Dear Prof. Michael Weintraub,

Thank you very much for your helpful comments to our MS, and we have carefully and thoroughly revised the MS according to your comments. Meanwhile, after discussing with co-authors, the MS was thoroughly revised according to the reviews from the four anonymous referees. The detailed responses to the comments are as follows.

Additionally, I found some of the wording to be confusing. For example, "interannual" vs. "year to year" in lines 338 and 339; and what does "incompletely consistent" mean?

Response: Yes, we revised "year to year" as "interannual" **(Page 2 line 6; Page 18 line 18; Page 20 line 2; Page 21 line 4)**; and the "incompletely consistent" was revised as "divergent" **(Page 20 line 4).**

Furthermore, it may be worth considering combining figures 2 and 3 into a single multipanel figure showing microbial biomass C and N temporal dynamics and growing season/non-growing season means.

Response: Yes, figures 3 and 4 (we thought) were combined to a single multi-panel figure in the revised MS **(Page 33 Fig. 4)**.

Also, in Figure 6, it would be nice to have every other season shaded in the top graph so it would be easier to see where each season ended.

Response: Yes, the sections of the growing season were shaded in Fig. 7 (we thought) **(Page 36 Fig. 7)**.

Please be sure that the clarifications provided in the response to reviewers are also incorporated into the manuscript text. Please also clearly describe how the text was revised according to the reviewer comments when submitting your revised manuscript.

Response: Yes, the responses to reviewers were incorporated into the revised manuscript, and the detailed responses are as follows.

Responses to Anonymous Referee #1

Overall, this is a largely descriptive project, but it is well presented and the overwinter data are valuable as those types of measurements are rare. The authors

might work on describing which parts of their study are most novel to help the study be better found and cited within the literature.

Response: We thank referee for the helpful comments. After discussing with co-authors, we thoroughly revised the manuscript.

I have some suggestions below on which topics to emphasize. The data are also remarkably "clean" for soil nutrient data with less heterogeneity of variance between dates than usual and no unusual "hot spots" of activity. The authors might discuss whether quality control measurements may have eliminated such points and if not, why the numbers are so consistent, which is not always the case for these types of studies. n = 15 is a reasonably large sample size so I do recognize that that is part of it.

Response: In our study, three adjacent sites approximately 100 m apart were sampled, and five replicates at each site were collected. So fifteen soil samples were collected at each sampling time, and then the mean values of soil nutrient were calculated (n=15) **(Page 6 lines 8-13)**. We thought the fifteen samples themselves would represent the heterogeneous soil nutrient status in the alpine meadow, and it might be the main reason that why you found the soil nutrient data with less heterogeneity of variance between dates. Actually, we did not take quality control to eliminate any points, and the numbers were so consistent because fifteen samples were collected at each sampling time.

Abstract is solid. No complaints.

Response: Thank you for your comment.

INTRODUCTION I recommend the authors work to define their knowledge gaps better. There are several possible areas to discuss including location of study (including why it may or may not be different from other sites), the rarity of the overwinter measurements (there are probably just a handful of studies with this type of data), and finally, the microbial cultures are not often done in association with these types of seasonal nutrient measurements so that is worth mentioning too and describing which other studies if any have done this. The authors do mention these topics, but don't zero in on specifically what is not currently known and why it is important that we know that. I'm not saying this wasn't done at all–

just that it can be done more and better.

Response: Yes, we revised the introduction according to your comments **(Page 3 lines 10-11; Page 4 lines 15-18)**, and we rewrote the research questions 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 the relationships between soil microbial biomass and available N pools in the seasonal frozen ecosystems?" **(Page 5 lines 2-7)**

L 15. I recommend removing these correction factors as it's widely understood that they are very ecosystem specific and hard to apply to sites in which they are not explicitly calibrated.

Response: Thank you for your comment. But, we did not know the L15 in which page.

Three parts of the meadow were measured. Some discussion is warranted as to the spatial configuration of the sampling and why they were pooled for analysis as a single site (n = 15).

Response: Considering the soil spatial heterogeneity in the alpine meadow, we selected three adjacent sites for soil sampling, and five replicates at each site were collected at each sampling time. Thus, fifteen soil samples were collected at each sampling time, and then statistical analyses of soil microbial and nutrient dynamics in the alpine meadow were performed on these samples at each sampling time (n = 15) **(Page 6 lines 8-13).**

Figure 3, Fig. 7. Fig. 6B. These figures all show results that are already shown in the more detailed time courses. The authors can maybe report some of those values in the text if needed and eliminate these figures. If the authors feel this leaves the paper a little thin on figures, I would recommend exploring the relationships among the measured variables and environmental covariates using an approach such as a scatterplot matrix of correlations on a per-sample basis (ie one data point per sample, not averaged by date). Along these lines, providing the raw data as a supplement or as a link to an online repository would add value to the study.

Response: We thank referee for the kindly and helpful suggestions. But we thought Fig.

3, Fig. 7, and Fig. 6B were indispensable for our study because they intuitively and detailedly showed the intra- and interannual patterns of microbial and nutrient dynamics in the alpine meadow.

I'm curious as to why the soil N numbers are so low-variance (particularly inorganic N). Were outliers eliminated before analysis? These types of measurements typically show substantial right skew and hot spots. Also TDN and MBN are often an order of magnitude higher than the inorganic constituents, but that is not the case here. These points warrant discussion.

Response: We did not eliminated any points before analysis. The standard error (s.e.) was used for figure drawing might be the reason why you found the soil N data with low-variance. In other ecosystems, the TDN and MBN are often an order of magnitude higher than the inorganic constituents, may because relatively high microbial activity will lead to high MBN and TDN accumulations in the soils. But in the alpine meadow ecosystems, low temperatures and N limitations may largely restrict microbial activity, causing relatively low MBN and TDN accumulations in the soils. Furthermore, alpine plants may largely uptake DON during the late growing season as the inorganic N is exhausted. We think these reasons may lead to the TDN and MBN are not an order of magnitude higher than the inorganic constituents in the alpine meadow.

The results section is serviceable but kind of boring with its descriptions of seasonal trends and what is "significant" or not sprinkled with uninsightful p-values. I'd like to see more of a narrative structure tied to some hypotheses (eg hypothesis that there will be a crash in N availability at beginning of season as seen in other studies, a hypothesis that would be supported).

Response: We thought you provided another paper writing habit that contain results and discussion together. But we preferred to separate the results from the discussion.

This study would benefit from a photograph of the sampled sites.

Response: Yes, a map of the study site was added into the revised manuscript **(Page 6 line 7 and Page 31 Fig. 1).**

The paper is completely readable and generally well written. Still, it could use a onceover by a native speaker to fix the most challenging issues for non-native

speakers such as proper preposition choice, a few cases of singluar/plural mismatch, etc.

Response: Yes, we have sent the revised manuscript to a professional language editing company for the language modification.

Conclusion: keep it focused on the seasonal questions and trends. Climate change is not really addressed in any way in this study and so it's not worth mentioning here. The study's value is in its contribution to basic understanding of soil nutrient cycling seasonality.

Response: In our study, we found that the interannual variations of soil temperature and water condition were the primary environmental factors driving the interannual dynamics of soil microbial biomass and available N pools. Furthermore, the alpine ecosystems are sensitive to the future climate change. So we thought it was necessary to mention the climate change in the conclusion.

Responses to Anonymous Referee #2

The paper deals with the seasonal and interannual dynamics of soil microbial biomass and available nitrogen in an alpine meadow in China. The subject is interesting but the poor english sometime let the comprehension of the text very difficult. I suggest some changes but I strongly recommend to check the english language through the assistance of a mother tongue.

Response: We thank referee for the helpful comments. After discussing with co-authors, we thoroughly revised the manuscript. Yes, the revised manuscript has been sent to a professional language editing company for the language modification during the final revised period.

Moreover the paper lacks of some information such as the measurement of the snowpack depth, the estimation of the depth of the active layer and the criteria that have been used to determine the growing season lenght.

Response: We are sorry for the lacks of detail information on the snowpack during the study years, and we only had some information on snowpack depth during the nongrowing season in 2012-2013 **(Page 5 lines 15-17)**. The definition of growing

season were added to the revised manuscript, i.e., "the growing season (i.e., during early May to late October according to the plant phenology observation in the alpine meadow from 2011 to 2013)" **(Page 6 lines 1)** and "The mean temperature of the growing season was calculated by the mean daily temperatures from 1 May to 31 October, and that of the nongrowing season was calculated by the mean daily temperatures from 1 November to 30 April." **(Page 7 lines 11-14)**.

Some specific points are listed below: Pag 1: lines 14/15: Did you collect topsoil samples? Please specify better line 16: add in (MBN) after and N.

Response: Yes, "Soil" was been changed to "Topsoil", and "(MBN)" was been added in the revised manuscript **(Page 1 lines 15-16)**.

Pag 2: line 12: With the term frozen soils do you mean permafrost soils?

Response: No, the "frozen soils" here refers to the seasonally frozen soils.

Pag 3: line 6: When you mention alpine ecosystems do you mean seasonally snow cover ecosystems?

Response: Yes, "alpine ecosystems" in our study refers to the seasonally snow covered ecosystems.

Pag 4: Lines 6: again, do you refer here to subnival microbial activity during winter? Line 9: correct seasnonal into seasonal.

