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Article

# Effects of Long-Term Rice-Crayfish Coculture Systems on Soil Nutrients, Carbon Pools, and Rice Yields in Northern Zhejiang Province, China

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**Abstract:** This research was to examine the impacts of long-term integrated rice-crayfish farming on soil nutrients, carbon pools, and rice yields in paddy fields. The aim was to establish a scientific basis for the sustainable development of RS in the northern region of Zhejiang. The results showed that the change from rice monoculture (CK) to rice-crayfish coculture systems (RS) led to a 24.99% increase in the 5-year average of soil ammonium nitrogen (AN), while the soil nitrate nitrogen (NN), available potassium (AK), and available phosphorus content (AP) decreased by 28.02%, 16.05%, and 28.76% respectively. Moreover, the total organic carbon (TOC), easily oxidizable organic carbon (EOC), dissolved organic carbon (DOC), and microbial biomass carbon (MBC) exhibited a reduction of 2.45 %, 8.82 %, 35.31 %, and 65.84 % respectively. Correlation analysis revealed a significant positive correlation between NN, EOC and MBC in the RS mode. In terms of rice yield, 5-year average of rice yield in RS has decreased by 8.40 % compared to CK. The mean yield of early-maturing rice varieties was reduced by 13.16 %, while that of late-maturing rice varieties was reduced by 6.00 %. These results shed light on the annual variation of soil nutrients, carbon pool and rice yield in the RS mode, providing insights for the sustainable development of RS in northern Zhejiang.

**Keywords:** red-claw crayfish; nutrients; carbon pool; yield; prolonged flooding

## 1. Introduction

Rice, as a primary crop in China, accounts for approximately 30% of the nation's total grain cultivation area [1]. China rice cultivation area had reached 29.45 million hectares in 2022 [2], which playing a crucial role in ensuring national food security. In recent years, the economic benefits of rice monoculture model has decreased due to various factors such as labor, land, and the increasing costs of agricultural materials [3]. At present, there is an increasing trend in utilizing the rice planting environment (temperature, light, water, air, heat) for aquaculture in paddy field compound aquaculture [4]. According to statistics, the area of rice-shrimp cultivation reached 156.67 million hectares in 2022 [5], representing 54.71% of the integrated rice-field aquaculture area in China [6]. Rice-crayfish cultivation represents a type of rice-shrimp model that has become a prominent mode of integrated rice-field aquaculture in the middle and lower regions of the Yangtze River [7,8].

Land-use type transforming is the main driver of changes in soil nutrient and carbon fractions [9,10]. Similarly, the land use shifts from rice monoculture to alternating rice-crayfish cultivation also faces this situation. Previous studies indicated that long-term rice-crayfish cultivation had significantly higher soil C, N and P concentrations than rice monoculture systems [11], and the soil

of total nitrogen and phosphorus increased due to the excessive use of nitrogen and phosphorus nutrients [12]. In terms of soil carbon fractions, the integrated rice-crayfish farming was found to significantly increase total organic carbon (TOC), dissolved organic carbon (DOC) content in all layers and microbial biomass carbon (MBC) in the 20-40 cm layer compared to paddy farming [13]. In addition, the organic matter content in each component of the soil aggregates had increased under the integrated rice-crayfish farming system compared to traditional paddy farming [14]. However, the unreasonable fertilizer and feed inputs [15,16], in addition to the flooding of the soil for extended periods [17], are significant variables that influence the soil characteristics of the rice-crayfish farming system. In recent years, an increasing number of studies indicate that long-term of rice-crayfish farming model has also raised a series of soil problems, such as soil fertility decline, soil acidification and soil microbial communities disruption [18–20]. Therefore, the impact of this aquaculture method on soil nutrients and carbon fractions needs to be further investigated for a more comprehensive view.

