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Persistent organic pollutants, metals, and the bacterial community composition associated with microplastics in Muskegon Lake (MI)

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ABSTRACT

Three different types of microplastics (MPs): low density polyethylene; polypropylene; and polyester, were incubated for one and three months at two sites (Channel and Lake) in Muskegon Lake (MI). After retrieval, MPs were analyzed for polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCs), select metals, and genomic characterization of the attached microbial biofilm. Polyaromatic hydrocarbons accumulated at similar levels on all microplastic types, but were ~one order of magnitude lower than sediment concentrations and ~three orders of magnitude lower than the Probable Effect Concentration (PEC): the concentration in sediment above which adverse biological effects are likely to occur. In contrast, PCB levels were 4–6× greater on polyethylene than on polypropylene or polyester, although all plastic-associated PCB concentrations were at least one order of magnitude less than the PEC. Organochlorine pesticide concentrations were very low (<1 ppb) on all microplastics and at all sites. Metal concentrations also were well below their respective PEC levels. The most abundant bacterial groups as part of the plastisphere were Burkholderiales, Rhodocyclaceae, Comamonadaceae, and Pseudomonadaceae. Polyester microplastics contained a higher number of bacterial families and the relative abundance of those families were more evenly distributed compared to the other plastic types. Overall, our results indicate that persistent organic pollutants are capable of accumulating on MPs but the concentrations generally were low; feeding trials are needed to determine if these environmentally realistic concentrations of pollutants attached to microplastics result in impacts to aquatic biota.

Introduction

The recent focus on microplastics (MPs), which are operationally defined as <5 mm in size (Driedger et al., 2015), in aquatic ecosystems can be characterized by several features, including a rapid increase in the number of published studies (Franzellitti et al., 2019), as well as a focus primarily on MP characterization (Burns and Boxall, 2018). Other commonalities among these studies include a significant lag in freshwater compared to marine studies, although the former are becoming more common (Wagner et al., 2014) and that many experimental studies use environmentally unrealistic concentrations; therefore, applying those results to the real world is problematic (Lenz et al., 2016; Phuong et al., 2016). One additional aspect of MP studies, and perhaps the most significant of all, is the lack of standardized methods in the analysis of MPs (cf. Burns and Boxall, 2018).

The Great Lakes are threatened by a variety of stressors, which vary in magnitude across time and space (Allan et al., 2013; Smith et al., 2015, 2019). Many of these stressors are well documented, such as invasive species, toxic pollutants, and excess nutrients leading to harmful algal blooms. Other stressors, such as plastic debris, are emerging issues. Hoffman and Hittinger (2017) estimate almost 10,000 metric tons of plastic debris enter the Great Lakes each year. A survey of 29 tributaries throughout the Great Lakes revealed that fibers were the most common type of microplastic (Baldwin et al., 2016); but in the open waters of the Great Lakes, plastic fragments were most common (Eriksen et al., 2014). These MPs appear to be a threat to the ecological health of the Great Lakes (Driedger et al., 2015; Hoffman and Hittinger 2017) although to what degree is still unknown. However, without a standardized method for analysis of microplastics, comparing microplastic

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abundance with previous studies in the Great Lakes is challenging and open to question, as we still lack a standardized method for analysis (Twiss and Stryszowska, 2016).

The abundance and type of MPs have potential direct and indirect impacts on aquatic organisms. Despite the abundance of recent studies on MP impacts, results are often inconsistent and in conflict with each other. Reasons for this include differences in MP concentrations, MP type, particle size, exposure times, biofilm development, and response organisms used in the studies (de Sá et al., 2018; Triebskorn et al., 2019; Menéndez-Pedriza and Jaumot, 2020). While direct ingestion may cause blockage of animals' digestive tract (Wright et al., 2013), plastic debris also can adsorb persistent organic pollutants (POPs) and select metals, and retain concentrations that are orders of magnitude greater than the surrounding water (Rochman et al., 2013; Ziccardi et al., 2016) which may indirectly impair organismal health (Ashton et al., 2010; de Sá et al., 2015; Gallo et al., 2018; Holmes et al., 2012; Mato et al., 2001; Ory et al., 2018; but see Koelmans et al., 2016). This adsorption can be directly on the microplastics or associated with biofouling of the plastic materials, which occurs within hours to days (Oberbeckmann et al., 2015, Rummel et al., 2017).

The food web in the Great Lakes has already undergone dramatic alteration due to invasive species (Bunnell et al., 2013; Ives et al., 2019) and MPs may exacerbate those trends by differential effects of toxicity. However, Burns and Boxall (2018) examined 18 studies that looked at MPs as a vector for contaminant transfer to organisms, with 14 studies showing no evidence and 4 studies showing inconclusive evidence that MPs were a significant exposure pathway for hydrophobic organic contaminants.

The goal of the current study was to investigate the role of plastic debris as a vector for POPs. In contrast to many of the studies that have focused on the type and density of MPs present in aquatic ecosystems, we adopted an experimental approach by incubating MPs in a lake at two sites and depths, for two different incubation periods, and then examining the MPs for POPs, metals and their associated bacterial biofilm (cf. Rummel et al., 2017). We posed four research questions: 1) to what extent do three common types of plastic (low density polyethylene [LDPE]; polypropylene [PP]; and polyethylene terephthalate (polyester [PET]; acronyms based on International Organization for Standardization) found in the environment act as a sink for select POPs and metals in a lake with a known history of environmental contamination (Muskegon Lake, Michigan); 2) does incubation duration (1-month vs. 3-month) in the lake influence accumulation of POPs and metals on the MPs; 3) does incubation depth (2-m surface vs. bottom) and location (Channel vs. Lake) influence POP and metal accumulation on the MPs; and 4) do bacterial biofilm communities found on the three plastics types differ after incubation? After each retrieval, samples were analyzed for: 1) three representative classes of POPs: polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCs); 2) select metals (chromium [Cr], manganese [Mn], nickel [Ni], copper [Cu], zinc [Zn], arsenic [As], selenium [Se], silver [Ag], cadmium [Cd], lead [Pb]); and 3) genomic characterization of the attached bacterial biofilm.

Materials and methods

Site description

Muskegon Lake is a drowned-river mouth system located on the west coast of Michigan's lower peninsula (Fig. 1). The Muskegon River serves as the primary inflow, while a navigation channel to Lake Michigan is the primary outflow. The lake covers ~17 km^2 with mean and maximum depths of 7 and 24 m, respectively. Mean hydraulic retention time is about 23 days (Freedman et al., 1979), and mid-summer hypoxia and anoxia are recurring events (Biddanda et al., 2018). Muskegon Lake has been impacted from industrial activity beginning in the 1800s with the logging industry and continuing into the 20th century with foundries and factories along the lakeshore (Steinman et al., 2008).

Two sites were selected for the deployment of the MPs (Fig. 1). One site was located in Muskegon Lake ("Lake") (43.23834 N, 86.27923 W; site depth = 12 m), which is influenced mostly by Muskegon River inflows but also can be affected by Lake Michigan when westerly winds push Lake Michigan water into Muskegon Lake (Liu et al., 2018). The second location is along the south side of the navigational channel ("CH") (43.22769 N, 86.33911 W; mean depth = 4.2 m), which is influenced heavily by Lake Michigan intrusions. For the channel site, plastics were incubated at two depths: two meters below the surface (CH 2-m) and on the sediment bottom (CH bottom). For the Lake site, plastics were incubated also at 2-m and on the sediment bottom, but the incubation tubes at Lake 2-m detached from the frame and were lost over both deployment durations, so no data are available from that location.

Microplastic type and incubation

Pellets of three different polymeric compositions were procured from Plastic Pellets 4 Fun (High Point, NC). Verification of polymeric composition was confirmed by pyrolysis gas chromatography mass spectrometry as LDPE, PP, and PET. The bulk material was then processed by sieving, resulting in a size range of 2–4 mm, washed in hexane, and subjected to end-over-end tumbling for 1 hr to remove surface contaminants. The hexane solvent was then removed by filtration and the process repeated two more times. Once the cleaning procedure was completed, the MPs were allowed to air dry in a desiccator overnight. The non-deployed and cleaned material was termed virgin microplastic (MP).

Microplastics were placed in incubation tubes 12 hr prior to deployment (Electronic Supplementary Material (ESM) Fig. S1). Each incubation tube was assigned and received ~42 g of one type of virgin MP. The 3 polymers of MP had 4 replicate tubes per frame, resulting in 12 incubation tubes per frame, and a total of 48 tubes per deployment time. The placement of the incubation tubes along each frame was assigned randomly. Incubation tubes were rinsed and soaked for 24 hr in deionized water and allowed to dry prior to MP additions.

The incubation tubes were made from 7.62 cm outer diameter (o.d.) Harvel Clear™ Rigid schedule PVC (ALSCO Industrial Products, Inc., Lithia Springs, GA, USA) cut into 15 cm-long sections, in which six evenly spaced holes (5 cm diameter) were drilled along the sides. The inside of the clear PVC pipe was lined with steel wire cloth (mesh size 0.96 mm; McMaster-Carr, Cleveland, OH, USA) formed into a sleeve to keep the MPs contained within the incubation tubes. Clear PVC slip caps were placed on each incubation tube end. A 0.64 cm o.d. threaded rod was placed through the center of each incubation tube and through holes drilled in the center of each slip cap. Washers and metal locknuts were used to secure both the slip cap and an automotive break line tab (part no.: AA-422-C; A&A Manufacturing, Inc., Spring Lake, MI, USA) to both sides of each tube. A metal quick link was then used to attach the assembled incubation tube via the break line tab to double loop chains along each attachment frame. This allowed the incubation tubes to move with wave motion within a frame and for easy removal at the end of the deployment.

