Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

Microplastic biofilm in fresh- and wastewater as a function of microparticle type and size class

AUTHOR NAMES Kathleen Parrish, and N.L. Fahrenfeld*

AUTHOR ADDRESS Department of Civil and Environmental Engineering, Rutgers, The State University of New Jersey, 500 Bartholomew Rd., Piscataway, New Jersey 08854, United States

Correspondence: nfahrenf@soe.rutgers.edu, (848) 445-8416

KEYWORDS polyethylene microplastic, polystyrene microspheres, ARG, fecal indicator, wastewater

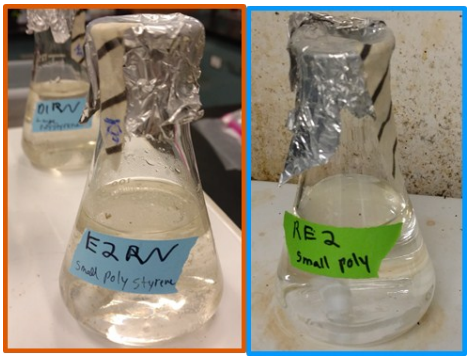

Fig. S1 Batch reactors with either Raritan River water or wastewater influent spiked with microparticles were continuously stirred, as described Methods, to simulate the shear expected for turbulent open channel flow.

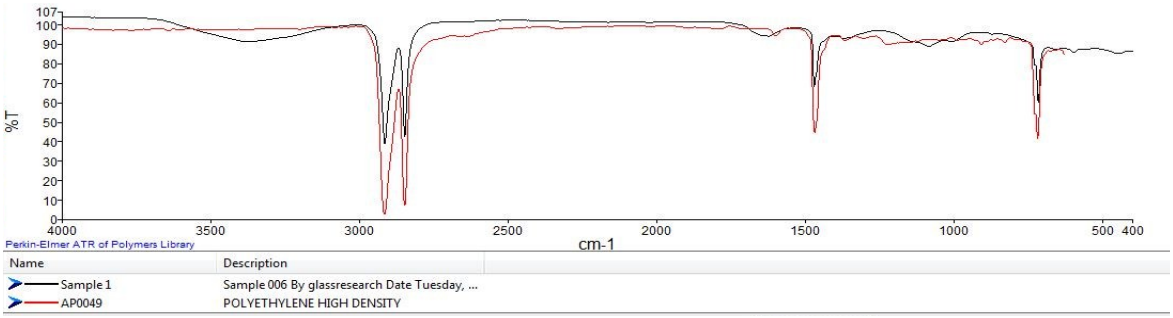

Fig. S2 FTIR spectra for the polyethylene particles extracted from the personal care product (black line) compared to high density polyethylene (red line). The resulting spectrum for the particles had the greatest similarity with the polyethylene standard in the Perkin Elmer spectral library.

Materials

A facial acne scrub was used as the source for the polyethylene particles used in this study. As described in the manuscript, the spectra for the particles was confirmed by ATF FTIR and was a X% match with polyethylene. The other ingredients in the PCP are as follows: water, cetyl alcohol, PPG-15 stearyl ether, polyethylene, glycolic acid, glycerin, polysorbate 60, steareth-21, C12-15 alkyl lactate, cetyl lactate, BHT, neopentyl glycol dicaprylate/dicaprate, cocamidopropyl

PG-dimonium chloride phosphate, potassium cetyl phosphate, diodium EDTA, xanthan gum, sodium benzotriazolyl butylphenol sulfonate, sodium hydroxide, agar, menthol, benzalkonium chloride, iron oxides, mica, titanium dioxide, red 40, red 33, yellow 5, and fragrance.

