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Microbiome and pollutants in the freshwater sponges *Ephydatia muelleri* (Lieberkühn, 1856) and *Spongilla lacustris* (Linnaeus, 1758) from the sub-Arctic Pasvik river (Northern Fennoscandia)

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ABSTRACT

Despite the ecosystem functions offered by sponges in freshwater habitats, fragmentary studies have targeted their microbiome and the bioaccumulation of legacy and emerging organic micropollutants, making it difficult to test hypotheses about sponge-microbe specificity and response to environmental factors and stressors. The sponge species *Ephydatia muelleri* and *Spongilla lacustris*, coexisting in two sites of the Pasvik River (northern Fennoscandia), were analyzed for persistent organic pollutant (POPs) and chemicals of emerging concern (CECs), along with quali-quantitative microbiological features. River water and sediment were similarly treated to establish if the obtained data were site- or sponge-specific. CECs mainly occurred in abiotic matrices, with trimethoprim and ciprofloxacin prevailing in water and sediment, respectively. Only ciprofloxacin and diclofenac were detected in sponges, with higher concentrations generally determined in *S. lacustris* than *E. muelleri*. Overall, POP concentrations were in the order polycyclic aromatic hydrocarbons > chlorobenzenes > polychlorobiphenyls > polychloronaphthalenes, with higher values in sponges with respect to abiotic matrices. Generally, POPs occurred at higher concentrations in *S. lacustris* than *E. muelleri*. Enzyme activity measurements displayed diverse trends across samples and sites, with *E. muelleri* displaying higher glycolytic activity than *S. lacustris*. Prokaryotic abundance in sponges generally exceeded that found in abiotic matrices. *Proteobacteria*, *Planctomycetota*, *Actinobacteriota*, *Verrucomicrobiota*, and *Cyanobacteria* predominated in sponge samples, with slight differences between sponge species and sampling sites, whereas *Desulfobacterota* and *Acidobacterota* were retrieved mostly in sediment samples. The sponge-associated bacterial communities appeared to be differently affected by pollutant concentration at the site level. Overall, this study highlights the ecological role of freshwater sponges, shedding light on their microbial associations, pollutant bioaccumulation, and potential as bioindicators of aquatic ecosystem health. The findings emphasize the importance of considering both microbial diversity and contaminant accumulation for a holistic understanding of the roles played by freshwater sponges in human-impacted environments.

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1. Introduction

Sponges (Phylum Porifera) play relevant roles in aquatic environments. Filtering large volumes of water, they are involved in the recycling of inorganic and organic nutrients, coupling benthic and pelagic ecosystems (de Goeij et al., 2013). Their complex three-dimensional structures create habitats and refugia from predators for several species, thus contributing to ecosystem biodiversity and functioning (Folkers and Rombouts, 2020). As it is well known, sponges host phylogenetically diverse prokaryotic communities, which are often sponge species-specific and highly different from prokaryotic assemblages retrieved from the surrounding environment. Nutrient acquisition, sponge skeleton stabilization, production of secondary metabolites, and processing of metabolic waste and xenobiotics are among the ecological functions played by microbial symbionts (Stabili et al., 2017). Several studies have shown that the sponge environment is among the main structuring factors of the sponge-associated microbiota (Laport et al., 2019; de Freitas et al., 2023). This means that alterations in the sponge environment (i.e., anthropogenic impact, environmental stressors) can determine changes in the structure and composition of the microbial community. Filtering large volumes of water, sponges are particularly vulnerable to water quality. They exhibit the unique capacity to hyperaccumulate, stabilize, and degrade pollutants (e.g., polychlorinated biphenyls, polycyclic aromatic hydrocarbons, heavy metals, pesticides) within their mesohyl matrix. Consequently, sponges emerge as potential bioindicators of environmental pollution levels (Stabili et al., 2008, 2017; Perez et al., 2023; Mahaut et al., 2013; Gentric et al., 2015; Masangkay et al., 2022; Lo Giudice et al., 2024b; Papale et al., 2024). Thus, sponge-associated bacteria are supposed to be adapted to the presence of pollutants in the host tissues and participate in detoxification processes (Selvin et al., 2009; Hoppers et al., 2015; Stabili et al., 2017).

Freshwater sponges (order Spongillida; family Demospongiae) can be found in lentic and lotic in-land water systems worldwide, except for Antarctica, covering both natural (e.g., wood, stones) and artificial surfaces (e.g., glass, metal, plastics), and assuming encrusting, branched, or cloddy bulk shapes. They can cope with highly variable environmental conditions (e.g., fluctuation in water level and temperature, long-period desiccation, and pollution events) thanks to molecular, physiological, and structural modifications (Manconi and Pronzato, 2008). Unlike marine sponges, which have been intensively targeted in microbiological studies, the association between microbes and freshwater sponges represents an under-investigated topic (Lo Giudice and Rizzo, 2024). To date, the application of culture-dependent and, more rarely, culture-independent approaches (e.g., clone libraries and next generation sequencing) has furnished scant and fragmentary information to make assumptions on environmental factors driving the associated microbiome structure, along with the sponge-microbe specific and metabolic relationships (Sugden et al., 2022). Most studies have been addressed to both healthy and diseased sponge individuals within the *Lubomirskiidae* family, endemic of the Lake Baikal, e.g. *Lubomirskia baicalensis*, *Baikalospongia bacillifera*, *B. intermedia* and *Swartschewskia papyracea* (e.g., Kaluzhnaya et al., 2012; Parfenova et al., 2008; Belikov et al., 2019; Chernogor et al., 2020). Few additional sponge species, spanning from European to American freshwater bodies and including *Corvospongilla lapidosa*, *Ephydatia* spp., *Eunapius* spp., *Spongilla lacustris*, and *Tubella variabilis* (Gernert et al., 2005; Costa et al., 2013; Keller-Costa et al., 2014; Gaikwad et al., 2016; Kenny et al., 2020; Sugden et al., 2022; Lo Giudice et al., 2024b) have been investigated for the associated bacterial communities.

Currently, there is a lack of studies on legacy and emerging organic micropollutants in freshwater sponges (Poliakova et al., 2000), while several studies have been focused on bioaccumulation of inorganic (e.g., metals) and organic (e.g. antibiotics, antifouling biocides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls) contaminants in marine sponges showing concentrations in the order of mg/Kg (Gentric et al., 2015; Girard et al., 2021; Varamogianni-Mamatsi et al., 2023). Recent research has addressed the use of sponge membrane bioreactors for the removal of legacy and emerging organic contaminants during the biological wastewater treatments from cities, catfish farms and hospitals (Nhut et al., 2020; Zhang X. et al., 2020; He et al., 2022; Sonwani et al., 2022; Shitu et al., 2023).

The encrusting sponge *Ephydatia muelleri* and the branching sponge *Spongilla lacustris*, which co-existed in two slow-flowing sectors along the sub-Arctic Pasvik River (Northern Fennoscandia), were targeted for the estimation of the associated prokaryotic community diversity (Bacteria, including Cyanobacteria, and Archaea), together with the potential accumulation of persistent organic pollutants and chemicals of emerging concern, including antibiotics in their mesohyl tissues. Results were compared to those obtained in sediment and water from the river. The study was mainly aimed at assessing inter-specific differences/similarities in the associated prokaryotic communities and bioaccumulation of pollutants, also considering data obtained from the analysis of the abiotic matrices.

2. Methods

2.1. Sampling area

The 145 km long Pasvik River (Norwegian: Pasvikelva; Russian: Paz or Patsoyoki) flows from Lake Inari in Finland into the Bøkfjorden (not far from the town of Kirkenes), marking the border between Norway and Russia. This northern Fennoscandian river system, which has a total area of 142 km^2, a catchment basin of 18.404 km^2, and a mean water flow of 175 m^3/s, is mostly shallow (1–8 m in depth), with water level oscillations of about 80 cm (Amundsen et al., 2020). The Pasvik River is sectioned by seven hydroelectric power dams. From late spring to mid-autumn, the river is typically free of ice, with a freezing period that is around 200 days long (Dauvalter and Kashulin, 2018). Snowmelt, substantial amounts of rainfalls, and aquifers are the main sources of water in the Pasvik River. Bedrock prevails in the region, and the watercourse is hedged in birch (*Betula* sp.) and pine (*Pinus sylvestris*) woods with patches of *Sphagnum* bogs.

2.2. Sample collection

Sampling was carried out at two sites in the Pasvik River (i.e. BIP1 and BIP5; coordinates 60°26′53″ N - 30°02′24″ E and 69°45′21.3″ N - 30°02′52.9″ E, respectively), which were selected following a preliminary survey of the area which revealed that the sponge species *Spongilla lacustris* (Linnaeus, 1759) and *Ephydatia muelleri* (Lieberkühn, 1856) (both class Demospongiae, order Spongillida, family Spongillidae) were simultaneously present only there. At both sites the river flowed slowly, showing features typical of wetlands, and meadows of the aquatic plant *Persicaria amphibia* (family Polygonaceae) occurred (Fig. 1). Wetlands are ecologically important transition areas between aquatic and terrestrial ecosystems characterized by high productivity and nutrient recycling capacities, that contribute to global greenhouse gas emissions (Dedysh and Ivanova, 2019).

Three specimens *per* sponge species were aseptically collected at a depth of 50–100 cm in summer 2021. Immediately after sampling, potential transient microorganisms, debris and sediment particles were carefully eliminated by rinsing the sponge surface with 0.22 μm filter-sterilized river water (Mangano et al., 2009). Sponge specimens were separately stored into sterile plastic bags and maintained at 4 °C until their arrival at the NIBIO Svanhovd Research Station (Svanvik, Pasvik Valley). Until in the laboratory, sponge specimens were placed on a sterile cutting surface and cut using a sterile scalpel blade for preliminary processing (within 2 h after sampling) and preservation, as described in sections 2.4-2.8. Water and sediment samples were contextually collected from both sites in the proximity of *S. lacustris* and *E. muelleri* individuals (in a 10-cm radius). Sponge fragments and

sediment samples for chemical analyses were stored in aluminium trays at −20 °C and then processed in Italy. Water samples were processed at NIBIO as reported below in sections 2.5-2.8.

Samples were named by the project acronym BIP, followed by Sl, Em, SED or WAT (to indicate *S. lacustris*, *E. muelleri*, sediment and water, respectively) and the site number (1 or 5) (for example, the label BIP-Em5 referred to *E. muelleri* collected from the site BIP5).

2.3. Taxonomic identification of sponges

For taxonomic identification, sponge fragments were preserved with 70% ethanol in sealed plastic containers. Until in Italy, the spicule complement and skeletal architecture were determined following the methodologies reported in Núñez-Pons et al. (2022) and examined under a light microscope. Taxonomic classifications were in accordance with the Systema Porifera (Hooper and van Soest, 2002) and the World Porifera Database (WPD) (de Voogd et al., 2023).

2.4. Persistent organic pollutants (POPs)

Polychlorobiphenyls (PCBs), polychloronaphthalenes (PCNs), chlorobenzenes (CBs) and polycyclic aromatic hydrocarbons (PAHs) were determined in sponge, water and sediment samples, following the extraction and analytical procedures previously reported by Papale et al. (2024). All details are given in the Supplementary Table S1. Pretreated samples were analyzed by GC-MS/MS.

