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WETLANDS AND CLIMATE CHANGE

Effect of Freezing–Thawing Cycle on Soil Active Organic Carbon Fractions and Enzyme Activities in the Wetland of Sanjiang Plain, Northeast China

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Abstract

To determine the response of soil active organic carbon fractions and enzyme activities to freezing-thawing cycles (FTCs), an FTC simulation experiment of *Deyeuxia angustifolia* wetland soil samples collected from Sanjiang Plain, Northeast China, were examined. The results showed that the water-extracted organic carbon (WEOC), microbial biomass carbon (MBC) concentration and three enzyme activities were affected by FTCs. FTCs increased WEOC concentration, and the small amplitude (0 °C ± 5 °C) and the low water content interaction had the maximum impact, which was nearly 9.0–70.4%, while the large amplitude and the low water content interaction had the minimum impact, which was about 3.6–50.7%. FTCs significantly decreased MBC concentration, amylase, invertase and catalase activities. During the large amplitude (0 °C ± 10 °C) and high water content interaction impacted heavily on MBC, which was approximately 4.2–41.45%. As the number of FTC increased, WEOC concentration increased followed by a gradually decline in contrast that MBC concentration and three enzyme activities showed an opposite tendency. There was a significant correlation between soil active organic carbon fractions and enzyme activities. In addition, negative correlation was observed between WEOC and MBC. This may suggested that increased WEOC by FTCs plays an important role in soil microbes.

Keywords Freezing–thawing cycle · Active organic carbon · Enzyme activities · Sanjiang plain

Introduction

Freezing-thawing cycles are the fluctuations of soil temperature across the 0 °C isotherm (Wang et al. 2013). As one climate-driven pedoturbation, freezing-thawing regularly happens in mid-high latitude and high altitude regions ecosystems (Zhao et al. 2008). In recent years, freezing-thawing events have caused more and more concern for their impacts on ecosystem processes, and their frequency and intensity changed by global climate change (Zhao et al. 2008). Freezing-thawing action can affect the activities of microorganisms, their population composition, and the biochemical cycling of carbon (Vestgarden and Austnes 2009). Freezing-thawing events of soil have been proved to destroy microbial cells and release their nutrients for usage by the surviving microbes which become highly active during soil thawing (Koponen et al. 2006). It was shown that a single FTC may kill up to 50% of the soil microbial biomass (Männistö et al. 2009). However, Herrmann and Witter (2002) also reported that only 5% of total soil microbial biomass was destroyed during FTC but this contributed to 65% of the total observed carbon increase. 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). Many studies found that FTCs could increase the concentration of dissolved organic carbon that was released from various types of soils (Feng et al. 2007; Matzner and Borken

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2008; Wang et al. 2014). However, the responses of microbial biomass carbon (MBC) to FTCs were different. Increased MBC was observed in wet Arctic sedge meadow (Edwards et al. 2006), while MBC decreased in the peatlands of the Da Xing'an Ling Mountains, Northeast China (Wang et al. 2014). MBC was not found to be influenced by FTCs in agricultural soils (Koponen et al. 2006), which could be due to different methodologies among studies and the various soil types that were used in the experiments (Henry 2007).

As a microbial breakdown product, the active organic carbon is also an essential substance that could be utilized by soil microbes (Miegroet et al. 2005). A large number of studies have shown that active organic carbon was significantly positive correlated with the soil microorganism and soil enzyme activity (e.g., Feng et al. 2007; Wan et al. 2008; Song et al. 2012). However, there are few reports about the relationships between soil active organic carbon fractions and enzyme activities during FTC period in the wetland. As the main mediator of soil biological processes, soil enzymes participated in all biochemical processes in soil and involved decomposition of organic matter, nutrient cycle, energy transfer and environmental quality, etc. (Yao et al. 2006; Xiao et al. 2017). Soil enzymes are mainly derived from microorganisms (Zornoza et al. 2006), and any factors that affect the activity of soil microorganisms will inevitably influence soil enzyme activities (Vallejo et al. 2010). Although the effect of FTC on soil microorganisms has been reported (e.g., Edwards et al. 2006; Koponen et al. 2006), there are few reports about effects of FTCs on the soil enzyme activities (Wang et al. 2014).

Wetlands in the Sanjiang Plain, northeast China, cover a concentrated distribution area of freshwater wetlands and one of the rare freshwater wetlands in the world, and are carbon sinks that store CO_2 from the atmosphere (Kayranli et al. 2010), and has been suggested that it is sensitive to climate change (Tan et al. 2004). Once the climate changes, it will affect the carbon balance of wetlands. Global warming make soil depth (Fitzhugh et al. 2001), the strength (Feng et al. 2007) cycle times changed during FTCs (Henry 2007), which can affect substrate availability, soil microbial activity, as well as nutrient transformation (Jefferies et al. 2010). Therefore, a laboratory-simulated FTC experiment was carried out by using soils collected from undisturbed *Deyeuxia angustifolia* wetland in Sanjiang Plain in northeastern China, with the objective to examine the impacts of different amplitudes, cycle frequencies, and water contents of FTCs on soil active organic carbon fractions, soil enzyme activities, across different active soil layers. This study provide a fundamental basis for understanding the mechanism of FTCs on soil ecological processes and the change of carbon emission in wetland of Sanjiang Plain.

