



**Full Length Article**

## Effects of Winter Snowpack on the Soil Bacterial Community in a Temperate Wetland in Northeastern China

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### Abstract

Global climate change is accompanied by changes in the amounts of ice and snow. However, the effects of snow coverage on the soil microbial community have been poorly studied. Here, the soil bacterial communities and diversities during winter in a marsh-meadow wetland were investigated in Sanjiang plain. In this study, there were four levels (no cover, 20 cm, 50 cm, 100 cm) snow cover were conducted in a field in the Sanjiang plain. Leakage of soil water into or from the treatment plots was prevented. Soil physicochemical properties were determined in early spring when the snow was removed, and the bacterial community structure and diversity in soil samples were determined by partial 16S rDNA gene amplification and sequencing. The results showed that with an increase of snow cover, the average soil temperature increased and the soil acidity and total nitrogen content were slightly decreased. A higher snow cover led to an increase in the soil bacterial alpha diversity and, influenced the relevant abundance of bacterial phyla in various ways. Interestingly, soil covered by 100 cm snow more strongly resembled the uncovered soil, in terms of phyla distribution, than a snow cover of 20 or 50 cm did. This demonstrates that a decrease of snow cover as a result of climate changes can have a serious impact on soil microbial community structure and diversity. In summary, these results showed that soil bacterial communities present in marsh meadows wetlands are sensitive to changes in winter snow coverage. © 2020 Friends Science Publishers

**Keywords:** High-throughput sequencing; Snowpack; Soil bacterial communities; Soil physicochemical property

### Introduction

Snowfall is common in mid-high and high altitudes around the world. The insulating properties of snow protect soil microorganisms against freezing (Hinkler *et al.* 2008), and the thickness of a snowpack influences the subsurface soil temperature and the metabolic activity of the soil microbial community (Wang *et al.* 2013; Freppaz *et al.* 2014). It has been predicted that local snowfall can decrease under global warming conditions (Kunkel *et al.* 2009; IPCC 2013), leading to a thinner snowpack in winter (Kunkel *et al.* 2009; Kapnick and Delworth 2013). Such a decrease in snow cover can affect the soil microbial community composition and diversity, triggering changes in essential ecosystem functions in unknown ways (Campbell *et al.* 2005; Zhang 2005). Allison *et al.* (2013) suggested that characterizing soil microbial mechanisms would be critical for understanding how ecosystem processes respond to changes in snowpack thickness under global climate change. Though rarely investigated, during winter such responses would affect soil carbon and nutrient cycles.

As an important contributor to soil ecological processes, microbes play an essential role in litter decomposition, carbon and nitrogen mineralization, and in soil nutrient conversion and circulation (Garcia *et al.* 2002). Natural fluctuation of external factors strongly affects soil microbial activity, but the existing research on these affects is sparse. During winter, the relatively stable water and temperature conditions that apply in soil covered by snow provide a suitable environment for growth and metabolic activity of microbes (Bogoev *et al.* 2002; Bombonato and Gerdon 2012). However, the alternate process of snow formation and melting directly affects soil temperature and moisture (Yang and Jin 2008; Wang *et al.* 2015), and repeated freeze-thawing cycles may alter the microbial community structure and function, possibly leading to microbial dormancy or decreased viability (Yan *et al.* 2018). Global warming has become an indisputable fact, with local changes in snowfall patterns as an inevitable consequence (Merino *et al.* 2014). Studies have shown that the reduction of snow caused by climate warming directly reduces the

local soil temperature, enhances soil freezing, and increases the frequency of freezing and thawing cycles (Price and Sowers 2004; Groffman *et al.* 2006; Monson *et al.* 2006). This causes mechanical disruption of animal and plant residues and can change microbial communities present in the upper layer of the soil. Under permissive temperatures nutrients released by cell rupture increase substrate availability and promote microbial growth, enhancing soil microbial activity (Wang *et al.* 2010). Soil microbial biomass is a repository of nutrients and these provide an important source of energy and nutrients for plant growth (Edwards *et al.* 2006). Microbial biomass represents the number of microorganisms involved in soil organic matter conversion and nutrient cycling and is sensitive to changes in soil environmental conditions (Drotz *et al.* 2010). Although the number of cultivable microorganisms is less than 1% of the presumed total number of microorganisms present in soil, the cultivable fraction can be studied directly, providing reliable evidence for responses to soil environmental changes. Therefore, to provide an in-depth understanding of the effects of climate warming-related snow reduction on soil microbial biomass and the cultivable microbial population, it is important to understand the response of soil ecosystems to climate changes in local settings.

