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Interactive comment on "Fungi regulate response of N_2O production to warming and grazing in a Tibetan grassland" *by* Lei Zhong et al.

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Thank you for you suggestions. We have revised our manuscript "Fungi regulate response of N2O production to warming and grazing in a Tibetan grassland", based on your comments. We have carefully addressed each comment and our responses to these comments are listed the below. The attachments are the manuscript which had improved as your suggestions. We hope that all necessary revisions have been made. However, we would be prepared to make further revisions and modifications if required.

Responses to the Reviewer's comments:

[Comment]- This is a concise and nicely written paper, focusing on fungal and bacterial contributions to potential N2O emissions in an alpine grassland in response to warming and grazing treatments in the field. The authors report several interesting observations, including an increased bacterial enzyme activity and a decreased fungal enzyme activity for N2O emissions under warming. The results have immediate implications for GHG emissions under the scenario of climate change. I have several suggestions for the authors to consider in order to improve the manuscript.

1. Although the authors showed that fungal and bacterial pathways for N2O emissions changed in different directions under warming, the underlying mechanisms or causes remain unknown. In Line 321-322, it is mentioned that increased NO3–N may inhibit fungal growth. Can you elaborate more? Also, did warming affect soil moisture contents or dynamics compared to the control? If so, how would moisture change affect fungal versus bacterial communities? In the end, I am interested in the driving force leading to the observed changes, it is direct warming effect or indirect effect mediated by other factors? Unless we know answers to these questions, we can hardly speculate on the future changes.

[Responses]- We thank the reviewer for the kind suggestion.

For "fungal and bacterial pathways for N2O emissions changed in different directions under warming, the underlying mechanisms or causes remain unknown." It is the two reasons that lead to the changes of fungal and bacterial pathways for N2O emissions by warming. Firstly, the increased of soil temperature directly reduce fungal activity but increase bacterial activity, because fungi prefer the cold environment compared with bacteria. Secondly, warming indirectly reduce fungal activity but increase bacterial activity through increased soil inorganic N and decreased soil organic N in our site, please see Lines 350-355, because fungi prefer higher organic C/N environment while bacteria prefer higher inorganic C/N environment. All these changes caused the fungal and bacterial pathways for N2O emissions changed in different directions under warming. We have improved the manuscript and make sure the underlying mechanisms is clearly, please see Lines 343-357.

For “In Line 321-322, it is mentioned that increased NO3–N may inhibit fungal growth. Can you elaborate more?”. We showed more data to support our findings, at our site, not only the soil inorganic N was increased, as reflected by soil NO3—N concentration (Fig. 2a and 2b); but also the soil dissolved organic nitrogen was significantly decreased from 48 to 41 mg kg-1 ($P<0.04$). Moreover the soil labile C and N was also found significantly decreased by warming(Rui et al., 2012). Warming indirectly reduce fungal activity but increase bacterial activity through increased soil inorganic N and decreased soil organic N in our site, please see Lines 349-355.

For “did warming affect soil moisture contents or dynamics compared to the control? If so, how would moisture change affect fungal versus bacterial communities?”. Yes, warming significantly decreased soil moisture at our site (Fig. 1), but we do not think warming affected fungal versus bacterial communities through the soil moisture. Although the fungi prefer the relative dry soil condition, the NEA and DEA from fungi were not increased, while the NEA and DEA from bacteria were not increased in the warming treatment. This might be duo to the fact that warming induced changes in in soil moisture is not great enough to affect the fungal and bacterial community.

For “I am interested in the driving force leading to the observed changes, it is direct warming effect or indirect effect mediated by other factors?” We believe that warming directly affected the fungal versus bacterial communities due to the increase of the temperature. Additionally, warming also indirectly mediated the fungal versus bacterial communities through the changes in the substrate. We had dicussed it in the first section, improved the manuscript and make sure the underlying mechanisms is clearly, please see Lines 343-357.

[Comment]- 2. Speaking of future predictions, I think it should be emphasized that measurements made here were potential rather than “real” emissions in the field. A critical requirement for denitrification to occur is anoxic or sub-oxic conditions. Therefore, I would think that N2O emissions more depend on the hydrological or redox conditions of the soil. Observations of fungal and bacterial enzyme activity changes in the lab may or may not apply to the field observations, depending on how warming affects soil moisture.

