Response of Soil Active Organic Carbon and Enzyme Activity to Freeze-Thaw Cycle in Wetlands

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Abstract

Freeze-thaw cycles (FTCs) are an important element of mid and high latitude ecosystems, and significantly influence soil physicochemical properties and microbial activities in the soil active layers. With the global warming, the effects of FTCs on the dissolved organic carbon (DOC) concentration and soil enzyme activity of different types of soil were still uncertain. In this study, soil of undisturbed *Deyeuxia angustifolia* wetland (UDAW), disturbed *Deyeuxia angustifolia* wetland (DDAW) and rice paddy field (RP) from three soil layers of (0–10, 10–20 and 20–30 cm) in Sanjiang Plain, 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. The results indicated that FTCs increased the soil DOC concentration but reduced the concentration of MBC and activities of cellulase, invertase and catalase. Increase in the freeze-thaw frequency, resulted in the DOC concentration increasing initially and then decreasing, and the MBC concentration and soil enzyme activities were opposite. The DOC concentration increment resulting from the freeze-thaw effects was different across different layers and soil type: as the soil depth increased, the average DOC increments decreased, and the average DOC increments varied across different soil types: UDAW > DDAW > RP. The average MBC concentration and soil enzyme activity decreased from 0-10 cm > 10-20 cm > 20-30 cm soil depth; MBC concentration and soil enzyme activities varied across the different soil types: UDAW > DDAW > RP. The freeze-thaw amplitude and soil moisture content interaction had an effect on soil active organic carbon fractions and enzymatic activity. Small amplitude FTCs and higher water content had the greatest effect on DOC concentration, while larger amplitude and higher water content had the greatest effect on MBC concentration and enzymatic activity. In wetland soil, the significant correlations between active organic carbon fractions and enzyme activities indicate that the increased DOC by FTCs plays an important role in soil microbes and enzyme activities. However, active organic carbon fractions and enzyme activities had little correlation in RP, indicating that FTCs has more influence on wetland than farmland.

1. Introduction

Freeze-thaw cycles (FTCs) are ubiquitous in middle to high latitudes and high altitudes (Grogan, 2004), and are important factors affecting the soil ecosystem in these areas, which can subsequently affect carbon, nitrogen and phosphorus cycling in soils and the leaching of nutrients through influencing biochemical and physicochemical processes. Global warming has resulted in

FTCs having profound effects on the carbon and nitrogen cycles, accelerating the loss and decomposition of carbon and nitrogen in the soil carbon pool (Yi et al., 2015). Dissolved organic carbon (DOC) and microbial biomass carbon (MBC) are constituents of soil active organic carbon which is active in the ecosystem and sensitive to changes in soil environmental factors (Wang et al., 1993). DOC, the substrate and product of microbial metabolism, is closely linked to microbial activity and provides a carbon source for microbial activity, particularly in frozen soil. Variations in soil DOC content results from the decomposition of DOC from dead microorganisms, which is released into the soil and dissolved by mineralization (Herrmann and Witter, 2002). The literature reports that FTCs can affect the DOC content, although different research methods and soil types has resulted in different findings (Feng et al., 2007; Grogan et al., 2004; Chaer et al., 2009). Soil MBC constitutes 2-3% of the total soil organic carbon and influences the dynamics of organic carbon (Jenkinson and Ladd, 1981). MBC has a role in the degradation and humidification of organic residues and the transfer of soil organic carbon between carbon pools. Nutrient elements secreted by microorganisms are available nutrients that can be directly used by plants. 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 have no significant effect on soil MBC. However, Schimel and Clei (1996) and Larsen et al. (2002) reported that the freeze-thaw effects on soil decreases the soil MBC concentration.

A number reports suggest that active organic carbon is correlated with 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 the author knowledge, there are few studies reported describing on the relationship between active organic carbon fractions and enzyme activities on FTC in wetland which experience seasonal freeze-thaw cycles. Soil enzymes, as the key players involved in the catalytic reactions, play have an essential role in the decomposition of organic matter (Dick 1994; Yao et al., 2006). 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). These enzymes are involved in various chemical reactions and biochemical processes in soil (Yang et al., 2002). So any factor that affects the soil microbial population will necessarily alter soil enzyme activities (Vallejo et al., 2010). Research by Purig and Ashman (1998) indicate temperature is an important factor affecting the activity of soil microorganisms. Early studies suggest that low winter temperatures can reduce or inhibit the activity of soil enzymes (Mikan, 2002). However, later studies using different methods report that FTCs may increase the activity of soil psychrophilic microorganisms (Hentschel, 2008). Although the impacts of FTC on the activity and composition of soil microbes has been extensively studied (e.g. Edwards et al., 2006; Koponen et al., 2006; Ma¨nnisto¨ et al., 2009; Sharma et al., 2006), little research is reported describing the changes in enzyme activities and active carbon fractions following FTC (Yergeau and Kowalchuk 2008), particularly in wetland soil.

Wetlands are important carbon storage sinks for 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 E et al., 1987). Due to intensification of human reclamation, wetlands have become one of the ecosystems most threatened by humanactivities (Lemly A D et al., 2000). Research on the impact of land use and changes in land cover due to the global carbon cycle and their feedback mechanism in wetland ecosystems have become hot topics in current scientific research.

Sanjiang Plain is located in a typical seasonal freeze-thaw area in northeast China, and is the most concentrated area of fresh water wetlands in China. It is one of the most important areas in the global wetlands due to its biodiversity conservation. It is also an important grain production base for China. In recent years, due to reclamation, overuse and other human economic activities, the area of wetlands in the Sanjiang Plain has been sharply reduced and their ecological functions have been weakened. The decomposition of soil organic matter, the growth and development of plants, the emission of greenhouse gases and the activity of soil microorganisms are affected. This has broken the original material cycle and energy balance of the ecosystem of wetlands. In order to study the effect of FTCs on soil active organic carbon fractions and soil enzyme activity after the change of land cover in the Sanjiang Plain, soil from undisturbed *Deyeuxia angustifolia* wetland (UDAW), disturbed *Deyeuxia angustifolia* wetland (DDAW) and adjacent rice paddy field (RP) was selected, based on the principle of “typicality and consistency”, and an laboratory-simulated FTCs experiment was carried out. This study provides a basis for understanding the influence of FTCs on soil ecological processes in seasonal freeze-thaw regions.

2. Materials And Methods

The sampling site was the Sanjiang Plain (N 45°01'-48°28', E 130°13'-135°05'), in northeast China. At an average elevation of 55.4-57.9 m, this area has a humid continental monsoon climate in the northern temperate zone. This are experiences long, cold winters and warm, humid summers. The annual average temperature is 1.9 ℃ and average annual precipitation is 600 mm. The lowest temperatures occur in January, with an average temperature of -23.4℃, and the highest temperatures occur in July, with an average temperature of 22.4℃. From late October to mid-November and from mid-March to mid-May, numerous FTCs occur. The majority of vegetation on the Sanjiang Plain is helophyte and hygrophyte, with some mesophyte, such as *Carex*, *Calamagrostis angustifolia*, low birch thicket and Salix brachypoda. Soil types are meadow-boggy soil, humus fen soil and submersible white pulp soil.

2.2 Sample collection and experiment design

At the end of October 2015, soil from undisturbed *Deyeuxia angustifolia* wetlands, disturbed *Deyeuxia angustifolia* wetlands outside the reserve and adjacent rice paddy fields on the Sanjiang Plain were sampled as follows: select three samples per type, with each plot measuring 10 × 10 m^2 at intervals of 30 m. Soil samples with three layers (0–10, 10–20 and 20–30 cm) were collected using the multipoint mixing method after removal of the surface weed and ground vegetation. Specific descriptions of the sites are shown in Table 1. The soil samples were sealed in plastic bags with headspace air removed and transported to the laboratory within 72 h and sieved using a 4 mm soil sieve, and stored at 4 ℃ for simulation experiments. Part of the sample was air-dried, crushed and sieved to 0.25 mm mesh for soil organic carbon (SOC) and total nitrogen (TN) analyses. Table 2 lists the basic physicochemical properties of the test soil.

