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Soil Bacteria Mediate Soil Organic Carbon Sequestration under Different Tillage and Straw Management in Rice-Wheat Cropping Systems

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Abstract: Soil organic carbon (SOC) largely influences soil quality and sustainability. The effects of no-till (NT) and crop straw return practices (SR) on soil organic carbon sequestration have been well documented. However, the mechanism of soil bacterial community in regulating soil organic carbon under NT and SR remains unclear. In this study, we investigated the impacts of tillage (conventional tillage (CT) and NT) and crop straw return practices (crop straw removal (NS) and SR) on topsoil layer (0–5 cm) bacterial community, CH_4 and CO_2 emissions and SOC fractions in rice-wheat cropping system. Overall, in the wheat season following the annual rice-wheat rotation in two cycles, NT significantly increased SOC by 4.4% for 1–2 mm aggregates in the 0–5 cm soil layer, but decreased CO_2 emissions by 7.4%. Compared with NS, SR notably increased the contents of SOC in the topsoil layer by 6.5% and in macro-aggregate by 17.4% in 0–5 cm soil layer, and promoted CH_4 emissions (by 22.3%) and CO_2 emissions (by 22.4%). The combination of NT and NS resulted in relatively high SOC and low CH_4 emissions along with high bacterial community abundance. The most abundant genus under different treatments was *Gp6*, which significant impacted SOC and MBC. Bacterial communities like Subdivision3 had the most impact on CH_4 emissions. Structural equation modeling further suggested that the soil bacterial community indirectly mediated the SOC through balancing SOC in 1–2 mm aggregates and CH_4 emissions. This study provides a new idea to reveal the mechanism of short-term tillage and straw return on SOC.

Keywords: no-till; straw return; soil organic carbon fractions; soil aggregate; bacterial diversity

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1. Introduction

Enhancing soil organic carbon storage is vital to achieving sustainable agriculture and alleviating the negative impacts of climate change [1–5]. Tillage and straw return practices greatly affect the storage of organic carbon in the soil [6–8]. Conventional intensive tillage (CT), which accompanies removing crop straw from the field, results in soil organic carbon decline, soil structural degradation, and greenhouse gas (GHG) emission increase [7,9,10]. On the contrary, no-till (NT) and crop straw return (SR) are regarded as effective ways to increase soil organic carbon (SOC) sequestration [11–13].

Compared with CT, NT can increase SOC content by improving soil aggregation and decreasing CH_4 and CO_2 emissions in the topsoil layer [14,15]. NT reduces soil disturbance, prevents the soil macro-aggregate from being destroyed, provides better physical protection for SOC, and slows down SOC decomposition [2,16]. Moreover, NT can increase the input of organic residues in soil surface, which can be broken down by soil microbes and thus provide microbial binding agents for macroscopic aggregates to form [17,18]. NT can also reduce soil temperature and enhance soil humidity, which leads to the decline in microbial activity and the emissions of CH_4 and CO_2, reducing the loss of SOC [19,20]. However, the effect of NT on SOC content was regulated by complex biochemical processes, such as the GHG emissions and the formation of soil aggregate, and thus consistent conclusions were not obtained [6,21]. The soil microbial regulation mechanism of NT affecting SOC content is still unclear.

Crop straw is a source of organic carbon, and crop straw return is shown to result in enhancing SOC sequestration. Compared to no straw (NS), SR may have positively influenced the SOC content in the topsoil layer by elevating organic carbon input and improving soil microbial community and aggregate stability [9,12,22]. However, straw return also stimulates GHG emissions [17,23], which may offset the positive effects of SR on SOC sequestration [12]. Studies reported that soil microbial community [21], soil aggregate size [7,18] and GHG emissions [24] are closely related to SOC sequestration [25]. Yet, no experiment has so far been conducted to reveal the relationships among soil microbial community, the SOC in soil aggregates, GHG emissions and SOC sequestration as influenced by SR. Further study is needed to reveal the soil microbial regulation mechanism of SR affecting SOC sequestration.

The objectives of this study were to evaluate the effects of NT and SR on SOC content and soil microbial communities in a rice-wheat cropping system and to reveal the mechanisms that enable soil bacterial communities to regulate SOC under NT and SR. Therefore, we studied the effects of tillage (CT and NT) and crop straw return practices (NS and SR) on soil bacterial communities, CH_4 and CO_2 emissions, crop yields, and SOC aggregates in rice-wheat cropping systems. During the experiment, we found that tillage and straw return management had significant effects on SOC content in the 0–5 cm soil layer after two cycles of the rice-wheat rotation, but no significant effect on soil SOC in the 5–10 cm and 10–20 cm soil layers. Therefore, we focused on the 0–5 cm soil layer in this study. We hypothesized that soil bacteria could mediate SOC content through affecting soil aggregate SOC and CH_4 emissions under tillage and straw return management.

2. Materials and Methods

2.1. Experimental Site

The experiment site lies at Dafashi Town (30°01′ N, 115°34′ E), Hubei province, China, and was established in June 2012. The area has a humid mid-subtropical monsoon climate, in which the annual mean air temperature is 16.8 °C, and the average annual precipitation from 2012 to 2014 is 1408.7 mm (Figure 1). The experimental soil (0–20 cm) is a silty clay loam (containing clay 40%, sandy 25%, and silt 40%), which is defined as Gleysol (FAO classification). Besides, the total organic carbon content is 1.64%, total nitrogen content is 0.24%, the pH is 5.9, and the bulk density is 1.20 g cm^{-3}. This site has been dominated by a cropping system of rice (HHZ, *Oryza sativa* L.) and wheat (ZM9023, *Triticum aestivum* L.).

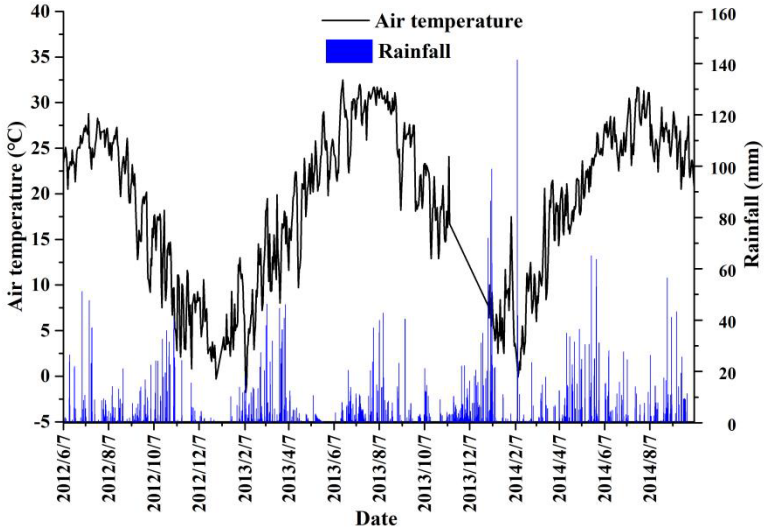

Figure 1. Average daily temperature and rainfall at the experimental site from 2012 to 2014 [2].

