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 is a conceptually straightforward incubation study seeking insight to the effects of soil parent material geochemistry on soil microbial biomass and extracellular enzyme activity in tropical Africa, where limited research has been conducted previously. The general results were that SOM and depth were the most important explanatory variables for MBC (and C-limitation), soils were consistently P-limited and P-limitations were strongly related to SOM, and not surprisingly, SOM was related to depth. The conclusion was that soil geochemistry affected MBC indirectly through affecting resource availability.

Although analyses were detailed and meticulous, the results provided little novel insight and are generally consistent with other studies of resource limitations in tropical soils. Thus, microbial systems in tropical soils in Africa appear to have similar constraints as elsewhere.

Authors' response 1. *Thank you for this comment. We agree that microbial processes in tropical soils of Africa have similar constraints as elsewhere (e.g., P but not N limitation). However, we showed that the geochemistry of the parent material shaped vertical patterns of C and P limitations in tropical systems. Overall, despite the long-lasting chemical weathering of investigated soils, the geochemistry of the parent material still affects resource availability which in turn shape patterns of* MBC_{Soil} *and EEA. This is an element that has so far not been highlighted enough in research on tropical biogeochemistry. Following changes will be made in the discussion section:*

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.

Referee's comment 2. The paper provides a solid background for this system, but the discussion could be much shorter with greater emphasis on similarities to other tropical systems.

Authors' response 2. *We will shorten the discussion as much as possible without constraining the interpretation and explanation of our results too much. Specifically, section 4.3. will be discussed together with section 4.1 as both sections discuss relationships between resource availability and microbial processes. To shorten the text as also suggested by Referee #2, we will move Fig. 2 to the supplement as it is cited only once in the discussion and remove Fig. 5 as its information could be contained in Table 2. We will emphasize 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. Comparison of our findings with other studies will be done as follow:*

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 the 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's comment 3. Section 2.5. The calculations of vector characteristics of extracellular enzyme activities were based on log ratios rather than proportions suggested by Moorhead et al. (2016). This choice affects results and warrants a brief explanation of choice.

Authors' response 3. *Thank you for this comment which was very helpful to improve our manuscript. We recalculated vector characteristics using untransformed proportions. This method yielded lower skewness and kurtosis than log-transformed ratios (Table 1).*

Table 1. Skewness and kurtosis of untransformed and log-transformed vector characteristics

	Vector length		Vector angle	
Untransformed proportions	Pre-incubation	Post-incubation	Pre-incubation	Post-incubation
Skewness	-0.95	-0.30	-0.36	-1.12
Kurtosis	0.61	-0.76	-0.65	1.15
Log-transformed ratios				
Skewness	5.24	6.02	1.79	2.14
Kurtosis	31.13	38.72	3.95	6.27

Moreover, untransformed proportional ratios of enzyme activity allowed to eliminate undefined values resulting from a zero in the denominator or in a logarithmic transformation (Moorhead et al. 2016). Therefore, we will use untransformed proportions in this study. Even though the obtained vector values differ between these two methods, the trend and main conclusions remain the same (Fig. 1).

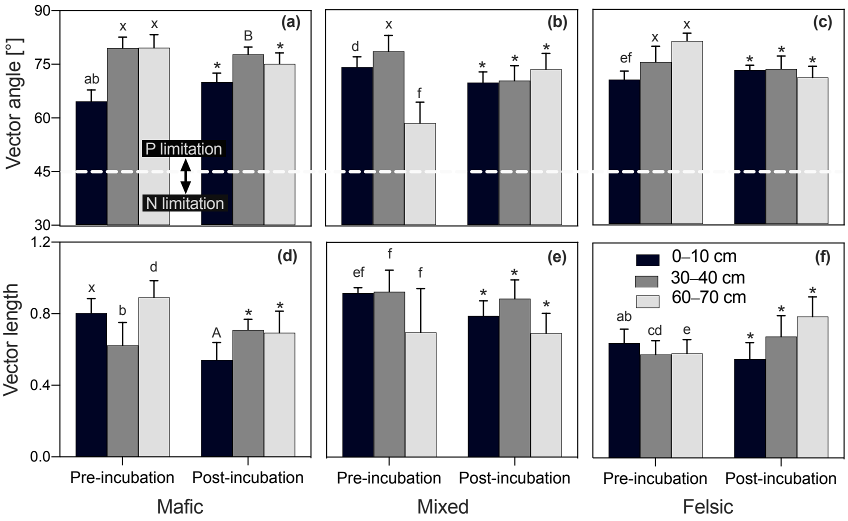

Figure 1. Vector analysis of microbial C and nutrient limitations. Error bars are standard errors of means (n=12). Vector length increases with microbial C limitation. Vector angle with <45° indicates N and >45° indicates P limitation. Lower-case letters compare geochemical regions per soil depth at pre-incubation. Upper-case letters and asterisks (*) compare geochemical regions per soil depth at post-incubation.