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

Site	UDAW	DDAW	Rice Paddies
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 angustifolia* wet grassland	*Deyeuxia angustifolia* wet grassland	Rice paddies
Hydrological features	Perennial flooded	Seasonal flooded	Seasonal flooded
Soil type	Humic Cryaquepts	Humic Cryaquepts	Aquandic Cryaquepts

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

Type	Layer(cm)	SOC(g/kg)	TN(g/kg)	C/N	pH	MWHC(%)
UDAW	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
DDAW	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)

Weighed 200g homogeneous soil sample and placed in a 250 mL culture bottle, respectively. The water content was adjusted using deionized water to the maximum water-holding capacity of 60% and 80%, and the sample cultured in a 20°C for one week to restore the microbial activity. The bottleneck was sealed using plastic film to minimize water evaporation, retaining a ventilation hole, and the sample weighed regularly and evaporated water was replaced. Based on the temperatures during FTCs in the Sanjiang Plain, we set a large (from -10 to 10°C) and a small (from -5 to 5°C) amplitudes of FTCs, the soil samples were frozen in a cryogenic incubator at -10°C and -5°C for 24 h, then thawed at 10°C and 5°C for 24 h. This cycle was repeated 15 times over 30 days. Simultaneously, some samples were kept at a constant temperature of 10°C and 5°C, without being subjected to the freeze-thaw treatment. To maintain constant water content, soils were sprayed with distilled water at the end of each FTC. DOC, MBC and enzyme activities were determined before FTC begun and after the 3rd, 5th, 10th and 15th cycle.

2.3 Analytical methods

Soil organic carbon: The Multi N/C 2100 TOC analyzer (Analytik Jena AG, Germany) high temperature combustion method was used to determine the soil organic carbon content. DOC: Fresh soil (10 g) and distilled water (50 mL) were combined in a triangular flask, which was 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. MBC: The improved chloroform fumigation-K_2SO_4 extraction method (Lu 2000) was used. Soil (20 g) was fumigated with trichloromethane for 24 h. Fumigated and non-fumigated samples were extracted using K_2SO_4 (0.5 M) for approximately 30 min and the total organic carbon concentration of leach liquor were determined using a Multi N/C 2100 TOC analyzer. MBC was calculated using:

MBC = (fumigated carbon-non fumigated carbon) / 0.38 (1)

Soil invertase and cellulase activities were measured using the methods by Ge et al. (2010) and Guan (1986), which use sucrose and carboxymethylated cellulose as substrates, respectively. These measurements were expressed as mg glucose. (g soil 24 h)$^{-1}$ for invertase activities, and mg glucose. (g soil 72 h)$^{-1}$ for cellulase activity. Catalase activity was determined using the permanganimetric method, using the volume of $KMnO_4$ (0.1 M) used by 1 g of soil in 20 min (Guan, 1986).

2.4 Statistical analysis

Statistical analysis was performed using SPSS version 19.0 for Windows (SPSS, Inc., USA), and graphics were produced using Microsoft Excel 2010 for Windows. Multivariate analysis of variance (MANOVA) was used to describe the effects of the water holding capacity, amplitude and frequency of FTCs on soil active organic carbon fractions and enzyme activities for each soil layer. A Pearson correlation was performed to determine the correlation between soil enzyme activities and active organic carbon. In all analyses, $P<0.05$ was considered statistically significant.

3. Results

3.1 Responses of active organic carbon fractions to FTCs

3.1.1 Responses of DOC to FTCs

The experimental data show that the freeze-thaw frequency and water-holding capacity had a significant affected on the soil DOC concentration. However, the amplitude of the freeze-thaw was only significant impact for the three types of soil at a depth of 0-10 cm DOC concentration (Table 3). The DOC concentrations were significantly affected by freeze-thaw amplitude × water-holding capacity interaction, amplitude × frequency and water-holding capacity × frequency interaction, but were not significantly affected by freeze-thaw amplitude × water-holding capacity × frequency interaction (Table 3). The DOC increments caused by freeze-thaw cycles show was consistent trend among various types or soil layers. The maximum DOC increment occurred at the sixth freeze-thaw frequency. The freeze-thaw frequency significantly increased the concentration of soil DOC, the minimum value as a result of FTCs greater than FTC (0) (Fig. 1).

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). Boldface values indicate significant effects at p < 0.05.

Variables	Type	Layer	A	WHC	F	A×WHC	A×F	F×WHC	A×F×WHC
DOC	UDAW	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**
	DDAW	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	UDAW	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
	DDAW	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

The average DOC increments after 15 FTCs that the DOC increment decreased with depth, and the increment between different soil types were UDAW > DDAW > RP (Fig. 6). The freeze-thaw amplitude and water content affected the soil DOC concentration. RP soil from 0-10 cm and 10-20 cm layers had a greater DOC increment at 60% water-holding capacity compared to 80%. Whereas the DOC concentration was greater at 80% water-holding capacity compared to 60% for all soil types and layers (Fig. 6). The greatest effect was the freeze-thaw cycle under the condition of small amplitude and high water content (Fig. 6).

3.1.2 Responses of MBC to FTCs

The freeze-thaw frequency significantly affected the soil MBC concentration. However, freeze-thaw amplitude and water-holding capacity had no significant effect on MBC concentration (Table 3). The freeze-thaw amplitude and water-holding capacity interaction had a significant impact on the MBC concentration across the three RP soil layers; the freeze-thaw amplitude × frequency interaction had a significant impact on UDAW 0-10 cm and 10-20 cm; Frequency ×water-holding capacity interaction had a significant impact on UDAW 0-10 cm and DDAW 0-10 cm and 10-20 cm (Table 3). Amplitude ×frequency × water-holding capacity interaction had no significant impact on the MBC concentration (Table 3). The test results show that FTCs reduced the MBC concentration in the three soil types, showed a consistent trend across different active layers and various soil types (Fig. 2). With an increase in freeze-thaw frequency, the soil MBC concentration in the three active layers decreased initially and then increased and the maximum values were smaller than that of FTC (0) (Fig. 2).

The average MBC of the 15 FTCs were compared across the different soil types and layers. As the soil depth increased, the influence of FTCs on MBC concentration decreased. For the smaller amplitude FTC, the influence of 60% water-holding capacity on MBC was greater than that of 80%. However, for larger amplitude FTC the influence of 80% water-holding capacity was greater than that of 60% (Fig. 7). For the smaller and larger amplitude FTC, the MBC concentration of the different soil types varied: DDAW > UDAW > RP (Fig. 7a), and UDAW > DDAW> RP, respectively (Fig. 7b). At the same time, the experimental results show that freeze-thaw amplitude and water content affected the soil MBC concentration. The larger amplitude and higher water content had the greatest influence on soil MBC (Fig. 7).

3.2 Responses of soil enzymes to FTCs

The freeze-thaw frequency had a significant impact on the activities of cellulase, invertase and catalase in all three soil types (Table 4). The freeze-thaw amplitude had no significant impact on invertase and catalase activity for DDAW. Water-holding capacity had a significant effect cellulose activity for DDAW and RP, and invertase activity for RP (Table 4). The freeze-thaw amplitude × water-holding capacity interaction had a significant impact on cellulase activity for DDAW, invertase activity for UDAW 0-10 cm and 10-20 cm, and RP 0-10 cm; and catalase activity for DDAW 20-30 cm and RP 10-20 cm. Amplitude ×frequency interaction had a significant effect on cellulase activity for DDAW only (Table 4). Frequency × water-holding capacity and the freeze-thaw amplitude × frequency × water-holding capacity interactions had no impact on soil enzyme activities (Table 4). The change in activity of the three soil enzyme activities caused by FTCs was consistent trend among various soil types or layers (Fig. 3, Fig. 4, Fig. 5). Changes in activity of three enzymes corresponded to changes in MBC concentration: the activity decreased early in the FTCs and then increased with an increase in freeze-thaw frequency. However, the maximum was still smaller than that for FTC (0) (Fig. 3, Fig. 4, Fig. 5).

