Wetlands (2022) 42: 36
https://doi.org/10.1007/s13157-022-01553-7

WETLAND SOILS

Responses of Soil Active Organic Carbon Fractions and Enzyme Activities to Freeze-thaw Cycles in Wetlands

Jinqiu Guan[1] · Chunxiang Song[1] · Yude Wu[1] · Xingtian Qi[1] · Rongjun Qu[1] · Fu Li[1] · Hongwei Ni[2]

Received: 9 November 2021 / Accepted: 28 March 2022 / Published online: 25 April 2022

Abstract

In order to clarify the response of soil active organic carbon fractions and enzyme activities in different type of wetlands to freeze-thaw cycles (FTCs), a FTCs simulation experiment of different type wetland soils were carried out. In the study, samples were collected from three soil layers of (0–10, 10–20 and 20–30 cm) undisturbed *Deyeuxia purpurea* wetland (UDPW), disturbed *Deyeuxia purpurea* wetland (DDPW) and rice paddy field (RP), and then exposed to FTCs at large (-10 to 10℃) or small (-5 to 5℃) amplitudes, respectively. The results showed that FTCs increased the soil dissolved organic carbon (DOC) concentration in the three soil layers of UDPW, DDPW and RP by approximately 4.7–45.1%, 3.8–41.9% and 1.1–32.7% at large amplitude, and 8.7–48.1%, 5.2–43.4% and 2.2–31.8% at small amplitude. The interaction between small amplitude and higher water content resulted in the maximum increment of DOC concentration. However, FTCs decreased microbial biomass carbon (MBC) concentration and cellulase, invertase and catalase activities, and particularly the interaction between the large amplitude and higher water content exerted the most significant effect. As the increase of freeze-thaw frequency, DOC concentrations increased firstly and then decreased, while MBC concentrations and the three enzyme activities were opposite to DOC. The average change in DOC and MBC concentrations and enzyme activities due to the effects of FTCs varied from soil type, and the variation of DOC, MBC and enzyme activities across different type wetlands were as follows: UDPW > DDPW > RP. As the soil depth increased, the FTCs effect gradually weakened, which was manifested as 0–10 cm > 10–20 cm > 20–30 cm. For the three wetland soils, the significant correlations between active organic carbon fractions and enzyme activities indicate that the increase in DOC due to FTCs plays an important role on soil microbes and enzyme activities. However, the correlation was weak in RP soil, which might be related to strong disturbance of human.

Keywords Sanjiang Plain · Wetland · Freeze–thaw cycles · Active organic carbon fractions · Enzyme activities

Introduction

Freeze-thaw cycles (FTCs) are ubiquitous in middle to high latitudes and high altitudes, which play important role in the soil ecosystem of these areas (Grogan et al. 2004). In the context of global warming, FTCs exert profound effects on the carbon and nitrogen cycles, thus accelerating the loss and decomposition of carbon and nitrogen in the soil carbon pool of terrestrial ecosystem (Yi et al. 2015). Dissolved organic carbon (DOC) and microbial biomass carbon (MBC) are fractions of soil active organic carbon, which are very active in the soil ecosystem and sensitive to changes in soil environmental factors (Wang and Bettany 1993). DOC, which is closely related to microbial activity, is not only the product of microbial metabolism but also the substrate that can be utilized by microbes, particularly in frozen soil, providing carbon source for living microbial activities. Variations in soil DOC content result from the composite effect of dead microorganisms, the new release in soil and the relative amount of DOC dissolved by mineralization (Herrmann and Witter 2002). More literature report that FTCs can affect the DOC concentration, although different research

---

✉ Fu Li
lifu0718_1999@163.com

✉ Hongwei Ni
nihongwei1964@163.com

[1] The Faculty of Science of Jiamusi University, Jiamusi 154007, China

[2] Heilongjiang Academy of Forestry, Harbin 150081, China

methods and soil types have resulted in different findings (Grogan et al. 2004; Feng et al. 2007; Chaer et al. 2009). Soil microbial biomass carbon (MBC) is an important soil labile C fraction that has been found to play a fundamental role in soil organic C dynamics (Grandy and Neff 2008; Liang et al. 2011) and serves as a useful indicator of changes in soil C stabilization and nutrient dynamics following soil management practices (Sparling 1992; Fierer et al. 2009). Different freeze-thaw conditions have different impacts on soil MBC, Koponen et al. (2006) and Grogan et al. (2004) found that freezing soil and low numbers of FTCs had no significant effect on soil MBC. However, Schimel and Clei (1996), Larsen et al. (2002) and Wang et al. (2014) reported that the freeze-thaw effects on soil decreased the soil MBC concentration. A number of articles reported that soil active organic carbon were correlated with soil microbes and enzyme activities (e.g., Feng et al. 2007; Wan et al. 2008; Song et al. 2012; Zaccone et al. 2012). However, to the best of the author knowledge, few studies have reported the relationship between soil active organic carbon fractions and enzyme activities in the different type wetlands which experience seasonal freeze-thaw cycles.

Soil enzymes mainly come from the secretion of soil microbes and plant roots, and the decomposition of plant material and soil fauna (Marx et al. 2001), which are involved in various chemical reactions and biochemical processes in soil (Yang and Wang 2002). Soil enzymes, acting as the key players involved in the catalytic reactions, play a pivotal role in the decomposition of organic matter (Dick 1994; Yao et al. 2006). Therefore, any factor that affects the soil microbial population will necessarily alter soil enzyme activities (Vallejo et al. 2010). Research carried out by Puri and Ashman (1998) indicate temperature is an important factor affecting the activity of soil microorganisms. Mikan et al. (2002) suggest that low winter temperatures can reduce or inhibit the activity of soil enzymes. However, the studies using different methods report revealed that FTCs might increase the activity of soil psychrophilic microorganisms (Hentschel et al. 2008). Although the effects of FTCs on the activity and composition of soil microbes has been extensively studied (e.g. Edwards et al. 2006; Koponen et al. 2006; Sharma et al. 2006; Männist et al. 2009), the changes in enzyme activities and active carbon fractions following FTCs (Yergeau and Kowalchuk 2008) are rarely reported, particularly in wetland soil. Wetlands are important carbon reservoir of terrestrial ecosystems. The turnover of soil carbon pools in wetland ecosystems, as well as carbon and carbon sink processes, play an extremely important role in global climate change (Matthews and Fung 1987). Especially, the carbon cycle has become hot topics in current scientific research during freeze-thaw period.

Global warming causes changes in soil patterns in seasonal freeze-thaw regions. We hypothesized that climate warming would lead to changes in soil freeze-thaw amplitude, frequency and soil water content, and their changes would stimulate the activity of soil enzyme activities and soil active organic carbon fractions change. To test this hypothesis, based on the principle of “typicality and consistency”, soil was selected from undisturbed *Deyeuxia purpurea* wetland (UDPW), disturbed *Deyeuxia purpurea* wetland (DDPW) and adjacent rice paddy field (RP) in the Sanjiang Piain, Northeast China for laboratory-simulated experiments of FTCs. The effects of different freeze-thaw frequency, water content and amplitude on soil active organic carbon and soil enzyme activities in different types and different active layers were emphatically discussed. This study aims to provide a fundamental basis for understanding the influence mechanism of FTCs for different type wetlands.

Materials and Methods

Description of the Study Area

The Sanjiang Plain is located in the northeastern part of Heilongjiang Province, China, ranging from 43°49′55″to 48°27′40″ in latitude and from 129°11′20″ to 135°05′26″ in longitude, with an elevation of lower than 200 m in most of the region (Wang et al. 2011). It is the most concentrated area of freshwater wetlands in China (Fu et al. 2020). The local climate is humid and semihumid continental monsoon-type climate, with annual rainfall varying from 500 to 600 mm, and the mean monthly temperature varying from − 18℃ in January to 22℃ in July (Fu et al. 2020). Dominant wetland plants include *Deyeuxia purpurea, Carex lasiocarpa*, *Carex meyeriana*, *Betula fruticosa*, *Salix brachypoda*, *Calamagrostis angustifolia* (Wang et al. 2011). Soil types are meadow-boggy soil, humus fen soil and submersible white pulp soil (Song et al. 2014).

Sample Collection and Experiment Design

At the end of October 2015, the soil samples from undisturbed *Deyeuxia purpurea* wetlands of the Honghe national nature reserve, disturbed *Deyeuxia purpurea* wetlands outside the reserve and adjacent rice paddy fields were collected on the Sanjiang Plain as follows: triplicate per type, collected from three sites, each site of 10×10 m^2, at an interval of 30 m. Specific location of sampling point are shown in Table 1. Soil samples were collected from three layers (0–10, 10–20 and 20–30 cm) using a soil core sampler after removal of the surface weed and ground vegetation. The soil samples were selected with multi point mixing methods, and

Table 1 Description of sampling sites in the Sanjiang Plain, Northeast China

Site	Undisturbed *Deyeuxia purpurea* wetland	Disturbed *Deyeuxia purpurea* wetland	Rice paddy
GPS coordinates	47°45′39"N 133°37′04"E	47°43′15″N 133°30′37″E	47°43′27″N 133°30′22″E
	47°45′42"N 133°37′04"E	47°39′08″N 133°29′03″E	47°39′11″N 133°29′02″E
	47°45′44"N 133°37′05"E	47°37′33″N 133°35′48″E	47°37′29"N 133°35′47"E
Wetland type	*Deyeuxia purpurea* wet grassland	*Deyeuxia purpurea* wet grassland	Rice paddy
Hydrological features	Perennial flooded	Seasonal flooded	Seasonal flooded
Soil type	Humic Cryaquepts	Humic Cryaquepts	Aquandic Cryaquepts

then put in sealed plastic bags with headspace air removed and transported to the laboratory within 72 h and screened using a 4 mm soil sieve, and stored at 4 ℃ for simulation experiments. 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. The soil physical-chemical properties are shown in Table 2.

