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Microplastic is an Abundant and Distinct Microbial Habitat in an Urban River

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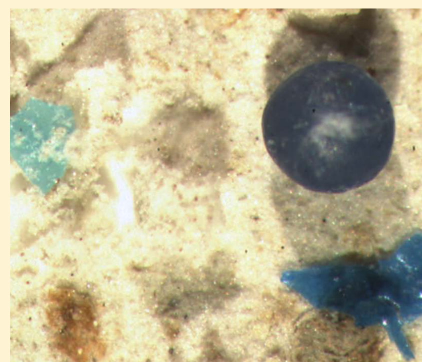
Supporting Information

ABSTRACT: Recent research has documented microplastic particles (< 5 mm in diameter) in ocean habitats worldwide and in the Laurentian Great Lakes. Microplastic interacts with biota, including microorganisms, in these habitats, raising concerns about its ecological effects. Rivers may transport microplastic to marine habitats and the Great Lakes, but data on microplastic in rivers is limited. In a highly urbanized river in Chicago, Illinois, USA, we measured concentrations of microplastic that met or exceeded those measured in oceans and the Great Lakes, and we demonstrated that wastewater treatment plant effluent was a point source of microplastic. Results from high-throughput sequencing showed that bacterial assemblages colonizing microplastic within the river were less diverse and were significantly different in taxonomic composition compared to those from the water column and suspended organic matter. Several taxa that include plastic decomposing organisms and pathogens were more abundant on microplastic. These results demonstrate that microplastic in rivers are a distinct microbial habitat and may be a novel vector for the downstream transport of unique bacterial assemblages. In addition, this study suggests that urban rivers are an overlooked and potentially significant component of the global microplastic life cycle.



INTRODUCTION

Global commerce relies heavily on the production of millions of metric tons of plastic per year,[1] and the abundance and ecological impacts of plastic litter are increasingly recognized as a critical field of study in marine ecology.[2−4] Recent research has found microplastic (i.e., plastic particles <5 mm in diameter[3]) in ocean habitats worldwide including pelagic zones,[5−8] coastal waters,[9−11] coastal sediments,[1,12,13] beaches,[14,15] and the deep ocean.[16] Microplastic sources include industrial resin pellets from manufacturing plants[17] and fragmentation of larger plastic through photolysis, abrasion, and microbial decomposition.[17,18] In addition, some personal care products and cleaners contain microplastic abrasives,[19,20] and washing machine effluent contains microplastic fibers from synthetic textiles.[13] The latter two sources enter the domestic wastewater infrastructure but are often not removed by wastewater treatment plants (WWTPs) due to their small size and buoyancy.[13,19,20] WWTPs have been identified as point sources for microplastic in marine environments. For example, in the United Kingdom, coastal WWTP disposal sites had >250% more microplastic than reference sites, despite over a decade passing since the termination of dumping UK sewage sludge at marine-disposal sites.[13]

Microplastic interacts with organisms in the ocean in multiple ways, including ingestion by consumers, facilitating accumulation of persistent organic pollutants (POPs) into food webs, and the selection of unique assemblages of colonizing microbes. Microplastic ingestion has been documented for marine organisms of varying sizes and trophic levels, from zooplankton to mammals,[17] and microplastic can be transferred from prey to predators.[21,22] Plastics can leach toxic chemicals such as polychlorinated biphenyl (PCB) and nonylphenols, which is a concern for water quality in general and for organisms that ingest plastic.[23] In addition, the hydrophobic surfaces of microplastic readily adsorb POPs that occur at low concentrations in the environment.[18] Plastic pollution adsorbs levels of POPs up to 1 million times higher than ambient concentrations,[23−25] and POPs can desorb inside organisms following ingestion, exacerbating POP bioaccumulation at higher trophic levels.[26,27] Sorption kinetics of POPs on microplastic in variable conditions have only recently been explored (i.e., across salinity gradients and within digestive organs), and vary according to chemical properties of individual POPs.[28,29]

Microplastic in the open ocean supports microbial biofilms that are distinct in taxonomic composition from the microbial assemblages of the surrounding water,[30] suggesting that microplastic surfaces represent a distinct microbial habitat and that processes carried out by microplastic-attached microbes might differ from those in the open water. Microplastic surfaces may also represent a novel mechanism

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for microbial species dispersal,[30] as plastic items can migrate rapidly among marine habitats.[7]

Whereas the ecological effects of microplastic have been documented in oceans, they have not been extensively measured in freshwaters.[31] Recent studies have found microplastic in a remote lake in Mongolia[32] and in Laurentian Great Lakes habitats including beaches[33] and surface waters at concentrations in the same range as marine studies.[7,8] Much of the microplastic in the Great Lakes surface waters was suspected to be microbeads originating from consumer products in wastewater effluent.[8]

Rivers share many of the same sources of microplastic as marine and Great Lake ecosystems, and have less water volume for dilution. Therefore, urban rivers which receive WWTP effluent are likely to have high microplastic concentrations. Rivers may retain microplastic or transport it to downstream lakes and coastal environments, with the potential for numerous biological interactions with river biota. Thus, microplastic could have significant effects on river ecosystems, and rivers may play a significant role in the global microplastic "life cycle".[34] Currently, the abundance of microplastic, its interaction with organisms, and its effects on ecosystem processes in rivers are unknown.

