ABSTRACT: In alpine zones, cold season processes, particularly those associated with snow accumulation and ablation, have a central role in ecosystem functioning. However, we know very little about soil carbon and nitrogen processes under the snowpack in these ecosystems, including the Tibetan Plateau. We conducted an experiment comparing three snow regimes (11 m × 1 m plots) of different snow depths and durations at an altitude of 4,100 m in the Minshan Range on the eastern Tibetan Plateau. The three snow regimes included a shallow and short duration snowpack (SS; depth <10 cm), a moderate snow depth and medium duration snowpack (MS; depth <20 cm), as well as a deep and long duration snowpack (DS; depth > 30 cm). This study explores the effects of different snow conditions on soil temperature, and further describes the sequence and timing of dissolved nutrients and microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) in soils under different snow regimes during the autumn-winter transition (i.e. November 7, 2008 – March 7, 2009). Three successive phases of temperature change were distinguished: I – initial decline – soil temperatures dropped steadily from 4°C to about 0°C at the same rate for all three snow regimes; II – moderate freezing – soil temperatures fluctuated between 0°C and –7°C under all three snow regimes; III – multiple freeze-thaw cycles took place in the SS and MS regimes, but permanent freezing occurred in the DS regime. Under moderate freezing, we found that soil temperature fluctuation was an essential factor for the transformation of soil C and N. Our results indicate that larger temperature fluctuations correlate with a greater increase in dissolved organic nitrogen (DON) content. Dissolved organic carbon (DOC) content increased markedly only under the most drastic temperature fluctuations. In contrast, MBC content increased significantly only when soil temperatures were relatively steady. Under the permanent freezing, only a large number of freeze-thaw cycles caused a significant decline of NO₃⁻–N and DOC concentrations. DON content declined markedly under permanent freezing and multiple freeze-thaw cycles. However, MBC content declined significantly only under permanent freezing. Ultimately, multiple freeze-thaw cycles resulted in the export of dissolved nutrients (organic and inorganic nitrogen) from the alpine ecosystem which had previously accumulated in the moderate freezing phase of the soil.

KEY WORDS: snowpack, soil freezing, freeze-thaw cycles, permanent freezing, microbial biomass, dissolved nutrients, Tibetan Plateau

1. INTRODUCTION

Alpine ecosystems are characterized by long winters where snow distribution is one of the most important variables controlling

Results from numerical simulations have demonstrated that snowpack significantly influences both ground-surface temperatures (Goodrich 1982). Shallow snowpack cannot provide effective insulation, which results in cold soil temperatures, extensive soil freezing and an increase in freeze-thaw cycles (Groffman et al. 1999). Late development of snowpack often results in soil freezing throughout the entire winter, in contrast to the unfrozen conditions that would likely prevail under early season snowpack development. Moreover, during the thaw stage of freeze-thaw cycles, melting of the snowpack often leads to wide fluctuations (including diurnal) in soil moisture and other local environmental conditions, such as pH and redox (Edwards et al. 2007). Therefore, different snow regimes have different impacts on the physicochemical and biological processes in subnivian soils. Thus, the reasons for changes in the amounts and chemical forms of available nutrients are numerous and complex under the snowpack. Both freezing and freeze-thaw cycles affect the nitrogen transformation and content in soils under the snowpack. Several studies have shown that freeze-thaw cycles increase nitrogen mineralization, but some others have found that nitrogen mineralization was unaffected, or even that nitrogen immobilization occurred under soil freezing (Schmidt and Lipson 2004). Previous studies have identified several ways in which soil nutrient processes are affected by snowpack. Firstly, both freezing and freeze-thaw cycles may cause a physical disruption of soil aggregates and cause a direct release of organic matter, fresh litter, and otherwise trapped dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) from micropores through fragmentation and biochemical reactions (Hinman and Frederick 1968, Bullock et al. 1988). Secondly, both freezing and freeze-thaw cycles increase mortality of fine roots and lysis of microbes in the field (Morley et al. 1983, Sakai and Larcher 1987, Skogland et al. 1988, Edwards and Cresser 1992, Schimel and Clein 1996, Neilson et al. 2001). At last, freeze-thaw cycles lead to rapid NO3− loss from soils – in contrast to NH4+, which is strongly retained by the soil cation exchange complex and selectively taken up by the soil microbial population (Brooks et al. 1995, Williams et al. 1995).

