

## ***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 #3,

Thank you for your comments and suggestions which help us greatly improve the quality of this manuscript. My co-authors and I agree with your comments, and we have made improvements on the manuscript according to 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. Thank you very much.

Anonymous Referee #3

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This paper studied the effects of N and P additions on N<sub>2</sub>O emission in two tropical forest soils. The authors claimed that this is the first study to exam how N and P interact to control soil N<sub>2</sub>O emission in tropical forests. As far as I can tell, the results are sound, but the conclusions might need to be further discussed. I also have several technical comments, detailed below, that should be addressed prior to publication.

Answer: Thank you for these comments.

1) In page 6, lines 7-9, it shows that natural atmospheric N deposition is ~50 kg N ha<sup>-1</sup> yr<sup>-1</sup> for this study region. Why did you add so much N (150 kg N ha<sup>-1</sup> yr<sup>-1</sup>) for your experiments?

Answer: Thank you for pointing out this question, and Referee #4 also mentioned the similar question that why we used the high rates of N and P fertilization.

In fact, we have another N addition experiment in the old-growth forest using different N gradients (50, 100, and 150 kg N ha<sup>-1</sup> yr<sup>-1</sup>) which are 1–3 folds of atmospheric N deposition rate (~50 kg N ha<sup>-1</sup> yr<sup>-1</sup>), and we found that many soil processes responded significantly only following high N addition (150 kg N ha<sup>-1</sup> yr<sup>-1</sup>) 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<sup>-1</sup> yr<sup>-1</sup>) 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<sup>-1</sup> yr<sup>-1</sup> and 150 kg P ha<sup>-1</sup> yr<sup>-1</sup>) 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).

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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).

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

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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.

2) In the introduction part, it would be useful to give some information about the differences between old-growth forest and younger forest, such as soil development, plant N utilization, soil N cycling, trees root. ...

Answer: Thank you for this good suggestion, and the information you provided will help our manuscript clarify why studied on different forest types (old-growth forest versus younger forest) more clearly.

Accordingly, we have followed your suggestion and added this information in the Introduction section: “However, the capacity of P to reduce N losses may relate to forest development. Despite many tropical forests have rich N in soils, several younger forests early in soil development are still N-limited (Vitousek et al., 1997a). Compared with the old-growth forests, younger forests often show the higher N demands and utilization of plants and microbes, but the lower rates of soil N cycling, such as mineralization, nitrification and leaching (Aber et al., 1998). In contrast, old-growth forests have the higher P demand because they are commonly depleted in P (Vitousek et al., 2010). For example, one of our previous study showed that soil microbes and/or tree roots released more phosphatase in the old-growth forest than in the younger one (Zheng et al., 2015).” (Please also see Page 4 Lines 16-23 in the revised manuscript).

Reference:

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., and Fernandez, I.: Nitrogen saturation in temperate forest ecosystems-Hypotheses revisited, *Bioscience*, 48, 921-934, 1998.

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Vitousek, P. M., and Farrington, H.: Nutrient limitation and soil development: experimental test of a biogeochemical theory, *Biogeochemistry*, 37, 63-75, 1997a.

Vitousek, P. M., Porder, S., Houlton, B. Z., and Chadwick, O. A.: Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions, *Ecol. Appl.*, 20, 5-15, 2010.

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.

3) In page 9, lines 8-9, 'soil WFPS decreased in summer'. But in page 6, lines 4-5, you wrote 75% of precipitation falls from March to August. Why is that? Do you have the rainfall data?

Answer: Thank you for pointing out this interesting question, and we have made some improvements in the text.

First, rainfall (precipitation) is not the main data supporting the mechanisms in our study, so we did not measure it. However, previous study showed that 75% of precipitation fell in spring (March to May) and summer (June to August) in the study forests (Huang and Fan, 1982), and our recent study also indicated a similar pattern (73.5%) (Lu et al., 2013), suggesting that precipitation pattern changes little in spite of slight fluctuation in the study region. Thus, to make this more clearly, we have replaced "75% of which falls from March to August. ..." with "about 75% of which falls from March to August. ... as reported by our previous studies (Huang and Fan, 1982; Lu et al., 2013)" in the text (Please also see Page 6 Lines 11-13 in the revised manuscript).

