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Effects of polycyclic aromatic hydrocarbon enrichment on microbial community structure in polar sediments

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Abstract Understanding microbial responses to polycyclic aromatic hydrocarbon (PAH) pollution is crucial for assessing the current status of PAH contamination in polar regions. In this study, intertidal and marine sediments were enriched with a mixture of PAHs (naphthalene, phenanthrene, fluorene, pyrene, and fluoranthene). Isolation of culturable bacteria, high-throughput sequencing, and functional prediction were combined to systematically analyze bacterial structural and predicted functional responses to PAH exposure. High-throughput sequencing results showed that the relative abundance of Proteobacteria was significantly increased after enrichment, and *Pseudomonas* and *Acinetobacter* were identified as dominant genera under PAH exposure. These findings were consistent with the 19 potential PAH-degrading strains (mainly *Pseudomonas*) that were successfully isolated from enrichment cultures. Distinct bacterial taxa between enriched marine and intertidal sediments indicated the existence of distinct PAH-degrading groups. PICRUSt2-based functional predictions suggested higher predicted abundances of PAH-degradation pathways in polar sediments, likely through the preferential degradation of parent PAH compounds in response to elevated concentrations. This study provides valuable data on microbial responses to PAH pollution in polar regions and offers new insights for evaluating ecological hazards induced by PAHs.

Keywords **polycyclic aromatic hydrocarbon, microbial community, Arctic, Antarctic**

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1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are classified as ubiquitous semi-volatile organic compounds (SVOCs) characterized by a rigid planar configuration of two or more fused aromatic rings. Their ecotoxicological effects, environmental persistence, long-range transport potential, and bioaccumulation capacity have been extensively documented (Latimer and Zheng, 2003). Based on molecular weight, PAHs are categorized into low-molecular-weight PAHs (LMW-PAHs, primarily 2–3 ring structures such as naphthalene and phenanthrene) exhibiting higher aqueous solubility, and high-molecular-weight PAHs (HMW-PAHs, ⩾4 ring structures exemplified by pyrene and benzo[a] pyrene)

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characterized by pronounced lipophilicity. HMW-PAHs undergo slower degradation under natural conditions compared to their LMW-PAHs (Alegbeleye et al., 2017). PAHs originate from both natural and anthropogenic sources, with natural emissions primarily attributed to forest fires, volcanic activities, and diagenetic processes, while anthropogenic releases are predominantly recognized as deriving from vehicular exhaust, coal-based residential heating, and industrial operations (Van Metre et al., 2000).

The Arctic and Antarctic play critical roles in regulating the Earth's climate and hosting unique and diverse polar ecosystems. However, numerous anthropogenic organic pollutants are being released into the environment, and abiotic factors driven by human activities are the primary drivers of changes in polar ecosystems (Hogg et al., 2006). These anthropogenic pollutants have damaged the relatively fragile polar ecosystems. PAHs have been reported as one of the most abundant anthropogenic pollutants in the Antarctic (Panicker et al., 2010). In addition to local direct emissions, PAHs can volatilize into the atmosphere and undergo long-range environmental transport. Through global migration mediated by marine biogeochemical processes and atmospheric circulation, they can be indirectly transported to polar regions, where they condense, deposit, and eventually accumulate (Hansen et al., 2006). This process may lead to polar regions becoming the ultimate sinks for global anthropogenic pollutants (Casal et al., 2019; Xie et al., 2022). Moreover, the low temperatures in polar environments prolong the chemical stability of PAHs and exacerbate their bioaccumulation by reducing desorption rates (Wania and MacKay, 1993). In addition, as a form of refractory dissolved organic carbon, PAHs inevitably bind with organic matter once released into aquatic systems and settle into deep waters and sediments.

Microbial communities serve as critical drivers of polar biogeochemical cycles and respond rapidly to environmental stress. In polar marine sediments, bacterial and archaeal communities constitute the primary contributors to taxonomic diversity and biomass, accounting for approximately 90% of total benthos (Jørgensen and Boetius, 2007). Structural shifts in microbial communities are exposed to PAHs through selective pressure on taxa with degradation capabilities. The development of high-throughput sequencing techniques has provided new perspectives for microbial ecology research and enabled direct examination of microbial responses to pollutant exposure. Current amplicon-based studies on polar microbial responses to PAHs primarily focus on structural and functional analyses under in-situ conditions, while comparative investigations of community divergence under artificially introduced PAH enrichment conditions are lacking (Ellis et al., 2022; Iriarte et al., 2023).

This study presents the comparative analysis of microbial taxa in polar sediments from both the Arctic and Antarctic regions. Community shifts were comparatively analyzed using high-throughput sequencing techniques under in-situ conditions and after artificial introduction of PAH enrichment. Bacterial strains with potential PAH-degrading capabilities were isolated from enriched cultures. Functional profiles of the bacterial communities within the study area were investigated using prediction methods. Understanding microbial responses to PAH enrichment in polar sediments is crucial for characterizing structural impacts of these pollutants on microbial communities. This knowledge is essential for developing bioremediation strategies using polar microorganisms to restore PAH-contaminated polar regions.

