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The impact of periodic waterlogging on biochemical characteristics and mineralization of soil organic carbon in straw-return farmland

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Abstract: Periodic waterlogging is more common due to more frequent extreme precipitation but its impact on soil organic carbon (SOC) loss is obscure in straw-return farmland. We compared soil properties and biochemical characteristics of SOC (compositions of non-cellulosic and amino polysaccharides) in adjacent periodic waterlogged farmland (PWF) and non-waterlogged farmland (NWF) in a semi-humid warm temperate region. SOC mineralization was also measured at 60% (aerobic) or 100% (anaerobic) of field capacity at 25 °C for 82 days. The negative effect of periodic waterlogging on SOC contents and soil aggregate stability were observed in the 20–80 cm depth but were offset in topsoil (0–20 cm) due to straw-return. Periodic waterlogging increased the non-cellulosic sugar content and amino sugar content in SOC and the mass ratio of (galactose plus mannose) to (arabinose plus xylose) at 40–80 cm depth except at 0–40 cm depth. By the end of 82 days' incubation, when aeration status changed from anaerobic to aerobic conditions, total C loss as CO_2 increased similarly (123.9%) in PWF and NWF soils in the top 40 cm, but more C loss occurred under PWF than under NWF (78.9% vs. 46.9%) in the 40–80 cm depth, which was probably ascribed to its higher non-cellulosic sugar and amino sugar content. Our result emphasized the importance of straw-return for maintaining soil quality under periodic waterlogged farmland.

Keywords: amino sugars; non-cellulosic sugars; periodic waterlogging; SOC mineralization; soil aggregate stability; straw return

With global warming, the extreme precipitation is more and more frequent (IPCC 2023), and probably influences the areas where had never experienced waterlogging in history (Trenberth 2011; Thorne 2014). The waterlogging caused by extreme precipitation may continue for several days or even months (Macé et al. 2016; Huang & Hall 2017), and threaten the ecological function of soil, such as changing soil physical and chemical properties and microbial community composition (Sánchez-Rodríguez et al. 2019a, b; Gschwend et al. 2020). Thus, extreme precipitation may reduce carbon storage in regional ecosystems (Reichstein et al. 2013), further exacerbating the greenhouse effect.

The effect of different durations of waterlogging on soil organic carbon (SOC) decomposition might be various. SOC decomposition would be restrained during short-term waterlogging period (hours-days) but promoted during long-term waterlogging period (weeks-months), because longer timescales anaerobic conditions could induce Fe^{3+} reduction by microorganism, and the protected C by Fe^{3+} complexation

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would be released (Huang & Hall 2017). The duration of waterlogging can also affect soil physical structure, such as stability of soil aggregates which physically protect SOC (Pu et al. 2022; Zhang et al. 2022). However, long-term or frequent waterlogging would also inhibit soil microbial activity and plant residue decomposition, leading to the storage of a large amount of SOC in soil, such as in paddy fields or wetlands (Nahlik & Fennessy 2016; Chen et al. 2021; Ren et al. 2022). Therefore, waterlogging inhibits the degradation rate of plant residues, but promotes the mineralization of metal- or mineral-associated C (Huang et al. 2020). However, many previous studies focused on the effect of one waterlogged phase on soil physical and chemical properties (Huang & Hall 2017; Sánchez-Rodríguez et al. 2019a, b; Liu et al. 2023; Ran et al. 2023). The impact of one waterlogged phase on soil properties might be limited, such as soil aggregates. The effects of many years of periodic waterlogging on soil properties and SOC decomposition are necessary to investigate due to increasing extreme precipitation.

The effect of periodic waterlogging on SOC decomposition between topsoil and subsoil were probably different in straw returned farmland. Crop residues were mainly input into topsoil in shallow cultivated farmland. Crop residues as external carbon could enhance the stability of soil aggregates and SOC content (Huang & Hall 2017; Xiang et al. 2018; Wang et al. 2021), which might offset the adverse effects of waterlogging on soil aggregates and SOC mineralization. Subsoil (below 30 cm) stored about half of the organic carbon (Jobbágy & Jackson 2000), but might be strongly influenced by periodic waterlogging due to little plant residues return. Therefore, the effect of periodic waterlogging on SOC mineralization in the subsoil should be focused.

In order to explore the impact of many years of periodic waterlogging on soil properties and the SOC loss in straw-return farmland, this study compared the SOC contents, stability of soil aggregates, non-cellulose sugars content and amino sugars content at 0–20, 20–40, and 40–80 cm soil layer between six different paired periodic waterlogged farmlands (PWF, about 1 to 2 months waterlogging period in summer at each year) and non-waterlogged farmlands (NWF) in a coal subsidence area in the east of Huaibei City, Anhui Province, China. This study also analysed the SOC decomposition potential by incubation in the dark at 25 °C for 85 days under two soil water content: 60% of field capacity (aerobic) and 100% of field capacity (anaerobic). Each paired PWF and NWF belonged to the same farmland which was separated by periodic waterlogging since 2005. Before obvious waterlogging, soil properties and SOC biochemical characteristics were speculated similarly due to the same type of crops and the same amount of fertilizer. We hypothesize that (*i*) SOC content and stable aggregates at topsoil (0–20 cm) weren't lower under PWF than under NWF due to abundant straw-return, but they may be smaller in the former than in the later at subsoil (20–80 cm) because of little plant residues input; (*ii*) C loss would be greater at subsoil under PWF than under NWF due to a decline of soil aggregate stability when aeration status changed from anaerobic to aerobic conditions. If these two hypotheses are true, we suggest that deep tillage might mitigate the negative impact of periodic waterlogging on SOC loss at subsoil in straw-return farmland.

