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Alterations in Soil Bacterial Community and Its Assembly Process within Paddy Field Induced by Integrated Rice–Giant River Prawn (*Macrobrachium rosenbergii*) Farming

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Abstract: Integrated rice–aquatic animal farming has become a vital strategy for enhancing food security. To assess the effects of integrated rice–giant river prawn (*Macrobrachium rosenbergii*) farming (IRPF) on agricultural ecosystems, we used 16S rRNA gene sequencing to analyze soil bacterial communities in comparison with traditional rice monoculture (RM). Our study revealed that the IRPF did not significantly affect the diversity of the soil bacterial community. However, during the initial culture stage, IRPF markedly increased the relative abundance of the phylum candidate division NC10 and the genus *Candidatus Methylomirabilis*, enhancing nitrogen-cycling-related functions within the bacterial community. Additionally, IRPF reduced the complexity and stability of these communities in the early to middle culture stages. While stochastic processes usually dominate the assembly of these communities, IRPF restricted bacterial migration and reduced the influence of these stochastic processes. Furthermore, IRPF had a significant impact on environmental factors within paddy soils, strongly correlating with shifts in bacterial communities, particularly through variations in soil nitrite concentration. In conclusion, the influence of IRPF on the bacterial community in paddy soils was primarily observed during the early and middle culture stages, and the impacts of IRPF on the soil bacterial community were primarily driven by environmental changes, especially soil nitrite concentration. These findings provide theoretical insights and a reference for understanding the microbiological impacts of different integrated rice–fish farming systems on agricultural ecosystems.

Keywords: paddy soil; giant river prawn; *Macrobrachium rosenbergii*; soil bacterial community; integrated rice–fish farming

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1. Introduction

Under the constraints of limited land and freshwater resources, meeting the growing population's food demands while ensuring environmental protection poses a significant challenge to global agriculture [1]. Integrated rice–aquatic animal farming has emerged as a viable solution to address these concerns, as it can enhance resource utilization efficiency and agricultural productivity by establishing a mutually beneficial relationship between rice and aquatic animals, thereby providing additional high-quality protein [2–5]. Integrated rice–fish farming has a long history in Asia, especially in China, dating back to as early as AD 220 [6]. In recent years, integrated rice–aquatic animal farming has gained popularity across China and other Asian countries [3,7–9]. In 2015, the total area devoted to integrated rice–fish farming in China was approximately 1.50 million hectares [10]. By 2022, this agricultural practice had expanded to 2.86 million hectares, with rice and aquatic product yields reaching 21.5 million tons and 3.87 million tons, respectively [7]. Additionally, Jiangsu province ranks fifth in China in terms of integrated rice–fish farming area, making it an important province for implementing this agricultural practice [7]. Giant river prawn,

Macrobrachium rosenbergii, is an important species in integrated rice–aquatic animal farming, known for its favorable taste, high nutritional value, and significant economic benefits, making it highly popular among consumers [7,11,12]. However, research on the ecological effects of the integrated rice–giant river prawn farming is still very limited.

Complex interrelationships and interactions occur in the integrated rice–aquatic animal farming systems among the rice, aquatic animals, environmental microbiome, and environment factors [13–15]. Paddy soil is the most complex habitat, harboring the richest and densest bacterial populations known to date [16–19]. Soil bacteria are part of a community where continuous interactions occur both within their species and with other species [20–22]. Bacteria play a crucial role in agricultural production by facilitating nutrient cycling, improving soil structure, enhancing soil fertility, promoting plant growth, strengthening plant immunity, and increasing plant resilience to adverse conditions [23–28]. Meanwhile, bacterial communities are an essential and indispensable indicator for assessing changes in agricultural ecosystems, helping to deepen our understanding of their ecological status [29–31]. In agricultural ecosystems, various production or management methods can cause a variety of environmental changes, thereby affecting bacterial communities [15,32–35]. Therefore, it is crucial to understand how agricultural ecosystems react and adjust to environmental changes caused by different production or management patterns, and to reveal the spatiotemporal dynamics of bacterial communities. Studies on the impact of integrated rice–aquatic animal farming on soil bacterial communities are relatively limited, especially for the integrated rice–giant river prawn farming system.

To address the need for more data on specific changes to soil bacterial communities under integrated farming, we utilized 16S rRNA gene sequencing technology to assess differences in soil bacterial community structure and soil physicochemical properties between integrated rice–prawn farming and traditional rice monoculture. Our objectives were (1) to elucidate the effects of the integrated rice–prawn farming on the composition, diversity, function, stability, and assembly process of the soil bacterial community and (2) to clarify the impact of this agricultural pattern on soil environmental factors and their inter-relationships with the soil bacterial community. We hope to provide scientific data that support the sustainable development of agricultural production patterns through insights into the soil microbiome.

2. Materials and Methods

2.1. Field Experiment and Sample Collection

This study was conducted at the scientific research base of the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences (Jingjiang, China; Figure 1). Previously, this experimental area was a single-crop rice paddy with no issues of degradation, over-fertilization, or anthropogenic pollution. To facilitate our experiment, we modified it. Ten newly constructed standardized paddy fields, each covering 700 m^2, were designated for integrated rice–prawn farming (IRPF) and traditional rice monoculture (RM), with five replicates per group (Figure 1). On 20 July, Nangeng 5055 rice seedlings were transplanted into all fields. Healthy giant freshwater prawns, *M. rosenbergii*, weighing about 29 g each, were introduced into the IRPF fields at a density of 2.25 prawns per square meter. Both rice and prawns were harvested at the beginning of November. Agricultural production management followed conventional local agricultural practices. The giant freshwater prawns were fed daily at 17:00 with commercial pellets from Zhejiang Haida Feed Co., Ltd. (Shaoxing, China). The daily feeding ratio was about 2% of their body weight.

Paddy soil samples were collected at the initial (25 August), middle (25 September), and final stages (25 October) of the integrated farming period, which are referred to as Stage I, Stage II, and Stage III in subsequent descriptions and diagrams. At each sampling, five soil samples were taken from each of the ten experimental paddy fields using the five-point sampling method. These samples were thoroughly mixed into a single composite sample for each paddy field, resulting in a total of 10 composite samples per sampling time. All samples were transported to the laboratory in portable ice boxes. Upon arrival,

samples for bacterial community analysis were immediately stored at −80 °C. Samples for assessing soil total nitrogen (TN), total phosphorus (TP), ammonium, nitrate, and nitrite concentrations were freeze-dried at −50 °C for 72 h using a lyophilizer (CHRIST LYO Alpha 1-4 LD plus, Lower Saxony, German), then ground and homogenized using a mortar.

