Environmental Research 277 (2025) 121563

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Do coastal bacterioplankton communities hold the molecular key to the rapid biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) from shipping scrubber effluent?

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ARTICLE INFO

Keywords:
Xenobiotics
Coast
Microbiota
Biodegradation
PAHs
-Omics
Scrubber washwater
IMO

ABSTRACT

Shipping scrubber effluents, containing a cocktail of Polycyclic Aromatic Hydrocarbons (PAHs), show undisputed effects at single-species experiments while PAHs fate in the marine environment after effluent discharge is still investigated. Bacterioplankton, composed of abundant diverse taxa with xenobiotic-degrading capabilities, are the first responders to scrubber emissions and can affect PAHs impacts on marine life. This work aims to examine the fate of scrubber effluent PAHs and alkyl-PAHs in mesocosms of coastal bacterioplankton communities from a pristine (phytoplankton carbon biomass was 8.16 μg C L^{-1}) and a eutrophic (105.35 μg C L^{-1}) coastal site. High-throughput 16S rRNA metabarcoding revealed differential responses of the bacterioplankton linked to their initial community structure and population abundances. Taxa known for their PAHs-degrading capacity were retrieved, including the genera *Roseobacter*, *Porticoccus*, *Marinomonas*, *Arcobacter*, *Lentibacter*, *Lacinutrix*, *Pseudospirillum*, *Glaciecola*, *Vibrio*, *Marivita*, and *Mycobacterium*, and were found to have increased roles in shifted communities by increasing their relative abundances at least 5-fold in treatments with high scrubber effluent additions. Additionally, metagenomic analysis of shotgun sequencing, indicated an increase on the number of Clusters of Orthologous Genes (COGs) associated with pathways involved in PAHs degradation. Up to 198 more COGs involved in signal transduction were retrieved in scrubber effluent enriched mesocosms compared to controls, while 15, 86, and 136 more COGs associated with naphthalene, aromatic compound, and benzoate degradation, respectively, were detected in the pristine mesocosms after effluent additions. In both experiments, bacterioplankton responses towards xenobiotic degradation under increased PAHs and alkyl-PAHs were coupled with a drop in their concentrations, below the limit of detection by Day 3 of the experiment in the eutrophic community, and by half in Day 6 in the pristine environment's community. Our findings indicate that PAHs and alkyl-PAHs impacts can be rapidly reduced in natural systems of high bacterial activity.

1. Introduction

One of the major environmental impacts of maritime transport was linked to the large emissions of sulfur oxides into the atmosphere. To mitigate their effects on the environment and human health, the International Maritime Organization (IMO) set a limit on the sulfur content in the fuel oil used on board ships to 0.5 % m/m, marking a significant milestone to improve air quality [IMO MARPOL Annex VI, MEPC.280(70), 2016]. However, naphthalene added to low sulfur oil as stabilizer can lead to higher atmospheric Polycyclic Aromatic

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https://doi.org/10.1016/j.envres.2025.121563
Received 9 December 2024; Received in revised form 1 April 2025; Accepted 6 April 2025
Available online 8 April 2025

Hydrocarbons (PAHs) emissions (Yeh et al., 2023). Alternatively, ships can be equipped with Exhaust Gas Cleaning Systems (EGCS), also known as scrubbers. In open-loop systems a wastewater is formed after spraying seawater into the EGCS and is directly released into the marine environment (Romero-Martínez et al., 2024). Scrubbers can remove up to 66 % of the SO_x (Andreasen and Mayer, 2007), reducing emissions to compliant levels (Grigoriadis et al., 2024). However, the use of the scrubbers' technologies result in a shift from atmospheric pollution to burdening marine ecosystems, as the washwater produced contains several contaminants, such as PAHs, metals, NO_x eutrophication agents, acidifying compounds (Teuchies et al., 2020), and nanoparticles in air with yet understudied costs to impacted systems (Grigoriadis et al., 2024). Furthermore, there is lack of data for alkyl-PAHs which can be even more toxic than parent PAHs (García-Gómez et al., 2023).

Even though the long-term consequences of the inflow of these pollutants in the diverse marine bio-communities are yet undetermined (Teuchies et al., 2020), and older reports expect small overall impact of scrubber use on pH and contaminant concentrations in seawater (e.g. Kjølholt et al., 2012), scientists are sounding alarm for "bad news for the marine environment" (Hassellöv, 2023) and are proposing open-loop scrubber discharge bans in coastal areas and ports, followed by a ban of bleed-off washwater discharge from closed-loop scrubbers by the end of the decade. This is fuelled by scenario-based models based on emission factors from conventional combustion and from combustion with scrubbers, which suggest that heavy fuel oil combustion with scrubbers increase the environmental load for most metals and PAHs (Hermansson et al., 2021). Furthermore, it has been estimated that open-loop scrubbers contribute with about 9 % of the total PAHs input in the Baltic Sea (Ytreberg et al., 2022). The impacts of various open-loop scrubber effluent dilutions were tested on single-species biological indicators and showed negative acute effects on the bioluminescence of the bacterium *Aliivibrio fischeri*, and on the growth and survival of plankton organisms at 10 % and 20 % of dilutions. Furthermore, low dilutions (<1 %) severely affected the developmental stages and reproductive success of the copepod *Acartia tonsa* (Picone et al., 2023).

On the other hand, Genitsaris et al. (2023, 2024) reported an acute bacterioplankton-based biodegradation of PAHs at various scrubber effluent concentrations in mesocosm experiments with natural planktonic multi-domain microbial communities. Their results agree with a previous study on the effects of scrubbing on microplankton communities, which showed cell-specific increase in bacterial productivity and differential phytoplankton group-specific responses with scrubber effluent additions due to elevated concentrations of eutrophying nitrogen compounds (Ytreberg et al., 2021). Such shifts in microalgal and bacterial communities' dominance with scrubber effluent inputs are not expected to affect higher trophic levels, since bacterioplankton assemblages can exhibit high remediation capacity (Genitsaris et al., 2024). For example, microalgae and bacteria efficiently removed PAHs in a wastewater treatment process (Lu et al., 2022). Although several studies agree that complex trophic interactions might increase ecosystem resilience in the face of perturbation (Vallina and Le Quéré, 2011; Genitsaris et al., 2016), there is little information on these interconnected food-web processes exposed to scrubber effluent discharge in the marine environment.

In the light of the growing concerns for the implications of open-loop scrubber discharge into areas with high marine traffic (Hassellöv, 2023), natural microbial communities can act as a buffering first level of contact with effluent contaminants such as PAHs (Genitsaris et al., 2024). The remediation capacity of marine bacteria by activating several genes that code for enzymes implicated in various xenobiotics degradation is long known (Miglani et al., 2022; Catania et al., 2020). Several taxa have been linked to PAHs rapid degradation, either acting alone or in multispecies consortia (Paniagua-Michel and Fathepure, 2018). Few of them have been isolated and characterized to possess hydrocarbon biodegradation abilities, belonging -among others-to the genera *Marinomonas* (Zannoti et al., 2023), *Porticoccus* (Gutierrez et al., 2012), *Mycobacterium* (Kim et al., 2008), and *Vibrio* (Smith et al., 2012). Recent culture-independent -omics technologies have broadened the detection potential for remediation-relevant taxa and their corresponding genetic pool. For example, 16S rRNA metabarcoding identified PAH-utilizing taxa including *Marinomonas*, *Porticoccus* and *Lutibacter* to increase their relative participation in bacterial communities after treatment with high-molecular weight PAHs (Mu et al., 2021). The biodegradation of PAHs by specific isolates and assemblages of synergistically acting bacteria involves a series of complex biochemical reactions typically initiated by multicomponent dioxygenase enzymes, followed by ring cleavage and further breakdown into simpler molecules like catechols, which can enter central metabolic pathways (Haritash and Kaushik, 2009).

The use of -omics tools is crucial to reveal key mechanisms and taxa involved in microbial degradation processes and help us understand the fate of pollutants in the marine environment. In this study, the capacity of coastal bacterioplankton communities to contribute to the biodegradation processes of contaminants in the scrubber effluent were examined in community mesocosm experiments using metabarcoding and shotgun sequencing. Potential taxonomic shifts towards hydrocarbon and aromatic compound bacterial degraders, and the presence of genes associated with xenobiotics, PAHs and alkyl-PAHs degradation pathways were targeted with sequencing tools, after low (1 % V/V) and high scrubber (5 % V/V) effluent inputs to natural seawater from two different coastal locations, representing pristine and eutrophic environments. These dilutions were selected to simulate environmentally relevant cases of high PAHs concentrations that are reported in busy shipping passages, such as the Aegean Sea (Valavanidis et al., 2008) and Alexandria coast in the Mediterranean Sea (El Nemr and Abd-Allah, 2003) and Haikou Bay in China (Li et al., 2015a,b). The findings from this study enhance our limited understanding of the environmental fate of PAHs discharged to coastal environment from the open-loop scrubbers of ships while the installation of these systems on both new and retrofitted ships is increasing globally.

