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Microplastic in Surface Waters of Urban Rivers: Concentration, Sources, and Associated Bacterial Assemblages

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Microplastic in surface waters of urban rivers: concentration, sources, and associated bacterial assemblages

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Abstract. The ecological dynamics of microplastic (<5 mm) are well documented in marine ecosystems, but the sources, abundance, and ecological role of microplastic in rivers are unknown and likely to be substantial. Microplastic fibers (e.g., synthetic fabrics) and pellets (e.g., abrasives in personal care products) are abundant in wastewater treatment plant (WWTP) effluent, and can serve as a point source of microplastic in rivers. The buoyancy, hydrophobic surface, and long transport distance of microplastic make it a novel substrate for the selection and dispersal of unique microbial assemblages. We measured microplastic concentration and bacterial assemblage composition on microplastic and natural surfaces upstream and downstream of WWTP effluent sites at nine rivers in Illinois, United States. Microplastic concentration was higher downstream of WWTP effluent outfall sites in all but two rivers. Pellets, fibers, and fragments were the dominant microplastic types, and polymers were identified as polypropylene, polyethylene, and polystyrene. Mean microplastic flux was 1,338,757 pieces per day, although the flux was highly variable among nine sites (min = 15,520 per day, max = 4,721,709 per day). High-throughput sequencing of 16S rRNA genes showed bacterial assemblage composition was significantly different among microplastic, seston, and water column substrates. Microplastic bacterial assemblages had lower taxon richness, diversity, and evenness than those on other substrates, and microplastic selected for taxa that may degrade plastic polymers (e.g., *Pseudomonas*) and those representing common human intestinal pathogens (e.g., *Arcobacter*). Effluent from WWTPs in rivers is an important component of the global plastic "life cycle," and microplastic serves as a novel substrate that selects and transports distinct bacterial assemblages in urban rivers. Rates of microplastic deposition, consumption by stream biota, and the metabolic capacity of microplastic biofilms in rivers are unknown and merit further research.

Key words: 16s rRNA; bacterial assemblages; microbial ecology; microplastic.

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Introduction

A growing field of research focuses on the abundance, sources, movement, and biological interactions of microplastic (<5-mm particles) in the environment (Thompson et al. 2004, Browne et al. 2011, Eriksen et al. 2014). Microplastic includes a diversity of polymer types (e.g., polyethylene, polypropylene) and shapes (e.g., fragments, pellets, and fibers), which can originate from different sources. Microplastic fragments form through breakdown of larger particulate plastic by photolysis, thermo-oxidation, thermo-degradation, and possibly via biodegradation

(Andrady 2011). "Microbeads" are plastic spheres contained in personal care products and production pellets used to manufacture plastic goods (Gregory 1996, Fendall and Sewell 2009, Cole et al. 2011). Additionally, washing synthetic textiles can generate high concentrations of microplastic fibers in washing machine effluent (Browne et al. 2011). Microplastic pellets and fibers enter the domestic wastewater infrastructure, but may not be captured by wastewater treatment plants (WWTPs) due to their small size (Fendall and Sewell 2009, Browne et al. 2011). Previous research has shown that WWTP effluent is a source of plastic fibers to marine sediment (Browne et al. 2011), fibers and particles to coastal waters (Talvitie et al. 2015), pellets to riverine sediment (Castañeda et al. 2014), and pellets, fragments, and fibers to river surface waters (McCormick et al. 2014).

A majority of microplastic research focuses on marine environments, and studies on microplastic in freshwaters and estuaries have only recently emerged (Wagner et al. 2014). Measurements of microplastic abundance in estuaries highlight the potential for rivers to transport microplastic to marine habitats (Dubaish and Liebezeit 2013, Lima et al. 2014, Sadri and Thompson 2014, Yonkos et al. 2014). Rivers are susceptible to the same sources of microplastic as marine environments and have relatively little water volume for microplastic dilution, which suggests they have high concentrations. Recent studies found high microplastic concentrations in riverine sediment (Castañeda et al. 2014) and surface waters (Moore et al. 2011, Lechner et al. 2014, McCormick et al. 2014). However, a greater understanding of the sources, accumulation sites, and movement of microplastic in rivers is needed to quantify global microplastic distribution and its role in river ecosystems.

The impacts of microplastic on freshwater biota remain largely unstudied. In marine environments, consumers may ingest microplastic, which can block digestion and transport contaminants to organisms (Rochman et al. 2013, Wright et al. 2013). Organisms of multiple trophic levels and feeding guilds (e.g., zooplankton, macroinvertebrates, fish, and marine mammals) consume microplastic (Browne et al. 2008, Lusher et al. 2012, 2015, Cole et al. 2013, Goldstein and Goodwin 2013, De Witte et al. 2014, Van Cauwenberghe and Janssen 2014), and microplastic can be transferred from prey to predators (Murray and Cowie 2011, Farrell and Nelson 2013, Setälä et al. 2014). Microplastic may also affect lower trophic levels by presenting a novel habitat for colonization by microbial biofilms in aquatic ecosystems. Biofilms are composed of bacteria, archaea, and microbial eukaryotes attached to surfaces and embedded in an extracellular matrix of polymeric substances (Fischer 2003). Biofilm microbes are essential for heterotrophic organic matter (OM) processing in aquatic systems and provide an energy input to food webs, as they may be ingested directly or through their association with larger particles (e.g., fine particulate OM, coarse particulate OM; Allan and Castillo 2007). The integral role of microorganisms to stream ecosystem functioning necessitates understanding the potential influence of microplastic on biofilm abundance and community composition.

Initial studies suggest microplastic selects for bacterial assemblages that are distinct in taxonomic composition from those on natural surfaces such as the water column in the Atlantic Ocean (Zettler et al. 2013), seston and the water column in an urban river (McCormick et al. 2014), and marine sediment (Harrison et al. 2014). Microplastic may select microbial biofilm constituents via several potential mechanisms: (1) the availability of a hard surface provides habitat for microbial attachment, (2) plastic has novel organic polymers, additives, and sorbed contaminants that can provide a carbon source for microbial metabolism, or (3) secondary microbial biofilm members may attach to biofilm polysaccharides or primary colonizers on plastic. Microplastic may represent a relatively stable, persistent, and buoyant surface in the water column (Cole et al. 2011), which otherwise lacks such colonization sites for biofilm-forming microorganisms. In rivers, naturally occurring seston and suspended sediment provide either organic surfaces that decompose, or inorganic surfaces (e.g., sand) that are periodically suspended and deposited. In contrast, microplastic can remain buoyant and resist decomposition over long distances and time periods, and thereby could support a unique consortium of biofilm constituents. Furthermore, the hydrophobic surface of plastic can stimulate biofilm formation in the water column (Zettler

et al. 2013) and the carbon polymers in plastic can be degraded by some groups of microorganisms (Shimao 2001, Shah et al. 2008, Yoshida et al. 2016). Microplastic biofilms may also increase the likelihood of consumer ingestion (Reisser et al. 2014) and contribute to microplastic deposition (Barnes et al. 2009) and decomposition (Yoshida et al. 2016). However, few studies have examined the capacity for microplastic biofilms to develop distinctive microbial consortia relative to natural habitats.

More studies on microplastic abundance, sources, and microbial interactions in rivers are needed. Our previous research showed WWTP effluent was a point source of microplastic that selected for unique bacterial assemblages in an urban river, which had not previously been documented (McCormick et al. 2014). However, that study was limited to one site with a large WWTP, and it is unclear whether the patterns apply to sites of variable WWTP types and river sizes. In addition, measurements of microplastic concentration and flux are needed from a wider variety of rivers to include lotic ecosystems in global budgets of plastic. Thus, the first objective of the current study was to compare microplastic concentrations at locations upstream and downstream of WWTP effluent outfalls in nine streams that span a range of sizes and represent a gradient of WWTP effluent relative to stream discharge. Our second objective was to analyze the bacterial assemblages on microplastic in these streams and compare these assemblages to those on natural substrates from the same streams (organic material [i.e., seston], upstream water column, and downstream water column) in order to identify dominant taxa within microplastic biofilms. We predicted that microplastic concentrations would be significantly higher downstream of WWTP effluent outfalls than upstream and that concentrations would show high variation among streams. We also hypothesized that bacterial assemblages on microplastic would be distinct from assemblages on natural habitats and bacterial composition on microplastic would be similar across rivers. In particular, we predicted microplastic would harbor a greater abundance of organisms with a preference for biofilm formation and the potential for plastic polymer degradation.

Methods

Study sites

Our study streams were in the Chicago metropolitan area of northeastern Illinois and northwestern Indiana ($n = 8$) and central Illinois ($n = 2$), and each received treated WWTP effluent (Table 1; Appendix S1: Table S1). Streams spanned a gradient in the relative contribution of

Table 1. Summary of stream sampling locations, wastewater treatment plant (WWTP) effluent volume, and tertiary treatment disinfection methods.