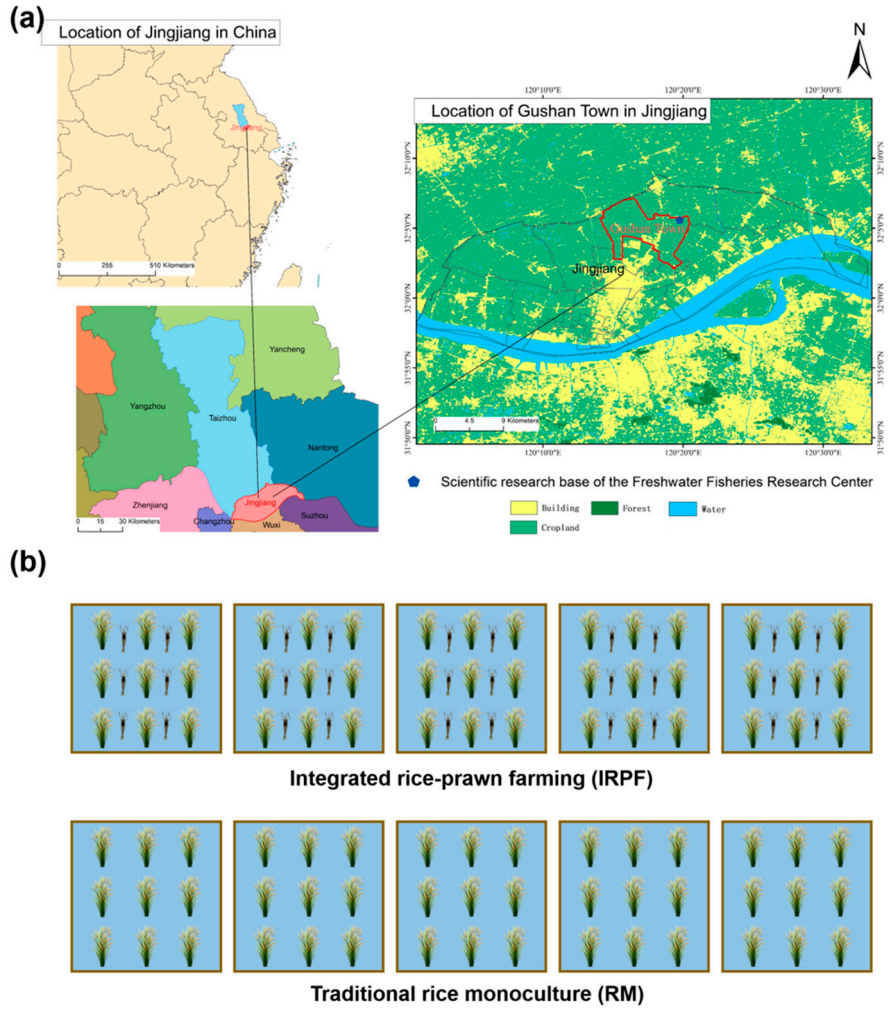

Figure 1. The geographic location of the experimental site (**a**) and the schematic diagram of the experimental design (**b**).

2.2. Paddy Soil Properties Determination

The concentrations of soil TN and TP were determined using the modified Kjeldahl method and the alkali fusion–molybdenum antimony (Mo-Sb) anti-spectrophotometric method, respectively. The soil ammonia, nitrate, and nitrite contents were quantified using spectrophotometric methods after extracting with potassium chloride solution. All the methods adhered to the Chinese national standards, including HJ 717-2014, HJ 632-2011, and HJ 634-2012 [36–38].

2.3. DNA Extraction

Soil bacterial DNA was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). Soil samples weighing 0.5 g were added to a centrifuge tube with 500 mg of magnetic beads and 1 mL of SLX-Mlus Buffer, then shook at 45 Hz for 250 s. Next, 100 µL of DS Buffer was added, thoroughly mixed, and then the mixture was incubated at 70 °C for 10 min and at 95 °C for 2 min before being centrifuged at room temperature for 5 min at 13,000 rpm. The supernatant was transferred to a new 2 mL tube, mixed with 270 µL of P2 Buffer and 100 µL of HTR Reagent, and incubated at −20 °C for 5 min before a subsequent centrifugation as above. The supernatant was then transferred to another tube,

to which an equal volume of XP5 Buffer and 40 μL of magnetic beads were added. After inverting to mix for 8 min, the residual liquid was discarded using a magnetic separation rack. Next, 500 μL of XP5 Buffer, 600 μL of PHB, and twice 600 μL of SPW Wash Buffer were added sequentially, removing the residual liquid between each addition. The sample was then centrifuged at room temperature for 10 s at 13,000 rpm, and the residual liquid was discarded. Afterwards, 100 μL of Elution Buffer was added and left to stand for 5 min. Finally, the supernatant was transferred to a new 1.5 mL centrifuge tube to obtain the total DNA.

2.4. Sequencing and Data Processing

For each sample, the V3–V4 regions of the 16S rRNA gene were amplified using primers 341F and 806R [39]. The resulting PCR products were purified, quantified, and pooled at equimolar ratios to prepare the sequencing library. Sequencing was performed on an Illumina NovaSeq 6000 platform with a 250 bp paired-end approach at BIOZERON Biotech. Co., Ltd., Shanghai, China.

Reads were filtered and screened according to the following criteria: an average Phred score < 20, ambiguous bases, more than eight primer mismatches in homopolymer runs, or a length shorter than 250 base pairs [40]. Subsequently, paired-end reads with an overlap exceeding 10 base pairs and devoid of mismatches were concatenated into tags by FLASH [41]. These tags underwent dereplication and were processed with the DADA2 within the QIIME 2 framework to detect indel mutations and substitutions, and were classified into amplicon sequence variants (ASVs) [42,43]. The ASVs were taxonomically classified utilizing the SILVA 138 reference database [44]. To facilitate comparisons across samples, the abundance tables of bacterial ASVs were normalized by scaling the tag counts to the minimum tag count (65,535 tags).

