Reviewer 2

Point-by-Point response

In this manuscript, the authors present the results of a 120 day soil incubation study of three soil types (mafic, felsic, and mixed sediment) sampled across topographic positions and investigate the biological and chemical controls on respiration rates and respired 14CO2. This manuscript is a significant contribution to the soil organic carbon research because the soils are from tropical Africa, a region where few studies have been carried out. Furthermore, this manuscript looks at the important C stabilization mechanisms in highly weathered soils, which are also poorly sampled and understood relative to less weathered soils often found in temperate regions. Another positive is that the researchers examined 3 depths within the soil profile. I found no glaring issues with the methods used in this study. I did have some questions about the way some of the data were presented. On one hand, this paper could be shorter and more streamlined, but on the other hand the lack of data from Tropical Africa does greatly increase the value of all the parameters reported here. I offer some suggestions on how to shorten the paper below.

Our response: We thank the reviewer for the overall positive evaluation, comments, and suggestions. In this response letter, the reviewer's comments are in italic, our responses listed always directly afterward. Suggested text that we will add or remove in the revised manuscript is stated between " " and new/changed text underlined.

Rev 2 Comment 1: *Lines 107-125: This whole paragraph would be better as a Table introducing the sites and their location and chemistry. A table would be easier to read than a paragraph and make it easier to compare the sites.*

Our response: We agree that this section can be shortened by adding a summary table. We will summarize the chemical composition of the sites in the form of a table (see table below) and revise lines 107-125 as described below.

"Study sites in the Democratic Republic of the Congo are located in Kahuzi-Biega National Park (-2.31439° S; 28.75246° E) where soils have developed from mafic magmatic rocks, a result of the active volcanism in the East African Rift System (Schlüter, 2006). Mafic magmatic rocks in the region are characterized by high Fe and Al and low Si content as well as a high content of rock-derived nutrients such as base cations, and phosphorus (P) (Table 1). Study sites in Uganda are located in Kibale National Park (0.46225° N; 30.37403° E) where soils have developed from felsic magmatic and metamorphic rocks. The felsic magmatic rocks in our study region are characterized by the gneissic-granulitic complex with low contents of Fe, and Al, and high Si content. Unlike mafic, felsic magmatic rocks in our study sites are characterized by low content of rock-derived nutrients (Table 1). Study sites in Rwanda are located in Nyungwe National Park (-2.463088° S; 29.103834° E) where soils have developed from a mixture of sedimentary rocks of varying geochemistry. These sediments are mostly dominated by quartz-rich sandstones and schist layers spanning along the Congo-Nile divide in the western province of Rwanda (Schlüter, 2006). Similarly to felsic magmatic rocks, sedimentary rocks in our study sites are characterized by low content of Fe and Al but high Si content and low amount of rock derived nutrients (Table 1). A specific feature of the sediment rocks in our study region is the presence of fossil organic carbon of up to 4% C. Fossil organic carbon in these sediments is further characterized by a high C:N ratio (153.9 ± 68.5), and depleted in N (Doetterl et al., 2021 in review; Reichenbach et al., 2021 in review)."

Table: Chemical composition of the unweathered rock samples representing the soil parent material in three geochemical regions. Values represent mean± standard errors.

Geochemical region	C[%]	Fe [%]	Al [%]	Si [%]	Ca [%]	K [%]	Mg [%]	P [%]
Mafic	0	8.98 ±0.75	6.26±1.15	14.22±0.82	0.58±0.23	0.08±0.03	1.25±0.13	0.36±0.05
Felic	0	1.08±0.5	0.51±0.38	37.28±1.87	0.01±0.004	0.01±0.006	0.01±0.005	0.005±0.002
Mixed sediment	4.03	2.32±0.99	0.61±0.23	36.11±4.04	0.005±0.005	0.07±0.03	0.01±0.005	0.02±0.009

Rev 2 Comment 2: Lines 154: 12 mm sieve seems like a rather large size when the usual is 2mm. It seems that the authors did not want to disrupt aggregates. That reasoning should be given here.