The objective of this study was to measure the microplastic concentration in an urban river and assess WWTP effluent as a potential point source. We hypothesized that microplastic concentrations would be significantly higher downstream of a WWTP effluent input than upstream. Further, we hypothesized that bacterial biofilms colonizing microplastic would differ in composition from bacterial assemblages in adjacent habitats (water column and suspended organic matter) and would include bacteria associated with domestic wastewater.

METHODS

Study Site. The North Shore Channel (NSC) in Chicago, Illinois (IL), USA (42.022, −87.710) is a 12-km man-made channel built in 1910 that receives water from Lake Michigan at Waukegan, IL and joins the North Branch of the Chicago River at Foster Ave in Chicago, IL. Treated wastewater effluent from the Terrence J. O'Brien Water Reclamation Plant flows into the NSC approximately 5.6 km upstream of its confluence with the Chicago River. The O'Brien Plant is an activated sludge plant that treats domestic wastewater. It has an average flow of 927 million liters per day and effluent is not disinfected prior to release. The NSC is part of the hydrologically complex Chicago Area Waterways System (CAWS), which is highly urbanized and contains several large WWTPs. CAWS drains into the Illinois River, then the Mississippi River, and may thereby introduce microplastic to downstream river and marine environments.

Microplastic Collection and Quantification. Microplastic was collected with two neuston nets (0.92 × 0.42 m and 0.36 × 0.41 m) of 333-μm mesh on September 13, 2013. The nets were deployed simultaneously behind a stationary boat. Water velocity was measured at the center of each net during each deployment (Marsh-McBirney Flo-Mate model 2000 Portable Flowmeter, Loveland, CO). After 20 min, all collected material was rinsed from the net into 1-L Nalgene containers (N = 4 downstream and 4 upstream) with ~250 mL of unfiltered site water, and then placed into a cooler on ice for transport to the laboratory where they were stored at 4 °C until measurement of microplastic concentrations.

To collect samples for bacterial measurements, additional net samples were collected (N = 4 downstream). Material from the nets was rinsed onto a sterile white tray. Individual microplastic particles were picked using sterilized forceps and placed in a 160-mL sterile specimen container with 20 mL of site water. Organic material from the sample was removed in the same fashion. To measure water column bacteria, 2-L samples of unfiltered site water from the water column at the upstream and downstream sites were collected. The specimen containers and 2-L water column samples were transported on ice to the laboratory where they were stored at 4 °C until processing. Samples for DNA extraction were processed within 72 h, and samples for microplastic counts were processed within 4−5 d. Also collected were triplicate, 20-mL filtered water samples (glass microfiber filter; GF/F; Sigma-Aldrich Co., St. Louis, MO) to measure dissolved nutrients at the upstream and downstream sites. Filtered water samples were frozen at −20 °C until solute analyses.

A protocol designed for the quantification of marine samples to measure microplastic concentrations was adapted for this study.[32,35] Samples from the net collections were first run through 2-mm and 330-μm stacked sieves. The remaining 0.330−2 mm fractions were stored in glass beakers in a drying oven at 75 °C for 48 h. Organic material was degraded through a wet peroxide oxidation (0.05 M Fe(II) and 30% hydrogen peroxide) at approximately 75 °C. Plastic is resistant to wet peroxide oxidation.[32,35] Samples then went through a salinity-based density separation using sodium chloride, where microplastic floated and heavier inorganic material was drained from the sample.[35] Microplastic was filtered and counted under a dissecting microscope. Because of the abundance of microplastic and the tendency of particles to stick to the filter (especially plastic fibers), particles were counted using a subsample approach. For each sample, 5 random subsamples of the filter were counted. Each subsample was 3% of the filter area. The microplastic type (i.e., fragment, pellet, foam, or fiber) was recorded for each particle in each field of view. The mean value from 5 subsamples was scaled up in proportion to the whole filter to determine microplastic abundance for the sample. Concentration was calculated by dividing the number of particles by water volume (no. items m^{-3}), or surface area (no. items km^{-2}). All reagents were checked for microplastic contamination, and none was found. Control samples were processed identically to environmental samples to measure procedural contamination (N = 4). No microplastic contamination of fragments, pellets, or foam was found. Mean (±SE) procedural contamination by microplastic fibers was 4.5(± 1.2) per sample, which was subtracted from each environmental sample. This represented 0.9% of fibers per sample from samples downstream of WWTP effluent and 9.3% from upstream.

