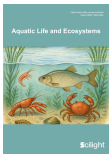
Scilight

Aquatic Life and Ecosystems
https://www.sciltp.com/journals/ale



Article

Diversity and Interactions of Bacterial Communities in the Gills of Kumamoto Oyster (*Crassostrea sikamea*) and Its Culture Environment

Shengli Fu [1,2], Tuo Yao [1,2], Jie Lu [1,2], Jinxia Peng [3] and Lingtong Ye [1,2,*]

[1] Key Laboratory of Aquatic Product Processing, Key Laboratory of South China Sea Fishery Resources Exploitation & Utilization, Ministry of Agriculture and Rural Affairs, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Science, Guangzhou 510300, China
[2] Sanya Tropical Fisheries Research Institute, Sanya 572426, China
[3] Guangxi Key Laboratory of Aquatic Genetic Breeding and Healthy Aquaculture, Academy of Fishery Sciences, Nanning 530021, China
* Correspondence: lingtong2753@126.com

How To Cite: Fu, S.; Yao, T.; Lu, J.; et al. Diversity and Interactions of Bacterial Communities in the Gills of Kumamoto Oyster (*Crassostrea sikamea*) and Its Culture Environment. *Aquatic Life and Ecosystems* **2025**. https://doi.org/10.53941/ale.2025.100005

Received: 27 August 2025
Revised: 17 September 2025
Accepted: 18 September 2025
Published: 19 September 2025

Abstract: The Kumamoto oyster (*Crassostrea sikamea*), an indigenous species in southern China, is ecologically and aquaculturally significant. To explore the associations between oyster-associated bacteria and their environment, we analyzed bacterial communities in oyster gills, seawater, and sediment from Techeng Island (TCCS, TCW, TCS) and Longtousha (LTSCS, LTSW, LTSS) using Illumina MiSeq sequencing of the 16S rRNA gene V3–V4 region. Alpha diversity showed the highest richness and diversity in sediments (Shannon index: 6.40–6.52), followed by seawater (4.15–4.58) and gills (2.64–2.99). Taxonomic analysis revealed 75 phyla, with Pseudomonadota (23.23–49.32%) dominant across all habitats. Habitat-specific patterns were observed: Spirochaetota was enriched in gills (45.31–46.43%), Bacteroidota in seawater (6.70–14.05%), and Thermodesulfobacteriota in sediments (0.31–0.91%). At the genus level, *norank_f_Spirochaetaceae*, *Marinococcus*, and *Woeseia* showed significant differences among groups ($p \leq 0.001$). Venn and PCoA analyses indicated closer similarity between gill and seawater communities than between gill and sediments, likely linked to oyster filter-feeding. This study clarifies the association between oyster gill bacteria and their environment, providing a basis for understanding microbial dynamics in oyster aquaculture.

Keywords: *Crassostrea sikamea*; 16S rRNA; bacterial community; culture environment; gills microbiota

1. Introduction

The association between microorganisms and their hosts represents a cornerstone of ecological research, underpinning the stability and functionality of ecosystems spanning terrestrial, freshwater, and marine biomes [1,2]. Marine invertebrates, including bivalves, generally lack sophisticated adaptive immune mechanisms [3]. Rather than existing in passive coexistence with microbes, they engage in highly dynamic, co-evolved interactions [4]. Host organisms provide microbial associates with sheltered niches and stable microenvironments, such as gill mucus [5]. They also supply nutrients like amino acids from epithelial secretions [6–8]. In return, microbial associates perform essential functions for their hosts, including modulating immune responses through the synthesis of antimicrobial peptides and producing essential nutrients like B vitamins, which bivalves cannot synthesize endogenously [8,9]. Additionally, microbial associates help maintain metabolic equilibrium by decomposing recalcitrant organic

compounds [10]. Aquatic organisms face more extreme and frequent environmental fluctuations than terrestrial counterparts. These fluctuations include diurnal temperature variations and tidal-induced salinity shifts [11,12]. Their associated microbial communities are governed by two sets of factors [13]. One set is host-specific factors, such as genetic predisposition and gill mucus composition [14–16]. The other set is external environmental conditions, including nutrient availability and hydrodynamic regimes [17]. This inherent plasticity allows aquatic microbiota to rapidly acclimatize to changing conditions. As a result, they serve as critical bioindicators of ecosystem health. They also play an indispensable role in the sustainability of aquaculture operations. In aquaculture, microbial dysbiosis often leads to disease outbreaks and economic losses.

The Kumamoto oyster (*Crassostrea sikamea*), an indigenous species endemic to the coastal waters of southern China (including Guangdong, Guangxi, and Hainan provinces), holds substantial ecological and aquacultural significance [18]. Economically, it is valued for its tender texture and savory flavor, emerging as a promising species in coastal aquaculture [19,20]. Ecologically, dense populations of Kumamoto oysters form complex reef structures. These reefs deliver vital ecosystem services. Individual oysters can filter 5 to 10 L of seawater per day [21]. This filtering action markedly improves water clarity by removing suspended particulates and phytoplankton. The calcium carbonate shells of these oysters create essential microhabitats. These habitats support numerous juvenile fish species, including the black sea bream (*Acanthopagrus schlegelii*) [22,23]. They also benefit crustaceans such as the mud crab (*Panopeus simpsoni*) [24]. Moreover, the physical architecture of oyster reefs attenuates wave energy. It can reduce wave energy by up to 40%. This helps mitigate coastal erosion [25–27]. Like other bivalves, the ecological performance of the Kumamoto oyster is closely tied to its associated bacterial communities. For example, Dai et al. (2022) showed that disruptions in the larval microbiota reduce metamorphosis success rates by up to 30%. This underscores the pivotal role of microorganisms in mediating oyster health and developmental fitness [28–31].

Despite growing academic and commercial interest, current research on the Kumamoto oyster has focused on several well-established domains. These domains include population genetics. Within population genetics, studies analyze mitochondrial DNA diversity across geographical isolates [32,33]. Another domain is reproductive biology. Here, techniques for tetraploid induction aim to augment growth. Larval development is also a key area. Research in this area emphasizes the impacts of temperature fluctuations on juvenile survival [34–36]. Recent investigations have begun to delineate the microbiota associated with this oyster species. For instance, Luis-Villasenor et al. (2018) described gut bacterial communities in wild populations from Mexico [37]. Dai et al. (2023) explored the larval microbiota under challenge with *Vibrio* infections [31]. However, most existing studies have concentrated on larval stages. Larval stages harbor distinct microbial assemblages due to divergent feeding strategies. Other studies focus on isolated organ systems like the gut [37]. Two critical gaps persist in existing research. First, studies focus on larval stages or isolated tissues such as the gut. Little attention is paid to the gill. The gill is a core organ that mediates oyster-environment interactions [38]. Second, no systematic comparison has been made between gill microbiota and the surrounding aquaculture environment. The environment includes seawater and sediment. This omission leaves environmental drivers of gill microbiota uncharacterized. The gill microbiota has been comparatively overlooked. This is despite its functional and applied importance. As a multifunctional organ, the gill is the primary interface between the oyster and its external milieu. It fulfills roles in gas exchange via thin-walled vasculature. It also captures food particles through ciliary-mucoid mechanisms for filter-feeding. Additionally, it secretes a pathogen-resistant mucous layer enriched with glycoproteins [29].

Functionally, disruptions to gill bacterial communities can directly impair filter-feeding efficiency. For example, altered mucus composition may affect particle adhesion. These disruptions can also increase susceptibility to pathogens like *Vibrio* [31]. From an applied perspective, Dai et al. (2023) linked gill microbiota dysbiosis to disease outbreaks in farmed oysters. This suggests that understanding gill-environment microbial relationships could inform management practices. One such practice is optimizing water exchange to maintain beneficial bacterial populations [31]. Consequently, the bacterial communities colonizing the gills are shaped by two influences. One is host-derived selective pressures, such as antimicrobial peptides embedded in the mucus [39]. The other is environmental inputs, including bacteria suspended in the surrounding seawater [40,41]. This makes the gill an exemplary model system for investigating tripartite host-microbe-environment interactions.

Furthermore, a systematic comparison between the gill microbiota of the Kumamoto oyster and bacterial communities in its immediate culture environment is lacking. The immediate environment includes seawater and sediment. This omission is particularly critical. Environmental bacteria serve as a principal inoculum for host-associated microbiota in filter-feeding bivalves. Fang et al. (2023) reported important findings for the Pacific oyster (*Crassostrea gigas*). Up to 40% of bacterial taxa in its gill microbiota could be traced to the ambient seawater. This pattern is chiefly ascribed to their filter-feeding activity [42]. Sediments harbor more diverse and stable microbial consortia than the water column. This is due to complex microhabitats generated by gradients. These gradients include oxygen tension, pH, and organic matter stratification [43]. Additionally, sediment

resuspension events can occur. These events are triggered by tidal action or aquaculture practices. They can liberate sediment-specific bacteria into the water column. These bacteria may subsequently invade or modulate the gill microbiota of resident bivalves [44]. A holistic comprehension of these associations is imperative. It is needed to elucidate how environmental perturbations affect the microbiota of the Kumamoto oyster. Eutrophication is one such perturbation. It is also necessary to unravel the mechanisms through which oysters regulate microbial communities within aquaculture settings.

