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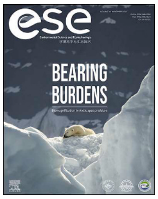

Short Communication

Tropical intertidal microbiome response to the 2024 Marine Honour oil spill

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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 Marine Honour 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. The dominant hydrocarbon-degrading bacteria differed markedly from those reported in other crude oil spills and in regions with different climates. 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.

1. 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 their intertidal supports lower baseline microbial diversity than other coastal habitats due to their oligotrophic and dynamic nature [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], benthic sediments [9,10], and coastal sediments [11–14] exhibited a similar pattern: a rapid

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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], while genes encoding alkane and aromatic hydrocarbon degradation 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 bacteria similar 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 oils are increasingly recognized as a source of accidental spills in marine environments [24]. The lack of mechanistic insight into 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 the microbial response to oil spills has been well-characterized in temperate systems [3,4,26], equatorial coastal environments present both potential challenges and opportunities for microbial degradation of spilled oil, due to the 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 Marine Honour oil spill in Singapore, which released Low-Sulfur 380 MFO that rapidly and extensively spread along the 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.

2. Materials and methods

2.1. Sampling design and sediment collection

Surface sandy sediment cores (0–10 cm depth, using sterile Falcon tubes) were sampled at 3 m spatial intervals from the mid-zone intertidal at Bendera Bay on St John's Island, Singapore (1°13′08″ N, 103°50′53″ E) following the Marine Honour oil spill at the Pasir Panjang Terminal on June 14, 2024. Sampling was carried out with three independent replicates for all abiotic and biotic measurements for each time point at 2, 17, 33, 48, 63, 93, 124, and 185 days post-deposition at low impact (no visible oil) and high impact (completely oil-covered) sites at low tide over six months (overall $N = 48$). For further description of the sampling sites, refer to Supplementary Fig. S1.

2.2. Hydrocarbons and physicochemical analysis

Hydrocarbons were extracted using United States Environmental Protection Agency (US EPA) methods 3540C/3545A [30,31] and quantified via gas chromatography–flame ionization detection (GC–FID; Agilent) for total petroleum hydrocarbons (TPH) following EPA method 8015C [32]. Semi-volatile organics and polycyclic aromatic hydrocarbons (PAHs) were analyzed by gas chromatography–mass spectrometry (GC–MS; Agilent) using EPA method 8270E [33]. Sediment pH and oxidation–reduction potential (ORP) were measured using ion-selective probes (Mettler Toledo). All aqueous measurements were performed in 1:2 (w/v) suspensions of sediment in deionized water. Total organic carbon was quantified using a total organic carbon analyzer (Shimadzu) using standardized methodology [34]. Nitrate, nitrite, phosphate, and sulphate were quantified by ion chromatography (Dionex) using EPA method 300 [35]. Metals were extracted from sediment using EPA method 3050B [36], and analyzed via inductively coupled plasma–optical emission spectrometry (ICP–OES; Agilent) using EPA method 200.7 [37]. Sediment toxicity was estimated using the Microtox acute toxicity assay (Modern Water) according to ISO 11348-3 [38]. This bioluminescence-based test measures viability in the bacterium *Vibrio fischeri*, where the sample dilution ratio (w/v, sediment in water; expressed as mg L^{-1}) that yields a 50% reduction of bacteria's light emission is expressed as the half-maximal inhibitory concentration [IC50]. Negative and positive controls were diluent (deionized water) and a 0–100 mg L^{-1} phenol positive control.

2.3. 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 [39], followed by bin analysis using MetaBAT [40] and Maxbin [41]. Metagenome-assembled genomes (MAGs) were dereplicated at ≥95% ANI using DAS Tools [42]. High-quality (>90% completeness, <5% contamination) and medium-quality (>50% completeness, <10% contamination) MAGs were retained for downstream analysis. The relative abundance of MAGs in the samples was estimated using CoverM [43]. Taxonomy was assigned via GTDB-Tk (release 220) [44], and phylogenomic trees were visualized in iTOL v6 [45]. Taxonomic diversity in metagenomes was estimated using SingleM [46]. Functional genes were annotated using eggNOG v.6.0 [47]. Functional annotation of genes associated with hydrocarbon degradation and aggregation into pathways was conducted using the curated HADEG database [48], and estimated using both metagenomes and MAGs. Gene abundances were quantified as transcripts per million, normalized for both gene length (in kilobases) and sequencing depth (per million reads), thus enabling standardized comparisons across samples.

