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ORIGINAL ARTICLE

Effects of freezing–thawing cycle on peatland active organic carbon fractions and enzyme activities in the Da Xing'anling Mountains, Northeast China

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Abstract Freezing–thawing cycle (FTC) is an important environmental factor affecting soil physicochemical properties and microbial activities. The effects of FTC at mid-high latitudes, especially in the permafrost regions impacted by global warming, have become a hot topic for research. However, the responses of active organic carbon fractions and soil enzyme activities to FTC in the active layers of permafrost regions remain far from certain. In this study, soil samples from three soil layers of (0–15, 15–30 and 30–45 cm) an undisturbed peatlands in Da Xing'anling Mountains, Northeast China, were collected, and then subjected to various FTCs with a large (10 to −10 °C) and a small (5 to −5 °C) amplitudes, respectively. Results showed that the soil active organic carbon fractions and enzyme activities were sensitive to FTCs. The FTCs significantly increased water-extracted organic carbon (WEOC) concentration in the three soil layers by approximately 5–28 % for the large amplitude and 22–36 % for the small amplitude. In contrast, FTCs significantly decreased microbial biomass carbon (MBC) concentration, cellulase, amylase and invertase activities. Overall, the damage of FTCs to soil enzymes was severe at the deeper soil depths and for the large amplitude. Interestingly, the soil WEOC concentration was lower at the large amplitude of FTC compared with the small amplitude. When the numbers of FTCs increased, WEOC concentration began to decrease and MBC concentration and enzyme activities began to increase. In addition, the significant correlations between active organic carbon fractions and enzyme activities indicate that the increased WEOC by FTCs plays an important role in soil microbes and enzyme activities.

Keywords Active organic carbon · Enzyme activities · Freezing–thawing cycle · Permafrost regions · Peatlands

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Introduction

Soil freezing–thawing, a phase transition of soil water, is a common phenomenon in boreal areas. Recently, the effect of freezing–thawing cycle (FTC) on soil biogeochemical processes became a subject of major ecological interest for the potential impact on microbial release of nutrients in plant available form (Grogan et al. 2004). The FTCs have shown strong effects on soil nutrient dynamics and microbial activity or composition (Feng et al. 2007; Henry 2007; Männistö et al. 2009; Matzner and Borken 2008). Previous studies indicated that active organic carbon, the most labile fraction of soil organic carbon with rapid turnover rates (Zou et al. 2005), was sensitive to FTCs (Feng et al. 2007; Henry 2007; Hentschel et al. 2008; Matzner and Borken 2008; Schmitt et al. 2008; Wang et al. 2013; Yu et al. 2011). It has been also observed that FTC can significantly increase dissolved organic carbon (DOC) release from soils of various ecosystems (e.g., Chaer et al. 2009; Feng et al. 2007; Grogan et al. 2004; Matzner and

Borken 2008; Wang et al. 2013; Yu et al. 2011). However, the responses of microbial biomass carbon (MBC) to FTC were different, with increased MBC in wet Arctic sedge meadow (Edwards et al. 2006), decreased MBC in coniferous forest and wetlands (Chaer et al. 2009; Wang et al. 2013), and unchanged MBC in agricultural soils (Koponen et al. 2006), partly due to the different methods among the studies and various soil characteristics (Henry 2007).

Active organic carbon is the important substrate that soil microbes can use (Zou et al. 2005). A number of papers reported that active organic carbon was correlated with soil microbes and enzyme activities (e.g., Feng et al. 2007; Song et al. 2012; Wan et al. 2008; Zaccone et al. 2012). However, to the best of our knowledge, there have been no studies on the relationships between active organic carbon fractions and enzyme activities upon FTC in the peatlands of permafrost regions. Soil enzymes, as the key players involved in the catalytic reactions, play an essential role in the decomposition of organic matter (Dick 1994; Yao et al. 2006). Since soil enzymes mainly originate from microorganisms, any factor that affects the soil microbial population will necessarily alter soil enzyme activities (Vallejo et al. 2010). Although the impacts of FTC on soil microbial activity or composition have been extensively studied (e.g., Edwards et al. 2006; Koponen et al. 2006; Männistö et al. 2009; Sharma et al. 2006), little research has been done about changes in enzyme activities following the FTC (Yergeau and Kowalchuk 2008).

