

Interactive comment on “Soil nitrogen transformation responses to seasonal precipitation changes are regulated by changes in functional microbial abundance in a subtropical forest” by Jie Chen et al.

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General Comment:

This manuscript uses an interesting design to explore the complex relationships between changes in precipitation regimens and soil N dynamics. The authors monitored changes in microbial functional genes, N-mineralization rates, and soil N₂O flux to understand how changes in precipitation regime, but not total precipitation amount, might affect soil biogeochemical cycles (i.e. N-cycle). Results indicated that 20% of total N-mineralization and nitrification rate were explained by microbial abundance (de-

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terminated via fumigation) and soil moisture content, and that amoA abundance also helped to explain a significant portion of variation in measured N pools. Together, the results indicate that predicted changes in seasonal precipitation associated with changing climate may result in increased NO₃- leaching from soils and decreases in N₂O emissions. Although the manuscript is scientifically sound and interesting, it should be heavily edited for proper grammar, spelling, and sentence structure. Response: Thanks to the reviewer for considering our work interesting. We carefully polished the English of the revised manuscript (e.g., lines 79-81, 102-118, 435-438, 537-540, and 574-582). Hopefully all language issues have been fixed. We acknowledged the reviewers for their constructive comments in the Acknowledgement section. The revised manuscript is attached as a Supplement to the point-by-point response to reviewers comments. All the changes have been marked in the revised version.

Specific Comments:

Comment #1: Line 35-37: the wording of this sentence is confusing, please clarify “main factor” (i.e. is it a statistical inference that is meant here?) Response: We thank the reviewer’s recommendation. This sentence has been reconstructed in a more clearly way: “More than 20% of the total variation of net nitrification and N mineralization could be explained by microbial abundance and soil water content (SWC). Noticeably, archaeal amoA abundance showed the highest correlation coefficients (≥ 0.35) with net N transformation rates, suggesting the critical role of archaeal amoA abundance in determining N transformations.” (see lines 36-39).

Comment #2: Line 39: do the authors mean “both dry and wet seasons”? this sentence should be clarified. Response: Yes, we meant to say “both dry and wet seasons”. The sentence has been clarified as “However, N₂O emission decreased moderately in both dry and wet seasons due to changes in nosZ gene abundance, MBC, net nitrification and SWC.” (see line 42).

Comment #3: Line 43: what was “determined by changes in DOC and NH₄+”? please

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clarify this conclusion. Response: This part of the sentence meant that functional genes abundance and MBC were mainly affected by changes in soil dissolved organic carbon (DOC) and NH₄⁺ based on the results of structure equation modeling (SEM) analyses. As displayed in Fig. 5, DOC and NH₄⁺ showed significantly direct effects on all the functional genes abundance and MBC content. As much as 15 - 64% of the variation in these genes abundance and MBC content could be explained by DOC and NH₄⁺. Thus, DOC and NH₄⁺ were the main factors in affecting changes in functional genes abundance and MBC in our study. The conclusion sentence has been revised for further clarity (see lines 45-47).

Comment #4: Line 49: this sentence is missing a word, are the authors indicating that precipitation changes have been observed as increasingly sever, or that they are predicted to be increasingly severe. Response: It's been predicted to be increasingly severe over this century. The sentence has been rewritten as "Precipitation changes caused by global climate change are predicted to be increasingly severe over the century" in the revised version (see line 52).

Comment #5: Line 54: how have changes in precipitation events have exceeded other climate change-related fluctuations? Response: We meant that precipitation changes are more spatially and temporally heterogeneous compared to the other climate change factors such as temperature and atmospheric CO₂ concentration. Therefore the complexity and unpredictability of future precipitation changes might exceed other climate changes (Beier et al., 2012). Furthermore, the direction and types of the changes in precipitation patterns are different among ecosystems. For instance, the annual precipitation amount will decrease in subtropical ecosystems, while it will increase in the mid- and high latitudes (Mc-Carthy, 2011). This will lead to more spatially heterogeneous and less predictable of precipitation change on ecosystems than for other major climate change drivers. A meta-analysis of experimental precipitation manipulation has revealed that precipitation change effects on ecosystems are less consistent than the effects of other climate changes, such as elevated CO₂ and warm-

