

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.

Anonymous Referee #3

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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.

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 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

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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 multi-season data in the same system. 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.

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, I17. Edwards and Jefferies is an arctic reference, not alpine P3, I1, 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 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 P4, I5, missing Buckeridge et al 2010 Biogeochemistry 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).

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P4, l11-14, #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?

Methods: P5, l12, 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? P5-6, what is the snow depth and timing at these sites? P6, l1-3, 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. P6, l4, are the 3 subsamples analytical replicates? P6, l8, how many iButtons for each temperature measurement? P6, l10, 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 – what was the threshold, and was it based on soil or air temp? 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. 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? 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.

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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. P11, l16, please clarify a 'significantly reducing process' – soil redox measured with mV, or personal observation based on what criteria?

Discussion: P12, l12, again, more refs here: Brooks 1998, Edwards 2006, Larsen 2007, Buckeridge 2010 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? P13, l1, 'even though' does not make sense here. P14, l9-10, the second half of this sentence is not useful P14, l18 & P16, 8, the frequency and number of freeze-thaw cycles was not stated in the results 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? 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

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