Short-term effects of snow cover manipulation on soil bacterial diversity and community composition

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Abstract

Winter snow cover is a major driver of soil microbial processes in high-latitude and high-altitude ecosystems. Warming-induced reduction in snow cover as predicted under future climate scenarios may shift soil bacterial communities with consequences for soil carbon and nutrient cycling. The underlying mechanisms, however, remain elusive. In the present study, we conducted a snow manipulation experiment in a Tibetan spruce forest to explore the immediate and intra-annual legacy effects of snow exclusion on soil bacterial communities. We analyzed bacterial diversity and community composition in the winter (i.e., the deep snow season), in the transitional thawing period, and in the middle of the growing season. Proteobacteria, Acidobacteria, and Actinobacteria were dominant phyla across the seasons and snow regimes. Bacterial diversity was generally not particularly sensitive to the absence of snow cover. However, snow exclusion positively affected Simpson diversity in the winter but not in the thawing period and the growing season. Bacterial diversity further tended to be higher in winter than in the growing season. In the winter, the taxonomic composition shifted in response to snow exclusion, while composition did not differ between exclusion and control plots in the thawing period and the growing season. Soil bacterial communities strongly varied across seasons, and the variations differed in specific groups. Both soil climatic factors (i.e., temperature and moisture) and soil biochemical variables partly accounted for the seasonal dynamics of bacterial communities. Taken together, our study indicates that soil bacterial communities in Tibetan forests are rather resilient to change in snow cover, at least at an intra-annual scale.

Keywords: Winter climate change; Snow cover; Bacteria; Community diversity; Community composition; Illumina sequencing.

1. Introduction

Reduced snow cover is one of the most dramatic changes under projected climate change scenarios for many cold-climate regions in the world (Stielstra et al., 2015). Snow cover change has major impacts on soil temperature, moisture, and the frequency of freeze-thaw cycles, during winter but especially also during the early growing season (Isobe et al., 2018; Potopová et al., 2016). Soil temperature and freeze-thaw cycles play critical roles in regulating soil microbial communities in cold-climate ecosystems (Groffman et al., 2011; Sharma et al., 2006). Hence, the lack of an insulating snow cover can have profound effects on the composition, diversity, and functioning of soil microbial communities (Gavazov et al., 2017; Robroek et al., 2013).

Soil bacteria play key roles in biogeochemical cycles in cold-climate ecosystems (Isobe et al., 2018; Zhang et al., 2014b). Previous studies have shown that some key drivers of soil bacterial communities, including soil temperature, moisture, and nutrient availability, vary in different ways due to changes in snow (Aanderud et al., 2013; Edwards et al., 2007; Lipson and Schmidt, 2004). Snowpack can prevent soil temperatures from falling much below freezing (Edwards et al., 2007). Conversely, the absence of snow cover may result in deep soil freezing and reduced soil moisture content (Groffman et al., 2011). Thus, changes in soil temperature and moisture associated with snow cover change may shift the structure of soil bacterial communities (Stark and Firestone, 1995; Lipson, 2007). In addition, snow cover change may also induce significant changes in soil biochemical properties (e.g., pH and N availability), and thereby mediate climate-induced shifts in soil bacterial communities (Kim et al., 2014; Ricketts et al., 2016).

The responses of soil microbes to environmental change may differ among seasons (Aanderud et al., 2013; Schimel et al., 2007; Zinger et al., 2009). Some studies have shown that snow cover change has immediate impacts on soil bacterial community composition in winter (Ricketts et al., 2016; Robroek

et al., 2013). Recent studies have also found that microbial response induced by snow cover change can carry over into the following snow-free growing season (Aanderud et al., 2013; Wubs et al., 2018). However, soil bacterial communities may also adapt and respond quickly to changing soil conditions, even in the short-term (Männistö et al., 2018; Schimel et al., 2007). Therefore, the post-winter legacy effects of changes in snow cover on soil bacterial communities may be limited. To our knowledge, soil bacterial responses to snow cover change have rarely been studied both in the winter and the following growing season (Aanderud et al. 2013). However, investigating the immediate and legacy effects of snow cover change on soil bacterial communities is important for better understanding microbial ecological processes in cold-climate areas.

The Tibetan Plateau has seen a significant warming trend over the last few decades (Wang et al., 2016). In this region, the temperature has been increasing at a rate of about 0.2℃/decade with the most pronounced warming occurring in winter (You et al., 2017). Climate warming may strongly impact on winter snowfall, thereby further affecting the seasonal snow accumulation and snowmelt time (Kapnick and Delworth, 2013). Our previous studies have shown that snow exclusion led to intensified soil freezing and increased soil nutrient availability, whereas snow exclusion suppressed winter soil respiration (Li et al., 2017; Yang et al., 2019). Future snow-free winters will alter winter soil conditions and, in turn, may have strong impacts on microbial communities in Tibetan forest soils. To test this, we conducted a snow-manipulation experiment in a Tibetan spruce forest to examine the effects of snow exclusion on the diversity and structure of soil bacterial communities in the winter, the transitional thawing period, and the subsequent growing season. Specifically, we tested the following hypotheses: (i) snow exclusion decreases the abundance and diversity of soil bacterial communities, and thereby changes the bacterial community composition; (ii) snow exclusion will cause both immediate effects (during winter) and legacy effects (during the thawing period and the following growing season) on soil bacterial communities; (iii) snow exclusion will impact soil

bacterial communities through changes in environmental and/or soil biochemical conditions.