Table 4

MANOVA results (*p* values) for cellulase, invertase and amylase concentration with two amplitudes of freeze–thaw, fifteen FTCs and two water holding capacities (n=3). Boldface values indicate significant effects at $p < 0.05$

Variables	**Type**	**Layer**	**A**	**WHC**	**F**	**A×WHC**	**A×F**	**F×WHC**	**A×F×WHC**
Cellulase	UDAW	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
	DDAW	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	UDAW	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
	DDAW	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
Amylase	UDAW	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
	DDAW	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

Variables	Type	Layer	A	WHC	F	A×WHC	A×F	F×WHC	A×F×WHC
		20-30	**0.001**	**0.000**	**0.000**	0.586	0.625	**0.004**	**0.027**

The average decrements for the 15 FTCs for the three soil types were calculated. The soil enzyme activities decrement of the three soil enzymes decreased with soil depth: 0-10 cm > 10-20 cm > 20-30 cm for the same soil type, and UDAW > DDAW > RP across different soil types. Freeze-thaw amplitude and soil water content affected the activities of the three soil enzymes, with larger amplitude and higher water content having a more significant impact on soil enzyme activities (Fig. 8, Fig. 9, Fig. 10).

3.3 Relationship between soil active organic carbon and soil enzymes

After 15 FTCs for both UDAW and DDAW, the following were found to be significantly correlated: DOC with MBC; MBC with each of the three enzymes cellulase, invertase and catalase; DOC with cellulase and invertase ($P < 0.01$; Table 5); DOC with catalase ($P < 0.05$; Table 5); and the three enzymes were significantly correlated ($P < 0.05$; Table 5). After 15 FTCs for the RP soil, there was significant correlation between DOC, MBC, cellulase and invertase ($P < 0.05$; Table 5); other variables were found to be not significantly correlated (Table 5).

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

		DOC	**MBC**	**Cellulase**	**Invertase**	**Catalase**
UDAW	DOC	1				
	MBC	0.981**	1			
	Cellulase	0.845**	0.792**	1		
	Invertase	0.936**	0.964**	0.895**	1	
	Amylase	0.679*	0.709**	0.911**	0.791**	1
DDAW		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	
	Amylase	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	
	Amylase	0.898	0.487	0.54	0.496	1

* Significant at 0.05 level ** Significant at 0.01 level

4. Discussion

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). An increase in the freeze-thaw frequency resulted in the DOC concentration increasing firstly and then decreasing, with a minimum value greater than that of soil sample FTC (0) (Fig. 1). This indicated that freeze-thaw action stimulated the release of soil DOC, possibly as a result of FTCs killing soil microorganisms, 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 content as well as soil DOC content (Staricka and Benoit 1995; Tierney et al. 2001).

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

Calculating the average increment of 15 FTCs, indicated that the soil DOC increment decreases gradually with the increase of soil depth (Fig. 6). This was possibly due to the number of soil microorganisms and fine roots decreasing as depth increased, and the influence of freezing-thawing was decreased with depth, thus reducing the DOC increment with depth. The DOC increment for different soil types was UDAW > DDAW > 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). Additionally, soil leaching and nutrient loss intensified gradually for DDAW and RP, resulting in decreased DOC. DOC is also related to the soil content of organic matter. Soil aggregate was more stable for soil having a high organic carbon content and better soil structure, gradually releasing organic carbon into the soil aggregates. In contrast, soil aggregates would decompose easily in soil with low organic carbon content, causing organic carbon to be released rapidly (Herrmann and Witter, 2002). The soil organic carbon content was reduced after the wetlands into farmland in Sanjiang Plain (Table 1). Therefore, the effect of FTCs on farmland was reduced.

It was found that the interaction of small amplitude and high water content had the greatest influence on DOC increment (Fig. 6), which is in agreement with the findings of Wang et al. (2009) and Oztas and Fayetorbay (2002). Wang et al. (2009) reported that with appropriate water content, aggregate stability was enhanced even if the freezing temperature fluctuated substantially. However, Oztas and Fayetorbay (2002) concluded that lower freezing temperatures can decompose aggregates more easily.

MBC is a component 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 soil types or active layers (Fig. 2). This finding was consistent with that reported by Chaer et al. (2009), Wang et al. (2014) and Larsen et al. (2002). Additionally, 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. As FTCs have an antimicrobial effect, they 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 can kill 50% of a microbial population and lead 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 concentration (Table 3), as soil microorganisms gradually adapted to the influence of the freezing and thawing conditions, decreasing microbe mortality as FTCs increased. The MBC concentration gradually increased, but was still smaller than that for FTC (0) (Fig. 2).

After calculating the average MBC decrements 15 FTCs, it was found that the decreased with soil depth for the three soil types. However, RP demonstrated 0-10 cm < 10-20 cm < 20-30 cm for the larger amplitude FTC and high water content soil. Across the different soil types, trend in MBC concentration was found to be UDAW≈DDAW > RP (Fig. 7), which is consistent with findings of Barbhuiya et al. (2004). MBC was less uniform as the soil depth increased in wetland soil. The freeze-thaw amplitude and soil water content also affected MBC concentration (Fig. 2), the larger amplitude and higher water content had the greatest effect on the change in soil MBC concentration (Fig. 7), although the amplitude of the freeze-thaw cycle was insignificant on the MBC concentration across the three soil types, with a larger freeze-thaw amplitude being more destructive to microorganisms and limiting of microbial activity (Schimel et al., 2004).

Soil enzymes are the key media of soil biological process and are involved in all biochemical soil processes (Dick 1994; Yao et al., 2006). Soil enzyme activity directly affects the rate of the carbon cycle in wetlands and plays an important role in the carbon balance and global climate change. The soil enzymes cellulase, sucrase and catalase are directly involved in the soil carbon cycle. Acting as the oxidation-reduction enzymes in soil, catalase activity can be used to characterize soil humidification 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 similar effect on the activity of soil enzymes and MBC concentration, i.e., reducing the activity of cellulase, invertase and catalase in the three soil types. The freeze-thaw frequency significantly affected soil enzyme activity (Table 4). Fewer FTCs reduced the activity of all three soil enzymes, and as the number of FTCs increased, the activity of these soil enzyme increased to a maximum value that was smaller than that of FTC (0) (Fig. 3). The influence of FTCs on cellulase, invertase and catalase activity decreased with an increase in soil depth, and differed across soil type: UDAW > DDAW > RP for each layer (Fig. 8, Fig. 9, Fig. 10). The freeze-thaw amplitude and water content had no significant effect on the activity of the three enzymes (Table 4),but it decreased the enzyme activities (Fig. 3, Fig. 4, Fig. 5). The larger freeze-thaw amplitude and higher water content interaction had the greatest influence on soil enzyme activity (Fig. 3, Fig. 4, Fig. 5). The effect of FTCs on soil enzymes was mainly due to the effects on soil microorganisms. Therefore, FTCs had a similar impact on MBC and soil enzyme activity. Correlation analysis indicated that MBC was significantly correlation with soil enzyme activity in UDAW and DDAW soils (Table 5). However, the change in the rates of enzyme activity were similar across all three layers of RP soil, with a slight correlation between MBC and soil enzyme activity, and between the enzymes (Table 5), possibly as a result of intense interference by agricultural reclamation. Previous studies have reported different responses in soil enzymes to FTCs, due to the use of different methods and soil properties. For example, Chaer et al. (2009) used Andrew deep forest soil from the USA and found that freeze and thawing reduced the activity of β-glucosidase, but phosphatase and phenoloxidase activities were unchanged. Yergeau and Kowalchuk (2008) used Antarctic soil found that freeze and thawing increased the activity of cellulase, but not significantly. Wang (2014) studied the permafrost from the Great Xingan Mountains and found that freeze and thawing reduced the activity of cellulase and cellulase and invertase. In fact, some enzymes in frozen soils are not fully passivated, especially the enzymes in the cold regions (Wang et al 2014). Soil FTCs firstly have damage effects on soil enzymes. However, FTCs destroys microbial cells which causes the release of carbon and nitrogen nutrients. These can be taken up by surviving microorganisms to support their activity.

In the context of Global warming, the frequency and amplitude of soil FTCs and thickness of the active layer will change. This will result in more carbon to be released from soil, affecting regional carbon changes and carbon balance. However, further studies are needed to determine how FTCs affect soil active organic carbon and microorganisms. Indoor simulation experiments cannot reflect the real situation occurring in the field and future studies should focus on field *in-situ* monitoring, to reveal the response of soil active organic carbon and enzymes to FTCs.

