Tropical Intertidal Microbiome Response to the 2024 Pasir Panjang Oil Spill

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SYNOPSIS

The 2024 Pasir Panjang marine fuel oil spill triggered proliferation of hydrocarbon-degrading bacteria and long-term functional priming of sediments, with implications for oil spill resilience in vulnerable tropical coastal ecosystems.

ABSTRACT

Marine fuel oil (MFO) spills in tropical coastal environments are under-characterized despite increasing risk from maritime activities. Microbial and geochemical responses to the June 2024 Pasir Panjang MFO spill on Singapore's intertidal sediments were analyzed in real time over 185 days. Using metagenomics and hydrocarbon profiling, microbial community shifts and hydrocarbon degradation were quantified across visibly oiled (high-impact) and clean (low-impact) sites. Microbiomes at all sites adapted rapidly to the spill through increased diversity and abundance of genes encoding alkane and aromatic compound degradation, detoxification, and biosurfactant production. Oil deposition intensity strongly influenced microbial succession and hydrocarbon-degrading gene profiles, and this reflected early toxicity constraints in heavily oiled areas. The persistence of hydrocarbon degradation genes beyond hydrocarbon detection in sediments suggested long-term functional priming may occur. The study provides novel genome-resolved insight into the microbial response to MFO pollution, advances understanding of marine environmental biodegradation, and provides urgently needed baseline data for oil spill response strategies in Southeast Asia and beyond.

INTRODUCTION

Tropical intertidal zones are dynamic and ecologically vital ecosystems that face threats from oil pollution due to dense maritime traffic and rapid coastal industrialization [1, 2]. An important part of oil spill attenuation is microbial biodegradation of hydrocarbons [3, 4]. Sandy shores are the most abundant tropical coastal habitat but its intertidal supports lower baseline microbial diversity than other coastal habitats [5, 6], raising concerns about the capacity for effective amelioration of oil spills.

The microbial response to crude oil spills in the marine environment has been extensively characterized, notably from the 2010 Deepwater Horizon spill. Metagenomic studies have revealed that oceanic and deep-sea water [7, 8], as well as benthic [9, 10], and coastal sediments [11-14], responded similarly in terms of the rapid proliferation of bacteria implicated in hydrocarbon degradation, including *Alcanivorax*, *Colwellia*, *Cycloclasticus*, and *Marinobacter* [7, 9, 10]. In coastal habitats oil deposition dramatically increased microbial cell abundance [13], genes encoding alkane and aromatic hydrocarbon degrading genes were detected [11], and expressed in microbiomes [14].

In contrast, the environmental response to marine fuel oils (MFO) including bunker fuel, marine diesel oil, and marine gas oil remains less well understood beyond broad descriptions of ecological impact [15-17]. Microcosm studies have indicated that similar bacteria to those arising from crude oil spills proliferate in response to bunker C [18], petro-diesel [19-21], and unidentified heavy fuel oils [22, 23]. These hydrocarbons are increasingly recognized as a source of accidental spills in marine environments [24]. The lack of mechanistic insight on microbial response to MFO represents a critical knowledge gap, especially given the unique physicochemical properties of the various MFO relative to crude oil [25].

While microbial response to oil spills has been well-characterized in temperate systems [3, 4, 26], equatorial coastal environments present potential challenges and opportunities for microbial degradation of spilled oil, due to heightened ecological vulnerability of tropical coastlines to oil spills [27]. Elevated temperatures and intense solar radiation may accelerate oil weathering but also influence microbial succession differently than in temperate systems. In highly urbanized equatorial tropical coastlines such as Singapore, chronic anthropogenic pressures may further compromise baseline ecological resilience [28].

The 2024 Pasir Panjang oil spill in Singapore, released Low Sulfur 380 MFO that rapidly and extensively spread along southern and eastern coastlines [29], presented a rare opportunity to study oil degradation dynamics in real time. Singapore's position as a major equatorial maritime hub and petrochemical refining center exacerbates its vulnerability to marine oil pollution yet also underscores the urgency of acquiring region-specific knowledge. This study characterized shifts in intertidal microbial community composition and functional hydrocarbon-degrading gene abundance following MFO exposure and assessed the persistence of hydrocarbons in sediments over time.

METHODS AND MATERIALS

Sampling Design and Sediment Collection. Surface sandy sediment cores (0–10 cm depth) were sampled from the mid-zone intertidal at Bendera Bay on St John's Island, Singapore (1°13′08″N, 103°50′53″E) following the Pasir Panjang oil spill on 14th June 2024. Sampling was carried out at three low impact (no visible oil) and three high impact (completely oil covered) sites at low tide over 6 months (N = 48).

Hydrocarbons and Physicochemical Analyses. Hydrocarbons were extracted using US EPA methods 3540C/3545A [30, 31], and quantified via GC-FID (Agilent) for total petroleum hydrocarbons (TPH) following EPA 8015C [32]. Semi-volatile organics and polycyclic aromatic hydrocarbons (PAHs) were analyzed by GC-MS (Agilent) using EPA 8270E [33]. Sediment pH and redox potential (ORP) were measured using ion-selective probes (Mettler Toledo). Total organic carbon was quantified using a TOC analyzer (Shimadzu). Nitrate, nitrite, phosphate, and sulphate were quantified by ion chromatography (Dionex). Metals were analyzed via ICP-OES (Agilent). Sediment toxicity was estimated using the Microtox acute toxicity assay (Modern Water).

Metagenomic Sequencing and Bioinformatic Analysis. DNA was extracted with DNeasy Powersoil Pro kits (Qiagen) and sequenced using the Illumina NovaSeq X Plus and PE150 sequencing kits. Quality-filtered reads were co-assembled using metaSPAdes [34], followed by bin analysis using MetaBAT [35]and Maxbin [36]. Metagenome-assembled genomes (MAGs) were dereplicated at ≥95% ANI using DAS Tools [37]. High-quality (>90 completeness, <5% contamination) and medium-quality (>50% completeness, <10% contamination) MAGs were retained for downstream analysis. Relative abundance of MAGs in samples was estimated using CoverM [38]. Taxonomy was assigned via GTDB-Tk (release 220) [39], and phylogenomic trees were visualized in iTOL v6 [40]. Taxonomic diversity in metagenomes was estimated using SingleM [41]. Functional annotation of genes associated with hydrocarbon degradation and aggregation into pathways was conducted using the curated HADEG database [42], and estimated using both metagenomes and MAGs. Gene abundances were quantified as transcripts per million (TPM), normalized for both gene length (in kilobases) and sequencing depth (per million reads), thus enabling standardized comparisons across samples.

Statistical Analysis. Data analysis was conducted in R using vegan [43], for estimation of diversity metrics, multivariate influence on community structure, and significance testing.

RESULTS AND DISCUSSION

Persistence of Hydrocarbons in Tropical Intertidal Sediments. Following the June 2024 MFO spill at Pasir Panjang, extensive hydrocarbon deposition was observed along Singapore's sandy intertidal zone (Supporting Information, Fig. S1). Total petroleum hydrocarbon (TPH) concentrations in high-impact sediments initially ranged from 1,810 to 9,408 mg/kg but stabilized within 17 days (1,349–1,965 mg/kg), then declined steadily up to 185 days post-spill while remaining elevated relative to low-impact sites (Fig. 1A). This initial deposition was comparable to those for crude oil at Pensacola Beach after the Deepwater Horizon oil spill, which reached 11,000 mg/kg, and with 4,500 mg/kg in sand, and was undetectable one year post-spill [13]. Low-impact sediments, although visually clean, exhibited detectable TPH (16.9–20.9 mg/kg) immediately post-spill, decreasing to undetectable levels by the study's end. No comparable studies for Low Sulfur MFO 380 are available but microcosm studies for other fuel oils (bunker C, petro-diesel) suggest they may deplete at similar rates in marine sediments [20, 44].

