RC2: 'Comment on egusphere-2023-210', Additional anonymous Referee #3, 9 January 2024

We would like to extend our sincere appreciation to Referee #3 for diligently reviewing our manuscript. We have thoroughly addressed all the comments and have integrated our responses to the previous referees' feedback. The key points from Referee #3 review are summarized below in italics, with our reply in normal font.

Reviewer comment to Authors

- *Angarife-Escobar et al studied the effect of temperature and moisture on CO_2 respiration to understand the stability of carbon in peatland and grassland. The paper presents interesting insights and is clearly well written. Nonetheless, I have few observations.*

General comments

- *Throughout the paper the Authors refer to heterotrophic CO_2 diffusion rate from the soil as CO_2 respiration. Please note that you did not measure CO_2 respiration but measured the diffusion rates of CO_2 from the soil. I am aware that many Scientists use this terminology but is not entirely correct as not all the respired CO_2 diffuses out of the soil. I would advise to acknowledge this in the beginning that you measured CO_2 diffusion rate as a close proxy of CO_2 respiration.*

We modified and added as recommended:

> The rate of accumulation of CO_2 in the headspace of our incubations represents the diffusion rate of heterotrophic CO_2 respiration released from the incubated soils. We refer throughout the manuscript to heterotrophic respiration, but we acknowledge that our measurements better capture how this heterotrophic CO_2 respiration flux diffuses out of the soil.

- *Further note that you did not measure autotrophic or total CO_2 diffusion rate but the incubation performed measured heterotrophic CO2 diffusion rate. It is useful to to refer to your respiration rate as heterotrophic CO2 respiration.*

> We changed the terminology to heterotrophic CO_2 respiration instead of soil respiration along the manuscript.

Specifics

- *Line 141: Incubations for each subset ended simultaneously until every sample had an estimated concentration of CO_2 -C in the headspace equivalent to ≥2mg of C, enough for radiocarbon analysis.*
  This statement is not clear to me. Further for how long did it take to achieve a CO_2 flux of ≥2 mg of C ? Was there pre-incubation period?

> We modified the sentence to avoid ambiguity:
>
> Incubations for each subset concluded concurrently once all the samples reached a C concentration (from CO_2) in the headspace estimated to be equal to or exceeding 2 mg, sufficient for subsequent radiocarbon analysis. This approach was not possible in

two of the 16 subsets due to lab material limitations and therefore, grassland samples were incubated between 15 and 67 days, while peatland samples were incubated for 13 days (Table A1).

Additionally, we added for clarification:

We conducted two sets of incubation experiments without pre-incubation period, one set with the grassland soil and a second set with the peatland soil.

- *144. For sampling headspace air, 50-ml vials were filled with 12 g of soil (± 1.5 g) and placed inside 0.5 L glass flasks along with 0.2 ml of water at the bottom of the flask (away from contact with the sample) to avoid possible drying (Dioumaeva et al., 2002); thereafter the flasks were sealed with rubber plugs and screwed with plastic caps. Flasks with samples were flushed with synthetic air (CO_2 free) to remove atmospheric CO_2. This flushing marked the starting day of the incubations.*
  How did you make sure the disturbed soils were repacked to a bulk density similar to that of undisturbed field soils?

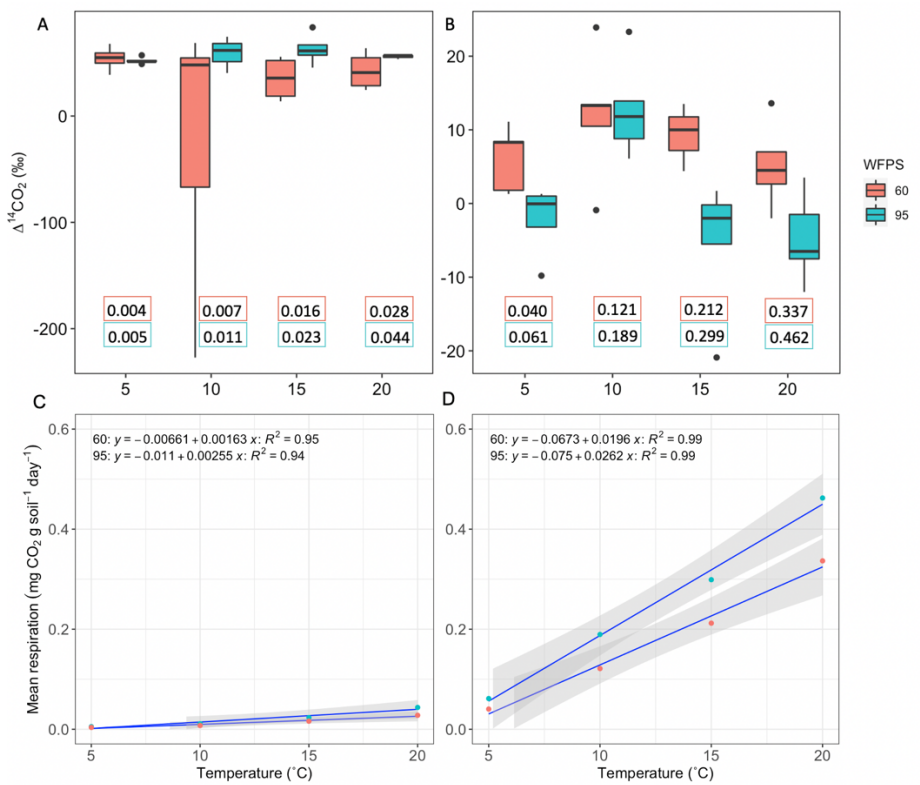
  Unfortunately, since the soils were disturbed, we did not try to match an exact value of bulk density during the repacking.