Materials and Methods

Research Area and Sampling

The research site (47°44′N, 133°31′E) is located in the Honghe National Nature Reserve of Sanjiang Plain, Northeast China (Fig. 1). The study area belongs to the freeze thaw zone, its annual average temperature is 1.9 °C and annual average rainfall is 585 mm. Average

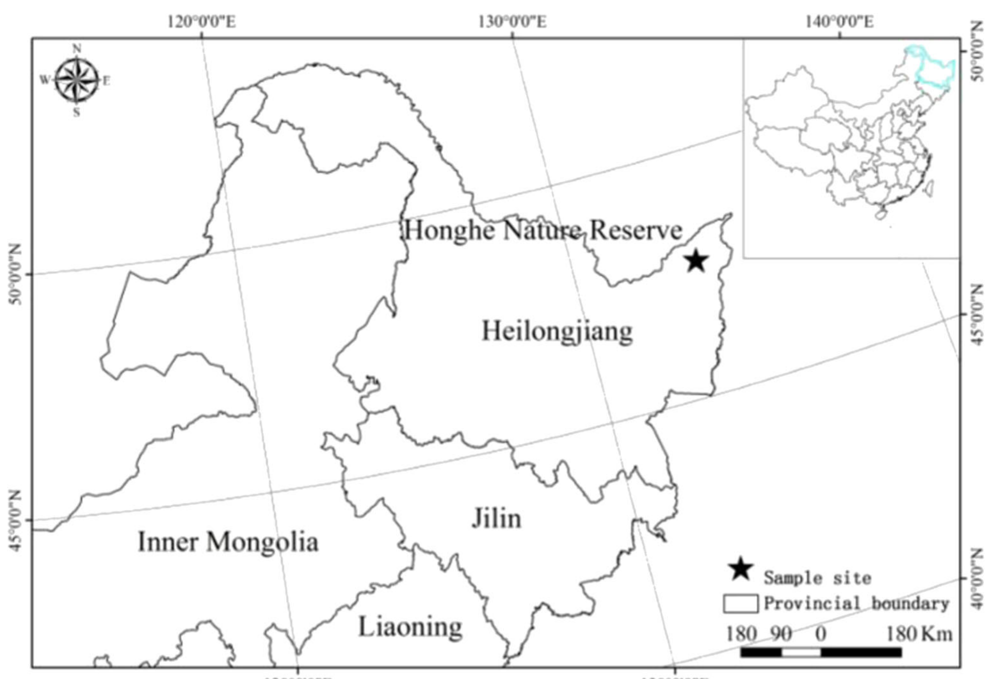


Fig. 1 Location of sampling site in the Sanjiang Plain, Northeast China

temperature in the coldest month is January, −23.4 °C, and average temperature in the warmest one is July, 22.4 °C. FTCs take place in late autumn and early spring. The study area is a typical wetlands ecosystem with a complex microtopography made of depression, low flat and flat ground. The depression ground always retains water and is associated with grassy plants such as *Carex pseudocraica* and *Carex lasiocapa.* The low flat ground and flat ground retains seasonal water and is continuously moist on the surface with grasses such as *Deyeuxia angustifolia* growing on it. In the higher terrain, *Quercus mongolica*, *Populous davidiana*, *Betula platphylla* and other shrubs can be found. The soils are transited by *Peat boggy soil* in depression ground to *Humus fen soil* and *Albic soil*. In this project's study area, soil, water content and vegetation distribution is based on the terrain and topography and is typical of the Sanjiang Plain.

Soil Samples

In late October 2015, undisturbed *Deyeuxia angustifolia* wetland was selected as the study area in the Honghe National Nature Reserve of Sanjiang Plain, in which three 10×10 m^2 plots were randomly chosen, and the distance between two plots was about 30 m. The soil was a meadow bog soil with *Deyeuxia angustifolia* mixed with a few *Carex appendiculata*. Soil samples with three layers (0–10, 10–20 and 20–30 cm) were collected using a soil core sampler after removal of the surface vegetation. The soil samples were selected by multipoint sampling, and each layer was homogenized sufficiently and put in sealed plastic bags with headspace air removed and immediately transported to Heilongjiang Academy of Sciences within 72 h and stored in a freezer at 4 °C. The soil samples were sieved with a 4 mm mesh for the incubation experiment. A portion of the samples were air-dried, crushed, sieved, and used for the analysis of soil organic carbon (SOC) and total nitrogen (TN) Soil physical-chemical properties are shown in Table 1.

Experimental Design

Homogenized soil samples (70 g equivalent to dry weight) from each layer were kept in 250 ml culture bottle in triplicates. Soil water content was adjusted to 60% and 80% of maximum water holding capacity (MWHC) by adding deionized water. All the bottles were put into incubators at 20 °C for a week to let microorganisms restore to normal status before the FTC experiment. Based on the actual temperature of the field during freezing–thawing periods, we created a small amplitude treatment (from −5 to 5 °C) and a large amplitude treatment (from −10 to 10 °C) for the FTC simulation. Each FTC consisted of freezing at −5 or −10 °C for 24 h and thawing at +5 or + 10 °C for 24 h. To keep soil water content constant, soil collections were sprayed with distilled water at the end of each FTC. Before FTC and after the 3rd, 6th, 10th and 15th FTC, the concentration of the soil organic carbon fractions and soil enzyme activities was analyzed.

