Variation of organic carbon and nitrogen in aggregate size fractions of a paddy soil under fertilisation practices from Tai Lake Region, China

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Abstract: Long-term changes in agricultural management can affect soil organic carbon (SOC) and nutrient dynamics, which can be monitored by determining the distribution of microbial activity and nutrient pools in soil aggregates of different size fractions. The objective of the present study was to investigate the changes in SOC and total nitrogen (N_t) distribution in aggregates of different size fractions from a paddy soil (Ferric-accumulic Stagnic Anthrosols) under a long-term fertilisation trial. Undisturbed samples of topsoil (0–5 and 5–15 cm depths) were collected from a field experiment farm located in Tai Lake Region, China, with the plots receiving no fertiliser (NF), chemical fertilisers (CF), chemical fertilisers with straw return (CFS) or chemical fertilisers plus pig manure (CFM). In the surface layer, SOC and N_t concentrations appeared as a bimodal peak in the 2000–250 and <2 µm fractions. SOC concentration increased by 38.6, 40.8 and 17.2% and N_t concentration by 30.0, 16.8 and 38.4% in the 2000–250 µm fractions under CFM, CFS and CF respectively as compared with NF treatment. There were slight changes in SOC and N_t in the <2 µm fractions from different fertilisation plots. Continuous addition of manure or straw increased storage of SOC and N_t mainly in the coarser aggregate fractions. SOC increases due to straw or pig manure application predominated in the 2000–250 µm fractions, with SOC seeming to be physically protected within macro-aggregates. Thus straw and manure are likely to play an important role in carbon and nitrogen storage in paddy soil under long-term combined chemical and organic fertilisation.

Keywords: aggregate; fertilisation practice; paddy soil; size fractions; soil organic carbon; total nitrogen

INTRODUCTION

In recent years, much attention has been focused on the potential of agricultural soils to mitigate the increase in atmospheric CO_2 through sequestration of carbon.[1–3] Soils can act as a source or sink of atmospheric CO_2, largely depending on the balance of soil organic carbon (SOC) formation and decomposition.[4] Biophysical processes associated with SOC fractionation and stabilisation in soil aggregates have been widely studied. Soil aggregate has been considered as the basic unit of soil entity in mediating mineral–organic interactions of a number of soil biophysical and biochemical processes at microscale, which are linked to decomposition and transformation of SOC through binding to various agents.[5,6] Hence carbon sequestration in soils involves storage and stabilisation of organic carbon compounds among various aggregate size fractions with regard to the turnover behaviour of SOC.[6–8] Physical protection of SOC associated with silt and clay particles in micro- and macro-aggregates has been investigated recently owing to the enhanced chemical resistance to decomposition. Some studies have shown that organic matter (OM) within soil aggregates has a lower decomposition rate than OM outside aggregates.[6,9] Several works have already evidenced that aggregate-occluded particulate organic matter (POM) has a slower turnover rate than OM protected by mineral affiliation.[10] In addition, aggregates of different sizes differ in microbial biomass and diversity, which may be linked to the varying degree of carbon accumulation and turnover in these size fractions.[11] Thus SOC variation in soil aggregates may be a crucial issue for better understanding the effect of soil management practices on carbon dynamics and sequestration in agricultural soils.[12]

In agricultural systems worldwide, organic manure and inorganic synthetic chemical fertilisers are commonly used for increasing crop productivity as well as improving the nutritional quality of food. Crop fertilisation and manuring generally increase OM input either through higher crop biomass residues or by direct addition of agricultural wastes such as animal manure.[13] Studies on changes in carbon and nitrogen distribution in different aggregate size fractions under

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different fertilisation practices have been documented for dry croplands of Europe and the USA.[14–16]

Paddy soils belong to a unique group of anthropogenic soils with a long history of rice cultivation under irrigation in China and have higher carbon sequestration potential than their dryland counterparts.[17,18] Tai Lake Region, where paddies dominate soil/land use, has played an important role in rice production and carbon sequestration as well as in the economic development of Jiangsu Province, China.[19] In the last two decades, however, chemical fertilisation has intensified to meet the demand for high yields; as a result, there has been a declining trend in manure application.[20] The objective of the present study was to determine the long-term effects of mineral fertilisation alone and combined mineral and organic fertilisation on SOC and total nitrogen (N_t) distribution within aggregate size fractions in a paddy soil from Tai Lake Region, China.