Stream	Plant	Date sampled M/D/Y	2013 effluent (MGD)	Contrib. of effluent to flow (%)	Sand bed (Y/N)	Disinfection method
Higgen's Cr.	James C. Kirie WRP	7/16/14	38.72	110.82	N	Chlor/dechl
Springbrook Cr.	Wheaton WWTP	10/13/14	7.39	86.18	Y	UV
L Kickapoo Cr.	Bloom. SE	7/10/14	4.24	78.93	Y	UV
Schererville Ditch	Schererville WWTP	8/08/14	4.32	70.22	N	Chlor/dechl
N. Shore Ch.	O'Brien WRP	8/07/14	225.00	70.00	N	None
Goose Cr.	Bloom. W Oakton	7/10/14	15.93	46.51	Y	Chlor/dechl
DuPage R.	Springbrook WRP	7/11/14	19.68	20.82	Y	Chlor/dechl
W Br DuPage R.	Bartlett WWTP	10/13/14	2.16	15.99	N	Chlor/dechl
Salt Cr.	Elmhurst WRP	8/04/14	7.03	13.17	N	UV
E Br DuPage R.	Woodridge Gr. WRP	9/19/14	10.00	13.24	Y	Chlor/dechl

Note: WRP, water reclamation plant; SE, southeast; Chlor/dechl, chlorination/dechlorination.

WWTP effluent to stream flow (Table 1). The WWTPs that discharge effluent into the sites spanned a range of municipality size, volume of effluent released per day, and treatment methods for effluent filtration and disinfection (Table 1). We note the North Shore Channel, Chicago, was a site of a previous study on microplastic conducted in September 2013 (McCormick et al. 2014), but samples from the North Shore Channel in the present study were collected in summer 2014.

Sample collection and microplastic quantification

We collected microplastic from surface water with neuston nets (0.52 × 0.36 m) of 333-μm mesh (McCormick et al. 2014). All sites were sampled in summer 2014, except one which was sampled in October (Table 1). In the North Shore Channel, nets were deployed behind a stationary boat. All other streams were shallower, so we waded in and held the nets in place manually at the water's surface. Each researcher held a net in front of them, perpendicular to the water flow, taking care not to disturb the net tail. We measured deployment time with a stopwatch (15–20 min per sample), water depth in the net, and water velocity at the center of each net (Marsh-McBirney Flo-Mate Model 2000 Portable Flowmeter, Loveland, Colorado). Separate net samples were collected upstream (n = 4) and downstream (n = 4) of the WWTP outfall site, in locations with well-mixed waters. Material was rinsed from the net into 1-L containers with unfiltered site water, and then placed into a cooler on ice for transport to the laboratory where they were stored at 4°C until processing for microplastic counts. At Schererville Ditch, very low water velocity upstream of the WWTP effluent site precluded analysis of microplastic concentrations, but samples were collected for bacterial assemblage analysis.

Additional samples from each stream were collected to assess bacterial assemblage composition. For microplastic and seston, we conducted subsequent net deployments downstream of the WWTP outfall site, as described above. Material from the nets was rinsed onto a white tray, which had been sterilized with ethanol. Individual microplastic particles were removed using sterilized forceps and placed in a 160-mL sterile specimen container with ~20 mL of site water. Particulate OM from the water column tended to flocculate and accumulate on the mesh tail of the net, which allowed us to remove seston from samples with sterilized forceps. Organic matter samples were placed in separate specimen containers. At three sites (Goose Creek, Little Kickapoo Creek, and East Branch of the DuPage River), we found no visible microplastic in the samples, so we did not have microplastic-associated bacteria from those sites. To analyze composition of water column bacterial assemblages, we collected 2 L of unfiltered site water from the water column (~10 cm below the water's surface) at the upstream and downstream sites using acid-washed containers. 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 (within 24 h). We note that storage conditions differed from those in the field (i.e., 4°C and dark relative to 21.6°C and light, respectively), which could affect bacterial assemblages. However, storage conditions were uniform across samples. We also recorded temperature, conductivity (YSI Model 30; YSI, Yellow Springs, Ohio, USA), and dissolved oxygen (DO; HQ40d portable meter with LDO101 DO probe; Hach Company, Loveland, Colorado, USA) at all upstream and downstream sampling locations. Finally, we collected triplicate 20 mL filtered water samples (0.45-μm glass microfiber filter; Sigma-Aldrich Co., St. Louis, Missouri, USA) to measure dissolved nutrients at the upstream and downstream sites. Filtered water samples were frozen at −20°C until solute analyses.

We adapted a protocol for quantifying microplastic from marine water column samples to measure microplastic concentration (Baker et al. 2011, McCormick et al. 2014). Samples were first run through 4.75-mm and 330-μm stacked sieves. The 0.330–4.75 mm fraction was stored in glass beakers in a drying oven at 75°C. Organic material was degraded through wet peroxide oxidation (0.05 mol/L Fe(II) and 30% hydrogen peroxide) at ~75°C. Plastic resists wet peroxide oxidation, while OM is degraded (Baker et al. 2011, Eriksen et al. 2013, Tagg et al. 2015). We added sodium chloride (final concentration = 6 mol/L) for a salinity-based density separation. The sample was placed in a glass funnel. Microplastic floated at the surface, and heavier

material was drained from the sample (Baker et al. 2011). Microplastic was filtered (Whatman glass fiber filters, 0.7 μm nominal pore size, filtered area = 5.23 cm^2; Whatman, Piscataway, New Jersey, USA) and counted under a dissecting microscope. Using the physical characteristics of microplastic particles, we recorded the classification type (i.e., fiber, film, fragment, pellet, foam, pellet, or fragment) for each item (Eriksen et al. 2014). Fibers included filament/line-shaped plastic pieces. Film pieces were typically irregular in shape, but were characteristically very thin relative to their surface area. Foam included polystyrene, which has a distinct sponge-like texture rather than a smooth surface. Pellets had a regular three-dimensional shape, which was typically round. Lastly, particles which had particularly jagged edges that suggested breakage of a larger plastic piece were classified as fragments. We counted all fragments, pellets, foam, and film particles individually. Fibers were very abundant and adhered to the filter, so we used a subsample approach (McCormick et al. 2014). For each sample, we counted three random subsamples for each quadrat on the filter (each subsample was 3% of filter area). The mean value from the 12 subsamples was scaled up in proportion to the whole filter to determine microplastic fiber abundance. We calculated microplastic concentration by dividing the number of particles by water volume (no. items/m^3) and surface area (no. items/km^2). We checked all reagents for microplastic contamination by filtering them and inspecting the filter under a dissecting microscope. We also processed control (deionized water) samples identically to environmental samples to measure procedural contamination (n = 5). We found no microplastic contamination of fragments, pellets, film, or foam. Average procedural contamination by microplastic fibers was 4.67 per sample, which we subtracted from each environmental sample.

Microplastic polymer analysis

A subset of microplastic from two streams was analyzed by pyrolysis–gas chromatography–mass spectrometry (py-GCMS; CDS Analytical 5200 pyroprobe and Varian 3800 gas chromatograph). Samples from all five categories were selected from the North Shore Channel and the DuPage River, which had high microplastic concentrations. Each sample was inserted into a quartz capillary tube between plugs of quartz wool, loaded into the pyroprobe, and heated to 750°C for 90 s. The transfer line to the GC and the injection port was held at 325°C with a split ratio of 10:1. Separation was performed on a Restek Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 μm df) with helium as the carrier gas at a flow rate of 2.0 mL/min. The oven ramped from 40° to 325°C at a rate of 10°C min^{-1} and held the final temperature for 20 min. The GC system was coupled to a Saturn 2000 ion trap mass spectrometer with the transfer line and ion trap held at 325° and 220°C, respectively. The mass spectrometer collected all mass to charge ions (m/z) from 35 to 550. Blanks were analyzed between samples to ensure that no carry-over occurred. Pyrograms for each sample were generated by averaging the mass spectra over the entire chromatogram and searched in the CDS Analytical 2013 pyrolysis library for the best match. The operating parameters chosen for the py-GCMS analysis were based on the parameters utilized by CDS Analytical to build the pyrogram database.

