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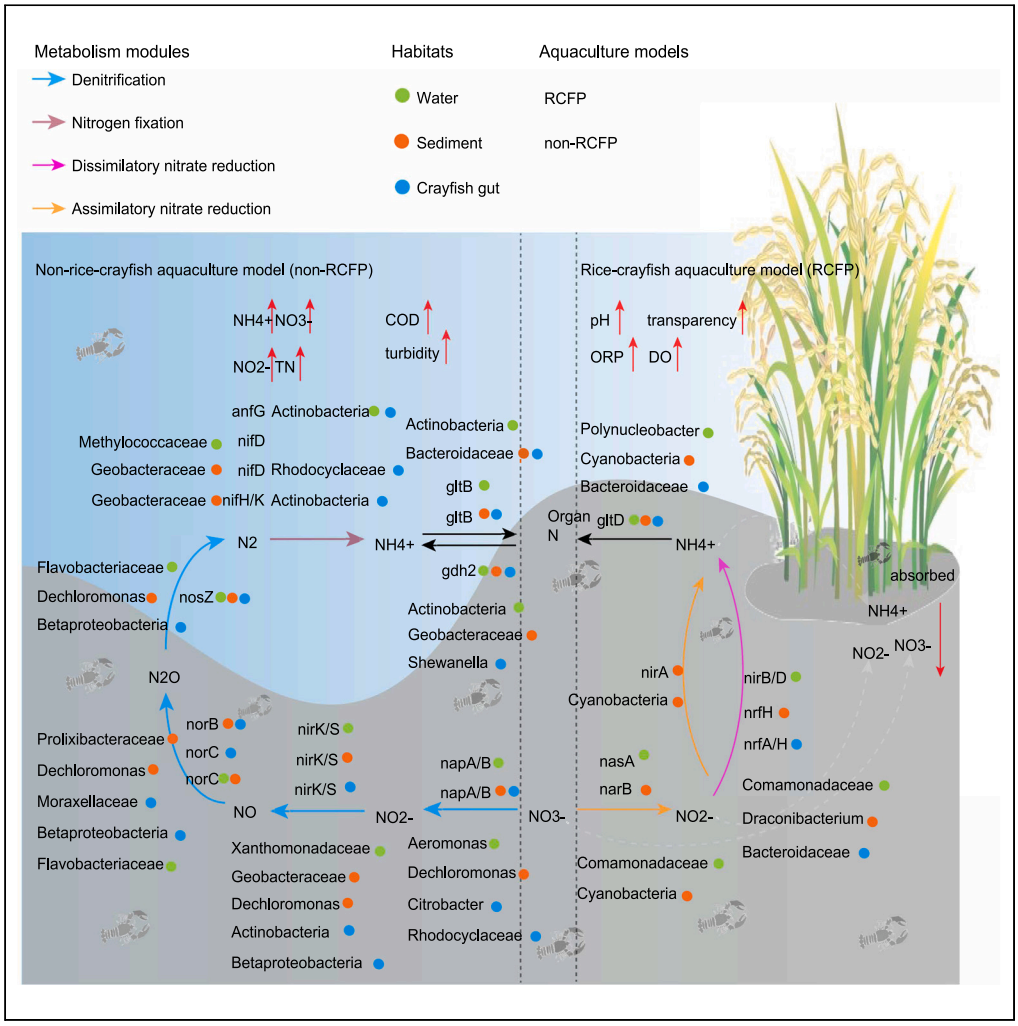

Xue Zhu, Pengshuo Yang, Guangzhou Xiong, Huimin Wei, Lu Zhang, Zhi Wang, Kang Ning

zwang@apm.ac.cn (Z.W.)
ningkang@hust.edu.cn (K.N.)

Highlights

A holistic conceptual model is proposed for microbial biogeochemical cycling

RCFP has high N and S pollutants removal abilities across habitats

This study evidentially supports the sustainability of RCFP aquaculture ecosystems

This study indicates RCFP might lead to the blue transformation of aquaculture

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Microbial biogeochemical cycling reveals the sustainability of the rice-crayfish co-culture model

Xue Zhu,[1] Pengshuo Yang,[1] Guangzhou Xiong,[1] Huimin Wei,[2,3] Lu Zhang,[2,3] Zhi Wang,[2,*] and Kang Ning[1,4,*]

SUMMARY

Aquaculture has great potential in nourishing the global growing population, while such staggering yields are coupled with environmental pollution. Rice-crayfish co-culture models (RCFP) have been widely adopted in China due to their eco-friendliness. However, little is known about RCFP's microbiome pattern, which hinders our understanding of its sustainability. This study has conducted metagenomic analysis across aquaculture models and habitats, which revealed aquaculture model-specific biogeochemical cycling pattern (e.g., nitrogen (N), sulfur (S), and carbon (C)): RCFP is advantageous in N-assimilation, N-contamination, and S-pollutants removal, while non-RCFP features N denitrification process and higher S metabolism ability, producing several hazardous pollutants in non-RCFP (e.g., nitric oxide, nitrogen monoxide, and sulfide). Moreover, RCFP has greater capacity for carbohydrate enzyme metabolism compared with non-RCFP in environmental habitats, but not in crayfish gut. Collectively, RCFP plays an indispensable role in balancing aquaculture productivity and environmental protection, which might be applied to the blue transformation of aquaculture.

INTRODUCTION

Aquaculture is an indispensable source of global food and nutrition supply, feeding approximately 12% of the population (1 billion people) around the world.[1–3] As one of the most important resources of nutrients, the aquaculture ecosystem contains diverse biologically available essential elements, such as carbon (C), nitrogen (N), and sulfur (S).[4,5] Microbes in this ecosystem represent one of the most important drivers of biogeochemical cycling, as they are responsible for the re-mineralization of organic matter and energy exchange.[6–10] For example, in aquaculture, under the transformation of the functional genes (i.e., *nor* family) from the microbiome, 75% of total N was lost through gas emissions or water exchange,[11] resulting in adverse effects on the global environment, such as water eutrophication, nitrate, and nitrite pollution, as well as greenhouse gas emissions.[1,12] Among the greenhouse gases, nitrous oxide is a major contributor, which has approximately 300 times the global warming potential than carbon dioxide,[13] thereby depleting the stratospheric ozone[12] and threatening the global climate.[14,15] Thus, it is crucial to further understand microbial-mediated biochemical cycling, which will promote the sustainable development of aquaculture.

