Sediment bacteria in an urban stream: Spatiotemporal patterns in community composition

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**Abstract**
Sediment bacterial communities play a critical role in biogeochemical cycling in lotic ecosystems. Despite their ecological significance, the effects of urban discharge on spatiotemporal distribution of bacterial communities are understudied. In this study, we examined the effect of urban discharge on the spatiotemporal distribution of stream sediment bacteria in a northeast Ohio stream. Water and sediment samples were collected after large storm events (discharge > 100 m³) from sites along a highly impacted stream (Tinkers Creek, Cuyahoga River watershed, Ohio, USA) and two reference streams. Although alpha (α) diversity was relatively constant spatially, multivariate analysis of bacterial community 16S rDNA profiles revealed significant spatial and temporal effects on beta (β) diversity and community composition and identified a number of significant correlating abiotic parameters. Clustering of upstream and reference sites from downstream sites of Tinkers Creek combined with the dominant families observed in specific locales suggests that environmentally-induced species sorting had a strong impact on the composition of sediment bacterial communities. Distinct groupings of bacterial families that are often associated with nutrient pollution (i.e., Comamonadaceae, Rhodobacteraceae, and Pirellulaceae) and other contaminants (i.e., Sphingomonadaceae and Phyllobacteriaceae) were more prominent at sites experiencing higher degrees of discharge associated with urbanization. Additionally, there were marked seasonal changes in community composition, with individual taxa exhibiting different seasonal abundance patterns. However, spatiotemporal variation in stream conditions did not affect bacterial community functional profiles. Together, these results suggest that local environmental drivers and niche filtering from discharge events associated with urbanization shape the bacterial community structure. However, dispersal limitations and interactions among other species likely play a role as well.

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**Keywords:** Bacterial community composition, Urban drainage, WWTPs, Spatiotemporal effects, Streams

### 1. Introduction

Urban discharge, consisting of stormwater run-off (SWR) and wastewater treatment plant (WWTP) effluent, is among the greatest source of diffuse pollution of surface waters (Paul and Meyer, 2001), including nutrients (PO₄-P and ammonia [NH₄-N]), carbon (Carey and Migliaccio, 2009), bacteria, organic pollutants, road salt, suspended solids, and metals (Gilliom et al., 2006; Lewis et al., 2007; Paul and Meyer, 2001; Poff et al., 2006; Wenger et al., 2009). Chemical degradation of these water bodies can have a negative effect on lotic ecosystem function, resulting in reduced nutrient retention efficiency, decreased biological diversity, and increased dominance of pollution-tolerant species (reviewed by House et al., 1993; Roy et al., 2014). Additionally, the altered hydrological regime and geomorphic adjustment from WWTPs and SWR can scour streambeds and increase erosion (Walsh et al., 2005), reducing habitat quality and altering ecosystem dynamics (Konrad et al., 2005; Roy et al., 2008). Although the severity of hydrogeomorphic (Fitzpatrick and Peppler, 2010), chemical (Beaulieu et al., 2014), and biological (Bryant and Carlisle, 2012) alterations from urban discharge depends on spatial and temporal differences within catchments, the overall effects on aquatic ecosystems are well documented (Coles et al., 2004; Cuffney et al., 2005; Paul and Meyer, 2001; Walker and Pan, 2006; Wenger et al., 2009). Thus, urban discharge can constitute as an environmental filter that potentially impacts benthic bacterial communities.

Benthic bacterial communities perform important functions in lotic ecosystems, such as biodegradation and biogeochemical
cycling (Zeglin, 2015), and thus are ideal candidates for monitoring ecological effects of urban discharge on functional characteristics of aquatic environments (Lear et al., 2009). Additionally, stream benthic bacteria are highly responsive to changes in the environment; they are the first to interact with dissolved substances and can be severely impacted by perturbations (Ancion et al., 2010; Beaulieu et al., 2014; Paerl et al., 2014). As a result of their fast growth rates and responses to small physical and chemical changes (Schwermer et al., 2008; Paerl et al., 2014), benthic bacterial community composition may differ temporally and spatially (i.e., longitudinally within a stream or among different streams) in response to environmental stimuli from urban discharge.

Overall, urban discharge impacts sediment bacterial communities in lotic ecosystems, and these impacts are spatiotemporally variable (Fisher et al., 2015; Drury et al., 2013; Newton et al., 2013; Parent-Raoult et al., 2005, Parent-Raoult and Boisson, 2007; Perryman et al., 2011). Yet, the majority of studies that have focused on microbial communities in urban aquatic ecosystems have studied the effects of urbanization on microbial-mediated nutrient cycling (Claessens et al., 2010; Groffman et al., 2004; Harbott and Grace, 2005; Imberger et al., 2008; Merbt et al., 2015; Perryman et al., 2008, 2011; Rosa et al., 2013) or sewage-derived bacteria (Baudart et al., 2000; Cha et al., 2010; Chigbu et al., 2004; Chu et al., 2014). Effects of urban discharge on native bacterial communities have largely been ignored (Goss et al., 2016). In this study, urban discharge impacts on spatiotemporal variation in benthic bacterial community composition and environmental drivers were examined in Tinkers Creek—a tributary of the Cuyahoga River in Northeast Ohio (USA). Effluent from WWTPs constitutes up to ~80% of streamflow in Tinkers Creek (Tertuliani et al., 2008) and input from nonpoint sources causes increased turbidity and sedimentation after heavy rain events (Ohio EPA, 2003). As a result, the stream is exposed to a wide range of physicochemical variation and various sources of inorganic and organic contamination.

