

Interactive comment on “Stem and soil nitrous oxide fluxes from rainforest and cacao agroforest on highly weathered soils in the Congo Basin” by Najeeb Al-Amin Iddris et al.

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GENERAL COMMENTS Stem-derived GHG emissions from tropical trees are a relatively understudied phenomena, and research on this topic has only really gained momentum in the last 5 years. The most comprehensive datasets are from organic soils in SE Asia (e.g. Indonesia), South and Central America (e.g. Brazilian Amazon, Panama); much less data is available from Africa or from well-drained mineral soils. The former is important because of the large areal extent which Africa accounts for, representing a major uncertainty in global atmospheric budgets of trace gases. The latter is critical because gas transport mechanisms through trees are thought to differ

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for wet, organic soils compared to mineral soils (i.e. aerenchymatous transport in wet soils versus xylem transport in well-drained soils). In addition, low redox conditions in wet, organic soils are likely to drive different patterns of trace gas production and consumption compared to well-drained mineral soils, which could affect the composition and magnitude of trace gas fluxes. This research addresses these knowledge gaps by quantifying tree stem and soil fluxes of N₂O from well-drained, mineral soil sites in the Congo. In addition to the emissions themselves, the authors have quantified the effects of land management (i.e. unmanaged tropical forest versus cacao agro-forestry), the influence of key environmental variables, and used stable isotopes to qualitatively assess the contribution of soil-produced N₂O to stem emissions. The paper was well-written and clearly argued; the bigger picture context of the research was clearly characterised, and neatly linked to the specific research questions posed in this study. The methods, results and discussion sections were also well-written and easy to understand. Sufficient information was provided in the methods such that other experts could replicate this study in other locations. The description of the statistical approach was thorough, and provided the reader with a complete picture of how the data were analysed. The experimental design was robust and well-replicated, taking care to account for potential site or treatment effects (e.g. edge effects) on the experimental results. The authors' extrapolation of their findings to larger spatial scales was thought provoking, as it provides the wider flux community with a baseline or starting point to discuss how mineral soil forests in tropical Africa could be influencing regional and global budgets of N₂O via tree stem emissions (see also my comments in point 8).

Overall, I support this paper for publication, given the rigour of the experimental design, the novelty of this dataset, and the high quality of the manuscript. I did, however, have a few questions and suggestions which I believe could improve this manuscript.

Reply: We greatly appreciate the thorough review and suggestions of Dr. Teh, which greatly improve our manuscript. Below, we specify how we propose to incorporate the comments into our revised manuscript.

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First, I was curious if the trees sampled in this study had similar or different functional traits (see points 5 and 6 below)? From the experimental design, the authors indicated that they sampled the dominant taxa in each cover type. I had wondered if the dominant trees were functionally similar to each other or if they were functionally different (e.g. do they fall within a similar “space” along the plant economic spectrum, or do the taxa span different life history strategies)? If the former, then the similarities in stem fluxes among taxa or between cover types may be partially explained by the similarity in the functional traits or ecophysiology of the sampled trees. This could mean that plant communities with very different functional traits could show different flux rates or responses to environmental variables. If the latter (i.e. the dominant trees include a mixture of plants with different functional traits), then the findings from this work could be more widely generalisable across communities at different successional stages or with different species compositions.

Reply: Thank you very much for this important observation. The tree species we measured at the study sites spanned different life history strategies and functional traits. We are currently working on a sister paper on stem CH₄ fluxes, which includes a table that summarises the ecological guilds and other functional traits of these trees. However, we will incorporate this important observation in the revised manuscript (see response to comment # 5 and 6).

Second, I was curious if the authors could use isotope mixing models or other data/techniques to infer how much of the N₂O was derived from the soil rather than from other sources, such as plant tissues (see point 7)? For example, if there are data from ex situ experiments (e.g. mesocosm or greenhouse experiments) that indicate how much N₂O could be produced from within plant tissues, then it may be possible to conservatively estimate what the potential flux rate was from this source under field conditions. Likewise, if plant-derived N₂O has a different stable isotope composition from soil-derived N₂O then it may be possible to use mixing models to ascertain how much N₂O was derived from each source.

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Reply: We agree that it will be interesting to separate plant-associated fluxes of N₂O from soils and other sources using stable isotope techniques, but there still haven't been enough studies to support an estimate of the potential flux rate from the tree source alone. We are still in the relatively early stages of tree stem flux measurements, and we think that it is perhaps more important to assess the magnitude of stem fluxes for unknown regions, and to ascertain the source of tree stem emissions, which is currently only speculated in the literature; these form part of the main focus of this study. We have provided a more specific answer below (see response to comment # 7).

