





## RESEARCH ARTICLE OPEN ACCESS

# Bacterial Microbiome Composition and Functional Potentials Across Digestive Regions of Wild New Zealand Abalone (*Haliotis iris*)

Jinchen Guo  | Andrea C. Alfaro  | Leonie Venter  | Donnabella C. Lacap-Bugler 

School of Science, Faculty of Health and Environmental Science, Auckland University of Technology, Auckland, New Zealand

**Correspondence:** Donnabella C. Lacap-Bugler ([donnabella.lacapbugler@aut.ac.nz](mailto:donnabella.lacapbugler@aut.ac.nz))

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## ABSTRACT

Abalone are valuable commercial marine gastropods, supporting both aquaculture and fishery markets. An important ecological aspect of their survival and physiological performance in a given habitat is a complex and balanced symbiotic relationship with microbes in their digestive system. 16S rRNA Illumina MiSeq sequencing was used to investigate the microbial composition of New Zealand abalone (*Haliotis iris*) digestive regions (buccal cavity, foregut, and hindgut), seaweed, and sediment samples from the Cook Strait, New Zealand. The findings revealed an overlap in the microbial communities in the foregut and hindgut samples which differed from buccal cavity samples. The foregut and hindgut were dominated by Fusobacteria, Firmicutes, and Proteobacteria. Proteobacteria were abundant in the buccal cavity, seaweed, and sediment samples. Despite distinct overall microbial compositions in the abalone digestive tract and environmental samples (seaweed and sediment), observed overlaps in bacterial richness and diversity suggest that surrounding habitats may serve as significant reservoirs for the abalone gut microbiome, reflecting potential microbial exchange and microbial functional adaptations. Establishing this microbial baseline for wild *H. iris* provides a reference for detecting microbiome shifts associated with environmental stress, dietary changes, and supports the development of microbiome-targeted feeds that can be used to enhance abalone growth in an aquaculture setting.

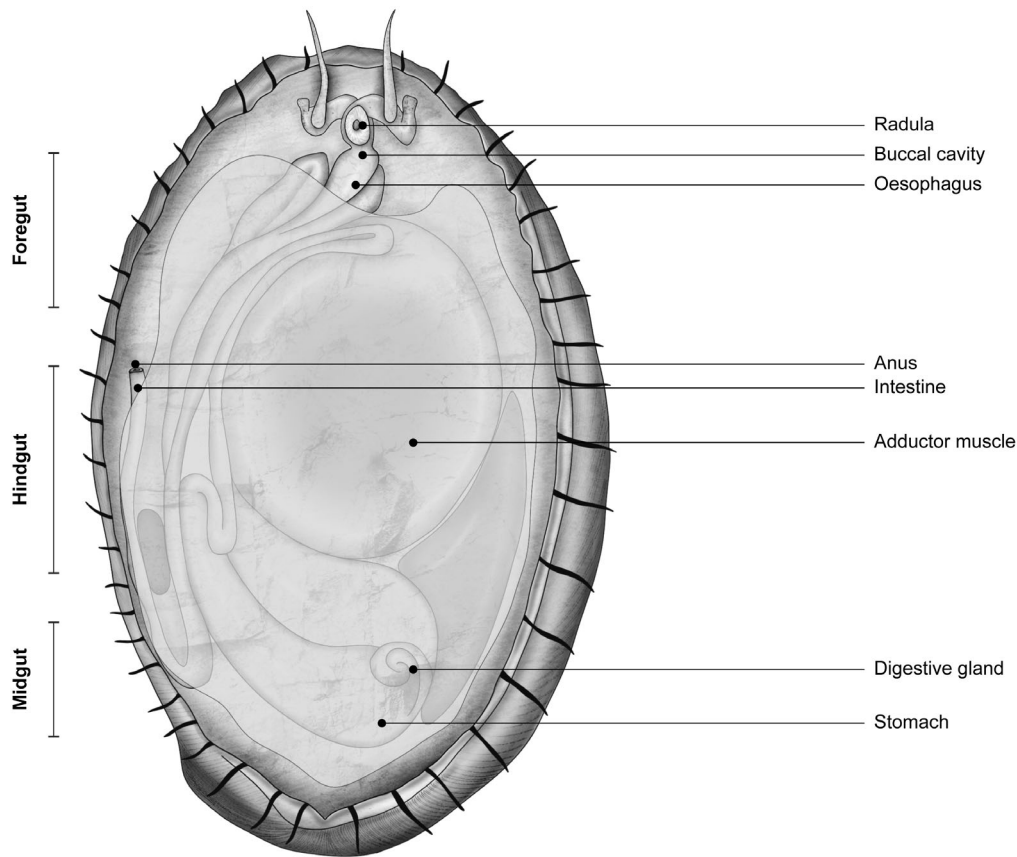
## 1 | Introduction

The New Zealand (NZ) black-foot abalone, *Haliotis iris*, is a herbivore that naturally inhabit rocky intertidal and subtidal reefs where they use radula (elongated ribbon-like structure with numerous chitinous teeth in buccal cavity) to graze mainly on drifting seaweed (Kuehl and Donovan 2021; Copedo et al. 2024). Besides wild populations, *H. iris* are also farmed in aquaculture facilities, where they consume a mixture of algae and formulated feed pellets (Bullon et al. 2023). This species is harvested from wild stocks to support recreational, customary, and commercial entities (Ryder et al. 2023). Various factors, such as environmental stress (Morash and Alter 2016), genetics (Van Der Merwe et al. 2011), diet and nutrition (Bullon et al. 2023),

diseases (Moore 2023), substrate, and habitat (Aguirre and McNaught 2011), have been reported to affect the growth and development of abalone. Growing evidence has also shown a close interaction between the microbes in the digestive tract and the host growth performance (Fan et al. 2019). These microbes, particularly bacteria, colonize the digestive tract of abalone (Nel et al. 2018) and support nutrient digestion, host health, metabolism, immune activity, and neural development (Yu et al. 2022). Depicting the composition and diversity of the bacterial communities in the digestive tract is a prerequisite for evaluating the gut microbial functions regarding the nutrient digestion and other physiological parameters.

