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RESEARCH ARTICLE

Earthworms promote the accumulation of rhizodeposit carbon to soil macroaggregate in a Mollisol of northeast China, primarily in long-term no-till soil

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HIGHLIGHTS

• Earthworms increased soil macroaggregate (>2 mm and 0.25–2 mm) formation.
• Maize roots and earthworms interact to produce soil macroaggregate.
• Earthworms were effective in transferring rhizodeposit carbon into macroaggregate, especially in soil derived from a long-term no-till system.
• Rhizodeposits were protected during soil aggregation.

GRAPHICAL ABSTRACT
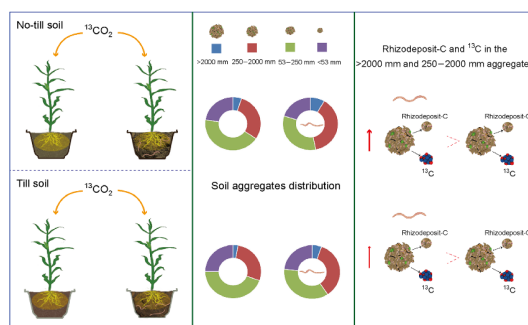
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ABSTRACT

As soil ecosystem engineers, earthworms are the main promoters of soil aggregation, a process that drives the production of ecosystem services by soils. A crucial factor in the ecosystem service of carbon sequestration is rhizodeposit carbon, which is the main energy source of soil food webs. The effects of earthworms on the distribution of rhizodeposit-carbon in soil aggregates remain unclear. Here, we conducted a $^{13}CO_2$ labeling experiment to determine the effects of earthworms on maize rhizodeposit carbon in soil aggregates after 14 years (2002–2016), in both conventional tillage (CT) and conservation tillage (no tillage, NT) soils. Four treatments were established in total: NTE (no tillage soil with earthworms), CTE (conventional tillage soil with earthworms), NTC (control, no tillage soil without earthworms), and CTC (control, conventional tillage soil without earthworms). Earthworms significantly enhanced the abundance of soil macroaggregates (>2000 μm and 250–2000 μm) on day 30 compared with day 2 (after labeling), especially in the NT soils. On day 30, in the presence of earthworms, the amounts of rhizodeposit carbon in the >2000 μm and 250–2000 μm soil aggregates in the NT soils were significantly higher than in those in the CT soils ($P < 0.05$), and higher $\delta^{13}C$ signatures in the same size aggregates were observed in the NT soils than in the CT soils ($P < 0.05$). These findings indicated that compared with the CT soils, with the involvement of earthworm activity, the NT soils promoted more rhizodeposit carbon transformation to the soil macroaggregates. Our results clearly indicate that soil macroaggregates formed in different tillage soils in the presence of 2 different engineers (earthworms and roots) significantly differ from those formed in the presence of only one organism (roots) in the long term.

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1 Introduction

Soil aggregates are the main component of soil structure, and their turnover is related to the activities of organisms that live within the soil (Six et al., 2004). Soil microaggregates bind more firmly than soil macroaggregates, thus providing more stable long-term storage for soil organic carbon (SOC) (Six et al., 2002). However, soil macroaggregates are key in providing sites for soil microaggregate formation (Six et al., 2000). The renewal of soil aggregates is a key process in the soil system which is increased by the activities of soil fauna (Six et al., 2000; Gong et al., 2019).

As ecosystem engineers, earthworms affect soil structure and nutrient cycling via their burrowing, feeding, and casting activities, which influence soil organisms and SOC decomposition (Brown et al., 2004; Fonte et al., 2012). The process of SOC stabilization is highly dependent on the formation and stability of soil aggregates, which are affected by the activities of earthworm (Bossuyt et al., 2005; Six and Paustian, 2014). Lubbers et al. (2017) reported that earthworms can increase the incorporation of residual carbon into soil aggregates in the short term, and aid in the decomposition of SOC in the long term. Soil and plant residues that are ingested by earthworms are mixed in their guts (Zangerlé et al., 2011), and the residual carbon is then rapidly protected by the microaggregates and macroaggregates in the soil (Bossuyt et al., 2005; Fonte et al., 2012). Although fresh leaf litter can contribute to the formation of soil aggregates through leachable organic compounds as aggregate binders, roots and rhizodeposits could be the main source of fresh organic matter that promotes the formation of soil macroaggregates (Fahey et al., 2011). An earlier investigation demonstrated that roots have a relatively larger effect on soil carbon pools, and the greater retention of root-derived carbon has been attributed to physical protection by soil aggregates (Wander and Yang, 2000). It has been suggested that increasing rhizodeposit carbon distribution could have positive or negative effects on soil carbon sequestration (Rogers et al., 1994; Wilson et al., 2009; Cheng et al., 2012). Additionally, Boone et al. (1998) found that live roots and rhizodeposition are key factors in regulating carbon sequestration rates. Most crops in agricultural soils supply much more leaf litter than root litter and, therefore, the soil aggregates have more leaf-derived carbon than root-derived carbon (Denef and Six, 2006). On the other hand, another investigation showed that root litter lasted longer in soil aggregates formed from crops (Kong and Six, 2010). Furthermore, recent studies have demonstrated that earthworms consume large amounts of root-derived carbon and incorporate it into soil aggregates (Gilbert et al., 2014; Sánchez-de León et al., 2014). Also, the labile substances released by living roots significantly increase SOC mineralization by up to 27% to 245% (Zhu et al., 2014). The relationships between these labile substances and earthworms are very close, and earthworms can change the fate of rhizodeposit carbon by increasing its accessibility to soil microbes (Huang et al., 2015). However, the effects of earthworms on the relatively more accessible carbon that is released into the rhizosphere and on the formation of soil aggregates have not been fully clarified. Therefore, a better understanding of the effects of earthworms on the transfer of rhizodeposit carbon to soil aggregates is needed.

