

Article

Effects of Aquatic Animals on Soil Organic Carbon Mineralization in a Paddy Ecosystem

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Abstract

Mineralization of soil organic carbon (SOC) is an important component of the soil carbon cycle. Studies have shown that rice–fish cocultures increased SOC; however, the question of whether raising aquatic animals with rice affects SOC mineralization remains unresolved. In this study, we conducted a field experiment to test SOC mineralization under three types of rice–aquatic animal cocultures (i.e., rice–fish coculture, RF; rice–crayfish coculture, RC; and rice–turtle coculture, RT) and rice monoculture (RM). SOC fractions, soil aggregate structure and SOC mineralization were determined in the experiment. Total SOC and microbial biomass carbon (MBC) under RT were significantly higher than under other treatments, and no significant differences in other SOC fractions were found among the treatments. For soil aggregates, there were significant differences in the proportions of large macroaggregates (LMA), microaggregates (MiA), and mean weight diameter (MWD) among the treatments. The LMA proportion significantly reduced while the MiA increased under the RT treatment compared to other treatments. SOC mineralization rates significantly differed among the treatments, where the RT and RC treatments had significantly higher mineralization ratios and daily average mineralization rates than the RF and RM treatments. RDA indicated that SOC fractions and soil aggregate structure influenced SOC mineralization. Our results demonstrate that aquatic animals exert differential effects on SOC mineralization, primarily through altering soil aggregate structure and SOC fractions. These findings highlight that the effects of aquatic animals on SOC dynamics should be considered in the assessment of coculture outcomes.

Keywords: aquatic animals; coculture system; soil organic carbon fraction; soil aggregate; soil organic carbon mineralization



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1. Introduction

Soil organic carbon (SOC) not only represents the largest carbon sink among terrestrial ecosystems but also functions as a critical nexus in the global carbon cycle [1]. Beyond its role in the global carbon cycle, the SOC content is also a critical measure of soil fertility in agricultural systems [2,3]. Therefore, maintaining the SOC pool is essential for climate regulation, soil quality improvement, and crop yield enhancement.

SOC mineralization, the conversion of organic carbon to inorganic forms (e.g., CO₂), is a key process in the soil carbon cycle [4]. This process is affected by SOC components, soil structure, environmental conditions, and human activities [4–6]. For SOC components, some SOC fractions, such as particulate organic carbon (POC), easily oxidizable organic

carbon (EOC), dissolved organic carbon (DOC), labile organic carbon (LOC), and microbial biomass carbon (MBC), are readily decomposed or utilized by microorganisms and have short residence times in the soil, whereas other fractions, such as recalcitrant organic carbon (ROC) and mineral-associated organic carbon (MAOC), are more stable and less susceptible to decomposition [6–9]. In addition to being influenced by its own chemical structure and composition, SOC mineralization is also affected by various factors such as soil chemical structure, aggregate fractions, iron or aluminum oxides, and exchangeable cation content [10,11]. For example, studies have shown that aggregates can enhance the persistence of organic carbon [12], and the disruption of aggregates promotes the carbon mineralization process [4].

SOC mineralization is also influenced by local climatic conditions, soil texture, and anthropogenic factors such as land use patterns, tillage practices, and cropping systems [4]. Elevated temperatures could promote SOC mineralization [13–16]. Moisture can affect the decomposition of organic carbon by shifting microbial community structure and altering oxygen availability in the soil [16]. Plants may influence SOC mineralization through root exudates, root growth, and litter inputs [17,18]. Compared with conventional tillage in agricultural systems, conservation tillage reduces soil disturbance; thus, soil aggregate structure and SOC stability are maintained, whereas excessive tillage may breakdown and promote the decomposition of soil organic matter [4,5]. Therefore, conservation tillage practices enhance carbon sequestration in the soil primarily by suppressing the mineralization and decomposition of the existing soil carbon pool.

Rice (*Oryza sativa* L.) is a staple food crop cultivated on approximately 9% of the world's arable land, and the paddy soil used for rice production plays a significant role in regional and global carbon dynamics [19]. Soil cycling processes in paddy soils, such as decomposition and mineralization, differ markedly from those in upland soils [20,21]. These processes are influenced by rice cropping systems, fertilization, straw returning, and water management strategies [16,19]. The rice–fish farming system is an important aspect of rice farming that rears a certain number of aquatic animals (e.g., fish, crayfish, crab, and turtle) concurrently with rice in paddy fields. It plays an important role in reducing the utilization of chemical fertilizers and pesticides, maintaining grain yield and soil fertility, and increasing farmers' income [22–27]. Studies have shown that rice–fish coculture can generally improve soil fertility and promote SOC accumulation [26,27]. Studies also showed that fish enhance the CH₄ oxidation rate of surface soil in the rice–fish system by increasing methanotrophs, where fish feeding interactions caused trophic cascades and drove N transfer [28]. However, the effects of rice–fish cocultures on SOC mineralization are not well known.

Through a field experiment on three rice–animal coculture systems (i.e., rice–fish, rice–crayfish, and rice–turtle), this study was designed to address two scientific questions: (1) Do different aquatic animals differentially affect SOC mineralization? (2) Are the observed effects on SOC mineralization mediated by changes in SOC fractions and soil aggregate structure?

2. Materials and Methods

2.1. Ethics Statement

In this study, all aquatic animals (i.e., fish, crayfish and softshell turtles) were cultured and measured in accordance with the approved guidelines of the Zhejiang University Experimental Animal Management Committee (approval code: ZJU20250140).

