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# Effect of snowpack on the soil bacteria of alpine meadows in the Qinghai-Tibetan Plateau of China

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ABSTRACT

Global climate change is accompanied by changes in the amounts of ice and snow. These changes have both a direct effect on the plant community structure, primary productivity and carbon cycle and an indirect influence on the belowground ecosystem. However, the effects of changes in snowpack on the soil environment and belowground ecological processes, particularly in soil microbial communities are still poorly understood in alpine meadows. We conducted a field study of controlled snowpack in the eastern margin of the Tibetan Plateau, where five treatments were set up, named as S0, S1, S2, S3, and S4 (S1: the amount of a natural snowpack; S2, S3, and S4 were twofold, threefold, and fourfold of S1, respectively; and S0: completely removed snow). Soil physicochemical properties, soil community structure and diversity measured by 16S rRNA gene amplicons were studied. The results indicated that 1) as snowpack increased, the average soil temperature decreased, but soil moisture and soil compaction increased; 2) soil chemical properties (pH, available nitrogen, available potassium, available phosphorus, total nitrogen, total potassium, total phosphorus and total soil organic carbon) all changed as snowpack changed; and 3) increasing snowpack led to a decrease in the relative abundance of *Acidobacteria*, but *Bacteroidetes* and *Actinobacteria* did not decline in response to increasing snowpack. In summary, these results showed that soil bacterial communities are sensitive to changes in snowpack in alpine meadows.

## 1. Introduction

Climate change is often accompanied by changes in the amounts of ice and snow (Li, 1995). Over the last 20 years, the Northern Hemisphere snow coverage has been significantly reduced and negatively correlated with temperature (Robinson, 1993). The study of snowpack in cold regions has become a hot issue in the field of global change study (Brown and Goodison, 1996; Hughes and Robinson, 1996). The accumulation and ablation of seasonal snow have profound impacts on soil properties (Mikan et al., 2002), soil microbial activities (Schimel and Mikan, 2005) and soil microbial community structure (Hu et al., 2013). Soil microorganisms are major components of belowground ecosystems and play important roles in element cycling, organic matter turnover, soil structure formation, and the regulation of ecosystem-scale productivity (Spedding et al., 2004; Yang et al., 2013). Due to logistical constraints imposed by the overwhelming taxonomic diversity of microbial communities, models of ecosystems often treat microbes as heavily parameterized “black boxes” (Strickland et al., 2009), with a soil microbial community considered as a single homogenously functioning entity (Parton et al., 1983).

In fact, the relation between soil microbes and the environment is very complex and diversified (Hua, 2004), and information on the functional diversity (metabolic potential) is essential for understanding the role of microbial communities in different environments (Preston-Mafham et al., 2002). Therefore, it is critical to explore the influence of environmental changes on the structure and function of soil microbial communities and to construct a model that explicitly considers microbial diversity. Aanderud et al. (2013) have evaluated the importance of snowpack depth on soil microbial communities in a temperate deciduous forest. Snow addition led to wetter, warmer, and relatively carbon substrate-rich soils, and changes in soil moisture and temperature resulted in soil microorganisms delaying response to increases in free-eater during soil thawing events. During the growing reason soil microbial structure were reset and the microorganisms were likely adapted to annual fluctuations in snowpack depth (Aanderud et al., 2013). Conversely, Tan et al. (2013) addressed how snow removal

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affected soil microbial biomass and enzyme activity related to soil carbon and nitrogen cycling and pools. They found that snow removal increased the daily variation of soil temperature, frequency of freeze-thaw cycle, and advanced the dates of soil freezing and melting, and the peak release of inorganic nitrogen, and meanwhile significantly decreased soil microbial biomass carbon and nitrogen. The above results demonstrated that snow removal would alter soil microbial activity and hence element biogeochemical cycling in alpine forest ecosystems (Tan et al., 2013). Gavazov et al. (2017) also assessed the effects of experimental snow removal on the soil microenvironments and soil microbial community. They found that snow removal led to a series of mild freeze-thaw cycles, meanwhile soil microbial biomass doubled under the snow, paralleled by a fivefold increase in its ratio of carbon to nitrogen, but no apparent changes in its bacteria-dominated community structure. Advanced spring conditions resulting from snow removal revealed an impaired microbial metabolism shortly. While bacteria showed a higher potential for uptake of plant-derived carbon substrates and the promotion of bacteria over fungi can likely impede winter soil organic matters cycling (Gavazov et al., 2017).

High altitude systems, such as the ecosystems in the Qinghai-Tibetan Plateau (QTP), are very sensitive to global climate changes and tend to be affected by climate change much earlier than those in the surrounding lower elevation areas (Zheng et al., 2002). Previous studies have demonstrated that the snow cover on the QTP quickly responds to environmental changes (Oechel, 2012). Unlike low latitude systems where global climate change leads to rapid change in snow cover (Shi and Wang, 2015), high altitude systems such as the QTP face the confounding factor of changes in precipitation. Recent studies have shown that in the QTP, especially the eastern portion, the duration and depth of snowfall were increasing, and these changes might further cascade to have global-scale impacts through modulation of the East Asian and South Asian Monsoon seasons (Verma et al., 1985; Wang et al., 2009; Zhu and Ding, 2009). Although there were significant changes in snowpack on the QTP, very little is known about the ecological impacts of these changes, particularly at the scale of microbial responses. Such an understanding is essential since the potential for ecological responses to have significant feedbacks on productivity, plant coverage, and thus temperature (Chu, 2013). Across the QTP, alpine meadows are the most important ecosystem type, covering > 66% of the whole territory (Jia et al., 2014). It is clear that climate change has direct effects on plant community structure, primary productivity, and the carbon cycle. In addition, changes in the plant community also influence the belowground ecosystem, particularly in terms of the abundance and community structure of soil microbes (Zhang et al., 2016). However, we know nothing about how snowpack influences soil microbial community composition and diversity, even though many regions have had obvious changes in snowpack (Chu, 2013).

A manipulation of the snowpack over the QTP winter would directly impact the soil temperature and moisture due to the snow cover preventing heat escaping, and then the freezing and thawing would impact soil physicochemical properties and soil bacterial community structure diversity. Thus we hypothesized that changes in snowpack depth would (1) change the soil temperature, soil moisture, and physicochemical properties; (2) the bacterial effects would be particularly large because of the dynamic natures of these communities.