2.2. Experimental Design

This experiment was set up in a split-plot design, where tillage (CT and NT) and straw treatment (NS and SR) were set as the main plot and subplots, respectively (Figure 2). Four treatments including: (i) CTNS; (ii) CTSR; (iii) NTNS; and (iv) NTSR were arranged, and each treatment was conducted in triplicates. The area of each plot was 90 m^2 (9 m × 10 m). Under CT treatment, the soil was moldboard plowed twice in one year at a depth of 20 cm before planting rice and wheat. Moldboard plowing was omitted in NT treatment. Crop straw was removed from the field for both CTNS and NTNS treatments. A 6 cm length of crop straw harvested from each plot, was covered on the soil surface under NTSR treatment and incorporated into the soil under CTSR treatment. For all treatments, crop stubbles were kept in the fields. Rice was thrown manually at the rate of 190,000 seedlings per hectare in June and reaped in October. Wheat was directly sown at 150 kg ha^{-1} in October and harvested in May the following year.

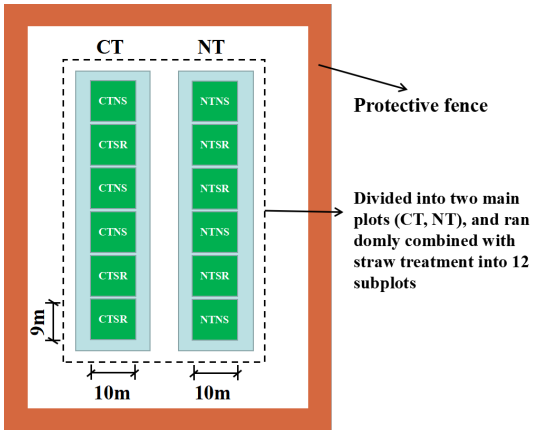

Figure 2. Design drawing of field experiment. Note: CT, conventional tillage; NT, no tillage; CTNS, conventional intensive tillage with straw removal; CTSR, conventional intensive tillage with straw return; NTNS, no tillage with straw removal; NTSR, no-tillage with straw return.

For all treatments, weeds were controlled by spraying 30% chlorpromazine emulsifiable oil containing 10% fenorim. The application rate of chemical fertilizer was 180 kg N ha^{-1}, 90 kg P_2O_5 ha^{-1} and 180 kg K_2O ha^{-1} in rice season and was 144 kg N ha^{-1}, 72 kg P_2O_5 ha^{-1}, and 144 kg K_2O ha^{-1} in wheat season. Commercial compound fertilizer (N:P_2O_5:K_2O = 15%:15%:15%) were used in rice and wheat seasons. P and K fertilizers were applied

immediately as basal fertilizers after throwing or sowing. N fertilizers were applied with four splits (seedling stages: tillering stages: jointing stages: booting stages = 25:10:6:9) for the rice season, and three splits (seedling stages: tillering stages: boosting stages = 5:3:2) for the wheat season. During the rice growing season, the depth of waterlogging was kept at 8 cm, except for during the tillering and maturing stages. The wheat season was not irrigated, except after sowing.

2.3. Soil Sampling and Physicochemical Analysis

Soil samples were collected in May and October after the rice and wheat harvests from 2012 to 2014. The soil samples were taken from eight locations in each plot at a depth of 0–20 cm using a soil sampler with a diameter of 5 cm, before being divided into three categories (0–5 cm, 5–10 cm and 10–20 cm depths). Then part of soil samples in 0–5 cm soil layer were separated into the 1–2 mm, 0.25–1 mm, 0.053–0.25 mm, and <0.053 mm aggregate with a nest of sieves mounted (including 1 mm, 0.25 mm, and 0.053 mm). These soil samples were used to measure SOC, soil microbial biomass carbon (MBC) and dissolved organic carbon (DOC). The remaining soil samples were placed at −20 °C for DNA extraction.

The dry-sieving method was used to separate soil aggregates following the descriptions of Garzia-Bengoetxea et al. [26]. In the present study, the dry sieving method was used as the mechanical pressure exerted from outside is the main cause of soil aggregate destruction, and compared with the wet sieving method, the dry sieving method is less destructive to the soil. Furthermore, drying at a low temperature of 4 °C minimizes the effect on the soil microbial community and activities. Retsch AS200 control (Retsch Technology, Düsseldorf, Germany) was used to separate soil aggregates. Air-dried soil fragments (5 mm) were prepared for separation, and soil samples were separated into 1–2 mm, 0.25–1 mm, 0.053–0.25 mm and <0.053 mm soil aggregates by mechanical shaking (amplitude 1.5 mm) for 2 min.

The SOC content was measured with a FlashEA 1112 elemental analyzer (Thermo Finnigan, Milan, Italy). Fumigation-extraction method was used to measure MBC. MBC was calculated as the ratio of differences in organic carbon extracted from fumigated and non-fumigated soil and the conversion coefficient was 0.38. DOC could be measured by the methods of Jiang et al. [27].

2.4. Phospholipid Fatty Acid Pattern

Phospholipid fatty acids were extracted from a 3 g freeze-dried soil sample using the methods of Frostegård et al. [28]. Briefly, lipids were extracted in a single-phase chloroform–methanol–phosphate buffer system in a ratio of 1:2:0.8 (*v/v/v*). A stream of N_2 was used for drying the different phases. Separation of extracts was performed on solid phase extraction columns (Supelco Inc., Bellefonte, PA, USA). The phospholipid fractions were saponified and methylated to fatty acid methyl esters. Internal standard 19:0 fatty acid methyl esters were added to calculate the absolute amount of fatty acid methyl esters before measurement. We employed a gas chromatograph/mass spectrometry system (6890–5973N series GC/MS Agilent Technologies, Palo Alto, CA, USA) outfitted with a Flame Ionization Detector and HP-5 capillary column (30 m × 0.25 mm × 0.25 μm) with ultra-purified helium as carrier gas for the extraction. The quantification of fatty acid methyl esters was performed.

2.5. Measurement of Crop Grain Yields

Crop grains harvested from the 2012 rice season to the 2014 rice season were measured at the central position in each plot using a 5 m^2 frame. The rice and wheat grains were air dried, weighed, and adjusted to 14.0% and 12.5% moisture content, respectively.

2.6. Measurement of CH_4 and CO_2 Emissions

Static closed steel chamber method was used to monitor soil CH_4 emission [14]. After the crop straw was returned to fields, continuous gas sampling was conducted until the crop harvest. The inner diameter of the chamber was 34 cm and the height of the chamber

was 50 or 120 cm (depending on the rice height). To mix the air in the chambers well, four fans were installed on the top of the chamber. During sampling, two rings were placed in each plot, the chambers were placed temporarily in the groove of rings, and water was added to create a sealed environment. After 0, 5, 10 and 15 min of chamber closure (according to the IGAC recommendations [29]), gas samples were gathered from each chamber. The gas samples were collected using a syringe (20 mL) at the chamber's headspace, and then transferred to 20 mL vacuum glass bottles. The gas samples were collected between 9:00 and 11:00 am once every 7–10 and 10–15 days (recommended by Buendia et al. [30]) in rice and wheat seasons, respectively. A chromatograph equipped with a flame ionization detector (Shimadzu GC-14B) was used to measure CH_4 fluxes [31].

