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Effects of snow absence on winter soil nitrogen dynamics in a subalpine spruce forest of southwestern China

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ABSTRACT

The lack of snow cover due to winter climate change has great potential to impact winter soil nitrogen cycling in boreal forests. A snow manipulation was conducted in a Tibetan spruce forest to explore the effects of snow absence on winter soil nitrogen dynamics by shelter method. Snow absence on average reduced soil temperatures at the depths of 0 cm and 5 cm by 1.44 °C and 0.33 °C, respectively, throughout the winter. Moreover, snow absence increased soil frost and freeze-thaw cycles. Soil net nitrogen mineralization and labile nitrogen pools (ammonium, nitrate and dissolved organic nitrogen) were higher in the snow absence plots compared to control plots. Snow absence increased soil microbial biomass carbon but did not affect microbial biomass nitrogen. Nevertheless, soil enzyme activities involved in nitrogen cycles were often lowered by snow absence over the winter. The results noted in this study suggest that warming-induced absence in seasonal snowpack may stimulate winter soil nitrogen availabilities by changing soil microhabitats, which has important implications for soil biogeochemical cycles in the subalpine forest ecosystems on the eastern Tibetan Plateau.

1. Introduction

At high latitudes and altitudes, winter precipitation is more likely to occur in the form of rain rather than snow as a result of climate change (IPCC, 2013; Wang et al., 2016). A reduction in winter snowfall could result in decreased snow depth and thereby enhance freeze-thaw cycles and soil frost as a result of the lack of insulation (Kreyling et al., 2012; Bokhorst et al., 2013), which could, in turn, have significant impacts on soil biogeochemical processes in boreal forests.

In order to get better insights into mechanisms underlying soil biochemical responses, a number of snow manipulation experiments have been carried out in snowy region (e.g., Groffman et al., 2001; Shibata et al., 2013; Tan et al., 2014). However, no consistent conclusions have emerged from these studies (Li et al., 2016a, 2016b). The lack of insulating snowpack could decrease soil temperatures, resulting in lower microbial metabolism and enzymes activities, thereby decreasing organic matter mineralization rates. However, a decrease in snow depth may enhance soil frost and freeze-thaw cycles, inducing higher root and microbial mortality (Cleavitt et al., 2008; Tierney et al., 2001), which are important available N sources and play critical role in soil N cycling processes under snow cover (Schimel et al., 2004). Therefore, the increase in substrate availability due to the enhanced root and microbial mortality may, in part, counteract the temperature effect under snow-free conditions. In cold biomes, winter soil freezing might affect the potential for interaction between nutrients and microbes, which is a critical regulator of nutrient cycling and retention during snow-covered period (Brooks and Williams, 1999). Thus, the lack of snow cover associated with climate change may exert complicated and important effects on soil biogeochemical cycles in cold ecosystem. Soil biochemical responses to snowpack reduction may be dependent on ecosystem traits (e.g., substrate quality and quantity) and site conditions (e.g., snowfall, albedo).

As the earth's ‘third pole’, the Tibetan Plateau has experienced a pronounced warming and winter snowfall has been decreasing over last decades (Chen et al., 2013; Wang et al., 2016). Moreover, snow cover in this region has its unique characters, including shorter snow duration and thinner snow depth relative to high latitudes. Furthermore, winter soil temperature is close to physical melting point and belowground processes are very susceptible to slight temperature changes (Wang et al., 2007). Hence, soil biogeochemical processes could be more sensitive to the alteration of snowpack. Up to now, most of existing studies in climate change biology have only focused on the growing season (Xu et al., 2010; Yin et al., 2013), while climate change in this region is more significant and soil biological activities are high over the wintertime (Chen et al., 2013; Tan et al., 2012). Thus, in this study, a snow manipulation experiment was conducted in a Tibetan spruce

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forest to assess the effects of snow absence on winter soil N dynamics. Specifically, we hypothesized that (1) snow absence would increase soil net N mineralization and N availability; (2) snow absence would increase soil microbial biomass and enzyme activities.