## 2. Materials and methods

### 2.1. Field site

The study site was located at the base of the Ecological Protection and Animal Husbandry High-Tech Research and Development of the Southwest University for Nationalities in Hongyuan County, Sichuan Province (32°49.823′ N, 102°35.237′ E). With an altitude of 3485 m, it sits at the lower end of the QTP (Xu et al., 2012). The climate is the typical continental plateau climate, with a large diurnal temperature range and a long frost period. The annual average temperature is 1.1 °C, the coldest monthly average temperature is −10.3 °C, and the average temperature of the hottest month is 10.9 °C. The annual average relative humidity is 60%–70%, with distinct wet and dry seasons. The average annual rainfall is 792 mm, which mostly occurs from May to October. The average annual evaporation capacity is 1262.5 mm. The hours of daylight are long and solar radiation is strong, with an average annual daylight time of 2159.7 h. The total annual solar radiation is 6194 MJ $m^{-2}$ (Gao et al., 2008). The average vegetation coverage is > 80%, and the highest vegetation height is 45–60 cm. The major plant species include Cyperaceae plants such as *Kobresia setchwanensis* and *K. pygmaea*, Gramineae plants such as *Agrostis clavata* and *Elymus nutans*, and forbs such as *Anemone trullifolia*, *Saussurea nigrescens* and *Potentilla* (Li et al., 2011). Soils were estimated to be Mat Cry-gelic Cambisols according to the Chinese soil classification (Gao et al., 2007).

### 2.2. Experimental design

The experiment was located in an alpine meadow area with relatively uniformly distributed vegetation. A field experiment of controlled snowpack was conducted from November 2013 to March 2014. Different snowpack depths were created by moving snow from plot to plot after every snowfall (Puma et al., 2007; Starr et al., 2008). The amount of natural snowfall during the study period is shown in Table 1. A randomized block experiment design was applied within a 30 m × 30 m area with 25 plots of 2 m × 2 m, and spacing between the plots of at least 1.5 m as a buffer. The artificial piles were used to produce the different snowpack depths in each sample area. There were five treatments, namely S0, S1, S2, S3, and S4 (S1: the amount of a natural snowpack; S2, S3, and S4 were two-fold, three-fold, and four-fold of S1, respectively; and S0: completely removed snow), with five replicates for each treatment, producing a total of 25 plots. A snowpack field was established in the surrounding plots, where multiple 2 m × 2 m waterproof tarps were laid out and fastened with nails. At the end of snowfall, the nails were pulled, and the collected snow on the tarps was uniformly piled for the S2, S3, and S4 treatments. Each piled snowpack in S2, S3, and S4 was one, two, and three tarps of snow, respectively. Before the snow began, the five S0 tarpaulins covering the five samples on the side were fastened with nails. After the snowfall, the snow tarps were all removed.

### 2.3. Soil sampling

Soil samples (composites of 5 cores) were collected from the 0–10 cm and 10–20 cm soil layers of each plot using a soil auger with an inner diameter of 5 cm on August 14th, 2014. For each snowpack treatment, soil samples from each 2 m × 2 m plot were combined, and three replicates were prepared. Each soil sample was divided into two portions that were separately labelled. One portion was immediately placed into 50-ml centrifuge tubes, which were placed in a box with ice bags, transported back to the laboratory, and stored at −20 °C for DNA extraction. Another portion was air-dried and passed through a 2-mm sieve to filter out stones and roots for the measurement of soil properties. For each treatment, there were three samples collected each at

**Table 1**
The average snowpack volume in the experimental region during the experiment.

| Experiment period | | 2013 | | 2014 | | |
|---|---|---|---|---|---|---|
| | | Nov. | Dec. | Jan. | Feb. | Mar. |
| Snowpack (mm) | S0 | 0 | 0 | 0 | 0 | 0 |
| | S1 | 12.5 | 1.1 | 4.5 | 17.4 | 36.2 |
| | S2 | 25 | 2.2 | 9 | 34.8 | 72.4 |
| | S3 | 37.5 | 3.3 | 13.5 | 52.2 | 108.6 |
| | S4 | 50 | 4.4 | 18 | 69.6 | 144.8 |

the 0–10 cm and 10–20 cm soil depths, for a total of 30 samples collected.

### 2.4. Soil analysis

There were multi-point soil temperature (ST) and soil moisture (SM) recorders (YM-01A, Handan, China) in the plots, recording the soil temperature and soil moisture, respectively, at the 0–10 cm and 10–20 cm soil layers with three replicates for each snowpack treatment. A soil compaction (SC) meter (SC900, Spectrum Technologies, Inc. USA) was used to measure the soil compaction at the 0–10 cm and 10–20 cm soil layers once a month, with three replicates for each snowpack treatment.

Soil pH was measured by a pH meter (FE20-Five Easy™ pH, Mettler Toledo, Germany) at a ratio of 1:5 (weight/volume) for soil versus distilled water. Total soil organic carbon (SOC) was measured by a TOC-5000A analyser (Shimadzu Corp., Kyoto, Japan). Total nitrogen (TN) was measured using the micro-Kjeldahl method (Koch and Mcmeekin, 1923). Available nitrogen (AN) was measured using the alkaline hydrolysis diffusion method. Total phosphorus (TP) and available phosphorus (AP) were analysed based on the molybdenum-antimony anti-spectrophotometric method. Total potassium (TK) was analysed based on the sodium hydroxide fusion-flame photometric method. Available potassium (AK) was analysed based on the ammonium acetate extraction-flame photometric method (Bao, 2000). All soil sample analyses were repeated three times.

A MoBio PowerSoil DNA Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) was used to extract the total DNA from 0.25 g soil samples according to the provided manufacturer's instructions (Version 02232016, 2014). The concentration and quality of DNA were determined using a NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The DNA samples were diluted to a concentration of 10 ng $\mu l^{-1}$ for subsequent experiments. The amplification of the V4–V5 hypervariable regions of the 16S rRNA gene was carried out with the universal primers 515F (5′-GTGCCAGCMGCCG CGG-3′) and 909R (5′-CCCCGYCAATTCMTTTRAGT-3′) with a 12 nt-long barcode (Tamaki et al., 2001). The polymerase chain reaction (PCR) system (25 μl) was composed of 10× Ex Taq buffer (2.5 μl), 25 mM $MgCl_2$ (2 μl), 2.5 mM dNTPs (2 μl), 1 μl each of the forward primer and reverse primer, 0.5 U Ex Taq polymerase (TaKaRa, Dalian, China) (0.2 μl), 10 ng $\mu l^{-1}$ of soil genomic DNA (5 μl), and $ddH_2O$ (11.3 μl). PCR amplification was performed using the Bio-Rad C1000 Touch™ Thermal Cycler (Bio-Rad, California, USA) according to the following procedure: denaturation at 94 °C for 5 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 50 s; and a final extension at 72 °C for 10 min. After the DNA samples were separated by gel electrophoresis, the 1% agarose gel was cut, and a SK8132 SanPrep DNA Gel Extraction Kit (Sangon Biotech, Shanghai, China) was used for the purification and recovery of DNA fragments. Subsequently, the recovered samples were tested. The procedure was repeated for the unqualified samples, and all of the qualified samples (in terms of concentration and quality) were mixed in equal molar proportions for high-throughput sequencing. High-throughput sequencing was performed using the Illumina MiSeq (Illumina, San Diego, USA) sequencing platform at the Chengdu Institute of Biology of the Chinese Academy of Sciences (www. Biobit.net.cn) using V2 reagent.

