Please find my review below for *Impacts of Drying and Rewetting on the Radiocarbon Signature of Respired CO2 and Implications for Incubating Archived Soils*, authored by Jeffrey Beem-Miller, Marion Schrumpf, Alison Hoyt, Georg Guggenberger, and Susan Trumbore.

The authors present an interesting study on the effects of drying + rewetting and storage on the radiocarbon signature of soils. The authors interrogated standard methodology and have demonstrated inherent flaws that will aid future researchers in soil carbon analyses. Due to constraints in funding and complicated logistics of organizing field sampling campaigns, archives of soils can be important for developing new scientific questions and hypotheses. Additionally, it is sometimes appropriate to re-analyze older samples as technology improves and general knowledge expands. Beem-Miller et al. have inherently asked an important and critical question: is it correct to assume that common soil processing and analysis techniques preserve the isotopic signature of soil carbon? Additionally, can we utilize stored samples to understand cycling of different carbon pools?

Interestingly, the authors find that the radiocarbon signature of the respired soil carbon is not significantly altered by storage duration, but is indeed altered by drying and rewetting. This finding suggests that results from other studies comparing rapidly analyzed and archived soils should be reconsidered to some degree.

Overall, the manuscript is well-written. The study design is logical, the analyses appear to be done with care, and the results have been effectively compiled. I do believe the manuscript needs some work before publication, particularly in the introduction and discussion, amounting to moderate revisions. The introduction needs some restructuring, and the discussion needs to be expanded to support claims of cycling of different carbon pools, directly related to a simple model. I have numbered major points below and line comments following. I have tried to provide sufficient detail (hence the length of this review) to guide the authors in their revisions. This is a good paper and just needs to be strengthened in a few key areas. I look forward to seeing it published.

Cheers,

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## **General comments:**

- 1) I appreciate that the authors have clearly stated their hypotheses in the introduction. However, the introduction needs a clearer structure and progression of information. For example, it is not until lines 134-137 where the study intent is indicated, and it is not clear until this point what the authors are interested in studying. Most of the necessary information is here but needs to be refocused. I recommend the authors shift their existing paragraphs into this general structure: carbon cycling, air-drying and rewetting, soil archives, radiocarbon dynamics, study objective and hypotheses. As I have already mentioned, all of these components are already in the introduction, but have been integrated in a way that is confusing in present form.
- 2) The methods are an integral section of the paper and need some more clarification. I found myself constantly flipping back between Table 2 and Section 2.3 to understand the different treatments for each of the samples and neither the table nor the text appear complete. For example, all of the information for Experiment 3 samples is included in a large supplementary table. The summarized information for all three experiments needs to be included in the manuscript.
  - There are several points where the motivation in the methods is unclear. For example, why change the equilibrium respiration for the different experiments (~line 247)? I worry that the authors created too many variables to be able to truly compare results from the different experiments without a proper normalization, which I understand can be difficult (e.g. lines 245-266). For experiment 3, it is unclear why the methods for experiments 1 and 2 could not be applied. Maybe I missed something here. These sections can be greatly strengthened by adding more justification.
- 3) Section 3.4 relies heavily on interpretations from Figure 4 and the finding that storage duration does not affect Δ<sup>14</sup>C is a critical conclusion for the paper. I generally agree with the authors' interpretations. However, they argue for a significant difference between control and treatment samples with differing storage durations for Oak Ridge. I do not see this same result. If you take the average of the samples/sites with consideration of error, there really is not a large statistical difference between the samples stored for around 5 years and those stored around 14 years. I recommend the authors instead focus on general observations. For example, one of the 14-year Oak Ridge sites has the lowest treatment-control value, etc.
- 4) These next few comments focus primarily on the discussion.
  - a. A few portions of the discussion are reiterations of the results (e.g. lines 610-617; 627-629). The authors should review to make sure the interpretations and applications of the results are the focus.
    - There are a few areas I really wish the authors expanded upon, particularly regarding slow and fast pool dynamics. In particular, I am curious how likely the scenario of pool reversal is on lines 577-581. Without more discussion in this section (4.2), it feels like a large leap to conclude that air-drying and rewetting results in different rates of carbon cycling between forests and grasslands and mobilizing different pools (lines 589-592). I would also like to see the authors expand the discussion on extracellular carbon on lines 641-643, if possible.