The red-claw crayfish (*Cherax quadricarinatus*), a native of Australia, is one of the most valuable freshwater economic shrimps in the world. The red-claw crayfish exhibits a number of advantageous traits, including a large size, a rapid growth rate, a high degree of adaptability, and an abundant nutrition source [21], which are highly beneficial in the context of rice field aquaculture. A substantial corpus of literature exists on the subject of rice-shrimp coculture [22–24]. However, the majority of the literature is focused on the rice-crayfish coculture model [25–27], with comparatively few reports on rice-red claw crayfish coculture. The co-cultivation system of rice and red-claw crayfish involves the cultivation of both species for an extended period. In order to facilitate the healthy development of red-claw crayfish, it is essential that the water level in the aquaculture system be maintained at a high level and that the rice remain submerged for an extended period until the time of harvest. Recent research indicates that rice-red claw crayfish coculture can significantly increase the secretion of organic acids and amino acids in rice root system, accelerate nutrient release, and promote rice yield [28]. He et al. (2019) [29] showed that the nitrogen, phosphorus, organic matter content and plankton density in rice-red claw crayfish coculture water were higher than those in traditional aquaculture ponds. However, many studies have shown that prolonged flooding of rice paddies can negatively affect the soil environment [30,31] and even crop yields [32,33].

North Zhejiang Province is the largest area to promote the rice-crayfish aquaculture model in Zhejiang Province, and is also an important food production area in Zhejiang Province. The impact of rice and crayfish cultivation on food security and farmland quality has always been a concern for local governments. Therefore, the aim of this study was to clarify the effects of rice-crayfish coculture model on soil quality and rice yield in northern Zhejiang. A comprehensive analysis of soil nutrients, carbon fractions and rice yield in two systems (rice monoculture and rice-crayfish coculture) was conducted from 2018 to 2022. The hypotheses were as follows: (1) the input of fertilizers and feed in rice-crayfish fields results in a significant accumulation of ammonium nitrogen due to the prolonged flooding conditions; (2) due to long-term flooding, soil microbial biomass carbon would decrease in the long-term rice-crayfish coculture systems; (3) rice yield would decrease in the long-term rice-crayfish coculture systems. The results showed that long-term rice-crayfish cultivation in paddy fields not only reduces soil nutrients (with the exception of ammonium nitrogen) and carbon fractions, but also reduces rice yield in comparison to conventional rice monoculture. In conclusion, it can be stated that improving water and nutrient management and cultivating late-maturing rice varieties are crucial strategies for the sustainable development of rice-crayfish coculture in northern Zhejiang.

## Materials and Methods

### 2.1. Experimental Site and Treatments

The field experiment was conducted in 2018-2022 at Gutang Experimental Station of Jiaying Academy of Agricultural Science (JXAAS), Jiaying, China (30°51'49.9"N, 120°42'14.5"E). The experimental site is located in the Hangjiahu Plain in the Yangtze River Delta, at an altitude of 10-15 m. The city has an East Asian monsoon climate [32], the climate is characterized by a comfortable average annual temperature of 17.3 °C and abundant rainfall, with an annual average of about 1,193

mm from 2018 to 2022. The annual average total sunshine and frost-free days are 1,920 h and 243 d, respectively.

The following trial rice varieties were adopted between 2018 and 2022: 'Jiahe 218' (early maturing), 'Zhehexiang No.2', 'Zhehexiang No.2', 'Xiushui 6545' (early maturing), and 'Jiahexiang No.1'. Rice transplanting was carried out in the middle and end of June each year. The rice planting density of 25 × 16 cm, with two plants per hole. The crayfish species cultivated is the red-claw crayfish. Before the start of the experiment, the basic physicochemical properties (0–20 cm depth) of soil as follows: soil organic carbon 15.65 g·kg<sup>-1</sup>, total nitrogen 1.63 g·kg<sup>-1</sup>, total phosphorus 0.50 g·kg<sup>-1</sup>, available phosphorus 7.63 mg·kg<sup>-1</sup>, available potassium 130.45 mg·kg<sup>-1</sup>, hydrolysable nitrogen 121.65 mg·kg<sup>-1</sup>, pH<sub>H2O</sub> 6.45.

The experiment included two treatments: rice monoculture (CK) and rice-crayfish coculture system (RS), each treatment covering an area of 0.2 ha. RS mode is derived from transformed conventional rice fields, which are excavated to create a circular ditch with a diameter of 2 meters and a depth of 1.5 meters around the rice field (with a circular ditch area accounting for about 10% of the total area of the paddy field). The red-claw crayfish were cultured within the circular ditch. RS began to prepare the soil in the middle of May each year, followed by the release red-claw crayfish flies (about 1 cm in body length) within the circular ditch. The density of crayfish placed in the ditch was 2,500 tails per hectare in the RS mode. In the initial stage of releasing crayfish seedlings, the quantity of food provided per 10,000 crayfish seedlings should be controlled at approximately 1,000 g. In the subsequent stage, the quantity of food provided should be gradually increased in accordance with the actual circumstances.

