Responses to Reviewer #1

We thank Reviewer #1 for their comments. Our responses are reported below in normal font, while the reviewer's comments are in *italic*.

This study proposes a phase-space model fitted to about 40 studies which estimates that ~75% of MAOM C and nearly all of MAOM N are stabilized by the in vivo pathway, in contrast to measurements which tend to be lower than this. The authors posit a few reasons for the discrepancy, including ex vivo stabilization pathways missing from the model, and the most interesting which is that necromass may be stabilized faster but turns over at a faster rate, resulting in less persistent MAOM from necromass.

We agree that some results might be surprising, but can still be explained in light of current conceptual understanding. Besides the different fraction of carbon (or nitrogen) stabilized through the in vivo pathway, in our view it is particularly interesting to see trends with soil texture and organic matter content that are consistent with the idea of saturation of mineral surfaces. Overall the fractions we estimated might be different due to methodological reasons, but the underlying trends are quite insightful.

I think in order to explore the hypotheses brought up by this study it is useful to run the model considering time. I assume that you would get the same curves that can be seen in the main results (Figs 2 and 4) when running the model forward as differential equations and plotting the model output as the relationship between pools, so I'm not sure what the phase space simplification really adds.

Solving the mass balance equations through time and then plotting one state variable as a function of the other will give the same results as directly solving the system in the phase space (see an example in the response to Reviewer #2). In practice the two approaches lead to the same modelled trajectories, and if the interest is in considering time dynamics, then the first approach should be used. However, the phase space approach has complementary advantages, such as: i) in the phase space, differences in the reaction kinetics do not interfere with the estimation of partitioning coefficients (in our model l and m) that provide information on the stabilization pathways, and ii) the analytical solutions in phase space are sufficiently compact to allow for mathematical insights that would not be possible when solving the model through time. Moreover, a fully analytical solution through time might not be possible if the decomposition kinetics are nonlinear, requiring numerical solutions. Here we do not need to make any assumptions on the decomposition kinetics (besides a proportionality argument), leading to very general analytical solutions in phase space. We discuss these advantages in Section 4.1. We will also address this reviewer's comment by revising Figure 4 (as reported below in Figure R1) and elaborating further on this point in the text in reference to the figure.

I think that retaining the ability to consider absolute values of MAOM and POM and track losses (C and N min) are relevant for addition scenarios in the field where total C retained in the system is as or more relevant than the form of C.

Normalizing the C and N contents in the modelled pools by the added residue C and N does not preclude the possibility to calculate absolute amounts of C and N in the soil pools. The analytical solutions can be simply multiplied by the added mass of C and N to obtain the absolute amounts, which would be expressed as contents, concentrations, or stocks depending on the units used for the added residue mass. We can explain by adding just before Eq. (10) where the normalization step is presented: "Moreover, the equations expressed in normalized form are independent of the units used to quantify inputs and mass in each compartment, and if needed it is easy to convert the normalized variables into absolute quantities by multiplying by the mass of added residue C."

There were similarly some places in the text (L357-359) and Fig. 4 where I was really curious how accumulation of N vs C in MAOM was evolving over time.

We are also curious about C and N accumulation in the MAOM compartment, but the data do not allow a consistent assessment across studies. Each study was designed differently, so sampling started and ended at different times (and different degrees of residue decomposition) and sampling frequency also varied (between 1 and 6 sampling times). Due to the low temporal resolution of the measurements, in most datasets the peak in residue-derived C in MAOM (before being decomposed) is not apparent from the data. Moreover, environmental conditions during the incubations (in the field or in the lab) also differed. Unfortunately, these differences among datasets and in general data limitations do not allow a systematic time series analysis to identify temporal trends in MAOM accumulation.