2. Materials and methods

2.1. Site description

The study was conducted in a dragon spruce (*Picea asperata*) stand at the Long-term Research Station of Alpine Forest Ecosystems of Sichuan Agricultural University, which is located at the eastern Tibetan Plateau of China (31°15′N, 102°53′E; 3021 m a.s.l.). The mean annual temperature is 3.0 °C, with maximum and minimum temperatures of 23.0 °C (July) and -18.0 °C (January), respectively. Annual precipitation is about 850 mm. In general, snow begins to accumulate in late November and melts in late March of the following year. The soil is classified as a Cambic Umbrisol (IUSS Working Group WRB, 2007). The organic carbon (C), nitrogen (N), and pH at the soil depth of 0-15 cm were 88.5 g C kg^{-1}, 5.4 g N kg^{-1}, and 6.4, respectively (Li et al., 2017). The understory is dominated by *Salix paraplesia*, *Rhododendron lapponicum*, *Cacalia* sp., *Carex* sp., and *Cyperus* sp. (Li et al., 2017).

2.2. Experimental design

Winter snowfall was excluded using shelters with the aim to manipulate the depth and intensity of soil frost. This shelter method is a useful tool for studying the responses of soil processes to winter climate change, as it effectively reduces snow cover and minimize unwanted environmental side effects (Li et al., 2016). Six wooden roofs (3 m × 3 m ground area) were installed in November 2015 to prevent the accumulation of snow on the ground. One control plot was randomly set up in the vicinity of each snow-exclusion plot. The snow removal manipulation began in late November 2015 and ended in early April 2016. See, Li et al. (2017) for further details.

2.3. Microclimate

Air temperature (2 m height) and soil temperature (5 cm depth) were measured using Thermochron DS1923-F5 iButtons (Maxim Dallas Semiconductor Corp, USA) every 1 h during the experimental period. Snow depth of the control plots was measured approximately every 2 weeks with a ruler. The minimum and maximum air temperatures were -14.1 ℃ and 18.1 ℃, respectively. The minimum daily mean soil temperatures were -2.2 ℃ and -0.5 ℃ in the snow exclusion plots and control plots, respectively (Fig. S1). Seasonal snow began to accumulate in late November 2015 and melted in late March 2016, with the maximum depth in the control plots of 40 cm in late February (Fig. S1). The number of freeze-thaw cycles over the winter period was 25 and 13 in the snow exclusion and control plots, respectively (Li et al., 2017). Snow exclusion resulted in more severe soil frost in the winter of 2015/2016 but did not affect winter soil moisture content. Additionally, soil temperature and moisture did not differ between control and snow exclusion plots during the growing season (Fig. S1).

2.4. Soil sampling

Three paired plots (snow exclusion vs. control) were randomly selected. Soils were sampled in the deep snow period (DSP, mid-February 2016), early thawing period (ETP, early April 2016) and in the middle of the growing season (MGS, mid-August 2016). At each sampling event, three soil cores were collected from each plot using an auger (15 cm long and 5 cm diameter) and were mixed into one composite sample per plot. The composite samples were passed through a 2 mm sieve, and any visible living plant material was removed from the sieved soil. Subsamples of the sieved soils were stored at -70°C and 4°C for molecular and biochemical analyses, respectively.

2.5. Soil physiochemical and biological analyses

Soil pH value was measured by a pH meter using a soil to water ratio of 1:2.5 (*m*/*v*). Soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) were extracted with 2 M KCl and then measured by colorimetry (Xu et al., 2010). Soil microbial biomass carbon (MBC) was measured by the fumigation-extraction method

125 (Vance et al., 1987).

126 We assessed the activities of seven enzymes which are involved in soil C, N, and P cycling:
127 β-glucosidase (BG), cellobiohydrolase (CBH), polyphenol oxidase (PPO), peroxidase (POD),
128 β-N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (AP). The
129 soil enzyme activities were assayed using the method described by Allison and Jastrow (2006).
130 Substrate solutions were 5 mM pNP-β-glucopyranoside for BG, 2 mM pNP-cellobioside for CBH, 50
131 mM pyrogallol and 50 mM EDTA for PPO, 5 mM L-DOPA and 10 μL of 0.3% H_2O_2 for POD, 2 mM
132 pNP-β-N-acetylglucosaminide for NAG, 5 mM leucine p-nitroanilide for LAP, and 5 mM
133 pNP-phosphate for AP. PPO and POD were incubated for 2 h at 20 °C, BG and AP were incubated for
134 1 h at 30 °C, and CBH, NAG, and LAP were incubated for 4 h at 30 °C. The absorbance of products
135 was read using a microplate spectrophotometer at 405 nm for the hydrolytic enzymes and 450 nm for
136 the oxidative enzymes. Enzyme activities were expressed in units of nmol of substrate and converted
137 per hour per gram of dry soil (nmol h^{-1} g^{-1}) (Yang et al., 2019).

