

Dear Prof. Michael Weintraub,

First of all, we are sorry for the ignoring of some important points made by the reviewers in the last round of review. Thank you very much for your helpful comments to our manuscript, and we have thoroughly revised the manuscript after carefully reconsidering every reviewers' comment. Furthermore, we have sent our paper to a professional language editing company to the language modification. The detailed responses to the comments are as follows:

Response to Anonymous Referee #1

The authors have made substantial progress. Here I note the some comments that are, in my view, still not addressed adequately.

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

Correction factors: it's the 0.45 and 0.54 (now page 8, L8). I recommend not correcting these numbers using these factors.

Response: Thank you for your comment. But, we thought correction factors of 0.45 for C and 0.54 for N were reasonable for the determination of MBC and MBN. First, using these extraction efficiency correction factors to correct the result was an important step in the MBC and MBN determinations according to the *Principles and Methods of Soil Microbial Research* written by Lin in 2010. Second, our previous research in the same study area found that it was necessary to using the extraction efficiency correction factors to correct the results for the determination of MBC and MBN (i.e., Wang J., Xu B., Wu Y., Gao J., and Shi F.: Flower litters of

alpine plants affect soil nitrogen and phosphorus rapidly in the eastern Tibetan Plateau. *Biogeosciences*, 13, 5619–5631, 2016.)

There is still no reason given for the pseudoreplicative design (ie three non-independent sets of 5 samples that are then treated as independent). It's not ideal, but there are often acceptable reasons for it and so it's not a fatal flaw in the study. However, I'd like to know the authors' thinking here. The way I see it there are two possibilities: (1) logistically it was not possible to randomly sample across the entire area (meadow or meadow system) of interest, but the authors wanted 15 samples so this is how they were able to achieve that. (2) they were originally planning on comparing the sites, but there were not a lot of interesting differences so they pooled them together in order to achieve a better overall understanding of seasonal trends, which were perhaps not visible with $n = 5$ at each site. This was a point brought up by multiple reviewers and the authors need to provide a justification on why such a design was used.

Response: Thank you for your comments. But, we did not thought it was reasonable that judging the 15 samples collected from 3 sites or locations were non-independent. Because, as you said, it was not possible to randomly sample across the entire alpine meadow. Thus, given the soil spatial heterogeneity in the alpine meadow, 3 independent sites or locations (i.e., located at the upper part, middle part, and lower part of the meadow) were randomly selected at each sampling time, and 5 samples were then randomly collected at each sites (**Page 6 lines 1-4**). Furthermore, we agreed with that the sample sets of season and year were dependent because they were repeatedly collected at different time in the same alpine meadow. It was the pseudo-replication if we treated the dependent samples as independent samples. However, we thought it was acceptable to use mixed-effects model to analyze the dependent variations (i.e., season and year) to

understand the seasonal and interannual dynamics of soil microbial biomass and available nitrogen in the alpine meadow across 3 years.

Figures: having the bar graphs below the line graphs further emphasizes that the information is redundant. I'll leave it up to authors and editor, but I still recommend removing the bar graphs.

Response: Thank you for your comment. But, we did not think having the bar graphs below the line graphs further emphasizes that the information is redundant. Because they showed different information, i.e., the line graphs showed detailed information on intra- and interannual patterns of microbial and nutrient dynamics; the bar graphs showed significant differences in microbial biomass and nutrients between seasons and years, and their interaction effects. Thus, these figures were indispensable for our study.

Inclusion of data in a publicly available repository: the authors did not address this request. I still recommend it.

Response: Yes, The data set related to this study has been provided as a supplement.

Re: TDN vs. MBN numbers. Some ok points made in the reviewer response, but it's not in the paper. It warrants discussion in the paper.

Response: Thank you for your comment. Actually, the important points made in the response have been stated in the discussion section (**Page 16 lines 3-6, 13-17**).

Photograph and map are not the same thing. It helps to see what the ecosystem looks like on the ground. I still recommend this, even if in the supp materials.

Response: Sorry, we did not have appropriate photographs to show the alpine meadow.

A comment from another reviewer about unsubstantiated statements in the discussion is still relevant: "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)" Some examples of these statements:

"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."

Response: Thank you for your comment. Here, we did not make any conclusion, but only cited some relevant results of previous researches in the similar regions. Moreover, they were potential reasons why “the soil MBC and available N pools both increased at the beginning of the early nongrowing season”. We revised “benefited from” as “might benefit from” (Page 14 line 2).

"Snow melting during this period is an important source of NH_4^+-N " This is only true with high deposition. Not sure if this region is susceptible to that.

Response: Thank you for your comment. Actually, this sentence was also a citation of previous research, which is a potential reason why “An obviously increased trend of NH_4^+-N content was found during the early soil thawing”. And we revised it as “On the other hand, snow melting during this period may be an important source of NH_4^+-N ” (Page 16 line 6).

"At the start of the growing season, NH_4^+-N content sharply decreased, partly because alpine meadow plants prefer NH_4^+-N " maybe change partly to possibly

Response: Yes, “partly” has been changed into “possibly” (Page 16 line 7).

"the seasonal dynamics of different available N pools showed significant complementarity with the nutrient supply process and play a crucial role in maintaining abundant biodiversity of alpine meadow ecosystem"

Response: Yes, “and play a crucial role in maintaining abundant biodiversity of alpine meadow ecosystem” was deleted (**Page 16 lines 16-17**).

"However, they showed a divergent interannual pattern during the growing season, partly because of the plant and microbe uptakes and leaching effects." same use of partly. maybe authors mean to use possibly in these cases.

Response: Yes, “partly” was changed into “possibly” (**Page 18 lines 4**).

"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." I would probably leave this out. It's something most would accept but at the same time, is not really shown by this study, which does not address mechanisms.

Response: Thank you for your comment. But, we thought this conclusion could be made according to our results (**e. g., Figures 2, 3; Tables 1, 2**), and we thought it very important for the study.

Finally, I would still axe the mentions of climate change from the conclusion. The contributions of this paper are on seasonal trends, not climate change. It's not a huge change. It's fine to mention it where it is mentioned just before the conclusion, but it should not be emphasized as the final statement in the paper. The authors justification in the response that because temperature and moisture appear to correlate with the other measured variables, climate change is important is not convincing. Temperature and moisture are always important for microbial processes, and it's a leap to then suggest that this paper provides particular insight on how this relationship will change with climate.

