

Effects of Substituting Manure for Fertilizer on Aggregation and Aggregate Associated Carbon and Nitrogen in a Vertisol

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ABSTRACT

Fertilizer management influences the accumulation of soil organic C and N in agricultural soils. The combined use of chemical and organic fertilizers is considered a good method to enhance soil fertility, but it is still unclear about the effects of substituting manure for fertilizer on aggregation and aggregate associated organic C and N. We selected a 4-yr-old soil fertility experiment to examine the aggregation and aggregate associated organic C and N in response to substituting manure for fertilizer. In comparison to control, no fertilizer added (CK); substituting manure for fertilizer significantly increased organic C and N by 11.61 to 47.97% and 21.05 to 32.63% in 0- to 20-cm bulk soil, respectively. It promoted organic C in the silt + clay fraction, microaggregates, small macroaggregates, and large macroaggregates by 3.17 to 17.15%, 12.94 to 34.09%, 10.99 to 50.20%, and 25.32 to 52.09%, respectively. Aggregate associated N also presented significant increases under substituting manure for fertilizer compared to CK. The mass distribution of small and large macroaggregates were 8.16 to 15.54% and 8.89 to 49.51% higher in the manure-substituted plots than CK, respectively. Organic C and labile C were generally greater in macro- than microaggregates in the manure-substituted plots; redundancy analysis confirmed that macroaggregates had positive effects on organic C and N sequestration in bulk soil and aggregates. Based on these results we suggested that substituting manure increases macroaggregate associated organic C and N, and the formation of small and large macroaggregates is accelerated by organic amendment in the test soil compared to CK.

Core ideas

- Manure improved soil aggregate-associated C and N sequestration.
- Fertilizer substituted with manure in increasing C and N concentrations used.
- Manure improved water stable soil aggregate stability.

SOIL ORGANIC C and total soil N are important components of soil C and N pools. Increasing C and N sequestration is important to control global climate warming and maintain sustainable agriculture (Rusco et al., 2003; Smith et al., 2007). Over the past few decades, optimizing fertilization has been proven beneficial to decrease cultivated land degradation and rebuild soil structure, thereby improving soil fertility (Six et al., 2000a; Campbell et al., 2001; Wei et al., 2016; Chaudhary et al., 2017).

Vertisol containing high amounts of 2:1 clay minerals swell and shrink regularly when subjected to moisture changes, but humus C could decrease the shearing stresses of montmorillonite minerals and promoted the formation of soil aggregates via cementing (Tisdall and Oades, 1982; Shi et al., 2016). Many studies have shown that the stability of soil aggregates influenced by soil organic C except for calcite or gypsum. The hierarchical conceptual model for soil aggregate structure proposed by Tisdall and Oades (1982), also indicates that soil aggregates depends on the concentration of persistent organic C in organic matters, while soil binding agents and macroaggregates enable organic C accumulation in turn (Six et al., 2000a). Thus, it can be seen that organic C has a close relation with soil aggregates. However, the application of chemical fertilizer barely maintains or even decreases organic C and has negative environmental impacts, such as acidification and nutrient loss. What is worse, organic C loss is bound to reduce the stability of soil aggregates, which are the physical carriers of organic C and N, thereby threatening the nutritional resources of soils. Fortunately, the combined application of organic amendments and fertilizers has been gaining increasing recognition as a feasible and practical approach in boosting crop yields in the short term and enhancing organic C in the long term. For example, Xia et al. (2017) and Xue et al. (2014) suggested that recycling of livestock manure in agroecosystems reduces the release of reactive N and increases organic C storage significantly, more than that caused by synthetic N fertilizer application. Six et al. (2000a) also observed that an increase in organic C in cultivated soils following manure could be attributed to physical protection of the newly added organic C by occlusion

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Abbreviations: CK, control, no fertilizer added; Cs, the stabilities of carbon; Ns, the stabilities of nitrogen; MWD, the mean weight diameter; RDA, redundancy analyses

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in microaggregates. In contrast, a few scholars suggested that long-term application of organic manure increased heavy metals, antibiotics, and the concentration of N and P in leaching water, which caused water or soil pollution (Hargreaves et al., 2008; Zhao et al., 2017). These inconsistent reports in the literature suggest that further work is needed to understand the organic C, N stability in soil, and to accurately explain the accumulation process of organic C, N in aggregates.

Generally, substituting manure for fertilizer alters N supply and organic C sequestration, which may stimulate mineral N immobilization, thereby decreasing N substrate and losing it as reactive N (Banerjee et al., 2002). Previous scholars have observed that there were differences in organic C and N concentrations among large aggregate size fractions and the structural stability was closely related to the concentrations of organic C and N from the particulate organic matter fraction (Cambardella and Elliott, 1993; Saroa and Lal, 2003). Craswell and Waring (1970) suggested that small aggregates contain a larger proportion of readily mineralizable organic N than larger aggregates, which revealed small aggregates contain more labile N than larger aggregates. However, Elliott (1986) concluded that mineralizable N was greater in macroaggregates (>250 μm) than microaggregate (<250 μm). These inconsistent results may be due to the variable conditions that include soil characteristics, management practices and climate conditions. And the knowledge about the mechanism of soil organic C and N accumulation in aggregates is still limited. To figure out the changes in the contents and vitality of organic C and N realistically and sensitively under various fertilization practices, therefore, scholars have chemically divided soil organic C and N into total and labile parts (Blair et al., 1995). Compared to total organic C, labile C is usually more sensitive to the available soil nutrients, soil physical properties, and techniques of soil management (Bremer et al., 1994). Short-term changes in labile C may be useful for predicting long-term changes in total organic C. Through a crop-rotation study, Bremer et al. (1994) emphasized that labile C, which was significantly enhanced by manure addition and the reductions in fallow frequency, was the most robust indicator of the management-induced effects on soil organic matter. Whitbread et al. (1998) found a highly significant correlation between labile C and the proportions of water stable macroaggregates in the red earth and gray clay, but this correlation was poor in the black earth soil. Previously, few studies have reported variations in the aggregate-associated labile C responses to substituting manure for fertilizer practice. Understanding the characteristics of variations in the labile C of soil aggregates is still unclear. To better understand the coupling effect between organic decomposition, soil mineralization potential, nutrient cycling, and soil aggregate formation, the present study was conducted to identify how organic C and N distributed among the different soil aggregate fractions, the stability of the formed aggregate fractions and the distributions of the accumulated nutrients in the newly formed aggregates, when manure, instead of fertilizer, is added to Vertisol.