Conservation tillage regimes, including no tillage (NT), have received attention because these practices can reduce soil erosion and increase the carbon sequestration of agricultural surface soils by increasing the stability of soil aggregates. The number of carbon-rich soil macroaggregates increases after implementation of NT, as the disturbance of soil decreases and soil fauna changes (Six et al., 2004). However, the soil organic matter turnover rate increases, the amount of soil aggregates decreases, and organic matter decomposition increases under intensive tillage (Six et al., 2000). The role of earthworms is particularly interesting in the long-term NT regime, because this practice can increase the number of earthworms and soil aggregate size (Bai et al., 2018). The number of soil surface-feeding earthworms that mix residues into the soil can be increased by increasing the utilization rate of surface soil residues and reducing the physical disturbance of NT soils, and this would alter the soil nutrient availability and soil structure. Furthermore, plant productivity (van Groenigen et al., 2014) and the proportion of photosynthesized carbon that is incorporated into the soil by earthworm activity is increased by earthworms (Nieminen et al., 2015; Huang et al., 2015). However, under different tillage regimes, the proportional contribution of earthworms to the relatively more accessible rhizospheric carbon, which is a vital factor in soil carbon sequestration, remains unclear. The development of rhizosphere management strategies for carbon sequestration and cropland soil fertility can be facilitated by an improved understanding.

In this investigation, a $^{13}CO_2$ labeling experiment was carried out to analyze the effects of earthworms on the mass of soil aggregates and the distribution of rhizodeposit carbon in soil aggregates under different tillage regimes. We hypothesized that 1) the presence of earthworms in either soil type would increase soil macroaggregate formation; and 2) compared to the CT soils with earthworms, increased biomass inputs related to earthworm activity in the NT soils would increase the contribution of rhizosphere carbon (root-derived carbon) to soil aggregate carbon storage.

2 Materials and methods

2.1 Site description and microcosm setup

The investigation site was established at the Experimental Station of the Northeast Institute of Geography and Agroecology (44°12′N, 125°33′E), Chinese Academy of Sciences, Dehui County, Jilin Province, China. Mean annual temperature is 4.4°C; the coldest month of the year is January (−19.5°C), and the warmest month is July (24.5°C). Mean annual precipitation is 520 mm, and more than 70% of the annual precipitation occurs from June to August. The soil type

Table 1 Soil physical and chemical properties under different tillage treatments before the experiment.

Tillage	NT	CT
SOC (g kg^{-1})	21.77±0.31	15.95±0.65
TN (g kg^{-1})	1.38±0.06	1.11±0.03
NO^{3-}–N (mg kg^{-1})	36.24±1.97	25.72±0.55
NH^{4+}–N (mg kg^{-1})	16.38±2.37	17.01±0.57
BD (g cm^{-3})	1.33±0.11	1.30±0.21
pH	5.89±0.21	5.97±0.19
C/N	15.82±0.43	14.32±0.68
^{13}C (‰)	−18.44±0.29	−19.55±0.27

is Mollisols soil (USDA soil taxonomy) with clay-loam texture (36% clay, 24% silt, and 40% sand) (Liang et al., 2007). The relevant soil properties prior to the experiment are presented in Table 1. In the NT system, the soil was not disturbed, except when the crops were planted with a KINZE-3000 NT planter. In the CT system, autumn moldboard plowing (18–20 cm) was followed by secondary seedbed preparation by disking (7.5–10 cm) in the spring. After harvesting, the maize straw in the NT system was cut into small pieces (approximately 15 cm), leaving stubble 15–20 cm long, and then returned to the soil surface. In the CT system, all maize residue on the soil surface was plowed below the soil surface during autumn tillage.

Two types of soil were used in this investigation—one from an NT system and the other from a CT system. Both systems had been planted with a maize monoculture and were cultivated for 14 years (2002–2016). The entire setup was presented in detail by Zhu et al. (2019). In short, a randomized block design was used in this investigation, with a total of 4 treatments (NTE (no tillage soil with earthworms), CTE (conventional tillage soil with earthworms), NTC (control, no tillage soil without earthworms), and CTC (control, conventional tillage soil without earthworms)), and each treatment had 5 replicates. In early September of 2016, 800 g of treated soil was placed in each nylon bag (mesh size ⩽ 0.3 mm, 30 cm long × 20 cm wide; nylon bag to prevent earthworms from escaping), and distilled water was added (150 mL/kg^{-1}) to reach 70% water-filled pore space (WFPS). Finally, each nylon bag was placed in a plastic pot (approximately 11.5 cm in diameter by 14 cm in height).