The Sanjiang Plain wetland in northeast China plays an important role in regional climate regulation, water conservation, and biodiversity conservation (Lipson *et al.* 2000). The annual period during which the soil is frozen typically lasts 5–6 months (Larsen *et al.* 2007), providing an excellent test opportunity to study the effect of snow cover. Cycles of freezing and thawing have been shown to affect soil nutrient characteristics, litter decomposition, soil biological activity and microbial diversity in winter (Larsen *et al.* 2007; Edwards and Jefferies 2013), but changes in snow cover over time may change the frequency and amplitude of soil freeze-thawing cycles. The soil microbial community structure and diversity in the area have been characterized, but changes in winter have so far not drawn much attention. Therefore, this work concentrated on the *Calamagrostis angustifolia* wetland in the Sanjiang Plain and investigated the soil microbial structure and function under a natural snow cover and, following snow removal, under artificially applied snow covers with defined thickness. This provided insights into the ecological processes taking place in wetland soils during winter and how these might respond to changed snow coverage patterns as a result of climate change.

## Materials and Methods

### Field site and experimental design

The study was conducted at the Sanjiang Wetland Experimental Station (47°35'N, 133°31'E), property of the Institute of Nature and Ecology of Heilongjiang Academy

of Sciences, China. Fig. 1 shows a map of the area. The local average temperature is 1.9°C and the monthly temperature ranges from –21.6°C in January to 21.5°C in July. The average annual precipitation in winter is about 200 mm, with approximately 80% occurring between November and March.

The experiments described here were performed in January–March 2018. Snow was collected and reapplied with a cover thickness of 0, 20, 50 and 100 cm. Depending on the thickness of the original snow, these covers were produced by means of reduction, or by repletion as necessary. Absence of a snow cover (0 cm treatment) was obtained by full clearance of the plots, and removal was repeated after fresh snowfall. For the other plots, excess snow was evenly removed to obtain the desired snow coverage levels in reduction treatments. Likewise, snow was uniformly deposited to the desired thickness using an 8-mesh sieve in the repletion treatment plots, and its thickness was regularly managed to correct for novel snowfall and windy weather to ensure a constant thickness over time. Each treatment was performed with 3 replicate plots, each plot having an area of 5 m × 5 m. In order to prevent different effects of melt water runoff, each treatment sample was sealed with a 5 mm thick, 50 cm high PVC plate that was buried to a depth of 30 cm to ensure that any melted snow or rainwater only infiltrated the treated plot directly. These enclosures had been put in place in the autumn of 2017.

### Soil sample collection

At the end of March 2018, when the snow started to melt, the snow cover was completely removed and the soil was sampled at three points randomly selected per plot. For each sample point, 10 cm depth of soil was extracted and transported to the laboratory for analysis. Plant roots and stones were removed, after which the fresh soil was sieved through a 4 mm mesh and divided into two parts. One part was used for the determination of soil bacteria, the other was air dried and passed through a 2 mm sieve to determine the soil pH and elemental content of carbon and of total nitrogen. Total carbon and total nitrogen content were assessed with an elemental analyzer (Vario Macro Cube). For determination of the soil pH, 10 g air dried soil was sieved (2 mm), mixed with 25 mL distilled water at room temperature, stirred for 2 min, and allowed to stand for 30 min before measuring the pH with a Sartorius PB-10 pH meter.