Our response: That is correct. We provided this information in lines 143-144. Initially, we thought that this information would fit better in the sample preparation section than in the experiment design. But we will move this information to section 2.3 and revise lines 154-155 as follows: "Briefly, 50 g of 12 mm sieved air-dried soil were weighed into a 100 ml beaker. Soil samples were sieved to 12 mm to homogenize the substrate while maintaining aggregate structure."

Rev 2 Comment 3: *Line 174: An average of the respiration rates over 120 days, when the rates usually decrease exponentially, seems like an odd metric. Why was this parameter chosen instead of say, cumulative C loss over 120 days?*

Our response: We certainly agree with this comment. Cumulative C loss is widely used as a proxy for soil C loss. Please note that we excluded four days as our pre-incubation period to give samples idle time to adjust to rewetting after being stored dry and prepared again for incubation. Thus, we thought that presenting cumulative values might give a biased impression since we were interested in the weighted averages rather than the absolute values of respired C. However, our weighted averages could also be easily converted into cumulative C loss, if the reviewer still considers this to be the better option. For clarification, we will revise lines 175-177 as follows: "As our aim was to compare average respiration between samples rather than the absolute values through the entire period of the experiment. We analyzed data as the weighted average of SPR and TPR over the entire length of the experiment after respiration leveled off. The weight was defined by how many days of the incubation experiment each observation represented."

Rev 2 Comment 4: *Line185: How was the 14C collected from three replicate jars into one evacuated container? Wasn't the vacuum in the container a different strength for each replicate so that they may not have been sampled equally?*

Our response: This would indeed yield different strengths for each replicate. Instead of connecting the evacuated container directly to the jars, we collected 120 ml from each replicate using a syringe and transferred the gas in the pre-evacuated container using a tube adapter. This approach is commonly used by the Max Planck Institute for Biogeochemistry in Jena. We will therefore clarify line 185 as follows:

"After accumulation, 120 ml of headspace gas from each field replicate incubation jar was sampled using a syringe. These replicate samples were transferred into a single 400 ml pre-evacuated Restek canister for composite analysis."

Rev 2 Comment 5: *Line 254: How did you evaluate the distinctness of the RCs based on F-values?*

Our response: The contributions to SPR and $\Delta^{14}C$ and distinctness of individual rPCs were evaluated based on their p-Values and standardized coefficients. We used the F-statistics to evaluate the explainability of RCs for the three models as reported in Table 2. We will revise line 254 as follows: "We used p-Values ($p<0.1$) and standardized coefficients to evaluate the explainability of individual RCs while the F-statistic was used to evaluate the overall relationship between RCs and SPR or $\Delta^{14}C$ for every model."

Rev 2 Comment 6: *Section 3.1: To help streamline the manuscript, I recommend getting rid of the discussion of TPR in the results, since SPR is the focus of the manuscript. Perhaps the TPR graphs and language could be in the supplement? I am not sure what additional understanding the TPR variable really adds here.*

Our response: While SPR provides important information in C-rich soils, in lower C soils especially subsoils in our study sites, TPR provides additional information that cannot be revealed by SPR. Nevertheless, we agree to revise Figure 1 and keep data related to SPR only. Data related to TPR will be reported in the appendices and only very briefly referred to in the text. Consequently, we will revise the results section so that it is aligned with the revised, more focused figure (See revised figure below).

