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# Soil aggregates response to tillage and residue management in a double paddy rice soil of the Southern China

Zhongdu Chen · Jin-song Ti · Fu Chen

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Abstract Soil aggregate stability is a key indicator of soil quality and environmental sustainability of agroecosystems. The protection of organic material within aggregates against microbial decomposition is regarded as an important process in soil organic carbon stabilization but detailed knowledge about this process is still lacking. The objective of our study was to examine the multiple year effects of plow tillage with residue removed (PT0), plow tillage with residue incorporation (PT), rotary tillage with residue retention (RT), and no-till with residue retention (NT) on soil water stable aggregates (WSA) under a double rice (Oryza sativa L.) cropping system in the Southern China. Results showed that the NT system increased the proportion of >2 mm aggregate fraction, and reduced the proportion of <0.053 mm aggregates at 0-5 cm depth in 2011. Compared with PT0 and PT,

Z. Chen

Center for Resources, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China

## J. Ti

#### F. Chen (🖂)

significantly higher large macroaggregate (>2 mm) associated-C contributions to TOC were observed in the surface layer (0–10 cm depth) under RT and NT. A significant positive correlation between TOC and macroaggregate (>2 and 2–0.25 mm) associated-C was observed at 0–20 cm soil depth in the paddy rice ecosystem. Therefore, conversion to NT, could enhance the formation of stable macroaggregate, macroaggregates associated-C, and total C contents in paddy soil of Southern China.

**Keywords** Soil organic carbon · Water stable aggregate · Conservation tillage · Residue retention

## Introduction

Soil aggregates are the arrangement of soil particles of different sizes joined by organic and inorganic materials, and their stability can be used as an index of soil structure (Bronick and Lal 2005). Soil aggregates physically protect soil organic carbon (SOC) from its degradation by soil microorganisms (Beare et al. 1994; Tisdall and Oades 1982). Interactions between SOC and soil structure significantly influence SOC sequestration in farmland ecosystems (Gupta Choudhury et al. 2014). The protection mechanism of soil aggregation may explain the effect of SOC sequestration and decomposition (Pan et al. 2008).

College of Agronomy and Biotechnology, Key Laboratory of Farming System, Ministry of Agriculture of China, China Agricultural University, Beijing 100193, China e-mail: tijinsong@cau.edu.cn

Key Laboratory of Farming System, Ministry of Agriculture of China, Beijing 100193, China e-mail: chenfu@cau.edu.cn

Soil management practices (e.g., tillage, fertilization, and wet-dry cycles), which directly affect soil physicochemical and microbiological properties, and impact the ability of soil aggregates and SOC content (Beare et al. 1994). PT generally destroys polymerization of clods, and reduces the formation and stability of macroaggregates, thereby exposing previously protected organic matter to microbial attack (Jiang and Xie 2009). In contrast, no-till (NT) greatly reduces the macroaggregate turnover rate and promotes the formation of C-enriched microaggregate within macroaggregates (Six et al. 2000). Furthermore, continuous NT practice can enhance the aggregate stability and increase total organic carbon (TOC) (Beare et al. 1994). Plaza-Bonilla et al. (2010) reported a strong relationship between increasing water-stable macroaggregates and SOC concentrations, with 1, 4, 11, and 20 years of NT practice in a Mediterranean semiarid agroecosystem. Similar results were also reported by Bhattacharyya et al. (2009), who indicated that continuous NT was favorable for increasing SOC in macroaggregate. Compared to the microaggregates, the macroaggregates contain mainly fresh labile SOC, and the turnover rates of SOC are therefore much faster (Gupta Choudhury et al. 2014). The use of tillage in crop production decreases the stability of soil and the decomposition of crop residues have also been shown to influence soil aggregate formation and stabilization (Lynch and Bragg 1985). Several researchers have reported that addition of readily available substrate causes a rapid stimulation of the soil microflora and this is accompanied by an increase in aggregate stability (Verhulst et al. 2011). Thus, it is crucial to understand the aggregate and its associated-C dynamics for SOC sequestration and soil quality enhancement in the tillage and residue management.

