Effects of substitution of chemical fertilizer by Chinese milk vetch on distribution and composition of aggregates-associated organic carbon fractions in paddy soils

Yanan Huang · Li Huang · Jun Nie · Mingjian Geng · Yanhong Lu · Yulin Liao · Bin Xue

Received: 1 June 2022 / Accepted: 19 August 2022 / Published online: 31 August 2022
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2022

Abstract

Purpose Green manure plays a key role in reducing chemical fertilizer applications and increasing soil organic carbon (SOC) stock. The effects of chemical fertilizer substitution by Chinese milk vetch (MV) on the distribution and composition of organic carbon fractions in macroaggregates and microaggregates and SOC stability mechanism were investigated in paddy soils in southern China.

Methods A 10-year (2008–2018) field experiment was conducted, including no fertilizer (CK), 100% NPK fertilizer (F100), MV with different percentages of chemical fertilizer (MV+F100, MV+F80, MV+F60 and MV+F40). The soil was separated into distinct organic carbon fractions using aggregate density fractionation and SOC chemical structure was analyzed by fourier-transform infrared and nuclear magnetic resonance.

Results Chemical fertilizer substitution by MV increased SOC contents in the bulk soil by 9.1% (MV+F80), 5.8% (MV+F60), 17.9% (MV+F40) compared to F100. Organic carbon fraction mainly existed in mineral associated organic carbon (mSOC), accounting for 70.3–83.7% and 69.4–84.0% of the relative mass of macroaggregates and microaggregates, respectively. Compared to F100, aromatic C increased by 11.6% and 29.1% under MV+F60 within mSOC in macroaggregates and microaggregates. Within the mSOC in macroaggregates, compared to CK, MV+F80 and MV+F60 promoted the proportion of alkyl C by 9.6% and 6.7%, and decreased the content of O-alkyl C by 14.1% and 11.0%, respectively, which correspondingly increased alkyl C/O-alkyl C ratio.

Conclusions Substitution of chemical fertilizer by MV (especially MV+F80 and MV+F60) improved the stability of SOC via increasing recalcitrant structure in mSOC, and was conducive to the sequestration of SOC in paddy soils.

Keywords Chinese milk vetch · Mineral associated organic carbon · Organic carbon · Organic carbon fraction · Soil aggregate
Introduction

Since rice is one of the most important cereal crops in China, it is necessary to improve paddy soils nutrient distribution, increase rice production and ensure food security (Zhang et al. 2021). Recently, the abuse of chemical fertilizers leads to soil degradation and soil acidification (Song et al. 2022). As a leguminous green manure, Chinese milk vetch (MV) fixes atmospheric nitrogen (N) and provides green and cheap N fertilizer (Voisin et al. 2014). Chemical fertilizer combined with MV has advantages in increasing total N and mineral N, and alters soil microbial community structure compared to single chemical fertilizer (Zhou et al. 2020). Green manure plays a key role in mitigating greenhouse gas emission (Kopittke et al. 2020; Song et al. 2021), reducing chemical fertilizer applications (Zhou et al. 2020), increasing soil organic carbon (SOC) stock (Yao et al. 2019; Khan et al. 2020).

Aggregate-protected SOC was critical to carbon stabilization (Okolo et al. 2020), and 0.25–2 mm aggregates protected SOC from degradation by preventing the effects of enzyme and microorganism on SOC (Chai et al. 2019). SOC associated with 0.053–0.25 and <0.053 mm aggregates were more stable and represented a long-term SOC storage (Torres-Sallan et al. 2017; Totsche et al. 2018). Long-term winter cultivation of MV significantly \(<0.05\) increased the content of SOC in >5, 2–5 and <0.25 mm aggregates compared with winter fallow treatment in paddy soils (Yang et al. 2014). The combined application of manure and NPK fertilizer consistently increased the SOC content in 0.25–2 and 0.053–0.25 mm aggregates, compared to NPK fertilizer (Duan et al. 2021). SOC concentrations of 0.25–2 and 0.053–0.25 mm under chemical fertilizer reduced by MV were 18.0% and 11.2% higher than control treatment in paddy soils (Kamran et al. 2021).

SOC existed in two physical fractions with distinct formation, persistence and function, as particulate organic carbon (POC) and as mineral associated organic carbon (mSOC) (Lavallee et al. 2020). The mSOC dominated soil carbon storage (Witzgall et al. 2021). The application of manure promoted the accumulation of SOC, which was attributed to the increase of SOC content in mSOC within macroaggregates and fine particulate organic carbon (fPOC) within microaggregates (Wen et al. 2021). The coarse particulate organic carbon (cPOC) was considered to be a more labile SOC pool and responded to management to a greater extent than fPOC in paddy soils (Benbi et al. 2012). Manure applications accelerated the formation of stable microaggregates within macroaggregates by promoting the transformation from cPOC to fPOC and the accumulation of the latter (Liang et al. 2014). In contrast, the addition of NPK fertilizer mainly increased the amount of SOC in cPOC and mSOC and had little effect on fPOC, which inhibited the formation of microaggregates within macroaggregates to a certain extent (Jiang et al. 2017). Macroaggregate turnover was the key process affecting the stability of SOC (Six et al. 2004). Green manure or reduce tillage had higher the ratio of fPOC to cPOC paralleled by shower turnover rate of macroaggregates, promoting organic carbon sequestration (Six et al. 2000; Garcia-Franco et al. 2015).

The evolution of organic carbon chemical structure varied among SOC fractions. The fPOC within macroaggregates under manure application was abundant in polysaccharide C and aliphatic C favorable for microbial growth (Wen et al. 2021). The addition of maize stalks induced O/N alkyl C to increase from about 50% to 70% in occluded POC, suggesting the dominance of polysaccharides (Witzgall et al. 2021). The mSOC contained higher ratio of alkyl C/O-alkyl C than POC under different land uses, showing that mSOC accumulated slightly advanced decomposed organic carbon (Yeasmin et al. 2020). In the POC and mSOC, the relative concentration of alkyl C increased under anaerobic conditions and straw incorporation, promoting the stability of SOC (Chen et al. 2018).