Response: Yes, "microbial activity" here refers to the subnival microbial activity during winter, and the "seasnonal" was corrected to "seasonal" in the revised manuscript.

Pag 5: Line 4: When you mention frost-free periods, do you refer to air temperature? What is the mean snow depth in the area?

Response: Yes, "frost-free periods" in our study refer to air temperatures. Some information on snow cover in the study area was added, 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 15-17)**.

Lines 9-10: Please add also the soil classification according to the Soil Taxonomy

Lines 12-13: Do you work in a catena of soils? What do you mean with the terms top, middle and bottom? Line 14: Does this soil horizon is a A horizon?

Response: The soil classification of the area was added, i.e. “mountain dark brown soil” **(Page 6 lines 5)**. Yes, serial soil samples were collected, and each sampling site was adjacent to each other at each sampling time. The terms of “top, middle and bottom” mean the locations of sampling sites, and we have revised this sentence as “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 selected. One site is located at the upper part of the alpine meadow, one at the middle part, and one at the lower part.” **(Page 6 lines 8-10)**. Yes, the 0-20 cm horizon in our study is the A horizon.

Pag 6: Line 1: In winter did you collect the soil samples under the snowpack? Lines 12-14: here you mention the chloroform fumigation technique. Why did you describe this method later at pag7 (lines 7-15)?

Response: Yes, the alpine meadow was snow covered in deep winter, and the snow was swept before soil sample collecting. Because the chloroform fumigation treatment was also used for the determination of TDN. 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 15 to Page 9 line 11)**.

Pag 8: Lines 1-4: Did you fumigate also some soil samples for the determination of extractable DOC in the measurement of the microbial C? Lines 10-11: What is the definition of growing season? Did you consider the air temperature to define this period? Did you consider the soil temperature?

Response: No, the fumigate treatment did not use for the determination of extractable DOC in the measurement of the microbial C. The definition of the growing season is according to the plant phenology observation in the alpine meadow from 2011 to 2013, which indicated that the growing season is during May to October **(Page 6 line 1)**.

Lines 12-13: Sorry I don’t understand this sentence.

Response: This sentence (Lines 12-13) was revised as “Pearson correlation analysis was then performed to analyze the correlation between MBC and SWC and that between MBC and DOC during the nongrowing and growing seasons.” **(Page 11 lines 2-4)**.

Pag 9: Line 2: Add respectively after 2012-2013 Lines 2-3: how do you define a freeze/thaw cycle event?

Response: Yes, "respectively" was added after 2012-2013. Actually, we did not measure the frequencies of freeze-thaw cycle events, and we inferred numbers of the freeze-thaw cycle event according to the mean soil temperature (0 ℃ 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.

Pag 10: Line 18: What do you mean from one another?

Response: "one another" was revised as "each other" **(Page 13 line 5)**.

Pag 11: Lines 3-4: I don't understand this sentence, in particular "but that significantly lower: : :.."

Response: This sentence was revised as "The DOC contents during the nongrowing season in 2011–2012 (174.27 mg kg^{-1} ± 32.59 mg kg^{-1}) and growing season in 2012–2013 (170.85 mg kg^{-1} ± 41.19 mg kg^{-1}) had no significant differences ($p > 0.05$), but those were significantly lower than that in other seasons ($p < 0.05$; Fig. 6B)" **(Page 13 lines 15-18).**

Pag 12: Line 11: Do you think is it necessary to specify "the beginning of the early non-growing season"? It's not possible to mention also the beginning of the early non-growing season?

Response: Yes, we thought it was necessary to specify and mention "the beginning of the early nongrowing season", because MBC contents showed different dynamics during different periods of the nongrowing season, i.e., MBC contents increased in early nongrowing season, but decreased in deeply cold period, and then increased in the late nongrowing season.

Pag 13: Lines 16: Do you mean the plant community? Please specify better this concept.

Response: Yes, the community productivity was mean the plant community productivity, and "community productivity" was been revised as "plant community productivity" **(Page 16 line 16)**.

Pag 14: Line 15: seasnon change into season Lines 17-18: Sorry but this sentence

is not clear. What do you mean with "increasing process of NH4-N"?

Response: Yes, "seasnon" was been changed into "season" in the revised manuscript. Actually, the "increasing process of NH_4^+–N" was mean "increasing trend of NH_4^+–N", and we revised this sentence as "An obviously trend of increasing NH_4^+–N content was found during the early soil thaw." **(Page 17 line 18 to Page 18 line 1)**.

Pag 15: Lines 2-3: change thawing with melting. Moreover, do you have data about snow chemistry in the area?

Response: Yes, "thawing" was changed into "melting". Sorry, we did not have the data on snow chemistry in the study area.

Lines 4: Preferred in comparison to what? NO3?

Response: Yes, alpine plant preferred NH_4^+–N compared to NO_3^-–N and DON.

Line 9: During the middle growing season do you expect a high plant uptake which cause the reduction of soil inorganic N?

Response: Yes, we do agree with that a high plant uptake causes the reduction of soil inorganic N.

Lines 10-11: Late in the growing season you observed a reduction in the soil inorganic N. But with the reduction of plant uptake you did not expect an opposite trend?

Response: Actually, some late-flowering plants such as *Gentiana sino-ornata* usually dominate the late growing season, and they need to uptake relatively high available N for growing. We found that the DON was an effective supplement of the available N pool during the late growing season.

Pag 16: Lines 7-8: Warmer and drier than 2012-2013? Moreover also a greater number of freeze/thaw cycles than 2012-2013?

Response: Yes, the nongrowing season in 2011-2012 was warmer and drier than that in 2012-2013. As we did not measure the frequencies of freeze–thaw cycle events, some similar literatures were cited in the revised manuscript. This sentence was revised as "Notably, the nongrowing season in 2011–2012 was warmer and drier 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 19 lines**

9-11).

Is the greater number of freeze/thaw cycles recorded in the drier season 2011-2012 related also to a thinner snowpack with a little insulation effect?

Response: Yes, we do agree with that the greater number of freeze–thaw cycles in the drier season may also related to a thinner snowpack with a little insulation effect. Unfortunately, we did not have detailed information on the snowpack during the study year.

Responses to Anonymous Referee #3

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.

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 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 13, 16; Page 3 lines 2, 5, 9, 15; Page 4 lines 4, 6, 14-17; Page 5 lines 2-8)**.

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. First, we introduced why 3 sites were selected and how we analysed the soil samples collected from the 3 sites, 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 selected. One site is located at the upper part of the alpine meadow, one at the middle part, and one at the lower part. Five replicates were collected from each site. The replicates from each site were 10 m apart from one another. The samples collected from the three sites (n = 15) at each sampling time were used for the statistical analyses" **(Page 6 lines 8-13).**

Second, "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 15 to Page 9 line 11)**.

Finally, we rewrote the "Statistical analyses" section, i.e., "The normal distribution and homogeneity of variance of the sample datum were analyzed with SAS 9.2 software (SAS Institute Inc., 2008). The results met the basic requirements of variance analysis. Microbial and nutrient variables were analyzed to test the intra-annual differences between the growing season (i.e., data from May to October were used as a sample set; n = 90) and nongrowing season (i.e., data from November to April were used as a sample set; n = 90). Their interannual differences were also tested. Two-way ANOVA

was performed via mixed-effects model, with season and year specified as fixed effects. For the analysis of the microbial community shifts during the transition between nongrowing and growing seasons, differences in the number of bacteria, fungi, and actinomycetes between the late nongrowing season (i.e., in March) and early growing season (i.e., in May) were determined via two-way ANOVA. This procedure was performed for 2 years (2012 and 2013), and season and year specified were used as fixed effects. Pearson correlation analysis was then performed to analyze the correlation between MBC and SWC and that between MBC and DOC during the nongrowing and growing seasons. Significant results were determined at the $p < 0.05$ level, and Duncan's test was performed to analyze the significant results of the multiple comparisons to the interaction effects between season and year (SAS Institute Inc., 2008)." **(Page 10 line 10 to Page 11 line 6).**

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 3 line 2).**

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 into the quotation **(Page 3 line 5-6)**.

P3, l11, 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, l17, 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 15-16)**.

P4, l5, missing Buckeridge et al 2010 Biogeochemistry

Response: Yes, "Buckeridge and Grogan, 2010" was added **(Page 4 line 12)**.

P4, l7-8, 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 that explore the changes in microbial and N pools in alpine ecosystems during summer and winter across several years are few." **(Page 4 lines 13-17).**

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?

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 the relationships between soil microbial biomass and available N pools in the seasonal frozen ecosystems?" **(Page 5 lines 2-7).**

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?

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 selected. One site is located at the upper part of the alpine meadow, one at the middle part, and one at the lower part." **(Page 6 lines 8-10)**. Fifteen

samples collected from the three sites at each sampling time were then performed together for statistical analyses (n = 15) **(Page 6 lines 11-13)**.

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 nongrowing season in 2012-2013, and the mean snow depth and timing were described in the "Site description" section, 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 15-17)**.

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.

Response: Yes, we added detailed months behind the cold periods and warm seasons, i.e., "the cold periods (i.e., November to April)" **(Page 7 line 1)** and "the warm seasons (i.e., May to October)" **(Page 7 line 4)**.