Second, why soil WFPS was high in spring but decreased in summer? We infer this may be caused by the higher plant uptake and transpiration in the summer. Plants may grow fast in the summer (growing season), and thus absorb more water directly from soils or the soil nutrients which are also carried by water. A recent study carried out

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by our colleagues found that dominant tree species in this forest generally showed the higher sap flow velocity and daily transpiration in the summer than in the dry season (Cheng et al., 2015). Thus, we have added this information in the Results section: "possibly due to the higher plant uptake and transpiration, despite the high amount of precipitation in summer" (Please also see Page 10 Line 7 in the revised manuscript), and also in the Discussion section: "In summer, N<sub>2</sub>O emission began to decrease given decreasing soil WFPS (Fig. 3) possibly caused by the higher plant uptake and transpiration (Cheng et al., 2015)." (Please also see Page 14 Lines 6-7 in the revised manuscript).

Reference:

Cheng J., Oyang X., Huang D. W., Liu S. Z., Zhang D. Q., Li Y. L.: Sap flow characteristics of four dominant tree species in a mixed conifer broadleaf forest in Dinghushan, *Acta Ecologica Sinica*, 35, 4097-4104, 2015.

Huang, Z. F., and Fan, Z. G.: The climate of Dinghushan (in Chinese with English abstract), *Tropical and Subtropical Forest Ecosystem*, 1, 11-16, 1982.

Lu, X., Gilliam, F. S., Yu, G., Li, L., Mao, Q., Chen, H., and Mo, J.: Long-term nitrogen addition decreases carbon leaching in nitrogen-rich forest ecosystems, *Biogeosciences*, 10, 3931-3941, 2013.

4) In page 12, lines 5-6, although higher MBC in old-growth forest soil, I am still not sure about the higher activity of (de)nitrifying bacteria as the low soil pH (~4.0). Chemodenitrification or other chemical processes might be more important than (de)nitrification.

Answer: Thank you. We agreed with your comments. We have looked up some relevant references, and understood that biological (de)nitrification are often active under neutral and slightly alkaline conditions, while chemo-denitrification is more important than biological (de)nitrification in acid conditions, especially when soil pH was lower than 4.0 (Tate, 1995; Chalk and Smith, 1983; Mørkved et al., 2007).

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Accordingly, the activity of (de)nitrifying bacteria may not be a proper explanation for the higher N<sub>2</sub>O emission in our old-growth forest soil which is acid, so we have replaced “Compared to the two younger forests, the old-growth forest had significantly higher soil dissolved organic C, total organic C, and microbial biomass C (Table 1), likely supporting a higher activity of nitrifying and denitrifying bacteria responsible for N<sub>2</sub>O production” with “Additionally, the old-growth forest had significantly higher soil dissolved organic C and total organic C (Table 1), which could provide more C energy for N<sub>2</sub>O production (Zhang et al., 2014).” in the text (Please also see Page 13 Lines 6-7 in the revised manuscript).

In addition, following your suggestion, we have also added another explanation: “Compared to the younger forests, the old-growth forest had more acid soil conditions (Table 1 and 2), likely supporting the higher chemo-denitrification (Tate, 1995; Chalk and Smith, 1983; Mørkved et al., 2007).” (Please also see Page 13 Lines 4-6 in the revised manuscript).

Reference:

Chalk, P., and Smith, C.: Chemodenitrification, in: Gaseous loss of nitrogen from plant-soil systems, Springer, 65-89, 1983.

Mørkved, P. T., Dörsch, P., and Bakken, L. R.: The N<sub>2</sub>O product ratio of nitrification and its dependence on long-term changes in soil pH, *Soil Biol. Biochem.*, 39, 2048-2057, 2007.

Tate, R. L.: *Soil microbiology*, John Wiley and Sons, 398, 1995.

Zhang, W., Zhu, X., Luo, Y., Rafique, R., Chen, H., Huang, J., and Mo, J.: Responses of nitrous oxide emissions to nitrogen and phosphorus additions in two tropical plantations with N-fixing vs. non-N-fixing tree species, *Biogeosciences*, 11, 4941-4951, 2014.