MATERIAL AND METHODS

Site description and sampling. Soil samples were collected from Liuyuan village, Huaibei city, Anhui province, China. This village has an area of 338 ha (34°0'13''N, 116°53'58''E, 65 m a.s.l.), with an annual mean air temperature and precipitation of 15.6 °C and 843.7 mm, respectively, in the last 15 years (China Statistical Yearbook). Besides, more than half of annual precipitation occurred from June to August. The crop planting patterns were maize-winter wheat rotation on an annual basis in this area. The terrain of farmland in village gradually descended due to the coal mining subsidence and partial farmland areas were often waterlogged from July to August. According to the local farmers, the periodic waterlogging had been going on more than 15 years because their village was moved to another place at the year of 2005. In the east of the village, there are about 35 hectares of farmlands (north-south direction, about 300 m long) and the terrain of north side of farmlands (about 100–180 m long) were relatively low compared with the south side of farmlands. Thus, the south side of farmlands were non-waterlogged farmlands (NWF) and the north side of farmlands were periodic waterlogged farmlands (PWF) in summer. The depth of water at PWF were about 1–22 cm from July to August in the year of 2019 and 2020. Maize was planted from June to October at NWF, but PWF in summer were dominated by *Lemna minor* L. during period

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of waterlogging and were dominated by *Cyperus cyperoides* (L.) Kuntze, *Echinochloa crus-galli* (L.) P. Beauv., *Erigeron canadensis* L. after waterlogging removal at the end of August. Winter wheat was normally planted at PWF and NWF from November to May of the following year. The straw and roots of crop (maize and wheat) or weeds were smashed by harvester or rotary tiller, and returned to a depth of 20 cm after seeds harvest. Urea (150 kg/ha of N) and compound fertilizer (75 kg/ha of P_2O_5 and 112.5 kg/ha of K_2O) were spread on each farmland before ploughing. PWF was unfertilized due to periodic waterlogging from June to August.

In September 12th 2020, we randomly selected six different farmlands (4–7 m wide) which contains PWF at the northside and NWF at the south side. Within each NWF or PWF, after removal of aboveground biomass, five subsamples were randomly taken using an auger (30 mm diameter) from each depth of 0–20, 20–40, 40–60 and 60–80 cm, respectively. Then the five subsamples were mixed together to produce one composite sample for each soil depth at each NWF or PWF. Thus, the experimental design consists of 2 types of farmlands (NWF or PWF) × 4 soil depths × 6 replications. Composite soil samples were sieved to < 5 mm immediately after removing roots. A small portion of fresh soil samples were stored in the refrigerator (4 °C) for soil moisture and soil enzyme activities measurement. The rest of soil samples were air-dried at room temperature and used for water stable aggregates measurement. Then about half of air-dried soil samples were sieved to < 2 mm for SOC mineralization, texture, pH value, carbonate content, available nutrient content and light fractions organic carbon determinations. A small portion of air-dried soil samples were ground to fine powders using a ball mill (Retsch MM 220, Hann, Germanny) for SOC, soil non-cellulosic neutral sugars and amino sugar content analyses. Soil moisture and water stable aggregates were measured again from the samples collected on June 1st 2021. Because the soil moisture might be higher under PWF due to its relatively lower terrain. Besides, during non-waterlogging period (from September to June of the following year), the returned straw and wheat planting might mitigate the negative impact of periodic waterlogging on the stability of water aggregates. Before harvest, aboveground biomass and belowground biomass (0–20 cm) of crops or weeds were collected and oven dried at 60 °C to constant weight.

Analyses of basic soil properties and β-glucosidase activity. Soil moisture was measured at 105 °C and soil bulk density was determined by cutting ring method (Lu 2000). Soil pH values were measured in a 1 : 2.5 soil : deionized water (w/w). Soil carbonate content was determined by a volumetric titration method (Lu 2000). Briefly, excess 0.5 M HCl was added to soil samples and was titrated with 0.25 M NaOH to a phenolphthalein point. Soil particle size distribution was estimated by the pipette method (Gee & Bauder 1986). In brief, after removing organic C and carbonates and Ca^{2+} in soil samples, clay and silt particles (< 0.002 and 0.002–0.05 mm) was collected with a pipette according to sedimentation time after using hexametaphosphate as dispersant, and then the sand particles (0.05–2 mm) were collected at a sieve of 0.05 mm mesh size after rinsing by deionized water. The total soil inorganic N and Olsen-P were extracted by 2 M KCl solution and 0.5 M $NaHCO_3$ solution, respectively, and analysed by Auto Discrete chemical Analyzer (Smartchem 200, AMS Alliance, Italy). The concentration of soil available potassium was extracted by 1 M ammonium acetate solution and determined by a flame photometer (FP6400A, Shanghai, China) (Lu 2000). Soil β-glucosidase activity was measured as the amount of *p*-nitrophenol (PNP) released from 1 g of fresh soil (< 2 mm) after incubation at 37 °C for 1 h in tris-hydroxymethyl-aminomethane buffer (pH 6.0) (Eivazi & Tabatabai 1988).

