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 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 compositions were measured by the dilution-plate method. Fungi and actinomycetes dominated the microbial community during the non-growing seasons, and the number of bacteria increased considerably during the early growing seasons. Trends of consistently increasing MBCConsistently increasing trends of MBC and available N pools were observed during the

non-growing seasons. MBC sharply declined during soil thaw and was accompanied by a peak of available N pool. Induced by soil temperatures, significant shifts in the structure and functions of microbial communities were found 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 interannual dynamics were observed between microbial biomass and available N pools, and soil temperature and water condition were the primary environmental factors driving these year-to-year fluctuations. Under the background of changing climate, the seasonal soil microbial activity and nutrient supply patterns will be further changed, having important implications to the productivity and biodiversity of alpine ecosystems.

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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 and nutrient cycles ~~during~~ in both ~~the~~ growing and non-growing seasons in Arctic and alpine ecosystems are limited. Particularly, the intraannual biogeochemical cycles affected by changing 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 −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 on the microbial activity during winter can improve the understanding nutrient supplies for plants and microbes during the subsequent growing season.

Previous studies suggested that the fungal/bacterial ratio of 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 structure and function 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 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 change from winter to summer. For example, winter microbes use dead plant materials, whereas plant root exudates supplied available C for summer microbes (Lipson et al., 2002; Schmidt et al., 2007). These microbial community changes bewteen winter and

summer might play a key role 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 were observed during winter time, followed by a reduction of microbial biomass during winter-spring transition when the soil thawed (Brooks et al., 1998; Lipson et al., 1999; Schmidt and Lipson, 2004; Miller et al., 2009). Moreover, the decrease of microbial biomass is linked to a pulse of N avilability when soils thaws, 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 avilable 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 on exploring the changes in microbial and N pools ~~during the summer growing seasons in these seasonal frozen ecosystems~~ during both summer and winter across several years in alpine ecosystems are few ~~(Edwards and Jefferies, 2013)~~. Thus, the annual patterns of microbial biomass and N pools and their responses to seas~~n~~onal 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 non-growing 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 relationships between soil microbial biomass and available N pools in the seasonal 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. 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 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~~ sampled, namely located at the upper, middle, and lower part of ~~in~~ the alpine meadow. ~~(top, middle, and bottom of the meadow), and f~~Five replicates at each site were collected~~. , and The~~ 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). Given that plant roots were 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 fifteen soil samples were collected ~~at each site~~ during each sampling time. The upper 1–2 cm of the surface materials (i.e., living plant roots and litter) were removed from the soil samples. During the ~~winter~~ cold periods (i.e., November to April), the samples were collected by using a portable permafrost drill. The frozen soil samples were cut into little pieces (< 1 cm^3) with a knife and hammer, and the large root and stick 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

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divided into three subsamples for further analysis. All the samples were processed at the laboratory of Chengdu Insititute of Biology, CAS, within two days of sampling.

3.3 Soil temperature measurement

Soil temperatures were measured at the central part of each location used for soil sampling. The soil temperature was recorded at 10 cm depth 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 by the datum of the nine loggers. The mean temperature of the growing season was calculated by the mean daily temperatures from 1th May to 31th October, and that of the non-growing season was calculated by the mean daily temperatures from 1th November to 30th April.

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

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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 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 Soil microbial biomass and community analyses

The 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 (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 the jar was covered with a sterile rubber plug and oscillated for 10 min to make a stock solution. Serial diluent was made from the stock solution. The 10^{-5} and 10^{-6} dilution ratios of

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the serial diluent were selected for 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 bacteria and actinomycetes, and at 25 ℃ for 3–5 days for the fungi. The CFUs of different microbes were counted under a microscope (Li, 1996).

