Microplastic selects for convergent microbiomes from distinct riverine sources

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Abstract: Microplastic is a contaminant of concern in freshwater ecosystems worldwide. Microplastic particles within aquatic habitats are colonized by dense microbial biofilms, and previous studies have shown that microplastic microbiomes are distinct in taxonomic composition from bacterial assemblages in the surrounding environment. However, questions remain about the degree to which microplastic selects for specific bacterial taxa across diverse aquatic habitats. We used laboratory microcosms inoculated with water from 3 rivers in northern Illinois watersheds with distinct land-use types to test the hypothesis that microbiomes present on microplastic would be similar even when the microplastic was incubated with microbial assemblages from different source waters. When microplastic from a commercial soap and ceramic tiles were incubated with water from each of the 3 rivers for ~1 mo, the bacterial assemblages that colonized the microplastic were remarkably consistent in taxonomic composition, whereas the planktonic and tile bacterial assemblages originating from the 3 rivers were distinct. The number of bacterial operational taxonomic units found within the microplastic microbiomes was consistent across source water treatments and ~3× lower than in the surrounding water and tiles. Some of the bacterial taxa that were over-represented in the microplastic microbiomes in our experiment can metabolize plastic or plastic-associated compounds. If microplastic microbiomes are metabolizing plastic polymers, this process could have significant implications for the long-term fate of microplastic in freshwater habitats.

Key words: microplastic, microbiome, bacteria, assemblage, freshwater, river

Microplastic particles <5 mm are contaminants in aquatic ecosystems worldwide, including both marine (Moore et al. 2001, 2002, Law et al. 2010, Doyle et al. 2011) and freshwater habitats (Eriksen et al. 2013, McCormick et al. 2014, 2016, Castaneda et al. 2014). Sources of microplastic to the environment include the accidental release of industrial resin pellets used in plastic manufacturing (Van Cauwenbergh et al. 2013) and fragmentation of larger discarded plastic items through photolysis, weathering, and physical abrasion (Cole et al. 2011, Van Cauwenbergh et al. 2013). In addition, some cleansers and personal care products contain microplastic abrasives (e.g., microbeads; Fendall and Sewell 2009, Andrady 2011). Washing synthetic textiles (e.g., fleece) can also release microplastic fibers (Gregory 1996, Murray and Cowie 2011). Microplastic from the latter 2 sources enters domestic wastewater and is not completely removed by wastewater treatment plants because of its small size and buoyancy (Gregory 1996, Fendall and Sewell 2009, Andrady 2011, Carr et al. 2016), resulting in the release of microplastic to the environment. Some of the highest microplastic concentrations in aquatic environments occur in urban rivers downstream from wastewater treatment plant effluent release points (McCormick et al. 2014, 2016, Mason et al. 2016). These rivers then carry microplastic to downstream habitats including lakes, estuaries, and oceans (GESAMP 2010, Hurley et al. 2018, Zhang et al. 2018).

Microplastic in aquatic environments can affect aquatic organisms. Specifically, microplastic can be ingested by consumers, bioaccumulate in food webs, and interact with microorganisms (Harrison et al. 2011, Cole et al. 2013, Wagner et al. 2014, McNeish et al. 2018). Ingestion and bioaccumulation of microplastic can be hazardous to organisms because microplastic can limit food uptake, damage digestive systems, and transmit harmful chemicals (Browne et al. 2008, Rios et al. 2010, Rochman et al. 2013, Wright et al. 2013). Recent studies have also found that microplastic particles are colonized by dense microbial biofilms in both marine (Carson et al. 2013, Zettler et al. 2013, Harrison et al. 2014, Reisser et al. 2016).
2014, Kirstein et al. 2016, Frère et al. 2018) and freshwater habitats (McCormick et al. 2014, 2016, Hoellein et al. 2017a). Some of these microplastic microbiomes included potentially pathogenic taxa (e.g., *Vibrio, Campylobacter*, and *Arcobacter*), which suggests that microplastic particles may be vectors of infectious diseases (Kirstein et al. 2016, Harrison et al. 2018). Some members of these microplastic microbiomes might also contribute to the breakdown of plastic polymers (e.g., Zettler et al. 2013, Reisser et al. 2014), which could have significant implications for the long-term fate of microplastic in the environment. In addition, the presence of microbial biofilms on microplastic may increase the likelihood of its ingestion by consumers (Reisser et al. 2014). Finally, the presence of microbial biofilms on microplastic particles can increase microplastic deposition from the water column (Fazey and Ryan 2016, Lagarde et al. 2016) and its retention in streams (Hoellein et al. 2019). Therefore, it is important to understand the composition of microplastic microbiomes in aquatic environments.