2 Materials and methods

2.1 Sampling sites and sample information

Antarctic samples comprised 5 marine sediment (C5_09A, CA_10A, CD_11A, DA03A, P1_12A) and 2 intertidal sediment (CJW02A, CJW04A) specimens obtained during 39th Chinese National Antarctic Research Expedition (2022), primarily covering waters adjacent to the Antarctic continent. Arctic samples consisted of 3 intertidal sediments (NCJW01A, NCJW02A, NCJW03A) collected from Ny-Ålesund (Svalbard Archipelago) during 13th Chinese National Arctic Research Expedition (2023). A total of 10 polar sediment samples were acquired. To minimize contamination risks, the upper 1 cm sediment layer was carefully removed using sterile spatulas, followed by the collection of surface sediments with flame-sterilized spatulas. All samples were stored in sterile sampling bags and preserved at –80 ℃ pending analysis. Sampling locations were visualized using Ocean Data View (Figure 1) (Schlitzer, 2022). For subsequent analyses, samples were grouped by habitat type, with marine sediments designated as MS and intertidal sediments designated as IS. All MS samples were derived exclusively from Antarctic marine sediments, whereas IS samples included both Antarctic and Arctic intertidal sediments.

2.2 Enrichment cultivation using PAHs as sole carbon sources

Microbial enrichment cultures were established using Antarctic marine, intertidal, and Arctic intertidal sediment samples with PAHs as the sole carbon source. A mineral salt liquid medium (MSM) was prepared comprising the following components: K_2HPO_4 (1 $g \cdot L^{-1}$), NaH_2PO_4 (1 $g \cdot L^{-1}$), $CaCl_2$ (0.02 $g \cdot L^{-1}$), $MgSO_4 \cdot 7H_2O$ (0.2 $g \cdot L^{-1}$), NaCl (0.5 $g \cdot L^{-1}$), $FeSO_4 \cdot 7H_2O$ (0.01 $g \cdot L^{-1}$), and NH_4Cl (1 $g \cdot L^{-1}$). The medium was then adjusted to pH 7.0 and sterilized at 121 ℃ for 20 minutes. A mixed PAH stock solution (500 $mg \cdot L^{-1}$) was generated by dissolving 25 mg each of naphthalene, fluorene, phenanthrene, pyrene, and fluoranthene in 50 mL of acetone, followed by sterile filtration through a 0.22 μm nylon syringe filter and light-protected storage. This stock solution was added to the

mineral salt medium to achieve a final concentration of approximately 25 m·L^{-1} per PAH, with the mixture allowed to stand until complete acetone evaporation. Under laminar flow conditions, 5 g of sediment was aseptically weighed and transferred into 50 mL of the PAHs-MSM medium. Cultures were incubated at 16 ℃ with orbital shaking (160 rpm) for one week, followed by subculturing (5 mL inoculum) into fresh PAHs-MSM medium for an additional week of enrichment. All glassware was pre-treated for organic contaminant removal prior to use.

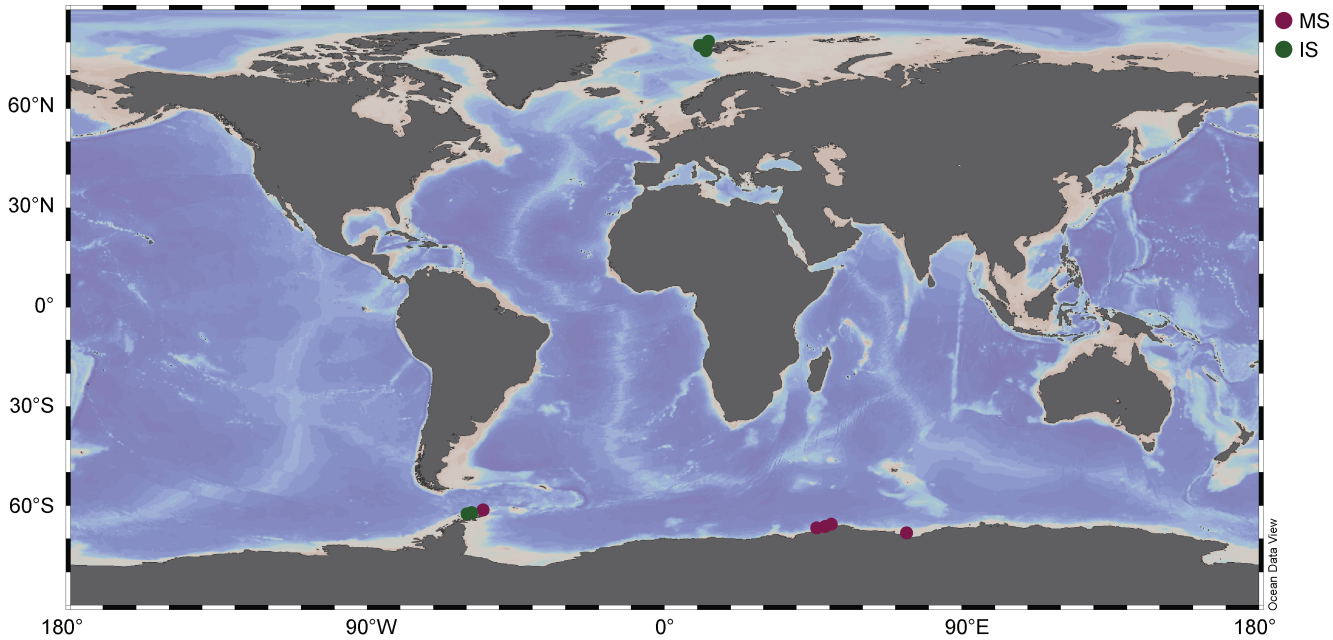

Figure 1 Map of the sampling sites using the Ocean Data View.