The management of fertilization and water irrigation in CK or RS mode was largely consistent throughout the period from 2018 to 2022. During the whole growth period, CK was fertilized at sowing with 84 kg N·ha<sup>-1</sup>, 45 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 135 kg K<sub>2</sub>O ha<sup>-1</sup>, respectively. The rice was fertilized at tillering stage and panicle initiation stage with 63 kg N·ha<sup>-1</sup>, respectively. RS was only fertilized at the tillering stage and panicle initiation stage with 43 kg N·ha<sup>-1</sup>, respectively. In accordance with the prevailing local planting and cultivation practices, the application of phosphorus fertilizer and potash was not applied every year.

In terms of water management, CK was managed in accordance with the local rice irrigation practice of early-stage irrigation, mid-stage sunshine, and late-stage alternating wet and dry conditions. In order to guarantee the optimal functioning of the RS mode, it is of the utmost importance to consider the depth of the water and the requirements for both crayfish aquaculture in the ring ditch and rice cultivation. The paddy water depth should be kept at about 10 cm for transplanting. In July, the depth of water in the paddy should be approximately 20 cm, with the paddy surface consistently flooded. From August to September, the paddy water depth should be about 40–45 cm, with the paddy flooded and irrigated. In early October, crayfish are caught using a cage, and by mid to early November, the field is drained and the catch is finished. The operational processes of the CK and RS modes are depicted in Table 1.

**Table 1.** The time of operation in different rice cultivation modes.

Mode	Crayfish fly release date	Rice planting date	Crayfish catch start date	Crayfish catch finish date	Rice harvest date
CK	-	19-Jun-2018	-	-	15-Nov-2018
	-	18-Jun-2019	-	-	26-Nov-2019
	-	24-Jun-2020	-	-	30-Nov-2020
	-	27-Jun-2021	-	-	20-Nov-2021
	-	22-Jun-2022	-	-	24-Nov-2022
RS	29-May-2018	19-Jun-2018	9-Oct-2018	5-Nov-2018	15-Nov-2018
	28-May-2019	18-Jun-2019	6-Oct-2019	16-Nov-2019	26-Nov-2019
	18-May-2020	24-Jun-2020	1-Oct-2020	20-Nov-2020	30-Nov-2020
	23-May-2021	27-Jun-2021	5-Oct-2021	10-Nov-2021	20-Nov-2021
	20-May-2022	22-Jun-2022	3-Oct-2022	14-Nov-2022	24-Nov-2022

(Note: CK, rice monoculture ; RS, rice- crayfish coculture system.).

## 2.2. Soil and Plant Measurements

The surface layer (0-20 cm) was sampled using a S-shaped sampling strategy in two distinct stages: prior to the release of crayfish flies (early May) and subsequent to the harvesting of rice (late November) in each year. Fresh soil samples were sieved (2 mm) and stored in the refrigerator at 4°C for determining dissolved organic carbon (DOC) and microbial biomass carbon(MBC). The remaining part samples were air dried, ground, and finally passed through 0.25 mm sieves for determining soil ammonium nitrogen(AN), soil nitrate nitrogen(NN), available phosphorus (AP), available potassium(AK), easily oxidizable organic carbon(EOC) and total organic carbon(TOC). NA, NN, AP, AK and TOC were determined by the methods of Lu (2000) [34]. DOC was measured by the method of Jiang et al. (2006) [35]. EOC was measured by the  $\text{KMnO}_4$  oxidation procedure with 333 mmol/L [36]. MBC was determined by chloroform fumigation-extraction method [37].

When the rice was ripe, it was harvested with the Kubota 688 and the moisture content of the paddy was determined and weighed on the spot. Actual rice yield was calculated using the Chinese national standard for safe storage moisture content for rice of 14.5%.