To address these research gaps, this study employed Illumina MiSeq sequencing. The sequencing targeted the V3–V4 region of the 16S rRNA gene. It comprehensively characterized bacterial communities inhabiting Kumamoto oyster gills. It also characterized communities in adjacent seawater and sediment. Samples were collected from two aquaculture sites in Zhanjiang: Techeng Island (TC) and Longtousha (LTS). This study had three specific objectives. First, it aimed to compare bacterial richness and diversity across distinct habitats. Second, it sought to identify dominant taxonomic groups and their habitat-specific distributions. Third, it evaluated the degree of similarity between gill-associated and environmental microbiota. The insights from this study are expected to advance the field of Kumamoto oyster microbiology. They are also anticipated to yield actionable guidance for the development of sustainable aquaculture practices.

2. Methods

2.1. Sample Collection

Samples of Kumamoto Oyster and their culture environment were collected on December 15, 2024, from two sites in Zhanjiang, Guangdong, China: Techeng Island (21°8′56″ N, 110°26′34″ E, a semi-sheltered area with low sediment resuspension) and Longtousha (21°27′24″ N, 109°48′55″ E, a mildly exposed area with frequent tidal sediment resuspension). At each site, environmental parameters were measured in situ: water temperature (28.5 ± 0.5 °C, measured with a YSI ProPlus meter), salinity (20.2 ± 1.1 psu), and dissolved oxygen (6.8 ± 0.3 mg/L). Healthy wild Kumamoto Oyster were sampled: 18 individuals per site (shell weight 32.45 ± 5.16 g, shell height 5.49 ± 2.41 cm, 36 total) were randomly selected using a random number table, covering 6 separate areas (≥ 20 m apart) to ensure spatial representativeness. Oysters were collected from the intertidal mid-tide zone (1–2 m water depth), their natural depth. Gill tissues were processed under sterile conditions to minimize contamination: each oyster was rinsed with 0.22-μm filtered seawater to remove surface sediments, then shucked with sterile stainless-steel tools. Approximately 0.5 g of gill tissue (from the middle filament, to ensure consistency) was dissected, rinsed three times with sterile seawater to eliminate loosely attached planktonic bacteria, and stored in 2 mL sterile tubes with 1 mL RNAlater (Thermo Scientific) to preserve microbial structure. For seawater samples, 2 L of surface water (0.5–1 m depth, the oyster's primary feeding layer) was collected from 6 random locations per site using sterile Nalgene bottles. The water was prefiltered through a 50-μm nylon mesh to remove large plankton (e.g., copepods) and debris, then vacuum-filtered through a 0.22-μm polycarbonate membrane (Millipore) to concentrate microbial cells. Membranes were stored at −80 °C until DNA extraction. Sediment samples were collected from the top 5 cm of surface sediment (influenced by oyster fecal deposition and resuspension) using sterile acrylic cores (5 cm diameter). 6 cores were taken per site (separated by ≥ 10 m for spatial representativeness), homogenized, and subsampled (10 g per replicate) into 50 mL sterile tubes. All samples (gills: Techeng Island *C. sikamea* gills (TCCS), Longtousha *C. sikamea* gills (LTSCS); seawater: Techeng Island seawater (TCW); Longtousha seawater (LTSW); sediment: Techeng Island sediment (TCS), Longtousha sediment (LTSS); 6 replicates per group) were frozen in liquid nitrogen for 30 min to halt microbial activity, then stored at −80 °C.

2.2. DNA Extraction, PCR Amplification and Illumina Sequencing

Total genomic DNA was extracted from gill tissues, seawater membranes, and sediment samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). This kit was selected for its ability to isolate high-quality DNA from complex samples, including humic acid-rich sediments and protein-rich host tissues. Gill tissues were homogenized in lysis buffer with a sterile pestle. For sediments samples, an additional proteinase K digestion step was incorporated to enhance organic matter degradation. Specifically, 50 μL of proteinase K (20 mg/mL) was added, followed by incubation at 56 °C for 30 min.

DNA quality was verified via 1.0% agarose gel electrophoresis (Beyotime, Shanghai, China) (clear band at ~20 kb indicated integrity) and NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA; A260/A280 ratio 1.8–2.0 for purity). All samples were standardized to 10 ng/μL using sterile ddH_2O to ensure consistent PCR amplification.

The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified using universal primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), each modified with a unique 8-base barcode to distinguish samples. PCR reactions (20 μL) contained 4 μL 5 × Fast Pfu buffer (TransGen Biotech), 2 μL 2.5 mM dNTPs, 0.8 μL each primer (5 μM), 0.4 μL Fast Pfu polymerase, 10 ng template DNA, and ddH_2O. Cycling conditions were optimized for bivalve microbiota: initial denaturation at 95 °C for 3 min; 27 cycles of 95 °C (30 s), 55 °C (30 s, primer specificity), 72 °C (45 s); final extension at 72 °C for 10 min.

PCR products were visualized on a 1.5% agarose gel (single band at ~ 460 bp confirmed success), purified using the PCR Clean-Up Kit (YuDa Bio-Technology, Guangzhou, China), and quantified with a Qubit 4.0 fluorometer (Thermo Fisher Scientific). Purified amplicons were pooled in equimolar amounts (200 ng per sample) and paired-end sequenced (2 × 300 bp) on an Illumina MiSeq platform (Majorbio Bio-Pharm Technology, Shanghai, China) following standard protocols.

2.3. Bioinformatic and Statistical Analysis

Raw sequencing reads were processed using a standardized pipeline to ensure high-quality data: Quality control: fastp v0.19.6 [45] truncated reads with Q20 < 50 bp over a sliding window and discarded reads < 50 bp post-truncation; adapters and barcodes were trimmed. Read merging: FLASH v1.2.7 [46] merged paired-end reads into contigs (overlap > 10 bp, mismatch ratio ≤ 0.2). OTU clustering: UPARSE v7.1 [47] clustered reads into Operational Taxonomic Units (OTUs) at 97% sequence similarity; chimeras were removed via UCHIME against the Silva v138 database. Taxonomic annotation: RDP Classifier v2.2 assigned taxonomy to representative OTU sequences (Silva v138, confidence threshold 0.8) [48].

Samples were rarefied to 20,000 reads to standardize sequencing depth [48]. Alpha diversity indices (Chao1 for richness, Shannon for diversity) were calculated using Mothur v1.30.1 [49]. Beta diversity was visualized via Principal Coordinate Analysis (PCoA) based on Bray-Curtis distances. Statistical differences were tested via one-way ANOVA (Tukey's post-hoc test, $p < 0.05$) for alpha diversity and taxa abundance, Two-way ANOVA to test for site-habitat interactions on community composition, and Analysis of Similarities (ANOSIM, R = 0–1, $p < 0.05$) for community composition. All analyses were performed in R v4.2.2 using the "vegan" and "ggplot2" packages.

3. Results

3.1. Characteristics of 16S rDNA Gene Sequencing

After quality filtering and chimera removal, 2,050,613 valid sequences were obtained from 36 samples (mean 56,961 reads per sample; range 44,634–88,980) (Tables 1 and S1). All samples had Good's coverage ≥ 97% (mean 98.2 ± 1.1%), indicating > 99% of bacterial taxa were detected. Rarefaction curves reached a plateau for all groups (Supplementary Figure S1), confirming sufficient sequencing depth to capture core microbial diversity.

Table 1. Composition of bacterial communities in different groups.

Groups	Phylum	Class	Order	Family	Genus	Species
TCCS	45	110	245	414	763	1139
TCW	55	121	287	494	913	1596
TCS	70	168	378	570	947	1715
LTSCS	49	113	260	434	800	1172
LTSW	54	114	261	450	850	1467
LTSS	63	157	362	572	971	1787
Total	75	194	466	796	1617	3163

At 97% sequence similarity, 56,589 unique OTUs were clustered across all samples. Venn analysis revealed 179 shared OTUs among the six groups (TCCS, TCW, TCS, LTSCS, LTSW, LTSS), representing the core microbiota of the Kumamoto oyster culture system. Unique OTUs were most abundant in sediment (TCS: 1333; LTSS: 1227), followed by seawater (TCW: 543; LTSW: 489), and least abundant in gills (TCCS: 189; LTSCS: 212) (Supplementary Figure S2).