Analytical Methods

Soil organic carbon concentrations were determined with a Multi N/C 2100 Analyzer (Analytik Jena AG, Germany). TN (Kjeldahl digestion) and pH (potentiometer method) were determined following the laboratory methods described by Zhang (2000). Soil water-extracted organic carbon (WEOC) was determined by the method of Jones and Willett (2006). Fresh soil samples were extracted with distilled water (soil: water ratio of 1:5) for 30 min at 25 °C using a shaker at approximately 250 rpm and then centrifuged for 10 min at 12,000 rpm. All liquid supernatant was filtered through a 0.45 μm membrane filter paper, which was used to analyze the organic carbon concentration with a Multi N/C 2100 Analyzer (Analytik Jena AG, Germany). Soil MBC was measured by a chloroform fumigation-K_2SO_4 extraction method (Zhang et al. 2007). The extracts were analyzed for carbon concentration with a Multi N/C 2100 (Analytik Jena AG, Germany) analyzer. MBC was calculated by the following equation:

$$MBC = (fumigatedcarbon - nonfumigatedcarbon)/0.45 \tag{1}$$

The invertase, amylase and catalase activities were determined by the methods of Guan (1986) and Ge et al. (2010), which used sucrose, starch and hydrogen peroxide as substrates respective. The results were shown as mg glucose (g soil*24 h)$^{-1}$ for invertase and amylase activities, and ml $KMnO_4$ (g soil*20 min)$^{-1}$for catalase activity.

Statistical Analysis

Statistical analysis was performed with SPSS for Windows, Release 18.0.0, Standard Version (SPSS Inc., US), and graphics were created with the Microsoft Excel 2010 for Windows. Multivariate analysis of variance (MANOVA) was used to analyze the effects of amplitude, water content and frequency of FTCs on soil active organic carbon fractions and enzyme activities in the three active soil layers studied. Pearson's correlation coefficients with two-tailed test of significance were used to examine the relationships between soil active organic carbon fractions and enzyme activities. When $P<0.05$, differences and correlations were considered statistically significant.

Table 1 Soil physical-chemical properties in Sanjiang Plain. Values shown are mean ± SD ($n = 3$)

SL(cm)	SOC(g kg^{-1})	TN(g kg^{-1})	C/N	pH	SWC(%)	MWHC(%)
0–10	47.76 ± 6.32	3.81 ± 0.52	12.54 ± 0.81	5.64 ± 0.16	71.82 ± 6.91	85.91 ± 9.08
10–20	36.52 ± 4.16	2.64 ± 0.39	13.83 ± 0.52	5.83 ± 0.11	44.68 ± 7.74	52.51 ± 6.81
20–30	20.43 ± 1.96	1.12 ± 0.32	18.45 ± 0.33	6.58 ± 0.18	39.35 ± 6.24	42.50 ± 3.79

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

Results

Effects of FTCs on Soil Active Organic Carbon Fractions

MANOVA results indicated that the frequency of FTC could significantly affect WEOC and MBC content in all soil layers ($P<0.05$; Table 2). But the response of WEOC and MBC concentration in each soil layer to amplitude and water content of FTC was different. For the WEOC, both large and small amplitudes of FTCs could significantly increase soil WEOC concentration ($P<0.05$; Fig. 2a, b). The highest WEOC values of both amplitude treatments appeared in 0–10 cm soil layer, with 708.5 mg kg^{-1} for the large amplitude, and 861.6 mg kg^{-1} for the small amplitude. The soil water content for both treatments was the high water content. Although the amplitude and water content affected the WEOC concentration and the result showed that the small amplitude had greater impacts under the same water content ($P<0.05$; Fig. 2a, b), MANOVA proved that the amplitude significantly affected the WEOC concentration in 0–10 and 10–20 cm soil layer, but 20–30 cm was not significantly affected ($P<0.05$; Table 2). As the frequency of FTCs increased, the WEOC released from the three soil layers gradually increased and then decreased. With the increase of soil depth, the effect of FTCs on the release rate of WEOC was reduced. The water content ($P = 0.006$; Table 2) and amplitude ($P = 0.007$; Table 2) had a significant impact on MBC concentration at 20–30 cm soil layer. FTCs significantly decreased MBC concentration (Fig. 2c, d) by approximately 27.5% (small amplitude and low water content) and 41.5% (large amplitude and high water content) compared with the unfrozen control sample FTC(0). As the soil depth increased, the influence of FTCs on MBC concentration decreased. Although soil MBC concentrations increased with the increased frequency of FTCs, all values were still lower than those of the unfrozen soil samples FTC(0) (Fig. 2c, d).

