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Effects of Snowpack and Nutrient Addition on Soil Microbial Growth and Activity in the Alpine Belt of the Eastern Tibetan Plateau*

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Microbial activity is rapidly reduced by decreasing temperature, but does not completely cease in the cold season. The deep snow in late winter insulates the soil, further increasing soil temperature and allowing microbes to remain active. Microbes need both carbon (C) and nitrogen (N) for their growth and metabolism and the scarcity of either will limit their activity. Previous results in the lowland and even arctic soils have suggested that continuous extreme C-limitation occurs toward the end of winter. Other studies demonstrated that microbial growth may actually be N-limited. However, whether or not microbial activity on the eastern Tibetan Plateau is limited by energy or nutrients remains unclear. In the current study, the effect of snowpack and nutrient addition on microbial growth and activity in the alpine belt of the eastern Tibetan Plateau was explored, through a field factorial design of litter addition and snow regimes in situ and in laboratory incubation with C and N addition. The results illustrated that labile C was the limiting factor for soil microbial activity and growth in late winter and this situation was strongly demonstrated under a regime with a deeper snow cover.

Key words: Snowpack; Litter; Soil microbe; Nutrient limitation; Tibetan Plateau.

Soil microbes are among the most important components of the terrestrial ecosystem, playing a key role in material cycles and energy flow in soil (Liu 2010). Microbes have long been assumed to be effectively frozen into dormancy during the winter, especially in arctic or alpine zones (Schimel and Mikan 2005). However, a number of studies over the last decade have convincingly demonstrated that microbial activity does not completely cease when soils freeze (Rivkina *et al*. 2000, Robinson 2001). Continuous liquid water films are present among soil particles even when temperature drops to -10 °C and possibly even -40 °C (Romanovsky and Osterkamp 2000, Price and Sowers 2004). These films could be used by microbes for physiological activity (Rivkina *et al*. 2000, Mikan *et al*. 2002), especially in alpine areas, where soils are covered by continuous seasonal snowpacks.

Although microbial activity is rapidly reduced by decreasing temperature (Mikan *et al*. 2002, Schimel and Mikan 2005), microbial cumulative metabolism can reach high levels in the cold season (Buckeridge and Grogan 2008). This activity is likely to be strongly sensitive to microenvironment variation, such as snow timing and depth, because this variation is the primary

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regulator of cold season soil temperature (Olsson *et al*. 2003, Brooks and Williams 1999). Deep snow insulates the soil, thereby increasing soil temperature, and allowing microbes to remain active, as well as inducing a shift in the nature of organic matter processing (Schimel *et al*. 2004).

Soil microbes need both Carbon (C) and Nitrogen (N) for growth and metabolism, and a scarcity of either will limit their activity (Holub *et al*. 2005). Previous results in alpine and arctic soils have suggested that continuous extreme C-limitation occurs toward the end of winter (Lipson *et al*. 2000, Brooks *et al*., 2004, Schmidt and Lipson 2004). In addition, deep snow, which is predicted to increase with climate change, will make C-limitation on microbial growth and activity even more severe, and could significantly effect N mineralization and thus soil N pools in the winter (Buckeridge and Grogan 2008). Under C-limited conditions, microbes have sufficient N to produce decomposing enzymes, which further results in faster gross N cycling (Holub *et al*. 2005). Furthermore, Pokarzhevskii *et al*. (2003) suggested that proteins and scarce minerals were more likely to limit microbial growth. However, Schimel and Weintraub (2003) proposed that soil respiration may be C-limited, whereas microbial growth may actually be N-limited. Under N-limited conditions, microbes do not have enough N to produce decomposing enzymes and therefore decomposition and thus gross N cycling should be slower (Holub *et al*. 2005). To date, a large number of studies have been conducted in areas with low soil organic matter (SOM), thus casting doubt on the applicability of the aforementioned conclusion to areas with high SOM content, which are prevalent around alpine regions on the Tibetan Plateau (Wu and Onipchenko 2005).

In the current study, in situ and in laboratory incubation were used to explore the effect of seasonal snowpack and litter or nutrient addition on microbial growth and activity in the alpine belt of the Tibetan Plateau. The objective of the current study was to assess the changes in soluble N pools and microbial growth and activity under different depths of snow cover in situ, as well as to investigate the effect of litter or available nutrient additions on soil mineral N pools and microbe biomass in situ and in the laboratory. Specifically, the following hypotheses for an alpine ecosystem on the Tibetan Plateau were tested, including (1) Microbial growth and activity was limited by available C toward the end of winter; (2) Soil-soluble N pools were limited by labile C availability; and (3) Deep snow cover increased soluble N pools and microbial count and exacerbated microbial C-limitation.