Previous studies regarding manure substitution and green manure returning mainly focused on SOC in the bulk soil and crop yield (Zhou et al. 2020; Lan et al. 2022; Song et al. 2022), and there were relatively few studies on the response of aggregates-associated organic carbon fractions to substitution of chemical fertilizer by MV. The decomposition and transformation of organic carbon in paddy soils were different from those in dry land soils due to anthropogenic disturbances and submerging conditions in paddy soils (Xu et al. 2007; Liu et al. 2018). Better knowledge of the organic carbon fractions and SOC functional groups in aggregates may contribute to the understanding of SOC stabilization in paddy soils. It was hypothesized that compared to microaggregates, the stable mSOC in macroaggregates and its chemical compositions more sensitively reflected the effect
of MV substitution chemical fertilizer on SOC pool in paddy soils. The objectives of this study were to investigate: i) the effect of MV with 40–100% chemical fertilizer applied on size distribution of soil aggregates and relate these to SOC dynamics; ii) explore organic carbon fractions contents and its chemical groups in macroaggregates and microaggregates; iii) illuminate the distribution, composition and stability mechanism of SOC in paddy soils aggregates under 10-year MV returning in southern China.

Materials and methods

Experimental site

The field experiment was conducted in 2008 in typical paddy soils at Sanxianhu Town (29°13′ N, 112°28′ E), Nan County, Hunan Province of China (Fig. 1). Elevation is about 28.8 m, climate in the region is subtropical monsoon humid with annual mean temperature 16.6 °C and annual mean precipitation 1238 mm. In the initial plough layer (0–20 cm), soil pH was 7.7, the organic carbon content was 27.1 g kg⁻¹, and the contents of total nitrogen, total phosphorus, total potassium were 3.28 g kg⁻¹, 1.28 g kg⁻¹, 22.2 g kg⁻¹, respectively. Soil was classified as purple clay soil developed from Dongting Lake sediment (Zhou et al. 2019), with texture consists of 7.8% sand, 72.6% silt and 19.6% clay.

Experimental design and sample collection

Six treatments were designed in a randomized complete block with three replicates for the long-term experiment: i) winter-fallowed, no fertilizer applied as the control (CK); ii) winter-fallowed, 100% recommended dose of chemical fertilizer (N, P and K) applied (F100); iii) 22.5 Mg ha⁻¹ MV returning to the field, 100% chemical fertilizer (N, P and K) applied (MV+F100); iv) 22.5 Mg ha⁻¹ MV returning to the field, 80% N and K, with 100% P applied (MV+F80); v) 22.5 Mg ha⁻¹ MV returning to the field, 60% N and K, with 100% P applied (MV+F60); vi) 22.5 Mg ha⁻¹ MV returning to the field, 40% N and K, with 100% P applied (MV+F40). Previous studies have shown that 22.5 Mg ha⁻¹ is the conventional MV returning amount in the practice of rice field production in southern China, which changes the bacterial community structure, improves yield and soil organic carbon accumulation (Zhou et al. 2019; Gao et al. 2021). To avoid the movement of water and nutrients between plots, each plot (4 m × 5 m) was surrounded by ridges (30 cm wide and 15 cm high aboveground) covered with polyethylene film. Soil samples were collected at 0–20 cm depths in April 2018 at S shaped pattern sampling in each treatment plot. After removal of visible plant residues, broken into <10 mm by hand and air-dried at room temperature.

The recommended amount of chemical application was: 150 kg ha⁻¹ N as urea (46% N), 75 kg ha⁻¹ as superphosphate (5% P), and 120 kg ha⁻¹ as potassium chloride (50% K). Both early and late rice applied the same amounts of chemical fertilizer under fertilized treatments. For N application, 50% urea was used as the basal application, with the remaining 50% top-dressed at the tillering stage. P and K fertilizers as the basal fertilizer were consistently applied at the recommended rates. Specifically, base fertilizers were applied one day before transplanting.

Early rice was planted (Yuanzao 1 in the first three years, and then replaced with Xiangzaoxian 25) after middle April and harvested in early July. Late rice (Huanghuazhan) was planted in middle and late July and harvested in early November. Areal density of 37.5 kg ha⁻¹ MV seeds (Xiangzi 1) were sown under green manure treatments, each plot applied 22.5 Mg ha⁻¹ fresh MV and ploughed back 10 days before early rice transplanting. The fresh MV had a water content of 90%, and the organic carbon content of MV (dry-weight basis) was 429 g kg⁻¹ (Zhou et al. 2019). Therefore, the estimated annual average soil organic carbon input of 22.5 Mg ha⁻¹ fresh MV was 965 kg ha⁻¹. The organic carbon release rate of MV was about 74%. All rice straw was removed from each plot after harvest of each crop.

Soil analysis

Soil pH was measured by a pH meter with a 1:2.5 ratio of soil to water, and soil organic carbon content was determined by the K₂Cr₂O₇–FeSO₄ oxidation method (Walkley and Black 1934). Total nitrogen, alkaline nitrogen, Olsen phosphorus and ammonium acetate extractable potassium were determined according to standard methods (Lu 2000). Water-stable aggregates of >5, 2–5, 0.25–2, 0.053–0.25
Fig. 1 Location of the investigation sites
and <0.053 mm in diameter were separated by wet sieving described by Elliott (1986).

Soil organic carbon fractions and analyses

According to the method described by Six et al. (1998), the macroaggregates (0.25–2 mm) and the microaggregates (0.053–0.25 mm) were respectively density-grouped (Fig. 2). Weigh 5.0 g of the sample into a 50 ml centrifuge tube, add 25 ml NaI solution with a density of 1.85 g cm⁻³ to make the sample and solution thoroughly mixed. The suspension was shaken using a horizontal shaker for 30 min, and the sample remaining on the lid was rinsed into the suspension with a small amount of NaI solution. After standing for 20 min, the suspension was centrifuged at 3000 r/min for 30 min. Immediately after centrifugation, the supernatant was filtered for 10 min under vacuum (138 kPa) using a microporous filter with a pore size of 0.45 μm. For complete extraction, the above steps were repeated 3 times, and the supernatant was separated and filtered to obtain free light fraction (LF).

After separation, the heavy fraction (HF) remaining in the centrifuge tube was washed three times with deionized water, and then 15 ml of a 0.5% (w/v) sodium hexametaphosphate (HMP) solution was added to the recombinant, shaken in a shaker for 18 h. After being completely dispersed, the samples were passed through 0.25 and 0.053 mm sieves in order to obtain coarse particulate organic carbon fraction (cPOC: >0.25 mm), fine particulate organic carbon fraction (fPOC: 0.053–0.25 mm), and mineral associated organic carbon fraction (mSOC: <0.053 mm), each fraction was dried and weighed at 40 °C, and the organic carbon content was measured. The SOC content in organic carbon fractions was expressed on an aggregate basis (Garcia-Franco et al. 2015; Jiang et al. 2017), and calculated as follows (1):

$$\text{SOC content (g C kg}^{-1}\text{aggregate)} = \text{SOC}_{\text{fraction}} \times M_{\text{fraction}}$$  

(1)

where SOC_{fraction} is the SOC concentration in each fraction and M_{fraction} is the mass percentage of organic carbon fractions in macroaggregates and microaggregates.

