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Impact of Microplastic on Freshwater Sediment Biogeochemistry and Microbial Communities Is Polymer Specific

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Abstract: Plastic debris is a growing threat in freshwater ecosystems and transport models predict that many plastics will sink to the benthos. Among the most common plastics found in the Laurentian Great Lakes sediments are polyethylene terephthalate (especially fibers; PET), polyvinylchloride (particles; PVC), and styrene-butadiene rubber resulting from tire wear ("crumb rubber"; SBR). These materials vary substantially in physical and chemical properties, and their impacts on benthic biogeochemistry and microbial community structure and function are largely unknown. We used a microcosm approach to evaluate the impact of these three plastics on benthic-pelagic coupling, sediment properties, and sediment microbial community structure and function using sediments from Irondequoit Bay, a major embayment of Lake Ontario in Rochester, New York, USA. Benthic metabolism and nitrogen and phosphorous cycling were all uniquely impacted by the different polymers. PET fibers and PVC particles demonstrated the most unique effects, with decreased ecosystem metabolism in sediments containing PET and greater nutrient uptake in sediments with PVC. Microbial diversity was reduced in all treatments containing plastic, but SBR had the most substantial impact on microbial community function, increasing the relative importance of metabolic pathways such as hydrocarbon degradation and sulfur metabolism. Our results suggest that individual polymers have unique impacts on the benthos, with divergent implications for ecosystem function. This provides deeper insight into the myriad ways plastic pollution may impact aquatic ecosystems and will help to inform risk assessment and policy interventions by highlighting which materials pose the greatest risk.

Keywords: microplastic; nutrient cycling; freshwater lakes; sediment; microbial community structure and function

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1. Introduction

With global plastic production surpassing 300 million tonnes each year [1], the accumulation of plastic debris in the environment is an increasingly critical issue with largely unknown implications for both ecosystem and public health. Plastic debris is now found in a diverse array of aquatic ecosystems [2–6], including the Laurentian Great Lakes [7,8]. These lakes comprise the world's largest surface freshwater system and millions of people depend on this resource for food, drinking water, and tourism [9]. As such, inputs and impacts of plastic pollution are a significant concern. The Great Lakes are also a gateway to the Atlantic Ocean through the St. Lawrence Seaway, and may act as a conduit to the oceans,

especially for buoyant plastic debris. Modeling studies [10–12] and field observations suggest that plastic debris behaves differently in freshwater systems than oceans: rather than accumulating in a large floating "patch" [7], debris distribution in the Great Lakes is determined by source location and transport [8,13,14]. Plastic ultimately accumulates nearshore in the benthos and on beaches, in concentrations that can be >10,000 particles kg^{-1} [15–18]. Further, plastic began accumulating in the benthos of Lake Ontario, the terminal lake of the Laurentian Great Lakes system, between 20 and 40 years ago [16], with largely unknown impacts to the ecosystem.

Freshwater benthic ecosystems are highly diverse and critical in regulating trophic dynamics and recycling and removing carbon and other nutrients [19]. These functions are largely driven by a diverse community of benthic microbes, but contamination to sediments may alter microbial community structure and function, with cascading effects [20–23] and the potential to disrupt ecosystem services like water purification and climate regulation. In the environment, plastics are subject to conditions that may alter their physical and chemical properties (e.g., microorganisms, UV exposure, water-borne contaminants), influencing fate and subsequent impacts. Microplastics impact microbial diversity and function in soils [24–26], marine sediments [27–34], and sewage sludge [35–38]. The distinct microbial composition on plastic surfaces often differs from communities in the surrounding environment [28,39], may accumulate toxins or pathogenic microbes [40–45], and shift ecosystem processes in the water column and sediment. Potential impacts to key processes, like carbon and nitrogen cycling [36,46–49], require further investigation to fully understand how microplastics alter ecosystem function and microbial community structure in the benthos of the Great Lakes.

While the impacts of plastic pollution on freshwater systems remain understudied compared to marine environments, recent evidence suggests that microplastics induce similar disruptions to microbial community structure and function by shifting microbial diversity [50–55]. For example, in riverine systems, increased relative abundance of nitrifying bacteria in plastic-associated bacteria [56] may increase nitrification in sediments and wastewater effluents [35], creating nitrification hotspots in affected streams and sediments. Alterations in nitrogen cycling from shifts in sediment microbial communities may cascade to impact primary production, trophic structure, and water quality, underscoring the importance of the benthos in whole-ecosystem dynamics. Further, because different plastics have unique impacts on nitrification and denitrification activity [57], it is critical to investigate multiple polymers rather than treating "plastic" as a single pollutant [58].

Plastics vary substantially in their chemical composition and physical properties, both of which influence fate and environmental impacts. While many types of plastics have been identified in freshwater benthos, polymers are not equally distributed and may therefore have different impacts on ecosystem function. Polymers with a higher material density, like synthetic microfibers, (e.g., polyester, nylon), polyvinyl chloride (PVC), and tire wear particles comprised of butylated rubber are among the most commonly reported materials in nearshore sediments [59–61]. Fibers have been found in quantities as high as 34,000 fibers kg-sediment dry $weight^{-1}$ (kg-dw^{-1}) [62] in the Great Lakes basin. Despite being one of the most commonly reported polymers and with a substantial recent investigation into microfiber transport pathways [5,61,63,64], little is known about ecosystem impacts in freshwater environments. PVC is another plastic of concern due to its high material density and frequent use in construction materials that may release significant microplastic particles over time [65]. PVC may negatively affect bioturbating organisms [66] and reduce denitrification [57] in marine ecosystems, and shift both denitrification and nitrogen fixation rates in freshwater systems [36,67]. Tire wear particles have been found at densities up to 5500 particles kg-dw^{-1} [60]. Despite rising concern about toxicity and ecosystem impacts [68–71], the full effects of butylated rubber particles on aquatic environments remain unclear.

