RC1: 'Comment on egusphere-2023-210', Anonymous Referee #1, 23 Mar 2023

We thank referee # 1 for his/her constructive comments which were highly appropriate and contributed significantly to the improvement of our manuscript. Here we quote his/her comments in *italics* and provide our answers below each of them.

- *Tangarife-Escobar et al. present a well-executed and thoughtful experiment carried out in an important geographic region. I enjoyed reading the work, and find it well argued and well composed for the most part. I would suggest that some additional data be included in the main text (which is now not given at all, or is relegated to the supplementary/appendix materials) to help clarify and strengthen arguments in the discussion. The modeling and empirical components are a bit disconnected from one another as currently presented. If the experimental results cannot be used directly in the soilR simulations, perhaps some of the data may be presented in merged figures to help the reader more directly understand the connections between these two components of the work (see detailed comments below).*

  We carefully addressed this issue to make sure that the connection between the experimental and the modelling approaches is clearer. Please see comments below.

  Detailed comments:

- *Abstract line 17: The statement that temperature is a significant variable contradicts the results stated earlier in the abstract.*

  We reinterpreted this assertion and corrected as follows:

  "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."

  Additionally, although temperature did not affect the $\Delta^{14}C$ values from bulk soil or CO_2 in the incubations, it does modulate the stability of carbon through changes in decomposition rates (we added Figure 5 C-D which clearly shows this pattern). The latter can be seen clearly in the results of the simulations, where the decomposition rates (k) affect the $\Delta^{14}C$ in both slow and fast cycling systems.

- *Lines 56-64: This is a very clear and succinct explanation of C pools and turnover. Nicely done!*

- *Line 110: How did the presence of inorganic C potentially affect the Delta 14CO2 values?*

  According to our calculations, the reported percentages of inorganic carbon of 0.05 and 0.06 as found in the analyzed peatlands and grassland soils, respectively, would affect the $\Delta^{14}C$ in less than 0.05 ‰, which we consider is negligible for the purpose of our study.

- *Methods: Soil incubation times… the duration of the incubations is cited many times in the discussion as a potential confounding variable in the interpretation of the Delta 14CO2 data. However, the length of the incubations is not given in the methods. The length of the incubations should be added to the manuscript along with a discussion of how variable lengths of incubations for the individual treatments might have influenced* *the Delta 14CO2 data. I’m assuming different treatments were incubated for different lengths of time since the methods indicate that they were incubated until a certain amount of CO2 was produced, and given the differences in respiration rates given in the appendix the length of incubation time might have varied by an order of magnitude? Could this have an influence on the age of C being respired (i.e. longer incubation times allowed for decomposition of more structurally and/or chemically "stabilized” substrates)?.*

The duration of the incubation is mentioned in the methodology (section 2.2). However, we added a column with the specific time of incubation for every treatment in the table A1 and reference it in section 2.2. We also corrected the mean CO_2 respiration values which were interchanged between peatland and grassland. As we emphasized in the discussion, the incubation duration could indeed have an effect on the type of C respired from the soil and therefore on the transit time of the respired CO_2 since “younger” SOM might have been oxidized faster than more stable “older” SOM.

We added:

“An important aspect that has been observed in soil incubations, is that CO_2 accumulation decreases or even stops after a certain period probably due to the CO_2 saturation of the limited headspace in the incubation flasks, which depends on the SOC content. From this we could deduce that a higher respiration rate is followed by fast saturation of the headspace in soils with high SOC and therefore only the carbon firstly decomposed (usually labile) will be present in the CO_2. For example, Azizi-Rad et al. (2022) found the respiration rate to decline after 14 days holding the soil at 10 C. In this sense, incubations with high TOC may run out of headspace soon and hinder the respiration of old carbon that requires longer times to be decomposed”

Complementarily, we have observed in recent incubations (Tangarife et al, in preparation) that the CO_2 respiration from soil stops after few days probably due to the saturation of the limited headspace in the incubation flasks. From this we could deduce that the isotopic signature of soil respiration can only be properly measured if the CO_2 accumulation is guaranteed.