FTIR analyses

Fourier-transform infrared spectra (FTIR) was used to analyze bulk soil and aggregate samples using a VERTEX70 FTIR spectrometer (Bruker, Hamburg, Germany). Dried pellets (1 mg soil sample with 100 mg⁻¹ KBr) were prepared for scanning range of 400–4000 cm⁻¹ and wavelength resolution of 4 cm⁻¹ (Xue et al. 2019). OPUS (Bruker, Hamburg, Germany) was used to analyze the spectrums. We assigned different FTIR bands based on the previous research (Xue et al. 2019; Pu et al. 2022). The 3390–3440 cm⁻¹ bands were ascribed to O–H stretching in hydroxyl C. The 2850–2920 cm⁻¹ absorption peaks were associated with C-H stretching in alkane C. The 1600–1640 cm⁻¹ bands reflected C=C=C stretching in aromatic C. The bands at 1413–1441 cm⁻¹ were attributed to the stretching of C=O in carboxylic C. The band at 1030–1080 cm⁻¹ were assigned to C-O vibration in polysaccharide C.

**Fig. 2** Scheme of the organic carbon density fraction. LF: free light fraction (<1.85 g cm⁻³), HF: heavy fraction (> 1.85 g cm⁻³), cPOC: coarse particulate organic carbon fraction (0.25–2 mm), fPOC: fine particulate organic carbon fraction (0.053–0.25 mm), mSOC: mineral associated organic carbon fraction (<0.053 mm), HMP: sodium hexametaphosphate.
Peaks at 760–820 cm\(^{-1}\) resulted from C-H deformation in alkene C.

**Solid state\(^{13}\)C NMR spectroscopy analyses**

The mSOC (\(< 0.053 \text{ mm}\)) in macroaggregates and microaggregates were selected as composite samples. Composites were repeated wash of 10% HF solution (120 rpm, 25 °C) for 1 h (four times), 12 h (three times) and 24 h (one time). Then, the residue was rinsed with deionized water for eight times, freeze-dried and ground to be measured (Xue et al. 2020). The experiments were conducted by cross polarization/total sideband suppression (CP/TOSS) using Bruker Ascend 500 MHz (Bruker, Karlsruhe, Germany). Samples were placed in a 4 mm diameter rotor spinning speed of 6 kHz, and \(^{13}\)C resonance frequency of 125.8 MHz. The sampling time for each spectrum was 0.01 s, meanwhile, a contact time of 3 s were set. The number of required scans was about 7000. Spectra were processed by Mestrenova 14.0 and integrated into six chemical shift regions: alkyl C (0–44 ppm), methoxyl C (44–63 ppm), O-alkyl C (63–110 ppm), aryl C (110–141 ppm), phenol C (141–161 ppm) and carboxyl C (161–220 ppm) (Spaccini and Piccolo 2009; Prietzel et al. 2018; Lan et al. 2022). Two indexes were used to assess the stability of soil organic carbon using Eqs. (2) and (3) (Pedersen et al. 2011; Xue et al. 2020):

\[
\frac{\text{AL/OA}}{\text{AR}} = \left( \frac{\text{alkyl C}}{\text{O} − \text{alkyl C}} \right) = \frac{\text{C}_{0–44 \text{ ppm}}}{\text{C}_{63–110 \text{ ppm}}} \tag{2}
\]

\[
\frac{\text{AL/AR}}{\text{AR}} = \left( \frac{\text{alkyl C} + \text{methoxyl C} + O − \text{alkyl C}}{\text{aryl C} + \text{phenol C}} \right) = \frac{\text{C}_{0–44 \text{ ppm}} + \text{C}_{44–63 \text{ ppm}} + \text{C}_{63–110 \text{ ppm}}}{\text{C}_{110–141 \text{ ppm}} + \text{C}_{141–161 \text{ ppm}}} \tag{3}
\]

**Statistical analysis**

Statistical analyses were conducted using SPSS 20. Analysis of variance (ANOVA) was used to test the effects of different treatments on aggregates size distribution and SOC dynamics. A one-way ANOVA was used to test for significant differences among different treatments according to the Duncan’s multiple range test calculations at 5% level. Principal component analysis (PCA) of the relative abundance of chemical composition in mSOC was conducted to determine the effects of different treatments using Origin 2022.

**Results**

The soil physical and chemical characteristics

The soil pH value in each treatment of MV returning to the field decreased slightly, with a decrease range of 2.4% to 3.6% (Table 1). The SOC concentration in bulk soil was significantly greater in MV + F40 than in F100 and CK. MV returning treatments (MV + F100, MV + F80, MV + F60 and MV + F40) had consistently higher amount of SOC and total nitrogen (TN) than without green manure treatments (F100 and CK). The C/N ratio ranged from 7.35 to 8.67, depending on the treatments, and MV + 40 had the widest value (8.67). Slightly higher contents of alkaline nitrogen (AN) were found at MV returning treatments compared to F100, and significant differences were found between CK with MV returning treatments. Olsen phosphorus (OP) and ammonium acetate extractable

