



# Organic Contaminant Mixture Significantly Changes Microbenthic Community Structure and Increases the Expression of PAH Degradation Genes

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Studying the effects of chemical contaminants on the structure and function of microbial and meiofauna communities have traditionally focused on specific effects of single contaminants on single species. This has left the complex interactions between mixtures of contaminants and its non-specific toxicity effects on the functions and structure of sediment microbial communities mostly overlooked. In order to improve our insights on such questions, we performed an experiment where Baltic Sea sediments were spiked with an ecologically relevant mixture of seven organic contaminants below specific toxicity levels and used 16S and 18S rRNA metabarcoding from RNA extracts to monitor changes in active microbial and meiofauna diversity and community structure in the spiked treatment compared to controls. In addition, we investigated the effects of exposure to this contaminant mixture on potential nitrification rates and on the expression of key-genes in the microbial nitrification and PAH degradation pathways with qPCR. There were significant differences in both eukaryotic and microbial community structures in sediments spiked with a mixture of organic contaminants. Nematoda showed a significant increase in overall relative abundance to the added contaminants ( $5.5 \pm 1.1\%$  higher in spiked), particularly taxa of the genus *Leptolaimus* (increased from  $10.2 \pm 5.4\%$  in the controls to  $32.5 \pm 10.2\%$  in the spiked treatment). Conversely, a significant decrease in relative abundance from  $18.2 \pm 5.6\%$  in control to  $7 \pm 3.4\%$  in of the genus *Paraplectana* was also detected. Additionally, while the abundance of active PAH degraders was significantly higher in spiked sediments than in the controls, no significant effect of our organic mixture was found on nitrification rates or the expression of *AmoA* (bacterial ammonia oxidizer gene). Our data indicate that mixtures of organic contaminants can have significant effects on microbenthic community structure even when its individual components are present at concentrations

below its specific toxicity. In addition, we suggest that eRNA-based metabarcoding can offer important insights in microbenthic community structure and activities, and further emphasizes the potential of meiofauna as bio-indicators of chemical contamination in benthic ecosystems.

**Keywords:** non-specific toxicity, meiobenthos, microbial, metabarcoding, polychlorinated biphenyl, polycyclic aromatic hydrocarbons

## INTRODUCTION

The marine benthos is the second largest ecosystem on Earth, harboring a substantial diversity of macro- and microorganisms (Snelgrove, 1999; Harley et al., 2006). These communities are crucial to global biogeochemical cycles, mineralizing organic matter and nutrients and serving as major nitrogen and carbon sinks through sedimentation processes (Kraaij et al., 2002; Sundbäck et al., 2004; Porubsky et al., 2009). The resilience and functioning of an ecosystem rely on its biodiversity, and highly diverse systems increase productivity by providing ecological niches and the ability to withstand disturbances through functional redundancy (Slootweg et al., 2009; Cardinale et al., 2012). In addition, benthic fauna like meiofauna (invertebrates smaller than 0.04 mm), which reside within sediments, continuously rework their habitat's structures, transport nutrients, and promote resource availability in sediment layers in which they are most active, through burrowing, particle resuspension, and bioturbation (Bonaglia et al., 2014b; Zeppilli et al., 2015; Schratzberger and Ingels, 2018). Meiofauna are highly diverse and abundant microscopic benthic microeukaryotes and are regarded as crucial players in sediment ecosystems (Sheppard, 2006; Nascimento et al., 2012; Bonaglia et al., 2014b; Schratzberger and Ingels, 2018). They play a key role in food webs on nearly all trophic levels and are highly responsive to environmental disturbances (Giery, 2009; Bik et al., 2012).

The human impact on coastal benthic ecosystems has been significant for several decades and as a result, coastal benthic communities are often exposed to organic contaminants. Many organic contaminants are hydrophobic and thus have a high affinity to particulate organic matter, which can accelerate their vertical transport to the sediment (Nizzetto et al., 2010; Zhang et al., 2015). Therefore, organic contaminants such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) can accumulate in marine sediments, leading to the exposure of sediment communities to complex mixtures of organic contaminants. Due to their hydrophobic nature, PAHs and PCBs are bioaccumulative (ECHA, 2017). They are often associated with higher mortality, lower reproduction (Lotufo, 1998) and feeding rates (Silva et al., 2009) of benthic populations and have been shown to interfere with oxygen and nutrient dynamics (Carman and Todaro, 1996; Fleeger et al., 2003; Quero et al., 2015). Acute exposure to high concentrations of PAHs can change the composition and ecological functions of benthic communities. For instance, Edlund and Jansson (2006) reported a rise in Gammaproteobacteria and Flavobacteria following bioremediation of polluted Baltic Sea sediments and Lindgren et al. (2012) found certain nematode taxa to decreased in

abundance, in response to the addition of diesel, while conversely Foraminifera increased in relative abundance. Additionally, Lindgren et al. (2014) show that exposure of marine sediments to organic contaminants impacts not only meiofauna and bacterial communities, but also important ecological processes related to benthic nitrogen cycling. These authors found sediment-to-water fluxes of nitrite and nitrate, and potential nitrification and denitrification to be reduced in sediment exposed to PAHs.

Biological communities in the environment are rarely exposed to a single, isolated compound, instead they are exposed to complex mixtures of contaminants (Mustajärvi et al., 2017b; Dulio et al., 2018; Jahnke et al., 2018). Our understanding of the effects of such mixtures on benthos is limited, particularly regarding higher levels of biological organization. This poses a significant obstacle for risk assessment of chemical contaminants, as there is evidence of negative effects of mixtures even if each of its components is present below a no-effect concentration (Silva et al., 2002; Kortenkamp et al., 2009). In general, concentrations of different chemicals cannot be added up to derive the overall toxicity of the mixture, because the relationship between toxicity and concentration is different for every chemical. Concentrations of single chemicals are often below what would cause compound specific toxicity (Inostroza et al., 2017). However, chemicals occur in the environment in complex mixtures, and mixtures can have a non-specific toxicity (Escher et al., 2002; Inostroza et al., 2017). Non-specific toxicity is induced by partitioning of contaminants to cell membranes, causing disturbance of the membrane. It is governed by the chemical's hydrophobicity, is additive and correlates to chemical activity (Mackay et al., 2011; Gobas et al., 2018). Chemical activity is a thermodynamic concept that relates the concentration ( $C$ ; mol L<sup>-1</sup>) of chemical  $i$  to its maximum solubility ( $S$ ; mol L<sup>-1</sup>) in the same environmental media (e.g., sediment porewater) (Schwarzenbach et al., 2002). Chemical activity can thus be used to quantify the total load and potency of environmental mixtures. In the case of non-specific toxicity, it is the chemical activity of the mixture that matters, and not the composition of the mixture (Mayer and Reichenberg, 2006; Mackay et al., 2009). However, how chemical activity as a proxy for non-specific toxicity, affects benthic community function and structure is not well understood, particularly regarding microbial and meiofauna communities. As previous work has demonstrated a baseline toxicity at the chemical activities level of 0.01 to 0.1 (Smith et al., 2013), we aimed for a total chemical activity of the contaminant mixture to be within that range.

Here we investigated the non-specific toxicity of a chemical mixture containing hexachlorobenzene (HCB), biphenyl, three PCB congeners (PCB52, PCB101, PCB153) and two PAHs (pyrene and phenanthrene), on the composition of pro-and

eukaryotic microbial and meiofaunal communities. For this, we utilized a metabarcoding approach and sequenced both the 16S and 18S regions of the ribosomal RNA to capture the diversity of benthic prokaryotes and eukaryotes, respectively. As extracellular DNA is generally stable and might persist in the environment (Corinaldesi et al., 2008), we targeted RNA to better target the active diversity. In addition, we used RT-qPCR to quantify the expression of the microbial ammonia monooxygenase (*AmoA*) and PAH-ring hydroxylating dioxygenase (*PAH-RHD*) genes (Cébron et al., 2008) and determined potential nitrification rates by oxalic slurry incubations (Bonaglia et al., 2014a). In brief, we hypothesized the following: i) the structure of benthic microbial and meiofauna communities is significantly changed in sediments containing a complex mixture of organic contaminants at non-specific toxicity levels; ii) exposure to this mixture of organic contaminants in sediments will significantly decrease the expression of microbial *AmoA* genes and nitrification rates, and iii) the expression of *PAH-RHDα* genes to significantly increase in contaminated sediments.

## MATERIALS AND METHODS

### Sediment and Animal Collection

The sediment and animals used in this experiment were collected at a depth of 37 m from the Skvallran pristine sampling station (58°47'25.0"N 17°43'42.2"E) near the Stockholm University Marine Field Station on the island of Askö, in the Northwestern Baltic Sea proper (Figure 1) using a benthic sled during the late spring (May 2nd) of 2017.