Alkanes dominated the hydrocarbon profile, with C18–C29 fractions most abundant. PAHs, including chrysene and phenanthrene, were also detected at high initial concentrations (Fig. 1B). Alkane depletion followed molecular weight trends: shorter-chain compounds (C14–C19) degraded rapidly, while heavier alkanes (C29–C36) persisted up to 185 days. Reappearance of high-molecular-weight alkanes after several months may indicate remobilization from deeper sediments or minor secondary spills. PAHs declined more rapidly, with 3-ring compounds such as phenanthrene dissipating faster than more complex 4–6 ring structures. PAHs were undetectable

in low-impact sites within two weeks but persisted for 48 days in high-impact areas (Fig. 1B). Overall, alkanes showed greater persistence than PAHs, likely reflecting their higher initial concentrations.

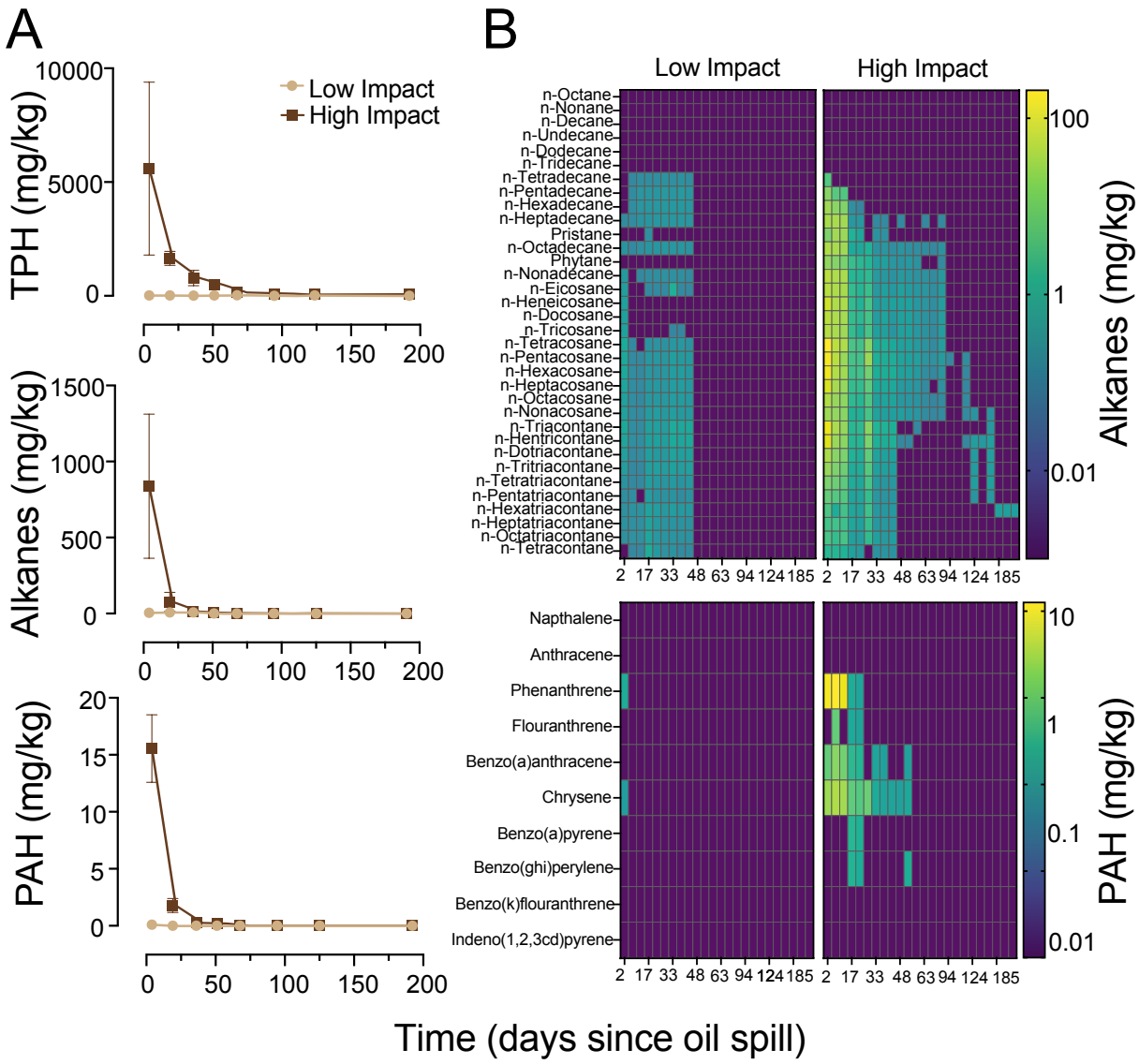

Figure 1. A) Concentrations of hydrocarbons in intertidal sediments following the oil spill. B) Composition profiles of individual alkanes and PAHs in post-spill sediments.

Genomic Inventory of Oil-Contaminated Tropical Sand Microorganisms. We recovered 43 MAGs (41 Bacteria, 2 Archaea) spanning eight microbial classes (Fig. 2A, Supporting Information, Fig. S2). This was consistent with Intertidal sand communities that support low diversity and abundance relative to other marine habitats, likely reflecting the oligotrophic and dynamic habitat [5, 13]. The dominant bacterial classes, Alphaproteobacteria and Gammaproteobacteria, were positively associated with hydrocarbon concentrations, while Acidimicrobiia and Bacteroidia were negatively associated. Archaeal groups Candidate Class E2 and Nitrosphaeria displayed a strong inverse relationship with hydrocarbons, suggesting potential roles as indicators of recovery, and matching the known role of Nitrosphaeria in undisturbed marine habitats [45]. Genomes indicating anaerobic *Thermoanaerobacula* (UBA5704) occurred with very low abundance, consistent with the oxic habitat.

The use of MAGs to infer dominant microbial community structure was validated by comparison to taxonomic composition profiles derived from the metagenomes (Supporting Information, Fig. S3). This confirmed that MAGs captured the important and abundant taxa in the sediments. Metagenomes also revealed several low abundance "rare" taxa that were not represented in genomes, and this suggests a potential reservoir of microbial diversity that facilitates ecological resilience under disturbance [46].

Microbial community patterns observed through Non-metric Multidimensional Scaling (NMDS) ordination of Bray-Curtis dissimilarity showed distinct microbiome differences between high- and low-impact sites (95% CI), with gradual convergence in community similarity over time (Fig. 2B). As expected, hydrocarbons were strong abiotic drivers of differences in community structure. Additional contributing variables that also displayed significant differences between

low- and high-impact sediments included pH, ORP, nutrients, and trace metals (envfit: p = < 0.05, Supporting Information, Fig. S4).