2. Materials and methods

2.1. Site description

The field experiment was conducted in a *Picea asperata* (Dragon spruce) stand at the Long-term Research Station of Alpine Forest Ecosystems, which is located on the eastern Tibetan Plateau, China (31°15′10″N,102°53′29″; 3021 m a.s.l). The annual mean precipitation and temperature are 850 mm and 3.0 °C, respectively. In general, snow cover begins to accumulate in late November and melts in late March of the following year. The understory is dominated by *Salix paraplesia*, *Rhododendron lapponicum*, *Cacalia spp*., *Carex spp*., and *Cyperus* spp. The soil is classified as Cambic Umbrisols (IUSS Working Group WRB, 2007). The basic physicochemical properties of soil (0–15 cm) are as follows: carbon 88.51 ± 10.52 g kg^{-1}, nitrogen 5.43 ± 0.51 g kg^{-1}, phosphorous 0.41 ± 0.01 g kg^{-1}, pH 6.41 ± 0.05 and bulk density 1.27 ± 0.03 g cm^{-3}, respectively.

2.2. Experimental design

In order to achieve a snow-free condition, six wooden roofs were set up in the spruce forest. One control plot was randomly established in the vicinity of each wooden roof. It was expected that all of the selected plots were identical in microhabitat characteristics. The wooden roof used in this study was 2 m height, with 3 m × 3 m in the ground area. A transparent plastic sheet on the top of roof was used to prevent snow accumulation on the ground. In late winter, the accumulated snow on the roof was added to the forest floor in order to ensure the similar water balance between the snow-free and control plots. The snow manipulation was started in late November 2015 and ended in late March 2016 when the seasonal snow in the control plots was melted.

2.3. Microclimate monitoring

Air temperature at 2 m height in the study site and soil temperatures 0 cm and 5 cm below the soil surface in the snow-free and control plots were measured by the Thermochron iButton DS1923–F5 Recorders (Maxim Dallas Semiconductor Corp., USA) every 1 h from mid November in 2015 to mid April in 2016. Soil moisture 5 cm below the soil surface was measured with a hand-held probe at about 2-week interval. Snow depth in the control plots and soil frost depth in each plot were measured approximately every 2 weeks. Soil frost depth was determined with soil tubes as described by Hardy et al. (2001). Briefly, the frost tubes consist of a permanently installed PVC tube and an inner tube. The inner tube was made of clear rubber and was filled with water and a dye. In winter, the inner dye-filled tubes were moved and the frost depth was measured. Freeze-thaw cycles were calculated as the number of occurrences when the soil temperature crosses the 0 °C isotherm and then returns to above-zero temperature more than 3 h (Konestabo et al., 2007).

2.4. Soil sampling and chemical analysis

Soil samples were collected from the top soil (0–15 cm) in the early snow cover (ESC), deep snow cover (DSC) and early snow melting (ESM) period, respectively. Three cores (5 cm in diameter, 0–15 cm deep) were randomly taken at each plot. The three soil cores from each plot were mixed to get one composite sample which was passed through a sieve (2 mm diameter), and any visible living plant material was manually removed from the sieved soil. The sieved soil was kept in the refrigerator at 4 °C (less than one week) for microbial properties, available nutrients and enzyme activities.

Inorganic soil N (ammonium and nitrate) was extracted with 2 M KCL extracting water solution and then measured by the Indophenol-blue and phenol disulphonic acid colorimetry, respectively (Xu et al., 2010). Soil microbial biomass carbon (MBC) and soil microbial biomass nitrogen (MBN) were measured by fumigation-extraction method. The released C and N were converted to MBC and MBN using *k*ec − 0.45 and *k*en − 0.54, respectively (Vance et al., 1987). Dissolved organic N (DON) was extracted by the method of Jones and Willett (2006). Total dissolved N (TDN) was measured using a total C & N analyzer (TOC-VcPH + TNM-1, Shimazu Inc., Japan). Dissolved organic N (DON) was calculated as DON = TDN − TIN (ammonium + nitrate). Soil nitrate and nitrite reductase activities were assayed as described by Xiong et al. (2014). For soil urease activity, we used the Kandeler and Gerber (1988) method.

Soil net N mineralization over the wintertime was determined from in situ incubations using the buried tube technique. The incubations were conducted using perforated PVC tubes (15 cm in height and 5 cm in diameter). Para film covered the top of each tube to avoid leaching of N. The wintertime net N mineralization (From mid November 2015 to early April 2016) was expressed as the difference in inorganic N (nitrate and ammonium) in the soil before and after incubation.

2.5. Statistical analysis

Repeated measures ANOVA was performed to test the effects of treatment, sampling date, and their interactions on measured parameters. For specific sampling dates, Student *t*-tests were used to compare the effect of the snow absence. Redundancy Analysis (RDA) was used to test the correlations between the measured N parameters and environmental factors. The statistical tests were considered significant at the $P < 0.05$ level. All statistical analyses are performed using 16.0 SPSS software package for Windows.