Bacterial assemblage composition

We extracted DNA from microplastic, suspended OM (seston), downstream water column, and upstream water column samples using MoBio Powersoil DNA extraction kits (MoBio Laboratories, Carlsbad, California, USA). For the microplastic and seston, we collected material manually from the specimen containers and placed it into 2-mL microcentrifuge tubes for DNA extraction. Sequencing analyses were performed for microplastic in bulk, with approximately 10 pieces used for each sample. We separated the 2 L water column samples into four 500 mL portions, and each was filtered with 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 (Crump et al. 2003). Bacterial assemblages were profiled via next-generation amplicon sequencing of 16S rRNA genes. PCR amplification was performed using primers 515F (5′GTGCCAGCMGCCGCG GTAA3′) and 806R (5′GGACTACHVGGGTW TCTAAT3′), which amplify the V4 hypervariable region of bacterial and archaeal 16S rRNA genes

(Caporaso et al. 2011). For all samples, we confirmed successful DNA amplification by agarose gel electrophoresis. Amplicons were sequenced in a 2 × 250 paired-end format using the Illumina MiSeq platform (Caporaso et al. 2012) by the DNA Services Facility, University of Illinois at Chicago. Sequences were processed using mothur v.1.33.0 as described by Schloss et al. (2011) and Kozich et al. (2013). Briefly, paired reads were assembled and demultiplexed, and any sequences with ambiguities or homopolymers longer than eight bases were removed. Sequences were aligned using the SILVA-compatible alignment database (based on SILVA release 119) available within mothur. Sequences were trimmed to a uniform length of 253 base pairs, and chimeric sequences were removed using Uchime (Edgar et al. 2011). 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 14,541 sequences per sample. All sequencing data analyzed in this study can be downloaded from the National Center for Biotechnology Information Sequence Read Archive, accession number SRP065321.

Water chemistry

Water samples were analyzed for soluble reactive phosphorus (SRP), ammonium (NH_4^+), and nitrate (NO_3^-) using an AutoAnalyzer 3 (Seal Analytical, Mequon, Wisconsin, USA). We used the phenol hypochlorite technique to measure NH_4^+ (Solorzano 1969), the antimonyl tartrate technique to measure SRP (Murphy and Riley 1962), and the cadmium reduction technique to measure NO_3^- (APHA 1998).

Data analysis

We used two-way analysis of variance (ANOVA) to compare total microplastic concentration among sites and relative to WWTP outfall location. We applied a natural log transformation to ensure data met the homoscedasticity and normality assumptions of ANOVA. Following a significant interaction in the two-way ANOVA, we compared upstream and downstream concentrations at each site individually, using a Bonferroni correction ($\alpha = 0.05/9 = 0.006$) for multiple pairwise comparisons (Zar 1999). After applying an $\ln(x + 0.5)$ transformation, we used two-way ANOVA to compare concentrations of each microplastic category (fragments, pellets, foam, film, and fibers). We calculated the ratio of downstream to upstream microplastic concentration to examine the WWTP effect among sites. One replicate each from downstream and upstream was randomly paired to calculate the ratio ($n = 4$ per site), and we used a one-way ANOVA on the natural log of the concentration ratio to detect differences among streams, followed by Tukey's multiple comparison test. We used Pearson's correlation coefficients to determine associations between the relative proportion of WWTP effluent in the river and downstream microplastic concentration across sites, and a Student's two-way t test to compare microplastic concentration at WWTPs with and without sand filtration. Finally, we used an F test for equality of variance to compare the variance of downstream and upstream concentrations for all sites combined. All ANOVAs, Tukey's tests, and t tests were completed in SYSTAT 13.0 (Systat, Chicago, Illinois, USA).

The composition of bacterial assemblages on microplastic, OM, upstream water column, and downstream water column samples 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 v.1.33.0 (Schloss et al. 2011, Kozich et al. 2013). This analysis was performed on raw OTU abundances. The statistical significance of differences in assemblages between sample types based on the Bray–Curtis index was assessed by the analysis of molecular variance (AMOVA) and analysis of similarities (ANOSIM) run within mothur. AMOVA is a nonparametric method used to test the hypothesis that diversity within two groups is not significantly different from that which would arise when pooling the groups together (Excoffier et al. 1992, Schloss 2008, http://www.mothur.org/wiki/Amova). Good's coverage estimate was calculated within mothur for each sample to estimate how well the sequence data sets captured the taxonomic richness of the communities (Good 1953). Microbial diversity, based on the observed

numbers of OTUs and Shannon–Wiener (H') and Shannon Evenness (E_H) indices, was also calculated for each sample using mothur. We used one-way ANOVA to assess the effects of substrate on diversity metrics followed by Tukey's multiple comparison test. Bacterial OTUs making the largest contributions to the dissimilarities between microplastic and OM samples (based on the Bray–Curtis index) were identified by a SIMPER analysis run in Primer 6 (Primer-E Ltd., Plymouth, UK). 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 sample types.

Results

Physical and chemical characteristics of study streams

Nutrients and conductivity were variable among study streams, but higher values downstream from WWTPs illustrated the influence of effluent on water chemistry (Appendix S1: Table S2). For example, NO_3^- concentrations were higher downstream than upstream at all sites, and at one site, NO_3^- concentration was 58 times higher downstream (Goose Creek). SRP concentration was higher downstream at all but one site (West Branch of the DuPage River). Conductivity was higher downstream than upstream at seven sites. Finally, there were no patterns for DO concentration upstream and downstream of WWTPs across sites.

Microplastic concentration and flux

Microplastic was found in every sample from both upstream and downstream of WWTP effluent sites, and mean (±SE) concentrations were 2.355 (±0.375) no./m^3 and 5.733 (±0.850) no./m^3, respectively. Microplastic concentration was higher downstream of the WWTP effluent site than upstream at all but two streams (Fig. 1, Table 2); however, there was a significant interaction between site and effluent effects ($P < 0.001$; Appendix S1: Table S3). Pairwise t tests with a Bonferroni correction indicated that two streams had significantly higher microplastic concentrations downstream than upstream (Higgen's Creek and Salt Creek; Fig. 1A). To examine relative differences between downstream and upstream locations, we compared the ratio of downstream to upstream concentrations (Fig. 1B). The ratio was >0 at seven of nine sites, and highest at Higgen's Creek, Springbrook Creek, the West Branch of the DuPage River, and Salt Creek, while Goose Creek was lowest (Fig. 1B). Among all study rivers, we estimated the average microplastic flux downstream of WWTPs was 1,338,757 pieces per day, with an estimated minimum of 15,520 pieces per day (Little Kickapoo Cr) and an estimated maximum of 4,732,709 pieces per day (North Shore Channel; Table 2).

We also examined patterns in the five microplastic categories. Pellets, fibers, and fragments were the most common microplastic types, while film and foam were uncommon (Appendix S1: Table S3, Fig. S1). All categories showed significant interactions between site and effluent input effects (Appendix S1: Table S4). Multiple comparison tests with a Bonferroni correction ($\alpha = 0.0056$) for each microplastic category at each site showed significantly higher concentrations of fragments (df = 6, $t = 10.93$, $P < 0.001$) and pellets (df = 6, $t = 16.89$, $P < 0.001$) downstream of the WWTP at Higgen's Creek and a higher concentration of pellets (df = 6, $t = 9.77$, $P < 0.001$) downstream at the West Branch of the DuPage River (Appendix S1: Table S3). Foam concentration was higher upstream than downstream (df = 6, $t = -6.50$, $P = 0.001$) in the DuPage River (Appendix S1: Table S3, Fig. S1).

Overall, the proportion of WWTP effluent in stream discharge and the use of sand filtration at the WWTP had no significant effect on microplastic concentrations. There was no correlation between the proportion of WWTP effluent in stream discharge and the mean ratio of downstream to upstream microplastic concentration (Pearson's correlation, $r = 0.19$, $P = 0.617$) or the mean difference between downstream and upstream microplastic concentration (Pearson's correlation, $r = 0.29$, $P = 0.443$). Sand filtration ($n = 5$ WWTPs with sand filters and $n = 4$ without; Table 1) had no effect on the ratio of downstream to upstream microplastic concentration (df = 6.96, $t = 2.18$, $P = 0.066$) or the difference between downstream and upstream microplastic concentrations (df = 5.13, $t = 0.43$, $P = 0.688$).