Peatlands in the Da Xing'anling Mountains, Northeast China, are located at the southern margin of the permafrost region of the Eurasian continent, and are one of the major soil carbon stocks on the planet (Zhou et al. 2000). In recent years, permafrost degeneration, including increased soil temperature and deepened active soil layer, has been observed in these areas (Jin et al. 2007). Considering the projected changes in the climate system in the twenty-first century, scientists predicted that permafrost regions will experience higher frequencies and larger amplitudes of FTC due to the reduced snowfall and warmer springs (Hollesen et al. 2011; Schmitt et al. 2008). However, how soil active organic carbon fractions and enzyme activities would respond to changed FTCs in the active soil layers remain far from certain. In this study, we carried out a laboratory-simulated FTCs experiment using soils collected from the peatlands in Da Xing'anling Mountains, Northeast China. The objectives were to examine the impacts of different amplitudes of FTCs on soil active organic carbon fractions and enzyme activities, and to determine whether the impacts are the same in different active soil layers. This study would provide a fundamental basis for understanding the influence mechanism of FTCs on soil ecological processes in the permafrost regions.

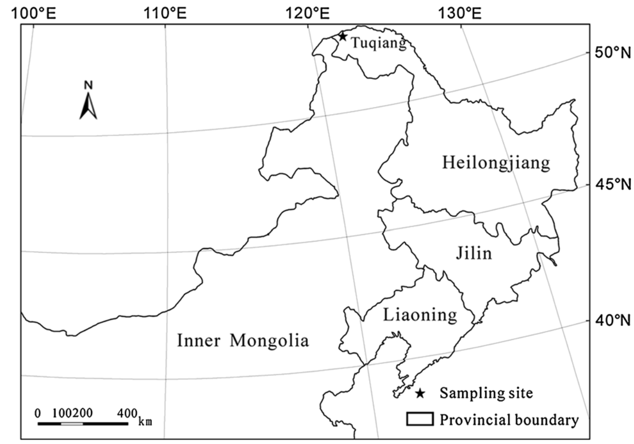

Fig. 1 The sketch map of location of the study area

Materials and method

Site description

The research site (52°25′N, 122°52′E) is located in a minerotrophic peatland of Tuqiang on the northwest slope of the Da Xing'anling Mountains, Northeast China (Fig. 1). The study site is situated in the continuous permafrost zone. The climate of this area is cool continental, with a 30-years (1980–2009) mean annual temperature of −3.9 °C and a mean annual precipitation of 452 mm. The coldest monthly mean temperature is −28.7 °C in January, and the warmest is 18.4 °C in July. Usually water and soils in the permafrost active layer (about 45–50 cm) remain frozen from October to the next April, and begin to melt in late April. The surface of the peatland site is a mosaic of microforms, which are divided into hummock, tussock and hollow. Plants usually grow from early May to late September and are dominated by dwarf shrubs (such as *Chamaedaphne calyculata*, *Ledum palustre*, *Vaccinium vitisidaea* and *Betula fruticosa*), sedges and mosses. Hummocks are covered by *Sphagnum* mosses (*S. capillifolium, S. magellanicum*), *Polytrichum commune* and previously mentioned shrubs. Tussocks support sedges (*Eriophorum vaginatum*) as the dominant vascular plant species, as well as sparse shrubs (*Vaccinium vitisidaea, Ledum palustre*). A scatter of bryophytes (*Polytrichum juniperinum*) is present in hollows (Miao et al. 2012). The soil type in this study site is classified as peat soil.