Along the length of Tinkers Creek, the extent of urban land use and the number of WWTPs increases with distance from the headwaters; physiochemical conditions were expected to reflect this pattern through higher nutrient loads and greater conductivity downstream (Tertuliani et al., 2008). We hypothesized there would be a longitudinal decrease in bacterial richness (α-diversity) concurrently with the urban gradient and that there would be high compositional dissimilarity (β-diversity) between Tinkers Creek and two reference streams. Further, we hypothesized that the urbanization gradient reflected in Tinkers Creek physicochemistry would result in increased compositional dissimilarity between upstream and downstream sites and that these changes would be reflected by fluctuations in specific functional traits. Additionally, we anticipated that seasonal variability in stream physicochemical parameters would result in a successional change in community composition over the course of the sampling season. Specifically, prior studies have shown seasonal fluctuations in temperature (Boyero et al., 2011; Silva and Williams, 2005; Zhang et al., 2012), nutrient concentrations (Dodds et al., 2002; Gessner and Chauvet, 1994; Findlay and Sinsabaugh, 2003), and streamflow (Chiaramonte et al., 2013; Fazi et al., 2013; Silva and Williams, 2005; Valett et al., 1997; Zoppini et al., 2010) to be selective forces for the temporal shifts observed in microbial communities.

2. Methods

2.1. Study site

Tinkers Creek, a 7th-order stream, drains a 250 km² watershed with a rural/agriculture to an urban land cover gradient that spans the length of the stream (Tertuliani et al., 2008). A small percentage (0.3%) of the watershed is classified as agricultural land use, while >70% is classified as commercial/industrial/transportation and residential, and 25.5% as wetlands, grasslands/pasture or forest (Tertuliani et al., 2008). The stream's flow is highly influenced by discharge from 8 WWTPs (Fig. S1) and stormwater run-off. The five sites selected for sampling were chosen to represent a wide range of physicochemical parameters and various sources of inorganic and organic contamination (Tertuliani et al., 2008), with only the most upstream site not receiving WWTP effluent. Qualitatively, substrate composition differed along Tinkers Creek, with silt/sand occurring at the most upstream locations, which shifted to pebbles/cobbles at downstream sampling locations. Additionally, single sampling sites were established in Furnace Run and Yellow Creek, 4th and 3rd-order tributaries of the Cuyahoga River, respectively, to serve as reference sites. Both streams are tributaries of the Cuyahoga River, and their watersheds are less developed compared to that of Tinkers Creek, and they lack WWTPs (Tertuliani et al., 2008; Table S1). Both streams meet biocriteria for attainment as specified by the Ohio Water Quality Standards (WQS; Ohio Administrative Code Chapter 3745-1) and Ohio EPA biological criteria (OAC Rule 3745-1-07; Ohio Environmental Protection Agency, 2003). In contrast, Tinkers Creek is impaired based on these metrics, with significant departures from biocriteria for fish and invertebrate communities.

2.2. Sample collection

Water (125 mL) and sediment (100 g) samples (N = 3) were collected from each of the seven study sites after large rain events (discharge > 100 m³/s; USGS discharge gauge at site 5 in Tinkers Creek) in October and November of 2012, and in April, May, June, July, August and September of 2013. Sampling after large rain events was performed so as to achieve maximum levels of urban discharge from WWTPs and stormwater. Samples were stored on ice for transport to the lab. Water samples were collected in polypropylene acid washed bottles. Sediments (top 10 cm) were collected with a scoop, homogenized, and divided into subsamples for nutrient analysis and DNA extraction. All samples were collected following standard USGS field collection procedures (Wagner et al., 2006).

2.3. Physicochemical variables

Dissolved oxygen (DO), conductivity, redox potential, pH, and turbidity were measured using a Hqd/IntelliCAL Rugged Field kit (Hach Company, Loveland, CO) and Hach turbidimeter model 2100P, respectively, during sample collection. Additionally, flow velocity (portable water flow meter model 201; Marsh-McBirney, Inc), and water depth and width were used to calculate discharge. Surface water was sub-sampled, filtered and acidified as appropriate before analysis. Dissolved organic carbon (DOC) and dissolved total nitrogen (TN) were measured from 50 mL subsamples using a Shimadzu TOC/TN analyzer (Eaton et al., 2005). Soluble reactive phosphorus (SRP) was determined from 50 mL subsamples following Eaton et al. (2005), while dissolved ammonium (NH₄-N), nitrate (NO₃-N), and nitrate (NO₂-N) were measured from 15 mL subsamples colorimetrically via a modified microplate analysis (Hood-Nowotny et al., 2010; Weatherburn, 1967).

For determination of nutrient content in sediments, subsamples (20 g) were treated with a 0.5M K₂SO₄ solution (1:5 ratio [soil: 0.5M K₂SO₄]) (Ettema et al., 1999), filtered, and nitrogen and P concentrations were measured colorimetrically as above (Eaton et al., 2005; Hood-Nowotny et al., 2010; Weatherburn, 1967). Benthic organic matter (BOM) was measured via combustion on 5 g sub-
samples of fresh sediments; percent organic matter was calculated based on the ratio of ash-free dry mass and dry mass.

