Point-by-point responses to Referee's comments

The Authors thank the Anonymous Referee #2 for the thorough review and valuable constructive comments. We addressed the comments and provided suggestions for the revision of the manuscript. New text to be added/modified in the manuscript is blue-coloured in this letter.

Referee comment 1. This manuscript explores how soil geochemistry (parent material) influences microbial functions in weathered tropical rainforest. To do so, the authors used the classical soil forming factors as an approach. They assume that time for soil development, climate, topography and biota are kept constant, and only parent material (geology) varies (mafic, mixed and felsic). However, as a reader I miss information on biota. Vegetation is "dense tropical forest" (line 91, Wilken 2021, missing in the references), but is soil fauna actually identical? Does the microbial community composition change? Do plant species differ? The authors should clarify why they believe that the factor "biota" actually is kept constant across the catena and different parent material. After all, the whole methodological approach is based on the assumption that all soil forming factors are kept identical, except parent material.

Authors' response 1. *Thank you for pointing this out. We did not analyse the microbial community composition. Our choice for the study sites has been motivated by a prior knowledge of vegetation structure, climate, and topography that are comparable. Overall, soil forming factors are similar among our sites except for the parent material. As different parent materials lead to different soil development which in turn affect soil biota, we can expect that microbial community differ as they have to adapt to soil conditions such as pH, nutrient availability, and texture (Fierer and Jackson, 2006; Deng et al., 2015; Fierer, 2017). Therefore, we assume that parent material geochemistry would shape the patterns of microbial processes in studied sites.*

Referee comment 2. Overall, I found it difficult to identify a key message of the manuscript. Maybe it would be good to simplify and shorten (39%) the text, show less figures, and focus the conclusions on the results on the results shown in the manuscript.

Authors' response 2. *Thank for this suggestion. We will shorten the manuscript (mainly the result, discussion, and conclusion sections) as much as possible without constraining the interpretation and explanation of our results too much. To shorten the text, we will move Fig. 2 to the supplement as it was cited only once in the discussion. Additionally, we will remove Fig. 5 as its information could be contained in Table 2. As also suggested by Referee #1, we will merge sections 4.1. and 4.3. as both sections discuss relationships between resource availability and microbial processes.*

Changes we will make mainly in the discussion and conclusion sections (following comments from the Referee #1 and #2) will allow us to improve the clarity of the key message. "Briefly, despite the advanced weathering stage of tropical rainforest soils, variability of the parent material still affects microbial processes through its effect on resource availability. This is an element that has so far not been highlighted enough in research on tropical biogeochemistry. Specifically, we emphasize on patterns of microbial resource limitations across contrasting geochemical regions and soil depths for understanding organic matter decomposition in tropical Africa where such studies are scarce. Furthermore, the manuscript reveals the weak effect of geochemical soil properties in controlling microbial C and P limitations indicating that microbial resource acquisition is complex".

Referee comment 3. There is some potential for clarifications

Introduction – briefly explain the differences between mafic and felsic.

Authors' response 3. *Thank for this suggestion. In addition to bedrock and soil samples description provided in the method section (Section 2.1), we will add the following sentences in the introduction:*

We used soil materials from three geochemically distinct parent materials (from mafic to felsic) and three soil depths (0−70 cm) and measured microbial biomass C and enzyme activity (i.e. C, N, and P acquiring enzymes) at the beginning and end of a 120-day incubation experiment. Mafic describes igneous rocks composed of minerals that are rich in iron and magnesium, dense, and typically dark in colour. It is contrasted with felsic which describes igneous rocks composed of minerals such as quartz and feldspar and are relatively light in colour and density (Panchuk, 2019).

Referee comment 4. Sample handling – the soil samples were air-dried (line 117), which typically should be avoided for reliable analysis soil microbial biomass and extracellular enzyme activity.

Authors' response 4. *Thank you for this comment. We agree that fresh soil samples are preferred for microbial analyses. However, logistic and working conditions in tropical Africa could not allow us to keep the samples under their field conditions (e.g., by freezing them or conducting analyses immediately after the sampling) as our analyses had to be conducted in Europe. As indicated in the manuscript (section 2.3), microbial activities were measured on re-wetted soils (i.e., both pre-incubated and incubated samples).*

Referee comment 5. Statistics and data presentation – The data presentation is difficult to follow, e.g., the choice of the vector analysis warrants an explanation. How can the p-value be 0.00 (line 277; Fig 5)?

