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Response of Rice Grain Yield and Soil Fertility to Fertilization Management under Three Rice-Based Cropping Systems in Reclaimed Soil

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Abstract: Reasonable cropping systems and fertilizer management are vital for improving the quality of barren soil. The effectiveness of different crop rotation methods and fertilizers in soil improvement depends on various factors, including soil type, climate conditions, and crop type. In the present study, based on three rice-based cropping systems, the effects of organic fertilizers combined with slow-release fertilizers on rice yield and soil fertility in reclaimed soil were analyzed. The results showed that the rice grain yield was highest under the rice-fallow rotation system (RF) with the application of rapeseed meal fertilizer. Available nutrients such as AN, N-NH₄⁺, TP, and AK showed a significant positive correlation with rice grain yield ($p < 0.05$). PCA and PERMANOVA analysis supported significant variation in CAZyme abundance among cropping systems ($R^2 = 0.60$, $p = 0.001$) and significant differences between slow-release fertilizer treatments and organic fertilizer treatments ($p < 0.05$), but not among the three organic fertilizer treatments. Network analysis indicated positive stronger correlations among all functional enzymes in organic fertilizer treatments compared to chemical fertilizer treatments. RDA and correlation heat map results showed that C/N ratios and N-NH₄⁺ were strongly related to CAZyme composition. PLS-PM analysis revealed that soil available nitrogen positively influenced several variables, while rice grain yield was negatively influenced by soil enzymes and TOC. These findings suggested that under appropriate cropping systems, partially substituting chemical fertilizers with organic fertilizers can effectively enhance the availability of nutrients in the soil, alter the activity of carbon-cycling microorganisms, and increase rice grain yield.

Keywords: reclaimed soil; cropping system; fertilization management; rice grain yield; microbial community; CAZyme



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1. Introduction

Effective fertilization management plays a critical role in sustainable agriculture by optimizing nutrient use efficiency, reducing environmental impacts, and ensuring long-term soil fertility [1]. In developed countries, various measures have been taken in fertilizer management to ensure that agricultural production is sustainable and environmentally friendly [2]. In contrast, although fertilizer management in the developing world is increasingly focused on sustainable agriculture and environmental protection, there are still some problems, such as traditional agricultural methods dealing with organic green farming [3]. Therefore, a comprehensive understanding of crop nutrient requirements, soil conditions, and careful monitoring and adjustment are required to meet the specific needs of the crops and maintain a balanced nutrient supply.

Fertilizer management includes soil testing and nitrogen assessment, making fertilization plans according to the growth stage of crops and nitrogen supply capacity, controlling fertilizer application amount, using slow-release fertilizers, combining organic and

green fertilizers, rational irrigation management, monitoring and adjusting fertilization strategies [4]. Controlled release fertilizer (CRF) or slow-release fertilizer products have tremendous potential and prospects in improving fertilizer application efficiency and mitigating environmental problems caused by poor fertilizer management [5]. Research has shown that in a one-year field experiment, the usage of controlled release nitrogen fertilizer (CRNF) resulted in a nitrogen use efficiency improvement as high as 43.96% compared to conventional urea fertilizer [6]. Alternatively, organic fertilizers as a route to controlled release of nutrients play an increasingly important role in fertilizer management [7]. The use of organic fertilizers helps improve soil fertility, maintain nutrient availability, increase soil biodiversity and crop yield, and reduce the risk of environmental pollution [8,9]. Organic fertilizers supply large amounts of inorganic N in both forms (i.e., NH_4^+ and NO_3^-) and labile C compounds, which increase the activity of heterotrophic denitrifying microorganisms and trigger the carbon and nitrogen cycles [10,11]. Organic fertilizer substitution significantly changed the soil bacterial community structure and increased the relative abundance of beneficial bacteria, such as *Bacilli* and *Flavobacteriales* [12]. Soil organic carbon and soil microbial biomass nitrogen were the most important contributors to the variance in microbial community composition, especially for non-dominant bacteria (*Gemmatimonadetes* and *Latescibacteria*) [13].

Organic fertilizer coupled with inorganic fertilizer is an approach for the sustainable production of crops [14–16]. However, some disadvantages should be considered. For example, when the basic agrochemical principles are not respected in the developing world, a high nitrogen content in chicken manure can lead to nitrogen overloading, causing negative environmental impacts. Rapeseed meal has a relatively high carbon-to-nitrogen (C/N) ratio, which means that more carbon compared to nitrogen can result in the immobilization of nitrogen in the soil [17,18]. Microorganisms in the soil require nitrogen to break down the carbon, which can temporarily reduce the availability of nitrogen for plant uptake, potentially leading to nitrogen deficiency in crops [19–21]. The nutrient content of vermicompost can vary depending on the feedstock materials used and the composting process [22]. This variability can make it challenging to accurately predict and control the nutrient levels, which may result in inconsistent nutrient availability for plants [23]. Thus, it's important to note that the type and quality of organic matter, as well as the application rates, should be carefully considered to ensure optimal results. The appropriate addition of organic matter should be determined based on the specific soil conditions, crop requirements, and environmental considerations [24].

Cropping systems have a significant impact on fertilization management in agriculture [25]. Different cropping systems have varying nutrient requirements, nutrient cycling processes, and interactions with the soil and environment. Monoculture offers simplified management and high crop uniformity, but reduces biodiversity and increases dependence on chemicals [26]. Crop rotation improves soil fertility and reduces pests, but requires careful planning [27]. Intercropping efficiently uses resources and enhances soil health, but involves complex management [28]. Farmers choose systems based on goals and local conditions to achieve sustainable agriculture.