2.5. Statistical Analysis

We employed the QIIME to calculate the Shannon, Simpson, Chao1, and Pielou_J indices to assess the bacterial community diversity in paddy soil [45]. Before quantifying the bacterial community diversity, rarefaction curves were generated to ensure the reasonableness of sequencing data. Principal component analysis (PCA), utilizing weighted Bray–Curtis distances and supplemented by a PERMANOVA test, was performed to assess the impact of IRPF and various culture stages on the bacterial communities and their functions within paddy fields. Tukey's honestly significant difference (HSD) test was used to statistically analyze differences in the diversity, composition, and function of the soil bacterial community, as well as environmental factors among groups [46]. Co-occurrence network analysis was employed to explore the co-occurrence patterns and potential biological interactions within the soil bacterial communities [47]. Co-occurrence events and statistically significant correlations were determined when the absolute correlation coefficient exceeded 0.6 and the p-value was below 0.05. The Functional Annotation of Prokaryotic Taxa (FAPROTAX) framework was used to identify the functional attributes of soil bacterial communities in the IRPF and RM groups [48,49]. The Neutral Community Model (NCM) was utilized to estimate the influence of neutral processes on the structure of bacterial communities in the paddy soils. The distance-based redundancy analysis (db-RDA) was utilized to reveal the relationships between bacterial communities and environmental factors in the paddy soils. The aggregated boosted tree (ABT) approach was implemented to ascertain the significance of environmental variables in influencing variations within the bacterial communities.

3. Results

3.1. Environmental Factors

Differences in the TN, TP, ammonium, nitrate, and nitrite concentrations in the paddy soil between the RM and IRPF groups at each specific sampling time are shown in Figure 2. During culture stage II, the TN content in the IRPF group was notably higher compared to

the RM group (Figure 2, $p < 0.05$). However, at stages I and III, no obvious differences in TN content between these groups were observed (Figure 2, $p < 0.05$). The RM group exhibited remarkably higher TP concentration than the IRPF group at stage I (Figure 2, $p < 0.05$), while the opposite was true at culture stage II (Figure 2, $p < 0.05$). There were no remarkable differences observed in TP concentrations between the IRPF and RM groups at the final stage (Figure 2, $p > 0.05$).

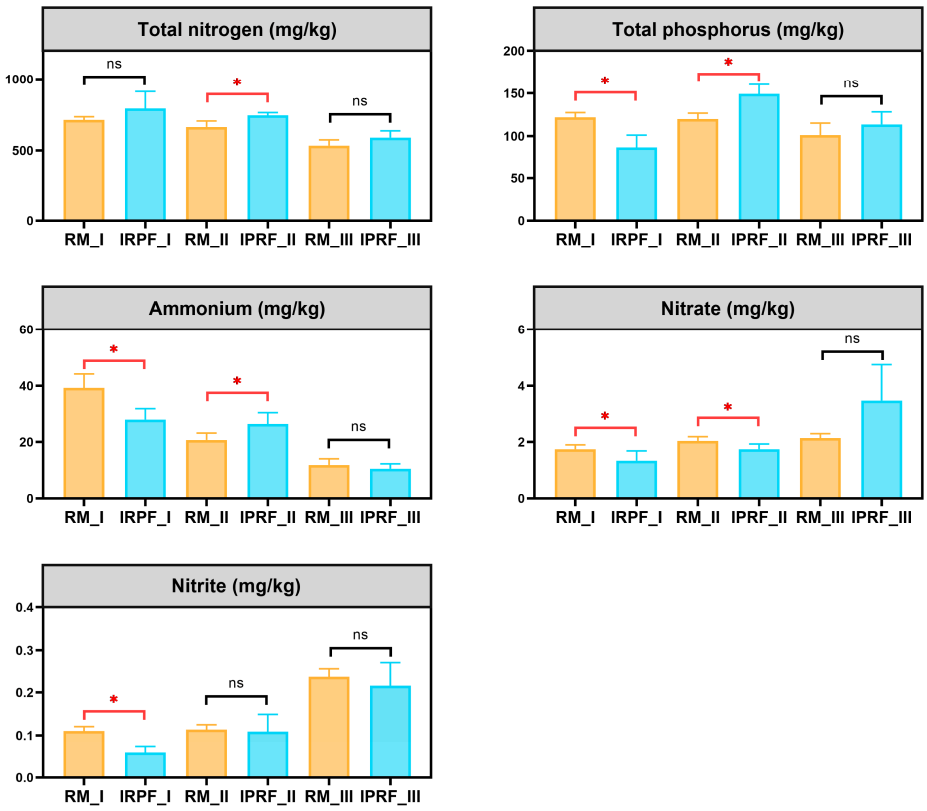

Figure 2. The total nitrogen (TN), total phosphorus (TP), ammonium, nitrate, and nitrite concentrations in the paddy soil at each specific sampling time. A red asterisk (*) indicates noticeable statistical discrepancy between the RM and IRPF groups ($p < 0.05$), and "ns" indicates no dramatic differences ($p > 0.05$). The "I", "II", and "III" represent the initial (25 August), middle (25 September), and final stages (25 October) of the integrated farming period.

As for the soil ammonium, the RM group had higher levels than the IRPF group at culture stage I (Figure 2, $p < 0.05$), but lower levels at culture stage II (Figure 2, $p < 0.05$), with no obvious differences at culture stage III (Figure 2, $p > 0.05$). The RM group's soil exhibited higher nitrate concentrations than the IRPF group during stages I and II (Figure 2, $p < 0.05$); however, no notable differences were observed at stage III (Figure 2, $p > 0.05$). Similarly, the nitrite concentration in the RM group was considerably higher at stage I (Figure 2, $p < 0.05$), but not at stages II and III (Figure 2, $p > 0.05$).

3.2. Soil Bacterial Community Diversities

The diversity values for the bacterial community in paddy soil during the experimental period are displayed in Figure 3. At culture stages I, II, and III, there were no statistical differences in the Simpson, Shannon, Chao 1, and Pielou_J indices between the RM and IRPF groups (Figure 3a, $p > 0.05$). In the PCA analysis, the PC1 axis accounted for 14.16% of the variations in the soil bacterial community, whereas the PC2 axis represented 8.9% (Figure 3b). Although PCA results indicated that effective visual separations between the RM and IRPF groups at various culture stages were not achievable, PERMANOVA test confirmed that both the culture stage and the integrated rice–giant river prawn farming

significantly impacted the bacterial community (Table S1, $p < 0.05$). As for the Bray–Curtis distances, the RM group at culture stage I showed a noticeably lower value than other groups (Figure 3c, $p < 0.05$). At culture stage II, the IRPF group exhibited dynamically higher Bray–Curtis distances than RM group (Figure 3c, $p < 0.05$). However, during culture stage III, no notable differences in the Bray–Curtis distances were detected between the RM and IRPF groups. (Figure 3c, $p > 0.05$).

