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Effects of snow absence on N pools and enzyme activities within soil aggregates in a spruce forest on the eastern Tibetan Plateau

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Abstract

Background

Snow cover change has a great potential to impact soil nitrogen (N) pools and enzyme activities in boreal forests. Yet, the nature of this biochemical processes within soil aggregates is still limited. We conducted a snow manipulation experiment to investigate the effects of snow absence on N pools and enzyme activities within soil aggregates in a subalpine spruce forest on the eastern Tibetan Plateau of China.

Results

Snow absence increased extractable inorganic N pools (ammonium and nitrate) and enzyme activities, accompanying with the improvement of N mineralization rate. Regardless of snow manipulations, both soil extractable inorganic N and net N mineralization was higher in macroaggregates than that in the other two aggregates. In contrast, microaggregates had higher urease and nitrite reductase activities compared to macroaggregates and large macroaggregates. Compared with small macroaggregates and large macroaggregates, N pools and enzymes within microaggregates is more sensitive to snow absence.

Conclusions

Our results indicated that the impacts of snow cover change on soil N dynamic depend on aggregate sizes and winter conditions (e.g., snow cover and temperature). Such findings have important implication for soil N cycling in snow-covered subalpine forests experiencing pronounced winter climate change.

Background

Snow, being an effective insulator, decouples the influence of air temperature on soil biochemical processes (Li et al., 2017; Stieglitz et al., 2003; Barnett et al., 2005). However, due to global warming, winter snow is more likely replaced by rain in the boreal forest (Ballesteros-Cánovas et al., 2018). It results in soil frost prior to snowpack accumulation, leading to unpredictable MB and community changes (Yang et al., 2019), largely affecting the carbon (C) and nitrogen (N) dynamics in soils (Brooks et al., 2011).

Snow absence directly drives soil temperature, moisture, and freeze-thaw cycles (FTCs), which are positively associated with microorganisms and enzyme activities (Allison et al., 2008; Xiao et al., 2020). For instance, the lack of insulating snowpack results in lower microbial metabolism and enzyme activities, thereby decreasing the net nitrification in forest soil in northern Japan (Shibata et al., 2013). However, snow absence simultaneously enhances fine root and microbial mortality, leading to the release of available N source for cold-resistant microorganisms (Cleavitt et al., 2008; Tierney et al., 2001). In the boreal forest ecosystem, a stable micro-environment in soil aggregates presents the obligatory condition for stable microbial biomass. Nevertheless, unstable soil aggregates may affect the organic matter (OM) and N mineralization and the exudation of microbial extracellular enzymes associated with the cycling of essential elements in the belowground ecosystem. Yet, these effects on snow absence induced by warming are less studied.

Previous studies have shown that snowpack-free, to some extent, led to the reduction of the strength and fractions of large-macroaggregates (Dagesse, 2011; Wang et al., 2012; Skvortsova et al., 2018). Oppositely, Lehrsch et al. (1991) indicated that the effect of snow absence on soil aggregate stability were dependent on the soil properties, e.g., texture, moisture content, and bulk density. Since its structural units is constituted by different soil texture levels, soil aggregate, is critical for soil microbial community structure and nutrient utilization (Edwards, 2013). The distribution of microorganisms depends on soil aggregate size classes; however, no consensus has yet been reached on the distribution of microorganisms. For instance, higher microbial biomass (MB) was in microaggregates in soils collected from a free air carbon dioxide enrichment facility (Dorodnikov et al., 2009; Monrozier et al., 1991). Oppositely, a higher MB in macroaggregates was found in a Karst soil ecosystem (Xiao et al., 2017), furthermore, Gupta and Germida (1988) presented microaggregates contained lower MB in both native and cultivated soils in Canada. Microorganisms and the relative enzyme activities are critical factors for N cycling, and the processes are sensitive to climate change (Li et al., 2017; Steinweg et al., 2012). Without snowpack isolation, exposed soils present significantly more soil frozen depth and FTCs (Li et al., 2017), which causes unpredicted effects on the soil ecosystem. However, this is still limited at the controlled conditions to assess the effect of warming-induced snow absence on the N dynamics, and microbial extracellular enzymes at the level of soil aggregates.