2. Materials and methods

2.1. Experimental design

Test community mesocosms were set up following the experimental design analytically described in Genitsaris et al. (2023). Briefly, scrubber effluent from the discharge outlet of an open-loop system of a container ship (the DANAOS *Leo C*) was obtained during an on-board campaign of the EMERGE project at the Mediterranean Sea south of the Peloponnese (36°16′25′′N, 22°02′51′′E) in November 2021. Scrubber effluent samples were collected when the ship was using the heavy fuel oil type HFO2. The pH of the scrubber effluent was 2.9 and the nitrate and nitrite nitrogen content were 55.6 μM (Genitsaris et al., 2023). The number of particles in the scrubber effluent (a mix of aggregates of micro- and nanoparticles) was 1.5 X 10^6 particles L^{-1} (Gondikas et al., 2025). The effluent was added in different volumes in natural seawater sampled from two different coastal sites in the Saronikos Gulf, Greece (Vouliagmeni: 37.799798N, 23.788917E, representing a pristine area, and Flisvos: 37.936514N, 23.685072E, a eutrophic site receiving high anthropogenic pressures; Supplementary Fig. S1). Surface seawater was sampled excluding larger zooplankton with a 200 μm mesh to avoid top-down pressure to the microbial communities. The seawater containing natural bacterioplankton communities was used for establishing three different treatments per sampling site. Triplicates of control (C: seawater of 12 L volume), low scrubber effluent (LS: 1 % V/V final concentration of the scrubber effluent to 12 L final volume), and high scrubber effluent (HS: 5 % V/V final concentration) experimental glass bottles were set in stable lab conditions for six days (Table 1, Supplementary Fig. S2). Another triplicate treatment of 2 % V/V scrubber effluent concentrations (MS) were set but were examined only with microscopy to determine bacterioplankton

Table 1
Details of the set-up of the experiment. Natural source-ecosystem, location of source, and sampling details for multifaceted analysis.

Source ecosystem	Coordinates	Codes	Description	Tools of analysis	Abiotic parameters
Vouliagmeni (V)	37.799798 (N)	V	Natural seawater from the pristine Vouliagmeni	16S rRNA gene metabarcoding	PAHs
	23.788917 (E)	VCd3 a-c	Control replicates, Day 3	Metagenomics	Alkyl-PAHs pH
		VCd6 a-c	Control replicates, Day 6		
		VLSd3 a-c	1 % v/v effluent concentration, Day 3		
		VLSd6 a-c	1 % v/v effluent concentration, Day 6		
		VHSd3 a-c	5 % v/v effluent concentration, Day 3		
		VHSd6 a-c	5 % v/v effluent concentration, Day 6		
		VMSd3 a-c	2 % v/v effluent concentration, Day 3	Microscopy	pH
		VMSd6 a-c	2 % v/v effluent concentration, Day 6		
Flisvos (F)	37.936514 (N)	F	Natural seawater from the eutrophic Flisvos	16S rRNA gene metabarcoding	PAHs
	23.685072 (E)	FCd3 a-c	Control replicates, Day 3	Metagenomics	Alkyl-PAHs pH
		FCd6 a-c	Control replicates, Day 6		
		FLSd3 a-c	1 % v/v effluent concentration, Day 3		
		FLSd6 a-c	1 % v/v effluent concentration, Day 6		
		FHSd3 a-c	5 % v/v effluent concentration, Day 3		
		FHSd6 a-c	5 % v/v effluent concentration, Day 6		
		FMSd3 a-c	2 % v/v effluent concentration, Day 3	Microscopy	pH
		FMSd6 a-c	2 % v/v effluent concentration, Day 6		

abundances (see methods below).

The bacterioplankton responses at multiple molecular endpoints were examined with metabarcoding and metagenomics in water subsamples taken from the initial seawater and at Day 3 and Day 6 from controls and HS bottles.

The temperature and salinity levels were controlled within the range of short-term fluctuations of the *in situ* conditions of the seawater at the time of sampling. The mesocosms were exposed to natural light conditions with the day–night cycle that corresponded to the time of the lab experiments (February, 10h light: 14h night). Measurements of pH were performed in the seawater from both sites and then in treatments daily using the 913 pH Meter (Metrohm). Concentrations of the PAHs, their alkyl derivatives (alkyl-PAHs), and metals were determined in the scrubber effluent and the seawater and at Day 3 and Day 6 -the final day of the experiment-in HS treatments. PAHs and alkyl-PAHs were analysed by solid-phase microextraction coupled with gas chromatography and tandem mass spectrometry, and metals were measured by inductively coupled plasma–mass spectrometry (for specific details, see Supplementary Material; Genitsaris et al., 2023). To highlight differences between the trophic state of the two coastal sites sampled, the pico-, nano- and micro-phytoplankton abundance and carbon biomass were calculated (see Supplementary Material for detailed methods).

2.2. Bacterioplankton abundances

Samples of 10 mL from the natural seawater and from the treatments were collected daily till Day 6 and were fixed with formaldehyde for bacterioplankton counts. The fixed samples were incubated for at least 24 h at 4 °C in the dark, filtered onto black nucleopore filters with 0.2 μm pore size, and stained with 4′,6-diamidino-2-phenylindole (DAPI) following the instructions in Porter and Feig (1980). The filters were observed using a Nikon ECLIPSE TE2000-S fluorescence microscope under UV and green excitation at 1000 × . At least 1000 bacterial cells were counted in optical fields per sample. Day 6 samples were not counted because of an algal wall-growth effect, while no wall-growth effect was observed before Day 6. The growth rate (r) was determined from changes in population abundance between the Day the population reached maximum abundance in Controls (considered as the Critical Time; CT) and seawater abundances divided by the number of days between the two to calculate the value per day according to the equation:

$$r = \frac{\ln B_{CT} - \ln B_0}{D_{CT} - D_0} \left[d^{-1}\right]$$

where B is the cell abundance of the population, CT the critical time (Day) of the maximum cell abundance of the population observed in Controls, and D the Day of the measurement.

2.3. Metabarcoding sequencing

Samples of 500 mL from the natural seawaters and from the replicates of each treatment were collected at Day 3 and at the end of the experiments at Day 6 and were immediately filtered through 0.2-μm nucleopore filters for molecular analyses. The environmental DNA (eDNA) from each filter was extracted using a Macherey–Nagel NucleoSpin® Soil, Genomic DNA Isolation Kit, according to the manufacturer's instructions. The concentration and quality of the recovered DNA was confirmed using Qubit fluorometric quantification.

The extracted DNA was subjected to PCR using primers (Klindworth et al., 2013) targeting the V3–V4 hypervariable regions of the 16S rRNA gene (S-DBact-0341-b-S-17 = CCTACGGGNGGCWGCAG; S-D-Bact-0785-a-A-21 = GACTACHVGGGTATCTAATCC). The amplicons were generated using Q5 High-Fidelity DNA Polymerase (New England Biolabs Ltd., Hitchin, UK) in a MiniAmp Thermal Cycler (Applied Biosystems™, Thermo Fisher Scientific Inc., Waltham, MA, USA), PCR-barcoded during their formation, electrophoresed on agarose gel, gel-extracted and purified using spin columns (Macherey-Nagel GmbH & Co. KG, Düren, Germany), and then equimolarly pooled. The concentration of mixed amplicons was evaluated using a Qubit 2.0 Fluorometer (Invitrogen™, Thermo Fisher Scientific Inc., Carlsbad, CA, USA). Amplicon mixes were used to generate barcoded sequencing libraries, using the MGIEasy Universal DNA Library Prep Set (MGI Tech Co. Ltd., Shenzhen, China), following the manufacturer's instructions. DNA-seq was conducted in the DNBSEQ-G400 platform (MGI Tech Co. Ltd.), using a DNBSEQ-G400RS High-throughput Rapid Sequencing Set (MGI Tech Co. Ltd.) providing paired-end 300 (PE300) chemistry. After sequencing barcode and adaptor trimming, sequencing data were further demultiplexed using Cutadapt (Martin, 2011). Amplicon raw reads were submitted to the NCBI sequence read archive (GenBank-SRA) under the accession number PRJNA903787.

Metabarcoding reads were subjected to downstream processing using the mothur V. 1.34.0 software (Schloss et al., 2009, 2011). Briefly, forward and reverse reads were joined, and contigs below 200 bp, with >8 bp homopolymers and ambiguous base calls, were removed from further analysis. The remaining reads were dereplicated to the unique sequences and aligned independently against the SILVA 138 database, containing 1,983,534 bacterial small subunit (SSU) rRNA gene sequences (Quast et al., 2013). Then the reads suspected of being chimeras were removed using the UCHIME software (Edgar, 2010). The remaining reads were clustered into Operational Taxonomic Units (OTUs) at a

97 % similarity level, using the average neighbor method in mothur. OTUs with a single read in the entire dataset -suspected of being erroneous sequences-were removed from the analysis (see Genitsaris et al., 2016). The resulting dataset was rarefied with the subsample command in mothur to the lowest number of reads in a sample (i.e., 138,745 reads). This course of action was considered the best compromise to retrieve most of the biodiversity detected by sequencing, and to maintain comparable effort among samples, reflected as similar total number of reads/sample. Taxonomic classification was assigned using SINA searches against the SILVA database for bacteria (Pruesse et al., 2012), by applying a lowest accepted level of >80 % similarity with a closest relative. The reads belonging to OTUs related to chloroplasts and unclassified reads were removed from the dataset.

To examine shifts in the bacterial composition and community structure after scrubber effluent additions the OTUs assemblages of the replicates at Day 3 and at the end of the experiment for each treatment were compared using the Plymouth routines in the multivariate ecological research software package PRIMER v.6 (Clarke and Gorley, 2006). The Bray–Curtis similarity coefficients were calculated, based on the untransformed number of bacterial reads to identify inter-relationships between samples and construct a cluster plot. For the determination of the OTUs responsible for the between-treatment dissimilarities, and in particular between HS and other treatments, the similarity percentage analysis (SIMPER) was used (Clarke and Warwick, 1994) in PRIMER v.6 (Clarke and Gorley, 2006).