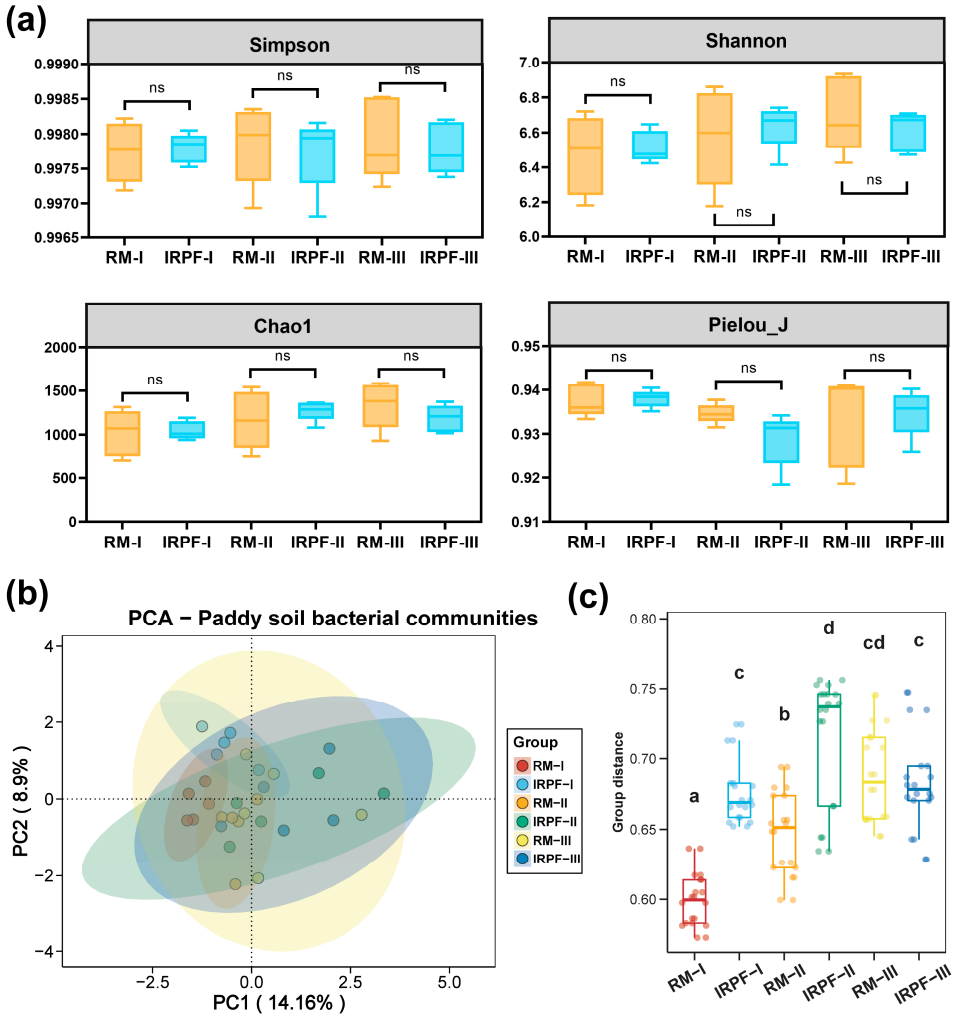

Figure 3. Differences in the α and β diversities of the soil bacterial communities between the RM and IRPF groups. (**a**) Statistical discrepancy in the α diversity indices for the soil bacterial communities between the RM and IRPF groups at each specific sampling time. (**b**) Principal components analysis (PCA) of the soil bacterial communities across all soil samples using Bray–Curtis distances. A red asterisk (*) indicates noticeable statistical discrepancy between the RM and IRPF groups ($p < 0.05$), and "ns" indicates no dramatic differences ($p > 0.05$). (**c**) Differences in the Bray–Curtis distances of the soil bacterial communities between the RM and IRPF groups. Distinct lowercase letters denote notable differences between the different groups ($p < 0.05$). The "I", "II", and "III" represent the initial (25 August), middle (25 September), and final stages (25 October) of the integrated farming period.

3.3. Soil Bacterial Community Compositions

The dominant bacterial phyla and genera in the paddy soil are shown in the Figure 4a,b. The top ten phyla by relative abundance in the paddy soil were Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Firmicutes, Bacteroidetes, Nitrospirae, candidate division NC10, Thaumarchaeota, and Euryarchaeota (Figure 4a). The top ten genera by relative abundance in the paddy soil were *Cronobacter*, *Escherichia*, *Salmonella*, *Luteitalea*, *Ralstonia*, *Defluviicoccus*, *Candidatus Methylomirabilis*, *Weissella*, *Desulfomonile*, and *Bellilinea* (Figure 4b).

The significant differences in the dominant phyla and genera between the IRPF and RM groups are shown in Figure 4c,d. During culture stages II and III, no obvious differences in any dominant phyla and genera between the IRPF and RM groups were observed ($p > 0.05$). Therefore, Figure 4c,d only display the significant discrepancy in the dominant phyla and genera between the IRPF and RM groups at culture stage I. At culture stage I, the IRPF group showed a remarkable reduction in the Actinobacteria and a notable rise in candidate division NC10 relative to the RM group (Figure 4c, $p < 0.05$). For the dominant genera, the IRPF group exhibited significantly decreased *Defluviicoccus* and increased *Candidatus Methylomirabilis* in contrast to RM group (Figure 4d, $p < 0.05$).