2.4. Bacterial community composition

DNA from stream sediment samples was extracted using PowerSoil DNA Isolation Kits following the manufacturer’s instructions (MoBio Laboratories, Carlsbad, CA). Bacterial community composition was assessed using Terminal Restriction Length Polymorphism (T-RFLP) of the 16S rRNA gene as described in Blackwood et al. (2003). PCR used primers Eub338F-II and Eub338F-I-III (forward), which were labeled at the 5′ termini with 6-carboxyfluorescein (6-FAM, Integrated Technologies) and 1392R (reverse) (Blackwood et al., 2003). Each reaction contained 0.5 μM of each primer, 400 ng BSA (New England Biolabs, Ipswich, MA), and approximately 10 ng of total DNA in 30 μl reaction volumes. Thermal cycling conditions were as follows: 1 cycle at 95 °C for 3 min followed by 40 cycles at 94 °C for 30s, 57 °C for 30s, and 72 °C for 1 min 30s, and 1 cycle at 72 °C for 7 min. PCR products were pooled from three reactions per sample and digested with endonuclease Hhal (New England Biolabs). Digested products were cleaned using E.Z.N.A DNA probe cleanup kit (Omega bio-tek, Norcross, Georgia) and were separated by automated capillary electrophoresis (3730 DNA analyzer; Applied Biosystems, Foster City, CA) at The Ohio State Plant-Microbe Genomics Facility to produce a community profile. Analysis of T-RFLP reads generated was performed with T-REX software (Culman et al., 2009), T-RFLPs were processed to remove peak noise and to align fragments before further analysis in R (see below).

Given that redundancy analysis of T-RFLPs revealed spatial and temporal differences (but no interaction effect) in bacterial communities, samples were pooled and subjected to 16S rRNA gene sequencing. DNA concentrations were standardized (10 ng μl⁻¹) and pooled by sampling location (samples were pooled across sampling dates for each site) and sampling date (samples were pooled across sites for each date). The V4-V5 hypervariable region of 16S rRNA genes was sequenced at The Ohio State Molecular and Cellular imaging center, via Illumina Miseq sequencing technology. iTags generated were processed in the QIIME pipeline v1.9.1 (Caporaso et al., 2010). Paired forward and reverse reads with ambiguities, homopolymers, as well as low-quality scores were removed using QCing in QIIME. Reads were then assembled into single contigs or iTags via PANDASeq (Masella et al., 2012). iTag primer sequences and barcodes were subsequently eliminated, contigs were edited to a uniform length of 250 bp, and then chimERIC reads detected and removed with USEARCH v 6.1 (Edgar, 2010). Quality-filtered contigs were then processed using the de novo and reference-based OTU clustering platforms in QIIME, and based on a >97% similarity to 16S rRNA sequences in the Green-genes reference database (Version 13.8) were assigned to operational taxonomic units (OTUs). Singletons were removed, and results were summarized at the phylum and family levels. Reads occurring in more than two samples with relative abundances greater than 1% were retained for α- and β-diversity analyses. Reads were then rarefied to 14,648 sequences per sample and used in further analysis.

PICRUSt (Langille et al., 2013) was used to predict functional characteristics of stream bacterial populations. PICRUSt utilized associations between 16S rRNA gene markers found in the Green-genes database and functional genes found in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to reconstruct potential functional gene families present in the sampled communities. De novo OTUs were removed and the remaining OTUs were normalized to create a closed reference OTU table consisting of samples with Greengenes IDs which were then used to predict metagenomics functional profiles. To compare differences in the functional profiles of communities among sampling sites and dates, the relative abundance of predicted KEGG Orthologs (KOs) were examined across three tiers of increasing functional resolution (tiers 1–3). KOs associated with tier 1 functions “organismal systems” and “human disease” were considered irrelevant to environmental samples and were discarded prior to further analysis. Accession numbers: Tinkers Creek Site 1: SAMN08245559; Tinkers Creek Site 2: SAMN08245560; Tinkers Creek Site 3: SAMN08245561; Tinkers Creek Site 4: SAMN08245562; Tinkers Creek Site 5: SAMN08245563; Reference Site Yellow Creek: SAMN08245564; Reference Site Furnace Run: SAMN08245565; April_pooled: SAMN08245566; May_pooled: SAMN08245567; June_pooled: SAMN08245568; July_pooled: SAMN08245569; August_pooled: SAMN08245570; September_pooled: SAMN08245571; October_pooled: SAMN08245527; November_pooled: SAMN08245537 can be found at NCBI BioSample database.

2.5. Statistical analysis

All statistical analyses were performed using R statistical software version 3.2.0 (R Development Core Team, 2014). Grubb’s tests (package: outliers [Luksza, 2015]), were used to identify outliers in stream physicochemical data, which were removed before further analysis. Physicochemical data failed to meet assumptions of normality and homoscedasticity, and attempts at data transformation were not successful. Consequently, Spearman’s rank correlations were used to explore general spatial trends in environmental conditions within Tinkers Creek and to identify seasonal patterns. In addition, permutational one-way analysis of variance (PERMANOVA) (coin package; Hothorn et al., 2008) followed by permutational multiple comparison tests (nparcomp package [Maintainer and Konietschke, 2015]) were used to identify site-specific differences in environmental conditions between Tinkers Creek and reference streams. All univariate P values were corrected following the Benjamini-Hochberg (B-H) procedure for reducing false discovery rates (Hochberg and Benjamini, 1995).

To evaluate differences in community composition among sampling sites and dates relative abundance data from T-RFLP profiles were Hellinger transformed and measures associated with α- (richness, evenness, Shannon’s entropy, and inverse Simpson’s index) and β-diversity (as Bray-Curtis distances) were calculated using the vegan package (Oksanen et al., 2007). Measures of α-diversity were log-transformed to reduce homoscedasticity and both α- and β-diversity data were tested using a permutational multivariate analysis of variance. PERMANOVA (coin package; Hothorn et al., 2008) followed by permutational multiple comparison tests (nparcomp package [Maintainer and Konietschke, 2015]) were used to identify site-specific or month-specific differences. All univariate P values were corrected following the Benjamini-Hochberg procedure for reducing false discovery rates (Hochberg and Benjamini, 1995). Nonmetric multidimensional scaling was used to compare and visualize β-diversity data (ggplot2 package; Wickham et al., 2016), Partial redundancy analysis (vegan package; Oksanen et al., 2007) was then performed on Hellinger transformed community T-RFLP relative abundance data to assess effects of sampling site and month on community composition. Additionally, α-diversity indices (Chao 1 richness and Shannon diversity index) were calculated to determine within sample diversity from rarefied sequences. A Bray-Curtis distance matrix was generated and used to generate multidimensional scaling axes to visualize trends in communities over time and among sampling sites. Finally, partial redundancy analyses were used to compare community KO profiles between sampling locations and between sampling months.
3. Results