5. Conclusion

The study demonstrated that freeze-thaw cycles can increase DOC concentration, and decrease MBC concentration and soil enzyme activities (cellulase, amylase and invertase) in the active layer of the soil. The freeze-thaw effects related to DOC, MBC concentrations and enzyme activities were apparent, further influenced by amplitude, moisture content and frequency. The effect of amplitude of FTC on soil DOC concentration was larger in the small amplitude. However, the responses of MBC and enzymes to FTCs generally presented greater damage at the large amplitude. The effects of freezing-thawing cycle on soil with different interference intensities were different. The higher the interference intensity, the lower the content of soil organic carbon and the number of microbes, and the smaller the effect of freeze-thaw, and with the increase of depth, the impact of freezing-thawing decreases gradually. The active organic carbon fractions during the freezing–thawing periods were significantly correlated with cellulase, amylase and invertase activities. The experimental approach used in this research can be generally applied in the study of soil solutions from disturbed or undisturbed soil systems. Our study might have important implications to carbon balance in the seasonal freezing-thawing zone.

Declarations

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Figures

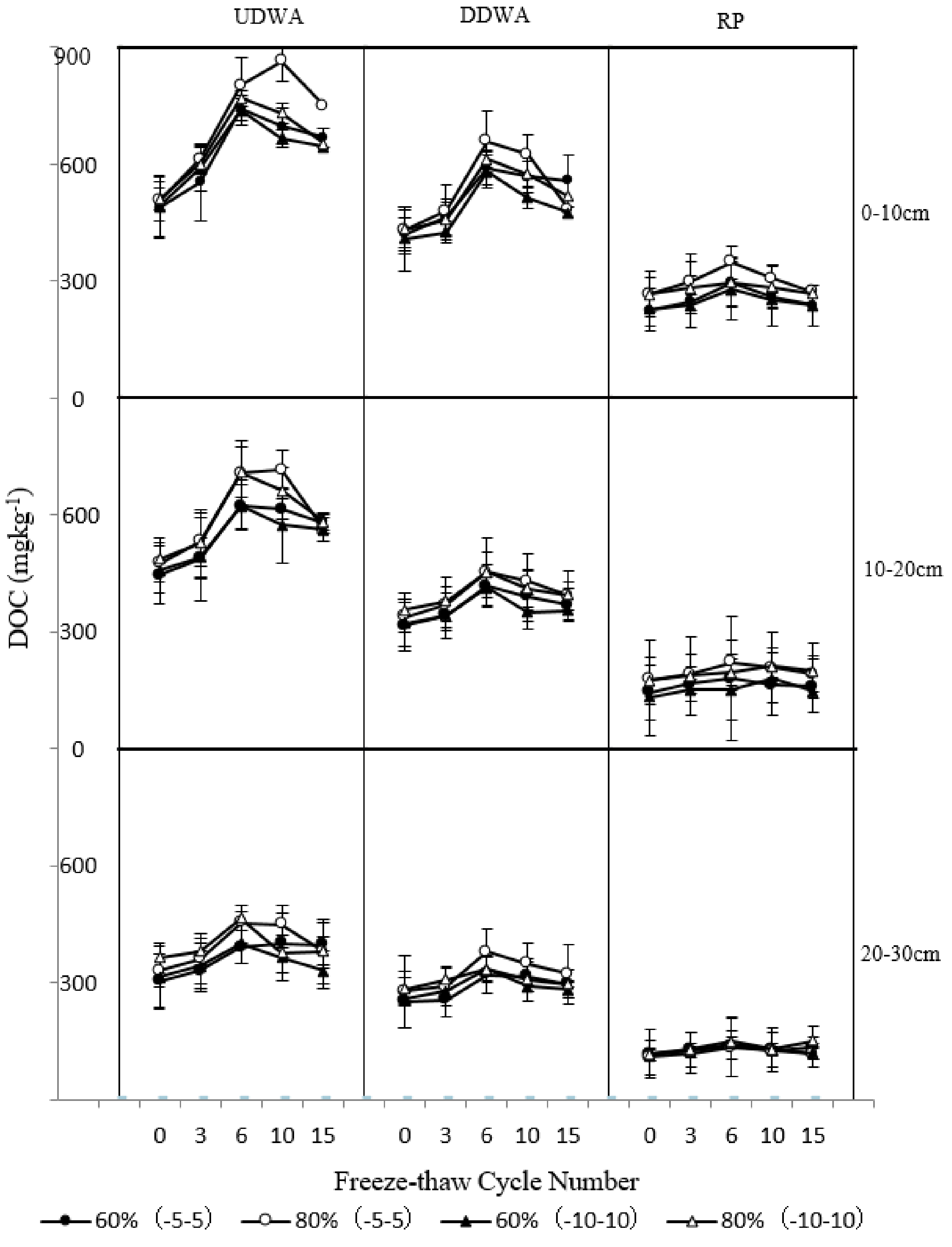

Figure 1