P6, l4, 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, l8, how many iButtons for each temperature measurement?

Response: The mean daily temperatures were then calculated by the data of nine iButtons, i.e., "Three iButton data loggers were placed at each site, and mean daily temperatures were then calculated from the data of the nine loggers." **(Page 7 lines 11-12)**.

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?

Response: The seasonal temperature was calculated by date, i.e., "the growing season was from 1 May to 31 October, and the nongrowing season was from 1 November to

30 April" **(Page 7 lines 12-14)**. 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 15 to Page 9 line 11)**.

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., data from May to October were used as a sample set; n = 90) and nongrowing season (i.e., data 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 nongrowing 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 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 ℃ 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 NO_3^-–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 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 nongrowing 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 nongrowing 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)**.

Responses to Anonymous Referee #4

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. See more specific comments below.

Response: We thank referee for the helpful comments. After discussing with co-authors, we thoroughly revised the manuscript.

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 ℃ 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, 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 15 to Page 9 line 11).**

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 ℃ in the revised manuscript **(Page 11 lines 12-13)**.

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; Igbinosa, 2015) **(Page 9 lines 6-8)**. 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 the analysis of the microbial community shifts during the transition between nongrowing and growing seasons, differences in the number of bacteria, fungi, and actinomycetes between the late nongrowing season (i.e., in March) and early growing season (i.e., in May) were determined via two-way ANOVA. This procedure was performed for 2 years (2012 and 2013), and season and year specified were used as fixed effects **(Page 10 lines 16-18 to Page 11 lines 1-2)**.

Statistics: Because the same sites/plots were sampled repeatedly, a repeated measures ANOVA would be more 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 r2 (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 **(Page 10 lines 15-16)**, and new statistical results were listed in Table 1 **(Page 29-30)**. Further, we revised the description of the Pearson correlation analysis as "Pearson correlation analysis was then performed to analyze the correlation between MBC and SWC and that between MBC and DOC

during the nongrowing and growing seasons." **(Page 11 lines 2-4)**. 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 12 lines 5, 16-17; Page 13 lines 12-13; Page 14 lines 2-3, 11-14, 18; Page 15 line 1)**, 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 nongrowing 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 16-17)**.

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 line 17)**.

Other conclusions require further elaboration. For 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 "Furthermore, the soil microorganism not only has a close correlation with the accumulation of inorganic N pools but also is an important soil organic N pool itself." **(Page 21 line 1-3)**

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 7)**, and the revised manuscript has been sent to a professional language editing company for the language modification.

Thank you again for your suggestion!

Best regards!

Bo Xu

Seasonal and interannual dynamics of soil microbial biomass and available nitrogen in an alpine meadow in the eastern part of Qinghai–~~-~~Tibet Plateau, China

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Abstract. Soil microbial activity occurs seasonally in frozen alpine soils during cold seasons and plays a crucial role in available N pool accumulation in soil. The intra- and interannual patterns of microbial and nutrient dynamics reflect the influences of changing weather factors, and thus provide important insights into the biogeochemical cycles and ecological functions of ecosystems. We documented the seasonal and interannual dynamics of soil microbial and available N in an alpine meadow in the eastern part of Qinghai–~~-~~Tibet Plateau, China between April 2011 and October 2013. ~~Soil~~ Topsoil samples were collected in the middle of each month and were analyzed for water content, microbial biomass C (MBC) and N (MBN), dissolved organic C and N, and inorganic N; soil microbial community composition~~s~~ ~~were~~ was measured by the dilution-plate method. Fungi and actinomycetes dominated the microbial community during the ~~non-growing~~nongrowing seasons, and the number of bacteria increased considerably during the early growing seasons. Trends of consistently increasing MBC~~Consistently increasing trends of MBC~~ and available N pools were

observed during the ~~non-growing~~nongrowing seasons. MBC sharply declined during soil thaw and was accompanied by a peak ~~of~~ in available N pool. Induced by soil temperatures, significant shifts in the structure and functions of microbial communities were ~~found~~ observed during the winter–~~-~~spring transition and largely contributed to microbial reduction. Divergent seasonal dynamics of different N forms showed a complementary nutrient supply pattern during the growing season. ~~Similar~~ Similarities between the interannual dynamics ~~were observed between~~of microbial biomass and that of available N pools were observed, and soil temperature and water condition were the primary environmental factors driving ~~these year-to-year~~interannual fluctuations. ~~Under the background of changing~~Owing to the changes in climate, ~~the~~ seasonal soil microbial ~~activity~~ activities and nutrient supply patterns ~~will be~~are expected to change further ~~changed~~, and these changes may have crucial ~~having important~~ implications ~~to~~ for the productivity and biodiversity of alpine ecosystems.

1 Copyright statement

We agree with the copyright policy of *Biogeosciences*.

2 Introduction

In Arctic and alpine ecosystems, soil microbial activity plays a crucial role in soil C and N cycles and nutrient transformation in frozen soils during cold seasons (Lipson et al., 1999; Murata et al., 1999; Panikov et al., 2006; Larsen et al., 2007; Matthew Robson et al., 2010). Unfortunately, information on belowground microbial ~~activity~~ activities and nutrient cycles ~~during~~ in both ~~the~~ growing and nongrowing seasons in such ~~alpine~~ ecosystems are limited. ~~Particularly~~Moreover, ~~the~~ intra-annual biogeochemical cycles affected by the ~~changing~~ changes in seasonal weather

factors in frozen regions are not fully understood. The integration between the intra- and interannual patterns in soil microbial and biogeochemical dynamics has important implications to the exploration of the current and future impacts of climate change on the functions of cold ~~alpine~~ ecosystems (Edwards and Jefferies, 2013).

Microorganisms in alpine environments covered seasonally with snow can survive in thin unfrozen water films when most of the soil water is frozen (Mikan et al., 2002~~; Edwards and Jefferies, 2013~~). Previous studies indicated that substantial microbial activity exists in the ~~alpine~~ frozen soils during cold seasons~~,~~ even at temperatures ~~of~~ lower than −5 ℃ ~~or lower~~ (Brooks et al., 1996; Lipson et al., 2002; Edwards et al., 2006; Panikov et al., 2006; Jefferies et al., 2010). Although microbial activity is limited by cold temperatures and substrate transport~~s~~ (Deming, 2002; Lipson et al., 2002; Oquist et al., 2009), its cumulative effects on organic matter decomposition in soil during long cold seasons significantly influence annual N pools in Arctic and alpine ecosystems (Lipson et al., 1999; Schmidt and Lipson, 2004; Schmidt et al., 2007; Buckeridge and Grogan, 2008). Thus, ~~knowledge~~ by understanding ~~on the~~ microbial ~~activity~~ activities ~~during~~ in winter, we can ~~improve~~ broaden our current knowledge regarding ~~the understanding~~ nutrient supplies for plants and microbes during the subsequent growing season.

Previous studies suggested that the fungal/bacterial ratio of a soil microbial community in winter is apparently higher than that in summer (Lipson et al., 2002; Schadt et al., 2003), and significant shifts in microbial community structures and functions occur during soil thawing in Arctic and alpine tundras (Lipson et al., 2002; Schadt et al., 2003; Lipson and Schmidt, 2004; Buckeridge et al., 2013). ~~Accompanied by~~Apart from these changes, the rate of microbial biomass turnover increases during winter-spring transition periods (Edwards et al., 2006; Schmidt et al., 2007; Edwards and

Jefferies, 2013; Buckeridge et al., 2013). Furthermore, available C substrates for ~~the~~ microbial ~~community~~ communities change from winter to summer. For example, winter microbes use dead plant materials, whereas plant root exudates ~~supplied~~ supply available C for summer microbes (Lipson et al., 2002; Schmidt et al., 2007). These changes in microbial ~~community~~ communities changes ~~bewteen winter and summer~~ might play ~~a~~ key roles in controlling annual patterns of nutrient cycling and plant N uptake in Arctic and alpine ecoysystems (Schmidt et al., 2007; Buckeridge and Grogan, 2008; Buckeridge et al., 2013).

In Arctic and alpine soils, ~~increasing~~ microbial biomass and avilable N pools increase in~~were observed during~~ winter ~~time~~, followed by ~~a~~ the reduction ~~of~~ in microbial biomass during winter–~~–~~spring transition when the soil ~~thawed~~ thaws (Brooks et al., 1998; Lipson et al., 1999; Schmidt and Lipson, 2004; Miller et al., 2009). ~~Moreover~~In alpine ecosystems, ~~the~~ decrease ~~of~~ in microbial biomass is linked to a ~~pulse of~~sudden rise in N avilability ~~when~~ during soils ~~thaws~~thawing, as observed in alpine ecosystems (Brooks et al., 1998; Lipson et al., 1999; Schmidt et al., 2007; Yang et al., 2016). The release of soluble N from microbial biomass during the soil thawing period provides an important available N source to plants, particularly in N–limited ecosystems (Lipson et al., 1999; Miller et al., 2009; Buckeridge and Grogan, 2010). 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 that ~~on exploring~~ explore the changes in microbial and N pools in alpine ecosystems ~~during the summer growing seasons in these seasonal frozen ecosystems~~ during summer and winter across several years are few ~~(Edwards and Jefferies, 2013)~~. Thus, the annual patterns of microbial biomass and N pools in alpine

ecosystems and their responses to seasnonal and interannual weather variations in alpine ecosysterms remain unclear. In this study, we documented the seasonal dynamics of soil microbial biomass and available N for three years in an alpine meadow in the eastern part of Qinghai–Tibet Plateau of China to address the following questions: 1) What are soil microbial and available N dynamics during the growing and nongrowing seasons in the alpine meadow?What are seasonal and interannual patterns of soil microbial and available N dynamics in the alpine meadow? 2) What are interannual patterns of soil microbial and available N dynamics in the alpine meadow? 23) What environmental factors affect these dynamics? 34) What are the relationships between soil microbial biomass and available N pools in seasonally frozen ecosystems?What are the nutrient supply patterns of different forms of available N pools in the alpine meadow soil?