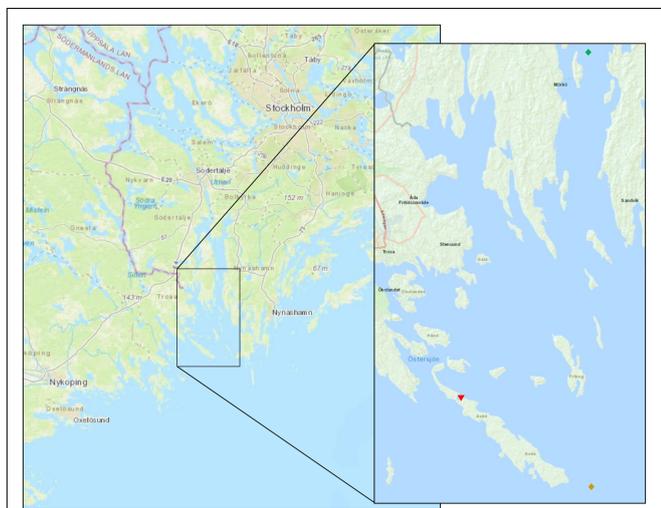
### Sediment Spiking and Chemical Analysis

Sediment was spiked with the chemical mixture (Table 1) by vertically rolling the sediment in 1 L amber glass spiking

jars (Ditsworth et al., 1990). Spiking jars were prepared with chemicals by adding stock solutions of 1 mg mL<sup>-1</sup> HCB in acetone:hexane 1:3 v/v to the empty jars, followed by a mixture of biphenyl 0.5 mg mL<sup>-1</sup>, PAHs 0.5 mg mL<sup>-1</sup> and PCBs 0.3 mg mL<sup>-1</sup> in acetone:dimethyl sulfoxide (DMSO) 10:1 v/v, after which the jars were left on a roller without lids to allow the solvent to evaporate. DMSO (1 mg kg<sup>-1</sup> sediment fresh weight) was added as a keeper to the stock solution to retain the more volatile compounds. The control jars were treated similarly by adding equivalent amounts of solvents and DMSO to those used for chemical spiking. Wet sediment was then added (800 g/jar) and the jars were covered with aluminum foil, sealed with a lid, and rolled directly for 20 min. The sediment was allowed to equilibrate with the chemical mixtures sorbed to the inner glass walls of the jars during 20 days at 5°C. Due to a limited space on the roller mixer, jars were rolled in three batches and were cycled equally between the roller and a vertical shaking table every 24 h. Jars were opened daily to avoid gas building up. After 20 days, the 800 g sub-batches of sediment were combined (total of 5.6 kg spiked and control sediment) and sediment samples were collected for chemical analysis.

Concentrations of the chemical mixture components were measured both in total sediment ( $C_{\text{sed}}$ ) and in sediment porewater ( $C_{\text{porewater}}$ ). Extraction of sediment was performed with accelerated solvent extraction (ASE, Dionex 200), with acetone:hexane (1:1 v/v) using two extraction cycles per sample and the instrument was set-up according to Josefsson et al. (2006). Dry sediment samples (0.1 g,  $n = 3$ ) were weighed to the extractions cells (34 mL), internal surrogate standards were added (PCB3, pyrene<sub>D10</sub>, phenanthrene<sub>D10</sub>, HCB<sub>C13</sub>), and the cells were filled with ISOLUTE® HM-N resin (furnished 450°C overnight prior to use). Method blanks were included, containing ISOLUTE® HM-N resin only. Sample extracts were then treated with activated Cu powder for sulfur removal. Due to the high expected concentrations in the extracts, the samples were diluted 1:20 with hexane before the second batch of surrogate standards were added (PCB52<sub>C13</sub>, PCB101<sub>C13</sub>, and PCB153<sub>C13</sub>). Dimethyl formamide (DMF) was used for sample cleanup, followed by 1 cm ø silica columns (10% deactivated). The cleanup method is described in detail by Mustajärvi et al. (2017a). Following clean-up, the samples were reduced in volume (200 µL), and internal recovery standards (PCB53 and aldrin) were added prior to GCMS analysis.

Porewater concentrations ( $C_{\text{porewater}}$ ) were measured in sediment by using equilibrium passive sampling. Sediment samples for  $C_{\text{porewater}}$  analysis were collected just before the start of the experiment and frozen until analysis. Passive equilibrium sampling was carried out by using glass jars coated internally with a 1, 3, or 5 µm silicone layer (DC1-2577- silicone; Dow Corning®) (Reichenberg et al., 2008). Approximately 10 g of wet sediment was added to the coated 20 ml glass jars. The glass jars were rolled horizontally for two weeks in room temperature in darkness, to allow the analytes to equilibrate between the sediment and the silicone. The sediment was then discarded, and the inside part of the glass vials was rinsed with cold tap water and dried carefully with a lint-free tissue. For extraction of the silicone, 2 ml acetone:hexane (1:1,



**FIGURE 1** | Sampling location of pristine sediment (brown diamond) near the Askö laboratory (red inverted triangle), South-West at the border of Stockholm County. Amphipods and diatoms were collected at station H4 (green diamond). Map was adjusted from ÅN Austin (2019) and OpenSeaMap (2019).

**TABLE 1** | Chemical mixture spiked to the sediment and analyzed sediment concentrations ( $C_{\text{sed}}$ ) before spiking and after the experiment ( $\text{mg kg}^{-1}$  sediment dry weight, average  $\pm$  standard deviation,  $n = 3$ ), as well concentrations in sediment porewater ( $C_{\text{porewater}}$ ) and chemical activity.

Chemical	Nominal $C_{\text{sed}}$ ( $\text{mg kg}^{-1}$ )	$C_{\text{sed}}$ after spiking ( $\text{mg kg}^{-1}$ )	$C_{\text{sed}}$ after experiment ( $\text{mg kg}^{-1}$ )	$C_{\text{porewater}}$ ( $\mu\text{g L}^{-1}$ ) and corresponding chemical activity (in brackets)	Surrogate standard recoveries for $C_{\text{porewater}}$
HCB	11	$5.32 \pm 0.13$	$0.74 \pm 0.12$	$1.15 \pm 1.2$ (0.003)	83%
Biphenyl	12	$0.15 \pm 0.1$	nd	nd	n/a
PCB52	4.4	$3.86 \pm 0.96$	$0.48 \pm 0.08$	$0.63 \pm 0.23$ (0.004)	59%
PCB101	2.3	$2.40 \pm 0.98$	$0.27 \pm 0.03$	$0.11 \pm 0.02$ (0.003)	59%
PCB153	9.2	$3.51 \pm 1.08$	$0.51 \pm 0.06$	$0.12 \pm 0.02$ (0.01)	59%
Pyrene	8.7	$5.79 \pm 0.99$	$0.83 \pm 0.08$	$1.16 \pm 0.36$ (0.002)	64%
Phenanthrene	10.9	$1.35 \pm 0.08$	$0.54 \pm 0.06$	$0.16 \pm 0.11$ (0.00004)	64%

Not detected = nd. Data on solubility (S) used to calculate chemical activity was taken from Li et al. (2003), Wang et al. (2008), Ma et al. (2010).

v:v) was added to the glass jars together with IS surrogate standards (CB3, pyrene<sub>D10</sub>, phenanthrene<sub>D10</sub>, HCB<sub>C13</sub>). The glass jars were rolled for 30 min and solvent was collected. The extraction of the silicone was repeated for a second time and the two extracts were pooled. The cleanup of the extracts followed the same procedure as described for the sediment samples. After cleanup all samples were reduced in volume (200  $\mu\text{l}$ ) and recovery standards (PCB53 and Aldrin) were added prior the gas chromatography mass spectrometry (GCMS) analysis.

Chemical concentrations in silicone ( $C_{\text{pol}}$ ) at equilibrium were converted to porewater concentrations by using polymer-water partition coefficients ( $K_{\text{pol/w}}$ ):

$$C_{\text{porewater}} = \frac{C_{\text{pol}}}{K_{\text{pol/w}}} \quad (1)$$

$K_{\text{pol/w}}$  from Gilbert et al. (2016) for PCBs and HCB,  $K_{\text{pol/w}}$  for phenanthrene and pyrene were extrapolated by plotting published  $K_{\text{pol/w}}$  (Gilbert et al., 2016) for PCBs against octanol-water coefficients ( $K_{\text{ow}}$ ) from Hawker and Connell (1988).

Limits of detections (LOD) were calculated from the method blanks (average plus three times standard deviation), and all samples were blank corrected. Average blank levels for  $C_{\text{porewater}}$  were 0.61 ng for PCBs, 0.90 for HCB and 0.21 ng for PAHs. Average blank levels for sediment samples were 0.40 to 0.99 ng for PCBs, 0.54 ng for HCB, and 1.22 ng and 2.45 ng for phenanthrene and pyrene, respectively. Surrogate standard recoveries for  $C_{\text{porewater}}$  were 59% PCBs, 83% HCB and 64% PAHs. For sediment samples the recoveries were 39% to 60 % for PCBs, 39% for HCB, and 64% and 74% for phenanthrene and pyrene, respectively.

## Microcosm Experiment

The experiment consisted of untreated sediments (control) and sediments spiked with chemical mixture ( $n = 5$ ) (spiked). Microcosms were prepared in 500 ml glass beakers with an inner diameter of 8 cm, by adding  $\sim 100$  g of spiked or control sediment per replicate to create a  $56 \text{ cm}^2$  layer of sediment  $\sim 3$  cm high. Microcosm jars were then filled up to the 400 mL marker with filtered brackish water collected at the Skvallran pristine sampling station (Figure 1). Meiofauna were isolated from an equivalent

volume of additional pristine sediment following the same method described in Nascimento et al. (2012), and added to each microcosm. To increase ecological relevance, 5 individuals of the amphipod *Monoporeia affinis* were added per microcosm. The collection of meiofauna, *Monoporeia* and diatoms are described in more detail in the **Supplementary Material** section.