The measurement of soil CO_2 flux was conducted according to the method proposed by Li et al. [31]. The CO_2 fluxes were measured three times for each plot with an 8100–103 short-term chamber connected to a LI-8100A soil CO_2 flux system (Li-Cor Inc., Lincoln, NE, USA). The final value of soil CO_2 flux was obtained by averaging the values of three separate measurements. The calculation of CH_4 and CO_2 fluxes was based on the linear variation in CH_4 and CO_2 fluxes [32]. The cumulative seasonal CH_4 and CO_2 emissions were derived by sequentially accumulating emissions from each of the two adjacent measurement intervals [31].

2.7. High-Throughput Sequencing

According to the instructions, the FastDNA kit for soil (MP Bio-medicals, Santa Ana, CA, USA) was used to extract DNA from the soil samples and then stored at −20 °C. The bacterial hypervariable regions, including V3, V4 and V5 of 16S rDNA, were amplified by PCR using primers 357F (5′-CCTACGGGAGGCAGCAG-3′) and 926R (5′-CCGTCAATTCMTTTRAGT-3′) [33]. The forward primer was modified to include the FLX-titanium adaptor "B" sequence (5′-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-3′), and the reverse primer was linked with the 454 FLX-titanium adaptor "A" (5′-CCATCTCATCCC TGCGTGTCTCCGACTCAG-3′) [21].

DNA samples (10 ng) were applied as templates in the polymerase chain reaction. Polymerase chain reaction was conducted at 95 °C for 3 min, 94 °C for 30 s, 55 °C for 45 s, 72 °C for 1 min with 25 cycles and a final extension step of 72 °C for 7 min. Polymerase Chain Reaction Purification Kit (Axygen Bio, Union City, California, CA, USA) was used for purification of polymerase chain reaction products. The amplitudes from each sample were then combined in equimolar concentrations into one tube prior to 454 pyrophosphate sequencing. Pyrophosphate sequencing was performed by Shanghai Personal Biotechnology Co., Ltd. using the 454 GS-FLX Titanium System (Roche, Switzerland). To ensure analytical accuracy, the Quantitative Insights into Microbial Ecology (QIIME) pipeline [34] was employed to fetch high quality sequences, following the descriptions of Fierer et al. [35]. The unique sequence set was classified into operational taxonomic units OTUs (a threshold of 97% pairwise identity) by the QIIME implementation. Extraction of the longest sequences of the most abundant OTUs was used as a proxy for taxonomic identification for comparison with the Green Gene Database (release 13.8 http://greengenes.secondgenome.com/ (accessed on 10 January 2012)).

2.8. Statistical Analysis

All data were expressed as means and standard deviations of three replicates. The main effects and interactions of tillage and straw returning were conducted using general linear model analysis of variance with SAS 9.0 (SAS Institute 1999) designed for split plot with tillage practice and straw returning methods as fixed factors and replicates as random factors. The least significant difference test was conducted to examine whether the influence of tillage practices, straw return practices, or their interactions were significant at the level of 0.05. To test the effect of experimental treatments on bacterial composition, redundancy analysis was performed using the "vegan" package in R v. 3.1.2 (R Development Core Team, 2014) [36].

Structural equation modeling was performed to reveal the influence paths of tillage and straw return practices on SOC content from the perspectives of the soil bacterial community, DOC, microbial biomass carbon, CH_4 and SOC in 1–2 mm aggregates. The use of structural equation modeling allowed the testing of complex path-relation networks. It should be noted that only the data for the 2013 rice season and 2014 wheat seasons were selected [37]. In the model, tillage (0 = no-till and 1 = tillage) and straw return (0 = straw removal and 1 = straw return) were considered as categorical variables. This approach allowed us to compare the effect of tillage and straw return practices on SOC content. Redundancy analysis results for bacterial communities in order level (relative abundance > 0.5%), were used as 'bacteria community' in the model. A 'robust' maximum likelihood estimation procedure of AMOS 20.0 (IBM SPSS, Chicago, IL, USA) software was conducted for the analysis. The chi-square test, goodness of fit index, comparative fit index, and root square mean error of approximation were used for testing the overall goodness of the fit of the model.

3. Results

3.1. Soil Organic Carbon

Tillage and straw return management significantly changed the SOC content in the 0–5 cm soil layer ($p < 0.05$, Table S1). Compared with CT treatment, NT treatment significantly increased SOC content in the 2013 wheat season (5.7%), 2013 rice season (15.3%) and 2014 rice season (4.4%). In comparison with NS treatment, SR treatment markedly enhanced SOC content in the 2012 rice season (6.6%), 2013 wheat season (8.3%), 2013 rice season (9.1%), 2014 wheat season (6.5%) and 2014 rice season (8.3%). Compared with CTNS, NTNS markedly increased SOC in the 2013 rice season, 2014 wheat season and 2014 rice season by 15.6%, 2.9% and 4.4%, respectively. In comparison with CTSR, NTSR significantly enhanced SOC in the 2013 wheat season, 2013 rice season and 2014 rice season by 7.1%, 15.1% and 4.5%, respectively. Interaction of tillage and straw return practices showed no remarkable effects on SOC content.

3.2. Distribution of Soil Aggregates

Tillage and straw returning methods had a significant effect on the distribution of soil aggregates in the soil layer within the topsoil layer (Table S2). Compared with CT treatment, NT treatment significantly increased the percentage of 1–2 mm soil aggregates in the 2014 wheat season (4.1%) ($p < 0.05$), whereas, there was a markedly reduced percentage of soil aggregates < 0.053 mm in the 2013 rice season (18.9%). Compared with NS treatment, SR treatment resulted in an increased the proportion of 1–2 mm soil aggregates in both wheat and rice seasons of 2013 (5.4%, 4.4%), and in the wheat season of 2014 (5.6%) ($p < 0.05$), but decreased the proportion of soil aggregates <0.053 mm in the 2014 wheat season (21%) ($p < 0.05$). NTNS significantly increased the proportion of 1–2 mm soil aggregates by 6.3% in the 2014 wheat season, and markedly reduced the percentage of soil aggregates < 0.053 mm in the 2013 rice season (7.6%) and 2014 wheat season (20.2%) compared to CTNS. NTSR, respectively, increased the proportion of 1–2 mm soil aggregates by 2.8%, 4.7%, 2.1% in the 2013 wheat season, 2013 rice season, 2014 wheat season, and markedly reduced the percentage of soil aggregates < 0.053 mm in 2013 rice season (29.9%) compared to CTSR. Interaction of tillage practices and straw returning methods showed no significant difference.

