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Water-Stable Aggregates and Associated Carbon in a Subtropical Rice Soil Under Variable Tillage

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ABSTRACT: Tillage systems can influence C sequestration by changing aggregate formation and C distribution within the aggregate. This study was undertaken to explore the impact of no-tillage without straw (NT-S) and with straw (NT+S), and moldboard plow without straw (MP-S) and with straw (MP+S), on soil aggregation and aggregate-associated C after six years of double rice planting in a Hydragric Anthrosol in Guangxi, southwest of China. Soil samples of 0.00-0.05, 0.05-0.20 and 0.20-0.30 m layers were wet-sieved and divided into four aggregate-size classes, >2 mm, 2.00-0.25 mm, 0.25-0.053 and <0.053 mm, respectively, for measuring aggregate associated C and humic and fulvic acids. Results showed that the soil organic carbon (SOC) stock in bulk soil was 40.2-51.1 % higher in the 0.00-0.05 m layer and 11.3-17.0 % lower in the 0.05-0.20 m layer in NT system (NT+S and NT-S) compared to the MP system (MP+S and MP-S), respectively. However, no statistical difference was found across the whole 0.00-0.30 m layer. The NT system increased the proportion of >2 mm aggregate fraction and reduced the proportion of <0.053 mm aggregates in both 0.00-0.05 and 0.05-0.20 m layers. The SOC concentration, SOC stock and humic and fulvic acids within the >0.25 mm macroaggregate fraction also significantly increased in the 0.00-0.5 m layer in NT system. However, those within the 2.00-0.25 mm aggregate fraction were significantly reduced in the 0.05-0.200 m layer under NT system. Straw incorporation increased not only the SOC stock in bulk soil, but also the proportion of macroaggregate, aggregate associated with SOC and humic and fulvic acids concentration within the aggregate. The effect of straw on C sequestration might be dependent on the location of straw incorporation. In conclusion, the NT system increased the total SOC accumulation and humic and fulvic acids within macroaggregates, thus contributing to C sequestration in the 0.00-0.05 m layer.

Keywords: Hydragric Anthrosol, soil organic carbon, straw incorporation, tillage system.



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INTRODUCTION

In agroecosystems, soil aggregation formation is considered an important process in soil organic carbon (SOC) stabilization by hindering decomposition of SOC and its interactions with mineral particles (Urbanek et al., 2011;Gunina and Kuzyakov, 2014). Generally, a more rapid loss of SOC may occur from macroaggregates than from microaggregates (Eynard et al., 2005). The SOC change under agricultural management may owe to the aggregate stability index (Nascente et al., 2015). Thus, soil aggregated fractionation has been widely applied to evaluate the SOC stability under contrasting tillage systems.

Tillage and, particularly, moldboard plow (MP) have been reported as a cause for macroaggregate disruption compared to a no-tillage (NT) system (Álvaro-Fuentes et al., 2008; Anders et al., 2010). In contrast, NT can promote soil surface macro-aggregation and improve structural stability due to lower soil disturbance and higher SOC, arbuscular mycorrhizal fungi, and microbial biomass and glomalin, which are more important driving factors for aggregate stability, and consequently enhance C retention (Castro et al., 1998; Blanco-Moure et al., 2012; Zhang et al., 2012, 2013).

Several studies has shown that significant SOC sequestration under NT is closely related to improved aggregation that protects C from mineralization (Six et al., 2002; Stewart et al., 2008; Nyamadzawo et al., 2009; Fuentes et al., 2012; Du et al., 2013).

The increased SOC concentration and SOC stock in surface soil under NT may be not only due to higher amount of C-rich macroaggregates, but also to a higher content of humic and fulvic acids under NT (Tang et al.,2011). Hayes and Clapp (2001) reported that humic and fulvic acids are relatively recalcitrant to microbial attack and, hence, are considered a SOC pool with high stability. Some studies have revealed that humic and fulvic acids are significantly linked to SOC stock (Ci et al., 2008; Ma et al., 2008). This provided some evidence that maintaining good soil humic and fulvic acids status favors SOC accumulation. Therefore, knowledge of the humic and fulvic acids distribution within an aggregate is useful to understand the stability mechanisms of C sequestration in soil under NT.

Rice is the major cereal crop in Guangxi, in the southwest of China. In this region, the old and children are the major laborers in rice production because most young people have shifted to cities for employment; thus, NT is widely adopted. Ou et al. (2010; 2011) analyzed the topsoil profile distribution (0.00-0.20 m) of SOC after shifting from MP to NT in this area, concluding that NT combined with or without straw incorporation increased the SOC concentration in bulk soil in the 0.00-0.05 m layer but not in 0.05-0.20 m layer compared to MP without straw incorporation. Aggregate-associated C has lower turnover rates in no-tillage than in the MP system (Six et al., 2000). Litter incorporation and soil mixing under MP could counterbalance the physical impact on macroaggregates (Andruschkewitscha et al., 2014). Meanwhile, as soil humic and fulvic acids are linked to SOC dynamics, it is hypothesized that the conversion from MP without straw incorporation to NT with or without straw incorporation may increase the C storage by increasing the stabilization of aggregate, physically protected C within the aggregate size fraction under the double rice system.