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**FIGURE 1** | Generalized abalone's digestive system. Illustration 2025, Anja Erasmus, Scientia illustration, adapted from Guo et al. 2025.

Abalone has a complete digestive system (Figure 1) that can be broadly divided into four regions: buccal cavity, foregut, midgut, and hindgut, each with its own physical and biochemical functions (Harris et al. 1998a). The buccal cavity mainly consists of the salivary gland and an elaborate odontophoral apparatus, including a ribbon-like structure called the radula and connective tissues (Voltzow 2023). The foregut refers to the esophagus pouch with additional glands. The midgut includes the stomach and digestive gland, which contain enzymes for digestion of food particles and conversion into macronutrients such as lipids, polysaccharides, and proteins. Lastly, the hindgut includes the intestine and anus, where most of the nutrient digestion and absorption take place (Johnston et al. 2005, Lobo-da-Cunha 2019). Since the buccal cavity and foregut are closer to the outer environments compared to the midgut and hindgut, they are mostly responsible for physically breaking down and hydrolyzing food particles, and as such the bacterial profiles of these two regions are hypothesized to be similar to the ambient environments (e.g., seawater, sediment, hard substrate surfaces, seaweed, etc.). The midgut and hindgut, on the other hand, are primarily involved in food digestion (e.g., macroalgae and formulated feeds) and absorption of nutrients. Moreover, the microenvironmental conditions (e.g., temperature, pH, dissolved oxygen concentration) of a specific region of the digestive system vary along the digestive tract and can influence the microbial composition and diversity (Escamilla-Montes et al. 2015). Hereby, the microbiota (the taxonomic composition and diversity of the entire microbial community) of the midgut and hindgut are expected to be different from those of the buccal cavity and foregut.

The abundance and diversity of the microorganisms observed in the digestive system correspond to the microenvironment that provides optimal biochemical conditions for the bacteria.

The differences in the bacterial composition and diversity along the digestive system regions have been described in different molluskan species. Like the bacterial profiles of the stomach and digestive gland of the Mediterranean mussel (*Mytilus galloprovincialis*) (Musella et al. 2020), the stomach and intestine of the Eastern oyster (*Crassostrea virginica*) were observed to have different community structures (King et al. 2012). In abalone, intestinal samples collected from *Haliotis midae* had a higher level of bacterial abundance and diversity compared to the post-esophagus–stomach region (Erasmus et al. 1997; Harris et al. 1998b), and the bacterial composition and diversity were also different among the buccal cavity, stomach, and intestinal samples collected in California red abalone *H. rufescens* under the same macroalgal diet (Guo 2017). Evaluation of the similarities or differences in the microbiota of different digestive tract regions has not been adequately documented, especially across the entire digestive system and specifically in *H. iris*. Since the digestive system of abalone is mainly responsible for food digestion and nutrient absorption, investigating the bacterial profiles in the buccal cavity, foregut, and hindgut of abalone will initiate an opportunity to investigate their gut microbiota and their potential role in modulating digestion of dietary nutrients. The potential shifts in the microbial profiles along the digestive tract can be valuable indications of potential microenvironmental, dietary, or health impacts, which will contribute

to the wild and aquaculture stock management of this ecologically and commercially important natural resource.

In addition to the microenvironmental influences, macroenvironmental substances such as seawater, seaweed, and sediment in the habitats of aquatic invertebrates contain diverse bacterial compositions and micronutrients (e.g., minerals) that can be microbial sources to host animals. Seaweed- and sediment-associated bacterial communities can have high bacterial concentrations and are primarily composed of Proteobacteria, Bacteroidetes, Firmicutes, Cyanobacteria, Planctomycetes, Actinobacteria, and Verrucomicrobia (Cragg et al. 1999; Selvarajan et al. 2019). Some of these bacterial taxa including *Psychrobacter haliotis*, *Vibrio midae*, and *Bacillus* spp. are known probiotics and were tested to show high efficiency in protein and monosaccharide digestion (Liu et al. 2023) and with antimicrobial potentials (Santiago and Mabuhay-Omar 2019) in mollusks. The trace metals (e.g., iron, zinc, magnesium, cobalt, etc.) that play an important role in the immune defense of *H. diversicolor* against pathogenic invasions were also observed in aquatic environments such as marine sediment (Marchetti et al. 2020). These natural reservoirs of probiotics and minerals can be bioactive compounds that could potentially benefit the gut health of mollusks and other invertebrates (Zhou et al. 2021; Salloum et al. 2025). Wild abalone are intertidal and subtidal algal grazers and constantly interact with the surrounding seawater, sediment, and seaweed. Consequently, environmental substances could supply microorganisms to the digestive system of gastropods and alter their bacterial profiles. Understanding the resemblance of the microbiota between the abalone digestive system and the environments can be helpful to identify potential sources of certain bacterial species, especially pathogenic strains that can cause shellfish infections and diseases in wild abalone populations as well as among farmed abalone stocks.