3. Results

3.1. Microclimates

The minimum air temperature was − 15.3 °C in late January 2016 (Fig. 1). Seasonal snow began to accumulate in late November 2015 and melted in late March 2016 with the maximum snow depth reaching approximately 40 cm in late February in the control plots (Fig. 2B). Compared to the control plots, snow absence lowered the average and minimal soil temperatures (Table 1). Snow absence on average reduced

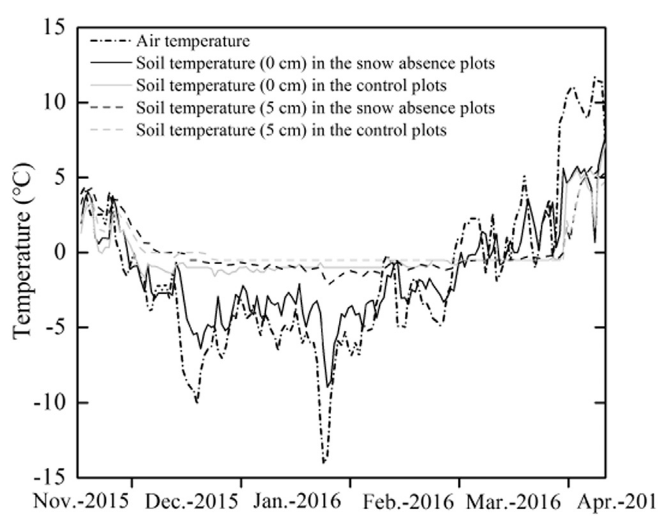

Fig. 1. Seasonal dynamics of air temperature (2 m high) and soil temperatures (0 cm and 5 cm) in the snow absence and the control plots in a subalpine forest of southwestern China.

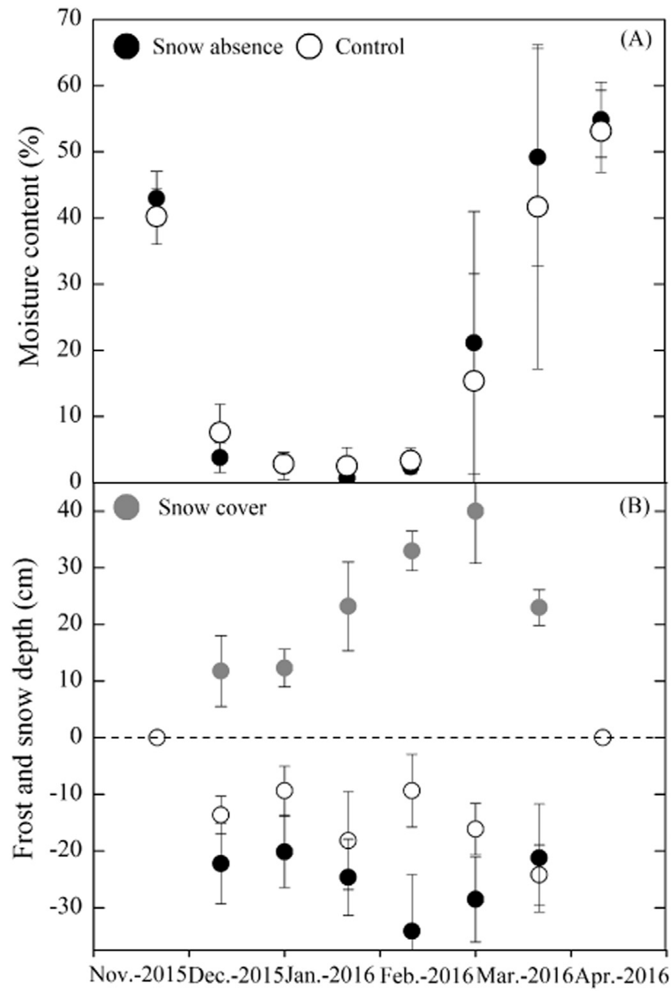

Fig. 2. Seasonal dynamics of moisture content (A), soil frost and snow depth (B) in the snow absence and the control plots in a subalpine forest of southwestern China over the wintertime.

Table 1
Soil temperature and soil frost in both treatments during the period of wintertime.