3.3. Soil Organic Carbon Content within Aggregates

Tillage and straw return practices greatly influenced the SOC content of aggregates in the 0–5 cm soil layer (Table 1). Compared to CT treatment, NT treatment increased the SOC content in 1–2 mm aggregates in the 2013 wheat season (17%), 2013 rice season (19.9%) ($p < 0.05$, Table 1). Higher SOC content in 0.25–1 mm aggregates was also observed under NT treatment than under CT treatment in the 2013 wheat (14.6%) and rice seasons (13.4%) ($p < 0.05$). Compared with NS treatment, SR treatment led to higher SOC content in

1–2 mm aggregates in the 2013 rice season (17.2%), and 2014 wheat season (17.4%) ($p < 0.05$). Moreover, higher SOC content in 0.25–1 mm aggregates was found in the 2013 rice season (7%) and 2014 wheat season (6.2%) under SR treatment than under NS treatment ($p < 0.05$). Compared to CTNS, NTNS significantly increased the SOC content in 1–2 mm aggregates in the 2013 rice season by 17.0%. Moreover, there were also significant differences in the SOC content of 0.25–1 mm aggregates in the 2013 rice season (16.5%), and in the 2014 wheat season (3.6%) between CTNS and NTNS. NTSR, respectively, increased the SOC content in 1–2 mm aggregates by 17.9%, 22.4%, 8.3% in the 2013 wheat season, 2013 rice season and 2014 wheat season, and enhanced SOC content in 0.25–1 mm aggregates in the 2013 rice season (10.6%) and 2014 wheat season (4.1%) relative to CTSR. In other soil layers, there was no remarkable difference between treatments. Interaction of tillage and straw return practices remarkably influenced the SOC content in 1–2 mm aggregates in the 2014 wheat season ($p < 0.05$).

Table 1. SOC contents (g kg^{-1}) of aggregate fractions under different tillage and straw return practices (2012–2014).

Crop Season	Soil Aggregate Fraction	CTNS	CTSR	NTNS	NTSR	T	SR	T × SR
2012 rice season	1–2 mm	17.03 ± 1.05 ^{a}	18.97 ± 0.60 ^{a}	18.61 ± 2.39 ^{a}	21.14 ± 1.70 ^{a}	ns	ns	ns
	0.25–1 mm	15.60 ± 0.32 ^{a}	17.07 ± 0.76 ^{a}	14.16 ± 0.03 ^{a}	16.94 ± 1.66 ^{a}	ns	ns	ns
	0.053–0.25 mm	19.67 ± 1.08 ^{a}	16.49 ± 1.72 ^{a}	20.62 ± 0.3 ^{a}	16.85 ± 2.00 ^{a}	ns	ns	ns
	<0.053 mm	17.97 ± 1.09 ^{a}	19.49 ± 1.04 ^{a}	18.72 ± 0.43 ^{a}	18.00 ± 0.38 ^{a}	ns	ns	ns
2013 wheat season	1–2 mm	16.32 ± 1.00 ^{c}	18.20 ± 0.29 ^{bc}	18.92 ± 0.91 b	21.45 ± 0.63 ^{a}	*	ns	ns
	0.25–1 mm	17.52 ± 0.55 ^{a}	18.52 ± 0.65 ^{a}	19.77 ± 0.12 ^{a}	21.53 ± 1.76 ^{a}	*	ns	ns
	0.053–0.25 mm	20.79 ± 1.62 ^{a}	21.59 ± 0.98 ^{a}	23.42 ± 1.91 ^{a}	26.14 ± 3.05 ^{a}	ns	ns	ns
	<0.053 mm	15.02 ± 1.34 ^{a}	16.59 ± 1.54 ^{a}	16.97 ± 1.75 ^{a}	19.79 ± 1.45 ^{a}	ns	ns	ns
2013 rice season	1–2 mm	16.14 ± 0.67 ^{c}	18.46 ± 0.28 ^{bc}	18.88 ± 0.43 ^{b}	22.60 ± 1.36 ^{a}	*	*	ns
	0.25–1 mm	18.06 ± 0.43 ^{c}	19.87 ± 0.18 b	21.04 ± 0.46 ^{ab}	21.98 ± 0.36 ^{a}	*	*	ns
	0.053–0.25 mm	16.90 ± 0.84 ^{a}	16.93 ± 1.82 ^{a}	18.23 ± 0.50 ^{a}	19.65 ± 1.00 ^{a}	ns	ns	ns
	<0.053 mm	15.51 ± 1.19 ^{a}	16.92 ± 1.95 ^{a}	20.18 ± 0.81 ^{a}	18.81 ± 0.77 ^{a}	ns	ns	ns
2014 wheat season	1–2 mm	16.25 ± 0.12 ^{c}	18.34 ± 0.18 ^{b}	16.30 ± 0.07 ^{c}	19.87 ± 0.34 ^{a}	ns	*	*
	0.25–1 mm	20.66 ± 0.33 ^{c}	21.89 ± 0.19 ^{ab}	21.40 ± 0.55 ^{bc}	22.78 ± 0.14 ^{a}	ns	*	ns
	0.053–0.25 mm	17.49 ± 0.88 ^{a}	18.22 ± 0.82 ^{a}	18.75 ± 0.85 ^{a}	19.63 ± 1.37 ^{a}	ns	ns	ns
	<0.053 mm	17.72 ± 4.40 ^{a}	17.83 ± 2.02 ^{a}	16.99 ± 3.11 ^{a}	17.67 ± 1.63 ^{a}	ns	ns	ns

Different letters in the columns denote statistical differences in the means of the variables between treatments by the least significant difference test ($p < 0.05$). * $p < 0.05$; ns, not significant. CTNS, conventional intensive tillage with straw removal; CTSR, conventional intensive tillage with straw return; NTNS, no tillage with straw removal; NTSR, no-tillage with straw return. T, tillage; SR, straw return practices. T × SR, the interactions between tillage and straw return. Values are mean ± standard deviation (n = 3).

3.4. Soil Dissolved Organic Carbon and Microbial Biomass Carbon

Tillage and straw returning methods had significant effects on DOC contents in the 0–5 cm soil layer (Table S3). Compared to CT treatment, NT treatment markedly increased the DOC contents in both wheat seasons and rice seasons in 2014 (12.3%, 8.8%) (Table S3, $p < 0.05$). Similarly, higher DOC contents were found in both wheat and rice seasons in 2013 (23.7%, 23.8%) and 2014 (18.5%, 13%) ($p < 0.05$) under the SR treatment than under the NS treatment. Compared with CTNS, NTNS showed a significant increase in the DOC contents in the 2013 rice season (3.3%) as well as in the 2014 wheat (13.4%) and rice seasons (10.4%). NTSR showed significantly higher DOC contents in both wheat and rice seasons in 2013 (16.5%, 13.8%) and 2014 (11.4%, 7.4%) relative to CTSR. The interaction between NT and SR remarkably influenced the DOC during the whole 2013 season.

Relative to CT treatment, NT treatment significantly increased the MBC contents in the 0–5 cm soil layer in both wheat and rice seasons in 2013 (15.1%, 14.3%) and 2014 (21.5%, 39.8%) (Table S4, $p < 0.05$). SR treatment also resulted in higher MBC contents in both wheat and rice seasons in 2013 (27.8%, 18.1%) and 2014 (26.6%, 20.1%) ($p < 0.05$) than NS treatment. Compared to CTNS, NTNS displayed a statistically significant improvement

in MBC contents for both the 2013 (19.2%, 6.9%) and 2014 wheat and rice seasons (5.7%, 33.2%). NTSR also showed an improvement in the 2013 wheat season (12.0%), 2013 rice season (21.0%), 2014 wheat season (35.8%) and 2014 rice season (45.5%) against CTSR. Interactions of tillage and straw returning practices had significant effects on MBC contents with the exception of the 2012 and 2013 wheat seasons ($p < 0.05$).

