

Fertilizer impacts on soil aggregation and aggregate-associated organic components

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ABSTRACT

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A 5-year (2012–2016) field experiment was conducted to investigate the impacts of different fertilizer treatments (no fertilizer, mineral and organic fertilizer) on organic carbon and soil water-stable aggregates in a North China Plain Vertisol. Compared with no fertilizer (control), single mineral fertilizer did not significantly ($P < 0.01$) affect organic carbon content or aggregate mass proportion in bulk soil. Small and large macroaggregate mass proportions increased, but applying organic manure significantly decreased the silt + clay fraction and microaggregates. Organic manure amendment significantly enhanced organic carbon concentrations in aggregates (large macroaggregates, $> 2000 \mu\text{m}$; small macroaggregates, $2000\text{--}250 \mu\text{m}$; microaggregates, $53\text{--}250 \mu\text{m}$; and free silt + clay fraction, $< 53 \mu\text{m}$) and aggregate subfractions, including intraparticulate organic matter and silt + clay subfractions ($< 53 \mu\text{m}$). Single mineral fertilizer amendment increased organic carbon concentrations in macroaggregates, particularly intraparticulate organic matter. The results indicated that the organic carbon increase in organic manure-amended soil were possibly due to enhanced silt + clay subfractions, which then promoted macroaggregates formation. Applying organic manure could improve organic carbon sequestration and maintain its stability in aggregates, whereas mineral fertilizer only enhanced organic carbon in large macroaggregates, but with low stability.

Keywords: soil quality; wet-sieving; residue layer; soil nutrients; soil structure

Increasing attention is being paid to potential benefits of organic manure in sequestering carbon (C), maintaining soil structure stability, and improving soil quality (Six et al. 1999). Previous studies involving several long-term field experiments show that applying organic manure increases organic carbon (OC) content (Nardi et al. 2004, Manna et al. 2005, Xin et al. 2016), which is attributed to resulting improvements in the biochemical properties of soil.

Microclimate changes affect the OC balance and mass structure of soil, directly or indirectly, in the surface residue layer. The influence of manure application is believed to relate to changes in the OC component accumulation, and the association with aggregate investigated in this study.

Manure-related increases in OC and mass macroaggregates fractions may be attributed to: (i) increased residue returns, which are considered the

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origin of manure-related OC sequestration compared with mineral fertilizers (Cai and Qin 2006); and (ii) changed chemical composition of soil solutions, which is highly related to the dispersion/flocculation of clay particles, and thus soil aggregation (Bronick and Lal 2005). OC is a binding agent and nucleus in soil aggregate formation, and the aggregation level and aggregate turnover (i.e., rate of aggregate formation and degradation) in turn influences OC (Six et al. 1998, Bronick and Lal 2005). Moreover, Monnier (1965) proposed a conceptual model in which aggregate stability increases immediately after organic amendment, and thereafter decreases as organic matter decomposes.

With continuous cultivation by manure application, organic debris is rendered relatively rapidly into particles, and accumulating organic matter is not highly processed. Although most accumulated OC occurs in mineral-associated macroaggregates fractions, a small part occurs as particulate organic matter (POM; density $\leq 1.85 \text{ g/cm}^3$) (Jastrow 1996). Formed from organic molecules attached to clay and polyvalent cations to form compound particles, microaggregates ($< 250 \mu\text{m}$) join with other particles to make macroaggregates; these can form around POM, becoming more stable as POM decomposes and microbial exudates are released (Edwards and Bremner 1967, Tisdall and Oades 1982, Bronick and Lal 2005, Totsche et al. 2018). OC can be stabilized by microaggregates, which form internally (Bronick and Lal 2005). Elucidating the relationship between different organic components in soil and OC accumulation in aggregates is thus vital to explore the mechanisms governing the latter, as well as macroaggregate formation.

In our study, physical fractionation procedures were used to separate the spatial arrangement of primary and secondary organic mineral particles, based on density and primary aggregate size, according to the method described by Six et al. (1998). Our objectives were to: (1) assess the effects of fertilization on OC sequestration and soil aggregation; and (2) understand OC accumulation processes following the application of organic manure-amended and mineral fertilizer.

