Article

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

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SUPPORTING INFORMATION

Sourcing and physical characterization of plastic polymers

Table S1. Table describing the physical attributes of the plastics used in the microcosm experiments, including shape, source, color, and average size. Size is reported in millimeters as the average ± std err of 10 representative particles.

Material	Source	Average Size (mm)	Color
PET	Polyester fleece fabric, sourced from JoAnn Fabrics, Rochester, New York	5.5 ± 0.46	Orange
SBR	Commercial crumb rubber particles purchased online from Al's Liner Systems (Product #: ALS-RC), Cloverdale, Indiana, USA	1.8 ± 0.12	Black
PVC	Particles sourced from Sigma Aldrich (Product #: 389293; CAS: 9002-86-2)	0.66 ± 0.06	White

Chemical characterization of plastic polymers

Methods.
Qualitative analysis on the additives in the three plastics (PET, SBR, and PVC) was performed using the method of Rani et al. (2015) as discussed in our previous publication (Chomiak et al. 2023). Briefly, 12 mL of dichloromethane was added to 500 mg of each plastic over three additions. After each addition, the samples were sonicated for 20 min and the liquid was transferred to a separate vial via vacuum filtration. The dichloromethane was evaporated to dryness under high purity nitrogen flow. The samples were reconstituted 1 mL of acetonitrile and analyzed by GCMS (Shimadzu QP 2020 with AOC 6000 autosampler) equipped with a Rtx-5MS (30 m x 0.25 mm I.D.) capillary column (Restek). The injection temperature was 300°C operating in split mode (50:1). The oven temperature ramp was held at 60°C for two min, ramped to 200°C at 8°C per min, held at 200°C for 10 min, and finally ramped to 320°C at 8°C per min. The quadrupole mass spectrometer was scanned m/z: 40-300. The mass spectrum from all major peaks in the chromatograms were compared with the NIST library within the software for positive identification. Medium and long chained hydrocarbons were expected to be present, especially for SBR. To check for hydrocarbons, extraction ion chromatograms at m/z 57, a fragment shared by most hydrocarbons, were constructed from each chromatogram.

Results.
Polymer identification followed expected material composition. The GCMS chromatograms of compounds extracted from the 3 polymers are shown in SI Figures 1-3. While PET fibers showed only three additives in our analysis, they appear to be in higher concentration than any additives found in SBR or PVC, shown by the strong GCMS signals. The three additives observed from the PET fibers included plasticizers, Bis(2-ethylhexyl)isophthalate and 2-ethylhexyl trioglycolate, and the dye that made the fibers orange, Coumarin 7.

SBR also shows notable additives, featuring many mid-length (C12-C16) branched hydrocarbons and many long, straight-chained hydrocarbons (C16-C28) alongside one long chained alcohol. These hydrocarbons and alcohols are a potential food source for microorganisms. Due to the similarity in mass spectra of varying hydrocarbons, we are hesitant to name these hydrocarbons without the accompanying authentic standards. It should also be noted that there is a possibility of additional longer chained hydrocarbons present in SBR that may not have been volatile enough to be determined by GC analysis. Overall, PVC did not show many additives, with only a set of mid-length (C10-C14) branched hydrocarbons observed.

