

Effects of Fishery Utilization on the Physicochemical Index and Microbial Community Composition in Saline-Alkaline Water

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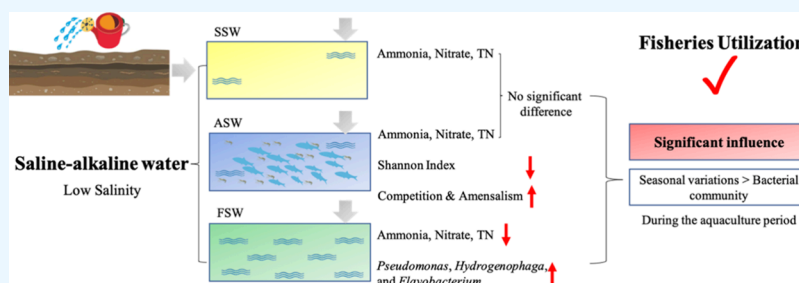
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ABSTRACT: Fishery utilization of idle saline-alkaline water resources offers various benefits including reducing surrounding soil salinity, improving the ecological environment, increasing arable land area, and providing economic advantages to the fishery industry. However, for decades, the characteristics and regulatory mechanisms of microbial communities that affect fishery utilization have not been clear, which restricts their application. In this study, high-throughput 16S rRNA amplicon sequencing was employed to analyze the bacterial community in these water resources. The sequencing yielded high-quality sequences (2,765,063), resulting in the identification of 18,761 bacterial operational taxonomic units (OTUs). Analysis revealed that the type of saline-alkaline water had a more significant influence on the bacterial community compared to seasonal variations within the aquaculture period. The Chao index for saline-alkaline ponds (ASW) was significantly lower ($P < 0.05$) than for still saline-alkaline water (SSW) and flowing saline-alkaline water (FSW), while the Shannon index for ASW was also significantly lower ($P < 0.05$) compared to FSW. When comparing ASW to nonaquaculture saline-alkaline water, a decrease in Proteobacteria to 26.87% was noted, particularly α -proteobacteria and γ -proteobacteria, accompanied by a rapid increase in Actinobacteria and Cyanobacteria to 28.60%. Network analysis further revealed that ASW significantly increased competition and amensalism from secondary saline-alkaline water microorganisms, resulting in a more solitary bacterial community composition as an adaptive strategy to cope with intense environmental pressures. Key bacterial species such as *Pseudomonas*, *Hydrogenophaga*, and *Flavobacterium* were found to be involved in hydrogen-cycling, nitrogen-cycling, and carbon-cycling, respectively, with all three exhibiting high abundance in FSW. Consequently, FSW demonstrates significant advantages in biogeochemical cycling, pollutant degradation, and the utilization of indigenous probiotic bacteria. Although the surface of abandoned secondary saline-alkaline land was covered with white salt particles, the fishery utilization of saline-alkaline water with low salinity levels (4.0–5.5), and the presence of nitrate and phosphate were identified as primary determinants of bacterial community composition. Nevertheless, a comparison of coastal high-salinity ponds indicated that salinity still selectively affects bacterial communities to some extent. Overall, our study provides valuable insights into the microbial regulation of nitrite during saline-alkaline water aquaculture, thereby aiding in the efficient utilization of secondary saline-alkaline water resources for fisheries.

1. INTRODUCTION

The process of soil salinization results in excessive accumulation of soluble salts in the soil. Saline soils were caused principally by human interferences, including poorly drained practices, inefficient water management leading to rising water tables, irrigation with saline and brackish water, surface seawater intrusion, overextraction of groundwater, and over-application of chemical fertilizers.¹ Northwest China occupied 71% of China's total area, of which 70% were saline-alkaline land.² Saline-alkaline water aquaculture, in addition to alleviating soil salinity, simultaneously provides a practical solution for the outlet of saline-alkaline water. Through

aquaculture, it confers economic dividends upon agrarians contending with saline-alkaline afflictions.³ Therefore, saline-alkaline water aquaculture has been widely applied in saline-alkaline areas with abundant water resources.

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Soils affected by salinization usually had low microbial biomass, but some specific microbial communities still existed. Low microbial biomass was a direct negative impact of salinity on microbes, while the lack of plant growth in soil, leading to reduced organic matter input, was also an indirect effect of low biomass.^{4,5} Simultaneously, the microbial composition was influenced by spatial heterogeneity. Zhao et al.² investigated hypersaline Ebinur Lake soils in Xinjiang and found that *Pseudomonas*, *Halomonas*, *Comamonas*, and *Smithella* were the indication genera over the salinity gradient. Yang et al.⁶ indicated that high salinity in coastal saline-alkaline lands reduced the abundance of Acidobacteria and Actinobacteria, and enhanced the abundance of Bacteroidetes and Gemmatimonadetes. However, these studies were not highly relevant to the artificial utilization of saline-alkaline water or aquaculture research.

The exploration of saline-alkaline water for aquaculture has emerged as an intriguing and compelling research field. Numerous research studies have delved into various aspects of aquacultural activities in high salt/alkaline water, encompassing the breeding of *Litopenaeus vannamei* and scrutinizing their impact on gut microorganisms, water microorganisms, and sedimentary microorganisms. However, the prevailing scholarly discourse predominantly focuses on the systematic simulation of the effects of saline-alkaline water on aquaculture organisms, emphasizing vitality, proliferation, and physiological-biochemical aspects.^{7,8} The emphasis persists on cultivating salt-tolerant varieties and elucidating the molecular mechanisms underlying adverse consequences resulting from saline-alkaline conditions.^{9,10}

Meanwhile, significant heterogeneity prevails in the chemical composition of saline-alkaline water across diverse geographical regions, contributing to a complex tapestry of distinct water quality typologies. While sulfate-dominant saline-alkaline water ubiquitously pervades Northwest China, the specific water quality varies markedly depending on regional idiosyncrasies. For instance, the eastward curvature of the Yellow River yields saline-alkaline water, characterized by a pronounced carbonate salt content. The Hetao region offers saline-alkaline water characterized by a low chloride content, manifesting in a discontinuous spatial distribution. In the Jin-Shaan salt lake precinct, high chloride-laden saline-alkaline water holds sway. Conversely, the Caowotan region principally showcases sulfate and chloride composite saline-alkaline water, manifesting a consequential potassium deficit alongside vulnerabilities to heightened nitrite, augmented ammonia nitrogen, and elevated pH.

Saline-alkaline water aquaculture, in addition to alleviating soil salinity, simultaneously provides a practical solution for the outlet of saline-alkaline water. Nevertheless, the intricate dynamics of piscatorial influence on the bacterial milieu in saline-alkaline vicinities and its potential jeopardy to the pristine quality of saline-alkaline water necessitate nuanced examination. In delineating the risk-benefit calculus governing piscatorial utilization and saline-alkaline land reclamation, this article seeks to proffer nuanced insights. It is thus envisaged that this research, leveraging the saline-alkaline water of Caowotan as a paragon, will illuminate the modulations in bacterial community structures resulting from piscatorial engagement, thereby furnishing a cogent scientific foundation for the efficacious exploitation of secondary saline-alkaline water.