MATERIAL AND METHODS

Field experiment. The field experiment was established in June 2012 in a well-drained field

where wheat (*Triticum aestivum* L.) was grown in winter and maize (*Zea mays* L.) in summer. The site is in a typical region of the North China Plain (33°33'N, 114°02'E). The average annual temperature and precipitation here are 14.8°C and 852 mm, respectively. The soil, derived from fluvio-lacustrine plain deposits, is a vertisol, with a loam texture of 45.60% sand, 40.70% silt and 13.70% clay. The OC and total nitrogen in June 2012 were 5.92 g/kg and 0.57 g/kg soil, respectively.

A randomized block design was used to prepare three replicates of each of four treatments: pre-soil; no fertilizer control (CK); NPK fertilizer (F), and 60% compost plus 40% nitrogen (N) fertilizer (FM). Calcium superphosphate (39 kg P/ha for NPK) and potassium sulphate (75 kg K/ha for NPK) were applied to each crop as basal fertilizers; compost (135 kg N/ha for FM) was applied annually as a basal fertilizer to maize. Urea totalled 225 and 240 kg N/ha in the maize and wheat NPK treatments, respectively. Organic manure, phosphorus, and potassium fertilizers were applied into the soil before sowing, and nitrogenous fertilizer was applied at the rates of 40% and 60% of the total amount before sowing and during the elongation stage, respectively. In the FM treatment, the compost addition rate was 6.32 t/ha, which corresponds to approximately 1.50 t OC/ha. Urea was applied as a supplemental fertilizer at a rate of 90 and 240 kg N/ha for maize and wheat, respectively. Calcium superphosphate and potassium sulphate were used to supplement insufficient levels of phosphorus and potassium. No fertilizer or compost was applied in the CK treatment.

Soil sampling and aggregate fractionation. In June 2016, immediately after the wheat harvest, four soil samples were randomly collected in the 0–20 cm soil layer at different locations in each plot, using a stainless-steel soil sampler. Pre-soil samples were also collected for testing in June 2012. Soil samples were wet-sieved into large macroaggregates ($> 2000 \mu\text{m}$); small macroaggregates (250–2000 μm); microaggregates (53–250 μm), and the silt + clay fraction ($< 53 \mu\text{m}$), at ambient temperature according to the method described by Elliott (1986). After separation, the aggregate fractions were dried at 40°C for soil property analysis or, using the density fractionation method, for further fractionation of macro- and microaggregates into different subfractions, including coarse intraparticulate POM (iPOM; $> 250 \mu\text{m}$), fine iPOM (53–250 μm), and SC (silt + clay subfraction; $< 53 \mu\text{m}$). Yu et al. (2012a) report the fractiona-

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tion method and its results in detail. OC content in the soil and aggregates was determined by the wet oxidation-redox titration method (Carter 1993).

Calculation and statistical analysis. Statistical analysis was performed using the SPSS v. 16.0 software (SPSS Inc., Chicago, USA). A one-way analysis of variance (ANOVA) followed by the least significant difference (*LSD*) test were used to compare differences between the pre-soil, CK, F, and FM treatments, at significance levels of $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

OC concentration and aggregate mass distribution. Application of organic manure significantly improved OC and macroaggregates levels. The analysis results showed that compared to pre-soil conditions, total OC was improved slightly (7.53%) and significantly enhanced (40.22%) by the F and FM treatments, respectively (Figure 1). Similar to other research results, applying mineral fertilizer and organic manure resulted in increased OC concentrations in bulk soil (Yu et al. 2012a, Xin et al. 2016). In the present study, the aggregate mass proportion ($< 250 \mu\text{m}$) was not significantly ($P < 0.01$) affected by the F treatment but, in contrast, it was significantly ($P < 0.01$) increased (including small and large macroaggregates) by the FM treatment, from 30.82–39.85% in pre-soil to 41–43.58%. Moreover, the silt + clay fraction and microaggregates were clearly observed to reduce

