Reply on RC2
Rachael Akinyede et al.

We appreciate Reviewer 2 for the helpful comments on our manuscript, "bg-2022-90". We are glad that the reviewer finds our study to be interesting with useful suggestions provided to further improve the manuscript. To meet the reviewer's concerns, we would include more information in the revised manuscript, on the role of microbial communities in dark CO₂ fixation in the introduction, elaborate on the gene abundance results in the discussion and tone down information on the effect of clay content on microbial biomass turnover. We would, in addition, clarify all confusing wordings and texts on the sampling design and statistical analysis done. We have provided a point-by-point response to each of the reviewer's comments below (see italicized bold text).

Reviewer 2: Comments to Author:

The authors compared the temperature sensitivity of dark CO₂ fixation and respiration in temperate forest soils of Germany. The fixed¹³C was traced into microbial biomass and SOC, allowing the authors to comment on potential microbial biomass turnover rates under the contrasting temperature treatments. The study is interesting and the design is overall simple, but effective; there is limited information on the potential changes in dark CO₂ fixation under climate change. However, several aspects of the analysis and the discussion could be further clarified. Some of the results pertaining to the microbial community response and gene abundance were not adequately addressed in the discussion.

- Line 26: This speculation around the role of clay content may not be appropriate for the abstract. Since the role of texture was not directly studied it is best not to highlight this as a possible mechanism in the abstract. Many other aspects of the systems may be able to explain differences in microbial biomass turnover. Similar comment for the last sentence of the abstract – “...variations in site-specific parameters might affect microbial biomass.”

  We agree and would limit our speculation on the role of clay content in microbial biomass turnover which we now describe as microbial residue formation (see reviewer 1 comment 2), to the discussion and remove this information from the abstract in the revised manuscript.

- Line 41-42: Add “through” so that it reads “through so-called dark CO₂ fixation...”. Also, what is meant by “which also affects CO₂ emissions from other soils”? This wording is unclear.

  Thank you. The word “through” would be added to the sentence as suggested. For
clarity and in line with our reply to reviewer 1 comment 6, we would remove the phrase, “which also affects CO\textsubscript{2} emissions from other soils” from the applied sentence in the revised manuscript.

- Line 66-67: What kind of ecosystems were included in this study by Nel and Cramer (2019)?
  This study was conducted for an afro-temperate forest and grassland ecosystems in Southern Africa, and this information would be included in the revised manuscript.

- Line 71: I suggest changing “can warm” to “projected to warm”
  This would be modified as suggested.

- Line 77-80: Please add a sentence or two to discuss the potential reasons why these processes would be expected to mirror each other.
  Thank you for this comment. In line with our reply to reviewer 1 comment 9, we would modify this section in the revised manuscript and change the wording “mirror” to clarify what we infer.

- In general, the introduction could have more discussion of the microbial community’s role in dark fixation.
  Following the reviewer’s advice, we would include more information on the role of microbial communities in dark CO\textsubscript{2} fixation to the introduction section of the revised manuscript.

- Hypotheses appear to be implied in the writing, but could be explicitly outlined in this last paragraph of the introduction.
  Thank you for this comment. This would be modified as suggested.

- Line 96-98: It is not clear what this is saying.
  We apologise for the confusion. Here we describe how the forest study site was established. We would modify the texts in this section to provide better clarity.

- The beech and spruce plots were not replicated, correct? I am not sure it is possible to comment on statistical differences between spruce and beech plots without further replication of the forest types.
  We did not replicate each forest plot. However, during sampling, we took six replicate soil cores each from the beech and spruce plot (line 118). We later refer to these plots as “soil” and not “plot” in the manuscript and this number of soil core replicates are sufficient to compare these soils. For this reason, we would tone down our wording (in the discussion section of the revised manuscript) on what our results might mean for other beech and spruce dominated soils.

- Line 126: What is a “biological replicate”?
  By using the term biological replicate, we refer to the replicates of the soil cores taken from each sample plot (either the beech or the spruce plot) and this is to account for variability within each of the sampled plots. However, for simplicity, we would only use term "replicate” in the revised manuscript.

- Line 183: Should $^{12}$C/$^{13}$C be $^{13}$C/$^{12}$C?
  We apologise for the oversight. This would be corrected in the revised manuscript.

- Line 279: What is the covariate in the ANCOVA?
  Thank you for this comment. When comparing some of the measured parameters between beech and spruce soil with ANCOVA, we included data from the whole soil profile for the beech and spruce soil. As a result, soil depth would also account for variability in these parameters. To account for this, soil depth was used as a covariate.
in the analysis. This would be clarified in the revised manuscript.

- Line 504-506: The turnover may be slower in the clay-rich soil, but there is a greater availability of mineral surfaces that could potentially interact with C. It was not entirely clear to us what the reviewer’s point is here. The spruce soil with higher clay content had slower biomass turnover or more accurately, lower residue formation compared to the beech soil. The original discussion focused mostly on the potential interactions with clay that could slow the transfer of residues from microbial biomass to SOM in the higher clay spruce soil, including the idea that more mineral surface area could also contribute to this. We agree that clay can act in additional ways to explain the reduced transfer of label from microbial residues. For example, association with clay surfaces can lower residue formation in SOC if the production of microbial residues would be diluted by a larger overall SOC inventory created by the higher clay content. Thus, the amount of label transferred would be more diluted resulting in a smaller proportion of the total SOC. However, it is always difficult to say the exact mechanisms for the patterns we observed especially since many parameters were not measured or tested experimentally. Nonetheless, we would add a respective sentence in the revised version of the manuscript.

- In general, the authors should elaborate on the gene abundance results. There appears to be no comment on these results in the discussion. Following the reviewer’s advice, we would provide more information about the gene abundance to discussion section of the revised manuscript.