2. MATERIALS AND METHODS

2.1. Sampling Site. Caowotan Fishery and Agriculture Comprehensive Utilization Demonstration Area, located in Jingtai County, Gansu Province (37°19' N, 104°07' E, altitude 1610 m above sea level). The study area is situated in the temperate arid desert climate zone in the upper reaches of the Yellow River, characterized by windy springs and dry-hot summers. The average annual temperature was 8.6 °C, with a maximum temperature of 35.6 °C and a minimum temperature of −27 °C, along with a large daily temperature difference. The precipitate was 185 mm, concentrated from April to September, and the annual average evaporation was 3038 mm. Jindian was China's largest high-lift irrigation project, which had a rich supply of water resources. As a result of the Jindian project being put into operation, the drought and water shortage problems were largely resolved, but soil salinization became the largest factor limiting agricultural production. Evaporation was 16 times greater than precipitation, and the low-lying terrain was poorly drained, resulting in a large area of secondary saline-alkaline land in the region. According to the local government, salt-affected soils reached 180 km² in 2014, of which 84.44% were severely abandoned.

2.2. Experimental Design. The study period was from June 2018 to October 2019, sampling at the preaquaculture period (April), the middle-aquaculture period (June and August), and the late-aquaculture period (October) in the Caowotan integrated Fisheries and Agriculture Demonstration Area. Three different management methods were set up:

1. SSW: still saline-alkaline water. The secondary saline-alkaline water was collected and stilled from the soil-washing process as a reserve for aquaculture water.
2. ASW: aquaculture saline-alkaline water. The secondary saline-alkaline water was collected from saline-alkaline aquaculture ponds used for the cultivation of shrimp.
3. FSW: flowing saline-alkaline water. The secondary saline-alkaline water was collected from the alkaline drainage ditch, and mixed by soil infiltration water and aquaculture pond discharge water.

SSW and ASW were supplemented with water according to the water level. If supplementation occurred, then sampling was delayed for more than 3 days. Each type of saline-alkaline water was collected in triplicate. The ASW pond covered an area of 6.86 hm² with a depth of 1.5–1.8 m, yielding about 400 kg per mu. It was primarily used for the cultivation of *Cyprinus carpio* and *Ctenopharyngodon idellus*, with *Penaeus vannamei* as an ecologically cocultivated species. Due to the relatively low overall yield, only an appropriate amount of feed was added during the aquaculture process, and no medications were used.

2.3. Physicochemical Property Determination. A total of 63 saline-alkaline water samples were collected from 9 to 12 am during 2018 to 2019. Water temperature (WT), redox potential (ORP), electrical conductivity (EC), DO, salinity, pH, illuminance (Lux), and chlorophyll-a (Chl-a) were measured in situ by a multiparameter water quality analyzer (YSI ProDSS, American). The waters were stored in polyethylene bottles at 4 °C. The physicochemical properties of water samples were detected at the Jingtai Center of the Engineering Technology Research Center of Saline-alkaline Water Fisheries, CAFS. Nine environment variables were analyzed using the standard methods¹¹ (SEPA, 2002), including total organic carbon (TOC), nitrite, ammonia, nitrate, suspended solids (SS), phosphate, total nitrogen (TN),

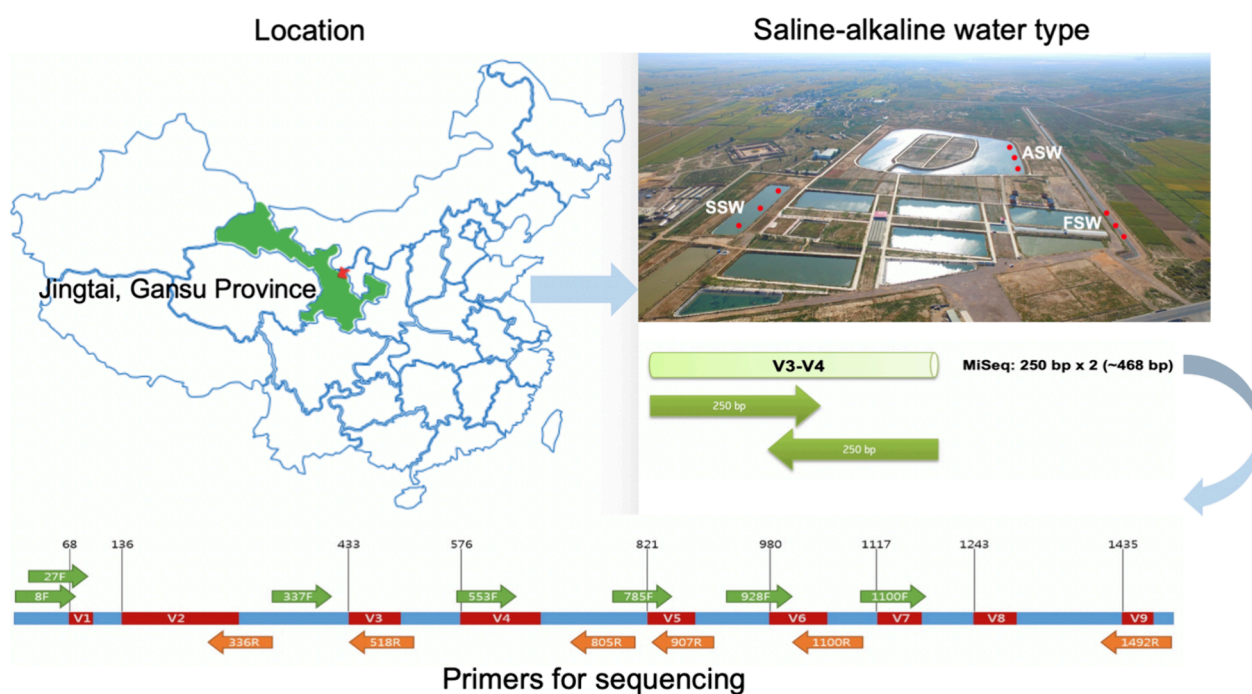


Figure 1. Flowchart of the experimental design, including location, type of saline-alkaline water, and primers for sequencing.