Analyses of total soil N and OC of soil and light-fraction and plant residue. Total soil N was determined by a semi-micro Kjeldahl digestion procedure (Lu 2000). The organic carbon (OC) content of soil or plant residue were measured with the Walkley and Black dichromate oxidation method (Nelson & Sommers 1982). The light-fraction organic carbon (LFOC, with a density of 1.6 g/cm^3) was measured with the method of Jia et al. (2020).

Analyses of water-stable aggregates. Water-stable aggregates were assessed according to an amendment to the procedure of Sarker et al. (2018). Briefly, different size of aggregates (1–5, 0.25–1 and 0.05–0.25 mm) was collected from a sequence of three sieves of 1.00, 0.25 and 0.05 mm mesh size after oscillating vertically in water 20 times at the rate of one oscillation per second using a 4 cm amplitude. The weight of aggregates < 0.05 mm was calculated by subtracting the total weight of aggregates > 0.05 mm. The stability of aggregate was expressed as mean weight diameter (MWD, mm), calculated as the sum of all products of proportion, multiplied by the mean diameter

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of each water-stable aggregate fraction (Equation 1) (Spaccini et al. 2004).

$$\text{MWD} = \sum_{i=1}^{n} X_i W_i \qquad (1)$$

where:

X_i – the mean diameter of i_{th} sieve size class (*i.e.* 5 mm > X_1 > 1.0 mm, 1.0 mm > X_2 > 0.25 mm, 0.25 mm > X_3 > 0.05 mm, 0.05 mm > X_4);

W_i – the proportional mass (%) of water-stable aggregates in i^{th} fraction.

The macroaggregates proportion (%) was the sum of proportion of aggregates > 0.25 mm.

Analyses of non-cellulosic and amino sugars in soil. Non-cellulosic sugars and amino sugars in soil were measured following the procedure described by Jia et al. (2020), with *Myo*-inositol as internal standard. Briefly, soil samples were hydrolysed with 4 M trifluoroacetic acid (TFA) for eight non-cellulosic sugar monomers (ribose, rhamnose, arabinose, xylose, fucose, mannose, glucose and galactose) measurement or with 10 M HCl for four amino sugars (glucosamine, mannosamine, galactosamine, and muramic acid) measurement. After purification and aldononitrile acetate derivatization, the gas chromatographic measurement of non-cellulosic or amino sugar monomers were performed on an Agilent 8860 gas chromatography (GC, Agilent Tech. Co. Ltd., USA) equipped with a flame ionization detector with a DB-1 column (60 m × 0.25 mm× 0.25 µm, Agilent Tech. Co. Ltd., USA). Soil microorganisms rarely synthesize pentose sugars (Schmidt et al. 2015). An increasing value of (galactose + mannose) to (arabinose + xylose) (GM/AX) reflects a larger relative contribution of microbial carbohydrate synthesis to SOC (Oades et al. 1970). Amino sugars couldn't be synthesized by plants (Amelung 2003). Glucosamine (GluN) is a common fungal chitin biomarker and muramic acid (MurA) is a unique component of bacterial cell walls, and then the GluN/MurA ratio could indicate the relative contribution of fungi and bacteria to microbial residues (Liang et al. 2009).

Determination of SOC mineralization. SOC mineralization was conducted in the dark at 25 °C for 85 days under two water regimes: aerobic (60% of field capacity, FC) and anaerobic (100% of FC). In brief, after adjusting soil moisture and equilibrium (4 °C, 1 week), soil samples were incubated in the dark at 25 °C in well-sealed jars with 10–20 mL 0.2 M NaOH solution. The CO_2-trapping NaOH solutions were collected on days 1, 4, 9, 15, 21, 30, 45, 58, 74, and 85 and were titrated with 0.1 M HCl to a phenolphthalein end point with excess $BaCl_2$. Soil moisture was controlled by weighing at every substitution of NaOH solutions during incubation.

Statistics. All data were expressed as the mean ± 1 standard error (SE) and tested for normality and homogeneity before statistical analyses. Paired sample *t*-tests were applied to evaluate the variation of the amounts of plant residual carbon input into PWF and NWF. One-way repeated measures analysis of variance (ANOVA) with farmland type (PWF or NWF) as a fixed factor and sampling depth as repeated measures was conducted on basic soil properties and biochemical parameters at each sampling depth within each site. Two-way repeated measures ANOVA with soil moisture and farmland type as two fixed factors and soil depth as the repeated measures factor were conducted on the total CO_2-C evolution for 85 days of incubation within each site. Duncan multiple range test was used to compare the means at $P < 0.05$.

RESULTS

The carbon of plant residue-return. The carbon of plant residue-return was 32.1% higher on June 2021, but was 42.5% smaller on September 2020 under PWF than under NWF ($P < 0.05$). However, there was no significant difference in the total carbon of plant residue-return between PWF and NWF for a whole year (1013.4 ± 40.6 vs. 944.7 ± 53.2 g/m^2) (Figure 1).

