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The Effect of Long-Term Organic Amendments on Soil Organic Carbon Accumulation via Regulating Microbial Traits in a Paddy Soil

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Abstract

Understanding how organic amendments affect microbial carbon use efficiency (CUE) and necromass C (MNC) is crucial for understanding soil organic C (SOC) formation and accrual in paddy fields, but the underlying mechanisms remain largely unclear. In this study, the microbial CUE, MNC, and microbial community composition, as well as SOC fractions and chemical composition, were measured under long-term organic amendments: rice straw (RS), green manure (GM), and pig manure (PM) in paddy soils. Four treatments were included: (1) chemical fertilizers (CF); (2) CF plus RS (CF + RS); (2) CF plus GM (CF + GM); and (4) CF plus PM (CF + PM). The CUE, MNC, and microbial community were determined by ^{18}O-H_2O incubation, amino sugars levels, and phospholipid fatty acids (PLFAs) content, respectively. Results showed that SOC, particulate organic C (POC), and mineral-associated organic C (MAOC) concentrations were significantly increased by organic amendments compared with chemical fertilization alone. The O-alkyl C decreased, but aromatic C increased with long-term organic amendments, suggesting enhanced SOC hydrophobicity. GM and PM inputs significantly enhanced microbial CUE, but straw return did not affect microbial CUE compared to CF. Microbial growth and C uptake increased by 25.2–42.4% and 19.8–30.0% under organic amendments relative with CF. Microbial respiration was increased by RS and GM amendments. Turnover time was more rapid in CF + RS and CF + GM than in CF and CF + PM. Compared to CF, organic amendments increased the MNC concentration due to the increase in microbial biomass. In addition, CF + RS and CF + GM enhanced the MNC contribution to SOC, but PM had no effect, suggesting that PM contributed more organic C from non-microbial sources. The SOC, POC, and MAOC increased with microbial CUE and MNC, indicating that microbial traits play a crucial role in SOC accrual. Higher microbial CUE and biomass explained the increased MNC accumulation under organic amendments. Our study highlights the crucial role of microbe-mediated processes in SOC accrual under long-term organic amendments in paddy soils. Our findings show that organic amendments are an effective management practice for accumulating more SOC in paddy soils.

Keywords: soil organic carbon fractions; organic fertilization; microbial carbon use efficiency; microbial necromass; paddy fields

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1. Introduction

Minor changes in soil organic carbon (SOC) inevitably affect global climate change and soil productivity by influencing soil fertility because SOC is the largest terrestrial soil C pool [1,2]. Therefore, a continuous increase in stable SOC pools could alleviate global climate change and ensure food security [3]. Rice paddies are agriculture systems consisting of anthropogenic soils with long-term rice cultivation under irrigation, producing 25% of the grain in China [4]. Paddy soils store 18 Pg of SOC in the 0–100 cm depth, which corresponds to 14.2% of SOC stock in global agricultural lands [5]. Enhancing the potential for stable SOC pools in paddy fields is important for addressing the challenges of climate change [4]. Amendment with organic materials is a key practice for improving SOC sequestration and enhancing crop yields [6,7]. Globally, organic materials application can increase 210–600 kg C ha^{-1} $year^{-1}$ of SOC in cropland soils [8]. Green manure (GM) has potential to increase SOC sequestration in paddy soils [9]. However, the microbial mechanisms underlying SOC accumulation in response to organic amendments are not yet fully understood.

Soil microbes control soil C cycling by converting exogenous C into their biomass C, and decomposing SOC into C and energy sources synchronous [10,11]. Microbial carbon use efficiency (CUE), defined as the proportion of substrate organic C allocated to the growth of microbial biomass relative to the total acquired C, is central to the formation and decomposition of SOC [12–14]. Generally, a higher CUE implies greater microbial growth and less C emissions via microbial respiration, whereas a lower CUE reflects larger C emissions through respiration [15–17]. In other words, higher CUE enhances microbial biomass production, ultimately resulting in higher microbial residues accumulation [14,18]. Microbial CUE depends on both abiotic and biotic factors [19,20], which are affected by organic amendments. For example, soil nitrogen (N) availability can influence microbial CUE [21]. In addition, microbial CUE is greatly associated with soil microbial community [22]. The application of organic fertilizers can directly and indirectly affect microbial CUE [22,23]. The quantity and quality of organic C inputs play essential roles in maintaining microbial CUE [24]. High-quality organic C, such as manure, can increase microbial CUE and improve microbial biomass, thereby promoting SOC accrual [25,26], while applying rice straw with low C/nutrient ratio can enhance soil microbial respiration, resulting in lower microbial CUE [24]. Meanwhile, organic amendments can affect microbial CUE by changing soil properties (e.g., soil pH, and available C and nitrogen contents) [23]. However, some studies found that organic fertilizers have no effect on microbial CUE [23]. Thus, how microbial CUE responds to long-term organic amendments in paddy soil is not well understood.

The SOC is composed of progressively decomposing plant-derived compounds and microbial necromass [27,28]. Generally, the SOC pools can be partitioned into particulate organic C (POC) and mineral-associated organic C (MAOC) based on their different turnover times and functions [2,29]. Compared with the bulk SOC components, the fractionation method yields greater mechanistic information [28]. POC represents a transient SOC pool that composes of partially degraded plant fragments [29,30]. Conversely, MAOC is formed by interacting relatively small and recognizable microbial byproducts with soil minerals [31,32]. The mean turnover time of POC (23 years) is shorter than that of MAOC (129 years) [33,34]. Consequently, POC exhibits greater susceptibility than MAOC to organic amendments [35]. A meta-analysis showed that POC and MAOC were promoted by 77% and 20% following organic materials application, respectively [36]. However, the responses of POC and MAOC to organic amendments are inconsistent [37]. Therefore, it is necessary to investigate the factors controlling the responses of the two SOC fractions to organic amendments in paddy fields.