Scanning Electron Microscope (SEM) Imaging. Microplastic pieces were placed in Karnovsky's Fixative for 24 h at 4 °C, followed by a buffer wash using 0.2 M sodium cacodylate. Samples were then postfixed with 2% osmium tetroxide, and dehydrated using a graded ethanol series (10−15 min each in 30%, 50%, 75%, 95%, followed by 3 × 10−15 min in 100% ethanol). Samples were dried using a Polarion E3000 Critical Point Dryer, mounted on aluminum SEM stubs using double-sided carbon sticky tabs. Samples were then coated with 30 nm of Gold Palladium using a Hummer 6.2 Sputter Coater. We viewed samples using a Cambridge Instruments S240 scanning electron microscope. Randomized fields of view (N = 5) were

photographed from microplastic fragments ($N = 3$) and pellets ($N = 3$) to quantify microbial cell densities.

DNA Extraction and Sequencing. DNA was extracted from samples of microplastic, suspended organic matter, downstream water column, and upstream water column using MoBio Powersoil DNA extraction kits (MoBio Laboratories, Carlsbad, CA). For the microplastic and organic matter samples, material collected manually from the net samples was placed into 2-mL microcentrifuge tubes for DNA extraction. For the water column samples, 500 mL of 2-L water samples was filtered using Millipore Sterivex 0.22-μm filter cartridges (N = 4 downstream and 4 upstream). The filters were removed from cartridges, cut with a sterilized razorblade, and placed into 2-mL microcentrifuge tubes for DNA extraction.[36] For all samples, successful DNA isolation was confirmed by agarose gel electrophoresis.

Bacterial assemblages were profiled via next-generation amplicon sequencing of 16S rRNA genes. PCR amplification was performed by the DNA Services Facility, University of Illinois at Chicago, using primers 515F and 806R, which amplify the V4 hypervariable region of bacterial and archaeal 16S rRNA genes.[37] Amplicons were sequenced in a paired end format using the Illumina MiSeq platform[38] by the Genomics Core Laboratory, Michigan State University. Sequences were processed by using MOTHUR v.1.33.0 as previously described.[39] Briefly, paired reads were assembled and demultiplexed, and any sequences with ambiguities or homopolymers longer than 8 bases were removed from the data set. Sequences were aligned using the SILVA-compatible alignment database available within MOTHUR. Sequences were trimmed to a uniform length of 253 base pairs, and chimeric sequences were removed using Uchime.[40] Sequences were classified using the MOTHUR-formatted version of the RDP training set (v.9), and any unknown (i.e., not identified as bacterial), chloroplast, mitochondrial, archaeal, and eukaryotic sequences were removed. Sequences were clustered into operational taxonomic units (OTUs) based on 97% sequence identity. To avoid biases associated with uneven numbers of sequences across samples, the entire data set was randomly subsampled to 101 845 sequences per sample. All of the sequence data analyzed in this paper can be downloaded from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) with accession number SRP042978.

Water Chemistry. Water samples were analyzed for soluble reactive phosphorus (SRP), ammonium (NH_4^+), and nitrate + nitrite (NO_x^-) using an AutoAnalyzer 3 (Seal Analytical, Inc., Mequon, WI, USA). SRP was measured using the antimonyl tartrate technique,[41] NH_4^+ was measuted with the phenol hypochlorite technique,[42] and NO_3^- was measured with the cadmium reduction technique.[43] Nitrite (NO_2^-) was measured without cadmium reduction, and nitrate (NO_3^-) was calculated as the difference between NO_x^- and NO_2^-.

