

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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The ms “Seasonal and interannual dynamics of soil microbial biomass and available nitrogen in an alpine meadow in the eastern part of Qinghai-Tibet Plateau, China” provides a nice dataset for microbial biomass and C and N pools at monthly intervals over 3 growing seasons and two winters in an alpine meadow. The duration of the dataset over such a long period with seasonally frozen alpine soils is quite valuable. Response: We thank referee for the helpful comments. After discussing with co-authors, we thoroughly revised the manuscript and listed in supplement. However, I have two important issues with this ms: 1. The justification for doing this study is not clearly formed because the research questions are not novel or clear. The background

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to these questions mixes Arctic references with alpine and yet is missing important references that have done very similar work in the Arctic (the Edwards 2013 paper on the long-term nutrients, which is cited, and the Buckeridge 2013 paper on the microbes, which is not cited). The authors could fix these problems in one of two ways a) narrow their scope to alpine research and tighten their research questions, or b) include the permafrost Arctic research that they are missing that is similar to theirs and then build research questions that addresses how this research is novel within this broader framework. The best version (in my opinion) would do a bit of both options and introduce the research in both Arctic and alpine, because they are historically mixed, and then focus the paper and RQs to just alpine. The value of this study is the multiseason data in the same system. Response: Yes, we selected the best version (in your opinion) to revise the introduction section that we introduced the research in both Arctic and alpine and then focused the paper and RQs to just alpine (Page 2 lines 12, 15, 18; Page 3 lines 4, 7, 12; Page 4 lines 3, 10-13, 17-18; Page 5 lines 1-4). 2. The methods are unclear (why 3 sites, when are these mentioned again? Is winter vs summer sample processing associated with seasonal shift in results? Description of fumigation is confusing) and the description of the statistics is missing important details (why and how bin into seasons, and why no random factor for time?). These issues can all be fixed (I think) and a bit more effort will make this a nice paper. Response: Yes, the methods were thoroughly revised according to your comments (Page 5 lines 15-16; Page 6 lines 5-9; Page 7 line 11 to page 10 line 18). Specific comments by line number, with a focus on introduction and methods since the rest may change once the introduction and methods are improved. Introduction: P2, l17. Edwards and Jefferies is an arctic reference, not alpine Response: Yes, we changed “alpine ecosystems” into “cold ecosystems” (Page 2 line 18). P3, l1, these papers show activity, but not mechanism, and not from alpine soils (which often do not freeze deeply) - perhaps remove ‘alpine’ and change/add a mechanistic or review ref, such as Panikov 2006 SBB, or Jefferies 2010 SBB Response: Yes, we changed “alpine” into “frozen”, and “Panikov et al., 2006; Jefferies et al., 2010” were added

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into the quotation (Page 3 line 4-5). P3, I11, missing Buckeridge SBB 2013 here and possibly in next line (although this is not an alpine ref, but the study is very similar to this one despite focus on one year only) – then in line 13 the refs are a mix of alpine and Arctic, so it is not clear why a mix of refs would be used in some places and not others, and why this very similar study is not cited. P3, I17, again, mix of alpine and Arctic refs when alpine stated Response: Yes, “Buckeridge et al., 2013” was added, and “alpine” was revised as “Arctic and alpine” (Page 3 lines 13-14). P4, I5, missing Buckeridge et al 2010 Biogeochemistry Response: Yes, “Buckeridge and Grogan, 2010” was added (Page 4 line 9). P4, I7-8, the lack of summer studies is surprising, and incorrect- there are lots of studies in the summer. Perhaps be more specific - the value of this study is a multi year investigation that encompasses both summer and winter, that is rare in alpine (Edwards and Jefferies 2013 already covered this in 2 Arctic systems). Response: Yes, we rewrote this sentence as “However, despite ample evidence of soil microbial activity and nutrient mineralization during the winter and/or summer months in Arctic and alpine regions (Edwards et al., 2006; Schmidt et al., 2007; Miller et al., 2009; Edwards and Jefferies, 2013; Buckeridge et al., 2013), studies on exploring the changes in microbial and N pools during both summer and winter across several years in alpine ecosystems are few” (Page 4 lines 10-13). P4, I11-14, #3 repeats #1, and how are these RQs novel? why do we need to have this information when Brooks (1998), Lipson (2002, 2004), Edwards (2006), Larsen (2007) and Buckeridge (2010, 2013) already showed this? These RQs need to be more specific about how this particular dataset advances the field. They should also be tied to the methods and results and the alpine setting – why compare seasons and years, what questions do the authors want to address by doing this? Response: Yes, we rewrote the RQs as “1) What are soil microbial and available N dynamics during the growing and non-growing seasons in the alpine meadow? 2) What are interannual patterns of soil microbial and available N dynamics in the alpine meadow? 3) What environmental factors affect these dynamics? 4) What are relationships between soil microbial biomass and available N pools in the seasonal frozen ecosystems?” (Page