2.2 Collection and inoculation of earthworms

Epigeic earthworms (*Esienia nordenskiodi pallida* Malevič, 1956) were hand-sorted in the field at the same location as the soils in late August 2016. Because the earthworm distribution was aggregated in the field, a slight population density of earthworms (4 ind. Pot^{-1}, approximately 385 m^{-2}) was used to make it easier to detect the effects of earthworms on soil carbon dynamics (Boag et al., 1994; Zhang et al., 2013; Zhu et al., 2016). The earthworms were placed on wet filter paper for 48 h so they could void their guts prior to being placed in the

pots when the maize seedlings were transplanted. Furthermore, 3 g of sterilized plant litter (a mixture of maize and weeds) previously collected from the same field site was added to the surface of each pot to provide sufficient food for the earthworms and to imitate the field environment.

2.3 Labeling chamber and ^{13}CO$_2$ labeling

A labeling chamber (1.8 m long × 1.2 m wide × 1.4 m high) was built in July of 2016 for the injection and monitoring of CO$_2$. It was made of acrylic glass (roof, 2 mm thick; walls, 5 mm thick) and contained two probes: a CO$_2$ concentration probe (Taishi TES 1370, China) and a high-precision temperature and relative humidity meter (Hengxin AZ8726, China). The modified CO$_2$ concentration detector probe was connected via a sensor to a ^{13}C-labeled and unlabeled CO$_2$ gas cylinder. A small air conditioner was placed in the labeling chamber to maintain the temperature at approximately 25°C. The plants (maize) in the chamber were watered every 3 or 4 days using a drip irrigation system.

Labeling started (in late September of 2016—15 days after the earthworm inoculation) after the earthworms had gradually adapted to their new environment. The specific methods of ^{13}CO$_2$ labeling we followed are described in Zhu et al. (2019). Briefly, ^{13}CO$_2$ (99 at. %^{13}C, Sigma Aldrich Corporation, USA) was delivered into the chamber for 4 consecutive days between 11:30 am and 1:00 pm (Kaštovská and Šsantrùčková, 2007). On cloudy days, a CO$_2$ concentration of approximately 370–400 ppm was delivered for 30 minutes, and on sunny days, a higher CO$_2$ concentration (approximately 500 ppm) was delivered for 1 h. Approximately 10 L of ^{13}CO$_2$ had been used at the end of the 4-day labeling period.

2.4 Sampling and analyses

Destructive sampling was conducted on days 2 and 30 after labeling. The main reason for the short duration was that we aimed to avoid any possible interference and wanted to focus on the effects of earthworms on the readily available rhizodeposit carbon (Gilbert et al., 2014). To obtain the soil samples, the whole maize root system was first dug out and gently shaken to remove large pieces of soil without root (bulk soil), and then all soil attached to the root surface was removed by shaking. Finally, the soil samples were carefully placed in bags by hand. This fraction is referred to thereafter as rhizospheric soil (Zhang et al., 2011). The soil samples were air-dried to analyze the δ^{13}C signatures in the soil aggregates. The maize was separated into roots and stems + leaves, which were cleaned with tap water and then rinsed with deionized water. All maize materials were oven-dried at 55°C and then used to analyze the δ^{13}C signatures and carbon content. The earthworms were counted by hand-sorting; then, all earthworms were rinsed with deionized water and euthanized by placing them in a freezer at −20°C for approximately 5 minutes. Next, the guts of the earthworms were dissected, and the contents were washed with deionized water and collected. The tissue and gut contents were then

freeze-dried to analyze the δ^{13}C signatures and carbon content.

Soil aggregate fractionation by wet-sieving was carried out following the methods adapted from Elliott (1986) and Six et al. (2000), and 4 aggregates were obtained: 1) large soil macroaggregates (>2000 µm), 2) small soil macroaggregates (250–2000 µm), 3) soil microaggregates (53–250 µm) and 4) silt and clay (<53 µm). Briefly, 80 g of air-dried soil was immersed in deionized water on a 2 mm sieve. After 5 minutes, the sieve was shaken up and down 50 times in 2 minutes. The soil remaining on the sieve was then rinsed into a pre-weighed aluminum pan, while the soil passing through the 2 mm sieve was transferred to a 250 µm sieve. The process was repeated with the 250 µm and 53 µm sieves until each of the 4 fractions was weighed in their respective pre-weighed pans. Each of these fractions was rinsed into pre-weighed aluminum pans and oven-dried at 60°C, and the δ^{13}C signatures were then measured as described below.

