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Geographic Structuring of Gut-Content Bacterial Communities in Invasive Blackchin Tilapia (*Sarotherodon melanotheron*) Across Thailand: Site-Level Environmental Associations and Implications for Invasion Ecology

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ABSTRACT

The blackchin tilapia (*Sarotherodon melanotheron*) has rapidly expanded across Thailand, but how environmental conditions relate to its gut microbiota during invasion remains unclear. We examined gut-content bacterial communities at six sampling sites along the Gulf of Thailand using full-length 16S rRNA gene sequencing of 68 fish, alongside site-level water analyses (one composite sample per site; $n = 6$) and helminth screening. Communities showed spatial structuring, with significant differences in alpha and beta diversity among locations. Distinct assemblages included dominance of *Enterovibrio nigricans* in Rayong and *Cetobacterium somerae* in Chumphon. Predicted metabolic functions showed site-specific enrichment. Taxon-covariation networks revealed location-associated patterns. Location explained 65% of community variation; pH, salinity, and orthophosphate together corresponded to about half of among-site variation. *Aeromonas* spp. and *Photobacterium damselae* showed positive associations with salinity, but these trends were not significant after multiple-testing correction. Helminths were almost entirely absent, with only incidental ingestion of free-living nematodes. Gut-content bacterial communities varied among locations and covaried with site-level water conditions. However, because environmental variables were measured at only six sites, their effects cannot be separated from other location-specific factors. These findings are consistent with a dual-assembly framework in which environmental microbial pools and host filtering shape gut-content bacterial communities during invasion.

1 | Introduction

Biological invasions are among the leading drivers of global biodiversity loss, ecosystem alteration, and economic costs worldwide. Freshwater systems are particularly vulnerable, as introduced species can rapidly alter trophic structure, nutrient cycling, and community composition. The blackchin tilapia (*Sarotherodon melanotheron*), native to West Africa, has been

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widely introduced to multiple continental regions for aquaculture due to its rapid growth and tolerance of a wide range of environmental conditions (Canonico et al. 2005; Suwannarat et al. 2025). Although widely cultured because of its aquaculture value, its establishment outside its native range has generated increasing ecological concern (Oluwale and Ugwumba 2022).

Several intrinsic biological traits likely contribute to the invasion success of *S. melanotheron*. Maternal mouthbrooding enhances offspring survival and may facilitate population establishment in newly colonized habitats, while its broad and opportunistic feeding strategy enables rapid exploitation of diverse food resources across heterogeneous ecosystems (Specker and Kishida 2000; Canonico et al. 2005; Ofori-Danson and Kumi 2009; Ndimele et al. 2010; U.S. Fish and Wildlife Service 2012). These behavioural traits are complemented by strong physiological adaptability, including tolerance of wide fluctuations in salinity, hypoxia, and high population densities (Ouattara et al. 2003; Lemarié et al. 2004; Tine et al. 2008; Chaianunporn et al. 2024). Together, these characteristics may enhance the species' ability to survive environmental variability, exploit available resources, and persist across newly invaded habitats.

In Thailand, this adaptability has resulted in widespread ecological concern. By early 2025, *S. melanotheron* had invaded at least 17 provinces surrounding the Gulf of Thailand, disrupting local aquatic food webs, negatively affecting native fish communities, and threatening both regional biodiversity and the economically important aquaculture sector (Guinto and Sricham 2024; Rujivanarom and Boonyakiat 2024). Its broad environmental tolerance and high reproductive capacity promote continued dispersal between coastal and inland habitats, increasing the likelihood of further spread and long-term ecosystem alteration. Reports from affected regions indicate shifts in community composition and growing concern among fishers regarding competition with commercially valuable native species (Suwannarat et al. 2025), underscoring the ecological and socioeconomic consequences of the invasion. Despite the scale and urgency of this problem, the biological mechanisms underlying its successful establishment in Southeast Asian ecosystems remain poorly understood.

Beyond intrinsic biological traits, host-associated microbial and parasitic communities may play critical yet underexplored roles in invasion success. The gut microbiome may contribute to host phenotypic plasticity by influencing metabolism, nutrient assimilation, and immune function, potentially supporting host responses to novel diets and fluctuating environmental conditions (Alberdi et al. 2016; Bettarel et al. 2018; Martinez-Porchas et al. 2023). Concurrently, the Enemy Release Hypothesis (ERH) posits that introduced species often experience reduced parasite pressure in invaded regions, potentially reallocating energy from immune defence towards growth and reproduction (Colautti et al. 2004; Marcogliese and Pietrock 2011). However, the interactive roles of gut microbiota and helminth communities in facilitating freshwater fish invasions remain largely unresolved.

Despite growing recognition of microbiome-mediated plasticity, most invasion studies have focused primarily on host intrinsic traits, with comparatively little attention given to the role of environmental context in shaping host-associated microbial communities (Legrand et al. 2018; Bell et al. 2024). In aquatic systems, physicochemical parameters such as salinity, pH, and nutrient availability are known to strongly structure free-living microbial communities (Zhu et al. 2022; Morshed and Lee 2023) and may indirectly influence host-associated microbiota through continuous environmental exposure (Fietz et al. 2018). Environmental filtering describes the process by which local abiotic and biotic conditions selectively favour microbial taxa that can persist under particular environmental conditions, thereby shaping community composition. In host-associated microbiomes, environmental filtering may interact with host selection to determine which microorganisms establish and persist within the gut (Araujo et al. 2026). For opportunistic benthic feeders such as *S. melanotheron*, ingestion of sediment and water-associated microbes may facilitate dynamic microbiome restructuring in response to local habitat conditions (Zhang et al. 2024). However, the extent to which environmental factors override host-specific filtering in invasive fish microbiomes remains poorly understood (Morshed and Lee 2023; Bell et al. 2024). Determining the relative importance of environmental filtering and host selection is therefore essential for understanding microbiome assembly and its potential contribution to invasion dynamics in rapidly changing aquatic ecosystems.

Here, we investigated gut-content bacterial communities and helminth communities of invasive *S. melanotheron* across six provincial sites in Thailand. We hypothesized that (i) gut microbial communities exhibit significant geographic structuring, (ii) variation in gut-content bacterial community composition is associated with site-level physicochemical conditions, and (iii) helminth diversity is reduced across invaded populations, consistent with the Enemy Release Hypothesis. To test these hypotheses, we integrated full-length 16S rRNA gene amplicon sequencing with parasitological screening and physicochemical analyses of aquatic environments. By linking microbial communities, parasite occurrence, and environmental conditions, this study aims to describe how gut-content bacterial communities vary among locations and with site-level environmental conditions during biological invasion.

2 | Materials and Methods

2.1 | Sample Collection and Preparation

Between March and August 2025, a total of 312 blackchin tilapia (*S. melanotheron*) were collected from six sites with documented high abundance across different provinces along the Gulf of Thailand: Rayong (RY), Phetchaburi (PB), Samut Sakhon (SS), Samut Prakan (SP), Songkhla (SK), and Chumphon (CP). Fresh fish specimens were captured by local fishers within a 5 km radius of each sampling location (Table S1). Each specimen was individually sealed in a plastic bag, immediately stored in ice boxes, and transported to the Applied Animal Science Laboratory, Department of Biology, Faculty of Science, Mahidol University. All collected specimens were dissected through a midline incision along the abdominal cavity following the protocol described by Ratnarathorn and Kongrit (Ratnarathorn and Kongrit 2025) to isolate the digestive organs, and the entire gastrointestinal tract was examined for parasites.

For microbiome analysis, 10–13 individuals were randomly selected from each site (68 individuals in total), and the proximal intestine was excised for DNA extraction and subsequent sequencing. The proximal intestine was consistently sampled across individuals to minimize variation associated with intestinal location, as gut microbial composition may differ along the intestinal tract (Gajardo et al. 2016). The remaining gastrointestinal tract was retained for parasitological examination. All 68 selected samples were successfully sequenced and included in the gut-content bacterial community analyses.