MATERIALS AND METHODS

Site description and experimental design

The soil for this study was collected from a long-term fertiliser experiment under continuous rice/rape rotation initiated in 1987 and located in Jinjiaba Township (31° 5′N, 120° 46′E), Wujiang Municipality, Suzhou City, Jiangsu Province, China. The soil was classified as Ferric-accumulic Stagnic Anthrosols.[17] It contained 302.9 g kg^{-1} of <2 µm clay and had a cation exchange capacity (CEC) of 20.5 cmol(+) kg^{-1} at 0–15 cm.

Fertiliser treatments were applied as listed in Table 1. The manure used in this study was from a pig feedlot. The manure and rice straw contained, on average, 134.8 g C, 5.2 g N and 2.1 g P kg^{-1} and 382.2 g C, 7.6 g N and 1.3 g P kg^{-1} respectively on a dry weight basis. Rice straw or manure was applied before rape transplantation. Mineral fertilisers were applied before planting rape or rice each year. No tillage was performed after rice harvest until rape harvest. Treatments were applied in a randomised block design with three replications. The treatments influenced soil nutrient pool, soil bulk density as well as soil pH. While combined fertilisation treatments enhanced both soil C and N contents, a significant increase in soil P content was observed only under CFM as a result of input from pig manure. However, SOC accumulation was not significant under CF alone.

Undisturbed soil samples were collected after rice harvest in November 2001 from 0–5 and 5–15 cm depths of each treated plot using an Eijkelkamp (Gelderland, The Netherlands) soil core sampler. All samples were stored in stainless cans at 4 °C prior to analysis.

Aggregate size fraction separation

Low-energy ultrasonic dispersion was used to obtain aggregate size fractions of the test soils. Bulk soil (50 g) was dispersed in distilled water (500 mL) using a tank disaggregator at ∼80 J mL^{-1}, following a procedure similar to that described by Tarchitzky *et al.*[21] The >2000 and 2000–250 µm fractions were obtained by wet sieving, while the <2 µm fraction was obtained by centrifugation. The other fractions (250–20 and 20–2 µm) were obtained by sedimentation. Finally, all samples of separated particle fractions were dried at 50 °C using infrared radiation.[22]

Table 1. Fertiliser treatments on studied paddy soil since 1987 (kg ha^{-1})

Treatment	Manure	Straw	N	P_2O_5	KCl	Chemical fertiliser ($N:P_2O_5:K_2O$)
NF	–	–	–	–	–	–
CF	–	–	427.5	45.0	54.0	1:0.11:0.20
CFS	–	4500	427.5	45.0	54.0	1:0.11:0.20
CFM	16 800	–	427.5	45.0	54.0	1:0.11:0.20

NF, no fertiliser; CF, chemical fertilisers; CFS, chemical fertilisers with straw return; CFM, chemical fertilisers plus pig manure.

Analytical methods

SOC content was determined by the wet combustion method,[23] N_t content by the Kjeldahl method[24] and free iron oxyhydrate content by the dithionite/citrate/bicarbonate (DCB) extraction method.[25] Carbohydrate content was determined by the following procedure. A 1 g subsample of a single particle size fraction (in duplicate) was first hydrolysed by shaking for 16 h with 10 mL of 0.25 mol L^{-1} H_2SO_4 solution in a rotary shaker. Interfering ions in the hydrolyte were reduced by elution through anion and cation exchange resins.[26] The carbohydrate content in the hydrolyte was measured by colorimetry as glucose equivalents using the phenol/H_2SO_4 method.[27]

Statistical methods

Statistical procedures were performed with the software package SPSS for Windows (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was used to compare differences among soil fractions and differences among soil fertilisation plots.