Response: Thank you for your comment. But, we still thought it was

necessary to mention the climate change in the conclusion. Because, according to this study, significant differences of interannual dynamics of soil microbial biomass and available nitrogen were observed, and that was a comprehensive influence of climate change across 3 years. Both the seasonal trend and interannual pattern of soil microbial biomass and available nitrogen were important in our study. Meanwhile, the alpine ecosystems are sensitive to the future climate change, such as temperature warming and uneven distribution of precipitation. Therefore, it was important to mention the climate change in the conclusion section.

minor:

"An obviously trend of increasing $\text{NH}_4^+ - \text{N}$ content was found during the early soil thaw" this revised sentence does not make sense

Response: Yes, this sentence was revised as “An obviously increased trend of $\text{NH}_4^+ - \text{N}$ content was found during the early soil thawing.” (Page 16 line 3)

Response to Anonymous Referee #2

I think that thanks to the suggestions of the referees the paper has considerably improved. However I suggest to the authors some further changes, listed below:

Pag. 27 line 7: change avilable into available. See also pag 27 line 10.

Response: Yes, “avilable” was changed into “available” (Page 4 lines 3).

Pag 28 lines 6-7: Did you add also the soil classification according to the Soil Taxonomy (Silty Loam Inceptisol)? If yes please add the proper reference: Soil Survey Staff. 2014. Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, Washington, DC.

Response: Yes, the reference that “Soil Survey Staff: Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, Washington, DC., 2014.” was added (Page 5 line 17; Page 25 lines 3-4).

Pag 28 lines 10-11: Please specify the elevation of the 3 sites

Response: Yes, the elevations of the 3 sites were added, i.e., “One site is located at the upper part (3984 m a.s.l.) of the alpine meadow, one at the middle part (3980 m a.s.l.), and one at the lower part (3975m a.s.l.)” (Page 6 lines 2-3).

Pag 29 line 1: add O horizon before living plant roots and litter

Response: Yes, “O horizon” was added before “living plant roots and litter” (Page 6 line 8).

Pag 35 line 14: sorry I don't understand this sentence. What do you mean with “from each other”

Response: “from each other” means that significant differences compared with each other. We have deleted “from each other” in the revised manuscript.

Pag 35 line 18: What do you mean with the term those

Response: Sorry, we could not find “those” in Page 35 line 18, and we were not sure which “those” in the manuscript did you refer.

Pag 37 line 10: delete early

Response: Sorry, we could not find “early” in Page 37 line 10, and we were not sure which “early” in the manuscript did you refer.

Pag 38 line 18: what do you mean with a-1

Response: The “a⁻¹” in the units for biomass refers to per year, and “a⁻¹” was revised as “per year” (Page 15 lines 3-5).

Pag 40 line 1: Why obviously?

Response: Sorry, we could not find “obviously” in Page 40 line 1, and we were not sure which “obviously” in the manuscript did you refer.

Pag 43 line 5: Delete monitoring

Response: Yes, “monitoring” was deleted (Page 17 line 14; Page 19 line 2).

Response to Anonymous Referee #4

This paper describes both seasonal and interannual variability in soil microbial biomass and soil available N in alpine tundra with monthly resolution over a 3 year time period. This is an impressive data set which is worthy of publication. I reviewed an earlier version of this manuscript and made a number of suggestions. The writing is much improved but still requires further work. Further, some of my previous comments have not been dealt with to my satisfaction, as described below.

Response: We thank referee for the helpful comments. After discussing with co-authors, we carefully revised the manuscript and listed in supplement.

In the previous version I had questions about the MBC/MBN methods as well as the statistics. Neither of these have been dealt with satisfactorily. The methods for determining TDN are still not clear. The procedure for TDN is described on page 7 line 11-16. Line 9-11 describes the chloroform fumigation procedure which is not the methods for TDN but are part of the methods for MBN. TDN is determined on both fumigated and non-fumigated samples (the non-fumigated sample analysis is currently not described in the paper) and the difference is MBN. The chloroform fumigation methods could be moved to the paragraph beginning on page 8 line 6.

Response: Yes, the methods for determining TDN (**Page 7 lines 5-11**), MBC and MBN (**Page 8 lines 1-13**) were rewrote according to your suggestion

For the statistics, the response to reviewer 4 indicates that sample ID was included in the model to account for the lack of independence of samples across time. However, the stastistical analysis section of the

manuscript does not describe any inclusion of sample ID in the model. Please clarify in the manuscript. Also, all F values throughout the manuscript should include the degrees of freedom.

Response: Yes. The sample ID was included in the statistical model, i.e., “For the repeat measure analysis with time-dependent covariate, two-way ANOVA was performed via mixed-effects model, with season and year specified as fixed effects, and the sample ID of each sampling time specified as the random effect”(Page 9 lines 11-13). The degree of freedom (df) was added behind the *F* value throughout the revised manuscript (Page 10 line 17; Page 11 lines 9-10; Page 12 lines 5-6, 13-14; Page 13 lines 2-3, 8-9).

Some parts of the result section are also not clear. For example, when describing DOC patterns, the seasons are described as being not significantly different from each other (page 11 line 18) and also significantly different (page 12 line 4).

Response: Thank you for your comment. Although, “the seasonal dynamics of DOC had no significant difference”, the interaction effects of DOC between season and year were significantly different. Thus, “the DOC contents during the nongrowing season in 2011–2012 ($174.27 \text{ mg kg}^{-1} \pm 32.59 \text{ mg kg}^{-1}$) and growing season in 2012–2013 ($170.85 \text{ mg kg}^{-1} \pm 41.19 \text{ mg kg}^{-1}$)” showed the result that “were significantly lower than those in other seasons”.

Also, the results section could be reduced – e.g. The two sentences from page 10 line 17 to page 11 line 1 say the same thing.

Response: Yes, “the MBC values in the nongrowing seasons were consistently higher than those in the growing seasons” was deleted.

Finally, just as a suggestion, figure 3 could also be presented with only part A and shading to indicate the different season – this way the data is only presented once rather than repeated in both parts of the figure.