2.2 | Parasitological Examination

After organ separation, each organ was placed in a Petri dish filled with tap water, and then opened longitudinally and examined under a stereomicroscope (Zeiss Stand K EDU, Germany) for helminths. Recovered worms were initially classified by major taxonomic group (i.e., nematodes) and preserved in 1.5 mL microcentrifuge tubes containing 70% ethanol for further identification. Worm morphology was examined in detail and photographed under an inverted microscope (Zeiss Primovert, Germany) equipped with a Zeiss Axiocam and ZEN 2 Blue Edition software (Charoennitiwat et al. 2023).

2.3 | Molecular Identification of Helminths

Based on Charoennitiwat, Chaisiri, et al. (2024); Charoennitiwat, Thaenkham, et al. (2024), genomic DNA was extracted using a Genomic DNA Mini Kit (Geneaid Biotech Ltd., New Taipei City, Taiwan) following the manufacturer's protocols. Due to the low DNA yield per individual, multiple helminth specimens were pooled into a single tube for extraction. Polymerase chain reaction (PCR) amplification was performed using a T100 thermocycler (Bio-Rad). The nuclear 18S ribosomal RNA gene (18S rDNA) was selected as the marker based on the availability of comparable sequences in the NCBI database. Each 30 μL reaction contained 15 μL of 2× i-Taq master mix (Biotechnology, Gyeonggi, South Korea), 10 μM of each primer, and 1 ng/μL of template DNA. Primers used were 1096F 5′-GGTAATTCTGGAGCTAATAC-3′ and 1916R 5′-TTTACGGTCAGAACTAGGG-3′ with expected amplicon length of 800 bp (Holterman et al. 2006). The thermocycling profile included an initial denaturation at 94°C for 5 min, followed by 5 cycles of 94°C for 30 s, 45°C for 30 s and 72°C for 70 s, and 35 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 70 s. The final extension was at 72°C for 5 min to conclude the reaction. PCR products were visualized on a 1% agarose gel stained with SYBR Safe (Thermo Fisher Scientific, USA). Successfully amplified products were sequenced using Fast Next-Generation Sequencing (Tsingke, Beijing, China). The retrieved 18S sequences were aligned with available sequences of other closely related helminths from NCBI using ClustalX 2.1. Phylogenetic analyses were conducted using Neighbour-Joining (NJ) in MEGA12.

2.4 | Full-Length 16S rRNA Gene Amplicon Sequencing for Bacterial

The gut contents of the proximal intestinal tract were collected and preserved in 1 mL of Nucleic Acid Preservation (NAP) buffer to stabilize microbial DNA (Menke et al. 2017). The samples were subjected to DNA extraction using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, USA). The full-length bacterial 16S rRNA gene was amplified with specific primers containing Nanopore adapters, following the previous protocol (Sawaswong et al. 2024). Each sample was uniquely barcoded with the PCR Barcoding Expansion 1–96 kit (Oxford Nanopore Technologies, UK), and the pooled amplicon library was purified using the QIAquick PCR Purification Kit (Qiagen, Germany), quantified with the Quant-iT dsDNA HS Assay Kit (Invitrogen, USA), and size-selected with Agencourt AMPure XP beads (Beckman Coulter, USA). The sequencing library was prepared using the Ligation Sequencing Kit (SQK-LSK114, Oxford Nanopore Technologies, UK) and run on a MinION Mk1B platform.

2.5 | Microbiome Data Analysis

Basecalling was performed with Guppy v6.1.2 (super-accuracy mode). The raw FASTQ reads were quality-filtered ($Q > 10$) to remove low-quality and short sequences (length < 1000 bp) before taxonomic classification. Quality assessment was conducted using MinIONQC and demultiplexing and adapter trimming were carried out with Porechop v0.2.4. Reads were clustered and polished through the NanoCLUST pipeline (Rodríguez-Pérez et al. 2021), and taxonomic classification was conducted using BLASTn against the Ribosomal Database Project (RDP) v11.5. Processed data were imported into QIIME2 v2021.2 to generate collapsed taxa tables, followed by alpha and beta diversity analyses, differential abundance testing using Linear Discriminant Analysis Effect Size (LEfSe), and taxon-covariation network analysis using the SparCC algorithm via MicrobiomeAnalyst. Pairwise comparisons of alpha diversity indices were performed using Dunn's multiple comparison test. The functional inference analysis was conducted using PICRUSt2. The results of differential abundance testing and functional inference analysis were visualized as heatmaps using Heatmapper (Babicki et al. 2016).

2.6 | Analysis of Physicochemical Parameters and Microbiome Correlation

Water samples were collected at each sampling site concurrently with fish collection and transported to the laboratory under cooled conditions for physicochemical analysis. Three water samples were collected from different points within the fish-collection area at each site and pooled to obtain one composite sample per site, thereby providing a site-level representation of the local water conditions while minimizing small-scale spatial variation. All samples were collected during fish sampling to minimize temporal variability. Measured parameters included pH, total dissolved solids (TDS), electrical conductivity (EC), and salinity using a HI285-7 multiparameter probe (Hanna

Instruments, USA). Orthophosphate concentration was quantified using the molybdenum blue colorimetric method following Murphy and Riley (1962).

Prior to statistical analysis, physicochemical variables were standardized using *z*-score transformation to account for differences in measurement scales. Because water chemistry was measured once per site ($n = 6$), whereas the microbiome data came from 68 fish nested within these sites, all environmental analyses accounted for this hierarchy. The effect of location on community composition was first tested by PERMANOVA (*adonis2*, vegan) on Bray–Curtis dissimilarities; this location R^2 is the maximum variation that any site-level variable can explain. The association of each physicochemical variable with community composition was then tested by PERMANOVA in which whole sites, rather than individual fish, were permuted (100 random subsamples of 10 fish per site). Variance partitioning (varpart) was used to describe the unique and shared contributions of the three environmental variables to community variation. Distance-based redundancy analysis (db-RDA) was used to visualize environmental gradients, and the significance of fitted vectors (*envfit*) was assessed with the same site-level permutation; collinearity was checked using variance inflation factors (VIF < 5 for all variables). Associations with individual taxa (prevalence $\geq 10\%$; 304 taxa) were explored using the default MaAsLin2 model (log2 transformation, linear model; Mallick et al. 2021) fitted to site-mean abundances ($n = 6$), with Benjamini–Hochberg false discovery rate correction ($q < 0.05$). Analyses were performed in R using vegan and permute packages.

3 | Results

3.1 | Helminths

During the field study period (March–August 2025), blackchin tilapia collected from Chumphon Province (CP) were the only specimens among the six study sites found to harbour an unidentified nematode species at high abundance in the gastrointestinal tract. The prevalence was 52% (26/50 fish), with infection intensity ranging from 10 to more than 100 individuals per fish. Morphological examination indicated that the worms exhibited a free-living rather than parasitic lifestyle, as evidenced by the presence of a large amphidial fovea, somatic setae, and the absence of structures adapted for tissue attachment (Figure 1). Based on these morphological characteristics, the specimens were assigned to the free-living superfamily Desmodoroidea Filipjev, 1922, as they closely match the diagnostic features of the superfamily according to the key to families provided by Tchesunov and Schmidt-Rhaesa (2014), including distinct cuticular annulation (Figure 1a), a smooth cephalic cuticle (Figure 1b), four cephalic setae (one pair on each lateral side of the cephalic region) (Figure 1b,c), spiral amphidial foveae (Figure 1b), and a conical tail tip (Figure 1d). Further classification to the family level was not possible because the unidentified desmodoroid formed a distinct monophyletic clade separate from other currently available Desmodoroidea reference sequences. Nevertheless, phylogenetic analysis consistently supported its placement within this superfamily (BP ≥ 50, Figure 1e).