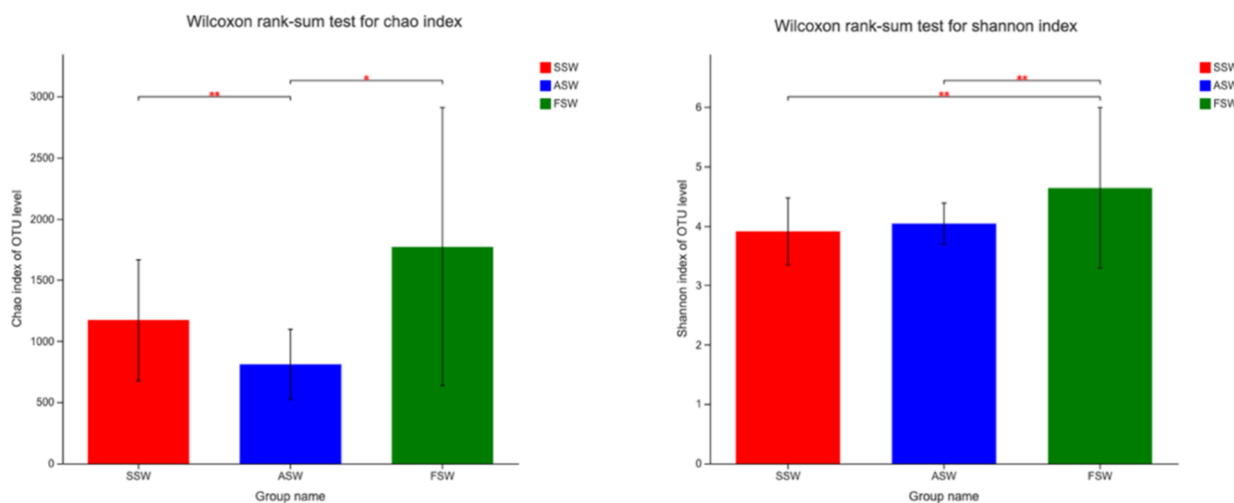


Figure 2. Alpha diversity analysis. (a) Chao indexes for SSW, ASW, and FSW from saline-alkaline water. (b) Shannon indexes of SSW, ASW, and FSW for saline-alkaline water. The statistical analysis was performed using the Wilcoxon rank-sum test. Significance was indicated by asterisks where $*P < 0.05$.

total phosphorus (TP), permanganate index (COD) (data are shown in [Supporting Information](#)).

2.4. DNA Extraction, Amplification, and Amplicon Sequencing. To collect microorganisms from saline-alkaline water, 500 mL of water was filtered by hydrophilic nucleopore filters (0.22 μm , Jingteng Laboratory Equipment Co. Ltd., China).¹² For the best result, the filter membrane samples were lysed at 6300 rpm on a Percellys Tissue Homogenizer for 10 s with 3 cycles. Filter membrane was used for total DNA extraction by E. Z. N. A. water DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA). DNA yield was measured by a nanodrop (ND-1000 spectrophotometer, NanoDrop Technology). Samples were confirmed to have intact high molecular weight DNA by agarose gel electrophoresis. Primers were used to amplify the V3–V4 hypervariable region of the bacterial 16S rRNA gene by primers 338F (5'- ACTCCTACGGGAGGCAGCAG-

3') and 806R (5'- GGACTACHVGGGTWTCTAAT-3').¹³ PCR products were purified by using the AxyPrepDNA purification kit (Axygen, Inc.).

Amplicons were pooled at equimolar concentrations, and paired-end sequencing (2×300) was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to standard protocols at Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Operational taxonomic units (OTUs) were clustered using UPARSE (version 7.1; <http://drive5.com/uparse/>) with a 97% similarity cutoff; chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA sequence was analyzed against the Silva (SSU123) 16S rRNA database using the RDP classifier algorithm (<http://rdp.cme.msu.edu/>) with a confidence threshold of 70%. In total, MiSeq sequencing of

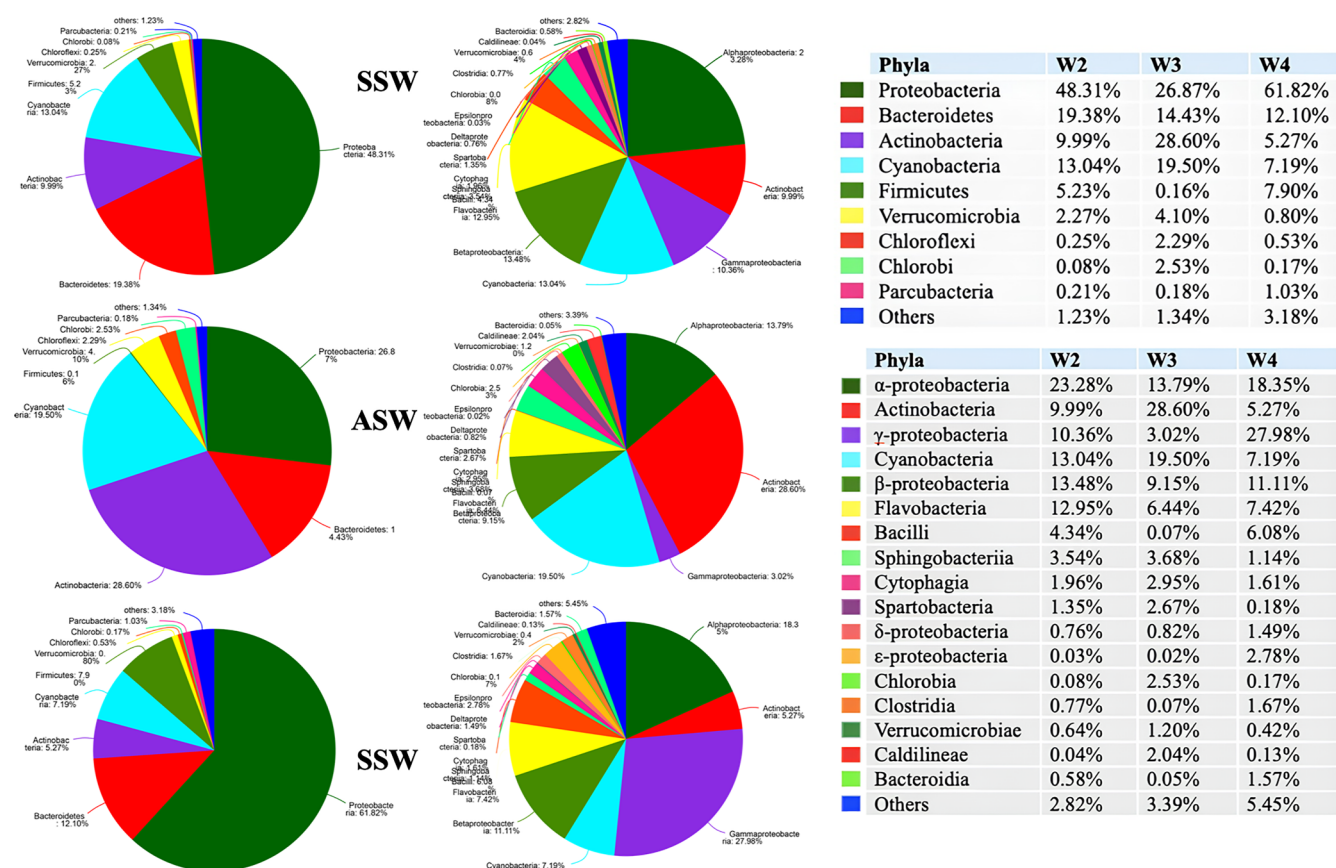


Figure 3. Microbial composition at the phylum and class levels. Pie charts show the proportion of reads in each phylum (left) and class (right) for SSW, ASW, and FSW. Phyla and class levels of microbial abundances are shown in the table.

bacterial 16S rRNA genes resulted in 2,765,063 high-quality reads, respectively.