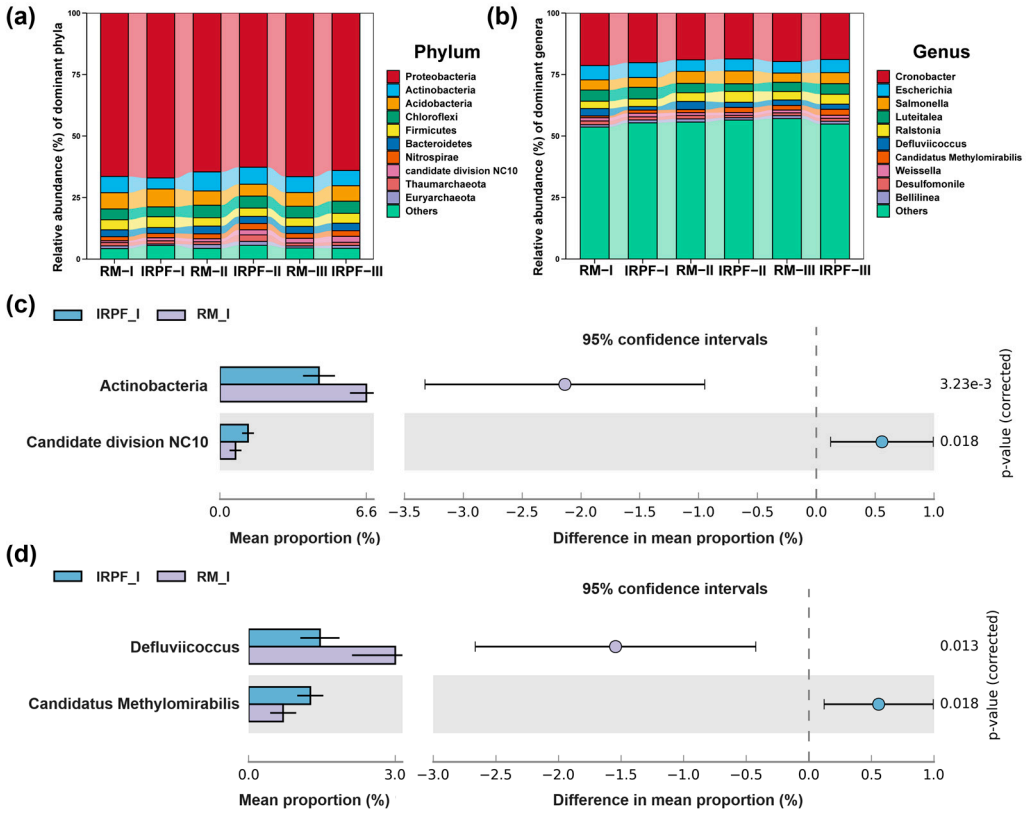

Figure 4. Bacterial community compositions in the IRPF and RM groups. (**a**) Composition of the top ten bacterial phyla in relative abundance across all the soil samples. (**b**) Composition of the top ten bacterial genera in relative abundance across all the soil samples. (**c**) Significant differences in the dominant phyla (top ten in relative abundance) in paddy soil between the RM and IRPF groups. (**d**) Significant differences in the dominant genera (top ten in relative abundance) in paddy soil between the RM and IRPF groups. The "I", "II", and "III" represent the initial (25 August), middle (25 September), and final stages (25 October) of the integrated farming period.

3.4. Function Prediction for Soil Bacterial Community

The results of the soil bacterial community function prediction are displayed in Figure 5. The top 10 most abundant functional groups for the soil bacterial community were animal parasites or symbionts, human-associated, chemoheterotrophy, all human pathogens, meningitis-related human pathogens, fermentation, aerobic chemoheterotrophy, sulfur compound respiration, nitrate reduction, and human gut (Figure 5). For the PCoA results, the PC_1 axis accounted for 46.0% of the variance in bacterial community functions, while the PC_2 axis accounted for 22.0% (Figure 5). The PERMANOVA test confirmed that the functions of the soil bacterial community were significantly impacted by the culture stage (Table S2, $p < 0.05$,), while not by the integrated rice–giant river prawn farming (Table S2, $p > 0.05$).

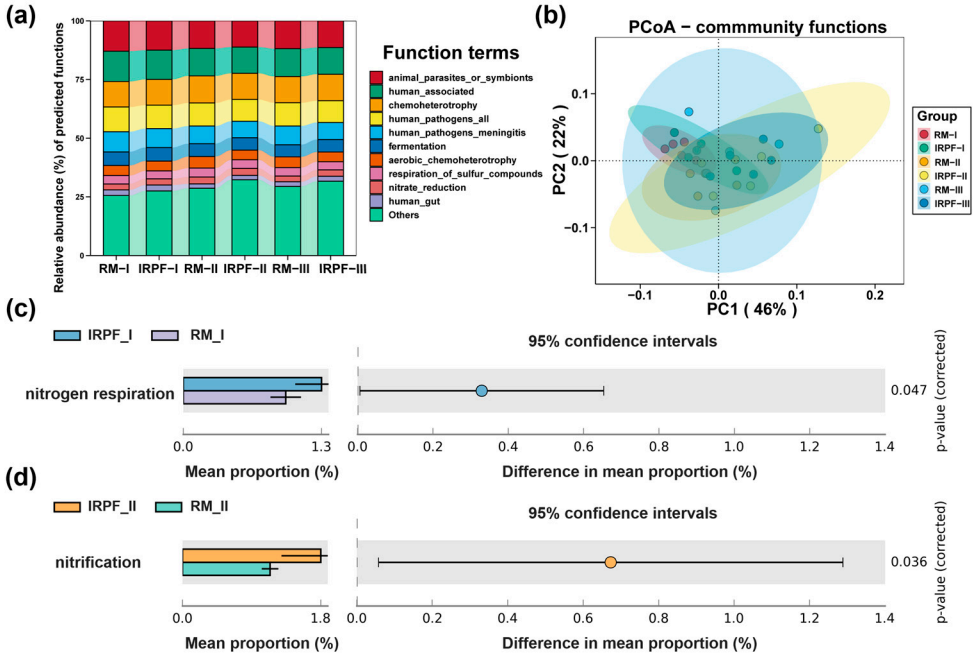

Figure 5. Function predictions for the soil bacterial communities. (**a**) Compositions of the dominant bacterial functions (top ten in relative abundance) across all the soil samples. (**b**) Principal coordinate analysis (PCoA) of the bacterial community functions in paddy soil. (**c**) Significantly different functional groups involved in organic matter decomposition and nutrient cycling for the soil bacterial community between the RM and IRPF groups at culture stage I. (**d**) Significantly different functional groups involved in organic matter degradation and elemental cycling for the soil bacterial community between the RM and IRPF groups at culture stage II. The "I", "II", and "III" represent the initial (25 August), middle (25 September), and final stages (25 October) of the integrated farming period.

To investigate the impact of the integrated rice–giant river prawn farming on the bacterial functions involved in organic matter decomposition and nutrient cycles, the top 10 most abundant functional groups related to organic matter decomposition and nutrient cycles were identified for subsequent analysis. These functional groups included chemoheterotrophy, fermentation, aerobic chemoheterotrophy, nitrate reduction, nitrogen respiration, nitrification, hydrocarbon degradation, methylotrophy, nitrite respiration, and methanotrophy. At the culture stage I, the IRPF group demonstrated dramatically higher nitrogen respiration relative to the RM group (Figure 5c, $p < 0.05$). At culture stage II, the relative abundance of nitrification in the IRPF group showed a substantial increase in contrast to RM group (Figure 5d, $p < 0.05$). However, at culture stage III, no noticeable discrepancies were observed in the dominant functional groups between the IRPF and RM groups ($p > 0.05$).