Therefore, we attempt this end using a six-year-old experiment with a double rice system in a Hydragric Anthrosol located in the subtropical region of China with the ultimate aim of identifying the impact of NT with or without straw incorporation on SOC accumulation and soil aggregation at depth, and of determining the distribution of SOC and humic and fulvic acids within the aggregate fraction.

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MATERIALS AND METHODS

Experimental site

The experiment was conducted on the farm (22° 51′ 18″ N, 108° 17′ 21″ E) of the College of Agriculture, Guangxi University, Nanning, China. The area has a subtropical continental climate with an average annual temperature of 21.6 °C and mean rainfall of 1,304.2 mm. The field experiment began in March 2008 on a Hydragric Anthrosol that developed from a Quaternary Red Soil. The basic soil chemical properties were measured prior to treatment implementation (Table 1). Soil OC was determined by the acid dichromate wet oxidation method (Nelson and Sommers, 1996). Alkali-soluble N was determined by the alkali solution diffusion method. The available P extracted by 0.5 mol L⁻¹ NaHCO₃ was measured by colorimetry. The available K extracted by 1 mol L⁻¹ NH₄OAC was measured by flame photometry. The pH (soil:water 1:2.5) was determined by the potential method. Sand, silt and clay were determined by the hydrometer method (Lv, 2000).

Experimental design

A randomized complete block design was employed with three replications and plot size of 3×7 m. The field had two crops per year. Four tillage treatments, no-tillage without straw (NT-S), moldboard plow without straw (MP-S), no-tillage with straw (NT+S) manually and evenly spread on soil surface and moldboard plow with straw (MP+S) incorporated into the soil using a moldboard plough, with a tillage layer of 0.20 m. For NT-S and MP-S, the above-ground residues were removed after harvest, and then the soil was puddled, harrowed and ploughed to a layer of 0.20 m before next planting in MP-S, while not-tillage prior to next planting in the NT-S. The straw incorporation rate was 6,000 kg ha⁻¹ of DW for both NT+S and MP+S. Planting density, chemical fertilization and irrigation were the same for all treatments. At the rice seedling stage, 58.5 kg ha⁻¹ N, 81 kg ha⁻¹ P₂O₅ and 75 kg ha⁻¹ K₂O were applied in the form of urea, single super phosphate and muriate of potash. In addition, 58.5 kg ha⁻¹ N was dressed by surface broadcast at the tillering stage, and 78 kg ha⁻¹ N and 75 kg ha⁻¹ K₂O were added for the topdressing stage.

Sampling and analysis

Soil samples of the 0.00-0.05, 0.05-0.20 and 0.20-0.30 m layers were collected with a soil drill after rice harvest in November 2013. Three soil cores per plot were pooled together to make a composite sample for each layer and replication, then divided into two parts. One part was air-dried at room temperature and sieved through a 0.149 mm sieve for determination of bulk SOC concentration. The other was gently broken by hand along the fractures of peds and passed through an 8 mm sieve and then air dried for aggregate analysis. Bulk density (Bd) was determined by collecting three additional triplicate soil cores from three layers using a stainless steel ring (0.05 m high and 0.05 m in diameter (Grossman and Reinsch, 2002).

Table 1. Soli properties before treatment									
Layer	SOC	Alkali-soluble N	Ρ	К	pH(H ₂ O)	Sand (2.00-0.02 mm)	Silt (0.02-0.002 mm)	Clay (<0.002 mm)	
m	g kg ⁻¹	mg k	g ⁻¹ —				—— g kg ⁻¹ ——		
0.00-0.05	19.1	173.6	61.7	215.4	6.48	350	250	400	
0.05-0.20	18.1	155.3	53.8	198.7	6.56	370	220	410	
0.20-0.30	17.3	131.6	40.6	162.5	7.10	370	230	400	

Table 1. Soil properties before treatment

SOC: soil organic carbon, determined by acid dichromate wet oxidation method (Nelson and Sommers, 1996). Alkali-soluble nitrogen: determined by alkali solution diffusion method. Available P: extracted by 0.5 mol L^1 NaHCO₃, and measured by colorimetry. Available K: extracted by 1 mol L^1 NH₄OAC, and measured by flame photometry, pH (soil:water 1:2.5) determined by potential method. Sand, silt and clay: determined by hydrometer method (Lv, 2000).

Aggregate separation

Fractionation of four aggregate size classes, >2 mm, 2.00-0.25 mm, 0.25-0.053 mm, and <0.053 mm, was achieved using a wet-sieving procedure (Elliott and Cambardella, 1991). The overall procedure yielded water-stable, large- (>2 mm), macro- (2.00-0.25 mm), micro- (0.25-0.053 mm), and silt+clay- (<0.053 mm) sized fractions. Before wet sieving, slaking pretreatment was applied. Briefly, 50 g of the subsample was submerged in water for 10 min on top of the 2 mm sieve, and then separated by mechanically oscillating the sieve 0.07 m up and down in water continuously for 30 min. The aggregates retained in each sieve were collected separately. The silt+clay fraction was collected after centrifugation. All aggregate fractions were separately kept in plastic containers and oven-dried at 40 °C for 48 h and weighed, and then further sieved through a 0.149 mm sieve for determination of aggregate-associate SOC and humic and fulvic acids concentration. Assuming that the sand contents were the same within aggregate-size classes, the water stable aggregates index, aggregate-associate SOC and humic and fulvic acids concentration were calculated without correcting sand content in all aggregate fractions by dispersion with sodium hexametaphosphate.