However, in a revised manuscript we can expand Figure 4 as shown below (Figure R1) to also illustrate the time series of the data, as suggested by the reviewer. As shown in panels A-F of Figure R1, the temporal trends in the data are highly variable (on purpose in this revised figure we would leave the same axis scaling in each row to highlight differences among studies), compared to the clearer patterns emerging in phase space (panels G-I). Other examples are shown below in response to another comment (Figure R3). We could use the expanded Figure 4 to illustrate the point that phase space representations allow to synthesize data more effectively than in the usual time domain, also addressing the reviewer's general comment above.
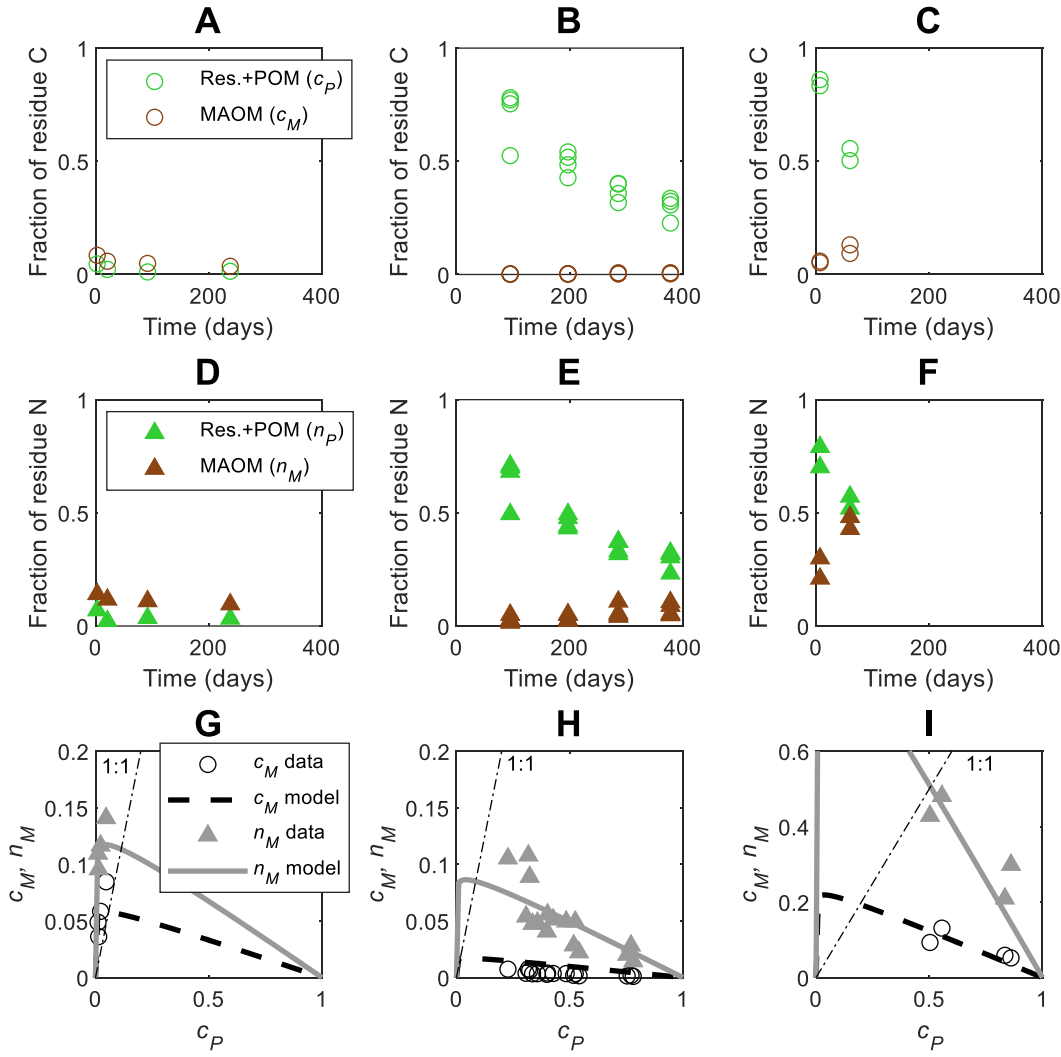
Figure R1. Examples of data time series from incubations (A-F) and model fitting in the phase space (G-I), for residues with increasing residue C:N from the left to the right column: A-C) fractions of added C in residues + POM (c_P, green circles) and MAOM (c_M, brown circles) as a function of time; D-F) fractions of added N in POM + residues (n_P, green triangles) and MAOM (n_M, brown triangles) as a function of time; G-I) c_M (black circles) and n_M (gray triangles) as a function of c_P. In G-I, we fitted parameters e and l in the functions $c_M(c_P)$ and $n_M(c_P)$ with $m = 1$ and $\kappa = 0.05$ (Table 2). Data are from: A, D, G) Buckeridge et al. (2022) (residues: *Escherichia coli* necromass, C:N=3.4), B, E, H) Mitchell et al. (2018) (residues: *Chloris gayana*, C:N=14.2), and C, F, I) Lavallee et al. (2018) (residues: *Andropogon gerardii*, silt soil, C:N=28.2). In G-I, residue decomposition progresses from right to left along the curves, as c_P decreases; the dot-dashed 1:1 lines represent equality between the fractions of added C or N shown on the y-axes and c_P shown on the x-axes.

