Effects of Rice Growth Stages on the Composition and Functional Potentialities of Rhizosphere Bacterial Community in Coupled Rice-Crab System

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ABSTRACT

Co-culture of rice with aquatic animals has garnered widespread interest for its potential to control soil-borne diseases, improve soil quality and increase rice yields. Therefore, it is necessary to further explore its mechanism. In this study, the samples of different growth stages of rice, including tillering stage (TS), jointing stage (JS), heading stage (HS) and mature stage (MS) were collected to analyze the impact on the community composition, diversity and functional potentialities of the rhizosphere bacterial in the coupled rice-crab system were investigated through 16S rRNA gene high-throughput sequencing. The results showed that, a total of 444,882 sequences from 12 samples were obtained, and 6637 bacterial operational taxonomic units (OTUs) with 97% sequence similarity were identified. The rhizosphere bacterial communities were more influenced by growth stages of rice than chemical properties. Principal component analysis (PCA) revealed the similarity of samples at four growth stages was significantly low (P <0.05). The dominant taxa phyla for bacterial community were similar in different growth stages. The LefSe analysis indicated that 32 distinctly taxa that were abundant among four stages with a linear discriminant analysis (LDA) score higher than 3.5. The α-diversity of the bacterial community in rhizosphere soil was not significantly different among different growth stages (P >0.05). Moreover, 8 functional potentials among 46 KEGG pathways showed notable differences for the rhizosphere bacterial community and HS had the largest number of species and strongest metabolic function potentials. Overall, the findings in this study provide valuable information for maintaining soil ecosystem balance and provide theoretical guidance for the practical application of this co-culture system.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most widely cultivated food crop worldwide, with the largest planting area in China. In recent decades, excessive application of chemical fertilizers to increase crop productivity has led to environmental and ecological concerns, such as soil acidification and soil health deterioration (Abán *et al.*, 2021), which poses a threat to the sustainable development of agriculture. These soil management and land-use practices seriously affect soil quality and health, and lead to nutrient loss, decreased diversity and abundance of soil biota, loss of functional biodiversity and deterioration of ecosystem services they regulate (de Vries *et al.*, 2013). In recent years, the co-culture of rice with ecologically friendly aquatic animals such as crabs, fish, frogs, river crabs, and turtle shrimps, has been proved to be a promising option for enhancing soil quality and soil health. Studies have shown that such animals have the ability to prevent soil-borne diseases, reduce the use of chemical fertilizers, minimize environmental pollution, and promote microbiological diversity and rice yield (Bashir *et al.*, 2020; Huang *et al.*, 2022; Hu *et al.*, 2020). The dominant bacterial phyla in paddy soil includes Chloroflexi, Actinobacteria, Proteobacteria, Acidobacteria, etc., which play a crucial role in soil nutrient cycling (Herlambang *et al.*, 2021; Arunrat *et al.*, 2022).

The rhizosphere is a critical component of the

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agroecosystem, comprising the soil surrounding plant roots that is influenced by plant root exudates, abscission, and plant residues. Plant roots secrete various metabolites, such as organic acids and carbohydrates, providing suitable conditions for growth and reproduction of soil microorganisms, which makes the rhizosphere contains an abundant and diverse microbial population. In the agroecosystems, rhizosphere microbiota has a profound impact on crop growth, nutrition and health (Philippot *et al.*, 2013). Rhizosphere bacteria play an important role in the decomposition of organic matter, nutrient transformation, disease control and plant immunity improvement, but are sensitive to environmental changes (Degens *et al.*, 2000; Mendes *et al.*, 2011; Zamioudis and Pieterse, 2012). Cultivable microorganisms under laboratory conditions represent only a small percentage of the soil microbial population, which limits the more detailed study of rhizosphere microorganisms. The emergence of high-throughput technologies makes it possible to study previously uncultivable and rare microbial populations (DeLong, 2004; Sogin *et al.*, 2006; Campbell *et al.*, 2011), not only can distinguish relative abundance and evolutionary relationships, but also can insight into the diversity and species composition of microbial communities, as well as their classification and phylogeny (McHugh and Schwartz, 2016; Si *et al.*, 2018; Li *et al.*, 2021).