Referee's comment 4. Moreover, both equations 1 and 2 seem to have mathematical errors. Equation 1 lists ln(BG+CB)/ln(LAP)ˆ2, that should be (ln(BG+CB)/ln(LAP))ˆ2, whereas equation 2 lists ln(BG+CB/ln(LAP), that should be ln(BG+CB)/ln(LAP), and ln(BG+CB/ln(NAG+LAP)), which should be ln(BG+CB)/ln(NAG+LAP). Please confirm and correct.

Authors' response 4. *Thank you for this hint. Since we will change the calculation of vector characteristics by using untransformed proportional ratios, the formula will be changed in the manuscript accordingly. The new formula will be:*

$$Vector\ length = \sqrt{X^2 + Y^2} \tag{1}$$

$$Vector\ angle\ (°) = Degrees(Atan2(X, Y)) \tag{2}$$

Where,

$$X = \frac{CB+BG}{(CB+BG)+(AP)},\ Y = \frac{CB+BG}{(CB+BG)+(NAG+LAP)}$$

Note. CB: Cellobiohydrolase, BG: β-glucosidase, NAG: N-acetylglucosaminidase, LAP: leucine-aminopeptidase

Referee's comment 5. Section 3.3. There is no absolute vector threshold for C, N or P limitation, only relative indications within a study. For example, vector (relative EEA) characteristics do not directly reflect availability of C, N or P that does not require enzyme action to acquire, only indirect evidence of such availability through relatively lower enzyme activity needed to acquire it from hydrolysable sources.

Authors' response 5. *We agree that vector characteristics reflect indirectly CNP availability and thus relative microbial C and nutrient limitations. We will clearly mention it throughout the manuscript as follow:*

"Exploiting the enzyme data in a comprehensive and cost-effective way for assessing nutrient limitations we used vector characteristics (e.g., vector length and vector angle) to evaluate relative microbial C and nutrient limitations (Moorhead et al., 2013; Hill et al., 2014; Moorhead et al., 2016)".

"Vector analysis reflects relative microbial investments in C, N, and P acquisition (Moorhead et al., 2013). Vector length increased with relative microbial C vs. nutrient limitation. Vector angle indicated relative P vs. N limitation, with angle <45° indicating N and angle >45° indicating P limitation (Moorhead et al., 2013; Chen et al., 2018; Cui et al., 2019)".

"Across all geochemical regions, the vector angle was >45° at all soil depths and indicated relative microbial P limitation rather than N limitation (Fig. 3 a–c). At pre-incubation, vector angle in topsoils was lowest in the mafic region and highest in mixed sedimentary region. The vector angle was higher in sub- (30-40cm) than topsoils at pre-incubation for all geochemical regions ($p<0.05$) but similar among soil depth and geochemical regions at post-incubation. At pre-incubation, vector length indicating relative microbial C limitation was highest in the mixed sedimentary region and lowest in the felsic region for both top- and subsoils ($p<0.05$). At post-incubation, microbial C limitation declined in topsoils and was similar among geochemical regions and soil depths ($p>0.05$)".

Referee's comment 6. The convergence in post incubation vector length patterns with depth across the three soils indicates a similar balance of enzyme-driven C, N and P acquisition despite differences in other soil characteristics. This is interesting in light of the substantial geo- chemical differences between soils.

Authors' response 6. *Thank you for this highlight. We will add the following sentences to mention it in the manuscript.*

Despite differences in soil geochemistry and resource availability, microbial C and P limitations were similar among geochemical regions and soil depths at post-incubation. This observation suggests a similar balance of enzyme-driven C, N and P acquisition in these soils likely caused by substrate depletion during incubation.

Referee's comment 7. Section 3.5. The correspondences between predictions and observations are not convincing.