The same change could be applied to the other figures. The two types of presentations are presented in the same figure sometimes (Figure 3) and as separate figures in others (Figure 7 and 8).

Response: Thank you for your comment. But, we thought these figures were indispensable for our study. Because, they showed different information, i.e., the line graphs showed detailed information on intra- and interannual patterns of microbial and nutrient dynamics; the bar graphs showed significant differences in microbial biomass and nutrients between seasons and years, and their interaction effects.

In the discussion, the authors still need to be cautious about implying causality for some of the patterns they have measured. Some examples are below:

Page 13 Line 14 – indicate that the mechanism for the increase in soil MBC and available N are speculative. Also, the conclusion of this paragraph describes an accumulation of organic N which is not described in the remainder of the paragraph.

Response: Yes, “Thus, an accumulation of inorganic and organic N pools occurred during the long and cold nongrowing seasons in these seasonally frozen ecosystems (Schimel and Mikan, 2005; Schmidt et al., 2007)” was deleted.

Page 15 Line 15 – Only describe what you have evidence for – e.g. an increase in microbial biomass and not activity

Response: Yes, the microbial activity was revised as “microbial biomass” (Page 16 line 1).

And lastly, a few clarifications are required in the discussion:

Page 15 line 9 – what do you mean by the “number of bacteria” increased just after thaw? The previous paragraph describes a crash in microbial biomass. Do you mean the proportion of bacteria to fungi? The number of bacterial phenotypes?

Response: Here, the “number of bacteria” mean the proportion of bacteria, and the “number of bacteria” was revised as “proportion of bacteria” (**Page 15 lines 13-14**).

Page 15 line 2-5 The units for soil organic matter need an area (per m²?) for this comparison to be relevant. Is this sentence implying that the SOM in tundra is limiting to microbial growth in some circumstances?

Response: Thank you for your comment. But, we thought the units for soil organic matter did not need an area, because we just need compare the differences of soil organic matter contents among different alpine meadows. Furthermore, we also did not know the values of SOM per square meter (m²) from the references. According to this sentence, we knew that the SOM contents in the alpine meadows of the Qinghai-Tibet Plateau were relatively higher than that in other alpine meadows. It was implied that the available C was relatively sufficient in this region and “might not have restricted microbial activity during the winter–spring transition”.

The writing in this version is much improved over the last version. However, the writing still needs to be improved for clarity and grammar. A number of examples follow:

Page 1 Line 10 – replace “occurs seasonally” with “varies seasonally” as the activity occurs all the time

Response: Yes, “occurs seasonally” was replaced with “varies seasonally” (**Page 1 line 10**).

Page 1 Line 15 – replace “Topsoil samples were” with “Soil was” and “and were analyzed” with “and analyzed”

Response: Yes, “Topsoil samples were” and “and were analyzed” were replaced with “Soil was” and “and analyzed”, respectively (**Page 1 line 15**).

Page 1 Line 17 – replace “was measured” with “as measured”

Response: Yes, “was measured” was replaced with “as measured” (**Page 1 line 16**).

Page 1 Line 18 – replace “the number of” with “the proportion of”

Response: Yes, “the number of” was replaced with “the proportion of” (**Page 1 line 18**).

Page 2 Line 1 – replace “induced by soil temperatures” with “induced by changes in soil temperatures”

Response: Yes, “induced by soil temperatures” was replaced with “induced by changes in soil temperatures” (**Page 2 line 1**).

Page 3 Line 11 – delete “apparently”

Response: Yes, “apparently” was deleted (**Page 3 line 11**).

Page 4 Line 5 Delete “In alpine systems” as it is repeated later in the sentence

Response: Yes, “In alpine systems” was deleted (**Page 4 line 5**).

Page 5 line 8 Delete “and”

Response: Yes, “and” was deleted (**Page 5 line 8**).

Page 6 line 10 – Delete “and 15 soil samples... time” as this information is repeated from a few sentences earlier

Response: Yes, “and 15 soil samples... time” was deleted (**Page 6 line 8**).

Page 9 line 4 – “analysis of variance” not “variance analysis”

Response: Yes, “variance analysis” was revised as “analysis of variance” (**Page 9 line 8**).

Page 14 line 17 – what does the a refer to in the units for biomass? Per year?

Response: Yes, the “a⁻¹” in the units for biomass means “per year”, and the “a⁻¹” was revised as “per year” (**Page 15 lines 3-5**).

Similarly, the use of appropriate references is also much improved. A

few more which need to be changed are:

Page 3, line 3: Mikan is not an alpine reference

Response: Yes, “Mikan et al., 2002” was deleted and two relevant alpine references were added, i.e., “Brooks et al., 1996; Jefferies et al., 2010”
(Page 3 line 3).

Page 16 line 13 – Qin and Petchey/Gaston are general biodiversity – ecosystem functioning researchers and not appropriate for use in this sentence.

Response: Yes, “Qin et al., 2003; Petchey and Gaston, 2006” were deleted, and this sentence was deleted **(Page 16 lines 17).**

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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10 **Abstract.** Soil microbial activity ~~varies~~ 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

15 2013. ~~Soil was~~ ~~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_i. ~~soil~~ Soil microbial community composition ~~was~~ as measured by the dilution-plate method. Fungi and actinomycetes dominated the microbial community during the nongrowing seasons, and the ~~proportion~~ number of bacteria increased considerably during the early growing seasons. Trends of consistently increasing MBC and available N pools were observed during the

nongrowing seasons. MBC sharply declined during soil thaw and was accompanied by a peak in available N pool.

Induced by [changes in](#) soil temperatures, significant shifts in the structures and functions of microbial communities were observed during the winter–spring transition and largely contributed to microbial reduction. [The Divergent-divergent](#) seasonal dynamics of different N forms showed a complementary nutrient supply pattern during the growing season.

5 Similarities between the interannual dynamics of microbial biomass and ~~that of~~ available N pools were observed, and soil temperature and water condition were the primary environmental factors driving interannual fluctuations. Owing to the changes in climate, seasonal soil microbial activities and nutrient supply patterns are expected to change further, and these changes may have crucial implications for the productivity and biodiversity of alpine ecosystems.