200 g homogeneous soil samples were weighed and placed in a 250 mL culture bottle, respectively. The water content was adjusted using deionized water to the highest water-holding capacity of 60% and 80%, and the samples were cultured in a 20℃ for one week to restore the microbial activity. The bottleneck was sealed using plastic film to minimize water evaporation, while retaining a ventilation hole. The samples were weighed regularly and kept at constant weight. Based on the temperatures during FTCs in the Sanjiang Plain, a large (from − 10 to 10 °C) and a small (from − 5 to 5 °C) amplitudes of FTCs were set. The soil samples were frozen in a cryogenic incubator (model: LRH-500CB) at -10 °C for 24 h and then thawed at 10 °C for 24 h as a freeze-thaw cycle. the small treatment was the same but freezing and thawing temperatures of -5 °C and 5 °C respectively. 15 freeze-thaw cycles were carried out for a total of 30 days. Simultaneously, the control samples were kept at a constant temperature of 10 °C and 5 °C, respectively, without the freeze-thaw treatment. Soil active organic carbon fractions (DOC and MBC) and enzyme activities (cellulase, invertase and catalase) were determined before FTC and after the 3rd, 5th, 10th and 15th FTC.

Analytical Methods

Soil organic carbon was determined by the Multi N/C 2100 TOC analyzer (Analytik Jena AG, Germany) high temperature combustion method. TN (Kjeldahl digestion) and pH (potentiometer method) were determined following the laboratory methods described by Zhang (2000). Soil DOC was determined by the method of Jones and Willett (2006). Fresh soil (10 g) and distilled water (100 mL) were mixed in a triangular flask and then oscillated for 30 min using a shaker at approximately 230 rpm, and then centrifuged for 20 min at 12,000 rpm at room temperature. The supernatant was filtered through a 0.45 µm filter membrane and analyzed using a Multi N/C 2100 TOC analyzer. Soil MBC was determined by a chloroform fumigation-K_2SO_4 extraction

Table 2 Soil physical and chemical properties for natural wetland and farmed soils in the Sanjiang Plain, northeast China

Type	Layer(cm)	Soil organic carbon(g/kg)	Total nitrogen(g/kg)	C/N	pH	Maximum water holding capacity (%)
UDPW	0–10	47.76 ± 6.32a	3.81 ± 0.52a	12.54 ± 0.81a	5.64 ± 0.16b	85.91 ± 9.08a
	10–20	36.52 ± 4.16a	2.64 ± 0.39b	13.83 ± 0.52a	5.83 ± 0.11ab	52.51 ± 6.81b
	20–30	20.43 ± 1.96b	1.12 ± 0.32c	18.45 ± 0.33b	6.58 ± 0.18a	42.50 ± 3.79b
DDPW	0–10	30.47 ± 4.62a	2.89 ± 0.45a	10.67 ± 2.1b	5.72 ± 0.18b	79.55 ± 5.12a
	10–20	20.03 ± 2.84b	2.07 ± 0.21b	9.67 ± 0.57ab	5.98 ± 0.21ab	47.20 ± 2.82b
	20–30	15.30 ± 3.05b	1.06 ± 0.19c	14.53 ± 2.91a	6.31 ± 0.16a	40.63 ± 1.77b
RP	0–10	28.03 ± 6.94a	3.96 ± 0.53a	7.10 ± 1.72b	5.70 ± 0.11b	53.27 ± 3.07a
	10–20	23.4 ± 4.06ab	3.44 ± 0.23a	9.67 ± 0.57b	5.93 ± 0.09b	43.65 ± 2.49b
	20–30	18.4 ± 1.39b	1.59 ± 0.27b	11.82 ± 2.14a	6.32 ± 0.14a	40.04 ± 1.38b

Values are indicative of the mean ± SE (n = 3). Different lower case letters denote significant differences among treatments ($P < 0.05$, Tukey's HSD test) *UDPW* undisturbed *Deyeuxia purpurea* wetland, *DDPW* disturbed *Deyeuxia purpurea* wetland, *RP* rice paddy, *pH* soil: water 1:5

method. Soil (20 g) was fumigated with trichloromethane for 24 h. Fumigated and non-fumigated samples were extracted using K_2SO_4 (0.5 mol/L) for approximately 30 min and the total organic carbon concentration of leach liquor were determined using a Multi N/C 2100 TOC analyzer. The MBC was calculated by the following equation (Zhang et al. 2007):

$$\mathrm{MBC} = (\mathrm{fumigated\ carbon} - \mathrm{non\ fumigated\ carbon})/0.45 \tag{1}$$

The cellulase and invertase activities were determined by the methods Ge et al. (2010) and Guan (1986), using carboxymethylated cellulose and sucrose as substrates, respectively. These measurements were expressed as mg glucose (g soil 72 h)$^{-1}$ and (g soil 24 h)$^{-1}$ for cellulase and invertase activities. The catalase activity was determined by the permanganimetric method (Guan 1986), using dhydrogen peroxide as substrate. The measurement was expressed as ml $KMnO_4$ (g soil 20 min)$^{-1}$ for catalase activity.

Statistical Analysis

Statistical analysis was performed using SPSS version 19.0 for Windows (SPSS, Inc., USA), and figures were plotted using Microsoft Excel 2010 and Origin8.0 for Windows. Multivariate analysis of variance (MANOVA) was used to describe the effects of the soil water content, amplitude and frequency of FTCs on soil active organic carbon fractions and enzyme activities of the three type wetlands for each soil layer. Pearson correlation test was used to examine the correlation 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

Responses of DOC to FTCs

The experimental data show that the freeze-thaw frequency and water content had a significant effect on the soil DOC concentration ($P < 0.05$; Table 3), while the amplitude of the freeze-thaw exerted only significant effect on the DOC concentration of three types of soil in the 0–10 cm depth ($P > 0.05$; Table 3). However, the freeze-thaw amplitude increased the soil DOC concentrations in each layer of the three types of wetland, all values were higher than FTC(0), and the highest values all appeared in the 0–10 cm soil layer of the UDPW, DDPW and RP at the sixth FTCs, which were about 718.87 mg kg^{-1}, 615.34 mg kg^{-1} and 347.18 mg kg^{-1} at small amplitude, and 688.85 mg kg^{-1}, 612.12 mg kg^{-1} and 293.55 mg kg^{-1} at large amplitude (Fig. 1). The DOC concentrations were significantly affected by the interaction between freeze-thaw amplitude and water content between amplitude and frequency, and between water content and frequency ($P < 0.05$; Table 3), but were not significantly affected by the interaction among amplitude, water content and frequency ($P > 0.05$; Table 3).

The DOC increments caused by freeze-thaw treatment varying with cycles showed consistent trend among various soil types or layers (Fig. 1). Therefore, we calculated the average increments of fifteen cycles, and compared the differences of DOC increments in various types and soil layers (Fig. 2). The average DOC increments varied greatly from types in the order of UDPW > DDPW > RP. For the same type wetland, with the increase of depth, the freeze - thaw effect weakens, and the DOC increment is the largest in 0-10 cm soil, followed by 10-20 cm and by 20-30 cm soil sequentially. Both freeze-thaw amplitude and water content affected the soil DOC concentration. The average DOC increments were greater at 80% water-holding capacity compared to those at 60% for all soil types and layers, except for RP soil layers from 0 to 10 cm and 10–20 cm (Fig. 2). The greatest effect was the freeze-thaw cycles under the condition of small amplitude and high water content interaction (Fig. 2a).

Responses of MBC to FTCs

The FTCs significantly decreased the soil MBC concentration in the three soil layers of UDPW, DDPW and RP by approximately 4.2–41.5%, 5.5–36.1% and 5.4–33.6% at the large amplitude, and 5.6–27.6%, 4.8–27.5% and 4.9–24.2% at the small amplitude, respectively (Fig. 3). The freeze-thaw frequency significantly decreased the soil MBC concentration of the three type wetlands ($P < 0.05$; Table 3). However, both freeze-thaw amplitude and water content had no significant effect on soil MBC concentrations ($P > 0.05$; Table 3). The freeze-thaw amplitude and water content interaction had a significant effect on the MBC concentration across the three RP soil layers; the freeze-thaw amplitude × frequency interaction had a significant effect on 0–10 cm and 10–20 cm UDPW ; Frequency ×water content interaction had a significant effect on 0–10 cm UDPW and 0–10 cm and 10–20 cm DDPW ($P < 0.05$; Table 3). The interaction among amplitude, frequency and water content had no significant effect on the MBC concentration ($P > 0.05$; Table 3). The test results indicated that the effects of FTCs on MBC concentration in soil active layer of different types of wetlands showed a consistent trend (Fig. 3): with an increase in freeze-thaw frequency, the soil MBC concentration in the three active layers decreased initially and

Table 3 MANOVA results (*p* values) for DOC and MBC concentrations with two amplitudes of freeze–thaw, fifteen FTCs and two water holding capacities (n = 3)

Variables	Type	Layer(cm)	Amplitude	Water content	Frequency	Amplitude×Water content	Amplitude×Frequency	Frequency×Water content	Amplitude×Frequency×Water content
DOC	UDPW	0—10	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.172
		10—20	**0.006**	**0.000**	**0.000**	**0.020**	**0.026**	**0.000**	0.625
		20—30	0.120	**0.000**	**0.000**	0.154	**0.000**	**0.031**	**0.017**
	DDPW	0—10	**0.001**	**0.000**	**0.001**	0.156	0.434	**0.035**	**0.047**
		10—20	0.186	**0.000**	**0.000**	**0.001**	**0.003**	**0.007**	**0.003**
		20—30	**0.017**	**0.000**	**0.000**	**0.049**	**0.000**	**0.000**	**0.000**
	RP	0—10	**0.000**	**0.002**	**0.000**	0.648	0.109	0.339	0.219
		10—20	0.377	**0.000**	**0.002**	0.124	**0.001**	**0.037**	**0.003**
		20—30	0.081	**0.012**	**0.009**	**0.026**	**0.002**	**0.011**	**0.000**
MBC	UDPW	0—10	0.719	0.440	**0.000**	0.256	**0.015**	**0.024**	0.635
		10—20	0.085	**0.027**	**0.000**	**0.049**	**0.005**	0.792	0.517
		20—30	**0.047**	**0.006**	**0.000**	0.075	0.291	0.656	0.245
	DDPW	0—10	**0.036**	0.720	**0.000**	0.531	0.249	0.158	0.907
		10—20	0.245	**0.047**	**0.000**	0.226	0.528	0.055	0.113
		20—30	0.326	0.181	**0.000**	0.730	0.326	0.772	0.895
	RP	0—10	**0.047**	**0.007**	**0.000**	**0.000**	0.099	**0.001**	0.326
		10—20	0.647	0.205	**0.000**	**0.002**	**0.007**	**0.041**	0.490
		20—30	0.747	0.092	**0.000**	**0.011**	0.059	0.793	0.794