Soil depth	Treatment	Mean temperature	Minimum temperature	Duration of frozen soil	Freeze-thaw cycle
		(°C)	(°C)	(days)	(No.)
0 cm	Snow absence	− 2.15	− 9.1	103	63
	Control	− 0.71	− 1.6	122	24
5 cm	Snow absence	− 0.61	− 2.2	83	25
	Control	− 0.28	− 0.5	99	13

soil temperatures at the depths of 0 cm and 5 cm by 1.44 °C and 0.33 °C, respectively, throughout the winter (Table 1). The minimum soil temperatures at the soil depth of 0 cm and 5 cm were − 9.1 °C and − 2.2 °C in the snow-free plots but were only − 1.6 °C and − 0.5 °C in the control plots, respectively (Table 1). Moreover, daily variations of soil temperatures remained relatively stable in the control plots but fluctuated strongly in the snow-free plots (Fig. 1). Soil moisture exhibited a similar seasonality over the winter in both treatments. In addition, there was no obvious difference in soil moisture between treatments (Fig. 2A). The soil frost depth was greater in the snow-free plots relative to control plots (Fig. 2B). Snow absence decreased the duration of soil frozen and increased the number of freeze-thaw cycle in both soil layers (Table 1).

3.2. Soil N pools and net N mineralization rate

In comparison with the control plots, snow absence tended to increase inorganic N (ammonium and nitrate) and DON concentrations (Fig. 3). Snow absence caused positive effects on ammonium and extractable inorganic N concentrations in the DSC and SCM, but not in the ESC period (Fig. 3A and C). Likewise, Snow absence also significantly increased nitrate concentrations in the ESC and SCM periods (Fig. 3B). In addition, soil DON was increased in the snow-free plots in each period (Fig. 3D). The ANOVA analyses showed that the interaction of snow absence and sampling dates had significant effects on nitrate, extractable inorganic N and DON (Table 2). Moreover, multivariate redundancy analysis (RDA) showed that soil N pools were correlated positively with freeze-thaw cycles, soil temperature and moisture but negatively with the soil frost depth (Fig. 4). In the incubation experiments, snow absence induced a higher net N mineralization rate during the wintertime (Fig. 5). Ammonification and nitrification contributed almost equally to the net N mineralization in both treatments (Fig. 5).

3.3. Soil microbial properties

Snow absence significantly increased MBC in the ESC and DSC periods (Fig. 6A). However, there were no significant snow absence effects on MBN among the sampling dates (Fig. 6B). Similar to MBC, the ratios of MBC:MBN were significantly higher in the snow-free plots than in the control plots both in ESC and DSC periods (Fig. 6C). RDA results suggested that soil frost depth had a positive relationship with microbial biomass C (Fig. 4). In addition, the ANOVA analyses showed that the effects of snow absence on MBC and MBC:MBN ratios were dependent on sampling seasons (Table 2).

3.4. Soil enzyme activities

Snow absence tended to reduce soil enzyme activities involved in N transformation (Fig. 7A, B, C). Snow absence decreased urease in the DSC period (Fig. 7A). Nitrate reductase was lowered by snow cover manipulations in the DSC and SCM periods (Fig. 7B). Similarly, obvious differences in nitrite reductase between treatments were detected in the ESC and DSC periods (Fig. 7C). RDA results indicated that urease was positively affected by soil frost depth (Fig. 4). Conversely, nitrate reductase was positively correlated with freeze-thaw cycles and soil moisture (Fig. 4). In addition, the statistical results showed that the interaction of snow absence and seasons had significant effects on urease activity but not on nitrate and nitrite reductase activities (Table 2).

4. Discussion

As a snow manipulation technique, shelters are considered to be a useful tool for studying the responses of soil biogeochemical processes to snow cover change associated with winter warming or snowfall reduction because they can effectively lead to snow-free conditions and minimize the changes in other unwanted environmental conditions (Fitzhugh et al., 2001; Tan et al., 2014; Li et al., 2016a, 2016b). Soil temperature and soil freeze-thaw cycles are correlated closely with the depth and duration of winter snow cover (Rixen et al., 2008; Groffman et al., 2011). For example, a snow cover with 30–40 cm depth can significantly decouple soils from air temperature (Cline, 1997; Steinweg et al., 2008). In the current case, snow absence reduced daily average soil temperature by 1.44 °C (0 cm) and 0.33 °C (5 cm), respectively, throughout the winter. The decrease in soil temperature caused by snow-free is similar to or less than those recorded in the temperate and boreal forests (Rockström et al., 2009; Wang et al., 2003). This may be due to the fact that there are significant differences in snow conditions (snow density and depth), air temperature, solar radiation and albedo among different experimental sites. In addition, snow absence increased the seasonal variations of soil temperatures, frost depth and freeze-thaw

