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

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Abstract. Soil microbial activity occurs seasonally in frozen alpine soils during cold seasons and plays a crucial role in available N pool accumulation in soil. The intra- and interannual patterns of microbial and nutrient dynamics reflect the influences of changing weather factors, and thus provide important insights into the biogeochemical cycles and ecological functions of ecosystems. We documented the seasonal and interannual dynamics of soil microbial and available N in an alpine meadow in the eastern part of Qinghai–Tibet Plateau, China between April 2011 and October 2013. Topsoil samples were collected in the middle of each month and were analyzed for water content, microbial biomass C (MBC) and N (MBN), dissolved organic C and N, and inorganic N; soil microbial community composition was measured by the dilution-plate method. Fungi and actinomycetes dominated the microbial community during the nongrowing seasons, and the 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 soil temperatures, significant shifts in the structure and functions of microbial communities were observed during the winter–spring transition and largely contributed to microbial reduction. Divergent seasonal dynamics of different N forms showed a complementary nutrient supply pattern during the growing season. 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

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 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 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 (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 ℃ (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 ecoysystems (Schmidt et al., 2007; Buckeridge and Grogan, 2008; Buckeridge et al., 2013).

In Arctic and alpine soils, microbial biomass and avilable 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 avilability 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 period provides an important available N source to plants, particularly in N-limited ecosystems (Lipson et al., 1999; Miller et al., 2009; Buckeridge and Grogan, 2010). However, despite ample evidence of soil microbial activity and nutrient mineralization during the winter and/or summer months in Arctic and alpine regions (Edwards et al., 2006; Schmidt et al., 2007; Miller et al., 2009; Edwards and Jefferies, 2013; Buckeridge et al., 2013), studies that 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

The study was performed in the alpine belt of Songpan County, which belongs to the Minshan Mountain in the eastern part of the Qinghai–Tibet Plateau, China. Accoding to the records 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 ℃ 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 during the growing season (i.e., during early May to late October according to the plant phenology observation in the alpine meadow from 2011 to 2013). Early flowering plants, such as *Primula sikkimensis*, *Androsace umbellate*, and *Caltha palustris*, dominate the community as soon as the snow melts; *Polygonum macrophyllum*, *Ranunculus tanguticus*, and *Carex melanocephala* dominate the middle growing season; and *Saussurea hieracioides* and *Gentiana sino-ornata* usually dominate the late growing season (Xu, unpublished data, collected 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; 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 the soil spatial heterogeneity, three adjacent sites, approximately 100 m apart (centered at 32°59′ N, 103°40′ E, 3980 m a.s.l.) were selected. One site is located at the upper part of the alpine meadow, one at the middle part, and one at the lower part. Five replicates were collected from each site. The replicates from each site were 10 m apart from one another. The samples collected from the three sites (n = 15) at each sampling time were used for the statistical analyses. 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 performed, and 15 soil samples were collected during each sampling time. The 1–2 cm layer of the surface material (i.e., 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 cm^3) 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 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 1 May to 31 October, and that of the nongrowing season was calculated by the mean daily temperatures from 1 November to 30 April.

3.4 Soil water content and nutrient analyses

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

and ultraviolet spectrophotometry (Norman et al., 1985), respectively. Dissolved organic N (DON) was calculated by subtracting dissolved inorganic N (NH_4^+–N and NO_3^-–N) from TDN.

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

3.5 Soil microbial biomass and community analyses

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; 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. 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; Igbinosa, 2015). Soil diluent (1 mL) and medium (10 mL) at 45–50 ℃ were injected into the plates and cultured at 28 ℃ for 7–10 days for the bacteria and actinomycetes. Another medium with same components was prepared at 25 ℃ 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 variance analysis. Microbial and nutrient variables were analyzed to test the intra-annual differences between the growing season (i.e., data from May to October were used as a sample set; n = 90) and nongrowing season (i.e., data from November to April were used as a sample set; n = 90). Their interannual differences were also tested. Two-way ANOVA was performed via mixed-effects model, with season and year specified as fixed effects. For the analysis of the microbial community shifts during the transition between nongrowing and growing seasons, differences in the number of bacteria, fungi, and actinomycetes between the late nongrowing season (i.e., in March) and early growing season (i.e., in May) were determined via two-way ANOVA. This procedure was performed for 2 years (2012 and 2013), and season and year specified were used as fixed effects. Pearson correlation analysis was then performed to analyze the correlation between MBC and SWC and that between MBC and DOC during the nongrowing and growing seasons. Significant results were determined at the $p < 0.05$ level, and Duncan's test was performed to analyze the significant results of the multiple comparisons to the interaction effects between season and year (SAS Institute Inc., 2008).