Studies in both marine and freshwater ecosystems have demonstrated that microplastic microbiomes are distinct in taxonomic composition from bacterial assemblages in the surrounding environment (Zettler et al. 2013, Harrison et al. 2014, McCormick et al. 2014, 2016, Frère et al. 2018, Oberbeckmann et al. 2018, Ogonowski et al. 2018). Thus, microplastic may be a distinct microbial habitat that selects for specific bacterial taxa. This selection may be based on the physical properties of microplastic (e.g., buoyancy, hydrophobicity, resistance to decomposition) or its distinct chemical composition, including the makeup of the plastic polymers or any associated additives or sorbed contaminants (Harrison et al. 2018). For example, several recent studies in marine habitats reported that the composition and diversity of microplastic microbiomes varied according to microplastic polymer type (Amaral-Zettler et al. 2015, Frère et al. 2018, Ogonowski et al. 2018).

Some bacterial taxa may be particularly well adapted to colonizing microplastic particles in aquatic systems. A recent paper analyzed 98 data sets that characterized microbial assemblages associated with polyethylene marine debris recovered from various locations across the Atlantic and Pacific Oceans and the North Sea (De T tender et al. 2017). That paper reported that most of the debris were colonized by the same set of taxa, suggesting that some marine bacterial taxa are better able to colonize plastic materials than other marine taxa (De T tender et al. 2017). We expect that similar patterns also exist in freshwater habitats but that different bacterial taxa will dominate plastic microbiomes in freshwaters. However, many more field studies of microbial colonization of microplastic across a range of freshwater ecosystems will be needed to address this hypothesis (Hoellein et al. 2014).

Here, we present the results of a lab-based study to assess the colonization of microplastic particles by bacterial assemblages originating from distinct rivers. Our hypotheses were that: 1) microplastic would support microbiomes that were different in composition from natural habitats (e.g., water column and rock surfaces), and 2) microplastic would be colonized by similar microbiomes even when incubated with microbial assemblages from different rivers. We used laboratory-based studies of microplastic colonization (e.g., Ogonowski et al. 2018) because lab studies can control for the confounding effects of particle type, age, source, route of entry, time in the water, and degree of weathering that cannot be avoided in field-based studies.

**Methods**

To test our hypotheses, we set up small microcosms (90 mL) containing microplastic beads and ceramic tiles as substrates for biofilm colonization. We inoculated replicate microcosms (*n* = 4) with water from 3 rivers that were chosen to provide distinct microbial assemblages for colonization of the substrates. After a 34-d incubation, we used DNA sequencing to compare the taxonomic composition of the bacterial assemblages in each of the 3 habitats within the microcosms (water, tile, and microplastic) and identified taxa that differed in abundance based both on the microbial assemblage source and on habitat.

**Field sites**

We collected water from 3 rivers in Illinois, USA on 1 July 2014. These rivers vary in physicochemical characteristics because of significant differences in watershed land use. The North Branch Chicago River watershed (42.007160, −87.791212) is predominantly urban (Cook and Hoellein 2016). The East Branch DuPage River watershed (41.827658, −88.052136) is predominantly suburban (Drury et al. 2013). Nippersink Creek (42.417964, −88.344610) is a woodland stream located in McHenry County, Illinois, with minimal urbanization (7.8% residential and 0.1% industrial) but high agriculture (63%) in its watershed (www.nippersink.org). Prior work at these field sites indicated that they differed significantly in nutrient chemistry (Cook and Hoellein 2016, McCormick and Hoellein 2016, Hoellein et al. 2017b) as well as microbial assemblage composition (Drury et al. 2013, McCormick et al. 2016).