Soil microorganisms synthesize their own biomass via acquiring organic compounds, and subsequently accumulate in soils as necromass via microbial anabolism and catabolism (known as "microbial C pump") [23,38,39]. Comprising microbial residues and byproducts, microbial necromass C (MNC) is an important contributor to SOC sequestration [40,41]. Increasing evidence has revealed that MNC can contribute up to 50% of the SOC pool in arable soils [42]. The application of organic materials changes the soil microbial structures, thereby affecting MNC formation and accumulation [43]. Previous studies have reported that organic amendments increase MNC accumulation and contribute to the SOC pool [44]. However, Ye et al. [43] reported that pig manure application decreased the MNC contribution to SOC in upland soils. Therefore, quantifying the MNC contribution to the SOC pool is vital for improving the sequestration of persistent SOC under organic amendment in paddy soils.

A 15-year field experiment was conducted using different organic materials [rice straw (RS), green manure (GM), and pig manure (PM)] in rice paddy soils. The objectives of this study were (1) to evaluate the effects of organic amendments on SOC fractions and chemical function, and (2) to assess the responses of microbial biomass, physiological traits, and MNC to long-term amendments in paddy soils. We hypothesized that (1) organic amendments would increase microbial CUE; (2) long-term organic fertilization would enhance MNC accumulation.

2. Materials and Methods

2.1. Study Sites and Experimental Design

A long-term field experiment was started in 2007 at Shaoxing City, Zhejiang Province, China (119°54′ E, 31°16′ N). The tested soil belongs to the Gleyi–Stagnic Anthrosol category according to the World Reference Base of Soil Resources [45]. The original soil physiochemical properties determined before the long-term experiment are as follows [46]: pH 6.27 (1:5 soil/water); soil organic carbon (SOC), 13.0 g kg^{-1}; total nitrogen (TN), 1.45 g kg^{-1}; available phosphorus (AP), 19.6 mg kg^{-1}; available potassium (AK), 128.2 mg kg^{-1}; sand, 11.1%; silt, 43.7%; clay, 45.2%.

The field experiment began in June 2007 and included four treatments:

(1) CF, chemical fertilizers;
(2) CF + RS, chemical fertilizers and rice straw;
(3) CF + GM, chemical fertilizers and green manure (milk vetch);
(4) CF + PM, chemical fertilizers and pig manure.

In each treatment, N fertilizer was split-applied at rates of 112.5 kg N ha^{-1} $year^{-1}$ (basal fertilizer) and 112.5 kg N ha^{-1} $year^{-1}$ (topdressing fertilizer), respectively. The P and potassium (K) fertilizers (Sinochem Group Co., Ltd., Beijing, China) were applied as basal fertilizers at 90 kg P_2O_5 ha^{-1} $year^{-1}$ and 120 kg K_2O ha^{-1} $year^{-1}$, respectively. The application rates of N, P, and K fertilizers were recommended by local farmers. The application rates of rice straw, green manure, and pig manure were 6300, 7900, and 7000 kg ha^{-1} $year^{-1}$, respectively, based on the equivalent C input annually (Table 1). The C:N ratio of GM was lower, but that of RS was higher compared with that of PM (Table 1). The green manure and rice straw were chopped to 5–10 cm, broadcast manually, and turned into the soil. Pig manure was broadcast manually and plowed into the soil via rotary tillage. Detailed information on the C and N in the RS, GM, and PM treatments is presented in Table S1. This study was conducted using a random block design, and each treatment had three replicates. The area size of each was 30 m^2 (5 × 6 m). A single rice-cropping system was adopted in this study, with rice planted in early June and harvested in late October. The rice straw was removed from each plot after the rice was harvested.

Table 1. The organic carbon and nitrogen contents of organic materials and their organic carbon and nitrogen input to soils.

Organic Materials	Application Rate (kg ha^{-1})	OC Content (g kg^{-1})	N Content (g kg^{-1})	C/N	C Input (kg ha^{-1})	N Input (kg ha^{-1})
Rice straw	6300	488	7.8	62.6	3074	49.1
Green manure	7900	392	28.2	13.9	3097	222.8
Pig manure	7000	439	17.5	25.1	3073	122.5

2.2. Soil Sampling and Analysis

After rice was harvested in December 2022, soil samples were collected from all plots. Five soil cores (0–20 cm) were collected randomly from each plot and combined into a single composite sample. These samples were then divided into three aliquots. One subsample was air-dried to determine the soil physicochemical properties. One was immediately used for soil mineral N and microbial metabolic traits; and the third soil subsample was stored at −20 °C for assessment of microbial traits.

Soil physicochemical properties were determined according to Lu [47]. The concentrations of SOC and TN were quantified using an elemental analyzer (Elementar, Vario Max, Langenselbold, Germany). Available N in the soil [ammonium- (NH_4^+) and nitrate-N (NO_3^-)] was measured using an auto-analyzer after extracting with 1 M KCl solution (Bran Lubbe, Norderstedt, Germany). Briefly, 10 g of fresh soil was mixed with 50 cm^3 of 2 M KCl, then shaken for 1 h at 25 °C on a shaker (Shanghai Instrument and Electronic, Shanghai, China), and finally the solution was filtered with a filter film. Soil AP was extracted with hydrochloric acid–ammonium fluoride (HCl-NH_4F) and analyzed using ICP-MS (Optima 2000DV, Waltham, MA, USA). Air-dried soil (5.0 g) was shaken with 35 cm^3 HCl-NH_4F for 5 min at 25 °C, and then the solution was filtered with a filter film. The soil pH was measured in a soil/water suspension of 1:5 (5 g air-dried soil mixed with 25 cm^3 water) using a pH electrode (Metter Toledo, Zurich, Switzerland). Soil microbial biomass carbon (MBC) was measured according to the method of chloroform fumigation-extraction [48]. Briefly, 15 g of fresh soil was fumigated with $CHCl_3$ for 24 h at 25 °C in the dark. After that, soil sample was extracted with 15 cm^3 0.5 M potassium sulfate (K_2SO_4) at 25 °C for 0.5 h. The MBC was determined using a TOC analyzer (Analytik Jena AG, Jena, Germany).

2.3. Soil Organic Carbon Fractions

Particle size fractionation was performed to separate the SOC pool into POC and MAOC [49]. Air-dried soil (20 g) was dispersed in 100 cm^3 sodium hexametaphosphate ($(NaPO_3)_6$) solution (0.5% w/v). The dispersed soil sample was rinsed through a 53 µm sieve with deionized water till the effluent ran clear. The fraction retained on the sieve was considered POC, while the fraction passing through the sieve was considered MAOC. The POC and MAOC were dried at 60 °C in a drying oven (ILin Instrument, Shanghai, China), ground, and measured using an elemental analyzer (Elementar, Vario Max, Langenselbold, Germany).