**Table 1** Soil properties in different treatments in 2018

|       | pH  | SOC (g kg\(^{-1}\)) | TN (g kg\(^{-1}\)) | C/N ratio | AN (mg kg\(^{-1}\)) | OP (mg kg\(^{-1}\)) | AK (mg kg\(^{-1}\)) |
|-------|-----|---------------------|-------------------|-----------|----------------------|---------------------|--------------------|
| CK    | 8.05 ± 0.06 a | 22.0 ± 0.62 c | 2.66 ± 0.17 c | 8.30 ± 0.58 ab | 167 ± 13.9 b | 6.80 ± 0.85 c | 72.5 ± 8.64 c |
| F100  | 7.98 ± 0.08 ab | 23.3 ± 1.75 bc | 2.97 ± 0.22 bc | 7.83 ± 0.18 bc | 191 ± 19.4 ab | 24.5 ± 2.38 a | 97.3 ± 11.9 a |
| MV + F100 | 7.83 ± 0.14 c | 26.4 ± 1.34 ab | 3.51 ± 0.24 a | 7.52 ± 0.27 c | 221 ± 7.26 a | 18.7 ± 1.95 b | 85.5 ± 7.50 b |
| MV + F80 | 7.76 ± 0.13 c | 25.4 ± 0.67 ab | 3.40 ± 0.23 ab | 7.50 ± 0.62 c | 204 ± 12.8 a | 22.4 ± 2.17 ab | 77.2 ± 5.43 bc |
| MV + F60 | 7.85 ± 0.09 bc | 24.6 ± 3.17 abc | 3.42 ± 0.75 ab | 7.35 ± 0.91 c | 202 ± 41.4 a | 22.1 ± 6.73 b | 79.7 ± 6.81 bc |
| MV + F40 | 7.86 ± 0.10 bc | 27.4 ± 4.49 a | 3.16 ± 0.51 abc | 8.67 ± 0.35 a | 211 ± 43.8 a | 21.7 ± 4.15 ab | 75.9 ± 1.26 bc |

Numerical values are means ± standard errors. SOC: soil organic carbon, TN: total nitrogen, AN: alkaline nitrogen, OP: Olsen phosphorus, AK: ammonium acetate extractable potassium. Different letters indicate significant differences among treatments at p < 0.05 according to Duncan’s multiple range test.
potassium (AK) contents were not significantly different between MV returning treatments, while F100 had significantly higher OP and AK contents than CK. It was indicated that the cultivation of MV was beneficial to increase soil nutrition. Reduced fertilizer and MV returning treatments significantly increased the contents of SOC, AN and OP, when compared with the CK.

Distribution of soil aggregates

Water-stable aggregates >0.25 mm constituted the largest proportion of all soil aggregates (68.0–87.6%), followed by <0.053 mm aggregates (7.9–21.6%), and 0.053–0.25 mm aggregates (3.6–10.4%) had the least (Fig. 3). The >5 mm aggregates accounted for 44.9–52.2% of all fractions, MV returning treatments were significantly higher than CK. MV + F80 had higher 2–5 and 0.25–2 mm aggregates than F100, adverse results for 0.053–0.25 and <0.053 mm aggregates. Compared to CK, >0.25 mm aggregates under MV + F40 and MV + F80 significantly increased by 14.4–19.5% after 10 years of MV application, whereas 0.053–0.25 and <0.053 mm aggregates decreased by 5.8–6.7% and 7.7–13.7%, respectively. The distribution of aggregates altered under chemical fertilizer reduced by MV, especially in terms of 2–5, 0.25–2 and <0.053 mm aggregates.

Organic carbon distribution in aggregates

The concentration of SOC distributed in aggregates varied among treatments. The content of SOC in soil aggregates was higher in >2 mm aggregates, and MV returning to the field had a significant effect (Table 2). The SOC content was lower in <2 mm aggregates, and various treatments had no significant difference. SOC concentrations were mainly concentrated in macroaggregates. Compared with CK, SOC content of each aggregate was increased to a certain extent with the application of reduced fertilizer with MV (except for >5 mm aggregates in MV + F60).

![Fig. 3](#) Effects of green manure on aggregate size distribution. CK, winter-fallowed, no fertilizer applied as the control; F100, winter-fallowed, 100% recommended dose of chemical fertilizer (N,P and K) applied; MV + F100, 22.5 Mg ha⁻¹ Chinese milk vetch (MV) returning to the field, 100% N,P and K applied; MV + F80, 22.5 Mg ha⁻¹ MV returning to the field, 80% N and K, with 100% P applied; MV + F60, 22.5 Mg ha⁻¹ MV returning to the field, 60% N and K, with 100% P applied; MV + F40, 22.5 Mg ha⁻¹ MV returning to the field, 40% N and K, with 100% P applied. Different capital letters indicate significant differences among different treatments for the same aggregate size (Duncan, p < 0.05), and lowercase letters indicate differences among different aggregate sizes of the same treatment (Duncan, p < 0.05)
Compared to F100, MV + F60 enhanced the SOC content in macroaggregates and microaggregates by 1.4 and 2.4 g kg$^{-1}$, respectively. MV + F40 significantly ($p < 0.05$) increased the SOC content in > 2 mm aggregates, meanwhile the effect of improving SOC content in bulk soil was most obvious. The content of organic carbon did not decrease significantly with the decrease in the amount of chemical fertilizer applied, which indicated that the replacement of chemical fertilizer by MV could satisfy the organic carbon supply in the soil.

Irrespective of treatments, mSOC was dominantly in macroaggregates and microaggregates (Fig. 4). The relative mass content of organic carbon fraction in macroaggregates ranged from high to low as follows: mSOC > fPOC > cPOC > LF. Organic carbon fraction mainly existed in the form of mSOC, accounting for 70.3–83.7% of macroaggregates. With the decrease of chemical fertilizer application, the relative mass of LF in macroaggregates increased significantly ($p < 0.05$). The relative mass of cPOC in macroaggregates was the highest under MV + F60, accounting for 12.3%, which was significantly ($p < 0.05$) higher than that of CK and F100. Compared with CK, relative mass of mSOC in macroaggregates under chemical fertilizer substitution by MV treatments decreased by 8.1–16.0%. In the microaggregates, the large majority of the organic carbon fraction was mSOC, accounting for 69.4–84.0%, followed by the fPOC, and the LF was the least. The distribution pattern of the organic carbon in the microaggregates was consistent with the macroaggregates.

The ranking order of SOC content of organic carbon fractions was mSOC > fPOC > cPOC > LF in macroaggregates (Fig. 5A). SOC content of mSOC and fPOC in macroaggregates under MV + F80, MV + F60 and MV + F40 showed no significant change, but it increased by 1.7–2.9 and 0.4–2.3 g kg$^{-1}$ when compared to F100. In the cPOC within macroaggregates, SOC content under MV + F60 and MV + F40 was significantly ($p < 0.05$) increased by 168% and 116% compared to CK. With the decrease of chemical fertilizer application, the SOC content of LF in macroaggregates increased significantly ($p < 0.05$). SOC content was significantly ($p < 0.05$) higher of mSOC and fPOC than that of LF in microaggregates (Fig. 5B). Under CK, F100 and MV + F60, the content of SOC in the mSOC was higher than that in fPOC within microaggregates, contrasting the results for other treatments. MV + F60 was the most effective treatment in improving SOC content of the mSOC and fPOC in macroaggregates and microaggregates. SOC accumulation in macroaggregates and microaggregates were mainly attributed to the increases of SOC content in the mSOC and fPOC.