3 Material and methods

3.1 Site description

The study was performed in the alpine belt of Songpan County, which belongs to the Minshan Mountain in the eastern part of the Qinghai–Tibet Plateau, China. Accoding to the Records records from a meteorological station (33°1′ N, 103°41′ E, 3600 m a.s.l.) near the study area, showed that the average monthly air temperatures range from −7.6 ℃ in January to 15.5 ℃ in August. The annual precipitation is 718 mm, and 70 % of which occurs from June to August. The region has no absolute frost-free period, and snowfall usually occurs from late September to early May. 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). The alpine vegetation community has rich species composition, and

dominated by different plant species ~~at different times of~~ during the growing season (i.e., during early May to late October according to the plant phenology observation in the alpine meadow from 2011 to 2013). Early flowering plants, such as *Primula sikkimensis*, *Androsace umbellate*, and *Caltha palustris*, dominate the community as soon as the snow melts; *Polygonum macrophyllum*, *Ranunculus tanguticus*, and *Carex melanocephala* dominate the middle growing season; and *Saussurea hieracioides* and *Gentiana sino-ornata* usually dominate the late growing season (Xu, unpublished data, collected ~~in~~ from 2011 ~~2012,~~to 2013). The predominant soil type is mountain dark brown soil and Mat Cry-gelic Cambisols (i.e., silty loam inceptisol ~~(~~; Chinese Soil Taxonomy Research Group, 1995; Wang et al., 2016). Study sites were located in an alpine meadow at Kaka Mountain (~~32°59′ N, 103°40′ E, 3980 m a.s.l.~~Fig. 1), which is a representative landscape in this region. Considering the soil spatial heterogeneity, ~~Three~~ three adjacent sites, approximately 100 m apart (centered at 32°59′ N, 103°40′ E, 3980 m a.s.l.) were selected. One site is located at the upper part of the alpine meadow, one at the middle part, and one at the lower part. ~~in the alpine meadow (top, middle, and bottom of the meadow), and f~~Five replicates ~~at each site~~ were collected from each site. The replicates from each site were 10 m apart from one another~~each other~~. The samples collected from the three sites (n = 15) at each sampling time were used for the statistical analyses. Given that plant roots ~~were~~ are mainly distributed at 0–20 cm soil depth, soil sampling was only focused on this soil layer.

3.2 Soil sampling

Soil samples were collected on the 15th day of each month from April 2011 to October 2013. Overall, 31 sampling times were performed~~. Five replicates~~, and 15 soil samples were collected ~~at each site~~ during each sampling time. The

~~upper~~ 1–2 cm layer of the surface materials (i.e., living plant roots and litter) of each soil sample ~~were~~ was removed ~~from the soil samples~~. During the ~~winter~~ cold periods (i.e., November to April), the samples were collected ~~by using~~with a portable permafrost drill. The frozen soil samples were cut into little pieces (< 1 cm^3) with a knife and hammer, and ~~the~~ large roots and sticks were removed ~~before further determination~~. The soil samples collected during the warm seasons (i.e., May to October) were sieved to separate the plant materials and other fragments greater than 2 mm in diameter. The soils were then mixed and divided into three subsamples for further analysis. All the samples were processed at the laboratory of Chengdu ~~Insititute~~ Institute of Biology, CAS, within ~~two~~ 2 days of sampling.

3.3 Soil temperature measurement

Soil temperatures were measured at the ~~central~~ center part of each sampled location ~~used for soil sampling~~. ~~The s~~Soil temperatures ~~was recorded~~ at 10 cm depth were recorded with DS1921G Thermochron iButton data loggers (DS1921G–F5, Maxim Integrated Products, Dallas Semiconductor Inc., Sunnyvale, CA, USA) at 1 h interval during the experimental period. Three iButton data loggers were placed at each site, and ~~The~~ mean daily temperatures ~~was~~ were then calculated from the data of the nine loggers. The mean temperature of the growing season was calculated by the mean daily temperatures from 1 May to 31 October, and that of the nongrowing season was calculated by the mean daily temperatures from 1 November to 30 April.

3.4 Soil water content~~, microbial~~ and nutrient analyses

One subsample was used to measure ~~the~~ gravimetric soil water content (SWC) after drying at 105 ℃ for 12 h. For the determination of total dissolved N (TDN) content, fresh soil subsamples (15 g) were measured into a beaker and placed

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into a sealed vacuum dryer along with another beaker containing 100 mL of chloroform. The samples were then subjected to vacuum treatment three times. A vacuum dryer was placed into the incubator under a temperature of 24 ℃ for 24 h and then subjected to vacuum treatment for approximately 30 min. K_2SO_4 (0.5 M) was added into the chloroform-treated soil samples with a soil weight-to-extractant volume (w/v) ratio of 1 : 5. The mixture was shaken for 1 h at 24 ℃. The extracted solution was filtered through filter paper (0.45 μm) and stored at −20 ℃ before determination (Lu, 2000; Jones and Willett, 2006). Then, 10 mL of the extracted solution was placed into a test tube containing 10 mL of oxidant (NaOH-$K_2S_2O_8$ mixed solution). The resulting solution was subjected to water bath treatment at 120 ℃ for 90 min. The TDN was then determined with an ultraviolet spectrophotometer. For the determination of available inorganic N (NH_4^+–N and NO_3^-–N), the extracted treatment solution used was similar to that used for the TDN, except that it was not subjected to chloroform fumigation. NH_4^+–N and NO_3^-–N contents were determined via the indophenol blue colorimetry (Sah, 1994) and ultraviolet spectrophotometry (Norman et al., 1985), respectively. Dissolved organic N (DON) was calculated by subtracting dissolved inorganic N (NH_4^+–N and NO_3^-–N) from TDN.

For the determination of the soil dissolved organic carbon (DOC), 10 g of fresh soil subsamples were shaken with 0.5 M K_2SO_4 at a 1: 5 w/v ratio for 1 h at 24 ℃, and the suspension was filtered at 0.45 μm under suction. The DOC values of the extracts were then measured through ultraviolet spectrophotometry (Lu, 2000; Jones and Willett, 2006).

3.5 Soil microbial biomass and community analyses

~~The s~~Soil microbial biomass C (MBC) and N (MBN) were determined via the chloroform-fumigation extraction method (Witt et al., 2000). Correction factors of 0.45 for C and 0.54 for N were used to convert the chloroform labile C and N

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to microbial C and N, respectively (Brookes et al., 1985; Wang et al., 2016).

The total colony-forming units (CFU) of bacteria, fungi, and actinomycetes were determined via the dilution-plate method (Li, 1996; Igbinosa, 2015). A total of 10 g of measured fresh soil subsamples were placed into a sterile jar, to which 90 mL of sterile distilled water was added~~,~~. ~~and then t~~The jar was then covered with a sterile rubber plug and oscillated for 10 min ~~to make a~~for stock solution preparation. Serial diluent was made from the stock solution. The 10^{-5} and 10^{-6} dilution ratios of the serial diluent were selected for the determination of bacteria and actinomycetes ~~determination~~, and 10^{-2} and 10^{-3} dilution ratios for fungi determination (Li, 1996). The selective mediums for bacteria, fungi, and actinomycetes were beef extract peptone agar, Sabouraud dextrose agar, and Gause synthetic agar medium, respectively (Li, 1996; Igbinosa, 2015). Soil diluent (1 mL) and medium (10 mL) at 45–50 ℃ were injected into the plates and cultured at 28 ℃ for 7–10 days for the bacteria and actinomycetes~~,~~. Another medium with same components was prepared~~and~~ at 25 ℃ for 3–5 days for the fungi. The CFUs of different microbes were counted under a microscope (Li, 1996).