Following the addition of both meiofauna and macrofauna individuals, each replicate received an input of food by adding 0.2  $\text{mg cm}^2$  of diatom bloom material (dominated by *Thalassiosira* sp.) with a pipette tip to the sediment surface (evenly spread over the entire surface area). Microcosms were supplied with gentle air bubbling and the experiment was started. It ran for a total of 5 weeks in a dark controlled climate room at 5°C. To assess microbial community structure, *PAH-RHD $\alpha$*  and *AmoA* gene expression over time, a total of 2 g of sediment from each experimental unit was sampled with a micro-spoon at 1, 3, and 5 weeks after the initial microcosm setup. Subsamples were taken from multiple random spots of each replicate and pooled into a 2 mL Eppendorf, thoroughly homogenized, and stored at  $-80^\circ\text{C}$  for later RNA extraction, qPCR, and 16S and 18S rRNA metabarcoding.

After 5 weeks, at the termination of the experiment, a small sediment sample ( $\sim 5$  g in a sterile tube) was collected from each replicate for potential nitrification rate measurements. Following this, meiofauna were isolated by quickly sieving the sediment using a 40  $\mu\text{m}$  mesh sieve, transferred to a screw-cap tube and further concentrated per replicate to a total volume of 10 mL. Collected meiofauna was then stored at  $-80^\circ\text{C}$  until RNA extraction.

Pooled samples from spiked sediments and control sediments were collected at termination for chemical analysis, and the samples were stored at  $-20^\circ\text{C}$  prior to analysis. The remaining sediment was sieved, *M. affinis* individuals were collected and counted. Oxygen conditions were stable during the duration of the experiment as indicated by the high survival rate of *M. affinis* (96.5%, **Supplementary Figure S4**), a hypoxia sensitive amphipod, in the control at the end of the experiment.

## RNA Extraction and cDNA Synthesis

From the collected meiofauna and the 1, 3, and 5 weeks subsampled sediment for bacterial community structure

analysis, a total of 2 g was transferred to a microcentrifuge tube. For the meiofauna suspension, a pipette with a cut-off tip was used. The procedure described below was followed for all samples. For total RNA extraction, the RNeasy PowerSoil (QIAGEN) kit was used with slight modifications to its provided instructions, namely, samples were mechanically lysed on a custom-made vortex adapter and incubation times following elution were increased to a minimum of 2 h. Following extraction, RNA was treated with DNase (TURBO DNA-free Kit, Invitrogen). To control for DNA carry-over, PCR using universal 16S primers (968F; 1401R, Felske et al., 1996) was performed on untreated RNA extractions for 35 cycles and showed no amplification through gel electrophoresis. RNA concentrations were normalized to 15 ng  $\mu\text{l}^{-1}$  and total RNA reverse transcription was carried out using the AccuScript First Strand Synthesis Kit (Agilent) on a GeneAmp PCR System (Applied Biosystems) following manufacturer's instructions. cDNA was then stored at  $-20^{\circ}\text{C}$  until metabarcoding library preparation.

## 16S and 18S rRNA Metabarcoding Library Preparation and Sequencing

Two libraries targeting the 16S and 18S ribosomal RNA gene on the synthesized cDNA were prepared, following the dual-index amplification methods adapted from Nascimento et al. (2018) and Andersson et al. (2008). The hypervariable variable region of the 16S and 18S rRNA marker was amplified using the 341F / 805R primer-pair (Herlemann et al., 2011) for bacteria and the TAREuk454FWD1/ TAREukREV3 primer-pair (Stoeck et al., 2010) for eukaryotes (Table 2). Thermocycler conditions for both the 16S and 18S primer-pairs in the first round were: 30 s at  $98^{\circ}\text{C}$ , followed by 12 cycles of 10 s at  $98^{\circ}\text{C}$ , 30 s at  $50^{\circ}\text{C}$ , 30 s at  $72^{\circ}\text{C}$ . All PCR reactions for the library preparation were carried out on a BioRad T100 Thermal Cycler (BioRad Laboratories) using Q5 HS High-Fidelity Master Mix (New England BioLabs) following the manufacturer's instructions. First round amplicons were cleaned by adding 0.1  $\mu\text{L}$  Exonuclease I (New England BioLabs) and 0.2  $\mu\text{L}$  Thermosensitive Alkaline Phosphatase (Promega), incubating for 15 min at  $37^{\circ}\text{C}$  followed by 15 min at  $74^{\circ}\text{C}$  to terminate the reaction. The second round of PCR was carried out using indexing primers as described by Andersson et al. (2008) to barcode each sample with a unique combination of forward and reverse index sequences avoid cross-contamination (Esling et al., 2015). Thermocycler conditions for the second round were 3 min at  $95^{\circ}\text{C}$ , 15 cycles of 30 s at  $95^{\circ}\text{C}$ , 30 s at  $55^{\circ}\text{C}$ , 30 s at  $72^{\circ}\text{C}$ , and a final elongation of 5 min at  $72^{\circ}\text{C}$ . The final barcoded amplification products were then cleaned using Agencourt AMPure XP magnetic beads (Beckman Coulter). Amplicon concentrations were measured using a Qubit 2.0 Fluorometer (dsDNA BR Assay Kit, Invitrogen), samples were then standardized for cDNA concentration, pooled, and sequenced on an Illumina MiSeq V3 system using a  $2 \times 300$  bp setup at the National Genomics Infrastructure in Stockholm, Sweden (NGI,

Stockholm). The sequences are available in the NCBI BioProject repository (PRJNA641540).

## Bioinformatics

Sequence reads were demultiplexed by the sequencing facility using `bcl2fastq_v2.20.0.422` from the CASAVA software suite. Further quality filtering and chimera removal was done in R (v3.5.2, R Core Team, 2014) using the DADA2 pipeline (Callahan et al., 2016). In more detail, forward and reverse paired-end reads were truncated at 290 and 210 bp, respectively, and trimmed at 8 bp using the following parameters: `truncLen = c(290,210)`, `maxEE = c(2,2)`, `trimLeft = c(8,8)`, `minFoldParentOverAbundance = 4`, and `allowoneoff = TRUE`. Taxonomic assignment for 16S rRNA amplicon sequence variants (ASVs) was carried out using the SILVA SSU database (r132) and the DECIPHER package (v 2.10.2, Wright et al., 2012). The ASVs generated by the DADA2 pipeline are higher-resolution alternative to the operational taxonomic units (OTUs) and report the number of times each amplicon is observed per sample (Callahan et al., 2017).

For eukaryotic ASVs, sequences were aligned against the NCBI NT (January 2019) database using BLAST (v2.7.0, Altschul et al., 1990) with an e-value threshold of 0.001. The output file was imported to MEGAN (v 6.14.2, Huson et al., 2016) that links NCBI NT association numbers with taxonomic classifications and uses the lowest common ancestor algorithm to further estimate taxonomic classifications. Sequences belonging to Thalassiosirophycidae, which accounted for the majority of diatoms that were added as food, were excluded from the dataset. Singletons were removed (ASVs occurring only once in the dataset) and the final read counts were normalized between samples as relative abundances.

Alpha diversities based on the 16S rRNA and 18S rRNA metabarcoding datasets were estimated using the Observed, ACE, and Shannon indices as implemented in the phyloseq package (v1.24.2; McMurdie and Holmes, 2013). The ACE index provides a rough estimate of abundance-based richness of ASVs and considers the abundance of rare taxa. The Shannon index accounts for both richness and evenness of ASVs present. A dissimilarity matrix was generated with the `vegdist` function of the `vegan` package (Oksanen, 2015) in R (v 3.4.2) using relative read counts with Bray-Curtis distances.

