Snow removal alters soil microbial biomass and enzyme activity in a Tibetan alpine forest

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A B S T R A C T

Projected future decreases in snow cover associated with global warming in alpine ecosystems could affect soil biochemical cycling. To address the objectives how an altered snow removal could affect soil microbial biomass and enzyme activity related to soil carbon and nitrogen cycling and pools, plastic film coverage and returning of melt snow water were applied to simulate the absence of snow cover in a Tibetan alpine forest of western China. Soil temperature and moisture, nutrient availability, microbial biomass and enzyme activity were measured at different periods (before snow cover, early snow cover, deep snow cover, snow cover melting and early growing season) over the entire 2009/2010 winter. Snow removal increased the daily variation of soil temperature, frequency of freeze–thaw cycle, soil frost depth, and advanced the dates of soil freezing and melting, and the peak release of inorganic N. Snow removal significantly decreased soil gravimetric water, ammonium and inorganic N, and activity of soil invertase and urease, but increased soil nitrate, dissolve organic C (DOC) and N (DON), and soil microbial biomass C (MBC) and N (MBN). Our results suggest that a decreased snow cover associated with global warming may advance the timing of soil freezing and thawing as well as the peak of releases of nutrients, leading to an enhanced nutrient leaching before plant become active. These results demonstrate that an absence of snow cover under global warming scenarios will alter soil microbial activities and hence element biogeochemical cycling in alpine forest ecosystems.

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1. Introduction

Soils in high-latitude or high-altitude ecosystems during winter experience extensive snow cover, freezing, thawing, and freeze-thaw cycle with the air temperature fluctuates above and below 0 °C (Edwards et al., 2007; Groffman et al., 2011). The global air temperature may increase 1.0–3.0 °C over this century (Lewis, 2013), with more pronounced warming at the high-latitude and high-altitude regions (IPCC, 2007). Warmer temperatures are predicted to decrease the depth and duration of snow cover and increase the frequency, severity, and spatial extent of soil freezing events (Freppaz et al., 2008; Groffman et al., 2011). Such changes could have profound repercussions for soil nutrient cycling and biological activity in high-latitude and high-altitude ecosystems during winter (Koch et al., 2007; Matzner and Borken, 2008; Steinweg et al., 2008; Groffman et al., 2011).

Soil temperature, moisture and soil freezing are closely related to the depth and duration of seasonal snow cover (Hardy et al., 2001; Groffman et al., 2011). In general, a 30–40 cm depth of snow cover could decouple soil temperature from air temperature, preventing the physical changes associated with soil freezing and thawing (Cline, 1997; Steinweg et al., 2008). Removing snow in the early winter could induce soils to remain frozen for most of the winter even after snow re-accumulation (Groffman et al., 2001b; Steinweg et al., 2008). Moreover, a later developed and earlier melt snow cover also could result in greater daily soil temperature variations, more frequent soil freezing and thawing events, deeper soil frost depth and lower soil moisture during winter (Hardy et al., 2001; Groffman et al., 2011). Therefore, decreases in snow cover may potentially affect soil nutrient availability and microbial properties by altering the environmental factors (i.e., soil temperature and freeze–thaw cycle) in cold ecosystems.

In general, soil nutrient availability, microbial biomass and enzyme activity are regulated by environmental factors (i.e., soil temperature, moisture). As mentioned above, snow removal could cause significant effects on soil temperature, moisture and
freeze–thaw cycles, which in turn affect soil biochemical processes (Freppez et al., 2008; Buckeridge et al., 2010; Groffman et al., 2011). Microbes play a critical role in carbon and nutrient transformation in forest soils (Lipson et al., 2002). Any changes in the microbial biomass or community structure may affect the cycling of C and N, and N availability to plants (Saffigna et al., 1989; Clein and Schimel, 1995). Soil enzyme activities are also used as sensors to study the influence of global changes on microbial functionality (Sardans et al., 2008; Allison and Treseder, 2008) and are greatly affected by changes in soil freeze–thaw (Groffman et al., 2009, 2011). In order to understand the overall effects of warming on soils, it is essential to investigate the effects of warming-induced seasonal snow decreases on soil enzymes involved in C and N mineralization (i.e., invertase promotes the hydrolysis of organic C and urease promotes the hydrolysis of organic N) in alpine ecosystems.

