Thank you for the opportunity to revise our manuscript titled "Diverse organic carbon dynamics captured by radiocarbon analysis of distinct compound classes in a grassland soil". We appreciate the feedback from the reviewers, as it has helped guide us in improving the clarity and impact of our manuscript. We have addressed the reviewer comments both in the final public response and here with point-by-point responses to each comment submitted by the reviewers. We include a marked-up revision with track-changes as well as a "clean" manuscript. The point-by-point comments from the reviewer responses are in red and our responses are in blue.

We described our revisions in the public response, which I summarize here. We fixed all typographical and grammatical errors and made additional minor changes for improved clarity and sentence flow as requested. We have added stronger language throughout the manuscript to better reflect the impact of these results. To improve the clarity of the 'bomb' radiocarbon interpretation we have added an additional subsection to the methods (2.8). We rearranged the Discussion to improve the flow as requested as follows: "4.1 Variability of 14C in compound classes in soils", "4.2 Differential OC cycling between the different "parent" fractions", "4.3 Variation in OC cycling throughout the depth profile", "4.4 Compound class Δ14C values in mineral-associated SOC", and "4.5 Persistent and Petrogenic OC". We have changed the figures as suggested by Reviewer 1. In addition, all line numbers we report are referencing the clean version of our revision. We agree with Reviewers 1 and 2 that these changes have strengthened the manuscript and thank them for their very constructive reviews.

We believe these revisions have improved the manuscript and we hope you now find it suitable for publication in *Biogeosciences.*

Sincerely,

Katherine Grant

Reviewer 1:

This paper by Grant et al presents a comprehensive study where they measured the 14C contents of many soil organic matter fractions with depth, from the specific grassland soil. These are very useful data to gain more insight in organic matter cycling in soils. A great amount of work lies behind it, for which I compliment the authors. Although the data are good, I think the paper would improve by a somewhat better presentation, and some more in depth discussions that can be structured better. I have given comments on the attached pdf, but some main and additional points here:

The influence of the bomb spike on 14C contents (especially important for the upper layer) is missing in the discussion.

Thank you for your comment. We have added to the revised manuscript a short paragraph under a new subheading "2.8 Interpretation of radiocarbon data" to introduce the influence of the bomb spike on ^{14}C contents in the methods section. This new subsection begins on Line 230 of the

clean version of the revision. As requested, we added the ^{14}C values for the time of sampling to Figure 2-3.

The authors discuss the relative differences between the various fractions (size, density, compound class), but not not discuss the depth aspect much, nor do they even try to quantify turnover times, which is one unique aspect that these 14C data allow. This is a missed chance.

The revised Discussion includes a subsection dedicated to the depth effect: "4.3 Variation in OC cycling throughout the depth profile", Lines 370-403. We have added a new paragraph (Lines 391-403) to this section to discuss trends with depth in the TLE, AA, and AI. This section clarifies the depth trends here and throughout the manuscript as requested and we thank the Reviewer for this suggested improvement to the manuscript.

We addressed the suggestion to quantify turnover times in the public author response. In brief, turnover times require applying a model with assumptions that further complicate their interpretation. Because of the bomb-curve, we have multiple turnover time solutions for the AA and TLE fractions in the surface. We prefer to focus on the 14C values here but intend to pursue modeling of turnover or transit times in a future manuscript.

A discussion of the source of the organic matter: besides leaf litter from above, which is then worked downwards by bioturbation and percolation, there should also be some root litter. Where does this go?

To clarify this point, we have added the following sentence to the site description (Lines 124-126): "The site is dominated by annual grasses, shallow rooted herbs, and forbs, and we did not observe roots below 10 cm. Thus, root derived inputs of OC are important in the surface horizons, but do not directly affect deeper soils at this site."

The structure of the discussion. This goes a little criss-cross through the data, size and compound fractions and I had to re-orient myself each time what figure and data to look at. One approach could be to try to answer certain questions/hypotheses using the data, and place this in perspective to data and insights from the literature. What is the most exciting news learned from this study?