Data Analysis. To compare microplastic abundance, cell density, and nutrient concentrations upstream and downstream of WWTP effluent, the nonparametric Mann−Whitney U-Test was used in SYSTAT 13.0 (Systat, Inc. Chicago, IL) because the data were not normally distributed and could not be transformed for normal distribution. The bacterial assemblages on samples of microplastic, organic matter, upstream water column, and downstream water column were compared by calculating the Bray−Curtis similarity index for each pair of samples and visualizing the resulting distance matrix using nonmetric multidimensional scaling (nMDS) run within MOTHUR. The statistical significance of differences in assemblages between sample types based on the Bray−Curtis index was assessed by AMOVA run within MOTHUR. Microbial diversity based on observed numbers of OTUs, Chao1 richness, and the inverse Simpson and Shannon−Weiner (H′) indices were calculated for each sample using MOTHUR. We used one-way ANOVA to assess the effects of sample type on microbial diversity followed by Tukey's multiple comparison test. Bacterial genera making the largest contributions to the dissimilarities between sample types (based on Bray−Curtis) were identified by a SIMPER analysis run in Primer 6 (Primer-E Ltd., Plymouth, United Kingdom). Two analyses were completed with SIMPER: comparing upstream to downstream water columns and comparing plastic to nonplastic downstream substrates (organic matter and downstream water column). For all genera identified as contributing to dissimilarities between sample types, a t test was completed to determine whether there were statistically significant differences in the relative abundances of the genera between samples types. All ANOVAs and t tests were completed in SYSTAT 13.0 (Systat, Inc. Chicago, IL).

RESULTS

Microplastic Concentration. Microplastic was found in each net sample, and concentration was higher downstream of WWTP effluent than upstream (Mann−Whitney U Test = 15.00; p-value = 0.043; Table 1; Figure 1). Mean (± SE) microplastic concentrations were 1.94 (0.81) m^{-3} upstream and 17.93 (11.05) m^{-3} downstream. Mean (± SE) upstream and downstream concentrations per unit area were 730 341 (279 341) km^{-2} and 6 698 264 (3 929 093) km^{-2}, respectively. Categories of microplastic were different between sites (Table 1). Foam and pellets were found only in downstream samples, and concentrations of foam and pellets were relatively low compared to fragments and fibers. All nutrient concentrations

Table 1. Mean (± SE) Microplastic Concentration and Water Column Nutrients Upstream and Downstream of the Terrence J. O'Brien Wastewater Treatment Plant in the North Shore Channel, Chicago[a]

parameter	upstream	downstream	U-value	p-value
Microplastic Concentration (no. m^{-3})				
total	1.94(0.81)	17.93(11.05)	15	0.043
fragments	0.73(0.24)	6.65(3.09)	15	0.043
pellets	0.00(0.00)	0.45(0.25)	14	0.047
styrofoam	0.00(0.00)	0.25(0.07)	16	0.014
fibers	1.21(0.59)	10.57(8.26)	15	0.043
Nutrient Concentrations (μg L^{-1})				
SRP	<4(<4)	693(19)	9	0.037
NH_4^+	58(2)	620(2)	9	0.050
NO_x^-	162(9)	7198(78)	9	0.050
NO_2^-	2(0)	160(0)	9	0.050
NO_3^-	160(9)	7038(77)	9	0.050
DIN	181(16)	7405(280)	9	0.050

[a] U-value and p-value data were determined by the Mann−Whitney test. Abbreviations: SRP = soluble reactive phosphorus, NH_4^+ = ammonium, NO_x^- = nitrite + nitrate, NO_2^- = nitrite, NO_3^- = nitrate, and DIN = dissolved inorganic nitrogen. For microplastic, N = 4 upstream 4 and downstream, and for solutes, N = 3 upstream and 3 downstream.

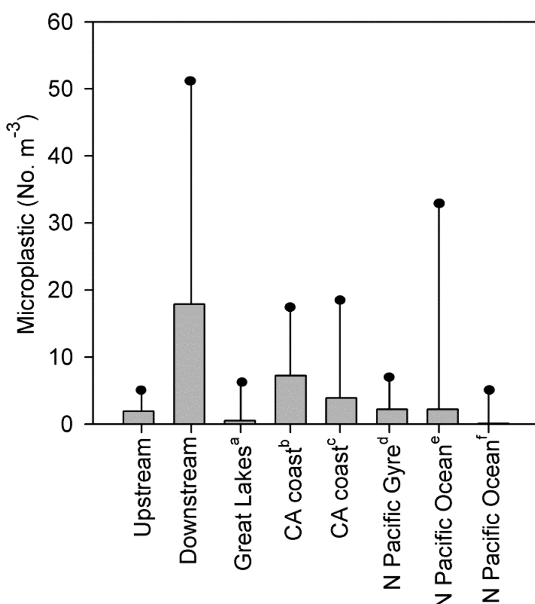

Figure 1. Mean and maximum microplastic concentrations at our study sites upstream and downstream of a WWTP in the North Shore Channel relative to values from the literature. Citations: a = Eriksen et al.,[8] b = Moore et al.,[9] c = Lattin et al.,[10] d = Moore et al.,[6] e and f = Goldstein et al.[44]

were significantly higher downstream of WWTP effluent (Table 1).