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4 line 17 to Page 5 line 4). Methods: P5, I12, I do not see these 3 sites again, just the seasonal data – where are the three sites? Were these samples pooled or only one used? Response: The locations 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” (Page 6 lines 5-7). Fifteen samples collected from the three sites at each sampling time were then performed together for statistical analyses (n=15) (Page 6 lines 7-8). P5-6, what is the snow depth and timing at these sites? Response: Sorry, we did not measure the snow depth and timing at the three sites in 2011 to 2013. But we investigated the snow depth of the alpine meadow during the non-growing season in 2012-2013, and the mean snow depth and timing were described in the section of “Site description”, i.e., “persistent snow cover usually occurs from late December to early April, and the mean snow depth is 16.58 cm in the study area (Xu, unpublished data, collected in 2012, 2013)” (Page 5 lines 2-4). P6, I1-3, the different treatment for winter (large roots removed) and summer (sieving 2mm) samples may explain different seasonal microbes and nutrient pool sizes - please indicate when this switch in handling occurred. Response: Yes, we added detailed months behind the cold periods and warm seasons, i.e., “the cold periods (i.e., November to April)” (Page 6 line 15) and “the warm seasons (i.e., May to October)” (Page 6 line 17). P6, I4, are the 3 subsamples analytical replicates? Response: NO, the 3 subsamples were analyzed for soil water content, nutrient, and microbial biomass and community, respectively. P6, I8, how many iButtons for each temperature measurement? Response: The mean daily temperatures were then calculated by the datum of nine iButtons, i.e., “Three iButton data loggers were placed at each site, and mean daily temperatures were then calculated by the datum of the nine loggers” (Page 7 lines 6-8). P6, I10, how was seasonal temperature calculated – by date or temperature? By date: A seasonal divide is needed – were all temp points used or were those near thaw and freeze excluded? How did the authors account for moving freeze and thaw dates across years? Or by temperature

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– what was the threshold, and was it based on soil or air temp? Response: The seasonal temperature was calculated by date, i.e., “the growing season was from 1th May to 31th October, and the non-growing season was from 1th November to 30th April” (Page 7 lines 8-10). All temperature points were used for calculating. P6, l11 to p8, l4, this section is very confusing, for several reasons: the content does not match the order of the title, the TDN paragraph includes the description of fumigation, probably because the authors used the fumigation control for measuring TDN, and so they are confusing their operational process with the description. However, the biomass calculations were introduced first in the section, before the biomass extraction protocol, which is backwards. Response: Yes, we rewrote this section, and the “3.4 Soil water content, 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). P7, l6, Does this CFU counting follow a standard protocol? Why no reference or brief protocol when so much explanation for the dilution and fumigation method? Response: Yes, the CFU counting followed a standard protocol, and references were added (Page 9 lines 2, 3, 6). P8, l6-14, there are a few problems with this section: 1. mentioned above already, how seasonal binning of data was performed, also, it is not clear why the specific months were selected for community analysis; 2. The analyses of the independent variables (season and year) on the dependent variables should utilise a mixed-effects model with sample ID as a random effect to account for the lack of independence of samples across time. Response: Yes, we clarified the criterion of seasonal binning of data, i.e., “the growing season (i.e., datum from May to October were used as a sample set; n=90) and non-growing season (i.e., datum from November to April were used as a sample set; n=90)” (Page 10 lines 8-9). We also clarified the reason why the specific months were selected for community analysis, i.e., “For analyses of the microbial community shifts during the transition between non-growing and growing seasons” (Page 10 lines 11-12). Finally, the mixed-effects model was performed for the analyses of the independent variables (season and year) on the dependent

