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Effects of Snow Thickness on Soil Cultivable Physiological Bacterial Groups in Late Soil-thawing Period in an Alpine Forest on the Eastern Tibetan Plateau of China

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The ongoing climate change plays an important role in changing snow pattern and its related ecological processes in alpine biomes. There are obvious natural snow patches with different snow thickness in the alpine forest at late freezing-thawing period between winter and growing season. These snow patches can have strong effects on soil microbial community structure and microbial activity by affecting soil temperature and humidity, but little information has been available on. Therefore, the effects of snow thickness on the characteristics of cultivable bacteria and bacterial physiological groups in the soil organic layer were studied in an alpine *Abies faxoniana* primary forest. The traditional plate culture method and the most probable number method were used to determine the numbers of specific microbial physiological groups, including ammonifying bacteria, denitrifying bacteria, nitrifying bacteria, aerobic cellulose decomposing bacteria, and anaerobic cellulose decomposing bacteria. The results indicated that snow thickness significantly influenced the numbers of bacteria and its physiological groups in the organic layer, although different bacterial physiological groups showed various responses to snow cover in different soil layers. Compared with others, ammonifying bacteria, nitrifying bacteria and anaerobic cellulose decomposing bacteria exhibited the higher number under the thickest snow patch than other snow patches. The number of denitrifying bacteria showed a decrease tendency with the increase of snow thickness, but few differences were observed in the number aerobic cellulose decomposing bacteria under different snow patches with different thickness. In addition, the numbers of bacterial physiological groups at the later freezing-thawing period in this alpine forest showed the order as: ammonifying bacteria> denitrifying bacteria > nitrifying bacteria > aerobic cellulose decomposing bacteria > anaerobic cellulose decomposing bacteria. The results here suggest that nitrogen cycling driven by ammonifying bacteria, denitrifying bacteria and nitrifying bacteria would be more sensitive to snow cover change compared with C cycling driven by cellulose decomposing bacteria in the scenario of climate change.

Key words: Snow cover, Soil organic layer, Cultivable bacteria, Bacterial physiological groups, Alpine forest.

Microorganisms not only play their roles as soil engineer and as driving force in accumulation of soil organic matter and the mineralization processes, but also act as a sensitive bio-indicator to environmental change (Li *et al*. 2011; Eugene 2011). As the direct embodiment of microbial activity in soil, the number of cultivable microorganisms can provide direct and reliable evidences for the change of soil environment, and can be one of the key indicators of physiological potential assessment of soil microorganisms (Feng *et al*. 2011; Yang *et al*. 2012a). Snow distribution is one of the most important variables controlling ecosystem properties such as soil temperature and

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moisture in seasonal freezing-thawing cycle (Rixen *et al*. 2004), and then profoundly influences soil microbial activity and community structure (Zhang *et al*. 2010; Andreas *et al*. 2013; Wang *et al*. 2013). Snow often melts with the increase of temperature in late soil-thawing season, and many researches documented that obvious snow patches with different thickness could be observed due to the effects of meteorological conditions, topography and vegetation (Colbeck 1983; Pomeroy *et al*. 1997). The heterogeneous snow patches can redistribute the available resources including light, heat, water and nutrients in the ecosystem, and then directly or indirectly affect soil microbial community structure, the related ecological processes (Lipson *et al*. 2002; Schadt *et al*. 2003). However, few attentions have been paid to the effects of snow thickness on soil microorganisms in the transition period between winter and spring.

Ammonifying microbes, denitrifying microbes, nitrifying microbes, aerobic cellulose decomposing microbes and anaerobic cellulose decomposing microbes, are involved in the numerous key processes, such as nitrogen mineralization, nutrient cycling and carbon fluxes (Kennedy and Smith 1995; Groffman *et al*. 1996, 2001; Qi *et al*. 2010; Jefferies *et al*. 2010; Liu *et al*. 2010b). Snow patches with different snow thickness in the late freezing-thawing season could regulate these important microbes. Moreover, snow cover is shrinking in many high altitude/latitude regions in the scenario of ongoing global warming (Ding and Qin 2009; Tan *et al*. 2014), which would affect the soil microbial community structure and the related soil ecological processes. However, these processes are still unclear.