Microbiota comparisons between the digestive systems of many aquatic invertebrates and the ambient environment have been previously documented (Danckert et al. 2021). Several studies have indicated that the microbiomes of hosts' digestive systems were similar to those of environmental samples (e.g., seawater, seaweed, sediment). Sponges can acquire bacteria horizontally from ambient seawater environment (Turon et al. 2018), while sediment played an important role in shaping the gut microbial communities in clams (Bernardini et al. 2023). However, there is evidence suggesting that the bacterial composition and diversity in hosts' digestive tracts are distinctive from those of the seawater and sediment samples. An investigation on nudibranchial species demonstrated that the gastropods' gut-associated bacterial profiles were different from those of the seawater and sediment (Stuij et al. 2023), and the bacterial community of freshwater mussels (*Pleurobema cordatum*) was less diverse than that of the surrounding river water and sediment samples (Aceves et al. 2020). Compared to their molluskan relatives, the association between the microbiomes of abalone's digestive tract and their surrounding environments has not been well documented. Investigating the wild abalone (*H. iris*) digestive tract will help understand the association between the microbial composition and diversity and the environmental microbial communities. Such explorations will contribute to future experimental designs, which will aid in understanding how wild abalone's gut microbiota can be influenced by the macroalgae.

Using the 16S ribosomal RNA (rRNA) amplicon sequencing technique, the present study aimed to conduct a baseline snapshot

microbiome survey to 1) define the bacterial community composition across the different regions of the digestive system (buccal cavity, foregut, and hindgut) in wild *H. iris* from the Cook Strait, New Zealand and 2) compare the host's digestive microbiome with the microbial communities of seaweed surface and sediment which are possible gut microbiome sources.

## 2 | Materials and Methods

### 2.1 | Abalone Collection and Dissection

Wild abalone samples ( $n = 20$ ) were collected from Cook Strait ( $41^{\circ} 11' 31.956''$  S,  $174^{\circ} 20' 39.48''$  E) with an average sea surface temperature of  $14.8^{\circ}\text{C}$  at the collection site by commercial divers under special permit (720, client number 9 791 209) issued by Fisheries New Zealand, Ministry of Primary Industries. Abalone samples were removed from rocks in the subtidal area with a blunt shucking knife and placed in polystyrene containers covered with wet hessian bags. Drifting brown seaweed ( $n = 5$ ) and sea sediment ( $n = 5$ ) samples were collected at the same collection site. All collected samples were airfreighted to Auckland and were weighed [wet weight =  $281.3 \pm 14.9$  g (mean  $\pm$  SE)] and measured [shell length =  $123.2 \pm 2.2$  cm (mean  $\pm$  SE)]. Abalone were shucked, and sex was determined based on the color of the gonad. The animals were dissected and buccal cavity was sampled, and the gut content was obtained by extruding the material from the esophagus pouch (foregut,  $n = 20$ ) and intestine (hindgut,  $n = 20$ ). All tissues were placed into individual sterile 2 mL cryo-vials, followed by snap freezing in liquid nitrogen and storing at  $-80^{\circ}\text{C}$  for downstream molecular applications.

### 2.2 | Amplicon Sequencing

The DNA was recovered from the animal's digestive regions (buccal cavity, foregut, and hindgut), seaweed, and sediment samples using the DNeasy PowerSoil Pro Kit (Qiagen, Catalog No. 47 014), quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Catalog No. Q32854), and standardized to  $3\text{ng}/\mu\text{L}$  for two-step polymerase chain reaction (PCR) assays. The first-step PCR amplification was conducted in triplicates with a pair of customized 16S ribosomal RNA (rRNA) primers (forward: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGG-GNGGCWGCAGG-3'; reverse: 5'-GTCTCGTGGGCTCGGAGATGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'). The total volume of each PCR reaction was  $25\mu\text{L}$  containing  $12.5\mu\text{L}$  of KAPA2G Robust HotStart ReadyMix polymerase mixture with dye (KAPA Biosystems, USA),  $1\mu\text{L}$  of each of the forward and reverse primers ( $10\mu\text{M}$ ),  $8.5\mu\text{L}$  of PCR-grade nuclease-free water, and  $2\mu\text{L}$  of each normalized gDNA template. Thermal conditions for the first-round PCR amplification were  $94^{\circ}\text{C}$  for 3 min followed by 25 cycles of  $94^{\circ}\text{C}$  for 45 s/ $60^{\circ}\text{C}$  for 60 s/ $72^{\circ}\text{C}$  for 90 s and a final extension period of 10 min at  $72^{\circ}\text{C}$ . The first-round PCR product in triplicate was pooled by sample, purified with a customized magnetic-bead-based purifying reagent, quantified with the Qubit assays, and normalized to  $3\text{ng}/\mu\text{L}$  with nuclease-free water. The second PCR amplification labeled samples with Illumina indexed primers, and the PCR product was purified with the same customized magnetic bead-based purifying reagent used in the previous step. After quantifying and

normalizing the purified, indexed PCR product to 10 nM, 5  $\mu$ L of each sample was pooled into a 2-mL microcentrifuge as a collection of 16S rRNA libraries. The 16S rRNA libraries were evaluated with the Bioanalyzer High Sensitivity DNA, and the quantified libraries were sequenced on an Illumina MiSeq platform using a v3 (600-cycle) sequencing kit following the manufacturer's protocol (the complete method can be viewed in the online Supporting Information).

### 2.3 | Data Processing and Statistical Analyses

Illumina sequences were processed through a modified DADA2 data processing pipeline (Archer et al. 2020) in R (version 4.2.2) to generate 16S rRNA amplicon sequence variants (ASVs), construct an ASV abundance table, and assign taxonomic information to the representative ASVs. ASV taxonomic assignments were based on SILVA high-quality ribosomal RNA database (Version 138) (Quast et al. 2012). Chloroplast ASVs were removed from the seaweed samples to account for the molecular interference from the macroalgal cells.