MATERIALS AND METHODS

Study area

The current study area is situated on the shady slope of a mountain belonging to the Minshan Mountain Range on the eastern Tibetan Plateau (32° 59' N, 103° 40' E, 3500 m a.s.l). The annual mean temperature is 2.8 °C with an average value of -7.6 °C in January and 9.7 °C in July. The annual precipitation in this area is 718 mm, 72% of which falls during June to August. Generally consistent snowpack does not occur until late November or early December. The average depth of snow cover is 25 cm to 40 cm, but sometimes can reach 80 cm to 90 cm during cold winter. Snowpacks usually melt in April or May of the following year.

The soil (pH 5.54 ~ 5.94, SOM 41.53~ 60.00 g kg^{-1} dry soil, total N 3.86~ 4.94 g kg^{-1} dry soil) is silty loam inceptisol, and plant roots are generally confined to the A-horizon (2 cm to 20 cm). The vegetation is dominated by mountain shrubs (*Sibiraea angustata Rhododendron zheguense* and *Salix zhegushanica*) and spruce trees (*Picea purpurea*) are distributed sparsely. A mumber of sedges and forbs (*Carex atrofusca* subsp. m*inor*, *Pedicularis longiflora* var. *tubiformis*, *Leontopodium longifolium,* and *Pyrethrum tatsienense*) are common on the ground.

Experiment 1: Effect of snowpack and litter addition on soil soluble N pools and microbial growth and activity in situ

Three 18 m×5 m sample areas were chosen at 5 m intervals. Each sampling area was divided into three equal 6 m×5 m plots, cleared of existing plants and litter, and marked as plots A, B, and C. Considering the difficulty in collecting soil samples from frozen soil, an in situ soil incubation method (DiStefano and Gholz 1986) was used during the deep winter. A total of 45 soil cores per plot (45 cores/plot×3 plot/sample area×3 sample areas = 405 cores) were installed by inserting 7.5 cm

diameter PVC tubes 20 cm into the ground on November 30, 2011.

Three litter input treatments were used in plots A to C, including one litter exclusion (NL) treatment (in which all ground litter was removed and the aboveground litter input was manually excluded), one light-litter (LL) addition treatment (in which 5 g of litter in a 0.147 mm aperture litter bag was placed on top of the soil core outside the tubes) and one medium-litter (ML) addition treatment (in which the same kind of bag contained 20 g litter, that reflects the natural litter input, was used). Leaf litter comprising Sibiraea angustata (deciduous shrubs), which is the dominant species in the current study area, was collected and dried at 40 °C on November 20, 2011.

Three different snow regimes were then established at different plots and manually controlled every day during winter. Plot A was kept without a snowpack (NS) by shoveling without disturbing the ground, plot B had 30 cm snowpacks (middle snow-depth, MS), and plot C had 100 cm snowpacks (deep snow-depth snowpacks, DS). Thus, the experiment involved nine treatments, with three snow regimes and three litter additions (in triplicate).

Soil temperatures were recorded 12 times a day in all the plots using button temperature data loggers (Onset Computer Corporation, Pocasset, MA), which were inserted 5 cm below the soil surface from November 30, 2011 to May 1, 2012.

Each soil sample was randomly taken from three PVC tubes of the same treatment and was then thoroughly homogenized (triplicate). A total of 81 PVC tubes (3 sample areas × 9 treatments/ sample area × 3 PVC tubes/treatment = 81 PVC tubes) were brought back to the laboratory on the first day of each month from January to May 2012. The roots and stones in the soil samples were carefully removed, and the NH_4^+-N, NO_3^--N, MBC, MBN, fungal and bacterial counts were measured.