2.4. Metagenomic shotgun sequencing

To identify the functional genetic diversity associated with biodegradation of xenobiotics' pathways, including the biodegradation of aromatic compounds, metagenomic HTS was performed on equimolar integrated DNA extracts from the triplicates of LS and HS treatments at Day 3 and at the end of the experiment, producing 12 metagenomes. Also, metagenomics was implemented on the initial seawater from the two sampled coastal sites, Vouliagmeni and Flisvos. Briefly, shotgun sequencing was done on a Illumina MiSeq device using 150 + 150 bp paired-end sequencing to a target depth of 3 Gbp per sample. The sequencing steps were performed at Eurofins Genomics, Germany, following the in-house protocols. Shotgun raw reads were submitted to GenBank-SRA under the same accession number as the amplicon raw reads (PRJNA903787).

The raw reads were first quality screened using the FastQC v0.12.0 quality control tool (Andrews, 2010), and then pretreated using the "Filter by quality" tool of Galaxy Ver. 0.72, by removing the low-quality reads (with a Phred score below 20) and those with <90 % high-quality score distribution. Forward and reverse reads were joined and then assembled into bigger contigs with the software MEGAHIT Ver. 1.084 (Li et al., 2015a,b) using a 60-bp overlap and a threshold of minimum 500 bp length in the produced contigs. The contigs were translated into amino-acid sequences, and the predicted genes were functionally annotated against the Kyoto Encyclopedia of Genes and Genomes orthology using GhostKOALA (Kanehisa et al., 2016) and further grouped into functional categories and potential pathways.

3. Results

Abiotic measurements included pH, PAHs, alkyl-PAHs and metals (Supplementary Tables S1–S5). Scrubber inputs in LS treatments slightly changed the pH of seawater (ranging from 7.92 ± 0.13 to 8 ± 0.1 in V and from 7.91 ± 0.07 to 7.97 ± 0.13 in F), while in HS, pH decreased between 7.68 ± 0.2 to 7.75 ± 0.14 in V treatments, and 7.73 ± 0.19 to 7.78 ± 0.12 in F (Supplementary Table S1). The scrubber effluent (including both dissolved and particulate fractions) contained high concentrations of several PAHs measured, with naphthalene (4810 ng L^{-1}), fluorene (1378 ng L^{-1}), and phenanthrene (3460 ng L^{-1}) exhibiting the highest concentrations. Alkyl-PAHs were also highly abundant in the effluent, with derivatives of the above-mentioned PAHs being the most conspicuous. Namely, naphthalene-2-methyl, naphthalene-1-methyl, naphthalene-C3, naphthalene-C3 and naphthalene-C4 were measured between 5839 ng L^{-1} to 1354 ng L^{-1}, fluorene-C2 was measured at 986 ng L^{-1}, and phenanthrene-C1 – phenanthrene-C4 were all >703 ng L^{-1} up to 4685 ng L^{-1} in the case of phenanthrene-C1. Low-molecular-weight (LMW) compounds' concentrations in F treatments were reduced acutely before Day 3 by 85 % of the initial concentrations, while in V treatments their removal was less efficient. However, between Days 3 and 6, both V and F mesocosms showed 25 % and 62 % decrease in LMW concentrations, respectively. High-molecular-weight PAHs and alkyl-PAHs showed high removal efficiencies in F, but <10 % of the initial concentrations in V, with their removal starting after Day 3 (Supplementary Fig. S3).

The phytoplankton abundance was different between V and F seawater (Supplementary Table S6). Pico-phytoplankton was the dominant component in V while micro-phytoplankton in F, exhibiting the highest total phytoplankton abundance together with nano-phytoplankton. As expected, micro-phytoplankton biomass was much higher in F (0.761 mg L^{-1}) compared to V (0.041 mg L^{-1}). Similarly, phytoplankton carbon biomass was much higher in F seawater (105.35 μg L^{-1} compared to 8.16 μg L^{-1} in V). Based on the high total phytoplankton carbon biomass ratio of F to V (12.91), the stoichiometric nitrogen and phosphorus biomass are expected to be higher in F compared to V, confirming that the F seawater is eutrophic in contrast to the V oligotrophic site.

Measurements in the seawater from both V and F sampling sites indicated values below the Limit of Detection (LOD) for all PAHs and alkyl-PAHs. High scrubber inputs in HS treatments were expected to increase PAHs contaminants in the natural seawater. In V, the concentrations of most PAHs [naphthalene, fluorene, phenanthrene, fluoranthene, pyrene, chrysene, and benzo(b)fluoranthene], and most Alkyl-PAHs dropped (Fig. 1, Supplementary Table S2) by Day 6. On the other hand, in F, only pyrene, chrysene, benzo(b)fluoranthene and phenanthrene-alkylated compounds exhibited values above the LOD in Day 3 with decreasing trends at Day 6 (Fig. 1, Supplementary Table S4). Concerning metals concentrations, the scrubber effluent contained considerably high V levels (226.73 μg L^{-1}) in a mixture of other metals, while the seawater of both sites had lower concentrations, apart from Zn (50.2 and 139.4 μg L^{-1} in V and F vs 25.6 μg L^{-1} in the scrubber), Cu (4.29 and 2.96 μg L^{-1} in V and F vs 1.41 μg L^{-1} in the scrubber) (Supplementary Tables S3 and S5). Temperature and salinity levels in the experimental bottles were controlled throughout the duration of the experiment within the range of short-term fluctuations of *in situ* conditions in the seawater sampling sites at the time of sampling, with ranges 39 ± 0.3 psμ and 14.6 ± 1 °C in V, and 36.1 ± 0.3 psμ and 13.6 ± 1 °C in F.

Bacterioplankton initial abundances were about 6 times higher in F, reaching 18 × 10^5 cells mL^{-1}. In both experimental set-ups, bacterioplankton showed no negative responses to the scrubber effluent inputs, even at the HS treatment, with net growth rates being always positive and similar to the controls. An apparent -but non-significant- increase was observed on Day 3 of the experiment in FHS treatments, when the population abundance was 1.5-fold higher than the controls (Fig. 2).

3.1. Metabarcoding

Overall, a total of 2758 and 3751 bacterial OTUs were retrieved from the Vouliagmeni's and Flisvos' mesocosms, respectively. Bray-Curtis comparisons based on OTU numbers of reads separated the bacterial communities of the two locations reflecting different spatial structure (Fig. 3a). Looking into the separate mesocosms, the HS treatments of V formed a distinct cluster compared to controls, based on the most dominant OTUs' relative abundances (OTUs with overall >0.01 % contribution to the total number of reads) (Fig. 3b). Similarly, bacterial

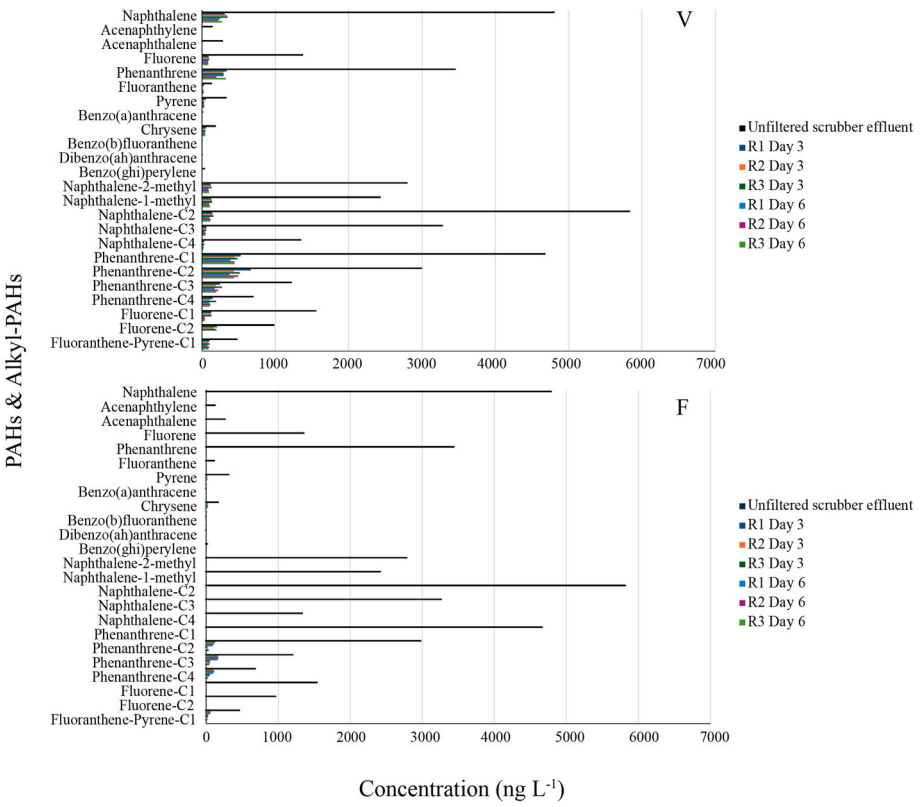

Fig. 1. PAH and Alkyl-PAH composition in the scrubber effluent and in the HS triplicate (R1, R2, R3) treatments (5 % v/v scrubber effluent dilutions) of Vouliagmeni (V) and Flisvos (F) experiments.