Several significant (Spearman rank test; \( P < 0.05 \)) correlations between environmental variables associated with urban discharge and the upstream-downstream Tinkers Creek sampling gradient were observed (Table S2). Dissolved nutrients (TN, NO\(_3\), NO\(_2\), and PO\(_4\)) as well as other abiotic variables (conductivity, pH, DO, and rates of discharge), increased from upstream to downstream within Tinkers Creek. In contrast, sediment nutrients (BOM, NH\(_4\), NO\(_3\), and PO\(_4\)) and turbidity decreased. Additionally, Tinkers Creek had significantly greater concentrations of dissolved and sediment (extractable) nutrients compared to reference streams (PERMANOVA [\( P < 0.001 \); Table S3]). Specifically, nearly all sites in Tinkers Creek had greater concentrations of DOC, TN, sediment NH\(_4\), and higher readings of conductivity, pH, redox, DO, and turbidity. Dissolved nitrogen species (NH\(_4\), NO\(_3\), and NO\(_2\)) and PO\(_4\), and rates of discharge were significantly higher in downstream Tinkers sites (2–5) compared to reference sites.

Seasonal changes in stream physiochemical characteristics demonstrated significant variation (\( P < 0.05 \)) (Table S4). Dissolved (NH\(_4\), NO\(_3\), PO\(_4\)), BOM, and sediment-extractable (NO\(_2\), NO\(_3\), PO\(_4\)) nutrients and DO exhibited significant increases over time; nutrients and higher DO measurements were observed during summer months as compared to autumn sampling periods. This trend was opposite for both nutrient (DOC, dissolved NO\(_3\), and sediment NH\(_4\)) and other physicochemical variables (conductivity, temperature, redox potential, turbidity, and discharge), which generally decreased as sampling dates approached September.

Bacterial community \( \alpha \)-diversity calculated from T-RFLP profiles did not differ significantly among sites, and no longitudinal pattern was observed in Tinkers Creek (Table 1). However, strong seasonal differences were observed (PERMANOVA \([ P < 0.001; \text{Table 1} \]); richness (\( P = 0.22 \)), evenness (\( P = 0.62 \)). Shannon entropy (\( P = -0.35 \)), and inverse Simpson (\( P = -0.32 \)) indices were all significantly, and negatively correlated with the month of sampling. There were significant increases in diversity between November and April, and then relatively stable values until a sharp, significant increase in August. Although there was a 5-month lag between the November and April sampling dates, the increase in diversity coincided with increased nutrient concentrations (data not shown). Sampling location and date significantly (\( P = 0.025 \) and \( P < 0.001 \), respectively) affected community \( \beta \)-diversity based on T-RFLP profiles. However, due to considerable variance at each site, it was difficult to discern clear spatial patterns in \( \beta \)-diversity (Fig. 1a). Seasonal effects on \( \beta \)-diversity were much more apparent, with a clear separation of October and November from other months, which exhibited far more overlap in community profiles (Fig. 1b).

Redundancy analysis of T-RFLP data revealed significant differences in community composition among sites (\( P = 0.02 \); Fig. 2a) and dates (\( P = 0.001 \); Fig. 2b); site and date interactions were not significant (data not shown). Therefore, site and date were considered separately. Sampling site explained 8% of the variance among communities when partitioning out the effects of sampling date. Ordination of the significant RDA axes showed most of the sites clustered relatively close to one another, except for the most upstream site in Tinkers Creek (TC1) and reference site 2 (Furnace Run) (Fig. 2a). Despite this clustering, there was little overlap in ordination space, as demonstrated by standard errors of community profile means, indicating significant compositional dissimilarity among sites. Of the environmental variables examined, BOM, dissolved and sediment nutrients, conductivity, pH, redox, DO, and discharge rates were all strong predictors of bacterial community composition (Table 2; \( P < 0.05 \)). Analysis of the community KO profiles revealed that there was no significant difference in functional groups regardless of functional resolution (e.g., levels 1 [Fig. 3], 2 or 3 [data not shown]) between sampling locations. However, the majority (\(-51\%\)) of functional genes were related to metabolic function.

Sampling date accounted for a greater percentage of described variance (14.5%) in community composition than did study site. This was apparent in the lack of overlap in community profiles by month and clear clustering that reflected seasonal environmental differences (Fig. 2b). For example, spring and summer months clustered near one another while mid to late fall communities (October and November) clustered together. These patterns were significantly correlated with seasonal changes in environmental conditions (i.e., dissolved N\(_4\), NO\(_3\), sediment NO\(_3\), conductivity, temperature, DO, turbidity, flow, and discharge [Table 2; \( P < 0.05 \))). September exhibited the greatest divergence from other community profiles, which correlated with elevated sediment NO\(_3\).