By evaluating how the ecological impacts of different plastics vary within the same system, we can better understand risks and generate a more targeted policy. In this

study, we use a microcosm approach to evaluate the impacts of three commonly identified consumer microplastics—polyethylene terephthalate microfibers from clothing (PET); styrene-butadiene rubber (SBR) "crumb rubber" from tires and athletic turf fields; and PVC particles from construction material—on carbon, nitrogen, and phosphorus cycling, community metabolism, benthic microalgal abundance, and microbial community structure and function. This work adds to our growing holistic understanding of microplastic pollution in freshwater systems.

2. Methods

2.1. Microcosm Design and Set-Up

Sediment was collected from the mouth of Irondequoit Bay, a major embayment on the south shore of Lake Ontario (43.2349° N, 77.5337° W), to a depth of approximately 10 cm using a 9.5 cm diameter polycarbonate core tube, separated into depth profiles (0–2, 2–5, 5–10 cm), and sieved through a 1-mm mesh to remove rocks, plants, and macroinvertebrates. Microcosms were created in clean polycarbonate tubing (9.5 inner diameter × 30 cm) sealed at the bottom with a leached butyl rubber stopper by reconstructing the sediment layers. To remove any easily leachable contaminants, all polycarbonate tubes and stoppers were soaked in water for 30 days prior to use in the collection of sediments or as microcosms. Tubes were wrapped with opaque plastic below the sediment–water interface to prevent light penetration to sediment below the water interface. The headspace of each microcosm was filled with approximately 1 L of artificial freshwater [72] and stored in a 416 L recirculating Living Stream tank (FrigidUnits, Inc., Toledo, OH, USA). The tank was held at 23 °C and illuminated with full-spectrum lights on a 14:10 h light:dark cycle to simulate summer conditions, and microcosms were individually aerated with room air using airline tubing attached to an aquarium pump. Climate data suggests that the water temperature of Irondequoit Bay can range from 21–26 °C during the summer months when sediment was collected [73], making 23 °C a realistic temperature for the conditions we aimed to simulate. Microcosms were acclimated in the tank for four weeks to restore microbial communities and porewater solute concentrations prior to imposing experimental conditions. During this time, the water in the tank was held above the level of the microcosms so that the headspace was continuous for all units.

2.2. Material Additions

Following the acclimation period, each treatment microcosm received microplastic additions at a value of 0.1% of whole sediment core dry weight. This value was selected using concentrations found in the literature for the Great Lakes [59–61]. Materials were sourced with the intention of using post-consumer items where possible. Ten representative particles of each were measured to determine the size distribution of each material. On average, the SBR crumb rubber and PVC particles used in this study were 1.8 mm and 0.66 mm, respectively. The fabric used for the PET fibers was sourced as a large sheet that was cut into smaller strips and then ground in a coffee grinder until they reached a size fraction of 5.5 mm average length. Additional characteristics of the polymers, including the sourcing of each material, are provided in the Supplemental Information Table S1. In addition, we assessed the potential leaching of additives from each polymerusing the methods of Rani et al. [74]; these results are also provided in the Supplemental Information (Figures S1–S3). PET fibers were sourced from a local fabric retailer JoAnn Fabrics, Rochester, NY, USA, though the exact brand is unknown. The SBR "crumb rubber" was sourced from Al's Liner Systems (Product #: ALS-RC), Cloverdale, IN, USA. Hard PVC particles were sourced from Sigma Aldrich, Burlington, MA, USA. (Product #: 389293).

Each plastic was thoroughly mixed into 150 g wet weight of additional surface layer (0–2 cm) sediment along with 0.75 g of Urtica powder to replenish organic matter, and this mixture was added to the top of each core. Control microcosms received a similar addition of plastic-free surface layer sediment (n = 4 per treatment). An additional 45 g of surface layer sediment was added on top of the treatment layer to prevent particle resuspension,

creating a total sediment height of 12.5 cm. Prepared microcosms were placed back in the tank and incubated for 30 d. The water level in the tank was drawn down 10 mm below the level of the microcosms to isolate the headspace while maintaining a consistent temperature; aerators were reinstalled in each unit to mix the water column and oxygenate the water column.

2.3. Oxygen and Nutrient Flux Measurements

After 30 d, sediment–water column fluxes of oxygen, nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^{3-}) were measured in the dark and light. These fluxes of nitrogen- and phosphorus-containing solutes were used to elucidate shifts in nutrient cycling. By measuring sediment uptake or release to the water column, and the variation between rates in the light and dark, we can infer changes in microbial and algal uptake and transformation [75]. Similarly, the sediment–water column flux of oxygen in the light and dark can be used as a proxy for benthic ecosystem metabolism (carbon cycling) and microalgal gross primary production [76]. Each microcosm was filled completely with artificial freshwater and tightly sealed with a clear polycarbonate lid fitted with a rubber o-ring. The rubber stopper inserted into the sampling port in the center of the lid was fitted with a small swivel that held a magnetic stir bar. During the flux measurements, microcosms were placed around a central core tube fitted with a motorized magnetic stir bar that spun each small magnet at approximately 60 rpm in order to prevent the build-up of a diffusion gradient at the sediment interface. Microcosms were tightly wrapped with aluminum foil to create a dark environment for the first half of the experiment.

Samples were taken every two hours, with the first three measurements in the dark and the last two in the light. Oxygen concentrations were measured using a self-stirring dissolved oxygen probe (Hach LDO-BOD with HQ40D meter), and water samples were taken using a 60-cc syringe. Water removed during sampling was replaced with a known volume of artificial freshwater, and this dilution was accounted for in flux calculations. Water samples were immediately filtered through a 0.45 µm PES membrane filter and stored at −20 °C until analysis. Nitrate was analyzed using a vanadium-based method [77], ammonium was measured using the phenol-hypochlorite method [78], and phosphate was measured using the ammonium molybdate method [79]. Flux rates were calculated from the change in concentration over time [75], with daily rates based on the sum of light and dark measurements scaled to a 14:10 h light:dark diel cycle. Gross primary production (GPP) was calculated using the difference between the hourly oxygen flux in the light and dark, assuming respiration is the only oxygen-consuming process occurring in the dark.

