Original Research

Comparison of Soil Microbial Biomass and Enzyme Activities among Three Alpine Grassland Types in Northern Tibet

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Received: 22 March 2012
Accepted: 2 October 2012

Abstract

Soil microbial biomass and enzyme activities have an important influence on nutrient cycling. The temporal variation in soil microbial biomass C, N, and enzyme activities during the growing season were determined under three different alpine grasslands in Northern Tibet. The results showed that soil microbial biomass C, and N contents and enzyme activities of the alpine meadow (AM) and the alpine meadow steppe (AMS) sites were much higher than those of the alpine steppe (AS) site. Soil microbial biomass C, N variations were not significantly correlated with the soil temperature and moisture, except that microbial biomass N seemed associated with the microsite where soil temperature was higher. Our results demonstrate that soil temperature was one of most important factors explaining the seasonal variation of microbial biomass N, but how the alpine grassland ecosystem's type affects microbial biomass C, N and enzyme activity are still needed to be clarified by determining other correlative ecological factors and covering prolonged observation periods.

Keywords: microbial biomass, enzyme activity, alpine grassland, Northern Tibet

Introduction

Soil microorganisms play an important role in regulating the litter and organic matter decomposition, biogeochemical cycle, and soil nutrient availability, consequently affecting plant nutrient uptake, growth, and productivity [1, 2]. Microorganisms responsible for the decomposition and mineralization of the organic matter fraction use part of the compounds contained in the residues as sources of nutrients and energy for their biomass formation [3]. Therefore, soil microbial biomass is the living portion of soil organic matter. It generally comprises about 1-5% of organic matter in soil [4]. Temperature, moisture, physical and chemical soil properties, substrate quality, and plant community composition all can affect soil microbial biomass level [5, 6].

Soil enzymes mainly originate from soil microorganisms and play an important role in organic matter decomposition and nutrient cycling [7]. This has been widely accepted as an indicator of changes in belowground processes and as an index of soil fertility [7, 8]. Soil enzyme activity is associated with viable cells and by soil colloids, which are primarily of microbial origin. Hence, any factor that affects soil microbial population will necessarily alter soil enzyme activity [8, 9]. The activity of enzymes is affected by soil environmental factors (e.g., temperature, moisture, soil pH, and oxygen content), by the chemical structure of organic matter and by its location in the soil strata [10].

The region of northern Tibet, generally more than 4,500 m above sea level and with higher than 6 km peaks, stands in the interior of the Tibetan Plateau. Alpine grassland is the dominant ecosystem, occupying about 94% of this region.

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It is not only the most important and largest ecosystem in this area, but also a key resource for supporting local people's subsistence [11]. Alpine ecosystems are critical for studies of global change and monitors of ecological change because they are fragile ecosystems sensitive to climate change and human activities due to the extremely harsh natural environment [12, 13]. The alpine ecosystems are characterized by low oxygen pressure, cold temperatures, low humidity, high levels of solar insolation and UV-B, and extreme temperature cycling across the freezing point [14, 15]. The soil is usually of low microbial activity in these extreme ecological conditions as a result of low temperatures leading to slow mineralization of organic compounds [16].

A number of recent studies examining soil microbial biomass characteristics in alpine ecosystems [17-19], include Djukic et al. [13], showing that the highest amounts of microbial biomass were found at sites with high pH and low C:N ratio, and the lowest amounts were found at sites with low pH and high C:N ratio in the Austrian Limestone Alps. In addition, Lipson et al. [17] and Jefferies et al. [18] found microbial biomass reaches a peak in late winter and then declines during the period when soil temperature rises to 0ºC at some alpine sites. Furthermore, Tscherko et al. [19] found that the increase in rhizosphere microbial biomass during succession might be related to a shift from vegetation dominated by annuals in early succession to vegetation dominated by perennials in late succession in the Ötz valley (Tyrol, Austria), whereas soil microbial biomass was found to be affected neither by elevated CO_2 [20] nor affected by the single freeze/thaw event [14, 15] in some alpine grassland ecosystems. Thus, the changes of microbial biomass were quite complex because of extreme environments in alpine regions.

