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# Returning Winter Cover Crop Residue Influences Soil Aggregation and Humic Substances under Double-cropped Rice Fields

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**ABSTRACT:** Residue management in cropping systems may improve soil quality. However, there are few studies on the effects of residue management on soil aggregation and carbon content in the humin (C-HUM), humic acid (C-HAF) and fulvic acid (C-FAF) fractions in South China. Therefore, the effects on soil aggregation and on the C-HUM, C-HAF, C-FAF from incorporating winter cover crop residues in a double-cropped rice (*Oryza sativa* L.) system in South China fields were studied. The experiment has been conducted since winter 2004. Five winter cropping systems were used: rice-rice-ryegrass (Ry-R-R), rice-rice-Chinese milk vetch (Mv-R-R), rice-rice-potato (Po-R-R), rice-rice-rape (Ra-R-R) and rice-rice with winter fallow (Fa-R-R). The results indicated that the organic C content in the paddy soil under the Ry-R-R, Mv-R-R, Po-R-R, and Ra-R-R systems was significantly higher than the content in the Fa-R-R system at the early rice and late rice maturity stages. The different sizes of aggregates under the five treatments showed similar trends. The Po-R-R systems had the highest percentage of soil aggregates in each size class and the Fa-R-R systems had the lowest percentage of soil aggregates in each size class in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m soil depth at the early rice and late rice maturity stages. The C-HUM, C-HAF, and C-FAF increased through long-term application of winter cover crop residues. Statistical analysis showed that the C-HAF under the Ra-R-R systems was significantly higher than that in the Fa-R-R systems at the early rice and late rice maturity stages. The C-FAF and C-HUM under the Mv-R-R systems was significantly higher than the C-FAF and C-HUM in the Fa-R-R systems at the early rice and late rice maturity stages. As a result, the soil organic C content, the soil aggregates in each size class, and the C-HUM, C-HAF, and C-FAF increased from application of winter cover crop residues in double-cropped rice systems.

**Keywords:** paddy, residue management, soil aggregation, soil carbon carbon.**\* Corresponding author:**

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## INTRODUCTION

Soil aggregation is an important facet of soil structure, providing resistance to wind and water erosion, physical protection of organic matter, and microsites for microbial activity. Plants affect structure at different scales and through various direct and indirect mechanisms, involving effects on different organic matter fractions (such as fungal biomass, hyphal length, labile polysaccharides, hydrophobic aliphatics, and microbial biomass) (Angers and Caron, 1998). Moreover, plant roots can enmesh and stabilize soil aggregates and can also promote soil aggregation by releasing material that can directly stabilize soil particles or by favoring microbial activity that, in turn, will affect soil aggregation (Tisdall and Oades, 1982). However, by penetrating the soil, roots create zones of failure, which contribute to fragmentation of the soil and formation of smaller aggregates (Angers and Caron, 1998).

Decomposing plant residues promote soil aggregation, and the magnitude of the effect is related to the decomposability of the material (Abiven et al., 2008). Potentials of less than -4 kPa prevented loss of plants that developed in aggregates of less than 2 mm diameter after a transitory period of waterlogging, although some shoot and root damage occurred (Shiel et al., 1987). Differences in soil aggregation and distribution of fulvic acid among experiment sites were attributed to the flooding effect (Kimura et al., 2017). Elevated O<sub>3</sub> influenced the formation and transformation of soil aggregates and the distribution of soil organic carbon (SOC) in the aggregates across soil layer classes (Kou et al., 2014). Soils under paddy cultivation displayed improved soil aggregate structure and altered the distribution patterns of P fractions in different aggregate size classes (Li et al., 2016).

