

## ***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:

In this M.S, the authors took advantage of in-situ two-year rainfall manipulation experiment combining with monitoring of soil chemical, biological properties and nitrogen mineralization rates and N<sub>2</sub>O fluxes to study the features and determinants of nitrogen transformation. They found that more than 20% of the soil net nitrification and N mineralization rates variation could be explained by the effects of microbial abundance, SWC, soil C and N substrates. AOA abundance was the main factor in regulating these

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two N transformation processes, while as much as 42% of the total variation in N<sub>2</sub>O emission was attributed to the total effects of SWC, nitrification rate, MBC and nosZ gene abundance. The results suggested that predicted seasonal precipitation changes in subtropical forests might result in less N<sub>2</sub>O emission while more NO<sub>3</sub><sup>-</sup> leaching. The study is of significant for efforts to understand the features and determinants of nitrogen transformation responses to the predicted precipitation change in subtropical area. Response: Thanks to the reviewer for the concise summary of our work. 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 responses to reviewers comments. All the changes have been marked in the revised version..

### Specific Comments:

Comment #1: Line 44-45: it is not a proper conclusion Response: We agree with the reviewer that the conclusion is too speculative and therefore has been deleted (see lines 47-48). The ending sentence of the abstract has also been revised accordingly.

Comment #2: Line 96: no verb of the sentence Response: The grammatical error has been fixed (see line 106).

Comment #3: Line123: reference? Need a brief introduction of SEM model. Response: Following the reviewer's suggestion, we revised the sentence and added two references. A brief introduction of the SEM model has been added in the statistical part (see lines 142, 332-337).

Comment #4: Line 127-129: add the reason for the third hypothesis Response: We revised the third hypothesis as “The responses of N transformation rates to precipitation change will be associated with the responses of functional microbial abundance” (see lines 146-147). The reason for proposing it has also been added (lines 148-149). According to Levy-Booth et al. (2014), soil N transformation processes are biologically catalyzed by specific enzymes coded by functional genes within functional microorganisms. For instance, ammonium oxidation is catalyzed by ammonium oxidase which

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coded by the *amoA* gene in ammonia-oxidizing archaea (AOA) and bacteria (AOB) (van der Heijden et al., 2008). It has been well documented that N transformation rates are significantly related to functional microbial abundance represented by functional gene abundance in various environments (Petersen et al., 2012; Wertz et al., 2012). Levy-Booth, D.J., Prescott, C.E., and Grayston, S.J.: Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems, *Soil Biol. Biochem.*, 75, 11-25, doi:10.1016/j.soilbio.2014.03.021, 2014. Petersen, D.G., Blazewicz, S.J., Firestone, M., Herman, D.J., Turetsky, M., Waldrop, M.: Abundance of microbial genes associated with nitrogen cycling as indices of biogeochemical process rates across a vegetation gradient in Alaska, *Environmental Microbiology*, 14, 993e1008, 2012. 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. Wertz, S., Leigh, A.K.K., Grayston, S.J.: Effects of long-term fertilization of forest soils on potential nitrification and on the abundance and community structure of ammonia oxidizers and nitrite oxidizers. *FEMS Microbiology Ecology* 79, 142-154. DOI: 10.1111/j.1574-6941.2011.01204.x, 2012.

Comment #5: Line 137-139: add the data or reference Response: A related reference has been added (line 159).

Comment #6: Line 174-175: why not add rainwater? Response: Yes, it is better to add rainwater. But in our precipitation manipulation experiment, it is practically and logistically difficult to do so mainly for two reasons. First, it requires a big tank to store the rainwater excluded during the dry season in order to add them back into the plots during the following wet season; even if we have such a facility the water quality might change tremendously after 6 months of storage. Second, if we collect rainwater during the wet season and add them into plots instantaneously, it would require a forest area several times larger than the experimental plot (~150 m<sup>2</sup>) and the corresponding exclusion facilities to collect the rainwater, which are costly and labor-intensive. We

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therefore used the water from a nearby pond. The chemistry of the throughfall and pond water were sampled and analyzed several times during the first year of the experiment. Results showed that total organic carbon and inorganic N contents were higher in the rainwater than in the pond water, which assures that we did not add nutrients while adding water. We added more descriptions to address this concern during this revision (see lines 205-208).

Comment #7: Line 183: in brackets, is that the instrument model of meteorological station? Response: Yes, that is the model of the rain gauge, an instrument used to measure rainfall.

Comment #8: Line 255-292: too many sentences for the introduction of Nitrous oxide (N<sub>2</sub>O) fluxes measurement Response: We eliminated several sentences that were very detailed and less important (see lines 287-293, 296-297, 300-303, 305-306, and 309-311). The remaining key descriptions are sufficient to understand the measurement method we used.