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 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

5   showed a decreasing trend during the growing season and increasing trend during 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 % ±2.22 %)

and those in the nongrowing season of 2012–2013 (65.68 % ±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

10   46.43 % ±2.28 % during 2012–2013.

4.2 Soil microbial biomass and community

Significant differences in MBC between seasons ($F = 860.28$, $p = 0.00$) and years ($F = 4.46$, $p = 0.01$) were observed in

the soils of the alpine meadow (Table 1). The annual peak of MBC occurred in the late nongrowing season (March) then

sharply decreased, indicating a diminishing trend during the growing season. The MBC reached a minimum value in the

15   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 °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 mg kg^{-1} ±80.01 mg kg^{-1}) was

significantly ($p < 0.05$) higher than those in the other seasons. Meanwhile, the mean MBC value during the growing season in 2012–2013 (i.e., 143.53 mg kg^{-1} ± 20.99 mg kg^{-1}) was the lowest (Fig. 4C). The MBC during the growing season had highly significant positive correlation with SWC ($p < 0.01$, $r = 0.62$; Table 2).

The soil MBN values had significant interannual differences ($F = 11.06$, $p = 0.00$), but the seasonal differences among MBN values were nonsignificant ($F = 0.06$, $p = 0.80$; Table 1). The seasonal and interannual dynamics of MBN were similar to those of MBC, and its annual peak generally occurs 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 mg kg^{-1} ± 5.93 mg kg^{-1}) was observed during the growing season in 2012–2013 (Fig. 4C).

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

4.3 Soil dissolved organic carbon

Significant interannual differences ($F = 5.50$, $p = 0.01$) in soil DOC contents were observed, and the seasonal dynamics of DOC had no significant difference from each other ($F = 0.04$, $p = 0.85$; 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 mg kg^{-1} ± 32.59 mg kg^{-1}) and growing season in

2012–2013 (170.85 mg kg^{-1} ± 41.19 mg kg^{-1}) had no significant differences ($p > 0.05$), but those were significantly

lower than that in other seasons ($p < 0.05$; Fig. 6B). Furthermore, the DOC during the growing season had highly

5   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 ($F = 28.3$, $p = 0.00$ and

$F = 3.20$, $p = 0.04$; Table 1). The annual peak of the NH$_4^+$–N content occurred in the late nongrowing season (April),

and then sharply reduced during the early growing season, and finally had an increasing trend during the nongrowing

10   season (Fig. 7A). The NH$_4^+$–N content in the nongrowing season was significantly higher ($p < 0.05$) than that in the

growing season. The NH$_4^+$–N content during the non-growing season in 2012–2013 (22.21 mg kg^{-1} ± 3.87 mg kg^{-1}) was

significantly higher than that in 2011–2012 (17.23 mg kg^{-1} ± 3.85 mg kg^{-1}), and no significant difference was observed

among the NH$_4^+$–N contents during the growing seasons in 2011–2013 ($p > 0.05$; Fig. 8).

Significant seasonal and interannual differences in soil nitrate N (NO$_3^-$–N) contents were observed ($F = 4.34$, $p = 0.04$

15   and $F = 3.28$, $p = 0.04$; Table 1). The NO$_3^-$–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 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 nongrowing season in 2011–2012 (7.64 mg kg^{-1} ± 1.12 mg kg^{-1}) was the lowest. No

significant difference was observed among the NO_3^-–N contents of the other seasons ($p > 0.05$; Fig. 8).

The DON contents had significant interannual differences ($F = 10.13$, $p = 0.00$), but their seasonal differences were not significant ($F = 0.63$, $p = 0.43$; 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).

5 Furthermore, the mean DON value during the growing season in 2012–2013 (7.53 mg kg^{-1} ± 1.74 mg kg^{-1}) was the lowest, and it was significantly lower than those in the other years ($p < 0.05$; Fig. 8).

