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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, the 5-year average of rice yield in RS 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 in soil nutrients, carbon pools, 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

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1. Introduction

Rice, as a primary crop in China, accounts for approximately 30% of the nation's total grain cultivation area [1]. The rice cultivation area in China reached 29.45 million hectares in 2022 [2], playing a crucial role in ensuring national food security. In recent years, the economic benefits of the rice monoculture model have 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, and heat) for 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 transformation is the main driver of changes in soil nutrient and carbon fractions [9,10]. Similarly, land use shifts from rice monoculture to alternating rice–crayfish cultivation also face 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 total nitrogen and phosphorus increased due to the excessive use of nitrogen and phosphorus nutrients [12]. In terms of soil carbon fractions, 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 increased under the integrated rice–crayfish farming system compared to traditional paddy farming [14]. However, 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 the long-term use of the rice–crayfish farming model has also caused a series of soil problems, such as soil fertility decline, soil acidification, and soil microbial community 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 and economic freshwater 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 body 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 the 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 the 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 the 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 long-term rice–crayfish coculture systems; and (3) rice yield would decrease in 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.

2. Materials and Methods

2.1. Experimental Site and Treatments

The field experiment was conducted in 2018–2022 at the Gutang Experimental Station of the Jiaxing Academy of Agricultural Science (JXAAS), Jiaxing, 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], which is characterized by a comfortable average annual temperature of 17.3 °C and abundant rainfall, with an annual average of about 1193 mm from 2018 to 2022. The annual average total sunshine and frost-free days are 1920 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 was 25 × 16 cm, with two plants per hole. The crayfish species cultivated is the red-claw crayfish (*Cherax quadricarinatus*). Before the start of the experiment, the basic physicochemical properties (0–20 cm depth) of soil were as follows: soil organic carbon 15.65 $g \cdot kg^{-1}$, total nitrogen 1.63 $g \cdot kg^{-1}$, total phosphorus 0.50 $g \cdot kg^{-1}$, available phosphorus 7.63 $mg \cdot kg^{-1}$, available potassium 130.45 $mg \cdot kg^{-1}$, hydrolysable nitrogen 121.65 $mg \cdot kg^{-1}$, and pH_{H2O} 6.45.

The experiment included two treatments: a rice monoculture (CK) and a rice–crayfish coculture system (RS), with each treatment covering an area of 0.2 ha. The RS mode is derived from transformed conventional rice fields, which are excavated to create a circular ditch with a diameter of 2 m and a depth of 1.5 m 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. For RS, the researchers began to prepare the soil in the middle of May each year, followed by the release of red-claw crayfish flies (about 1 cm in body length) within the circular ditch. The density of crayfish placed in the ditch was 2500 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 1000 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 the 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 $N \cdot ha^{-1}$, 45 kg P_2O_5 ha^{-1}, and 135 kg K_2O ha^{-1}. The rice was fertilized at the tillering stage and panicle initiation stage with 63 kg $N \cdot ha^{-1}$. The RS was only fertilized at the tillering stage and panicle initiation stage with 43 kg $N \cdot ha^{-1}$. In accordance with the prevailing local planting and cultivation practices, phosphorus fertilizer and potash were 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 complete. 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 June 2018	-	-	15 November 2018
	-	18 June 2019	-	-	26 November 2019
	-	24 June 2020	-	-	30 November 2020
	-	27 June 2021	-	-	20 November 2021
	-	22 June 2022	-	-	24 November 2022

Table 1. *Cont.*

Mode	Crayfish Fly Release Date	Rice Planting Date	Crayfish Catch Start Date	Crayfish Catch Finish Date	Rice Harvest Date
RS	29 May 2018	19 June 2018	9 October 2018	5 November 2018	15 November 2018
	28 May 2019	18 June 2019	6 October 2019	16 November 2019	26 November 2019
	18 May 2020	24 June 2020	1 October 2020	20 November 2020	30 November 2020
	23 May 2021	27 June 2021	5 October 2021	10 November 2021	20 November 2021
	20 May 2022	22 June 2022	3 October 2022	14 November 2022	24 November 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 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 using the methods of Lu (2000) [34]. DOC was measured using the method of Jiang et al. (2006) [35]. EOC was measured using the $KMnO_4$ oxidation procedure with 333 mmol/L [36]. MBC was determined using the chloroform fumigation–extraction method [37].

