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Application of earthworm cast improves soil aggregation and aggregate-associated carbon stability in typical soils from Loess Plateau

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ABSTRACT

Earthworm casts exhibit remarkable fertility and have been widely used as an organic fertilizer. This study focused on the effects of earthworm cast application on soil aggregates and aggregate-associated carbon in typical soils from the Loess Plateau (China). Soil column experiments were conducted in the laboratory using cultivated loessial soil (CS), dark loessial soil (DS), and aeolian soil (AS). Application of earthworm casts significantly reduced the content of aggregates sized <0.5 mm but increased the content of water-stable aggregates. Compared to without-cast treatment, earthworm cast application increased the organic carbon content by 13.4–58.3%, 14.4–51.1%, 17.9–45.3%, 16.7–62.4%, 18.4–43.3%, and 19.8–62.9% in soil aggregate fractions of sizes <0.25, 0.25–0.5, 0.5–1, 1–2, 2–5, and >5 mm, respectively. The application of earthworm casts significantly increased heavy fraction organic carbon (HFOC), $CaCO_3$, and exchangeable Ca contents in soil by 14.5–69.4%, 12.8–51.9%, and 33.3–63.2%, respectively. Compared with macroaggregates, microaggregates had higher $CaCO_3$ contents but smaller light-fraction organic carbon (LFOC) to HFOC ratios, indicating that earthworm cast application improved the organic carbon stability more in microaggregates than macroaggregates. Comparison analysis of the three soils showed AS performed better in aggregation and aggregate-associated carbon stability than CS and DS after applying earthworm casts. The findings improve our understanding of the effects of earthworm cast application on soil aggregate distribution and aggregate-associated carbon stability, which will help improve the application efficiency of earthworm casts as an organic fertilizer in the Loess Plateau area.

1. Introduction

Understanding the quantitative and stability characteristics of soil aggregates is crucial for soil structure and property improvements. Soil organic carbon (SOC) plays a predominant role in aggregate formation and stabilization. The SOC modifies the cohesiveness of soil particles and then influences soil structural stability. On the one hand, clay minerals in soil have a strong adsorption capacity due to their large specific surface area and cation exchange capacity; thus, they readily absorb hydrophobic organic carbon and protect it from degradation (Krull et al., 2003). On the other hand, some fine amorphous minerals and organic carbon can be replaced by ligands or form ionic bonds to form stable organic-inorganic complexes (Merino et al., 2017). Changes in SOC content alter the size distribution of soil aggregates (Jat et al., 2019). Moreover, studies have shown that exogenous organic materials can significantly promote the macroaggregate formation (Long et al., 2015; Dai et al., 2019). Furthermore, soil organisms can accelerate the turnover and utilization of organic matter and accumulate refractory organic carbon in soil aggregates by improving their activities (Eckschmitt et al., 2005). Studies have demonstrated that soil agglomeration surrounds unprotected granular organic carbon dispersed outside soil microaggregates with additional soil aggregates, resulting in a more intensive combination of organic carbon and mineral particles (Six et al., 2000). Therefore, the organic carbon in large macroaggregates exhibits a lower decomposition rate and becomes more stable (Oades and Waters, 1991; Six et al., 2002). The stability of aggregate-associated carbon

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varies with aggregate size due to the different cementation processes of different-sized aggregates (Puget et al., 2000).

Furthermore, the soil carbon stability also depends on the composition of different fractions of soil organic carbon (SOC) and their environmental interactions (Six et al., 2002; Leite et al., 2004). Soil heavy fraction organic carbon (HFOC) is organic matter combined with minerals that mainly exists in organic-inorganic complexes and has a low mineralization rate (Grandy and Robertson, 2007). Soil light fraction organic carbon (LFOC) is unstable SOC that is sensitive to changes in soil management (Wu et al., 2003). Content changes in these two organic carbon fractions affect the stability of aggregate-associated carbon. Many studies have shown that application of organic fertilizer can significantly increase the content of soil organic matter, affect the organic carbon fractions of soil, improve the content of soil water-stable aggregates, and promote the formation of macroaggregates with a size >0.25 mm (Yu et al., 2012; Dai et al., 2019).

As a pollution-free, sustainable, and efficient organic fertilizer, earthworm cast has been widely used in agricultural production (Park et al., 2008; Zhou et al., 2019). Studies have shown that earthworm casts contain abundant humus, conducive to the formation of soil aggregates (Tejada and Gonzalez, 2009; Molina et al., 2013). Moreover, earthworm casts have higher water stability than the general soil (Lipiec et al., 2015), and the amount and stability of soil aggregates increase with the application of earthworm casts (Scullion and Malik, 2000). Through a one-year culture experiment, Martin (1991) demonstrated that earthworm cast applications could effectively reduce the mineralization rate of SOC.

The low fertility of soils in the Loess Plateau (China) limits agricultural production in this area; hence, appropriate amendment or fertilizer is essential for sustainable development. Based on the beneficial nature of earthworm casts, we aimed to investigate the impact of applying earthworm cast on: (1) the distribution and stability of soil aggregates; (2) the variations in aggregate-associated carbon content; and (3) the stability of aggregate-associated carbon. The study provides a scientific basis for using earthworm casts to improve the soil structure and quality of typical soils from the Loess Plateau.

2. Materials and methods

2.1. Soil and earthworm cast preparation

Three typical soils from the farmlands of Ansai (109°19′E, 36°51′N), Changwu (107°78′E, 35°21′N), and Shenmu (110°52′E, 38°83′N) in the Loess Plateau were used, namely, cultivated loessial soil (CS), dark loessial soil (DS), and aeolian soil (AS). The CS, DS, and AS samples were collected from the 0–20 cm depth soil layer of wheat fields. Undisturbed soil samples were taken with 100-cm^2 cutting rings for determination of bulk density and field capacity. Basic physicochemical properties were measured in uniformly mixed soil samples from each location. The remaining soil samples were then air-dried and passed through a 2-mm sieve prior to the microcosm experiment.

The earthworm cast used in this study was obtained by incubating a native epigeic species in the laboratory and handpicking regularly. *Eisenia fetida* (Oligochaeta, Lumbricidae), one of the dominant earthworm species in the middle part of the Loess Plateau, were collected from farmland using an ultrasonic trap. Grated chinar leaves (3 kg), apple pomace (1 kg), and corn stalk (15 kg) were mixed and used to fill up a cylindrical plastic container (35 cm in diameter × 30 cm in height). The feed was then fermented for 20 days at room temperature until it turned black. Forty adult earthworms were inoculated, and the container was maintained at a temperature of 20 °C and 60%–70% humidity. Earthworm casts were collected from the substrate surface every 7 days for a total of 3 months, and all earthworm casts were mixed after incubation. Earthworm-cast samples were also analyzed for physical and chemical properties. The measured typical property indicators of soil and earthworm casts are presented in Tables 1 and 2, respectively.

2.2. Experimental design

The microcosm experiment was conducted in a laboratory at the Institute of Soil and Water Conservation, Chinese Academy of Sciences, in Yangling, China. The experiment used a completely randomized design involving two factors: two earthworm cast treatments – with cast and without cast (CK); and three soils – CS, DS, and AS. Each treatment was repeated in triplicate. The microcosm was constructed from cylindrical plexiglass vessels (20 cm in diameter × 25 cm in height, Fig. 1) packed with sieved soil to a height of 20 cm based on pre-measured bulk density in the field; thus, the weight of soil used in each vessel was 27,516.5 g, 25,977.1 g, and 32,904.3 g for CS, DS, and AS, respectively. Based on the pre-measured nutrient content of earthworm cast and local traditional organic fertilizer application amount, 5% of earthworm cast on a weight/soil weight (w/w) basis was mixed with sieved soil before packing for the treatments with earthworm cast. Afterward, the water content of each treatment was adjusted to field capacity; 17.3%, 23.6%, and 11.5% for CS, DS, and AS, respectively. All microcosms were stored in the laboratory for 4 months at a relative humidity of 35 ± 3% and a temperature of 22 ± 3 °C. Water loss was determined by weighing the microcosms every 3 days, and water was added to the surface to maintain soil moisture to field capacity.