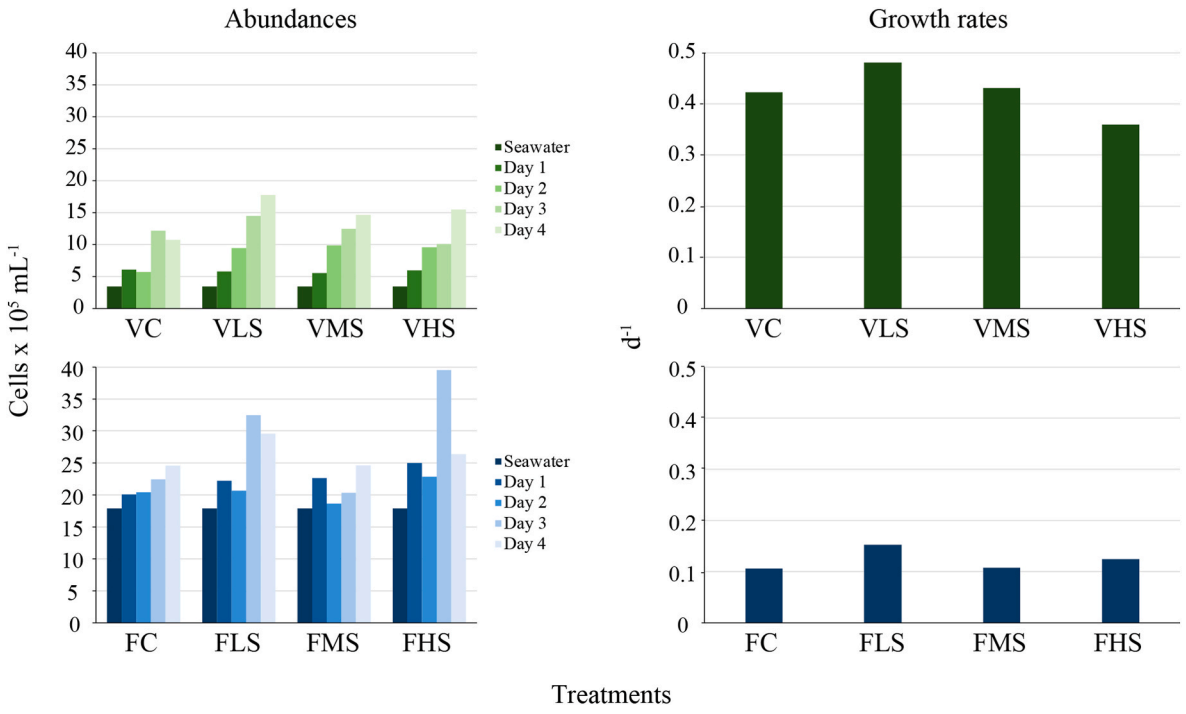

Fig. 2. Left panel: Bacterioplankton abundances measured every 24h in the mesocosms from Vouliagmeni (V) and Flisvos (F). Right panel: Growth rates at critical time. C: Controls, LS: 1 % v/v scrubber dilutions, MS: 2 % v/v scrubber dilutions, HS: 5 % v/v scrubber dilutions. Due to glass wall-growth effects and the appearance of aggregates after Day 4, bacterial abundances of Day 6 are not presented.

communities of F were clustered based on the day of the experiment forming two clusters. Within each cluster, HS treatments were grouped together and were clearly separated from the other treatments (Fig. 3c).

Simper analysis indicated an average dissimilarity of about 30 % between HS and controls in V, and 31 % and 44 % between HS treatments and other treatments at Day 3 and Day 6, respectively. The OTUs

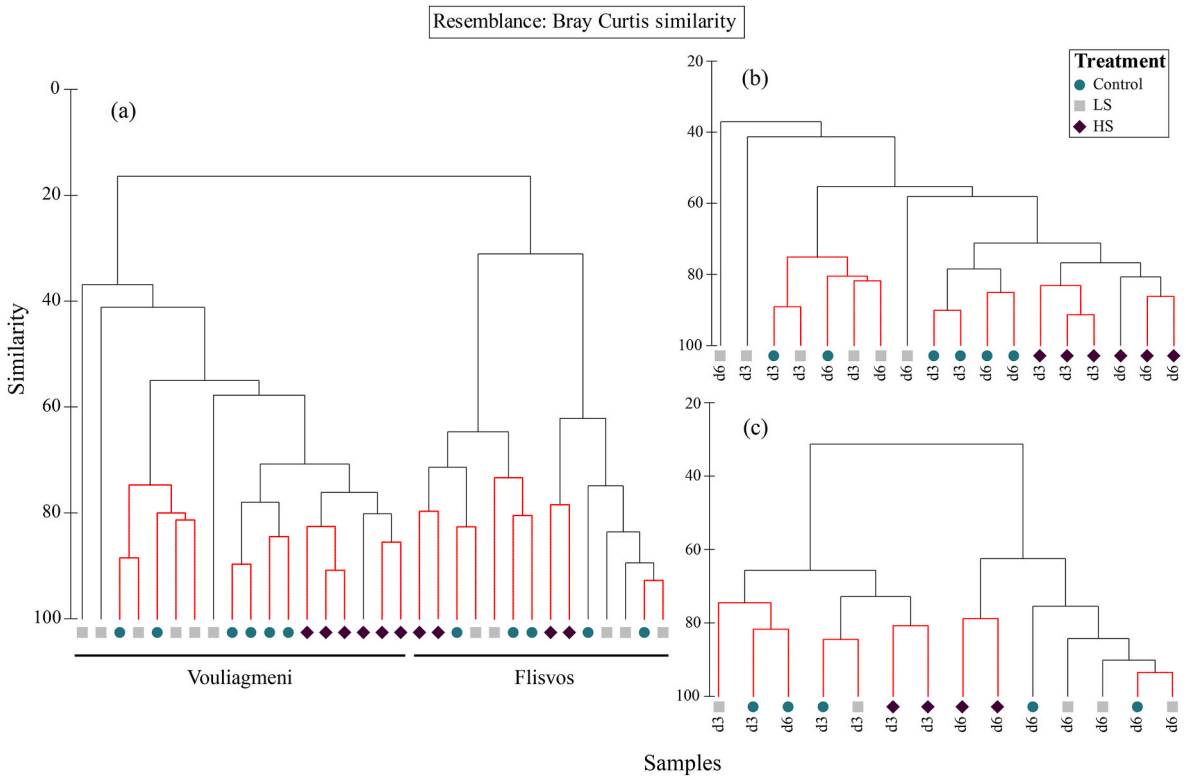

Fig. 3. Cluster diagrams based on Bray–Curtis dissimilarities calculated based on the metabarcoding's non-transformed number of reads of OTUs. Red clades in the dendrogram indicate significant similarities ($P < 0.05$) between bifurcations, based on the SIMPROF significance test. (a) All samples from both mesocosms; (b) Vouliagmeni's mesocosms; (c) Flisvos' mesocosms. d3 indicate samples taken at Day 3, and d6 indicate samples at the end of the experiment at Day 6. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

responsible for the between-treatment clustering dissimilarities included several OTUs affiliated with bacteria with various xenobiotics, PAHs and oil degradation capabilities (Table 2) that were detected with high number of reads in both areas. Several of the above-mentioned OTUs increased their abundances at least 5-fold compared to controls and/or LS treatments with 5 % v/v scrubber effluent concentrations in HS (Fig. 4). In Vouliagmeni's mesocosms, HS effluent additions caused the proliferation in terms of number of reads of the OTUs affiliated to *Roseobacter*, *Porticoccus*, *Marinomonas*, *Arcobacter*, *Lentibacter*, *Lacinutrix* and *Pseudospirillum* (Fig. 4a), especially at Day 6. These genera that have been identified as potential xenobiotics, PAHs, oil and hydrocarbon degraders (Table 2). In Flisvos, a conspicuous increase in abundances of a consortium of bacterial OTUs was observed in both LS and HS compared to controls for (Fig. 4b). These OTUs were associated with the xenobiotics and PAHs degraders *Glaciecola*, *Vibrio*, *Roseobacter*, *Porticoccus*, *Marinomonas*, *Lacinutrix*, *Pseudospirillum*, *Marivita* and *Mycobacterium*.

3.2. Metagenomes

All metagenomes produced per base high-quality reads (Supplementary Figs. S4 and S5). Overall, the number of total sequences produced ranged between 10,012,432 at Day 3 of the LS treatment in Flisvos' communities (FLSd3) and 15,086,476 reads at Day 6 in the control treatments of Vouliagmeni's communities (VCd6). The number of annotated COGs to microbial functions was highest on Day 6 in the LS treatments of V (Table 3). In V, this number ranged between 4847 on Day 3 of the control experiment, and 8086 in the LS treatments on Day 6. In F, the highest number of microbial COGs retrieved was from control treatments at Day 3, and the lowest in HS treatments on Day 6. In all samples, >75 % of the expected diversity was retrieved according to Nonpareil v3.5 software (Rodriguez-R and Konstantinidis, 2014).

Changes in the number of COGs associated with major metabolic pathways in the treatments with scrubber effluent inputs (LS and HS) compared to the controls in V and F mesocosms, suggested different responses of the two bacterial communities. In LS treatments of V, bacterioplankton exhibited an increase of 54 and 170 more COGs of signal transduction functions compared to controls at Days 3 and 6, respectively. Bacterioplankton in HS treatments demonstrated different responses at Day 3 and Day 6, as reflected by the variations in the number of COGs per major metabolic pathways (Fig. 5a). While on Day 3, the predominant increase in the number of COGs was observed for signal transduction (198 more COGs compared to controls), and transport and catabolism (105 more COGs compared to controls), on Day 6 higher number of COGs compared to the controls was detected for energy metabolism, carbohydrate metabolism, amino acid metabolism, and xenobiotics degradation (Fig. 5a). On the other hand, in HS treatments of F, an increase in COGs associated with xenobiotics degradation and membrane transport was evident. However, signal transduction pathways included 3 % and 1.5 % fewer number of COGs compared to the controls at Day 3 and at Day 6, respectively (Fig. 5b).