5) In page 14, lines 15-16, you only measured N<sub>2</sub>O emissions and nitrate leaching, but didn't measure other gases lost (NH<sub>3</sub>, NO, HONO, NO<sub>2</sub>) and also didn't measure

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nitrogen utilization by plant. Thus, it is hard to say that ‘N continues to be utilized and was not lost...’, and also hard to support the hypothesis in the following sentence.

Answer: Thank you very much for pointing out this question, and we agreed with this constructive comment. Although we did not measure other gases lost (NH<sub>3</sub>, NO, HONO, NO<sub>2</sub>) and the nitrogen utilization by plant, our previous studies have showed that the N input was mainly lost via leaching or retained for plant biomass and litter increment in the two younger forests, as explained below.

First, our previous survey showed that atmospheric N deposition via precipitation was 49.5 kg N ha<sup>-1</sup> yr<sup>-1</sup> for this region, and total dissolve N leaching losses (surface runoff plus seepage leaching in soil solution) from the upper 20 cm soil was 29 and 22 kg N ha<sup>-1</sup> yr<sup>-1</sup> for the pine and mixed forest, respectively, and 21 and 28 kg N ha<sup>-1</sup> yr<sup>-1</sup> (for the pine and mixed forest, respectively) was retained in the upper 20cm soil and through plant uptake (Fang et al., 2008). These retention estimates based on input-output budgets also account for the potential gaseous N loss by (de)nitrification (Fang et al., 2008).

Second, in the pine forest, previous estimate suggests that the canopy tree, the understory plants and standing floor litter accumulated 9.1, 6.0 and 6.5 kg N ha<sup>-1</sup> yr<sup>-1</sup>, respectively, during the period from 1990 to 2000 (Mo et al., 2004), and these total N accumulation approximates to the observed 21 kg N ha<sup>-1</sup> yr<sup>-1</sup> that was retained above the upper 20 cm soil (Fang et al., 2008). In the mixed forest, N accumulation in the plant biomass and the increasing litter layer were probably higher than in the pine forest, due to higher litter production and higher foliar N concentration (Mo et al., 2007), and might as well account for the 28 kg N ha<sup>-1</sup> yr<sup>-1</sup> retained in this forest (Fang et al., 2008). Thus, under N deposition, the N retention in two younger forests was in accordance with the estimates of N accumulation in biomass and litter increment (Fang et al., 2008), suggesting that the N input had less effect on gaseous N loss in the two younger forests.

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Third, our N addition study showed that N addition had no effects on nitrification rate and N<sub>2</sub>O emission in the younger forests (Fig. 4 and 6), further suggesting that the N retention was mainly used for plant growth rather than for gaseous N loss.

The above evidences may support “N continues to be utilized following N addition”, but we admit that “N was not lost” is not true because parts of the added N were lost via leaching (Fang et al., 2008). Thus, we have made some improvements in the text: (1) we have added this information: “In addition, our previous study showed that under atmospheric N deposition, the N retention in the two forests was in accordance with the estimates of N accumulation in plant biomass and litter increment (Mo et al., 2004, 2007a; Fang et al., 2008), suggesting that the N retention was mainly used for plant growth rather than gaseous N loss.” (Please also see Page 15 Lines 15-18 in the revised manuscript); (2) we have added this statement: “In this study, despite we did not measure other gases losses (such as NH<sub>3</sub>, NO, HONO and NO<sub>2</sub>) which are also important in forest soils, . . .” (Please also see Page 15 Lines 18-20 in the revised manuscript).; (3) we have replaced this statement “N continues to be utilized and was not lost” with “N continues to be utilized rather than N<sub>2</sub>O emission” (Please also see Page 15 Lines 22-23 in the revised manuscript).; (4) we have deleted this hypothesis in the text: “This confirms our hypothesis that soil N<sub>2</sub>O emission shows no response to N addition in N-limited forests (Zhang et al., 2008).”; and (5) we have added this information: “Further studies are needed to examine whether N addition increases other nitrogenous gases loss in the N-limited forests.” (Please also see Page 15 Lines 23-24 in the revised manuscript).

Reference:

Fang, Y. T., Gundersen, P., Mo, J. M., and Zhu, W. X.: Input and output of dissolved organic and inorganic nitrogen in subtropical forests of South China under high air pollution, *Biogeosciences*, 5, 339-352, 2008.

