

## Urban stream microbial communities show resistance to pharmaceutical exposure

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**Abstract.** Residues of pharmaceuticals are increasingly detected in surface waters throughout the world. In four streams in Baltimore, Maryland, USA, we detected analgesics, stimulants, antihistamines, and antibiotics using passive organic samplers. We exposed biofilm communities in these streams to the common drugs caffeine, cimetidine, ciprofloxacin, and diphenhydramine. Respiration rates in the least urban stream were suppressed when exposed to these drugs, but biofilm functioning in the most urban stream was resistant to drug exposure. Exposure to the antibiotic ciprofloxacin altered bacterial community composition at all sites, with the greatest change occurring in the most urban stream. These results indicated that continuous exposure to drugs in urban streams may select for sub-populations of highly resistant bacteria that maintain community function in response to urban contaminants.

**Key words:** biofilms; ecological function; pharmaceuticals and personal care products; urban land use.

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### INTRODUCTION

Microbial biofilms, which are mixed assemblages of microorganisms, that is, bacteria and algae, encased in an extracellular matrix and attached to a surface, are ubiquitous in aquatic habitats and are frequently both structurally and taxonomically complex (Davey and O'Toole 2000). Biofilms are key components of stream ecosystems because they are major drivers of nitrogen and carbon cycling (Battin et al. 2003), they are the base of stream food webs (Hall and Meyer 1998, Pusch et al. 1998), and they contribute to important ecosystem services, such as decreasing nutrient pollution and bioremediation of organic pollutants (Davey and O'Toole 2000). The composition and activity of stream biofilms have been shown to be sensitive to a variety of ecological perturbations. In particular, recent

research illustrates that exposure to anthropogenic contaminants such as pharmaceuticals and personal care products (hereafter referred to as drugs) can influence stream biofilm function, for example, by suppressing processes such as primary production and respiration (Bunch and Bernot 2011, Rosi-Marshall et al. 2013, Lee et al. 2016, Richmond et al. 2016). In addition, continuous exposure to drugs can alter the diversity and taxonomic composition of microbial communities in freshwater ecosystems by selecting for organisms that can tolerate or in some cases digest the contaminant (Drury et al. 2013a, Rosi-Marshall et al. 2013, Lee et al. 2016, Zhang et al. 2016). Changes in the diversity and taxonomic composition of stream biofilms may have important implications for the structure and function of these communities and the ecosystem services they provide.

Human-dominated landscapes, such as urban rivers and streams, contain a host of anthropogenic contaminants that can influence both the structure and function of aquatic biofilms. Within urban ecosystems, contaminants including drugs enter streams through point sources such as discharge from septic fields, leaky sewage infrastructure, combined sewer overflows, and wastewater treatment plant effluent (Rosi-Marshall and Royer 2012). In addition, non-point sources such as runoff from impervious surfaces can introduce contaminants to urban streams, including road salt, inorganic nutrients, oil and grease, trace metals, and polyaromatic hydrocarbons. As the global human population increases and our world becomes more urban (Grimm et al. 2008), the concentrations of these contaminants in surface waters will continue to increase. Understanding the extent to which contaminants influence aquatic biofilm structure and function will be essential for maintaining the ecosystem services that streams provide. Currently, the effects of these urban contaminants, which generally occur as complex mixtures, on stream ecosystems are not well understood. We posit that these contaminants will exert significant pressure on the taxonomic composition and function of aquatic biofilms, which will have major consequences for the sustainability of the function and structure of these ecosystems.

In Baltimore, Maryland, USA, the suburban and urban streams draining the metropolitan area have high concentrations of nutrients (Kaushal et al. 2011) and metals (Kaushal and Belt 2012), and indicators of sewage inputs, mainly due to leaking sanitary infrastructure and impervious surface runoff (Kaushal and Belt 2012). This urban sewage input delivers a range of drugs to these streams, with higher concentrations in more urbanized catchments (Lee et al. 2016). Therefore, these streams offer an opportunity to assess the impacts of dispersed anthropogenic contaminants associated with urbanization on the structure and function of stream biofilms. We hypothesized that residues of biologically active drugs are present in streams in Baltimore and that drug concentrations are highest in the most urbanized streams, similar to other research at these sites (Lee et al. 2016). Furthermore, we hypothesized that biofilms in the most urban streams are resistant, in terms of both taxonomic composition and function, to the

drugs due to previous exposure. In contrast, we hypothesized that biofilms in suburban streams are sensitive, in terms of both taxonomic composition and function, to drugs due to less previous exposure. To test these hypotheses, we used polar organic chemical integrative samplers (POCIS) (Alvarez et al. 2005) to identify and quantify a suite of drugs in four streams in Baltimore ranging from suburban to highly urban (Fig. 1). We also used contaminant exposure substrates (CES; Rosi-Marshall et al. 2013, Costello et al. 2016) to experimentally expose biofilms in the same four streams to four drugs (caffeine, the antibiotic ciprofloxacin, and the antihistamines cimetidine and diphenhydramine) and measured both the ecological function (as respiration) and the bacterial community composition (based on high-throughput sequencing of 16S rRNA genes) of

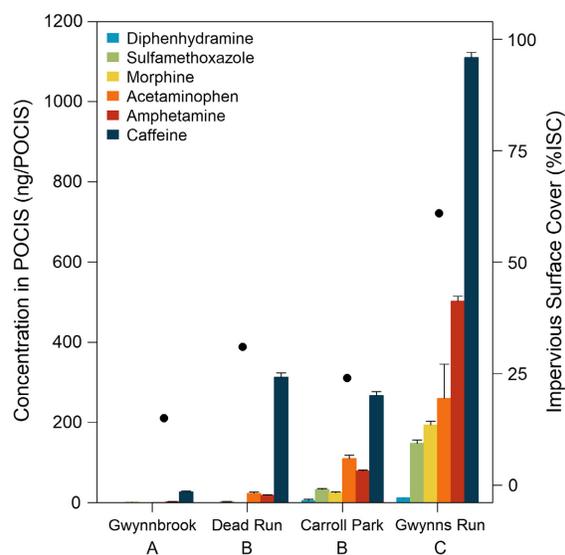


Fig. 1. Concentrations of pharmaceuticals that accumulated in passive samplers during a two-week deployment at four stream sites along a suburban to urban gradient. Impervious surface area in the watershed as indicated by the dots aligned with the second *y*-axis provides information on the extent of urbanization of the four sites. Letters below sites indicate significant differences in concentrations across sites (Tukey's honestly significant difference [HSD];  $P < 0.05$ ). Significant differences were seen across all compounds (Tukey's HSD;  $P < 0.05$ ) except the concentrations of sulfamethoxazole and morphine were similar at the sites (Tukey's HSD;  $P = 0.99$ ).

biofilms. Functional resistance and changes in composition were assessed based on comparisons to control substrates not exposed to drugs. Results generally confirmed our hypotheses and indicated that urban streams had higher concentrations of drugs and contained biofilms with greater functional resistance to drugs than biofilms from less urban streams. However, in contrast to our hypothesis, biofilms in urban streams had significantly altered community composition in response to drugs. This indicates that large shifts in community taxonomic composition may be responsible for maintaining function. Our results provide novel insights into the ability of stream microbial communities to maintain ecosystem services in the face of exposure to increasing levels of anthropogenic contaminants.

## METHODS

### *Study sites*

Work was conducted at four stream sites in Maryland, USA. These streams are in the Baltimore metropolitan area and are long-term research sites of the Baltimore Ecosystem Study ([beslter.org](http://beslter.org)) representing a suburban to urban gradient. The streams are part of the Gwynns Falls watershed (76°30', 39°15'), which lies predominantly within the Piedmont physiographic province in Baltimore County and Baltimore City. The watershed drains a total area of 17,150 ha and flows to the Northwest Branch of the Patapsco River, which discharges into the Chesapeake Bay. The main channel of the Gwynns Falls watershed extends from urban Baltimore City at its mouth to suburban and rural portions of Baltimore County. The watershed is classified as being mainly urbanized with a population of 356,000 people. Detailed descriptions of the watershed, streams, land-use classifications, hydrologic monitoring, and nitrogen dynamics can be found in Groffman et al. (2005), Kaushal et al. (2008, 2011), and at [www.beslter.org](http://www.beslter.org). The four stream sites used in this study were Gwynnbrook (GB), Dead Run (DR), Carroll Park (CP), and Gwynns Run (GR). These sites represent a distinct gradient in sewage contamination and nutrient levels (Groffman et al. 2005, Kaushal et al. 2011, Rosi-Marshall et al. 2013). Gwynns Run is the most urbanized site and has documented sewage contamination (Kaushal et al.

2011, Duan et al. 2012). Gwynnbrook, which is located in the upper watershed, is considered rural and suburban. Carroll Park and DR are located between GB and GR and are intermediate in terms of anthropogenic inputs.