3.5. Co-Occurrence Network for Soil Bacterial Community

The co-occurrence network patterns for the soil bacterial community are shown in Figure 6. At culture stage I, the RM group had 163 nodes; 1241 edges, with 40.37% negative and 59.63% positive edges; and a clustering coefficient of 0.53 (Figure 6a). In contrast, the IRPF group had 134 nodes; 809 edges, with 49.81% negative and 50.19% positive edges; and a clustering coefficient of 0.58 (Figure 6a). As for culture stage II, the RM group showed 142 nodes; 997 edges, with 36.51% negative and 63.49% positive edges; and a clustering coefficient of 0.58 (Figure 6a). The IRPF group had 120 nodes; 673 edges, with 50.22% negative and 49.78% positive edges; and a clustering coefficient of 0.63 (Figure 6a). At culture stage III, the RM group consisted of 125 nodes; 734 edges, with 35.56% negative and 64.44% positive edges; and a clustering coefficient of 0.59, whereas the IRPF group had

118 nodes; 557 edges, with 43.27% negative and 56.73% positive edges; and a clustering coefficient of 0.54 (Figure 6a).

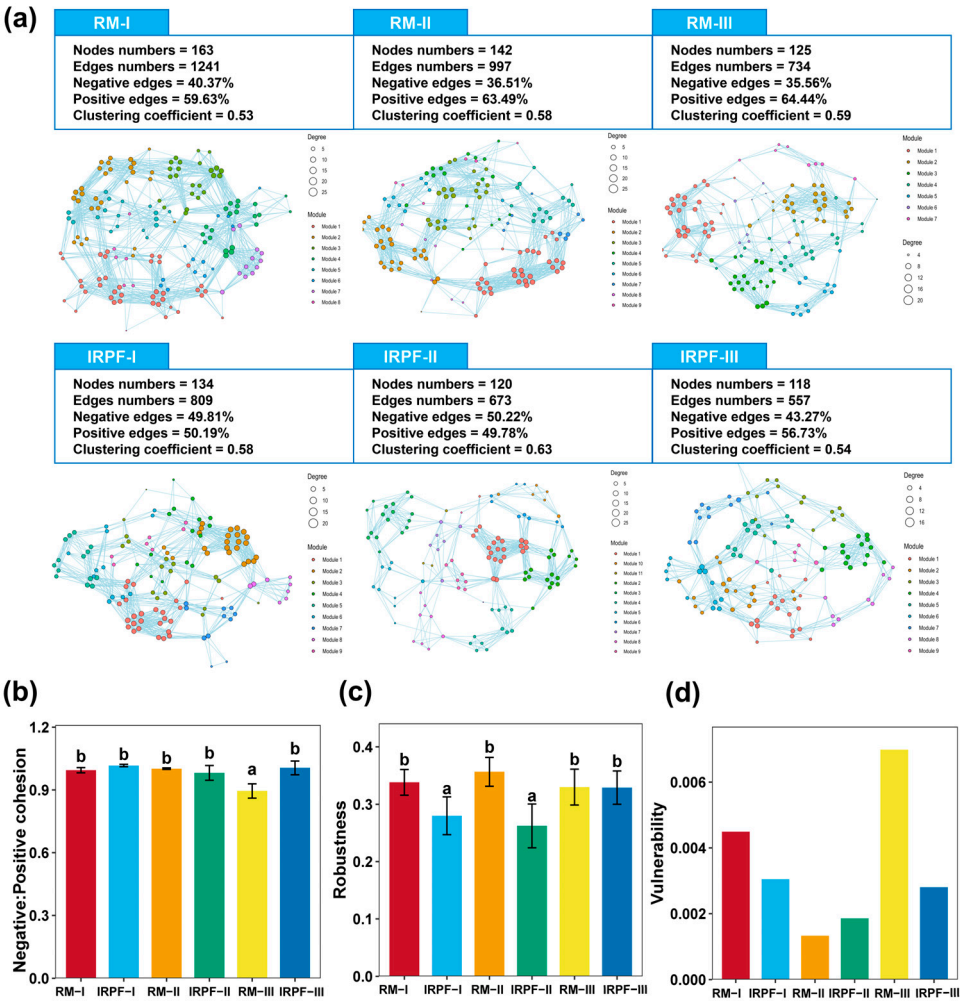

Figure 6. Co-occurrence network analysis assessing the interactions among soil bacterial communities. (**a**) Bacterial co-occurrence networks with topological parameters in RM and IRPF groups during the experimental period. Modules in the networks are visually differentiated by labels in various colors. (**b**) Differences in the negative/positive cohesion values between the RM and IRPF groups at the different culture stages. (**c**) Differences in the robustness values between the RM and IRPF groups at the different culture stages. (**d**) The values of vulnerability in the RM and IRPF groups at the different culture stages. The "I", "II", and "III" represent the initial (25 August), middle (25 September), and final stages (25 October) of the integrated farming period. Distinct lowercase letters denote notable differences between the different groups.

There were no significant differences in the negative/positive cohesion between the IRPF and RM groups at stages I and II (Figure 6b, $p > 0.05$). The negative/positive cohesion of the RM group at the culture stage III was dramatically lower relative to any other groups (Figure 6b, $p < 0.05$). The robustness values in the IRPF group at culture stages I and II were markedly less than the RM group (Figure 6c, $p < 0.05$). No notable differences in the robustness values between the IRPF and RM groups at culture stage III were observed (Figure 6c, $p > 0.05$). At culture stages I and III, the RM group showed increased vulnerability values compared to the IRPF groups, and the vulnerability value of the RM group at culture stage III was the maximum value over the entire experimental period (Figure 6c).

3.6. Assembly Processes Shaping the Soil Bacterial Community

Assembly processes shaping the soil bacterial community in the RM and IRPF groups are shown in Figure 7. To improve the accuracy of the NCM fitting, we no longer differentiated based on the culture stage but instead present an overall fitting results for the IRPF and RM groups throughout the entire experimental process, respectively. During the culture period, 66.0–70.3% of the variations in the bacterial community could be explained by NCM (Figure 7). Specifically, the R^2 values for the RM and IRPF groups were 0.703 and 0.66, respectively, while the m values were 0.02 for the RM group and 0.018 for the IRPF group (Figure 7). The IRPF group displayed slightly lower R^2 and m values compared to the RM group (Figure 7).