Looking closer at the xenobiotics' degradation pathways, HS treatments in V caused an increased response in naphthalene, aromatic compound, and benzoate degradation, which are connected to PAHs degradation pathways, compared to controls. The increase was already pronounced at Day 3, when 15, 86, and 136 more COGs were associated to naphthalene, aromatic compound, and benzoate degradation, respectively, while most xenobiotics degradation pathways also contained more COGs than in controls (Fig. 6a). At the end of experiments, these pathways maintained a higher number of COGs compared to controls, indicating a prolonged presence. Furthermore, an increase was observed in LS treatments of F at Day 6; in HS treatments a high bacterioplankton response to scrubber effluent inputs by up to 137 more COGs linked to benzoate degradation compared to controls was

Table 2
Operational Taxonomic Units (OTUs) that are closely related to the hydrocarbon- and polycyclic hydrocarbon-degrading bacteria responsible for dissimilarities among control/low scrubber effluent treatments (C/LS) and high scrubber effluent treatments (HS) in the mesocosms with seawater from the two sampled sites, Vouliagmeni (V) and Flisvos (F). Closest relatives were retrieved after BLAST searches against NCBI database. (+) denotes OTUs that are responsible for between-clusters dissimilarities according to SIMPER analysis.

OTU	High-level taxonomic affiliation	Closest identified relative (% similarity) [NCBI accession number]	Dissimilarities among C/LS and HS		Hydrocarbon-degrading potential	Indicative references
			Vouliagmeni	Flisvos		
OTU1	Gammaproteobacteria	*Glaciecola amylolytica* (98.4 %) [NR_171501]	+	+	The genus exhibits Oil-degradation properties	Chronopoulou et al. (2015)
OTU2	Gammaproteobacteria	*Vibrio crassostreae* (98.8 %) [MK224657]	+	+	The genus exhibits oil/PAH degrading properties	Graziano et al. (2016)
OTU6	Alphaproteobacteria	Uncultured *Roseobacter* sp. (98.8 %) [EU600623]	+	+	The genus exhibits PAH degrading capabilities	Zhou et al. (2020)
OTU7	Gammaproteobacteria	*Porticoccus hydrocarbonoclasticus* (98.4 %) [NR_118247]	+	+	A specialized hydrocarbon-degrading bacterium	Gutierrez et al. (2012)
OTU8	Gammaproteobacteria	*Marinomonas* sp. (99.1 %) [KP027824]	+	+	Hydrocarbon/PAH degrader	Dong et al. (2014)
OTU9	Epsilonproteobacteria	*Arcobacter cloacae* (99 %) [CP053833]		+	The genus exhibits oil/PAH degrading properties	Li et al. (2020)
OTU10	Epsilonproteobacteria	Uncultured *Arcobacter* sp. (100 %) [HQ821621]	+	+	The genus exhibits oil/PAH degrading properties	Li et al. (2020)
OTU11	Gammaproteobacteria	*Vibrio* sp. (99.3 %) [MK102607]	+		The genus exhibits oil/PAH degrading properties	Graziano et al. (2016)
OTU14	Alphaproteobacteria	*Lentibacter algarum* (99.5 %) [MN746224]	+	+	Key oil degrader	Zhou et al. (2023)
OTU19	Bacteroidota	Uncultured *Lacinutrix* sp. (98.6 %) [JQ217330]	+	+	The genus includes strains with hydrocarbon degradation-related genes	Aleman et al. (2024)
OTU21	Gammaproteobacteria	Uncultured *Pseudospirillum* sp. (98.5 %) [JX530489]	+	+	The genus exhibits hydrocarbon metabolic properties	Doyle et al. (2018)
OTU23	Alphaproteobacteria	*Marivita litorea* (100 %) [MN746209]		+	Key oil degrader	Zhou et al. (2023)
OTU29	Gammaproteobacteria	Uncultured *Oleispira* sp. (98.4 %) [JX525106]		+	Oil degrader	Gentile et al. (2016)
OTU35	Epsilonproteobacteria	*Arcobacter* sp. (100 %) [OR418053]		+	The genus exhibits oil/PAH degrading properties	Li et al. (2020)
OTU45	Gammaproteobacteria	Uncultured *Colwellia* sp. (98.4 %) [KF721891]		+	The genus exhibits oil/PAH degrading properties	Dong et al. (2015)
OTU50	Alphaproteobacteria	*Marivita* sp. (97.3 %) [OR234740]		+	Key oil degrader	Zhou et al. (2023)
OTU57	Bacteroidota	*Polaribacter dokdonensis* (99.8 %) [KU173582]		+	Oil degrader	Wang et al. (2014)
OTU64	Gammaproteobacteria	*Pseudoalteromonas haloplanktis* (99.1 %) [MT114591]		+	Hydrocarbon/PAH degrader	Martínez-Varela et al. (2022)
OTU75	Epsilonproteobacteria	*Arcobacter defluvii* (98.8 %) [CP053835]		+	The genus exhibits oil/PAH degrading properties	Li et al. (2020)
OTU79	Actinomycetes	*Mycobacterium* sp. (98.8 %) [MK585227]		+	PAH degrader	Zeng et al. (2010)
OTU145	Gammaproteobacteria	*Pseudoalteromonas* sp. (98.4 %) [MN889131]		+	Hydrocarbon/PAH degrader	Martínez-Varela et al. (2022)
OTU148	Alphaproteobacteria	*Roseobacter* sp. (98.3 %) [AB822604]		+	The genus exhibits PAH degrading capabilities	Zhou et al. (2020)
OTU153	Gammaproteobacteria	*Colwellia* sp. (99.1 %) [MK577365]		+	The genus exhibits oil/PAH-degradation properties	Dong et al. (2015)
OTU314	Gammaproteobacteria	*Marinomonas pontica* (99.5 %) [MK967189]		+	Hydrocarbon/PAH degrader	Dong et al. (2014)

recorded (Fig. 6b). Overall, the natural bacterioplankton communities of the eutrophic Flisvos (F), exhibited an increase in numbers of cells (Fig. 2), reads of OTUs linked to PAHs degraders (Fig. 4b), and COGs linked to xenobiotic degradation pathways, including COGs that encode enzymes that participate in various PAHs degradation (Fig. 6b). These responses were coupled with an acute decrease in the most conspicuous PAHs of scrubber effluent below the LOD already from Day 3 of the Flisvos mesocosms (Fig. 1).

Furthermore, regarding xenobiotics degradation modules, in HS treatments of the F mesocosms the module of phthalate degradation linked to PAHs degradation was complete in addition to benzoate degradation and the catechol pathway to acetyl-CoA that were complete (no gene blocks missing from the pathway) in all treatments in both mesocosms, including controls (Supplementary Fig. S6).

4. Discussion

Two natural bacterioplankton communities from coastal areas receiving different anthropogenic pressures were tested to scrubber effluent inputs in a mesocosm study. The pH changes observed in the treatments are within the natural short-term variability in the marine environment. According to Ytreberg et al. (2021), pH alone could not explain the observed results of scrubber effluent effects on a microplankton community in the Baltic Sea. Several metals were also detected in the scrubber effluent, with vanadium being the most prevalent. Vanadium can act as either a synergistic of antagonistic factor influencing toxicity and tends to promote microbial cooperative interactions in aquatic systems when vanadium reducing bacteria with detoxification/tolerance genes are present (Wang et al., 2022). In addition, the high NO_3^- and NO_2^- concentrations of the scrubber effluent (55.6 μM; Genitsaris et al., 2023) may stimulate the release of reduced V (Wang et al., 2023). However, concentrations measured in our scrubber