Basic soil properties and soil organic matter and LFOC and β-glucosidase activity. Soil moistures at each soil depth were all significantly higher under PWF than NWF on Sep 12th 2020 and June 1st 2021 (Figure 2). Soil bulk density in the 0-20 cm was higher under PWF compared with NWF ($P < 0.1$), but it was not affected by period waterlogging in the 20–80 cm (Table 1). pH values only decreased in the top 40 cm and the content of carbonate decreased at 40–80 cm depth under PWF compared with NWF (Table 1). The soil texture was silt loam with smaller silt content and higher clay content at depth of 0–20 cm under PWF compared with NWF (Table 1). PWF had lower inorganic N content and Olsen-P content in the top 80 cm, but greater available K at depth of 0–20 and 60–80 cm than NWF (Table 1), indicating that much more available K was absorbed by maize under NWF at depth of 0–20 cm due to no fertilizer under PWF during the growth period of maize.

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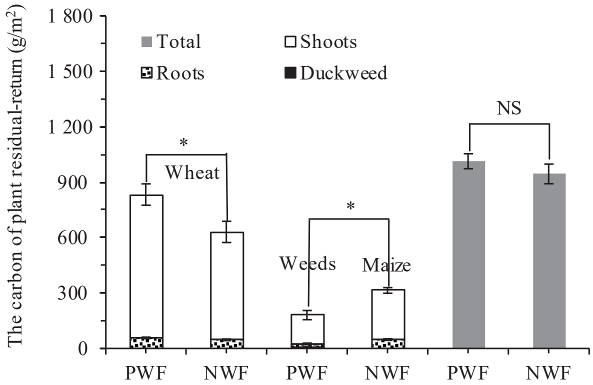

Figure 1. The carbon of plant residue-return under PWF and NWF in June (the shoots and roots of wheat) or September (the shoots and roots of weeds or maize) or a whole year (total)

PWF – periodic waterlogged farmland; NWF – non-waterlogged farmland; *significant at $P < 0.05$; NS – not significant ($P > 0.05$)

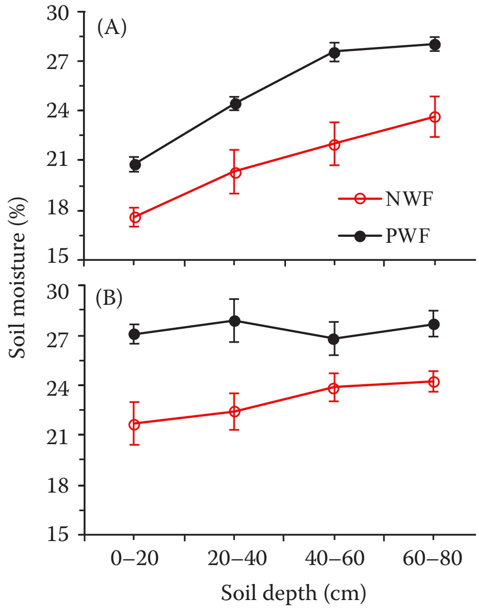

Figure 2. Soil moisture at 0–20, 20–40, 40–60 and 60–80 cm soil depth under PWF and NWF on Sep 12th 2020 (A) and June 1st 2021 (B)

PWF – periodic waterlogged farmland; NWF – non-waterlogged farmland

Periodic waterlogging significantly affected the SOC contents and C/N ratios except soil N contents. The SOC content in the 0–20 cm was 8.4% higher ($P < 0.1$) under PWF than under NWF. However, the contents of SOC were 16.4%, 32.6% and 34.3% smaller ($P < 0.05$) in the 20–40, 40–60 and 60–80 cm under PWF than under NWF, respectively (Table 2). The contents of soil N were similar at each soil depth between PWF and NWF (Table 2). Consequently, the C/N ratio of soil organic matter were 9.2%, 11.6% and 18.3% lower in the 20–40, 40–60 and 60–80 cm under PWF than under NWF, respectively (Table 2). LFOC contents were 66.7% and 72.0% greater in PWF than in NWF at depth of 0–20 and 20–40 cm, respectively (Table 2), but light fraction was not detected

Table 1. Soil physicochemical properties and soil available nutrient contents at depths of 0–20, 20–40, 40–60 and 0–80 cm under PWF and NWF on Sep 12th 2020

Depth (cm)	Farmland	Bulk density (g/cm^3)	pH	$CaCO_3$ (%)	Particle size composition (%)			Available nutrient (mg/kg)		
					2–0.05 mm	0.05 to 0.002 mm	< 0.002 mm	Inorganic N	Olsen-P	K
0–20	PWF	1.23 (0.04)	8.06 (0.03)	5.4 (0.2)	4.4 (0.5)	58.6 (2.4)	37.0 (2.2)	35 (4)	4.2 (0.9)	318 (15)
	NWF	1.06 (0.06)	8.25 (0.02)	5.6 (0.1)	5.4 (0.6)	68.8 (2.2)	25.9 (1.8)	103 (9)	9.2 (0.2)	242 (15)
20–40	PWF	1.35 (0.03)	8.33 (0.04)	5.9 (0.1)	3.9 (0.9)	64.2 (3.1)	31.9 (3.7)	30 (2)	2.9 (0.5)	183 (16)
	NWF	1.34 (0.01)	8.48 (0.03)	6.0 (0.3)	3.6 (1.2)	61.8 (4.5)	34.6 (5.5)	51 (8)	6.3 (0.7)	145 (11)
40–60	PWF	1.38 (0.03)	8.55 (0.04)	5.0 (0.2)	4.5 (1.3)	81.8 (0.9)	13.6 (1.4)	16 (2)	2.6 (0.3)	127 (9)
	NWF	1.40 (0.01)	8.62 (0.04)	5.7 (0.1)	7.9 (2.9)	72.5 (3.1)	19.6 (3.7)	50 (11)	5.4 (0.6)	101 (11)
60–80	PWF	1.40 (0.02)	8.67 (0.04)	4.6 (0.2)	5.2 (1.5)	82.2 (0.8)	12.6 (1.6)	18 (4)	3.0 (0.2)	112 (2)
	NWF	1.42 (0.03)	8.72 (0.03)	5.1 (0.1)	11.0 (3.6)	73.6 (3.4)	15.4 (3.7)	54 (6)	5.7 (0.6)	83 (5)
Farmland		NS	*	*	NS	NS	NS	***	***	**
Depth		**	***	***	NS	***	***	**	**	***
Farmland × depth		NS	*	NS	NS	**	**	*	NS	*