Microbial Colonization of Microplastic. SEM imaging revealed extensive microbial colonization of microplastic pellets and fragments (Supporting Information (SI) Figure S1). No fungal hyphae or algal cells were observed under SEM, suggesting that the cells colonizing the microplastic were mainly prokaryotic. Cell density varied from 0.000 to 0.304 cells μm^{-2} in the randomized SEM fields of view, and cells were found in aggregates on microplastic. Mean ($\pm$ SE) cell density colonizing downstream microplastic was 0.037 (0.012) cells μm^{-2} on pellet particles and 0.063 (0.032) cells μm^{-2} on fragments. There was no significant difference in cell densities on pellets relative to fragments (Mann−Whitney $U = 5.5$, $p = 0.658$).

Diverse bacterial assemblages were found on microplastic, as well as within upstream water column, downstream water column, and on downstream organic material, with a mean number of observed OTUs of 3023, 2795, 3630, and 4264, respectively. Mean coverage of sampling, calculated by dividing the number of observed OTUs by the Chao1 richness estimator, for the upstream water column, downstream water column, organic material, and microplastic was 72.5%, 78.5%, 73.2%, and 72.1%, respectively. Downstream water column and organic material samples had a higher number of observed OTUs, Chao1 richness index, and diversity (both as inverse Simpson's and Shannon−Weiner) than the upstream water column and microplastic samples (Figure 2).

Bacterial assemblages were significantly different among sample types (Figure 3). Bray−Curtis index scores were significantly different when comparing all sample types (p-value <0.001) and when comparing any one category to another (SI Table S1). There were clear differences among the 4 sample types in the relative abundance of bacteria OTUs at the family level (Figure 4). The 3 most common bacteria families were different in each sample type. The most common

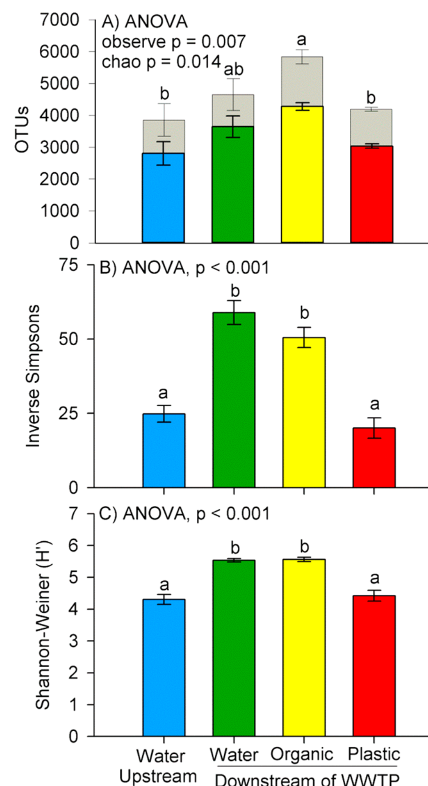

Figure 2. (A) Number of observed bacterial operational taxonomic units (OTUs) (colored) and estimate of total number of bacterial OTUs based on Chao1 richness estimator (gray); (B) inverse Simpson diversity index, and (C) Shannon−Weiner diversity index (H′) for North Shore Channel bacterial assemblages. P-values are from 1-way ANOVA comparing among 4 categories, letters show Tukey's test results.

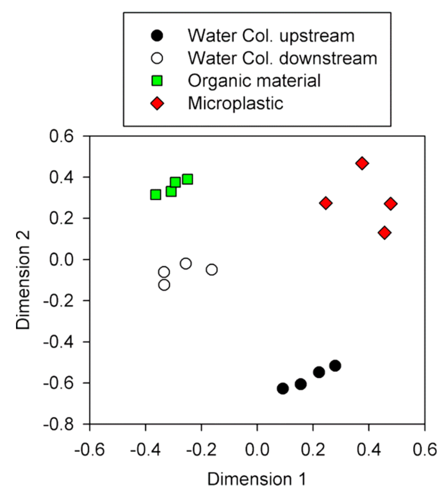

Figure 3. Nonmetric multidimensional scaling (nMDS) ordination of 16S sequencing data (Bray−Curtis dissimilarity) comparing assemblages of bacteria collected in the North Shore Channel.

in the upstream water column were Actinomycetales, Proteobacteria, and unclassified bacteria, and in the downstream water column the most common were unclassified bacteria, Moraxellaceae, and Comamonadaceae. The most common families in the organic material included Rhodocyclaceae, unclassified bacteria, and Thiotrichaceae, and on plastic the most common were Pseudomonadaceae, Proteobacteria,