Reclaimed soil, which refers to soil that has undergone restoration and improvement processes, plays a crucial role in revitalizing degraded landscapes and ensuring the sustainable use of land resources [29]. In this study, we aimed to investigate the effects of different organic fertilizers, including chicken manure, vermicompost derived from cow dung, and rapeseed meal fertilizer, on rice grain yield and soil fertility in reclaimed soils. Specifically, we focused on three rice-based cropping systems: rice-wheat rotation, rice-green manure rotation, and rice-fallow rotation. It is hypothesized that in a rice green manure rotation system, the ample application of organic fertilizers would result in the highest soil fertility and rice grain yield and increased microbial activity associated with carbon [30]. Additionally, we proposed that using organic fertilizers with lower carbon-to-nitrogen ratios would further enhance these effects. To test this hypothesis, we performed an analysis comparing different treatments, examining the physicochemical properties of

the soil, enzyme activity, and microbial factors related to carbohydrate-active enzymes (CAZymes). CAZymes encompass a variety of enzymes involved in carbohydrate synthesis and degradation, and their presence could potentially reflect the carbon cycling potential of the microbial community [31]. This research holds significant importance as it has the potential to provide valuable scientific insights and practical guidance for improving soil quality, enhancing crop production, and promoting sustainable agricultural practices in infertile or reclaimed soils.

2. Materials and Methods

2.1. Experimental Site

The experimental site is located at the northern edge of China's subtropical zone, in the Yangzhou Gongdao rice planting area (E 119°19'10", N 32°34'13"). The region has an average annual temperature of 16.6 °C, an average annual precipitation of 1600 mm, an average annual sunshine duration of 1902 h, and an average frost-free period of 223 days [32]. Before 2012, the area was a rural residential site, and in 2012, it was reclaimed as a paddy field. Subsequently, research on soil fertility improvement using a combination of organic and inorganic fertilizers began. The basic physicochemical properties of the soil before the experiment were as follows: pH 6.58, total organic carbon (TOC) 6.86 g kg⁻¹, total nitrogen content (TN) 0.42 g kg⁻¹, available phosphorus content (AP) 11.93 mg kg⁻¹, and available potassium content (AK) 191 mg kg⁻¹.

2.2. Experimental Materials

The rice variety used is Nanjing 3908, a late-maturing medium japonica rice commonly used in the local area. The wheat (Zhenmai 18) and green manure (*Astragalus sinicus*) are also used. The tested fertilizers were slow-release fertilizers of ternary compound fertilizer (26-10-15, 90 days; 15-15-15, 90 days) and binary compound fertilizer (30-0-5, 90 days), commercial organic manure fertilizers derived from chicken (average C 16.6%, N 1.6%, P 1.2%, K 0.7%), vermicompost (C, 14.5%, N 2.0%, P 1.7%, K 1.0%) derived from cow dung and rapeseed meal fertilizer (C 38.1%, N 4.2%, P 2.3%, K 1.6%), respectively (Table S1). Slow-release fertilizers added with urea formaldehyde, nitrification inhibitor (2-chloro, 6-trichloromethyl pyridine), and urease inhibitor (N-butyl thiophosphoryl triamine) were from Jiangsu Huachang Chemical Co., Ltd. (Zhangjiagang, China). Commercial organic fertilizers were from Jiangsu Dili Organic Fertilizer Technology Co., Ltd. (Yangzhou, China).

2.3. Cropping System

The area has three rice-based cropping systems: rice-wheat rotation (RW), rice-green manure rotation (RG), and rice-fallow rotation (RF). Rice is planted in late June every year, transplanting seedlings at the age of 3.5–4.0 leaves using a rice transplanter, with a row spacing of 30 cm × 12.5 cm, and 267,000 holes per hectare. In the RW system, wheat was planted in late October each year with 270 seedlings per hectare. In the RG system, a seeding density of 120 kg per hectare for *Astragalus sinicus* was planted after the rice harvest each year. In the RF system, after the rice is harvested, the land is left uncultivated until June of the following year.

2.4. Fertilization Management

Slow-release fertilizer combined with organic fertilizer was used to conduct the experiment; traditional fertilization management was 300 kg ha⁻¹ N, 75 kg ha⁻¹ P, and 150 kg ha⁻¹ K, with the total dose of N in all fertilized variants, regardless of whether it is in mineral or organic form. In each cropping system, five treatments with three replications in a randomized block design were performed in this experiment. The treatments were CK1 (no N input), CK2 (pure slow-release fertilizer), Chicken (60% slow-release fertilizer + 40% chicken manure fertilizer N), Vermicompost (60% slow-release fertilizer + 40% Vermicompost N), and Rapeseed (60% slow-release fertilizer + 40% rapeseed meal fertilizer N). The area of each replicated plot was 36 m² (4.5 m × 8 m). The same N fertilizer application

amount was adopted in the treatments, except CK1. The detailed treatments were shown in Table 1. The experiment was conducted in the same plot for two consecutive years. Rice was harvested on 26 October 2020.

Table 1. Fertilization management of different treatments in each cropping system. The dose for N, P, and K were the total dose of N, P, K in all fertilized variants, regardless of whether they were in mineral or organic form.

Treatment	N (kg ha ⁻¹)		P (kg ha ⁻¹)		K (kg ha ⁻¹)		Total (kg ha ⁻¹)		
	Slow-Release Fertilizer	Organic Fertilizer	Slow-Release Fertilizer	Organic Fertilizer	Slow-Release Fertilizer	Organic Fertilizer	N	P	K
CK1	0	0	75	0	150	0	0	75	150
CK2	300	0	75	0	150	0	300	75	150
Chicken	180	120	0	90	97	53	300	90	150
Vermicompost	180	120	0	102	90	60	300	102	150
Rapeseed	180	120	9	66	104	46	300	75	150

Note: CK1, No N input; CK2, pure slow-release fertilizer N; Chicken, 60% slow-release fertilizer N + 40% Chicken manure fertilizer N; 60% slow-release fertilizer N + 40% Vermicompost N, 60% slow-release fertilizer N + 40% Rapeseed meal N.