The depth and duration of snow cover are often substantially lower in temperate alpine regions than in the subarctic and boreal regions (Edwards et al., 2007). Under moderate snow cover soil organic carbon and nitrogen are significantly greater than under deep or shallow snow (Freppez et al., 2012). Inorganic nitrogen stored in snow may range between 0.2 and 0.8 kg N ha−1 corresponding to about five of the over–winter–N mineralization (Filippa et al., 2010). The predicted changes in soil frost and snow cover in the subarctic and boreal regions have shown variable effects on above- and below-ground processes in different ecosystems (i.e., Steinweg et al., 2008; Wipf et al., 2009; Bombonato and Gerdol, 2012). As compared to the arctic tundra and boreal forest, the duration of seasonal snow cover on the eastern Tibetan Plateau is shorter and the snow depth is relatively thinner (Qin et al., 2006). Moreover, snow on the forest floor in these forests was often close to its melting point and might thus rapidly respond to minor changes in temperature (Wang et al., 2007). Therefore, responses of soil biochemical process in alpine forests on the Tibetan Plateau might be more sensitive to a decreased snow cover compared to other cold ecosystems. However, information is limited on how soil microbial activities and nutrient biogeochemical cycling could be affected under a projected snow cover decrease on the Tibetan Plateau.

The alpine forests of western China between the Tibetan Plateau and the Sichuan Basin are typical alpine forests at low altitude, with important consequences for regional C and N balances (Taylor et al., 1996; Shi et al., 2006). The dynamics surrounding freezing and snowpack development and subsequent thawing often last about half a year (Wu et al., 2010). Moreover, the magnitude of global warming on the Tibetan Plateau is projected to be larger relative to other temperate regions at the same latitude (IPCC, 2007). Thus, the depth and duration of snow cover in alpine forests of western China could be greatly decreased by future climate change. To test these hypotheses, we conducted an experiment to manipulate the depth and duration of snow cover throughout a whole winter season (October 2009 to May 2010) in a fir–spruce dominated alpine forest by an artificial snow removal manipulation with transparency plastic film coverage and returning of melt snow water, our objectives were thus to address how an altered snow cover depth and duration could affect (1) soil microbial properties related to C and N cycling and (2) soil C and N pools under a Tibetan alpine forest.

2. Materials and methods

2.1. Study site

The study site locates in a 120 years old natural alpine forest in the Bipenggou Nature Reserve of Lixian County, Sichuan, China (31°15′28.10″N, 102°53′29.34″E, 3580 m a.s.l. in the Eastern Tibetan Plateau). Canopy vegetation is dominated by fir (Abies faxoniana) and dragon spruce (Picea asperata) with some under-story shrubs (i.e., Salix paraplesia, Rhododendron spp.) and grasses (i.e., Cacalia auriculata, Cystopteris montana, Carex capilliformis) (Tan et al., 2010). Annual mean precipitation is 850 mm. Annual mean temperature is 3.0 ± 0.5 °C with maximum of 23.1 ± 1.1 °C (July) and minimum of −18.0 ± 1.3 °C (January), respectively. Soil temperature goes down below 0°C and remains frozen during the whole cold snow season from late-November to mid-April (Wu et al., 2010). Soil is classified as Cambic Umbrisols (IUSS Working Group WRB, 2007) with a ~15 cm deep organic matter layer. The basic chemical properties of soils (0–15 cm) are as follows: pH 6.1 ± 0.5, 150.3 ± 15.9 g total organic C kg−1, 9.7 ± 0.9 g total N kg−1, 1.2 ± 0.2 g total P kg−1 and 13.4 ± 1.0 g total K kg−1.