We hypothesized that substituting manure for fertilizer will accelerate soil macroaggregation by improving the stability of organic C and N, thereby promoting the accumulation of organic C, and N and their components. Organic C and N accumulation, in turn, will drive further formation of macroaggregates. In this study, based on a 4-yr-long field experiment that

was established to monitor the dynamics of organic C, and N and soil structures in response to substituting manure for fertilizer practices, we aimed: (i) to assess the overall effects of substituting manure for fertilizer on soil aggregation, organic C and N sequestration in agroecosystems, (ii) to quantitatively analyze the responses of labile C and alkalyzable N, and the stabilities of carbon (C_c) and nitrogen (N_c) to substitution with manure.

MATERIALS AND METHODS

Experimental Site

The study was conducted at a fertilization experimental field (33°33' N, 114°02' E), situated in Xiping County of Henan Province, China. The experiment started on June 2012 on a well-drained field, a typical irrigable cropland in the North China Plain. The average annual temperature and precipitation here are 14.8°C and 852 mm, respectively. The average sunshine hours and the average frost-free period are 2659 h and 121 d, respectively. In June 2012 the total organic C and N were 5.92 g kg⁻¹ soil and 0.57 g kg⁻¹ soil, respectively. The soil, derived from a fluvio-lacustrine deposit plain, has a loam texture, with 0.46 kg sand kg⁻¹ soil, 0.41 kg silt kg⁻¹ soil, and 0.14 kg clay kg⁻¹ soil.

Experimental Design

The experiment included two crops per year, winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.), and six treatments: control (CK; no fertilizer added) plots, plots treated with chemical fertilizer (225 kg N ha⁻¹ + 90 kg P₂O₅ ha⁻¹ + 90 kg K₂O ha⁻¹) applied at a standard rate (FR), and plots treated chemical fertilizer in which 20, 40, 60, and 80% of the amount of N fertilizer was replaced by organic manure (designated M₁, M₂, M₃, and M₄, respectively). The organic manure was produced by chicken excrement. While in the maize season, the amount of nitrogenous, phosphorus (P₂O₅), and potassium (K₂O) fertilizers in all treatments were 240 kg N ha⁻¹, 90 kg ha⁻¹, and 90 kg ha⁻¹, respectively. The total inputs of N, P₂O₅, K₂O were 465 kg ha⁻¹, 180 kg ha⁻¹, and 180 kg ha⁻¹ in all treatments (M₁, M₂, M₃, and M₄) by chemical fertilizer and organic manure. Organic manure, phosphorus (P₂O₅), and potassium (K₂O) 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. Mature wheat and maize were harvested in June and October, respectively.

Soil Sampling and Analysis

Immediately after wheat harvest on 1 June 2016, four samples were taken at depth of 0 to 20 cm at three positions in each plot. All samples from each plot were carefully mixed to form a composite. The samples were immediately transferred in hard-plastic boxes to the laboratory.

Moist soil samples were gently broken apart along natural break points and passed through a 10-mm sieve. Plant and organic debris in the sieved soil were carefully identified, visually, and removed with forceps. After mixing thoroughly, a subsample of the sieved soil was air-dried and used for soil fractionation analyses and four classes of aggregates (>2000 μm , 250–2000 μm , 53–250 μm , and <53 μm) were obtained. After separation, the aggregate fractions and another subsample were used to determine total organic C, N, labile C, and alkalyzable

N. Total organic C and labile C were measured by the wet oxidation-redox titration method and K permanganate oxidation methods, respectively (Carter and Gregorich, 2007; Loginow et al., 1987). Nitrogen and alkalyzable N were determined by the semi-micro Kjeldahl and alkaline hydrolysis diffusion methods, respectively (Carter and Gregorich, 2007).

Soil Aggregation and Analysis

Determination of soil aggregate physical fractions was in accordance with Elliott (1986). A sample of 100 g of each the first air-dried subsample mentioned above was immersed in deionized water on top of a 2000 μm sieve. After 5 min of slaking, the sieve was manually moved 60 times up and down, through a distance of 3 cm, over a 2-min period. Water plus soil <2000 μm was poured through a 250 μm , then a 53 μm sieves. The fractions remaining on the 2000, 250, and 53 μm sieves were collected. Soil smaller than 53 μm (silt + clay fraction) was allowed to settle and centrifuged. Thus, four aggregates-size classes were obtained: (i) large macroaggregate (>2000 μm), (ii) small macroaggregate (250–2000 μm), (iii) microaggregate (53–250 μm), and (iv) silt + clay fraction (<53 μm). After separation, the aggregate fractions were dried at 40°C for the analysis of soil properties.

Data Analysis

The contribution of the water stable aggregates to organic C or N accumulation in the silt + clay fractions, microaggregates, small macroaggregates, and large macroaggregates was calculated by dividing the contents of organic C or N in each of the four fractions by the contents of organic C or N in the 0 to 20 cm bulk soil. Organic C or N storage in the aggregates was calculated by the organic C or N contents in the silt + clay fractions, microaggregates, small macroaggregates, and large macroaggregates, multiplied by the mass proportions of the silt + clay fractions, microaggregates, small macroaggregates, and large macroaggregates, respectively. Accumulated net organic C or N in the silt + clay fractions, microaggregates, small macroaggregates, and large macroaggregates was calculated by subtracting the contents of organic C or N in each of the four fractions by the contents of organic C or N in the CK plot, respectively. Contribution rates of the accumulated net organic C or N in the silt + clay fractions, microaggregates, small macroaggregates, and large macroaggregates to the organic C or N in 0 to 20 cm bulk soil were determined by dividing the increased amounts of organic C or N storage in each of these four fractions by the increased amounts of organic C or N content in the 0 to 20 cm bulk soil of the CK plot. The difference between total and labile C is termed as the non-labile C, and the difference between total and alkalyzable N is termed as the non-labile N. The C_S was calculated by dividing the non-labile C by the total organic C content in aggregate and the N_S was calculated by dividing the non-labile N by the total organic N content in aggregate (Blair et al., 1995).

Following van Bavel's method (van Bavel, 1950), as modified by Kemper et al. (1986), the mean weight diameter (MWD) was calculated using the following equation:

$$\text{MWD} = \sum_i^n W_i X_i$$

where X_i is the mean diameter of each aggregate-size fraction i , and W_i is the proportion of the total sample weight occurring in the size fraction i .