PWF – periodic waterlogged farmland; NWF – non-waterlogged farmland; values in brackets are ± 1 standard error; *,**,***significant at $P < 0.05$, 0.01, 0.001; NS – not significant ($P > 0.05$)

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Table 2. SOC and N contents and C/N ratios and LFOC contents and β-glucosidase activities at depths of 0–20, 20–40, 40–60 cm and 60–80 cm under PWF and NWF on Sep 12th 2020

Depth (cm)	Farmland	Soil organic matter			LFOC (mg/g SOC)	β-glucosidase (mg/g·h)
		SOC (g/kg)	N (g/kg)	C/N		
0–20	PWF	16.7 (0.5)	1.52 (0.03)	11.0 (0.3)	136.4 (15.0)	2.1 (0.1)
	NWF	15.4 (0.6)	1.45 (0.05)	10.7 (0.2)	81.8 (7.0)	2.4 (0.1)
20–40	PWF	6.1 (0.4)	0.73 (0.04)	8.9 (0.2)	88.4 (9.0)	1.9 (0.2)
	NWF	7.3 (0.2)	0.75 (0.03)	9.8 (0.4)	51.4 (7.1)	2.4 (0.2)
40–60	PWF	3.1 (0.4)	0.37 (0.04)	8.4 (0.5)	–	2.4 (0.1)
	NWF	4.6 (0.4)	0.48 (0.04)	9.5 (0.3)	–	3.2 (0.3)
60–80	PWF	2.3 (0.3)	0.30 (0.03)	7.6 (0.4)	–	3.1 (0.2)
	NWF	3.5 (0.2)	0.38 (0.01)	9.3 (0.3)	–	3.2 (0.3)
Treatment effects						
Farmland		*	NS	**	***	NS
Depth		***	***	***	**	NS
Farmland × depth		***	*	*	NS	NS

SOC – soil organic carbon; N – nitrogen; LFOC – light-fraction organic carbon; values in brackets are ± 1 standard error; *,**,***significant at $P < 0.05$, 0.01, 0.001; NS – not significant ($P > 0.05$)

at depth of 40–80 cm. β-glucosidase activities were not affected by periodic waterlogging at each soil depth (Table 2).

Water-stable aggregates. Periodic waterlogging didn't affect the stability of aggregates in the 0–20 cm but decreased aggregate stability in the soil depth of 20–80 cm (Figure 3). Averaging over sampling date, the MWD at depth of 20–40, 40–60 and 60–80 cm were 37.0%, 53.1% and 48.1% lower in PWF than in NWF, respectively (Figure 3A, B). Consequently, macroaggregate proportions at the 20–40, 40–60 and 60–80 cm were 42.2%, 76.9% and 84.3% smaller

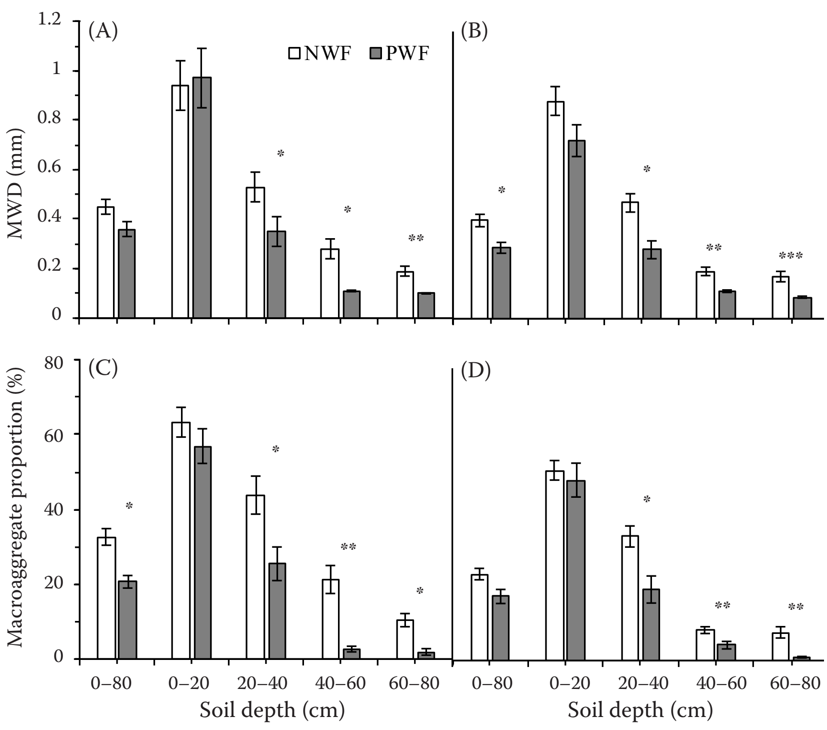

Figure 3. MWD of water stable aggregates and macroaggregates proportion on Sep 12th 2020 (A, C) and June 1st 2021 (B, D) at NWF and PWF at 0–80, 0–20, 20–40, 40–60 and 60–80 cm soil depth