Modified Table A1.

Table A1. Mean daily CO_2 respiration for incubated soils under temperature and WFPS variation.

Ecosystem	Temperature (°C)	WFPS (%)	Mean CO_2 respiration (mg CO_2 g soil $^{-1}$ day $^{-1}$)	σ	Incubation time (days) n = number of samples
Grassland	20	95	0.040	0.035	2n = 32, 1n = 19
	20	60	0.028	0.016	4n = 19
	15	95	0.024	0.008	3n = 30, 1n = 15
	15	60	0.016	0.004	4n = 30
	10	95	0.011	0.011	3n = 66
	10	60	0.008	0.023	6n = 66
	5	95	0.005	0.003	5n = 67
	5	60	0.004	0.001	4n = 67
Peatland	20	95	0.510	0.001	5n = 13
	20	60	0.371	0.003	4n = 13
	15	95	0.330	0.004	5n = 13
	15	60	0.234	0.003	3n = 13
	10	95	0.209	0.002	5n = 13
	10	60	0.134	0.001	5n = 13
	5	95	0.066	0.000	4n = 13
	5	60	0.044	0.001	5n = 13

- *Methods: What is the reasoning behind the choice of WFPS values? Ninety-five percent is very high. Doesn't this value inhibit evolution of gases from the soil matrix? How/why were 65% and 95% chosen?*

  We choose 60 and 95% WFPS because of several reasons. First, to compare our results to Sierra et al. (2017) and Azizi-Rad et al. (2022) who had conducted previous incubation experiments on sensitivity of soil respiration to temperature and moisture. Azizi-Rad et al. (2022) analyzed the same grassland soil of our study and found that temperature was the factor limiting soil decomposition provided that moisture and oxygen were sufficiently available. Hence, we were aware that our WFPS levels must contain enough water and oxygen for ensuring soil respiration. Finally, one of the main objectives of our study was to observe the behavior of $\Delta^{14}C$ and transit times of C in soils under degradation processes such as from high saturation towards drying in peatland soils and from seasonally frozen to water saturated in grasslands.

  We added the next sentences to the section 2.2 in methodology:

  "Each of the sets was placed at two different WFPS levels (60 and 95 %) which were selected in order to reassemble the thaw and consequent water saturation of seasonally frozen soils in grasslands; and for peatland soils, the process of drying (through artificial desiccation) after high water saturation"

  On the other hand, 95% of WFPS is certainly a high level of moisture saturation, nonetheless, the incubated soil had still wide contact with oxygen, allowing SOM oxidation and CO_2 accumulation.

- *Table 2: Unclear what is being compared here. Is the anova between grasslands and peatlands at each treatment level of temp/moisture? Or is it comparing different levels of temp within each soil category? It's confusing because the soils weren't radiocarbon dated *after* incubation, correct?*

  We apologize for the misunderstanding. We are comparing here the variation of $\Delta^{14}C$ values of bulk soil and CO_2 for each type of ecosystem with the objective to evaluate the effect of temperature, WFPS and WFPS x temperature treatments. $\Delta^{14}C$ values from bulk and CO_2 were obtained after the incubations. We reformulated the caption of Table 2 as follows:

  "Summary of *p*-values obtained from ANOVA tests for the $\Delta^{14}C$ of bulk soil and the $\Delta^{14}CO_2$ of grassland and peatland soils after incubation. *p*-values are given for the independent effect of temperature (T) and WFPS as well as the integrated effect of temperature and WFPS (T • WFPS)."

  And also added a sentence in the methodology to clarify:

  "Radiocarbon analysis were conducted in the bulk soil of each sample after the incubation."