When the rice was ripe, it was harvested with a 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 pools (TOC, EOC, DOC, and MBC). Duncan's multiple comparisons was used to test the significance of differences between treatment means. Specifically, soil data were collected on a biannual basis, in early May (before crayfish fly release) and late November (after rice harvest) in each year. Subsequently, the data were employed to compare the differences between the RS and CK modes on soil nutrients and carbon pools in each year. Furthermore, the soil data obtained twice a year for different time periods of RC or CK are used to analyze the differences in annual soil nutrients and carbon pools. Pearson correlation analysis was performed on the annual soil nutrient, carbon pool, and yield data of the different treatments. The data were processed and tabulated with WPS 2010 software and plotted with Origin 2021 software.

3. Results

3.1. Effect of Rice–Crayfish Coculture System on Soil Nutrients

The content of AN in the 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 CK. The content of NN in RS in 2018, 2019, and 2022 was significantly lower ($p \leq 0.05$) than that of the 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.

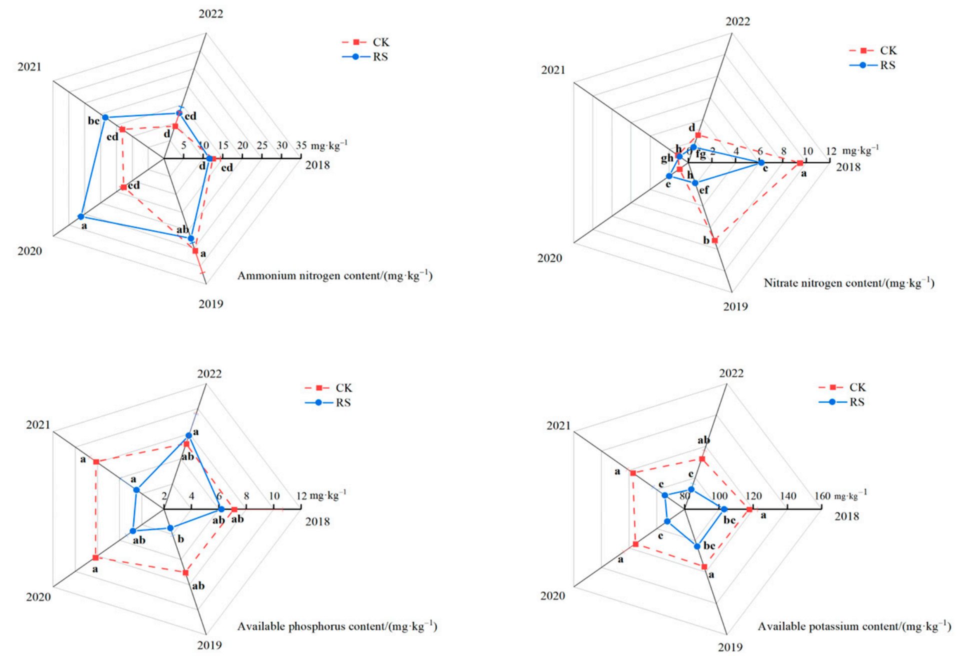

Figure 1. The annual variation characteristics of soil available nutrients under different rice cultivation modes from 2018 to 2022. (Note: CK: rice monoculture; RS: rice–crayfish coculture system. Different letters indicate significant differences at $p \leq 0.05$).

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

The content of DOC and MBC in the RS mode exhibited a decline from 2018 to 2022, compared with the CK mode (Figure 2). The content of DOC in the 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 the 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 the CK mode. The 5-year average contents of TOC, EOC, DOC, and MBC in the RS mode were decreased by 2.45%, 8.82%, 35.31%, and 65.84%, respectively, compared with the CK mode.

