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Indicator species characterization and removal in a detention pond in the Plaster Creek watershed

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ABSTRACT

Microbial pathogen contamination is a leading cause of impairment for urban rivers and streams in Michigan. Reports on the ability of green infrastructure best management practices to remove microbial pathogens have been highly variable. This study evaluated the influence of a detention basin (Kreiser Pond) on microbial dynamics in the Plaster Creek watershed in West Michigan. High levels of fecal indicator bacteria and coliphage were documented in influent and effluent water, with significant increases in indicator microbe concentrations during storm events. In dry conditions, Kreiser Pond efficiently reduced the number of indicator microbes flowing through the basin. Rainfall volume had a greater influence on the diversity of bacteria than sampling location. Antibiotic resistance was prevalent in culturable *E. coli* from Kreiser Pond, demonstrating a potential public health risk and highlighting the need for identifying the ultimate sources of microbial pollution.

1. Introduction

Stormwater runoff from rain events poses physical, environmental, and health hazards to urban communities. Urbanization has led to an increase in impervious surfaces and has been shown to increase chemical pollutants, toxicants, and microbes flowing from these surfaces into local waterways (Brabec et al., 2002; Falbo et al., 2013). Water volume surges can lead to erosion and high sediment levels in surface waters. An increase in stormwater runoff has also been correlated with increases in fecal indicator bacteria (FIB) in local water sources (Liao et al., 2015; Ahmed et al., 2019). FIB such as *E. coli* and other coliform bacteria may enter urban waterways from septic tank leaks, stormwater runoff, or wildlife and domestic animal waste and can pose significant health risks for communities through which the contaminated water flows (Marsalek and Rochfort, 2004). FIB are also correlated with high levels of additional human pathogens, such as viruses and protozoan parasites and large precipitation events have been associated with increases in waterborne illnesses (Curriero et al., 2001). FIB are routinely quantified in surface water as indicators of potential health impacts, and elevated FIB concentrations are a primary cause of surface water impairment in the United States ("Water Quality Assessment and TMDL Information| US EPA," 2017). Bacteriophage prevalence has also been correlated with human enteric viruses such as norovirus and human adenovirus and is another useful microbial indicator of pathogen pollution (Dias et al., 2018). Pathogen pollution is currently the third leading cause of impairment for rivers and streams in Michigan, with over nine thousand miles of rivers and streams impaired by FIB ("Michigan Water Quality Assessment Report | US EPA," n.d.). For this reason, Michigan has set a water quality standard for acceptable levels of *E. coli* in surface waters of the state, with a daily maximum for partial body contact of 1000 *E. coli* per 100 mL of water. Stormwater runoff has been shown to regularly exceed this level of *E. coli* in many of Michigan's urban waterways (Resources Division, 2019).

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Abbreviations

FIB	fecal indicator bacteria
BMPs	best management practices
ARGs	antibiotic resistance genes
US EPA	United States Environmental Protection Agency
LF	linear feet
RCP	reinforced concrete pipe
Cfs	cubic feet per second
E. coli	*Escherichia coli*
TMDL:	Total Maximum Daily Load
GIS	Geographic Information System
SAL:	Single Agar Layer
PFU	Plaque Forming Units
RCP	Reinforced Concrete Pipes
TSB	Tryptic Soy Broth

Stormwater Best Management Practices (BMPs) are increasingly being used to mitigate the negative effects of stormwater runoff in urban communities. Structural BMPs, some of which are classified as green infrastructure, are designed for a number of purposes, depending on the volume and characteristics of water flow during storm events in a specific region (Deeb et al., 2018). BMPs provide a combination of physical, chemical and biological processes that contribute to the removal or transformation of pollutants, decreasing their prevalence downstream. Significant increases in FIB have been noted during wet weather conditions compared to dry weather conditions (Liao et al., 2015; Ahmed et al., 2019). The ability of BMPs to eliminate this dangerous microbial challenge remains unclear (Clary et al., 2008; Ahmed et al., 2019). Though some studies have demonstrated that BMPs decrease the concentration of FIB (Karim et al., 2004; Karpiscak et al., 1996; Gannon et al., 2005; Kadlec and Knight, 1996; Struck et al., 2008; Davis et al., 2009), others have documented situations where BMPs act as a source, rather than a sink for FIB (Serrano and DeLorenzo, 2008; Wolfand et al., 2018; Clary et al., 2008). This variability is likely due in part to the diversity of BMP designs and may also reflect animal sources of FIB within the BMP. It has been suggested that during a storm event, turbulent water may release FIB and bacteriophage that were previously