Thank you for this suggestion. In the revision, we rearranged the Discussion to improve the flow as requested as follows: "4.1 Variability of 14C in compound classes in soils", "4.2 Differential OC cycling between the different "parent" fractions", "4.3 Variation in OC cycling throughout the depth profile", "4.4 Compound class Δ14C values in mineral-associated SOC", and "4.5 Persistent and Petrogenic OC".

First, we have added a paragraph to the subsection 4.1, so that all the compound class extraction results are addressed in this first section and clarify that here we address the questions "are there

differences in cycling time/age between the various compounds in the bulk soils? Do the differences in cycling time change with depth?" (See Lines 328-335.)

Next, subsection "4.2 Differential OC cycling between the different 'parent' fractions" includes the comparison of the sample extractions from the bulk soil, silt+clay, sand, and dense fraction. This enables comparison of physical fractionation methodologies earlier and clarifies what we refer to as the "mineral associated fraction" later in the Discussion. This allows for a more natural flow into the following subsections. (See Lines 343-368.)

Then, subsection "4.3 Variation in OC cycling throughout the depth profile" more directly addresses patterns with depth in the quickly cycling OC fractions (WEOC, POC, and FLF) as well as in the compound classes (TLE, AA, and AI). (See Lines 370-403.)

These changes allow a clearer discussion of compound classes from the mineral-associated fractions in subsection 4.4 and petrogenic OC in subsection 4.5.

In addition to the changes outlined above, we have included the following as suggested and noted in the marked pdf:
-We have made requested grammatical, typo, and word choice corrections.
-In Figure 1, we have added a third panel to show the density fractionation scheme to the figure and modified the figure caption accordingly. The density fractionation scheme is extensive and has been published previously (McFarlane et al. 2009), so the detailed methods remain in the SI.
-In Figures 2 and 3, the radiocarbon age scale has been fixed. Thank you for noting the incorrect scale. Additionally, for Figures 2 and 3 we have added the atmospheric value at the year of sampling, which was approximately 0‰.
-We added references to the manuscript as requested in lines 47-50, and in 67.
-We clarified in section 4.5 that the biospheric endmember we used was the measured TLE and bulk 14C values, which are listed in Table 1. We have clarified that we use both to provide a range of estimates for the petrogenic contribution to the SOC. (See Lines 454-458.)

Reviewer 2:

In this manuscript, the authors describe radiocarbon data collected from a wide variety of soil carbon fractions following various definitional methods. This was done in an attempt to identify faster and slower cycling carbon pools following our understanding of bioavailability and persistence of soil carbon. The data are compelling and clearly presented, and the interpretation is well-founded. Thank you for the opportunity to review this research. This is a very strong manuscript that should be published following minor corrections.

Throughout the manuscript, there is some lack of narrative and propulsive sentence structure. The data here are novel and very important to the current understanding of soil carbon cycling, especially in the "mineral-associated/heavy" fraction and the diverse cycling rates therein. If these data can be presented in a stronger, more assertive fashion, these data could support a strong narrative. I may sound like a broken record on this, but it is the primary thing that could

be improved on in an otherwise very strong manuscript. If the writing is tightened up and a bit more forward-leaning, this could be a high-impact paper.

Something that could possibly strengthen the discussion throughout is how "bulk soil" is referred to. Since we're discussing radiocarbon, the "bulk soil" 14C is the mean of all C atoms in the soil. If an extracted C fraction is enriched, than it is cycling faster than the average C atom, or the average of the bulk soil. Of course, this is not a normal distribution. But if an extracted C pool has a higher radiocarbon content, that pool cycles more quickly than the soil C on average. Perhaps I haven't articulated it well here either, but the framing of these C pools and being faster or slower than the mean i.e. bulk soil could support the narrative.

Thank you for your positive comments and constructive suggestions. We have corrected grammatical errors and edited the manuscript to improve flow and clarity. We have added stronger language throughout the manuscript to better reflect the impact of these results.