The contribution rates of accumulated net organic C, N in various sizes of aggregation to the organic C or N in bulk soil (0–20 cm) were calculated using the following equation:

The contribution rates of accumulated net organic C = $C_{\text{Treatment}} - C_{\text{control}}$

The contribution rates of accumulated net N = $N_{\text{Treatment}} - N_{\text{control}}$

where $C_{\text{Treatment}}$, $N_{\text{Treatment}}$ are the accumulated net organic C and N of aggregates in fertilizer treatments, respectively. C_{control} and N_{control} are the aggregate associated organic C and N in control treatment, respectively.

One-way ANOVA was used to test for significant differences of the various measured parameters ($P < 0.05$ and 0.01) among the different treatments, soils, or aggregates using Tukey's HSD to compare between means. A stepwise method was used in linear regression analysis to identify the relationships between the aggregate associated organic C or N contents and MWD in the macroaggregates, using the SPSS 17.0 software package for Windows (SPSS Inc., Chicago, IL). Redundancy analyses (RDA) (Braak and Smilauer, 1998) was used to evaluate the relationships between soil macroaggregation and the organic C and N contents.

RESULTS

Soil Organic Carbon and Nitrogen concentration

After 4 yr of experiment, the contents of organic C, N, labile C, and alkalyzable N, which increased with the increase in the amounts of manure applied, were significantly increased in the plots treated with fertilizers substituted with manure than in the CK plot. Especially, organic C in the M_1 , M_2 , M_3 , and M_4 treatments were found to be 9.61, 10.56, 12.15 and 12.74 g kg^{-1} , an increase of 11.61, 22.65, 41.11 and 47.97%, respectively, compared with that in CK. What's more, the N concentration was 21.05, 24.21, 29.47 and 32.63% higher in the M_1 , M_2 , M_3 , and M_4 treatments than CK. And, labile C and alkalyzable N presented similar increases (Table 1). But applying more manure led to the decline in C_S , which decreased from 74.9 to 61.88%. However, N_S improved slightly with more manure application.

Aggregation Mass Distribution and Aggregate Associated Organic Carbon and Nitrogen Contents

The mass percentage of small and large macroaggregates (>250 μm) was much higher than that of microaggregates (53–250 μm) or silt + clay fraction (<53 μm). And, the organic manure practice had a significant effect on aggregation mass distribution (Table 2). The aggregate mass distribution of the silt + clay fractions and microaggregates were 3.11 to 12.55% and 11.41 to 20.50%, respectively. The small macroaggregates and large macroaggregates accounted for 38.60 to 44.60% and 28.34 to 42.37% of the total soil mass in all the treatments, respectively (Table 2). Substituting manure significantly increased ($P < 0.05$) the mass proportions of small and large

Table 1. Organic C and N, and their labile components for six fertilizer or manure treatments.

Treatment†	C		N		Labile C		Alkalyzable N		C _s ‡		N _s	
	g kg ⁻¹		g kg ⁻¹		mg kg ⁻¹		mg kg ⁻¹		%		%	
CK	8.61 ± 0.23d§	0.95 ± 0.01c	1.17 ± 0.65e	111.79 ± 0.71d	86.55 ± 7.19a	88.26 ± 0.13c						
FR	9.32 ± 0.29c	1.13 ± 0.02b	2.19 ± 0.14d	133.93 ± 2.32b	76.51 ± 0.77b	88.11 ± 0.02c						
M ₁	9.61 ± 0.35c	1.15 ± 0.02b	2.43 ± 0.02d	119.70 ± 0.77c	74.90 ± 0.66b	89.55 ± 0.11a						
M ₂	10.56 ± 0.19b	1.18 ± 0.06b	3.09 ± 0.33c	123.81 ± 3.62c	70.82 ± 2.58bc	89.50 ± 0.26a						
M ₃	12.15 ± 0.46a	1.23 ± 0.04ab	4.17 ± 0.23b	137.34 ± 1.44ab	65.70 ± 0.60c	88.83 ± 0.25b						
M ₄	12.74 ± 0.35a	1.26 ± 0.01a	4.86 ± 0.28a	141.15 ± 5.58a	61.88 ± 1.14c	88.81 ± 0.53b						

† CK, control; FR, fertilizer nitrogen, phosphorus, potassium; and M₁, M₂, M₃, M₄ denote plots treated with chemical fertilizer in which 20, 40, 60, and 80% of the amount of nitrogen fertilizer was replaced by organic manure, respectively.

‡ C_s, the stabilities of carbon; N_s, the stabilities of nitrogen.

§ Values are means ± standard error (n = 3). Different lowercase letters in the same column denote significant differences at the 0.05 level, within the same aggregate fraction.

Table 2. Mass proportion (%) of soil aggregates in 0- to 20-cm bulk soil affected by substituting manure for fertilizer.

Treatment†	Silt + clay fraction (< 53 μm)	Microaggregate (53–250 μm)	Small macroaggregate (250–2000 μm)	Large macroaggregate (> 2000 μm)
CK	12.55 ± 1.51a‡	20.50 ± 1.06a	38.60 ± 0.69c	28.34 ± 0.24e
FR	8.80 ± 2.58b	18.58 ± 0.87b	41.75 ± 1.76b	30.86 ± 0.05d
M ₁	6.18 ± 0.58c	16.42 ± 0.63c	42.99 ± 1.13ab	34.41 ± 0.73c
M ₂	4.15 ± 0.05cd	13.21 ± 0.41d	43.25 ± 1.47ab	39.39 ± 1.93b
M ₃	4.01 ± 1.02cd	11.41 ± 0.42e	43.58 ± 1.91ab	41.00 ± 1.81ab
M ₄	3.11 ± 0.41d	9.92 ± 0.48f	44.60 ± 2.15a	42.37 ± 1.34a

† CK, control; FR, fertilizer nitrogen, phosphorus, potassium; and M₁, M₂, M₃, M₄ denote plots treated with chemical fertilizer in which 20, 40, 60, and 80% of the amount of nitrogen fertilizer was replaced by organic manure, respectively.

‡ Values are means ± standard error (n = 3). Different lowercase letters in the same column denote significant differences at the 0.05 level, within the same aggregate fraction.

Table 3. The variation of organic C, N concentration in aggregates affected by substituting manure for fertilizer.