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139 2.6. DNA extraction, PCR amplification and Illumina MiSeq sequencing

140 Microbial DNA was extracted from 18 samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio Inc.
141 Norcross, GA, USA) according to manufacturer's protocols. The final DNA concentration and
142 purification were determined by Nanodrop® ND-1000 UV-Vis spectrophotometer (Nano-Drop
143 Technologies, Wilmington, DE, USA), and DNA quality was checked by 1% agarose gel
144 electrophoresis. The bacterial 16S rDNA gene was amplified with the primers 338F_806R (Dennis et
145 al., 2013) by a thermocycler PCR system (GeneAmp 9700, ABI, USA). The PCR reactions were
146 conducted using the following program: 3 min of denaturation at 95℃, 35 cycles of 30 s at 95℃, 30 s
147 for annealing at 55℃, and 45 s for elongation at 72℃, and a final extension at 72℃ for 10 min. PCR
148 reactions were performed in triplicate using a 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2
149 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of

template DNA.

The PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol. The purified amplicons were pooled in equimolar and then paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw reads were deposited in the NCBI Sequence Read Archive (SRA) database with accession number PRJNA633722.

2.7. Processing of sequencing data

Raw FASTQ files were demultiplexed and quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (1) The reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window. (2) Primers were matched allowing two-nucleotide mismatching, and reads containing ambiguous bases were removed. (3) Sequences whose overlap was longer than 10 bp were merged. Operational taxonomic units (OTUs) were clustered based on a 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME (Edgar et al., 2011). The taxonomy of each 16S rDNA gene sequence was analyzed by the RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva 132/16S_bacteria database using a confidence threshold of 70%. Alpha-diversity indices were calculated by Mothur 1.30.1 to test differences in bacterial diversity among samples (Schloss et al., 2009).

2.8. Statistical analysis

Alpha diversity metrics, including the Shannon-Wiener, Simpson, and Chao indices, were calculated using the "diversity" and "richness" functions of the bacterial community. Repeated measures

ANOVAs were performed to test the effects of snow exclusion, sampling date (deep snow period, thawing period, growing season), and their interactions on the bacterial indices. One-way ANOVA was performed to test the effects of sampling date on bacterial community structure within the same treatment. For individual sampling dates, Wilcoxon rank-sum test was used to compare the effects of snow exclusion on bacterial community structure. Effects of snow exclusion on bacterial community structure was further tested by Principal Coordinate Analysis (PCoA) using OTUs. Spearman correlation analysis was used to assess the relationships between the relative abundance of bacterial taxa and biochemical properties (i.e., soil physicochemical properties and enzyme activities). Both PCoA and Spearman analyses were performed using the VEGAN package (Oksanen et al., 2013) in R (R Development Core Team, 2015). Other statistical analyses were performed using SPSS 20.0 (IBM Corporation, Armonk, NY, USA). The statistical tests were considered significant at the $P < 0.05$ level.

3. Results

3.1. Sequence data characteristics and bacterial community diversity

Across all soil samples, a total of 664576 high-quality sequences were identified. Each library had 18127 reads and 1512 OTUs were obtained in all. All rarefaction curves tended to approach the saturation plateau, indicating that the data volumes of the sequenced reads were reasonable (Fig. S2). There were significant differences in diversity (Shannon and Simpson) and richness (Chao) indices among different sampling dates (Fig. 1). In the growing season, Shannon and Chao indices were lower, while the Simpson index was higher, compared to the deep snow period and the thawing period. Importantly, while the Shannon and Chao indices were unaffected by snow exclusion, we found a significant interactive effect between snow exclusion and sampling date for the Simpson index with positive effects of snow exclusion on bacterial diversity during the deep snow period and negative effects during the thawing period and the growing season (Fig. 1b).

3.2. Taxonomic composition of bacteria

Sequences that could not be classified into any known group were assigned as unclassified and groups with an average relative abundance of less than 1% were classified as ‘others’ (Fig. 2). At the phylum level, the classified sequences were affiliated to 28 groups. Proteobacteria (29.21-48.60%), Acidobacteria (19.50-25.59%), Actinobacteria (5.92-13.16%), and Chloroflexi (6.31-10.36%) were the most dominant bacterial phyla. The relative abundances of the Bacteroidetes (0.91-5.72%), Firmicutes (1.43-5.22%), Gemmatimonadetes (1.84-4.67%), Rokubacteria (1.27-6.68%), Nitrospirae (0.86-4.36%), Verrucomicrobia (1.36-2.54%), Latescibacteria (0.81-1.89%), Planctomycetes (0.74-1.29%), and Patescibacteria (0.31-1.00%) were relatively low across seasons (Fig. 2a). Bacterial community composition varied among seasons (Fig. S3) but was largely unaffected by snow exclusion (Fig. S5). For instance, regardless of snow manipulation treatment, the relative abundance of Nitrospirae was significantly increased in the growing season, whereas the opposite was found for

Bacteroidetes. The relative abundance of Firmicutes was higher in the thawing period than in the other two periods, while the opposite patterns was found for Gemmatimonadetes. The relative abundance of Actinobacteria was lowest in the deep snow period (Fig. S3). However, no significant differences were found in bacterial phyla between snow regimes for all sampling events (Fig. S5).