2.2. Study Site and Rice–Aquatic Animal Coculture Systems

This study was carried out at a rice–aquatic animal coculture experimental site of Tongxiang City (Figure 1A, 30°34' N, 120°24' E) in northern Zhejiang Province, China, during the 2022–2025 period. The study area is situated in a typical subtropical monsoon climate zone, where the mean annual temperature ranges from 16 to 17 °C and annual precipitation averages 1200–1300 mm. The weather conditions (i.e., air temperature and rainfall) during the experimental period in 2023 are shown in Figure 1B. The soil in the experimental site consists of sediments from rivers, seas and lakes, which belong to paddy soil and fluvo-aquic soil. The soil is fertile and suitable for planting rice and economic crops. The soil is classified as Hapli-Stagnic Hydragric Anthrosols in the WRB system (IUSS Working Group WRB, 2022; World Reference Base for Soil Resources) [29].

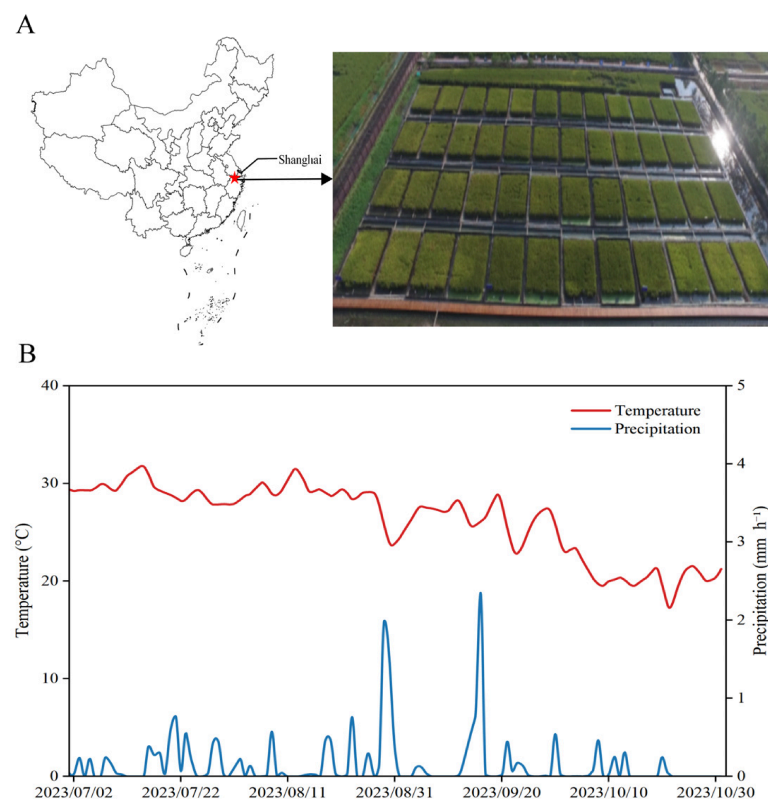


Figure 1. Location of experimental site (A) and weather conditions during experimental period (B).

Three types of coculture systems were included in the study, i.e., rice–fish coculture, rice–crayfish coculture and rice–turtle coculture. These coculture systems have been developed and adapted to different rice-growing regions in China [27]. In our study area, farmers culture rice and aquatic animals together, and they harvest rice and aquatic animal products from the coculture systems. The rice-growing season in the experimental site is usually from early June to late October (ca. 140 days). The aquatic animals are cultured with the rice during the whole rice-growing period. Farmers usually apply feed to achieve high aquatic yields. Aquatic animals were harvested in early November after the rice was harvested.

2.3. Experimental Design

The experiment was a randomized block design with four treatments and four replicates. The four treatments are: (1) rice monoculture (RM); (2) rice–crayfish coculture (RC); (3) rice–turtle coculture (RT); and (4) rice–fish coculture (RF). Crayfish (*Procambarus clarkii*), turtle (*Pelodiscus sinensis*) and fish (*Channa argus*) were used in the experiment. Each

experimental plot covered an area of 108 m² (9 m × 12 m), of which 90% was used for rice cultivation and the remaining 10% was a ditch for the temporary rearing of aquatic animals. Both rice cultivation and aquatic animal rearing were carried out following local conventional practices. Detailed field cultivation and rearing procedures are provided in Supplementary Information Table S1.

2.4. Measurement

2.4.1. Collection of Soil Samples

Surface soil samples (0–5 cm) were collected one day before the harvest of rice and aquatic animals in the second year of the experiment (2023). In each experimental plot, soil samples were taken using a PVC tube (10 cm in diameter, 5 cm in length) following a five-point sampling method. The collected soil was mixed thoroughly to form a composite sample, placed into a sample bag, and immediately transported to the laboratory in a container with ice packs. After removing visible roots and stones, each soil sample was evenly divided into three portions. One portion was air-dried in a shaded, cool place for the determination of total soil organic carbon (SOC), particulate organic carbon (POC), mineral-associated organic carbon (MAOC), recalcitrant organic carbon (ROC), labile organic carbon (LOC), easily oxidizable organic carbon (EOC), and microbial necromass carbon (MNC). The second portion was stored at 4 °C for the prompt measurement of soil dissolved organic carbon (DOC) and microbial biomass carbon (MBC). The third portion, consisting of fresh soil, was used for aggregate analysis and SOC mineralization measurement.

2.4.2. SOC Components and Microbial Necromass Carbon

SOC content was measured using the K₂Cr₂O₇-H₂SO₄ oxidation method [30,31]. Particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) were quantified using a combined density and size fractionation approach [5,31]. Soil recalcitrant organic carbon (ROC) and labile organic carbon (LOC) were measured using the acid hydrolysis method [32]. Soil easily oxidizable organic carbon (EOC) was determined using the potassium permanganate oxidation method [33]. Soil microbial biomass carbon (MBC) was measured using the chloroform fumigation–extraction method [34,35]. The organic carbon extracted from non-fumigated soil was taken as soil dissolved organic carbon (DOC) [36].

