Responses to Reviewer #2

We thank Reviewer #2 for their comments. Our responses are reported below in normal font, while the reviewer's comments are in *italic*.

Manzoni and Cotrufo propose a two-compartment model (POM + residues and MAOM), which can be used to diagnose through which pathway (in vivo vs. ex vivo) C and N are stabilized. The simplicity of the model reduces the number of parameters and allows the authors to solve their equations analytically in the so-called space phase, i.e. they focus on relations between compartments instead of analyzing temporal trajectories to detect underlaying mechanisms. The model suggests that stabilization via the in vivo pathway is more relevant in clay-rich soils and less relevant in C-rich soils, whereby the calculated fractions seem to be higher than reported by earlier studies. Overall, the paper is well (and lively!) written and the chosen approach is an interesting method to explain potentially appearing inconsistencies in data sets. E.g. the model is able to capture MAOM stabilization during early decomposition and destabilization of MAOM in late decomposition by the same mechanism. From that perspective and because it adds a new insight into the process of C and N stabilization, the paper is a highly relevant for the BG community. Also, discrepancies with earlier studies are mentioned and discussed appropriately.

We thank the reviewer for their supportive comments—both on our writing style and on the content of the manuscript.

However, I'm concerned about the assumptions and approximations, which are made during the derivation of the solution, and that are a bit loosely justified (see below), and would ask the authors to elaborate these parts a bit further.

We agree that assumptions need to be spelled out clearly—please see our responses below.

As mentioned before, I'd ask the authors to explain used assumptions and approximations in more detail. Especially the assumption of (quasi-)equilibrium needs more explanation and discussion. Why (under which circumstances) can quasi-equilibrium be assumed?

Reviewer #3 had a similar comment regarding our assumption that microbes at quasi-equilibrium, so we respond to both comments in the same way.

To assess the consequences of our quasi-equilibrium assumption, we modelled (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.