### 2.5. Sequence and statistical analysis

High-throughput sequencing data processing was mainly performed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline version 1.7.0 (Caporaso et al., 2010a). The various samples were separated according to barcodes, and the low-quality sequences, primers and sequences for sample identification were removed. The UCHIME algorithm was employed to conduct chimaera checks, and the sequences containing chimaeras were removed (Edgar et al., 2011). Sequences were divided into OTUs based on a sequence similarity threshold of 97%. Information was classified using the Ribosomal Database Project (RDP) classifier (Wang et al., 2007), and the typical sequence was compared with the local reference database (Greengenes core set). Sequence alignment was performed using the Python Nearest Alignment Space Termination (PyNAST) algorithm (Caporaso et al., 2010b). The quality of the sequence alignment was checked before building the phylogenetic trees, and the phylogenetic trees were reconstructed using general time-reversible (GRT) models, the maximum likelihood (ML) method and FastTree 2.1.1 (Price et al., 2010). QIIME was used to build the OTU table, followed by α (Shannon-Wiener index, Phylogenetic-Diversity, Observed-Species and Chao 1 index) and β diversity (PCoA, UniFrac) analyses.

One-way analysis of variance was performed to test the difference in soil physicochemical properties (SM, ST, SC, pH, AN, AK, AP, TN, TK, TP and SOC), diversity indexes (Shannon-Wiener index, Phylogenetic-Diversity, Observed-Species and Chao 1 index) and soil bacterial phyla along the snowpack gradient. When significant differences between treatments ($P < 0.05$) were observed, the least significant difference (LSD) test was performed. These statistical analyses were conducted using SPSS (SPSS Inc., version 19.0). Structural changes in the bacterial communities were evaluated by principal coordination analysis (PCoA) based on Bray-Curtis distances using the relative abundances of OTUs without singletons (Li et al., 2015). Redundancy analysis (RDA) and Pearson correlation analysis were used to determine the correlation between soil physicochemical properties and dominant bacteria phyla. RDA were performed using CANOCO in Windows (4.5), and Pearson correlation analyses were performed using SPSS. The Mantel test was applied to evaluate the correlations between microbial communities and physicochemical properties using Passage (Yao et al., 2014). Aggregated boosted tree analysis (ABT) was carried out using R.2.9.1 (gbmplus package) to quantitatively evaluate the relative influences of soil physicochemical factors on soil bacterial community diversity (Shannon-Wiener index, Phylogenetic-Diversity, Observed-Species and Chao 1 index) (Kuang et al., 2013).

## 3. Results

### 3.1. Response of soil physicochemical properties to snowpack

Snowpack changed the soil physical (Fig. 1) and chemical properties (Table 2) of the different soil layers. In the 0–10 cm soil layer, the soil moisture increased as snowpack increased (Fig. 1a). There was a significant ($P < 0.05$) difference between treatments, except for S0 and S1, S2 and S3, and S3 and S4. In the 10–20 cm soil layer, there were significant ($P < 0.05$) differences in the treatments. Soil temperature was significantly ($P < 0.05$) decreased in both soil layers (0–10 and 10–20 cm) with an increase in accumulated snowpack (Fig. 1b). Soil compaction was significantly ($P < 0.05$) decreased in S0, while there were no significant differences among other treatments in the 0–10 cm soil layer. In the 10–20 cm soil layer, soil compaction increased with the increase in snowpack. S3 and S4 showed significantly ($P < 0.05$) higher compaction than the other treatments (Fig. 1c).

In the 0–10 cm soil layer, changes in snowpack resulted in increases in pH, AP, AK, TN, TP and SOC, but AN was significantly decreased in S0 ($P < 0.05$). In the 10–20 cm soil layer, AK and TN were significantly increased ($P < 0.05$) along the snowpack, but TK was significantly decreased ($P < 0.05$). SOC and AP were significantly increased ($P < 0.05$) with the increase in snowpack. Soil pH, AN and TP varied greatly along the snowpack (Table 2).

In the 10–20 cm soil layer, we found that soil moisture, soil compaction and AN were significantly ($P < 0.05$) positively correlated with snowpack, but soil temperature and TK were significantly ($P < 0.05$) negatively correlated with snowpack. In the 10–20 cm soil layer, SM, SC, AP, TN, TP and SOC were significantly ($P < 0.05$)

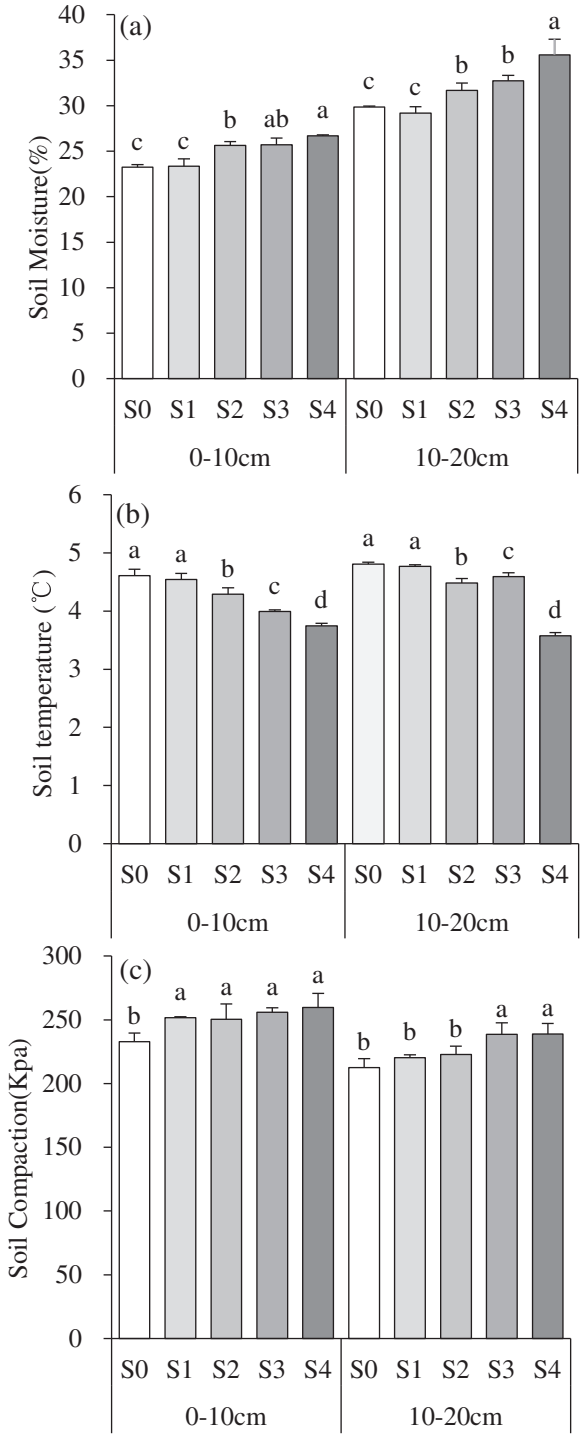


**Fig. 1.** Change of soil physical property of different soil layer under different snowpack volume treatment in Alpine meadow (2013–2014) (means ± SE, $n = 3$). The soil moisture (a), soil temperature (b) and soil compaction (c) were the average value of all measured data in the rest of this article. S0 represents removing snow treatment, S1 is natural snowpack volume treatment, S2, S3, and S4 are twofold, threefold, and fourfold as S1, respectively. Different letters on the pillar indicate significant differences between treatments at 0.05 level, the same below.

positively correlated with snowpack, while soil temperature, pH and TK were significantly ($P < 0.05$) negatively correlated with snowpack (Table 2).