3.2. Alpha and Beta Diversity of Bacterial Communities

Alpha diversity indices differed significantly by habitat ($p < 0.001$, ANOVA) (Supplementary Table S2, Figure 1): Sediment: Highest richness (Chao1: 3284.9 ± 166.12 in TCS; 3497.4 ± 59.12 in LTSS) and diversity (Shannon: 6.52 ± 0.08 in TCS; 6.40 ± 0.05 in LTSS). Post-hoc tests confirmed sediment had significantly higher

Chao1 and Shannon indices than seawater ($p < 0.001$) and gills ($p < 0.001$). No significant inter-site differences in were observed ($p > 0.05$). Seawater: Intermediate richness (Chao1: 1970.30 ± 92.53 in TCW; 1787.40 ± 199.73 in LTSW) and diversity (Shannon: 4.58 ± 0.33 in TCW; 4.15 ± 0.54 in LTSW). Significant inter-site differences in Shannon index were detected ($p < 0.05$). Gills: Lowest diversity (Shannon: 2.64 ± 1.12 in TCCS; 2.99 ± 0.87 in LTSCS) and richness (Chao1: 428.93 ± 306.54 in TCCS; 751.65 ± 218.18 in LTSCS). No significant differences in gill bacterial diversity were observed between sites ($p > 0.05$), indicating host factors override site effects.

Beta diversity analysis confirmed habitat-driven community separation. Hierarchical clustering and principal coordinate analysis (PCoA) based on Bray-Curtis distances showed clear separation of microbial communities by habitat (gills, seawater, sediment), with samples from the same habitat clustering closely (Figure 2). PCoA explained 35.04% (PC1) and 36.61% (PC2) of total variation (Figure 2B), revealing stronger similarity between gills and seawater communities than between gills and sediments. Statistical analysis confirmed significant differences in community composition across habitats (ANOSIM: R = 0.9259, $p < 0.01$).

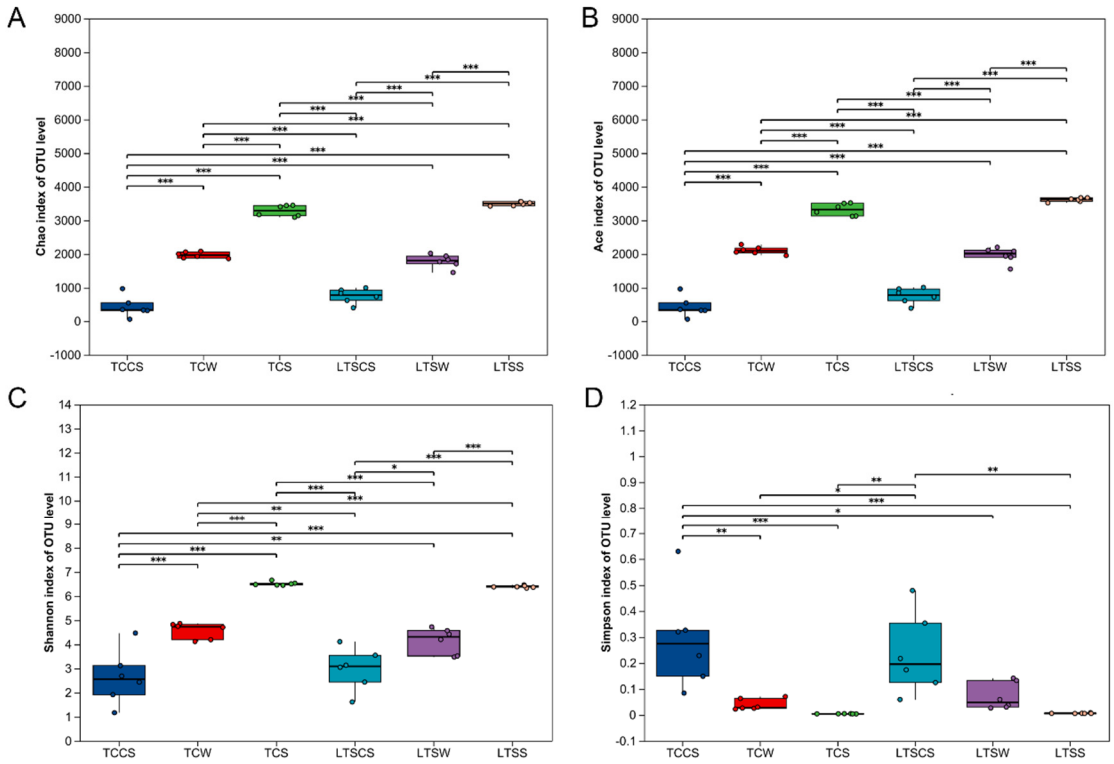


Figure 1. Alpha diversity indices of bacterial communities across habitats. (**A**) Chao index; (**B**) Ace index; (**C**) Shannon index; (**D**) Simpson index. Data are mean ± SD (n = 6). Asterisks indicate significant differences: * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, and *** $p \leq 0.001$.

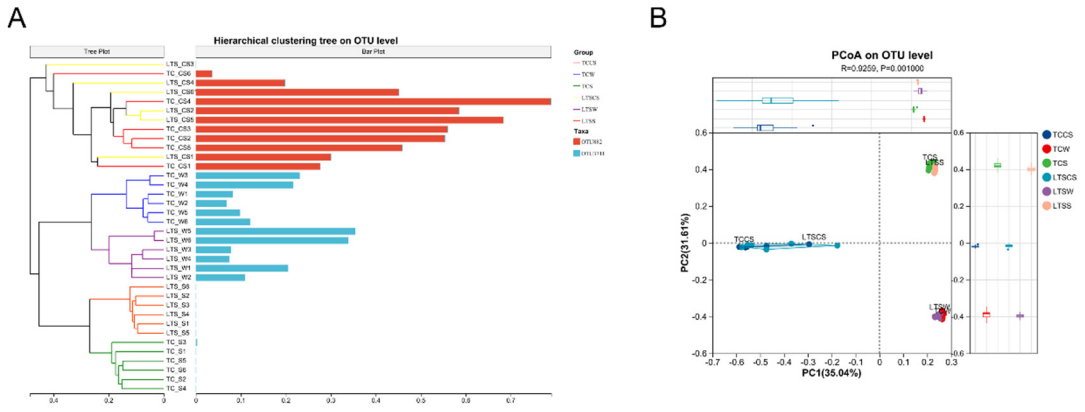


Figure 2. Beta diversity of bacterial communities. (**A**) Hierarchical clustering tree; (**B**) Principal co-ordinates analysis (PCoA, Bray-Curtis distances). TCCS (TC gills), TCW (TC seawater), TCS (TC sediment), LTSCS (LTS gills), LTSW (LTS seawater), LTSS (LTS sediment).

3.3. Taxonomic Composition of Bacterial Communities

At the phylum level, 75 bacterial phyla were detected, with 6 dominant phyla (relative abundance ≥ 5% in at least one group) accounting for 85.6–92.3% of total abundance (Figure 3A): Pseudomonadota (23.23–49.32%), Spirochaetota (0.004–46.43%), Bacillota (0.68–23.34%), Bacteroidota (2.94–14.05%), Actinomycetota (0.77–21.07%), and Cyanobacteriota (1.74–21.72%). Clear habitat-specific patterns emerged (Figure 4A): Gills were enriched in Spirochaetota (TCCS: 45.31 ± 26.33%; LTSCS: 46.43 ± 28.46%; $p < 0.001$ vs. seawater/sediment), followed by Pseudomonadota (28.56–32.14%); Seawater had higher abundances of Pseudomonadota (TCW: 49.32 ± 3.65%; LTSW: 42.18 ± 4.01%), Bacillota (LTSW: 23.34 ± 4.66%; TCW: 18.32 ± 2.15%), and Actinomycetota (LTSW: 21.07 ± 4.66%; TCW: 10.03 ± 0.99%); Sediments were dominated by Thermodesulfobacteriota (TCS: 0.91 ± 0.18%; LTSS: 0.31 ± 0.11%; $p < 0.05$ vs. gills/seawater). Moreover, distinct differences were observed in microbial composition among groups (Figure 5A).

At the genus level, 1617 genera were identified, with 10 dominant genera (relative abundance ≥ 1%) showing significant intergroup differences ($p < 0.001$) (Figure 3B). These genera were strongly habitat-specific (Figure 5B): *norank_f_Spirochaetaceae* (14.09–15.23%) and *Spirochaeta* (8.76–9.12%) were dominant in gills; *Marinococcus* (4.87–5.49%) and *Halomonas* (3.21–3.65%) were most abundant in seawater. *Woeseia* (2.18–2.43%) and *Geitlerinema_PCC-7105* (1.87%) were prevalent in sediment.