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."
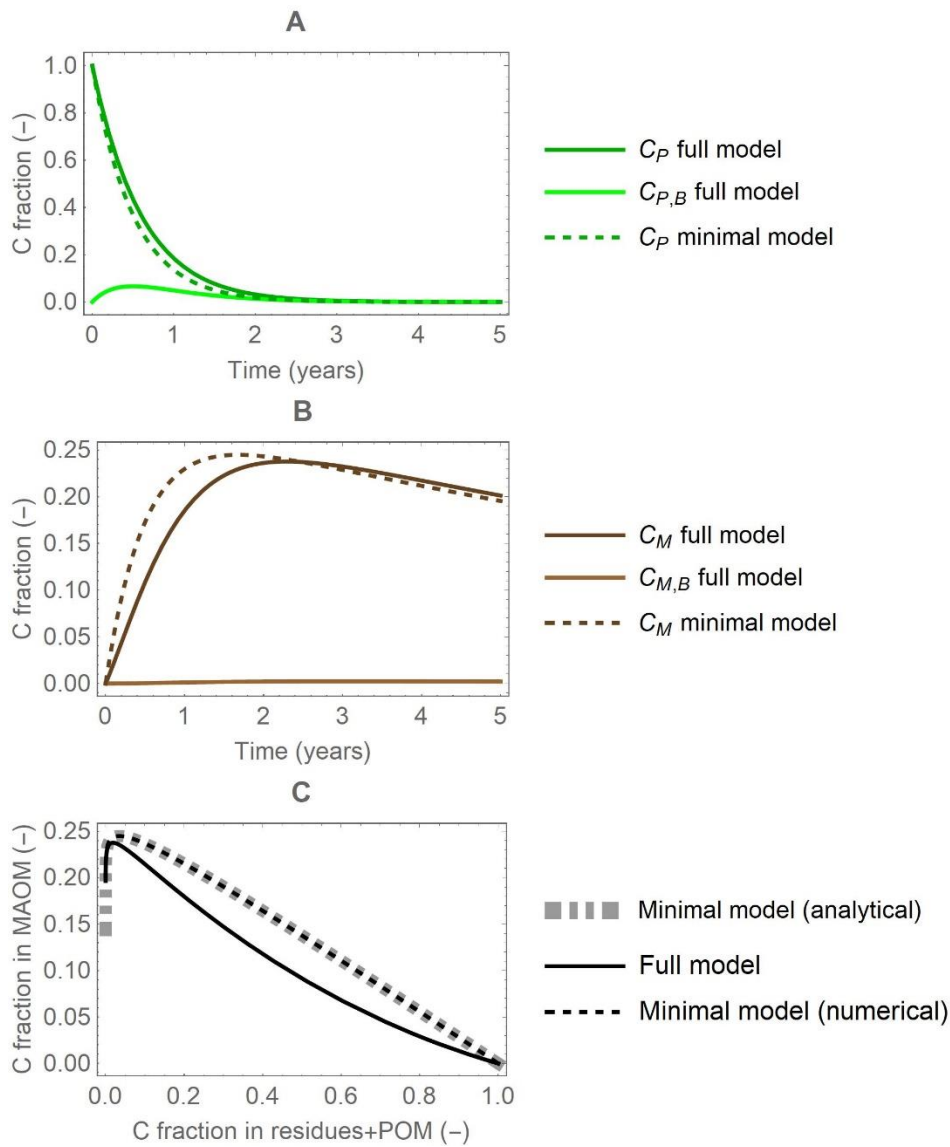
Figure R1. Temporal trends in the fractions of added C in: A) residues + POM (microbial biomass, c_{PB}, and total, c_P) and B) MAOM (microbial biomass, c_{MB}, and total, c_M). C) Phase space representation of the same time trajectories—i.e., c_M is plotted as a function of c_P. In all panels, curves 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}.
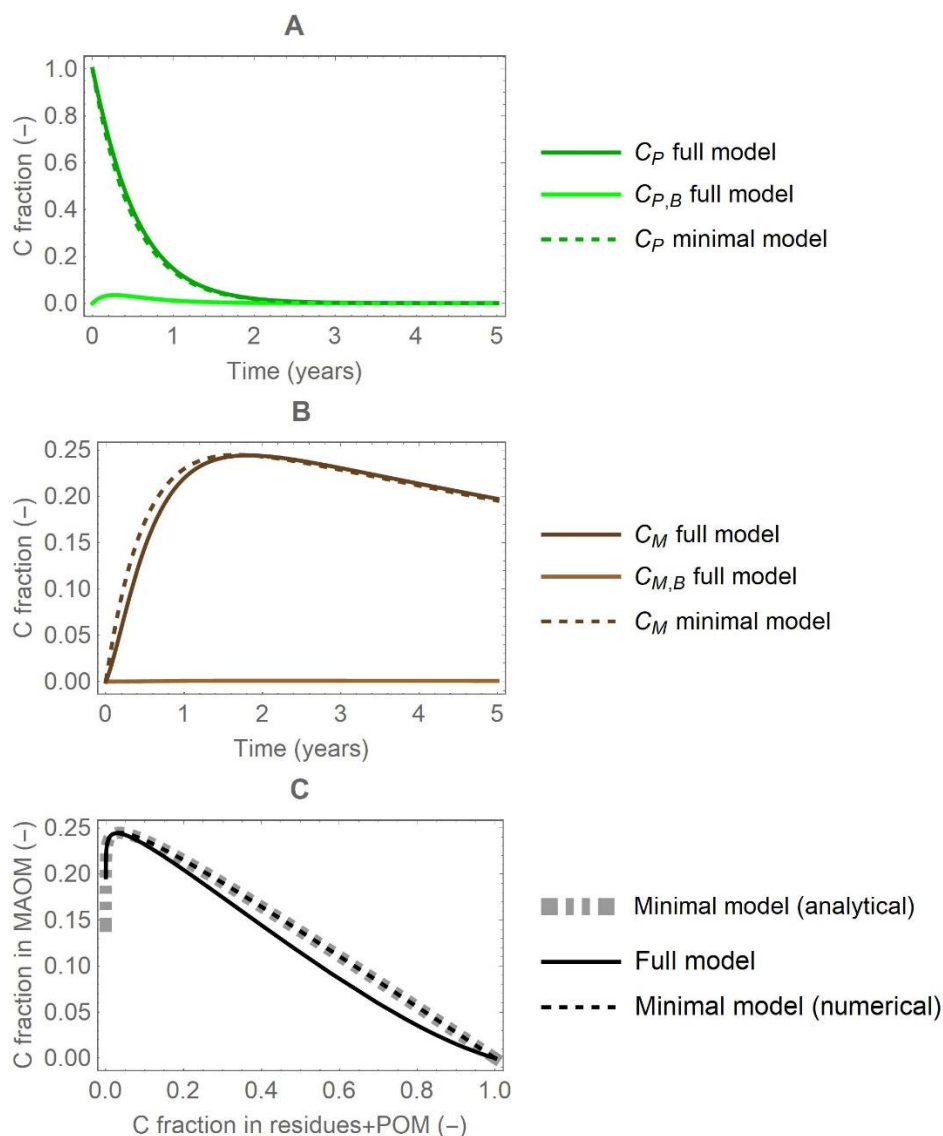
Figure R2. Same as Figure R1, but with a microbial mortality rate constant of 6 y^{-1}.