Within replicate net samples, microplastic concentrations were variable. At six of nine sites, the coefficient of variation (CV) was higher

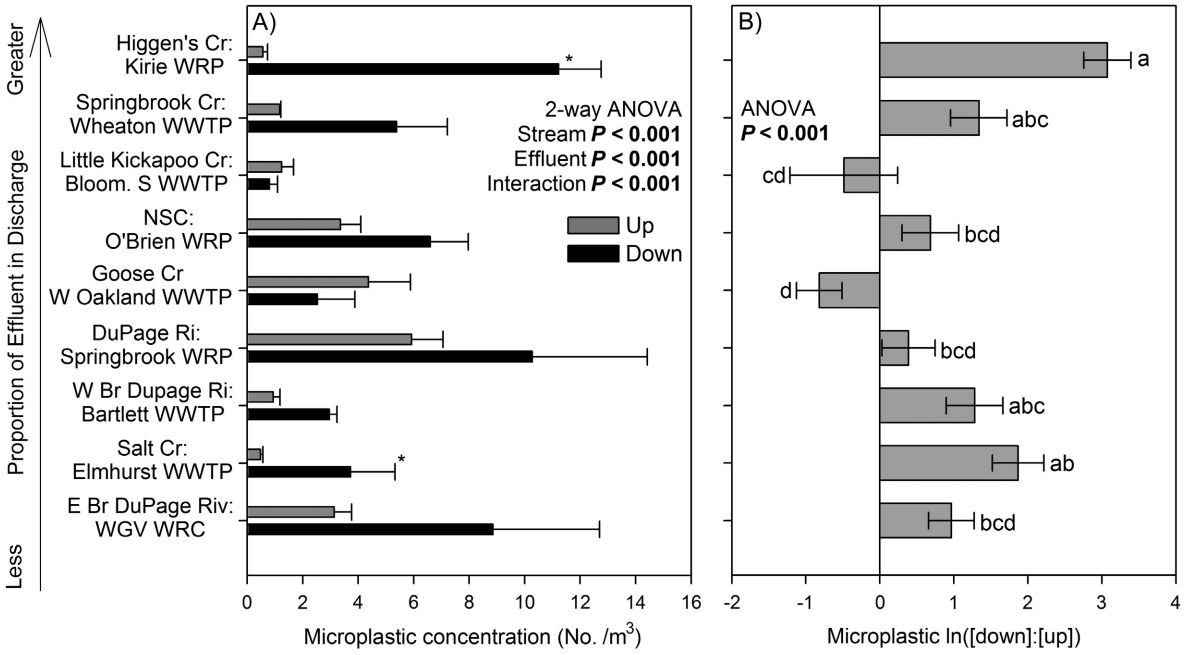

Fig. 1. (A) Mean (±SE) microplastic concentration upstream and downstream of wastewater treatment plants (WWTP), water reclamation plants (WRP), or water reclamation centers (WRC) at nine streams in Illinois (n = 4 per mean). (B) Mean (±SE) ratio of microplastic concentration downstream and upstream at each site (n = 4 per mean). *Significant difference in downstream and upstream concentrations with a Bonferroni correction. Letters represent differences in the ratio of downstream to upstream microplastic concentrations among sites, determined by Tukey's test results. Cr, creek; Bloom, Bloomington; NSC, North Shore Channel; S, south; W, west; E, east; Ri, river; Br, Branch; WGV, Woodridge Green Valley.

Table 2. Mean (±SE) microplastic concentrations upstream and downstream of WWTPs and downstream flux in each sampling stream.

Stream	Plant	Microplastic (no./m^3)		Downstream flux (no./d)
		Upstream	Downstream	
Higgen's Cr.	James C. Kirie WRP	0.57 (0.16)	11.22 (1.53)	857,758
Springbrook Cr.	Wheaton WWTP	1.17 (0.05)	5.39 (1.82)	185,317
L Kickapoo Cr.	Bloom. SE	1.24 (0.43)	0.80 (0.30)	15,520
N. Shore Ch.	O'Brien WRP	3.36 (0.74)	6.60 (1.37)	4,721,709
Goose Cr.	Bloom. W Oakton	4.37 (1.52)	2.53 (1.36)	214,449
DuPage R.	Springbrook WRP	5.92 (1.14)	10.28 (4.14)	3,520,277
W Br DuPage R.	Bartlett WWTP	0.93 (0.25)	2.96 (0.27)	217,570
Salt Cr.	Elmhurst WRP	0.48 (0.09)	3.73 (1.60)	364,692
E Br DuPage R.	Woodridge Gr. WRP	3.14 (0.62)	8.86 (3.83)	1,951,522

Note: Abbreviations are as in Table 1.

for downstream samples than for upstream (Appendix S1: Table S5), but there was no statistical difference in the CV between all upstream and downstream locations (df = 13.82, t = 1.44, P = 0.176). Sites and sampling location showed a wide range of variance in upstream and downstream microplastic concentrations (Appendix S1: Table S5). An F test for equality of variance for downstream and upstream samples (combined for all sites) indicated that the variances of the two groups were unequal ($F_{35,34}$ = 0.200, P < 0.001) and greater for downstream samples.

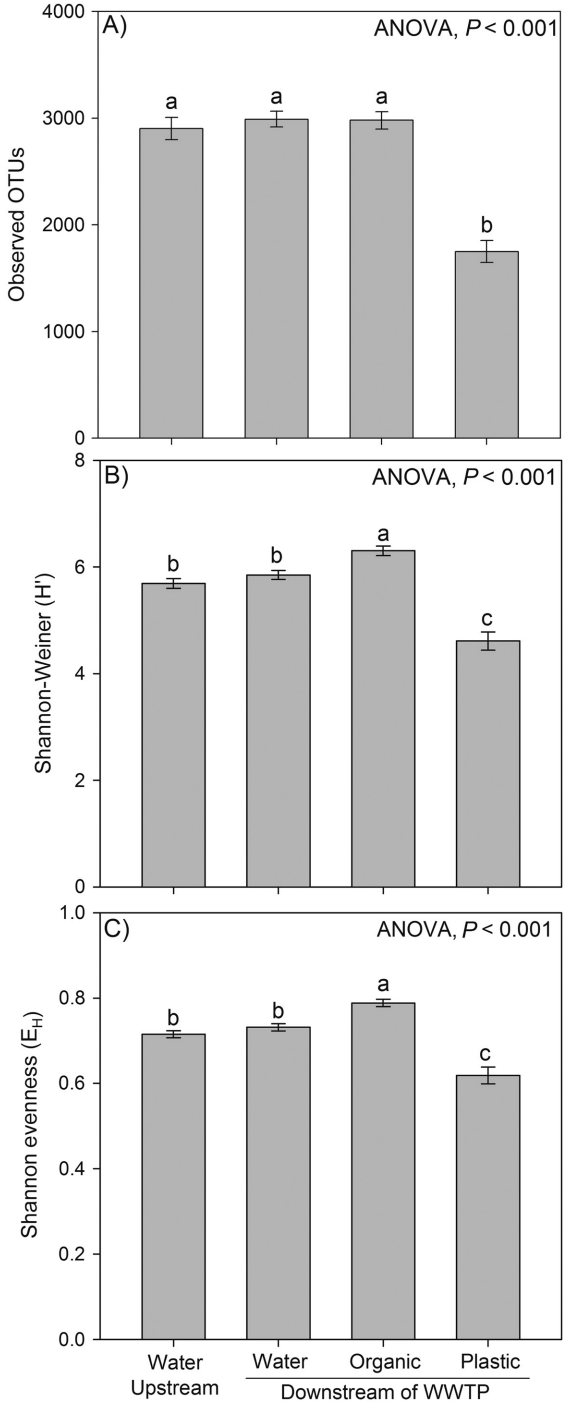

Fig. 2. Mean (±SE) (A) number of observed bacterial operational taxonomic units (OTUs), (B) Shannon–Wiener diversity index (*H*′), and (C) Shannon Evenness index (E_H) for bacterial assemblages from all study sites. *P*-values are from one-way ANOVA comparing measurements among the four sample types. Letters show Tukey's test results. WWTP, wastewater treatment plant.

Microplastic polymer analysis

Polymer analysis indicated isolated material was composed of commonly occurring plastic compounds. Our samples consisted of the polymers polyethylene, polypropylene (low density), polystyrene, and ethylene (Appendix S1: Table S6). Two fiber samples were lost during shipment, and one fiber sample had a pyrogram with no matching spectra.

Bacterial assemblages across substrates

We found diverse bacterial assemblages associated with all four substrates: upstream water column, downstream water column, downstream organic material, and microplastic, with mean (±SE) numbers of observed OTUs of 2902 (±105), 2989 (±74), 2979 (±81), and 1748 (±103), respectively. Mean coverage of sampling, measured by Good's coverage estimate, for the upstream water column, downstream water column, organic material, and microplastic was 86.4%, 86.5%, 87.9%, and 92.5%, respectively, indicating that the sequencing depth was adequate to assess the composition and diversity of these assemblages. Microplastic bacterial assemblages had significantly lower taxon richness (ANOVA, $F_{3,135} = 25.44$, $P < 0.001$), community diversity (*H*′ index, ANOVA, $F_{3,135} = 38.79$, $P < 0.001$), and community evenness (E_H index, ANOVA, $F_{3,135} = 35.95$, $P < 0.001$) than the other substrates (Fig. 2). Downstream organic material had significantly higher diversity and evenness measured by the Shannon–Wiener (*H*′) index and Shannon Evenness (E_H) indices than other substrates (Fig. 2B, C).