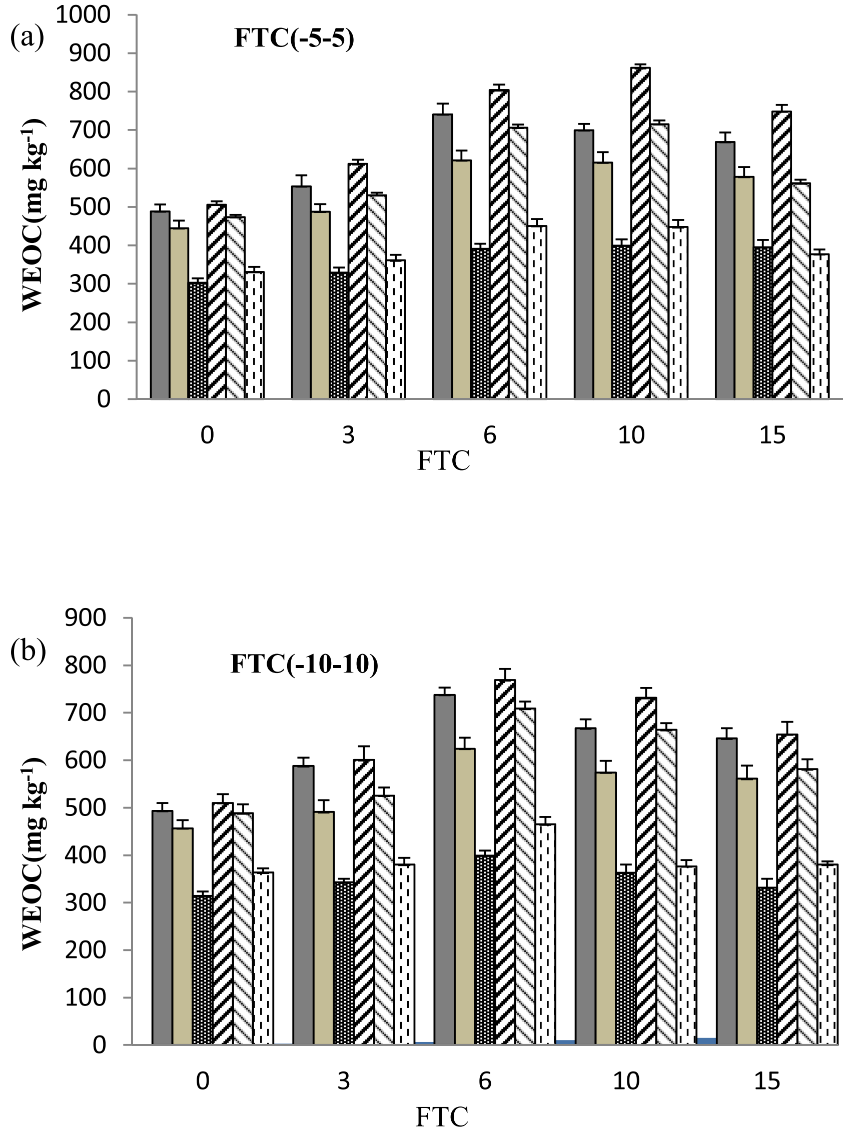
Table 2 MANOVA results (*P* values) for WEOC, MBC concentration and amylase, invertase, catalase activity in three soil layers with two water content (WC), two amplitudes (A) and fifteen frequencies (F)of Sanjiang Wetland

Source	Layer	WC	A	F	WC × A	MC × F	A × F	WC × A × F
WEOC	0–10	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.172
	10–20	**0.000**	**0.006**	**0.000**	**0.020**	**0.000**	**0.026**	0.625
	20–30	**0.000**	0.120	**0.000**	0.154	**0.031**	**0.000**	**0.017**
MBC	0–10	0.440	0.719	**0.000**	0.256	**0.024**	**0.015**	0.635
	10–20	0.270	0.085	**0.000**	**0.049**	0.792	**0.005**	0.517
	20–30	**0.006**	**0.007**	**0.000**	0.075	0.656	0.291	0.245
Amylase	0–10	0.052	0.051	**0.000**	0.109	0.848	0.246	0.894
	10–20	**0.003**	**0.002**	**0.000**	0.021	0.972	0.467	0.969
	20–30	**0.002**	**0.009**	**0.000**	0.918	0.973	0.383	0.989
Invertase	0–10	0.450	**0.004**	**0.033**	**0.000**	**0.000**	0.682	**0.001**
	10–20	**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.045**	0.072
	20–30	0.065	**0.000**	**0.000**	0.890	0.566	0.829	0.299
Catalase	0–10	0.483	**0.000**	**0.000**	0.661	0.054	0.314	0.992
	10–20	**0.001**	**0.024**	**0.000**	0.360	0.303	0.425	0.873
	20–30	**0.017**	**0.031**	**0.000**	0.221	0.769	0.166	0.528

Boldface values indicate significant effects at $P < 0.05$

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

Fig. 2 Effects of FTCs on active organic carbon fractions in Sanjiang Wetland, Northeast China. The number of FTC is identified on *x*- axis, *WEOC* and *MBC* concentration is identified on *y*-axis. 60% and 80% refer to the 60% and 80% of maximum water holding capacity respectively. Values are mean values and standard deviation *bars* ($n = 3$) *WEOC* water-extracted organic carbon, *MBC* microbial biomass carbon



