Freezing–thawing cycles effect on the water soluble organic carbon, nitrogen and microbial biomass of alpine grassland soil in Northern Tibet

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Soil freezing-thawing cycle may substantially influence soil physical properties, microbial activity, and the rates of carbon and nitrogen cycling in soils. In this study, the soil water soluble organic C, N (WSOC, WSON) as well as microbial biomass C, N (MBC, MBN) of two alpine grassland types, alpine meadow and alpine steppe, were investigated after freezing-thawing cycles in a grassland landscape of Northern Tibet. The results showed that with froze at −15°C, the WSOC and WSON contents of alpine grassland soils observably increased after 1 freezing-thawing cycle and decreased after 2 and 4 cycles, and with froze at −25°C, the WSOC and WSON contents showed a gradually increasing trend. The MBC and MBN contents of both alpine meadow and alpine steppe soils generally exhibited a similar “low-high-low” pattern during the process of freezing-thawing cycles. Furthermore, the alpine grassland type had a significant effect on the WSOC and MBC concentrations, and the freeze temperature, freezing-thawing cycle times had significant effects on the WSOC, WSON, MBC, and MBN concentrations.

Key words: Freezing–thawing cycle, water soluble organic C, microbial biomass, alpine grassland

INTRODUCTION

Freezing and thawing of soils is a common phenomenon during cold season, freezing of soils in autumn and thawing in spring are typical in higher latitudes and altitudes regions. The frequency and intensity depend on regional climate and the thickness of the insulating snow cover. Freezing and thawing may substantially influence the rates of carbon and nitrogen cycling in soils due to physicochemical and/or biological effects (Hentschel et al., 2008), and thus has a profound influence on the overall functioning of ecosystems (Larsen et al., 2002). For instance, freezing–thawing increases the release of carbon dioxide (CO₂), dissolved organic C (DOC), dissolved organic N (DON) and dissolved inorganic N (DIN) for soils below Molinia and Sphagnum in Storgama, Telemark county, southern Norway (Vestgarden and Austnes, 2009) and increases DOC, NH₄⁺-N and NO₃⁻-N concentrations in wetland soil solutions at the Sanjiang Plain in northeast China (Yu et al., 2011). In a well-drained, pristine grassland soil in south of Edmonton, Canada, the labile soil organic matter were most influenced by increased freezing-thawing cycles and free lipids may contribute indirectly to the freeze–thaw-induced CO₂ flush from the soil (Feng et al., 2007).

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 (Sugihara et al., 2010). Soil freezing-thawing events have been suggested to destroy microbial cells, releasing nutrients from the destroyed cells for the surviving microbes, which then are highly active during soil thawing (Koponen et al., 2006). During freezing-thawing cycle process, the easily decomposable material becomes available and microbial biomass C contributes 65% of the

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C flush in Ultuna Long-Term Soil Organic Matter Experiment (Herrmann and Witter, 2002). However, Larsen et al. (2002) found that microbial biomass C decreased and microbial biomass N did not change in freezing-thawing cycle, leading to decreased microbial C to N ratio in two arctic ecosystem types. Further, due to freezing stress and competition for freeze-thaw-induced substrate release soil microbial community maybe shifts in some ecosystems. Stres et al. (2002) found that the microbial communities from the high-altitude Himalayan noncontinuous permafrost soils were largely freezing-thawing resistant and microbial populations active increased with the ambient Himalayan temperature fluctuations. In contrast, the temperate soils subjected to freezing-thawing showed an initial decrease in microbial abundance and activity (Stres et al., 2002).

The Qinghai–Tibet Plateau with a mean altitude over 4,000 m is the highest plateau in the world and it covers about 2.5 million km². The permafrost and seasonal frozen soil are widely distributed (Hu et al., 2009). In the world, the Qinghai–Tibet plateau is the only region where the permafrost is present in mid latitude and the seasonal frozen soil and permafrost has already been impacted by global warming and changing now (Wang et al., 2009; Zhang et al., 2011). From the 1970s to the 1990s, the ground temperature of seasonal frozen soil and of sporadic permafrost raised 0.3-0.5°C; mean annual ground temperature of continuous permafrost raised 0.1-0.3°C, the degradation of frozen soil is ubiquitous and serious (Wu et al., 2006; Hu et al., 2009). Previous studies in Qinghai-Tibet Plateau mainly focused on the environmental changes of permafrost (Wu et al., 2010; Cao et al., 2011). Little attention has been focused on the effects of freezing and thawing on alpine ecosystems process. The objectives of this study were (1) to determine the effects of repeated freezing-thawing events on microbial biomass C, N (MBC, MBN), water soluble organic C, N (WSOC, WSON) concentrations in alpine grassland soil and (2) to compare the effects of different alpine grassland types, alpine meadow and alpine steppe in Northern Tibet.