Bacterial assemblage OTU composition was significantly different among the four substrates. Bray–Curtis indices were significantly different when comparing all four substrates (AMOVA, df = 3,135, *F*s = 9.35, $P < 0.001$) and when comparing any one category to another (AMOVA, all $P < 0.001$). Results from the ANOSIM also indicated significant differences in assemblage composition among all substrates ($R = 0.585$, $P < 0.001$). Additionally, there were also significant differences in bacterial assemblage

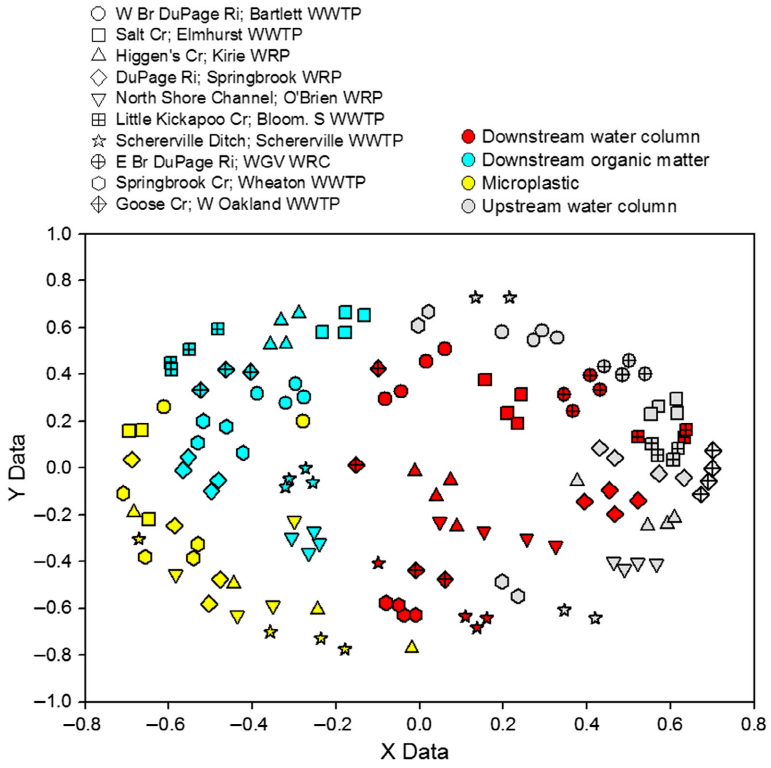

Fig. 3. Nonmetric multidimensional scaling (nMDS) ordination of 16S rRNA gene sequencing data (Bray–Curtis dissimilarity) comparing bacterial assemblages collected in 10 study streams. Note: microplastic was not visible at three sites (Little Kickapoo Cr, Goose Cr, and E Br DuPage Ri); thus, there were no microplastic sample types from these sites for bacterial analysis. Abbreviations are as in Fig. 1.

composition in pairwise comparison between sites (Appendix S1: Table S7). The nMDS ordination of Bray–Curtis indices depicts separation in bacterial assemblage composition among the four substrates (Fig. 3). The stress value for the nMDS plot is 0.379.

When all sites were combined, there were clear differences among the four substrates in the relative abundance of bacterial phyla (Fig. 4). The relative abundance of Bacteriodetes decreased from the upstream water column (44.1%), downstream water column (31.8%), organic material (23.6%), and plastic (9.5%). In contrast, the relative abundance of Proteobacteria increased across the upstream water column (33.7%), downstream water column (46.8%), organic material (56.9%), and plastic (74.9%). Within Proteobacteria, Betaproteobacteria had a higher relative abundance on plastic (32.1%), than in the upstream water column (23.2%), downstream water column (25.1%), and organic material (25.0%). The relative abundance of Gammaproteobacteria was also higher on plastic (32.5%) than in the upstream water column (5.0%), downstream water column (12.3%), and organic material (15.0%). Finally, the phylum Actinobacteria was more abundant in the water column samples than in organic material and plastic, and Firmicutes had a higher relative abundance on plastic than other substrates (Fig. 4).

Family-level resolution of bacterial assemblages also showed differences among the four substrates. The three most common families were different on each substrate. The most common in the upstream water column were Flavobacteriaceae, unclassified Actinomycetales, and Cytophagaceae, and in the downstream water column, the most common were Flavobacteriaceae, unclassified Betaproteobacteria, and unclassified bacteria (Fig. 5; Appendix S1: Table S8). The most common families in the organic material included

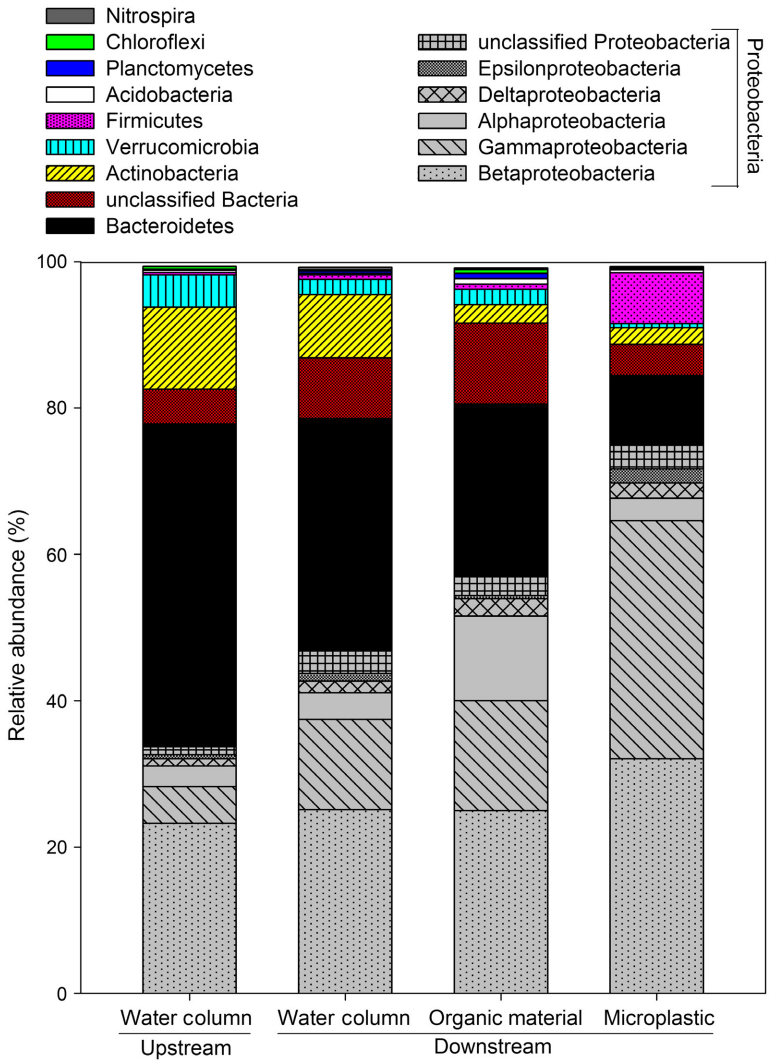

Fig. 4. Relative mean abundance of the 10 most abundant phyla based on 16S rRNA gene sequencing data for bacterial assemblages collected in 10 study streams. Proteobacteria is represented by relative abundance of classes.

unclassified bacteria, Comamonadaceae, and Flavobacteriaceae, and on plastic the most common were Pseudomonadaceae, unclassified Gammaproteobacteria, and Comamonadaceae (Fig. 5; Appendix S1: Table S8).

Several bacterial families were more abundant on microplastic compared with the other substrates. Pseudomonadaceae was significantly more abundant on plastic, and it accounted for 12.2% of total sequences on the plastic, but only 0.8% of the total sequences from the upstream water column and 2.0% and 2.5% of total sequences from the downstream water column and OM, respectively (Fig. 5; Appendix S1: Table S8). Similarly, unclassified Gammaproteobacteria represented 9.3% of sequences on plastic, but <2% of the total sequences on all other substrates (Fig. 5; Appendix S1: Table S8). On plastic, Burkholderiales incertae sedis comprised 5.5% of sequences, but only 1.2% on organic material and <1% in the upstream and downstream water columns (Fig. 5; Appendix S1: Table S8). Finally, Veillonellaceae and Campylobacteraceae accounted for 4.2% and 1.7% of total sequences on the plastic, respectively, but <1% of the total sequences in the other three substrates; however,