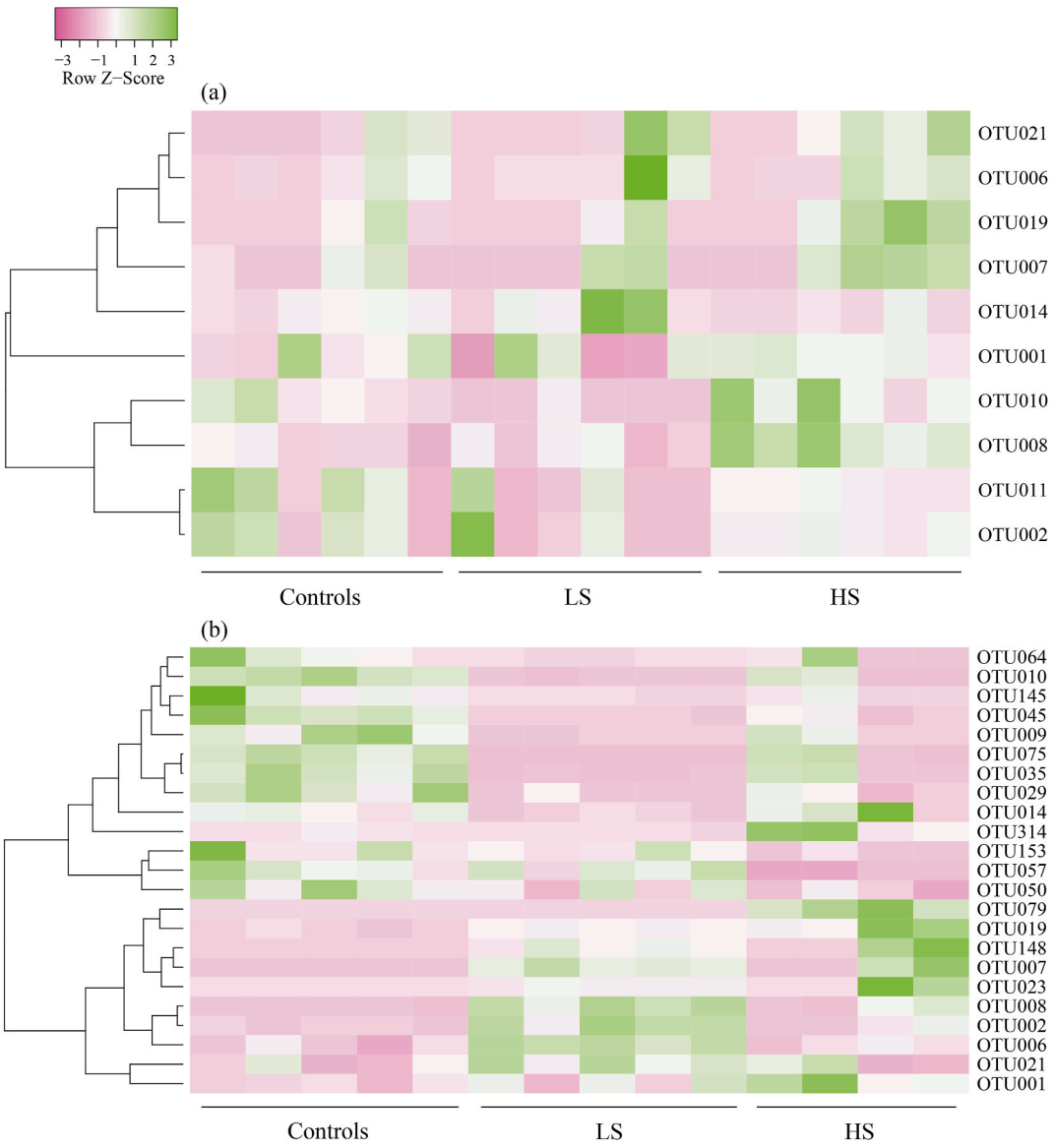

Fig. 4. Heatmaps of the abundances, in terms of number of reads, of the bacterial OTUs closely affiliated to taxa with various xenobiotics, PAHs and oil degradation capabilities (see Table 2) that are responsible for the between-treatment samples dissimilarities. Low scrubber effluent (LS): 1 % v/v scrubber effluent dilutions; high scrubber effluent (HS): 5 % v/v scrubber effluent dilutions. When the z-score is 0, it indicates that the abundance at the data point is identical to the mean score, and a z-score difference of 1 indicates abundances with 1 SD variation from the mean. (a) Vouliagmeni's mesocosms; (b) Flisvos' mesocosms.

Table 3
FastQC quality control reports of metagenomic outputs from Vouliagmeni (V) and Flisvos (F) mesocosms. See Table 1 for treatments' code annotations. COGs denote Clusters of Orthologous Genes.

Sample codes	Total sequences	Total bases (Gbp)	Total number of COGs associated with microbial functions	% GC	Total deduplicated %
VCd3	10,450,970	1.5	4847	42	88.4
VCd6	15,086,476	2.2	7816	45	87.2
VLSd3	10,868,396	1.6	4930	43	88.4
VLSd6	13,334,489	1.9	8086	45	88
VHSd3	14,503,671	2.1	5751	43	82.7
VHSd6	10,803,717	1.6	6892	41	86.5
FCd3	14,704,183	2.2	7914	45	87.7
FCd6	12,060,416	1.8	6887	44	88.7
FLSd3	10,012,432	1.5	7127	45	89.1
FLSd6	12,942,871	1.9	6789	44	88.4
FHSd3	10,143,598	1.5	7067	46	88.5
FHSd6	10,582,305	1.5	6454	45	87.6

effluent treatments were much lower than those reported by Wang et al. (2022, 2023) and no vanadium nitrogenase genes associated with the reduction of vanadium from V^{+5} to V^{+4}, which is less toxic, were retrieved. Both acute and chronic toxicity of V on phytoplankton and zooplankton was observed in higher concentrations than those in our HS treatments (Schiffer and Liber, 2017). The relevance of PAHs in scrubber effluent for environmental risk assessment and regulation policies is evident as the maximum allowable discharge limit from the scrubber effluent is set at 50 μg L^{-1} of the net phenanthrene equivalence with flow rate normalized at 45 m^3 MWh^{-1} for open-loop scrubbers (Genitsaris et al., 2023). In this discussion, we first examine the bacterial responses to scrubber effluent at the community structure level, followed by the analysis of the detected PAHs-degrading OTUs in our datasets and the associated metabolic pathways. Finally, we provide an outlook on the broader implications of our findings for PAHs ecotoxicological research.

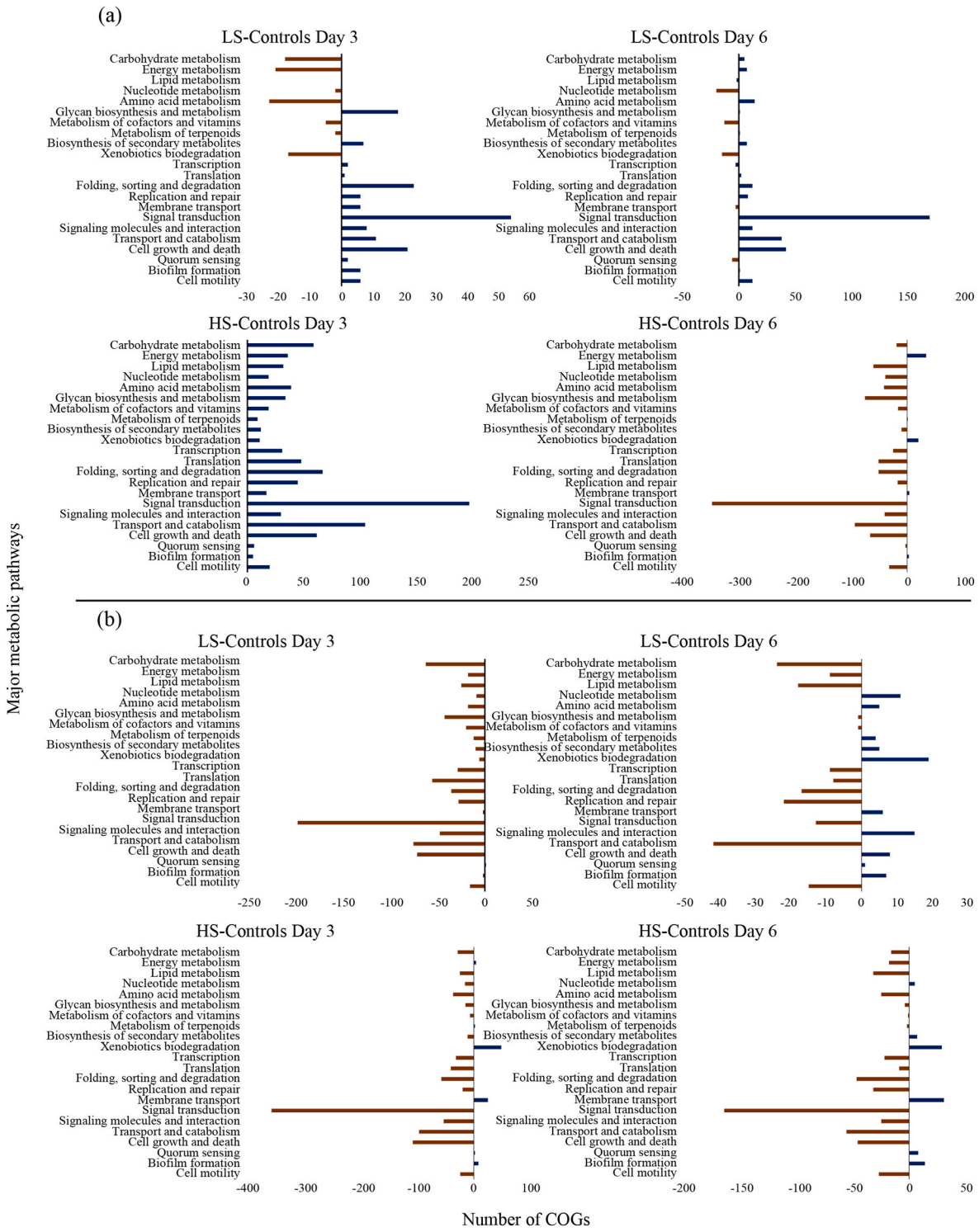

Fig. 5. Differences on the number of Clusters of Orthologous Genes (COGs) linked with microbial functional pathways between LS and controls (left panels) and HS and controls (right panels) in Vouliagmeni's (a) and Flisvos' (b) mesocosms.