Effects of FTCs on Soil Enzyme Activities

The frequency of FTCs had a significant effect on soil amylase, invertase and catalase activities ($P<0.05$; Table 2). However, the significant effects of the amplitude of FTC on three enzyme activities appeared in all soil layers, except that 0–10 cm for amylase was not significant ($P = 0.051$; Table 2). The significant effects of soil water content of FTCs on three enzyme activities appeared in the layers 10–20 cm and 20–30 cm for amylase, 10–20 cm and 20–30 cm for catalase, and 10–20 cm for invertase ($P<0.05$; Table 2). Compared with the unfrozen control soil sample FTC(0), FTCs decreased these three enzyme activities (Fig. 3). For amylase, the large amplitude decreased activity by approximately 17.3–28.6% (high water content), 14.6–28.6% (low water content) and the small amplitude decreased by about 2.6–18.6% (high water content) and 3.2–18.6% (low water content). For invertase, the large amplitude decreased activity by approximately 8.4–35.5% (high water content), 3.5–32.9% (low water content) and the small amplitude decreased by approximately 7.1–41.6% (high water content) and 14.7–41.1% (low water content). For catalase, the large amplitude decreased activity by about 1.9–25.9% (high water content), 2.8–25.2% (low water content) and the small amplitude decreased activity by about 1.3–25.2% (high water content) and 2.5–25.6% (low water content). Result analysis showed that large amplitude and the high water content interaction had greater effects on FTCs (Fig. 3). With the increase of soil depth, the effect of FTCs of the three

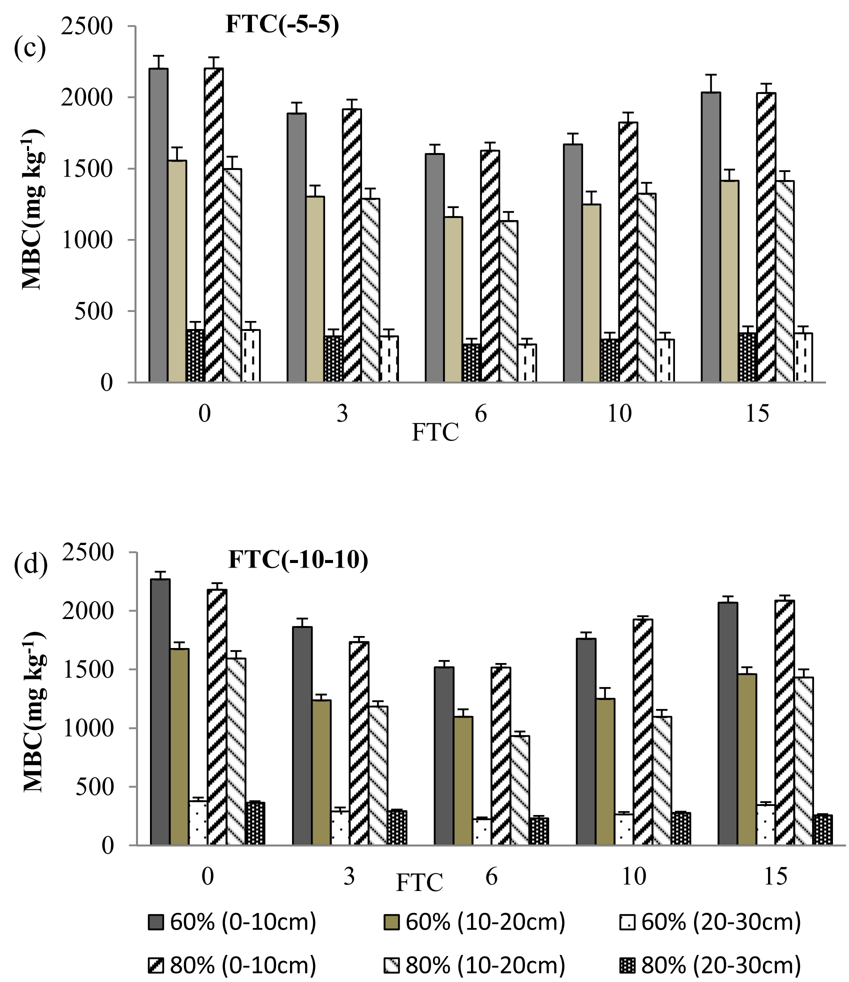


Fig. 2 (continued)

enzyme activities decreased. As the frequency of FTCs increased, the three enzyme activities gradually decreased and then increased, but their highest value was still lower than that of the unfrozen soil samples (Fig. 3).

Relationships Between Soil Active Carbon Fractions and Enzyme Activities

After the two amplitudes, two water gradients and fifteen freezing–thawing cycles, the correlation analysis showed that WEOC concentration had a significantly negative correlation with MBC concentration ($R = -0.812$; $P<0.05$) and three enzyme activities ($R = -0.450$ for amylase, $R = -0.515$ for invertase, $R = -0.674$ for catalase $P<0.05$) (Table 3). Meanwhile, the MBC was significantly correlated with the three enzyme activities ($R = 0.469$ for amylase and $R = 0.882$ for catalase, $P<0.05$; $R = 0.774$ for invertase, $P<0.01$) (Table 3). Furthermore, Table 3 shows the significant correlations among three enzyme activities ($R = 0.491$ for amylase and invertase; 0.453 for amylase and catalase; 0.345 for invertase and catalase, respectively; $P<0.05$).

