

## Reviewer #2 (Comments to Author)

### 1. General comments:

- 1.1. 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.

Author's response:

We thank Reviewer #2 for both the general and specific comments on the manuscript as these have helped us further improve the clarity and overall quality of our manuscript. In addition, we appreciate the reviewer's acknowledgement of the tangible contribution our study makes towards the better understanding of the tropical forest responses to changes in ecosystem nutrient dynamics (particularly nitrogen and phosphorus). Below (in blue) are our point-by-point responses to Reviewer #2 comments.

- 1.2. (Part 1 of 4) However, the experimental design is vague and needs additional clarifications.

Author's response:

This nutrient manipulation experiment (NME), uses a completely randomized design where N, P and K were applied individually and in all possible combinations (N, P, K, N+P, N+K, P+K, N+P+K), and compared with an unamended control plot. Each of the eight treatments was replicated four times (hence,  $n = 32$  plots; 8 treatments  $\times$  4 replications). All plots were established in a compact geographical area where soil properties (physical, chemical and moisture regimes) were similar. The completely randomized design was the most appropriate for this ecosystem-scale NME because the NME involved only a single independent variable — the macronutrients, and several response variables (ecosystem processes). All the treatments were randomly assigned to the experimental units (plots) in order to minimize any possible confounding between the desired treatment effects (macronutrients) and other unknown effects. This experimental design is both statistically sound and also robust to measure the effect of macronutrients (independent variable) on ecosystem processes (dependent/response variables). The study on soil greenhouse gas fluxes — the basis for this manuscript, was conducted on only N, P, N+P, and the unamended control treatment plots ( $n = 16$  plots) because N and P availability has been demonstrated to alter soil greenhouse gas fluxes from tropical forest biomes.

For the benefit of clarity, the text below and in double quotation marks will be added to the experimental design subsection of the materials and methods section of the revised manuscript as follows:

*“The study was carried out in the framework of a running nutrient manipulation experiment (NME). The NME used a completely randomized design to investigate how the three macronutrients (applied individually (N, P, K) and in all possible combination (N+P, N+K, P+K, N+P+K) as treatments) constrained key ecosystem processes (particularly nutrient cycling, net primary productivity, carbon sequestration, and soil greenhouse gas fluxes) in comparison to the unamended control. Each of the eight*

*treatments was replicated four times (hence, n = 32 plots; 8 treatments x 4 replications). However, the soil greenhouse gas study—the basis for this manuscript, was conducted on only N, P, N+P, and the unamended control treatment plots (n = 16 plots) because N and P availability have been shown to limit soil greenhouse gas fluxes from tropical forest biomes.”*

1.2 (Part 2 of 4): 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).

Author’s response:

To clarify, this experiment was not designed to simulate the effects of future N deposition on greenhouse gas fluxes. As the reviewer notes, the nutrient application rates used in this study far exceed any realistic future N deposition for this relatively remote area of central Africa. Instead, the aim of our study was to learn how macronutrients regulate background (long-term) soil greenhouse gas fluxes, and specifically to identify the role these macronutrients have in soil GHG production and consumption when ecosystem nutrient limitations are alleviated. These application rates (125 kg N ha<sup>-1</sup> yr<sup>-1</sup> and 50 kg P ha<sup>-1</sup> yr<sup>-1</sup>) are in line with all other NMEs currently ongoing across the tropics (see our response to Reviewer #1 and Table 1) which aim to understand constraints regulating ecosystem processes.

*Table 1. Ongoing nutrient manipulation experiments in the tropical forest ecosystems*

Site name	Country	N (kg N ha <sup>-1</sup> yr <sup>-1</sup> )	P (kg P ha <sup>-1</sup> yr <sup>-1</sup> )
<b>This study</b>	<b>Uganda</b>	<b>125 - Urea</b>	<b>50 - Triple super phosphate</b>
Gigante	Panama	125 - Urea	50 - Triple super phosphate
NITROF	Panama	125 - Urea	-
EFFEX	Costa Rica	100 - Urea	47 - Triple super phosphate
Sabah	Malaysia	100 - Urea	50 - Triple super phosphate
Nouragues	French Guiana	125 - Urea	50 - Triple super phosphate
Paracou	French Guiana,	125 - Urea	50 - Triple super phosphate
AFEX	Brazil	125 - Urea	50 - Triple super phosphate

We have carefully adjusted the wording in LN 89 and 90 of the original manuscript to ensure that readers are aware this is not an N deposition simulation experiment. LN 89 and 90 will now read as follows:

*“However, a NME study in an African tropical forest would offer valuable insights on the soil GHG flux feedbacks of these understudied biomes in case of alleviations of N and P limitations.”*

1.2 (Part 3 of 4) I also have concerns about the greenhouse gas sampling frequency and the time between sample collection and measurement in the lab.

