Effect of exogenous carbon addition and the freeze-thaw cycle on soil microbes and mineral nitrogen pools1

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Abstract. To elucidate the alpine soil process in winter, the response mechanism of soil mineral nitrogen and soil microbes to exogenous carbon (0 mg C, 1 mg C, 2 mg C, 4 mg C and 8 mg C·g^{-1} dry soil) and the freeze-thaw cycle (-2 ℃, -2 ~ 2 ℃, -20 ~2℃) were studied by laboratory simulation. The freeze-thaw treatment had no significant effect on microbial biomass nitrogen and the number of bacteria. The soil mineral N pool, the number of fungi, and enzyme activities were obviously affected by the freeze-thaw cycle. A mild freeze-thaw cycle (-2~2℃) significantly increased the number of fungi and catalase activity, while severe freeze-thaw cycle (-20~2℃) obviously decreased invertase activity. The results suggested that both the freeze-thaw rate and freeze-thaw temperature amplitudes have a strong effect on soil microbial dynamics in the alpine zone in winter. The results showed that exogenous carbon addition significantly decreased soil NO_3-N and NH_4^+-N contents, increased soil microbial biomass, the number of microbes, and soil enzyme activities. The results showed that microbial growth in the eastern Tibetan Plateau was somewhat limited by available C. It may represent a larger potential pulse of soil nutrient for alpine plants in the next spring, and may be instrumental for plant community shifts under future climate change predictions due to the possible increased litter addition.

1. Introduction

Microbes perform essential role in soil and act as marker of soil health and quality (Abbas et al., 2015). Microbes have long been assumed to be frozen into a hibernation state during the winter, especially in arctic or alpine zones (Schimel and Mikan, 2005). However, many studies over the last decade have convincingly showed that microbial activity decreased with decreasing soil temperature (Mikan et al., 2002) but does not cease completely even if the soil freezes (Rivkina et al. 2000, Robinson 2001). Larsen et al. (2002) found that the number of live bacteria under freeze-thaw cycles was one of the tens of thousands of constant temperatures culture, and the species diversity was significantly reduced. These results were attributed to the increase in carbon consumption throughout the winter, and the resulting carbon supply deficit led to a decrease in the number of microbes and microbial activity (Schmidt & Lipson, 2004), thereby affecting the process of soil nitrogen mineralization and mineral nitrogen pools (Schimel and Mikan, 2005).

"Carbon deficit theory" in winter in the alpine areas has gradually been recognized, and confirmed by previous studies in the arctic tundra and high latitude regions (Lipson et al., 2000; Schmidt & Lipson, 2004). However, the eastern Tibetan Plateau is at low latitude and high elevation, with high organic matter contents (69.7～112.4 $g.kg^{-1}$) (Wu and Onipchenko, 2005) and more soil freeze-thaw cycle. Therefore, the existence of carbon deficit in the eastern Tibetan Plateau region needs to be determined.

The objective of this research was to characterize the response mechanism of soil mineral nitrogen pools and microbes to exogenous carbon and freeze-thaw cycle in the eastern Tibetan Plateau by laboratory simulation. This work aimed to elucidate soil microbes and nitrogen dynamics, providing the basis for further study on soil nutrient transformation and plant nutrient supply.

2. Material and Methods

In the spring of 2013, soil samples were collected from Minshan Mountain Range on the eastern Tibetan Plateau（33°59′ N, 102°40′ E, 3520 m a.s.l). The soil pH was 5.42~5.83, the organic matter content was 69.7~112.4 $g\cdot kg^{-1}$, and the total N content was 3.51~5.12 $g\cdot kg^{-1}$ dry soil.

The freshly soil samples were thoroughly blended, visible roots and stones were removed. The water capacity of the soil was adjusted to about 70%. A total of 45 sample cups with polyethylene film (containing 250 g dry soil) were placed at 25℃ for a week. After a week, 0 g, 0.25 g, 0.5 g, 1 g, and 2 g glucose were separately added into sample cups (Buckeridge and Grogan 2008). These C additions were approximately equal to 0 mg C, 1 mg C, 2 mg C, 4 mg C, and 8 mg $C\cdot g^{-1}$ dry soil.