The attachment frames were created using schedule 40 PVC with 2.54 cm o.d. measuring 0.6 m × 1.0 m and bisected with an additional 1.0 m pipe (ESM Fig. S1) to support the incubation tubes. All frames were deployed on June 4, 2018. Frames were retrieved on July 3, 2018 and September 11, 2018 for the 1-month and

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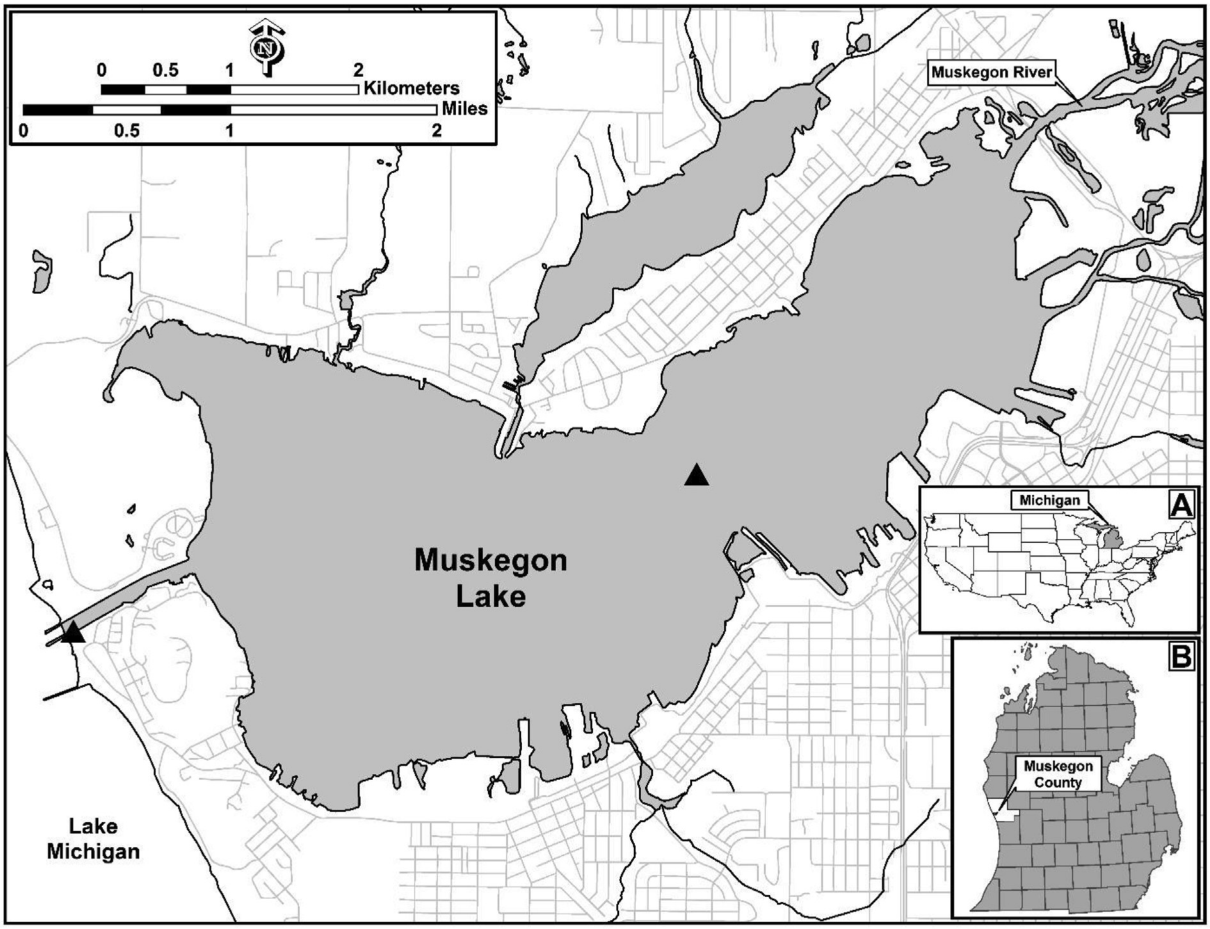

Fig. 1. Map of Muskegon Lake with locations (triangles) of microplastic deployment in the channel (far left) and mid-lake. Insets: A) location of Michigan (shaded) within the US; B) location of Muskegon Lake and County in lower peninsula of Michigan.

3-month deployments, respectively. The frames for the 2-m depth were deployed vertically in the water column, hanging via loose chain at two anchor points at the top and weighted with 0.64 cm (1/4 in.) o.d. chain. The frames sitting on the sediment bottom had four anchor points, one at each corner, with chain allowing it to lay horizontally once deployed. Galvanized 0.64 cm o.d. steel rope was used at the center point along the chain anchor points to attach the frame either to a buoy at Lake site or to the seawall at Channel site. At the Lake 2-m location, the frame was attached in-line with the mooring weight while the CH 2-m frame hung against the sea wall.

The frames from the three incubation locations with intact tubes (CH 2-m; CH bottom; Lake bottom) were brought back to the lab after each deployment duration, typically within 4 hr of retrieval, where the incubation tubes were disassembled and MPs processed for further analysis. Materials were transported back to the lab in coolers, and all MPs were stored at 4 °C in amber glass jars with Teflon lined plastic caps.

Water quality

At each location, just prior to deploying the frames, general water quality including water temperature, pH, dissolved oxygen (DO), specific conductivity (Sp Cond), total dissolved solids (TDS), and turbidity were measured at 2 m and bottom with a Yellow Springs Instruments (YSI) 6600 multi-sensor sonde (ESM Table S1). Waters and sediments were sampled only at the time of initial deployment, June 4th, 2018. Water samples were taken at 2-m and near-bottom depths using a Teflon lined General Oceanics Niskin bottle. Sediment samples were taken using a petite Ponar.

Microplastic identification by py-GCMS (Panno et al., 2019)

Microplastics were identified by pyrolysis gas chromatography mass spectrometry (py-GCMS). A small sub-sample was cut from several representative microplastic materials and inserted into a quartz capillary tube with quartz wool plugs. The tube was loaded into a CDS Analytical 5200 series pyroprobe and heated to 600 °C for 90 s. The temperatures of the GC injection port and transfer line were constant at 300 °C (split ratio of 100:1). Separation was performed on a capillary column (30 m × 0.25-mm × 0.25 μm film thickness; Rtx-5MS) with a helium carrier gas (flow rate of 1.0 ml/min). The oven was held at 40 °C for 2 min and then heated at a rate of 10 °C/min to 300 °C, which was then held for 10 min.

Sample preparation

The retrieved MPs were allowed to air dry in a desiccator until water was no longer visible. Both virgin plastics and deployed plastics were extracted by a modified US EPA Method 3545 (US EPA, 1998b) utilizing a Dionex ASE 300 system. Plastic samples (10 g) were mixed with diatomaceous earth and loaded into 33 ml stainless steel extraction cells. At this time all surrogates and spikes were added to the appropriate cells to serve as quality control. For plastic materials, hexane was employed as the extraction

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solvent at 60 °C and 1500 psi. Each extraction cell was filled to 60% volume with extraction fluid, with a static hold time of 5 min. After flushing into a collection bottle, the process was repeated 4×. All extracts were then exchanged to hexane and concentrated to a final volume of 5.0 ml using a Zymark TurboVap 500 system and processed by US EPA Method 3630C (US EPA, 1996a). The 5.0 ml extract was added to a pre-conditioned silica gel column and three fractions were collected for each sample. A silica gel quality control column was also processed with each batch that involved spiking of all target compounds on a column and processing in parallel with the samples. The elution volumes and solvent types for each fraction were as follows: fraction 1: 130 ml hexane and 60 ml dichloromethane; fraction 2: 100 ml dichloromethane; fraction 3: 100 ml of acetone (25%) /dichloromethane (75%). Finally, fractions were exchanged to hexane and concentrated down to 1.0 ml.

Sediment samples were homogenized with a spatula before processing. Sub-samples of each sample type were removed for moisture determination and were found to contain 83.2% solids and 17.3% solids, channel and lake sediments respectively. Additional sediment sub-samples were then extracted wet by the same ASE method as plastics materials; however, hexane–acetone (1:1) replaced hexane as the extraction solvent. Following ASE, the sediment extracts contained visible water and therefore were dried with sodium sulfate before further processing. After drying, the extracts were processed in the identical manner as the plastic materials.

Aqueous samples were prepared by a liquid–liquid extraction method, US EPA Method 3510 (US EPA, 2007). In brief, 500 ml of sample was added to a separatory funnel, spiked with the appropriate surrogates and/or spikes, and then followed by addition of 500 ml of dichloromethane. The funnel was shaken and allowed to settle three consecutive times. Afterwards, the bottom dichloromethane layer was transferred to a collection bottle and the process was repeated two more times. The pooled organic extracts were then concentrated to a 5.0 ml final volume and processed in the identical manner as the plastic materials and sediments.

Plastic and sediment samples were prepared for metals analysis by US EPA Method 3051A (US EPA, 1998a, 2014). Plastic materials (0.5 g) were brought completely in solution with nitric acid (10 ml), hydrogen peroxide (3.0 ml) and digestion with Milestone Ethos microwave system. Wet sediment samples (0.5 g) were also prepared in the similar manner, however the undigested residue (silicates) were filtered after digestion. Therefore, sediment results reflect a strong acid leach as opposed to a total concentration. All digestates were then diluted to a final volume of 50 ml with deionized water. Aqueous samples were analyzed as received.

POP analysis by GC–MS

Sixty-three representative compounds were selected from three classes of POPs: 1) PAHs; 2) PCBs; and 3) OCs. These contaminants were selected because they are known stressors in the Great Lakes region and are known to be persistent in the environment. Target POPs are listed in Tables S11–13.

The POP analysis was performed per US EPA Method 8270C (US EPA, 1996b). Prior to each assay, the instruments were calibrated with the appropriate reference materials procured from Accustandard (New Haven, CT) or Wellington Laboratories (Ontario, Canada). Calibration was verified by analysis of independent reference materials. During analysis, the mass spectrometer was operated in select ion mode (SIM/SIR). This technique allows greater sensitivity and is less prone to interference since only select ions are captured and measured for each target compound. The SIM programs were developed beforehand using reference materials to determine the appropriate retention time windows and most sensitive quantification ions.