Because there was no site by date interaction from the T-RFLP data, samples for 16S rRNA gene sequencing were pooled by date and by site. Nonmetric multidimensional scaling (NMDS) of sequencing data revealed marked differences in bacterial community composition among sites and dates at phylum (Figs. S2a and S2b), order (Figs. S3a and S3b), and family (Fig. 4a and b) levels. Nutrients (water and sediment), and other stream abiotic properties (e.g., conductivity, redox, DO, turbidity, pH, and discharge) were related to differences in composition among sampling site (Fig. 4a; Tables S5a and S5b). When comparing the relative abundances of specific families of bacteria across all sampling locations, the most abundant sequences were classified as a members of the Betaproteobacteria (Burkholderiales Comamonadaceae), which made up \(-16.4\%\) of the sequencing reads, followed by and Bacteriodia ([Saprospirales] Chitinophagaceae) (10.9% of the reads), Planctomycetia (Pirellulales Pirellulaceae) and Verrucomicrobia (Verrucomicrobia Verrucomicrobia) (7.1% and 6.9% of the reads, respectively), and Alphaproteobacteria (Rhodobacteriales Rhodobacteraceae and Rhizobiales Phyllobacteriaceae) (6.8% and 4.7% of the reads, respectively).

Communities in Tinkers Creek were more similar among sites with greater spatial proximity to each other (e.g., site 1 was more similar to site 2 than to sites 3–5; Fig. 5a and b, Fig. S3a). Upstream sites (TC1–2) had a higher prevalence of families in the class Acidobacteria-6 (iii 1–15 unassigned), Bacteroidia (unassigned Bacteroidales), BSV26, Ignavibacteria, Anaerolineae, Gemm-1, Nitrospira, Verrucomicrobia, Betaproteobacteria (SC-I-84 unassigned), Deltaactebacteria, Gammaproteobacteria \( p < 0.05 \); Fig. 5a and b). In contrast, downstream sites (TC 3–5) were dominated by families from the class [Chloracidobacteria], Acidobacteria-6 (iib 15 mb2424), Solbacteres, [Saprospirae], Flavobacteria, Planctomycetia

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Sampling date(^a) and location(^b) effect on ( \alpha )-diversity of Hellinger transformed 16S T-RFLP OTU profiles. Mean ± SE.</th>
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<tr>
<td>Date(^a)</td>
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<tr>
<td>Richness</td>
<td>10.67 (1.00)</td>
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<tr>
<td>Evenness</td>
<td>0.95 (0.006)</td>
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<td>Location(^b)</td>
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<tr>
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<td>15.67 (2.03)</td>
</tr>
<tr>
<td>Evenness</td>
<td>0.96 (0.006)</td>
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proportional to their correlations with the ordination. (Fig. 4b), significant parameters to correlate with July community composition (Figs. S2b and S3b; Fig. 4b). Although dissolved NH₄⁺ was the only abiotic parameter to correlate with dissolved nutrients positively and negatively, respectively, correlated with abundance within the phylum Acidobacteria, whereas Gammaproteobacteria negatively correlated with dissolved nutrients and/or sediment nutrients (Table S6a). Additionally, all nutrient stream measurements (Table S6b) positively correlated with different taxa over time, except for DO concentration which was negatively correlated to Nitrospirae abundance.

Seasonal differences in the prevalence of dominant families (abundances >3% of sequence reads) were apparent for the Chitinophagaceae, Pirellulaceae, Verrucomicrobiaceae (Fig. 5a), Rhodobacteraceae, and Comamonadaceae (Fig. 5b). The Comamonadaceae was the most dominant sediment community member over the course of this study (except during October); with abundances peaking in June at 10.3% of sequencing reads. In October, Verrucomicrobiaceae and Rhodobacteraceae were the most prevalent groups, making up 10% of the reads. Both families peaked again in abundance (7.4% and 4.6% of reads, respectively) during spring (April). Similarly, Chitinopagaceae made up a large proportion (7.3%
of the sequence reads) of the community. In July, Chitinopagaceae was the most prevalent sediment community member, making up 8.2% of the sequence read. Pirellulaceae taxa demonstrated a cyclic peaking pattern, with increases in abundance in October, May, and July followed by decreases in abundance in November, June, and August.

Less prominent families also demonstrated peaks in abundance over the course of the study. Acidobacteria peaked in abundance during April and July, making up 4.5% and 5.7% of sequencing reads, respectively (Fig. 6a). The Bacteroidia, Cytophagales, and Deltaproteobacteria taxa peaked in abundance in May, making up 8% and 4.7% of sequence reads. However, seasonal changes in community composition did not result in distinct changes in community function, as redundancy analysis of PICRUSt results showed no differences in major functional profiles over time (Fig. 7).

The majority (~51%) of functional genes were related to metabolic function, followed by genetic information processing (~20%).

4. Discussion

Urban discharge is a primary source of stream degradation in urban areas (Parr et al., 2015). Alterations in microbial-mediated nutrient cycling processes (Merbt et al., 2015) and increases in fecal bacterial indicator contamination (Baudart et al., 2000; Cha et al., 2010; Chigbu et al., 2004; Chu et al., 2014) as a result of urban discharge have been widely documented. However, there are few reports detailing spatiotemporal variations in bacterial sediment assemblages in streams dominated by urban discharge (Staley et al., 2013; Wang et al., 2011; Zhang et al., 2016). By analyzing the sediments of Tinkers Creek and two references...
streams, we demonstrated that bacterial community composition was correlated with local environmental conditions (e.g., conductivity and nutrient concentrations), which were directly influenced by surrounding sub-watershed land use, suggesting that land use and local stream properties influence bacterial sediment communities.