SOC analysis

The bulk and SOC content were determined by the acid dichromate wet oxidation method (Nelson and Sommers, 1996). The concentration of SOC was expressed on a per unit aggregate weight (g C kg⁻¹ aggregate). To account for the differences in soil mass produced by bulk change, we also compared the soil C stock based on equivalent soil mass (Ellert and Bettany, 1995). The heaviest soil mass was selected as the equivalent soil mass; then the equivalent SOC stock was calculated using the following equation:

 $M_{c} = \rho_{b} \times \left[T + (M_{\text{soil, equiv}} - M_{\text{soil}}) \times 0.0001 / \rho_{b}\right] \times Conc_{c} \times 10$

where M_c = equivalent SOC mass per unit area (kg ha⁻¹), ρ_b = bulk density (Mg m⁻³), T = soil thickness (m), $M_{soil, equiv}$ = equivalent soil mass (kg ha⁻¹), M_{soil} = soil mass (kg ha⁻¹) and Conc_c = SOC concentration (g kg⁻¹). The total mass of SOC on equivalent basis was calculated by summing the values of the 0.00-0.30 m layers. The SOC stock in the aggregate fractions, that is, the SOC concentration, expressed as g kg⁻¹ C microaggregate, was estimated by the mass proportion (g kg⁻¹ C) of the fraction in bulk soil and the corresponding equivalent soil mass.

Humic and fulvic acids analysis

The humic and fulvic acids within the aggregate fractions were extracted with 0.1 mol L⁻¹ sodium pyrophosphate (1:50 w/v) in a Dubnoff bath at 100 °C for 1 h and separated from the suspended material by centrifuging at 4,000 rpm for 15 min. Here, the terms humic and fulvic acids were applied to the alkali-soluble fraction. Humic and fulvic acids extracts (15 mL) were transferred into a hard tube and adjusted the pH to neutral by adding 0.5 mol L⁻¹ sulfuric acid, then evaporated in a boiling water bath and determined by acid dichromate wet oxidation, as described by Nelson and Sommers (1996).

Statistical analyses

The data obtained without angular transformation were subjected to ANOVA and the mean values were separated using Duncan's test at p<0.05. All statistical analyses were performed using the DPS 7.05 statistical package.

RESULTS

SOC concentration and distribution in bulk soil

The bulk density (Bd) had the order of NT+S = NT-S < MP+S = MP-S in the soil of the 0.00-0.05 m layer, whereas the NT system decreased the Bd in contrast to the MP system (Table 2). There were no significant differences between NT+S and NT-S as well

Laver	Treatment	Bd	SOC concentration	SOC stock
m		Mg m ⁻³	g kg ⁻¹	mg ha ⁻¹
	MP-S	1.17 ± 0.08 a	27.5 ± 0.8 c	17.4 ± 0.5 c
0.00.0.05	MP+S	1.08 ± 0.10 a	28.7 ± 2.0 c	18.2 ± 1.3 c
0.00-0.05	NT-S	0.73 ± 0.10 b	38.6 ± 1.3 b	24.4 ± 0.8 b
	NT+S	0.64 ± 0.04 b	43.4 ± 2.0 a	27.5 ± 1.3 a
	MP-S	1.33 ± 0.02 a	21.3 ± 1.3 b	45.7 ± 3.2 b
0.05.0.00	MP+S	1.32 ± 0.01 a	$23.0 \pm 1.0 a$	49.4 ± 2.1 a
0.05-0.20	NT-S	1.37 ± 0.07 a	18.9 ± 0.9 c	40.5 ± 1.8 c
	NT+S	1.36 ± 0.05 a	19.1 ± 0.9 c	41.0 ± 2.0 c
	MP-S	1.55 ± 0.10 a	18.4 ± 0.7 a	29.6 ± 1.6 a
0.00.0.00	MP+S	1.44 ± 0.03 a	17.1 ± 1.0 a	30.6 ± 1.1 a
0.20-0.30	NT-S	1.48 ± 0.04 a	18.8 ± 0.6 a	28.9 ± 1.0 a
	NT+S	1.49 ± 0.10 a	17.4 ± 0.6 a	31.3 ± 1.0 a
	MP-S	-	-	92.7 ± 1.4 b
0 00 0 00	MP+S	-	-	98.2 ± 2.3 a
0.00-0.30	NT-S	-	-	93.9 ± 1.7 b
	NT+S	-	-	99.8 ± 1.2 a

Table 2. Bulk density (Bd), soil organic carbon (SOC) concentration and stock (\pm standard deviation, n = 3) on an equivalent soil mass basis in the 0.00-0.05, 0.05-0.20 and 0.20-0.30 m layers, as affected by tillage

MP-S: moldboard plow without straw; MP+S: moldboard plow with straw; NT-S: no-tillage without straw; NT+S: no-tillage with straw. Different lower-case letters in the same soil layer mark significant differences between treatments at p < 0.05 in Duncan's test.

as between MP+S and MP-S. In the soil of 0.05-0.20 and 0.20-0.30 m layers, Bd showed no significant differences among all the tillage treatments.