The experiment had two treatments: with and without the snow removal from the forest floor. Each treatment had five plots or replicates (5 m × 5 m each) with ≥50 m distance away from each other. Based on Suklava and Huhta (2003), five roofs (0.8 m above the soil surface at the eaves and 1.5 m at the top) with plastic film coverage were used to prevent snow accumulation on the forest floor. The film is made of low density polyethylene that transmits ~80% photosynthetically active radiation. With minimal vegetation damage and soil disturbance, this method differs from the snow removal manipulation by shovel at the Hubbard Brook Experimental Forest (Groffman et al., 2001a, b). The snow removal was started in early November 2009 and ended in late April 2010 when the snow cover in the snow treatment was completely melted. Meanwhile, the forest floor under the plastic film cover was watered three times (21 December 2009, 21 January and 22 March 2010) to simulate rain showers and melting of snow during thaw periods in winter and the melted snow water from the plastic film cover was returned to the forest floor under the plastic film cover, and the irrigation was performed when the air temperature was above 0 °C in winter. On the other hand, five polyethylene roofs with 1.2 m cuts every 1.3 m range within the 5 m × 5 m plot plastic coverage were also supplied to the no-snow removal treatment for the drop-off of snow into the forest floor.

2.2. Microclimate monitoring

Temperature of both forest floor air and soil (5 cm depth) were recorded by buried Thermochron iButton DS1923–F5 Recorders (Maxim Dallas Semiconductor Corp., USA) every 1 h in all plots between October 2009 and May 2010. The start of soil freezing or thawing is defined as the soil temperature is continually dropped below 0 or above 0°C for more than 3 days (Jones, 2001). Meanwhile, a freeze–thaw cycle is defined as whenever the soil temperature is dropped below 0°C for at least 3 h and followed by a rise above 0°C for at least 3 h, and vice versa (Konestabo et al., 2007). Soil gravimetrical water, snow depth (no-snow removal plots) and soil frost depth in all plots were routinely measured.

2.3. Soil sampling

The winter season in the study site had been divided into five periods: (1) early snow, (2) early cold, (3) deep cold, (4) late cold and (5) thaw (Wu et al., 2010). However, during this study no obvious transition was observed between the early snow and early cold or between the late cold and thaw. As a result, in this study only three coverage periods for the winter was divided: (1) early snow, (2) deep snow and (3) snow melting. Meanwhile, two other periods as the before snow cover and the early growing were included for a total five periods in this study.

A total of seven samplings were performed on (1) 19 October for the before snow cover (BSC, 19 October to 9 November 2009), (2) 21 December for the early snow cover (ESC, 9 November to 31
3.1. December 36

3.2. B. ANOVA

3.3. January 10 March 2010, (4) 22 March and 18 April for the snow cover melting (SCM, 11 March to 30 April 2010) and

3.4. 18 May for the early growing period (EGS, 1 May to 18 May 2010). On each sampling date, three soil cores (~150 g each) from
each plot were collected using a soil auger (15 cm depth and 5 cm
diameter) and mixed as one composite sample after the removal of
visible debris and fresh litter. Soil samples were stored in freezer
boxes, and transported to the laboratory within 24h. Fresh soils
were passed through a 2.0 mm sieve and then kept in the refrigera-
tor at 4 •C within less than one week for microbial and chemical
analysis.

2.4. Soil chemical analysis

Available N (ammonium and nitrate) was extracted with a
2 M KCl and then measured by the Indophenol-blue and phenol
disulphonic acid colorimetry, respectively (Lu, 1999). Analysis of
soil microbial biomass C (MBC) and N (MBN) were extracted by
0.5 M K2SO4 and then determined followed by the chloroform
fumigation extraction method with a conversion factor of 0.45
for MBC and 0.54 for MBN (Brookes et al., 1985; Vance et al.,
1987). Soil extractable organic C and total N in these fumigated
and unfumigated extracts were also determined by the dichro-
ate oxidation-ferrous sulphate titration and semi-micro Kjeldahl
method, respectively (Brookes et al., 1985; Vance et al., 1987).