### Extraction of total DNA and high-throughput analysis

A soil DNA extraction kit (MOBIO Power Soil® DNA Isolation Kit, U.S.A.) was used to extract total DNA for characterization of soil microbial genomic DNA. DNA was quantified and its purity was checked using a nucleic acid quantifier (NanoDrop ND-1000).

Specific primers with bar codes (338F, 5'-actcctacgggagcgagca-3' and 806R, 5'-ggactachvvgggtwctaat-3') were used to amplify the V3-V4 region of 16S rDNA

(Zhong *et al.* 2010). The PCR amplification was done with the TransGen AP221-02 kit and TransStart Fast-*Pfu*DNA Polymerase (TransGen, China), on an ABI GeneAmp® 9700. All samples were amplified 3 times. The 20- $\mu$ L PCR reaction contained 4  $\mu$ L of 5 $\times$ FastPfu Buffer, 2  $\mu$ L of 2.5 mmol L<sup>-1</sup> dNTPs, 0.4  $\mu$ L of forward and reverse primer (5  $\mu$ mol L<sup>-1</sup>) each, 2  $\mu$ L of template (10 ng DNA), 0.4  $\mu$ L of Polymerase, and 10.8  $\mu$ L of ddH<sub>2</sub>O. PCR amplification steps were 95°C pre-denaturation for 2 min, then 30 cycles of 30 s at 90°C, 30 s at 50°C and 30 s at 72°C, with a final extension time of 10 min at 72°C. The PCR products of the three sample replicates were combined and rechecked by 2% agarose gel electrophoresis. According to these results, PCR products were quantified more precisely with the QuantiFluor™-ST blue fluorescence quantitative system (Promega). The DNA concentration of the sample amplicons was adjusted and the DNA was externally sequenced on a HiSeq Illumina platform (Beijing Biomaker Company).

### High-throughput sequencing data analysis

The DNA was pair-end sequenced. First, quality control of the raw data was conducted, and the sequences were connected with the software Flash, while unconnected sequences were discarded. Bases below the read value of the tail of the read were filtered with a window set to 50 bp. When the average read value within this window was below 20 bp, the base downstream of the window was cut off, and the reads below 50 bp were filtered after quality control. The paired reads were merged for which the minimum overlap length had to be 10 bp. The maximum error ratio allowed in the overlapping region was 0.2, and the non-conformance sequence was screened. The tag sequence at the end of the sequence was detected, and the minimum mismatch number was 0. The sequence containing tag at the beginning was inversely complemented, and the tag was removed. The barcodes were used without mismatches, the maximum primer mismatches were set at 2, and the final sequence for analysis was obtained as previously described (Edgar *et al.* 2011).

With the application of QIIME (Quantitative Insights into Microbial Ecology), the sequences were classified into multiple OTUs (operational taxonomic units) according to similarities between the sequences. The OTUs contained in each sample and the number of sequences contained in each OTU was recorded. The uparse OTU (version 7.1; <http://drive5.com/uparse/>) method was used for clustering, with the OTU sequence similarity set at 97% to obtain representative OTU sequences (Edgar *et al.* 2011). Using uchime (version 4.2.40; [http://drive5.com/usearch/manual/uchime\\_algo.html](http://drive5.com/usearch/manual/uchime_algo.html)), chimeric sequences were detected and removed (Quast *et al.* 2013). Using the usearch\_global method, the map of the optimized sequence was compared back to the OTU representative sequence, and an abundance statistics table of each sample sequence of an OTU was generated.

To obtain each OTU corresponding species classification information, as an RDP classifier the Bayesian algorithm was used with a 97% similarity level for all levels (phylum, class, genus). For the statistical community composition of each sample, we specified the dominant population at the door, and steel level relative abundance had to be greater than 10%, or for the genus level it had to be greater than 1%. The Silva database (Release115 <http://www.arb-silva.de>) was used for comparison (Chao 1984). The OTUs with a similarity at or above 97% were selected to generate the expected dilution curve, and mothur was used to calculate the abundance indices Chao1 and ACE (for bacterial community richness) and the diversity indices Shannon and Simpson (for community species richness). The Shannon index reflects the degree of diversity of a sample while the Simpson index reflects the dominance of species.