Experiment 2: Effect of C×N additions on soil biochemical properties and microbial counts

The soil samples near the Experiment 1 soils (the in situ study) were processed. Visible plant and live roots were removed. A total of seven subsamples (containing 250 g dry soil per subsample) were prepared in sample cups with polyethylene film (three replicates), and the water capacity was adjusted to about 70%. Nutrients were then added to each subsample, including a control check (CK, no addition), four C additions treatments (0.25 g Glu, 0.5 g Glu, 1 g Glu, and 2 g Glu /100g dry soil), and two N additions treatments (0.047 g $(NH_4)_2SO_4$ and 0.189 g $(NH_4)_2SO_4$/100g dry soil). These C and N additions were approximately equal to 1 mg C, 2 mg C, 4 mg C, 8 mg C, 0.1 mg N, and 0.4 mg N of each gram of dry soil, respectively.

All subsamples (7 factorial additions treatment ×3 replications= 21) were preprocessed in the low temperature incubator (LT-36VL, PERCIVAL, USA) using the method by Sulkava and Huhta (2003). The samples were then kept at -2 °C for 6 weeks (simulating soil temperature under normal snowpack). Subsequently, the temperature was increased gradually (+2 °C for 2 d, then +5 °C for 3 d, and finally +15 °C for 1 week). The incubation temperature was kept sufficiently low to allow psychrophilic microbes to be active, but not extremely low as to constrain microbial activity during the incubation period. After incubation, soil NH_4-N, NO_3-N, microbial biomass carbon (MBC) and nitrogen (MBN), fungal, and bacterial counts were measured immediately.

Chemical determination

All chemical soil analyses were conducted on the ground of soil fraction finer than 2 mm. MBC and MBN contents were determined using the chloroform-fumigation direct-extraction (CFE) technique (Brookes *et al*. 1985). NH_4^+-N and NO_3^--N were analyzed using the method of indophenol blue colorimetric and ultraviolet spectrophotometry respectively (Mulvaney 1996) The total dissolved N (TDN) content was determined using fresh soil by the multi N/C 2100/ 2100S instrument (Analytic Jena Co. Ltd., Germany). Dissolved organic N (DON) was calculated by subtracting soluble inorganic N (NH_4^+-N and NO_3^--N) from TDN (Jones and Willett 2006). The numbers of bacteria and fungi were determined using the serial dilution plate count method (Microbiological Department, Institute of Soil Science, Chinese Academy of Sciences 1985). Soil CO_2 production was measured in situ by attaching each sample cup to an infrared gas analyzer system (LI-6400, LI-COR, USA) within a modified chamber in a closed circulation loop ((Buckeridge and Grogan 2008).

Statistical analysis

Experiment 1 (the in situ study) used a completely randomized design with three replications. Results were analyzed by one-way ANOVA to verify the significance of difference of litter addition (0 g, 5 g, and 20g litter addition) and snow regime (0 cm, 30 cm, and 100cm snow depth) treatments to the state of soil nutrients (NH_4^+-N, NO_3-N, MBC, and MBN) and soil microbial counts (bacteria and fungi) in winter (n=3).

Experiment 2 (the incubation study in laboratory) also used a completely randomized design with three replications. One-way ANOVA was used to show the significance of the differences in Glucose (C source) and $(NH_4)_2SO_4$ (N source) addition to the soil nutrients and soil microbe counts (n=3). Results with $P < 0.05$ were regarded as statistically significant.

RESULTS

Effect of deepened snow and litter addition on soil biochemistry and microbial count in situ in late winter

The soil mean temperature in NS fluctuated significantly, with the minimum temperature at -10.9 °C occurring in January and the maximum value at 5.7 °C occurring in May. The soil mean temperature was relatively stable in MS and DS, between -2 °C to 0 °C for the duration of winter (Fig. 1). No freeze-thaw cycle occurred because of the insulation of the snow cover in MS and DS, but the soil in NS underwent approximately 50 freeze-thaw cycles from late February to early May (data not shown).

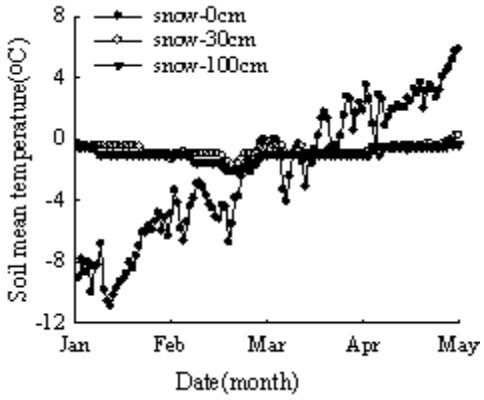

Fig. 1. Daily soil mean temperatures at the depth of 5cm below soil surface under different snow regimes from December 2011 to May 2012