Authors' response 7. *Thank you for this comment. We would like to point out that correspondences between predictions and observations showing a quite low prediction power of our independent variables are a result of high variability in the dataset (i.e., differing strength of correlations between independent and dependent variables across geochemical regions). In the mixed sedimentary region, for instance, C was poorly correlated to absolute microbial biomass due to the high content of geogenic organic C which is poorly accessible to microbes (Reichenbach et al. 2021, in review) while strong correlations were observed in the felsic and mafic regions. Therefore, observed correspondences between predicted and observed MBC resulted in relatively low R^2. Concerning predictions of relative C and P limitations, our data are an indication that microbial resource acquisition is complex and could strongly depend on other factors such as microbial physiology and substrate stoichiometry.*

Referee's comment 8. Section 4.1. It was hard to follow this discussion, but the general relationship between MBC and resource availability (C and N) seems to contradict the following paragraph stating that microbes were P-limited throughout. Also, the implication that EEA is not responsive to short term variations in resource availability questions those parts of this study.

Authors' response 8. *Thank you for this comment. We will re-write this part of the discussion in order to address comment 2 and 9. Concerning the relationship between microbial biomass C (MBC_{Soil}) and resource availability, we show that MBC_{Soil} follows not only patterns in C and N abundance but also C quality. Higher C content was not always associated with higher MBC_{Soil}. This was the case in the mixed sedimentary region where poorly available geogenic organic C was found. Microbial biomass differed between studied soils due to differences in C and N resource quantity and quality. Concerning P limitation, the fact that microbes are P-limited elsewhere is a general observation for studied soils. However, the magnitude of P limitation still differs between compared soils particularly at pre-incubation in topsoils. This observation is in line with the observed relationship between MBC_{Soil} and resource availability. Please see below the proposed revision of the first section of the discussion:*

Investigated geochemical regions showed contrasting C and N contents that defined patterns of MBC_{Soil} and EEA with the highest values in the mafic region (Fig. 1-2, Fig. A1). This is in line with our multiple regression analysis indicating that organic matter is the main explanatory variable driving MBC_{Soil} at both pre- and post-incubation (Table 2). Observed patterns in C and N contents among geochemical regions likely reflect differences in organic matter input and its interactions with geochemical soil properties regulating its stabilization. Highest C stabilization potential, favoured by highest pedogenic oxides was reported as a strong driver of C stock in the mafic region (Reichenbach et al., 2021). Conversely, low pedogenic oxide contents in the mixed sedimentary region limit C stabilization potential and C stock (Reichenbach et al. (2021), leading to lower MBC_{Soil} and EEA. In the mixed sedimentary region, poor C quality limited MBC_{Soil} independently of C quantity. The mixed sedimentary region showed over 2 times more DOC than the felsic region, however over 0.5 times less MBC_{Soil} in topsoils (Fig. 1 a–f). This observation is due to the presence of geogenic organic C (up to 52 %) which is poorly available to microbes due to its chemical composition (Reichenbach et al., 2021). Low C availability in the mixed sedimentary region is supported by the observed lowest MBC_{DOC} (Fig. 1). Indeed, small amounts of MBC within a large pool of C mean that the average availability of C sources must be low (Insam and Domsch, 1988). In contrast MBC_{Soil}, MBC_{SOC} was highest in the felsic region which indicates high C availability in these soils and supports a lack of strong C stabilization mechanisms. The most important controls on MBC_{SOC} were soil acidity and exchangeable cations, solid phase geochemistry and substrate quality at post-incubation but not at pre-incubation (Table 2). We propose that depletion in labile C over the course of incubation led to less abundant but more adapted microbial communities with wide stoichiometric requirements and able to live in environments with reduced resources. This microbial community established in environments with little available resources could thus be the most affected by soil geochemistry. Whereas the more abundant microbial biomass at pre-incubation was likely driven by higher moisture, temperature and oxygen conditions associated with a relatively higher amount of labile C. Overall, despite the long-lasting chemical weathering of investigated soils, the geochemistry of the parent material still affects resource availability which in turn shape patterns of MBC_{Soil} and EEA.

Soil microbes were P-limited rather than N-limited throughout all three regions (Fig. 3). The lowest P limitation was observed in the mafic region particularly in topsoils. This is in line with the highest P content, which is due to higher organic matter content (Doetterl et al., 2021). However, the similar magnitude of P limitation among geochemical regions in subsoils indicates that within these undisturbed forest subsoils, processes regulating the P availability seem to be comparable between soils of contrasting geochemical origin.

Referee's comment 9. Section 4.3. This paragraph reiterated the relationship between MBC and SOM, mentioned earlier, and responsive to soil characteristics that vary with site and depth. This is consistent with many other studies. The most important point arrives at lines 389- 391, i.e., relative resource limitation is complicated.

Authors' response 9. *To address this comment, we will merge sections 4.1. and 4.3. as both sections discuss relationships between resource availability and microbial processes.*

References

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