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ARTICLE

Effects of Microbial Fertilizer Application on Cadmium-Contaminated Soil Remediation and Cadmium Content in Rice under Chemical Fertilizer Reduction Combined with Organic Fertilizer

Cheng Qu[1,2], Fen Liu[3], Dongping Yao[1], Zhijun Li[1], Yuanhuan Li[1], Tangxing Liu[1], Yue Wang[2] and Lili Yang[1,*]

[1]College of Plant Science and Technology, Hunan Biological Electromechanical Vocational Technical College, Changsha, China
[2]College of Agronomy, Hunan Agricultural University, Changsha, China
[3]College of Agriculture and Horticulture, Huaihua Vocational and Technical College, Huaihua, China
*Corresponding Author: Lili Yang. Email: yanglili@hnbemc.edu.cn

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ABSTRACT: To clarify the remediation effect of microbial inoculant fertilizer on cadmium (Cd) pollution following the application of pig manure organic fertilizer, a field experiment was conducted. No fertilization (CK1) and conventional fertilization (CK2) were used as controls. The treatments included conventional fertilization + pig manure organic fertilizer (PM, 2800 kg/hm^2) and four levels of microbial inoculant fertilizer (7.5, 15, 22.5, 30 L/hm^2) combined with the PM treatment. The study compared the effects of different treatments on rice economic traits, yield, Cd content in grains and plants, soil available Cd, total Cd, soil pH, and soil enzyme activities. The results showed that conventional fertilization + pig manure organic fertilizer significantly increased the effective panicle number per hectare and yield of rice. However, it concurrently led to a significant increase in rice grain Cd content, soil available Cd, and total Cd by 26.08%, 13.35%, and 16.67%, respectively. Membership function analysis for comprehensive evaluation of economic traits and quality indicated that the treatment with 70% chemical fertilizer + pig manure organic fertilizer received the highest score. Compared with the application of pig manure organic fertilizer alone, the combined application of microbial inoculant fertilizer and pig manure organic fertilizer reduced rice grain Cd content, plant Cd content, total soil Cd, and available Cd by 20.50%~28.42%, 4.48%~12.54%, 4.98%~10.25%, and 43.28%~49.59%, respectively. Further analysis revealed that the combined application increased soil pH by 0.10~0.24 units, and enhanced the activities of soil sucrase, catalase, urease, and alkaline phosphatase by 7.49%~17.85%, 4.19%~10.18%, 0.39%~15.69%, and 16.35%~48.08%, respectively. Based on the comprehensive score from principal component analysis, the treatment with microbial inoculant fertilizer at 15 L/hm^2 + pig manure organic fertilizer + 70% chemical fertilizer achieved the highest score. Considering the production economic value, the fertilization model of microbial inoculant fertilizer at 15 L/hm^2 + pig manure organic fertilizer + 70% chemical fertilizer is recommended for application and promotion in local production.

KEYWORDS: Cadmium; microbial inoculant fertilizer; pig manure organic fertilizer; availability

1 Introduction

Cadmium (Cd), as a highly toxic, mobile, and bioaccumulative heavy metal pollutant, has become a major environmental issue threatening global agricultural ecological security and human health [1,2]. The overall soil environment in China is generally stable, but in specific areas, particularly in major rice-producing regions of the south, the rate of Cd contamination in arable land exceeds standards at certain sites. Research indicates that the area of arable land at risk of Cd contamination in some southern

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provinces exceeds 200,000 hectares [3–5]. Rice, the staple food for over 60% of China's population, exhibits Cd accumulation characteristics that lead to frequent incidents of Cd levels exceeding the standard in rice grains. Monitoring results from production areas in Hunan, Jiangxi, Guangxi, etc., show that Cd content in rice from some paddy fields reaches as high as 0.45 mg/kg, far exceeding the national standard [6,7]. Long-term consumption of rice with excessive Cd can cause itai-itai disease, renal failure, cardiovascular diseases, and even affect fetal development by crossing the placental barrier [8,9]. Therefore, the safe utilization of Cd-contaminated paddy fields and the quality control of rice have become critical components of the national food security strategy.

China is the world's largest pig producer and has abundant resources of pig manure organic fertilizer [10,11]. However, pig manure itself contains a certain amount of cadmium. Long-term or excessive application can lead to Cd accumulation in the soil [12]. Studies have shown that long-term application of pig manure may result in the accumulation of heavy metals in soil, threatening food safety [13]. Long-term positioning experiments indicate that the total Cd content in the topsoil of paddy fields where pig manure organic fertilizer was applied continuously for five years significantly increased by 1.25 times compared to the initial value, significantly raising the risk of Cd content exceeding standards in rice grains [14,15]. However, other studies suggest that the regulatory effect of organic fertilizer on Cd bioavailability exhibits a "dual nature". On one hand, organic fertilizer input can increase soil organic matter content and pH, promoting the formation of stable complexes between Cd and humic or fulvic acids, thereby reducing its bioavailability [16,17]. On the other hand, low-molecular-weight organic acids produced by the decomposition of organic matter may compete with Cd for adsorption sites on soil colloid surfaces, or dissolve Cd bound to iron-manganese oxides, conversely promoting the reactivation of Cd. This effect is related to the initial soil physicochemical properties, the type of organic fertilizer, and the application rate [18–20].