Dissolved organic C (DOC) and total dissolved N (TDN) were
extracted by the method of Jones and Willett (2006). The total C
and N in the extracts were measured using a TOC-VCPH + TNM–1 C/N
analyzer (Shimadzu Inc., Kyoto, Japan). Dissolved organic N (DON)
was calculated as DON = TDN – (ammonium + nitrate). Analysis of
soil invertebrate activity (mg glucose g–1 soil DW d–1) or soil urease
activity (mg NH4+ – N g–1 soil DW d–1) was accorded to Wang et al.

2.5. Statistical analysis

Data (means ± SD, n = 5) were subjected to repeated measures
ANOVA and significant differences between treatment means
for each variable were compared by the Tukey’s HSD post hoc test
or the Student’s independent-sample t-test at P < 0.05. All statisti-
cal analyses were performed using 18.0 SPSS software package
for Windows (SPSS Inc., IL, USA).

3. Results

3.1. Microclimate

Snow cover of snow fall started on 5 October 2009, gradually
accumulated to 19.0 cm on 19 December 2009, reached the max-
imum 36.4 cm in 5 March 2010 and then dropped to 2.4 cm on 22
March 2010 in the no-snow removal plots (Fig. 1). The minimum air
temperature was reached at – 10.29 °C on 18 February 2010, which
was lower than temperature at 5 cm soil depth under both the snow
removal and no-snow removal. Snow removal caused freezing
events in the snow removal plots (Fig. 1). Compared to the no-
snow removal plot, snow removal significantly (P < 0.05) decreased
the average and minimal soil temperature and increased the daily
variation of soil temperature (Table 1). Moreover, the respective
timing of soil freezing and melting advanced by 18 and 38 days,
and the number of freeze–thaw cycle was respectively increased by
12 and 34 times during the early snow cover (ESC) and the deep
snow cover (DSC) period. Snow removal also significantly (P < 0.05)
increased the soil frost duration and frost depth (24.2 ± 3.2 cm),
compared with the no-snow removal (16.7 ± 2.1 cm) (Table 1). In
addition, soil gravimetric water at 0–15 cm depth during the ESC,
DSC and SCM period was significantly increased under the no-snow removal than under the snow removal (Table 1).

3.2. Soil inorganic N pools

Compared with the no-snow removal, under the snow removal ammonium and total inorganic N (ammonium plus nitrate) concentrations were significantly increased in the ESC period, and then significantly decreased in the DSC, SCM, and EGS periods (Fig. 2A and C, \( P = 0.05 \)). In contrast, nitrate concentration was significantly increased by the snow removal during the whole experiment period, except in the EGS (Fig. 2B, \( P = 0.05 \)).

3.3. Dissolved organic C and N in soil

Compared to the no-snow removal, both the DOC and DON were significantly lower in the ESC, but significantly higher in the DSC, SCM and EGS under the snow removal, except for DON in the SCM period (Fig. 2D and E, \( P = 0.05 \)). Meanwhile, DOC showed a similar dynamical change trend to DON under both the snow removal and no-snow removal, i.e., a decrease from the BSC to the ESC and then an increase trend till the EGS period. In addition, both DOC and DON reached highest in the DSC period.

3.4. Soil microbial biomass carbon and nitrogen

Compared to the no-snow removal, both MBC and MBN under the snow removal were significantly lower in the ESC, but generally significantly higher in the DSC, SCM and EGS period, except MBC in the DSC and MBN in the EGS period (Fig. 3A and B, \( P = 0.05 \)). In addition, compared to the no-snow removal, the ratio of MBC to MBN under the snow removal was significantly higher in the ESC, but significantly lower in the DSC, SCM and EGS period (Fig. 3C, \( P = 0.05 \)).