The goal of this study was to evaluate long-term effects of N and P additions, rather than the short-term peaks caused by fertilization or precipitation events/episodes. It is the long-term measurements (made >28 days after fertilization), that represent the new equilibrium established with elevated N or (and) P levels that are particularly relevant for the objectives of this study. A similar approach was reported in the Köhler et al. (2009) study.

Next, monthly measurements are very common when measuring GHG fluxes in the tropics, as they give a relatively high data resolution of temporal trends through the year, while balancing the expenses of the fieldwork. Monthly measurements have been reported in numerous publications in NMEs (e.g. Köhler et al., 2009) and in other GHG studies across the tropics (e.g. Iddris et al., 2020, Hassler et al., 2015, Hassler et al., 2017, Lontsi et al., 2020).

Finally, Labco Exetainers® with the Labco Grey Chlorobutyl Septum can reliably store gas samples for many months before measurement on the gas chromatography. According to Hassler et al. (2015), these Exetainers could reliably store gas samples for periods of up to six months. In our study, all collected gas samples were analyzed within 12 weeks of collection. Furthermore, all plastic caps were screwed on to the gas vials/Exetainers by hand and ‘quarter turned’ to ensure that they were all airtight (this procedure is outlined in the methodological paper of Pavelka et al. (2018)).

1.2 (Part 4 of 4) 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.

Author’s response:

As addressed earlier, the objectives of this study were not to simulate N deposition processes or highlight the cascade of effects this reactive N addition may have on soil greenhouse gas fluxes. Instead, our objective (as stated in the Introduction, LN 91-92 of the original manuscript) was to explore the role elevated background N and P availability has in driving soil GHG fluxes when different nutrient limitations in this ecosystem are lifted. While it may appear minor, we believe there is a very important distinction, which accordingly affects the results we report. Furthermore, the nutrient application rates far exceed any potential future N deposition so that drawing conclusions on N deposition is, in our opinion, not correct.

## Specific comments

2. General abstract comments:

2.1. 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.

Author's response:

As indicated in section 1.2, the aim of the study was not to simulate elevated atmospheric N and P deposition, but instead to investigate the role nutrients have in soil GHG production and consumption when nutrient limitations are alleviated. Atmospheric N and P deposition rates over our tropical forest site have been very low (Galloway et al., 2004), remain low based on our onsite measurements ( $8.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , and  $0.03 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ ), and are expected to only marginally increase in the next 30 years (to about  $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) (Galloway et al., 2004). It is for this reason that we framed our study as a macronutrient enrichment experiment rather than an N or (and P) deposition simulation study. Furthermore, as Reviewer #2 rightly observed, the fertilizer application rates used in our study would be too high for an N or P deposition simulation study. If our objective had been to simulate atmospheric N and P deposition, we would have applied far lower quantities of nutrient to reflect more realistic future deposition. Fertilizer application rates we used in our study were in line with almost all ongoing NMEs in the tropics currently investigating ecosystem responses to nutrient limitations (see Table 1).

Following the reviewer's advice, we will include upfront (in the abstract) that the study was conducted in a forest reserve, and all the p values used in the text will be reduced to two significant values in the revised version of the manuscript.

3. General introduction comments:

- 3.1. 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.

Author's response:

We would like to clarify that this was not N or and P deposition simulation study but rather an ecosystem scale study underpinning the soil greenhouse gas response from tropical forest biomes following lifting N or (and) P limitations on soil microbial communities. Consistent with the aim of the study, a strong nutrient pulse (in form of fertilization) was introduced to the forest ecosystem and the soil greenhouse gas fluxes (among other ecosystem responses) measured on a monthly basis. In this respect, we believe that both the literature review and subsequent contextualization of our study were thoroughly done.

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

Author's response:

We think that we adequately dealt with the effect of P availability on soil GHG budget in the preprint version (please see LN68-LN69 of the pre-print version of the manuscript). However, following the reviewer's advice, we have elaborated on how P availability further opens up the N cycle in the revised manuscript. We will additionally write in the revised manuscript as follows:

*“P availability opens up the N cycle by stimulating increased mineralization of soil organic matter availing more N for soil nitrification or (both) denitrification processes (Mori et al., 2010)”.*

- 3.3. 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.