3.2 | Bacteria

3.2.1 | Bacterial Diversity

Rarefaction analysis of full-length 16S rRNA gene amplicon sequencing data indicated that sequencing depth was sufficient to capture the majority of bacterial taxa present in the gut-content bacterial communities of invasive blackchin tilapia across all sampled locations in Thailand (Figure S1). Alpha diversity was assessed using the Chao1 richness estimator and the Shannon diversity index. Both indices revealed significant differences in alpha diversity among locations (Kruskal–Wallis test with post hoc analysis; Chao1: $p = 3.39 \times 10^{-7}$; Shannon: $p = 2.09 \times 10^{-7}$). The Chao1 index showed that SK had the highest richness while RY exhibited significantly lower species richness compared with all other locations except CP (Figure 2a). For Shannon index, PB had the highest Shannon diversity, reflecting greater species richness and evenness, compared to RY and CP. Similarly, RY showed the lowest Shannon diversity and differed significantly from all locations except CP. (Figure 2b and Table S2).

Beta diversity was evaluated using the Bray–Curtis dissimilarity index. Overall gut-content bacterial community composition differed significantly among locations (Permutational multivariate analysis of variance [PERMANOVA], $p = 0.001$) (Figure 2c). Notably, both dispersion (PERMDISP, $p = 1.80 \times 10^{-8}$) and community composition (ANOSIM, $p < 0.001$) differed significantly among locations. Pairwise comparisons between all pairs of locations were described in Table S3. Pairwise PERMDISP analysis indicated significantly higher dispersion in Samut Sakhon and Chumphon relative to other locations. Ordination analysis revealed three primary clustering patterns: Songkhla and Samut Prakan grouped together; Phetchaburi, Chumphon, and Samut Sakhon formed a second cluster; whereas Rayong was distinct from all other locations, forming an isolated group.

3.2.2 | Relative Abundance and Differential Abundance

At the phylum level, marked variation in gut-content bacterial community composition was observed among sampling locations (Figure 3a). Proteobacteria was the dominant phylum across all sites, with an overall mean relative abundance of $44\% \pm 19\%$. The relative abundances of other major phyla varied geographically. SK, PB, and SP were characterized by relatively high abundances of Firmicutes (26%–33%) and Planctomycetes (15%–37%). In contrast, RY and CP were distinguished by greater dominance of Fusobacteria, with mean relative abundances of $12\% \pm 10\%$ and $34\% \pm 28\%$, respectively. SS exhibited a heterogeneous community profile, displaying characteristics overlapping with multiple locations. Differential abundance analysis using LEfSe identified location-specific discriminatory taxa (LDA score > 4, $p < 0.05$) (Figure 4a). At the phylum level, Proteobacteria was a discriminatory taxon for RY, whereas Fusobacteria and Verrucomicrobia were associated with CP and SP, respectively. SK was characterized by Planctomycetes, Actinobacteria, and Firmicutes, while Firmicutes and Actinobacteria were discriminatory for PB. SS was characterized by Bacteroidetes.

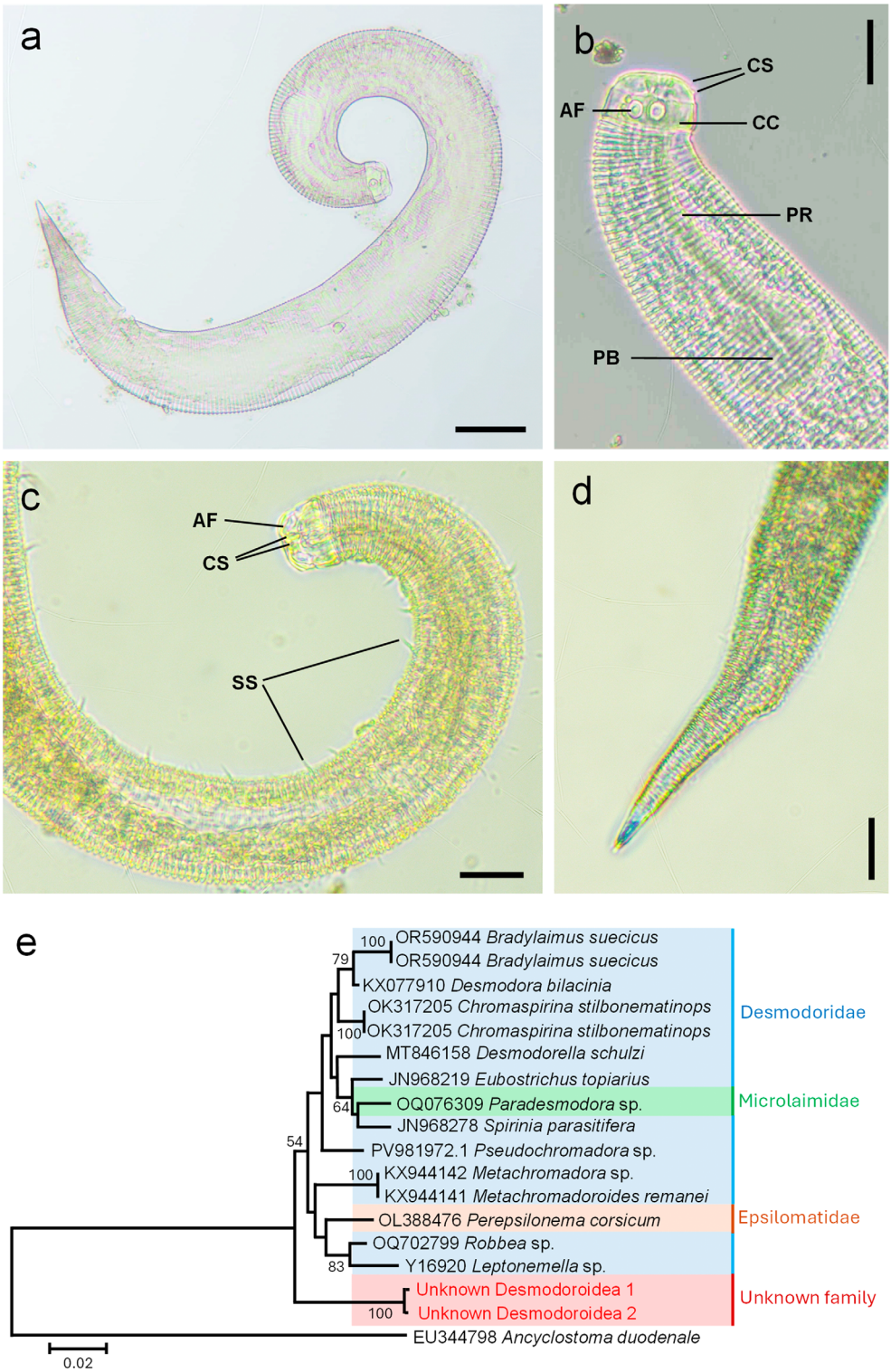

FIGURE 1 | Morphological and molecular phylogenetic placement of a free-living desmodorid nematode recovered from the gastrointestinal tract of blackchin tilapia collected in Chumphon, Thailand: whole body, dorsal view (a); cephalic region, dorsal view (b); cephalic region, lateral view (c); tail, lateral view (d). CS, cephalic setae; CC, cephalic cuticle; AP, amphidial fovea; PR, pharynx; PB, pharyngeal bulb; SS, somatic setae. Scale bars: 50 μm (a); 20 μm (b–d). Neighbour-joining phylogenetic tree (e) based on partial 18S rRNA sequences showing the desmodorid (red box/font). Bootstrap values > 50% are shown at nodes.