More in general, in a revised manuscript we can assess to what degree C and N in MAOM increase or decrease in relation to the fraction of remaining C in residues + POM. This analysis would show accumulation pathways as the reviewer suggests, but without (or with less) variability induced by environmental conditions across studies. In practice, such trends can be evaluated numerically from pairs of data points acquired in subsequent times, by computing the change in MAOM (C or N) per unit change in residues + POM (C or N). These local slopes of the MAOM vs. residues + POM relations can then be plotted as a function of residues + POM to identify MAOM accumulation and depletion phases. A negative slope indicates accumulation of MAOM as residues + POM are depleted, whereas a positive slope indicates a depletion of both MAOM and residues + POM. Overall, the slopes are negative at high values of the fraction of residues + POM (early decomposition phase), but they turn negative at low values of the fraction of residues + POM (late decomposition phase) (Figure R2). These trends are consistent for C (Figure R2A) and N (Figure R2B). The turning points when MAOM starts being depleted are at a fraction of remaining C in residues + POM of ≈0.26 and at a fraction of remaining N in residues + POM of ≈0.18. This new figure would be included in the Appendix and referred to in Section 3.3, where examples of time series will be shown. We hope that such an analysis can at least partly satisfy readers interested in residue-derived MAOM accumulation and depletion.
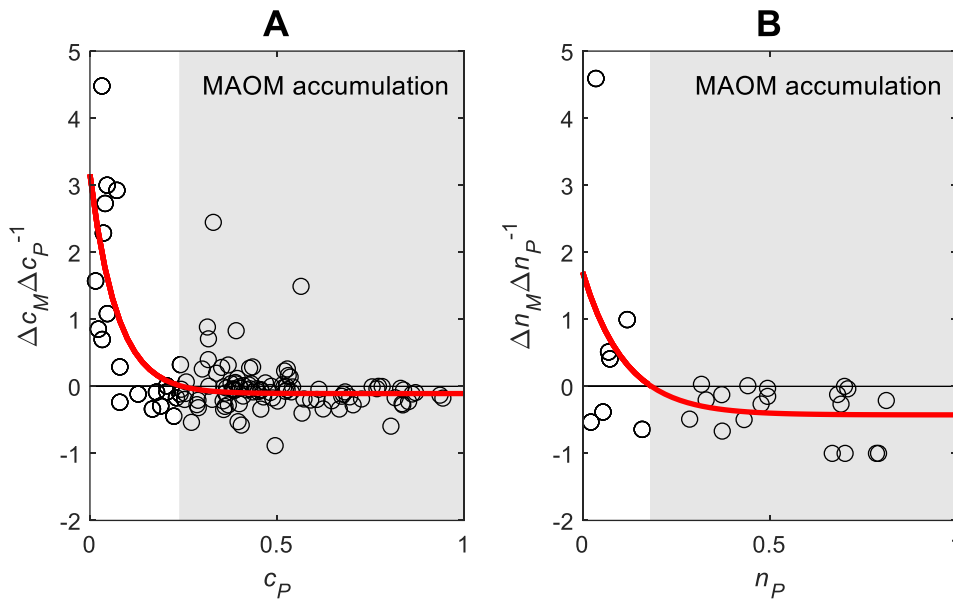
Figure R2. Changes in MAOM per unit change in residues + POM, as a function of remaining residues + POM, for A) residue C and B) residue N. As in the submitted manuscript, c_P and c_M (n_P and n_M) denote the fractions of remaining C (respectively N) in residues + POM and MAOM. Time progresses from right to left as c_P and n_P decrease. Data points are from all datasets containing at least two subsequent measurements for the same treatment, site, and residue type. Solid curves are fitting exponential functions with an asymptote (we excluded from the regression outliers defined as values lower than the 3th and higher than the 97th percentile). The MAOM accumulation phase is highlighted in light gray.