~~The total dissolved N (TDN) content was determined. Fresh soil subsamples (15 g) were measured into a beaker and placed into a sealed vacuum dryer together with another beaker with 100 mL of chloroform. The samples were then subjected to vacuum treatments thrice. The vacuum dryer was placed into an incubator under a temperature of 24 ℃ for 24 h and then subjected to vacuum treatment for approximately 30 min. K_2SO_4 (0.5 M) was added into the chloroform-treated soil samples with a soil weight-to-extractant volume (w/v) ratio of 1 : 5 and then shaken for 1 h at 24 ℃. The extracted solution was filtered through filter paper (0.45 μm) and stored at −20 ℃ before determination (Lu, 2000; Jones~~

~~and Willett, 2006). Then, 10 mL of the extracted solution was placed into a test tube, in which 10 mL of oxidant (NaOH-$K_2S_2O_8$ mixed solution) was added. The resulting solution was subjected to water bath treatment at 120 ℃ for 90 min. The TDN was then determined with an ultraviolet spectrophotometer. For the determination of available inorganic N (NH_4^+-N and NO_3^--N), the extracted treatment solution used was similar to that used for the TDN, except that it was not subjected to chloroform fumigation. NH_4^+-N and NO_3^--N contents were determined via the indophenol blue colorimetry (Sah, 1994) and ultraviolet spectrophotometry (Norman et al., 1985), respectively. Dissolved organic N (DON) was calculated by subtracting dissolved inorganic N (NH_4^+-N and NO_3^--N) from TDN. For the determination of the soil dissolved organic carbon (DOC), 10 g of fresh soil subsamples were shaken with 0.5 M K_2SO_4 at a 1: 5 w/v ratio for 1 h at 24 ℃, and the suspension was filtered at 0.45 μm under suction. The DOC in the extracts was then measured through ultraviolet spectrophotometry (Lu, 2000; Jones and Willett, 2006).~~

3.~~5~~ 6 Statistical analyses

The normal distribution and homogeneity of variance of the sample datum were analyzed with SAS 9.2 software (SAS Institute Inc., 2008). The results met the basic requirements of variance analysis. Microbial and nutrient variables were analyzed to test the intra-annual differences between the growing season (i.e., data from May to October were used as a sample set; n = 90) and nongrowing season (i.e., data from November to April were used as a sample set; n = 90~~),~~). ~~and~~ Their interannual differences ~~among three years~~were also tested. Two-way ANOVA was performed via mixed-effects model, with season and year ~~as~~ specified as fixed effects~~fixed factors~~. For the analysis of the microbial community shifts during the transition between nongrowing and growing seasons, ~~Differences~~ differences in the number of bacteria, fungi,

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and actinomycetes between the late ~~non-growing~~nongrowing season (i.e., in March) and early growing season (i.e., in May) ~~for two years (2012 and 2013)~~ were determined via two-way ANOVA. This procedure was performed for 2 years (2012 and 2013), and season and year specified were used as fixed effects. Pearson correlation analysis was then performed to analyze the correlation ~~of the~~between MBC ~~of~~ and SWC ~~with~~ and that between MBC and ~~of the~~ DOC during the ~~non-growing~~ nongrowing and growing seasons. ~~Significances~~ Significant results were determined at the $p < 0.05$ level, and Duncan's test was performed to analyze the significant results of the multiple comparisons to the interaction effects between season and year (SAS Institute Inc., 2008).

4 Results

4.1 Soil temperature and water content

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In the alpine meadow, the mean soil temperatures (at 10 cm depth) were 6.01 ℃, 7.61 ℃, and 7.06 ℃ during the three growing seasons (May to October) from 2011 to 2013 and −1.76 °C and −2.17 °C during the two ~~non-growing~~nongrowing seasons (November to April, Fig. ~~1~~2). In addition, the soil was frozen (below 0 ℃) for 125 days ~~and 165 days during~~on 2011–2012 and 165 days on 2012–2013.~~,~~ The soil was deeply frozen (below −5 °C) for 32 days on 2011–2012 and 36 days on 2012–2013. ~~and the early non-growing season (November to December) of 2011–2012 had more freeze-thaw cycle events than those of 2012–2013.~~

Significant seasonal and inter-annual differences in ~~the~~ topsoil water contents (0–20 cm depth, SWC) were observed (Table 1). The SWC showed a decreasing trend during the growing season and increasing trend during ~~non-growing~~ nongrowing season (Fig. ~~2A~~3A), and SWC in the nongrowing~~non-growing~~ season was significantly higher than that in

the growing season (Fig. ~~2B~~3B). No significant difference was observed between the SWC mean values in the nongrowing~~non-growing~~ season of 2011–2012 (64.73 % ±2.22 %) and those in the nongrowing~~non-growing~~ season of 2012–2013 (65.68 % ±4.03 %; $p > 0.05$; Fig. ~~2B~~3B). However, the SWC mean values in the growing seasons on 2011–2013 were significantly different ($p < 0.05$; Fig. ~~2B~~3B), and the lowest SWC was 46.43 % ±2.28 % during 2012–2013.

4.2 Soil microbial biomass and community

Significant differences ~~($p < 0.05$)~~ in MBC between seasons ($F = 860.28$, $p = 0.00$) and years ($F = 4.46$, $p = 0.01$) were observed in the soils of the alpine meadow ~~in terms of MBC~~ (Table 1). The annual peak of MBC occurred in the late nongrowing~~non-growing~~ season (March) then sharply decreased, indicating a diminishing trend during the growing season. The MBC reached a minimum value in the late growing season (September) then showed an increasing trend during the nongrowing~~non-growing~~ season (Fig. ~~3A~~4A). However, a trend of significant decreas~~e~~~~ing trend~~ in MBC was observed in February when the soil temperatures were the lowest (below −5 °C). In addition, the MBC values in the nongrowing~~non-growing~~ seasons were consistently higher than those in the growing seasons. The mean MBC value during the nongrowing~~non-growing~~ season in 2012–2013 (i.e., 943.93 mg kg^{-1} ± 80.01 mg kg^{-1}) was significantly ($p < 0.05$) higher than those in the other seasons. Meanwhile, the mean MBC value during the growing season in 2012–2013 (i.e., 143.53 mg kg^{-1} ±20.99 mg kg^{-1}) was the lowest (Fig. 4C). The MBC during the growing season had highly significant positive correlation with ~~the~~ SWC ($p < 0.01$, $r = 0.62$; Table 2).

The soil MBN values had significant interannual differences ($F = 11.06$, $p = 0.00$~~$p < 0.05$~~), but the seasonal differences ~~of~~ among MBN values were~~was not~~ nonsignificant ($F = 0.06$, $p = 0.80$~~$p > 0.05$~~; Table 1). ~~Its~~ The seasonal and interannual

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dynamics of MBN were similar to those of ~~the~~ MBC, and its annual peak generally ~~occurred~~ occurs in April or May. Furthermore, no significant difference was observed between the mean MBN values ~~during~~ in the growing season of 2013 and those ~~of~~ in 2011–2012 ($p > 0.05$). The lowest MBN value (72.06 mg kg^{-1} ± 5.93 mg kg^{-1}) was observed during the growing season in 2012–2013 (Fig. 4C).

Additionally, the microbial community comprised bacteria, fungi, and actinomycetes, showing a significant shift during the winter–~~–~~spring transition (March to May; $p < 0.05$; Fig. 5). The number of bacteria in May was significantly higher ($p < 0.05$) than that in March, and the number of bacteria in May 2013 (i.e., 8.25 × 10^6 CFU g^{-1}) was significantly higher ($p < 0.05$) than that in 2012 (i.e., 7.22 × 10^6 CFU g^{-1}). The numbers of fungi and actinomycetes in March were significantly higher than that in May ($p < 0.05$). The number of fungi in March 2013 (4.33 × 10^4 CFU g^{-1}) was the highest, and no significant difference was observed between the number of actinomycetes in March 2012 and that in March 2013 ($p > 0.05$; Fig. 5).

4.3 Soil dissolved organic carbon

Significant interannual differences ($F = 5.50$, $p = 0.01$) in soil DOC contents were observed, and the seasonal dynamics of DOC had no significant difference from each other~~one another~~ ($F = 0.04$, $p = 0.85$~~$p > 0.05$~~; Table 1). ~~The~~ DOC ~~peaked~~ peaks annually ~~occurred~~ in May and ~~showed~~ shows a diminishing trend during the growing season and increasing trend during the non-growing season (Fig. 6A). ~~No significant difference ($p > 0.05$) was observed between t~~The DOC contents during the non-growing season in 2011–2012 (174.27 mg kg^{-1} ± 32.59 mg kg^{-1}) and growing season in 2012–2013 (170.85 mg kg^{-1} ± 41.19 mg kg^{-1}) had no significant differences ($p > 0.05$), but those were~~that~~ significantly lower than

those that in other seasons ($p < 0.05$; Fig. 6B). Furthermore, the DOC during the growing season had highly significant positive correlation with MBC ($p < 0.01$, $r = 0.64$; Table 2).

4.4 Soil available nitrogen

Soil ammonium N (NH_4^+–N) contents showed significant seasonal and interannual differences ($p < 0.05$; $F = 28.3$, $p = 0.00$ and $F = 3.20$, $p = 0.04$; Table 1). The annual peak of the NH_4^+–N content occurred in the late non-growingnongrowing season (April), and then sharply reduced during the early growing season, and finally had an increasing trend during the nongrowingnon-growing season (Fig. 7A). The NH_4^+–N content in the nongrowingnon-growing season was significantly higher ($p < 0.05$) than that in the growing season. The NH_4^+–N content during the non-growing season in 2012–2013 (22.21 mg kg^{-1} ± 3.87 mg kg^{-1}) was significantly higher than that in 2011–2012 (17.23 mg kg^{-1} ± 3.85 mg kg^{-1}), and no significant difference was observed among the NH_4^+–N contents during the growing seasons in 2011–2013 ($p > 0.05$; Fig. 8).