Soil amino sugar content was determined using gas chromatography as a tracer for microbial necromass [37–40]. Based on the measured contents of glucosamine (GluN) and muramic acid (MurA), the contents of fungal necromass C (FNC) and bacterial necromass C (BNC) were calculated. The sum of bacterial and fungal necromass C contents represents the microbial necromass C (MNC) content.

$$\text{FNC (g} \cdot \text{kg}^{-1}) = (\text{GluN}/179.2 - 2 \times \text{MurA}/251.2) \times 179.2 \times 9/1000 \quad (1)$$

$$\text{BNC (g} \cdot \text{kg}^{-1}) = \text{MurA} \times 45/1000 \quad (2)$$

$$\text{MNC (g} \cdot \text{kg}^{-1}) = \text{FNC} + \text{BNC} \quad (3)$$

In the equations, 179.2 and 251.2 are the molecular weights (g·mol^{−1}) of GluN and MurA, respectively. A molar ratio of GluN to MurA in bacterial cells is assumed to be 2:1. A conversion factor of 9 is used to convert fungal GluN into FNC content, and a conversion factor of 45 is used to convert bacterial MurA into BNC content. The value of 1000 is the conversion factor from mg·kg^{−1} to g·kg^{−1}.

2.4.3. Soil Aggregates

Soil aggregates were separated using a modified wet-sieving method [41–43]. Briefly, a nested set of sieves (with mesh sizes of 2 mm, 0.25 mm, and 0.053 mm from top to bottom) was attached to a soil aggregate analyzer (TPF-100, TPN, Hangzhou, China). A 100–200 g fresh soil sample was placed on top of the 2 mm sieve and gently immersed in deionized water for 5 min. The instrument was then adjusted for vertical oscillation frequency to obtain aggregates of different-sized fractions. Floating organic material (density $< 1 \text{ g}\cdot\text{cm}^{-3}$) was considered plant residue and removed from the $> 2 \text{ mm}$ fraction. Ultimately, four fractions were obtained through sieving: large macroaggregates ($> 2 \text{ mm}$), small macroaggregates (250 μm –2 mm), microaggregates (53–250 μm), and silt + clay aggregates ($< 53 \mu\text{m}$). The unsettled portion of the suspension that passed through the 53 μm sieve was decanted and centrifuged to obtain the precipitate, which was then combined with the other portion (soil that had settled in the bucket) to obtain the silt- or clay-sized fraction ($< 53 \mu\text{m}$).

Soil aggregate stability was expressed as mean weight diameter (MWD, mm), geometric mean diameter (GMD, mm), and fractal dimension (D) [44]. MWD, GMD, and D were calculated using the following equations:

$$\text{MWD} = \sum_{i=1}^n X_i W_i \quad (4)$$

$$\text{GMD} = \exp\left(\sum_{i=1}^n W_i \ln X_i\right) \quad (5)$$

$$(3 - D) \lg\left(\frac{X_i}{X_{\max}}\right) = \lg\left(\frac{W(\delta \leq X_i)}{W_0}\right) \quad (6)$$

where X_i is the mean diameter of the aggregates, W_i is the percentage of soil aggregate dry weight within that size fraction, $X_{\max}$ is the mean diameter of the largest aggregates, $W(\delta \leq X_i)$ is the cumulative mass of soil particles with diameters less than X_i , and W_0 is the total mass of all size fractions. $i = 1, 2, 3$, and 4 represent the size fractions of $> 2 \text{ mm}$, 0.25–2 mm, 0.053–0.25 mm, and $< 0.053 \text{ mm}$, respectively.

2.4.4. SOC Mineralization

SOC mineralization was examined using an incubation experiment. Soil samples collected from each treatment (RM, RC, RT, and RF) as described in Section 2.4.1 were prepared before the incubation experiment. After all visible roots were removed, soil water content was determined using the oven-drying gravimetric method. Then, 50 g of fresh soil from each soil sample was weighed and put into a 250 mL glass bottle that was wrapped with sterile sealing film. The mean water content of the soil samples varied between 47.95% and 56.00%. SOC mineralization rate was calculated based on dry soil weight.

After the soil was pre-cultured in the dark at 25 °C for a week to active microorganisms, the sterile sealing film was removed, and all bottles were covered with bottle caps that had two bidirectional valves. The two-way valves passed through the bottle cap and were connected by rubber tubes. The connection points were glued together to ensure a seal. High-purity air (79% N_2 , 21% O_2) without CO_2 was introduced into all the bottles for 5 min, and then the soil was placed in a 25 °C dark environment for 31 days of cultivation. During incubation, the soil was irrigated with deionized water, maintaining a 2 cm water layer on the soil surface.

The SOC mineralization rate was represented by the CO_2 accumulation rate over the headspace of each jar. During the cultivation period, gas samples were collected from the glass bottles using 50 mL syringes at 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 20, 25 and 31 days, with approximately 30 mL collected each time and stored in aluminum foil gas bags. After each

measurement, high-purity air (79% N₂; 21% O₂) was continuously introduced into the bottle for 5 min to avoid anaerobic activity, and then the two-way valve was closed for further incubation. Meanwhile, deionized water was regularly supplemented by weighing to make up for the loss of water. The collected gas samples were analyzed for CO₂ concentration using an Agilent GC 8860 gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA).