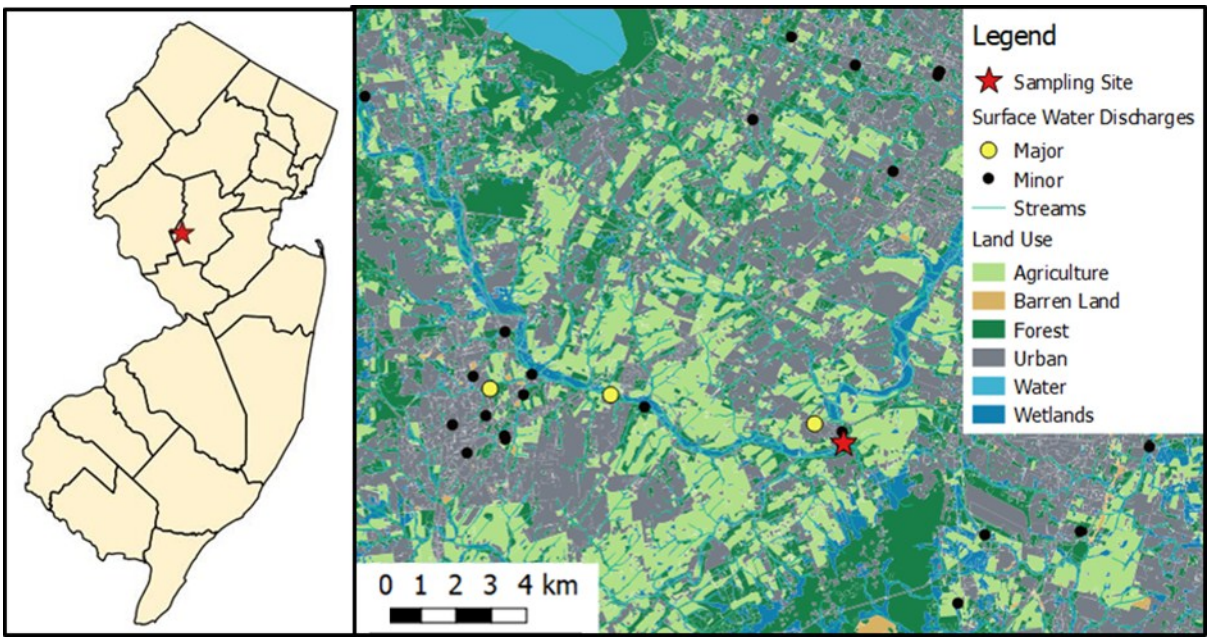

Fig. S3 Map illustrating land use and location of Raritan River water sampling in New Jersey, USA. Major surface water discharges have a permitted discharge of >3.8 million liters per day (>1 million gallons per day).

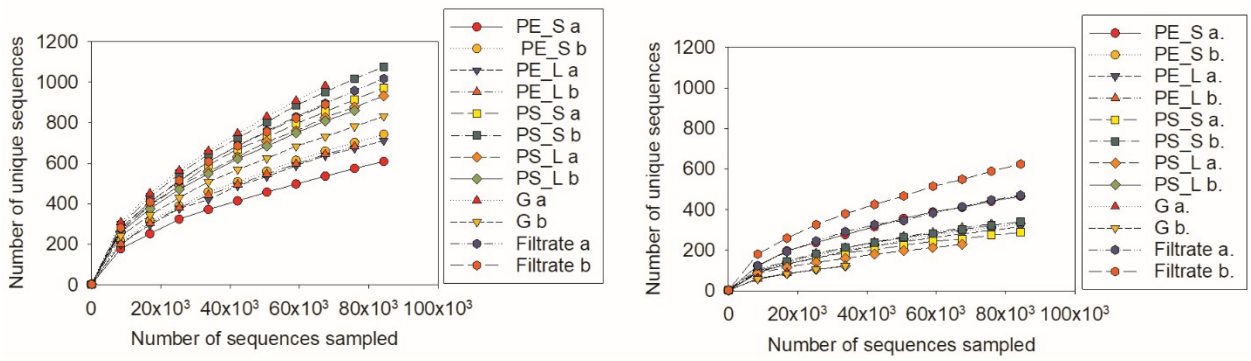

Fig. S4 Rarefaction curves for samples incubated in (a) wastewater or (b) river water. Samples include biofilm from three materials (PE=polyethylene, PS=polystyrene, and G= glass), up to two sizes (S=small, L=large), or filtrate. Samples were analyzed in replicate (indicated by "a" or "b")