1 Copyright statement

10 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 activities and nutrient
15 cycles in both growing and nongrowing seasons in such ecosystems are limited. Moreover, intra-annual biogeochemical cycles affected by the 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~~ [for](#) the exploration of the current and future impacts of climate change on the functions of cold 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 (Brooks et al., 1996; Jefferies et al., 2010; Mikan et al., 2002). Previous studies indicated that substantial microbial activity exists in the frozen soils during cold seasons even at temperatures lower than $-5\text{ }^{\circ}\text{C}$ (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 (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, by understanding microbial activities in winter, we can broaden our current knowledge regarding 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). 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 microbial communities change from winter to summer. For example, winter microbes use dead plant materials, whereas plant root exudates supply available C for summer microbes (Lipson et al., 2002; Schmidt et al., 2007). These changes in microbial communities changes might play key

roles in controlling annual patterns of nutrient cycling and plant N uptake in Arctic and alpine ecosystems (Schmidt et al., 2007; Buckeridge and Grogan, 2008; Buckeridge et al., 2013).

In Arctic and alpine soils, microbial biomass and available N pools increase in winter, followed by the reduction in microbial biomass during winter–spring transition when the soil thaws (Brooks et al., 1998; Lipson et al., 1999; Schmidt and Lipson, 2004; Miller et al., 2009). In alpine ecosystems, decrease in microbial biomass is linked to a sudden rise in N availability during soils 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 periods 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 explore the changes in microbial and N pools in alpine ecosystems during summer and winter across several years are few. Thus, the annual patterns of microbial biomass and N pools in alpine ecosystems and their responses to seasonal and interannual weather variations 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? 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 seasonally frozen ecosystems?

3 Material and methods

3.1 Site description

5 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. According to the records from a meteorological station (33°1' N, 103°41' E, 3600 m a.s.l.) near the study area, the average monthly air temperatures range from –7.6 °C in January to 15.5 °C 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, 10 collected in 2012, 2013). The alpine vegetation community has rich species composition; and is dominated by different plant species 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*, 15 *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 from 2011 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; [Soil Survey Staff, 2014](#); Wang et al., 2016).

Study sites were located in an alpine meadow at Kaka Mountain (Fig. 1), which is a representative landscape in this region. ~~Considering~~ Given the soil spatial heterogeneity in the area, three adjacent sites, approximately 100 m apart (centered at 32°59' N, 103°40' E, ~~3980 m a.s.l.~~) were randomly selected. One site is located at the upper part (3984 m a.s.l.) of the alpine meadow, one at the middle part (3980 m a.s.l.), and one at the lower part (3975m a.s.l.). Five replicates were randomly 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. Given that plant roots 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 was~~ performed 31 times, and ~~15 soil samples were collected during each sampling time~~. The 1–2 cm layer of the surface material (i.e., O horizon, living plant roots and litter) of each soil sample was removed. During the cold periods (i.e., November to April), the samples were collected with a portable permafrost drill. The frozen soil samples were cut into little pieces (< 1 cm³) with a knife and hammer, and large roots and sticks were removed. 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 samples were then mixed and divided into three subsamples for further analysis. All the samples were processed at the laboratory of Chengdu Institute of Biology, CAS, within 2 days of sampling.

3.3 Soil temperature measurement

Soil temperatures were measured at the center of each sampled location. Soil temperatures 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 mean daily temperatures 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 ~~4~~ May 1 to ~~31~~ October 31, and that of the nongrowing season was calculated by the mean daily temperatures from ~~1~~ November 1 to ~~30~~ April 30.

3.4 Soil water content and nutrient analyses

One subsample was used to measure gravimetric soil water content (SWC) after drying at 105 °C for 12 h. For the determination of total dissolved N (TDN) content, fresh soil subsamples (15 g) were measured into a beaker, and ~~placed 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 °C for 24 h and then subjected to vacuum treatment for approximately 30 min.~~ K₂SO₄ (0.5 M) was then 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 °C.

The extracted solution was filtered through filter paper (0.45 µm) and stored at -20 °C before determination (Lu, 2000; Jones and Willett, 2006). ~~Then, 10 mL of the extracted solution was transferred to a test tube containing 10 mL of oxidant (NaOH-K₂S₂O₈ mixed solution). The resulting solution was subjected to water bath treatment at 120 °C for 1 h. The TDN was then determined with an ultraviolet spectrophotometer (Lu, 2000). For the determination of available inorganic N (NH₄⁺-N and NO₃⁻-N), the extracted treatment solution used was similar to that used for the TDN, except that it was not subjected to chloroform fumigation.~~ The NH₄⁺-N and NO₃⁻-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 ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) 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 °C, 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

For the determination of soil microbial biomass C (MBC) and N (MBN), fresh soil subsamples (15 g) were measured into a beaker and placed 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 °C 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 °C. The extracted solution was filtered through filter paper (0.45 µm) and stored at -20 °C before determination (Lu, 2000; Jones and Willett, 2006). The extracted solution of non-chloroform-treated samples was made similar to that of chloroform-treated samples, except that it was not subjected to chloroform fumigation. The contents of C and N in the extracted solution were then measured through ultraviolet spectrophotometry (Lu, 2000; Jones and Willett, 2006). The MBC and MBN were then calculated by subtracting the C and N contents of non-chloroform-treated samples from that of chloroform-treated samples, respectively. 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 to microbial C and N, respectively (Brookes et al., 1985; [Lin, 2010](#); Wang et al., 2016).

The total colony-forming units (CFU) of bacteria, fungi, and actinomycetes were determined via the dilution-plate method (Li, 1996; Igbinsosa, 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. The jar was then covered with a sterile rubber plug and oscillated for 10 min 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, 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; Igbinsosa, 2015). Soil diluent (1 mL) and medium (10 mL) at 45–50 °C were injected into the plates and cultured at 28 °C for 7–10 days for the bacteria and actinomycetes. Another medium with same components was prepared at 25 °C for 3–5 days for the fungi. The CFUs of different microbes were counted under a microscope (Li, 1996).

3.6 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 [analysis of variance](#). Microbial and nutrient variables were analyzed ~~to~~ [for the testing of](#) 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. [For the repeat measure analysis with time-](#)

~~dependent covariate.~~ Two-way ANOVA was performed via a mixed-effects model, with season and year specified as fixed effects, ~~and the sample ID of each sampling time specified as the random effect.~~ 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. ~~Then, we performed~~ 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).