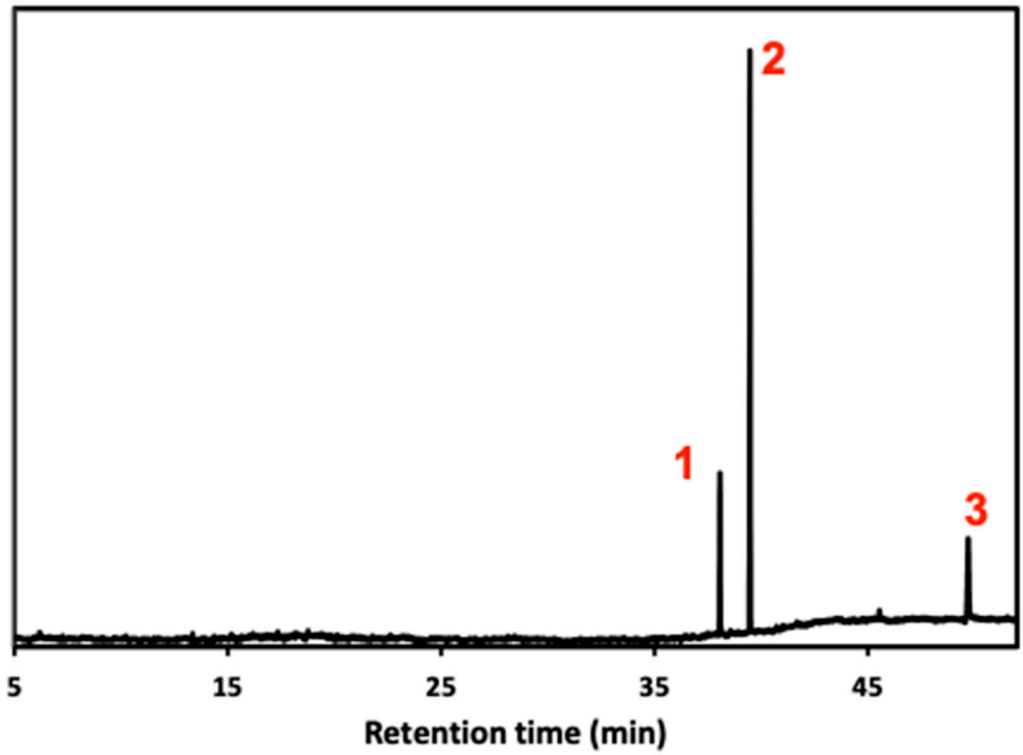


Figure S1. GCMS total ion chromatogram of additives extracted from polyethylene terephthalate using dichloromethane. The three identified peaks are 1) 2-ethylhexyl trioglycolate, 2) Bis(2-ethylhexyl)isophthalate, and 3) coumarin 7. No hydrocarbons were observed from extraction of m/z 57.