Soil OC stock in bulk soil was also impacted by tillage systems. In the 0.00-0.05 m soil layer, the SOC stock in MP-S was similar to that in MP+S, and significantly increased by 40.2 % in NT-S and 58.0 % in NT+S compared to that in MP-S. The addition of straw resulted in 12.7 and 4.6 % higher SOC stock in the NT and MP systems, respectively. The SOC stock showed the order of MP+S>MP-S>NT+S = NT-S in the 0.05-0.20 m soil layer, and no difference was found between any treatments in the 0.20-0.30 m soil layer. For the whole 0.00-0.30 m soil layer, the difference in SOC stock was not significant between NT-S and MP-S as well as between NT+S and MP+S, but significantly higher in NT+S and MP+S than in NT-S and MP-S.

Distribution of soil aggregates with different sizes

Tillage systems obviously affected the distribution of soil aggregates with different sizes (Figure 1). The proportion of the >2 mm aggregate fraction in NT+S was 7.1 % higher than that in NT-S in the 0.00-0.05 m layer. There was no significant difference in the total amount of all the aggregate fractions between NT+S and NT-S in both the 0.05-0.20 and 0.20-0.30 m layers. Both NT+S and NT-S showed higher proportions of >2 mm aggregate and lower proportions of <0.053 mm aggregate compared to the MP system for the 0.00-0.20 m layer. The proportion of >0.25 mm macroaggregate was significantly higher in MP+S than in MP-S in most cases, but the proportion of <0.053 mm aggregate was 11.5-20.5 % lower in MP+S than in MP-S for all the soil layers.

Aggregate-associated SOC concentration

The aggregate-associated SOC concentration in different soil layers was influenced by tillage systems (Figure 2). In the 0.00-0.05 m layer, SOC concentration in macroaggregates showed the order of NT+S>MP+S = NT-S>MP-S, whereas the NT system was superior to the MP system. However, the NT system significantly reduced the SOC concentration in the 2.00-0.25 mm



Figure 1. Distribution (%) of water-stable aggregates with different sizes in different soil layers as influenced by tillage treatments. (a) 0.00-0.05 m; (b) 0.05-0.20 m; (c) 0.20-0.30 m. MP-S: moldboard plow without straw; MP+S: moldboard plow with straw; NT-S: no-tillage without straw; NT+S: no-tillage with straw. Error bars represent standard errors of the means (n = 3). Different lower-case letters within an aggregate size indicate significant differences between treatments according to Duncan's test (p<0.05).

> fraction in the 0.05-0.20 m layer. A similar trend was observed in the 0.25-0.053 mm fraction in the 0.20-0.30 m layer. Across all the soil layers, there was no difference in the <0.053 mm fraction between NT-S and MP-S, as well as between NT+S and MP+S, indicating that the NT system did not affect the SOC concentration in the silt+clay fraction. In average across the soil layers, the SOC concentration in the macroaggregate was increased by 13.5 % in MP+S, 4.4 % in NT-S and 19.3 % in NT+S, and those in the microaggegrate (<0.25 mm) were increased by 6.1 % in MP+S and 7.0 % in NT+S compared to MP-S. For all the soil layers, the SOC concentration in all the aggregate size classes was increased with straw incorporation, by 20.0, 3.8 and 5.7 % under the MP system, and 20.2, 6.3 and 8.8 % under the NT system.

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Figure 2. Aggregate-associated SOC concentration in different layer intervals as influenced by tillage treatments. (a) 0.00-0.05 m; (b) 0.05-0.20 m; (c) 0.20-0.30 m. MP-S: moldboard plow without straw; MP+S: moldboard plow with straw; NT-S: no-tillage without straw; NT+S: no-tillage with straw. Error bars represent standard errors of the means (n = 3). Different lower-case letters within an aggregate size indicate significant differences between treatments according to Duncan's test (p < 0.05).

SOC storage in different aggregate size fractions

Tillage systems also affected the distribution of SOC stock in different aggregate fractions (Table 3). Compared to MP-S, the SOC stock in the >2 mm aggregate fraction increased and that in the <0.053 mm fraction declined in MP+S, NT-Sand NT+S in the 0.00-0.05 and 0.05-0.20 m layers. Within the 0.00-0.20 m layer, the SOC stock in the >2 mm aggregate fraction was increased by 28.1, 56.1 and 88.4 %, and that in the <0.053 mm aggregate fraction decreased by 17.7, 30.3 and 34.2 % in MP+S, NT-S and NT+S than in MP-S. The SOC stock in the 2.00-0.25 mm aggregate fraction did not differ among the MP+S, NT-S and NT+S treatments, but was significantly increased compared to the 0.00-0.05 m layer