The impacts of the urban discharge on physicochemical variables were evident in downstream sites along Tinkers Creek and were hypothesized to result in a longitudinal decrease in bacterial species richness. However, despite large longitudinal differences in water and sediment physicochemical properties, bacterial communities exposed to higher urban drainage in Tinkers Creek did not differ in species richness or evenness relative to upstream and reference stream communities. These results are
inconsistent with other studies that have shown that higher concentrations of organic and inorganic nutrients associated with anthropogenic activity may either increase (Staley et al., 2014; Marti and Balcázar, 2014; Wakelin et al., 2008) or decrease (Lu and Lu, 2014; Drury et al., 2013) species richness in stream sediment bacterial assemblages. The observed similarity in species richness and evenness may imply that many of the taxa are generalists, capable of utilizing a wide variety of nutrients (Wittebolle et al., 2009), and/or capable of withstanding non-extreme environmental perturbations (Staley et al., 2014; Wittebolle et al., 2009). Along the same lines, Staley et al. (2014) observed a significant difference in diversity between forested and urban sites, but not between agricultural and urban sites, and suggested that the lack of differences was attributable to the
similarity in anthropogenic disturbance between the sites (i.e., similar nutrient and contaminant loads). Alternatively, our results may reflect the large number of dormant cells within these communities, which has been documented to affect species richness, as dormant individuals are capable of withstanding environmental perturbations (Lennon and Jones, 2011).

In spite of similarities in species richness among sites, bacterial community composition differed among sites. Longitudinal differences in composition in Tinkers Creek were strongly connected to environmental conditions, including nutrient concentrations, DO, conductivity, redox, and discharge. This suggests that species sorting had a strong impact on sediment bacterial communities,
with local habitat conditions selecting for specific groups of bacteria (Gibbons et al., 2014; Heino et al., 2014; Staley et al., 2013). The degree of connectivity between lotic systems and adjacent terrestrial systems are influenced by drainage density and hydrological exchange, which can have a direct impact on microbial communities in streams (Hullar et al., 2006). The bacterial communities in our study encompassed microbes of terrestrial, aquatic, and human origins, with the mixture of bacteria from different putative sources varying among sampling locations.

More urbanized sites (TC 3–5) included taxa associated with nutrient pollution and other anthropogenic disturbance. The families Comamonadaceae, Rhodobacteraceae, and Pirellulaceae were
among the most dominant groups at downstream sites—accounting for up to 29% of sequence reads at these sites. Members of these families are commonly found in freshwater environments (Puigal et al., 2014; Willems, 2014; Youssef and Elshahed, 2014), but are most known for being dominant groups in nutrient-rich environments (Rosenberg et al., 2013; Tang et al., 2017; Vetterli et al., 2015; Yu et al., 2017; Youssef and Elshahed, 2014). Other taxa with elevated abundance at sites with more urban impact included *Phyllobacteriaceae* (6.13% of sequences) and *Sphingomonadaceae* (5.7% of sequences) and *Acidobacteria* (6.13% of sequences). *Phyllobacteriaceae* taxa have most commonly been studied with regard to degradation of xenobiotic and recalcitrant compounds, such as thiophene (Bambauer et al., 1998), phenols (Fritsche et al., 1999), naphthalenesulfonates (Ghosh and Dam, 2009), ethylenediaminetetraacetic acid (Doronina et al., 2010), and thiophene-2-carboxylate (Bambauer et al., 1998). Likewise, *Sphingomonadaceae* are often found in high proportions in habitats contaminated with recalcitrant (poly) aromatic compounds of natural (Glaeser et al., 2010; Rosenberg et al., 2013) or anthropogenic origin (Basta et al., 2005; Romine et al., 1999; Sprenger, 1993; Rosenberg et al., 2013). The relative dominance of these two families in downstream sites may reflect increased industrial contaminants present in WWTP effluent, as the treatment facilities receive chemicals and other wastes from industrial sources. Alternatively, these taxa may serve as an indicator of the degree of urbanization occurring upstream of these sites as stormwater runoff from street drains enters this stream from a variety of locations.

Other families that were prevalent at downstream sites are not well characterized in terms of their ecological role in aquatic systems, including *Ellin 6077* RB411 (*Chloracidobacteria*), mb2424 (*Acidobacteria*-6 iii–15), *Cryomorphaceae* and *Chitinophagaceae*. Taxa within the phylum *Acidobacteria* are known for their resistance to pollutants like petroleum compounds (Abed et al., 2002), *p*-nitrophenol (Paul et al., 2006), linear alklybenzene sulfonate (Sánchez-Peinado et al., 2010), and uranium (Ellis et al., 2003; Gremon et al., 2003; Barns et al., 2007), whereas, *Bacteroidetes* are prevalent in organic-rich systems (Crump and Hobbie, 2005; Huang et al., 2008; Obernosterer et al., 2011; Wang et al., 2011).

Therefore, the dominance of *Acidobacteria* and *Bacteroidetes* in more urbanized sites may indicate the prevalence of organic wastewater compounds within Tinkers Creek (Tertuliani et al., 2008). In general, the variety of taxa found in high abundance in downstream sites of Tinkers Creek suggests that effluent from the WWTPs plays a strong role in shaping the stream bacterial communities, as different contaminants from these WWTPs may have different selective forces on community composition.