Third, it was not clear if forest age or size structure could play a role in influencing rates of stem flux. The data presented in Table A1 tends to imply that the forests and cacao agroforestry have a similar size structure (i.e. see basal area data). However, it is not clear if there could be an effect of stem size on flux rates (i.e. would stem emissions be similar or different for stands with smaller or larger stems?). If there is an effect of stem size on flux this could have implications for stands of different successional stages or ages.

Reply: We did not find an effect of stem diameter size on stem fluxes, probably due to the small diameter range of our measured trees (10–18 cm DBH for cacao trees and 10–30 cm DBH for the forest trees), which mirrored the average DBH of trees in our study sites (see Table A1 of the original manuscript). We suggest incorporating this comment by including the following paragraph in the revised manuscript: “We did not find an effect of tree diameter sizes on stem N₂O fluxes at our study sites, in contrast to the findings of other studies (references). This was due to the narrow range between the DBH of our measured trees (10–18 cm DBH for cacao trees and 10–30 cm DBH for the forest trees), which reflected the mean stem diameter of trees in our sites (Table A1). Future studies should incorporate trees of variable diameter size classes in stem flux measurements, as they may influence N₂O flux estimates at the ecosystem-scale.” We propose to add this in the discussion section, at line 427.

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Specific questions are outlined in the section below.

SPECIFIC COMMENTS

1. Lines 68-70: The literature on the effects of soil N availability, fertilizer and farm management practices is relatively well-developed, and I recommend adding a few more references here to add weight to your statement. To keep the referencing concise, you could cite one or two of the excellent review or synthesis papers published by colleagues such as Eric Davidson, Pam Matson or Peter Groffman?

Reply: We will revise this in the manuscript by adding the following references: Davidson et al. (2000) Testing the hole-in-the-pipe model of nitric and nitrous oxide emissions from soils using the TRAGNET database and Groffman et al. (2000) Evaluating annual nitrous oxide fluxes at the ecosystem scale.

2. Lines 86-94: What techniques can be used to determine the main transport mechanism for N₂O for the trees in your study site? For example, are their differences in the isotopic fractionation for N₂O transported via aerenchyma versus xylem sap?

Reply: This is a very interesting question; isotopic labelling experiments will be useful for unravelling the source and main transport mechanism of stem-emitted N₂O. But to the best of our knowledge, there has been no measurements on the isotopic composition of N₂O emitted via the different transport mechanisms (either xylem sap or aerenchyma) to enable a definite assessment of the dominant transport medium in our site. However, because the trees in our study sites typically lacked aerenchyma tissues, N₂O is more likely to move in its dissolved form through the xylem via the transpiration stream of the trees, where it is then emitted to the atmosphere via the stomata (Machacova et al., 2013, 2019; Wen et al., 2017).

3. Lines 95-106: For prior stem flux studies on wet soils (i.e. Sunitha Pangala & Vince Gauci's work), wood density was found to be predictor for stem flux rates. Was this a variable measured here, or was wood density thought to be unimportant given that flux

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is likely to be via xylem transport (rather than aerenchymatic tissues)?

Reply: This is also a very interesting point. Wood density is important to measure as tree physiological traits have been shown to affect stem fluxes. However, this has mostly been related to trees having aerenchyma tissues, as the increased pore spaces of such trees (low wood density) suggest for greater transport of water from the soil (e.g. Pangala et al., 2013; Wang et al., 2017). However, we did not measure the wood density of trees in our study, because we expected from our review of the literature, that stem flux emissions would most likely occur via xylem transport. Our findings of similar N₂O fluxes between the different species we measured would also suggest that wood density may not influence stem fluxes in our study sites.

4. Line 109: To give readers a bit more insight into how you selected tree species for study, you may consider adding a sentence or phrase indicating that the trees measured represented the most dominant species in each plot.

Reply: Thank you for the suggestion. We already expounded this in detail in the Materials and Methods (lines 154-163) and therefore suggest maintaining line 109 as it is in the introduction.

5. Line 154-156: The only issue to be aware of here is that the most dominant species may have similar characteristics to each other because they may occupy a similar "space" along the plant economic spectrum and possess similar functional traits (e.g. in old-growth systems, the dominant species tend to show similar traits such as slow growth, high wood density, low tissue turnover times, higher N-use efficiency, shade tolerance, etc.). It's possible that plants with different functional traits (e.g. fast-growing species) may show slightly different physiological characteristics and consequently show differences in stem fluxes. 6. Lines 411-412: I think it is significant that there do not appear to be any statistically significant, species-specific differences in N₂O flux in either forest or agro-forestry systems, suggesting that the mean or median N₂O flux may be similar for trees growing on well-drained soils. The only potential issue

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to be aware of is whether or not this may be because the dominant trees sampled in this study possessed similar functional traits (assuming that they may occupy the same “space” along the plant economic spectrum; see point 5 above). This may be something worthwhile discussing further in the paper.