Discussion

WEOC and MBC are soil active organic carbon fractions and can be affected by climate change (Wang et al. 2014). This study showed that FTCs could significantly increase WEOC concentration in every active layer (Fig. 2a, b), the result consistent with that of other researches (e.g., Feng et al. 2007; Matzner and Borken 2008; Chaer et al. 2009; Wang et al. 2014). FTCs could affect the effectiveness of water content, movement, direction, and death and decomposition of soil microorganisms, possibly leading to the release of small molecular sugar, amino acids, increasing the content of medium and small molecular compounds in soil, resulting in the increase of WEOC concentration. The small amplitude and high water content brought about the largest increased in WEOC, while the large amplitude and low water content brought about the smallest increase in WEOC (Fig. 2a, b). The reason might be attributed to different water contents and freezing temperatures damaged the soil aggregation to different extents (Staricka and Benoit 1995), lower water content could enhance the stability of aggregation even if the freezing

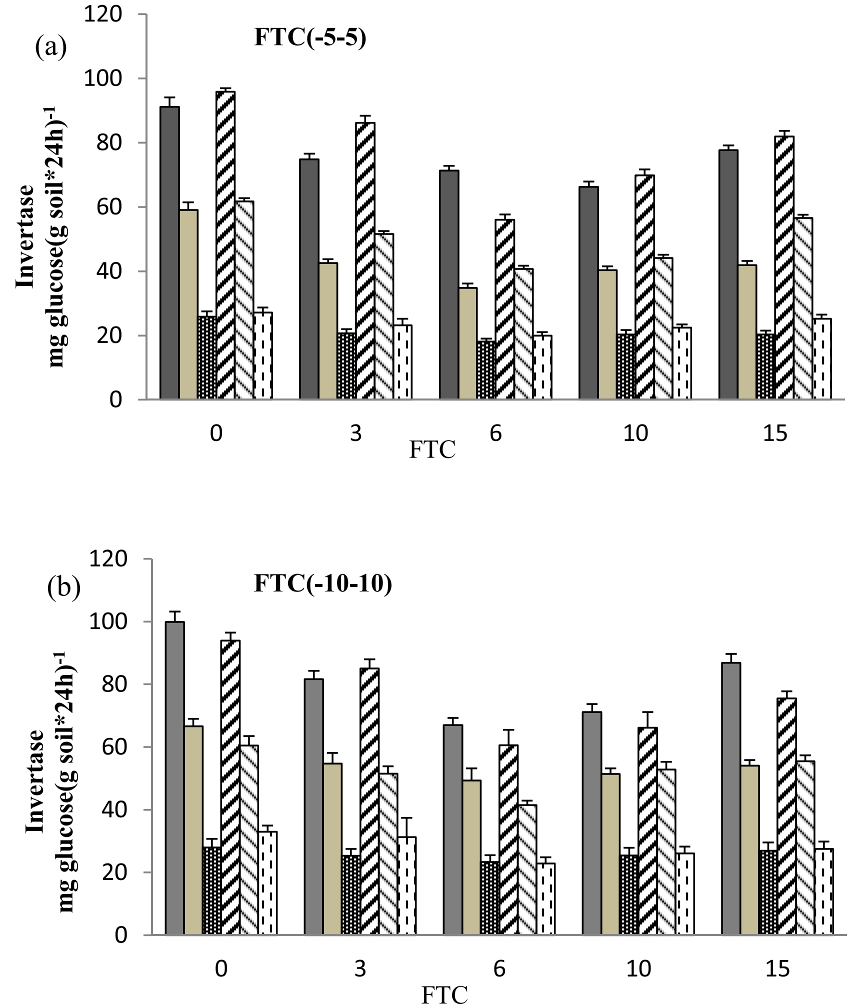


Fig. 3 Effects of FTCs to soil enzyme activities in Sanjiang Wetland, Northeast China. The number of FTC is identified on the *x*-axis, Invertase, Amylase and Catalase activity is identified on the *y*-axis. 60% and 80% refer to the 60% and 80% of maximum water holding capacity respectively. Values are mean values and standard deviation *bars* ($n = 3$)

temperature fluctuated within a wide range. At the same freezing temperature, approaching a saturated water content, was the most efficient way to destroy soil aggregation. Oztas and Fayetorbay (2003) reported that lower freezing temperatures were more likely to break the aggregation. In addition, large amplitude FTCs would accelerate the consumption of WEOC during high-temperature thawing, due to the mineralization rate increased as the temperature rose. The number of FTCs, amplitude and water content increased WEOC concentration in active soil layer. As the number of FTCs increased, all values were still higher compared with those of unfrozen control sample FTC(0) (Fig. 2a, b), suggesting that short-term FTCs could stimulate the releases of WEOC from soil, as with the increased frequency of FTCs, soil microorganisms adapted gradually, and with the increased FTCs, the amount of microbial death decreased, and the WEOC released from dead microorganisms was reduced. On the other hand, in the preliminary stage of FTC, the released WEOC was constantly decomposed and made use of by live microorganisms in soil, thus reducing the WEOC concentration. This conclusion was consistent with other research findings (Herrmann and Witter 2002; Grogan et al. 2004; Wang et al. 2014).