The soil organic matter (SOM) fractions are more sensitive for revealing changes caused in the soil coming from the different management systems compared to the total organic carbon (TOC) (Briedis et al., 2012). Some studies showed that the decomposition of organic matter was reduced in flooded soil, such as in submerged rice paddy soils (Bell, 1969). Results from Bechtold and Naiman (2009) suggest that basic influences on SOM retention in these paddy soils are not functionally different than those that apply to upland soils. Other research results showed that SOM sequestration improved with the addition of organic matter and straw incorporated into paddy soils (Shan et al., 2008; Kader et al., 2011). Returning winter cover crop residues to the soil as fertilizer affected the soil physical properties.

Soil humus substances are relatively stable and resistant to biological decomposition. However, they may be significantly modified by agrotechnologies of different intensities. Fertilizer application systems should improve the quality of the soil humus. The effect of different fertilizer systems on the group and fractional composition of the humus should be studied. At present, despite numerous investigations on that composition, there are different opinions concerning the effect of fertilizers on the qualitative composition of humus. The effect of organic fertilizers on the transformation of soil humus has been the subject of less study. Most scientists point to their favorable effect on the group and fractional composition of soil humus (Matveeva et al., 1976). However, some scientists (Brodowski et al., 2007) argue that the application of organic fertilizers cannot considerably change the qualitative composition of soil humus. According to Matveeva et al. (1976), the long-term application of mineral fertilizers does not significantly affect humus quality. Other researchers (Donskikh et al., 1997) argue that mineral fertilizers cause unfavorable changes in the qualitative composition of humus, with an increase in the content of the aggressive fractions of fulvic acids.

Winter cover crops, which are grown during an otherwise fallow period, are a possible means of improving nutrient dynamics in the surface layer of intensively managed cropping systems. Hermawan and Bomke (1997) suggested that growing winter cover crops such as annual ryegrass may protect aggregate breakdown during winter and result in a

better soil structure after spring tillage, as opposed to leaving soil bare. Other potential benefits of winter cover crops are prevention of nitrate leaching and weed infestation, and improvement in soil water retention, SOM content, and microbial activity (Powlson et al., 1987). Recycling of crop residues has been suggested to improve overall soil conditions, reduce the requirement for nitrogen (N) fertilizers, and support sustainable rice yield. Chinese milk vetch, ryegrass, and rape are the main winter cover crops in China.

In recent years, many studies have shown that C accumulation, aggregation, and humus substances in soil are affected by soil tillage and residue management, application of fertilizer and organic matter, crop rotations, and field management (Wei-Xiang et al., 2004). However, relatively few studies on soil C accumulation, soil aggregation, and soil humus substances in a double-rice cropping system with winter cover crop rotations have been conducted in southern China.

There is a growing concern that the soil aggregation and chemical SOM fraction in soil were change under different residue management systems. Does application with winter cover crop residue increase the different sizes of soil aggregates and SOM content? Is winter cover crop residue application a viable option to maintain SOM content and physical quality? The objectives of this study were to determine the effects on soil aggregation and chemical SOM fractions of returning different winter cover crop residues to the soil in a double-cropping rice system.

## MATERIALS AND METHODS

### Site description

The experiment was conducted at the experimental station of the Institute of Soil and Fertilizer Research, Hunan Academy of Agricultural Science, China (28° 11' 58" N, 113° 04' 47" E) from winter 2004 to winter 2015. The typical cropping system in this area is double-cropped rice. The soil is a silty light clayey paddy soil from Quaternary red earth, which is classified as Fe-Typic Hapli-Stagnic Anthrosols (Soil Survey Staff, 2014). The properties of the surface soil (0.00-0.20 m) are pH(H<sub>2</sub>O) 5.40, soil organic carbon (SOC) 13.30 g kg<sup>-1</sup>, total N 1.46 g kg<sup>-1</sup>, available N 154.5 mg kg<sup>-1</sup>, total P 0.81 g kg<sup>-1</sup>, available P 39.2 mg kg<sup>-1</sup>, total K 13.0 g kg<sup>-1</sup>, and available K 57.0 mg kg<sup>-1</sup>. The principal physical and chemical properties of surface soil was determined according to the method described by Klose et al. (2001). All these values were determined before the experiment in 2004. This region has a subtropical monsoonal humid climate, with a long hot period and short cold period. The average annual rainfall is approximately 1,500 mm and the annual mean temperature is 17.1 °C. The annual frost-free period ranges from 270 to 310 days.