3.5. Greenhouse Gas Emissions

Relative to CT treatment, NT treatment remarkably decreased CH_4 emissions during the 2014 rice seasons (15.6%) ($p < 0.05$) (Table 2). SR treatment resulted in higher CH_4 emissions during the rice (by 34.0–91.2%) and wheat (by 22.1–22.3%) seasons throughout all three experimental years ($p < 0.05$). Compared with CTSR, NTSR showed a significant reduction in CH_4 emissions in the 2013 wheat season (18.0%), 2014 wheat season (10.8%), and 2014 rice season (16.6%). However, the NTNS showed the lowest CH_4 emissions among all treatments in whole seasons. Interaction of tillage and straw return practices showed no significant effects on CH_4 emissions.

Table 2. Seasonal CH_4 emissions (kg hm^{-2}) under different tillage and straw return practices (2012–2014) (has been published by Guo et al. [2]).

Treatment	2012	2013		2014	
	Rice Season	Wheat Season	Rice Season	Wheat Season	Rice Season
CTNS	400 ± 7.51 ^{b}	4.86 ± 0.98 ^{c}	475 ± 21.7 ^{b}	5.39 ± 0.54 ^{c}	167 ± 11.37 ^{b}
CTSR	560 ± 30.73 ^{a}	16.91 ± 0.37 ^{a}	645 ± 12.0 ^{a}	15.95 ± 0.99 ^{a}	202 ± 13.68 ^{a}
NTNS	391 ± 21.16 ^{b}	3.99 ± 0.42 ^{c}	445 ± 7.7 ^{b}	4.81 ± 0.46 ^{c}	140 ± 10.60 ^{b}
NTSR	632 ± 27.09 ^{a}	12.53 ± 2.23 ^{b}	610 ± 9.7 ^{a}	12.33 ± 0.60 ^{b}	162 ± 2.35 ^{b}
T	ns	ns	ns	ns	*
SR	*	*	*	*	*
T × SR	ns	ns	ns	ns	ns

Different letters in the columns denote statistical differences in the means of the variables between treatments by the least sign difference test ($p < 0.05$). * $p < 0.05$; ns, not significant. CTNS, conventional intensive tillage with straw removal; CTSR, conventional intensive tillage with straw return; NTNS, no tillage with straw removal; NTSR, no-tillage with straw return. T, tillage; SR, straw return practices. T × SR, the interactions between tillage and straw return. Values are mean ± standard deviation (n = 3).

Compared with CT treatment, NT treatment lowered CO_2 emissions in the 2014 wheat (7.2%) and rice reasons (21.4%) ($p < 0.05$) (Table 3). SR treatment induced more CO_2 emissions ($p < 0.05$) than NS treatment in the 2012 rice season (91.2%), 2013 wheat (22.1%) and rice seasons (40.8%), and 2014 wheat (22.3%) and rice seasons (34.0%). Compared with CTNS, NTNS markedly elevated CO_2 emissions in the 2012 rice season by 19.3%, whereas, it reduced CO_2 emissions in the 2013 rice season by 22.7% and 2014 rice season by 10.9%. NTSR had lower CO_2 emissions in the 2012 rice season, 2013 rice season, 2014 rice season (by 9.7%, 14.6% and 28.5%, respectively) against CTSR. Interaction of tillage and straw return practices showed a significant effect on CO_2 emissions only in the 2012 rice seasons and 2014 rice seasons ($p < 0.05$). Meanwhile, the combination of NT and NS can reduce CO_2 emissions compared to other treatments.

Table 3. Seasonal CO_2 emissions (kg hm^{-2}) under different tillage and straw return practices (2012–2014) (has been published by Guo et al. [2]).

Treatment	2012	2013		2014	
	Rice Season	Wheat Season	Rice Season	Wheat Season	Rice Season
CTNS	2230 ± 92 ^{d}	5282 ± 123 ^{b}	4833 ± 397 ^{b}	3982 ± 84 ^{bc}	4283 ± 50 ^{c}
CTSR	4914 ± 27 ^{a}	6695 ± 408 ^{ab}	6503 ± 308 ^{a}	4989 ± 123 ^{a}	6332 ± 154 ^{a}
NTNS	2660 ± 86 ^{c}	6090 ± 298 ^{ab}	3734 ± 116 ^{c}	3799 ± 124 ^{c}	3817 ± 88 ^{d}
NTSR	4438 ± 47 ^{b}	7193 ± 939 ^{a}	5557 ± 265 ^{b}	4525 ± 452 ^{ab}	4525 ± 162 ^{b}
T	ns	ns	ns	*	*
SR	*	*	*	*	*
T × SR	*	ns	ns	ns	*

Different letters in the columns denote statistical differences in the means of the variables between treatments by the least sign difference test ($p < 0.05$). *, $p < 0.05$; ns, not significant. CTNS, conventional intensive tillage with straw removal; CTSR, conventional intensive tillage with straw return; NTNS, no tillage with straw removal; NTSR, no-tillage with straw return. T, tillage; SR, straw return practices. T × SR, the interactions between tillage and straw return. Values are mean ± standard deviation (n = 3).

3.6. Soil Microbial Community

Tillage and straw returning methods significantly influenced bacterial biomass in 0–5 cm soil layer (Table S5). Compared with CT treatment, NT treatment increased the bacterial biomass in the 2013 rice seasons (35.6%) and 2014 wheat seasons (56.2%) ($p < 0.05$). SR treatment led to higher bacterial biomass than NS treatment in the 2013 wheat season (76.5%), 2013 rice season (54.9%), 2014 wheat season (75.7%), 2014 rice season (59.7%) ($p < 0.05$). Compared with CTNS, NTNS had a greater impact on bacterial biomass in the 2013 rice season (33.1%), and in the 2014 wheat season (38.3%), while for fungi, NTNS had a significant reduction in the 2013 wheat season (25.2%). Compared to CTSR, NTSR had a greater effect on microorganisms in both wheat and rice seasons in 2013 (70.1%, 37.1%) and 2014 (67%,75%), while for fungi, NTSR showed a significant improvement in the 2013 wheat season (23.8%). The interaction of tillage and straw return practices had a significant effect on bacterial biomass in the 2014 wheat season ($p < 0.05$). Tillage and straw returning practices had no significant effects on fungal biomass in the 2013 wheat seasons (Table S5). The fungal PLFAs were not detected in the 2013–2014 rice seasons.

3.7. Soil Bacterial Community

Soil bacterial community was mainly composed of phylum Acidobacteria, Verrucomicrobia and Proteobacteria in the 2013 rice season (Table S6), while it was mainly composed of phylum Acidobacteria, Chloroflexi and Proteobacteria in the 2014 wheat season (Table S7).