2.4. Statistical analysis

Data analysis was conducted in R using the ‘vegan’ package for estimation of alpha diversity (Shannon's Index, H), beta diversity (non-metric multidimensional scaling [NMDS] ordination of Bray-Curtis dissimilarity distances), Analysis of variance [ANOVA], Tukey's honestly significant difference [HSD], multivariate analysis of abiotic influences on community structure (envfit), and t-tests for Microtox data [49]. Correlations for abiotic and biotic variables (using generalised linear models, GLM) were performed in base R.

3. Results and discussion

3.1. Oil hydrocarbons were rapidly depleted in tropical intertidal sediments

Following the Marine Honour oil spill, extensive hydrocarbon deposition was observed along Singapore's sandy intertidal zone (Supplementary Fig. S1). Total petroleum hydrocarbon (TPH) concentrations in high-impact sediments initially ranged from 1810 to 9408 mg kg^{-1} but stabilized within 17 days (1349–1965 mg kg^{-1}), then declined steadily up to 185 days post-spill while remaining elevated relative to low-impact sites (Fig. 1a).

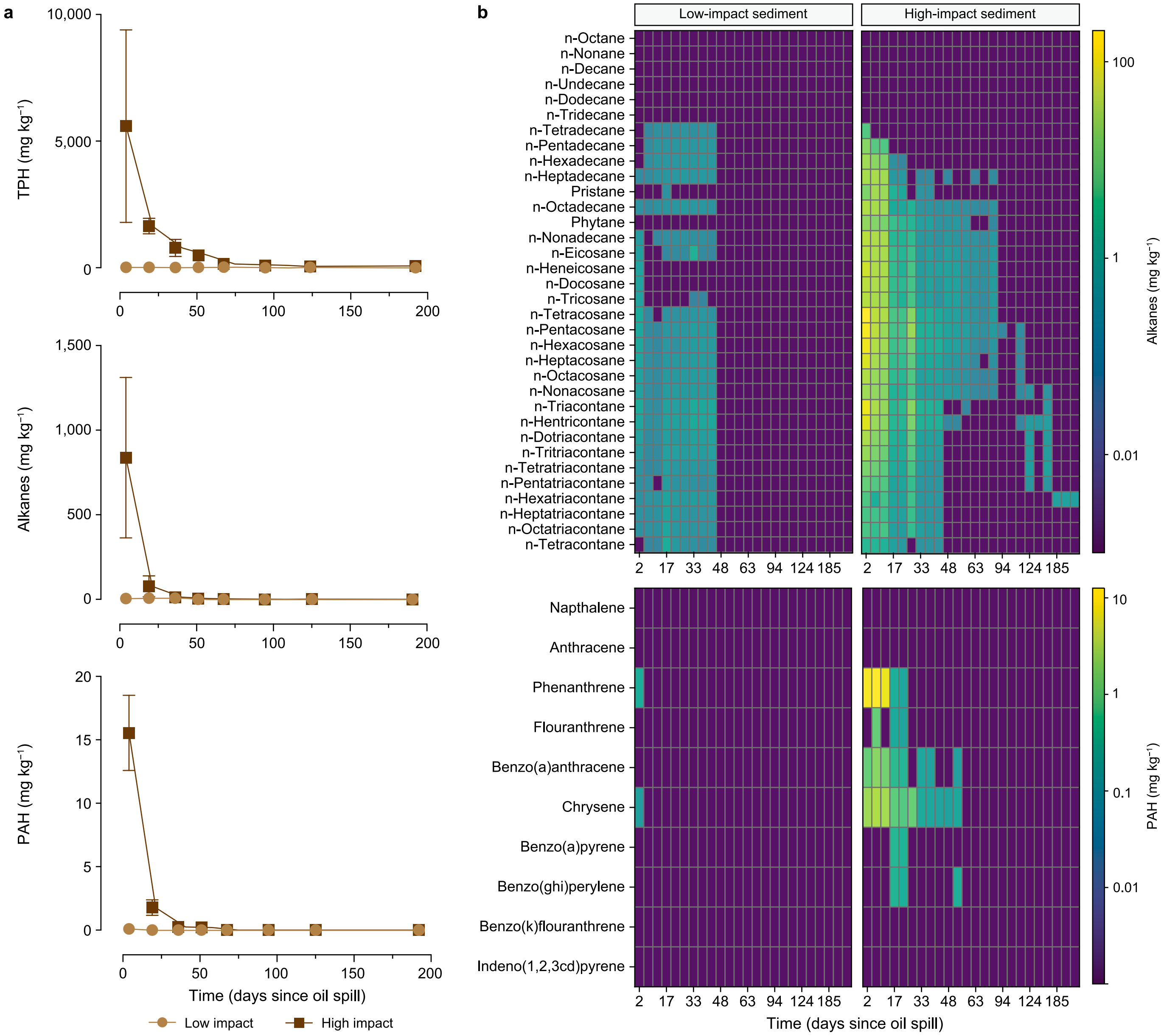

Fig. 1. **a**, Concentrations of hydrocarbons in intertidal sediments following the oil spill. **b**, Composition profiles of individual alkanes and polycyclic aromatic hydrocarbons (PAHs) in post-spill sediments. Hydrocarbon values are displayed as mg kg^{-1} dry sediment. All measures were performed on three independent replicates, and mean values were reported, with error bars indicating the standard deviation. Low-impact sediments returned very low hydrocarbon values; these are replotted with an expanded scale in Supplementary Fig. S2. TPH, total petroleum hydrocarbons.

This initial deposition was comparable to that for crude oil at Pensacola Beach after the Deepwater Horizon oil spill (4500–11,000 mg kg^{-1} in sand), where hydrocarbons were undetectable one year post-spill [13]. Low-impact sediments, although visually clean, exhibited detectable TPH (16.9–20.9 mg kg^{-1}) immediately post-spill, decreasing to undetectable levels by the study's end. No comparable studies for Low-Sulfur MFO 380 are available; however, microcosm studies for other fuel oils (bunker C, petro-diesel) suggest that they may deplete at similar rates in marine sediments and seawater [20,50], despite different environmental conditions.