Peat sampling and experimental design

In late August 2010, three 10 × 10 m^2 plots including the microforms of hummock, tussock and hollow were randomly established in the study area. Soil samples with

Table 1 The basic peat physicochemical properties in permafrost peatlands, Northeast China

Peat layer (cm)	SOC (%)	TN (%)	C/N	pH	MWHC (%)
0–15	46.20	2.10	22.00	4.54	565.61
15–30	44.97	2.01	22.37	4.58	489.97
30–45	40.11	1.64	24.46	4.60	359.08

SOC soil organic carbon, *TN* total nitrogen, *pH* soil:water 1:5, *MWHC* maximum water holding capacity

three layers (0–15, 15–30 and 30–45 cm) of peat were collected using a soil core sampler after removal of the surface litter and aboveground vegetation. The soil samples were sealed in plastic bags with headspace air removed and immediately transported to the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences within 48–72 h. The soils from each layer were homogenized sufficiently and sieved with a 4-mm mesh for incubation experiment. Part of the samples were air-dried, crushed and sieved to 0.25 mm mesh for soil organic carbon (SOC) and total nitrogen (TN) analyses. Soil physicochemical properties are shown in Table 1.

The homogenized soil samples (approximately 100 g fresh soil) from each layer were kept in 500-ml glass jars in triplicates. They were wetted with distilled water to the optimal soil moisture of 60 % by maximum water holding capacity (MWHC) (Rey et al. 2005), and equilibrated at 20 °C for 4 days before the FTC experiment. Based on the field surface temperatures during spring freezing–thawing periods, we set a large (from −10 to 10 °C) and a small (from −5 to 5 °C) amplitudes of FTCs, respectively. Each FTC consisted of freezing at −10 or −5 °C for 24 h and thawing at 10 or 5 °C for 24 h. To maintain constant water content, soils were sprayed with distilled water at the end of each FTC. Before FTC and after the 3rd, 5th, 10th and 15th FTC, DOC, MBC and enzyme activities were determined. We acknowledge that the sieving process may have disrupted soil aggregates and microbial responses, and the soil from the deeper layers may have been exposed to unrealistically large temperature fluctuations. However, the main objective of this study was to compare the effects of different amplitudes of FTCs on the active organic carbon and enzyme activities at multiple depths. All the samples were handled equally, and thus, handling errors are assumed to be equivalent for each sample.

Analytical methods

Soil organic carbon (potassium dichromate-external heating method), TN (Kjeldahl digestion) and pH (potentiometer method) were determined following the laboratory methods described by Zhang (2000). Soil MBC was determined by a chloroform fumigation-K_2SO_4 extraction method, and the extracts were analyzed for carbon concentration with a Multi N/C 2100 Analyzer (Analytik Jena AG, Germany). The MBC was calculated by the following equation (Zhang et al. 2007):

$$\text{MBC} = (\text{fumigated carbon} - \text{non fumigated carbon})/0.45 \quad (1)$$

Soil DOC (water extracted) was determined by the method of Jones and Willett (2006). Moist soil samples were extracted with distilled water (water:soil ratio of 10:1) for 30 min at 25 °C using a shaker at approximately 230 rpm, and then centrifuged for 20 min at 8,000 rpm. The filtrate through a 0.45 μm filter was analyzed for carbon concentration with a Multi N/C 2100 Analyzer (Analytik Jena AG, Germany). This proportion of carbon is referred to as water-extracted organic carbon (WEOC). The invertase, amylase and cellulase activities in soil samples were determined by the methods of Ge et al. (2010) and Guan (1986), which use sucrose, starch and carboxymethylated cellulose as substrates, respectively. These measurements were expressed as mg glucose (g soil 24 h)$^{-1}$ for invertase and amylase activities, and mg glucose (g soil 72 h)$^{-1}$ for cellulase activity.

Statistical analysis

Statistical analysis was performed with the SPSS version 13.0 for Windows (SPSS, Inc., USA), and figures were conducted with the Microsoft Excel 2010 for Windows. Multivariate analysis of variance (MANOVA) was used to describe the effects of the amplitude and frequency of FTCs on soil active organic carbon fractions and enzyme activities at each soil layer. Pearson correlation test was used to examine the relationships between soil enzyme activities and active organic carbon. In all analyses, when $P < 0.05$, differences and correlations were considered statistically significant.