Currently little is known about the microbial biomass and enzyme activity, and the factors affecting them in alpine grassland soil in Northern Tibet. The present study includes three types of alpine grasslands: alpine meadow (AM), where vegetation coverage is 70% with wet soil condition, alpine meadow steppe (AMS), where vegetation coverage is 40% with moderate wet soil condition; and alpine steppe (AS), where vegetation coverage was less than 20% with dry soil conditions. They were selected to compare the variations in microbial biomass C, N, and enzyme activity during the growing season, and to analyze their relationships with soil environmental factors, including soil temperature, soil moisture, and so on.

Materials and Methods

Site Description

This study was carried out in the permanent plots of the Alpine Steppe and Wetland Ecosystem Observation and Experiment Station (30º57′N, 88º42′E, 4675 m a.s.l.) in Shenzha county, northern Tibet, China. This area is located in a cold and semi-arid plateau monsoon climate region. The natural environment is extremely harsh, and the soil is generally quite poor in nutrients. According to the data from the meteorological station near the study site, the annual mean air temperature was 0ºC, the mean air temperature during January was -10.1ºC, and the mean air temperature during July was 9.6ºC. There was no absolute frost-free season and the frosty period was up to 279 days. The annual mean time of solar radiation was 2,916 hours. Average annual precipitation was 300 mm, most of which occurred from May to September.

The region of the Shenzha Alpine Steppe and the Wetland Ecosystem Observation and Experiment Station is located in a typical alpine grassland ecotone in Northern Tibet, and different types of land use coexist in the study area. There are nearly 4,200 ha grassland developed into a typical alpine meadow (AM) because of the adequate water supply from snow melt, and approximately 600 ha grassland developed into a typical alpine steppe (AS) due to drought. And the transition zone between alpine meadow and alpine steppe developed into alpine meadow steppe (AMS) because of the moderately wet soil conditions. Vegetation coverage of AM was dominated by *Kobresia humilis* and occasionally distributed with individuals of *Oxytropis* spp., *Gentiana squarrosa* and *Aster tataricus* L. In AMS, the distributed species were similar to AM, but coverage was lower. And the AS was mainly *Stipa purpurea*, *Artemisia capillaris* Thunb, and *Rhodiola rotundaia* assemblages. These three different types of alpine grasslands were selected to identify microbial biomass C, N, and enzyme activity in this study.

Soil Sampling and Analyses

Soils were sampled from each type of grassland during May-September to capture the dynamic variabilities during the 2010 growing season. Three replicate samples with a depth of 0-15 cm were collected per month at each grassland site for measuring the microbial biomass. And three replicate samples at each grassland site were collected in August for measuring enzyme activity. Soil samples were kept at 4ºC in cool boxes during transport to the laboratory. Soil samples for microbial biomass and enzyme activity were stored in a refrigerator at 4ºC and processed within 10 days. In addition, soil samples for chemical and physical properties were determined during the first sampling. Soil physical and chemical properties were determined using regular analysis methods [21]. The details on soil physical and chemical properties among three alpine grassland sites have been described in our previous study [22]. Soil temperature and moisture were automatically monitored by HOBO weather stations (Onset Corp., Pocasset, MA, USA) every 30 min.

Measurement of Microbial Biomass C, N

Soil microbial biomass C and N were determined by the chloroform fumigation extraction method with some modifications [23, 24]. Briefly, two portions equivalent to 10 g soil were taken from the soil samples. One portion was placed in 50 ml beakers and kept in a vacuum desiccator containing a 100 ml beaker with 25 ml alcohol-free chloroform. Another desiccator was maintained without chloroform and both kept for 24 h at 25ºC in the dark. The fumigated samples were then evacuated with a vacuum pump until the chloroform had completely evaporated. Following fumigant removal, the soil was extracted by horizontal shaking at 300 rpm with 50 ml 0.5 M K_2SO_4 for 30 min. Simultaneously, the non-fumigated portion was extracted under the same conditions. Organic C and total N in fumigated and unfumigated extracts was measured by a Vario TOC cube total organic carbon analyzer (Elementar Analysensysteme GmbH., German). We calculated soil microbial biomass C and N by dividing the difference of total extractable C and N between fumigated and unfumigated samples by the conversion factors 0.45 for biomass C and 0.54 for biomass N [23-25].