- *Table 3: This is really a lot of different conditions... and on top of that you discuss the type I, type II or type III systems. How do these three things relate to one another ("fast/slow", "parallel/series", "type I/II/II")? Also, please add to the "System" column "grassland" and "peatland" in addition to "fast" and "slow". I know it's in the text directly below, but it would help the reader keep on top of all the modeling approaches.*

  The classification in type of system (I, II and III) indicates the relationship between age and transit time. We established the possible type of systems for specific ecosystems and environmental conditions based on the $\Delta^{14}C$ values obtained from the incubations. Additionally, from the modelling exercise we observed how the soils moved along these types of system when modifying the internal characteristics and the model structure.

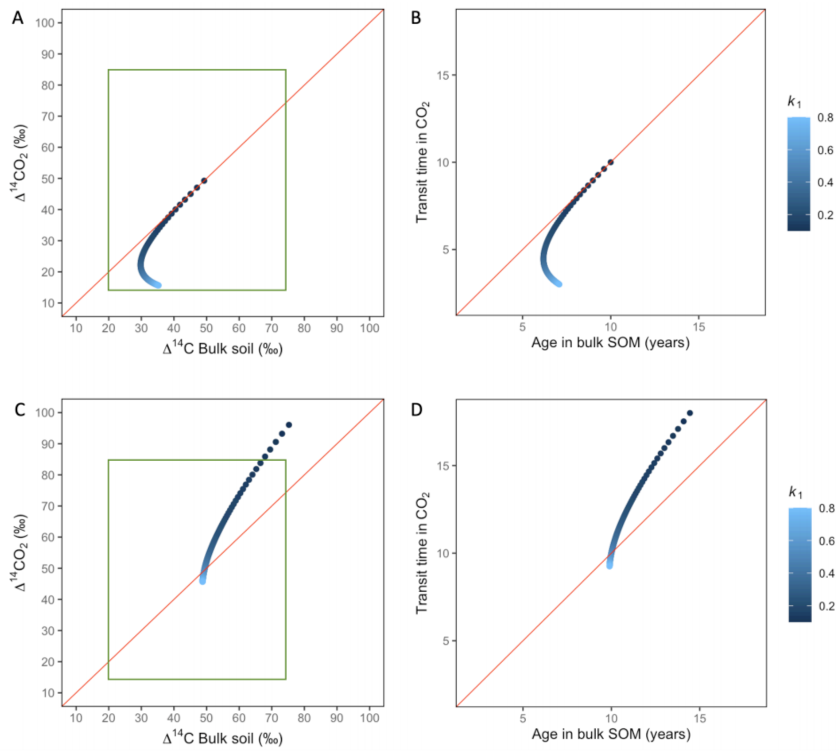
  We added a sentence to clarify this relationship:

  "Our simulations mimicked a fast cycling grassland and a slow cycling peatland by differentiating the ranges of decomposition rates (Table 3) and showed how the modelled conditions affected the type of system (I, II and III).

  We would prefer not adding "grassland" and "peatland" to the "System" column in Table 3. Our modelling approach reassembles the conditions for slow and fast cycling systems and targets the $\Delta^{14}C$ values found in our incubations. As we do not fit the models to any observed values from the incubated grassland and peatland soils but rather explore which ranges of conditions would be useful to describe the $\Delta^{14}C$ values, we decided not to indicate in the table the names "grassland" and "peatland" to not to mislead the reader. Instead, we clarified in caption of Table 3 that the parameters used for the simulations might be helpful to understand $\Delta^{14}C$ values as well as age and transit time in grasslands and peatlands.

  "Range of parameters used for simulations in a SOC decomposition model for fast and slow cycling systems (Tangarife-Escobar et al. 2023). These ranges explore the required conditions to describe the $\Delta^{14}C$ variation found in the incubated grassland and peatland soils and their equivalent age and transit time relationship. The target $\Delta^{14}C$ intervals are shown as boxes in the Figures 6 to 9."

Additionally, to establish a more accurate connection between the $\Delta^{14}C$ values from the experiments and the simulations, we added some panels indicating the target region on the Figure 6-9 and A1 to A6 (see comment below).