### *Passive organic contaminant integrative samplers*

Within each stream, we placed POCIS (Alvarez et al. 2004, 2005, Petty et al. 2004). Polar organic chemical integrative samplers were deployed in March 2012 and were left in place for two weeks. At the completion of the sampling period, POCIS were removed from the streams, transported back to the laboratory on ice, and shipped on ice to University of Nebraska for extraction and quantification of recovered compounds. Polar organic chemical integrative samplers were processed as described previously (Bartelt-Hunt et al. 2011, Brown et al. 2015, Jaimes-Correa et al. 2015). Briefly, drugs were eluted from the POCIS with methanol and were identified and quantified by liquid chromatography–tandem mass spectrometry. The results are expressed as ng/POCIS to avoid complications or assumptions required in estimating time-weighted average water column concentrations (Alvarez et al. 2005). In this study, the following compounds were assayed: acetaminophen (analgesic), caffeine (stimulant), sulfamethoxazole (antibiotic), diphenhydramine (antihistamine), amphetamine (stimulant), and morphine (opioid).

### *In situ contaminant exposure substrates*

We used CES (Rosi-Marshall et al. 2013, Costello et al. 2016) to measure the responses of biofilms in each of the four streams to caffeine, cimetidine, ciprofloxacin, and diphenhydramine. This contaminant exposure method allowed us to test the effects of drugs on the structure and function of microbial communities in situ. CES consisted of 30-mL polyethylene cups filled with a 2% (by weight) agarose gel amended with caffeine, ciprofloxacin, cimetidine, diphenhydramine, or no pharmaceutical. The compounds employed in the CES did not completely overlap with the compounds assayed for in the passive samplers. However, ciprofloxacin and cimetidine are commonly detected in surface waters (Kolpin et al. 2002) and were investigated in a previous CES study (Rosi-Marshall et al. 2013) so we included them in the CES experiment to allow

comparison. The concentration of drug in each cup was 0.015 mol/L. The goal in selecting this drug concentration was not to mimic the concentration of drugs found in the field, but rather to create enough selective pressure on the developing biofilms to enable us to discriminate the degree of drug resistance of microbial communities from the four sites used in this study. This specific concentration was chosen because we had previously demonstrated that all four of these drugs applied at this concentration in CES resulted in significant decreases in respiration rates in three suburban streams in New York, Indiana, and Maryland, USA, including the DR site used in the current study (Rosi-Marshall et al. 2013). Each cup was capped with a cellulose sponge to promote colonization by heterotrophic microorganisms. Five replicate CES of each drug treatment and an additional control treatment containing no drugs were secured to the stream bottom on plastic L-bars, with treatments and replicates arranged randomly. The CES were deployed in March 2012 and were left in place for two weeks over the same incubation period as POCIS. CES were then collected and transported to the laboratory on ice.

#### *Biofilm activity*

We measured community respiration ( $R$ ) for biofilms colonizing the cellulose sponges from each CES. Each sponge was placed in a 50-mL centrifuge tube, and each tube was filled with filtered stream water with known initial dissolved oxygen (DO) concentration. All stream water used was filtered through GN-6 Metrical Membrane (Pall Corporation, Port Washington, New York, USA) filters with a pore size of 0.45  $\mu\text{m}$ , and after filtration, we measured DO with an optical meter (ProODO meter; YSI, Yellow Springs, Ohio, USA). Each tube was capped underwater to remove all air bubbles and was incubated in the dark for 2–4 h. We included “blank” tubes, which were filled with filtered stream water only, to correct for changes in background DO. After the incubation period, we measured the DO concentration in each of the tubes. We calculated  $R$  as the change in DO per substratum area per time (Hill et al. 2002). After completion of the respiration assay, cellulose sponges were immediately frozen at  $-20^{\circ}\text{C}$  for subsequent DNA extraction.

#### *Microbial community composition*

We measured the diversity and composition of the biofilm bacterial communities colonizing the CES cellulose sponges using high-throughput amplicon sequencing. We extracted DNA directly from the cellulose sponges using the MoBio Power Biofilm DNA Isolation Kit (MoBio, Carlsbad, California, USA) according to the manufacturer’s instructions. The extracted DNA was stored at  $-20^{\circ}\text{C}$  and shipped on dry ice to Argonne National Lab for amplification and sequencing of 16S rRNA genes. PCR amplification of the V4 region of the 16S rRNA gene was performed using primers 515F and 806R (Caporaso et al. 2012) and the following PCR cycling parameters: 5-min denaturation at  $95^{\circ}\text{C}$ ; 28 cycles of  $95^{\circ}\text{C}$  for 30 s,  $55^{\circ}\text{C}$  for 45 s, and  $72^{\circ}\text{C}$  for 30 s; and 7-min elongation at  $68^{\circ}\text{C}$ . The V4 hypervariable region was chosen because it has been shown to provide species richness estimates comparable to those obtained with the nearly full-length 16S rRNA gene (Youssef et al. 2009). Sequencing was conducted as previously described (Caporaso et al. 2012) using the Illumina MiSeq platform in a  $2 \times 250$  bp paired-end format. All sequence data produced in this study can be downloaded from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) with accession number SRP092627.

DNA sequences were processed using mothur v.1.30.2 (Schloss et al. 2009) following the MiSeq Standard Operating Procedure (Kozich et al. 2013). Briefly, paired reads were assembled and any sequences longer than 275 base pairs or sequences with ambiguities or homopolymers longer than 8 base pairs were removed from the data set. Sequences were aligned using the SILVA-compatible alignment database (Quast et al. 2013) available within mothur. Aligned sequences were screened to remove poorly aligned sequences, and chimeric sequences were identified and removed using Uchime (Edgar et al. 2011). The remaining 4.9 million high-quality sequences were trimmed to a uniform length of 253 base pairs. Sequences were assigned to taxa using the mothur-formatted version of the RDP training set (v.9) and the method described by Wang et al. (2007) with a bootstrap value of 80% for the classification cutoff. All unknown (i.e., not identified as bacterial), chloroplast, mitochondrial, archaeal, and eukaryotic sequences were removed. Sequences were also

clustered into operational taxonomic units (OTUs) based on 97% sequence identity using the average neighbor algorithm. In order to avoid biases associated with uneven numbers of sequences across samples, the entire data set was randomly subsampled to 10,218 sequences per sample. Good's coverage was calculated for each sample to estimate the sequence coverage of the data set (Good 1953) and indicated >80% coverage of bacterial OTUs in all samples. Chao1 richness was calculated to provide an estimate of the total number of bacterial taxa within each of the samples (Chao 1984). Some sequences that were assigned to unclassified genera by mothur were compared to the NCBI database of bacterial and archaeal 16S rRNA sequences (<ftp://ftp.ncbi.nlm.nih.gov/blast/db/>) using blastn version 2.6.0 (<ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/>).

### Statistics

Drug concentrations were compared across sites using two-way ANOVA with site and drug as the factors, followed by Tukey's multiple comparison test on main effects when ANOVA results were significant. Respiration rates were analyzed by first calculating response ratios as:

$$\text{Response Ratio} = R_{\text{tft}}/R_{\text{mean.ctl}}$$

where  $R_{\text{tft}}$  is respiration rate for each replicate treatment CES, and  $R_{\text{mean.ctl}}$  is the mean of control CES from the same deployment. Response ratios were then compared across sites using one-way ANOVA followed by Tukey's multiple comparison test when ANOVA results were significant. ANOVA and Tukey's analyses were performed using R (version 3.3.1; R Development Core Team 2016) with a level of statistical significance set at  $\alpha < 0.05$ . The composition of the bacterial communities in the samples was compared by calculating Bray–Curtis dissimilarities between samples (Bray and Curtis 1957) based on the OTU abundance data and ordinating the resulting distance matrix using non-metric multidimensional scaling (nMDS) run within mothur. The significance of differences between treatment groups in the nMDS ordination was determined using analysis of molecular variance (AMOVA) calculated with mothur. The SIMPER routine in the Primer V.5 software package (Primer-E, Plymouth, UK) was used to identify the bacterial genera (identified by comparison with the RDP training set v.9)

making the largest contributions to differences in composition between treatments.