2.3 Bacterial isolation and 16S rRNA gene sequencing

Diluted enrichment cultures were spread onto solid PAHs-MSM medium and incubated at 16 ℃ for 3–5 days. Colonies were selected based on morphological characteristics and subjected to 16S rRNA gene amplification. The 16S rRNA gene was amplified using universal bacterial primers B8F (5'-AGAGTTTGATCCTGGCTCAG-3') and B1510R (5'-GGTTACCTTGTTACGACTT-3'). The thermal cycling protocol comprised initial denaturation at 94 ℃ for 5 min; 30 cycles of denaturation (94 ℃, 1 min), annealing (55 ℃, 1 min), and extension (72℃, 1.5 min); followed by final extension at 72 ℃ for 10 min. PCR products were examined by 1% agarose gel electrophoresis, and qualified amplicons were submitted for sequencing analysis (Majorbio Bio-Technology Company (China)).

2.4 DNA extraction and sequencing

Environmental DNA was extracted using the FastDNA Spin Kit for Soil (MP Biomedicals, USA). Specifically, 1 g of in-situ sediment and the pellet obtained from 1 mL of a 3-week enriched culture after high-speed centrifugation were processed. Briefly, samples were homogenized by bead beating with Lysing Matrix E, DNA was adsorbed onto a silica matrix, washed with SEWS-M buffer, and finally eluted in 100 μL of DES buffer. DNA concentration and purity were determined with a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Qualified samples were stored at −80 ℃ for subsequent analysis. For both in-situ sediment samples and 3-week enriched cultures, the V3–V4 region of the 16S rRNA gene was amplified using primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Caporaso et al., 2011) following the PCR protocol by Chen et al (2022). High-throughput amplicon sequencing was performed on the Illumina NovaSeq platform by Majorbio Bio-Technology Company (China). Sequences were processed using the VSEARCH plugin in QIIME2 (Rognes et al., 2016) for paired-end joining and dereplication. Amplicon Sequence Variants (ASVs) were generated via DADA2 denoising in QIIME2 to eliminate effects of random sequencing errors, low-quality reads, and chimeric sequences. Taxonomic assignment of ASVs was conducted using a self-trained classifier based on the SILVA database v138/16S rRNA gene reference. Raw data have been archived in the NCBI BioProject database (BioProject number: PRJNA1293735)

2.5 Statistical analysis

Alpha diversity indices (Chao1, Shannon and Simpson) were calculated using Mothur (v1.30.1) (Schloss et al., 2009). Interrelationships among α-diversity metrics were assessed via Spearman correlation analysis (psych R

package), while differences between sample groups were evaluated by ANOSIM (vegan R package). Changes in community composition based on relative abundance patterns were statistically analyzed and visualized using SPSS (v22.0) (Xu et al., 2014). Beta diversity patterns were visualized through non-metric multidimensional scaling (NMDS) (vegan R package) (Hoshino et al., 2020). Hierarchical clustering of phylum-level species composition was performed based on Bray-Curtis dissimilarity (vegan), with dendrogram visualization implemented using "ggtree" (Xu et al., 2022).

2.6 Predictive functional analysis

Functional genes and pathways were predicted using PICRUSt2 with the Integrated Microbial Genomes (IMG) database (Douglas et al., 2020). A phylogenetic workflow based on 16S rRNA marker gene sequences was implemented to derive relative abundances of gene families through alignment against the KEGG database. Taxonomic ASVs were initially normalized by 16S rRNA gene copy number, achieved by dividing each ASV by its known gene copy abundance. Normalized ASVs were then utilized to infer gene content for uncultured taxa based on genomic annotations of phylogenetically proximate reference species, enabling prediction of community-wide pathway abundances via KEGG mappings. The Nearest Sequenced Taxon Index (NSTI) was applied to validate the reliability of predicted functional pathways. Differential abundance analysis of these pathways was subsequently performed using STAMP to identify statistically significant alterations in bacterial gene and pathway profiles following PAH exposure (Parks et al., 2014).

3 Results

3.1 Isolation of PAH-degrading bacterial strains

Culturable PAH-degrading bacteria were obtained from enrichment cultures exposed to PAHs (naphthalene, phenanthrene, fluoranthene, pyrene, and fluorene), as tabulated. Thirty-one bacterial isolates were acquired from polar sediment samples and identified through cultural characteristics and 16S rRNA gene sequencing. Identification revealed 19 known species, with 13 strains isolated from marine sediments, affiliated with Proteobacteria, Actinobacteria, and Firmicutes (Table 1). The MS isolates were predominantly composed of Gammaproteobacteria, including 5 *Pseudomonas* strains, 2 *Acinetobacter* strains, 2 *Psychrobacter* strains, and 1 *Stenotrophomonas* strain. All IS isolates were assigned to Gammaproteobacteria, comprising 5 *Pseudomonas* strains and 1 *Acinetobacter* strain.

Table 1 Cultivable bacteria from polar sediments obtained from PAH enrichment cultures.

Group	Phylum	Order	Family	Species
MS	Proteobacteria	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas shirazensis*
				Pseudomonas kermanshahensis
				Pseudomonas alloputida
				Pseudomonas hibiscicola
				Pseudomonas putida
			Moraxellaceae	*Acinetobacter lwoffii*
				Acinetobacter radioresistens
				Psychrobacter cibarius
				Psychrobacter vallis
			Lysobacteraceae	*Stenotrophomonas maltophilia*
	Actinobacteria	Actinomycetia	Nocardiaceae	*Rhodococcus qingshengii*
			Microbacteriaceae	*Microbacterium liquefaciens*
	Firmicutes	Bacilli	Bacillaceae	*Peribacillus muralis*
IS	Proteobacteria	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas nunensis*
				Pseudomonas mandelii
				Pseudomonas iridis
				Pseudomonas frederiksbergensis
				Pseudomonas moorei
			Moraxellaceae	*Acinetobacter johnsonii*