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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 intraannual differences between the growing season (i.e., datum from May to October were used as a sample set; n=90) and non-growing season (i.e., datum from November to April were used as a sample set; n=90), and interannual differences among three years. Two-way ANOVA was performed via mixed-effects model, with season and year ~~as~~ specified as fixed effects~~fixed factors~~. For analyses of the microbial community shifts during the transition between non-growing and growing seasons, ~~Differences~~ differences in the number of bacteria, fungi, and actinomycetes between the late non-growing season (i.e., in March) and early growing season (i.e., in May) for two years (2012 and 2013) were determined via two-way ANOVA, with season and year specified as fixed effects. Pearson correlation analysis was then performed to analyze the correlation of the MBC ~~of~~ with SWC ~~with~~ and of that ~~of~~ with the DOC during the non-growing and growing seasons. Significances 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.

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4 Results

4.1 Soil temperature and water content

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 seasons (November to April, Fig. ~~1~~2). In addition, the soil was frozen (below 0 ℃) for 125 days and 165 days during 2011–2012 and 2012–2013, respectively; the soil was deeply frozen (below −5 ℃) for 32 days and 36 days during 2011–2012 and 2012–2013, respectively. ~~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 season (Fig. ~~2A~~3A), and SWC in the 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 non-growing season of 2011–2012 (64.73 % ± 2.22 %) and those in the 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 ~~($p < 0.05$)~~ 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 non-growing

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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 non-growing season (Fig. ~~3A~~4A). However, a significant decreasing trend was observed in February when the soil temperatures were the lowest (below −5 °C). In addition, the MBC values in the non-growing seasons were consistently higher than those in the growing seasons. The mean MBC value during the 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. ~~4~~5). 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 difference of MBN was not significant ($F = 0.06$, $p = 0.80$~~$p > 0.05$~~; Table 1). Its seasonal and interannual dynamics were similar to those of the MBC, and its annual peak generally occurred in April or May. Furthermore, no significant difference was observed between the mean MBN values during the growing season of 2013 and those of 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. ~~4~~5).

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~~6). 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~~6).

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 annually occurred in May and showed a diminishing trend during the growing season and increasing trend during the non-growing season (Fig. ~~6A~~7A). ~~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~~7B). 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-growing season (April), and then sharply reduced during the early growing season, and finally had an increasing trend during the non-growing season (Fig. ~~7A~~8A). The NH_4^+–N content in the non-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~~9).

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 non-growing seasons and increased initially before decreasing during the growing seasons (Fig. ~~7B~~8B). Furthermore, ~~a significantly~~an 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 non-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~~9).

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 non-growing season (Fig. ~~7C~~8C). 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~~9).

5 Discussion

5.1 Seasonal microbial biomass and available nitrogen dynamics

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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 and 7). Generally, the soil MBC and available N pools both increased at the beginning of the early 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 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 ℃~~, and these communities retained their high activity in alpine soils during the cold periods~~. Thus, an accumulation of inorganic and organic N pools occurred during the long and cold non-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 non-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 non-growing and growing seasons was similar to the changes of MBC in other ~~arctic~~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

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(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 anaerobic soil conditions are established as soils become flooded with liquid water during the late soil thaw. However, increasing DOC and inorganic N (NH_4^+–N and NO_3^-–N) contents were observed in our study during the non-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 the microbial community compositions in the non-growing seasons and those in the growing seasons (Fig. ~~5~~6). Similar to other alpine meadows, the 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 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 the soils completely thawed but 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 study, the inorganic N and DON contents both showed an increasing trend during the non-growing seasnon, 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. 78). An obviouslysignificantly increasing process trend of NH_4^+–N was found during the early soil thaw. On the one hand, 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 snow meltingthawing of this period is an important source of NH_4^+–N (Williams and Tonnessen, 2000). At the beginning of the growing season, the NH_4^+–N content sharply decreased partly because of the alpine meadow plants preferred 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, playing a crucial role in maintaining the rich biodiversity of the alpine meadow ecosystem (Qin et al., 2003; Petchey and Gaston, 2006).