In contrast to the more urbanized sites, a large percentage (~32%) of the taxa prevalent in the less urbanized sites (Tinkers Creek 1 and 2, Ref 1 and 2) has syntrophic and fermentative lifestyles. Members of the family *Syntrophaceae* are commonly found in anaerobic freshwater sediments (Jackson et al., 1999; Shelton and Tiedje, 1984; Wallrabenstein and Schink, 1994), and are capable of fermenting substrates that are utilized by H2/formate-utilizing partners (Kuever et al., 2005; Schink, 1997). The *Desulfobulbaceae*, *Syntrophobacteraeaceae*, and *Geoacteriaeaceae* are sulfate/sulfur-reducing bacteria (Kuever et al., 2005; Muyzer and Stams, 2008) and/or other metal-reducing bacteria (Holmes et al., 2004, 2004a, 2004b; Kuever, 2005; Röling, 2014), respectively. Although mostly known for their ability to utilize sulfur or other metal compounds as their terminal electron acceptors, genera in these families have an important role in the anaerobic fermentation oxidation of organic compounds (Akhujkar et al., 2012; reviewed in Muyzer and Stams, 2008; Röling, 2014). *Thermodesulfovibrionaceae*, such as *Deltaproteobacteria* detected in this study, are sulfate reducers with chemooorganoheterotrophic or chemolithoheterotrophic lifestyles. During chemolithoheterotrophic growth, genera of this family use H2 as electron donor and acetate as a carbon source (Henry et al., 1994). Additionally, *Crenothrichaceae* (*Gammaproteobacteria*), which was only found in upstream sites of Tinkers, is a type Ib methanotroph group. These organisms are facultative aerobes that utilize methane and methanol, or other C1 compounds as substrates (Bowman, 2014; Stein et al., 2012; Stoecker et al., 2006). When considering the relatively high diversity of fermenters and sulfur/sulfate-reducing taxa found in the upstream sites and reference streams, the microbial communities may be structured by inputs from groundwater and the hyporheic zone (Griebler and
Lueders, 2009; Storey et al., 1999). Although we lack data on the source of water at the different sites, the difference in community composition and the differences in sediment composition (with fine sediment at upstream sites and pebbles/cobblestones at downstream sites) suggests a switch from deep to shallow water flow-paths longitudinally, thus supporting the notion that local sampling environment can influence the structure of the microbial community within the stream (Heino et al., 2014; Cloutier et al., 2015).

Overall, the dissimilarities in bacterial community composition among upstream, downstream, and reference sites reflect OTU-specific environmental tolerances to local conditions (Comte et al., 2014; Comte and Del Giorgio, 2009; Newton et al., 2011; Philippot et al., 2010; Wang et al., 2011). However, it is difficult to ascertain how much of these differences are due to site characteristics (land use and/or physical-chemical) that differ along the longitudinal gradients in and among these streams, and how much is due to dispersal limitations (Crump et al., 1999; Astorga et al., 2012; Lindström and Langenheder, 2012), interactions among species (Fortunato and Crump, 2011; Gilbert et al., 2012), or some combination of the three (Astorga et al., 2012). Future work to tease apart the contributions of spatial proximity and shared environmental characteristics will be required. Nevertheless, the results suggest that differences in water chemistry attributable to urban discharge served as a selective force on bacterial taxa in these streams (Astorga et al., 2012; Beier et al., 2008; McArthur and Richardson, 2002).

Seasonal changes in the dominant sediment bacterial populations were correlated with changes in aqueous physicochemistry across all sites, as predicted. These results are consistent with previous studies that have discovered shifts in microbial composition linked to seasonal variation of water physicochemical properties (Duarte et al., 2016; Moss et al., 2006; Yannarell et al., 2003) and allochthonous inputs (Dann et al., 2017). Alterations in stream temperature, light penetration, organic and inorganic concentrations in the water column and sediments over the annual period may have shaped the changes observed in the community composition of sediment bacteria in these systems.

Distinct groupings of bacterial families became more prominent on particular dates, revealing the highly dynamic nature of the bacteria in these streams. Fall-dominant families contained known degraders of recalcitrant litter (Verrucomicrobia; Stevenson et al., 2004; Wymore et al., 2016) and humic substances (Sphingomonadaeae; Glaeser et al., 2010; Glaeser and Kämpfer, 2014). Specifically, these groups are important for their utilization of humic substances, and ability to degrade recalcitrant high-molecular weight compounds (Glaeser et al., 2010; Wymore et al., 2016), suggesting that these organisms play an important role in carbon cycling in our streams.

Spring-dominant families belonged to phyla that are often found associated with algae (Planctomycetes; Bengtsson and Övreläs, 2010; Boborquez et al., 2017), and microbial mats (Allen et al., 2009; Baumgartner et al., 2009) or biofilms (Bacteroidetes; Bartrons et al., 2012; Boborquez et al., 2017) during high levels of algal activity. Taxa within Planctomycetes have the ability to degrade sulfated polysaccharides of algal origin (Kim et al., 2016; Lage and Bondoso, 2014), whereas members of Bacteroidetes can degrade organic compounds that may be released from algae; thus suggesting that they have an ecological role in the degradation of polysaccharides produced by algae in streams. Alternatively, the high prevalence of Bacteroidetes and Phyllobacteriaceae may indicate a higher prevalence of discharge from WWTPs, or runoff during the rainy season (Bambauer et al., 1998; Drury et al., 2013; Doronina et al., 2010; Eichmiller et al., 2013; Fritsche et al., 1999; Kämpfer, 1999). In comparison, summer was dominated by taxa in families that have wide genetic diversity, such as Comamonadaceae. Taxa within this group are known denitrifiers (Adav et al., 2010; Etcheberhe et al., 2001; Khan et al., 2002; Wu et al., 2013), fermenters (Chen et al., 2013; Finneran et al., 2003; Kim et al., 2012), aerobic organotrophs (Kim et al., 2012; Liang et al., 2011), photoheterotrophs (Hiraishi et al., 1991; Madigan et al., 2000) and photoautotrophs (Zeng et al., 2012), which suggests that these organisms are involved in a variety of biogeochemical processes in aquatic ecosystems (reviewed by Willems, 2014). It should be noted, however, that more detailed phylogenetic work needs to be done to link function with phylogeny, especially with largely uncultured groups such as Verrucomicrobia, or for groups with unknown ecologies (i.e., Chitinophagaceae). However, our results suggest that in stream systems, seasonal changes allow for different and distinct combinations of bacterial populations to become prominent members of the community at different times of the year (Gilbert et al., 2012, 2009; Shade et al., 2013; Portillo et al., 2012).