**Field sampling**

We collected 1 L of water in sterile glass bottles at each field site and stored it on ice in a cooler for transport to the lab. Water samples were stored in the lab over night at 4°C to limit microbial activity prior to initiation of the incubations the next day. We also collected 4 replicate 50-mL water samples at each field site for nutrient analysis. We filtered these samples on site with a 0.2-μm syringe filter (Thermo Fisher Scientific, Rockwood, Tennessee), placed them in sterile scintillation vials, stored them on ice in a cooler for transport to the lab, and then stored them at −20°C in the lab until analysis. We measured conductivity at each field site with a Yellow Springs Instruments™
We measured SRP with the antimonyl tartrate technique an Auto Analyzer 3 (SEAL Analytical, Mequon, Wisconsin). We analyzed water samples for soluble reactive phosphorus (SRP), ammonium (NH$_4^+$), and nitrate (NO$_3^-$) with an Auto Analyzer 3 (SEAL Analytical, Mequon, Wisconsin). We measured SRP with the antimonyl tartrate technique (Murphy and Riley 1962), NH$_4^+$ with the phenol hypochlorite technique (Solorzano 1969), and NO$_3^-$ with the cadmium reduction technique (Rice et al. 2012). Chemical analyses were completed within 4 mo of collection. We followed quality control and assurance checks recommended by the manufacturer (Seal Analytical) including equipment blanks, carryover tests, and drift correction. All standard curves showed $r^2 \geq 0.999$. Finally, analytical results were within expected ranges based on our extensive prior work at these field sites (Turek and Hoellien 2015, Cook and Hoellien 2016, McCormick and Hoellien 2016, Hoellien et al. 2017b).

**Microplastic collection**

We obtained the microplastic used in our incubation experiment from a facial scrub produced and marketed by an international manufacturer (Deep Clean® Invigorating Foaming Scrub, Neutrogena®, Los Angeles, California) that we purchased at a retail store in Illinois, USA in June 2014. The microplastic component of the product consisted of both spherical beads ranging from 0.5 to 1 mm in diameter and irregularly-shaped fragments ranging from 0.25 to 0.5 mm in diameter. The product label identified the microplastic polymer as polyethylene, and we confirmed that the composition of both the beads and fragments was polyethylene via pyrolysis–gas chromatography–mass spectrometry (as described in McCormick et al. 2016). The size and composition of the microplastic in this soap is consistent with microplastic previously identified in personal care products (Fendall and Sewell 2009) and in environmental samples (e.g., Erikson et al. 2013, McCormick et al. 2014, 2016). We collected microplastic from the soap with a 125-μm sieve and rinsed the microplastic with tap water until all visible soap bubbles were removed. We stored 3 replicate samples of this microplastic (0.5 mL each) in 2-mL microcentrifuge tubes at −20°C. These samples served as pre-incubation controls to determine if there was detectable microbial colonization of microplastic prior to incubation. The remaining microplastic was placed in a 90-ml sterile plastic specimen cup (Parter Medical Products™, Carson, California) and air dried in a sterile hood for 48 h prior to addition to the microcosms.

**Microcosm incubation**

We set up 12 microcosms and inoculated each with water from 1 of 3 sources (Chicago River, DuPage River, or Nippersink Creek) for a total of 4 replicate microcosms for each of the 3 rivers. Each microcosm consisted of a 90-ml sterile plastic specimen cup (Parter Medical Products) filled with 80 mL of river water. We then added 2.5 mL of microplastic collected from the facial scrub and 1 sterile 2 x 2-cm unglazed ceramic tile to each microcosm. The unglazed ceramic tiles served as a surrogate for a rock substrate (Hoellien et al. 2014) and were sterilized by autoclaving before they were placed in the microcosms.