RESULTS

Aggregate size fraction distribution

Some basic physical and chemical properties of the topsoil under the different treatments are listed in Table 2. The distribution of particle size fractions of the treated plots is shown in Table 3. The aggregate size fraction distribution was significantly ($P < 0.05$) influenced by fertiliser treatments. Aggregates of size 20–2 µm were the dominant fraction, accounting for ∼35 and ∼40% of the total soil at 0–5 and 5–15 cm depths respectively, followed by aggregates of size 250–20, 2000–250, >2000 and <2 µm. The proportion of aggregates of size 2000–250 µm increased and

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that of aggregates of size <20 μm decreased in the surface layer compared with the subsurface layer. CFM and CFS treatments significantly increased aggregates of size 2000–250 μm at 0–5 cm depth as compared with NF treatment. However, fertiliser treatments had hardly any effect on the aggregate distribution at 5–15 cm depth.

Soil organic carbon and total nitrogen concentrations in aggregate size fractions

SOC concentrations in the aggregate size fractions are given in Table 4. The SOC concentration in both surface and subsurface samples decreased in the following order: 2000–250, <2, >2000, 250–20 and 20–2 μm. SOC in aggregates of different sizes was significantly affected by fertiliser treatments. The greatest variation in SOC was found in the 2000–250 μm fractions of different treatment plots. At 0–5 cm depth, SOC increased by 38.6, 40.8 and 17.2% under CFM, CFS and CF respectively as compared with NF treatment. Compared with NF treatment, SOC in the 250–20 μm fraction increased significantly by 13.8% under CFM. Furthermore, at 5–15 cm depth, SOC in the 2000–250 μm fraction increased significantly by 6.0% under CFM and that in the 250–20 μm fraction increased by 13.1% under CFS as compared with NF treatment. There appeared to be no significant differences in SOC in other aggregate size fractions among the different treatments. Changes in carbohydrate concentration in aggregate size fractions from different treatment plots at 0–5 cm depth are shown in Fig. 1. There were hardly any differences in carbohydrate concentration in different size fractions between NF and CF treatments. However, CFS and CFM treatments significantly increased the carbohydrate concentration in the 2000–250 μm fraction by 82.9 and 55.0% respectively.

As shown in Table 5, the variation pattern of N_t in aggregate size fractions was different from that of SOC. Generally, at both 0–5 and 5–15 cm depths

Table 2. Basic properties of treated plots at different depths of topsoil

Treatment	Depth (cm)	pH (H_2O)	Bulk density (g cm^{-3})	SOC (g kg^{-1})	N_t (g kg^{-1})	P_t (g kg^{-1})
NF	0–5	6.13	1.17	16.12	1.72	0.243
	5–15			15.86	1.14	0.300
CF	0–5	5.93	1.18	16.57	1.80	0.369
	5–15			14.83	1.54	0.308
CFM	0–5	5.74	1.14	17.00	2.05	0.718
	5–15			16.26	1.58	0.511
CFS	0–5	5.88	1.18	16.95	2.06	0.371
	5–15			15.08	1.59	0.352

P_t, soil total phosphorus.