The Tibetan Plateau has been in substantial warming over the last few decades. In this region, the average air temperature has increased at about 0.2 °C per decade, with the majority happening in winter. Climate change induces snow absence, coupled with FCTs (Li et al., 2017, Yang et al., 2019), may affect soil aggregation, microbial extracellular enzymes and their resulting in N dynamics. This study aimed to evaluate the effects of manual caused snow removal on soil aggregate stability and its relative N dynamics. Here, we hypothesized that (1) snow absence increased soil N availability and OM mineralization of; (2) snow absence stimulated soil microbial enzyme activities.

Materials And Methods

Site description and Experimental design

Snowpack manipulation experiment was carried out in a *Picea asperata* (Dragon spruce) stand at the Long-term Research Station of Alpine Forest Ecosystems, which was located at the eastern Tibetan Plateau, China (31° 15' N, 102° 53' E; 3021 m a.s.l.). The mean annual temperature (MAT) and precipitation are 3.0 °C and 850 mm, respectively. Snow cover begins to accumulate in late November and melts in later March of the following year. The soil is classified as Cambic Umbrisols (IUSS Working Group WRB, 2007). Basic soil properties (0–15 cm) as follows: soil organic C 88.5 g C kg^{-1}, total N 5.4 g N kg^{-1}, and pH 6.4 (Li et al. 2017).

In order to exclude winter snowfall and minimize the unwanted interference, six wooden roofs with 3 m × 3 m in ground area were installed in November 2015 to prevent snow accumulation on the ground. The control plot was established in the vicinity of each treatment (Li et al., 2017). Our previous study indicates that excluding snow successfully induced more severe soil freezing in the winters of 2015/2016 and 2016/2017 but did not affect soil moisture content (Yang et al., 2019)

Soil sampling and aggregate size fractionation

Two soil cores from each plot were collected from the 0–15 cm layer using an auger 10 cm in diameter in the early thawing periods of 2015/ 2016 and 2016/2017 winters. Aggregates were isolated as described by Kristiansen et al., (2006). Large macroaggregates (> 2 mm), small macroaggregates (0.25–2 mm) and microaggregates (< 0.25 mm) were collected. See, Yang et al. (2019) for further details. Our previous study showed that the snow absence did not affect the distribution of aggregates in the size classes in the transitional thawing period of 2016 and 2017 (Yang et al., 2019).

Soil chemical analysis

We analyzed the selected chemical properties of all treated soil samples and aggregate fractions as follows. Nitrate (NO_3^--N) and ammonium (NH_4^+-N) were extracted with 2 M KCl (1:5 soil:solution), and then determined using Indophenolblue and Phenol-disulphonic acid colorimetry, respectively (Xu et al., 2010). Here, soil net N mineralization was determined from in situ incubations using the buried tube technique, which were carried out using perforated PVC tubes (15 cm in height and 5 cm in diameter). The top of each tube was covered by para film to avoid leaching of N. The wintertime net N mineralization was expressed as the difference in inorganic in the soil before and after snow accumulation (from mid-November to early April next year) in years of 2016 and 2017. Soil nitrate (NARA) and nitrite reductase (NIRA) activities were analyzed using the method described by Xiong et al., (2014). We followed the method of Kandeler and Gerber (1988) to analyze soil urease activity (URA).

Statistical analysis

Repeated measures ANOVA was performed to test the effects of treatment, sampling date, aggregate size, and their interactions on measured parameters. One-way ANOVA was used to test the significant differences for specific parameters. All statistical analyses were considered as a significant level at $P < 0.05$. All statistical analyses were performed using SPSS 23.0 (IBM Deutschland GmbH, Ehningen, Germany) software package for Windows.

Results

Soil N pools

Overall, soil aggregate size, sampling year, and their interactions significantly affected soil inorganic N pools (Table 1). Snow absence tended to increase NH_4^+-N content and caused a profound difference in microaggregates (Fig. 1a), and macroaggregates presented a significant higher NH_4^+-N content than that of both microaggregates and large- macroaggregates in 2016 (Fig. 1a). Moreover, soil NH_4^+-N was significantly higher in 2016 than in 2017 (Fig.1a). Snow removal increased NO_3^--N content in microaggregates in 2016 significantly (Fig. 1b). However, there was no profound difference of NO_3^--N between soils with various aggregates among the sampling years (Fig. 1b). Soil with/without snow cover had a similar extractable inorganic N during these years, except of the large-macroaggregates in the snowpack cover plots (Table 2c).