Boldface values indicate significant effects at $p < 0.05$

DOC dissolved organic carbon, *MBC* microbial biomass carbon, *UDPW* undisturbed *Deyeuxia purpurea* wetland, *DDPW* disturbed *Deyeuxia purpurea* wetland, *RP* rice paddy

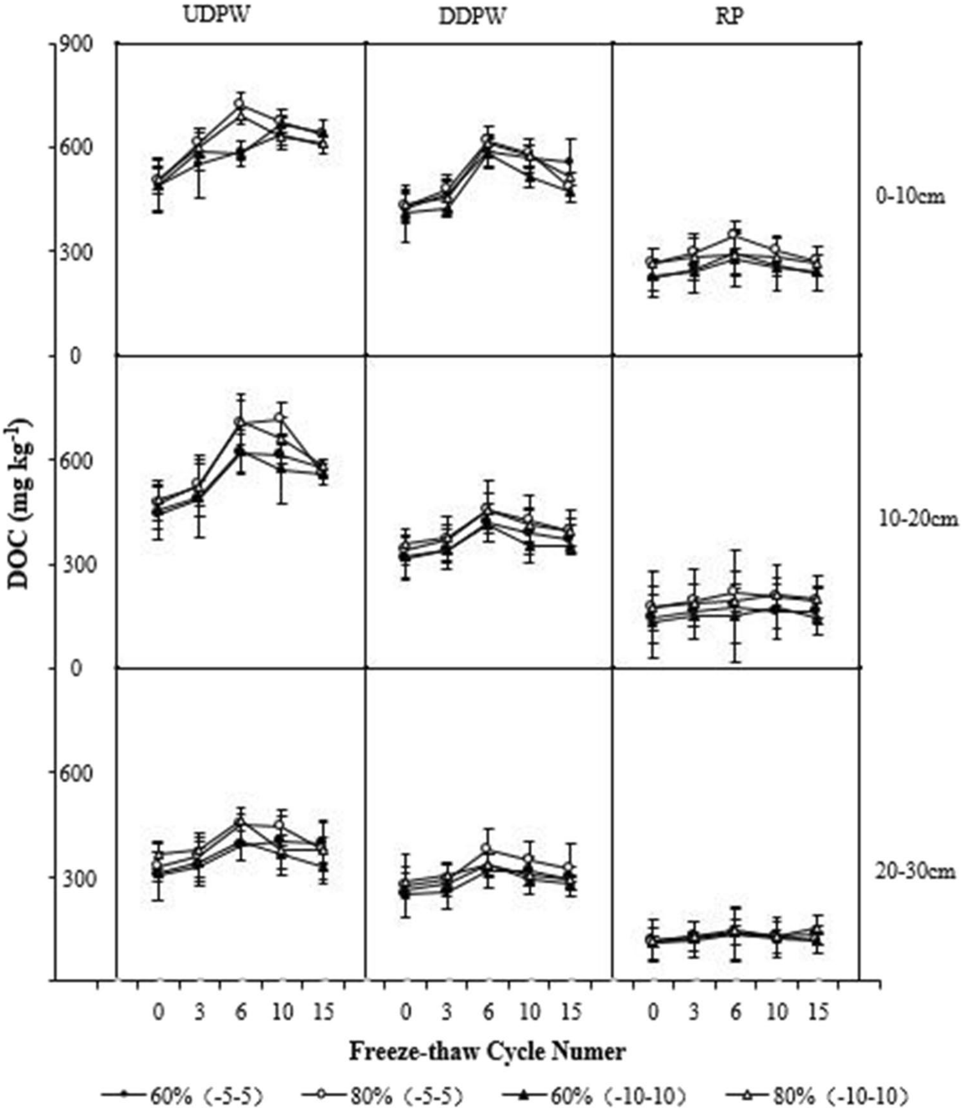


Fig. 1 Dissolved organic carbon (DOC) concentrations in soil. The number of freeze thaw cycle is identified on the *x*-axis, the DOC concentration in the soil is identified on the *y*-axis. 60% and 80% mean the 60% and 80% of maximum water holding capacity, respectively. (-5-5) and (-10-10) mean (-5 to 5 °C) and (-10 to 10 °C), respectively. Error bars represent the standard error of the mean of three parallel samples

then increased and the highest values were smaller than that of the unfrozen control sample at FTC (0) (Fig. 3).

The MBC decrements caused by FTCs treatment varying with cycles showed a consistent trend among various soil types or layers (Fig. 3). Therefore, we calculated the average MBC decrements of fifteen cycles, and compared the differences in various wetlands and soil layers (Fig. 3). The average MBC decrements caused by FTCs decreased with the increase of soil depth, in the order of 0–10 cm > 10–20 cm > 20–30 cm (Fig. 3). At the smaller amplitude of FTC, the influence of 60% water-holding capacity on MBC was greater than that of 80%. However, at larger amplitude of FTC, the influence of 80% water-holding capacity was greater than that of 60% (Fig. 4). For the smaller and larger amplitude FTC, the MBC concentration of the different soil types varied as follows: DDPW > UDPW > RP (Fig. 4a), and UDPW > DDPW > RP, respectively (Fig. 4b). At the same time, the experimental results showed that freeze-thaw amplitude and water content interaction affected the soil MBC concentration. The larger amplitude and higher water content had the greatest influence on soil MBC (Fig. 4).

Responses of Soil Enzyme to FTCs

Consistent with the change of MBC concentration, FTCs decreased activities of cellulase, invertase and catalase in the three soil layers, UDPW, DDPW and RP (Figs. 5, 6 and 7). The freeze-thaw frequency had

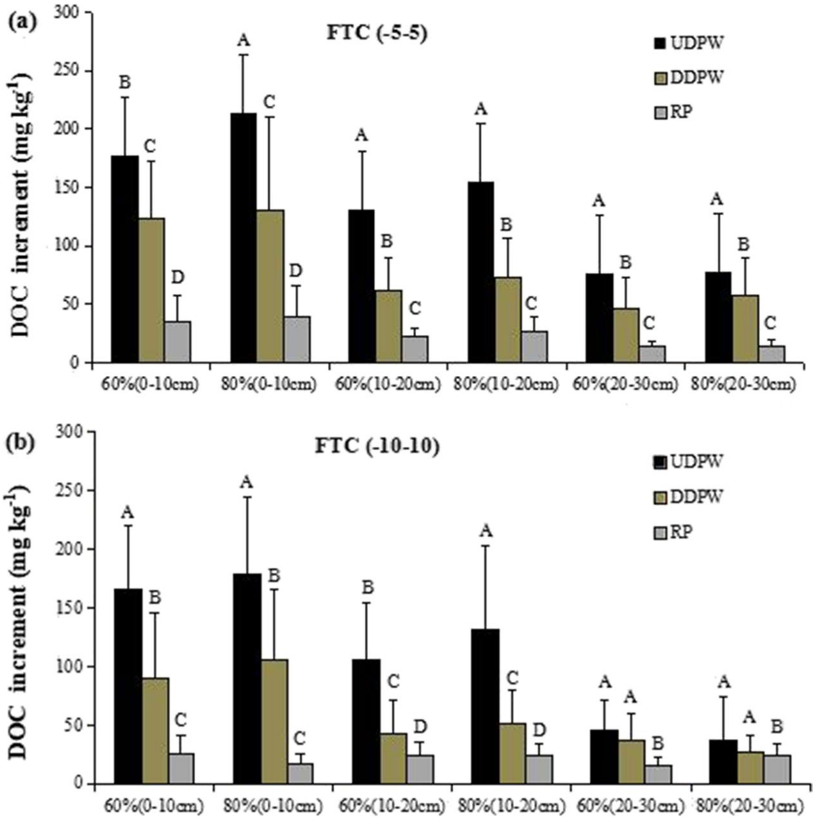


Fig. 2 Average increment of dissolved organic carbon (DOC) concentration of 15 cycles caused by freeze-thaw treatment (mean ± standard error, n = 3). The soil layer and type are identified on the *x-axis*, and the average increment percentage of DOC concentrations of fifteen cycles caused by freeze-thaw treatment is identified on the *y-axis*. 60% and 80% mean the 60% and 80% of maximum water holding capacity, respectively. (-5-5) and (-10-10) mean (-5 to 5 °C) and (-10 to 10 °C), respectively

a significant effect on the three enzyme activities in all three type wetland soils ($P < 0.05$; Table 4). The freeze-thaw amplitude had no significant effect on invertase and catalase activity for DDPW ($P > 0.05$; Table 4). The water content had a significant effect on cellulose activity in DDPW and RP, and invertase activity in RP ($P < 0.05$; Table 4). The interaction between freeze-thaw amplitude and water content had a significant impact on cellulase activity of DDPW, invertase activity of 0–10 cm and 10–20 cm layers of UDPW, and 0–10 cm layer of RP; and catalase activity for 20–30 cm layer of DDPW and 10–20 cm layer of RP. The interaction between amplitude and frequency had a significant effect on cellulase activity for only DDPW($P < 0.05$; Table 4). The frequency×water content, and amplitude × frequency × water conten t interactions all had no impact on soil enzyme activities ($P > 0.05$; Table 4). The change in enzyme activities of the three types of soil caused by FTCs was consistent among various soil types or layers (Figs. 5, 6 and 7). The changes in three enzyme activities corresponded to those in the MBC concentrations: the three enzyme activities decreased early in the FTCs and then increased with an increase in freeze-thaw frequency. However, the highest value was still smaller than that of the unfrozen control sample at FTC (0) (Figs. 5, 6 and 7).

The three enzyme activities decrements caused by FTCs showed a consistent trend among various soil types or layers (Figs. 8, 9 and 10). Therefore, we calculated the average enzyme activities decrements of fifteen cycles, and compared the differences in various wetlands and soil layers. The three soil enzyme activities decreased with soil depth in the following order: 0–10 cm > 10–20 cm > 20–30 cm for the same soil type, and UDPW > DDPW > RP across different types soil. The freeze-thaw amplitude and water content affected the activities of the three soil enzymes, and larger amplitude and higher water content resulted in a more significant impact on soil enzyme activities (Figs. 8, 9 and 10).