Prior to the isotopic and carbon content analyses, all dried materials were ground using a ball mill and sieved through a < 0.15 mm mesh. Then, the δ^{13}C signatures in the earthworm tissues, soil aggregates, and maize were determined following standard procedures using stable isotope ratio mass spectrometry (MAT 253, Thermo Fisher, USA) after the samples were concentrated, centrifuged, and dried. The results were calculated as follows: δ^{13}C (‰) = $[(^{13}R_{sample}/^{13}R_{standard}) - 1] \times 1000$, where $^{13}R = {}^{13}C/{}^{12}C$; Pee Dee Belenite (PDB) was used as the standard for ^{13}C. Jones et al. (2009) reported that plant roots are generally assumed to be responsible for a large fraction of the rhizodeposition process and that the δ^{13}C signature of roots reflects the δ^{13}C value of both root respiration and rhizodeposit-related respiration. In our investigation, the δ^{13}C value was used as an indicator of the δ^{13}C signature in rhizodeposits, including both the ^{13}C-labeled and non-labeled newly photosynthetic carbon. Therefore, according to Huang et al. (2015), rhizodeposit carbon in the soil aggregates and earthworms was calculated as follows:

$$C_{soil} = (\delta^{13}C_{SOC-t0} - \delta^{13}C_{SOC-t1})/(\delta^{13}C_{SOC-t0} - \delta^{13}C_{root})$$

$$C_{aggregates} = (\delta^{13}C_{aggregates-t0} - \delta^{13}C_{aggregates-t1})$$
$$/(\delta^{13}C_{aggregates-t0} - \delta^{13}C_{root})$$

$$f_{aggregates} = C_{TOC-aggregates} \times C_{aggregates}$$

$$C_{gut\ content} = (\delta^{13}C_{SOC-t0} - \delta^{13}C_{gut\ content})/(\delta^{13}C_{SOC-t0} - \delta^{13}C_{root})$$

Where, C_{soil} is the fraction of rhizodeposit carbon in the soil, $\delta^{13}C_{root}$ is the δ^{13}C in the plant roots, and $\delta^{13}C_{SOC-t0}$ and $\delta^{13}C_{SOC-t1}$ are δ^{13}C of the soil prior to and after labeling, respectively. $C_{aggregates}$ is the fraction of rhizodeposit carbon in the large macroaggregates (>2000 µm), small macroaggregates (250–2000 µm), soil microaggregates (53–250 µm), and silt and clay (<53 µm) respectively. $\delta^{13}C_{aggregates-t0}$ and $\delta^{13}C_{aggregates-t1}$ are δ^{13}C of the given soil aggregates prior to

and after labeling, respectively. $f_{aggregates}$ is the amount of rhizodeposit carbon in the soil aggregates, and $C_{TOC-aggregates}$ is the total organic carbon in aggregates. $C_{gut\ content}$ is the fraction of the rhizodeposit carbon in the earthworm gut contents, and $\delta^{13}C_{gut\ content}$ is the δ^{13}C of the gut contents after labeling.

2.5 Statistical analysis

The influence of soils and earthworms on soil aggregates and δ^{13}C signatures, as well as rhizodeposit carbon in the soil aggregate fractions, was examined using analysis of variance (ANOVA). The earthworms, soils (NT and CT), and earthworm × soil interaction were considered the main effects. Where the homogeneity of variance was satisfied, a least-significant difference (LSD) test was used to compare the means; otherwise, the data were log10 or square root-transformed. All statistical analyses were conducted with SPSS 16.0 (SPSS Inc., Chicago, IL, USA), and statistical significance was evaluated at $P < 0.05$.

3 Results

3.1 Mass of soil aggregates

Soil aggregates >2000 µm were more abundant in the NTE treatment than in the CTE treatment both on days 2 ($P < 0.01$) and 30 ($P < 0.05$) (Fig. 1A and B). On day 30, soil aggregates that were 250–2000 µm in the treatments with earthworms (NTE and CTE) were more abundant than those without earthworms (NTC and CTC), and there were significant differences between the NTE and NTC treatments ($F = 23.888$, $P = 0.035$) (Fig. 1B). The presence of earthworms significantly decreased the content of soil aggregates measuring 53–250 µm ($F = 7.310$, $P = 0.011$). Compared to the result on day 2, the content of soil aggregates >2000 µm in the NTE treatment significantly increased on day 30 ($F = 9.834$, $P = 0.035$). On day 30, the abundances of soil aggregates 250–2000 µm in the NTE ($F = 13.932$, $P = 0.020$) and CTE ($F = 9.570$, $P = 0.036$) treatments were significantly higher than those on day 2. The contents of soil aggregates 53–250 µm in the NTE ($F = 32.392$, $P = 0.005$) and CTE ($F = 14.336$, $P = 0.019$) treatments on day 30 significantly decreased compared to those on day 2.