In the 2013 rice season, compared with CT treatment, NT treatment affected the abundance of *Gp1* (−17.7%), *Gp18* (−19.4%), *Gp4* (65.2%), *Gp16* (21.6%), *Dehalogenimonas* (29.1%), *Caulobacterales* (27.6%), *Desulfuromonadales* (33.0%), *Myxococcales* (42.8%), and *Legionellale* (−9.7%) (Table S6, $p < 0.05$). Compared with NS treatment, SR treatment significantly affected the abundance of *Gp18* (−16.3%), *Gp4* (48.1%), *Gp17* (16.4%), *Chlamydiales* (−26.0%), *Caulobacterales* (19.7%), *Burkholderiales* (−37.6%) ($p < 0.05$). Compared with CTNS, NTNS significantly increased the abundance of *Rhizobiales* (7.0%), *Burkholderiales* (13.4%), and *Spartobacteria_genera_incertae_sedis* (46.2%), whereas, it markedly decreased the abundance of order *Gp1* (1.5%), *Gp18* (25.2%), *Gp4* (38.3%), and *Dehalogenimonas* (17.2%). Compared with CTSR, NTSR significantly elevated the abundance of *Gp4* (267%), *Holophagales* (85.4%), *Dehalogenimonas* (103.4%), *Chlamydiales* (33.9%), *Caulobacterales* (50.9%), *Burkholderiales* (27.8%), and *Spartobacteria_genera_incertae_sedis* (30.5%), whereas it significantly reduced the abundance of order *Gp1* (32.0%) and *Rhizobiales* (17.2%). Interaction of tillage and straw return practices significantly affected the abundance of *Gp1*, *Gp18*, *Gp4*, *Gp17*, *Holophagales*, *Dehalogenimonas*, *Chlamydiales*, *Caulobacterales*, *Syntrophobacterales*,

and *Spartobacteria_genera_incertae_sedis* ($p < 0.05$). NTSR showed the highest or the lowest bacterial community richness compared with the NTNS and CTSR.

In the 2014 wheat season, NT treatment led to a higher abundance of *Myxococcales* (206.3%) than CT treatment (Table S7, $p < 0.05$). In comparison with NS treatment, SR treatment brought out higher enrichment of *Gp4* (573.9%), *Gp10* (502.4%), *Gp18* (97.5%), *Sphingobacteriales* (164.7%) *Gp7* (303.8%), *Sphingobacteriales* (164.7%), *Flavobacteriales* (89.4%), and *Myxococcales* (173.2%), while it decreased the abundance of *Gemmatimonadales* (47.8%) and *Rhodospirillales* (42.0%) ($p < 0.05$). Compared with CTNS, NTNS significantly increased the abundance of order *Myxococcales* (176.0%), whereas it markedly decreased the abundance of order *Xanthomonadales* (5.5%). Compared with CTSR, NTSR significantly increased the abundance of *Gp4* (161.9%), *Gp10* (429.1%), *Gp18* (106.2%), *Gp7* (145.9%), *Xanthomonadales* (17.7%), and *Myxococcales* (218.1%). The interplay of tillage and straw return practices significantly influenced the abundance of *Gp18* and *Flavobacteriales* ($p < 0.05$).

3.8. Crop Grain Yields and Their Relationship with Soil Properties

Crop grain yields in this study were reported in our previous study (Table S8) [2]. NT treatment significantly reduced crop yields by 8.8% in the 2014 wheat season compared to CT treatment ($p < 0.05$). SR treatment showed no significant difference relative to NS. There was no significant difference in grain yields between NTNS and CTNS. NTSR had a remarkable increase in crop yields in the 2014 wheat season compared to CTSR (19.1%, $p < 0.05$). Interaction of tillage and straw return practices showed a significant effect on crop yields in the 2014 wheat season ($p < 0.05$). A significant correlation was observed between DOC and crop yields (Table S9).

3.9. Relationship of Bacterial Community with Yield, Soil Aggregates and Soil Organic Carbon Fractions

Redundancy analysis (RDA) showed that soil bacterial community was considerably influenced by SOC content in 1–2 mm aggregates, MBC and CH_4 emissions (Figure 3, $p < 0.05$). MBC and SOC in 1–2 mm aggregates were closely related to *Gp6*, *Burkholderiales*, *Gp10*, *Sphingobacteriales*, *Myxococcales*, *Gp16*, *Flavobacteriales*, *Gp2*, *Gp3*, and *Xanthomonadales*. CH_4 emissions were closely related to *Subdivision3_genera_incertae_sedis*, *Gp18*, *Caulobacterales*, *Gp16*, and *Chlamydiales*. Besides, no significant correlation were found between crop yield and microbial community.

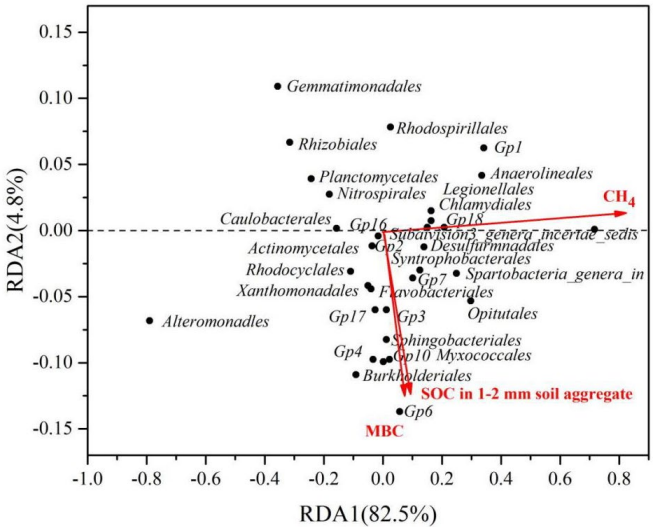

Figure 3. Redundancy analysis (RDA) ordination plot showing changes in bacterial community composition in 0–5 cm soil layer at order level (relative abundance > 0.5%) during the 2013 rice season and 2014 wheat season. SOC, soil organic C; MBC, microbial biomass C.

The structural equation modeling revealed that the predictors could explain 85.0% of the variances in SOC content (Figure 4). Soil bacterial community mediated SOC under tillage and straw systems through affecting SOC in 1–2 mm aggregates and CH_4 emissions.

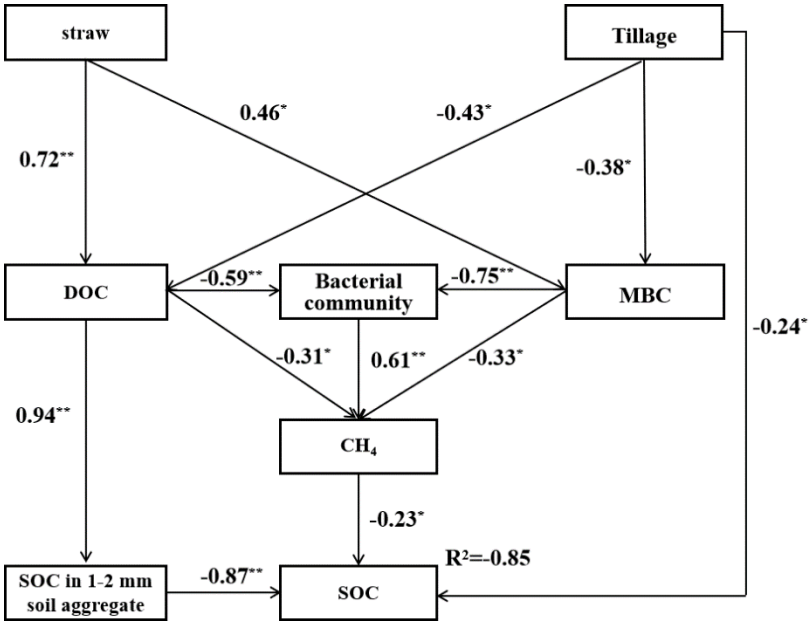

Figure 4. Selected structural equation modeling (data from 2013 rice season and 2014 wheat season were selected) for SOC in 0–5 cm soil layer (The chi-square test = 9.91; Goodness of fit index = 1.00; Comparative fit index = 0.91; Root square mean error of approximation = 0.00), based on the impact of tillage and straw return practices and SOC fractions. Values related to the solid arrows stand for the path coefficients. R^2 indicates the proportion of variance explained. Significance levels are as follows: * $p < 0.05$; ** $p < 0.01$. Straw indicates straw systems; DOC indicates dissolved organic carbon; MBC indicates microbial biomass carbon; SOC indicates soil organic carbon.