3.5. Soil enzyme activities

Both the soil invertase and urease activity were generally decreased by the snow removal over the whole winter (Fig. 4A and B). In detail, snow removal significantly decreased the invertase activity in the ESC, DSC and EGS period, and the urease activity in the ESC and DSC period. In contrast, the invertase activity in the SCM and the urease activity in both the SCM and EGS period were similar between the no-snow removal and snow removal.

4. Discussion

4.1. Effects of snow removal on soil microclimate

A 30–40 cm depth of snow cover is well known as being critical for decoupling soil thermal changes from the air environment, preventing the soil microenvironment changes associated with the air temperature fluctuates above and below 0 °C (Cline, 1997; Steinweg et al., 2008). This snow depth may offer an ideal combination of moister and warmer soil conditions that can be very favorable to microbial growth and C and N mineralization (Freppaz et al., 2012). Our results suggest that the snow removal has important implications for soil microclimate. During the winter (November to April), the minimal and averaged soil temperatures were higher under the no-snow removal than under the snow removal (Table 1), indicating an insulate effect of snow cover under the snow removal. Snow removal also resulted in relatively mild freezing events in manipulated alpine soils in northwest Italy (Freppaz et al., 2008). Soil temperature was not extremely as low as \(< -3 \) °C, but the greater frequency of freezing and thawing events was observed under the snow removal, particularly during the DSC period (Table 1). The temperature fluctuations, around 0 °C, could have cumulative effects on plant, soil and microbial activities and nutrients losses in winter (Freppaz et al., 2008; Wipf et al., 2009; Groffman et al., 2011). A significant increase of soil frost depth was also observed under snow removal in the Hubbard Brook Experimental Forest, New Hampshire, USA (Groffman et al., 2001a, b; Hardy et al., 2001). Moreover, the date of soil freezing and melting started earlier under the snow removal than under the no-snow removal (Fig. 1). Indeed, the changes in soil freezing and subsequent thawing in winter might have affected aboveground and belowground ecosystem processes in alpine forest in various ways (Wipf et al., 2009; Bombonato and Gerdol, 2012; Comerford et al., 2013). In addition, the higher soil temperature under the snow removal than under the no-snow removal during the early snow cover and
the deep snow cover periods (Fig. 1) might be attributed to greater solar radiation (Freppaz et al., 2008).

On the other hand, soil gravimetical water was also generally significantly lower under the snow removal than under the no-snow removal (Table 1). The decrease of soil moisture may have profound influences on beloground ecological processes because soil moisture is closely related to the physical change of frozen soils and the unfrozen water has tight relation to activities of heterotrophic microorganisms and relevant solute release and transformations (Groffman et al., 2009, 2011).

4.2. Effects of snow removal on soil C and N

Dissolve organic matter (DOM) is an important labile fraction and valuable C source for nutrients cycling that rapidly respond to environmental changes (Fitzhugh et al., 2001; Jones and Willett, 2006). The decrease of temperature associated with snow removal could affect the DOM budget of soil ecosystems through the enhancement of physical disruption of soil aggregates and DOM decomposition (Larsen et al., 2002; Steinweg et al., 2008). Snow removal also stimulated the release and loss of DOM (Joseph and Henry, 2008; Matzner and Borken, 2008), and did greatly increase both DOC and DON from the deep snow cover till the early growing period (Fig. 2D and E). There were several possible underlying mechanisms resulting in such changes. First, the daily variation of soil temperature was remarkably greater under the snow removal than the under no-snow removal (Fig. 1), which would accelerate the release of DOM from fresh litter and soil aggregates (Steinweg et al., 2008; Wu et al., 2010; Freppaz et al., 2012). Second, increased freezing and thawing cycles could often exert greater damage on soil organisms (fauna and microbes) and fine roots (Tierney et al., 2001; Koponen et al., 2006; Conerford et al., 2013). As a consequence, the lysis of these organisms and fine roots would provide larger amounts of lable or dissolved organic matter. Third, in comparison with the no-snow removal, the snow removal decreased the loss of DOM through leaching during snow melting, and thus favored the accumulation of DOM in soil (Hardy et al., 2001). These results indicate that snow removal may change the dynamic of DOC and DON in winter and subsequently affect the soil C and N mineralization in an alpine forest.