Table 3. Distribution of soil particle size fractions from treatment plots of paddy soil (%)

Treatment	>2000 μm	2000–250 μm	250–20 μm	20–2 μm	<2 μm
0–5 cm					
NF	10.55 ± 0.73b	20.15 ± 1.61b	21.25 ± 0.53a	39.15 ± 1.93a	8.61 ± 0.99a
CF	14.70 ± 0.73a	18.87 ± 0.92b	21.97 ± 1.28a	35.27 ± 0.77b	7.81 ± 1.01a
CFS	10.42 ± 0.69b	23.48 ± 0.69a	22.05 ± 1.07a	35.40 ± 0.67b	8.38 ± 0.77a
CFM	13.06 ± 1.38a	23.62 ± 1.38a	23.09 ± 0.41a	31.21 ± 1.20c	8.81 ± 0.15a
5–15 cm					
NF	10.99 ± 1.06a	10.42 ± 1.36b	21.21 ± 2.73b	42.49 ± 1.42a	11.27 ± 0.65b
CF	9.44 ± 2.39a	11.51 ± 1.26b	24.04 ± 0.36a	42.25 ± 2.84a	12.32 ± 0.76b
CFS	10.21 ± 1.23a	11.97 ± 1.73b	23.97 ± 0.98a	44.03 ± 2.04a	9.70 ± 0.29c
CFM	6.49 ± 0.89b	14.11 ± 1.26a	21.09 ± 0.18b	40.02 ± 1.66b	14.03 ± 0.30a

Comparison between means was made with the LSD test. Within each column, means followed by different letters are significantly different at $P < 0.05$.

Table 4. SOC contents in particle size fractions of paddy soil under different fertilisation treatments (g kg^{-1})

Treatment	>2000 μm	2000–250 μm	250–20 μm	20–2 μm	<2 μm
0–5 cm					
NF	18.05 ± 0.92a	19.17 ± 0.53c	14.96 ± 0.13b	14.22 ± 0.64a	19.46 ± 0.42a
CF	17.32 ± 1.01a	22.47 ± 0.42b	15.83 ± 1.19b	13.82 ± 0.47a	20.08 ± 0.49a
CFS	17.26 ± 0.74a	27.00 ± 0.44a	16.12 ± 0.65ab	14.77 ± 0.96a	20.49 ± 0.89a
CFM	17.54 ± 1.12a	26.57 ± 0.72a	17.03 ± 0.32a	14.70 ± 0.20a	20.50 ± 0.60a
5–15 cm					
NF	16.17 ± 0.57b	27.35 ± 0.38b	13.35 ± 0.73b	12.99 ± 0.68a	17.88 ± 0.30a
CF	12.94 ± 0.28c	25.57 ± 0.98c	13.51 ± 1.65b	13.20 ± 0.44a	18.29 ± 1.23a
CFS	15.65 ± 0.75b	27.63 ± 0.42ab	15.10 ± 1.04a	13.82 ± 0.34a	18.38 ± 1.06a
CFM	18.66 ± 0.98a	29.00 ± 0.66a	13.77 ± 0.72ab	13.35 ± 1.16a	17.54 ± 1.08a

Comparison between means was made with the LSD test. Within each column, means followed by different letters are significantly different at $P < 0.05$.

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Table 5. N_t contents in particle size fractions of paddy soil under different fertilisation treatments (g kg^{-1})

Treatment	>2000 µm	2000–250 µm	250–20 µm	20–2 µm	<2 µm
0–5 cm					
NF	1.75 ± 0.11a	1.90 ± 0.05c	1.39 ± 0.05b	1.64 ± 0.07a	2.40 ± 0.05b
CF	1.74 ± 0.07a	2.63 ± 0.20a	1.61 ± 0.14a	1.65 ± 0.09a	2.69 ± 0.11a
CFS	1.89 ± 0.11a	2.22 ± 0.12b	1.64 ± 0.10a	1.65 ± 0.08a	2.72 ± 0.20a
CFM	1.85 ± 0.06a	2.47 ± 0.13b	1.79 ± 0.14a	1.59 ± 0.11a	2.82 ± 0.16a
5–15 cm					
NF	1.70 ± 0.05a	1.86 ± 0.09b	1.30 ± 0.13b	1.60 ± 0.11a	2.20 ± 0.05b
CF	1.51 ± 0.12a	2.05 ± 0.17b	1.20 ± 0.09b	1.35 ± 0.19b	2.30 ± 0.05b
CFS	1.59 ± 0.20a	2.61 ± 0.11a	1.50 ± 0.15a	1.59 ± 0.08a	2.50 ± 0.14a
CFM	1.53 ± 0.17a	2.61 ± 0.16a	1.40 ± 0.13a	1.37 ± 0.11b	2.30 ± 0.19b

Comparison between means was made with the LSD test. Within each column, means followed by different letters are significantly different at $P < 0.05$.

