



Comment on soil-2020-94

Anonymous Referee #2

Referee comment on "Nutrient limitations regulate soil greenhouse gas fluxes from tropical forests: evidence from an ecosystem-scale nutrient manipulation experiment in Uganda" by Joseph Tamale et al., SOIL Discuss., <https://doi.org/10.5194/soil-2020-94-RC2>, 2021

General comments

This manuscript presents the results of large-scale nutrient manipulation experiment in a tropical forest in Uganda. Four treatments were considered in this experiment including an unamended control and three different nutrient applications (N, P, and N+P). Greenhouse gas fluxes and other soil data were collected over a fourteen-month experiment. The findings of this manuscript will help advance our understanding of GHG fluxes in African tropical forest ecosystems and how these ecosystems may respond to increases in nitrogen and phosphorus availability.

However, the experimental design is vague and needs additional clarifications. The manuscript is also framed as a global change experiment (i.e., increased nutrient deposition), but the amount of N and P applied is not justified and exceeds reasonable nutrient additions in similar ecosystems examining the effects of N and P deposition (e.g., Lu et al. 2018, Van Langehove et al. 2020). I also have concerns about the greenhouse gas sampling frequency and the time between sample collection and measurement in the lab. And, in general, the primary findings of the experiment are not effectively placed into the context of global changes and the consequences of increasing reactive nitrogen in the environment.

Van Langenhove, L., Verryckt, L.T., Bréchet, L. et al. Atmospheric deposition of elements and its relevance for nutrient budgets of tropical forests. *Biogeochemistry* 149, 175–193 (2020). <https://doi.org/10.1007/s10533-020-00673-8>

Lu, X., Vitousek, P. M., Mao, Q., Gilliam, F. S., Luo, Y., Zhou, G., ... & Mo, J. (2018). Plant acclimation to long-term high nitrogen deposition in an N-rich tropical forest. *Proceedings of the National Academy of Sciences*, 115(20), 5187-5192.

Specific comments

General abstract comments: Consider framing this experiment in the context of global changes, i.e., increased N and P deposition in natural ecosystems. It is not clear from the initial framing if this study concerns managed forests or native forest ecosystems. It is later explained that the experiment occurred in a forest reserve, and this should be

clarified for the reader.

LN 30: Listing p-values to three significant figures unnecessary. Consider reducing to two significant figures and changing elsewhere in the text.

General introduction comments: The impacts of climate change and alterations to the global N and P cycle should be discussed to contextualize this work, particularly in relation to changing N and P dynamics in forested ecosystems. The authors present other NMEs in tropical forests and the lack of experimentation in tropical Africa, but these studies were largely conducted to understand forest responses to N and P deposition. While the authors mention N deposition in LN 96, this global change driver is not presented earlier in the text, and it is an important consideration and rationale for this work.

LN 96: What about phosphorus? Please provide additional justification for how changes in P deposition could impact tropical forest and GHG budgets.

LN 104: Why would P stimulate N release from organic matter? This is mentioned, but not described in detail, in LN 75-84. Perhaps part of my confusion is from the use of organic matter. Do the authors mean soil organic matter or litter? These terms are used interchangeably in LN 81-84.

General methods comments: The materials and methods section needs substantial clarifications, including: the rationale for the treatment application rates, when the applications occurred over the course of the experiment, details about the experimental design, and clarification about the GHG flux measurements. Please refer to the detailed comments below.

LN 113: Please use a more appropriate citation. The authors might consider the WorldClim dataset.

LN 121-125: Additional information about the NME needs to be described. Please add a citation if one exists of previously published work from this site. At a minimum, the text should provide additional clarification regarding the experimental design, i.e., was it randomized? It is also unclear what the number of replicates is in each treatment. Please include in the text that there were four blocks or four replicated plots per treatment.

LN 127-128: The nitrogen and phosphorus additions rates need justification. These rates are unusually high for N and P deposition experiments, and the rates align more closely with those common in agricultural fertilization experiments. This is one of my primary concerns with the framing of this experiment; the applications rates seem far too high to justify as N or P deposition.

LN 135: How were these soil samples collected, i.e., shovel or core?

LN 148: I have concerns regarding this sampling frequency and the subsequent calculations of GHG annual fluxes. This measurement frequency is far too coarse to capture the sensitivity of N₂O to precipitation events. From Figure 2, it appears like there were many pulses in precipitation over the experimental period, which may have resulted in substantial N₂O release. While I acknowledge the difficulty in sampling at a twice weekly or weekly sampling frequency, the manuscript should describe why this monthly interval was selected for measurement.

LN 151-152: Was litter/residue left inside the chamber or was the soil kept bare?

LN 149-150: I have concerns about the area of the chambers and the sampling times used in this experiment. Carbon dioxide fluxes are usually orders of magnitude greater

than N₂O or CH₄; a larger chamber area is usually necessary to estimate these fluxes from soil. Furthermore, while the sampling times for N₂O and CH₄ make sense, I am concerned that CO₂ may have plateaued during this interval, impacting CO₂ diffusion, and the CO₂ concentration measured. Did the authors test for a linear relationship in their pooled and unpooled approach? How representative do the authors feel the chambers were of the overall plot GHG fluxes given the small size of these chambers?

LN 159: The duration between sample collection and measurement needs additional information. How long was the duration between sample collection and measurement? While generally stable for period of days to a couple of weeks, exetainers are not ideal for long-term storage of gas samples, which should ideally be measured immediately (up to 72 hours) after collection. Please describe the care that was taken to ensure there was no degradation to the gas samples over time.

LN 187: Please provide a citation for this method.

LN 190-204: The manuscript should include additional details about a) the frequency of measurements, chamber size, etc. for the trenching experiment, and b) how the authors portioned CO₂ to autotrophic and heterotrophic sources and a citation for their methodology.

More information about the estimation of root biomass (number of cores, how samples were processed) should also be included, especially because these data are discussed in the results and discussion.

LN 212: Is it common to refer to MANOVA as LMEMS?

LN 219: A description of the interpolation method used to calculate annual GHG fluxes should be described here. I am also confused why the authors present these data but did not do any statistical analyses with them? If these data are included in the results, they should be analyzed statistically.

LN 231: Please include the R packages used in the analyses.

General results comments: There are several occurrences in tables and figures where analyses are referenced, but they were not described in the text. This information is more appropriate to include at length in the methods section, and it is inappropriate to only provide as footnotes.

Table 1: If the authors present isotope data, they should describe how these data were collected.

Figure 2: Why were these climatic data not used to estimate 30-yr mean annual temperature and precipitation? The use of this weather stations should be described in the methods section.

General discussion comments: I do not find the claim that the ecosystem is "complex" a compelling argument for interpreting the results of the study. The manuscript should omit this language. I also recommend the manuscript include an addition section in the discussion placing the findings of this study in context – how do these results fit into findings of other tropical forest NME and changing N and P deposition rates in forested ecosystems? The broader impact and relevance to the science and policy communities would strengthen the framing of the manuscript.

LN 357: See previous comments about CO₂ measurement and sampling frequency concerns.

LN 433: Please provide additional information about P availability would open the N cycle.

General conclusion comments: Please clarify the rationale of this experiment: increased nutrient deposition or fertilization for enhanced forest production? Again, all ecosystems are complex, and this is a weak interpretation of the findings of this study.