Results

Responses of active organic carbon fractions to FTCs

In this study, the frequency of FTC significantly affected soil WEOC and MBC concentrations ($P < 0.05$; Table 2). However, their responses to the amplitude of FTC were different in various soil layers. For the WEOC, both large and small amplitudes of FTCs significantly increased soil WEOC releases ($P < 0.000$; Fig. 2a, b), and the highest values appeared in the 0–15 cm soil layer, being about 648.06 mg kg^{-1} for the large amplitude and 831.91 mg kg^{-1}

Table 2 MANOVA results (P values) for active organic carbon fractions and soil enzymes with two amplitudes of freeze–thaw and fifteen cycles

Source	Soil layer (cm)	Amplitude	Frequency	Amplitude × Frequency
WEOC	0–15	**0.000**	**0.000**	**0.000**
	15–30	**0.000**	**0.000**	**0.000**
	30–45	0.071	**0.000**	**0.007**
MBC	0–15	0.289	**0.000**	0.302
	15–30	0.119	**0.000**	0.361
	30–45	**0.008**	**0.000**	0.497
Cellulase	0–15	0.939	**0.004**	0.095
	15–30	**0.000**	**0.000**	**0.000**
	30–45	**0.000**	**0.000**	**0.002**
Invertase	0–15	0.164	**0.000**	0.072
	15–30	0.328	**0.000**	**0.022**
	30–45	**0.000**	**0.000**	0.079
Amylase	0–15	**0.000**	**0.000**	**0.014**
	15–30	**0.006**	**0.000**	**0.000**
	30–45	**0.008**	**0.000**	0.256