Item	Treatment†	Silt + clay fraction (<53 μm)	Microaggregate (53–250 μm)	Small macroaggregate (250–2000 μm)	Large macroaggregate (>2000 μm)
Organic C, g kg ⁻¹	CK	7.12 ± 0.10b‡	7.42 ± 0.24d	7.66 ± 0.17d	6.96 ± 0.83d
	FR	7.34 ± 0.03b	8.38 ± 0.27c	8.50 ± 0.37c	8.72 ± 0.43c
	M ₁	8.11 ± 0.51a	9.00 ± 0.46b	9.75 ± 0.14b	9.64 ± 0.35b
	M ₂	8.35 ± 0.36a	9.26 ± 0.29b	10.16 ± 0.36b	9.94 ± 0.09ab
	M ₃	8.29 ± 0.16a	9.87 ± 0.01a	10.22 ± 0.39b	10.33 ± 0.40ab
	M ₄	8.34 ± 0.30a	9.95 ± 0.28a	11.50 ± 0.53a	10.59 ± 0.46a
N, g kg ⁻¹	CK	0.84 ± 0.05c	1.07 ± 0.03c	1.17 ± 0.04c	1.02 ± 0.06b
	FR	0.93 ± 0.07b	1.14 ± 0.04bc	1.19 ± 0.01c	1.05 ± 0.03b
	M ₁	0.96 ± 0.05b	1.18 ± 0.04b	1.26 ± 0.03b	1.08 ± 0.03b
	M ₂	1.03 ± 0.04ab	1.18 ± 0.05b	1.25 ± 0.04b	1.09 ± 0.01ab
	M ₃	1.03 ± 0.04ab	1.20 ± 0.03b	1.31 ± 0.01a	1.10 ± 0.02ab
	M ₄	1.07 ± 0.04a	1.27 ± 0.05a	1.32 ± 0.00a	1.15 ± 0.01a
C/N	CK	8.50 ± 0.35a	6.93 ± 0.04e	6.57 ± 0.08e	6.78 ± 0.41d
	FR	7.89 ± 0.59a	7.34 ± 0.05d	7.17 ± 0.26d	8.33 ± 0.17c
	M ₁	8.45 ± 0.06a	7.61 ± 0.15c	7.73 ± 0.04c	8.96 ± 0.05b
	M ₂	8.08 ± 0.00a	7.84 ± 0.01b	8.15 ± 0.03b	9.12 ± 0.04ab
	M ₃	8.04 ± 0.13a	8.25 ± 0.22a	7.79 ± 0.21bc	9.42 ± 0.23a
	M ₄	7.81 ± 0.03a	7.83 ± 0.15bc	8.70 ± 0.40a	9.23 ± 0.32ab

† CK, control; FR, fertilizer nitrogen, phosphorus, potassium; and M₁, M₂, M₃, M₄ denote plots treated with chemical fertilizer in which 20, 40, 60, and 80% of the amount of N fertilizer was replaced by organic manure, respectively.

‡ Values are means ± standard error (n = 3). Different lowercase letters in the same column denote significant differences at the 0.05 level, within the same aggregate fraction.

macroaggregates, by 8.16 to 15.54% and 8.89 to 49.51%, respectively, and reduced the proportions of microaggregates and free silt + clay fractions, compared with those in CK. Applying mineral fertilizers only (in FR) also significantly affect the proportions of the small and large macroaggregates ($P < 0.05$).

Organic C and N concentrations, which increased with the increasing amounts of applied manure, in the macroaggregates

increased obviously after the application of manure-substituted fertilizer. In comparison to CK, substituting manure significantly increased organic C in the silt + clay fraction, microaggregates, small macroaggregates, and large macroaggregates by 3.17 to 17.15%, 12.94 to 34.09%, 10.99 to 50.20%, and 25.32 to 52.09%, respectively (Table 3). Substituting manure also increased N in the different aggregate-size classes somewhat.

Table 4. The variation of labile C and alkalyzable N in aggregates as affected by substituting manure for fertilizer.

Item	Treatment†	Silt + clay fraction (< 53µm)	Microaggregate (53–250 µm)	Small macroaggregate (250–2000 µm)	Large macroaggregate (>2000 µm)
Labile C, g kg ⁻¹	CK	4.83 ± 0.64c‡	7.13 ± 0.41b	7.56 ± 0.06b	5.51 ± 0.43d
	FR	6.08 ± 0.64b	7.23 ± 0.12ab	7.34 ± 0.41b	6.78 ± 0.39c
	M ₁	6.61 ± 0.39ab	7.63 ± 0.25ab	7.65 ± 0.14b	6.70 ± 0.61c
	M ₂	6.82 ± 0.41ab	7.55 ± 0.42ab	8.34 ± 0.43a	7.58 ± 0.13b
	M ₃	6.95 ± 0.63ab	7.45 ± 0.36ab	8.66 ± 0.46a	7.94 ± 0.50ab
Alkalyzable N, mg kg ⁻¹	M ₄	7.52 ± 0.98a	7.83 ± 0.52a	8.34 ± 0.45a	8.54 ± 0.43a
	CK	131.00 ± 3.46b	129.90 ± 0.47c	154.46 ± 3.31c	135.56 ± 2.36c
	FR	143.12 ± 12.75b	154.46 ± 3.31b	147.37 ± 3.40d	132.26 ± 2.83c
	M ₁	142.65 ± 9.45b	166.74 ± 8.03a	162.02 ± 0.47b	137.61 ± 7.56c
	M ₂	166.74 ± 1.42a	170.05 ± 9.45a	166.90 ± 5.04b	147.69 ± 3.82b
M ₃	168.16 ± 1.89a	170.05 ± 9.45a	169.10 ± 1.89ab	155.88 ± 4.72a	
M ₄	179.49 ± 7.87a	169.10 ± 2.83a	172.88 ± 2.83a	162.49 ± 4.09a	

† CK, control; FR, fertilizer nitrogen, phosphorus, potassium; and M₁, M₂, M₃, M₄ denote plots treated with chemical fertilizer in which 20, 40, 60, and 80% of the amount of nitrogen fertilizer was replaced by organic manure, respectively.

‡ Values are means ± standard error (n = 3). Different lowercase letters in the same column denote significant differences at the 0.05 level, within the same aggregate fraction.

Table 5. The stabilities of organic carbon (C_S) and nitrogen (N_S) effected by substituting manure for fertilizer.