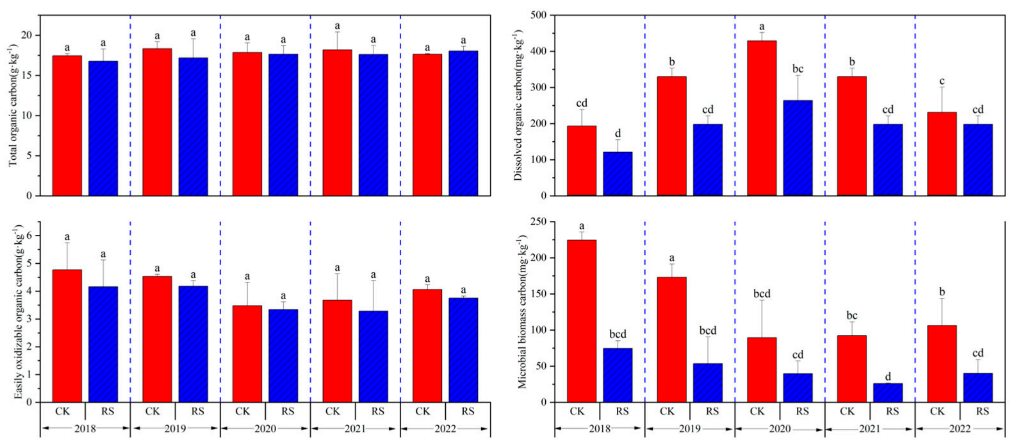

Figure 2. The annual variation characteristics of soil carbon pools under different rice cultivation modes from 2018 to 2022. (Note: CK: rice monoculture; RS: rice–crayfish coculture system. Different letters indicate significant differences at $p \leq 0.05$).

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.

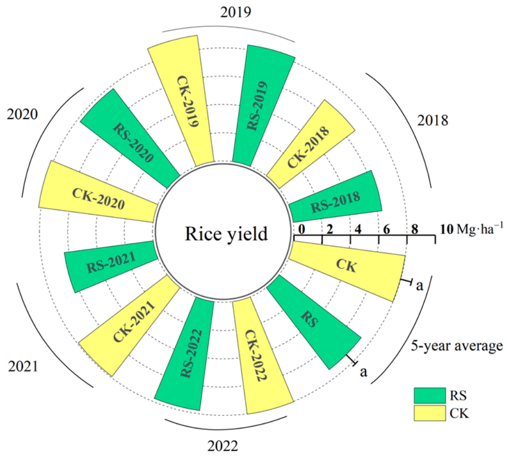

Figure 3. The annual variation characteristics of rice yield under different rice cultivation modes from 2018 to 2022. (Note: CK: rice monoculture; RS: rice–crayfish coculture system. Different letters indicate significant differences at $p \leq 0.05$).

3.4. Correlation Analysis

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

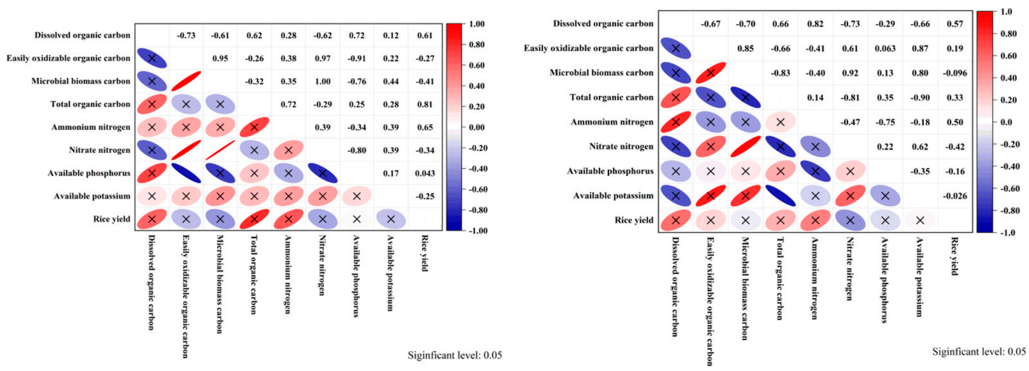

Figure 4. Correlation analysis of soil available nutrients, carbon pools, and rice yield in RS (**a**) and CK (**b**). × indicates not significantly correlated.

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 soil NN was reduced in the RS system. These results are in line with our first hypothesis, emphasizing that the soil AN content was accumulated in the RS mode due to the application of N fertilizer [38], artificial feed [39], and floodwater [40]. Moreover, the decomposition of dead crayfish may also incorporate nitrogen nutrients 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 the RS mode have shown that the increased soil pH under flooding resulted in the significant increase in Fe^{2+} and 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 the RS and CK systems, but the 5-year average content of AP in the RS mode is lower than in the CK mode (Figure 1). This might be explained by the non-application of phosphate fertilizer in the RS mode from 2018 to 2022, although the addition of organic matter (such as feed debris, rice straw, and organic fertilizer) could increase the proportion of stable phosphorus fractions in RS systems [22]. However, these forms are not readily available to rice, leading to a decrease in AP. Moreover, prolonged soil flooding can accelerate soil potential phosphorus losses [43]. Similar to soil AP, the non-application of potash fertilizer in RS systems resulted in significantly lower soil readily AK content in the RS mode than in the CK mode in different years [44].

