

Authors' Comment in response to Anonymous Reviewer 2.

Authors: Kayler Z.E., Kaiser M., Gessler A., Ellerbrock R.H., Sommer M

Reviewer's comments italicized.

General comment

In this paper, the authors aimed to gain insights into the level of microbial transformation of stable SOM and its type of interaction with minerals. To do so, they explored the natural abundances in ^{13}C and ^{15}N from a range of soils under arable and forest land use, after isolation of the fraction assumed to contained the stable OM by removal of particulate OM and water extractable OM. In this fraction they separated OM extractable by Na-pyrophosphate from OM non extractable by Na-pyrophosphate as they expected different stabilisation mechanisms to operate in these two fractions. They treated their data with partial least square regression and interpreted their results using a model for OM interaction with mineral surfaces.

The approach of the authors is quite interesting. The paper is very well written and easy to follow. However, the dataset is not really appropriate to reach the goal of the authors. Indeed there initial assumption is that stable isotopes can be used to determine the frations that are microbially processed, which is not exact. Neither enrichment in ^{13}C or in ^{15}N can alone indicate a microbial transformation of OM.

Authors' response: We thank the reviewer for his helpful comments and suggestions that have ultimately improved the manuscript. We also agree, that our statement describing an objective as detecting OM fractions that are microbially processed was presumptive. We have since edited the text describing our goals as follows:

"We analyzed the isotopic signal of OM fractions sequentially separated from a range of soil types under arable and forest land use to investigate patterns of isotopic enrichment in different OM fractions and to determine the type of interaction between OM and soil minerals."

*^{13}C enrichment could reflect other processes such as the Suess effect (as mentioned by the authors), or an enrichment in molecules such as pectin (Glaser, 2005), whereas various ^{15}N enrichment may reveal different patterns for nitrogen mineralisation (see for example *Stable Isotope in Ecology and Environmental Science* for a synthesis or the pioneer studies of Mariotti for ^{15}N). To conclude on the presence of microbial processed OM by the use of isotopes, both ^{13}C and ^{15}N must show the same trend, possibly confirmed by other proxies, such as the C:N ratio for example.*

Authors' response: Perhaps the only conclusive experiment that proves certain molecules in the soil are definitively from microbial products would require direct isotopic labeling in laboratory incubations. This approach would most likely preclude many investigations of long-term field applications and land use; furthermore, questions would arise concerning the applicability of laboratory results to field conditions. Without this information, researchers are left with proxies of hypothesized processes of which stable isotopes is one such proxy.

We agree with the reviewer that stable isotopes alone cannot detect microbially processed OM. We also use proxies of mineral characteristics to aid in our investigation of organo-mineral associations. The combination of the two, isotopes and mineral characteristics, provides a robust basis for interpretation.

The difference between this study and other studies of OM using isotopes, is that we use very specific fractions of OM for our investigation: fractions that are hypothesized to be stabilized through organo-mineral interactions. The consensus from the results from a limited number of studies finds that products found in stabilized OM are processed by microbes prior to stabilization. Once bound to a mineral surface there are chemical processes that can lead to exchange of Carbon, this has been proven with ^{14}C studies. Thus, the patterns in the ^{13}C , as we discuss in the current paper, are not always stable, and one would not necessarily expect similar patterns between ^{13}C and ^{15}N to show the presence of microbially processed OM.

But of more concern is the interpretation of isotope trends in term of microbial processing for arable lands having being submitted to fertilizers input and crop rotation (Kaiser et al., 2011). A maize cropping in the rotation may strongly impact the $d^{13}\text{C}$ value, whereas fertilizers exhibit a broad range of ^{15}N composition depending on their nature (manure, $\text{NH}_4\text{...}$) and impact the soil $d^{15}\text{N}$ for decades, even centuries. Taking this major issue into account, the paper is not currently acceptable for publication.

Authors' Response: We added a table (table 2) of the different crop rotations and fertilization regimes. We further addressed the potential effects of different sources with respect to our interpretation. We added the following text in the discussion:

“Past land management effects are difficult to assess; however, tillage practices are generally thought to destabilize OM occluded in aggregates thus freeing OM for microbial decomposition. In this study, we separated the more labile physically uncomplexed, macro- and micro-aggregate occluded organic particles as well as water extractable OM (Kaiser et al., 2011) prior to separating the OM(PY) fraction. Thus, the effect due to plowing should be negligible. Management practices extended to fertilization application at our sites. There were different fertilizers applications over the past 100yrs (table 2) that could lead to a misinterpretation of the data. Effects due to different land use practices are often unavoidable with investigations that attempt to understand processes that occur over multiple time scales, such as OM stabilization in soil. We sought to limit these effects by centering our hypothesis around the organo-mineral interactions that occur on two very specific OM fractions. This approach reduces the uncertainty associated with the analysis of multiple isotopic sources represented in bulk OM. Furthermore; our results are similar to previous studies that found a consistency in isotopic signals within OM fractions that identified microbial processing as a precursor to deposition (Bol et al., 2005; Lobe et al., 2005). Nitrogenous compounds are increasingly seen as important for OM stabilization and only with further study can we realize the impact of varying nitrogen fertilization practices on the subsequent ^{15}N isotopic signature of stabilized OM.”

As their approach is very interesting, I strongly encourage the authors to focus their study on forest sites, maybe adding a couple of sites for a more robust analysis.