All C addition treatment soil samples were randomly divided into three temperature treatment subgroups (A, B, and C subgroups). First, the soil sample was pretreated: 15℃ for three weeks → 5℃ for a week → 2℃ for two weeks → -2℃ for a week. Then the temperature gradient simulation test was performed (Sulkava and Huhta, 2003). Subgroup A, CK, -2 ℃ for six weeks; Subgroup B, freeze-thaw group, -2～2 ℃ for six weeks; Subgroup C, severe freeze group, -20℃～2℃ for six weeks. After treatment, the three subgroups were cultured at 2℃ for two days, 5℃ for three days, and 15℃ for a week. Soil NH_4^+-N content, NO_3-N content, microbial biomass C and N contents, the numbers of bacteria and fungi, cellulose activity, invertase activity and catalase activity were determined after soil culture.

The NH_4^+-N and NO_3-N contents were determined by indophenol blue colorimetric assay and ultraviolet spectrophotometry, respectively (Mulvaney, 1996). Microbial biomass C and N were determined by the fumigation-extraction method (Brookes et al., 1985). The number of bacteria and fungi were determined by the serial dilution plate count method (Hu et al., 2014). Soil enzyme activities were assayed as described by Guan (1986).

The results were analyzed by two-way ANOVA, showing the significance of the difference of freeze-thaw cycle (-2 ℃, -2 ~ 2 ℃, -20 ~ 2℃) and exogenous C addition (0 mg C, 1 mg C, 2 mg C, 4 mg C and 8 mg $C\cdot g^{-1}$ dry soil) to the soil mineral N, the number of soil microbes and soil enzyme activities. Effects with $P < 0.05$ were regarded as statistically significant. After two way ANOVA revealed statically significant interaction between freeze-thaw cycle and exogenous C addition, the effect of the exogenous C addition was analyzed using Student's t-test for independent samples for each freeze-thaw cycle.

3. Results and Analysis

3.1. Effects of simulation freeze-thaw and C addition on soil mineral N pools

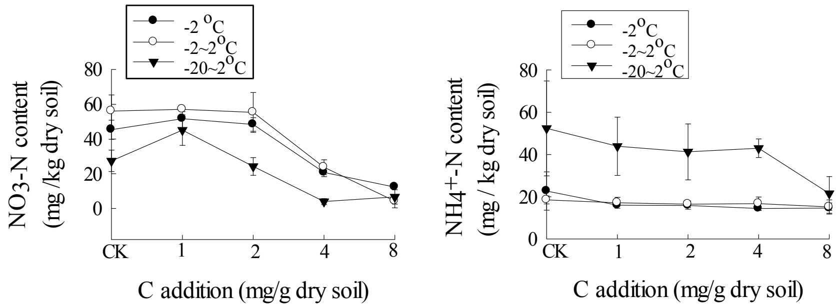

Fig. 1 Effects of simulation freeze-thaw and C addition on soil mineral N pools

C addition significantly decreased soil NH_4^+-N and NO_3-N contents. In particular, the NH_4^+-N content of soil rapidly decreased by 30~40 % upon addition of 4 mg or 8 mg C/g·dry soil. A rapid decrease in the NO_3-N content of soil was observed upon addition of 8 mg C/g·dry soil (Fig. 1). AVOVA analysis results show that the severe freeze-thaw cycle (-20 ~ 2℃) decreased the NO_3-N content and increased the NH_4^+-N content (Tab. 1). Compared with the steady freezing (-2 ℃), the severe freeze-thaw cycle reduced the NO_3-N content by 39~81%, and increased the NH_4^+-N content by 47~197%.

3.2. Effects of simulation freeze-thaw and C addition on soil microbial biomass and the number of microbes

A significant effect ($P<0.01$) of C addition on both microbial biomass and the number of microbes was observed. Both MBC and MBN showed a gradual increase with the increase of C addition (Tab.1). Compared to the control, the contents of MBC and MBN increased respectively by 40% and 94% upon addition of 8 mg C/ g dry soil (Fig. 2).

The variation trend in the number of microbes was somewhat different from the trend in microbial biomass, with a small effect for a small amount of C input (1 or 2 mg C/g dry soil) and a significant increase for a large amount of C input (4 or 8 mg C/g dry soil). When 8 mg C/g dry soil was input, the number of bacteria and fungi respectively increased to 1.19~4.27 times and 7.23~22.27 times the number present in the control.