Litter addition and snow-cover significantly affected the NH_4^+-N and NO_3^--N contents of soil during winter (Table 2). NH_4^+-N in NS was significantly higher than that in snow-covered plots, although the content of NH_4^+-N was slightly decreased in NS during the winter (Fig. 2). NO_3^--N gradually increased before March and then decreased, especially in ML in the modest snow-depth regime (Fig. 3). Both NH_4^+-N and NO_3^--N concentrations in the soil solution were affected by the litter addition. The addition of 20 g litter (ML) significantly increased the NO_3^--N content in soil, but reduced the NH_4^+-N content (Table. 2).

The number of bacteria in soil decreased gradually from January to May of 2012, whereas the fungi count increased (Table. 1). The number of fungi and bacteria in the snow-covered area was bigger than that in NS. Although differences in the soil microbial community may occur, no difference was observed in MBC and MBN between the different snow regimes ($P = 0.176, 0.125 > 0.05$). Medium litter addition increased the number of bacteria and fungi, especially in snow covered soils, indicating that the microbial nutrient limit was partly eliminated by an adequate energy

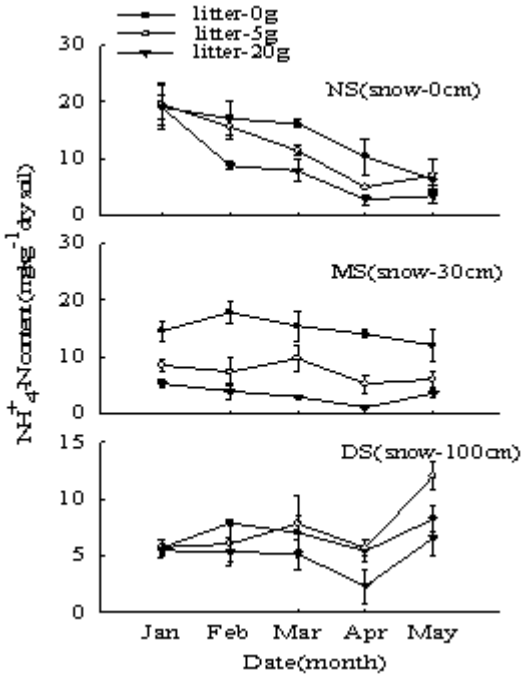

Fig. 2 Dynamics of NH_4^+-N content with different litter additions in three snow regimes from January to May 2012

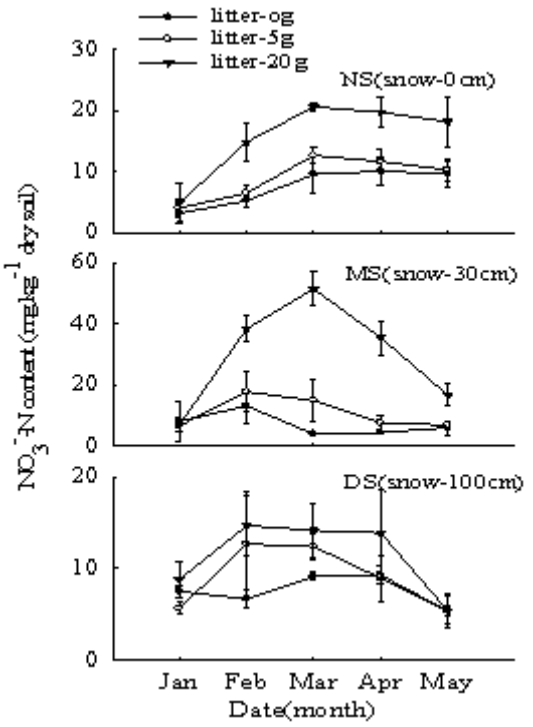

Fig. 3. Dynamics of NO_3^--N content with different litter additions in three snow regimes from January to May 2012