4. Discussion

4.1. Impact of NT and Straw Return on SOC Content in Aggregates

NT can enhance SOC content by promoting the SOC sequestration in macro- aggregate [7,38,39]. In this study, a higher proportion of 1–2 mm soil aggregates (Table S2), and more SOC content in 1–2 mm aggregates, were found under NT than under CT (Table 1). Tillage operations breaks soil macro-aggregates and results in SOC losses [40]. Conversely, NT keeps soil undisturbed, which is conducive for accelerating the formation of macro-aggregate, and reduces the degradation rate of SOC [41]. Moreover, NT can provide more physical protection for soil aggregates and promote the longevity of newly-formed macro-aggregates, leading to stabilization of SOC in the micro-aggregates formed within stable macro-aggregates [42,43].

As an essential organic matter source, SR can promote the formation of soil macro-aggregates and increase SOC content. Previous studies have well reported that SR can increase SOC content by increasing the input of organic carbon input [12,43]. In this work, higher SOC content in the topsoil layer (0–5 cm) was observed under SR than under NS (Table S1), which may be due to higher SOC sequestrated in 1–2 mm aggregates (Table S2). Straw degradation generates a large number of organic matter particles, contributing to the formation of macro-aggregates and the accumulation of SOC in macro-aggregate [7,17,44,45].

Some studies reported that interaction of tillage and straw return practices significantly affected SOC, possibly as NT can promote the accumulation of straw on the soil surface, thus enhancing SOC sequestration in the topsoil [21]. Similarly, we also found that both under NS or SR conditions, NT caused higher SOC in 1–2 mm and 0.25–1 mm aggregates than CT (Table 1). Moreover, the interaction of tillage and straw return practices significantly

affected SOC in 1–2 mm aggregates in the 2014 wheat season (Table 1), suggesting that under straw return condition, NT can further promote SOC sequestration in 1–2 mm aggregates [7,21]. However, the interaction of tillage and straw return practices had no significant effect on SOC in other aggregates sizes (Table 1), possibly as 1–2 mm soil aggregates are more sensitive than soil aggregates of other smaller sizes [2,21].

4.2. Effect of NT and Straw Return on Greenhouse Gas Emissions

Emissions of CH_4 and CO_2 are important pathways of carbon loss from agricultural soil [24,31,46]. In this study, NT treatment reduced CH_4 and CO_2 emissions compared with CT treatment (Tables 2 and 3). CH_4 emissions are primarily affected by the availability of organic matter and oxygen [12,17,46]. NT decreases soil disturbance and improves gas diffusion, inhibiting the growth of methanogenic bacteria and reducing the production of CH_4 [23]. NT also can enhance soil moisture, reduce soil temperature, slow the organic residue degradation, and reduce the activity of soil microorganisms, thus reducing CH_4 and CO_2 emissions [12,19,20].

In contrast, straw return promoted CH_4 and CO_2 emissions (Tables 2 and 3) mainly by providing a large number of organic carbon for soil microorganisms [12,46]. Moreover, anaerobic degradation of crop residues can reduce soil Eh, thus increasing methanogenic populations and enhancing CH_4 emissions [17,47,48]. Nevertheless, a large number of straw-derived carbon can be sequestrated in soil by the formation of resistant organic matter, which may offset the losses of SOC caused by CH_4 and CO_2 emissions.

In this study, the interaction of tillage and straw return practices had no effect on CH_4 and CO_2 emissions in the experiment (Tables 2 and 3), which may be due to SR and NT having the opposite effect on CH_4 emissions. SR significantly enhanced CH_4 and CO_2 emissions, whereas, NT was found to have reduced CH_4 and CO_2 (Table 2). We also found that CTNS had no significant effect on CH_4 emissions relative to NTNS, while NTSR had lower CH_4 emissions than NTNS (Table 2). The reason may be the fact that NT leads to more straw being accumulated in the soil surface, which has better oxygen available than topsoil layers, thus inhibiting the production of CH_4 from the soil [17,48]. Besides, CTNS had higher CO_2 emissions than NTNS, and CTSR also had more CO_2 emissions than NTSR. This can be attributed to there being a lower soil temperature under NT than under CT, thus leading to a decrease in the activity of soil microorganisms, and subsequently to a decrease in CO_2 emissions [19,20].

4.3. Effects of NT and Straw Return on Bacterial Community

Microorganisms play a key role in regulating SOC turnover and sequestration [49]. The bacterial community accounts for the majority of soil microorganisms in the rice-wheat cropping system (Table S5) [50], which is probably due to the long-term flooding of the field during the rice season resulting in the formation of an anaerobic environment, inhibiting the growth of the soil fungal community [21]. The bacterial community is sensitive to tillage and straw management [51]. Common dominant bacteria such as Phylum Actinobacteria, Proteobacteria and Actinobacteria are recognized to be remarkable plant biomass decomposers [52–54]. In this study, Phylum Acidobacteria, Verrucomicrobia and Proteobacteria phylum were dominated in the 2013 rice season (Table S5) and phylum Acidobacteria, Chloroflexi and Proteobacteria phylum were dominated in the 2014 wheat season (Table S6).

We found that NT significantly affected the bacterial community in the 2013 rice season and 2014 wheat season (Tables S5 and S6). NT can enhance some bacterial abundance related to the decomposition of crop residue [52,54,55], for example, phylum Actinobacteria (including order *Gp4*, *Gp16*), phylum Chloroflex (including order *Dehalococcoidetes*), phylum Proteobacteria (including order *Myxococcales*), phylum Alphaproteobacteria (including order *Caulobacterales*), phylum Chloroflexi (including order Dehalococcoidates), and phylum (including *Desulfuromonadales* and *Myxococcales*) (Tables S5 and S6). This is probably due to the fact that NT can provide more available substrates and nutrients for

soil microorganisms [56]. However, we also found NT decreased the abundance of bacteria such as order *GP1* and *Gp18* in 2013 rice season (Table S5), which may be due to *Gp1* and *Gp18* benefitting from a poor nutrition condition [57].

Straw return can input a large quantity of straw-derived carbon into soil, and thus affect the bacterial community [56,58,59]. In this study, straw return observably affected the bacterial community in the 2013 rice season and 2014 wheat season (Tables S6 and S7). Generally, SR can tend to increase the abundance of the bacterial community as SR can provide more metabolic substrates for bacteria [12]. SR can improve soil properties, such as soil permeability and water holding capacity, and provide comfortable habitat conditions for bacteria, thus improving the bacterial community [12,59]. However, in this study, SR decreased the abundance of some within the bacterial community, such as order *Caulobacterales*, which may be due to SR increasing the availability of oxygen and thus inhibiting the growth of *Caulobacterales* in the 2013 rice season [60]. SR also decreased the abundance of order *Gp18* in the 2013 rice season, which is probably due to the fact that order *Gp1* and *Gp18* could benefit from a poor nutrition condition [57].