2.5. Statistical and Bioinformatics Analysis. Differences between physicochemical properties, richness and diversity estimators, and relative abundances of major phyla or genera were analyzed using the Kruskal–Wallis test and Wilcoxon rank-sum test (Past v 3.17). Spearman's correlation analysis between physicochemical properties and salinity of saline-alkaline water samples was calculated and visualized using the Hmisc package in R.¹⁴ Network analysis was carried out using Networkx software.¹⁵ Variance inflation factor (VIF), principal coordinates analysis (PCoA), canonical correlation analysis (CCA), and variance partitioning analysis (VPA) were performed using the Vegan package in R.¹⁶ The difference significance level P was 0.05, and the extreme significance level P was 0.01. The mean data result is expressed as the mean \pm standard deviation (mean \pm SD).

3. RESULTS

3.1. Diversity of the Saline-Alkaline Water Microbiota. To explore the bacterial structure and diversity in different saline-alkaline water, we detected saline-alkaline water from the Caowotan Integrated Fisheries and Agriculture Demonstration Area (Jingtai, Gansu Province). Accordingly, SSW, ASW, and FSW samples were investigated as described in Figure 1. In total, 2,765,063 high-quality, nonchimeric sequences were obtained, with an average sequence of 43,890 (range from 21,037 to 69,796) per sample (Table S1). Based on the OTU numbers, rarefaction curves of saline-alkaline water samples are shown in Figure S1. 18,761 bacterial OTUs

were identified in a single sample by rarefying to the lowest number of reads. The Good's coverage for the observed OTUs was $98.99 \pm 0.01\%$ (mean \pm s.e.m.) (Figure S1). A crucial difference was observed in Chao index values between compartment samples except SSW and FSW samples. α -diversity analysis (Shannon index, Shannon-even index, etc.) showed significant differences between saline-alkaline water microbial communities except ASW and SSW (Figure 2 and Table S2).

3.2. Bacterial Community Composition. The dominant bacterial communities across were Proteobacteria, Bacteroidetes, Actinobacteria, and Cyanobacteria (Figures 3 and S2). Compared to nonaquaculture saline-alkaline water, Proteobacteria decreased to 26.87% in aquaculture saline-alkaline water. The abundance of α -Proteobacteria and γ -Proteobacteria decreased sharply, while the abundance of Actinobacteria and Cyanobacteria increased rapidly to 28.60 and 19.50%, respectively. The differences were even greater for Proteobacteria, Bacteroidetes, Cyanobacteria, and Actinobacteria in the four seasons of the SSW (Figure S3a). However, Actinobacteria were most abundant in the ASW (Figure S3b) samples. The relative abundance of major microbial phyla, including Cyanobacteria, unclassified norank was significantly higher in August than those in April. Furthermore, Chloroflexi were more abundant in October than in April (Figure S3). Differences in SSW bacterial composition by season were also demonstrated at the genera level; the change was similar to that at the phylum level (Figure S4). Among the top first ten genera, five of the SSW samples (Figure S4a), six of the ASW samples (Figure S4b), and seven of the FSW samples (Figure

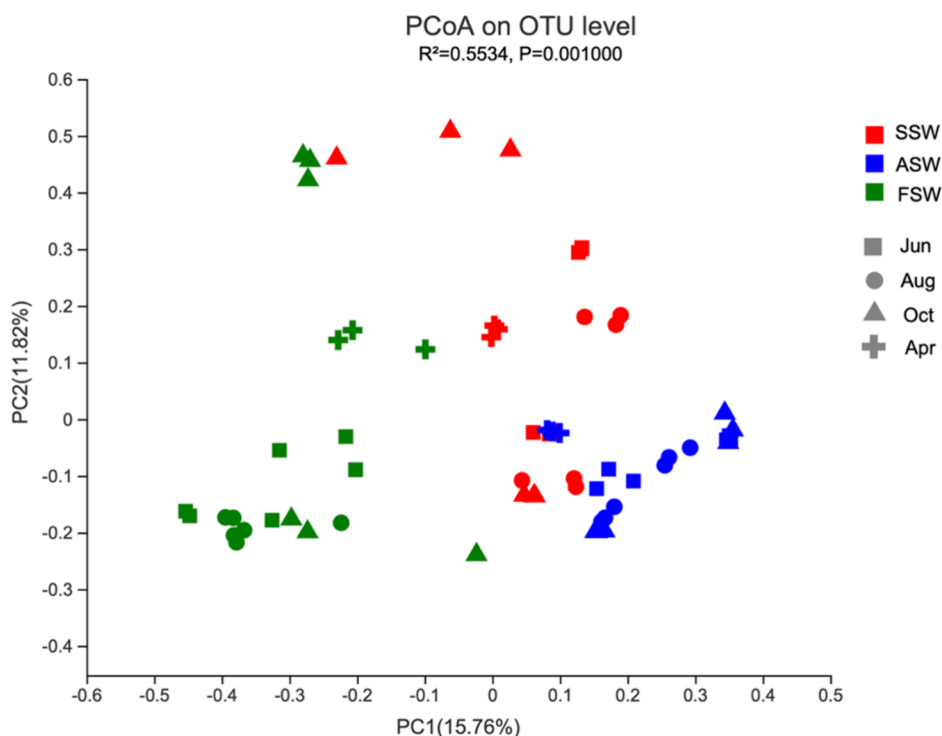


Figure 4. Beta-diversity of microbes in three types of saline-alkaline water. PCoA of Bray–Curtis distances indicate that saline-alkaline water type was a major source of bacterial community variation. Adonis calculate the significance of clustering according to the type of saline-alkaline water ($Pr(>F) = 0.001$). Every point represents a different sample, and each compartment is represented by a different shape and color.

S4c) were significantly different and belonged to Proteobacteria and Cyanobacteria. Moreover, *Pseudomonas*, *Hydrogenophaga*, and *Flavobacterium* occupied higher abundance in FSW (Figure S4c).

A PCoA of Bray–Curtis distances revealed a clear separation of SSW (red circle), ASW (blue triangle), and FSW (green rhombus) microbiota in three types of water (Figures 4 and S5). As an example, PERMANOVA (Table S3) and weighted UniFrac distances (Table S4) demonstrated that the microorganisms in the four seasons of saline-alkaline water were significantly different. The type of saline-alkaline water had a deeper influence on microbial communities than seasonal changes within the aquaculture period (Figure S5). Fishery utilization of saline-alkaline water had a significant impact on microbial diversity.

3.3. Microbial Interaction Networks of Saline-Alkaline Water. Microbial interaction was one of the major driving factors that shaped population structure and dynamics, as microbes could coexist or exclude each other.^{17,18} Our studies showed an advanced level of node connectivity of the bacterial community. We selected the top 50 bacteria at the genus level for analysis. The results demonstrated a total of 347 correlations in the SSW (Figure 5a and Table S5), 320 correlations in the ASW (Figure 5b and Table S6), and 401 correlations in the FSW (Figure 5c and Table S7) samples. The interaction networks in the ASW and SSW are relatively simple contrasted to that in FSW. In ASW samples, bacteria correlation increased, while network complexity declined. In FSW samples, *Pseudomonas* was negatively correlated with *Sphingomonas* (−0.816764), and unclassified f Comamonadaceae (−0.709321). *Wolbachia* was negatively correlated with *Limnohabitans* (−0.65083), *Fluviicola* (−0.614271) *Loktanella* (−0.616279), and *Ketogulonicigenium* (−0.6047). *Rikenella*

ceae RC9 gut group was negatively correlated with *Fluviicola* (−0.650814), and norank p Parcubacteria (−0.611933). *Lactococcus* was negatively correlated with *Limnohabitans* (−0.606909). All the other taxa had a positive relationship ($|$ Coefficient ≥ 0.6 , Figure 5c), which suggests taxa can co-occur with saline-alkaline water in FSW and will not exclude other species. Additionally, the negative relationship between *Wolbachia* and *Limnohabitans* (−0.645884) was demonstrated in SSW. *Wolbachia* was correlated with 12 genera in SSW samples, while *Limnohabitans* were correlated with 17 (Figure 5a and Table S5).