The subalpine/alpine forest of western Sichuan, sensitive to climate change, is the second biggest forest in China, which locates on the eastern Tibetan Plateau and plays an important role in regulating regional climate, conserving water and soil, and nursing biodiversity (Feng *et al*. 2006; Liu *et al*. 2010a; Tan *et al*.2011). There were obvious seasonal freeze-thaw cycles and snow patches formed with different thickness during the alternating process of seasonal snow cover and melt in this region (Wu *et al*. 2010; Tan *et al*.2011; He *et al*. 2013). The previous studies mainly focused on soil enzyme under seasonal snow cover, soil microbial biomass carbon and nitrogen and soil fauna communities during soil thawing period (Yang *et al*. 2012a; Yang *et al*. 2012b; Tan *et al*. 2011;Tan *et al*. 2012), but the effects of snow patches with different thickness on soil microorganism and its physiological groups in soil organic layer has not been understood. This lacking knowledge would limit the understanding in the transition of soil ecological processes from non-growing season to growing season in the scenario of the climate change. Therefore, the objective here was aimed to reveal the changes of the cultivable bacteria and bacterial physiological groups as affected by snow patches with different thickness in late soil-thawing period

MATERIALS AND METHODS

The study region, attention for years (Wu *et al*. 2010; Yang *et al*. 2012a; He *et al*. 2013), is located in the Bipenggou Nature Reserve (E102°532 –102°572 , N31°142 –31°192 ; 2458–4619 m above sea level), Li County, western China, which is the transition zone of the Qinghai-Tibet Plateau and the Sichuan basin. The mean annual temperature ranges from 2 to 4 °C, with maximum and minimum temperatures of 23 and -18 °C, respectively. The annual precipitation is approximately 850 mm. The snow cover season is from November to April of the following year. The main forest vegetation is called as fir (*Abies faxoniana*) primary forest. Fir, birch (*Betula albo-sinensis*), larch (*Larix mastersiana*) and cypress (*Sabina saltuaria*) are representative forest canopy species. The understory plants are dominated by *Festuca ovin*, *Rhododendron delavayi*, *Carex* spp., *Cystopteris montana* and *Berberis sargentiana* (Wu *et al*. 2010). The seasonal soil freezing-thawing period is observed begin in early November after the first snow fall and the soil remains frozen for 5 to 6 months. Markedly different thickness of natural snow patches come into being during this period (He *et al*. 2013). Soil physicochemical properties have been researched with a detailed study by our team (Yang *et al*. 2012a).

Based on the previous research on the dynamic monitoring results of soil temperature in the study region (Yang *et al*. 2012a; He *et al*. 2013), five snow packs with different snow thickness (none snow cover: NS; thinner snow cover: TS; middle thick snow cover: MS; thicker snow cover:

CS; thickest snow cover: HS) were selected according to the distribution pattern of snow (He *et al*. 2013) in the forest on April 25, 2013 in late soil-thawing period. Under each snow pack three replicates soils were sampled from the litter layer (LL), the semi decomposed layer (FL) and the humus layer (HL), respectively, according to Papamichos (1990) and Yang *et al*. (2007).

The traditional plate culture method and the most probable number method were used to determine the numbers of specific microbial physiological groups (Dinamarca *et al*. 2007; Vicky *et al*. 2010; Xu *et al*. 2012). The determination results indicated in the way of colony forming unit per gram dry soil (CFU g^{-1}). The quantity of bacteria, ammonifying bacteria and aerobic cellulose decomposing bacteria were determined by plate counting method (Xu and Zheng 1986; Lu 1990; Zhao and He 2002; Xu *et al*. 2012). At the same time, the quantity of nitrifying bacteria, denitrifying bacteria and anaerobic cellulose decomposing bacteria were determined by most probable number (MPN) method (Xu and Zheng 1986; Lu 1990; Zhao and He 2002; Lin 2010; Xu *et al*. 2012). The experiment followed a completely randomized design, with three replications. Statistical analyses were performed using SPSS software package (Standard released version19.0 for Windows, SPSS Inc., IL. USA). The results were analyzed via one-way ANOVA (Analysis of variance), showing the significance of the difference of the effects of snow patches on cultivable bacteria and bacterial physiological groups. Changes with $P<0.05$ were regarded as statistically significant.