Item	Treatment†	Silt+clay fraction (<53 µm)	Microaggregate (53–250 µm)	Small macroaggregate (250–2000 µm)	Large macroaggregate (>2000 µm)
C _S , %	CK	32.17 ± 8.06a‡	3.95 ± 2.40d	1.32 ± 1.38e	20.6 ± 3.40bc
	FR	17.23 ± 8.30b	13.73 ± 1.35c	13.65 ± 1.03d	22.36 ± 0.55bc
	M ₁	18.14 ± 8.92b	15.13 ± 2.04bc	21.49 ± 2.54b	30.62 ± 3.78a
	M ₂	18.39 ± 1.45b	18.57 ± 2.07b	17.95 ± 1.33c	23.70 ± 0.67b
	M ₃	16.17 ± 6.00b	24.55 ± 3.58a	15.3 ± 1.23d	23.15 ± 1.90bc
N _S , %	M ₄	10.02 ± 8.50b	21.42 ± 3.67ab	27.51 ± 0.62a	19.33 ± 0.56c
	CK	84.36 ± 0.46b	87.87 ± 0.28a	86.78 ± 0.16c	86.74 ± 0.59b
	FR	84.69 ± 0.16ab	86.48 ± 0.23b	87.58 ± 0.19a	87.36 ± 0.10a
	M ₁	85.13 ± 0.15a	85.90 ± 0.01c	87.16 ± 0.22b	87.21 ± 0.32ab
	M ₂	83.85 ± 0.55bc	85.61 ± 0.37c	86.61 ± 0.02c	86.45 ± 0.29b
M ₃	83.69 ± 0.39c	85.80 ± 0.40c	87.10 ± 0.00b	85.78 ± 0.22c	
M ₄	83.19 ± 0.08c	86.70 ± 0.29b	86.93 ± 0.21bc	85.84 ± 0.24c	

† CK, control; FR, fertilizer nitrogen, phosphorus, potassium; and M₁, M₂, M₃, M₄ denote plots treated with chemical fertilizer in which 20, 40, 60, and 80% of the amount of nitrogen fertilizer was replaced by organic manure, respectively.

‡ Values are means ± standard error (n = 3). Different lowercase letters in the same column denote significant differences at the 0.05 level, within the same aggregate fraction.

The C/N ratio significantly increased in the microaggregates and macroaggregates in the manure-substitution treatments than in the corresponding soil aggregates in CK, but the C/N ratio in the silt + clay fractions changed little after 4 yr of substituting manure for fertilizer. The improvement in the C/N ratio of the large macroaggregates was the most significant than those of the silt + clay fractions, microaggregates, and small macroaggregates in the four manure-substitution treatments.

Labile C in the microaggregates and small macroaggregates varied from 7.13 to 7.83 mg kg⁻¹ and 7.56 to 8.34 mg kg⁻¹, respectively, and the values were higher than those for the silt + clay fractions and large macroaggregates under the same treatment (Table 4). Chemical fertilizer application alone (FR) and manure substitution (M₁, M₂, M₃, and M₄) all observably increased the labile C in the silt + clay fraction. The manure-substitution treatments, M₁, M₂, M₃, and M₄, had a significantly increased labile C ($P < 0.05$). Similar to labile C, alkalyzable N in the four different size aggregates was enhanced significantly ($P < 0.05$) compared to that in CK.

Variations in Stabilities of Organic Carbon and Nitrogen, and Correlation Analysis

The C_S in microaggregate or small macroaggregate in organic manure treatments (M₁, M₂, M₃, and M₄) was significantly higher than that in CK, but not in silt+clay fraction. While, manure-substituted fertilizer practice presented no significant effect on the N_S. The C_S in the microaggregates and small macroaggregates in organic manure treatments (M₁, M₂, M₃, and M₄) was 2.83 to 4.42 and 15.28 to 19.84 times higher, respectively, than that in CK (Table 5). However, in comparison to CK, substituting manure reduced C_S in the silt + clay fractions by 43.61 to 68.85%. But, C_S did not vary significantly in the large macroaggregates under different fertilizer treatments. N_S, which did not show a clear change in the manure substitution treatments, showed a different pattern from that of C_S. The values of N_S were 83.19 to 87.87% across all the tested treatments and presented no significant change after 4 yr of fertilization.

Regression analysis showed that MWD was significantly, positively, and linearly correlated with organic C, labile C, N, and alkalyzable N in the different aggregate-size classes ($P < 0.05$ and 0.01) across the tested treatments (Fig. 1). Furthermore,

Table 6. Contribution (%) of aggregate associated organic C and N to the 0- to 20-cm bulk soil.

Item	Treatment†	Silt+clay fraction Microaggregate		Sum‡	Small macroaggregate (250–2000 µm)	Large macroaggregate (>2000 µm)	Sum§
		(<53 µm)	(53–250 µm)				
Organic C	CK	10.37 ± 1.12a¶	17.67 ± 0.82a	28.04 ± 0.31a	34.34 ± 0.77c	22.89 ± 2.32c	57.23 ± 1.55e
	FR	6.90 ± 1.85b	16.72 ± 0.77ba	23.62 ± 1.08b	38.05 ± 1.14bc	28.88 ± 0.58b	66.94 ± 0.56d
	M ₁	5.22 ± 0.63b	15.38 ± 0.87b	20.60 ± 1.04c	43.67 ± 2.07a	34.51 ± 0.74a	78.18 ± 1.47a
	M ₂	3.28 ± 0.04c	11.58 ± 0.21c	14.86 ± 0.17d	41.59 ± 0.69ab	37.06 ± 1.47a	78.65 ± 0.78a
	M ₃	2.73 ± 0.70c	9.27 ± 0.37d	12.01 ± 0.49e	36.65 ± 1.59c	34.85 ± 1.56a	71.50 ± 0.54c
N	M ₄	2.04 ± 0.28c	7.75 ± 0.32e	9.78 ± 0.56f	40.22 ± 1.21b	35.21 ± 1.66a	75.43 ± 0.5b
	CK	11.09 ± 1.89a	23.04 ± 0.77a	34.14 ± 1.22a	47.34 ± 0.69a	30.5 ± 1.98bc	77.84 ± 2.55b
	FR	7.38 ± 2.59b	18.84 ± 0.45b	26.23 ± 2.14b	43.97 ± 2.21a	28.68 ± 0.43c	72.65 ± 1.78c
	M ₁	5.18 ± 0.69bc	16.94 ± 0.72c	22.12 ± 1.20c	47.34 ± 1.11a	32.29 ± 1.09b	79.63 ± 0.55b
	M ₂	3.64 ± 0.06c	13.24 ± 0.65d	16.88 ± 0.71d	45.74 ± 2.27a	36.38 ± 0.23a	82.12 ± 2.05ab
	M ₃	3.36 ± 0.85c	11.10 ± 0.38e	14.46 ± 0.49e	46.48 ± 3.05a	36.52 ± 0.94a	83.00 ± 2.21a
	M ₄	2.64 ± 0.44c	10.02 ± 0.95e	12.66 ± 1.35e	46.74 ± 1.87a	38.54 ± 1.87a	85.29 ± 0.27a

† CK, control; FR, fertilizer nitrogen, phosphorus, potassium; and M₁, M₂, M₃, M₄ denote plots treated with chemical fertilizer in which 20, 40, 60, and 80% of the amount of N fertilizer was replaced by organic manure, respectively.

