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Comment on bg-2022-37

Anonymous Referee #1

Referee comment on "Temperature sensitivity of soil organic carbon respiration along a forested elevation gradient in the Rwenzori Mountains, Uganda" by Joseph Okello et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2022-37-RC1, 2022

The manuscript by Okello et al. presents a potentially interesting dataset examining the sensitivity of soil organic carbon stocks to projected temperature changes. The work fits well in the scope of BG, and I much appreciate the important work done – but the presentation of the data and the interpretation does require a substantial amount of clarification and improvement before being reconsidered. Detailed comments below.

Main comments & suggestions

-Terminology:

*throughout the manuscript, the terminology related to stable isotopes is really not OK. For example, the authors refer to "the 13C depletion factor" or "isotopic depletion factor" (L167) – that is not an accepted term in the literature, what you are referring to is termed fractionation (epsilon).

*other examples: L 321 "13C and content of soil organic carbon relatively increased"

*Keeling mass balance approach (line 155-162): this is just a mass balance approach, a Keeling plot is something quite different; equation 1 does not appear in the Keeling (1958) paper you refer to.

*L 146, 190, 289: "d13C isotopic composition": again, this is not appropriately phrased. Use either "d13C values" or "the C stable isotope composition" but not combinations of the

two.

*enrichment in 13C isotope (L478): enrichment in 13C

*L373: d13CO2 --> d13C-CO2 or d13C of CO2

-PLFA data: there is a short section in the Methods outlining the extraction and derivatization of PLFA, and then basically nothing. No info on how PLFA were identified and quantified, no information on how the resulting data were treated – assigned to microbial groups etc. The data are presented later on as concentrations of PLFA representing gram-positive, gram-negative bacteria, fungi, etc. but no information or references are given; no mention of this in the Methods, and very little real discussion of these data. Either add all this info, or remove them if the data don't contribute much to the story.

-precipitation: L101 : 7000 mm should be 700mm probably or something in that order of magnitude at least. The study sites cover a wide range of precipitation, and there is also a clear difference between Kibale and Rwenzori (as in: precipitation is higher in Kibale than at the lowest elevation along the Rwenzori transect)- however, the effect of precipitation on the data from the translocation experiments is not discussed at all; this should be worth some discussion.

-L111-116: No mention is made on whether samples were acidified to remove potential carbonates (or carbonates precipitation from the soil solution during soil drying). C/N ratios are reported but you need to specify whether these are weight/weight or molar ratios. For a proper interpretation of data, specify the reproducibility of your measurements (e.g. for d13C) and mention which standards were used. In Table 1, specify then if these concentrations refer to organic or total C. If total C, then you may want to add a note of caution in the interpretation of differences between d13C of soil C and CO2 produced.

-section 2.3: provide a description of how temperature was controlled during these incubations.

-section 2.3: mention how you coped with removing a 45 mL gas sample from your incubation jars: was this volume replaced with air while taking these samples, if not how was the pressure difference accounted for in your measurements?

-L208: why refer to the "slope of the CO2 concentration in function of time" ? If I understand well, you simply have measurement at the start and end of the incubation ?

-L199 and further: any reason to go for 50 mL jars here instead of 1 L jars (as in section 2.3) ? For the d13C measurements, it's important to convince the reader that the data you collected from these experiments are valid: you are in a closed system, where you sample gaseous CO2 for d13C analysis, but you also have an aqueous phase. CO2 will equilibrate between the two, and there is a (small) degree of isotope fractionation involved. The smaller the headspace volume compared to the volume of soil (and thus water), the higher the possible bias in resulting d13C-CO2 data if not accounted for. It might be negligible in your setup, but you need to provide arguments to show this.

-Section 2.5: while I am aware that much of the literature refers to soil CO2 flux measurements using closed chambers as "soil respiration", one should avoid keeping using this terminology in use; what is measured is not total in situ soil respiration but the diffusive flux of CO2 from the soil. This diffusive flux is governed by the gradient of CO2 concentrations / partial pressures and is thus influenced by e.g. porosity, water content etc. Part of the CO2 produced by soil respiration is lost via percolation and groundwater losses.

-Chamber deployment time (L224): 90 minutes seems excessively long for chamber closures, especially given the low chamber surface area. Pavelka et al. (2018, doi: 10.1515/intag-2017-0045) and other recommend much lower chamber closure times, in the order of 5 minutes. Were the chambers equipped with a fan to ensure proper mixing within the chambers ? If the CO2 increase was not linear, this has implications for your fluxes as well as d13C data interpretation. Not clear, by the way, if all d13C-CO2 measurements were made using a Picarro G2113, this is only mentioned for the t0 samples in section 2.3. If other CO2 samples were measured using other methods, add the necessary info (equipment, standards, reproducibility) to your Methods section.