Compared with the results on day 2, the absolute mass of soil aggregates >2000 µm ($P = 0.035$) and 250–2000 µm ($P = 0.020$) in the NTE treatments and the absolute mass of soil aggregates >2000 µm ($P = 0.036$) in the CTE treatment significantly increased on day 30 (Fig. 2A and B). On day 30, earthworms significantly increased the absolute mass of soil aggregates >2000 µm ($P = 0.048$) and 250–2000 µm ($P = 0.008$) in NT soils (Fig. 2B). However, in the CT soils, the presence of earthworms only increased the absolute mass of soil aggregates >2000 µm ($P = 0.041$). In the treatments with earthworms, tillage significantly affected the masses of >2000 µm soil aggregates ($P = 0.024$) (Fig. 2B).
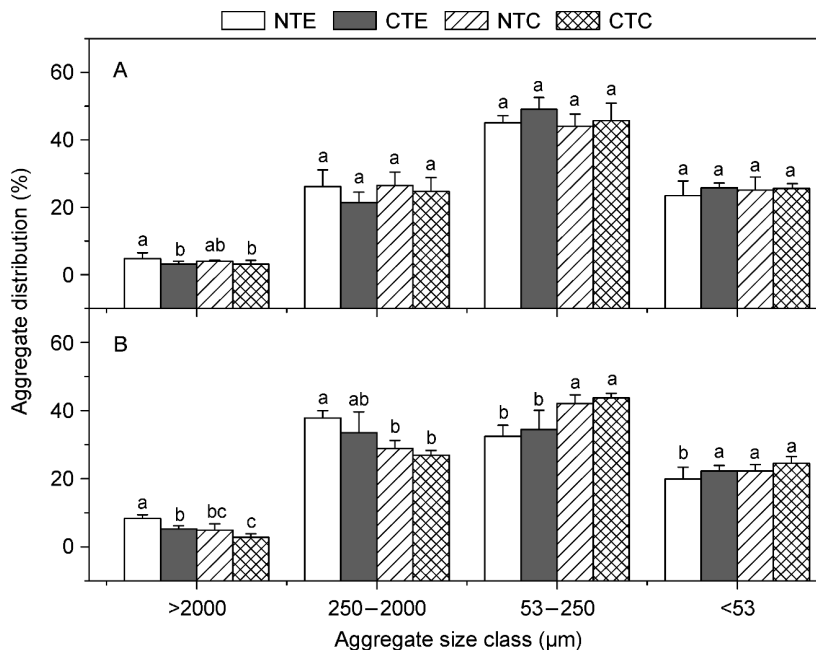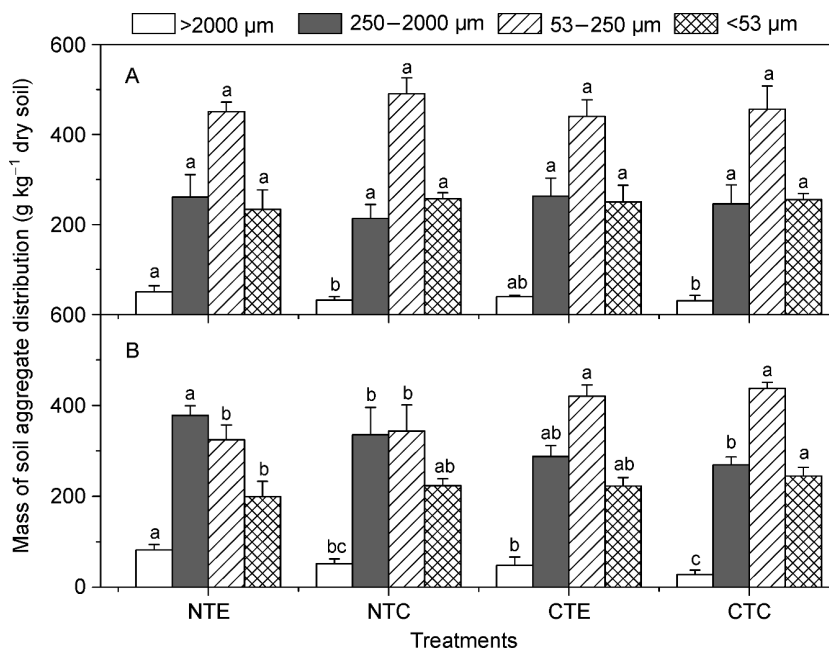
Fig. 1 Mass distribution of soil aggregate under different tillage regimes on day 2 (A) and on day 30 (B). Different letters indicate significant differences between treatments.
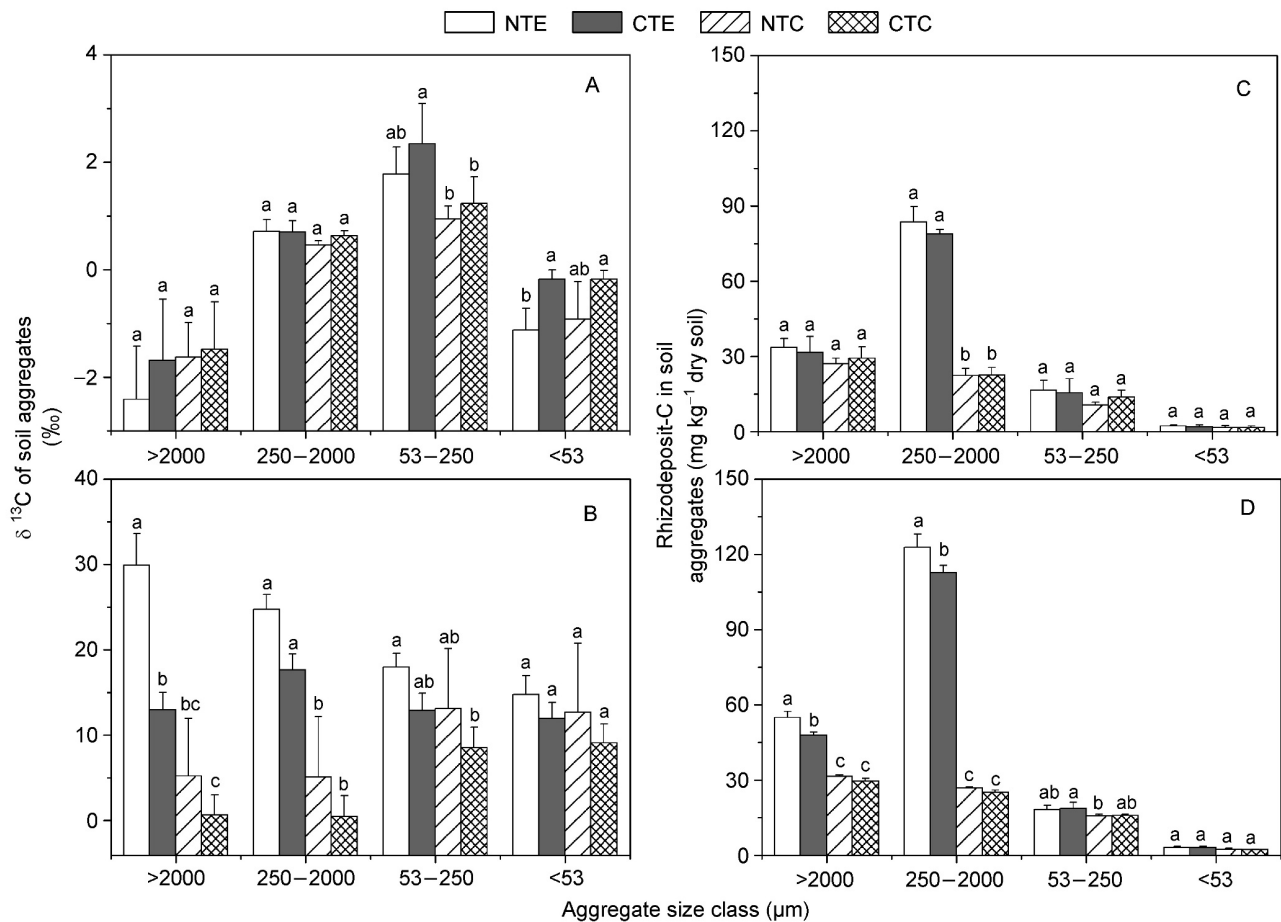
3.2 $\delta^{13}C$ signatures and rhizodeposit carbon retained in the soil aggregates

