Current Science
CS 5 (4), 3735-3746 (2025)
Received 20 April 2025 | **Revised 20 May** 2025 | **Accepted 22 June**. 2025 | **Online Available 28 July** 2025
https://doi.org/ 10.52845/CS/2025-5-4-34

Current Science
Current Science

OPEN ACCESS
ISSN(O)2795-8639

Original Article

Study on the Bacterial Community Structure of Sediment in Different Habitats of *Penaeus Orientalis*

Yazi Li[†1], Guoxing Ma[†*1, 2], Ying Bao[1], Ling Fu[1], Xueting Li, Jingjie Zhang[1], Yunfeng Zhang[1], Yongshan Fan[1]

[1]Department of Life Sciences, Tangshan Normal University, Tangshan 063000, China

[2]Engineering Research Center of Molecular Medicine of Ministry of Education, Huaqiao University, Xiamen 361021, China

[*]Corresponding Author: Guoxing Ma

Abstract:
The microbial communities in *Penaeus orientalis*' habitats are important to the quality of the species. This study utilized the PacBio sequencing platform to analyze the microbial diversity in the different habitats of the species. The analysis indicates that the shrimp pond sediment samples have a greater number of features compared to the intertidal gully sediment samples, with a total of 1910 feature sequences overlapping between the two. According to the results of beta diversity analysis showed that the sediment samples in the intertidal gully were far away from the sediment samples in the shrimp pond, indicating a great difference in microbial flora species. Sediment samples from shrimp ponds showed the presence of dominant bacteria genera such as *Sulfurovum* and *Lactobacillus*. According to KEGG functional prediction analysis, the relative abundance of the Translation metabolic pathway in sediment samples was significantly higher compared to that of intertidal gullies sediment samples. In both samples, the highest abundance of metabolic pathways was Translation, whereas the abundance of metabolic pathways related to Signaling molecules and interaction metabolic pathways was relatively low. The COG function prediction analysis revealed that the abundance of Signal transduction mechanisms in the sediment samples from intertidal gullies was higher than those in the samples from shrimp pools. The results of this study analyzed the environmental adaptability of *P. orientalis* from the perspective of habitat environment and provided a basis for the development of strains that have special functions and natural metabolites in the sediment.

Keywords: Intertidal gully, Shrimp pond, Microbial diversity, Function

Introduction

The bottom sediments of shrimp ponds and intertidal gullies harbors a diverse community of microorganisms including prokaryotic, photo oxygen, and heterotrophic eukaryotic microorganisms. Microorganisms play a vital role in the circulation of matter and energy within ocean and lake ecosystems, and are essential to maintaining the quality of prawns[1-4]. In the process of artificially cultivating prawns, it is common practice to provide them with an external diet to maintain their growth and metabolic functions. However, the utilization of diet by prawns is limited, which usually results in the retention and enrichment of a large amount of organic matter in the sediment at the bottom of the prawn pond[5]. A large amount of nitrogen, phosphorus, organic matter, and microorganisms accumulated in the sediment under the influence of aquaculture bait and excrement[2, 3, 5]. The sediment at the bottom of a shrimp pond contains organic matter that, when disturbed by external factors, can release excess nutrients into the water. This can lead to eutrophication, which disrupts the balance and circulation of the entire culture system and its microbial homeostasis [6]. The microbial community in sediment is an important part of an aquatic ecosystem. Microorganisms have the ability to impact the distribution and transformation of nutrients in sediments, as well as the intestinal microbial homeostasis of prawns through processes such as assimilation, alienation, and adaptation to changing environmental conditions[7, 8]. To gain a better understanding of the distribution of microorganisms in aquatic ecosystems and their impact on prawn health, it is crucial to study the changes in major microbial communities in sediments from various habitats.

Currently, microbial species identification and community richness analysis are predominantly conducted through traditional culture and molecular biology methods. However, these methods have limitations in identifying the total number of microbial populations, thereby failing to provide an accurate reflection of the microbial groups and diversity present in the environment[9]. The advancement of molecular biology technology towards high throughput and high precision has resulted in the emergence of sequencing technology with several technical advantages. This technology does not require in vitro culture and exhibits high sensitivity, enabling the detection of many microorganisms that cannot be cultured. This discovery paves the way for the development of related functional microorganisms and natural products[10]. Intertidal gullies are located in the area connecting shrimp ponds and marine tidal flats, being significantly influenced by human activities (industrial and agricultural), which is of great significance for studying the impact of wild environments on the quality of penaeid shrimp.

In this study, sediment (0~2 cm) was collected from shrimp ponds and different parts of adjacent intertidal gullies in Liuzan town (latitude: 39.222750, longitude: 118.661660), Tangshan city. Illumina Miseq high-throughput sequencing technology based on 16S rRNA gene was used to study microbial communities in sediment under different environments, explore the potential relationship between microbial community structure and environment, and provide a theoretical basis for a high-quality culture of *P. orientalis* shrimp.

Materials and Methods

Sample Collection and Preservation

Sediment samples were taken from various locations of shrimp ponds (C1, C2 and C3) and intertidal gullies (D1, D2 and D3). Under aseptic conditions in the laboratory, an appropriate amount of sediment was removed from a super-clean workbench and immediately placed in a 1.5 mL aseptic freeze-storage tube. After the liquid nitrogen was frozen, it was placed in a -80°C refrigerator.

Extraction and high-throughput sequencing of total DNA of samples

TGuide S96 soil/fecal genome DNA extraction kit (TianGen, Beijing) was used to extract total DNA from shrimp pond and intertidal gully mud samples. Full-length amplification of 16S rDNA gene was performed using primers 27F~1492R (27F: 5' -AGRGTTTGATYNTGGCTCAG-3', 1492R: 5'-TASGGHTACCTTGTTASGACTT-3'). The amplification conditions were as follows: 95°C (predenaturation) for 5 min; 95°C (denaturation) 30 s, 50°C (annealing) 30 s, 72°C (extension) 60 s, 20 cycles; 72°C (extended) for 5 min. The purity of the samples was determined by agarose gel electrophoresis (gel concentration: 1.8%; voltage: 70 v; electrophoresis time: 40 min). The 16S rDNA gene was sequenced on the PacBio platform by Beijing Biomarker Technologies (Beijing, China). Three replicates per sample.