## Quantitative PCR

cDNA synthesized from each replicate was used for the gene expression quantification of *AmoA*, PAH-ring hydroxylating dioxygenase G-, and 16S rRNA. For this purpose, the primer sets *PAH-RHD GN F* and *PAH-RHD GN R*, R968, and F1401 and *amoA1F* and *amoA2R* were used (Table 2). The *PAH-RHD $\alpha$*  primer set was designed by Cébron et al. (2008) and targets a wider selection of PAH-degraders than other sets (Baldwin et al., 2003; Nyyssönen et al., 2006; Park and Crowley, 2006). This primer-set was shown to be highly specific toward the target bacteria and resulted in a clear single band on 1% agarose gels. Quantification of 16S and *PAH-RHD $\alpha$*  copy numbers were normalized through gBlocks gene fragments (IDT) at  $4.48 \times 10^6$

**TABLE 2** | List of primers used in the study.

Primer	Sequence (5' → 3')	Target	References
<i>PAH-RHD GN Fw</i>	GAGATGCATACCACGTKGGTTGGA	PAH-ring hydroxylating dioxygenase	Cébron et al., 2008
<i>PAH-RHD GN Rv</i>	AGCTGTTGTTCCGGGAAGAYWGTGCMGTT		
<i>968F</i>	AACGCGAAGAACCTTAC	Bacterial 16S rRNA	Felske et al., 1996
<i>1401R</i>	CGGTGTGTACAAGACCC		
<i>amoA 1F</i>	GGGGTTTCTACTGGTGGT	Ammonia mono-oxygenase	Rotthauwe et al., 1997
<i>amoA 2R</i>	CCCCTCKGSAAAGCCTTCTTC		
<i>341F</i>	CCTACGGGNGGCWGCAG	Bacterial 16S rRNA	Herlemann et al., 2011
<i>805R</i>	GGACTACHVGGGTWTCTAAT		
<i>TAReuk454FWD1</i>	CCAGCASCYCGGTAATTCC	Eukaryote 18S rRNA	Stoeck et al., 2010
<i>TAReukREV3</i>	ACTTTCGTTCTTGATYRA		
<i>PCR1 Fw adapter</i>	AGACTCTTCCCTACACGACGCTCTCCGATCT NNNNN		
<i>PCR1 Rv adapter</i>	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCT		

and  $4.70 \times 10^6$  copies  $\mu\text{L}^{-1}$ , respectively. Quantitative PCR was then carried out in duplicate, on a Roche LightCycler 480 II Real-Time PCR System in reaction volumes of 20  $\mu\text{L}$ , containing SYBR Green I Master Mix (Roche), 0.10  $\mu\text{M}$  of each primer, and 1  $\mu\text{L}$  of template at approximately 5 ng  $\mu\text{L}^{-1}$  DNA. Thermocycler conditions were set to 95°C for 10 min as an initial denaturation step, followed by 40 cycles of 95°C for 20 s, 55°C for 20 s annealing, and 72°C for 15 s, followed by a final continuous melt curve analysis step at 60°C. Standard curves for each primer set were generated in duplicates using gBlocks Gene Fragments (IDT) at concentrations of  $5 \times 10^8$ – $5 \times 10^1$ . Copy numbers and melting curves were validated through a standard curve for each primer set and the amplification efficiency of each run ( $1.95 \geq 2$ ) using the LC480 software. Data analysis was carried out using the LightCycler 480 device software (v1.5.1) and converted to Excel for further analysis and visualization. Gene copy numbers for *PAH-RHD $\alpha$*  and *AmoA* were normalized against 16S cDNA amplification data for each sample.

## Potential Nitrification Rates

Potential nitrification rates (PNR) were measured by  $\text{NO}_3^-$  production in  $\text{NH}_4^+$  amended sediment slurries at *in situ* temperature (5°C) with shaking as previously described by Bonaglia et al. (2014b). This was done by creating slurries and transferring 2 g of sediment from each replicate to a 50 mL centrifuge tube after the termination of the experiment using a cut-off syringe. This was followed by adding 40 mL of filtered *in situ* bottom water using a disposable 0.2  $\mu\text{m}$  filter. All slurries were then amended with a stock solution of 20 mM  $\text{NH}_4\text{Cl}$  to a final concentration of 100  $\mu\text{M}$   $\text{NH}_4^+$  and incubated in the dark for 15 min on a shaker table at *in situ* temperature. Subsamples of 15 mL were then taken after 15 min (T0), 24 h (T1), and 48 h (T2) for analysis of dissolved inorganic nitrogen (DIN, i.e.,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$ ). DIN was determined on a segmented flow AutoAnalyzer system (ALPKEM, Flow Solution IV). The precision was  $\pm 0.036 \mu\text{mol L}^{-1}$  for  $\text{NH}_4^+$ ,  $\pm 0.021 \mu\text{mol L}^{-1}$  for  $\text{NO}_3^-$ , and  $\pm 0.014 \mu\text{mol L}^{-1}$  for  $\text{NO}_2^-$ – $\text{NO}_3^-$ . The concentrations in each slurry were

plotted against the sampling time, and individual slopes of the linear regression were used to calculate the net PNR according to Caffrey et al. (2019).

## Statistical Analysis

Repeated measures ANOVA was used to test the effects of the chemical mixture (i.e., treatment), time and interaction of the two factors on the alpha-diversity metrics for bacteria, more specifically ACE (Abundance-based Coverage Estimate) and Shannon diversity indices. In the cases where time and the interaction of time and treatment had significant effects, one-way ANOVA was used to test the effect of treatment on diversity at each time point. The differences in bacterial community composition between the treatments were visualized using Non-metric Multidimensional Scaling (NMDS) ordination based on Bray-Curtis dissimilarity matrix. Effects of the contaminant mixture (treatment), time and their interaction on bacterial community structure were analyzed using repeated measures PERMANOVA test with PERMANOVA+ package in PRIMERE (v6) (Clarke et al., 2006). As meiofauna were collected only at the end of the experiment, differences in alpha diversity and community structures for the 18S dataset were tested with One Way ANOVA. PERMANOVA was used to test the effects of treatment on the community composition with the *adonis* function of the vegan package (Oksanen, 2015) in R (v 3.4.2). Best-fit analyses based on the Bray-Curtis dissimilarity index and Spearman correlations were conducted using the BIO-ENV method (Clarke and Ainsworth, 1993). This made it possible to get an indication of the best subset of taxa that changed in relative abundance in response to the addition the contaminant mixture. Differences in *AmoA* and *PAH-RHD $\alpha$*  gene abundances and nitrification rates were tested using repeated measures ANOVA. Prior to all ANOVA tests, the assumptions of normality of distribution was tested with Shapiro Wilk tests and homogeneity of variances with the Levene test. Differences in relative abundances of the top 20 bacterial and eukaryotic taxa between treatments at the phylum and genus level were tested with Kruskal-Wallis non-parametric test as relative abundances were not normally distributed. The 'betadisper' function in the

R vegan package was used to test for differences in multivariate homogeneity of beta diversity variance between treatments.

## RESULTS

### Exposure Concentrations of the Contaminant Mixture in Sediment

The concentration of each single contaminant in sediment porewater ( $C_{\text{porewater}}$ ) of the spiked sediment was 0.11 to 1.15  $\mu\text{g L}^{-1}$ , corresponding to chemical activities of 0.00004 to 0.01 and a total chemical activity of the mixture of 0.024 (Table 1). The total exposure of the contaminant mixture used in this study is similar to what has previously been observed in contaminated areas in the Baltic Sea (Löf et al., 2016), but is higher than at National monitoring stations in background areas (Swedish Geology Survey, apps.sgu.se/kartvisare). The exposure level is a tradeoff between having a mixture with a high enough chemical activity (i.e., 0.01 to 0.1 according to literature) that causes non-specific toxicity, alongside the concentration of each mixture-constituent below any concentration threshold for specific toxicity. The chemical analyses of the sediment showed a loss of chemicals during spiking (nominal spiked versus measured concentrations prior experiment), and during the experiment (measured concentrations prior and after the experiment). At the end of the experiment less than 15% of PCBs, HCB and pyrene, and 40% of phenanthrene were retained compared to the measured concentrations prior experiment (Table 1). Biphenyl was no longer detected at the end of the experiment. Despite the loss of chemicals during the spiking and the experiment, the concentrations in the spiked sediment were high compared to control. In control sediment, all chemicals were below detection limits (Table 1). The loss of chemicals during the experiment is likely due to the chemicals not reaching equilibrium with the sediment during spiking, which in turn, likely is an effect of the high mass of sediment ( $\sim 800$  g) per jar.

### Amplicon Sequencing Output

Amplicon sequencing yielded a total of 3,000,277 and 1,127,063 reads after quality trimming and filtering, with an average of 32,063 and 112,706 sequences per sample for the 16S rRNA and 18S rRNA marker, respectively. After removing singletons, a total of 5,369 bacterial ASVs and 4,543 eukaryotic ASVs remained.

### Bacterial Diversity and Community Structure

Of the total number of ASVs from all samples, the majority belonged to the *Proteobacteria* ( $71.4 \pm 5.5\%$ , mean  $\pm$  one standard deviation), *Bacteroidetes* ( $9.65 \pm 2.6\%$ ), *Actinobacteria* ( $5.74 \pm 1.65\%$ ) and *Acidobacteria* phyla ( $3.43 \pm 1.4\%$ ) (Supplementary Figure S1A). Within the *Proteobacteria* phylum, ASVs belonged mainly to the classes *Gammaproteobacteria* ( $51 \pm 9\%$ ) and *Deltaproteobacteria* ( $15 \pm 3\%$ ). The top 20 genera with the highest relative abundance belonged to 17 distinct families (Figure 2). The relative abundance of *Methylophagaceae* was notably high in samples

across all timepoints, averaging at  $23 \pm 14\%$  and  $26 \pm 16\%$  in control and spiked sediments, respectively. *Desulfobacteraceae* followed with  $11 \pm 1\%$  in control and  $10.4 \pm 1.8\%$  in spiked and *Ilumatobacteraceae* with  $5.4 \pm 0.5\%$  and  $6.4 \pm 1.6\%$ , respectively.