Measurement of Enzyme Activity

Selected soil enzyme activities were assayed on moist samples throughout. Invertase (EC 3.2.1.26) was measured by incubating 5 g soil with 15 ml of 8% sucrose for 24 h at 37ºC. The suspension reacted with 3,5-dinitrosalicylic acid for the colorimetric assay and absorbance was read at 508 nm [26]. Urease (EC 3.5.1.5) and protease (EC 3.4.21.19) activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M Na-benzoylargininamide (BAA) were used as substrates, respectively. Two ml of buffer and 0.5 ml of substrate were added to 0.5 g of the soil sample, which was incubated at 30ºC (urease) or 37ºC (protease) for 90 min. Both activities were determined by the NH_3-N released [27]. Dehydrogenase (EC 1.1) activity was determined by reduction of triphenyltetrazolium chloride to triphenylformazone using the method of Yao and Huang [26]. Enzyme activities were determined in duplicate per sample and expressed by the dry weight equivalent soil.

Statistical Analyses

Two-way ANOVA adapted the alpine grassland type and the sampling time as the main factors for analyzing the following variables: microbial biomass C, microbial biomass N and microbial biomass C/N ratio. One-way ANOVA was used to test the differences of soil invertase, urease, protease and dehydrogenase activities among the three alpine grasslands, and an LSD test was used to distinguish difference at P=0.05. The relationship between microbial biomass C and N, and soil environmental factors was tested using linear correlation analysis. All analyses were performed using the SPSS 11.5 statistical software package (SPSS Inc., USA).

Results

Soil Temperature, Moisture

Monthly soil temperature (ranging from 7.4ºC to 15.5ºC) and moisture (ranging from 2.6% to 29.6%) showed seasonal variations across all alpine grassland sites and sampling times (Fig. 1). Over the period of field sampling (May-September, 2010), the average soil temperature at 10 cm depth were 10.3ºC in the AM site, 11.7ºC in the AMS site, and 13.6ºC in the AS site, respectively. And the mean value of soil moisture at 10 cm depth were 20.0% in the AM site, 9.6% in the AMS site, and 6.9% in the AS site, respectively.

Microbial Biomass C, N

Monthly soil microbial biomass C also showed seasonal variations ranging from 103.3 mg·kg^{-1} to 285.5 mg·kg^{-1} across all alpine grassland sites and sampling times (Fig. 2a). The highest microbial biomass C was obtained in July in AM and AMS site, were 260.6 and 285.5 mg·kg^{-1}, respectively. And the highest microbial biomass C (137.9 mg·kg^{-1}) in AS site was obtained in August. At all three sites the dynamics of soil microbial biomass N (ranging from 17.7 to 46.8 mg·kg^{-1}) exhibited a similar "low-high-low" pattern during the growing season (Fig. 2b). The amounts of microbial biomass N increased from May and reached maximum in July for the AM site (45.0 mg·kg^{-1}) and in August for both AMS (46.8 mg·kg^{-1}) and AS (36.0 mg·kg^{-1}) sites, and subsequently decreased quickly in September. Among the three