3.4. Environmental Factors Affect the Formation of the Bacterial Community. VIF was evaluated to judge the collinearity among different factors. WT, DO, and Nitrite with VIF > 7 were eliminated (Table S8). Bacterial communities varied significantly from different saline-alkaline water (Figures 6a and 4). CCA analysis was used to investigate the potential hydrochemical drivers in bacterial communities. Hydrochemical parameters explained the 19.14% variance of the bacterial community (Figure 6a and Table S9). Interestingly, nitrate concentration, which separated FSW from other wells, had a significant impact on the bacterial community composition (Figure 6a). Phosphate ($r^2 = 0.45$) and nitrate ($r^2 = 0.39$) were the primary factors determining the composition of the bacterial community. Nitrogen compounds and organic matter were the key drivers of bacterial compositional differences.

The contribution of physiochemical properties to bacterial community variation is illustrated by VPA (Figure 6b). All the variation partitioning fractions were conspicuous in an ANOVA permutation test ($P < 0.05$). All physiochemical properties explained a total of 22.57% of the variation in the bacterial communities. Nutrients were clearly contributed the

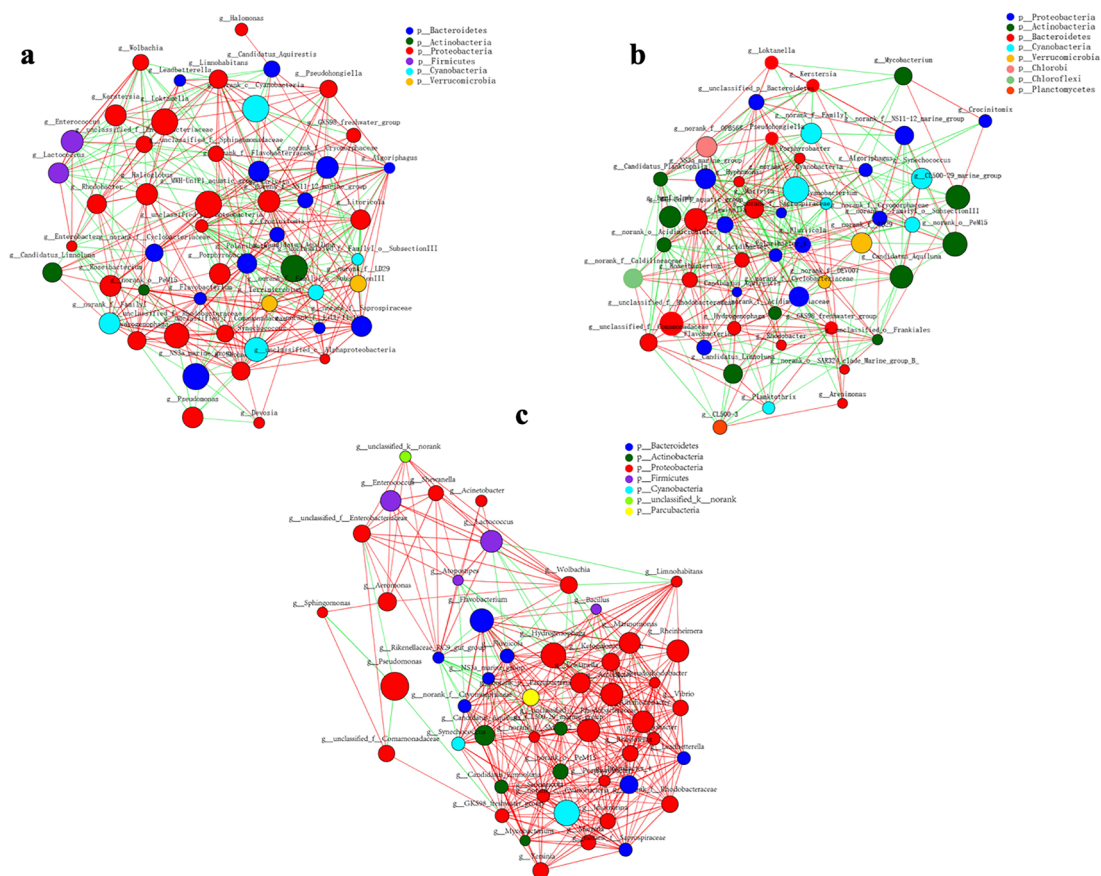


Figure 5. Networks of microbial interactions in different saline-alkaline water. Interaction network of dominant microbiota at the genus level (top 50) in SSW (a), ASW (b), and FSW (c). The size of the nodes indicates the abundance of OTUs, while the different colors indicate the corresponding taxonomic assignment at the phylum level. Positive (red) and negative (green) correlations are indicated by edge color. The edge thickness indicates the correlation values; only significant interactions are indicated ($r \geq 0.5$; $P < 0.05$).