Significant seasonal and interannual differences in soil nitrate N (NO_3^-–N) contents were observed ($F = 4.34$, $p = 0.04$ and $F = 3.28$, $p = 0.04$$p < 0.05$; Table 1). The NO_3^-–N content showed an increasing trend during nongrowingnon-growing seasons and increased initially before decreasing during the growing seasons (Fig. 7B). Furthermore, a significantlyan obviously reducing process decreasing trend of NO_3^-–N contents was observed during the soil thawing period (April to May). The NO_3^-–N contents peaked annually in June while that during the nongrowingnon-growing season in 2011–2012 (7.64 mg kg^{-1} ± 1.12 mg kg^{-1}) was the lowest. No significant difference was observed among the NO_3^-–N contents of the other seasons ($p > 0.05$; Fig. 8).

The DON contents had significant interannual differences ($F = 10.13$, $p = 0.00$~~$p < 0.05$~~), but their seasonal differences were not significant ($F = 0.63$, $p = 0.43$~~$p > 0.05$~~; Table 1). In general, the peak DON content was observed in April or May, then sharply decreased during the middle and late growing season, and finally increased during the nongrowing~~non-growing~~ season (Fig. 7C). Furthermore, the mean DON value during the growing season in 2012–2013 (7.53 mg kg^{-1} ± 1.74 mg kg^{-1}) was the lowest, and it was significantly lower than those in the other years ($p < 0.05$; Fig. 8).

5 Discussion

5.1 Seasonal microbial biomass and available nitrogen dynamics

The ~~Significant~~ significant seasonal dynamics of the soil microbial biomass and available N pools were observed in the alpine meadow located in the eastern part of the Qinghai–~~–~~Tibet Plateau for three years (Table 1; Figs. ~~3~~ 4 and 7). Generally, the soil MBC and available N pools both increased at the beginning of the early nongrowing~~non-growing~~ season, and this finding is consistent with the results of ~~the~~ previous studies conducted in other ~~arctic~~ Arctic and alpine ecosystems (Brooks et al., 1998; Lipson et al., 1999; Lipson et al., 2002; Edwards et al., 2006; Larsen et al., 2007, Buckeridge et al., 2010; Edwards and Jefferies, 2013). This period of active microbial activity and N mineralization benefited from substrates conducive for microbial growth, particularly those supplied by the fresh plant litter inputs in autumn (Lipson et al., 1999; Nemergut et al., 2005). However, a decline ~~of~~ in soil MBC was observed during the deeply cold period (i.e., in February when soil temperatures were below − 5 ℃). This decline implied that the temperature threshold of the survival of these cold-adapted microbial communities was at least −5 °C~~, and these communities retained their high activity in alpine soils during the cold periods~~. Thus, an accumulation of inorganic and organic N pools

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occurred during the long and cold nongrowingnon-growing seasons in these seasonally frozen ecosystems even though the N uptakes of plants were degraded (Schimel and Mikan, 2005; Schmidt et al., 2007; Edwards and Jefferies, 2013). The annual peak of MBC generally occurred during the late nongrowingnon-growing season while the mean soil temperatures were below 0 ℃. A modest reduction in MBC was observed in the onset of early soil thaw, and a steep decline in MBC occurred during the late soil-thawing period while the mean soil temperatures exceeded 0 ℃. This sharp decrease in MBC during the transition between nongrowingnon-growing and growing seasons was similar to the changes of MBC in other arcticArctic and alpine meadows during late winter and early spring (Lipson et al., 2002; Edwards et al., 2006). Previous studies suggested several factors that contribute to the decline of MBC during the soil thawing period. First, physical changes in soil during thawing can result in microbial cell death and release of solutes (Jefferies et al., 2010; Edwards and Jefferies, 2013). Second, depletion of soil available C and N can also lead to microbial reductions during soil thawing (Edwards et al., 2006; Buckeridge and Grogan, 2008). Furthermore, Edwards and Jefferies (2013) hypothesized that the oxygen availability in soils may lead to MBC reductions, because although aerobic microbial growth can still be supported in winter, . the aAnaerobic soil conditions are established as soils become flooded with liquid water during the late soil thaw. However, in our study, increasing increases in DOC and inorganic N (NH_4^+–N and NO_3^-–N) contents were observed in our studywas observed during the nongrowingnon-growing season, implying that available C and N were relatively sufficient and might not restrict the microbial activity during the winter–spring transition. This phenomenon may be closely related to the high plant community productivity in the eastern part of the Qinghai-Tibet Plateau. The aboveground biomass ranges from 299.8 g m^{-2} a^{-1} to 475.8 g m^{-2} a^{-1} in the alpine meadows

on this region (Gao et.al, 2008; Yang et al., 2014) but 198 ±73.8 g m^{-2} a^{-1} in the paramo grassland of Colombia (Hofstede et al., 1995) and ranges from 160 g m^{-2} a^{-1} to 230 g m^{-2} a^{-1} in the alpine meadows of the central Rocky mountains (Walker et al., 1994; Körner, 2003). Furthermore, the soil organic matter content in the alpine meadows of this region ranges from 69.7 g kg^{-1} to 112.4 g kg^{-1} (Wu and Onipchenko, 2005) but 12.8 g kg^{-1} in the Alaskan tundra (Körner, 2003) and ranges from 20.3 g kg^{-1} to 34.7 g kg^{-1} in the alpine meadows of the Alps and Colorado (Billings and Bliss, 1959; Körner, 2003).

Additionally, a significant difference was observed ~~in~~ between the microbial community composition~~s~~ in the nongrowing~~non-growing~~ seasons and those in the growing seasons (Fig. 5). ~~Similar to other alpine meadows, the w~~Winter microbial community was dominated by fungi, which is more adapted to cold temperatures and utilizes complex substrates (Lipson et al., 2002; Schadt et al., 2003). Apart from the fungi community, another important microbial community in winter soils ~~was~~ is the actinomycetes~~, which might contribute to the seasonal dynamics of the microbial biomass~~. Furthermore, the number of bacteria significantly increased during the early growing season ~~as~~ after the soils completely thawed. ~~but~~ By contrast, number of fungi and that of actinomycetes declined considerably. This shift in the microbial community may lead to the sharp decline in MBC during soil thaw, partly because of the C investment per unit volume in fungal cells were threefold larger than that in bacteria cells (Buckeridge and Grogan, 2008).

In ~~this~~ the present study, ~~the~~ inorganic N and DON contents both showed an increasing trend during the ~~non-growing~~nongrowing seas~~n~~on, and this trend was closely related to high microbial activity in the soils of this region (Lipson et al., 1999; Matthew Robson et al., 2010). However, divergent dynamics among different forms of available N

were observed during the growing season (Fig. ~~7~~8). An obviously~~significantly increasing process~~ trend of increasing NH_4^+–N content was found during the early soil thaw. ~~On the one hand~~Furthermore, frequent and strong freeze--thaw cycles during this period may contribute to the release of unavailable NH_4^+–N from the organic and inorganic colloids in alpine soils (Freppaz et al., 2007). ~~On the other hand, the s~~Snow melting~~thawing of~~ during this period is an important source of NH_4^+–N (Williams and Tonnessen, 2000). At the ~~beginning~~ start of the growing season, ~~the~~ NH_4^+–N content sharply decreased, partly because ~~of the~~ alpine meadow plants prefer~~red~~ NH_4^+–N (Jaeger et al., 1999; ~~Henry and Jefferies, 2003;~~ Gherardi et al., 2013). Moreover, strong microbial activity in the soil requires a large amount of NH_4^+–N at increasing temperature (Bowman, 1992; Schmidt and Lipson, 2004). As observed in other alpine regions (Brooks et al., 1997; Edwards et al., 2007), the NO_3^-–N had a sharp decline during the soil thaw in our study, mostly because a massive amount of NO_3^-–N might have run off with the snow melt water. The NO_3^-–N content first increased during the early growing season and then decreased during the middle growing season as the NH_4^+–N content decreased. Meanwhile, ~~the~~ DON content slightly decreased during the early and middle growing season and sharply decreased during the late growing season as both NH_4^+–N and NO_3^-–N were exhausted. These results implied that although the DON may not be the main source of N pools for plants, it is an effective supplement of the available N pool. Furthermore, the seasonal dynamics of different available N pools showed ~~a~~ significant complementarity with the nutrient supply process~~,~~ and play~~ing~~ a crucial role in maintaining ~~the rich~~ abundant biodiversity of ~~the~~ alpine meadow ecosystem (Qin et al., 2003; Petchey and Gaston, 2006).