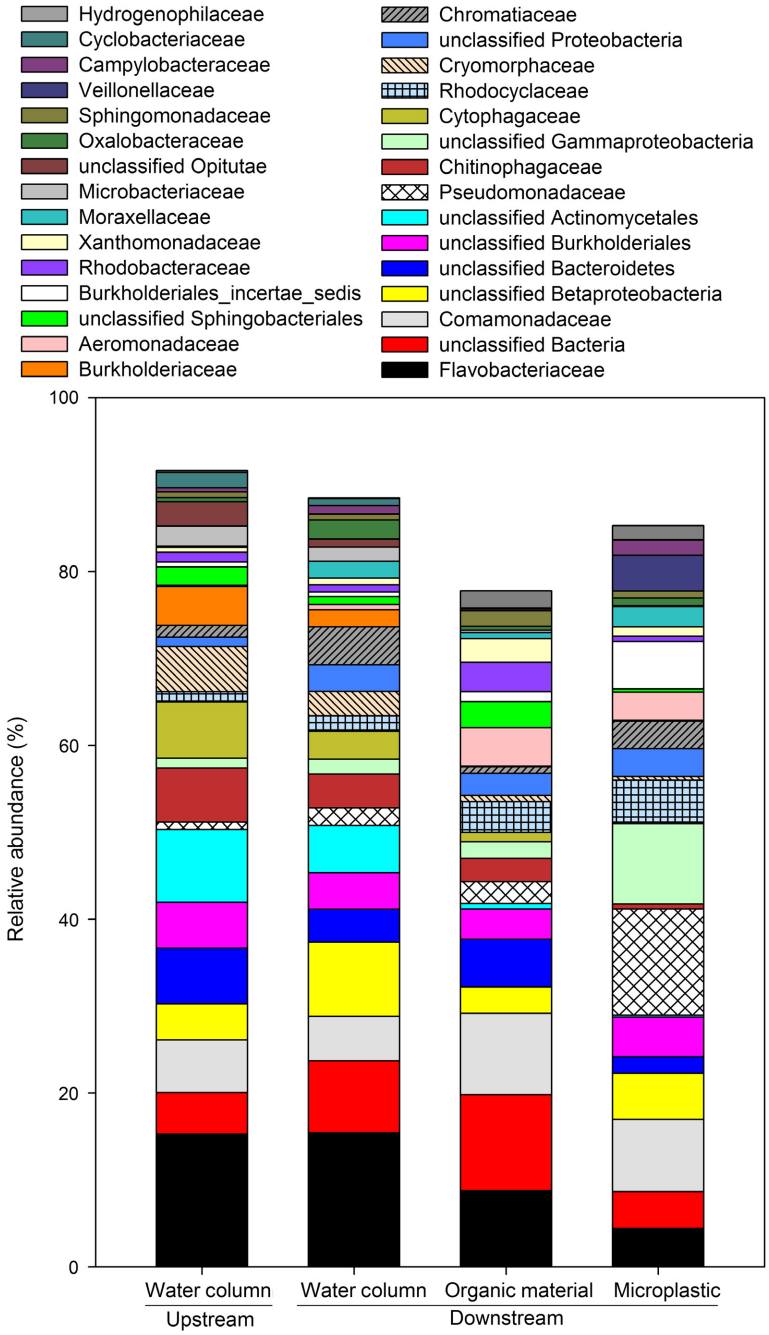

Fig. 5. Relative mean abundance of the 30 most abundant families based on 16S rRNA gene sequencing data for bacterial assemblages collected in 10 study streams.

this increased abundance on plastic was not statistically significant for these two families (Fig. 5; Appendix S1: Table S8).

There were 60 OTUs that accounted for 60.7% of the variation between plastic and downstream organic material (Table 3). The taxa contributing most to this variation were unclassified Gammaproteobacteria (6.9%), which were 5.3 times more abundant on plastic than on organic material, and unclassified bacteria (6.2%), which

were 2.9 times more abundant on organic material than on plastic (Table 3). *Pseudomonas* and *Aquabacterium* were 8.7 and 14.5 times more abundant on plastic than on organic material, respectively. Other groups that were significantly more abundant on plastic than on organic material were unclassified Pseudomonadaceae, unclassified Betaproteobacteria, *Rheinheimera*, *Acinetobacter*, *Arcobacter*, and *Azospira*. *Flavobacterium* and unclassified genera from Bacteroidetes, Sphingobacteriales, Rhodobacteraceae, Rhizobiales, Chitinophagaceae, and Alphaproteobacteria were significantly higher on the organic material than on plastic (Table 3).

Microplastic bacterial assemblages among streams

The relative composition of microplastic-associated taxa showed variation among study streams. For instance, unclassified Gammaproteobacteria was the most dominant bacterial group on plastic from Schererville Ditch (28.7%) and the DuPage River (13.8%), but its relative abundance at other sites was 0.9–11.2% (Appendix S1: Fig. S2). *Pseudomonas* were present on plastic from all streams, and their relative abundance ranged from 1.2 to 14.6% (Appendix S1: Fig. S2). Unclassified Betaproteobacteria was the most prevalent group in Springbrook Creek (10.8%), and *Aquabacterium* was the most common genus in Higgen's Creek (18.3%; Appendix S1: Fig. S2). The dominant genera on plastic from the North Shore Channel, Salt Creek, and the West Branch of the DuPage River were *Zymophilus* (19.1%), *Rheinheimera* (10.9%), and *Thiobacillus* (11.0%), respectively (Appendix S1: Fig. S2). Across streams, unclassified Pseudomonadaceae, *Acinetobacter*, *Arcobacter*, and *Azospira* had relative abundances on microplastic samples of 0.7–8.6%, 0.3–4.3%, 0.2–3.5%, and 0.1–4.9%, respectively.

Discussion

Microplastic concentration and flux in urban rivers

Our results for microplastic concentration and the composition of microplastic types suggest that WWTP effluent is an important source of microplastic to urban rivers, and rivers represent a substantial flux of plastic to downstream ecosystems. Microplastic concentrations were higher downstream of WWTPs than upstream at all but two sites. Pellets, which are associated with personal care products that enter WWTPs (Fendall and Sewell 2009), had a higher relative abundance downstream, and their concentration was higher downstream at all but one site. Fibers made up a large proportion of microplastic from both upstream and downstream locations, and the concentration of fibers was higher downstream at all but two sites.

Although WWTP effluent influenced microplastic concentrations at almost all of our sites, it had no effect at two streams in Bloomington, IL (Goose and Little Kickapoo Creeks; Fig. 1). We propose two possible explanations for this pattern: sand filtration and upstream hydrology. Sand filtration is a tertiary treatment step used in some WWTPs in which pretreated wastewater flows over and percolates through a sand layer or similar media. Sand filtration is designed to remove contaminants (e.g., suspended solids, ammonia, and fecal coliform bacteria) from wastewater (Environmental Protection Agency 2002). Specifications for sand grain size in sand filters are 0.25–1.00 mm, and <3% of the media is recommended to contain fine grains, classified as <0.074 mm (Environmental Protection Agency 2002). The effectiveness of the sand media is dependent on the size and uniformity of grains (Environmental Protection Agency 2002), and an initial study on microplastic retention in sand filters found low effectiveness for plastic size fractions of 20–300 and >300 μm (Magnusson and Wahlberg 2014 as reported in Storck and Kools 2015). However, other recent studies have demonstrated that microplastic concentrations in effluent can be relatively low compared with those in influent. For instance, at a small WWTP in Sweden, Magnusson and Norén (2014) found that >99% of microplastic in sewage influent was retained in sludge during the treatment process. Similarly, at WWTPs using tertiary treatment, a majority of microplastic was found to mix with sludge and settle, and very few plastic particles were contained in effluent (Carr et al. 2016).

Our study locations with sand filters ($n = 5$) and without sand filters ($n = 4$) had mean downstream to upstream microplastic concentration ratios of 0.43 and 1.71, respectively. While the mean ratio was lower at sites using sand filtration, there was no statistical difference because the ratios were highly variable among sites. Also, at three of the sites with sand filters (Goose Creek, Little

Table 3. Bacterial operational taxonomic units (OTUs) making the most significant contribution to variation between assemblages from plastic and organic material collected downstream of WWTPs.

