## RC2: 'Comment on egusphere-2023-210', Anonymous Referee #2, 25 Jul 2023

We would like to thank referee # 2 for providing a review on our manuscript. All the comments were thoroughly addressed and complemented with the replies to the first referee. The main comments of his/her review are provided below in *italics*, with our reply in normal font.

- In this study, Tangarife-Escobar and co-authors incubated peatland and grassland soils at four different temperatures and two water-filled pore spaces to better understand temperature and moisture effects on the 14C signature of bulk and respired soil carbon. They also used a mathematical model to analyze how decomposition rates and other soil parameters affect the ∆14C of bulk and respired C and their relationship. Papers that investigate the relationship between carbon age and transit time are crucial in order to better understand carbon destabilization with changing environmental conditions. Although this paper has the potential to be impactful, it needs to be revised to address important flaws in the interpretation of the incubation results and to improve the link between the incubation and mathematical model.
- Overall, there are major issues with the interpretation of the incubation results and this is my main concern about this paper. The authors stated that temperature and soil manipulations caused a response in the direction of the Δ14C (Abstract and Lines 233-234); however, these statements are not supported by the statistical analysis, which show that temperature had no effect on the Δ14C of bulk soil or respiration. The authors do not present any information on the statistical model that was used to interpret these results in the methods section, and as such, it becomes difficult to understand how they arrive at some conclusions related to the Δ14C and CO2 flux (e.g., was site included in the stats model? What was the stats model for the CO2 flux?). Additionally, the text in the results section rarely includes wording on whether a main effect was significant, and sometimes the authors will discuss the interactive effect of moisture and temperature on Δ14C, even though the interaction was not significant.

We apologize for the misunderstanding. Certainly, temperature did not show any effect on the  $\Delta^{14}$ C values, but rather on the magnitude of fluxes, which directly affects the release of "old" carbon if the moisture conditions allow it. To illustrate this, we added Figure 5 (C-D) which provide a clearer picture on the relationship between CO<sub>2</sub> fluxes and treatments and we indicate the respiration rates for each treatment inside small panels. It is relevant to point out that although our experiment did not find significant evidence of a correlation between temperature variation and  $\Delta^{14}$ C values as assessed by a two-way ANOVA (type III), other authors have found that temperature is the main driver of SOC decomposition. Hence, we clarified in the abstract and along the manuscript that the influence of the temperature occurs only on the fluxes.

Additionally, we added information on the statistical model we used for the interpretation (see comment below).

Regarding the measurement of the  $CO_2$  fluxes, we added a paragraph in the methodology explaining its calculation and added Figure 5 (C-D) to provide a deeper interpretation on  $CO_2$  fluxes patterns at different treatments. A more detailed answer can be seen in "reply to referee #1".

Finally, the interaction effect of temperature and soil moisture manipulations on  $\Delta^{14}$ C values was evaluated from the perspective of the incubation results and no significant

difference was found by the two-way ANOVA (type III), which is appropriate for unbalanced data since our results did not have equal number of values (see comment on section 2.3). Nonetheless, we argue based on our observations of temperature variation effect on CO<sub>2</sub> fluxes and the studies of other authors, that temperature and soil moisture strongly influence the decomposition of SOM and therefore would have an influence on  $\Delta^{14}$ C values, age and transit time.  $\Delta^{14}$ C, as opposed to CO<sub>2</sub> fluxes, is a variable that may show more complex dynamics due to the trends of the atmospheric bomb curve, motivating our subsequent modelling analysis.

 It is also unclear how the incubation informed the SOC decomposition model, for example did the CO2 flux response to temperature and moisture get used in the model to influence the decomposition constants, or were these based on Manzoni et al. 2009 (Lines 182-185)? If the incubation did not influence the model, then what was the reason to include the incubation results in the paper? The authors should expand on the link between the incubation and model and how they influenced each other.

As previously mentioned,  $\Delta^{14}$ C is a variable that can show much more complex behaviors than CO<sub>2</sub> fluxes because of the dynamics of the atmospheric bomb curve, which can potentially lead to non-statistical differences among treatments even though decomposition rates and transit times may respond strongly to changes in temperature and moisture.