Currently, selecting low-Cd accumulation rice varieties [21], water management [22], and applying amendments [23] are widely used methods to manage soil Cd pollution. Among these, applying amendments offers significant advantages, such as notable effectiveness and environmental friendliness, making it the mainstream technology for moderately and lightly polluted paddy fields. Soil amendments reduce Cd bioavailability by converting it into residual or iron-manganese oxide-bound forms, which is a current research hotspot. Amendments are generally categorized into three types: inorganic, organic, and microbial. Inorganic amendments like lime promote Cd precipitation by increasing soil pH, but their long-term application can easily lead to soil compaction and secondary salinization [24]. Single organic fertilizers, such as pig manure, can reduce Cd availability through complexation with organic matter, but some organic fertilizers carry Cd themselves, which may conversely increase the risk of Cd exceeding standards in rice grains [25]. Low-molecular-weight organic acids produced by the decomposition of single organic fertilizers might promote Cd activation. Microbial fertilizer, as a novel functional material, contains functional microorganisms such as *Bacillus*, *Pseudomonas*, and *Actinomycetes* that can immobilize Cd through extracellular adsorption, biotransformation, or secreting siderophores, while also improving soil fertility [26–28]. Research shows that applying phosphate-solubilizing bacterial fertilizer can reduce soil available Cd by 28.3% and decrease Cd uptake by rice by 35.7% [29].

Therefore, this study conducted a field experiment in acidic Cd-contaminated paddy fields by applying different concentrations of microbial fertilizer during the rice growth period. The aim was to investigate the effects of microbial fertilizer application on rice yield, Cd content in grains and plants, soil pH, and soil enzyme activities in Cd-contaminated farmland. This research aims to provide a theoretical basis for improving acidic paddy soil and promoting green and sustainable agricultural development.

2 Materials and Methods

2.1 Test Materials and Site

The experiment was conducted in 2024 at the Hedong Farm, Liuyang City, Hunan Province (E 113°48′23″, N 28°30′30″). The soil at the test site was an acidic soil developed from Quaternary red clay, with the following baseline fertility characteristics: pH 5.82, organic matter 10.81 g/kg, total nitrogen 1.05 g/kg, total phosphorus 1.12 g/kg, total potassium 13.43 g/kg; alkali-hydrolyzable nitrogen 114.01 mg/kg, available phosphorus 48.19 mg/kg, available potassium 185 mg/kg. The initial total cadmium concentration of the experimental soil was 0.53 mg/kg, and the initial available cadmium concentration was 0.29 mg/kg. According to the Chinese Soil Environmental Quality Standard (GB 15618-2018), given the soil pH of 5.82, the risk screening value for cadmium in paddy soil is 0.4 mg/kg. Therefore, the soil used in this study is classified as lightly to moderately contaminated with cadmium.

The tested rice variety was the indica two-line hybrid rice "Jingliangyou 1377" (National Approved Rice 2016608), provided by Yuan Longping High-Tech Agriculture Co., Ltd. The tested chemical fertilizer was Longke Agricultural Supplies Compound Fertilizer (N-P_2O_5-K_2O content ratio 20-10-15), produced by Hunan Longke Ecological Agriculture Service Co., Ltd. The tested organic fertilizer was pig manure, provided by a large-scale pig farm in Liuyang City (mass fractions of organic matter, total nitrogen, total phosphorus, and total potassium were 15.52%, 1.81%, 1.77%, and 1.34%, respectively; cadmium content was 0.0006 mg/kg). The microbial fertilizer [containing a total effective viable count of 4.46×10^8 cfu/mL (including 3.15×10^8 cfu/mL of *Bacillus subtilis* and 1.31×10^8 cfu/mL of *Bacillus licheniformis*)] was provided by Hunan Yirun Biotechnology Co., Ltd.

2.2 Experimental Design

The doses of microbial fertilizer were selected based on the results of preliminary trials, which tested four concentration gradients (7.5, 15, 22.5, and 30 L/hm^2) to assess their effects on rice growth and cadmium uptake. The pig manure organic fertilizer rate (2800 kg/hm^2) was determined according to local agricultural practices and previous studies on cadmium immobilization in paddy soil in Hunan Province. The experiment employed a randomized block design with a total of 7 treatments: ① CK1 (blank control, no fertilizer application); ② CK2 (conventional fertilization); ③ T1: 70% chemical fertilizer + pig manure organic fertilizer; ④ T2: 70% chemical fertilizer + pig manure organic fertilizer + microbial fertilizer 7.5 L/hm^2; ⑤ T3: 70% chemical fertilizer + pig manure organic fertilizer + microbial fertilizer 15 L/hm^2; ⑥ T4: 70% chemical fertilizer + pig manure organic fertilizer + microbial fertilizer 22.5 L/hm^2; ⑦ T5: 70% chemical fertilizer + pig manure organic fertilizer + microbial fertilizer 30 L/hm^2. Conventional fertilization consisted of a basal application of 750 kg/hm^2 compound fertilizer and a tillering topdressing of 150 kg/hm^2 compound fertilizer. The 70% chemical fertilizer treatment applied a basal dose of 525 kg/hm^2 compound fertilizer and a tillering topdressing of 105 kg/hm^2 compound fertilizer. The organic fertilizer (pig manure) was applied entirely as a one-time basal dressing at a rate of 2800 kg/hm^2. The microbial fertilizer was applied three times: as basal fertilizer, tillering fertilizer, and panicle fertilizer (65%:15%:20%). The application method for the microbial fertilizer was to dilute the stock solution 100 times with water and spray evenly. Rice was sown on 25 April 2024, transplanted on 27 May, and harvested on 29 September, with a transplanting density of 20 cm × 26 cm and 2 seedlings per hill. Each treatment had 3 replicates, arranged randomly. Each plot area was 50 m^2, with separate irrigation and drainage to prevent water and fertilizer cross-contamination between plots.