5.2 Interannual microbial biomass and available nitrogen dynamics

Significant year-to-year 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 season in 2012–2013 were significantly higher than that 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, a warmer and ~~dry~~ drier non-growing season was observed ~~during~~ 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)~~in late autumn and early winter~~. These environmental variations might

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contribute to the reduction in soil microbial biomass during the 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 long cold season will play a crucial role in the accumulation 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 change of soil N pool. Soil NH_4^+–N and DON had a consistent interannual variation with soil MBC during the non-growing season. However, they showed an incompletely consistent 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

An increasing trend of soil MBC and available N pools was found in non-growing seasons compared with growing seasons, with a sharp decline of MBC 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 of 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 with~~plays a crucial role in~~ the accumulation of inorganic N pools but also is an important soil organic N pool itself. Thus, the interannual dynamic of soil microbial biomass substantially affects the year-to-year differences in 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. Given the changing climate of alpine ecosystems, the soil microbial activity and nutrient supply patterns will be further changed, 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 and their responses to the environmental change. 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.

10 Acknowledgements

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Table

Table 1. Results from two-way ANOVA comparing growing season (May to October) and non-growing 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
$NH4^{+}$–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 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

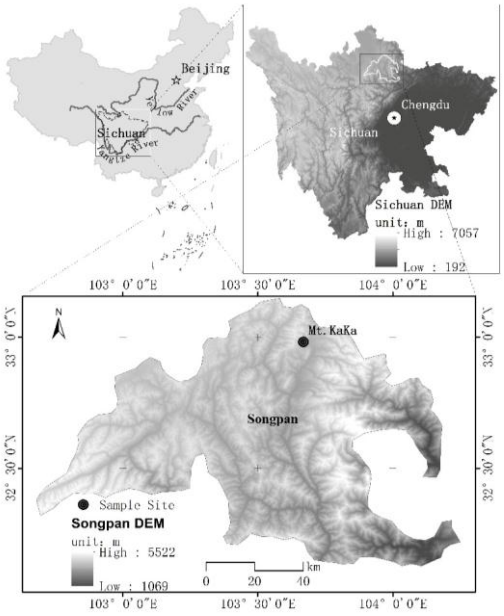

Fig. 1. Location of the study site

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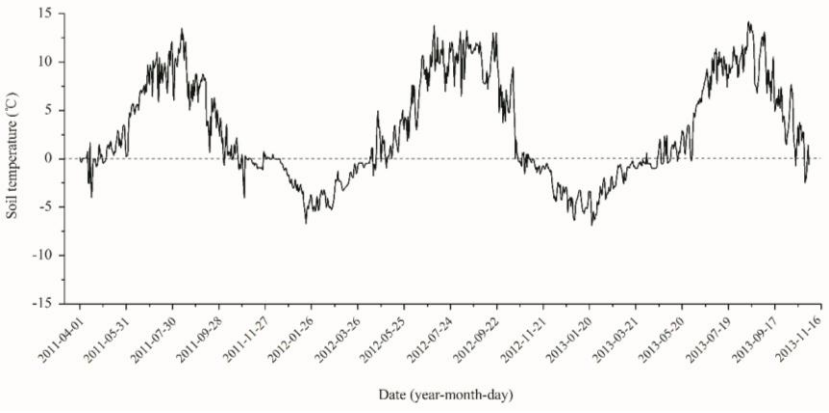

Fig. ~~1~~2. Mean daily soil temperature in the alpine meadow from April 2011 to October 2013. Thermochron iButton data loggers were placed at 10 cm soil depth to obtain automatic readings every 60 minutes, and the mean daily soil temperature was calculated every day.

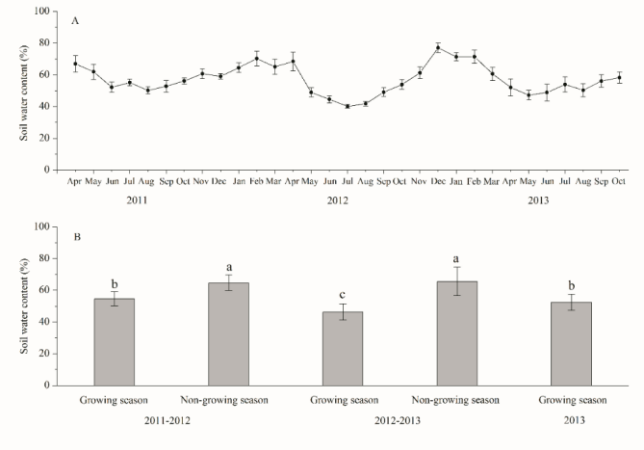

Fig. ~~2~~3. Dynamics of soil water content (A; mean ± s.e.; n = 15) and its seasonal and interannual changes (B; mean ± s.e.; n = 90) from 2011 to 2013.