We further elaborated on the reasons to use a mathematical model and how it relates to the incubation data (section 3.2 and comments to the first referee). The CO<sub>2</sub> results were not used explicitly in the mathematical model since the objective was to explore behaviors in the dynamics of  $\Delta^{14}$ C as decomposition rates change, and how these behaviours differ from those of age and transit time. Because we aimed at exploring a wide range of values of decomposition rates, we didn't use the specific results of the incubation experiment, but we rather tried to explore the directions in which  $\Delta^{14}$ C would change as decomposition rates change. The incubation results were useful to show the type of storage system of each of the ecosystems as well as to gain knowledge on the influence of different treatments on the  $\Delta^{14}$ C values. The lack of clear response to these treatments observed in Fig. 4 along with the presence of outliers were approached by the model simulation, which contrasted slow and fast cycling systems to observe what other additional parameters could modulate the behavior of  $\Delta^{14}$ C values along with the age and transit time metrics. Specifically, the  $\Delta^{14}$ C data obtained from the incubations were used as the target space of our simulations, for what we added some panels on the Figures 6 to 9 to indicate the aimed  $\Delta^{14}$ C range. By doing that, we hope the connection between the incubations and the modelling results be more substantial.

• Finally, the writing can be improved and the authors should check the manuscript for typos and inconsistency in terminology.

We carefully addressed this comment by proofreading and adjusting terminology consistently along the manuscript.

### Detailed comments: Abstract

• The abstract does not currently satisfactorily connect the results to the big takeaway statements and implications for the net carbon balance of the area.

We elaborated on the implications of our results for the ecosystems future and stated them in the abstract and conclusions.

• Line 10: Is this bulk soil 14C?

We clarified the sentence as follows:

"From our incubations, we found that <sup>14</sup>C values in bulk and CO<sub>2</sub> from peatland were significantly more depleted (old) than from grassland soil."

• Line 14: Consider not using terms like k in the discussion of results, but instead refer to the decomposition rates. To a general audience saying what the effect of a 'low k value' may not carry a lot of meaning; however, discussing the impacts of 'low or high decomposition rates' will aid in the interpretability of the results.

We changed "k" for "decomposition rates" in the discussion when appropriate, to help the readability and interpretation of results.

Lines 13-16: It is not clear how these two sentences are linked, or how the first statement leads to the second. It seems like they should be switched: The correspondence between Δ14C and age and transit time strongly depended on the internal dynamics of the soil (k, α, γ and number of pools) as well as on model structure. When decomposition rates were low (low k values), the (replace "modified Δ14C" by the direction in which the Δ14C changed, did it increased or decreased the age of bulk/respired CO2?) due to the incorporation of 14C-bomb in soil (does the incorporation of 14C-bomb mean anything to the reader at this point in the abstract? Consider writing what this means (e.g., the proportion of C cycling on decadal timescales increased). What does this result mean for carbon cycling in wet/dry or cold/wet systems? The abstract is missing the implication of the results.

We reformulated these sentences for a better connection between them and added the specific implications of our results as follows:

"In our models, the correspondence between  $\Delta 14C$ , age and transit time highly depended on the internal dynamics of the soil (*k*, *a*, *y* and number of pools) as well as on model structure. We observed large differences between slow and fast cycling systems, where low values of decomposition rates modified the  $\Delta^{14}C$  values in a non-linear pattern due to the incorporation of modern carbon (<sup>14</sup>C-bomb) in the soil. We conclude that the stability of carbon in the peatland and grassland soils of the QTP depends strongly on the direction of change in moisture and how it affects the rates of SOM decomposition while temperature regulates the amount of fluxes. Current land cover modification (desiccation) in Zoige peatlands and climate change occurring on the QTP, might largely increase CO<sub>2</sub> fluxes along with the release of old carbon to the atmosphere potentially shifting carbon sinks into sources."

• Lines 16-18: Why are the authors concluding that the stability of carbon depends strongly on changes in temperature if this did not affect the ∆14C of respired CO2 in either soil?

We reinterpreted this assertion and modified accordingly along the manuscript (see previous comment).

• Lines 18-19: It is not clear how modeling improved predictions on interactions between terrestrial and atmospheric carbon.

We modified the end of the abstract to highlight the implications of our results (see comment above).

### Introduction

• The introduction is a bit choppy. Some of the paragraphs can be combined and the order should be reconsidered to improve the readability of the paper. For example, paragraphs 3 and 6 are both about carbon stabilization/de-stabilization, yet they are broken up by paragraphs 4 and 5.

As paragraph 3 introduces the concept of stabilization mechanisms and their relation to SOM persistence and paragraph 6 explains how <sup>14</sup>C can be altered by stabilization and destabilization mechanisms, we consider that a bridge (paragraph 4 and 5) that introduces both age and transit time and how they are studied through radiocarbon is necessary to keep the logic sequence of the introduction.