To balance high productivity and environmental sustainability,[16] different aquaculture models have been developed, especially the co-culture models, which are important forms of aquaculture due to their high productivity and lower environmental pollution.[17–19] In recent years, rice-crayfish co-culture model (RCFP) is widely implemented in China due to its eco-friendliness.[20] In this aquatic-rice co-culture aquaculture model, paddy provides sufficient space and food for crayfish growth, whereas crayfish preys on pests and provides organic fertilizer for rice, which limits the inputs of antibiotics and chemical residues considerably.[19,20] Utilizing these multi-directional interactions of co-culture species,[20] this model yields 90% of total crayfish production in China, while is relatively environmentally friendly.[18,19,21–23] However, how microbial communities, which surely contribute greatly, contribute to this balance of productivity and sustainability remains unclear.

Previous research has deciphered that aquaculture-related microbial communities (e.g., lake, soil, and animal gut microbiome) are important for crayfish growth and rice yields.[20,22,24,25] Comparing the microbial communities of RCFP and other aquaculture models revealed that the RCFP aquaculture model possesses

[1]Key Laboratory of Molecular Biophysics of the Ministry of Education, Hubei Key Laboratory of Bioinformatics and Molecular-imaging, Center of AI Biology, Department of Bioinformatics and Systems Biology, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, Hubei 430074, China

[2]Key Laboratory for Environment and Disaster Monitoring and Evaluation of Hubei, Innovation Academy for Precision Measurement Science and Technology, Chinese Academy of Sciences, Wuhan 430077, China

[3]University of Chinese Academy of Sciences, Beijing 100049, China

[4]Lead contact

*Correspondence: zwang@apm.ac.cn (Z.W.), ningkang@hust.edu.cn (K.N.)

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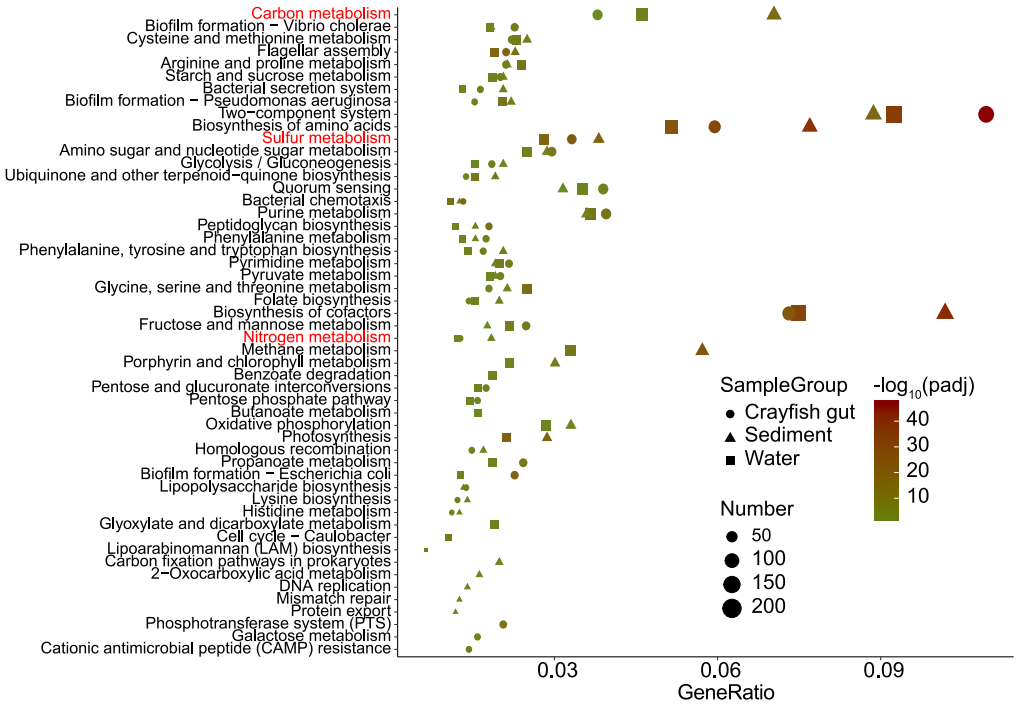

Figure 1. KO enrichment for functional genes across habitats and aquaculture models

Only the top 40 enriched KEGG pathways (adjusted p value <0.05) were visualized. The size and color of these shapes represent the count number of functional genes and their adjusted p value. Square: water; Triangle: sediment; Circle: crayfish gut.

a distinct set of microbes, robust microbial community, lower ARG content, and HGT events, and is less affected by environmental factors.[16,26,27] All of these findings have demonstrated the sustainability of the rice-crayfish aquaculture model. However, as major drivers of biogeochemical cycling,[28,29] there lacks of research on analyzing the role of microbiome in biogeochemical cycling between the RCFP and non-RCFP, which hinders our deeper understanding of the sustainability of RCFP from the perspective of microbial biogeochemical cycling.

Therefore, in this study, we profiled the microbial community collected from water, sediment, and crayfish gut samples, as well as proposed a holistic conceptual model for a deeper understanding of the functional role of microbial communities in driving the biogeochemical cycling processes between RCFP and non-RCFP. From the perspective of biogeochemical cycling, we have found RCFP and non-RCFP are responsible for biogeochemical cycling through different N, S, and C metabolic modules coupled with different sets of microbial genes. Besides, RCFP microbial community has higher abilities in converting mineral N into organ N, and N contamination removal. While non-RCFP microbial community is responsible for N cycling through denitrification and N decomposition modules, coupled with a series of environmental pollutants (nitrite, nitric oxide, and nitrous oxide). Moreover, RCFP microbial community has less S pollution compared to non-RCFP. Furthermore, we also found that RCFP has a higher carbohydrate enzyme metabolism than that of non-RCFP in environmental habitat, but not in animal gut habitat. Taken together, these findings demonstrated that RCFP is a sustainable aquaculture model from biogeochemical cycling, thereby shedding important insights into sustainable aquaculture and environmental protection.