4 Results

4.1 Soil temperature and water content

In the alpine meadow, the mean soil temperatures (at 10 cm depth) were 6.01 °C, 7.61 °C, and 7.06 °C during the three growing seasons (May to October) from 2011 to 2013 and -1.76 °C and -2.17 °C during the two nongrowing seasons (November to April, Fig. 2). In addition, the soil was frozen (below 0 °C) for 125 days 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. Significant seasonal and interannual differences in 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 ~~the~~ nongrowing season (Fig. 3A), and SWC in the nongrowing season was significantly higher than that in the growing season (Fig. 3B). No significant

difference was observed between the SWC mean values in the nongrowing season of 2011–2012 ($64.73\% \pm 2.22\%$) and those in the nongrowing season of 2012–2013 ($65.68\% \pm 4.03\%$; $p > 0.05$; Fig. 3B). However, the SWC mean values in the growing seasons on 2011–2013 were significantly different ($p < 0.05$; Fig. 3B), and the lowest SWC was $46.43\% \pm 2.28\%$ ~~during in~~ 2012–2013.

5 4.2 Soil microbial biomass and community

Significant differences in MBC between seasons ($F = 860.28$, df = 1, $p = 0.00$) and years ($F = 4.46$, df = 2; $p = 0.01$) were observed in the soils of the alpine meadow (Table 1). The annual peak of MBC occurred in the late nongrowing season (March) ~~then sharply but eventually~~ decreased sharply, 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 season (Fig. 4A). However, a trend of significant decrease in MBC was observed in February when the soil temperatures were the lowest (below $-5\text{ }^{\circ}\text{C}$). In addition, the ~~MBC values in the nongrowing seasons were consistently higher than those in the growing seasons. The~~ mean MBC value during the nongrowing season in 2012–2013 (i.e., $943.93\text{ mg kg}^{-1} \pm 80.01\text{ mg kg}^{-1}$) 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., $143.53\text{ mg kg}^{-1} \pm 20.99\text{ mg kg}^{-1}$) was the lowest (Fig. 4C). The

15 MBC during the growing season had highly significant positive correlation with SWC ($p < 0.01$, $r = 0.62$; Table 2).

The soil MBN values had significant interannual differences ($F = 11.06$, df = 2; $p = 0.00$), but the seasonal differences among MBN values were nonsignificant ($F = 0.06$, df = 1; $p = 0.80$; Table 1). The seasonal and interannual dynamics of MBN were similar to those of MBC, and its annual peak generally ~~occurs-occurred~~ in April or May. Furthermore, no

significant difference was observed between the mean MBN values in the growing season of 2013 and those in 2011–2012 ($p > 0.05$). The lowest MBN value ($72.06 \text{ mg kg}^{-1} \pm 5.93 \text{ mg kg}^{-1}$) 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., $8.25 \times 10^6 \text{ CFU g}^{-1}$) was significantly higher ($p < 0.05$) than that in 2012 (i.e., $7.22 \times 10^6 \text{ CFU g}^{-1}$). 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 ($4.33 \times 10^4 \text{ CFU g}^{-1}$) 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 ($F = 5.50$, df = 2; $p = 0.01$) in soil DOC contents were observed, and the seasonal dynamics of DOC had no significant difference ~~from each other~~ ($F = 0.04$, df = 1; $p = 0.85$; Table 1). DOC peaks annually in May and shows a diminishing trend during the growing season and increasing trend during the nongrowing season (Fig. 6A). The DOC contents during the nongrowing season in 2011–2012 ($174.27 \text{ mg kg}^{-1} \pm 32.59 \text{ mg kg}^{-1}$) and growing season in 2012–2013 ($170.85 \text{ mg kg}^{-1} \pm 41.19 \text{ mg kg}^{-1}$) had no significant differences ($p > 0.05$), ~~but those~~ were significantly lower than ~~that those~~ 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 ($\text{NH}_4^+\text{-N}$) contents showed significant seasonal and interannual differences ($F = 28.3$, $\text{df} = 1$; $p = 0.00$ and $F = 3.20$, $\text{df} = 2$; $p = 0.04$; Table 1). The annual peak of the $\text{NH}_4^+\text{-N}$ content occurred in the late nongrowing season (April), and then sharply ~~reduced-declined~~ during the early growing season ~~before, and finally had an~~ showing an increasing trend during the nongrowing season (Fig. 7A). The $\text{NH}_4^+\text{-N}$ content in the nongrowing season was significantly higher ($p < 0.05$) than that in the growing season. The $\text{NH}_4^+\text{-N}$ content during the non-growing season in 2012–2013 ($22.21 \text{ mg kg}^{-1} \pm 3.87 \text{ mg kg}^{-1}$) was significantly higher than that in 2011–2012 ($17.23 \text{ mg kg}^{-1} \pm 3.85 \text{ mg kg}^{-1}$), and no significant difference was observed among the $\text{NH}_4^+\text{-N}$ contents during the growing seasons in 2011–2013 ($p > 0.05$; Fig. 8).

Significant seasonal and interannual differences in soil nitrate N ($\text{NO}_3^-\text{-N}$) contents were observed ($F = 4.34$, $\text{df} = 1$; $p = 0.04$ and $F = 3.28$, $\text{df} = 2$; $p = 0.04$; Table 1). The $\text{NO}_3^-\text{-N}$ content showed an increasing trend during nongrowing seasons and increased initially before decreasing during the growing seasons (Fig. 7B). Furthermore, ~~an obviously~~ decreasing trend ~~of in~~ $\text{NO}_3^-\text{-N}$ contents was observed during the soil thawing period (April to May). The $\text{NO}_3^-\text{-N}$ contents peaked annually in June while that during the nongrowing season in 2011–2012 ($7.64 \text{ mg kg}^{-1} \pm 1.12 \text{ mg kg}^{-1}$) was the lowest. No significant difference was observed among the $\text{NO}_3^-\text{-N}$ contents of the other seasons ($p > 0.05$; Fig. 8).