At the genus and species levels, gut-content bacterial community composition differed markedly among locations (Figure 3b,c). SK and SP shared similar dominant taxa, particularly *Thermogutta hypogea* and *Laceyella sacchari*. In SK, these species accounted for approximately 19% and 17% of the total community, respectively, whereas SP exhibited a more even distribution, with each contributing approximately 9%. Additional taxa, including *Cyanobacterium* TDX16 and *Roseimicrobium*

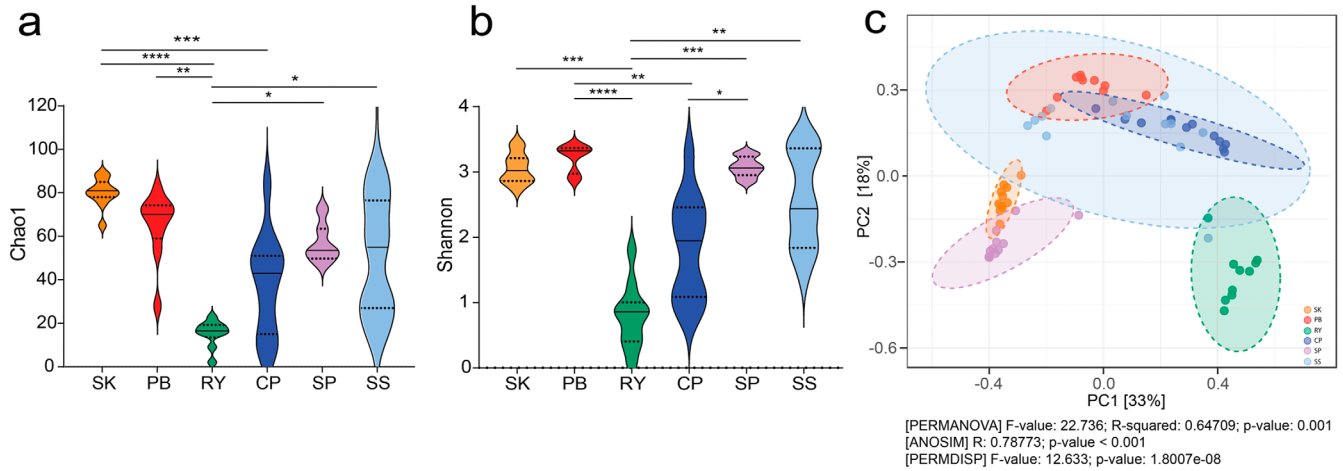

FIGURE 2 | Alpha and beta diversity of gut microbiota in invasive blackchin tilapia across sampling locations in Thailand. Alpha diversity was assessed using the Chao1 richness estimator (a) and the Shannon diversity index (b). Statistical differences among locations were evaluated using the Kruskal–Wallis test ($p < 0.05$). Beta diversity was assessed based on the Bray–Curtis dissimilarity index and visualized by principal coordinate analysis (PCoA) (c). Differences in community composition among locations were tested using PERMANOVA ($p < 0.05$). Asterisks indicate statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

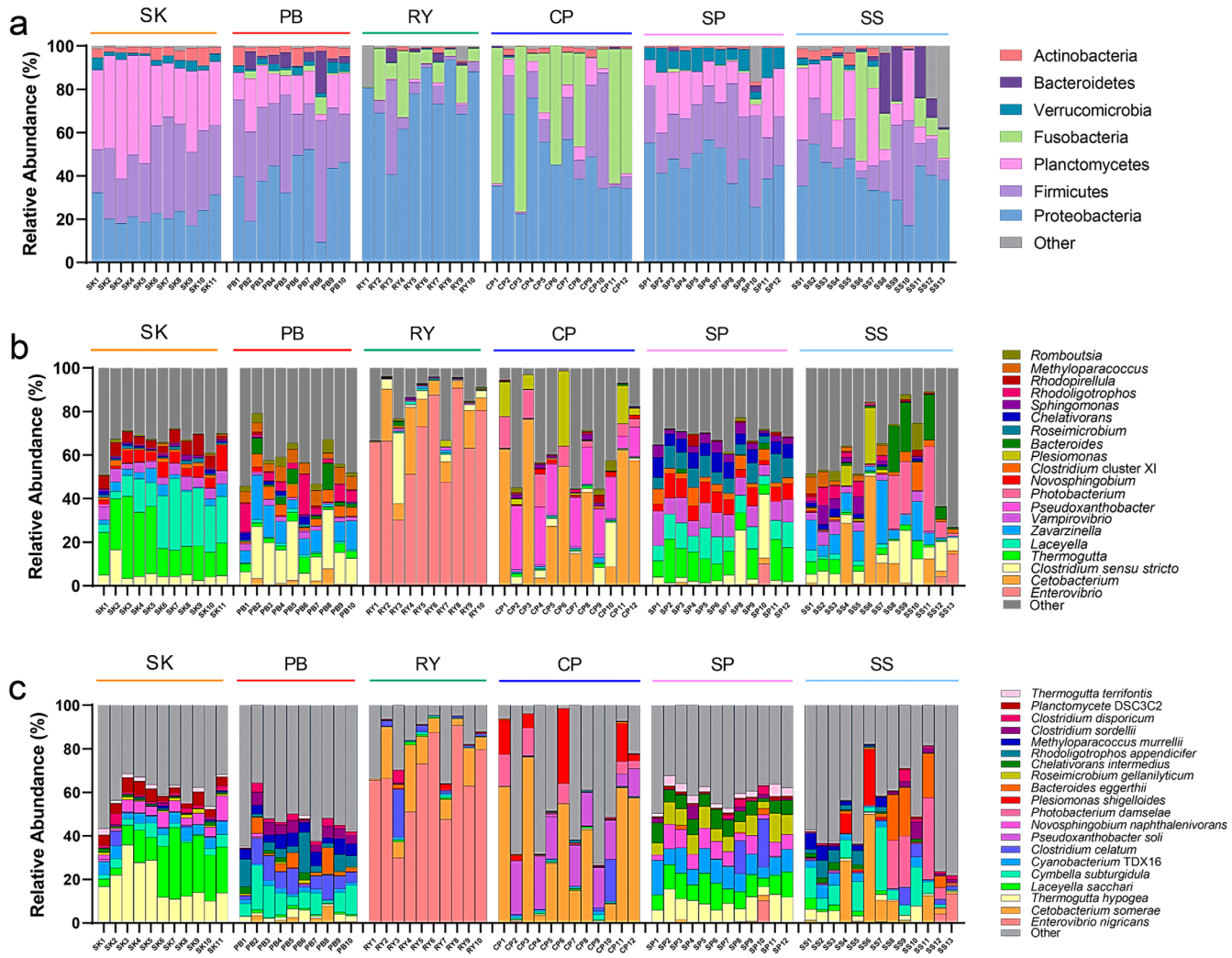

FIGURE 3 | Relative abundance of gut microbiota in invasive blackchin tilapia across sampling locations in Thailand. Relative abundances (%) based on full-length 16S rRNA gene amplicon sequencing are presented as stacked bar plots at three taxonomic levels: Phylum (a), genus (b), and species (c).