Authors' response 5. *We will provide the explanation for the choice of vector analysis in the manuscript as follow:*

Exploiting the enzyme data in a comprehensive and cost-effective way for assessing nutrient limitations we used vector characteristics to evaluate relative microbial C and nutrient limitations (Moorhead et al., 2013; Hill et al., 2014).

Concerning p-value, we will correct the sentence as follow: Soil depth together with physico-chemical properties explained significantly ($p<0.05$) the variance in MBC_{Soil} and MBC_{SOC} at pre- and post-incubation and only at pre-incubation for C and P limitations (Table 2).

Referee comment 6. Results - The results are consistent with general knowledge that tropical soils are P- limited. The authors should emphasis on similarities/differences to other tropical systems.

Authors' response 6. *We will emphasize on both similarities and differences of our findings to other tropical regions. We will focus our comparisons on studies that assessed microbial C and nutrient limitations using vector analysis. These changes will be made as follow:*

In contrast to the lowest C limitation in subsoils, microbes were more P-limited than their topsoil counterparts (Fig. 3a–c). Most probably, recycling of organic matter is the major source of P in these deeply weathered tropical forest soils where subsoils are depleted in rock-derived nutrients (Chadwick and Asner, 2017). Hence, it is reasonable to find a high P limitation in subsoil where organic matter content is low. Furthermore, relative to N and P, DOC was highest in subsoils (Table A2) indicating an increasing amount of potentially available C relative to nutrients. Comparable depth gradient of P limitation was reported by Jing et al. (2020) in tropical forests of China. However, Lui et al. (2020) showed similar or lower P limitation in top- compared to subsoils in subtropical forests of China. Overall, while this study confirms the well-established P limitation rather than N in the humid Tropics, it reveals different vertical patterns of C and P limitations compared to other tropical regions likely due to differences in the geochemistry of the parent material. Moreover, the common understanding for younger (temperate zone) soils suggesting an increase in fresh minerals with soil depth that may favour the release of mineral P likely does not apply to these deeply weathered tropical forest soils.

Physico-chemical soil properties measured in this study did not greatly explain variance in C and P limitations (Table 2 suggesting that microbial resource acquisition is complex and could strongly depend on microbial physiology and substrate stoichiometry (Stone et al., 2014; Cui et al., 2019). In *subtropical forests of China, Lui et al. (2020) revealed that soil C, N, and P stoichiometry was an important factor defining microbial C and nutrient limitations. Phosphorous rather than N limitation as observed in this study is usually attributed to weathering losses of P-containing primary minerals and P occlusion by secondary minerals in tropical soils (Chadwick et al., 1999). Also, due to long-lasting efficient recycling of organic matter, tropical forest soils are rarely N-limited (Chadwick and Asner, 2017). Although this finding is not new; it corroborates several studies in tropical and subtropical forest soils that found microbial P limitation rather than N limitation (e.g., Camenzind et al., 2018; Wang et al., 2019; Jing et al., 2020). Across all geochemical regions, soil microbes remained P-limited at post-incubation (Fig. 3). Even a possible release of P due to organic matter decomposition could not alleviate microbial P limitation in a 120-day incubation experiment. According to Jing et al. (2020), microbial P limitation in tropical soils results from long-term adaptation of soil microbial communities to site-specific soil and environmental conditions. We argue that the alleviation of microbial P limitation would require a longer observation time than a 120-day incubation experiment with repeated inputs of organic matter. In the study by Wang et al. (2020) a 10-year P addition experiment was necessary to reduce microbial investments towards enzyme production and thus alleviated microbial P limitation in tropical forest soils.*

Referee comment 7. Discussion - Typically manuscripts point out potential methodological limitations, and discuss how these limitations affect the outcome. The addition of such a section would strengthen the credibility of the discussed implications.