Are the data filtered by that?

No, there is no way from the data available to filter datasets according to the turnover rate of microbial biomass or DOC. Our arguments for model simplification are general and not data-driven.

How does this relate to the later discussion about "early" and "late" decomposition?

Early and late decomposition phases are discussed based on residue + POM decomposition and MAOM accumulation and subsequent decomposition, which are both evident from the data and model results even when assuming that microorganisms are in quasi-equilibrium.

How do this assumption and the other assumptions and approximations, which are used to derive the final analytically-solvable model, i.e. the first-order decay with a constant decomposition rate or the "similar" C:N ratios, affect model results and limit their transferability, e.g. under a changing climate?

We would like to first clarify that we did not assume first-order decay, but only that the decay rate constants for residue + POM and MAOM decomposition are proportional. This assumption is less strict than assuming first-order decay for these processes. Regarding our assumption of a single value for microbial biomass C:N ratio, we could not find evidence in the literature of different microbial C:N between POM and MAOM fractions. It is possible that microbial communities associated with POM are more fungal dominated and thus have higher C:N, but we can only speculate about the extent of this difference. We can elaborate on the various model assumptions—including these ones—in a new Discussion section "4.2. Model limitations."

In this new section we would explain: "microorganisms growing on POM are likely different from those feeding on organic matter desorbed from minerals. For example, we could expect higher fungal to bacterial ratio in POM, with higher microbial biomass C:N and possibly lower CUE (Soares and Rousk, 2019), but lack of specific information on microbial traits within the soil fractions does not allow us to parameterize these communities in the model (though soil fraction-specific traits are retained in the general solutions of the mass balance equations)."

Figure 2: I think, it would help the reader, if you could add a reference figure (base values used) to understand the overall model behavior and the direction of the changes. I would also like to see the varied values, i.e. the values that are used to derive the solid and dashed lines in each sub-figure, in the figure description.