We agree that the terminology regarding the bulk soil was somewhat unclear. We have now referred to the soil from which the fractions were extracted as the "parent" soil for the chemical extractions. We have changed this throughout the text and agree it has made the manuscript clearer. Namely this is reflected in the figure axis label change in Figure 4 and in a subsection in the reorganized Discussion: "4.2 Differential OC cycling between the different 'parent' fractions" (See Lines 343-368).

Below are line-by-line comments:

L43-50: I think this paragraph could better introduce why you want to specifically refer to compounds. I think a couple more references here and more active justification would serve this paragraph well.

Thank you for the suggestion. We changed the wording in Line 43 to "do not satisfactorily demonstrate" instead of "have not fully demonstrated". We added three additional citations to Lines 47-48 "Lehmann and Kleber, 2015, Schmidt et al. 2011, Kleber et al., 2021". We added a clarifying sentence to line 49-51: "Thus, specific compounds isolated from soils, such as amino acids and lipids (Rethemeyer et al., 2004) can provide information on how OC is stabilized in different environments."

L58-60: Switching this sentence to more active voice would clarify it and more strongly identify the persistent research question.

We changed the sentence, so it currently reads "Therefore, physically isolated mineral-associated OC is still a heterogenous mixture of OC molecules that have a distribution of turnover times, rather than a single homogenous and intrinsically stable SOC pool (Stoner et al., 2023; Van Der Voort et al., 2017)." (See Lines 60-62.)

L70: The sentence that starts with “Each of these…” could better introduce these compounds as biologically valuable C pools, both of which we might assume to be quite fast-cycling. I think this sentence and the one on line 73 could add to the story of C decomposition in soil; a competition between microbial decomposition and stabilization, and the processes and soil characteristics that may determine those outcomes. I know this paragraph ends with a sentence along these lines, so maybe this could be reshuffled to create more narrative.

We have rephrased and moved the sentence to the beginning of the paragraph.

L73: I think there is something missing from this sentence; that, or the “not only” can be removed

We removed the “not only”.

L81: “can be” is repeated.

We removed the repeated phrase.

L81: While “wholistic” is correct, “holistic” is more commonly used. Your choice.

We changed to “holistic”.

L83: “of” is missing between “understanding” and “carbon”

We added “of”.

L91: This probably was supposed to be “coastal”.

Changed. Thank you.

L246, 252: Add a + before positive radiocarbon values or remove them elsewhere, in the interest of consistency.

Thank you for this suggestion to keep things as simple as possible, we removed all the + signs from the radiocarbon values.

L272: “each of which” may flow better than just “which” on its own

We have added “each of which” to this sentence in L 293 of the revision.

L279-281: This sentence is doesn’t pack the punch that the last sentence of this first discussion paragraph could have to propel the discussion. Tie it in with the data directly, either why you see this, or why you don’t. This information is available from the figures, but it should be clear whether the data is behaving as you’d expect or otherwise.

We have changed the sentence to begin with “furthermore” (Ln 302) and in addition we reworded the last sentence to read “Furthermore, at deeper depths, new vegetation inputs should be less readily available, which results in more depleted $\Delta^{14}C$ signatures at depth and could necessitate microbial use and recycling of older SOC.”

L283: Maybe personal preference, but prepositions at the end of sentences should be avoided, “from which they were extracted”.

Changed to “from which they were extracted”.

L284-285: This sentence could be tidied up to be easier to read and more propulsive.

We have combined these two sentences to make this more concise.

L288: Maybe add “preferentially” here, to highlight that they are microbially active because of their N content making them more appealing

We have added the term “preferentially”.

292-307: This is well reasoned, well done.

Thank you.

319: This is an great description of what WEOC is

Thank you.

L323: Maybe add “mobile” or something similar to this list

We added “mobile” to the sentence.

L324: Could this also be that the FLF fraction is the remainder of more “recalcitrant” C that persists after the more bioavailable C is decomposed (and possibly shows up in WEOC and POC)?

Yes, we agree. That makes sense.

L326-328: These first two sentences could be streamlined for readability.

