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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