Alkanes are the most abundant compounds in MFO and dominate its hydrocarbon profile, with C_{18}–C_{29} fractions (n-octadecane to n-nonacosane, Fig. 1b) being most abundant. PAHs form a minor component of MFO but are also important due to their toxicity to marine life and as general indicators of persistent marine pollution [51]. We measured ten representative size classes of PAH and notably observed fuel oil-associated compounds phenanthrene, benzo(a)anthracene, and chrysene were elevated post-spill (Fig. 1b). Alkane depletion followed molecular weight trends: shorter-chain compounds (C_{14}–C_{19}, n-tetradecane to n-nonadecane, Fig. 1b) degraded rapidly, while heavier alkanes (C_{29}–C_{36}, n-nonacosane to n-hexatriacontane, Fig. 1b) 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 (Fig. 1b). 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.

3.2. *Sediment microbiomes displayed an immediate response to oil hydrocarbons*

We recovered 43 MAGs (41 Bacteria, 2 Archaea) spanning eight microbial classes (Fig. 2a, Supplementary Fig. S3). This was consistent with Intertidal sand communities, which support low diversity and abundance relative to other marine habitats, likely reflecting the oligotrophic and dynamic nature of the habitat [5,13]. The dominant bacterial classes, Alphaproteobacteria and Gammaproteobacteria, were more abundant and diverse in samples with higher hydrocarbon values, while Acidimicrobiia and Bacteroidia were less abundant. Archaea, such as Candidate Class E2 and Nitrosphaeria, displayed lower diversity and abundance in high-impact sediments, consistent with the established role of Nitrosphaeria in undisturbed marine habitats [52], and suggesting potential roles as indicators of recovery. Genomes indicating anaerobic Thermoanaerobaculia (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 (Supplementary Fig. S4). 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, suggesting a potential reservoir of microbial diversity that facilitates ecological resilience under disturbance [53].