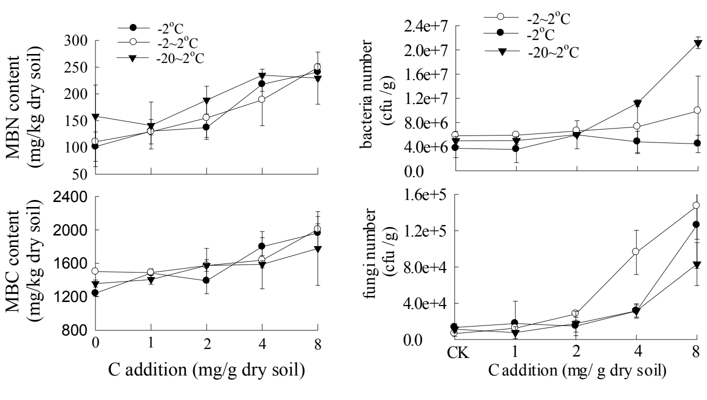

Fig. 2 Effects of freeze-thaw simulation and C addition on soil microbial biomass and the number of microbe

There was no significant effect of the freeze-thaw cycle on MBN contents and the number of bacteria, but there was a significant effect on MBC and the number of fungi. According to the statistical analysis (Tab.1), the mild freeze-thaw treatment (-2～2℃) was conducive to MBC contents and the number of fungi, suggesting that the fungi were more adapted to the harsh environment in winter than bacteria.

The interaction effect of C addition and freeze-thaw cycle on the number of bacteria was significant. When the freeze-thaw cycle was tested separately for exogenous C addition effects, the value of the number of bacteria were significantly higher in the 8 mg C/g dry soil than the other subjected to a -20~2℃ freeze-thaw cycle.

3.3. Effects of simulation freeze-thaw and C addition on soil enzyme activity

The activities of cellulose and invertase showed a nearly consistent change trend under the three freeze-thaw treatments, increasing gradually with C input and increasing suddenly at higher addition amounts (Fig.3). Statistical analysis showed that C addition significantly increased the cellulose activity. Freeze-thaw events had different effects on the activity of the three enzymes. The activities of cellulose and catalase were increased significantly under -2~2 ℃ freeze-thaw treatment, while invertase activity was reduced significantly under -20~-2℃ freeze-thaw treatment. At the same time, the combination of freeze-thaw and C input on invertase activity was also significant. Under -2℃ freeze-thaw treatment, the invertase activity with 8 g C addition was significantly greater than that for other amounts of C addition (Tab.1).

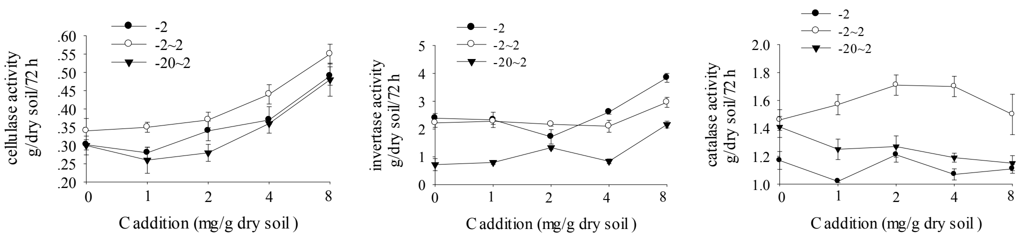

Fig. 3 Effects of simulated freeze-thaw and C addition on soil enzyme activity

Tab. 1 The statistical analysis results of simulation freeze-thaw and C addition on soil mineral N pools, microbial numbers and enzyme activity

Category	Variable	Factor	P	Response
N pool	NH_4^+-N	C	0.000	decrease
		Freeze-thaw	0.000	increase
		C×Freeze-thaw	0.575	ns
	NO_3-N	C	0.000	decrease
		Freeze-thaw	0.001	decrease
		C×Freeze-thaw	0.623	ns
Microbial pool	MBC	C	0.000	increase
		Freeze-thaw	0.285	ns
		C×Freeze-thaw	0.394	ns
	MBN	C	0.000	increase
		Freeze-thaw	0.084	ns
		C×Freeze-thaw	0.540	ns
Microbial count	Bacteria	C	0.000	increase
		Freeze-thaw	0.205	ns
		C×Freeze-thaw	0.006	
	Fungi	C	0.000	increase
Enzyme activity		Freeze-thaw	0.023	-2~2 increase
		C×Freeze-thaw	0.057	ns
		C	1.102	ns
	Catalase enzyme	Freeze-thaw	0.020	-2~2 increase
		C×Freeze-thaw	0.640	ns
	Cellulose enzyme	C	0.000	increase
		Freeze-thaw	0.049	-2~2 increase
	Invertase enzyme	C×Freeze-thaw	0.760	ns
		C	0.046	increase
		Freeze-thaw	0.001	-20~-2 decrease
		C×Freeze-thaw	0.055	ns