RESULTS

N, S, and C cycles are the representative biogeochemical processes in Honghu farm

Three major biogeochemical cycling pathways were the significantly enriched KEGG pathways across both aquaculture models and habitats. The annotated KO genes in each MAG were assessed by clusterProfiler to further select the significantly enriched genes in KEGG pathways (Figure 1). As the representative biogeochemical process, N metabolism (ko00910; water: p = 2.61586E-05, sediment: p = 5.98E-08, crayfish

gut: p = 5.51222E-05), S metabolism (ko00920; water: p = 3.85E-19, sediment: p = 5.29E-17, crayfish gut: p = 1.55E-23), and C metabolism (ko01200; water: p = 4.81E-07, sediment: p = 4.78E-21, crayfish gut: p = 0.013) were detected as enriched KO pathways across habitats and aquaculture (Figure 1). Then, the functional genes involved in the three major biogeochemical cycling pathways were extracted, including 32 KO genes, 78 KO genes, and 140 KO genes in N, S, and C metabolism pathways, respectively. Thus, we mainly concentrated on the metabolic pathways of these three biogeochemical cycling pathways.

RCFP possesses higher abilities in N-assimilation and N pollutant removal

RCFP is advantageous in N-assimilation and N contamination removal, whereas non-RCFP possesses higher N decomposition and denitrification capacities. A total of 32 N-cycling genes differed significantly between RCFP and non-RCFP (Wilcoxon test, $p < 0.1$; Figure 2A), which refers to 5 N metabolism modules, including nitrification, denitrification, assimilatory nitrate reductase (ANR), dissimilatory nitrate reductase (DNR), and nitrogen fixation (Figure 2). These modules were consistently observed across water (Figure 2B), sediment (Figure 2C), and crayfish gut habitats (Figure 2D), but exhibited different N metabolism modules between RCFP and non-RCFP (Figure 2).

In water habitat, the RCFP microbial community played the N-cycling pathways through DNR process (*nasA*) to convert nitrate into nitrite, then the nitrite was transformed into ammonium through ANR process (*nirB*: $p < 0.1$; *nirD*: $p < 0.01$), and the ammonium was converted as organic N through N assimilation (*gltD* gene), which promotes the biosynthesis of organic N and significantly decreased the content of nitrate, nitrite, and ammonium (Figure 2B). These processes promote N pollutants removal, which is beneficial to aquatic animals in aquaculture.[30] While the lower dissolved oxygen (DO; Figure S1A) and higher chemical oxygen demand (COD; Figure S1B) promote the denitrification process in non-RCFP aquaculture model, the nitrate (Figure S1C) was first transformed into nitrite by *napA* and *napB* genes (Figure 2B), resulting in the accumulation of nitrite in non-RCFP (Figure S1D). While the high level of nitrite is noxious to aquatic animals.[31,32] And the denitrification process also produced a series of gaseous N-compounds (nitric oxide: *nirK* ($p < 0.05$) and *nirS*; nitrous oxide: *norB*; nitrogen: *nosZ*; Figure 2B); nitrogen was further fixed as ammonium through nitrogen fixation (*nifD* and *anfG* genes). Though the ammonium was transformed to organ N under *gltB* genes (Figure 2B), the organ N was significantly converted into ammonium by *gdh2* gene ($p < 0.1$), which resulted in the accumulation of ammonium in non-RCFP (Figure S1E). The higher organic decomposition abilities in non-RCFP might be caused by the higher fertilizer inputs in non-RCFP,[16,20] which was also supported by our analyzed data (Figures S1C–S1F). These phenomena were also observed in sediment (Figure 2C) and crayfish gut habitats (Figure 2D). Besides, the higher temperature also inhibited the release of ammonium from sediment, resulting in the accumulation of ammonium.[33] Excessive inorganic N compounds in non-RCFP (Figures S1C–S1F and S2B-C) are adverse for aquatic animals.[12,30,34] Collectively, these findings indicated that RCFP has higher N assimilation and N contamination removal abilities.

RCFP has fewer S-related pollutants

RCFP has less S-related compound contamination. The microbial community in different aquaculture models exerted S-cycling pathways through different S-related genes either in the environmental habitat or in the animal gut habitat (Figure 3). The distribution of S-related genes varied across aquaculture and habitats (Figure 3A; gene number: water: 26, sediment: 23, and crayfish gut: 25). Among these habitats, these S-cycling gene distributions varied greatly (Figure 3). Besides, a total of three S-metabolism modules were consistently detected across habitats and aquaculture models, which included assimilatory sulfate reduction (ASR), dissimilatory sulfate reduction (DSR), and thiosulfate oxidation by SOX complex (SOX complex) pathway across water (Figure 3B), sediment (Figure 3C), and crayfish gut (Figure 3D) habitats, but the genes involved in these modules were distinct between RCFP and non-RCFP (Figure 3), indicating aquaculture model-dependent variation in S-cycling pathways. Compared with RCFP, non-RCFP microbial community has more abundant genes involved in S metabolism, promoting the accumulation of sulfide, which is hazardous to aquatic animals and the environment, especially in the unique condition (i.e., higher pH and N levels, lower DO) of non-RCFP (Figures S1 and S2).

RCFP has greater carbohydrate metabolism ability

RCFP aquaculture model has a higher carbohydrate enzyme metabolism activity than that of non-RCFP in environmental habitats. To better understand the carbon metabolism, the non-redundancy genes in each sample were annotated via the CAZy database (Figure 4). After the coverage of each gene in carbohydrate metabolism categories (Figures 4A–4F) was summed, the abundance of each category was plotted