3.2 Alpha diversity of each sampling site before and after PAH enrichment

Following sequence quality control and clustering, 12,172 ASVs were obtained across 4 sample groups: 7,389 in MS_Control, 215 in MS_Treat, 4,318 in IS_Control, and 250 in IS_Treat. Chao1 indices ranged from 22 to 1,588.56 (with 99% coverage), Shannon indices from 0.16 to 1.42, and Simpson indices from 0.05 to 0.100 (Figure 2). Alpha diversity was assessed via Chao1, revealing maximum values at Co_DA03A (MS) and Co_NCJW03A (IS), with minima at Tr_DA03A (MS) and Tr_CJW02A (IS). Significant reductions in Chao1 indices (p<0.001) were observed between Control and Treatment groups in both sediment types, indicating substantially altered community richness. Simpson diversity peaked at Co_P1_12A (MS) and Co_NCJW03A (IS), with minima at Tr_CA_10A (MS) and Tr_CJW02A (IS). While Simpson indices showed significant differences only in IS, Shannon indices exhibited statistically significant alterations (p<0.05) across all comparisons. These results demonstrate progressive reductions in microbial diversity and richness under PAH enrichment.

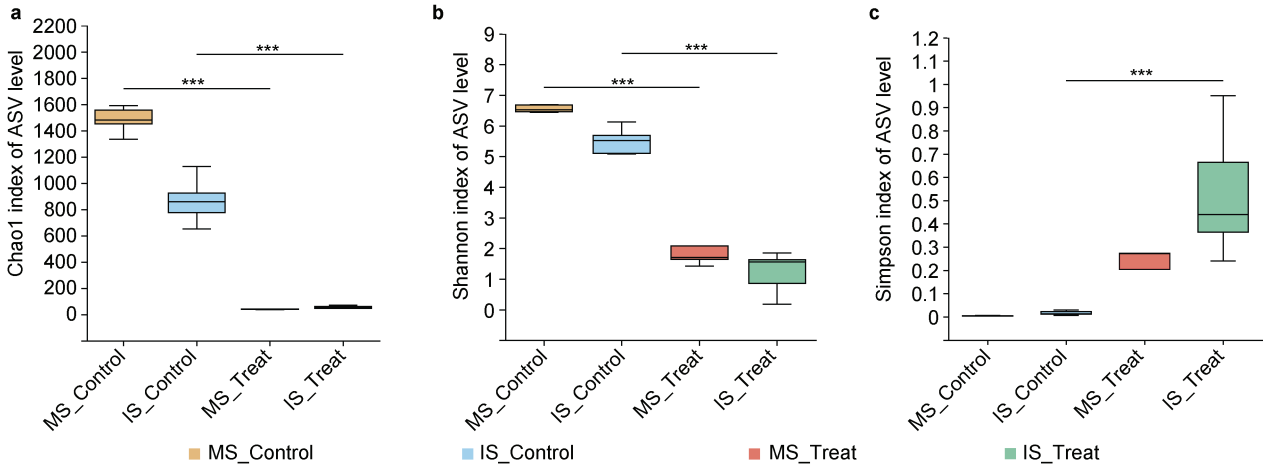

Figure 2 The α-diversity indices in the marine sediments (MS) and intertidal sediments (IS). **a**, Chao1 index; **b**, Shannon index; **c**, Simpson index. The significant difference in α-diversity indices between groups was calculated using the Kruskal-Wallis test (***, p<0.001).

3.3 Composition and beta diversity of the community

NMDS revealed significant segregation of Control and Treatment samples from both MS and IS into 4 distinct clusters. ANOSIM analysis further confirmed substantial inter-group differences (R=0.8380, p<0.001) (Figure 3a). Hierarchical clustering based on phylum-level relative abundances demonstrated divergent species composition between MS and IS under control conditions, whereas convergent compositional profiles were observed in Treat groups following PAH exposure (Figure 3b).

Proteobacteria constituted the most abundant phylum across polar sediments and exhibited significantly enhanced dominance under PAH enrichment, with increasing relative abundances observed in all samples. The most pronounced increase occurred at station C5_09A (33.92% to 97.10%), while minimal change was recorded at DA03A (0.7% increase). In IS, Proteobacteria abundances increased by >35% universally. In contrast, Actinobacteriota—the second most abundant phylum—generally declined, though station DA03A showed a 40.22% increase.

At the genus level, the top 30 most abundant genera were selected for compositional analysis, with the remaining genera categorized as "Others" (Figure 3c). Distinct differences were observed. Dominant genera in in-situ marine sediments included *Woeseia*, norank_o__Actinomarinales, norank_p__NB1-j, and unclassified_c__Gammaproteobacteria. In PAHs-enriched communities, *Pseudomonas* exhibited significantly higher relative abundance. However, this shift was not observed in samples CA_10A, P1_12A, or DA03A. Instead, *Acinetobacter* dominated in CA_10A and P1_12A, while DA03A was characterized by unclassified_f__Micrococcaceae. Additional dominant genera in MS_Treat included *Sphingobium* and *Stenotrophomonas*, *Brevundimonas*, and *Rhodococcus*. Similar trends were identified in intertidal sediments: pre-treatment dominants (*Granulosicoccus*, *Ilumatobacter*, unclassified_f__Flavobacteriaceae) were replaced by *Pseudomonas* as the most abundant genus post-PAH enrichment, with *Janthinobacterium*, *Methylobacterium*, and *Acinetobacter* also demonstrating growth advantages.