Polyaromatic hydrocarbons and PCBs were analyzed with a Shimadzu QP2010 SE gas chromatograph mass spectrometer (GC–MS). Separation was achieved on a SH-Rxi-5Sil MS capillary column (30 m × 0.25 mm × 0.25 μm df) with helium as a carrier gas at 2.0 ml/min. For PAHs, a 1-μL injection was performed in splitless mode at 280 °C with the transfer line and ion source held at 300 °C and 250 °C, respectively. At the time of injection, the oven was held at 45 °C for 1 min, then increased to 130 °C at a rate of 43 °C/min, then increased to 180 °C at a rate of 12 °C/min, then increased to 240 °C at a rate of 7 °C/min, and then finally increased to 320 °C at a rate of 12 °C/min and was held at that temperature for 4 min. For PCBs, a 1-μL injection was performed splitless at 280 °C and the transfer line and ion source were held at 250 °C. At the time of injection, the oven was held at 45 °C for 4 min and then increased to 320 °C at a rate of 10 °C/min.

Organochlorine pesticides were analyzed with an Agilent 6890 gas chromatograph coupled to an AutoSpec Ultima high-resolution mass spectrometer. Separations were achieved on a Stx-500 capillary column (15 m × 0.25 mm × 0.15 μm df) with helium as a carrier gas at 2.0 ml/min. A 1-μL injection was performed in splitless mode at 280 °C and the transfer line and ion source held at 280 °C. At the time of injection for OCs, the oven was held at 45 °C for 4 min and then increased to 320 °C at a rate of 10 °C/min.

Metals analysis

Metals analysis was performed per US EPA Method 6020B (US EPA 2013). Measurements were performed with a VG Elemental PQ-Excel ICP-MS instrument and calibrations were performed daily with reference materials procured from Spex Certiprep. Calibration was also verified by analysis of independent reference materials.

Quality control

Seven surrogates, representative of the three POP classes, were spiked into all samples before processing for organics. They included: Terphenyl-d14, ^{13}C Dieldrin, ^{13}C 4,4′-DDD, PCB congeners #14, #65, #155, and #204. Data were reported only for samples with surrogate recoveries in the 50% to 150% range. For virgin plastics each sample type was prepared and analyzed in duplicate. For aqueous, sediment, and deployed plastics each sample was processed and analyzed in triplicate.

Additional quality control associated with each extraction batch included a reagent blank, a reagent blank spike, and duplicate sample matrix spike. All spikes performed contained all the target POPs in addition to surrogates. During each silica gel fractionation batch, a silica gel QC column was also spiked with all target compounds/-surrogates and processed alongside samples in each batch.

Prior to each POP assay, internal standards were added to all calibration standards, check standards, and samples. For PAH analysis, Naphthalene-d8, Acenaphthene-d10, Phenanthrene-d10, Acenaphthene-d10, Chrysene-d12, and Perylene-d12 were utilized. For PCB and OC analysis, pentachloronitrobenzene and PCB congener 204 were utilized, respectively. Independent check standards containing all target compounds and surrogates were also prepared and analyzed for each POP assay post-calibration, after every 10 injections, and at the end of each run. Additional instrumental quality control parameters included an analytical duplicate sample and an analytical duplicate sample spiked with the target compounds for each analysis batch.

Aqueous, sediment, and plastic materials were prepared and analyzed in duplicate for metals. With each digestion batch, quality control parameters included a reagent blank and a matrix sample spike with target metals. For sediments, a NIST Montana Soil

(SRM 2710) was also prepared and analyzed. Scandium, yttrium, rhodium, and thorium were utilized as internal standards. Independent check standards containing all target metals were also prepared and analyzed during each assay post-calibration, after every 10 injections, and at the end of each run. Additional instrumental quality control parameters included an analytical duplicate sample and an analytical duplicate sample spiked with the target metals for each analysis batch. Quality control results are presented in supplemental Tables S14-S25.

16S amplicon sequencing and analysis

Microplastics were stored at −80 °C until DNA extraction. Prior to extraction, all microplastics were gently washed with nuclease-free water to remove any sediment particles attached. DNA was extracted using a Macherey-Nagel NucleoSpin Soil DNA extraction kit (Macherey-Nagel, Bethlehem, PA), and the 16S v4 region was amplified using the 515F/806R primer set (Caporaso et al., 2012). The region was amplified using a two-step PCR process with the conditions outlined in the Illumina 16S Amplicon Sequencing Library Preparation protocol (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf).

Sample libraries were pooled and the amplicons were sequenced using a 2 × 300 bp format, along with a 15% spike-in of Phi-X, on the Illumina MiSeq System (Illumina, San Diego, CA). Sequence alignment, chimera removal, taxonomic identification, OTU identification, and abundance information were obtained using the program MOTHUR v1.42.3 (Schloss et al., 2009). Briefly, reverse and forward reads were assembled and sequences with ambiguous calls or homopolymers longer than 8 bp were removed. Sequences were aligned to the full Silva bacteria reference database v132. Chimeras were identified using VSEARCH and removed. Sequences were classified based on the Silva trainset9_03212 files provided by MOTHUR, and sequences identified as "chloroplast", "mitochondria", "Archaea", "Eukaryota", and "unknown" were removed. Because we were interested in examining bacterial communities at the family-level, OTUs were binned by phylotype using taxonomic information, rather than by clustering via 97% sequence similarity. To avoid biases due to unequal sequencing depth among samples, all sequences were randomly subsampled to 4724 sequences per sample.

Data analysis

All results associated with organic surrogate recoveries lower than 50% were not included in the data analysis. In addition, the results presented are an average of duplicate or triplicate results. In cases where a target compound was detected in only one of the replicates, then the single detection has been reported as the average. Statistically significant differences in contaminants were determined using 2 separate 3-way ANOVAs. Due to the loss of MPs from the Lake 2-m frames, there were 3 locations available for analysis: 1) CH 2-m; 2) CH bottom; and 3) Lake bottom. The first 3-way ANOVA compared MP type (3 levels), 2-m vs. bottom at CH, and 1- vs. 3-month deployment; the second 3-way ANOVA compared MP type (3 levels), site location (CH bottom vs. Lake bottom), and 1- vs. 3-month deployment. Residual plots were examined to evaluate homoscedasticity, and normality was tested with Shapiro-Wilk tests. Trends in data were also examined with non-metric multidimensional scaling (NMDS). Data were transformed if necessary.

Stacked bar plots were created to identify bacterial family OTUs whose relative abundance surpassed at least 2.5% of the total community across each polymer type, sampling location, and deployment time. Amplicon data were examined with NMDS of OTUs using the R statistical package phyloseq v1.28.0 (McMurdie and Holmes, 2013). Genetic distances were calculated using the Bray-Curtis method (Bray and Curtis, 1957) in phyloseq, which included a square root transformation and Wisconsin double standardization. A permanova using the distance matrix was performed using the adonis function in vegan v2.5-6 (Oksanen et al., 2019) to examine significant differences in centroid location in relation to polymer-type (PET, LDPE, and PP), location (CH vs. Lake), and deployment time (1- vs. 3-months).

Measures of alpha diversity (i.e., diversity of organisms at an individual site representing the local species pool) for family-level bacterial OTUs were obtained to assess OTU richness (i.e., the number of bacterial OTUs present on each plastic polymer), and OTU evenness (i.e., the relative distribution of each OTU for each plastic polymer). These were quantified by subsampling each sample with replacement to a minimum sequence depth (equal to the sequence depth of our smallest library) 100 times to gain estimates of species abundance. Observed richness and evenness, based on inverse Simpson's index (Sun, 1992), were quantified for the three plastic-types, as well as the channel and lake locations, using phyloseq v1.28.0 (McMurdie and Holmes, 2013). A 3-way ANOVA was then used to examine the effects of polymer type, location, and the interaction between polymer type and location on richness and evenness estimates. Tukey's multiple comparison was then used to identify pairwise differences between samples.

Data Accessibility

All raw sequence reads were deposited to the Sequence Read Archive (SRA) database (Bioproject accession #: PRJNA642182.

Results

Microplastics quickly became coated with a biofilm (plastisphere; cf. Zettler et al., 2013), as evidenced by the changes to the appearance of polyethylene (LDPE) beads from their virgin condition through the 1-month and 3-month incubation periods (Fig. S2). Similar changes occurred to the polyester (PET) and polypropylene (PP) beads.

PAHs

Although total PAH concentrations in the water column were very low (<1 μg/L; CH 2-m: 0.05 ± 0.04 μg/L, CH bottom: 0.12 ± 0.04 μg/L, Lake 2-m: 0.07 ± 0.03 μg/L, Lake bottom: 0.12 ± 0.04 μg/L) at all sites at the time of deployment, concentrations near the sediment were about twice as high as those higher in the water column, presumably due to sediment-related contamination (Fig. 2). Mean sediment concentrations of PAH were low in the channel (<10 ng/g; 10.0 ± 6.9 ng/g) but about 20× greater in the lake (254 ± 130 ng/g) presumably because of the higher organic content (Table S1; Fig. 2). PAH concentrations were detectable on all virgin plastic types but at low concentrations (PET: <0.20 ng/g, PP: <0.20–0.24 ng/g, LDPE: 0.21–0.35 ng/g). At all sites and on all polymer types, PAH concentrations after a 1-month incubation were greater than on the virgin plastics and underwent a modest but statistically significant increase (Fig. 2). Mean PAH concentrations declined modestly between 1 and 3 months at both CH 2-m and bottom on all MP types; at the CH 2-m location, the decline was statistically significant only on PET, whereas at CH bottom the declines were significant on all polymer types (Fig. 2). In contrast, mean PAH concentrations increased modestly from 1 to 3 months at Lake bottom on both PET and PP but remained constant on LDPE. When the 1- and 3-month PAHs were pooled by site, concentrations were significantly greater at CH 2-m (Fig. 2, uppercase A)

