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Interactive comment on "Effects of nitrogen and phosphorus additions on nitrous oxide emission in a nitrogen-rich and two nitrogen-limited tropical forests" *by* M. H. Zheng et al.

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Dear referee #4,

Thank you for your comments and suggestions which help us further improve our manuscript. My co-authors and I agree with your comments and have followed your suggestions. Below are our point-by-point responses. All the revised portions are marked in red in the revised manuscript, and the page and line numbers of the revised manuscript are also provided.

Anonymous Referee #4

This is a very well written paper on the impact of N and P on N2O emissions from

young and old tropical forest soils. The authors carried out a statistically designed plot experiments and applied either N, P or N+P to just water to the plots and measured the N2O fluxes, soil DIN, P, SOC and microbial biomass. Their general findings, that P addition reduced the N induced N2O emissions is interesting and as the authors suggested will warrant further investigation. This paper is certainly suitable for publication in BG. There are a few mainly technical points the authors should address (see below).

Answer: Thank you very much for these positive comments.

1. My only main concern is the large rates of N & P application (150 kg N / ha / y and 150 kg P / ha/ y). The N applied is $\sim$5 times larger than the atmospheric N deposition rate at the site. The authors need to justify these unrealistic large rates. Would the results of the paper be different if slightly more realistic rates of N and P would be have been applied?

Answer: Thank you for pointing out this question. In our study region, inorganic N deposition is about 24$-$34 kg N ha-1yr-1, with an additional input of 15$-$20 kg N ha-1yr-1 as dissolved organic N, so atmospheric N deposition is about 50 kg N ha-1yr-1 (Page 6 Line 15$-$16 in the revised manuscript).

First, we have another N addition experiment in the old-growth forest using different N gradients (50, 100, and 150 kg N ha-1 yr-1) which are 1$-$3 folds of atmospheric N deposition rate ($\sim$50 kg N ha-1 yr-1), and we found that many soil processes responded significantly only following high N addition (150 kg N ha-1 yr-1) in this forest. For example, our previous studies found that only high N addition significantly decreased soil respiration rates (Mo et al., 2008), methane uptake rates (Zhang et al., 2008), fine root biomass and soil pH (Lu et al., 2010) in the old-growth forest. These results suggest that soil processes may have a high N threshold in this N-rich forest. Although the two younger forests are N-limited, we used a similar N gradient (150 kg N ha-1 yr-1) for the main purpose of comparison among the three forests (Zheng et al., 2015; Zhu et al., 2013). Secondly, we used the high P addition rate because of the high P

demand of soil microbes in our old-growth forest (Liu et al., 2012). The high fertilization rates (150 kg N ha-1 yr-1 and 150 kg P ha-1 yr-1) can remove all possible N and P constraints in both young and old-growth forests (Cleveland and Townsend, 2006). Finally, our experiment design (including the plot size and fertilizer level) also refers to the experiment in a tropical forest in Costa Rica (Cleveland and Townsend, 2006).

Thus, to clearly clarify why we used the high fertilization rates, we have added these information in the Materials and Method section: "We used the high N gradient, about 3 folds of atmospheric N deposition rate, because many soil processes responded significantly only under this gradient in the old-growth forest (Mo et al., 2008; Zhang et al., 2008a; Lu et al., 2010). High P gradient was used because of the high P demand of soil microbes in the old-growth forest (Liu et al., 2012). Although the two younger forests are N-limited, we used the similar N and P gradients for the main purpose of comparison among the forests (Zheng et al., 2015; Zhu et al., 2013a). High fertilization rates can remove all possible N and P constraints in both young and old-growth forests (Cleveland and Townsend, 2006). In addition, plot size and fertilizer level in our forests were also the same as those in Costa Rica by Cleveland and Townsend (2006)." (Please also see Page 6 Line 25 and Page 7 Lines 1-7 in the revised manuscript).