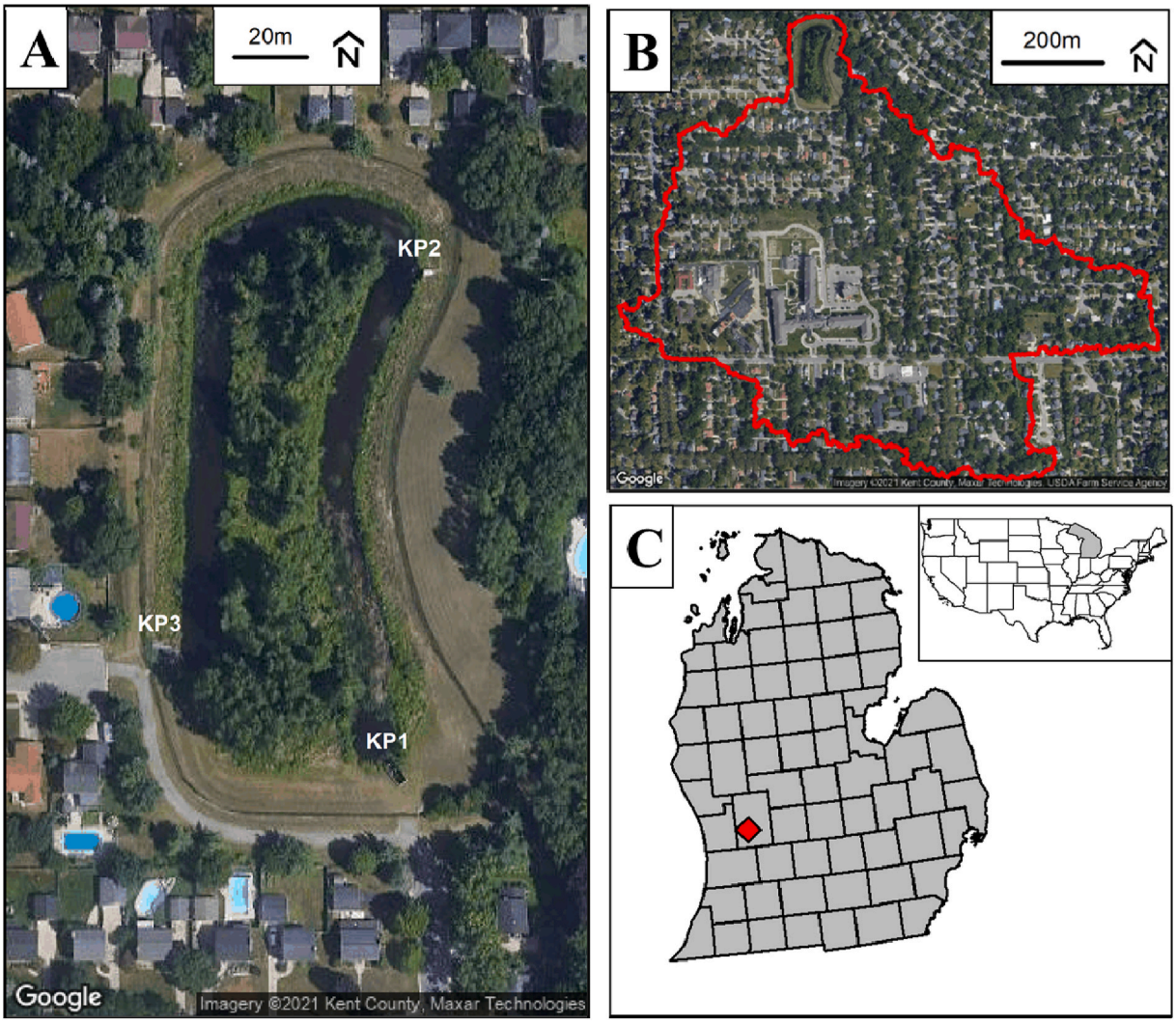

Fig. 1. Location of Kreiser pond Sampling Sites. (A) Aerial view of Kreiser pond. KP1, KP2, and KP3 represent the three locations sampled in this study. Water flow begins at KP1 from two 24″ reinforced concrete pipes (RCP). Water flows towards KP2 where more incoming water enters the pond through a single 54″ RCP. Flow moves around the horseshoe channel to the outlet structure KP3. **(B) Aerial view of the Kreiser pond drainage area.** The star denotes the location of Kreiser Pond. Land-use within the 51.42-ha drainage area is predominately low-intensity, residential development. **(C) Location of Kreiser pond in the Lower Peninsula of Michigan.** Kreiser pond is located within the city of Grand Rapids in Kent County. Michigan is highlighted in gray on the inlay map of the United States.

sequestered within BMP sediment (Struck et al, 2007, 2008, 2007; Karim et al., 2004). Indeed, elevated suspended solids are often observed during storm events and *E. coli* is frequently bound to sediment particles that can be resuspended from stream bottoms and transported downstream (Jamieson et al., 2005; Falbo et al., 2013; Krometis et al., 2007).