We incubated the microcosms with their lids on at room temperature for 34 d in a glass top incubator with circular horizontal shaking at 80 revolutions/min. We included shaking to improve gas exchange between the water and the headspace and to mimic the constant movement of the microplastic that would occur in a river. Microcosms were positioned randomly within the incubator and exposed to sunlight via an adjacent window. Each microcosm was aerated every 48 h by removing it from the incubator, lifting its lid ~1.5 cm directly above the microcosm for 10 s, and then replacing its lid. We aerated the microcosms individually to prevent cross-contamination between microcosms. However, some airborne microbes from the ambient air in the lab might have entered the microcosms during the aeration step. After aeration, we rearranged the microcosms randomly within the incubator to minimize positional effects.

Incubations lasted for 34 d. At the end of the experiment we used sterile forceps to remove the tile from each microcosm. We collected the biofilm from each tile by swabbing the entire tile surface with a sterile cotton swab, then stored the swab in a sterile 2-ml microcentrifuge tube at −20°C for subsequent DNA extraction. Three unused sterile cotton swabs were also placed in microcentrifuge tubes and stored at −20°C as controls to confirm that the swabs themselves were not contaminated.

We removed the water from each microcosm at the end of the experiment with a sterile syringe and collected suspended bacteria by filtering the water through Sterivex™ 0.22-μm filter cartridges (Millipore, Burlington, Massachusetts). Each filter was removed from its cartridge and cut into 5 equally-sized pieces with a sterile razorblade. The pieces from each filter were stored in 1 sterile 2-ml microcentrifuge tube at −20°C before subsequent DNA extraction (Crump et al. 2003). We also cut 3 unused Sterivex filters into 5 pieces, placed them in sterile 2-ml microcentrifuge tubes, and stored them at −20°C as controls to confirm that the filters themselves were not contaminated.

After removing the water from each microcosm (as described above), we collected the microplastic and homogenized it with a sterile spatula. We stored a 0.5-mL subsample of microplastic from each microcosm in a sterile 2-ml microcentrifuge tube at −20°C for subsequent DNA extraction. We extracted DNA from all cotton swabs, Sterivex filters, and microplastic samples with a PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, California) following the manufacturer’s instructions and stored the DNA at −20°C.
Polymerase chain reaction amplification and sequencing

Partial 16S rRNA genes were amplified from all DNA samples with primers 515F and 806R, which amplify the V4 hypervariable region of bacterial 16S rRNA genes (Caporaso et al. 2011). We amplified the V4 hypervariable region because it provides species richness estimates similar to those obtained with nearly full-length 16S rRNA genes (Youssef et al. 2009). We confirmed polymerase chain reaction amplification with agarose gel electrophoresis and observed appropriately-sized bands for all incubated tile, water, and microplastic samples. No bands were observed for controls (sterile swabs, unused Sterivex filters, or unincubated microplastic), confirming these items were not contaminated. Amplicons were sequenced in a 2 × 250 paired-end format with the MiSeq platform (illumina®, San Diego, California; Caporaso et al. 2012) by the DNA Services Facility, University of Illinois at Chicago. All sequence data analyzed in this paper can be downloaded from the National Center for Biotechnology Information Sequence Read Archive with accession number PRJNA541508.

Analysis of sequence data

Sequences were processed with mothur v.1.31.2 (Schloss et al. 2009) following the MiSeq Standard Operating Procedure (Kozich et al. 2013; see Supplementary Material for a full list of commands). Briefly, paired reads were assembled and demultiplexed, and any sequences with ambiguities or homopolymers >8 bases were removed from the data set. Sequences were aligned with the SILVA-compatible alignment database available within mothur. Chimeric sequences were identified with UCHIME (Edgar et al. 2011) and removed from the data set. Sequences were classified with 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 then clustered into operational taxonomic units (OTUs) based on 97% sequence identity (hereafter referred to as species). We randomly subsampled the entire dataset to 35,312 sequences/sample to avoid biases associated with uneven numbers of sequences across samples. We grouped each of the OTUs into genera by comparison to the mothur-formatted version of the RDP training set. We estimated Good’s coverage (Good 1953) for each sample to quantify the percentage of each of the assemblages that was covered by the subsampled sequence data set, and this metric indicated that coverage averaged 75% across all samples (range = 56–88%).