### Experimental design and soil sampling

The winter cover crops in the cropping systems were planted after harvesting double cropped rice every year. The study was continuously conducted for 12 years after recycling straw from the winter cover crop. The five cropping systems used were: rice-rice-ryegrass (Ry-R-R), rice-rice-Chinese milk vetch (Mv-R-R), rice-rice-potato (Po-R-R), rice-rice-rape (Ra-R-R), and rice-rice with winter fallow (Fa-R-R). A randomized block design was adopted in the plots, with three replications. The plot area was 1.1 m<sup>2</sup> (1 × 1.1 m). Before sowing the winter cover crops, 75.0 kg ha<sup>-1</sup> of N (163.0 kg urea) and 45.0 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> (375.0 kg diammonium phosphate) were applied as basal fertilizer. The winter cover crops planted in winter 2004 were cut and incorporated into the soil by means of a disk plow (conventional tillage) in the following spring. When winter cover crops were harvested, the straw residue of ryegrass, Chinese milk vetch, potato, and rape straw residue were weighed and returned at the same quantity of 22,500 kg ha<sup>-1</sup> to the soil surface. The plots were plowed once to a depth of 0.20 m with a moldboard plow fifteen days before transplanting rice seedlings.

One-month-old seedlings were transplanted at a density of 150,000 plants ha<sup>-1</sup> (one seed per 0.16 × 0.16 m, with 2-3 plants per hill). For both early and late rice, basal fertilizer was applied at the rate of 150.0 kg ha<sup>-1</sup> of N as urea (60 %, basal; 40 %, top-dressed at the tillering stage), 75.0 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> as diammonium phosphate, and 120.0 kg ha<sup>-1</sup> of K<sub>2</sub>O as potassium sulfate.

Data were collected during the early and late rice harvest in 2015. Soil samples in the plowed layer (0.00-0.05, 0.05-0.10, and 0.10-0.20 m) were collected from a point centered among four hills of rice plants by using a soil auger at early and late rice harvest.

### Soil organic carbon analysis

Soil samples were air dried and crushed with a wood roller to pass through a 2 mm mesh, and a subsample of approximately 20 g was further crushed in a mortar to pass through a 250 μm mesh. About 400 mg was analyzed in a dry combustion Shimadzu TOC-VCSH analyzer. To calculate the total organic C stock in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m layers, the equivalent soil mass approach was adopted, following the procedure employed by Ellert and Bettany (1995).

### Aggregate size distribution

Soil sample blocks with preserved structure were manually and gently disrupted in the planes of weakness to obtain aggregates of <9.51 mm diameter, which were air dried. Duplicates of about 50 g of these aggregates were capillary wetted overnight in funnel-folded filter paper, gently transferred into a 1000 mL cylinder (0.072 m diameter × 0.235 m height) containing 500 mL water, and end-over-end shaken for 2 min under 16 rotations per minute, according to an adaptation of the method proposed by Tisdall et al. (1978).

### Soil humus composition

Humic substances of SOM were fractionated into the fulvic acid fraction (FAF), humic acid fraction (HAF), and humin (HUM) using differential solubilization, as proposed by the International Humic Substance Society and adapted by Benites et al. (2003). Quantification of organic carbon in the fractions (C-HUM, C-FAF, and C-HAF) was performed in accordance with Yeomans and Bremner (1988).

### Statistical analysis

All data were expressed as mean ± standard error. The data were analyzed as a randomized complete block, using the analysis of variance (Anova) procedure of SAS (2003). The Tukey-HSD was calculated for comparison of the treatment means. Mean values were compared using the least significant difference (LSD) test, and a probability value of 0.05 was considered to indicate statistical significance.