5 Discussion

5.1 Seasonal microbial biomass and available nitrogen dynamics

The significant seasonal dynamics of the soil microbial biomass and available N pools were observed in the alpine

10 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; 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

15 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 ℃). This decline implied that the temperature threshold of the survival of these cold-adapted microbial communities was at least −5 °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 ℃. 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 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 (NH_4^+–N and NO_3^-–N) contents was observed during the nongrowing season, implying that available C and N were relatively sufficient and might not restrict 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

5 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

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 is the actinomycetes. Furthermore, the number

10 of bacteria significantly increased during the early growing season after the soils completely thawed. By contrast, number

of fungi and that of actinomycetes declined considerably. This shift in the microbial community may lead to the sharp

decline in MBC during soil thaw, partly because of the C investment per unit volume in fungal cells were threefold larger

than that in bacteria cells (Buckeridge and Grogan, 2008).

In the present study, inorganic N and DON contents both showed an increasing trend during the nongrowing season, and

15 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. 8). An obviously trend of increasing NH_4^+–N content was found during the early soil thaw. Furthermore,

frequent and strong freeze–thaw cycles during this period may contribute to the release of unavailable NH_4^+–N from the

organic and inorganic colloids in alpine soils (Freppaz et al., 2007). Snow melting during this period is an important source of NH_4^+–N (Williams and Tonnessen, 2000). At the start of the growing season, NH_4^+–N content sharply decreased, partly because alpine meadow plants prefer NH_4^+–N (Jaeger et al., 1999; Gherardi et al., 2013). Moreover, strong microbial activity in the soil requires a large amount of NH_4^+–N at increasing temperature (Bowman, 1992;

5   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, 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^-–

10   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 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

15   Significant interannual differences in microbial biomass and available N were observed across the study years. For example, the MBC and NH_4^+–N contents during the 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 soil water conditions is an important environmental driver that affects the microbial biomass in alpine meadows. First, low soil moisture in the growing season causes a decline in plant productivity (Körner, 2003), resulting in a decline of C substrates supplied by plant root exudates and litters. Second, low soil moisture in summer leads to an increased oxidation in the surface soil, thus exerting significant influence on the microbial communities (Blodau et al., 2004), and some of these influences are retained during winter (Edwards and Jefferies, 2013). Notably, the nongrowing season in 2011–2012 was warmer and drier than that in 2012–2013, which might accompanied with more frequent freeze–thaw cycles during the early period of this season (Mellander et al., 2007; Henry, 2008). These environmental variations might contribute to the reduction in soil microbial biomass during the nongrowing 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 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 a long cold season play a crucial role in accumulating 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 interannual change of 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, 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

5   and nutrient dynamics may become more common and pronounced in the alpine meadow in the eastern part of the Qinghai–Tibet Plateau as a result of multiple impacts of climate change, particularly increasing extreme weather events, such as winter warming and heterogeneous precipitation (Edwards and Jefferies, 2013).

6 Conclusions

A trend of increasing soil MBC and available N pools was found in nongrowing seasons. A sharp decline in MBC was

10   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 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 the accumulation of inorganic N pools but also

15   is an important soil organic N pool 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 (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	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 nongrowing seasons