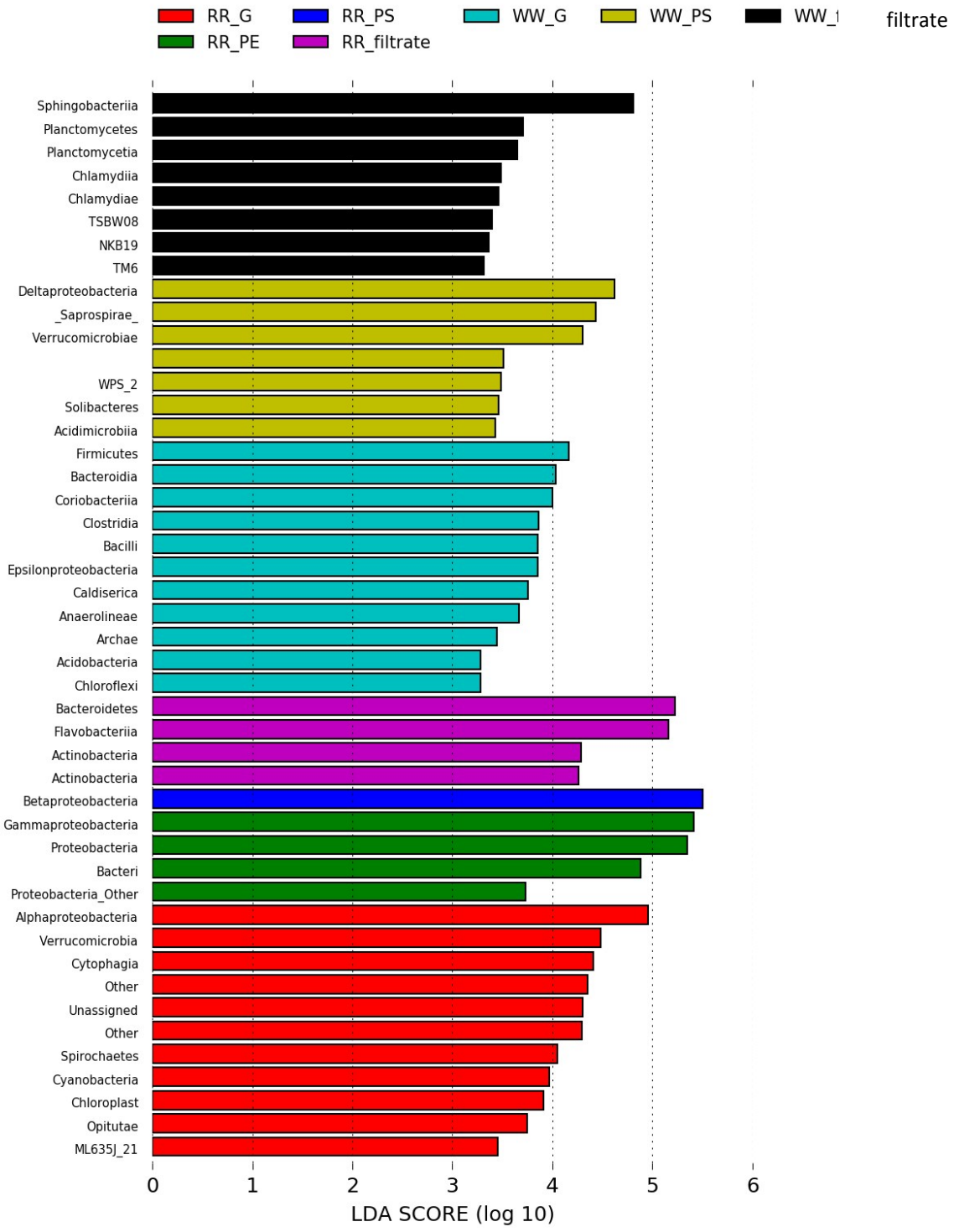

Fig. S5 Significantly discriminative features displayed based on LEfSE biomarker analysis (LDA score >2). Particle material is denoted by PE (polyethylene), PS (polystyrene), or G (glass), unless the sample is of filtrate. Water source is denoted by WW (wastewater) or RR (Raritan River water).

Table S1 Microparticles were measured with a 210 µl scoop for incubation in batch reactors. Particles per scoop were counted.

	Particles per 210 µl sample	Mass per 210 µl sample (g)
Glass	4.03×10^3	0.305
Small PS	1.06×10^5	0.125
Large PS	4.94×10^3	0.123
Small PE	5.73×10^3	0.045
Large PE	1.64×10^3	0.067

Table S2 Primers, annealing temperatures, and amplicon lengths of each gene measured using qPCR.

Gene	Primer sequence	Annealing Temp.(T_a) (°C)	Amplicon length (bp)	Reference
*sul*1	CGCACCGGAAACATCGCTGCAC TGAAGTTCCGCCGCAAGGCTCG	65	163	[1]
BacHum	TGA GTT CAC ATG TCC GCA TGA CGT TAC CCC GCC TAC TAT CTA ATG /56-FAM/TCC GGT AGA CGA TGG GGA TGC GTT /36-TAMSp/	60	81	[2]
16S rRNA	CCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	65	202	[3]

.

Table S3 Chi-squared, degrees of freedom, and p-values for Kruskal-Wallis tests. Where significant differences were observed, p-values corresponding to the output of the posthoc test are reported in the manuscript text.

	Chi-squared	Degrees of freedom	p-value
16S rRNA gene copies (per volume)	20.2	11	0.043
16S rRNA gene copies (per bead)	18.2	9	0.032
sul1 gene copies	18.6	11	0.069
BacHum gene copies	7	11	0.8
Shannon diversity	21.5	11	0.029

Table S4 Average Shannon Diversity Index of each sample reported with the standard deviation. Shannon diversity index is commonly used to characterize species diversity in a community based on the abundance and evenness of species present.

	Treatment	H'(loge) and Standard Deviation
Wastewater	Glass	2.3± 0.17
	Large PE	1.8±0.01
	Small PE	1.8±0.24
	Large PS	2.3±0.01
	Small PS	2.3±0.00
	Filtrate	2.3±0.09
Raritan River Water	Glass	2.0±0.10
	Large PE	1.0±0.14
	Small PE	1.1±0.03
	Large PS	0.99±0.09
	Small PS	0.96±0.07
	Filtrate	1.8±0.06

References

1. Pei, R.; Kim, S.-C.; Carlson, K. H.; Pruden, A., Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Water Research.* **2006,** *40*, (12), 2427-2435.
2. Kildare, B. J.; Leutenegger, C. M.; McSwain, B. S.; Bambic, D. G.; Rajal, V. B.; Wuertz, S., 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific fecal Bacteroidales: A Bayesian approach. *Water Research.* **2007,** *41*, (16), 3701-3715.
3. Muyzer, G.; De Waal, E. C.; Uitterlinden, A. G., Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology* **1993,** *59*, (3), 695-700.