3.4 Distinct bacterial taxa of the polar sediments

LEfSe analysis was employed to identify statistically differentiated, distinct bacterial taxa among sediment samples and characterize specialized communities. As illustrated in Figure 4a, 86 bacterial clades exhibited

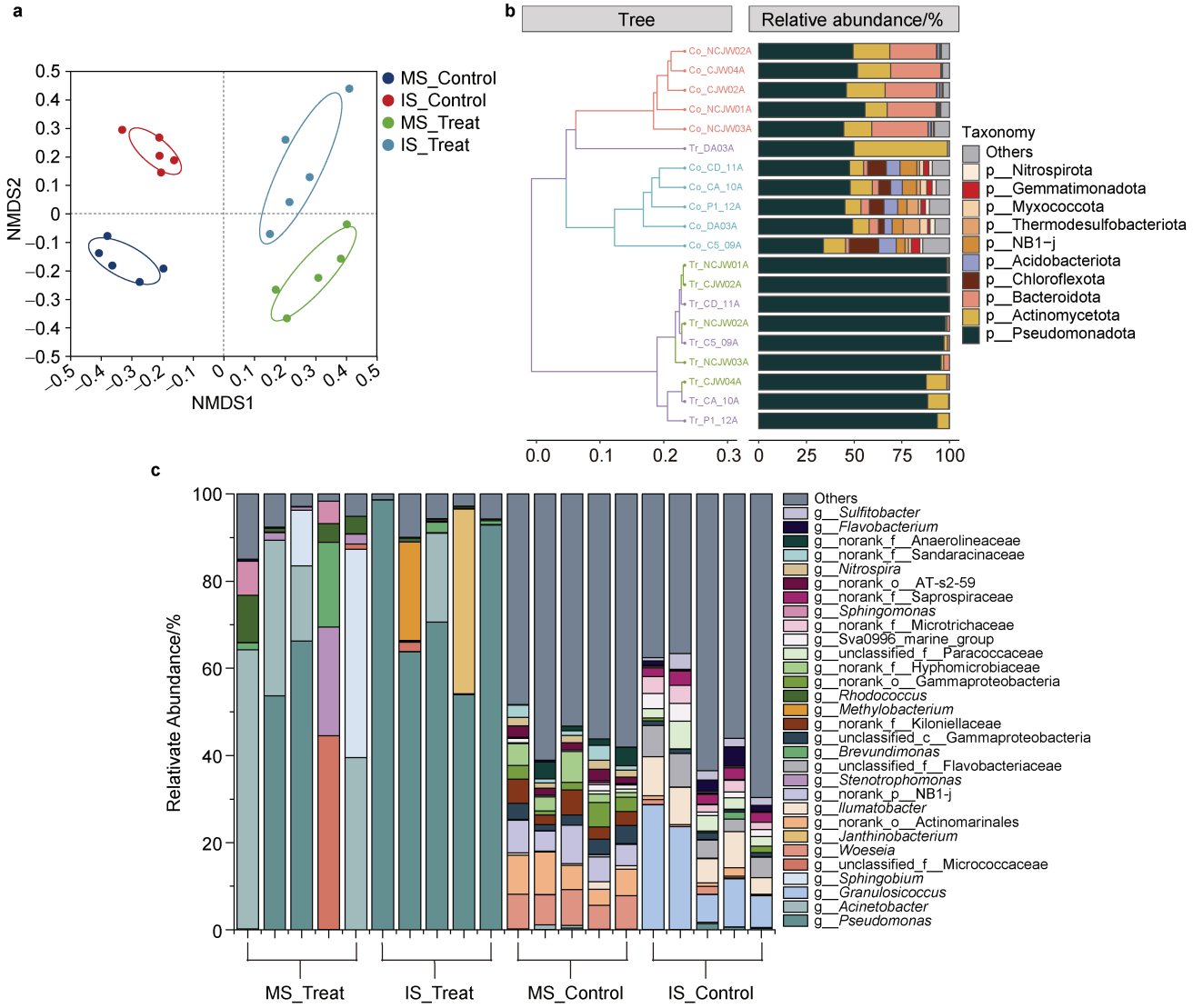

Figure 3 Multivariate analyses of microbial community structure across the MS and IS. **a**, non-metric multidimensional scaling (NMDS); **b**, phylum-level hierarchical clustering; **c**, genus-level species composition.

significant differences across groups (LDA>3, p<0.05), with MS yielding more distinct bacterial taxa (44) than IS (42). In the Control group, 29 distinct bacterial taxa were identified for MS_Control, predominantly affiliated with Acidobacteriota, Actinomycetota, Pseudomonadota, Thermodesulfobacteriota, Nitrospirota, Bacillota, Bacteroidota, and unclassified bacterial phyla, of which Acidobacteriota made the predominant contribution (Figure 4b). IS_Control contained the highest number of distinct bacterial taxa (35), primarily comprising *Bacteroidota*, *Pseudomonadota*, *Actinomycetota*, *Patescibacteria*, and *Verrucomicrobiota*, with *Bacteroidota* contributing most significantly. Following PAH exposure, a distinct bacterial taxa profile underwent substantial alterations: MS_Treat contained 15 differentially abundant taxa, 3 assigned to Actinomycetota and the remainder to Proteobacteria (predominantly Alphaproteobacteria), with the Sphingomonadaceae contributing most substantially at the family level and *Sphingobium* at the genus level. IS_Treat exhibited only 7 distinct bacterial taxa, 2 belonging to Bacteroidota and 5 to Gammaproteobacteria; despite lower distinct bacterial taxa counts, this group contained the highest number of taxa with LDA>5, where the Pseudomonadales order contributed most significantly overall and the genus *Pseudomonas* dominated at the genus level.