2.4. Sediment Properties

The following day, sediments were sampled for oxygen penetration depth, benthic chlorophyll *a* (Chl *a*), sediment microbial community structure, and potential denitrification. Fecal mounds from tubificid worms were evident at the surface, likely from juveniles not removed during sediment preparation. Before destructive sampling, the number of fecal mounds was recorded. Following all destructive measures, the remaining sediment was sieved through a 1-mm mesh to isolate and quantify tubificid worms. The depth of oxygen penetration into the sediment was assessed based on the visible color change of the sediment (n = 3 depths averaged per microcosm). Sediment anoxic depth was measured similarly, using visual assessment of the different coloration (n = 5 depths averaged per microcosm). Benthic Chl *a* and microbial samples were taken in duplicate using clean 5-cc syringe corers to 1 cm depths and placed in 15 mL centrifuge tubes to be stored at −80 °C until further analysis. Syringe corers designated for sediment microbial samples were rinsed with 70% ethanol and allowed to dry before and between sampling to prevent contamination.

Chl *a* samples were immediately wrapped in aluminum foil to prevent light exposure, frozen at −80 °C, and analyzed within 30 d. Pigments were extracted by sonication in 90% acetone, followed by a 24 h extraction at −20 °C. Samples were then centrifuged, and absorbance of the supernatant was measured at 665 nm and 750 nm on a Shimadzu UV-1800

spectrometer (Shimadzu Scientific Instruments; Columbia, MD, USA). spectrophotometer before and after acidification using 1N HCl [80]. Chl *a* and phaeopigment concentrations were calculated using equations described by Lorenzen (1967) [81].

2.5. Potential Denitrification

Sediment for potential denitrification was collected using a 60-cc syringe corer to a depth of 2 cm, immediately placed in 50 mL centrifuge tubes, and refrigerated until the following day. Potential denitrification was measured using the acetylene inhibition method [82,83] in 160 mL serum bottles with septa lids. After adding 20 g of soil, 10 mL of sparged nanopure water and 10 mL of media (nitrate 100 mg^{-1} kg^{-1} + dextrose 40 mg^{-1} kg^{-1} + chloramphenicol 10 mg^{-1} kg^{-1}), anaerobic conditions were ensured by flushing each serum bottle with N_2 gas for 3 cycles of 2 min each, shaking the bottles in-between flushes. Using a gas-tight syringe, 11 mL of acetylene was added to each bottle. Gas samples were taken immediately after adding acetylene and injected into an evacuated gas-tight vial. Bottles were placed on an orbital shaker (125 RPM), and additional samples were taken after 30, 60, and 120 min. Gas samples were analyzed using a Shimadzu Gas Auto Analyzer Gas Chromatograph (Shimadzu Scientific Instruments; Columbia, MD, USA). Potential denitrification rates were calculated based on the increase in N_2O over time.

2.6. Microbial Community Structure

Bacterial community structure was evaluated using the Illumina 16S amplicon sequencing protocol [84,85]. Genomic DNA was extracted from soil samples using the DNeasy Powersoil Kit (Qiagen; Germantown, MD, USA) following the manufacturer's protocol. Two µL genomic DNA from each sample was assessed for DNA concentration using a DNA High Sensitivity Kit on a Qubit 3.0 fluorometer (Thermo Fisher Scientific; Waltham, MA, USA) diluted to a concentration of 5 ng/µL in molecular grade nuclease free water, and 2.5 µL DNA from each extraction was used to amplify the variable 3 and 4 regions of the 16S rDNA using a GoTaq Green PCR (Promega Corporation; Madison, WI, USA) kit with the V3/V4 primers. Samples were then run on two 1% agarose gels to check for the presence of 16S rDNA V3/V4 amplicon at 550 bp. Then, 2.5 µL DNA at 5 ng/µL from each library was amplified with a KapHiFi PCR kit (Qiagen; Germantown, MD,USA), cleaned with AMPureXP (Beckman Coulter; Brea, CA, USA) beads, indexed using Illumina Nextera XT index adapters (Illumina; San Diego, CA, USA), and cleaned again with AMPureXP beads before checking for fragment size distribution using DNA 1000 chips on the Agilent 2100 bioanalyzer (Agilent Technologies; Santa Clara, CA, USA). Fragment size distribution and DNA concentrations from a final Qubit 3.0 check were combined to determine the molarity of sequencing-ready libraries, and libraries were diluted to a concentration of 4 nM. Samples were pooled, denatured, and diluted to a final loading concentration of 6 pM with a 10% PhiX spike-in. The pooled libraries were then run on an Illumina V3 (Illumina; San Diego, CA, USA). 600 cycle cartridge for 2 × 250 bp [84,85].

QIIME2-2022.8 software was used to demultiplex the 16S rRNA sequences in 29 samples (three samples were eliminated in the final analyses due to quality) [86], remove chimeras, and the final 8,140,642 sequences were denoised using the DADA2 1.22.0 plugin for QIIME2-2022.8 software [87]. The SILVA SSU 138.1 database was used to assign taxonomy and formatted by using RESCRIPt 2022.8.0 software [88,89]. Artifacts were converted into a phyloseq object using R-Studio 2020.03.01 and R version 4.2.3 [90–92] and then transformed into a 'microtable' for the Microeco 1.3.0 package [93]. All ASVs (amplicon sequence variants) not assigned to Archaea or Bacteria, and those assigned to "mitochondria" or "chloroplast" were removed. The 'tidy dataset' function was used to trim the dataset to eliminate samples with 0 artifacts, and the 'microtable' was rarefied to a sample size of 100,000 sequences per sample [94].

