

## Reviewer\_#1 (Comments to Author)

Dear Reviewer #1, Thank you for the time investment in reading our manuscript and making suggestions for its improvement. We have incorporated the suggestions and made changes accordingly. These are reflected in our point-by-point reply below in blue.

1. It is not clear in the abstract or materials and methods that the fertilizer was split applied. I suggest that the authors work on making this clear from the reader upfront. I also mention the rates and frequency of fertilizer application.

### Author's response:

We would like to clarify that nitrogen was applied at a rate of 125 kg N ha<sup>-1</sup> yr<sup>-1</sup> as urea ((NH<sub>2</sub>)<sub>2</sub>CO) and phosphorous at a rate of 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>) which is already mentioned in the materials and methods section (see LN 121 to 122 of the original manuscript). Likewise, we also mention that the fertilizers were split into four equal doses annually. This means that, 31.25 kg N ha<sup>-1</sup> yr<sup>-1</sup> and 12.5 kg P ha<sup>-1</sup> yr<sup>-1</sup> were applied either individually (N or P) or in combination (N + P) to the replicate plots of the nutrient manipulation experiment on a quarterly basis (every three months). We will subsequently include the quarterly rates in the materials and methods sections of the revised manuscript as suggested by the reviewer.

2. Ln 48: The word “geochemsitsry” was supposed to be “geochemistry”

### Author's response:

Indeed, the word geochemistry was misspelt and has been corrected in the revised manuscript.

3. Ln 121-122: why where these fertilization rates chosen? What was the rationale?

### Author's response:

The rationale behind these application rates was to produce comparable results with other nutrient manipulation experiments (NME) in the humid tropics. The table below shows the fertilizer application rates used in other ongoing NMEs across the tropics.

*Table 1. Ongoing nutrient manipulation experiments in 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

Specifically, our experiment was all modeled after the Gigante nutrient manipulation experiment in Panama (Wright et al 2011) and accordingly, we applied the same types of fertilizer, the same fertilizer quantities and applied them in four equal doses.

The premise of these fertilization rates was to create nutrient enriched conditions through application of a relatively large dose of N ( $125 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ), and P ( $50 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ ), and measure the ecosystem response including the soil greenhouse gas fluxes. The fertilizer application rates used in our study represent 92 % ( $136 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) of annual N inputs and about 470 % (according to Wright et al., 2011) of the annual P inputs from tropical forests' litter. For ecosystem-scale studies premised in the tropics, it is very important to use a large P dose relative to the annual P litter input in order to overcome the known strong P fixation capacity of tropical soils (Yavitt et al., 2010).

4. Ln 125: How much fertilizer was applied at different times. Consider mentioning the rates of N and P application.

Author's response:

We applied  $31.25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  and  $12.5 \text{ kg P ha}^{-1} \text{ yr}^{-1}$  either individually (for N or P) or in combination (N + P) to the replicate plots of the nutrient manipulation experiment each quarter (every three months). Hence, the fertilizers were applied four times each year. As suggested, we will write in the revised manuscript that:

*“ $31.25 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  and  $12.5 \text{ kg P ha}^{-1} \text{ yr}^{-1}$  were added to the replicate plots of the nutrient manipulation experiment each quarter (every three months) either individually (for N or P) or in combination (N + P).”*

5. Ln 127-128: How was the soil sampled collected in a pit? Or using augers? Be clearer on what was done.

Author's response:

Soil samples were taken at ten different locations within the plot for the 0-10 cm depth. Here, soil monoliths (20 cm (L) x 20 cm (W) x 10 cm (D)) were carefully taken out using a spade. For the depths; 10-30 cm, and 30- 50 cm, soil samples were obtained from five of the ten different locations within each plots using an auger. This will be clearly stated in the methods section of the revised manuscript.

6. Ln 142: Considering the expected peak in GHG emission following fertilizer application, why was the intensity of GHG monitoring not increased immediately after fertilization?

Author's response:

It is a known fact that addition of N will increase  $\text{N}_2\text{O}$  fluxes immediately after fertilization (short-term response), but this was not the aim of the study. The aim was to evaluate the long-term effects of N enrichment on ecosystem response (including soil greenhouse gas fluxes). The long-term response reflects the new equilibrium established with elevated N levels. It is also against this background that the respective GHG flux and soil-environmental control datasets were divided into short-term response (transitory phase, < 28 days from fertilization) and long-term response (background phase, > 28 days after fertilization) in order to tease apart the short-term and long-term responses to N addition.