3.5 Predictive microbial community functional response

To assess potential functional responses of bacterial communities to PAH exposure, predictive metagenomic profiling was performed with PICRUSt2 based on taxa classified in IMG. Across all samples, 7,066 KEGG orthologs were predicted (mean detected abundance: 6,821.89). KEGG pathway profiles were compared between Control and Treatment

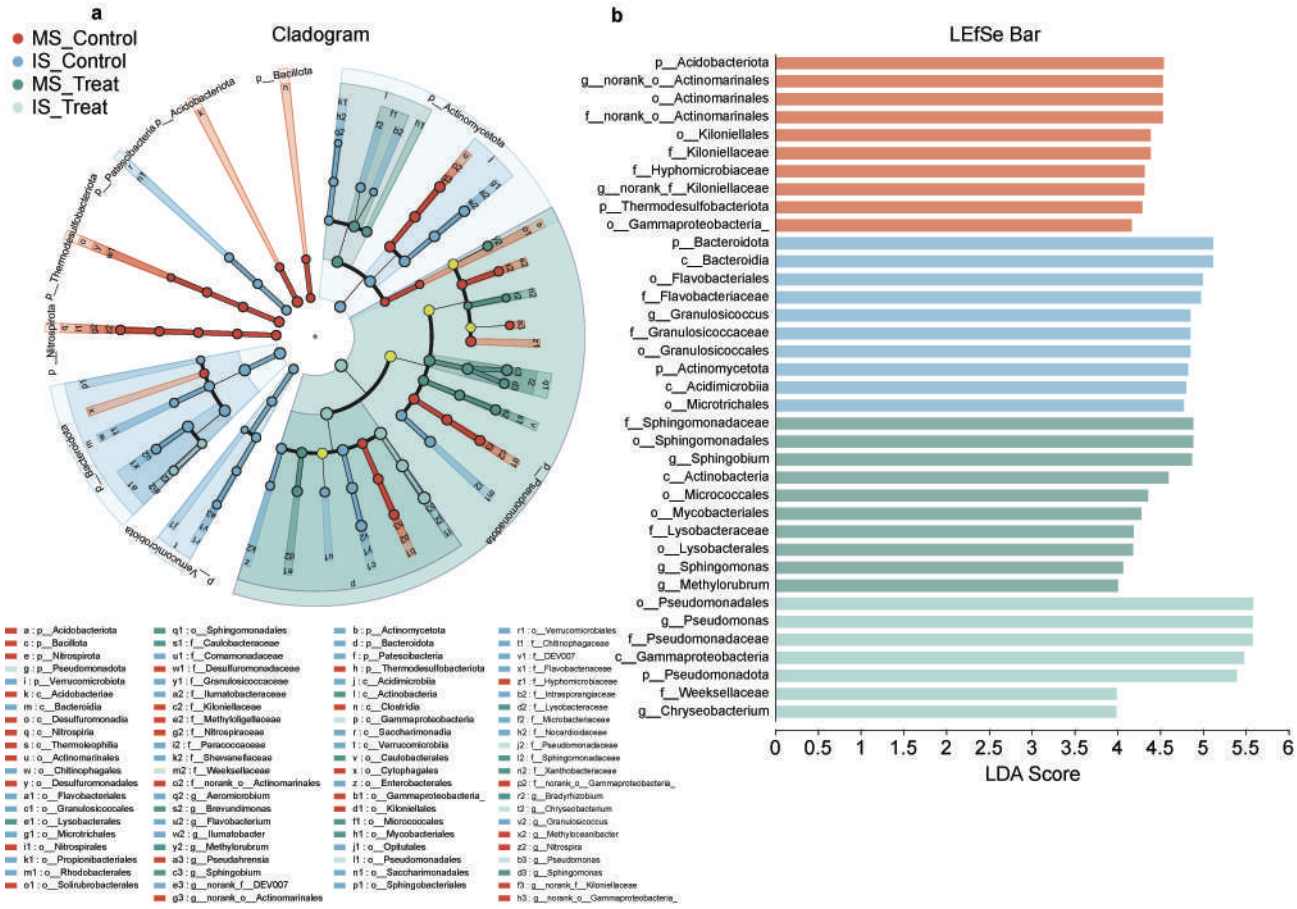

Figure 4 LEfSe analysis of the microbial communities in the samples. **a**, cladogram showing the taxonomic hierarchy from phylum to genus, with circles radiating from the center outward representing different taxonomic levels. Each small circle denotes a specific taxon, and its size reflects its relative abundance. Colored nodes indicate taxa significantly enriched in the corresponding group, while yellow nodes represent taxa without significant differences. **b**, LEfSe bar chart displaying taxa with significant differential abundance among groups, with an LDA score greater than 3. A higher LDA score indicates a greater contribution of that taxon to the observed differences. Taxonomic levels are abbreviated as follows: p, phylum; c, class; o, order; f, family; g, genus; s, species.

within MS and IS. Five PAH-related pathways (naphthalene degradation, polycyclic aromatic hydrocarbon degradation, benzoate degradation, aminobenzoate degradation, and aromatic compound degradation) were examined (Figures 5a and 5b), and the 30 most abundant core metabolic pathways were also evaluated (Figures 5c and 5d). STAMP indicated higher predicted abundances (p<0.05) of the polycyclic aromatic hydrocarbon degradation and aromatic compound degradation pathways in MS_Treat, whereas the aminobenzoate degradation pathway showed lower predicted abundance. In IS, similar increases were detected for the polycyclic aromatic hydrocarbon and aromatic compound degradation pathways, while both aminobenzoate and benzoate degradation pathways were lower in Treatment (p<0.05). The naphthalene degradation pathway did not differ significantly between conditions; however, several constituent KOs displayed higher predicted abundances in Treatment, which did not translate into a pathway-level difference. Among high-abundance pathways, 18 differentially predicted pathways were detected in MS; Treatment showed higher values (p<0.05) for microbial metabolism in diverse environments, metabolic pathways, valine leucine and isoleucine degradation, propanoate metabolism, two-component system, and butanoate metabolism. In IS, 20 pathways differed, with ABC transporters, two-component system, microbial metabolism in diverse environments, and glyoxylate/dicarboxylate metabolism showing higher predicted abundances in Treatment (p<0.05).