Author’s response:

We meant to say that P availability has been shown to stimulate increased mineralization of soil organic matter availing more N for soil nitrification or (both) denitrification processes (Mori et al., 2010). For clarity, the details in double quotation in comment 3.2. will be added to revised manuscript .

Next, we would also like to indicate that mineralization was used with respect to soil organic matter and not litter. This will be adjusted in the revised manuscript.

4. General methods comments:

- 4.1. 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.

Author’s response:

As suggested by reviewer #2, we will clarify the rationale of the treatment application rates, the timing of the split fertilizer doses, and GHG flux measurements in the text of the methods section of the revised manuscript.

Specifically, we will write in the revised manuscript:

*“The study was carried out in the framework of a running nutrient manipulation experiment (NME). The NME used a completely randomized design to investigate how the three macronutrients (applied individually (N, P, K) and in all possible combination (N+P, N+K, P+K, N+P+K) as treatments) constrained key ecosystem processes (particularly nutrient cycling, net primary productivity, carbon sequestration, (and) soil greenhouse gas fluxes) in comparison to the unamended control. Each of the eight treatments was replicated four times (hence, n = 32 plots; 8 treatments x 4 replications). However, the soil greenhouse gas study — the basis for this manuscript, was conducted on only N, P, N+P, and the unamended control treatment plots (n = 16 plots) because N and P availability have been shown to limit soil greenhouse gases from tropical forest biomes. Each treatment plot measured 40 m x 40 m in size with an inner core measurement zone (30 m x 30 m) to avoid boundary effects. A spacing of at least 40 m between experimental plots was ensured to prevent spillover of applied nutrients from the neighboring plots. To achieve N and P enriched conditions, Nitrogen was applied at a rate of 125 kg N ha<sup>-1</sup> yr<sup>-1</sup> in form of urea ((NH<sub>2</sub>)<sub>2</sub>CO), and P at 50 kg P ha<sup>-1</sup> yr<sup>-1</sup> as triple super phosphate (Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>), with these fertilizers split into four dozes annually. The fertilizer application rates used in this study represent 92 % (136 kg N ha<sup>-1</sup>yr<sup>-1</sup>) of annual N inputs and about 470 % (according to Wright et al., 2011) of the annual P inputs from the litter. Additionally, the rates were comparable to those used in Wright et al. (2011) allowing us to stretch our conclusions beyond the Ugandan tropical forest site”.*

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

Author response:

The citation has been changed to Lukwago et al. (2020) because their study was conducted in Budongo Forest reserve and they reported an average temperature (of about 25 °C) and precipitation (of about 1700 mm) for this region.

4.3. 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.

Author's response:

Additional information about the NME will be provided (as highlighted in the response to comment 4.1.) including the detailed description of the experiment (i.e. completely randomized experimental design, consisting of eight treatments, with each treatment replicated four times). None of the work from the study site has been published yet.

4.4. 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.

Author's response:

As stated in comment 2.1 and 3.1, we would like to reiterate that the aim of the study was to understand how soil greenhouse gas fluxes respond to macronutrient enrichment in tropical forests and not simulate gradual effects of N or P deposition on soil GHG fluxes. Accordingly, we created an N and P enriched environment in this tropical forest by applying a relatively large dose of N (125 kg N ha<sup>-1</sup> yr<sup>-1</sup> as urea) and P (50 kg P ha<sup>-1</sup> yr<sup>-1</sup> as triple super phosphate). The N and P fertilization rates of 125 kg N ha<sup>-1</sup> yr<sup>-1</sup> and 50 kg P ha<sup>-1</sup> yr<sup>-1</sup> represented 92 % (136 kg N ha<sup>-1</sup> yr<sup>-1</sup>) about 470 % (according to Wright et al. (2011)) of the annual N and P inputs in the litter respectively at our tropical forest site. It is worth mentioning that for ecosystem-scale studies premised in the tropics, a large P dose is applied relative to the annual P litter input in order to overcome the known strong P fixation capacity of tropical soils (Yavitt et al., 2011). The fertilizer application rates used in our study have not only been used by Wright et al. (2011) but also in ongoing NMEs listed in Table 1.