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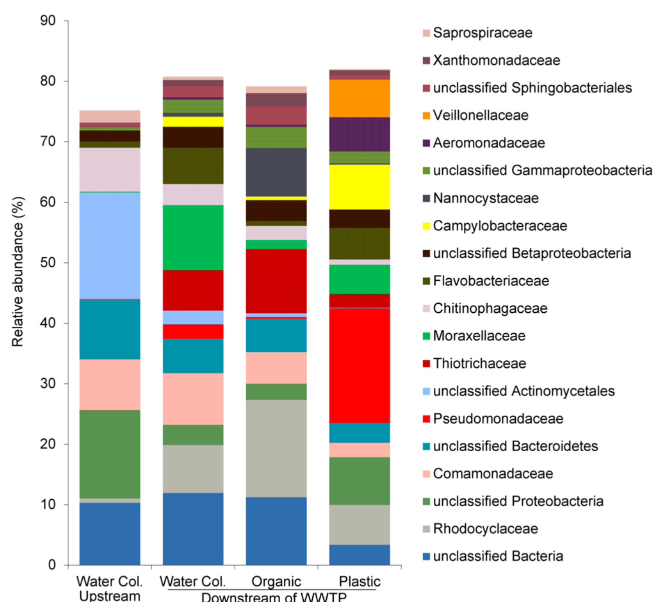

Figure 4. Relative mean abundance of 20 most abundant bacterial families based on 16S sequencing data for samples collected in the North Shore Channel.

and Campylobacteraceae (Figure 4). The large abundances of Pseudomonadaceae and Campylobacteraceae on plastic were especially notable. Pseudomonadaceae accounted for 19.0% of total sequences on the plastic but <1% of the total sequences from the upstream water column and organic material and 2.4% of total sequences from the downstream water column. Similarly, Campylobacteraceae accounted for 7.4% of total sequences on the plastic but <1% of the total sequences from the upstream water column and organic material and 1.7% of total sequences from the downstream water column.

There were 46 OTUs that accounted for 66.6% of the variation between plastic and nonplastic downstream substrates (Table 2). The genus contributing most to this variation was *Pseudomonas* (14.0% of variation), as *Pseudomonas* sequences were 15 times more abundant on plastic than nonplastic. Other groups significantly higher on plastic than nonplastic substrates were *Arcobacter*, *Aeromonas*, and unclassified Veillonellaceae, while *Thiothrix*, *Zoogloea*, Bacteroidetes, Comamonadaceae, and Sphingobacteriales abundances were significantly higher on the nonplastic substrates than plastic. There were 52 OTUs that contributed to 87.8% of the variation in downstream water column and upstream water columns bacterial assemblages (SI Table S2). Actinomycetales and Proteobacteria were significantly more abundant in the upstream water column than downstream. Whereas mean abundance of *Zoogloea*, *Acinetobacter*, Moraxellaceae, *Flavobacterium*, and *Thiothrix* in the downstream water column ranged from 4.9 to 6.7%, the mean abundance upstream for all of these groups was <1% (SI Table S2).

DISCUSSION

Recent research has revealed that microplastic is widespread in marine environments and the Laurentian Great Lakes,[8,13,17] and researchers documenting microplastic in these environments have suggested the potential for rivers to serve as a microplastic source.[8,17] However, data to confirm this suggestion are lacking. To compare microplastic concentrations from this urban river to published values, we performed a literature review using many studies referenced in a recent review paper[17] and studies identified by a Google scholar search (search date = December 2013, search terms = "microplastic concentration", "surface water", "ocean", "river", and "lake"). We narrowed the selection to only those studies which used similar nets and reported measurements as number of items per water volume or surface area. Microplastic abundance downstream of the WWTP in this study was higher than concentrations from several studies in the open ocean,[6,44] and the maximum concentration in this study was above the maximum values from other studies (Figure 1). In addition, microplastic concentration downstream of the WWTP in this study was comparable to maximum coastal concentrations after storms (Figure 1), which are acknowledged to be periods of high microplastic abundance.[9,10] Results demonstrate that this site had microplastic abundance equal to or higher than the oceans and Great Lakes, which has important implications for riverine biota and ecosystem processes in urban rivers.