Net N mineralization rate

Snow absence showed a no significant effect on net N mineralization (Fig. 2). Macroaggregates had a significant higher mineralization than that of both microaggregates and large-macroaggregates in snowpack-free plots in 2016, while soil aggregates caused a non-significant effect on net N mineralization in 2017 (Fig. 2). The ANOVA analyses showed that snow absence affected N mineralization, but dependent on the sampling year (Table 1). The net N mineralization of macroaggregates presented a significant decrease, while it showed an opposite trend in both microaggregates and large-macroaggregates in snow cover plots during the period. However, there was no significant difference in net N mineralization between the first and second years.

Pearson correlation coefficient

The Pearson correlation between N mineralization and N pools was positive, while it was only significant in extractable inorganic N in control plots (Fig. 3). Extractable inorganic N had a significant relationship with NH_4^+ and NO_3^-. Snow absence changed the pattern between soil N pools and enzyme activities. The relationship between NARA with soil N pools and N mineralization rate was significant in plots with snowpack (Fig. 3b). Yet, it was insignificant with NO_3^- and N mineralization rate in snow absence plots (Fig. 3a). The relationship between NIRA, URA and soil N pools or N mineralization, to some extent, changed after artificial snow removal.

Soil enzyme activities

Snow absence tended to increase soil enzyme activities which were involved in N transformation in 2016, while the difference was not significant (Fig. 4). The ANOVA analyses showed that aggregates and sampling year affected URA significantly (Table 1). Microaggregates had a substantial higher URA than that of large-macroaggregates in control in 2016. Moreover, microaggregates and macroaggregates had a significant higher URA in 2016 than that in 2017 (Fig. 4a). The statistical results showed that the interaction between snow absence and sampling year had a significant effect on both NARA and NIRA. The significant effect of soil aggregates on NARA and NIRA only occurred in 2016 (Fig. 4a and b). Macroaggregates had a significant higher NARA than that of both microaggregates and large-macroaggregates (Fig. 4b). In addition, NIRA was in the order of microaggregates, macroaggregates, and large-macroaggregates. NIRA in microaggregates had a significant difference between in 2016 and in 2017 (Table 1).

Discussion

Climate change alters the belowground ecosystem structure on the eastern Tibetan Plateau (Li et al., 2017; Yang et al., 2019). We conducted a two-year field manipulated experiment for a deep understanding on soil N cycling induced by snow absence. Soil frozen physical effects the binding between soil aggregates and OM derived from plant debris, root exudates, and microbial secretions (Six et al., 2004). However, snowpack-free treatment did not significantly disrupt soil aggregates in this studied area after two years experiment (Yang et al., 2019). This is in contrast to that from Steinweg et al. (2008), indicating that snow absence significantly effects soil aggregate distribution, but largely dependent on complex factors such as soil water content, OM concentration, and the frequency of FTCs (Bisal and Nielsen, 1964; Bryan, 1971; Lehrsch et al., 1991).