At the class level, 20 bacterial classes (>1%) were observed across seasons (Fig. 2b). There were also significant seasonal dynamics in the classes of soil bacteria (Fig. S4), while snow exclusion had little effect (Fig. S6). For example, across the snow manipulation treatments, Bacilli had higher relative abundance in the thawing period than in the deep snow period and the growing season. However, Deltaproteobacteria, Acidobacteriia, and Gemmatimonadetes showed opposite trends. For NC10 and Nitrospira, the relative abundances were highest in the growing season whereas the relative abundances of Bacteroidia and Alphaproteobacteria were lowest in the growing season. For Gammaproteobacteria, the relative abundance was highest in the deep snow period whereas the relative abundance of Actinobacteria was lowest in the deep snow period. Consistent with the phylum level, no significant differences were detected between control and snow exclusion plots at the class level (Fig. S6).

The bacterial composition was further analyzed with principal coordinate analysis (PCoA) at the OTU level (Fig. 3). The first and second principle component axes together explained as much as 64.71% of the variance in bacterial communities. The results of PCoA showed that soil bacterial communities were different among the deep snow period, the early thawing period, and the growing season. In the deep snow period, bacterial communities were clearly different between control and snow exclusion plots, while for the other periods snow exclusion did not have any effects.

3.3. Relationships between bacterial communities and biochemical or environmental factors

Spearman correlation heatmap analysis was performed to explore the relationships between bacterial communities and biochemical or environmental factors (Fig. 4). At the phylum level, soil moisture, ammonium nitrogen, and activities of polyphenol oxidase, leucine aminopeptidase, cellobiohydrolase, β-N-acetyl-glucosaminidase, acid phosphatase, and β-glucosidase were positively correlated with Firmicutes, Verrucomicrobia, Bacteroidetes, Actinobacteria, and Patescibacteria but negatively correlated with Chloroflexi, Rokubacteria, Latescibacteria, Gemmatimonadetes, Nitrospirae, and Planctomycetes (Fig. 4a). However, microbial biomass C, pH, nitrate, temperature, and peroxidase were negatively correlated with Firmicutes, Verrucomicrobia, and Bacteroidetes, but positively correlated with Chloroflexi, Rokubacteria, Latescibacteria, Gemmatimonadetes, Nitrospirae, and Planctomycetes (Fig. 4a). At the class level, Bacteroidia and Verrucomicrobiae were positively correlated with ammonium, moisture, polyphenol oxidase, leucine aminopeptidase, acid phosphatase, β-glucosidase, β-N-acetyl-glucosaminidase, and cellobiohydrolase, but negatively correlated with soil temperature, nitrate, pH, microbial biomass C, and peroxidase (Fig. 4b). Conversely, Nitrospira, Gemmatimonadetes, NC10, AD3, Latescibacteria, and Anaerdineae were negatively correlated with ammonium, soil moisture, polyphenol oxidase, leucine aminopeptidase, acid phosphatase, β-glucosidase, β-N-acetyl-glucosaminidase, and cellobiohydrolase, but positively correlated with soil temperature, nitrate, pH, microbial biomass C, and peroxidase (Fig. 4b). In addition, some classes belonging to the Proteobacteria (i.e., Alphaproteobacteria and Gammaproteobacteria) showed significant negative correlations with soil temperature and peroxidase, while the opposite was found for Deltaproteobacteria (Fig. 4b). Among the phylum of Firmicutes, the class of Clostridia was negatively correlated with soil temperature and peroxidase, while Bacilli was positively correlated with soil temperature (Fig. 4b).

4. Discussion

Across treatments and seasons, the predominant bacterial phyla in our experimental site in a Tibetan spruce forest were Proteobacteria, Acidobacteria, and Actinobacteria, which is consistent with observations in the Swiss and Australian Alps (Wunderlin et al., 2016) and the Qinghai-Tibetan Plateau (Wu et al., 2017). Alphaproteobacteria and Gammaproteobacteria were the most dominant classes of Proteobacteria, which agrees well with observations in shrub-dominated tundra heaths of northern Finland (Männistö et al., 2018) and subarctic tundra in Alaska (Kim et al., 2014). These findings together indicate that soil bacteria share common dominant higher taxa in high-altitude and high-latitude ecosystems. Previous studies have reported that Chloroflexi, Bacteroidetes, Firmicutes, Gemmatimonadetes, Nitrospirae, Verrucomicrobia, and Planctomycetes are often found in snow-covered ecosystems (Lipson, 2007; Lipson and Schmidt, 2004; Männistö et al., 2013; Ricketts et al., 2016; Wunderlin et al., 2016). However, some bacterial phyla found in our experimental site, such as Rokubacteria, Latescibacteria, and Patescibacteria, has rarely been reported in other snowy soils (Ricketts et al., 2016). This may be partially attributable to technological differences among studies (e.g., DNA extraction, primer specificities, and taxonomic classification of downstream analysis). In addition, it is important to note that the detected Chloroflexi is not a dominant phylum in alpine ecosystems, but its active role in biogeochemical cycles of cold soils has been long established (Costello and Schmidt, 2006; Zhang et al., 2014a).