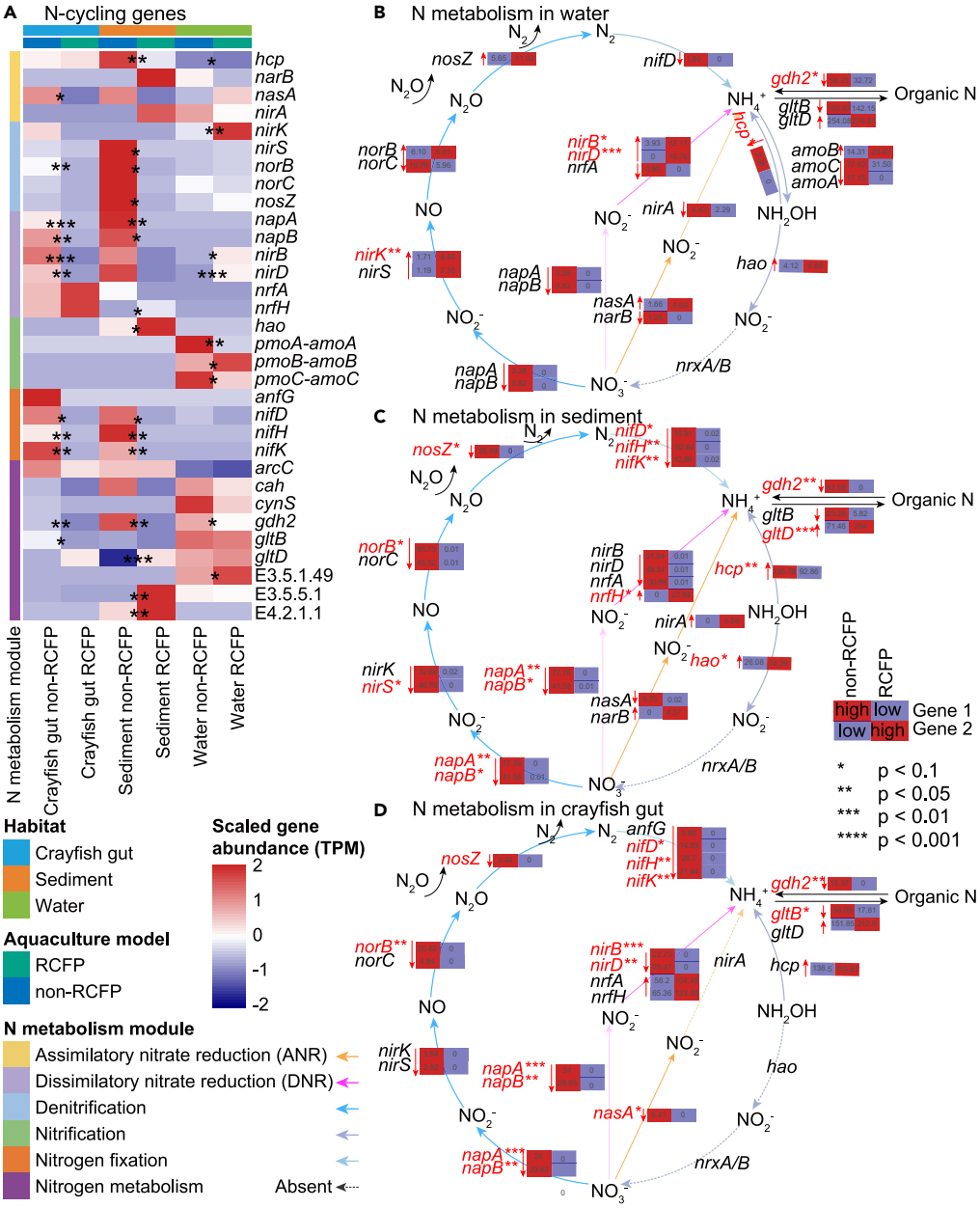

Figure 2. Functional profiles of N-cycling pathways between RCFP and non-RCFP across habitats

(A–D) The relative abundance (TPM, transcripts per million) of genes in N-cycling pathways across aquaculture models and habitats. (A) Wilcoxon test was used to identify differences of microbial functional genes between RCFP and non-RCFP in each habitat. Gene distribution in N-cycling pathways between RCFP and non-RCFP in water habitat (B), sediment (C), and crayfish gut habitats (D), respectively. Arrows in lilac, blue, wathet, purple, and orange represent nitrification, denitrification, nitrogen fixation, dissimilatory nitrate reduction (DNR), and assimilatory nitrate reduction (ANR), respectively. The number in the heatmap, from left to right, indicates the gene abundance in RCFP and non-RCFP, respectively. And red arrows represent gene that is more (upward) or less (downward) abundant in RCFP. Significant differences in gene abundance (TPM, transcripts per million) between RCFP and non-RCFP are marked with asterisks (genes colored in red; Wilcoxon test, *: $p < 0.1$, **: $p < 0.05$, ***: $p < 0.01$; ****: $p < 0.001$). NO_3^-: nitrate; NO_2^-: nitrite; NH_4^+: ammonium; NO: nitric oxide; N_2O: nitrous oxide; N_2: nitrogen.

(Figures 4G–4L). Compared with non-RCFP, higher carbohydrate metabolism abilities were observed in RCFP microbial communities across water and sediment habitats, but not in crayfish gut, which may promote matter accumulation in crayfish (Figure 4).