## RESULTS

The abundance of six common drugs (acetaminophen, caffeine, sulfamethoxazole, diphenhydramine, amphetamine, and morphine) was measured in four streams in Baltimore, Maryland, that were known to vary in degree of anthropogenic inputs (Kaushal and Belt 2012). Polar organic chemical integrative samplers were used to measure the cumulative loads of these compounds in the streams during a two-week period. All six compounds were detected in samples from at least one of the sites, with the most urban site (GR) having the highest relative concentrations of all detected drugs and the more suburban site (GB) having the lowest relative concentrations (two-way ANOVA and subsequent Tukey's honestly significant difference [HSD];  $df = 3, 48$ ;  $F = 485.32$ ;  $P < 0.05$ ; Fig. 1). Caffeine was the most abundant drug recovered at all sites (two-way ANOVA and subsequent Tukey's HSD;  $df = 5, 48$ ;  $F = 288.11$ ;  $P < 0.05$ ), and it was highest at GR (1110 ng/POCIS) and lowest at GB (27 ng/POCIS). Amphetamine was quite abundant at GR (503 ng/POCIS) but was much lower at all other sites (between 3 and 80 ng/POCIS). Finally, the widely used pain reliever acetaminophen was abundant at GR (261 ng/POCIS) and tended to decrease with decreasing urbanization of the sites (Fig. 1). Sulfamethoxazole, a commonly used antibiotic, was detected at all sites, but the concentrations were higher in the two more urban sites, and the antihistamine diphenhydramine was lower than all other drugs, but was highest at the most urban site. Morphine, the breakdown product of heroin, was detected at all sites, but was highest at the most urban site and reflects the use of illicit drugs in Baltimore. Illicit drugs such as heroin may have ecological consequences because they are potent compounds (Rosi-Marshall et al. 2015).

The potential of drug exposure to affect the composition and function of stream microbial communities was assessed at the same four sites in Baltimore using CES (Rosi-Marshall et al. 2013, Costello et al. 2016). Caffeine ( $df = 1, 52$ ;  $F = 6.59$ ;  $P < 0.05$ ), cimetidine ( $df = 1, 51$ ;  $F = 7.02$ ;  $P < 0.05$ ), and ciprofloxacin ( $df = 1, 52$ ;  $F = 12.76$ ;

$P < 0.05$ ) all significantly reduced biofilm respiration across all sites, whereas diphenhydramine had a marginal effect ( $df = 1, 52; F = 3.34; P = 0.07$ ). Looking across all sites, the greatest relative reduction in biofilm respiration based upon response ratios was found at the least urban site (GB; Fig. 2) across all four pharmaceuticals, with the antibiotic ciprofloxacin having the largest negative effect on respiration rates (Fig. 2).

The CES data indicated that the functional responses of biofilm communities to the four drugs varied by site (Fig. 2). Specifically, the effects of the drugs on respiration rates tended to be greater at

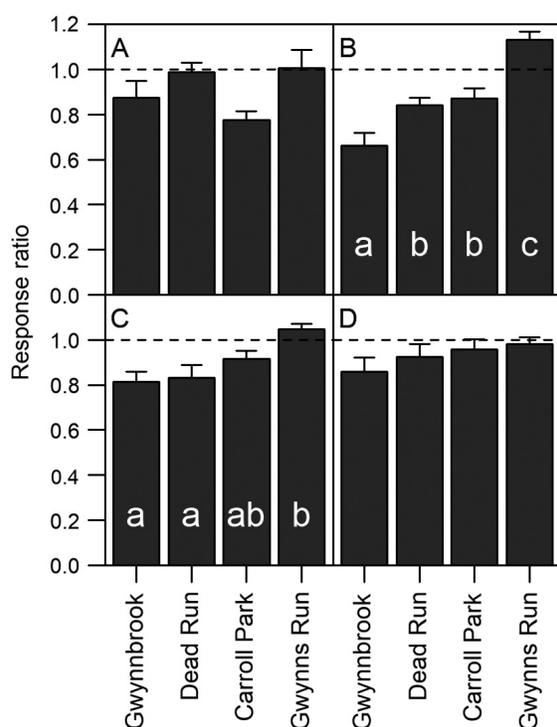


Fig. 2. Respiration rates of biofilm communities in streams along a suburban to urban gradient grown on organic substrates and exposed to pharmaceuticals (A) caffeine, (B) ciprofloxacin, (C) cimetidine, and (D) diphenhydramine. Bars represent response ratios of respiration rates for pharmaceutical treatments: no pharmaceutical controls. The dashed line indicates a response ratio of 1, indicating no effect of exposure to the drug. Lowercase letters indicate significant differences between sites (Tukey's honestly significant difference;  $P < 0.05$ ). Error bars represent standard error. No significant differences were seen between sites for A or D.

less urbanized sites (GB, DR), which had the lowest background drug concentrations (Fig. 1). Response ratios were significantly lower for CES from GB than GR for both ciprofloxacin ( $df = 3, 16; F = 20.02; P < 0.05$ ) and cimetidine ( $df = 3, 15; F = 6.64; P < 0.05$ ). Although there were no significant differences ( $df = 3, 16; F = 1.19; P > 0.1$ ), a similar pattern was observed for diphenhydramine, that is, greater effects in the non-urban stream than in the urban stream. For caffeine, two of the less urban streams indicated a slight but non-significant response to caffeine ( $df = 3, 16; F = 2.98; P = 0.06$ ) while the most urban stream had no response to this drug. Biofilms from the most urbanized site (GR), which had the highest background drug concentrations (Fig. 1), showed no significant reductions, and in two cases, respiration rates were elevated with increasing exposure to drugs (Fig. 2).

The bacterial taxonomic composition of biofilms formed on the CES was assessed via a culture-independent molecular approach based on high-throughput sequencing of 16S rRNA genes. Biofilm bacterial communities from the four field sites were distinct in composition, as demonstrated by clustering of the control treatments by site on the nMDS plot (Fig. 3) and AMOVA (all comparisons of control treatments by site had  $P < 0.001$ ; Table 1). On the control substrates with no drugs, the largest difference in community composition (based on Bray–Curtis dissimilarity scores) occurred between the least urbanized site (GB) and the most urbanized site (GR), with DR and CP communities intermediate between them (Fig. 3). SIMPER analysis was used to identify the bacterial genera making the most significant contributions to differences in composition between the urbanized and less urbanized sites (Table 2). Some examples included *Aeromonas* and *Flavobacterium*, both of which were more abundant at the more urbanized sites. Biofilm bacterial communities from the four field sites also varied significantly ( $F_3 = 13.138; P < 0.001$ ) in taxonomic richness, with the most urbanized site (GR) having a significantly higher ( $P < 0.05$ ) estimated number of bacterial taxa ( $6329 \pm 357$ ) as compared to GB ( $4821 \pm 349$ ), DR ( $3295 \pm 346$ ), and CP ( $4024 \pm 355$ ).

Exposure to the antibiotic ciprofloxacin resulted in a significant shift in the taxonomic composition of biofilms from all four sites, as indicated by the

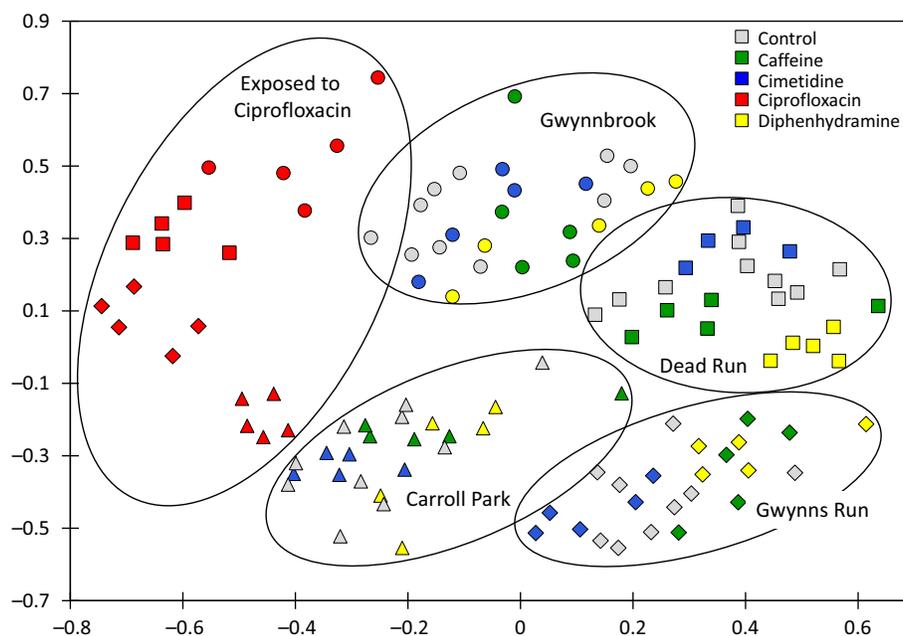


Fig. 3. Non-metric multidimensional scaling ordination of biofilm bacterial communities in streams along a suburban to urban gradient grown on organic substrates and exposed to pharmaceuticals. Stress value of ordination 0.316. Ellipses were drawn by hand to highlight each of the treatments.

separation between the control and ciprofloxacin-treated samples on the nMDS plot (Fig. 3) and AMOVA (all comparisons of control and ciprofloxacin-treated samples had  $P \leq 0.001$ ; Table 1). It is noteworthy that the ciprofloxacin-treated samples from all four sites clustered together on the nMDS plot (Fig. 3). However, ciprofloxacin-treated samples from each individual site could still be discriminated on the nMDS plot (Fig. 3) and were significantly different based on AMOVA (all comparisons of ciprofloxacin-treated samples by site had  $P < 0.05$ ; Table 1). Comparison of the ciprofloxacin amended and control substrates also indicated that ciprofloxacin exposure resulted in a decrease in taxonomic richness for biofilm communities from all four sites. The most urbanized site (GR) had a significantly greater ( $F = 5.284$ ;  $P < 0.010$ ) decrease in estimated total taxa in the ciprofloxacin-exposed biofilms (52% decrease) than the other sites (36%, 38%, and 33% decreases for GB, DR, and CP, respectively).