2.3. Data collection and analysis

After incubation for 4 months, soil samples were collected from each microcosm. Soil cores were sampled every 5 cm in the vertical direction using a 100 cm^3 cutting ring, then oven-dried and averaged to determine bulk density. After this, the vessels were carefully split, and all soil samples were successively sieved through 0.25, 0.5, 1, 2, and 5 mm to determine dry aggregate size distribution (Cambardella and Elliott, 1993) and obtain aggregates samples of all sizes. The aggregate samples were then used to determine LFOC and HFOC, $CaCO_3$, exchangeable Ca content, and CO_2 emission amounts. Soil aggregates were quantitatively characterized by determining dry aggregate size distribution and the content of water-stable aggregate. Aggregates with sizes >0.25 mm were defined as macroaggregates, and those <0.25 mm were considered microaggregates (Tisdall and Oades, 1982). The CO_2 emission was determined by incubating aggregates with different sizes in a small jar with an alkali trap (0.5 mol L^{-1} NaOH) to absorb the released CO_2 for a month (Anderson, 1982). At 10, 20 and 30 days of the incubation, the NaOH solution were titrated with 0.2 mol L^{-1} HCl to determine the amount of CO_2 emissions. The cumulative CO_2 emissions were the sum of the three measured results. The content of water-stable aggregates of different grain sizes was determined with Yoder-type wet sieving

Table 1
Basic physicochemical characteristics of soils used in this study.

Soils	Soil type[a]	Soil texture	Particle size distribution (%)			Organic matter (g kg^{-1})	Bulk density (g cm^{-3})	pH
			≥2 mm	0.002–0.02 mm	<0.002 mm			
Cultivated loessial soil	Anthrosols	Loam clay	23.57 ± 0.72c	59.89 ± 1.07a	16.54 ± 0.35b	4.96 ± 0.03b	1.43 ± 0.02b	7.13 ± 0.02b
Dark loessial soil	Anthrosols	Loam clay	42.22 ± 1.13b	37.42 ± 1,22b	20.36 ± 0.27a	6.27 ± 0.06a	1.35 ± 0.03c	7.21 ± 0.02b
Aeolian soil	Cambisols	Sandy loam	67.22 ± 1.21a	30.74 ± 1.18c	2.04 ± 0.33c	1.14 ± 0.02c	1.71 ± 0.02a	7.73 ± 0.01a

[a] Soil type was identified according to WRB (2014).

Table 2
Basic physicochemical characteristics of earthworm casts used in this study.

Particle size distribution (%)			Organic matter (g kg^{-1})	Bulk density (g cm^{-3})	pH	Total nitrogen (g kg^{-1})	Total phosphorus (g kg^{-1})
≥2 mm	0.002–0.02 mm	<0.002 mm					
19.78 ± 0.23	59.69 ± 1.13	20.53 ± 0.67	19.82 ± 0.27	0.62 ± 0.01	7.32 ± 0.02	12.75 ± 0.13	11.16 ± 0.09

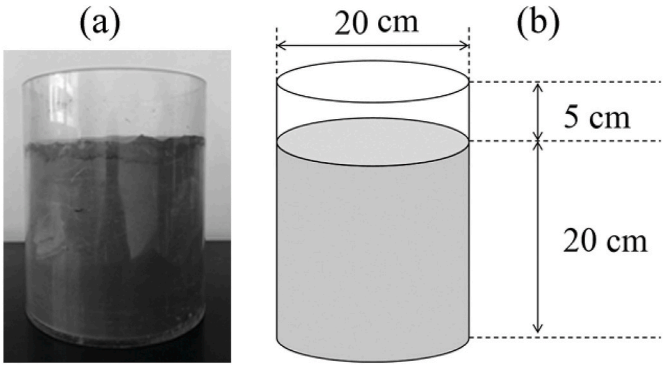

Fig. 1. The schematic diagram of the experimental microcosm.

apparatus (Nimmo and Perkins, 2002). The stability of soil aggregates was described by mean weight diameter (MWD), which was calculated using the following equation (Mazurak, 1950):

$$MWD = \sum_{i=1}^{n} (\overline{x_i} w_i) \tag{1}$$

where: $\overline{x_i}$ is the mean diameter of each sized fraction, w_i is the proportion of the total sample weight.

Soil organic carbon content was determined by the external heating potassium dichromate method. The LFOC and HFOC contents in soil aggregates were measured by the method reported by Freixo et al. (2002). The $CaCO_3$ content was determined using acid-base titration, and the exchangeable Ca content was determined by extracting the soil with 1 mol L^{-1} ammonium chloride solution at pH 7.0 followed by analysis using an ICP-OES Optima 7300 DV spectrophotometer (PerkinElmer, Inc., Waltham, MA, USA). The contribution of organic carbon in each aggregate fraction to soil total organic carbon can be used to explore the distribution of SOC in aggregates and soil-carbon sequestration capacity, thus revealing the protection mechanism of SOC, which was estimated by the following equation (Sun et al., 2005):

$$\begin{aligned}&\text{The contribution of aggregate}\\&-\text{associated organic carbon to SOC} = a_i b_i / c \times 100\%\end{aligned} \tag{2}$$

where: a_i is the organic carbon content of each size fraction, b_i is the content of soil aggregates of different particle fractions, and c is the SOC content.

2.4. Statistical analysis

Data were subjected to one-way analysis of variance followed by Fisher's least significant difference (LSD) test ($p < 0.05$) in order to determine the effects of earthworm cast application on soil aggregation, and the quantity and stability of aggregate-associated carbon. All statistical analyses were performed using IBM SPSS Statistics v20 software (IBM Corp., Armonk, NY, USA). GraphPad Prism version 8 for Windows (GraphPad Software, La Jolla, CA, USA) was used to create the figures.

3. Results

3.1. Soil aggregate properties under earthworm cast application

The dry aggregate size distributions of the three soil types with earthworm cast treatment are presented in Fig. 2. The largest aggregate content was observed in the <0.25-mm size in CK for all three soil types. Applying earthworm casts increased the content of aggregates sized 0.5–1, 1–2, 2–5, and >5 mm by 14.6–25.2%, 20.3–56.3%, 8.7–58.2%, and 17.9–50.1%, compared to CK. For aggregate fractions sized <0.25 and 0.25–0.5 mm, however, the dry aggregate content decreased by 14.5–35.8% and 23.7–38.3%, respectively. Of the three soil types, the smallest change in aggregate-size distribution with earthworm-cast application was in the DS group.

For all soil types, significant MWD differences existed between earthworm cast treatment and CK (Table 3), indicating earthworm cast application had significant effects on soil aggregate stability. The MWD values of soil aggregates were 22.6%, 20.8%, and 56.6% higher with cast treatment than without cast treatment for CS, DS, and AS, respectively. The MWD values with earthworm cast application were in the order: DS > CS > AS. The application of earthworm casts significantly increased the soil content of water-stable aggregates, and these significantly increased for each size fraction of all three soil types (Fig. 3, $p < 0.05$), indicating that earthworm cast application led to increased water stability of soil aggregates. Compared with CK, application of earthworm casts increased the soil content of water-stable aggregates by 9.7%–27.2% in CS, 10.8%–22.1% in DS, and 16.9%–35.7% in AS. The minimum increase in water-stable aggregates occurred in the 2–5 mm aggregate fraction with the application of earthworm casts. Meanwhile, the maximum increase in water-stable aggregates occurred in the 0.25–0.5 mm aggregate fraction among the CS and DS groups with earthworm cast application, and in the <0.25 mm aggregate fraction in the AS group.

3.2. Effects of earthworm cast on aggregate-associated carbon

The SOC of soil with earthworm cast treatment was significantly higher than CK for all soil types ($p < 0.05$, Table 3). The order of SOC content in the treatments was as follows: DS > CS > AS. Application of earthworm casts increased SOC content by 29.5%, 32.7%, and 66.9% in CS, DS, and AS, respectively.