RESULTS

Snow thickness significantly influenced the number of cultivable bacteria in the litter layer and the semi decomposed litter layer in this forest ($P<0.01$) (Tab.1). The number of cultivable bacteria in the litter layer increased with the increase of snow thickness, but no significant difference among snow patches with different thickness (Fig.1). In contrast, the number of cultivable bacteria in the semi decomposed litter layer decreased with the increase of snow thickness. In this layer, the numbers of cultivable bacteria were relatively high both under the thinner snow cover and the thicker snow cover, having no significant difference, and the same as in the humified layer. In addition, the number of cultivable bacteria was higher in the humified layer compared with the other two soil layers under the same snow patches.

Snow thickness significantly influenced ammonifying bacteria regardless of soil layers in the forest ($P<0.01$), and significantly influenced denitrifying bacteria and nitrifying bacteria in the litter layer ($P<0.01$) (Tab.1). The higher number of ammonifying bacteria was noted both under the thicker snow cover and the thickest snow cover, while the number of nitrifying bacteria decreased from no snow cover to middle thick snow cover and increased from middle thick snow cover to thickest snow cover (Fig. 2). Meanwhile, the number of denitrifying bacteria in each layer decreased as snow thickness increased and this decreasing trend enhanced gradually from the humified layer to the litter layer.

Table 1. F values in statistics analysis of snow thickness to the numbers of bacteria and its physiological groups in different soil organic layers in an alpine forest

	The soil organic layer		
	LL	FL	HL
Bacteria number	14.92**	15.636**	2.95
Ammonifying bacteria number	10.71**	12.49**	27.99**
Nitro bacteria number	12.52**	1.16	2.41
Denitrifying bacteria number	18.47**	2.10	1.26
Aerobic cellulose decomposing bacteria number	0.37	0.43	1.37
Anaerobic cellulose decomposing bacteria number	1.71	6.14**	4.48*

* : $P<0.05$ **: $P<0.001$, n=3

Snow thickness did not significantly affect aerobic and anaerobic cellulose decomposing bacteria in the organic layer in alpine forest, but had significant influence on anaerobic cellulose decomposing bacteria in the semi decomposed litter layer and the humified layer (Tab.1). The number of aerobic cellulose decomposing bacteria had no significant change

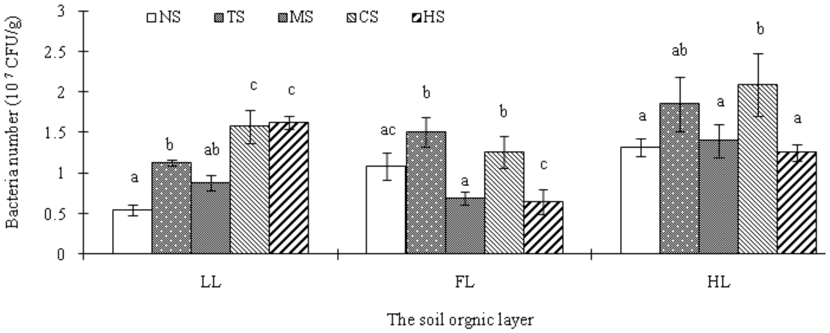

Fig. 1. The cultivable bacteria number in the soil organic layer covered by different snow thickness (mean±SD)

NS: none snow cover; TS: the thinner snow cover; MS: the middle thick snow cover; CS: the thicker snow cover; HS: the thickest snow cover; LL: the litter layer; FL: the semi decomposed layer; HL: the humified layer, Different letters meant significant difference within the snow of the same soil layer at 0.05 level. The same below