The calculation formula for the soil CO₂ mineralization rate, measured each time, is as follows:

$$R = \frac{\Delta c}{\Delta t} \times \frac{273 \times M \times V}{(273 + T) \times 22.4 \times m} \times \alpha \quad (7)$$

where R is the mineralization rate (mg CO₂-C·kg⁻¹ dry soil·day⁻¹); $\frac{\Delta c}{\Delta t}$ is the change in CO₂ concentration per unit time (ppm·day⁻¹); V is the volume of the incubation bottle (L); M is the relative molecular mass of CO₂ (44 g·mol⁻¹); 22.4 (L·mol⁻¹) is the molar volume of gas at 273 K; T is the incubation temperature (25 °C); m is the dry soil mass (kg); and α is the conversion factor from CO₂ to C, which is 12/44.

The calculation formula for soil metabolic quotient ($q\text{CO}_2$) is as follows:

$$q\text{CO}_2 = \sum R_i / (MBC \times \text{days}) \quad (8)$$

where $q\text{CO}_2$ is the soil metabolic quotient (mg CO₂-C·g⁻¹ MBC·day⁻¹); $\sum R_i$ is the cumulative CO₂-C emissions (mg CO₂-C·kg⁻¹ dry soil); MBC is the soil microbial biomass carbon content (g·kg⁻¹); and days is the total number of incubation days.

The calculation formula for the soil organic carbon mineralization ratio is as follows:

$$A = \sum R_i / \text{SOC} \quad (9)$$

where A is the soil organic carbon mineralization ratio (mg CO₂-C·g⁻¹ SOC); $\sum R_i$ is the cumulative CO₂-C emission (mg CO₂-C·kg⁻¹ dry soil); and SOC is the soil organic carbon content (g·kg⁻¹).

2.5. Statistical Analysis

Statistical analyses were performed using SPSS (V.29.0). One-way analysis of variance (ANOVA) was used to compare the SOC content and its fraction contents, water-stable aggregate proportions, mean weight diameter (MWD), geometric mean diameter GMD), fractal dimension (D), and average daily soil organic carbon mineralization rates among the RM, RF, RC and RT treatments. Repeated-measures ANOVA was used to compare the cumulative CO₂ emissions among the treatments during the incubation period. Redundancy analysis (RDA) was conducted to identify the key predictors of SOC content and SOC mineralization among the treatments and to evaluate the relative importance of each factor. Prior to RDA, variance inflation factors (VIFs) were calculated to detect multicollinearity among environmental predictors. Variables with VIF > 10 were removed. Redundancy analysis (RDA) was performed in Canoco 5.0 (Microcomputer Power, Ithaca, NY, USA).

3. Results

3.1. SOC Fraction Contents

After the experiment, the contents of SOC and soil microbial biomass carbon (MBC) in the surface soil (0–5 cm) differed among the treatments (Figure 2, SOC: $p = 0.0233$; MBC: $p = 0.0464$), while other SOC fractions did not significantly differ among the treatments (Figure 2). Both SOC and MBC contents under the RT treatment were significantly higher than under the RF, RC and RM treatments (Figure 2, $p < 0.05$).

For microbial necromass carbon, no significant differences in fungal necromass C (FNC), bacterial necromass C (BNC) and microbial necromass C (MNC) were found among the treatments (Figure 3A–C; FNC: $p = 0.1408$; BNC: $p = 0.0549$; and MNC: $p = 0.0787$). The ratio of FNC to BNC was also not significant among the treatments (Figure 3D, $p = 0.9026$).

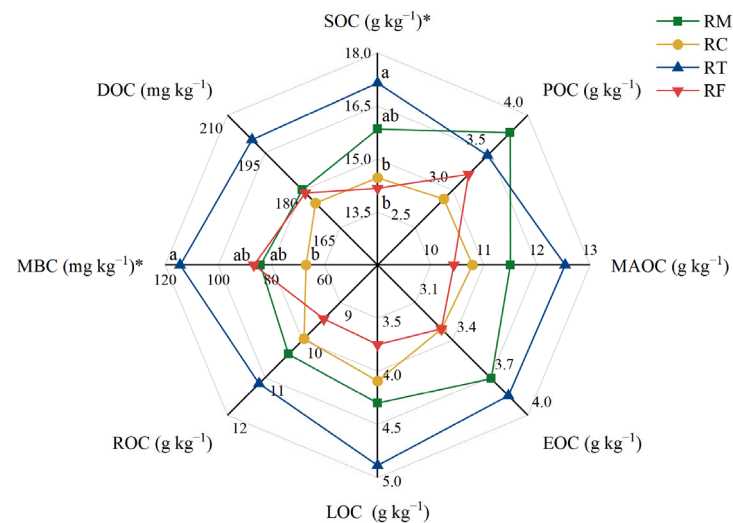


Figure 2. Fractions of soil organic carbon contents. SOC: soil organic carbon; POC: particulate organic carbon; MAOC: mineral-associated organic carbon; EOC: easily oxidizable organic carbon; LOC: labile organic carbon; ROC: recalcitrant organic carbon; MBC: microbial biomass carbon; DOC: dissolved organic carbon. RM: rice monoculture; RC: rice–crayfish coculture; RT: rice–turtle coculture; RF: rice–fish coculture. Values are shown as mean $\pm$ SE. Different lowercase letters represent significant differences among treatments at $p < 0.05$. * represents significant difference at the level of 0.05.