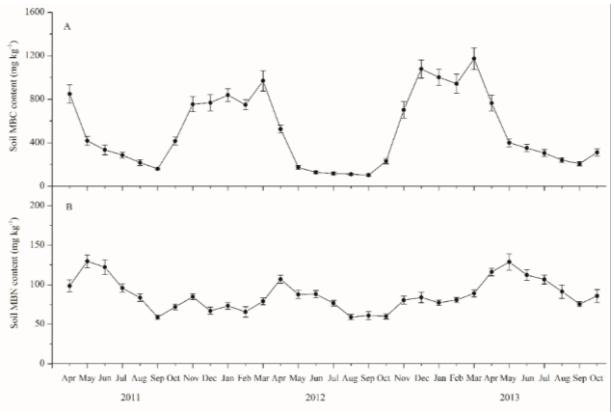

Fig. ~~3~~4. Dynamics of microbial biomass C (A) and N (B) in soils of the alpine meadow from April 2011 to October 2013

(mean ± s.e.; n = 15).

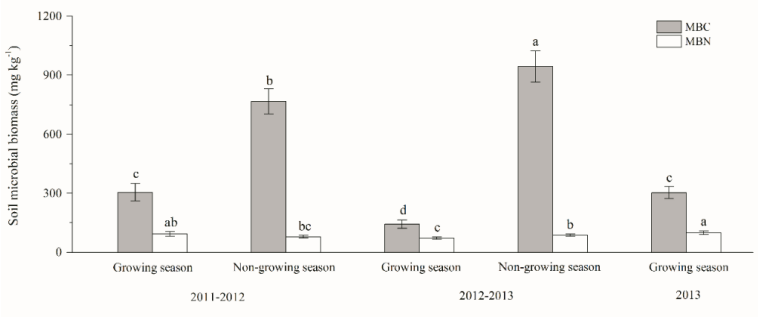

Fig. 45. Changes in microbial biomass C (MBC) and N (MBN) in the growing and non-growing seasons from 2011 to 2013 (mean ± s.e.; n =90). The sampling time was on the 15th day of each month during the growing season from May to October, and during the non-growing season from November to April next year. Seasons and years were compared using two-way ANOVA, and different lowercase letters indicate significant differences of the interaction effects between season and year ~~($p < 0.05$)~~ determined via Duncan test ($p < 0.05$).

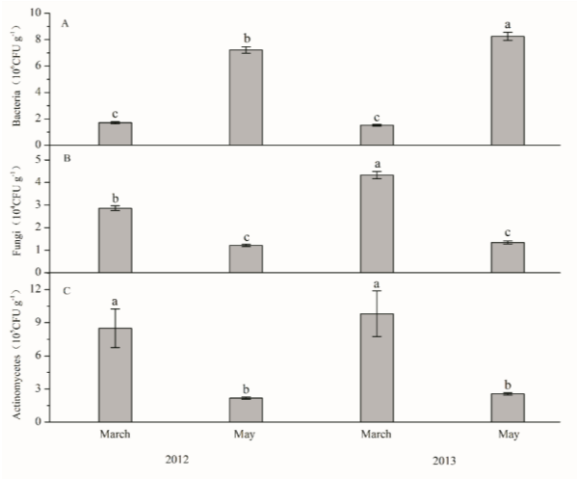

Fig. ~~5.~~6 Changes in the number of bacteria (A), fungi (B), and actinomycetes (C) during the transition between freezing and thawing periods (mean ± s.e.; n = 15). The sampling time during the freezing period was on 15 March and during the thawing period was on 15 May each year. Different lowercase letters indicate significant differences of the interaction effects between season and year ~~($p < 0.05$)~~ according to two-way ANOVA ($p < 0.05$).