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 (30°57'N, 88°42'E, 4675 m a.s.l.) which located in Shenzha County, Northern Tibet, China. Northern Tibet is the headwater of many important rivers and high mountain lakes in China, such as the Yangtze, Nu (Salween River), Lancang (Mekong River). It is also a major livestock production centre in Tibet which is one of the China's five key livestock raising provinces. Northern Tibet belongs to type cold and semi-arid plateau monsoon climate. The natural environment is extremely harsh with very thin soil. According to the history data from the meteorological station near the study site, the annual mean air temperature is 0°C, the mean air temperature in January is -10.1°C, and the mean air temperature in July is 9.6°C. There is no absolute frost-free season, and the frost period is up to 279.1 d. The annual mean time of solar radiation is 2915.5 h. The average annual precipitation is 300 mm and most of which occurs 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 4200 ha grassland developed into a typical alpine meadow because of the adequate water supply from snow melt and approximately 600 ha grassland developed into a typical alpine steppe due to drought. These two types of alpine grassland were selected in this study to identify on alpine ecosystems process. The alpine meadow had about 70% vegetation coverage and was dominated by Kobresia humilis with scattered Oxytrops spp., G. squarros and Aster tataricus L. The alpine steppe had less than 20% vegetation coverage, mainly Stipa purpurea, Artemisia capillaris Thunb and Rhodiola rotundata assemblages.

Soil sampling and incubation

Soils were sampled from each type of grassland in July 2010 using polyacrylic cylinders with the diameter of 8.1 cm and the depth of 0-15 cm. Soil samples were kept at 4°C in cool boxes during transport to the laboratory. The laboratory incubation experiment was conducted to examine the effects of freezing temperature, freezing-thawing frequency on MBC, MBN and WSOC, WSON concentrations. The polyacrylic cylinders were frozen at either -15 or -25°C for 1 day, and then thawed at 5°C for 1 day. That process defined one cycle of freezing-thawing. Three polyacrylic cylinders of soil (n = 3) from each treatment were taken for analysis after 1, 2 and 4 freeze-thaw cycles. The polyacrylic cylinders of soil which did not come through freezing-thawing process, and directly determined after sampling from field was defined as 0 cycle. In addition, the bulk density (BD), pH, soil organic carbon (SOC), total nitrogen, total phosphorus and total potassium of the soil samples were determined on the sampling date. Soil BD and total K levels differed significantly between the alpine meadow and alpine steppe site, but the differences of soil pH, SOC, total N and total P were not statistically significant. The details on soil physical and chemical properties between the two alpine grassland have been described in previous study (Lu et al., 2011).

Soil analysis

After a defined incubation time of freezing-thawing cycling, fresh sample of each soil was treated with deionized water using soil/water ratios 1:5 (W/V) for 30 min under agitation (130 times/min) in a flask. After the extraction, samples were centrifuged at 6500 r/min for 15 min. Supernatants were filtrated with a 0.22 μm Millipore membrane. Water extraction organic carbon and nitrogen of the filtrate was determined by a Vario TOC cube total organic carbon analyzer (Elementar Analysensysteme GmbH., Germany). Soil microbial biomass C and N were determined by the chloroform fumigation extraction method (Vance et al., 1987). We calculated soil microbial biomass C and N by identifying the difference of total extractable C, N between fumigated and unfumigated samples by the conversion factors 0.45 for biomass C and 0.54 for biomass N (Joergensen, 1996; Joergensen and Mueller, 1996).

Statistical analyses

One-way ANOVA was used to test the differences of soil microbial biomass C, N and water soluble organic C, N concentrations between the two alpine grasslands. A Least Significant Difference (LSD) test was used to distinguish difference. Three-way ANOVA
which adapted on the alpine grassland type, freezing-thawing cycle times and the freezing temperature as the main factors were selected to analyze the following variables: soil microbial biomass C, N and water soluble organic C, N concentrations. All analyses were performed using the SPSS 11.5 statistical software package (SPSS Inc., USA).

