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A Symbiotic System of Irrigated Rice–Earthworm Improves Soil Properties and Rice Growth in Southern China

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Abstract: Earthworms have been studied in many ecosystems, demonstrating their high ecological value. However, there are few studies on the coupling of earthworms to irrigated paddy fields. On this basis, a symbiotic system of irrigated rice and earthworms was established with a wide-ridge cultivation model, and a combination of field experiments and pot experiments was carried out in Southern China. The results showed that the symbiosis of irrigated rice and earthworms in the pot experiment significantly loosened the soil by 5–10 cm, compacted the soil by 10–20 cm, increased the soil pH value by 0–10 cm, and increased the content of soil organic matter by 5–20 cm compared with rice monoculture. Due to the significant increase in leaf area index and grain weight at the mature stage, the white root at the heading and grain filling stages improved significantly, and the yield of irrigated rice also increased significantly by 15.39%. However, in the field experiment, due to the low survival rate of earthworms, the effect of inoculating earthworms was not significant. This study confirmed the beneficial effect of earthworm inoculation on the paddy field ecosystem, and provided a research basis for introducing earthworms into the paddy field ecosystem, realizing the sustainable development of rice cultivation, and ensuring world food security.

Keywords: irrigated rice; earthworm; symbiosis; wide-ridge cultivation; rice growth; soil properties

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1. Introduction

Rice is one of the three major food crops in the world, with a global planting area of about 145 million hectares, feeding more than half of the world's population [1]. China is the birthplace of rice cultivation and the most populous country in the world. Rice is the main source of food for the Chinese people. In recent years, China's total rice output in 2019 ranked first in the world with 2.12×10^8 t [2], which is of great significance to maintaining food security in China and the world. However, high yields go hand in hand with large fertilizer inputs. According to statistics, China's fertilizer consumption accounts for 35% of the world's total, which is the sum of the consumption in the United States and India [3]. Extensive use of chemical fertilizers leads to the degradation of arable land, especially soil compaction, acidification and reduction in organic matter content [4,5].

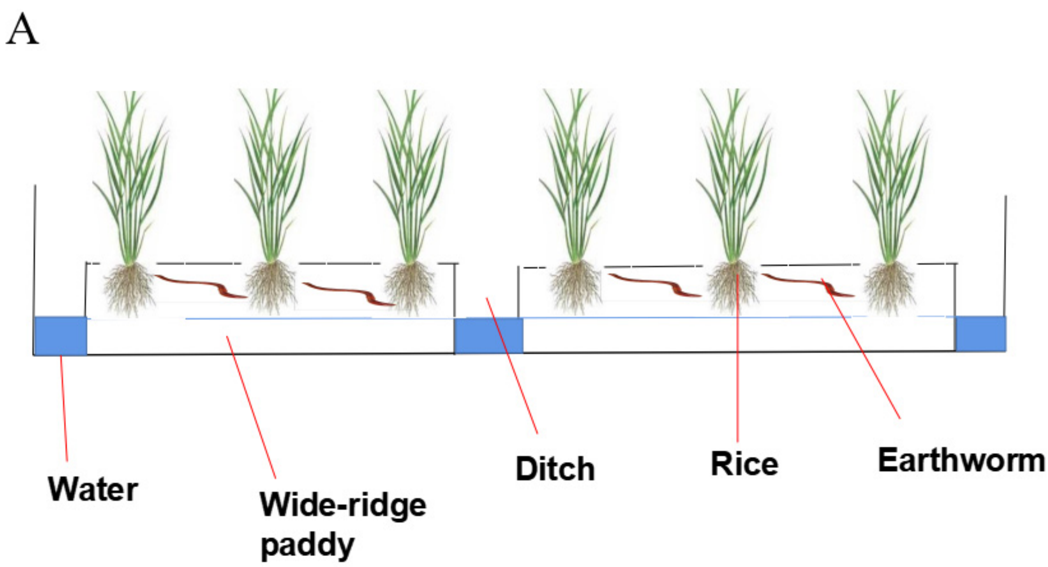
Rice paddy planting and breeding is a classic agronomy of world agriculture and an important part of modern ecological agriculture, by both planting rice and breeding animals in paddy fields. In this system, the complementary effect of the ecological niche of animals and plants is used to create a double coupling of the time dimension and the space dimension, so as to realize the effect of "dual use of one water and double harvest of one field" and achieve the sustainable development of agriculture. At present, typical rice farming models include rice–fish symbiosis, rice–duck symbiosis, rice–crayfish symbiosis, etc., which have positive effects on improving soil fertility, reducing drug use, preventing rice diseases, insects and weeds, and promoting rice growth [6–10]. In these models, the pathways of action of animals in the paddy ecosystem mainly depend on the direct activities of the animals, such as walking, predation and defecation. However, there are many straw resources in paddy fields, and straw is an important form of organic matter in paddy fields. Therefore, if a new rice planting and breeding system can be established to promote the development and utilization of rice straw resources, the soil properties of paddy fields will be continuously improved and the sustainable development of paddy fields will be realized.

Earthworms are large soil animals that prefer dark, moist, organic soil. They are omnivorous with extensive dietary diversity and are known as "ecosystem engineers". Studies have shown that inoculation of earthworms in dryland ecosystems can improve physical properties [11,12], chemical properties [13,14] and biological properties [15,16] of soil, accelerate the decomposition of soil pollutants [17], reduce heavy metal activity [18] and promote crop growth [19–21]. However, studies on the symbiotic relationship between earthworms and rice are mainly concentrated in the laboratory [22,23], upland paddy fields [24–26] or rainfed paddy fields [27,28]; studies under irrigated paddy fields are scarce with the exception of Liang et al. [29]. The biggest technical difficulty is that the traditionally irrigated paddy environment during rice planting is not suitable for the growth of earthworms.

Southern China is the main rice-producing area in China and even the world, accounting for more than 75% of China's total output [30]. The paddy fields are mainly irrigated paddy fields. The symbiosis between rice and earthworms is not possible in shallowly irrigated paddy fields during most of the rice-growing season. With the straw returning to the field, the wide-ridge no-tillage direct-seeding rice cultivation model creates ideal conditions for the symbiosis of rice and earthworms (Figure 1A). Firstly, ditch irrigation is carried out in the wide-ridge paddy field, and water permeates from the furrow to the rhizosphere of the rice. There is no water on the surface of the paddy field, providing basic living space for earthworms. Secondly, the soil organic matter is rich in the paddies with straw returning, which provides nutrition for earthworms. Thirdly, the combination of a rice canopy and straw layer provides a relatively dark environment for earthworms. Fourthly, the suitable soil pH for rice growth is 6.0–7.5, which is consistent with that of earthworms. Fifthly, no-tillage direct seeding provides a guarantee for earthworms to continuously survive in paddy fields.

However, studies on the symbiotic system of irrigated rice and earthworms under a no-till wide-ridge cultivation model have not been reported yet. So, what happens when earthworms are inoculated into this ideal rice farming system? What will happen to paddy soil and rice growth? We hypothesized that earthworms could quickly adapt to this environment and reproduce, thereby improving soil physicochemical properties and rice growth. In this case, there would be theoretical support for introducing earthworms into irrigated paddy fields, and farmers in irrigated paddy fields would benefit from this sustainable rice farming technology.

In this study, we combined field and pot experiments, aimed to explore the adaptability of earthworms, soil properties (bulk density, porosity, organic matter content and pH) and rice growth characteristics (leaf area index, root number, dry matter weight and rice yield) in this symbiosis system relative to mono-cropping systems.



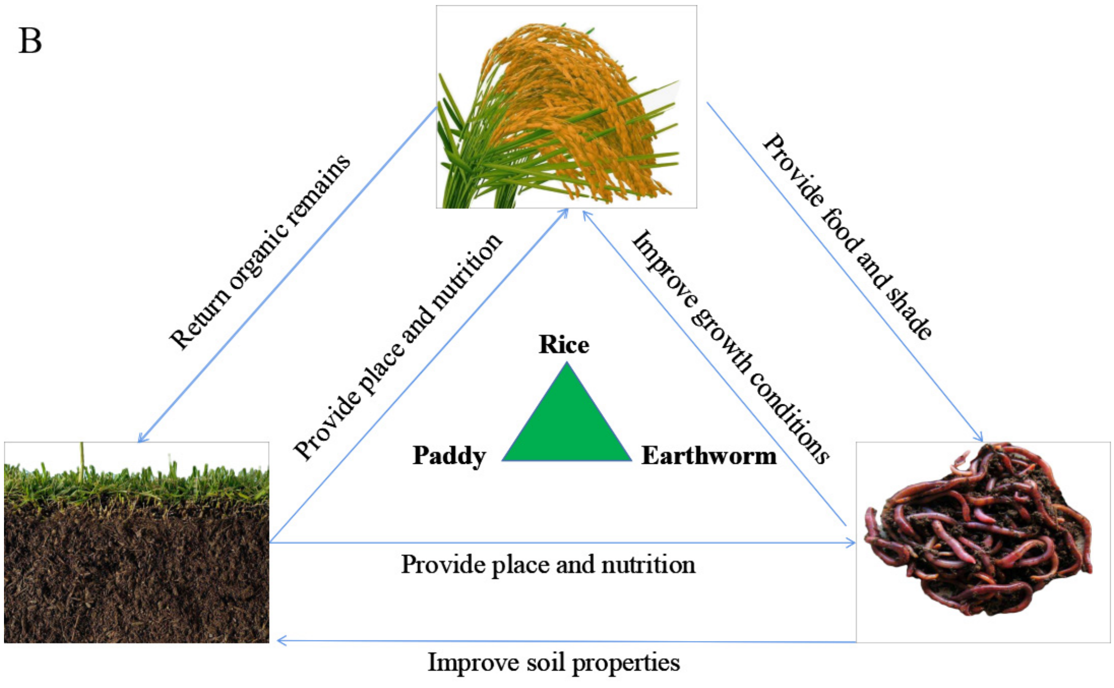


Figure 1. Rice–earthworm symbiosis system (**A**) and relationship between rice, earthworm and paddy soil (**B**).