MWD – mean weight diameter; PWF – periodic waterlogged farmland; NWF – non-waterlogged farmland; *, **, ***$P < 0.05$, 0.01, 0.001 are significantly different between NWF and PWF at each soil depth

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Table 3. Amounts of SOC-normalized non-cellulosic neutral sugar and amino sugar contents and mass ratios of GM/AX and GluN/MurA at 0–20, 20–40 and 40–80 cm soil depth under PWF and NWF on Sep 12th 2020

Depth (cm)	Farmland	Non-cellulosic neutral sugars (mg/g)	GM/AX	Amino sugar content (mg/g)	GluN/MurA
0–20	PWF	92.0 (5.9)	0.89 (0.04)	110.3 (9.3)	31.5 (2.6)
	NWF	117.4 (6.6)	0.89 (0.03)	115.5 (9.0)	29.9 (2.2)
20–40	PWF	117.5 (17.5)	0.88 (0.05)	161.3 (41.5)	16.3 (2.3)
	NWF	100.1 (6.9)	0.85 (0.03)	109.9 (16.4)	13.8 (2.3)
40–80	PWF	85.8 (10.6)	0.80 (0.09)	109.9 (16.4)	–
	NWF	39.2 (3.6)	0.54 (0.06)	53.5 (4.5)	–
Treatment effects					
Farmland		**	NS	**	NS
Depth		*	***	*	***
Farmland × depth		*	**	*	NS

SOC – soil organic carbon; GM/AX – (galactose + mannose) to (arabinose + xylose); GluN – glusocamine; MurA – muramic acid; PWF – periodic waterlogged farmland; NWF – non-waterlogged farmland; values in brackets are ± 1 standard error; *,**,***significant at $P < 0.05$, 0.01, 0.001; NS – not significant ($P > 0.05$)

under PWF compared with NWF, respectively (Figure 3C, D). Across soil depths and sampling date, the MWD and macroaggregate proportion in the top 80 cm were 23.6% and 32.2% smaller under PWF than under NWF, respectively (Figure 3).

Soil non-cellulosic and amino sugar contents. Across farmland, SOC normalized total non-cellulose sugars and the mass ratio of GM/AX were 41.6% and 23.6% lower at 40–80 cm depth than at 0–40 cm depth, respectively (Table 3). There were two-way (depth

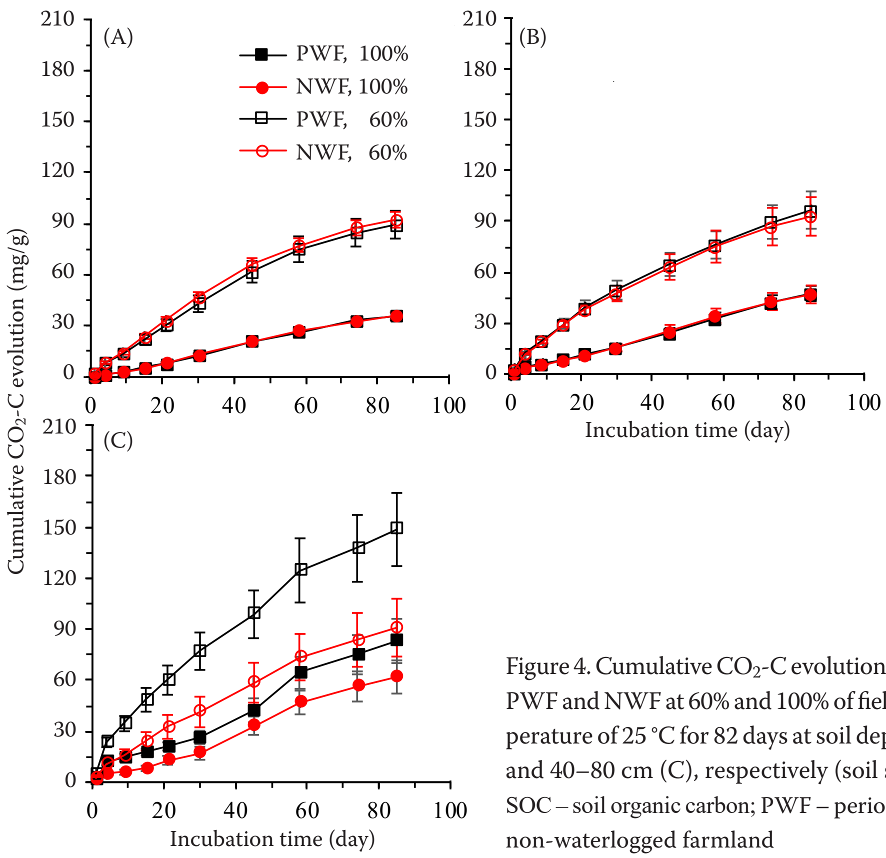

Figure 4. Cumulative CO_2-C evolutions per g SOC in soils sampled from PWF and NWF at 60% and 100% of field capacity under incubation temperature of 25 °C for 82 days at soil depth of 0–20 cm (A), 20–40 cm (B) and 40–80 cm (C), respectively (soil sampled on Sep 12th 2020)