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

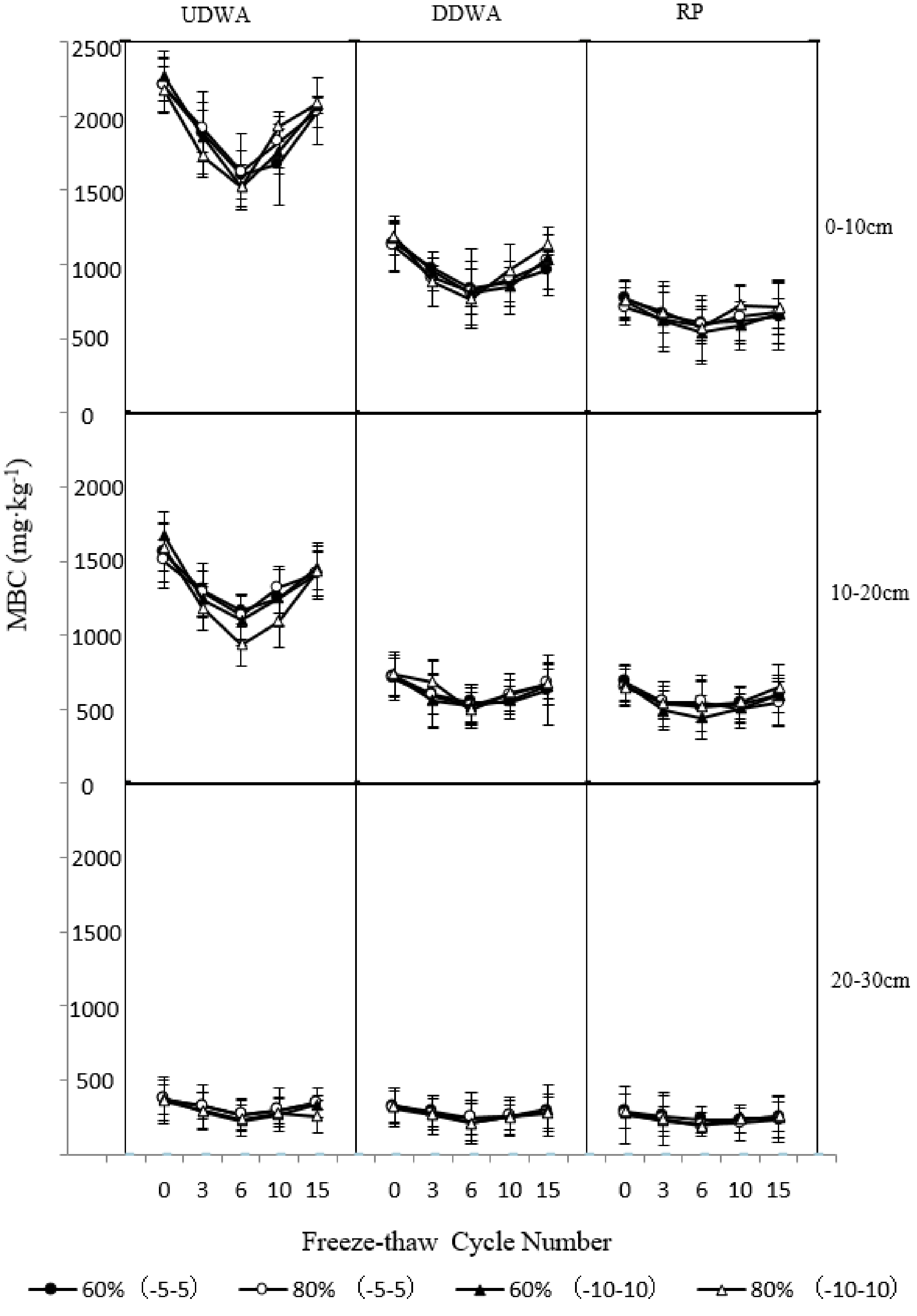

Figure 2

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

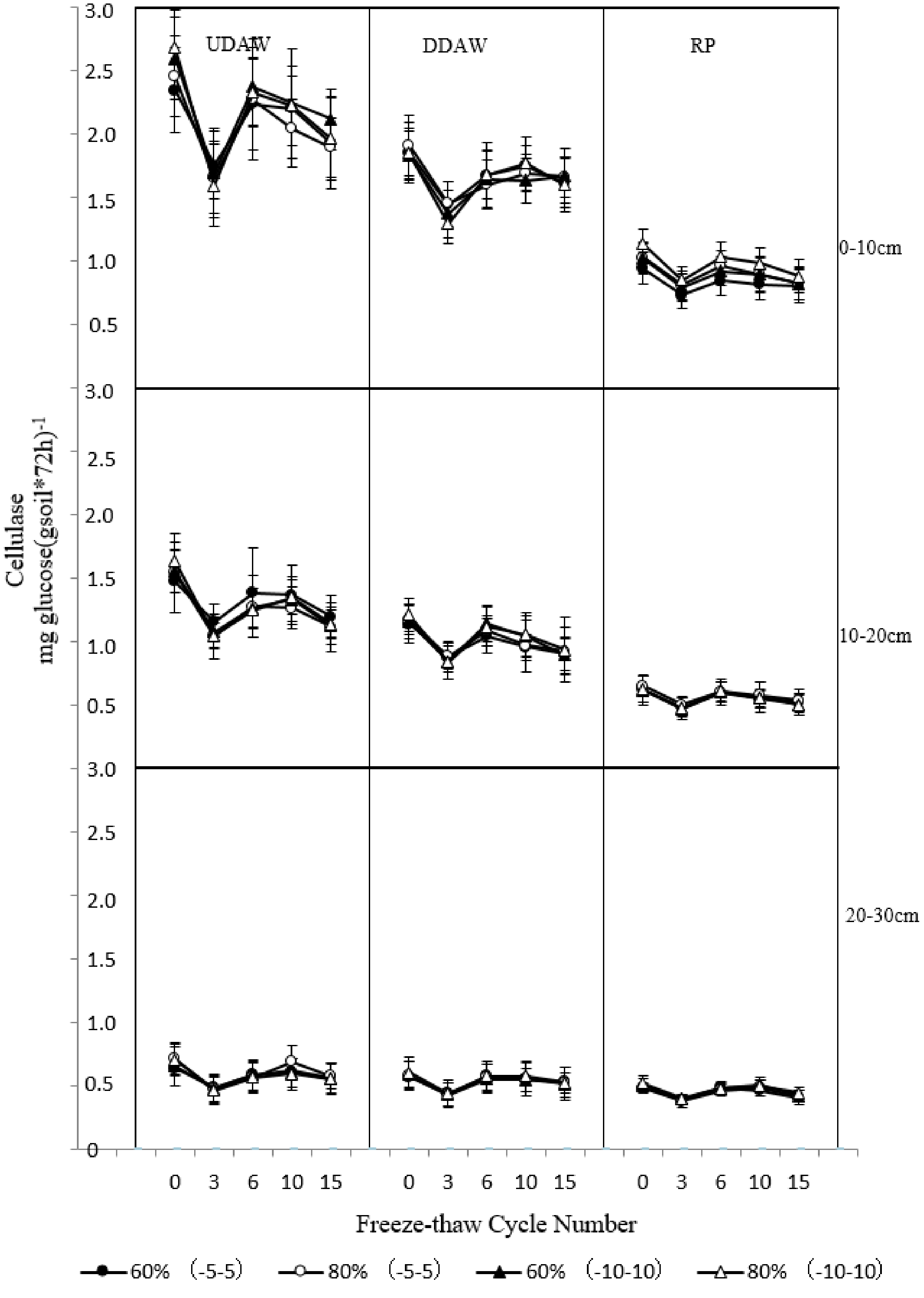

Figure 3

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

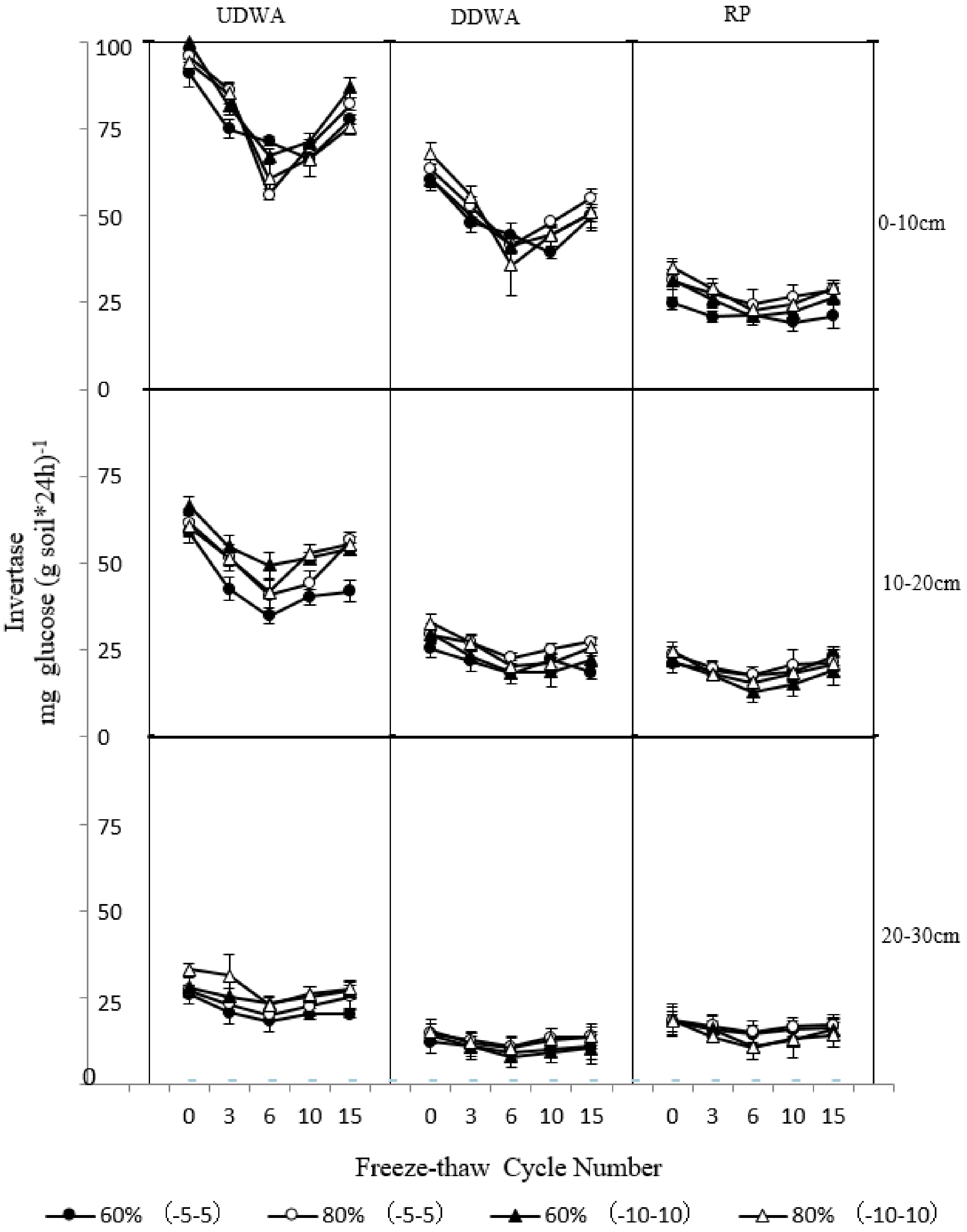

Figure 4

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

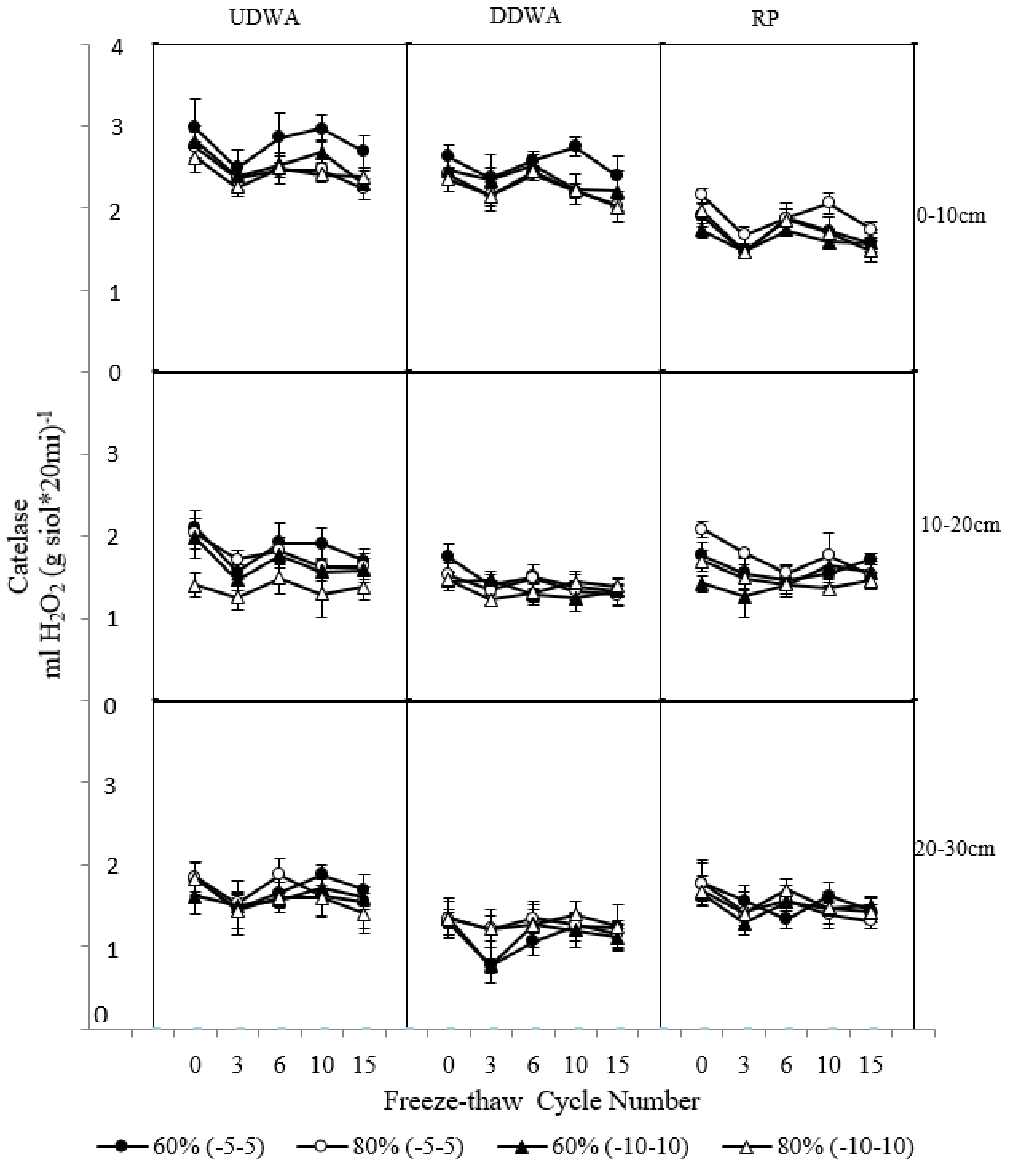

Figure 5

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

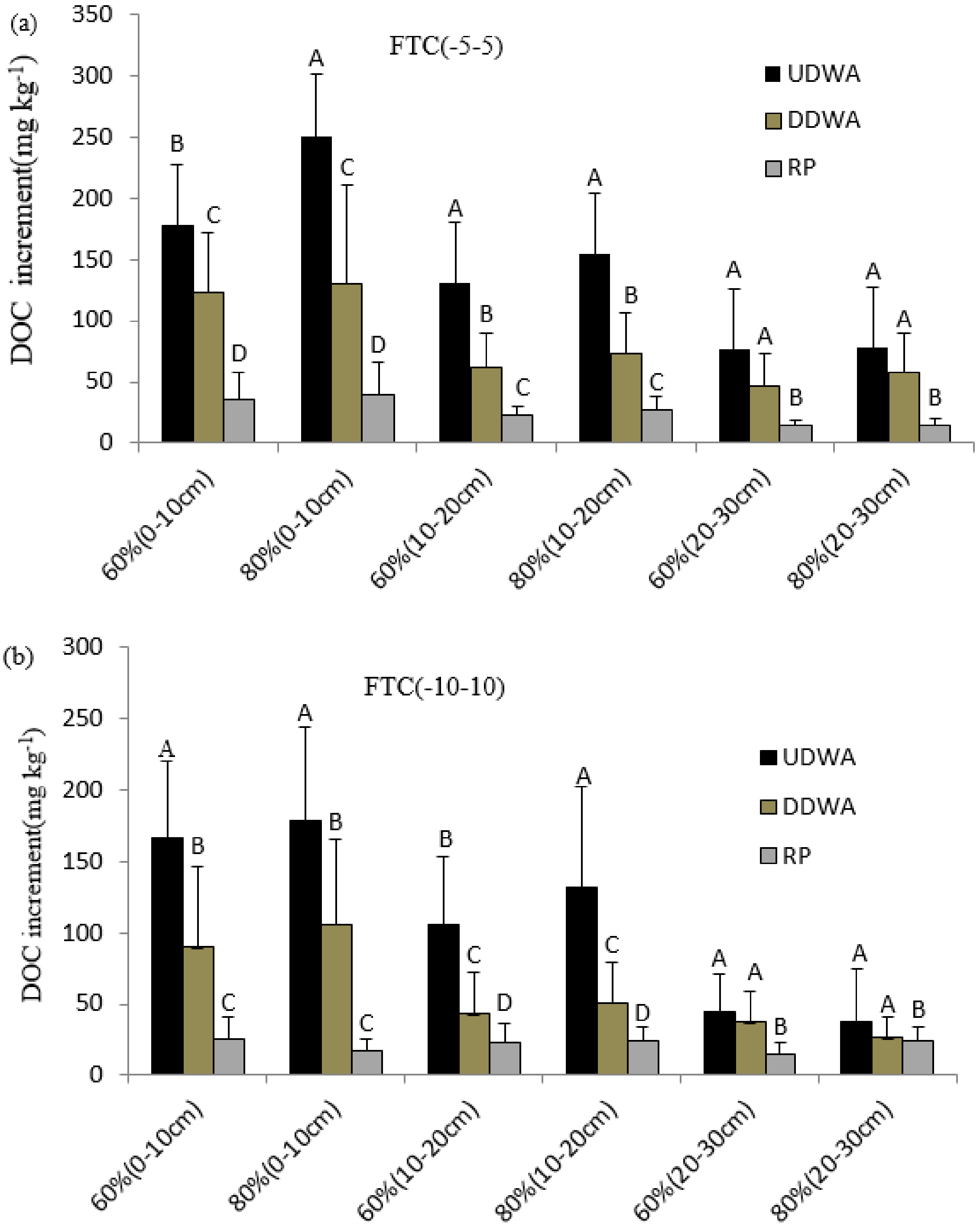

Figure 6

Average increment of 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% refer to the 60% and 80% of maximum water holding capacity, respectively. (-5-5) and (-10-10) refer to (-5 to 5°C) and (-10 to 10°C).