4 Discussion

4.1 PAHs-degrading bacteria in polar sediments

Bacterial isolation results confirmed the crucial role of *Pseudomonas* in PAH degradation, indicating its broad substrate adaptability. Numerous marine sediment-derived PAH-degrading bacteria have been identified, predominantly affiliated with the phylum Proteobacteria, with representative genera including *Pseudomonas*, *Alteromonas*, *Cycloclasticus*,

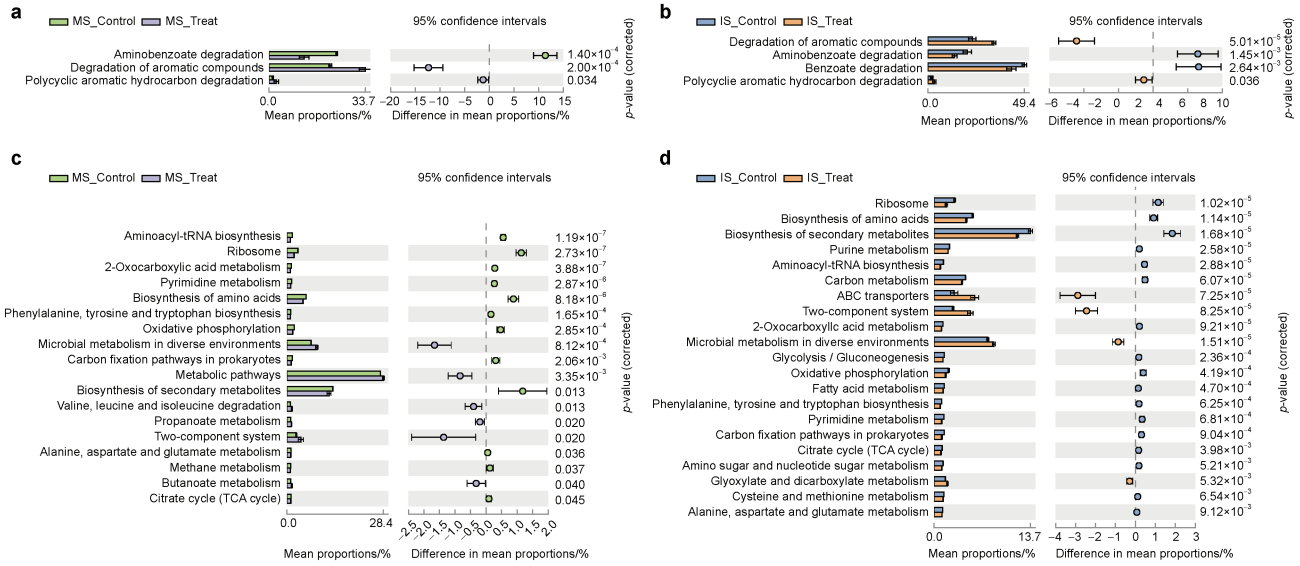

Figure 5 Significantly different KEGG pathways between MS and IS before and after PAH enrichment. **a**, PAH-metabolism-related pathways in MS; **b**, PAH-metabolism-related pathways in IS; **c**, basic metabolic pathways in MS; **d**, basic metabolic pathways in IS.

Halomonas, *Marinobacter*, *Marinomonas*, *Neptunomonas*, *Novosphingobium*, and *Pseudoalteromonas* (Yakimov et al., 2007). In this study, *Pseudomonas* isolates comprised 50% of the total culturable bacteria. This genus metabolizes diverse complex hydrocarbons through versatile enzymatic pathways, enabling PAH utilization as carbon and energy sources (Sivasamy et al., 2025). *Pseudomonas putida* represents a well-characterized model strain, while the isolated strain NBRC 14164 from polar marine sediments demonstrated high degradation potential for heavily PAH-contaminated sediments (Acer et al., 2021). Cultivation experiments established *Pseudomonas* as a keystone taxon in polar microbial communities responding to contamination, suggesting its applicability for bioremediation of escalating polar PAH pollution. Furthermore, *Acinetobacter* was confirmed as a potent PAH degrader, previously identified as a key player in PAH-impacted diesel-degrading consortia (Czarny et al., 2020). The isolated *Acinetobacter johnsonii* strain from intertidal sediments exhibited multi-substrate activity, excelling in co-biodegradation of anthracene, phenanthrene, pyrene, and naphthalene (Jiang et al., 2018).

Psychrobacter spp. may accelerate the bioremediation of PAHs contamination in polar regions through their low-temperature PAH-degrading capabilities. Existing studies indicate that certain *Psychrobacter* can utilize diesel and exhibit pyrene tolerance (de Melo Carlos et al., 2024; Rizzo et al., 2020). The capacity of *Psychrobacter* to degrade multiple PAHs was further demonstrated in this study. Given the vulnerability of polar ecosystems, utilization of cryophilic PAH-degrading bacteria, represented primarily by *Psychrobacter*, is considered essential for in situ bioremediation of PAH-contaminated polar regions. Moreover, low-temperature conditions enhance PAHs degradation efficiency by polar microbial communities. Several species within *Rhodococcus* and *Microbacterium* were identified as potential PAH-degrading candidates in this study.