SOC – soil organic carbon; PWF – periodic waterlogged farmland; NWF – non-waterlogged farmland

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× farmland) interaction effects on SOC normalized total non-cellulose sugars and the mass ratio of GluN/MurA (Table 3). Total non-cellulose sugars in SOC and the mass ratio of GM/AX at 0–40 cm depth were all similar between PWF and NWF, but they were 118.9% and 48.1% greater at 40–80 cm depth under PWF compared with NWF, respectively (Table 3). Across farmland, SOC normalized amino sugars contents were 34.2% smaller at 40–80 cm depth than at 0–40 cm depth, respectively (Table 3). SOC contained similar amino sugars contents at 0–40 cm depth between PWF and NWF, however, SOC at 40–80 cm depth was 105.4% richer in amino sugars under PWF than under NWF (Table 3). Muramic acid wasn't detected at 40–80 depth. The mass ratio of GluN/MurA in the 0–20 cm was 104.0 % higher than in the 20–40 cm (Table 3).

Decomposability of SOC. Total CO_2 evolution over 85 days of incubation was only significantly affected by soil moisture and soil depth (Figure 4, Table 4). Across farmland and soil depth, increase in soil moisture from 60% to 100% of FC deceased total CO_2-C evolution (mg CO_2-C per g SOC) by 96.4% (101.8 ± 5.8 vs. 51.9 ± 3.7 mg/g). Across farmland and soil moisture, total CO_2-C evolution in the 0–20 cm were similar with the depth 20–40 cm (63.4 ± 6.7 vs. 71.0 ± 6.8 mg/g), but significantly lower than the total CO_2-C evolution in the 40–80 cm (96.1 ± 10.4 mg/g) (Figure 4, Table 4). In particular, in the 40–80 cm depth, the C loss per unit SOC in NWF increased 46.9% but in PWF increased 78.9% under anaerobic conditions compared with aerobic conditions (Figure 4C), indicating a much more C loss at 40–80 cm when aeration status changed from anaerobic to aerobic conditions in PWF than in NWF.

Table 4. Effects of farmland (NWF, and PWF), soil moisture (60% and 100% of field capacity), and soil depth (0–20, 20–40 and 40–80 cm) on total SOC mineralization (evolved as CO_2-C, mg/g) over the 85 days at 25 °C

Source	df	*F* value	*P* value
Moisture	1	14.1	< 0.001
Farmland	1	0.5	0.472
Depth	2	50.0	< 0.001
Moisture × farmland	1	0.1	0.765
Moisture × depth	2	2.2	0.127
Farmland × depth	2	0.3	0.761
Moisture × farmland × depth	2	1.9	0.163
Error	36		
Total	48		

SOC – soil organic carbon; PWF – periodic waterlogged farmland; NWF – non-waterlogged farmland; df – degrees of freedom

DISCUSSION

Soil organic carbon content and soil aggregate stability. Our first hypothesis that many years of periodic waterlogging induced the decrease of SOC contents and soil aggregate stability in the 20–80 cm except topsoil (0–20 cm) was confirmed. Before periodic waterlogging, SOC contents in each farmland were speculated similarly because of the long-term same treatment (i.e. crops, fertilizer and straw-return). There wasn't significant difference of the carbon of plant residue-return to the depth of 0–20 cm in a whole year between PWF and NWF (Figure 1), the higher concentration of SOC in the 0–20 cm under PWF than under NWF was probable due to the constraint on the decomposition of plant and microbial residues during waterlogging (Nahlik & Fennessy 2016; Chen et al. 2021; Wang et al. 2024). Greater LFOC at 0–40 cm depth in PWF (Table 2) also implies that decomposition of plant residues was suppressed under saturated conditions. Although higher dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) contents during waterlogging would accelerate SOC mineralization when waterlogging recedes (Sánchez-Rodríguez et al. 2019a, b), the excessive DOC and DON contents were consumed in a few days after waterlogging (Sánchez-Rodríguez et al. 2019a) and then might not offset the suppression on decomposition of plant and microbial residues during waterlogging (about 1-month). However, lower content of SOC at the depth of 20–80 cm under PWF than NWF was possible mainly due to lower soil aggregate stability and higher non-cellulosic neutral content and amino sugar content (Figure 3, Table 3). The SOC would be easily consumed by microorganism along with a decline of soil aggregate stability (Huang et al. 2020; Pu et al. 2022) and its mineralization was related with SOC biochemistry (Liu et al. 2023). We didn't measure root biomass below the top 20 cm of soil because more than 90% of roots biomass occurred in the topsoil (0–20 cm) (Wang 2018). Little plant roots were observed during soil sampling at 40–80 cm depth which was consistent with little LFOC under PWF and NWF as well (Table 2). The roots biomass in the 20–40 cm may be lower under PWF than under

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NWF according to lower roots biomass in the 0–20 cm in the former than in the later (Figure 1). Therefore, lower SOC content in the 20–40 cm under PWF than NWF was possible due to lower roots biomass return and soil aggregate stability.