- *Figure 5: I feel that it is important to have an additional two panels in this figure showing the total amount of C respired by each of the treatments for a given length of time or the respiration rates. This information is referenced in the discussion, but I don't see it anywhere. In the discussion, the manuscript makes a point about the relative importance of the age vs. the amount of respired C, so the amounts should be shown. See additional comment regarding appendix table A1 below.*

This is an excellent suggestion. We added panels with the respiration rates, calculated over the total length of the incubation for each treatment. The times of incubation are specified in table A1. Here the correlation between ecosystem, temperature, WFPS and CO_2 respiration rates becomes more evident. In that sense, we modified Figure 5 and its caption, see comment below where Figure 5 is also addressed.

Additionally, we presented the results on respiration rates more in detail in section 3.1:

"Higher temperature and WFPS caused an increase of CO_2 fluxes from respiration in the treated incubated soils (Table A1, Fig. 5 C-D). In both ecosystems, wetter conditions showed higher respiration rates and higher slopes as the temperature increased. The absolute amounts of CO_2 produced from peatland soil was in average 14 times higher than from grassland soils for every independent treatment."

- *Figures 4 and 5: The 10 deg C thing… something unique seems to be happening at this temperature in the peatland soils. Do you have some explanatory hypotheses? This temperature also has strong outliers in both soil types (Figure 4), would you please comment on this?*

  The appearance of outliers in the 10 ˚C treatments for both of the ecosystems was one of the main questions emerging from our experiments. After discarding any measurement issue, we conducted the simulations to evaluate if such values belonged to the range in which $\Delta^{14}C$ moved for specific soil characteristics. However, the $\Delta^{14}C$ curves obtained from the simulations did not describe the behavior of the outliers.

  We elaborated on the outliers explanation as follows:

  "Finally, although extremely depleted $\Delta^{14}C$ values are rare, they are probable since the measured carbon particles represent one value, which can fall on the tail of the system-age probability distribution. We interpret that outlier results belong to carbon particles that have remained for very long time in the system (out of the mean values) whose specific soil characteristics were not captured in our model structure or internal soil conditions. The reasons behind the occurrence of these outliers at the specific 10 ˚C remain to be investigated."

- *Figures 6-9: Please label all these panels of figures as "grass vs peat" and "fast vs slow". Preferably in the figure itself, but at least in the caption. This will help the reader more easily keep track of what they're looking at.*

  Every figure from 6 to 9 corresponds to only one set of conditions. For example, Figure 6 shows the simulation for a fast cycling system, which targets the $\Delta^{14}C$ values of the incubated grassland soil. Therefore, we added this information in the caption of each figure (Fig. 6-9 and Fig. A1-A6) which were accordingly adapted as follows:

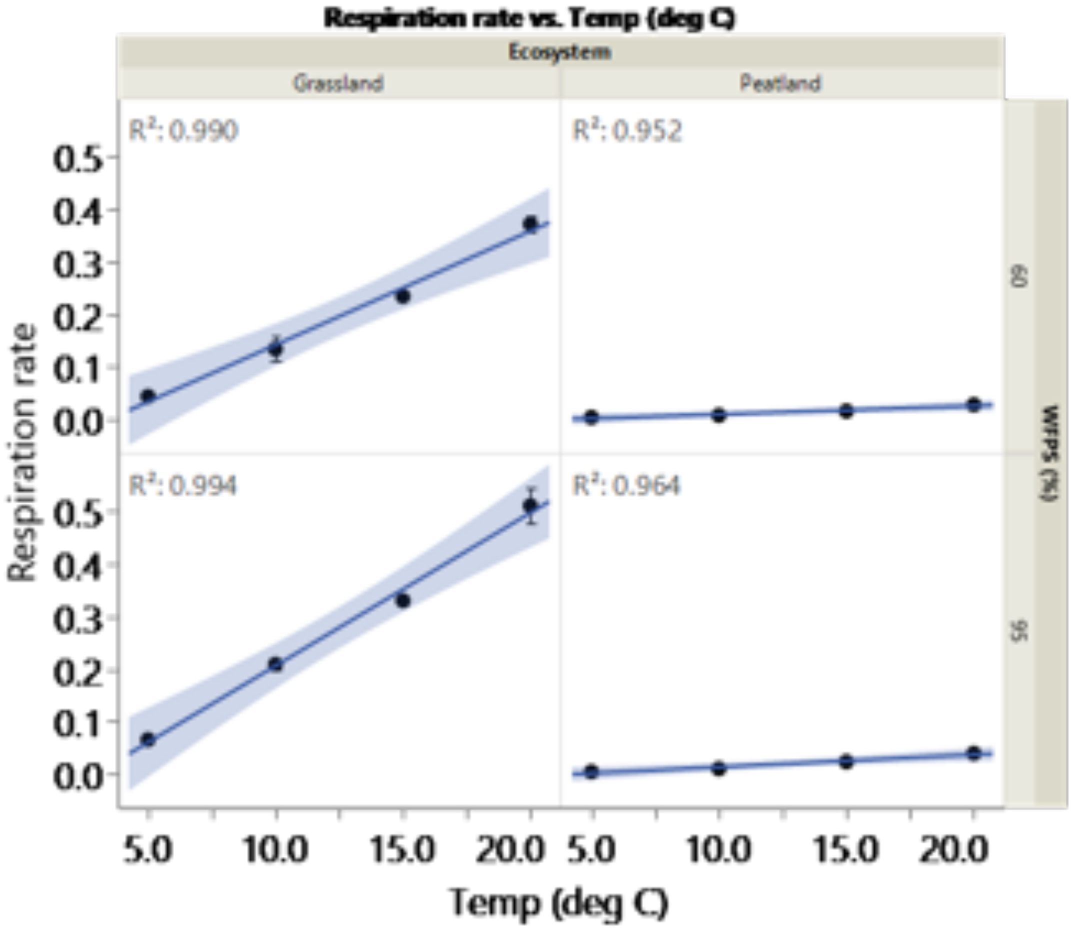
  "Predictions of $\Delta^{14}C$ in bulk soil vs $\Delta^{14}CO_2$ with their equivalent simulation of mean age in bulk soil vs mean transit time in CO_2 for parallel (panel A and B) and series model structure (C and D) for a fast cycling system. k_1 = 0.8, k_2 = 0.1, with α = 0 - 1 and γ = 0 - 1. Green box represents the range of measured $\Delta^{14}C$ values obtained from incubated grassland soils for bulk (20 to 74 ‰) and CO_2 (10 to 85 ‰), excluding outliers."

  "Predictions of $\Delta^{14}C$ in bulk soil vs $\Delta^{14}CO_2$ with their equivalent simulation of mean age in bulk soil vs mean transit time in CO_2 for parallel (panel A and B) and series model structure (C and D) for a slow cycling system. Variation of k_1 with α = 0.1 and γ = 0.2. Brown box represents the range of measured $\Delta^{14}C$ values obtained from incubated peatland soils for bulk (-90 to -65 ‰) and CO_2 (-18 to 25 ‰), excluding outliers."