Contrary to our hypothesis, snow exclusion had weak effects on bacterial diversity and snow exclusion only affected community composition in wintertime. This is inconsistent with previous

findings in tundra and temperate deciduous forest (Aanderud et al., 2013; Ricketts et al., 2016). The following reasons may account for the differences found. Firstly, the magnitude of soil freezing caused by snow exclusion in our study is smaller than those reported in temperate and arctic ecosystems (Aanderud et al., 2013; Ricketts et al., 2016). This could be due to differences winter conditions between low-latitude alpine ecosystems and high-latitude boreal ecosystems (e.g., snow density, depth, and albedo). Short-term mild frost, therefore, may not be severe enough to cause lasting changes in the diversity and composition of soil bacterial communities. In addition, most of the dominant bacterial taxa in frozen soils may have strong adaptive capabilities and resistance, thereby maintaining the stability of community structure via diverse ecological strategies (Männistö et al., 2018; Ricketts et al., 2016). For example, some specific traits (e.g., mycelium structures and sporing formations) of Actinobacteria help them to survive in the extreme cold and low-nutrient alpine conditions (Embley and Stackebrandt, 1994; Zhang et al., 2016). Further, high substrate affinities and extracellular enzyme production favor the effective establishment of defense structure of Alphaproteobacteria to resist extreme conditions, such as soil freezing (Fontaine et al., 2003). On the other hand, the measured biotic and abiotic soil properties did not differ between snow regimes in the following growing season (Yang et al., 2019). This can explain why we did not find legacy effects of snow exclusion on soil bacterial communities.

The diversity of soil bacterial community varied with seasons largely independent of snow regimes. Both the diversity indices of Shannon and Chao were lower but Simpson index was higher in the growing season than in the winter season, indicating a detectable decrease in bacterial diversity and richness from winter to growing season. The relatively high diversity in the winter may be because of bacterial adaptation to and survival in frozen conditions. Winter bacterial communities may mainly utilize complex and recalcitrant substrates (i.e., cellulose and salicylate), to resist the frost stress. Thus, different functional responses to environmental stress may result in the separation of bacterial

communities (Schimel et al., 2007). Low-resource winter conditions may favor oligotrophic K-strategists, such as Alphaproteobacteria and Acidobacteria (Fierer et al., 2007; Lauro et al., 2009). These members usually have high functional diversity to break down complex C substrates (Lladó et al., 2016; Männistö et al., 2013). In contrast, rich-resource growing season conditions, may favor copiotrophic r-strategists, such Gammaproteobacteria and Actinobacteria. These R-strategists reproduce fast, thereby gaining a quantitative advantage and suppressing other groups, which in turn leads to a decrease in diversity (Fierer et al., 2007).

In general, different bacterial populations have different preference for specific conditions, which can induce the shifts in community structure among seasons (McMahon et al., 2011). Previous studies have shown that the relative abundances of some specific groups of bacteria (e.g., Actinobacteria and Bacteroidetes) differed among seasons in snowy ecosystems (Lipson, 2007; Lipson and Schmidt, 2004; Männistö et al., 2018; Zinger et al., 2009). Some studies have found that season-related changes in environmental variables profoundly affected microbial communities, in contrast to the transient and mild influences of change in snow cover (Lipson and Schmidt, 2004; Männistö et al., 2018). In our study, we found that shifts in bacterial community composition across seasons. Several possible mechanisms may account for the phenomenon.

Firstly, bacterial community composition is often driven by the thermal adaptability of the total community in a specific season (Aanderud et al., 2013; Lipson, 2007). Winter communities may be driven by physiological stress associated with freeze-thaw cycles, which could select for frost-resistant taxonomic units (Sharma et al., 2006). In our study, Bacteroidetes, Alphaproteobacteria, and Gammaproteobacteria had negative correlations with soil temperature. Although mineralization rate were low in winter due to low temperature, some bacteria can still survive by utilizing recalcitrant substrates (Zhang et al., 2014b; Straza et al., 2010; Tada et al., 2013). Secondly, soil

moisture is also an important factor affecting bacterial communities. Microbial communities may respond to changes in soil moisture through their capability of inherent acclimation for the reasonable allocation of limited resources (Schimel et al., 2007). In our study, Nitrospirae, Gemmatimonadetes, and Latescibacteria were negatively correlated with soil moisture. It has previously been demonstrated that Gemmatimonadetes can survive at low humidity conditions (DeBruyn et al., 2011). Besides, since substrate diffusion mainly relies on the presence of a water film, the access of bacteria to nutrients may increase during the (warm) growing season. As a result, bacterial taxa grow and reproduce rapidly in warm and humid conditions (Stark and Firestone, 1995). On the other hand, intense hydrological activity during the early thawing period can cause considerable leaching losses of nutrients (Edwards et al., 2007), which may further shift microbial composition due to nutrient competition (Walker et al., 1999).