I recommend to complete their dataset with C:N ratio, and if possible some molecular characterisation of the fractions so as to confirm the microbial trend they suspect. I also recommend to not over interpret their data. In my opinion, the current dataset is too weak to provide any evidence or to contradict the model developed by Kleber et al., 2007.

Authors' Response: The Kleber et al. model is a conceptual model, a tool to understand how organo-mineral associations are assembled. Our intention was to use the model to understand how the mechanisms we identified from the PLS analysis manifest in possible configurations of OM bound to mineral surfaces. Our intention was not to prove or disprove the model. We accept the model as the state-of-the-art understanding of organo-mineral associations.

Other specific comments:

1. Abstract

Some sentences in the abstract are a bit disconnected from the manuscript (eg p1986, l.17: undisturbed soil - l.22 pooled OM fractions) p1986, l.18: "The $\delta^{15}N$ signature of OM fractions served as a reliable indicator for microbial processed carbon in both arable and forest land use types" - It does for organic matter rather than for C; not really demonstrated in the manuscript.

Authors' Response: To reiterate, in our analysis we used both isotopes and proxies of mineral surface properties, and only through the analysis of both were we able to provide strong evidence that the OM was microbially processed.

2. Introduction:

p1987, l.10: C storage would indeed increase when MRT increases but also if the input increases.

Authors' Response: We attempted to bring a simplified view of how carbon storage increases belowground and within this simplified context an increase of C inputs with a constant loss rate will indeed increase storage. However, this depends on the availability of these inputs for microbial degradation. This is where we lead the reader to in the subsequent text of the introduction. We present a view of stabilization based on how long it stays in the system, rather than just how much is input into the system. We follow this discussion of what factors lead to the increased time a molecule of carbon resides.

p1988, l.7: C3C4 experiments are design to interpret ^{13}C composition in term of MRT. ^{15}N analyses are complementary analyses, possibly used to infer mechanisms, but not to determine MRT. Huygens et al., 2008: Many others before him provide evidences of microbial processing of OM in microaggregate. It would be nice to cite some of them.

Authors' Response: Besides Huygens et al., we have included work by Sollins et al., Kleber et al., Six et al., Marin-Spiotta et al., Moni et al., and Chenu and Plante in the manuscript.

3. Material & Methods:

Step 1: A bit more details would be useful

Step 2: Not clear to me whether the data you present are from the Na-Py extract after particle removal and water extraction or after particle removal, water extraction and HCl extraction

Authors' Response: We have added a figure (figure 1) that depicts the OM fractionation process.

PLS: very well explained.

4. Results and Discussion:

As previously mentioned: impossible to conclude anything with the current dataset.

Remove the data from arable land sites

Authors' Response: We contend that we have presented results that are consistent with the hypotheses we set to investigate and relevant to the subject of stabilized OM belowground. We have also fully addressed all the concerns the reviewer has concerning different sources and possible transformations. We have also placed caveats where we deem necessary so as to point out further directions of this type of research.

PLS: Why don't you show the 2 first components to demonstrate they are related to particle-size? I do not know anything about PLS, but as I follow your explanation, I understand that the 3rd component is orthogonal to the two first ones, and should allow investigating other relationships than particle-size. However, Fig 6 and 7 indicate a contribution of clay, silt and sand for component #3.

Authors' Response: We have included figures in the supplementary materials section that show the PLS weights of the different parameters we measured on components 1 and 2. We have also included the following text:

“In this supplementary material we report the weight of influence by the examined parameters on the first two components calculated from the partial least square regression analysis (PLS). Based on the results presented below, we argue that the first two components for both arable and forest land uses are primarily representative of soil textural properties (i.e. the proportion of sand, silt and clay). The efficiency of the PLS algorithm of ensuring components that are orthogonal to each other is illustrated by comparing the weights of the textural properties below and the much smaller values in the third component which we present in the main text. Stabilized organic matter (OM) is known to be distributed non-randomly in different soil particles, particularly silt and clay fractions. Thus, it is important to, in effect, control for this known relationship when comparing mechanisms across different soil types as we do in the present study. Consequently, we can be assured that the subsequent analysis of the third component is largely dependent on the soil mineral properties we set out to investigate initially. “

Is the patchy distribution of OM on mineral compatible with the zonal model?

Authors' Response: We think that OM bound to mineral surfaces in a patchy distribution is not inconsistent with the zonal model. The Kleber model works as well for „islands“ of OM and doesn't require the existence of layers surrounding mineral particles.

If you

focus your interpretation on a molecular model, then try to get some data on the molecular nature of your fractions. The difference in isotopic composition between distinct specific compounds can be larger than the one related to microbial fractionation Outline inconsistent in section 4.

Authors' Response: We used proxies of molecular binding to mineral surfaces, for example, the oxalate extractable Iron, to indicate what type of bonds might form (sensu Kleber et al. 2007). We agree with the reviewer that compound specific analysis is the next step for such investigations,

5. Tables and Figures:

Fig 1: Be consistent in the names in Fig 1 and Tab 3.

Authors' Response: We have fixed this in the revised manuscript.

Fig 1: HCf is lacking.

Authors' Response: We have corrected this in the revised manuscript.

Fig 2, 3, 4: Better use the histogram representation for quantity or proportion. For values, prefer dotplot. Combine some figures, for example fig 2 and 3

Fig 4: Not clear to me what it is: forest, arable, both combined?

Authors' response: We kept figure 2 in the main text so that readers may understand our results initially with only viewing the tables and graphs. We placed figures 3 and 4 in the supplementary material (S.1) so that readers can view the results compiled by OM fraction and soil type.