Soil MBC is another important active organic carbon fraction released from the lysed microbial cells (Wang et al. 2014). Therefore, soil MBC is an important indicator to evaluate soil microbial biomass (Nsabimana et al. 2004). Compared with the control group, FTCs significantly decreased MBC concentration in the active soil layer (Fig. 2c, d), which was consistent with the discoveries of Larsen et al. (2002), Chaer et al. (2009), and Wang et al. (2014). With the increased frequency of FTCs, soil MBC concentration first decreased and then increased with WEOC in every active layer (Fig. 2c, d). Perhaps FTCs killed a large number of microorganisms in the initial phase, hence MBC concentration was reduced, but with the increased frequency of FTCs, soil microbes adapted to the environment of FTCs, whose response was waning to soil MBC, and MBC concentration gradually increased. Although the amplitude and water content did not significantly affect MBC concentration during FTCs, the large

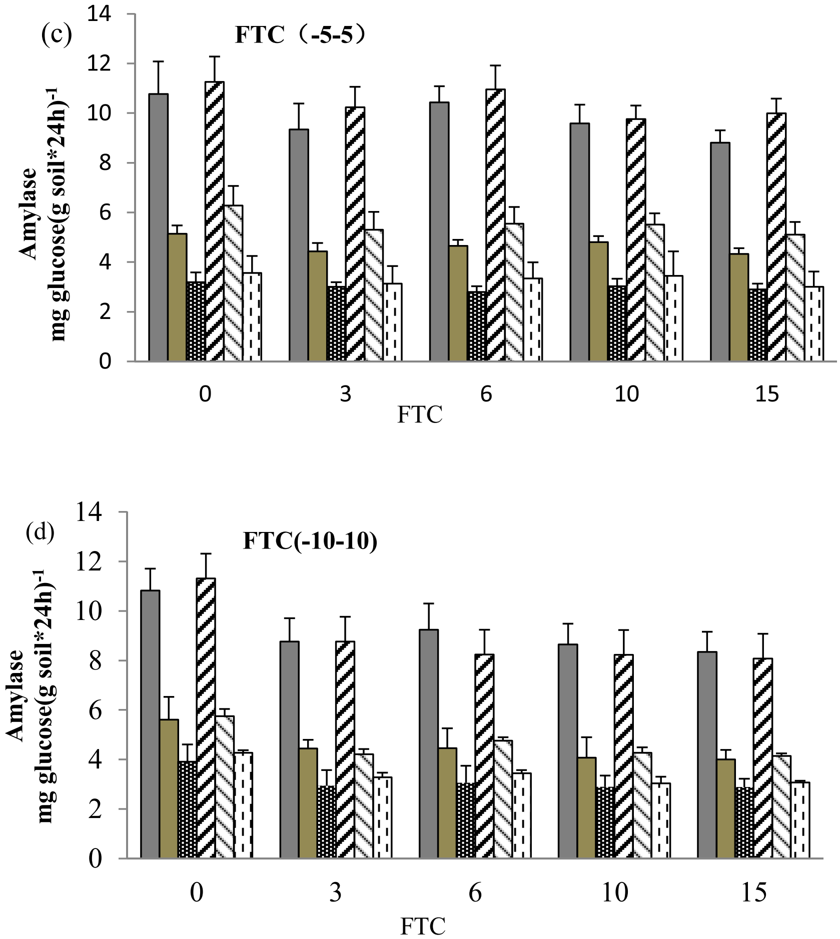


Fig. 3 (continued)

amplitude and low water content interaction had a greater effect on MBC concentration, indicating that the damage might be stronger with the large amplitude and suitable moisture content to microbial biomass since the large amplitude of FTC may decrease the amount of unfrozen water, thus can kill even more microbes. This study showed WEOC and MBC concentration were negatively correlated with each other. Meanwhile, the loss of MBC might have also contributed to the increase of WEOC in soil layers, but its relevant contribution to the increase of WEOC concentration in FTC-treated soils was not found.

As the key factor of soil biological processes and involved in all biochemical processes in soil, soil enzyme activity is closely related to organic decomposition, nutrient cycle and energy transfer (Yao et al. 2006). Soil enzymes mainly originate from soil microorganisms and plant root secretions. Therefore, any factor that affects soil microbial communities will affect soil enzyme activities. (Vallejo et al. 2010). In this study, it was found that FTCs significantly reduced the activities of amylase, invertase and catalase in the three active layers. With the increased frequency of FTCs, three enzyme activities were consistent with MBC, which decreased at first and then increased, and significantly correlated with MBC since it was mainly derived from microbes. 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 small in the deeper soil depths. Most researches have shown that the responses of soil enzyme activities to FTCs were different due to different methods and various soil characteristics in studies. For example, Tan's analysis of the soil of Eastern Tibetan Plateau showed that the invertase activity increased then decreased during the thawing (Tan et al. 2012). Meanwhile, Wang et al. (2014) found freeze and thawing cycles reduced the activity of cellulase and amylase and invertase in the peatlands of the Da Xing'an Ling Mountains, China. Qiao et al. (2008) research showed that freezing and thawing FTCs can promote the activity of catalase of Liaohe oilfield soil. Other studies found that completely frozen soil also had a higher enzyme activity (Wallenstein et al. 2009), which might be related to the active microorganisms in frozen soil possessing a unique mechanism to adapt to the low temperature (Mikan et al. 2002). In fact, the enzymes were not completely inactivated in frozen soil, once the frozen soil melted, various enzyme activities were enhanced, and