Authors' response 7. *Thank you for this hint. Since our discussion is already quite long, we will add the following section as a supplement and refer to it in the method section.*

Limits of vector analysis in assessing microbial resource limitations

Our method for assessing microbial C and nutrient limitations (i.e., vector analysis) is based on stoichiometric and metabolic theories of ecological systems (Allison et al., 2011). Here, EEA is assumed to reflect microbial resource demand as enzyme production is regulated by microbial stoichiometric and metabolic requirements for resources (i.e., C, N, and P) and their environmental availability. However, a substantial fraction of soil enzymes could be constitutively produced (or stabilized on mineral surfaces) and thus obscures short-term shifts in microbial enzyme allocation in response to changes in nutrient availability (Allison and Vitousek, 2005; Geisseler et al., 2010). In this study, we observed that despite the increase in N content (Fig. A1), N acquiring enzyme activity remained high (Fig. 2), which likely affected the magnitude of P limitation relative to N as observed at post-incubation (Fig. 3). This observation implies that when significant amounts of enzymes can be out of prompt microbial regulation, the interpretation of microbial resource limitation may require caution, which could be the case for our post-incubation results. The vector analysis reveals C limitation relative to N and P and P vs. N limitations rather than absolute limitations of these nutrients. Furthermore, readily available C, N, and P that can be assimilated without enzyme actions cannot be directly reflected by vector angle and length but only indirectly by lower EEA. The vector analysis is useful for understanding microbial resource acquisition and organic matter decomposition although it provides only indirect evidences of environmental resource availability and relative microbial resource limitations (Hill et al., 2014). Another limit of the method is that N acquiring enzymes (e.g., NAG and LAP) can also generate C that is not reflected in the calculation of vector analysis and thus affect C/N enzymatic ratio differently from C/P enzymatic ratio (Moorhead et al., 2016). This issue is usually reported in studies that measured only BG as C acquiring enzyme (e.g., Moorhead et al., 2016; Wang et al., 2018; Mori et al., 2018). Including CB together with BG in the calculation of vector characteristics as done in this study could reduce the error arising from C acquired by N acquiring enzymes.

Referee comment 8. Literature citations – some of the citations used are not include in the reference. Unpublished works are referenced multiple times but it is difficult to assess the statements in the manuscripts based on unpublished works.

Authors' response 8. *Thanks a lot for this hint. We apologize for this and we will carefully go through the manuscript and include all missing citations in the reference list. The missing references are related studies to be published in the same special issues with our study that got no DOI yet. All of these studies are already posted as preprints in SOIL discussion (e.g., Bukombe et al., 2021; Reichenbach et al., 2021; Wilken et al., 2021) and GFZ Data Services (Doetterl et al., 2021) and got their DOIs in the meanwhile. Please see references below:*

Doetterl, S.; Bukombe, B.; Cooper, M.; Kidinda, L.; Muhindo, D.; Reichenbach, M.; Stegmann, A.; Summerauer, L.; Wilken, F.; Fiener, P. TropSOC Database. Version 1.0. GFZ Data Services. https://doi.org/10.5880/fidgeo.2021.009, 2021.

Bukombe, B., Fiener, P., Alison M. Hoyt, A. M., Doetterl, S.: Controls on heterotrophic soil respiration and carbon cycling in geochemically distinct African tropical forest soils. SOIL Discuss. [pre-print], doi:10.5194/soil-2020-96, in review, 2021.

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 Discussion [pre-print], doi:10.5194/soil-2020-96, in review, 2021.

Wilken, F., Fiener, P., Ketterer, M., Meusburger, K., Muhindo, D. I., van Oost, K., and Doetterl, S.: Assessing soil erosion of forest and cropland sites in wet tropical Africa using $^{239+240}$Pu fallout radionuclides, SOIL Discuss. [preprint], https://doi.org/10.5194/soil-2020-95, in review, 2020.

Referee comment 9. Conclusion section – much of this is a stretch from the data. The conclusion section should be refocused on the data that is presented within the manuscript.

Authors' response 9. *Thank you for this comment. That was our ambition i.e., using the data to show what we learned. Considering your comment, the conclusion will be refocused on the results presented in the manuscript as follow:*

5 Conclusions

Do patterns of microbial processes in deeply weathered tropical forest soils reflect the geochemical gradient of parent material under similar climatic conditions?

We conclude that patterns of microbial biomass (MBC_{Soil}) and enzyme activity in tropical soils of contrasting geochemical origin are markedly distinct due to differences in resource availability. Similar patterns and magnitude of P limitation in these deeply weathered, undisturbed systems particularly in subsoils indicated that processes regulating P availability do not differ between soils of contrasting geochemical origin. Because of long-lasting efficient recycling of organic matter in humid tropics, none of the investigated regions and soil depths showed signs of N limitation for microbial processes.