Standard solution analysis was carried out to optimize instrumental parameters in TIC (Total Ion Current) mode. Then, a Multiple Reaction Monitoring (MRM) program was created. Retention times and selected transitions are given in Supplementary Table S2. The instrument used was an Agilent GC 7890B coupled with a MS 7010 triple quadrupole mass spectrometer, equipped with an Agilent ALS autosampler and an MMI (Multi Mode Injector) injection port, used in the solvent vent mode. The column used was an HP-5MS UI (95% dimethyl – 5% phenyl-polysiloxane, 30 m × 0.250 mm, film thickness 0.25 μm). The mobile phase was helium, with a flow of 1.2 mL/min, a pressure of 11,052 psi. For the collision cell, helium was used with a flow of 4.0 mL/min, while nitrogen was used with a flow of 1.5 mL/min with a pressure of 10 psi. All details for chromatographic conditions are given in the Supplementary Table S3. All analyses were performed by injecting 1 μL of sample. The data system contains all the software required for calibration, GC/MS-MS spectra collection and data processing for qualitative and quantitative analysis. All the calibration curves were linear in the observed concentration range, with a typically value of r^2 of 0.999, always better than 0.997. Recoveries (ranging from 70 to 105%) were measured for each target compound. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated for each compound as three times and ten times, respectively, the standard deviation of the

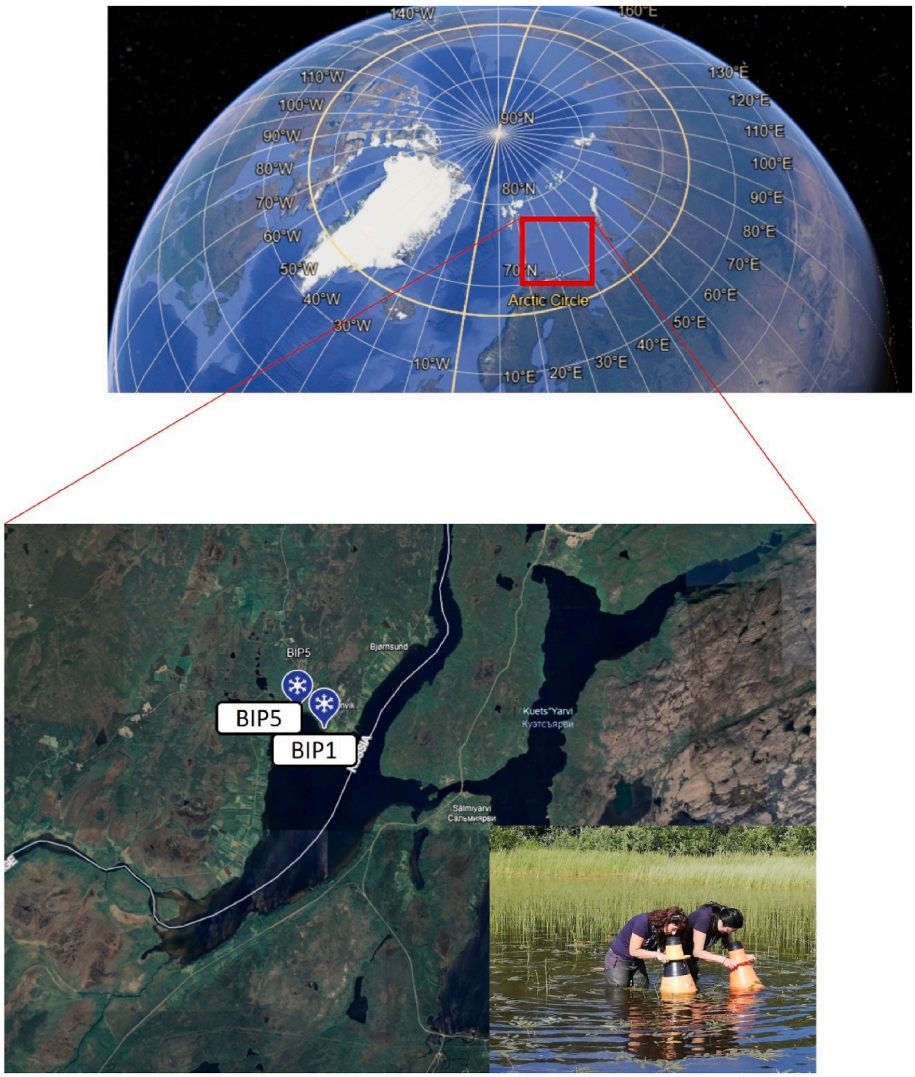

Fig. 1. Sampling sites BIP1 and BIP5 in the Pasvik River (in the picture: the site BIP1).

blank (calculated on seven replicate blanks).

The LOD and LOQ values for each analyte class were respectively 0.02 and 0.07 ng/g for PAHs, 0.01 and 0.02 ng/g for CBs, 0.02 and 0.07 ng/g for PCNs, 0.01 and 0.05 ng/g for PCBs.

2.5. Determination of chemicals of emerging concern (CECs)

The pharmaceutical products trimethoprim, ciprofloxacin, tetracycline, sulfamethoxazole, diclofenac, and ibuprofen were extracted and pre-concentrated from water samples (in triplicate) by SPE (Solid Phase Extraction) using the method detailed in Spataro et al. (2019). This method, previously optimised to extract antibiotic residues from liquid samples, was tested for the contaminants investigated in the present work and recoveries (ranging from 75 to 100%) were measured for each target compound (Supplementary Table S4).

The extraction of target CECs from solid samples (i.e., sediment and sponges *S. lacustris* and *E. muelleri*) was performed by applying the method detailed in Visca et al. (2021). Like what was done for the SPE method, the extraction recoveries from solid samples were measured for all target compounds (Supplementary Table S1) and ranged between 74 and 102%.

The quantification of target compounds from liquid and solid phase samples s was performed by HPLC (High Performance Liquid Chromatography, PerkinElmer Serie 200 micropump) connected with a triple quadrupole mass spectrometer detector (mod. API 3000, AB Sciex, Germany) using the chromatographic Gemini column 150 × 4.6 mm (5 μm RP C18, Phenomenex, France) with a guard column packed with the same stationary phase, both maintained at 25 °C. The injection volume was 20 μL. The gradient elution of the target pharmaceuticals (trimethoprim, ciprofloxacin, tetracycline, sulfamethoxazole, diclofenac and ibuprofen) was performed at the flow rate of 0.3 mL/min with the mobile phase composed by a phase A (Acetonitrile-ACN) and phase B (ultrapure water acidified with 0.1% formic acid). The elution profile was as follows: for the first 5 min, phase A was at 45%, after this, phase A increased to 80% in 10 min and finally, it returned to the initial condition in 15 min. The elution was performed at the flow rate of 0.3 mL/min using the following gradient profile: phase B started at 5 %, increased linearly to 95% at 20 min and this condition was maintained for further 3 min. The ESI and MS/MS acquisition parameters used for the analysis of pharmaceuticals are detailed in Supplementary Table S5.

Nitrogen was the collision and carrier gas. The MS/MS detector was set in multiple reaction monitoring (MRM) mode. The identification of analytes was based on the *m/z* ion ratios, which were the intensity ratios of qualifier and quantifier ion transitions.

Quality assurance (QA) and quality control (QC) protocols were applied through matrix spikes, laboratory blanks and continuous calibration verifications. Analyzing laboratory blanks for natural river water samples helped to identify potential contamination during handling. Efforts were made to prevent contact between the environmental samples and plastic materials to minimize any potential alteration of the samples.

Standard solutions were used to obtain two calibration curves at lower (0.25, 0.5, 1, 2 and 5 ng/L) and higher (5, 20, 50, 100 and 500 ng/L) concentration range of the target analytes. These calibration curves always showed a good linearity ($R \geq 0.99$) for both concentration ranges. The reproducibility of the LC-MS/MS method was expressed by the coefficient of variance (CV, from triplicate analysis of the working standard solutions and CVs were <15% for all the analytes.

The limits of detection (LODs) for liquid and solid matrices were calculated, according to the IUPAC Method (Thompson et al., 2002), from the lowest analyte concentrations producing a peak that can be reliably distinguished to the noise (three-time signal to noise ratio) (Supplementary Table S2). Instead, the limits of quantification (LOQs) were set at 3 times the LOD (Supplementary Table S2).

2.6. Estimation of prokaryotic cell abundance and activities

2.6.1. Extracellular enzymatic activity

Sponge fragments, sediments and water samples collected for the enzymatic activity measurements were treated according to the standard analytical procedures in use at CNR laboratory for these assays. Particularly, sponge fragments were weighed under aseptic conditions, diluted in a decimal ratio (weight/volume) in a sterile 0.22 μm prefiltered distilled water and homogenized using a Potter Elvehjem tissue grinder. The supernatant obtained after pelleting was used as the enzyme extract, as described in a previous study (Caruso et al., 2022). Sediments were weighed and re-suspended in sterile distilled water in the same ratio and further diluted in a 1:10 ratio (weight/volume) in the same medium, while water samples were directly processed according to the protocol of fluorogenic assay (Caruso, 2010). Three enzymes, leucine aminopeptidase (LAP), alkaline phosphatase (AP) and beta-glucosidase (GLU) as markers of proteolytic, phosphatasic, and glycolytic activities respectively, were assessed. Sponge homogenates were incubated with L-Leucine-7-amido-4-Methylcoumarin hydrochloride (Leu-MCA), 4-methylumbelliferone (MUF)-beta-D-glucopyranoside (MUF-glu) and MUF-phosphate (5 mM stock solutions) to obtain final concentrations of 10, 30, and 100 nM. Using MCA and MUF as standards for calibration, a fluorimeter (Jenway model 6280, London, UK) measured the amount of fluorescence released after a 2h-incubation at in situ temperature (Caruso et al., 2022). To water and sediments samples, fluorogenic substrates were added to obtain final concentrations ranging from 10 to 30 nM and from 10 to 100 nM respectively. The Lineweaver-Burke transformation was used to compute the maximum velocity (*Vmax*) of the enzyme reaction, considering the initial dilution of the sponge and sediment samples. For water samples the results were reported in nmol/l/h, while for sediments and sponge samples in nmol/g/h.

2.6.2. Total prokaryotic cell abundance

Sponge fragments, sediments and water for the estimation of total prokaryotic cell abundance were immediately frozen at −20 °C in sterile polyethylene containers. Sponge and sediment samples were defrosted at 4 °C, weighted, manually homogenized in sterile physiologic water (0.9% NaCl) and diluted up to a final volume of 5 mL. Homogenates were immersed in ice/water, and prokaryotic cells were detached from the particles by 3 cycles of 1 min sonication (frequency 46 KHz), 1 min in ice, and vortexing for 1 min. Samples were prefiltered on polycarbonate membranes (porosity 0.8 μm; diameter 25 mm) to avoid the interference of the coarse fraction when counting. Total prokaryotic cell abundance was estimated according to Porter and Feig (1980). After sample fixation with formaldehyde (2% final concentration), followed by filtration on polycarbonate black membranes (porosity 0.22 μm; diameter 25 mm) and the subsequent cell staining with 4′,6-diamidino-2-phenylindole (DAPI, Sigma, final concentration 10 μg/mL) for 20 min, samples were observed under the epifluorescence microscope. Total prokaryotic cell abundance was expressed as cells/g and cells/mL for sediment/sponge and water samples, respectively.

2.6.3. Cyanobacteria abundance

The number of cyanobacterial cells (Nedoma et al., 2001) and the taxonomical composition (*Chroococcales*, *Oscillatoriales*, *Nostocales*) of communities were determined using light and epifluorescence microscope (Olympus BX 60). Blue excitation (MWG filter cube green excitation 510–550, emission 590+ for *Cyanobacteria*) was used. The amount of *Cyanobacteria* was expressed as cells/g of dry weight of sediment and sponges.

2.7. Taxonomic composition of the prokaryotic communities

Water samples (between 500 and 800 mL) were prior filtered onto polycarbonate membranes (porosity 0.22 μm). Filters, aliquots of sponge

specimens and sediment samples were preserved at −20 °C in sterile polyethylene containers. Total DNA was extracted in triplicates from sponges, sediment and membranes by using Power Soil DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. A BioSpec Nano (Shimadzu Corporation) was used to check spectrophotometrically the quality and concentration of all DNA extracts. Control amplification step was carried out by using the universal primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 907R (5′-CCG TCA ATT CCT TTR AGT TT-3′) on a BioRad C100 touch thermocycler (BioRad, Laboratories) in a final volume of 50 μl using MyTaq HS (Meridian) (program: denaturation of 15 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s annealing at 51 °C, and 30 s extension at 72 °C, and a final extension 5 min at 72 °C). All amplification products were verified on a 0.8% agarose-TAE gel. The 16S rDNA V3-V4 region was amplified with two loci specific primers with Illumina overhangs 16S-341 5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGG GNBGCASCAG -3′ and 16S-805R 5′GTCTCGTGGGCTCGGAGATGT GTATAAGAGACAGGACTACNVGGGTATCTAATCC -3′ following the standard Illumina protocol (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). Thirty cycles were used in the first PCR amplification with locus-specific PCR primers, while subsequent amplification that integrates relevant flow-cell binding domains and unique indices (NexteraXT Index Kit, FC-131-1001/FC-131-1002) was performed according to the protocol. Sequencing was performed using the Illumina MiSeq platforms, following the standard protocols of the company IGA Technology Services Srl (Udine, Italy).