Aggregate-associated carbon concentrations of each aggregate fraction for the three soil types are presented in Table 4. The application of earthworm casts significantly improved soil aggregate-associated organic carbon for all soil types, which was significantly increased for each aggregate fraction ($p < 0.05$). Earthworm cast application increased the organic carbon content by 13.4–58.3%, 14.4–51.1%, 17.9–45.3%, 16.7–62.4%, 18.4–43.3%, and 19.8–62.9% in aggregate fractions with sizes <0.25, 0.25–0.5, 0.5–1, 1–2, 2–5, and >5 mm, respectively. Larger-sized aggregates generally had higher associated organic carbon content than smaller-sized aggregates. For CS, the greatest increase in aggregate-associated carbon was in the 0.25–1 mm aggregate fraction, and the application of earthworm casts led to an increase of 41.9% compared to CK. For DS, the aggregate-associated carbon content increased by 11.23%–16.68%, and the maximum and minimum increases occurred in aggregates sized >5 mm and <0.25 mm, respectively. However, in AS, the highest organic carbon content was obtained in the 0.5–1 mm aggregate fraction with an improvement of

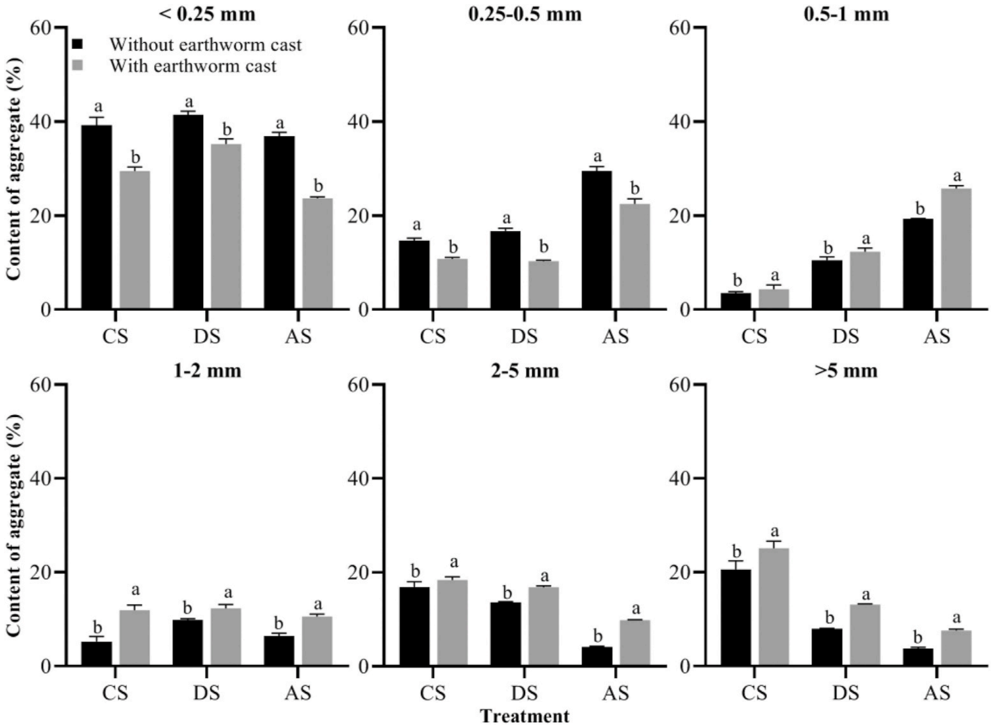

Fig. 2. Effects of earthworm cast application on dry aggregate size distribution. The letters show differences at $p < 0.05$ between earthworm casts application and control in the same soil.

Table 3

MWD and soil organic carbon (SOC) of earthworm casts application treatment and control. The data represent the arithmetic mean of three replicates, and the letters in columns show differences at $p < 0.05$ between earthworm casts application and control in the same soil.

Soil	Treatment	MWD (mm)	SOC (g kg^{-1})
CS	without cast	0.47 ± 0.03b	4.72 ± 0.23b
	with cast	0.61 ± 0.03a	7.44 ± 0.41a
DS	without cast	0.57 ± 0.03b	5.88 ± 0.65b
	with cast	0.72 ± 0.03a	9.52 ± 0.54a
AS	without cast	0.13 ± 0.01b	1.24 ± 0.02b
	with cast	0.32 ± 0.01a	2.75 ± 0.05a

62.2%, while the lowest organic carbon content was obtained in the 2–5 mm aggregate fraction. The largest increase in aggregate-associated carbon content was in the AS group among the three soil types.

A significant difference in the contribution of organic carbon in each aggregate fraction between earthworm-cast treatment and CK was observed (Fig. 4, $p < 0.05$). Aggregate-associated carbon content was mainly distributed in aggregate fractions sized >5 mm, 2–5 mm, and 0.25–0.5 mm in CS, DS, and AS, respectively. Compared with CK, earthworm cast application significantly improved the contribution of organic carbon in aggregate fractions 2–5 mm and >5 mm in CS with variabilities of 3.34% and 4.85%. The variability of the contribution of aggregate-associated organic carbon for fractions sized 1–2 mm and 2–5 mm was 3.4% and 3.15% in DS, respectively, and 2.83% and 2.09% for aggregate fractions sized <0.25 mm and 0.25–0.5 mm in AS, respectively.

3.3. CO_2 emission, LFOC, HFOC, $CaCO_3$ and exchangeable Ca within aggregates

The cumulative CO_2 emission amounts in treatments with earthworm cast were significantly higher than treatments without casts for all aggregate fractions (Fig. 5, $p < 0.05$), which increased by 4.6–5.1%, 4.0–6.3%, 3.7–8.7%, 4.9%–10.1%, 4.0–9.3%, and 5.0–12.8% for aggregate fractions sized 0.25–0.5, 0.5–1, 1–2, 2–5, and >5 mm for treatments with earthworm cast, respectively, compared with CK. Application of earthworm casts significantly increased the content of HFOC and LFOC in soil aggregates (Fig. 6, $p < 0.05$), with a greater effect on HFOC than on LFOC. In comparison with CK, the maximum and minimum increases of HFOC content were obtained from aggregate fractions sized 0.5–1 mm (28.6%) and <0.25 mm (17.2%) in CS with earthworm-cast treatment. In DS, aggregates sized 2–5 mm retained maximum (22.9%) increases of HFOC content. Aggregates sized 2–5 mm and >5 mm had maximum (69.4%) and minimum (44.3%) increases for HFOC content in AS, which were highest of the three soil types. The maximum (19.8–62.9%) increase of LFOC content was obtained in the aggregate fraction sized >5 mm, compared to CK. The LFOC content in the aggregate fraction sized <0.25 mm significantly increased by 13.4–55.4%, while 14.4–51.1%, 17.9–45.3%, 16.7–62.4%, and 18.4–43.3% increases were observed in aggregate fractions sized 0.25–0.5 mm, 0.5–1 mm, 1–2 mm, and 2–5 mm, respectively.

For all soil types, the application of earthworm casts significantly increased the $CaCO_3$ content in soil aggregates, which increased with increasing aggregate size ($p < 0.05$, Fig. 7). Compared with CK, the $CaCO_3$ content of soil aggregates subjected to earthworm cast treatment increased by 13.7–30.8%, 14.3–32.4%, 14.9–34.3%, 12.8–43.8%, 13.3–43.8%, and 12.3–51.9% for aggregate fractions sized <0.25, 0.25–0.5, 0.5–1, 1–2, 2–5, and >5 mm, respectively. The exchangeable Ca content in soil aggregates increased significantly after applying earthworm casts, and the maximum exchangeable Ca content was obtained from the aggregate fraction sized >5 mm. The exchangeable Ca content among earthworm cast treatments increased by 34.3–63.2%, 34.6–61.8%, 37.5–61.3%, 38.1%–61.9%, 33.3–57.6%, and 35.2–51.8% for aggregate fractions sized 0.25–0.5, 0.5–1, 1–2, 2–5, and >5 mm, respectively. Of the three soil types, the increases in $CaCO_3$ and exchangeable Ca content were highest in the AS group after treatment with earthworm casts.