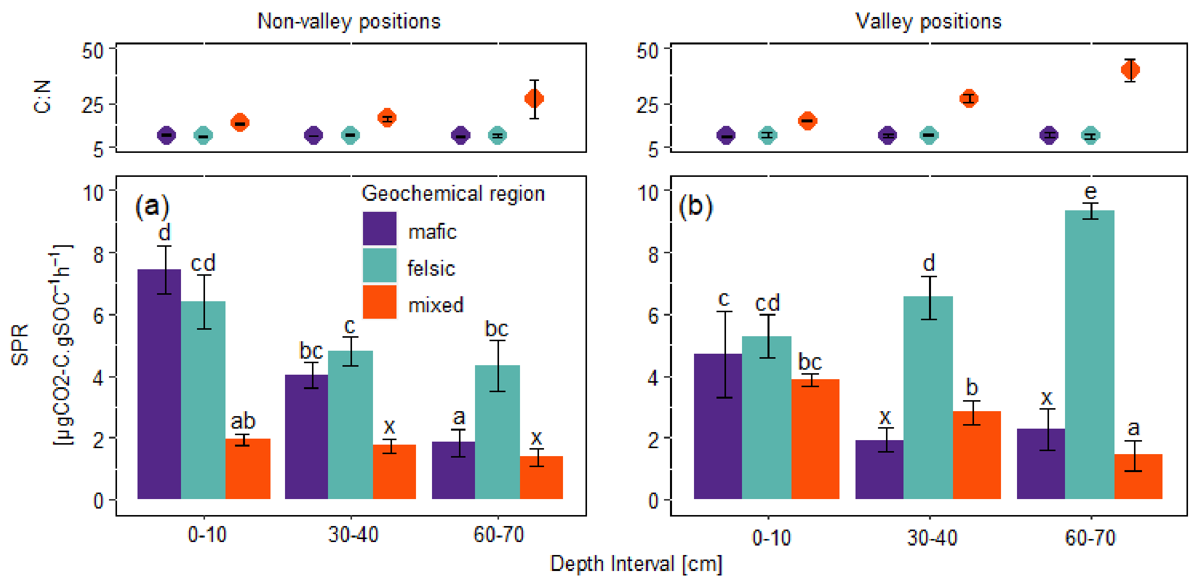

Figure 1. Average and standard errors based on field replicates for specific potential respiration (SPR) as bars (lower panel) and the C:N ratio as points on top of the bars (upper panel), for non-valley positions (**a**) and valley position (**b**). (N=9 for non-valleys, and N=3 valleys). The same letters on top of bars indicate no significant difference in SPR following ANOVA tested for differences between geochemical regions and depth intervals."x" indicates no significant difference between depth intervals within geochemical regions. ANOVA tests were performed separately for non-valley and valley positions.

Rev 2 Comment 7: *Lines 280-281: I think this sentence is basically a repeat of the first sentence*

Our response: This is indeed redundant. In the revised manuscript, we will exclude the following sentence in that paragraph. "For non-valley positions, no statistically significant differences for SPR, TPR, and $\Delta^{14}C$ were found between sloping and plateau positions"

Rev 2 Comment 8: *Fig 1. Are the standard errors based on the replicates or the measurement times since all were averaged to get these values.*

Our response: That is correct, the standard errors presented here are based on the field replicates. We will add this information and revise the figure caption as follows: "Figure 1. Average and standard errors based on the field replicates for specific potential respiration (SPR)......."

Rev 2 Comment 9: *Fig 2. I think these graphs could better show the differences between the bulk and respired 14C based on how you discuss the results in section 3.2. It would be easier to compare bulk and respired 14C if they were put on the same graph. The way they are now it is hard to see when they are similar and when they are not.*

Our response: Thank you for this suggestion, we agree to revise Figure 2 and merge the panels as suggested. See the revised figure and corresponding caption below:

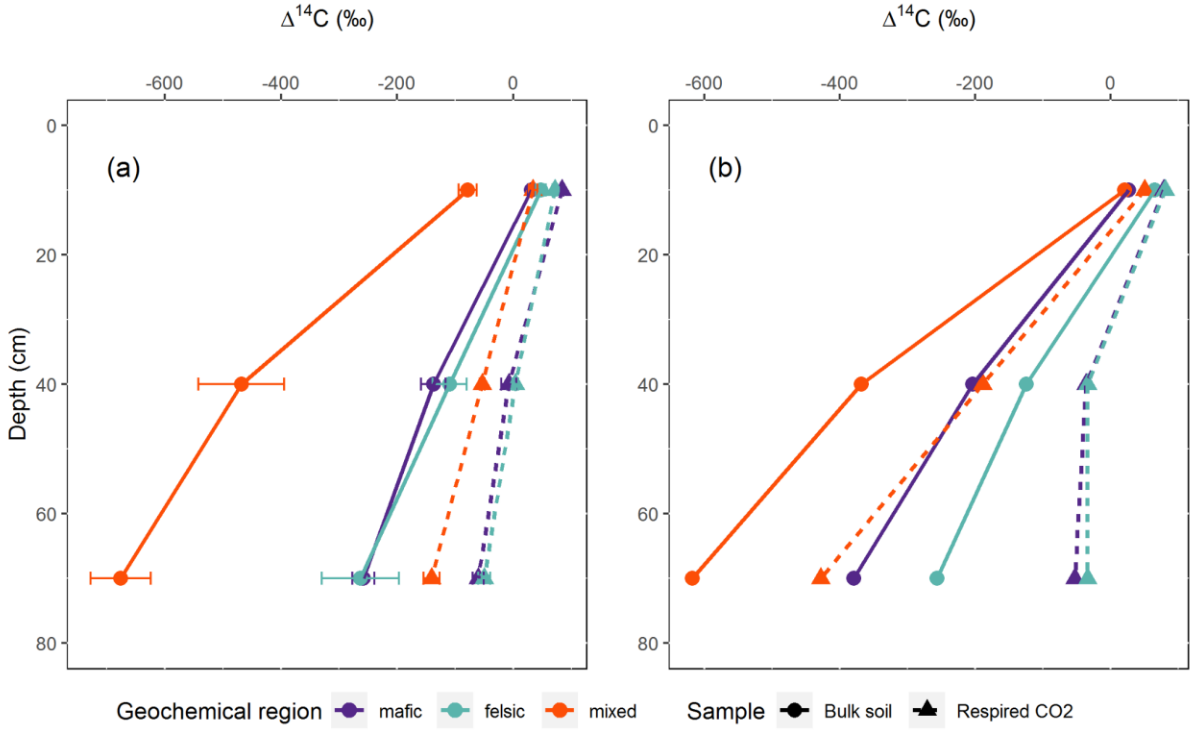

Figure 2: Average and standard errors for (a) radiocarbon content ($\Delta^{14}C$) of the bulk soil and respired CO_2 for non-valley positions, (b) $\Delta^{14}C$ of the bulk soil and respired CO_2 for valley positions, (n=27 for non-valleys, and n=9 valleys for each depth interval).

Rev 2 Comment 10: *Lines 345-349: I am not sure what the extrapolation of the respiration rates of the fossil organic C add here and in Table 1. Given the caveats, which you mention in the discussion, it would be better to leave these numbers to the discussion only.*

Our response: We agree that highlighting this information in the discussion section would suffice. We will remove lines 345-348 (see the underlined text below) in the results section and revise Table1 as shown below.

"Considering the measured respiration, under the conditions of our lab incubation experiments, we calculate all fossil organic C in non-valley positions would be mineralized in approximately 450 years from topsoil and in 387-440 years from the subsoil. In valley positions, fossil organic C in topsoil would be mineralized after 61 years. In valley subsoil, fossil organic C would be mineralized after 50-100 years."

Table 1. Biogenic and fossil organic carbon contribution in the mixed sediment rock region to SOC and respired CO_2 as % of total C and ratio bulk soil / respired C for both parameters. Values are displayed separately for non-valley and valley positions per soil depth. (one observation per position due to merging of replicates into composites prior to analysis).

		Biogenic [%]			Fossil [%]		
Position	Depth [cm]	Bulk soil	Respired gas	Bulk/Respired	Bulk soil	Respired gas	Bulk/Respired
Non-valley	0-10	89	96	0.9	11	4	2.8
	30-40	61	93	0.6	39	7	6.0
	60-70	48	91	0.5	52	9	5.8
Valley	0-10	98	97	1.0	2	3	0.7
	30-40	72	81	0.9	28	19	1.5
	60-70	57	61	0.9	43	39	1.1

Rev 2 Comment 11: *Fig 4. After all the data that is presented in the results, it is odd that the discussion starts off with yet more data! I find figure 4 overwhelming. It has 8 graphs, each with three correlations, with a total of 24 to examine! Many of these are not significant. I suggest saving the whole figure for the supplement and choosing 1-3 graphs to highlight in the discussion. Furthermore, something should indicate which relationships are significant here, maybe make the r and p values bold where they are significant?*

Our response: Thank you for this comment. We certainly agree to revise Figure 4. We also agree that having this information in the appendices section is better and could shorten the discussion. We propose the following:

1. We will revise Figure 4 and keep those variables that are largely discussed.
2. We will move Figure 4 into the appendices section and shorten the discussion related to it to the most essential parts (shown in the figure).
3. See the revised figure below.

