

## ***Interactive comment on “Seasonal and interannual dynamics of soil microbial biomass and available nitrogen in an alpine meadow in the eastern part of Qinghai-Tibet Plateau, China” by Bo Xu et al.***

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General comments: This paper describes intra-annual and inter-annual patterns in soil nutrient availability (inorganic and organic N) as well as microbial biomass and community structure in alpine tundra. The investigators sampled soils monthly over a 3 year period, including both the frozen and unfrozen periods. This is an impressive data set and I'm not aware of another published data set that is nearly as comprehensive. For this reason alone I encourage the authors to continue to work towards the publication of this data set. There are some aspects of both the methods and the interpretation of the results which I question and these aspects in particular require more attention by the authors before publication of this paper.

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See more specific comments below. Response: We thank referee for the helpful comments. After discussing with co-authors, we thoroughly revised the manuscript and listed in supplement. Specific comments: Referencing: Some of the references are inappropriate. Specifically, there are many citations which are used to support statements about alpine systems which were not conducted in alpine ecosystems (E.g. Page 2 line 17 and Page 4 line 8 Edwards and Jefferies, Page 3 line 6 Buckeridge and Grogan, Page 15 line 4 Henry and Jefferies). Some references are missing (Page 14 line 3: reference for Alaskan tundra is missing) and others did not examine the phenomena they are used to support (e.g. Edwards and Jefferies did not examine the survival of microorganisms surviving in thin water films (Page 3 line 1). Response: Yes, we carefully revised these inappropriate references one by one in the new manuscript (Page 2 lines 12, 15, 18; Page 3 lines 3, 4, 7, 12; Page 4 lines 3, 10-13). The methods are lacking some necessary details. The description of the 3 sites were vague: The sites are described as being at the “top middle and bottom of the meadow”. Were there elevational differences between the sites? How far is the distance between them? Response: Yes, the details of the 3 sites were added, i.e., “Considering the soil spatial heterogeneity, three adjacent sites approximately 100 m apart (centered at 32°59′ N, 103°40′ E, 3980 m a.s.l.) were sampled, namely located at the upper, middle, and lower part of the alpine meadow. Five replicates at each site were collected, and the replicates from each site were 10 m apart from each other. Fifteen samples collected from the three sites at each sampling time were then performed together for statistical analyses (n=15).” (Page 6 lines 5-9). Further, were the soils collected in the winter kept frozen into analysis? Response: Yes, the soil samples collected in the winter were stored at 0 °C before analysis, and all the samples were processed at the laboratory of Chengdu Institute of Biology, CAS, within two days of sampling (Page 7 lines 1-2). Finally, was TDN measured only after chloroform fumigation? This is how it is described, but then it would be impossible to measure MBC and MBN. Response: No, different subsamples were used for the determinations of TDN, MBC and MBN. We rewrote this section, and the “3.4 Soil water content,

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microbial and nutrient analyses” section was divided into two sections, i.e., “3.4 Soil water content and nutrient analyses” and “3.5 Soil microbial biomass and community analyses” (Page 7 line 11 to page 9 line 5). It would also be good to report days below -5C rather than just below 0C: -5C is often reported as when microbial activity significantly slows. Response: Yes, we added the results of the number of days below -5 °C in the revised manuscript (Page 11 lines 6-7). I also question the methods used to determine changes in microbial community structure. The authors used total colony forming units of bacteria, fungi and actinomycetes using a plate dilution method. However, this only allows culturable bacteria to be counted. Further, they were all incubated at 25C regardless of season, when the winter samples likely should have been incubated at colder temperatures. Also, how were these #s compared over time? The results state which dates are significantly different from each other – were they pairwise comparisons? If the authors plan to use these methods to describe microbial community structure I would like to see citations indicating they are appropriate, as well as further description of the limitations of these methods. Response: Actually, the dilution-plate method can be used to counting the CFU of bacteria, fungi, and actinomycetes by different selective mediums, i.e., beef extract peptone agar, Sabouraud dextrose agar, and Gause synthetic agar medium for the cultivation of bacteria, fungi, and actinomycetes, respectively (Li, 1996; Igbinsosa, 2015) (Page 9 line 3). We thought if the cultivation temperature was too low, the visible microbial colony might hard to forming. So we referred to the methods of Li (1996), and measured the CFUs of bacteria, fungi, and actinomycetes. For analyses of the microbial community shifts during the transition between non-growing and growing seasons, the numbers of bacteria, fungi, and actinomycetes between the late non-growing season (i.e., in March) and early growing season (i.e., in May) for two years (2012 and 2013) were measured. These differences in the number of bacteria, fungi, and actinomycetes between season and year were then determined via two-way ANOVA, with season and year specified as fixed effects (Page 10 line 14). Statistics: Because the same sites/plots were sampled repeatedly, a repeated measures ANOVA would be more