During the rice growing season, an intermittent irrigation regime was implemented as follows: a 5 cm water layer was maintained in the field for 7 days, followed by 3 days of natural drainage. This cycle was repeated throughout the growing period until 7 days prior to harvest, at which point irrigation was discontinued and the field was left to dry under natural conditions.

2.3 Sample Analysis and Data Determination

2.3.1 Determination of Yield and Yield Components

At maturity, 30 hills per plot were surveyed to calculate the number of effective panicles per plant. Aboveground rice plant samples were collected. Five representative plant hills were sampled from each plot using a five-point sampling method, and yield components such as 1000-grain weight and seed setting rate were investigated. All plants from each plot were harvested, threshed, and weighed separately, and the actual grain yield was calculated after adjusting to standard moisture content.

2.3.2 Determination of Cadmium Content

Rice grains were ground into flour, sieved, and dried in an oven at 80°C. A 0.3000 g sample was taken for analysis. At maturity, whole rice plants were collected, cleaned, and dried at 75°C. For determination of plant Cd content, the aboveground parts (stems and leaves) and rice grains were analyzed separately. Soil sampling: Soil samples were collected from each plot at 0–20 cm depth using a five-point sampling method. From each plot, five soil cores were collected and mixed thoroughly to form one composite sample. The soil analyzed was bulk soil (non-rhizosphere soil). Soil samples were air-dried, ground, and passed through a 0.15 mm sieve. Rice grain samples were digested according to the "Determination of Lead and Cadmium in Rice—Graphite Furnace Atomic Absorption Spectrometry" method. Plant samples were digested using a wet digestion method with a mixed acid of HNO_3:$HClO_4$ at a volume ratio of 4:1 at 180°C for 3 h. Soil samples were digested with a mixed acid of HNO_3:$HClO_4$:HF at a volume ratio of 5:1:1 at 200°C for 4 h using a hot plate. Total cadmium content in rice plants and soil was determined using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7800, USA). Soil available cadmium was extracted using DTPA-$CaCl_2$ and determined by ICP-MS. Rice grain cadmium content was determined using graphite furnace atomic absorption spectrometry (GFAAS, PerkinElmer AAnalyst 600, USA). Calibration was performed using multi-element standard solutions (Agilent, USA). Reagent blanks and sample duplicates were included in each analytical batch. Certified reference materials (rice flour GBW10043 and soil GSS-5) were used for quality assurance. The recovery rate for cadmium ranged from 92% to 105%. The detection limit was 0.002 mg/kg for ICP-MS and 0.0005 mg/kg for GFAAS.

2.3.3 Determination of Soil pH

Soil pH was measured using a pH meter (soil:water ratio 1:5).

2.3.4 Determination of Soil Enzyme Activities

Soil urease activity was assayed using the indophenol blue colorimetric method, with results expressed as milligrams of NH_3-N released per gram of soil over a 24-h period. Sucrase activity was evaluated via the 3,5-dinitrosalicylic acid colorimetric approach, and the values were expressed as milligrams of glucose generated per gram of soil within 24 h [30]. Alkaline phosphatase activity was measured by the disodium phenyl phosphate colorimetric method, and the activity was expressed as milligrams of phenol produced per gram of soil after 24 h of incubation. Catalase activity was determined through titration using 0.1 mol/L $KMnO_4$ solution.

2.3.5 Comprehensive Evaluation of Rice Yield Traits under Different Fertilization Treatments

The fuzzy mathematics membership function method was employed to perform a comprehensive evaluation of the different fertilization treatments. For each measured indicator, the corresponding membership function value was calculated by substituting the data into the appropriate formula. The average membership value was then determined for each treatment, with a higher average value indicating better overall quality. Because rice grain Cd content and plant Cd content are negatively associated with the comprehensive assessment of rice yield traits, the reverse membership function was applied to calculate their membership degrees [31].

Evaluation formula: X(u) = (X − Xmin)/(Xmax − Xmin)

where X is the measured value of a certain indicator for a given treatment, Xmax is the maximum value of that indicator among all treatments, and Xmin is the minimum value of that indicator among all treatments.

2.4 Data Processing and Analysis

Data analysis was performed by taking the average of three replicates. Excel 2019 and DPS 9.01 were used for data calculation and correlation analysis. Duncan's multiple range test was used for significance analysis ($p < 0.05$). Graphs were plotted using GraphPad Prism 10.0.