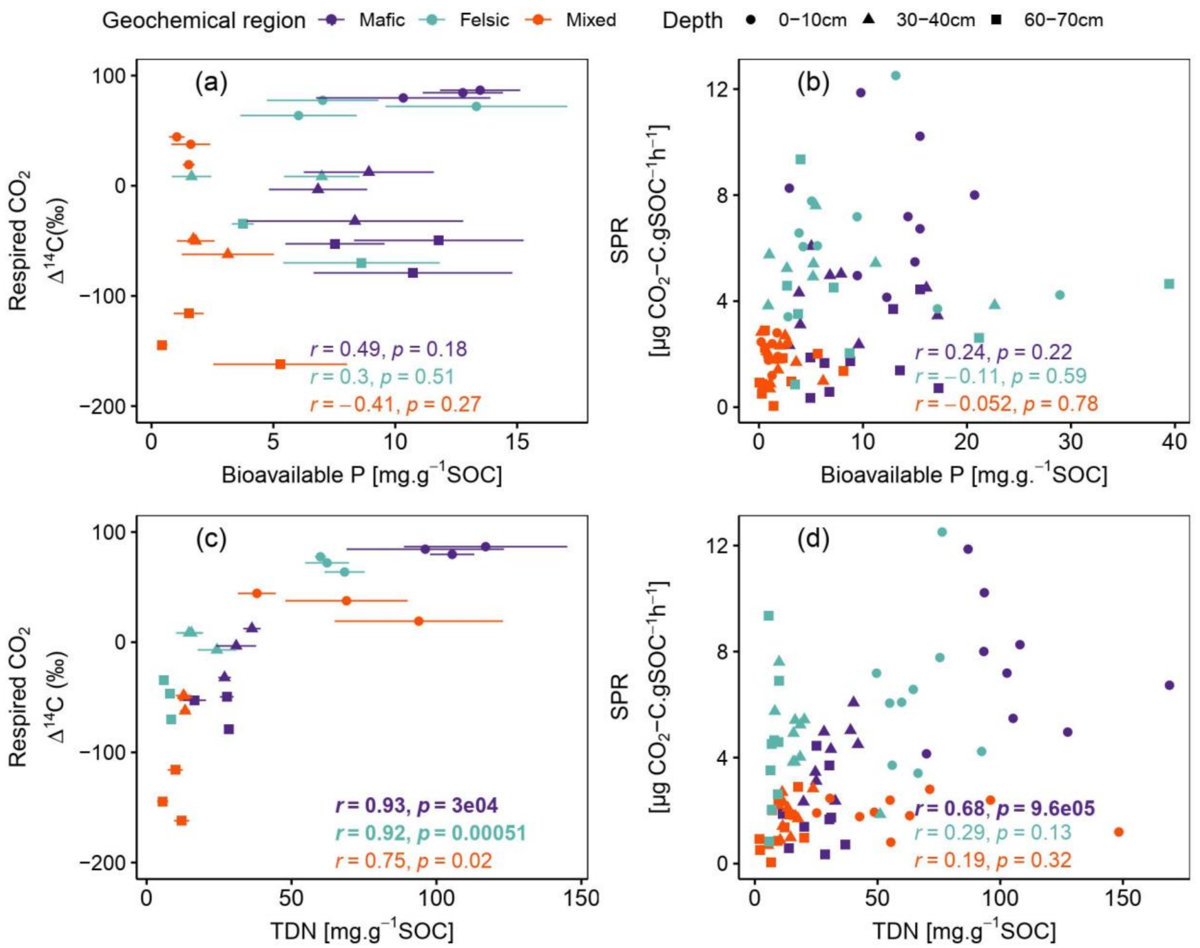

Figure 4. Pearson correlation between composite of corresponding replicates of $\Delta^{14}C$ of respired CO_2 and SPR to P (panels, a-b), and N (panels, c-d) available nutrient data reported by Kidinda et al. (2020 in review) normalized to SOC content for non-valley positions. Bioavailable P = Bray-P, TDN = Total dissolved nitrogen. Data displayed in panels a, and c, are averages plus standard errors of three field replicates. Panels b, and d, show all individual field replicates. Note that two outliers (artifacts) with high bioavailable P values in subsoil were removed from panels a, and b. Pearson correlations and p-values in bold font indicate significant results at $p<0.05$

Rev 2 Comment 12: *Line 474: I am confused by the attribution of mineral stabilization mechanisms to controlling SPR here as amorphous and crystallized oxides had no relationship to SPR and pyrophosphate-extractable had a positive relationship indicating it was not stabilizing the Carbon.*

Our response: We certainly agree. While pedogenic oxides did not show effect on SPR, they control SOC stocks. In fact, a detailed analysis conducted on the same samples (Reichenbach et al., 2021) shows that SOC stocks significantly depend on the amount of pedogenic oxides. "Our data (Fig. A1) suggests that carbon associated with pyrophosphate extractable oxides is readily available to microbial decomposers even in a short-term respiration experiment. In contrast, the presence of oxalate or DCB extractable oxides (and the carbon associated with it) does not relate to the short-term C respiration in our study. Instead, its effects on the long-term SOC stability are more likely related to the formation of stable aggregates (Oades, 1988, Kleber et al., 2005, Reichenbach et al. 2021). This is the main message we wanted to share in Fig. A1."

In the revised manuscript we proposed to add the above statement between lines (464 and 465) in order to clarify this relationship.