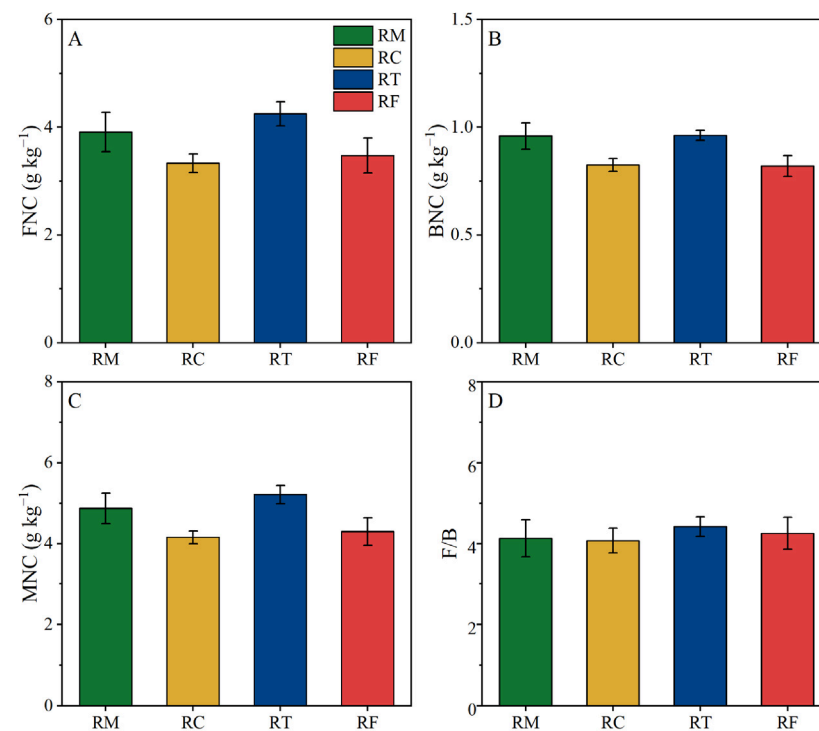


Figure 3. Microbial necromass carbon. (A) FNC: fungal necromass C; (B) BNC: bacterial necromass C; (C) MNC: microbial necromass C; and (D) F/B: ratio of fungal necromass C to bacterial necromass C. RM: rice monoculture; RC: rice–crayfish coculture; RT: rice–turtle coculture; RF: rice–fish coculture. Values are shown as mean $\pm$ SE.

3.2. Soil Aggregate Compositions and Properties

There were significant differences in the proportion of large macroaggregates (LMA), the proportion of microaggregates (MiA), and the mean weight diameter (MWD) among different treatments (Figure 4A,B; LMA: $p = 0.0030$; MiA: $p = 0.0466$; and MWD: $p = 0.0076$). Specifically, the LMA proportion in the RT treatment was significantly lower than that in the rice monoculture (RM), RC, and RF treatments (Figure 4A, $p < 0.05$), while the MiA proportion was significantly higher than that in the RC treatment (Figure 4A, $p < 0.05$). Meanwhile, the MWD in the RT treatment was also significantly lower than that in the other three treatments (Figure 4B, $p < 0.05$). Regarding the geometric mean diameter (GMD) and fractal dimension (D), no significant difference was found among the treatments (Figure 4C,D).

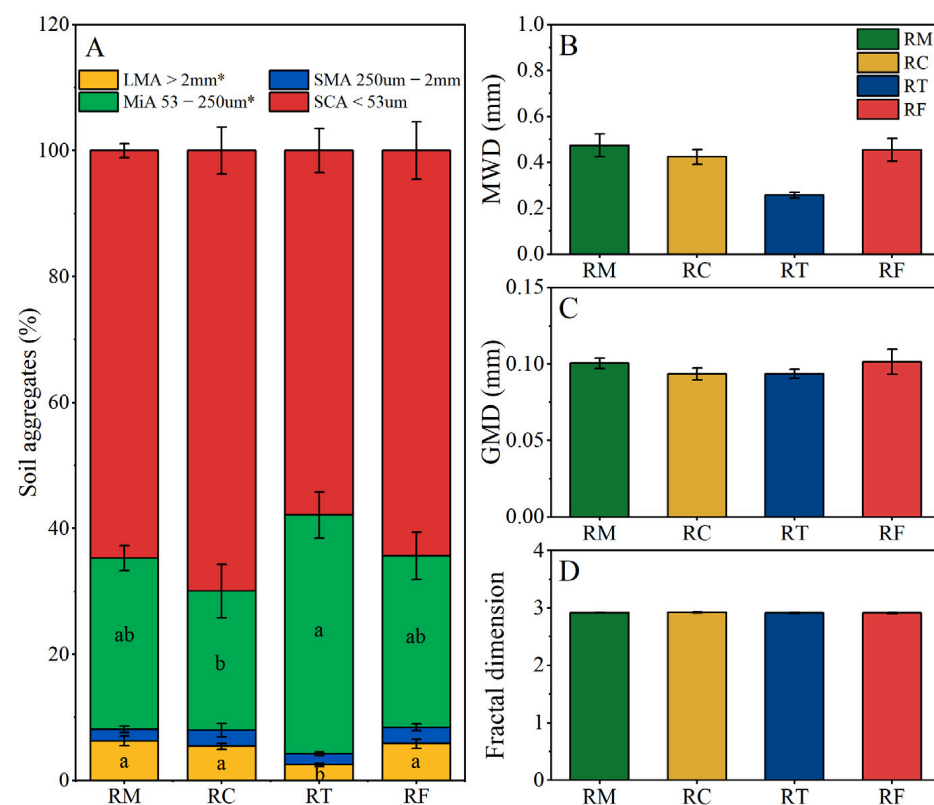


Figure 4. Soil aggregates. (A) Soil aggregate percentage content, (B) mean weight diameter, (C) geometric mean diameter and (D) fractal dimension. RM: rice monoculture; RC: rice–crayfish coculture; RT: rice–turtle coculture; RF: rice–fish coculture. LMA (large macroaggregates): >2 mm; SMA (small macroaggregates): $250\ \mu\text{m} - 2$ mm; MiA (microaggregates): $53 - 250\ \mu\text{m}$; SCA (particle sized silt + clay fractions): $<53\ \mu\text{m}$. MWD: mean weight diameter; GMD: geometric mean diameter. Values are shown as mean $\pm$ SE. Different lowercase letters indicate significant differences among treatments ($p < 0.05$). * represents significant difference at the level of 0.05.