2. Materials and Methods

2.1. Experiment Site

Both the field experiment and the pot experiment were conducted from May to September 2020 in Mingyue Village, Lukou Town, Changsha County, Hunan Province, China (28°40′38″ N, 113°29′48″ E). The region belongs to the subtropical monsoon climate, with an average annual temperature of 16–20 °C, sunshine duration of 1600–1800 h and frost-free period of 260–300 d. The average annual rainfall is 1472.9 mm, and the rainfall is concentrated from April to August. The paddy soil is clay type (Chinese classification) and the cultivation system is rice-rapeseed double cropping. The basic physical and chemical properties of soil can be seen in Table 1.

Table 1. Basic physical and chemical properties of soil before planting in the field experiment and pot experiment.

Experiment Type	Soil Depth (cm)	pH	Total Nitrogen (g/kg)	Total Phosphorus (g/kg)	Total Potassium (g/kg)	Available Nitrogen (mg/kg)	Available Phosphorus (mg/kg)	Available Potassium (mg/kg)	Organic Matter (g/kg)
Field experiment	0–5	5.23	0.14	0.36	7.8	151.73	5.86	130.0	23.18
	5–10	5.53	0.15	0.36	7.8	130.43	4.69	83.3	21.28
	10–20	5.65	0.14	0.32	8.0	139.77	6.38	70.0	18.44
Pot experiment	0–5	5.68	0.22	0.65	7.5	128.10	18.41	96.7	22.29
	5–10	5.63	0.19	0.61	7.7	137.78	16.38	120.0	22.01
	10–20	5.47	0.18	0.68	7.7	145.60	15.86	136.7	25.04

2.2. Experiment Design

2.2.1. Field Experiment

Randomized block design was used in the field experiment (Figure 2A). Three rice–earthworm symbiosis treatments were set up: low density (L: 30 g/m^2, about 92 earthworms), medium density (M: 60 g/m^2, about 184 earthworms) and high density (H: 90 g/m^2, about 276 earthworms); rice monoculture (CK) was used as the control treatment, and each treatment was repeated three times.

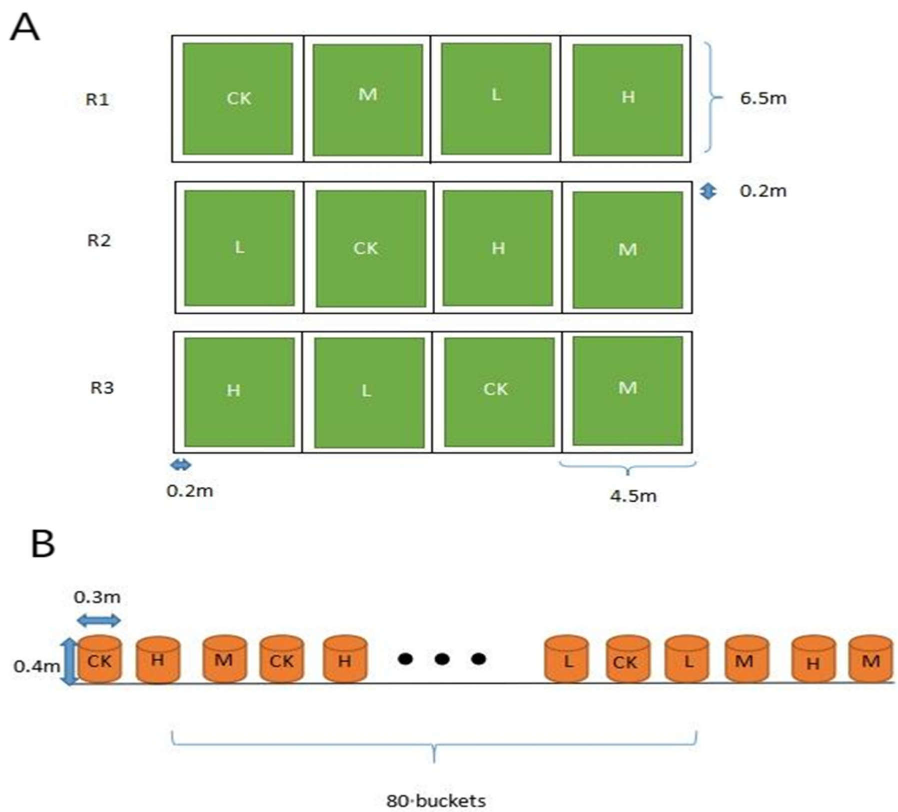


Figure 2. Experiment design: Randomized block design in the field experiment (**A**), completely randomized design in the pot experiment (**B**).

The preceding crop of the experiment area was rapeseed, and straw was fully covered and returned to the field. Each experiment plot was 6.5 m long and 4.5 m wide, drained and irrigated separately. Ditches with a width of 20 cm and a depth of 20 cm were excavated around each experiment plot. A bird-proof reflective tape was set above the experiment area.

No-tillage and wide-ridge were adopted for rice cultivation (Figures 1A and 3A). Conventional rice Nongxiang 32 was selected as the research material, which belongs to medium ripe medium indica rice, with whole growth period of 137.5 days and plant height of 126.4 cm. Rice was transplanted with row spacing of 20 cm and plant spacing of 25 cm, and 4 seedlings of rice were planted in each hole. The water level in the plot retreated 10 cm below the wide-ridge surface until the rice harvest a week after rice planting. Compound fertilizer (N:P_2O_5:K_2O = 15:15:15) of 562.5 kg/ha was applied as base fertilizer, urea was applied at 75 kg/ha as tillering fertilizer, foliar fertilizer of potassium dihydrogen phosphate was applied as grain fertilizer. *Bacillus thuringiensis* (8000 IU/UL, diluted 200 times) was applied to control *Cnaphalocrocis medinalis*.

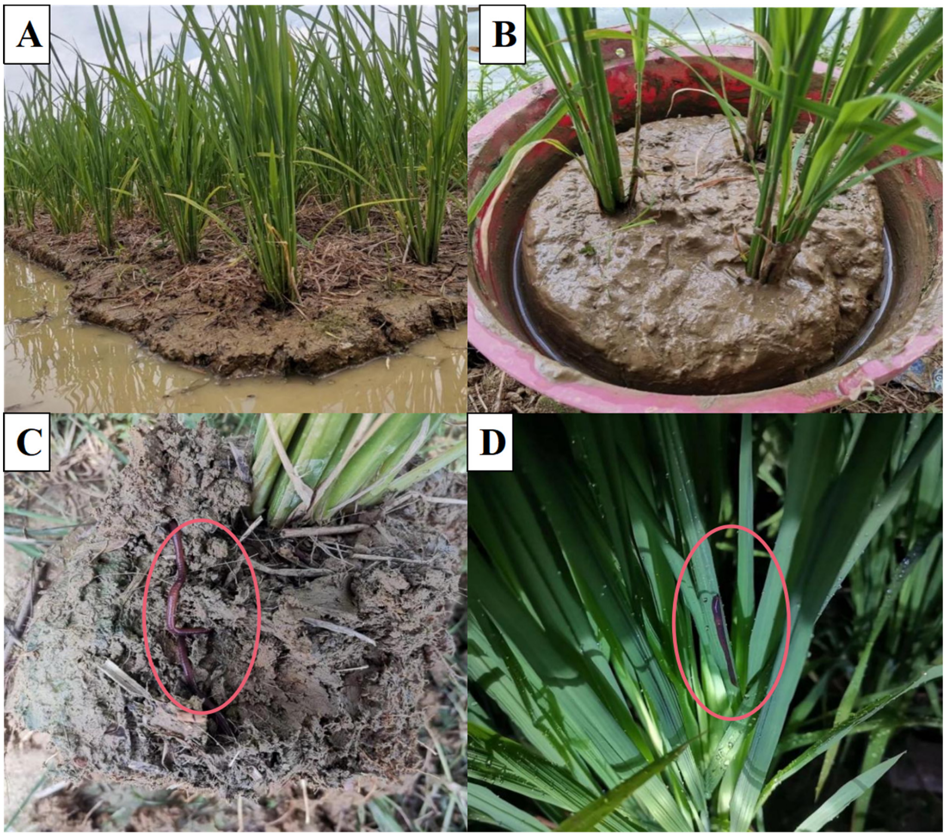


Figure 3. Wide-ridge cultivation and earthworms activities: wide-ridge cultivation in the field experiment (**A**), model of wide-ridge cultivation in the pot experiment (**B**), earthworms inhabit the rhizosphere of rice (**C**), earthworms climb onto rice leaves after rain (**D**).