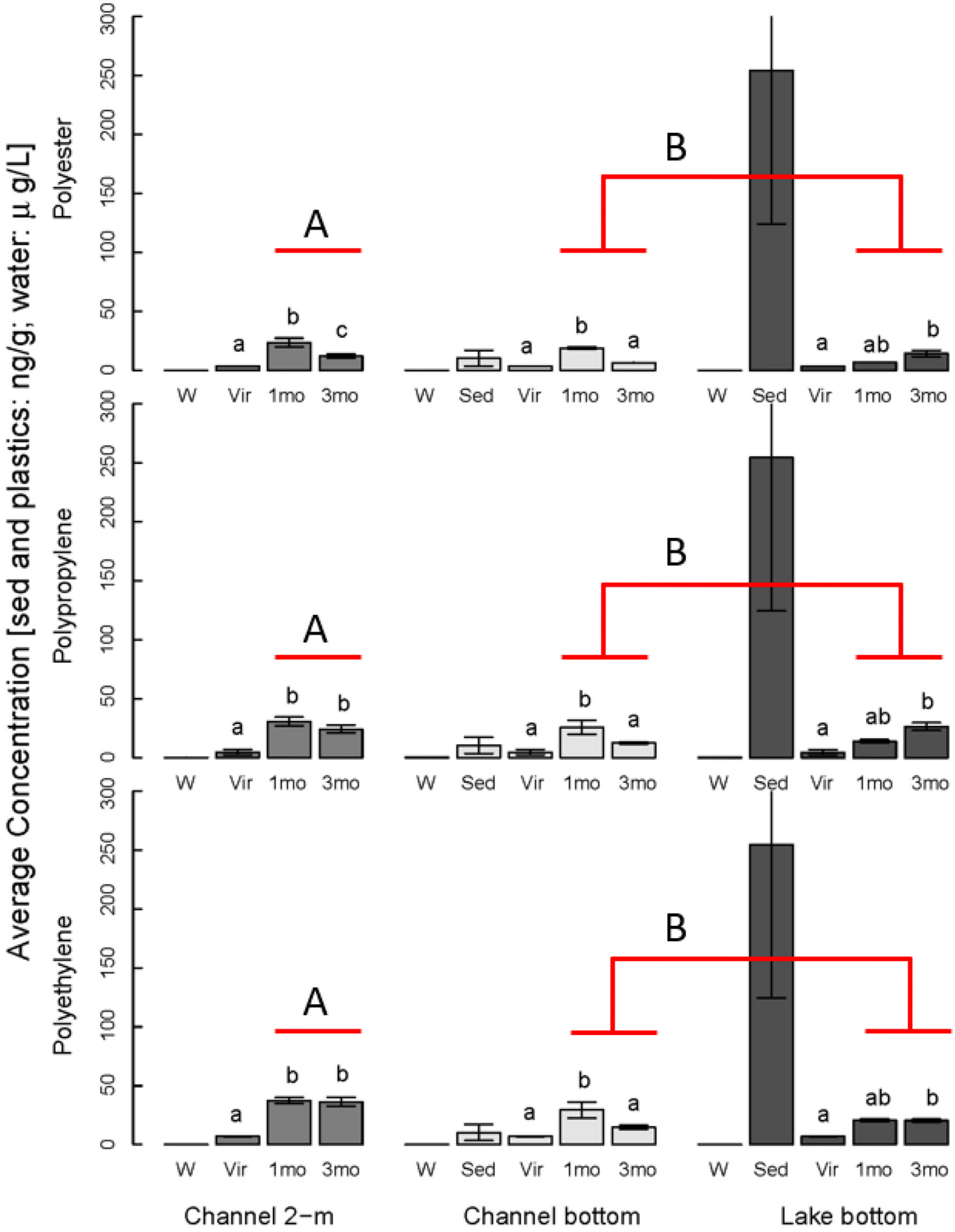

Fig. 2. Average concentrations of total PAHs for three polymer types at the three sampling locations: Channel 2-m depth, Channel bottom, and Lake bottom. PAH values provided for lake water (µg/L), sediment (ng/g), and plastic (ng/g). 2-way ANOVAs were conducted on three plastic incubation duration levels: virgin plastic (never been incubated), one month (1 mo), and three month (3 mo). Different lowercase letters denote statistical differences within a site; different uppercase letters denote statistical differences among sites for 1 mo and 3 mo only, as virgin plastic values are the same across sites.

than the other two locations (Fig. 2, uppercase B), irrespective of polymer type; there was no significant difference in concentration between Lake bottom and CH bottom.

When PAHs were pooled across all 3 sampling locations, concentrations increased from the virgin plastic to the 1-month incubation at all locations and on all polymer types, although increases were greater in the channel than in the lake (Tables 1–3). Polyaromatic hydrocarbons concentrations then declined by ¼ to ⅔ at the channel sites but doubled at Lake between the 1- and 3-month deployments on PET and PP (Tables 1 and 2). On LDPE, PAH concentrations remained constant at the CH 2-m and Lake bottom locations but declined by half at CH bottom (Table 3). The most abundant PAH compounds were very similar on the different polymer types, and included fluoranthene (5-ring), naphthalene

Table 1
Mean ± 1SD concentrations (ng/g) of summed PAH, PCB, and OC compounds attached to *polyester* microplastics pooled from Channel 2-m, Channel bottom, Lake bottom after 1- and 3- month (mo) deployments in Muskegon Lake. ND = non-detectable; NC = not calculated.

Deployment Site and Time	PAH	PCB	OC
Virgin Plastic	3.6 ± 0.11	1.4 ± 0.71	ND
Channel 2-m: 1-mo	24 ± 3.7	1.1 ± 0.53	0.11 ± NC
Channel 2-m: 3-mo	12 ± 1.8	0.80 ± 0.49	0.12 ± NC
Channel bottom: 1-mo	19 ± 0.83	1.1 ± 0.08	ND
Channel bottom: 3-mo	6.6 ± 0.17	0.89 ± 0.61	0.35 ± 0.08
Lake bottom: 1-mo	7.2 ± NC	1.4 ± 0.33	ND ± NC
Lake bottom: 3-mo	14 ± 2.7	0.46 ± 0.42	ND ± NC

Table 2
Mean ± 1SD concentrations (ng/g) of summed PAH, PCB, and OC compounds attached to *polypropylene* microplastics pooled from Channel 2-m, Channel bottom, Lake bottom after 1- and 3- month (mo) deployments in Muskegon Lake. ND = non-detectable; NC = not calculated.

Deployment Site and Time	PAH	PCB	OC
Virgin Plastic	4.7 ± 2.3	0.89 ± 0.04	ND
Channel 2-m: 1-mo	31 ± 3.7	1.0 ± 0.29	0.55 ± 0.22
Channel 2-m: 3-mo	24 ± 3.2	1.3 ± 0.29	0.33 ± 0.04
Channel bottom: 1-mo	26 ± 5.9	1.1 ± 0.38	ND
Channel bottom: 3-mo	13 ± 0.90	1.1 ± 0.53	0.06 ± NC
Lake bottom: 1-mo	14 ± 1.4	1.2 ± 0.23	ND ± NC
Lake bottom: 3-mo	26 ± 3.2	0.60 ± 0.27	ND ± NC

Table 3
Mean ± 1SD concentrations (ng/g) of summed PAH, PCB, and OC compounds attached to *polyethylene* microplastics pooled from Channel 2-m, Channel bottom, Lake bottom after 1- and 3- month (mo) deployments in Muskegon Lake. ND = non-detectable.

Deployment Site and Time	PAH	PCB	OC
Virgin Plastic	7.1 ± 0.15	0.86 ± 0.19	ND
Channel 2-m: 1-mo	37 ± 2.7	13 ± 2.3	0.56 ± 0.09
Channel 2-m: 3-mo	36 ± 3.6	2.0 ± 0.13	1.9 ± 0.24
Channel bottom: 1-mo	29 ± 6.8	7.8 ± 2.8	ND
Channel bottom: 3-mo	15 ± 1.7	1.3 ± 0.40	0.22 ± 0.02
Lake bottom: 1-mo	21 ± 1.2	11 ± 2.6	0.19 ± NA
Lake bottom: 3-mo	21 ± 1.2	3.4 ± 0.40	0.09 ± 0.10

(2-ring), phenanthrene (3-ring), chrysene (4-ring), and pyrene (5-ring) (Tables S2-4). In general, 3- and 4-ring PAH compounds showed the greatest sorption and 6-ring analogues the least.

PCBs

Similar to PAHs, 1) total PCB concentrations (ESM Table S5-7) were very low in the water column (<1 μg/L; CH 2-m: 0.009 ± 0.003 μg/L, CH bottom: 0.076 ± 0.034 μg/L, Lake 2-m: 0.004 ± 0.007 μg/L, Lake bottom: 0.022 ± 0.012 μg/L) at all sites at the time of deployment and 2) sediment PCB concentrations were low in the Channel (0.13 ± 0.01 ng/g) but greater by more than an order of magnitude at the Lake site (3.20 ± 0.56 ng/g) (Fig. 3), likely due to its greater sediment organic matter content. PCB concentrations averaged ~1 ng/g on the three virgin polymer types (PET: 1.40 ± 0.71 ng/g, PP: 0.89 ± 0.04 ng/g, LDPE: 0.86 ± 0.19 ng/g) and did not change significantly on PET or PP at any location after the 1-month incubation (Tables 1 and 2; Fig. 3). However, PCB concentrations increased significantly, about 10-fold, on LDPE after 1 month at all three locations (Table 3; Fig. 3). After 3 months, concentrations on PET and PP remained generally stable and low at the two channel locations but declined by 50–70% at Lake bottom (Tables 1 and 2; Fig. 3). PCB concentrations on LDPE declined significantly, by 3- to 6-fold between 1 and 3 months at all locations (Table 3; Fig. 3).

When the 1- and 3-month PCBs were pooled by sampling location, mean concentrations were similar among locations for PET and PP (Tables 1 and 2; Fig. 3); however, for LDPE, concentrations of PCB were significantly greater at CH 2-m and Lake bottom than at CH bottom, due largely to the differences after the 1-month incubation (Fig. 3). The most dominant PCB congener was PCB 8 (2,4′-Dichlorobiphenyl), followed by PCB 156 (2,3,3′,4,4′,5-Hexachlorobiphenyl) and 44 (2,2′,3,5′-Tetrachlorobiphenyl), which were about 10-fold lower (Table S4).