The microbial composition and diversity at the genus level across the different regions of the digestive tract and between the gut content and environmental samples were compared. Briefly, the read abundance data were not rarefied but normalized using the total sum scaling method to retain all biological information. Alpha-diversity estimators (e.g., rarefaction curves, observed richness, chao1 richness, evenness, and Shannon's diversity index) were calculated using the MicrobiomeAnalyst portal (Lu et al. 2023), with appropriate statistical tests performed in R (version 4.2.2). To illustrate beta-diversity among the digestive tract regions and the environmental samples, the normalized ASV abundance data at the genus level were converted to a Bray–Curtis dissimilarity matrix. Bacterial composition among the sample groups was visualized in nonmetric multidimensional scaling (nMDS) plots, and permutational multivariate analysis of variance (PERMANOVA) tests were conducted using the Plymouth Routines in Multivariate Ecological Research (PRIMER) software (Version 7) (Clarke and Gorley 2015) to detect statistical significance. In addition, core microbiota with a sample prevalence of at least 20% and a detection threshold of 0.1% across all sample groups was also identified and presented in a cluster heat map, along with a differential abundance analysis based on linear discriminant analysis (LDA) effect size (LEfSe) (Peng et al. 2018) to detect essential bacterial genera that differentiated the microbiota among the groups. Microbial functions were predicted, based on the 16S rRNA amplicon sequences, using the 'Tax4Fun2' function (Wemheuer et al. 2020) in MicrobiomeAnalyst. The functional composition was predicted from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al. 2012). More details about the alpha- and beta-diversity statistical analysis can be found in the supplementary material.

## 3 | Results

A total of 9,739,864 pre-quality check reads were generated from the different regions of the digestive tract, seaweed, and sediment samples. After passing through the DADA2 bioinformatic pipeline and data curation, 8,673,449 quality reads were produced,

**TABLE 1** | Summary of the 16S rRNA genetic amplicon reads and the observed amplicon sequence variant (ASV) richness following the DADA2 bioinformatic pipeline.

Sample Type	Sample Size	Post-quality-check Reads	ASVs
All samples	70	8,673,449	5,837
Buccal cavity	20	2,734,445	1,065
Foregut	20	2,276,557	1,023
Hindgut	20	3,304,348	1,179
Seaweed	5	158,617	217
Sediment	5	199,482	3,657

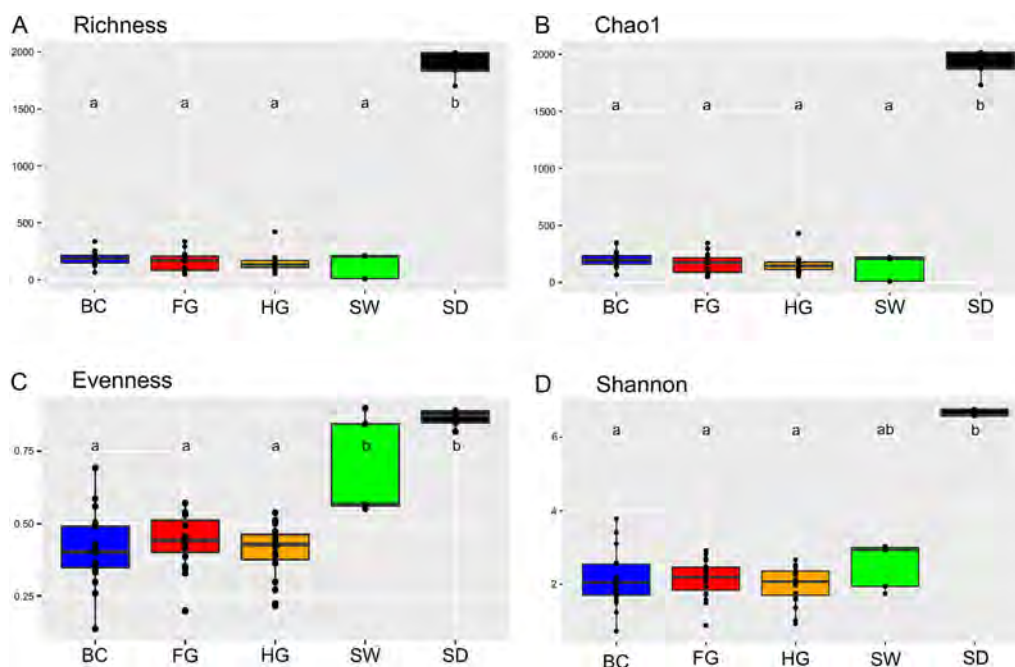
with 5,837 representative 16S rRNA ASVs observed from all samples (Table 1). While the sediment samples had the highest number of ASVs, the hindgut and sediment samples showed the highest observed unique ASV richness, and there were only 16 ASVs shared among all sample groups (Figure S1). The prokaryotic ASVs were assigned to 96 classes, 220 orders, 370 families, and 676 genera. The sequencing depths were adequate for all sample groups to carry out the subsequent analyses except for the sediment samples (Figure S2). Alpha-diversity estimators were based on the ASV level, and comparisons of the microbial composition, beta-diversity, and predicted microbial functions were conducted at the bacterial genus level.