Organic carbon functional groups in SOC fractions

The FTIR spectra of different fractions in the aggregate and bulk soil were relatively similar (Fig. 6). The major organic carbon functional groups were hydroxyl C, alkane C, aromatic C, carboxylic C, polysaccharide C and alkene C. Polysaccharide C dominated functional groups in aggregates and bulk soil. Under MV returning treatments, the peak intensity values of all functional groups in fPOC fraction in macroaggregates tended to increase. Although the peak intensity values were higher, the differences among FTIR spectra in soil organic carbon of bulk soil were slight. Compared with F100, signals corresponding to polysaccharide C under MV + F80 and

### Table 2 Effects of green manure on SOC concentration in different aggregate sizes (g C kg$^{-1}$ aggregate)

| Treatment | > 5 mm   | 2–5 mm   | 0.25–2 mm | 0.053–0.25 mm | < 0.053 mm |
|-----------|----------|----------|-----------|---------------|------------|
| CK        | 32.4 ± 1.89 c | 33.6 ± 2.26 b | 26.6 ± 2.72 a | 21.2 ± 3.83 a | 22.2 ± 4.21 a |
| F100      | 37.0 ± 1.84 ab | 36.7 ± 0.46 ab | 30.4 ± 1.69 a | 26.4 ± 1.88 a | 23.6 ± 4.98 a |
| MV + F100 | 35.7 ± 2.77 bc | 35.8 ± 1.87 ab | 32.2 ± 0.58 a | 26.6 ± 2.16 a | 25.5 ± 4.18 a |
| MV + F80  | 35.1 ± 1.93 bc | 34.2 ± 2.94 b | 28.0 ± 2.32 a | 26.6 ± 5.86 a | 24.0 ± 2.76 a |
| MV + F60  | 32.2 ± 4.12 c | 35.9 ± 5.76 ab | 31.8 ± 8.57 a | 28.8 ± 11.9 a | 25.9 ± 8.19 a |
| MV + F40  | 39.9 ± 3.50 a | 38.4 ± 2.39 a | 30.8 ± 4.90 a | 25.5 ± 8.53 a | 25.8 ± 8.33 a |

Numerical values are means (n = 3) with standard errors. Different lowercase letters in rows indicate significant differences among treatments (Duncan, $p < 0.05$).
MV+F60 increased by 21.6% and 17.7% in cPOC fraction, respectively (Fig. 7). From fPOC to mSOC, the proportion of alkane C decreased and the relative content of alkene C increased in macroaggregates and microaggregates.

Six carbon groups were typically assigned in CP/TOSS NMR spectra (Fig. 8). The peak intensity of macroaggregates were higher than that of microaggregates, and the latter was less variable. Differences in the relative quantities of functional groups were discovered (Fig. 9). Dominant signals were attributed to O-alkyl C and alkyl C, accounting for 26.0–32.3% and 26.4–29.7%, respectively. Relative intensity of phenol C was less variable and accounted for 4.0–5.3% of total spectra across all samples. The relative contents of functional groups of mSOC altered in response to substitution of chemical fertilizer by MV. Within the mSOC in macroaggregates, when compared with CK, MV+F80 and MV+F60 promoted the proportion of alkyl C by 9.6% and 6.7%, and decreased the content of O-alkyl C by 14.1% and 11.0%, respectively, which correspondingly increased the AL/
OA ratio. MV+F100 decreased alkyl C abundance and the AL/OA ratio within mSOC in macroaggregates compared to F100. The relative content of carbonyl C was higher under MV+F80, MV+F60, MV+F40 than under CK and F100 within mSOC in macroaggregates and microaggregates.

PCA was used to analyze the changes in chemical groups of mSOC under chemical fertilizer substitution by MV. The first (PC1) and second components (PC2) represented 42.8% and 33.2% of the variability in chemical composition of mSOC, respectively (Fig. 10A). The difference of chemical composition of mSOC among various treatments of macroaggregates was more obvious than that of microaggregates. Inspection of PC loadings suggested that carbonyl C and alkyl C were important in PC1 and phenol C and O-alkyl C were dominated in PC2 (Fig. 10B).

**Discussion**

The organic carbon fraction in the tested soil was mainly mSOC in macroaggregates and microaggregates (Fig. 4), accounting for 70.3–83.7% and 69.4–84.0%, respectively, which was a stable soil organic matter pool. The $^{13}$C-labelled litter decomposition study indicated that mSOC could be directly formed with fresh litter input in a short period of time (within 6 months) and stored in stable SOC pool (Zhao et al. 2021). There was a positive correlation ($p<0.05$) between LF and total mSOC (from 0.25–2 and 0.063–0.25 mm) under green manure treatment, suggesting that fresh SOC was stabilized by forming complexes with mineral particles (Garcia-Franco et al. 2015). This study showed that compared with CK, polysaccharide C in mSOC was increased by 3.6–6.6% and 0.4–4.3% in macroaggregates and microaggregates, respectively, under MV+F60 and MV+F40 (Fig. 7), indicating that after MV application the polysaccharide C easily bind with mineral particles (Wen et al. 2021) and facilitated the aggregation of particles. Polysaccharide was an unstable component of organic carbon, which was easy to be preferentially utilized by microorganisms, and can also be stabilized by adsorption to the mineral surface (Rumpel et al. 2010). Meanwhile, the aromatic C relative content increased by 11.6% and 29.1% under MV+F60 compared to F100 within mSOC in macroaggregates and microaggregates, respectively, indicating that MV application could promote the aromaticity and stability of the mSOC. Progressive extent of SOC decomposition was characterized by increased aromaticity in agricultural soils (Wagai et al. 2013). The organic carbon content
Fig. 6 Fourier transform infrared (FTIR) spectra of organic carbon fractions in aggregates and bulk soil. A-C cPOC, fPOC and mSOC of macroaggregates, D-E fPOC and mSOC of microaggregates, F soil organic carbon of bulk soil.
in bulk soil and mSOC under MV+F60 were higher than those under F100. Consistent with previous studies, higher SOC content promoted the SOC concentrations in aggregates, which was further conducive to the formation and persistence of mSOC (Fulton-Smith and Cotrufo 2019).