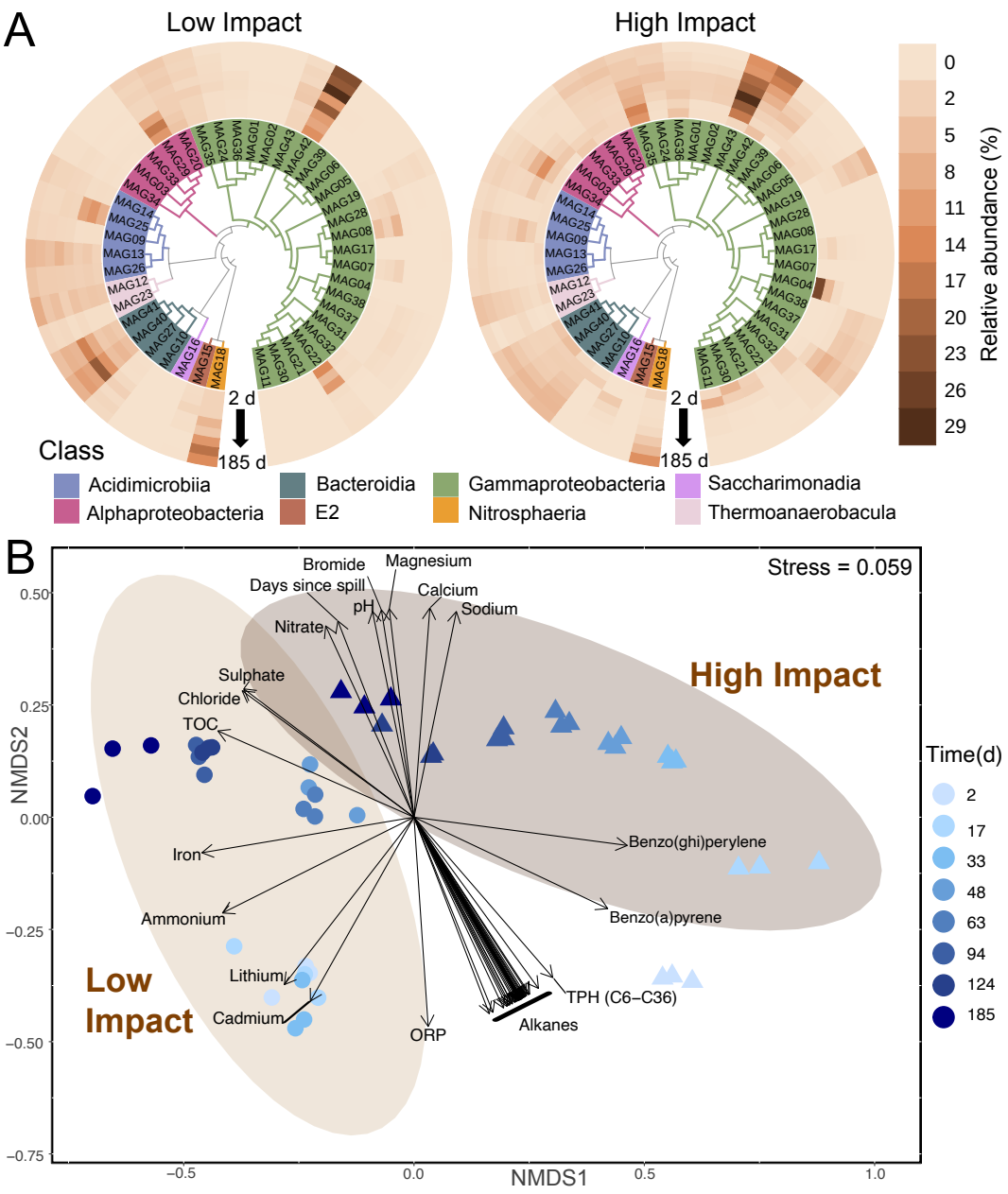

Figure 2. A) Phylogenomic diversity and distribution of MAGs across sites over time. B) Beta diversity of microbial communities in low-impact (circles) versus high-impact (triangles) sediments.

MFO Selects for a Specialized Hydrocarbon Degrading Microbiome. Genomes were screened against the HADEG database, a curated database which includes 259 protein sequences related to aerobic hydrocarbon degradation and 32 associated with biosurfactant production, to infer gene presence and metabolic pathway participation (Fig. 3A, Supporting Information, Fig. S5). All MAGs in the classes Acidimicrobiia, Alphaproteobacteria, Bacteroidia, Gammaproteobacteria possessed genes enabling alkane and/or aromatic hydrocarbon degradation. Hydrocarbon degrading genes were not detected in Archaeal genomes or the Saccharimonadia which belong to the nanobacterial phylum Patescibacteria.

Several genomes, particularly from the Gammaproteobacteria, Alphaproteobacteria, and Acidimicrobiia encoded genes for both alkane and aromatic degradation. For the alkanes the gene combinations revealed that terminal/biterminal oxidation and Finnerty pathway reactions were the most represented (Supporting Information, Fig. S6). Ring cleavage pathways were also widespread but some such as phenol degradation occurred only in genomes of *Cycloclasticus* sp. (MAGs 05,06). Similarly, those for p-cumate degradation were specific to genomes of Immundisolibacteraceae (MAGs 01,02,24,35,36). Overall, the most comprehensive suites of aromatic degradation pathways also belonged to these latter two bacterial groups (Supporting Information, Fig. S6).

Gammaproteobacteria genomes exhibited the most abundant alkane degradation genes, notably in *Oleiobacter* sp (MAGs 11,21,30) [47], *Macondimonas* sp (MAG 43) [48], *Marinobacter* sp

(MAGs 22,32) [49], plus other candidate lineages of bacteria previously associated with oil spills, e.g. HB2-32-21 sp. (MAG 31) [10]. Genes for aromatic compound degradation were also most abundant in Gammaproteobacteria, including novel members of the Immundisolibacteraceae [50], as well as *Alteromonas* sp (MAG 08) [51], and *Cycloclasticus* sp. [52]. Surprisingly, the well-documented alkane degrader *Alcanivorax* and aromatic degrader *Colwellia*, prominent following the Deepwater Horizon crude oil spill, were not recovered in our genome set, and reads assigned to *Alcanivorax* were present only at very low abundance in metagenomes. Instead, *Macondimonas diazotrophica* (MAG43), a known oil-degrading and nitrogen-fixing bacterium [48], emerged as the most abundant taxon in response to the MFO spill in high-impact locations. This may in part reflect hydrocarbon substrate preferences, nutrient-poor tropical waters, or biogeographic differences between tropical and other marine habitats. In contrast, the abundant *Cycloclasticus* genomes encountered in this study, coupled with its occurrence also in temperate [53] and polar [54] oil spill locations, as well as deep ocean oil seeps [55], suggests it may be a cosmopolitan oil-degrading genus.

Alphaproteobacteria represented the second most diverse group of MAGs, with genomes including *Erythrobacter* sp. (MAG 34), a known aromatic degrader [56], and other taxa such as *Hyphomonas* sp. (MAG 03), *Roseivivax* sp. (MAG 20), and novel Rhodobacteraceae (MAGs 29,33) implicated in degradation of aromatics and asphaltenes [57]. Within the Acidimicrobiia that were the third most diverse clade, *Ilumatobacter* sp. (MAG 09), a bacterium associated with beach sand after oil contamination, although this taxon is also encountered in pristine coastal environments [58].

Microbiome Responses Vary by Oil Deposition Intensity. The abundance of genes implicated in microbial alkane and aromatic compound degradation, detoxification, and biosurfactant production responded rapidly in sediments in response to the oil spill (Fig. 3B; Supporting

Information, Fig. S7). Mapping gene abundances to catabolic pathways confirmed that complete degradation of hydrocarbons could be achieved (Supporting Information, Fig. S8).