Soil N availability is a crucial limiting factor to net primary productivity (NPP) in terrestrial ecosystems, especially in cold biomes (Lavoie et al., 2011). Thus, soil available N pools induced by climate change and snowpack plays a key role in N cycling and NPP. Indeed, snow absence affects soil N dynamics through physic-chemical routes. Overall, this is in line with our hypothesis that snow absence increased NH_4^+-N, NO_3^--N, and extractable inorganic N content (Fig. 1). This observation is also consistent with previous snow-free manipulation experiments in boreal forests (Steinweg et al., 2008, Shibata et al., 2013). Snow absence caused a higher root and microbial mortality, leading to the release of OM for the cellular metabolism of the cold-tolerance microorganisms, and accelerating the transformation of soil substrates from organic to inorganic status (Li et al., 2017). This likely results from that the composition of microbial communities alters in micro-environment with high amplitudes or frequencies (Finlay et al., 1997). In particular, both ammonification and nitrification to the temperature sensibility affecting N dynamics is largely different (Schütt et al., 2014). In particular, this study found that URA in all soil aggregates significantly reduced with increasing temperature from 2016 to 2017. As being recently reported by Yang et al. (2019), ammonifiers had a significantly higher positive relationship with soil temperature, especially when soil temperature between – 2.1 and 8 °C. In this study, most of the same NH_4^+-N content was in both macroaggregates and large-macroaggregates in 2017 with a mean air temperature of 1.2 °C higher than that of the first year. This finding positively highlights that unexpected warming stimulates the ammonification process in the subalpine conifer forest in 2017. Being consistent with Steinweg et al. (2008), snow absence significantly stimulated soil N availability within macroaggregates. Macroaggregates bound by microaggregates contain comparable higher easily accessible C for the life of fungi and bacteria, which control OM mineralization (Gupta and Germida, 1988). On the other side, warming-induced FTCs decreased mean weight diameter (MWD) by disrupting larger-macroaggregates, stimulating the fragmentation of OM by microorganisms due to the larger surface and more contact points (Grogan et al., 2004).

Net N mineralization, a biochemical process, is influenced by the available substrate and MB (Zaman and Chang, 2004; Li et al., 2019). In line with our hypothesis, compared to control, snow absence led to a specific higher net N mineralization than that of control (Fig. 2). This result indicated a more generous amounts of readily decomposable OM and vibrant microbial activities present in snow absence plots (Friedel et al., 1996; Klose et al., 1999). Unexpectedly, there was no noticeable increase in net N mineralization in 2017. This is attributed to that, extremely warm winter mitigated the influence of snow absence on soil ecosystem biochemical processes due to the unobvious environmental difference (Yang et al., 2019). The net N mineralization was comparable higher in macroaggregates than that in microaggregates or large-macroaggregates in 2016. There are some potential explanations for this phenomenon. First, the distribution of MB has an apparent preference, mainly being concentrated in macroaggregates (Miller and Dick, 1995). Indeed, macroaggregates featured with stable MWD retain the microbial community stability resist to the destruction associated with snow absence (Šimanský et al., 2008). Second, microaggregates constructed by primary particles bounded with the plant and microbial debris, increase the stability of soil available substrates against the decomposition of OM even without snowpack cover (Denef et al., 2001; Bossuyt et al., 2002; Six and Jastrow, 2002). In contrast, macroaggregates contain higher easily accessible C with strong characteristics of readily lost upon snow-free than that of microaggregates associated with more recalcitrant C (Gupta and Germida, 1988). In this study, it was observed that N mineralization presented a positive relationship with NH_4^+-N, NO_3^--N, and extractable inorganic N (Fig. 2). In a belowground ecosystem, microorganisms are limited by SOC, while N is usually of secondary importance. Available N released caused by snow absence can be assimilated by specific frigostable organisms, improving soil MB if the SOC is sufficient, which, in turn, enhances the net N mineralization.

Soil enzyme activities are essential sensors for terrestrial elements cycling and served as indicators of various changes in plant-soil systems (Aon and Colaneri, 2001). Therefore, a greater knowing about enzyme activities related to N transfer is necessary to better predicate the N cycling in boreal forest. In this experiment, soil enzyme activities presented a significant relationship with soil N pools, under both snow absence and control plots (Fig. 3). This finding suggests that soil N pools is an important factor controlling enzyme activities in the belowground systems. Soil enzyme activities are firmly temperature-dependent. Thus, the appropriate warm temperature can stimulate enzyme activities (Wallenstein et al., 2009). However, Fang et al. (2016) presented that soil warming did not or negatively influence hydrolase activities and the ratios of soil hydrolase activities to soil MBC. These discrepancies reveal a significant different response to environmental change for enzyme activities. For instance, the activities of URA, NARA, and NIRA showed some various reactions to snow absence (Fig. 3). In details, the response of NARA to snow absence is much more sensitive than NIRA, indicating that the enzyme activities of microorganism associated to climate change are different. In line with our initial hypotheses, snow absence, to some extent, increased URA, NARA, and NIRA. There are two potential explanations for these findings. First, snow absence caused a low temperature, in turn leading to the mortality of fine root and frigolabile MB. The released labile OM immobilized by live microorganisms could increase the specific MB and enzyme activities. However, the nutrient utilization of MB is significantly different (Schimel et al., 2004). Second, the increase of both MB and frozen pore water caused by snow absence in this studied region can restrict oxygen (O_2) availability and intensify the anaerobic level in pores of the soil, which stimulated enzyme activities for denitrification processes parallelly (Bollmann and Conrad, 1998).