Lover	Treatment	Aggregate size					
Layer	Ireatment	>2 mm	2.00-0.25 mm	0.25-0.053 mm	<0.053 mm		
m		mg ha ⁻¹					
	MP-S	3.26 ± 0.25 d	3.11 ± 0.21b	2.52 ± 0.18 ab	2.74 ± 0.21a		
0.00.0.05	MP+S	4.40 ± 0.55 c	4.11 ± 0.32 a	2.69 ± 0.16 a	2.30 ± 0.28 b		
0.00-0.05	NT-S	5.69 ± 0.29 b	4.04 ± 0.06 a	1.87 ± 0.18 c	$1.92 \pm 0.06b$		
	NT+S	7.13 ± 0.18 a	4.48 ± 0.10 a	2.23 ± 0.24 bc	$1.96 \pm 0.1 \text{ b}$		
	MP-S	7.73 ± 0.36 c	11.69 ± 0.35 b	7.59 ± 0.86 a	11.90 ± 2.17 a		
0.05.0.20	MP+S	9.36 ± 0.81 b	14.49 ± 0.64 a	7.31 ± 0.76 a	9.62 ± 0.47 b		
0.05-0.20	NT-S	10.64 ± 1.11 b	10.65 ± 0.36 c	8.03 ± 0.51 a	8.24 ± 0.45 bc		
	NT+S	12.23 ± 0.44 a	12.54 ± 0.48 b	8.64 ± 0.67 a	7.14 ± 0.46 c		
	MP-S	2.55 ± 0.38 a	4.54 ± 0.24 b	6.65 ± 0.34 a	7.19 ± 0.39 a		
0 20 0 20	MP+S	2.73 ± 0.85 a	6.92 ± 1.04 a	6.84 ± 1.03 a	5.93 ± 0.76 bc		
0.20-0.30	NT-S	3.28 ± 0.36 a	4.78 ± 0.13 b	4.97 ± 0.60 b	6.77 ± 0.23 ab		
	NT+S	3.78 ± 0.43 a	6.52 ± 0.14 a	5.38 ± 0.24 b	5.83 ± 0.39 c		

Table 3. Soil organic carbon (SOC) storage (\pm standard deviation, n = 3) in varied aggregate size fractions in different soil layers as influenced by tillage

MP-S: moldboard plow without straw; MP+S: moldboard plow with straw; NT-S: no-tillage without straw; NT+S: no-tillage with straw. Different lower-case letters in the same soil layer mark significant differences between treatments at p < 0.05 in Duncan's test.

for MP-S treatment. There was a significant increase in SOC stock of macroaggregate in MP+S than in MP-S as well as in NT+S than in NT-S in the 0.05-0.20 and 0.20-0.30 m layers.

Humic and fulvic acids concentrations in different aggregate size fractions

In the 0.00-0.05 m layer, the humic and fulvic acids concentrations was higher in NT-S than in MP-S, as well as in NT+S than in MP-S for all the aggregate size fractions, especially the macroaggreate fraction (Figure 3). The average humic and fulvic acids concentrations for all the aggregate size fractions was43.9 % higher in NT-S than in MP-S, and 20.5 % higher in NT+S than in MP+S in the 0.00-0.05 m layer. However, an opposite trend was observed in the 0.05-0.20 and 0.20-0.30 m layers. For all the soil layers, the humic and fulvic acids concentrations in all the aggregate size fractions was increased with straw incorporation, by 36.5, 33.3 and 46.8 % in the 0.00-0.05, 0.05-0.20 and 0.20-0.30 m layers for the MP system, respectively, while 14.2, 32.1 and 24.7 %, respectively, for the NT system. When compared to MP+S, NT-S only significantly increased the humic and fulvic acids concentrations in the 2.00-0.25 mm aggregate fraction in the 0.00-0.05 m layer, but decreased it in all the aggregate fractions in the 0.05-0.20 and 0.20-0.30 m layers.

DISCUSSION

Changes in SOC distribution in soil profile

In this study, plots subjected to the NT system had greater SOC concentration for the 0.00-0.05 m layer than for the 0.05-0.20 and 0.20-0.30 m intervals. However, this effect was not so obvious for the MP system due to disturbance. This may be due to the greater root biomass in the 0.00-0.05 m layer under NT system. Other workers (Souza Nunes et al., 2011) have also reported that the NT system resulted in stratification of SOC, while the MP system resulted in a more homogeneous distribution in the 0.00-0.20 m layer.

When compared to MP-S, the SOC concentration and stock in NT-S was higher in the 0.00-0.05 m layer but lower in the 0.05-0.20 m layer. The same trend was also observed between NT+S and MP+S. These results paralleled those of Conceição et al. (2015). When considering the whole 0.00-0.30 m layer, however, the differences in SOC stock were not significant between NT-S and MP-S as well as between NT+S and MP+S (Table 2).



Figure 3. Humic and fulvic acids concentration in varied aggregate size fractions in different soil layers as influenced by tillage treatments.(a) 0.00-0.05 m; (b) 0.05-0.20 m; (c) 0.20-0.30 m. MP-S: moldboard plow without straw; MP+S: moldboard plow with straw; NT-S: no-tillage without straw; NT+S: no-tillage with straw. Error bars represent standard errors of the means (n = 3). Different lower-case letters within an aggregate size indicate significant differences between treatments according to Duncan's test (p<0.05).

This indicates that the NT system did affect the SOC stock distribution in the soil profile but not the total quantity, as suggested by Du et al. (2013).

In this study, straw incorporation treatment significantly increased the SOC stock in the soil profile under both NT and MP systems, and there existed a difference in SOC stock distribution in the soil profile between the NT and MP systems, which may be due to the difference in C input returned to the soil. The straw mainly concentrated on the soil surface under the NT system, but was distributed relatively evenly within the plough layer under the MP system. Consequently, C accumulation was significantly concentrated in the 0.00-0.05 m soil layer under the NT system and in the 0.00-0.20 m layer under the MP system.