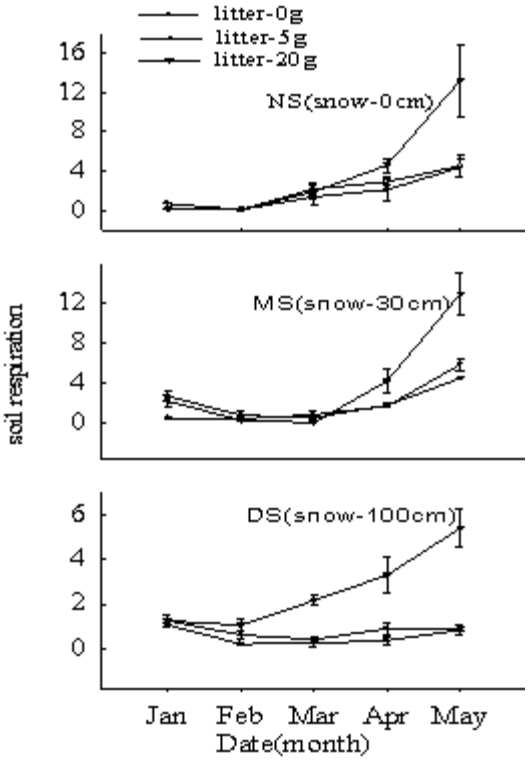

Fig. 4. Dynamics of soil respiration with different litter additions in three snow regimes from January to May 2012

supply. However, a significant decrease in MBN content and a stable MBC (P = 0.111 - 0.05) concentration resulted (Table. 2). Soil respiration gradually increased from January to April, and abruptly increased in May because of snow melt (Fig. 4). Deep snow and litter addition resulted in significant increases in soil respiration (Table 2). Specially, compared with the snow-free plot, CO_2 respirations in ML in the snow-covered plots increased by 30 – 40 %.

Table 1. Summary of effects of deepened snow and litter addition on *in situ* soil biochemical properties and microbial counts in late winter

Variable	Sample time	Treatment								
		NS (0cm snow depth)			MS (30cm snow depth)			DS (100cm snow depth)		
		NL	LL	ML	NL	LL	ML	NL	LL	ML
Bacteria	2012-01	5.0×10^7	7.0×10^7	9.0×10^7	9.0×10^7	8.0×10^7	8.0×10^7	5.0×10^7	4.0×10^7	6.0×10^7
	2012-02	5.0×10^7	2.0×10^8	6.0×10^8	6.0×10^7	7.0×10^7	9.0×10^7	4.0×10^7	8.0×10^7	4.0×10^7
	2012-03	2.0×10^7	1.0×10^8	1.0×10^8	3.0×10^7	4.0×10^7	2.0×10^7	1.0×10^7	1.0×10^7	3.0×10^7
	2012-04	3.0×10^7	3.0×10^7	4.0×10^7	5.0×10^7	6.0×10^7	3.0×10^7	3.0×10^7	4.0×10^7	5.0×10^7
	2012-05	2.0×10^7	3.0×10^7	3.0×10^7	4.0×10^7	2.0×10^7	2.0×10^7	1.0×10^7	4.0×10^7	4.0×10^7
Fungi	2012-01	4.9×10^4	5.3×10^4	2.5×10^4	5.1×10^4	2.9×10^4	3.1×10^4	2.3×10^4	3.5×10^4	3.8×10^4
	2012-02	1.2×10^5	4.5×10^4	4.5×10^4	6.7×10^4	4.0×10^4	4.7×10^4	3.3×10^4	7.8×10^4	4.4×10^4
	2012-03	1.1×10^5	1.3×10^5	8.1×10^4	8.1×10^4	6.9×10^4	9.8×10^4	9.8×10^4	9.2×10^4	9.9×10^4
	2012-04	5.1×10^4	7.8×10^4	6.3×10^4	8.7×10^4	9.1×10^4	1.0×10^5	6.0×10^4	5.7×10^4	7.7×10^4
	2012-05	1.7×10^5	1.9×10^5	1.7×10^5	9.1×10^4	1.1×10^5	1.1×10^5	1.1×10^5	1.3×10^5	1.6×10^5

Table 2. Summary of statistically significant effects of snow cover and litter inputs on soil biochemical properties in situ

Category	variable	Factor	P	Response
Soil solution	NH_4-N	Snow	0.000	Decrease
		Litter	0.000	Decrease
	NO_3-N	Litter	0.000	increase
Microbial pool	MBN	Litter	0.000	decrease
Microbial counts	Bacteria	Snow	0.000	increase
		Litter	0.000	increase
	Fungi	Snow	0.000	increase
		Litter	0.003	increase
Microbial activity	CO_2	Snow	0.000	increase
		Litter	0.000	increase