Initial response showed that a hydrocarbon-degrading microbiome was recoverable within two days of the oil spill. Overall, alpha diversity estimates revealed that high-impact sites (mean $H = 2.975$) and low-impact sites (mean $H = 2.665$) were significantly different from day 17 onwards (ANOVA, $p \leq 0.001$; Tukey HSD $p \leq 0.01$). Microbial beta diversity community patterns observed through NMDS ordination of Bray-Curtis dissimilarities (95% confidence interval) showed distinct microbiome differences between high- and low-impact sites. Microbial communities at low- and high-impact sites exhibited increasing convergence in composition over time, likely driven by the progressive depletion of oil hydrocarbons resulting in more similar environmental conditions across sites (Fig. 2b). Hydrocarbons emerged as significant abiotic drivers of differences in community structure between low- and high-impact sediments, in addition to other abiotic variables including pH, ORP, nutrients, and trace metals (envfit, $p \leq 0.05$; Supplementary Fig. S5).

3.3. *The oil spill selected for a specialized hydrocarbon-degrading microbiome*

Genomes were screened against the HADEG database, a curated database that 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, Supplementary Fig. S6). All MAGs in the classes Acidimicrobiia, Alphaproteobacteria, Bacteroidia, and 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 (Supplementary Fig. S6). Ring cleavage pathways were also widespread; however, some, such as phenol degradation, occurred only in genomes of *Cycloclasticus* sp. (MAGs 05 and 06). Similarly, genes for *p*-cumate degradation were specific to genomes of Immundisolibacteraceae (MAGs 01, 02, 24, 35, and 36). Overall, the most comprehensive suites of aromatic degradation pathways also belonged to these latter two bacterial groups (Supplementary Fig. S7).

Gammaproteobacteria genomes exhibited the most abundant alkane degradation genes, notably in *Oleiobacter* sp. (MAGs 11, 21, and 30) [54], *Macondimonas* sp. (MAG 43) [55], *Marinobacter* sp. (MAGs 22 and 32) [56], 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 [57], as well as *Alteromonas* sp. (MAG 08) [58], and *Cycloclasticus* sp. [59]. Oil-degrading taxa such as *Alcanivorax*, which was prominent following the Deepwater Horizon crude oil spill in oceanic water and beaches [13], and *Colwellia*, which has been associated with cold deepwater [60] and polar environments [61], were not recovered in our genome library and occurred with very low read abundance in metagenomes, thus reinforcing the notion that oil-degrading microbiomes may be habitat-specific and that tropical environments support a distinct oil-degrading microbiome. Instead, *Macondimonas diazotrophica* (MAG 43), a known oil-degrading and nitrogen-fixing bacterium [55], emerged as the most abundant taxon in response to the MFO spill in high-impact locations. This may in part reflect hydrocarbon substrate preferences, habitat preference for coastal sand and/or 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 their occurrence in temperate [62] and polar [63] oil spill locations, as well as deep ocean oil seeps [64], suggest that 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 [65], and other taxa such as *Hyphomonas* sp. (MAG 03), *Roseivivax* sp. (MAG 20), and novel Rhodobacteraceae (MAGs 29 and 33) implicated in degradation of aromatics and asphaltenes [66]. Within the Acidimicrobiia, which is the third most diverse clade, *Ilumatobacter* sp. (MAG 09) is a bacterium associated with beach sand after oil contamination; however, this taxon is also encountered in pristine coastal environments [67].

3.4. *Microbiome responses varied with oil deposition intensity*

The abundance of genes implicated in microbial alkane and aromatic compound degradation, detoxification, and biosurfactant production responded rapidly to the oil spill (Fig. 3b; Supplementary Fig. S8). Overall diversity and abundance of genes involved in hydrocarbon degradation were positively correlated with the level of oil hydrocarbons in sediments (GLM, alkanes $p \leq 0.001$, PAH $p \leq 0.001$). Mapping gene abundances to catabolic pathways confirmed that complete degradation of hydrocarbons could be achieved (Supplementary Fig. S9). Biosurfactant genes (*wzc*) increased in abundance relative to the 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 [68].