I suppose my ask to the authors is to justify to readers the utility of phase space modeling compared to running the time-dependent form of the model, or to use time-dependent modeling to more completely address some of the hypotheses advanced, especially about N vs C accumulation in MAOM.

Thanks for this suggestion, we are happy to provide this justification as also explained above. However, we believe that analyzing soil fraction data in phase space helps focusing on intrinsic properties. To illustrate them for the interest of the reviewer we provide a couple of examples (Figure R3). In one example, chemically different litter types are incubated in the same conditions (*Sorghum* residues), whereas in the second example the same litter type is incubated with or without additional mineral N (*Miscanthus* residues). In both cases, the temporal dynamics of residues + POM (Figure R3A) and MAOM (Figure R3B) exhibit large variability due to either the different litter chemistry or the incubation conditions, but the trajectories in the phase space converge to similar patterns (see orange and blue lines or yellow and green lines in Figure R3C). We would not include Figure R3 in a revised manuscript, as the same point is already illustrated in the proposed Figures R1 and R2.

In synthesis, we agree with the reviewer that the temporal dynamics contain additional information, but that will mostly reflect incubation conditions rather than the intrinsic capacity of the soil and its microbial community to promote organic matter stabilization in MAOM through the in vivo or ex vivo pathway. A study focusing on the temporal dynamics would be useful to test the effect of soil moisture or temperature on the kinetics of the stabilization, or to compare field-based to lab-based incubations, but these questions are outside our scope in this contribution. The two more conceptual questions we stated in the Introduction (numbered ii and iii) focus on C and N partitioning between pathways rather than on the kinetics of the process: ii) What is the dominant pathway of C and N stabilization in MAOM? iii) What are the drivers of the stabilization pathway as represented by model parameters?