Boldface values indicate significant effects at $P < 0.05$

WEOC water-extracted organic carbon, *MBC* microbial biomass carbon

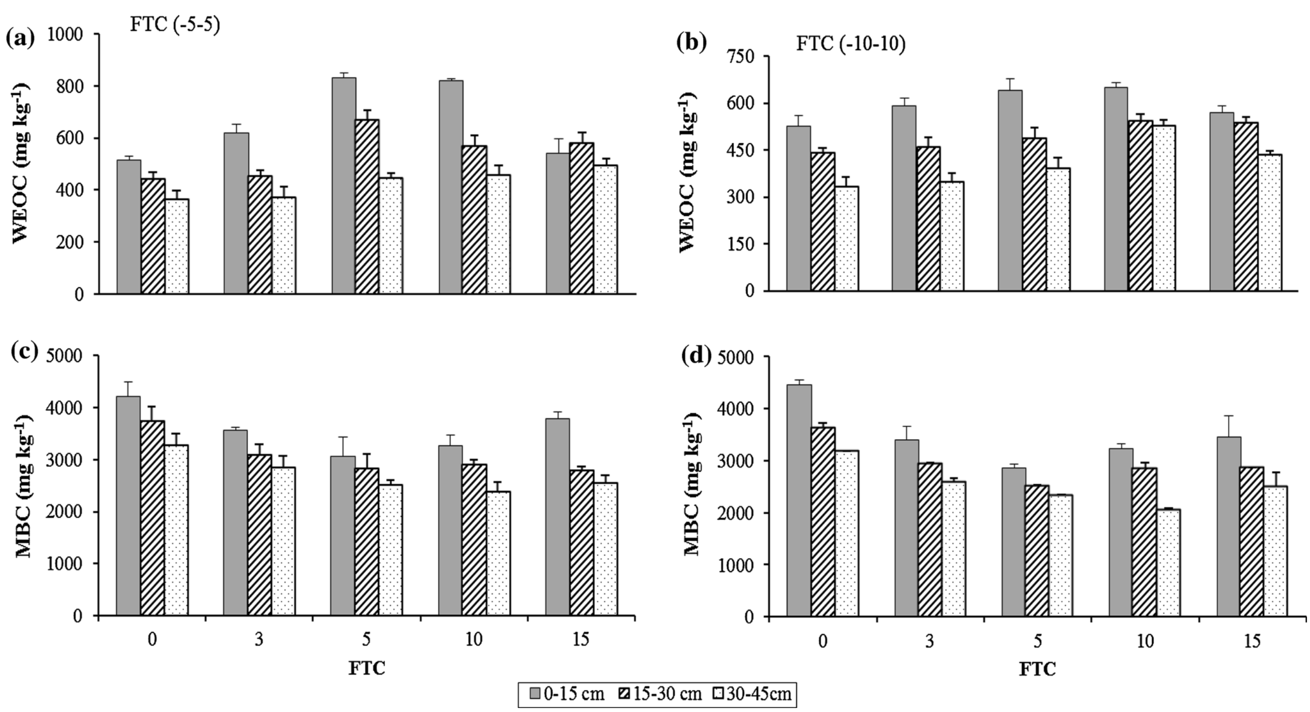

Fig. 2 Response of soil active organic carbon fractions to FTCs in permafrost peatlands, Northeast China. Values are means and standard deviation *bars* ($n = 3$); *FTCs* freezing–thawing cycles, *WEOC* water-extracted organic carbon, *MBC* microbial biomass carbon

for the small amplitude. Although the amplitude of FTC significantly affected the WEOC concentration and presented greater impacts at the small amplitude of FTC, the values in the 30–45 cm soil layer did not appear significant ($P = 0.071$; Table 2). When the numbers of FTCs increased, the amount of WEOC released from soils gradually decreased. Contrary to the WEOC, the significant effect of amplitude of FTC on MBC only appeared in the 30–45 cm soil layer ($P = 0.008$; Table 2). Soil FTCs significantly decreased the MBC concentration ($P < 0.05$; Fig. 2c, d) by approximately 25 % (large amplitude) and 21 % (small amplitude) compared to the unfrozen control sample (FTC0). Although soil MBC concentrations gradually increased with repeated FTCs, yet the values were still lower than the unfrozen control sample.