3 Results

3.1 Effects of Microbial Fertilizer on Rice Yield and Yield Components under Chemical Fertilizer Reduction Combined with Organic Fertilizer Application

As shown in Table 1, fertilization methods significantly affected rice yield and yield components. Compared with CK2, CK1 significantly reduced the effective panicle number, grains per panicle, seed setting rate, theoretical yield, and actual yield of rice. Compared with CK2, treatments T1-T5 significantly increased the effective panicle number by 5.00%, 6.53%, 8.04%, 11.15%, and 5.69%, respectively; theoretical yield increased by 11.22%, 13.93%, 19.85%, 16.55%, and 13.29%, respectively; and actual yield significantly increased by 12.41%, 15.78%, 16.63%, 16.23%, and 17.06%, respectively. The effective panicle number in treatment T4 was significantly higher than in T1 and T5 by 5.86% and 5.16%, respectively. Among treatments T2–T5, both actual yield and theoretical yield showed a trend of initially increasing and then decreasing with the increase in microbial fertilizer application rate, with treatment T3 achieving the highest values for both.

Table 1: Effects of different treatments on rice yield and yield components.

Treatment	Effective Panicles ($\times 10^4$ Panicles/hm^2)	Filled Grains per Panicle (Grains)	Seed Setting Rate (%)	1000-Grain Weight (g)	Theoretical Yield (kg/hm^2)	Actual Yield (kg/hm^2)
CK1	205.64 ± 8.42d	162.23 ± 10.85b	74.23 ± 3.64b	19.45 ± 1.95a	6511.27 ± 1273.77c	7462.78 ± 445.33c
CK2	251.23 ± 6.52c	212.42 ± 10.56a	82.65 ± 3.81a	21.32 ± 1.05a	11,389.57 ± 1279.86b	9123.45 ± 541.73b
T1	263.78 ± 7.13b	219.45 ± 9.38a	85.87 ± 2.52a	21.78 ± 2.06a	12,667.39 ± 2337.40ab	10,255.98 ± 771.65a
T2	267.63 ± 7.19ab	220.87 ± 12.77a	86.23 ± 3.60a	21.90 ± 1.45a	12,976.66 ± 2159.39ab	10,563.18 ± 209.17a
T3	271.42 ± 11.17ab	225.23 ± 8.52a	86.45 ± 4.02a	22.43 ± 2.53a	13,650.37 ± 2477.29a	10,640.93 ± 199.50a
T4	279.23 ± 0.49a	223.90 ± 6.59a	88.98 ± 4.56a	21.23 ± 0.66a	13,274.77 ± 1073.76ab	10,603.93 ± 323.55a
T5	265.53 ± 2.35b	226.12 ± 8.43a	88.12 ± 2.54a	21.52 ± 1.54a	12,902.76 ± 1433.15ab	10,625.18 ± 280.19a

Data are presented as means + SDs ($n = 3$). Different lowercase letters indicate that the data are significantly different at the 0.05 level.

3.2 Effects of Microbial Fertilizer on Cadmium Content in Rice Grains and Plants under Chemical Fertilizer Reduction Combined with Organic Fertilizer Application

As shown in Fig. 1A, compared with CK1, the cadmium content in rice grains under CK2 significantly increased by 22.84%, and as shown in Fig. 1B, the cadmium content in plants increased by 7.35%. Compared with CK2, treatment T1 significantly increased the cadmium content in rice grains by 26.08% (Fig. 1A) and the cadmium content in plants by 3.35% (Fig. 1B). Compared with treatment T1, treatments T2-T5 reduced the cadmium content in both rice grains and plants. Specifically, as shown in Fig. 1A, the cadmium content in rice grains under treatments T2-T5 was significantly reduced by 22.48%, 23.20%, 28.42%, and 20.50%, respectively. As shown in Fig. 1B, the cadmium content in plants under treatments T2-T4 was significantly reduced by 8.91%, 11.02%, and 12.54%, respectively. Treatment T4 showed the most significant reduction effect on cadmium content in both rice grains and plants.

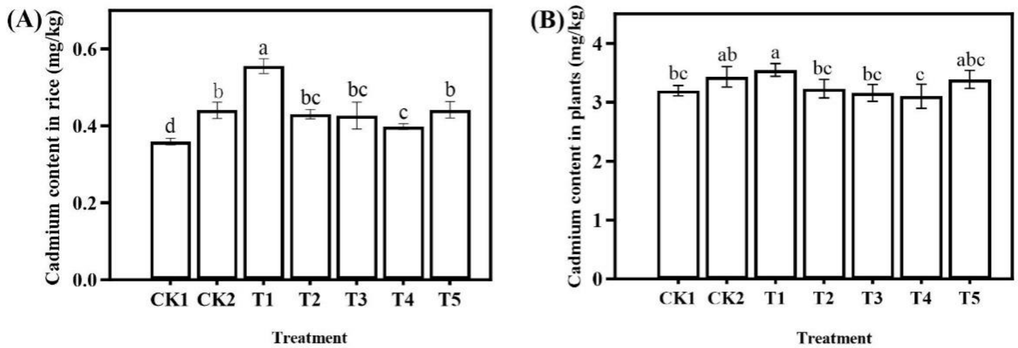

Figure 1: Effects of microbial fertilizer on cadmium content in rice grains and plants under chemical fertilizer reduction combined with organic fertilizer application. In the figure, (**A**) represents cadmium content in rice; (**B**) represents cadmium content in rice. Means ± Ses (n = 3) with different letters denote significant statistical differences (p < 0.05).