### Results

After keeping snow cover levels of isolated plots constant at various levels for 3 months (4 treatments with different snow cover levels and 3 replicates for each treatment), the snow was removed and from all 12 plots and soil samples were collected and analyzed. The obtained physicochemical properties are summarized in Table 1. As expected, the soil temperature was lower in absence of a snow cover. The thickness of the snow cover correlated negatively with acidity (least acidic pH was found with the thickest snow). Soil carbon content only significantly differed for the uncovered soil. More variation was observed for the nitrogen content of the soil, which was significantly higher in the uncovered soil, lowest for soil covered by 10 and 100 cm of snow, and medium for the sample covered by 20 cm.

### Sequencing results of soil samples and validation of sampling depth

By sequencing amplicons of the bacterial 16S rDNA V3–V4 region, after filtering out low quality sequences, a total of 210,383 valid sequences and 183,232 reads were obtained. The three replicate treatments were combined here, as were the amplicon triplicates. The reads were clustered at 97% similarity to classify the corresponding species into OTUs. A total of 25,843 different OTUs were identified. As shown in Table 2, the highest number of reads were obtained from uncovered soil, but the number of predicted OTUs varied only slightly from 1886 (0 cm) to 1896 (100 cm). This represented an increase of 0.9% of number of OTUs, and this increase seemed to depend on snow thickness, as the numbers for the 20 and 50 cm samples suggest.

### Analysis of soil bacterial community richness and alpha diversity

The richness of the detected bacterial communities was assessed by determination of Chao1 and ACE indices, but

**Table 1:** Physicochemical properties of the soil after treatment (averages of triplicate samples)

Snow cover (cm)	Soil temperature (°C)	pH	Soil organic carbon (g kg <sup>-1</sup> )	Soil nitrogen (g kg <sup>-1</sup> )
0	-9.9 ± 0.02a	5.62	44.17 ± 2.52a	4.62 ± 0.21a
20	-5.4 ± 0.02b	5.75 ± 0.08ab	42.64 ± 2.80b	4.18 ± 0.18c
50	-4.4 ± 0.01c	5.83 ± 0.03ab	42.15 ± 1.58b	4.42 ± 0.28b
100	-4.1 ± 0.04c	5.89 ± 0.07a	42.37 ± 1.82b	4.18 ± 0.23c

Different letters in a column represent statistical significance ( $P < 0.05$ ) as calculated by Least-significant difference (LSD), one-way ANOVA

**Table 2:** Number of sequence reads and OUT of soil bacterial sequences after the different treatments

Snow cover (cm)	Number of reads <sup>a</sup>	Number of OTUs <sup>a</sup>
0	53,967	1880
20	52,018	1886
50	51,889	1892
100	52,640	1896

<sup>a</sup>No significant differences were observed ( $P > 0.05$ )

**Fig. 1:** Map of the research site in the Sanjiang Plain, China

no statistically significant differences were observed between the treatments (Table 3). This would indicate that neither presence nor absence of snow, nor snow cover thickness, significantly affected the richness of the bacterial OTUs. In contrast, the Shannon index varied between the treatments, with a significantly lower value obtained from uncovered soil, and a significantly higher value for the soil covered by 100 cm of snow (Table 3), which represented an increase of 3.53% compared to the 0 cm sample. There was no significant variation in the Simpson index of the treatments. These results indicate that snow cover only significantly increased the soil bacterial diversity.

### Analysis of soil bacterial groups

At the phylum level, bacteria were distributed into 15 known phyla, in addition to 11 candidate phyla and one unclassified group. Members of the following phyla were detected (at >1%, in order of decreasing abundance): Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Bacteroidetes, Verrucomicrobia, Gemmatimonadetes, Planctomycetes, Nitrospirae, Firmicutes, Candidatus,

Saccharibacteria, Elusimicrobia, Cyanobacteria, Ingavibacteriae, and Candidatus Parcubacteria. The relative abundance of these 14 bacterial phyla is shown in Fig. 2. The sum of the relative abundances of all detected phyla accounted for more than 95% of the total amount of OTUs identified, in all the twelve soil samples.