In addition to microbial contaminants, metagenomic analyses have identified the presence of antibiotic resistance genes (ARGs) in surface waters (Liu et al., 2019). A small number of studies have demonstrated an increase in ARG levels in urban BMP waters following storm events (Baral et al., 2018a, 2018b). These findings signal the possibility that storm events spread antibiotic resistance through environmental microbial communities, which could elevate public health risks. Monitoring FIB and ARGs is an important aspect of maintaining the health of aquatic environments, mitigating negative public health effects, and understanding the function of BMPs. However, such monitoring is not an effective way to understand the microbial community structure of an aquatic environment and may miss significant variations in the microbial milieu (Joyner et al., 2019). For example, significant restructuring of microbial communities has been noted in proximity to effluent from wastewater facilities, suggesting that water runoff from urban communities may also affect microbial community structure (Hladilek et al., 2016).

The Plaster Creek watershed in Kent County, Michigan, a roughly 58-square-mile region, contains several bodies of water listed as "contaminated" by the US EPA ("My Waterway," n.d.). Section 303(d) of the federal Clean Water Act and the United States Environmental Protection Agency's Water Quality Planning and Management Regulations require states to develop Total Maximum Daily Loads (TMDLs) for waterbodies that are not meeting Water Quality Standards (US EPA, n.d.). This was done for Plaster Creek in 2002 (Total Maximum Daily Load for Escherichia Coli in Plaster Creek, 2002). The report mentioned part of the watershed containing residential, industrial, and light commercial land that drains into Silver Creek, one of two main tributaries of Plaster Creek as a location containing four combined sewer outflows, to be eliminated by the year 2019. The majority of the approximately 4-mile waterway of Silver Creek was relocated underground in the 1940s. An in-line detention basin named Kreiser Pond was installed in the Silver Creek sub-watershed to reduce stormwater runoff volume and relieve pressure in the storm sewers during major rain events. Detention ponds are a widely used BMP for this purpose and have proven an efficient method of reducing downstream stormwater runoff, erosion and pollutant flow. However, the effect of this BMP installation on the significant microbial pollution observed in the Plaster Creek watershed had not been assessed.

We evaluated the influence of the Kreiser Pond detention basin on microbial population dynamics and community structure. Given that peak FIB concentrations are typically observed during summer months (Schnabel et al., 2010) and the public health impacts of urban stormwater runoff are of particular interest during times when water-related recreational activities are most common (Hathaway and Hunt, 2012), samples were collected from June to August in the years 2018 and 2019 (Table S1). This study provides microbial characterization of influent and effluent water collected at Kreiser Pond during both dry-weather and wet-weather conditions. *E. coli*, total coliform, F + RNA coliphage, and somatic coliphage levels were analyzed as indicators of pathogen presence. 16 S rRNA gene amplicon sequencing was used to describe the bacterial community and determine the effects of this BMP on bacterial community structure. Antibiotic resistance was explored in culturable *E. coli*. Studies like this that analyze the effect of BMPs on indicator microbe levels and the microbial community structure are important to ensure that BMP installations are not further contributing to harmful microbial watershed contamination. This work will aid in the informed planning of future BMP installations in the Plaster Creek watershed as well as other urban residential stormwater detention ponds.

2. Materials and methods

2.1. Sampling sites

Samples were collected from two inlets and one outlet of Kreiser Pond (42°56′23.5″N 85°37′15.0″W) (Fig. 1a). Inlet samples were collected directly from inlet pipes, as these were above the water level of the pond. Outlet samples were collected from water in the outlet structure of KP3. When water levels were below the level of the outlet, KP3 water samples were collected from water directly in front of the outlet structure.

2.2. Water collection

Triplicate grab samples were collected from sampling sites in 500-mL sterile polypropylene bottles and transported directly to the lab for processing. Water samples were stored at 4 °C within 1 h of collection and were processed within 24 h of collection.