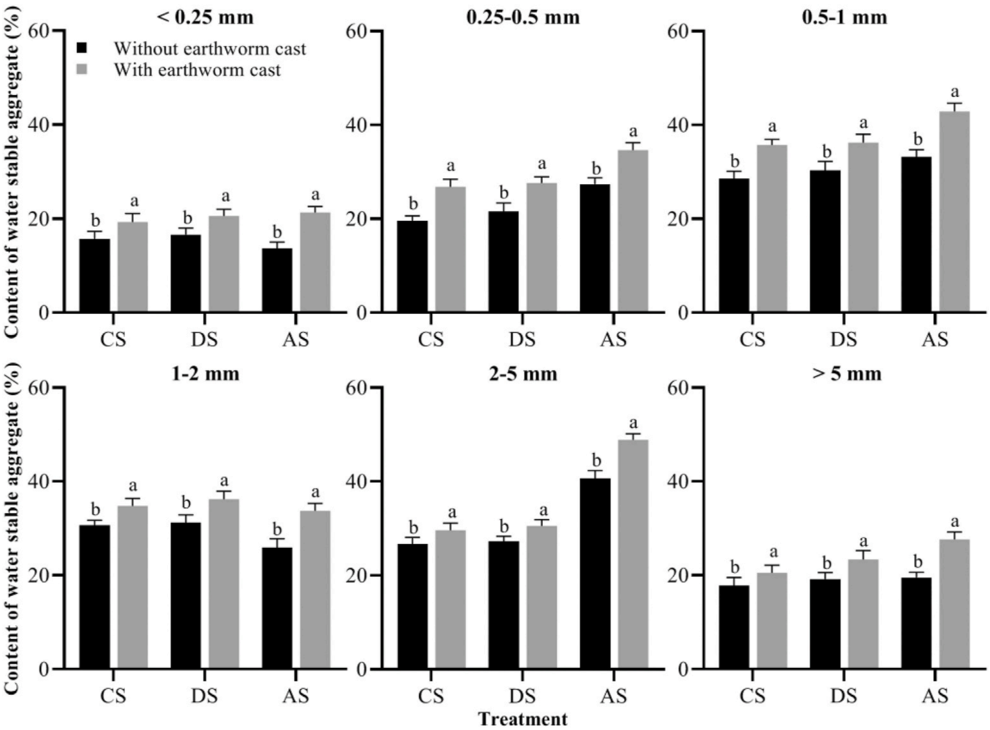

Fig. 3. Effects of earthworm cast application on wet aggregate stability of soils. The letters show differences at $p < 0.05$ between earthworm casts application and control in the same soil.

Table 4

Organic carbon concentration (C_{con}, g kg^{-1}) and rates of increase (R_{inc}, %) for aggregate sizes by earthworm cast and controls. The data represent the arithmetic mean of three replicates, and the letters in columns show differences at $p < 0.05$ between earthworm casts application and control in the same soil.

Treatment		<0.25 mm		0.25–0.5 mm		0.5–1 mm		1–2 mm		2–5 mm		>5 mm	
		C_{con}	R_{inc}	C_{con}	R_{inc}	C_{con}	R_{inc}	C_{con}	R_{inc}	C_{con}	R_{inc}	C_{con}	R_{inc}
CS	without cast	3.84 ± 0.41b	–	3.93 ± 0.34b	–	4.17 ± 0.19b	–	4.85 ± 0.43b	–	5.03 ± 0.36b	–	5.17 ± 0.22b	–
	with cast	4.58 ± 0.32a	16.2	4.77 ± 0.29a	17.6	5.62 ± 0.11a	25.8	6.02 ± 0.23a	19.4	6.36 ± 0.41a	20.9	6.63 ± 0.15a	22.1
DS	without cast	5.43 ± 0.25b	–	5.55 ± 0.18b	–	5.81 ± 0.78b	–	6.13 ± 0.18b	–	6.16 ± 0.31b	–	6.34 ± 0.57b	–
	with cast	6.27 ± 0.72a	13.4	6.48 ± 0.41a	14.4	7.08 ± 0.25a	17.9	7.36 ± 0.37a	16.7	7.55 ± 0.63a	18.4	7.91 ± 0.33a	19.8
AS	without cast	0.78 ± 0.02b	–	0.94 ± 0.04b	–	1.22 ± 0.05b	–	0.53 ± 0.01b	–	0.59 ± 0.04b	–	0.62 ± 0.03b	–
	with cast	1.87 ± 0.09a	58.3	1.92 ± 0.03a	51.1	2.23 ± 0.07a	45.3	1.41 ± 0.17a	62.4	1.04 ± 0.13a	43.3	1.67 ± 0.15a	62.9

4. Discussion

The addition of organic matter to soil affects soil aggregates and their stability, and the degree of aggregation and aggregate stability increase with increasing macroaggregate content in the soil (Nimmo and Perkins, 2002; Abiven et al., 2009). Moreover, organo-mineral associations in soil, as nanoscale components of microaggregates, are also affected by the addition of organic materials (Totsche et al., 2018). In the present study, the application of earthworm casts significantly increased the content of soil aggregates sized >0.25 mm, indicating a positive effect on soil aggregate stability. The decreased content of aggregates sized <0.25 mm may be attributed to the high Ca content of the soil with earthworm cast application, which promoted the cementation of soil aggregates with soil particle sizes >0.5 mm (Chai et al., 2019). In addition, Lim et al. (2014) noted that polysaccharides contained in earthworm casts positively affected the cementation of aggregates in soil with particle sizes <0.25 mm. Manivannan et al. (2009) also reported that the addition of earthworm casts could increase the quantity and activity of microorganisms in the soil, thereby leading to the formation of soil macroaggregates. The MWD evaluates the condition of aggregates based on diameter, which could better reflect the content change of large aggregates (van Bavel, 1950). In our study, the value of MWD increased with earthworm cast application. This result is consistent with that of Yilmaz and Snmez (2017), who determined that organic fertilizer application significantly increased soil macroaggregate content and increased the MWD value. However, Aksakal et al. (2016) suggested that application of earthworm casts could also reduce the value of MWD, which could be attributed to the fact that small aggregates are generally more stable than large aggregates due to their larger internal pores and higher packing density of mineral particles (Horn and Dexter, 1989).

The SOC content is very important in the formation and stability of soil aggregates (Six et al., 2002). Studies have reported that enhanced SOC benefits the formation of macroaggregates due to the accretion of SOC linked to minerals and a steady supply of freshly decomposed C-containing materials (He et al., 2018; Adnan et al., 2020). Therefore, the increase in SOC after applying earthworm casts may be one of the main reasons for the increase in macroaggregates. The stability of soil aggregates is reportedly strongly related to organic matter content, and increased SOC improves the stability of soil aggregates (Alagöz and Yilmaz, 2009). Tisdall and Oades (1982) also stated that all types of organic matter are important cementation materials and favor the formation of macroaggregates. Earthworm casts have an excellent aggregate structure, which can effectively protect organic carbon, improve the long-term stability of carbon in the soil, and increase soil carbon storage (Bossuyt et al., 2005). The organic carbon content of earthworm casts in the present study was as high as 19.82 g kg^{-1}, which was