For the bioinformatics analysis, the quality of raw sequences was tested by FastQC. The bioinformatics workflow included quality filtering, trimming, de-noising and merging by using R package DADA2 to infer amplicon sequence variants (ASVs), i.e., biologically relevant variants. Reduction of replicate, length, and chimera errors was carried out by applying adequate filters. Bacterial taxonomy annotation was performed using Silva database formatted for DADA2, offering an updated framework for annotating microbial taxonomy (silva_nr99_v138.1_wSpecies_train_set.fa.gz and silva_species_assignment_v138.1.fa.gz). A taxa filter was used for the decontamination from eukaryotic, chloroplast, and mitochondrial sequences. The analysis was carried out with the support of IGA Technology Services Srl (Udine, Italy).

2.8. Statistical analyses

The Pearson correlation matrix calculation and plotting were carried out on the dataset of pollutant concentrations and bacterial phyla abundance, by considering results statistically significant when $p < 0.05$ (Past, version 3.22). Relative abundances obtained from the taxonomic analysis at family level were transformed and then processed to calculate Bray–Curtis similarity and perform the cluster analysis. The obtained matrix was used to perform a Non-Metric Multidimensional Analysis (nMDS). PERMANOVA analysis was carried out to verify the occurrence of significant differences between the bacterial communities associated with the three matrices (i.e., water, sediment and sponges) and between the two sampling sites (namely, BIP1 and BIP5) by setting as factors the sample and the sampling site. The principal component analysis (PCA) was computed by using environmental data after transformation, and by superimposing the bacterial abundance at family levels to observe potential correlations (correlation >0.7). All the analyses were performed by using the software Primer 7 (Plymouth Marine Laboratory, Rodborough, United Kingdom).

3. Results

3.1. Determination of persistent organic pollutants (POPs)

Overall, POP concentrations were in the order PAHs > CBs > PCBs > PCNs with higher values in sponges (Fig. 2) than abiotic matrices (Table 1). At site BIP5 sediment was richer in PAHs than BIP1 (range 0.09–17.71 and 0.11–2.59 pg/g, respectively). Conversely, with few exceptions, the concentrations of PAHs were often comparable between water samples from the two sites (range 0.1–15 and 0.1–8.5 pg/L at BIP1 and BIP5, respectively). CBs, PCNs and PCBs were determined in water from BIP1, and only seldom detected in water from BIP5.

With regard to sponges, PAHs were generally more concentrated in *S. lacustris* (range 150–5806 pg/g) than *E. muelleri* (range 50–11030 pg/g), with higher values found at site BIP5 for both sponge species. Exceptions were naphthalene (NAP), 2-methylnaphthalene (NAP 2-Me), acenaphthylene (ACY), acenaphthene (ACE), fluoranthene (F), phenanthrene (PHE), and anthracene (ANT) that were more concentrated in BIP-Em1 than BIP-Em5 (Fig. 2a). Phenanthrene amounts were particularly high in *S. lacustris* (26100 and 58060 pg/g in BIP-Sl1 and BIP-Sl5, respectively).

CBs were always more concentrated in *S. lacustris* (range 28–3971 pg/g) than *E. muelleri* (3.6–164 pg/g) at both sites. Higher CB values were determined in BIP-Em1 than BIP-Em5, and in BIP-Sl5 than BIP-Sl1 (Fig. 2b).

PCBs were generally more concentrated in *S. lacustris* (range 0.1–1655 pg/g) than *E. muelleri* (0.1–75 pg/g). Higher PCB values were generally determined in BIP-Em1 than BIP-Em5, and in BIP-Sl5 than BIP-Sl1 (Fig. 2c).

Finally, PCNs ranged between 8 and 322 pg/g in *S. lacustris*, with higher values determined at BIP5 than BIP1, and between 0.8 and 9.1 pg/g in *E. muelleri*, with similar concentrations at BIP1 and BIP5 (Fig. 2d).

3.2. Determination of chemicals of emerging concern (CECs)

Target contaminants were detected in both abiotic matrices (i.e., water and sediment), except for tetracycline which was always < LOD (Table 1). No statistically-significant differences (*t*-test, $p > 0.05$) were found among CECs measured at sampling points BIP1 and BIP5 in river water and sediment. In general, the antibiotic trimethoprim was found in higher amounts than other CECs in river water samples, while ciprofloxacin prevailed over other target compounds in sediment samples.

As shown in Fig. 2e, among target contaminants, only ciprofloxacin and diclofenac were measured in sponges with a concentration range of 2.89–3.86 ng/g dry weight and 1.12–2.78 ng/g dry weight for ciprofloxacin and diclofenac, respectively. Ciprofloxacin was found in a higher amount (*t*-test, $p < 0.05$) than diclofenac. The comparison of the CEC content in both the sponge species *S. lacustris* and *E. muelleri* at the BIP1 and BIP5 sampling points indicated no statistical differences (*t*-test, $p > 0.05$). Ciprofloxacin was bioaccumulated in sponges in a higher amount (*t*-test, $p < 0.05$) than diclofenac. At the BIP5 sampling point, both ciprofloxacin and diclofenac were measured at higher amounts in *S. lacustris* than *E. muelleri*.

3.3. Estimation of prokaryotic cell abundance and activities

3.3.1. Enzyme activity measurements

The trends recorded for LAP, GLU and AP activities were different depending on the assayed sample (water, sediment or sponges). Enzyme levels measured in water followed the order LAP > AP > GLU, while in sediments LAP and GLU prevailed; sponges displayed higher GLU and AP, while very low levels of proteolytic activity were noticed (Fig. 3).

Generally, both in water and sediment higher activities were recorded at BIP1 than BIP5, except for LAP that in water reached high activity rates at BIP5. Differences in the enzyme activity levels were also recorded among the two sponge species, with sponge *E. muelleri* characterized by high glycolytic activity (especially at BIP5) and moderate AP activity, and sponge *S. lacustris* showing peaks of LAP and AP at BIP5 and BIP1, respectively, consistent with those recorded in water.

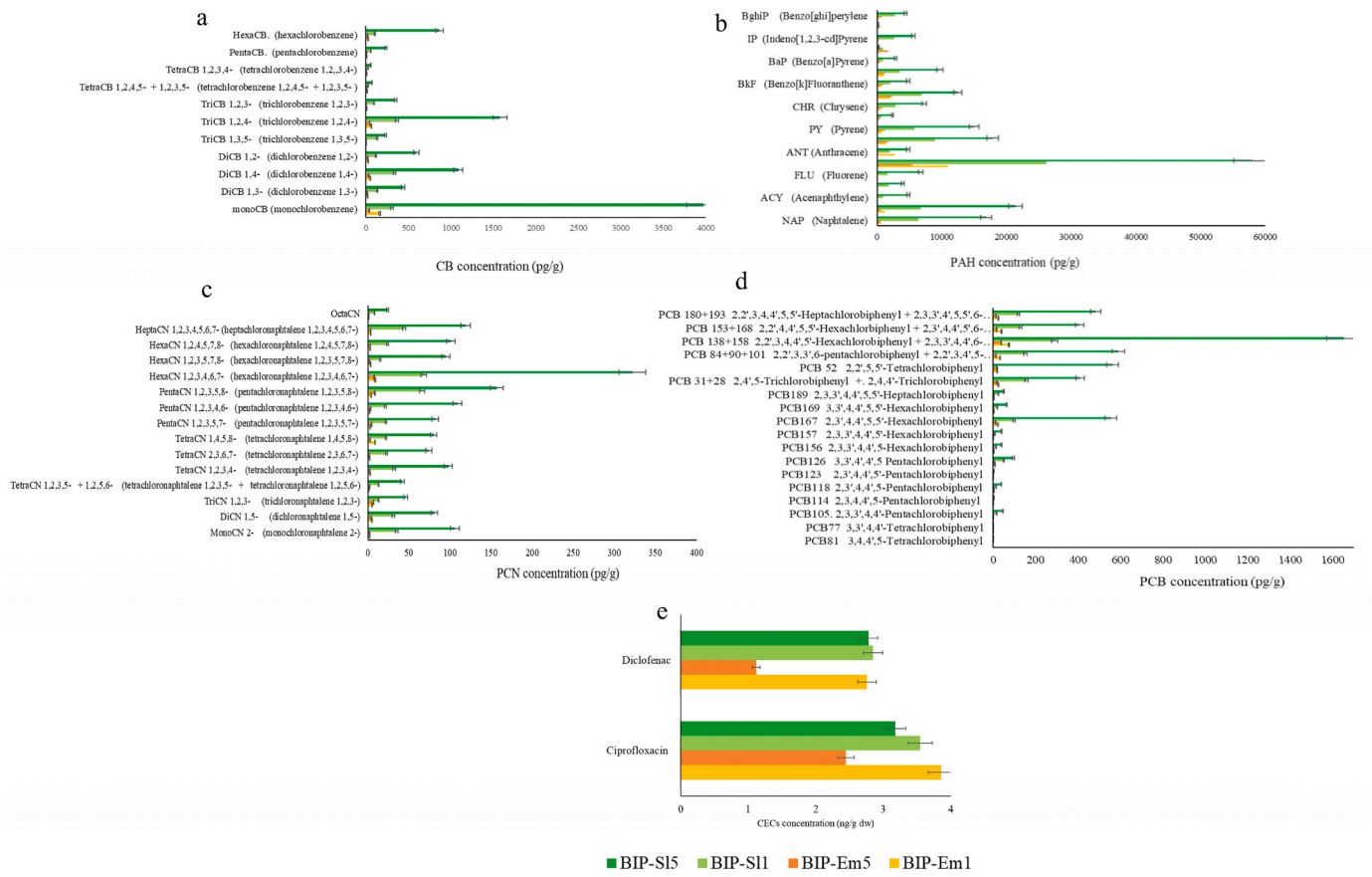

Fig. 2. Mean POP and CEC concentrations measured in sponge samples (Em: *E. muelleri*; Sl: *S. lacustris*) collected at sampling sites BIP1 and BIP5 in the Pasvik River. **a)** Chlorobenzenes (CBs); **b)** Polycyclic Aromatic Hydrocarbons (PAHs); **c)** Polychloronaphthalenes (PCNs); **d)** Polychlorobiphenyls (PCBs); **e)** Chemicals of Emerging Concern (CECs). *Please, note the different scales.*

3.3.2. Total prokaryotic cell abundance

Overall, prokaryotic abundances were higher in sponges than abiotic matrices (i.e., water and sediment). The two sponge species hosted a similar number of prokaryotes at site BIP1 (namely $4.8 \times 10^8 \pm 5.81 \times 10^5$ and $3.3 \times 10^8 \pm 4.51 \times 10^5$ cells/g in BIP-Sl1 and BIP-Em1, respectively). At site BIP5 prokaryotic abundance was one order of magnitude higher in BIP-Em5 than BIP-Sl5 ($6.3 \times 10^8 \pm 4.35 \times 10^5$ and $3.7 \times 10^7 \pm 4.32 \times 10^4$ cells/g, respectively). Finally, the abundance of prokaryotic cells in sediment was $8.9 \times 10^5 \pm 1.2 \times 10^4$ cells/g and $7.74 \times 10^5 \pm 7.92 \times 10^3$ cells/g for BIP-SED1 and BIP-SED5, respectively. In water samples prokaryotes accounted for $9.17 \times 10^4 \pm 1.32 \times 10^4$ and $1.86 \times 10^5 \pm 2.70 \times 10^4$ cells/mL in BIP-WAT1 and BIP-WAT5, respectively.