• Please be consistent with word choices, e.g., de-stabilization, destabilization, (de)stabilization, and the use of carbon or 'C.'

We changed to the terms "destabilization" and "carbon" consistently throughout the entire manuscript.

• Please add text in the introduction on how the nuclear weapons test enriched atmospheric 14C to aid in the interpretation of positive vs negative values.

Thanks for the recommendation. We added some sentences to explain the relationship between nuclear weapons and  $\Delta^{14}$ C values.

• Line 4: add comma for non-essential clause: ", and in consequence,"

We corrected along the entire manuscript as suggested.

• Line 34: Comma before non-essential clause ", which store"

We corrected along the entire manuscript as suggested.

• Line 37: Driving the net carbon balance to become a C source? Is it already a source of C?

We changed the verb "driving" to "modulating" since the sense of the sentence is to indicate that according to the referenced authors, climate change and land cover change are affecting the carbon balance through effluxes. The direction of such change from sink to source or vice versa differs across different studies and sub-regions of the QTP.

• Line 80-81: Add Pegoraro et al. 2021 to the list of citations for release of old C from deep soil layer after drainage.

Very useful recommendation. We added as suggested.

• Lines 81-82: Is this a perennial or seasonal frozen layer?

We modified the sentence to clarify as follows:

"Such a process might be occurring in the Zoige peatland soils due to the presence of seasonal frozen layers (Liu et al., 2021; Yang et al., 2022)."

• Line 86: What is the impetus for the older C increase with increasing moisture? One would think that higher soil moisture would decrease the decomposition of SOC, and thus preserve older C, based on the citations in the previous paragraph.

This is a crucial observation whose reasoning relies on the starting moisture point of the soil. According to Sierra et al. (2017) and Azizi-Rad et al. (2022), SOM decomposition is limited at extreme values of soil moisture and oxygen levels (0 % or 100 %). So, as long as oxygen and moisture are available (as in our 95 % WFPS level), SOM decomposition and  $CO_2$  release (old in the case of the peatlands) would occur, while if the moisture decreases to the extreme, decomposition would be highly restricted independently of temperature and oxygen levels.

Therefore, we rephrased the hypothesis as follows:

"Changes in temperature and moisture contribute to the destabilization of carbon in soils from the QTP. Hence, we hypothesize that higher temperature would increase the age of respired  $CO_2$  as well as changes in soil moisture would increase or decrease (depending on the direction of moisture change) the age of respired  $CO_2$  in soils subjected to controlled manipulations."

• Line 93: replace 'their' by 'the'

We changed as suggested.

• Line 94: The part about the model seems to have been thrown in at the end without much explanation. Why does the model help the interpretation? What are the challenges of understanding age and transit time and how does the model tackle that? The model results are a big part of the results section.

We elaborated on the relevance of the model for the interpretation of our results along the manuscript to explain the connection between the experimental and the modelling approaches as well as to explain its advantages as follows:

"In addition, we used a mathematical model to better interpret the interaction between decomposition rates change, expressed through internal dynamics of the soil (*k*, *a*, *y* and number of pools) and the  $\Delta^{14}$ C values by targeting the range found in the incubations. Thus, our observations and models strengthen each other to gain a deeper comprehension on the relationship between soil carbon stability,  $\Delta^{14}$ C, age and transit time."

### Methods

• Please provide coordinates for sites.

We added the missing coordinates for the Nam Co site.

• How was the soil collected, with an auger, or another method?

We specified the method for both of the sites.

• The site and soil parameters would be nicer if presented on a table to so both sites can be easily compared.

As the same information could not be recovered for both sites: CEC, pH and EC are missing for the Zoige peatland, we think that a table would burden the readability of the method section and would not contribute significantly to a proper comparison.

• Line 129: These are analytical replicates, not field replicates?

That is correct, these are analytical replicates, meaning that the same treatment was repeated in 3-6 incubation bottles to avoid ending up with few radiocarbon data due to eventual failures in gas extraction, CO<sub>2</sub> separation, C graphitization, etc.

We specified in the description of the replicates that they are analytical.

• Table 1 has an exclamation point that seems out of place

## Corrected

• The methods are missing information on statistical analyses to discuss the differences in the respired and bulk 14CO2 in the results. This is a big missing component that seriously concerns me, especially since there are issues in the interpretation of the results.