2.4. Analysis of SC Chemical Composition

The SOC chemical composition was determined using ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Briefly, air-dried soil was pretreated with 10% hydrofluoric acid (HF) to prevent interference from manganese (II) ion (Mn^{2+}) and iron (III) ion (Fe^{3+}) and then was subjected to the ^{13}C solid-state NMR spectroscopy (AVANCE II 300, Bruker BioSpin, Fällanden, Switzerland). The chemical shift regions of the SOC corresponded to the following C structures: alkyl C at 0–45 ppm; O-alkyl C at 45–110 ppm; aromatic C at 110–160 ppm; and carbonyl C at 160–220 ppm. The extent of SOC aliphaticity was

estimated using ratio of aliphatic C to aromatic C [(alkyl C + O-alkyl C C)/aromatic C] [50]. The hydrophobicity index [(alkyl C + aromatic C)/(O-alkyl C + carbonyl C)] was used to assess the chemical stability of SOC [51,52].

2.5. Phospholipid Fatty Acids (PLFAs) Analysis

The soil phospholipid fatty acids (PLFAs) were used to indicate soil microbial community composition and measured using the method described by Wu et al. [53]. The extraction was performed on 3 g of freeze-dried soil with a citrate–chloroform–methanol mixture (0.8:1:2 $v/v/v$). Following the extraction, phospholipids were separated using a silica column. Subsequently, they were converted to fatty acid methyl esters (FAMEs). Methyl ester concentrations were determined using Agilent gas chromatograph (GC, Agilent Technologies, Santa Clara, CA, USA) combined with MIDI identification software 4.5 (MIDI Sherlock, Newark, NJ, USA). The PLFA concentration was quantified using an in internal standard (19:0). The representative Gram-positive and Gram-negative bacterial and fungal PLFAs were identified according to previous studies [54–56], and the specific PLFAs are shown in Table S1.

2.6. Measurement of Microbial Carbon Use Efficiency

An ^{18}O-H_2O method was used to determine microbial carbon use efficiency (CUE) [57]. In brief, two replicates of 0.3 g fresh soil sample from each treatment were loaded into 2 cm^3 vials and pre-incubated for 14 days at 25 °C. Subsequently, ^{18}O-H_2O (98% atom% ^{18}O) was added to one sample, resulting in 20% atom% ^{18}O in the final soil water. The unlabeled H_2O was added to another soil sample as a control. The vials were then incubated at 25 °C for 48 h in the dark. CO_2 emissions were determined using an Agilent 7890A GC (Agilent Technologies, Santa Clara, CA, USA). Soil DNA was extracted using the PowerSoil Isolation kit (MoBio Laboratories, Carlsbad, CA, USA). The DNA concentration was detected using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The extracted DNA was dried in silver capsules for 2 days at 60 °C. An isotope ratio mass spectrometer (IsoPrime 100, Langenselbold, Germany) was used to quantify the ^{18}O abundance in the extracted DNA. Microbial CUE, growth, respiration, C uptake, and turnover time were calculated as described by Spohn et al. [23,57] and Mganga et al. [58]. Briefly, microbial growth was calculated by multiplying the conversion factor and production rate of soil DNA. Microbial respiration was calculated based on the CO_2 emissions during 48 h incubation. Microbial C uptake was calculated as the sum of microbial growth and respiration. Microbial CUE was calculated as the proportion of microbial growth to C uptake.

2.7. Soil Amino Sugars Analysis

Amino sugar concentration was measured according to a previous study [59]. Soil sample (<0.15 mm) was subjected to acid hydrolysis for 8 h at 105 °C (6 M hydrochloric acid (HCl), 10 cm^3). After cooling, the hydrolysate was purified through a series of steps including filtration, centrifugation, and drying under a stream of nitrogen (N) gas. The resulting dried residue was then derivatized and dissolved in dichloromethane. The derivatives were determined using Agilent GC (Agilent Technologies, Santa Clara, CA, USA).

Soil fungal and bacterial necromass C (FNC and BNC) content was estimated using the methods adopted by Liang et al. [40].

$$\text{Bacterial necromass C } \left(\text{g kg}^{-1}\right) = 45 \times \text{ Muramic acid} \quad (1)$$

$$\text{Fungal necromass C } \left(\text{g kg}^{-1}\right) = \left(\frac{\text{Glucosamine}}{179.17} - 2 \times \frac{\text{Muramic acid}}{251.23}\right) \times 179.17 \times 9 \quad (2)$$

where the molecular weights of glucosamine (GlcN) and muramic acid (MurA), represented by 179.17 and 251.23, respectively. Forty-five and nine are the conversion factors. The microbial necromass C (MNC) was the sum of the FNC and BNC.

The necromass accumulation coefficient (NAC) was calculated according to references [60,61] as follows:

$$\text{Necromass accumulation coefficient } = \frac{\text{Microbial necromass C}}{\text{Microbial biomass C}} \quad (3)$$

2.8. Statistical Analysis

The influence of different treatments on variables including SOC fractions (SOC, POC, and MAOC), SOC chemical composition, soil microbial community (total PLFAs, fungi, bacteria, and actinomycetes), and microbial CUE and MNC was examined using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test at $p < 0.05$. Pearson's correlations were used to assess the relationships between CUE, MNC, MNC/SOC, and environmental factors ($p < 0.05$). The relationships between microbial physiology and MNC, MNC/SOC, and NAC were determined using Pearson's correlation analysis at $p < 0.05$. To assess the direct and indirect effects of organic amendments on MNC, the structural equation model (SEM) was applied using AMOS 22.0 (Smallwaters Corporation, Chicago, IL, USA). Microbial biomass is indicated by the total microbial PLFAs in the model. All statistical analyses were carried out using SPSS software 22.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Soil Properties, Organic C Fractions, and Chemical Composition

The soil pH was increased by organic amendments compared to CF ($p < 0.05$; Table S2). The soil TN and AN were significantly higher in organic amendments treatments than those in CF ($p < 0.05$). A significant increase in soil AP was observed after organic amendments compared to CF ($p < 0.05$). The MBC content was significantly higher in CF + RS, CF + GM and CF + PM than in CF.