Table 3. Summary of effects of factorial combinations of C and N additions on soil biochemical properties under laboratory incubation

Treatment		Variable				
		NH_4^+-N	NO_3^--N	DON	MBC	MBN
CK		22.7	45.3	93.9	1241.7	101.3
C addition(Glucose)	1mg	16.0	51.7	73.0	1483.3	130.3
	2mg	15.9	48.4	17.8	1391.8	136.9
	4mg	14.5	20.7	2.3	1798.9	217.9
	8mg	14.7	12.3	11.5	1963.5	240.8
N addition(NH_4SO_4)	0.1mg	36.5	58.8	101.2	1422.3	150.9
	0.4mg	160.9	40.9	105.9	1447.3	465.8
C×N additions	4mgC+0.1mgN	34.2	48.7	64.2	1768.2	209.9
	4mgC+0.4mgN	140.8	55.6	74.1	1751.7	692.1
	8mgC+0.1mgN	25.1	17.0	13.2	1963.9	271.1
	8mgC+0.4mgN	109.3	43.8	20.1	1864.0	518.9

DON, MBC and MBN mean values are in mg kg^{-1} dry soil. The addition of Glucose and NH_4SO_4 are in g^{-1} dry mass of soil.

Table 4. Summary of statistically significant effects of factorial addition of C and N on soil biochemical properties under laboratory incubation

Category	variable	Factor	P	Response
Soil solution	NH_4-N	C	0.000	decrease
		N	0.025	increase
	NO_3-N	C	0.000	decrease
Microbial pool	MBC	C	0.000	increase
	MBN	C	0.048	increase
		N	0.001	increase
Microbial counts	Bacteria	C	0.000	increase
		N	0.000	increase
	Fungi	C	0.000	increase

Physiological responses of soil biochemistry and microbial count to C×N additions in the laboratory incubation

Both NH_4^+-N and NO_3-N concentrations significantly decreased by C addition. Along with the increase of added C, NH_4-N concentration reduced by 30 – 40 % (Table 3). A prompt decrease in NO_3-N was found after the large C addition, which decreased by 73% when 2 % Glu was added into soil. NH_4^+-N and NO_3-N responded differently when N was added. NO_3-N content was not significantly affected (P = 0.075 > 0.05) and NH_4^+-N significantly increased by the addition of N (Table 4).

Soil bacteria count increased when both C and N were added (Table 4). Bacteria count increased by 5.4 % - 68.64 % when C was added, whereas the addition of N elicited a 1.55 to 2.03 – fold increase (Table 3). Soil fungi count increased only with the addition of available C (Table 4), which was up to 9.4 times as CK when 2 % Glu was added (Table 3). N addition did not significantly affect soil fungi count (P = 0.275 ÿ 0.05). MBC responded in a manner similar to that of fungi count, increasing with C addition, but showing no significant change with N addition (P = 0.125 ÿ 0.05). Both MBN in soils increased significantly with the addition of C and N (Table 4).

DISCUSSION

Effect of snow cover and litter addition on soil biochemistry and microbial counts in situ in late winter T

The NH_4^+-N content in NS was higher than that in MS and DS, contrary to the finding by Larsen *et al*. (2002) in the arctic ecosystem. This result can be attributed to the litter decomposition dynamics, that is, the microbial immobilization of soil N in NS was slower than that in snow-covered areas (data is not shown). Contrary to expectations, the newly available C from the litter input induced the heterotrophic microbes to absorb mineral N, further decreasing the NH_4^+ pools, so soil ammonification in the current study was reduced with the addition of organic matter, which supports previous studies in the temperate forests of Northern America and Central Europe (Holub *et al*. 2005).

The accumulation of soil NO_3^--N is generally related to either severe freeze-thaw cycles (Elliott and Henry 2009) or long-term deep freezing (Austnes and Vestgarden 2008). An increasing amount of snowmelt water is flushed into the soil system with the rise in air temperature and the gradual thawing of snow from March to the end of May, resulting in more N losses (Schimel and Mikan 2005), especially in snow cover plots. Similar to the result of Fisk and Fahey (2001) in the White Mountain National Forest, litter input could stimulate the decomposition of an organic substance (Nadelhoffer *et al*. 2004), providing an ideal condition for microbial growth and benefitting net nitrification. Thus, a significant increase in the NO_3^--N content in ML was found in the current study. The data indicated that both warmer soil under deeper snow and medium litter addition result in an increase in the amount of bacteria and fungi as expected. However, MBC and MBN concentration did not show the same trend, that is, no change in different snow regimes, a stable MBC concentration, and a decrease of MBN concentration under medium litter input. The greater mismatches between microbial biomass and microbial count in the current treatments might have resulted from a flaw of dilution plate count and chloroform-fumigation methods (Bloem *et al*. 1994, Frey *et al*. 1999). And it suggested that deepened snow and litter inputs over winter may have resulted in a change in the soil microbial community by altering the physiological response of fungi to available nutrients (Buckeridge and Grogan 2008).