The DON contents had significant interannual differences ($F = 10.13$, $\text{df} = 2$; $p = 0.00$), but their seasonal differences were ~~not~~ significant ($F = 0.63$, $\text{df} = 1$; $p = 0.43$; 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 season (Fig. 7C). Furthermore, the mean DON value during the growing season in 2012–2013 ($7.53 \text{ mg kg}^{-1} \pm 1.74 \text{ mg kg}^{-1}$) 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 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. 4 and 7). Generally, the soil MBC and available N pools both increased at the beginning of the early nongrowing season, and this finding is consistent with the results of previous studies conducted in other Arctic and alpine ecosystems (Brooks et al., 1998;

10 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 ~~might benefited~~ from substrates conducive ~~for-to~~ microbial growth, particularly those supplied by the fresh plant litter inputs in autumn (Lipson et al., 1999; Nemergut et al., 2005). However, a decline in soil MBC was observed during the deeply cold period (i.e., in February when soil temperatures were below $-5 \text{ }^{\circ}\text{C}$). This decline implied that the temperature threshold of the survival of these
15 cold-adapted microbial communities was at least $-5 \text{ }^{\circ}\text{C}$. ~~Thus, an accumulation of inorganic and organic N pools occurred during the long and cold nongrowing seasons in these seasonally frozen ecosystems (Schimel and Mikan, 2005; Schmidt et al., 2007).~~

The annual peak of MBC generally occurred during the late nongrowing season while the mean soil temperatures were

below 0 °C. 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 °C. This sharp decrease in MBC during the transition between nongrowing and growing seasons was similar to the changes of MBC in other Arctic 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 oxygen availability in soils may lead to MBC reductions because aerobic microbial growth can still be supported in winter. Anaerobic soil conditions are established as soils become flooded with liquid water during the late soil thaw. However, in our study, increases in DOC and inorganic N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) contents was observed during the nongrowing season, implying that available C and N were relatively sufficient and might not have restricted 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⁻¹per year to 475.8 g m⁻² a⁻¹per year in the alpine meadows on this region (Gao et al., 2008; Yang et al., 2014) but 198 ± 73.8 g m⁻² a⁻¹per year in the paramo grassland of Colombia (Hofstede et al., 1995) and ranges from 160 g m⁻² a⁻¹per year to 230 g m⁻² a⁻¹per year 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 between the microbial community composition in the nongrowing seasons and those in the growing seasons (Fig. 5). Winter microbial community was dominated by fungi, which is more

5 adapted to cold temperatures and utilizes complex substrates (Lipson et al., 2002; Schadt et al., 2003). Apart from the fungi community, actinomycetes are another important microbial community in winter soils ~~is the actinomycetes~~.

Furthermore, the proportion number of bacteria significantly increased during the early growing season after the soils ~~was~~ completely thawed. By contrast, the proportion 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, possibly partly because of
10 the C investment per unit volume in fungal cells were threefold larger than that in bacteria cells (Buckeridge and Grogan, 2008).

In the present study, inorganic N and DON contents both showed an increasing trend during the nongrowing season, and this trend was closely related to high microbial biomass 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
15 growing season (Fig. 8). An obviously increased trend of ~~increasing~~ $\text{NH}_4^+\text{-N}$ content was found during the early soil

thawing. The potential reasons of this phenomenon are as follows: Furthermore On the one hand, frequent and strong freeze-thaw cycles during this period may contribute to the release of unavailable $\text{NH}_4^+\text{-N}$ from the organic and inorganic colloids in alpine soils (Freppaz et al., 2007). On the other hand, Snow-snow melting during this period is may

be an important source of $\text{NH}_4^+\text{-N}$ (Williams and Tonnesen, 2000). At the start of the growing season, $\text{NH}_4^+\text{-N}$ content sharply decreased; ~~possibly partly~~ because alpine meadow plants prefer $\text{NH}_4^+\text{-N}$ (Jaeger et al., 1999; Gherardi et al., 2013). Moreover, strong microbial activity in ~~the~~ soil requires a large amount of $\text{NH}_4^+\text{-N}$ at increasing temperatures (Bowman, 1992; Schmidt and Lipson, 2004). As observed in other alpine regions (Brooks et al., 1997; Edwards et al., 2007), ~~the~~ $\text{NO}_3^-\text{-N}$ ~~content had a sharply~~ declined during the soil thawing in our study; mostly because a massive amount of $\text{NO}_3^-\text{-N}$ might have run off with the snow melt water. The $\text{NO}_3^-\text{-N}$ content first increased during the early growing season and then decreased during the middle growing season as the $\text{NH}_4^+\text{-N}$ content decreased. Meanwhile, DON content slightly decreased during the early and middle growing seasons and sharply decreased during the late growing season as both $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ 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 ~~supplement~~. Furthermore, the seasonal dynamics of different available N pools showed significant complementarity with the nutrient supply process ~~and play a crucial role in maintaining abundant biodiversity of alpine meadow ecosystem (Qin et al., 2003; Petchey and Gaston, 2006)~~.

5.2 Interannual microbial biomass and available nitrogen dynamics

Significant interannual differences in microbial biomass and available N were observed across the study years. For example, the MBC and $\text{NH}_4^+\text{-N}$ contents during the nongrowing season in 2012–2013 were significantly higher than those in 2011–2012, and 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~~ in 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~~ the 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

5 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 season in 2011–2012 was warmer and drier than that in 2012–2013, ~~which and~~ might have been accompanied ~~with more~~ by frequent freeze–thaw cycles during the early period of this season (Mellander et al., 2007; Henry, 2008). These environmental variations might contribute to the reduction in soil microbial biomass during ~~the~~ nongrowing seasons (Larsen et al., 2002; Yanai et al., 2004; Mellander et al., 2007;

10 Henry, 2008). Although the extent of the influence of these environmental factors on soil microbial biomass cannot be verified, our ~~monitoring~~ results suggested that soil moisture and temperature are two important environmental factors influencing the interannual dynamics of soil microbial biomass.