2.3. GIS

The drainage area for Kreiser Pond and average surface imperviousness within the drainage area were determined using a Geographic Information System. Albers equal area conical projection was used for all geospatial data to minimize area distortion. Drainage area was estimated using the Hydrology toolset of ArcMap (version 10.4.1; ESRI, Redlands, CA, USA). Flow direction and accumulation were determined based on a 1-m resolution digital elevation model (created using 3D Elevation Program dataset; courtesy of the U.S. Geological Survey, Reston, VA, USA). The Watershed tool (NHD Watershed Tool) was used to delineate the drainage area for Kreiser Pond. The pour point was set to the point of highest flow accumulation within 15 m of the outlet structure. Percent imperviousness within the drainage area was extracted from the 2016 National Land Cover Database Percent Developed Imperviousness raster (30-m resolution; Homer et al., 2020), using the raster R package (version 3.3–13; Hijmans, 2020).

2.4. E. coli and total coliform enumeration

Samples were collected and processed for fecal coliform bacteria and *E. coli* enumeration following the protocols of the U.S. Environmental Protection Agency (Method 1604: Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium), 2002). Water samples were diluted 10, 100, and 1000 X in working solution (0.3112 mM KH2PO4, 0.1995 mM MgCl2, pH 7.2) and filtered through a sterile 0.45 μm membrane filter (Hach). Filters were incubated on MI agar for 16–20 h. *E. coli* were differentiated by color and counted. Total coliform bacteria were enumerated under UV light. A positive control (*E. coli*) and a negative control (working solution) were run before and after each set of samples processed.

2.5. Coliphage enumeration

Samples were collected and processed for coliphage enumeration following the protocols of the U.S. Environmental Protection Agency (Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure, 2007). Water samples were mixed with tryptic soy agar (TSA) containing either F^{amp} (ATCC 700891) or CN-13 (ATCC 700609) *E. coli*. Plaque forming units (PFU) were enumerated after 24 h. Negative controls (reagent grade water) and positive controls [MS2 (ATCC 15597-B1) with F^{amp} *E. coli* and PhiX174 (ATCC 13706-B1) with CN-13 *E. Coli*] were run alongside each set of samples.

2.6. Statistics

2.6.1. Wilcoxon-Mann-Whitney test

The Wilcoxon-Mann-Whitney test was used to compare microbial concentrations (Marx et al., 2016).

2.6.2. Probability plots

Probability plots were developed in R Studio (version 3.6.2, www.rstudio.com) using the empirical cumulative distribution function.

2.7. Flow model

As-built construction plans were reviewed and coupled with field survey to determine the elevation, slope, and dimensions of the inlet and outlet structures (Fig. S1). According to the as-built plans for the 2011 improvements, the main inlet to the pond, KP1 consists of 32 LF of two parallel, 24″ diameter reinforced concrete pipes (RCP) originating at a manhole and flowing to a concrete baffle structure and then to a scour pool which connects to the dredged channel (Fig. S1b, Fig. S2a, Fig. S2b). The lower east pipe is at 6.19 % slope while the west pipe is at 6.00 % slope. These steep slopes result in supercritical flow with depth approaching normal depth. Therefore, Manning's equations can be used to estimate the flow in these pipes using a measured depth of flow in the pipe. Based on a culvert analysis performed in HY-8, the culvert rating curve shows a USGS flow type of 1-S2n with normal depth at the outlet for flow rates 0 to 26 cfs with a 5-S2n USGS flow type for flows 27 cfs or greater. Manning's n coefficient was selected as 0.015 for rough concrete pipes.

There are no detailed engineering plans for the baffle inlet structure. Field survey and pictures were used to better understand and model the structure (Fig. S2b). Upon exiting the 24" RCP, water flows over a sloped section of concrete with a 10.4-inch drop to the top of the first row of baffles which are also 12-inches high, with a total of 1.87-foot drop from pipe invert to basin invert. There are two additional rows of baffles in the structure. Water must pond 1.36 feet in the basin before spilling out into the scour pool/channel.

The secondary inlet to the pond, KP2, is a 54″ RCP that conveys runoff from a manhole to the pond at a 0.2 % slope (Fig. S1b, Fig. S2c). 2018 field observations suggest that the KP2 pipe may have settled into a steeper slope due to a crack in the upstream portion of the pipe (Fig. S2d). However, when Manning's equation was used with the original slope the resulting water width was very close to field measurements. This pipe also leads to a scour pool along the channel.

The horseshoe channel is 30-feet wide with 4H:1 V side slopes (Fig. S1a). The channel is typically inundated, having a 40-foot water surface width, suggesting a depth of 1.25 feet. It follows the outer side of the basin, creating a 960-foot-long flow path and has an elevated area in the center of the pond. The horseshoe channel conveys runoff from KP1 and KP2 to the outlet, KP3.