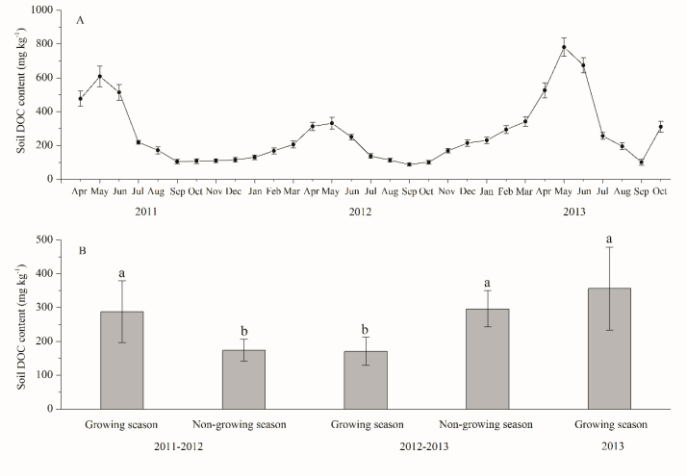

Fig. ~~6~~7. Dynamics of dissolved organic C (A; mean ± s.e.; n = 15) and its seasonal and interannual changes (B; mean ± s.e.; n = 90) from 2011 to 2013.

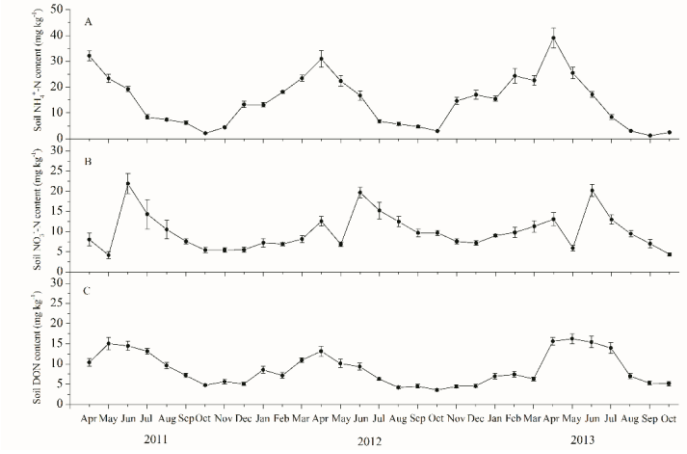

Fig. ~~7~~8. Dynamics of NH_4^+–N(A), NO_3^-–N(B), and DON(C) in soils of the alpine meadow from April 2011 to October 2013 (mean ± s.e.; n = 15).

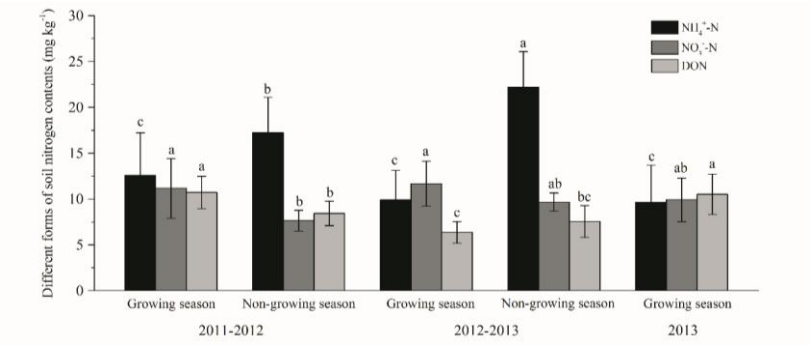

Fig. ~~8~~9. Changes in NH_4^+–N, NO_3^-–N, and DON of growing and non-growing seasons from 2011 to 2013 (mean ± s.e.; n =90). The sampling time was on the 15th day of each month from May to October during the growing season and during the non-growing season from November to April next year. Seasonal 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 ($p < 0.05$).