Changes in snowpack led to a great decrease in the soil N/P and significant increase in the soil C/N in the 0–10 cm soil layer. The soil N/P increased with the change in snowpack, and the maximum value of

**Table 2**
Effects of different snowpack gradient on soil chemical property in alpine meadow (means ± SE, n = 3).

| Soil layer (cm) | Treatments | pH | AN (mg/kg) | AP (mg/kg) | AK (mg/kg) | TN (g/kg) | TP (g/kg) | TK (g/kg) | SOC (g/kg) | C/N | N/P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0–10 | S0 | 5.81 ± 0.02ab | 102.48 ± 4.45b | 4.57 ± 0.21b | 209 ± 19.47a | 4.91 ± 0.21a | 0.87 ± 0.04a | 15.38 ± 0.08a | 52.17 ± 2.72a | 5.32 ± 0.34a | 5.64 ± 0.34b |
| | S1 | 5.62 ± 0.17b | 206.04 ± 6.87a | 2.90 ± 0.56c | 136.33 ± 35.44b | 4.18 ± 0.35b | 0.39 ± 0.06b | 15.46 ± 0.07a | 37.03 ± 1.69b | 4.44 ± 0.20b | 10.90 ± 1.73a |
| | S2 | 5.72 ± 0.02ab | 208.57 ± 11.8a | 6.40 ± 0.56a | 178.67 ± 9.07ab | 4.20 ± 0.21b | 0.83 ± 0.07a | 15.5 ± 0.16a | 44.57 ± 3.08c | 5.33 ± 0.54a | 5.07 ± 0.49b |
| | S3 | 5.91 ± 0.07a | 193.53 ± 6.27a | 4.30 ± 0.36b | 183.33 ± 4.16ab | 4.67 ± 0.31ab | 0.89 ± 0.03a | 15.69 ± 0.05a | 43.17 ± 0.87bc | 4.63 ± 0.17ab | 5.28 ± 0.42b |
| | S4 | 5.89 ± 0.08a | 213.28 ± 10.5a | 4.30 ± 0.36b | 165.33 ± 13.2ab | 4.43 ± 0.21ab | 0.76 ± 0.04a | 14.31 ± 0.22b | 42.47 ± 2.61bc | 4.80 ± 0.31ab | 5.86 ± 0.23b |
| 10–20 | S0 | 5.71 ± 0.23ab | 176.37 ± 10.39b | 2.23 ± 0.31c | 92.33 ± 20.74ab | 3.52 ± 0.44b | 0.79 ± 0.09b | 15.46 ± 0.19a | 28.78 ± 1.35b | 4.14 ± 0.56b | 4.54 ± 0.85ab |
| | S1 | 5.81 ± 0.03ab | 178.55 ± 9.73b | 2.87 ± 0.65bc | 65.33 ± 11.02b | 3.22 ± 0.1b | 0.82 ± 0.05b | 15.49 ± 0.14a | 31.77 ± 1.43b | 4.93 ± 0.30ab | 3.93 ± 0.17b |
| | S2 | 5.94 ± 0.05a | 153.09 ± 4.83bc | 4.3 ± 0.2a | 90.33 ± 4.93ab | 3.47 ± 0.09b | 0.86 ± 0.04ab | 15.46 ± 0.15a | 32.68 ± 1.67b | 4.71 ± 0.17ab | 4.03 ± 0.05b |
| | S3 | 5.94 ± 0.04a | 128.97 ± 2.62c | 3.2 ± 0.23bc | 95.67 ± 5.51ab | 3.72 ± 0.3b | 0.76 ± 0.03b | 14.45 ± 0.18b | 32.27 ± 1.88b | 4.35 ± 0.27b | 4.92 ± 0.37ab |
| | S4 | 5.55 ± 0.12b | 272.3 ± 16.25a | 3.56 ± 0.2ab | 104.67 ± 8.08a | 5.39 ± 0.25a | 0.98 ± 0.02a | 14.6 ± 0.27b | 57.09 ± 1.24a | 5.30 ± 0.22a | 5.51 ± 0.12a |

Note: pH: Soil pH; AN: Soil available nitrogen; AP: Soil available phosphorus; AK: Soil available potassium; TN: Soil total nitrogen; TP: Soil total phosphorus; TK: Soil total potassium; SOC: Soil organic carbon. Different letters mean significant differences at the 0.05 level, the same below.

**Table 3**
Effects of different snowpack gradient on soil bacterial community diversity in alpine meadow. (means ± SE, n = 3).

| Soil layer | Treatment | Phylogenetic-diversity | Observed-species | Chao 1 | Shannon-Wiener index |
|---|---|---|---|---|---|
| 0–10 cm | S0 | 69.41 ± 3.71a | 1022.67 ± 48.75ab | 1527.38 ± 71.77a | 8.76 ± 0.11a |
| | S1 | 70.06 ± 1.47a | 985.33 ± 63.52b | 1505.37 ± 76.02a | 8.75 ± 0.06a |
| | S2 | 72.01 ± 1.7a | 1073 ± 33.66a | 1569.82 ± 79.28a | 8.87 ± 0.11a |
| | S3 | 72.27 ± 1.92a | 1051.5 ± 18.19ab | 1577.28 ± 78.07a | 8.86 ± 0.04a |
| | S4 | 71.95 ± 0.9a | 1035 ± 48.08ab | 1561.65 ± 69.49a | 8.81 ± 0.07a |
| 10–20 cm | S0 | 69.83 ± 1.84a | 924 ± 31.95a | 1477.34 ± 75.34a | 8.45 ± 0.13a |
| | S1 | 65.81 ± 4.9a | 900.67 ± 66.58a | 1313.98 ± 59.12a | 8.34 ± 0.21a |
| | S2 | 67.98 ± 3.09a | 950.33 ± 61.08a | 1486.85 ± 69.14a | 8.54 ± 0.21a |
| | S3 | 67.1 ± 3.25a | 932.33 ± 64.37a | 1422.26 ± 61.87a | 8.52 ± 0.10a |
| | S4 | 66.99 ± 3.61a | 938.33 ± 65.05a | 1373.38 ± 71.42a | 8.56 ± 0.23a |

soil C/N appeared in S4 in the 10–20 cm soil layer (Table 2).