Compared with the results from day 2, the $\delta^{13}C$ signatures in each soil aggregate increased to different degrees on day 30, but there were significant differences in the treatments with

earthworms (Fig. 3A and B). On day 30, the earthworms had significant effects on the $\delta^{13}C$ signatures of the soil macro-aggregate fractions (>2000 μm, $F = 59.666$, $P < 0.001$; 250–2000 μm, $F = 64.946$, $P < 0.001$) (Fig. 3B, Table 2). Compared to the CT soils, the NT soils with earthworms had significant effects on the $\delta^{13}C$ signatures of soil aggregates >2000 μm

Fig. 2 Absolute mass of soil aggregates on day 2(A) and on day 30 (B). Different letters indicate significant differences between treatments.

Fig. 3 δ^{13}C signatures (A, day 2; B, day 30) and rhizodeposit carbon (C, day2; D, day 30) in the soil aggregates. Different letters indicate significant differences between treatments.

(F = 48.312, P = 0.002). The interaction between earthworms and the different tillage soils (E × S) only had a significant effect on the δ^{13}C signatures of soil aggregates >2000 μm (F = 6.603, P = 0.033).

On day 2, there were no significant differences in rhizodeposit carbon among other soil aggregates except for the >2000 μm soil aggregates (Fig. 3C). More rhizodeposit carbon was retained in the >2000 μm and 250–2000 μm soil aggregates in the treatments containing earthworms than in those without earthworms (day 30, P < 0.001) (Fig. 3D). NT soils with earthworms retained more rhizodeposit carbon in the >2000 μm (F = 21.009, P = 0.010) and 250–2000 μm (F = 8.551, P = 0.043) soil aggregates than in the CT soils (Fig. 3D). Moreover, the interaction between earthworms and the different tillage soils had significant effects on the rhizodeposit carbon in the soil macroaggregate fractions (>2000 μm, F = 9.118, P = 0.017; 250–2000 μm, F = 5.627, P = 0.045) (Table 2). Compared to the results of day 2, the rhizodeposit carbon in the >2000 μm (NT soils, P = 0.001; CT soils, P = 0.013) and 250–2000 μm (NT soils, P = 0.001; CT soils, P < 0) soil aggregates on day 30 significantly increased both in NT and CT soils in the presence of earthworms.

4 Discussion

4.1 Soil aggregate distribution

The earthworms significantly macroaggregated the soils. In just over one month, compared to the results of day 2, large soil macroaggregates (>2000 μm) and small soil macroaggregates (250–2000 μm) increased significantly on day 30 in the treatments with earthworms (Fig. 1). These results confirm the capacity of these organisms to transform large amounts of soil in relatively short periods of time. This observation, which has been made for earthworms in several previous studies (Zangerlé et al., 2011; Arai et al., 2013), had not been made for the coexistence of earthworms with roots, especially in soils with different tillage regimes. This finding indicates that ecosystem engineers can macroaggregate soil very rapidly when the conditions are favorable.