Eisenia foetida was selected as research materials, with average body weight of 0.325 g, average body length of 5.623 cm and average body width of 0.239 cm. Earthworms were inoculated into the rice field two weeks after rice planting, and the inoculation time was about six o'clock pm. Earthworms were evenly scattered on the wide-ridge surface, then they could drill into the wide-ridge surface or the straw layer by themselves. Earthworms grow and reproduce naturally in paddy fields without artificial feeding.

2.2.2. Pot Experiment

The pot experiment used a completely randomized design, and the treatment was the same as the field experiment (Figure 2B). The number of earthworms in low density, medium density and high-density treatments was 6, 12 and 18 earthworms per bucket (diameter of 30 cm and height of 40 cm), respectively, and 20 buckets were set for each treatment.

Soil was collected from paddy fields, mixed and loaded into buckets; each bucket of soil was about 15 kg. A surrounding ditch with a depth of 15 cm and a width of 3 cm was excavated inside each bucket to simulate wide-ridge cultivation (Figure 3B). Each bucket planted 3 holes of rice, with equilateral triangle distribution. Earthworm inoculation, rice variety, rice transplanting time, water and fertilizer management and other cultivation measures were consistent with the field experiment.

2.3. Sampling and Measurement

2.3.1. Number and Distribution of Earthworms

In the field experiment, three soil samples (both length and width are 20 cm) were randomly selected from each plot at tillering stage (12 July), booting stage (31 July), full heading stage (16 August), filling stage (26 August) and maturity stage (13 September), and the number of earthworms in the 0–5 cm, 5–10 cm and 10–20 cm soil layers was recorded, respectively. By the way, only the number of adult earthworms was recorded. (Soil depth was determined by measuring with a ruler.) Earthworm density can be calculated through the below Equation (1). Twenty-five was the coefficient of soil sample area (0.04 m^2) converted into unit area (1 m^2):

$$\text{Earthworm density } (/\text{m}^2) = 25 \times \text{earthworm number in soil sample} \quad (1)$$

In pot experiment, three buckets were randomly selected from each treatment at tillering stage (15 July), booting stage (2 August), full heading stage (27 August), filling stage (4 September) and maturity stage (20 September), and the number of earthworms in the 0–5 cm, 5–10 cm and 10–20 cm soil layers was recorded, respectively. Earthworm density can be calculated through the below Equation (2). Fourteen was the coefficient of pot bucket area (0.07065 m^2) converted into unit area (0.04 m^2).

$$\text{Earthworm density } (/\text{m}^2) = 14 \times \text{earthworm number in bucket} \quad (2)$$

2.3.2. Physical and Chemical Properties of Soil

In the field experiment, soil samples of 0–5 cm, 5–10 cm and 10–20 cm were taken from each plot at each growth stage of rice under the five-point sampling method, and dried in the shade. Soil pH was measured by potentiometric method. Soil organic matter content was measured by potassium dichromate volumetric method [31]. After rice harvest, three points in each plot were selected to measure the soil bulk density of 0–5 cm, 5–10 cm and 10–20 cm by cutting ring method [32]. Soil porosity can be calculated through the below Equation (3).

$$\text{Soil porosity } (\%) = (1 - \text{soil bulk density}/2.65) \times 100\% \quad (3)$$

In the pot experiment, three buckets of rice were randomly taken from each treatment at each growth stage of rice. Soil samples of 0–5 cm, 5–10 cm and 10–20 cm were respectively taken and dried in the shade. Soil pH and organic matter were measured by the same method as the field experiment. At maturity stage of rice, three buckets of rice were randomly taken from each treatment to measure the soil bulk density and porosity of 0–5 cm, 5–10 cm and 10–20 cm by the same method as the field experiment.

2.3.3. Growth Characteristics of Rice

In the field experiment, three representative plants were randomly selected according to tiller dynamics in each plot at each growth stage of rice. We used a spade to grab the rice plants, cleaned them and brought them indoors. Total root number (/plant) and white root number (/plant) were measured by manual counting method. The leaf area meter (LICOR-3000, LI-COR Biotechnology, 4647 Superior Street, Lincoln, NE, USA) was used to measure the leaf area (cm^2/plant) of rice. The dry matter weight of each organ was weighed (g/plant) after the rice plants were dried to constant weight. At the maturity stage, three places were randomly selected from each plot, five consecutive rice plants were selected from each place to measure effective panicle number ($\times 10^4$/ha), and three representative plants were randomly selected and brought indoors. The total number of grains per panicle and seed-setting rate (%) was calculated by threshing rice plants separately and separating filled grains from empty grains. Then 1000 grains were randomly selected from the filled grains and dried to constant weight in an oven at 80 °C to calculate 1000-grain weight (g). Finally, theoretical yield (t/ha) can be calculated through the below Equation (4) [33]. At the same time, three places were randomly selected from each plot, and 1 m^2 rice was cut from each place and threshed, respectively. After drying, the rice was weighed and measured for moisture content, so as to calculate the actual yield (t/ha) with 13.5% moisture content.

$$\text{Theoretical yield (t/ha)} = \text{Effective panicles } (\times 10^4/\text{ha}) \times \text{Grain number per panicle} \times \text{Seed setting rate (\%)} \times 1000 - \text{grain weight (g)} \times 10^{-7} \quad (4)$$

In the pot experiment, three buckets of rice were randomly taken from each treatment at each growth stage, and the rice plant was cleaned and brought indoors. The leaf area, total root number, white root number and dry matter weight of each organ were measured by the same method as the field experiment. The theoretical yield of rice was measured with three buckets of samples taken at maturity stage.

2.4. Data Processing

Data were analyzed using SPSS statistical software (version 23). Data are expressed as mean plus or minus one standard error. Treatment differences were assessed using one-way ANOVAs, followed by Fisher's least significant difference post hoc tests to identify significant ANOVA results. Data at different soil depths and different rice growth stages were analyzed separately.

3. Results

3.1. Number and Distribution of Earthworms

It can be seen from Table 2 that earthworms were mainly distributed in the 0–5 cm soil layer in both field and pot experiments. The number of earthworms both showed a decreasing trend, and the biggest decreasing range was both from the tillering stage to the booting stage, the decreasing range from large to small was L, H and M, the decreasing ranges were 78.2–89.7% in the field experiment and 37.5–75.0% in the pot experiment.

The survival condition of earthworms in the pot experiment was better than that in the field experiment. In the pot experiment, earthworms co-existed with rice in L the treatment until the filling stage, and in the M and H treatment until the maturity stage. While in the field experiment, earthworm and rice co-existed only until the booting stage. The distribution of earthworms in the pot experiment was wider than that in the field experiment (a small number of earthworms burrow into the 5–10 cm soil layer in the pot experiment).

Table 2. The number of earthworms in the field experiment and pot experiment at each growth stage of rice (/m^2).