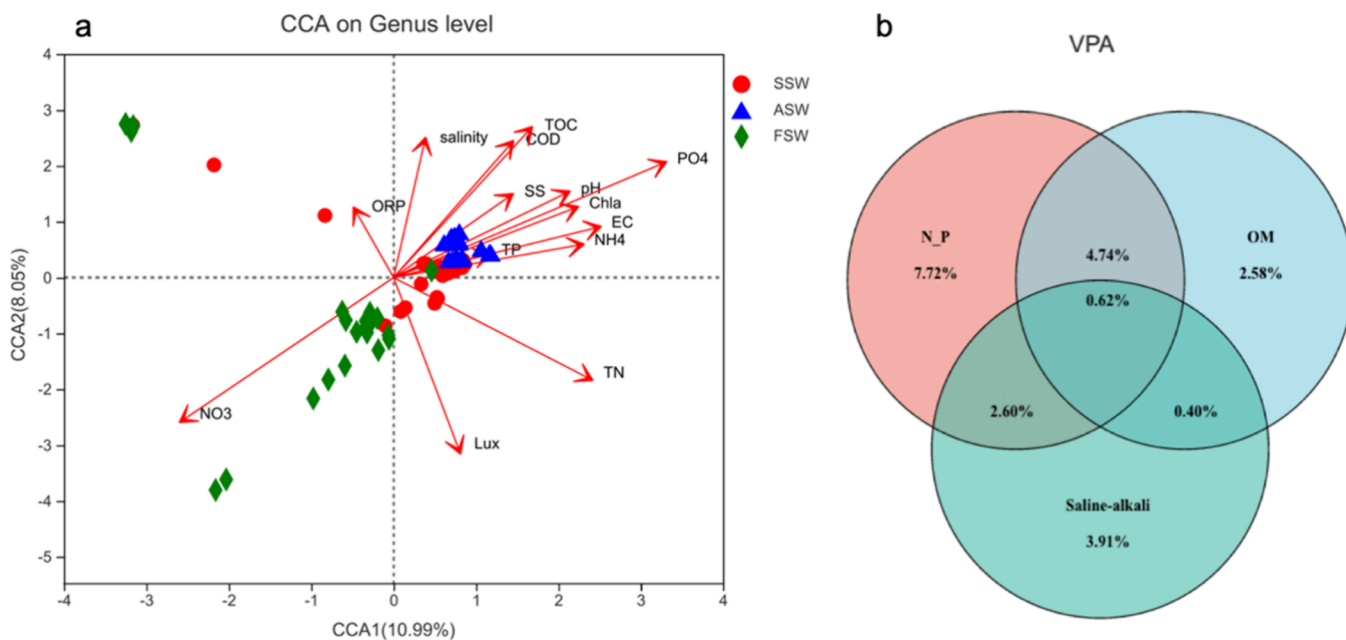


Figure 6. Drivers of bacterial β -diversity in saline-alkaline water samples. (a) Canonical correlation analysis (CCA) reveal hydrochemical parameters that influenced bacterial community composition. (b) Variation partitioning analysis (VPA) of the relative contributions of nitrogen and phosphorus nutrients, saline-alkali variables and organic matter variables to the variation in bacterial β -diversity. By using Adonis, clustering significance by saline-alkaline water type was determined ($Pr > F = 0.001$). Each point corresponds to a different sample, and each compartment is represented by a different color and shape. NO_3 , nitrate; NH_4 , ammonium; PO_4 , phosphate; Chla, chlorophyll-a.

most. Moreover, six segmentation points were set for salinity, with 71.43% of the samples from both SSW and ASW concentrated in the range of 4.6 to 5.1. For FSW, 80.96% of the samples were concentrated in the same salinity range (4.6 to 5.1). Additionally, 19.05% of FSW samples had a salinity not exceeding 4.3, while SSW had 4.76% of samples with a salinity not exceeding 4.3 (Table 1). Soil salinity was moderately

Table 1. Cumulative Percentage Analysis of Salinity

salinity point	accumulation (%)		
	SSW	ASW	FSW
3	0	0	0
4.3	4.76	0	19.05
4.6	14.29	4.76	42.86
4.8	23.81	28.57	19.05
5.1	33.33	38.10	19.05
6	23.81	28.57	0
min	4.29	4.32	4.03
max	5.3	5.5	5.08

correlated ($r = 0.5$) with pH, weakly correlated ($r < 0.4$), or uncorrelated with other factors (Table S10). Consequently, low salinity was not the main factor determining the bacterial community in our study.

4. DISCUSSION

Existing studies on microbial community diversity in saline-alkaline land focused on soils. High salinity increased the relative abundance of Gemmatimonadetes and Bacteroidetes and reduced the relative abundance of Actinobacteria and Acidobacteria in the Yellow River Delta.⁶ Firmicutes and Bacteroidetes were positively correlated with salinity in Xinjiang.¹⁹ Saline-alkaline soil and saline-alkaline water in the study region were dominated by the $\text{SO}_4\text{-Na}$ 2 type, which differed from the coastal Cl-Na type. The type of saline-alkaline water largely affected the physiology and biochemistry of aquaculture animals. Furthermore, the study of microbial diversity in saline-alkaline soils was not replaced by the study of microbial diversity in saline-alkaline waters. For example, a study on a soda lake in India²⁰ indicated that Proteobacteria and Firmicutes were significantly higher in sediment samples, whereas Actinobacteria, Candidate division TM7, and Cyanobacteria were significantly abundant in water. To the best of our knowledge, this is the first work to analyze the bacterial structure variation by different aquaculture management of the saline-alkaline water zone of Northwest China by water amplicon sequencing.

Although the dominant genus in saline-alkaline water differed significantly between fishery utilization types and seasons (Figure S4), the effect of the fishery utilization type of bacterial composition was greater compared to season within the aquaculture period (Figures 4 and S5). ASW had a lower bacterial richness and diversity than FSW, the opposite for evenness. Aquaculture management significantly enhanced microbial interactions from different types of saline-alkaline water (Figure 5 and Tables S5–S7). Positive connections implied collaborative engagements involving cross-feeding and niche overlap, while negative connections signified instances of amensalism and competition with deleterious effects within the network.^{15,21} In essence, positive correlations suggested symbiotic cooperation, whereas negative correlations hinted at antagonistic competition.

In the general discourse, the compromised water quality in aquaculture ponds arises from the simultaneous waste products of aquaculture organisms and constrained resource dynamics. This accentuated interspecific competition among bacterial communities for limited resources, consequently diminishing the requisites for pollutant biodegradation. However, contrary to these presumptions, our previous investigations revealed no significant disparities in the concentrations of ammonia, nitrite, and TN between SSW and ASW.²² This elucidation posited that microbial modulatory mechanisms constitute pivotal adaptive strategies. Moreover, bacterial communities in FSW of saline-alkaline water exhibited a preference for cooperative synergism in fostering the degradation of contaminants, thereby fostering heightened stability in the microbial community.

Therefore, the key factor for more negative connections in ASW and positive ones in FSW lies in the limited resources and collaborative competition among bacteria. It is noteworthy that the impact of aquaculture activities on nutrient dynamics in saline-alkaline water remains relatively inconsequential. Nevertheless, prudence dictates the periodic supplementation of indigenous probiotics and the diligent removal of organic sediment despite the comparatively lower stocking density in secondary saline-alkaline water, which is imperative to preempt potential failure in aquaculture endeavors.

The abundance of *Pseudomonas*, *Hydrogenophaga*, and *Flavobacterium* was higher in the FSW. *Pseudomonas*, *Hydrogenophaga*, and *Flavobacterium* belong to hydrogen-cycling, nitrogen-cycling, and carbon-cycling bacteria, respectively.²³ *Pseudomonas* was previously reported as key nitrifiers belonging to the nitrite-oxidizing bacterial group in natural ecosystems, which can participate in the competition for nitrite.²⁴ Our previous study demonstrated that high nitrite became a technical bottleneck limiting the saline-alkaline water fishery utilization for the nitrite concentration in FSW was significantly lower than ASW and SSW.²² Moreover, a high abundance of *Flavobacterium* promoted the uptake and degradation of dissolved organic matter.²⁵ Therefore, FSW has great advantages in biogeochemical cycling, pollutant degradation, and the excavation of indigenous probiotic bacteria.