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appropriate than the 2-way ANOVA. Further, the description of the Pearson correlation analysis is not clear. I would like to see more of the results for this correlation described than just the  $r^2$  (Table 2). Response: We thought the analyses of the independent variables (season and year) on the dependent variables should utilize a mixed-effects model with sample ID as a random effect to account for the lack of independence of samples across time. So, the mixed-effects model was performed for the analyses of the independent variables (season and year) on the dependent variables, and new statistical results were listed in Table 1 (Page 10 lines 10-11; Page 29). Further, we revised the description of the Pearson correlation analysis as “Pearson correlation analysis was then performed to analyze the correlation of the MBC with SWC and of that with the DOC during the non-growing and growing seasons” (Page 10 line 15). In Table 2, information on  $r$  and  $p$  values was listed, we thought it was enough to describe the results of the correlation analysis. Also, throughout the results section I would like to see the actual statistics stated rather than just  $p < 0.05$ . Finally, is it possible to define a “peak” time for MBN or DON in the season when MBN did not vary seasonally? (Page 9 line 5). Response: Yes, we added the actual statistics results in the two-way ANOVA analysis throughout the results section (Page 11 line 17; Page 12 lines 9-10; Page 13 lines 4-5; 13-14; Page 14 lines 2-3, 9-10), but the description of “ $p < 0.05$ ” was retained in the sections of the multiple comparison and Pearson correlation analysis. Finally, it is possible to define a “peak” time for MBN or DON according to their monthly values, and the MBN or DON had no significant seasonal differences just compared between growing and non-growing seasons. Interpretation: Some of the interpretation of the results goes beyond what the results actually indicate. For example (Page 12 line 17) High microbial biomass does not mean there is high activity. Response: Yes, the sentence “and these communities retained their high activity in alpine soils during the cold periods” was deleted in the revised manuscript (Page 15 lines 7-8). Also see a reference to activity on page 14 line 16: this study did not contain any tests of microbial activity. Response: Yes, “Lipson et al., 1999; Matthew Robson et al., 2010” were added (Page 17 lines 6-7). Other conclusions require further elaboration. For

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example, the section on page 13 line 16 needs elaboration – Why would the decrease in MBC at thaw be related to the higher productivity and SOM in this site compared with others? Response: Actually, we did not get the conclusion that the decrease in MBC at thaw be related to the higher productivity and SOM in this site compared with others. But, we inferred that available C and N were relatively sufficient and might not restrict the microbial activity during the winter-spring transition, and this phenomenon may be closely related to the high plant community productivity and SOM in our study compared with others. Finally, there isn't direct support for many of the overall conclusions of the paper – this study can describe correlations, but not the types of conclusions described (e.g. soil microorganisms play a crucial role in accumulation of inorganic N pools) Response: Yes, we revised it as “soil microorganism not only has a close correlation with the accumulation of inorganic N pools” (Page 20 line 6) Technical comments: The paper could use a thorough editing for English grammar: E.g. Community compositions should be community composition (Page 1 line 16) E.g. Change “Consistently increasing trends of MBC” to “Trends of consistently increasing MBC” E.g. Substrate transports should be substrate transport (Page 2 line 4)? Response: Yes, we revised them one by one according to your comments (Page 1 lines 16, 18-19; Page 3 line 6), and the revised manuscript will be send to a professional language editing company for the language modification during the final revised period.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/bg-2017-66/bg-2017-66-AC4-supplement.pdf>

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