3.3. Soil Organic Carbon Mineralization

During the incubation period, the SOC mineralization rate of all treatments showed a trend of first increasing and then decreasing. Repeated-measures ANOVA results indicated that there were significant differences in the SOC mineralization rate among different treatments over the incubation period (Figure 5A, $p = 0.0035$).

The one-way ANOVA results show that there were significant differences in the average daily mineralization among different treatments (Figure 5B, $p = 0.0025$). The average daily mineralization of the RT treatment ($8.842 \pm 0.5612\ \text{mg CO}_2\text{-C}\cdot\text{kg}^{-1}\ \text{dry soil}\cdot\text{day}^{-1}$) and the RC treatment ($7.615 \pm 0.4573\ \text{mg CO}_2\text{-C}\cdot\text{kg}^{-1}\ \text{dry soil}\cdot\text{day}^{-1}$) showed no signifi-

cant difference, but both were significantly higher than those of the rice monoculture (RM) treatment and the RF treatment (RM: $5.612 \pm 0.4363 \text{ mg CO}_2\text{-C}\cdot\text{kg}^{-1} \text{ dry soil}\cdot\text{day}^{-1}$; RF: $6.012 \pm 0.5578 \text{ mg CO}_2\text{-C}\cdot\text{kg}^{-1} \text{ dry soil}\cdot\text{day}^{-1}$).

Significant differences in the soil metabolic quotient were found among different treatments (Figure 6A, $p = 0.0147$). The soil metabolic quotient of the RC treatment was the highest, at $116.80 \pm 13.02 \text{ mg CO}_2\text{-C}\cdot\text{g}^{-1} \text{ MBC}\cdot\text{day}^{-1}$, which is significantly higher than those of the other three treatments.

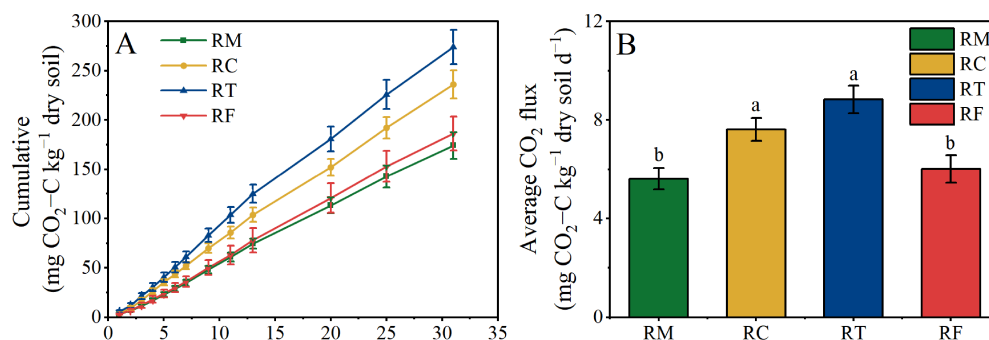


Figure 5. Soil organic carbon mineralization. (A) The cumulative CO₂ flux over the incubation experiment. (B) The average daily mineralization rate. RM: rice monoculture; RC: rice–crayfish coculture; RT: rice–turtle coculture; RF: rice–fish coculture. Values are shown as mean $\pm$ SE. Different lowercase letters represent significant differences among treatments at $p < 0.05$.

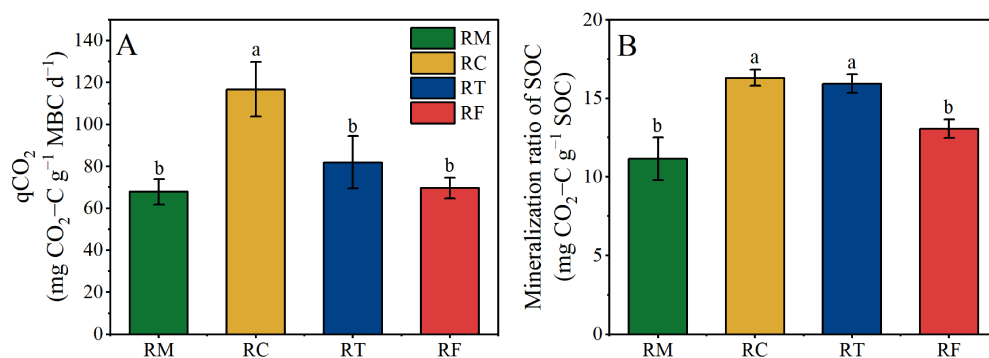


Figure 6. Soil metabolic quotient (A) and mineralization ratio of SOC (B). RM: rice monoculture; RC: rice–crayfish coculture; RT: rice–turtle coculture; RF: rice–fish coculture. Values are shown as mean $\pm$ SE. Different lowercase letters represent significant differences among treatments at $p < 0.05$.

Significant differences in SOC mineralization ratios were also found among different treatments (Figure 6B, $p = 0.0029$). The RT treatment ($15.93 \pm 0.5997 \text{ mg CO}_2\text{-C}\cdot\text{g}^{-1} \text{ SOC}$) and the RC treatment ($16.31 \pm 0.5213 \text{ mg CO}_2\text{-C}\cdot\text{g}^{-1} \text{ SOC}$) showed no significant difference, but both had significantly higher SOC ratios than the RM treatment and the RF treatment (RM: $11.16 \pm 1.3690 \text{ mg CO}_2\text{-C}\cdot\text{g}^{-1} \text{ SOC}$; RF: $13.07 \pm 0.5985 \text{ mg CO}_2\text{-C}\cdot\text{g}^{-1} \text{ SOC}$).