Mo, J. M., Peng, S. L., Brown, S., Kong, G. H., and Fang, Y. T.: Nutrient dynamics in

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response to harvesting practices in a pine forest of subtropical China, *Acta Phytoecol. Sin.*, 28, 810–822, 2004.

Mo, J. M., Brown, S., Xue, J. H., Fang, Y. T., Li, Z. A., Li, D. J., and Dong, S. F.: Response of nutrient dynamics of decomposing pine (*Pinus massoniana*) needles to simulated N deposition in a disturbed and a rehabilitated forest in tropical China, *Ecol. Res.*, 22, 649–658, 2007a

6) One way to check the mechanism of P alleviation of N<sub>2</sub>O emissions is to compare soil microbial community in Control and P addition treatments. This might give you a clue in microbiological level.

Answer: Thank you for this good suggestion, and we have added the relevant information on soil microbial community to support the mechanism of P alleviation of N<sub>2</sub>O emissions. We have added: “P addition likely alleviated the P limitation on soil microbes in our old-growth forest, because our previous study showed that P addition significantly increased soil microbial biomass and soil respiration (Liu et al., 2012). Compared with the controls, P addition changed soil microbial community, including the increases in biomass of bacteria and AM fungi (Liu et al., 2012, 2013). The increases in AM fungi may help plants acquire more N and P nutrients (Tresede and Vitousek, 2001), because they are more efficient in obtaining nutrients from the soil than the plant roots (Liu et al., 2013). In addition, the increases in soil bacterial and fungal biomass may potentially increase total N acquirement, as evidenced by our previous study showing that 4 years of P- and NP-addition tended to increase soil microbial biomass N (Liu et al., 2013).” (Please also see Page 17 Lines 22-25 and Page 18 Lines 1-4 in the revised manuscript).

Reference:

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.

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Liu, L., Zhang, T., Gilliam, F. S., Gundersen, P., Zhang, W., Chen, H., and Mo, J. M.: Interactive effects of nitrogen and phosphorus on soil microbial communities in a tropical forest, *PLoS One*, 8, e61188, 2013.

Treseder, K. K., and Vitousek, P. M.: Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests, *Ecology*, 82, 946-954, 2001.

7) For my understanding, your control experiment is under natural atmospheric N deposition? Compared with control treatment, P addition treatment didn't decrease N<sub>2</sub>O flux (Fig. 3 and 4). So it is not possible to get the conclusion that 'P fertilization can be used to reduce soil N<sub>2</sub>O emission in N-rich forests under atmospheric N deposition'. Even P addition treatment decreased N<sub>2</sub>O flux compared with high N (150 kg N ha<sup>-1</sup> yr<sup>-1</sup>) addition treatment, how do you know P addition will also decrease N<sub>2</sub>O flux under low N addition or atmospheric N deposition (50 kg N ha<sup>-1</sup> yr<sup>-1</sup>)? Especially you explained that N<sub>2</sub>O emissions are caused by high N content or N-rich soil.

Answer: Thank you for pointing out this excellent question, and we agreed with your comments. It is interesting that P addition treatment decreased N<sub>2</sub>O emission compared with high N addition treatment (150 kg N ha<sup>-1</sup> yr<sup>-1</sup>), but not when compared with natural atmospheric N deposition (50 kg N ha<sup>-1</sup> yr<sup>-1</sup>). We suggested two following reasons accounting for this phenomenon.

First, this may be related to the levels of N addition. It is possible that low N addition (or natural atmospheric N deposition) may not cause a significant increase in N<sub>2</sub>O emission in this N-rich forest soil. Our previous study showed that N loss via leaching in our N-rich forest was higher than the N input via atmospheric deposition (~50 kg N ha<sup>-1</sup> yr<sup>-1</sup>), suggesting a net N loss under this low N input conditions and thus the less N retained for N<sub>2</sub>O production (Fang et al., 2008). Accordingly, it is possible that low N addition fail to increase soil N<sub>2</sub>O emission in this N-rich forest, and P addition may show no alleviated effect. In contrast, under high N addition (150 kg N ha<sup>-1</sup> yr<sup>-1</sup>), apart from N leaching, parts of the N input may be used for increasing N<sub>2</sub>O emission, and

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thus P addition may show the alleviated effect.