Among the ten most abundant alkane-associated genes, rubredoxin (*rdx*), an electron carrier in alkane oxidation, was the

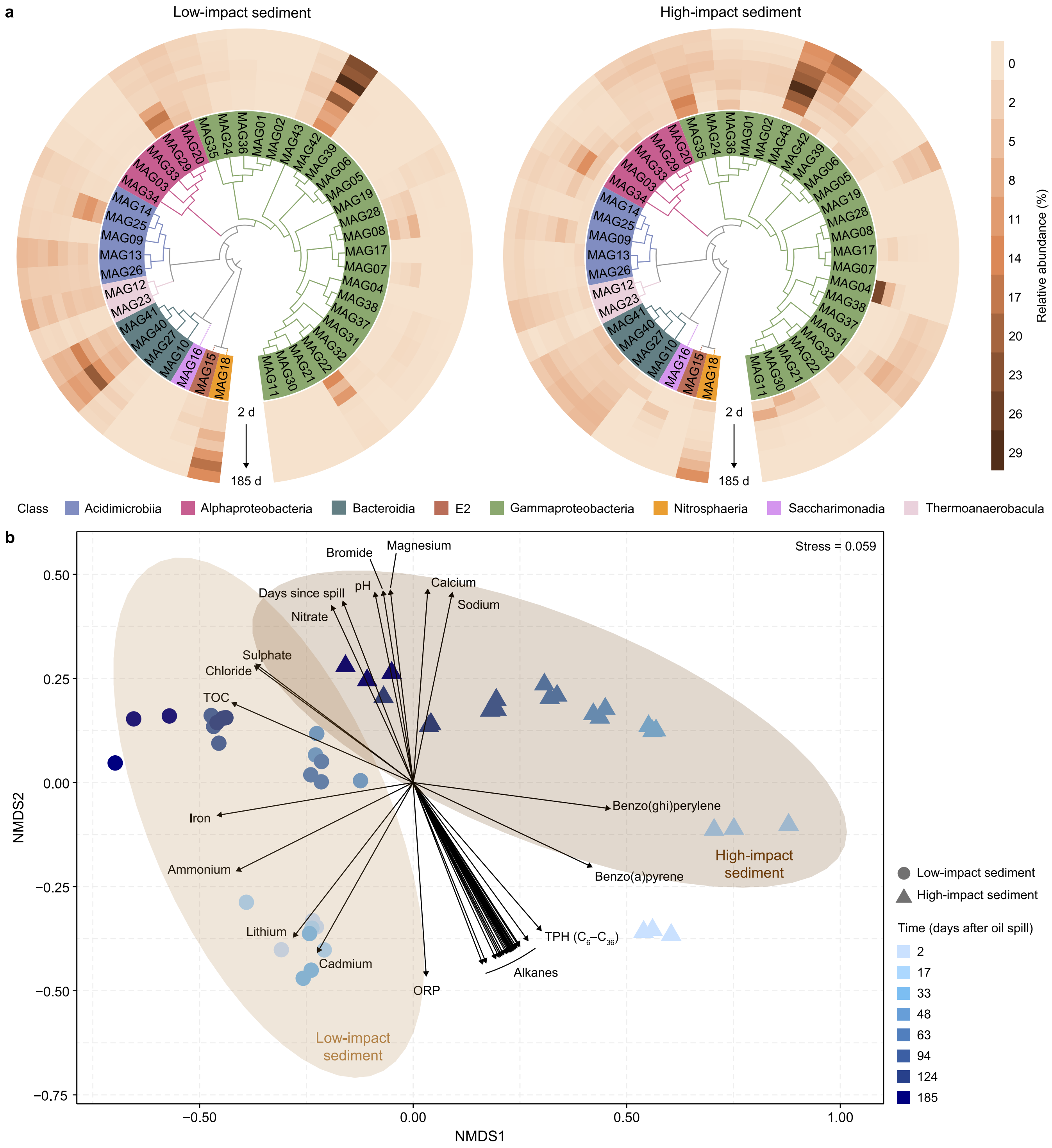

Fig. 2. **a**, Phylogenomic diversity and distribution of metagenome-assembled genomes (MAGs) across sites over time. The arrows for time indicate the sequential display of data for each sampled time point at 2, 17, 33, 48, 63, 93, 124, and 185 days after oil deposition in sediments. Full taxonomic resolution for all MAGs is shown in Supplementary Fig. S3 **b**, Beta diversity of microbial communities in low-impact (circles) versus high-impact (triangles) sediments. Biotic data were plotted as Bray-Curtis dissimilarities using non-metric multidimensional scaling (NMDS), and the influence of abiotic variables was estimated using multiple regression analysis (envfit, $p \leq 0.05$). Ellipses denote samples clustering at a 95% confidence interval. TOC, total organic carbon; ORP, oxidation–reduction potential; TPH, total petroleum hydrocarbons.