The integrated agri-aquaculture system (IAAS) ensures food security and promots sustainable agricultural development by connecting aquaculture with agricultural systems (Lansing and Kremer, 2011; Hu *et al.*, 2016). Such system can reduce green gas emissions, increase farm productivity, and improve resource utilization efficiencies (Bashir *et al.*, 2020). Therefore, as one of the most important aquatic ecosystem, the coupled rice-crab system has the potential to reduce the need for herbicides, pesticides and chemical fertilizer, and to stimulate the growth of rice through their uninterrupted activities. Therefore, it has gained increasing attentions due to its ability to increase soil nutrient levels and improve nutrient accumulation (Xu *et al.*, 2019; Song *et al.*, 2019), and has been applied in northern China.

Many studies have investigated the diversity and composition of bacterial community in paddy soil. Previous study indicated that straw returning in the rice-crayfish integrated system could improve microbial community functional diversity, contributing to the improvement of soil quality and the long-term sustainable development of the rice-crayfish integrated system (Zhu *et al.*, 2022). Chen *et al.* (2021) demonstrated that the rice-turtle integrated system could effectively improve soil nutrients, and paddy field with low stocking density of Chinese soft-shelled turtles exhibited relatively high soil microbial diversity. Fernández-Baca *et al.* (2021) studied changes in rhizosphere soil microbial communities during plant developmental stages of high and low methane-emitting rice genotypes, contributing to determine the period of high methane emission and reduce methane emission from rice fields by screening rice varieties with low abundance methanogenic bacteria and high abundance methanogenic bacteria during this period. However, the effects of different growth stages on the structure and functional potentialities of bacterial communities in the rhizosphere soil of coupled rice-crab system is yet to be adequately investigated. The Gaijiaba organic planting farm in Beijingzi town, Donggang city, Liaoning province has been producing organic rice by culturing paddy fields with aquatic animals in recent years. Previous study have confirmed that river-crab coculture paddy fields have a better ecological basis for soil bacterial (Song *et al.*, 2020).

In this study, the influences of different growth stages of rice on the structure and functional potentialities of bacterial communities in rhizosphere soil of the coupled rice-crab system was analysed. This study aims to identify the main driving rhizosphere bacterial communities in the coupled rice-crab system, to evaluate the diversity, structure and potential functions of the rhizosphere soil bacterial community at the different growth stages, and to distinguish the dominant bacterial population, the main functional potentials and the growth stage with the highest potential functions in the rhizosphere soil. This study provides valuable information for the practical application of this co-culture system and contributes to the understanding of maintaining soil ecosystem balance.

MATERIALS AND METHODS

Study area

The field study was conducted at the Gaijiaba Organic Planting Farm, located in Beijingzi, Donggang city, Liaoning province (39°54' N, 123°50' E), China from May to October 2020. This area has a continental monsoon climate, with an average annual precipitation of 888 mm and temperature of 8.4°C. The soil type was coastal saline paddy soil, with a pH of 7.1, total nitrogen (TN) of 1460 mg/kg, available phosphorus (AP) of 31.39 mg/kg, and available potassium (AK) of 126.7 mg/kg.

The main experimental plot (about 1000 m²) (Supplementary Fig. S1) was divided into 3 plots, each with a width of 15 m and length of 20 m. The rice variety used in this study was the Japanese high quality rice variety Yueguang. Rice seedlings were transplanted on May 10, 2020, with a plant to plant and row to row spacing of 15cm × 30cm. A week following transplantation,

Chinese mitten crabs (*Eriocheir sinensis*) with an average weight of 23.1 ± 0.8 g for females and 20.8 ± 0.7 g for males, sourced from the Panjin river crab breeding base, were randomly distributed in the paddy fields at a ratio of 1:1 and the throwing density was about 450 crabs/667m^2 (Zheng *et al*., 2019). The paddy field was surrounded by blue polyethylene escape film and equipped with a circular ladder-shaped temporary breeding pond, which could provide fish and shrimp, green feed or high-protein concentrate feed for river crabs. The seaweed biological organic fertilizer produced by Yikang Organic Agricultural Science and Technology Development Co., Ltd. (Rizhao, China) was applied in March and July respectively to provide nutrients for paddy field, regulate water quality of paddy field and enhance rice disease resistance. The crabs would be co-cultured with rice plants until the harvest on October 25, 2020.