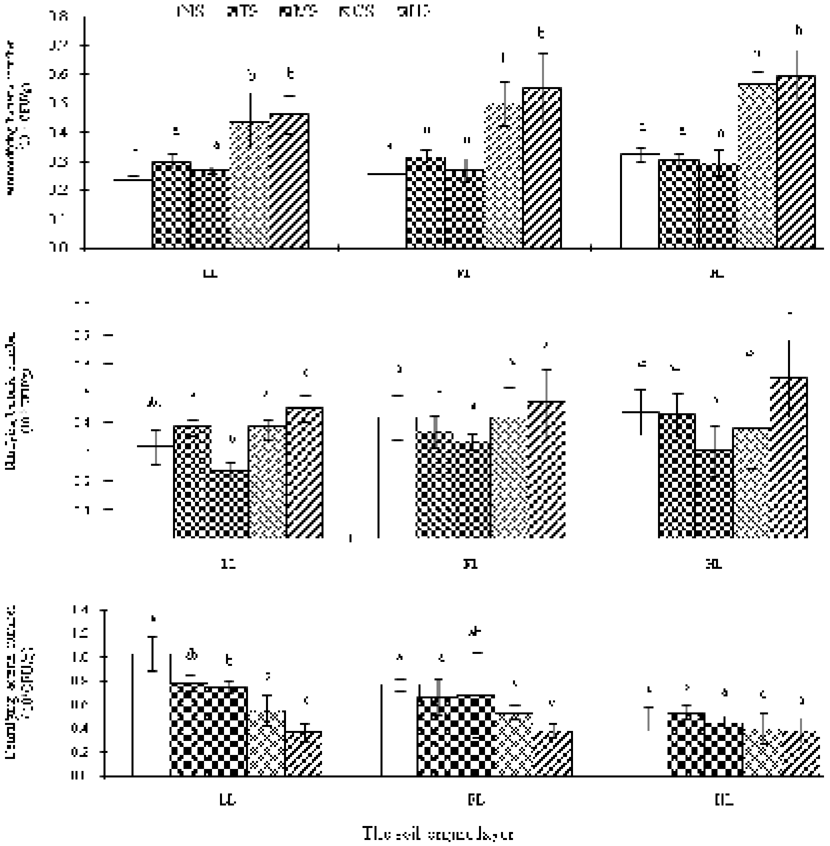

Fig. 2. The numbers of ammonifying bacteria, nitrifying bacteria and denitrifying bacteria in the organic layer covered by different snow thickness (mean±SD)

as snow increased in the 3 soil layers, but it had an increasing trend in the litter layer and a decreasing trend in the semi decomposed litter layer and the humified layer with the increase of snow thickness (Fig.3). On the other hand, anaerobic cellulose decomposing bacteria in the 3 layers increased with the increase of snow thickness, and this increasing trend was especially obvious.

The relative number of each functional bacterium was obvious difference in soil original layer under different snow thickness (Fig.4). Ammonifying bacteria was the most abundant of physiological group, which accounted for 62.70%~84.36% in the litter layer, 67.46%~86.11% in the semi decomposed litter layer and 77.19%~87.49% in the humified layer, respectively,