Relationships Between Soil Active Organic Carbon and Enzyme activities

After 15 FTCs for both UDPW and DDPW of soil, the correlation analysis showed that the DOC concentration was significantly correlated with the MBC concentration ($P < 0.01$; Table 5) and the activities of cellulase and invertase ($P < 0.05$; Table 5). Meanwhile, the positive relationships

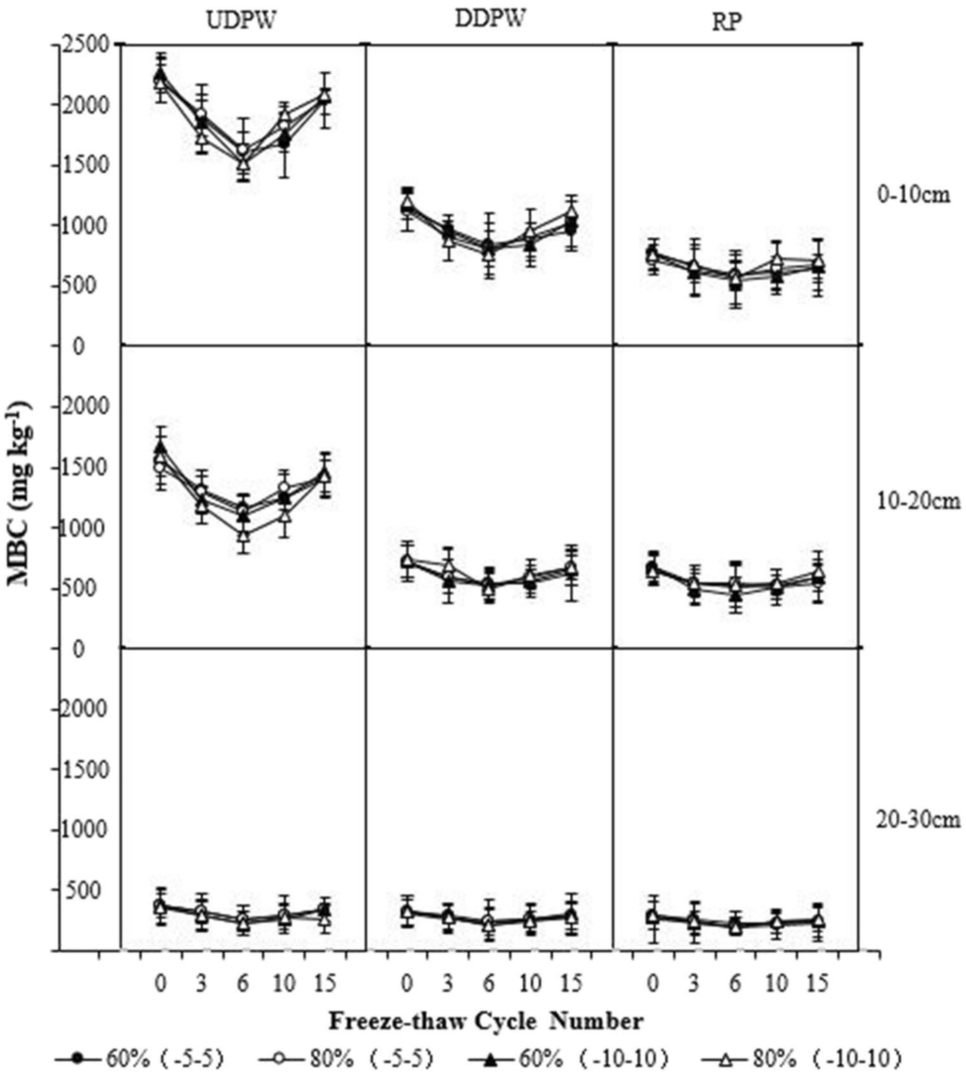


Fig. 3 Microbial biomass carbon (MBC) concentrations in soil. The number of freeze thaw cycle is identified on the *x*-axis, and the MBC concentration in the soil is identified on the *y*-axis. 60% and 80% mean the 60% and 80% of maximum water holding capacity, respectively. (-5-5) and (-10-10) mean (-5 to 5 °C) and (-10 to 10 °C), respectively. Error bars represent the standard error of the mean of three parallel samples

between the MBC concentration and the activities of the three enzymes were also significant ($P < 0.01$; Table 5). In addition, the significant correlations were observed between the three soil enzymes ($P < 0.01$; Table 5). However, after 15 FTCs for the RP soil, there were significantly correlation between DOC, MBC, cellulase and invertase ($P < 0.05$; Table 5), and other variables between them were not correlated ($P > 0.05$; Table 5).

Discussion

Responses of Active Organic Carbon Fractions to FTCs

As a component of soil active organic carbon, DOC is an important substrate that can be used by microorganisms and is closely related to the activity of microbes in the soil (Marschner and Kalbitz 2003; Matzner and Borken 2008). Experimental results indicated that both the freeze-thaw amplitude and water content significantly affected the concentration of soil DOC (Table 3). With the increase in the freeze-thaw frequency firstly resulted in an increase in DOC concentration followed by a decrease, with the lowest value greater than that of the unfrozen control sample FTC(0) (Fig. 1). This indicated that freeze-thaw cycles stimulated the release of soil DOC. This may be attributed to the fact that freeze-thaw cycles kill microorganisms just like chloroform fumigation, causing microorganism death and root decomposition. During this process, small molecular sugars and amino acids were released, which would improve the soil organic matter and thus increase the DOC content (Staricka and Benoit 1995; Tierney et al. 2001). The

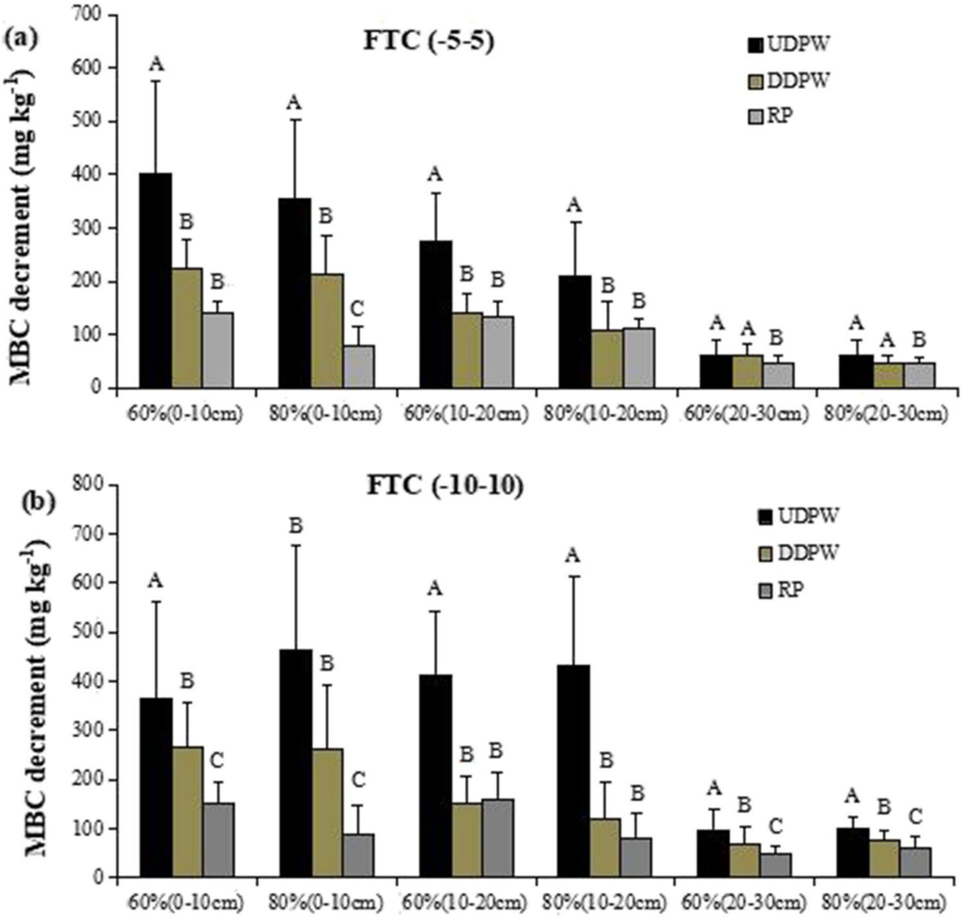


Fig. 4 Average decrement of microbial biomass carbon (MBC) concentration of fifteen cycles caused by freeze-thaw treatment (mean ± standard error, n = 3). The soil layer and type are identified on the *x-axis*, and the average decrement percentage of MBC concentrations of fifteen cycles caused by freeze-thaw treatment is identified on the *y-axis*. 60% and 80% mean the 60% and 80% of maximum water holding capacity, respectively. (-5-5) and (-10-10) mean (-5 to 5 °C) and (-10 to 10 °C), respectively

freeze-thaw frequency significantly affected the soil DOC concentration (Table 3). With an increase in the freeze-thaw frequency, soil microorganisms were gradually adapted to the changing conditions, which resulted in less microorganism deaths, thus decreasing DOC release, and the original soil DOC was constantly consumed and decomposed by microorganisms, therefore the soil DOC content decreased after several FTCs. This result was in agreement with other reported studies (Grogan et al. 2004; Feng et al. 2007; Matzner and Borken 2008; Chaer et al. 2009).

The soil DOC increment decreases gradually with the increase of soil depth (Fig. 2). This was possibly due to the number of soil microorganisms and fine roots decreasing as depth increased, and the influence of freeze-thaw was decreased with depth, thus reducing the DOC increment with depth. The DOC increment for different soil type wetlands were as follows: UDPW > DDPW > RP. This may be due to changes in soil nutrients, permeability, temperature and other physicochemical properties when the wetlands are reclaimed for farmland (Mailapalli et al. 2010), which affects the plant growth environment and the quantity of litter, and leads to the change and decrease of soil microbial population. Soil leaching and nutrient loss gradually increase for DDAW and RP due to human disturbance, resulting in decreased DOC. Additionally, DOC is also related to the soil content of organic matter. The soil aggregate is more stable for soil with a high organic carbon content and better soil structure, so that the organic carbon wrapped in soil aggregates will be released gradually; in contrast, soil aggregates with lower organic carbon content are easier to depolymerize and the soil organic carbon to be released rapidly (Herrmann and Witter 2002). The soil organic carbon content is reduced by half and the number of soil microorganisms and fine roots all decreased after the wetland is reclaimed for farmland in the Sanjiang Plain (Table 1). Therefore, the freeze-thaw cycle has the lowest influence on DOC concentration in farmland.