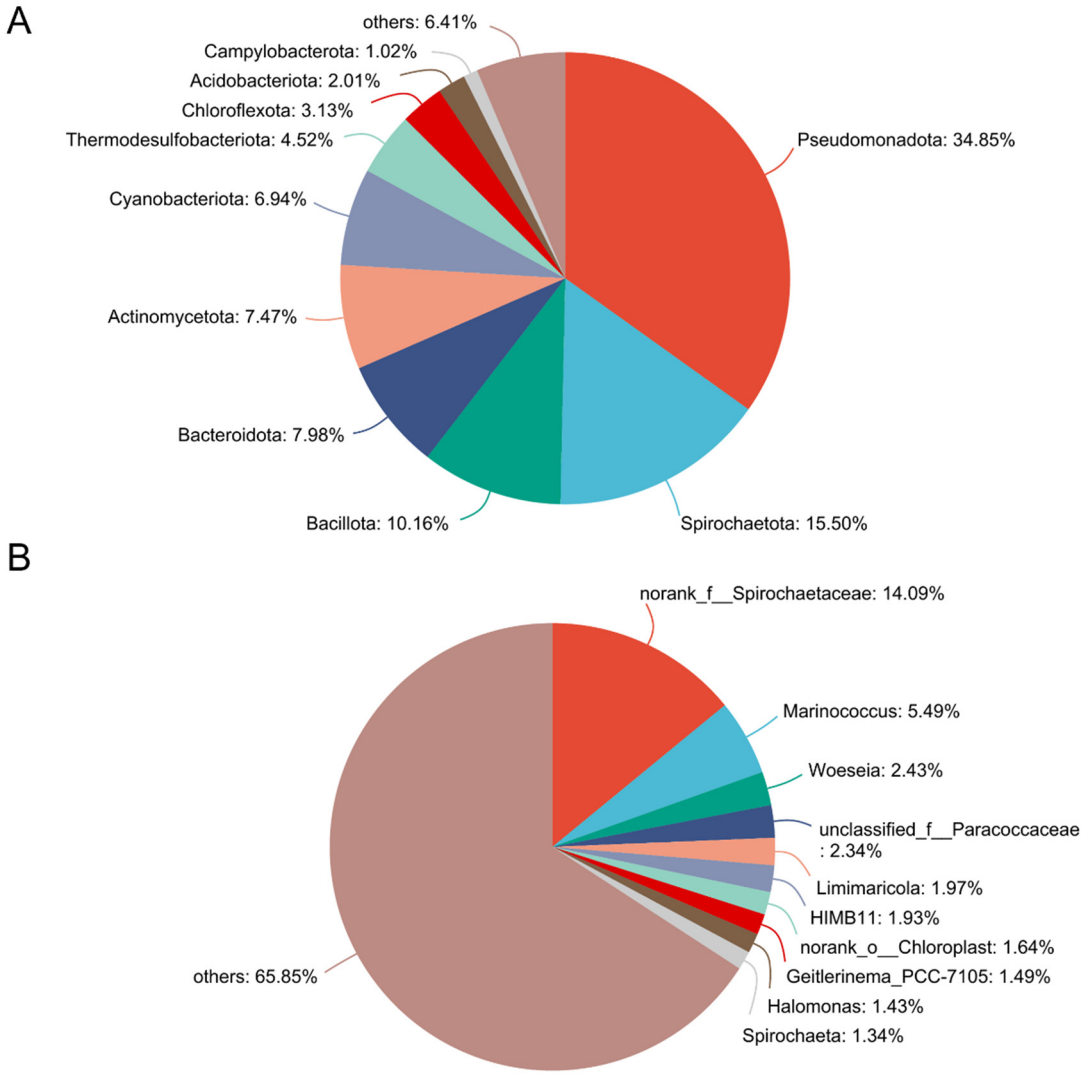


Figure 3. Dominant bacterial taxa across all samples. (**A**) Phylum level (relative abundance ≥ 1%); (**B**) Genus level (relative abundance ≥ 1%). "Others" include taxa with relative abundance < 1%.

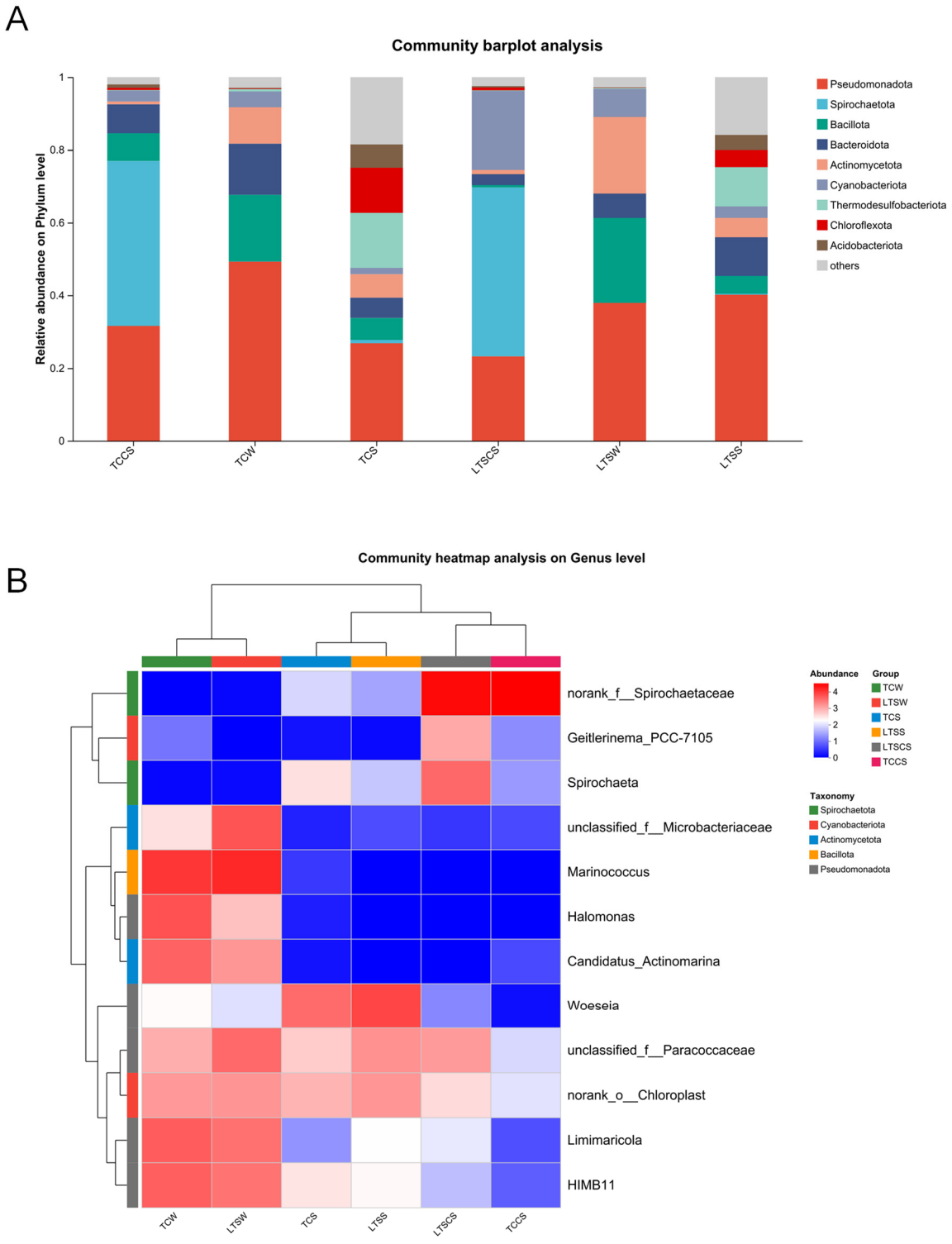


Figure 4. Habitat-specific distribution of dominant taxa. (**A**) Bar plot of phylum-level relative abundance; (**B**) Heatmap of genus-level relative abundance (top 10 dominant genera).