Biosurfactant genes (*wzc*) increased in abundance relative to hydrocarbon load of sediments (Fig. 3B). The microbial production of biosurfactants has been shown to facilitate biofilm formation on oil droplets and uptake of hydrocarbons by bacterial cells [59].

Among the ten most abundant alkane-associated genes rubredoxin (*rdx*), an electron carrier in alkane oxidation, was the most abundant gene across all samples. The C5-C16 alkane monooxygenase degradation genes (*alkB*, *alkJ*, *alkN*) peaked early in both impact zones reflecting early use of smaller chain length alkanes. The Baeyer-Villiger monooxygenase *BVMO* peaked early only in high-impact sediments and may reflect adaptation to high hydrocarbon levels. The long-chain ≥C20 alkane monooxygenase *almA* were enriched in high-impact sediments and during the early phase of low-impact sediments. Detoxification-related genes (*ahpC–ahpF*, *CYP450*) were prevalent and positively associated with elevated hydrocarbon concentrations. Once alkane levels approached background levels in low-impact sites the alkane-degrading genes became depleted, although some such as the *prmD* propane monooxygenase gene involved in downstream metabolism of short chain C2-C4 alkanes remained abundant in all samples. The *rdx* gene remained abundant although this may in part reflect that its product also performs other roles in microbial metabolism.

Aromatic degradation genes showed a proliferation of dioxygenases (*paa* and *pca* operons) across all samples. Some striking differences also occurred between low and high impact sites. In low-impact sediments, degradation genes *catC* and *andAb* were abundant early, whilst in high-impact sediments the initially more abundant genes were associated with detoxification and

metabolism of methylated and chlorinated aromatics (*bph*, *cmt*, and *xyl* operons) High-impact sediments also harbored additional genes (*xyl* and *tod* operons) associated with monoaromatic and PAH degradation that were absent at low-impact sites.

Overall, oil spill severity influenced the pace and structure of microbial succession and patterns of hydrocarbon genes. This may in part reflect selection imposed by the level of PAH levels in sediments. Toxicity may also be a factor, and significant differences in acute toxicity between low-impact versus high-impact sites lend tentative evidence for this (IC50 = 24 x 10^3 vs. 9 x 10^3 mg/l, t-test: $t(4) = 7.23$, $p = 0.0019$). Our findings are consistent with observations for crude oil spills where low-level contamination promotes transient shifts and rapid recovery [13, 60], while higher-impact spills select for specialist degraders and sustain long-term catabolic potential [26, 61]. This suggests that spill severity and hydrocarbon type shape the persistence of microbial degradation potential in sediments.

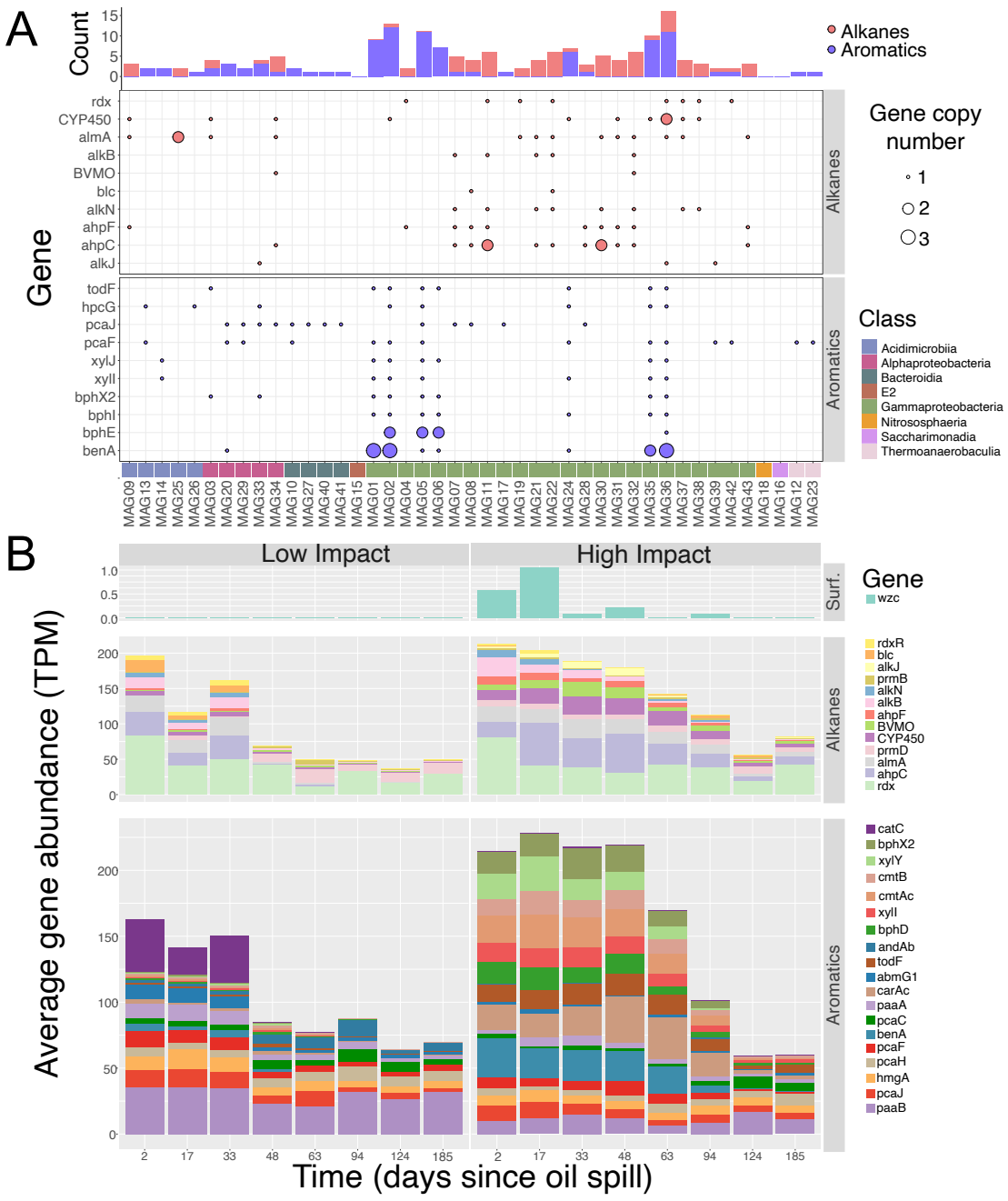

Figure 3. Distribution of the ten most abundant hydrocarbon degradation genes in A) MAGs, and B) environmental samples (Surf. = biosurfactants). Abundance and pathway mapping for all genes and samples are shown in Supporting Figs S7 and S8.

Oil-Degrading Microbiomes Endure Six Months Post-Spill. Temporal trends showed that hydrocarbon gene abundance peaked earlier (≤33 days) in low-impact sites, whereas high-impact sites exhibited longer duration and more diverse hydrocarbonoclastic pathway representation (Supporting Information, Fig. S7 and S8). Oil-degrading microorganisms are typically rare in pre-spill communities [26, 61], yet hydrocarbon degradation is now understood as a widespread trait among environmental microorganisms [7, 54, 62]. Chronic but low-level hydrocarbon exposure in marine locations such as Singapore which is a global maritime and petrochemical hub, may have selected for latent microbial communities pre-adapted to such disturbances.