Snow cover also is a key factor to regulate soil N turnover and transformation in the cold season in alpine regions (Groffman et al., 2001a,b). For example, soil NO$_3^-$ concentrations were significantly increased by snow removal as seen in the Fig. 2B, which might be either through stimulating nitrification rates or decreasing root uptake (Groffman et al., 2001b). Moreover, studies had demonstrated that soil N availability (ammonium and inorganic N) was increased under snow removal (Fitzhugh et al., 2001; Hardy et al., 2001). However, soil N availability only increased during the early snow cover period in this study (Fig. 2C). This was mainly because snow removal increased the physical disruption of fresh litter, and fine root and microbial mortality during the early snow cover with
the increase of freeze-thaw cycles (Tierney et al., 2001; Koponen et al., 2006; Comerford et al., 2013). In contrast, snow removal decreased soil N availability during both the deep snow cover and snow cover melting period (Fig. 2C). Freppaz et al. (2012) have shown that soils typically do not experience severe freeze/thaw events and free water is available throughout the winter under moderate snow cover condition, resulting in heterotrophic activity, microbial growth and C and N mineralization continue through the winter and N retention is relatively high. Thus, the disappearance of snow insulating associated with the increase in freeze-thaw cycles in the snow removal plots decreased soil N availability during both the deep snow cover and snow cover melting period. In addition, nutrient losses typically peaked in the early spring when microbial mineralization and nitrification could precede nutrient uptake by fine roots in northern hardwood forests of New Hampshire, USA (Muller and Bormann, 1976). Our results showed that the peak time of the available N (ammonium, nitrate and inorganic N) was earlier (the deep snow cover period vs. the snow cover melting period) under the snow removal than under the no-snow removal (Fig. 2A–C). This may lead to a certain amount of N loss through leaching during the late winter and early spring when plants remain being dormant as available N may not be taken up by fine roots (Groffman et al., 2001b, 2011; Edwards et al., 2007; Freppaz et al., 2007; Joseph and Henry, 2008).

4.3. Effects of snow removal on soil microbial activities

Soil microbes play critical roles in C and nutrient transformation in forest soils (Lipson et al., 2002). Studies in the arctic tundra and alpine ecosystem with persistent snow cover have demonstrated

Fig. 3. Effect of snow removal during a whole winter on soil microbial biomass C (MBC, A), soil microbial N (MBN, B) and the ratio of MBC to MBN (C) at 0–15 cm in a natural alpine forest in the eastern Tibetan Plateau. Error bars indicate standard error (n = 5). *P < 0.05, ns: not significant. Abbreviations: BSC, before snow cover; ESC, early snow cover; DSC, deep snow cover; SCM, snow cover melting; EGS, early growing period.

Fig. 4. Effect of snow removal during a whole winter on the activity of soil invertase (A) and urease at 0–15 cm (B) in a natural alpine forest in the eastern Tibetan Plateau. Error bars indicate standard error (n = 5). *P < 0.05, ns: not significant. Abbreviations: BSC, before snow cover; ESC, early snow cover; DSC, deep snow cover; SCM, snow cover melting; EGS, early growing period.
that soil microbial biomass often kept at an annual peak value during the winter (Edwards et al., 2007). Slight changes in the microbial biomass or community structure could affect the cycling of C and N (Saffigna et al., 1989; Clein and Schimel, 1995). Lipson and Monson (1998) and Lipson et al. (2000) reported that multiple moderate freeze-thaw fluctuations appeared to have only a minor influence on microbial biomass in alpine meadows. In this study, snow removal significant increased freeze-thaw cycles (Table 1), which had different effects on soil microbial biomass. For instance, the increased freeze-thaw fluctuations showed negative effect on soil microbial biomass during the early snow cover period, but positive effects during the deep snow cover period. On the one hand, during the onset of soil freezing (i.e., in the early snow cover period), an increase of freeze-thaw cycles might damage soil microbial biomass (Clein and Schimel, 1995). Subsequently, nutrients released from the cells of the senescing microbes could be utilized by surviving microbes. On the other hand, the surviving microbes could be stimulated by the input of C and N substrates (i.e. DOC and DON), leading to a marked increase in the soil microbial biomass. The C and nutrients in cells of microbes then acted as important nutrient sources in the early spring when snow was melting (Schmidt and Lipson, 2004). Furthermore, MBN was significantly increased under the snow removal during both the deep snow and snow melting period, implying that the released available N and DON could have been immobilized by microbes. Therefore, the partial immobilization of nutrients as microbial biomass might represent the immediate input of available nutrients into these forest soils for plant growth after snowmelt in the early spring (Edwards et al., 2007; Buckk, 2010).