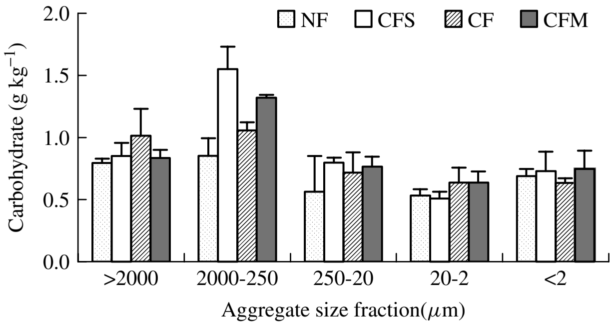

Figure 1. Carbohydrate content in aggregate size fractions at 0–5 cm depth from treated plots.

the <2 µm fraction had the highest N_t concentration, followed by the 2000–250 µm fraction, while the 250–20 µm fraction had the lowest concentration of N_t. Although the N_t concentration in all size fractions from the NF plot was significantly lower than in those from the fertilised plots, significant variation of N_t with fertilisation treatments was detected only in the 2000–250 µm fraction from the surface soil. However, in the subsoil, significant differences in N_t among different treatments were found in all size fractions except the coarsest (>2000 µm). Compared with the N_t variation of size fractions from the surface soil, CFM and CFS treatments tended to increase N_t in the 2000–250 and 250–20 µm fractions.

Figures 2–5 show the amount (g kg^{-1} soil) and storage (% of bulk soil) of SOC and N_t in different size fractions. In the surface layer the highest amounts of SOC and N_t were found in the 2000–250 µm (from 3.86 to 6.34 g C kg^{-1} and from 0.38 to 0.58 g N kg^{-1}) and 20–2 µm (from 4.59 to 5.57 g C kg^{-1} and from 0.50 to 0.64 g N kg^{-1}) fractions (Figs 2 and 3). The contributions of SOC in the 2000–250 µm fraction to the bulk soil were 34.4 and 34.5% and those of N_t were 30.7 and 27.9% under CFM and CFS respectively (Figs 4 and 5). In the subsurface layer the 20–2 µm fraction had the highest amount of SOC (from 5.34 to 6.08 g C kg^{-1}) among all size fractions under different treatments (Fig. 2). Significant increases in SOC and N_t storage occurred in the 2000–250 µm fraction only under CFM (Figs 4 and 5).

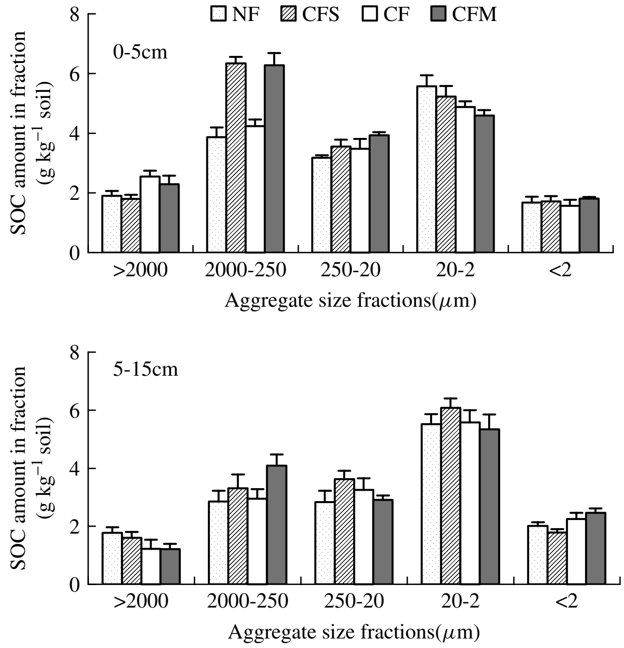

Figure 2. SOC amount in aggregate size fractions from treated plots.