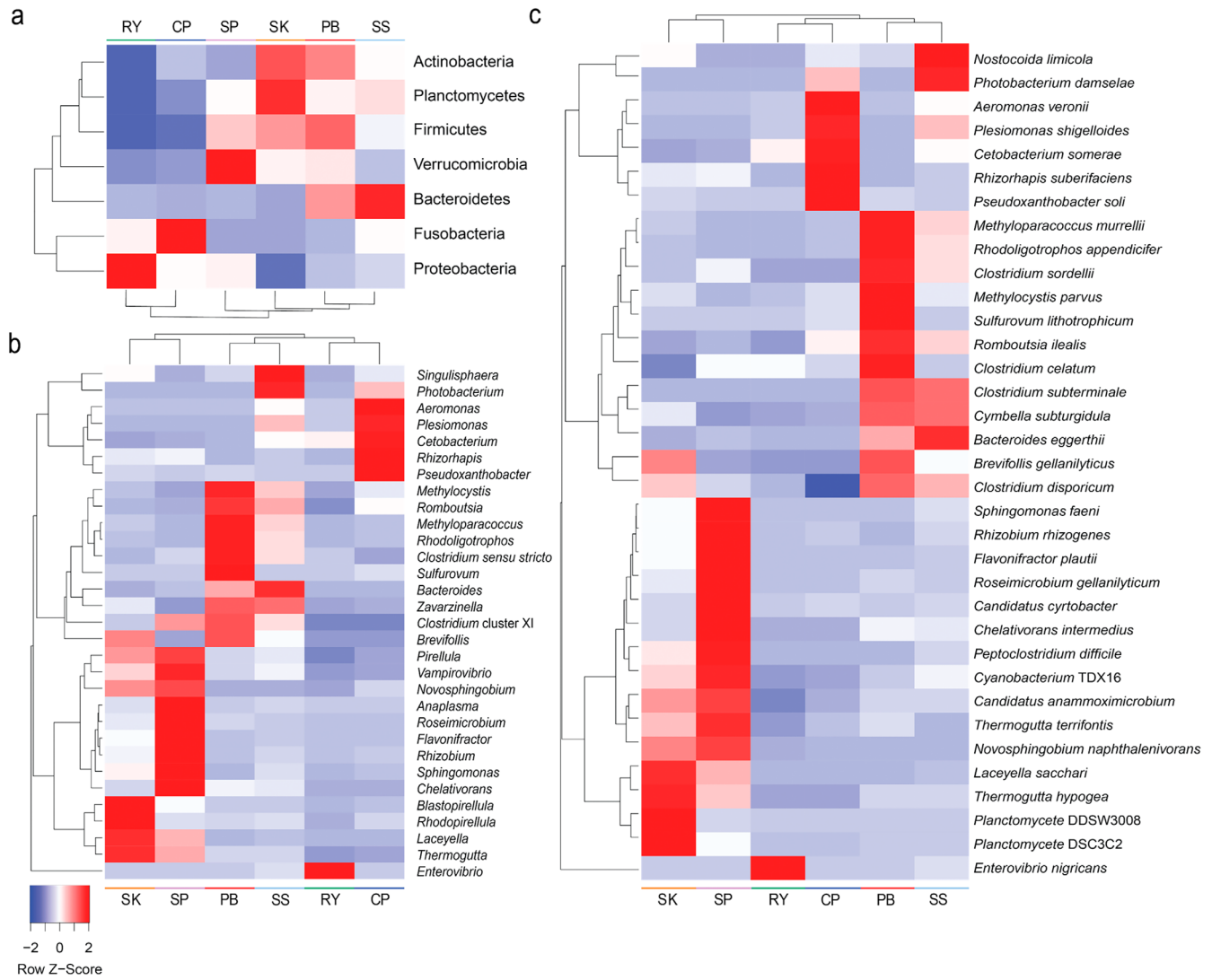

FIGURE 4 | Heatmap of differentially enriched gut microbiota taxa in different sampling locations, identified by linear discriminant analysis effect size (LEfSe) with an LDA score > 4 and $p < 0.05$. The heatmap was generated using normalized *z*-scores from LDA scores and visualized using Heatmapper. Results are shown at three taxonomic levels: Phylum (a), genus (b), and species (c).

gellanilyticum, also contributed substantially in SP. LEfSe analysis identified *Novosphingobium naphthalenivorans* and *L. sacchari* as discriminatory taxa associated with both SK and SP (Figure 4b,c).

In contrast, RY was strongly dominated by *Enterovibrio nigricans*, comprising approximately 65% of total relative abundance, and this species was the only significantly enriched taxon identified for this location. CP was primarily characterized by the enrichment of *Cetobacterium somerae*, although some individuals exhibited higher abundances of *Pseudoxanthobacter soli*. LEfSe analysis revealed multiple uniquely enriched taxa in CP, including *Aeromonas veronii*, *Plesiomonas shigelloides*, *C. somerae*, and *P. soli*. Microbial composition in SS varied substantially among individuals, with some profiles resembling CP-associated taxa (e.g., *C. somerae*, *P. shigelloides*) and others resembling PB-associated taxa (e.g., *Rhodoligotrophos appendicifer*, *Methyloparacoccus murrellii*, and *Cymbella subturgidula*). Consistent with this pattern, LEfSe identified *Clostridium subterminale* and *C. subturgidula* as discriminatory taxa shared between PB and SS.

3.2.3 | Taxon-Covariation Network

Taxon-covariation network analysis revealed structured correlation patterns among bacterial taxa (Figure 5). Several clusters of correlated taxa broadly corresponded to location-specific bacterial distributions. Taxa predominantly associated with SP and SK formed a cluster characterized by positive correlations, including *Candidatus* spp., *Sphingomonas* spp., and *N. naphthalenivorans*. Taxa primarily associated with PB and SS formed another cluster of correlated taxa, including *R. appendicifer* and *M. murrellii*. Additionally, taxa predominantly associated with SS and CP, including *P. shigelloides*, *A. veronii*, and *Aeromonas hydrophila*, formed a closely connected group characterized by positive correlation. Overall, the network revealed location-associated patterns of taxon covariation, with taxa sharing