Additionally, biochemical variables can further mediate soil bacterial communities. Firstly, some biogeochemical reactions catalyzed by microbial enzymes may be closely related certain bacterial taxa (Langenheder et al., 2006). For example, it is well known that some members of Bacteroidetes secrete enzymes (e.g. acid phosphatase) to decompose complex organic matter, such as chitin, cellulose, and other high-molecular organic compounds (Wolińska et al., 2017). Likewise, some cellulose-degrading bacteria phylotypes affiliated to Firmicutes, such as the classes of Bacilli and Clostridia (Rastogi et al., 2009), may be positively correlated with some hydrolytic enzymes (e.g., β-glucosidase, β-N-acetyl-glucosaminidase, and acid phosphatase) (Menon et al., 2013). Similar findings were also revealed in our study, suggesting that the production of soil enzymes can partially reflect seasonal changes in the diversity and composition of bacterial communities. Secondly, nutrient availability is one of most the important factors that regulate soil bacterial communities (Gavazov et al., 2017). For example, soil bacterial community diversity is largely affected by ammonium availability in some forest ecosystems (Zeng et al., 2016). Previous studies have suggested that

ammonium concentration in soil is usually high in the thawing period due to increased nitrate leaching (Li et al., 2017). Thus, higher ammonium availability may partly account for richer bacterial communities in the early thawing period. Lastly, it has been well recognized that pH is a key driver of soil bacterial community composition (Lauber et al., 2009; Ricketts et al., 2016). In cold regions, soil thawing-induced rapid changes in nitrogen availability can significantly influence soil pH during the early thawing period (Edwards et al., 2007). In our study, some dominant bacterial phyla (e.g., Gemmatimonadetes, and Nitrospirea) showed strong dependence on soil pH. This result is consistent with observations in other cold snowy ecosystems (DeBruyn et al., 2011; Lauber et al., 2009).

5. Conclusions

We examined the immediate and legacy effects of a short-term change in snow cover on the diversity and composition of bacterial communities in an alpine spruce forest on the Tibetan Plateau of China. Our results showed that snow exclusion had some effects on soil bacterial communities in snow-covered winter season, but not during the thawing period and the following growing season. This suggests that soil bacterial communities in Tibetan forests are resilient to the short-term change of snow cover. In addition, soil bacterial communities strongly varied with seasons, showing a significant shift from winter to the growing season. Season-related changes in environmental factors (e.g., temperature and moisture) and biochemical variables (e.g., soil N availability and enzyme activities) accounted to some extent for the seasonal variation in bacterial communities. Considering the already-observed strong winter warming in the Tibetan region, the ecological responses of soil microorganisms to winter climate change require long-term experimental studies.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Figure legends

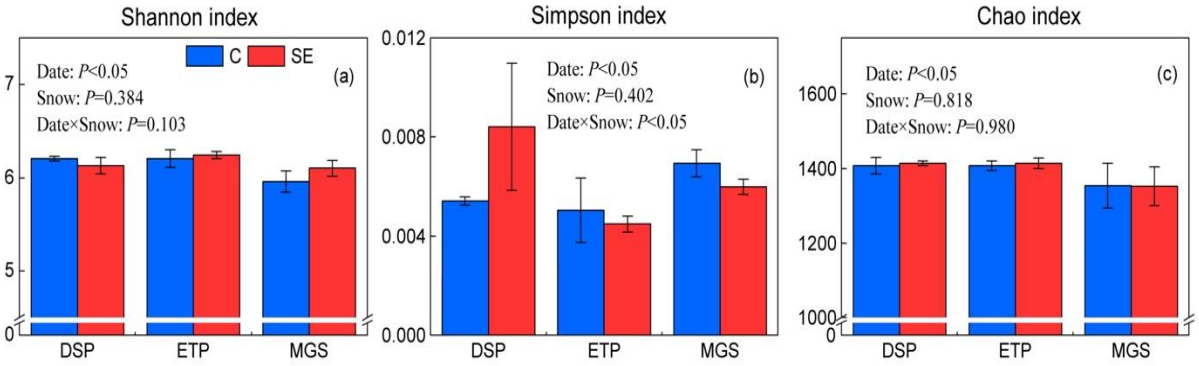
Fig. 1. Shannon diversity index(a), Simpson index (b) and Chao index(c) of soil bacterial community in control (C) and snow exclusion (SE) plots in the deep snow period (DSP), early thawing period (ETP), and in the middle of the growing season (MGS). Data shown are mean ± s.e.

Fig. 2. Taxonomic profiles of bacterial community composition at the phylum level (a) and the class level (b) in control (C) and snow exclusion (SE) plots in the deep snow period (DSP), early thawing period (ETP), and in the middle of the growing season (MGS). Shown are group accounting for >1% of the relative abundance, while groups accounting for <1% are integrated into 'others'.