It has been reported that paddy ecosystem with long-term waterlogging can suppresses microbial respiration rate and reduce microbial biomass, eventually slow down the decomposition of soil aggregate (Pan et al. 2008). Aggregate stability corresponds to the ability of an aggregate not to break up into smaller fragments (Algayer et al. 2014). The strong aggregate stability of paddy soil enhances the SOC conservation and the enrichment of SOC in macroaggregates, resulting in a higher carbon (C) sequestration potential in paddy soil (Pan et al. 2008; Wissing et al. 2013). Li et al. (2000) future reported that soil water stable aggregates (WSA, 0.25–2 mm) was the main carrier of SOC in paddy soils. Whereas, some studies also indicate that alternate dry-wet process in paddy soils can generally cause macroaggregate disruption paralleled by enhanced decomposition of SOC (Van Veen and kuikman 1990). Therefore, understanding the characteristic of soil aggregates in paddy soils is critical to mainining soil quatiliy and enhancing SOC sequestration capicity.

Southern paddy fields refer to the region south of the Yangtze River, which is the dominant rice growing region of China. Expectedly, PT was the dominant tillage in traditional farming. Although PT has greatly contributed to China's historic crop production, it requires numerous steps prior to sowing, such as residue removal, plow tillage, harrowing, land leveling, and seeding. Indeed, the complex process adversely affects soil and the environment by accelerating erosion, reducing aggregation, increasing SOC loss, decreasing profit and prolonging the time involved (Zhang et al. 2014). Thus, conversion to CT systems could have both resource and environmental benefits, such as increasing soil aggregation and physical stabilization of SOC (Wang et al. 2011). However, there is lack of information regarding continuous use of NT on soil aggregation and SOC protection in paddy rice systems. Therefore, it is important to understand soil aggregates response to different tillage/residue-management practice in paddy soils.

Thus, the objectives of study were to assess (1) the impact of tillage systems on aggregate size distribution and aggregate stability indices; and (2) temporal changes in the distribution of SOC in different WSA fractions under diverse tillage and residue management in the double paddy rice (*Oryza sativa* L.) of the Southern China.

# Materials and methods

### Site description

The experimental site was located at a farm in Ningxiang County, Hunan Province, China (28°07′N, 112°18′E). Ningxiang County is located in the middle reaches of the Yangtze Plain at an elevation of 36.1 m above sea level. This region represents a typical subtropical agricultural region in central South

China, with an average annual precipitation of 1358.3 mm and average annual temperature of 16.8 °C. The region has approximately 1739.2 h of sunshine with nearly 300 frost free days, about 9 months with mean temperatures of >10 °C and is characterized by a double rice cropping system. The soil is characterized as silty clay loam, which is Ultisol with (2.00-0.02 mm),35% 43% sand silt (0.02–0.002 mm), and 22% clay (<0.002 mm) in the United States Department of Agriculture (USDA) soil taxonomy and Haplic Acrisol in the Food and Agriculture Organization (FAO) soil classification system (Das et al. 2014a, b). Table 1 presents the basic physical and chemical properties of the soil at 0-20 cm depth.

#### Experimental design

The experiment included four tillage treatments: (1) plow tillage with residue removed (PT0), involving ploughing to 15 cm depth with mouldboard plough and rotavating twice to 8 cm depth; (2) plow tilllage with residue incorporation (PT), similar to PTO, but with residue incorporation in the 15 cm layer on the day of transplanting the rice seedlings; (3) rotary tillage with rice residue incorporation (RT), involving four passes by the rotary tiller to a depth of 8 cm with residues incorporated into the 0-8 cm layer on the day of transplanting of rice seedling; (4) no-till with rice residues retention on the soil surface (NT) throughout the entire growing season. The field experiment was laid out as a randomized complete block design, with replications of a plot of  $67.2 \text{ m}^2$ three  $(8.2 \text{ m} \times 8.2 \text{ m})$ . The residues of the early and late

**Table 1** Basic physical and chemical properties of studied soilat 0-20 cm depth before treatment in 2005

Items	
Bulk density (g cm <sup>-3</sup> )	1.21
Soil organic matter (g kg <sup>-1</sup> )	34.9
Available N (mg kg <sup>-1</sup> )	224.1
Available $P (mg kg^{-1})$	4.38
Available k (mg kg <sup>-1</sup> )	97.1
pH (H <sub>2</sub> O)	6.26
Sand (2.00–0.02 mm) (g kg <sup>-1</sup> )	365
Silt (0.02–0.002 mm) (g kg <sup>-1</sup> )	230
Clay (<0.002 mm) (g kg <sup>-1</sup> )	405

rice were crushed to about 5 cm, and totally retained to the filed after rice harvests (fresh weight,  $\sim 10,500 \text{ kg ha}^{-1} \text{ year}^{-1}$  of the early rice residue, and 12,500 kg ha<sup>-1</sup> year<sup>-1</sup> of the late rice residue) under PT, RT, and NT.