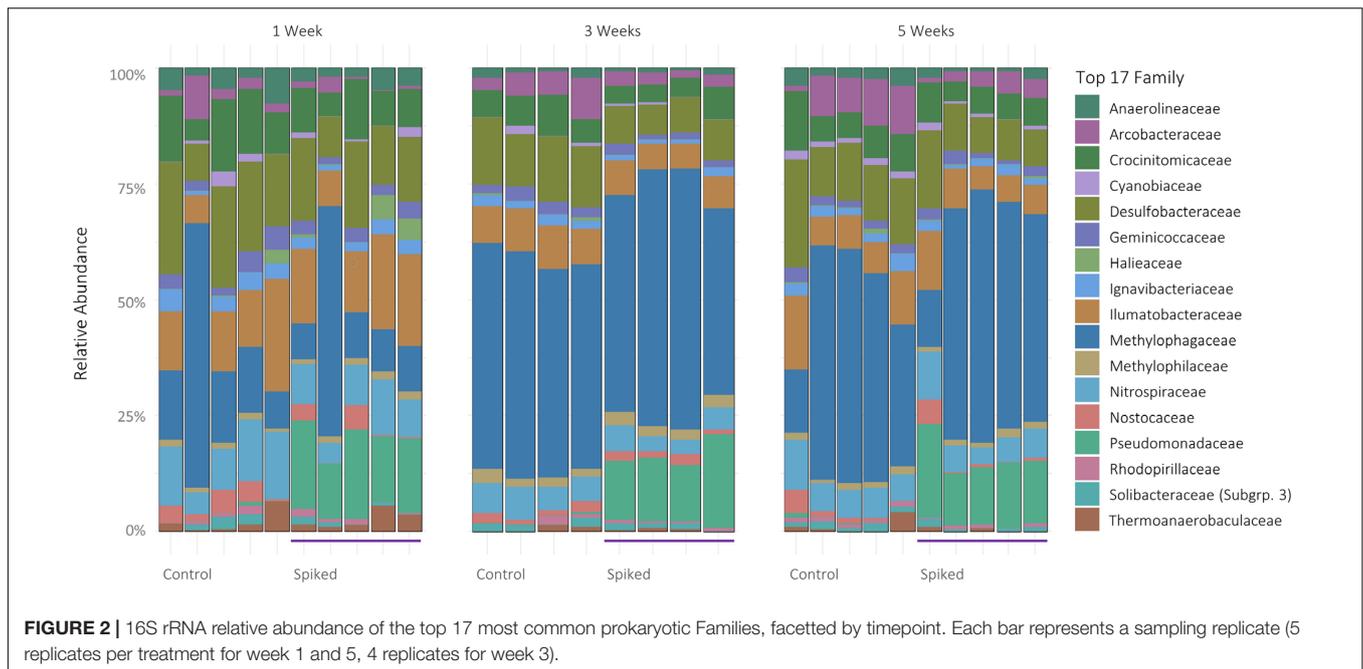
Focusing on the changes between treatments, our results show that the relative abundance of *Gammaproteobacteria* increased in the spiked sediment after 5 weeks of incubation (week 1,  $41 \pm 8\%$ ; compared to week 5,  $53 \pm 11\%$ ;  $n = 5$  for both weeks, Kruskal-Wallis test,  $df = 1$ ,  $H = 6.91$ ,  $p = 0.031$ ) (Supplementary Figure S1B). Notably, *Pseudomonadaceae* were significantly higher in the spiked treatment ( $5.2 \pm 0.05\%$  *Pseudomonas* spp.,  $1.4 \pm 0.02\%$  other unclassified *Pseudomonadaceae*) when compared to control ( $0.06 \pm 0.01\%$  and  $0.02 \pm 0.01\%$ , respectively) ( $n = 5$ , KW,  $p < 0.001$ ,  $df = 1$ ,  $H = 21.2$ ) across all timepoints. Finally, averaged over all timepoints, the relative abundances of *Desulfobacteraceae*, *Crocinitomicaceae*, and *Anaerolineaceae*, were significantly lower in the spiked sediment (KW,  $p < 0.05$ ,  $df = 1$ ,  $H = 13.8$ ,  $6.8$ ,  $7.4$ , respectively). The bio.env analysis indicated that changes in *Pseudomonas* and *Desulfobacteraceae* (*Desulfobacterales*) best explained the differences between treatments in community structure (Spearman's rho 0.84; Figure 3).

There was no effect of treatment on alpha diversity of bacterial communities calculated by ACE or Shannon indices (repeated measures ANOVA:  $F_{1,9} = 0.66$ ,  $p = 0.44$  and  $F_{1,9} = 0.32$ ,  $p = 0.59$ , respectively) (Supplementary Figure S2). On the other hand, there was an effect of treatment on the bacterial community structure (repeated measures PERMANOVA:  $\text{pseudo}F_{1,9} = 4.68$ ,  $p = 0.008$ ). The NMDS ordination on bacterial ASVs showed that the majority of the sampling sites formed two significantly different clusters; one for control and one for the spiked replicates (Figure 4). Additionally, repeated measure PERMANOVA showed a significant difference of time on the bacterial community structure ( $\text{pseudo}F_{1,9} = 4.4$ ,  $p = 0.0026$ ) (Figure 4).

### Microeukaryotic Diversity and Community Structure

Across all samples, *Ciliophora* (average read abundance  $30.2 \pm 11\%$ ), *Metazoa* ( $24.3 \pm 6\%$ ) and *Bacillariophyta* ( $8 \pm 2\%$ ) were the most represented taxa (Figure 5A). Zooming in the differences among treatments at the class level, our results show that the relative abundance of diatoms (*Bacillariophyta*) was higher in controls compared to spiked sediment (KW,  $p = 0.009$ ,  $df = 1$ ,  $H = 6.8$ ). Conversely, ciliates were found at a lower relative abundance in the controls compared to spiked sediments ( $21 \pm 1.2\%$ ,  $27 \pm 3.3\%$ , respectively), with the class *Prostomatea* being dominant in this treatment (KW,  $p = 0.021$ ,  $df = 1$ ,  $H = 0.02$ ).

For meiofauna, the relative abundance of nematodes was significantly higher in spiked replicates ( $53 \pm 14\%$ ) when compared to control ( $22 \pm 8\%$ ; Figure 5). There was a significant increase in relative abundance amongst taxa in the *Leptolaimidae* family (KW,  $p = 0.02$ ,  $df = 1$ ,  $H = 5.12$ ) and. Particularly, *Leptolaimus* spp. showed a significant increase in abundance in spiked sediments (KW,  $p = 0.013$ ,  $df = 1$ ,  $H = 6.14$ ), whereas *Paraplectonema*, *Adoncholaimus*, and



**FIGURE 2 |** 16S rRNA relative abundance of the top 17 most common prokaryotic Families, faceted by timepoint. Each bar represents a sampling replicate (5 replicates per treatment for week 1 and 5, 4 replicates for week 3).

*Bathylaimus* spp. showed a decrease (KW,  $p = 0.046$ ,  $df = 1$ ,  $H = 5.12$ ;  $p = 0.05$ ,  $H = 3.7$ , and  $p = 0.027$ ,  $H = 4.8$ , respectively). The bio.env analysis of the top 30 reported metazoa, showed that changes in *Leptolaimus* and Microlaimidae relative abundance explained most of the variation between treatments (Spearman's  $\rho = 0.72$ ). Similar to the 16S rRNA dataset, eukaryotic alpha diversity was not affected by the chemical mixture (One Way ANOVA,  $F_{1,9} = 0.5425$ ,  $p = 0.48$ ) (Supplementary Figure S3), but there was a significant effect on the microeukaryotic community structure (PERMANOVA, pseudo  $F_{1,9} = 2.77$ ,  $p = 0.009$ ). An NMDS plot based on relative abundances for the 18S rRNA showed separate clustering between the controls and spiked sediment (Figure 6). A similar pattern was found using presence/absence matrix and Sørensen differences (Supplementary Figure S5, PERMANOVA, pseudo  $F_{1,9} = 2.57$ ,  $p = 0.03$ ).

### Quantification of PAH-RHD and *AmoA* cDNA

*AmoA* expression levels did not show any significant changes between treatment and timepoints (repeated measures ANOVA,  $F_{1,9} = 0.55$ ,  $p = 0.46$  and  $F_{1,9} = 2.35$ ,  $p = 0.12$ , respectively) (Figure 7). However, there was a significant difference between treatments in the expression levels of the *PAH-RHD* gene (repeated measures ANOVA,  $F_{1,9} = 42.98$ ,  $p < 0.0001$ ). It was detected in spiked sediment samples from all three timepoints but remained under detection limit in the control samples (Figure 7).