The peak intensity of macroaggregates were stronger than that of microaggregates, and the latter was less variable according to NMR and PCA results (Fig. 8, Fig. 10). Microaggregate was less dependent on agricultural management, and had higher stability than macroaggregate (Zhao et al. 2021). Macroaggregate prevented SOC from microbial decomposition, and the protection ability increased with increased SOC and clay contents (Balesdent et al. 2000). Decomposition of MV resulted in the decrease of O-alkyl C and the increase of alkyl C relative content (Fig. 9). Similar phenomenon was observed in decomposition of spruced litter (Helfrich et al. 2006). Alkyl C originated from lipids, amino acids and cutin (Prietzel et al. 2018). The content of O-alkyl C decreased, indicating that labile constitutes such as carbohydrates and peptides in SOC decreased (Pedersen et al. 2011; Rodriguez et al. 2021). The possible explanation would be that MV contained labile organic carbon forms firstly utilized by microorganisms and O-alkyl C further degraded to produced alkyl C (Kopittke et al. 2020; Lan et al. 2022). Previous studies have shown that a kind of microbial derived carbon (glomalin related soil protein) contained high alkyl C and low O-alkyl C and its recalcitrant structure contributed to a stable SOC pool (Wang et al. 2021). In addition, the abundance of O-alkyl C was higher in mSOC of macroaggregates than that of microaggregates, suggesting than the significance of O-alkyl C in SOC of macroaggregates. MV+F80 contained the highest alkyl C (29.7%) of the mSOC in macroaggregates, indicating that connection between long-chain alkyl constituents and minerals (Clemente et al. 2012). The more aliphatic components enriched, the stronger the resistance of SOC to microbial degradation (Chen et al. 2018). In mSOC fraction, relative proportions of carbonyl C increased from 11.7% (CK) to 18.5% (MV+F80) in macroaggregates and from 11.9% (CK) to 15.7% (MV+F40) in microaggregates. Carbonyl C was significantly related to microbial biomass and total

![Fig. 7](https://example.com/fig7.png) Percentage of organic carbon composition in the SOC fractions and bulk soil by FTIR (%). A-C cPOC, fPOC and mSOC of macroaggregates, D-E fPOC and mSOC of microaggregates, F soil organic carbon of bulk soil

![Fig. 8](https://example.com/fig8.png) Solid state $^{13}$C NMR spectrums of the mSOC in macroaggregates (A) and microaggregates (B)
Compared with NPK fertilizer, green manure application increased microbial biomass C and hydrolase activities (Khan et al. 2020). The balance between microbial necromass input and microbial decomposition output determined the change of mSOC (Chen et al. 2021). Therefore, the accumulation of carbonyl C in mSOC may be due to the enhancement of microbial activities and products by MV returning.

In the mSOC, chemical fertilizer reduced by MV (MV + F80, MV + F60, MV + F40) contained higher AL/OA ratio and higher degree of SOC humification than treatments without MV (F100 and CK) in macroaggregates and microaggregates, respectively. The results showed that the returning of MV to the field increased the proportion of resistant organic carbon and was conducive to the accumulation of SOC. Regarding mSOC of macroaggregates, compared to

![Fig. 9](image-url)
F100, MV + F100 decreased the alkyl C abundance and AL/OA ratio by 2.2% and 2.3%, respectively. MV had the capacity to fix air-borne N by symbiosis with bacteria (Voisin et al. 2014) and sufficient N promoted the decomposition of resistant organic carbon, fading carbon sequestration potential. In addition, MV had highest decomposition at early time and released 73.6% of the original carbon (Zhou et al. 2020). Therefore, the application of chemical fertilizer should be appropriately reduced when MV returning to the field. The AL/AR ratio of F100 was higher than that of MV + F60 and MV + F40 in macroaggregates, indicating that the application of chemical fertilizer resulted in less aromatic structure and smaller extent of condensation. Consequently, MV incorporation promoted the complexity of molecular structure within the mSOC and enhanced the SOC contents in macroaggregates.

During the decomposition of plant residues, the particle size gradually decreased with polar and ionizable groups increased, meanwhile, mineral complexes or aggregates may be formed to prevent further decomposition (Lehmann and Kleber 2015). The percentage of 13C-labelled residual carbon allocated to LF and cPOC decreased, while these to fPOC and mSOC extended with in-situ incubation time (Li et al. 2016). High contribution of plant-derived polysaccharide was observed in bulk soil (Rumpel et al. 2010). Compared with F100, polysaccharide C originating from MV decomposition under MV + F80 and MV + F60 increased by 21.6% and 17.7% in cPOC fraction, respectively (Fig. 7). Polysaccharide C acted as binding agent in macroaggregates (Wen et al. 2021), and increased significantly (p < 0.05) under manure (Chen et al. 2022). From fPOC to mSOC, the proportion of alkane C decreased and the relative content of alkene C increased in macroaggregates and microaggregates, as expected increasing SOC humification (Artz et al. 2008; Xue et al. 2019), suggesting that mSOC...
had advanced decomposed and stable SOC than fPOC.

A schematic was provided to better understand the impact of chemical fertilizer substitution by MV on SOC formation in different fractions (Fig. 11). Chemical fertilizer significantly increased SOC content of fPOC in macroaggregates. Chemical fertilizer increased root biomass, promoted the mineralization and decomposition of soil organic carbon and was not conducive to the combination of organic carbon and mineral particles to form mSOC (Jiang et al. 2017). Hence, particles with higher decomposition (fPOC) were formed under F100 within macroaggregates. F100 increased the content of soil organic carbon compared with CK, offset the destructive effect of paddy soils cultivation on aggregates to a certain extent. Macroaggregate turnover was decreased, resulting in accumulation of fPOC and formation of new microaggregates within macroaggregates (Six et al. 2004). Chemical fertilizer substitution by MV promoted SOC content in all organic carbon fractions in macroaggregates, and increased SOC content of fPOC in microaggregates. According to research fruits (Six et al. 2000), we proposed that green manure residues induced the formation of macroaggregates, then cPOC in macroaggregates was further decomposed to form fPOC, which was wrapped by clay particles and microbial products to form newly microaggregates within macroaggregates. With the degradation of binding agents in macroaggregates, stable microaggregates released. The fPOC within macroaggregates was crucial for the formation of microaggregates. A higher organic carbon content and AL/AR ratio was in macroaggregates than that in microaggregates. After 10 years chemical fertilizer reduced by MV, SOC composition of mSOC in macroaggregates was highly responsive to various treatments compared to that of microaggregates, influencing the stabilization of SOC.