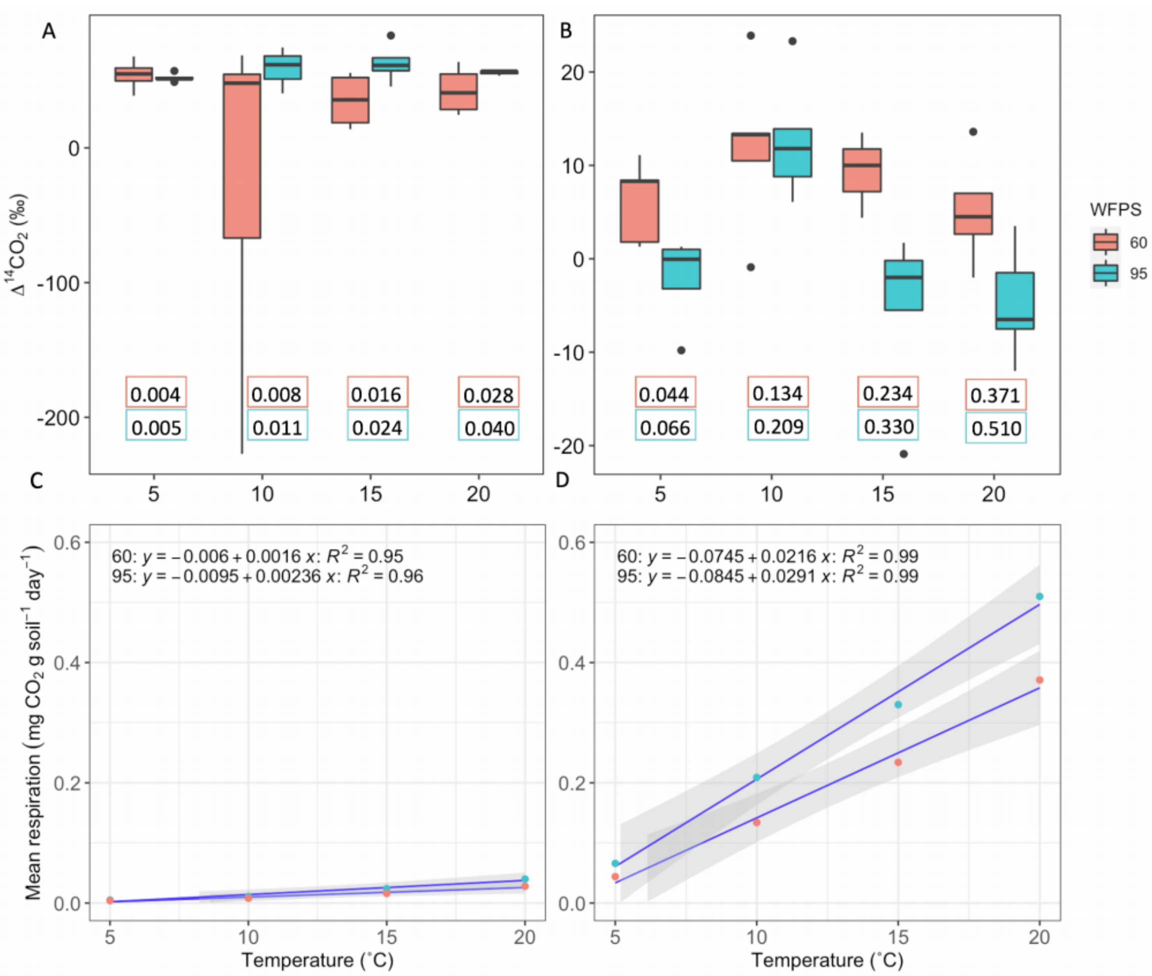
- *Model/data fusion: Can soilR not use the Delta 14C of the respired C to constrain alpha/gamma and k1/k2 values? Or is that too computationally intensive at this point? I find the paper to be well written, but there is not a lot of integration of the incubation data with the modeling exercise. What would help me understand the connections would be plotting (some? All?) the data from figure 4 onto figures 6-9. This would directly show me how the experimental results map onto the different modeled scenarios. This would really the reader more quickly understand the connections between the type I/II/III systems and the model parameters (gamma/alpha and decomposition rate constants).*

Please see comment above. Our objective was not to fit a model to the observed incubated data but rather exploring how certain set of conditions (internal characteristics of soil) related to SOM decomposition rates affected the $\Delta^{14}C$ values and their corresponding mean age and mean transit time (as added in section 2.3). Our experimental results provided radiocarbon data with relatively large variance, which is challenging to model in a classical, parameter optimization, sense. Nonetheless, the $\Delta^{14}C$ data in the bulk soil and respiration obtained from the incubations were used as the target x-y-coordinate-space of our simulations. To show this connection in a more precise way we modified the captions and added boxes to the simulation figures as explained in the previous comments. We hope that in this way, the link between the experimental and the modelling sections of our manuscript becomes more tangible.