A significant increase in SOC concentration was observed after organic amendments compared to CF ($p < 0.05$; Figure 1). Amendments of three types of organic materials increased POC and MAOC concentrations by 14.7–24.3% and 17.0–32.1%, respectively, relative to CF (Table S3). The POC/MAOC ratio was lower in CF + RS and CF + GM than in CF ($p < 0.05$), but there was no difference between CF and CF + PM ($p > 0.05$).

No change was noted in alkyl C among the four treatments ($p > 0.05$; Figure 2). The O-alkyl C was decreased by 3.29–4.02% under organic fertilization compared to CF (Table S4). However, the aromatic C was increased by 15.9–24.7% ($p < 0.05$; Table S4). Carboxyl C levels were similar among treatments ($p > 0.05$). Hydrophobicity was higher in the organic treatments than in CF, whereas aliphaticity showed the opposite trend ($p > 0.05$).

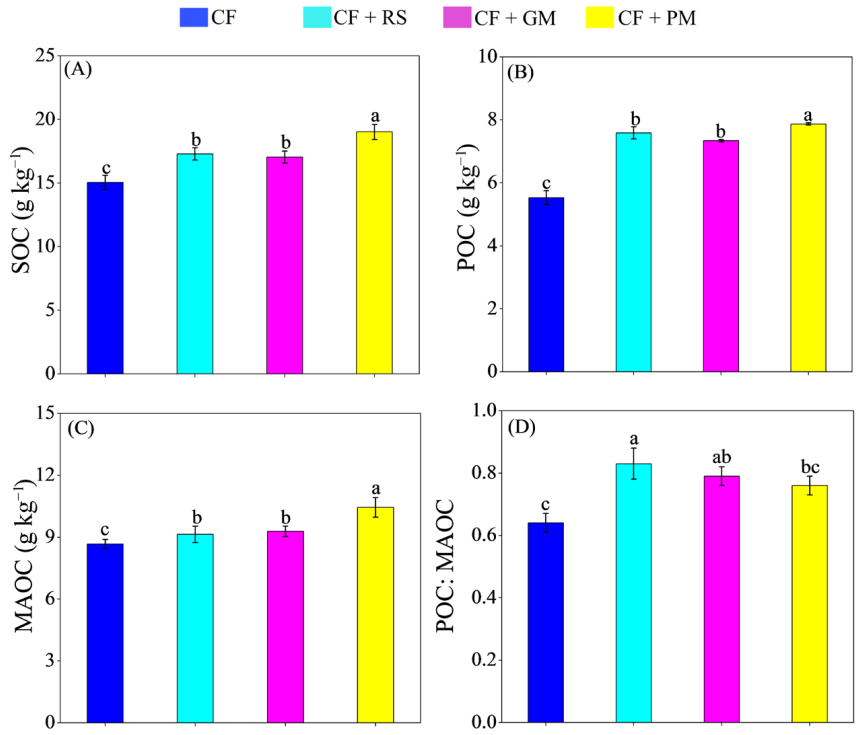

Figure 1. Soil organic carbon fractions under treatments with chemical fertilizers (CF), chemical fertilizers plus rice straw (CF + RS), chemical fertilizers plus green manure (CF + GM), and chemical fertilizers plus pig manure (CF + PM). (**A**) Soil organic carbon (SOC) concentration; (**B**) particulate organic carbon (POC) concentration; (**C**) mineral-associated organic carbon (MAOC) concentration; (**D**) ratio of POC and MAOC. Different letters indicate significant differences between different treatments based on one-way ANOVA with least significant difference (LSD) at $p < 0.05$.

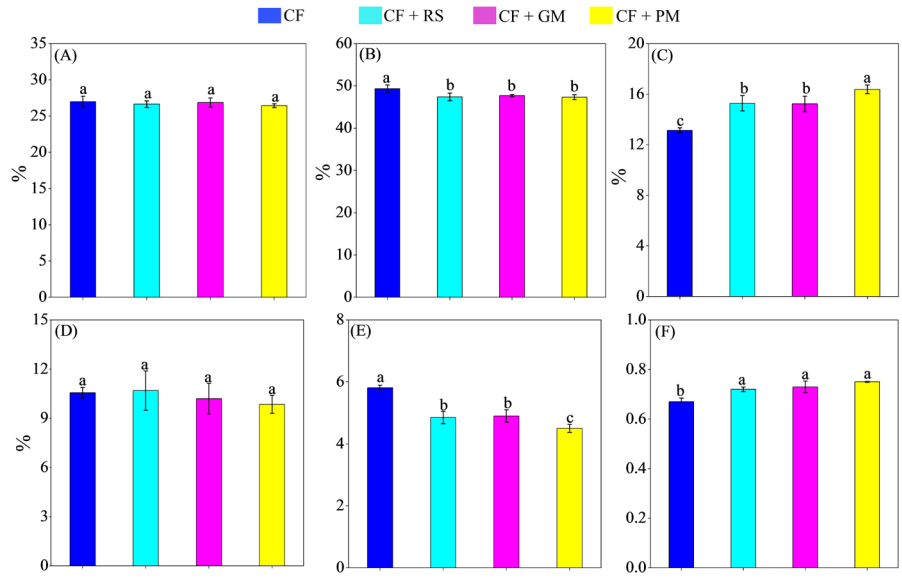

Figure 2. The proportions of soil organic carbon functional groups under treatments with chemical fertilizers (CF), chemical fertilizers plus rice straw (CF + RS), chemical fertilizers plus green manure (CF + GM), and chemical fertilizers plus pig manure (CF + PM). (**A**) Alkyl carbon; (**B**) O-alkyl carbon; (**C**) aromatic carbon; (**D**) carboxyl carbon; (**E**) aliphaticity; (**F**) hydrophobicity. Different letters indicate significant differences between different treatments based on one-way ANOVA with least significant difference (LSD) at $p < 0.05$.