Soil samples collection

Rhizosphere soil samples were collected from each plot using a five-point sampling method at four growth stages: the tillering stage (June 29, 2020), jointing stage (July 31, 2020), heading stage (September 2, 2020) and mature stage (October 15, 2020), which were named as TS, JS, HS and MS, respectively. The rice plants were carefully dug out to keep the intact roots and bulk soil shaken off, the rhizosphere soil closely adhering to the root surface of about 2 mm was collected using a sterile brush. Three biological replicates were collected at each growth stage and each replicate was mixed by five sub-samples. The soil samples were then divided into two parts, with one part utilized for soil chemical property determination, and the other part stored at -80°C for DNA extraction.

Determination of chemical properties of rhizosphere soil

The soil samples were air-dried, crushed, and sieved through a 2 mm sieve. Soil pH was determined using a 1:5 soil/water suspension. The content of soil organic matter (SOM) was measured by potassium dichromate oxidation method (Liu *et al*., 2020). The content of AK was extracted using ammonium acetate solution and determined by flame photometry (Zhao *et al*., 2004). The content of soil AP was determined by NaHCO$_3$ extraction molybdenum antimony colorimetry method (Zhang *et al*., 2019). The content of soil TN was determined using the Kjeldahl method (Bremner, 1960).

DNA extraction, PCR amplification and high-throughput sequencing

Rhizospheric soil DNA extraction was extracted from 0.5 g of each soil sample using the E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, USA).

The V3-V4 hypervariable region of the bacterial 16S rRNA gene were amplified with the primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) using an ABI GeneAmp® 9700 PCR system (Applied Biosystems, Foster City, CA, USA). The amplification procedure was: pre-denaturation at 95°C for 2 min; denatured at 95°C for 30 s, annealed at 55°C for 30 s, extended at 72°C for 30 s, lasting for 25 cycles; finally, extended at 72°C for 5 min. PCR products were obtained and then quantified using the QuantiFluor ST blue fluorescence quantitative system (Promega, Madison, WI, USA). Finally, the DNA was mixed in corresponding proportions and sequenced using the Illumina MiSeq 300 platform (Meiji biology Co., Ltd., Shanghai, China). The paired-end reads for each sample were merged using FLASH v1.2.11 (Magoč and Salzberg, 2011), the filtered sequences were clustered into operational taxonomic units (OTUs) with a 97% similarity threshold using UPARSE 7.0.1090 (Edgar, 2013). The representative OUT sequence was annotated using the SILVA database v.138 (https://www.arb-silva.de/).

Data analysis

Venn diagram of the common and specific OTUs among groups were drawn using R software (v3.3.1, http://www.R-project.org/). Alpha diversity of rhizosphere soil bacterial communities, including Chao1 and Shannon indices, was analyzed using Mothur software (v1.30.2, https://www.mothur.org/). Statistical significance determined by one-way analysis of variance (ANOVA) for α-diversity. QIIME software (v1.9.1) was used to conduct PCA analysis to show the beta diversity of the bacterial community at different stages (Caporaso *et al*., 2010). The linear discriminant analysis (LDA) effect size (LEfSe) was applied to identify taxa with statistical differences (LDA score >3.5, *P* <0.01), and the correlations between the rhizosphere soil bacterial communities and soil chemical properties (SOM, pH, TN, AP, AK) were evaluated by Mantel tests (Dixon, 2003). Adonis statistical method was employed to determine the significant difference between different groups, the impact of different stages on the rhizosphere soil bacterial community was valued by Permutational multivariate analysis of variance (PERMANOVA). The cluster of orthologous groups (COG) family and KO (KEGG ortholog) information corresponding to each OTU were obtained using the PICRUST software (v1.1.0) and PICRUSt program with 3 levels of metabolic pathways was used to forecast the metabolic function of bacterial community in consideration of its effectiveness in obtaining functional predictions (Langille *et al*., 2013).