In 2007, local rice cultivars, Zhongjiazao32 and Xiangwanshan13, were used for the early and late ricecropping seasons, respectively. In 2011, the early and late rice cultivars were Zhongjiazao32 and Zhuliangyou2, respectively. The intervals between the two ricecropping seasons were about 3–5 days. All plots received Sakefu compound fertilizer (N:  $P_2O_5$ :  $K_2O = 20$ : 12: 14) at 375 kg ha<sup>-1</sup> as basal fertilizer applied 2 days before rice transplanting. The plots were top-dressed with urea (46% N) one week after the transplanting at 150 kg ha<sup>-1</sup> for the early rice and 75 kg ha<sup>-1</sup> for the late rice. Pesticide and herbicide were applied 7 days before rice transplanting in all treatments followed the local practices.

In the early rice season, flooding was initiated 1 week before early rice transplanting, and drained at the aeration period and 7–10 days prior to rice harvesting. The water logging depth was maintained at about 5 cm with an irrigation-drainage system. The water regime was similar to that in the early rice season during the late rice-cropping season. All field plots were drained out in the winter season. The hight of ridge was built to 30 cm with the plastic mulch coverd to the soil of a 10 cm depth, to prevent the interaction of each plot.

## Soil sampling and analysis

Soil samples from each plot were composited depth wise were obtained using a flat spade in three soil layers from 0 to 20 cm depth (0-5, 5-10, and 10-20 cm), and analyzed immediately after the late rice harvest in October 2007 (~2 years) and 2011 ( $\sim 6$  years). Samples were stored in crush-resistant airtight containers, and were air-dried at room temperature. A portion of samples was passed through a 2 mm sieve and the other portion was passed through a 8 mm sieve. The <2 mm size fraction of the soil samples were grounded to pass a 0.2 mm sieve and analyzed for the TOC, while the other fractions were used for aggregate size distribution and aggregate stability indices, which were determined for each composite sample using a modified wet sieving method (Elliott 1986). Briefly, 25 g air-dried (8 mm sieved) soil sample was placed on the top of a 2 mm sieve and submerged for 10 min in deionized water at room temperature. The nest of sieves were oscillated under water 250 times in 10 min with a 30 mm amplitude to separate aggregate fractions. After the 10 min cycle, WSA (>2 mm) were gently backwashed off the sieve into an aluminum pan.

A nest of three sieves (2, 0.25, and 0.053 mm) were used to obtain four aggregate fractions: (1) large macroaggregate (>2 mm), (2) small macroaggregate (0.25-2 mm), (3) microaggregate (0.053-0.25 mm), and (4) silt-plus clay-sized particle (<0.053 mm). All aggregate size fractions were oven-dried at 105 °C (24 h) in an aluminium pan and weighed. Sand content of the aggregate classes (>0.053 mm) was determined by dispersing a 5 g subsample in sodium hexametaphosphate solution (5 g  $L^{-1}$ ) using a reciprocal shaker, and the suspension poured through a sieve with the same mesh size as the one from which the aggregates were collected. The sand remaining on each sieve was washed onto an aluminum pan, ovendried at 105 °C for 24 h, and weighed. Sand correction was performed for each aggregate size class because it was not considered to be a part of the aggregates (Elliott 1986). The proportion of WSA in each size fraction was calculated from the following expression (Alvaro-Fuentes et al. 2009):

$$WSA_{i} = \frac{Total_{i} - Sand_{i}}{\left\{\frac{Soil}{(1 + Moisture)}\right\} - \sum_{i=1}^{n} Sand_{i}}$$
(1)

where i is the ith size fraction (2–8, 0.25–2, and 0.053–0.25 mm); Total is the oven-dry mass of total, non-dispersed aggregates collected on each sieve; Sand is the oven-dry mass of sand collected on each sieve; Soil is the oven-dry mass of the remoistened, sieved (<8 mm) soil; and Moisture is the gravimetric moisture content of the remoistened, sieved (<8 mm) soil.

The mean weight diameter (MWD) and geometric mean diameter (GMD) were calculated taking into account the sand content in each aggregate size fraction, by using Eqs. (2) and (3) (Van Bavel 1949):

$$MWD = \sum_{i=1}^{n} X_i WSA_i \tag{2}$$

where i is the ith size fraction (2–8, 0.25–2, 0.053– 0.25, and <0.053 mm) and *X* is the mean diameter of each size fraction, based on the mean intersieve size.