### Potential Nitrification Rates

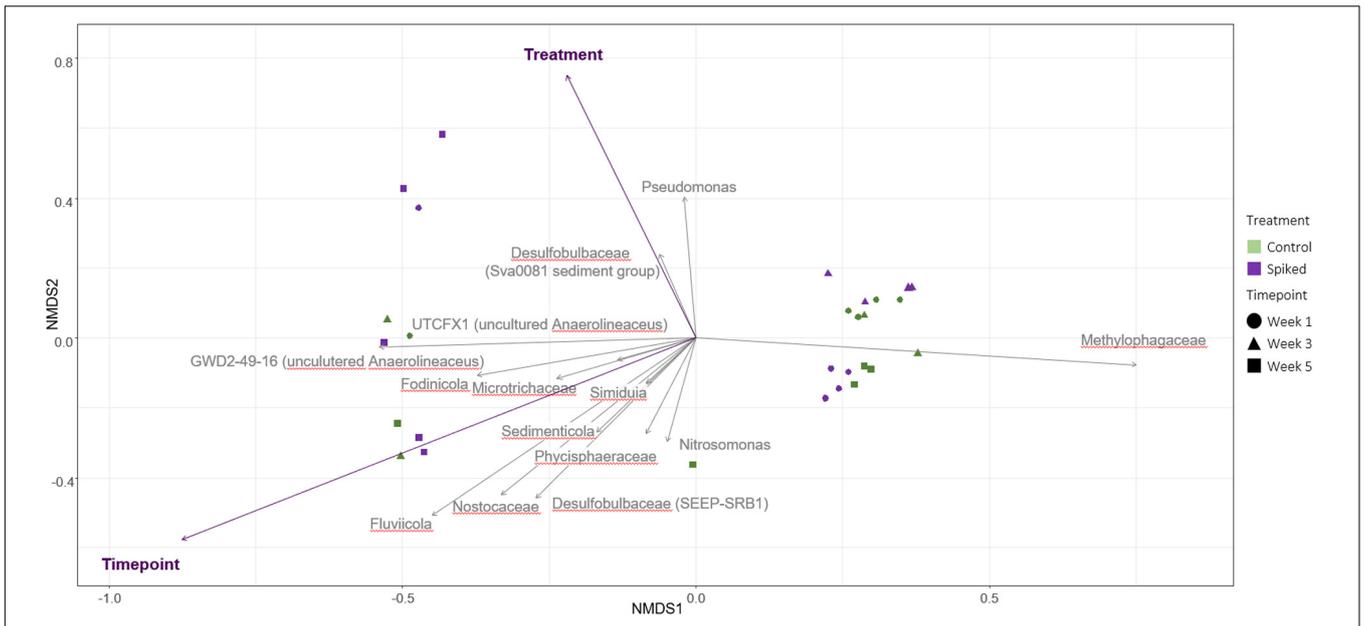
All incubated sediment slurries showed linear nitrate production with time (with  $R^2$  ranging between 0.87–0.98). Average potential nitrification rates (PNR) calculated

from these slopes were  $21.1 \pm 4.6 \text{ nmol cm}^{-3} \text{ d}^{-1}$  in control and  $23.5 \pm 1.5 \text{ nmol cm}^{-3} \text{ d}^{-1}$  in spiked treatment. Exposure to the contaminant mixture did not significantly affect PNR at the end of the experiment (Figure 8, ANOVA,  $F_{1,9} = 1.26$ ;  $p = 0.293$ ). However, average PNR was lower in the controls compared to spiked treatment.

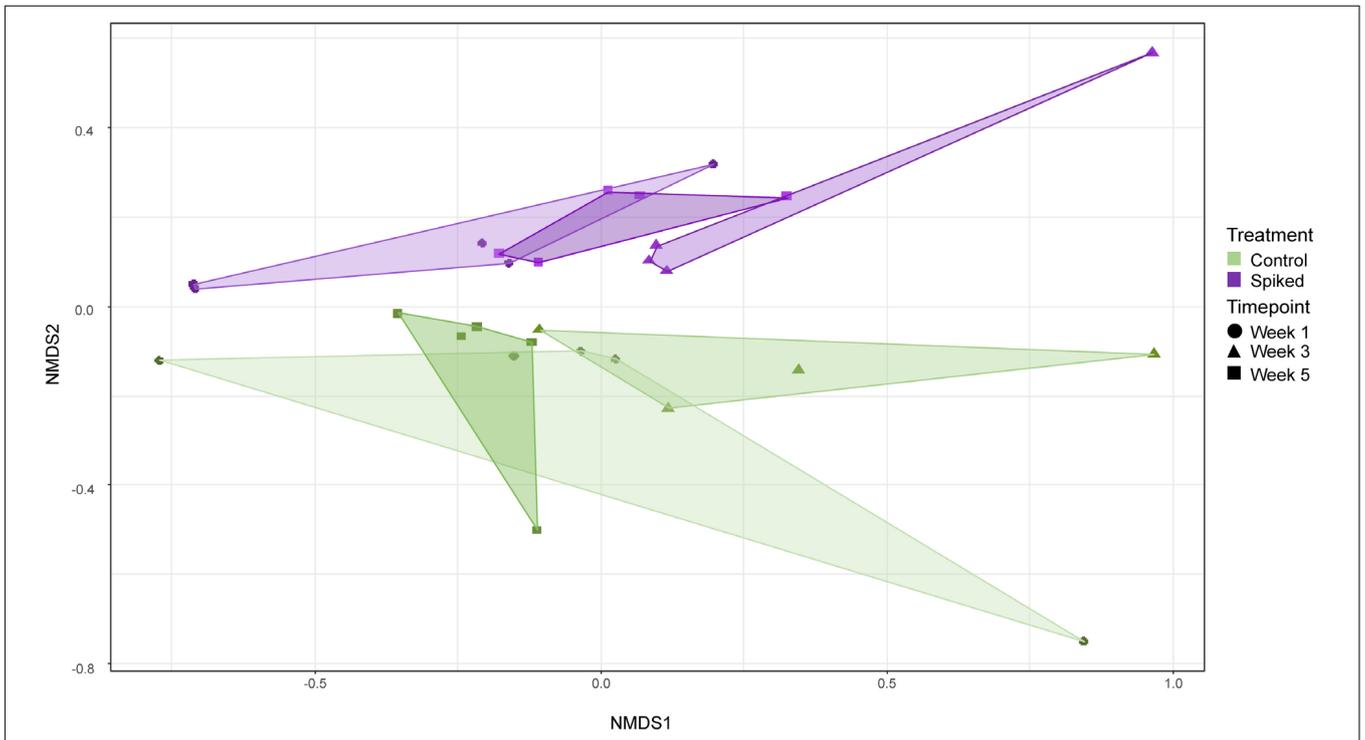
## DISCUSSION

### 16S rRNA Microbial Community Structure Analysis

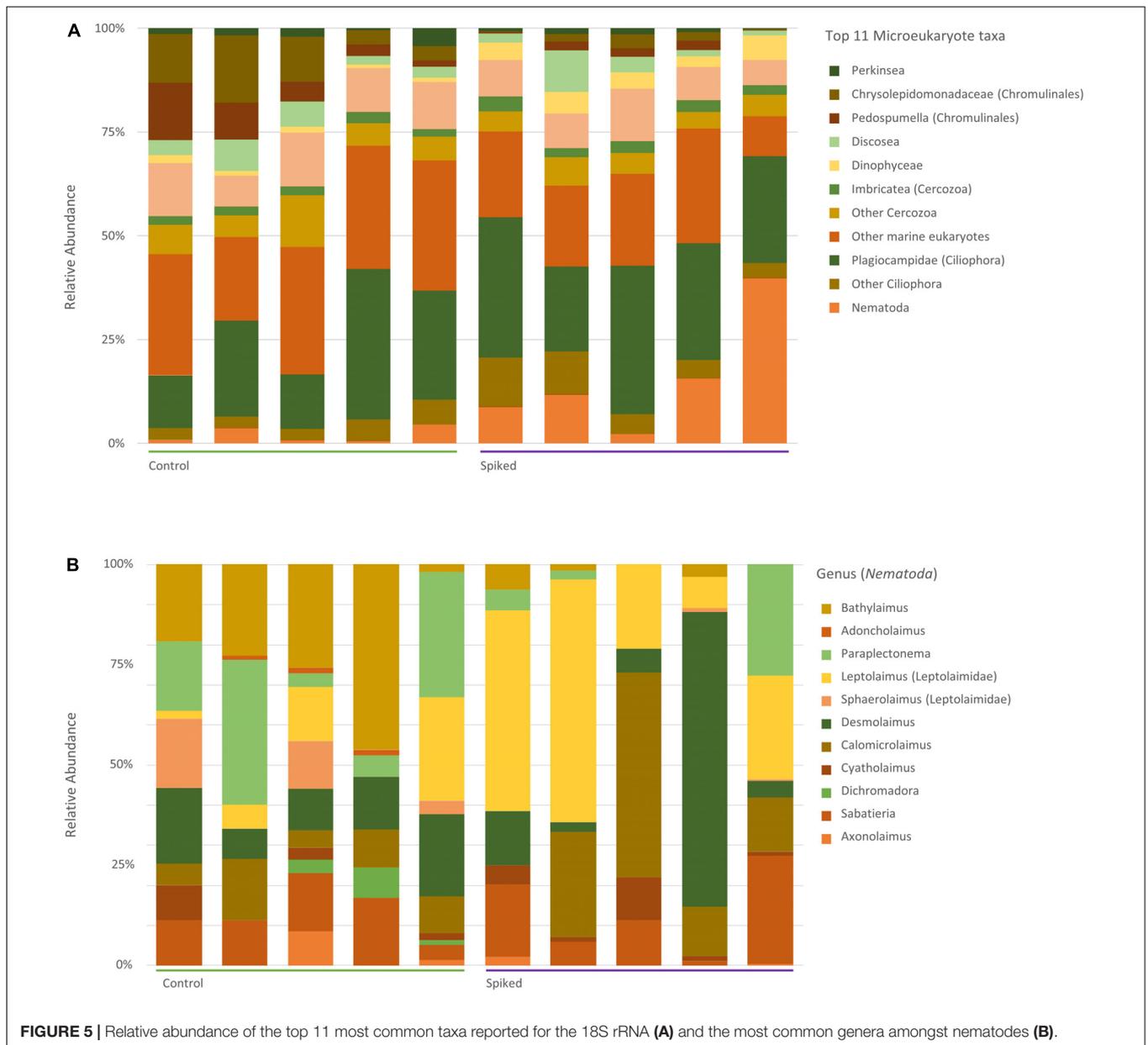
Our results showed that there was a notable shift in the relative abundance of several microbial taxa in spiked sediments. *Pseudomonas* (amongst the Gammaproteobacteria) showed a clear increase in relative abundance in treated sediments (from  $0.23 \pm 0.13\%$  to  $11 \pm 2.7\%$ ). Species belonging to the *Pseudomonas* genus have regularly been found at various contaminated sites (Ridgway et al., 1990; Aitken et al., 1998; Romero et al., 1998) and shown to be able to degrade a variety of PAHs (amongst other contaminants) in previous pure-culture studies (Müller et al., 1999; Rockne et al., 2000; Wasi et al., 2011). This increase in abundance of PAH-degrading taxa was again visible from the high PAH-dioxygenase expression levels measured in the contaminated sediments. Active PAH degradation, degradation pathways of biphenyl, PCB and other halogenated aromatic degradation, by *Pseudomonas* spp. has been widely reported (Hofer et al., 1993; Commandeur and Parsons, 1994), even under aerobic conditions (Abramowicz, 1990). This action by *Pseudomonas* spp. could partially explain the lower contaminant levels measured at the end of our experiment. Several strains of *Pseudomonas* have been