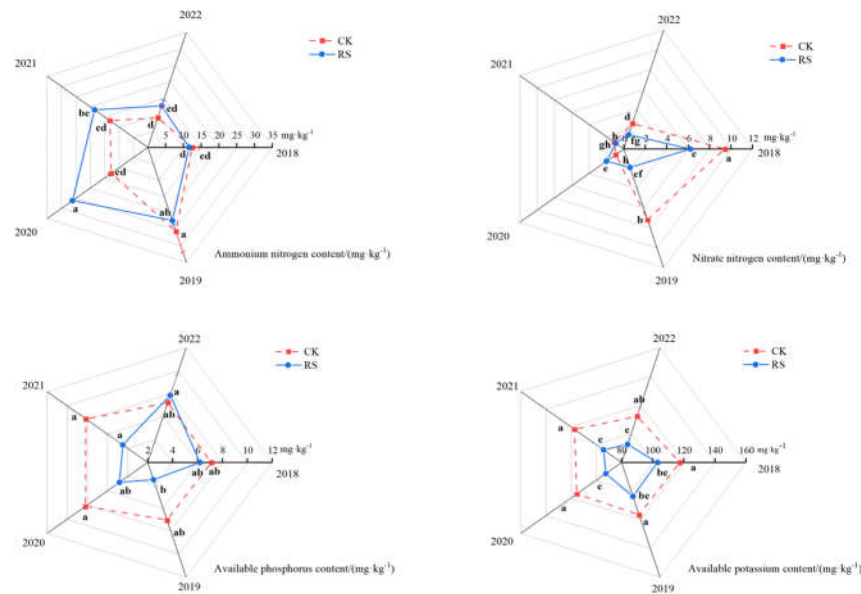
## 2.3. Methods of Statistical Analysis of Data

Two-way analysis of variance (ANOVA) was performed using SPSS 25.0 software to examine the interactive effects of different years (2018, 2019, 2020, 2021 and 2022) and rice cultivation mode (RS and CK) on soil available nutrients(AN, NN, AP and AK) and carbon pool(TOC, EOC, DOC and MBC). The Duncan's multiple comparisons was used to test the significance of differences between treatment means. Specifically, soil data is collected on a biannual basis, at the times of early May(before crayfish flies release) and late November (after rice harvest) in each year. Subsequently, the data is employed to compare the differences between the RS and CK modes on soil nutrients and carbon pool in each year. Furthermore, the soil data obtained twice a year for different time periods of RC or CK is used to analysis the differences in annual soil nutrient and carbon pool. Pearson correlation analysis was performed on the annual soil nutrient, carbon pool and yield data of the different treatments, and the data were processed and tabulated using WPS 2010 software. The data were processed and tabulated with WPS 2010 software, and the data were plotted with Origin 2021 software.

## 3. Results

### 3.1. Effect of Rice-Crayfish Coculture System on Soil Nutrients

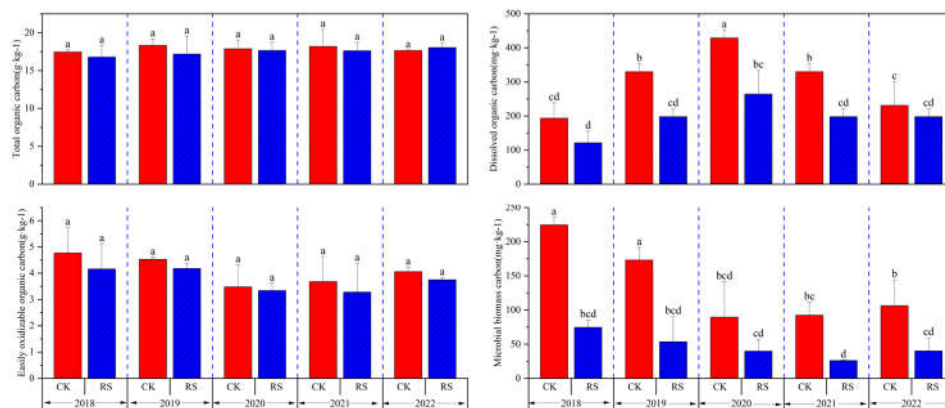
The content of AN in RS mode was higher than CK from 2020 to 2022 with the extension of the years of experimental conduct, in which the RS soil AN significantly increased by 105.42 % ( $p \leq 0.05$ ) in 2020 (Figure 1). However, the content of AK in RS was significantly decreased by 12.29 %, 10.96 %, 19.94 %, 19.68 %, and 17.46 % ( $p \leq 0.05$ ), respectively, from 2018 to 2022 compared with the CK. The content of NN in RS in 2018, 2019, and 2022 were all significantly lower ( $p \leq 0.05$ ) than CK treatment. Compared with CK, the 5-year average content of soil AN in RS increased by 24.99 %, and the content of soil NN, AK and AP decreased by 28.02 %, 16.05 % and 28.76 %, respectively.