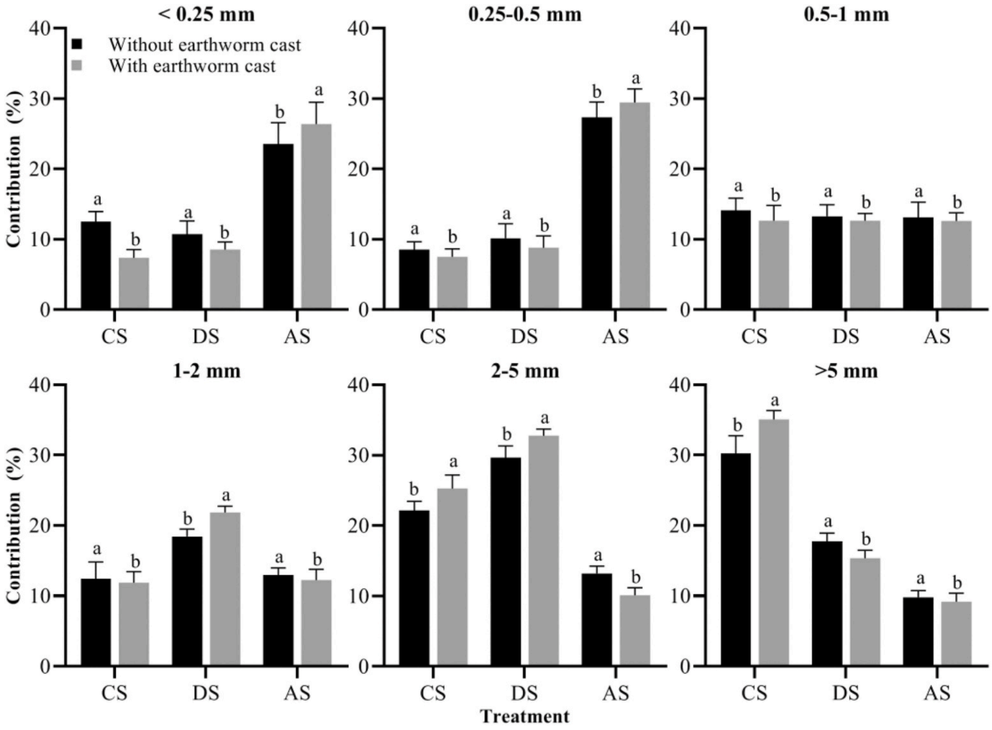

Fig. 4. The contribution of aggregate-associated organic carbon to soil organic carbon. The letters show differences at $p < 0.05$ between earthworm casts application and control in the same soil.

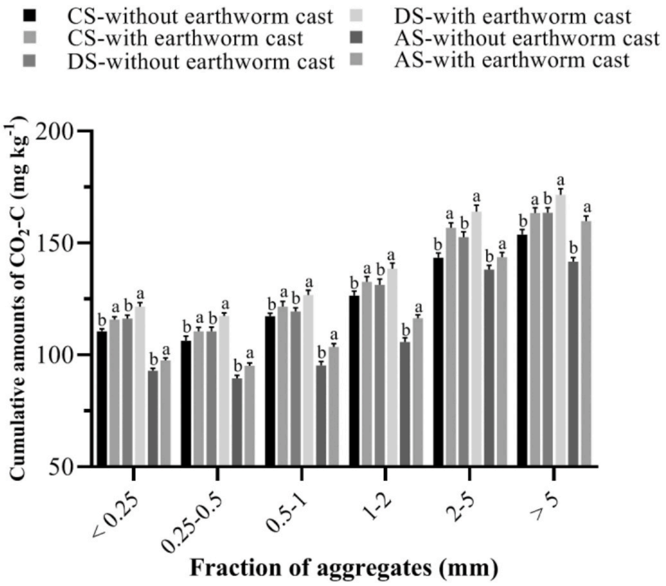

Fig. 5. Effects of earthworm cast application on the decomposition rate of soil organic carbon of soil aggregates. The letters show differences at $p < 0.05$ between earthworm casts application and control in the same soil.

68.4–94.2% higher than that of bulk soil for the three soils. Similar results were found by Lim et al. (2014), who reported that the organic matter content in soil with earthworm casts was significantly higher than in general soil. Compared to CK, higher soil content of water-stable aggregates was observed with earthworm cast treatment for different sized aggregates of the three soil types. Sui et al. (2012) found that the application of organic fertilizer in eroded black soil from northeast China significantly increased the soil content of water-stable aggregates. Additionally, the responses of aggregate stability to earthworm cast application also varied between soil types. More stable aggregates were noted in AS than in DS and CS. Differences between soils are related to soil texture, and the soil parent material will affect the content and stability of soil aggregates and SOC content (Gryze et al., 2006; Mamedov et al., 2017). Šimanský et al. (2019) also reported that a higher sand content resulted in lower aggregate stability.

The distribution of aggregate-associated carbon helps reveal the sequestration mechanism of organic carbon in soil aggregates (Six et al., 2000). In our study, the application of earthworm casts increased the aggregate-associated carbon content, and the positive effect increased with aggregate size. Yu et al. (2012) and Guan et al. (2019) reported that aggregate-associated carbon content was positively correlated with soil aggregate particle size under the application of exogenous carbon. The contribution of soil aggregate organic carbon to SOC was mainly in macroaggregates for DS and CS. Macroaggregates are proven to have more SOC content due to stronger physicochemical and biological protection than microaggregates (Post and Kwon, 2000; Ghosh et al., 2018). Kong et al. (2005) also observed that carbon input was first reflected in large soil aggregates while microaggregates remained at a relatively stable level. The greatest contribution of organic carbon in aggregates for AS, however, was in microaggregates. The result could be relevant to the lower organic content of macroaggregates formed by sand. Sand in the soil has a smaller specific surface area than clay and fewer reactive sites where SOC can be stabilized (Ding et al., 2014).

Aggregate-associated carbon increased 13.40–62.90% after applying earthworm cast; the cumulative CO_2 emission from aggregates, however, increased only 3.67–12.77%. Also the CO_2 emissions in macroaggregates were higher than in microaggregates. Positive correlations between cumulative CO_2 emission and soil organic C was also found by Ding et al. (2007), who reported the addition of organic manure contributed to the increase in soil CO_2 emission. The relatively lower increase in CO_2 emissions suggested an enhancing stability of aggregate-associated carbon. Huang et al. (2019) studied the effects of organic fertilization on carbon sequestration and aggregate-associated organic carbon stability and found CO_2 emission is positively correlated with the stability of SOC. Tan et al. (2007) reported that LFOC and HFOC play important roles in SOC stability. LFOC is an important

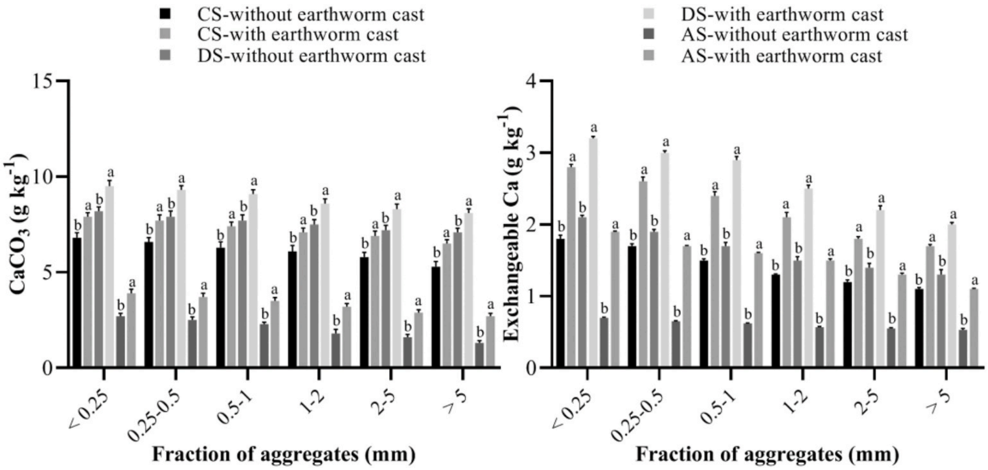

Fig. 6. Effects of earthworm cast application on light (LFOC) and heavy fractions organic carbon (HFOC) of soil aggregates. The letters show differences at $p < 0.05$ between earthworm casts application and control in the same soil.