Taxon	Organic material	Plastic	df	*t*-value	*P*-value	Contrib. to variation (%)	Cumulative contrib. to variation (%)
Unclassified Gammaproteobacteria	1.90	10.12	24.10	−2.93	0.007	6.92	6.92
Unclassified Bacteria	11.12	3.81	50.44	5.32	<0.001	6.23	13.15
Pseudomonas	0.87	7.58	24.22	−4.00	0.001	5.21	18.36
Flavobacterium	7.97	4.04	42.17	3.73	0.001	4.30	22.66
Aquabacterium	0.92	5.28	24.28	−2.61	0.015	3.60	26.26
Unclassified Pseudomonadaceae	0.86	4.85	24.29	−2.91	0.008	3.22	29.48
Unclassified Betaproteobacteria	3.02	5.47	26.43	−2.31	0.029	3.01	32.49
Unclassified Bacteroidetes	5.51	1.82	41.01	9.51	<0.001	2.90	35.39
Unclassified Sphingobacteriales	3.04	0.31	40.93	8.92	<0.001	2.09	37.48
Rheinheimera	0.75	2.56	25.60	−2.19	0.038	1.92	39.40
Unclassified Rhodobacteraceae	2.40	0.40	36.45	4.08	<0.001	1.55	40.95
Acinetobacter	0.31	2.05	24.93	−2.94	0.007	1.47	42.42
Unclassified Rhizobiales	1.86	0.35	39.87	5.28	<0.001	1.19	43.61
Unclassified Chitinophagaceae	1.88	0.38	58.00	8.26	<0.001	1.18	44.79
Unclassified Alphaproteobacteria	1.67	0.39	52.71	7.64	<0.001	1.01	45.80
Arcobacter	0.22	1.42	24.73	−3.45	0.002	0.98	46.78
Azospira	0.07	1.30	24.15	−2.79	0.010	0.97	47.75
Unclassified Xanthomonadaceae	1.42	0.64	43.18	3.88	<0.001	0.84	48.59
Unclassified Sphingomonadaceae	1.17	0.41	57.95	3.20	0.002	0.82	49.41
Cellvibrio	0.82	0.39	57.48	2.23	0.030	0.63	50.04
Arenimonas	0.80	0.11	36.00	3.11	0.004	0.56	50.60
Unclassified Cytophagaceae	0.72	0.07	35.27	4.31	<0.001	0.52	51.12
Prosthecobacter	0.71	0.08	37.29	4.10	<0.001	0.52	51.64
Rhodobacter	0.71	0.08	35.70	4.18	<0.001	0.50	52.14
Methylophilus	0.70	0.14	40.02	4.09	<0.001	0.49	52.63
Unclassified Flavobacteriaceae	0.67	0.06	34.17	2.24	0.032	0.47	53.10
Deefgea	0.65	0.18	43.68	2.89	0.006	0.47	53.57
Thiothrix	0.52	0.09	41.31	2.48	0.017	0.41	53.98
Unclassified Actinomycetales	0.62	0.22	58.00	2.53	0.014	0.40	54.38
Unclassified Saprospiraceae	0.45	0.03	58.00	5.63	<0.001	0.32	54.70
Unclassified Planctomycetaceae	0.46	0.09	50.05	5.44	<0.001	0.32	55.02
Sulfurospirillum	0.02	0.41	24.07	−3.39	0.002	0.31	55.33
Haliea	0.38	0.06	51.38	5.06	<0.001	0.28	55.61
Haliscomenobacter	0.39	0.05	58.00	6.53	<0.001	0.27	55.88
3 genus incertae sedis	0.41	0.09	51.92	5.89	<0.001	0.26	56.14
Unclassified Sphingomonadales	0.33	0.10	54.87	2.38	0.021	0.26	56.40
Unclassified Hyphomicrobiaceae	0.33	0.04	37.55	4.43	<0.001	0.24	56.64
Unclassified Deltaproteobacteria	0.35	0.12	52.67	3.74	<0.001	0.23	56.87
Ohtaekwangia	0.32	0.03	58.00	4.80	<0.001	0.23	57.10
Unclassified Burkholderiales incertae sedis	0.05	0.32	24.35	−3.40	0.002	0.22	57.32
Ferruginibacter	0.30	0.04	58.00	4.30	<0.001	0.21	57.53
Unclassified Actinobacteria	0.29	0.04	58.00	4.65	<0.001	0.21	57.74
Bacteroides	0.05	0.24	58.00	−2.32	0.024	0.20	57.94
Sediminibacterium	0.29	0.08	58.00	3.85	<0.001	0.19	58.13
Unclassified Verrucomicrobiaceae	0.27	0.03	38.60	5.39	<0.001	0.19	58.32
Porphyrobacter	0.25	0.05	38.10	3.11	0.004	0.18	58.50
Catellibacterium	0.24	0.07	48.73	2.46	0.018	0.18	58.68
Unclassified Methylococcaceae	0.22	0.06	49.38	2.32	0.025	0.17	58.85
Unclassified Acidimicrobiales	0.25	0.03	58.00	4.49	<0.001	0.17	59.02
Nitrospira	0.22	0.06	55.17	3.71	<0.001	0.17	59.19

Table 3. Continued.

Taxon	Organic material	Plastic	df	*t*-value	*P*-value	Contrib. to variation (%)	Cumulative contrib. to variation (%)
Unclassified Verrucomicrobia	0.25	0.03	51.68	9.36	<0.001	0.17	59.36
Caldilinea	0.23	0.02	36.92	5.31	<0.001	0.16	59.52
Unclassified Microbacteriaceae	0.24	0.06	58.00	3.60	0.001	0.16	59.68
Bosea	0.23	0.04	37.06	3.40	0.002	0.16	59.84
Sphingomonas	0.21	0.06	54.33	3.20	0.002	0.15	59.99
Gp4	0.21	0.04	58.00	3.66	0.001	0.15	60.14
Novosphingobium	0.23	0.06	54.43	4.70	<0.001	0.15	60.29
Cloacibacterium	0.04	0.20	25.04	−2.64	0.014	0.14	60.43
Byssovorax	0.17	0.04	51.85	2.66	0.010	0.14	60.57
Silanimonas	0.18	0.03	36.45	2.80	0.008	0.13	60.70

Notes: Each data point for organic material and plastic is the mean relative abundance of the respective taxon across all sites. *P*-value is based on a *t* test comparison of raw abundance data from plastic and organic material samples.

Kickapoo Creek, and East Branch of the DuPage River), we could not detect visible microplastic while collecting pieces for bacterial analyses, suggesting that sand filters may remove the larger microplastic items from the effluent. However, we did not separate microplastic into additional size classes, so we cannot address this hypothesis quantitatively. Furthermore, our study was not explicitly designed to test the effect of tertiary treatment methods, such as sand filtration, on microplastic concentrations or particle types in WWTP effluent. To do this effectively, microplastic abundance in both raw sewage and treated effluent would have to be measured. While that was beyond the scope of the current study, additional studies comparing microplastic concentrations in sewage influent, WWTP effluent, and various steps in the wastewater treatment process, including sand filters, are warranted and would illustrate effective methods for microplastic retention across size classes and polymer types.

Microplastic concentrations were variable among replicate net samples within each sampling site. Because net samples were collected simultaneously or in direct sequence, these data show microplastic distribution within a stream is spatially and temporally heterogeneous. Microplastic pieces collected in surface water may be recently suspended from sediment, in the processes of deposition, or floating in the water column as relatively low-density materials (i.e., polystyrene). To our knowledge, no previous work has measured distribution of microplastic at multiple sites through the water column simultaneously to determine the extent to which a surface net accurately represents the instantaneous microplastic flux throughout the water column. These assessments represent an important line of questioning for future research.

Microplastic concentration and flux were variable among streams, which is consistent with previous research showing microplastic concentrations are spatially and temporally heterogeneous in the environment (Gilfillan et al. 2009, Dubaish and Liebezeit 2013, Goldstein et al. 2013, Yonkos et al. 2014). Differences in microplastic concentrations among streams could be explained by variation in landscape features such as the number of WWTPs, combined sewer overflows, impervious surface cover, dams, and stream geomorphology. These features could enhance microplastic concentration or promote deposition. For instance, the DuPage River in Naperville, Illinois, and the North Shore Channel in Chicago, Illinois had relatively high microplastic concentrations downstream and upstream of WWTPs. The East and West Branches of the DuPage River contain several WWTPs, and they join to form the DuPage River ~730 m upstream of the Springbrook Water Reclamation Plant. Additionally, water from Lake Michigan, which contains treated effluent from various municipalities including Milwaukee, Wisconsin, flows into the North Shore Channel. Eriksen et al. (2013) measured microplastic concentrations in three of the Great Lakes and found higher concentrations near urban centers, so it is likely that the nearshore waters of Lake Michigan are a source of microplastic to the North Shore Channel.

During heavy rainfall, the North Shore Channel also receives untreated wastewater via combined sewer overflows that can contribute to microplastic accumulation.

Our study was designed to address the role of WWTPs influencing microplastic abundance using paired upstream and downstream sites. Future longitudinal studies on microplastic concentration would benefit from incorporation of land-use and stream geomorphology data (Mani et al. 2015). Detailed hydrologic environmental data would also assist in determining what factors may influence the differences in bacterial assemblage composition among streams.