‡ Indicates the total contribution of silt + clay fraction and microaggregate C and N to C and N in 0–20cm bulk soil.

§ Indicates the total contribution of small macroaggregate and large macroaggregate C and N to C and N in 0- to 20-cm bulk soil.

¶ Values are means ± standard error (n = 3). Different lowercase letters in the same column denote significant differences at the 0.05 level, within the same aggregate fraction.

regression equations were obtained between MWD and the total organic C, labile C, N, alkalyzable N contents in the (A) silt+clay fraction, (B) microaggregates, (C) small macroaggregates, and (D) large macroaggregates, indicated by A_C , B_C , C_C , D_C , $A_{labile\ C}$, $B_{labile\ C}$, $C_{labile\ C}$, $D_{labile\ C}$, A_N , B_N , C_N , D_N , $A_{alkalyzable\ N}$, $B_{alkalyzable\ N}$, $C_{alkalyzable\ N}$, and $D_{alkalyzable\ N}$ respectively, as follows:

$$MWD = -0.33A_C + 9.10B_C + 5.00C_C - 2.39D_C + 17.63 \quad (R^2 = 0.92, P < 0.001) \quad [1]$$

$$MWD = 1.90A_{labile\ C} - 7.64B_{labile\ C} + 4.83C_{labile\ C} + 8.53D_{labile\ C} + 67.56 \quad (R^2 = 0.86, P < 0.001) \quad [2]$$

$$MWD = 103.08A_N - 48.80B_N + 144.54C_N - 78.65D_N - 16.58 \quad (R^2 = 0.86, P < 0.001) \quad [3]$$

$$MWD = 0.18A_{alkalyzable\ N} + 0.28B_{alkalyzable\ N} + 0.34C_{alkalyzable\ N} + 0.14D_{alkalyzable\ N} - 24.64 \quad (R^2 = 0.91, P < 0.001) \quad [4]$$

Effects of Substituting Manure on the Accumulated Organic Carbon and Nitrogen in Soil Aggregates

Substituting manure practice increased the contribution of macroaggregate associated organic C and N, however, decreased the contribution of silt + clay fraction and microaggregate associated organic C and N to the total organic C and N of topsoil (0–20 cm). The contribution of macroaggregate organic C to the organic C of topsoil (0–20 cm) was generally higher in the plots treated with chemical fertilizer alone and with fertilizer substituted with manure than in CK (Table 6). The total contribution of the small macroaggregate and large macroaggregate organic C in the plots substituted with manure was 71.5 to 78.65%; the silt + clay fraction and microaggregate organic

C contributed to the topsoil organic C by 9.78 to 20.60% in the manure substitution treatments. However, compared to CK, substituting fertilizer with manure reduced the contribution of the silt + clay fraction and microaggregate organic C to topsoil organic C by 49.66 to 80.35% and 12.99 to 56.16%, respectively. Additionally, substituting manure for fertilizer significantly increased the contribution of the large macroaggregate N to the N of bulk soil, in comparison to that in CK, by 5.87 to 26.38%, however, there was no significant change in the contribution of the small macroaggregate associated N to topsoil N in the manure substituted treatments than in CK, and the contributions of the silt + clay fraction and microaggregate N to the N of bulk soil decreased by 53.30 to 76.18% and 26.49 to 56.51%, respectively, compared to that in CK.

The contribution rates of the accumulated net organic C and N in the microaggregates across the manure substitution treatments had negative values, and they did not significantly change in the silt + clay fractions (Fig. 2 and 3), but, compared to FR, the contribution rates of accumulated net organic C and N in microaggregates decreased significantly under the manure-substitution treatments. In addition, in comparison with FR, the manure-substitution treatment M₁ enhanced the contribution rates of accumulated net organic C and N in small and large macroaggregate, although, the contribution rates of accumulated net organic C decreased slightly in the manure substitution treatments M₃ and M₄.

DISCUSSION

After 4 yr of substituting manure for fertilizer, the C and N concentrations linearly increased with the increasing rates of manure application. And, aggregate associated organic C and N increased linearly with substituting manure amount among all the soil aggregate-size classes (Tables 1 and 3). Many studies have also reached similar conclusions. Smith et al. (1997) and Whalen and Chang (2002) also suggested that organic manure not only supplies organic C and N to the soil directly, but also favors soil aggregate stability, due to the binding action of the humic substances and other microbial by-products. Thus, the