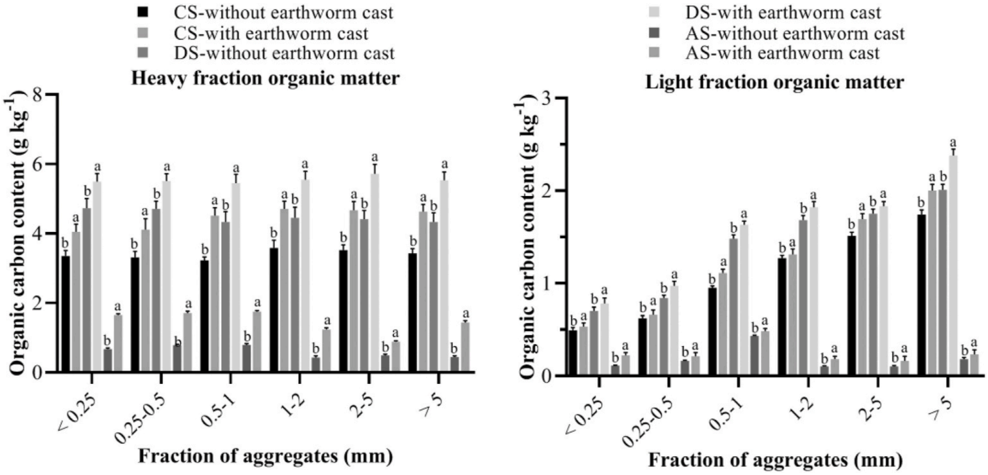

Fig. 7. The $CaCO_3$ and exchangeable Ca content to each aggregate fraction. The letters show differences at $p < 0.05$ between earthworm casts application and control in the same soil.

component of the soil's unstable organic carbon pool; and HFOC represents the comparatively decomposed fraction, which consists of organo-mineral complexes and reflects the ability of soil to maintain organic carbon under different degrees of physical and chemical protection by soil minerals (Barrios et al., 1997). Our study revealed that both LFOC and HFOC contents increased with aggregate-associated carbon content, and LFOC content in aggregates increased with increasing aggregate size. The LFOC is a main component of SOC. It has a rapid turnover rate and can be an important and rapid source of plant nutrients (Wander and Traina, 1996; Li et al., 2012). In contrast, HFOC is a higher density organic carbon with a lower mineralization rate and stronger stability (Gong et al., 2009). According to Fig. 3, the LFOC to HFOC ratio decreased with earthworm cast treatment and increased with increasing aggregate size. The minimum LFOC/HFOC ratio (0.131–0.142) obtained in the <0.25 mm aggregate fraction for all soil types indicated higher stability of organic carbon in microaggregates compared with macroaggregates. Similarly, Yu et al. (2012) suggested that the microenvironment associated with microaggregates could effectively protect organic carbon from microbial and enzyme attack. Huang et al. (2019) also discovered that the specific carbon mineralization rate (carbon mineralization rate per unit SOC) in microaggregates was lower than in macroaggregates with organic fertilizer applications. Overall, the application of earthworm casts significantly improved soil aggregate-associated carbon stability, which was conducive to soil-carbon sequestration. The largest LFOC/HFOC among the three soils was found in AS, which further proved the lower stability of aggregates in this soil type.

The $CaCO_3$ content in soil aggregates is a critical index for evaluating aggregate stability. The reason is that $CaCO_3$ is an important binding agent in soil aggregation and helps form concretions or coatings on the surface of aggregates, effectively reducing the decomposition of organic carbon (Bronick and Lal. 2005; Xie et al., 2015). Our results showed that the application of earthworm casts significantly increased the content of $CaCO_3$ in soil. One possible reason is that the synthesis of calcite granules by earthworm activities leads to higher total calcium carbonate content in casts, resulting in higher Ca and total carbonate levels in earthworm cast than soils (Henrot and Brussaard, 1997; García-Montero et al., 2013). The present data further revealed that the $CaCO_3$ content decreased with increasing aggregate size, while the SOC content increased with increasing aggregate size. This finding suggests that the protection of organic carbon by $CaCO_3$ in macroaggregates is lower than in microaggregates. Six et al. (2000) also suggested that the turnover rate of macroaggregates was faster than that of microaggregates. Therefore microaggregates offer greater physical protection to organic carbon than macroaggregates, and this is further proven by the increased stability of organic carbon in microaggregates compared with macroaggregates. Furthermore, the application of earthworm casts significantly increased the exchangeable Ca content in soil (Fig. 5).

These findings are consistent with those of Alekseeva et al. (2010), who indicated that digestion by earthworms could change soil properties and that casts were characterized by larger exchangeable Ca contents. Exchangeable Ca in soil and SOC form an organic calcium complex that increases SOC sequestration and improves organic carbon stability (Huang et al., 2019). The exchangeable Ca content in microaggregates was higher than in macroaggregates, indicating that the protective effect on organic carbon in microaggregates was stronger than in macroaggregates.

5. Conclusions

Earthworm cast treatment significantly influenced aggregate distribution by promoting macroaggregate formation and increased the content of water-stable aggregates in soil. The content of water-stable macroaggregates was higher than that of water-stable microaggregates with earthworm cast application. At the same time, the application of earthworm casts significantly increased aggregate-associated carbon content of different-sized aggregates in soil, especially for macroaggregates. The positive effects of earthworm cast application on soil aggregation were greater in AS than CS and DS. The increased HFOC, $CaCO_3$, and exchangeable Ca contents in soil aggregates with earthworm cast treatment improved the physical protection of aggregate-associated carbon and hence its stability. Meanwhile, the stability of organic carbon in microaggregates was higher than in macroaggregates after applying earthworm casts. The study findings will provide scientific reference for the more effective use of earthworm casts to improve soil fertility for agricultural production in the Loess Plateau.

Credit author statement

Yanpei Li: Investigation, Formal analysis, Performing the experiments, Data curation, Writing - original draft. Jiao Wang: Methodology, Writing - review & editing. Ming'an Shao: Project administration, Supervision.

Declaration of competing interest

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

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References

Abiven, S., Menasseri, S., Chenu, C., 2009. The effects of organic inputs over time on soil aggregate stability - a literature analysis. Soil Biol. Biochem. 41, 1–12. https://doi.org/10.1016/j.soilbio.2008.09.015.

Adnan, M., Xu, M.G., Syed, A.A.S., Muhammad, M.A., Sun, N., Wang, B.R., Cai, Z.J., Qudsia, S., Muhammad, N., Khalid, M., Avelino, N.D., 2020. Soil aggregation and soil aggregate stability regulate organic carbon and nitrogen storage in a red soil of southern China. J. Environ. Manag. 270, 110894. https://doi.org/10.1016/j.jenvman.2020.110894.

Aksakal, E.L., Sari, S., Angin, I., 2016. Effects of vermicompost application on soil aggregation and certain physical properties. Land Degrad. Dev. 27, 983–995. https://doi.org/10.1002/ldr.2350.

Alagöz, Z., Yilmaz, E., 2009. Effects of different sources of organic matter on soil aggregate formation and stability: a laboratory study on a Lithic Rhodoxeralf from Turkey. Soil Tillage Res. 103, 419–424. https://doi.org/10.1016/j.still.2008.12.006.

Alekseeva, T., Besse, P., Binet, Françoise, Delort, A.M., Tixier, C., 2010. Effect of earthworm activity (aporrectodea giardi) on atrazine adsorption and biodegradation. Eur. J. Soil Sci. 57, 295–307. https://doi.org/10.1111/j.1365-2389.2005.00738.x.

Anderson, J.P.E., 1982. Soil respiration. In: Page, A.L. (Ed.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Methods. Amer. Soc. Agron, pp. 837–871.

Barrios, E., Buresh, R.J., Kwesiga, F., Sprent, J.I., 1997. Light fraction soil organic matter and available nitrogen following trees and maize. Soil Sci. Soc. Am. J. 61, 826–831. https://doi.org/10.2136/sssaj1997.03615995006100030016x.

Bossuyt, H., Six, J., Hendrix, P.F., 2005. Protection of soil carbon by microaggregates within earthworm casts. Soil Biol. Biochem. 37, 251–258. https://doi.org/10.1016/j.soilbio.2004.07.035.

Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. Geoderma 124, 3–22. https://doi.org/10.1016/j.geoderma.2004.03.005.

Cambardella, A.C., Elliott, T.E., 1993. Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. Soil Sci. Soc. Am. J. 57, 1071–1076. https://doi.org/10.2136/sssaj1993.03615995005700040032x.

Chai, Y.J., Zeng, X.B., E, S.Z., Che, Z.X., Bai, L.Y., Su, S.M., Wang, Y.N., 2019. The stability mechanism for organic carbon of aggregate fractions in the irrigated desert soil based on the long-term fertilizer experiment of China. Catena 173, 312–320. https://doi.org/10.1016/j.catena.2018.10.026.

Dai, H.C., Chen, Y.Q., Liu, K.C., Li, Z.X., Qian, X., Zang, H.D., Yang, X.L., Zhao, Y.X., Shen, Y.S., Li, Z.J., Sui, P., 2019. Water-stable aggregates and carbon accumulation in barren sandy soil depend on organic amendment method: a three-year field study. J. Clean. Prod. 212, 393–400. https://doi.org/10.1016/j.jclepro.2018.12.013.

Ding, W.X., Meng, L., Yin, Y.F., Cai, Z.C., Zheng, H.Y., 2007. CO2 emission in an intensively cultivated loam as affected by long-term application of organic manure and nitrogen fertilizer. Soil Biol. Biochem. 39, 669–679. https://doi.org/10.1016/j.soilbio.2006.09.024.

Ding, F., Huang, Y., Sun, W., Jiang, G., Chen, Y., 2014. Decomposition of organic carbon in fine soil particles is likely more sensitive to warming than in coarse particles: an incubation study with temperate grassland and forest soils in northern China. PloS One 9, e95348. https://doi.org/10.1371/journal.pone.0095348.

Ekschmitt, K., Liu, M., Vetter, S., Fox, O., Wolters, V., 2005. Strategies used by soil biota to overcome soil organic matter stability - why is dead organic matter left over in the soil? Geoderma 128, 167–176. https://doi.org/10.1016/j.geoderma.2004.12.024.

Freixo, A.A., Machado, P.L.O.D.A., Santos, H.P.D., Silva, C.A., Fadigas, F.D.S., 2002. Soil organic carbon and fractions of a rhodic ferralsol under the influence of tillage and crop rotation systems in southern Brazil. Soil. Till. Res. 64, 221–230. https://doi.org/10.1016/s0167-1987(01)00262-8.

García-Montero, L.G., Valverde-Asenjo, I., Grande-Ortíz, M.A., Menta, C., Hernando, I., 2013. Impact of earthworm casts on soil pH and calcium carbonate in black truffle burns. Agrofor. Syst. 87, 815–826. https://doi.org/10.1007/s10457-013-9598-9.

Ghosh, A., Bhattacharyya, R., Meena, M., Dwivedi, B., Singh, G., Agnihotri, R., Sharma, C., 2018. Long-term fertilization effects on soil organic carbon sequestration in an Inceptisol. Soil. Till. Res. 177, 134–144. https://doi.org/10.1016/j.still.2017.12.006.

Gong, W., Yan, X., Wang, J., Hu, T., Gong, Y., 2009. Long-term manure and fertilizer effects on soil organic matter fractions and microbes under a wheat-maize cropping system in northern China. Geoderma 149, 318–324. https://doi.org/10.1016/j.geoderma.2008.12.010.

Grandy, A.S., Robertson, G.P., 2007. Land-use intensity effects on soil organic carbon accumulation rates and mechanisms. Ecosystems 10, 59–74. https://doi.org/10.1007/s10021-006-9010-y.

Gryze, S.D., Jassogne, L., Bossuyt, H., Merckx, R., 2006. Water repellence and soil aggregate dynamics in a loamy grassland soil as affected by texture. Eur. J. Soil Sci. 57, 235–246. https://doi.org/10.1111/j.1365-2389.2005.00733.x.

Guan, S., Liu, S.J., Liu, R.Y., Zhang, J.J., Ren, J., Cai, H.J., Lin, X.X., 2019. Soil organic carbon associated with aggregate-size and density fractions in a Mollisol amended with charred and uncharred maize straw. J. Integr. Agr. 18, 1496–1507. https://doi.org/10.1016/S2095-3119(19)62643-2.

He, Y., Xu, C., Gu, F., Wang, Y., Chen, J., 2018. Soil aggregate stability improves greatly in response to soil water dynamics under natural rains in long-term organic fertilization. Soil. Till. Res. 184, 281–290. https://doi.org/10.1016/j.still.2018.08.008.

Henrot, J., Brussaard, L., 1997. Abundance casting activity, and cast quality of earthworms in an acid Ultisol under alley-cropping in the humid tropics. Appl. Soil Ecol. 6, 169–179. https://doi.org/10.1016/S0929-1393(97)00007-3.

Horn, R., Dexter, A.R., 1989. Dynamics of soil aggregation in an irrigated desert loess. Soil. Till. Res. 13, 253–266. https://doi.org/10.1016/0167-1987(89)90002-0.

Huang, X.L., Jia, Z.X., Guo, J.J., Li, T.L., Sun, D.S., Meng, H.S., Yu, G.H., He, X.H., Ran, W., Zhang, S.S., Hong, J.P., Shen, Q.R., 2019. Ten-year long-term organic fertilization enhances carbon sequestration and calcium-mediated stabilization of aggregate-associated organic carbon in a reclaimed Cambisol. Geoderma 355, e113880. https://doi.org/10.1016/j.geoderma.2019.113880.

Jat, H.S., Datta, A., Choudhary, M., Yadav, A.K., Choudhary, V., Sharma, P.C., Gathala, M.K., Jat, M.L., McDonald, A., 2019. Effects of tillage, crop establishment and diversification on soil organic carbon, aggregation, aggregate associated carbon and productivity in cereal systems of semi-arid northwest India. Soil. Till. Res. 190, 128–138. https://doi.org/10.1016/j.still.2019.03.005.

Kong, A.Y.Y., Six, J., Bryant, D.C., Denison, R.F., van Kessel, C., 2005. The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. Soil Sci. Soc. Am. J. 69, 1078–1085. https://doi.org/10.2136/sssaj2004.0215.

Krull, E.S., Baldock, J.A., Skjemstad, J.O., 2003. Importance of mechanisms and processes of the stabilisation of soil organic matter for modelling carbon turnover. Funct. Plant Biol. 30, 207–222. https://doi.org/10.1071/fp02085.

Leite, L.F.C., Machado, E.D.S., Machado, P.L.O.D.A., Filho, E.I.F., Neves, J.C.L., 2004. Simulating trends in soil organic carbon of an acrisol under no-tillage and disc-plow systems using the century model. Geoderma 120, 283–295. https://doi.org/10.1016/j.geoderma.2003.09.010.

Li, X.H., Han, X.Z., Li, H.B., Song, C., Yan, J., Liang, Y., 2012. Soil chemical and biological properties affected by 21-year application of composted manure with chemical fertilizers in a Chinese mollisol. Can. J. Soil Sci. 92, 419–428. https://doi.org/10.4141/cjss2010-046.

Lim, S.L., Wu, T.Y., Clarke, C., Lim, P.N., Shak, K.P.Y., 2014. The use of vermicompost in organic farming: overview, effects on soil and economics. J. Sci. Food Agric. 62, 691–698. https://doi.org/10.1002/jsfa.6849.

Lipiec, J., Turski, M., Hajnos, M., Swieboda, R., 2015. Pore structure, stability and water repellency of earthworm casts and natural aggregates in loess soil. Geoderma 243–244, 124–129. https://doi.org/10.1016/j.geoderma.2014.12.026.

Long, P., Sui, P., Gao, W., Wang, B., Huang, J., Yan, P., Zou, J., Yan, L.L., Chen, Y.Q., 2015. Aggregate stability and associated C and N in a silty loam soil as affected by organic material inputs. J. Integr. Agric. 14, 774–787. https://doi.org/10.1016/S2095-3119(14)60796-6.