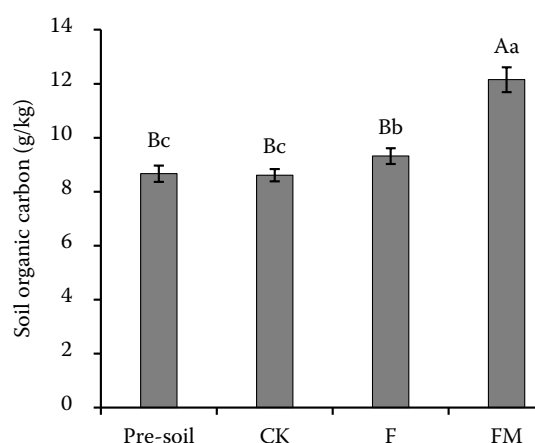


Figure 1. Soil organic carbon in bulk soil (0–20 cm) in four different experimental treatments: pre-soil – samples were collected before the test; CK – no fertilizer (control); F – NPK fertilizer; FM – nitrogen fertilizer. Vertical bars represent the standard deviation of the mean ($n = 3$). Different lowercase and capital letter indicate significant differences between treatments ($P < 0.05$ and $P < 0.01$)

in the FM treatment (Figure 2); this might result from cementing or binding agents (which may be inorganic, organo-mineral associations, or organic matter added by organic manure) forming larger aggregates from stabilized silt + clay fractions or microaggregates (Tisdall and Oades 1982). The result provided direct evidence in the support of organic manure rather than mineral fertilizer promoting large aggregate formation.

Water-stable aggregate-associated OC. In the pre-soil and CK treatments, all aggregate size

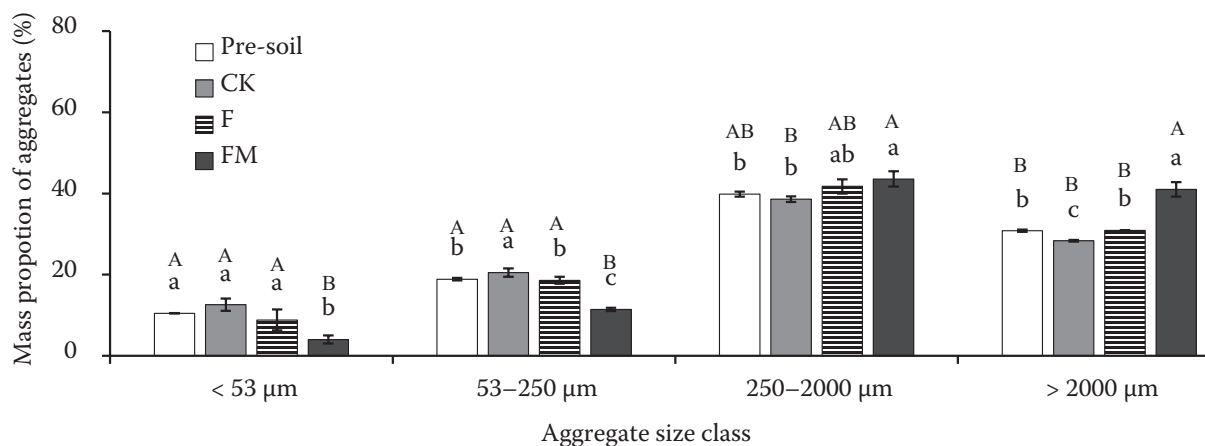


Figure 2. Soil aggregate size fractions of four different experimental treatments: pre-soil – samples were collected before the test; CK – no fertilizer (control); F – NPK fertilizer; FM – nitrogen fertilizer. Vertical bars represent the standard deviation of the mean ($n = 3$). Different lowercase letters and capitals indicate significant differences between treatments ($P < 0.05$ and $P < 0.01$)

Table 1. Organic carbon (OC, g/kg) concentration and a rate of increase (%) in aggregates in the 0–20 cm soil layer