2.5. Plant and Soil Analysis

At the maturity stage, twenty-eight rice plants from each plot were randomly chosen to calculate the number of panicles per hole. Additionally, three representative rice plants were randomly selected to determine the number of spikelets per panicle, the percentage of filled grains, and the 1000-grain weight (g). The rice grain yield was computed using the following formula:

$$\text{rice grain yield (t ha}^{-1}\text{)} = \text{panicle number per hole} \times 267,000 \text{ holes per ha}^{-1} \times \text{spikelets number per panicle} \times \text{filling grain percentage} \times \text{thousand grain weight}/10^{11}$$

Rice grain yield was calculated based on grain dry matter.

After the rice harvest, soil samples were collected from the 0–20 cm layer of each plot using a five-point sampling method. These samples were combined, creating a composite sample with impurities removed. A portion of the fresh soil was passed through a 2 mm nylon sieve and sent to Suzhou Keming Biotechnology Co., Ltd. (Suzhou, China). for soil enzyme activity analysis. The remaining soil was taken to the lab for drying and grinding. Soil samples for total nutrient content testing were screened with 100-mesh sieves, while those for available nutrient content testing were screened with 20-mesh sieves.

Soil total carbon (TC) and total nitrogen (TN) were measured using an element analyzer (Vario EL cube, Elementar, Germany). Soil organic carbon (TOC) was measured with a modified Walkley-Black Wet Oxidation Method [33]. Soil total phosphorus (TP) was determined by alkaline fusion-molybdenum antimony spectrophotometry. Alkali-hydrolyzable nitrogen (AN) was determined using the alkaline N-proliferation method [34]. Soil samples were extracted with 1 mol L⁻¹ KCl to measure two kinds of available nitrogen, i.e., ammoniacal nitrogen (N-NH₄⁺) and nitrate nitrogen (N-NO₃⁻) content. N-NH₄⁺ in the soil was analyzed using the semi-micro Kjeldahl method [35], while N-NO₃⁻ was measured using ultraviolet spectrophotometry. Available phosphorus (AP) was extracted with 0.5 mol L⁻¹ NaHCO₃ and determined by the molybdenum blue colorimetric method [36]. Available potassium (AK) was extracted using 1 mol L⁻¹ NH₄OAc and determined by flame photometry [37]. Cation exchange capacity (CEC) was extracted with 1 mol L⁻¹ NH₄OAc and determined by hydrochloric acid titration [38].

Soil sucrase (S_SC), nitrate reductase (S_NR), catalase (S_CAT), cellulase (S_EC), and alkaline phosphatase (S_AKP) enzyme activities were measured using corresponding reagent kits [39,40]. In brief, soil sucrase was determined by the 3,5-dinitrosalicylic acid colorimetric method using a Solid-Sucrase Assay Kit. Soil nitrate reductase activity was measured with α -naphthylamine-sulfanilic acid using NaNO₂ as a substrate and incubating the soil slurry anaerobically for 24 h at 30 °C in a gyratory shaker (180 rpm) [41,42]. Soil

catalase was determined by measuring the change in absorbance at 240 nm after the reaction of H₂O₂ with soil using a Solid-Catalase Assay Kit. Alkaline phosphatase was determined using sodium phenolphthalein phosphate as a substrate; after incubation at 37 °C for 24 h, the liberated phenol was measured.

Rhizosphere soil metagenomes were sequenced with high-throughput sequencing technology based on the Illumina platform. The experimental procedure was carried out according to the standard protocol provided by Illumina, including sample quality inspection, library construction, library quality inspection, and library sequencing. Clean reads were assembled, and coding genes were predicted. Finally, a non-redundant gene set was constructed. Functional annotations for the non-redundant gene set were performed using both general and specialized databases. Taxonomic analysis was conducted to determine the species composition and abundance information of the samples. The analysis CAZyme (Carbohydrate-active enzymes) was performed with the help of Majorbio (<https://cloud.majorbio.com> (accessed on 1 March 2022)), including alpha diversity, beta diversity, functional comparison, environmental factor correlation analysis, and correlation network analysis.

CAZyme (Carbohydrate-Active enZYme) including glycoside hydrolases (GHs), polysaccharide lyases (PLs), glycosyltransferases (GTs), carbohydrate esterases (CEs), auxiliary activities (AAs) dependent on redox cofactors, and carbohydrate-binding modules (CBMs) were annotated [43]. The principal component analysis (PCA) and PERMANOVA (Permutational Multivariate Analysis Of Variance) were used to explore the differences among treatments. LEfSe (Linear discriminant analysis Effect Size) was a software for discovering high-dimensional biomarkers and revealing functional features. LEfSe used a non-parametric factorial Kruskal-Wallis sum-rank test to detect whether there were significant abundance differences in functional enzymes between slow-release fertilizer treatments and three organic fertilizer treatments. Linear discriminant analysis (LDA = 2) was used to estimate the size of the effect of the abundance of each functional enzyme on the differences. By calculating the correlation coefficient between functions, the function correlation network was constructed to analyze the function correlation.