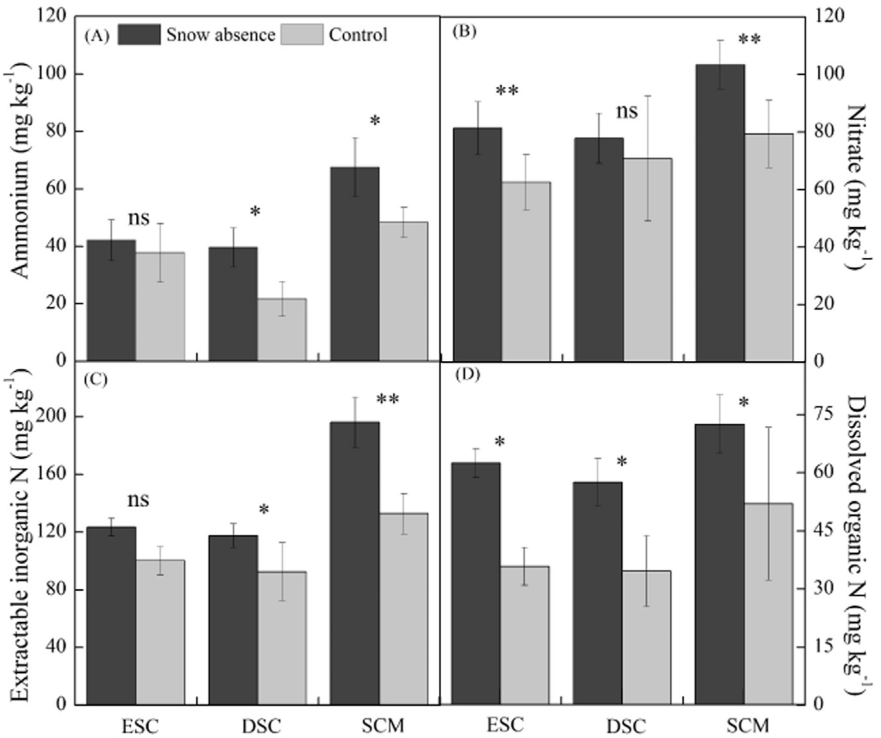

Fig. 3. Effect of snow absence on ammonium (A), nitrate (B), extractable inorganic N (C) and dissolved organic N (D) concentrations. Asterisks denote significant differences between treatments in same sampling date. $*p < 0.05$, $**p < 0.01$, ns $p > 0.05$; ESC, early snow cover; DSC, deep snow cover; SCM, snow cover melting.

Table 2
Summary results of repeated measure ANOVA for the effects of sampling date, snow absence and their interaction on soil N pools, microbial biomass and enzyme activities.

Variables	NH_4^+-N	NO_3^--N	TIN	DON	MBC	MBN	MBC:MBN	URA	NARA	NIRA
SA	0.321	< 0.001	< 0.001	0.002	0.050	0.911	0.018	0.140	< 0.001	0.349
SD	< 0.001	0.048	< 0.001	0.052	< 0.001	0.014	< 0.001	< 0.001	< 0.001	< 0.001
SD × SA	0.753	< 0.001	< 0.001	0.037	0.033	0.872	0.019	0.001	0.373	0.243

SA: snow absence; SD: sampling date; TIN: total inorganic nitrogen; DON: dissolved organic nitrogen; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; URA: urease activity; NARA: nitrate reductase activity; NIRA: nitrite reductase activity.

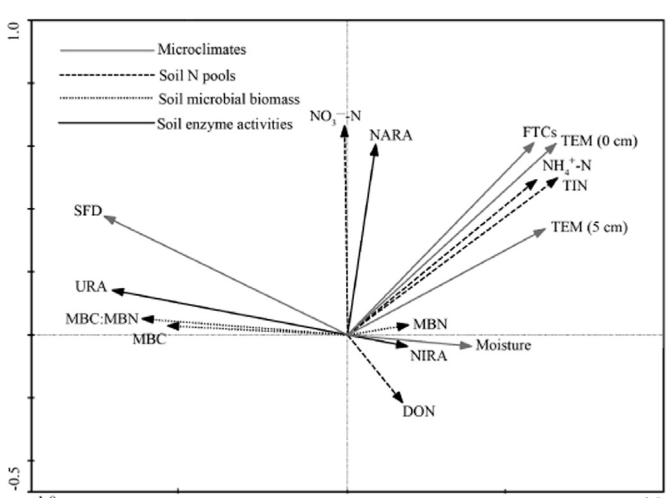

Fig. 4. Redundancy analyses (RDA; axes 1 and 2) biplot of the soil N pools, soil microbial biomass and soil N enzyme activities constrained by the soil microclimates. TEM (5 cm): soil temperature at the 5 cm depth; TEM (0 cm): soil temperature at the surface; SFD: soil frost depth; FTCs: freeze-thaw cycles; TIN: total inorganic nitrogen; DON: dissolved organic nitrogen; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; URA: urease activity; NARA: nitrate reductase activity; NIRA: nitrite reductase activity.