Mamedov, A.I., Huang, C.H., Aliev, F.A., Levy, G.J., 2017. Aggregate stability and water retention near saturation characteristics as affected by soil texture, aggregate size and polyacrylamide application. Land Degrad. Dev. 28, 543–552. https://doi.org/10.1002/ldr.2509.

Manivannan, S., Balamurugan, M., Parthasarathi, K., Gunasekaran, G., Ranganathan, L. S., 2009. Effect of vermicompost on soil fertility and crop productivity - beans (Phaseolus vulgaris). J. Environ. Biol. 30, 275–281.

Martin, A., 1991. Short and long-term effects of the endogeic earthworm Millsonia anomala (Omodeo) (Megaseoleeldae, Oligochaeta) of tropical savannas, on soil organic matter. Biol. Fertil. Soils 11, 234–238. https://doi.org/10.1007/BF00335774.

Mazurak, A.P., 1950. Effect of gaseous phase on water -stable synthetic aggregates. Soil Sci. 69, 135–148. https://doi.org/10.1097/00010694-195002000-00005.

Merino, C., Fontaine, S., Palma, G., Matus, F., 2017. Effect of aluminium on mineralization of water extractable organic matter and microbial respiration in southern temperate rainforest soils. Eur. J. Soil Biol. 82, 56–65. https://doi.org/10.1016/j.ejsobi.2017.08.003.

Molina, M.J., Soriano, M.D., Ingelmo, F., Llinares, J., 2013. Stabilisation of sewage sludge and vinasse bio-wastes by vermicomposting with rabbit manure using eisenia fetida. Bioresour. Technol. 137, 88–97. https://doi.org/10.1016/j.biortech.2013.03.029.

Nimmo, J.R., Perkins, K.S., 2002. Aggregate stability and size distribution. In: Dane, J.H., Topp, G.C. (Eds.), Methods of Soil Analysis, Part 4, Physical Methods. SSSA Inc., Madison, pp. 317–328.

Oades, J.M., Waters, A.G., 1991. Aggregate hierarchy in soils. Aust. J. Soil Res. 29, 815–828. https://doi.org/10.1071/SR9910815.

Park, S., Lee, I., Cho, C., Sung, K., 2008. Effects of earthworm cast and powdered activated carbon on methane removal capacity of landfill cover soils. Chemosphere 70, 1117–1123. https://doi.org/10.1016/j.chemosphere.2007.07.034.

Post, W.M., Kwon, K.C., 2000. Soil carbon sequestration and land-use change: processes and potential. Global Change Biol. 6, 317–327. https://doi.org/10.1046/j.1365-2486.2000.00308.x.

Puget, P., Chenu, C., Balesdent, J., 2000. Dynamics of soil organic matter associated with particle-size fractions of water-stable aggregates. Eur. J. Soil Sci. 51, 595–605. https://doi.org/10.1111/j.1365-2389.2000.00353.x.

Scullion, J., Malik, A., 2000. Earthworm activity affecting organic matter, aggregation and microbial activity in soils restored after opencast mining for coal. Soil Biol. Biochem. 32, 119–126. https://doi.org/10.1016/s0038-0717(99)00142-x.

Šimanský, V., Juriga, M., Jonczak, J., Uzarowicz, Ł., Stępień, W., 2019. How relationships between soil organic matter parameters and soil structure characteristics are aected by the long-term fertilization of a sandy soil. Geoderma 342, 75–84. https://doi.org/10.1016/j.geoderma.2019.02.020.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C- saturation of soils. Plant Soil 241, 155–176. https://doi.org/10.1023/a:1016125726789.

Six, J., Paustian, K., Elliott, T.T., Combrink, C., 2000. Soil structure and organic matter: i. distribution of aggregate-size classes and aggregate-associated carbon. Soil Sci. Soc. Am. J. 64, 681–689. https://doi.org/10.2136/sssaj2000.642681x.

Sui, Y.Y., Jiao, X.G., Liu, X.B., Zhang, X.Y., Ding, G.W., 2012. Water-stable aggregates and their organic carbon distribution after five years of chemical fertilizer and manure treatments on eroded farmland of Chinese mollisols. Can. J. Soil Sci. 92, 551–557. https://doi.org/10.4141/cjss2010-005.

Sun, T.C., Li, S.Q., Shao, M.A., 2005. Effects of long-term fertilization on distribution of organic matters and nitrogen in Cinnamon soil aggregates. Sci. Agric. Sin. 38, 1841–1848. https://doi.org/10.1016/j.fct.2013.07.067 (in Chinese).

Tan, Z., Lal, R., Owens, L., Izaurralde, R.C., 2007. Distribution of light and heavy fractions of soil organic carbon as related to land use and tillage practice. Soil. Till. Res. 92, 53–59. https://doi.org/10.1016/j.still.2006.01.003.

Tejada, M., Gonzalez, J.L., 2009. Application of two vermicomposts on a rice crop: effects on soil biological properties and rice quality and yield. Agron. J. 101, 336–344. https://doi.org/10.2134/agronj2008.0211.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Eur. J. Soil Sci. 33, 141–163. https://doi.org/10.1111/j.1365-2389.1982.tb01755.x.

Totsche, K.U., Amelung, W., Gerzabek, M.H., Guggenberger, G., Klumpp, E., Knief, C., Lehndorff, E., Mikutta, R., Peth, S., Prechtel, A., Ray, N., Kögel-Knabner, I., 2018. Microaggregates in soils. J. Plant Nutr. Soil Sci. 181, 104–136. https://doi.org/10.1002/jpln.201600451.

van Bavel, C.H.M., 1950. Mean weight-diameter of soil aggregates as a statistical index of aggregation. Soil. Sci. Soc. AM. J. 14, 20–23. https://doi.org/10.2136/sssaj1950.036159950014000C0005x.

Wander, M.M., Traina, S.J., 1996. Organic matter fractions from organically and conventionally managed soils: i. carbon and nitrogen distribution. Soil Sci. Soc. Am. J. 60, 1081–1087. https://doi.org/10.2136/sssaj1996.03615995006000040017x.

WRB, 2014. World Reference Base for soil resources 2014: international soil classification system for naming soils and creating legends for soil maps. Food and Agriculture Organization of the United Nation (FAO).

Wu, T., Schoenau, J., Li, F., Qian, P., Malhi, S., Shi, Y., 2003. Effect of tillage and rotation on organic carbon forms of chernozemic soils in saskatchewan. J. Plant Nutr. Soil Sci. 166, 328–335. https://doi.org/10.1002/jpln.200390051.

Xie, J.Y., Xu, M.G., Qiangjiu, C.R., Yang, Y., Zhang, S.L., Sun, B.H., Yang, X.Y., 2015. Soil aggregation and aggregate associated organic carbon and total nitrogen under long-term contrasting soil management regimes in loess soil. J. Integr. Agric. 14, 2405–2416. https://doi.org/10.1016/S2095-3119(15)61205-9.

Yilmaz, E., Snmez, M., 2017. The role of organic/bio–fertilizer amendment on aggregate stability and organic carbon content in different aggregate scales. Soil. Till. Res. 168, 118–124. https://doi.org/10.1016/j.still.2017.01.003.

Yu, H., Ding, W., Luo, J., Geng, R., Cai, Z., 2012. Long-term application of organic manure and mineral fertilizers on aggregation and aggregate-associated carbon in a sandy loam soil. Soil. Till. Res. 124, 170–177. https://doi.org/10.1016/j.still.2012.06.011.

Zhou, G.X., Xiu, W., Chen, L., Zhang, C.Z., Ma, D.H., Zhang, J.B., 2019. Succession of organics metabolic function of bacterial community in response to addition of earthworm casts and zeolite in maize straw composting. Bioresour. Technol. 280, 229–238. https://doi.org/10.1016/j.biortech.2019.02.015.