Furthermore, soil enzyme activities associated to MB are largely controlled by the stabilization of aggregates. Although a mass of researches have been conducted, while, so for, there is no a concordant conclusion about the relationship between soil aggregates particles and enzyme activity. For instance, Fang et al. (2016) found that the enzyme activities associated to C cycling increases as aggregate size decreases in subtropical forest. However, Fansler et al. (2005) had presented that microaggregates fraction did not contribute much to the enzyme activities, despite its high activity for both enzymes. These conflicting results indicate that though macroaggregate structures provide appropriate habitat for soil microorganisms and enzyme activities, but microorganisms and their associated enzymes have a comparable high spatial variation (Chotte et al. 1998; Guggenberger et al. 1999). Moreover, these inconsistent conclusions can be the evidence for our current results that the enzyme activities about denitrification processes had distinct differences in specific sizes of aggregates, that micro- and macroaggregates had a comparable higher NIRA and NARA, respectively (Fig. 4b and c). One part of the differences can be explained that macroaggregate structures provide suitable habitat for aerobic microorganisms (Miller and Dick, 1995). Furthermore, compared with macroaggregates, microaggregates characterized by smaller particle size, provided low O2 concentration for specific microorganisms (Gupta and Germida, 1988), which benefit for retaining relatively high enzyme activities of microorganisms preferring anaerobic conditions.

Conclusion

This study examined the effect of snow absence on soil N cycling within aggregates in a subalpine spruce forest on the Tibetan Plateau of China. Our results presented that snow absence to some extent increased soil available N pools, N mineralization, and enzyme activities. However, this process was limited by specific soil and climate conditions, e.g., soil moisture and OM content, soil aggregate size, and temperature. In addition, the soil inorganic N pool and N-converse enzyme activities in microaggregates were more sensitive to snow absence than that in macroaggregates and large macroaggregates. Macroaggregates had a comparable higher soil inorganic N pool and N mineralization rate than microaggregates and large macroaggregates. Yet, the N converse enzyme activities within microaggregates were similar to macroaggregates. Thus, we highlight that, in the subalpine spruce forest, macroaggregates is the main sink of soil inorganic N pool and the conversion from organic to inorganic status is also majority carried out in macroaggregates. Moreover, the influence of snow absence on soil N dynamics may decrease due to a gradual warming of soil surface. These findings improve our understanding of soil N dynamics responding to climate change on the Tibet plateau of China, whereas especially large decreases in winter snowfall.

Declarations

Ethics approval and consent to perticipate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZFX conceived the study. KJY, LZ, BT and RY performed the research and analyzed the data. ZJL wrote the manuscript. ZML, RR and ZFX contributed to editing. All authors contributed to the work and gave final approval for publication.

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Tables

Table 1 Summered results regarding the effects of snow absence, aggregate size, sampling date and their interaction of soil N pools, mineralization, and enzyme activities under the repeated measure ANOVA.

	NH_4^+-N	NO_3^--N	EIN	Mineralization	URA	NARA	NIRA
Snow manipulation (SM)	0.277	0.579	**0.006**	0.525	0.263	0.245	0.197
Soil aggregate (SA)	**0.017**	0.751	**0.007**	0.105	**0.035**	0.113	**0.001**
Sampling year (SY)	**0.014**	0.116	0.506	0.070	**0.002**	0.737	**0.001**
SM × SA	0.278	0.891	0.379	0.802	0.952	0.781	**0.050**
SM × SA	0.236	0.730	0.472	0.440	0.357	**0.050**	**0.006**
SA × Sy	**0.002**	**0.049**	**0.004**	**0.008**	0.100	0.105	**0.010**
SM × SA ×SY	0.466	0.575	0.124	0.991	0.919	0.927	0.592

Abbreviation: EIN: extractable inorganic nitrogen; URA: urease activity; NARA: nitrate reductase activity; NIRA: nitrite reductase activity.