3.3 Evaluation of Microbial Fertilizer on Rice under Chemical Fertilizer Reduction Combined with Organic Fertilizer Application Based on Membership Function Analysis

Eight indicators, including effective panicle number, filled grains per panicle, seed setting rate, and yield, are key criteria for evaluating rice quality. However, a single indicator cannot provide an objective assessment, necessitating a comprehensive analysis. As shown in Table 2, the comprehensive scores of treatments T1–T5 were higher than that of CK2, and the scores of treatments T2–T5 were higher than that of T1. Treatment T4 achieved the highest score, followed by treatment T3.

Table 2: Evaluation of microbial fertilizer on rice under chemical fertilizer reduction combined with organic fertilizer application based on membership function analysis.

Indicator	CK1	CK2	T1	T2	T3	T4	T5
Effective Panicles	0.00	0.62	0.79	0.84	0.89	1.00	0.81
Filled Grains per Panicle	0.00	0.79	0.90	0.92	0.99	0.97	1.00
Seed Setting Rate	0.00	0.57	0.79	0.81	0.83	1.00	0.94
1000-Grain Weight	0.00	0.63	0.78	0.82	1.00	0.60	0.69
Theoretical Yield	0.00	0.68	0.86	0.91	1.00	0.95	0.90
Actual Yield	0.00	0.52	0.88	0.98	1.00	0.99	1.00
Rice Grain Cd Content	1.00	0.58	0.00	0.63	0.65	0.80	0.58
Plant Cd Content	0.79	0.26	0.00	0.71	0.88	1.00	0.36
Average Membership Degree	0.22	0.58	0.62	0.83	0.90	0.91	0.78
Rank	7	6	5	3	2	1	4

3.4 Effects of Microbial Fertilizer on Soil Cadmium Content and Soil pH under Chemical Fertilizer Reduction Combined with Organic Fertilizer Application

As shown in Fig. 2, fertilization methods significantly affected soil cadmium content and soil pH. As shown in Fig. 2A, compared with CK1, the total cadmium content in soil under CK2 increased by 0.95%, and as shown in Fig. 2B, the available cadmium content increased by 5.88%, but these differences were not statistically significant. Compared with CK1 and CK2, treatment T1 significantly increased the total cadmium content in soil by 14.42% and 13.35%, respectively (Fig. 2A), and significantly increased the available cadmium content by 23.53% and 16.67%, respectively (Fig. 2B). Compared with treatment T1, treatments T2-T5 significantly reduced the total cadmium content in soil by 4.98%, 4.98%, 10.25%, and 9.78%, respectively (Fig. 2A). The total cadmium content and available cadmium content in soil under treatments T4 and T5 were significantly lower than those under treatments T2 and T3 (Fig. 2A,B). As shown in Fig. 2C, the soil pH under CK2 was 1.40% lower than that under CK1, but the difference was not statistically significant. Compared with CK1 and CK2, treatment T1 significantly reduced soil pH by 6.81% and 5.49%, respectively (Fig. 2C). Compared with treatment T1, treatments T2-T5 increased soil pH. Specifically, soil pH under treatments T4 and T5 was significantly higher than that under treatment T1 by 4.49% and 3.93%, respectively (Fig. 2C).

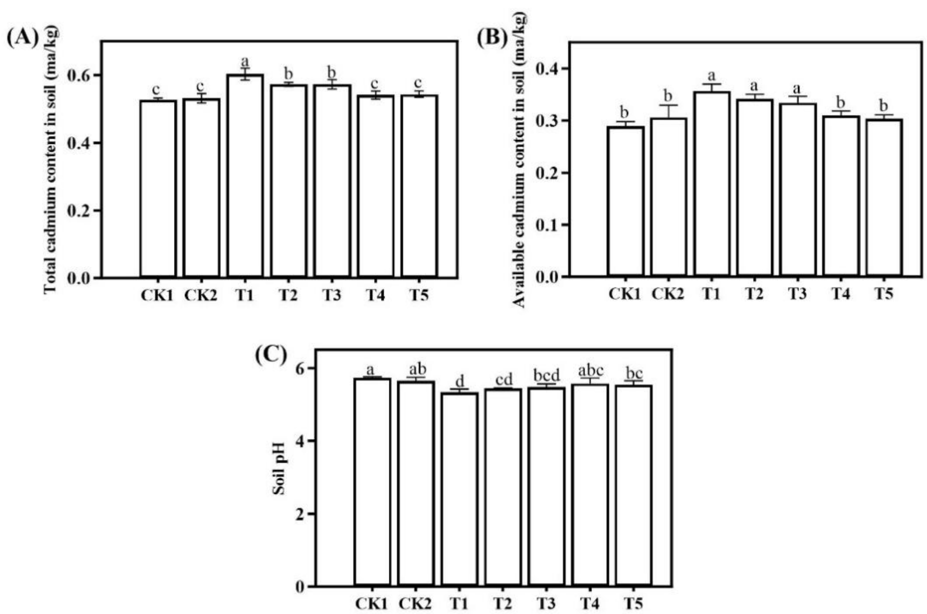

Figure 2: Effects of microbial fertilizer on soil cadmium content and soil pH under chemical fertilizer reduction combined with organic fertilizer application. In the figure, (**A**) represents total cadmium content insoil; (**B**) represents available cadmium content in soil; and (**C**) represents soil pH. Means ± SEs (n = 3) with different letters denote significant statistical differences ($p < 0.05$).