In both the snow removal and no-snow removal soils, the MBC: MBN ratios were relatively low before snowfall, indicating that bacteria contributed most to the microbial biomass (Freppaz et al., 2008). However, the MBC: MBN ratios were significantly increased after the formation of snow cover, also indicating that a potential shift in the contribution of fungi to the microbial biomass (Lipson et al., 2002). The increase of the MBC: MBN ratios were greater under the snow removal than under the no-snow removal during the early snow cover, but not after the early snow cover period (Fig. 3C). This is in conflict with a low MBC: MBN ratio after snow removal in alpine soils and subarctic soils at higher latitudes (Larsen et al., 2002; Freppaz et al., 2008). These results suggested that the decrease of snow cover might have more complex effects on microbial community structure in alpine soils at low latitudes, which have important implications for soil C and N mineralization in winter (Schmidt and Lipson, 2004; Groffman et al., 2011).

Soil enzyme activities are also considered as important indicators of microbial activities (Allison and Treseder, 2008; Sardans et al., 2008). Studies revealed that soil enzyme activities maintained relatively high under snow cover (Lipson et al., 2002; Koch et al., 2007), and snow removal often induced lower soil enzyme activities (Larsen et al., 2002; Koponen et al., 2006). In our study, snow removal significantly decreased both the soil invertebrate and urease activity during most of the whole winter (Fig. 4A and B). These were generally accompanied by a decrease of ammonium under snow removal plots (Fig. 2A). The decreases of soil enzyme activities could mainly be attributed to the lack of available water and substrates (Groffman et al., 2009, 2011). Therefore, snow removal could be negatively to accelerate soil C and nutrient cycling. However, a significant increase in DOC and DON under the snow removal (Fig. 2D and E) could compensate for the shortage of C and N in the deeper snow and late winter, resulting in the increase of soil invertebrate and urease activities during the snow cover melting period. This might be in favor of C and N mineralization during the late winter and early spring period.

5. Conclusions

The absence of persistent snow cover resulted in earlier freezing and melting during the winter. In particular, the loss of thermal buffering under snow removal induced a deeper soil frost depth and more frequency of mild freezing and thawing cycles or events. These changes caused a significant increase of nitrate and earlier peak accumulation of soil inorganic N during the winter. Moreover, during most of the sampling dates, snow removal decreased soil ammonium, inorganic N and invertase and urease activities but increased MBC and MBN, DOC and DON. These results suggested that the decrease of snow cover associated with global warming might advance the timing of soil freezing and thawing and peak releases of nutrients. Such changes might result in the loss of nutrients by leaching before plant become active. In addition, a significant increase in the MBC: MBN ratio also indicated a potential shift from bacterial dominated to fungal dominated communities. Our results suggest that a decreased snow cover associated with global warming may advance the timing of soil freezing and thawing as well as the peak of releases of nutrients, leading to an enhanced nutrient leaching before plant become active. Our results demonstrate that an absence of snow cover under global warming scenarios will alter soil microbial activities and hence element biogeochemical cycling in alpine forest ecosystems.

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