DISCUSSION

Our results showed that combined application of chemical fertilisers with organic supplements of manure and straw increased the proportion of aggregates larger than 250 µm in size, particularly the 2000–250 µm fraction (Table 3). Angers and N'Dayegamiye[28] and Aoyama *et al*.[29,30] reported that the proportion of water-stable macro-aggregates (>250 µm) increased in soils that received cattle manure applications annually for more than a decade, as input residues enhanced macro-aggregate formation via enhancement of microbial activity and accumulation of binding agents.[10,31] Organic residues could be a catalyst for microbial activity and induce binding of soil particles into macro-aggregates.[8,32] The surface layer of the studied paddy soil had a higher proportion of macro-aggregates (>250 µm) and a lower proportion of micro-aggregates (<250 µm) than the subsurface soil, because under minimum tillage the accumulation of manure and straw occurs in the surface soil.

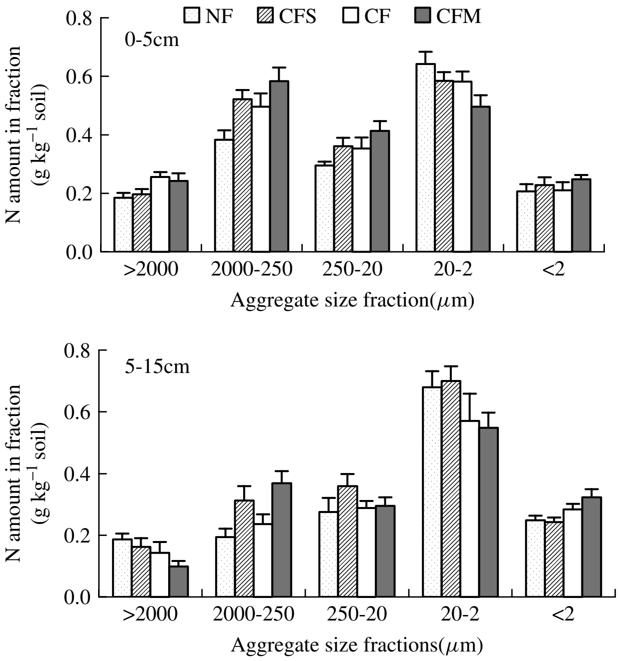

Figure 3. Total N amount in aggregate size fractions from treated plots.