After 185 days, hydrocarbon-degrading genes remained recoverable in intertidal sand despite the very low or undetectable levels of hydrocarbons (Fig. 3B), suggesting a potential "priming" effect whereby sediments retain latent catabolic capacity (Supporting Information, Fig. S8). While biosurfactant-related genes declined sharply following hydrocarbon dissipation, their rapid initial appearance after the spill implies recruitment from the rare low abundance taxa. Residual alkane-related genes were primarily associated with electron transfer and oxidative stress mitigation (*rdx*, *ahpC*). In contrast, PAH degradation pathway genes involved in ring cleavage (*paa* and *pca* operons) persisted across both impact categories, indicating potential longer-term functional imprinting for PAH degradation.

Tropical Coastal Management Lessons from Singapore's Oil Spill. The Pasir Panjang spill offered a rare opportunity to observe real-time microbial responses to a MFO spill in a tropical intertidal setting. While Singapore's rapid response removed oiled sand from public beaches, our protected study sites remained untouched, enabling direct assessment of *in situ* biodegradation. Findings show that, even without sand removal, microbial communities in Singapore's intertidal zones can support recovery from moderate spills within six months. However, this biological

capacity does not replace the need for remediation driven by economic, ecological, and aesthetic concerns. Additionally, infrastructure to facilitate large-scale sand removal is not widely available in other tropical regions, highlighting the broader value of our findings.

The study provides novel insight that the intensity of oil deposition critically shapes microbial responses, driving divergent microbial responses in terms of hydrocarbon degradation and biosurfactant production. Notably, the oil spill induced long-term functional priming of sediment microbiomes. These findings emphasize the rapid adaptive capacity of intertidal microbial communities and draw attention to distinct biogeochemical strategies employed in response to MFO, a pollutant class that remains insufficiently explored in the current literature.

Unresolved processes and the interaction of biotic degradation with abiotic factors such as photochemical weathering, tidal redistribution, and conversion to persistent sinks such as tar balls or deeper subsurface deposits [63, 64], as well as potential impact of dispersants used in oil spill control [10, 65], require further study.

Overall, these results provide a much-needed baseline for estimating microbial resilience, supporting recovery modeling and spill preparedness across Southeast Asia.

ASSOCIATED CONTENT

Supporting Information.

Fig. S1. Map and photos showing the extent of the oil spill.

Fig. S2. Phylogenomic tree and metadata for MAGs.

Fig. S3. Comparison of diversity estimates using MAGs and metagenomes.

Fig. S4. Abiotic variables at low-impact and high-impact sites.

Fig. S5. Distribution of all hydrocarbon-degrading genes in MAGs.

Fig. S6. Estimation of hydrocarbon-degrading pathways in MAGs.

Fig. S7. Distribution of all hydrocarbon-degrading genes in environmental samples.

Fig. S8. Estimation of hydrocarbon-degrading pathways in environmental samples.

Data Availability Statement.

All DNA sequence data has been deposited in the NCBI sequence read archive (https://www.ncbi.nlm.nih.gov) under BioProject PRJNA1263040.

Notes.

The authors declare no competing financial interests.

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Author Contributions

Fieldwork was conducted by HMD, LD, SLMT, and SBP. Laboratory analysis was performed by CG, LD, JL, and YW. Bioinformatic and statistical analysis were conducted by CG, LD, and YQ. All authors contributed to data interpretation. SBP wrote the manuscript with input from all authors.

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ABBREVIATIONS

MFO, marine fuel oil

TPH, total petroleum hydrocarbons

PAH, polycyclic aromatic hydrocarbons

ORP, oxidation-reduction potential (redox potential)

MAG, metagenome-assembled genome

TPM, transcripts per million

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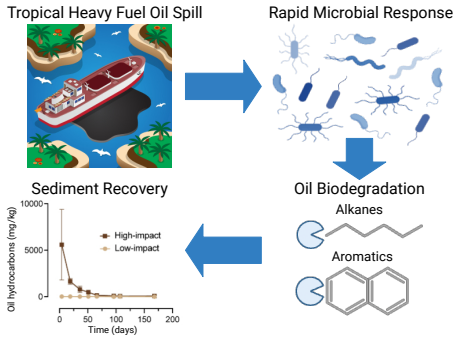
TOC GRAPHIC



Tropical Intertidal Microbiome Response to the 2024 Pasir Panjang Oil Spill

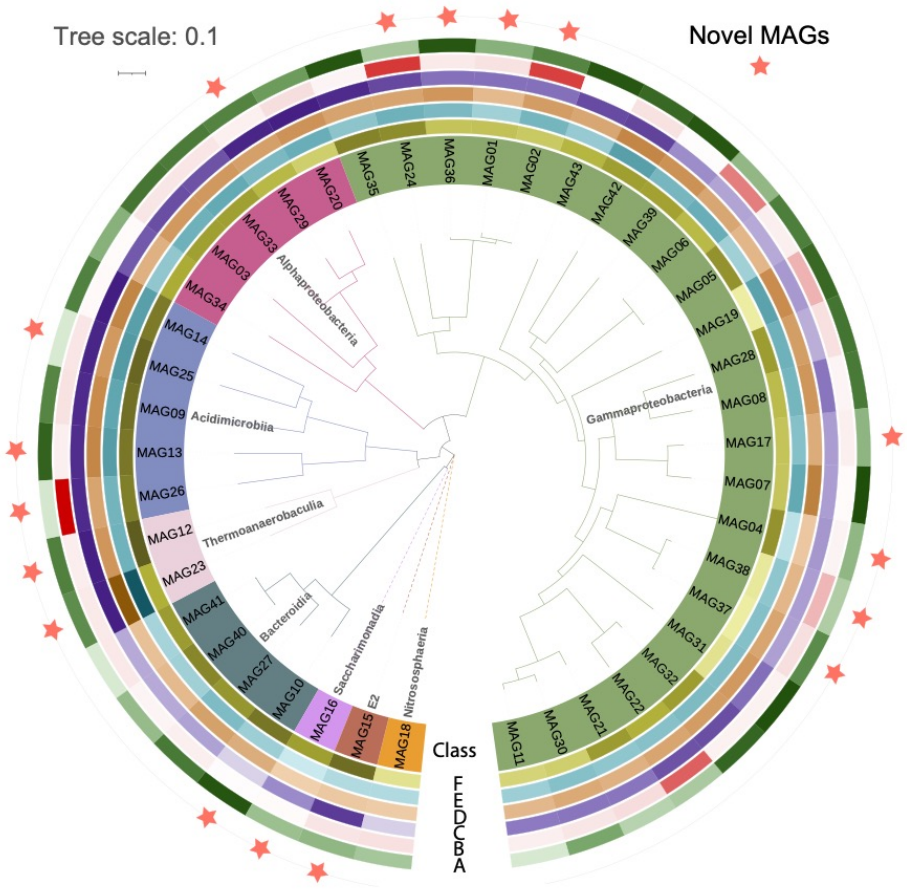
Supporting Information

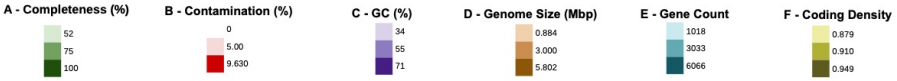
Table of Contents

Fig. S1. Map and photo showing the extent of the oil spill.

A) The spill originated at Pasir Panjang terminal on 14th June 2024 and was first observed on St John's Island where sampling sites were located on 16th June 2025. B) Clean-up of oiled beaches.

Fig. S2. Phylogenomic tree and metadata for MAGs.