Redundancy analysis (RDA) was used to reveal the explanation of environmental factors to abundance variation among all treatments. Before RDA analysis, VIF (variance inflation factor) was used to quantify the impact of multicollinearity between all variables on linear regression models. Variables with VIF < 10 were used to perform RDA analysis. Finally, Partial Least Squares Path Modeling (PLS-PM) was used to analyze complex relationships between latent variables [44]. GOF (goodness of fit) was an indicator used to assess the goodness of fit of a model, ranging from 0 to 1; the closer it is to 1, the better the model fits the data.

2.6. Statistical Analysis

The data were analyzed using Excel software and SPSS Statistics 23. The normal distribution of the data was assessed using the Shapiro–Wilk test and transformed accordingly. One-way analysis of variance (ANOVA) was used to analyze the data, and Duncan analysis was performed to determine the significance of differences between treatments. PLS-PM was performed in R version 4.1.0 with package “plsppm Version 0.5.0” [45].

3. Results

3.1. Rice Grain Field and Soil Physic-Chemical Properties

Among the three cropping systems, the rice grain yield is the highest under the rice-fallow rotation system (Figure 1). Compared to RW and RG, the five treatments increased by 28.6%, 26.7%, 37.1%, 47.1%, 53.6%, and 38.2%, 31.6%, 32.2%, 35.8%, 46.7%, respectively. In the RW system, compared to CK2, the combined application of organic and slow-release fertilizers did not significantly increase rice grain yield; in the RG system, compared to CK2, the combined application of organic and slow-release fertilizers increased rice grain yield, with Chicken, Vermicompost, and Rapeseed increasing by 4.0%, 14.0%, and 10.9%,

respectively, but the effects were not significant; in the RF system, compared to CK2, the combined application of organic and slow-release fertilizers increased rice grain yield, with Chicken, Vermicompost, and Rapeseed increasing by 4.9%, 21.4%, and 42.0%, respectively, and the application of Rapeseed meal fertilizer having the most significant effect ($p < 0.05$).

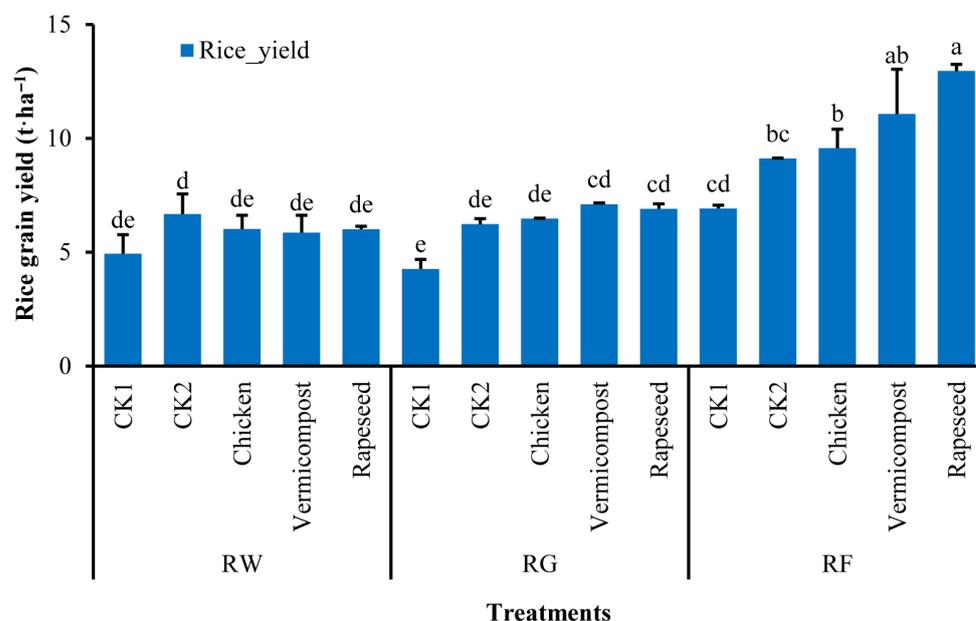


Figure 1. Effect of fertilization management on average rice grain yield under three rice-based cropping systems. RW, rice-wheat rotation; RG, rice-green manure rotation; RF, rice-fallow rotation; CK1, No N fertilizer; CK2, 100% slow-release fertilizer N; Chicken, 40% Chicken manure N + 60% slow-release fertilizer N; Vermicompost, 40% Vermicompost N + 60% slow-release fertilizer N; Rapeseed, 40% Rapeseed meal N + 60% slow-release fertilizer N. Error bar represents standard deviation. The histograms with the same letter at each treatment are not significantly different by the Duncan's significant difference test ($p < 0.05$).

All soil chemical properties and enzymes were used to reveal the relationship between rice grain yield and soil fertility; however (Table S2, Figure S1), only four properties were significantly correlated to rice grain yield, while they all exhibited a certain degree of autocorrelation. Rice grain yield was positively correlated with the contents of soil AN ($R = 0.479$, $p < 0.01$), $N_{-}NH_4^+$ ($R = 0.697$, $p < 0.001$), TP ($R = 0.502$, $p < 0.01$), and AK ($R = 0.726$, $p < 0.001$).

3.2. Functional Composition of Carbohydrate-Active Enzymes

In all treatments, a total of six classes and 541 CAZyme gene families were annotated, with each treatment varying between 479 and 503 (Figure 2). The following analysis results are based on families. In the RW and RF systems, the Chicken treatment had the most specific CAZymes, with four and seven, respectively; in the RG system, Vermicompost had the most specific enzymes, with four, followed by Rapeseed treatment, with three. In the three systems, the top 10 enzyme categories in abundance were the same, but there were slight differences in abundance, including GT41, GT4, GT2_Glycos_transf_2, CE1, CE10, GT83, GH74, PL22, CE9, and AA3.