Experiment Type	Treatment	Soil Depth (cm)	TS	BS	FHS	FS	MS
Field experiment	CK	0–5	0	0	0	0	0
		5–10	0	0	0	0	0
		10–20	0	0	0	0	0
	L	0–5	97	10	0	0	0
		5–10	0	0	0	0	0
		10–20	0	0	0	0	0
	M	0–5	206	45	0	0	0
		5–10	0	0	0	0	0
		10–20	0	0	0	0	0
	H	0–5	265	55	0	0	0
		5–10	0	0	0	0	0
		10–20	0	0	0	0	0

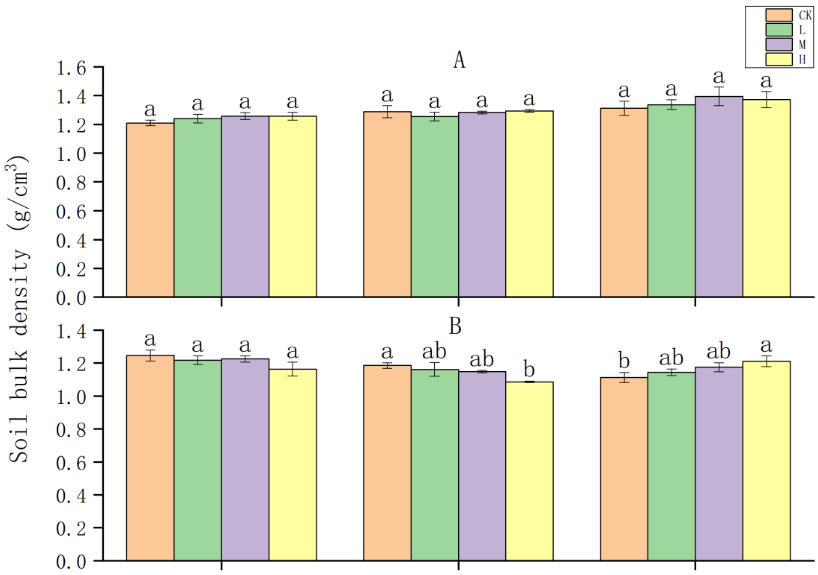
Table 2. *Cont.*

Experiment Type	Treatment	Soil Depth (cm)	TS	BS	FHS	FS	MS
Pot experiment	CK	0–5	0	0	0	0	0
		5–10	0	0	0	0	0
		10–20	0	0	0	0	0
	L	0–5	57	14	14	14	0
		5–10	0	0	0	0	0
		10–20	0	0	0	0	0
	M	0–5	113	71	14	14	14
		5–10	14	0	0	0	0
		10–20	0	0	0	0	0
	H	0–5	198	71	57	28	28
		5–10	14	0	0	0	0
		10–20	0	0	0	0	0

Note: CK, L, M and H represent 0 g/m^2, 30 g/m^2, 60 g/m^2 and 90 g/m^2 earthworm inoculation treatments, respectively. TS, BS, FHS, FS and MS represent tillering stage, booting stage, full heading stage., filling stage and maturity stage of rice, respectively.

3.2. Effects of Earthworm Inoculation on Soil Physical and Chemical Properties

It can be seen from Figure 4 that there was no significant difference in soil bulk density and porosity of all soil layers in the field experiment (Figure 4A,C). In the pot experiment, the soil bulk density of H treatment in 5–10 cm was significantly lower than that under the CK treatment, but the soil bulk density of H treatment in 10–20 cm was significantly higher than that under the CK treatment (Figure 4B). Meanwhile, the soil porosity of H treatment in 5–10 cm was significantly higher than that under the CK treatment, but the soil porosity of H treatment in 10–20 cm was significantly lower than that under the CK treatment (Figure 4D).



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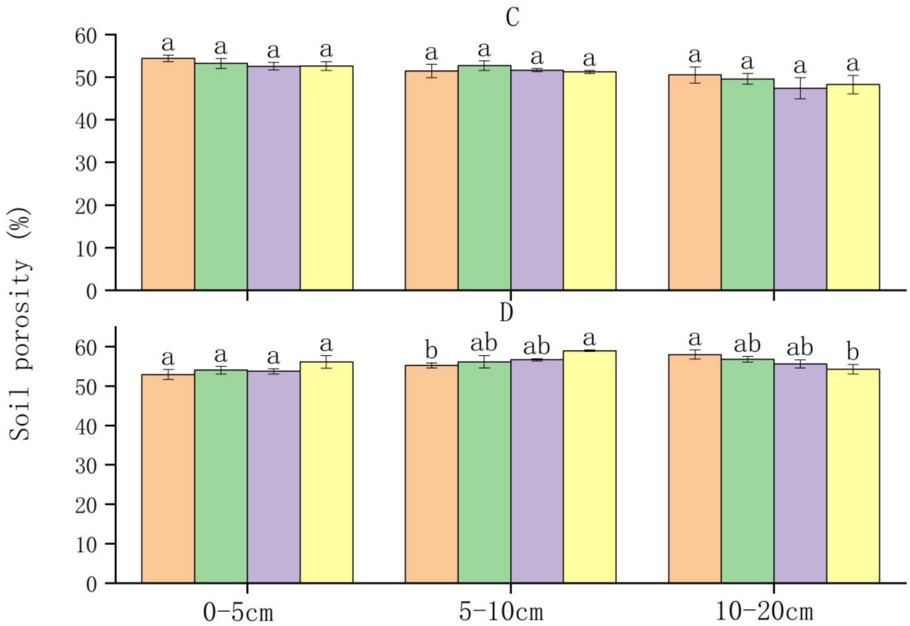
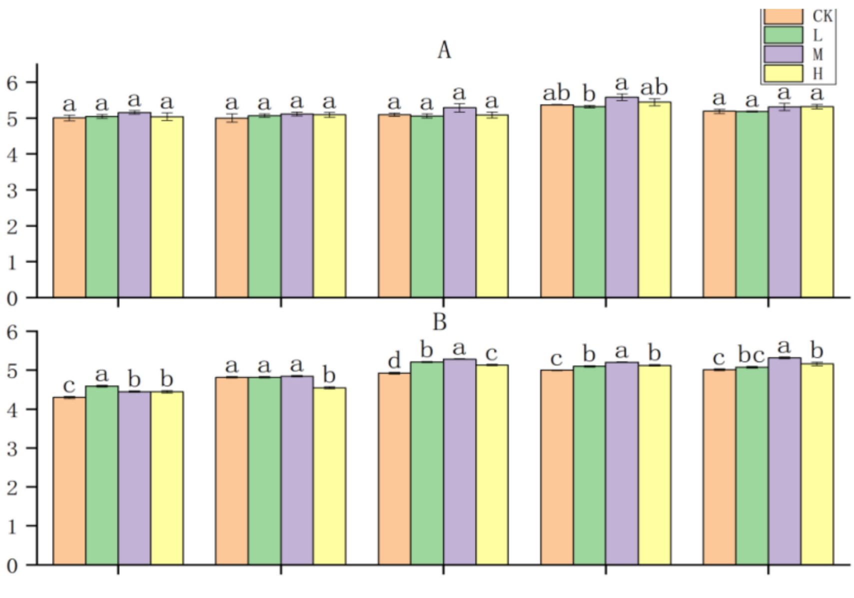


Figure 4. Bulk density (**A**,**B**) and porosity (**C**,**D**) at each soil depth under each treatment after rice harvest in the field experiment (**A**,**C**) and pot experiment (**B**,**D**): CK, L, M and H represent 0 g/m^2, 30 g/m^2, 60 g/m^2 and 90 g/m^2 earthworm inoculation treatments, respectively. Labels on the x-axes represent different soil depths. The bars are standard errors of the mean. Different lowercase letters above the bars indicate that the two means are significantly different from each other (Fisher's LSD; $p < 0.05$). The same is seen below.

As can be seen from Figure 5, in the field experiment (Figure 5A,C,E), although there was no significant difference in the pH at the rice maturity stage, the pH of 0–5 cm, 5–10 cm and 10–20 cm soil layers under M and H treatments increased by 2.31–2.50%, 3.62–6.16% and 6.87–7.78%, respectively, compared with that under CK treatment. While in the pot experiment (Figure 5B,D,F), the pH of the rice–earthworm symbiosis treatment and CK treatment were significantly different in most soil layers at most growth stages of rice. At the maturity stage of rice, the pH of the rice–earthworm symbiosis treatment in 0–5 cm and 5–10 cm soil layers was significantly higher than that of CK treatment, among which the effect of M and H treatment is more obvious.