**Figure 1.** The annual variation characteristics of soil available nutrients under different rice cultivation modes from 2018 to 2022. (Note: CK: rice monoculture; RS: rice-crayfish coculture system. Different letters indicate significant differences at  $p \leq 0.05$ ).

### 3.2. Effects of Rice- Crayfish Coculture System on Soil Carbon Pools

The content of DOC and MBC in RS mode exhibited a decline from 2018 to 2022, compared with the CK mode (Figure 2). The content of DOC in RS mode was significantly decreased by 40.00 %, 38.46 %, and 40.00 % in 2019-2021, respectively ( $p \leq 0.05$ ), in comparison to the CK mode. And, the content of MBC in RS mode was significantly decreased by 66.76 %, 69.01 %, 55.56 % and 71.75 % in 2018, 2019, 2021 and 2022, respectively ( $p \leq 0.05$ ), compared with CK mode. The 5-year average contents of TOC, EOC, DOC and MBC in RS mode were decreased by 2.45 %, 8.82 %, 35.31% and 65.84%, respectively, compared with CK mode.

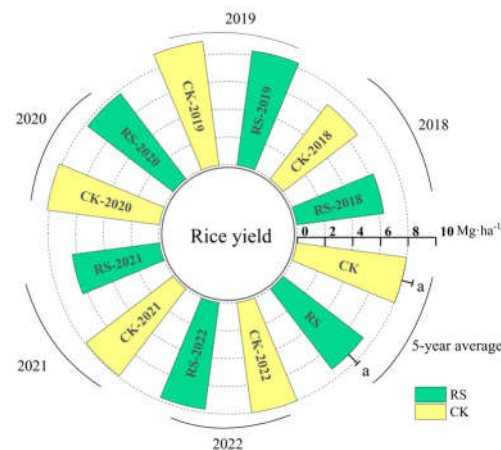


**Figure 2.** The annual variation characteristics of soil carbon pool under different rice cultivation modes from 2018 to 2022. (Note: CK: rice monoculture; RS: rice-crayfish coculture system. Different letters indicate significant differences at  $p \leq 0.05$ ).

### 3.3. Effect of Rice-Crayfish Coculture System on Rice Yield

The rice yield of the RS treatment was found to be lower than that of the CK treatment in the same year (Figure 3). From 2018 to 2022, the rice yield of the RS treatment was found to decrease by 6.37 %, 7.62 %, 4.80 %, 17.96 % and 3.22 % in comparison with the CK mode, respectively.

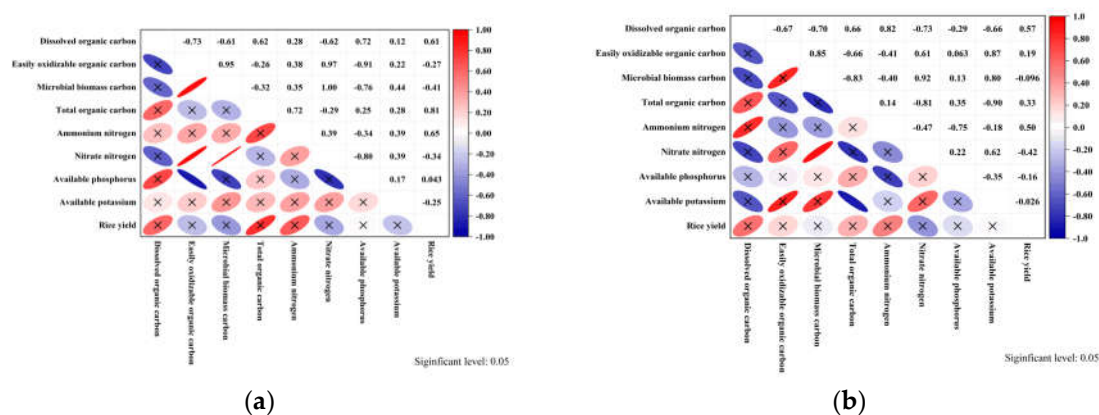
Furthermore, the five-year average yield of the RS mode was found to be 8.40% lower than that of the CK mode, although this difference was not statistically significant.



**Figure 3.** The annual variation characteristics of rice yield under different rice cultivation modes from 2018 to 2022. (Note: CK: rice monoculture; RS: rice-crayfish coculture system. Different letters indicate significant differences at  $p \leq 0.05$ ).