2.7. Statistical Analysis

Statistical analyses pertaining to metrics of ecosystem function (nutrient fluxes and sediment properties) were completed using JMP 15.0 Pro software; analysis of sediment microbial communities was completed using JMP Pro 16.0 software and RStudio 2020.03.01.

Prior to analysis, all data were assessed for normality and heterogeneity of variance to verify assumptions for analysis of variance (ANOVA). For the analysis of nutrient fluxes and sediment properties, one-way ANOVA was used to compare plastic types for all analyses, apart from ammonium and nitrate fluxes, and to compare the presence of worms among the different treatments. When significant effects were found (p-value ≤ 0.05), Dunnett's post-hoc tests were used to identify if treatment groups differed significantly from the control. Ammonium and nitrate flux data could not be transformed before analysis to meet assumptions of ANOVA, and Kruskal–Wallis tests were used. Dunn's tests were used to analyze if treatment groups differed significantly from the control group when significant effects were found. To compare hourly nutrient fluxes in the light and dark for each treatment, paired t-tests were used. To further evaluate the whole suite of biogeochemical variables, a principal component analysis (PCA) was run.

The relative abundance at the Kingdom, Phylum, Class, Order, Family, Genus, and Species levels was calculated, and data visualizations were performed in R using the *ggplot2* package [95]. Here we present only results for Phylum and Family. The relative abundance of each family was compared using the non-parametric Kruskal–Wallis test to evaluate differences among treatments. When a difference was observed, post hoc evaluation was used to compare each treatment to the control using Dunn's test with control for joint ranks. Diversity metrics for each plastic treatment and control were calculated using the Microeco package for R, including Total Observed Features, Faith's Phylogenetic Diversity (PD), and Shannon (H') Diversity Indices. Beta diversity analyses were performed in Microeco to evaluate community structure among treatments. Dissimilarity was visualized using non-metric multidimensional scaling and pair-wise Bray-Curtis dissimilarity estimates. The top metabolic functions (by percent) were determined using the FAPROTAX package in Microeco [93,96]. The relative abundance of each functional group was compared between each plastic type and the control using student's t-test.

3. Results

3.1. Nutrient Fluxes and Sediment Properties

Plastics significantly and uniquely impacted key ecosystem processes (Table 1 and Figure 1). While GPP did not differ among treatments (Table 1, Figure 1A, $p = 0.31$) and all sediments were net heterotrophic and generally similar, PET-containing sediments had a lower NEM than the control (Table 1, Figure 1B, $p = 0.02$). Along with decreased NEM, sediments containing PET had marginally deeper sediment oxygen penetration (Table 1, Figure 1C). Sediments containing plastic had a more developed and distinct anoxic layer beneath the oxidized surface layer, especially in treatments containing SBR and PVC (Table 1, Figure 1D).

The presence of SBR and PVC was also associated with unique effects on nutrient cycling: nitrate fluxes were negligible in all treatments except for SBR (Figure 1E, $p = 0.04$), and both ammonium (Figure 1F, $p = 0.03$) and phosphate (Figure 1G, $p = 0.03$) were released from the sediments to the water column in all treatments except for PVC, where sediment uptake was significant. Sediments containing PVC had decreased potential denitrification (Figure 1H; $p = 0.58$) and slightly higher benthic Chl *a* content (Figure 1I, $p = 0.08$). Tubificid worm abundance was substantially higher in PET treatments (Figure 1J, $p = 0.01$).

Table 1. Results of one-way analysis of variance or Kruskal–Wallis tests examining the effect of plastic on net ecosystem metabolism (NEM), gross primary production (GPP), sediment oxygen penetration (oxic depth), sediment anoxic depth (anoxic depth), daily fluxes of nitrate, ammonium and phosphate, colonization of sediments by tubificid worms (Worm Count), microalgal chlorophyll *a* (Chl *a*) content, and potential denitrification (PDNF). Significant effects ($p < 0.05$) are bolded. Values with asterisks indicate a Chi-square value from Kruskal–Wallis tests used for non-parametric data. All degrees of freedom are 3.

Variable	Plastic Type	
	F/X^2	***p***
GPP	1.32	0.31
NEM	4.97	**0.02**
Oxic Depth	3.38	0.05
Anoxic Depth	2.82	0.08
Daily NO_3^-	7.91 *	**0.04**
Daily NH_4^+	8.93	**0.03**
Daily PO_4^{3-}	3.93 *	**0.03**
PDNF	2.84	0.58
Chl *a*	2.84	0.08
Worm Count	5.59	**0.01**