Conclusions

Chemical fertilizer substitution by MV altered the SOC concentration and distribution in aggregates and influenced chemical constitution of organic carbon fractions. MV + F80, MV + F60 and MV + F40 significantly increased the contents of SOC, AN and OP compared to CK. A tendency of relative mass and SOC content of LF in macroaggregates increased significantly with the decrease
of chemical fertilizer application (from MV + F80 to MV + F40), and similar trend (no statistically significant) was found for microaggregates. F100 significantly increased SOC content of fPOC in macroaggregates relative to CK. Chemical fertilizer substitution by MV promoted SOC content in all organic carbon fractions in macroaggregates, and increased SOC content of fPOC in microaggregates, compared to CK.

Compared with F100, polysaccharide C originating from MV decomposition under MV + F80 and MV + F60 increased in cPOC fraction. From fPOC to mSOC, alkane C decreased and alkene C increased in macroaggregates and microaggregates, suggesting that mSOC had advanced decomposed and stable organic carbon than fPOC. The major form of organic carbon was the mSOC in macroaggregates and microaggregates. PCA results indicated that SOC composition of mSOC in macroaggregates was highly responsive to various fertilizer treatments compared to that of microaggregates. In the mSOC, chemical fertilizer substitution by MV (MV + F80, MV + F60, MV + F40) contained higher AL/OA ratio and higher degree of SOC humification than treatments without MV (F100 and CK) in macroaggregates and microaggregates, respectively. MV incorporation promoted the complexity of molecular structure within the mSOC and enhanced the SOC stability in macroaggregates. Substitution of chemical fertilizer by MV improved the stability of SOC via increasing recalcitrant structure (alkyl C and aromatic C) in mSOC, and was conducive to the sequestration of SOC in paddy soils. In the case of MV returning, 60–80% of the fertilizer application rate was recommended in paddy soils in southern China. These results provided a better understanding of SOC persistence and stability mechanism under green manure application.

Acknowledgements We would like to acknowledge all the staff of the long-term experiment at Nan County, Hunan Province of China. This research was supported by National Natural Science Foundation of People’s Republic of China (Grant No. 41977020) and China Agriculture Research System of MOF and MARA (CARS-22). Dr. Yuedong Liu helped in measuring NMR datas.

Declarations

Conflicts of interest The authors declare no conflict of interest.

References

Artz RRE, Chapman SJ, Jean Robertson AH et al (2008) FTIR spectroscopy can be used as a screening tool for organic matter quality in regenerating cutover peatlands. Soil Biol Biochem 40:515–527. https://doi.org/10.1016/j.soilbio.2007.09.019

Balesdent J, Chenu C, Balabane M (2000) Relationship of soil organic matter dynamics to physical protection and tillage. Soil Tillage Res 53:215–230. https://doi.org/10.1016/S0167-1987(99)00107-5

Benbi DK, Toor AS, Kumar S (2012) Management of organic amendments in rice-wheat cropping system determines the pool where carbon is sequestered. Plant Soil 360:145–162. https://doi.org/10.1007/s11104-012-1226-3

Chai Y, Zeng X, E S, et al (2019) The stability mechanism for organic carbon of aggregate fractions in the irrigated desert soil based on the long-term fertilizer experiment of China. Catena 173:312–320. https://doi.org/10.1016/j.catena.2018.10.026

Chen M, Zhang S, Liu L et al (2022) Organic fertilization increased soil organic carbon stability and sequestration by improving aggregate stability and iron oxide transformation in saline-alkaline soil. Plant Soil. https://doi.org/10.1007/s11104-022-05326-3

Chen X, Xu Y, Gao H et al (2018) Biochemical stabilization of soil organic matter in straw-amended, anaerobic and aerobic soils. Sci Total Environ 625:1065–1073. https://doi.org/10.1016/j.scitotenv.2017.12.293

Chen Y, Liu X, Hou Y et al (2021) Particulate organic carbon is more vulnerable to nitrogen addition than mineral-associated organic carbon in soil of an alpine meadow. Plant Soil 458:93–103. https://doi.org/10.1007/s11104-019-04279-4

Clemente JS, Gregorich EG, Simpson AJ et al (2012) Comparison of nuclear magnetic resonance methods for the analysis of organic matter composition from soil density and particle fractions. Environ Chem 9:97. https://doi.org/10.1071/EN11096

Duan Y, Chen L, Zhang J et al (2021) Long-term fertilisation reveals close associations between soil organic carbon composition and microbial traits at aggregate scales. Agric Ecosyst Environ 306:107169. https://doi.org/10.1016/j.agee.2020.107169

Elliott ET (1986) Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci Soc Am J 50:627–633. https://doi.org/10.2136/ssaj1986.03615995005000030017x

Fulton-Smith S, Cotrufo MF (2019) Pathways of soil organic matter formation from above and belowground inputs in a Sorghum bicolor bioenergy crop. GCB Bioenergy 11:971–987. https://doi.org/10.1111/gcbb.12598

Gao S, Cao W, Zhou G, Rees RM (2021) Bacterial communities in paddy soils changed by milk vetch as green manure: A study conducted across six provinces in South China. Pedosphere 31:521–530. https://doi.org/10.1016/S1002-0160(21)60002-4

García-Franco N, Alhalad Je8 J, Almagro M, Martínez-Mena M (2015) Beneficial effects of reduced tillage and green manure on soil aggregation and stabilization of organic carbon in a Mediterranean agroecosystem. Soil Tillage
Pedersen JA, Simpson MA, Bockheim JG, Kumar K (2011) Characterization of soil organic carbon in drained thaw-lake basins of Arctic Alaska using NMR and FTIR photoacoustic spectroscopy. Org Geochem 42:947–954. https://doi.org/10.1016/j.orggeochem.2011.04.003

Prietzel J, Müller S, Kögel-Knabner I et al (2018) Comparison of soil organic carbon speciation using C NEXAFS and CPMAS 13C NMR spectroscopy. Sci Total Environ 628–629:906–918. https://doi.org/10.1016/j.scitotenv.2018.02.121

Pu Y, Lang S, Wang A et al (2022) Distribution and functional groups of soil aggregate-associated organic carbon along a marsh degradation gradient on the Zoige Plateau. China Catena 209:105811. https://doi.org/10.1016/j.catena.2021.105811