OCs

Total OC concentrations were low (CH 2-m: 0.027 ± 0.035 μg/L, CH bottom: 0.010 ± 0.008 μg/L, Lake 2-m: 0.003 ± NC μg/L, Lake bottom: 0.006 ± 0.006 μg/L) in the water column at all sites at the time of deployment; concentrations in the sediment were low in the channel (0.006 ± NC ng/g) but somewhat higher in the

Table 4
Mean ± % RSD (relative standard deviation: virgin, sediment, and water only) and % RPD (relative percent difference) concentrations (μg/g) of most abundant* metals attached to *polyester* microplastics pooled from Channel 2-m, Channel bottom, Lake bottom after 1- and 3-month (mo) deployments in Muskegon Lake, as well as in the sediment and water column (μg/L) at each location. NC2 = one or more replicates with a less than value. Sediment and water values are the same for Tables 4-6. Missing copper values for sediment channel and water samples due to high copper concentrations found in blanks. PEC = probable effect concentration (see text).

Metal	Microplastic Substrate							Sediment Substrate		Water Matrix			PEC
	Virgin Plastic	Channel 2-m: 1-mo	Channel 2-m: 3-mo	Channel bottom: 1-mo	Channel bottom: 3-mo	Lake bottom: 1-mo	Lake bottom: 3-mo	Sediment: Channel	Sediment: Lake	Water: Channel 2-m	Water: Channel bottom	Water: Lake bottom	
Chromium	0.34 (49%)	0.64 (20%)	3.5 (0.4%)	0.78 (22%)	0.32 (40%)	<0.1 (NC2)	<0.1 (NC2)	1.9 (17%)	63 (0.93%)	1.7 (0.9%)	1.4 (7%)	1.5 (2.9%)	111
Copper	<0.1 (NC2)	0.87 (2%)	1.5 (24%)	0.61 (7%)	0.57 (19%)	0.78 (74%)	0.23 (19%)	–	47 (5.5%)	–	–	–	149
Lead	<0.1 (NC2)	0.22 (1%)	0.32 (18%)	0.36 (26%)	0.12 (NC2)	0.14 (NC2)	<0.1 (NC2)	1.6 (7.7%)	66 (6.1%)	3.8 (26%)	<0.1 (NC2)	1.3 (43%)	128
Manganese	40 (0.9%)	101 (15%)	19 (28%)	93 (35%)	54 (29%)	46 (1%)	46 (8.5%)	1.9 (17%)	63 (0.93%)	1.7 (0.9%)	1.4 (7%)	<0.2 (NC2)	1100
Nickel	<0.1 (NC2)	<1 (NC2)	1.23 (30%)	<1 (NC2)	<1 (NC2)	<1 (NC2)	<1 (NC2)	1.6 (22%)	25 (5.5%)	<10 (NC2)	<10 (NC2)	<10 (NC2)	48.6
Zinc	8.2 (NC2)	23 (17%)	17 (38%)	16 (61%)	8.1 (24%)	7.9 (1%)	41 (22%)	5.4 (44%)	147 (3.9%)	<10 (NC2)	<10 (NC2)	<10 (NC2)	459

[*] Concentrations of arsenic, cadmium, selenium, and silver were < 1 μg/g.

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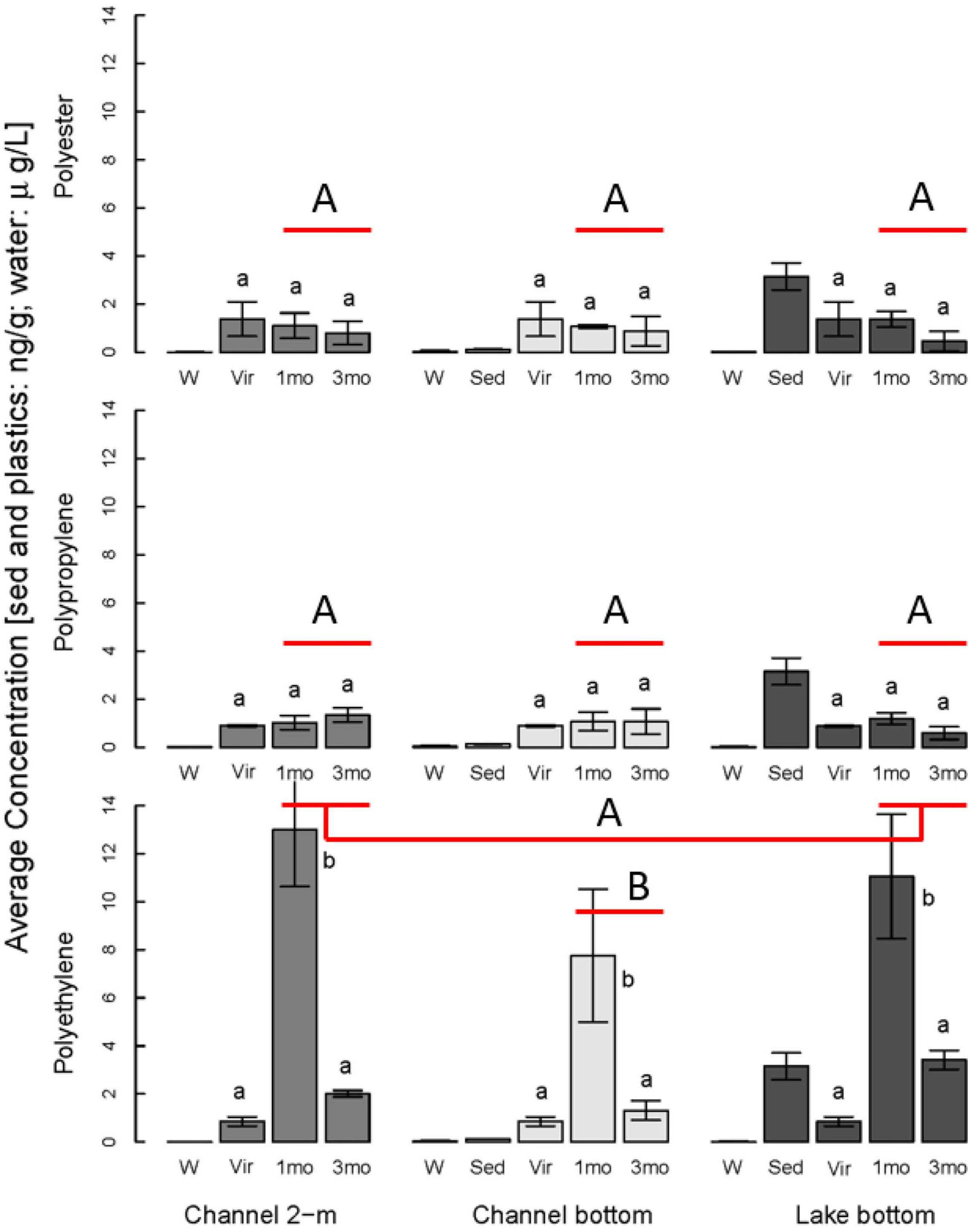

Fig. 3. Average concentrations of total PCBs. Abbreviations and statistical analyses as in Fig. 2.

lake (0.086 ± 0.035 ng/g) (Fig. 4). OC concentrations were either extremely low or undetectable on all three virgin polymer types (PET: ND – 0.12 ng/g, PP: ND, LDPE: ND – 0.12 ng/g), and after one month, remained undetectable for all MPs at CH bottom and for PET and PP at Lake bottom (Tables 1 and 2; Fig. 4), limiting the ability to perform statistical tests. After 3 months, mean OC concentrations increased on PP and LDPE at CH 2-m and on all three MPs at CH bottom, but remained undetectable on PET and PP at Lake bottom (Tables 1–3; Fig. 4). The dominant organochlorine pesticides were γ-chlordane, α-chlordane, and endrin on all three MP types (Tables S8–10).

NMDS: The NMDS plot of contaminants (PAHs, PCBs, and OCs) on the three MPs revealed separation along NMDS1 (Fig. 5). Among polymer types, POPs on polyester (PET; red) clustered on the positive side of NMDS1, with the 3-month incubations (closed red symbols) most responsible for the separation. With respect to sampling locations, CH bottom (circles) separated along NMDS1 from the cluster of CH 2-m (squares) or Lake bottom (triangles). There