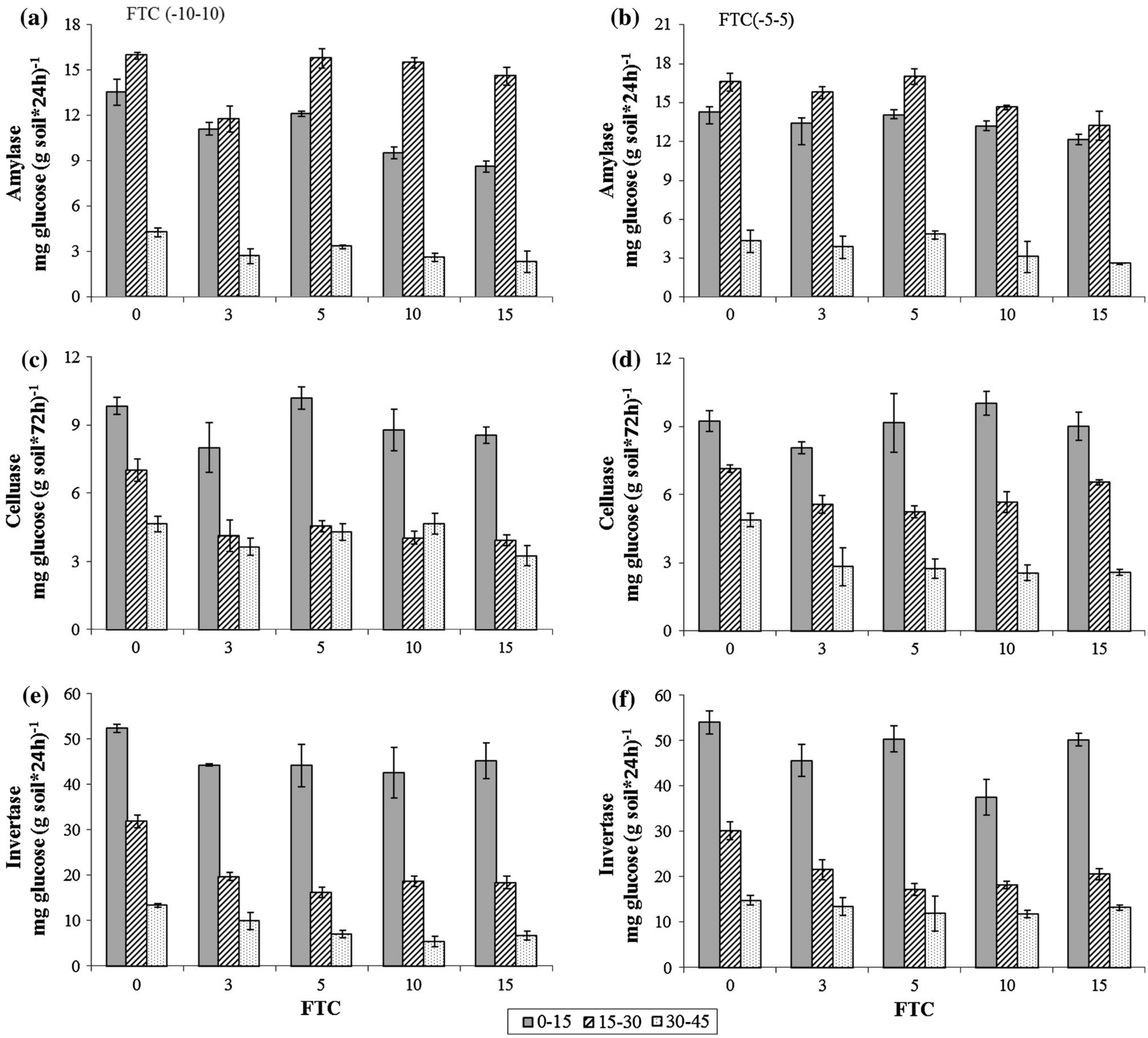

Fig. 3 Response of soil enzyme activities to FTCs in permafrost peatlands, Northeast China. Values are mean values and standard deviation *bars* ($n = 3$); *FTCs* freezing–thawing cycles

Responses of soil enzyme activities to FTCs

The frequency of FTC had a significant effect on soil cellulase, amylase and invertase activities in the active layer of permafrost region ($P < 0.05$). However, the significant effects of amplitude of FTC on these three enzymes appeared in 15–30 and 30–45 cm soil layers for cellulase, 30–45 cm soil layer for invertase, and three soil depths for amylase ($P < 0.05$; Table 2). Compared to the unfrozen control sample, both large and small amplitudes of FTCs significantly decreased the activities of these three enzymes ($P < 0.05$) by approximately 13–44 % (large amplitude) and 2–46 % (small amplitude) for cellulase, 10–36 % (large amplitude) and 8–17 % (small amplitude) for amylase, and 14–50 % (large amplitude) and 7–32 % (small amplitude) for invertase, respectively, presenting greater impacts at the large amplitude of FTC (Fig. 3). Like MBC concentrations, the activities of these three enzymes gradually increased when the numbers of FTCs increased. However, their values were still lower than the unfrozen control sample (Fig. 3).

Relationships between soil active organic carbon and enzyme activities

Under the two amplitudes and 15 cycles of freezing–thawing, the correlation analysis showed that the WEOC concentration in the active soil layer of permafrost region

Table 3 The correlations between active organic carbon fractions and soil enzyme activities

	WEOC	MBC	Cellulase	Invertase	Amylase
WEOC	1.000				
MBC	0.431**	1.000			
Cellulase	0.753**	0.669**	1.000		
Invertase	0.671**	0.803**	0.883**	1.000	
Amylase	0.556**	0.499**	0.475**	0.464**	1.000

WEOC water-extracted organic carbon, *MBC* microbial biomass carbon, *FTC* freezing–thawing cycle

* Significant at 0.05 level

** Significant at 0.01 level

was significantly correlated with the MBC concentration ($R = 0.431$; $P < 0.01$) and the activities of three enzymes ($R = 0.753$ for cellulase, 0.671 for invertase and 0.556 for amylase, respectively; $P < 0.01$) (Table 3). Meanwhile, the positive relationships between the MBC concentration and the activities of the three enzymes were also significant ($R = 0.669$ for cellulase, 0.803 for invertase and 0.499 for amylase, respectively; $P < 0.01$). In addition, the significant correlations were observed between the three soil enzymes ($R = 0.883$ for cellulase and invertase, 0.475 for cellulase and amylase, 0.475 for amylase and invertase, respectively; $P < 0.01$).