During the non-growing season, soil microbes under the snowpack may play a key role in alpine carbon and nitrogen cycling (Brooks et al. 1996, Brooks et al. 1997). Even if bulk soil water freezes when soil temperatures remain below 0°C, liquid water films remain around soil particles, at least down to temperatures of −8°C (Romanovsky and Osterkamp 2000, Price and Sowers 2004). As long as there is unfrozen water, microbes can remain physiologically active (Coxson and Parkinson 1987, Rivkina et al. 2000, Mikan et al. 2002). This also may be a reason why some studies have indicated that microbial biomass was unaffected after single or multiple moderate freeze-thaw events or moderate freezing in alpine meadows (Morley et al. 1983, Lipson and Monson 1998, Lipson et al. 2000). However, microbial biomass declines rapidly if soil temperatures fall below −10°C in winter (Mikan et al. 2002). It also has been reported that up to 50% of the viable soil microbes were killed by a single freeze-thaw event in experimental studies (Edwards et al. 2006). Nutrients released from the cells of the senescing microbes can be utilized by surviving microbial flora, but additional freeze-thaw cycles can lead to a steep decline to the cold-adapted microbial population (Skogland et al. 1988, Deluca et al. 1992).

Many areas of the Tibetan Plateau are seasonally snow-covered (Yang et al. 2008), which is seriously influenced by global
warming (Wu et al. 2007, Tomonori 2009). However, we know little about the dynamics of dissolved nutrients and microbial biomass in soils under seasonal snowpack in this region until now. In order to explore the effect of seasonal snowpack on soil carbon and nitrogen biogeochemical processes on the Tibetan Plateau, we designed an experiment to answer the following three questions: (a) If different snow conditions cause different soil freeze/thaw situations? (b) If soils accumulate more dissolved C and N nutrients under deeper snowpack due to the relatively high and steady soil temperature? (c) What are the effects of soil freezing and multiple freeze-thaw cycles on MBC and MBN under different snow regimes?

2. MATERIALS AND METHODS

2.1. Study area

The research was conducted on Mount Kaka (32°59’ N, 103°40’ E) of the Minshan Range on the eastern Tibetan Plateau, at an elevation of 4,100 m. The annual mean temperature at this location was 2.8°C with a mean value of −7.6°C in January and 9.7 °C in July. The annual mean solar radiation was 1,827 hours. Annual mean precipitation was 718 mm, with 72% of that that distributing during June to August. Snow can fall in any month but consistent snowpack usually does not occur until late December or early January.

The soil (pH 5.54–5.94, SOM 41.53–60.00 g kg⁻¹ dry soil, TN 3.86–4.94 g kg⁻¹ dry soil) is silty loam Inceptisol, and plant roots in this ecosystem are generally confined to the surface A-horizon (2–20 cm). The meadow is dominated by some forbs (Polygonum macrophyllum D Don., Pedicularis davidii Franch., Caltha scaposa Hook. f. et Thoms., Thalictrum alpinum Linn.var. elatum Ulbr.) and graminoid species (Carex atrofusca subsp. minor (Boott) T. Koyama, Juncus thomsonii Buchen. var. thomsonii). A few dwarf shrubs are scattered sporadically in the meadow, most commonly Rhododendron zheguense Ching et H. P. Yang and Salix zhegushanica N. Chao. Mosses are abundant and cover most of the ground.

2.2. In situ experimental design and treatments

The natural gradient of snowpack covers an area of 50 m from the top of the ridge on south-facing slopes (a difference in perpendicular height of 0.51 m) due to the direction of the prevailing wind in winter. The three rectangular snow regime plots (approximately 11 m × 1 m) were constructed in the field, and were covered by shallow and short duration snowpack (SS), moderate depth and duration of snowpack (MS), as well as deep and long duration snowpack (DS) from the top to the bottom of the slope, respectively. The distance to adjacent snow regime plots was 10 m. Five square replicated plots (each plot was approximately 1 m × 1 m) were built in each snow regime.

Twenty PVC tubes 75 mm in diameter and 200 mm long (surface soil depth) were buried randomly in situ at each plot on November 7, 2008. Soil samples from the PVC tubes were taken on 7 November and 3 December of 2008, and 4 January and 7 March of 2009. Each sample was taken randomly from the five PVC tubes of the same plot and than homogenized thoroughly, and the fifteen samples were then analyzed in lab. From each sample, NH₄⁺–N, NO₃⁻–N, DOC, DON, MBC (microbial biomass carbon) and MBN (microbial biomass nitrogen) contents were measured. All of these variables were calculated by dry weight of the soil. We also determined soil moisture during each measurement. Soil pH, organic matter and total nitrogen and available nitrogen were determined once in each snow regime in November 7, 2008. Soil temperatures were recorded hourly in the middle plot of each snow regime by using HOBO temperature data loggers (Onset Computer Corporation, Pocaset, MA) inserted 5 cm below the soil surface from 1 November, 2008 to 7 March, 2009.