Statistical analyses

We compared water nutrient chemistry from each of the 3 rivers at the time and location of sampling to confirm that they represented distinct habitats. We first assessed if these water chemistry data were normally distributed with the Shapiro–Wilk test. Normally-distributed data (SRP concentrations) were analyzed with a 1-way analysis of variance (ANOVA) with river as the independent variable followed by Tukey’s post-hoc test for all pairwise comparisons (n = 4). Non-normally distributed data (NH₄⁺ and NO₃⁻ concentrations) were analyzed with the Kruskal–Wallis 1-way ANOVA with river as the independent variable followed by the Dwass–Steel–Christlows–Fligner test for all pairwise comparisons (n = 4). All of the tests were done in SYSTAT (version 13.0; SYSTAT Software, Chicago, Illinois).

The microcosm experiment was designed with microbial assemblage source (i.e., the river that was used as the source of water for the microcosm) and microbiome habitat (water, tile, and microplastic) as the experimental variables. At the completion of the microcosm incubation, we assessed the effects of these experimental variables on the number of OTUs. The numbers of observed bacterial species were normally distributed according to the Shapiro–Wilk test, so these data were analyzed with a 2-way ANOVA followed by Tukey’s post-hoc test (n = 4). Because the effect of microbial assemblage source varied for the different habitats, we also ran 1-way ANOVAs for each habitat type with microbial assemblage source as the experimental variable.

The composition of bacterial assemblages from the post-incubation samples were further compared by calculating dissimilarities based on the Bray–Curtis dissimilarity index (Bray and Curtis 1957) for every possible pair of samples and visualizing the resulting dissimilarity matrix with non-metric multidimensional scaling in mothur. Differences in microbiomes between habitat types and assemblage sources based on the Bray–Curtis dissimilarity index were assessed by analysis of molecular variance (Excoffier et al. 1992), a nonparametric analog of traditional ANOVAs, which was run in mothur. We used metasats analysis (White et al. 2009) run within mothur to identify bacterial genera that were differentially abundant between plastic and non-plastic samples and between microbial assemblage sources, and we used ANOVA and Tukey’s tests run in Systat to assess the significance of differences in the relative abundances of these genera.

RESULTS

Differences in nutrient chemistry among 3 microbial assemblage sources

Water from the 3 field sites (Chicago River, DuPage River, and Nippersink Creek) differed in conductivity, SRP, NH₄⁺, and NO₃⁻ concentrations (Table 1). The Chicago and DuPage Rivers had similar nutrient concentrations. Nippersink Creek had higher conductivity, ~7× lower SRP, and ~2× higher NH₄⁺ and NO₃⁻ than did the other 2 sites.

Effects of habitat and microbial assemblage source on species richness of microcosm microbiomes

Both habitat and microbial assemblage source affected the composition of the microcosm microbiomes. After the
34-d incubation, the number of bacterial species within the microcosm microbiomes differed based on both habitat (water, tile, and microplastic) and microbial assemblage source. Microplastic microbiomes included ~3 × fewer bacterial species (OTUs) than either water or tile habitats, with no difference in species richness between the water and tile microbiomes (Fig. 1A). There were also differences in species richness in the microcosm microbiomes originating from each of the 3 microbial assemblage sources, with the Chicago River producing the most, Nippersink Creek the fewest, and the DuPage River an intermediate number of species (Fig. 1B). When the effect of microbial assemblage source on species richness was assessed for each of the habitats individually, the species richness of the water and tile microbiomes differed based on microbial assemblage source, with the Chicago River producing the most species and Nippersink Creek the fewest in both the water and tile habitats (Fig. 2A, B). In contrast, the species richness of the microplastic microbiomes was consistent across the 3 microbial assemblage sources (Fig. 2C).