3.3.3. Abundance of cyanobacterial cells

Three different groups of cyanobacteria were recognized under epifluorescence microscopy according to their morphological features, and were calculated as separate groups: unicellular *Chroococcales*, simple filamentous *Oscillatoriales*, and filamentous *Nostocales* with heterocytes and akinetes (Table 2). Overall, the number of *Chroococcales* was at least one order of magnitude higher than other detected groups in both sponges and sediment, with highest values determined in association with BIP-Sl1 (i.e., 8.9×10^8 cells/g, respectively), where they were the sole observed cyanobacterial group. *Oscillatoriales* occurred exclusively in BIP-SED1 (3.5×10^4 cells/g). Finally, *Nostocales* were observed in all samples (except for BIP-Sl1) with higher abundances in association with both sponges at BIP5 than in other samples. However, it has to be mentioned that during samples storage and transportation *Nostocales* filaments could break down into single cells and *Chroococcales* can be overestimated.

3.4. Taxonomic composition of the prokaryotic communities

Total sequence reads, expressed as Amplicon Sequence Variants (ASVs), and diversity indices per each sample are reported in Supplementary Table S6. As shown by the Shannon index values, diversity level in microbial communities was higher in sediment samples, followed by sponges, and decreased in water samples. We found a total of ASVs ranging from 140391 in BIP-Sl1 samples to 848199 in BIP-SED5. The dataset was represented by 67 phyla, 165 classes, 415 orders, 553 families, and 1551 genera.

The analysis of the taxonomic composition of bacterial assemblages at phylum level revealed the overall predominance of *Proteobacteria, Planctomycetota, Actinobacteriota, Verrucomicrobiota, and Cyanobacteria* in sponge samples, with slight differences between sponge species and sampling sites, whereas *Desulfobacterota* and *Acidobacterota* were retrieved predominantly in sediment samples. Less represented taxonomic groups occurred at relative percentages between 0.01% and 0.34% (Fig. 4).

The abundance of main phyla associated with *E. muelleri* was in the order: *Proteobacteria* (34.9%) > *Planctomycetota* (23.0%) > *Cyanobacteria* (9.7%) > *Verrucomicrobiota* (8%) > *Actinobacteriota* (7.1%) in BIP-Em1; *Proteobacteria* (28.4%) > *Planctomycetota* (22.2%) > *Actinobacteriota* (13.4%) > *Cyanobacteria* (12.3%) > *Verrucomicrobiota* (8%) in BIP-Em5. The abundance of main phyla associated with *S. lacustris* was in the order: *Planctomycetota* (47.7%) > *Proteobacteria* (19.2%) > *Cyanobacteria* (9.4%) > *Verrucomicrobiota* (8%) > *Actinobacteriota* (7.5%) in BIP-Sl1; *Planctomycetota* (34.6%) > *Proteobacteria* (27.0%) > *Actinobacteriota* (11.8%) > *Cyanobacteria* (9.7%) > *Verrucomicrobiota* (8%) in BIP-Sl5.

In sediment phyla predominated in the following magnitude order:

Table 1
Concentrations of legacy contaminants in water (WAT; pg/mL) and sediment (SED; pg/g) samples at sites BIP1 and BIP5 in the Pasvik River. Concentrations of emerging contaminants (CECs) are reported in water (WAT; ng/L) and sediment (SED; ng/g d.w.) samples. *n.q.: not quantified; n.d.: not detected.*

Typology[a]	Contaminant (acronym)	BIP-WAT1	BIP-WAT5	BIP-SED1	BIP-SED5
CBs	*Monochlorobenzene* (monoCB)	2.9 ± 0.2	0.7 ± 0.0	n.q.	n.q.
	Dichlorobenzene (1,3-DiCB1.3)	0.3 ± 0.0	0.4 ± 0.0	n.q.	n.q.
	Dichlorobenzene (1,4-DiCB 1.4)	0.1 ± 0.0	0.1 ± 0.0	n.q.	n.q.
	Dichlorobenzene (1,2-DiCB 1.2)	n.d.	n.d.	n.d.	n.d.
	Trichlorobenzene (1,3,5- TriCB 1.3.5)	0.4 ± 0.0	0.8 ± 0.0	n.q.	n.q.
	Trichlorobenzene (1,2,4-TriCB 1.2.4)	1.2 ± 0.0	2.7	n.q.	n.q.
	Trichlorobenzene (1,2,3-TriCB 1.2.3)	0.5 ± 0.1	0.9 ± 0.1	n.q.	n.q.
	Tetrachlorobenzene 1,2,4,5- + 1,2,3,5 (TetraCB 1245 + 1235)	0.4 ± 0.0	0.4	n.q.	n.q.
	Tetrachlorobenzene 1,2,3,4- (TetraCB 1.2.3.4)	0.4 ± 0.0	0.4	n.q.	n.q.
	Pentachlorobenzene (PentaCB)	0.4 ± 0.0	0.5	n.q.	n.q.
	Hexachlorobenzene (HexaCB)	0.4 ± 0.0	0.6	0.07 ± 0.03	0.09 ± 0.05
PAHs	*Naphthalene* (NAP)	0.5 ± 0.2	5.0 ± 0.0	0.16 ± 0.11	0.09 ± 0.08
	Naphthalene-2 methyl (NAP 2-Me)	1.1 ± 0.1	3.5 ± 0.2	0.90 ± 0.20	1.05 ± 0.40
	Acenaphthylene (ACY)	0.5 ± 0.0	0.7 ± 0.0	n.q.	0.27 ± 0.37
	Acenaphthene (ACE)	0.6 ± 0.1	2.7 ± 0.0	0.11 ± 0.01	0.15 ± 0.13
	Fluoranthene (F)	0.6 ± 0.1	2.4 ± 0.1	0.20 ± 0.04	0.28 ± 0.20
	Phenanthrene (PHE)	7.8 ± 0.0	8.5 ± 0.4	1.33 ± 0.48	5.20 ± 4.63
	Anthracene (ANT)	15.0 ± 1.3	0.7 ± 0.1	n.q.	1.04 ± 0.91
	Fluorene (FLU)	0.8 ± 0.2	1.5 ± 0.2	1.23 ± 1.59	17.71 ± 12.62
	Pyrene (PY)	0.3 ± 0.0	1.4 ± 0.1	0.93 ± 0.98	12.73 ± 9.41
	Benzo[a]anthracene (B[a]A)	0.3 ± 0.0	0.6 ± 0.0	1.15 ± 1.49	5.37 ± 4.03
	Chrysene (CHRY)	0.5 ± 0.1	0.7 ± 0.1	1.91 ± 1.63	8.70 ± 8.47
	Benzo[b]Fluoranthene (B[b]F)	0.4 ± 0.2	0.9 ± 0.1	2.59 ± 2.33	15.47 ± 9.44
	Benzo[k]Fluoranthene (B[k]F)	0.6 ± 0.3	0.8 ± 0.0	1.57 ± 0.99	6.47 ± 5.09
	Benzo[e]Pyrene (B[e]P)	0.1 ± 0.1	0.1 ± 0.1	0.86 ± 1.33	7.62 ± 5.06
	Benzo[a]Pyrene (B[a]P)	0.1 ± 0.0	0.7 ± 0.4	2.52 ± 2.40	11.22 ± 7.77
	Perylene (PE)	0.1 ± 0.0	0.1 ± 0.0	2.14 ± 0.76	9.72 ± 3.49
	Indeno[1,2,3-cd]Pyrene (I[1.2.3-cd]PY)	0.3 ± 0.1	0.5 ± 0.1	n.q.	2.30 ± 1.50
	Dibenz[a,h]anthracene (DB[a.h]A)	0.4 ± 0.0	0.4 ± 0.0	n.q.	8.67 ± 2.43
	Benzo[ghi]perylene (B[g.h.i]PE)	0.1 ± 0.0	0.1 ± 0.0	0.50 + 0.89	10.14 ± 8.75
PCNs	*Monochloronaphtalene* (2-MonoCN 2)	0.3 ± 0.01	0.5 ± 0.03	n.q.	n.q.
	Dichloronaphtalene 1,5- (DiCN 1.5)	0.4 ± 0.01	0.4 ± 0.00	n.q.	n.q.
	Trichloronaphtalene 1,2,3- (TriCN 1.2.3)	2.0 ± 2.4	0.6 ± 0.09	n.q.	n.q.
	Tetrachloronaphtalene 1,2,3,5-+1,2,5,6- (TetraCN 1235 + 1256)	0.6 ± 0.5	0.2 ± 0.35	0.41 ± 0.62	0.35 ± 0.57
	Tetrachloronaphtalene 1,2,3,4- (TetraCN 1.2.3.4)	1.0 ± 0.9	0.3 ± 0.05	n.q.	n.q.
	Tetrachloronaphtalene 2,3,6,7- (TetraCN 2.3.6.7)	0.4 ± 0.01	0.6 ± 0.32	n.q.	n.q.
	Tetrachloronaphtalene 1,4,5,8- (TetraCN 1.4.5.8)	0.3 ± 0.01	0.4 ± 0.10	0.39 ± 0.59	n.q.
	Pentachloronaphtalene 1,2,3,5,7- (PentaCN 1.2.3.5.7)	0.3 ± 0.03	0.4 ± 0.02	n.q.	n.q.
	Pentachloronaphtalene 1,2,3,4,6- (PentaCN 1.2.3.4.6)	0.2 ± 0.01	0.3 ± 0.01	n.q.	n.q.
	Pentachloronaphtalene 1,2,3,5,8- (PentaCN 1.2.3.5.8)	0.3 ± 0.01	0.3 ± 0.00	n.q.	n.q.
	Hexachloronaphtalene 1,2,3,4,6,7- (HexaCN 1.2.3.4.6.7)	0.2 ± 0.01	0.2 ± 0.00	n.q.	n.q.
	Hexachloronaphtalene 1,2,3,5,7,8- (HexaCN 1.2.3.5.7.8)	0.3 ± 0.01	0.3 ± 0.01	n.q.	0.07 ± 0.03
	Hexachloronaphtalene 1,2,4,5,7,8- (HexaCN 1.2.4.5.7.8)	0.3 ± 0.01	0.3 ± 0.02	n.q.	0.06 ± 0.02
	Heptachloronaphtalene 1,2,3,4,5,6,7- (HeptaCN 1.2.3.4.5.6.7)	0.3 ± 0.01	0.3 ± 0.04	0.07 ± 0.05	0.08 ± 0.08
	Octachloronaphthalene (OctaCN)	0.5 ± 0.05	0.6 ± 0.12	n.q.	n.q.
PCBs (dioxine-like)	*3,4,4′,5-Tetrachlorobiphenyl* (PCB81)	0.2 ± 0.01	0.3 ± 0.02	n.q.	n.q.
	3,3′,4,4′-Tetrachlorobiphenyl (PCB77)	0.2 ± 0.07	0.2 ± 0.01	n.q.	n.q.
	2,3′,4,4′,5′-Pentachlorobiphenyl (PCB123)	0.3 ± 0.02	0.4 ± 0.00	n.q.	n.q.
	2,3′,4,4′,5-Pentachlorobiphenyl (PCB118)	0.3 ± 0.02	0.3 ± 0.02	n.q.	n.q.
	2,3,4,4′,5-Pentachlorobiphenyl (PCB114)	0.4 ± 0.03	0.3 ± 0.04	n.q.	n.q.
	2,3,3′,4,4′,5,5′-Heptachlorobiphenyl (PCB189)	0.4 ± 0.00	0.4 ± 0.01	n.q.	n.q.
	2,3,3′,4,4′-Pentachlorobiphenyl (PCB105)	0.3 ± 0.03	0.4 ± 0.02	n.q.	n.q.
	3,3′,4′,4′,5 Pentachlorobiphenyl (PCB126)	0.2 ± 0.01	0.2 ± 0.01	n.q.	n.q.
	2,3′,4,4′,5,5′-Hexachlorobiphenyl (PCB167)	0.2 ± 0.01	0.2 ± 0.03	n.q.	n.q.
	2,3,3′,4,4′,5-Hexachlorobiphenyl (PCB156)	0.3 ± 0.01	0.4 ± 0.05	n.q.	n.q.
	2,3,3′,4,4′,5′-Hexachlorobiphenyl (PCB157)	0.3 ± 0.02	0.3 ± 0.01	n.q.	n.q.
	3,3′,4,4′,5,5′-Hexachlorobiphenyl (PCB169)	0.3 ± 0.01	0.4 ± 0.04	n.q.	n.q.
PCBs (markers)	*2,4′,5-Trichlorobiphenyl + 2,4,4′-Trichlorobiphenyl* (PCB 31 + 28)	0.4 ± 0.01	0.4 ± 0.01	0.056 ± 0.032	n.q.
	2,2′,5,5′-Tetrachlorobiphenyl (PCB 52)	0.3 ± 0.00	0.3 ± 0.02	n.q.	n.q.
	2,2′,3,3′,6-Pentachlorobiphenyl + 2,2′,3,4′,5-Pentachlorobiphenyl + 2,2′,4,5,5′-Pentachlorobiphenyl (PCB 84 + 90+101)	0.3 ± 0.00	0.4 ± 0.00	n.q.	n.q.
	2,2′,4,4′,5,5′-Hexachlorbiphenyl + 2,3′,4,4′,5′,6-Hexachlorobiphenyl (PCB 153 + 168)	0.2 ± 0.00	0.4 ± 0.01	n.q.	n.q.
	2,2′,3,4,4′,5′-Hexachlorobiphenyl + 2,3,3′,4,4′,6-Hexachlorobiphenyl (PCB 138 + 158)	0.4 ± 0.00	0.4 ± 0.01	n.q.	0.079 ± 0.008
	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl + 2,3,3′,4′,5,5′,6-Heptachlorobiphenyl (PCB 180 + 193)	0.3 ± 0.01	0.3 ± 0.01	0.095 ± 0.004	0.109 ± 0.018