2.3. Chemical determination

Soil samples were kept at 4°C in cool boxes before the laboratory measurements were conducted. Soil samples for NH₄⁺–N, NO₃⁻–N, DOC and DON were processed on the day after arrival. Subsamples for MBC and MBN were frozen and processed within 15 days of
sampling. Soil samples were oven dried for 10 hours at 70°C, until they remained at a consistent mass, to determine the moisture content. MBC and MBN contents were determined by the chloroform-fumigation direct-extraction (CFE) technique (Brookes et al. 1985). C and N concentrations in the fumigated and non-fumigated samples were determined by multi N/C 2100/2100S (Analytic Jena Co. Ltd., Germany). MBC and MBN were calculated as the difference between fumigated and non-fumigated extractable C and N samples. Differences were divided by a correction factor to account for MBC or MBN that is not susceptible to chloroform fumigation (kC = 0.35; kN = 0.4) (Jonasson et al. 1996). DOC and total dissolved N (TDN) contents were determined using unsieved fresh moist soil subsamples by the above-mentioned instrument. Soil subsample extractions were shaken for 1 hour with 2M KCl at a temperature of approximately 20°C and a soil-to-solution ratio of 1:5 (w/v), and subsequently the extracted solution was filtered using Whatman No 42 filter paper before further determination (Jones and Willett 2006). NH4+–N and NO3–N were analyzed with the indophenol blue colourimetric (Mulfaneey 1996) and ultraviolet spectrophotometry methods (Cawse 1967), respectively. DON was calculated by subtracting dissolved inorganic N (NH4+–N and NO3–N) from TDN. The net ammonification, nitrification, and N mineralization rates were calculated as the difference in NH4+–N, NO3–N and inorganic N contents, respectively, between adjacent sampling dates. A negative value of net N mineralization rate indicates a reduction of inorganic N and suggests that inorganic N has been immobilized by the end of the phase.

2.4. Statistical analysis

Treatment effects were analyzed by comparing results for different snow regimes (SS, MS and DS; N = 3) on single sampling days or by comparing differences between sampling dates (7 November and 3 December of 2008, and 4 January and 7 March of 2009; N = 4) for a single regime. Differences between all the indices were assessed using one-way analysis of variance (ANOVA), except for net ammonification, nitrification and mineralization rates. Because it was not possible to pair the cores for initial and final harvests through time, net ammonification, nitrification and net N mineralization rates were calculated using the means for NH4+–N, NO3–N and total inorganic N from each harvest. Therefore, no statistical analyses were conducted on ammonification, nitrification and mineralization rates. Within our study, all statistical comparisons were assessed at a = 0.05 using SPSS version 13.0 (SPSS Inc., Chicago, IL).

3. RESULTS

3.1. Snow depth, soil temperature and soil freeze-thaw cycles

Due to differences in the depth and timing of snowpack accumulation, soil temperatures at a depth of 5 cm were significantly different among the three snow regimes (Fig. 1 and Fig. 2). The first snowfall occurred in early November of 2008. The changes in soil temperature during the autumn-winter transition of 2008 can be partitioned into three phases (Fig. 2): I – Initial decline: before early December of 2008, the soil daily mean temperature steadily declined from 4°C to 0°C in the three snow regimes, due to the shallow and ephemeral snowpack.
II – Moderate freezing: until early January of 2009, soil temperature remained between 0°C and –7°C in the three snow regimes, due to the shallow and consistent snowpack. Nevertheless, soil temperature fluctuations had been the greatest in the SS regime.

III – Permanent freezing occurred in the DS regime, but multiple freeze-thaw cycles occurred in the SS and MS regimes. From early January until early March 2009, surface soils experienced twenty-one freeze-thaw cycles in the SS regime and nine freeze-thaw cycles in the MS regime (freeze-thaw numbers are based on detailed soil temperature data; the data are not shown), under snow depths of <10 cm in the SS regime and <20 cm in the MS regime. Soil ice formation persisted in the DS regime as a result of the late formation of insulating snowpack, although snow depth consistently remained at or above 30 cm.