**Fig. 1** Water-stable aggregate size distribution at 0–5, 5–10 and 10–20 cm soil depths in 2007 and 2011 as affected by tillage. PT0, plow tillage with residue removed; PT, plow tillage with residue incorporation; RT, rotary tillage with residue incorpration; NT, no tillage with residue retention. *Different lower-case letters* in the same soil layer mark significant differences between treatments at P < 0.05 level. The *error bars* indicate the standard error of the means (n = 3)

Thus, four aggregate size fractions were obtained: WSA (2–8 mm), WSA (0.25–2 mm), WSA (0.053–0.25 mm), and WSA (<0.053 mm).

$$GMD = \exp\left[\left(\sum_{i=1}^{n} WSA_i \lg X_i\right) \middle/ \sum_{i=1}^{n} WSA_i\right] \quad (3)$$

where WSA<sub>i</sub> is the weight of aggregates whose average diameter is X<sub>i</sub>, and  $\sum_{i=1}^{n} WSA_i$  is the total weight of soil samples.

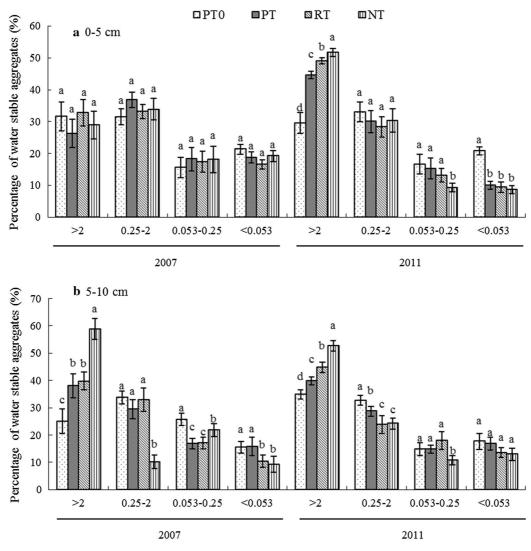
Subsamples from each aggregate size fraction were then grounded to pass a 0.2 mm sieve (Wright and Hons 2005) and analyzed for SOC from each water-stable aggregate size (aggregate associated-C). The TOC and aggregate associated-C were determined using the wet oxidation of the Walkley–Black method described by Nelson and Sommers (1996). The contribution of aggregate size fractions C to TOC (CR) was calculated by using Eq. (4) (Qiu et al. 2009):

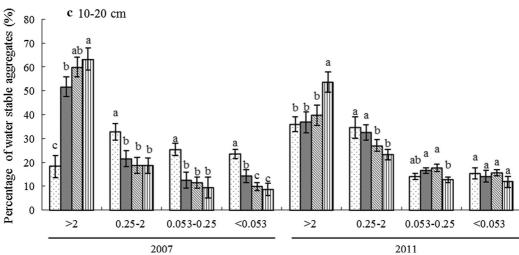
$$CR = \frac{C_F \times WSA_i}{C_T} \tag{4}$$

where  $C_F$  is the concentration of aggregate associated-C, and  $C_T$  is the concentration of TOC.

### Statistical analyses

Statistical analyses were conducted using a normal randomized block design with SPSS 17.0 (SPSS Inc., Chicago, IL, US). Differences in seasonal MWD and GMD of Water stable aggregates, aggregate associated-C and contribution of aggregate associated-C from double rice-cropping systems as affected by tillage, year and their interaction were examined by using a two-way analysis of variance (ANOVA, Tables 3, 4, 5). Differences in waterstable aggregate size distribution among tillage treatments were further examined by using the least square means test (LSD) (Fig. 1). Statistical





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significance was evaluated at P < 0.05 unless otherwise stated.

## Results

Distribution of water-stable soil aggregate

The depth distribution of percentage of WSA with different sizes varied significantly among tillage systems (Fig. 1). The percentage of WSA (>2 mm) under NT was significantly higher than those under other tillage systems at 0-5, 5-10 and 10-20 cmsoil layer (P < 0.05) in 2011, but without significant different among all tillage systems at 0-5 cm soil layer in 2007. Meanwhile, both NT and RT showed lower proportions of WSA (0.25-2 mm) compared to the PT0 system for the 5-10 and 10-20 m layer in 2007 and 2011 (P < 0.05). Across all the soil layers, the percentage of WSA (0.053-0.25 mm) significant decreased in soil under NT compared other tillage practices in 2011, followed with were reduced by 47.1, 40.0, and 30.8% compared with that under PTO, PT, and RT at 0-5 cm depth, respectively (Fig. 1). There was no significant difference in the <0.053 mm fraction among NT, RT and PT, but those were 51.5–58.0% significantly lower than that under PT0 at 0–5 cm soil layer. With an increase in the duration of these treatments, PT, RT, and NT practices in 2011 significant increased the percentage of WSA (>2 mm), but decreased the percentage of WSA (<0.053 mm) at 0–5 cm depth compared with those in 2007. The main increased can be obseared on the the percentage of WSA (>2 mm) under PT0 in 2011 compared with that in 2007 at 5–10 cm and 10–20 cm depth.