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

Author's response:

In every plot, soil samples from 0 - 10 cm depth were collected using a spade, which we used to remove soil monoliths (20 x 20 x 10 cm) from 10 randomly located spots per plot. These soil monolith samples were subsequently mixed together in a large basin, from where we removed approximately a 500 g homogenized soil sample for laboratory analysis. For deeper soils (10 - 30, and 30 - 50 cm), soil samples

were obtained from five of the 10 sampling locations using a heavy-duty gouge auger. Here too we collected a composite (pooled) sample for each respective depth.

- 4.6. 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 N<sub>2</sub>O 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 N<sub>2</sub>O 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.

Author's response:

While we recognize that the reviewer is correct, and that by only measuring monthly we may not capture the small-scale variability in N<sub>2</sub>O fluxes (including some precipitation induced flushes), our interest in this study was to observe longer-term background controls, namely how nutrient availability regulates GHG fluxes. It is for the same reason that we divided the soil GHG flux dataset into transitory and background phases during statistical analysis in order to tease apart the immediate responses to fertilization from the long-term ones. The transitory phase included all measurements taken between 0-28 days following fertilization while background fluxes included all the GHG fluxes measured more than 28 days from fertilization (i.e. after the disappearance of the fertilization induced GHG flushes/peaks). As mentioned above in response to comment 1.2 (part 3 of 4), the same sampling frequency has been used in many other studies in the humid tropics (Iddris et al., 2020, Hassler et al., 2015, Hassler et al., 2017, and Lontsi et al., 2020).

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

Author's response:

Litter/residue was left inside the chamber. However, the chamber was always maintained vegetation free in order to avoid measuring night respiration of the plants during chamber closure.

- 4.8. 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 N<sub>2</sub>O or CH<sub>4</sub>; a larger chamber area is usually necessary to estimate these fluxes from soil. Furthermore, while the sampling times for N<sub>2</sub>O and CH<sub>4</sub> make sense, I am concerned that CO<sub>2</sub> may have plateaued during this interval, impacting CO<sub>2</sub> diffusion, and the CO<sub>2</sub> 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?

Author's response:

Chamber design: Up until now, there is still no standard chamber design because the design of the chamber is dictated by the nature of the ecosystem under investigation (Pavelka et al., 2018). In this methodological paper, Pavelka et al. (2018) indicated that the chamber design should at least cover a minimum ground area of 0.2 m x 0.2 m (0.04 m<sup>2</sup>). In our experiment, we used a circular chamber with a diameter of 0.237 m that covered a ground area of 0.044 m<sup>2</sup>. Therefore, our chamber design was well

within the recommendation confines of Pavelka et al. (2018). Koehler et al. (2009) and Matson et al. (2017), too, used a similar chamber design to measure soil greenhouse gas fluxes (including carbon dioxide fluxes) from their tropical forest sites.

Sampling times used in this experiment: Our sampling times were consistent with those used in the separate studies of Koehler et al. (2009) and Matson et al. (2017) to estimate soil carbon dioxide (among other soil greenhouse gas) fluxes from tropical forest ecosystems. Moreover, in all these studies, the used sampling times were well below the 45-minute maximum chamber closure period recommendation by Pavelka et al. (2018). Wanyama et al. (2019) used the 45-minute maximum chamber closure period and still obtained decent estimates for soil CO<sub>2</sub> fluxes. They did not report any incidences of plateauing in the measured CO<sub>2</sub> fluxes. Furthermore, we inspected the linear increase of CO<sub>2</sub> concentration during chamber closure for all the batches of gas samples as a quality control check. Here, the R<sup>2</sup> was typically above 0.95, with no evidence of plateauing.

Linear relationship in their pooled and unpooled approach: We collected both pooled and unpooled gas samples for the month of February 2020 and tested how the pooled approach compared to the unpooled for all the three gases. There was no significant difference in the concentration of all the three gases between the pooled and unpooled approaches (see Fig. 1 below). We also mentioned this in the original manuscript (see LN 150) but we will include this figure as supplementary material for the revised manuscript.