The most abundant bacterial phyla in the four treatments were Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi and Bacteroidetes, which in combination accounted for 85% in all treatments (Fig. 2). However, the relative abundance of these phyla varied with treatments, although there was no common trend visible related to of snow-cover thickness. The largest differences in relative phylum abundance were observed between plots without snow cover versus those with a snow cover ( $P < 0.05$ ). For instance, compared to uncovered soil, a snow layer of 20 cm increased the relative abundance of Proteobacteria by 8% though their abundance decreased again when the snow cover increased from 50 to 100 cm. Conversely, a decrease in abundance of Chloroflexi was observed between 0 cm and 20 cm, but the 100 cm sample contained more Chloriflexi than the 0 cm sample did.

At the class level, a total of 41 known classes of bacteria were obtained, in addition to unclassified classes (which accounted for approximately 25% of the OTUs) and 40 candidate classes (lumped as 'others' in Fig. 3). At an abundance >5%, we identified (in decreasing order for uncovered soil): Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Solibacteres, Actinobacteria, Thermoleophilia, Acidobacteria, Anaerolineae, Sphingobacteriia, Holophagae, and Acidimicrobiia which in combination accounted for 63% (100 cm samples) to 70% (20 cm samples) of the total amount of soil bacterial classes.

The most abundant bacterial classes were Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Thermoleophilia and Solibacteres, for all treatments, but with variable proportions. Notably, the sum of these four was highest for the 20 cm treatment, while uncovered soil contained relatively more members of the Thermoleophilia class. Compared with the 0 cm, presence of a snow cover consistently increased the relative abundance of Betaproteobacteria, while Deltaproteobacteria were higher for the 20 and 50 cm samples.

The relative abundance level of the top 20 bacterial genera was used to build a hierarchical clustering figure; this is shown in Fig. 4 together with a heat map. The hierarchical clustering is based on relative abundance of these genera was visualized by colour in the heat map. The tree to the top clusters the 12 soil samples, with regard to presence of these genera only. As can be seen, the different treatment replicate were not grouping together. Rather, based on these representatives, there were two groups of sample types, with the three uncovered soil samples grouping together with two 100 cm and one 50 cm sample. Moreover, the represented genera clustered according to their abundance (tree to the right) that in part related to the

**Table 3:** Richness and diversity indices of the soil bacterial communities in the different treatments

Snow cover (cm)	ACE index	Chao1 index	Shannon index	Simpson index
0	1859.03 ± 16.00a	1879.29 ± 15.64a	6.23 ± 0.06b	0.0053 ± 0.0005a
20	1865.66 ± 13.28a	1875.45 ± 10.41a	6.31 ± 0.08ab	0.0041 ± 0.0003a
50	1869.05 ± 22.76a	1880.43 ± 24.47a	6.42 ± 0.02ab	0.0039 ± 0.0004a
100	1889.89 ± 13.75a	1900.96 ± 10.71a	6.45 ± 0.04a	0.0046 ± 0.0005a

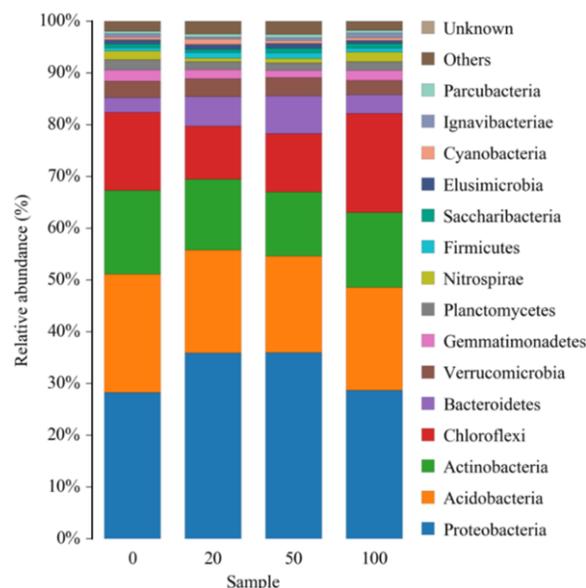
Different letters in a column represent statistical significance ( $P < 0.05$ ) as calculated by Least-significant difference (LSD), one-way ANOVA