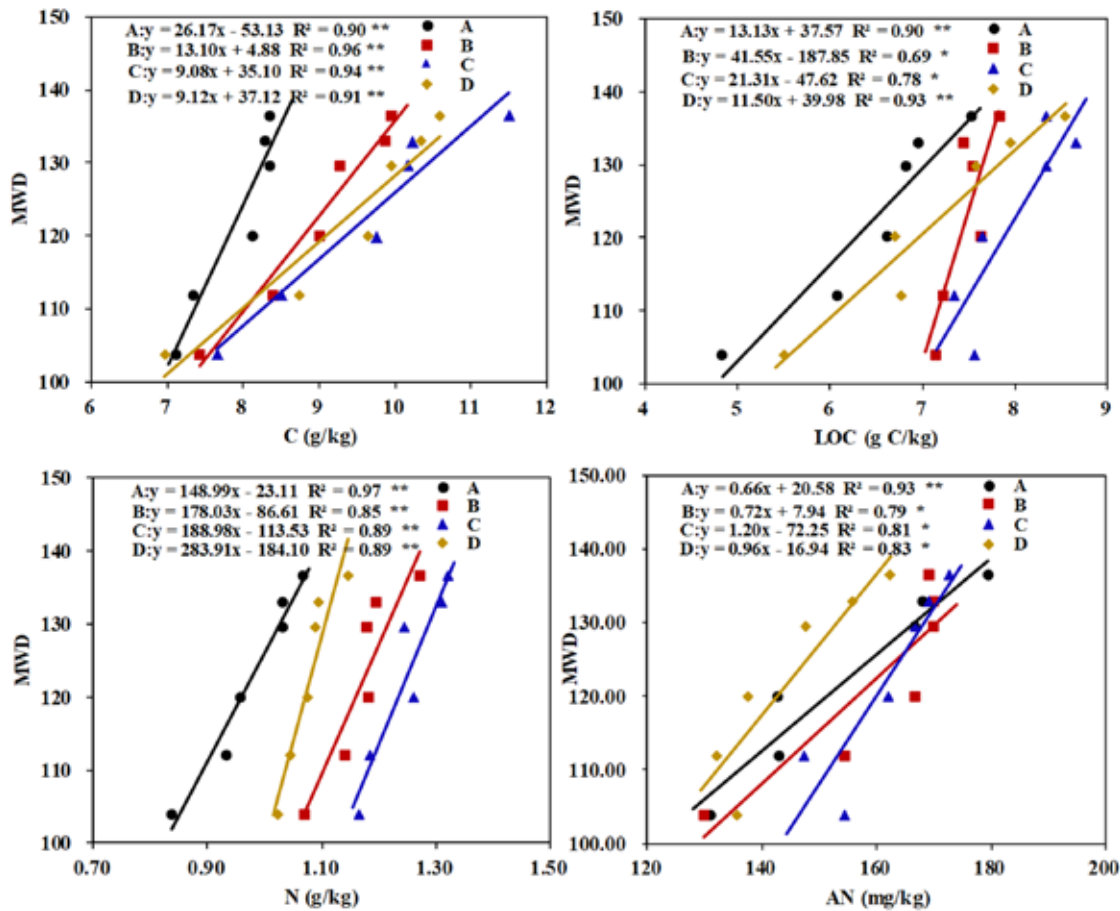


Fig. 1. Correlations analyses between C, N, and mean weight diameter (MWD). A, B, C, and D indicate silt + clay fraction, microaggregate, small macroaggregate, and large macroaggregate, respectively. Equations, followed A, B, C and D, indicate the regression equations between MWD and values of silt + clay fraction, microaggregate, small macroaggregate, and large macroaggregate, respectively. * and ** indicate regression analysis reached significant at the 0.01 and 0.05 level, respectively

source of C and N accumulation might be attributed to the largest input of humic substances and aggregate stability in the tested soil. However, Triberti et al. (2016) pointed out that the organic C sequestrations can be ascribed to the low N content in its roots and stubbles that delays residue decomposition by limiting soil microbial activity. Disagreed with previous reports, we detected that N and alkalyzable N increase in the same pace as organic C and labile C in the soil. This result was likely due to the fact that the application of organic manure inputs organic C substrates and stimulates mineral N immobilization directly, which is helpful to increase the stability of soil structure, and the aggregate stability helps C, N accumulation in turn, because C, N is physically protected from microbial attack (Campbell et al., 2001). What is more, N_C did not increase with the increase of manure application in the tested soil, while C_C decreased significantly (Table 1), which might be explained by the fact that organic manure, supplying highly reactive humus and maintaining high labile C concentration, promotes the proportion of labile C for organic C, where N is entrapped in slowly degradable compounds. The lagging of mineralized N is beneficial to promote the build-up of stable organic C. It may therefore be said that substituting manure for fertilizer strengthened the synergistic effect between organic C and N in the tested soil.

What is more, a faster accumulation of organic C, N and their labile components in small and large macroaggregates than

silt+clay fraction and microaggregates was also observed in the present research (Tables 3 and 4), which might be because that microaggregates are induced to bind together into macroaggregates by organic binding agents after substituting manure for fertilizer and the turnover rate of macroaggregates is slower in the manure-substituted plots than in the control plot (Six et al., 2000b). Correlations analyses between organic C, N and their labile components and MWD also proved that the stability of aggregates was closely related to aggregates associated organic C and N (Fig. 1). Thus, the stability of small and large macroaggregates protected organic C, N and their labile components from erosion physically. When simultaneously considering the increase of labile C and alkalyzable N in aggregates and the variation in relative distribution of aggregate mass in present study, the application of substituting manure for fertilizer enhanced aggregate associated labile C and alkalyzable N, and the increased labile C and alkalyzable N in aggregates following substituting manure for fertilizer was closely associated with the formation of the macroaggregates. However, Yu et al. (2015) declared that only recalcitrant C functional groups were enriched in fertilizer-amended soils and the recalcitrant C functional groups was mainly distributed in macroaggregates. These inconsistencies might be due to organic manure practice, which promoted the buildup of organic matter and entrapped N in slowly degradable compounds by supplying stable humus

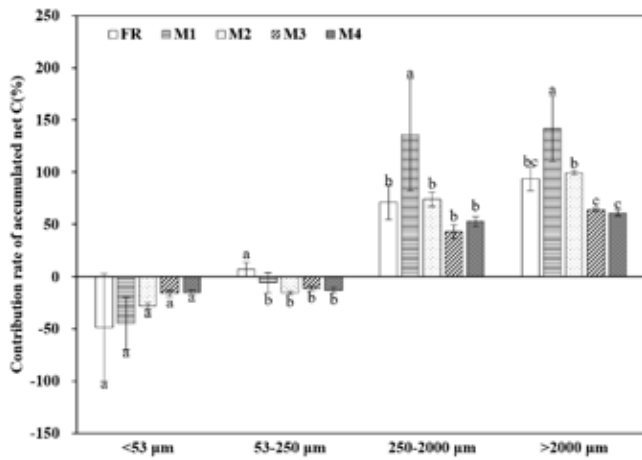


Fig. 2. Contribution rate of accumulated net organic C in various sizes of aggregates to 0- to 20-cm bulk soil after 4 yr substituting manure for fertilizer treatment.

(Triberti et al., 2016). In brief, application of organic manure increases the organic C and N concentrations in two ways: (i) by adding C and N already in the manure, and (ii) by adding organic C and N released from crop residues by increasing the decomposition rates of plant litter, due to higher crop yields in the soils receiving manure (Whalen and Chang, 2002). As a result of a fine dynamic balance, macroaggregates stocked more organic C, N and their labile components than microaggregates, with the help of the physical protection accorded by the macroaggregates (Fig. 2 and 3). Redundancy analysis in our study, relating the mass proportions of water stable aggregations to soil organic C and N contents, also confirmed that macroaggregates had a positive impact on both the bulk soil-associated and aggregate-associated organic C and N (Fig. 4 and 5).