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variables, and new statistical results were listed in Table 1 (Page 10 lines 10-11; Page 29). Results: P8, l18-P9,l3, this passage describes a good reason why alpine and Arctic studies should be differentiated: these are not permafrost soils and they are not very cold. These mean ‘freezing’ soil temperatures are probably not experienced as freezing to a microbe full of osmolytes or a soil full of salts: : .although perhaps they are during extreme lows – these extreme lows should be described, in timing, depth and frequency. Are how are freeze-thaw cycles defined? What is ‘more cycles’ – number and dates of FT cycles should be stated for each year. Response: Yes, we totally agreed with your comments, and we added the number of extreme freezing days (below -5°C) (Page 11 lines 6-7). Actually, we did not measure the frequencies of freeze-thaw cycle events, and we speculated the freeze-thaw cycle event according to the mean soil temperature (0°C or thereabout). It is unreasonable to define a freeze-thaw cycle event just according to soil temperature. So, this result was deleted in the revised manuscript. P11, l16, please clarify a ‘significantly reducing process’ – soil redox measured with mV, or personal observation based on what criteria? Response: Yes, this sentence was revised as “Furthermore, an obviously decreasing trend of $\text{NO}_3\text{--N}$ contents was observed during the soil thawing period (April to May)” (Page 14 line 5). Discussion: P12, l12, again, more refs here: Brooks 1998, Edwards 2006, Larsen 2007, Buckeridge 2010. Response: Yes, these references were added into the revised manuscript (Page 15 lines 1-2). P12, l16, ‘temperature threshold’ for what specifically? Survival, lysis? And how does the MBC decline imply high activity in cold periods? Are the authors inferring mid-winter predation? Response: The “temperature threshold of these cold-adapted microbial communities” was revised as “temperature threshold of the survival of these cold-adapted microbial communities” (Page 15 line 6). It is unreasonable to inferring that the decline of MBC imply high activity in cold periods. So, 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). P13, l1, ‘even though’ does not make sense here. Response: Yes, the sentence “even though the N uptakes of plants were degraded” was deleted in the

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revised manuscript (Page 15 line 9). P14, l9-10, the second half of this sentence is not useful Response: Yes, the sentence “which might contribute to the seasonal dynamics of the microbial biomass” was deleted in the revised manuscript (Page 16 line 18). P14, l18 & P16, 8, the frequency and number of freeze-thaw cycles was not stated in the results Response: Yes, as we did not measure the frequencies of freeze-thaw cycle events, some similar literatures were cited in the revised manuscript. The sentence was revised as “Notably, a warmer and drier non-growing season was observed in 2011–2012 than that in 2012–2013, which might accompanied with more frequent freeze-thaw cycles during the early period of this season (Mellander et al., 2007; Henry, 2008)” (Page 18 lines 16-18). P16, l1-5, is this discussion based on gravimetric water content? Can the authors comment on why gravimetric content would correlate with non-growing season biomass if this water was frozen and unavailable? Fig.2 and associated data: are these values for gravimetric water content? How meaningful are the conclusions drawn from water pool sizes and correlations if the frozen soil water is not removed from the calculations? Response: Yes, we agree with your comments. Actually, the discussion was based on the gravimetric water content during the growing season. Furthermore, low correlation ($r = 0.35$) between MBC and SWC was observed during the non-growing season. We thought the frozen soil water might be correlated with the MBC during the soil thawing period. Fig.4 the lowercase letters represent the post-hoc test for which effect? The interaction? Fig.8, again not clear which main effect test the post-hoc letters are representing. Response: In Fig.4 and Fig.8, the lowercase letters represented the post-hoc test for the interaction effects between season and year, and we clarified it in the revised manuscript (Page 10 lines 17-18; Page 33 lines 11-12; Page 36 lines 15-16).

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/bg-2017-66/bg-2017-66-AC3-supplement.pdf>

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