Data Processing

Sediment microbial sample data processing. After obtaining sequencing data of all samples, lima v1.7.0 software was first used to remove barcode for CCS identification and obtain Raw-CCS sequence data. Cutadapt 1.9.1 software was used to identify and remove primer sequences and perform length filtering to obtain Clean-CCS sequences without primer sequences. Using UCHIME v 4.2 software, the chimera sequence was identified and removed to obtain the final valid data. The species were compared with SILVA database and classified. QIIME software was used to generate species abundance tables at different taxonomic levels. Then R language tool was used to draw community structure maps of samples at different levels. The Alpha diversity index of samples was evaluated using QIIME2 software. PICRUSt2 software was used to predict the functional abundance of samples based on the sequence abundance of marker genes.

Statistical Analysis

QIIME2 2020.6 software was used to calculate the alpha diversity index of samples, including observed species (ACE), Chao1 index, Simpson index, and Shannon index[11]. Excel 2010 was used to analyze and process the data and make corresponding tables. SPSS 22.0 software was used for one-way ANOVA analysis and multiple comparisons.

Results

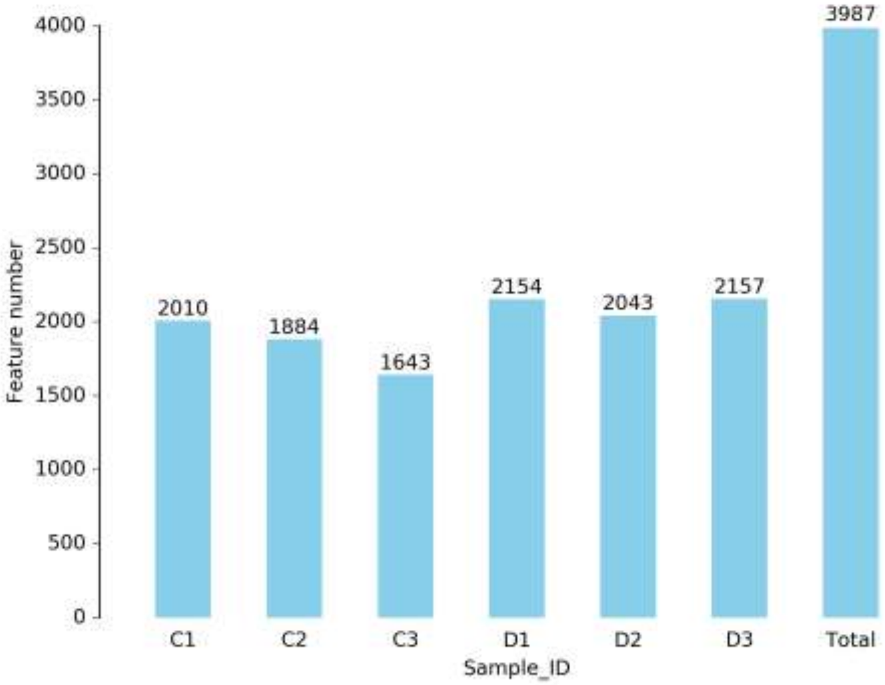
Sample Sequencing and Valid Data Statistics

Following the sequencing of sediment samples from shrimp ponds (C1, C2 and C3) and intertidal gullies (D1, D2 and D3), 145,593 CCS sequences were obtained by barcode identification (Genebank: PRJNA1011836). Each sample produced at least 22,717 CCS sequences, with an average of 24,266 CCS sequences. The average length of the sequence was 1461.5 bp. Among them, the sediment samples of shrimp pond and intertidal gully had effective CCS values of 66,756 and 76,423, respectively. In addition, the whole sediment sample quality control effectiveness was 98.29% on average (**Table 1**).

Table 1 Statistics of sequencing data processing results in soil sample

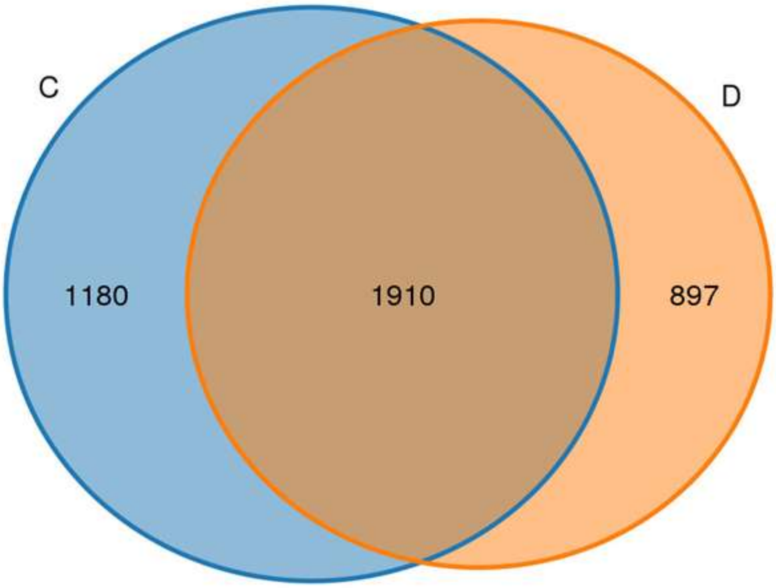
Name	Results
Raw CCS	145,593
Average raw CCS	24,266
Clean CCS	145,472
Effective CCS	143,179
Average length (bp)	1461.5
Average effective percent (%)	98.29

After sequencing, cluster analysis was used to identify 2010, 1884, and 1643 OTUs from the sediment samples of shrimp ponds (C1, C2 and C3). 2154, 2043 and 2157 OTUs were obtained from the intertidal gully bottom mud samples (D1, D2 and D3) (**Supplementary Fig.1**).



