Answer to the comments of reviewer 2

In this preprint the authors use carbon (C) isotopes (^{14}C and ^{13}C) to determine the source and age of respired soil carbon in three sites along a climatic gradient in the Coastal Cordillera of Chile. They find that respired CO2-C was of more recent origin than soil organic C and that C in deeper soils was older than surface C. They then use these results along with total DNA extracted from the soils to conclude that microbial decomposition is primarily of new carbon, rather than old in these soils. I found this manuscript to be an important contribution to our understanding of the origin and age of microbially-processed soil carbon and generally an interesting well-written piece. My chief concerns are to do with the authors' use of total DNA as equivalent to microbial DNA, and therefor a proxy for microbial activity (Line 137), in some of the authors statistical methods relating respired total ^{14}C to respired ^{14}C (Figure 2c), and the interpretations regarding the influence of primary productivity (Line 228, conclusions, and final line in abstract), which I have detailed below.

We thank the reviewer very much for the positive and constructive review of our manuscript. Our detailed answers can be found below.

Specific comments below:

Line 49-50: It would be nice to include an explanation for why the arid site was dug less deeply than the other two, perhaps in the methods section 2.2. We added the following sentence in section 2.2. "The soils profiles were chosen to be less deep at the arid site because the soils at this site are less deeply developed than at the other two sites (Bernhard et al., 2018)."

Line 73: Do the authors have any estimation of the differences in isotopes that might be due to the differences in sampling year and how that compares to the ranges seen in their results? This is particularly important for the ^{13}C-isotope results. I could see those as being particularly sensitive to differences in temperature and moisture conditions between the two sampling years. The samples were collected in the same month (March) of two consecutive years. Therefore, we have no reason to believe that the difference in sampling year affects our (^{13}C-isotope) results. The ^{14}C ratios are corrected for sampling year (as stated in the method description).

Line 114: For methodological transparency, the authors should include a brief explanation about how they determined the pre-incubation period for each environment. The pre-incubation varied between 3 (arid site) to 4 (mediterranean and humid sites) days for practical reasons. Given that the experiment lasted for six weeks, and that the soils of the sites differ in very many respects this difference should be of very minor importance.

Line 137: Rather than referring to this measurement as microbial DNA, the authors should refer to it as total DNA. It likely not only includes DNA from microbial sources (e.g. microbial eukaryotes, fungi, and prokaryotes), but also DNA from non-microbial sources such as plant roots or soil arthropods. Although proportionally the non-microbial DNA is likely to be low in comparison, it is likely to vary among soil types (based on the amount of vegetation and moisture of the soil) and depth. Therefore it likely that the soils not only contain to contain different proportions of microbial:non-microbial DNA in different soils but also at different depths. The authors therefore should be cautious in their interpretation of total DNA as a proxy for microbial activity and should adjust their discussion accordingly. One relatively easy experimental way around this, would be to use qPCR to quantify the abundance of 16S and ITS gene copies in each soil sample's DNA. These numbers would be a more accurate quantification of microbial abundance at least, even though not all organisms possessing those genes are likely to have been active during incubation. We changed microbial DNA to total DNA throughout the manuscript, as also recommended by the first reviewer.

Section 2.7 – not having much disciplinary expertise in this area, I defer to others who do in evaluation of the methods. However, I appreciate the explanation in the last paragraph about accurate estimation of ages, which is helpful for non-experts in ^{14}C dating such as myself. It is also helpful that the authors imply that the carbon they observe likely is derived from the last 1000 years, and would be further helpful for their non-expert audience to know where the estimate of 1000 years comes from. Furthermore in their discussion, the authors describe a similar study in permafrost that was able to estimate ages using ^{14}C, if there is any way to get at least a range of ages from this data to compare to that study, I would find it very useful in interpreting the results. We are aware that much confusion exists in the literature regarding the reporting of ages from soil radiocarbon data. It is true that many previous studies have reported a conventional or a calibrated radiocarbon age, but only recently there has been more awareness in the soil radiocarbon community about this issue and lack of consistency in reporting ages (e.g. Trombone et al. 2016, Sierra et al. 2017, https://doi.org/10.1111/gcb.13556). The explanation we give at the end of section 2.7 is the main reason we do not provide an age estimate, even though some previous studies have done so. We do not want to continue reproducing this practice of reporting radiocarbon ages when we know that in an open system such as a soil all the organic matter is a mix of carbon from a wide spectrum of ages (e.g. Chanca et al. 2022, https://doi.org/10.1029/2021JG006673). We mention that we would need data of carbon inputs from at least the last 1000 years to accurately model this system in the method description. The mentioned 1000 years is only a rough estimate and by no means an accurate estimate of how old the carbon in these soils may be.

Figure 2c – The R^2 value of the arid linear model is quite low compared to the mediterranean and humid sites. I wonder if a simple linear model is even appropriate for this relationship as it seems that depth, along with other co-correlates are at play. In fact, for the humid site as well, the mean and variance appear to be related with higher mean values having less variance at more shallow sites which violates the assumption of equal variance of residuals which means that their estimated slopes may be incorrect. The authors should address this low fit in the text and potentially may find some helpful solutions in this guide: https://academic.macewan.ca/burok/Stat378/notes/remedies.pdf The linear models provide a reasonable representation of the relationship. The main purpose of these regression lines is to underline that the datapoints (almost) all plot below the 1:1 line.

Line 228, conclusions, and final line in abstract: I'm not sure the authors have made a very strong argument that processes of the soil are highly dependent on primary productivity aboveground. Although the results do indicate that newer carbon is being respired, an alternative explanation could be that the newer carbon is simply being re-cycled among the microbial community as the community turns over. The incubations are not directly measuring the influence of primary productivity since they are plant-free incubations and there aren't measurements for primary productivity at each site. I recommend a more conservative interpretation. We replaced "primary production aboveground" by "recent carbon inputs from plants" and "carbon that recently entered the ecosystem through CO_2 fixation." in abstract and conclusion. Even though we cannot exclude that the C has already cycled through the microbial biomass before being respired, we know that it has recently been fixed from the atmosphere.

Section 4.2: The authors may also want to consider the process of priming in their interpretation of this observation (see for example Bernard et al. 2022 for a nice review on the subject - https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/1365-2435.14038). We added the following sentence "Further, it could be that recently fixed carbon that enters the soil in the upper decimeters also leads to priming (Bernard et al., 2022)."

Technical corrections:

Line 98 – Typo, I believe "weight" should be "weighed" Corrected

Line 118 – typo in the specification of volume of gas: there are extra spaces between the number and the units. Additionally the units of liters should be abbreviated with a capital "L" rather than lowercase "l". Corrected.