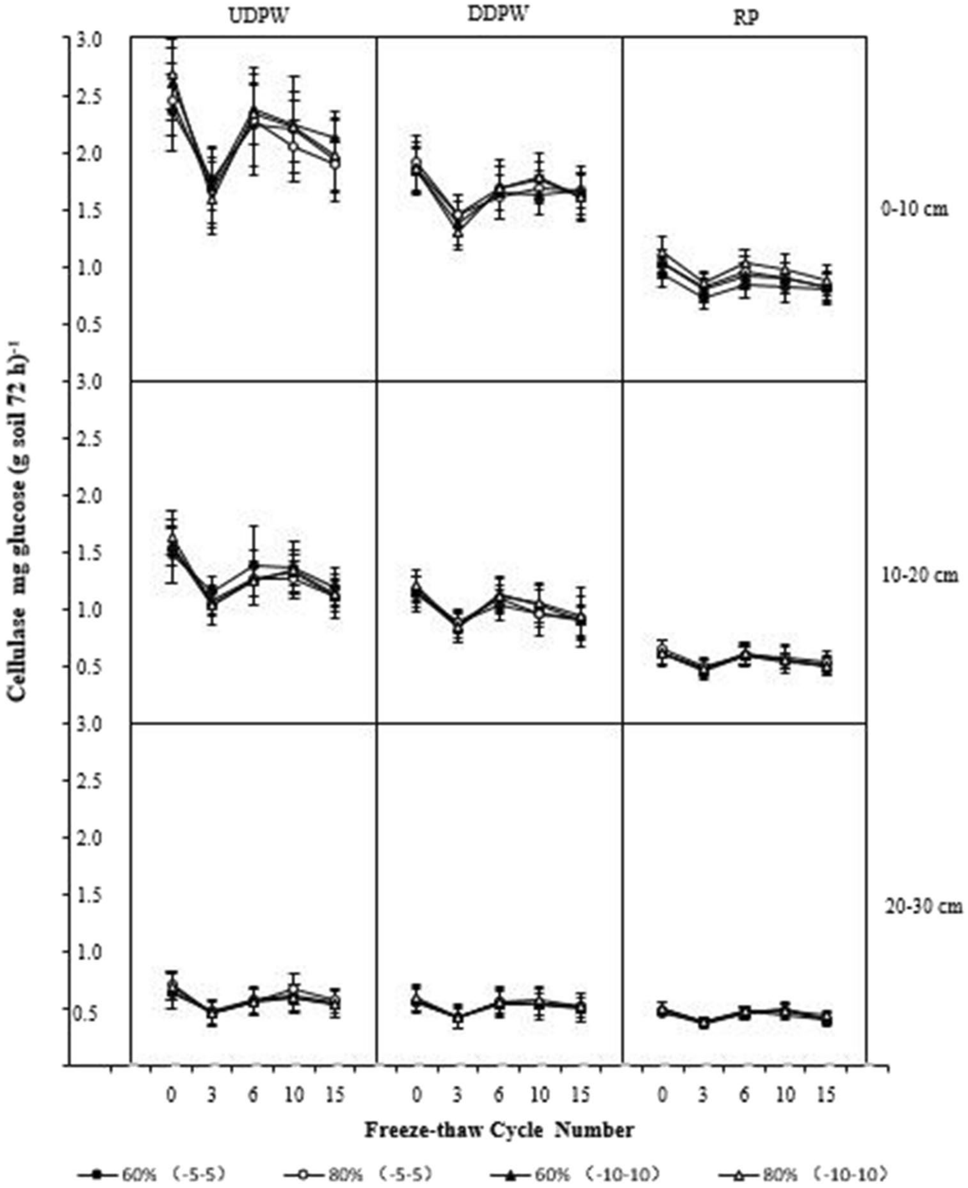


Fig. 5 Cellulase activity in soil. The number of freeze-thaw cycle is identified on the *x-axis*, and the cellulose activity in the soil is identified on the *y-axis*. 60% and 80% mean the 60% and 80% of maximum water holding capacity, respectively. (-5-5) and (-10-10) mean (-5 to 5 °C) and (-10 to 10 °C), respectively. Error bars represent the standard error of the mean of three parallel samples

It was found that the interaction of small amplitude and high water content had the greatest influence on DOC increment (Fig. 2), which was in agreement with the findings of Oztas and Fayetorbay (2002), Wang et al. (2009) and Wang et al. (2014). Wang et al. (2009) reported that with appropriate water content, the stability of aggregates could be enhanced even if freezing temperature fluctuated in a wide range. However, Oztas and Fayetorbay (2002) concluded that lower freezing temperatures decomposed aggregates more easily.

The soil MBC is a fraction of active organic carbon released from ruptured cells, which can provide an indication of soil microbial biomass and activity (Poelson et al. 1987). In contrast to the change in DOC concentration, FTCs significantly lowered the MBC concentration in various type soils or active layers (Fig. 3). This finding was consistent with that reported by Larsen et al. (2002), Chaer et al. (2009) and Wang et al. (2014). Additionally, the correlation analysis indicated that soil DOC was significantly related to soil MBC (Table 5), possibly due to the reduced MBC increasing the DOC content. However, the relative contribution of MBC to the increase in DOC concentration in FTC-treated soils remains unclear. Due to

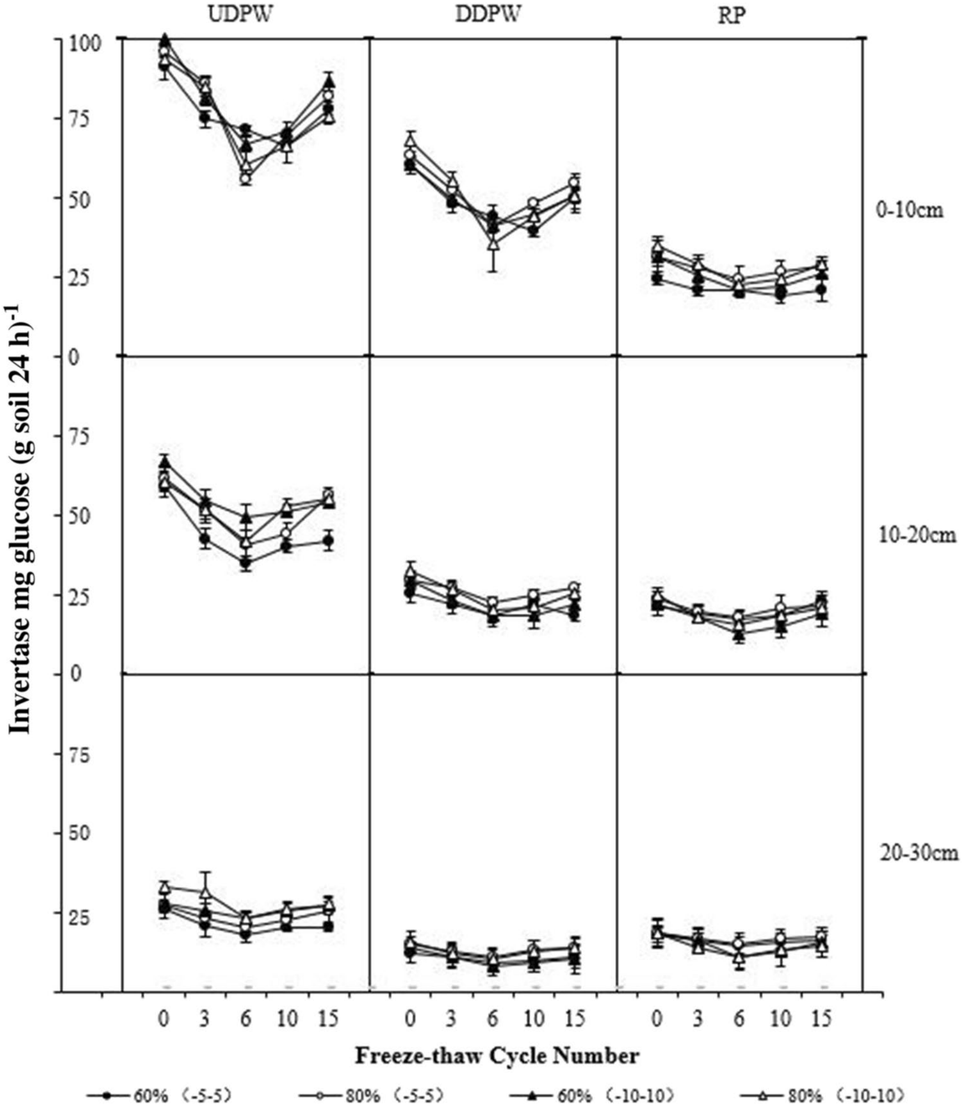


Fig. 6 Invertase activity in soil. The number of freeze-thaw cycle is identified on the *x-axis*, and the invertase activity in the soil is identified on the *y-axis*. 60% and 80% mean the 60% and 80% of maximum water holding capacity, respectively. (-5-5) and (-10-10) mean (-5 to 5 °C) and (-10 to 10 °C), respectively. Error bars represent the standard error of the mean of three parallel samples

the bactericidal effect, FTCs,can destroy microbial cells and cause mass microorganism mortality (Larsen et al. 2002). Skogland et al. (1988) and DeLuca et al. (1992) reported that FTC killed 50% of a microbial population and led to the release of intracellular substances, including DOC and MBC. This could explain the decrease in soil MBC concentration and increase in DOC concentration in the early stage of the FTCs. The freeze-thaw frequency had a significant impact on MBC concentrations of the three type wetland soils (Table 3), as soil microorganisms gradually were adapted to the influence of the freeze- thaw conditions, thus decreasing microbe mortality as FTCs increased, MBC concentrations gradually increased, but were still smaller than the unfrozen control sample FTC (0) (Fig. 3).