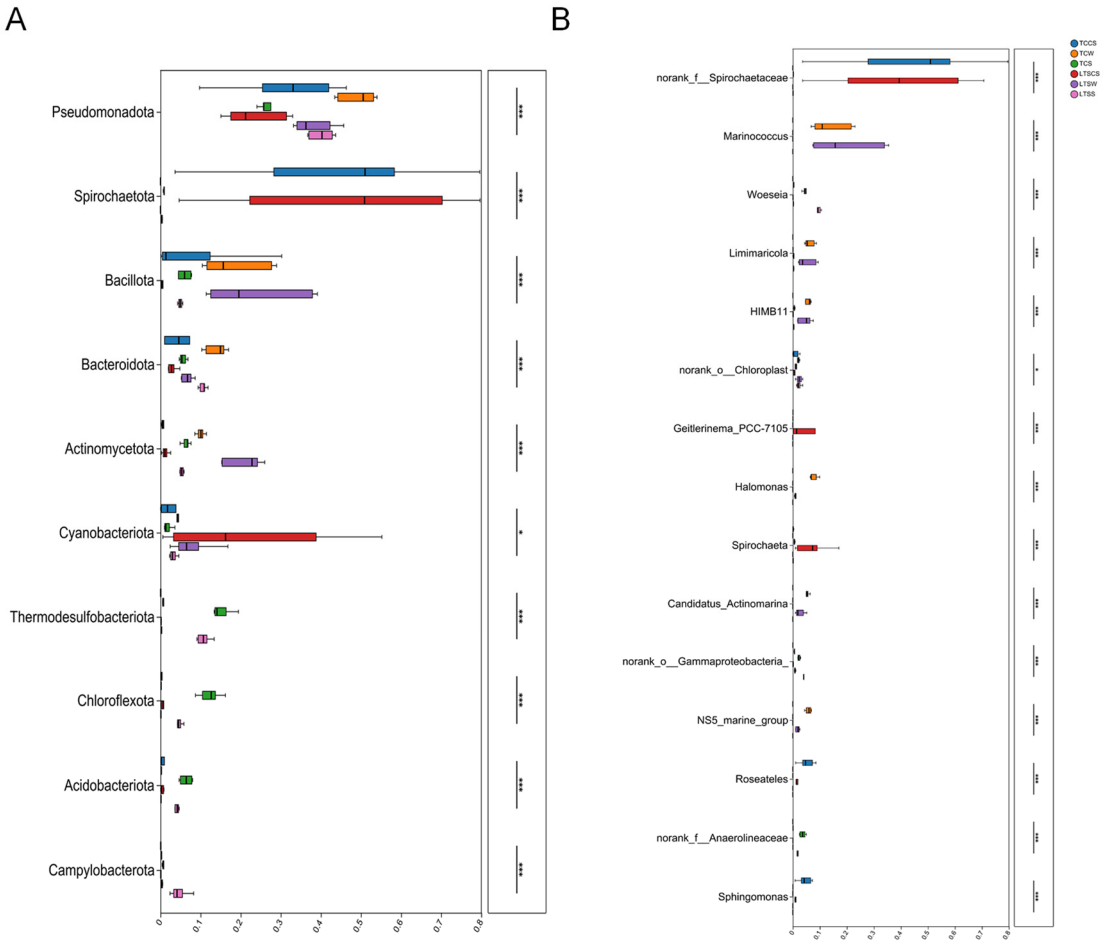


Figure 5. Intergroup comparisons of dominant taxa. (**A**) Phylum level; (**B**) Genus level. Asterisks indicate significant differences: * $0.01 < p \leq 0.05$, $0.001 < p \leq 0.01$, and *** $p \leq 0.001$.

3.4. Comparison of Microbial Community

Comparisons between sites and habitats revealed: Gills vs. seawater: Spirochaetota was 100-fold more abundant in gills ($p < 0.001$), while Pseudomonadota was more abundant in seawater ($p < 0.01$); Seawater vs. sediment: Bacillota (seawater: 18.32–23.34%) and Actinomycetota (seawater: 10.03–21.07%) were more abundant in seawater, whereas Thermodesulfobacteriota and Chloroflexota were enriched in sediment ($p < 0.05$); Site differences: LTSW had higher Actinomycetota (21.07 ± 4.66%) than TCW (10.03 ± 0.99%; $p < 0.01$), while TCS had higher Thermodesulfobacteriota than LTSS ($p < 0.05$) (Figures 6 and 7).

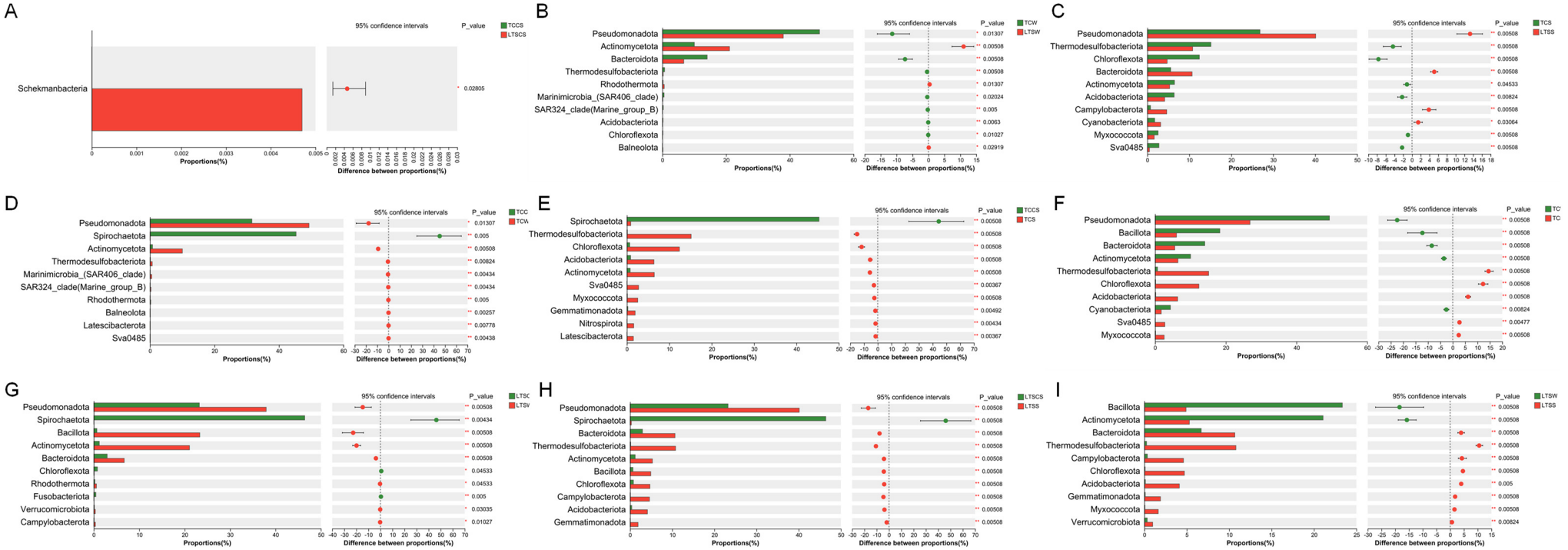


Figure 6. Inter-habitat and inter-site comparisons of bacterial communities (phylum level). (**A**) TCCS vs. LTSCS; (**B**) TCW vs. LTSW; (**C**) TCS vs. LTSS; (**D**) TCCS vs. TCW; (**E**) TCCS vs. LCS; (**F**) TCW vs. TCS; (**G**) LTSCS vs. LTSW; (**H**) LTSCS vs. LTSS; (**I**) LTSW vs. LTSS. Asterisks indicate significant differences: * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, and $p \leq 0.001$.