Treatment	Silt + clay fraction ($< 53 \mu\text{m}$)		Microaggregates ($53\text{--}250 \mu\text{m}$)		Small macroaggregates ($250\text{--}2000 \mu\text{m}$)		Large macroaggregates ($> 2000 \mu\text{m}$)	
	OC	increase rate	OC	increase rate	OC	increase rate	OC	increase rate
Pre-soil	$7.03 \pm 0.19^{\text{Bc}}$	–	$6.70 \pm 0.62^{\text{Cd}}$	–	$7.31 \pm 0.35^{\text{Cc}}$	–	$7.01 \pm 0.46^{\text{Cc}}$	–
CK	$7.12 \pm 0.10^{\text{Bbc}}$	1.25	$7.42 \pm 0.24^{\text{BCc}}$	10.72	$7.66 \pm 0.17^{\text{BCc}}$	4.79	$6.96 \pm 0.83^{\text{Cc}}$	–0.65
F	$7.34 \pm 0.03^{\text{Bb}}$	4.46	$8.38 \pm 0.27^{\text{Bb}}$	25.05	$8.50 \pm 0.37^{\text{Bb}}$	16.30	$8.72 \pm 0.43^{\text{Bb}}$	24.50
FM	$8.29 \pm 0.16^{\text{Aa}}$	17.87	$9.87 \pm 0.01^{\text{Aa}}$	47.22	$10.22 \pm 0.39^{\text{Aa}}$	39.86	$10.33 \pm 0.4^{\text{Aa}}$	47.40

Pre-soil – samples were collected before the test; CK – no fertilizer (control); F – NPK fertilizer; FM – nitrogen fertilizer. Values are means ($n = 3$), with standard error. Different lowercase letters in the same column indicate significant differences between treatments at $P < 0.05$, and different capital letters in the same row indicate significant differences between fractions at $P < 0.01$

fractions had similar OC values. However, OC concentrations in small and large macroaggregates varied from 8.5 to 10.33 g/kg, values that were apparently higher than those for microaggregates and