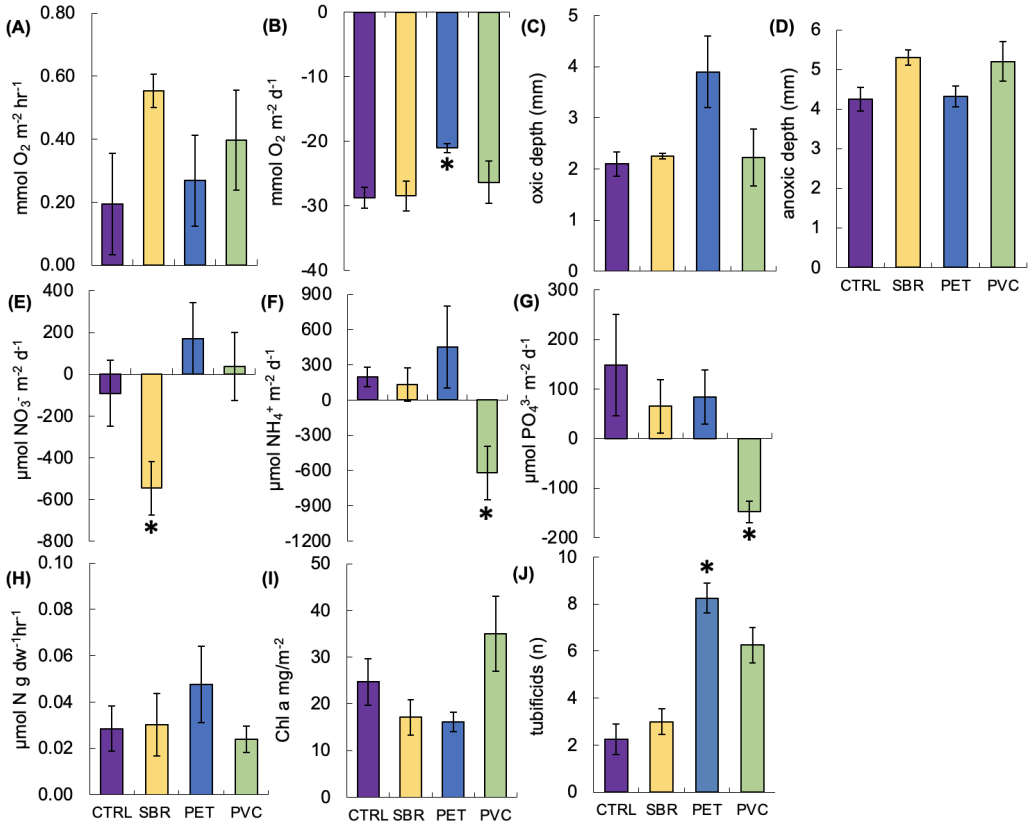

Figure 1. Gross primary production (GPP; **A**); net ecosystem metabolism (NEM; **B**); sediment oxygen penetration (**C**); sediment anoxic depth (**D**); daily sediment–water column fluxes for NO_3^- (**E**); NH_4^+ (**F**); and PO_4^{3-} (**G**); potential denitrification (**H**), benthic microalgal Chl *a* (**I**), and tubificid worm abundance (**J**), measured after 30 d. Values are mean ± SE, n = 4. Negative flux values indicate the uptake of the solute by the sediments. Asterisks represent significant differences compared to the control based on post hoc testing.

The impact of microalgal activity was demonstrated in some cases by differences in flux rates between light and dark. In control treatments only, the uptake of nitrate in the light was modestly higher than in the dark ($p = 0.07$). Ammonium uptake in the PVC treatment occurred only in the light, with release in the dark (Table 2, $p = 0.08$). No differences were observed between light and dark fluxes of phosphate ($p > 0.1$ for all).

Table 2. Hourly fluxes of nitrate (µmol m^{-2} h^{-1}), ammonium (µmol m^{-2} h^{-1}), phosphate (µmol m^{-2} h^{-1}) in the light and dark after 30 day incubations (mean ± SE, n = 4). Negative values indicate the uptake of the solute by the sediments. Values in bold are significantly different between light and dark at $p < 0.1$.

Variable	Light	CTRL	SBR	PET	PVC
NO_3^-	Light	**−43.8 ± 18.3**	−24.6 ± 14.0	26.4 ± 9.7	−23.6 ± 12.5
	Dark	**52.1 ± 17.5**	−20.2 ± 27.7	−19.9 ± 23.1	36.8 ± 24.4
NH_4^+	Light	−7.1 ± 12.6	21.3 ± 11.6	9.1 ± 20.0	**−68.0 ± 31.7**
	Dark	29.9 ± 13.7	−16.5 ± 8.7	30.7 ± 10.2	**33.2 ± 24.3**
PO_4^{3-}	Light	11.6 ± 9.1	6.1 ± 5.4	9.7 ± 4.6	−2.9 ± 1.3
	Dark	3.9 ± 3.3	1.7 ± 4.5	0.5 ± 4.6	−7.0 ± 1.3

The PCA resulted in two main components that together explained 49% of the data variability (28.7% Component 1; 20.3% Component 2). The biplot of the PCA shows distinct grouping based on the plastic type, with PVC and PET separating from the control and SBR treatments (Figure 2). PET separates primarily on Component 1, which is defined primarily by reduced NEM, a deeper oxic layer, and worm abundance (Table 3). PVC separates on Component 2, which is associated with high GPP and Chl *a*, and sediment uptake of ammonium and phosphate (Table 3).

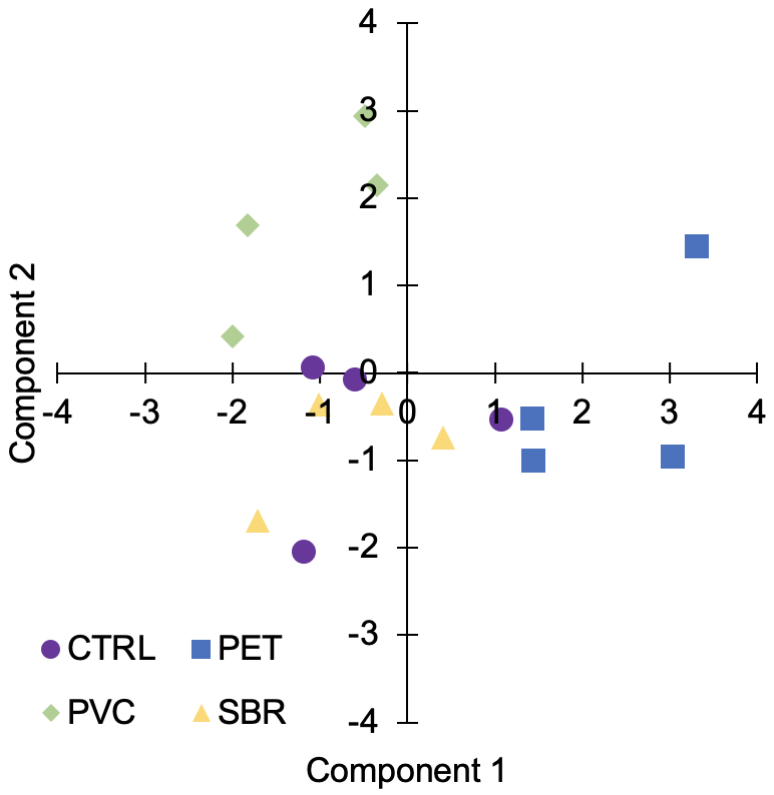

Figure 2. Biplot of factor scores from two strongest principal components. Components 1 and 2 explain 28.7% and 20.3% of variability in the data, respectively.