Second, a lack of response of N<sub>2</sub>O emission to P addition compared with the control may also be related to fertilization period. Because nutrients (N and P) addition in our study was only applied for about 2 years, we did not observe the alleviated effect of P addition on N<sub>2</sub>O emission under natural N deposition (Fig. 3 and 4). However, our recent study in the same forest found that long-term (6 years) P addition (150 kg P ha<sup>-1</sup> yr<sup>-1</sup>) significantly decreased soil N<sub>2</sub>O emission compared with the control (natural N deposition) (Chen et al., 2015). This in part suggests that fertilization period is also an important factor affecting the alleviated effect of P addition on N<sub>2</sub>O emission in this N-rich forest.

Thus, based on your suggestion and the above two possible reasons, we have made some improvements in the text: (1) we have added this information: "It is interesting that soil N<sub>2</sub>O emission reduced after P addition compared with that after N addition (150 kg N ha<sup>-1</sup> yr<sup>-1</sup>), but not when compared with that under atmospheric N deposition (~50 kg N ha<sup>-1</sup> yr<sup>-1</sup>). We infer this may be related to the levels of N addition and/or the period of P addition. First, it is possible that low N addition, such as atmospheric N deposition in our study, may not cause a significant increase in soil N<sub>2</sub>O emission in this N-rich forest. Our previous study showed that under atmospheric N deposition (49.5 kg N ha<sup>-1</sup> yr<sup>-1</sup>), soil had higher N leaching (59.8 kg N ha<sup>-1</sup> yr<sup>-1</sup>) in this N-rich forest, suggesting a net N loss under atmospheric N deposition (low N input), and thus the less N retained for N<sub>2</sub>O production (Fang et al., 2008). Accordingly, it is possible that low N addition fail to increase soil N<sub>2</sub>O emission in the N-rich forest, and thus P addition may show no alleviated effect. Second, a lack of response of N<sub>2</sub>O emission to P addition compared with the control may also be related to the P fertilization period. Nutrients (N and P) addition in our study was only applied for about 2 years, and we did not observe the alleviated effect of P addition on soil N<sub>2</sub>O emission under atmospheric N deposition (Fig. 3 and 4). However, our recent study in the same forest found that long-term (6 years) P addition significantly decreased soil N<sub>2</sub>O emission compared with the control

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(atmospheric N deposition) (Chen et al., 2015). This suggests that fertilization period is also an important factor affecting the alleviated effect of P addition on N<sub>2</sub>O emission in this N-rich forest.” (Please also see Page 18 Lines 9-23 in the revised manuscript); (2) we have replaced this statement “Therefore, our findings suggest that P addition will alleviate the stimulating effects of N on N<sub>2</sub>O emission in the N-rich forest.” with “Therefore, our findings suggest that P addition will alleviate the stimulating effects of N on N<sub>2</sub>O emission in the N-rich forest, but this effect may only occur under high N addition and/or long-term P addition.” (Please also see Page 18 Lines 24-25 in the revised manuscript); and (3) we have also added this sentence “this effect may only occur under high N deposition and/or long-term P addition,” in the Abstract and Conclusions section (Please also see Page 2 Lines 8-9 and Page 19 Lines 8-9 in the revised manuscript).

Reference:

Fang, Y. T., Gundersen, P., Mo, J. M., and Zhu, W. X.: Input and output of dissolved organic and inorganic nitrogen in subtropical forests of South China under high air pollution, *Biogeosciences*, 5, 339-352, 2008.

Chen, H., Gurmessa, G. A., Zhang, W., Zhu, X., Zheng, M., Mao, Q., Zhang, T., and Mo, J.: Nitrogen saturation in humid tropical forests after 6 years of nitrogen and phosphorus addition: Hypothesis testing, *Funct. Ecol.*, doi: 10.1111/1365-2435.12475, 2015.

Again, thank you very much for your constructive comments and suggestions.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/bg-2015-552/bg-2015-552-AC2-supplement.pdf>

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Interactive comment on *Biogeosciences Discuss.*, doi:10.5194/bg-2015-552, 2016.