



# 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 m) from sites along a highly impacted stream (Tinkers Creek, Cuyahoga River watershed, Ohio, USA) and two reference streams. Although alpha ( $\alpha$ ) diversity was relatively constant spatially, multivariate analysis of bacterial community 16S rDNA profiles revealed significant spatial and temporal effects on beta ( $\beta$ ) diversity and community composition and identified a number of significant correlative 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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## 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 (PO<sub>4</sub>-P and ammonia [NH<sub>4</sub>-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

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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., 2008; 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 (Gosset 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; physicochemical 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 ( $\alpha$ -diversity) concurrently with the urban gradient and that there would be high compositional dissimilarity ( $\beta$ -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 (Boyer et al., 2011; Sliva and Williams, 2005; Zhang et al., 2012), nutrient concentrations (Dodds et al., 2002; Gessner and Chauvet, 1994; Findlay and Sinsabaugh, 2003), and streamflow (Chiaromonte et al., 2013; Fazi et al., 2013; Sliva 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 7<sup>th</sup>-order stream, drains a 250 km<sup>2</sup> 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, 4<sup>th</sup> and 3<sup>rd</sup>-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 m<sup>3</sup>/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 (NH<sub>4</sub><sup>+</sup>-N), nitrate (NO<sub>3</sub><sup>-</sup>-N), and nitrite (NO<sub>2</sub><sup>-</sup>-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 K<sub>2</sub>SO<sub>4</sub> solution (1:5 ratio [soil: 0.5M K<sub>2</sub>SO<sub>4</sub>]) (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-

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