### 3.1 | Microbial Composition across Samples

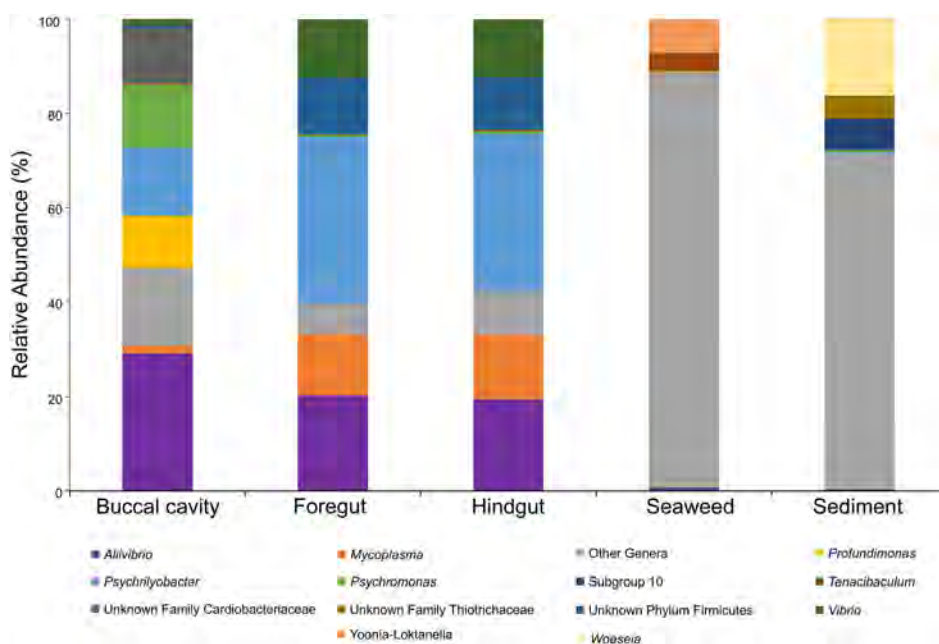
At the ASV level, the alpha-diversity matrices revealed that the microbial communities within samples were similar across the abalone digestive tract and environmental samples (Figure 2). However, the variances in the ASV evenness among the digestive tract samples were relatively larger, indicating possible differences among the abalone individuals (Figure 2C,D).

The bacterial compositions differed among the three digestive tract regions (Figure 3). The foregut and hindgut shared a similar bacterial composition, with *Psychrobacter* as the most abundant genus. While the buccal cavity bacterial composition was significantly different from the gut samples (Tables S1 and S2) with *Profundimonas* observed to be more abundant, *Mycoplasma* and *Vibrio* were less abundant compared with the gut samples (Figure 3).

The alpha-diversity comparisons showed significant difference in observed ASV richness (Figure 2A; Kruskal–Wallis test,  $X^2_{(4, 67)} = 18.05$ ,  $p < 0.01$ ), chao1 richness (Figure 2B; Kruskal–Wallis test,  $X^2_{(4, 67)} = 19.04$ ,  $p < 0.01$ ), evenness (Figure 2C; Kruskal–Wallis test,  $X^2_{(4, 67)} = 25.01$ ,  $p < 0.01$ ), and Shannon's diversity index (Figure 2D; Kruskal–Wallis test,  $X^2_{(4, 67)} = 16.03$ ,  $p < 0.01$ ) between the digestive tract samples and the seaweed and sediment samples, and the Dunn's tests indicated that the sediment samples were significantly different from all abalone digestive tract samples (Figure 2). In contrast, the alpha-diversity estimators of the seaweed samples were not significantly different from those of the digestive tract samples except for the evenness (Figure 2C).



**FIGURE 2** | Alpha diversity metrics showing the observed (A) richness, (B) Chao1 richness, (C) evenness, and (D) Shannon's indices (D) of the prokaryotic amplicon sequence variants (ASVs) across all sample types: buccal cavity (BC,  $n = 20$ ), foregut (FG,  $n = 20$ ), hindgut (HG), seaweed (SW), and sediment (SD). Letters indicate statistical significance based on the Dunn's tests.

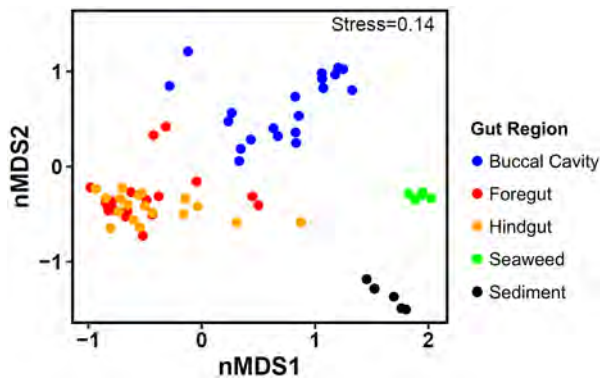


**FIGURE 3** | Relative abundance of the bacterial genera with at least 5% prevalence across the five sample groups: buccal cavity ( $n = 20$ ), foregut ( $n = 20$ ), hindgut ( $n = 20$ ), seaweed ( $n = 5$ ), and sediment ( $n = 5$ ).

The microbial composition of the seaweed and sediment samples was significantly different from that of the digestive tract samples (Tables S1 and S2), as clearly indicated in the clustering of samples in the nMDS plots at different taxonomic levels (Figure 4). However, approximately 80% of the observed bacteria recovered from the seaweed and sediment samples were assigned to unclassified taxa (Figure 3).

### 3.2 | Core Microbiome across Samples

The core microbiome of all samples collected included *Psychrilyobacter*, *Mycoplasma*, *Vibrio*, *Aliivibrio*, *Psychromonas*, and other unassigned bacterial genera (Figure S3). The samples collected from different regions of the digestive system, seaweed, and sediment also showed distinctive bacterial profiles: bacteria identified as *Psychromonas* and *Profundimonas* were mostly



**FIGURE 4** | nMDS plot of the bacterial composition at genus level of the buccal cavity, foregut, hindgut, seaweed, and sediment samples. Data were normalized using the total sum scaling method and converted to Bray-Curtis dissimilarity matrices.

observed in the buccal cavity samples; the foregut and hindgut samples had a similar bacterial profile of which *Psychrilyobacter*, *Mycoplasma*, and *Vibrio* were relatively more abundant; and the seaweed and sediment samples differed from the digestive tract samples with *Blastopirellula*, *Propionigenium*, *Sphingomonas*, and *Photobacterium* being more abundant (Figure 5). A further LDA supported that the listed bacterial genera were major taxa that contributed to the partition of the sample types (Figure S4).