SIMPER analysis was used to identify the bacterial genera making the most significant contributions to differences in composition between the control and ciprofloxacin-treated samples. One

notable example was the bacterial genus *Pedobacter*, which predominated in ciprofloxacin-treated biofilms, representing on average 23% of total sequences, but was a minor component of biofilms without ciprofloxacin exposure, representing on average <0.5% of sequences (Fig. 4, Table 3). Ciprofloxacin also significantly increased the relative abundance of an unclassified genus from the family Bradyrhizobiaceae, which represented 4% of total sequences in ciprofloxacin-treated biofilms but 0.2% or less in biofilms without ciprofloxacin exposure (Fig. 4, Table 3). Comparison of the sequences from this unclassified genus of Bradyrhizobiaceae with the NCBI 16S rRNA database revealed that the closest match for 78% of the sequences was the genus *Rhodopseudomonas*, with all of these matches having >98% coverage and >92% identity. Moreover, 74% of the sequences matching the genus *Rhodopseudomonas* had their closest match to the species *pseudopalustris*, with all of these matches having >94% coverage and >97% identity. Finally, ciprofloxacin exposure also dramatically decreased the relative abundance of sequences from the bacterial genus *Pseudomonas* (Fig. 4, Table 3).

Table 1. Analysis of molecular variance results for comparisons of bacterial community composition between treatments.

Comparison	<i>F</i>	<i>P</i>
Gwynnbrook Control vs. Dead Run Control	11.592	<0.001
Gwynnbrook Control vs. Carroll Park Control	13.125	<0.001
Gwynnbrook Control vs. Gwynns Run Control	12.730	<0.001
Dead Run Control vs. Carroll Park Control	15.663	<0.001
Dead Run Control vs. Gwynns Run Control	14.780	<0.001
Carroll Park Control vs. Gwynns Run Control	15.667	<0.001
Gwynnbrook Control vs. Gwynnbrook Ciprofloxacin	6.328	<0.001
Dead Run Control vs. Dead Run Ciprofloxacin	15.829	<0.001
Carroll Park Control vs. Carroll Park Ciprofloxacin	3.794	0.001
Gwynns Run Control vs. Gwynns Run Ciprofloxacin	11.444	<0.001
Gwynnbrook Ciprofloxacin vs. Dead Run Ciprofloxacin	3.753	0.021
Gwynnbrook Ciprofloxacin vs. Carroll Park Ciprofloxacin	7.076	0.001
Gwynnbrook Ciprofloxacin vs. Gwynns Run Ciprofloxacin	4.095	0.009
Dead Run Ciprofloxacin vs. Carroll Park Ciprofloxacin	12.841	0.002
Dead Run Ciprofloxacin vs. Gwynns Run Ciprofloxacin	3.100	0.009
Carroll Park Ciprofloxacin vs. Gwynns Run Ciprofloxacin	9.981	0.004
Gwynnbrook Control vs. Gwynnbrook Cimetidine	1.635	0.052
Dead Run Control vs. Dead Run Cimetidine	3.006	0.051
Carroll Park Control vs. Carroll Park Cimetidine	1.650	0.086
Gwynns Run Control vs. Gwynns Run Cimetidine	2.343	<0.001
Gwynnbrook Control vs. Gwynnbrook Caffeine	1.508	0.071
Dead Run Control vs. Dead Run Caffeine	1.676	0.078
Carroll Park Control vs. Carroll Park Caffeine	0.990	0.383
Gwynns Run Control vs. Gwynns Run Caffeine	1.816	0.028
Gwynnbrook Control vs. Gwynnbrook Diphenhydramine	2.320	0.057
Dead Run Control vs. Dead Run Diphenhydramine	6.470	<0.001
Carroll Park Control vs. Carroll Park Diphenhydramine	1.915	0.036
Gwynns Run Control vs. Gwynns Run Diphenhydramine	4.697	0.001

Table 2. Relative abundance† of bacterial genera making the most significant contributions to differences in community composition between the control substrates from the least urbanized site (Gwynnbrook [GB]) and the most urbanized site (Gwynns Run) as identified by SIMPER (mean ± standard error).

Bacterial genus	GB	Dead Run	Carroll Park	Gwynn's Run	<i>F</i> ‡	<i>P</i> ‡
Janthinobacterium	5.26 ± 2.24 <sup>A§</sup>	2.52 ± 1.69 <sup>A</sup>	0.43 ± 0.22 <sup>A</sup>	4.87 ± 2.42 <sup>A</sup>	1.53	0.22
Methylophilus	8.44 ± 1.22 <sup>A</sup>	4.99 ± 0.73 <sup>AB</sup>	2.87 ± 1.05 <sup>B</sup>	2.67 ± 0.53 <sup>B</sup>	8.13	0.00
Pseudomonas	6.35 ± 1.77 <sup>A</sup>	19.55 ± 3.57 <sup>B</sup>	2.98 ± 0.89 <sup>A</sup>	8.30 ± 1.11 <sup>A</sup>	11.37	0.00
Aeromonas	0.83 ± 0.59 <sup>A</sup>	1.16 ± 0.22 <sup>A</sup>	1.15 ± 0.44 <sup>A</sup>	5.12 ± 1.09 <sup>B</sup>	9.71	0.00
Flavobacterium	4.57 ± 0.50 <sup>A</sup>	3.35 ± 0.74 <sup>A</sup>	7.55 ± 1.03 <sup>B</sup>	9.01 ± 0.76 <sup>B</sup>	10.98	0.00
Oxalobacteraceae¶	5.09 ± 1.23 <sup>A</sup>	0.81 ± 0.41 <sup>B</sup>	0.15 ± 0.04 <sup>B</sup>	0.79 ± 0.30 <sup>B</sup>	11.46	0.00
Methylophilaceae¶	4.17 ± 0.60 <sup>A</sup>	0.13 ± 0.03 <sup>B</sup>	0.14 ± 0.08 <sup>B</sup>	0.25 ± 0.06 <sup>B</sup>	41.57	0.00
Acidovorax	0.13 ± 0.02 <sup>A</sup>	0.18 ± 0.05 <sup>A</sup>	0.32 ± 0.04 <sup>A</sup>	3.84 ± 0.43 <sup>B</sup>	77.83	0.00
Rhizobium	0.59 ± 0.07 <sup>A</sup>	2.59 ± 0.32 <sup>B</sup>	1.10 ± 0.16 <sup>A</sup>	3.03 ± 0.48 <sup>B</sup>	15.88	0.00
Rhodocyclaceae¶	0.10 ± 0.01 <sup>A</sup>	0.24 ± 0.04 <sup>A</sup>	0.19 ± 0.05 <sup>A</sup>	2.48 ± 0.30 <sup>B</sup>	61.36	0.00
Comamonadaceae¶	7.97 ± 0.82 <sup>A</sup>	5.88 ± 0.91 <sup>A</sup>	7.80 ± 0.49 <sup>A</sup>	7.74 ± 0.49 <sup>A</sup>	1.92	0.14
Albidiferax	2.43 ± 0.24 <sup>A</sup>	0.05 ± 0.01 <sup>B</sup>	4.60 ± 0.54 <sup>C</sup>	0.10 ± 0.02 <sup>B</sup>	52.08	0.00
Gammaproteobacteria¶	1.24 ± 0.11 <sup>A</sup>	1.98 ± 0.37 <sup>AB</sup>	4.67 ± 0.45 <sup>C</sup>	3.19 ± 0.30 <sup>B</sup>	20.74	0.00
Burkholderiales¶	3.00 ± 0.33 <sup>A</sup>	3.07 ± 1.34 <sup>A</sup>	2.04 ± 0.20 <sup>A</sup>	4.64 ± 0.53 <sup>A</sup>	1.93	0.14
Chitinophagaceae¶	2.83 ± 0.39 <sup>A</sup>	0.39 ± 0.08 <sup>B</sup>	0.62 ± 0.09 <sup>B</sup>	0.95 ± 0.15 <sup>B</sup>	25.83	0.00

† Number of sequences assigned to a genus as a percentage of the total number of sequences analyzed for that sample.