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Changes in aggregation distribution in soil profile

We observed that tillage regimes obviously influenced soil aggregation distribution in the soil profile. In the upper 0.00-0.05 and 0.05-0.20 m layers, the NT system improved the formation level of the >2 mm aggregate but reduced the formation level of <0.053 mm aggregates, compared to the MP system, suggesting that mechanical operation reduced large-macroaggregate formation and disrupted soil macroaggregates into individual particles. Similar results have also been reported by Wohlenberg et al. (2004), Veiga et al. (2009), Huang et al. (2010) and Jiang et al. (2011). The higher proportion of >2 mm aggregates and lower proportion of <0.053 mm aggregates under NT systems might be the result of the higher soil hydrophobicity, low intensity of wetting and drying cycles, higher soil C concentration or the physical and chemical characteristics of large macroaggregates making them more resistant to breaking up (Balesdent et al., 2000; Eynard et al., 2006; Vogelmann et al., 2013).

When the straw was incorporated, C supply to the soil increased, resulting in production of microbial polysaccharides that increase aggregate cohesion; this could explain the progressive increase in large-macroaggregates in the 0.00-0.05 m layer under the NT system and macroaggregates in the 0.00-0.30 m layer under the MP system. Positive effects of straw application on macroaggregate formation and stability have also been reported by other workers (Singh et al., 2007; Veiga et al., 2009; Bandyopadhyay et al., 2010).

Changes in SOC distribution in different aggregate fractions

We found that the NT system had a greater SOC concentration in the macroaggregate fraction than the MP system in the 0.00-0.05 m layer. However, the NT system significantly reduced the SOC concentration of 2.00-0.25 mm aggregates in the 0.05-0.20 m layer, indicating that the tillage system particularly affected C storage and dynamics in macroaggregates. Because macroaggregates contained mostly labile C (Abiven et al., 2007), intensive tillage such as MP may cause a larger loss in labile C by destroying macroaggregates and exposing the protected SOC to soil organisms (Six et al., 2000; Jiang et al., 2011; Fuentes et al., 2012), thus reducing C accumulation. In contrast, the lower SOC concentration under NT system may be due to lower root biomass and straw incorporation in the subsoil.

For all the soil layers in this study, the SOC concentration and SOC stock in the macroaggregate fraction increased with straw incorporation, suggesting that straw incorporation favored C sequestration in aggregates in the top soil. This can be explained by the concept of aggregate hierarchy (Oades, 1984; Calonego et al., 2008) which states that fresh organic matter is the precursor to the formation and stabilization of macroaggregates. Moreover, the high content of polysaccharides in straw could lead to production of higher labile SOC in the macroaggregate fraction (Bandyopadhyay et al., 2010).

Humic and fulvic acids distribution in different aggregate fractions

Our data showed that humic and fulvic acids distribution in different aggregate fractions was similar to the SOC. The NT system promoted humic and fulvic acids concentrations in all the aggregate fractions compared to the MP system in the 0.00-0.05 m layer. However, opposite results were found in the 0.05-0.20 and 0.20-0.30 m layers. For all the soil layers, humic and fulvic acids in all the aggregate size fractions increased with straw incorporation. This can be explained by the fact that humic and fulvic acids are mainly derived from the humification of SOC.

Humic and fulvic acids are naturally resistant to microorganisms or are physically protected by adsorption on the mineral surfaces or bound inside the aggregates (Theng et al., 1989). Thus, it appears that the NT system favored an increase in the SOC stability in all the aggregate fractions in the 0.00-0.05 m layer, but not in the 0.05-0.20 and 0.20-0.30 m layers, while the use of straw favored an increase for all layers. Higher humic and fulvic acids concentrations in the aggregate fractions, particularly in the macroaggregate fraction, would benefit C sequestration.



CONCLUSIONS

Soil organic carbon (SOC) stock in bulk soil significantly increased in the 0.00-0.05 m layer but decreased in the 0.05-0.20 m layer under the no-tillage (NT) system in a double rice system.

No-tillage system promoted large macroaggregation in both the 0.00-0.05 and 0.05-0.20 m layers. Accordingly, associated SOC concentration, SOC stock and humic and fulvic acids concentrations within the >0.25 mm macroaggregate fraction also significantly increased in the 0.00-0.05 m layer in the NT system, while those within the 2.00-0.25 mm aggregate fraction were significantly reduced in the 0.05-0.20 m layer under the NT system. In conclusion, it may be stated that the adoption of the NT system that increases SOC stock in the 0.00-0.05 m soil profile might be dependent on the macroaggregate fractions and the high amount of associated SOC and humic and fulvic acids.

Straw incorporation increased not only the SOC stock in bulk soil, but also the proportion of the macroaggregate, aggregate-associated SOC and humic and fulvic acids concentrations within the aggregate, thus contributing to SOC accumulation.