Table 3. Factor loading for the first two Principal Components.

Variable	Component 1	Component 2
NEM	0.42	0.28
GPP	−0.07	0.44
Oxic Depth	0.45	0.33
Daily NO_3^-	0.23	0.30
Daily NH_4^+	0.38	−0.37
Daily PO_4^{3-}	0.23	−0.38
PDNF	0.18	−0.20
Chl *a*	−0.18	0.45
Worm Count	0.54	0.07

3.2. Microbial Community Structure: Diversity and Function

The analysis of relative abundance within the microbial community across different treatments at the taxonomic level of the family showed trends in microbial community structure within the three treatments compared to the control (Figure 3, Table S1). The families of *Clostridiaceae* and *Erysipelotrichaceae* in the phyla Firmicutes were consistent across all treatments ($X^2 = 2.05$ $p = 0.56$ and $X^2 = 1.21$ and $p = 0.75$), although both families were slightly higher (7–10%) in sediments containing SBR.

Desulfocapsaceae was slightly, but not significantly, greater than the control in PET treatments ($X^2 = 3.07$, $p = 0.38$).

Relative abundance of *Rhodocyclaceae* was also 52% higher in sediments containing PVC compared to the control and was the overall highest among the three treatments. While there were few differences among the individual families across treatments, the trends in alpha diversity were more clear.

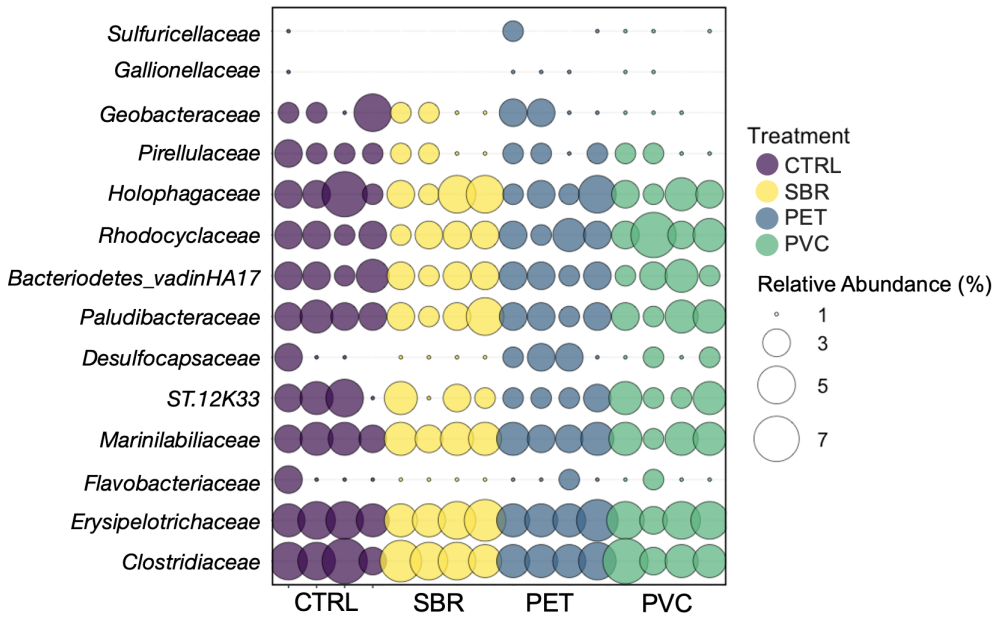

Figure 3. Bubble graph depicting relative abundances of the top 14 bacterial families among the four treatments. The size of the bubble is proportional to the relative abundance of each bacterial family, indicated by the relative abundance legend.

Alpha diversity was reduced to some extent in all three polymer treatments relative to the control (Figure 4 and Table 4). Total observed features were 28 % to 43 % higher in the control compared to all plastic treatments, with sediments containing PET having the lowest value ($F = 4.76$, $p < 0.01$). While there was no difference in the Shannon index (H')

across treatments, Faith's phylogenetic diversity was 16-23% greater in the control than SBR and PET, but not different from PVC ($F = 4.76$, $p < 0.05$) (Figure 4, Table S2).

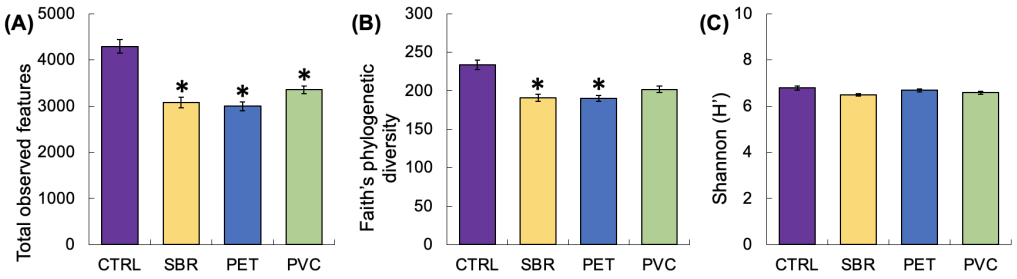

Figure 4. Alpha diversity including total observed features (**A**), Faith's phylogenetic diversity (**B**), and Shannon's diversity (**C**). Asterisks indicate significant differences from the control identified in Dunnett's post-hoc tests.

Table 4. Results of one-way analysis of variance examining three different alpha diversity indices of microbial communities identified in each treatment group. All degrees of freedom are 3. Values in bold indicate significant differences at $p < 0.05$.