- *The headspace volume is not mentioned. What headspace volume were left when the 12g of soil were packed in 50 mL. Were the headspace left uniform for all samples throughout? Were headspace volume corrected for in the flux calculations?*

  We appreciate this helpful comment. We have now corrected the flux calculation considering the change of headspace when adding the soil. Consequently, we added the following in the methodology:

  The headspace volume of the incubation flasks was measured as 587 ml, which was corrected after adding the soil. In average, the final headspace was 575.6 and 533 ml for the incubated grassland and peatland soils, respectively. These values were used to calculate the fluxes of heterotrophic CO_2 respiration.

  Additionally, we modified Figure 5:



- *The CO_2 respiration was measured within what time interval? every minute, 10 minutes or what exactly?*

  Although we measured fluxes at several time intervals for the different subsets of incubation, the fluxes calculation was made based on the last measurement of heterotrophic CO_2 concentration. Here we complemented this information by adding the column “flux duration (day)” in Table A1, which is referenced along the manuscript when referring to the flux accumulation.

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	Flux duration (day)	Incubation time (day) n = number of samples
Grassland	20	95	0.044	0.009	19	2n = 19, 1n = 32
	20	60	0.028	0.003	19	4n = 19
	15	95	0.023	0.004	15	3n = 30, 1n = 15
	15	60	0.016	0.003	15	4n = 30
	10	95	0.011	0.001	60	3n = 66
	10	60	0.007	0.001	60	6n = 66
	5	95	0.005	0.000	60	5n = 67
	5	60	0.004	0.001	60	4n = 67
Peatland	20	95	0.462	0.032	7	5n = 13
	20	60	0.337	0.014	7	4n = 13
	15	95	0.299	0.007	7	5n = 13
	15	60	0.212	0.004	7	3n = 13
	10	95	0.189	0.010	9	5n = 13
	10	60	0.121	0.020	9	5n = 13
	5	95	0.061	0.004	7	4n = 13
	5	60	0.040	0.001	7	5n = 13

We included the explanation on the role of time interval of the heterotrophic CO_2 fluxes in the comment below.

- *How were the CO_2 concentrations converted into fluxes? This should be stated in the methods.*