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

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

Figure legends
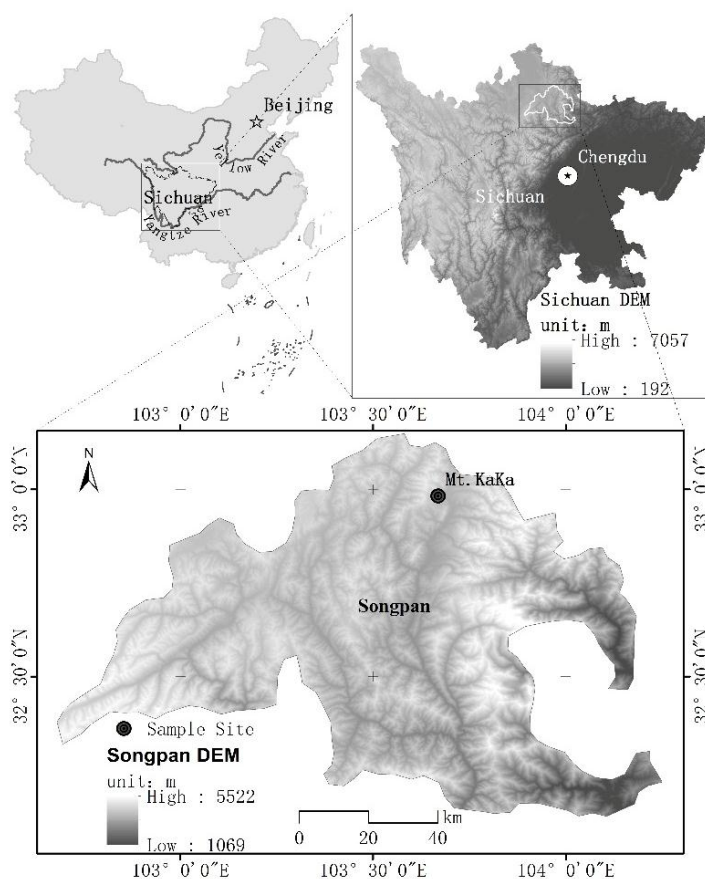
Fig. 1. Location of the study site
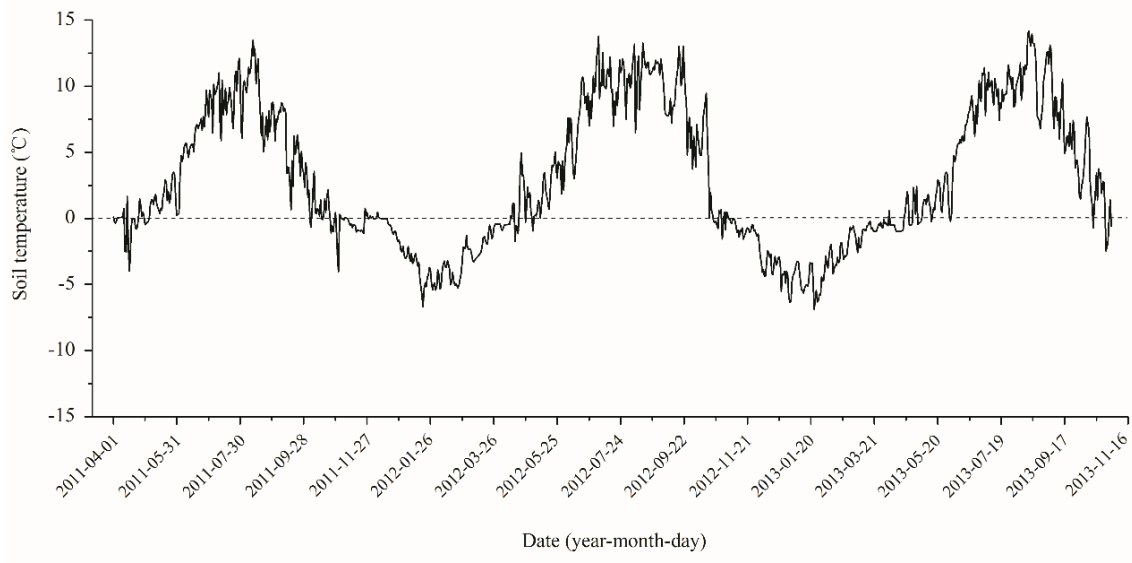
Fig. 2. Mean daily soil temperature in the alpine meadow from April 2011 to October 2013. Thermochron iButton data loggers were placed at 10 cm soil depth to obtain automatic readings every 1 h, and the mean daily soil temperature was calculated every day.
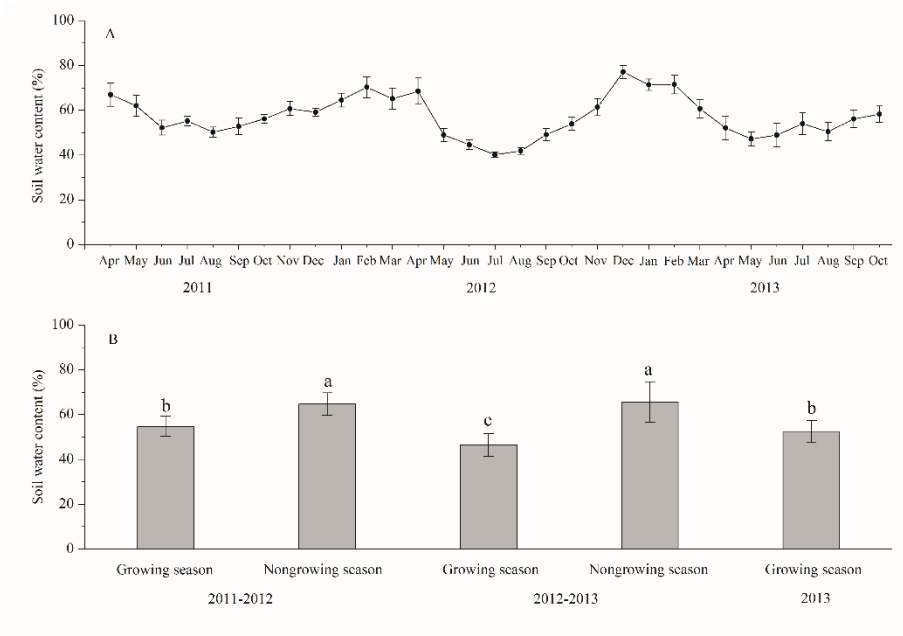
Fig. 3. Dynamics of soil water content (A; mean ± s.e.; n = 15) and its seasonal and interannual differences (B; mean ± s.e.; n = 90) from 2011 to 2013.
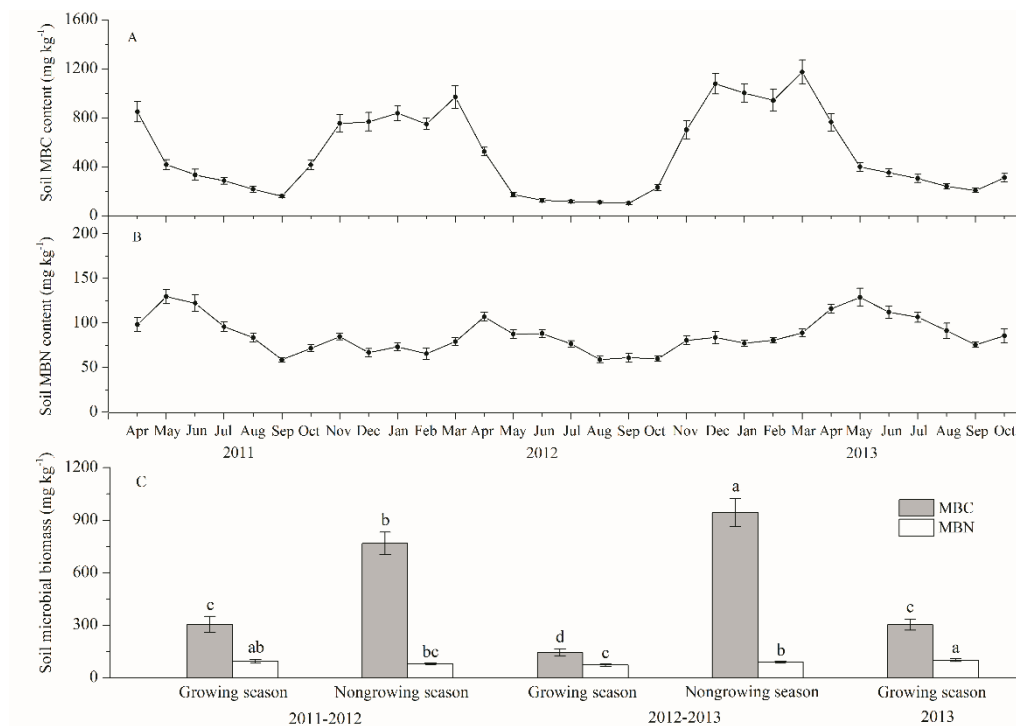