In the alpine meadow, organic matter decomposition and nutrient mineralization caused by soil microbial activity during a long cold season play a crucial role in accumulating soil inorganic N pool (Hidy, 2003; Rinnan et al., 2007), and the

15 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~~ interannual changes of in soil N pool. Soil NH_4^+ -N and DON had a consistent interannual variation with soil MBC during the nongrowing season. However, they showed a divergent interannual pattern during the growing season; ~~possibly 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 periods~~ (Brooks et al., 1997; Edwards et al., 2007). Therefore, such interannual variations in the microbial and nutrient dynamics may become ~~more increasingly~~ 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 trend of increasing soil MBC and available N pools was ~~found-observed~~ in nongrowing seasons-, and ~~A-a~~ sharp decline 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, a shift in microbial community induced by changing temperatures may largely contribute to ~~this-the~~ decline ~~in-of~~ 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~~ microorganisms not only ~~has-ahave~~ close correlation with the accumulation of inorganic N pools but also ~~is-anare~~ important soil organic N pools ~~itself~~. Thus, the interannual dynamics of soil microbial biomass substantially affects the interannual differences 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 the changing climates of alpine ecosystems, soil microbial activities and nutrient supply patterns are expected to change further. These changes play 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 ecosystem functions and their responses to 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 nongrowing season

10 (November to April) values across three years of study for SWC, MBC, MBN, DOC, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and DON in

the alpine meadow.

Variable	Source	df	<i>F</i>	<i>p</i>
SWC	Year	2	6.79	0.00
	Season	1	180.62	0.00
	Year \times season	2	18.29	0.00
MBC	Year	2	4.46	0.01
	Season	1	860.28	0.00
	Year \times season	2	61.67	0.00
MBN	Year	2	11.06	0.00
	Season	1	0.06	0.80
	Year \times season	2	20.79	0.00
DOC	Year	2	5.50	0.01
	Season	1	0.04	0.85
	Year \times season	2	14.73	0.00
$\text{NH}_4^+\text{-N}$	Year	2	3.20	0.04
	Season	1	28.3	0.00
	Year \times 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 seasons

MBC	SWC	DOC
Growing season	0.62 **	0.64 **
Nongrowing season	0.35 **	0.12 ns

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

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Figure legends

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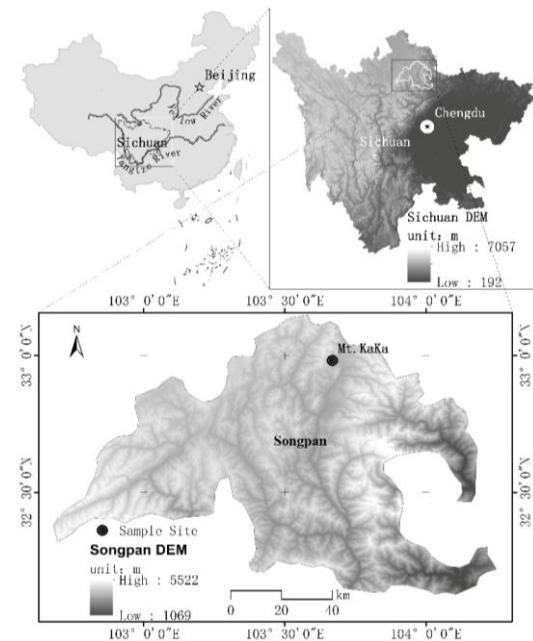


Fig. 1. Location of the study site

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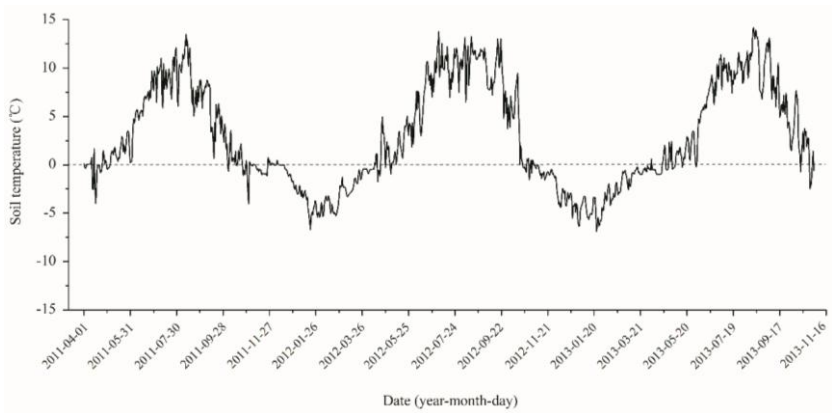
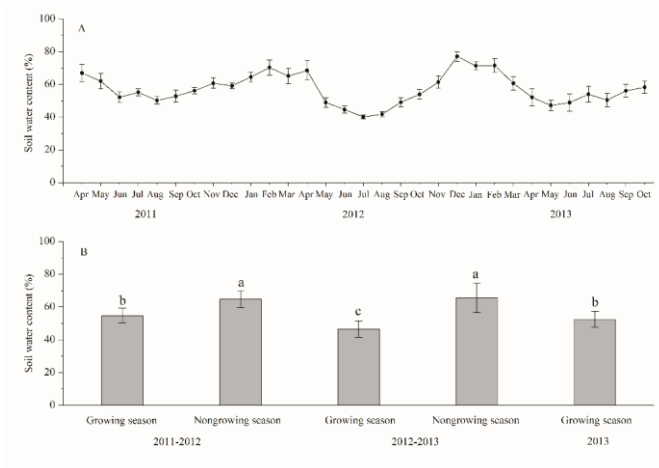


Fig. 2. Mean daily soil temperature in the alpine meadow from April 2011 to October 2013. Thermochron iButton data

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loggers were placed at 10 cm soil depth to obtain automatic readings every 1 h, and the mean daily soil temperature was calculated every day.



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Fig. 3. Dynamics of soil water content (A; mean \pm s.e.; $n = 15$) and its seasonal and interannual differences (B; mean \pm s.e.; $n = 90$) from 2011 to 2013.