### 3.2. Response of soil bacterial community diversity to snowpack change

After removal of the chimaera and redundant sequences, a total of 142,723 sequences were obtained. The OTU number in each sample was 4643 to 4770 sequences (4757.43 ± 24.31). On the basis of a sequence similarity of 97%, the number of OTUs in each sample was between 691 and 1096 (972.23 ± 91.66). The coverage of OTUs for all taxa was between 90.01% − 92.82% (91.18% ± 0.07%), indicating that the majority of communities with a high abundance of non-units could be covered, except for some species with low proportions. At the phylum level, a total of eight soil bacterial populations were detected, including *Acidobacteria* (26.55%), *Proteobacteria* (21.39%), *Bacteroidetes* (15.94%), *Actinobacteria* (6.43%), *Planctomycetes* (4.60%), *Crenarchaeota* (4.33%), *Firmicutes* (4.28%), and *Chloroflexi* (4.07%).

The Shannon-Wiener index, Phylogenetic-Diversity, Observed-Species and Chao 1 index reflected the α-diversity of the soil bacterial community (Table 3). The Shannon-Wiener index in the S1 bacterial community was the smallest. When the snowpack changed, the Shannon-Wiener index increased slightly, but there were no significant differences among the treatments in S1. The variation of the Chao 1 index of the soil bacterial community was similar to that of the Shannon-Wiener index. The Phylogenetic-Diversity first increased and then decreased when the temperatures were raised in the 0–10 cm soil layer. The same trend was observed in the 10–20 cm soil layer, except for S0, which had the maximal Phylogenetic-Diversity value. In the different soil layers, the change in snowpack promoted the increase in the Observed-Species of the soil bacterial community. A significant ($P < 0.05$) difference was found between S1 and S2 in the 0–10 cm soil layer.

PCoA showed the β-diversity of the soil bacterial community from the principal coordinate analysis, both UniFrac weighted and non-weighted (Fig. 2). The contributions of the first principal component and the second principal component were 22.63% and 12.77%, respectively. A trend of isolation occurred among the different snow treatments, with complete isolation between S2 and S1 (Fig. 2b) and different soil layers (Fig. 2a).

After the treatments were applied, the taxonomic composition of 60 samples was analysed. There were 38 phyla, of which 95.60% of the taxa were bacteria and 4.40% were Archaea (Fig. 3). In all treatments, *Acidobacteria* took the dominant position in the bacterial communities, but the proportion varied greatly among different soil layers. In the 0–10 cm soil layer, the proportions of *Acidobacteria* varied greatly among the different treatments of S1 (28.45%), S0 (27.15%), S3 (25.41%), S4 (21.41%) and S2 (17.21%). In the 10–20 cm soil layer, they were S0 (35.61%), S1 (31.66%), S3 (24.75%), S4 (30.01%) and S2 (26.45%). Overall, with the accumulation of snowpack, the dominance of *Acidobacteria* in the soil bacterial community declined. In the 0–10 cm soil layer, there was no significant difference among all of the treatments, except that the *Proteobacteria* proportion was significantly lower in S4 than that in S0 ($P < 0.05$). The proportion of *Bacteroides* in the microbial community composition showed a positive correlation with the amount of snow accumulation. When the amount of snow cover changed, there were obvious changes in the 0–10 cm soil layer. The proportions of *Actinomycetes* were 4.74%–7.65% across all of the treatments, increasing along the snowpack, especially in the 0–10 cm soil layer. Additional, the proportions of *Planctomycetes* were 3.25%–5.58% across all of the treatments, and the proportion of *Planctomycetes* in the 0–10 cm soil layer of S4 was lower than those of other treatments. There were significant differences between S4 and S0, S4 and S3, and S4 and S1 ($P < 0.05$). Overall, the changes in snowpack led to a decline in the proportion of *Crenarchaeota* and an increase in the proportion of *Firmicutes*. In different treatments, the proportion of *Chloroflexi* varied between 3.23% and 5.41%, with no difference among the treatments in different layers (see the Supplementary Table 1).

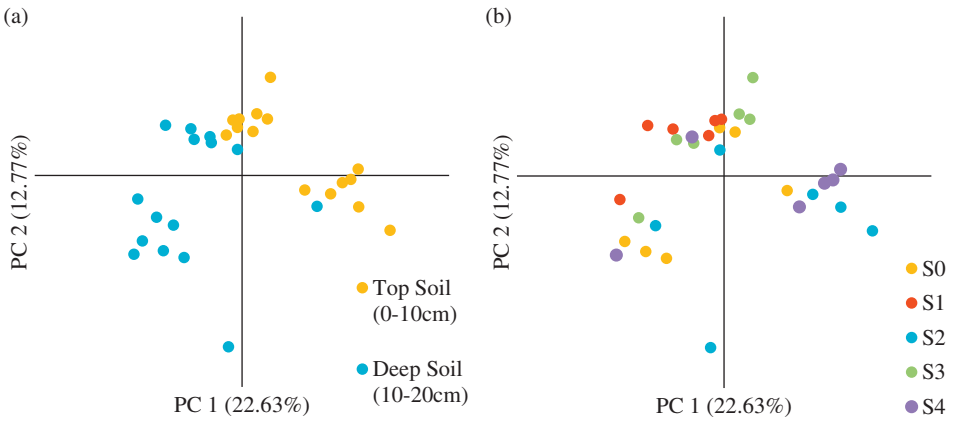


**Fig. 2.** Principal coordinate analysis (PCoA) on bacterial community of different soil layers (a) and different snowpack volume treatments (b).

| | 0-10cm | | | | | 10-20cm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Relative Abundance (%) | S0 | S1 | S2 | S3 | S4 | S0 | S1 | S2 | S3 | S4 |
| Acidobacteria | 27 | 28 | 17 | 25 | 21 | 36 | 32 | 25 | 30 | 26 |
| Proteobacteria | 25 | 23 | 21 | 23 | 19 | 21 | 19 | 21 | 22 | 22 |
| Bacteroidetes | 13 | 19 | 23 | 16 | 31 | 10 | 10 | 16 | 11 | 15 |
| Actinobacteria | 8 | 5 | 8 | 8 | 6 | 6 | 5 | 7 | 7 | 7 |
| Firmicutes | 4 | 2 | 9 | 2 | 5 | 6 | 2 | 5 | 3 | 5 |
| Crenarchaeota | 2 | 5 | 3 | 6 | 1 | 2 | 11 | 4 | 6 | 4 |
| Planctomycetes | 5 | 5 | 4 | 5 | 3 | 4 | 5 | 4 | 4 | 5 |
| Chloroflexi | 4 | 3 | 3 | 5 | 3 | 3 | 5 | 4 | 5 | 4 |
| Verrucomicrobia | 2 | 1 | 2 | 1 | 1 | 3 | 1 | 6 | 2 | 3 |
| Cyanobacteria | 3 | 2 | 4 | 2 | 3 | 1 | 2 | 2 | 2 | 2 |
| Nitrospirae | 2 | 2 | 1 | 2 | 2 | 2 | 3 | 2 | 3 | 2 |
| Gemmatimonadetes | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 1 | 2 | 2 |
| Other | 3 | 3 | 4 | 3 | 2 | 3 | 3 | 3 | 3 | 3 |

**Fig. 3.** Effects of different snowpack volume treatments on the structure of soil bacterial community in alpine meadow (means, n = 3).