Microplastic concentration in urban rivers is high relative to other ecosystems

We compared our data to global microplastic concentrations from a variety of ecosystems that used the same size range for microplastic collection and found that riverine microplastic concentrations are comparable to or exceed many previously documented values. Mean upstream and downstream microplastic concentrations from this study were higher than mean concentrations from several studies in the open ocean, and our maximum concentration was higher than almost all measurements from the open ocean (Appendix S1: Table S9). Coastal regions are considered areas of high microplastic concentration, and mean riverine microplastic concentrations from this study were comparable to mean coastal measurements. Our riverine measurements were also higher than estuarine studies, and equal to or higher than concentrations from lakes (Appendix S1: Table S9). The mean downstream microplastic concentration was similar to maximum concentrations reported in the Great Lakes (Eriksen et al. 2013), and riverine concentrations were 15–40 times higher than the maximum concentration from a remote lake in Mongolia (Free et al. 2014). Finally, our results were in the range of other riverine microplastic data (Lechner et al. 2014, Dris et al. 2015, Mani et al. 2015). However, during the wet season, Moore et al. (2011) documented higher microplastic concentrations in the San Gabriel River. Additionally, the maximum concentration from the Danube River (Lechner et al. 2014) was six times higher than our maximum measurement. Some studies sampled a larger size range of microplastic than our equipment: 0.08–0.33 mm in the Seine River (Dris et al. 2015) and >0.112 mm in Three Gorges Reservoir (Zhang et al. 2015). The maximum concentrations from these two studies were higher than our measurements.

Bacterial assemblages colonizing microplastic are distinct from natural substrates

Structure and composition of bacterial assemblages differed among sample sites and substrate types. Few studies have examined microplastic microbial assemblages, but our results showing microplastic selects for a unique assemblage of bacteria are consistent with results from other earlier studies (Zettler et al. 2013, Harrison et al. 2014, McCormick et al. 2014). In particular, community richness and diversity on microplastic were low compared with natural substrates, as shown in the North Shore Channel one year prior to data collection for this study (McCormick et al. 2014).

The differences between the bacterial assemblages on organic material and plastic are of particular interest as the substrates exist in close proximity in rivers and were collected simultaneously in the same net. The identity of the taxa that were more abundant on plastic offers support for both mechanisms of taxa selection by plastic: the availability of a hard surface and the organic carbon source in plastic polymers.

Among the most notable distinctions between plastic and other substrates was the relatively high abundance of sequences representing Pseudomonadaceae and unclassified Gammaproteobacteria. Previous research has shown Gammaproteobacteria are early biofilm colonizers of nonnatural substrates in marine habitats (Lee et al. 2008), and these bacteria also are prevalent in biofilms located downstream of WWTPs (Marti et al. 2013). In particular, the Gammaproteobacterial genus *Pseudomonas* had significantly higher abundance on microplastic than organic material. *Pseudomonas* sequences were also prevalent on microplastic-associated bacterial assemblages from previous work in an urban river (McCormick et al. 2014), and *Pseudomonas* is a common genus in other urban waterways (Ibekwe et al. 2013). *Pseudomonas* spp. have been associated with degradation of plastic polymers such as high-density polyethylene (HDPE; Balasubramanian et al. 2010),

low-density polyethylene (Tribedi et al. 2015), polyethylene (Kathiresan 2003), polypropylene (Cacciari et al. 1993, Arkatkar et al. 2010), and polyvinyl alcohol (Shimao 2001). Some strains of *Pseudomonas* produce enzymes such as serine hydrolases, esterases, and lipases, which assist in plastic biodegradation (Bhardwaj et al. 2013). Furthermore, previous studies have shown plastic degradation by some *Pseudomonas* strains is rapid. For example, a *Pseudomonas* strain isolated from a plastic waste disposal site contributed to a 15% weight loss of HDPE after a 30-d incubation (Balasubramanian et al. 2010), and another *Pseudomonas* isolate degraded over 20% of polyethylene in 30 d (Kathiresan 2003). While *Pseudomonas* is a metabolically diverse bacterial genus containing over 200 species (Euzéby 1997) and our data do not permit identification of specific species or strains of this genus, its consistent presence on microplastic substrates suggests there is a mechanism selecting for this group's colonization of plastic.

Another notable feature of the microplastic-associated microbial assemblages was the significantly higher relative abundance of the bacterial family Burkholderiales incertae sedis (order Burkholderiales). A recent survey of bacterial diversity in 14 wastewater treatment systems in China reported that Burkholderiales incertae sedis were found in all of the systems (Wang et al. 2012). In wastewater treatment processes using moving bed biofilm reactors, Pal et al. (2012) found that Burkholderiales incertae sedis was an abundant group in bacterial communities on biofilm substrates composed of polypropylene, polyethylene, and polyvinyl chloride (PVC). Another member of the Burkholderiales order, the genus *Aquabacterium* (family Comamonadaceae), also had a high relative abundance on microplastic, and previous research identified this taxon as a dominant member of biofilms formed on plastic in drinking water facilities (Kalmbach et al. 2000). Drinking water is oligotrophic and dark in comparison with WWTP effluent and the water column of urban rivers, so their abundance may be related to the presence of plastic rather than nutrient availability in effluent. Some members of the *Aquabacterium* genus metabolize plasticizers used in soft PVC (Kalmbach et al. 1999), so it is possible that these taxa have plastic-degrading capabilities.

In addition to biofilm-forming and putatively plastic-degrading bacteria, some of the taxa common to microplastic assemblages are associated with pathogenic bacteria, supporting a wastewater origin of microplastic in the rivers. For instance, while not statistically significant, Campylobacteraceae had the highest relative abundance on microplastic substrates, and this family includes several pathogens (On 2001, Lu and Lu 2014). In particular, sequences representing the genus *Arcobacter* (family Campylobacteraceae) were significantly more abundant on microplastic than organic material, and this genus contains pathogenic species (Engberg et al. 2000, Lu and Lu 2014) and is abundant in sewage (Newton et al. 2013). Recent evidence suggests survival and growth of *Campylobacter jejuni*, a common cause of human gastroenteritis, is greater on biofilms containing *Pseudomonas aeruginosa* (Culotti and Packman 2015), which may offer some explanation for the abundance of both *Pseudomonas* spp. and Campylobacteraceae taxa on microplastic biofilms. The capacity of microplastic to support biofilms that transport pathogenic bacteria from WWTPs into rivers poses a potential threat to human and ecosystem health. Pathogenic bacteria abundance may be relatively high on microplastic recently emerging from the WWTP, and it is unknown whether they may persist after long exposure to the environmental conditions in rivers. Research on the capacity of microplastic to transport pathogenic bacteria longer distances downstream is needed.

Composition of bacterial assemblages on microplastic samples varied among sites. Previous studies on microplastic-associated bacterial assemblages also show variation in composition. For instance, Zettler et al. (2013) described a diverse "plastisphere" assemblage on microplastic in the marine pelagic environment, where *Vibrio* was a dominant member of bacterial assemblages. With an incubation experiment using marine sediment, Harrison et al. (2014) found that after 14 d, bacterial communities on low-density polyethylene were dominated by two genera: *Arcobacter* and *Colwellia*. We found no *Vibrio* or *Colwellia* sequences in our samples, but the genus *Arcobacter* was significantly more abundant on microplastic than suspended OM. There are few studies on the interactions between

microbes and microplastic (Harrison et al. 2011), and further research is necessary to understand microplastic's ecological impacts via microbial community composition and function. In particular, research that identifies plastic degradation metabolism and persistence of pathogens on microplastic is needed.

The fate of riverine microplastic

Urban rivers contain high microplastic concentrations in surface waters compared with other habitats, and rivers in our study transport an estimated average of 1,338,757 microplastic pieces per day (Table 2). If concentrations in the rivers are consistent across seasons, this represents 488 million pieces of plastic per year per river (min: 5.6 million pieces per year, max: 1.7 billion pieces per year). We note these annual estimates are very preliminary, and seasonal analyses will be required to generate more robust calculations of annual flux. These data support a major role for rivers in the global microplastic "life cycle." However, we know little about the downstream movement and deposition of microplastic in rivers. Microplastic can be transported long distances, as several recent studies reported high concentrations of microplastic in estuaries and other coastal habitats and implicated rivers as major microplastic sources to the ocean (Moore et al. 2002, Dubaish and Liebezeit 2013, Lima et al. 2014, Sadri and Thompson 2014, Yonkos et al. 2014). Some microplastic is deposited into river sediments, as microplastic concentrations in St. Lawrence River sediments were ~137,590 no./m^3 (Castañeda et al. 2014) and microplastic concentrations in sediment were up to 150,000 times higher than surface water samples in the North Shore Channel (T. J. Hoellein, *unpublished data*). In addition, biofilm formation may decrease the buoyancy of microplastic and thus contribute to its accumulation in sediments (Castañeda et al. 2014). We suspect that deposition is also driven by hydrology (i.e., storms), geomorphology (e.g., dams), and location within a river network (e.g., headwater streams to large rivers).

Conclusions

Our data demonstrate that microplastic is an abundant substrate in urban rivers, which have the potential to retain and move the material to downstream habitats. However, further studies on the rates of microplastic deposition, export, and degradation in rivers are needed. Additionally, while our data indicate that microplastic-associated bacterial assemblages are consistently different from those on natural substrates, additional research is needed to elucidate the mechanisms for this selection. Finally, the effects of microplastic on other microorganisms and freshwater consumers are relatively unknown and warrant further investigation.

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Supporting Information

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