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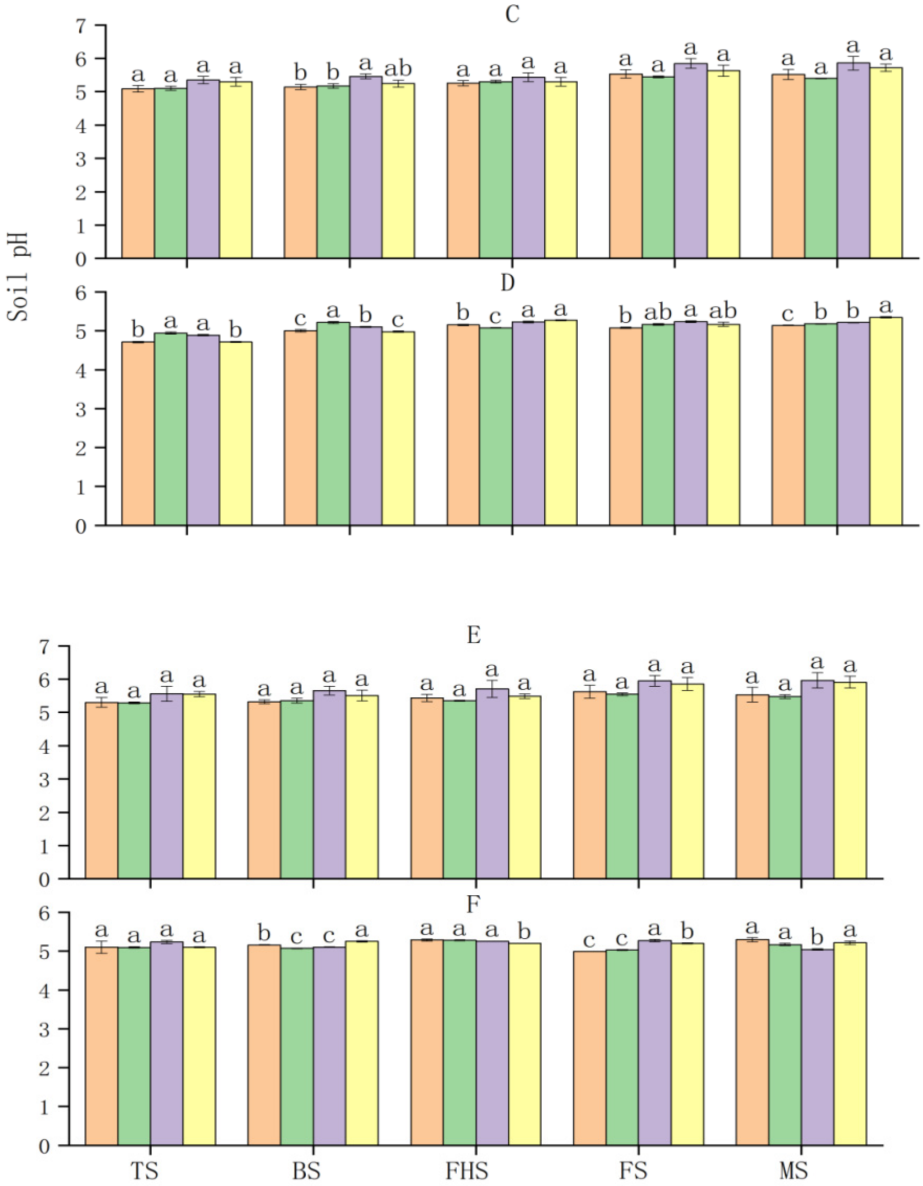

Figure 5. pH of 0–5 cm soil depth (**A**,**B**), 5–10 cm soil depth (**C**,**D**) and 10–20 cm soil depth (**E**,**F**) under each treatment at each growth stage of rice in the field experiment (**A**,**C**,**E**) and pot experiment (**B**,**D**,**F**). Different lowercase letters above the bars indicate that the two means are significantly different from each other (Fisher's LSD; $p < 0.05$).

It can be seen from Figure 6 that in the field experiment (Figure 6A,C,E), there was no significant difference in organic matter content of all soil layers in all treatments and stages. While in the pot experiment (Figure 6B,D,F), the organic matter content in 5–10 cm and 10–20 cm soil layers under the rice–earthworm symbiosis treatment was higher than that under the CK treatment at the maturity stage of rice, and significant differences were achieved in the 5–10 cm soil layer under the L treatment, H treatment and the 10–20 cm soil layer under the L treatment.

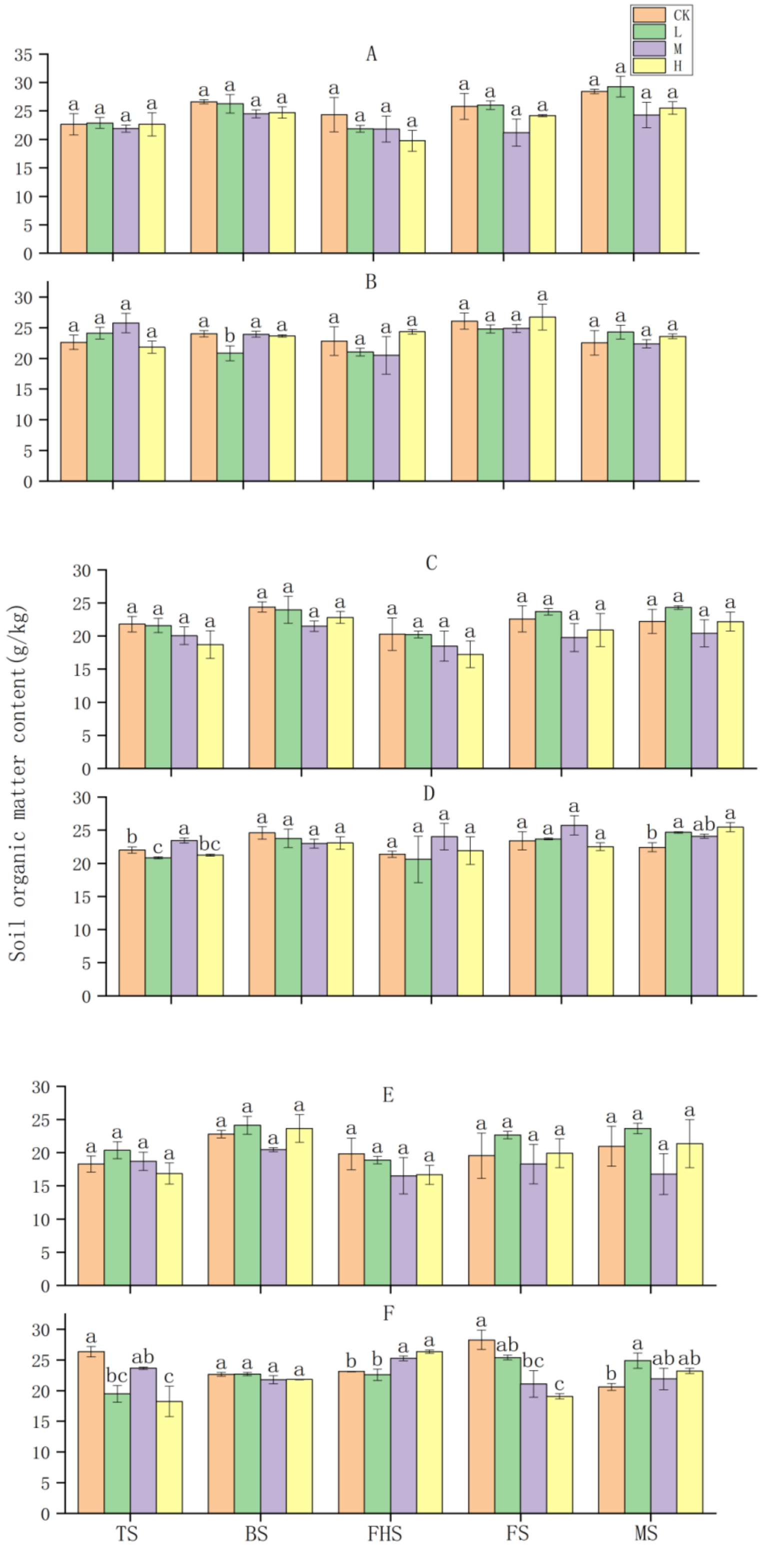

Figure 6. Organic matter content of 0–5 cm soil depth (**A**,**B**), 5–10 cm soil depth (**C**,**D**) and 10–20 cm soil depth (**E**,**F**) under each treatment at each growth stage of rice in the field experiment (**A**,**C**,**E**) and pot experiment (**B**,**D**,**F**). Different lowercase letters above the bars indicate that the two means are significantly different from each other (Fisher's LSD; $p < 0.05$).

3.3. Effects of Earthworm Inoculation on Growth Characteristics of Rice

It can be seen from Figure 7 that in both the field experiment (Figure 7A) and the pot experiment (Figure 7B), the leaf area index (LAI) of all treatments showed a trend of increasing first and then decreasing, and reached the peak at booting stage or full heading stage. In the field experiment, although there was no significant difference in the LAI at all stages, the LAI of rice–earthworm symbiosis treatment was 27.8–52.0% higher than that of CK treatment at the filling stage and maturity stage. In the pot experiment, the LAI of each treatment had little difference from tillering stage to the filling stage. However, from the filling stage to the maturity stage, the LAI of CK treatment decreased significantly, resulting in the LAI of CK treatment at the maturity stage being significantly lower than that of other treatments.