### 3.3. Relative influence of physicochemical properties on alpha diversity

Aggregated Boosted Tree analysis showed that soil moisture showed the greatest relative influence on the Chao 1 index (Fig. 4a), Observed-Species (Fig. 4b) and Phylogenetic-Diversity (Fig. 4c) of the soil bacterial community, which accounted for 28.02%, 25.28% and 23.05%, respectively. The pH showed the greatest relative influence (29.59%) on the Shannon-Wiener index (Fig. 4d). AP, AK, TK and SOC showed a high relative influence (> 10%) on each diversity index of the soil bacterial community.

### 3.4. Correlation analysis between the relative abundance of soil bacterial phyla and physicochemical factors

The RDA analysis showed that the soil physical property of axes 1 and 2 represented 35.1% and 7.3%, respectively, in the 0–10 cm soil

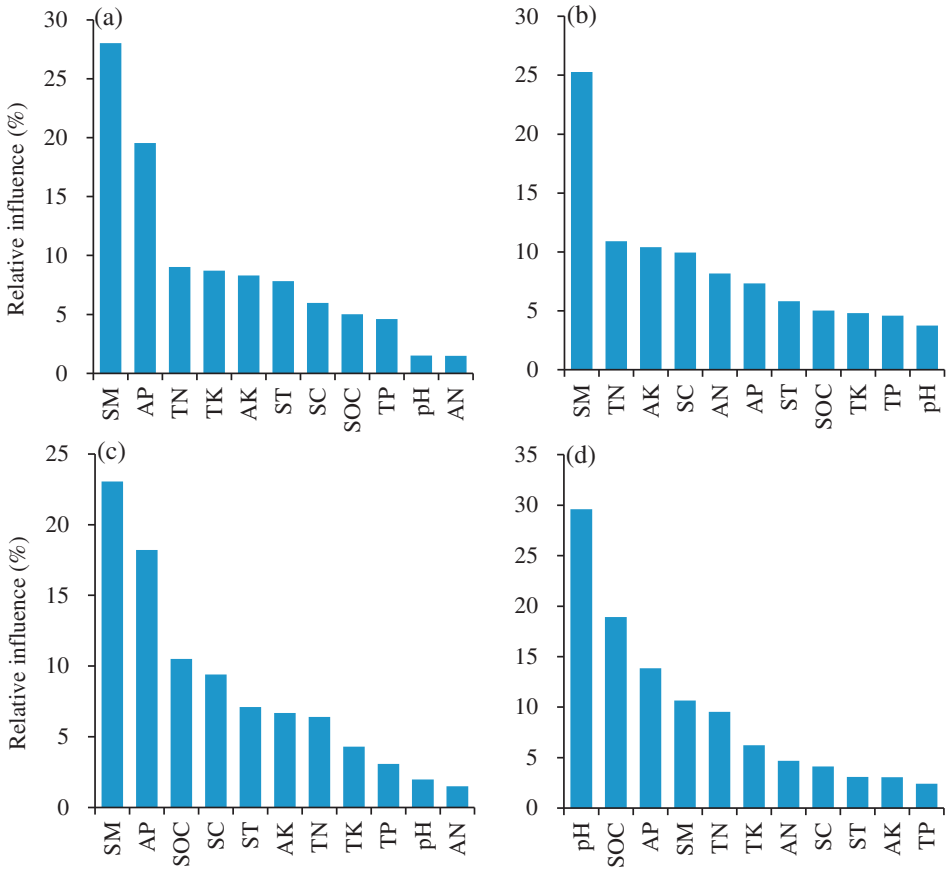


**Fig. 4.** Relative influence (%) for the soil physicochemical properties to Chao 1 index (a), Observed-species (b), Phylogenetic-diversity (c) and Shannon-Wiener index (d) of soil bacterial community. SM: Soil moisture, ST: Soil temperature, SC: Soil compaction, SOC: Soil organic carbon, TN: Soil total nitrogen, AN: Soil available nitrogen, TP: Soil total phosphorus, AP: Soil available phosphorus, TK: Soil total potassium, AK: Soil available potassium, the same below.