Table I. Chemical factors of rhizosphere soil at different growth stages of the coupled rice-crab system.

Stage	pH	AP (mg/kg)	AK (g/kg)	SOM (g/kg)	TN (g/kg)
TS	6.66±0.09[a]	31.39±0.56[ab]	126.7±5.81[a]	25.98±3.54[a]	1.46±0.20[ab]
JS	6.03±0.19[b]	35.68±2.82[a]	112.18±36.79[a]	26.70±2.67[ab]	1.50±0.15[ab]
HS	6.31±0.2[ab]	29.55±0.86[b]	94.75±13.55[a]	31.85±2.6[a]	1.78±0.15[a]
MS	6.69±0.11[a]	25.76±0.65[b]	75.39±3.23[a]	20.48±1.52[b]	1.15±0.08[b]

TS, tillering stage; JS, jointing stage; HS, heading stage; MS, maturity stage; AP, available phosphorus; AK, available potassium; SOM, soil organic matter; TN, total nitrogen. a-b: Small letters in the same column indicate significant difference among different growth stages at P <0.05.

RESULTS

Rhizosphere soil chemical properties at different growth stages of coupled rice-crab system

The chemical factors of rhizosphere soil in the coupled rice-crab system changed at different growth stages (Table I). The pH at TS was significantly different from that at JS (P <0.05). The AP content decreased significantly by 20.74% at HS and 38.51% at MS compared to that at JS (P <0.05). The AK content gradually decreased with the growth and development of rice, but there was no significant difference among different stages (P >0.05). The content of SOM and TN showed a trend of increasing first and then decreasing, with the highest at HS and the lowest at MS. Compared with HS, the content of SOM and TN at MS significantly decreased 55.52% and 54.78% respectively (P <0.05).

α-Diversity of bacterial community in rhizosphere soil at different growth stages

After quality control, a total of 444,882 clean sequences from 12 samples were obtained and 6637 rhizosphere soil bacterial OTUs with 97% sequence similarity were identified. 4690, 5056, 5139 and 5048 bacterial OTUs were detected at TS, JS, HS and MS, respectively (Supplementary Fig. SII). The numbers of common bacterial OTUs among the four groups were 3122; and the numbers of unique bacterial OTUs were 273, 294, 264 and 328 at TS, JS, HS and MS, respectively.

The results of the analyzed soil samples collected at different growth stages showed an insignificant change in rhizosphere soil bacterial community α-diversity indicies (ANOVA, P >0.05). The highest bacterial Chao1 index (4784.3 ± 165.89) was detected at HS, while the Chao1 indices at TS, JS and MS were 4373.45 ± 199.03, 4770.05 ± 274.37 and 4689.98 ± 303.17, respectively. Furthermore, the highest Shannon index (6.87 ± 0.06) was observed at MS, while the Shannon indices at TS, JS and HS were 6.85 ± 0.04, 6.78 ± 0.11 and 6.80 ± 0.09, respectively (Table II).

Table II. Chao1 and Shannon indices of rhizosphere soil bacterial community at four different stages in the coupled rice-crab system.

Indices	TS	JS	HS	MS
Chao1	4373.45± 199.03	4770.05± 274.37	4784.3± 165.89	4689.98± 303.17
Shannon	6.85±0.04	6.78±0.11	6.80±0.09	6.87±0.06

Notes: Values are mean ± standard deviations (SD).

Factors driving rhizosphere soil bacterial communities in different stages of paddy fields