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ing (Wu et al., 2011). This may reflect the more fluctuation and uncertainty of precipitation changes. Beier, C., Beierkuhnlein, C., Wohlgemuth, T., Penuelas, J., Emmett, B., Körner, C., de Boeck, H.J., Christensen, J.H., Leuzinger, S., Janssens, I.A., and Hansen, K.: Precipitation manipulation experiments - challenges and recommendations for the future, *Ecol. Lett.*, 15, 899-911, doi:10.1111/j.1461-0248.2012.01793.x, 2012. Wu, Z.T., Dijkstra, P., Koch, G.W., Penuelas, J., and Hungate, B.A.: Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation, *Glob. Change Biol.*, 17, 927-942, doi:10.1111/j.1365-2486.2010.02302.x, 2011. McCarthy, J.J.: Climate change 2001: impacts, adaptation, and vulnerability: contribution of Working Group II to the third assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, 2001.

Comment #6: Line 57: seasonal redistribution of what metric? Response: We meant to say “redistribution of seasonal precipitation” (e.g., reduced dry-season precipitation but increased wet-season precipitation). The whole sentence has been revised for clarity (see line 62).

Comment #7: Line 123: reference for the SEM? Also, please introduce SEM and how/ if they have been previously used in a similar manner. Response: Two relevant references for the SEM analyses have been added (see line 141). Meanwhile, an introduction of SEM including previous examples of using this method have been added in the statistical analysis part (see lines 332-337).

Comment #8: Line 130: please clarify “easily” Response: It was not an accurate word. We have revised it as “strongly” to clarify the sentence without changing the meaning to it (see line 151).

Comment #9: Line 135-145: please add a reference for these data, or indicate how the authors collected the data. Response: A reference containing this information has been cited (see line 159). A brief description of the vegetation investigation method is also provided (see lines 162-164).

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Comment #10: Line 174: was this water also examined for microbial community structure and functional genes? Is it possible that this water acted as a source of microbial change? Response: It is a very intriguing question! No, we did not examine the microbial community structure and functional genes in the water. But we suspect that the microbial community composition and functional gene abundance should be different in the rainwater and the pond water. It is difficult to speculate that whether such difference can cause soil N transformation and soil microbial community changes. Our experiment is still running, we will take the reviewer's suggestion seriously and examine whether the microbial community composition differ between the two sources of water in future studies.

Comment #11: Line 231: why did the authors not also quantify bacterial and archaeal 16S rRNA genes? The assay would independently confirm results indicated by chloroform fumigation techniques, and may potentially help to shed light on the negative results associated with amoB gene quantification. Response: The 16S rRNA and ITS genes have been quantified and reported in Zhao et al. (2017). This work was mainly focused on the roles of the key functional microorganisms in driving soil N transformations under precipitation changes. As suggested by previous studies (van der Heijden et al., 2008), ammonia-oxidizing archaea (AOA) and bacteria (AOB) marked by the amoA functional gene drive the central and rate-limiting step in N cycle: ammonium oxidation. The denitrification process is mainly driven by nitrite-reducing bacteria marked by the nirK and nirS genes and nitrous oxide-reducing bacteria marked by nosZ gene (Chan et al., 1997; Billings, 2008). Thus, we only quantified these functional microorganisms in our study. The negative results of AOB community abundance were mainly caused by the low pH value (4.08 ± 0.05) in our study soil, which has been evidenced previously (Isobe et al., 2012) and described in the manuscript (see lines 394-397). Except for the functional microorganisms, the chloroform fumigation extracted microbial biomass carbon (MBC) was also investigated and evidenced as an important factor in driving N transformation in our study. There are two seasons: firstly, the chloroform fumigation techniques can measure the total microbial biomass including bacteria, ar-