The average decrements of MBC caused by freeze-thaw cycles is UDPW ≈ DDPW > RP in different type wetlands, and 0–10 cm > 10–20 cm > 20–30 cm in varied soil layers, which may be due to the different microbial and plant roots in different types of wetlands and different soils, so the effect of freeze-thaw is not obvious. In addition, Mostaghimi et al. (1988) and Wang et al. (2009) reported that the freeze-thaw action was also related to soil type, organic matter, water content, initial aggregate size, freezing temperature and other factors. However, under the interaction of large amplitude and high water content, the RP showed

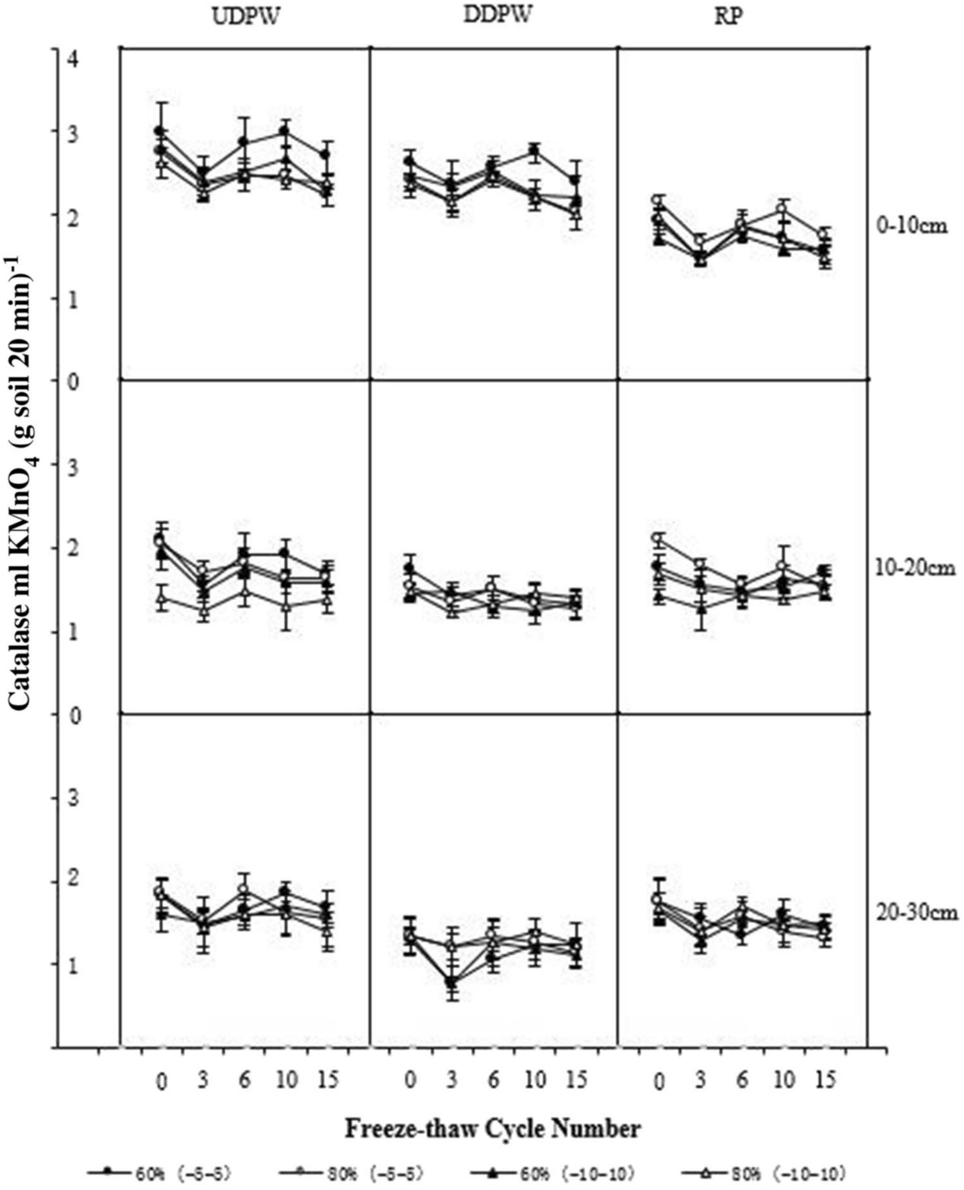


Fig. 7 Catalase activity in soil. The number of freeze-thaw cycle is identified on the *x-axis*, and the catalase activity in the soil is identified on the *y-axis*. 60% and 80% mean the 60% and 80% of maximum water holding capacity, respectively. (-5-5) and (-10-10) mean (-5 to 5 °C) and (-10 to 10 °C), respectively. Error bars represent the standard error of the mean of three parallel samples

0–10 cm < 10–20 cm < 20–30 cm, which was consistent with findings of Barbhuiya et al. (2004). MBC was less uniform as the soil depth increased in wetland soil. At the same time, the rice paddy fields are ploughed every year, leading to big changes in the upper and lower layers of the soil, which may also be responsible for this result. The freeze-thaw amplitude and soil water content also affected MBC concentrations (Fig. 3), and the larger amplitude and higher water content interaction had the greatest effect on soil MBC concentrations (Fig. 4). Although the effect of the amplitude of FTCs was insignificant on the MBC concentrations of the three type wetland soils, but a larger freeze-thaw amplitude causes more damage to microorganisms, because large amplitude of FTCs could kill more microorganisms and limiting of microbial activity (Schimel et al. 2004).

Responses of Soil Enzyme Activities to FTCs

Soil enzymes are the key media of soil biological process and are involved in all soil biochemical processes (Dick 1994; Yao et al. 2006), and soil enzyme activities directly affect the rate of the carbon cycle in wetlands and play an important role in the carbon balance and global climate

Table 4 MANOVA results (*p* values) for cellulase, invertase and catalase concentration with two amplitudes of freeze–thaw, fifteen FTCs and two water holding capacities (n = 3)

Variables	Type	Layer (cm)	Amplitude	Water content	Frequency	Amplitude×Water content	Amplitude×Frequency	Frequency×Water content	Amplitude×Frequency×Water content
Cellulase	UDPW	0—10	**0.001**	0.056	**0.000**	0.245	0.334	0.671	0.753
		10—20	**0.000**	**0.003**	**0.000**	**0.033**	0.612	0.894	0.947
		20—30	0.256	**0.039**	**0.041**	0.921	0.351	0.957	0.914
	DDPW	0—10	**0.004**	0.832	**0.000**	0.326	**0.001**	0727	0.937
		10—20	**0.005**	0.127	**0.000**	0.226	**0.000**	0.155	0.207
		20—30	**0.001**	0.254	**0.000**	0.623	**0.001**	0.729	0.873
	RP	0—10	**0.002**	0.566	**0.000**	0.953	0.271	0.956	0.987
		10—20	0.217	0.267	**0.000**	0.853	0.192	0.898	0.916
		20—30	**0.035**	**0.001**	**0.003**	0.079	0.846	0.212	0.735
Invertase	UDPW	0—10	**0.004**	**0.045**	**0.000**	**0.000**	0.682	**0.000**	**0.001**
		10—20	**0.000**	**0.003**	**0.000**	**0.000**	**0.045**	**0.000**	0.072
		20—30	**0.000**	**0.002**	**0.000**	0.890	0.829	0.566	0.299
	DDPW	0—10	0.867	**0.023**	**0.000**	0.306	0.228	**0.038**	0.304
		10—20	0.880	**0.000**	**0.000**	0.126	**0.022**	0.505	0.813
		20—30	0.445	**0.000**	**0.000**	0.994	0.208	0.164	0.796
	RP	0—10	**0.000**	**0.000**	**0.000**	**0.001**	**0.002**	0.323	0.715
		10—20	**0.050**	0.150	**0.034**	0.273	0.456	0.398	0.420
		20—30	**0.000**	0.191	**0.000**	0.617	**0.016**	0.283	0.275
Catalase	UDPW	0—10	**0.004**	0.483	**0.000**	0.661	0.314	0.054	0.992
		10—20	**0.024**	**0.001**	**0.000**	0.360	0.425	0.303	0.873
		20—30	**0.031**	**0.017**	**0.000**	0.221	0.166	0.769	0.528
	DDPW	0—10	0.241	**0.015**	**0.000**	0.526	0.838	0.854	0.998
		10—20	**0.000**	0.226	**0.000**	0.065	0.112	0.644	0.586
		20—30	0.110	**0.000**	**0.000**	**0.035**	0.167	0.680	0.412
	RP	0—10	**0.000**	**0.000**	**0.000**	0.058	0.562	0.783	0.770
		10—20	**0.000**	**0.000**	**0.000**	**0.006**	0.302	0.180	0.459
		20-30	**0.001**	**0.000**	**0.000**	0.586	0.625	**0.004**	**0.027**

Boldface values indicate significant effects at $p < 0.05$

UDPW undisturbed *Deyeuxia purpurea* wetland, *DDPW* disturbed *Deyeuxia purpurea* wetland, *RP* rice paddy

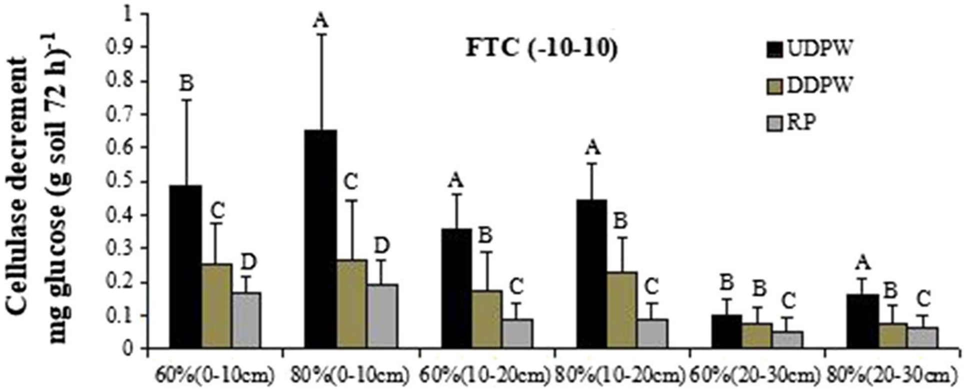
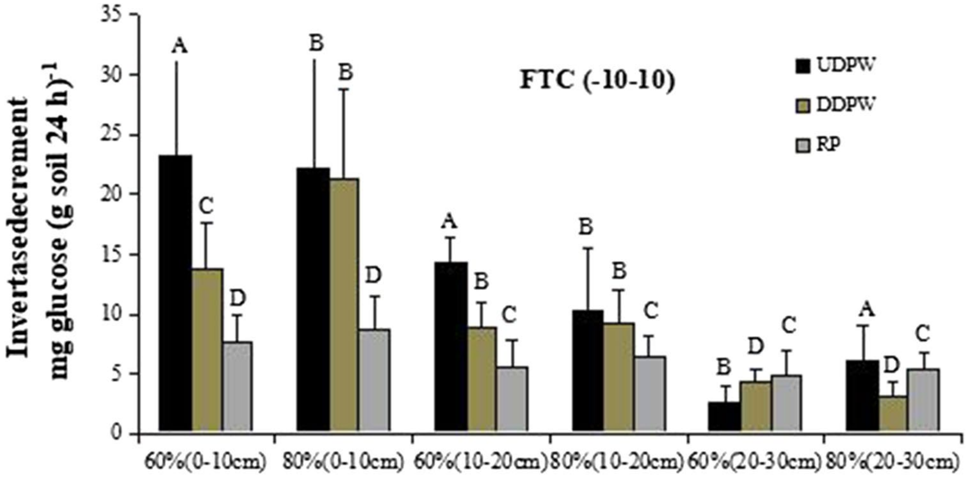