3.5 Effects of Microbial Fertilizer on Soil Enzyme Activities under Chemical Fertilizer Reduction Combined with Organic Fertilizer Application

As shown in Table 3, the changes in the activities of soil sucrase, catalase, urease, and alkaline phosphatase are presented. Compared with CK1, treatment CK2 increased the activities of soil sucrase, catalase, urease, and alkaline phosphatase by 23.55%, 6.21%, 19.51%, and 1.05%, respectively. Compared with CK2, treatment T1 increased the activities of soil catalase, urease, and alkaline phosphatase by 8.44%, 4.08%, and 8.33%, respectively. Compared with T1, treatments T2-T5 all increased the activities of soil catalase, urease, and alkaline phosphatase, with treatment T4 exhibiting the highest soil enzyme activities. Compared with treatment T1, treatment T4 significantly increased the activities of soil sucrase, catalase, urease, and alkaline phosphatase by 17.85%, 10.18%, 15.69%, and 48.08%, respectively.

Table 3: Effects of microbial fertilizer on soil enzyme activities under chemical fertilizer reduction combined with organic fertilizer application.

Treatment	Sucrase ($mg \cdot g^{-1}$, 24 h)	Catalase (0.1 mol L^{-1} $KMnO_4$ mL g^{-1})	Urease (NH_3-N, mg(g, 24 h)$^{-1}$)	Alkaline Phosphatase (mg g^{-1}, 24 h)
CK1	6.03 ± 0.09e	1.45 ± 0.1c	2.05 ± 0.18c	0.95 ± 0.17d
CK2	7.45 ± 0.09cd	1.54 ± 0.07c	2.45 ± 0.16b	0.96 ± 0.13d
T1	7.34 ± 0.19d	1.67 ± 0.03b	2.55 ± 0.04b	1.04 ± 0.19cd
T2	7.89 ± 0.23bcd	1.75 ± 0.07ab	2.64 ± 0.09b	1.21 ± 0.22bcd
T3	8.03 ± 0.85abc	1.83 ± 0.04a	2.86 ± 0.08a	1.32 ± 0.22abc
T4	8.65 ± 0.13a	1.84 ± 0.1a	2.95 ± 0.09a	1.54 ± 0.13a
T5	8.45 ± 0.2ab	1.75 ± 0.07ab	2.56 ± 0.11b	1.45 ± 0.11ab

Data are presented as means + SDs (n = 3). Different lowercase letters indicate that the data are significantly different at the 0.05 level.

3.6 Correlation Analysis

Correlation analysis between yield, yield components, and rice Cd content, soil Cd content, and soil pH (Fig. 3): The correlations between effective panicles, grains per panicle, seed setting rate, 1000-grain weight, yield and rice grain Cd content, and plant Cd content were not significant. The correlations between effective panicles, grains per panicle, seed setting rate, yield and soil total Cd content, and soil available Cd content were significant or highly significant. Rice yield and yield components were negatively correlated with soil pH, but the correlations were not significant.

Correlation analysis between yield, yield components, and soil enzyme activities: The correlations between rice effective panicles, grains per panicle, seed setting rate, yield and soil sucrase, catalase, and urease activities were significant or highly significant. However, the correlations between rice effective panicles, grains per panicle, seed setting rate, yield and alkaline phosphatase activity were not significant. The correlations between 1000-grain weight and soil enzyme activities were not significant.

Correlation analysis between soil enzyme activities, rice Cd content, soil Cd content, and soil pH: Soil enzyme activities were negatively correlated with rice grain Cd content, plant Cd content, and soil pH, but the correlations were not significant. Soil enzyme activities were positively correlated with soil total Cd content and soil available Cd content, but the correlations were not significant.

3.7 Principal Component Analysis

Principal component analysis was conducted on seven indicators, including soil total cadmium content, available cadmium content, and soil pH, across the seven treatments. The eigenvalues, variance contribution rates, and cumulative variance contribution rates of the principal components were obtained, as detailed in Table 4. Principal components with a cumulative variance contribution rate greater than 95% were selected for analysis. Principal component 1 had an eigenvalue of 4.44, accounting for 63.42% of the variance. Principal component 2 had an eigenvalue of 2.26, accounting for 32.35% of the variance. These two principal components together represented 95.77% of the information from the original data and could substitute for the seven indicators from the seven treatments in comprehensive analysis and evaluation.