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chaea and fungi (Wu et al., 1990). Therefore, the MBC determined by the chloroform fumigation method is more appropriate than the specific gene copy numbers to reflect the changes in overall microbial community size in soil. Secondly, since soil microbial biomass can be either a source or sink of available nutrients, it usually plays an important role in soil nutrient transformations (Singh et al., 1989). Chan, Y.K., McCormick, W.A., Watson, R.J: A new nos gene downstream from nosDFY is essential for dissimilatory reduction of nitrous oxide by rhizobium (*sinorhizobium*) meliloti. *Microbiology* 143, 2817-2824, doi:10.1099/00221287-143-8-2817, 1997. Billings, S.A.: Biogeochemistry: nitrous oxide in flux. *Nature* 456, 888-889, 2008. Isobe, K., Koba, K., Suwa, Y., Ikutani, J., Fang, Y.T., Yoh, M., Mo, J.M., Otsuka, S., and Senoo, K.: High abundance of ammonia-oxidizing archaea in acidified subtropical forest soils in southern China after long-term N deposition, *Fems Microbiol. Ecol.*, 80, 193-203, doi:10.1111/j.1574-6941.2011.01294.x, 2012. Singh, J.S., Raghubanshi, A.S., Singh, R.S., and Srivastava, S.C.: Microbial biomass acts as a source of plant nutrients in dry tropical forest and savanna, *Nature*, 338, 499-500, doi:10.1038/338499a0, 1989. van der Heijden, M.G.A., Bardgett, R.D., and van Straalen, N.M.: The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems, *Ecol. Lett.*, 11, 296-310, doi:10.1111/j.1461- 0248.2007.01139.x, 2008. Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brooks, P.C.: Measurement of Soil Microbial Biomass C by Fumigation- Extraction - An Automated Procedure, *Soil Biol. Biochem.*, 22, 1167-1169, 1990. Zhao, Q., Jian, S., Nunan, N., Maestre, F. T., Tedersoo, L., He, J., Wei, H., Tan, X., and Shen, W.: Altered precipitation seasonality impacts the dominant fungal but rare bacterial taxa in subtropical forest soils. *Biol. Fertil. Soils*, 53, 231-245, doi: 10.1007/s00374-016-1171-z, 2017.

Comment #12: Line 381: could the opposite also be true here? That alterations in DOC and N are due to changes in functional gene abundance? Response: Yes, we agree with the reviewer that soil DOC and N could be affected by changes in functional gene abundance. In our study, the changes in NH₄⁺ and NO₃⁻ availabilities were mainly affected by soil water content (SWC) and precipitation events, while the DOC

changes could not be explained by the measured variables (see Fig. 5). However, the alterations in functional gene abundance were largely explained by soil DOC and N changes (see Fig. 5). Thus, the effects of functional gene abundance on soil DOC and N were not emphasized in the discussion part.

Comment #13: Line 398: reference? Response: The reference (Gao et al., 2017) has been added (see line 453).

Comment #14: Line 424: is there a figure or table that would also demonstrate this statement? Response: Yes, this statement could be demonstrated by the changes in SWC (Fig. 2d), MBC (Fig. 2h), N transformation rates (Fig. 3b, d) and archaeal amoA gene abundance (Fig. 4b), and the relationships between net N transformation rates and these variables (i.e., SWC, MBC and amoA gene abundance) in Fig. S2. We have added the Figure numbers behind the corresponding text (see lines 485-488).

Comment #15: Line 433: please clarify this sentence, how are microorganism responsible for reducing microbial abundance? Response: This sentence has been clarified (see lines 495-501). When the microorganisms are limited by substrate resources, such as available C and N, one part of microorganisms may die from starvation or competition. Since microbial biomass C and N can be either a sink or source of available nutrients (Yang et al., 2010), the other part of microorganisms which survived from starvation and competition can reuse the C and N released from the dead microorganisms (Borken and Matzner, 2009). Finally, the community size of soil microorganisms may reduce by the death of some microorganisms after substrate decrease. Borken, W., and Matzner, E.: Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils, *Glob. Change Biol.*, 15, 808-824, doi:10.1111/j.1365-2486.2008.01681.x, 2009. Yang, K., Zhu, J.J., Zhang, M., Yan, Q.L., and Sun, J.X.: Soil microbial biomass carbon and nitrogen in forest ecosystems of Northeast China: a comparison between natural secondary forest and larch plantation, *Journal of Plant Ecology*, 3, 175-182, doi:10.1093/jpe/rtq022, 2010.

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Comment #16: Line 444: are there other appropriate references available here? Response: Yes, we added one more reference here (Gao et al., 2016, see line 513) as suggested by the reviewer.

Comment #17: Figures 2-5 would benefit from the addition of panel indicators (i.e. "Fig1 A") to help clarify to the reader exactly which data the authors are discussing in the MS. Fig 5: this is a nicely illustrated complex figure. Response: We thank the reviewer's positive comment on Fig. 5. The panel indicators have been added for all the figures throughout the manuscript.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/bg-2017-3/bg-2017-3-AC2-supplement.pdf>

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2017-3, 2017.

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