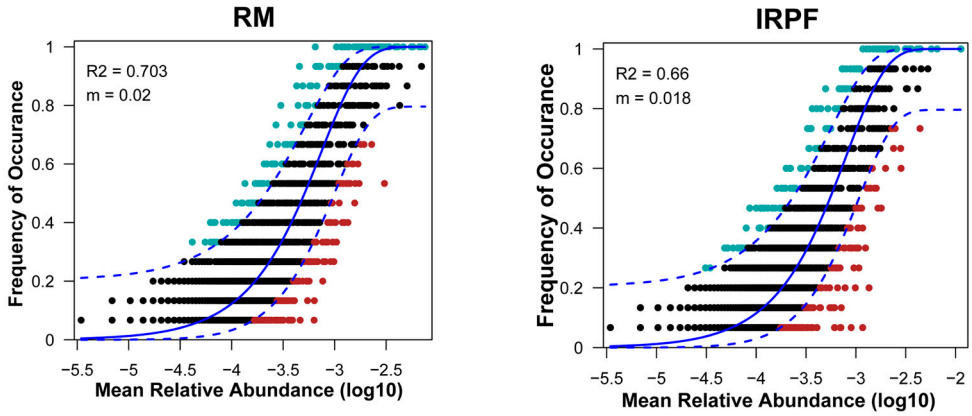

Figure 7. The neutral community model (NCM) revealing assembly processes shaping soil bacterial communities. The solid lines indicating the best fit of the NCM, and dashed lines showing the 95% confidence interval around the model predictions. The parameter "m" represents migration rate, and the "R^2" value indicates the model's goodness of fit.

3.7. Correlations of Environmental Factors with Soil Bacterial Communities

According to the db-RDA results, 54.28% of the variance in the soil bacterial communities could be explained by the environmental factors identified in this study (Figure 8a). All environmental factors, including ammonia, nitrate, nitrite, TN, and TP, were significantly correlated with the bacterial community structure (Figure 8a and Table S3, $p < 0.05$). The correlations between environmental factors and variations in soil bacterial communities was evaluated using ABT, which identified nitrite concentration as having the maximum impacts on the soil bacterial community, followed by TP, nitrate, ammonium, and TN (Figure 8b).

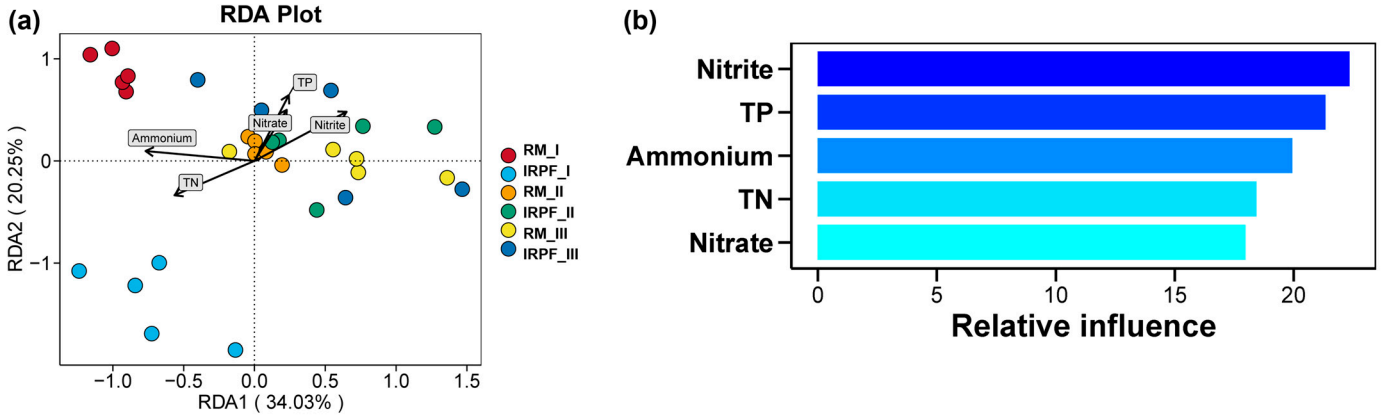

Figure 8. Associations between soil properties and bacterial communities. (**a**) Redundancy analysis (RDA) for examining associations of soil bacterial communities with soil properties. (**b**) Aggregated

boosted tree (ABT) for determining the contributions of environmental factors to variations in soil bacterial communities. The "I", "II", and "III" represent the initial (25 August), middle (25 September), and final stages (25 October) of the integrated farming period.

4. Discussion

Revealing the effects of integrated rice–prawn farming on the soil bacterial community and soil physicochemical properties is crucial to understand the broad impact of integrated rice–prawn farming on the agricultural ecosystems. In this study, we analyzed the differences in the composition, diversity, function, stability, and assembly processes of the soil bacterial community between IRPF and RM groups. Our findings clearly indicated that the impacts of IRPF on soil bacterial community were mainly observed in the initial and middle stages of integrated farming period. While IRPF did not significantly affect the bacterial community diversity, it markedly influenced the composition, functionality, co-occurrence network, and assembly process of the soil bacterial community. Additionally, IRPF substantially affected environmental factors in paddy soil, which were intimately corelated with the variations in soil bacterial community.

4.1. Variations in the Soil Bacterial Community Composition, Diversity, and Function

Overall, the IRPF has a very limited impact on the composition and diversity of bacterial communities in paddy soil. The bacterial community diversity within paddy soil in the IRPF exhibited no significant differences compared to traditional rice monoculture, aligning with findings from studies on various integrated rice–fish farming systems, such as rice–frog, rice–crab, rice–fish, and rice–crayfish [50–55]. The impact of integrated rice–fish farming on soil bacterial diversity is not immediately evident within short production cycles [53,54,56]. Zhao et al. [54] found that changes in soil bacterial community diversity occur only after five years of implementing integrated rice–fish farming; implementing this agricultural production pattern for just one year has no significant impact on soil bacterial diversity. Meanwhile, Li et al. [53] demonstrated that twelve years of integrated rice–turtle farming significantly altered the soil bacterial community diversity, whereas seven years of the same practice did not alter the soil bacterial community diversity. Therefore, it was reasonable for our study, which covered only one normal production cycle, to find that the IRPF delivered no remarkable impacts on soil bacterial diversity.