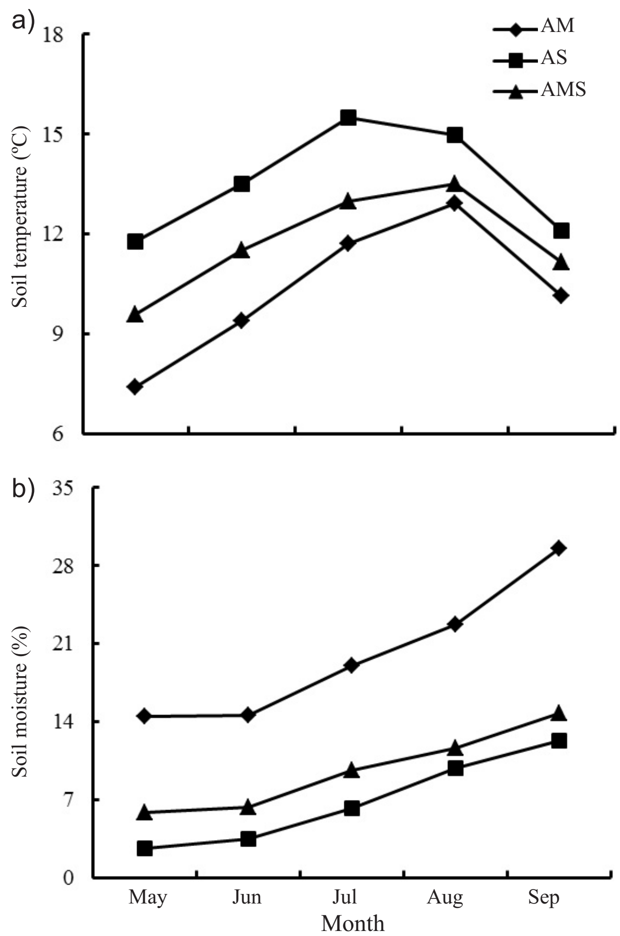

Fig. 1. Temporal variations in soil (a) temperature and (b) moisture under three contrasting alpine grassland ecosystems across the plant growing season.

Table 1. Two-way ANOVA for the soil Microbial biomass C, N, and C/N with significant effects in italics.

Main effects	d.f.	Microbial biomass C		Microbial biomass N		Microbial biomass C/N	
		F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
AGT	2	*17.95*	*0.000*	*7.14*	*0.003*	1.23	0.307
ST	4	1.00	0.422	*7.12*	*0.000*	*3.23*	*0.026*
AGT×ST	8	0.24	0.979	0.37	0.927	0.64	0.736

AGT – Alpine grassland type, ST – Sampling time

alpine sites, the mean values of microbial biomass C and N during the growing season of the AS site were much lower than those of the AM and AMS sites. The microbial biomass C of the AM and AMS were 1.8 and 1.9 times higher, and the microbial biomass N of the AM and AMS were 1.4 and 1.5 times higher, respectively, compared to the AS site. Results from two-way ANOVA demonstrate that alpine grassland types had a significant effect on the microbial biomass C and N concentrations, the sampling time had a significant effect on microbial biomass N ratios, and their interaction had no significant effect on microbial biomass C, and N contents (Table 1).

Soil microbial biomass C accounted for 1.8%, 1.7%, and 1.1% of the SOC content, and the microbial biomass N contributed 3.2%, 2.3%, and 2.8% to total N in AM, AMS, and AS sites, respectively (Table 2). The ranges of soil microbial biomass C/N ratios for the AM, AMS, and AS sites were 4.6-11.6, 3.8-7.4, and 4.6-9.2, respectively. In general, the ratios were low from June to August for the three sites, and high in May and September (Fig. 2c). The mean soil microbial biomass C/N values during the growing season were on the order of AM>AMS>AS, were 6.5, 6.4, and 5.1, respectively (Table 2). In addition, linear regression analyses showed that soil microbial biomass C was significantly correlated with microbial biomass N (r^2 = 0.33, P < 0.05) (Fig. 3).