Diversity Index	F	*p*
Total observed features	7.06	**0.005**
Faith's phylogenetic diversity	4.76	**0.02**
Shannon index (H')	0.83	0.50

Differences in specific metabolic functions may further reflect changes in community structure (Figure 5). Sediments containing SBR had the most notable differences in metabolic functions when compared to the control ($p < 0.05$), including greater sulfate reduction, methanotrophy, hydrocarbon degradation, respiration of sulfur compounds, methylotrophy, fermentation, and anaerobic chemoheterotrophy. PET treatments also demonstrated higher anaerobic chemoheterotrophy than the control ($p < 0.05$). PVC was slightly higher for anaerobic chemoheterotrophy and methylotrophy ($p < 0.1$).

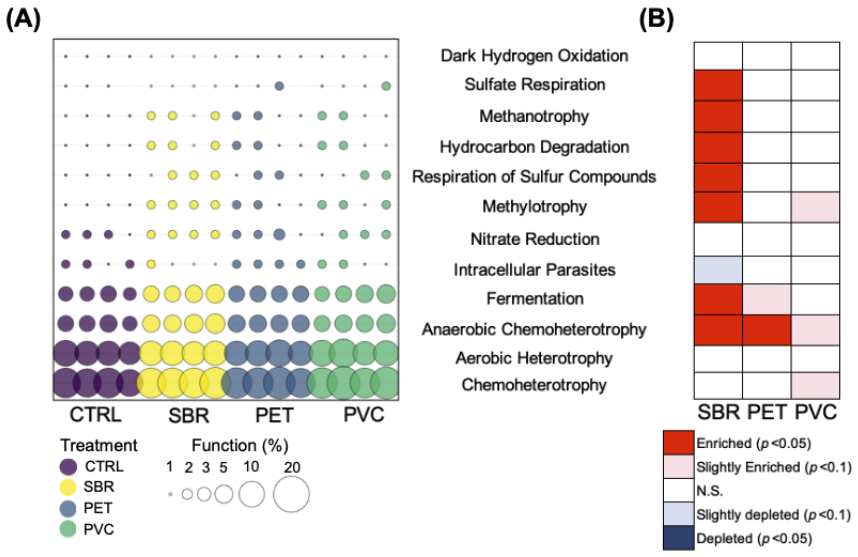

Figure 5. Top 12 metabolic function inferred from taxonomic analyses represented by bubble plots depicting the relative proportion of functional attributes across the four treatments (**A**); heat map illustrating the statistical significance for functional enrichment (red) or depletion (blue) compared to the control sediments (**B**).

4. Discussion

In this study, we show the unique impacts of three commonly encountered plastics on freshwater benthic ecosystem function, including primary production and nutrient cycling. While others have investigated the impacts of microplastics on freshwater ecosystem function [97–101], this is the first study, to our knowledge, to examine this specific set of diverse plastics under such a comprehensive combination of metrics in freshwater sediments, and the results point to a clear need for additional work to better understand the potential risk that microplastic poses to benthic ecosystems. The ubiquity of both use (i.e., PET clothing, tire debris from vehicles, and PVC in construction/tools) and the environmental presence of the polymers selected for this study suggest a broad applicability of the work and also highlight the extraordinary complexity of the "plastic problem".

Each product has a characteristic chemical composition and set of physical properties that provide utility during the use phase and also give rise to the unique ecological impacts observed here. While the use of plastic polycarbonate tubing for sediment collection and containment of the microcosms could introduce confounding impacts, the divergent results observed here relative to the control also stored in these same tubes, seem to suggest that the polycarbonate tubing had negligible effects. Microbial diversity was reduced to varying degrees in all plastic treatments (Figure 4). Overall diversity, measured as total observed features (similar to species richness) was lower for all plastics. In addition, Faith's PD, a measure of how each of the observed ASVs may be evolutionarily related to one another, was reduced with SBR and PET, suggesting a contraction in overall community structure and more similarity among microbes within each plastic-associated community [102,103]. The shift in the microbial community, combined with other direct and indirect effects on the benthos, led to distinct ecosystem characteristics and functions associated with each polymer. PET fibers altered ecosystem metabolism, whereas PVC altered nitrogen and phosphorus cycling. SBR enhanced sediment uptake of nitrate and fostered the most unique microbial community structure and function relative to all other treatments. These results provide deeper insight into the myriad ways plastic particles may alter the benthic environment and will help to inform risk assessment and policy interventions by highlighting which materials pose the greatest risk.

4.1. PET Impacts Ecosystem Metabolism

PET fibers, which separate on the first component of the PCA, shifted metabolism and increased oxygenation of surface sediments (Figure 2). In addition, there was an increase in anaerobic chemoheterotrophy (Figure 5), potentially related to sulfur metabolism associated with a minor shift in Desulfocapsaceae and Sulfuricellaceae (Figure 3). The substantial tubificid worm populations, found only in the microcosms containing PET (Figure 1I), likely contributed to the impact by mixing surface sediment and consuming organic matter. While the presence of invertebrates typically increases NEM [104–110], we suspect that bioturbation of surface sediments along with organic matter consumption during the 30 d experiment led to the exhaustion of organic matter reserves and ultimately reduced NEM and increased oxygen penetration. However, we also observed worms entangled in fibers, suggesting a strong direct interaction. Toxicity was not directly measured here; however, in other work, we observed both physical and chemical impacts of plastic on similar benthic species [111]. Here, we observed worms as a significant potential driver of the effects observed in PET microcosms (Figure 2). It is unclear whether physical interactions (entanglement) alone or in concert with chemical toxicity drove the combined direct and indirect effects of fibers on ecosystem function. Analysis of chemical additives in the polymers studied here identified three main additives in the PET, including two plasticizers (Bis(2-ethylhexyl)isophthalate; DEHP, and ethylhexyl thioglycolate) and the dye used to color the fabric orange (Coumarin 7). Both plasticizers identified have been noted as significant environmental and human toxins [112–115] and likely play a role in the impacts observed to tubificid worms. Given the critical role of benthic organisms as ecosystem engineers (e.g., [19]), an additional study investigating the mechanism of toxicity across

polymers (i.e., morphology, chemical composition, ingestion, entanglement) is needed to fully understand the impact of plastic pollution in waterways.