Supplementary Fig.1 Distribution of the number of characteristics of each sample

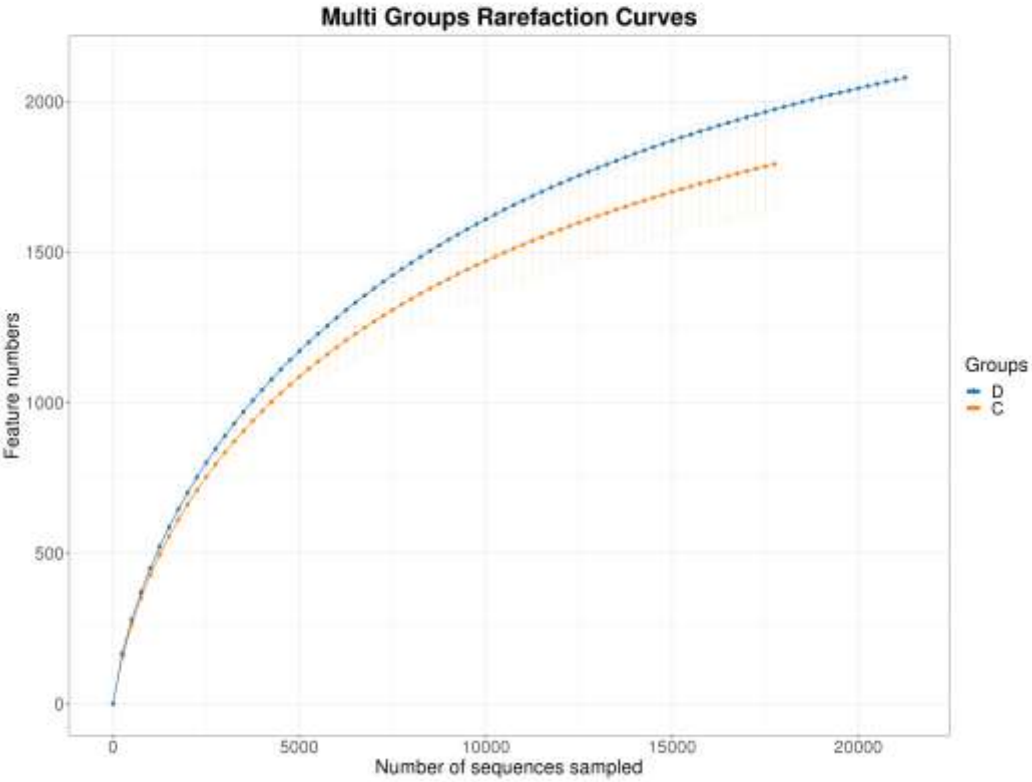
The shrimp pond bottom mud samples have more feature sequences than the intertidal gully bottom mud samples, and 1910 feature sequences of those features overlap (**Supplementary Fig.2**).



Supplementary Fig.2 Feature venn diagram

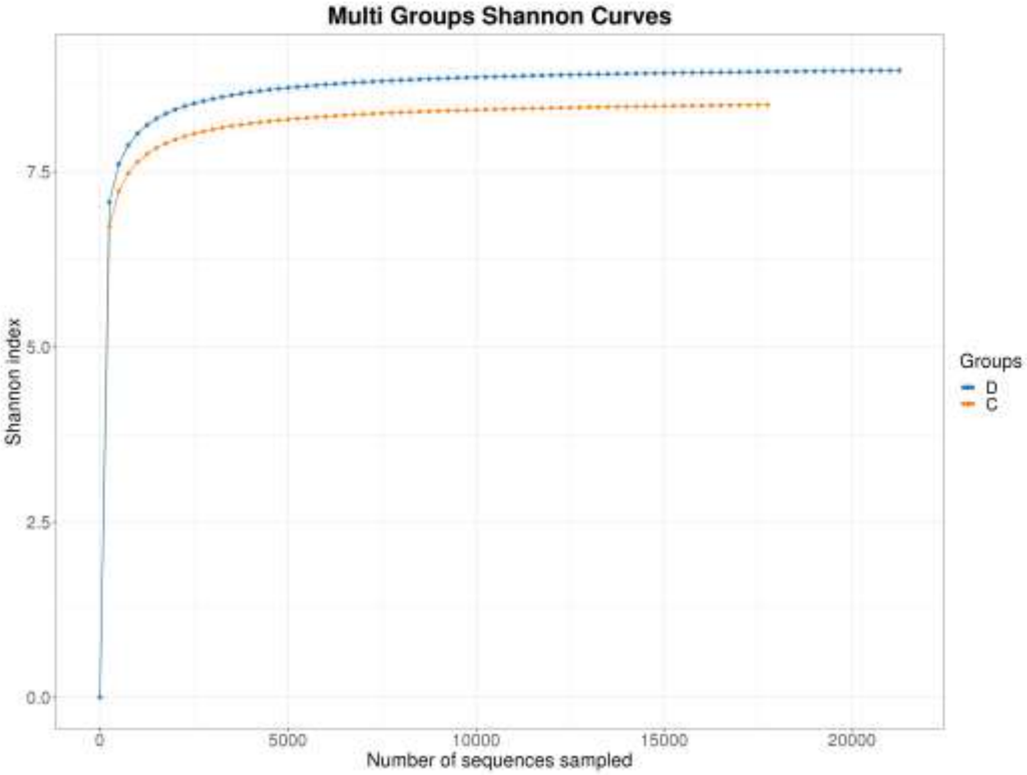
Alpha Diversity Analysis

Alpha diversity reflects richness and richness mainly through ACE, Chao1, Simpson, Shannon, PD_whole_tree and Coverage indexes. In this study, the Chao1 index of the shrimp pond sediment sample is the highest, ranging from 2,249.0698 to 2,510.9914, and the richness is the lowest. The Chao1 index of the intertidal gully sediment samples is 2,382.0452~2,542.2173, with the highest richness. The dilution curve of observed species shows that the number of detected feature sequences from high to low are intertidal gully bottom mud samples and shrimp pond bottom mud samples (**Supplementary Fig.3**).



Supplementary Fig.3 Dilution curve of observed species

Shannon index dilution curve showed that the species diversity of intertidal gully bottom mud samples was higher than that of shrimp pool bottom mud samples, and all curves tended to be flat, indicating that the sequencing quantity was large enough and the sequencing results were reliable (**Supplementary Fig.4**).