4.2. Rice–Crayfish Coculture System Weakened Soil Carbon Pools

Soil carbon pools are 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 in soil carbon pools, which can reflect the small changes in 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 increases 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 the RS mode compared to the 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], and persistently high soil moisture promotes the reductive dissolution of soil iron oxides, which promotes microbial exposure to previously protected iron oxides. It also 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, and 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 the RS mode, which was 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 the RS mode, compared with the CK mode (Figure 2). The input of excessive carbon sources in the 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]. Moreover, flooding was the dominant driver of microbial C limitations [57]. Long-term integrated rice–crayfish coculture was significantly reduced microbial richness and diversity [17]. Some invertebrates, protists, and bacteria were reduced in the integrated rice–crayfish farming systems [58]. These findings support our second hypothesis and emphasize the important driver of flooding in the RS mode for soil MBC.

4.3. Rice–Crayfish Coculture System Reduced the Rice Yield

In previous studies, there was 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 the RS mode was 8.4% lower than that of the 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. 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 Mg·ha^{-1}), the average yield of conventional late-maturing varieties in RS systems is 7.95 Mg·ha^{-1}, which can achieve the target of a steady yield of 7.50 Mg·ha^{-1} of rice in the northern part of Zhejiang Province.

4.4. 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 the RS mode (Figure 4). These results are in accordance with previous observations, emphasizing the effects of long-term flooding on soil nutrients and carbon pools in rice–crayfish coculture fields. This is due to the fact that the 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 the 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 the RS mode. Consequently, the unique long-term anaerobic conditions and the input of production materials (such as fertilizers and crayfish feeds) under RS mode alter the soil nutrient status and 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 the RS mode in northern Zhejiang.