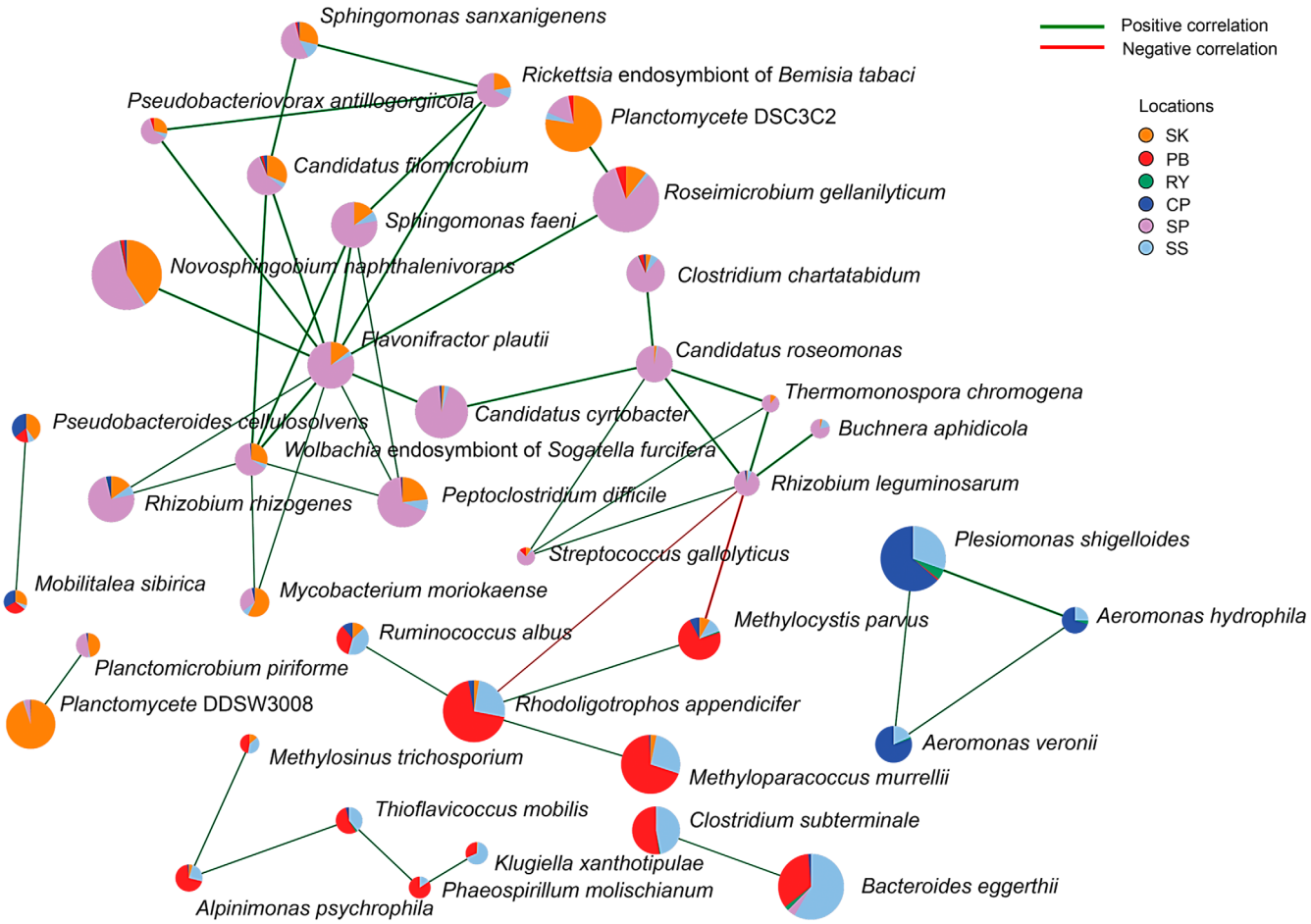

FIGURE 5 | Taxon-covariation network of gut microbiota in invasive blackchin tilapia across sampling locations in Thailand. The correlation network was constructed using the Sparse Correlations for Compositional Data (SparCC) method to represent correlations among bacterial species in the gut microbiota ($r > 0.7$, $p < 0.05$). Nodes are shown as pie charts indicating the relative abundance of each species across sampling locations. Edge colours indicate correlation type, with positive correlations shown in green and negative correlations shown in red.

similar geographic distributions showing stronger correlations in the observed dataset. These correlations represent patterns of taxon covariation and do not by themselves demonstrate direct microbial interactions, causal relationships, or environmental filtering.

3.2.4 | Functional Inference of Gut-Content Bacterial Communities

Functional prediction of the gut-content bacterial communities revealed clear differences in metabolic potential among sampling sites (Figure S2). Linear Discriminant Analysis Effect Size (LEfSe; LDA > 3, $p < 0.05$) identified site-specific differences in predicted pathways. Microbiota from RY formed a distinct functional cluster characterized by enrichment of inferred pathways associated with metabolic flexibility such as nitrogen metabolism, amino acid degradation, alternative carbon utilization, and osmotic stress responses. SK and SP exhibited similar predicted functional profiles, enriched in pathways related to central carbon metabolism, including both aerobic respiration (TCA cycle-associated pathways) and fermentative processes. These sites also showed enrichment in nitrogen and sulfur assimilation pathways supporting amino acid biosynthesis, as well as light-responsive and photosynthesis-associated pathways. CP was distinguished by strong enrichment of vitamin and cofactor biosynthesis pathways, including thiamine, folate, biotin, and cobalamin metabolism.

Additionally, CP showed potential enrichment of fermentation and anaerobic metabolic pathways, along with pathways supporting carbohydrate utilization and nitrogen metabolism. PB and SS shared enrichment of electron transport and redox-related pathways, particularly ubiquinone biosynthesis. However, PB exhibited higher enrichment of C1 compound utilization and fermentative metabolism, whereas SS was more enriched in core biosynthetic processes, including amino acid, nucleotide, and cell envelope biosynthesis pathways.

3.3 | Associations Between Site-Level Aquatic Parameters and Microbiome Composition

Location explained 65% of the variation in gut-content bacterial community composition among the 68 fish (PERMANOVA, $R^2 = 0.65$, $p < 0.001$). Because the physicochemical variables were measured once per site, this value is the upper limit for any site-level variable; together, pH, salinity, and orthophosphate concentration accounted for $R^2 = 0.305$ (Table S4), approximately half of the among-site variation. When the six sites were treated

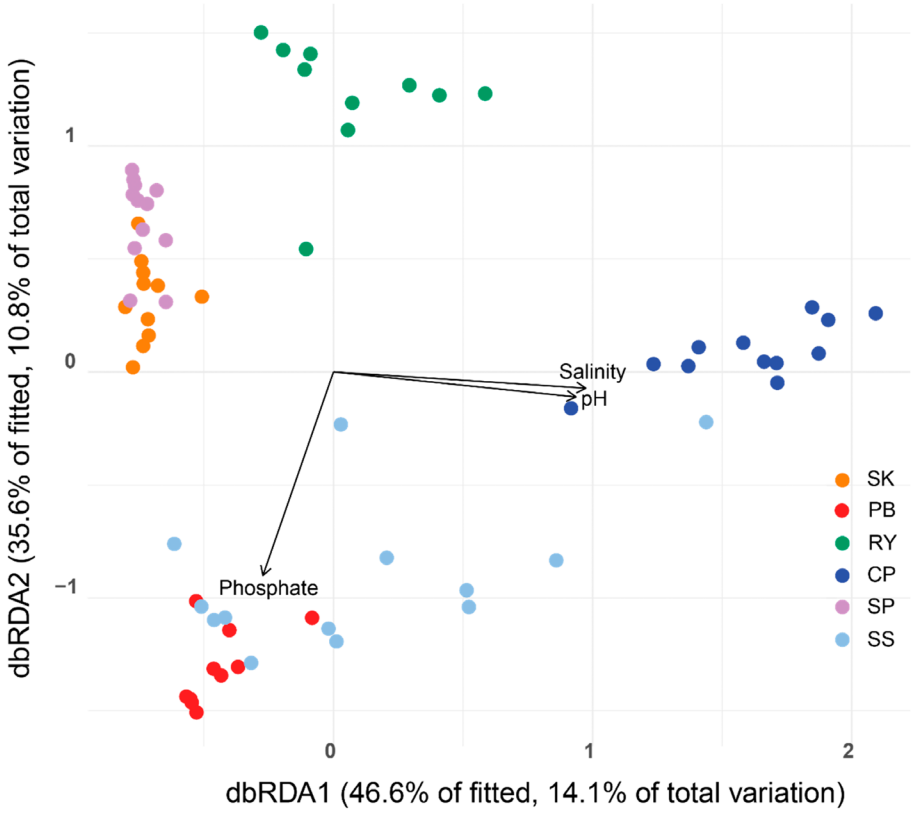

FIGURE 6 | Distance-based redundancy analysis (db-RDA) of microbial community composition across sampling locations based on Bray–Curtis dissimilarity. Physicochemical parameters (arrows) are site-level variables (one composite water sample per site; $n = 6$) fitted onto the ordination space for visualization, with arrow direction indicating the gradient of increasing values and arrow length reflecting the strength of the relationship with community structure. The first two db-RDA axes explain 47% and 36% of the fitted variation, corresponding to 14% and 11% of the total variation, respectively.

as the independent units, pH ($R^2 = 0.126$), salinity ($R^2 = 0.130$), and orthophosphate concentration ($R^2 = 0.113$) were not significantly associated with community composition (PERMANOVA with site-level permutation, median $p = 0.49$, 0.40, and 0.53, respectively; $p < 0.05$; Table S5). Variance partitioning indicated that the three variables together corresponded to 27% of the variation in community composition (Adj. $R^2 = 0.272$), with unique adjusted R^2 values of 0.027, 0.046, and 0.109 for pH, salinity, and orthophosphate concentration, respectively, and a further 0.117 shared between pH and salinity (68 fish from six sites; environmental predictors, $n = 6$; Table S4).