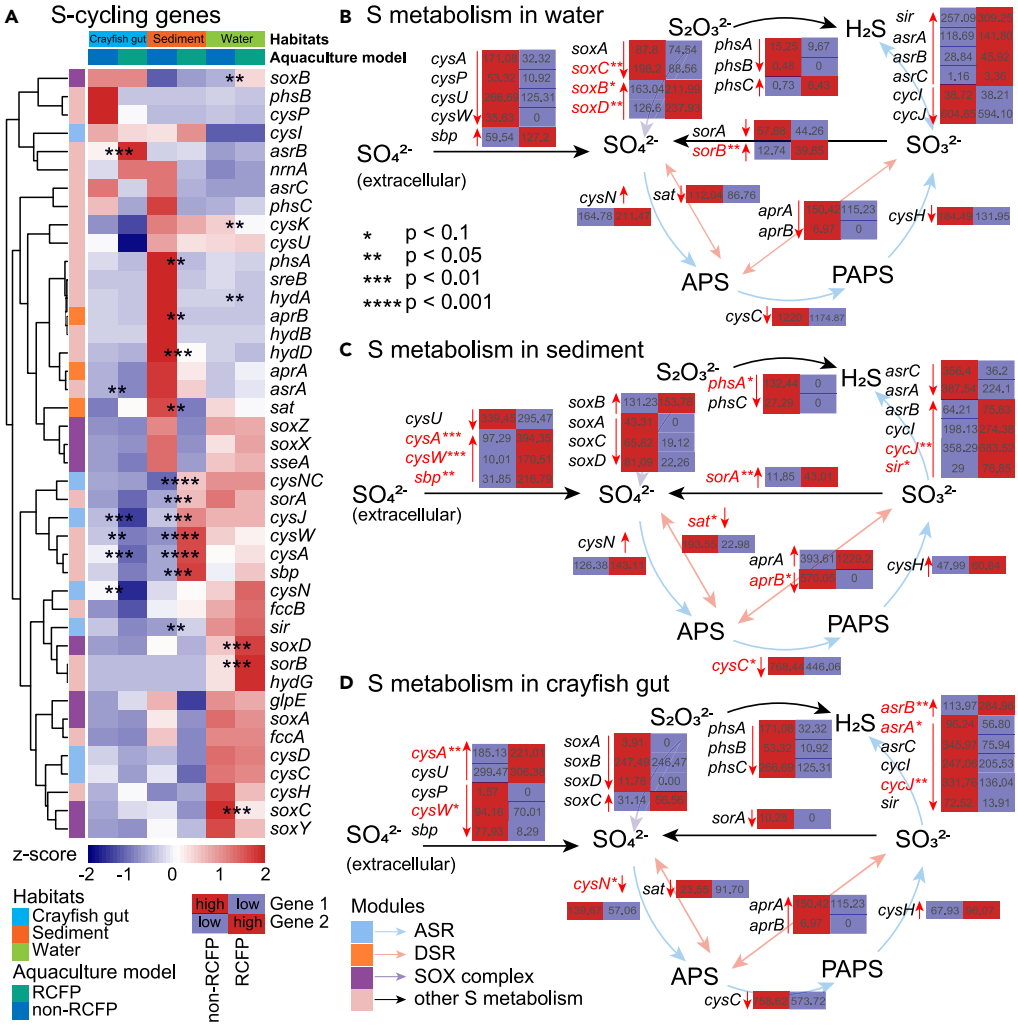

Figure 3. Functional profile of S-cycling across habitats and aquaculture models

(A–C) The relative abundance of genes related to S-cycling pathways in RCFP and non-RCFP across water, sediment, and crayfish gut habitats. A schematic model illustrating the distribution of S-cycling pathways between RCFP and non-RCFP in water (B), sediment (C), and crayfish gut habitats.

(D) Arrows in wathet, orange, purple, and pink represent assimilatory sulfate reduction (ASR), dissimilatory sulfate reduction (DSR), thiosulfate oxidation by SOX complex (SOX complex), and other S metabolism modules. The number in the heatmap, from left to right, indicates the gene abundance in RCFP and non-RCFP, respectively. Red arrows represent gene that is more (upward) or less (downward) abundant in RCFP. Significant differences in gene abundance (TPM, transcripts per million) between RCFP and non-RCFP are marked with asterisks (genes colored in red; Wilcoxon test, *: $p < 0.1$, **: $p < 0.05$, ***: $p < 0.01$; ****: $p < 0.001$). SO_4^{2-}: sulfate; SO_3^{2-}: sulfite; H_2S: sulfide; $S_2O_3^{2-}$: thiosulfate.

DISCUSSION

Aquaculture has great potential to feed and nourish the world's growing population,[5] yet the expansion of aquaculture has often occurred at the expense of environmental disruption. Sustainable aquaculture development remains critical to balancing aquaculture sustainability and the supply of the growing demand for aquatic foods.[5,35] Biogeochemical cycling is the primary pathway for nutrient assimilation, recycling, and reutilizing in aquaculture to satisfy the high demand for nutrients.[36,37] As important members of ecosystems, microbial biogeochemical cycles contribute profoundly to these processes. While rice-crayfish co-culture model has been implemented at the largest scale in China due to its high productivity and low environmental impact,[16,19,20,22,23,26] the underline microbial biogeochemical cycles remain uncharted. A few previous research studies have reported the potential

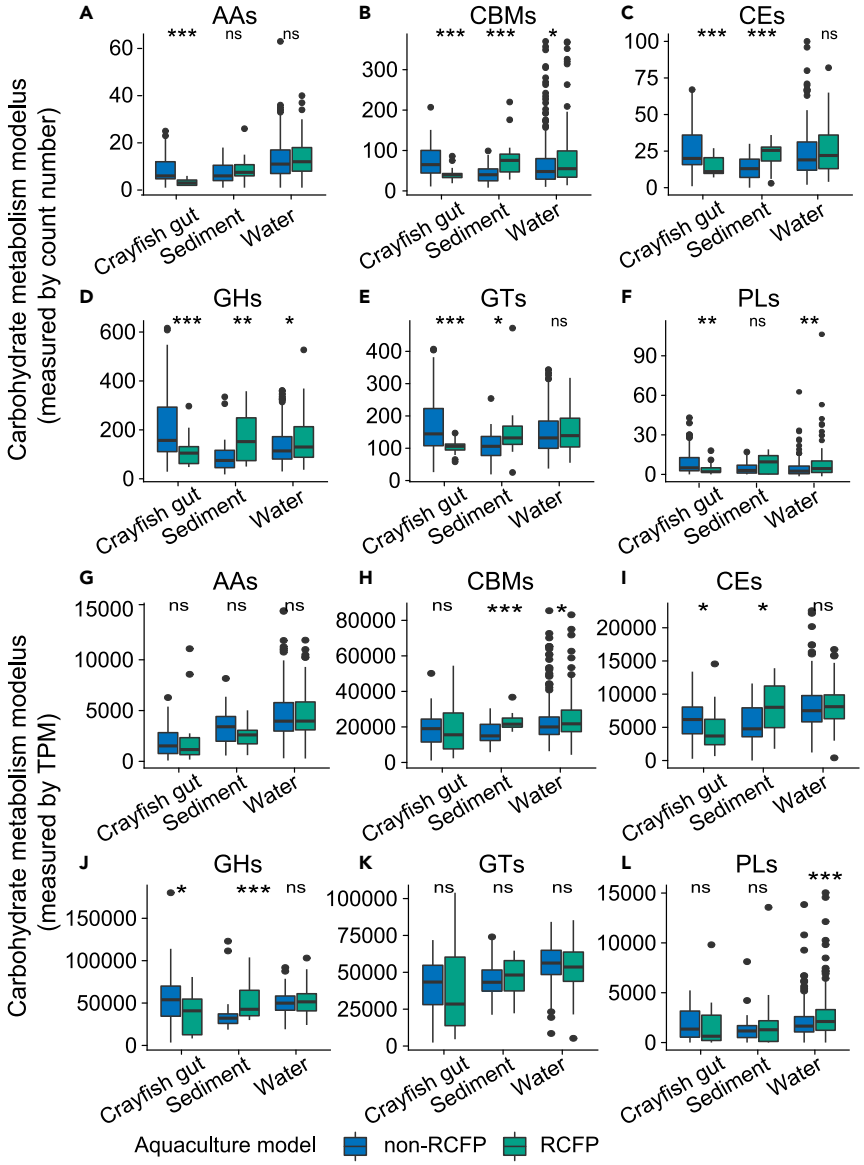

Figure 4. Distribution of genes related to carbohydrate enzyme metabolism

The count number of genes participating in carbohydrate enzyme metabolism between RCFP and non-RCFP in water, sediment, and crayfish gut habitats.

(A) AAs: auxiliary activities.

(B) CBMs: carbohydrate-binding modules.

(C) CEs: carbohydrate esterases; (D) GHs: glycoside hydrolases.