The influence of all driving factors including rhizosphere soil chemical properties and different stages on the bacterial communities was investigated. The result of Mantel test (Supplementary Table SI) indicated that the rhizosphere soil chemical properties had little correlation with bacterial communities (Mantel test, R^2 = 0.227, P >0.05), whereas the different stages explained 55.7% of variance in bacteria (PERMANOVAs, R^2 = 0.557, P = 0.034 <0.05). The PCA results showed that the structure of bacterial communities at four growth stages were significantly different (Fig. 1, R^2 = 0.5663, Adonis test, P = 0.001).
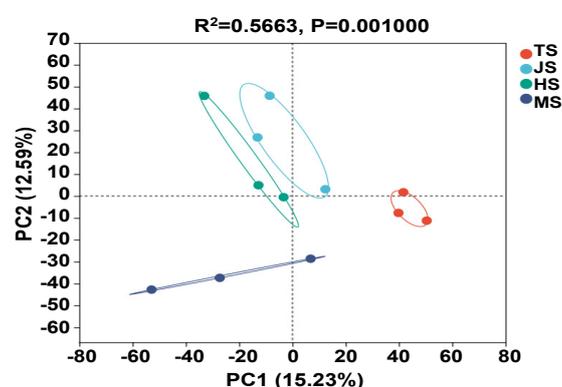

Fig. 1. Principal component analysis (PCA) based on Bray-Curtis Distance Matrix for soil samples collected from the rhizosphere of the four different stages.

Composition of the rhizosphere soil bacterial community

Dynamic changes of species at different classification levels were assessed (Fig. 2). There were 10 dominant bacterial phyla accounted for 91% of the whole rhizosphere soil bacterial composition. Chloroflexi, Actinobacteriota, Proteobacteria and Acidobacteriota were the top four predominant phyla in all samples, accounting for 18.72~28.25%, 13.20~22.61%, 12.27~19.41%, 11.29~14.04% respectively (Fig. 2A). Chloroflexi was the most dominant bacterial phylum in all four samples, but with no significant difference at four stages. In addition, Actinobacteriota, Proteobacteria, Desulfobacterota, Firmicutes and Genmatimonadota were significantly different among four different stages (ANOVA, *P* <0.05) (Supplementary Fig. S3A). The most abundant classes were Anaerolineae (12.56%), Gammaproteobacteria (9.18%), Actinobacteria (8.99%), Alphaproteobacteria (6.56%) and KD-4-96 (6.33%) (Fig. 2B).

Across the four stages in the main bacterial class, Gammaproteobacteria, Actinobacteria, Alphaproteobacteria and Bacteroidia were remarkably different (ANOVA, *P* <0.05) (Supplementary Fig. S3B). The *norank_fnorank_o_norank_c_KD4-96* (3.86%~8.38%), *norank_f_norank_o_RBG-13-54-9* (2.43%~4.10%), *norank_f_norank_o_Gaiellales* (2.73%~3.97%), *norank_f_norank_o_Vicinamibacterales* (2.03%~3.36%), *norank_f_Anaerolineaceae* (1.84%~3.48%) were the top 5 genus at four stages in all samples (Fig. 2C) and *norank_f_ norank_O_RBG-13-54-9* was the only genus with notable difference among the four stages (ANOVA, *P* <0.05) (Supplementary Fig. S3C).