most abundant gene across all samples. The C_5–C_{16} 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 peaked early only in high-impact sediments and may reflect adaptation to high hydrocarbon levels. The long-chain ($\geq C_{20}$) alkane monooxygenase *almA* were enriched in high-impact sediments and during the early phase of low-impact sediments. Detoxification-related genes

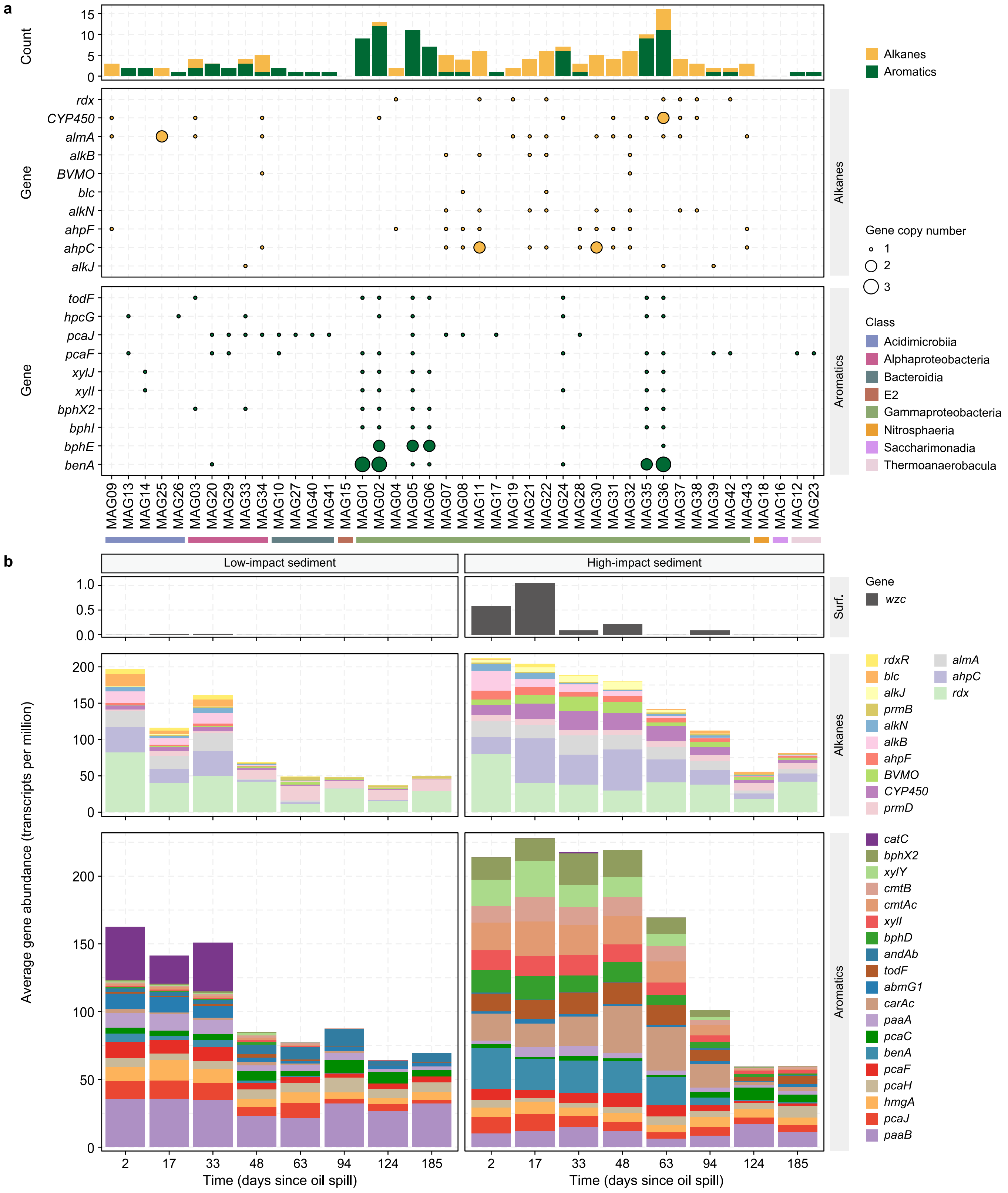

Fig. 3. Distribution of the ten most abundant hydrocarbon degradation genes from the HADEG database across metagenome-assembled genomes (MAGs; **a**) and environmental samples (**b**). Complete gene abundance profiles and pathway mapping are provided for MAGs in Supplementary Fig. S6 and S7, and for environmental samples in Supplementary Fig. S8 and S9. Surf., biosurfactant 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 C_2–C_4 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 [69].

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 the 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: 24×10^3 versus 9×10^3 mg L^{-1}; *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 [26,70]. This suggests that spill severity and hydrocarbon type shape the persistence of microbial degradation potential in sediments.

3.5. Oil-degrading microbiomes endured six months post-spill

Temporal trends showed that hydrocarbon gene abundance peaked earlier ($\leq$33 days) in low-impact sites, whereas high-impact sites exhibited longer duration and more diverse hydrocarbonoclastic pathway representation (Supplementary Fig. S8 and S9). Oil-degrading microorganisms are typically rare in pre-spill communities [26,70], yet hydrocarbon degradation is now understood as a widespread trait among environmental microorganisms [7,63,71]. Chronic but low-level hydrocarbon exposure in marine locations such as Singapore, which has long been a global maritime and petrochemical hub, may have selected for latent microbial communities pre-adapted to such disturbances. There has, however, been no other major oil spill to Singapore's coastlines since the Mt Bunga Kelana 3 crude oil tanker spill in 2010 [72].

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 (Supplementary Fig. S9). 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. It was not possible to obtain pre-spill baselines in this real-time response to the oil spill, and this limitation is acknowledged. However, we showed that when sediments returned to a background/undetectable level of hydrocarbons, the microbiomes in previously heavily oiled sediment retained greater diversity and abundance of hydrocarbon-degrading genes than those in low-impact sediments without oil deposition.