Therefore, we will be happy to revise the manuscript as suggested above to better motivate our choice to focus on the phase space rather than the time domain, but we would be reluctant to fit the model to the time series as interpreting patterns in both kinetic constants (which we now do not consider) and partitioning coefficients (our current focus) would be difficult and results would depend on the selected kinetics.
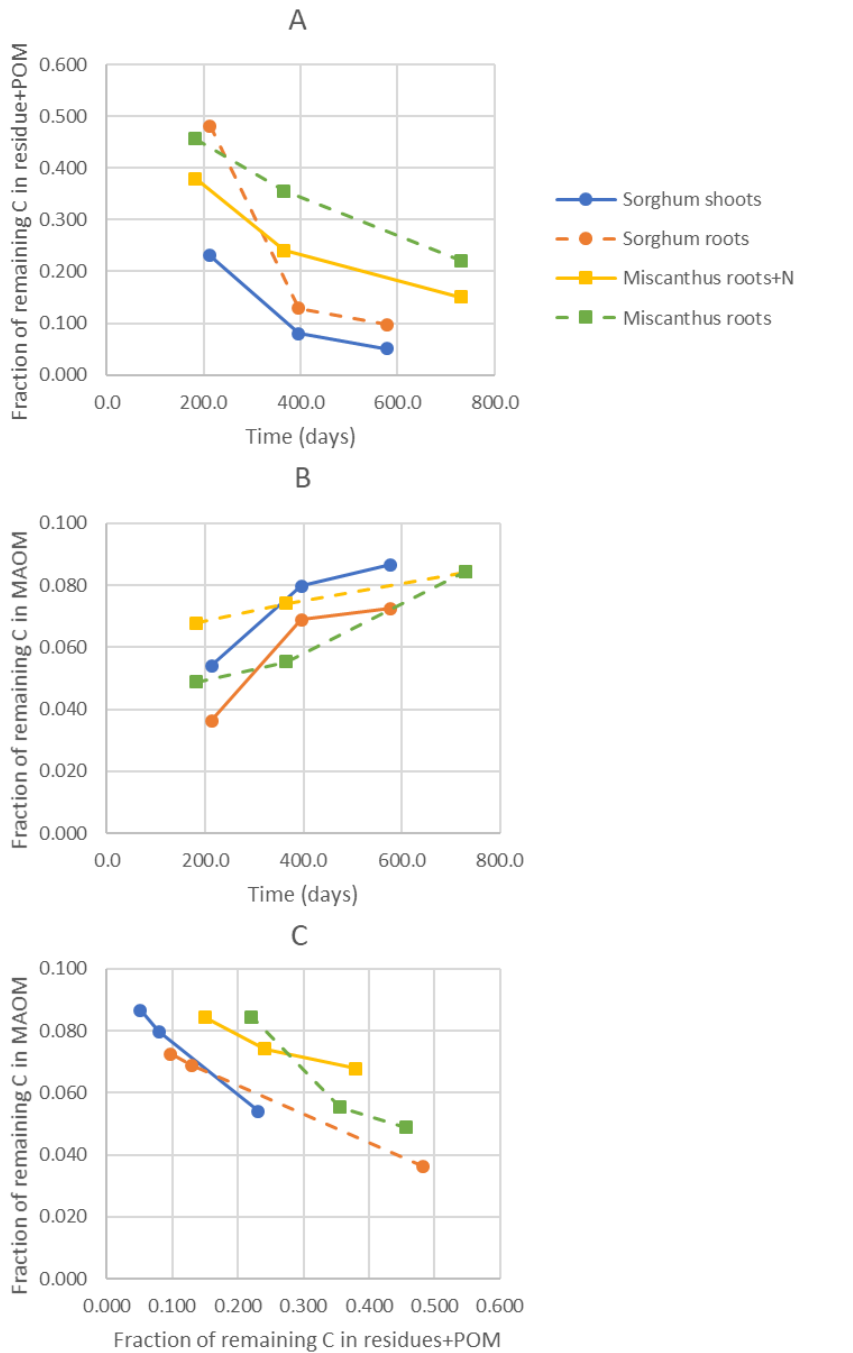
Figure R3. Examples of time trajectories of the fractions of remaining C in either A) residues + POM or B) MAOM; C) phase space representation of the same data—i.e., fraction of remaining C in MAOM as a function of the fraction of remaining C in residues + POM. Colors and symbols refer to different datasets: circles refer to shoots (blue) and roots (orange) of *Sorghum bicolor* (Fulton-Smith and Cotrufo, 2019); squares refer to roots of *Miscanthus sacchariflorus* incubated with additional mineral N (yellow) or without mineral N (green) (Poeplau et al., 2023).

L59-60: I think this sentence needs a main clause verb.