Nevertheless, the IRPF did influence the composition of the soil bacterial community to some extent. During the culture stage I, the IRPF exhibited noticeably increased phylum candidate division NC10 and genus *Candidatus Methylomirabilis* within paddy soil compared to traditional rice monoculture. Additionally, remarkably decreased relative abundances of Actinobacteria and *Defluviicoccus* were observed in paddy soil induced by the IRPF. The candidate division NC10 has been found to significantly influence the biogeochemical cycles by mediating the coupling between anaerobic methane oxidation (AOM) and nitrite reduction, thus integrating the carbon and nitrogen cycles in soils [57–60]. Genus *Candidatus Methylomirabilis*, as a member in this division, can perform nitrite-dependent anaerobic methane oxidation in anoxic conditions [57,58,61]. Phylum Actinobacteria are widely distributed in terrestrial ecosystems, capable of metabolizing complex organic substances, and are especially capable of degrading xenobiotic compounds [62–65]. Consequently, they serve a vital function in soil material cycling and ecological functions [62]. The genus *Defluviicoccus* participates in degrading organic matter and facilitates phosphorus removal [66–68]. Meanwhile, functional prediction results based on FAPROTAX have confirmed that IRPF significantly enhanced nitrogen-related functions within the soil bacterial community, primarily nitrification and nitrogen respiration [48,69]. Hence, the differences in the composition and functionality of soil bacterial community observed in this study suggested that the IRPF might have facilitated the bacterial anaerobic methane oxidation process coupled with nitrogen cycling, but reduced the capacity of bacteria to break down organic material. Nonetheless, the influence of IRPF on the composition of soil bacterial community was limited to the early and middle culture stages.

4.2. Variations in the Soil Bacterial Co-Occurrence Network Pattern and Community Assembly

In natural ecosystems, bacteria are not isolated units but are intricately linked, forming complex bacterial communities [70,71]. Interactions across microbes can influence the bacterial community structure [70,71]. The co-occurrence network among bacteria can be used to assess the interconnection pattern within complex bacterial communities, thereby providing a deeper understanding of bacterial community structure and characteristics [71,72]. Different agricultural production patterns could affect the bacterial interconnection patterns and alter the bacterial co-occurrence networks [51,73–76]. For our study, throughout the experimental process, co-occurrence networks of the bacterial community in IRPF obviously had fewer edges and nodes compared to rice monoculture. The lower numbers of edges and nodes indicated that the IRPF resulted in the weaker interactions among the soil bacterial community, lower resistance to disturbances, and greater instability [77,78]. Furthermore, the robustness indicator also confirmed the adverse effects of IRPF on the stability of the soil community and its resilience to environmental changes [79,80]. These findings differed with previous studies by Hou et al. [15,51], which demonstrated that integrated rice–fish and rice–crayfish farming systems enhance the complexity and stability of soil bacterial communities, evidenced by the increased edges and nodes. In our study, the reduction in the complexity and stability of the bacterial community affected by IRPF could primarily be attributed to the different farmed aquatic species.

Soil bacterial community assembly is governed by both niche-based deterministic processes and stochastic processes [81]. The NCM results revealed that the stochastic processes predominantly shaped the bacterial communities across the entire experimental period. Higher R^2 values suggest that the community assembly is dominated by neutral processes, where random events and stochastic processes significantly affect species abundance and distribution [82]. The migration rate (m) can be explained as the dispersal limitation, indicating the likelihood that the random loss of an individual in a local community will be offset by dispersal from the metacommunity [82,83]. In our study, the IRPF exhibited the lower R^2 and m values for the soil bacterial community than the traditional rice monoculture, which might suggest that the migration of the soil bacterial community was limited by the IRPF and the prevalence of stochastic processes in the soil bacterial community assembly was declining.

4.3. Assiciations of Environmental Factors with Bacterial Communities in Paddy Soil

In our study, the IRPF system significantly influenced the environmental factors within paddy soil during the initial and middle culture stages. Initially, the IRPF exhibited significantly lower TP and ammonia concentrations in paddy soil in contrast to rice monoculture. By the middle culture stage, however, the TN, TP, and ammonia concentrations in paddy soil of the IRPF group were significantly increased compared to traditional rice monoculture. Previous researchers on other integrated rice–aquatic animals farming systems reported increased nitrogen and phosphorus contents in paddy soil, primarily due to the exogenous feed input and the accumulations of residual feed and feces [50,53,54,81]. Environmental changes critically affect the composition and distribution of bacterial communities [84,85]. Aquatic animal cultivation, exogenous feed input, and production management practices can alter the environmental factors, which subsequently change the environmental bacterial communities [15,53,86–89]. In our findings, the TN, TP, ammonium, nitrate, and nitrite concentrations were significantly associated with the soil bacterial communities. Notably, soil nitrite concentration had the most substantial influence on bacterial community differences, followed by TP. This significant impact of nitrite on the soil bacterial community might be associated with the high presence of the genus *Candidatus Methylomirabilis* in IRPF paddy soil. As noted above, the nitrite-dependent anaerobic methane oxidation process mediated by *Candidatus Methylomirabilis*, which uses methane as an electron donor and nitrite as an electron acceptor, converts methane to CO_2 and reduces nitrite to N_2 under anaerobic conditions [90,91]. Therefore, in this study, the impacts of IRPF on the soil

bacterial community were primarily driven by these environmental changes, especially soil nitrite concentration.

5. Conclusions

In conclusion, the influence of IRPF on the bacterial community in paddy soils was primarily observed during the early and middle stages of cultivation. IRPF did not significantly affect the diversity of the soil bacterial community. However, during the initial culture stage, IRPF markedly increased the phylum candidate division NC10 and the genus *Candidatus Methylomirabilis*, enhancing nitrogen cycling-related functions within the bacterial community. IRPF significantly reduced the complexity and stability of the bacterial community at the initial and middle stages of cultivation. Stochastic processes were predominant in assembly processes shaping soil bacterial communities, yet IRPF limited the bacterial migration rates and diminished the dominance of the stochastic processes. Moreover, IRPF significantly influenced the environmental factors in paddy soils, which correlated strongly with bacterial communities; notably, nitrite contributed the most to variations in the soil bacterial community. Consequently, the impacts of IRPF on the soil bacterial community were primarily driven by these environmental changes, especially soil nitrite concentration.

These findings provide a solid data foundation to understand the broad impact of integrated rice–prawn farming on the agricultural ecosystems from the perspective of the soil microbiome. Although this agricultural pattern initially reduced the soil bacterial stability, it also enhanced bacterial-mediated nitrogen cycling, potentially benefiting rice growth. However, as this study spanned only one growth cycle, further research across multiple growth cycles is necessary to determine how to adjust the fertilization strategies or optimize the agricultural pattern according to the variations in soil bacterial community by integrated rice–prawn farming.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14081600/s1, Table S1. Two-way PERMANOVA assessing the effects of culture stages and integrated rice-giant river prawn farming on soil bacterial community based on the Bray-Curtis distance; Table S2. Two-way PERMANOVA assessing the effects of culture stages and integrated rice-giant river prawn farming on the functions of soil bacterial community based on the Bray-Curtis distance; Table S3. Significance tests of environmental factors in Redundancy analysis (RDA).

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