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REFERENCES

Abiven S, Menasseri S, Angers DA, Leterme P. Dynamics of aggregate stability and biological binding agents during decomposition of organic materials. Eur J Soil Sci. 2007;58:239-47. doi:10.1111/j.1365-2389.2006.00833.x

Álvaro-Fuentes J, Arrúe JL, Gracia R, López MV. Tillage and cropping intensification effects on soil aggregation: Temporal dynamics and controlling factors under semiarid conditions. Geoderma. 2008;145:390-6. doi:10.1016/j.geoderma.2008.04.005

Anders MM, Brye KR, Olk DC, Schmid BT. Rice rotation and tillage effects on soil aggregation and aggregate carbon and nitrogen dynamics. Soil Sci Soc Am J. 2010;76:994-1004. doi:10.2136/sssaj2010.0436

Andruschkewitscha R, Kochb HJ, Ludwiga B. Effect of long-term tillage treatments on the temporal dynamics of water-stable aggregates and on macro-aggregate turnover at three German sites. Geoderma. 2014;217-218:57-64. doi:10.1016/j.geoderma.2013.10.022

Balesdent J, Chenu C, Balabane M. Relationship of soil organic matter dynamics to physical protection and tillage. Soil Till Res. 2000;53:215-30. doi:10.1016/S0167-1987(99)00107-5

Bandyopadhyay PK, Saha S, Mani PK, Mandal B. Effect of organic inputs on aggregate associated organic carbon concentration under long-term rice-wheat cropping system. Geoderma. 2010;154:379-86. doi:10.1016/j.geoderma.2009.11.011

Blanco-Moure N, Moret-Fernández D, López MV. Dynamics of aggregate destabilization by water in soils under long-term conservation tillage in semiarid Spain. Catena. 2012;99:34-41. doi:10.1016/j.catena.2012.07.010

Calonego JG, Rosolem CA. Soil aggregate stability after management with crop rotation and chiseling. Rev Bras Cienc Solo. 2008;32:1399-407. doi:10.1590/S0100-06832008000400004

Castro FC, Muzilli O, Podanoschi AL. Soil aggregate stability and its relation with organic carbon in a typic Haplorthox, as a function of tillage systems, crop rotations and soil sample preparation. Rev Bras Cienc Solo. 1998;22:527-38. doi:10.1590/S0100-06831998000300019

Ci E, Yang LZ, Cheng YQ, Shi LL, Yin SX. Effect of cultivation history on distribution of organic carbon and structure of humus in paddy soils. Acta Pedol Sin. 2008;45:950-6. doi:10.3321/j.issn:0564-3929.2008.05.022

Conceição PC, Boeni M, Bayer C, Dieckow J, Salton JC, Reis CES. Efficiency of the dense solutions in physical fractionation of soil organic matter. Rev Bras Cienc Solo. 2015;39:490-7. doi:10.1590/01000683rbcs20140447

Du ZL, Ren TS, Hu CS, Zhang QZ, Humberto BC. Soil aggregate stability and aggregateassociated carbon under different tillage systems in the north China plain. J Integr Agric. 2013;12:2114-23. doi:10.1016/S2095-3119 (13)60428-1

Ellert BH, Bettany JR. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. Can J Soil Sci. 1995;75:529-38. doi:10.4141/cjss95-075

Elliott ET, Cambardella CA. Physical separation of soil organic matter. Agric Ecosyst Environ. 1991;34:407-19. doi:10.1016/0167-8809(91)90124-G

Eynard A, Schumacher TE, Lindstrom MJ, Malo DD, Kohl RA. Effects of aggregate structure and organic C on wettability of Ustolls. Soil Till Res. 2006;88:205-16. doi:10.1016/j.still.2005.06.002

Eynard A, Schumacher TE, Lindstrom MJ, Malo DD. Effects of agricultural management systems on soil organic carbon in aggregates of Ustolls and Usterts. Soil Till Res. 2005;81:253-63. doi:10.1016/j.still.2004.09.012

FAO. World reference base for soil resources. ISSS-AISS-IBG, ISBN 92-5-104141-5; 1998.

Fuentes M, Hidalgo C, Etchevers J, De León F, Guerrero A, Dendooven L, Verhulst N, Govaerts B. Conservation agriculture, increased organic carbon in the topsoil macro-aggregates and reduced soil CO₂ emissions. Plant Soil. 2012;355:183-97. doi:10.1007/s11104-011-1092-4

Grossman RB, Reinsch TG. Bulk density and linear extensibility. In: Dane JH, Topp GC, editors. Methods of soil analysis; Physical method. Madison: SSSA; 2002. Pt.4. p. 201-28.

Gunina A, Kuzyakov Y. Pathways of litter C by formation of aggregates and SOM density fractions: implications from ¹³C natural abundance. Soil Biol Biochem. 2014;71:95-104. doi:10.1016/j.soilbio.2014.01.011

Hayes MHB, Clapp CE. Humic substances: considerations of compositions, aspects of structure, and environmental influences. Soil Sci. 2001;166:723-7. doi:10.1097/00010694-200111000-00002

Huang S, Sun YN, Rui WY, Liu WR, Zhang WJ. Long-term effect of no-tillage on soil organic carbon fractions in a continuous maize cropping system of northeast China. Pedosphere. 2010;20:285-92. doi:10.1016/S1002-0160(10)60016-1

Jiang X, Hu Y, Bedell JH, Xie D, Wright AL. Soil organic carbon and nutrient content in aggregate-size fractions of a subtropical rice soil under variable tillage. Soil Use Manage. 2011;27:28-35. doi:10.1111/j.1475-2743.2010.00308.x

Lv RK. Soil agricultural chemical analysis method. Beijing: China's Agricultural Science and Technology; 2000.