The disturbance from drouth-flood would break soil aggregates (Bi et al. 2020) and this disaggregation increased with the increasing wetting-drying cycle number (Bazzoffi & Nieddu 2011). However, the stability of aggregate in the 0–20 cm was not affected by many years of periodic waterlogging in this study (Figure 1). This was possibly ascribed to the input of abundant plant residue which could improve soil aggregate stability (Wang et al. 2017; Pu et al. 2022), because microbial-derived binders (i.e. extracellular) were produced when microorganism decompose plant residues (Ayoubi et al. 2012; Six et al. 2020). Therefore, lower stability in the 20–80 cm under PWF than NWF was due to little of plant residue-return. Thus, the negative effect of periodic waterlogging on soil aggregates stability wouldn't be counteracted due to the lack of sufficient binder.

Soil non-cellulosic and amino sugar pools. Our results showed similar GM/AX ratios in the 0–40 cm between PWF and NWF (Table 3) which were different with the lower GM/AX ratios in frequently waterlogged or paddy soils compared with meadow or upland soils (Jia et al. 2020; Chen et al. 2021). This might be due to a shorter period of waterlogging (about 1 month) in this study. The effect of about 1 month of waterlogging on the accumulation of microbial synthesis might be minor compared with non-waterlogging period (more than 10 months) every year. However, larger non-cellulosic carbohydrate pool and higher GM/AX ratios under PWF than under NWF in the 40–80 cm reflected a relatively higher production of microbial-derived substances in PWF soils. This might be ascribed to its lower aggregate stability and then the SOC was easily utilized by microorganisms in the absence of plant residue return.

PWF had a significantly increased total amino sugar content in SOC in the 40–80 cm compared with NWF (Table 3), reflecting an increased contribution of microbial necromass to SOC. This also might be related with its lower soil aggregate stability. Fungi mainly decompose organic polymers, which is easily inhibited in anaerobic environment and bacteria preferentially decompose small molecule substances and are insensitive to anaerobic conditions (Sánchez-Rodríguez et al. 2019a). Similar GluN/MurA ratios at 0–20 or 20–40 cm depth between PWF and NWF, indicating similar relative contribution of bacteria and fungi to microbial residues. The effect of waterlogging on fungi growth was possibly eliminated by long-term non-waterlogging period each year. Besides, lower GluN/MurA ratio in the 20–40 cm than in the 0–20 cm reflected an increased contribution of bacteria to necromass along with increased soil depth which was related with the proportions of microaggregates or macroaggregates. The contribution of bacterial residues to organic C was higher in microaggregates while the contribution of fungal residue was higher in macroaggregates (Xu et al. 2022).

Decomposition of soil organic carbon. Our second hypothesis that the decline of soil aggregate stability would increase C loss at subsoil in PWF soil when aeration status changed from anaerobic to aerobic conditions was confirmed in the 40–80 cm soil depth. The impact of waterlogging on SOC mineralization was positive related with SOC biochemistry (i.e. total non-cellulosic sugar contents and amino sugar contents) (Zhao et al. 2014; Jia et al. 2020; Liu et al. 2023). The proportion of SOC mineralized for 82 days' incubation were similar between PWF and NWF at the depth of 0–20 or 20–40 cm (Figure 4, Table 4). This was consistent with their similar contents of non-cellulosic sugars and amino sugars (Table 3). However, in the 40–80 cm soil depth, the cumulative CO_2-C evolution from anaerobic to aerobic conditions was significantly higher under PWF than under NWF, indicating an increase of C loss in this soil layer after many years of periodic waterlogging, which was consistent with the research of Schindlbacher et al. (2022). This may be because their higher contents of non-cellulosic sugar and amino sugar under PWF than NWF in the 40–80 cm. Although the stability of aggregate was lower under PWF than under NWF in the 20–40 cm or in the 40–80 cm, SOC decomposition presented different changes (Figure 4). Therefore, waterlogging stimulated SOC decomposition might don't necessarily result from the decreased stability of aggregate. Besides, lower SOC mineralization under anaerobic conditions than under aerobic reflected the inhibition of O_2 limitation on SOC decomposition which was consistent with previous studies (Yang et al. 2013; Liu et al. 2019; Qu et al. 2024). However, waterlogging promoted SOC mineralization in meadows or farmland or coastal marshes (Huang & Hall 2017; Jia et al. 2020; Li et al. 2022; Liu et al. 2023). This might be due to their different soil characteristics and microbial community composition, but further research is needed. Our results highlighted that the degree of SOC degradation is an important factor affecting SOC mineralization in farmland.

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CONCLUSION

As expected, abundant straw-return could offset the adverse effects of periodic waterlogging on soil aggregate stability and SOC contents in the 0–20 cm. Therefore, periodic waterlogging resulted in the decrease of soil aggregate stability and SOC contents in the 20–80 cm due to little straw-return. However, periodic waterlogging only increased the non-cellulosic sugar contents, GM/AX ratios and amino sugars contents in the 40–80 cm, resulting in a much more C loss from anaerobic to aerobic conditions at 40–80 cm depth. Our result emphasized the importance of straw-return under periodic waterlogged farmland and might implied that deep ploughing could reduce the C loss in the subsoil in periodic waterlogged farmland.

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