The predicted microbial functions across all sample types were classified into the categories: metabolism, BRITE hierarchies (a structured framework for classifying and analyzing microbial functions based on the biological functions and related pathways of microbial genes), cellular processes, environmental information processing, and genetic information processing. Among the top specific microbial functions identified based on the

KO (KEGG Orthology) abundance data, the bacteria observed in the buccal cavity samples were mostly involved in synthesizing 3-oxoacyl-[acyl-carrier protein] reductase, putative adenosine triphosphate (ATP)-binding-cassette (ABC) transport system ATP-binding protein, and RNA polymerase sigma-70 factor. The bacteria observed among the seaweed samples mostly participated in the processing of 3-oxoacyl-[acyl-carrier protein] reductase and iron complex outer membrane receptor protein. The microorganisms observed in the foregut, hindgut, and sediment samples had a similar predicted functional profile, and they were mostly involved in the processing of putative ABC transport system ATP-binding protein, peptide/nickel transport system ATP-binding protein, methyl-accepting chemotaxis protein, and diguanylate cyclase (Figure 6).

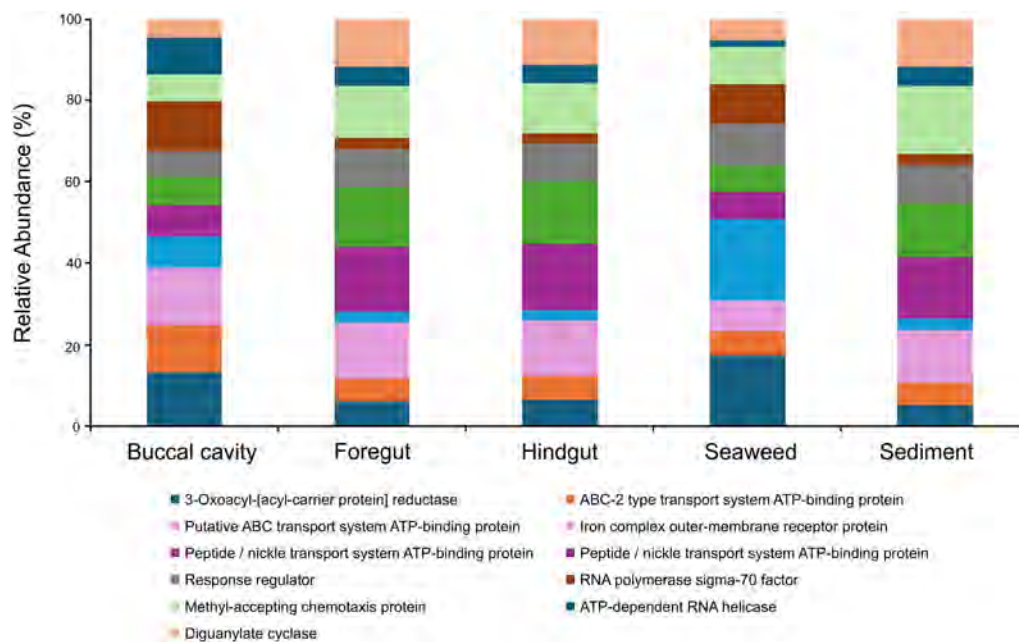
## 4 | Discussion

### 4.1 | Microbiome Comparisons across the Digestive System Regions and Seaweed

The results from the microbiomes of the buccal cavity, foregut, and hindgut samples of wild *H. iris*, which were specific to the Cook Strait at the time of sampling, showed that the bacterial composition and the between-group microbial diversity were significantly different in the buccal cavity and gut samples. However, in *H. rufesens*, these digestive regions showed similar microbial diversity (Guo 2017). In mussel and oyster, gut bacterial compositions and diversity differed from gill tissue, where food particles were initially collected and ingested in bivalves (King et al. 2020, Li et al. 2022). Since abalone's buccal cavity opens to external environments and their hindgut primarily functions to digest food (therefore has different microenvironmental conditions from the buccal cavity), it was expected that



**FIGURE 5** | Cluster heatmap showing the hierarchical clusters of the most abundant bacterial genera observed among all samples. The bacterial clusters were generated using the Minkovski distances and complete clustering algorithm in MicrobiomeAnalyst. “SW” denotes “seaweed” and “SD” denotes “sediment”.



**FIGURE 6** | The predicted Kyoto Encyclopedia of Genes and Genomes (KEGG) specific functions across the five sample groups: buccal cavity ( $n = 20$ ), foregut ( $n = 20$ ), hindgut ( $n = 20$ ), seaweed ( $n = 5$ ), and sediment ( $n = 5$ ). The predicted bacterial functions of the samples collected from the digestive tract (buccal cavity, foregut, and hindgut) of wild *Haliotis iris*, seaweed, and sediment samples were represented as relative abundance of the KEGG orthology.

the microbial profiles between the two regions would be different.

Interestingly, similar bacterial profiles, both compositionally and functionally, between the foregut and hindgut samples were observed in the present study. The foregut samples were collected from the lower esophagus pouch, while the hindgut samples were collected from the lower intestine. The observed microbiome similarities indicate that both digestive tract regions might share similar microenvironmental conditions, which are not commonly noted in mollusks (Escamilla-Montes et al. 2015). It was reported that the microenvironmental conditions, such as pH and dissolved oxygen levels, in the foregut and hindgut regions of *H. rubra* were differentiated and, therefore, shaped the bacterial profiles at these two regions (Johnston et al. 2005). The unexpected similar microbiome profile between the foregut and hindgut regions, the differences between the gut and buccal cavity samples, and the observed within-group bacterial diversity variations indicate the bacterial profiles might be influenced by certain unknown influencers, such as habitat ambient macroenvironmental conditions or the digested seaweed.