Figures

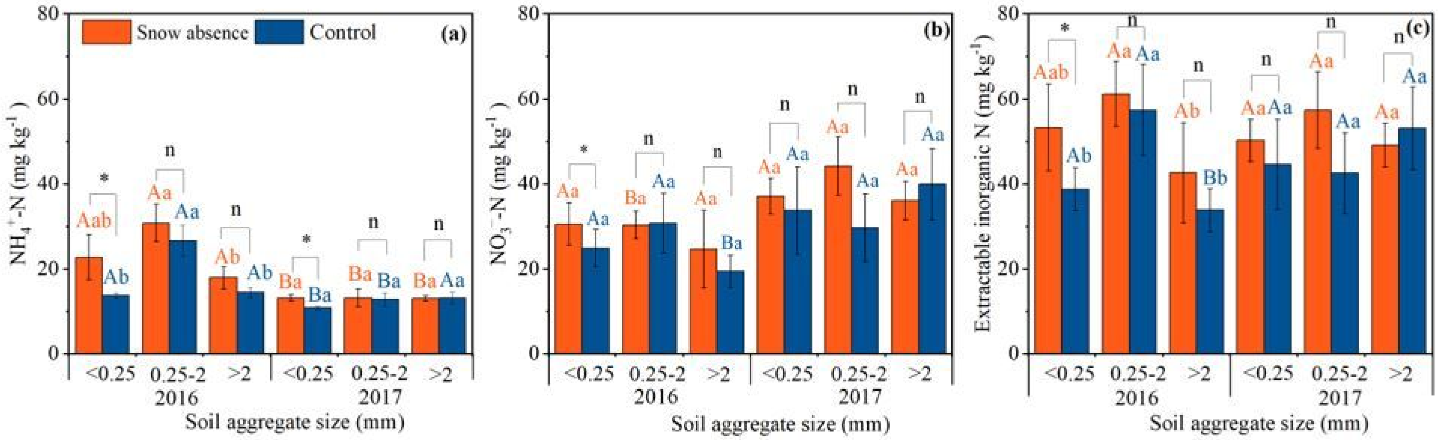

Figure 1

Soil NH4+-N (a), NO3−-N (b), and extractable inorganic N (c) concentrations in snow absence and control plots. The significant difference between snow absence and control in the same soil aggregate and sampling date is indicated by asterisks (P < 0.05) or n (P > 0.05). Different lowercases denote significant differences between 2016 and 2017 in the same aggregate at the same condition (P < 0.05).

