern is surprising. We are familiar with the CIDET dataset mentioned by the reviewer (Moore et al., 2006), and other empirical studies showed the same pattern (Parton et al., 2007). In our own work—though from a more theoretical perspective—we also found faster loss of C compared to N or P during litter decomposition (Manzoni et al., 2008, 2010). Yet, the data is clear, indicating that when mixed in the soil, residues lose C and N at rates proportional to their initial C:N ratio—i.e., with no preferential retention of N. As the reviewer suggests, from a purely stoichiometric perspective, this pattern could be explained by N-rich necromass being preferentially associated with soil minerals and thus being recovered in the MAOM fraction. This would be different from litterbag studies where necromass is recycled locally contributing to litter N enrichment during decomposition. We could elaborate these points at the end of Section 4.3 "Reconciling contrasting decomposition patterns in phase space."

I was further wondering, how the transformation from organic to inorganic N and the potential stabilization of ammonium on minerals was considered in the cited experiments? If N accumulation on minerals by sorption as inorganic N is not considered,

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could that bias the results in soils rich in clay minerals? Could this have contributed to the observation, that the in vivo pathway is more important in clay rich soils?

The experiments did not distinguish between N stabilized in MAOM in organic vs. inorganic forms. Indeed, both processes might have occurred—stabilization of organic N or of mineral N from mineralization of the residues, even though inorganic N form are typically negligible when compared to the organic forms. The N from the added residues recovered in the MAOM fraction is the result of both processes. Therefore—if we understand the reviewer's comment correctly—there would be no bias due to some N remaining unaccounted for.

It also surprised me, that the in vivo pathway should be more sensitive to saturation than the ex-vivo pathway (e.g. line 561). Microbes can also live and die directly attached to mineral surfaces. Probably more microbes can potentially live on mineral surfaces if these provide more substrate at higher OM-loading, so I am wondering what the mechanism behind the expected higher sensitivity of the in vivo pathway to saturation should be.

Thanks for this curiosity. While there are no definitive explanations for this finding, it is consistent with the current understanding that the organic matter directly bonded to minerals is N-(and P-)rich (Spohn, 2024), and therefore more likely of direct microbial origin. This would be the organic matter susceptible to saturation, being dependent on the active mineral surface availability. The C-rich organic matter (i.e., more likely derived from the ex-vivo pathway) appears to be indirectly bonded to minerals, via organic matter-organic matter interactions (Spohn, 2024). This "vertical structure" of OM on minerals is advocated to not be constrained by saturation (Begill et al., 2023). We can summarize these arguments in Section 4.5: "This finding is consistent with N-rich organic matter—likely of microbial origin—directly bonding to minerals (Spohn, 2024) and thus being dependent on the availability of active mineral surfaces. In contrast, C-rich organic matter from the ex-vivo pathway tends to indirectly bond to minerals through organic matter-organic matter interactions (Spohn, 2024) and is thus less constrained by saturation of the mineral surfaces (Begill et al., 2023)."

The results presented in Figures 5 and 6 regarding the relation between microbial CUE and CN ratios seem contradictory: while it is positive in Figure 5, it is negative in Figure 6. What is the difference? This should be clarified, since in the discussion the focus is on the observed negative relation (line 565 ff).

The change in the slope from positive in the univariate regression to negative in the multivariate regression may be caused by i) the effects of data grouping (the multivariate regression is performed using a linear mixed effect model, with data source as a random factor) and ii) the effects introduced by interactions involving residue C:N (even though such effects are not statistically significant). This comment made us realize that the univariate relations might be confusing, because they offer an incomplete view on the actual drivers of each parameter. Therefore, we would remove them from a revised manuscript, leaving only the box plots (left column in Figure 5) to illustrate the parameter ranges and distributions. The statistical analysis would then focus on the more complete linear mixed effect model. Moreover, data points could be added in Figure 6B-C, to show the spread of the data in a scatter-plot format.

It was not clear to me, why the authors always use the term "residues + POM" throughout the manuscript – what is the difference? Does the term "residue" stand for the labelled material added and traced into MAOM? Why is the same distinction then not also necessary for MAOM when it is composed of both, residue- and POM-derived OM?

We used the term "residues + POM" to indicate C or N originated from the added residues that is either still in the form of "residue" (fragments larger than 2 mm or separated by hand) or POM (obtained via size or density fractionation). In many datasets, residues were not removed prior to fractionation, so it was not possible to distinguish between residues and POM. Therefore, we chose to model residues and POM combined, and MAOM in a separate compartment. We can clarify in Section 2.1.1 "The choice of merging these two fractions in one model compartment is motivated by the fact that in many datasets residues and POM were not separated." Further details on the type of data used and data processing are given in Section 2.2.1.

In line 19 of the abstract, the authors state that MAOM is fuelled by microbes decomposing POM – since there were two microbial pools in the model – can a distinction be made how much MAOM is from each microbial pool? Similar to direct input by microbes in the MAOM, also plant/ex vivo-derived MAOM is subsequently recycled in microbial biomass – is this not also a kind of in vivo OM transformation, since it will also affect MAOM CN ratios?

The statement in the Abstract is accurate, as the flow of C from residues + POM to MAOM is dominated by the in vivo pathway according to our results. Within MAOM there is indeed recycling of microbial necromass, but that does not contribute to MAOM accumulation—only to C and N cycling within the MAOM compartment. With time, more and more of the MAOM will be composed of necromass from MAOM microbes. In a sense we agree that this is also an in vivo transformation, but we would use this term to refer specifically to stabilization into MAOM of necromass from microbes decomposing residues + POM.

Mathematically, the fraction of MAOM originated from either one or the other microbial group could be calculated, but we are not sure it would add to our discussion about the transfer of C and N from residues + POM to MAOM.

Line 539: remove "tend" or adapt accordingly

Thanks, that was a leftover from a text edit.

References

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