We’ve combined the sentence and made it one.

330-331: Why could this be the case? Due to closer contact with microbes and dissolved C under lower water conditions? Of course, if water content decreases enough then this could de facto preserve C as it cannot be accessed by microbes. Does this also imply abiotic oxidation? This sentence could be strengthened with one or two references and more context for why this observation would be expected.

We have added the reference (Heckman et al, 2022) and added the clarification "With prolonged dry periods, water soluble OC may be more susceptible to microbial decomposition or oxidation because anaerobic preservation is removed (Heckman et al. 2022)"

332-335: Great!

Thank you.

350-351: This is true, but slightly redundant. What may be more interesting is that the sand size fraction is older (in order to produce a mean value in the bulk soil, if bulk – silt – clay = sand). We could assume the silt+clay to be more microbially active due to higher C content etc, and thus have enriched 14C, but the sand story is also interesting.

We have added this phrase to open this paragraph and it now reads "While the TLE from the silt+clay and bulk soil had similar $\Delta^{14}C$ values, the AA from the silt+clay size fraction was enriched in ^{14}C compared to the AA from bulk soil ($r^2 = 0.98$, $p<0.05$). This suggests that AAs cycle faster in the silt+clay mineral pool than in the bulk soils. While mineral surfaces usually are thought to promote stability and persistence of OC, in some soil systems, mineral associations may not be the single defining factor of OC persistence (Rocci et al., 2021) and could have a more nuanced role influencing OC cycling in soils." Lines 422-428.

354-360: This is the first time AI is discussed, which is not inherently a problem but it is slightly jarring since the interpretation of AI data hasn't been established yet.

We have moved the entire first paragraph of this section to the first part of the discussion and then we have discussed all the different compounds previously. We feel this flows better now. (See Lines 328-335)

363-366: What do you think is the source of the older TLE that is implied by the more depleted 14C in bulk soil TLE compared with DF TLE?

This was an error in the text and has been fixed.

Upon looking at the figure, this may have been written incorrectly. It appears that DF TLE is always more depleted than bulk soil TLE except at the surface. This makes much more sense. One would not expect a source of older TLE in the FLF of OLF fractions, e.g. Be sure to correct this in the text.

We have fixed this error.

I think this is a crucial paragraph, and is important in pushing our understanding of diverse, multi-level pools and highlights the benefits of compound-specific 14C analysis. Make sure it comes across with a strong statement.

We have rearranged and revised this paragraph (See Lines 429-436.) It now reads “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 $\Delta^{14}C$ is significantly older than the silt+clay TLE (Fig. 6b) and the TLE of the bulk soil at depth (Fig. 6). This suggests that lipids in mineral-associated OC pools vary in cycling rates. This is complementary to findings from other studies where ^{14}C values from different lipid biomarkers are divergent from the bulk soils (Gies et al., 2021) and indicates the necessity of looking at entire compound class pools for understanding soil carbon persistence. Further investigation into the composition and age-distribution of compounds within mineral associated-OC is needed to better quantify the distribution of cycling rates within mineral associated OC pools.”

379: The comma after “these” can be removed.

This has been fixed.

387: Indicative *of* aromatics

This has been fixed.

393-394: Interesting that the petrogenic rocks are still relatively modern. Do you think this a mixture of rock and more modern sources? Or rock-derived C being incorporated the biologic C cycle on the rock surface? There is some evidence of this occurring.

Yes, that is exactly what we assume.

412-414: Good explanation.

Thank you.

415-425: Discussion of methodology limitations is often lacking, and this serves as a nice comparison of methodologies.

Thank you.

Section 4.5: If there is a recommendation to be made about size vs density separation, I think this is a good place to put it. Obviously there are pros and cons to each, but don’t shy away from suggesting one is better for research question X vs Y.

We think it is important to consider the objectives of individual studies and select methods according to those needs. Thus, we prefer not to suggest one is better than the other but for researchers to carefully consider the implications their choices of methodologies have on their results.

436: Abbreviate to SOC.
Fixed this.