RESULTS

Water soluble organic C, N (WSOC, WSON)

The WSOC contents of both alpine meadow and alpine steppe soils observably increased after 1 freezing-thawing cycle and then presented a decreasing trend after 2 and 4 freezing-thawing cycles with froze at −15°C (Figure 1a and b). With froze at −25°C, the WSOC content of alpine meadow soil showed an increasing trend and the WSOC content of alpine steppe soil changed a little after freezing-thawing cycles. For alpine meadow soil, the WSON content presented the similar change trend with the WSOC content, with froze at −15°C, the WSON increased to 39.92 mg kg⁻¹ after 1 freezing-thawing cycle and then decreased to 25.68 mg kg⁻¹ after 4 freezing-thawing cycles (Figure 1c). And for alpine steppe soil with froze at −15°C, the WSON increased to 30.50 mg kg⁻¹ after 1 freezing-thawing cycle and with froze at −25°C, the WSON reached the maximal value (27.09 mg kg⁻¹) after 2 cycles (Figure 1d). Results from the three-way ANOVA demonstrated that, sampling time alpine grassland type (AGT), freeze temperature (FT), freezing-thawing cycle times (FCT) and their interaction had significant effects on the WSOC concentrations, while the
alpine grassland type and the interaction of freeze temperature and freezing-thawing cycle times had no significant effect on the WSON concentrations (Table 1).

**Microbial biomass C, N (MBC, MBN)**

The MBC and MBN content of both alpine meadow and alpine steppe soils generally exhibited a similar “low-high-low” pattern after freezing-thawing cycles (Figure 2). For the alpine meadow soil, the MBC content increased slightly and peaked (192.54 mg kg$^{-1}$) after 1 freezing-thawing cycle with froze at $-15^\circ$C, subsequently decreased to the minimum (101.71 mg kg$^{-1}$) after 4 cycles, whereas for the alpine steppe soil, the highest MBC contents were obtained after 2 cycles, with values as 183.64 (froze at $-15^\circ$C) and 180.06 mg kg$^{-1}$ (froze at $-25^\circ$C), respectively. The MBN contents of alpine grassland soils reached maximum after 1 freezing-thawing cycle, were 78.23 (froze at $-15^\circ$C) and 81.90 mg kg$^{-1}$ (froze at $-25^\circ$C) for alpine meadow soils, and 92.50 (froze at $-15^\circ$C) and 58.39 mg kg$^{-1}$ (froze at $-25^\circ$C) for alpine steppe soils, respectively. Statistical analyses showed that the MBC content were significantly different between the two alpine grasslands as well as the two freeze temperature and among freezing-thawing cycle times. However, the MBN content were only significantly different between the two freeze temperature and among freezing-thawing cycle times not significantly different between the two alpine grassland types (Table 1).

**DISCUSSION**

Alpine grassland is not only the most important and largest ecosystem in Northern Tibet but also a key resource that supports the local people’s subsistence. As fragile ecosystems, the alpine grassland ecosystem in Northern Tibet is extremely sensitive to climate change and human activities (Gao et al., 2009). This study determines the effects of repeated freezing-thawing cycles on MBC, MBN, WSOC, and WSON concentrations of alpine grassland soil in Northern Tibet. After freezing-thawing processes, the WSOC and WSON contents of both alpine meadow soil and alpine steppe soil were generally higher than the soils without freezing-thawing events (Figure 1), indicating that freezing-thawing cycles could increase WSOC and WSON production of soils. These results generally agree with the previous findings that freezing-thawing cycles could increase DOC concentrations in wetland soil solutions in the Sanjiang Plain, northeast China (Yu et al., 2011) and the total soluble organic nitrogen loss from intact soil-plant mesocosms which were collected from a 5 ha temperate oldfield north of London, Ontario, Canada remained high after 11 freezing-thawing cycles (Joseph and Henry, 2008). It has been reported that WSOC and WSON were released by killing of the microbial cells, physical disruption of soil aggregates and the breakdown of plant material during the freezing period (Freppaz et al., 2007; Vestgarden and Austnes, 2009; Yu et al., 2011). These could be responsible for the increase of WSOC and WSON concentrations after freezing-thawing cycles.

The freezing–thawing process promoted the release of MBC and MBN of alpine meadow soil after the 1 freezing–thawing cycle, and then the MBC and MBN contents decreased after 2 and 4 cycles. Whereas the MBC content of alpine steppe soil reached the maximum after 2 cycles and the MBN reached the maximum after 1 cycle (Figure 2). These maybe because that the freezing–thawing process of the early days stimulated the alpine grassland soil microbes and brought the higher microbial biomass. Then, after 2 or 4 cycles the cell content was released from microbes that died and their cells lysed, while most released labile C was respired as CO$_2$ or leaching as WSOC, N was assimilated together with the initial soil organic nitrogen, resulting in reduced microbial C and N (Larsen et al., 2002; Schmidt et al., 2009). However, previous research showed that, there were no changes or some decline in microbial biomass C, N due to soil freezing-thawing cycles. Kopen et al. (2006) analyzed soil microbial biomass of Finnish agricultural soil with lipid biomarkers and found that the MBC contents were not affected by freezing-thawing cycles. Lipson et al. (2000) also found the soil microbial biomass of the alpine dry meadows in the front range of the Colorado Rocky

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**Table 1.** Results of three-way ANOVAs (F-values) testing the effects of alpine grassland type (AGT), freeze temperature (FT) and freezing-thawing cycle times (FCT) on soil C and N characteristic in Northern Tibet (* P < 0.05; ** P < 0.01; *** P < 0.001).