PERMANOVA further supported that CAZyme composition varied significantly among cropping systems ($R^2 = 0.60, p = 0.001$) (Table 2). In each cropping system, CAZyme abundance varied significantly between slow-release fertilizer treatments and three organic fertilizers treatments (RW: $R^2 = 0.50, p = 0.014$; RG: $R^2 = 0.73, p = 0.007$; RF: $R^2 = 0.72, p = 0.004$), but not significantly among three organic fertilizer treatments (RW: $R^2 = 0.19, p = 0.54$; RG: $R^2 = 0.47, p = 0.13$; RF: $R^2 = 0.16, p = 0.72$).

Table 2. PERMANOVA analysis of CAZyme on functional family level in soil microbial community. C-O, among slow-release fertilizer and organic fertilizer in each cropping system; O-O, among organic fertilizers in each cropping system.

Characteristics	Sums of Sq	Mean Sq	F_Model	R ²	p Value
Cropping systems	0.18	0.09	20.48	0.60	0.001 ***
RW (C-O)	0.02	0.02	7.91	0.50	0.014 *
RW (O-O)	0.01	0.00	0.70	0.19	0.54
RG (C-O)	0.04	0.04	21.41	0.73	0.007 **
RG (O-O)	0.02	0.01	2.67	0.47	0.13
RF (C-O)	0.02	0.02	20.17	0.72	0.004 **
RF (O-O)	0.00	0.00	0.58	0.16	0.72

Note: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.

The LefSe analysis shows that there were significant differences in the differentiated species under the three cropping systems (Figure 4). In the RW, the difference in functional enzymes in the organic fertilizer treatments was GT41, CE9, and PL22, while the difference in functional enzymes in the slow-release fertilizer treatments was GH2 and GH94. In the RG and RF, the difference in functional enzymes in the organic fertilizer treatments were more like each other, e.g., GT87, GT89, GH42, CBM50, CBM66.

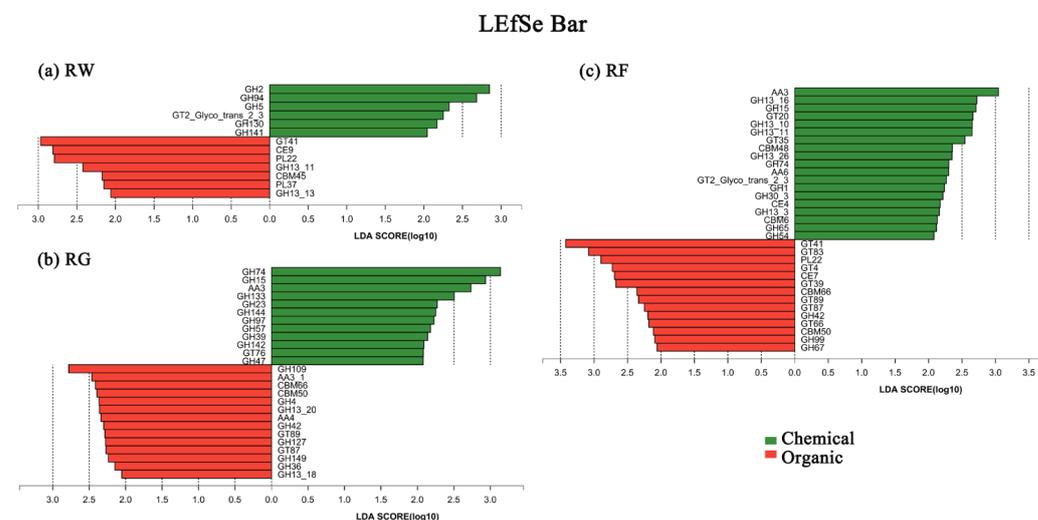


Figure 4. LefSe analysis among slow-release fertilizer treatments (Chemical) and organic fertilizer treatments (Organic). (a) RW: rice-wheat rotation, (b) RG: rice-green manure rotation, and (c) RF: rice-fallow rotation.

Network analysis suggested that all functional enzymes were positively related, but exhibited varied correlation connections (Figure S2). In the three cropping systems, the thickness of the lines indicates that the functional interactions among the top 10 abundant functional enzymes vary greatly, and the relationships between various functional enzymes in the organic fertilizer treatments were significantly stronger than those in the chemical fertilizer treatment group. For example, the number of co-working lines of AA3 and PL22 in each treatment is inconsistent, and the intensity differences were also significant. In the RF, only eight functional enzymes in the chemical fertilizer group have interactive

relationships, while nine functional enzymes in the organic fertilizer group have interactive relationships.

RDA results indicated that the first two axes contribute 58.60% and 0.66%, respectively, with the variables C/N, N_NH₄⁺, and TOC making significant large contributions to the composition of functional enzymes between different treatments (*p* < 0.05) (Figure 5). C/N and TOC were negatively related to CAZymes composition, while N_NH₄⁺ was negatively related to CAZymes diversity.