5.2 Interannual microbial biomass and available nitrogen dynamics

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Significant ~~year-to-year~~interannual differences in microbial biomass and available N were observed across the study years. For example, the MBC and NH_4^+–N contents during the ~~non-growing~~nongrowing season in 2012–2013 were significantly higher than ~~that~~ those in 2011–2012, and ~~the~~ MBC during the growing season in 2012–2013 was the lowest among the growing seasons (Figs. 4 and 8). Furthermore, significant positive correlation between MBC and SWC was observed during the growing season (Table 2). This result suggested that interannual variability of soil water conditions is an important environmental driver that affects the microbial biomass in alpine meadows. First, low soil moisture in the growing season causes a decline in plant productivity (Körner, 2003), resulting in a decline of C substrates supplied by plant root exudates and litters. Second, low soil moisture in summer leads to an increased oxidation in the surface soil, thus exerting significant influence on the microbial communities (Blodau et al., 2004), and some of these influences are retained during winter (Edwards and Jefferies, 2013). Notably, the nongrowing ~~a warm and dry non-growing~~ season ~~was observed during~~ in 2011–2012 was warmer and drier 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)~~in late autumn and early winter~~. These environmental variations might contribute to the reduction in soil microbial biomass during the nongrowing~~non-growing~~ season (Larsen et al., 2002; Yanai et al., 2004; Mellander et al., 2007; Henry, 2008). Although the extent of the influence of these environmental factors on soil microbial biomass cannot be verified, our monitoring results suggested that ~~the~~ soil moisture and temperature are two important environmental factors influencing the interannual dynamic of soil microbial biomass.

In the alpine meadow, organic matter decomposition and nutrient mineralization caused by soil microbial activity during

~~the~~ a long cold season ~~will~~ play a crucial role in ~~the accumulation~~ accumulating ~~of~~ soil inorganic N pool (Hidy, 2003; Rinnan et al., 2007), and the microorganism itself is also an important soil organic N pool (Lipson et al., 2002). Thus, the interannual pattern of the soil microbial biomass largely affects the ~~year-to-year~~interannual change of soil N pool. Soil NH_4^+–N and DON had a consistent interannual variation with soil MBC during the nongrowing~~non-growing~~ season. However, they showed ~~an~~ a ~~incompletely consistent~~divergent interannual pattern during the growing season, partly because of the plant and microbe uptakes and leaching effects. Meanwhile, for the NO_3^-–N, relatively small interannual variability was observed. In addition, the interannual variability of precipitation affected the interannual pattern of available inorganic N pool in the soil. The snow melt is not only an important supplement for the NH_4^+–N pool (Williams and Tonnessen, 2000) but also a cause of a mass of NO_3^-–N losses during the soil-thawing period (Brooks et al., 1997; Edwards et al., 2007). Therefore, such interannual variations in the microbial and nutrient dynamics may become more common and pronounced in the alpine meadow in the eastern part of the Qinghai~~–~~–Tibet Plateau as a result of multiple impacts of climate change, particularly increasing extreme weather events, such as winter warming and heterogeneous precipitation (Edwards and Jefferies, 2013).

6 Conclusions

A~~n increasing~~ trend of increasing soil MBC and available N pools was found in nongrowing~~non-growing~~ seasons ~~compared with growing seasons, with~~. ~~a~~ A sharp decline ~~of~~ in MBC was also observed during the soil~~–~~–thawing period. Microbial activity may not be restricted by the soil available C and N in the time of soil thaw~~;~~. ~~however~~However, a shift ~~of~~ in microbial community induced by changing temperatures may largely contribute to this decline in MBC. Different

forms of available N pools showed a divergent decreasing pattern during the growing season, suggesting that a significantly complementary pattern of nutrient supply exists among different N pools. Furthermore, the soil microorganism not only has a close correlation withplays a crucial role in the accumulation of inorganic N pools but also is an important soil organic N pool itself. Thus, the interannual dynamics of soil microbial biomass substantially affects the year-to-yearinterannual differences in among soil available N pools. According to our monitoring results, soil temperature and water condition are the primary environmental factors driving the seasonal and interannual dynamics of soil microbial biomass and available N pools. Owing to Given the changing climates of alpine ecosystems, the soil microbial activity activities and nutrient supply patterns will be are expected to change further changed, . These changes playing an important role in the productivity and biodiversity of these regions. Long-term integrative studies on intra- and interannual variations of microbial and nutrient dynamics have important implications for understanding functions of ecosystems functions and their responses to the environmental changes. Combined with some objective experimental studies, these research results can provide crucial insights into the biogeochemical cycles and functions of ecosystems in the eastern part of the Qinghai – Tibet Plateau, and their potential responses to the future climate change.

7 Data availability

The data set related to this study has been provided as a supplement.

8 Author contribution

Fusun Shi, Ning Wu, and Yan Wu designed the experiments; Bo Xu and Jinniu Wang carried field experiments out; Bo Xu prepared the manuscript with contributions from all co-authors.

9 Competing interests

The authors declare that they have no conflict of interest.

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Table

Table 1. Results from two-way ANOVA comparing growing season (May to October) and non-growingnongrowing season (November to April) values across three years of study for -SWC, -MBC, -MBN, -DOC, $NH4^+$–N, NO_3^-–N, and DON in the alpine meadow.

Variable	Source	df	*F*	*p*
SWC	Year	2	15.68	< 0.01
	Season	1	180.62	< 0.01
	Year × season	2	18.29	< 0.01
MBC	Year	2	48.74	< 0.01
	Season	1	860.28	< 0.01
	Year × season	2	61.67	< 0.01
MBN	Year	2	12.35	< 0.01
	Season	1	0.06	0.80
	Year × season	2	20.79	< 0.01
DOC	Year	2	6.30	0.00
	Season	1	0.04	0.85
	Year × season	2	14.73	0.00
$NH4^+$–N	Year	2	7.70	< 0.01
	Season	1	28.30	< 0.01
	Year × season	2	0.39	0.53
NO_3^-–N	Year	2	3.78	0.03
	Season	1	4.34	0.04
	Year × season	2	0.18	0.67
DON	Year	2	11.67	< 0.01
	Season	1	0.63	0.43
—	Year × season	2	6.40	0.01

Variable	Source	df	*F*	*p*

SWC	Year	2	6.79	0.00
	Season	1	180.62	0.00
	Year × season	2	18.29	0.00
MBC	Year	2	4.46	0.01
	Season	1	860.28	0.00
	Year × season	2	61.67	0.00
MBN	Year	2	11.06	0.00
	Season	1	0.06	0.80
	Year × season	2	20.79	0.00
DOC	Year	2	5.50	0.01
	Season	1	0.04	0.85
	Year × season	2	14.73	0.00
NH_4^+–N	Year	2	3.20	0.04
	Season	1	28.3	0.00
	Year × season	2	0.39	0.53
NO_3^-–N	Year	2	3.28	0.04
	Season	1	4.34	0.04
	Year × season	2	0.18	0.67
DON	Year	2	10.13	0.00
	Season	1	0.63	0.43
__	Year × season	2	6.40	0.01

Table 2. Pearson correlations of MBC between SWC and DOC during growing and nongrowing ~~non-growing~~ seasons

MBC	SWC	DOC
Growing season	0.62 **	0.64 **
Non-growing season	0.35 **	0.12 ns

Note: ns, no significant difference; **, $p < 0.01$.

Figure legends

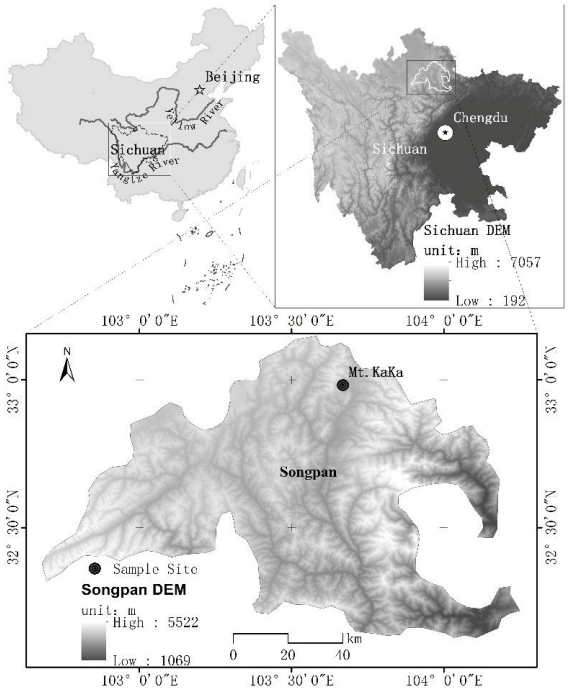

Fig. 1. Location of the study site

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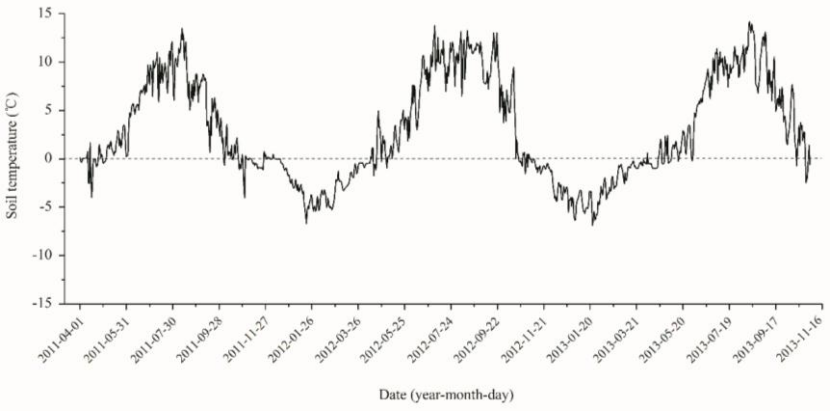

Fig. ~~1~~2. Mean daily soil temperature in the alpine meadow from April 2011 to October 2013. Thermochron iButton data loggers were placed at 10 cm soil depth to obtain automatic readings every ~~60~~ 1 ~~minutesh~~, and the mean daily soil temperature was calculated every day.