Comment #9: Line 297-298: why not use paired sample T test? Response: Thanks for the reviewer's suggestion. The paired sample T test is also called dependent T test, and the objective of this statistical method is to compare values from the same individuals at different conditions or different times. The independent sample T test aims at comparing the values from two different sets of individuals at different experimental conditions (Field, 2009). In our study, we focused on the comparison of one variable values from two different plots (i.e., precipitation change plot and control plot) at the same time. Although the soil properties and vegetation features have no significant differences between the two plots before our experiment, they are still separated from each other, and the soil environmental heterogeneity still exist between the two plots. Therefore, the compared plots cannot be assumed as identical as one plot, and the independent sample T test should be more appropriate in our situation to compare the values between treatment plot and control plot. Field, A.: *Discovering Statistics Using SPSS*, SAGE Publications Ltd (the third edition), London, 2009.

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Comment #10: Line 395-402: Need further explanation why the precipitation addition treatment decreased soil water contents. How this SWC change will affect functional gene results. Response: The most possible reason is that the trees in the precipitation treatment plots (height:  $10.2 \pm 5.0$  m, DBH:  $10.7 \pm 6.3$  cm) are bigger than those in the control plot (height:  $7.7 \pm 3.5$  m, DBH:  $9.5 \pm 5.2$  cm), which might have caused greater soil water loss via tree transpiration in the precipitation manipulation plots during the wet season. Another reason might be the large size of precipitation events added (55 mm/day).. Large-sized precipitation is similar to flood-irrigation that can break the soil pore and lead to soil structural decline (Barber et al., 2001; Murray and Grant, 2007) and consequently affect the water retention capacity (Loll and Moldrup, 2000). More discussions have been added for the explanation of this result (lines 445-458). From our results, we did not find any direct effects of SWC or precipitation events on functional gene abundance. Instead, changes in SWC and precipitation events showed direct effects on  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations, which might indirectly influence functional genes abundance. For instance, large precipitation events could cause large soil  $\text{NO}_3^-$  loss by leaching, and lower SWC could result in decreased soil  $\text{NH}_4^+$  by higher ammonium volatilization (Mcgarry et al., 1987). Consequently, decreased  $\text{NO}_3^-$  and  $\text{NH}_4^+$  availabilities could restrict the growth of functional microorganisms and therefore result in decreases of functional microbial abundance (see Fig. 2 and 4). Barber, S.A., Katupitiya, A., and Hickey, M.: Effects of long-term subsurface drip irrigation on soil structure, Proceedings of the 10th Australian Agronomy Conference, Hobart 2001. Loll, P., and Moldrup, P.: Soil characterization and polluted soil assessment, Aalborg University, 2000. Mcgarry, S.J., O'Toole, P., and Morgan, M.A.: Effects of soil temperature and moisture content on ammonia volatilization from urea-treated pasture and tillage soils. *Ir. J. agric. Res.*, 26(2/3):173-182, 1987. Murray, R.S., and Grant, C.D.: The impact of irrigation on soil structure, The national program for sustainable irrigation (Land & Water Australia), Braddon, 2007.

Comment #11: Fig. 1: precipitation change Fig. 2 mark the meaning of the blue bar; why the SWC is significantly lower under the precipitation addition treatment than the

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control in wet season? Response: We have changed "precip-change" to "precipitation change" in Fig. 1 as suggested by the reviewer. In Fig. 2, the blue bar represents "precipitation not excluded". We have added the symbol description in the panel. The lower SWC in the precipitation addition plots was most likely due to the greater soil water loss caused by greater transpiration from relatively larger trees in the precipitation addition plots. Another possible reason was that we might have missed capturing the SWC increase after water addition since our soil sampling was at least 1 week later from the water addition date. More explanations on this concern are also provided in the Response to Reviewers Comment #10 and in the revised discussions (lines 445-458).

Comment #12: Fig. 4 why not use log transformed number? Response: Actually, we also drew the figure with log transformed data of functional microorganisms during the data processing. However, the changes in gene abundance with sampling time and the differences of gene abundance between the precipitation change treatment and control were not so obvious in visual sense as the figure displayed with original data. In order to display our data more straightforward, the figure drawn by original data was used in the current version.

Comment #13: Fig. 5 add symbols a,b,c in the figure and M.S. Response: Thanks for the recommendation. Symbols have been added to all the figures to indicate different panels. Specific figure panel symbols (e.g. Fig. 2a) have been cited in the main text as well.

Comment #14: Fig. S2 why analyze the relationships between nirK+nirS and archaeal amoA; between nosZ and nirK+nirS? Response: The objective of the correlation analyses in Fig. S2 was to detect the significant relationships between each two variables before the structural equation modeling (SEM) analyses. Based on these primary correlation results, the structural equation models could be constructed, thereafter, the casual relationships among these variables were further detected and testified. We analyzed the relationships between nirK+nirS and archaeal amoA gene

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abundance; between nosZ and nirK+nirS gene abundance in Fig. S2, and found significant positive correlation between nosZ and nirK+nirS gene abundance. Thus, the relationship between nosZ and nirK+nirS was added in the SEM analyses (see Fig. 5c), and the casual relationship between the two variables was further examined. A brief description of Fig. S2 have been added in the statistical analysis part to clarify this confusion (lines 347-349).

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/bg-2017-3/bg-2017-3-AC1-supplement.pdf>

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