

Dear Editor,

Thank you very much for the constructive comments and for requesting a revision of our manuscript so that we may address them. We thank you for your time and effort in handling this manuscript and agree that the requested changes have improved it. We have made all the minor changes requested and have addressed the scientific questions you posed. Following (in red) are our responses to each of your comments. We have included a marked-up version as well as the clean, revised version of the manuscript.

Sincerely,  
Katherine Grant

AE Comments:

Thank you for submitting a revised version of your manuscript, which I have reviewed. I have provided my AE comments on this version. In addition to the editorial comments, I have listed below, there is two research questions that I would invite you to discuss in more depth in your manuscript, or address in a short rebuttal.

The first concerns the difference in compound-specific ages from the MAOM fraction, using two different methods. You describe the proposed mechanisms that can result in the offset, however, stop short of providing a recommendation. Based on your results, which of the methods give the more realistic estimates of MAOM in soils?

Thank you for your comment. This is an important issue within the entire soil community. We strive to understand what carbon compounds exist within *the in situ* soil environment, but even in collecting samples of the soil we have disturbed and isolated it from its environment. In our research, we try to alter our samples as little is possible to minimize artifacts associated with sample collection and processing. However, soil physical and chemical fractionation protocols, the approaches we use to study the physical and chemical components of soil, are inherently altering of the samples themselves. Thus, we have tried in our study to minimize these alterations as much as possible and to qualify and quantify those alterations. We think the more realistic results will come from the approach with the “least” alteration of the soil chemistry after physical separation of the mineral-associated organic matter (MAOM).

In our study, we encountered two issues that challenged our approaches to physical fractionation of MAOM. First, we think the grinding of the dense fraction with a ball mill prior to the total lipid extraction liberated a significant pool of old OC (likely OCpetro from sand fraction) because DCM extracts include n-alkanes, which can be from rock sources. We suspect this explains the presence of older lipids in the dense fraction than the bulk soil or silt+clay fraction. In future work, we will reassess homogenization after physical fractionation and chemical extraction (e.g., by starting with a sample that has not been dried to necessitate grinding or gentler grinding and crushing approaches). Second, the size separation of particulate organic matter (POM) from the silt+clay-associated MAOM posed different challenges for our study soil. The presence of petrogenic organic carbon in the sand fraction required the physical removal of particulate organic matter (POM) by

floating in water. A similar floating of POM from the silt+clay fraction was not possible because of the presence of colloids, which would either be lost or require flocculant or extended filtration to recover. In our soil this silt+clay-POM fraction is likely very small, but could be an important carbon pool in a different soil. Thus, in our study the silt+clay fraction was likely the best representation of MAOM formed from soil biological processes.

However, how to separate a soil into operationally defined fractions that represent functionally important pools has been debated and tested for many years. The advent of techniques (e.g., FTIR, DRIFT, and pyGC-MS) to fully analyze the chemistry of OM before and after fractionation or may clarify the advantages and disadvantages of different protocols – this is absolutely called for in future work. Thus, we are hesitant to say that any single fractionation protocol is the “best” across soil types and experiments.

We have added a few sentences to reflect this thought to section 4.2 LN 372 “We hesitate to definitively choose a best method for fractionation because each soil environment and experiment require careful methodological consideration and selection. However, given the clear differences in results between MAOM derived from size and density fractionation, it appears grinding the samples prior to extraction has significant effects on the age of the resulting TLE.”

The second concerns the petrogenic OC, and the impact this can have on your results. How does the amount compare with other soils that have been studied. Could you outline whether its presence can explain some of your results? For instance, if we know that the bulk soil contains substantial petrogenic OC, how does this influence your interpretation of the offset bulk soil – TLE – AA?

Thank you for the interest in understanding the contribution of petrogenic OC (OCpetro) to the soil. There is very little information on the influence of OCpetro in soil carbon pools and cycling. There has been some suggestion that OCpetro can be important for soil carbon (Petsch et al., 2001, Hemingway et al., 2018, Grant et al. 2023), but a comprehensive study of OCpetro on soils has not been done. I am currently working on a comprehensive assessment of the amount of OCpetro in soils in the US under a different project both in terms of its importance on soil carbon pool sizes and on soil carbon  $^{14}\text{C}$  values.

With regards to our manuscript, the majority of OCpetro is likely in highly condensed lipid-like compounds such as kerogen or n-alkanes (Petsch et al. 2001). These lipids are likely a small fraction of the total lipids in our study soils that could result in an apparent decrease in  $^{14}\text{C}$  of the AI, sand, and dense fractions. Importantly, we found this OCpetro contribution was a small percent even in the AI through a mixing model analysis, which was the oldest extracted fraction. We think that the influence of OCpetro on the AA and TLE extractions from the silt+clay and bulk soil was minimal considering these extractions had relatively young  $^{14}\text{C}$  ages, and that the OCpetro influence is highest in the AI, sand, and dense fraction  $^{14}\text{C}$  ages (of the source sample and the TLE extracted from that source).

Minor comments:

L 50: The link with the previous sentence is not clear, mainly because “in-situ OC chemical

compounds” is not easily understood. Then at L51, it’s not clear what’s meant with multiple approaches.

We’ve changed this first sentence to read “without altering OC molecular chemistry”. We have added “such as a physical separation followed by a chemical separation” to line 51.

L 93: Would you need to add Wang and Druffel (2001) to this list of references as well?

Yes, thank you. We’ve included the reference.

L 110: ‘should result in”.

Added missing word “in”

L111. TLE hypothesis is proposed as ‘to be older’, without reference to the papers that the hypothesis is based on. AA hypothesis refers to ‘cycling speed’, and the reference is included. For clarity: consider using the same structure for each of the compound classes.