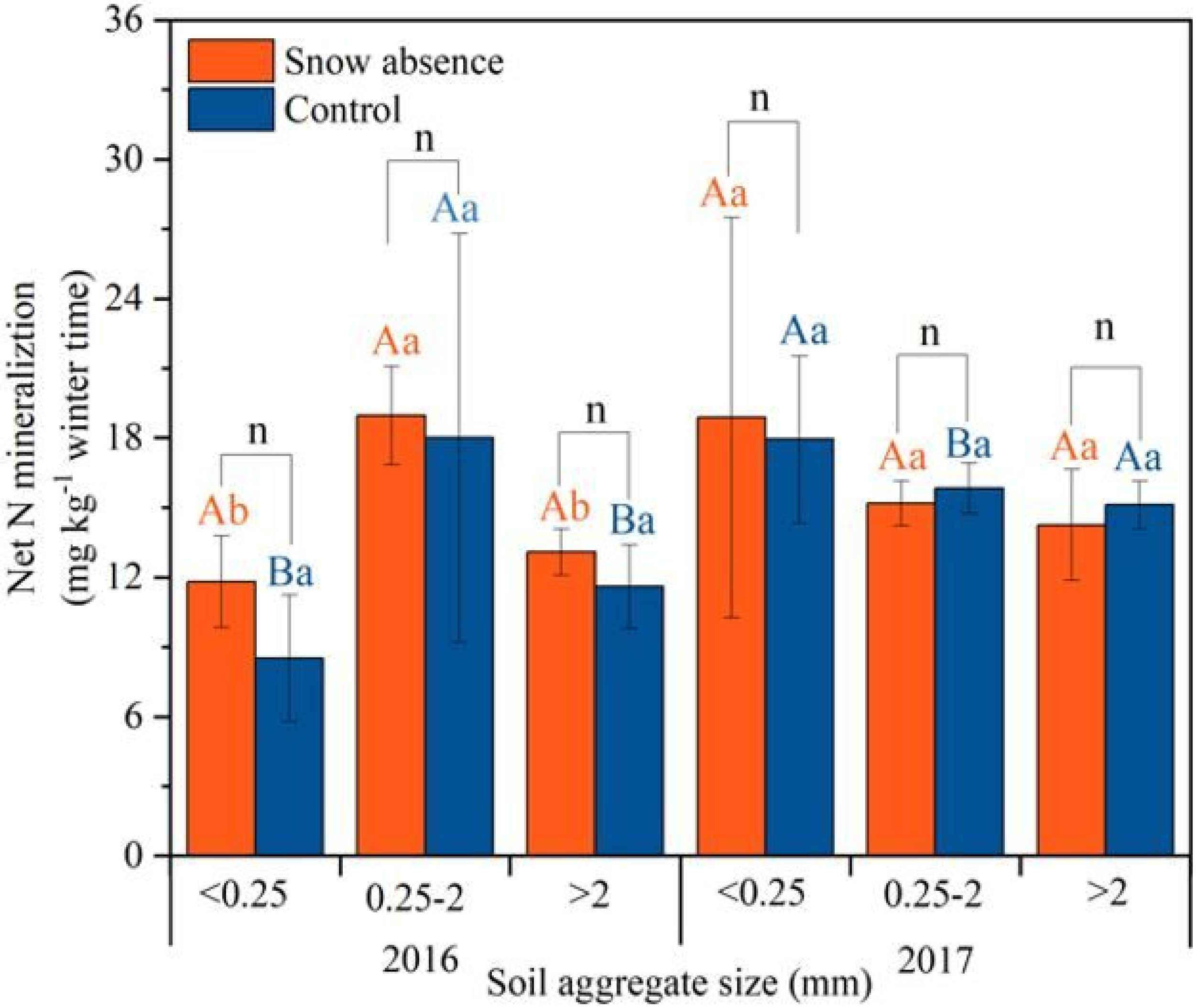

Figure 2

Net N mineralization in snow absence and control plots. Different lowercases denote significant differences between 2016 and 2017 in the same aggregates under the same snow condition and sampling date (P < 0.05). The significant difference between snow absence and control in the same soil aggregate and sampling date is indicated by asterisks (P < 0.05) or n (P > 0.05).