Soil temperature fluctuations in the DS regime were consistently the smallest of the three snow regimes throughout the experimental process (Fig. 2).

3.2. NH$_4^+$–N, NO$_3^-$–N, net ammonification, nitrification and N mineralization

The fluctuation of NH$_4^+$–N contents showed similar patterns in the SS and MS regimes. Fluctuations increased under moderate freezing, but declined under multiple freeze-thaw
cycles; however, none of the changes were significant (Fig. 3 and Table 1). NH\textsubscript{4}\textsuperscript{+} was the dominant source (approximately 80%) of inorganic N in the soil; NO\textsubscript{3}\textsuperscript{–} content was much lower than NH\textsubscript{4}\textsuperscript{+} during the entire experiment. NO\textsubscript{3}\textsuperscript{–} content declined significantly only after multiple freeze-thaw cycles in the SS regime (Fig. 3 and Table 1).

Both the ammonification and N mineralization rates ascended to peak values under moderate freezing, but then turned negative after multiple freeze-thaw cycles in the SS and MS regimes (Fig. 4). In contrast, there were the contrary changes in ammonification and N mineralization rates in the DS regime, suggesting that microbial immobilization of NH\textsubscript{4} had occurred under moderate freezing and stable temperatures. In each snow regime, net nitrification rate first ascended to peak values after II – phase and then turned negative in the III – phase (Fig. 4).

### 3.3. DOC and DON

Variation in DOC content was similar in the three snow regimes (Fig. 5). In the SS regime, DOC content increased significantly after II – phase, and then declined after twenty-one freeze-thaw cycles (Table 1). DON content first declined markedly after I – phase (initial decline) and then increased significantly during II – phase (Table 1) in the three snow regimes. DON content declined
Table 1. Changes in soil parameters (mg kg⁻¹ dry soil) throughout the study period in the three snow regimes.

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Nov. 7</th>
<th>Dec. 3</th>
<th>Jan. 4</th>
<th>Mar. 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺-N SS</td>
<td>9.23±0.49</td>
<td>10.41±0.49</td>
<td>14.57±4.89</td>
<td>9.5±0.97</td>
</tr>
<tr>
<td>MS</td>
<td>8.19±0.59</td>
<td>9.41±0.36</td>
<td>12.49±3.52</td>
<td>8.88±0.63</td>
</tr>
<tr>
<td>DS</td>
<td>8.44±0.70</td>
<td>10.43±0.79</td>
<td>7.56±0.47</td>
<td>10.02±0.88</td>
</tr>
<tr>
<td>NO₃⁻-N SS</td>
<td>1.96±0.17a</td>
<td>2.05±0.24a</td>
<td>3.42±0.41a</td>
<td>1.49±0.44b</td>
</tr>
<tr>
<td>MS</td>
<td>2.44±0.34</td>
<td>2.03±0.15</td>
<td>3.42±0.84</td>
<td>2.38±0.55</td>
</tr>
<tr>
<td>DS</td>
<td>3.04±0.36</td>
<td>2.14±0.22</td>
<td>3.12±0.40</td>
<td>2.21±0.14</td>
</tr>
<tr>
<td>DOC SS</td>
<td>152.42±15.00b</td>
<td>152.60±24.82b</td>
<td>319.64±62.79a</td>
<td>184.00±27.78b</td>
</tr>
<tr>
<td>MS</td>
<td>112.17±6.84</td>
<td>137.13±21.58</td>
<td>176.24±18.61</td>
<td>145.16±26.54</td>
</tr>
<tr>
<td>DS</td>
<td>93.36±7.67</td>
<td>102.90±11.80</td>
<td>125.60±7.97</td>
<td>90.29±4.19</td>
</tr>
<tr>
<td>DON SS</td>
<td>28.12±2.46b</td>
<td>8.48±1.96c</td>
<td>43.54±6.14a</td>
<td>11.68±2.58c</td>
</tr>
<tr>
<td>MS</td>
<td>17.03±1.55a</td>
<td>5.17±1.74b</td>
<td>20.29±4.04a</td>
<td>4.85±1.50b</td>
</tr>
<tr>
<td>DS</td>
<td>14.75±2.66a</td>
<td>3.75±0.87b</td>
<td>17.90±2.73a</td>
<td>3.56±1.30b</td>
</tr>
<tr>
<td>MBC SS</td>
<td>857.23±70.51</td>
<td>859.83±178.56</td>
<td>886.70±252.08</td>
<td>859.81±156.72</td>
</tr>
<tr>
<td>MS</td>
<td>933.28±75.87</td>
<td>876.31±140.89</td>
<td>1152.48±88.18</td>
<td>919.86±114.84</td>
</tr>
<tr>
<td>DS</td>
<td>1115.92±96.34ab</td>
<td>839.87±137.62b</td>
<td>1269.23±127.16a</td>
<td>807.15±72.81b</td>
</tr>
<tr>
<td>MBN SS</td>
<td>40.91±8.29</td>
<td>61.61±15.82</td>
<td>64.31±21.96</td>
<td>78.27±18.68</td>
</tr>
<tr>
<td>MS</td>
<td>72.94±11.92</td>
<td>73.89±12.18</td>
<td>102.16±12.66</td>
<td>79.45±16.19</td>
</tr>
<tr>
<td>DS</td>
<td>95.02±10.35</td>
<td>81.06±13.91</td>
<td>133.12±17.94</td>
<td>88.44±9.15</td>
</tr>
</tbody>
</table>