‡ Based on one-way ANOVA.

§ Within each genus, data points followed by different letters are significantly different based on Tukey's post hoc test.

¶ Taxon assignments above the genus level indicate that these sequences were assigned to that taxon but could not be assigned to a genus within that taxon with >80% confidence.

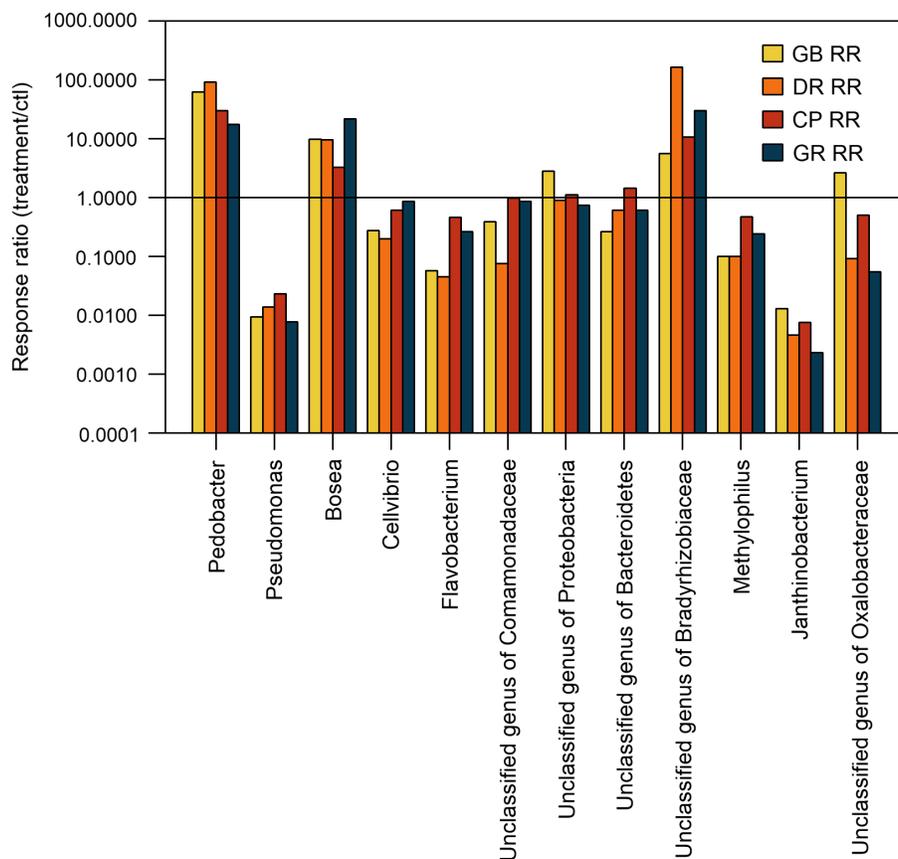


Fig. 4. Response ratios of bacterial genera making the most significant contributions to differences in community composition between ciprofloxacin-treated and non-ciprofloxacin-treated samples as identified by SIMPER. Bars above the 1 line indicate an increase in representation in the ciprofloxacin-treated biofilm relative to the control biofilms, and bars below the 1 line indicate reductions relative to control. Study sites include GB (suburban), DR (intermediate urban), CP (more urban), and GR (most urban). CP, Carroll Park; DR, Dead Run; GB, Gwynnbrook; GR, Gwynns Run.

## DISCUSSION

In general, our data demonstrate that there are higher concentrations of pharmaceuticals and personal care products in more urbanized streams and that common drugs can affect respiration rates and bacterial composition of stream biofilms. The POCIS method for drug detection was chosen because it has been shown to detect a wider range of organic contaminants than standard water column sampling. Polar organic chemical integrative samplers detect compounds that are intermittently present or present in low concentrations, and because this approach is very well suited to measuring relative time-weighted

average chemical exposure to aquatic organisms (Alvarez et al. 2005). Our data demonstrate that a suite of commonly used drugs occurs in these urban streams, as has been shown elsewhere (Lee et al. 2016).

Caffeine was the most abundant drug detected at all sites. Caffeine is a popular stimulant that is present in a wide range of beverages (e.g., coffee, tea, soft drinks, and energy drinks) and many prescription and over-the-counter drugs (Lawrence et al. 2012). Caffeine has been detected previously in surface waters receiving wastewater inputs (Alvarez et al. 2005, Aufdenkampe et al. 2006, Choi et al. 2008) and is commonly used as a marker for wastewater contamination of

Table 3. Relative abundance† of bacterial genera making the most significant contributions to differences in community composition between ciprofloxacin-treated and non-ciprofloxacin-treated samples as identified by SIMPER (mean ± standard error).

Bacterial genus	Ciprofloxacin-treated samples	Gwynnbrook non-cipro samples	Dead Run non-cipro samples	Carroll Park non-cipro samples	Gwynn's Run non-cipro samples	F‡	P‡
Pedobacter	23.01 ± 4.48 <sup>A§</sup>	0.37 ± 0.12 <sup>B</sup>	0.46 ± 0.19 <sup>B</sup>	0.09 ± 0.02 <sup>B</sup>	1.36 ± 0.21 <sup>B</sup>	30.9	0.000
Pseudomonas	0.14 ± 0.05 <sup>A</sup>	9.15 ± 1.43 <sup>B</sup>	20.63 ± 2.89 <sup>C</sup>	4.29 ± 1.14 <sup>B</sup>	11.30 ± 1.73 <sup>B</sup>	18.8	0.000
Bosea	6.39 ± 1.05 <sup>A</sup>	0.24 ± 0.03 <sup>B</sup>	0.93 ± 0.11 <sup>B</sup>	1.24 ± 0.11 <sup>B</sup>	0.47 ± 0.05 <sup>B</sup>	35.7	0.000
Cellvibrio	2.92 ± 1.01 <sup>A</sup>	0.60 ± 0.13 <sup>A</sup>	0.64 ± 0.09 <sup>A</sup>	16.43 ± 1.64 <sup>B</sup>	1.64 ± 0.10 <sup>A</sup>	63.4	0.000
Flavobacterium	1.50 ± 0.34 <sup>A</sup>	6.48 ± 0.95 <sup>B</sup>	3.21 ± 0.53 <sup>A</sup>	7.40 ± 0.56 <sup>B</sup>	7.96 ± 0.68 <sup>B</sup>	17.4	0.000
Comamonadaceae¶	4.05 ± 0.95 <sup>A</sup>	5.97 ± 0.55 <sup>AB</sup>	6.54 ± 0.72 <sup>B</sup>	6.62 ± 0.33 <sup>B</sup>	8.00 ± 0.55 <sup>B</sup>	5.1	0.001
Proteobacteria¶	4.93 ± 2.92 <sup>A</sup>	4.86 ± 2.29 <sup>A</sup>	2.11 ± 0.70 <sup>A</sup>	2.05 ± 0.11 <sup>A</sup>	2.48 ± 0.15 <sup>A</sup>	0.9	0.493
Bacteroidetes¶	3.47 ± 1.30 <sup>A</sup>	1.98 ± 0.16 <sup>A</sup>	0.99 ± 0.14 <sup>A</sup>	8.32 ± 1.27 <sup>B</sup>	1.24 ± 0.15 <sup>A</sup>	15.6	0.000
Bradyrhizobiaceae¶	3.85 ± 1.10 <sup>A</sup>	0.20 ± 0.03 <sup>B</sup>	0.05 ± 0.01 <sup>B</sup>	0.04 ± 0.01 <sup>B</sup>	0.17 ± 0.03 <sup>B</sup>	14.2	0.000
Methylophilus	0.73 ± 0.10 <sup>A</sup>	7.83 ± 0.82 <sup>C</sup>	4.77 ± 0.63 <sup>B</sup>	2.35 ± 0.44 <sup>A</sup>	2.35 ± 0.32 <sup>A</sup>	25.6	0.000
Janthinobacterium	0.03 ± 0.01 <sup>A</sup>	6.32 ± 1.52 <sup>B</sup>	2.52 ± 0.87 <sup>AB</sup>	2.06 ± 0.87 <sup>A</sup>	3.34 ± 0.94 <sup>AB</sup>	4.9	0.001
Oxalobacteraceae¶	2.74 ± 1.69 <sup>AB</sup>	4.08 ± 0.68 <sup>B</sup>	0.43 ± 0.18 <sup>A</sup>	0.18 ± 0.04 <sup>A</sup>	0.57 ± 0.12 <sup>A</sup>	5.9	0.000

† Number of sequences assigned to a genus as a percentage of the total number of sequences analyzed for that sample.

‡ Based on one-way ANOVA.

§ Within each genus, data points followed by different letters are significantly different based on Tukey's post hoc test.