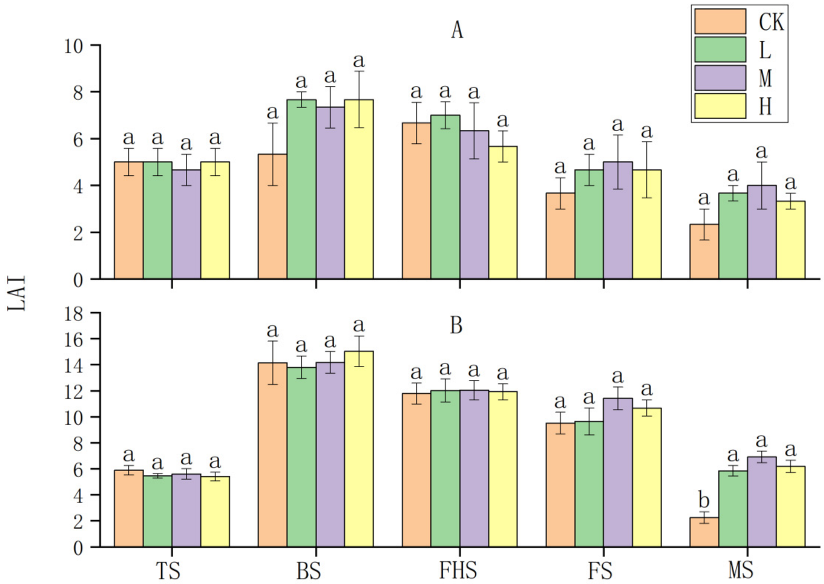

Figure 7. Leaf area index (LAI) under each treatment at each growth stage of rice in the field experiment (**A**) and pot experiment (**B**). Different lowercase letters above the bars indicate that the two means are significantly different from each other (Fisher's LSD; $p < 0.05$).

It can be seen from Figure 8 that the total root number of rice in both the field experiment (Figure 8A) and the pot experiment (Figure 8B) increased first and then tended to be stable. There was no significant difference in total root number except at the full heading stage of the pot experiment. In the field experiment, there was no significant difference in white roots number between each treatment in each stage (Figure 9A). While in the pot experiment, the white root number of M and H treatments from the full heading stage to the filling stage was significantly higher than CK (Figure 9B).

As can be seen from Figures 10–13, no matter whether the field experiment (Figures 10A, 11A, 12A and 13A) or pot experiment (Figures 10B, 11B, 12B and 13B), there was no significant difference in the dry matter weight of each rice organ under each treatment except at the tillering stage of field experiment and the filling stage of the pot experiment. In the field experiment, the dry weight of stem and leaf under the M treatment at the tillering stage was significantly higher than that of CK. In the pot experiment, the dry weight of root and stem under the M treatment at the filling stage was significantly higher than that of CK.

As can be seen from Table 3, in the field experiment, although there was no significant difference, the theoretical yield of L treatment increased by 24.4% compared with CK, and the actual yield of L treatment and M treatment increased by 35.1% and 38.8% compared with CK, respectively. In the pot experiment, the theoretical yield of L, M and H treatments increased by 6.7%, 6.7% and 15.4%, respectively, compared with CK, among which H treatment reached a significant difference. The 1000-grain weight of M treatment was significantly higher than that of CK. Compared with CK, the seed setting rate of L, M and H treatments increased by 8.6%, 9.6% and 11.5%, respectively.

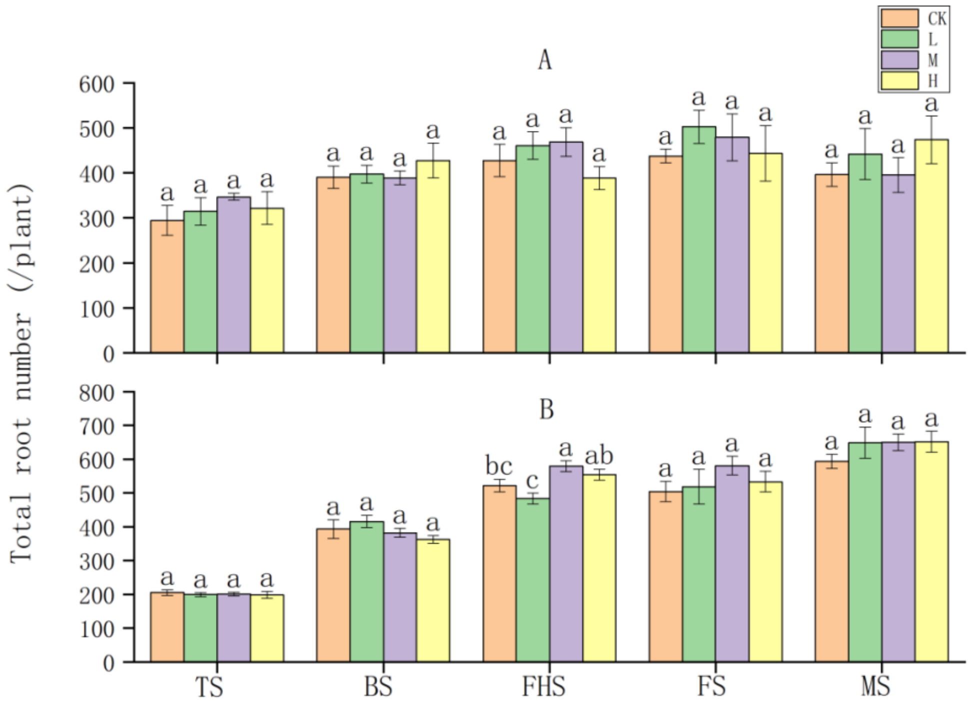

Figure 8. Total root number (/plant) under each treatment at each growth stage of rice in the field experiment (**A**) and pot experiment (**B**). Different lowercase letters above the bars indicate that the two means are significantly different from each other (Fisher's LSD; $p < 0.05$).

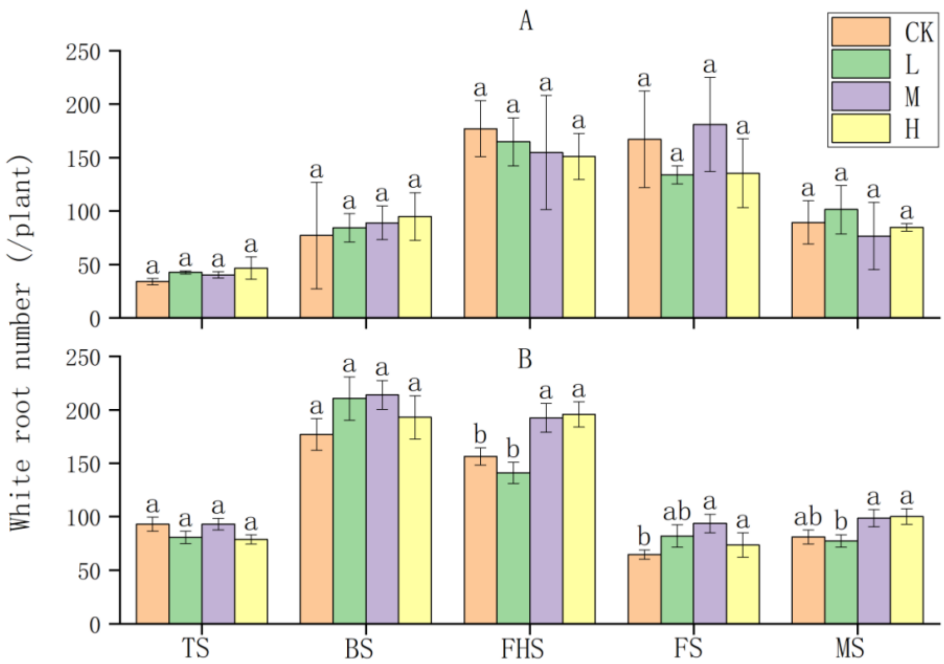

Figure 9. White root number under each treatment at each growth stage of rice in the field experiment (**A**) and pot experiment (**B**). Different lowercase letters above the bars indicate that the two means are significantly different from each other (Fisher's LSD; $p < 0.05$).

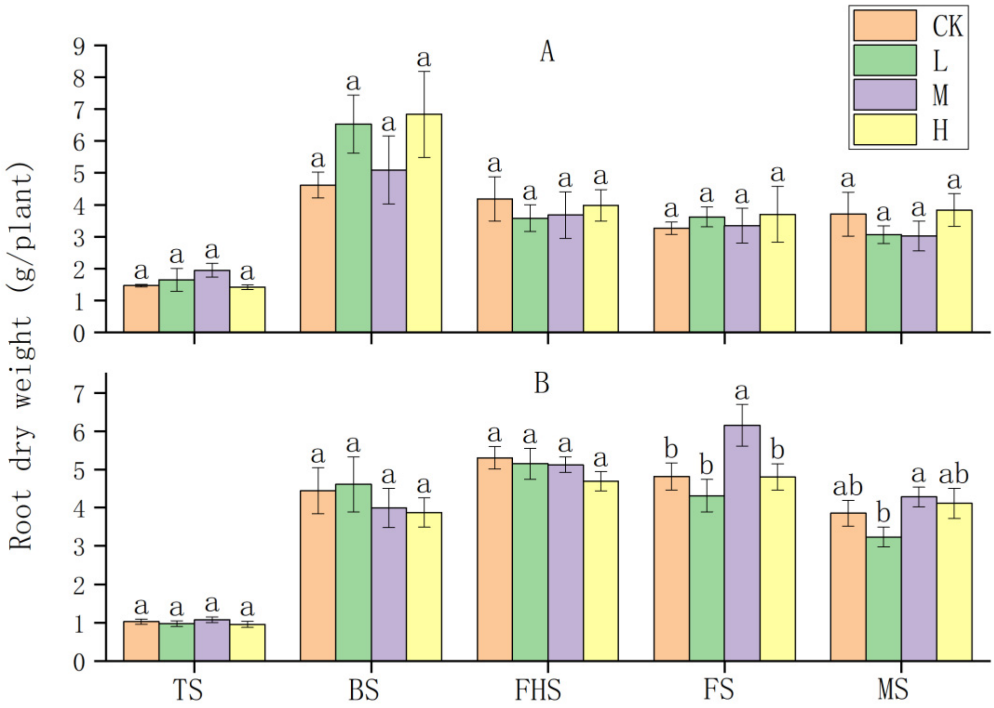

Figure 10. Root dry weight under each treatment at each growth stage of rice in the field experiment (**A**) and pot experiment (**B**). Different lowercase letters above the bars indicate that the two means are significantly different from each other (Fisher's LSD; $p < 0.05$).