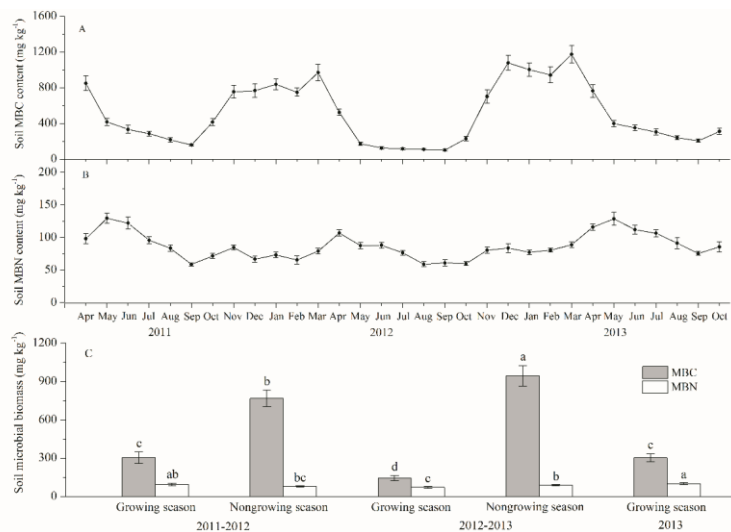


Fig. 4. Dynamics of microbial biomass C and N (A and B; mean \pm s.e.; $n = 15$), and their seasonal and interannual differences (C; mean \pm s.e.; $n = 90$) from April 2011 to October 2013 (mean \pm s.e.; $n = 90$). The sampling time was on the 15th day of each month during the growing season from May 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 ($p < 0.05$).

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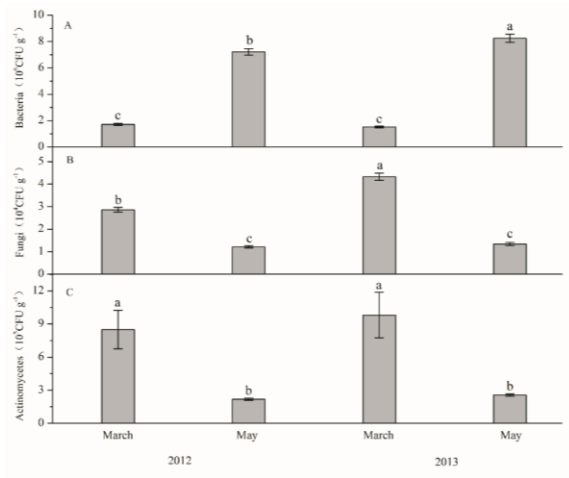
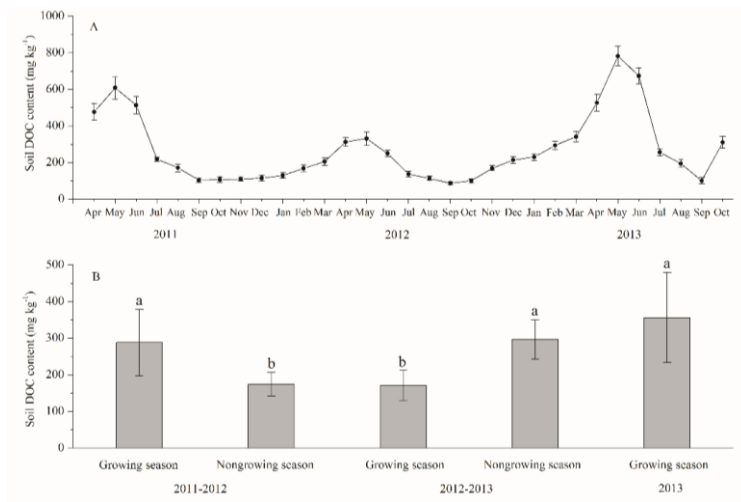


Fig. 5. Changes in the number of bacteria (A), fungi (B), and actinomycetes (C) during the transition between freezing and thawing periods (mean \pm s.e.; $n = 15$). The sampling time during the freezing period was on 15 March and during the thawing period was on 15 May each year. Different lowercase letters indicate significant differences of the interaction effects between season and year according to two-way ANOVA ($p < 0.05$).

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

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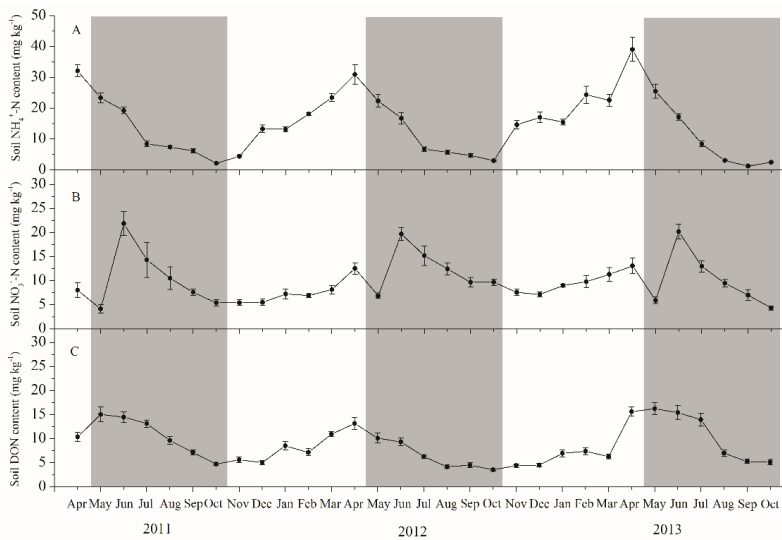
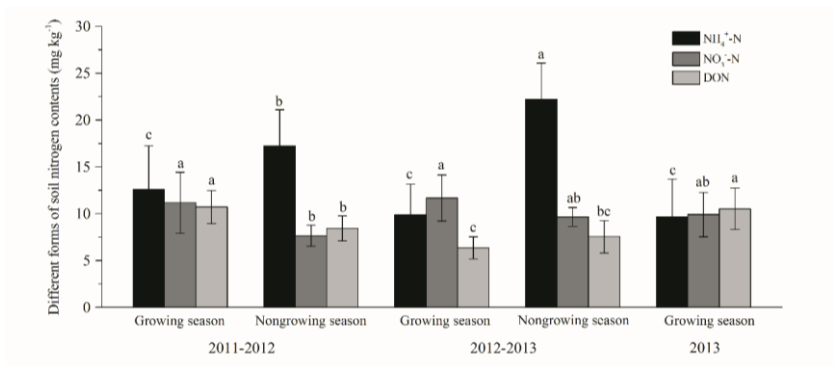


Fig. 7. Dynamics of NH₄⁺-N(A), NO₃⁻-N(B), and DON(C) in soils of the alpine meadow from April 2011 to October 2013 (mean ± s.e.; n = 15).

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10 Fig. 8. Changes in $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and DON of growing and nongrowing seasons from 2011 to 2013 (mean \pm 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 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 determined via Duncan test ($p < 0.05$).

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