(E) GTs: glycosyl transferases.

(F) PLs: polysaccharide lyases. The relative abundance (TPM, transcripts per million) of genes participating in carbohydrate enzyme metabolism between RCFP and non-RCFP in water, sediment, and crayfish gut habitats.

(G) AAs: auxiliary activities.

(H) CBMs: carbohydrate-binding modules.

(I) CEs: carbohydrate esterases; (J) GHs: glycoside hydrolases.

(K) GTs: glycosyl transferases.

(L) PLs: polysaccharide lyases. Wilcoxon test, *: $p < 0.1$, **: $p < 0.05$, ***: $p < 0.01$; ****: $p < 0.001$.

role of the microbiome in biogeochemical cycling in natural marine and freshwater ecosystems,[8,10,38,39] while fewer studies have explored the microbial community, functions, and metabolic pathways involved in biogeochemical cycling in an engineered ecosystem, such as RCFP aquaculture model.

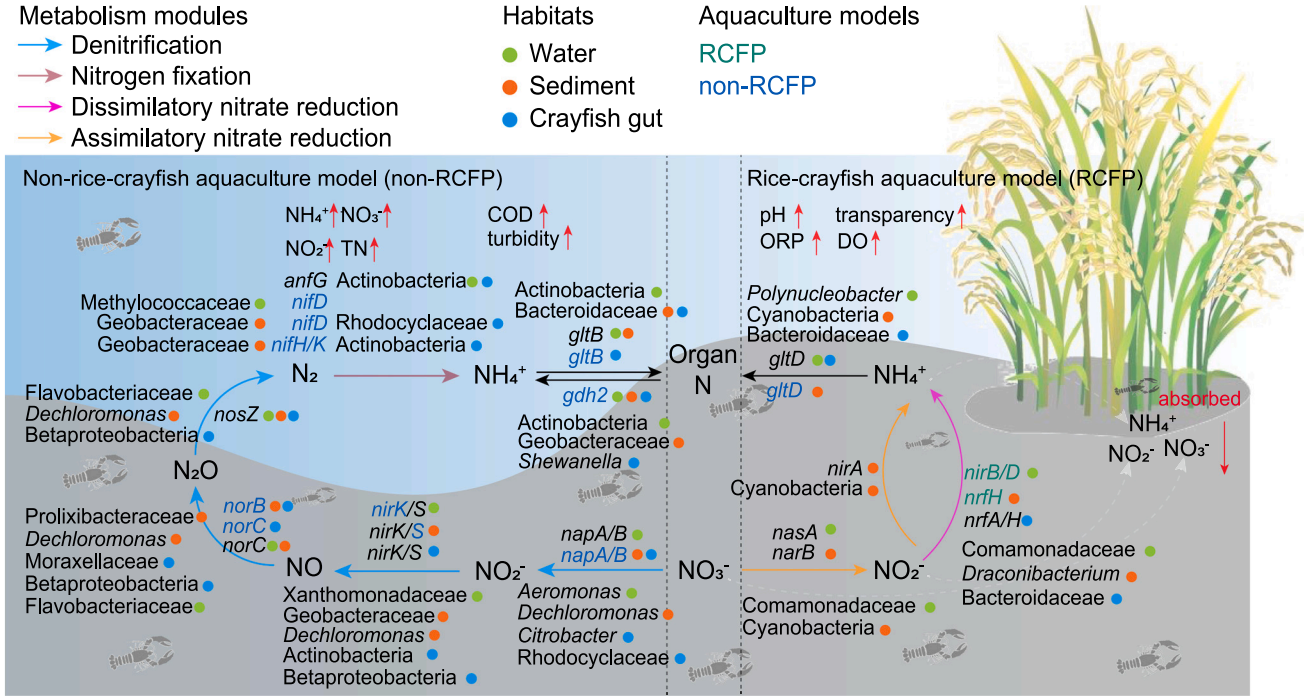

Figure 5. A conceptual model for describing the microbiome in driving N-cycling across aquaculture models and habitats

Arrows in blue, red, purple, and yellow represent denitrification, N-fixation, dissimilatory nitrate reduction, and assimilatory nitrate reduction, respectively. While circles in green, orange, and blue are water, sediment, and crayfish gut habitats, respectively. The genes colored in dark green and blue are more abundant in RCFP and non-RCFP, respectively. Red arrows represent the environmental factors (such as NH_4^+, NO_3^-, NO_2^-, pH, DO). Among them, RCFP has lower N levels and high pH, DO, and ORP compared to non-RCFP. RCFP: rice-crayfish co-culture model; non-RCFP: non-rice-crayfish co-culture model; NO_3^-: nitrate; NO_2^-: nitrite; NH_4^+: ammonium; TN: total nitrogen; DO: dissolved oxygen; COD: chemical oxygen demand; ORP: oxidation-reduction potential. See Figures S1 and S2 for more detailed information of environmental factors.

This study has focused on a well-adopted aquaculture model RCFP, aiming to gain a deep understanding of the sustainability of RCFP from the perspective of biogeochemical cycling. Our study has shed light on metagenomic binning analysis and revealed the functional genes were enriched in the N, S, and C metabolic pathways across habitats aquaculture models in Honghu farm. While the microbial communities drive the N, S, and C cycling pathways through distinct metabolism modules and functional genes between RCFP and non-RCFP.

For deeper understanding of the sustainability and eco-friendliness of RCFP, a conceptual model was proposed for describing the microbiome in driving N-cycling across aquaculture models and habitats (Figure 5), which took water, sediment, crayfish gut, rice, and environmental factors into a holistic consideration. For N-cycling, redox reaction (ANR and DNR) and denitrification were the predominant N-cycling pathway in RCFP and non-RCFP (Figures 2 and 5), respectively. Compared to non-RCFP, the DO content in RCFP (Figure S1A) is sufficient for the COD (Figure S1F), which provides sufficient conditions for redox reaction. And thus, redox reaction (Figure 5) was the predominant N-cycling pathway in this model. The nitrate was revivified to ammonium under the nitrate-reducing bacteria: Comamonadaceae (ANR: *nasA*; DNR: *nirB/D* genes) in water habitats, Cyanobacteria (ANR: *narB* and *nirA*) and Draconibacterium (DNR: *nrfH*) in sediment, and Bacteroidaceae (DNR: *nrfA/H*) in crayfish gut habitats (the right part of Figure 5), which could potentially reduce the content of nitrate, nitric oxide, and nitrous oxide, providing a more friendly environment for aquatic animals.[30] And then ammonium was subsequently converted as organ N by N-assimilating bacteria (water: *Polynucleobacter*; sediment: Cyanobacteria; crayfish gut: Bacteroidaceae; gltD gene). These biogeochemical processes substantially decreased the concentrations of inorganic N compounds across habitats (Figures S1B–S1E and 2B-C). In addition, ammonium and nitrate are two important N sources for plants,[40] whereas rice is an ammonium-preference species that partially absorbs the N-related compounds, particularly ammonium, and improves the surrounding environment.[24,41,42]