### 3.4. Correlation Analysis

There was a significant positive correlation ( $p \leq 0.05$ ) between soil NN, EOC and MBC in RS mode. NN and MBC were significantly positively correlated ( $p \leq 0.05$ ) in CK mode (Figure 4).



**Figure 4.** Correlation analysis of soil available nutrients, carbon pools and rice yield in RS (a) and CK (b).

## 4. Discussion

### 4.1. Rice-Crayfish Coculture System Altered Soil Nutrient Status

In this study, the 5-year average content of AN has to be improved for the RS mode compared to the CK systems (Figure 1). It was also observed that the soil of NN was reduced in the RS system. These results are in line with our first hypothesis, emphasizing that the soil of AN content was accumulated in the RS mode due to the application of N fertilizer [38], artificial feed [39] and floodwater [40]. Besides, the decomposition of dead crayfish may also incorporate nitrogen nutrient into the paddy field [16]. This is supported by a higher level of AN in RS (Figure 1). A lower level of NN could be explained by the fact that the anoxic situation of prolonged anaerobic flooding in RS systems might weaken soil nitrification function, resulting in lower soil NN in RS than in CK systems, which is consistent with those of previous research [41].

Phosphorus (P) comes from the deposition of crayfish, feed debris and fertilizer in RS systems [42]. Previous studies of RS mode have shown that the increased soil pH under flooding state resulted in the significant increase of  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  content, which could adsorb and fix soil P, thus promoting soil P accumulation [11]. Moreover, Our data showed that there is no significant difference in AP between RS and CK systems, but the 5-year average content of AP in RS mode is lower than in CK mode (Figure 1). This might be explained by the non-application of phosphate fertilizer in RS mode from 2018 to 2022. Although, the addition of organic matter (such as feed debris, rice straw and organic fertilizer) could increase proportion of stable phosphorus fractions in RS systems [22]. However, these forms are not readily available to rice, leading to a decrease in AP. Besides, prolonged soil flooding can accelerate soil potential phosphorus losses [43]. The similar to soil AP, due to the non-application of potash fertilizer in RS systems, resulted in significantly lower soil readily AK content in RS mode than in the CK mode among different years [44].

#### 4.2. Rice-Crayfish Coculture System Weakened Soil Carbon Pools

Soil carbon pool is an important part of the global carbon cycle. Among them, soil labile organic carbon (such as MBC, DOC and EOC) is a sensitive indicator reflecting the dynamic changes of soil carbon pool, which can reflect the small changes of soil organic carbon caused by farmland management measures such as fertilizer application and irrigation [45,46]. Previous studies have indicated that flooding in rice-crayfish systems increase soil TOC in a short period of time by reducing microbial activity and oxygen supply from plant roots [47,48]. Contrary to these findings, this study observed a decrease in the 5-year average TOC content in RS mode compared to CK mode. This may be attributable to the following reasons. First, the flooding period in our rice-red claw crayfish systems is about 120 days longer than in other rice-crayfish systems [14,39], persistently high soil moisture promotes reductive dissolution of soil iron oxides, which promotes microbial exposure to previously protected iron oxides. And it promotes microbial access to previously protected unstable carbon, thereby increasing carbon loss from the soil [49]. Second, labile C inputs (such as feed debris) could activate microorganisms, further stimulating soil organic matter decomposition [50,51]. Third, DOC is a potential source of total soil organic carbon, prolonged soil flooding can accelerate soil water-soluble organic carbon leaching [43]. Before harvesting rice, it is necessary to drain the rice fields that have been flooded for a long time, which leads to a significant loss of carbon from the soil [52]. The 5-year average contents of EOC and DOC were decreased in RS mode, which also supported by carbon loss from prolonged inundation (Figure 2).

Numerous studies have indicated that integrated rice-aquaculture farming could change the MBC because of its rapid response to field management practices [53,54]. In this study, the 5-year average contents of MBC was decreased in RS mode, compared with CK mode (Figure 2). The input of excessive carbon sources into RS mode might enhance the soil C/N ratio, result in soil loss, restrict the development of soil microorganisms and reduce MBC content [55,56]. Besides, flooding was the dominant driver of microbial C limitations [57]. Long-term integrated rice-crayfish culture has significantly reduced microbial richness and diversity [17]. Some invertebrates, protists and bacteria have reduced in the integrated rice-crayfish farming systems [58]. These findings support our second hypothesis and emphasize the important driver of flooding in RS mode for soil MBC.