Discussion

The WEOC in soil, as one of the active organic carbon fractions, is considered an important substrate that microbes can utilize (Marschner and Kalbitz 2003; Matzner and Borken 2008). In this study, both large and small amplitudes of FTCs significantly increased the WEOC concentration in the three soil depths of active layer, which was consistent with other studies (e.g., Chaer et al. 2009; Feng et al. 2007; Grogan et al. 2004; Matzner and Borken 2008; Wang et al. 2013; Yu et al. 2011). Soils in permafrost peatlands contain a vast amount of undecomposed root litter and half-decomposed root litter due to the cold and wet environments. Freezing–thawing process can fragment litter and increase fine root (<1 mm diameter) mortality, and subsequently release available nutrients (Hobbie and Chapin 1996; Matzner and Borken 2008). Meanwhile, the loss of MBC (indicated by the reduced MBC concentration) upon FTCs in this study may have also contributed to the increased amount of WEOC in soils (Larsen et al. 2002; Schimel and Clein 1996). However, the relative contribution of these two sources of WEOC to the increased WEOC concentration in FTC-treated soils is unclear. When the numbers of FTCs increased, the released WEOC concentration gradually decreased, but the values were still higher compared to the unfrozen control sample (Fig. 2a, b), indicating that the initial FTC can stimulate the massive releases of WEOC from soils but the amount became smaller in the late stage. In addition, the continuing depletion by microbes during the incubation period also contributed to the decreasing WEOC concentration. Unexpectedly, the WEOC concentration in the 0–15 and 15–30 cm soil layers was significantly lower at the large amplitude of FTC than that at the small amplitude (Table 2; Fig. 2), which is inconsistent with the hypothesis that the large amplitude of FTC may release more WEOC due to the severe damage to soil aggregates and microbial and fine root cells (Bechmann et al. 2005; Sharma et al. 2006). However, the relatively greater effect of large amplitude of FTC on MBC concentration in these two soil depths indirectly suggested that the contribution of cell lysis under FTCs to the increased WEOC was small in this study. In addition, compared with the small amplitude of FTC, the higher temperature in the thawing period of large amplitude of FTC may accelerate WEOC depletion since soil carbon mineralization increases with temperature (Wang et al. 2012, 2010). Under global warming and permafrost degeneration, the amplitude of FTC may shift, and the frequency of FTC will increase, indicating that more WEOC may be lost in the soils but whether the loss is trough leachate or substrate of gas still requires further research.

Soil MBC is another active organic carbon fraction released from the lysed cells, so it also represents the soil microbial biomass. In this study, FTCs significantly decreased the MBC concentration in the three soil depths of active layer, which was consistent with the findings of Chaer et al. (2009), Wang et al. (2013) and Larsen et al. (2002). The decreased MBC concentration is attributed to the fact that FTCs have a sterilization function, which can disrupt soil microbial cell structure, resulting in a mass of microbial deaths (Larsen et al. 2002). Interestingly, the extent of the FTC impacts on MBC was similar in various soil depths. The active layer in the permafrost regions frequently undergoes thawing (from the surface layer to the permafrost layer) and freezing processes (from both the surface layer and the permafrost layer to the middle part). Therefore, one possible explanation is that the frequent freezing–thawing process in the field has allowed soil microbes in the active layer to better adapt to this environmental change, thus resulting in the similar extent of the FTC impacts on MBC concentration in various soil depths. Although the effect of amplitude of FTC on MBC concentration was not significant in the 0–15 and 15–30 cm soil layers, the relatively greater effect in the large amplitude of FTC suggested that the damage of the large amplitude of FTC to microbial biomass might be larger since the large amplitude of FTC may decrease the amount of unfrozen water, thus limiting microbial activities (Schimel et al. 2004).