4.1. Bacterial responses at community structure level

Bacterioplankton responses, reflected by shifts in compositional dominance and a variable change in the number of genes involved in relevant to xenobiotic degradation responses, were linked to PAHs input and were site-specific and possibly dependent on the source populations' abundances and the presence of PAH-degrading taxa. The initial bacterial population abundances were $>16 \times 10^5$ cells mL^{-1} in the eutrophic seawater (Flisvos, F), and less than one-sixth in the pristine site (Vouliagmeni, V). Flisvos' communities are exposed to multiple sources of cultural eutrophication and pollution while Vouliagmeni's communities experience oligotrophic pristine conditions. Most of the organic carbon in the sea originates from phytoplankton, which influences the nutrient environment of bacteria (Biddanda and Benner, 1997). The significantly higher phytoplankton biomass in F seawater compared to V, and the high ratio of F/V carbon content - leading to expected higher stoichiometric N and P in F - supports the higher diversity of PAH-degrading functional bacteria detected in F, as well as the

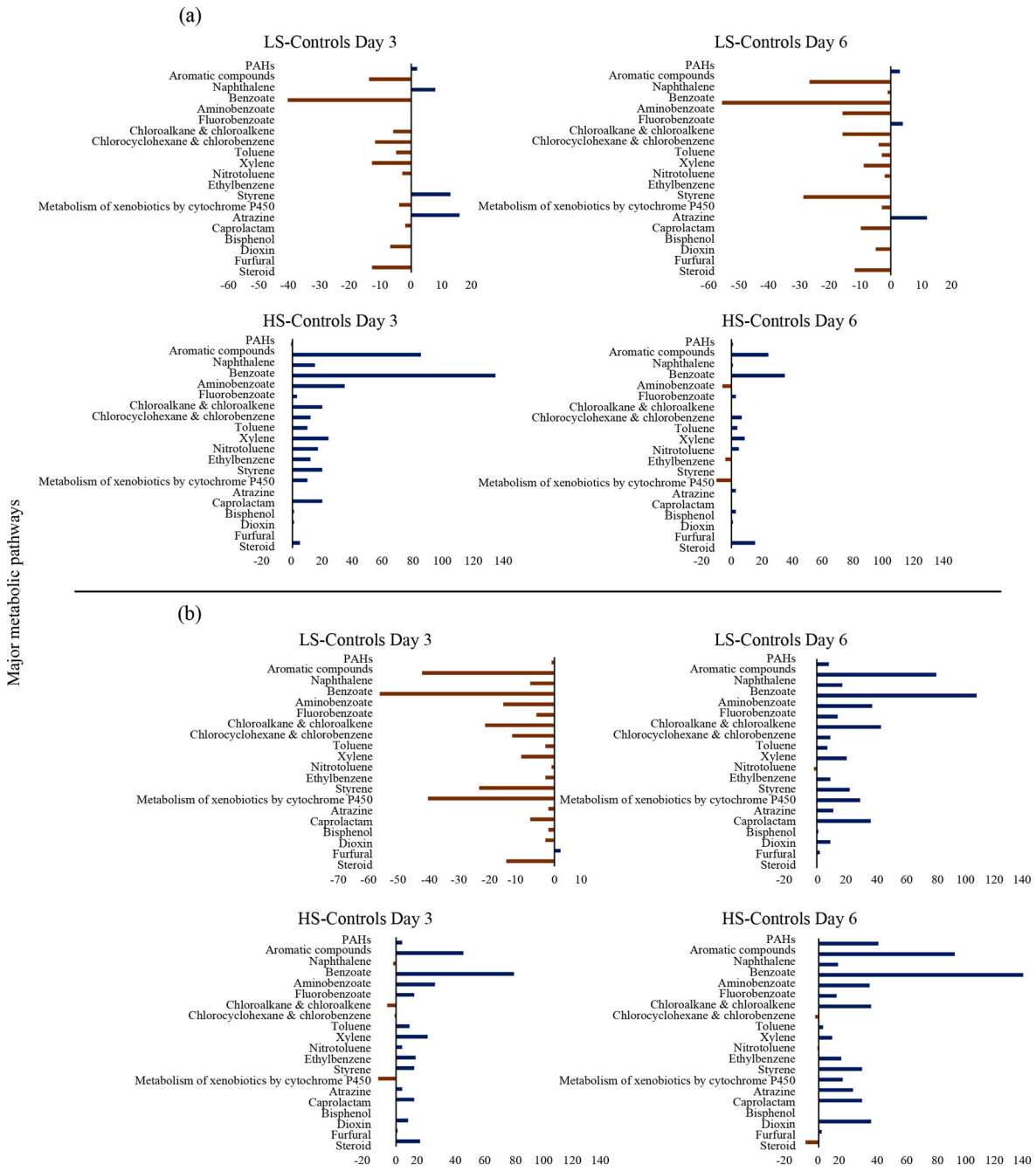

Fig. 6. Differences on the number of Clusters of Orthologous Genes (COGs) linked with xenobiotic degradation pathways between LS and controls (left panels) and HS and controls (right panels) in Vouliagmeni's (a) and Flisvos' (b) mesocosms.

subsequent higher PAH degradation rates. The clear difference in the trophic state of the two sites is mirrored in bacterial total abundances. In a similar experimental set-up, Genitsaris et al. (2023), report a decrease of open-loop scrubber effluent PAHs to below LOD concentrations during a 6-day exposure period of natural bacterioplankton communities of initial abundances of $>8 \times 10^5$ cells mL^{-1} from other coastal sites in Greece. Indeed, biodegradation of various xenobiotics in natural systems is influenced by microbial communities' dynamics, reflected by population numbers and community composition and function (Cui et al., 2020). This is further supported in the present experiments, where micro-phytoplankton dominance in F seawater, compared to pico-phytoplankton dominance in V, suggests a larger individual size/radius and thus bigger phycosphere radius in F (Seymour et al., 2017).

The high diversity and abundance of specific microbial taxa that contain genes that decode the key metabolic pathways to break down complex compounds to simpler and non-toxic molecules can regulate degradation processes, leading to more efficient and rapid biodegradation endpoints (Maila and Cloete, 2005). In other words, higher abundances of diverse bacterioplankton increase the likelihood that some members retain the genetic capacity to rapidly degrade a wide range of xenobiotics, coupling taxonomic and functional diversity (Philippot et al., 2010). To highlight the importance of the presence of phylogenetically diverse bacterial assemblages, it has been noted that the synergistic activity of bacterial consortia induces higher degradation rates of xenobiotics than single species (Dell'Anno et al., 2020). A contaminant may also be fortuitously degraded by an enzyme or cofactor produced during microbial metabolism of another compound (Nzila, 2013;

Ellegaard-Jensen et al., 2014) a process known as cometabolism. This mechanism is critical for the complete mineralization of complex molecules. Recent advancements in microbial bioremediation strategies have demonstrated removal efficiencies of 80–90 %, comparable to our results, when using microbial communities which can cometabolize PAHs into non-toxic compounds (Ragini et al., 2024).

It is noteworthy that, in the mesocosms of the pristine area, PAHs and alkyl-PAHs concentrations remained high at the three-day mark of the experiment but were found to have decreased by Day 6. The lower diversity and abundances of V communities likely contributed to lower PAHs and alkyl-PAHs removal efficiencies. Less complex bacterial communities may result in slower or incomplete degradation and accumulation of intermediates, thereby prolonging the overall time required to mineralize PAHs (Kanaly and Harayama, 2000). One additional factor that explains the observed differential response of the eutrophic and pristine communities is associated with the impact of the different carbon and nutrient content in each experiment, since microbes release oxidative enzymes based on organic and nutrient substrate content (Vijayanand et al., 2023). PAHs degradation rate-limiting factors, related to imbalanced nutrient availability (Vijayanand et al., 2023), may act in the V experiment but not in the eutrophic F treatments. The stronger coupling of phytoplankton and bacterioplankton interactions governed by the bigger phycosphere in F could control the nutrient provision to bacteria. Nutrient biostimulation as a remediation strategy has been shown to enhance microbial biodegradation of complex hydrocarbons, even in nutrient-rich habitats (Howland et al., 2024). Additionally, a balanced C/N/P ratio has been demonstrated to facilitate rapid PAHs metabolization by bacterial strains in soil experiments (Leys et al., 2005). Conversely, the excess of carbon sources due to PAH inputs is more pronounced in the oligotrophic V mesocosms and may result in limitation of other nutrients (Fuentes et al., 2014). Second, impacted natural systems, such as the eutrophic F, may lead to adapted microbial populations with complex interrelationships that have evolved mechanisms to efficiently degrade pollutants (Singh and Ward, 2004).

Higher scrubber effluent inputs (5 % v/v) were associated with a rapid (within the first three days of the experiments) increase of the relative abundance of a high diversity of PAHs degraders at the eutrophic F mesocosms. This response was coupled with an acute decrease -much faster than their half-lives- in 2 and 3-ring PAHs concentrations below the LOD. On the other hand, the oligtrophic Vouliagmeni's mesocosms showed a gradual response with a slower increase in the relative abundance increase of fewer PAHs-degrading OTUs and a simultaneous slow decrease of the concentrations of added PAHs after a few exposure days towards the end of the experiment (Day 6). This slower response is indicative of the lower complexity and lower initial abundances of the site's bacterial inhabitants. Bacosa et al. (2021) showed that initial oil concentrations affected biodegradation rates of hydrocarbons in laboratory incubation studies. Indicators of ultimate biodegradation are critical for the estimation of PAHs half-lives for potential toxicity effects assessment (Genitsaris et al., 2024). Our experimental results suggest that the most abundant PAHs of the scrubber effluent, such as naphthalene, phenanthrene, and fluorene, exhibited shorter half-lives, within the range of few days, than those used in models for the chemical fate of scrubber effluent PAHs (Aghito et al., 2023).