¶ Taxon assignments above the genus level indicate that these sequences were assigned to that taxon but could not be assigned to a genus within that taxon with >80% confidence.

surface waters (Standley et al. 2000, Buerge et al. 2003). Therefore, the presence of caffeine at our field sites and the relationship between its average concentration and the degree of urbanization of the sites were expected. Amphetamine is another stimulant used in the treatment of various human disorders, including attention-deficit disorder, hyperactivity, and narcolepsy, but is also widely used illegally as a recreational or performance-enhancing drug (www.nih.gov). Several studies have detected amphetamine in wastewater (Boles and Wells 2010), but only a few studies have detected amphetamine in streams (e.g., Huerta-Fontela et al. 2008, Lee et al. 2016). Acetaminophen has also been detected in other rivers and its presence linked to inputs of effluent from a sewage treatment plant (Choi et al. 2008). In total, these results support our hypotheses that drugs are present in stream water at our field sites in Baltimore and that the average concentrations of drugs increase with the degree of urbanization of the sites.

The compounds used in the CES were chosen because they represent a range of drug classes and modes of action. We amended CES with caffeine, ciprofloxacin, cimetidine, and diphenhydramine; these compounds represent a stimulant, antibiotic, antihistamine used to treat heartburn, and antihistamine for allergens, respectively. These drugs are

commonly used and have all been previously detected in aquatic habitats throughout the United States and Europe (Kolpin et al. 2002, Monteiro and Boxall 2010). In addition, both caffeine and diphenhydramine were detected at our field sites in this study (Fig. 1). Ciprofloxacin and cimetidine were not quantified in the POCIS assay, so it is unknown whether they were present at our field sites. Our results suggest that microbial communities were differentially sensitive to these four compounds. The function at the less urban sites was sensitive to exposure. In contrast, the microbial communities at the most urban site were highly resistant to these drugs and were able to form biofilms with high function (i.e., respiration rates) even on the substrates treated with drugs. This adaptation may be the result of prior exposure and selection for resistance to the drugs tested, which has significant implications for the stability of stream ecosystem processes in urbanized streams in spite of contaminant exposure.

The microbial communities were significantly different among streams as evidenced by the composition of biofilms that grew on the control substrates, that is, where no drugs were added. Bacteria from the genus *Aeromonas* were common in our most urbanized sites and have frequently been reported in polluted waters and wastewater (Martin-Carnahan and Joseph 2005), so the

abundance of this genus in biofilms from our urbanized sites fits the profile of this taxon. Most of the species within the *Aeromonas* genus are associated with human disease, especially gastrointestinal disease (Janda and Abbott 2010), so the prevalence of this organism in our urbanized site raises concerns about water quality and public health. *Flavobacterium* is another widely distributed bacterial genus, occurring mostly in aquatic ecosystems (Bernardet and Bowman 2006), and they are commonly associated with biofilms (Basson et al. 2008). Within aquatic habitats *Flavobacterium* are involved in the metabolism of various organic compounds, including carbohydrates and polysaccharides (Bernardet and Bowman 2006), cellulose derivatives (Bernardet and Bowman 2006), and cellulose (Lednicka et al. 2000), which could explain their high abundance in the most urban streams and their high abundance on the CES cellulose substrates. *Flavobacterium* have also been linked to organic enrichment/pollution (Bissett et al. 2008) and to the breakdown of complex aromatic compounds (Trzesicka-Mlynarz and Ward 1995), both of which can be associated with urbanized streams. Therefore, the significantly higher abundance of *Flavobacterium* in biofilms from these urbanized sites fits the profile of this taxon.

Although function (i.e., respiration rate) was maintained at the most urban site when exposed to drugs, function at the least urban site was reduced with exposure to drugs, with ciprofloxacin resulting in the largest decrease. These results agree with previous findings that exposure to these four compounds reduced biofilm respiration rates for CES incubated in streams in New York, Indiana, and Maryland, USA, and that ciprofloxacin resulted in the greatest decrease in respiration rates (Rosi-Marshall et al. 2013). Ciprofloxacin is an antibiotic, so its inhibitory effect on respiration is likely due to its toxicity to bacteria. Diphenhydramine has also been reported to have some antibacterial activity (Dastidar et al. 1976), which may explain its negative effect on respiration. The mechanisms by which caffeine and cimetidine inhibit respiration are less clear, as we are not aware of any reports of direct interactions between these compounds and bacterial cells. However, one study found that caffeine exposure of river biofilm communities resulted in decreased biofilm biomass coupled with increased density of

protozoa and micrometazoa within the biofilms, and suggested that the decreased biofilm mass with caffeine exposure may have been the result of enhanced grazing (Lawrence et al. 2012). These types of trophic interactions may also have played a role in our study.

Exposure to the antibiotic ciprofloxacin resulted in a dramatic shift in bacterial community composition. These results indicate that this drug exerted a strong selective pressure on the biofilm communities, presumably toward more ciprofloxacin-resistant taxa. It is noteworthy that the ciprofloxacin-treated samples from all four sites clustered together on the nMDS plot (Fig. 3). These data suggest that ciprofloxacin is selecting for similar taxa from all four sites, although the ciprofloxacin-treated samples from each individual site could still be discriminated on the nMDS plot (Fig. 3) and were significantly different based on AMOVA (all comparisons of ciprofloxacin-treated samples by site had  $P < 0.05$ ; Table 1). SIMPER analysis was used to identify the bacterial genera making the most significant contributions to differences in composition between the control and ciprofloxacin-treated samples. One notable example was the bacterial genus *Pedobacter*, which predominated in ciprofloxacin-treated biofilms, representing on average 23% of total sequences, but was a minor component of biofilms without ciprofloxacin exposure, representing on average <0.5% of sequences (Fig. 4, Table 3). The genus *Pedobacter* belongs to the order *Sphingobacteriales*, which are Gram-negative bacteria that are found in a wide array of habitats and are known for their ability to utilize unusual compounds, including herbicides and antimicrobial compounds (Kampfer 2010). In a prior study, we also observed elevated abundance of bacteria from the order *Sphingobacteriales* in the benthic habitat of a river downstream of the input of wastewater treatment plant effluent (Drury et al. 2013b), suggesting that sewage input may select for this taxon. *Pedobacter* was also identified as one of the predominant antibiotic-resistant taxa isolated from a sewage-contaminated river in Brussels, with several isolated strains showing multi-drug resistance (Garcia-Armisen et al. 2011), and *Pedobacter* was identified through isolation of antibiotic-resistant strains from a river in Estonia (Voolaid et al. 2012). The potential for antibiotic resistance and the potential ability to digest antimicrobial compounds suggest possible

mechanisms that may explain the predominance of *Pedobacter* in the ciprofloxacin-exposed biofilms.

Ciprofloxacin exposure also significantly increased the relative abundance of an unclassified genus from the family Bradyrhizobiaceae. Comparison of sequences from this unclassified genus with the NCBI 16S rRNA database indicated that the majority matched most closely with *Rhodopseudomonas pseudopalustris*. *Rhodopseudomonas pseudopalustris* was only recently proposed as a species, having previously been considered a strain of *Rhodopseudomonas palustris* (Ramana et al. 2012). *Rhodopseudomonas palustris* is a member of the alpha proteobacteria that is widely distributed in nature and is considered to be one of the most metabolically versatile bacterial species (Harwood and Gibson 1988). Several strains of *R. palustris* with the ability to metabolize halogenated aromatic compounds have been isolated from a range of environments, including soils, aquatic sediments, and sewage sludge (Kamal and Wyndham 1990, van der Woude et al. 1994, Eglund et al. 2001). Ciprofloxacin is a halogenated aromatic compound, so the increased relative abundance of genes matching closely with *R. pseudopalustris* on the ciprofloxacin amended substrates in our study may reflect this organism's ability to degrade the drug. In addition, the complete genome of *R. palustris* was sequenced and revealed the presence of genes encoding an unusually large number of transport systems, including 20 transport systems classified as heavy metal and drug efflux pumps (Larimer et al. 2004). The presence of multiple drug efflux pumps suggests that *R. palustris* may be resistant to a range of antibiotics, and indeed, a strain of *R. palustris* that was resistant to at least four antibiotics (including ciprofloxacin) was recently isolated from a sewage-contaminated river (Schreiber and Kistemann 2012). Therefore, the high relative abundance of *R. pseudopalustris* on the ciprofloxacin amended substrates in our study may also reflect this organism's intrinsic antibiotic resistance.

Ciprofloxacin exposure also dramatically decreased the relative abundance of sequences from the bacterial genus *Pseudomonas* (Fig. 4, Table 3). Organisms from the *Pseudomonas* genus are common in freshwater ecosystems and are known for their ability to produce biofilms (Palleroni 2010) and for their ability to degrade a large variety of organic molecules (Palleroni 2010).