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References

1. Mi, S.X.; Tan, X.L.; Tan, J.Y.; Jiang, L.X.; Wang, Z.K. Analysis of influencing factors of rice planting area evolution in Dongting Lake Area during 1987–2017. *J. Nat. Resour.* **2020**, *35*, 2499–2510. [CrossRef]
2. Xu, C.C.; Ji, L.; Chen, Z.D.; Fang, F.P. Analysis of China's Rice Industry in 2022 and the Outlook for 2023. *China Rice* **2023**, *29*, 1–4.
3. Wang, Y.C.; Li, J.; Wang, X.D. Change Law and Trend of Grain Production in China Since the Founding of the People's Republic of China. *J. Agric. Sci. Technol.* **2021**, *23*, 1–11.
4. Cai, C.; Li, G.; Zhu, J.Q.; Peng, L.; Li, J.F.; Wu, Q.X. Effects of Rice-crawfish Rotation on Soil Physicochemical Properties in Jianghan Plain. *Acta Pedol. Sin.* **2019**, *56*, 217–226.
5. Che, Y.; Cheng, S.; Tian, J.Y.; Tao, Y.; Liu, Q.Y.; Xing, Z.P.; Dou, Z.; Xu, Q.; Hu, Y.J.; Guo, B.W.; et al. Characteristics and differences of rice yield, quality, and economic benefits under different modes of comprehensive planting-breeding in paddy fields. *Acta Agron. Sin.* **2021**, *47*, 1953–1965.
6. Yu, X.J.; Hao, X.J.; Dang, Z.Q.; Yang, L.K. Report on the Development of China's Integrated Rice and Fishery Breeding Industry. *Chin. Fish.* **2023**, *8*, 19–26.
7. Jiang, R.; Xu, Q.; Li, J.Y.; Dai, L.X.; Ao, D.C.; Dou, Z.; Gao, H. Sensitivity and uncertainty analysis of carbon footprint evaluation: A case study of rice-crayfish coculture in China. *Chin. J. Eco-Agric.* **2022**, *30*, 1577–1587.
8. Wang, R.; Zhu, J.; Jin, T.; Liu, Z.Y. Characteristics of ammonia oxidation microbial abundance and community structure in paddy soils of rice–crayfish symbiosis farming system. *J. Plant Nutr. Fertil.* **2019**, *25*, 1887–1899.
9. Chen, D.; Wei, W.; Chen, L.D.; Ma, B.J.; Li, H. Response of soil nutrients to terracing and environmental factors in the Loess Plateau of China. *Geogr. Sustain.* **2024**, *5*, 230–240. [CrossRef]
10. Huang, B.; Zhang, L.; Cao, Y.; Yang, Y.; Wang, P.; Li, Z.; Lin, Y. Effects of land-use type on soil organic carbon and carbon pool management index through arbuscular mycorrhizal fungi pathways. *Glob. Ecol. Conserv.* **2023**, *43*, e02432. [CrossRef]
11. Linsen, D.; Wang, H.; Zhang, Z.; Zhou, Y.Y.; Xiao, Z.Y.; Muhammad, S.; Avelino, N.D.; Sun, G.; Yu, Y.L.; Xiao, Z.H.; et al. Effects of long-term rice-crayfish farming on soil CNP storage and stoichiometry. *Soil Tillage Res.* **2024**, *235*, 105882.
12. Hou, J.; Zhang, D.Y.; Zhu, J.Q. Nutrient accumulation from excessive nutrient surplus caused by shifting from rice monoculture to rice-crayfish rotation. *Environ. Pollut.* **2021**, *271*, 116367. [CrossRef] [PubMed]
13. Si, G.; Peng, C.; Yuan, J.; Xu, X.; Zhao, S.; Xu, D.; Wu, J. Changes in soil microbial community composition and organic carbon fractions in an integrated rice-crayfish farming system in subtropical China. *Sci. Rep.* **2017**, *7*, 2856. [CrossRef]
14. Lv, T.; Wang, C.; Xu, Y.; Zhou, X.; Huang, F.; Yu, L. Impact of Integrated rice-crayfish farming on soil aggregates and organic matter distribution. *Agronomy* **2024**, *14*, 16. [CrossRef]
15. Bashir, M.A.; Liu, J.; Geng, Y.; Wang, H.; Pan, J.; Zhang, D.; Abdur, R.; Muhammad, A.; Liu, H. Co-culture of rice and aquatic animals: An integrated system to achieve production and environmental sustainability. *J. Clean. Prod.* **2020**, *249*, 119310. [CrossRef]
16. Wu, Y.Y.; Li, Y.; Niu, L.H.; Wang, L.F.; Zhang, H.J. Nutrient status of integrated rice-crayfish system impacts the microbial nitrogen-transformation processes in paddy fields and rice yields. *Sci. Total Environ.* **2022**, *836*, 155706. [CrossRef]
17. Zhang, C.M.; Mi, W.J.; Xu, Y.Z.; Zhou, W.Z.; Bi, Y.H. Long-term integrated rice-crayfish culture disrupts the microbial communities in paddy soil. *Aquac. Rep.* **2023**, *29*, 101515. [CrossRef]
18. Li, Q.; Xu, L.; Xu, L.; Qian, Y.; Jiao, Y.; Bi, Y.; Zhang, T.; Zhang, W.; Liu, Y. Influence of consecutive integrated rice-crayfish culture on phosphorus fertility of paddy soils. *Land Degrad. Dev.* **2018**, *29*, 3413–3422. [CrossRef]
19. Zhou, H.; Ge, T.; Li, H.; Fang, T.; Li, H.; Shi, W.; Zhang, R.; Dong, X. A multi-Medium analysis of human health risk of toxic elements in rice-crayfish system: A case study from Middle reach of Yangtze River, China. *Foods* **2022**, *11*, 1160. [CrossRef]
20. Zhang, Y.; Chen, M.; Zhao, Y.Y.; Zhang, A.Y.; Peng, D.H.; Lu, F.; Dai, C.C. Destruction of the soil microbial ecological environment caused by the over-utilization of the rice-crayfish co-cropping pattern. *Sci. Total Environ.* **2021**, *788*, 147794. [CrossRef]
21. Luo, W.; Zhou, Z.L.; Zhao, Y.L.; Yang, Z.B.; Zhang, M.F. Analysis on the Contents of Protein and Amino Acids in *Cherax quadricarinatus* during different embryonic development stage. *J. East China Norm. Univ. (Nat. Sci.)* **2004**, *1*, 88–92.

