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Interactive comment on "Soil microbial nutrient constraints along a tropical forest elevation gradient: a belowground test of a biogeochemical paradigm" *by* A. T. Nottingham et al.

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Referee comment:

It was pleasant to read this nicely written paper which suggests, along a tropical forest elevation gradient, a shift in microbial nutrient acquisition from P to N with elevation. This finding improves our understanding of nutrient limitation of tropical forests which is generally based on the responses of aboveground production.

Author response: We thank the reviewer for these positive comments.

Referee comment: The main limitations of the study are: 1) the uncertainty of con-

clusions inherent to correlative approaches: are these nutrient constrains the driving force of plant-soil functioning or the consequences of other processes not studied in this work (rhizosphere processes, allelopathy. . .)?

Author response:

We are aware that our correlative approach means we cannot isolate the influence of individual driving forces. However, we have attempted to reflect this in our discussion by not overstating the role of nutrients or discounting other potential factors not assessed in our study. Although many factors might contribute to the observed patterns, we feel that our results provide convincing evidence that microbes shift investment in nutrient acquisition from P to N with increasing elevation along this transect.

Referee comment:

2) to use an elevation gradient along which many environmental factors vary in the same time: temperature, rainfall, soil type. . .

Author response:

We agree that co-variation of environmental factors is a limitation in this and many other studies along environmental gradients. However, we note that several key environmental factors are constrained along this gradient. For example, rainfall does not vary linearly and soil pH is consistently low throughout. We attempted to constrain the differences in soil type in our analyses by performing analyses for separate soil types (mineral and organic horizons). Temperature is the main factor that varies along this gradient and might therefore drive differences in nutrient availability, for example by lowering decomposition and N-fixation rates in montane forests. Overall, we have tried to be clear in our study that our findings are specific to this elevation gradient and require consideration of the co-varying factors that change with elevation.

Referee comment:

3) to consider the pool of soil organic matter (SOM) as a homogeneous pool entirely

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available to microbial uptake. It's well known that SOM are composed of different pools with different level of accessibility (some are not accessible at all) and different quality. Consequently, the total soil C:N:P ratio is a poor predictor of stoichiometric constrains of decomposers: this is also shown by your results since the high soil C:P ratio opposes to the high P availability in mountains.

4) a lack of discussions of some results that seem to contradict the theory: 1) if there is no P limitation in mountains, why soil C:P increases with elevation?

Author response:

With regards to the heterogeneity of SOM, we agree with the reviewer. However, we assume that total element ratios provide a sufficient level of detail to assess stoichiometric constraints on decomposers, especially because the variation in these total elements is very large across this gradient, and that they are correlated to the abundance of elements available for microbial uptake. We know that total soil C is strongly correlated to the relative abundance of labile C (both physically and chemically unprotected) (Zimmermann, M. et al. 2012. Biogeochemistry 107:423-436). We also know that that total organic N and P (which are generally considered available to decomposers) are correlated to total N and P (equivalent for N). The high soil C:P ratio in the higher elevation sites does not necessarily reflect P limitation; it reflects the larger increase in total C relative to total P, although both total C and P increase with elevation (Table 2). Soil C:P may increase with elevation due to the accumulation of soil C because of increasing N limitation.

Thus, although the use of total C:N:P ratios may not precisely represent the 'decomposable' C:N:P ratios, we argue that they are correlated to labile forms and can be used to estimate differences in substrate availability along the gradient. Total elemental ratios are commonly used in broad studies of stoichiometry and microbial processes (Cleveland and Liptzin 2007, Sinsabaugh et al. 2009). In general we cautiously infer N or P constraints to decomposers by considering multiple lines of evidence from total,

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microbial and enzymatic stoichiometry.

Referee comment:

2) if nutrient constrains of microbes increase with elevation, why microbial biomass increase with elevation?