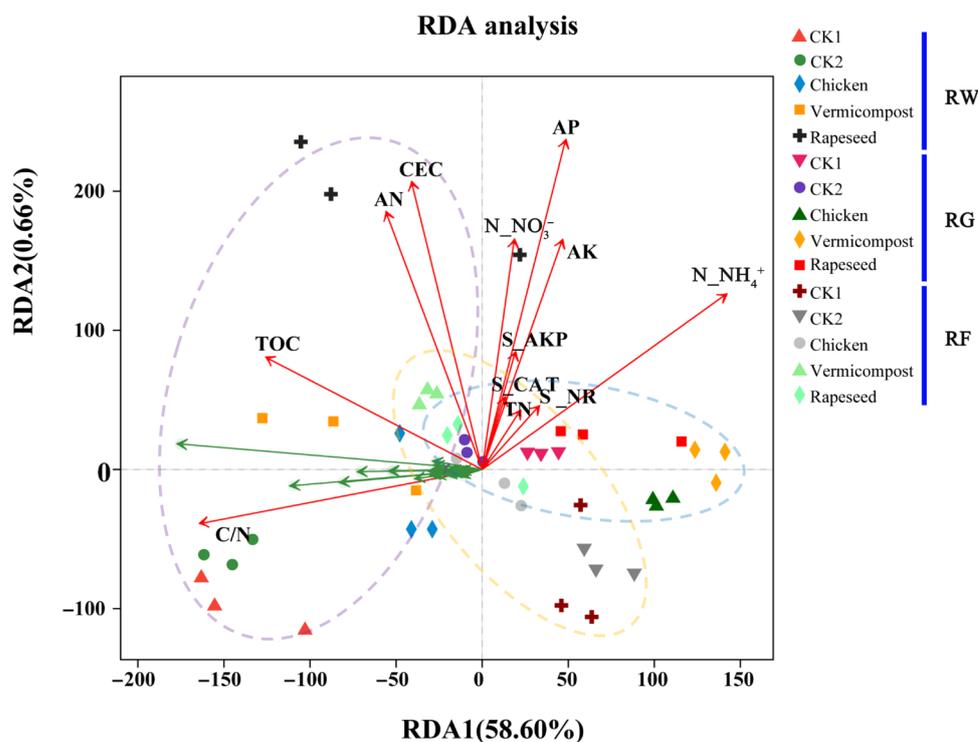


Figure 5. RDA analysis with variables VIF < 10 and CAZyme in family level. Only significant properties are presented. TOC, total organic carbon; TN, total nitrogen; AN, Alkali-hydrolyzable N; N_NH₄⁺, ammoniacal N; N_NO₃⁻, nitrate nitrogen; C/N, carbon to nitrogen ratio; AK; available K; CEC, cation exchange capacity; S_AKP, alkaline phosphatase; S_CAT, catalase; S_NR, nitrate reductase; the same below.

The correlation heat map further indicated that C/N, TOC, and S_EC were all positively correlated with the majority of dominant abundance functional enzymes, while N_NH₄⁺ was negatively correlated with the majority of dominant abundance functional enzymes. As for dominant abundance functional enzymes, GH15 was negatively correlated with the majority of soil properties, soil enzyme, and rice grain yield (Figure 6).

Partial Least Squares Path Modeling (PLS-PM) indicated that the goodness of fit was 0.517, suggesting that the model had a relatively good fit (Figure 7). The explained variances of Soil_enzyme, Soil_C, and C_microbe were 43.1%, 59.8%, 22.6%, and 35.4%, respectively. Soil_N was positively correlated with Soil_enzyme (R = 0.671), Soil_C (R = 0.774), and Rice_yield (R = 0.775), but negatively correlated with C_microbe (R = -0.685). Soil_C had a strong positive correlation with C_microbe (R = 0.619), but weak negative correlations with Soil_enzyme (R = -0.018) and Rice_yield (R = -0.012). Rice_yield was negatively correlated with both Soil_enzyme (R = -0.438) and C_microbe (R = -0.073).

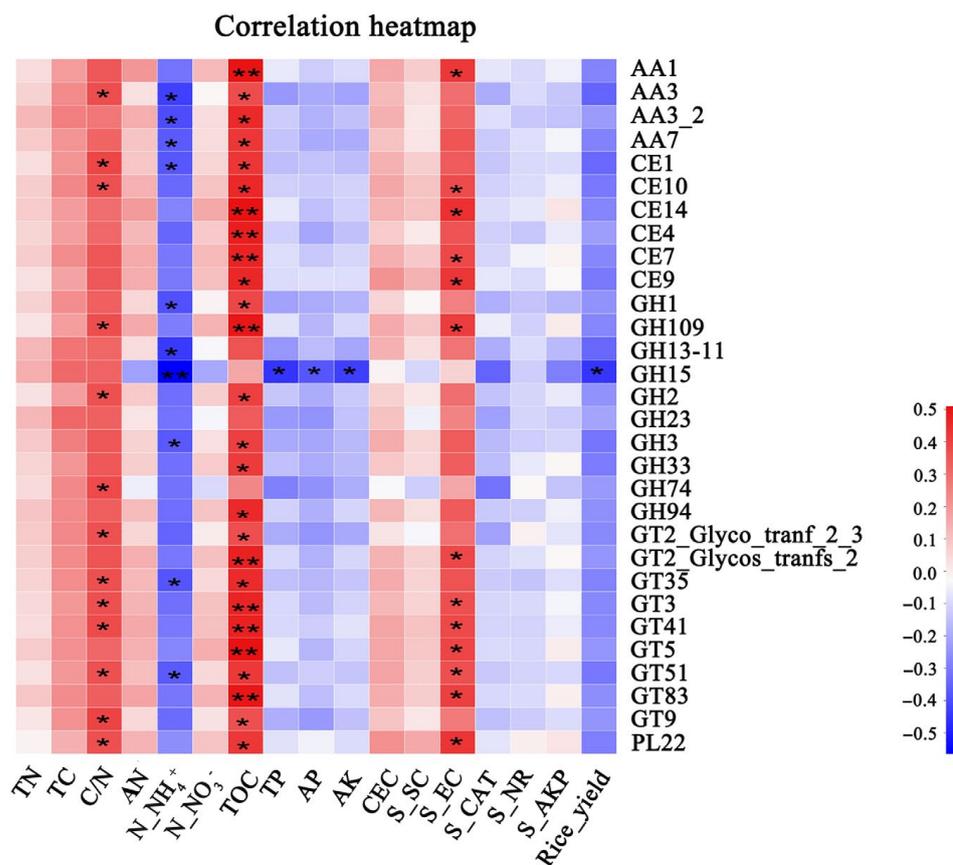


Figure 6. Correlation heatmap with all treatments on family level. TP, total P; S_SC, sucrose, S_EC, cellulase; Rice_yield, rice grain yield. * $p < 0.05$, ** $p < 0.01$.