Fig. 4. Dynamics of microbial biomass C and N (A and B; mean ± s.e.; n = 15), and their seasonal and interannual differences (C; mean ± s.e.; n = 90) from April 2011 to October 2013 (mean ± s.e.; n = 90). The sampling time was on the 15th day of each month during the growing season from May to October, and during the nongrowing season from November to April next year. Seasons and years were compared using two-way ANOVA, and different lowercase letters indicate significant differences of the interaction effects between season and year determined via Duncan test (p < 0.05).
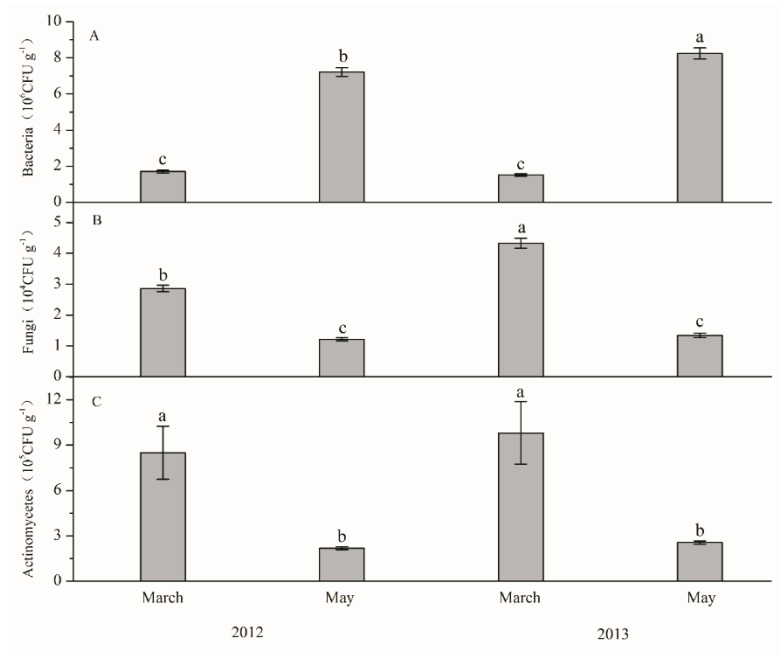
Fig. 5. Changes in the number of bacteria (A), fungi (B), and actinomycetes (C) during the transition between freezing