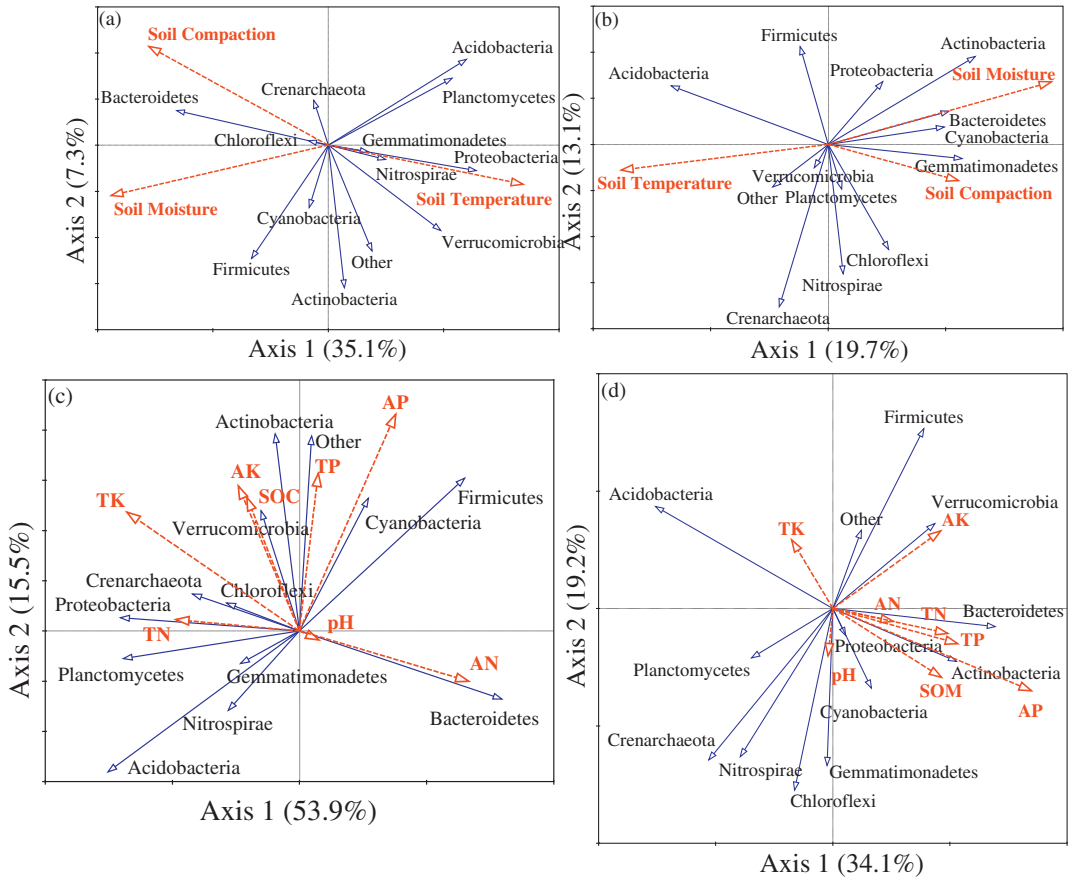


**Fig. 5.** Redundancy analysis (RDA) for soil physicochemical properties and soil bacterial phylum across the change of snowpack volume. (a) and (c) indicated the 0–10 cm soil layer, (b) and (d) 10–20 indicated cm soil layer.

layer (Fig. 5a), and soil temperature had a negative correlation with soil compaction and soil moisture. Fig. 6 shows that *Bacteroidetes*, *Firmicutes*, *Crenarchaeota* and *Chloroflexi* had positive ($P < 0.05$) correlations with soil compaction. *Bacteroidetes*, *Actinobacteria*, *Cyanobacteria*, *Firmicutes* and *Chloroflexi* had positive ($P < 0.05$) correlations with soil moisture. *Proteobacteria, Acidobacteria*, *Planctomycetes* and some other phylum of soil bacteria had positive ($P < 0.05$) correlations with soil temperature. In the 10–20 cm soil layer, the soil physical property of axes 1 and 2 represented 19.7% and 13.1%, respectively (Fig. 5b). *Acidobacteria*, *Crenarchaeota* and *Nitrospirae* had positive ($P < 0.05$) correlations with soil temperature. There was a significant positive ($P < 0.05$) correlation between *Actinobacteria*, *Gemmatimonadetes* and soil moisture.

In the 0–10 cm soil layer, the RDA analysis showed that the soil chemistry property of axes 1 and 2 represented 53.9% and 15.5%, respectively (Fig. 5c). *Actinobacteria* had a positive ($P < 0.01$) correlation with AP, AK, TP and SOC, while *Acidobacteria* and *Bacteroidetes* had negative ($P < 0.01$) correlations with AP and TK. In the 10–20 cm soil layer, axes 1 and 2 represented 34.1% and 19.2%, respectively (Fig. 5d). *Acidobacteria* and *Gemmatimonadetes* had negative ($P < 0.01$) correlations with AP and TK.

## 4. Discussion

### 4.1. Impact of snowpack change on soil physical factors

Snow cover is a poor conductor of heat, with low thermal conductivity and a high thermal capacity (Wei et al., 2007), and it influences soil temperature by changing the energy balance, atmospheric circulation, evaporation from the soil, etc. (Brooks et al., 1996; Gong et al., 2003; Osterkamp, 2005). The duration and depth of the snow cover and snow density can strongly influence the freezing rate of the freezing period and the soil temperature conditions of the ice melting period (Mellander et al., 2005). Snow cover induces soil temperatures that are higher than the atmospheric temperature by preventing the loss of heat in winter, resulting in the lag of soil temperature recovery during the warm temperature in spring (Chang et al., 2012). In this study, we found that the warming effects of snow cover were more significant than expected because of the high availability of radiant energy on the QTP (Chen et al., 2006). These high energy inputs led to increased snowmelt, which allowed water infiltration into the ground. This meltwater could then freeze (Zhou et al., 2000). This ice, and the energy involved in its freeze-thaw cycles, resulted in increases in soil temperature because of the latent heat of melting (Fig. 1a and b). The large changes in temperature from day to night induced the multi-gelation of soil, also contributing to the delayed rise of the soil temperature (Zhu et al., 2006). In addition, the extension of soil freezing led to an increase in soil compaction (Fig. 1c). In alpine meadows, snowpack is an important climatic factor that may change the soil nutrients (Table 2) which are important regulators (Wang et al., 2005) and associated with several soil factors, including climate, organisms, topography, parent material, and time (Muzuku et al., 2005). Snowpack is an important climatic factor that can change the soil nutrients in alpine meadows (Table 2). Soil C/N is often considered an important index of soil nitrogen mineralization. A lower soil C/N is conducive to the nutrient release of microorganisms during organic matter decomposition (Wang et al., 2014). Soil N/P is also an important factor in the trophic structure, biodiversity, and geochemical cycles (Smith, 1992). Although this pattern shown in the soil nutrient ratio were not in order from S0 to S4, it showed that at the top soil depth the soil C/N significantly