(*continued on next page*)

Table 1 (*continued*)

Typology[a]	Contaminant (acronym)	BIP-WAT1	BIP-WAT5	BIP-SED1	BIP-SED5
CECs	*Ciprofloxacin*	0.30 ± 0.03	0.4 ± 0.1	12.0 ± 3.7	14.8 ± 16.1
	Sulfamethoxazole	0.09 ± 0.01	0.13 ± 0.03	4.0 ± 0.2	3.8 ± 0.2
	Trimethoprim	0.3 ± 0.3	0.6 ± 0.1	8.2 ± 1.5	5.0 ± 0.5
	Tetracycline	n.d.	n.d.	n.d.	n.d.
	Ibuprofen	0.1 ± 0.2	0.30 ± 0.04	1.7 ± 0.9	1.5 ± 1.1
	Diclofenac	0.16 ± 0.03	0.18 ± 0.03	4.7 ± 1.9	7.0 ± 6.8

[a] CBs, Chlorobenzenes; PAHs, Polycyclic Aromatic Hydrocarbons; PCNs, Polychloronaphthalenes; PCBs, Polochlorinated Bibenyls; CECs, Contaminants of Emerging Concern.

Proteobacteria (19.7%) > *Planctomycetota* (13.3%) > *Actinobacteriota* = *Verrucomicrobiota* (11.5%) > *Cyanobacteria* (1.7%) in BIP-SED1; *Proteobacteria* (27.0%) > *Verrucomicrobiota* (7.9%) > *Planctomycetota* (7.5%) > *Actinobacteriota* (6.8%) > *Cyanobacteria* (1.8%) in BIP-SED5. Finally, the abundance of main associated phyla in water samples was: *Proteobacteria* = *Actinobacteriota* (27.9%) > *Verrucomicrobiota* (21.6%) > *Planctomycetota* (5.6%) > *Cyanobacteria* (2.5%) in BIP-WAT1; *Actinobacteriota* (22.7%) > *Verrucomicrobiota* (17.1%) > *Proteobacteria* (14.6%) > *Planctomycetota* (2.9%) > *Cyanobacteria* (2.4%) in BIP-WAT5.

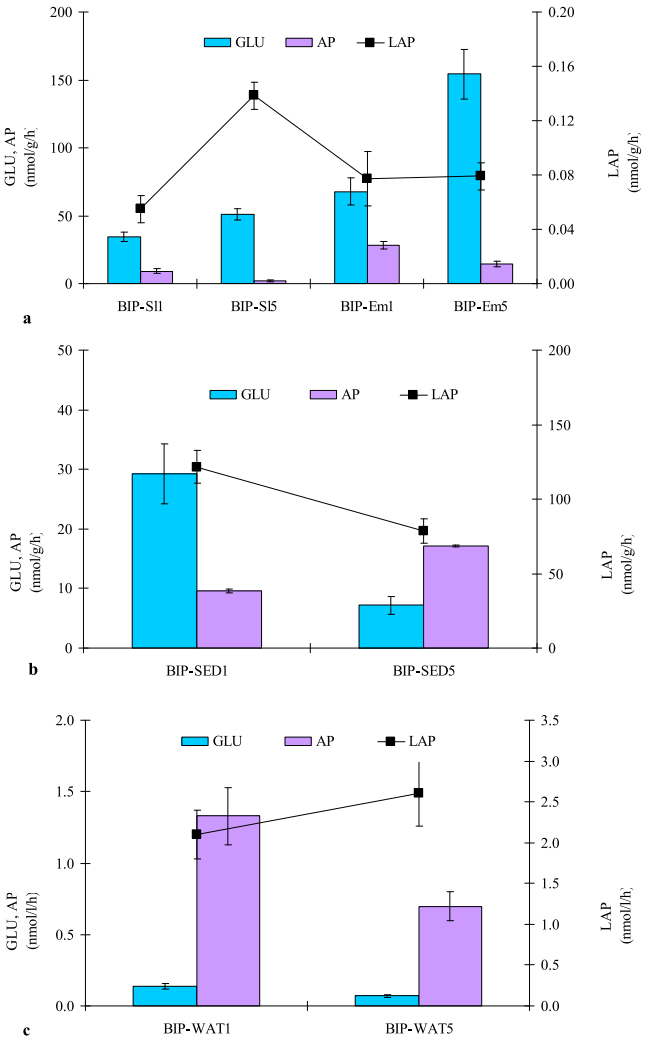

Fig. 3. Microbial enzymatic activities in **a)** sponge, **b)** sediment and **c)** water samples from the Pasvik River. *Please, note the different scales.*

Marked differences among samples were instead observed at family level (Fig. 5). Bacterial communities inhabiting Pasvik water were mainly composed of *Sporichthyaceae* (18.4 and 15% of the total bacterial community in BIP-WAT1 and BIP-WAT5, respectively), *Comamonadaceae* (13.7 and 13% in BIP-WAT1 and BIP-WAT5, respectively) and *Terrimicrobiaceae* (9.6 and 5.6% in BIP-WAT1 and BIP-WAT5, respectively), which occurred at similar relative percentages in both investigated sites. Conversely, river sediment hosted a more diversified bacterial communities, with differences encountered between the two sampling sites. In particular, the predominance of *Chthoniobacteraceae* and *Gemmataceae* (relative abundance of 5.1 and 5% on the total bacterial community, respectively) was retrieved only in BIP-SED1, whereas *Anaerolineaceae* (4.8%) and *Clostridiaceae* (3.7%) were more abundant in BIP-SED5. In comparison with abiotic matrices, *Pirellulaceae* were markedly more abundant, with higher values in *S. lacustris* (i.e., 28.3 and 22.4% in BIP-Sl1 and BIP-Sl5, respectively) than *E. muelleri* (i.e., 9.5 and 13.3% in BIP-Em1 and BIP-Em5, respectively). *Cyanobiaceae* (from 5.3% in BIP-Em1 to 8.9% in BIP-Sl5) and *Gemmataceae* (from 5% in BIP-Em5 to 11.3% in BIP-Sl1) were also the most abundant bacterial families in sponge mesohyl.

Overall, when considering the site as a factor, all samples shared 66.5 and 66.9% of ASVs at family level at BIP1 (Supplementary Fig. S1a) and BIP5 (Supplementary Fig. S1b), respectively. At BIP1 the sharing level was higher between *E. muelleri* and water samples than sediment samples, while at BIP5 a higher number of families was shared between sponge and sediment samples. Among families detected as exclusive of sponges, *S. lacustris* specimens from the two sites shared the group of *Fervidobacteriaceae,* while *E. muelleri* individuals shared the group of *Caminicellaceae.*

The relative abundances at genus level are shown in Fig. 6. Several genera (e.g., *Gemmata*, *Arenimonas*, *Mycobacterium* and *Zavarzinella*) were retrieved in both sponge samples (even if more markedly in *S. lacustris*) at higher abundances than abiotic matrices. For instance, the relative abundance of the genus *Gemmata* ranged between 0.9 and 1.8% (in BIP-Em1 and BIP-Sl5, respectively) in sponges, with lower value that were determined in water (0.04%; both sampling sites) and sediment (0.4% and 0.1% in BIP-SED1 and BIP-SED5, respectively). *Cyanobium*

Table 2
Number of cyanobacterial cells in sponge and sediment samples.

Matrix	Sample ID	Chroococcales (cells/g)	Oscillatoriales (cells/g)	Nostocales (cells/g)
S. lacustris	BIP-Sl1	8.9×10^8	–	–
	BIP-Sl5	1.4×10^8	–	4.6×10^7
E. muelleri	BIP-Em1	3.2×10^8	–	1.8×10^6
	BIP-Em5	1.4×10^8	–	4.6×10^7
Sediment	BIP-SED1	1.4×10^8	3.5×10^4	3.5×10^5
	BIP-SED5	0.3×10^8	–	4.4×10^5

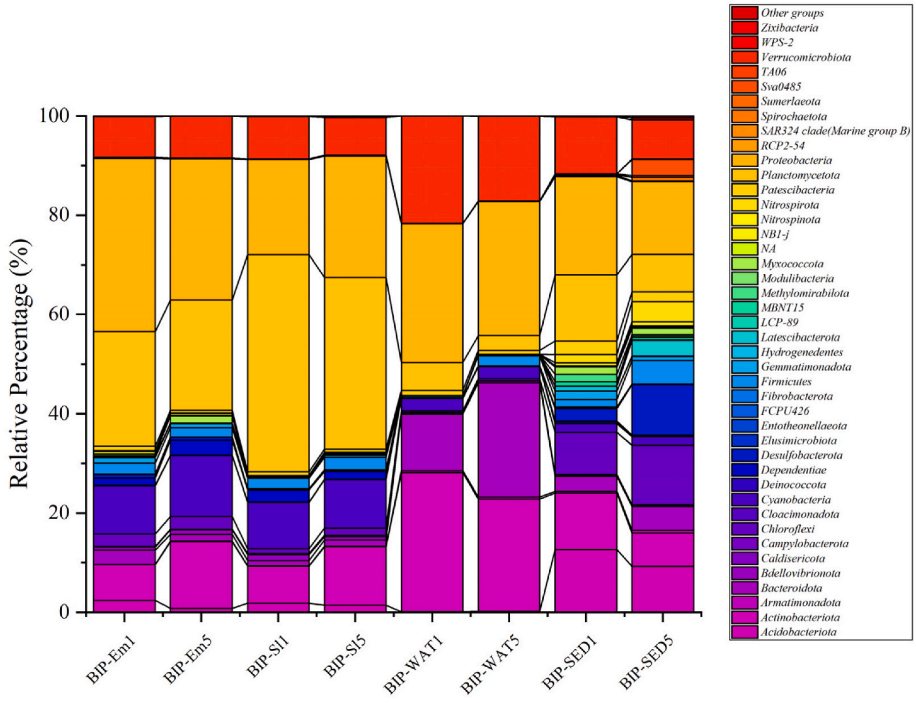

Fig. 4. Distribution of bacterial phyla in sponge-associated, water and sediment communities.