Our results showed that the NT soil facilitated more soil macroaggregates with the activities of earthworms compared to the CT soils (Fig. 1B). Studies have demonstrated that many factors can affect soil aggregate formation, such as soil fauna (especially earthworms), tillage regimes, and soil

Table 2 *F*- and *P*-values of ANOVA (analysis of variance) on the effects of earthworms (with and without) and different tillage soils (NT and CT) on the $\delta^{13}C$ signatures and rhizodeposit-carbon across soil aggregates.

Index	Soil aggregates	Earthworms (E)		Soils (S)		Earthworms × soils (E × S)	
		F	*P*	*F*	*P*	*F*	*P*
$\delta^{13}C$ signatures	>2000	59.666	**0.000**	20.095	**0.002**	6.603	**0.033**
	250–2000	64.946	**0.000**	6.593	**0.033**	0.300	0.599
	53–250	4.126	0.077	4.558	0.065	0.011	0.921
	<53	0.934	0.362	1.591	0.243	0.023	0.883
Rhizodeposit-carbon	>2000	592.978	**0.000**	27.698	**0.001**	9.118	**0.017**
	250–2000	2662.057	**0.000**	11.468	**0.010**	5.627	**0.045**
	53–250	8.823	**0.018**	0.112	0.746	0.001	0.982
	<53	4.912	0.058	0.033	0.860	0.147	0.711

Significant effects are highlighted in bold ($P < 0.05$).

microorganisms (Pinheiro et al., 2004; Pulleman et al., 2005; Arai et al., 2013). The growth and turnover of roots and their exudates, factors that drove belowground productivity in this investigation area (Zhang et al., 2013; Zhang et al., 2018), are probably important soil aggregate binding agents. As binding agents, soil microbial biomass plays a crucial role in the formation and stabilization of soil aggregates (Six et al., 2004). Reduced tillage and increased crop residues could improve the physical, chemical, and nutrient habitat characteristics of soil microbes, thereby promoting microbial growth (Rillig, 2004). Furthermore, under conservation tillage, earthworm activities become prominent and their abundance was almost always higher than that under CT (Capowiez et al., 2009; Ayuke et al., 2011; Briones and Schmidt, 2017). In our previous investigation, the survival rates of earthworms were higher in NT soil than in CT soil (Zhu et al., 2019) and it is presumed that earthworm guts can provide binding materials for soil macroaggregate formation (Judas, 1992). Studies showed that earthworms increased soil microbial density and mixed crop residues and soil, which increased the resource supply of soil microbes, thereby favoring microbial density (Groffman et al., 2015; Gong et al., 2019).

Our results also indicated that the beneficial influence of maize on soil aggregation could depend on the presence of earthworms and/or the carbon input related to maize roots (Zhu et al., 2019). Some studies have suggested that the stability of earthworm casts and soil macroaggregates is correlated with soil carbon content (Jouquet et al., 2008; Kong and Six, 2010; Fonte et al., 2012), as organic matter forms the basis of stable bonding agents that hold aggregates (including casts) together (Guggenberger et al., 1996). Therefore, the beneficial effects of plant roots on the observed soil aggregates could be related to earthworm activity, the interaction between earthworms and soil microbes, and the aggregation of roots on soil aggregates (Angers and Caron, 1998). The reduction in soil microaggregates might be because new macroaggregates were formed because of the rearrangement of these microaggregate fractions. In a

microcosm experiment, Bossuyt et al. (2005) found that more soil microaggregates were stabilized within earthworm-formed large soil macroaggregates (>2000 µm) in treatments with earthworms, than in treatments without earthworms. Our findings indicate that earthworms are likely to consume existing soil structures of different sizes and can rapidly rearrange them to produce new soil macroaggregates, which could be more pronounced in long-term conservation tillage soils.

4.2 ^{13}C and rhizodeposit carbon in the soil aggregates

Our most striking results may have come from the comparison of the $\delta^{13}C$ signatures and the fate of rhizodeposit carbon between soil aggregates, with and without earthworms, under different soil tillage treatments. In the presence of earthworms, more $\delta^{13}C$ signatures and rhizodeposit carbon were incorporated into the soil macroaggregates (>2000 µm and 250–2000 µm) than in the treatments without earthworms, and the effects were more obvious in the NT soils (Fig. 3). Yavitt et al. (2015) found that earthworms greatly increased the proportion of root-derived carbon incorporated into soil macroaggregates and then accelerated the process of root carbon incorporation into soil aggregates. Furthermore, earthworms can directly absorb carbon recently fixed by plants (Huang et al., 2015) or the newly fixed fraction contained in soil aggregates (Fonte et al., 2012). Arai et al. (2013) found that earthworm activity and plants rapidly increased soil carbon content by incorporating fresh carbon into the soil macroaggregtes in a no-tillage system. Our previous investigation showed that earthworms increased maize biomass, and the $\delta^{13}C$ signatures in the maize roots reflected the input of ^{13}C-labeled photosynthetic carbon into the soil. In the NT soils, with the higher maize biomass and the activity of soil microbes, earthworms could allocate relatively more ^{13}C to the soil (Zhu et al., 2019). Therefore, due to earthworm activities, such as mixing the soil and feeding, the increased ^{13}C values in the treatments with earthworms were largely due to the addition of new photo-

synthetic carbon. These findings indicate that earthworms promote the incorporation of fresh organic matter into soil macroaggregates. Moreover, the findings indicate that plant roots and earthworms are closely related in the formation of soil aggregates, and the proper management of soils by these two organisms should be considered simultaneously.