Most studies focus on the effects of organic manure on the aggregate associated organic C, but there are few studies on the C/N ratio in aggregates over the past few decades (Puget et al., 2000; Yu et al., 2015). In our study, the C/N ratio in large macroaggregates was higher than that in microaggregates or silt + clay fractions after 4 yr-old application of substituting manure for fertilizer (Table 3). This result revealed that the level of decomposition of organic C in large macroaggregates

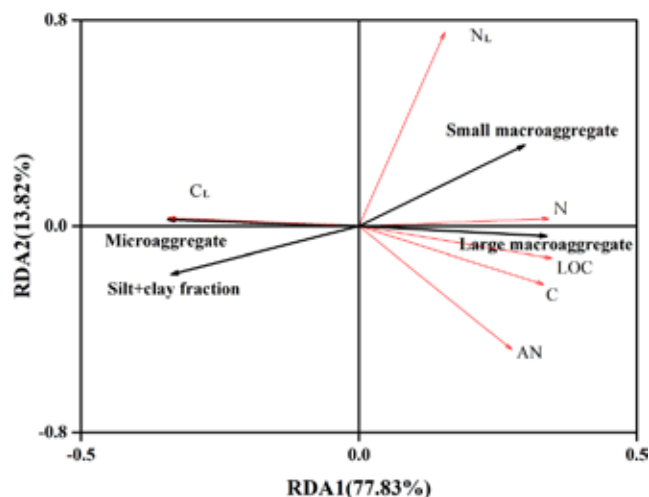


Fig. 3. Contribution rate of accumulated net N in various sizes of aggregates to 0- to 20-cm bulk soil after 4 yr substituting manure for fertilizer treatment.

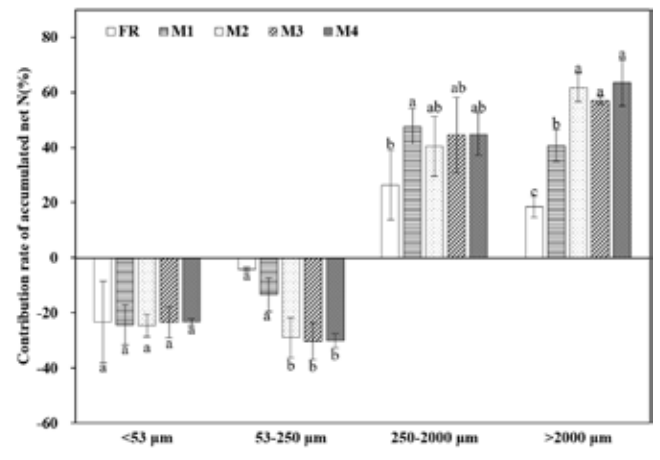


Fig. 4. Redundancy analysis (RDA) relating mass proportion of water stable aggregates to organic C, N concentration in bulk soils in the 0- to 20-cm layers.

was lower than that in microaggregates or silt + clay fractions (Springob and Kirchmann, 2003). According to the schematic composition of aggregates proposed by Puget et al. (2000), large macroaggregates were formed by the binding of microaggregates by additional young organic C because that young organic C was preferentially incorporated and was responsible for large macroaggregates. Therefore, it can be deduced that more organic C with low decomposition degree were involved in the formation of large macroaggregates. Furthermore, organic manure can release large quantities of organic C and N for soil microorganisms, which produce extracellular polysaccharides known to flocculate soil minerals into aggregates. And, polysaccharides and other aliphatic and aromatic compounds brought by organic manure could bind soil particles and organo-mineral complexes to form macroaggregates (Lee, 2010; Tisdall and Oades, 1982). As a result, the application of substituting manure for fertilizer might accelerate the formation of small and large macroaggregates through altering the supply of organic C and N.

Under increasing intensive cultivation, the decreased stability of water stable aggregations is paralleled by the reductions

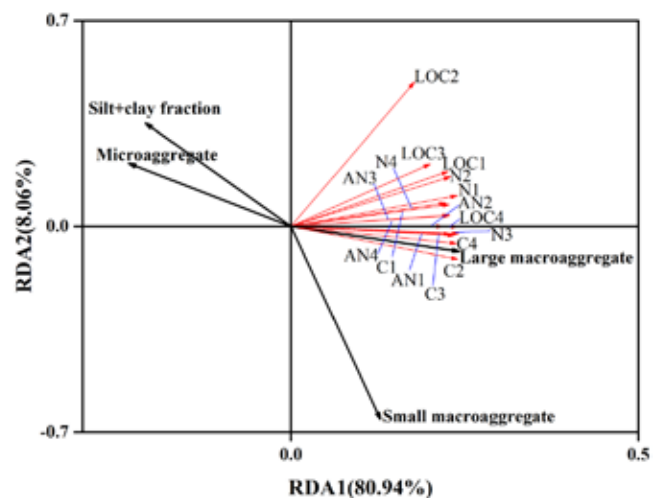


Fig. 5. Redundancy analysis (RDA) relating mass proportion of water stable aggregation to aggregate associated organic C, N concentration in 0- to 20-cm bulk soils. The numbers 1, 2, 3, and 4 behind the letters represent silt+clay fraction, microaggregate, small macroaggregate, large macroaggregate, respectively.

in organic C concentrations and increases in the proportions of microaggregates. Consequently, a greater proportion of the soil mass is at a risk of physical breakage, following many years of intense, continuous tillage. Healthy and sustainable soils will be increasingly threatened (Six et al., 1999). Fortunately, organic C and labile C, as the basic indicators of soil fertility, were found to be generally greater for the macro- than microaggregates, in the plots treated with manure substituted for fertilizer, N and alkalyzable N, as sources of soil activation, were always the highest in all aggregate-size classes, following the substitution of manure for fertilizer. Macroaggregate associated organic C was more abundant and more stable than microaggregate associated organic C, under the manure substitution treatment. These findings suggest that the application of manure-substituted fertilizer is beneficial to build up better soil structure for reserving more nutrition in comparison to traditional fertilization.

CONCLUSION

Macroaggregates play a key role in organic C and N accumulation in our tested soils. Substituting manure for fertilizer significantly increased the macroaggregates formation and the contribution of macroaggregates to organic C and N accumulation at the 0- to 20-cm depths. As a result, the formation of soil macroaggregates improved total organic C content level and strengthen the stability of organic C in soil following manure-substituted fertilizer practice. The magnitude of contribution from the increased amounts of organic C and N in aggregates varied with fertilizer practices. The accumulation of organic C and N in macroaggregates might be derived by the increase mount manure-substituted fertilizer. Therefore, it is recommended that substituting manure for fertilizer should be adopted as one of the optimal components in an overall strategy to promote soil macroaggregate formation and organic C and N sequestration in the North China Plain.

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