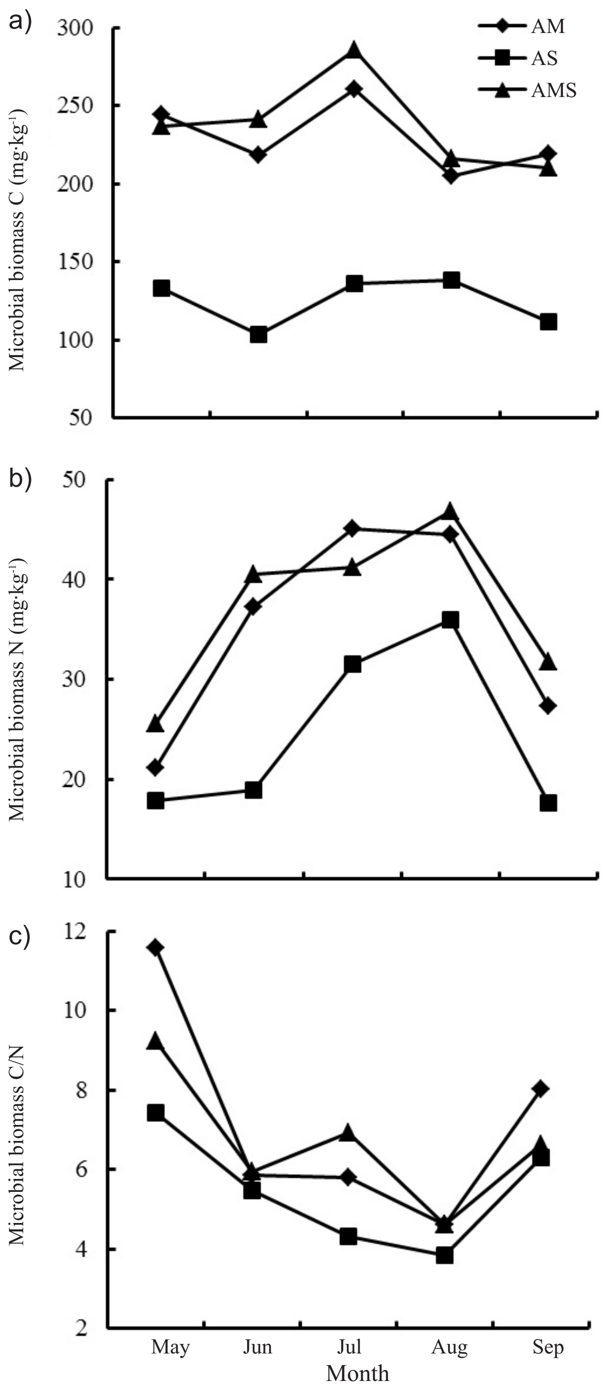

Fig. 2. Temporal variations in soil (a) microbial biomass C, (b) microbial biomass N and (c) microbial biomass C/N ratios under three contrasting alpine grassland ecosystems across the plant growing season.

Enzyme Activities

The average of soil invertase, urease, protease, and dehydrogenase activities in August grossly was in the order of AMS>AM>AS (Table 3). Furthermore, one-way ANOVA analysis showed that soil invertase and dehydrogenase activities had no significant difference among the three alpine grassland sites. Soil urease activity of the AS site was significantly lower than that of the AMS and AM sites, and soil protease activity of the AMS site was significantly higher than that of the AM and AS sites.

Discussion

The grassland ecosystem type can profoundly impact soil microbial biomass through the alteration of abiotic and biotic characteristics of soils and soil physical and chemical properties [28, 29]. The region of the Shenzha Alpine Steppe and the Wetland Ecosystem Observation and

Table 2. Soil microbial biomass C (MBC), microbial biomass N (MBN), soil organic carbon (SOC), and total nitrogen (TN) in three alpine grasslands during the 2010 growing season.

Alpine grassland type	MBC $mg \cdot kg^{-1}$	MBN $mg \cdot kg^{-1}$	SOC $g \cdot kg^{-1}$	TN $g \cdot kg^{-1}$	MBC/MBN	MBC/SOC	MBN/TN
AM	229.10	35.03	13.11	1.09	6.54	0.02	0.03
AMS	237.85	37.19	13.68	1.63	6.40	0.02	0.02
AS	124.38	24.38	11.12	1.03	5.10	0.01	0.02

Experiment Station provided a good background for comparison of the grassland type effects on soil microbial biomass C and N and enzyme activity, because it is located in a typical alpine grassland ecotone and different types of land use coexist. Thus, we selected three different types of alpine grasslands, including alpine meadow (AM), alpine meadow steppe (AMS), and alpine steppe (AS), whose difference in vegetation and soil environmental conditions to compare the variations in microbial biomass C and N, and enzyme activity during the growing season, and to analyze the influences of soil environmental factors, including soil temperature, soil moisture, and so on.