Although abandoned secondary saline-alkaline land was covered with white salt particles, fishery utilization of saline-alkaline water had a low salinity range (4.0–5.5). It resulted in no significant effect of salinity on bacterial community richness and diversity in our study (Table 1). Consistently, the same salinity range was also not a major influence in determining bacteria community composition. To further analyze the effect of salinity on bacterial diversity in secondary saline-alkaline water, the saline-alkaline ponds in this study were compared with coastal ponds with salinity ranging from 28 to 38.^{26–28} 80% of the bacterial sequences in both coastal ponds and secondary saline-alkaline ponds contained Bacteroidetes, α -Proteobacteria, γ -Proteobacteria, Actinobacteria, and Cyanobacteria. Various research studies have previously proved that Bacteroidetes were widespread in different highly saline-alkaline conditions and resisted high salinity.²⁹ Bacterial richness and diversity were significantly lower in saline-alkaline ponds compared to those in coastal ponds, with differences greater than 75 and 40%, respectively, in Chao richness and Shannon diversity. ASW did not include Planctomycetes or Acidobacteria, but Chlorobi were present. Salinity was still

selective and affected bacterial communities, despite the low salinity range.

A strong correlation between bacterial communities and nutrients such as nitrogen, phosphorus, or carbon, approximately other physiochemical properties like soil EC, was discovered in research studies among different scales.² In addition, studies in coastal high-salinity shrimp pond waters indicated that salinity, pH, TN, temperature, or chlorophyll may drive bacterial community changes.^{26–28} Nevertheless, there was no significant relationship between the bacterial community of the West Greenland estuary and some environmental factors.³⁰ Bacteria communities from different ecosystems or salinity ranges were affected by diverse physiochemical properties. With the purpose of defining whether the distribution of bacteria was controlled more by environmental conditions, we explored the relationship between the bacterial community and physiochemical properties. Consistent with the hypothesis, bacterial community structure different between ASW and FSW, the community structure of ASW and SSW were driven by phosphate, while the FSW community structure was driven by nitrate (Figure 6a). The above environmental variables were the primary influences determining bacterial community composition in a low salinity range (3 to 6). Therefore, during the fishery utilization of secondary saline-alkaline water in Northwest China, the influence of nutrients, such as nitrogen and phosphorus, should be focused more.

5. CONCLUSIONS

Microbial diversity analysis has accumulated a vast amount of data on currently uncultivable microorganisms, expanding our capacity for microbial data collection far beyond the capability of cultivating microorganisms. Some researchers argue that microbial diversity analysis has limitations due to the challenging accurate classification of microbes and the feasibility issues with isolation and purification. Another ongoing study we are conducting involves screening salt-tolerant bacteria with good ecological functions in saline-alkaline environments to complement the limitations of microbial diversity studies in isolation and cultivation. Strains such as *Pseudomonas alcaliphila* and *Halomonas shizuishanensis* DWK9 (CMCC27980) that have been screened exhibit excellent performance in salt tolerance and denitrification rates. The nitrogen metabolism mechanisms of SSW, ASW, and FSW require further investigation. In the future, microbial diversity analysis should be combined with nitrogen metabolism functional genes such as *amoA*, *nxrA*, *nirS*, *narG*, *hzsA*, etc. Additionally, to further elucidate the role of key species in nitrogen metabolism, metagenome techniques can be employed to explain the metabolic regulatory mechanisms of microbes on a molecular biology pathway.

This study provides a comprehensive overview of microbial communities in saline-alkaline water utilized for fisheries, particularly comparing the microbial community structures in common fisheries configurations, including saline-alkaline ponds, aquaculture ponds, and alkaline drainage canals. The study clearly delineates the differences in water quality and microbial community structures among various types of saline-alkaline water along with identifying key environmental factors influencing microbial communities. It is the first to analyze changes in bacterial structure using high-throughput sequencing for the management of fisheries in the saline-alkaline regions of Northwest China. Saline-alkaline water aquaculture

enhances microbial competition and promotes a more homogeneous microbial community structure, which can also impact water quality through nutrient levels. However, compared with uncultured saline-alkaline water, cultured saline-alkaline water does not significantly increase levels of total nitrogen (TN), ammonia, and nitrite. Additionally, the unique secondary saline-alkaline water contains a diverse range of microbial resources. The use of alkaline drainage canals during fishery utilization helps identify indigenous probiotic bacteria that excel in biogeochemical cycling and pollutant degradation. The potential risks associated with fishery utilization in saline-alkaline waters are therefore much lower than the ecological and economic benefits that can be obtained from this strategy. This study helps to initiate artificially controlled improvement of saline alkaline water quality through microbial populations for improved aquaculture inclusivity and diversity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c08437>.

Sampling depths of SSW, ASW and FSW samples from saline-alkaline water; structural composition of the saline-alkaline water microbiota in different seasons at phylum level; different relative abundances of major Phyla (top 10) among the different compartments; different relative abundances of major genera (top 10) among the different compartments; PCoA analyses based on Bray–Curtis distance; sequence number in saline-alkaline water samples; alpha diversity analysis of samples; PERMANOVA analysis of the microbial community in different saline-alkaline water type composition based on Bray–Curtis; PERMANOVA analysis of the microbial community in different saline-alkaline water type composition based on UniFrac; correlation coefficient of microorganisms (genus level) in SSW samples; Correlation coefficient of microorganisms (genus level) in ASW samples; correlation coefficient of microorganisms (genus level) in FSW samples; variance inflation factor (VIF) to judge the collinearity among different physicochemical parameters; table of environmental factors of CCA; and Pearson correlation coefficient (ρ) between physicochemical parameters and salinity (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Hassani, A.; Azapagic, A.; Shokri, N. Global predictions of primary soil salinization under changing climate in the 21st century. *Nat. Commun.* **2021**, *12*, 6663–6680.
- (2) Zhao, S.; Liu, J. J.; Banerjee, S.; Zhou, N.; Zhao, Z. Y.; Zhang, K.; Tian, C. Y. Soil pH is equally important as salinity in shaping bacterial communities in saline soils under halophytic vegetation. *Sci. Rep.* **2018**, *8*, 4550.
- (3) Rozema, J.; Flowers, T. Crops for a salinized world. *Science* **2008**, *322* (5907), 1478–1480.
- (4) Yuan, B. C.; Li, Z. Z.; Liu, H.; Gao, M.; Zhang, Y. Y. Microbial biomass and activity in salt affected soils under arid conditions. *Applied Soil Ecology* **2007**, *35*, 319–328.
- (5) Rath, K. M.; Rousk, J. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: A review. *Soil Biol. Biochem.* **2015**, *81*, 108–123.
- (6) Yang, C.; Li, K. J.; Lv, D. T.; Jiang, S. Y.; Sun, J. Q.; Lin, H.; Sun, J. Inconsistent response of bacterial phyla diversity and abundance to soil salinity in a Chinese delta. *Sci. Rep.* **2021**, *11*, 12870.
- (7) Zeng, F. Y.; Luo, K.; Luan, S.; Cao, B. X.; Lu, X.; Tan, J.; Chen, B. L.; Meng, X. H.; Kong, J. Analysis of growth and survival among different families of *Litopenaeus vannamei* in the chloride typed alkaline water. *Journal of Fishery Sciences of China* **2018**, *25* (2), 308–315. In Chinese.
- (8) Peng, M. X.; Li, Z.; Liu, X. J.; Niu, D. H.; Li, J. L. Inland alkaline brackish water aquaculture of juvenile razor clam: Survival, growth, physiology and immune responses. *Aquaculture Reports* **2020**, *18*, No. 100463.
- (9) Wang, F.; Zhu, L.; Wei, Y. X.; Gao, P. C.; Liu, Y. M.; Zhou, K.; Sun, Z.; Lai, Q. F.; Yao, Z. L. Intestinal ion regulation exhibits a daily rhythm in *Gymnocypris przewalskii* exposed to high saline and alkaline water. *Scientific Reports* **2022**, *12*, 807.
- (10) Liu, Y. J.; Yao, M. Z.; Li, S. W.; Wei, X. F.; Ding, L.; Han, S. C.; Wang, P.; Lv, B. C.; Chen, Z. X.; Sun, Y. C. Integrated application of multi-omics approach and biochemical assays provides insights into physiological responses to saline-alkaline stress in the gills of crucian

carp (*Carassius auratus*). *Science of The Total Environment* **2022**, *822*, No. 153622.