A

B

Fig. 3. Cladogram showing the phylogenetic distribution of the bacterial lineages associated with rhizosphere soil from four growth stages in the rice-crab paddy field. (A) Indicator bacteria with LDA scores of 3.5 or greater in bacterial communities associated with soil from four stages in the coupled rice-crab paddy field. (B) Different coloured regions represent different constituents. Circles indicate phylogenetic levels from phylum to genus. The diameter of each circle is proportional to the abundance of the group.
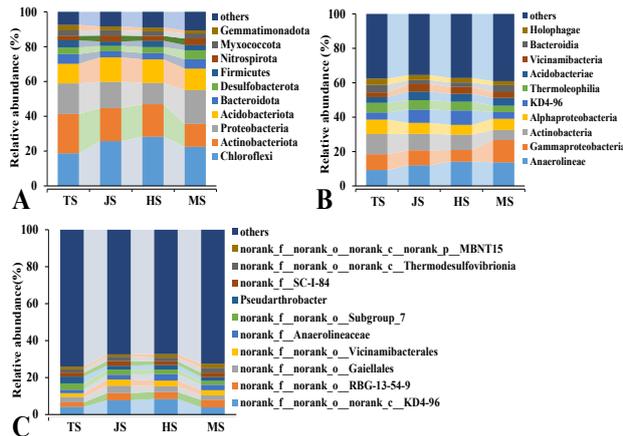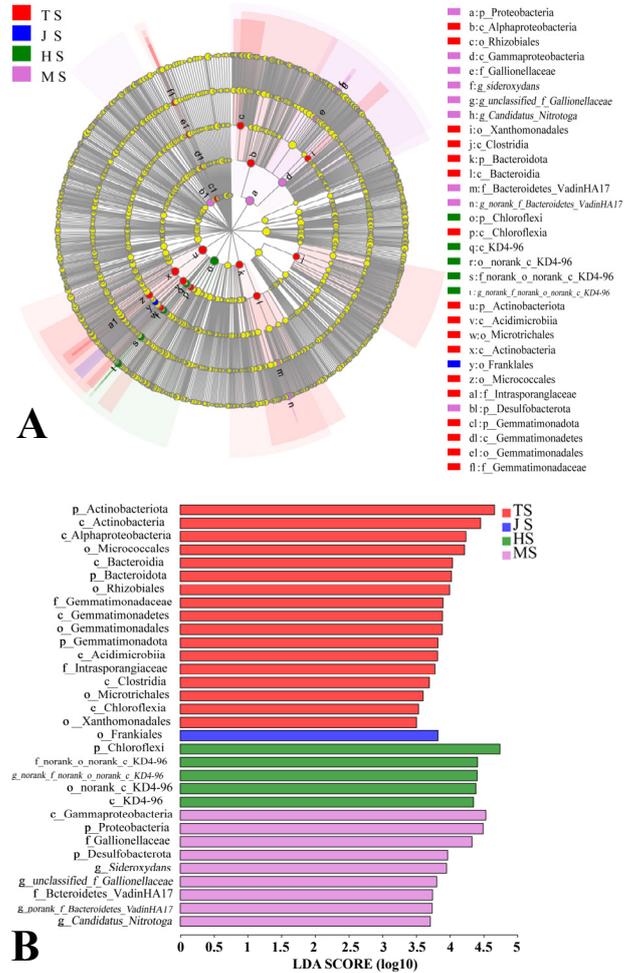

Fig. 2. Rhizosphere soil bacterial taxonomic composition at phylum (A), class (B) and genus (C) levels in four growth stages.
Note: The top 10 relative abundances were shown, while the remaining abundance were indicated as others.

LEfSe of the bacterial communities in the rhizosphere of the four stages in the crab-rice paddy fields

Taxa with significant differences among TS, JS, HS and MS were detected by LEfSe (Fig. 3). The LEfSe results indicated that 32 taxa were enriched among the four stages when the LDA score was higher than 3.5. 17 were distinctly abundant at TS, notably the phylum Actinobacteriota and Bacteroidota, the class Actinobacteria, Alphaproteobacteria and Bacteroidia and the order Micrococcales. The differentially abundant taxa

at JS was the order Frankiales. The phylum Chloroflexi and the class, order, family and genus of KD4-96 were enriched taxa in HS. The class Gammaproteobacteria, the phylum Proteobacteria and the family Gallionellaceae were amplified in MS.

Function prediction of rhizosphere soil bacterial community at different growth stages of rice-crab paddy field

We used PICRUSt to predict KEGG pathways based on the OTU data. In total, 46 KEGG pathways at the 2nd level were found by PICRUSt analysis. The results demonstrated that the relative abundance of 43 pathways at HS were higher than other three stages, and the relative abundance of 8 metabolic pathways was dramatically different such as amino acid metabolism, biosynthesis of other secondary metabolites, energy metabolism, translation, replication and repair, signal transduction, lipid metabolism, cellular community-prokaryotes at four different stages (Fig. 4).
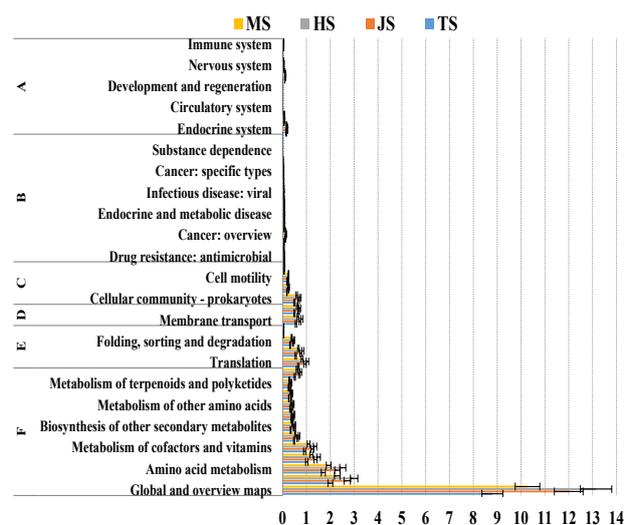