3.2. Microbial Community Composition

The organic amendment significantly increased the total microbial PLFAs by 44.2–50.1%, compared to the control ($p < 0.05$; Figure 3; Table S5). No differences were detected among the three organic amendment treatments ($p > 0.05$). CF + RS, CF + GM, and CF + PM increased bacterial PLFAs by 27.4%, 44.8%, and 43.9%, respectively, compared to CF ($p < 0.05$; Table S5). The highest fungal PLFAs were observed in CF + RS ($p < 0.05$), which were higher than that observed in the other three treatments ($p < 0.05$). The fungal-to-bacterial PLFAs ratio was higher in CF + RS and CF + PM than in CK and CF + GM ($p < 0.05$), indicating that the organic amendments changed the microbial community composition.

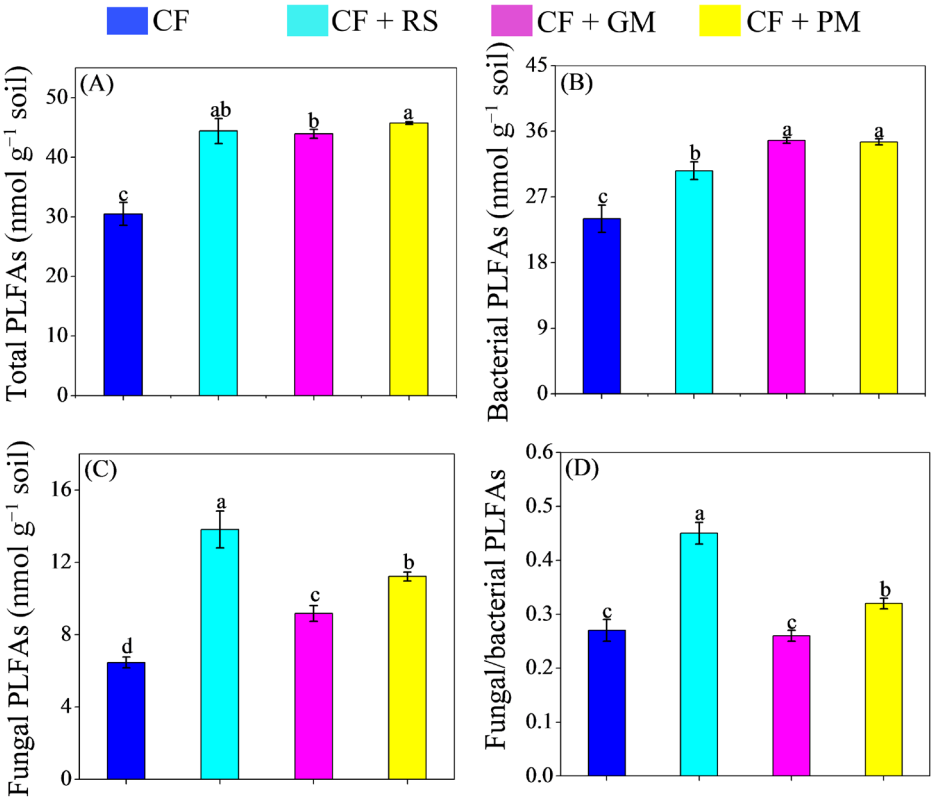

Figure 3. Soil phospholipid fatty acids under treatments with chemical fertilizers (CF), chemical fertilizers plus rice straw (CF + RS), chemical fertilizers plus green manure (CF + GM), and chemical fertilizers plus pig manure (CF + PM). (**A**) Total phospholipid fatty acids (PLFAs) concentration; (**B**) bacterial phospholipid fatty acids (PLFAs) concentration; (**C**) fungal phospholipid fatty acids (PLFAs) concentration; (**D**) the ratio of fungal and bacterial phospholipid fatty acids (PLFAs). Different letters indicate significant differences between different treatments based on one-way ANOVA with least significant difference (LSD) at $p < 0.05$.

3.3. Microbial C Use Efficiency

Organic amendments increased microbial CUE by 4.54–10.7% when compared with CF (Figure 4; Table S6). No significant differences were noted in microbial CUE among the three types of organic materials ($p > 0.05$). CF + GM had the highest microbial growth compared to the other treatments ($p < 0.05$), and CF + RS and CF + PM had higher microbial growth than CF ($p < 0.05$). Microbial respiration was higher in CF + RS and CF + GM than in CF. Organic material amendments increased microbial C uptake by 19.8–30.0% compared to CF. The turnover time was higher in CF + RS and CF + GM than in CF ($p < 0.05$), but it was similar between CF + PM and CF ($p > 0.05$).