(11) State Environmental Protection Administration. *Water and wastewater monitoring analysis method of editorial committee: Water and wastewater monitoring analysis method*, 4th ed.; China Environmental Science Press: Beijing, 2002.

(12) Sun, Z.; Li, G. P.; Wang, C. W.; Jing, Y. H.; Zhu, Y. P.; Zhang, S. M.; Liu, Y. Community dynamics of prokaryotic and eukaryotic microbes in an estuary reservoir. *Sci. Rep.* **2014**, *4*, 6966.

(13) Mori, H.; Maruyama, F.; Kato, H.; Toyoda, A.; Dozono, A.; Ohtsubo, Y.; Nagata, Y.; Fujiyama, A.; Tsuda, M.; Kurokawa, K. Design and experimental application of a novel non-degenerate universal primer set that amplifies prokaryotic 16S rRNA Genes with a low possibility to amplify eukaryotic rRNA Genes. *DNA Research* **2014**, *21*, 217–227.

(14) Kalwasinska, A.; Deja-Sikora, E.; Szabo, A.; Felfoldi, T.; Kosobucki, P.; Brzezinska, M. S.; Walczak. Salino-alkaline lime of anthropogenic origin a reservoir of diverse microbial communities. *Sci. Total Environ.* **2019**, *655*, 842–854.

(15) Hartmann, M.; Frey, B.; Mayer, J.; Mader, P.; Widmer, F. Distinct soil microbial diversity under long-term organic and conventional farming. *ISME journal* **2015**, *9*, 1177.

(16) Oksanen, J.; Blanchet, F. G.; Kindt, R.; Legendre, P.; Minchin, P. R.; O'Hara, R.; Simpson, G. L.; Solymos, P.; Stevens, M.; Wagner, H. *Vegan: community ecology package. R Package version 2.3-0*, 2015.

(17) Falony, G.; Joossens, M.; Vieira-Silva, S.; Wang, J.; Darzi, Y.; Faust, K.; Kurilshikov, A.; Bonder, M. J.; Valles-Colomer, M.; Vandeputte, D.; Tito, R. Y.; Chaffron, S.; Rymenans, L.; Verspecht, C.; de Sutter, L.; Lima-Mendez, G.; d'Hoe, K.; Jonckheere, K.; Homola, D.; Garcia, R.; Tigchelaar, E. F.; Eeckhaut, L.; Fu, J.; Henckaerts, L.; Zhernakova, A.; Wijmenga, C.; Raes, J. Population-level analysis of gut microbiome variation. *Science* **2016**, *352*, 560–564.

(18) Han, Q.; Ma, Q.; Chen, Y.; Tian, B.; Xu, L. X.; Bai, Y.; Chen, W. F.; Li, X. Variation in rhizosphere microbial communities and its association with the symbiotic efficiency of rhizobia in soybean. *ISME Journal* **2020**, *14*, 1915–1928.

(19) Hou, Y. L.; Zeng, W. Z.; Hou, M. L.; Wang, Z.; Luo, Y.; Lei, G. Q.; Zhou, B.; Huang, J. S. Responses of the soil microbial community to salinity stress in Maize fields. *Biology* **2021**, *10*, 1114.

(20) Paul, D.; Kumbhare, S. V.; Mhatre, S. S.; Chowdhury, S. P.; Shetty, S. A.; Marathe, N. P.; Bhute, S.; Shouche, Y. S. Exploration of microbial diversity and community structure of lonar lake: the only hypersaline meteorite crater lake within basaltic rock. *Frontiers in Microbiology* **2016**, *6*, 1533.

(21) Zheng, W.; Xue, D. M.; Li, X. Z.; Deng, Y.; Rui, J. P.; Feng, K.; Wang, Z. L. The responses and adaptations of microbial communities to salinity in farmland soils: a molecular ecological network analysis. *Applied Soil Ecology* **2017**, *120*, 239–246.

(22) Zhuang, Y. R.; Sun, Z.; Zhou, K.; Zhu, X. L.; Gao, B. D.; Gao, P. C.; Lai, Q. F. Correlation between inorganic nitrogen transformation and environmental factors in secondary saline-alkali water in northwest China. *J. Fish. Sci. China* **2020**, *27* (12), 1438–1447. In Chinese.

(23) Zhang, H. X.; Zheng, S. L.; Ding, J. W.; Wang, O. M.; Liu, F. G. Spatial variation in bacterial community in natural wetland-river-sea ecosystems. *J. Basic Microbiol.* **2017**, *57*, 536–546.

(24) Kuypers, M. M. M.; Marchant, H. K.; Kartal, B. The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* **2018**, *16*, 263–276.

(25) Kirchman, D. L. The ecology of Cytophaga-Flavobacteria in aquatic environments. *FEMS Microbiology Ecology* **2002**, *39*, 91–100.

(26) Zhang, D. M.; Wang, X.; Xiong, J. B.; Zhu, J. L.; Chen, H. P.; Guo, A. N.; Wu, J. F.; Dai, H. P. Bacterioplankton assemblages as biological indicators of shrimp health status. *Ecol. Indic.* **2014**, *38*, 218–224.

(27) Huang, F.; Pan, L. Q.; Song, M. S.; Tian, C. C.; Gao, S. Microbiota assemblages of water, sediment, and intestine and their associations with environmental factors and shrimp physiological health. *Appl. Microbiol. Biotechnol.* **2018**, *102* (19), 8585–8598.

(28) Alfiansah, Y. R.; Hassenruck, C.; Kunzmann, A.; Taslihan, A.; Harder, J.; Gardes, A. Bacterial abundance and community composition in pond water from shrimp aquaculture systems with different stocking densities. *Frontiers in Microbiology* **2018**, *9*, 2457.

(29) Keshri, J.; Mody, K.; Jha, B. Bacterial community structure in a semi-arid haloalkaline soil using culture independent method. *Geomicrobiology Journal* **2013**, *30*, 517–529.

(30) Hauptmann, A. L.; Markussen, T. N.; Stibal, M.; Olsen, N. S.; Elberling, B.; Bælum, J.; Sicheritz-Pomten, T.; Jacobsen, C. S. Upstream freshwater and terrestrial sources are differentially reflected in the bacterial community structure along a small arctic river and its estuary. *Front. Microbiol.* **2016**, *7*, 1474.