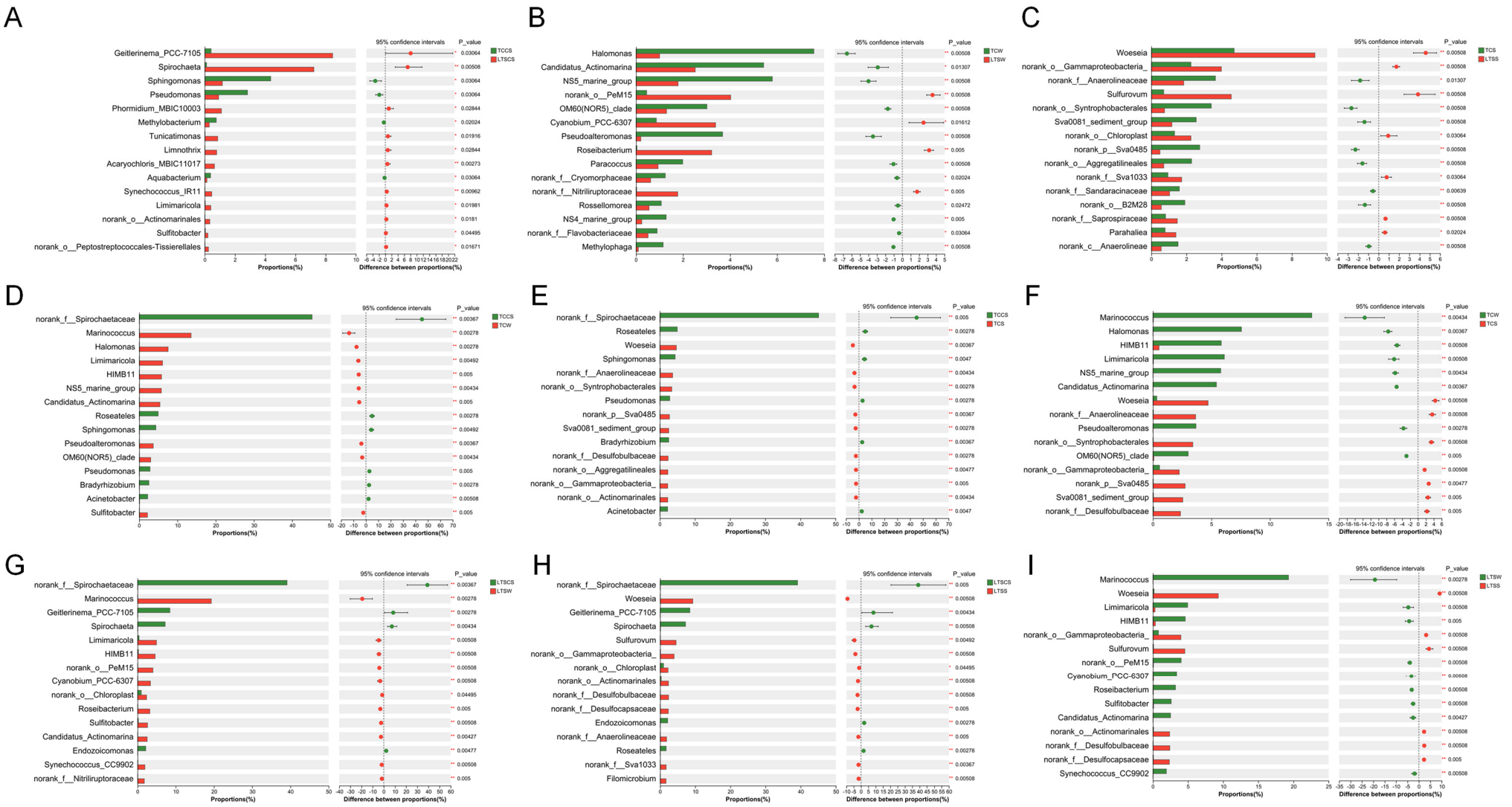


Figure 7. Inter-habitat and inter-site comparisons of bacterial communities (genus level). (**A**) TCCS vs. LTSCS; (**B**) TCW vs. LTSW; (**C**) TCS vs. LTSS; (**D**) TCCS vs. TCW; (**E**) TCCS vs. LCS; (**F**) TCW vs. TCS; (**G**) LTSCS vs. LTSW; (**H**) LTSCS vs. LTSS; (**I**) LTSW vs. LTSS. Asterisks indicate significant differences: * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, and $p \leq 0.001$.

4. Discussion

This study provides a comprehensive characterization of bacterial communities. It focuses on communities associated with the gills of the Kumamoto oyster. It also delineates their relationships with microbiota in surrounding seawater and sediment. Our results demonstrate clear, habitat-specific microbial distributions. These distributions are influenced by two sets of factors. One set is host biological traits, such as filter-feeding behavior. The other set is environmental ecological properties. These findings extend the current understanding of microbiota-environment interactions in marine invertebrates [42,50]. They also offer mechanistic insights into the assembly and dynamics of microbial communities within oyster aquaculture systems.

The observed diversity gradient is a key finding. Sediment samples harbored the highest microbial richness and diversity. Seawater samples followed. Gill tissues had the lowest values. The Shannon indices were 6.40–6.52 for sediments, 4.15–4.58 for seawater, and 2.64–2.99 for gills. This gradient aligns with established ecological principles [42,51]. Sediments act as microbial hotspots. This is due to their pronounced micro-environmental heterogeneity. Vertical gradients in oxygen concentration, pH, and organic matter availability create numerous ecological niches. These niches support a wide array of specialized taxa. For instance, aerobic *Actinomycetota* tends to dominate surface sediment layers. Anaerobic *Thermodesulfobacteriota* prevails in deeper, anoxic zones [52]. The physical stability of sedimentary environments supports slower-growing, specialist bacteria. *Woeseia* is an example of such a bacterium. In contrast, the water column's dynamic nature favors fast-growing generalist bacteria. *Marinococcus* is an example here [44]. This finding is consistent with observations in other marine invertebrates. It has been seen in polychaetes [52] and sea cucumbers (*Apostichopus japonicus*) [44]. This underscores the generality of this ecological phenomenon. The reduced diversity in gill tissues is likely due to host-mediated selective filtering. The gill mucus layer is a key factor here. It is composed of glycoproteins like MUC5AC, lectins, and antimicrobial peptides [29,53]. This layer fosters the establishment of symbiotic bacteria. Spirochaetota is one such group. It metabolizes mucus-derived polysaccharides [54]. At the same time, the layer inhibits colonization by potential pathogens. Notably, gill bacterial diversity did not differ significantly between the two geographic sites ($p > 0.05$). This is despite measurable variations in the seawater microbial communities at TC and LTS. This consistency has been previously reported in other oyster species. It was seen in the Pacific oyster [42] and the pearl oyster (*Pinctada fucata martensii*) [55]. This emphasizes host physiology as a primary determinant of gill microbiota assembly in bivalves. This host-dominant pattern has important implications. It indicates that host physiology exerts stronger filtering effects than environmental variation. For example, antimicrobial peptides in gill mucus and selective filter-feeding play key roles. This does not mean environmental effects are negligible. Oyster physiology ensures gill microbiota primarily originates from seawater, not sediment. Oysters filter 5–10 L of seawater per day [21]. The mucus layer then screens and retains beneficial taxa like *Spirochaetota*. This forms a balance between environmental input and host selection. The closer phylogenetic similarity between gill and seawater communities is another key result. PCoA revealed this similarity. It is directly linked to the filter-feeding ecology of the Kumamoto oyster [21]. During this process, water-column bacteria like *Marinococcus* spp. adhere to the gill mucus. This leads to considerable overlap between planktonic and gill-associated microbial communities [42]. In contrast, sediment-derived microbial communities are predominantly composed of obligate anaerobes. *Thermodesulfobacteriota* is an example. These taxa are unable to survive under the oxygen levels present in gill tissues or seawater. Their strict anaerobic nature explains the significant clustering observed between sediment and other sample types, as confirmed by ANOSIM analysis ($R = 0.96$, $p < 0.01$).

The dominance of Pseudomonadota across all habitats highlights its metabolic versatility. Its relative abundance ranges from 23.23% to 49.32%. This phylum encompasses taxa adapted to a wide range of niches. In seawater, Alphaproteobacteria and Gammaproteobacteria decompose phytoplankton exudates. This helps maintain nutrient balance [56]. In gills, symbiotic Gammaproteobacteria like *Halomonas* produce siderophores. These siderophores facilitate host iron uptake [55]. In sediments, Deltaproteobacteria mediate sulfate reduction. This recycles sulfur back into the food web [9]. This broad distribution confirms Pseudomonadota as a keystone phylum. It connects pelagic, benthic, and host-associated nutrient cycles. The high abundance of Spirochaetota in gill tissues carries important functional implications. Its relative abundance ranges from 45.31% to 46.43%. Marine invertebrate-associated Spirochaetota possess genetic machinery for chitinase and cellulase production [50,57]. These enzymes facilitate the degradation of phytoplankton exoskeletons. Phytoplankton is a major component of the oyster diet. The enzymes also break down refractory organic matter. Within Kumamoto oyster gills, these bacteria likely convert captured food particles. They turn them into absorbable short-chain fatty acids like acetate [58,59]. This enhances the host's digestive efficiency. This function is especially crucial in turbid coastal environments. In these environments, elevated sedimentary organic matter increases the proportion of recalcitrant

dietary substrates. Our results align with those of van de Water et al. (2016). They found that Spirochaetota accounted for 40% of the microbial community in red coral mucus. These bacteria had chitinase activity. This activity decomposed refractory organic matter. This is consistent with our hypothesis that gill Spirochaetota aids food decomposition [57]. This enhances gill-related digestive efficiency. The near-absence of Spirochaetota in seawater and sediment (<0.1%) further confirms its strong adaptation to a host-associated lifestyle. The high abundance of Bacillota in seawater has practical implications for aquaculture health management. Its relative abundance ranges from 18.32% to 23.34%. This phylum includes genera like *Bacillus* and *Marinococcus*. These genera are known producers of bacteriocins. Bacteriocins are antimicrobial compounds that can effectively suppress *Vibrio* growth [60]. The absence of detectable *Vibrio* in LTSW samples is notable. LTSW contained 23.34 ± 4.66% Bacillota. This suggests a natural mechanism of pathogen suppression. Balcázar & Rojas-Luna (2007) provided supporting evidence [60]. They demonstrated that *Bacillus subtilis* isolated from shrimp aquaculture reduced *Vibrio* counts by 70%. This reduction was via bacteriocin production. This supports our observation that LTSW, with high Bacillota abundance, had no detectable *Vibrio*. The higher abundance of Bacillota at the LTS site may be linked to water exchange. LTS has stronger water exchange. This imports oligotrophic members of this phylum from adjacent coastal waters.