Soil microbial biomass C and N exhibited obvious seasonal variation trends among different alpine grassland ecosystem types during the 2010 growing season (Fig. 2). Our results generally agree with Barbhuiya et al. [30], Goberna et al. [31], and Sugihara et al. [32], who also observed the seasonal fluctuations of microbial biomass C and N from different regions. A comparison of the magnitude of microbial biomass C and N among the three alpine grassland sites showed that mean values of microbial biomass C and N of the AM and AMS sites were much higher than those of the AS site during the growing season. Furthermore, statistical analyses showed that alpine grassland types had a significant effect on microbial biomass C and N concentration (Table 1). Significant correlations were observed between microbial biomass and activities of

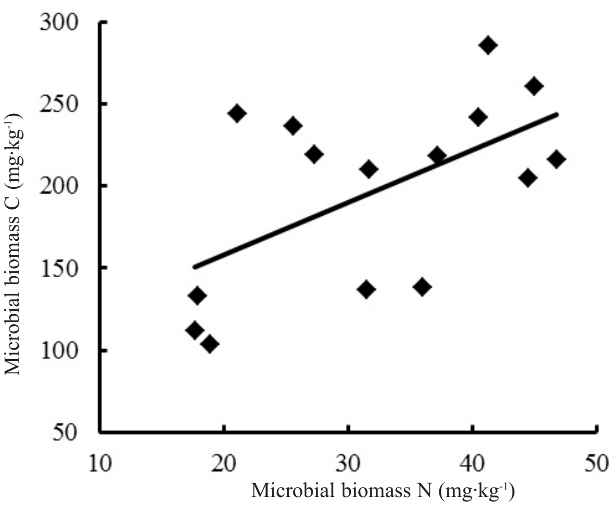

Fig. 3. Relationship between soil microbial biomass C and microbial biomass N under three alpine grassland ecosystems based on data from five sampling dates.

soil enzyme in previous studies, and soil enzymes may be good indicators of microbial activities [33]. In this study, the average of soil invertase, urease, protease, and dehydrogenase activites were on the order of AMS>AM>AS, which kept similar trends with microbial biomass C, N.

The ratio of microbial biomass C to SOC indicates the proportion of the organic carbon that may be readily metabolized [34]. The microbial biomass C value obtained in the present study falls well within the ranges (46-2000 $mg \cdot kg^{-1}$) reported by Wardle [28] and Kaschuk [4] for various agricultural, grassland, and forest soils. The soil microbial biomass C account for the SOC contents (AM: 1.8%, AMS: 1.7%, AS: 1.1%) in present study were in agreement with those reported by Li and Chen [35] and Kaschuk et al. [4], who found that microbial biomass C generally comprised 1.0-4.0% of SOC. The ratio of microbial biomass N to total N represents the mineralizable N fraction, i.e. it expresses the potential of inorganic N available in the soil [36]. The ratios of microbial biomass N to total N in alpine grasslands (AM: 3.2%, AMS: 2.3%, AS: 2.4%) also were generally within the ranges in grassland ecosystems, which were usually 2.0-5.0% [2, 35, 36].

The ratio of microbial biomass C/N is often used to describe the structure and the state of the microbial community. A high microbial biomass C/N ratio indicates that the microbial biomass contains a higher proportion of fungi, whereas a low value suggests that bacteria predominate in the microbial population [37]. The mean soil microbial biomass C/N values during the growing season were 6.5, 6.4, and 5.1 in three alpine grassland sites, respectively (Table 2). The ratios were similar to the ratios reported by Turner et al. [38] and Watanabe et al. [39], less than ratios reported by Barbhuiya et al. [30]. In addition, soil microbial biomass C of the alpine grassland ecosystem was significantly correlated with microbial biomass N. The similar correlation also was found in other ecosystems [37, 39].