Based on our explanation above, if we use the slightly more realistic rates of N and P ($\sim$50 kg ha-1 yr-1), we guess that the low fertilization rates may be insufficient to affect soil N2O emission in the old-growth forest, but it may have the same effects as our present study using the high rates in the two younger forests. Future studies will be carried out to test this case.

Reference:

Cleveland, C. C., and Townsend, A. R.: Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere, P. Natl. Acad. Sci. USA, 103, 10316-10321, 2006.

Liu, L., Gundersen, P., Zhang, T., and Mo, J. M.: Effects of phosphorus addition on soil

microbial biomass and community composition in three forest types in tropical China, Soil Biol. Biochem., 44, 31-38, 2012.

Mo, J., Zhang, W., Zhu, W., Gundersen, P., Fang, Y., Li, D., and Wang, H.: Nitrogen addition reduces soil respiration in a mature tropical forest in southern China, Global Change Biol., 14, 403-412, 2008.

Zhang, W., Mo, J., Zhou, G., Gundersen, P., Fang, Y., Lu, X., Zhang, T., and Dong, S.: Methane uptake responses to nitrogen deposition in three tropical forests in southern China, J. Geophys. Res, 113, 2008.

Zhu, F. F., Yoh, M., Gilliam, F. S., Lu, X. K., and Mo, J. M.: Nutrient limitation in three lowland tropical forests in southern China receiving high nitrogen deposition: insights from fine root responses to nutrient additions, PLoS One, 8, e82661, 2013.

Zheng, M., Huang, J., Chen, H., Wang, H., and Mo, J.: Responses of soil acid phosphatase and beta-glucosidase to nitrogen and phosphorus addition in two subtropical forests in southern China, Eur. J. Soil Biol., 68, 77-84, 2015.

Technical comments:

1) P7 line 1-5: you need to include a bit more detail on the chamber design: dimensions of the baseframe and lid (or chamber). Did you use a stiring fan, pressure valve? How did you seal the chamber to the lid?

Answer: Thank you for this good suggestion, and we have added this information in the text: "Each static chamber consisted of an anchor ring and a removable cover chamber. The anchor ring was a PVC pipe (25 cm diameter and 16 cm height) permanently anchored into the soil to 8 cm depth. During gas collection, a removable cover chamber (25 cm diameter and 30 cm height) was attached tightly to the anchor ring using a rubber O-ring seal." (Please also see Page 7 Line 16-19 in the revised manuscript).

In this study, we did not use a stiring fan, but we used the syringes to flush chamber gas three times to mix the headspace before each sampling. So, we have added this infor-

mation: “Before each sampling, syringes were flushed three times with chamber gas to mix the headspace.” (Please also see Page 7 Lines 22-23 in the revised manuscript).

2) P7 line 5: Change sentence to: ‘. . .and analyzed within 12 h on the gas chromatograph (Agilent 4890D) fitted. . .’ (replaced ‘in’ with ‘on’).

Answer: Thank you, and we have replaced “in” with “on” in this sentence. (Please also see Page 7 Line 23 in the revised manuscript).

3) P7 line 10: ‘The calculation of N2O fluxes followed the method of Holland et al. (1999), based on linear regression of’ chamber gas concentration across time (changed ‘across’ to ‘with’).

Answer: Thank you. We have replaced “across” with “with” in this sentence. (Please also see Page 8 Line 2 in the revised manuscript).

4) P7 Line 11: was the soil temperature measured inside the chamber?

Answer: Yes, both soil temperature and soil moisture were measured inside the chamber. To make this clear, we have added this information in the text: “soil temperature (at 5 cm depth) and moisture ($0-10$ cm depth) inside each chamber, were measured. . ..” (Please also see Page 8 Line 5 in the revised manuscript).

5) P7: line 16: I am not certain that the very general particle density value of 2.65 g/cm3 is appropriate to be used for your forest soils? Would you not expect a different particle density in the OG forest compared to the mixed/pine forests?