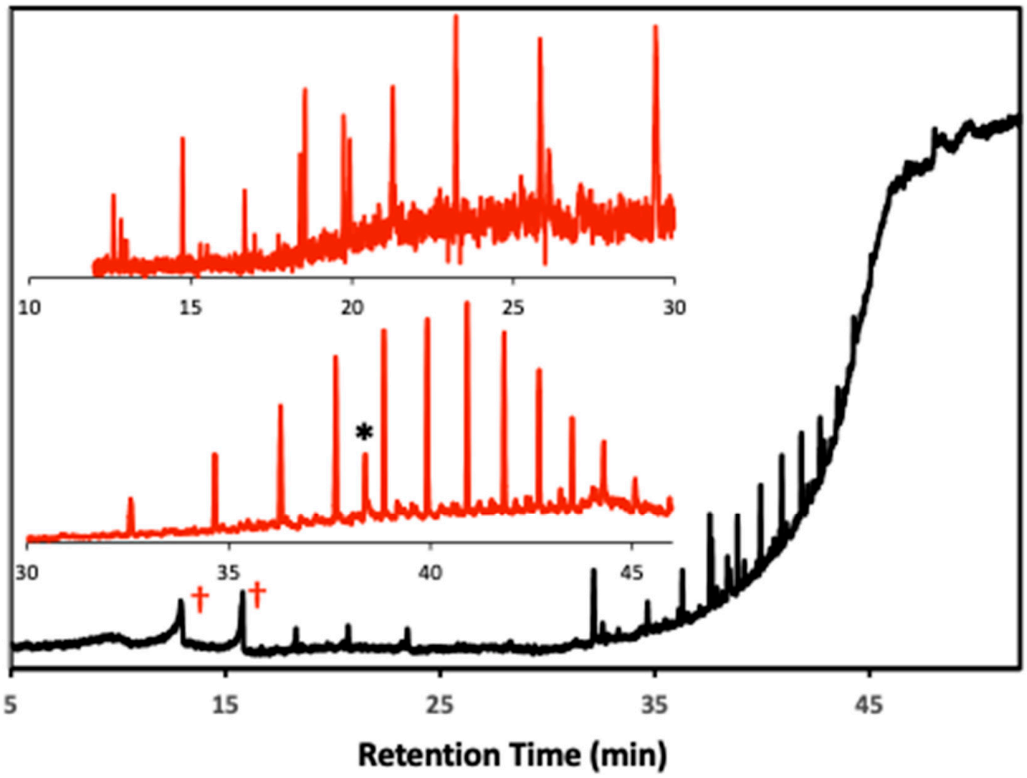


Figure S2. GCMS total ion chromatogram of additives extracted from SBR "crumb rubber" using dichloromethane. The inset chromatograms (red) are extraction ion chromatograms (m/z 57, a fragment shared by most hydrocarbons). Peaks from 11-30 minutes are all branched alkanes (C12-C16) and the larger peaks from 31-45 minutes are from long, mostly straight chained hydrocarbons (C16-C28). The starred peak at 38.4 minutes is from a branched, long-chained alcohol. The two peaks around 13 and 15 minutes (marked with a †) are contaminants from the GC column.

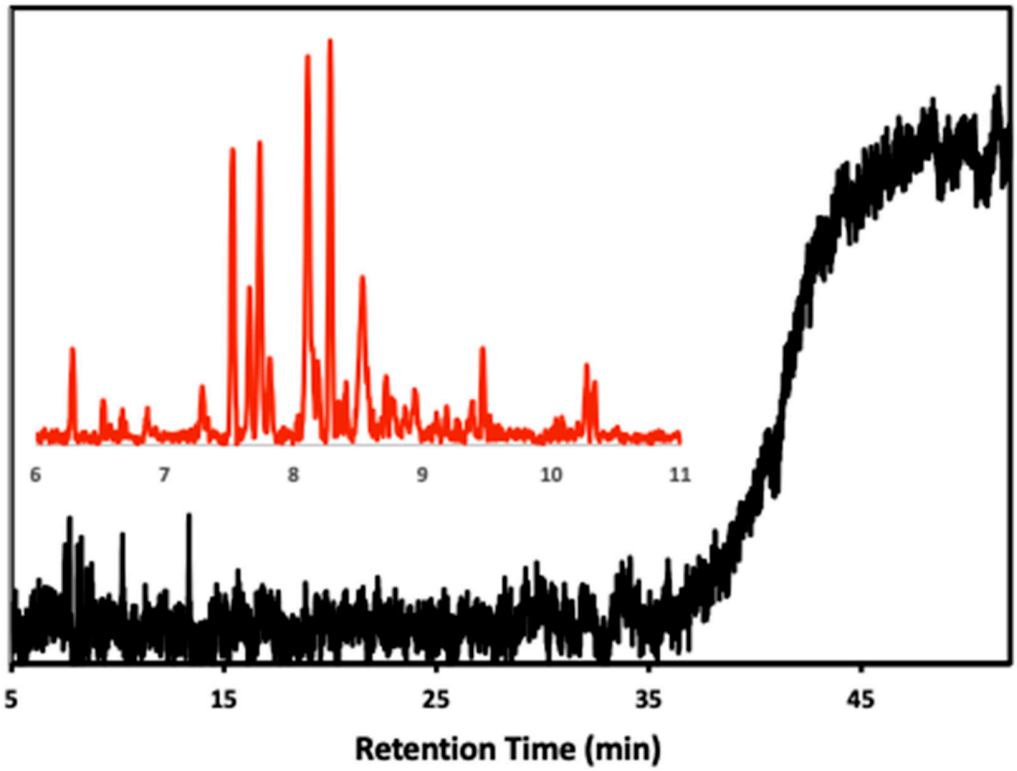


Figure S3. GCMS total ion chromatogram of additives extracted from polyvinyl chloride using dichloromethane. The inset chromatograms (red) are extraction ion chromatograms (m/z 57, a fragment shared by most hydrocarbons). Peaks from 6-11 minutes are all branched alkanes (C10-C14).