Class

Acidimicrobiia
MAG14 - SZUA-35 sp.
MAG25 - Unassigned JACDCH01 (family)
MAG09 - Ilumatobacter sp.
MAG13 - Unassigned SZUA-232 (family)
MAG26 - Unassigned Acidimicrobiia (class)

Alphaproteobacteria
MAG20 - Roseivivax sp.
MAG29 - Unassigned Rhodobacteraceae (family)
MAG33 - SAT37 sp.
MAG03 - Hyphomonas sp.
MAG34 - Erythrobacter sp.

Bacteroidia
MAG41 - HDS-28 sp.
MAG40 - HDS-28 sp.
MAG27 - HDS28 sp.
MAG10 - Unassigned Flavobacteriaceae (family)

E2 (Domain - Archaea)
MAG15 - Unassigned UBA9212 (order)

Gammaproteobacteria
MAG35 - Immundisolibacter sp.
MAG24 - Unassigned Immundisolibacteraceae (family)
MAG36 - Unassigned Immundisolibacteraceae (family)
MAG01 - Unassigned Immundisolibacteraceae (family)
MAG02 - Unassigned Immundisolibacteraceae (family)
MAG43 - Macondimonas diazotrophica
MAG42 - Unassigned GCA-001735895 (genus)
MAG39 - UBA5861 sp.
MAG06 - Cycloclasticus sp.
MAG05 - Cycloclasticus sp.
MAG19 - UBA5859 sp.
MAG28 - Psychrosphaera sp.
MAG08 - Alteromonas sp.
MAG17 - Unassigned Kangiellaceae (family)
MAG07 - Pleionea sp.
MAG04 - Unassigned JADFDG01 (family)
MAG38 - Unassigned UBA3067 (family)
MAG37 - Unassigned UBA3067 (family)
MAG31 - HB2-32-21 sp.
MAG32 - Marinobacter sp.
MAG22 - Marinobacter sp.
MAG21 - Oleibacter sp.
MAG30 - Oleibacter sp.
MAG11 - Oleibacter sp.

Nitrososphaeria (Domain - Archaea)
MAG18 - PXYB01 sp.

Saccharimonadia
MAG16 - Unassigned Saccharimonadales (order)

Thermoanaerobaculia
MAG12 - Unassigned UBA5704 (family)
MAG23 - Unassigned UBA5704 (family)

Fig. S3. Comparison of diversity estimates using MAGs and metagenomes. Relative abundance for the top 50 most abundant Classes is shown.

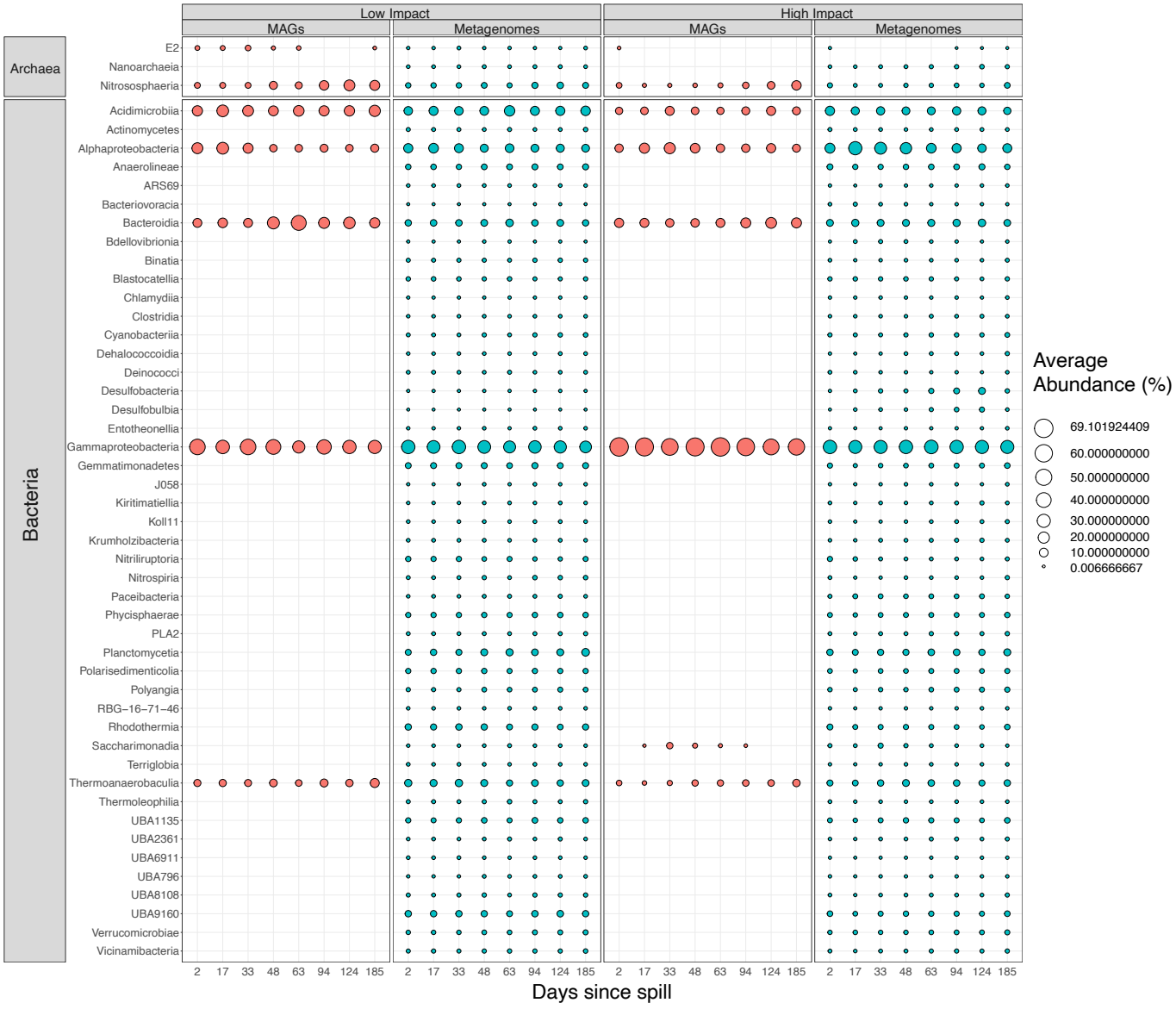


Fig. S4. Abiotic variables at low-impact and high-impact sites.
P-values indicate significance between sample groups.