7. Ln 152: The gas measuring window 9 am-4 pm is too wide. Wouldn't air temperature be different at 9 am and at 3 pm for instance?

Author's response:

While temperature plays an important role in regulating GHG fluxes in the soil, the diurnal air temperature variability at this tropical forest was minimal ( $0.6 \pm 0.04$  °C; mean  $\pm$  SE). Correspondingly, soil temperatures also had minimal diurnal variability ( $0.2 \pm 0.03$  °C; mean  $\pm$  SE). In addition, we de-trended any effects of diurnal temperature effects may have on the soil GHG fluxes by randomly selecting the plot to be measured. This ensured that all the plots had an equal chance of being measured either in the morning or mid-afternoon or late afternoon. We are therefore confident that the negligible diurnal variation in both air and soil temperature during the time of sampling did not affect the measured soil GHG fluxes (this can also be seen in Fig. 1).

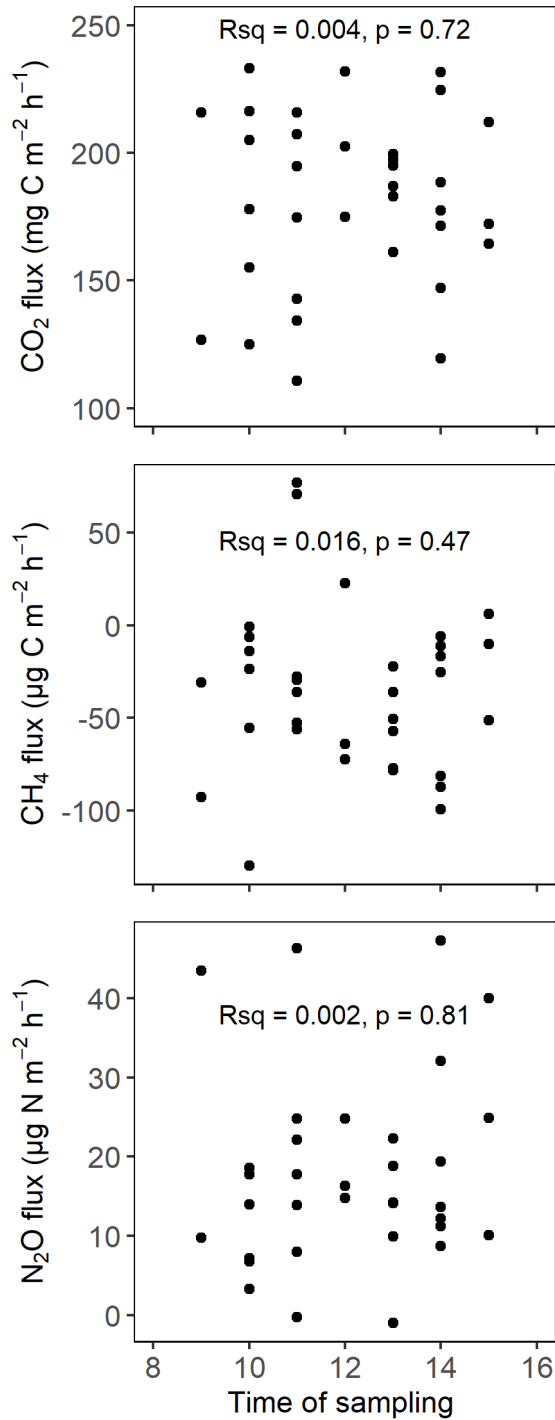


Figure 1. Linear relationship between the measured soil greenhouse gas fluxes (CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O) and the time of sampling from the control plots of the NME premises in Budongo Forest Reserve.

8. Ln 173 Ammonia or Ammonium?

[Author's response:](#)

This was a typo. We wrote ammonia in the text but it is supposed to be ammonium. This has been corrected in the revised manuscript.

9. Ln 264: In Fig 3a it does not appear CO<sub>2</sub> fluxes ever went above 250 mg C m<sup>-2</sup> h<sup>-1</sup> yet here you give the range as 60 to 330 mg C m<sup>-2</sup> h<sup>-1</sup>? Please explain or correct.