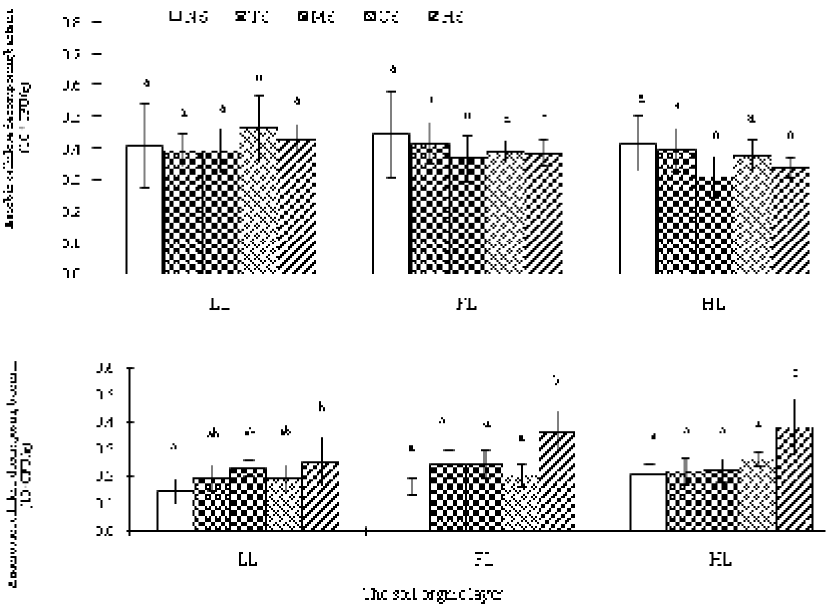

Fig. 3. The aerobic cellulose decomposing bacteria and anaerobic cellulose decomposing bacteria number in the organic layer covered by different snow thickness (mean±SD)

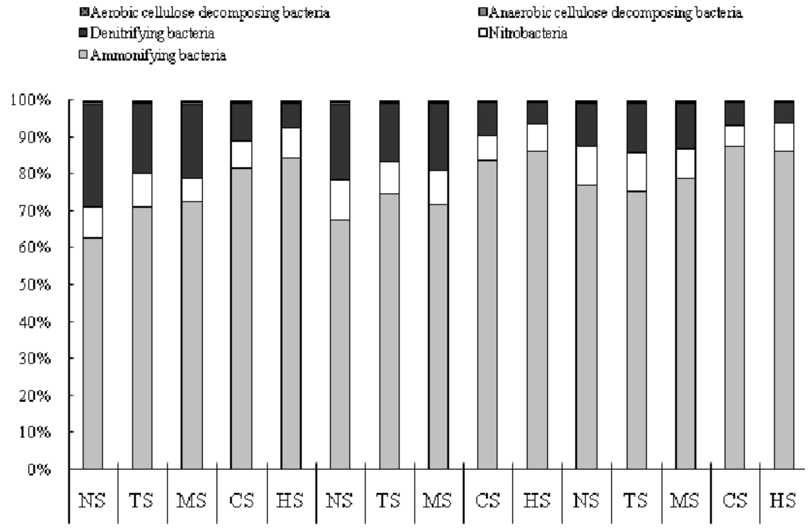

Fig. 4. The ratios among different soil bacteria number of physiological groups in the soil organic layer covered by different snow thickness

and its proportion increased with the increase of snow thickness in each layer. Denitrifying bacteria accounted for 27.69%~6.68% in the litter layer, 11.00%~7.26% in the semi decomposed litter layer and 10.29%~7.89% in the humified layer, respectively, and its proportion decreased as snow thickness increased in each layer. In addition, nitrifying bacteria and two kinds of cellulose decomposing bacteria (less than 1%) were few in number but stable in proportion.

DISCUSSION

The ongoing global warming is changing the seasonal snow patches and the associated soil temperature in alpine region and which would surely affect the soil microbial community and the soil processes (Jefferies *et al*. 2010). The similar results were got in our study, which indicated that snow thickness significantly influenced the numbers of cultivable bacteria and its physiological groups in the organic layer, although these groups showed various responses to snow thickness in different soil layers. Many previous studies have assumed that there were no further changes in the microbial community after soil completely frozen in the winter (Sulkava and Huhta *et al*. 2003; Edwards *et al*. 2007; Henry 2007). However, many recent results (Schmidt *et al*. 2009; Liu *et al*. 2010a; Wang *et al*. 2012) showed that there were a considerable amount of microbial groups, which showed certain physiological activity after soil completely frozen. The results here also showed many changes in the number of cultivable bacteria and its physiological groups with the changes of snow thickness at the later freezing-thawing season. In all microbes studied, cultivable bacteria, ammonifying bacteria, nitrifying bacteria and anaerobic cellulose decomposing bacteria exhibited the higher number under the thickest snow patches than under the other snow patches. The number of denitrifying bacteria showed a decrease tendency with the increase of snow thickness, but aerobic cellulose decomposing bacteria was less affected by snow patches. Moreover, the results also implied that nitrogen cycle was likely to be affected by pattern changes of snow patches relative to the carbon cycle associated with cellulose.