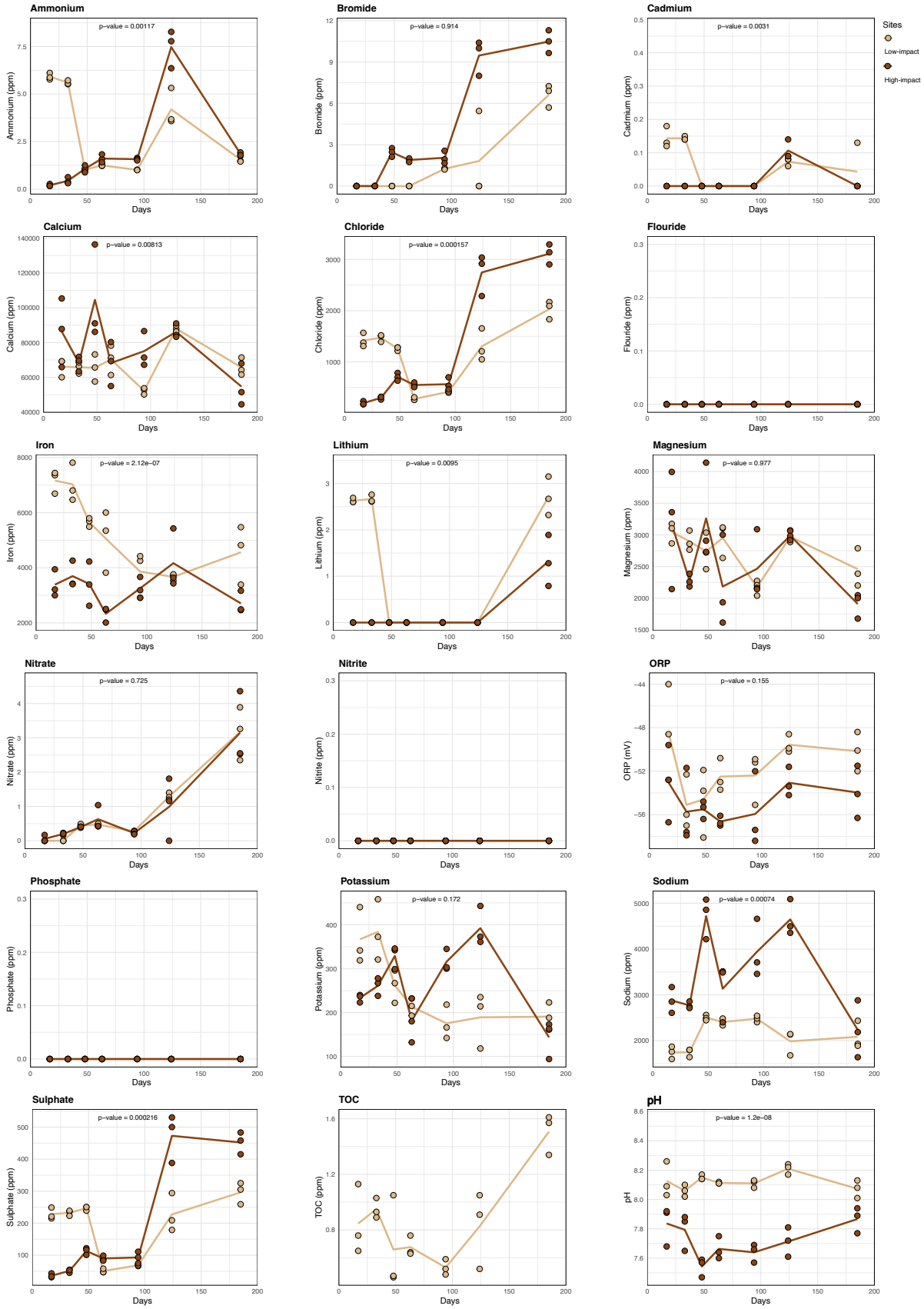


Fig. S5. Distribution of all hydrocarbon-degrading genes in MAGs.

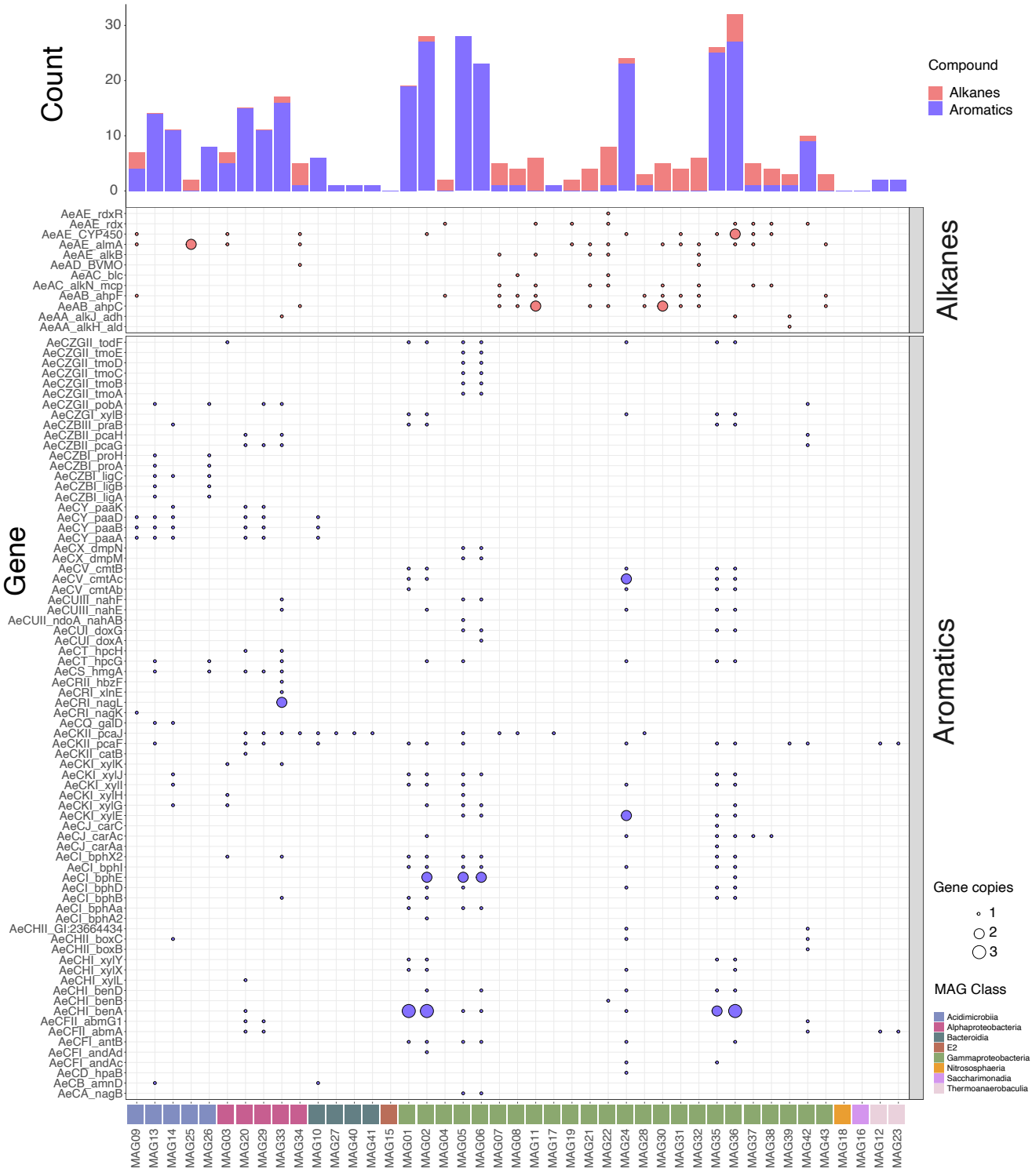


Fig. S6. Estimation of hydrocarbon-degrading pathways in MAGs.