#### MWD and GMD of water-stable aggregates

MWD and GMD at 0–20 cm at the late rice harvest significantly varied with tillage, but independent of year and their interaction (Table 2). The value of MWD and GMD in 2007 ranged from 1.51–4.03 and 0.76–1.42 mm, respectively, followed with ranged from 2.18–3.50 and 0.81–1.23 mm in 2011. MWD and GMD under NT were higher than those under another tillage treatments with significant differences (P < 0.05) in 2007 and 2011 (Table 2), which illustrated that NT had larger size aggregates. During the experimental period, the value of MWD and GMD under NT at 0–5 cm soil depth in 2011 were significantly higher than those in 2007 (P < 0.05), but lower

Table 2 MWD and GMD of water stable aggregates under different tillage systems in 2007 and 2011

Year	Treatment	MWD (mn	n)		GMD (mm)			
		0–5 cm	5-10 cm	10–20 cm	0–5 cm	5-10 cm	10–20 cm	
2007	PT0 <sup>\$</sup>	2.28a <sup>#</sup>	1.92c	1.51c	0.96b	0.81b	0.76b	
	PT	1.67b	2.64b	3.36b	0.83b	0.95b	1.22a	
	RT	2.49a	2.78b	3.83b	1.00b	1.06a	1.33a	
	NT	2.77a	3.68a	4.03a	1.04a	1.19a	1.42a	
2011	PT0	2.18c	2.46c	2.57b	0.81c	0.85b	0.93b	
	PT	3.04b	2.75b	2.65b	1.08b	0.94b	1.10a	
	RT	3.29a	2.98b	2.72b	1.15b	1.00a	1.14a	
	NT	3.46a	3.45a	3.50a	1.23a	1.15a	1.17a	
Significant								
Treatment			*			*		
Year			NS			NS		
Treatment $\times$ year			NS			NS		

NS is meaning not significant

\*, \*\* Significant correlation at 0.05 and 0.01 levels, respectively

<sup>\$</sup>PT0, conventional tillage with residue removed; PT, conventional tillage with residue incorporation; RT, rotary tillage with residue incorporation; NT, no-till with residue retention

<sup>#</sup>Different lower-case letters in the same soil layer mark significant differences between treatments at P < 0.05 level

Table 3 Aggregate associated-C under different tillage treatments in 2007 and 2011

Depth (cm)	Treatment	Aggregate associated-C (g kg <sup>-1</sup> )								
		>2 mm		0.25–2 mm		0.053–0.25 mm		<0.053 mm		
		2007	2011	2007	2011	2007	2011	2007	2011	
0–5	PT0 <sup>\$</sup>	26.53b <sup>#</sup>	23.17b	28.32b	23.88c	22.72b	22.37b	14.35a	12.18b	
	PT	22.68b	30.40b	25.36b	24.64c	30.26a	31.89a	14.08a	15.18a	
	RT	24.40b	31.93b	20.66c	30.36b	30.55a	29.43a	15.85a	18.18a	
	NT	33.08a	40.94a	32.45a	38.06a	30.66a	32.95a	17.53a	19.46a	
5-10	PT0	29.34a	20.44b	23.15b	23.76b	24.05c	24.72b	15.01a	14.14b	
	РТ	29.18a	18.92b	26.13a	24.49b	28.57a	27.54b	13.89a	17.75a	
	RT	27.98a	30.57a	27.85a	28.91a	30.10a	32.64a	13.31a	19.92a	
	NT	27.18a	21.25b	27.18a	19.42c	27.19b	25.19b	13.44a	16.91a	
10–20	PT0	25.82a	18.19b	23.16a	20.69a	24.65a	21.28a	14.30a	11.52b	
	PT	22.72a	23.95a	19.59b	25.87a	22.16a	26.94a	16.08a	16.48a	
	RT	19.32b	20.20b	20.99b	19.46b	19.66b	19.20b	12.34a	15.60a	
	NT	18.01b	17.80b	19.84b	13.48c	18.88b	15.83b	13.76a	15.87a	
Significant		*		**		*		*		
Treatment		**		NS		*		NS		
Year		*		NS		NS		NS		
Treatment $\times$ year										

NS is meaning not significant

\*, \*\* Significant correlation at 0.05 and 0.01 levels, respectively

<sup>\$</sup>PT0, conventional tillage with residue removed; PT, conventional tillage with residue incorporation; RT, rotary tillage with residue incorporation; NT, no-till with residue retention

<sup>#</sup>Different lower-case letters in the same soil layer mark significant differences between treatments at P < 0.05 level

at 5–10 and 10–20 cm depths, PT0 just appear the opposite trend. The range of the change in MWD was larger than that of the change in GMD from 2007 to 2011.