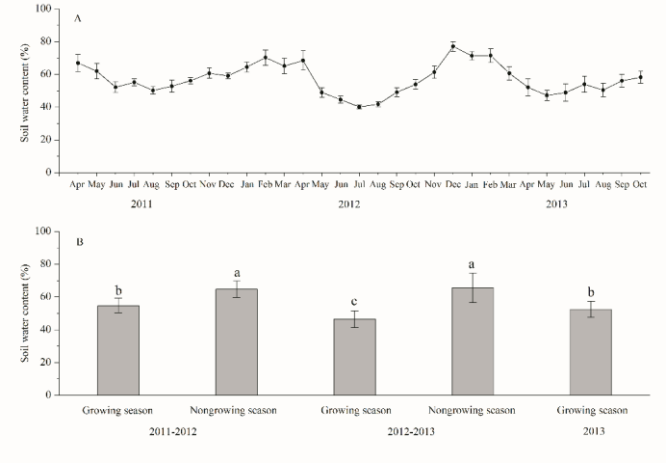

Fig. ~~2~~3. Dynamics of soil water content (A; mean ± s.e.; *n* = 15) and its seasonal and interannual ~~changes~~ differences (B; mean ± s.e.; *n* = 90) from 2011 to 2013.

~~Fig. 3. Dynamics of microbial biomass C (A) and N (B) in soils of the alpine meadow from April 2011 to October 2013 (mean ± s.e.; *n* = 15).~~

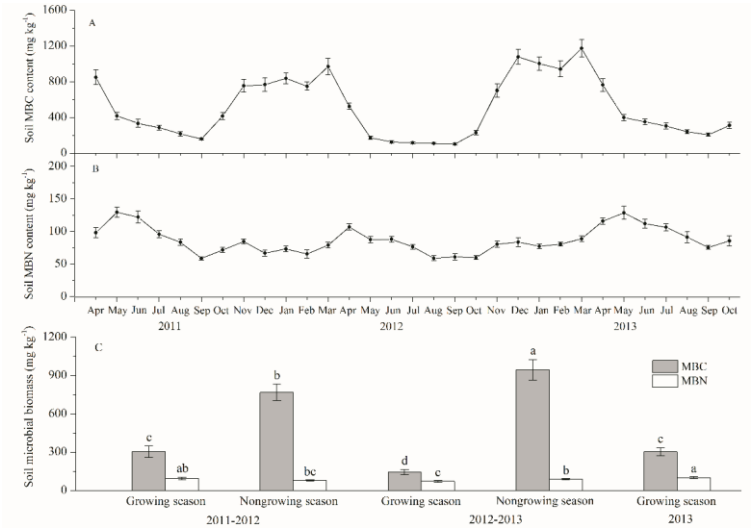

Fig. 4. Dynamics of microbial biomass C and N (A and B; mean ± s.e.; *n* = 15), and their seasonal and interannual differences (C; mean ± s.e.; *n* = 90) from April 2011 to October 2013 (mean ± s.e.; *n* = 90). The sampling time was on the 15th day of each month during the growing season from May to October, and during the nongrowing season from November to April next year. Seasons and years were compared using two-way ANOVA, and different lowercase letters

indicate significant differences of the interaction effects between season and year determined via Duncan test ($p < 0.05$).

~~Fig. 4. Changes in microbial biomass C (MBC) and N (MBN) in the growing and non-growing seasons from 2011 to 2013 (mean ±s.e.; n =90). The sampling time was on the 15th day of each month during the growing season from May to October, and during the non-growing season from November to April next year. Seasons and years were compared using two-way ANOVA, and different lowercase letters indicate significant differences ($p < 0.05$) determined via Duncan test.~~

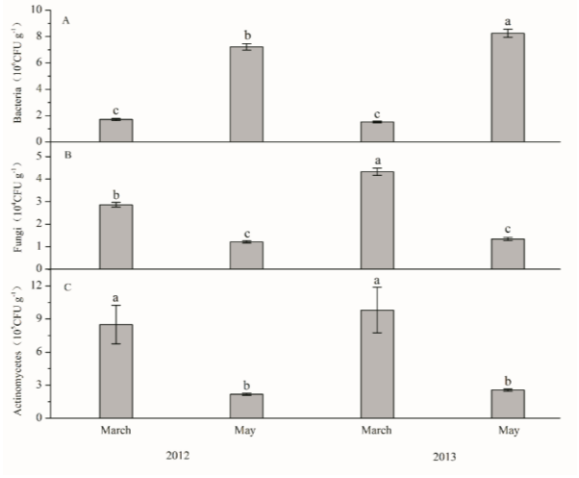

Fig. 5. Changes in the number of bacteria (A), fungi (B), and actinomycetes (C) during the transition between freezing and thawing periods (mean ±s.e.; n = 15). The sampling time during the freezing period was on 15 March and during the thawing period was on 15 May each year. Different lowercase letters indicate significant differences of the interaction

effects between season and year ~~($p < 0.05$)~~ according to two-way ANOVA ($p < 0.05$).

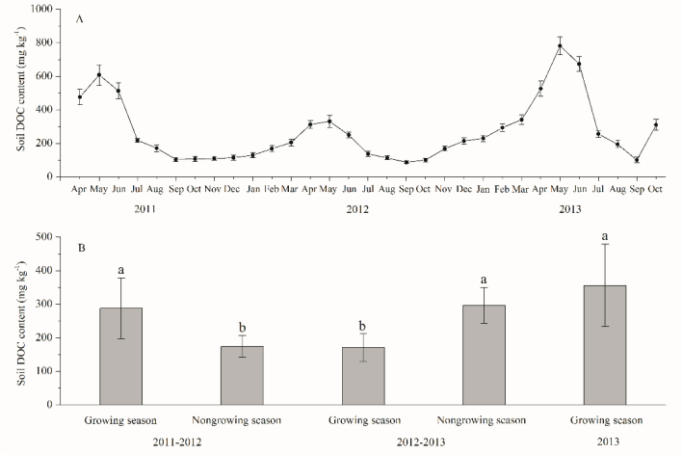

Fig. 6. Dynamics of dissolved organic C (A; mean ± s.e.; $n = 15$) and its seasonal and interannual ~~changes~~ differences (B; mean ± s.e.; $n = 90$) from 2011 to 2013.

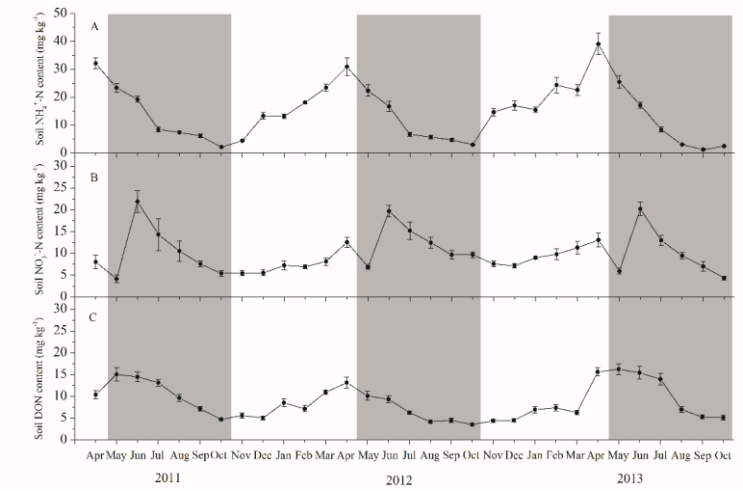

Fig. 7. Dynamics of NH_4^+–N(A), NO_3^-–N(B), and DON(C) in soils of the alpine meadow from April 2011 to October 2013 (mean ± s.e.; n = 15).

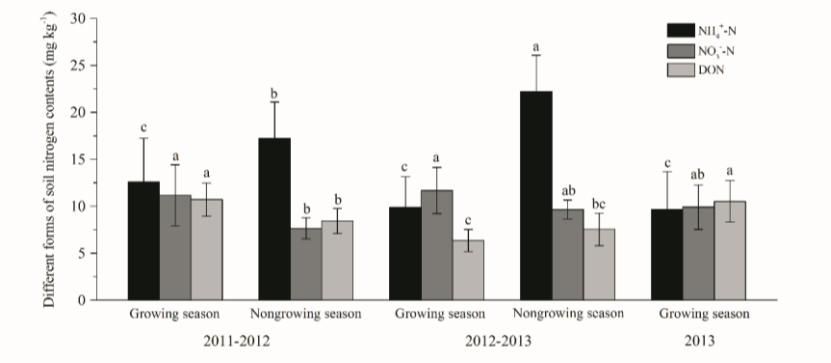

Fig. 8. Changes in NH_4^+–N, NO_3^-–N, and DON of growing and ~~non-growing~~nongrowing seasons from 2011 to 2013 (mean ± s.e.; n =90). The sampling time was on the 15th day of each month from May to October during the growing season and during the ~~non-growing~~nongrowing season from November to April next year. Seasonal and interannual differences were compared using two-way ANOVA. Different lowercase letters indicate significant differences of the interaction effects between season and year ~~($p < 0.05$)~~ determined via Duncan test ($p < 0.05$).