Literature Cited – Chemical characterization of plastic polymers

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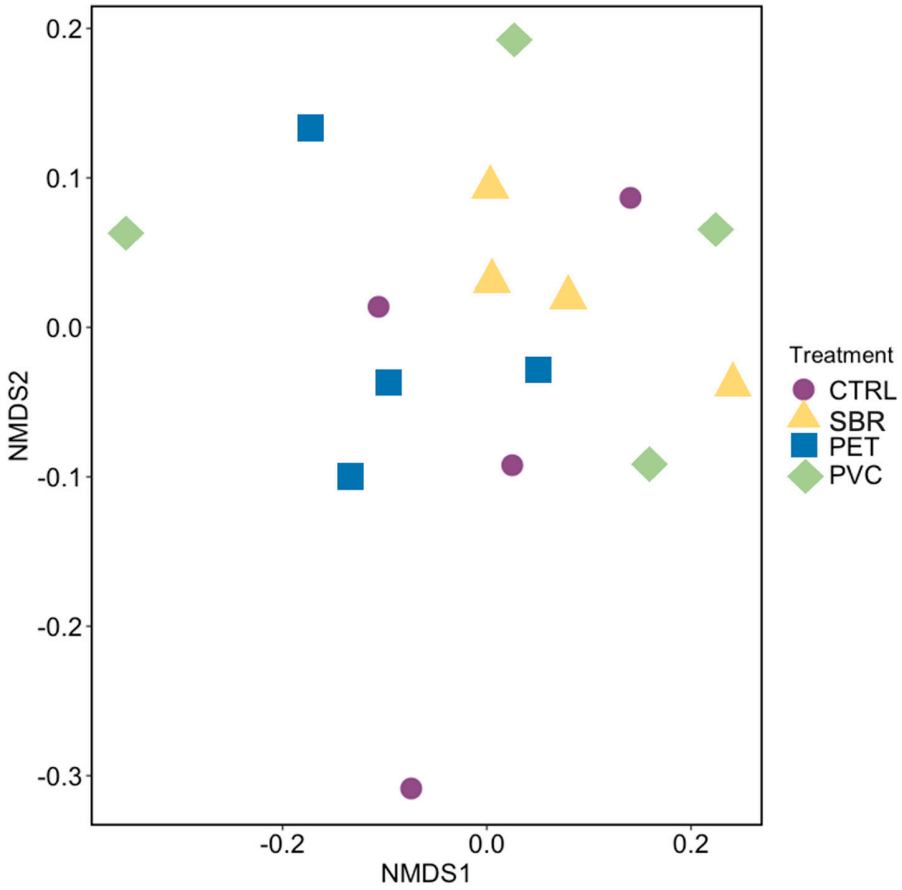


Figure S4. Non-metric multidimensional scaling (NMDS) of the Bray-Curtis Dissimilarity distances for sediment microbial communities. PERMANOVA analysis within all groups and between CTRL show no significance in groupings ($p = 0.342$, $R^2 = 0.029$)