Effects of habitat on taxonomic composition of microcosm microbiomes

Taxonomic composition of the bacterial assemblages within the microcosms varied based on habitat type. After the 34-d incubation, microplastic microbiomes clustered separately from tile and water microbiomes in the ordination (Fig. 3) and differed from tile and water microbiomes based on the Bray–Curtis dissimilarity index (Table 2). In contrast, tile and water microbiomes did not form distinct clusters in the ordination (Fig. 3) and were not different from each other based on the Bray–Curtis dissimilarity index (Table 2). These results indicate that microplastic supported a distinct microbiome when incubated with water from 3 different sources, whereas tile and water did not support distinct bacterial assemblages. Several bacterial genera differed in relative abundance between the microplastic and non-plastic habitats (Table 3). For example, relative abundances of Burkholderiales, Sinobacteraceae, and Aquabacterium were higher within the microplastic microbiomes than in the non-plastic microbiomes. Further, relative abundances of Sphingomonas, unclassified Betaproteobacteria, and Xanthobacteraceae were lower on the microplastic than on either the tiles or in the water column.

Effects of microbial assemblage source on taxonomic composition of microcosm microbiomes

Taxonomic composition of the bacterial assemblages within the microcosms also varied based on microbial assemblage source. Bacterial assemblages originating from each of the 3 water sources formed distinct clusters in the non-metric multidimensional scaling ordination (Fig. 3) and differed based on the Bray–Curtis dissimilarity index (Table 2). Both the ordination and the Bray–Curtis scores indicated that the bacterial assemblages originating from the Chicago and DuPage Rivers were similar, whereas assemblages originating from Nippersink Creek were more distinct. Several bacterial genera differed in relative abundance based on microbial assemblage source (Table 4). Sinobacteraceae and Sphingomonas were both more abundant in microcosms seeded with water from the Chicago and DuPage Rivers than in microcosms seeded with water from Nippersink Creek. Conversely, Xanthobacteraceae, Azospirillum, and Acinetobacter were significantly more abundant in microcosms seeded with water from Nippersink Creek than in microcosms seeded with water from the other 2 sources.

DISCUSSION

Similar and simpler bacterial assemblages colonize microplastic

The goal of this study was to test the hypotheses that 1) the bacterial assemblages present on microplastic would be similar regardless of the water source, and 2) that these assemblages would differ from those in natural habitats (e.g., water column and rock surfaces). We used laboratory microcosms to test these hypotheses because it allowed us to control for potentially confounding factors that would be difficult to control for with field-collected plastic. We collected water from 3 rivers in northern Illinois, USA, that
differed significantly in watershed land use and that we knew from prior work differed in nutrient chemistry and microbial assemblage composition. When microplastic from a commercial soap was incubated with water from each of the 3 rivers for ~1 mo in laboratory microcosms, the taxonomic composition of the bacterial assemblages that colonized the microplastic were remarkably consistent across the 3 microbial assemblage sources, even though the planktonic and tile microbiomes originating from the different microbial assemblage sources were distinct. In addition, the number of bacterial species found on the microplastic was the same across all 3 microbial assemblage sources and was almost 3× lower than in the water or tile habitats. Several previous field studies have also reported lower taxonomic richness on microplastic collected from aquatic habitats relative to the surrounding native assemblages (Zettler et al. 2013, McCormick et al. 2014, 2016). Further, a previous laboratory study found lower bacterial diversity on
This result strongly suggests that some specific taxa capable of metabolizing plastic and plastic-associated compounds are abundant on microplastic. Further research on this topic should include measurements of plastic decomposition rates by freshwater microbial consortia. In addition, metagenomic and metatranscriptomic analyses of microplastic microbiomes could provide valuable insight into the presence and activity of specific metabolic pathways linked to the breakdown of plastic and plastic-associated compounds. This type of information could greatly improve our understanding of the long-term fate of microplastic in aquatic environments.
freshwater habitats and could be valuable in the development of strategies to limit and remediate microplastic contamination of aquatic ecosystems.