- *Table A1: Ok. Here is where the significant temperature effect is... this looks like a pretty linear response of respiration rate to increasing temperatures. Why not include this result in the main text? I think it's alluded to in the abstract, but there's no actual evidence of a temperature effect included in the main text at this point (maybe add to figure 5).*



This is a very appropriate idea. We added panel C and D to figure 5 to illustrate the response of mean respiration rates to temperature and WFPS. In consequence we modified Figure 5 and its caption as follows:



"Figure 5: Comparison between $\Delta^{14}C$ of respiration from incubated grassland (A) and peatland (B) soil at different temperature levels under WFPS = 60 and 95 %. Black points represent minimum and maximum values out of the range between quartile 1 and 3 (25 to 75 % of the data). The quartile 50 (median) represented by the line inside the box indicates the midpoint value in the frequency distribution. Box for the treatment WFPS= 60 % and T= 10 ˚C shows a large dispersion of the 50 % of the data, which is explained by the outliers observed in Fig. 4. Additional panels indicate the respiration rates (mg CO_2 • g soil -1 • day -1) for each treatment based on the total duration of incubation (see Table. A1). Response of mean respiration rates to temperature and WFPS treatments in grassland (C) and peatland (D) soils."

And added the next lines in the text:

"Higher temperature and WFPS caused an increase of CO_2 fluxes from respiration in the treated incubated soils (Table A1, Fig. 5 C-D). In both ecosystems, wetter conditions showed higher respiration rates and higher slopes as the temperature increased. The absolute amounts of CO_2 produced from peatland soil was in average 14 times higher than from grassland soils for every independent treatment."

- *Conclusions: The conclusions lack punch. What are the broader implications of this work? How do the experimental treatments relate to current climate projects for the QTP? Will the peatlands dry out and change this from a type X to a type X system? Will the grasslands get hotter and therefore respire X more gigatons of C on an annual basis? The introduction states that these soils are being studied because of they hold* *vast stores of C. What do your experiments suggest for the fate of these C stores under future climate scenarios? .*

We complemented and modified the conclusions as follows:

"From our modelling approach, we conclude that radiocarbon can be used as a tool to understand SOM persistence through the use of the concepts of mean age and mean transit time and their mutual relation. Our simulations were able to reassemble the $\Delta^{14}C$ values obtained from incubations and showed that modelled $\Delta^{14}C$ values differed widely between slow cycling systems and fast cycling systems. We found that low values of decomposition rates, more common in slow cycling systems, modified the behavior of $\Delta^{14}C$ patterns due to the incorporation of ^{14}C-bomb in the soil system. Hence, the correspondence between these mutual relations strongly depended on the internal dynamics of the soil and its interaction with the environment. For this reason, the acquisition of empirical data from soils (number of pools, I, C, k, γ and α) along with the correct setting of model structure will improve our understanding on the stability of carbon in the soils of a changing QTP. In this way, current changes in climate patterns and land cover alteration may have a larger impact on the Zoige peatlands than on the grasslands given the vulnerability of large carbon stocks to be destabilized by changes in temperature. Nevertheless, the interaction with moisture may dampen or amplify the temperature effect, adding uncertainty on the future trajectories of soil carbon in the Qinghai-Tibetan Plateau."

New references:

Sierra, C. A., Malghani, S., and Loescher, H. W.: Interactions among temperature, moisture, and oxygen concentrations in controlling decomposition rates in a boreal forest soil, Biogeosciences, 14, 703–710, 2017a.