We inserted the next paragraph in the methods section (Section 2.3):

"The effects of soil moisture and temperature manipulation on  $\Delta^{14}$ C were evaluated for the bulk and CO<sub>2</sub> fractions in each ecosystem separately through two-way ANOVA tests (type III). This type of ANOVA is also referred to as Partial Sum of Squares and is appropriate for unbalanced data since it does not depend on the sampling structure or the particular order in the model (Shaw and Mitchell-Olds, 1993); hence, this approach adjusts best to our data set where treatments did not have equal amount of values. As for the CO<sub>2</sub> fluxes, rates were measured regularly for every treatment and mean CO<sub>2</sub> respiration rates (mg CO<sub>2</sub> g soil -1 day -1) were calculated based on the total duration of the incubation (Fig. A1)."

### Results

• Line 217: Remove duplicate 'to' and add comma before 'which'

### Corrected

• Line 219: Was the difference, or maybe lack thereof, statistically significant?

We reformulated this sentence as follows:

"In contrast, for the grassland soil, the  $\Delta^{14}$ C of bulk soil (21.1 to 73.9 ‰, mean=43.3, n=33) fell similarly around the 1:1 line compared to the  $\Delta^{14}$ CO<sub>2</sub> (13.9 to 83.4 ‰,

mean=38.5, n=33, including outliers of -227.1 and -105.1) indicating that the samples behaved mostly as a well-mixed homogeneous system (type I)."

• Figure 3: Are these results for all moisture and temperature levels? Why combine them? Having a graph that shows the effect of moisture would aid in the interpretation of the results. Figure 5 is not appropriate to show the significant result of the moisture effect on the 14CO2 signature since it splits it by temperature levels, and there were no significant temperature and moisture interactions.

Yes, the results are the combination of all moisture and temperature treatments. This Figure 3 allows to understand the magnitude of the difference between  $\Delta^{14}$ C values between the respired CO<sub>2</sub> and the bulk soil for each ecosystem, therefore its relevance. The specific effect of moisture can also be clearly seen in Figure 5 if the boxplots (60 and 95 WFPS) levels are taken for each temperature individually. From this Figure, we can conclude that the  $\Delta^{14}$ C-CO<sub>2</sub> values in peatlands are more depleted as moisture increases.

• Line 227: Please add the p-value for the CO2 model and discuss the results as significant or not. The statistical model needs to be added to the methods.

See comment above on the methods (section 2.3).

 Line 233: It is not clear from the stats results or the graph that temperature and moisture manipulations caused a response in the vertical and horizontal direction in the ∆14C of bulk versus CO2 space. Ecosystem type seems to be the driver of the clustering, please explain how this conclusion was made.

Excellent point. We adjusted this affirmation as follows (section 3.2):

"Changes in the vertical and horizontal direction of the  $\Delta^{14}$ C-bulk versus  $\Delta^{14}$ C-CO<sub>2</sub> space is more evident across ecosystem type, which at the same time implies specific environmental conditions for the stability of SOM."

# Discussion

• Line 291: Please discuss the moisture results as whether they are significant or not for each ecosystem. Additionally, since there was not a significant moisture x temperature interaction, it is not accurate to discuss the moisture effect on different temperature treatments. It is also unclear whether the sites were added in the model structure; therefore, I'm not sure if the moisture effect can even be discussed separately for each site. All of this needs to be addressed in the methods and results sections.

We modified this section to discuss treatment variation in terms of significance as follows:

"Nevertheless, changes in WFPS had a significant effect on the  $\Delta^{14}CO_2$  and  $\Delta^{14}C$ -bulk of grassland soils and only on the  $\Delta^{14}CO_2$  of peatland soils."

Besides, the contrasting conditions between grassland and peatland were adapted in the model by differentiating the ranges of decomposition rates in both slow (peatland) and fast (grassland) cycling systems. This was added in the section 2.4 of the methods and 3.2 of the results.

Since SOM stability depends on the direction of moisture change, we consider that approaching to this discussion has to differentiate grasslands from peatlands as SOM would react different when moisture thresholds are crossed. Therefore, we emphasize on the importance of understanding not only the values of WFPS but also the direction in which it changes (e.g., from dry to wet or wet to dry) as well as its interplay with temperature as modulator of SOM decomposition rates.

New references:

Pegoraro, E. F., Mauritz, M. E., Ogle, K., Ebert, C. H., & Schuur, E. A. (2021). Lower soil moisture and deep soil temperatures in thermokarst features increase old soil carbon loss after 10 years of experimental permafrost warming. Global change biology, 27(6), 1293-1308.

Shaw, R. G., & Mitchell-Olds, T. (1993). ANOVA for unbalanced data: an overview. Ecology, 74(6), 1638-1645.