**Differences in microcosm bacterial assemblages originating from different sources**

Microbial assemblage source, i.e., which of the rivers provided the seed assemblage for the microcosms, also had significant effects on the microcosm microbiomes. The Chicago and DuPage Rivers, which have predominantly urban and suburban watersheds, respectively, produced microcosm microbiomes that were similar in species richness and taxonomic composition, while Nippersink Creek, which has a watershed dominated by agriculture, produced microcosm microbiomes with lower species richness and distinct taxonomic composition. Several bacterial taxa were significantly more abundant in the microcosms established from the Chicago and DuPage Rivers, including *Burkholderiales* and *Sinobacteraceae*, both of which have been linked to the decomposition of complex organic compounds (Juhasz et al. 1997, Watanabe 2001, Wang et al. 2017) and both of which were also abundant on the microplastic incubated in our study. In addition, bacteria from the genus *Sphingomonas* were ~25× more abundant in the microcosms incubated with waters from the Chicago and DuPage Rivers than with water from Nippersink Creek. *Sphingomonas* are highly metabolically versatile, commonly isolated from contaminated environments, and able to degrade complex organic compounds including antimicrobial compounds (Kämpfer 2010). Therefore, the differences in bacterial assemblage composition that we observed between microcosms incubated with waters from different rivers likely reflect the presence of anthropogenic compounds in the urban and suburban Chicago and DuPage Rivers, including plastic-related and wastewater-associated chemicals typical of more urbanized ecosystems. The taxa that were more abundant in the microcosms seeded with water from Nippersink Creek, specifically *Azospirillum* and *Acinetobacter*, are common soil organisms (Steenhoudt and Vanderleyden 2000, Doughari et al. 2011). The high relative abundance of these genera within the Nippersink Creek microcosms may reflect the rural nature of this site’s watershed.

| Experimental variable | p-value | Pairwise comparison | p-value |
|-----------------------|---------|---------------------|---------|
| Habitat               | <0.001  | Microplastic-Tile   | <0.001  |
|                       |         | Microplastic-Water  | <0.001  |
|                       |         | Tile-Water          | 0.245   |
| Assemblage source     | <0.001  | Chicago River-DuPage River | <0.001 |
|                       |         | Chicago River-Nippersink Creek | <0.001 |
|                       |         | DuPage River-Nippersink Creek | <0.001 |

Table 2. Analysis of molecular variance results for comparisons of bacterial assemblage composition based on the Bray-Curtis index.

| Experimental variable | p-value | Pairwise comparison | p-value |
|-----------------------|---------|---------------------|---------|
| Habitat               |         | Microplastic-Tile   | <0.001  |
|                       |         | Microplastic-Water  | <0.001  |
|                       |         | Tile-Water          | 0.245   |

Table 3. Bacterial genera with the largest differences in relative abundance between microplastic and non-plastic habitats (water or tile).

| Genus                      | Microplastic (%) | Non-plastic (%) | p-value |
|----------------------------|------------------|-----------------|---------|
| Unclassified Bacteria      | 8.89 ± 0.83      | 21.06 ± 2.10    | 0.001   |
| Unclassified Burkholderiales | 17.55 ± 1.52     | 9.63 ± 1.25     | 0.002   |
| Unclassified Sinobacteraceae | 9.91 ± 2.40      | 3.63 ± 0.75     | 0.015   |
| Aquabacterium              | 4.54 ± 1.71      | 0.22 ± 0.05     | 0.001   |
| Unclassified Alphaproteobacteria | 7.61 ± 0.72      | 4.44 ± 0.33     | 0.001   |
| Sphingomonas               | 1.41 ± 0.37      | 3.81 ± 0.76     | 0.017   |
| Unclassified Betaproteobacteria | 0.77 ± 0.08      | 2.71 ± 0.29     | 0.001   |
| Unclassified Xanthobacteraceae | 0.21 ± 0.08      | 2.12 ± 1.11     | 0.053   |
| Brevundimonas             | 2.96 ± 0.32      | 1.06 ± 0.43     | 0.002   |
| Unclassified Verrucomicrobia | 0.47 ± 0.07      | 1.63 ± 0.38     | 0.006   |
| Unclassified Rhizobiales  | 1.21 ± 0.09      | 2.33 ± 0.37     | 0.003   |

a Genera are listed in order of decreasing differences in relative abundance between plastic and non-plastic microbiomes. All genera with differences in relative abundance greater than 1% are included.

b Data represent mean relative abundance for each genus (±SE; n = 12 for microplastic and n = 24 for non-plastic).
Table 4. Bacterial genera with the largest differences in relative abundance within microcosm microbiomes based on microbial assemblage source.