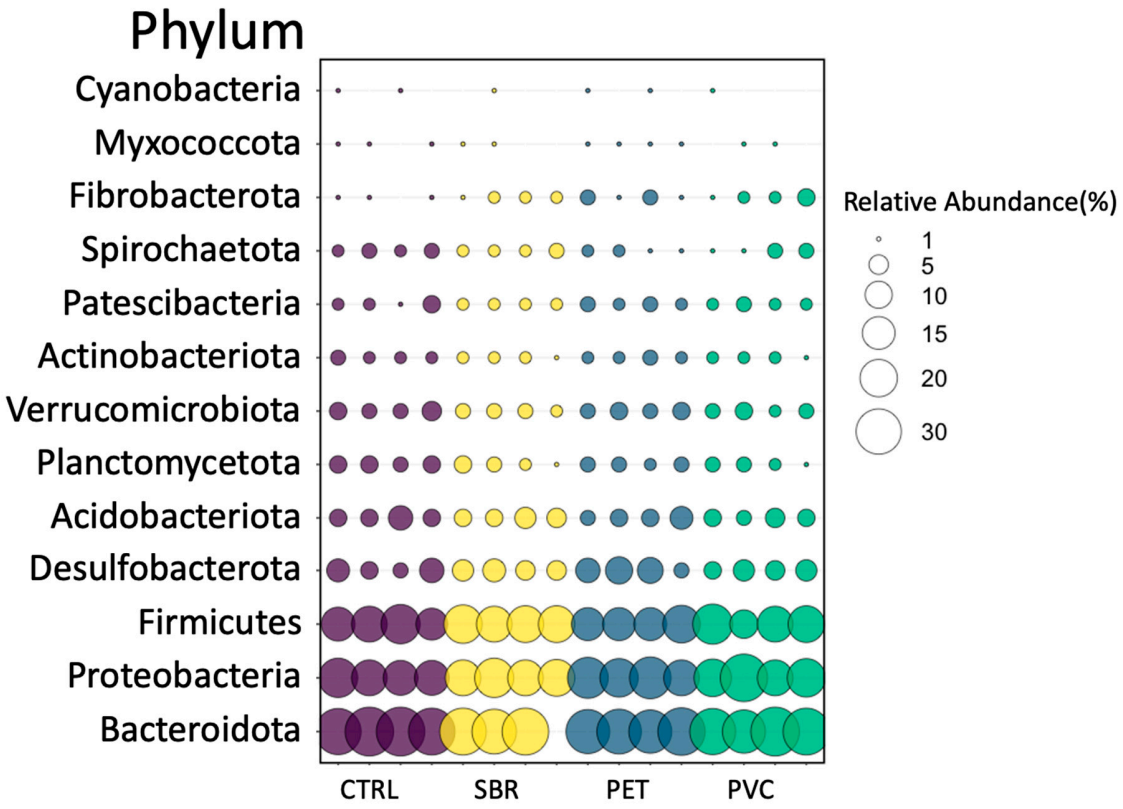


Figure S5. Bubble graph depicting the relative abundances of the top 13 Phyla. Size of the bubble is proportional to relative abundance of each phylum. **SI Table 2.** Table describing the relative abundances by percent of microbial communities at the family level and statistical output from Kruskal-Wallis nonparametric testing evaluating for comparison with the control group.

Table S2. Table describing the relative abundance of microbial families, and the statistical output of differences in relative abundance of each listed family among the different treatment groups, as revealed by Chi-square tests. Statistically significant differences are noted by bold font in the p-value column.

Family Relative Abundance	Treatment	Percent	Chi-square	*p*
Clostridiaceae	CTRL	4.84±0.9	2.05	0.56
	SBR	5.30±0.5		
	PET	4.28±0.4		
	PVC	4.75±0.9		
Erysipelotrichaceae	CTRL	4.51±0.4	1.21	0.75
	SBR	4.83±0.4		
	PET	4.28±0.5		
	PVC	4.67±0.6		
Flavobacteriaceae	CTRL	1.93±0.5	4.5	0.21
	SBR	1.09±0.1		
	PET	1.22±0.1		
	PVC	1.32±0.4		
Marinilabiliaceae	CTRL	3.66±0.2	0.73	0.87
	SBR	3.52±0.4		
	PET	3.40±0.2		
	PVC	3.29±0.4		
ST-12K33	CTRL	3.19±0.8	1.28	0.73
	SBR	2.73±0.5		
	PET	2.39±0.1		
	PVC	3.07±0.6		
Desulfocapsaceae	CTRL	1.25±0.6	3.07	0.38
	SBR	1.17±0.1		
	PET	2.11±0.6		
	PVC	1.48±0.2		
Paludibacteraceae	CTRL	3.35±0.3	1.08	0.78
	SBR	3.25±0.5		
	PET	2.86±0.3		
	PVC	3.00±0.6		
Bacteroidetes_vadinHA17	CTRL	2.92±0.3	0.82	0.85
	SBR	2.76±0.2		
	PET	2.84±0.2		
	PVC	2.65±0.5		
Rhodocyclaceae	CTRL	2.81±0.2	2.27	0.52
	SBR	2.88±0.2		
	PET	2.93±0.4		
	PVC	4.26±1.1		
Holophagaceae	CTRL	3.56±1.1	0.33	0.95

	SBR	3.57±0.71		
	PET	3.10±0.7		
	PVC	2.98±0.5		
Pirellulaceae	CTRL	2.16±0.2	3.93	0.27
	SBR	1.48±0.4		
	PET	1.81±0.1		
	PVC	1.44±0.3		
Geobacteraceae	CTRL	2.34±0.8	2.16	0.54
	SBR	1.53±0.4		
	PET	1.87±0.4		
	PVC	1.08±0.2		
Gallionellaceae	CTRL	0.36±0.2	2.56	0.47
	SBR	0.33±0.2		
	PET	0.64±0.2		
	PVC	0.55±0.2		
Sulfirocellaceae	CTRL	0.33±0.2	2.54	0.47
	SBR	0.31±0.1		
	PET	0.75±0.4		
	PVC	0.81±0.3		

Table S3. Table describing microbial function percentages, and the statistical output of differences of each group from the control as revealed by t-tests. Statistically significant differences are noted by bold font in the p-value column.