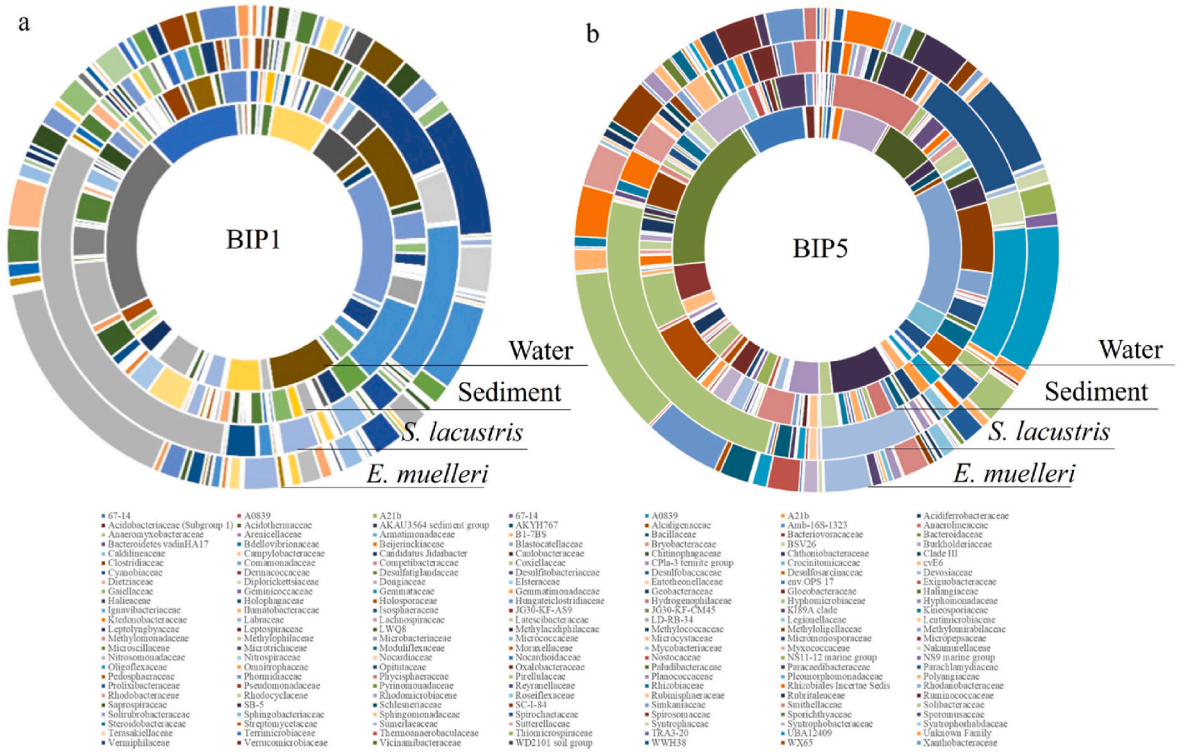

Fig. 5. Taxonomic composition at family level of the bacterial communities in biotic and abiotic matrices collected from site **a)** BIP1 and **b)** BIP5.

affiliates were particularly abundant in sponge tissues (8.2 and 8.8% in BIP-Sl1 and BIP-Sl5, respectively; 7.4 and 5.3% in BIP-Em1 and BIP-Em5, respectively), whereas they ranged between 0.7 and 2.4% in abiotic matrices. *Fimbriglobus* members were mainly retrieved in association with *S. lacustris* (1.9 and 2.3% in BIP-Sl1 and BIP-Sl5, respectively) and, at a lesser extent, *E. muelleri* (0.9 and 1.6% in BIP-Em1 and BIP-Em5, respectively) than in water and sediment (range 0.04–0.3%). Finally, *Mycobacterium* sequences were particularly enriched in BIP-Sl5 with respect to both BIP-Sl1 (6.8 and 2.6%, respectively) and *E. muelleri* (about 2% at both sites), whereas they occurred in the range 0.7–1.9% in abiotic matrices.

Bacterial communities in water were dominated by *Limnohabitans* affiliates (10.3 and 9.1% in BIP-WAT1 and BIP-WAT5, respectively) and hgcI clade (13.2 and 10.8% in BIP1 and BIP5, respectively), followed by

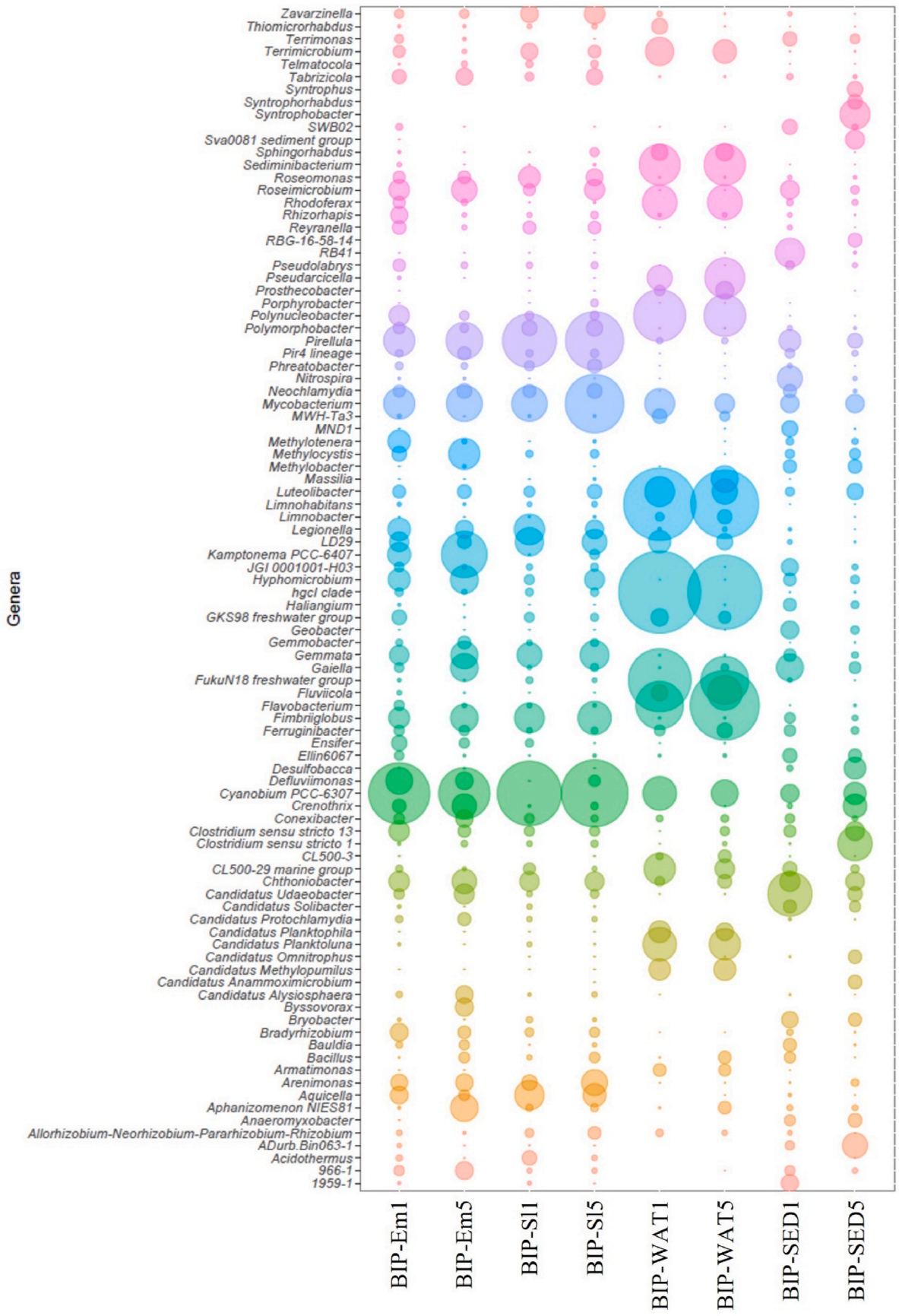

Fig. 6. Relative abundances at genus level in bacterial communities associated with sponges, water and sediment from the sampling sites BIP1 and BIP5 (*only genera with a relative abundance >0.3% in at least one sample are represented*).

Flavobacterium (4.6% and 9.5% respectively in the sites BIP-WAT1 and BIP-WAT5), FukuN18 freshwater group (7.9 and 4.6% in BIP-WAT1 and BIP-WAT5, respectively), and *Polynucleobacter* (5.5 and 3.6% in BIP-WAT1 and BIP-WAT5, respectively). Conversely, the sediment bacterial communities differed at genus level between the two sites, being *Clostridium sensu stricto* 1 (2.5%) and *Syntrophobacter* (1.9%) more abundant in BIP-SED5, and *Candidatus Udaeobacter*, RB41, *Gaiella* and *Nitrospira* (average relative abundance 4, 1.8, 1.6, and 1.4%, respectively) better represented in BIP-SED1.

The archaeal communities, accounting for 0.01–1% (BIP-SED1 and BIP-SED5, respectively) of total ASVs, were overall dominated by the group of *Halobacterota*, with relative abundance of about 59% of total archaeal sequences in *E. muelleri* from both sites, and 64.7% and 57.1% in BIP-Sl1 and BIP-Sl5, respectively. Similar *Halobacterota* abundances were determined in water and sediment from sites BIP1 (28% vs 20.1%) and BIP5 (73.7% vs 77.8%). *Halobacterota* were mainly represented by *Methanoregula, Methanosaeta* and *Methanosarcina* members, with higher abundance detected for *Methanosaeta,* accounting for: 9.4 and 38.7% in BIP-WAT1 and BIP-WAT5, 2.5 and 37.9% in BIP-SED1 and BIP-SED5; 42.1 and 22.5% in BIP-Em1 and BIP-Em5; 47.9 and 14.8% in BIP-Sl1 and BIP-Sl5, respectively. The second most abundant archaeal group was *Crenarchaeota* showing higher abundance in water and sediment samples from the sampling site BIP1 (32 and 67.6% in BIP-WAT1 and BIP-SED1, respectively). Sponge-associated *Crenarchaeota* were detected at averaged relative abundance of 20.9 and 12.2% in BIP-Em1 and BIP-Em5, respectively, and 23.5 and 22.8% in BIP-Sl1 and BIP-ESl5, respectively. *Euryarchaeota* and *Thermoplasmatota* seldom occurred at lower percentages (Supplementary Fig. S2).

3.5. Statistical analyses

Correlations between the relative abundances of different bacterial phyla and pollutant concentrations were calculated (Supplementary Fig. S3). A strong positive correlation was detected between CBs, PAHs, PCNs concentration and *Campylobacterota* abundance ($p \leq 0.001$), while *Armatimonadota* were negatively correlated with DB[a,h]A ($p \leq 0.05$). Strong positive correlations were detected between *Campylobacterota* and the groups WPS-2 and PCBs. A positive correlation was generally observed between most of the investigated CECs, (i.e. ciprofloxacin, sulfamethoxazole, trimethoprim, ibuprofen and diclofenac) and the occurrence of *Acidobacteriota*, *Chloroflexi*, *Nitrospinota* and *Sumerlacota*. Negative correlations were instead detected between *Proteobacteria* and ciprofloxacin, and between *Actinobacteriota* and diclofenac.

The nMDS performed on the relative abundances at family level showed a separation among sponges, water and sediment samples, with three main clusters: a first cluster included the bacterial communities of water from BIP1 and BIP5 with a similarity of 60%; a second cluster included the bacterial communities of sediment from BIP1 and BIP5 with similarity of 60%; finally, a bigger cluster included two subclusters sharing a 80% similarity, each composed of the *S. lacustris* and *E. muelleri* samples, respectively (Fig. 7). PERMANOVA analysis revealed significant differences between microbial assemblages of water and sponges and between sediment and sponges from both sampling sites. Moreover, significant differences were detected in the bacterial communities within level 'Sediment' between the two sampling sites, but not within the levels 'water', '*E. muelleri*' and '*S. lacustris*' (Supplementary Table S7).

Overall, the microbial communities of water and sediment samples at family level were significantly different, mainly due to NA (not assigned; contribution 4.87%) and *Sporichthyaceae* (contribution 3.78%), as shown by the SIMPER analysis. Significant differences between bacterial communities of water and those associated with *S. lacustris* and *E. muelleri* were attributed to *Sporichthyaceae* (6.66 and 5.61%, respectively) and *Comamonadaceae* (4.90 and 4.67%, respectively), while between sediment and *S. lacustris* to NA (7.14%) and *Pedosphaeraceae* (1.90%), and between sediment and *E. muelleri* to NA (6.13%) and *Anaerolineaceae* (1.93%). Finally, the differences between the two sponge species were due to the relative abundance of NA (3.69%) and *Phormidiaceae* (2.67%). Not assigned taxonomic groups at family level, as well as *Pirellulaceae*, were responsible for dissimilarities between sediment microbial communities of BIP1 and BIP5 sites (Supplementary Table S8).