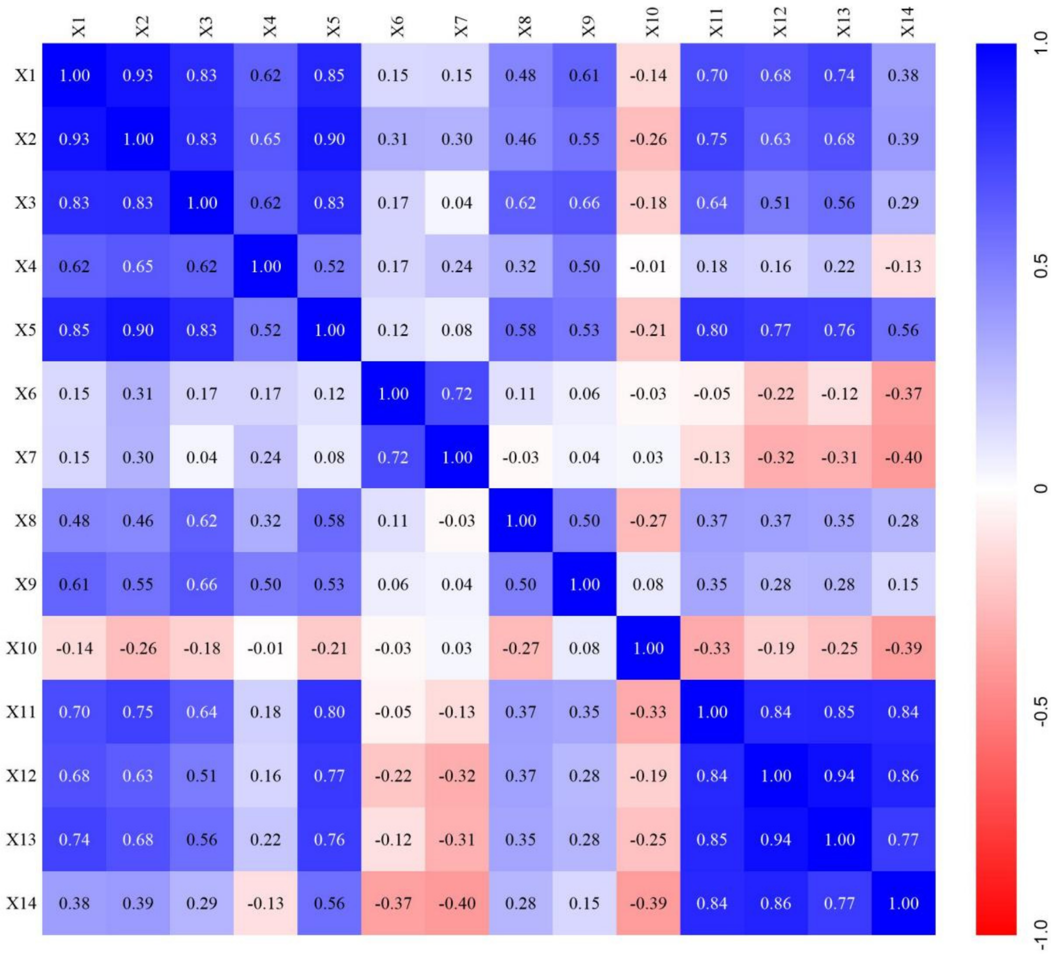

Figure 3: Pearson correlation coefficients among yield, yield components, soil enzyme activities, cadmium content, and soil pH (n = 21). X1, X2, X3, X4, X5, X6, X7, X8, X9, X10, X11, X12, X13, X14 respectively represent the number of effective panicles, number of filled grains per panicle, seed setting rate, thousand-grain weight, yield, cadmium content in rice, cadmium content in plants, total cadmium content in soil, available cadmium content in soil, soil pH, invertase activity, catalase activity, urease activity, and alkaline phosphatase activity.

Table 4: Principal component eigenvalues and variance contribution rates.

Principal Component	Eigenvalue	Variance Contribution Rate (%)	Cumulative Variance Contribution Rate (%)
1	4.44	63.42	63.42
2	2.26	32.35	95.77

From the loading values of each indicator in Table 5, the expression for each principal component can be obtained using the function Ui = Ai/ $\sqrt{\lambda i}$ (where Ui represents the principal component score coefficient, Ai represents the factor loading matrix, and λi represents the eigenvalue):

$$F1 = 0.45X_1 + 0.43X_2 + 0.39X_3 - 0.38X_4 + 0.33X_5 + 0.33X_6 + 0.32X_7$$

$F2 = 0.20X_1 + 0.21X_2 + 0.34X_3 + 0.38X_4 - 0.47X_5 + 0.45X_6 - 0.49X_7$

where $X_1, X_2, X_3 \ldots X_7$ represent the standardized data values of the soil total cadmium content, soil available cadmium content, soil pH, sucrase activity, catalase activity, urease activity, and alkaline phosphatase activity, respectively.

Table 5: Loading value matrix of soil indicators.

Indicator	Load Value	
	Principal Component 1	Principal Component 2
Total Cadmium Content in Soil	0.151	−0.324
Available Cadmium Content in Soil	0.159	−0.309
Soil pH	−0.181	0.256
Sucrase	0.185	0.223
Catalase	0.212	0.135
Ureas	0.204	0.137
Alkaline Phosphatase	0.155	0.299

Comprehensive score = $(0.63F_1 + 0.32F_2)/(0.63 + 0.32)$. Calculated and ranked using the principal component comprehensive score function model (Table 6), the comprehensive scores and rankings of different fertilization treatments were T3 > T1 > T2 > T4 > T5 > CK2 > CK1.

Table 6: Comprehensive scores and rankings.