## RESULTS

### Total organic carbon

Recycling the straw from winter cover crops significantly affected total organic carbon (TOC) content in the soil (Table 1). In the 0.00-0.20 m soil depth, the TOC content under the Ry-R-R, Mv-R-R, Po-R-R, and Ra-R-R systems was significantly higher than that of the Fa-R-R system at the early rice and late rice maturity stages. The TOC content in the paddy soil under the Ry-R-R, Mv-R-R, Po-R-R, and Ra-R-R systems was significantly higher than the TOC content in the Fa-R-R system. The TOC content was nearly constant within the 0.00-0.20 m layer of Ry-R-R, Mv-R-R, Po-R-R, Ra-R-R, and Fa-R-R soil, while stratification with higher contents in the 0.00-0.05 m layer occurred in Ry-R-R, Mv-R-R, Po-R-R, Ra-R-R, and Fa-R-R soils, compared to 0.05-0.10 m and 0.10-0.20 m (Table 1).

**Table 1.** Total organic carbon content in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m layers at the early rice and later rice maturity stages

Treatment	Early rice			Later rice		
	0.00-0.05 m	0.05-0.10 m	0.10-0.20 m	0.00-0.05 m	0.05-0.10 m	0.10-0.20 m
	g kg <sup>-1</sup>					
Ry-R-R	20.54±0.60b	18.74±0.59c	17.14±0.51ab	21.07±0.59ab	19.67±0.55b	16.60±0.48ab
Mv-R-R	20.67±0.56b	19.07±0.55b	17.41±0.50ab	21.79±0.61a	20.49±0.59ab	16.66±0.47ab
Po-R-R	23.37±0.59a	20.55±0.54a	17.76±0.47a	22.37±0.65a	21.72±0.63a	17.93±0.48a
Ra-R-R	19.56±0.67b	18.55±0.54d	16.13±0.49b	20.60±0.63b	19.19±0.57b	16.39±0.52b
Fa-R-R	18.87±0.54c	17.96±0.52e	15.68±0.45c	19.60±0.57c	18.76±0.55c	15.70±0.47c

Ry-R-R: rice-rice-ryegrass cropping system; Mv-R-R: rice-rice-Chinese milk vetch cropping system; Po-R-R: rice-rice-potato cropping system; Ra-R-R: rice-rice-rape cropping system; Fa-R-R: rice-rice cropping system with winter fallow. Different letters indicate significance at  $p < 0.05$ , according to the least significant difference test.

### Soil aggregation

For different treatments, the size classes of soil aggregates followed the sequence: 0.05-0.01 mm > 0.25-0.05 mm > 1-0.25 mm > 0.01-0.005 mm > 0.005-0.001 mm > 0.001 mm at the early rice and late rice maturity stages. With the addition of the winter cover crop residues, the percentage of soil aggregates in each size class in the Po-R-R, Mv-R-R, Ry-R-R, and Ra-R-R soils was higher than the percentage in the Fa-R-R (Figure 1). In other words, the percentage of soil aggregates of each size class increased from the application of cover crop residues in the rice growing season. At the 0.00-0.20 m soil depth, the percentage of soil aggregates in each size class decreased as follows: Po-R-R > Mv-R-R > Ry-R-R > Ra-R-R > Fa-R-R systems at the early rice and late rice maturity stages.