Supplementary Fig.4 Dilution curve of Shannon index

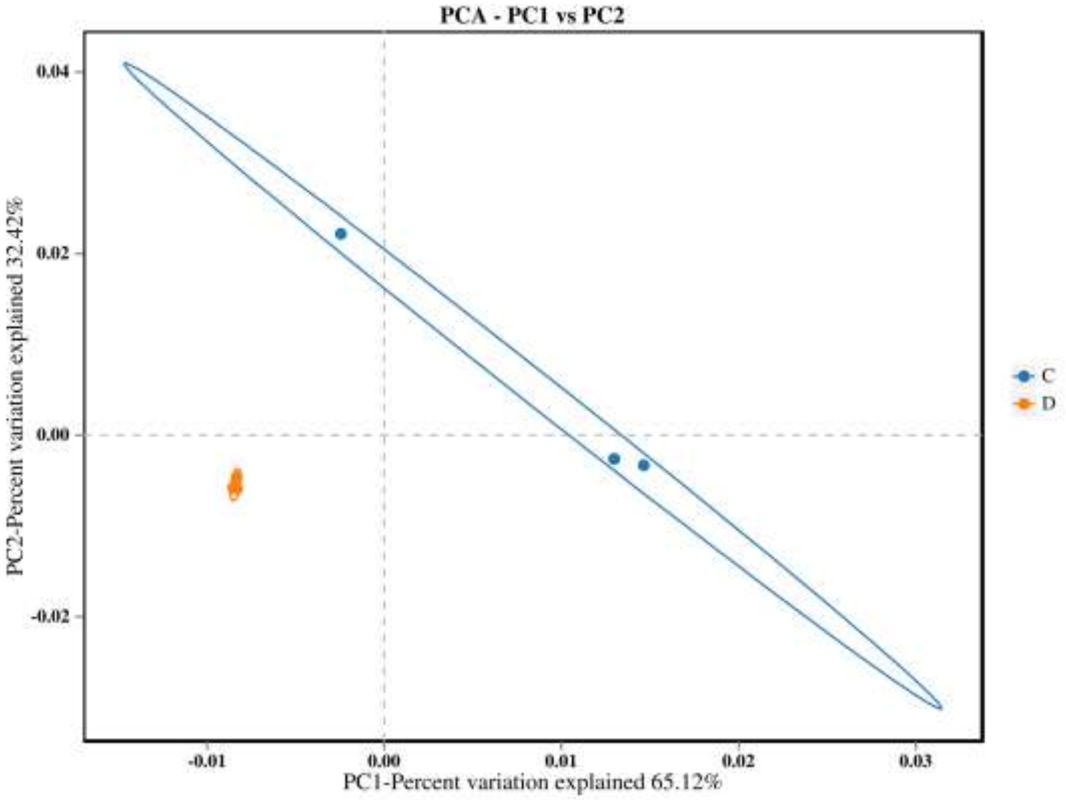
Coverage refers to microbial coverage. The higher the value, the lower the probability that the new species in the sample was not detected. In this study, the coverage index of all samples was close to 1, indicating that the sequencing results were true and reliable (**Table 2**).

Table 2 Alpha diversity index statistics

Sample	Feature	ACE	Chao1	Simpson	Shannon	PD_whole_tree	Coverage
C1	2010	2508.5545	2510.9914	0.9834	8.5378	144.9226	0.9709
C2	1884	2294.6765	2287.5213	0.9807	8.367	115.3356	0.9748
C3	1643	2113.2326	2249.0698	0.9889	8.4941	136.0704	0.9716
D1	2154	2605.156	2519.2365	0.9938	8.9336	145.7536	0.9757
D2	2043	2423.6161	2382.0452	0.9937	8.9069	144.5814	0.9757
D3	2157	2567.3622	2542.2173	0.9938	9.0256	151.066	0.977

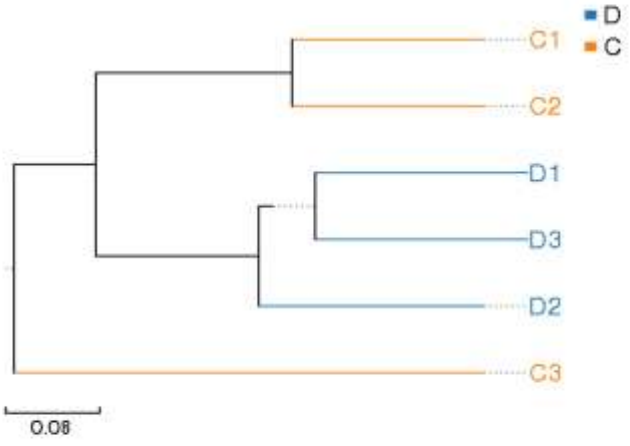
Beta Diversity Analysis

Based on Bray-Curtis distance, PCA (Principal components analysis) analysis was carried out, and the principal coordinate combination with the largest contribution rate was selected for drawing display. Differences between sample groups were observed through PCA, and different colors represented different groups in the results. The closer the samples were, the more similar the microbial composition and structure were between the samples; otherwise, the greater the difference was. The distance between the sediment samples from the intertidal gully and the sediment samples from the shrimp pond indicates a large difference in microbial flora species (**Supplementary Fig.5**).



Supplementary Fig.5 Schematic diagram of PCA analysis

UPGMA (Unweighted Pair-group Method with Arithmetic Mean) cluster analysis can also fully support the above results. In UPGMA, different color branches represent different groups. The clustering tree shows the similarity between samples. The shorter the branch length between samples, the more similar the two samples are; otherwise, the greater the difference is (**Supplementary Fig.6**).



Supplementary Fig.6 UPGMA hierarchical cluster analysis

Analysis of Species Composition

The statistical results of the species in the samples showed that there were 1,111 species, 82 classes, 230 orders, 384 families, 774 genera, 38 phyla of bacteria in shrimp pond sediment samples. The intertidal gully sediment samples belong to 34 phyla, 80 classes, 224 orders, 355 families, 671 genera, 914 species. The alterations in sediment samples at the phyla, genus, and species levels are the main focus of this investigation (**Table 3**).

Table 3 High-throughput sequencing results in soil sample

Sample	Kindom	Phylum	Class	Order	Family	Genus	Species
C1	2	34	74	190	296	498	618
C2	1	33	71	182	272	446	560
C3	2	34	72	164	298	591	844
D1	2	33	75	197	312	554	711
D2	2	33	75	196	302	521	664
D3	2	33	74	195	309	550	714
Total	2	38	86	253	424	888	1316

Phylums-Level Composition of the Flora

At the phylum level, a total of 38 phyla were obtained from the species composition analysis. The top ten species in terms of abundance were chosen for classification, and stacked bar charts were used to show the relative abundance of each category (**Fig.1**). Bacteroidota (17.231%), Proteobacteria (16.995%) and Desulfobacterota (14.009%) are the most common bacteria in the bottom mud samples of the shrimp pond. In the intertidal gully bottom mud samples, Desulfobacterota (30.575%), Proteobacteria (28.032%) and Bacteroidota (13.395%) are predominant bacteria. Need to explain is unclassified_Bacteria appear here refers to the match in the database on the target sequence from the culture identification of microorganisms.