| Genus                        | Chicago River (%) | DuPage River (%) | Nippersink Creek (%) | p-value |
|------------------------------|-------------------|------------------|----------------------|---------|
| Unclassified Sinobacteraceae | 10.66 ± 1.86      | 6.22 ± 1.37      | 0.01 ± 0.00          | <0.001  |
| Unclassified Xanthobacteraceae | 0.07 ± 0.01      | 0.20 ± 0.07      | 4.38 ± 2.07          | 0.024   |
| Azospirillum                  | 0.04 ± 0.02      | 0.01 ± 0.00      | 4.17 ± 1.35          | 0.001   |
| Sphingomonas                  | 4.15 ± 0.82      | 4.43 ± 1.04      | 0.16 ± 0.06          | 0.001   |
| Aquabacterium                 | 0.74 ± 0.19      | 0.39 ± 0.12      | 4.19 ± 1.89          | 0.037   |
| Unclassified Burkholderiales  | 10.28 ± 1.02     | 13.87 ± 1.37     | 12.97 ± 3.07         | 0.425   |
| Unclassified Saprospiraceae   | 0.99 ± 0.16      | 1.30 ± 1.05      | 3.34 ± 1.06          | 0.140   |
| Unclassified Verrucomicrobia  | 2.30 ± 0.61      | 1.13 ± 0.24      | 0.17 ± 0.03          | 0.002   |
| Unclassified Opitutae        | 1.85 ± 1.28      | 0.36 ± 0.23      | 0.00 ± 0.00          | 0.213   |
| Unclassified Bacteria         | 14.75 ± 1.85     | 19.50 ± 4.28     | 16.41 ± 2.02         | 0.522   |
| Unclassified Alphaproteobacteria | 6.14 ± 0.77   | 5.78 ± 0.80      | 4.59 ± 0.45          | 0.290   |
| Unclassified Sphingomonadaceae | 3.72 ± 0.37     | 3.53 ± 0.51      | 2.19 ± 0.42          | 0.045   |
| Acinetobacter                 | 0.71 ± 0.18      | 0.37 ± 0.13      | 2.23 ± 0.68          | 0.007   |
| Unclassified Proteobacteria   | 2.76 ± 0.23      | 2.66 ± 0.18      | 4.25 ± 0.46          | 0.002   |
| Zoogloea                      | 0.15 ± 0.04      | 0.20 ± 0.05      | 1.57 ± 0.80          | 0.061   |
| Unclassified Gammaproteobacteria | 2.50 ± 0.24   | 2.11 ± 0.13      | 1.11 ± 0.27          | <0.001  |
| Unclassified Chlamydiales     | 0.13 ± 0.04      | 0.24 ± 0.06      | 1.50 ± 1.28          | 0.361   |
| Novosphingobium               | 0.89 ± 0.10      | 1.33 ± 0.46      | 2.19 ± 0.30          | 0.028   |
| Rhizobiales                   | 1.62 ± 0.14      | 1.38 ± 0.14      | 2.92 ± 0.70          | 0.031   |

* Genera are listed in order of decreasing differences in relative abundance between Chicago River and Nippersink Creek assemblages. All genera with differences in relative abundance greater than 1% are included.

b Data represent mean relative abundance for each genus ± standard error (n = 12). Lowercase letters indicate significant differences between microbial assemblage sources based on Tukey’s post-hoc test (p < 0.05).

Considerations for future work

This study was based on a simple microcosm system, and while it did not perfectly match the physical and chemical conditions that would have existed in the field, it enabled us to maintain consistent incubation conditions for all of our treatments, which would not have been possible in the field. Another advantage of this simple system is that it required no advanced equipment and could easily be replicated by virtually any lab. Further experiments under more realistic field conditions would help to assess the relevance of our findings to actual rivers. Furthermore, our study did not include an analysis of the bacterial assemblages in the water from each of the field sites at the time of collection. We cannot definitively state that the initial bacterial assemblages from each of the rivers were different at the time of collection.

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Author contributions: JJK and TJH designed the experiment. JJK, NO, and AO carried out the experiment. MGL and JJK analyzed the data. JJK and TJH wrote the manuscript.

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