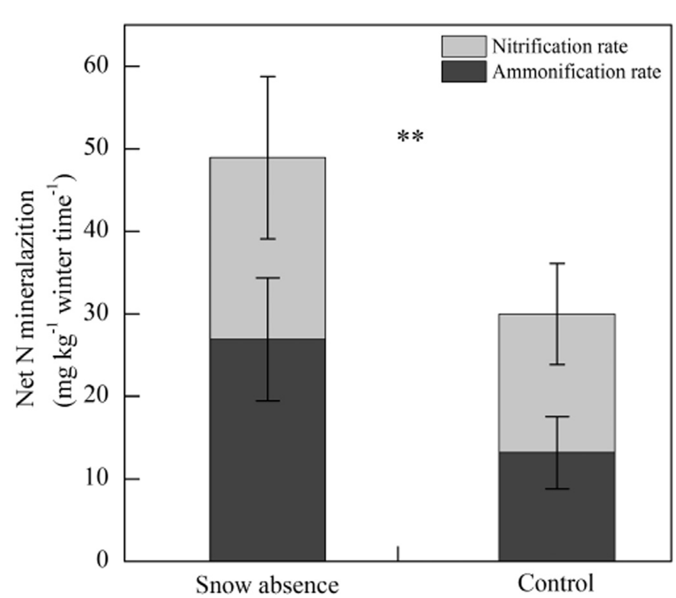

Fig. 5. Effect of snow absence on soil net N mineralization. Asterisks denote significant differences between treatments. $**p < 0.01$.

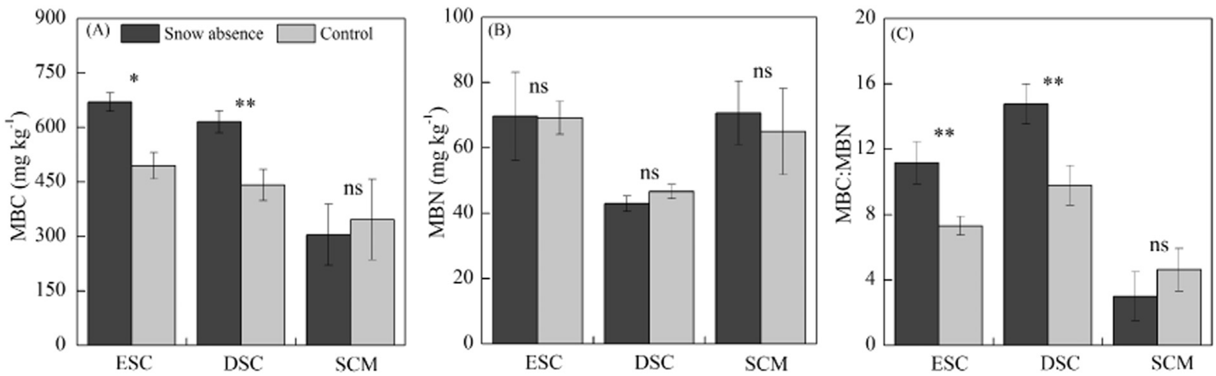

Fig. 6. Effect of snow absence on MBC (A), MBC (B) and MBC:MBN (C). Asterisks denote significant differences between treatments in same sampling date. $*p < 0.05$, $**p < 0.01$, ns $p > 0.05$; ESC, early snow cover; DSC, deep snow cover; SCM, snow cover melting.

cycles, suggesting that seasonal snow cover, as an insulator, can profoundly prevent forest soils from frigid winter climate in the study area. In this study, the wooden roofs did not affect soil moisture over the experimental period. This result was different from the snow manipulations by shovel, which often resulted in lower soil moisture as compared to control plots (Hardy et al., 2001; Wipf and Rixen, 2010).