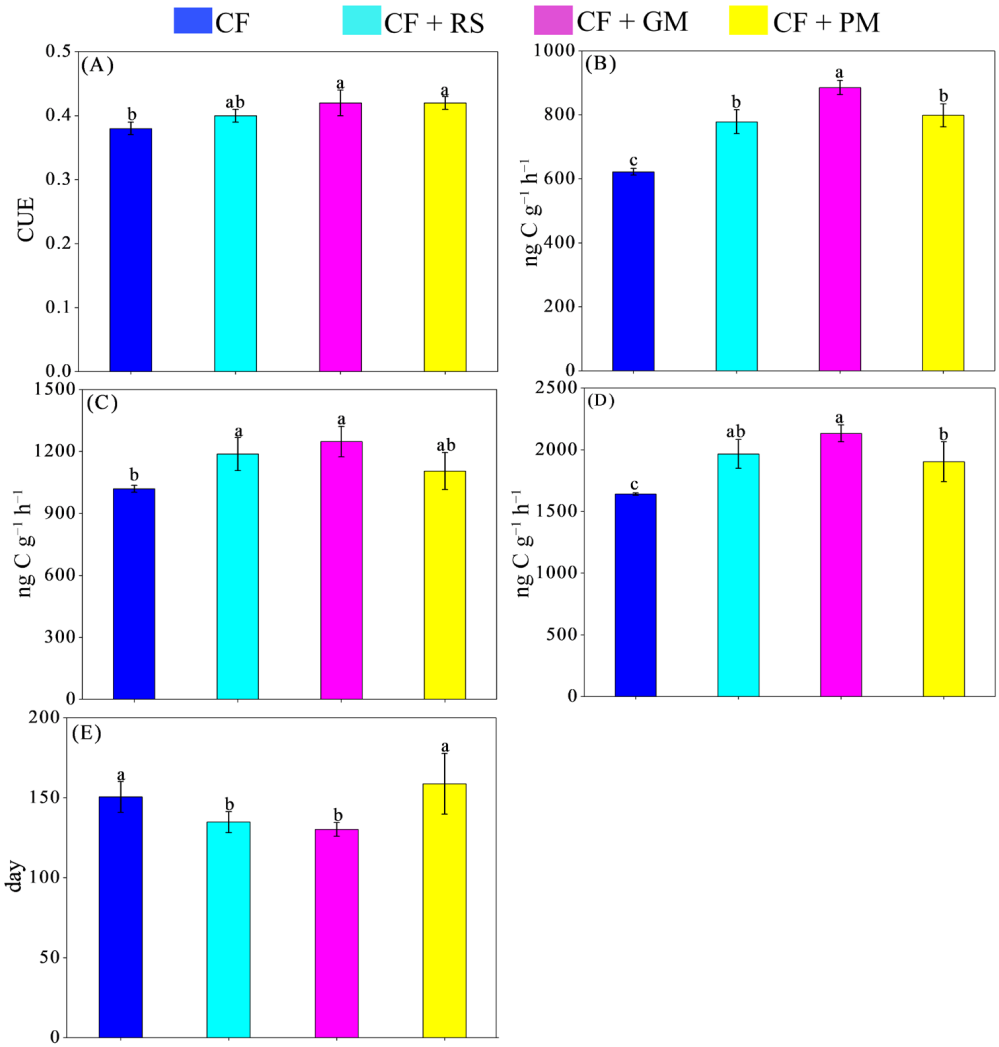

Figure 4. Microbial physiology traits under treatments with chemical fertilizers (CF), chemical fertilizers plus rice straw (CF + RS), chemical fertilizers plus green manure (CF + GM), and chemical fertilizers plus pig manure (CF + PM). (**A**) Microbial carbon use efficiency (CUE); (**B**) microbial growth; (**C**) microbial respiration; (**D**) microbial carbon uptake; (**E**) turnover time. Different letters indicate significant differences between different treatments based on one-way ANOVA with least significant difference (LSD) at $p < 0.05$.

3.4. Microbial Necromass C

Compared with CF, organic fertilization increased MNC, FNC, and BNC by 26.7–31.9%, 24.6–33.4%, and 26.7–31.9%, respectively (Figure 5; Table S7). The FNC-to-BNC ratio was higher in the CF + RS and CF + GM than in the CF and CF + PM ($p < 0.05$). CF + RS and CF + GM increased the MNC contribution to SOC by 10.3% and 13.6%, respectively, compared to CF. However, no change was detected between CF + PM and CK ($p > 0.05$). CF + GM had the highest BNC contribution to SOC compared with the other three treatments ($p < 0.05$). CF + PM had a similar FNC contribution to SOC as CF ($p > 0.05$), whereas CF + RS and CF + GM enhanced the FNC contribution to SOC compared to CF ($p < 0.05$). NAC was higher in CF + RS than in CF and CF + PM ($p < 0.05$), but was similar to that in CF + GM ($p > 0.05$).

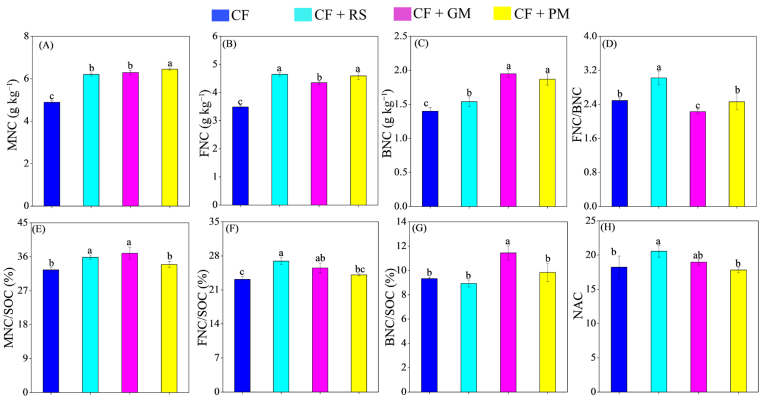

Figure 5. Soil microbial necromass carbon under treatments with chemical fertilizers (CF), chemical fertilizers plus rice straw (CF + RS), chemical fertilizers plus green manure (CF + GM), and chemical fertilizers plus pig manure (CF + PM). (**A**) Microbial necromass crabon (MNC); (**B**) fungal necromass carbon (FNC); (**C**) bacterial necromass carbon (BNC); (**D**) FNC:BNC ratio; (**E**) microbial necromass carbon/soil organic carbon (MNC/SOC); (**F**) fungal necromass carbon/soil organic carbon (FNC/SOC); (**G**) bacterial necromass carbon/soil organic carbon (BNC/SOC); (**H**) necromass accumulation coefficient (NAC). Different letters indicate significant differences between different treatments based on one-way ANOVA with least significant difference (LSD) at $p < 0.05$.

3.5. Correlations Among Soil Properties, Microbial Metabolic Traits, and Microbial Necromass

Pearson's correlation analysis revealed that both CUE and MNC were significantly associated with the soil chemical properties and SOC fractions ($p < 0.05$; Figure 6A). Microbial CUE increased with increasing aromatic C and SOC hydrophobicity ($p < 0.01$) but decreasing SOC aliphaticity ($p < 0.01$). In addition, when the PLFAs of bacteria and total microbes increased, microbial CUE increased ($p < 0.001$). As O-alkyl C and aliphaticity increased, MNC decreased ($p < 0.01$), but MNC increased with increasing aromatic C and hydrophobicity ($p < 0.01$). Additionally, MNC increased when microbial biomass increased ($p < 0.01$). Furthermore, MNC concentration, including FNC and BNC, increased with increasing microbial CUE, growth, and C uptake ($p < 0.05$; Figure 6B). Microbial growth increased as the soil chemical properties and SOC fractions increased ($p < 0.05$; Figure S1). Microbial respiration increased with increasing soil AN, and total and bacterial PLFAs, but with decreasing O-alkyl C ($p < 0.05$).