Thanks for catching this mistake. Deleting "and" fixes the problem.

L120: Probably good to acknowledge that some C will be lost as DOC leaching, even if it is small and you don't consider it here.

We can clarify by adding that "Leaching of dissolved organic matter is neglected."

Fig 1: A fraction of necromass is sorbed and the rest goes back into particulate C... what is that fraction based on?

The fraction of necromass that is sorbed is quantified by the parameter m, which is estimated by fitting the model to the data. Therefore, we do not make any assumptions about this fraction, but rather let the data tell if it is large or small. We would not add a clarification on this comment, because the estimation approach for parameter m is presented in Section 2.2.2.

At L322, Section 0 is referenced as where you explain how m is constrained but I could not find this section.

Sorry about this, there was an issue with a section referencing. It should read "Section 3.2"

Eq.10 is the same as Eq. 9 but with a different boundary condition. I would suggest referencing Eq. 9 instead of printing the equation again.

Equation (10) looks similar, but besides the different boundary conditions, it also includes normalized variables (small c instead of capital C), so we would prefer to keep it.

N mineralization assumes a one-way flow where N is mineralized but not taken up again. This is similar to a lot of soil models but not necessarily realistic, so perhaps worth acknowledging that in text somewhere.

We understand the concern, but the reviewer missed that this is not necessarily the case in our formulation of net N mineralization, which accounts for possible net N immobilization when microbial N demand is higher than the N supply from organic matter decomposition. We can add a clarification below Eq. (17): "These formulations for net N mineralization allow capturing both net N release if substrates are sufficiently rich in N ($N_{PS}/C_{PS} > e_P r_{PB}$, $N_{MS}/C_{MS} > e_M r_{MB}$) and net N immobilization when they cannot provide enough N for microorganisms ($N_{PS}/C_{PS} < e_P r_{PB}$, $N_{MS}/C_{MS} < e_M r_{MB}$)."

Table S1: If POM+residues are considered as one category in the model (Fig 1) then why are they separated out here? Please clarify how each category is allocated when used in the model.

The dataset contains data on residues alone, POM alone, or combined residues + POM. As most datasets report combined residues + POM, we chose to use this as a state variable in the model. 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.

L266 seems to imply that those studies without POM+residues reported were not used, does this change the reported sample size used in analyses?

Six studies reporting only POM or only residue C were not used. The reviewer is correct that the total number of studies mentioned in the Abstract and Methods should reflect only the number of studies actually used. We can update those values after we include the additional dataset suggested below.

L357-359: I found it curious that a higher C:N ratio causes lower C accumulation in MAOM but higher N accumulation. Does the C:N of MAOM really decrease in this scenario or is this an artifact of only viewing Cm as relative to Cp which is also changing over time?

Higher residue C:N does not affect C accumulation in MAOM (dashed and solid curves overlap in Figure 4, top left panel); however, higher C:N does promote N accumulation in MAOM because more N is immobilized by microorganisms, converted into necromass, and eventually transferred from the residues + POM to the MAOM compartment.

In Figure 4, it is also interesting that N seems to accumulate faster than C in MAOM, but again we are not looking at time here so travel along either curve could occur at different speeds. Can you discuss this or provide some information about the time component?

The reviewer is correct—we are not looking at temporal trajectories, so it is not possible to read reaction speed in these phase spaces. As suggested above, we can add panels in Figure 4 to illustrate the temporal changes (Figure R1) and comment on those patterns, for example by highlighting that "more N than C accumulate in MAOM at a given time or c_P value (compare Figure 4B and 4E or Figure 4C and 4F), indicating preferential retention and stabilization of N when residues with high C:N are decomposed (as also shown in Figure 2)."

Following from the understanding that in vivo is more important for N than C, I wonder if this can tell us something about the rate of the in vivo pathway vs ex vivo.