Added a reference to two papers (Grant et al., 2022; Van der vort et al., 2017), which have all found long chain lipids to be older (ie cycle more slowly) than the bulk soil 14C values. We have changed the “cycle faster” to “younger” in line 111.

L 115: WEOC and AI are not mentioned any more, perhaps this is because the relative abundance will not be reported? Including a line on how the age of WEOC and AI will be used would make this paragraph more complete.

Thank you for pointing this out. We have changed the sentence to include “total lipid, amino acid, and acid insoluble compound class extracts” and we specify that the WEOC is only measured in the bulk soil.

L 140. Typo, fraction.

Fixed. Thank you.

L 138. Still add a few words to specify the ‘water density’ separation approach.

We’ve added “by suspending the sand fraction in 18.2 MΩ and removing the floating OC”

L 144. Has this chemical alteration been described? If yes add a reference, if not outline why you would expect a chemical alteration.

Thank you, we do not know of a citation, but we expect because SPT is a heavy liquid with a high ionic strength and a low pH there could be significant interaction with the organic matter which could alter the chemical reactivity after fractionation. We’ve added a clause to L144 “since SPT has a high ionic strength and low pH”.

L 155. I place this comment here at the first use of the ‘parent soil’ term. In my opinion it is confusing, especially as the term ‘parent material’ is already in use. At least, the term ‘parent soil’ should be introduced and include this in the methods section. Perhaps, the authors can consider a different term, “extracted soil/fraction”, ‘source soil/fraction’ come to mind.

Thank you, yes, we have been struggling to find the appropriate way to consistently describe this relationship between extractions and the soil they are extracted from. We have decided to use source soil/fraction and will change this reference throughout the MS including in Figure 1 and Figure 4. The changes occur at L 155, 341, 345, 346, 395, 404, 460

L 212. Consider referring to section 2.8 here for additional background information on the interpretation of  $^{14}\text{C}$  ages? I would perhaps move the current section 2.8 before the current section 2.7.

Thank you for the suggestion. We have included the reference “(see Section 2.8)” to Ln 213. However, we think the point on interpreting the results for  $^{14}\text{C}$  makes more logical sense to go after we describe how the data is being analyzed. We have decided to keep the current sections 2.7 and 2.8.

Title 4.2: As you compare the impact of different fractionation methods on the size fraction  $^{14}\text{C}$  ages, could this be reflected in the title already?

I am sorry, but we don't fully understand what the reviewer means by this statement. The title now reads:

“Differential OC cycling between fractionation methods.” We believe this gives a clearer understanding of the section's content.

L 360: write DF in full.

We have written out “dense fraction (DF)” in line 369 in the revision.

L 361: I would write FLF in full, the abbreviation has not been introduced in the text (only in a Figure).

We have written out “free light fraction (FLF)” in line 371 of the revision.

L 367: ‘with minerals’ is redundant

Thank you, we deleted this from line 376.

L 392-402: it is not clear how this review relates to the results presented in the manuscript. Make the connection more clear or remove from the discussion.

We present this discussion because the younger WEOC, POC, and FLF at all depths in the profile, but we agree, this further discussion can be removed. We added “potentially shifting the composition or amount of OC that percolates down the soil column, which could shift the age of the OC that the microbial community accesses at depth.” This makes it clear that the youngest “fractions” of the soil OC can be significantly influenced by the water balance.

L 408. Omitted word: ‘from the silt and clay ... fraction’.

Fixed the omitted word.

L 410-414: Would these lines fit better in the section 4.2., where you compare the age of different compound classes between size fractions?

We were asked by a previous reviewer to comment on importance of depth between the age of the different compounds in this section. Here, we discuss the different ages in that context and think that we should keep the discussion of depth here.

L 441-443. This has been introduced in section 4.2. already, I would remove here to avoid repetition.

We removed this from section 4.4. :“We also compared the TLE extracted from the silt+clay to that extracted from the DF because both fractions are often considered mineral associated. Across studies, the mineral-associated OC is not a uniformly defined pool, and the observed results are a consequence of the methodology used to separate the samples (Fig. 6). The DF TLE  $\Delta^{14}\text{C}$  is significantly older than the silt+clay TLE (Fig. 6b) and the TLE of the bulk soil at depth (Fig. 6).”

In addition, we moved the sentence “Across studies, the mineral-associated OC is not a uniformly defined pool, and the observed results are a consequence of the methodology used to separate the samples (Fig. 6).” to section 4.2 to improve clarity here.

L 466, “The chemical structure of the AI fraction has been difficult to characterize”, please rephrase slightly to indicate clearly that this refers to earlier studies.

Added the word “Historically” to clarify this is taken from other studies.

L 467: Wang and Druffel, typo.

No, there is a publication from Hwang and Druffel 2003, which we are referring.  
Hwang, J. and Druffel, E. R. M.: Lipid-Like Material as the Source of the Uncharacterized Organic Carbon in the Ocean?, Science, 299, 881-884, doi:10.1126/science.1078508, 2003.

L 479: Does this value reflect carbon dead material? Maybe add a few words to explain why the value is chosen.

We added “which is by definition  $^{14}\text{C}$  free” to indicate this is the defined value of OCpetro.

L 517: the evidence for the presence of slowly cycling OM in the AI fraction was not clear to me yet, is this not an assumption made?

The evidence is that the AI is the oldest fraction measured, but it is not  $^{14}\text{C}$  dead, so it must contain a source of OC from the “biospheric OC” and likely has the influence of the OCpetro.

Fig. 1b; has ‘remove floating material and ...’, is a line of text missing here?

Thank you for that catch, it is just a typo.

In general, for captions: use the caption to explain the abbreviations used in the Figure. For instance, FLF, POC, WEOC.

In each of the figure captions, we have added an explanation of each of the abbreviations used in the figure.