Soil aggregate associated-C

At 0–20 cm soil depth, the concentration of aggregate associated-C at the late rice harvest was significantly varied with tillage and residue management at each size fraction, significantly varied with tillage at >2 mm and 0.053–0.25 mm, but independent of their interaction except at >2 mm size fraction (Table 3). NT practices has the highest the aggregate associated-C (>2 and 0.25–2 mm) at 0–5 cm depth compared with another tillage practices with significantly different (P < 0.05) in 2007 and 2011. However, the NT system significantly reduced the SOC concentration in the >0.25 mm fraction in the 10–20 cm layer in 2011. Both PT0 and PT showed higher aggregate

SOC concentration in aggregate (0.053–0.25 mm) compared to RT and NT for the 10–20 m layer. For all the soil layers, there was no difference in the <0.053 mm fraction between NT, RT and PT, indicating that the tillage system did not affect the SOC concentration in the silt + clay fraction, but the SOC concentration was increased with residue incorporation in the silt + clay fraction, by 6.2–26.2% under the PT system compared with those under the PT0 system in 2011. Compared with the samples obtained in 2007, the aggregate associated-C (>2 mm) increased in soil under NT by 23.8% at 0–5 cm depth in 2011, but decreased at 5–10 and 10–20 cm depths (Table 3).

Contribution of aggregate associated-C to TOC

The distribution of TOC concentration (g kg<sup>-1</sup>) across soil depths significantly varied among tillage systems (Table 4). The concentration of TOC at 0–5 cm depth

Depth (cm)	Treatment	TOC (g $kg^{-1}$ )		Contribution of aggregate associated-C to TOC							
				>2 mm		0.25–2 mm		0.053–0.25 mm		<0.053 mm	
		2007	2011	2007	2011	2007	2011	2007	2011	2007	2011
0–5	PT0 <sup>\$</sup>	27.00c <sup>#</sup>	29.37d	0.37a	0.38b	0.39a	0.30a	0.15b	0.21a	0.13a	0.12a
	PT	26.83c	34.62c	0.20b	0.53a	0.40a	0.29a	0.28a	0.19a	0.13a	0.06b
	RT	28.30b	38.62b	0.37a	0.55a	0.30a	0.34a	0.23a	0.14b	0.10a	0.06b
	NT	31.26a	41.06a	0.46a	0.53a	0.38a	0.35a	0.19a	0.11b	0.06a	0.05b
5-10	PT0	24.48b	28.82b	0.32b	0.34b	0.34a	0.35a	0.27a	0.15b	0.10a	0.15a
	PT	27.4a	33.10a	0.45b	0.32b	0.31a	0.38a	0.20a	0.13b	0.09a	0.14a
	RT	28.92a	35.98a	0.42b	0.51a	0.38a	0.22b	0.21a	0.22a	0.06a	0.10b
	NT	24.38b	29.51b	0.67a	0.56a	0.12b	0.24b	0.25a	0.14b	0.05a	0.09b
10–20	PT0	21.41b	21.45b	0.22b	0.39b	0.35a	0.34b	0.29a	0.18a	0.15a	0.10a
	PT	24.53a	22.33b	0.58a	0.38b	0.21b	0.41a	0.14b	0.17a	0.11a	0.09a
	RT	25.99a	23.02a	0.64a	0.46b	0.22b	0.30b	0.12b	0.19a	0.07a	0.10a
	NT	16.35c	21.69b	0.65a	0.65a	0.21b	0.21c	0.10b	0.14a	0.07a	0.10a
Significant											
Treatment				*		**		*		*	
Year				**		*		NS		NS	
Treatment $\times$ year				*		*		NS		NS	

Table 4 Total soil organic C (TOC) and contribution of aggregate associated-C to TOC under different tillage treatments in 2007 and 2011

NS is meaning not significant

\*, \*\* Significant correlation at 0.05 and 0.01 levels, respectively

<sup>\$</sup>PT0, conventional tillage with residue removed; PT, conventional tillage with residue incorporation; RT, rotary tillage with residue incorporation; NT, no-till with residue retention

<sup>#</sup>Different lower-case letters in the same soil layer and same year mark significant differences between treatments at P < 0.05 level

followed the order of NT > RT > PT > PT0 in 2007 and 2011. At 5–10 and 10–20 cm depths, the TOC concentration under RT was higher than compared to the other treatments (P < 0.05). In comparison with PT0, the concentration of TOC under PT at 0–5, 5–10, and 10–20 cm depths was increased by 17.9, 14.9, and 4.1% in 2011, respectively (P < 0.05) (Table 4), and thus indicating the importance of residue retention in increase the rate of TOC concentration.