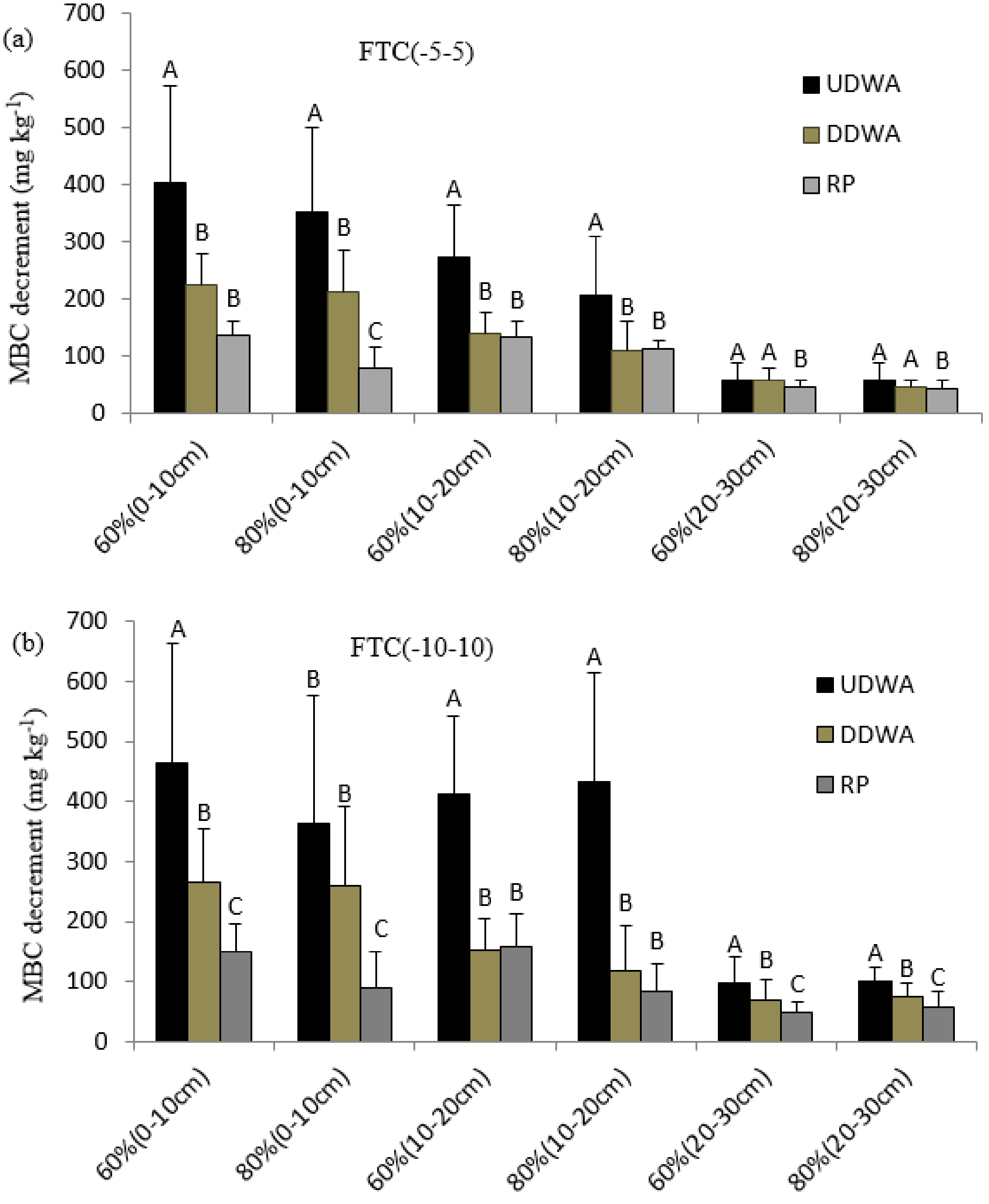

Figure 7

Average decrement of 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% refer to the 60% and 80% of maximum water holding capacity, respectively. (-5-5) and (-10-10) refer to (-5 to 5°C) and (-10 to 10°C).

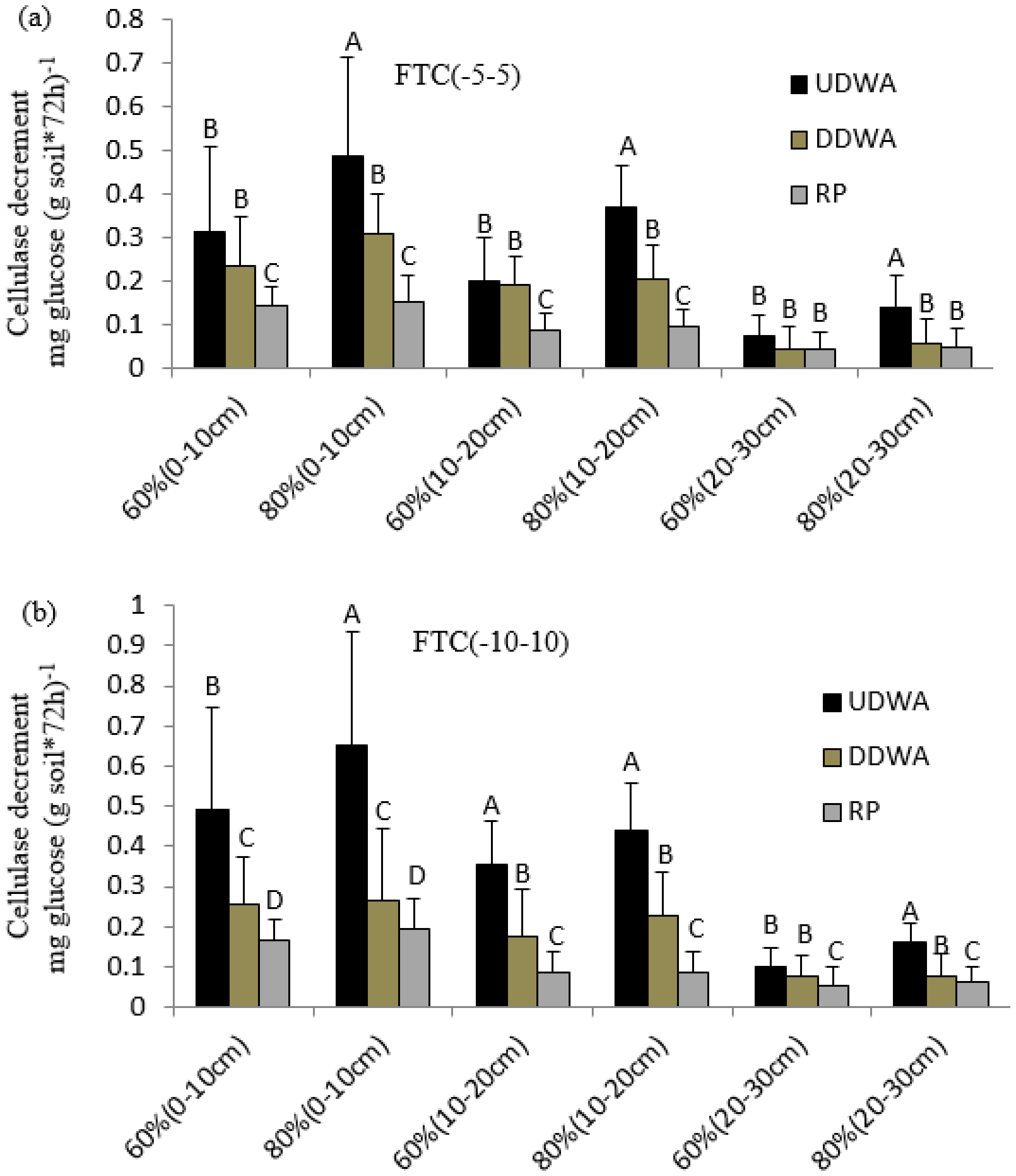

Figure 8

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 cellulase activity of fifteen cycles caused by freeze-thaw treatment is identified on the y-axis. 60% and 80% refer to the 60% and 80% of maximum water holding capacity, respectively. (-5-5) and (-10-10) refer to (-5 to 5°C) and (-10 to 10°C).