[Responses]-For "Speaking of future predictions, I think it should be emphasized that measurements made here were potential rather than "real" emissions in the field." We fully agree with the referee that the fungal and bacterial enzyme activities cannot be shown as the result of N2O emissions. The measurements under laboratory incubation reflected the potential ability of the soil fungal and bacterial activities in nitrification and denitrification because such laboratory incubation could avoid the impacts of various confounding factors and well clarify the mechanism responsible for N2O emission. At revised version, we clarified that our measurements in the laboratory indicated the potential emission.

For "A critical requirement for denitrification to occur is anoxic or sub-oxic conditions. Therefore, I would think that N2O emissions more depend on the hydrological or redox conditions of the soil." Yes, we also fully agree with the referee that anoxic or sub-oxic conditions and soil moisture is very important for N2O emissions. For hydrological or redox conditions, because we did not measure it, so it is hard to dicussed it directly, but it was mainly influenced by soil moisture, The soil moisture was showed in Fig. 1c.

For "Observations of fungal and bacterial enzyme activity changes in the lab may or may not apply to the field observations, depending on how warming affects soil moisture." The observations of fungal and bacterial enzyme activities were also not appled as the field emissions, they were used to clarify the mechanism responsible for N2O emission. In our stie, the filed N2O emission in 2011-2012 was shown in the manuscript. And the laboratory measurements of the total nitrication and denitrification enzyme activities all were the same with the filed N2O emission at our site (Zhu et al. 2015; Fig. 4c and 4f), which showed it could well clarify the mechanism responsible for N2O emission.

For "depending on how warming affects soil moisture". Although warming significantly

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decreased the soil moisture at our site, the field N2O emission, total nitrification and denitrification enzyme activity did not change as a result of warming (Zhu et al. 2015; Fig. 4c and 4f). It might be due to the fact that the changes in soil moisture by warming was not great enough to lead to a detectable difference in field N2O emission, total nitrification and denitrification enzyme activity.

[Comment]-Some minor points: Line 163: I notice that there was no field replicate for the measurement?

[Responses]- In this study, we used in field replicates. There were four replicates for each of four treatments. Therefore, twe had 16 plots in total. We collected soil samples from each plot. We made detailed description on how to collect soil in the revised version, please see lines 139-143.

[Comment]-Line 223: N2 not N.

[Responses]- Corrected

[Comment]-Line 227: Why only three time points for the denitrification measurement versus 5 points for nitrification?

[Responses]-For DEA incubation experiment, we collected at least 12 ml gas for N2O concentration measuring. If too many times were used to collect N2O, it would change the incubation pressure and influence the responsibility of the experiment. So, we only collected 3 times in the incubation experiment. But for NEA incubation experiment, it does not matter. Additionally, different sampling times for NEA and DEA should have little effect on the reliability of our results because this study did not aim to distinguish the contribution of total nitrification and denitrification to N2O emissions. Here we just estimated nitrification enzyme activity by analyzing the change of NO2-+NO3- concentration after incubation, see lines 212-214 and denitrification enzyme activity by analyzing the change of N2O concentration after incubation, see lines 236-240. Overall. we only compared NEA and DEA among all treatments, respectively.

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[Comment]-Lines 285 and 292: NEA, DEA, FDEA, BDEA: : :not used in the previous text.

[Responses]-For NEA, DEA, we changed it to TNEA and TDEA. They were used in the previous version, please see lines 279-288.

[Comment]-Line 304: I don't think IC is much higher in Haibei soils than some temperate grassland soils in Mongolia. IC contents are dependent on soil pHs. . . [Responses]-Thank you for your suggestion. We corrected it in the new version.

References

Rui, Y., Wang, Y., Chen, C., Zhou, X., Wang, S., Xu, Z., Duan, J., Kang, X., Lu, S., and Luo, C.: Warming and grazing increase mineralization of organic P in an alpine meadow ecosystem of Qinghai-Tibet Plateau, China, Plant Soil, 357, 73-87, 2012. Zhu, X., Luo, C., Wang, S., Zhang, Z., Cui, S., Bao, X., Jiang, L., Li, Y., Li, X., and Wang, Q.: Effects of warming, grazing/cutting and nitrogen fertilization on greenhouse gas fluxes during growing seasons in an alpine meadow on the Tibetan Plateau, Agr. Forest Meteorol., 214, 506-514, 2015.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2017-552/bg-2017-552-AC1-supplement.pdf

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