22. Jens, K.; Maximilian, K.; Chau, M.K.; Gianna, B.; Zita, S.; Wulf, A. Land use change from permanent rice to alternating rice-shrimp or permanent shrimp in the coastal Mekong Delta, Vietnam: Changes in the nutrient status and binding forms. *Sci. Total Environ.* **2020**, *703*, 134758.
23. Xu, R.; Yang, T.; Han, G.M.; Wu, L.M.; Zhang, J.H.; Kou, X.M.; Wang, S.H. Effect of rice-crayfish cultivation mode on the accumulation of soil reducing substances and nutrients. *Chin. J. Ecol.* **2022**, *41*, 925–932.
24. Chen, S.W.; Jiang, Y.; Wang, J.P.; Cao, C.G. Situation and countermeasures of integrated rice-crayfish farming in Hubei Province. *J. Huazhong Agric. Univ.* **2020**, *39*, 1–7.
25. Li, L.N.; Yan, L.L.; Cao, C.G.; Jiang, Y.; Liu, J.P. Effects of straw returning and crayfish feeding on rice growth and nutrient uptake in rice-crayfish ecosystem. *J. Huangzhong Agric. Univ.* **2020**, *39*, 8–16.
26. Chen, L.; Wan, W.T.; Liu, B.; Xu, W.J.; Gu, Z.M. Effects of rice-crayfish integrated system on microbial diversity and communitystructurein paddy water. *J. Huazhong Agric. Univ.* **2022**, *41*, 141–151.
27. Xu, X.Y.; Zhang, M.M.; Peng, C.L.; Si, G.H.; Zhou, J.X.; Xie, Y.Y.; Yuan, J.F. Effect of rice-crayfish co-culture on greenhouse gases emission in straw-puddled paddy fields. *Chin. J. Eco-Agric.* **2017**, *25*, 1591–1603.
28. Tao, X.F.; Li, B.; Yu, Z.X.; Hou, Y.R.; Wang, L.; Zhu, J. Effects of rice-crayfish integrated model on root exudates and microorganisms of rice during grain filling. *J. Fish. China* **2022**, *46*, 2122–2133.
29. He, J.; Zhang, X.Z.; Jiang, Z.J.; Jiang, Y.; Luo, S.H. Effects of rice-crayfish integrated mode on water environment. *Jiangsu Agric. Sci.* **2019**, *47*, 213–215.
30. Cao, C.G.; Jiang, Y.; Wang, J.P.; Yuan, P.L.; Chen, S.W. "Dual character" of rice-crayfish culture and strategies for its sustainable development. *Chin. J. Eco-Agric.* **2017**, *25*, 1245–1253.
31. Si, G.H.; Peng, C.L.; Xu, X.Y.; Xu, D.B.; Yuan, J.F.; Li, J.H. Effect of integrated rice-crayfish farming system on soil physico-chemical properties in waterlogged paddy soils. *Chin. J. Eco-Agric.* **2017**, *25*, 61–68.
32. Li, B.X.; Wang, B.J.; Huai, Y.; Shen, Y.Q.; Zhang, H.M.; Cheng, W.D. Effects of integrated rice-Redclaw Crayfish farming system on soil nutrients, carbon pool and rice quality. *Acta Agric. Zhejiangensis* **2021**, *33*, 688–696.
33. Zhang, Y.; Wu, X.B. Research on the impact of the "crayfish-rice cultivation" model on national food security: Based on asurvey in Qianjiang City, Hubei Province. *Hubei Agric. Sci.* **2021**, *60*, 201–204.
34. Lu, R.K. *The Analysis Method of Soil Agricultural Chemistry*; China Agricultural Science and Technology Press: Beijing, China, 2000; pp. 146–190.
35. Jiang, P.K.; Xu, Q.F.; Xu, Z.H.; Cao, Z.H. Seasonal changes in soil labile organic carbon pools within a Phyllostachyspraecox stand under high rate fertilization and winter mulch in subtropical China. *For. Ecol. Manag.* **2006**, *236*, 30–36. [CrossRef]
36. Blair, G.J.; Lefroy, R.D.B.; Lisle, L. Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. *Aust. J. Agric. Res.* **1995**, *46*, 1459–1466. [CrossRef]
37. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. Microbial biomass measurements in forest soils: The use of the chloroform fumigation-incubation method in strongly acid soils. *Soil. Biol. Biochem.* **1987**, *19*, 697–702. [CrossRef]
38. Nielsen, S.N.; Anastácio, P.M.; Frias, A.F.; Marques, J.C. CRISP-crayfish rice integrated system of production. 5. Simulation of nitrogen dynamics. *Ecol. Model.* **1999**, *123*, 41–52. [CrossRef]
39. Si, G.H.; Yuan, J.F.; Peng, C.L.; Xia, X.G.; Cheng, J.P.; Xu, X.Y.; Jia, P.A.; Xie, Y.Y.; Zhou, J.X. Nitrogen and phosphorus cycling characteristics and balance of the integrated rice-crayfish system. *Chin. J. Eco-Agric.* **2019**, *27*, 1309–1318.
40. Liu, X.X.; Zhang, H.H.; Zhu, Q.Y.; Ye, J.Y.; Zhu, Y.X.; Jing, X.T.; Du, Y.X.; Zhou, M.; Lin, X.Y.; Zheng, S.J.; et al. Phloem iron remodels root development in response to ammonium as the major nitrogen source. *Nat. Commun.* **2022**, *13*, 561. [CrossRef]
41. Wang, A.; Hao, X.L.; Chen, W.L.; Luo, X.S.; Huang, Q.Y. Rice-crayfish co-culture increases microbial necromass' contribution to the soil nitrogen pool. *Environ. Res.* **2023**, *216*, 114708. [CrossRef]
42. Wu, X.C.; Jiang, Q.Q.; Ma, T.; Wu, Y.X. Release risk of soil phosphorus under different farming systems: Indoor experiments and in-situ measurement. *Soil. Tillage Res.* **2024**, *240*, 106106. [CrossRef]
43. Antonio, R.S.R.; Paul, W.H.D.C.; Davey, L.J. typology of extreme flood events leads to differential impacts on soil functioning. *Soil. Biol. Biochem.* **2019**, *129*, 153–168.
44. Yu, Y.; Zhao, Y.T.; Chang, Q.Z. Spatial-temporal variability of soil readily available nutrients incultivated land of Weibei tableland area. *Acta Pedol. Sin.* **2015**, *52*, 1251–1261.
45. Ball, K.R.; Malik, A.A.; Muscarella, C.; Blankinship, J.C. Irrigation alters biogeochemical processes to increase both inorganic and organic carbon in arid-calcic cropland soils. *Soil Biol. Biochem.* **2023**, *187*, 109189. [CrossRef]
46. Hu, Q.Y.; Liu, T.Q.; Ding, H.N.; Li, C.F.; Yu, M.; Liu, J.; Cao, C.G. The effects of straw returning and nitrogen fertilizer application on soil labile organic carbon fractions and carbon pool management index in a rice-wheat rotation system. *Pedobiologia* **2023**, *101*, 150913. [CrossRef]
47. Zhang, Z.; Du, L.S.; Xiao, Z.Y.; Li, C.W.; Wang, Z.C.; Zhou, P.Y.; Sun, G.; Ye, Y.Y.; Hu, T.; Wang, H. Rice-crayfish farming increases soil organic carbon. *Agric. Ecosyst. Environ.* **2022**, *329*, 107857. [CrossRef]
48. Sun, G.; Sun, M.; Du, L.S.; Zhang, Z.; Wang, Z.C.; Zhang, G.B.; Nie, S.A.; Xu, H.Q.; Wang, H. Ecological rice-cropping systems mitigate global warming A meta-analysis. *Sci. Total Environ.* **2021**, *789*, 147900. [CrossRef]
49. Wei, L.; Ge, T.D.; Zhu, Z.K.; Luo, Y.; Yang, Y.H.; Xiao, M.Y.; Yan, Z.F.; Li, Y.H.; Wu, J.S.; Yokoa, K. Comparing carbon and nitrogen stocks in paddy and upland soils: Accumulation, stabilisation mechanisms, and environmental drivers. *Geoderma* **2021**, *398*, 115121. [CrossRef]