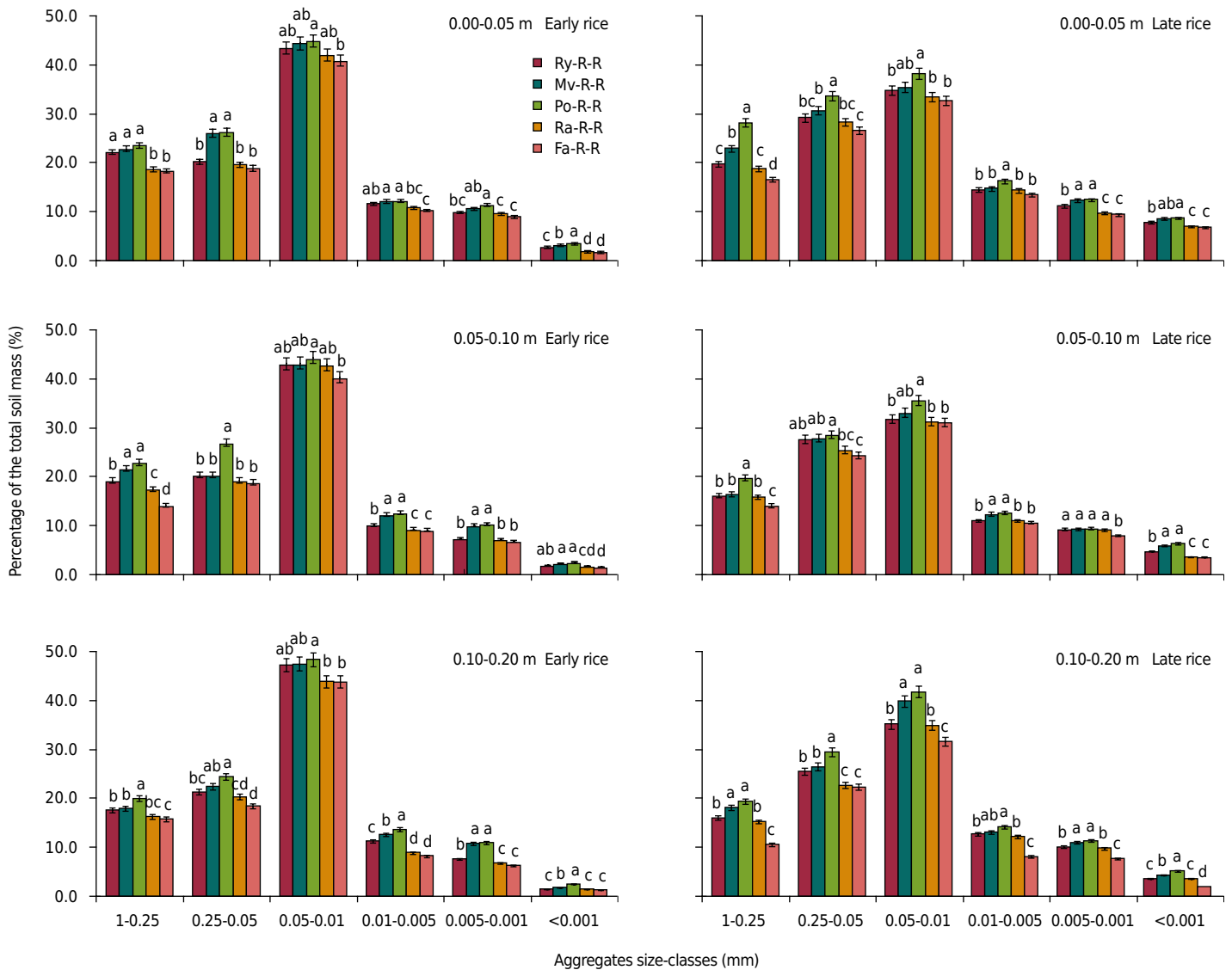
The distribution patterns of different sizes of soil aggregates were similar under the Po-R-R, Mv-R-R, Ry-R-R, Ra-R-R, and Fa-R-R systems. The Po-R-R system had the highest percentage of soil aggregates in each size class and the Fa-R-R system had the lowest percentage of soil aggregates in each size class in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m soil depths at the early rice and late rice maturity stages. Statistical analysis showed that the Po-R-R system had significantly higher ( $p < 0.05$ ) amounts of all aggregate size fractions than the Ra-R-R and Fa-R-R systems. There were no significant differences ( $p > 0.05$ ) for any aggregate size fractions under the Ra-R-R and Fa-R-R systems at the early rice maturity stages.