Fig. 8 Average decrement of cellulase activity of fifteen cycles caused by freeze-thaw treatment (mean ± standard error, n = 3). The soil layer and type are identified on the *x-axis*, and the average decrement percentage of MBC concentrations of fifteen cycles caused by freeze-thaw treatment is identified on the *y-axis*. 60% and 80% mean the 60% and 80% of maximum water holding capacity, respectively. (-10-10) mean (-10 to 10 °C)

change (Yao et al. 2006). Of them, acting as the oxidation-reduction enzymes in soil, catalase activity can be used to characterize soil humus degree and organic matter conversion rate (Zhou 1987). Cellulase and sucrase as hydrolase, are primarily involved in the decomposition of the compounds in the soil. They hydrolyze the high molecular weight compounds into simple small molecules, and are important enzymes involved in the mineralization of organic matter (Guan 1986).

Experimental data suggested that FTCs had a consistent effect on the soil enzyme activities and MBC concentration, i.e., short-term FTCs decrease the three soil enzyme activities. This result is consistent with most conclusions (Wang et al. 2014; Sorensen et al. 2018; Miura et al. 2019). The effect of FTCs on the soil enzyme activities mainly came from the effect on soil microbes (Vallejo et al. 2010). Therefore, FTCs had a similar impact on soil MBC and enzyme activities. In UDPW and DDPW soil, MBC was significantly positively correlated with three enzymes, which also supported the viewpoints ($P < 0.05$; Table 5). However, the weak correlation between MBC and soil enzyme activity, and between the enzymes (Table 5) in RP soil is possibly the result of intense interference by agricultural reclamation. 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, Chaer et al. (2009) used Andrew forest soil from the USA and found that freeze-thaw reduced the activity of β-glucosidase, but phosphatase and phenoloxidase activities were unchanged. The studies on the Antarctic soil column carried out by Yergeau and Kowalchuk (2008)

Fig. 9 Average decrement of invertase activity of fifteen cycles caused by freeze-thaw treatment (mean ± standard error, n = 3). The soil layer and type are identified on the *x-axis*, and the average decrement percentage of MBC concentrations of fifteen cycles caused by freeze-thaw treatment is identified on the *y-axis*. 60% and 80% mean the 60% and 80% of maximum water holding capacity, respectively. (-10-10) mean (-10 to 10 °C)



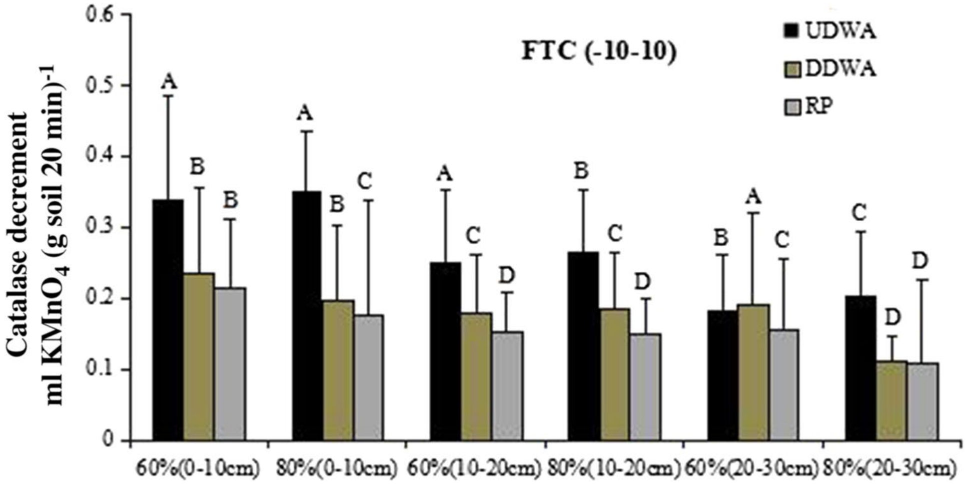


Fig. 10 Average decrement of catalase activity concentration of fifteen cycles caused by freeze-thaw treatment (mean ± standard error, n = 3). The soil layer and type are identified on the *x-axis*, and the average decrement percentage of MBC concentrations of fifteen cycles caused by freeze-thaw treatment is identified on the *y-axis*. 60% and 80% mean the 60% and 80% of maximum water holding capacity, respectively. (-10-10) mean (-10 to 10 °C)

revealed that freeze and thawing increased the activity of cellulase, but the effect was not significant. Wang et al. (2014) studied the permafrost from the Great Xingan Mountains and found that freeze-thaw reduced the activity of cellulase, amylase and invertase in the active layers. As the frequency of FTCs increased, these soil enzyme activities increased, but the highest value was still smaller than that of FTC(0) (Figs. 5, 6 and 7). In fact, some enzymes in frozen soils are not fully passivated, especially the enzymes in the cold regions. FTCs destroys microbial cells to release carbon and nitrogen nutrients for surviving microbes, thus increasing their activities (Koponen et al. 2006). In addition, the temperature, time and frequency of freeze-thaw and soil moisture before freezing all affected soil microbial activity and population structure (Koponen et al. 2006; Tan et al. 2014; Meisner et al. 2021). The freeze-thaw frequency had a significant effect on the three enzyme activities, while the amplitude had no significant effect on them. However, a large amplitude of FTCs could kill more microorganisms and decrease soil enzyme activities.

Table 5 The correlations between active organic carbon fractions and soil enzyme activities

		DOC	MBC	Cellulase	Invertase	Catalase
UDPW	DOC	1				
	MBC	0.981**	1			
	Cellulase	0.845**	0.792**	1		
	Invertase	0.936**	0.964**	0.895**	1	
	Catalase	0.679*	0.709**	0.911**	0.791**	1
DDPW		DOC	MBC	Cellulase	Invertase	Catalase
	DOC	1				
	MBC	0.934**	1			
	Cellulase	0.853**	0.861*	1		
	Invertase	0.942**	0.942**	0.906**	1	
	Catalase	0.960*	0.949**	0.912**	0.957**	1
RP		DOC	MBC	Cellulase	Invertase	Catalase
	DOC	1				
	MBC	0.872*	1			
	Cellulase	0.905*	0.605*	1		
	Invertase	0.808	0.870*	0.62	1	
	Catalase	0.898	0.487	0.54	0.496	1

* Significant at the 0.05 level ** Significant at the 0.01 level, *DOC* dissolved organic carbon, *MBC* microbial biomass carbon, *UDPW* undisturbed *Deyeuxia purpurea* wetland, *DDPW* disturbed *Deyeuxia purpurea* wetland, *RP* rice paddy

Conclusion

The sensitive responses of active organic carbon fractions and enzyme activities to FTCs were clearly observed in this laboratory-simulated experiment of three type wetland soils in the Sanjiang Plain. The freeze-thaw cycles significantly increased DOC concentration but decreased MBC concentration and soil enzyme activities (cellulase, invertase and catalase) in the active layer of the Sanjiang Plain. The freeze-thaw frequency had significant effects on active organic carbon fractions and enzyme activities. The freeze-thaw amplitude and water content affect soil active organic carbon concentration and soil enzyme activities. NoTably, the interaction between the small amplitude and high water content resulted in the greatest effect on soil DOC concentration. However, the responses of MBC concentration and enzyme activities to FTCs generally presented greater effects at the large amplitude and high water content interaction. The effects of freezing and thawing on different types of soil were different. The lower the content of soil organic carbon and the number of microbes, the smaller the effect of freeze-thaw. The correlation analysis showed that the DOC and MBC and enzymes of soils were significantly correlated, but the correlation between them was affected by human interference.

In the context of global warming and wetlands degeneration, we anticipate that more dissolved forms of carbon will release from wetlands if the frequency of FTCs and active layer depth increase. This will decrease the carbon storage of wetlands in the seasonal frozen regions. However, further studies are still needed to investigate how FTCs affect carbon cycles and enzyme activities. At present, most freeze-thaw cycle experiments are based on indoor simulation, which cannot fully reflect the actual change of active organic carbon fractions and enzyme activities. Therefore, future research should focus on field monitoring and reveal the response of soil organic carbon to the change in FTCs under the warming climate in wetlands.

Acknowledgements We gratefully acknowledge "National Natural Science Foundation of China" (No. 31670489); "Doctoral Fund project of Jiamusi University" (No. JMSUBZ2020-12) for financial support.

Author Contributions Jinqiu Guan, Fu Li and Hongwei Ni developed the idea of the study, participated in its design and coordination and helped to draft the manuscript. Chunxiang Song and Yude Wu contributed to the acquisition and interpretation of data. Xingtian Qi and Rongjun Qu provided critical review and substantially revised the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by [National Natural Science Foundation of China] (Grant numbers. No. 31670489); and [Doctoral Fund project of Jiamusi University] (Grant numbers No. JMSUBZ2020-12).