Distance-based redundancy analysis (db-RDA) (Figure 6) further illustrated the relationship between environmental gradients and bacterial community composition. Salinity and pH exhibited partial collinearity and were associated primarily with variation in Chumphon and Samut Sakhon, whereas orthophosphate concentration was more strongly aligned with variation in Samut Sakhon and Phetchaburi. Because these vectors are based on six site-level values, none was significant under site-level permutation (*envfit*, median $p = 0.08$–0.19; Table S5).

At the taxon level, site-mean regressions ($n = 6$) identified no taxa whose abundance was significantly associated with any physicochemical variable after FDR correction (all $q \geq 0.28$). Before correction, several taxa showed site-level trends: *Aeromonas veronii*, *A. hydrophila*, *A. allosaccharophila*, *A. salmonicida*, and *Photobacterium damselae* were more abundant at higher-salinity sites (unadjusted $p = 0.002$–0.013), and *Kaistia geumhonensis* and *Anaerobaculum thermoterrenum* were more abundant at higher-phosphate sites (unadjusted $p = 0.015$ and 0.017).

4 | Discussion

All blackchin tilapia examined across the six sampling sites harboured only a single non-parasitic helminth species, detected exclusively at CP with high prevalence and intensity. This contrasts with a previous survey from the same locality, in which no helminths were recovered from 164 individuals (Suwannarat et al. 2025). Despite the high diversity of helminths reported from native African populations (e.g., Echi et al. 2009; Euphrasie et al. 2021; Bouko et al. 2023), the relatively recent introduction of this species into Thailand (approximately 4–8 years ago) (Chaianunporn et al. 2024) may have limited parasite acquisition. Such a time-lag is expected, as many helminths possess complex life cycles involving multiple hosts, requiring ecological fitting and/or co-evolution before successful establishment in a novel host (Bennett et al. 2023; Latif et al. 2023; Suwannarat et al. 2025).

Host specificity may further constrain colonization. For instance, the zoonotic trematode *Haplorchis taichui* readily infects carp (*Cyprinus* spp.) but is absent in *Oreochromis* spp. under

experimental conditions (Kopolrat et al. 2015), suggesting reduced susceptibility of tilapia to certain local parasites. Together, these findings are consistent with predictions of the enemy release hypothesis (ERH), whereby reduced parasite pressure may facilitate the spread of invasive populations (Colautti et al. 2004; Marcogliese and Pietrock 2011; Chalkowski et al. 2018). However, the observed low helminth diversity does not directly demonstrate ERH, and alternative explanations, including the limited sampling period, local environmental conditions, and host specificity, should also be considered. There is currently no evidence that this species has altered local helminth transmission dynamics.

Interestingly, a high prevalence of free-living nematodes (Desmodoroidea) was also observed at CP. Although the taxonomy of this group remains incompletely resolved, its placement within Desmodorida de Coninck, 1965—a lineage composed entirely of free-living taxa—strongly indicates that these nematodes are non-parasitic. The specimens exhibited diagnostic features of free-living nematodes and lacked morphological traits typical of parasitic taxa (*sensu* Tchesunov and Schmidt-Rhaesa 2014). Their presence is therefore best explained by incidental ingestion during benthic feeding, as desmodorids are common sediment-dwelling organisms (Armenteros et al. 2014). As such, they are unlikely to affect host health or contribute to helminth infection dynamics in coastal ecosystems.

The gut-content bacterial community analyses revealed clear spatial differentiation among sampling locations. RY formed a distinct cluster, whereas SK and SP grouped together, and PB, SS, and CP showed closer similarity. Such spatial structuring suggests that local environmental conditions, dietary resources, and habitat characteristics contribute to shaping gut-content bacterial community composition (Bensch et al. 2023). Blackchin tilapia are opportunistic benthic feeders consuming zooplankton, phytoplankton, and sediment-associated materials (Ofori-Danson and Kumi 2009; Ndimele et al. 2010). Accidental ingestion of sediment or water during feeding may introduce environmentally derived microorganisms into the gut, some of which may represent transient rather than established members of the gut community. This possibility is particularly relevant for environmentally associated taxa detected in the present study, including *T. hypogea* and *L. sacchari* in SK and SP, and taxa associated with sediments or oligotrophic environments in PB and SS. Their detection may therefore reflect local dietary or environmental exposure rather than persistent colonization. The greater inter-individual variation observed in CP and SS may also reflect environmental heterogeneity within these sampling areas, where fish were collected across zones spanning estuarine to upstream habitats (Sompongchaiyakul and Sirinawin 2007). RY was characterized by a high relative abundance of *E. nigricans*, a taxon commonly associated with marine environments and marine fish hosts (Pujalte et al. 2015), whereas CP was dominated by *C. somerae*, a bacterium commonly associated with freshwater fish (Qi et al. 2023; Tsuchiya and Sugita 2023; Zhao et al. 2023). These location-specific patterns are therefore compatible with differences in environmental exposure and dietary resources, although the present data cannot distinguish stable colonization from transient passage.

The physicochemical data indicate that site-level water conditions covaried with the among-site differences in gut-content bacterial community composition. Together, salinity, pH, and orthophosphate concentration corresponded to about half of the variation attributable to location, which is consistent with their established roles in structuring aquatic microbial communities through osmotic stress, nutrient availability, and metabolic constraints (Uren Webster et al. 2020; Zhu et al. 2022). At the taxon level, several *Aeromonas* spp. and *P. damselae* tended to be more abundant at higher-salinity sites, particularly Chumphon, although these trends did not remain significant after FDR correction; salinity gradients may therefore contribute to the distribution of these taxa in the gut contents, but this requires confirmation (Morshed and Lee 2023). Similarly, exploratory associations between orthophosphate and specific taxa are consistent with the possibility that nutrient availability contributes to variation in microbial composition through bottom-up ecological processes (Zhu et al. 2022). However, the measured environmental variables corresponded to only approximately 27% of the variation in microbiome composition (Adj. R^2), indicating that other factors, including diet, microhabitat heterogeneity, host characteristics, and stochastic processes, may also contribute to microbiome assembly (Uren Webster et al. 2020; Zhang et al. 2024). Moreover, partial collinearity between pH and salinity indicates that these environmental variables may act in combination rather than independently. Importantly, because water chemistry was measured once at each of six sites, the environmental predictors have only six independent observations, and none of the community-level associations was statistically significant when sites were treated as the units of replication. With so few sites, statistical power is low, and the environmental variables are completely confounded with location; their effects therefore cannot be separated from other unmeasured location-specific factors, such as diet, sediment characteristics, habitat structure, and sampling date. The observed relationships should thus be interpreted as descriptive site-level associations rather than as evidence of environmental causation or environmental filtering.

Notably, taxa showing site-level trends in the taxon-level models did not always correspond to the most abundant taxa in relative-abundance profiles. This discrepancy likely reflects differences between analytical approaches: relative-abundance data are compositional and may obscure biological variation, particularly among dominant taxa (Lin and Peddada 2020), whereas the log-transformed linear models used here (following MaAsLin2) evaluate each taxon on a relative scale, independently of its overall abundance (Mallick et al. 2021). Thus, taxa showing site-level trends with environmental variables need not be the most abundant members of the community.