### Soil humus composition

Recycling the straw from winter cover crops significantly affected soil humus composition (Table 2). In the 0-20 m soil depth, the soil humic acid fraction (C-HAF), fulvic acid fraction (C-FAF), and humin (C-HUM) under the Ry-R-R, Mv-R-R, Po-R-R, and Ra-R-R systems were higher than that of Fa-R-R system at the early rice and late rice maturity stages.

The Ra-R-R system had the highest C-HAF, and the Fa-R-R system had the lowest C-HAF in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m soil depths at the early rice maturity stage. The Mv-R-R system had the highest C-FAF and the Fa-R-R system had the lowest C-FAF in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m soil depth at the early rice maturity stage. The Ry-R-R system had the highest C-HUM, and the Fa-R-R system had the lowest C-HUM in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m soil depth at the early rice maturity stage. Statistical analysis showed that the C-HAF under the Ra-R-R system was significantly higher than the C-HAF in the Fa-R-R system. The C-FAF under the Mv-R-R system was significantly higher than the C-FAF in the Fa-R-R system. The C-HUM under the Ry-R-R system was significantly higher than the C-HUM in the Fa-R-R system.

The Ra-R-R system had the highest C-HAF and the Fa-R-R system had the lowest C-HAF in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m soil depth at the late rice maturity stage. The Ry-R-R system had the highest C-FAF and C-HUM, and the Fa-R-R system had the



**Figure 1.** Aggregate size class distribution in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m layers at the early rice and later rice maturity stages. Ry-R-R: rice-rice-ryegrass cropping system; Mv-R-R: rice-rice-Chinese milk vetch cropping system; Po-R-R: rice-rice-potato cropping system; Ra-R-R: rice-rice-rape cropping system; Fa-R-R: rice-rice cropping system with winter fallow. The lowercase letters above the bars compare the five treatments within the same cropping system according to the Tukey test ( $p < 0.05$ ).

**Table 2.** Soil humus composition in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m layers at the early and later rice maturity stage

Treatment	C-HAF			C-FAF			C-HUM		
	0.00-0.05 m	0.05-0.10 m	0.10-0.20 m	0.00-0.05 m	0.05-0.10 m	0.10-0.20 m	0.00-0.05 m	0.05-0.10 m	0.10-0.20 m
g kg <sup>-1</sup>									
Early rice maturity stage									
Ry-R-R	3.69±0.10b	3.42±0.10b	2.98±0.08b	5.88±0.17a	5.01±0.14a	4.04±0.12b	13.8±0.40a	12.4±0.35a	10.0±0.29a
Mv-R-R	3.69±0.10b	3.51±0.10a	3.31±0.10a	5.47±0.16a	4.96±0.14a	4.81±0.14a	11.5±0.35bc	10.5±0.30a	8.8±0.28bc
Po-R-R	3.60±0.12b	3.42±0.10b	2.72±0.10bc	4.51±0.13b	4.15±0.12b	3.34±0.11c	11.9±0.34b	11.1±0.35b	9.4±0.27ab
Ra-R-R	4.39±0.10a	3.51±0.10a	3.42±0.09a	4.54±0.13b	4.30±0.12b	4.01±0.10b	12.1±0.33b	12.2±0.32b	9.8±0.25a
Fa-R-R	3.51±0.10b	3.33±0.10b	2.68±0.08c	4.42±0.12b	4.14±0.12b	3.15±0.09c	10.8±0.31c	10.1±0.29b	8.3±0.24c
Later rice maturity stage									
Ry-R-R	2.73±0.08b	2.55±0.07ab	2.00±0.05a	4.41±0.13a	3.41±0.10a	3.26±0.10a	16.28±0.47a	16.00±0.46a	13.15±0.38a
Mv-R-R	2.73±0.07b	2.59±0.07ab	2.05±0.06a	3.47±0.10b	3.30±0.10a	3.23±0.10a	13.99±0.43c	13.66±0.42b	11.13±0.35b
Po-R-R	2.39±0.10c	2.41±0.08b	1.68±0.06b	3.40±0.10b	2.93±0.09b	2.71±0.09b	14.84±0.41bc	13.75±0.40b	11.99±0.35b
Ra-R-R	3.14±0.08a	2.69±0.08a	2.05±0.06a	3.43±0.10b	3.15±0.09ab	3.11±0.08a	15.65±0.45ab	14.61±0.39b	12.09±0.32b
Fa-R-R	2.05±0.06d	2.05±0.06c	1.68±0.06b	3.03±0.09c	2.52±0.07c	2.51±0.08b	13.99±0.40c	13.53±0.39b	3.26±0.10c