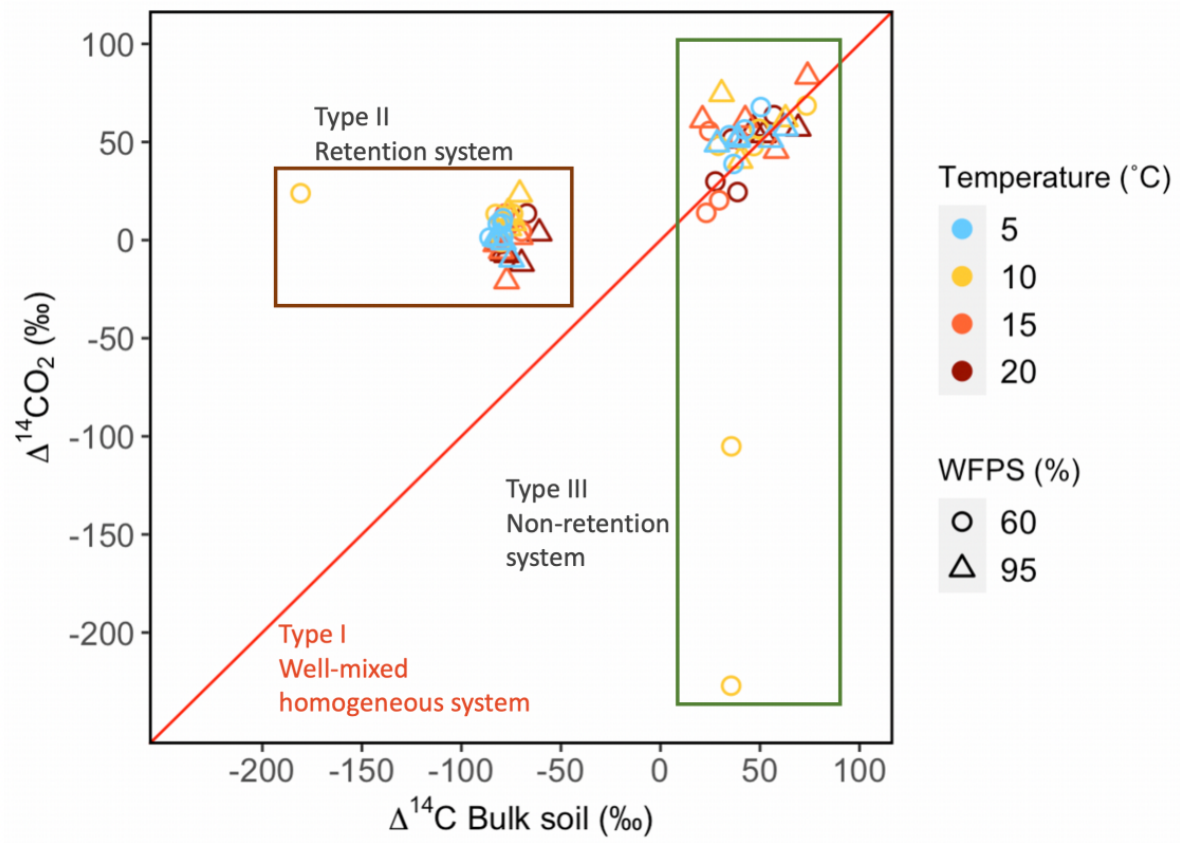
  We added in the methodology:

  Rates were measured at intervals of 1 to 2 weeks using a CO_2 analyzer LI-COR 6262 for every treatment and mean heterotrophic CO_2 respiration rates (mg CO_2 g soil^{-1} day^{-1}) were calculated through the division of CO_2 concentration in the headspace by the product of the accumulation duration (days) (Table A1) and the mass of the introduced soil (g).

Results

- *Fig. 4: The symbols for WFPS at 60 and 95 % are not visible in the graph.*

  The symbols for the samples in Fig 4 indicate a combined treatment, where WFPS is represented by shape and temperature by color. Thus, the shapes for WFPS can be seen in the graph in different colors depending on the temperature. To deal with the ambiguity of the black color in WFPS, we have modified the Fig 4 and changed the legend to empty shapes.



- *At such a high WFPS of 95%, doesn't C emission shift to CH4 pathway rather than CO2?*

  As we had mentioned in the reply to referee#1 and 2, "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." Unfortunately, we did not measure CH_4 levels and cannot elaborate on this topic.

  We added in the methodology:

  Despite the high WFPS, the soil samples had still contact with air inside the vials, which guaranteed microbial decomposition of the organic matter and accumulation of heterotrophic CO_2 respiration.

- *Fig 5 C and D: You measured the CO_2 respiration at four different temperatures. With this result you can derive and compare an important parameter of C transformation. i.e. the coefficient of temperature sensitivity of CO_2 respiration Q_{10}.*

  Although the Q_{10} parameter has been widely used as an indicator of biochemical processes, we consider it is not useful for our approach due to several reasons. First, there is a large debate on the usefulness of Q_{10} as a metric of temperature sensitivity of SOM. Although Q_{10} is conceptually clearly defined, there are different formulas to calculate it (Fang et al., 2005, Fierer et al., 2005, Conant et al., 2008, Wetterstedt et al., 2010) that impose limitations and even biases on their intercomparison.

Furthermore, various methods frequently employed to assess the temperature sensitivity of different substrates can produce contradictory results, despite being based on the same fundamental principles (Sierra 2012). For example, theoretical analysis on the "quality-temperature" hypothesis (Bosatta & Ågren 1999) have been contradicted by empirical mixed results with no conclusive evidence (von Lützow and Kögel-Knabner 2009). In consequence, some authors have discouraged the use of Q_{10}s (Davidson *et al*., 2006, Sierra 2012) and we prefer not to perpetuate the use of this metric. Second, our methodological setting intended to measure C concentrations from heterotrophic respiration for ^{14}C analysis instead of conducting a comprehensive temperature sensitivity analysis. Hence, we consider that our data are not well suited to this approach. Finally, our study is focused on analyzing how ^{14}C reacts to changes in temperature and soil moisture, and the calculation of the Q_{10} value does not contribute to answer our research questions.

References

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