**FIGURE 3 |** NMDS of the 16S rRNA data with Bray-Curtis distance, based on data for all timepoints. The overlying triplot shows the results of the bio.env analysis. The circles denote control replicates (green) and sediment spiked with the chemical mixture (purple), (stress = 0.07, Spearman's rho = 0.84).

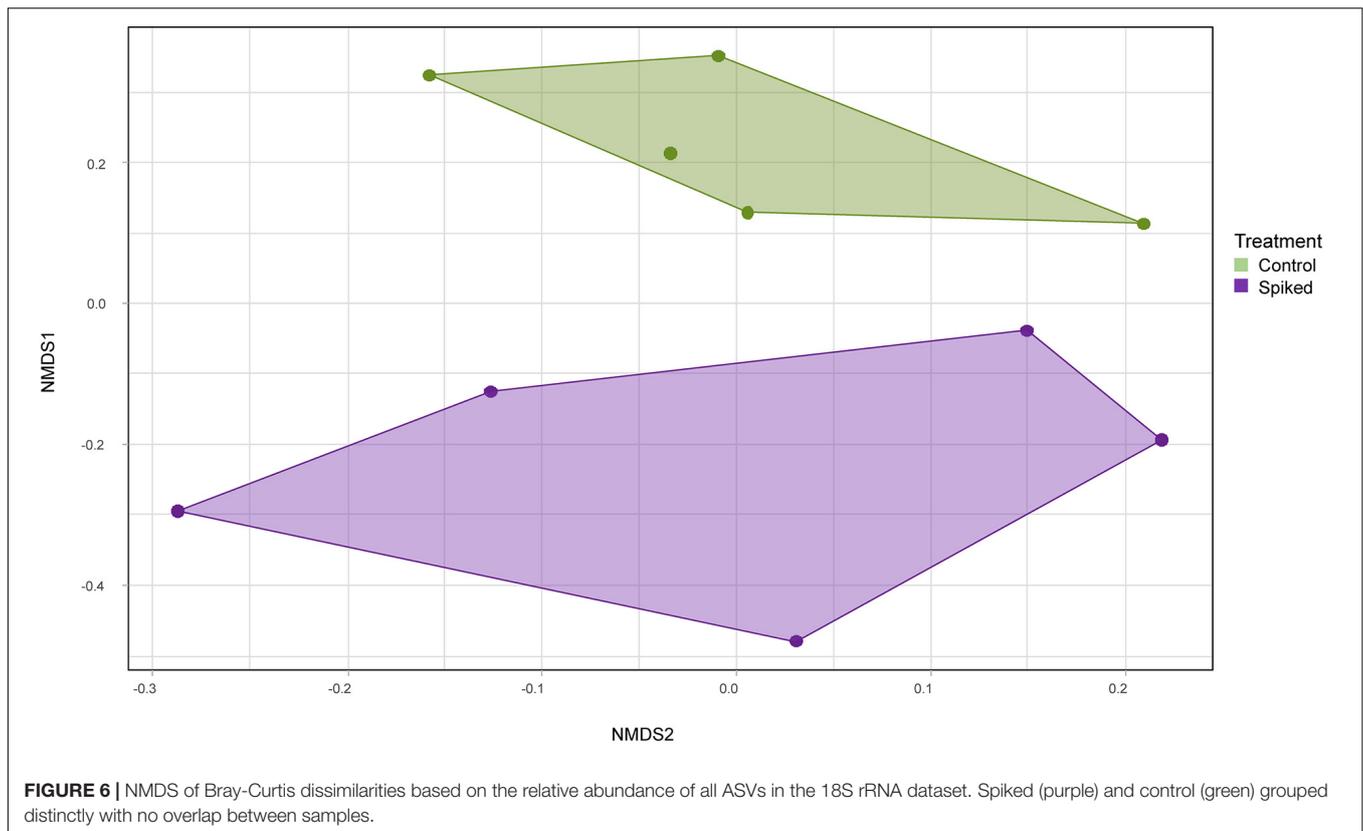


**FIGURE 4 |** NMDS of Bray-Curtis dissimilarities based on the relative abundance of all ASVs classified as bacteria (stress = 0.02). Different colors represent the two experimental treatments (with green denoting controls, and purple sediment spiked with the chemical mixture). The shaped points denote samples taken in the different timepoints.



suggested as viable candidates for use in bioremediation or bioaugmentation scenarios (Wasi et al., 2013; Kahlon, 2016; Huang et al., 2017; Liu et al., 2017). The high *Pseudomonas* relative abundance as well as *PAH-RHD* gene expression in our contaminated microcosms further suggests its viability in such applications. Additionally, Epsilonproteobacteria, particularly Helicobacteraceae, have been reported to increase in relative abundance in sediments contaminated with a complex mixture of PCBs, PAHs and heavy metals (Quero et al., 2015), but contrary to *Pseudomonas*, Heliobacteria do not seem to be able to metabolize PAHs or PCBs. They appear to be abundant in contaminated sediments, but not essential for the degradation of organic contaminants (Starke et al., 2016). Obi et al. (2016) suggested that Epsilonproteobacteria

may be syntrophs of potential PAH and PCB metabolizing species. Nevertheless, the most common Epsilonproteobacteria in our spiked sediment, Arcobacteraceae, did not increase but in fact significantly decreased in relative abundance in the spiked treatment when compared to the controls. Alongside *Pseudomonas*, Desulfobacteraceae has also been associated with anaerobic degradation of naphthalene (Kümmel et al., 2015; Bonaglia et al., 2020). Accordingly, in our study the genus *Sva0081* of the Desulfobacteraceae family was shown by the bio.env analysis to drive an important portion of the differences between treatments in microbial community composition. This minor shift in our findings may be due to the well-aerated conditions, partially aided by the *M. affinis* (Karlson et al., 2010) in our microcosms,

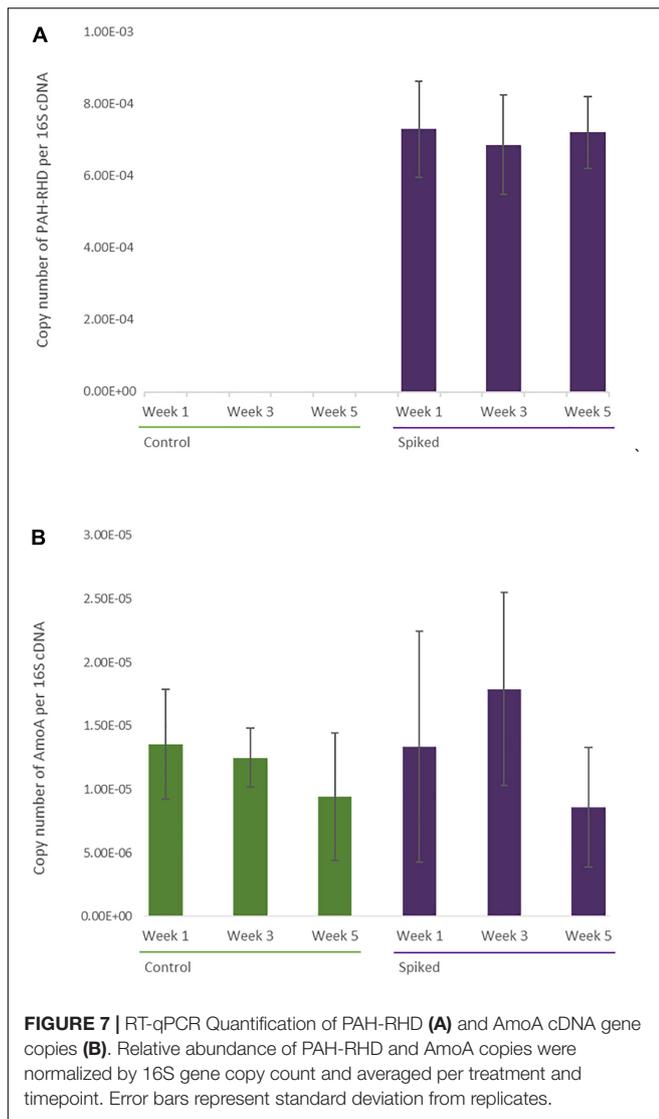


that tends to favor aerobic degraders like *Pseudomonas* (Lu et al., 2011).