The outlet structure is located above the channel, meaning the channel must be full of flow to begin to allow any runoff out of the pond through the outlet structure (Fig. S1c, Fig. S2e). Flow leaves the basin in the 42″ pipe. The 42" RCP is sloped upwards at 0.19 % for 177 LF until it reaches a catch basin located on Kreiser Avenue where it is then conveyed at a downward slope of 0.6 %. The water surface elevation of the pond controls the amount of flow leaving the pond.

The system configuration and measured depth of flow in the inlet pipes were used to determine a flow rate entering the pond at KP1 and KP2. By measuring both the depth and top width, we could verify the as-built dimensions and determine the manning's n that best fit the data. These initial estimates were used to calibrate the HEC-RAS model for the entire system in a one-dimensional steady flow analysis instead of isolated pipes. This approach was critical because the flow rate leaving the pond is controlled by water surface elevation as the outlet pipe flows slightly uphill and could have a tailwater effect on the inlet pipes. In all cases, depth of flow at KP1 resulted in less than 26 cfs so Manning's equation was valid for headwater conditions.

Table 1
Indicator Microbe Reductions Under Dry and Wet Conditions. Concentrations of indicator microbes were measured in influent and effluent water under dry and wet weather conditions. The percent reduction from influent to effluent water was calculated for inlet and for each date due to high variability. For each inlet, the percent reduction was calculated for each indicator microbe (see Tables S2-5). For each indicator microbe, sampling dates were scored as either a positive or negative reduction. A positive reduction was a date where there was a positive reduction for both KP1 and KP2. *For coliphage, the percent reduction could not be calculated on most dry days due to low numbers. See Table S4 and Table S5.

INDICATOR MICROBE	Dry condition dates with positive reduction	Wet condition dates with positive reduction
E. COLI (CFU/100 ML)	2/5	0/5
TOTAL COLIFORM BACTERIA (CFU/100 ML)	1/5	1/5
F + RNA COLIPHAGE (PFU/100 ML)*	2/6	1/6
SOMATIC COLIPHAGE (PFU/100 ML)*	0/6	2/6

Table 2
Estimated flow rates of influent and effluent water on sampling dates. The Kreiser inlet, channel, and outlet configuration was modeled on HEC-RAS. Measured flow depths at KP1, KP2, and KP3 were used to calibrate the model and establish a rating curve for the flow at each location. *Flow at KP2 on October 6, 2019 is out of the range of expected flow due to high water levels and was excluded from statistical analyses.

DATE	LOCATION	WIDTH (CM)	DEPTH (CM)	HEC-RAS ESTIMATED FLOWS (CFS)
April 6, 2019	KP1	21	**1.5**	0.12
October 6, 2019	KP1	24	**3**	0.44
6/17/2019	KP1	21.5	**2.5**	0.21
April 6, 2019	KP2	80	**12**	23
October 6, 2019*	KP2	131*	**41***	22.2*
6/17/2019	KP2	55	**8**	1
April 6, 2019	KP3	58	**7**	0.000011
October 6, 2019	KP3	80	**18**	0.21
6/17/2019	KP3	38	**3.5**	0.00001

The depth of flow in the 54" pipe at KP2 on June 10, 2019 was too high to make sense with the model. Field observations suggest that the water level in Kreiser Pond was above the level of the inlet pipe at KP2 on this date, confounding flow measurements. This date was excluded from flow model analysis. Evapotranspiration and infiltration reduce the quantity of water in the pond resulting in little to no flow leaving the basin through the outlet pipes in dry weather. The resulting flow rates at each of the three collection points are listed in Table 2.

2.8. Sample preparation for metagenomic sequencing

Triplicate 100 mL water samples were filtered through a sterile 0.45 μm membrane filter (Hach). Filters were fragmented with sterilized scissors. Genomic DNA was isolated from filters using the E. Z.N.A.® Water DNA Kit (Omega Biosciences). 16sRNA gene hypervariable V4 regions were amplified using barcoded 515 F-806 R primers (Caporaso et al., 2011) using DreamTaq DNA Polymerase Mastermix (Thermo Fisher Scientific). 515 F: **ACACTGACGACATGGTTCTACA**GTGCCAGCMGCCGCGGTAA and 806 R: **TACGGTAGCAGAGACTTGGTCT**GGACTACHVGGGTWTCTAAT (bold regions indicate