4. Conclusion: tropical coastal management lessons from Singapore's oil spill

The Marine Honour oil spill offered a rare opportunity to observe real-time consequences of an 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* metagenomic responses. Findings show that, even without sand removal, microbial communities in Singapore's intertidal zones rapidly adapt the ability to degrade hydrocarbons and potentially assist in recovery from 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. This pollutant class remains insufficiently explored in the current literature.

The relative contribution of biodegradation versus abiotic processes remains unresolved, and we predict that mobilization of oil from any given intertidal location may be the largest factor accounting for hydrocarbon depletion. 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 [73,74], as well as the potential impact of dispersants used in oil spill control [10,75], require further study.

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

CRediT authorship contribution statement

Christaline George: Writing - Review & Editing, Software, Methodology, Investigation, Formal Analysis, Data Curation, Conceptualization. **Hashani M. Dharan:** Writing - Review & Editing, Investigation. **Lynn Drescher:** Investigation, Formal Analysis. **Jenelle Lee:** Writing - Review & Editing, Methodology. **Yan Qi:** Writing - Review & Editing, Formal Analysis. **Yijin Wang:** Writing - Review & Editing, Investigation. **Ying Chang:** Writing - Review & Editing, Formal Analysis. **Serena Lay Ming Teo:** Writing - Review & Editing, Methodology, Formal Analysis, Conceptualization. **Benjamin J. Wainwright:** Writing - Review & Editing, Methodology, Formal Analysis, Conceptualization. **Charmaine Yung:** Writing - Review & Editing, Methodology, Formal Analysis. **Federico M. Lauro:** Writing - Review & Editing, Methodology, Investigation, Funding Acquisition, Conceptualization. **Terry C. Hazen:** Writing - Review & Editing, Formal Analysis. **Stephen B. Pointing:** Writing - Review & Editing, Writing - Original Draft, Supervision, Resources, Project Administration, Methodology, Investigation, Funding Acquisition, Formal Analysis, Conceptualization.

Data availability statement

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

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ese.2025.100623.

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