#### 4.3. Rice-Crayfish Coculture System Reduced the Rice Yield

In previous studies, there is no unified conclusion on rice yield in rice-crayfish coculture systems. Chen et al. (2024)[59] showed that rice-crayfish coculture promoted economic benefits but threatened grain production. However, Liu et al. (2022)[60] showed that rice-crayfish coculture increased rice nitrogen uptake and yield relative to rice monoculture. According to our results, the 5-year average yield of RS mode was 8.4 % lower than that of CK mode. Among these, the yield of early maturing varieties was reduced by 13.16 %, and the yield of late maturing varieties was reduced by 6.00 % in RS systems (Figure 3), supporting our third hypothesis. Fertilization [61], flood control [62] and rice variety selection [63] are important reasons for differing yields in rice-crayfish coculture systems. A favorable nutrient conditions would create good microbial and physicochemical environments for

rice growth in rice-crayfish coculture systems [16]. Inadequate fertilization and prolonged flooding deplete soil nutrients (Figure 1), resulting in reduced yields in our RS mode. In addition, the high intensity of waterlogging in RS systems leads to a high accumulation of ammonium nitrogen in the soil, which inhibits primary root growth and yield [40]. Combined with the technical indicators and requirements of the Chinese Aquaculture Industry Standard “Technical Specifications for Integrated Rice-Fishery Farming Part 1: General Rules” (the proportion of ditch pits should not be more than 10% of the total area under cultivation, and the rice yield in the plain area should not be less than  $7.50 \text{ Mg}\cdot\text{ha}^{-1}$ ), the average yield of conventional late-maturing varieties in RS systems is  $7.95 \text{ Mg}\cdot\text{ha}^{-1}$ , which can achieve the target of a steady yield of  $7.50 \text{ Mg}\cdot\text{ha}^{-1}$  of rice in the northern part of Zhejiang Province.

#### 4.4. The Relationships between Soil Nutrient Status and Carbon Pools

Taylor et al. (2010) [64] showed that soil NN was non-linearly and significantly negatively correlated with DOC. The present study showed a linear negative correlation between NN and DOC under different rice cropping patterns. In addition, soil NN, DOC and MBC were positively correlated ( $p \leq 0.05$ ) in RS mode (Figure 4). These results are in accordance with previous observations, emphasizing that effects of long-term flooding on soil nutrients and carbon pools in rice-crayfish coculture fields. This is due to the fact that RS mode inhibits soil nitrification and reduces soil NN [65], and also decreases MBC due to poor soil aeration and weakened aerobic microbial activity under long-term flooding anaerobic conditions [66]. Furthermore, prolonged and persistent flooding results in reductive dissolution of iron oxides in the soil, which in turn promotes microbial exposure to previously protected unstable carbon. This results in a reduction in the concentration of EOC in the soil [49], which provides an additional rationale for the observation that the 5-year average content of soil TOC in the RS mode is lower than that in CK.

## 5. Conclusions

The long-term rice-crayfish coculture system (RS) was found to increase the available nitrogen (AN) content in paddy soil, while the content of nitrate nitrogen (NN), available phosphorus (AP), soil organic carbon pools (including total organic carbon (TOC), easily oxidizable organic carbon (EOC), dissolved organic carbon (DOC) and microbial biomass carbon (MBC)) and rice yield decreased compared to rice monoculture. On the one hand, the long-term flooding and anaerobic conditions under the RS mode stimulate the dissolution of available nutrients and carbon fractions, which results in the loss of nutrients and carbon fractions by excessive drainage before the harvest of the rice crop. Furthermore, long-term anaerobic conditions in flooded soil under RS mode suppress nitrification and respiration, resulting in the accumulation of AN and a reduction in the content of NN and MBC. On the other hand, the introduction of crayfish feed could facilitate the decomposition of TOC. The absence of phosphorus and potassium fertilizers could result in a reduction in the content of soil AP and AK in RS mode. Consequently, the unique long-term anaerobic conditions and input of production materials (such as fertilizers and crayfish feeds) under RS mode alter the soil nutrient status, carbon pools, which collectively contribute to a reduction in rice yield. In the future, it is essential to implement strategies to enhance water and fertilizer management and cultivate late-maturing rice varieties in order to achieve the sustainable development of RS mode in northern Zhejiang.

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