4.2. PVC Impacts Nutrient Cycling

Measured ecosystem function in sediments containing PVC was most distinct from both the control and other plastic treatments, particularly in the enhancement of benthic nutrient uptake (Figure 1E,F). Greater uptake of ammonium in the presence of microplastic is consistent with findings in intertidal systems [116], but not other marine sediments [66]. PVC separates from other treatments on the second component of the PCA (Figure 2), where GPP and Chl *a* are positively loaded and phosphate and ammonium flux are negatively loaded (Figure 2). This suggests a link between benthic microalgal abundance and GPP and sediment nutrient uptake, which is further corroborated by the greater ammonium uptake in the light and release in the dark (Table 2). Combined, these results suggest a PVC-enhanced microalgal production and ammonium uptake. Alternatively, photosynthesis may create oxic zones that facilitate nitrification and also lead to the observed pattern in ammonium uptake. The increased uptake of ammonium and slight release of nitrate to the water column (relative to the control) is consistent with an increase in nitrification activity in freshwater sediment in the presence of PVC [35]. A third potential pathway for ammonium uptake is via anammox (anaerobic ammonium oxidation) in the anoxic layer, which was not assessed here. One route for increased microalgal production is through the provision of substrate for microalgal growth. Other explanations may include the role of additives leaching from PVC to the environment.

Prior studies also suggest that plastics have direct impacts on nitrogen cycling in sediments and soils [57,117–119] where the presence of PVC and accompanying suite of additives may further disrupt nitrogen removal. PVC is typically produced with a number of additives that have been shown to leach into the environment [120,121], presenting the potential for additional ecotoxicological impacts to microbial communities. For example, titanium dioxide is a common UV stabilizer and colorant used in plastic manufacturing [122], and a concerning emerging contaminant [123]. Titanium dioxide has negative impacts on nitrification and denitrification activity in soils [124], and may have contributed to the slightly suppressed denitrifying activity in the sediment containing PVC here. Our analysis of other additives (see Supplemental Information) did not uncover additional clues to support a clear explanation of drivers, suggesting a need for further evaluation of the impact of this very common material on aquatic ecosystems.

4.3. SBR Promotes Unique Microbial Communities

Sediments containing SBR had few measured functional changes, with only an increase in sediment nitrate uptake. However, this treatment had the greatest alteration in potential microbial function, with enhanced sulfate respiration/respiration of sulfur compounds, hydrocarbon degradation, methanotrophy, methylotrophy, fermentation, and anaerobic chemoheterotrophy (Figure 5). These results suggest a substantial shift in community structure, likely prompted by a change in organic and inorganic substrates. Our additives analysis revealed that the SBR used in this study features 12 branched hydrocarbons, and 12 long straight changed hydrocarbons; other hydrocarbons may be present but not detected with the methods used here. This enrichment of sediments with hydrocarbons may explain the enhanced hydrocarbon degradation. Several genera of microbes are known hydrocarbon degraders, including *Acinetobacter*, *Pseudomonas*, [125–127] and *Claocibacterium* are among the dominant hydrocarbon degraders found in aquatic systems, including freshwater [126]. Analysis at the phylum level (Supplemental Information, Figure S5) identified a greater presence of Firmicutes in sediments containing SBR, which may be attributed to the enhanced fermentation of hydrocarbons (Figure 5). There is growing evidence and concern surrounding the environmental impacts of SBR and similar rubbers used in turf fields and tire manufacturing [128–132]. Many of the additives present in materials like SBR are hazardous to aquatic life, particularly quinones [133–136]. Polycyclic aromatic hydrocarbons

(PAHs), especially pyrenes, are also common chemicals released from rubbers [137]. Pyrene may enhance methylotrophy and methanol oxidation, and suppress nitrogen fixation and ammonia oxidation in soils [55]. We observed enhanced methylotrophy and uptake of nitrate by sediments perhaps promoted by suppressed ammonia oxidation. Pyrene may also suppress rare microbes [55], leading to the compression of the diversity illustrated by Faith's PD (Figure 4). Further, many rubbers are vulcanized with sulfur to enhance elasticity. Microbes have long been investigated as a potential mechanism for the desulfurization of tire waste to promote reuse and recycling (e.g., [138]), and this enhancement of sulfur availability may also explain the impacts on sulfur and nitrogen cycling.

5. Conclusions

Plastics are a highly diverse group of materials that are critically important to modern society. The vast array of different polymers, each with unique ecological impacts, creates a complex environmental issue. This work provides a glimpse into the potential cascading environmental effects of plastic pollution in freshwater ecosystems and highlights the complexity and need to investigate the impacts of plastic pollution as a multi-faceted issue. Plastics cannot be treated equally when evaluating ecological risk and require a more targeted approach when generating policy. As plastic debris continues to enter the environment, understanding these unique differences among polymers and across systems will aid in developing more strategic and achievable policies surrounding plastic pollution in order to protect vital aquatic resources like the Great Lakes.

Supplementary Materials: The following supporting information can be found at: https://www.mdpi.com/article/10.3390/w16020348/s1. Figure S1: GCMS chromatogram of additives extracted from PET. Figure S2: GCMS chromatogram of additives extracted from SBR. Figure S3: GCMS chromatogram of additives extracted from PVC. Figure S4: NMDS of the Bray-Curtis dissimilarity distances for sediment microbial communities. Figure S5: Bubble graph depicting the relative abundances of the top 13 phyla. Table S1: Physical attributes and sourcing of plastics used in microcosm experiments. Table S2: Descriptions and statistical output of the relative abundance of microbial families compared across all treatment groups. Table S3: Descriptions and statistical output of microbial function percentages as compared to control sediments.

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