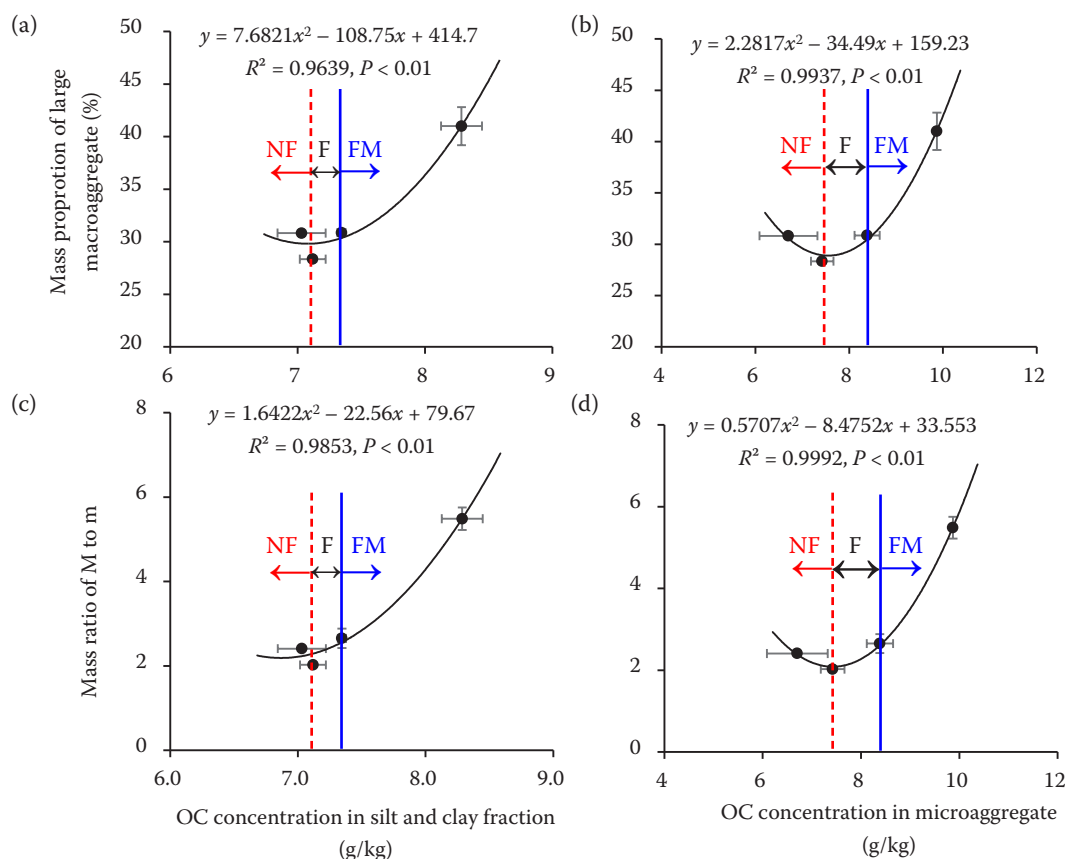


Figure 3. Relationships between the macroaggregate mass proportion and organic carbon (OC) concentration in the free silt + clay fraction (a) or microaggregates (b); and relationships between the mass ratio of macro- to microaggregates and the OC concentration in the free silt + clay fraction (c) or microaggregates (d). Vertical and horizontal bars denote the standard error of means ($n = 3$); NF – no fertilizer; F – single mineral fertilizer; FM – mixed mineral fertilizer with organic manure; M – macroaggregates; m – microaggregates. Left side of the red dashed line indicates no fertilizer treatment, the middle area between red dashed and blue line indicates single mineral fertilizer treatment and the right side of blue line indicates the treatment of mixed mineral fertilizer with organic manure

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Table 2. Organic carbon (OC) amount in aggregates in the 0–20 cm soil layer

Treatment	Microaggregates (53–250 μm)		Macroaggregates (> 250 μm)		
	SC (silt + clay subfraction, < 53 μm)	Fine iPOM (53–250 μm)	SC (silt + clay subfraction, < 53 μm)	Fine iPOM (53–250 μm)	Coarse iPOM (> 250 μm)
Pre-soil	7.84 \pm 0.53 ^{Bb}	10.54 \pm 0.11 ^{Cc}	8.92 \pm 0.19 ^{Bb}	7.25 \pm 0.29 ^{Cc}	11.15 \pm 0.09 ^{Bb}
CK	7.31 \pm 0.36 ^{Bb}	11.05 \pm 0.59 ^{Cc}	8.76 \pm 0.57 ^{Bb}	7.62 \pm 0.33 ^{Cc}	11.64 \pm 0.3 ^{ABa}
F	7.86 \pm 0.37 ^{Bb}	12.38 \pm 0.28 ^{Bb}	9.39 \pm 0.3 ^{Bb}	9.02 \pm 0.1 ^{Bb}	11.8 \pm 0.19 ^{ABa}
FM	9.14 \pm 0.53 ^{Aa}	13.71 \pm 0.28 ^{Aa}	11.43 \pm 0.13 ^{Aa}	12.37 \pm 0.22 ^{Aa}	12.02 \pm 0.31 ^{Aa}

Pre-soil – samples were collected before the test; CK – no fertilizer (control); F – NPK fertilizer; FM – nitrogen fertilizer. Values are means ($n = 3$), with standard error. Different lowercase letters in the same column indicate significant differences between treatments at $P < 0.05$, and different capital letters in the same row indicate significant differences between fractions at $P < 0.01$; iPOM – intraparticulate particulate organic matter

free silt + clay fractions in the F and FM treatments (Table 1). Compared with pre-soil conditions, the FM treatment significantly ($P < 0.01$) increased OC in the free silt + clay fraction, microaggregates, and small and large macroaggregates by 17.87, 47.22, 39.86, and 47.70%, respectively. The mineral fertilizer amendment, compared with pre-soil conditions, also significantly ($P < 0.01$) increased OC in large macroaggregates, while the F treatment enhanced OC in microaggregates and small macroaggregates, but not significantly ($P < 0.01$). Furthermore, OC concentrations increased with increasing aggregate size (Table 1). These results suggested that the F and FM treatments might primarily increase OC concentrations in smaller size aggregates and then influence the formation of macro- from microaggregates; in turn, soil aggregation could physically protect OC from decomposition (Jastrow 1996, Bronick and Lal 2005).