Microplastic particles at our study site were colonized by dense bacterial biofilms, and the taxonomic composition of these biofilms was distinct from the bacteria in the water column and suspended organic matter, even though microplastic and organic samples were collected simultaneously in the same nets and thus were in intimate physical contact. The microplastic biofilms were also significantly less diverse than the bacterial assembalges in the downstream water column and suspended organic matter. These results indicate that that microplastic surfaces select for a unique suite of bacteria. Recent research focused on freshwater systems has found that larger fragments of inert anthropogenic litter material (i.e., plastic, glass, metal) also support distinct microbial biofilms as compared to those on organic substrates.[34]

It is unclear if the microplastic bacterial assemblage was selected simply by the hard surface of the microplastic or by the chemical composition of the microplastic (i.e., plastic-degrading organisms). Plastic polymers such as polyethylene and polystystrene are considered to be nonbiodegradable, but photo- or thermo-oxidation can facilitate biodegradation.[45] Under microscopic inspection, many of our microplastic particles contained jagged edges, which suggests they may have formed from the fragmentation of larger plastic pieces.[17,18] One of the dominant bacterial taxa within the microplastic biofilm assemblages, which was present at very low abundance in the nonplastic bacterial assemblages, was the genus *Pseudomonas*, which has been highlighted in previous studies of plastic biodegradation. For example, strains of *Pseudomonas* can degrade poly(vinyl alcohol) (PVA) and utilize it as a carbon source.[46] Under laboratory conditions, *Pseudomonas* spp. degraded over 20% of polythene sample mass in 1 month.[47] Polypropylene has also been biodegraded by *Pseudomonas*.[48] Therefore, the high abundance of *Pseudomonas* spp. on the microplastic from our study site suggests that the microplastic may be selecting for bacteria capable of decomposing the plastic compounds. Some fungal taxa are also known to be capable of plastic degradation.[49] We did not observe any fungal hyphae on the microplastic from our study sites, but the use of molecular approaches to assess possible fungal colonization of microplastic should be considered in future studies.

The high abundance of microplastic at our downstream sampling site compared to the upstream site indicates that WWTP effluent was a point source of microplastic to this river. Microplastic types included fibers and pellets associated with synthetic textiles and personal care and cleaning products,

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Table 2. Bacterial OTUs Making the Most Significant Contribution to Variation between Communities from Plastic and Non-Plastic Sample Types Downstream of the WWTP[a]

taxon	nonplastic	plastic	*p*-value	contribution to variation (%)	cumulative contribution (%)
Pseudomonas	1.13	17.81	<0.001	13.95	13.95
unclassified Bacteria	11.57	3.34	<0.001	6.88	20.83
Thiothrix	8.67	2.27	<0.001	5.34	26.17
Arcobacter	1.02	6.57	<0.001	4.64	30.81
Aeromonas	0.38	5.27	<0.001	4.09	34.90
unclassified Veillonellaceae	0.02	4.04	0.003	3.37	38.27
Zoogloea	5.83	2.00	<0.001	3.20	41.47
unclassified Bacteroidetes	5.59	3.29	0.001	1.92	43.39
unclassified Comamonadaceae	3.27	1.12	<0.001	1.80	45.19
unclassified Sphingobacteriales	2.39	0.75	0.003	1.38	46.57
Zymophilus	0.00	1.60	0.003	1.33	47.90
Hydrogenophaga	1.67	0.10	0.008	1.31	49.21
unclassified Actinomycetales	1.46	0.08	0.013	1.15	50.36
unclassified Myxococcales	1.42	0.18	0.005	1.04	51.40
Aquabacterium	0.41	1.52	0.027	0.99	52.39
unclassified Chitinophagaceae	1.72	0.60	<0.001	0.94	53.33
unclassfied Pseudomonadaceae	0.12	1.10	0.001	0.82	54.15
Thauera	1.05	0.13	<0.001	0.77	54.92
unclassified Xanthomonadaceae	1.21	0.30	0.017	0.75	55.67
Sediminibacterium	1.13	0.23	0.024	0.75	56.42
unclassified Oxalobacteraceae	0.21	1.08	<0.001	0.73	57.15
Desulfovibrio	0.03	0.85	0.002	0.69	57.84
Albidiferax	0.98	0.16	0.002	0.68	58.52
Sulfurospirillum	0.09	0.83	<0.001	0.62	59.14
Shewanella	0.02	0.70	<0.001	0.56	59.70
Nitrospira	0.86	0.23	0.016	0.53	60.23
unclassified Chlamydiales	0.56	0.04	0.047	0.43	60.66
unclassified Alphaproteobacteria	0.93	0.43	0.001	0.42	61.08
Prosthecobacter	0.60	0.09	0.011	0.42	61.50
unclassified Ruminococcaceae	0.04	0.51	<0.001	0.39	61.89
unclassified Porphyromonadaceae	0.06	0.51	<0.001	0.37	62.26
3_genus_incertae_sedis	0.63	0.19	0.043	0.37	62.63
unclassified Burkholderiales	0.88	0.47	0.042	0.36	62.99
Bacteroides	0.21	0.64	0.002	0.35	63.34
unclassified Rhodobacteraceae	0.55	0.14	<0.001	0.35	63.69
Prevotella	0.03	0.45	<0.001	0.35	64.04
unclassified Saprospiraceae	0.51	0.12	0.002	0.33	64.37
Anaerosinus	0.00	0.37	0.002	0.31	64.68
Desulfobulbus	0.05	0.39	0.001	0.29	64.97
unclassified Rhizobiales	0.45	0.13	0.005	0.27	65.24
unclassified Deltaproteobacteria	0.36	0.06	0.004	0.25	65.49
Mycobacterium	0.33	0.05	0.004	0.24	65.73
unclassified Acidimicrobiales	0.30	0.03	<0.001	0.23	65.96
Haliscomenobacter	0.33	0.07	0.01	0.22	66.18
Turneriella	0.32	0.08	0.004	0.21	66.39
unclassified Bacteroidales	0.03	0.26	<0.001	0.20	66.59