Earthworms regulate carbon dynamics mainly through their gut processes to protect the carbon from the effects of their digested aggregates. Annually, earthworms generate from a few hundred to thousands of milligrams of casts (dry weight) per ha (Lavelle et al., 1992). Once these casts dry, they become compact and can be kept intact for several years without being destroyed by physical processes or ingested by other invertebrate ecosystem engineers (Blanchart et al., 1999). This process can promote carbon stabilization in the soil aggregates that are formed in earthworm casts (Bossuyt et al., 2005; Pulleman et al., 2005). Therefore, if earthworms regulate or protect rhizodeposit carbon, more rhizodeposit carbon would be observed in their gut contents than in the control soils (Huang et al., 2015). Our previous research results showed that the amounts of rhizodeposit carbon in earthworm guts were significantly higher than those in the control soils (Zhu et al., 2019). Furthermore, compared with the CT soils, NT soils had higher rhizodeposit carbon in the earthworm guts. Additionally, in this area, the 14-year (2002–2016) NT system could have enhanced the structure and function of the soil food web via bottom-up effects (Zhang et al., 2015), forming a stable soil ecosystem (Kong and Six, 2010; Zhang et al., 2013). Gong et al. (2019) reported that the assemblage of soil microbial communities changed drastically between soil aggregate fractions and with the presence of the earthworms. Additionally, the microbial communities in bulk soil were more similar to those in large macroaggregates (>2 mm) or small aggregates (< 0.053 mm) (Wieland et al., 2001; Gong et al., 2019). These observations indicate that the effects of earthworms on rhizodeposit carbon transformation depend on the earthworms on rhizosphere microbiota (Huang et al., 2015), whereas the absence of earthworm, by the effects the activities of soil microbes, could limit the transformation of rhizodeposit carbon. Another investigation indicated that the amount of root-derived CO_2 was significantly higher under the CT system than under the NT system (Mo et al., 2019). Therefore, in NT soils, earthworms could have allocated relatively large amounts of fixed ^{13}C belowground and the earthworms are more likely to transfer the root-derived carbon and protect it in the soil macroaggregates.

Furthermore, earthworms increase plant productivity (van Groenigen et al., 2014) and the proportion of photosynthesized carbon that is incorporated into the soil by earthworm activity (Nieminen et al., 2015; Huang et al., 2015). Roots and associated rhizodeposits are presumably the main sources of fresh organic matter facilitating soil macroaggregate formation (Miller and Jastrow, 1990; Wilson et al., 2009). Root-derived carbon was better than leaf and other litter carbon for macroaggregate incorporation (Sánchez-de León et al.,

2014). Recent evidence has shown that earthworms consume significant amounts of root-derived carbon (i.e., root exudates or other labile carbon) (Gilbert et al., 2014) which is immediately incorporated into the soil macroaggregate fractions of rhizosphere soil (Sánchez-de León et al., 2014). Root feeding by earthworms has been rarely reported, and most earthworms seem to avoid feeding on roots, at least in the early stages of root decomposition. However, Decaëns et al. (1999) observed freshly deposited casts on fine roots, thus indicating that roots often utilize the nutrients in casts. Our results clearly showed interactions regarding soil macroaggregate formation given that the soil macroaggregates that were formed in the presence of 2 different engineers (earthworms and roots) were significantly different from the macroaggregates that were formed in the presence of only one organism (roots). Furthermore, the presence of earthworms or the interactions of earthworms with soils had a significant effect on the soil macroaggregates (Table 2). More rhizodeposit carbon and ^{13}C were present in the soil macroaggregates in the treatments with earthworms than in the treatments without earthworms (Fig. 3). This finding seemed to indicate that rhizodeposition was stimulated by earthworm activities and that the earthworms probably fed on root exudates and rhizodeposits (Zangerlé et al., 2011). Another explanation could be that the soil macroaggregates formed by the earthworms were further colonized by roots, and the roots added their rhizodeposits to the soil in specific organic compounds that were then mixed by the earthworms. These results indicate that roots and earthworms do not produce soil macroaggregates in isolation; instead, their additive effects impact the mixed source structure. Our findings also indicated that there was an important interaction between the earthworms and roots in soils under different tillage regimes, that not only helped improve the stability of aggregates, but also helped stabilize the organic residues in the soil aggregates.

5 Conclusions

The presence of earthworms stimulated the immobilization of rhizodeposit carbon, and the earthworms in the NT soils promoted more rhizodeposit carbon retention in soil macroaggregates than those in the CT soils. Our results showed that earthworms have a significant effect on soil aggregation that increased the abundance of soil macroaggregates and negatively impacted the formation of soil microaggregates. The interaction between rhizodeposits and earthworms could have a major effect on soil function regulation during plant growth, such as soil aggregation, soil organic matter priming, and different functional groups of earthworms. Furthermore, earthworms could affect rhizosphere soil microbes, leading to an increase in the retention of rhizodeposit carbon in the soil macroaggregates, which is more obvious in the long-term no-till soil. These interactions require further research.

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