Results were analyzed by comparing differences between sampling days for a single regime using one-way analysis of variance (ANOVA) (P <0.05). Bold indicates a significant difference. SS, MS and DS denote the shallow and short duration of snow cover, moderate depth and medium duration of snow cover, as well as deep and long duration of snow cover, respectively. DOC, DON, MBC and MBN denote dissolved organic carbon, dissolved organic nitrogen, microbial biomass carbon, and microbial biomass nitrogen, respectively.

significantly after III – phase (permanent freezing and multiple freeze-thaw cycles; Table 1). Moreover, DOC and DON content were the greatest in the SS regime throughout the experiment (Fig. 5).

3.4. MBC and MBN

Under moderate freezing, MBC and MBN content increased in all the three snow regimes. Moreover, MBN content in the DS regime was significantly greater than in the SS regime. During III – phase, MBC and MBN contents declined in all the three snow regimes, with the exception of MBN content in the SS regime, which increased slightly. Only in the DS regime were the changes in MBC content statistically significant. None of changes in MBN content were significant during the entire experiment (Fig. 6 and Table 1).

4. DISCUSSION

It was reported by Cline (1995) that certain snow depths (i.e. 30–40 cm) could effectively decouple soil temperature from air temperature during the non-growing season. Similarly, we observed that during III – phase, soil temperature fluctuations were weaker in the DS regime than in the SS and MS regimes because the snow was deep enough (> 30 cm) to insulate the soil from air temperature, while snow depth in the SS and MS regimes was always less than 20 cm. However, soil in the DS regime remained frozen because the insulating snowpack developed after air temperatures had dropped to below 0°C. This finding is in accordance with the opinion of Groffman et al. (2001a), who projected a ‘colder soils in a warmer world’ scenario due to the late development of snowpack as a result of warming. In the SS and MS regimes, the relatively shallow snowpack did not provide effective insulation and air temperatures rose above zero at intervals during III – phase. As a result, the soil went through multiple freeze-thaw cycles, resembling the results of Decker et al. (2003). The frequency of surface soil freeze-thaw cycles was once a day at the eastern edge of the Tibetan
Plateau during the autumn-winter transition. This finding is similar to the report of Yang et al. (2006) in Nagqu in the central Tibetan Plateau.