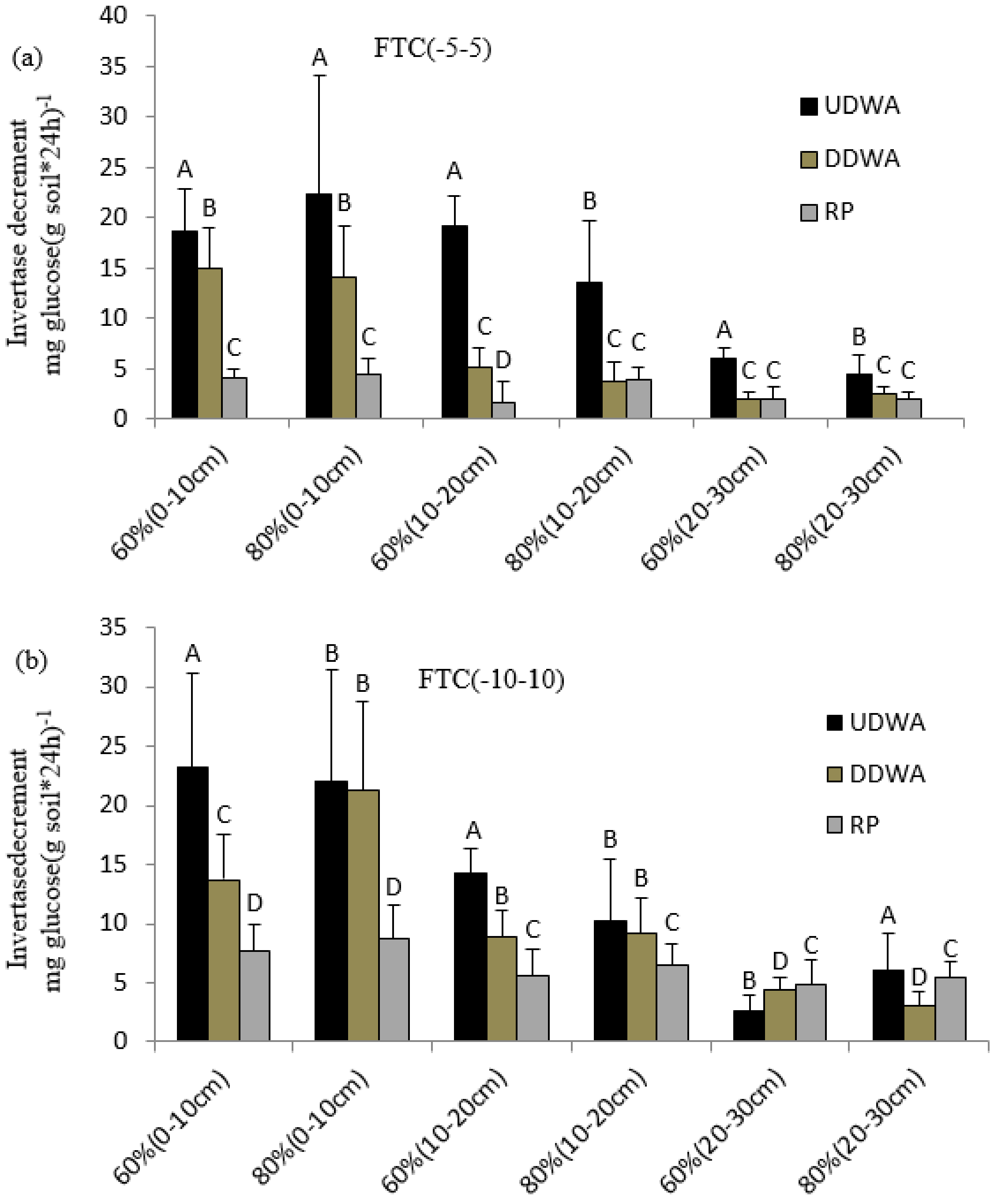

Figure 9

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 invertase activity of fifteen cycles caused by freeze-thaw treatment is identified on the y-axis. 60% and 80% refer to the 60% and 80% of maximum water holding capacity, respectively. (-5-5) and (-10-10) refer to (-5 to 5°C) and (-10 to 10).

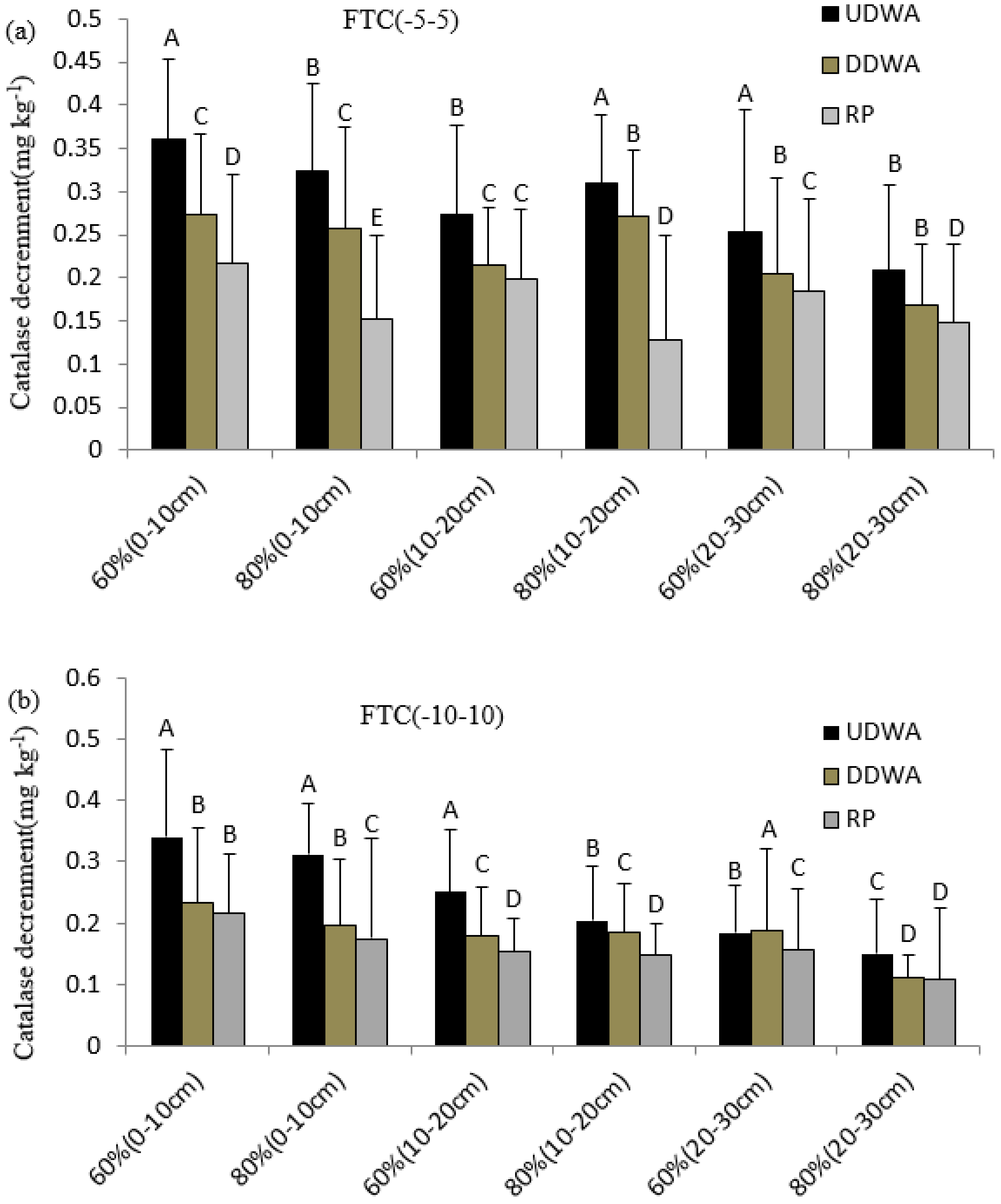

Figure 10

Average decrement of catalase 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 catalase activity of fifteen cycles caused by freeze-thaw treatment is identified on the y-axis. 60% and 80% refer to the 60% and 80% of maximum water holding capacity, respectively. (-5-5) and (-10-10) refer to (-5 to 5°C) and (-10 to 10°C).