The prevalence of Thermodesulfobacteriota in sediments reflects its role in iron and sulfur cycling [9]. Its relative abundance ranges from 0.31% to 0.91%. These chemolithoautotrophic bacteria oxidize toxic hydrogen sulfide. Hydrogen sulfide is a byproduct of organic matter decomposition. The bacteria convert it into harmless sulfate. This prevents the accumulation of sulfide, which is detrimental to oyster health. The significantly higher relative abundance of Thermodesulfobacteriota in TCS is noteworthy. It was 0.91 ± 0.18%, compared to LTSS. This correlates with the higher sediment organic matter content at TC. TC had 2.1%, while LTS had 1.2%. Enhanced organic matter decomposition promotes hydrogen sulfide production. This creates favorable conditions for these sulfate-reducing bacteria. Their absence in both gill and seawater samples further confirms their strict anaerobic specialization [40,52].

The closer similarity between gill and seawater communities is consistent with oyster filter-feeding ecology. PCoA plots visualized this similarity. During feeding, the Kumamoto oyster filters plankton and organic particles from the water column. This facilitates the colonization of gill surfaces by seawater bacteria. It results in overlapping community structures [42]. In contrast, sediment-associated microbial communities are distinct. They are shaped largely by benthic processes. These processes include anaerobic metabolism and mineral cycling. They are less directly influenced by oyster physiology [61].

5. Conclusions

This study characterized bacterial communities in the Kumamoto oyster gills and their surrounding seawater/sediment from two Zhanjiang aquaculture sites, with key findings: bacterial richness and diversity followed a habitat-specific pattern (sediments > seawater > gills), shaped by environmental heterogeneity and host selective filtering; gill bacterial communities were more similar to seawater (linked to oyster filter-feeding) than to sediments; dominant taxa showed habitat specialization. The study supplements knowledge of the Kumamoto oyster gills microbiota, offers practical aquaculture guidance, and clarifies environmental drivers of gill microbiota, laying a foundation for marine bivalve microbiology research and sustainable aquaculture.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/2509191540538454/ALE-25080332-Supplementary-Materials.pdf.

Author Contributions

S.F.: conceptualization, methodology, writing—original draft preparation; T.Y.: software, data curation; J.L.: visualization, investigation; J.P.: supervision; L.Y.: writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Funding

This project was funded by the Central Public-interest Scientific Institution Basal Research Fund, South China Sea Fisheries Research Institute, CAFS (NO. 2023TS03, 2023TD44 and 2021SD05),Key Laboratory of South China Sea Fishery Resources Exploitation & Utilization, Ministry of Agriculture and Rural Affairs, P. R. China (FREU2024-06) and the earmarked fund for CARS (CARS-49).

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the South China Sea Fisheries Research Institute (protocol code: NHDF2025-31 and Date of Approval: 22 October 2024).

Data Availability Statement

The data presented in the study are deposited in the NCBI Sequence Read Archive repository, accession number PRJNA1242002.

Conflicts of Interest

Authors have no conflicts of interest.

Use of AI and AI-assisted Technologies

No AI tools were utilized for this paper.

References

1. Lokmer, A.; Kuenzel, S.; Baines, J.F.; et al. The role of tissue-specific microbiota in initial establishment success of Pacific oysters. *Environ. Microbiol.* **2016**, *18*, 970–987.
2. Ghosh, A.; Rathore, A.; Gaba, S.; et al. The Chinese mitten crab (*Eriocheir sinensis*) and its microbiome: A review. *Aquaculture* **2025**, *595*, 741518.
3. Guryanova, S.V.; Ovchinnikova, T.V. Innate immunity mechanisms in marine multicellular organisms. *Mar. Drugs* **2022**, *20*, 549.
4. O'Brien, P.A.; Webster, N.S.; Miller, D.J.; et al. Host-microbe coevolution: Applying evidence from model systems to complex marine invertebrate holobionts. *mBio* **2019**, *10*. https://doi.org/10.1128/mbio.02241-18.
5. Yu, J.; Kang, M.; Park, M.; et al. Microbial community structure and functional characteristics across the mucosal surfaces of olive flounder (*Paralichthys olivaceus*). *Front. Microbiol.* **2025**, *16*, 1587288.
6. Deka, D.; Sonowal, S.; Chikkaputtaiah, C.; et al. Symbiotic associations: Key factors that determine physiology and lipid accumulation in oleaginous microorganisms. *Front. Microbiol.* **2020**, *11*, 555312.
7. Men, Y.; Yang, Z.; Luo, J.; et al. Symbiotic microorganisms and their different association types in aquatic and semiaquatic bugs. *Microbiol. Spectr.* **2022**, *10*, e02794-22.
8. Sharma, S.; Chaubey, K.K.; Singh, S.V.; et al. Symbiotic microbiota: A class of potent immunomodulators. *Scienceasia* **2022**, *48*, 855–865.
9. Shulga, N.; Abramov, S.; Klyukina, A.; et al. Fast-growing Arctic Fe-Mn deposits from the Kara Sea as the refuges for cosmopolitan marine microorganisms. *Sci. Rep.* **2022**, *12*, 21967.
10. Langdon, C.J.; Newell, R.I.E. Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. *Mar. Ecol. Prog. Ser.* **1990**, *58*, 299–310.
11. Bal, A.; Panda, F.; Pati, S.G.; et al. Modulation of physiological oxidative stress and antioxidant status by abiotic factors especially salinity in aquatic organisms. *Comp. Biochem. Phys. C* **2021**, *241*, 108971.
12. Wall, C.B.; Perreault, M.G.; Demmel, M.Y.; et al. Geography and host identity shape intraseasonal variation of free-living and zooplankton associated microbial communities in alpine lakes. *Mol. Ecol.* **2025**, e70069. https://doi.org/10.1111/mec.70069.
13. Adair, K.L.; Douglas, A.E. Making a microbiome: The many determinants of host-associated microbial community composition. *Curr. Opin. Microbiol.* **2017**, *35*, 23–29.
14. Slinger, J.; Adams, M.B.; Wynne, J.W. Comparison of bacterial diversity and distribution on the gills of Atlantic salmon (*Salmo salar* L.): An evaluation of sampling techniques. *J. Appl. Microbiol.* **2021**, *131*, 80–92.
15. Amill, F.; Gauthier, J.; Rautio, M.; et al. Characterization of gill bacterial microbiota in wild Arctic char (*Salvelinus alpinus*) across lakes, rivers, and bays in the Canadian Arctic ecosystems. *Microbiol. Spectr.* **2024**, *12*, e02943-23.
16. Li, J.; Wang, J.; Wei, H. Strengthening the functional research on the interaction between host genes and microbiota. *Sci. China Life Sci.* **2020**, *63*, 929–932.
17. Zeglin, L.H. Stream microbial diversity in response to environmental changes: Review and synthesis of existing research. *Front. Microbiol.* **2015**, *6*, 454.
18. Qin, Y.; Noor, Z.; Li, X.; et al. Tetraploid induction of *Crassostrea hongkongensis* and *C. sikamea* by inhibiting the polar body 1 release in diploid fertilized eggs. *Mar. Life Sci. Tech.* **2021**, *3*, 463–473.
19. Wang, C.; Li, Q.; Kong, L.; et al. Establishment of single oyster (*Crassostrea sikamea*) seeds. *J. Ocean U. China* **2016**, *46*, 136–142+186.

20. Wang, T.; Li, Q. Effects of salinity and temperature on growth and survival of juvenile of Kumamoto oyster (*Crassostrea sikamea*). *Oceanol. Limnol. Sin.* **2017**, *48*, 297–302.