Data Availability The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Conflict of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Barbhuiya AR, Arunachalam A, Pandey HN, Arunachalam K, Khan ML, Nath PC (2004) Dynamics of soil microbial biomass C, N and P in disturbed and undisturbed stands of a tropical wet-evergreen forest. Eur J Soil Biol 40:113–121

Chaer GM, Myrold DD, Bottomley PJ (2009) A soil quality index based on the equilibrium between soil organic matter and biochemical properties of undisturbed coniferous forest soils of the Pacific Northwest. Soil Biology andBiochemistry 41:822–830

DeLuca TH, Keeney DR, McCarty GW (1992) Effect of freezing-thawing events on mineralization of soil nitrogen. Biology and Fertility ofSoils 14:116–120

Dick RP (1994) Soil enzyme activities as indicators of soil quality. In: Doran JW, Coleman DC, Bezdicek DF, Stewart BA (eds) Defining soil quality for a sustainable environment. Madison, WI, pp107–124

Edwards KA, McCulloch J, Kershaw GP, Jefferies RL (2006) Soil microbial and nutrient dynamics in a wet Arctic sedge meadow in late winter and early spring. Soil Biology andBiochemistry 38:2843–2851

Feng XJ, Nielsen LL, Simpson MJ (2007) Responses of soil organic matter and microorganisms to freeze–thaw cycles. Soil Biology andBiochemistry 39:2027–2037

Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC (2009) Global patterns in belowground communities. Ecological Letters 12:1238–1249

Fu J, Liu J, Wang XW, Zhang MD, Chen WC, Chen B (2020) Ecological risk assessment of wetland vegetation under projected climate scenarios in the Sanjiang Plain, China. Journal of EnvironmentalManagement. https://doi.org/10.1016/j.jenvman.2020.111108

Ge GF, Li ZJ, Fan FL, Chu GX, Hou ZN, Liang YC (2010) Soil biological activity and their seasonal variations in response to long-term application of organic and inorganic fertilizers. Plant Soil 326:31–44

Grandy AS, Neff JC (2008) Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. Science of the TotalEnvironment 404:297–307

Grogan P, Michelsen A, Ambus P, Jonasson S (2004) Freeze–thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. Soil Biology andBiochemistry 36:641–654

Guan SY (1986) Soil enzymology and research method. Agricultural Press, 274–323. (In Chinese)

Hentschel K, Borken W, Matzner E (2008) Repeated freeze-thaw events affect leaching losses of nitrogen and dissolved organic matter in a forest soil. Journal of Plant Nutritionand Soil Science 171:699–706

Herrmann A, Witter E (2002) Sources of C and N contributing to the flush in mineralization upon freeze-thaw cycles in soils. Soil Biology andBiochemistry 34:1495–1505

Jones DL, Willett VB (2006) Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biology andBiochemistry 38:991–999

Koponen HT, Jaakkola T, Keinänen-Toivola MM, Kaipainen S, Tuomainen J, Servomaa K, Martikainen PJ (2006) Microbial communities, biomass, and activities in soils as affected by freeze thaw cycles. Soil Biology andBiochemistry 38:1861–1871

Larsen KS, Jonasson S, Michelsen A (2002) Repeated freeze-thaw cycles and their effects on biological processes in two arctic ecosystem types. Appl Soil Ecol 21:187–195

Liang B, Yang XY, He XH, Zhou JB (2011) Effects of 17-year fertilization on soil microbial biomass C and N and soluble organic C and N in loessial soil during maize growth. Biology and Fertility ofSoils 47:121–128

Männistö MK, Tiirola M, Häggblom MM (2009) Effect of freeze-thaw cycles on bacterial communities of arctic tundra soil. Microb Ecol 58:621–631

Mailapalli DR, Wallender WW, Burger M, Horwath WR (2010) Effects of field length and management practices on dissolved organic carbon export in furrow irrigation. Agriculture WaterManagement 98:29–37

Marschner B, Kalbitz K (2003) Controls of bioavailability and biodegradability of dissolved organic matter in soils. Geoderma 113:211–235

Marx MC, Wood M, Jarvis SC (2001) A microplate flurimetric assay for the study of enzyme diversity in soils. Soil Biology andBiochemistry 33:1633–1640

Matthews E, Fung I (1987) Methane emissions from natural wetland lands: global distribution, area and environmental characteristics of sources. Global BiogeochemicalCycles 1:61–86

Matzner E, Borken W (2008) Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review. European-Journal of Soil Science 59:274–284

Meisner A, Snoek BL, Nesme J, Dent E, Jacquiod S, Classen AT, Priemé (2021) Soil microbial legacies differ following drying-rewetting and freezing-thawing cycles. The ISME Journal 15:1207–1221

Mikan CJ, Schimel JP, Doyle AP (2002) Temperature controls of microbial respiration in arctic tundra soils above and below freezing. Soil Biology andBiochemistry 34:1785–1795

Miura M, Jones TG, Hill PW, Jones DL (2019) Freeze-thaw and dry-wet events reduce microbial extracellular enzyme activity, but not organic matter turnover in an agricultural grassland soil. Appl Soil Ecol 144:196–199

Mostaghimi S, Young RA, Wilts AR, Kenier AL (1988) Effects of frost action on soil aggregate sTableility. American Society ofAgricultural Engineers 31:435–439

Oztas T, Fayetorbay F (2002) Effect of freezing and thawing processes on soil aggregate sTableility. Catena 52:1–8

Poelson DS, Prookes PC, Christensen BT (1987) Measurement of soil microbial biomass provides an early indication of changes in total organic matter due to straw incorporation. Soil Biology andBiochemistry 19:159–164

Puri G, Ashman MR (1998) Relationship between soil microbial biomass and gross N mineralisation. Soil Biology andBiochemistry 30:251–256

Schimel JP, Bilbrough C, Welker JM (2004) Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. Soil Biology andBiochemistry 36:217–227

Schimel JP, Clein JS (1996) Microbial response to freeze-thaw cycles in tundra and taiga soils. Soil Biology andBiochemistry 28:1061–1066

Sharma S, Szele Z, Schilling R, Munch JC, Schloter M (2006) Influence of freeze–thaw stress on the structure and function of microbial communities and denitrifying populations in soil. Appl Environ Microbiol 72:2148–2154

Skogland T, Lomeland S, Goksøyr J (1988) Respiratory burst after freezing and thawing of soil: experiments with soil bacteria. Soil Biology andBiochemistry 20:851–856

Song KS, Wang ZM, Du J, Liu L, Zeng L, Ren CY (2014) Wetland degradation: its driving forces and environmental impacts in the Sanjiang Plain, China. Environ Manage 54:255–271

Song YY, Song CC, Yang GS, Miao YQ, Wang JY, Guo YD (2012) Changes in labile organic carbon fractions and soil enzyme activities after marshland reclamation and restoration in the Sanjiang Plain in Northeast China. Environ Manage 50:418–426

Sorensen PO, Finzi AC, Giasson MA, Reinmann AB, Demott RS, Templer PH (2018) Winter soil freeze-thaw cycles lead to reductions in soil microbial biomass and activity not compensated for by soil warming. Soil Biology andBiochemistry 116:39–47

Sparling GP (1992) Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. Australia Journal of SoilResearch 30:195–207

Staricka JA, Benoit GR (1995) Freeze-drying effect on wet and dry soil aggregate sTableility. Soil Science Society ofAmerica 599:218–223

Tan B, Wu FZ, Yang WQ, He XH (2014) Snow removal alters soil microbial biomass and enzyme activity in a Tibetan alpine forest. Appl Soil Ecol 76:34–41

Tierney GL, Fahey TJ, Groffman PM, Hardy JP, Fitzhugh RD, Driscoll CT (2001) Soil freezing alters fine root dynamics in a northern hardwood forest. Soil Biology andBiochemistry 56:175–190

Vallejo VE, Roldan F, Dick RP (2010) Soil enzymatic activities and microbial biomass in an integrated agro forestry chronosequence compared to monoculture and a native forest of Colombia. Biology and Fertility ofSoils 46:577–587

Wan ZM, Song CC, Guo YD, Wang L, Huang JY (2008) Effects of water gradients on soil enzyme activity and active organic carbon composition under *Carex lasiocarpa* marsh. Acta Ecol Sin 28:5980–5986

Wang F, Han XZ, Li LH, Zhang KQ (2009) The effect of freezing and thawing processes on black soil aggregateSTableility.Journal of Glaciology and Geocryology31:915–919 (In Chinese)

Wang FL, Bettany JR (1993) Influence of freezing-thawing and flooding on the loss of soluble organic carbon and carbon dioxide from soil. Journal of EnvironmentalQuality 22:709–714

Wang JY, Song CC, Hou AX, Miao YQ, Yang GS, Zhang J (2014) Effects of freezing–thawing cycle on peatland active organic carbon fractions and enzyme activities in the Da Xing'anling Mountains, Northeast China. Environmental Earth Sciences 72:1853–1860

Wang ZM, Song KS, Ma WH, Ren CY, Zhang B, Liu DW, Chen JM, Song CC (2011) Loss and fragmentation of marshes in the Sanjiang Plain, Northeast China, 1954–2005. Wetlands 31:945–954

Yang WQ, Wang KY (2002) Advances on soil enzymology. Chinese Journal of Applied andEnvironmental Biology 8:564–570

Yao XH, Min H, LÜ ZH, Yuan HP (2006) Influence of acetamiprid on soilenzymatic activities and respiration. European Journal of SoilBiology 42:120–126

Yergeau W, Kowalchuk GA (2008) Responses of Antarctic soil microbial communities and associated functions to temperature and freezing-thawing cycle frequency. Environment Microbiology 10:2223–2235

Yi Y, Kimball JS, Rawlins MA, Moghaddam M, Euskirchen ES (2015) The role of snow cover affecting boreal-arctic soil freeze-thaw and carbon dynamics. Soil Biology andBiochemistry 12:5811–5829

Zaccone R, Boldrin A, Caruso G, Ferla RL (2012) Enzymatic activities and prokaryotic abundance in relation to organic matter along a west–east mediterranean transect (TRANSMED Cruise). Microb Ecol 64:54–66

Zhang JB, Song CC, Wang SM (2007) Dynamics of soil organic carbon and its fractions after abandonment of cultivated wetlands in Northeast China. Soil and Tillage Research 96:350–360

Zhang ZY (2000) Development and utilization of peat resources. Chinese Jilin Science and Technology Press, Changchun, pp 191–202 ((**in Chinese**))

Zhou LK (1987) Soil enzymology. Science Press, 266–270 (In Chinese)