Answer: Thank you, and we agreed with your comment. However, in our study, we cautiously used the particle density value of 2.65 g cm-3 just as an assumption value. This value has been suggested to applied in mineral soils of forests (Linn et al., 1984), and has been widely used in many tropical forests (Koehler et al., 2009; Rowlings et al., 2012; Zhu et al., 2013) This value was also used in our old-growth forest in a previous study (Zhang et al., 2012).

It is possible that the value may be different between forest types (old-growth vs. younger forests). However, the case that the same value (2.65 g cm-3) was used in different ages of forest soils can also be found in other forest studies (Riley et al., 1995; Werner et al., 2006). In addition, because we using the WFPS focus on the comparison between treatments rather than between forest types in this study, whether or not using different particle density values to calculate WFPS may be of minor importance.

To make it more clear to the readers, we have replaced “. . ..2.65 is the density of soil particles (g cm-3)” with “. . ..2.65 g cm-3 is the assumed particle density in mineral soil of forests (Linn et al., 1984). It is possible that the particle density value may be different between forest types (old-growth vs. younger forests), but we focused on the comparison between treatments in this study, so this case is of minor importance.” (Please also see Page 8 Lines 9-12 in the revised manuscript).

Reference:

Koehler, B., Corre, M. D., Veldkamp, E., Wullaert, H., and Wright, S. J.: Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input, Global Change Biol., 15, 2049-2066, 2009.

Linn, D., and Doran, J.: Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils, Soil Sci. Soc. Am. J., 48, 1267-1272, 1984.

Riley, R. H., and Vitousek, P. M.: Nutrient dynamics and nitrogen trace gas flux during ecosystem development in montane rain forest, Ecology, 292-304, 1995.

Rowlings, D., Grace, P., Kiese, R., and Weier, K.: Environmental factors controlling temporal and spatial variability in the soil-atmosphere exchange of CO2, CH4 and N2O from an Australian subtropical rainforest, Global Change Biol., 18, 726-738, 2012.

Werner, C., Zheng, X. H., Tang, J. W., Xie, B. H., Liu, C. Y., Kiese, R., and Butterbach-Bahl, K.: N2O, CH4 and CO2 emissions from seasonal tropical rainforests and a rubber

plantation in Southwest China, Plant Soil, 289, 335-353, 2006.

Zhang, T., Zhu, W., Mo, J., Liu, L., Dong, S., and Wang, X.: Increased phosphorus availability mitigates the inhibition of nitrogen deposition on CH4 uptake in an old-growth tropical forest, southern China, Biogeosciences, 8, 2011.

Zhu, J., Mulder, J., Wu, L., Meng, X., Wang, Y., and Dörsch, P.: Spatial and temporal variability of N 2 O emissions in a subtropical forest catchment in China, Biogeosciences, 10, 1309-1321, 2013.

6) P8 line 3: How was NH4 extracted from the soil?

Answer: Thank you for pointing out this question, and we have added this information in the text: "....after extraction with potassium chloride solution". (Please also see Page 9 Lines 1-2 in the revised manuscript).

7) P8: line 8 & 10: NO3âĂŤN. '-' should not be a superscript

Answer: Thank you for this careful suggestion, and we have replaced "NO3âĂŤN" with "NO3-−N" in the text. (Please also see Page 9 Line 6 and Line 8 in the revised manuscript)

8) P10 line 3: change to: 'mixed, and pine forests, respectively (Fig. 4), with being significantly higher (P = 0.001) in the old-growth forest.

Answer: Thank you, and we have followed your suggestion to change the sentence to "mixed, and pine forests, respectively (Fig. 4), with being significantly higher (P = 0.001) in the old-growth forest" in the text. (Please also see Page 11 Line 2 in the revised manuscript)

9) Page 9 line 16 delete 'were' and line 21: delete 'was'.