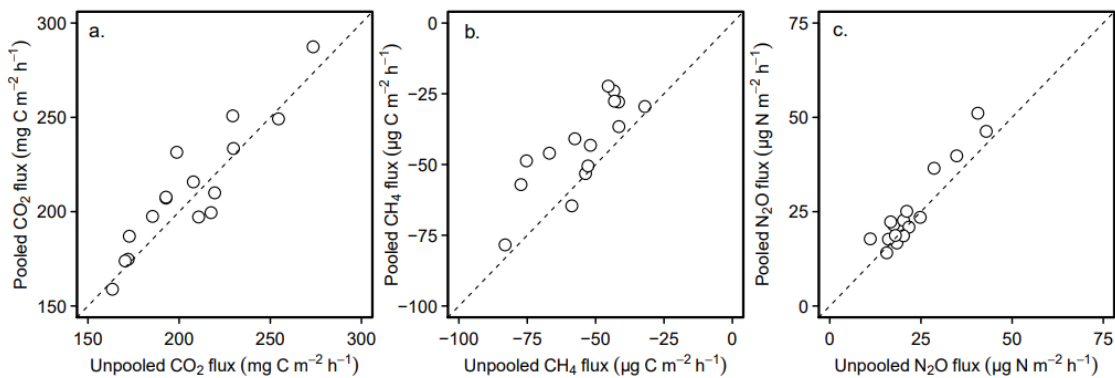


Figure 1. Comparison of the soil CO<sub>2</sub> fluxes (a), soil CH<sub>4</sub> fluxes (b), and soil N<sub>2</sub>O fluxes (c) measured with the pooled and unpooled approaches during the month of February 2020 in Budongo Forest reserve.

How representative were the measurements given the size of the chamber used: We are confident that the flux measurements presented in our manuscript are representative of the fluxes from the different plots of the NME in this tropical forest biome. This is because our chamber design was in conformity with the minimum ground area requirement for representative terrestrial GHG flux measurements (Pavelka et al., 2018). Additionally, we minimized any plot level spatial variability by randomly deploying the four chambers within the plot. All the pooled gas samples in every plot (at each time interval during chamber closure) were always a composite of the respective head air spaces of the four chambers.



4.9. 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.

Author's response:

Potential gas leakages in exetainers are mainly due to the type of exetainers used and the fact that the exetainers are dispatched from the factory with loose caps (not airtight). We purposely selected Labco exetainers (Labco Limited, Lempeter, UK) with screw-on plastic caps fitted with Labco Grey Chlorobutyl Septum for this study because they have been demonstrated to remain airtight for periods spanning up to six months (Hassler et al., 2015). Moreover, all plastic caps were screwed on to the gas vials by hand, and then 'quarter turned' to ensure that they were all airtight (see this procedure in Pavelka et al. (2018)). Although, the Labco exetainers can remain airtight for periods up to six months (Hassler et al., 2015), we ensured that on average, all collected gas samples were analyzed under 12 weeks. We submitted all the samples to the laboratory at ETH Zürich in five batches across the 14 months of field gas sampling.

LN 187: Please provide a citation for this method.

Author's response:

As suggested, we have provided a citation for the method.

4.10. 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 CO<sub>2</sub> to autotrophic and heterotrophic sources and a citation for their methodology.

Author's response:

As suggested by reviewer #2, additional details about the trenching experiment will be provided in the revised manuscript including the citation for methodology used. We will specifically provide the following additional details in the revised manuscript:

- *“The measurements to disentangle the sources of soil CO<sub>2</sub> effluxes spanned over a period of four months (starting in November 2019 and ending in February 2020) and were done on a monthly basis. We purposely selected this measurement period because it represented the transition from the wet season to the long dry season allowing us to capture how soil moisture constrained the different soil CO<sub>2</sub> efflux sources.*
- *Both the trenched and reference chamber bases had a design (area = 0.044 m<sup>2</sup>, and volume = about 12 L) identical to the one used in the nutrient manipulation soil GHG flux study.*
- *The different soil CO<sub>2</sub> source were calculated as follows:  
Heterotrophic (microbial) respiration = CO<sub>2</sub> effluxes from the trenched chamber  
Autotrophic (root) respiration = CO<sub>2</sub> effluxes from the reference chamber – Heterotrophic respiration”.*

- 4.11. 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.

Author's response:

As suggested by the reviewer, the details on estimation of root biomass will be included in the revised version of the manuscript. We will specifically write:

*“Root biomass distribution with depth was determined by digging three profile pits (measuring 1 m x 1 m x 1 m) at the forest site. In every dug pit, ten soil monoliths (each measuring: 20 cm (L) x 20 cm (W)) were carefully cut out (using a spade and hoe) following a 10 cm depth interval from the surface down to 1 m. The soil monolith were thoroughly washed to isolate the roots from the bulk soil. The root samples were oven dried at 60 °C for 48 hours and weighed to determine the root biomass per depth increment. The root biomass for each depth interval was calculated as the mean of the root biomass from the three pits for that interval”.*

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

Author's response:

Not exactly, because multivariate analysis of variance (MANOVA) is used when you have two or more response variables in an experiment and you jointly treat them as one multivariate response variable. The MANOVA structure cannot support multi stratum analysis of variance and its formula is always without an error term. Contrary to the MANOVAs, linear mixed effects models (LMEMs), like majority of the statistical approaches, deal with a single response variable, support multi stratum analysis of variance, and their formula always includes an error term.