Although site and date differences were observed in sediment bacterial community composition, we did not see any significant differences in functional profiles over space or time. Functional redundancy to disturbances is possible if the microbial community contains individuals that have versatile physiologies (Evans and Hofmann, 2012). Core communities found in these systems were comprised of families that had a vast network of genera capable of performing a wide array of biogeochemical cycles. For example, genera in the family Comamonadaceae and Rhodocactraceae have metabolic capabilities that span a wide variety of cycles, such as organotrophs, denitrifiers, hydrogen oxidizers, photoheterotrophs, photoautotrophs, fermenters, Fe3+-reducers (Baldani et al., 2014; Willems, 2014; Pujalte et al., 2014). Most genera in these families have an aerobic heterotrophic metabolism but are capable of switching to other metabolic forms depending on the substrate or electron acceptor availability (Willems, 2014). One such group, purple nonsulfur bacteria, can be heterotrophic under aerobic conditions and phototrophs under anoxic conditions (Hiraishi and Imhoff, 2005; Pujalte et al., 2014). Through altering metabolic capabilities (Meyer et al., 2004; Swingley et al., 2007) or genetic change (Evans and Hofmann, 2012; Lenski, 2017; De Meester et al., 2016), bacteria can often overcome detrimental environmental change by exploiting previously unavailable resources. Thus, changes in community composition may not correspond with the response, or the lack thereof, in community functional profiles (Allison and Martiny, 2008).

Alternatively, a large fraction of the community may be dormant (Lennon and Jones, 2011), which is common among communities living in temporally and spatially dynamic environments (Lennon and Jones, 2011; Pedrós-Alió, 2006; Rehman et al., 2010). In fact, dormant individuals of bacterial communities directly affect species diversity (Chesson, 2000) by acting as seed banks. Seed banks can contribute to the stability of ecosystem processes through the facilitation of niche complementation and/or functional redundancy (Loreau et al., 2001; Petchev and Gaston, 2002). This can occur as previously dormant groups become more prevalent under certain conditions while functionally complimentary groups or those functionally similar, but less tolerant to current environmental conditions and phototrophs under anoxic conditions (Hiraishi and Imhoff, 2005; Pujalte et al., 2014). Through altering metabolic capabilities (Meyer et al., 2004; Swingley et al., 2007) or genetic change (Evans and Hofmann, 2012; Lenski, 2017; De Meester et al., 2016), bacteria can often overcome detrimental environmental change by exploiting previously unavailable resources. Thus, changes in community composition may not correspond with the response, or the lack thereof, in community functional profiles (Allison and Martiny, 2008).
et al., 2016). As such, adaptation of local communities to changing environmental patterns can be independent of functional change in communities (Bier et al., 2015; Frost et al., 1995; Fernandez-Gonzalez et al., 2016; Ostman et al., 2010; Reiss et al., 2009).

Overall, the predicted functional profile of the microbial community—as determined by PICRUSt's algorithm—provides a coarse overview of the functional potential present within the community; however, these results must be interpreted with caution. Rarefaction of pooled DNA samples fails to capture the full extent of diversity present within the system, which is likely reflected in the predicted functional profile. Additionally, individual functional genes may not necessarily be correlated with community structure (Fierer et al., 2012), as the placement of novel diversity cannot accurately be mapped into a phylogenetic context due to the fact that a large proportion of bacterial phylogeny is poorly identified (Harriss et al., 2013). To adequately assess gene categories deeper sequencing would be required (Fierer et al., 2012). PICRUSt can neither preclude or outperform deep metagenomic sequencing (Langille et al., 2013); the algorithm is significantly affected by the phylogenetic dissimilarity among environmental samples and sequenced genomes (Langille et al., 2013). Thus, we suggest that further studies that utilize both metagenomic sequencing and marker gene studies are needed in this system and that more samples are required to adequately assess intra- and inter-stream variability.

5. Conclusion

Although α-diversity was relatively constant both spatially, we found that urban drainage impacts bacterial community structure in streams, with greater prevalence of bacteria associated with urban discharge in downstream sites in Tinkers Creek. Moreover, we found evidence for indirect seasonal effects, as nutrient and hydrologic characteristics influenced bacterial community assemblage within our streams. However, there were no spatial or temporal effects on the core community function. Our results suggest that determinist forces are important for community assembly and that differences in β-diversity between sites and over time are predominantly due to changes in the relative abundance of a core community. This work demonstrates that urban drainage has a marked impact on shaping benthic bacterial communities; yet, these changes seem not to have an impact on sediment bacteria function. This suggests that communities in urban environments may be more resilient to disturbance via versatile physiologies and/or functional redundancy. Although there were no changes in the sediment bacteria functional profiles, changes in the composition of microbial communities may affect the energy requirements and expenditures of the communities (Bier et al., 2015; Canedo-Argüelles et al., 2014; Nieuwdorp et al., 2014; de Ruiter et al., 1995), in which turn may affect the trophic transfer of energy in stream food webs.

Declaration of interest

All authors have seen and approved the final version of the manuscript, and warrant that the article is the author's original work, is not considered for publication elsewhere, and has not received the prior publication. Additionally, authors declare no conflicts of interest.

Contributors

The study was conceived of and designed by AR and LL. Samples were collected by AR and JVG and processed by AR. Statistical analysis was done by AR and JVG. Interpretation of data and writing of the manuscript was done by AR, JVG, and LL.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2018.01.045.

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