The PCA computed on dataset of chemical contamination (Supplementary Fig. S4) showed the occurrence of two main clusters: the first one including the abiotic matrices with two smaller subclusters composed of water and sediment samples, respectively; the second one including all sponge samples, with two subclusters composed of *S. lacustris* and *E. muelleri*, respectively. PC1 and PC2 components explained the 97.6% of the total variance: PC1 with a contribution of 93.8% mainly influenced by trimethoprim concentration (positive correlation) and NAP concentration (negative correlation), and PC2 with a contribution of 3.8% mainly influenced by ciprofloxacin and diclofenac concentration (positive correlation) and B[b]F (negative correlation).

4. Discussion

Extremely diverse marine sponges, representing more than 95% of all known sponge species, have been the primary subject of investigations on sponge microbiome (e.g., Webster and Taylor, 2014; Lo Giudice et al., 2024a; Mazzella et al., 2024) and pollutant bioaccumulation, mainly focusing on trace elements and heavy metals (e.g., Krikech et al., 2022; Roveta et al., 2022; Pala et al., 2023; Papale et al., 2024). Conversely, the about 250 currently known species of freshwater sponges have received relatively less attention while providing similar ecosystem services in a variety of freshwater systems (Lo Giudice and Rizzo, 2024). Moreover, sponge-associated prokaryotes have been seldom compared between freshwater sponge species, as well as with those from freshwater or sediment (Sugden et al., 2022; Lo Giudice et al., 2024b; Paix et al., 2024). In this study, the microbiome of the sponges *S. lacustris* and *E. muelleri,* inhabiting the same sectors of the sub-Arctic Pasvik River, was characterized, together with the accumulation of both legacy (i.e., polychlorobiphenyls, polychloronaphthalenes, chlorobenzenes and polycyclic aromatic hydrocarbons) and emerging (including pharmaceutical drugs) pollutants in the sponge mesohyl.

Freshwater sponges have been previously reported as suitable bioindicators of water pollution and exploitable candidates for monitoring studies, thanks to their active bioaccumulation process (Yakhnenko et al., 2022; Lo Giudice et al., 2024b). Chemical analyses revealed an alarming environmental issue, due to the evidence of high concentrations of contaminants in the sponge tissues. Targeted contaminants, mainly deriving from anthropogenic (e.g., industry, mining, agriculture, small house agglomerates) sources in the investigated area, were generally determined at lower concentrations in the abiotic matrices than in the sponge mesohyl, thus indicating a strong accumulation capability by sponges. For instance, almost all POPs generally showed the highest affinity with the mesohyl of the branched *S. lacustris*.

Furthermore, POP levels were always higher in water than in sediment, where CBs, PCBs and PCNs were generally below LOD. This finding suggests a transient occurrence of such pollutants, which generally accumulate in sediment, in the investigated area. Conversely, PAHs were determined, even if at low concentrations, both in water and sediment. This class of pollutants shows equal or greater affinity for sediments than water depending on the relative Log K_{ow} of the individuals. PAHs with three or more aromatic rings are more adsorbed on sediment particles due to their low vapor pressure and high hydrophobicity (Abdel-Shafy and Mansour, 2016), a trend found in our samples. Significant differences were encountered between both sponge species and sites, with *S. lacustris* specimens from BIP5 that showed impressive bioaccumulation properties, as they accumulated CBs, PCNs, PCBs and PAHs at higher levels compared to *E. muelleri*. This finding needs to be further elucidated considering additional factors, such as biological traits, chemical and environmental characteristics, trophic interactions.

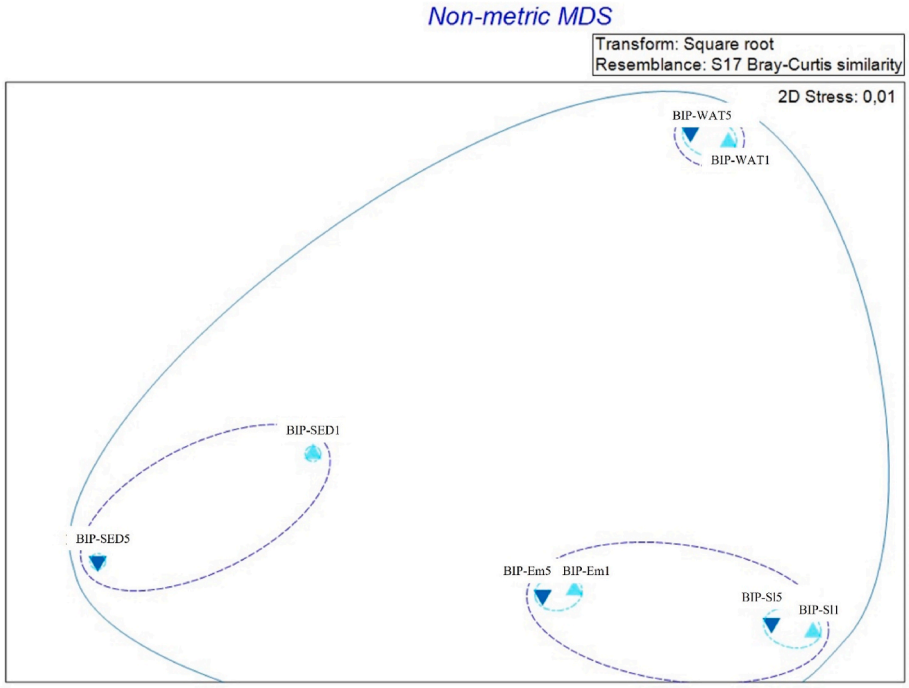

Fig. 7. nMDS showing the similarity level between bacterial communities of samples at family level.

To date, what influences the mechanisms of transfer of POPs and how ecological consequences cascade across trophic levels are poorly understood (Windsor et al., 2019).

A different trend was observed for CECs as they were more concentrated in sediment samples than in water. Among them, only diclofenac and ciprofloxacin were accumulated at a comparable rate by the two sponges, with sulfamethoxazole, trimethoprim and ibuprofen that were not detected. The trophic transfer of ciprofloxacin and diclofenac in food webs (i.e. macrobenthic algae and invertebrate species) has been previously reported (Sokołowski et al., 2024), highlighting a certain biomagnification behaviour of these chemicals especially for ciprofloxacin than diclofenac (Sokołowski et al., 2024), in agreement with the results obtained in the present study. The bioaccumulation of CECs in marine organisms is mainly linked to a small subset of species that exhibit a strong affinity for lipophilic substances in the aqueous phase. This process can also involve the uptake of pollutants in the dissolved phase or those adsorbed onto colloids via the digestive tract (Fabbri and Franzellitti, 2016). The highest bioconcentration factors in organisms, including sponges, are generally observed for compounds with relatively high octanol-water partition coefficients (K_{ow}). This mechanism explains the accumulation of diclofenac (Log $K_{ow} = 4.51$) observed in the present study.

However, the bioconcentration of CECs in sponges is not solely determined by the chemical's hydrophobicity (Rizzi et al., 2023). For example, caffeine can bioaccumulate even if it is characterised by a low K_{ow} (−0.07), suggesting that lipid-independent mechanisms may also play a role in the bioconcentration process. Our study also found that ciprofloxacin (Log $K_{ow} = 0.28$) accumulated in sponges, likely explained by its tendency to participate in complexation reactions (Zhang et al., 2023).

Host-microbe specificity and metabolic linkages, as well as the environmental determinants driving the associated microbiome structure, remain inadequately investigated in freshwater sponges, particularly in remote cold areas. The present study is a contribute to knowledge the microbiota associated to *S. lacustris* and *E. muelleri* from a sub-Arctic riverine system, by estimating quali-quantitative parameters, such as prokaryotic abundance and diversity and enzymatic activities.

Microbial abundance and enzymatic activities. To the best of our knowledge, data on the abundance of associated prokaryotic cells have so far been lacking for freshwater sponges. The abundance values obtained in our investigation were one order of magnitude higher than those measured in the water (10^6 cells/mL) and similar to those retrieved in the sediment (10^7 cells/g), as previously observed (Schläppy et al., 2010). Results confirm previous observations on *S. lacustris* from the Pasvik River (Lo Giudice et al., 2024b). A recent study by Hustus et al. (2023) on algal symbionts of *E. muelleri* showed that bacterial communities are stably associated with *Auxenochrorella pyrenoidosa* (Division Chlorophyta). These bacterial communities are significantly more abundant within the sponge than in the water, consistently with observations on *S. lacustris* (Lo Giudice et al., 2024b). In addition, a study by Sugden and coworkers (2022) on the microbiome of *E. muelleri*, showed that the microbial communities are distinct from communities in ambient water and surrounding biofilms, reinforcing the idea that this sponge could provide a microhabitat for bacterial communities, where prokaryotic abundance and diversity are higher than in the surrounding environment.

The determination of microbial activity rates revealed higher values in the sponge and sediment samples compared to water. GLU activity was particularly higher in sponge tissues, while in sediment samples AP was more represented. Both in water and sediment, the microbial community populating site BIP1 was generally metabolically more active than at site BIP5. This pattern indicated that BIP1 was enriched in organic polymers supporting microbial metabolism. Moreover, sediments are also known to act as a sink for organic detritus coming from the overlaying water (Gooday and Turley, 1990). Enzymatic activities measured in sponges played a role in the nutrition of these benthic organisms, as the breakdown of organic substrates mediated by all these molecules (LAP, GLU and AP) allowed the release of simple molecules that can provide a trophic resource for the organisms living in the area. GLU activity peaks observed in BIP-Em5 suggested the presence of polysaccharides at that site. The similar trends observed between the metabolic patterns of the sponges and of the sediment and water samples provided evidence of the similar origin of the enzymes in the matrices.

Prokaryotic diversity. To date, the number of papers dealing with next-generation sequencing of freshwater sponge-associated prokaryotic communities remains still scant (*see* Lo Giudice and Rizzo, 2024 *for a*

review), resulting in a dearth of high-resolution taxonomic data on these communities. Similarly to marine sponges, literature data highlight that freshwater sponges host microbiomes dominated by *Proteobacteria* and *Actinobacteria*, whereas *Bacteroidetes*, *Planctomycetes*, *Verrucomicrobia*, *Acidobacteria* and *Armatimonadetes* are generally detected at a lower abundance and only seldom reported among dominant bacterial phyla (e.g., Kaluzhnaya et al., 2012; Costa et al., 2013; Gladkikh et al., 2014). In contrast with this general trend, the prokaryotic communities associated with both *E. muelleri* and *S. lacustris* from the sub-arctic Pasvik River were dominated by *Proteobacteria* and *Plancomycetota*, followed by a conspicuous presence of *Cyanobacteria* and *Verrucomicrobiota* (both generally occurring at abundances similar to those of *Actinobacteriota*). Archaeal communities in freshwater sponges (as well as in water and sediment samples) were mainly constituted by *Halobacterota* and *Crenarchaeota*, especially in *E. muelleri* specimens.

Overall, the phylogenetic structures of the prokaryotic communities associated with *S. lacustris* and *E. muelleri* were highly similar at phylum level, showing also similarities with abiotic matrices. This finding suggests that the sponge surroundings might strongly contribute to shape the prokaryotic community structure in association with sponges. Sugden et al., (2022) suggested horizontal acquisition from ambient bacteria as a crucial factor explaining rivers-specific microbiome. Later on, Paix et al. (2024) demonstrated the microbiome acquisition during the first development stages of *S. lacustris* (vertical transmission), highlighting the importance of gemmule epibacteria for the holobiont (sponge *plus* microbiome) stability. Interestingly, the authors excluded the horizontal recruitment to happen during the first stages of development. In this study, some dissimilarities observed between *E. muelleri* and S. *lacustris*, between sampling sites and between sponges and abiotic matrices could be attributed to several families, as detailed in the results section. Some distinctive features were also observed at genus level within *Cyanobacteria* and *Planctomycetota*, with genera (e.g., *Cyanobium*, *Gemmata*, *Pirellula*) that were significantly more abundant in sponges than in water and sediment, suggesting the ability of sponges to selectively acquire microbes from the bulk environment.