While in non-RCFP (the left part of Figure 5), the increased inputs of organic matter (OM) (Figure S2A) and the high consumption of DO (Figure S1A) also induced the hypoxic reduction state, resulting in the oxidization of OMs by nitrate and nitrite. This process also promotes denitrification and inhibits nitrification,[43,44] which hindered the reproduction of aerobic nitrobacteria, but increased the accumulation of nitrate, nitrite, and ammonium (Figures S1C–S1F, and S2B–S2C), as well as increased the emission of nitrous oxide.[45] Previous research has reported that accumulated inorganic nitrogen compounds at the highest stocking density inhibit innate immunity and induce oxidative stress in red seabream,[30] whereas the high levels of ammonium and nitrite in non-RCFP (Figures S1D–S1E, S2B, and S2C) might impair the immune system of aquatic animals, making them more susceptible to infections, as well as affecting their growth and yield.[34,46,47] Moreover, previous studies have also reported that non-RCFP has higher level of antibiotics compared with RCFP,[26] such as tetracycline, which may increase nitrous oxide emissions.[48] In addition, nitrous oxide is also an important greenhouse gas with approximately 300 times global warming potential compared to carbon dioxide.[13] Coupled with stratospheric ozone depletion,[12] its increased emission in aquaculture, especially in non-RCFP, is detrimental to the global climate,[14,15] thereby impeding the sustainable development of the agricultural industry. The increased denitrification process and N decomposition in non-RCFP (Figures 2 and 5) may also be attributed to a lower oxidation-reduction potential (Figure S1G). More importantly, these N-cycling genes were influenced by environmental factors, such as the association between *nirB/D* and salinity/SpCond ($p < 0.05$; Figure S3A), as well as *gltD* and OM, moisture content ($p < 0.05$; Figure S3B). While *nirB/D* could transform nitrate into ammonium, and *gltD* could transform ammonium into organ N (Figures 2 and 5). And thus, we speculated through regulating the environmental factors (i.e., temperature, salinity, and antibiotic usage) to govern aquaculture, such as the biogeochemical cycling process, we could improve the method of RCFP and promote the blue transformation of aquaculture.

For S metabolism, the higher sulfide level in non-RCFP might be due to the higher water temperature in non-RCFP (Figure S1H), which can promote the consumption of DO (Figure S1A) and the reproduction of sulfate-reducing bacteria.[49] While the product sulfide of ASR and DSR in non-RCFP is highly toxic under acidic conditions in sediment (median pH: 6.5; Figure S2D), its oxidization process could incorporate hydrogen ions, sulfate, and metals into the aquatic environment, allowing the fixation of pollutants in the aquatic food chain.[50] Chronic exposure to sulfide would induce high mortality and impair the health of crayfish.[51]

For carbohydrate metabolism, RCFP microbial community has higher carbohydrate enzyme metabolism captivity compared with non-RFCP in environmental habitats, but this result was not observed in crayfish gut habitat. This phenomenon is probably due to the fact that enough COD in RCFP in environmental habitats (Figures S1A and S1F) promotes carbohydrate metabolism and relaxes energy,[52] which provides enough nutriment for aquatic animals and plants. While animal gut is an oxygen-free environment,[53] the lower carbohydrate enzyme metabolism in RCFP could reduce the content of lactic acid and ethyl alcohol during carbohydrate metabolism under an aerobic condition,[52] and promotes carbohydrate storage in aquatic animals, which could potentially promote the growth of aquatic animals in aquaculture.[54]

Collectively, this study has proposed a holistic conceptual model for microbial functions in driving biogeochemical cycling across aquaculture models and habitats. This model has evidentially supported the sustainability of RCFP aquaculture ecosystems from the perspective of microbial biogeochemical cycling: RCFP microbial community possesses ANR, DNR, and organic N assimilation, which promotes N assimilation and N pollutants removal, while the denitrification and decomposition processes were the dominance of N metabolism pathways in the non-RCFP aquaculture model, producing a series of N pollutants (i.e., ammonium, nitrite, nitric oxide, and nitrous oxide). Moreover, non-RCFP has higher sulfate reduction capacities, which produces several hazardous S pollutants (i.e., sulfide) compared with RCFP. Furthermore, higher carbohydrate enzyme metabolism abilities were observed in RCFP compared to non-RCFP across water and sediment habitats, but not in crayfish gut habitat, which may contribute to the growth of crayfish. All of these findings illustrated the sustainability of RCFP from the aspect of biogeochemical cycling, suggesting RCFP might be applied for the blue transformation of aquaculture.[5]

Environmental implication

Aquaculture has great potential in providing food and nutrition for global growing population. According to Food and Agriculture Organization, aquaculture production could grow ~40% by 2030. Such staggering

yields are also coupled with environmental pollution. To balance high productivity and environmental sustainability, co-culture models (e.g. RCFP) were widely developed in China due to their eco-friendliness. This study proposed a holistic conceptual model that supports the sustainability of RCFP from microbial biogeochemical cycling. This is especially important under the current carbon neutralization goal on earth. More importantly, the strong association between environmental factors and biogeochemical cycling genes indicated we might regulate the environmental factors (i.e., temperature and salinity) to govern the process of biogeochemical cycling in aquaculture.[16] The higher abilities of RCFP in N-assimilation, N-pollutants, and S-pollutants removal could promote nutrient bioavailability and environmental sustainability, suggesting RCFP might lead to the blue transformation of aquaculture.

Limitations of the study

It is worth noting that the limitation of sample size also hinders the extrapolation of our findings to generally aquatic-rice co-culture models. With further explorations on other possible aquatic-rice co-culture models, we might gain a more complete understanding of the aquatic-rice co-culture models, which could help us to step forward for a better blue transformation of aquaculture.