Soil respiration responded more strongly with deep snow cover under litter addition. This result supported prior findings in the arctic birch hummock tundra. For example, Buckeridge and Grogan (2008) reported that soil respiration in the late winter responded positively to increased C availability and tended to be higher from the deepened snow soils. Moreover, soil microorganisms appeared to be C-limited for activity. Respiration from late winter soils responded positively to the increased C availability and tended to be higher in the snow covered soils, a result consistent with our hypothesis, that is, snow cover exacerbates the C-limitation of microbial activity in late winter.

Physiological responses of soil biochemistry and microbial count to C×N additions in laboratory incubation

A previous study suggested that soil

food webs and microbial activity are energy limited (Richards 1987) and that macronutrient and micronutrient fluxes in soils were associated with the availability of C (Buckiedge and Grogan 2008). However, Pokarzhevskii *et al*. (2003) presented that proteins and scarce minerals were more likely to limit microbial and macrofaunal growth. In the present work, the generous additions of C and N in the laboratory incubation raised soil MBC, MBN, bacteria, and fungi count, indicating that several potential limitations to microbial growth or activity had been removed.

Despite an increase in the bacterial count with the factorial addition of C or N, no change was observed in fungi count with N addition alone except when C was added. This finding indicated that both available C and N could stimulate bacterial reproduction. However, only the N nutrient was insufficient for fungi growth, which requires large energy material (C source). Fungal rather than bacterial metabolism may be more likely to become C-limited in late winter, as a result of the threefold larger C investment per unit volume in the fungal cell structure (Buckeridge and Grogan 2008). Furthermore, C addition also stimulated the increases in MBC and MBN, indicating that microbial growth was restricted by available C.

In addition, previous study demonstrated that if microbes were C limited, soil mineral N pools should be large and when available C increase (e.g. following addition of labile C or high C: N material) then mineral N pools should decrease as microbes immobilize N (Holub *et al*. 2005). Similar to the result of Experiment 1 in the current study, more available C addition resulted in a decrease in the soil mineral N pool in the laboratory incubation study, further suggesting that microbial activity relevant to the soil N cycle was limited by available C. Late winter C-limitation of the microbial growth was consistent with the alpine tundra ecosystem (Brooks *et al*. 2004) and arctic tundra research (Buckeridge and Grogan 2008), generating the hypothesis that depleted available organic pools in late winter facilitate the mortality of soil microbes (Lipson *et al*. 2000), resulting in a release of cytoplasmic soluble nutrients into the soil solution at the time of spring thaw (Buckeridge and Grogan 2008).

CONCLUSIONS

The current study demonstrated the potential for soil microbial activity and growth in the alpine belt of the Tibetan Plateau with low levels of labile C in late winter. Under deeper snow-cover, the microbial count will increase and the C-limitation of microbial activity will be even more severe, which may exacerbate microbial cytoplasmic release during the thaw period, and may represent a larger potential pulse of N for alpine plants in the spring, and may therefore be instrumental for plant community shifts under future climate change predictions because of the possible shortening of snow-cover accumulation and changes in snowpack depth.

In addition to the microbial biomass response to C addition, soil mineral N pools were found to be strongly limited by C availability in late winter in situ and in the laboratory. The soil nitrogen dynamic may have been primarily controlled by available C in alpine soil with high organic matter content in the eastern Tibetan Plateau. Although clear response patterns of soil nitrogen cycles to available C were not shown, functional microbial community and activity (e.g. ammonifiers, nitrobacterium, and denitrificans *et al*.) were found to be the most important components of N transformation in alpine soils. Therefore, future studies on alpine functional microbes in related to energy or nutrient addition are needed to facilitate better understanding of the specific effect of microbial nutrient limitation on N dynamics.

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