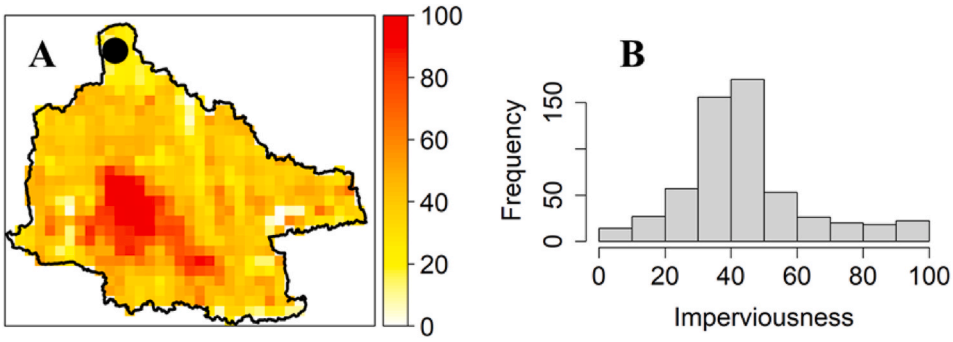

Fig. 2. Characterization of Kreiser pond drainage area. (A) Percent imperviousness within the Kreiser pond drainage area. The black dot denotes the location of Kreiser Pond. Percent imperviousness is summarized at a 30-m spatial resolution. **(B) Histogram of percent imperviousness values extracted within the Kreiser pond drainage area.**

sequence adapters). PCR products were purified using the HighPrep™ PCR Clean-up System (MagBio Genomics) and quantified using the Quant-iT™ dsDNA Assay Kit (Thermo Fisher Scientific).

2.9. Metagenomic analysis

Libraries were prepared from primary PCR products by performing secondary PCR using dual indexed, illumina compatible primers targeting the Fluidigm CS1/CS2 universal oligomers at the ends of the primary PCR products. Complete libraries were batch normalized using a SequalPrep DNA Normalization plate (Invitrogen) and pooled. The pool of completed libraries was QC'd and quantified and then sequenced using an Illumina MiSeq v2 Standard flow cell and reagent cartridge. Raw fastq files from the MiSeq instrument were demultiplexed and processed using the QIIME 2 Core 2018.11 distribution (Bolyen et al., 2019). In brief, DADA2 (Callahan et al., 2016) (dada2 denoise-paired) was used to trim, quality filter and denoise sequences, remove chimeric sequences and obtain ASVs. Taxonomic assignment of each ASV was performed using a Naïve Bayes classifier (Werner et al., 2012) pre-trained on the Greengenes 16 S rRNA gene reference database (version 13_8). Unassigned sequences or sequences identified as plant chloroplast or mitochondria were removed. Diversity analyses were performed within QIIME 2. Samples were rarified to 37,502 reads for calculating alpha diversity metrics, the highest number of read which retained all samples. The entire sequence analysis workflow is available on GitHub (https://github.com/bradleycp/Kreiser-Pond). Raw source 16 S rRNA gene sequences from this project are available in the Sequence Read Archive database under BioProject PRJNA717986, accession numbers SRR14086939 to SRR14087008.

2.10. Kirby-Bauer assay

Individual *E. coli* colonies were selected and grown overnight in TSB medium at 37 °C. A lawn of each *E. coli* isolate was grown on Mueller-Hinton agar in the presence of antibiotic discs (BD Diagnostic Systems) containing 10 μg ampicillin, 30 μg tetracycline, 10 μg streptomycin, 5 μg ciprofloxacin, or 30 μg doxycycline for 16–18 h at 37 °C. Zone of inhibition diameters were measured and isolates were classified as susceptible, intermediate, or resistant based on published values (Clinical and Laboratory Standards Institute (CLSI), 2017).

3. Results and discussion

3.1. Characterization of the Kreiser Pond drainage area

Kreiser Pond is owned and managed by the Kent County, Michigan Drain Commission and is the first pond in a series of regional detention ponds located within the Silver Creek-Hall Drain (Fig. 1c). Originally designed as a typical detention pond, it has two inlet locations, one outlet, and serves to reduce stormwater runoff peaks and relieve pressure in the receiving pipes. In 2011, construction plans were drafted to modify the pond to further reduce stormwater runoff and prevent downstream flooding (Fig. S1). Among other improvements, these plans included adding stilling basins at the outlets of the inlet pipes, a serpentine channel, and a staged discharge outlet structure (Fig. 1a, Fig. S1). The drainage area for Kreiser Pond, estimated from surface elevation data, is 51.42 ha (Fig. 1b). Average imperviousness within the drainage area was 44.57 % (standard deviation = 18.69 %; Fig. 2), indicating a predominate pattern of low-intensity development within the drainage area consistent with residential land-use (Homer et al., 2020).