Fig. 4. The relative abundance of rhizosphere soil bacterial community functions at four different stages in the coupled rice-crab system. A, organismal system; B, human diseases; C, cellular processes; D, environmental information; E, genetic information processing; F, metabolism.

DISCUSSION

Many studies have been conducted on various co-culture systems for a long time, which bring many positive effects on both crops and the environment, showing significant sustainability (Herlambang *et al*., 2021; Bashir *et al*., 2020; Fierro-Sañudo *et al*., 2020; Feng *et al*., 2016; Hu *et al*., 2016). The rice-crab co-culture

system effectively enhances rice yield and quality, while concurrently reducing the use of fertilizers and pesticides; it also improves soil quality, optimizes land use, and safeguards biodiversity (Hu *et al*., 2020; Jin *et al*., 2020; Bao *et al*., 2022). The co-culture of crabs can impact the composition of soil microbial communities in rice paddies (Li *et al*., 2021). To gain a better understanding of the dynamic changes associated with this co-culture system, this study investigated the chemical properties and bacterial communities in the rhizosphere soil, clustered at different growth stages in the coupled rice-crab paddy field. The results showed that there was no significant correlation between chemical properties and bacterial communities in rhizosphere soil. Furthermore, PERMANOVAs result showed that the different growth stages of rice explained 55.7% of the variance in bacteria. Rasche *et al*. (2006) indicated that bacterial community in rhizosphere of *Solanum tuberosum* varied significantly at different vegetation stages. Zhao *et al*. (2022) proved that rhizosphere bacterial community in orchard soil under a cover crop at different periods was obviously different. Thus, we deduced that the growth stage of rice may be the main factor driving rhizosphere bacterial community of the coupled rice crab system. Different growth stage had different bacterial diversity in the rhizosphere soil of the coupled rice-crab system (Chen *et al*., 2019; Wang *et al*., 2021). Previous study demonstrated that the number, species and diversity of rhizosphere microorganisms showed a dynamic change trend (Lauber *et al*., 2009). In our study, soil samples of the same kind of rice in the rice-crab paddy field were collected at the four different stages, to invest whether the growth stage was the main factor influencing rhizosphere bacterial diversity. The results showed that, there were the most OTUs and Chao1 index at the heading stage, indicating there were the largest number of species, meanwhile, Shannon index at maturity is the highest, showing that the species are most abundant in this period. Similarly, Xu *et al*. (2009) demonstrated that diversity of rhizosphere bacterial community in soybean changed with a progression in growth stage and full maturity had the highest diversity. This may be due to that the component and content of plant root exudates in different growth stages were different, which might prohibit specific rhizosphere bacterial groups, consequently affecting the diversity of bacterial community in the rhizosphere. Despite slight differences in the α-diversity indices across the different growth stages, no significant differences were observed among them, which is consistent with the results of other rice co-culture studies, including rice-fish (Arunrat *et al*., 2022), rice-frog (Yi *et al*., 2019), and rice-crab (Li *et al*., 2021).