<table>
<thead>
<tr>
<th>Source</th>
<th>WSOC</th>
<th>WSON</th>
<th>MBC</th>
<th>MBN</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGT</td>
<td>43.77***</td>
<td>0.99</td>
<td>44.22***</td>
<td>0.01</td>
</tr>
<tr>
<td>FT</td>
<td>12.27**</td>
<td>7.28*</td>
<td>11.08**</td>
<td>52.74***</td>
</tr>
<tr>
<td>FCT</td>
<td>15.87***</td>
<td>23.69***</td>
<td>40.88***</td>
<td>138.73***</td>
</tr>
<tr>
<td>AGT×FT</td>
<td>16.55**</td>
<td>6.81*</td>
<td>0.69</td>
<td>171.51***</td>
</tr>
<tr>
<td>AGT×FCT</td>
<td>7.91**</td>
<td>4.13*</td>
<td>114.55***</td>
<td>38.99***</td>
</tr>
<tr>
<td>FT×FCT</td>
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<td>2.31</td>
<td>17.21***</td>
<td>28.25***</td>
</tr>
<tr>
<td>AGT×FT×FCT</td>
<td>6.06**</td>
<td>2.52</td>
<td>1.25</td>
<td>49.03***</td>
</tr>
</tbody>
</table>

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After freezing-thawing cycles on MBC, MBN, WSOC, and WSON concentrations of alpine grassland soil in Northern Tibet. After freezing-thawing cycles on MBC, MBN, WSOC, and WSON concentrations of alpine grassland soil in Northern Tibet. After
Mountains was not affected by a single freeze-thaw event. But Larsen et al. (2002) reported that microbial biomass C in subarctic heath decreased after 18 freezing-thawing cycles.

The freeze temperature (FT) had a significant effect on the WSOC, WSON, MBC, and MBN concentrations of both alpine meadow and alpine steppe soils. The freeze temperature had an intensive effect on soil microbes, including the death of more soil microbes, and organic matter from non-microbial origin also contributed to the increase of soil mineral N under the lower temperature condition (Sulkava and Huhta, 2003; Freppaz et al., 2007). Freezing-thawing cycles might cause the release of C, N from the soil inorganic and organic colloids that would have been previously unavailable, and the lower freeze temperature would lead to worse damage to soil aggregates (Kvarno and Oygarden, 2006; Zhou et al., 2011). The freezing-thawing cycle times (FCT) also affected the release of C and N during the process of freezing-thawing of these two alpine grassland soils. At first, lower temperature stimulated the alpine grassland soil microbes. Nevertheless, with increasing freezing-thawing cycling, the residual microbes adapted gradually to the low temperature conditions and soil aggregates became increasingly stable (Larsen et al., 2002; Zhou et al., 2011). The influence of freeze temperature and freezing-thawing cycle times on releasing of C, N has been found in previous studies (Teepe et al., 2001; Feng et al., 2007; Hentschel et al., 2008; Joseph and Henry, 2008). The grassland ecosystems type can profoundly impact soil biogeochemical characteristic through the alteration of abiotic and biotic characteristics of soils and

Figure 2. The Microbial biomass C (MBC) contents of alpine meadow soils (a), alpine steppe soils (b) and the Microbial biomass N (MBN) contents of alpine meadow soils (c), alpine steppe soils (d) following the process of freezing-thawing cycles in Northern Tibet.
soil physical and chemical properties (Jones et al., 2004; St Clair et al., 2009). In this study, the MBC and WSON content which came through freezing–thawing process which froze at 15 or 25°C were significantly different between the two alpine grassland soils.

The soil WSON and WSON as well as MBC, MBN after freezing–thawing cycles were studied in two different alpine grasslands, alpine meadow and alpine steppe, in Northern Tibet. The WSON and WSON contents of alpine grassland soils increased clearly after 1 freezing–thawing cycle and decreased after 2 and 4 cycles with froze at −15°C, and with froze at −25°C the WSON and WSON contents showed gradually increasing trend. The MBC and MBN contents of both types of alpine grassland soils generally exhibited a similar “low-high-low” pattern during the process of freezing–thawing cycles. Furthermore, the WSON and MBC concentrations were significantly different between the two alpine grasslands, and the WSON, WSON, MBC, and MBN concentrations were significantly different among different freezing temperature and the different freezing–thawing cycle times.

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