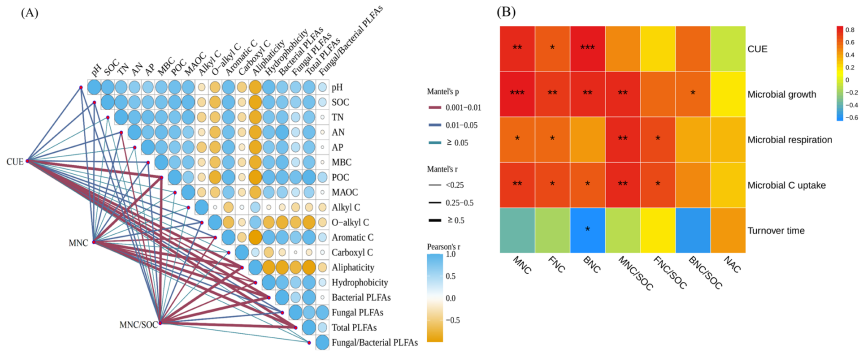

Figure 6. Correlation analysis between environmental factors and microbial carbon use efficiency, and microbial necromass carbon and its contribution to soil organic carbon (**A**). Pearson correlation analysis between microbial physiology and microbial necromass carbon (**B**). CUE, carbon use efficiency; MNC, microbial necromass carbon; SOC, soil organic carbon; TN, total nitrogen; AN, available nitrogen; AP, available phosphor; MBC, microbial biomass carbon; POC, particulate organic carbon; MAOC, mineral-associated organic carbon; PLFAs, phospholipid fatty acids; FNC, fungal necromass carbon; BNC, bacterial necromass carbon; NAC, necromass accumulation coefficient. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The structural equation modeling (SEM) showed that the model explained 97% of microbial necromass variance (Figure 7). Organic fertilization indirectly promotes MNC accumulation through its direct positive effect on microbial biomass (Figure 7). In addition, organic amendments indirectly enhanced soil C quality by affecting soil pH and nutrients, increasing microbial biomass, and consequently accumulating more MNC. Microbial CUE indirectly affects MNC accumulation through its direct effect on microbial biomass.

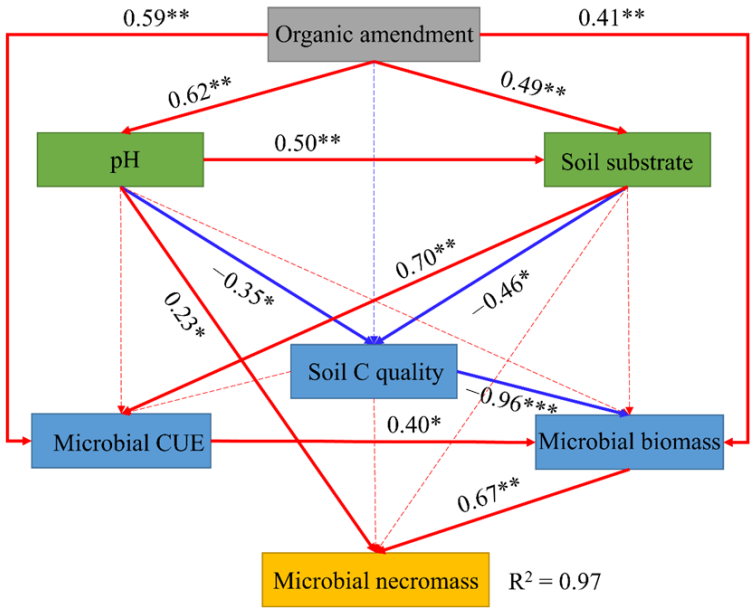

Figure 7. SEM of the effects of organic amendments on microbial carbon use efficiency and necromass carbon in rice paddy soils. The red and blue solid arrows indicate positive and negative relationships, respectively. Dotted line arrows indicate no significant relationships. Numbers next to the arrows are standardized path coefficients. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

4.1. Effects of Organic Amendments Soil Organic Carbon

SOC accrual depends on the balance between the exogenous OC input and decomposition [1,62]. In this study, the SOC significantly increased by organic amendments ($p < 0.05$), which aligns with previous reports [6,63]. Organic fertilizers, such as green manure, crop straw, and organic manure, supply exogenous C to soils, resulting in SOC accumulation [64]. Moreover, long-term organic amendments affect soil microbial communities and functions, and microbial-mediated C processes have a crucial effect on SOC sequestration [25,65]. The SOC concentration was positively correlated with soil microbial CUE and MNC, suggesting that organic amendments increased microbial CUE and accumulated more MNC, ultimately leading to higher SOC accrual. A high microbial CUE indicates a relatively high microbial biomass, which is beneficial for microbe-derived SOC formation and accumulation in soils [66]. Among the three types of organic materials, PM had the strongest effect on SOC enhancement in paddy soils, although the three treatments had similar organic C inputs. The contrasting effects of different organic materials on SOC accrual are attributed to the interrelated responses of soil nutrient availability, microbial metabolic traits, and MNC accumulation [67]. The composting PM has more recalcitrant organic C than RS and GM [43], thereby directly contributing to SOC accumulation. The data also showed that aromatic C was higher in PM than in RS and GM, which supports the previous finding.

The POC and MAOC were increased by organic amendments ($p < 0.05$). The increased POC under organic amendments was attributed to the direct input of organic C derived from straw, green manure or pig manure. This is because POC mainly consists of partially decomposed plant residues [29,68]. MOAC is largely composed of microbial residues, such as microbial necromasses, and bonds with soil mineral particles [35,69]. Therefore, MAOC has a longer turnover time than POC and plays a more important role in SOC accruals [35]. Microbial necromasses play a vital role in the formation of MAOC [69]. Organic amendments significantly increased the MNC and promoted MAOC formation and accumulation. Organic amendments decreased O-alkyl C but increased aromatic C compared with CF, indicating that organic amendments enhance the stability of SOC. This is because O-alkyl C mainly originates from carbohydrates that are easily decomposable [70], whereas aromatic C is primarily derived from plant lignin and microbial byproducts that are chemically recalcitrant [70]. In addition, the decreased POC/MAOC ratios in the GM and PM treatments indicate that organic amendments increased SOC stability.