Soil N availability is a key limiting factor for plant productivity in terrestrial ecosystems, especially in cold biomes (Lavoie et al., 2011). It has been believed that warming-induced reduction in snow cover might directly and/or indirectly impact soil N dynamics in cold ecosystems through altering biotic and abiotic factors, including soil temperature and moisture, freeze-thaw cycle, substrate quality and quantity and microbial properties (e.g., Hardy et al., 2001; Steinweg et al., 2008). In line with our first hypothesis, snow-free manipulation resulted in significant increases in soil N availabilities in the subalpine spruce forest over the wintertime. The increases in N availabilities in the snow-free plots were consistent with other studies conducted in temperate or boreal forests (Ozawa et al., 2001; Sulkava and Huhta, 2003; Viglietti et al., 2014). This observation noted in our case was mainly because soil net N mineralization rate was significantly stimulated by snow absence treatment during the wintertime. The higher DON concentration in the treatment plots could be partially responsible for observed results because the increased DON may provide more high-quality N source for N mineralization. There are several underlying possible mechanisms for our observations. First, previous studies have demonstrated that freeze-thaw cycle is favorable to litter fragmentation of subalpine forests over the wintertime in our study site (Deng et al., 2010; Zhu et al., 2012). Thus, the increased freeze-thaw cycle in the snow-free plots, to some extent, may increase winter soil N availabilities by releasing greater amount of high-quality organic matter (potential source for soil N mineralization) from aboveground and belowground litters relative to the control plots. Second, previous studies have observed that a relatively mild freezing caused by snow removal significantly increased the mortality of plant fine root or soil microbes, implying that larger labile organic matter may be added in these soils (Robroek et al., 2013; Yin et al., 2014). This may also partly favor the production of extractable soil N in the snow-free plots. Lastly, freezing-induced disruption in soil aggregates made organic substrates more accessible to soil microbes, suggesting that larger amount of inorganic N could be released by mineralization (Steinweg et al., 2008).

On the other hand, the enhanced N availability induced by snow absence is likely to favor primary production early in the growing season, especially in cold ecosystems where N is limiting plant growth. However, if soil N mineralization was much higher than immobilization by the living biomass, thus soil N may leach to ground causing soil acidification. Both soil DON and nitrate are generally easier to be leached as compared to soil ammonium. Therefore, in the present case, freezing-induced increases in extractable N pools (DON and nitrate) throughout the winter could also result in certain N loss by leaching early over the growing season.

Considerable evidence is now available indicating that microbes play key roles in soil N cycling in terrestrial ecosystems. Slight changes in microbial biomass or community structure are likely to impact the cycling of soil N (Robroek et al., 2013; Jusselme et al., 2016). Consequently, soil microorganisms have been thought to be the potential indicator of the effects of global change on forest soils (Aanderud et al., 2013). It has been demonstrated that a considerable number of soil microbes can survive and maintain relatively high activities under snow cover. Moreover, soil microbial growth and activities are very vulnerable to soil frost (Groffman et al., 2011; Bokhorst et al., 2013). In the present study, snow absence increased soil MBC on most of sampling dates, implying that soil microorganisms themselves may, to some extent, profit directly from the higher N availability. However, the lack of a clear effect on MBN suggested that microbial immobilization is not

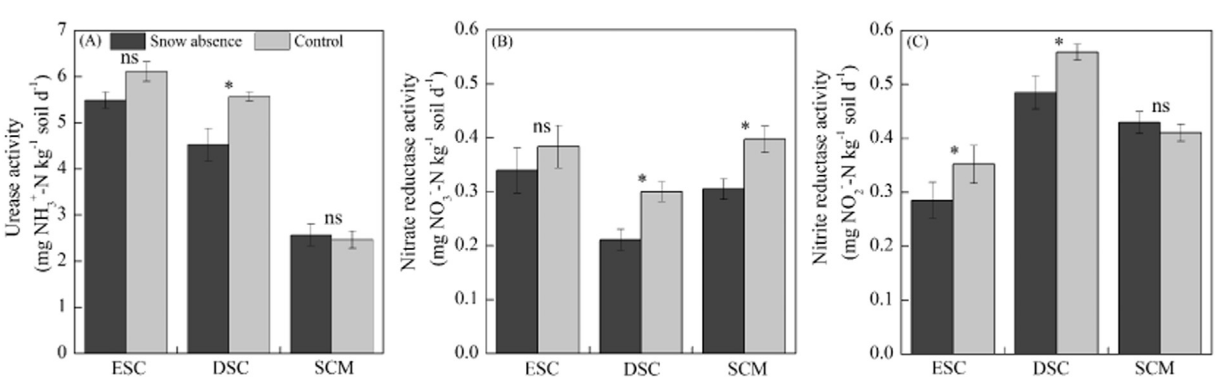

Fig. 7. Effect of snow absence on urease (A), nitrate reductase (B) and nitrite reductase (C) activities. Asterisks denote significant differences between treatments in same sampling date. $*p < 0.05$, ns $p > 0.05$; ESC, early snow cover; DSC, deep snow cover; SCM, snow cover melting.