21. Sekino, M.; Sato, S.; Hong, J.-S.; et al. Contrasting pattern of mitochondrial population diversity between an estuarine bivalve, the Kumamoto oyster *Crassostrea sikamea*, and the closely related Pacific oyster *C. gigas*. *Mar. Biol.* **2012**, *159*, 2757–2776.
22. Tsuyuki, A.; Umino, T. Spatial movement of black sea bream *Acanthopagrus schlegelii* around the oyster farming area in Hiroshima Bay, Japan. *Fish. Sci.* **2017**, *83*, 235–244.
23. Kawai, K.; Fujita, H.; Sanchez, G.; et al. Oyster farms are the main spawning grounds of the black sea bream *Acanthopagrus schlegelii* in Hiroshima Bay, Japan. *PeerJ* **2021**, *9*, e11475.
24. Laroche, R.A.S.; Doan, T.M.; Hanke, M.H. Habitat characteristics of artificial oyster reefs influence female oystershell mud crab *Panopeus simpsoni* Rathbun, 1930 (Decapoda: Brachyura: Panopeidae). *J. Crustac. Biol.* **2022**, *42*, ruac033.
25. Quan, W.; Feng, M.; Zhou, Z.; et al. Ecological assessment of the oyster *Crassostrea sikamea* population and associated benthic communities on restored oyster reefs along Jiangsu Province coast, China. *Acta Ecol. Sin.* **2017**, *37*, 1709–1718.
26. Cressman, K.A.; Posey, M.H.; Mallin, M.A.; et al. Effects of oyster reefs on water quality in a tidal creek estuary. *J. Shellfish Res.* **2003**, *22*, 753–762.
27. Piazza, B.P.; Banks, P.D.; La Peyre, M.K. The potential for created oyster shell reefs as a sustainable shoreline protection strategy in Louisiana. *Restor. Ecol.* **2005**, *13*, 499–506.
28. Kong, W.; Cheng, G.; Cao, J.; et al. Ocular mucosal homeostasis of teleost fish provides insight into the coevolution between microbiome and mucosal immunity. *Microbiome* **2024**, *12*, 10.
29. Destoumieux-Garzon, D.; Montagnani, C.; Dantan, L.; et al. Cross-talk and mutual shaping between the immune system and the microbiota during an oyster's life. *Philos. T. R. Soc. B* **2024**, *379*, 20230065.
30. Dai, W.; Ye, J.; Liu, S.; et al. Bacterial community dynamics in kumamoto oyster *Crassostrea sikamea* hatchery during larval development. *Front. Microbiol.* **2022**, *13*, 933941.
31. Dai, W.; Ye, J.; Xue, Q.; et al. Changes in bacterial communities of kumamoto oyster larvae during their early development and following *Vibrio* infection resulting in a mass mortality event. *Mar. Biotechnol.* **2023**, *25*, 30–44.
32. Liu, S.; Xue, Q.; Xu, H.; et al. Identification of main oyster species and comparison of their genetic diversity in Zhejiang coast, south of Yangtze river estuary. *Front. Mar. Sci.* **2021**, *8*, 662515.
33. Xuan, Y.; Chang, G.; Liu, S.; et al. A comparison of the mitochondrial DNA-based genetic diversity of Kumamoto oyster populations from China, Japan, and the United States. *Mar. Sci.* **2024**, *48*, 24–33.
34. Wu, X.; Zhang, Y.; Xiao, S.; et al. Comparative studies of the growth, survival, and reproduction of diploid and triploid Kumamoto oyster, *Crassostrea sikamea*. *J. World Aquacult. Soc.* **2019**, *50*, 866–877.
35. Ma, H.; Lv, W.; Qin, Y.; et al. Aquaculture potential of two Kumamoto oyster (*Crassostrea sikamea*) populations and their reciprocal hybrids in southern China. *Aquaculture* **2022**, *546*, 737301.
36. Zhang, Y.; Qin, Y.; Ma, L.; et al. Gametogenesis from the early history life stages of the Kumamoto oyster *Crassostrea sikamea* and their breeding potential evaluation. *Front. Physiol.* **2019**, *10*, 524.
37. Luis-Villasenor, I.E.; Zamudio-Armenta, O.O.; Voltolina, D.; et al. Bacterial communities of the oysters *Crassostrea corteziensis* and *C. sikamea* of cospita bay, Sinaloa, Mexico. *Rev. Int. Contam. Ambie.* **2018**, *34*, 203–213.
38. Zhang, E.; Li, Z.; Dong, L.; et al. Exploration of molecular mechanisms of immunity in the Pacific oyster (*Crassostrea gigas*) in response to *Vibrio alginolyticus* invasion. *Animals* **2024**, *14*, 1707.
39. Munoz, K.; Flores-Herrera, P.; Goncalves, A.T.; et al. The immune response of the scallop *Argopecten purpuratus* is associated with changes in the host microbiota structure and diversity. *Fish Shellfish Immunol.* **2019**, *91*, 241–250.
40. Zardinoni, G.; Deb, S.; Ravi, S.; et al. Difference in composition and functional analysis of bacterial communities between *Mytilus galloprovincialis* gills and surrounding water in a brackish inshore bay, analyzed by 16S rDNA multi-amplicon sequencing. *Ann. Microbiol.* **2024**, *74*, 3.
41. Varela, J.L.; Nikouli, E.; Medina, A.; et al. The gills and skin microbiota of five pelagic fish species from the Atlantic Ocean. *Int. Microbiol.* **2025**, *28*, 95–105.
42. Fang, G.; Yu, H.; Zhang, Y.; et al. Diversities and shifts of microbial communities associated with farmed oysters (*Crassostrea gigas*) and their surrounding environments in Laoshan Bay marine ranching, China. *Microorganisms* **2023**, *11*, 1167.
43. An, L.; Yan, Y.-C.; Tian, H.-L.; et al. Roles of sulfate-reducing bacteria in sustaining the diversity and stability of marine bacterial community. *Front. Microbiol.* **2023**, *14*, 1218828.
44. Zhao, Z.; Jiang, J.; Pan, Y.; et al. Temporal dynamics of bacterial communities in the water and sediments of sea cucumber (*Apostichopus japonicus*) culture ponds. *Aquaculture* **2020**, *528*, 735498.
45. Chen, S.; Zhou, Y.; Chen, Y.; et al. fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **2018**, *34*, 884–890.
46. Magoc, T.; Salzberg, S.L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27*, 2957–2963.
47. Edgar, R.C. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **2013**, *10*, 996.
48. Wang, Q.; Garrity, G.M.; Tiedje, J.M.; et al. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microb.* **2007**, *73*, 5261–5267.

49. Douglas, G.M.; Maffei, V.J.; Zaneveld, J.R.; et al. PICRUSt2 for prediction of metagenome functions. *Nat. Biotechnol.* **2020**, *38*, 685–688.
50. Lin, Y.-T.; Kiel, S.; Xu, T.; et al. Phylogenetic placement, morphology and gill-associated bacteria of a new genus and species of deep-sea mussel (Mytilidae: Bathymodiolinae) from the South China Sea. *Deep-Sea Res. Pt. I* **2022**, *190*, 103894.
51. Kunselman, E.; Minich, J.J.; Horwith, M.; et al. Variation in survival and gut microbiome composition of hatchery-grown native oysters at various locations within the puget sound. *Microbiol. Spectr.* **2022**, *10*, e01982-21.
52. Dale, H.; Solan, M.; Lam, P.; et al. Sediment microbial assemblage structure is modified by marine polychaete gut passage. *FEMS Microbiol. Ecol.* **2019**, *95*, fiz047.
53. Benhamed, S.; Guardiola, F.A.; Mars, M.; et al. Pathogen bacteria adhesion to skin mucus of fishes. *Vet. Microbiol.* **2014**, *171*, 1–12.
54. Zhou, S.; Luo, R.; Gong, G.; et al. Characterization of metagenome-assembled genomes and carbohydrate-degrading genes in the gut microbiota of Tibetan pig. *Front. Microbiol.* **2020**, *11*, 595066.
55. Zheng, Z.; Liao, Y.; Ye, J.; et al. Microbiota diversity in Pearl Oyster *Pinctada fucata martensii* intestine and its aquaculture environment. *Front. Mar. Sci*. **2021**, *8*, 114202.
56. Wang, C.; Wang, Y.; Liu, P.; et al. Characteristics of bacterial community structure and function associated with nutrients and heavy metals in coastal aquaculture area. *Environ. Pollut.* **2021**, *275*, 116639.
57. van de Water, J.A.J.M.; Melkonian, R.; Junca, H.; et al. Spirochaetes dominate the microbial community associated with the red coral *Corallium rubrum* on a broad geographic scale. *Sci. Rep.* **2016**, *6*, 27277.
58. Francois, C.; Haure, J.; Billy, J-C.; et al. Toward reference intervals for shellfish: An illustrative case of feeding and respiratory activities in the Pacific cupped oyster, *Crassostrea gigas*. *Vet. Clin. Pathol.* **2020**, *49*, 345–353.
59. Ehrich, M.K.; Harris, L.A. A review of existing eastern oyster filtration rate models. *Ecol. Model.* **2015**, *297*, 201–212.
60. Balcazar, J.L.; Rojas-Luna, T. Inhibitory activity of probiotic *Bacillus subtilis* UTM 126 against *Vibrio* species confers protection against vibriosis in juvenile shrimp (*Litopenaeus vannamei*). *Curr. Microbiol.* **2007**, *55*, 409–412.
61. Zhong, R.; Huang, J.; Liao, Y.; et al. Insights into the bacterial community compositions of peanut worm (*Sipunculus nudus*) and their association with the surrounding environment. *Front. Mar. Sci*. **2022**, *9*, 1076804.