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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:

[Comments] This manuscript presents an interesting study on the response of an alpine grassland ecosystem to warming and grazing in the period of 10 years. N2O production via variable microbial components was the main focus. It is written concisely and easy to understand. However, regarding the experiment design and interpretation of the data set, I believe that there is still more to improve before it could be published. Despite their investigation into multiple treatments and parameters, the authors need to provide more field evidence and literature comparison to reach a convincing conclusion. Throughout the whole manuscript, the authors seem to mix up denitrification enzymatic activity and N2O production. If the inhibitors applied in the experiments to determine denitrification rates also inhibit N2O reduction to N2, the N2O production should rather represent potential denitrification rates. If N2O reduction was not inhibited during the experiment, the results could not be noted as "denitrification rates". Please clarify this key point and make revision accordingly. The methods determining these rates should be described in more details in M&M.

[Responses] Based on the referee #1 's suggestions, we provided more field data and literature to support our conclusion. The filed N2O emission in 2011-2012 at our site (Zhu et al. 2015) was referenced in our manuscript, please see lines 338-339 and lines 378-379. We also added the mean temperature and rainfall data during the sampling year and months; the soil dissolved organic nitrogen data in our manuscript, please see lines 130-132 and lines 346-349. Because these data were obtained by other colleagues, we cannot present them as figures in the current study. The filed N2O emission supported our conclusion of warming had no effect on total nitrification and potential of N2O production from denitrification. The soil dissolved organic nitrogen data supported our conclusion of warming reduced the potential of N2O from fungi because of the reduction of organic substrates. We also showed more references to supports our conclusions, e.g. Zhu et al. (2015) to support our conclusion of warming had no effect on total nitrification and potential of N2O production from denitrification; the results of Zhu et al. (2015), Krümmelbein et al. (2009) and Steffens et al. (2008) supported our conclusion of winter grazing had little effect on environment because the soil is frozen in winter and often covered with snow and grazing has little effect on soil conditions, please see lines 338-339 and 374. To determine potential denitrifica-

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tion rates, we incubated soil samples under anaerobic condition and did not add any inhibitor to inhibit N2O reduction to N2 process. Therefore, our results only can be presented as the potential of N2O emission from denitrification. We have clarified this in M&M, please see lines 196-251.

[Comments] Line 111: “To clarify whether fungi control the N2O production process” is misleading as Fungi contributes anyway; I assume that the authors wish to clarify the “role of fungi in N2O production process”

[Responses] Done as your suggestion. please see lines 112-113.

[Comments] Line 161-162: Please explain this; why do you see the effects on ecosystem level despite that plot size are 3 m? Any data to support this?

[Responses] This is really good question. The plot size used for warming treatments are generally small, less than 1 m2 (Cantarel et al. 2012) to more than 10 m2 (Long et al. 2015). These studies well showed the effects of treatments on ecosystem (Cantarel et al. 2012; Long et al. 2015). In this study, the size of our plots was considered according to three points: 1) A little big size was used because grazing was involved. Although the size of plot might affect the animal feeding activities, all experimental sheep were fenced into three additional 5*5 m fenced plots for one day before the beginning of the grazing experiment to help them adapt to small plots for reducing the experimental error. 2) The warming efficiency and cost (we used the infrared heaters in warming treatments for increasing soil temperature) was another factor; and 3) the species composition and vegetation coverage is even in this grassland. Previous publications (Wang et al. 2012 Ecology, Luo et al. 2010 Global Change Biology, Luo et al. 2009 Soil Biology and Biochemistry, Rui et al. 2012 Journal of Soils and Sediments) from this study have demonstrated that the plot size can show the effects on ecosystem level.

[Comments] Line 165: If 10 years’ warming and grazing treatment was done, why was only one sampling of soils by the end of 10 years’ treatment? Have you considered

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the soil heterogeneity between control and treatment plots since the beginning of treatments?

[Responses] Only one sampling of soils was done by the end of 10 years treatment. The reason is that this is the first time for us to pay attention to the contribution of fungi and bacteria to N2O production based on recent research advances and fresh soil is required for microbial analysis especially for the incubation experiment. A thorough understanding about the long-term impact of warming and grazing on soil fungal nitrification and denitrification from alpine meadow grassland requires further investigation through multi-sampling during a long period. We mentioned this limitation in Discussion, please see lines 383-386. Additionally, we considered the soil heterogeneity between control and treatment plots since the beginning of treatments. There is no difference between treatments the beginning of this experiment. To reduce the soil heterogeneity, all the plots were asigned in a complete randomized block. For "soil heterogeneity between control and treatment plots since the beginning of treatments?". We think the spatial heterogeneity was exit in everywhere.

[Comments] Line 166: Including or excluding organic layer? Please specify.

[Responses] Done as your suggestion. please see lines 167.

[Comments] Line 225-226: 100% of water-holding capacity could favor denitrification; however, it may not likely represent field condition, which is usually drier. Please justify your choice of such incubation condition.

[Responses] The incubation experiment was used to show the potential of N2O produce from denitrification of soil, it cannot be represented as the N2O production of field. The 100% of water-holding capacity was provided an relative good environment for denitrification so that can inspire the activities of denitrifying microorganism and show the ability N2O produce by denitrifying microorganism in soils. The method and the incubation condition was commonly used to measure the denitrification enzyme activity and proved to be useful (Smith and Tiedje, 1979; Simek and Hopkins, 1999;

Chroňáková et al. 2009; Cantarel et al. 2012).

[Comments] Line 294-298: Use present tense: use “is” to replace “was”.

[Responses] Done as your suggestion. Please see lines 299-230

[Comments] Line 298: Change “who” to “whom”.

[Responses] Done as your suggestion. Please see lines 303

[Comments] Line 314 to 315: When comparing the studied alpine grassland to temperate grassland, how do come to the conclusion that the lower inorganic C and N contents in soil were due to larger fungal contribution to N2O production? What about the higher mineralization rates in the temperate systems? In addition, the control of inorganic C or N levels in soil could be also related to biomass uptake and turnover. Please clarify it and avoid such speculation.

[Responses] We fully agree with the referee that the lower inorganic C and N contents in soil based on observations from alpine grassland to temperate grassland cannot come to the conclusion. In the new version, we removed the sentence and improved this part to avoid such speculation. Please see lines 319-320

[Comments] Line 324: “common” and “globally” do not fit together; please revise.

[Responses] Done as your suggestion. Please see lines 325

[Comments] Line 348-349: “gene abundance of fungi was not changed” against treatments; how do you reconcile your finding with the hypothesis?

[Responses] The gene abundance of fungi was not changed by warming, but warming changed FNEA and FDEA. Such inconsistency between gene abundance of fungi and FNEA/FDEA might be explained by the fungal gene abundance not providing information on real-time process rates. The reason is that process rates are largely dependent on environmental conditions. Fluctuations in environmental conditions can cause rapid changes in real-time process rates, but do not necessarily affect gene abundance

(Zhong et al. 2014). We have improved it in the new version, please see lines 353-358

Reference

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Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2017-552/bg-2017-552-AC3-supplement.pdf

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