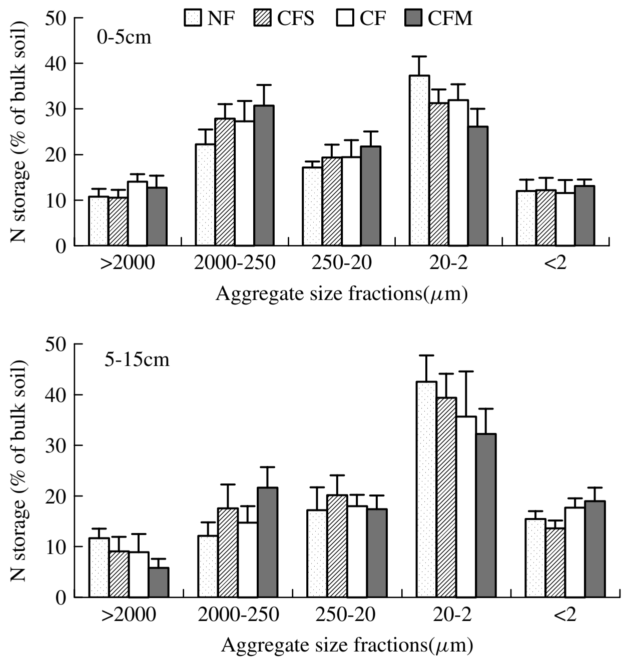

Figure 5. Total N storage by aggregate size fractions (total N contents of bulk soil under NF, CF, CFS and CFM were 1.72, 1.82, 1.87 and 1.89 g kg^{-1} at 0–5 cm depth and 1.68, 1.68, 1.78 and 1.75 g kg^{-1} at 5–15 cm depth respectively).

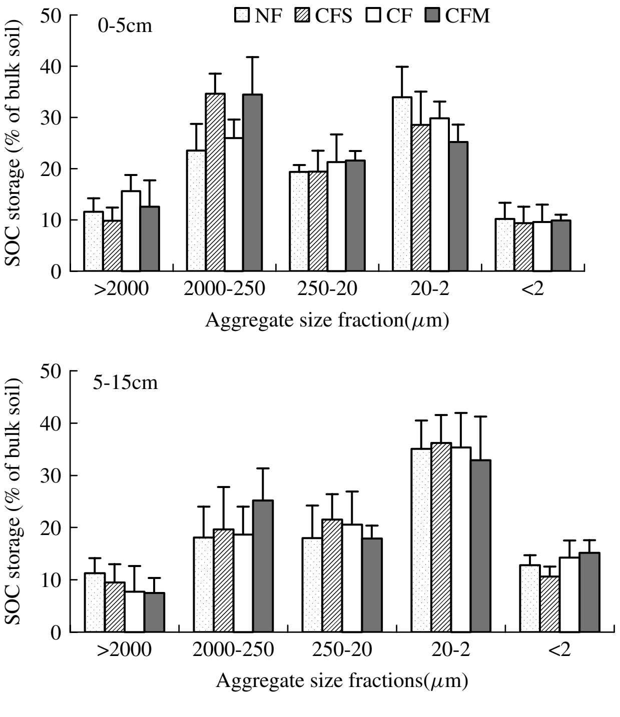

Figure 4. SOC storage by aggregate size fractions (SOC contents of bulk soil under NF, CF, CFS and CFM were 16.40, 16.32, 18.30 and 18.21 g kg^{-1} at 0–5cm depth and 5.73, 15.78, 16.81 and 16.24 g kg^{-1} at 5–15 cm depth respectively).

Compared with their concentrations in bulk soil, SOC and N_t in the paddy soil were much enriched in the 2000–250 and <2 µm fractions. Fourteen years of continuous addition of manure and straw greatly increased the amount of SOC and N_t in macro-aggregates, especially in the 2000–250 µm fraction. In contrast, application of compound mineral fertilisers increased the pool of macro-aggregate-protected N but had hardly any effect on macro-aggregate-protected C. Similar studies by Six *et al.*,[6] Puget *et al.*[8] and Singh and Singh[33] also showed that higher contents of SOC and N were generally associated with macro-aggregates. Aoyama *et al.*[29] concluded that manure application increased the pools of protected C and N located in small macro-aggregates of size 1000–250 µm. The increase in SOC and N_t preferentially in the 2000–250 µm fractions under CFS and CFM observed here may be attributed to the annual input of fresh straw residue and pig manure. This is similar to the findings of Six *et al.*[6] and Spaccini *et al.*[34] that greater amounts of fresh plant materials were enriched in larger aggregates under OM input. It has been described that higher carbohydrate content in macro-aggregates or sand-sized particles is mainly due to plant residue decomposition.[35,36] In the present study the carbohydrate content was much higher in the 2000–250 µm fractions under annual addition of pig manure and straw (Fig. 1), which could be an indicator of younger OM being preferentially associated with macro-aggregates. Relative high concentration of OM in the clay fractions could be attributed to the fact that clay minerals have large specific surfaces susceptible to reactive OM from root exudates or microbial metabolites.[34] Thus SOC in the clay fractions was likely to be less influenced by fertilisation treatments than that in the coarser fractions of the paddy soil (Tables 4 and 5). On the other hand, the results also showed that the clay-sized fraction was more enriched in N_t than the 2000–250 µm fraction. This effect may be partly attributed to adsorption of ammonium by clay minerals.