At 0–20 cm soil depth, the concentration of aggregate associated-C at the late rice harvest was significantly varied with tillage and residue management at each size fraction, significantly varied with tillage at >2 mm and 0.053–0.25 mm, but independent of their interaction except at >2 mm size fraction (Table 4). Contribution of macroaggregate associated-C to TOC was greater than that of microaggregate in the same year and within the same soil depth (Table 4), which was consistent with the distribution of soil aggregate. At the harvest of late rice in 2007 and 2011, more than 80% of C was concentrated in WSA (>0.25 mm) in soil under NT at 0-5 cm depth (Table 4), and the contribution of aggregates associated-C (>2 mm) under NT were higher than those under another tillage practice in 2007. The trend was reversed in the contribution of aggregates associated-C (<0.053 mm) (NT < RT < PT < PT0). At 5–10 and 10–20 cm depths, the contribution of all tillage treatments regarding aggregates associated-C to TOC followed the same trend. Compared with sampling in 2007, the contribution of aggregates associated-C (>2 mm) to TOC in soil under NT increased by 15.2% at the harvest of late rice in 2011 at 0-5 cm soil layer, but decreased in 5-10 cm layer. The contribution of aggregate associated-C (>2 mm) to TOC under RT and PT were mainly increased at 0-5 and 5-10 cm depth, and those under PTO were increased at 10-20 cm depth. In fact, the contribution of aggregate

<b>Table 5</b> Correlationsbetween soil aggregateassociated-C and TOCunder tillage practices in2007 and 2011	Year	Depth (cm)	Correlations between soil aggregate associated-C and TOC					
			>2 mm	0.25–2 mm	0.053–0.25 mm	<0.053 mm		
	2007	0–5	0.72*	0.87**	0.35	-0.61*		
		5-10	0.68*	0.80*	0.24	0.25		
		10-20	0.64*	0.75*	0.32	0.41		
	2011	0–5	0.89**	0.93**	0.49	-0.68*		
*, ** Significant correlation at 0.05 and 0.01 levels, respectively		5-10	0.76*	0.81*	-0.12	0.16		
		10–20	0.62*	0.77*	0.49	0.23		

associated-C for different size fractions tended to balance one another.

Correlations between soil aggregate associated-C and TOC

Table 5 showed macroaggregate associated-C had a significant positive correlation with TOC. At harvest of the late rice in 2007 and 2011, the aggregates associated-C (>2 and 0.25-2 mm) existed a significant positive correlation to TOC (P < 0.05) at each soil layers, and reached extremely significant correlation (P < 0.01) in the surface layer. Compared to 2007, the correlation coefficient between TOC and macroaggregate associated-C (>2 and 0.25-2 mm) was increased in soil in 2011. There is no significant positive correlation between aggregates associated-C (0.25–0.053 mm) and TOC at each soil layer depth in 2007 and 2011, except at 5-10 cm depth in 2011. In addition, a significant negtive correlation between TOC and the aggregates associated-C (<0.053 mm) was found at 0-5 cm depth in 2007 and 2011 (P < 0.05).

## Discussion

Tillage impact on the soil aggregate stability in paddy soil

It is widely accepted that binding agents (e.g.  $Fe^{2+}$ , SOC, microbial biomass, and glomalin-related soil proteins) plays a key role in stabilizing soil aggregate (Six et al. 2000). The data presented herein show that C both in bulk soil and in most aggregates, is strongly correlated with the distribution of water-stable aggregate. This trend suggests that aggregate stability is related to the distribution of SOC in different