The microbiome associated with *E. muelleri* has been only recently investigated by culture-independent approaches, revealing a level of diversity comparable to that of the several marine demosponges. Kenny et al. (2020) analyzed the microbiome of *E. muelleri* from six different locations across in the Northern hemisphere and observed the predominance of *Proteobacteria* and *Bacteroidetes*. However, differences were determined by geographic location, with high or moderate abundances of *Firmicutes*, *Campylobacteria* and *Cyanobacteria* that were seldom recorded. The same authors detected few ASVs (affiliated with *Betaproteobacteriales* and *Chitinophagales*) that were shared among all samples (range <1% to >20% of total sequences), opening questions on the role they may play within the holobiont ecology. Differently from Kenny et al. (2020), *E. muelleri* from the Pasvik River did not host *Betaproteobacteria*, whereas *Chitinophagales* were more abundant in abiotic matrices than in sponges. In addition, in our sponge samples *Bacteroidota* were scarcely represented within the associated bacterial communities. Sugden et al. (2022) demonstrated that *E. muelleri* core microbiome was conserved among different Canadian rivers (British Columbia), even if sponges distinguished by their origin due to a few microbial differences. These differences were not reflected in the water, indicating that environmental or host-specific factors may drive the observed geographic variability. Finally, comparing the microbiomes associated with *E. muelleri* and *E. muelleri* algal symbionts, Hustus et al. (2023) found that *Cyanobacteria* were generally dominant in the *E. muelleri* microbiome, followed by *Proteobacteria*, with species from the orders *Nostocales*, *Synechococcales*, *Oscillatoriales*, and *Pleurocapsales* found in all samples. In addition, *E. muelleri* harbored a high abundance of *Bacteroidetes*, that were not especially abundant in the algal microbiomes, and medium abundances of *Actinobacteria*, *Verrucomicrobia* and *Spirochaetes*, that decreased in the algal samples.

To date the microbiome of *S. lacustris* has been only rarely characterized by adopting advanced culture independent methods. Our findings are in line with those recently obtained for specimens from the Austrian Lake Pichlinger See (Graffius et al., 2023). Based on the 16S rRNA gene amplicon sequencing outputs, sequences belonging to six main phyla representing *Cyanobacteria*, *Proteobacteria*, *Bacteroidota*, *Verrucomicrobiota*, *Planctomycetota*, and *Patescibacteria* were found, with a large portion of ASVs that were strongly related to uncultured and unclassified *Bacteroidota*, *Alphaproteobacteria*, *Gammaproteobacteria*, and *Betaproteobacteria*. It is noteworthy that a preliminary report on the bacterial community associated with *S. lacustris* from the Pasvik River, collected in 2014, revealed the predominance of *Alphaproteobacteria* and *Gammaproteobacteria* (among *Proteobacteria*), followed by *Chloroflexi* and *Acidobacteria*, which were instead less represented in 2021 sponge samples (Lo Giudice et al., 2024b). While functions and roles of *Chloroflexi* remain largely unknow in natural environments, *Acidobacteria* are involved in the oxidation of iron and sulfur compounds, as well as in the nitrogen cycle. This discrepancy between 2014 and 2021 might derive from potential changes in environmental conditions, probably linked to the closing of the Pechenganickel smelter in December 2020 (with sulfur emissions that were annually reduced from 80.000 tons to zero), or lower eutrophication levels in the catchment area of the river.

Overall, the high abundance of *Proteobacteria* (mainly affiliated to Alpha- and Gammaproteobacteria) observed in both sponges, even if lineages (e.g., *Rhodobacterales* and *Rhodospirilles*) occurred at differential relative percentages between *E. muelleri* and *S. lacustris*, was in accordance with previous reports on marine and freshwater sponges. Among *Gammaproteobacteria*, sequences affiliated to the genus *Arenimonas* were particularly abundant in association with *E. muelleri* and *S. lacustris*. Such genus is frequently reported in industrially, agriculturally and domestically polluted rivers (Zhang L. et al., 2020). For instance, *Arenimonas* members are often detected in heavy metal-contaminated environment, enough to be considered as a bacterium with a bioremediation potential in chromium-polluted environments (Li et al., 2020a; Wang et al., 2021). Finally, *Arenimonas* was among the main genera for denitrification in wastewater treatment (Yang et al., 2024).

Planctomycetota are commonly reported at highest abundances in water-saturated environments, such as wetlands (which are rich in plant-derived organic matter). *Plantomycetota* are also known to reside permanently in marine sponges, suggesting the establishment of highly integrated symbiotic relationships (Pimentel-Elardo et al., 2003). However, to date few information is available on potential functions played by its members in the environment. They are reported described as resistant to environmental contaminants, able to degrade organic matter and producers of biotechnologically relevant biomolecules (e.g. antibiotics and antifungal compounds) (Kallscheuer and Jogler, 2021). A high abundance of *Planctomycetota* was previously reported in association with the cosmopolitan freshwater-brackish sponge *Ephydatia fluviatilis* (Costa et al., 2013). They were also detected, even if at a lesser extent, in other freshwater sponges, such as *Eunapius carteri* and *Corvospongilla lapidosa* (Gaikwad et al., 2016), the endemic Baikal species *Lubomirskia baicalensis* (Seo et al., 2016), *Baikalospongia* sp. (Gladkikh et al., 2014), and *Swartschewskia papyracea* (Kaluzhnaya and Itskovich, 2016). In our study, among *Planctomycetota*, members in the genus *Pirellula* (family *Pirellulaceae*) were particularly abundant (especially in association with *S. lacustris*) suggesting the occurrence of ammonium oxidation (Mohamed et al., 2010). Within the family *Gemmataceae*, member in the genus *Gemmata* have been previously reported in symbiosis (by mechanical or nutritional relationships) with marine sponges. Most of *Gemmata* like-related *Planctomycetota* (e.g., *Zavarzinella* and *Fimbriiglobus*) have been previously recovered from *Sphagnum* moss-dominated wetlands (Kulichevskaya et al., 2009, 2017), playing a crucial role in the decomposition of *Sphagnum*-derived litter in these ecosystems. *Fimbriiglobus* was among *Planctomycetota* retrieved in boreal and subarctic wetlands (Dedysh and Ivanova, 2019).

Cyanobacteria, including both coccoid and filamentous

morphologies, are abundant in both freshwater and marine sponges, providing them UV radiation protection and chemical defence (by production of bioactive compounds) (Webster and Taylor, 2014; Kulakova et al., 2014; Gladkikh et al., 2014; Mutalipassi et al., 2021), and playing crucial roles in host adaptation by expanding metabolic function via photoautotrophy and nitrogen fixation (Hudspith et al., 2022).

It is of significant scientific interest to understand the diversity of *Cyanobacteria* associated with sponges to comprehend the ecology and evolutionary history of these symbiotic associations (Usher, 2008), particularly in regions that have not been extensively examined from this perspective. A high abundance of *Cyanobacteria*, comparable with the abundance of *Cyanobacteria* found in marine sponges, was reported by Gaikwad et al. (2016) in association with *E. carteri* and *C. lapidosa*, with the predominance of *Synechococcus*. Conversely, *Cyanobium gracile* PCC6307 prevailed in *E. muelleri* and *S. lacustris*, as previously reported (Kulakova et al., 2014; Burgsdorf et al., 2015; Kaluzhnaya and Itskovich, 2017). *Cyanobium* and *Synechococcus* are both among the dominant picocyanobacteria in freshwater systems (Ivanikova et al., 2007; Callieri, 2008). Results from next-generation sequencing were supported by microscopic observations, revealing a high abundance of *Chroococcales*. Several studies applying both methodological approaches obtained complementary results (e.g., Pushkareva et al., 2015; 2022).

Differently from other studies on freshwater sponges, *Actinobacteriota* did not predominate within the prokaryotic communities associated with *E. muelleri* and *S. lacustris* from the Pasvik River. However, our study provided evidence for the moderate enrichment of *Mycobacterium* in sponge mesohyl (especially in *S. lacustris* from the site BIP5) than in water and sediment samples. Data on mycobacteriosis in freshwater and marine aquatic invertebrates have increased during the past few years (Davidovich et al., 2020). The application of novel diagnostic techniques, along with increased awareness and surveillance, has certainly contributed to an increase in the number of reported cases. Furthermore, freshwater and marine aquatic habitats are being impacted by climate change. Numerous *Mycobacterium* species have been identified in aquatic invertebrates, including sponges (Izumi et al., 2010; Pidot et al., 2024). Future studies exploring the occurrence of pathogenic bacteria in freshwater sponges from remote areas will shed more light on the impact of climate change on the growth and survival rates of microorganisms, the spread of pathogens, and the vulnerability of hosts to infection, which are neglected so far. *Methanosaeta* members were detected as the most representative archaeal groups in both abiotic and biotic compartments. They are known as the most active aceticlastic methanogens in wetlands, producing an extensive amount of methane on Earth (Brauer et al., 2020). This aspect is particularly interesting, as methane strongly contributes to the greenhouse effect, by influencing the global climate.

Some significant correlations were observed between microbial and chemical data. While some contaminants were correlated with one or two bacterial taxonomic groups, as in the case of CBs, PAHs, PCNs and PCBs, the CEC concentrations globally seemed to affect the entire bacterial community, by correlating with significance to different bacterial phyla and, in some cases, by showing a strong negative correlation, as observed for diclofenac and ciprofloxacin. A clear impact of pollutants on the bacterial communities was demonstrated in the area of the Pasvik River by using cultivable approach, with occurrence of HM-tolerant and PCB-oxidizing bacteria in correlation with the presence of these contaminants. The capacity of psychrotolerant bacteria to withstand various chemical stressors has been suggested as an optimal criterion for utilizing them as valuable bioindicators and or substrate for biosensors implementation (Tomova et al., 2014; Caputo et al., 2019; Rappazzo et al., 2019).

5. Conclusion

Overall, this study represents a substantial contribute to the existing knowledge of the ecology and microbiology of freshwater sponges. Chemical analysis revealed an alarming environmental issue, due to the evidence of CEC in the sponge tissues, especially from the sampling site BIP5. As revealed by the high bioaccumulation factor for the investigated contaminants, sponges are here confirmed as optimal bioindicators of environmental pollution, reinforcing previous observations in the marine environment. This means that the study of their distribution and wellness could be included in monitoring plans of aquatic environments also in the sub-Arctic area, which deserves special attention due to its vulnerability. Between the two investigated sponge species, *S. lacustris* was more affected by chemical contamination, probably explaining the lower abundance and irregular distribution of this species in the study area observed during sampling activities. The phylogenetic structure of the associated microbial communities showed high similarities between the sponge species, and also high similarities with abiotic matrices both at phylum and genus level. However, a different representation of some taxa in specimens from BIP5, which overall appeared more impacted, suggested an influence of environmental conditions. This was particularly evident in the microbial communities associated with the *S. lacustris* specimens inhabiting the sampling site BIP5, probably due to higher bioaccumulation and to different filtration rates of the two sponge species. The obtained results encourage the planning of continuous survey to monitor the distribution of these sponges and of their associated microbial communities along the Pasvik River, by extending the investigations also to different areas, taking into account the influence of seasonal changes and weather conditions. Some aspects, as well as the occurrence of potential pathogens and archaeal methanogens in the sponge tissues need to be further addressed in future studies, in a cause/effect climate change perspective.

CRediT authorship contribution statement

Carmen Rizzo: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Gabriella Caruso:** Writing – review & editing, Formal analysis. **Giovanna Maimone:** Writing – review & editing, Formal analysis. **Luisa Patrolecco:** Writing – review & editing, Formal analysis, Data curation. **Marco Termine:** Formal analysis. **Marco Bertolino:** Writing – review & editing, Formal analysis. **Stefania Giannarelli:** Writing – review & editing, Formal analysis, Data curation. **Alessandro Ciro Rappazzo:** Writing – review & editing, Formal analysis. **Josef Elster:** Writing – review & editing, Funding acquisition, Formal analysis, Data curation. **Alessio Lena:** Writing – review & editing, Formal analysis. **Maria Papale:** Writing – review & editing. **Tanita Pescatore:** Formal analysis. **Jasmin Rauseo:** Writing – review & editing, Formal analysis. **Rosamaria Soldano:** Writing – review & editing, Formal analysis. **Francesca Spataro:** Writing – review & editing, Formal analysis. **Paul Eric Aspholm:** Writing – review & editing, Investigation, Formal analysis. **Maurizio Azzaro:** Writing – review & editing, Formal analysis. **Angelina Lo Giudice:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.121126.

Data availability

Data will be made available on request.

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