Fig. 3. Principal Coordinate Analysis (PCoA) of bacterial community composition at the OTU level in control (C) and snow exclusion (SE) plots in the deep snow period (DSP), early thawing period (ETP), and in the middle of the growing season (MGS).

Fig. 4. Correlation heatmap of environment factors, biochemical properties and bacterial gene read numbers at the phylum (a) and class (b) levels. Tm: temperature, MBC: microbial biomass carbon, BG: β-glucosidase, CBH: cellobiohydrolase, PPO: polyphenol oxidase, POD: peroxidase, NAG: β-N-acetyl-glucosaminidase, LAP: leucine aminopeptidase, AP: acid phosphatase. The color intensity in each panel indicates the relative correlation between soil properties and read numbers of each group. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

596 **Figure 1**



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598 **Figure 2**

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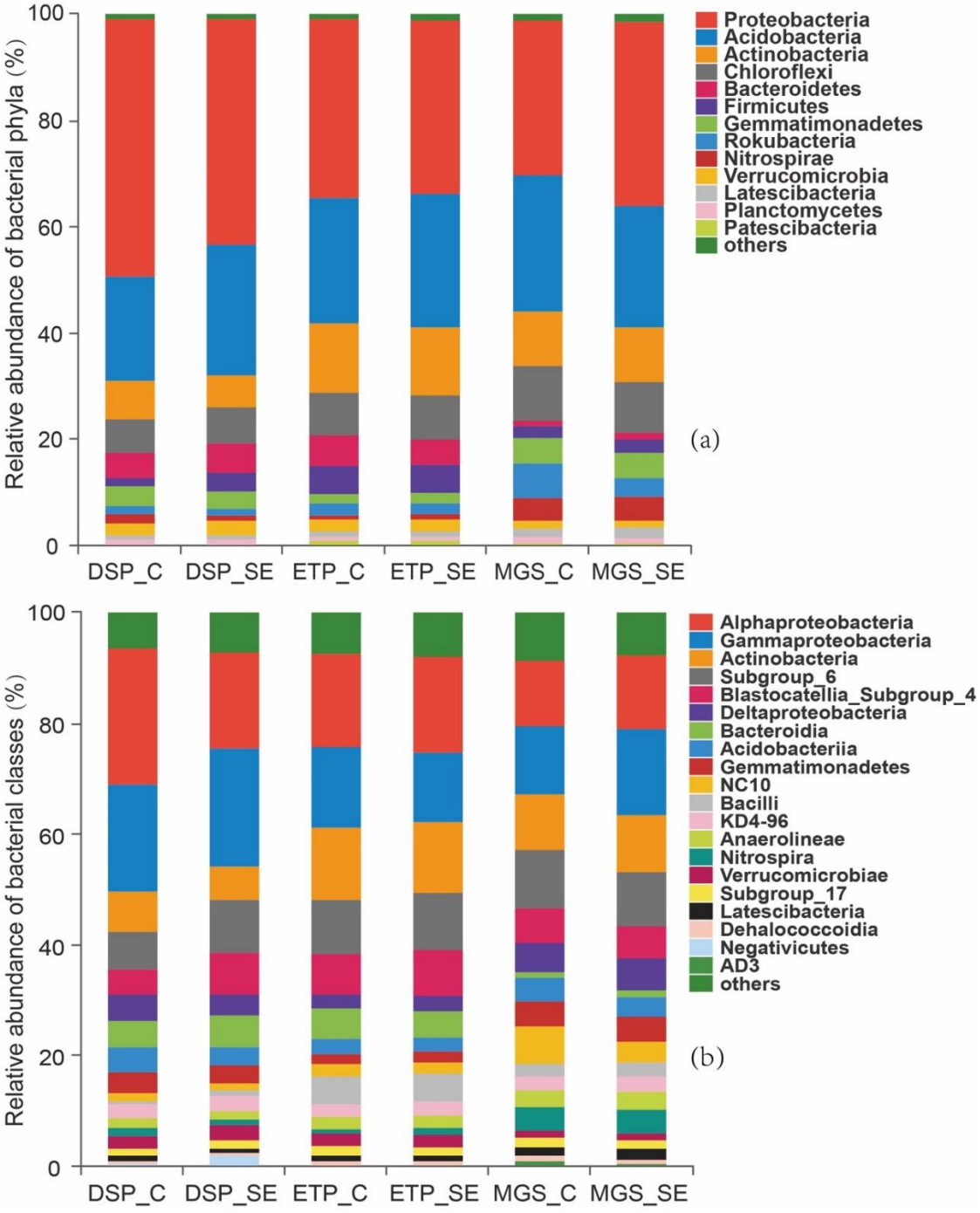