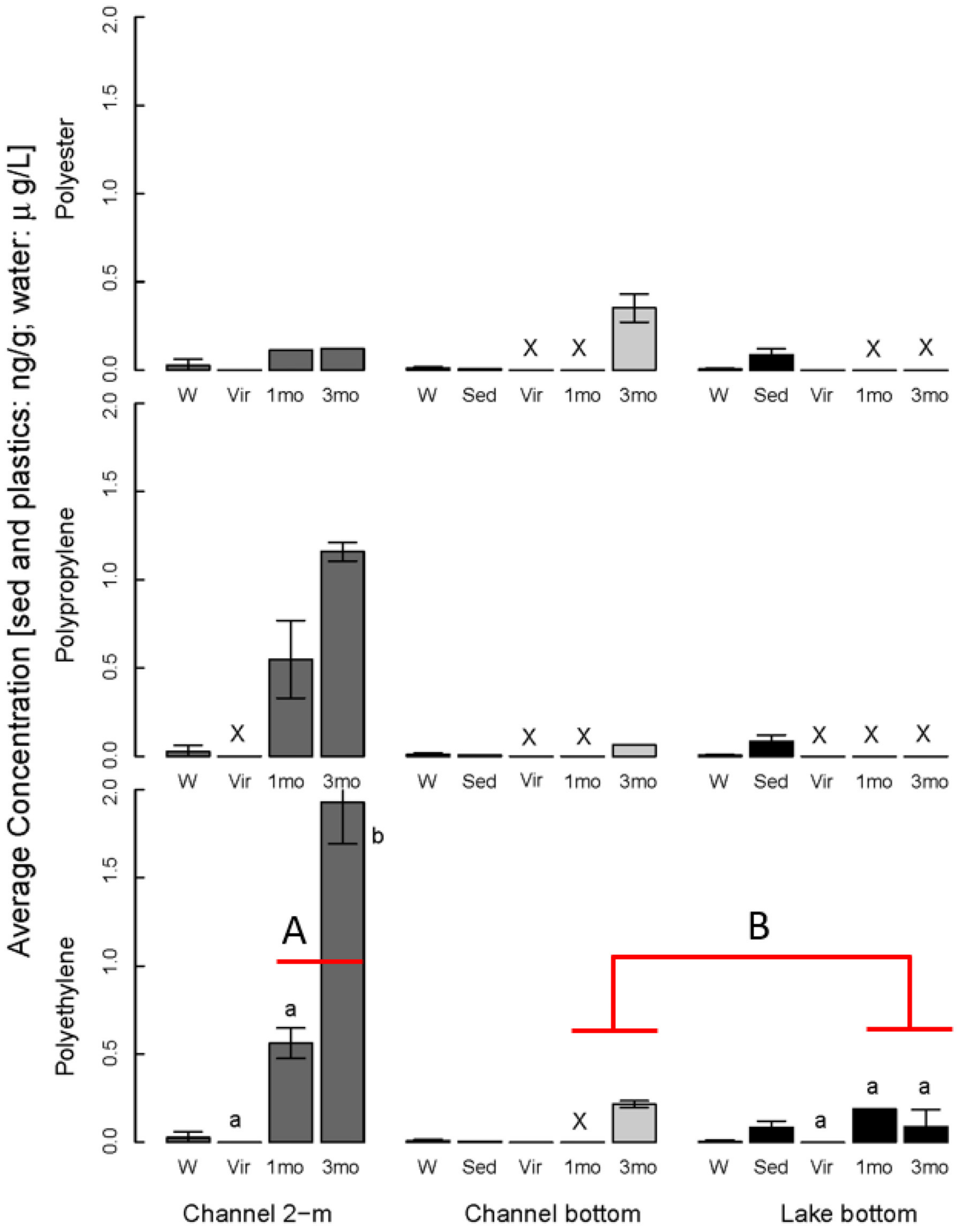

Fig. 4. Average concentrations of total OCs. Abbreviations as in Fig. 2. Large Xs represent all data below the detection limit. Statistical comparisons could not be conducted due to too many below detection values, with the exception of Channel 2-m vs. Channel bottom (p = 0.01).

was slight separation between POPs attached to PP and LDPE along NMDS2 (Fig. 5).

Metals

Chromium, copper, and lead concentrations were ≤3.5 µg/g on all MP types, irrespective of site or time (Tables 4–6). In contrast, mean concentrations of these metals ranged from 47 to 66 µg/g in the sediment at Lake bottom but were <2 µg/g in sediment at CH bottom (Tables 4–6). Nickel also had very low concentrations (<2 µg/g) on all MPs except PP and LDPE after 1-month at CH 2-m (Tables 5 and 6); nickel was below detection in the water. Manganese and zinc concentrations were variable in time and space, but both metals had much higher concentrations than the other four metals on average, including surprisingly high concentrations with the virgin PET (Table 4). Zinc concentrations increased 5- to 10-fold on all MPs between 1 and 3 months, irrespective of type. Arsenic, cadmium, selenium, and silver concentrations were below

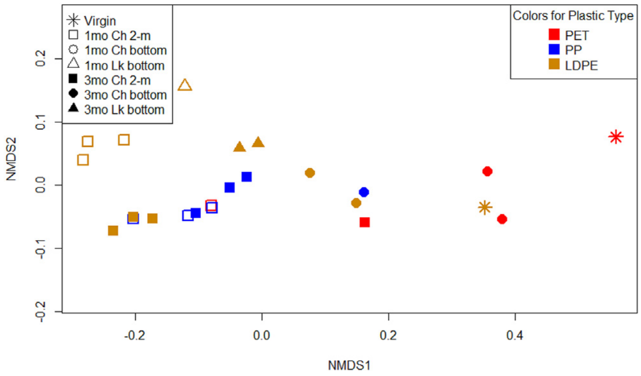

Fig. 5. NMDS of metals concentrations in 3 types of polymer from 3 different incubation periods. Metals included are Cr, Mn, Ni, and Zn. PET = polyester, PP = polypropylene, LDPE = low density polyethylene, virgin = no incubation, 1 mo = 1 month incubation, 3 mo = 3 month incubation, CH = Channel, LK = Lake.

detection (<1 μg/g) in all samples and are not reported. Metals in water were quite low, often below the detection limits (Tables 4–6). As was the case for all metals, Mn and Zn concentrations were at least one order of magnitude greater in Lake sediment than Channel sediment (Tables 4–6).

Genomics

We identified 25 different bacterial families attached to microplastics that accounted for at least 2.5% of the total relative abundance; the most abundant were Burkholderiales, Rhodocyclaceae, Comamonadaceae, Pseudomonadaceae, and unclassified bacteria (Fig. 6); cyanobacteria were excluded from this analysis although they are clearly present in Muskegon Lake (Gillett and Steinman, 2010). There were relatively few differences in bacteria relative abundance between the 1- and 3-month incubations for biofilms attached to PP and PET. However, differences were more evident over time for bacteria families attached to LDPE, with a decrease in relative abundance in Burkholderiales_incertae_sedis by 3-months in samples from the channel for both the bottom and 2-m locations. The relative abundance of both Rhodocyclaceae and Pseudomonadaceae were greater in the Lake than the Channel, irrespective of duration or polymer type (Fig. 6). Note that the relative abundance in Fig. 6 does not total 100% as only those families that represent at least 2.5% of the bacterial community are represented.

The NMDS plot showed that the location of the three plastics within Muskegon Lake played a role in influencing the bacterial microfilm community, with polymers incubated in the channel showing clear separation from polymers that were incubated in the lake along NMDS1 (Fig. 7). The LDPE-1 month and PP-1 month within the channels also tended to cluster together and separately from the other polymer types and deployment times. Permanova results suggest that polymer-type, location in the lake (CH vs. Lake), and deployment time (1-month vs. 3-month) had a significant effect on centroid locations for the NMDS (polymer-type: df = 2, Sum of Squares (SS) = 1.31, F = 10.88, R^2 = 0.19, p = 0.001; location: df = 1, SS = 1.99, F = 33.16, R^2 = 0.29, p = 0.001; deployment time: df = 1, SS = 0.65, F = 10.74, R^2 = 0.095, p = 0.001), with location having the strongest effect.

Alpha diversity estimates identified a significant difference in the observed richness of OTUs between the three MPs and a significant interaction between the type of MP and location (polymer-type: df = 2, SS = 7400, F = 19.04, $p = 8 \times 10^{-7}$; location: df = 2, SS = 2901, F = 7.46, p = 0.002), but not for location alone (df = 1, SS = 691, F = 3.56, p = 0.066) (Fig. 8). For evenness, based on the inverse Simpson's index, there were significant differences between MPs and between locations (polymer-type: df = 2, SS = 533.40, F = 26.34, $p = 2.13 \times 10^{-8}$; location: df = 1, SS = 431.06, F = 42.56, $p = 4.32 \times 10^{-8}$), but there was no MP by location interaction (df = 2, SS = 20.02, F = 0.99, p = 0.397) (Fig. 8). OTU richness and evenness were significantly higher for biofilms found on PET polymers relative to PP or LDPE, regardless of location (Fig. 8). In addition, richness and evenness tended to be lower in the Lake compared to the Channel, although this was not always the case (e.g., richness on PET) (Fig. 8).

Discussion

Recent reviews on MPs have emphasized their role as a potential source of pollutants via additives leaching from the polymers themselves (Franzellitti et al., 2019) or pollutants attached to the microplastics directly and/or to the biofilm surrounding the MPs (de Sá et al., 2018). Contaminants such as organochlorines, polycyclic aromatic hydrocarbons, and metals (Rios et al., 2007;

Table 5

Mean ± % RSD (relative standard deviation: virgin, sediment, and water only) and % RPD (relative percent difference) concentrations (μg/g) of most abundant* metals attached to *polypropylene* microplastics pooled from Channel 2-m, Channel bottom, Lake bottom after 1- and 3-month (mo) deployments in Muskegon Lake, as well as in the sediment and the water column (μg/L) at each location. NC2 = one or more replicates with a less than value. Sediment and water values are the same for Tables 4-6. Missing copper values for sediment channel and water samples due to high copper concentrations found in blanks.

Metal	Microplastic Substrate							Sediment Substrate		Water Matrix			PEC
	Virgin Plastic	Channel 2-m: 1-mo	Channel 2-m: 3-mo	Channel bottom: 1-mo	Channel bottom: 3-mo	Lake bottom: 1-mo	Lake bottom: 3-mo	Sediment: Channel	Sediment: Lake	Water: Channel 2-m	Water: Channel bottom	Water: Lake bottom	
Chromium	0.19 (17%)	0.40 (47%)	2.1 (48%)	0.75 (64%)	0.25 (1%)	<0.1 (NC2)	<0.1 (NC2)	1.9 (17%)	63 (0.93%)	1.7 (0.9%)	1.4 (7%)	1.5 (2.9%)	111
Copper	0.12 (14%)	0.55 (23%)	1.8 (66%)	0.47 (57%)	0.73 (50%)	0.41 (28%)	0.42 (11%)	–	47 (5.5%)	–	–	–	149
Lead	0.25 (NC2)	0.16 (NC2)	0.17 (60%)	0.35 (101%)	<0.1 (NC2)	<0.1 (NC2)	<0.1 (NC2)	1.6 (7.7%)	66 (6.1%)	3.8 (26%)	<0.1 (NC2)	1.3 (43%)	128
Manganese	<0.1 (NC2)	38 (47%)	74 (31%)	53 (44%)	18 (16%)	3.9 (45%)	9.8 (12%)	1.9 (17%)	63 (0.93%)	1.7 (0.9%)	1.4 (7%)	<0.2 (NC2)	
Nickel	<1 (NC2)	4.9 (31%)	<0.1 (NC2)	<1 (NC2)	<1 (NC2)	<1 (NC2)	<1 (NC2)	1.6 (22%)	25 (5.5%)	<10 (NC2)	<10 (NC2)	<10 (NC2)	48.6
Zinc	<5 (NC2)	20 (34%)	6.4 (10%)	10 (67%)	14 (0.9%)	8.0 (4%)	89 (10%)	5.4 (44%)	147 (3.9%)	<10 (NC2)	<10 (NC2)	<10 (NC2)	459

[*] Concentrations of arsenic, cadmium, selenium, and silver were < 1 μg/g.