Collectively, these findings reveal the potential of diverse bacterial taxa in PAHs degradation, particularly highlighting the bioremediation role of *Pseudomonas* and related genera in polar environments. This study provides essential microbial resources and theoretical foundations for developing targeted bioremediation strategies against PAHs contamination in polar regions. However, it should be noted that future work needs to incorporate culture-based degradation analyses of isolated taxa to confirm their specific degradation capabilities.

4.2 Effects of PAH enrichment on microbial community composition in polar regions

Significant variations in α-diversity indices demonstrate that PAH enrichment substantially reduces bacterial diversity and richness in polar sediments, with both parameters exhibiting progressive declines. Proteobacteria constituted the most abundant phylum across all samples and displayed increased relative abundance following PAH exposure, while Actinobacteriota and Bacteroidota decreased—a pattern consistent with microbial responses to petroleum-derived PAH contamination in soils and sediments (Lin et al., 2023). This trend is validated in polar environments, where Proteobacteria abundance escalates with pollution intensity (Jurelevicius et al., 2022). By simulating contamination escalation through artificial PAH amendment, we reveal the significant bioremediation potential of proteobacterial taxa for restoring polluted polar ecosystems.

At the genus level, ASVs assigned to *Pseudomonas* and *Acinetobacter* exhibited elevated relative abundances in polar sediments, aligning with enrichment culture results

and confirming their broad PAH-removal capabilities. Although MS and IS shared similar dominant taxa post-PAH enrichment, significant abundance differences were observed. These shifts highlight microbial groups actively participating in contaminant biodegradation. Distinct bacterial taxa analysis further revealed habitat-specific responses: Pseudomonadales dominated in intertidal sediments, with successful enrichment from both sediment types, confirming their survival advantage and biodegradation potential under PAH enrichment. Conversely, Sphingomonadaceae contributed most significantly in marine sediments based on LEfSe. This family—recognized for metabolizing aromatic compounds via versatile ortho- and meta-cleavage pathways—degrades diverse PAHs, including phenanthrene and HMW-PAHs (Waigi et al., 2015; Zhang et al., 2022). Although diesel-degrading Sphingomonadaceae isolates have been recovered from polluted polar soils (Gran-Scheuch et al., 2017), their absence in this study may be attributed to slow growth kinetics in oligotrophic low-temperature sedimentary environments. Collectively, artificial PAH enrichment induced significant bacterial community restructuring across polar sediments, reflecting the profound influence of PAHs on shaping microbial communities. However, the comparison between MS and IS does not fully distinguish habitat effects from geographical influences, because all marine sediments are collected from Antarctica, whereas intertidal sediments include samples from both Antarctica and the Arctic. To mitigate the imbalance caused by the limited number of Arctic samples (only 3 intertidal samples and no marine sediments), a grouping strategy based on habitat type is applied. Although this limitation should be considered when interpreting the results, the comparisons still reveal differences in taxa responding to PAHs across sediment types. Future studies that incorporate Arctic marine sediments are needed to more clearly disentangle the relative contributions of habitat and geography to the distribution of degradative taxa.

4.3 Effects of PAH enrichment on microbial community function in polar regions

Following PAH enrichment, PICRUSt2-based predictions suggested higher relative abundances of pathways associated with polycyclic aromatic hydrocarbon and aromatic compound degradation in both MS and IS, accompanied by lower predicted abundances of aminobenzoate (and, in IS, benzoate) degradation. In intertidal sediments, enrichment of ABC transporters was consistent with a greater predicted capacity for nutrient uptake and efflux activity by microbial communities, facilitating the uptake of degradation products and essential nutrients while exporting intracellular PAHs or toxic intermediates to mitigate cellular stress (Poretsky et al., 2010). Enrichment of two-component systems in both habitats was compatible with enhanced environmental signal sensing and regulatory responses under PAH exposure (Papon and Stock, 2019). Differences observed in short-chain fatty acid metabolism (MS) and the glyoxylate cycle (IS) pointed to potential adjustments in central metabolic pathways under the experimental conditions.

Overall, these patterns were interpreted as shifts in predicted functional potential rather than as direct evidence of gene expression or enzymatic activity, and were considered in light of NSTI values; lower NSTI in Treatment groups supported higher confidence relative to Controls, whereas, given the higher NSTI in MS_Control, interpretations were treated with greater caution (IS_Control, 0.178; IS_Treat, 0.023; MS_Control, 0.301; MS_Treat, 0.023).

5 Conclusion

This study demonstrates that artificial PAH enrichment significantly alters both the composition and function of microbial communities. Nineteen bacterial strains with potential degradation capabilities—predominantly *Pseudomonas* and *Acinetobacter*—were isolated from mixed-PAH enrichments, indicating their bioremediation potential in polar environments. High-throughput sequencing analysis revealed substantial differences in diversity and richness between marine and intertidal sediment communities after PAH enrichment. *Proteobacteria* dominated the enriched microbial taxa, while genus-level analysis showed elevated relative abundances of *Pseudomonas* and *Acinetobacter* ASVs, consistent with enrichment culture results. LEfSe analysis identified Pseudomonadales as key distinct bacterial taxa in marine sediments and Sphingomonadaceae in intertidal sediments post-PAHs exposure. Functional prediction suggests polar sediment microbial communities respond to PAH enrichment not only through parent compound degradation but potentially via synergistic multi-mechanism pathways, though further experimental validation is required. Collectively, these findings provide insights for assessing PAHs pollution impacts on native microbial structures/functions in polar regions, while the isolated degraders support development of bioremediation agents.

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