4.2. Effects of Organic Amendments on Microbial C Use Efficiency

Microbial CUE is considered a key factor governing SOC formation and storage [70,71]. Several studies have shown that microbial CUE is significantly correlated with SOC [16,72]. In the present study, microbial CUE in paddy soils ranged from 0.38 to 0.42 across soil samples, which are similar with previous studies [24,73]. A meta-analysis conducted by Hu et al. [74] observed that the mean value of microbial CUE is 0.37 across global 100-paired observation, which is lower than the results from this study. Through collecting paddy soils across eastern China, Duan et al. [75] reported that microbial CUE ranged from 0.3 to 0.5 in paddy fields, which is similar with our study.

The microbial CUE was significantly enhanced by organic amendments ($p < 0.05$). Many environmental factors, such as C sources, soil water, and nutrient availability strongly affect microbial CUE in soils [76–78]. Among the factors, nutrient availability may be one of the most crucial factors affecting the microbial CUE [56,79]. Increased nutrient availability reduces microbial C allocation to additional enzymes and increases microbial CUE, whereas low soil substrate availability increases microbial C investment in extracellular enzymes, resulting in lower microbial CUE [80]. Soil microorganisms acquire nutrients by secreting extracellular enzymes [80]. The application of organic materials increased soil nutrient availability, and thus enhanced microbial CUE. In addition, organic amendments enhanced SOC quality, leading to a higher microbial CUE. A forest soil survey conducted by Soares and Rousk [81] revealed that higher SOC quality can increase microbial CUE in 33 Swedish soil samples. Previous studies have demonstrated that organic material application enhances microbial CUE and reduces turnover time, thereby promoting SOC accrual [82]. Moreover, microbial community composition is closely related to microbial CUE [83,84]. Microbial CUE is negatively correlated with fungal abundance [85]. Meanwhile, our data showed that microbial CUE was positively associated with total microbial and bacterial biomass, but not with fungal biomass.

Organic materials would supply sufficient soil nutrients for microbial growth [80]. In this study, microbial growth was significantly increased by organic amendments compared to the CF ($p < 0.05$) because organic materials supply sufficient soil nutrients for microbial growth [21]. Microbial growth can govern both the sequestration and loss of SOC [34,86,87]. However, soil microbial growth was not correlated with SOC and MAOC, and was only significantly correlated with POC, indicating that microbial growth was coupled with POC but decoupled from SOC and MAOC. POC is easily decomposed by soil microorganisms and can supply C and energy sources for microbial growth [28]. Microbial growth was significantly correlated with MNC, FNC, and BNC, indicating that microbial growth plays

a vital role in MNC formation and accumulation. High microbial growth means high microbial biomass accumulation, which then increases MNC accumulation [84].

4.3. Effects of Organic Amendments on Microbial Necromass Carbon

In this study, MNC accumulation, as well as FNC and BNC, was enhanced by organic amendments compared to CF. Organic materials incorporation provides additional C and energy sources for microbial proliferation, thereby promoting MNC accumulation [43,44]. Organic amendments improved soil nutrients, thereby stimulating microbial growth, and ultimately facilitating MNC accumulation. Our data showed that a significant increase in microbial biomass was detected under organic amendments, which supports this finding. Moreover, the SEM result showed that organic amendments increased microbial CUE and then enhanced microbial biomass, ultimately facilitating MNC accumulation. Higher microbial CUE indicates higher microbial biomass production, which can accumulate more MNC in soils [16,79]. In the present study, we showed that CF + RS and CF + GM increased the MNC contribution to SOC, but CF + PM had no effect compared to CF. In addition, the contribution of MNCs was higher in CF + RS and CF + GM than in CF + PM. Similarly, Ye et al. [43] found that PM application increased MNC accumulation, but decreased its contribution compared to RS, because PM can directly contribute to SOC accrual, owing to its abundant recalcitrant organic C. CF + RS increased the contribution of FNC to SOC, resulting from higher fungal PLFAs in CF + RS than in CF or PM. RS had a higher C/N ratio than GM or PM, which stimulated fungal growth. Fungi utilize organic materials with higher C/N ratios than bacteria because they can release a broad range of extracellular enzymes that decompose recalcitrant organic compounds [75,88].

The present data demonstrated that the contribution of FNC to SOC (23.2–26.9%) was significantly higher than the contribution of BNC (8.9–11.4%; $p < 0.05$). This result indicates the more vital role of FNC in the SOC accrual, which aligns with previous reports [89]. Fungal necromass in soils is less prone to decomposition than bacterial necromass due to its higher C/N ratio and more recalcitrant composition. In contrast, bacterial necromass is preferentially reutilized by soil microbes [90]. Previous study has indicated that fungi can acquire a carbon-N source for biomass growth by decomposing bacterial necromass and consequently accumulating as FNC [91]. Furthermore, fungal hyphae facilitate the soil aggregates formation, which physically shields the fungal necromass from microbial degradation and, ultimately, thus enhances its accumulation over bacterial necromass [92].

5. Conclusions

Organic amendments have the potential to sequester more soil organic carbon (SOC) into paddy soils. We found that organic amendments could accumulate more SOC, particulate organic C (POC) and mineral-associated organic C (MAOC) in paddy fields, and that pig manure (PM) favored greater SOC concentrations than rice straw (RS) and green manure (GM). The organic materials application enhanced microbial C use efficiency (CUE), resulting in an increase in microbial necromass C (MNC) and translating into the formation and accrual of POC and MAOC. Moreover, MNC was positively correlated with microbial CUE, growth, and respiration. We provide evidence of the causal links among SOC accrual, and microbial physiology, biomass, and necromass under long-term organic amendments in rice paddy soils. Our results highlight the crucial role of microbe-mediated processes in the accrual of SOC under long-term organic amendment in rice paddies, and assist in organic fertilizers' management for SOC sequestration. Further studies are needed to investigate the regulation of microbial functions on microbial CUE and MNC under long-term organic amendments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture15212308/s1. Table S1: The phospholipid fatty acids detected in our study; Table S2: The soil properties under different treatments; Table S3: The soil organic carbon fractions under different treatments; Table S4: The soil organic carbon functional groups under different treatments; Table S5: The soil phospholipid fatty acids under different treatments; Table S6: The soil microbial physiology traits under different treatments; Table S7: The soil microbial necromass carbon under different treatments; Figure S1: Correlation between environmental factors, and soil organic carbon fractions and chemical composition.

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