3.2. Enumeration of fecal indicator bacteria and bacteriophage

Water sampling from Kreiser Pond was performed on fourteen separate occasions from June 2018 to August 2019 (Table S1) at each of three locations: two inlets (KP1 and KP2, Fig. 1b) and one outlet (KP3, Fig. 1b) in dry and wet conditions. We defined a dry sample as a sample collected within a 48-h period after 0.1 inches of rain or less. A wet sample was defined as a sample collected within a 48-h period receiving at least 0.2 inches of rain (Table S1). Mean concentrations of FIB (Fig. S3; Tables S2 and S3) and coliphage (Fig. S4; Tables S4 and S5) were calculated after quantification of triplicate samples. High levels of FIB were observed in most samples, with *E. coli* concentration exceeding the Michigan recreational-water quality standard in 29 of 30 samples (Fig. S3). The relationship between amount of rainfall and concentration of indicator microbes was investigated. Using the Wilcoxon-Mann-Whitney test, we determined that samples collected on dry days ($n = 42$) contained significantly fewer culturable indicator microbes than samples collected on wet days ($n = 42$) across all sampling sites ($p = 5.77^{e-25}$ for *E. coli*, $p = 4.47^{e-17}$ for total coliform bacteria, $p = 1.57^{e-12}$ for male-specific coliphage, $p = 7.90^{e-12}$ for somatic coliphage) (Marx et al., 2016). This stormwater-driven increase in indicator microbes has been demonstrated for other green infrastructure installations (Krometis et al., 2007; Liao et al., 2015; Marsalek and Rochfort, 2004; Wilkes et al., 2009). The difference may be due to increased pollution runoff from the urban environment, microbial resuspension due to turbulent flow, or a combination of both factors (Ahmed et al., 2019; Falbo et al., 2013; Struck et al., 2008). Total indicator microbe concentrations did not vary significantly between 2018 and 2019 (p value > 0.05, data not shown).

3.3. Microbial reduction efficiency of Kreiser Pond

We calculated the mean microbe concentration of influent samples (KP1 and KP2) and the mean of effluent samples (KP3) for each date and calculated the removal efficiency for each date, microbe, and inlet (Tables S2-S5). We used these calculations to determine the dates with an overall positive reduction (positive reduction seen for both KP1 and

Table 3
Indicator Microbe Reductions Under Dry Conditions. An indicator microbe flow rate was calculated for each location and date analyzed from triplicate samples. KP1 and KP2 measurements were added to calculate an influent microbe flow rate. The geometric means of influent and effluent measurements for the two dates analyzed were used to calculate the percent reduction in the microbial flow rate from the influent to effluent sample sites.

INDICATOR MICROBE	Influent Mean	Effluent Mean	Reduction (%)
E. COLI (CFU/S) April 6, 2019	1,971,820.9 ± 330,886.2	6.0 ± 0.3	**>99.9**
E. COLI (CFU/S) 6/17/2019	235,067.9 ± 38,275.0	2.2 ± 0.4	**>99.9**
TOTAL COLIFORM (CFU/S) April 6, 2019	10,657,673.6 ± 4,855,256.9	1225.6 ± 29.4	**>99.9**
TOTAL COLIFORM (CFU/S) 6/17/2019	6,269,701.6 ± 2,276,657.0	23.2 ± 4.4	**>99.9**
F + RNA COLIPHAGE (PFU/S) April 6, 2019	0.0 ± 0.0	0.0 ± 0.0	N/A
F + RNA COLIPHAGE (PFU/S) 6/17/2019	14.4 ± 28.0	0.003 ± 0.004	**>99.9**
SOMATIC COLIPHAGE (PFU/S) April 6, 2019	147,457.0 ± 21,094.9	2.9 ± 0.4	**>99.9**
SOMATIC COLIPHAGE (PFU/S) 6/17/2019	37,293.6 ± 10,177.0	0.4 ± 0.1	**>99.9**

KP2, Table 1). Our results suggest that in Kreiser Pond, there is no significant reduction in indicator microbe concentrations between the influent water and the effluent water. Rather, the concentration of some indicator microbes increased in effluent water compared to influent water under wet conditions. This is most striking for FIB, as demonstrated by probability plots showing concentrations of *E. coli* and FIB (Fig. S5) and F + RNA and somatic coliphage (Fig. S6) in samples from an inlet or samples from the outlet of Kreiser Pond. Wet ponds, like Kreiser Pond, have shown varied levels of removal of indicator microbes, with some demonstrating poor performance and negative concentration reductions (Krometis et al., 2007) and others demonstrating significant removal (Gannon et al., 2005; Hathaway and Hunt, 2012). For detention basins, an increase in effluent FIB has been documented (Clary et al., 2008). Our observations of FIB concentrations in Kreiser Pond supports the conclusion that detention ponds have low effectiveness in reducing the concentration of bacteria. Possible contributing factors include increased temperature in pooled water, nutrient runoff from residential lawn fertilizers, septic system leakage, and pet and wildlife waste runoff. Increased levels of these contributors have been correlated with increased concentrations of FIB (Serrano and DeLorenzo, 2008; Wolfand et al., 2018). In Kreiser Pond we observed water temperatures ranging from 14 to 20 °C, which did not exceed the Michigan water quality standards (Michigan Water Quality Assessment Report). We observed many waterfowl resident in the pond and given the predominantly residential land use (Fig. 2), we can speculate that nutrient runoff from lawns may create an environment in Kreiser Pond that is conducive to bacterial growth. Since sedimentation has been suggested as a primary mechanism by which green infrastructure reduces FIB concentrations (Karim et al., 2004), turbidity caused by stormwater surges may be an important mechanism for increasing effluent indicator microbe concentrations in wet conditions. Future directions involve measuring water turbidity, temperature, and nitrogen levels during storm surges to determine if increases in these factors correlate with the observed increase in FIB.