In this study, in the rice-crab co-culture system, the

relative abundance of the bacterial community varied with the growth stage at different taxonomic levels, and that of some predominant bacterial groups showed remarkable difference at the four stages. Distinct differences were found in the abundances of dominant bacterial phyla, Actinobacteriota was significantly abundant at TS, and the abundance of Proteobacteria at MS was high. Similarly, Ding et al. (2021) demonstrated that Proteobacteria and Actinobacteriota were the rhizosphere donimant phyla in the rice-turtle co-culture system. Moreover, Desulfobacterota was significantly enriched at MS, Firmicutes and Gemmatimonadota were also more abundant at TS than in the other stages., Actinobacteria, widely existed in soil and water ecosystems, played a key role in decomposition and humus formation, controlling plant disease, promoting plant growth and regulating the biological interactions among the microenvironment, plants, and pathogens (Buée et al., 2009; Gao et al., 2019). Johnston-Monje et al. (2016) had confirmed that environment with rich nutrient was suitable for the rapid growth of Proteobacteria. Most of Proteobacteria are gram negative bacillus, many of which are responsible for nitrogen fixation and polycyclic aromatic hydrocarbon degradation. Desulfobacterota is a kind of sulfate-reducing bacteria which is widely existed in the paddy fields. It can not only reduce sulfate to sulfide, participating in the biogeochemical cycle of sulfur soil, but also degrade cresol, biphenyl and other pollutants in paddy soil (Shibata et al., 2007; Yang et al., 2008). Firmicutes can inhibit the production and emission of methane by inhibiting the activity of methanogens and improving the redox potential of methanogenic environment through Fe(III) reduction process. (Cheng et al., 2007; Frenzel et al., 1999). Bay et al. (2021) identified phylum Gemmatimonadeota potentially capable of aerobic methanotrophy. Significant enrichment in rhizosphere soils at different stages suggested the rhizosphere could attrack specific bacteria in the different growth stages. All these results showed that growth stages could influence the composition of the rhizospheric bacterial community. LEfSe analysis showed that there were more bacterial taxa with significant differences at TS than that in other samples, which might be related to the vigorous metabolic activity of rice roots at TS (Zhang et al., 2015). PCA analysis showed that the structure of bacterial communities at four growth stages were significantly different, but the community structure between JS and HS was similar, indicating the bacterial community structure inclined to be stable and the variation was small in the middle stage of rice development. The community structure between TS and MS was a little different. Moreover, the community structure of TS and MS was different from that of JS and HS, explaining that growth stage of rice had an impact on the rhizosphere bacterial community structure.

Soil microorganisms can affect soil nutrient cycling and regulate soil function by participating in the decomposition and mineralization of soil organic matter (Romaniuk et al., 2011). Yuan et al. (2015) deduced that the continuous changes of some physicochemical factors such as the change of root structure and rhizosphere secretion caused the succession of bacterial functional structure. In this study, the PICRUSt results showed metabolic pathways at 3 levels in different growth stages of rice-crab paddy field were similar, and the metabolic function of bacteria changed in four different stages of rice development. Furthermore, the abundance of 43 metabolic pathways at the 2nd level was higher in HS than those in the other three stages and there were significant differences in 8 metabolic pathways. The rice HS is an essential phase of rice production because it can affect the rice yield directly (Bai et al., 2018). Previous study demonstrated that the community structure in the middle and late stages of rice development was relatively stable, which was different from that in the early stage (Noll et al., 2005). In this paper, the number of OTU and Chao1 index at HS were also the highest among the four growth stages, revealing that the total number of species in this period was the largest. Taken together, HS had the highest number of species and the strongest metabolic functional potentials, indicating the interrelation between bacterial community composition and function. Future studies may further explore the correlation between rhizosphere bacterial communities structure and function and rice yield in the coupled rice-crab system.

CONCLUSION

In conclusion, the results showed that the rhizosphere bacterial communities were more influenced by growth stage than chemical properties, and the bacterial community structure differed significantly among the four growth stages. At the heading stage, the bacterial community had the largest number of species and strongest metabolic function potentials. Specifically, with the OTU number and Chao1 index of 5139 and 4784.3, respectively, which were the highest at the four stages. Moreover, the relative abundance of 43 among the total 46 metabolic pathways at heading stage was higher than that of the other three stages. This study highlights the impact of rice growth stages on the composition and functional potentialities of rhizosphere bacterial communities in the coupled rice-crab system. These results provide data reference for the practical application of this co-culture system, and essential insights for maintaining soil ecosystem balance.

IRB approval

This study did not involve human subjects.

Ethical statement

Not applicable.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20220222030200

Statement of conflict of interest

The authors have declared no conflict of interest.

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