Coastal aquatic environmental conditions can have a substantial influence on molluscan physiology, including abalone's gut microbiome. This is mostly because the intertidal and subtidal habitats where the host mollusks dwell have fluctuating seawater temperatures, salinity levels, and dissolved oxygen concentrations. Previous studies have demonstrated that macroenvironmental fluctuations can have a significant impact on the general physiology of abalone, such as impeding larval settlement (Naylor and McShane 2001, Onitsuka et al. 2008), reducing growth rates (Cummings et al. 2019), inhibiting shell formation (Auzoux-Bordenave et al. 2020), and modifying hemocyte proteomic profiles (Wessel et al. 2018). In addition to the aquatic physical and chemical properties, seawater itself is an environmental

microbial reservoir that could influence the microbial communities associated with marine mollusks, including abalone. For instance, Pierce and Ward (2019) noted that gut microbiome of the eastern oyster (*Crassostrea virginica*) was closely linked to the microbial communities in the seawater samples, and similar results were also observed in the giant abalone (*H. gigantea*), whose gill microbiome significantly overlapped with that of seawater (Mizutani et al. 2020). The current study however was designed to capture solid environmental matrices (drifting algae and sediment) that are directly associated with the abalone's feeding strategy and the benthic interactions, which are considered as the primary abalone gut microbial sources. Seawater microbiome comparisons maybe included in future study of the New Zealand pāua microbiome investigations to better resolve microbial connectivity between the host and their surrounding environments.

Another potential explanation for the observed difference in microbiome across the digestive tract is the species of seaweed being ingested and digested. It is clear from this study that the microbiome compositions and diversity of the abalone hindgut were significantly different from the seaweed surface microbiome, indicating that the seaweed bacterial communities did not influence the host's gut microbiota. Hereby, implying other factors such as specific digested food type could shape the bacterial communities in the abalone digestive system. Abalone may consume red algae (Rhodophyta), brown algae (Phaeophyta), and green algae (Chlorophyta). Species such as *Gracilaria chilensis*, *Ecklonia radiata*, *Lessonia variegata*, *Sargassum sinclairii*, *Ulva lactuca* and *Ulva intestinalis* have also been linked to abalone diet (Erasmus et al. 1997, Tanaka et al. 2015, Guo 2017, Nel et al. 2018, Guo et al. 2025). In fact, previous studies have documented that different digested seaweed types can influence abalone's gut microbiome. For example, alginate-degrading bacteria such as *Psychrilyobacter*,

*Mycoplasma*, and *Vibrio* are prevalent in the gut of abalone fed with red macroalgae (Tanaka et al. 2016, Gobet et al. 2018), whereas *Formosa* and *Clostridia* were commonly found in abalone's gut under brown algal diets in addition to the common bacteria associated with red seaweed (Tanaka et al. 2015, Guo 2017, Nel et al. 2018). While *Psychrilyobacter*, *Mycoplasma*, and *Vibrio* were part of the core microbiome observed in the present study and were relatively stable across time and developmental stages of various abalone species (Tanaka et al. 2016, Guo et al. 2017, Gobet et al. 2018), they were also among the discriminative bacterial genera that partitioned the microbial profiles across the digestive tract regions, indicating the observed bacterial communities along the digestive tract of *H. iris* might have resulted from the ingested seaweed that was rich in algininate. While these findings also provide a baseline for further investigations on how wild abalone's gut microbiome would respond to different macroalgal diets, brown macroalgae was the dominant species of algae during the season when the abalone samples were collected. Future abalone gut microbiome research may incorporate a broader algal species, longitudinal (i.e., multiple seasons), and cross-life-stage sampling scheme to better understand diet–microbiome linkages.

#### 4.2 | Microbiome and Predicted Functions in the Hindgut, Seaweed, and Sediment

The present study revealed differentiated bacterial composition and significantly high bacterial diversity in the sediment samples compared to the other sample groups at different taxonomic levels. These findings are in line with previous microbiome studies conducted in other mollusks, including *Crassostrea virginica* and *Mytilus edulis* (Pierce and Ward, 2019), *Ruditapes philippinarum* (Offret et al. 2020), and *Littorina* spp. (Maltseva et al. 2021). The difference in the bacterial composition and diversity observed between the hindgut and the surrounding substrate (mostly sediment) indicates that bacterial taxa are unlikely to be directly transported to the digestive tract of mollusks including abalone.

Although there was a significant difference in the bacterial composition and diversity between the hindgut and sediment samples, it is interesting to discover that both sample types shared similar predicted microbial functions. Our results of the microbial functional predictions indicated that bacteria found in the foregut, hindgut, and sediment samples were likely involved in synthesizing signaling proteins that bind and transport various physical and chemical substances like peptides and nickel. There are three implications resulting from these findings. First, the microbial compositional and functional resemblance between the hindgut and sediment samples supports the concept of 'functional redundancy' among microbial communities, where taxonomically unrelated bacteria could perform similar ecological functions (Xenophontos et al. 2021). This suggests that microbiome investigations in abalone and other shellfish hosts could focus more on microbial functions, which can provide ecologically significant information. Second, the presence of peptide- and nickel-transporter-synthesizing bacteria in the hindgut samples indicates that the bacterial communities in abalone's hindgut are likely to be selected by the nutrient metabolism needs of the hosts, which supports the idea that the foregut and hindgut microbiome of the wild abalone in this study might be affected by dietary nutrients that needs to be further investigated. Lastly, the

predicted microbial functions from the 16S rRNA genetic amplicons suggest that bacteria found in the digestive tract of wild *H. iris* were involved in diverse functions other than nutrient metabolism. While the predicted microbial functions were solely based on one genetic marker, future investigations should utilize more advanced technologies, such as metagenomic shotgun sequencing, to make the microbial functional predictions more robust.