phylum to which they belong. This indicates that different bacterial species that are taxonomically related may respond similar to the tested conditions, increasing the overall findings per phyla, as shown in Fig. 2.

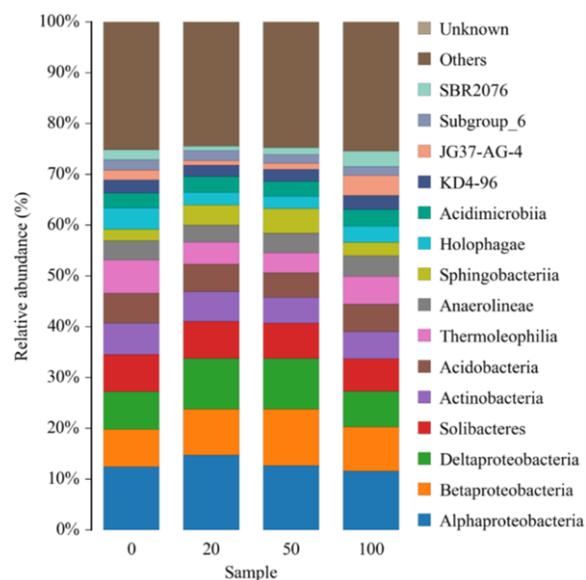
## Discussion

Multiple studies in alpine and arctic tundra ecosystems have reported active microbial metabolism under snow-cover during winter (Schadt *et al.* 2003; Schmidt *et al.* 2007). However, until now little has been known about winter microbial biogeochemical processes in temperate areas. This study determined the soil microbial diversity and community composition in a variety of seasonally snow-covered temperate wetland ecosystems. Following exposure to various temporarily maintained levels of snow cover (from 0 cm to 100 cm) under otherwise natural conditions, the Shannon diversity of naturally occurring soil bacteria increased with snow height, indicating that snow thickness increases soil microbial diversity, probably as a result of insulation and less severe freezing soil temperatures (Brookes *et al.* 1985). While any water contained in the soil remains frozen, local water shortness in combination with low temperatures can kill bacteria or induce their dormancy, resulting in a decline in soil microbial biodiversity (Ross 1990) as observed here in the uncovered soil. We have demonstrated that with an increase of snow cover thickness, the soil is better insulated and reaches less low temperatures. This probably explains why snow cover thickness correlates with soil bacterial diversity. All main bacteria phyla remained present in the soil under the different snow cover conditions, so that snow cover (or absence of it) did not change the soil microbial community composition completely, but it did change the relative abundance of phyla and members therein, which is consistent with previous studies (Fierer *et al.* 2003).

The present study showed that the soil bacterial community structure at the phylum level significantly differed with snowpack changes observed in an alpine meadow. There, Acidobacteria were dominant (Barns 1999) while in the wetland investigated here, Acidobacteria were only the second-most abundant phylum. A previous study in this wetland reported that the local bacterial community is affected by soil factors such as pH, organic carbon, C/N ratio, soil temperature, and soil moisture (Naether *et al.* 2012). Those studies were performed at temperatures above freezing, and here we complete these findings for winter conditions. Acidobacteria in forest soil have been shown to respond to these environmental factors as well (Naether *et*