Under moderate freezing (i.e. between \(-7^\circ\text{C}\) and 0°C), soil temperature fluctuation has a strong impact on changes in the forms of dissolved C and N nutrients; relatively large temperature fluctuations appear to accelerate the production of DOC and DON. However, moderate freezing caused an increase in NH\(_4^+\)-N and NO\(_3^-\)-N content. The slight changes in ammonification and nitrification rates indicate that both ammonifiers and nitrifiers can adapt to moderate freezing by degrees. Interestingly, negative ammonification rates in the DS regime showed that NH\(_4^+\)-N immobilization dominated under weaker temperature fluctuations. In contrast, ammonification had an advantage over NH\(_4^+\)-N immobilization due to the large temperature difference in the SS and MS regimes. Because temperature differences were seldom focused on soil freezing in the past, a number of studies in forest or arctic soils have reported inconsistent results about N mineralization after soil freezing (Groffman et al. 2001b, Larsen et al. 2002, Schimel et al. 2004, Hentschel et al. 2008). There have been few systematic studies on the effects of freezing temperature, and the effects of snowpack and soil freezing on N mineralization has rarely been reported. Under moderate freezing, weaker temperature fluctuations led to a greater increase in microbial biomass. In the DS regime, which had the smallest temperature fluctuation, there was a significant increase in MBC content. Similarly, Larsen et al. (2002) found that microbial biomass is strongly affected by temperature fluctuations around the freezing point. Therefore, microbial populations could adapt to moderate freezing and osmosis stress.
in the region if frozen temperatures remained relatively steady and both multiple freeze-thaw cycles and permanent freezing lead to losses of dissolved C and N nutrients in the alpine meadow. The significant decline to \( \text{NO}_3^- \) content after twenty-one thaw cycles is likely due to three probable mechanisms: stronger denitrification (Sharma et al. 2006), export of \( \text{NO}_3^- \) with meltwater during thaw (Rascher et al. 1987, Kaste et al. 2008, Matzner and Borken 2008) and microbial immobilization. The low \( \text{NH}_4^+ \) content in the SS regime corresponds to the findings of Larsen et al. (2002), who reported smaller amounts of extractable \( \text{NH}_4^+ \) in freeze-thaw (\(-4^\circ\text{C}\)) experiments with arctic soil mesocosms relative to permanently frozen samples. Further, in the SS and MS regimes, multiple freeze-thaw cycles led to negative rates of net ammonification, nitrification and N mineralization, likely due to the death of some of specific microbial communities (e.g. ammonifiers, nitrifiers) and meltwater run-off during snowmelt. In the DS regime, the net nitrification rate was negative, but the net ammonification rate was positive, which indicates that nitrifiers are less resistant to permanent freezing than ammonifiers. DOC content decreased significantly only after twenty-one freeze-thaw cycles, while DON content decreased significantly under both permanent freezing and multiple freeze-thaw cycles. Furthermore, our results from the SS and MS regimes indicate that the longer the duration of thaw during the multiple freeze-thaw cycles, the greater decline to dissolved nutrients. More frequent freeze-thaw cycles could have led to a greater loss of dissolved nutrients from surface soil. Moreover, coupled with hydrological activity during snowmelt, a considerable loss of dissolved C and N occurred. If the total amount of N infiltrated or leached during and after thawing is larger than the amount trapped during the freezing period, net loss will result and vice versa. Frequent freeze-thaw cycles in the alpine soil led to the export of the organic and inorganic forms of dissolved nitrogen which had previously accumulated in the stable and moderate freezing phase. A significant decline to DON content under permanent freezing could have been due to the transformation of a portion of the DON to fixed nitrogen by way of adsorption (Kalbitz et al. 2000). Another possibility is that surviving microbes shift their substrate use pattern from C rich material (i.e., plant detritus) to using more N rich material (i.e., dissolved material, including dissolved organic matter) and recycled microbial biomass (Larsen et al. 2002, Schimel and Mikan 2005).

Numerous previous studies in alpine and tundra soils have revealed no freeze-thaw (Lipson and Monson 1998, Lipson et al. 2000, Grogan et al. 2004, Sharma et al. 2006) or strong freezing (Stenberg et al. 1998, Pesaro et al. 2003) effects on microbial biomass. Our results indicate that multiple freeze-thaw cycles had an only minor effect on microbial biomass, while permanent freezing caused a significant decline to MBC content. We deduce that a sufficient duration of soil freezing may also be one of threshold values for soil microbial biomass in alpine meadow on the Tibetan Plateau. This could be due primarily to two processes: 1) permanent freezing leads to a reduction to available water in soils because microbes had almost exhausted unfrozen water with freezing postponement. 2) the insulating properties of long-duration snowpack leads to a lack of oxygen in the soil.

5. CONCLUSION

In alpine meadows on the Tibetan Plateau, shallow (<20 cm) snowpack leads to more freeze-thaw cycles and greater soil temperature fluctuation, while late development of insulating snowpack (> 30 cm) results in lower soil freezing temperature and less temperature fluctuation.

1) Under moderate freezing, greater temperature fluctuations are associated with more significant increases in DOC and DON contents. Only relatively steady soil temperatures promote a significant increase in MBC content. MBN content unchanged significantly regardless of the degree of fluctuation in soil temperature.

2) Both \( \text{NO}_3^- \) and DOC contents declined significantly only after multiple freeze-thaw cycles. Both organic and inorganic forms of nitrogen which had previously accumulated during the stable and moderate
freezing phase of the soil were exported from the alpine meadow ecosystem with meltwater runoff during the autumn-winter transition. Overall, the effect of long-term soil freezing on microbial biomass was more profound than freeze-thaw cycles.

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