-L237: linear regression: if you have 2 datapoints only, then avoid referring to this as 'fitting a linear regression to the concentrations over time'.

-L402-403: 'the SOC contents of warmed soil were relatively lower than those of control (soils) along the elevational transect': While Figure 4e may indeed suggest this, this does not appear to be the case for the lower elevation sites + even for the higher elevations the large error bars do not suggest that this difference is significant. Quantifying small changes in SOC stocks is challenging – if the difference is not statistically significant, then avoid phrasing in the way it is currently done. If you do feel confident that these are robust differences, you need to provide statistical justification + provide an estimated analytical error on your bulk density and %C (or OC, see elsewhere) data. The same comes back on L 484 where you claim that SOC was relatively lower in warmed as compared to control [samples] – if these differences are not significant then such statements should be rephrased; this is what others will pick out as conclusions in subsequent work.

-L403-404: the data presented in Figure 4f show a surprisingly large difference in d13C values , albeit with relatively high standard deviations. It would be good to provide statistics for this: for which elevations are these differences significant or not ? Again, I

assume that for the high elevation sites, they are not statistically different – which should not be a surprise, as you have very organic soils here for which you would need to have a very high turnover rate to see any differences in d13C of the SOC pool after 2 years. You could likely do some back-of-the-envelope calculations here.

-Discussion, section 4.1. The discussion on differences in d13C between CO2 and soil (organic) carbon needs to be reconsidered. The current discussion assumes that there should be a relationship between rates of mineralization and isotope fractionation during respiration. On line 480, you refer to Amundson et al. (2003) to back up this idea – but this is a paper that only discusses nitrogen stable isotope ratios in soils. As far as I'm aware, there is no sound evidence in the literature that the degree of isotope fractionation (if any) during respiration would be related to either respiration rates, or temperature -as you hint at in the first paragraph of section 4.1. You also refer to Andrews et al. (2000) and Natelhoffer & Fry (1988) here, but these do not really back up such statements: (i) Natelhoffer & Fry merely demonstrate that the SOC pool is typically enriched in 13C as mineralization progresses, without unambiguously demonstrating via which mechanisms (selective mineralization or degradation, Suess effect etc – for an updated discussion see e.g. Ehleringer et al. 2000 Ecological Applications 10: 412-422 and subsequent literature); and (ii) Andrews et al. (2000) should be interpreted carefully here. Granted, they observed similar patterns for soils from FACE experiments and control soils, but note that they do not invoke kinetics in offering an explanation to their data: "The increase in respiration rate across the entire temperature range and the enrichment in 13C only at 4°C rule out a strictly kinetic explanation for the observed carbon isotope fractionation. In addition, there is no theory that suggests a very different ratio of reaction rates of 13C compared to 12C in slow versus fast reactions (Agren et al., 1996). We suggest that the shift in carbon isotopic ratios in respired CO2 is the result of a shift in the use of carbon substrates in the soil". Hence, likely better to refer to a shift in d13C or to "apparent fractionation" then to fractionation. Note also that they observed a strong change between the first days of incubations and subsequent days, and that there are some methodological aspects to consider when interpreting their data: high volume of soil (and water) compared to headspace, and complete flushing of the headspace with CO2-free air (which implies that CO2 dissolved in soil water remained and will re-equilibrate, this dissolved CO2 has a different d13C value that the headspace CO2, etc).

In short, this entire section at the moment lacks a solid empirical or theoretical basis to interpret differences in d13C data observed to the influence of temperature or higher respiration rates. The same holds true for how some of the conclusions are expressed, e.g. L 536-539: the statements made are not convincingly supported at the moment.

-L511-512: 'low soil moisture content limited microbial CO2 respiration at high elevations': I do not see such lower moisture content anywhere in the data. Given the strong gradient in precipitation, I would expect to see rather the opposite ?

-L528-530: "we showed that…": rephrase this, limit to what you really unambiguously demonstrate, relationships are not necessarily causal. For example, I do not see strong direct evidence that soil moisture or pH had a direct effect on soil respiration along your gradient.

Minor / textual comments

-L20: insight into temperature sensitivity: insight into the temperature sensitivity:

-L25 and further: temperature sensitivity: make it explicit that you are referring to Q10 values here.

-L38: make it explicit that you refer to terrestrial primary production, not global (terrestrial + marine).

-L61: delete "of the CO2 respiration from soil"

-L89: in the eastern slope: on the eastern slope.

-Equation 1: bit of an odd choice of symbols – (F, f, I, i)

-L172: why "increment" ? I think this can be deleted.