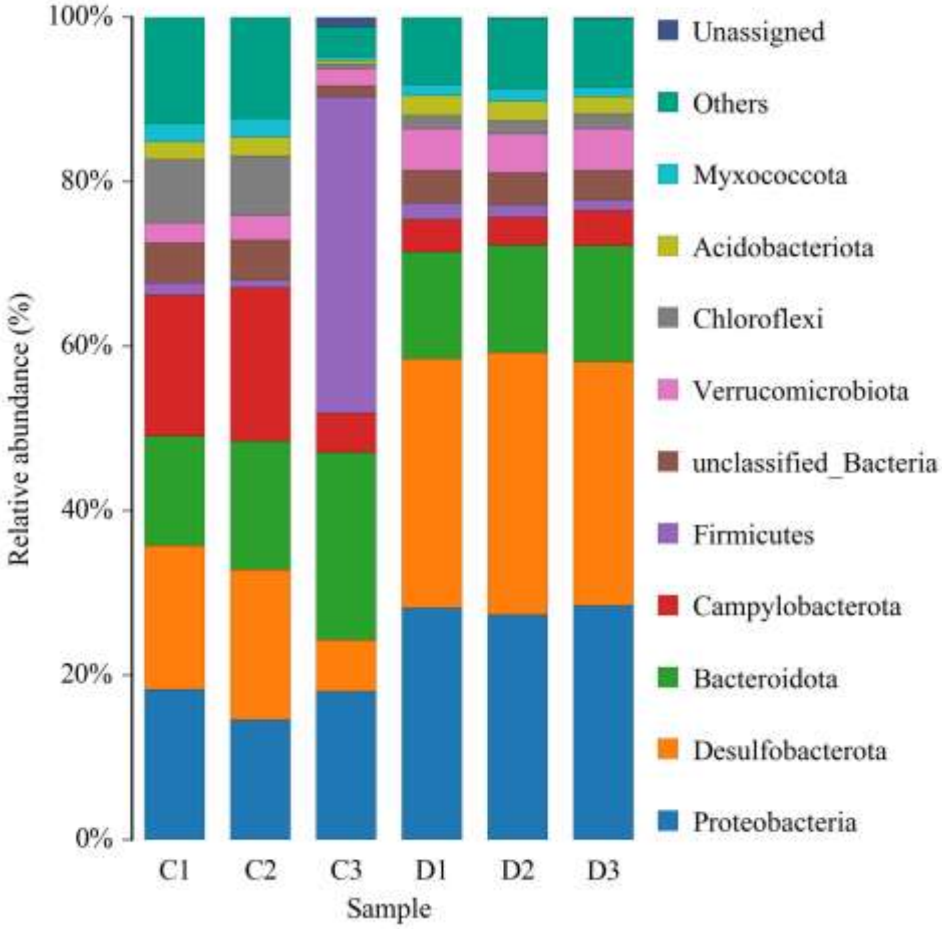


Figure 1 Bar chart of the relative abundances of bacteria at the phylum level

Genus-Level Composition of the Flora

At the genus level, a total of 888 genera were obtained by species composition analysis. The 10 species with the highest abundance were selected to be classified, and the relative abundance of each group was displayed in stacked bar charts (**Fig.2**). The shrimp ponds the dominant bacteria genera in sediment samples for *Sulfurovum* (12.599%) and *Lactobacillus* (4.552%). intertidal gullies are the dominant bacteria genera in sediment samples for unclassified_*Desulfobulbaceae* (6.630%), uncultured_delta_proteobacterium (3.856%) and unclassified_Bacteria (3.808%).

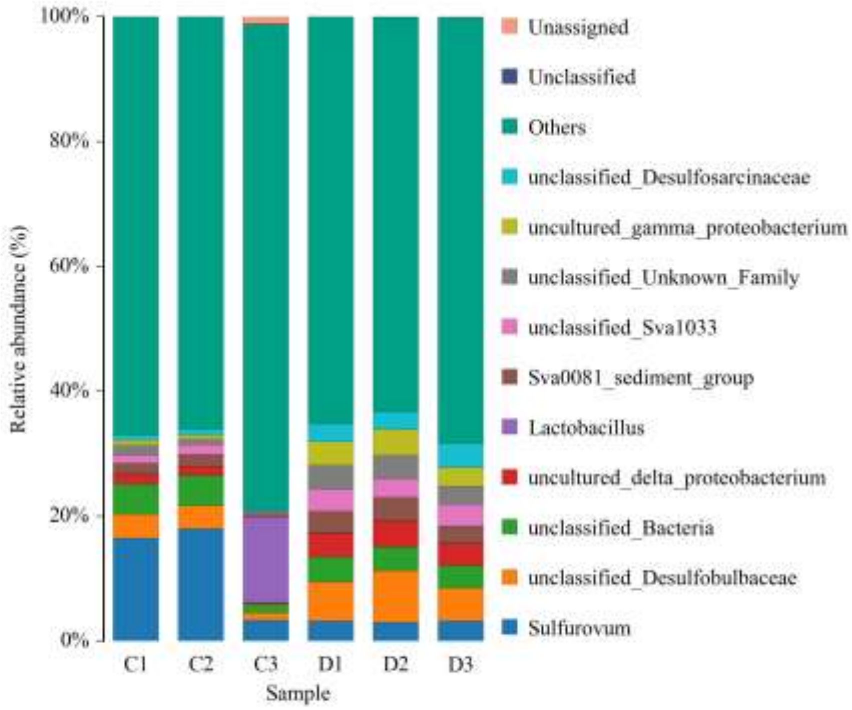
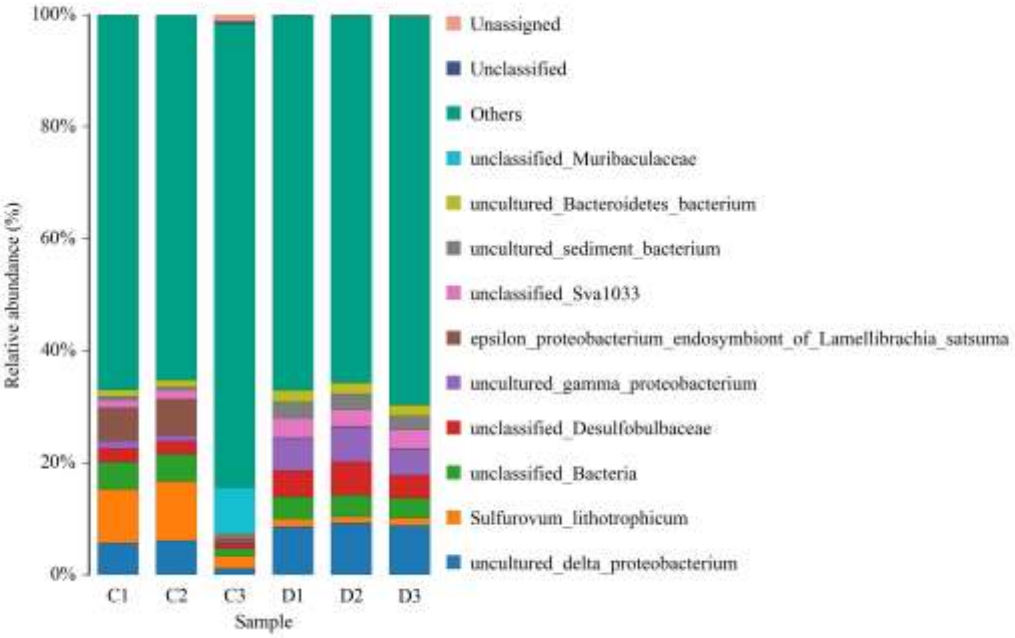