Treatment	F1	F1 Rank	F2	F2 Rank	Comprehensive Score	Comprehensive Rank
CK1	−1.22	7	−0.24	5	−0.89	7
CK2	−1.03	6	0.67	1	−0.46	6
T1	0.66	2	0.36	2	0.56	2
T2	0.48	3	0.04	3	0.33	3
T3	0.97	1	0.00	4	0.64	1
T4	0.48	4	−0.26	6	0.23	4
T5	−0.34	5	−0.58	7	−0.42	5

4 Discussion

The combined application of microbial fertilizer and pig manure organic fertilizer exhibits a significant synergistic effect in paddy fields. Microbial fertilizer contains functional microorganisms such as Bacillus, Pseudomonas, and Actinomycetes, which can improve the soil environment, promote rice growth, and reduce dependence on chemical fertilizers. Pig manure is rich in complex organic matter such as cellulose, hemicellulose, and proteins, whose decomposition relies on microbial degradation. The combined application of these two fertilizers can reduce the bioavailability of cadmium through multiple pathways, including regulating soil pH and forming stable complexes with cadmium.

4.1 Microbial Fertilizer Increases Rice Yield under Chemical Fertilizer Reduction Combined with Organic Fertilizer Application

Similar positive effects of organic fertilizer substitution on crop yield have been widely reported. Previous studies have shown that the combined application of organic and inorganic fertilizers can effectively regulate soil fertility and increase yield in red soil paddy fields [32]. The greater yield improvement observed with the addition of microbial fertilizer can be attributed to enhanced nutrient availability and improved soil biological activity. These findings are consistent with reports that biofertilizers can promote crop growth, enhance stress resistance, and improve nutritional quality [33], suggesting that microbial fertilizers have important application value in sustainable agricultural development.

4.2 Effects of Microbial Fertilizer on Rice Cadmium Content, Soil Cadmium Content, and Soil pH under Chemical Fertilizer Reduction Combined with Organic Fertilizer Application

Previous studies have shown that the combined application of microbial fertilizer containing *Bacillus subtilis* and decomposed cow manure can improve wheat growth by enriching the abundance of nitrate-reducing bacteria, thereby enhancing soil nutrients and reducing soil available cadmium content [34]. The application of biochar and organic fertilizer can alter the rhizosphere bacterial community in heavy metal-contaminated farmland soil, promote the growth of soil bacteria, and reduce the content of available cadmium in soil and cadmium in rice grains [35].

The reduction in cadmium uptake under combined application observed in this study aligns with previous findings [36]. Functional groups such as carboxyl and amino groups on the surface of microbial cell walls can directly adsorb Cd^{2+}, forming stable complexes [37]. Although the application of pig manure alone increased soil total and available cadmium content, the combined application of organic and microbial fertilizers significantly reduced both parameters. This may be because the active components in microbial fertilizer can alter soil pH and redox potential, promoting the transformation of Cd from the exchangeable state to the residual or organic-bound state [38]. This study found that soil pH decreased significantly after the application of pig manure organic fertilizer, while the combined application of microbial fertilizer and organic fertilizer increased soil pH, which may indirectly reduce cadmium availability by promoting cadmium adsorption and complexation in the soil.

4.3 Microbial Fertilizer Increases Soil Enzyme Activities under Chemical Fertilizer Reduction Combined with Organic Fertilizer Application

Soil enzyme activity is an important indicator for assessing soil health. Urease reflects the soil's nitrogen supply capacity, sucrase reflects the degree of soil maturation, catalase levels can reflect the soil's self-purification capacity against biological toxicity [39], and cellulase reflects the state of change in the activity and content of the soil carbon pool [40]. The enhancement of soil enzyme activities under combined application suggests improved soil biological functioning. Previous studies have shown that microbial fertilizers increase the diversity of bacterial and fungal communities and influence catalase, urease, and sucrase, thereby promoting soil nutrient release, enhancing soil fertility, and indirectly affecting the yield of sweet sorghum [41].

The combined application of pig manure organic fertilizer and chemical fertilizer can increase the abundance of soil Nitrospira, Bacillus, and Actinobacteria, promote soil enzyme activity, enhance soil digestion and organic matter decomposition, and consequently increase crop yield [42]. The functional microorganisms in microbial fertilizer can directly secrete various extracellular enzymes, supplementing the soil enzyme pool [43]. Additionally, microorganisms decompose the macromolecular organic matter in pig manure organic fertilizer, generating small molecule substances such as amino acids and sugars, which provide reaction substrates for soil enzymes and activate enzyme activity [44].

Therefore, considering the comprehensive evaluation of yield, cadmium reduction, and soil enzyme activities, the treatment with 15 L/hm^2 microbial fertilizer combined with pig manure organic fertilizer and 70% chemical fertilizer is recommended for local agricultural practice.

5 Conclusion

Based on conventional fertilization, replacing chemical fertilizer with pig manure organic fertilizer, as well as the combined application of pig manure organic fertilizer and microbial fertilizer, can significantly improve the economic traits and yield of rice. The application of microbial fertilizer can significantly reduce the total cadmium content and available cadmium content in the soil, significantly reduce the cadmium content in rice grains and plants, and mitigate the risk of cadmium contamination in soil and rice caused by the application of pig manure organic fertilizer. According to the comprehensive score from principal component analysis, the treatment with microbial fertilizer at 15 L/hm^2 + pig manure organic fertilizer + 70% chemical fertilizer achieved the highest score. However, the comprehensive evaluation of economic traits and quality using the membership function method showed that treatment T4 had the highest score, followed by treatment T3. Considering the production economic value, the fertilization model of microbial fertilizer at 15 L/hm^2 + pig manure organic fertilizer + 70% chemical fertilizer is recommended for application and promotion in local production.

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