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 ($p < 0.05$).
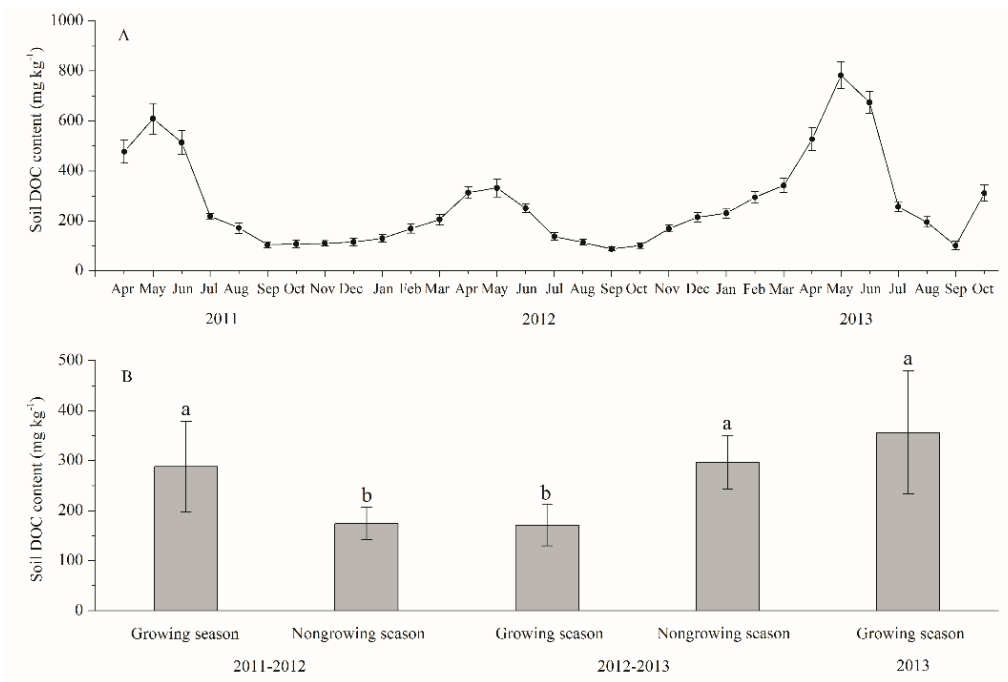
Fig. 6. Dynamics of dissolved organic C (A; mean ± s.e.; n = 15) and its seasonal and interannual differences (B; mean ± s.e.; n = 90) from 2011 to 2013.
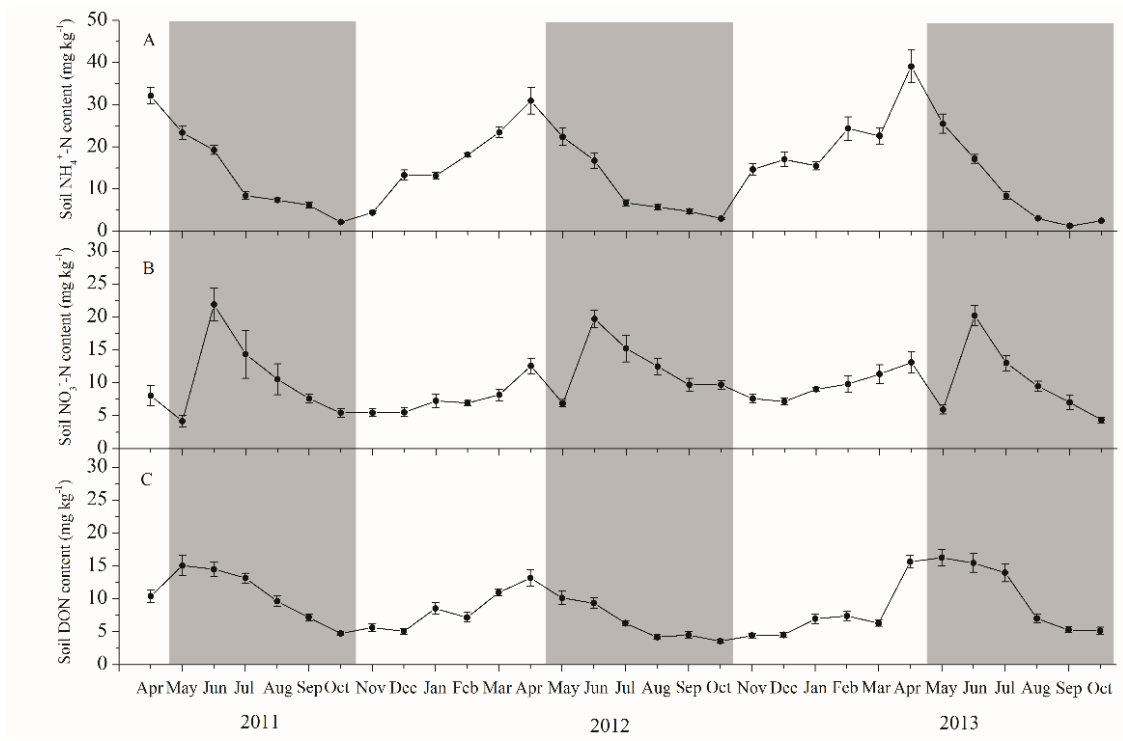
Fig. 7. Dynamics of NH_4^+–N(A), NO_3^-–N(B), and DON(C) in soils of the alpine meadow from April 2011 to October 2013 (mean $\pm$ s.e.; $n = 15$).