Bacteria are usually far more dominant than the other microorganisms in the soil, which can transform organic matter into CO_2, biomass, thermo-energy (heat) and humus-like end-product (Jefferies *et al*. 2010; Zhang *et al*. 2010). In agreement with many other studies that snow cover can maintain a stable microenvironment that protects decomposer communities and insulates soil organisms from extremely adverse environments (Massman *et al*. 1995; Cline 1995; Lipon *et al*. 2000; He *et al*. 2013). What's more, athermic effect of snow patches kept microorganisms a relatively high number, which was further proved as follows. On the one hand, the cultivable bacteria were influenced by snow patches more significantly in the semi decomposed layer and the litter layer than in the humus layer. On the other hand, the number of cultivable bacteria increased as snow thickness increased in the litter layer while fluctuated in the semi decomposed layer and the humified layer. This might be related to harm-avoiding of biology, soil environment and the physic-chemical properties of the two layers.

Seasonal snow patches had profound influence on soil temperature and moisture, which could make the physical structure of litter destroyed directly because soil freezing during snow cover period, and which could make the microbial substrate availability reduced because of freezing events (Groffman *et al*. 2001; Liu *et al*. 2010b). Accordingly, there were several obvious differences among the key physiological groups in soil organic layer. For one thing, the number and structure of groups related to carbon cycle was affected relatively small by the snow thickness, leading to the slow decomposition of animal and plant residues in soil and the weakening cycle of nutrient elements and energy flow. Then the carbon cycle process would be influenced deeply. For another, absolute predominance of physiological groups associated with nitrogen cycle showed that snow cover accelerated the mineralization of soil N and increased the NO_3-N content during seasonal freezing-thawing period in alpine forest and the cold season might be a critical period for N dynamics (Liu *et al*. 2012). Obviously, there was a positive correlation between the nitrogen mineralization rate and temperature changes caused by snow thickness (Wang *et al*. 2010). Further, seasonal snow patches had effects on these groups also by influencing the soil pH and redox potential (Edwards *et al*. 2007). It was because of acid soil in study region (Groffman *et al*. 1996; Teepe *et al*. 2001; Aanderud *et al*. 2008) that the numbers of the nitrifying and denitrifying bacteria were less than that of ammonifying bacteria. The oxygen partial pressure, one of the limiting factors to microbial growth, played a large restrictive role in denitrifying group (Groffman *et al*. 1996; Lu 1999), so that there was a sharp drop in the number of denitrifying bacteria in superficial layer as snow thickness increased. All the results illustrated that microorganisms were affected more significantly in the litter layer or the semi decomposed litter layer than in the relatively stable humus layer. Besides, the results did not show a linear relationship between microbial population and snow thickness (As mentioned previously, the number of nitrifying bacteria decreased from no snow cover to middle thick snow cover and increased from middle thick snow cover to thickest snow cover.), which was related to non-linear relationship between the snow thickness and the most factors influencing the number and distribution of microorganisms.

In conclusion, snow thickness significantly influenced the numbers and structure of bacteria and its physiological groups in the organic layer in alpine forest at latter freezing-thawing season and all groups had different responses to the changes of temperature and humidity caused by snow thickness. In general, the numbers of bacterial physiological groups at the later freezing-thawing season in the alpine forest showed the order as: ammonifying bacteria> denitrifying bacteria > nitrifying bacteria > aerobic cellulose decomposing bacteria > anaerobic cellulose decomposing bacteria. In a changing climate, the results of our study had important implications for microbial communities and ecology processes associated in alpine forest soils.

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