Conclusions

Taken together, this study has provided evidence and possible model to support the sustainability of RCFP aquaculture ecosystems, from the aspect of microbial biogeochemical cycling. The unique environment of RCFP creates a win-win situation for environmental protection and economic benefits, which is especially important under the current carbon neutralization goal on earth, suggesting RCFP might be deemed as a cost-efficient and eco-friendly aquaculture model and might be broadly adopted toward sustainable aquaculture.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- METHOD DETAILS
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- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.106769.

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AUTHOR CONTRIBUTIONS

Conceptualization, K.N. and Z.W.; Formal Analysis, X.Z. and P.S.Y.; Resources, K.N., Z.W., X.Z., H.W., and L.Z.; Writing – Original Draft, X.Z.; Writing – Review & Editing, K.N., Z.W., X.Z., P.Y., G.X., and L.Z.; Funding Acquisition, K.N. and Z.W.; Supervision, K.N. and Z.W.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCES	SOURCE	IDENTIFIER
Software and algorithms		
MEGAHIT	Li et al.[55]	v1.1.2
QUAST	Gurevich et al.[56]	v5.0.2
metaWRAP	Uritskiy et al.[57]	v1.2.2
CheckM	Parks et al.[58]	v1.0.18
Taxator-tk	Dröge et al.[59]	v1.3.3
Prodigal	Hyatt et al.[60]	v2.6.3
CD-HIT	Huang et al.[61]	v4.8.1
Salmon	Patro et al.[62]	v1.3.0
KofamKOALA	Aramaki et al.[63]	v1.3.0
clusterProfiler	Wu et al.[64]	v4.0
DIAMOND	Buchfink et al.[65]	v2.0.14.152
Deposited data		
Raw metagenomic sequence data	This paper	GSA: PRJCA009514

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to the corresponding author Kang Ning (e-mail: ningkang@hust.edu.cn).

Materials availability

This study did not generate new unique materials.

Data and code availability

- The raw metagenomic sequence data used in this study are available in the Genome Sequence Archive (GSA; https://ngdc.cncb.ac.cn/gsub/) database (GSA accession number: PRJCA009514).
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon reasonable request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The experimental animal model was crayfish, a freshwater crustacean with an average weight of 25.89 ± 8.12 grams. Besides crayfish samples, we also collected the water and sediment samples in the same aquaculture model to investigate the sustainability of RCFP. The samples were collected from Honghu farm in Hubei province, China, and were immediately placed on drikold after collection to preserve their quality. To extract the intestinal contents, we followed conventional anatomical methods under aseptic conditions, and placed the samples in sterile centrifuge tubes (5 mL) in the bioinformatic laboratory. The samples were then immediately frozen at −80 °C before sequencing. It is important to note that the use of crayfish in research is subject to ethical considerations and regulations to ensure their welfare and proper treatment.

METHOD DETAILS

Sample description

Water, sediment, and crayfish gut samples were from the RCFP and other aquaculture models (non-RCFP) in November 2019 at Honghu farm (29.92° N, 113.49° E), Hubei province, China. Here, water and sediment

samples were considered as environmental samples, whereas crayfish gut samples were referred to as animal gut samples. RCFP refers to an aquatic-rice co-culture model (e.g., rice-crayfish co-culture in paddy fields) in aquaculture, which utilized the multi-directional interactions of co-culture species to realize the economic and environmental benefits.[16,20,26] While non-RCFP aquaculture models are usually implemented with a high-density monoculture or mixed culture of aquatic animals, such as crayfish, crab, and fish monoculture, crab-crayfish mixed culture.[66,67] This dataset includes 19 water samples, 19 sediment samples, and 11 crayfish gut samples. The sample distribution, DNA extraction, and quality control for these metagenomic samples were described in our previous work.[16] Totally, 49 samples were collected and stored at −80 °C before sequencing. We also measured the water and sediment environmental factors of RCFP and non-RFCP, including DO, COD, nitrate, nitrite, ammonium, TN, COM, DO, temperature, ORP, turbidity, pH, OM, and antibiotics (Figures S1–S3).

Metagenome assembly and function analysis

The high-quality reads from each sample were individually assembled into contigs using MEGAHIT (v1.1.2)[55] with a minimum contig length of 1,000, and then these assemblies were evaluated using QUAST (v5.0.2)[56]. The high-quality contigs were grouped into metagenome assembly genes (MAGs) using the maxbin2, metabat2, and concoct algorithms in metaWRAP (v1.2.2)[57]. Then, the output results from these three algorithms were integrated using metaWRAP software to calculate an optimized set of MAGs from a single assembly. As a result, a total of 466 MAGs were produced, and then these MAGs were assessed by CheckM (v1.0.18).[58] The taxonomy of each MAG was annotated by Taxator-tk with nt database.[59] After that, the genes in each MAG were predicted using Prodigal (v2.6.3)[60]. The genes were clustered by CD-HIT(v4.8.1) with at least 95% global sequence identity.[61] The microbial gene abundance (measured by transcripts per million (TPM)) was quantified by Salmon (v1.3.0)[62]. The non-redundancy protein-coding genes were annotated against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database using KofamKOALA (v1.3.0)[63]. The KEGG pathways enrichment was performed for all annotated KEGG orthology terms using the R (v4.1.2) "clusterProfiler" package (v4.0)[64]. The non-redundancy genes were aligned to NCycDB,[68] SCycDB,[69] and Carbohydrate Active Enzymes database (CAZyDB)[70] for further profiling the N-cycling, S-cycling, and C-cycling gene families across aquaculture models and habitats, using DIAMOND (v2.0.14.152) with "-e 1e-5".[65] These annotated genes were also extracted from KEGG pathways (N: ko00910; S: ko00920; C: ko01200) for deeper understanding the biogeochemical cycling. For C-cycling genes, they were grouped according to six carbohydrate enzyme categories: auxiliary activities (AAs), carbohydrate esterases (CEs), glycoside hydrolases (GHs), glycosyl transferases (GTs), polysaccharide lyases (PLs), and carbohydrate-binding modules (CBMs), which that catalyze the breakdown, biosynthesis or modification of carbohydrates and glycoconjugates, and adhesion to carbohydrates.

QUANTIFICATION AND STATISTICAL ANALYSIS

In this study, we detected differences in microbes, microbial genes, as well as environmental factors between RCFP and non-RCFP across water, sediment, and crayfish gut habitats using Wilcoxon test, and all p value were adjusted by Benjamini-Hochberg (BH) methods. Additionally, we performed KEGG enrichment analysis using Fisher's precision probability test and adjusted the p values using BH methods. Significances were determined at an adjusted p value <0.05 between groups.