[a]Each data point is the mean abundance. *P*-value is based on a *t*-test comparison of plastic downstream samples and non-plastic downstream samples.

respectively. These microplastic particles can enter the domestic wastewater stream through normal use of these products. Many common wastewater treatment methods, including the activated sludge system in use at our study site, are not designed to remove nonbiodegradable particles in the microplastic size range, resulting in their release to the environment.[8,20] The transport of microplastic through the domestic wastewater stream creates the opportunity for colonization by wastewater-associated microorganisms, and might provide a vehicle for the transport of these organisms within aquatic ecosystems.

The family Campylobacteraceae includes multiple taxa associated with human gastrointestinal infections such as gastroenteritis[50,51] and Campylobacteraceae was one of the most predominant families on microplastic at our study site (7.4%). Campylobacteraceae sequences were more than 4 times more abundant on the microplastic than in the downstream water column and more than 13 times more abundant on the microplastic than in the suspended organic matter, demonstrat-

ing that Campylobacteraceae have a strong affinity for microplastic. Several other genera that contain pathogenic taxa (e.g., *Aeromonas*, *Arcobacter*, and *Pseudomonas*)[52] were also significantly higher on microplastic compared to nonplastic samples (Table 2). We note that not all members of these genera or the family Campylobacteraceae are pathogenic, but their high abundance on microplastic originating from the WWTP at our study site suggests signficant colonization of microplastic by wastewater-associated organisms and indicates that microplastic may be a novel pathway for transporting disease-causing bacteria into waterways. Further research will be needed to assess the transport of microplastic within river ecosystems and the persistence of potentially pathogenic, wastewater-associated organisms.

The influence of wastewater effluent on the composition of the downstream microbial assemblages was also demonstrated by the more than 200-fold higher abundance of *Zooglea* sequences in the downstream water compared to the upstream water. *Zooglea* is a genus of aerobic chemoorganotrophic bacteria that are key players in aerobic wastewater treatment systems due to their ability to degrade organic carbon and promote floc formation.[53] *Zooglea* sequences were abundant in all of the downstream samples, including water column, organic matter, and microplastic, but in contrast to Campylobacteraceae, *Zooglea* were significantly more abundant on nonplastic samples compared to microplastic.

Our results represent the first exploration of microbial assemblages colonizing microplastic in a freshwater ecosystem. The fact that WWTP effluent was a point source of microplastic is significant because transport through the wastewater system creates the opportunity for colonization by pathogenic bacteria common in wastewater, and our results suggest that potentially pathogenic bacteria may have an affinity for microplastic. The link between microplastic and WWTP effluent is also significant because WWTP effluent typically contains higher concentrations of inorganic nutrients than receiving waters, and indeed at our study site the downstream water had dramatically higher concentrations of inorganic nutrients than the upstream water. Elevated levels of nutrients in effluent are likely to stimulate bacterial biofilm growth, suggesting that microplastic entering the environment via WWTP effluent may support more biofilm biomass than microplastic entering via other pathways. Higher biofilm mass on wastewater-associated microplastics could have significant implications for bacterially driven ecosystem processes such as C and N cycling. Biofilm mass may also impact interactions of microplastic with higher trophic levels, as aquatic invertebrates have been shown to prefer to feed on detritus that has been extensively colonized by microbes.[54−56]

Our results suggest that urban rivers represent an overlooked and potentially important source of microplastic to downstream environments. We acknowledge the limited geographic range of this study, and stress that more studies are needed to assess the abundance, movement, retention, and ecological effects of microplastic in rivers. Results from this study and future research will contribute to public and ecological health by informing mitigation and prevention strategies which can reduce microplastic accumulation and biological impacts in rivers.

■ ASSOCIATED CONTENT

Supporting Information

Figure showing SEM images from microplastic samples; 3 tables illustrating results from pair-wise comparisons of the entire bacterial communities among 4 sample types and showing which genera explained the most variation in microbial community composition among sample types. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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