Some *Pseudomonas* strains can degrade common urban pollutants such as benzene and toluene (Reardon et al. 2000), polycyclic aromatic hydrocarbons (Deziel et al. 1996), and polychlorinated biphenyls (Hickey and Focht 1990). Therefore, the decrease in abundance of *Pseudomonas* within biofilms in response to ciprofloxacin exposure that was observed in our study could have significant implications for the ability of urban stream biofilms to process a number of urban contaminants. However, care should be taken when attempting to assign functional roles to bacterial genera identified based on 16S rRNA gene sequence data, as many bacterial genera, including *Pseudomonas*, are functionally diverse. Further work would be required to identify specific linkages between pharmaceutical exposure and changes in the functional capabilities of bacterial communities.

While ciprofloxacin exposure caused a shift in community composition for biofilms from all four streams, it is noteworthy that the largest shift in composition occurred for the most urbanized site (GR), which had not shown any reduction in respiration rates with ciprofloxacin exposure. This result directly contradicted our hypothesis that biofilms in the most urban streams are resistant, in terms of both taxonomic composition and function, to the drugs due to previous exposure. As discussed above, the biofilms from GR were resistant to ciprofloxacin exposure in terms of community function (i.e., respiration) (Fig. 2), but these communities showed the largest shift in taxonomic composition in response to ciprofloxacin (Fig. 3). Gwynns Run communities on the control substrates had the highest taxonomic richness, but showed the largest decrease in richness when exposed to ciprofloxacin. These results suggest that prior exposure to drugs at GR selected for a bacterial community that included a sub-population of organisms with a high level of resistance to ciprofloxacin. It is possible that these organisms were able to rapidly colonize the ciprofloxacin CES, producing a biofilm community with significantly different taxonomic composition that was able to maintain a level of function equivalent to the communities on the control substrates. In contrast, the microbial communities from the less urbanized sites, which had less prior exposure to drugs, may not have had as large a sub-population of organisms with a high level of resistance

to ciprofloxacin, so they were less able to successfully colonize the ciprofloxacin CES and were thus unable to maintain a level of function equivalent to the communities on the control substrates. These results indicate that large shifts in community taxonomic composition may be responsible for maintaining function in urban streams, and provide novel insights into the potential biological effects of common anthropogenic contaminants on the stability, health, and long-term productivity of stream ecosystems in urban watersheds.

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## LITERATURE CITED

- Alvarez, D. A., J. Petty, J. N. Huckins, T. L. Jones-Lepp, D. T. Getting, J. P. Goddard, and S. E. Manahan. 2004. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. *Environmental Toxicology and Chemistry* 23:1640–1648.
- Alvarez, D. A., P. E. Stackelberg, J. D. Petty, J. N. Huckins, E. T. Furlong, S. D. Zaugg, and M. T. Meyer. 2005. Comparison of a novel passive sampler to standard water-column sampling for organic contaminants associated with wastewater effluents entering a New Jersey stream. *Chemosphere* 61:610–622.
- Aufdenkampe, A. K., D. E. Arscott, C. L. Dow, and L. J. Standley. 2006. Molecular tracers of soot and sewage contamination in streams supplying New York City drinking water. *Journal of the North American Benthological Society* 25:928–953.
- Bartelt-Hunt, S. L., D. D. Snow, T. Damon-Powell, D. L. Brown, G. Prasai, M. Schwarz, and A. S. Kolok. 2011. Quantitative evaluation of laboratory uptake rates for pesticides, pharmaceuticals, and steroid hormones using POCIS. *Environmental Toxicology and Chemistry* 30:1412–1420.
- Basson, A., L. A. Flemming, and H. Y. Chenia. 2008. Evaluation of adherence, hydrophobicity, aggregation, and biofilm development of *Flavobacterium johnsoniae*-like isolates. *Microbial Ecology* 55:1–14.
- Battin, T. J., L. A. Kaplan, J. D. Newbold, and C. M. Hansen. 2003. Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature* 426:439–442.
- Bernardet, J.-F., and J. P. Bowman. 2006. The genus *Flavobacterium*. *Prokaryotes* 7:481–531.
- Bissett, A., J. P. Bowman, and C. M. Burke. 2008. Flavobacterial response to organic pollution. *Aquatic Microbial Ecology* 51:31.
- Boles, T. H., and M. J. Wells. 2010. Analysis of amphetamine and methamphetamine as emerging pollutants in wastewater and wastewater-impacted streams. *Journal of Chromatography A* 1217:2561–2568.
- Bray, J. R., and J. T. Curtis. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs* 27:325–349.
- Brown, D., D. D. Snow, G. Hunt, and S. L. Bartelt-Hunt. 2015. Persistence of pharmaceuticals in effluent-dominated surface waters. *Journal of Environmental Quality* 44:299–304.
- Buerge, I. J., T. Poiger, M. D. Muller, and H. R. Buser. 2003. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. *Environmental Science & Technology* 37:691–700.
- Bunch, A. R., and M. J. Bernot. 2011. Distribution of nonprescription pharmaceuticals in central Indiana streams and effects on sediment microbial activity. *Ecotoxicology* 20:97–109.
- Caporaso, J. G., et al. 2012. Ultra-high throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME Journal* 6:1621–1624.
- Chao, A. 1984. Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics* 11:265–270.
- Choi, K., Y. Kim, J. Park, C. K. Park, M. Kim, H. S. Kim, and P. Kim. 2008. Seasonal variations of several pharmaceutical residues in surface water and sewage treatment plants of Han River, Korea. *Science of the Total Environment* 405:120–128.
- Costello, D. M., E. J. Rosi-Marshall, L. E. Shaw, M. R. Grace, and J. J. Kelly. 2016. A novel method to assess the effects of chemical stressors on natural biofilm structure and function. *Freshwater Biology* 61:2129–2140.
- Dastidar, S. G., P. K. Saha, B. Sanyamat, and A. N. Chakrabarty. 1976. Antibacterial activity of ambo-dryl and benadryl. *Journal of Applied Microbiology* 41:209–214.

- Davey, M. E., and G. A. O'Toole. 2000. Microbial biofilms: from ecology to molecular genetics. *Microbiology and Molecular Biology Reviews* 64:847–867.
- Deziel, E., G. Paquette, R. Villemur, F. Lepine, and J. Bisaillon. 1996. Biosurfactant production by a soil *Pseudomonas* strain growing on polycyclic aromatic hydrocarbons. *Applied and Environmental Microbiology* 62:1908–1912.
- Drury, B., E. J. Rosi-Marshall, and J. J. Kelly. 2013b. Wastewater treatment effluent reduces the abundance and diversity of benthic bacterial communities in urban and suburban rivers. *Applied Environmental Microbiology* 79:1897–1905.
- Drury, B., J. Scott, E. J. Rosi-Marshall, and J. J. Kelly. 2013a. Triclosan exposure increases triclosan resistance and influences taxonomic composition of benthic bacterial communities. *Environmental Science & Technology* 47:8923–8930.
- Duan, S., S. S. Kaushal, P. M. Groffman, L. E. Band, and K. T. Belt. 2012. Phosphorus export across an urban to rural gradient in the Chesapeake Bay watershed. *Journal of Geophysical Research: Biogeosciences* 117:G01025.
- Edgar, R. C., B. J. Haas, J. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27:2194–2200.
- Egland, P. G., J. Gibson, and C. S. Harwood. 2001. Reductive, coenzyme A-mediated pathway for 3-chlorobenzoate degradation in the phototrophic bacterium *Rhodospseudomonas palustris*. *Applied and Environmental Microbiology* 67:1396–1399.
- Garcia-Armisen, T., K. Vercammen, J. Passerat, D. Triest, P. Servais, and P. Cornelis. 2011. Antimicrobial resistance of heterotrophic bacteria in sewage-contaminated rivers. *Water Research* 45:788–796.
- Good, I. J. 1953. The population frequencies of species and the estimation of population parameters. *Biometrika* 40:237–264.
- Grimm, N. B., S. H. Faeth, N. E. Golubiewski, C. L. Redman, J. Wu, X. Bai, and J. M. Briggs. 2008. Global change and the ecology of cities. *Science* 319:756–760.
- Groffman, P. M., A. M. Dorsey, and P. M. Mayer. 2005. N processing within geomorphic structures in urban streams. *Journal of the North American Benthological Society* 24:613–625.
- Hall Jr., R. O., and J. L. Meyer. 1998. The trophic significance of bacteria in a detritus-based stream food web. *Ecology* 79:1995–2012.
- Harwood, C. S., and J. Gibson. 1988. Anaerobic and aerobic metabolism of diverse aromatic compounds by the photosynthetic bacterium *Rhodospseudomonas palustris*. *Applied and Environmental Microbiology* 54:712–717.
- Hickey, W. J., and D. D. Focht. 1990. Degradation of mono-, di-, and trihalogenated benzoic acids by *Pseudomonas aeruginosa* JB2. *Applied and Environmental Microbiology* 56:3842–3850.
- Hill, B., A. Herlihy, and P. Kaufmann. 2002. Benthic microbial respiration in Appalachian Mountain, Piedmont, and Coastal Plains streams of the eastern USA. *Freshwater Biology* 47:185–194.
- Huerta-Fontela, M., M. T. Galceran, and F. Ventura. 2008. Stimulatory drugs of abuse in surface waters and their removal in a conventional drinking water treatment plant. *Environmental Science & Technology* 42:6809–6816.
- Jaimes-Correa, J. C., D. D. Snow, and S. L. Bartelt-Hunt. 2015. Seasonal occurrence of antibiotics and a beta agonist in an agriculturally-intensive watershed. *Environmental Pollution* 205:87–96.
- Janda, J. M., and S. L. Abbott. 2010. The genus *Aeromonas*: taxonomy, pathogenicity, and infection. *Clinical Microbiology Reviews* 23:35–73.
- Kamal, V. S., and R. C. Wyndham. 1990. Anaerobic phototrophic metabolism of 3-chlorobenzoate by *Rhodospseudomonas palustris* WS17. *Applied and Environmental Microbiology* 56:3871–3873.
- Kampfer, P. T. 2010. Order Sphingobacteriales. Pages 330–351 in N. R. Krieg, W. Ludwig, W. B. Whitman, B. P. Hedlund, B. J. Paster, J. T. Staley, N. Ward, and D. Brown, editors. *Bergey's manual of systematic bacteriology: the Bacteroidetes*. Second edition. Springer, New York, New York, USA.
- Kaushal, S. S., and K. T. Belt. 2012. The urban watershed continuum: evolving spatial and temporal dimensions. *Urban Ecosystems* 15:409–435.
- Kaushal, S. S., P. M. Groffman, L. E. Band, E. M. Elliott, C. A. Shields, and C. Kendall. 2011. Tracking non-point source nitrogen pollution in human-impacted watersheds. *Environmental Science & Technology* 19:8225–8232.
- Kaushal, S. S., P. M. Groffman, L. E. Band, C. A. Shields, R. P. Morgan, M. A. Palmer, K. T. Belt, C. M. Swan, S. E. G. Findlay, and G. T. Fisher. 2008. Interaction between urbanization and climate variability amplifies watershed nitrate export in Maryland. *Environmental Science & Technology* 42:5872–5878.
- Kolpin, D. W., E. Furlong, E. Meyer, E. M. Thurman, and S. Zaugg. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. *Environmental Science & Technology* 36:1202–1211.
- Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander, and P. D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the

- MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology* 79:5112–5120.
- Larimer, F. W., et al. 2004. Complete genome sequence of the metabolically versatile photosynthetic bacterium *Rhodopseudomonas palustris*. *Nature Biotechnology* 22:55–61.
- Lawrence, J. R., B. Zhu, G. D. Swerhone, J. Roy, V. Tumber, M. J. Waiser, E. Topp, and D. R. Korber. 2012. Molecular and microscopic assessment of the effects of caffeine, acetaminophen, diclofenac, and their mixtures on river biofilm communities. *Environmental Toxicology and Chemistry* 31:508–517.
- Lednicka, D., J. Mergaert, M. C. Cnockaert, and J. Swings. 2000. Isolation and identification of cellulolytic bacteria involved in the degradation of natural cellulosic fibres. *Systematic and Applied Microbiology* 23:292–299.
- Lee, S. S., A. Paspalof, D. Snow, E. K. Richmond, E. J. Rosi-Marshall, and J. J. Kelly. 2016. Occurrence and potential biological effects of amphetamine in stream ecosystems. *Environmental Science & Technology* 50:9727–9735.
- Martin-Carnahan, A., and S. W. Joseph. 2005. Genus I. *Aeromonas*. Pages 557–578 in D. J. Brenner, N. R. Krieg, and T. J. Staley, editors. *Bergey's manual of systematic bacteriology*. Springer, New York, New York, USA.
- Monteiro, S. C., and A. B. A. Boxall. 2010. Occurrence and fate of human pharmaceuticals in the environment. *Reviews of Environmental Contamination and Toxicology* 202:53–154.
- Palleroni, N. J. 2010. The *Pseudomonas* story. *Environmental Microbiology* 12:1377–1383.
- Petty, J., J. Huckins, D. Alvarez, W. Brumbaugh, W. Cranor, R. Gale, A. Rastall, T. Jones-Lepp, T. Leiker, and C. Rostad. 2004. A holistic passive integrative sampling approach for assessing the presence and potential impacts of waterborne environmental contaminants. *Chemosphere* 54: 695–705.
- Pusch, M., D. Fiebig, I. Brettar, H. Eisenmann, B. K. Ellis, L. A. Kaplan, M. A. Lock, M. W. Naegeli, and W. Traunspurger. 1998. The role of micro-organisms in the ecological connectivity of running waters. *Freshwater Biology* 40:453–495.
- Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41:D590–D596.
- R Development Core Team. 2016. R: a language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria.
- Ramana, V. V., S. K. Chakravarthy, P. S. Raj, B. V. Kumar, E. Shobha, E. V. V. Ramaprasad, C. Sasikala, and C. V. Ramana. 2012. Descriptions of *Rhodopseudomonas parapalustris* sp. nov., *Rhodopseudomonas harwoodiae* sp. nov. and *Rhodopseudomonas pseudopalustris* sp. nov., and emended description of *Rhodopseudomonas palustris*. *International Journal of Systematic and Evolutionary Microbiology* 62: 1790–1798.
- Reardon, K. F., D. C. Mosteller, and J. D. Bull Rogers. 2000. Biodegradation kinetics of benzene, toluene, and phenol as single and mixed substrates for *Pseudomonas putida* F1. *Biotechnology and Bioengineering* 69:385–400.
- Richmond, E. K., E. J. Rosi-Marshall, S. S. Lee, R. M. Thompson, and M. R. Grace. 2016. Antidepressants affect stream ecosystems: selective serotonin reuptake inhibitors (SSRIs) decrease algal production, but increase insect emergence. *Freshwater Science* 35:845–855.
- Rosi-Marshall, E. J., D. Kincaid, H. Bechtold, T. V. Royer, M. Rojas, and J. J. Kelly. 2013. Pharmaceuticals suppress algal growth and microbial respiration and alter bacterial communities in stream biofilms. *Ecological Applications* 23:583–593.
- Rosi-Marshall, E. J., and T. V. Royer. 2012. Pharmaceutical compounds and ecosystem function: an emerging research challenge for aquatic ecologists. *Ecosystems* 15:867–880.
- Rosi-Marshall, E. J., D. Snow, S. L. Bartelt-Hunt, A. Paspalof, and J. L. Tank. 2015. A review of ecological effects and environmental fate of illicit drugs in aquatic ecosystems. *Journal of Hazardous Materials* 282:18–25.
- Schloss, P. D., et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75:7537–7541.
- Schreiber, C., and T. Kistemann. 2012. Antibiotic resistance among autochthonous aquatic environmental bacteria. *Water Science and Technology* 67:117–123.
- Standley, L. J., L. A. Kaplan, and D. Smith. 2000. Molecular tracers of organic matter sources to surface water resources. *Environmental Science & Technology* 34:3124–3130.
- Trzesicka-Mlynarz, D., and O. P. Ward. 1995. Degradation of polycyclic aromatic hydrocarbons (PAHs) by a mixed culture and its component pure cultures, obtained from PAH-contaminated soil. *Canadian Journal of Microbiology* 41:470–476.
- van der Woude, B. J., M. de Boer, N. M. J. van der Put, F. M. van der Geld, R. A. Prins, and J. C. Gottschal. 1994. Anaerobic degradation of halogenated benzoic acids by photoheterotrophic bacteria. *FEMS Microbiology Letters* 119:199–208.

- Voolaid, V., A. Jõers, V. Kisand, and T. Tenson. 2012. Co-occurrence of resistance to different antibiotics among aquatic bacteria. *BMC Microbiology* 12:225.
- Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* 73:5261–5267.
- Youssef, N., C. S. Sheik, L. R. Krumholz, F. Z. Najjar, B. A. Roe, and M. S. Elshahed. 2009. Comparison of species richness estimates obtained using nearly complete fragments and simulated pyrosequencing-generated fragments in 16S rRNA gene-based environmental surveys. *Applied and Environmental Microbiology* 75:5227–5236.
- Zhang, D., J. Luo, Z. M. P. Lee, Y. Maspolim, R. M. Gersberg, Y. Liu, S. K. Tan, and W. J. Ng. 2016. Characterization of bacterial communities in wetland mesocosms receiving pharmaceutical-enriched wastewater. *Ecological Engineering* 90:215–224.