We appreciate the reviewer curiosity, but as extensively explained above with the current analysis, we cannot evaluate and rank the rates of C and N stabilization, but only the relative proportions.

Figure 3b: I know there are many data sources, but can the data source color bar be discretized?

Yes, we can replace the existing Figure 3B with Figure R4. However, the difference with respect to the original figure is minimal given the number of discrete levels.
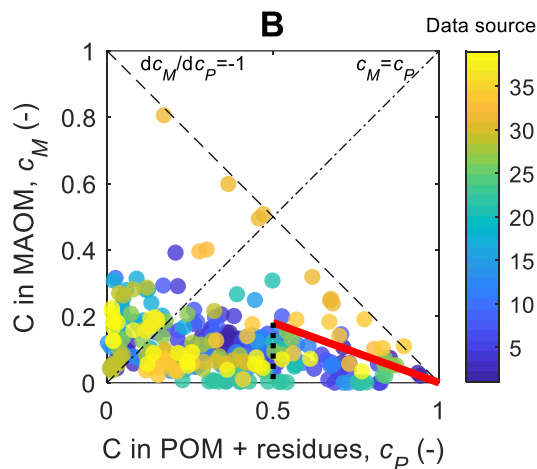

Figure R4. Revised panel B of Figure 4 in the original manuscript, with discretized colorbar.

L466: I was a little surprised to see such a strong prediction of clay trends in B and C but no trend in univariate space (Fig 5). Do you feel confident in the LME prediction?

P-values for the clay effects on the fraction of C and N following the in vivo pathway are low (<0.05) and coefficients are relatively large, so yes, we are confident there is a trend. If invited to revise the manuscript, we can also strengthen our analysis by adding some datasets we found during the review phase (Hilscher and Knicker, 2011; Córdova et al., 2018). To address this comment and similar one by Reviewer #3, we propose to remove the univariate analysis—it is probably more confusing than helpful.

References

Buckeridge, K., Mason, K., Ostle, N., McNamara, N., Grant, H., Whitaker, J., 2022. Microbial necromass carbon and nitrogen persistence are decoupled in agricultural grassland soils. Communications Earth & Environment 3. doi:10.1038/s43247-022-00439-0

Córdova, S., Olk, D., Dietzel, R., Mueller, K., Archontouilis, S., Castellano, M., 2018. Plant litter quality affects the accumulation rate, composition, and stability of mineral-associated soil organic matter. Soil Biology & Biochemistry 125, 115–124. doi:10.1016/j.soilbio.2018.07.010

Fulton-Smith, S., Cotrufo, M., 2019. Pathways of soil organic matter formation from above and belowground inputs in a Sorghum bicolor bioenergy crop. Global Change Biology Bioenergy 11, 971–987. doi:10.1111/gcbb.12598

Hilscher, A., Knicker, H., 2011. Degradation of grass-derived pyrogenic organic material, transport of the residues within a soil column and distribution in soil organic matter fractions during a 28 month microcosm experiment. Organic Geochemistry 42, 42–54. doi:10.1016/j.orggeochem.2010.10.005

Lavallee, J., Conant, R., Paul, E., Cotrufo, M., 2018. Incorporation of shoot versus root-derived 13C and 15N into mineral-associated organic matter fractions: results of a soil slurry incubation with dual-labelled plant material. Biogeochemistry 137, 379–393. doi:10.1007/s10533-018-0428-z

Mitchell, E., Scheer, C., Rowlings, D., Conant, R., Cotrufo, M., Grace, P., 2018. Amount and incorporation of plant residue inputs modify residue stabilisation dynamics in soil organic matter fractions. Agriculture Ecosystems & Environment 256, 82–91. doi:10.1016/j.agee.2017.12.006

Poeplau, C., Begill, N., Liang, Z., Schiedung, M., 2023. Root litter quality drives the dynamic of native mineral-associated organic carbon in a temperate agricultural soil. Plant and Soil. doi:10.1007/s11104-023-06127-y