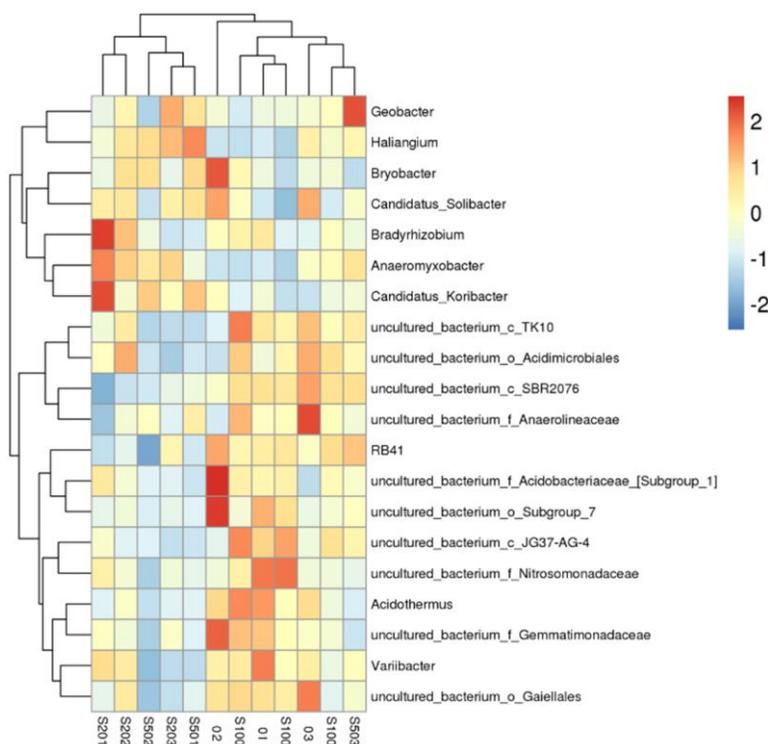


**Fig. 2:** Comparison of bacterial groups at the phylum level after different snow pack treatments. The phyla are sorted for relative abundance in the uncovered soil samples



**Fig. 3:** Comparison of bacteria groups at the class level after different treatments

*al.* 2012). We found that moderate snow cover decreased the relative abundance of Acidobacteria, compared to zero coverage which was positively correlated with soil



**Fig. 4:** Hierarchical clustering diagram of bacteria at the genus level after different soil treatments for the 12 individual samples. The codes to the bottom start with 'S' to indicate snow cover, followed by '10', '20' or '100' to indicate its thickness, and '1', '2' or '3' for the replicate samples. The tree to the left shows the hierarchical clustering based on abundance of the genera identified to the right

temperature and pH. Our results further identified that different snow cover depth caused variation in relative abundance of Proteobacteria, but a general trend correlating with cover thickness was not observed. This phylum covers very extensively different species with different metabolic activities (Song *et al.* 2016). Bacteria within the phylum Bacteroidetes are widely distributed across ecological niches (Garrity and Holt 2001), and although they were not the most abundant phylum, their abundance increased with moderate snow cover, to decrease again at 100 cm snow coverage. Gram-positive Actinobacteria constitute one of the largest phyla among bacteria and typical soil members have high guanine and cytosine contents in their DNA (Ventura *et al.* 2007). Only limited variation was observed in their relative abundance between the various treatments. More extensive variation was seen for the Chloroflexi. In combination, the relative abundance of all phyla in combination showed resemblance of the 100 cm covered soil with uncovered soil, while snow coverage of 20 and 50 cm were most different. This demonstrates that relatively minor changes in snow coverage can have severe effects on the soil microbial community. In conclusion the diversity of the soil bacterial community varied inconsistently with the snowpack gradient, with different changes observed for individual phyla of the soil bacterial community, most likely resulting in functional differences in the soil bacterial community.

## Conclusion

Results of this study proved that the change of snowpack caused significant changes in soil physical-chemical parameters and the bacterial community in the temperate wetland in winter. The relative abundances of Proteobacteria and Acidobacteria were increased by the increasing of snowpack. In general, snowpack might potentially affect soil bacterial structure and composition, but not changed the alpha diversity in temperate wetland systems under global change scenarios. Over the long term, investigating the snowpack change is important to accurately track and predict the responses of global climate change in wetland ecosystems.

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