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Table 6
Mean ± % RSD (relative standard deviation: virgin, sediment, and water only) and % RPD (relative percent difference) concentrations (μg/g) of most abundant[*] metals attached to *low density polyethylene* microplastics pooled from Channel 2-m, Channel bottom, Lake bottom after 1- and 3-month (mo) deployments in Muskegon Lake, as well as in the sediment and water column (μg/L) at each location. NC2 = one or more replicates with a less than value. Sediment and water values are the same for Tables 4-6. Missing copper values for sediment channel and water samples due to high copper concentrations found in blanks.

Metal	Microplastic Substrate							Sediment Substrate		Water Matrix			PEC
	Virgin Plastic	Channel 2-m: 1-mo	Channel 2-m: 3-mo	Channel bottom: 1-mo	Channel bottom: 3-mo	Lake bottom: 1-mo	Lake bottom: 3-mo	Sediment Channel	Sediment Lake	Water Channel: 2-m	Water Channel: bottom	Water Lake: bottom	
Chromium	0.17 (6%)	0.25 (12%)	0.67 (29%)	0.77 (58%)	0.21 (48%)	<0.1 (NC2)	<0.1 (NC2)	1.9 (17%)	63 (0.93%)	1.7 (0.9%)	1.4 (7%)	1.5 (2.9%)	111
Copper	<0.1 (NC2)	1.2 (34%)	0.48 (5%)	0.81 (126%)	0.83 (26%)	0.46 (31%)	0.33 (3.3%)	–	47 (5.5%)	–	–	–	149
Lead	<0.1 (NC2)	<0.1 (NC2)	<0.1 (NC2)	0.79 (NC2)	0.12 (6.7%)	<0.1 (NC2)	<0.1 (NC2)	1.6 (7.7%)	66 (6.1%)	3.8 (26%)	<0.1 (NC2)	1.3 (43%)	128
Manganese	0.18 (42%)	27 (15%)	41 (25%)	124 (156%)	29 (23%)	3.3 (14%)	5.0 (13%)	1.9 (17%)	63 (0.93%)	1.7 (0.9%)	1.4 (7%)	<0.2 (NC2)	
Nickel	<0.1 (NC2)	145[**] (34%)	<0.1 (NC2)	<1 (NC2)	7.9 (NC2)	<1 (NC2)	<1 (NC2)	1.6 (22%)	25 (5.5%)	<10 (NC2)	<10 (NC2)	<10 (NC2)	48.6
Zinc	<5 (NC2)	8.1 (21%)	<5 (NC2)	11 (NC2)	6.2 (34%)	10 (6%)	55 (20%)	5.4 (44%)	147 (3.9%)	<10 (NC2)	<10 (NC2)	<10 (NC2)	459

[*] Concentrations of arsenic, cadmium, selenium, and silver were <1 μg/g.

[**] Potential contamination.

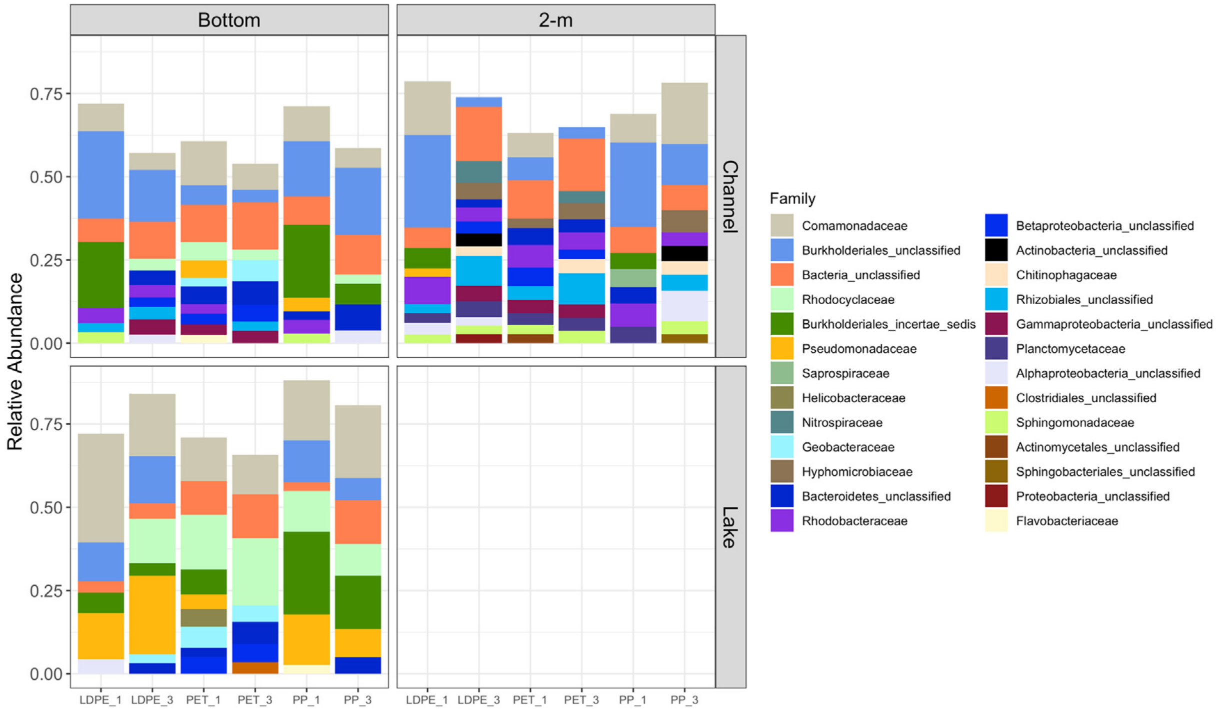

Fig. 6. Relative abundance of bacterial family-level OTUs at the three sampling sites classified by MP polymer type and length of incubation (1 = 1 mo; 3 = 3 mo). Samples from the 2-m depth in the lake were unrecoverable. MP abbreviations as in Fig. 5 caption. These include only bacterial families composing at least 2.5% of total abundance, thus values do not reach a total sum of 100%.

Ashton et al., 2010) can accumulate on plastics and possibly induce toxicity to organisms that ingest them (Rochman et al., 2013; Gallo et al., 2018; Menéndez-Pedriza and Jaumot, 2020).

Our study adopted an experimental approach by incubating three types of MPs for two different incubation durations in a drowned-river mouth lake that connects to Lake Michigan. Our results clearly indicate that all three MP types have the potential to accumulate POPs, although the degree of accumulation varied depending on the MP type, pollutant type, and sampling location, indicating that caution is needed when generalizing about the role of plastics as a source for organic pollutants. Certain organic pollutants, such as PCBs and PAHs, were found to concentrate up to 380 times the background aqueous concentrations within one month. Given the current estimates that 50% of microplastics found in waterways are 17 years in age or older, the timescale for our experiments is relatively short (Koelmans et al., 2016). The concentration factors observed in these experiments for POP and metals may be far from equilibrium and hence, more conservative than those found for microplastics in the environment with longer residence times. It is expected that longer residence times would also incur more degradation resulting in greater surface area, and a greater capacity for adsorption. Furthermore, the microplastic

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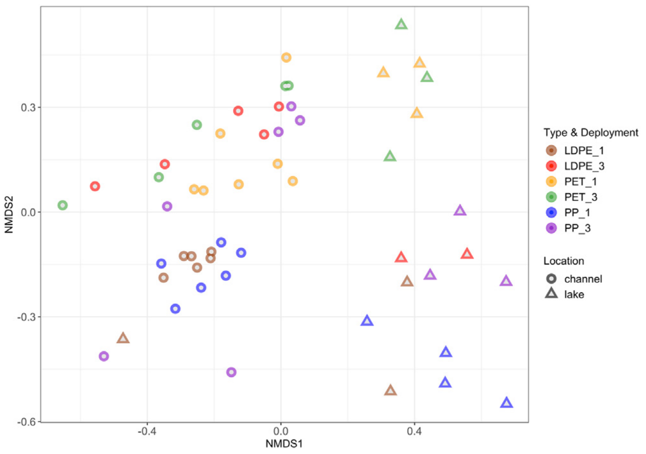

Fig. 7. NMDS of Muskegon Lake bacterial communities associated with microplastics. Abbreviations as in Fig. 5 caption.

materials used for this study were on the larger end of the size spectrum and the smaller sized materials typically found in the environment may also have a larger adsorption capacity due to an increase in surface area.

Although PAH compounds accumulated on all MP types and at all sites, the concentrations still were approximately one order of magnitude lower than those measured in the lake sediment. The transfer of PAH compounds from water to plastic debris is thermodynamically driven and based on fugacity where higher molecular weight compounds accumulate at slower rates (Lee et al., 2017). Our results followed a similar trend where 6-ring PAH compounds showed lower levels of accumulation than 3 and 4-ring structures. The mean PAH lake sediment concentration in our study (255 ng/g) was generally similar to the highest concentration measured at any of the Muskegon Lake sites sampled in 1999 (143 ng/g; Carter et al., 2006). These concentrations are much less than the PEC (22,900 ng/g), which is the pollutant concentration above which sediment-residing detritivores (e.g., oligochaetes and chironomids) are adversely impacted (MacDonald et al., 2000). However, a more recent study at a nearshore area in Muskegon Lake, close to former sources of industrial discharges, found very high PAH concentrations (maximum of ~770,000 ng/g; EA Engineering, Science, and Technology 2013), with 7 of 15 sampling locations having sediment PAH levels exceeding the PEC. The variability in sediment PAH concentrations in Muskegon Lake is a function of the different locations of sources around the lakeshore, as well as several sediment remediation projects in this Great Lakes Area of Concern. The sites used in the current study were not located near any of the former industrial discharge sites, and even after 1 and 3 months of incubation, PAH concentrations on plastic remained several orders of magnitude lower than the PEC. Given that the lake sediment had up to 10× the PAH levels than the microplastics, it is unlikely that the microplastics used in our study serve as a significant additional source for the sediment fauna, although smaller sized micro- or nanoplastics may have a negative impact; in addition, planktivores filtering large volumes of water for food may be negatively affected (Setälä et al., 2014).