#### 4.3 | Implications to Abalone Feed Optimizations in Aquaculture

The similar predicted microbial functional profiles between the abalone gut and the surrounding sediment samples in the present study provide an implication of formulating beneficial constituents, such as bacterial strains as probiotics and micronutrients (e.g., minerals), into abalone feeds to optimize food digestion and nutrient absorption and promote immunological responses against pathogenic infections. Coastal marine sediment (mostly sand, clay, and silts) contains loads of diverse microorganisms (e.g., viruses, bacteria, fungi, protozoans, microscopic metazoans, etc.) specializing in a variety of ecological functions such as sulfate reduction (Wasmund 2023) and toxin decontamination (Wasmund et al. 2021). Several studies have illustrated that bacterial strains including *Psychrilyobacter haliotis*, *Vibrio midae*, and *Bacillus* spp. that were previously discovered in marine sediment have been tested to show high efficiency on protein and monosaccharide digestion (Liu et al. 2023) and antimicrobial potentials (Czarina et al. 2019). Furthermore, some of the bacterial strains found in the sediment can also synthesize vitamin B12, which is essential in the metabolism of abalone (Fang et al. 2017, Watanabe and Bito 2018).

Minerals or trace metals are another group of marine sediment components that can be beneficial to abalone. Trace metals are essential chemical elements that naturally accumulate in marine sediment in very small quantities. Despite their low concentrations, they are considered micronutrients in the dietary nutritional profiles of many animals and play crucial roles in multiple physiological processes. For instance, iron is a limiting micronutrient in the open oceans (Sharada et al. 2020, Schallenberg et al. 2022), but it is a vital component of hemoglobin for oxygen transportation (Hediger et al. 2002) and enzymes for macronutrient metabolisms (Andrews 2000) in shellfish. Besides the dietary benefits, minerals like iron (in the form of ferritin), zinc, and magnesium were observed to induce immunological responses in abalone *H. diversicolor* during pathogenic challenges, suggesting a role in immune defense against pathogenic invasions (Marchetti et al. 2020). Geothermally active areas like New Zealand have freshwater run-offs that constantly bring trace metals (e.g., iron (in the form of ferritin), zinc, magnesium) from chemically weathered volcanic rocks to nearby coastal embayment as a part of local marine sediment (Carey et al. 2002). The presence of the iron receptor-synthesizing bacteria in the seaweed samples, along with the nickel receptor-synthesizing bacteria observed in the gut and sediment samples, indicates that the habitat of wild *H. iris* in the Cook Strait may contain loads of trace metals that could be essential to promote food digestion and gut health in the local abalone populations.

The utilization of beneficial bacterial strains as ingredients of formulated abalone feeds has been previously conducted in several

abalone species. For instance, Wang et al. (2025) demonstrated that the inclusion of probiotic strains like *Bacillus* and *Lactobacillus* increased the intestinal microbial diversity and body weight of *H. diversicolor*. In another feeding experiment, the abalone feed fermented by *Lactobacillus plantarum* improved the growth performance and diversified the gut microbiota of the Pacific abalone (*H. discus hannai*; Ke et al. 2025). Regarding the black-footed abalone *H. iris*, probiotic strains of *Vibrio* sp., *Exiguobacterium* sp., and *Enterococcus* sp. were microencapsulated and delivered to the digestive system of *H. iris* demonstrated growth and immunity improvements as well as higher feed conversion rates (Masoomi Dezfouli et al. 2021, Dezfouli et al. 2023). With the advanced and developed probiotic delivery methods, the incorporation of the beneficial bacteria and minerals into abalone formulated feeds can be further researched and executed to improve abalone production at aquaculture facilities and better protect the wild populations from microbial diseases.

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### Conflicts of Interest

No potential conflict of interest was reported by the authors.

### Data Availability Statement

The raw sequencing data generated in this study have been deposited in the European Nucleotide Archive (ENA) under accession number [PRJEB89187]. These data can be accessed at: <https://www.ebi.ac.uk/ena/browser/view/PRJEB89187>. All relevant metadata are also included in the submission.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Supporting Fig. S1:** Venn diagram showing the

number of shared and unique 16S rRNA amplicon sequence variants (ASVs) among the five sample groups (buccal cavity, foregut, hindgut, seaweed, and sediment) collected from Cook Strait in May 2021. **Supporting Fig. S2:** Rarefaction curves showing the number of observed amplicon sequence variants (ASVs) per sequenced samples collected from the buccal cavity (n=20), foregut (n=20), and hindgut (n=20) samples of wild *Haliotis iris*, seaweed (n=5), and sediment (n=5) samples. **Supporting Fig. S3:** Heat map of the bacterial core microbiome at the genus level. The sample prevalence and detection threshold were set to 20% and 0.1%, respectively. **Supporting Fig. S4:** Linear discriminant analysis Effect Size (LEfSe) identifying the top bacterial genera explaining the differences across the sample types (i.e., buccal cavity, foregut, hindgut, seaweed, and sediment). The Linear Discriminate Analysis (LDA) threshold was set to 2.0 with an adjusted p-value cut-off of 0.01 in the Kruskal-Wallis rank test. **Supporting Table S1:** Permutational multivariate analysis of variance (PERMANOVA) test results of the multivariate microbial diversity analysis across the sample types (buccal cavity, foregut, hindgut, seaweed, and sediment) collected in Cook Strait in May 2021. The sequencing data were normalized and transformed to Bray-Curtis dissimilarities. **Supporting Table S2:** Pairwise permutational multivariate analysis of variance (PERMANOVA) test results of the multivariate bacterial diversity analysis across the sample types (buccal cavity, foregut, hindgut, seaweed, and sediment) collected in Cook Strait in May 2021. The sequencing data were normalized and transformed to Bray-Curtis dissimilarities. Asterisk indicates the Monte Carlo p-values were used due to low number of permutations.