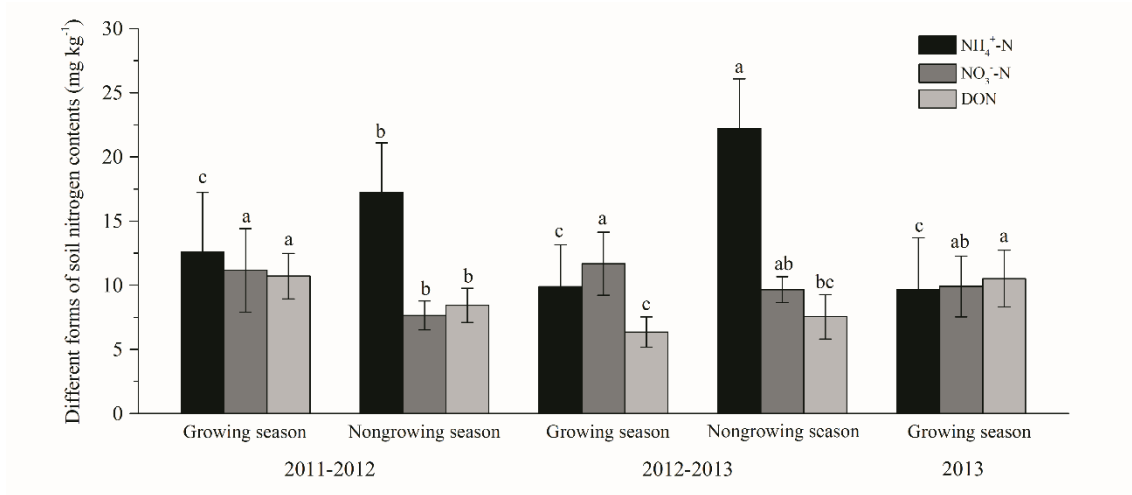
Fig. 8. Changes in NH_4^+–N, NO_3^- –N, and DON of growing and nongrowing seasons from 2011 to 2013 (mean ±s.e.; n =90). The sampling time was on the 15th day of each month from May to October during the growing season and during the nongrowing season from November to April next year. Seasonal and interannual 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 ($p < 0.05$).