4. Discussions

A temporary change in soil carbon input may regulate the number of soil microbes, microbial activity, and the extracellular enzymes that are released into the soil by the action of microbes (Bossio et al., 1998). Several studies have observed changes in the number and activity of microorganisms in the soil associated with changes in C input (Martyniuk and Wagner, 1978; Powlson et al., 1987; Fraser et al., 1988; Knudsen et al., 1999). Consistent with the results of this study, a previous study showed that high-OM soil had consistently higher microbial numbers and microbial biomass levels than low-OM soil in arable soils in Denmark (Kasia et al., 1999). Some studies suggested that the activities of cellulases and invertase were closely related to the input of fresh organic materials, but others seemed to be more sensitive to soil temperature and moisture (Bolton et al., 1985; Martens et al., 1992; Dick, 1994). Our study also approved that the nimeral nitrogen and microbe were closed related to the available C levels. A previous study showed that the growth of microorganisms in the soil was limited by the available C in the alpine and Arctic tundra ecosystems (Brooks *et al.*, 2005; Buckeridge and Grogan, 2008). In this study, exogenous C addition significantly increased microbial numbers and activities, suggesting removal of a potential limiting effect of microbial growth. In winter, fungi are more likely to be restricted by available C because the C requirement of fungi is three times higher than bacteria (Buckeridge and Grogan, 2008). This result was also confirmed by our results, as exogenous C caused an extreme increase in the number of fungi (up to 7.23-22.27 times the number present in the control), indicating that the addition of exogenous C eliminated the effect of fungi nutrient limitation. Seasonal freezing and thawing of soil is an important climatic characteristic in the alpine areas. The freeze-thaw action can change the physical, chemical, and biological properties of soil, including soil porosity, the soil enzyme activity, and the mineralization levels of organic matter. The frequency, rate, and temperature range of freeze-thaw events can have a strong effect on soil microbial status. In general, there is a small effect of rapid freezing and melting on microbial activity. However, the effect of freeze-thaw speed must be combined with final temperature of soil freeze. If the final temperature is still higher than the critical temperature of what can be tolerated by cells, then the melting rate will not significantly affect cell survival, but if the final temperature is below the critical temperature of cells, the melting rate will become the main factor to determine cell survival (Dumont, 2006). Some study results showed that microbial populations were significantly reduced by repeated freeze-thaw cycles. Larsen et al. (2002) found that the number of live bacteria under freeze-thaw treatment was one of the tens of thousands under constant temperature culture, and the species diversity was significantly reduced. However, studies have also shown that soil microorganisms have strong resistance to a mild freeze-thaw (-3～4℃). The soil microbes are affected slightly when the soil temperature is maintained at 0 ℃, but are decreased significantly under a freeze-thaw cycle with a larger temperature range. Our results in this study supported this conclusion, as microbial biomass carbon, fungi counts, and catalase and cellulose activities increased significantly in response to a mild freeze-thaw (-2~2℃), and invertase activity decreased in response to a severe freeze-thaw (-20~2℃).

Soil microorganisms play an important role in converting essential nutrients in soil from organic to inorganic forms (Rubenecia et al., 2015) and this decomposition of soil organic N is affected by soil microbes. The newly exogenous C input stimulated the growth and activity of soil microbes, leading to more heterotrophic microbes involved in the fixation of mineral nitrogen, both the NH4+-N and NO3-N contents decreased with the addition of C in the current study, which supports previous studies in the temperate forests of Northern America and Central Europe (Holub et al., 2005). On the other hand, The accumulation of NO3-N in soil is related to either severe freeze-thaw cycles or long-term deep freezing (Elliott and Henry, 2009; Austnes and Vestgarden, 2008). The soil invertase activity which plays a key role in nitrification was decreased obviously under severe freeze-thaw cycle (-20~2℃), so the NO3-N content was decreased significantly in this study.

5. Conclusion

Although the soil temperature and water content of the alpine soil were both low on the eastern Tibetan Plateau, many soil microorganisms were present. A mild freeze-thaw cycle increased the number of fungi and catalase activity, which showed that a mild freeze-thaw could be beneficial to fungi, and could play an active role in the transformation of soil nutrients. The addition of exogenous carbon significantly decreased the mineral N pool and increased the number of microorganisms and enzyme activities, which indicated that the microbial limit of carbon was greatly eliminated. This may represent a larger potential pulse of soil nutrient for alpine plants in the next spring, and may be instrumental for plant community shifts under future climate change predictions due to the possible increased litter addition.

Acknowledgments

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