References added:

Oades, J. M.: The retention of organic matter in soils, Biogeochemistry, 5, 35-70, https://doi.org/10.1007/BF02180317,1988.

Martinez, P., and Souza, I. F.: Genesis of pseudo-sand structure in Oxisols from Brazil – a review, Geoderma Regional [pre-proof], https://doi.org/10.1016/j.geodrs.2020.e00292, 2019.

Reichenbach, M., Fiener, P., Garland, G., Griepentrog, M., Six, J. and Doetterl, S.: The role of geochemistry in organic carbon stabilization in tropical rainforest soils, SOIL [in review], 1–35, doi:10.5194/soil-2020-92, 2021.

Kirsten, M., Mikutta, R., Vogel, C., Thompson, A., Mueller, C. W., Kimaro, D. N., Bergsma, H. L. T., Feger, K. H. and Kalbitz, K.: Iron oxides and aluminous clays selectively control soil carbon storage and stability in the humid tropics, Sci. Rep., 11(1), 1–12, doi:10.1038/s41598-021-84777-7, 2021.

Rev 2 Comment 13: Fig 5. Can you bold the p values for what is significant here? Same for the similar graphs in the Appendix.

Our response: Thank you for this suggestion. We will revise Figure 5, and Figure A2 and put in bold significant correlations and their corresponding p-values, and amend the figure captions accordingly.

Rev 2 Comment 14: *502: specify high C:N here*

Our response: We will revise this paragraph as follow:

“Despite similar SOC stocks, SPR and TPR were lowest in soils of the mixed sediment region, which also had the lowest bulk soil and respired $\Delta^{14}C$ of the three geochemical regions (Fig. 1, 2). <u>The depletion of N and high C:N values (153.9 ± 68.5) of fossil organic C likely contributed to reducing soil respiration rates in the mixed sediment region (Whitaker et al., 2014).”</u>

Reference added:

Whitaker, J., Ostle, N., McNamara, N. P., Nottingham, A. T., Stott, A. W., Bardgett, R. D., Salinas, N., Ccahuana, A. J. Q. and Meir, P.: Microbial carbon mineralization in tropical lowland and montane forest soils of Peru, Front. Microbiol. , 5, 720: https://www.frontiersin.org/article/10.3389/fmicb.2014.00720, 2014.