Figure 2 Bar chart of the relative abundances of bacteria at the genera level

Species-Level of Composition of Flora

At the species level, a total of 788 species were obtained from the species composition analysis. The 10 species with the highest abundance were selected to be classified, and the relative abundance of each group was displayed in stacked bar charts (**Supplementary Fig.7**).



Supplementary Fig.7 Community analysis at species level

The dominant bacteria in the sediment samples of shrimp pools are *Sulfurovum_lithotrophicum* (7.409%) and epsilon_ proteobacterium_ endosymbiont_of_*Lamellibrachia_satsuma* (4.4 60%) and uncultured_delta_proteobacterium (4.36 2%). intertidal gully sediment samples of dominant species for uncultured_ delta_ proteobacterium (8.918%), uncultured_ gamma_ proteobac-terium (5.601%) and unclassified_ *Desulfobu lbaceae* (4.857%)

Predictive Analysis of Functional Genes

PICRUSt2 software was used to annotate the 16S rRNA gene sequences in the sediment samples of prawn survival in KEGG and COG databases.

Analysis of KEGG Function Prediction

By comparing the sediment samples from prawn ponds and intertidal gullies by gene annotation, five distinctly different KEGG metabolic pathways were discovered ($P<0.05$) (**Fig.3**). In comparison to intertidal gully sediment samples, shrimp pond sediment samples had a much greater relative abundance of the Translation metabolic pathway. Translation was the metabolic pathway with the largest abundance in both samples, while Signaling molecules and interaction metabolic pathways was very low.

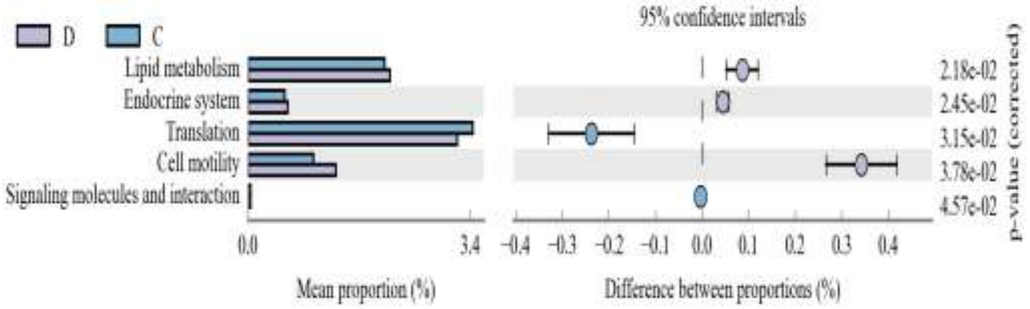


Figure3 Histogram of KEGG metabolic pathway

Analysis of COG Function Prediction

COG is a prokaryotic homologous protein cluster database, which is commonly used for functional classification of prokaryotic proteins. One functional annotation with significant difference was obtained between the shrimp pond and intertidal gully sediment sample treatment group ($P<0.05$) (**Fig.4**). The function abundance of Signal transduction mechanisms in intertidal gully sediment samples is higher than that in shrimp pond sediment samples. Signal transduction mechanisms had the highest abundance of functional annotation.

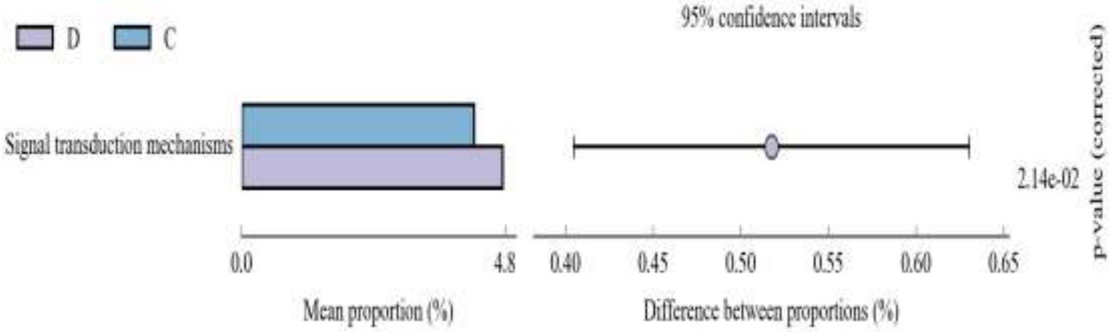


Figure 4 Histogram of COG metabolic pathway

Discussion

Numerous bacteria live in the muck at the aquatic ecosystem's bottom. It is engaged in numerous biological processes, including nutrient metabolism, energy balance, and the enhancement of water quality, thanks to its varied species and complex community structures[12-17]. At the moment, molecular biological techniques are mostly used for the identification and functional analysis of sediment microorganisms. High-throughput sequencing technology has recently offered a potent way to fully comprehend the microbial community structure in the muck at the bottom of prawn cultivation ponds. In this study, Illumina Miseq sequencing technology was used to detect the 16S rRNA sequence of bacteria, and the species diversity and community structure of bacteria in the sediment of prawns reared in different species were systematically analyzed.