In this study, the interaction of tillage and straw return practices significantly influenced the abundance of the bacterial community in 0–5 cm soil layer, such as order *Gp1, Gp18*, *Gp4, Gp17, Holophagales, Dehalogenimonas, Chlamydiales, Caulobacterales, Syntrophobacterales,* and *Spartobacteria_genera_incertae_sedis* (Tables S6 and S7). SR and NT tended to increase the abundance of the soil bacterial community, and the combination of SR and NT can provide better habitat conditions, such as higher availability of oxygen and greater organic carbon for the soil bacterial community [12,59]. However, some within the bacterial community, such as order *G16* and *Rhodospirillales*, were not significantly affected by the interaction (Tables S6 and S7). This can be attributed to high diversity of soil bacterial community, and the difference in the preference of soil microorganisms regarding habitat conditions, such as oxygen availability and carbon and nitrogen sources. Moreover, crop rotation can also reduce the interaction effect of tillage and straw return practices on the soil bacterial community [21,26].

4.4. Effect of NT and Straw Return on Crop Yields

The effect of NT and SR on crop yields was discussed in our previous study [2]. In this study, NT had no significant effect on grain yields during 2012–2014, except that NT significantly reduced crop yields in the 2014 wheat season (Table S8). In general, less than five years of continuous NT is not enough to change crop yields [2,61]. Lower yields under NT than under CT in the 2014 wheat season can be attributed to high rainfall during the growth season of wheat (Figure 1). NT can promote the accumulation of straw residue on the soil surface, and enhance the soil anaerobic condition in the case of high rainfall, inhabiting the growth of wheat under NT [2,62–64]. Moreover, NT can decrease crop yield and may be due to decreased productive tillers and increased weed growth [2].

On the contrary, straw return often increases crop yields, as SR can enhance the input level of organic matter, thus improving soil nutrient conditions [61]. In this study, SR had no effect on crop yields (Table S8). The reason may be the fact that a long time is required, usually, for straw to be degraded and then change soil physical-chemical properties. Therefore, short-term straw return may have little effect on crop yields [2].

In this study, the interaction of tillage and straw return practices had no effect on crop yields, except in the 2014 wheat season (Table S8). Generally, the interaction of long-term tillage and straw return can increase crop yield, as long-term NT or SR can promote straw residue input into the soil, thus providing more nutrition for crops [3]. However, short-term NT and SR cannot significantly change crop yields [11].

4.5. Relationships between Soil Organic Carbon and Bacterial Community under Different Tillage and Straw Return Practices

The soil bacterial community largely contributes to aggregates stabilization and SOC sequestration [25,41]. In this study, the bacterial community (such as *Gp6*, *Gp10*, *Gp16*,

Gp18), Planctomycetes (including *Burkholderiales* and *Subdivision3_genera_incertae_sedis*) and Actinobacteria (such as *Sphingobacteriales*) were significantly affected by MBC and SOC in 1–2 mm macro-aggregates (Figure 3), which may be due to the fact that bacteria can metabolize organic matter and be stabilized as microbial residues in organic mineral complexes [53,54]. During the process of the decomposition of organic matter, a large quantity of broken organic carbon is released, contributing to the formation of soil macro-aggregates [64].

We found that the bacterial community, such as *Subdivision3_genera_incertae_sedis*, *Gp18*, and *Caulobacterales*, observably affected CH_4 emissions (Figure 3). It was reported that the bacterial community could affect CH_4 emissions through changing the availability of oxygen and organic carbon for methanogens [65–67]. The bacterial community can provide organic carbon for methanogens by degrading crop residue and thus enhance CH_4 emissions [67,68]. Besides, methanotrophic bacteria are important mediators for CH_4 consumption, which plays a significant role in controlling CH_4 emissions [69]. Therefore, the bacterial community may contribute to the shift in SOC content in macro-aggregates and CH_4 emissions, thus affecting the dynamics of SOC [17,41].

In this study, structural equation modeling analysis showed that SOC in 1–2 mm aggregates and CH_4 emissions jointly affected SOC sequestration under tillage and straw return systems (Figure 4), suggesting that SOC content was regulated by the balance between the SOC sequestration in 1–2 mm aggregates and the SOC losses induced by CH_4 emissions. Compared with CT, NT enhanced the formation of macro-aggregates (Table S2) and the accumulation of SOC in 1–2 mm aggregates (Table 1), while it reduced CH_4 emissions (Table 2), resulting in an increase in SOC content in the topsoil layer [32,41,42]. Compared with NS, SR promoted the losses of SOC induced by CH_4 emissions compared with NS (Table 2), and accelerated an increase in SOC sequestration in 1–2 mm aggregates (Table 1). Moreover, part of the straw could be sequestrated in soil by forming recalcitrant organic matter [7], which leads to increase in SOC content. Therefore, it can be concluded that both NT and SR increased SOC content, which may be the results of the balance between SOC accumulation in 1–2 mm aggregates and CH_4 emissions.

5. Conclusions

Both NT and SR increased SOC content in 0–5 cm topsoil layers in a rice-wheat cropping system. Our study indicates that NT and SR increased SOC content in 1–2 mm soil aggregates. NT resulted in lower CO_2 and CH_4 emissions compared with CT. However, SR increased CO_2 and CH_4 emissions compared to NS. Bacterial communities (such as Gp6, Gp10, Gp16 and Gp18), had significant relationships with SOC in 1–2 mm aggregates and MBC. Bacterial communities like *Subdivision3_genera_incertae_sedis*, *Gp18*, and *Caulobacterales* had *the most effect on* CH_4 emissions. Our study highlights that 4.4–15.3% of increase in SOC contents under NT and straw return were mainly due to the balance between SOC accumulation in 1–2 mm soil aggregates and CH_4 emissions in rice and wheat cropping systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12101552/s1, Table S1: Changes of soil organic carbon contents (g kg^{-1}) under different tillage and straw return practices from (2012–2014); Table S2: Changes in aggregate composition (%) under different tillage and straw return practices in 0–5 cm soil layer (2012–2014); Table S3: Changes of dissolved organic carbon (g kg^{-1}) contents in 0–5 cm soil layer under different tillage and straw return practices during 2012–2014; Table S4: Changes of soil microbial biomass carbon (mg kg^{-1}) contents in 0–5 cm soil layer under different tillage and straw return practices during 2012–2014; Table S5: Soil bacterial and fungal PLFA under different tillage practices and residue returning methods in 0–5 cm soil layer (2013–2014); Table S6: The change in bacterial community at order level (relative abundance > 0.5%) under different tillage and straw return practice in 2013 rice season; Table S7: The change in bacterial community at order level (relative abundance > 0.5%) under different tillage and straw return practice in 2014 wheat season. Bacterial community; Table S8: The change in bacterial community at order level (relative abundance > 0.5%)

under different tillage and straw return practice in 2014 wheat season. Bacterial community; Table S9: The relationship between crop yield and soil properties.

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