We can revise Figure 2 according to this suggestion, by adding a column with model results obtained using the baseline parameter values (Figure R2). The values of the parameters used to make each panel are listed in the legends at the bottom of the figure, so it seems redundant to also report them in the caption.
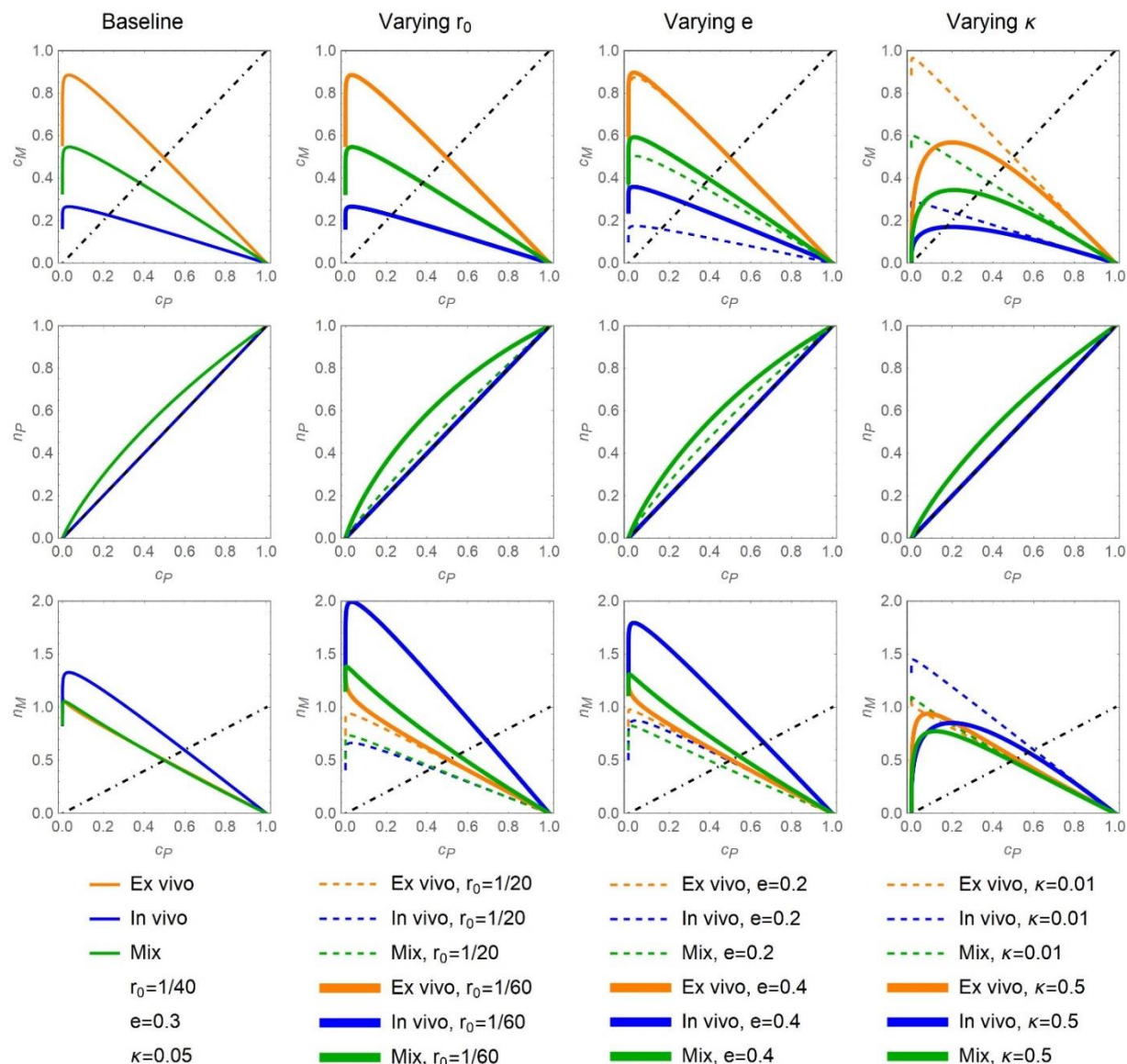

Figure 2. Fraction of added C in MAOM, c_M (top row), fraction of added N in residues + POM, n_P (center row), and fraction of added N in MAOM, n_M (bottom row), as a function of the fraction of added C in residues + POM, c_P, under different stabilization pathway scenarios (colors) and when varying the values of model parameters around baseline values shown in the 1st column: residue N:C ratio, r_0 (2nd column), microbial carbon use efficiency, e (3rd column), and ratio between the decay constants of MAOM and residues + POM decomposition, κ (4th column). Three stabilization scenarios are considered: dominant ex vivo stabilization ($l = 1$; orange), dominant in vivo stabilization ($l = 0, m = 1$; blue), and a combination of pathways denoted by 'mix' ($l = 1/2, m = 1/2$; green). In all panels, residue decomposition progresses from right to left along the curves, as c_P decreases. The dot-dashed black lines indicate 1:1 lines, which represent equality between the fractions of added C or N shown on the y-axes and c_P shown on the x-axes; the added residues are assumed to be insoluble ($b = 1$).

What is "Section 0"? (L322, L416)

These are issues with section referencing. They should read "Section 3.2" and "Section 3.4", respectively.

L34 Please check the unit.

Font issue—it should be µm.

Table 2: I personally would like to have the Parameter/Symbol entries all centered (or maybe all right-bounded), but not changing within lines, which seems to happen for all entries that do not have subscripts. Or is there I reason for that?

No particular reason, but it seems equation objects sit in the center by default despite normal text being aligned to the left. The table can be easily formatted so that all symbols are centered.

References

Soares, M., Rousk, J., 2019. Microbial growth and carbon use efficiency in soil: Links to fungal-bacterial dominance, SOC-quality and stoichiometry. Soil Biology and Biochemistry 131, 195–205. doi:10.1016/j.soilbio.2019.01.010

Spohn, M., Pötsch, E.M., Eichorst, S.A., Woebken, D., Wanek, W., Richter, A., 2016. Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland. Soil Biology and Biochemistry 97, 168–175. doi:10.1016/j.soilbio.2016.03.008