Rodriguez AF, Gerber S, Inglett PW et al (2021) Soil carbon characterization in a subtropical drained peatland. Geoderma 382:114758. https://doi.org/10.1016/j.geoderma.2020.114758

Rumpel C, Eusterhues K, Kögel-Knabner I (2010) Non-cellulosic neutral sugar contribution to mineral associated organic matter in top- and subsoil horizons of two acid forest soils. Soil Biol Biochem 42:379–382. https://doi.org/10.1016/j.soilbio.2009.11.004

Six J, Bossuyt H, Degryze S, Denef K (2004) A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res 79:7–31. https://doi.org/10.1016/j.still.2004.03.008

Six J, Elliott E, Paustian K, Doran JW, (1998) Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Sci Soc Am J 62:1367–1377. https://doi.org/10.2136/sssaj1998.03615995006200050032x

Six J, Elliott ET, Paustian K (2000) Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biol Biochem 32:2099–2103. https://doi.org/10.1016/S0038-0717(00)00179-6

Song HJ, Lee JH, Canoty RC et al (2021) Strong mitigation of greenhouse gas emission impact via aerobic short pre-digestion of green manure amended soils during rice cropping. Sci Total Environ 761:143193. https://doi.org/10.1016/j.scitotenv.2020.143193

Song W, Shu A, Liu J et al (2022) Effects of long-term fertilization with different substitution ratios of organic fertilizer on paddy soil. Pedosphere 32:637–648. https://doi.org/10.1007/s13118-021-00604-4

Spaccini R, Piccolo A (2009) Molecular characteristics of humic acids extracted from compost at increasing maturity stages. Soil Biol Biochem 41:1164–1172. https://doi.org/10.1016/j.soilbio.2009.02.026

Torres-Sallan G, Schulte RPO, Lanigan GJ et al (2017) Clay illuviation provides a long-term sink for C sequestration in subsoils. Sci Rep 7:45635. https://doi.org/10.1038/srep45635

Totsche KU, Amelung W, Gerzabek MH et al (2018) Micro-aggregates in soils. J Plant Nutr Soil Sci 181:104–136. https://doi.org/10.1002/jpln.201600451

Voisin A-S, Guéguen J, Huyge C et al (2014) Legumes for feed, food, biomaterials and bioenergy in Europe: a review. Agron Sustain Dev 34:361–380. https://doi.org/10.1007/s13593-013-0189-y
Wagai R, Kishimoto-Mo AW, Yonemura S et al (2013) Linking temperature sensitivity of soil organic matter decomposition to its molecular structure, accessibility, and microbial physiology. Glob Change Biol 19:1114–1125. https://doi.org/10.1111/gcb.12112

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 37:29–38. https://doi.org/10.1097/00010694-193401000-00003

Wang Q, Wen Y, Zhao B et al (2021) Coastal soil texture controls soil organic carbon distribution and storage of mangroves in China. Catena 207:105709. https://doi.org/10.1016/j.catena.2021.105709

Wen Y, Tang Y, Wen J et al (2021) Variation of intra-aggregate organic carbon affects aggregate formation and stability during organic manure fertilization in a fluvo-aquic soil. Soil Use Manag 37:151–163. https://doi.org/10.1111/sum.12676

Witzgall K, Vidal A, Schubert DI et al (2021) Particulate organic matter as a functional soil component for persistent soil organic carbon. Nat Commun 12:4115. https://doi.org/10.1038/s41467-021-24192-8

Xu Q, Rui W, Bian X, Zhang W (2007) Regional differences and characteristics of soil organic carbon density between dry land and paddy field in China. Agric Sci China 6:981–987. https://doi.org/10.1016/S1671-2927(07)60137-0

Xue B, Huang L, Huang Y et al (2019) Effects of organic carbon and iron oxides on soil aggregate stability under different tillage systems in a rice–rape cropping system. Catena 177:1–12. https://doi.org/10.1016/j.catena.2019.01.035

Xue B, Huang L, Huang Y et al (2020) Straw management influences the stabilization of organic carbon by Fe (oxyhydr)oxides in soil aggregates. Geoderma 358:113987. https://doi.org/10.1016/j.geoderma.2019.113987

Yang Z, Zheng S, Nie J et al (2014) Effects of long-term winter planted green manure on distribution and storage of organic carbon and nitrogen in water-stable aggregates of reddish paddy soil under a double-rice cropping system. J Integr Agric 13:1772–1781. https://doi.org/10.1016/S2095-3119(13)60565-1

Yao Z, Zhang D, Liu N et al (2019) Dynamics and sequestration potential of soil organic carbon and total nitrogen stocks of leguminous green manure-based cropping systems on the loess plateau of China. Soil Tillage Res 191:108–116. https://doi.org/10.1016/j.still.2019.03.022

Yeasmin S, Singh B, Smernik RJ, Johnston CT (2020) Effect of land use on organic matter composition in density fractions of contrasting soils: A comparative study using 13C NMR and DRIFT spectroscopy. Sci Total Environ 726:138395. https://doi.org/10.1016/j.scitotenv.2020.138395

Zhang H, Wang D, Su B et al (2021) Distribution and determinants of organic carbon and available nutrients in tropical paddy soils revealed by high-resolution sampling. Agric Ecosyst Environ 320:107580. https://doi.org/10.1016/j.agee.2021.107580

Zhao Z, Gao S, Lu C et al (2021) Effects of different tillage and fertilization management practices on soil organic carbon and aggregates under the rice–wheat rotation system. Soil Tillage Res 212:105071. https://doi.org/10.1016/j.still.2021.105071

Zhou G, Chang D, Gao S et al (2021) Co-incorporating leguminous green manure and rice straw drives the synergistic release of carbon and nitrogen, increases hydrolase activities, and changes the composition of main microbial groups. Biol Fertil Soils 57:547–561. https://doi.org/10.1007/s00374-021-01547-3

Zhou G, Gao S, Lu Y et al (2020) Co-incorporation of green manure and rice straw improves rice production, soil chemical, biochemical and microbiological properties in a typical paddy field in southern China. Soil Tillage Res 197:104499. https://doi.org/10.1016/j.still.2019.104499

Zhou X, Lu Y, Liao Y et al (2019) Substitution of chemical fertilizer by Chinese milk vetch improves the sustainability of yield and accumulation of soil organic carbon in a double-rice cropping system. J Integr Agric 18:2381–2392. https://doi.org/10.1007/s00374-021-01547-3

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.