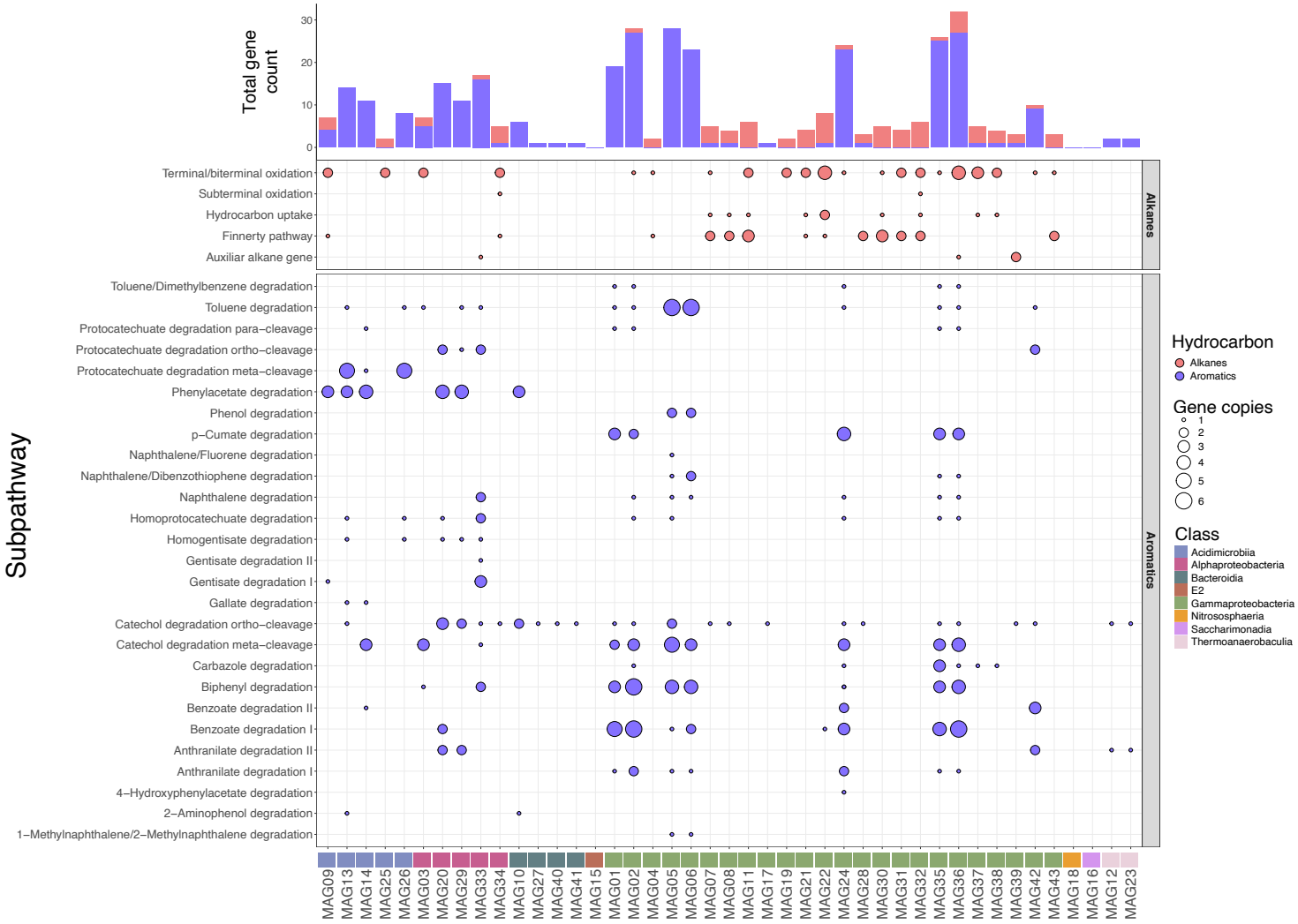


Fig. S7. Distribution of all hydrocarbon-degrading genes in environmental samples.

Gene abundances were quantified as transcripts per million (TPM), normalized for both gene length (in kilobases) and sequencing depth (per million reads), thus enabling standardized comparisons across samples (*N* = 48).

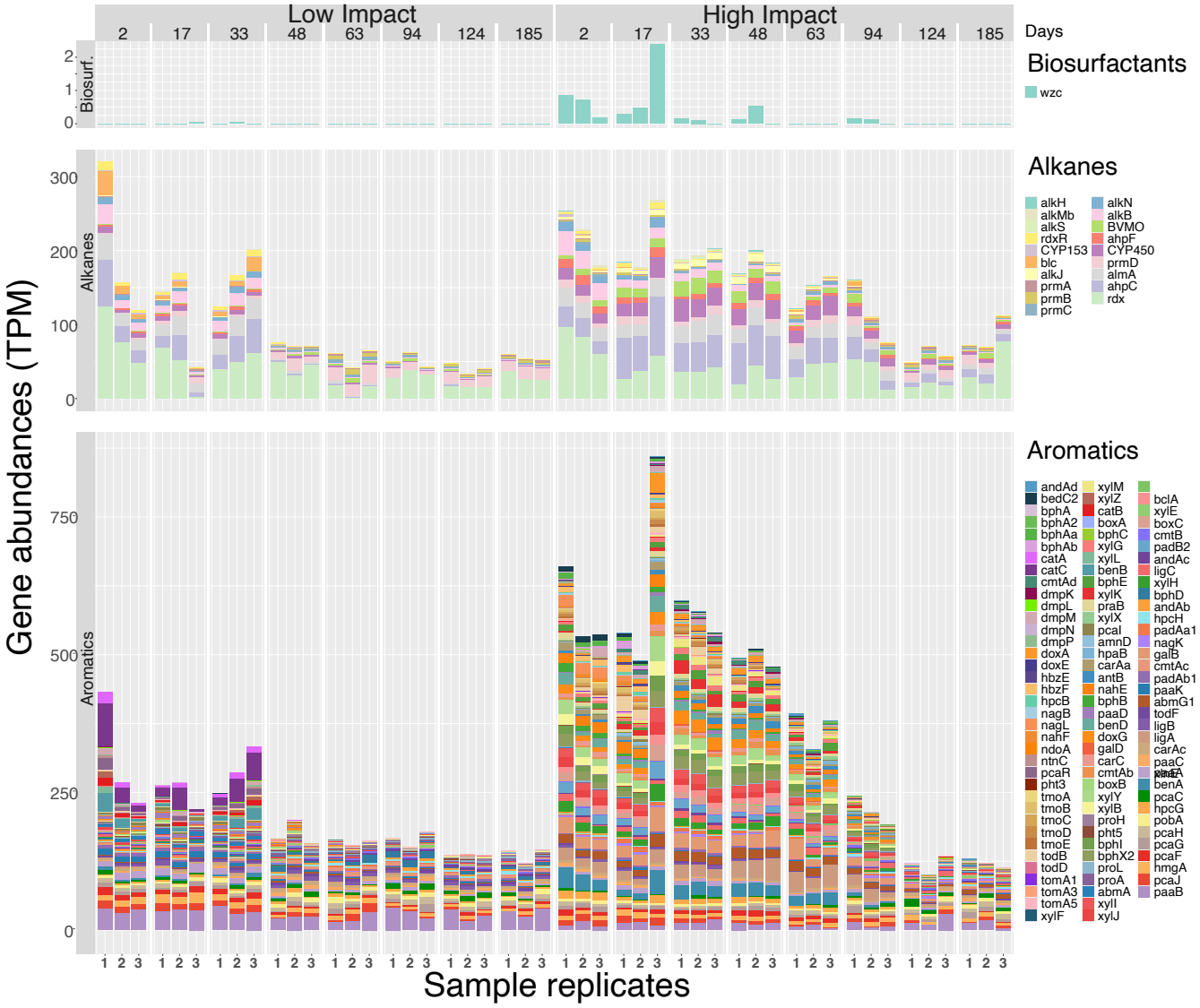


Fig. S8. Estimation of hydrocarbon-degrading pathways in environmental samples. Pathway contributions based upon gene occurrences in Fig. S7.