C-HAF: humic acid fraction; C-FAF: fulvic acid fraction; C-HUM: humin. Ry-R-R: rice-rice-ryegrass cropping system; Mv-R-R: rice-rice-Chinese milk vetch cropping system; Po-R-R: rice-rice-potato cropping system; Ra-R-R: rice-rice-rape cropping system; Fa-R-R: rice-rice cropping system with winter fallow. Different letters indicate significance at  $p < 0.05$ , according to the least significant difference test.

lowest C-FAF and C-HUM in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m soil depth at the late rice maturity stage. Statistical analysis showed that the C-HAF under the Ra-R-R system was significantly higher than the C-HAF in the Fa-R-R system. The C-FAF and C-HUM under the Mv-R-R system was significantly higher than those in the Fa-R-R system.

## DISCUSSION

### Soil aggregation and winter cover crop residues applied

In this study, these differences among the treatments in soil aggregates in each size class are due to the different direct and indirect mechanisms of effects on soil aggregation of winter cover crop residues applied, such as the release of different organic matter fractions, influences on microbial activity, soil drying, and enmeshing of soil particles by roots (Angers and Caron, 1998). Furthermore, the crop sequences may have influenced the magnitude and frequency of the wetting and drying cycles, which influence aggregate stability (Caron et al., 1992b). Moreover, the drying of soil by the roots may also act synergistically with the aggregate binding material produced in the rhizosphere and increase the water-stability of aggregates. The drying that occurs in the mucilage production zone increases the efficiency of the cementing agents, which increases the sorption of the binding material onto mineral surfaces (Caron et al., 1992a).

Another important factor related to the influence of crop sequence on the stability of soil aggregates is the quantity of winter cover crop residues incorporated into the soil. In this study, the potato straw residues also contributed to greater stability of soil aggregates in the Po-R-R system. Considering the importance of roots in soil aggregates (Tisdall and Oades, 1982), the difference in the quantity and level of the renewal rate of roots through application of different winter cover crop residues may have contributed to the differences observed in soil aggregates in the soil. The highest percentage of soil aggregates in each size class in the Po-R-R system may be due to the root system of rice promoted by application of potato straw residues.

In this study, the highest organic carbon contents were in the Po-R-R system and the lowest organic carbon contents were in the Fa-R-R system. Other studies also found the lowest organic carbon contents and lowest percentage of the total soil mass in the aggregates <0.25 mm (Liu et al., 2005). The variation in the organic C contents and aggregates of different size classes may be due to an accumulation of decomposition products from winter cover crop residues in certain size classes of aggregates and also to the greater presence of fungal hyphae and small roots in the macroaggregates (Tisdall and Oades, 1982).

### Soil humus composition and winter cover crop residues applied

The formation of humic substances in natural environments is related to microbial activity (Machado and Gerzabeck, 1993) and humification over time is therefore a final product of microbiological processes. The C-HUM, C-HAF, and C-FAF increased through application of winter cover crop residues. It should be noted that the different winter cover crop residue treatments generally had a positive effect on transformation of the group and composition of the soil humus, with an increase in the C-HUM, C-HAF, and C-FAF, and this could be attributable to slow accumulation rates of these fractions in the soils without winter cover crop residues. The application of winter cover crop residues may exert a mobilizing effect on the soil humus, this can be attributed to the increase of C-HUM, C-HAF, and C-FAF. Higher humic fractions in the soils with winter cover crop residues could be attributable to fine roots, which serve as an important source of stable fractions of SOM. In contrast with the rates of loss of humic substances, climate did not seem to influence rates of gain of these humic substances (Matveeva et al., 1976). Therefore, we inferred that applications of winter cover crop residues in the paddy field played a key role. Returning winter cover crop residues to the soil as fertilizers can



improve plant biomass, the primary source of organic matter, and/or reduce or improve microbial activity and decomposition rates, which ultimately influence organic matter content in soils (Gregorich et al., 1994).