Ma L, Yang LZ, Ci E, Wang Y, Yin SX, Shen MX. Humus composition and stable carbon isotope natural abundance in paddy soil under long-term fertilization. Chinese J Appl Ecol. 2008;19:1951-8.

Nascente AS, Li Y, Crusciol CAC. Soil aggregation, organic carbon concentration, and soil bulk density as affected by cover crop species in a no-tillage system. Rev Bras Cienc Solo. 2015;39:871-9. doi:10.1590/01000683rbcs20140388

Nelson DW, Sommers LE. Total carbon, organic carbon, and organic matter. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Summer ME, editors. Methods of soil analysis; chemical methods. Madison: Soil Science Society of America; 1996. Pt 3. p.961-1010. (Book Series, 5). Nyamadzawo G, Nyamangara J, Nyamugafata P, Muzulu A. Soil microbial biomass and mineralization of aggregate protected carbon in fallow-maize systems under conventional and no-tillage in Central Zimbabwe. Soil Till Res. 2009;102:151-7. doi:10.1016/j.still.2008.08.007

Oades JM. Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil. 1984;76:319-37. doi:10.1007/BF02205590

Ou H, He MJ, Huang J, Zhu GY, Gu MH, Li XF, Shen FK. Effect of no-tillage and rice straw manuring on the combined forms of humus and microbial activities in paddy soil. Acta Ecol Sin. 2010;30:6812-20.

Ou HP, He MJ, Zhu GY, Huang J, Gu MH, Li XF, Shen FK. Effect of tillage on conversion of soil organic carbon in paddy soil. J South China Agric University. 2011;32:1-6. doi:10.3969/j.issn.1001-411X.2011.01.001

Singh G, Jalota SK, Singh Y. Manuring and residue management effects on physical properties of a soil under the rice-wheat system in Punjab, India. Soil Till Res. 2007;94:229-38. doi:10.1016/j.still.2006.07.020

Six J, Elliott ET, Paustian K. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. 2000;2:2099-103. doi:10.1016/S0038-0717(00)00179-6

Six J, Feller C, Denef K, Ogle SM, Morales Sá JC, Albrecht A. Soil organic matter, biota and aggregation in temperate and tropical soils effects of no tillage. Agronomie. 2002;22:755-75. doi:10.1051/agro:2002043

Souza Nunes R, Castro Lopes AA, Souza DMG, Carvalho Mendes I. Sistemas de manejo e os estoques de carbono e nitrogênio em Latossolo de cerrado com a sucessão soja-milho. Rev Bras Cienc Solo. 2011;35:1407-19. doi:10.1590 /S0100-06832011000400035

Stewart C, Plante A, Paustian K, Conant R, Six J. Soil carbon saturation: Linking concept and measurable carbon pools. Soil Sci Soc Am J. 2008;72:379-92. doi:10.2136/sssaj2007.0104

Tang XH, Luo YJ, Ren ZJ, Lü JK, Wei CF. Distribution characteristics of soil humus fractions stable carbon isotope natural abundance (δ13C) in paddy field under long-term ridge culture. Chinese J Appl Ecol. 2011;22:986-91. doi:10.13287/j.1001-9332.2011.0098

Theng BKG, Tate KR, Sollins P. Constituents of organic matter in temperate and tropical soils. In: Coleman DC, editor. Dynamics of soil organic matter in tropical ecosystems. Honolulu: University of Hawaii Press; 1989. p.5-31.

Urbanek E, Smucker AJM, Horn R. Total and fresh organic carbon distribution in aggregate size classes and single aggregate region using natural $^{13}C/^{12}C$ tracer. Geoderma. 2011;164:164-71. doi:10.1016/j.geoderma.2011.05.020

Veiga M, Reinert DJ, Reichert JM. Aggregate stability as affected by short and long-term tillage systems and nutrient sources of a Hapludox in southern Brazil. Rev Bras Cienc Solo. 2009;33:767-77. doi:10.1590/S0100-06832009000400003

Vogelmann ES, Reichert JM, Prevedello J, Awe GO, Mataix-Solera J. Can occurrence of soil hydrophobicity promote the increase of aggregates stability? Catena, 2013;110:24-31. doi:10.1016/j.catena.2013.06.009

Wohlenberg EV, Reichert JM, Reinert DJ, Blume E.Dinâmica da agregação de um solo franco-arenoso em cinco sistemas de culturas em rotação e em sucessão. Rev Bras Cienc Solo. 2004;28:891-900. doi:10.1590/S0100-06832004000500011

Zhang SX, Li Q, Lü Y, Zhang XP, Liang WJ. Contributions of soil biota to C sequestration varied with aggregate fractions under different tillage systems. Soil Biol Biochem. 2013;62:147-56. doi:10.1016/j.soilbio.2013.03.023

Zhang SX, Li Q, Zhang XP, Wei K, Chen LJ, Liang WJ. Effects of conservation tillage on soil aggregation and aggregate binding agents in black soil of Northeast China. Soil Till Res. 2012;124:196-202. doi:10.1016/j.still.2012.06.007

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