The pronounced spatial structuring observed in taxonomic composition, taxon-covariation patterns, and predicted functional pathways indicates that geographic context is associated with variation in gut-content bacterial community composition in invasive *S. melanotheron* (Sullam et al. 2012; Burns et al. 2016). Because all individuals belonged to the same host species and were sampled within a similar period, some host-related variation was reduced; nevertheless, clear geographic differences persisted. The conserved core microbiota suggests that host-associated factors may

contribute to maintaining a common component of the bacterial community (Shade and Handelsman 2012). Similar core microbiota patterns have been reported in Nile tilapia, suggesting that host physiology may constrain bacterial community composition to some extent (Sullam et al. 2012). Together, these observations are compatible with a dual-assembly framework in which gut-content bacterial communities reflect both environmental exposure and host-associated processes (Costello et al. 2012). However, because host filtering was not directly quantified and the present study did not experimentally separate environmental from host effects, the relative contributions of these processes cannot be determined.

The observed geographic variation in gut-content bacterial communities may be relevant to the ecological flexibility of invasive *S. melanotheron*, but whether such variation contributes to invasion success remains unresolved. As *S. melanotheron* disperses across heterogeneous aquatic systems, differences in gut-content bacterial communities associated with local conditions may potentially reflect microbial responses accompanying environmental acclimatization (Hauffe and Barelli 2019; Henry et al. 2021). However, the present study did not measure host performance, physiological fitness, or invasion-related traits, and therefore cannot determine whether the observed microbial variation provides a functional advantage to the host. Similarly, the near absence of parasitic helminths across most sites is consistent with predictions of the Enemy Release Hypothesis (Torchin et al. 2003), but does not establish reduced parasite pressure as a mechanism of invasion success. Gut-associated bacterial communities may potentially contribute to resistance against parasite establishment because microbial communities can influence pathogen colonization and host immune responses (Gómez and Balcázar 2013; Llewellyn et al. 2014). Alternatively, environmental conditions may independently constrain parasite survival or transmission, with microbiome composition representing a response to the same environmental gradients rather than a direct defensive mechanism (Poulin 2006; Marcogliese 2008; Sullam et al. 2012). Comparative and experimental studies will therefore be required to determine whether microbiome characteristics contribute causally to parasite exclusion or invasion performance.

The strong geographic signal observed in the gut-content bacterial community suggests that these communities may reflect differences in local environmental conditions, although their value as environmental indicators requires further validation (Suzzi et al. 2022). Taxa such as *C. somerae*, which have previously been associated with host nutrition and health in fish, may also have potential relevance to aquaculture (Huavas et al. 2024). More broadly, the findings are compatible with a metacommunity perspective in which spatially structured environmental microbial pools contribute to variation in host-associated microbiomes (Leibold et al. 2004; Costello et al. 2012). Environmental management of factors such as nutrient loading, salinity, and habitat structure may therefore influence microbial communities associated with invasive fish, although whether such changes affect invasion performance remains to be tested. Future studies incorporating a larger number of sites, replicated environmental sampling within sites, temporal monitoring, comparative host sampling, and experimental manipulation will be important for distinguishing environmental associations from causal mechanisms.

5 | Conclusions

This study demonstrates that gut-content bacterial communities differed among the six sampled locations of invasive *S. melanotheron* across Thailand, with clear geographic variation in community composition, dominant taxa, taxon-covariation patterns, and predicted functional profiles. Site-level water characteristics, particularly salinity, pH, and orthophosphate concentration, covaried with these among-site differences; however, with only six independent environmental observations, these associations were not statistically significant and cannot be separated from other location-specific factors. These relationships should therefore be interpreted as descriptive site-level associations rather than evidence of environmental causation, and the measured variables accounted for only part of the observed variation. The remaining variation may reflect unmeasured environmental conditions, dietary differences, host characteristics, temporal variation, and other ecological processes. Parasitological screening revealed a very low occurrence of parasitic helminths across the sampled populations, although the presence of free-living nematodes at CP highlights the importance of distinguishing incidental ingestion from true parasitism. The low helminth diversity is consistent with predictions of the Enemy Release Hypothesis but does not provide a direct test of this hypothesis. Together, these findings support a dual-assembly framework in which geographic context is associated with marked variation in gut-content bacterial communities at the regional scale, while host-associated processes may still contribute to the maintenance of a conserved bacterial community. This raises the possibility that variation in gut-content bacterial communities, together with low parasite burden, may support functional adaptation to local environments, ecological resilience, and ultimately facilitate invasion success in this species. However, whether these microbial patterns directly contribute to host performance or invasion success remains to be determined.

Author Contributions

Vorthon Sawaswong: conceptualization, methodology, validation, formal analysis, investigation, software, writing – review and editing, supervision, project administration, funding acquisition. **Wattanapol Nuangjakjoy:** methodology, formal analysis, investigation, data curation, visualization, writing – original draft. **Vachirapong Charoennitiwat:** methodology, formal analysis, investigation, data curation, visualization, writing – original draft. **Sila Viriyautsahakul:** methodology, formal analysis, investigation, data curation, visualization, writing – original draft. **Suthida Visedthorn:** methodology, formal analysis. **Toemthip Poolpak:** methodology, funding acquisition. **Sakonwan Kuhaudomlarp:** validation, supervision, writing – review and editing. **Nisamanee Charoenchon:** validation, writing – review and editing, supervision. **Sunchai Payungporn:** supervision, resources. **Urusa Thaenkham:** supervision, funding acquisition. **Napat Ratnarathorn:** conceptualization, methodology, validation, investigation, resources, visualization, writing – original draft, writing – review and editing, supervision, project administration, funding acquisition.

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Ethics Statement

All animal procedures were conducted in accordance with institutional and national guidelines for the care and use of laboratory animals. Ethical approval was granted by the Institute Animal Care and Use Committee (MUSC-CAF IACUC), Faculty of Science, Mahidol University (protocol no. MUSC68-002-772). This study did not involve human participants, human data, or human tissue.

Consent

The authors have nothing to report. The manuscript does not contain data from any identifiable individual.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The full-length 16S rRNA gene amplicon sequencing datasets generated during this study will be deposited in the NCBI Sequence Read Archive (SRA). Accession numbers and persistent links will be provided upon acceptance of the manuscript. All other data generated or analysed during this study are included in this published article and its supplementary information files. Additional materials are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Rarefaction curve of gut microbiota classified based on 16S rRNA full-length sequencing using Oxford Nanopore Technologies. The result of rarefaction analysis was represented by a line graph plotting the number of observed taxa against the sequencing depth. **Figure S2:** Heatmap of differentially enriched functional pathways of the gut microbiota in invasive blackchin tilapia across sampling locations in Thailand. Functional pathways were inferred using PICRUSt2 and identified by Linear Discriminant Analysis Effect Size (LEfSe) with and LDA score >3 and $p<0.05$. Heatmap values represent z-score normalized pathway abundances. **Table S1:** Geographic coordinates of blackchin tilapia sampling sites. Coordinates represent center points of collection areas, with specimens obtained within a 5 km radius of each location. **Table S2:** Pairwise comparisons of alpha diversity among sampling locations based on Chao1 and Shannon indices. Pairwise comparisons were performed using Dunn's multiple comparisons test following the Kruskal–Wallis test. p-values were adjusted for multiple comparisons, with statistical significance defined as $p<0.05$. **Table S3:** Pairwise comparisons of microbial community composition and dispersion among locations were conducted using PERMANOVA, ANOSIM, and PERMDISP based on Bray–Curtis dissimilarity. Values shown represent p-values adjusted for multiple testing using the Benjamini–Hochberg (false discovery rate, FDR) method. **Table S4:** Variance partitioning of microbial community composition (relative-abundance data) among three site-level environmental variables using RDA (68 fish from six sites; environmental predictors measured once per site, $n=6$). Values shown are R^2 and adjusted R^2 (Adj. R^2). Unique Adj. R^2 represents the independent contribution of each variable after controlling for the other two. **Table S5:** Associations between site-level environmental variables and gut-content bacterial community composition (Bray–Curtis), analysed with the six sites as the independent units of replication. Microbiome data: 68 fish from six sites; environmental predictors: one composite water sample per site ($n=6$).