Are subsoil microbes in highly weathered tropical forest soils more C- and nutrient-limited than their topsoil counterparts?

Less microbial C limitation in sub- compared to topsoils indicated that subsoil microbes can significantly participate in C cycling and limit C storage if high oxygen availability is prevalent. This is an observation that has so far not been highlighted enough in research on tropical biogeochemistry.

Furthermore, enhanced P limitation in sub- compared to topsoils indicated that the recycling of organic matter is the major P source as studied soils are depleted in rock-derived nutrients.

What are important controls on soil microbial biomass, C and nutrient limitations?

We conclude that microbial biomass C (MBC_{Soil}) is predominantly driven by organic matter. Control of organic matter over MBC_{Soil} is strongly influenced by soil depth which could define the quality and quantity of organic matter as well as its interaction with other geochemical soil properties. In contrast, soil geochemistry and substrate quality are significant controls for MBC_{SOC} particularly under conditions of less labile C. Strong controls of microbial C and P limitations could not be identified in this study referring to the large complexity of microbial resource acquisition.

Referee comment 10. Some more thoughts and questions: How much C was respired as CO2 over the incubation across treatments? (LINE 125) Did this vary statistically across treatments, and if so, how? Line 96. Spelling of schist Line 190. Spelling of bestNormalize Line 190.

Authors' response 10. *As requested, we calculated the cumulative CO_2 release over the course of the incubation for different treatments (Fig. 1). Please note that respiration data in Bukombe et al. (2021, same special issue) is presented as weighted average of 12 CO2 sampling points during incubation. The weighing factor was defined as the number of incubation days represented by each CO2 collection point.*

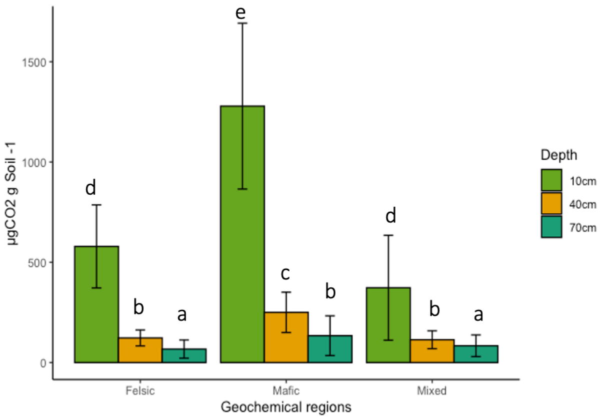

Figure 1. Cumulative CO_2 respiration during the 120-day incubation. Error bars are standard deviations (n=12). Letters compare geochemical regions per soil depth.

- Total CO_2 respired over the incubation was highest in the mafic region regardless of soil depth. Mixed sedimentary and felsic regions had statistically similar CO_2 release.
- We will correct the spelling of schist and bestNormalize in the manuscript.

Referee comment 11. Which transformation was performed on which variables? Line 216. Define MBCDOC Line 314. EAA or EEA? Fig 1. The figures should be set up in more comprehensive way, with abbreviations defined and treatments given in the same order and with legends. Fig 5. Are these p values significantly different?

Authors' response 11. *Thank you for these hints. As suggested, we will indicate in the manuscript the transformations used for each variable as follow:*
In case of deviation from ANOVA assumptions (due to the natural variability of samples), we performed data transformation using the bestNormilize package in R. Log transformation was applied on EEA, orderNorm transformation was applied on vector characteristics, DOC and TDN, square root transformation was applied on MBCSoil, MBCSOC and MBCDOC (Peterson and Cavanaugh, 2019).
-As suggested, MBC_{DOC} will be defined in the manuscript as follow: Increased MBC_{SOC} and MBC_{DOC}

(microbial biomass C normalized to DOC) with soil depth were determined in the felsic region at pre-incubation.
-EAA was a mistake, the appropriate abbreviation (EEA for extracellular enzyme activity) will be provided throughout the discussion.
-For each figure we will define all abbreviations in the legend. Treatments will be presented in the same order throughout the result section.
- We compared all models (Fig. 5 or Table 2) and they are significantly different.

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