Unlike PAH concentrations that showed very little variation among polymer types, PCB levels were 4–6× greater on LDPE than on PET or PP, with the maximum mean concentrations on LDPE reaching ~13 ng/g. PCB concentrations in Lake sediment were ~4 ng/g, which were greater than the concentrations associated with PET and PP, but were less than those on LDPE. The greater PCB levels associated with LDPE are not due to levels contained in the virgin plastic, which are similar to those in the other plastic types; there appears to be differences in the biofilm composition among the plastic types (see below), which may account for the variability. The PEC for PCBs is 676 ng/g, indicating the PCB concentrations in both the sampled sediment and on the incubated microplastics are unlikely to present a significant toxicity threat if consumed by aquatic organisms. Depending on their bioavailability, PCBs associated with microplastics are in the range that may be of concern for bioaccumulation (Kelly et al., 2007). How-

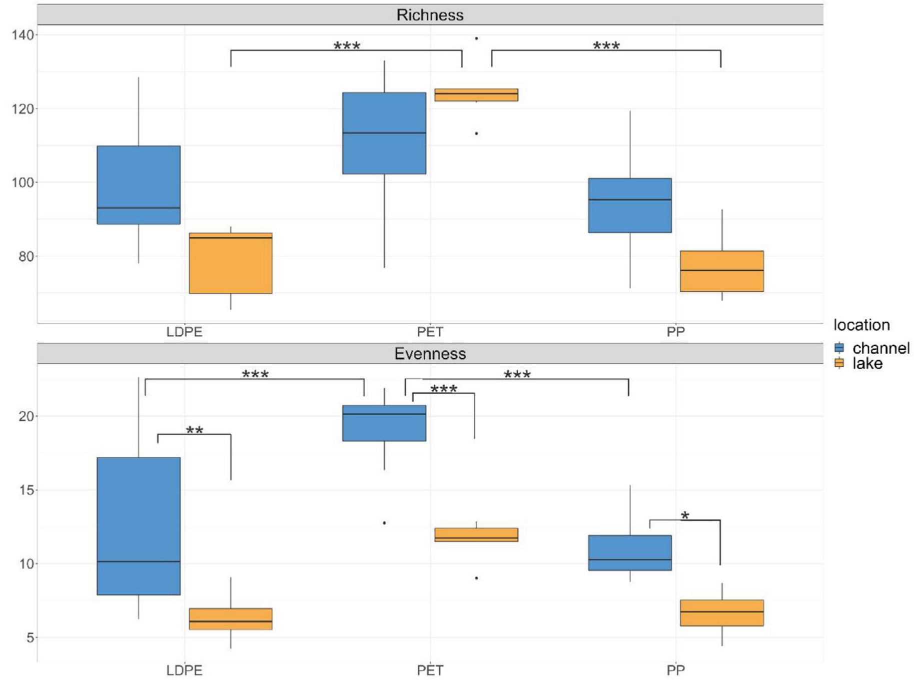

Fig. 8. Bacterial family-level alpha diversity (richness and evenness) estimates for the three MPs incubated at two locations in Muskegon Lake. Asterisks indicate level of significance: *** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$.

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ever, other studies measuring PCB levels at Muskegon Lake sites closer to contamination sources have exceeded the PEC (e.g., 1870 and 2045 ng/g; EA Engineering, Science, and Technology, 2013).

Total OCs are synthetic pesticides that belong to the group of chlorinated hydrocarbon derivatives; they are known for their high toxicity, slow degradation, and bioaccumulation (Jayaraj et al., 2016). Overall, their average concentrations were very low (<2.0 ng/g), which is not surprising given that most OCs were banned in the USA and other regions in the 1970s and 1980s. Nonetheless, our data show that OC concentrations were greater on the microplastics than on the sediment, especially on polyethylene (LDPE) and at CH 2-m. Several OCs can still be detected in the environment due to their environmental persistence (Yang et al., 2015). MacDonald et al. (1996) identified a PEC for the sum of dichlorodiphenyltrichloroethanes (DDTs) at 51.7 ng/g, which is much higher than even the elevated concentrations on PP and LDPE at CH 2-m in our study. The question of OC bioavailability needs to be evaluated as bioaccumulation may be possible (Kelly et al., 2007).

With very few exceptions, metals accumulation was minimal on the three MP types over time. There was elevated Mn after 1 month at the two channel sites on all MPs; in the case of PET, this may have been attributable to the high Mn concentration in the virgin plastic, but this was not the case for the other two MP types. With the exception of Mn, metal concentrations on all MPs were much lower than that in Lake sediments or their respective PEC levels; metal concentrations in Lake sediment continue to decline (Nelson and Steinman, 2013), and the concentrations in this study are equivalent to or lower than those reported in Carter et al. (2006). These results suggest that metal accumulation on MPs is unlikely to have a strong negative effect on organisms in Muskegon Lake, but may be a source for bioaccumulation.

Bacterial colonization of MPs can influence sorption and release of contaminants, as well as their trophic transfer. Hence, characterizing the biofilm is an important step in understanding its role in microplastic ecology in the real world (Rummel et al., 2017). Several freshwater studies have shown differing levels of fidelity between bacteria community composition and substrate type. McCormick et al. (2016) found that bacterial composition differed among microplastic, seston, and the water column at sites upstream and downstream of wastewater treatment plants in Illinois (USA), while Fujimoto et al. (2016) found both seasonal and spatial variability in the plankton composition, with variation higher among particle-associated than free living bacterioplankton along a transect from Muskegon Lake into Lake Michigan. Similarly, we found differences in bacterial alpha diversity estimates (both richness and evenness) between the three MPs, as well as differences in evenness among plastics incubated in Muskegon Lake and the channel that leads to Lake Michigan. We also found that bacterial alpha diversity estimates appear to be higher on PET relative to the other polymer types, particularly for the lake location.

The relatively coarse level of taxonomic discrimination in the current study compared to Fujimoto et al. (2016) limits the value of any direct comparisons, but in general we saw little variation in bacterial composition from 1 to 3 months (with the exception of CH 2-m on LDPE). This difference among studies may be a result of a more homogenous substrate surface (MPs) than a variety of particle types in the open water or our coarser level of taxonomic resolution. Studies in marine systems have shown that the Rhodobacteraceae are often early colonizers of surfaces (Zettler et al., 2013); similarly, taxa from the same family were more abundant at 1- vs. 3-months on all polymer types at both depths in the channel, suggesting that taxa from this family are early colonizers in both fresh and salt water. Rhodobacteraceae are also capable of degrading PAH compounds with small numbers of aromatic rings (Gutierrez et al., 2011). Although we did not detect the family on any MP at Lake bottom, it may have been present but below our 2.5% abundance threshold for analysis.

Fujimoto et al. (2016) found that the most abundant particle-associated taxa were chemoorganoheterotrophs (i.e., organisms that require organic substrates to obtain the carbon required for growth and development). In our samples, the functional attributes of the most abundant groups were variable. The family Comamonadaceae, which were present at all sites and on all MPs, is a large and diverse bacterial family that form a phylogenetically related cluster but also have tremendous functional diversity that includes aerobic organotrophs, anaerobic denitrifiers and Fe (III)-reducing bacteria, hydrogen oxidizers, photoautotrophic and photoheterotrophic bacteria, and fermentative bacteria (Willems et al., 2014). Similar to the Comamonadaceae, the Burkholderiales family is phenotypically, metabolically, and ecologically diverse, including strictly aerobic and facultatively anaerobic chemoorganotrophs, obligate and facultative chemolithotrophs, and nitrogen-fixing organisms among others. Its presence was more abundant on LDPE and PP than on PET, irrespective of location, perhaps due to the greater diversity on PET, limiting dominance of any one taxon. Previous work examining microbial colonization of poly(3-hydroxybu tyrate-co-3-hydroxyhexanoate) (PHBH) in freshwater systems also found Burkholderiales to be one of the dominant families present within the biofilm community (Morohoshi et al., 2018) and it is a primary degrader of certain biodegradable plastics, such as polyhydroxyalkanoate (PHA) (Boyandin et al., 2013). Similarly, biofilms composed of both Comamonadaceae and Burkholderiales families have been associated with the degradation of PCBs (Macedo et al., 2007; Cupples, 2016). While our experiments indicate that POPs accumulate on microplastics, longer term studies are needed to determine if the attached microbial community is capable of degrading PAH, PCB, and OC compounds. Finally, the greater relative abundances of Rhodocyclaceae and Pseudomonadaceae, which are both in the phylum Proteobacteria, at Lake compared to CH may be related to the much lower DO concentrations at the lake bottom site compared to either channel site. Unfortunately, the loss of samples at the Lake 2-m site, where DO would be greater, precludes any definitive conclusions regarding the role of dissolved oxygen in the distribution of these bacteria families.

Conclusions

Our results indicate that persistent organic pollutants are capable of accumulating on microplastics, but the concentrations generally were lower than their corresponding PEC within our relatively short time scale (3-months). It is unclear from these results if the contaminants found adsorbed to the microplastics were associated with the plastic material, the biofilm present, or both. Further studies would have to be performed to address this issue. With regards to the total sum of all POPs measured in this study, polyethylene concentrated the most total POPs followed by polypropylene, then polyester. There were no consistent differences in bacteria biofilm community structure among MP types and between lake and channel sites, perhaps because of short hydrologic retention times and thorough mixing (Liu et al., 2018) in Muskegon Lake. However, one cannot rule out potential synergistic effects resulting from the presence of many legacy, emerging, and inorganic contaminants on the same material. Furthermore, the presence of exotic bacteria, pathogens, and even organic/inorganic plastic additives could further exacerbate the potential adverse effects to organisms exposed to these materials through ingestion. The findings of this study highlight the importance of considering not only the potential adverse effects of microplastic

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exposure alone to aquatic organisms but also the chemical and biological materials associated with them.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2020.07.012.

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