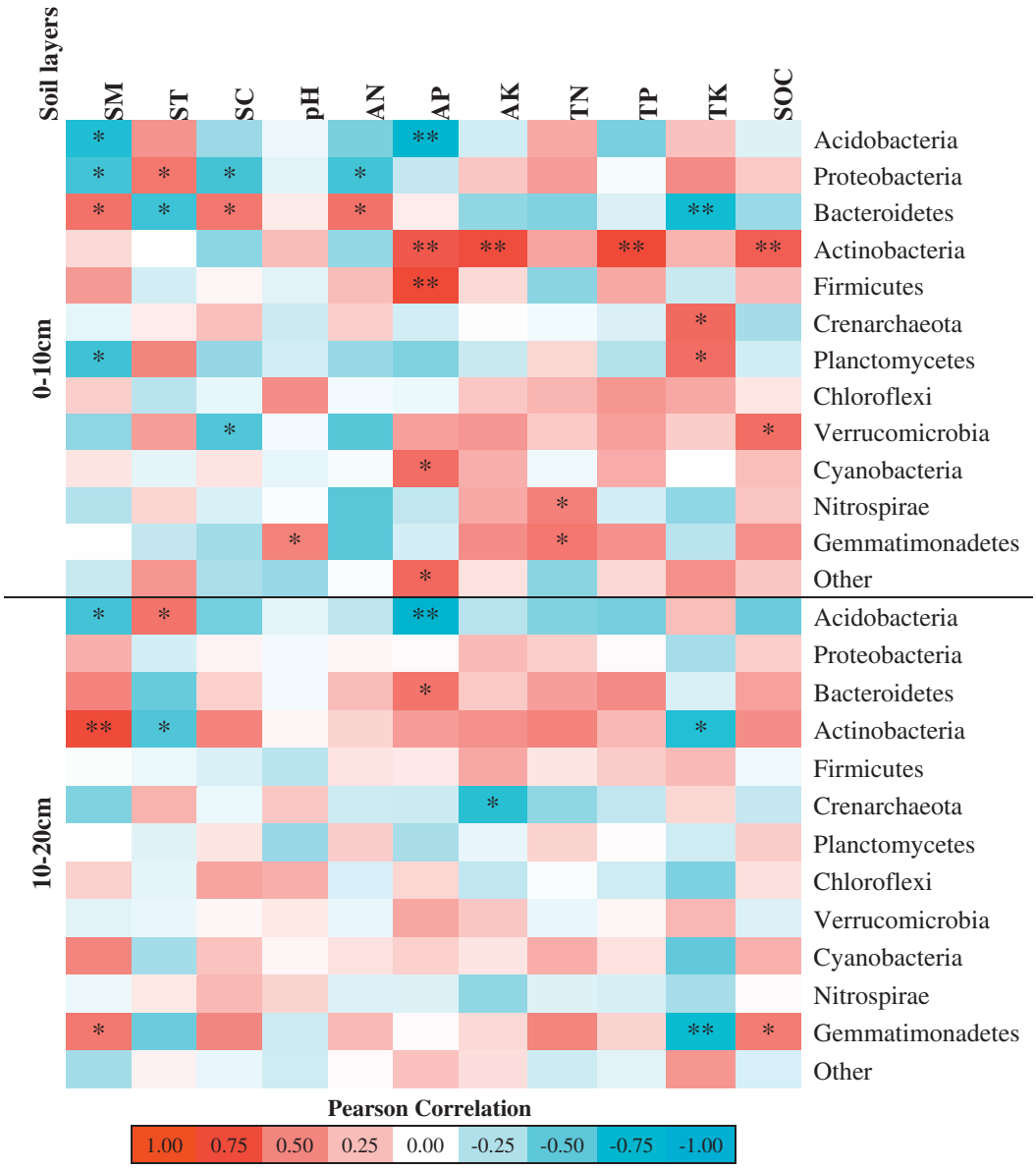


**Fig. 6.** The Pearson correlations between the soil physicochemical property and relative abundance of soil bacteria. $^{**}P < 0.01$,$^{*}P < 0.05$.

increased but N/P declined. The mechanism for this change is not clear; the change will definitely influence the structure and function of the soil microbial community.

### *4.2. Effect of snowpack change on the diversity of the soil bacterial community*

The results of the present study showed that the α-diversity of the soil bacterial community changed with snowpack depth. The α-diversity of the soil bacterial community in all treatments increased in comparison to S1. The β-diversity showed no significant difference among treatments, whereas it showed a significant difference between different soil layers.

Although soil microorganisms in the harsh winter permafrost environment are able to maintain a certain degree of activity (Gilichinsky et al., 2007), low temperature or soil freezing will cause soil microbial death or dormancy (Wang, 2004). In the S0 treatment, soil freezing was weak and the soil temperature maintained a relatively higher level. Soil freezing was stronger when the snowpack increased. In the alpine meadow ecosystem, the bacteria are dominant in summer (Lipson et al., 2002), whereas the main ingredient of the microbial community in winter is fungal due to the death of bacteria (Katem and Paul, 2008; Schadt et al., 2003). Therefore, the effect of a snow-mediated decrease in soil temperature may decrease the soil bacterial diversity. Microbial activity was present in soils below 0 °C because small amounts of water remained unfrozen, allowing the diffusion of microbial substrates and consumption of products (Ostroumov and Siegert, 1996). In spring, soil microorganisms can rapidly propagate under appropriate hydrothermal conditions, leading to the significant increase in populations and diversity (Fierer, 2003; Rietz and Haynes, 2003). The results also indicated that the optimum hydrothermal conditions were in S2, based on the diversity of the soil bacterial community (Table 3), but it decreased when the snowpack further increased and the hydrothermal conditions changed.

Soil depth represented a strong environmental factor, with multiple changed edaphic factors (Fierer, 2003). Previous studies have shown that soil depth was an important spatial factor in determining the microbial community (Eilers et al., 2012; Hansel et al., 2008). Our results showed that the diversity of soil bacterial communities was obviously different in the different soil layers. This difference resulted from the

different soil properties (Fig. 1 and Table 2) at different soil depths, which is consistent with other researchers' findings on soil temperature (Wilkinson et al., 2002) and soil nutrients (Fanin et al., 2015). In addition, bioturbation (Postma-Blaauw et al., 2006) can have an effect on the soil bacterial community in different soil layers.

### 4.3. Effect of snowpack change on the structure of the soil bacterial community

The present study showed that the soil bacterial community structure at the phylum level significantly differed with snowpack change in the alpine meadow. *Acidobacteria* was dominant in soil microorganisms (Barns, 1999) of the alpine meadow. A previous study found that the *Acidobacteria* community was affected by many soil factors, such as pH, organic carbon, C/N ratio, soil temperature, soil moisture, etc. (Naether et al., 2012). The increasing snowpack decreased the relative abundance of *Acidobacteria*, which was positively correlated with soil temperature and AP. *Proteobacteria* is named because of its plentiful morphology, and all members of *Proteobacteria* are gram-negative bacteria (Madigan et al., 2008). Our results indicated that the change in the relative abundance of *Proteobacteria* is irregular because the members of *Proteobacteria* cover very extensive metabolic types (Song et al., 2016). Bacteria within the phylum *Bacteroidetes* are widely distributed across ecological niches (Garrity and Holt, 2001), and it is likely that some are well adapted to increased snowpack. *Actinobacteria* constitute one of the largest phyla among bacteria and represent gram-positive bacteria with high guanine and cytosine contents in their DNA (Ventura et al., 2007). The change in *Actinobacteria* showed a positive correlation with soil temperature. Similar results have been found in previous studies (Jia et al., 2014). The diversity of the soil bacterial community was changed with regularity along the snowpack gradient, and the changes in phyla in the soil bacterial community were different, resulting in the functional differences in the soil bacterial community.

## 5. Conclusions

These results have important implications for our understanding of soil physical properties and soil bacterial communities in relation to snowpack depths in the alpine meadow ecosystem of the QTP. The results of our study showed that the increase in the snowpack may decrease the average soil temperature and increase soil moisture and soil compaction, temporarily changing the soil chemistry properties. Changes in soil physicochemical properties lead to the increase in the diversity of the soil bacterial community, especially soil temperature. The relative abundance of *Acidobacteria* decreased in cold environments, whereas *Bacteroidetes* was better adapted to cold environments. *Actinobacteria* preferred environments with higher moisture in comparison with other phyla in alpine meadows. Future experiments should focus on the relationship between the plant community composition and the soil microbial community composition (e.g., bacteria, fungi) under changing snowpack.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catena.2018.01.004.

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