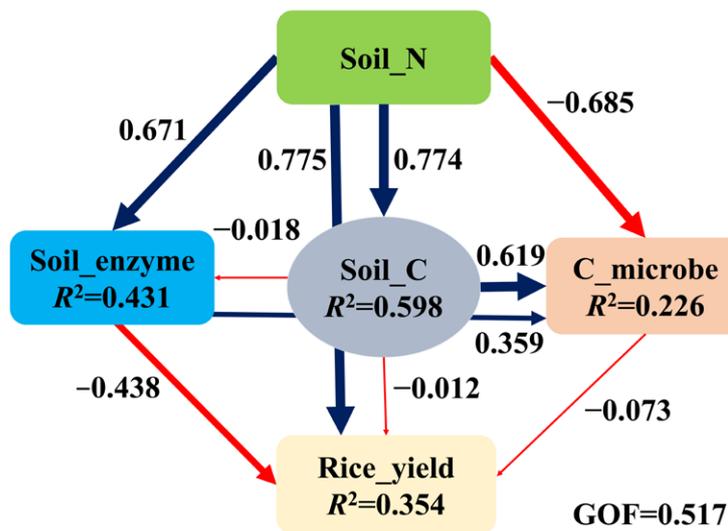


Figure 7. Partial Least Squares Path Modeling based on rice grain yield and Carbohydrate-active enzymes. Path coefficients: These represent the strength of the relationship between two latent variables. A positive value beside a blue arrow indicates a positive correlation, while a negative value beside a red arrow indicates a negative correlation. The closer the value is to ± 1 , the stronger the relationship, and the bolder the arrow. The R^2 value represents the proportion of explained variance in the dependent variable; the closer it is to 1, the higher the degree of explanation. Soil_N: available nitrogen in soil, Soil_C: organic carbon in soil, C_microbe: carbohydrate-active enzymes.

4. Discussion

4.1. Effects of Cropping Systems and Organic Fertilizers on Soil Properties and Rice Grain Yield

Numerous studies have indicated the benefits of green manure rotation in enhancing soil fertility and increasing crop yields [30,46]. However, in this study, the rice grain yield was found to be highest in the rice-fallow rotation system, which contradicts our hypothesis regarding the highest yield in the rice-green manure rotation system. Research indicated that the advantages of rice-fallow rotation include soil nutrient restoration, pest and disease control, water resource management, soil conservation and improvement, as well as economic benefits [47]. During the fallow period, the soil in rice fields can accumulate and restore nutrients effectively for the next rice planting season. The fallow period provides an opportunity for soil improvement measures such as organic matter addition and tillage, which contribute to enhancing and preserving soil structure. Proper rice-fallow rotation can increase rice grain yield and quality, thereby increasing farmers' income [48]. These advantages were not present in the other two rotation systems.

Among the three types of organic fertilizers, vermicompost and rapeseed meal demonstrated superior effects on rice grain yield. The results of this study indicate that there is no direct correlation between rice grain yield and the carbon-to-nitrogen ratio, which is also inconsistent with our hypothesis. Specifically, the C/N ratios for the organic fertilizers derived from chicken manure, vermicompost, and rapeseed meal were 10.37, 7.25, and 9.07, respectively. Organic fertilizers directly increased soil organic matter and availability of nutrients and promoted increased yield [7,49]. Therefore, the total carbon-to-nitrogen ratio in the soil is not directly correlated with rice grain yield. Correlation analysis revealed a significant positive correlation between rice grain yield and available nutrients, while the relationship with soil enzymes was not significant, suggesting that the activity of the selected enzymes in this study is not a direct factor affecting yield. The application of organic fertilizers, especially rapeseed meal fertilizer, can comprehensively enhance soil fertility and improve soil structure. A similar study by Ju et al. (2022) revealed that the partial application of organic N could improve soil fertility and rice grain yield in reclaimed soils, with rapeseed meal fertilizer the most effective organic N source [32]. Zhou et al. (2022) revealed that the application of rapeseed meal fertilizer could synergistically improve nitrogen use efficiency and grain quality, including the structure and physicochemical properties of starch, contents of high value protein and amino acids, contents of microelements, and cooking and eating quality [50]. The appropriate application of inorganic fertilizers along with organic manure increased soil fertility more than that by organic or inorganic fertilizers separately [51].