- 4.13. 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.

Author's response:

As suggested, the description of the interpolation method will be included in the methods section of the revised manuscript. As you will also read in similar studies, for instance; Koehler et al. (2009), Veldkamp et al. (2013), and Iddris et al. (2020), it is recommended that statistical analyses be conducted on only actual (monthly) measurements and not the annual fluxes because the latter are obtained through interpolation of measured actual soil GHG fluxes over the sampling time period. Annual GHG fluxes are however, an important result to present, so as to allow inter-comparability between different forests and experiments.

We will additionally write in the materials and methods section of the revised manuscript that:

*“Annual soil GHG fluxes were obtained through conducting a trapezoidal interpolation on the measured monthly soil GHG fluxes, assuming constant flux rates per day. It is worth mentioning that the annual soil GHG fluxes from the different treatments were not statistically analyzed because they were not actual measurements but rather interpolations.”*

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

Author's response:

As suggested, the key R packages used in the statistical analyses will be added to the methods section of the revised manuscript. As indicated in the original manuscript, statistical analyses were mainly accomplished with linear mixed effects model (LMEMs) and one-way analysis of variance (ANOVA). We used the 'nlme' and 'car' packages for LMEMs and one-way ANOVA tests respectively.

We will specifically add a sentence in the revised manuscript stating that:

*"For statistical analyses, we used the 'nlme' and 'car' packages for LMEMs and one-way ANOVA tests respectively".*

5. 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.

Author's response:

As suggested by the reviewer, we will make adjustment in the text of the revised manuscript. See the proposed revised texts (in italics and double quotation marks in comment 4.10, 4.11, and 4.13) to be added to the methods and materials section of the revised manuscript.

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

Author's response:

The isotope data presented in this manuscript is from a sister study and we will clarify this by adding a footnote to Table 1.

5.2. 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.

Author's response:

The weather station data presented in our manuscript was only available for the period of the experiment (about 2 years). This data was beneficial to understand how for instance precipitation constrained soil greenhouse gas fluxes given its direct control on water filled pore space. For the long-term average of the study region, we will cite the Lukwago et al. (2020) study (because they reported long-term temperature and precipitation for this forest site), instead of extrapolating of our 2-year climatic snap shot data to the 30-year climatic average which could be misleading. As suggested by the reviewer, we write in the materials and methods section that:

*"The weather data from our field station was only available for the period of the experiment, and therefore, was used to understand how precipitation constrained soil greenhouse gas fluxes given its direct control water filled pore space".*

6. 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.

Author's response:

We will rephrase the argument on the complexity of the ecosystem in the revised manuscript.

Next, it is evident throughout the discussion section that on top of explaining how the soil GHG production and consumption processes at this tropical forest site were directly or indirectly affected by the alleviation of nutrient limitations, we fit our NME findings in the context of other tropical forest NME (for instance see LN 352-356, LN391-396, LN420-421, LN424-426, e.t.c. of the preprint version of manuscript). Like we already elaborated in the preceding comment sections, we think that introducing a subsection in the discussion, putting our study findings in the context of increasing N or (and) P deposition would be quite misleading. Why? Because the N or P deposition rates over our tropical forest site are quite low. Additionally, the large fertilizer application rates used in our study only served the purpose of achieving a nutrient enriched environment at our tropical forest site to measure a soil GHG flux response. These fertilizer application rates were too high to reflect any realistic projection in N or P deposition over this tropical region.

6.1. LN 357: See previous comments about CO<sub>2</sub> measurement and sampling frequency concerns.

Author's response:

Please refer to our response to comment 4.8, for details on the chamber design and the sampling times used in our study.

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

Author's response:

As suggested by the reviewer, additional information on how P availability would open up the N cycle will be added to the revised manuscript. Please see our response to comment 3.3.

7. 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.

Author's response:

We sought to understand the soil greenhouse gas flux response of tropical forest biomes under enriched N and P soil conditions and not necessarily simulate the effects of N and P deposition on soil greenhouse gas fluxes from these biomes. Again, the statement on the complexity of the ecosystem has been rephrased in the conclusion section.

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