Author response:

In our manuscript we do not suggest microbes at higher elevation are any more or less constrained by nutrients than those at lower elevation. Rather, we suggest that the relative investment of microbes into nutrient acquisition shifts from P towards N with increased elevation. The increase in microbial biomass with elevation is much more likely to be due to the increase in labile carbon substrate with elevation. For example, we know that the total abundance of C and relative proportion of C in chemically and physically labile forms increase with elevation along this gradient (Zimmermann, M. et al. 2012. Biogeochemistry 107:423-436). As we state in our introduction, the microbial biomass is primarily C limited – and C availability drives microbial abundance (with N and P occurring as secondary constraints).

Referee comment:

Specific comments The statement 3) is not clear to me: which ratio are you talking about? Why should it be enzymes involved in the release of N AND P? given you are talking about the decreasing N availability and increasing P availability with increasing elevation, I would expect an increase investment in enzymes releasing N only.

Author response:

We thank the reviewer for identifying a poorly worded and ambiguous statement. We have changed the statement to clarify the hypothesis as follows: “increased ratios of enzymes involved in the degradation of compounds containing N and P” has been changed to: “increased activity of enzymes involved in the degradation of compounds containing N relative to those containing P (increased N:P enzymatic ratio)”

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Referee comment:

L4-18 Quantifying enzyme activities with only one measurement time and not in kinetics is not advisable (though it's usual in soil science). Have you checked that the substrate was still in excess at the end of incubation?

Author response:

With regards to making measurements at one time only: If the reviewer is referring to measuring activity at a single time during the assay, we can confirm that we checked linearity of enzyme activity with time during the assay. If the reviewer is referring to a measuring activity at a single seasonal time point, because there is no significant seasonal temperature variation in the tropics we predict variation due to seasonality of rainfall only. Therefore, we were careful to make our measurements during the wet season for all sites. It is possible that there was seasonal variation in enzyme activity relating to rainfall, but soil moisture measurements (Zimmermann et al., Global Biogeochem Cy, 24, 2010.) have shown that none of the sites appear to suffer significant seasonal moisture stress, suggesting that our sampling, though limited by access to a remote location, will be representative of the prevailing conditions at other times of the year. With regards to enzyme kinetics, we performed kinetics assays for a subset of these soils and found that the substrate was saturated throughout (therefore still in excess at the end of the incubation).

Referee comment:

I disagree with the fact that enzyme activities need to be normalized by soil organic C (it's not clear whether you are talking about C stock or C concentrations, this should be clarified). It is well known that a large part of SOC is not accessible to microbes and does not fuel enzymatic activities: SOC can be linked to minerals or occluded in soil pore not accessible to microbes, some SOC compounds are too poor in energy to sustain microbial activity. . . This "normalization" can lead to important biases since the amounts of SOC vary substantially between sites. If you wish to conserve this

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way of presenting data, non-normalized activities must also be presented and must not challenge your main statements.

Author response:

We normalized enzyme activities to SOC to avoid bias, because SOC contents vary widely among sites. We have made it clearer in our methods section that we normalized enzyme activities to soil organic C concentrations as follows: “Enzyme activities were expressed on the basis of soil organic C (nmol MU g C-1 min-1), to allow for direct comparisons among our sites with widely different bulk density and organic C concentrations.)”

Although the SOC is not entirely accessible to microbes, the total SOC concentration correlate strongly with ‘bio-available’ soil C along this gradient; total SOC is strongly correlated with the relative abundance of C in particulate organic fractions and in O-alkyl groups (Zimmermann, M. et al. 2012. Biogeochemistry 107:423-436.). Presumably for this reason, enzyme activity is commonly standardized to soil C concentration in the literature (e.g. Sinsabaugh, R. L et al., Ecol Lett, 11, 1252-1264, 2008.)

We can also refer the reviewer to the magnitude of difference in enzyme activities along this gradient (∼500 fold) compared to the magnitude of difference in SOC concentration along this gradient (∼30 fold) – indicating that the patterns we found and our conclusions are unaffected by normalization of enzyme data to SOC. Finally, and importantly, we note that our main conclusions are drawn from ratios of enzyme activities, which remain unchanged regardless of whether (and to what) enzyme activities are normalized.

Interactive comment on Biogeosciences Discuss., 12, 6489, 2015.