Data analysis showed that the microbial diversity and richness of the intertidal gully bottom mud samples were higher than those of the shrimp pool bottom mud samples. The results indicated that the microbial diversity and richness of sediment were affected by the environment in which the prawn was cultured. In addition, there were abundant species of sediment bacteria in prawn culture, and the 16S rRNA sequences were annotated into 2 kingdoms, 38 phyla, 86 classes, 253 orders, 424 families, 888 genera, and 1316 species. The microbial diversity of shrimp pond and intertidal gully bottom mud is Bacteroidota, Proteobacteria, and Desulfobacterota, but the proportion is slightly different. This is similar to previous studies on major phyla in shrimp intestines and sediments[18] (**Fig.1**). A total of 888 genus bacteria were identified in the sediment samples, which was much higher than the current results based on the traditional plate culture technique and library construction method[19]. This research not only isolated from sediment samples to the dominant bacteria genera in *Sulfurovum*, *Lactobacillus* and separation of culture or not bacteria genera unclassified_*Desulfobulbaceae*, uncultured_delta_proteob appraisement of advantage *Acterium* and unclassified_Bacteria, unclassified_Sva1033, unclassified_Bacteria, and unclassified_*Desulfosarcinaceae*. It can be seen that Illumina MiSeq high-throughput sequencing technology based on 16S rRNA can more comprehensively reveal the structure composition and diversity of intestinal microorganisms of *P. orientalis* (**Fig.2**).

In this study, *Sulfurovum_lithotrophicum* (7.409%) of the genus *Sulfurovum* was found to be the dominant bacterial species in the bottom mud of shrimp ponds (**Supplementary Fig.7**). The bacterium *Sulfurovum* is commonly found in seawater, surface sediments and dominant animals. The genus *Sulfurovum* plays an important role in the carbon, sulfur and nitrogen cycles of the ocean[20]. Given the culturable characteristics of this genus, subsequent studies on its physical and chemical properties, location distribution, quantitative dynamics, mode of transmission and biological functions will become feasible. In addition, a certain percentage of *Lactobacillus* was also found in the sediment (**Supplementary Fig.7**). The probiotics can be used as feed additives to regulate intestinal flora, improve the physiological function and cognitive ability of animals, and help the host to resist the infection of foreign pathogens[21, 22]. In addition, through the comparison of gene annotation, it was found that five significantly different pathways of KEGG metabolism were obtained in the sediment sample treatment groups of shrimp pond and intertidal gully prawn survival (P<0.05) (**Fig.3**). The relative abundance of Translation metabolic pathway in shrimp pond sediment samples was significantly higher than that in intertidal gully sediment samples. The highest abundance of metabolic pathways in both samples was Translation, while the relative abundance of Signaling molecules and interaction metabolic pathways was low. COG functional prediction analysis found that the functional abundance of Signal transduction mechanisms in intertidal gully sediment samples was significantly higher than that in shrimp pond sediment samples (**Fig.3-4**). The function prediction of these sediment microorganisms is helpful to the development and application of subsequent bacterial functions.

To sum up, the microorganism in the bottom mud of prawn culture tank is rich and diverse. It is an optimal strategy to use high-throughput sequencing to identify microbial species in sediment, and the culturable microorganisms with a small amount are an important resource that has not yet been developed. In this study, based on the 16S r RNA meta-genomic strategy for the first time, the community structure and diversity of microorganisms in the bottom mud of prawn aquaculture ponds were comprehensively analyzed and their functions were predicted. The results of this study will provide guidance for the subsequent revelation of the relationship between the microbial community characteristics of prawn and sediments, and provide guidance for healthy aquaculture in freshwater and marine aquaculture environments.

Conclusion

The study found that the species and abundance of mud samples in the intertidal gully were greater than those in the shrimp pond. This suggests that the microbial diversity and abundance in the bottom mud of prawns are influenced by the surrounding environment. In addition, the microbial structure and composition of the sediment in culture environments showed roughly the same function, so it was speculated that there were some microbial groups with specific functions in the bottom mud, which might be the result of long-term co-evolution with their living environment. In addition, there are a variety of probiotics in the sediment of cultured prawn, such as Sulfurovum and Lactobacillus, which play an important role in decomposing organic waste, maintaining the health of the host and improving the immunity of prawn. Therefore, the identification and community analysis of these functional probiotics in different habitats are helpful to provide a theoretical basis for the healthy culture of prawn and the development of related natural products.

Author Contributions: Yazi Li and Guoxing Ma writing—original draft; Jingjie Zhang and Xueting Li investigation, resources; Yunfeng Zhang, Ling Fu and Ying Bao writing—review and editing; Yongshan Fan conceptualization, supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: We are grateful to all lab members for helpful discussions and suggestions in this review writing. This study was financially supported by the Key Research and Development Plan Project of Tangshan (24150202C), the Science Research Project of Hebei Education Department (QN2025283), Scientific Research Foundation of Tangshan Normal University (20256129068) and Central Government Guides Local Funds for Science and Technology Development (246Z3610G).

Conflicts of Interest: The authors declare no conflict of interest.

Ethical Statements: “Not applicable” for studies not involving humans or animals.

Data Availability Statement: We didn't create the data.