4.2. PAHs-degrading OTUs

The PAHs-degrading OTUs that were retrieved and identified as having increased roles in shifted communities due to scrubber effluent inputs belonged to the genera *Mycobacterium*, *Vibrio*, *Roseobacter*, *Lentibacter*, *Marivita*, *Glaciecola*, *Porticoccus*, *Marinomonas*, *Arcobacter*, *Lacinutrix* and *Pseudospirillum*. For example, several *Mycobacterium* and *Vibrio* species are long known to have the ability to mineralize PAHs, such as naphthalene, pyrene, fluoranthene, phenanthrene, anthracene and benzo[*a*]pyrene (Hedlund and Staley, 2001; Cerniglia, 2003). Molecular analyses have demonstrated that *Mycobacterium* species enhance hydrocarbonoclastic protein production in response to PAHs, further highlighting their biodegradation potential in aquatic environments (Cerniglia, 2003). Members of the *Roseobacter* clade have exhibited genetic and phenotypic evidence that render them capable of utilizing aromatic compounds as primary growth substrates (Buchan et al., 2001, 2005). Furthermore, *Lentibacter*, *Marivita*, and *Glaciecola,* along with *Roseobacter,* were key degradation genera at *in situ* microcosm experiments with surface petroleum contaminated seawater in an oil trading port. Metagenomic analyses suggested that bacterial consortia including these genera displayed a higher biodegradation potential of PAHs and other aromatic compounds after oil spills (Zhou et al., 2023). The species *Porticoccus hydrocarbonoclasticus*, also retrieved in our experiments with increased participation in the bacterial communities of the HS treatments, was identified as a highly specialized hydrocarbon-degrading bacterium based on its phenotypic and genotypic characteristics (Gutierrez et al., 2012). Its increased abundance stressed its potential role in PAHs cometabolism. In a study of PAH-contaminated sediments in the high-latitude Arctic Ocean, several bacterial taxa were characterized as efficient PAH degraders, and among them, bacteria of *Marinomonas* were suggested to play the most important role in PAH mineralization *in situ* (Dong et al., 2015). Similarly, *Arcobacter* was suggested to be involved in PAHs degradation, as members of the genus proliferate in PAH-contaminated systems (Isaac et al., 2013) and were the most dominant bacterial genus in Phenanthrene-degrading culture experiments (Ye et al., 2019). Apart from the above well-known oil--associated and hydrocarbonoclastic bacteria, recent comprehensive population mapping studies in controlled oil-spill microcosms and mesocosms revealed an enrichment of unexpected genera, such as *Lacinutrix* (Aleman et al., 2024) and *Pseudospirillum* (Doyle et al., 2018), which both responded positively in the scrubber effluent inputs of our mesocosms. Even though *Lacinutrix* members have not yet been directly linked to hydrocarbon biodegradation, its genomic traits and phylogenetic proximity to hydrocarbon-degrading taxa suggest a potential role in this process (Aleman et al., 2024).

4.3. Metabolic pathways

The differential responses of the two bacterioplankton communities regarding shifts in overall structure and prevalence of PAH-degrading taxa in variable scrubber effluent treatments coupled with the variable decrease in PAHs concentrations, indicate that variations would also be observed at the gene level. Indeed, the number of COGs associated with major metabolic pathways changed differently in each community depending on the level of scrubber effluent exposure. Indicatively, in LS treatments of V bacterioplankton exhibited an increase of signal transduction functions, whereas in HS treatments of F, an increase of COGs linked to xenobiotics degradation and membrane transport was evident. Two-component signal transduction systems comprise the key mechanisms by which bacteria sense environmental signals and regulate the expression of catabolic pathways (Tropel and van der Meer, 2004). Several bacterial strains have been described to activate such regulatory systems in the presence of xenobiotic compounds, triggering the gene expression of detoxification and degradation enzymes (see Mosquéda et al., 1999; Leuthner and Heider, 1998; Labbé et al., 1997). The interplay between signal transduction and xenobiotic metabolism is evident through pathways involving gene expression regulation, stress response, and enzyme activation. Furthermore, membrane transport functions are commonly highlighted with high xenobiotic occurrence, since the transport of compounds across cellular membranes is a critical step towards their metabolism. Membrane transporters ensure the initial recognition, uptake, and efflux of xenobiotic compounds (Mutanda et al., 2022). In bacteria, the uptake of most aromatic compounds is mediated by an adaptable and complex network of specialized transporter systems, such as outer membrane channels for the transport of

benzoate (Choudhary et al., 2017), naphthalene (Neher and Lueking, 2009) and benzo[a]pyrene and phenanthrene (Liang et al., 2020).

In HS treatments of both areas' mesocosms, a high increase in COGs associated to benzoate and aromatic compound degradation was detected. At the same time, the concentrations of key and abundant PAHs, including naphthalene and phenanthrene, dropped rapidly below the LOD at Day 3 of the F mesocosms. Links between naphthalene and phenanthrene degradation, and benzoate degradation via catechol formation (Macchi et al., 2018) have been also identified in similar mesocosms with natural bacterioplankton communities (Genitsaris et al., 2024).

Microbes that can degrade various aromatic compounds often possess the genetic pool towards the production of the enzymes, such as dioxygenases and dehydrogenases, that effectively break down PAHs to simpler minerals (Banerjee et al., 2024). The genetic and enzymatic versatility enables multi-species bacterial assemblages to utilize similar pathways to metabolize structurally related compounds. For example, in HS treatments of the F mesocosms, the degradation pathway of phthalates, a group of chemical pollutants primarily used as plasticizers (Giam et al., 1978), was complete (no gene blocks missing from the relevant module). Dibutyl phthalate was found to be the main accumulated metabolite during the degradation of phenanthrene and other PAHs by a mixture of bacterial strains (Xu et al., 2021). Strains of the genus *Mycobacterium* – identified as key players in scrubber effluent treatments – facilitate certain PAHs degradation via the phthalate pathway (Imam et al., 2022). This pathway is now recognized as the dominant route to phenanthrene degradation (Sun et al., 2019). Phthalates degradation follows a series of enzymatic steps, where phthalic acid is converted under aerobic conditions to protocatechuate (Boll et al., 2019), which is further degraded by ring-cleavage dioxygenases into metabolites that eventually enter the citric acid cycle (Kamimura and Masai, 2014). This is a key metabolic pathway and the final common route for the oxidative degradation of amino acids, fatty acids and carbohydrates, reinforcing its ecological relevance.

Thus, the proposed degradation pathway that is possibly employed by the microbial communities in the treatments with scrubber effluent inputs includes the initial breaking of complex aromatic compounds by several dioxygenases and dehydrogenases to the production of dihydroxy-, hydroxy- and carboxy-compounds, and the activity of hydratases-aldolases towards salicylate production. Further hydroxylases and aldolases can lead to the production of catechol and phthalates, which also are linked to the catechol complete module through the production of benzoate with the activity of several specific enzymes. The endpoint of the catechol metabolic pathways is either the formation of pyruvate, or acetyl-CoA, leading to simpler molecules through glycolysis and the citrate cycle, respectively. It is evident that the degradation mechanisms of a wide range of xenobiotics -including PAHs-by diverse bacterial assemblages share metabolic similarities, involving the breakdown of complex aromatic structures into simpler molecules that can be further metabolized through commonly found genetic and enzymatic machinery in microbial communities of distant evolutionary relations. Understanding these molecular mechanisms is crucial for assessing how microbial communities sense, respond and detoxify pollutants in the marine environment.

5. Conclusions

Our findings, at both community and genetic structure endpoints, highlight the key role of the rapid bacterial degradation in the detoxification of PAH and alkyl-PAH pollutants cocktail of scrubber effluents in seawater. Microbial communities from areas of different trophic states exhibited adapted rates of PAHs and alkyl-PAHs removal, coupled with an increase in different COGs associated with xenobiotic and PAHs biodegradation processes. Thus, we suggest that the billions (cells L^{-1}) of marine bacteria that represent the first life entry points in aquatic systems can act as pollutant natural absorbers reducing PAHs concentrations in the marine environment and subsequently their potential toxic impact on other organisms. Furthermore, we emphasize the need to include natural microbes in ecotoxicological experiments of single-species to add relevance and realism in environmental risk assessment and regulation policies. In such assessments, it is important to consider site-specific microbial communities and their attributes, background environmental conditions, trophic state, and open-loop scrubber effluent origin and characteristics, since its impacts can largely depend on engine type, operation and fuel and lube oil specifications. As there is a significant increase in the installation of open-loop scrubbers on both new and retrofitted ships globally and given the high health risks associated with PAHs emitted into the air by ships, it is important to understand whether this technology can mitigate air pollution issues associated with maritime operations. Our work contributes to the ongoing IMO policy discussions on the role of scrubbers as an alternative or transitional technology toward sustainable fuels.

CRediT authorship contribution statement

Savvas Genitsaris: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Natassa Stefanidou:** Writing – review & editing, Methodology. **Polyxeni Kourkoutmani:** Methodology. **Evangelia Michaloudi:** Writing – review & editing, Methodology. **Meritxell Gros:** Writing – review & editing, Validation, Methodology. **Elisa García-Gómez:** Writing – review & editing, Validation, Methodology. **Mira Petrović:** Writing – review & editing, Validation, Methodology. **Leonidas Ntziachristos:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition. **Maria Moustaka-Gouni:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

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 paper.

Acknowledgements

This work was conducted in the framework of EMERGE project that received funding from the European Union's Horizon 2020 research and innovation programme, under grant agreement Nr. 874990. Furthermore, it was partially funded by the European Union – Next Generation EU - National Recovery and Resilience Plan (NRRP) – Greece 2.0. Project "NAVGREEN – Green Shipping of Zero Carbon Footprint" (Project Code: TAEDR-0534767). M. Gros acknowledges her Ramon y Cajal contract (RYC2020-030324-I) funded by the MCIN/AEI 10.13039/501100011033 and by "ESF Investing in your future".

We thank the members of the ecotox group of EMERGE for helpful discussions. We are grateful to the AUTh and IVL leaders, Leonidas Ntziachristos and Jana Moldanova, and members of EMERGE for organizing and running the onboard shipping measurement campaign, from which the scrubber effluent was supplied.

We would like to thank the two anonymous reviewers that helped improve the original text.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.121563.

Data availability

The data that support the findings of this study are openly available

in NCBI-SRA depository at https://www.ncbi.nlm.nih.gov/sra/?term=PRJNA903787, reference number PRJNA903787.

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