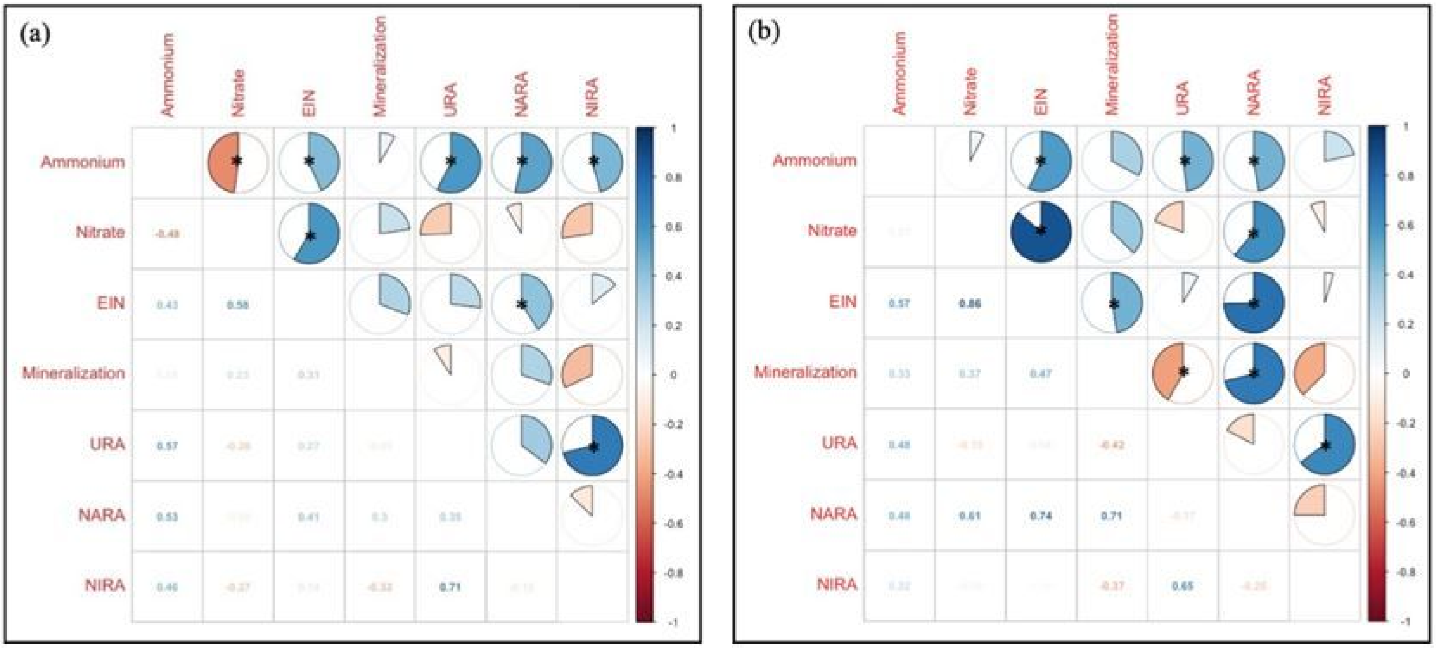

Figure 3

The Pearson correlation coefficient between soil N pools, N mineralization, and enzyme activities in snow absence (a), and control (b) plots. Abbreviations: EIN: extractable inorganic nitrogen; URA: urease activity; NARA: nitrate reductase activity; NIRA: nitrite reductase activity.

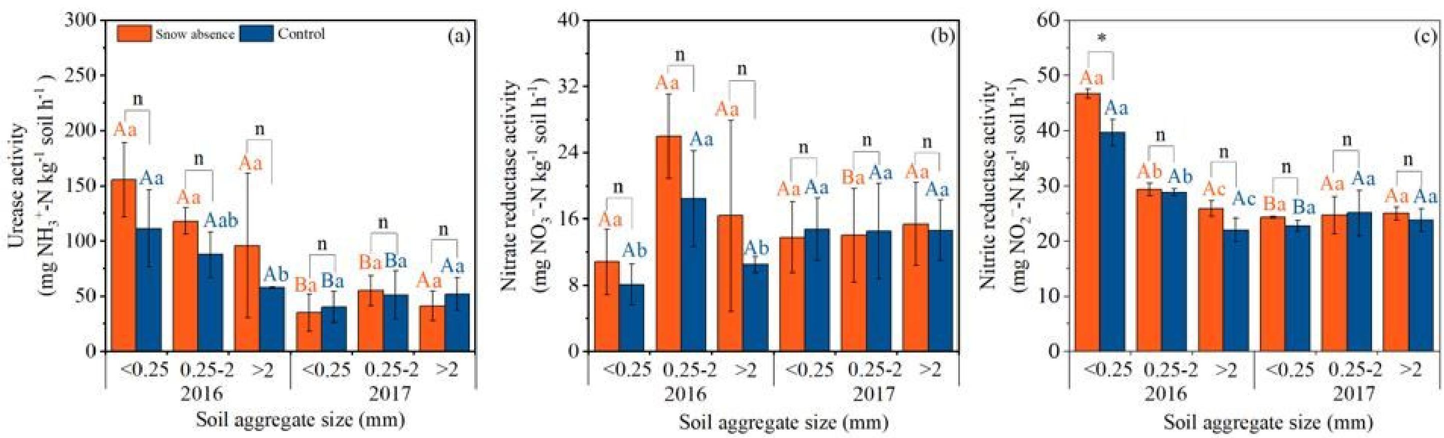

Figure 4

Soil urease (a), nitrate reductase (b) and nitrite reductase (c) activities in snow absence and control plots. The significant difference between snow absence and control in the same soil aggregate and sampling year is indicated by asterisks ($P < 0.05$). Different lowercases denote significant differences between aggregates in the same snow condition and sampling year ($P < 0.05$) or n ($P > 0.05$).