50. Fontaine, S.; Barot, S.; Barré, P.; Mary, B.; Rumpel, C. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* **2007**, *450*, 277–280. [CrossRef] [PubMed]
51. Chen, L.; Liu, L.; Qin, S.; Yang, G.B.; Fang, K.; Zhu, B.; Yakov, K.; Chen, P.D.; Xu, Y.P.; Yang, Y.H. Regulation of priming effect by soil organic matter stability over a broad geographic scale. *Nat. Commun.* **2019**, *10*, 5112. [CrossRef] [PubMed]
52. Dien, L.D.; Hiep, L.H.; Hao, N.V.; Sammut, J.; Burford, M.A. Comparing nutrient budgets in integrated rice-shrimp ponds and shrimp grow-out ponds. *Aquaculture* **2018**, *484*, 250–258. [CrossRef]
53. Si, G.H.; Yuan, J.F.; Xu, X.Y.; Zhao, S.J.; Peng, C.L.; Wu, J.S.; Zhou, Z.Q. Effects of an integrated rice-crayfish farming system on soil organic carbon, enzyme activity, and microbial diversity in waterlogged paddy soil. *Acta Ecol. Sin.* **2018**, *38*, 29–35. [CrossRef]
54. Cai, S.; Xu, S.; Zhang, D.; Geisen, S.; Zhu, H. Soil microbial biomass and bacterial diversity enhanced through fallow cover cropping in rice–fish coculture. *Agronomy* **2024**, *14*, 456. [CrossRef]
55. Li, J.H.; Zhang, R.; Cheng, B.H.; Ye, L.F.; Li, W.J.; Shi, X.M. Effects of nitrogen and phosphorus additions on decomposition and accumulation of soil organic carbon in alpine meadows on the Tibetan Plateau. *Land Degrad. Dev.* **2021**, *32*, 1467–1477. [CrossRef]
56. Lu, X.; Hou, E.; Guo, J.; Gilliam, F.S.; Li, J.; Tang, S.; Kuang, Y. Nitrogen addition stimulates soil aggregation and enhances carbon storage in terrestrial ecosystems of China: A meta-analysis. *Glob. Change Biol.* **2021**, *27*, 2780–2792. [CrossRef]
57. Huang, X.Y.; Li, Y.X.; Lin, H.Y.; Wen, X.T.; Liu, J.; Yuan, Z.F.; Fu, C.; Zheng, B.F.; Gong, L.Q.; Zhan, H.; et al. Flooding dominates soil microbial carbon and phosphorus limitations in Poyang Lake wetland, China. *Catena* **2023**, *232*, 107468. [CrossRef]
58. Feng, J.; Pan, R.; Hu, H.W.; Huang, Q.Y.; Zheng, J.L.; Tan, W.F.; Liu, Y.R.; Manuel, D. Effects of integrated rice-crayfish farming on soil biodiversity and functions. *Sci. Bull.* **2023**, *68*, 2311–2315. [CrossRef]
59. Chen, Y.L.; Yu, P.H.; Wang, L.; Chen, Y.Y.; Edwin, H.W.C. The impact of rice-crayfish field on socio-ecological system in traditional farming areas: Implications for sustainable agricultural landscape transformation. *J. Clean. Prod.* **2024**, *434*, 139625. [CrossRef]
60. Liu, T.Q.; Li, C.F.; Tan, W.F.; Wang, J.P.; Feng, J.H.; Hu, Q.Y.; Cao, C.G. Rice-crayfish co-culture reduces ammonia volatilization and increases rice nitrogen uptake in central China. *Agric. Ecosyst. Environ.* **2022**, *330*, 107869. [CrossRef]
61. Xu, Q.; Dai, L.X.; Zhou, Y.; Dou, Z.; Gao, W.Y.; Yuan, X.C.; Gao, H.; Zhang, H.C. Effect of nitrogen application on greenhouse gas emissions and nitrogen uptake by plants in integrated rice-crayfish farming. *Sci. Total Environ.* **2023**, *905*, 167629. [CrossRef] [PubMed]
62. Guilherme, M.T.; Luan, C.; Paula, S.A.; Estéfani, S.; Carlos, H.P.M.; Catarine, M.; Aldo, M.J. Variability to flooding tolerance in barnyardgrass and early flooding benefits on weed management and rice grain yield. *Field Crops Res.* **2023**, *300*, 108999.
63. Wang, X.; Jing, Z.H.; He, C.; Liu, Q.Y.; Jia, H.; Qi, J.Y.; Zhang, H.L. Breeding rice varieties provides an effective approach to improve productivity and yield sensitivity to climate resources. *Eur. J. Agron.* **2021**, *124*, 126239. [CrossRef]
64. Taylor, P.G.; Townsend, A.R. Stoichiometric control of organic carbon-nitrate relationships from soils to the sea. *Nature* **2010**, *464*, 1178–1181. [CrossRef]
65. Zhao, X.; Xu, C.M.; Wang, D.Y.; Chen, S.; Ji, C.L.; Chen, L.P.; Zhang, X.F. Effect of rhizosphere dissolved oxygen on nitrogen utilisation of rice. *Chin. Rice Sci.* **2013**, *27*, 647–652.
66. Chen, X.B.; Hu, Y.J.; Xia, Y.H.; Zheng, S.M.; Ma, C.; Rui, Y.C.; He, H.B.; Huang, D.Y.; Zhang, Z.H.; Ge, T.D.; et al. Contrasting pathways of carbon sequestration in paddy and upland soils. *Glob. Change Biol.* **2021**, *27*, 2478–2490. [CrossRef]