References:

1. Zhou N, Zhang GL, Liu SM. (2022). Nutrient exchanges at the sediment-water interface and the responses to environmental changes in the Yellow Sea and East China Sea. Mar Pollut Bull 176: 113420. http://doi.org/10.1016/j.marpolbul.2022.113420
2. Towatana P, Voradaj C, Panapitukkul N. (2002). Changes in soil properties of abandoned shrimp ponds in southern Thailand. Environ Monit Assess 74(1): 45-65. http://doi.org/10.1023/a:1013802704889
3. Lopez-Cortes A, Garcia-Pichel F, Nubel U, Vazquez-Juarez R. (2001). Cyanobacterial diversity in extreme environments in Baja California, Mexico: a polyphasic study. Int Microbiol 4(4):227-236. http://doi.org/10.1007/s10123-001-0042-z
4. Palmer MA, Covich AP, Sam Lake, Peter Biro, Brooks JJ, Jonathan Cole, Cliff Dahm, Janine Gibert, Willem Goedkoop, Koen Martens, Jos Verhoeven, Van De Bund WJ. (2000). Linkages between aquatic sediment biota and life above sediments as potential drivers of biodiversity and ecological processes: A disruption or intensification of the direct and indirect chemical, physical, or biological interactions between aquatic sediment biota and biota living above the sediments may accelerate biodiversity loss and contribute to the degradation of aquatic and riparian habitats. BioScience 50(12): 1062-1075. https://doi.org/10.1641/0006-3568(2000)050[1062:LBASBA]2.0.CO;2
5. 5.Chen Y, Dong S, Wang F, Gao Q, Tian X. (2016). Carbon dioxide and methane fluxes from feeding and no-feeding mariculture ponds. Environ Pollut 212:489-497. https://doi.org/10.1016/j.envpol. 2016. 02.039
6. Qin BQ, Zhu GW. (2006). The nutrient forms, cycling and exchange flux in the sediment and overlying water system in lakes from the middle and lower reaches of Yangtze River. Sci China Ser D 49: 1-13. https://doi.org/10.1007/s11430-006-8101-0
7. Findlay S, Tank J, Dye S, Valett HM, Mulholland PJ, McDowell WH, Johnson SL, Hamilton SK, Edmonds J, Dodds WK, Bowden WB. (2002). A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. Microb Ecol 43(1):55-66. https://doi.org/10.1007/s00248-001-1020-x
8. Landsman A, St-Pierre B, Rosales-Leija M, Brown M, Gibbons W. (2019). Impact of

aquaculture practices on intestinal bacterial profiles of pacific whiteleg shrimp litopenaeus vannamei. Microorganisms 7(4): 93. https://doi.org/10.3390/microorganisms7040093

9. Amjad M. (2020). An overview of the molecular methods in the diagnosis of gastrointestinal infectious diseases. Int J Microbiol 2020,8135724. https://doi.org/10.1155/2020/8135724
10. Vos M, Wolf AB, Jennings SJ, Kowalchuk GA. (2013). Micro-scale determinants of bacterial diversity in soil. FEMS Microbiol Rev 37(6):936-954. https://doi.org/10.1111/1574-6976.12023
11. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, ... Caporaso G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37(8): 852-857. https://doi.org/10.1038/s41587-019-0209-9
12. Fan L, Li F, Chen X, Shen L, Chu Y, Qiu L, Hu G, Song C, Li D, Meng S, Chen J. (2023). Responses of bacterial and three sub-microeukaryote communities in the water of white shrimp *Penaeus vannamei* aquaculture ponds in two polyculture models. Can J Microbiol http://doi.org/10.1139/cjm-2022-0178
13. Hou D, Huang Z, Zeng S, Liu J, Wei D, Deng X, Weng S, He Z, He J. (2017). Environmental factors shape water microbial community structure and function in shrimp cultural enclosure ecosystems. Front Microbiol 8:2359. http://doi.org/10.3389/fmicb.2017.02359
14. Ji Y, Angel R, Klose M, Claus P, Marotta H, Pinho L, Enrich-Prast A, Conrad R. (2016). Structure and function of methanogenic microbial communities in sediments of Amazonian lakes with different water types. Environ Microbiol 18(12): 5082-5100. http://doi.org/10.1111/1462-2920.13491
15. Liu Y, Li S, Wang X, An Y, Wang R. (2020). Dynamic interception effect of internal and external nitrogen and phosphorus migration of ecological ditches. Water 12(9): 2553. https://doi.org/10.3390/w12092553
16. Xu Y, Li L, Lou S, Tian J, Sun S, Li X, Li Y. (2022). Effects of nano-aerators on microbial communities and functions in the water, sediment, and shrimp intestine in litopenaeus vannamei aquaculture ponds. Microorganisms 10(7): 1302. https://doi.org/10.3390/microorganisms10071302
17. Zhang L, Chen M, Chen X, Wang J, Zhang Y, Xiao X, Hu C, Liu J, Zhang R, Xu D, Jiao N, Zhang Y. (2021). Nitrifiers drive successions of particulate organic matter and microbial community composition in a starved macrocosm. Environ Int 157: 106776. http://doi.org/10.1016/j.envint.2021.106776
18. Fan L, Wang Z, Chen M, Qu Y, Li J, Zhou A, Xie S, Zeng F, Zou J. (2019). Microbiota comparison of Pacific white shrimp intestine and sediment at freshwater and marine cultured environment. Sci Total Environ 657: 1194-1204. http://doi.org/10.1016/j.scitotenv.2018.12.069
19. Claesson MJ, O'Toole PW. (2010). Evaluating the latest high-throughput molecular techniques for the exploration of microbial gut communities. Gut Microbes 1(4): 277-278. http://doi.org/10.4161/gmic.1.4.12306
20. Sun QL, Zhang J, Wang MX, Cao L, Du ZF, Sun YY, Liu SQ, Li CL, Sun L. (2020). High-throughput sequencing reveals a potentially novel *Sulfurovum* species dominating the microbial communities of the seawater-sediment interface of a deep-sea cold seep in south china sea. Microorganisms 8(5): 687. http://doi.org/10.3390/microorganisms8050687
21. Ni Y, Yang X, Zheng L, Wang Z, Wu L, Jiang J, Yang T, Ma L, Fu Z. (2019). *Lactobacillus* and *Bifidobacterium* improves physiological function and cognitive ability in aged mice by the regulation of gut microbiota. Mol Nutr Food Res 63(22): e1900603. http://doi.org/10.1002/mnfr.201900603
22. Huan W, Jing G, Wenfeng W, Yanqin L, Xiaochao F, Yu F, Wei Q, Xiaohua H. (2017). Are there any different effects of *Bifidobacterium*, *Lactobacillus* and *Streptococcus* on intestinal sensation, barrier function and intestinal immunity in PI-IBS mouse model? PLoS One 9(3): e90153. http://doi.org/10.1371/journal.pone.0090153