synchronous with higher N availability. The ratio of MBC:MBN can partly characterized soil microbial structure. In our experiment, snow-free induced a higher MBC:MBN ratio in the ESC and DSC periods. This finding is different from the previous studies derived from temperate hardwood forest ecosystems (northeastern USA) and a subalpine forest ecosystem (northwestern Italy), where the ratio of MBC:MBN lowered or did not change after snow-free treatment (Viglietti et al., 2014; Sorensen et al., 2016). Such response differences imply that the effects of seasonal snow cover on microbial structure may vary with snow conditions or forest types. Some studies have demonstrated that a great number of bacterial lysis was observed after freeze–thaw events and soil fungi could be more stable during the seasonal freeze–thaw stage (Freppaz et al., 2008; Lipson et al., 2002; Zhang et al., 2005). The differences in cold-tolerance between soil bacteria and fungi may also partially reflect a potential shift of contribution to microbial biomass.

Soil enzyme activities, as important environmental sensors and microbial indicators, are often used in global change biology (Xu et al., 2010; Groffman et al., 2011). Therefore, knowing soil enzymes involved in N transformation is very helpful to understand winter soil N cycle in boreal soils. Several studies have revealed that soil enzyme activities are sensitive to soil frost and freeze-thaw cycle (Tan et al., 2012; Sorensen et al., 2016). This case found that soil enzymes still kept relatively high activities over the winter in the spruce forest. However, in line with our second hypothesis, snow absence on average decreased soil urease, nitrate and nitrite reductase activities by 11.04%, 20.89% and 9.25%, respectively, over the whole winter. It is widely accepted that soil N_2O fluxes are associated strongly with soil denitrification enzyme activities which are regulated by environmental changes at large (Groffman and Tiedje, 1989; Groffman et al., 2011). Consistent with this deduction, lower denitrification enzyme activities in the snow-free plots may imply that soil N_2O fluxes were decreased by soil freezing. In comparison with snow-free plots, seasonal snow cover in the control plots may decouple oxygen flux, leading to a lower soil oxygen concentration. The anaerobic conditions could, to some extent, favor the denitrification enzyme activities (Wang et al., 2007). Moreover, freezing-caused changes in available N and microbial biomass may also be beneficial to soil denitrification enzyme activities.

5. Conclusion and uncertainty

In summary, this study explored the potential effects of snow-free associated with climate change on soil N availabilities and enzyme activities in a subalpine forest of southwest China over the wintertime. The results noted in this case revealed that the lack of snow cover significantly lowered soil temperatures but increased soil frost and freeze-thaw cycle. In addition, snow absence increased soil N availabilities and net N mineralization but decreased soil enzyme activities during the winter season. The freezing-increased soil labile N pools over the winter may favor the plant growth, but also increase the risk of N loss through leaching early in the growing season. Winter soil N cycling is very susceptible to direct and/or indirect effects derived from snow conditions in this specific area.

Seasonal snow cover is key factor that regulates soil biogeochemical process during the winter in cold ecosystems. However, warming-induced reduction in snow cover could have complex impacts on soil biogeochemical processes in cold terrestrial ecosystems in different scenarios. On the one hand, if winter snowfall would reduce significantly and air temperature would not change or change slightly in the near future, the lack of snow cover could lower soil temperatures but increase variations of topsoil temperature, and further alter belowground biogeochemical processes. On the other hand, if snow-free winter would be directly caused by strong winter warming in the coming decades, the increased air temperature could, to some extent, offset the negative effects of snow-free on soil temperature. However, to our knowledge, seasonal snow cover plays a fundamental and significant role in decoupling soil from frigid air temperature. Nevertheless, soil temperature generally is not sensitive to small change in air temperature. Therefore, the decreased winter snowfall associated with climate change in Tibetan forests could have a stronger impact on soil biotic and abiotic processes than global warming itself. Certainly, long-term monitoring is very important to explore the winter belowground dynamics and underlying mechanisms of the observed phenomena. Moreover, additional delicate work need to integrate relevant variations and explore their potential relative importance would help us to better understand and predict the belowground dynamics of cold biomes in a warmer world.

Acknowledgements

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