Author's response:

Referring to Fig 3a was erroneous and an oversight on the part of the authors because the values mentioned in the text were ranges and not means (presented in Fig 3a). This has been corrected.

10. Ln 288: In Fig 3b, it does not appear CH<sub>4</sub> uptake was ever above -200 mg C m<sup>-2</sup> h<sup>-1</sup>, yet here you have it as -278 mg C m<sup>-2</sup> h<sup>-1</sup>? Please explain or correct.

Author's response:

Referring to Fig 3b was equally erroneous and an oversight on the part of the authors because the mentioned values in the text were ranges and not means (presented in Fig 3b). This has also been corrected in the text.

11. Ln 353: I think "mirobial" was supposed to be "microbial"

Author's response:

This was a typo and has been corrected in the revised version of the manuscript.

12. Ln 400-402: Does the relationship not depend on the form of mineral N (NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup>)? Also, see: Banger, K.; Tian, H.; Lu, C. Do nitrogen fertilizers stimulate or inhibit methane emissions from rice fields? *Glob. Chang. Biol.* 2012, 18, 3259–3267; for insights on the mechanisms.

Author's response:

Yes, whereas it has been shown that the relationship between CH<sub>4</sub> uptake and soil mineral N depends the form of N (NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup>), the proportion of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> in the soil is key in shaping this relationship. In terrestrial ecosystems, the proportion of the forms of mineral N is influenced by the type of fertilizer used (urea or ammonium based fertilizer) and the nature of the N cycle. The dominant ecosystem N cycles are either closed (soil mineral N dominated by NH<sub>4</sub><sup>+</sup> compared NO<sub>3</sub><sup>-</sup>, Hassler et al., 2015) or open/leaky (soil mineral N dominated by NO<sub>3</sub><sup>-</sup> compared NH<sub>4</sub><sup>+</sup>, e.g. at our study site and that of Koehler et al., 2009). In either N cycles (i.e. closed or open), aggregation of the datasets for the different forms of mineral N during statistical analysis is very common (see Hassler et al., 2015). We used the same approach in our statistical analysis because the NO<sub>3</sub><sup>-</sup> ion concentration in the soil at our tropical forest site was significantly and consistently higher than the NH<sub>4</sub><sup>+</sup> ions throughout the gas measurement campaign. It is also imperative to note that CH<sub>4</sub> uptake was mainly limited by NO<sub>3</sub><sup>-</sup> ion content but there was no meaningful correlation between CH<sub>4</sub> uptake and NH<sub>4</sub><sup>+</sup> content. Similarly, even after aggregation of the NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> contents together (to get total soil mineral N), the relationship between CH<sub>4</sub> uptake and soil mineral N still went in the direction of NO<sub>3</sub><sup>-</sup>.

13. Ln 430: What do the results look like when you correlate N<sub>2</sub>O with either NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup>

Author's response:

There was a relatively weak negative correlation between N<sub>2</sub>O and NO<sub>3</sub><sup>-</sup>, and no correlation at all between N<sub>2</sub>O and NH<sub>4</sub><sup>+</sup>. The correlation between CH<sub>4</sub> uptake and soil mineral N (NO<sub>3</sub><sup>-</sup> content plus NH<sub>4</sub><sup>+</sup> content)

was still negative like it were in the case of  $\text{NO}_3^-$ , simply because  $\text{NO}_3^-$  ions dominated the soil mineral N at our tropical forest study site.

#### References:

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2. Lukwago, W., Behangana, M., Mwavu, E. N., & Hughes, D. F. (2020). Effects of selective timber harvest on amphibian species diversity in Budongo forest Reserve, Uganda. *Forest Ecology and Management*, *458*, 117809.
3. Koehler, B., Corre, M. D., Veldkamp, E., Wullaert, H., & Wright, S. J. (2009). Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input. *Global Change Biology*, *15*(8), 2049-2066.
4. Wright, S. J., Yavitt, J. B., Wurzburger, N., Turner, B. L., Tanner, E. V., Sayer, E. J., ... & Corre, M. D. (2011). Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology*, *92*(8), 1616-1625.
5. Yavitt, J. B., Harms, K. E., Garcia, M. N., Mirabello, M. J., & Wright, S. J. (2011). Soil fertility and fine root dynamics in response to 4 years of nutrient (N, P, K) fertilization in a lowland tropical moist forest, Panama. *Austral Ecology*, *36*(4), 433-445.