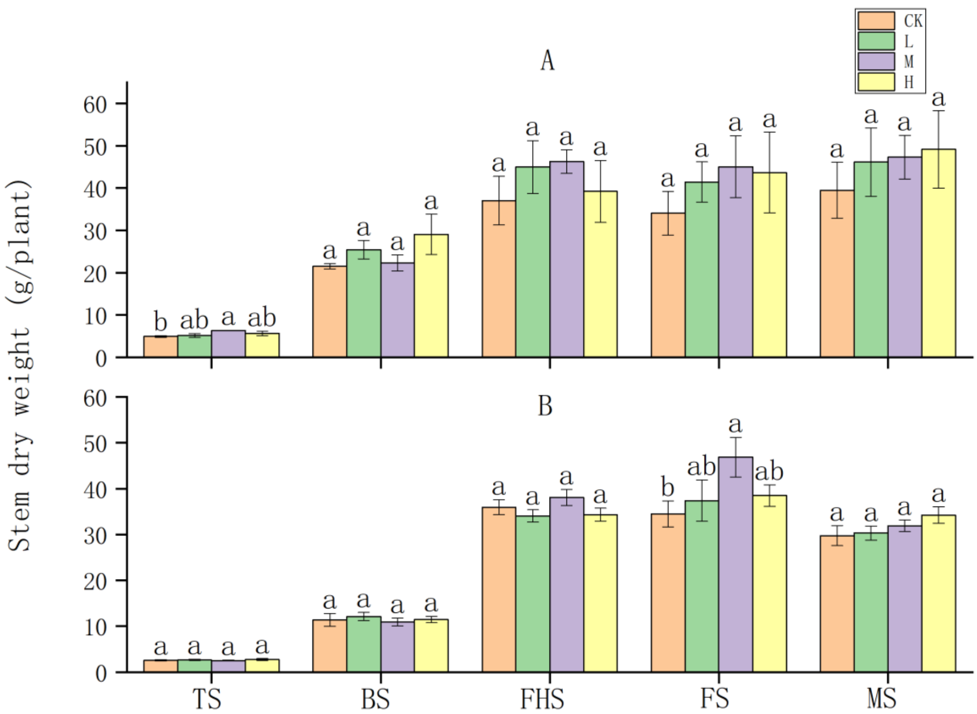

Figure 11. Stem dry weight under each treatment at each growth stage of rice in the field experiment (**A**) and pot experiment (**B**). Different lowercase letters above the bars indicate that the two means are significantly different from each other (Fisher's LSD; $p < 0.05$).

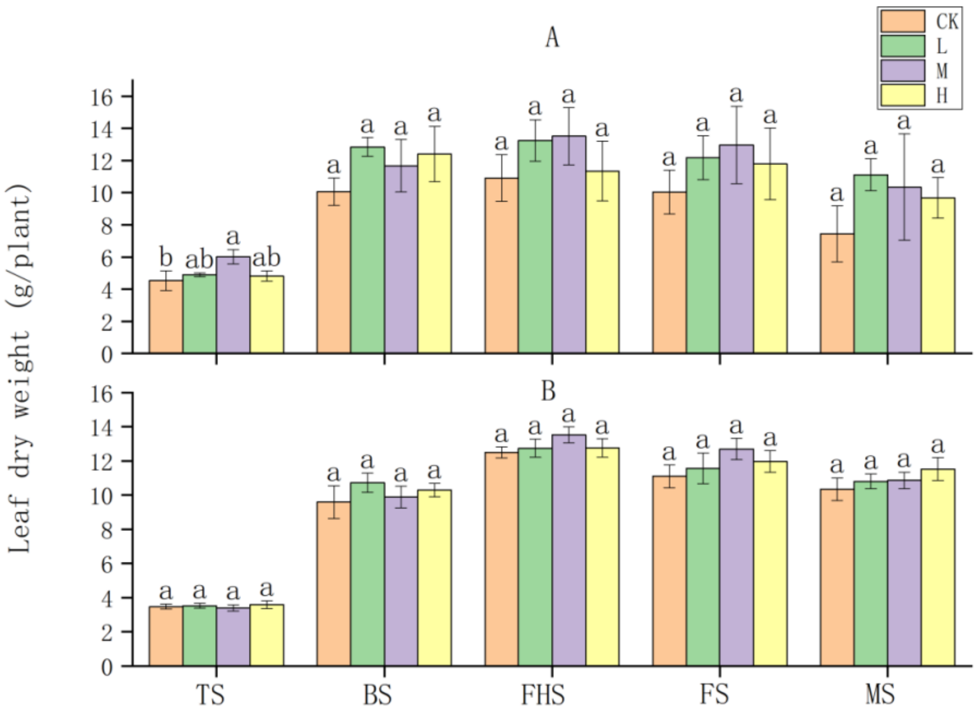

Figure 12. Leaf dry weight under each treatment at each growth stage of rice in the field experiment (**A**) and pot experiment (**B**). Different lowercase letters above the bars indicate that the two means are significantly different from each other (Fisher's LSD; $p < 0.05$).

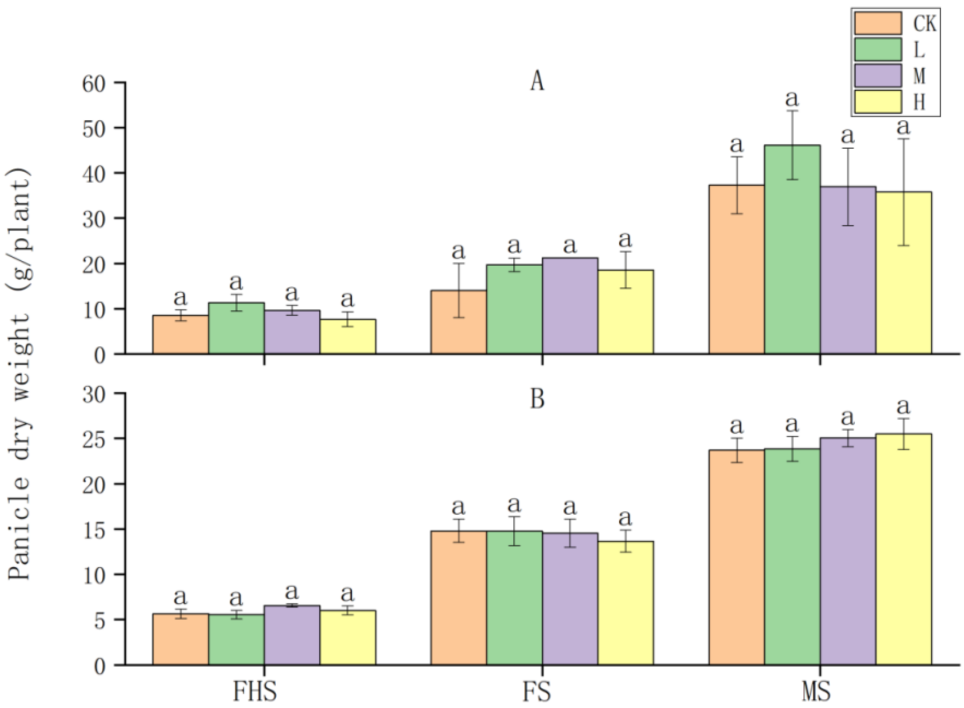

Figure 13. Panicle dry weight under each treatment at each growth stage of rice in the field experiment (**A**) and pot experiment (**B**). Different lowercase letters above the bars indicate that the two means are significantly different from each other (Fisher's LSD; $p < 0.05$).

Table 3. Rice yield and yield composition under each treatment in the field experiment and pot experiment.