600 **Figure 3**

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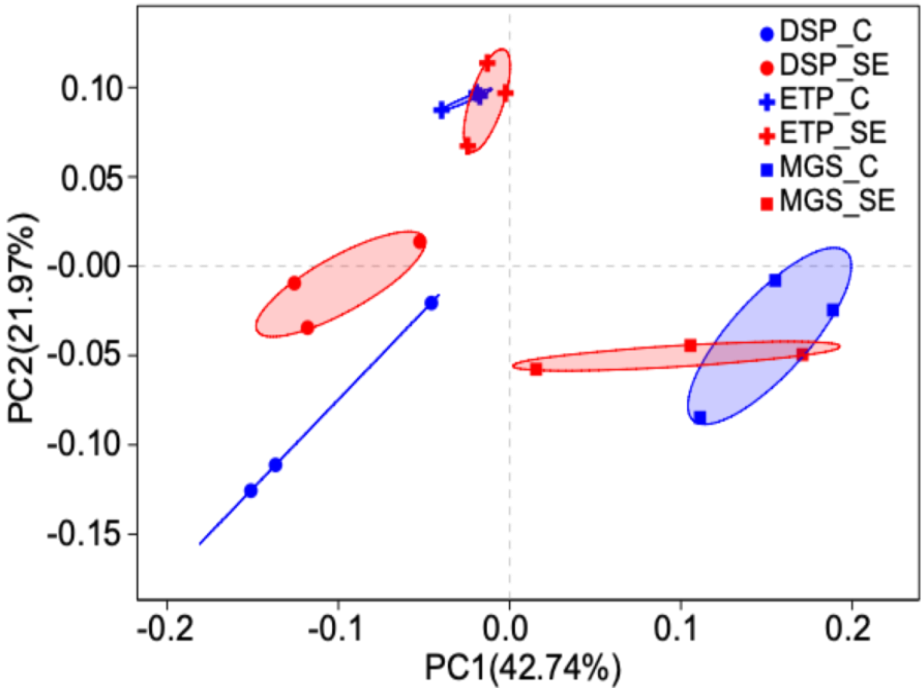

602 **Figure 4**

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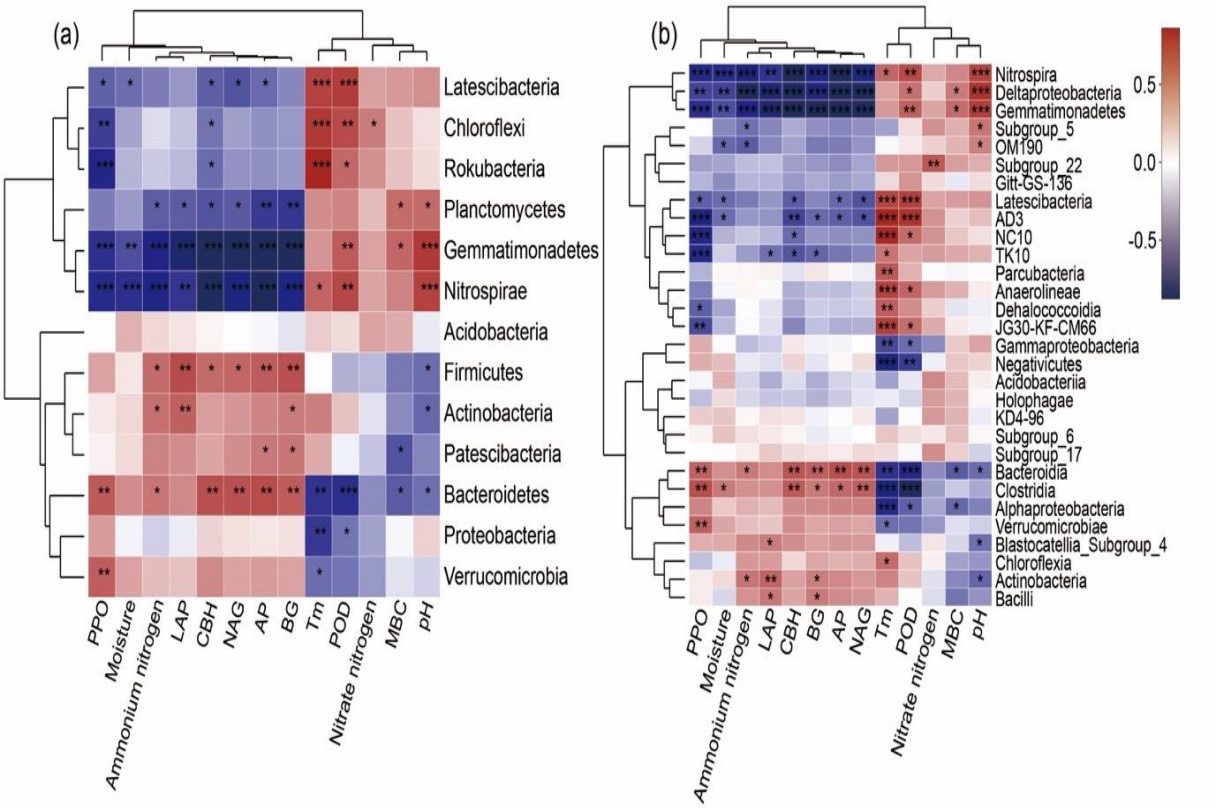

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