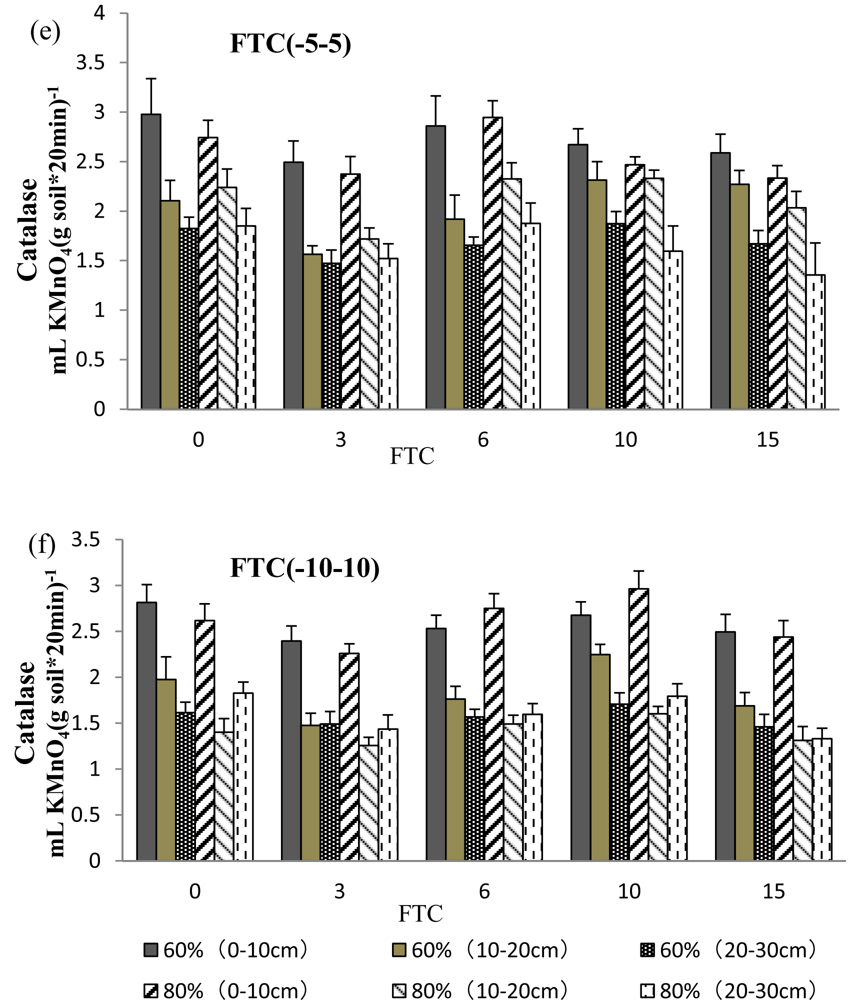


Fig. 3 (continued)

mineralization rate of organic matter was accelerated as well, which was supported by the significant correlations between three enzyme activities and active organic carbon fractions (WEOC, MBC) in this study.

Global warming and permafrost degradation are two indisputable facts around the globe. As temperature rises, more carbon will be released from frozen soil and cause increased frequency and thickness of active layer in seasonal permafrost region, and the reserve of soil organic carbon will decline. Therefore, researching the soil enzyme activity during seasonal FTCs will help us better understand the effect of global climate change on the mechanism of action with the middle-high latitude and high elevation region ecosystem. At present, there are few studies on the changes of soil enzyme activities during seasonal freezing-thawing period, and the simulation experiment cannot fully reflect the soil active organic carbon fractions and enzyme activities. Therefore, in future, the research should emphasize more on field monitoring and different types of soils, and use incubation studies combining with field experiment to reveal the mechanism of seasonal FTC, responses of soil active organic carbon fractions and enzyme activities to freezing-thawing changes.

Table 3 Correlations between soil active organic carbon fractions and enzyme activities

	WEOC	MBC	Amylase	Invertase	Catalase
WEOC	1.000				
MBC	−0.812*	1.000			
Amylase	−0.450*	0.469*	1.000		
Invertase	−0.515*	0.774**	0.491*	1.000	
Catalase	−0.674*	0.882*	0.453*	0.345*	1.000

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

* Significant at 0.05 level, ** Significant at 0.01 level

Conclusions

The sensitive responses of active organic carbon fractions and enzyme activities to FTCs were observed in this laboratory-simulated experiment. The FTCs significantly increased WEOC concentration but decreased MBC concentration and activities of invertase, amylase and catalase in wetland of Sanjiang. With the increase of depth, the impact of freezing-thawing decreases gradually. Frequency of FTCs could significantly affect active organic carbon fractions and three enzyme activities in the active layer and showed short-term effects. Amplitude of FTCs affects soil active organic carbon concentration and enzyme activities, among which small amplitude and low water content interaction impacted WEOC concentration was larger. However, large amplitude and the high water content interaction impacted MBC and three enzyme activities the most severely. Correlation analysis showed that WEOC was significantly negatively correlated with MBC and three enzyme activities, while MBC had a significantly positive correlation with the three enzyme activities. 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.

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