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Increased storage of SOC and N_t under continuous addition of manure and straw was found mainly in the coarse fractions. A significant positive linear correlation was found between SOC in bulk soil and that in the 2000–250 µm fraction (Fig. 6), which indicated that SOC enhancement in bulk soil under fertiliser treatments was dominated by the increase in SOC protected within macro-aggregates of size 2000–250 µm. Hence the 2000–250 µm fraction could be regarded as a sensitive indicator of soil C storage changes under fertilisation practices. In contrast to the study of Leinweber and Schulten,[37] in which the composition and stability of OM were shown to be significantly influenced by pedogenic oxides, no significant correlation was observed between SOC content and DCB-extractable Fe in the 2000–250 µm fraction in the present work (Fig. 7). Thus binding with iron oxyhydrates could not account for the enhanced SOC storage in macro-aggregates in the present study. Many studies have demonstrated that micro-aggregates are bound together by young OM into larger macro-aggregates and that OM associated with macro-aggregates is more labile than that associated with micro-aggregates.[6,10,38] Six *et al.*[12] found that the capacity of micro-aggregates occluded within macro-aggregates to protect POM C in the longer term was crucial for SOC sequestration in forested systems. Maysoon and Rice[39] also showed that manure application increased macro-aggregate-protected labile C and N as compared with mineral fertiliser. It seems that physical protection of labile C in macro-aggregates is a dominant factor for SOC enhancement and thus for C sequestration in paddy soils.

In conclusion, physical protection of SOC in macro-aggregates can be a mechanism for the enhancement of SOC in a paddy soil under combined application of chemical and organic fertilisers. Manure and straw application to the paddy soil contributed to the accumulation of macro-aggregate-protected C and N, whereas mineral fertilisation increased only macro-aggregate-protected N. The mechanism of protection of SOC in macro-aggregates plays an important role in C and N storage, and long-term manure and straw application can improve this process in paddy soils.

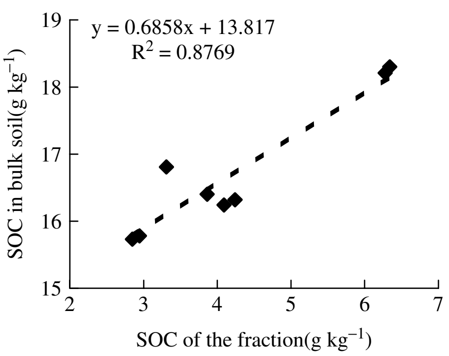

Figure 6. Correlation of SOC of bulk soil with SOC of 2000–250 µm fraction from all plots.

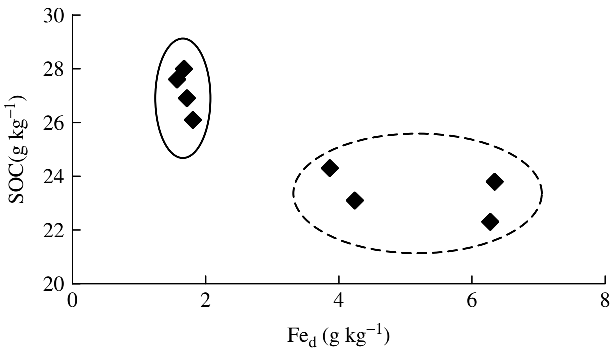

Figure 7. Correlation of SOC with DCB-extractable Fe (Fe_d) in 2000–250 µm fraction at 0–5 cm depth (broken ellipse) and 5–15 cm depth (full ellipse).

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