### Microeukaryotic Community Structure Analysis

Among the microeukaryotes, nematodes and ciliates, with the majority belonging to the Chromadorea class and Plagiocampidae family, respectively, were found in higher relative abundances in the contaminated sediments compared to the controls. The relative abundances of a number of taxa at the family level within *Nematoda* (Desmodoridae, Monhysteridae, Plectidae, and Araeolaimidae) did not differ significantly between control and spiked sediments. The lack of response of benthic metazoans to exposure to organic contaminants contrasts with previous results reported by Bik et al. (2012) that found nematodes to decrease in relative abundance in response to the Deep-Water Horizon Oil spill. The exposure of nematodes to organic contaminants in this study was not measured, but it is likely to have been much higher than what was seen in our experimental sediments, providing a possible explanation for the contrasting responses. Nevertheless, at the genus level we found in our study important shifts in some taxa, namely *Leptolaimus* and *Bathylaimus*, and to a lesser extent, *Paraplectonema*, showed a higher relative abundance in spiked sediments, indicating that these nematode taxa were tolerant to non-specific toxicity of contaminant mixture at the levels here tested. The striking dominance of *Leptolaimus* in our sediments contaminated with PCBs/PAHs

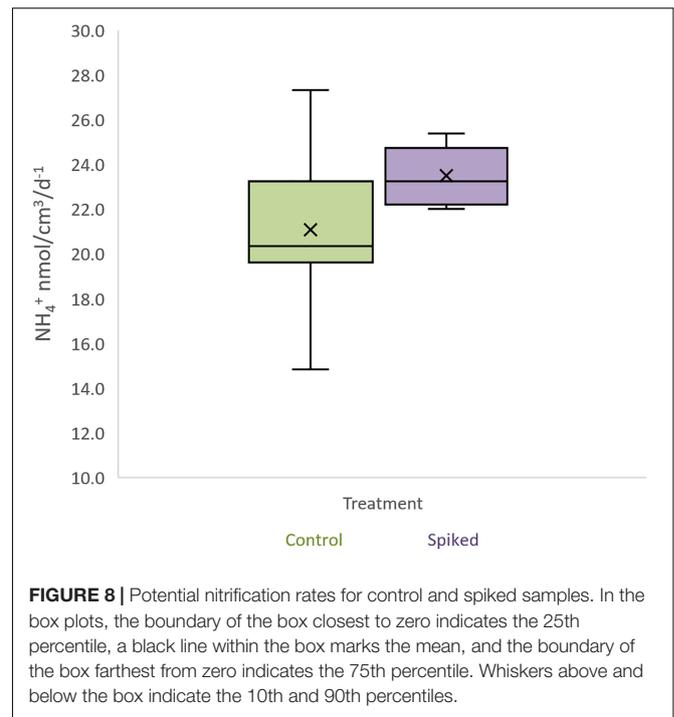
detected with metabarcoding, is in accordance with previous morphological studies by Wang et al. (2009) and Moreno et al. (2008a), despite the different methods utilized. From a wide range of contaminants tested, Moreno et al. (2008a) found *Leptolaimus* to only be present in highly contaminated areas where PAH content measured up to  $\sim 145 \text{ mg kg}^{-1}$ . Moreover, *Leptolaimus* appears to be particularly resilient toward a wider variety of environmental stressors, from physical disturbances (Schratzberger and Warwick, 1998), hypoxia/anoxia (Modig and Ólafsson, 1998) to heavy metals (Somerfield et al., 1994; Moreno et al., 2008b) and other organic contaminants (Moreno et al., 2008a; Wang et al., 2009). As such, their general resilience might allow them to avoid food competitors and, in this case, thrive in relatively toxic shallow sediment. Nematodes of the genus *Sabatieria* have also been found in our study to be either unaffected by the exposure to contaminated sediment or to even increase in relative abundance therein (Louati et al., 2015). They have been reported for their tolerance to petrochemical and heavy metal contamination (Hedfi et al., 2007; Beyrem et al., 2010) or high levels of PAHs. The PAH/PCB content in the sediment most likely exerted a negative pressure on potential food competitors, benefitting the more tolerant and opportunistic feeders. As in most studies using high throughput DNA sequencing to investigate changes in bacterial communities our data is compositional, presenting data in relative abundances. Detecting changes in microbial communities with compositional data has been shown to present a number of important drawbacks (Morton et al.,



2019). Alternative methods of normalization like log-ratio or reference frames (Gloor et al., 2017; Quinn et al., 2019) should be considered in the future to facilitate the detection of reproducible changes of microbial communities from compositional data.

### Potential Nitrification Rates (PNR) and Expression of Ammonia Monooxygenase (AmoA) and PAH-Dioxygenase (PAH-RHD) Marker Genes

PNR rates were several orders of magnitude lower when compared to those found in previous works in the Baltic Sea (Bonaglia et al., 2014a; Caffrey et al., 2019). Our rates ranged only between 14.8 and 27.3 nmol N mL<sup>-1</sup> d<sup>-1</sup>, while the cited studies reported rates ranged between 283.2–640.8 nmol N mL<sup>-1</sup> d<sup>-1</sup> (Bonaglia et al., 2014a) and 108–1154 nmol N mL<sup>-1</sup> h<sup>-1</sup> (Caffrey et al., 2019). The



lower nitrification rates found in our study compared to the enormous ones reported by Caffrey and co-authors were likely due to differences in temperature between our experimental conditions (5 °C) and the referenced study (room temperature, approximately 21 °C). Additionally, the manipulation of our sediments might have contributed to such differences. Our microcosms were well aerated, and the sieving of the sediment prior to the microcosm setup might have resulted in substrate limitation and consequent lower nitrifying activity (Bernhard and Bollmann, 2010). Expression of the bacterial ammonia monooxygenase marker gene (*AmoA*) measured through qPCR and PNR did not show a significant change between contaminated and control sediment. However, although not statistically significant, both *AmoA* expression levels and PNR-rates were indicative of a net increase of nitrification activity in spiked sediment. This appears to conflict with recent work by Lindgren et al., 2014, 2012, which showed a reduced capability for nitrification as a response to PAH exposure. While many *Pseudomonas* members (Gammaproteobacteria) are known denitrifiers, some species (e.g., *P. stutzeri*, *P. tolaasii*, *P. aeruginosa*) exhibit heterotrophic nitrification capabilities (i.e., He et al., 2015). Paired with the high expression of PAH-RHD, their nitrifying activity, consequentially, may have been inhibited. In addition, our experimental setup only allowed us to assess effects on PNR at the end of the experiment. It is possible that exposure to contaminants had an initial effect on this benthic process that was not detectable at the termination of our experiment. Nevertheless, our results suggest that such a potential negative effect on nitrification was not present 5 weeks after exposure. Future work should aim to investigate such

temporal dynamics of effects of non-specific toxicity on benthic ecosystem processes.

## CONCLUSION

Our results reveal a complex response of benthic interactions to non-specific toxicity of organic contaminant mixtures and calls for further research with a broader range of chronic chemical exposure to identify potential taxa that can serve as bioindicators of non-specific toxicity. Nevertheless, we report on effects of non-specific toxicity of organic contaminant mixtures at a community level of biological organization for both bacteria and microeukaryotes. We found that the tested levels of the chemical mixture can significantly decrease the relative abundance of sensitive taxa like *Paraplectonema* and increase the dominance of resistant nematodes like *Leptolaimus*. In addition, our results indicate a functional response of bacterial communities with the levels of microbial PAH-degradation gene expression increasing in spiked microcosms aligned clearly with a significant rise in the relative abundance of active *Pseudomonas* genus. Furthermore, our study indicates that utilizing metabarcoding, next-generation sequencing and quantitative methods on RNA gene expression has shown to be an effective approach to assess effects of disturbance on active meiofauna and microbial community structures in experiments with a short time frame. As (e)DNA-based metabarcoding has become a versatile and robust method for community-wide studies and assessing biodiversity, using eRNA can provide additional insights to how benthic communities can respond to environmental changes over a short period of time.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI BioProject, accession no: PRJNA641540.

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## AUTHOR CONTRIBUTIONS

SI performed the experiment, molecular analysis and bioinformatics, and drafted the manuscripts. IN performed the sediment spiking and chemical analysis, and provided feedback on the manuscript. SB performed biogeochemistry and data analysis, and provided feedback on the manuscript. AK and AS helped to design the experiment and provided feedback on the manuscript. FN designed the experiment, finances and coordinated the project and helped draft the manuscript. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2020.00128/full#supplementary-material>

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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