Answer: Thank you for this suggestion. We have deleted "were" in Page 9 line 16 and deleted "was" in Page 9 Line 21.

10) Page 10: line 3: change to 'mixed, and pine forests, respectively (Fig. 4), with being significantly higher (P = 0.001) in the old-growth forest'.

Answer: Thank you, and we have followed your suggestion to change the sentence to "mixed, and pine forests, respectively (Fig. 4), with being significantly higher (P = 0.001) in the old-growth forest" in the text. (Please also see Page 11 Line 2 in the revised manuscript)

11) P 11 section 4.1 first paragraph: you may like to add that the variability of the data available could be due to soil type and also variability in climate.

Answer: It is a good suggestion, and we have added this information in this section: "Taken together, these data suggest a high variation in N2O emission among different study regions, possibly due to the difference in soil types and/or climatic conditions." (Please also see Page 12 Lines 12-13 in the revised manuscript)

12) P11 line 23-24: is this the same forest as in your study? If this is the case, replace with: ...in this old-growth forest, investigated previously by Fang et al (2008).

Answer: Yes, it is the same forest as in our study. According to your suggestion, we have replaced the sentence "....in the old-growth forest (Fang et al., 2008)" with ".... in this old-growth forest, investigated previously by Fang et al (2008)". (Please also see Page 12 Line 24 in the revised manuscript)

13) P13 line 12 'In spring, forest soil was enriched with inorganic N (accumulated during non-growing seasons)'you need to say that the non growing season is due to the lack of rainfall. Also comment on the pulsing effect (wetting dry soil triggers N2O emissions and other gases.

Answer: Thank you for this good suggestion. (1) We have added: "accumulated during non-growing seasons mainly due to the lack of rainfall" (Please also see Page 14 Lines 2-3 in the revised manuscript). (2) We have replaced the sentence "conditions that would increase microbial consumption of soil NH4+ and/or NO3- (Davidson et al.,

2000), and thus greatly increase N2O production (Davidson et al., 2000; Butterbach-Bahl et al., 2004; Werner et al., 2006)." with "conditions that would generate a pulsing effect, because wetting dry soil will trigger emissions of N2O and other nitrogenous gases by stimulating microbial consumption of soil NH4+ and/or NO3- (Davidson et al., 2000; Butterbach-Bahl et al., 2004; Werner et al., 2006)." (Please also see Page 14 Lines 3-7 in the revised manuscript)

14) P14 line 22: change to 'allowing us to reject the hypothesis that P addition causes greater decrease in N2O emission'.

Answer: Thank you, and we have followed your suggestion to change the sentence to "allowing us to reject the hypothesis that P addition causes greater decrease in N2O emission. . .." in the text. (Please also see Page 16 Line 3 in the revised manuscript)

15) P15 line 1-2: Under laboratory conditions, Sundareshwar et al. (2003) found a negative response of sediment N2O emission to nitrate addition. This sentence should be moved to the nitrogen section 4.3

Answer: Thank you for this suggestion, but we would like to make an explanation for this sentence. "nitrate addition" is a typo made by mistake, and we intended to use the phase "phosphate addition". We have replaced "nitrate addition" with "phosphate addition" in this sentence, and thus, this sentence may be appropriate to remain in the section "4.4 Effects of P addition on N2O emission". (Please also see Page 16 Line 9 in the revised manuscript)

16) Fig 3 & 4 legend line 3: delete 'before analysis' Fig 5 legend change to. . ." in the three control plots of the study forest. . .;

Answer: Thank you for these suggestions. We have deleted "before analysis" in both Fig.3 and Fig. 4 legend in line 3. Because we have five control plots in each forest, we replaced ". . ..in the study forests" with ". . ..in the five control plots of the study forests" in Fig. 5 legend.

Again, thank you very much for the above comments and suggestions.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/bg-2015-552/bg-2015-552-AC3-supplement.pdf

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