Function	**Treatment**	**Percent**	**SE**	**T-ratio**	***p***
Chemoheterotrophy	CTRL	19.67	0.95		
	SBR	21.50	0.66	1.6	0.16
	PET	21.93	0.78	1.8	0.12
	PVC	23.07	1.06	2.4	0.05
Aerobic Chemoheterotrophy	CTRL	13.93	0.63		
	SBR	13.61	0.33	-0.4	0.67
	PET	15.01	0.76	1.1	0.31
	PVC	15.90	1.17	1.5	0.19
Anaerobic Chemoheterotrophy	CTRL	5.74	0.41		
	SBR	7.89	0.43	3.6	**0.01**
	PET	6.93	0.14	2.7	**0.03**

	PVC	7.17	0.43	2.4	0.05
Fermentation	CTRL	5.18	0.37		
	SBR	6.91	0.42	3.1	**0.02**
	PET	6.09	0.13	2.3	0.06
	PVC	6.33	0.55	1.7	0.13
Intracellular Parasites	CTRL	1.75	0.14		
	SBR	1.33	0.13	-2.2	0.07
	PET	1.60	0.02	-1.1	0.32
	PVC	1.42	0.21	-1.3	0.23
Nitrate Reduction	CTRL	1.66	0.10		
	SBR	1.86	0.13	1.2	0.26
	PET	1.91	0.25	0.9	0.38
	PVC	1.92	0.20	1.2	0.28
Methylotrophy	CTRL	1.32	0.11		
	SBR	1.98	0.15	3.5	**0.01**
	PET	1.76	0.23	1.7	0.13
	PVC	1.76	0.15	2.3	0.06
Respiration of Sulfur Compounds	CTRL	1.12	0.12		
	SBR	1.61	0.05	3.6	**0.01**
	PET	1.50	0.23	1.4	0.20
	PVC	1.52	0.21	1.6	0.15
Hydrocarbon Degradation	CTRL	1.07	0.11		
	SBR	1.68	0.12	3.8	**0.008**
	PET	1.49	0.20	1.9	0.11
	PVC	1.41	0.17	1.7	0.14
Dark Hydrogen Oxidation	CTRL	0.96	0.07		
	SBR	0.91	0.05	-0.5	0.62
	PET	0.89	0.04	-0.9	0.40
	PVC	0.93	0.03	-0.3	0.74
Methanotrophy	CTRL	1.05	0.11		
	SBR	1.59	0.12	3.3	**0.01**
	PET	1.45	0.20	1.8	0.13
	PVC	1.35	0.16	1.6	0.16
Sulfate Respiration	CTRL	0.73	0.14		
	SBR	1.13	0.07	2.6	**0.04**
	PET	1.07	0.24	1.2	0.27

	PVC	1.07	0.18	1.5	0.19
Animal Parasites or Symbionts	CTRL	1.01	0.03		
	SBR	1.15	0.11	1.3	0.25
	PET	1.10	0.11	0.7	0.48
	PVC	1.04	0.04	0.6	0.55
Nitrate Respiration	CTRL	0.97	0.06		
	SBR	1.06	0.09	0.8	0.45
	PET	1.13	0.20	0.7	0.49
	PVC	1.22	0.14	1.6	0.16
Nitrogen Respiration	CTRL	0.97	0.06		
	SBR	1.06	0.09	0.8	0.45
	PET	1.13	0.20	0.7	0.49
	PVC	1.22	0.14	1.6	0.16
Human Pathogens	CTRL	0.86	0.05		
	SBR	0.91	0.09	0.5	0.64
	PET	0.88	0.02	0.2	0.82
	PVC	0.91	0.11	1.0	0.34