- I do not believe the discussion will be significantly longer with the suggestions above once the redundant portions from the results are removed/condensed.
- b. On lines 544-545, the authors state that the decomposition rates they selected were arbitrary. They use this rate to create a simple model shown in Figure 6. Either sound justification for the selected rates need to be provided, or the authors need to be much more general in their interpretations. If the decomposition rate is arbitrary, then only trends can be discussed in lines 550-555, and not specifics as the authors have currently written. On line 566, the authors state that the carbon pool becomes enriched in Δ<sup>14</sup>C in the mid-1970s based on Figure 6. This is similar for line 569. Again, if the decomposition rates are arbitrary, the year is not accurate/important. It's fine to mention years for the figure, but it needs to be clearly stated that these are not representative (or if they are representative, more justification needs to be provided).

I understand that a complete model is not within the scope of this study. However, the authors base much of the discussion on a model with arbitrary values. Moving forward, I recommend the authors provide clear justification for their model inputs and dial back their interpretations to focus more on trends and general pool behavior. The current text on lines 570-572 is a great example.

5) The figures generally look great and the abstract and plain language summary read well.

## Line comments:

Line 74: Use of attack is odd; define artifacts.

Lines 76-77: See comment above.

Line 84: I would remove this topic sentence.

Lines 91-92: Can this be expanded? It is not clear what is meant.

Line 151: This study did not really "assess the feasibility of measuring  $\Delta^{14}$ C", correct? I understood it as quantifying post-collection alteration.

Line 173: Delete the second "within the".

Lines 201 and 205: What are the number of control samples for the three experiments/treatments? Can these numbers be included?

Line 235: Was there a target range for the soil carbon content?

Line 238: How did you adjust the soil moisture to 60%?

Lines 245-247: Which study did the authors reference for the incubation duration?

Line 266: Why not measure equilibrium for all samples?

Line 268: As closely to what?

Line 280: How was the moisture adjustment determined?

Line 297: Can a sample number be listed instead of "a few"?

Lines 319-322: Important information, but not methods.

Line 335: A familywise error correction would be helpful to make sure the authors are not committing a type-1 error.

Line 340: What is meant by "field-moist"?

Lines 361-362: Are those really peaks?

Lines 369-370: Maybe I'm missing something because the chart is in days, but I don't see peaks at the interval mentioned.

Line 371: Why is this in the supplements? Is it not possible to include experiment 3 in Figure 1?

Lines 386 & 391: Which tests were used to determine significance? What are the values? Information is missing.

Line 411: I don't agree that this is a significant depletion since the values do have overlap.

Line 415: Typo, should read "not measured".

Line 502: I interpreted this as a slightly lower mean for the forested sites.

Lines 521-524: This is confusing and I'm not sure what is meant.

Line 524: A significant change relative to what?

Line 533: Shouldn't this be incubation practice, not technique?

Line 550: Maybe I'm missing something, but I don't see the shift towards the slow pool curve. It looks like the respiration line to me.

Lines 605-608: How would you determine if the pool curves did indeed cross?

Lines 622-624: This sentence is hard to understand.

Line 645: I would just start this section with "The consistent enrichment...".

Line 646: Instead of microbes, is it possible that the enrichment could be due to decomposition?

Line 654: "a process [that] has also..."

Lines 665-669: Is there actually evidence at the study sites for mineral-carbon release? Are there limestone/calcite/dolomite beds?

Line 680: I disagree that the Oak Ridge data provide \*strong\* evidence of different carbon pools. A couple of typos here too: "data provides..."; "fixed from the atmosphere in over the...".

Line 689: This explanation seems fine, but I'm not sure it explains the variability for the rest of the samples.

Line 709: Sorry if this is a silly question, but what is meant by protected carbon in this context?

Lines 734-737: I wonder if a correction could also be appropriate here.

- Figure 1: All results are discussed relative to hours. It would be more helpful for the x-axis to include hours as well.
- Figure 3: Can the different treatments be shown somehow?
- Figure 4: The caption says all three experiments, but I believe only experiments 1 and 3 are shown.
- Figure 5: Can the grey line be made black instead? Can the x-axis include 2011 as well? The last line of the caption should read "radiocarbon data are...".
- Table 2: I really like this table, but it needs a little more detail. I mention this previously, but instead of saying "various" for experiment 3, can the authors include ranges with standard deviations?