3.4. Relationship Between Soil Organic Carbon Mineralization and Soil Organic Carbon Fractions and Aggregate Structure

The RDA results show that the SOC fractions and soil aggregate structure well explained the CO₂ emissions and SOC content (Figure 7 and Table 1, $p = 0.01$). MAOC, ROC, POC and LMA proportions were significant explanatory variables ($p < 0.01$), with explanation rates of 41.89%, 16.03%, 15.77%, and 8.41%, respectively.

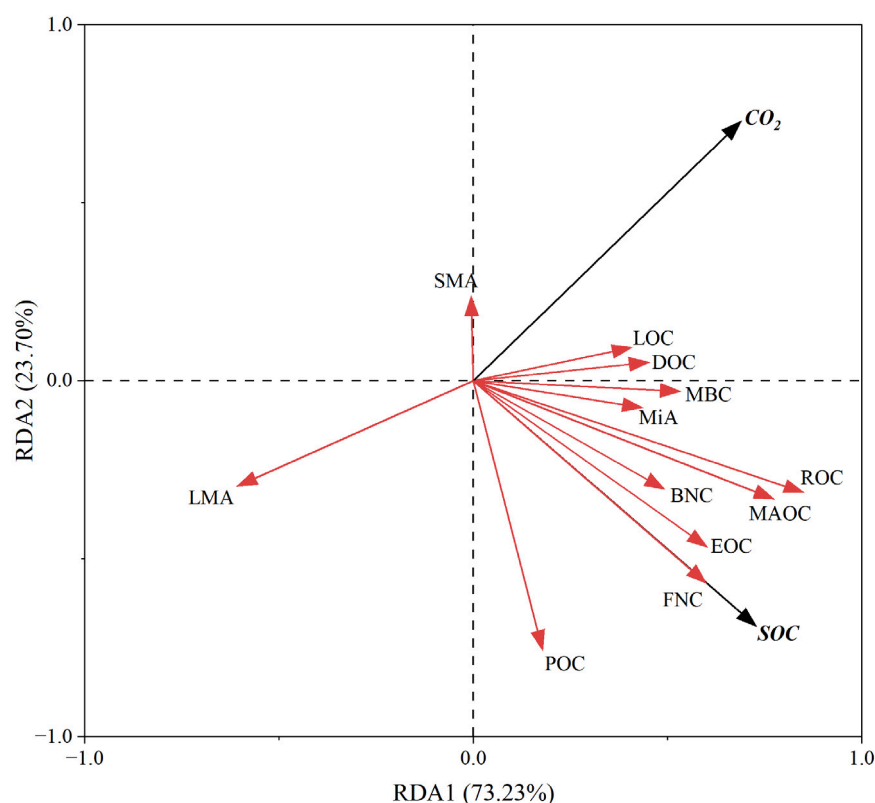


Figure 7. Redundancy analysis (RDA) of the effects of soil organic carbon fractions and aggregate structure (explanatory variable, red arrows) on soil CO_2 emissions and SOC content (response variables, black arrows). SOC: soil organic carbon; POC: particulate organic carbon; MAOC: mineral-associated organic carbon; EOC: easily oxidizable organic carbon; LOC: labile organic carbon; ROC: recalcitrant organic carbon; MBC: microbial biomass carbon; DOC: dissolved organic carbon. FNC: fungal necromass C; BNC: bacterial necromass C; LMA: large macroaggregates; SMA: small macroaggregates; MiA: microaggregates.

Table 1. The results of the redundancy analysis.

	Explains %	Contribution %	<i>p</i>
MAOC	41.89	43.21	0.002 **
ROC	16.03	16.54	0.011 *
POC	15.77	16.27	0.007 **
LMA	8.41	8.68	0.03 *
SMA	3.3	3.4	0.132
LOC	3.14	3.24	0.167
MBC	2.65	2.73	0.182
FNC	2.37	2.44	0.193
EOC	1.3	1.34	0.361
DOC	1.25	1.29	0.374
MiA	0.47	0.48	0.664
BNC	0.35	0.36	0.693

* and ** represent significant differences at the levels of 0.05 and 0.01, respectively. The abbreviations used in Table 1 are consistent with those in Figure 7.

4. Discussion

4.1. Aquatic Animals Differentially Affecting SOC and Soil Aggregate Structure

The role of animals in ecosystem carbon cycling has received increasing attention [45–48]. Animals influence carbon cycling in terrestrial and aquatic ecosystems through their feeding, bioturbation, and excretion/egestion activities [45–48]. Experiments

have shown that the coculture of rice and aquatic animals in paddy ecosystems can significantly increase SOC [24,27,49]. In this study, we found that the effects of aquatic animals on SOC content and fractions varied. Among the three types of coculture, soil under the rice–turtle coculture showed the highest values for all organic carbon fractions, with the total organic carbon (SOC) and microbial biomass carbon (MBC) reaching significant levels (Figure 2). Moreover, the contents of total SOC, MAOC, EOC, LOC, ROC, MBC, DOC and MNC in the rice–turtle system were all higher than those in the rice monoculture (Figure 3), suggesting that the coculture of rice and turtle has the potential to achieve soil carbon sequestration. Different types of aquatic animals exhibit differences in feeding and other activities within the paddy ecosystem [22,23,28]. Experimental evidence indicates that turtles perform better than other aquatic animals in promoting the decomposition of soil organic matter and driving nitrogen cycling in the paddy ecosystem [22]. Studies have also shown that turtles have the greatest beneficial effects on stabilizing rice yields and controlling pests [27,49]. In natural ecosystems, animal feces are mostly stored in the soil in the form of organic matter. The different biological characteristics (body size and life form) of aquatic animals and their varying assimilation and utilization efficiencies of field natural resources and feed [22,26] may lead to differences in the mass and carbon content of organic matter excreted as feces, thereby resulting in different amounts of carbon input to the soil.