Under the F and FM treatments, OC concentrations in the free silt + clay fraction and microaggregates were positively correlated with the large macroaggregates mass proportion, and with the mass ratio of macro- to microaggregates ($P < 0.01$). However, under the CK treatment, OC concentrations in the free silt + clay fraction and microaggregates increased with decreasing macroaggregates accumulation (Figure 3). Our findings showed OC increases in the free silt + clay fraction could not promote macroaggregates formation under the CK treatment; however, OC concentrations in the free silt + clay fraction probably play a key role in macroaggregates formation after applying fertilizer, especially organic manure. The relationship between the macroaggregates

mass proportion and OC concentration in the free silt + clay fraction and microaggregates indicated that OC concentrations in the latter two control macroaggregates. Thus, it was considered that low OC concentrations in microaggregates (< 250 μm) possibly limited macroaggregates (> 250 μm) formation in soils with added mineral or organic manure fertilizer, compared with no fertilizer.

Unlike stable SC (silt + clay subfraction; < 53 μm), iPOMs within macroaggregates are unstable, and correlate significantly with C-cycling enzyme activity (Yu et al. 2012b). Yu et al. (2012a) report that increments in OC content are not dependent on increases in iPOM entrapped in aggregates. However, this finding was very different from an earlier study. OC accumulation in macroaggregates was mainly attributed to increased OC in coarse iPOM in the F treatment (Table 2). It could be concluded that OC increments in macroaggregates via applied mineral fertilizer were unstable, and could not be maintained for long periods. Meanwhile, application of organic manure (FM) enhanced SC and iPOMs in aggregates equally (Table 2). Therefore, applying FM could result in a sustainable and stable OC increase in aggregates. Moreover, the grain yield was significantly influenced by the different fertilization treatments (Figure 4). Regardless of the wheat and maize, the grain yields under FM treatment were significantly higher than that of the CK. Applying compost, compared to F treatment, improved wheat significantly (8.84%) and maize slightly (7.04%).

In conclusion, compared to the no fertilizer treatment, fertilizer application could enhance OC content and large macroaggregates. However,

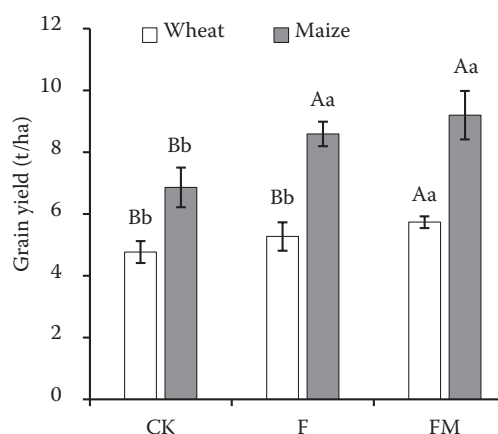


Figure 4. The oven-dried grain yield of wheat and maize under different experimental treatments after four years: CK – no fertilizer (control); F – NPK fertilizer; FM – nitrogen fertilizer. Vertical bars represent the standard deviation of the mean ($n = 3$). Different lowercase and capital letter indicate significant differences between treatments ($P < 0.05$ and $P < 0.01$)

OC and macroaggregates remained unstable and could not be maintained by single mineral fertilizer applications. OC in the microaggregates and silt + clay fractions played a key role in OC sequestration in organic manure-amended soil. Applying organic manure significantly increased OC by enhancing OC concentrations in the free silt + clay fraction, which then promoted macroaggregates formation by binding the silt + clay fraction into macroaggregates. Therefore, it is concluded that applying organic manure as a soil amendment can not only increase grain yield but also induce OC concentrations, in particular facilitating the formation of water-stable macroaggregates, and simultaneously improving the SC and iPOM content in aggregates.

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