Soil enzymes play an important role in organic matter decomposition and nutrient cycling (Dick 1994; Yao et al. 2006). The FTCs significantly decreased cellulase, amylase and invertase activities in the three soil depths of the active layer, which was in agreement with the decreased MBC impacted by FTCs in this study. It is not surprising that the patterns of these three enzymes activities were also reflected in the MBC since soil enzymes mainly originate from microbes (Vallejo et al. 2010). Previous studies showed that the responses of soil enzymes to FTCs were different partly due to the different methods among the studies and various soil characteristics. For example, in undisturbed coniferous forest soils of the Pacific Northwest, Chaer et al. (2009) found that FTCs significantly reduced β-glucosidase activity but phosphatase and phenol oxidase did not respond to FTCs. In the maritime Antarctic soil cores, a lower FTC frequency numerically increased cellulose and laccase activities (Yergeau and Kowalchuk 2008). In the Eastern Tibetan Plateau, invertase, urease and acid phosphatase activities suddenly declined as the thawing proceeded (Tan et al. 2012). With the increasing numbers of FTCs, cellulase and amylase activities presented a rising trend. In fact, some enzymes in frozen soils are not fully passivated, especially the enzymes in the cold regions. Soil FTCs firstly have damage effects on soil enzymes. However, the broken soil aggregates and lysed cells impacted by FTCs increased the contact of labile organic matter with microbes, thus stimulating soil enzyme activities (Cheng et al. 1971; Pelletier et al. 1999). The significant correlations between the three enzymes activities (cellulase, amylase and invertase) and the active organic carbon fractions (WEOC, MBC) in this study also supported the viewpoints above. In addition, the damage of the large amplitude of FTC on the activities of three soil enzymes was severe, and the response of enzyme activities to FTC was larger in the deeper soil depths. If the increased frequency and the larger amplitude of FTCs following global warming do happen, the decreased activities of the enzymes involved in carbon cycling would predict that the total organic carbon decomposition in the peatlands of permafrost regions may not increase during the initial freezing–thawing period. However, the enduring microbes and enzymes can use the increased active organic carbon, leading to carbon loss in the form of gas and/or leachate.

Conclusions

The sensitive responses of active organic carbon fractions and enzyme activities to FTCs were clearly observed in this laboratory-simulated experiment. The FTCs significantly increased WEOC concentration but decreased MBC concentration and activities of three enzymes (cellulase, invertase and amylase) in the active layer of permafrost regions. The effect of amplitude of FTC on soil WEOC concentration was larger in the small amplitude. However, the responses of MBC and enzymes to FTCs generally presented greater damage at the large amplitude, and the large extent of FTC effect on soil enzymes appeared in the deeper soil depths. Meanwhile, the active organic carbon fractions during the freezing–thawing periods were significantly correlated with cellulase, amylase and invertase activities. The study indicates that frequency and amplitude of FTCs have an effect on soil WEOC release capacity and enzymes activities. The increased WEOC impacted by FTC plays an important role in surviving microbes and enzyme activities.

In the context of global warming and permafrost degeneration, we anticipate that more dissolved forms of carbon (WEOC) will export from peatlands of permafrost regions if the frequency of FTC and active layer thickness increase. This will decrease the peatland carbon storage in permafrost regions. However, the changed FTCs will also decrease some enzymes activities in carbon cycling during the initial freezing–thawing periods, especially in the deeper soil depths. Thus, how FTCs impact on peatland carbon cycles still needs further studies. On account of that, there is little research about changes in enzyme activities following the FTC and incubation studies cannot fully reflect the actual active organic carbon and enzyme activities during the seasonal freezing–thawing periods. Therefore, future research should focus on field monitoring and revealing how soil enzymes respond and adapt to the changed FTCs impacted by the warming climate in permafrost regions.

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