Experiment Type	Treatment	Effective Panicles (×104/ha)	Grain Number per Panicle	Seed Setting Rate (%)	1000-Grain Weight (g)	Theoretical Yield (t/ha)	Actual Yield (t/ha)
Field experiment	CK	206.77 ± 6.67 a	176.83 ± 6.67 a	73.07 ± 3.41 a	24.14 ± 0.55 a	6.57 ± 1.19 a	5.33 ± 0.77 a
	L	246.80 ± 26.70 a	197.67 ± 26.70 a	73.93 ± 2.62 a	22.67 ± 0.90 a	8.17 ± 1.33 a	7.20 ± 1.04 a
	M	226.77 ± 24.04 a	179.23 ± 24.04 a	66.87 ± 8.90 a	23.56 ± 0.45 a	6.70 ± 1.89 a	7.40 ± 0.38 a
	H	213.43 ± 29.06 a	174.70 ± 29.06 a	66.47 ± 10.44 a	23.37 ± 0.56 a	6.17 ± 2.28 a	5.27 ± 2.02 a
Pot experiment	CK	448.22 ± 16.00 a	130.33 ± 8.08 a	65.47 ± 4.51 a	23.67 ± 0.27 b	8.90 ± 0.06 b	
	L	448.22 ± 17.50 a	126.47 ± 4.51 a	71.13 ± 0.67 a	23.70 ± 0.15 b	9.50 ± 0.47 ab	
	M	424.63 ± 15.82 a	129.17 ± 3.07 a	71.77 ± 1.09 a	24.40 ± 0.25 a	9.50 ± 0.31 ab	
	H	471.81 ± 17.97 a	129.23 ± 8.65 a	73.00 ± 5.76 a	23.53 ± 0.12 b	10.27 ± 0.32 a	

Note: CK, L, M and H represent 0 g/m^2, 30 g/m^2, 60 g/m^2 and 90 g/m^2 earthworm inoculation treatments, respectively. The lowercase letters indicate that the two means are significantly different from each other. (Fisher's LSD; $p < 0.05$.)

4. Discussion

4.1. Adaptability of Earthworm

In the rice–earthworm symbiosis system, the growth, reproduction and continuation of earthworms are the most important steps to maximize the benefit of the system, and it is also the most difficult step. In this study, the number of earthworms in both pot experiment and field experiment showed a decreasing trend, but the decrease in the pot experiment was smaller. Meanwhile, earthworms were mainly distributed in the 0–5 cm soil layer.

These circumstances are the result of both earthworm species and habitat. The earthworm in this study is *Eisenia foetida*, which belongs to the surface habitat type and likes to eat animal manure [34]. However, plant residue was more common in rice paddy soil, so the number of earthworms decreased continuously and was mainly distributed in the 0–5 cm soil layer. The no-tillage soil in the field experiment was relatively compact, while the soil in the pot experiment was dug up and loose, which was more favorable for earthworms to burrow into the soil and avoid predators on the surface. Accordingly, the survival condition of earthworms in the pot experiment was better than that in the field experiment.

Therefore, it is necessary to optimize the species and habitat of earthworms to realize the maximum benefit of rice–earthworm symbiosis. Firstly, soil-feeding earthworms, such as *Pheretima guillemi*, should be given priority [35]. Secondly, soil should be properly tilled before earthworm inoculation, to help earthworms better burrow into the soil and settle down.

4.2. Earthworm Inoculation Improved Paddy Soil Properties

Soil degradation is a serious challenge for rice paddy in China and even the world, which seriously affects crop growth and is not conducive to ensuring food security. Earthworms are a kind of large soil animal, known as "excellent underground workers", and play a positive role in soil quality regulation. A large number of previous studies have shown that earthworm inoculation in dryland ecosystem can loosen soil [36], alleviate soil acidification [37] and salinization [12], make soil pH tend to be neutral, and also increase soil organic matter content [37], greatly improving soil quality. Meantime, studies on earthworm inoculation in rice paddy soil also show similar effects [27,28].

Our study showed that earthworm inoculation in the pot experiment significantly loosened soil of 5–10 cm but tightened soil of 10–20 cm, increased soil pH of 0–10 cm, and increased soil organic matter content of 5–20 cm, but there was no significant effect in the field experiment. These results are consistent with the condition of earthworms in the pot experiment and field experiment—earthworms survived better in the pot experiment and were mainly inhibited in the surface soil. Therefore, significant effects mainly occurred in the pot experiment and positive effects mainly occurred in the surface soil. Our results are similar to previous studies but are more detailed at different soil depths.

The burrowing behavior of earthworms and the good structure of earthworm manure are very beneficial to loosening soil [38] The optimum pH of most earthworm varieties is nearly neutral [37] (*Eisenia foetida* is 6.5 [39]), and most earthworms have calcium glands in their bodies.

Meanwhile, the surface mucus and vermicompost of earthworms contain a large number of active groups and adhesives [37]. Therefore, earthworms will utilize the function of calcium glands in their bodies, together with the secretion of mucus and vermicompost, to regulate the acidic soil until the pH is suitable for their survival and reproduction. Earthworms feed on soil mixtures and excrete them in the form of vermicompost, which is rich in organic matter [38,40]; it also provides materials for the propagation of soil microorganisms, further accelerating the degradation of animal and plant residues, and greatly replenishing the soil organic matter.

However, studies have found that different species of earthworms have different effects on soil [41]. Research has shown that earthworms in paddy fields may drill broke the hardpan. At the same time, earthworms may damage the structure of the soil structure near the root of rice, which potentially causes the rice to lodge when a disaster occurs [42]. Therefore, when selecting earthworm species for a rice–earthworm symbiosis system, their effects on soil quality in paddy fields should also be considered.

4.3. Earthworm Inoculation Improved Irrigated Rice Growth

Numerous previous studies have shown that earthworm inoculation increases the yield of spinach [43], peanut [44], upland rice [26], rainfed rice [27,28] and other crops. However, only Liang et al. confirmed the effect of earthworm inoculation on irrigated rice yield increasing through the rice ridge planting experiment [29].

Our study showed that earthworm inoculation in the pot experiment significantly increased the irrigated rice yield by 15.39%, because of a significant increase in the LAI and grain weight at the maturity stage, and a significant improvement in white root at the full heading and filling stage. However, in the field experiment, earthworm inoculation did not generate a significant effect on rice yield owing to the earthworms' low survival rate. A study by Liang et al. showed increased rice yield with earthworm inoculation benefiting from a significant improvement in dry matter weight and total root number [29], which are different from our results. This may be due to differences in fertilizer application and tillage methods.

After earthworms are inoculated in paddy soil, they burrow, feed, defecate and secrete body fluids in soil, which are beneficial for loosening soil [41], decomposing animal and plant residues [45], releasing soil nutrients [19,46] and promoting microbial reproduction [44]. At the same time, vermicompost also contains a large number of phytohormone substances [38], which are beneficial for stimulating the better growth of rice. In a word, earthworms provide a good soil environment for rice growth, which is beneficial to rice yield formation.

The various activities of earthworms, such as the decomposition of plant and animal residues and the release of soil nutrients, are all carried out by microorganisms as vectors, and these activities, in turn, affect the abundance and diversity of microorganisms. Therefore, it is necessary to explore the mechanism of rice yield formation in the rice–earthworm symbiosis system from the perspective of microorganisms. In addition, in order to optimize the benefits of this ecosystem, it is necessary to study the species and density of earthworms that are suitable for paddy soil, the daily management of earthworms after inoculation and how they cope with adversity.

4.4. Prospect of Irrigated Rice–Earthworm Symbiotic System

With the development of irrigated rice farming, light and water-saving agriculture is in demand. Therefore, no-tillage cultivation is an important trend in China and across the world [47]. However, how to effectively solve the problems of crop straw disposal and soil compaction under no-tillage cultivation is the key. A good strategy is to change the flat paddy into wide-ridge paddy and to inoculate earthworms to realize rice–earthworm symbiosis.

At the same time, the favorable structure formed by the wide-ridge also creates the possibility to improve the biodiversity and the economic benefit of the rice field [48]. On the one hand, the ditch could provide a space for small fish, such as crucian carp, carp and so on. On the other hand, the shady and waterless wide-ridge surface is suitable for a natural chicken and duck farm. Chickens and ducks also reward the rice field by catching insects, eating grass and defecating. In this way, a diversified paddy ecosystem with rice, chickens, ducks, earthworms and fishes can be formed, so as to achieve a high yield, high quality and sustainable paddy field.

5. Conclusions

Under the wide-ridge cultivation pattern, irrigated rice and earthworm symbiosis significantly improved soil properties and rice growth in the pot experiment. However, in the field experiment, earthworm inoculation did not generate a significant effect owing to the earthworms' low survival rate. Therefore, we suspect that the survival status of earthworms plays an important role in the overall benefits of the symbiotic system, and we propose that earthworm species optimization and habitat improvement should be the main direction of follow-up research.

This study confirmed the beneficial effect of earthworm inoculation on an irrigated rice paddy ecosystem, which could provide a research basis for introducing earthworms into this ecosystem, realizing sustainable development of rice cultivation, and guaranteeing world food security. However, this study was only conducted for one year, which is one of its deficiencies. Therefore, a long-term positioning experiment is required for subsequent research.

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