As observed for TOC stocks, similarity in humic fractions among cropping systems shows that the crop rotation used in different cropping systems induces similar increases in C and in humic substances in the subsurface soil layers. Root systems of Po-R-R, Mv-R-R, and Ry-R-R may account for this result.

With regard to cropped areas in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m soil depths, the Mv-R-R system had the highest C-HUM and C-FAF. The C-HAF was also greater in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m depths in the Ra-R-R system (Table 2). These results may be due to the use of winter cover crops, which produce plant residues with a high C/N ratio. Moreover, slower decomposition of Ra-R-R residues raises C-HAF production. Similar results were reported by Rossi et al. (2011), who found that the content of the humic fraction and the humic and fulvic acid fractions increased by application of soybean straw.

The highest C-FAF was found in the Mv-R-R system in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m depths. Compared to the C-HAF, the C-FAF has lower molecular weight and higher mobility in the soil, favored by greater plant residue intake, and this results in a higher tendency of humification. In the Fa-R-R system, in addition to lack of winter cover crop residues, lower dry matter production (Table 2) and lower Ca contents and stocks may compromise C-HAF formation, promoting a relative rise in C-FAF. Similar results were found by Rossi et al. (2011), who reported that the C-FAF increased by application of soybean straw.

Since the humin fraction corresponds to nearly 70 % of the TOC, this may be a result of higher TOC stocks in the Ry-R-R system in the 0.00-0.05, 0.05-0.10, and 0.10-0.20 m depths. Regarding the depth factor, for C-HUM, all systems evaluated showed the same pattern, with values of C-HUM decreasing with increasing depth. This indicates that, regardless of the double-cropping rice system, the addition of winter cover crop residues from crop rotation and the dry matter of cover crops in double-cropping rice areas favors greater interaction with the mineral fraction and the organic fraction in the surface layer. This leads to higher concentrations of C-HUM, which has greater chemical stability and less variation in depth, as other studies have reported (Rossi et al., 2011).

In double-cropping rice areas, differences in the C-FAF in depth may result from migration of organic acids of low molecular weight (i.e., citric, malic, oxalic, and tartaric acid), which favors the migration of C-FAF in depth, due to its greater mobility (Franchini et al., 2003). This results in homogenization of the C-FAF in the different cropping systems. In the double-cropping rice area, the contribution of more lignified plant material, mainly derived from winter cover crop residues, probably through their decomposition, should release smaller amounts of low molecular weight organic acids. This larger pattern in the surface is due to the accumulation of plant material as depth decreases (Table 2). These results are similar to studies by Rossi et al. (2011), working with soils and crop rotation, including millet, sorghum, and brachiaria grass.

## CONCLUSIONS

Soil organic carbon content, soil aggregation, and soil humus composition were affected by winter cover crop residues applied under a double-cropping rice system.

The potato straw residues applied under a double-cropping rice system in a South China paddy field provided the highest water-stability of soil aggregates. This crop sequence and winter cover crop residue provided the highest total organic carbon content and soil aggregates with a diameter of 0.05-0.01 mm.

The regular application of different winter cover crop residues resulted in the transformation of the composition of the humus in the paddy soil. The C-HUM, C-HAF, and C-FAF increased through long-term application of winter cover crop residues.

There were significant differences among effects of the winter cover crops (ryegrass, Chinese milk vetch, potato, rape) on the soil aggregation and soil humus composition.

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