Reply: We will combine addressing the comments 5 & 6 in our revision since they both centered on the same point. As we mentioned in our answer above, the tree species we measured at our study sites have different life history strategies, including a mixture of pioneers, non-pioneer light demanders, and shade bearers. We will incorporate these excellent suggestions by expanding our discussions in the implication section as follows: “Our measured tree species spanned different life history strategies and functional traits (a mixture of pioneers, non-pioneer light demanders, and shade tolerants); the lack of species-specific differences suggest that our findings could be more widely generalisable across communities with different species compositions, at least from highly weathered soils. However, the narrow range of tree DBH classes of our measured trees may have important implications for stands of different successional stages or ages, as stem diameter size, wood density and other physiological characteristics may affect stem N₂O fluxes (Machacova et al., 2019; Welch et al., 2019). Also, the possibility for large N₂O fluxes at the stem base near the ground (Barba et al., 2019; Welch et al., 2019), which we could not measure due to irregular surface of buttresses, warrants further investigation. All these combined may imply that our quantified stem N₂O emissions result in a conservative estimate of the overall stem N₂O budget from this important region”.

7. Lines 451-460: I understand the logic behind this statement and broadly agree with the interpretation; the soil does seem to be the most likely source of N₂O, given that the turnover of N in soil is probably significantly greater than N turnover in plant tissues, on roots (the rhizoplane) or within roots. My one question here is whether or not there is a way to use mixing models to infer how much of the N₂O was derived from the soil versus to N₂O produced within the plant? Does the isotope value of N₂O derived from

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in-tree processes differ enough from soil-produced N₂O that you could estimate how much N₂O is coming from each process? If this is possible, this would lend weight to the authors’ argument.

Reply: This is another intriguing question. If there would be enough information on the isotopic fingerprint of stem-derived N₂O, then we could estimate how much N₂O is been emitted by the stem itself. To the best of our knowledge, only one study has investigated stable isotopes of plant-emitted N₂O from leaves of a single species (Lenhart et al., 2019). Although the isotopic values of plant-emitted N₂O were different from the range of known dual isotopic values of N₂O from chemical and microbial production, the range of the isotopic values of plant-emitted N₂O were relatively small and the pathway and extent to which it contributed to total N₂O flux was unknown. While we did carryout a ¹⁵N-isotope tracing experiment, our purpose was just to ascertain if N₂O produced in the soil can be detected from the stem emissions, which is currently unknown and has been speculated as one of the mechanisms in the literature but without any field-based measurements.

8. Lines 493-505: I like that the authors have been bold enough to report annualised, upscaled estimates of N₂O flux from their study sites, as not all investigators would have been confident to do so. Given how little data exists for African systems (and for stem fluxes in general), these kinds of upscaling exercises enable the wider flux community to understand how stem fluxes may fit into the bigger picture of regional and global N₂O cycling. Even if these numbers are refined or improved upon by future field experiments, we now have a starting point or baseline to compare against. My recommendation here is that it may be worthwhile to briefly expand this section of the text to discuss the other ways this kind of upscaling could be done to derive annualised fluxes. For example, for landscapes that are spatially structured due factors such as agricultural/forestry planting patterns, topography, soil moisture, fertility, differences in soil type) spatially weighted upscaling may be another approach that could be used. This would not only signal to the reader that the authors are aware of the

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assumptions/potential limitations of their approach, but also provide food for thought for colleagues who might be interested in conducting similar types of studies in other regions.

Reply: Point well taken. We will add a summarized topic on extrapolation method in this paragraph: "The most important consideration in bottom-up spatial extrapolation approach is to recognize at the outset that the design of the field quantification must reflect the landscape-scale drivers of the studied process, e.g. land-use types (reflecting management), soil texture (as a surrogate of parent material) and climate are landscape-scale controllers of soil N, C and GHG fluxes (e.g. Corre et al., 1999; Hassler et al., 2017; Silver et al., 2000; Veldkamp et al., 2008, 2013), and topography (reflecting soil types, moisture regimes, fertility) is the main driver within a landscape (e.g. (Corre et al., 1996, 2002; Groffman and Tiedje, 1989; Pennock and Corre, 2001). Process-based models and geographic information system database can be combined with field-based measurements for improved extrapolation.

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