Our results also show that the aquatic animals in the coculture systems differentially affected soil aggregate structure (Figure 4). The proportion of large macroaggregates (LMA) in the rice–turtle coculture was significantly lower than that in the rice monoculture, rice–crayfish coculture, and rice–fish coculture (Figure 4A). Meanwhile, the mean weight diameter (MWD) of the soil in the rice–turtle coculture treatment was also significantly lower than that in the other three treatments (Figure 4B). In a grazing system, body shape and the activities of animals generally affect soil porosity and soil aggregate structure [50,51]. Turtles have short, flat limbs and move by alternately pushing against the soil with their limbs. Their range of activity is relatively large, but their movement trajectory is concentrated in the surface soil layer (generally 0–15 cm depth), and their crawling compacts the soil. Crayfish use their chelipeds to dig into the sediment to construct burrows (generally 30–50 cm depth); their range of activity is relatively small, but their vertical disturbance is significant. In contrast, fish primarily swim rapidly in the water layer; although their activity range covers the entire paddy field, their impact on the soil is relatively minor, thereby reducing the degree of aggregate disruption.

4.2. Aquatic Animals Affecting SOC Mineralization by Shifting SOC Fractions and Soil Aggregate Structure

The SOC composition and soil aggregate structure directly influence the decomposition and accumulation of organic matter [5,6]. For SOC composition, the labile organic carbon pool is highly active, sensitive to environmental changes, and readily utilized and decomposed by microorganisms [6,7]. In contrast, the stable organic carbon pool turns over slowly in the soil, resists decomposition, and can generally persist for hundreds to thousands of years [6,7]. In our study, the RDA indicated that SOC fractions have a significant effect on the SOC mineralization rate (Figure 7; Table 1). Compared with the rice monoculture, the rice–turtle system showed increases in both soil labile carbon fractions (EOC, LOC, MBC, and DOC) and stable carbon fractions (MAOC, ROC, and MNC); however, the LOC and DOC contents positively contributed to SOC mineralization in the redundancy analysis.

The RDA also showed that the soil aggregate structure contributed to the SOC mineralization rate in our study (Figure 7; Table 1). The soil aggregate structure has an important influence on the retention and stabilization of organic carbon. The physical protection pro-

vided by macroaggregates can reduce SOC mineralization [52]. Macroaggregates contain large amounts of unstable labile organic carbon [53,54], and their breakdown can increase SOC mineralization [55]. In the coculture systems in this study, the bioturbation by aquatic animals (e.g., soft-shelled turtles) reduced the proportion of soil macroaggregates. On the one hand, the physical protection provided by aggregates decreased. On the other hand, the breakdown of macroaggregates into microaggregates released more protected labile carbon, which was subsequently utilized by microorganisms and promoted SOC mineralization [41,42]. Thus, the disturbance of soil by aquatic animals alters the structure of soil aggregates, thereby influencing SOC mineralization.

4.3. Implication and the Future Study

Studies have shown that coculturing rice with a certain kind of aquatic animals (e.g., fish, crayfish, crab, or turtle) can help maintain rice yield while reducing chemical fertilizers and pesticides and increasing the SOC content [22–27,56,57]. Studies have also shown that these outcomes of cocultures varied among coculture types [22,27,49]. In our study, although all the cocultures had stable rice yields comparable to the rice monoculture, different types of aquatic animals exert distinct effects on SOC composition and aggregate structure, thereby influencing SOC mineralization differentially. This highlights the importance of aquatic animals for the success of coculture systems during their establishment. As one of the approaches to sustainable agricultural development, rice–fish coculture systems have been rapidly expanding in rice-growing regions worldwide, with 47 out of 117 rice-producing countries having adopted and promoted integrated rice–aquaculture systems [58]. Understanding the effects of aquatic animals in paddy ecosystems would help in the development of sustainable rice–aquatic animal cocultures worldwide.

In this study, we focused on surface soil (0–5 cm) that is considered the most active interface for SOC turnover and mineralization because this zone serves as the primary receiving layer for organic matter inputs (e.g., crop residues, root exudates, and animal excreta) [28,59]. Our previous research on rice–fish coculture indicated that nitrogen released by the fish mainly accumulates in the soil surface layer (0–1 cm), and its influence on soil CH₄ oxidation also predominantly occurs in the surface soil layer [28]. However, the burrowing and bioturbation behavior of certain aquatic animals, such as turtles and crayfish, may influence or reach deeper soil layers. Therefore, future research on SOC dynamics in rice–aquatic coculture systems should extend across multiple soil layers.

5. Conclusions

This study demonstrates that raising aquatic animals with rice in paddy fields significantly alters soil organic carbon (SOC) mineralization, yet the direction and magnitude of this effect are highly dependent on aquatic animal species. Among the three coculture systems, rice–turtle and rice–crayfish systems exhibited the strongest enhancement in SOC mineralization, primarily driven by their distinct modifications to soil carbon fractions and aggregate structure. Specifically, the increase in mineralization under these systems was closely associated with shifts in soil recalcitrant organic carbon (ROC), particulate organic carbon (POC), and the redistribution of aggregate size classes. These findings enhance our understanding of how rice–aquatic animal coculture systems affect SOC dynamics. However, our results are derived from a short-term field experiment and a surface soil measurement. The underlying mechanisms (e.g., the metabolic and behavioral effects of aquatic animals) remain to be validated through long-term experiments across multiple soil layers.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agriculture16151619/s1>.

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