Many factors have been suggested to explain the effects of vegetation type on microbial biomass in soils. For instance, soil microbial biomass C and N were reported by several studies that were significantly and positively related with soil temperature and moisture content in different ecosystems [12, 40]. In this study soil temperature significantly impacted microbial biomass N (r^2 = 0.76 for AM, r^2 = 0.89 for AMS, r^2 = 0.80 for AS) and microbial biomass C/N ratio in AS site (r^2 = 0.91), and had no significant impact on microbial biomass C and microbial biomass C/N ratios in AM and AMS. In three alpine grassland sites soil moisture content had no significant impact on microbial

Table 3. Comparisons of average enzyme activities in three alpine grassland sites during the 2010 growing season.

Alpine grassland type	Invertase	Urease	Protease	Dehydrogenase
	mg glucose released 24 $h^{-1}\cdot g^{-1}$	mg NH_4^+-N released 24 $h^{-1}\cdot g^{-1}$	mg NH_4^+-N released 24 $h^{-1}\cdot g^{-1}$	µl H^+ released 24 $h^{-1}\cdot g^{-1}$
AM	91.14 a	0.19 a	0.97 b	12519.70 a
AMS	131.20 a	0.18 a	1.79 a	18358.07 a
AS	82.64 a	0.04 b	0.67 b	8184.50 a

Mean values followed by different letters (a and b) across different grassland types are different from each other at $P \leq 0.05$ level.

biomass C and N, and C/N ratios. These results are consistent with Jin et al. [41], who also found that soil water content was not significantly related with microbial variables such as microbial biomass C and N, and microbial C/N ratio. The absence of correlations between microbial biomass C and soil temperature, and between microbial biomass C and N and water content indicates that environmental factors did not influence the seasonal variation of microbial biomass C, N in alpine grassland ecosystems of Northern Tibet, except that microbial biomass N was significantly correlated with soil temperature. Based on the present study, the increased soil temperature therefore appears to be one most important factor explaining the seasonal variation of microbial biomass N in three alpine grassland sites. Nevertheless, how the alpine grassland ecosystem's type affects microbial biomass C and N, and enzyme activity, and what are the crucial factors that regulate the soil microbial biomass in alpine grassland ecosystems remains to be clarified. This may be the same with other ecosystems [2, 41], litterfalls, translocations of carbohydrates from aboveground to belowground organs were primary factors regulating seasonal variation of microbial biomass C and N in this region. But to support this hypothesis it would be necessary to determine the quantities of aboveground and belowground litter, the nutrition transformation from aboveground to belowground of three alpine grassland sites, and covering prolonged observation periods to further elucidate the underlying mechanisms.

Conclusions

In conclusion, soil microbial biomass C and N exhibited clearly seasonal variation trends in three different alpine grasslands, including alpine meadow (AM), alpine meadow steppe (AMS), and alpine steppe (AS) during the 2010 growing season. The microbial biomass C and N contents and enzyme activities of the AM and AMS sites were much higher than those of the AS site. Soil temperature had significant impacts on microbial biomass N, but had no significant impacts on microbial biomass C. In addition, soil moisture content did not influence the seasonal variation of microbial biomass C and N and C/N ratios. Based on the present study, the increased soil temperature therefore appears to be one of most important factors explaining the seasonal variation of microbial biomass N in alpine grassland ecosystems.

Acknowledgements

This study is funded by the National Natural Science Foundation of China (41001177 and 41201053), the One Hundred Young Persons Project of the Institute of Mountain Hazards and Environment (SDSQB-2010-02), the National Key Technologies R&D Program of China (2011BAC09B03) and the “Strategic Priority Research Program – Climate Change: Carbon Budget and Related Issues” of the Chinese Academy of Sciences (XDA05050506).

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