aggregates size fractions. Macroaggregates are generally formed around fresh SOC that constitutes a C source for microbial activity, which produces microbial-derived binding agents (Six et al. 2000). This transient C source contributes to the higher SOC content of macroaggregates compared with microaggregates (Messiga et al. 2011). Similarly, Plaza-Bonilla et al. (2010) reported that higher SOC content in the surface layer of NT system may lead to more and stable aggregation in large macroaggregates, thus promoting aggregate stability. Plow tillage disrupts the network of fungal hyphae by mechanical, breakdown of macroaggregates which releases particulate organic carbon, thus reducing aggregate stability (Ashagrie et al. 2007). The results presented herein were similar to previous studies, the percentage of WSA (>2 mm) under NT and RT were nearly 2 times higher than that under PT. In addition, humus from the conversion of crop residue acts as an important cementing material conducive to the formation of large aggregates (Gupta Choudhury et al. 2014). Meanwhile, crop residues decompose rapidly under wetting and drying in paddy soils, release the nutrients in the decomposition process and promotes roots growth, which cements the clay particles into larger water-stable aggregates (Zhou and Pan 2007).

Tillage impact on the water stable aggregates-C distribution in paddy soil

The data here indicate that long-term double rice cultivation can lead to a gain of C-rich macroaggregates in paddy soil. These results are similar to Li et al. (2000) who indicated the added SOC mainly fixed in the WSA (>2 mm) in paddy soil, and the chemical composition of iron oxides in paddy soil increased the SOC by promoting formation of water-stable aggregates. Jastrow et al. (1996) future used stable C isotope analysis, and observed that large macroaggregates have greater stability, and enrichment of C in macroaggregate reduces the loss of soil C. In addition, Bhattacharyya et al. (2009) indicated that changes in tillage system can significant affect C dynamics within aggregate. The large stable aggregates were more abundant and stable in the surface samples of a kaolinitic soil under NT management, and contained higher concentrations of C than did water stable aggregates in soil under PT0 management (Beare et al. 1994). NT in the paddy fields changes the flooded or drained water plant in traditional tillage system, eliminates the effects of gravitational water, and the soil is infiltrated by capillary water, thus contributing to the frequent exchange of water, heat and gas in the soil, eventually leading to the soil can automatically shrink and form a stable soil structure, finally physically protects SOC from its degradation (Xie and Chen 2002). On the other hand, higher C concentration was observed in each aggregate size fractions in soil under PT at 10–20 cm depth in 2011 compared that in PT0, which was probably due to the incorporation of crop residue into the subsoil through tillage (Wright and Hons 2005). The retained crop residue in the paddy field promotes microbial activity, which enhances the aggregates cohesion and hydrophobicity (Wallis and Horne 1992). Meanwhile, crop residue also hinders the soil water infiltration rate and releases air in the soil pore slowly, resulting in gelatinization stress reduction and soil aggregate stability improvement, and thus increasing the content of C (Wallis and Horne 1992).

Correlations between soil aggregate associated-C and TOC

During the 6-year of this experiment, the TOC was significantly correlated with macroaggregate associated-C maybe due to a large amount of C associated within macroaggregates in soil (P < 0.05) (Table 5). Mikha and Rice (2004) also indicated SOC concentrations was more pronounced in macroaggregates than that in microaggregates. This trend is consistent with Lei (2012) who reported that WSA (>2 mm) was the main carrier of new SOC in paddy soil, which was an important bonding material to form large aggregates in soil. Soil under long-term flooding enhance the percentage and stability of large aggregates, which avoid the loss of C protected by the large aggregates, to some extent increase the correlation between large

aggregate-C and TOC in paddy soil (Pan et al. 2008). Consequently, in the paddy soil of the Southern China, the macroaggregate associated-Cmaybe a better indicator to characterize SOC dynamics due to the larger correlations coefficient between soil macaggregate associated-C and TOC (Table 5). However, Liang et al. (2008) also observed that TOC was strongly correlated with soil C in all aggregate size fractions in virgin and cultivated black soil. Differences in these results may be attributed to the different agroecosystem. In addition, crop residue is the key to soil structural development and stability since organic matter is an important factor in soil aggregation (Verhulst et al. 2011).

## Conclusions

The data presented herein indicates the temporal change caused by different tillage and residue management system on soil aggregates in a doublecropped paddy field. Our results showed that maintenance of NT over time enhanced the percentage of large macroaggregate (>2 mm) and decreased WSA (<0.053 mm) in paddy soil surface. Large macroaggregate significantly increased the contribution of aggregate associated-C to TOC compared with that by other aggregate size fractions. Macroaggregate associated-C (>2 and 2-0.25 mm) could be a better indicator on bulk SOC dynamics due to a extremely significantly positive correlation with TOC in a paddy rice ecosystem. Therefore, NT can enhance the formation of stable soil structure, and the enrichment of C concentration of macroaggregates is the main mechanisms of SOC protection in the double-rice fields of the Southern China.

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