4.2. Responses of Carbohydrate-Active Enzymes

CAZymes play a crucial role in organisms, participating in biological processes such as cell wall biosynthesis, degradation, restructuring, and modification [52,53]. PERMANOVA analysis showed that the differences in whether organic fertilizers were applied were more significant than the differences observed when the same type of organic fertilizer was applied in different planting systems. Furthermore, the LEfSe analysis indicated that the differences in CAZymes were not observed in dominant enzymes, but rather in some specific enzymes unique to the application and non-application of organic fertilizers. Both PCA and LEfSe results demonstrated that the functional composition of CAZymes in the RG and RF systems were more similar. For example, both systems exhibited specific enzymes such as GT87, GT89, GH42, CBM50, among others, when organic fertilizers were applied. CBM50 modules were also found in a multitude of other enzymes targeting the peptidoglycan, such as peptidases and amidases [54]. In the RW system, specific functional enzymes such as PL_22, GT41, and CE9 were all included in many biological processes. PL_22 is oligogalacturonate lyase, which contributes to the remodeling and modification of plant cell walls [55]. GT41 is peptide beta-N-acetylglucosaminyltransferase. The glucosamine glycosylation modification plays an important role in regulating cellular processes, signal transduction, and gene expression [56]. The activity and specific catalysis of peptide

beta-N-acetylglucosaminyltransferase were crucial for understanding the mechanisms and functions of protein modifications in these biological processes. CE9 is N-acetylglucosamine 6-phosphate deacetylase, and plays a crucial role in various biological processes, including glucosamine metabolism, cell wall synthesis, and ubiquitin metabolism. It is essential for maintaining normal cellular function and metabolic balance [57]. These enzymes play important roles in the structure and functional regulation of plant cell walls, signal transduction, and metabolic balance. A thorough understanding of their activity and catalytic mechanisms will help uncover the regulatory mechanisms and functions of these biological processes.

As network revealed, carbohydrate-related enzymes typically exhibit positive correlations because they cooperate in carbohydrate metabolism pathways to ensure smooth metabolic processes and energy balance [58]. In the regulation of the structure and function of plant cell walls, enzymes such as PL_22, GT41, and CE9 may interact with each other, and their activities and catalytic capabilities may mutually influence each other. This positive correlation helps maintain the balance and coordination of carbohydrate metabolism to meet the energy requirements and physiological functions of cells [59]. By gaining a deep understanding of the interactions and regulatory mechanisms between these enzymes, we can better reveal the regulatory networks and functions of carbohydrate metabolism pathways. In organic fertilizer treatments, there is a strong correlation among carbohydrate-related enzymes, whereas in chemical fertilizer treatments, these relationships are weaker, which may indicate differences in nutrient sources and influence of microbial activity. On one hand, organic fertilizers and chemical fertilizers provide different types of nutrients [60]. Organic fertilizers typically contain carbohydrates, organic acids, and other organic compounds, which can directly serve as substrates or modulators influencing enzyme activity and interactions [61]. Chemical fertilizers, on the other hand, mainly provide inorganic salts and nutrients, which may have a weaker direct regulation on carbohydrate enzymes [62]. Additionally, organic fertilizers were often rich in organic matter, providing a suitable growth environment for microorganisms. Microorganisms play a significant role in organic fertilizer treatments by decomposing organic matter and participating in carbon cycling [63]. These microorganisms can produce enzymes or metabolites that affect the interactions between carbohydrate-related enzymes. In chemical fertilizer treatments, microbial activity is typically lower, resulting in weaker interactions between carbohydrate enzymes [62].

The redundancy analysis indicates that functional diversity was positively correlated with ammonium nitrogen, and negatively correlated with the carbon-to-nitrogen ratio and soil organic carbon. In contrast, the results of the heatmap correlation analysis showed the opposite trend, where ammonium nitrogen was negatively correlated with dominant functional enzymes, while the carbon-to-nitrogen ratio and soil organic carbon were positively correlated. The opposite results obtained from RDA and heatmap correlation analysis may be due to their focus on different aspects. RDA emphasizes the relationship between functional enzyme structure and environmental factors, while heatmap correlation analysis focuses on the relationship between dominant functional enzyme families and environmental factors. This difference could be attributed to variations in the analysis methods and the different ways in which environmental factors affect functional enzyme composition. The PLS-PM analysis results demonstrated that the effectiveness of nitrogen in the soil directly determines rice grain yield. Other environmental factors had a negative impact on yield. In addition, there was a negative correlation between rice grain yield and carbohydrate enzyme classes. This may be because the higher activity of carbohydrate enzymes requires increased nitrogen consumption [64]. This relationship was supported by the positive correlation between soil nitrogen availability and carbohydrate enzyme activity. Consequently, the reduced availability of nitrogen in an absorbable form for plant roots led to a negative effect on rice grain yield.

5. Conclusions

The study emphasized the importance of reasonable cropping systems and fertilizer management in improving barren soil quality. The rice-fallow rotation system with the application of rapeseed meal fertilizer resulted in the highest yield increase. Rice grain yield was positively correlated with available nutrients. Differences in the CAZyme family's compositions were observed among cropping systems and fertilizer treatments. Organic fertilizer treatments showed stronger correlations among functional enzymes compared to chemical fertilizer treatments. Enzymes such as GT87, GT89, GH42, and CBM50 play important roles in the structure and functional regulation of plant cell walls, signal transduction, and metabolic balance. Functional enzymes were positively correlated with ammonium nitrogen and negatively correlated with the carbon-to-nitrogen ratio and soil organic carbon. C/N ratios, soil total organic carbon, and available nitrogen had significant impacts on soil enzymes and rice grain yield. The study suggested that using organic fertilizers in appropriate cropping systems can enhance nutrient availability, influence carbon-cycling microorganisms, and increase rice grain yield. Farmers should choose appropriate methods based on soil conditions, crop requirements, and sustainability goals, and continuously monitor and adjust their soil management practices. Seeking guidance and advice from agricultural experts can provide more specific and personalized soil improvement solutions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13071840/s1>, Figure S1: Relationships between rice grain yield and soil fertility; Figure S2: Network analysis within slow-release fertilizer treatments (Chemical) and organic fertilizer treatments (Organic), respectively; red line indicates positive relationship; Table S1: Parameters for organic fertilizers used in the present study; Table S2: Soil chemical properties and enzymes.

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