3.4. Flow modeling for microbial reduction calculations

While no significant reduction in the concentration of indicator microbes was observed for Kreiser Pond, on many of the sampling dates the water level in the pond was low and very little effluent water was leaving the pond at KP3 (Fig. S2f, Fig. S2g). Therefore, while water containing indicator microbes was flowing into Kreiser Pond, negligible microbial contamination was carried downstream, out of Kreiser Pond. We expected that this reduction in flow may lead to significant reductions in indicator microbe numbers between influent and effluent water, even when the concentration of indicator microbes did not significantly change. To analyze this, the depth and width of the water in the pipes flowing into Kreiser Pond at KP1 and KP2 and flowing out of Kreiser Pond at KP3 were measured for all sampling dates in June 2019. This information was coupled with the 2011 as-built engineering design plans (Fig. S1) for the modifications of Kreiser Pond and used to estimate a flow rate at KP1, KP2, and KP3 for each sampling date (Table 2). Due to pooling water in the inlet, we were unable to accurately measure the flow rate for KP2 on 6/10/19 and this data point was excluded from statistical analysis. We used the calculated estimated water flow rates at KP1, KP2, and KP3 to transform our concentration data to a flow rate of indicator microbes per second for 6/4/19 and 6/17/19. The geometric means of microbial flow rates at KP1 and KP2 were added together to calculate the total flow of microbes in Kreiser Pond per second. We then calculated the reduction in microbes per second entering or leaving Kreiser Pond in dry conditions (Table 3). When considering the differential flow rates in influent and effluent water in Kreiser Pond, the overall microbial reduction is significant in dry conditions. This result suggests that detention ponds may significantly decrease the number of microbes in effluent water even in the absence of a significant reduction in microbial concentration. Preliminary work suggests that the flow rate differential between inlets and the outlet of Kreiser Pond is not significant during wet conditions. We would expect reduction efficiency to decrease or even disappear when the flow rates do not differ between the inlets and the outlet.

3.5. 16 S rRNA gene-based metagenomic analysis of Kreiser Pond microbial communities

While analyzing indicator microbe numbers can inform us of the capability of Kreiser Pond to sequester and remove these microbes, we also wanted to investigate whether this detention pond influences the composition of the resident bacterial community. Analysis of the microbial communities associated with green infrastructure is necessary to fully understand the extent to which human activity has influenced the aquatic and soil environments of green infrastructure installations (Hladilek et al., 2016; Joyner et al., 2019). To this end, we isolated genomic DNA from influent (KP1 and KP2) and effluent (KP3) water on each sampling day and performed metagenomic analyses. Taxonomic analysis of the V4 16 S rRNA gene amplicon reads yielded a total of 4,418,841 classifiable bacterial classes for samples from KP1, KP2, and KP3 (Fig. 3a). The families *Comamonadaceae (Hydrogenophaga* was the most common genus) and *Flavobacteriaceae* (*Flavobacterium* was the most common genus) were the most common families and were present in all Kreiser Pond samples. These families and genera are commonly found in the soil and sludge of aquatic environments (Jooste and Hugo, 1999; Willems et al., 1989). The family *Enterobacteriaceae*, which contains species of interest to public health such as *E. coli* and *K. pneumoniae*, is the third most abundant family observed in Kreiser Pond samples. Interestingly, this group appears to be more enriched in samples from KP1 than from KP2 or KP3, which may suggest that potentially pathogenic bacterial contamination is entering Kreiser Pond principally from KP1. This finding may aid in the determination of contamination sources. Another notable family present in these waters is the *Rhodocyclaciae*, which is known to be dominant in activated sludge samples from wastewater treatment facilities and can enhance water treatment with several functions, including nitrogen fixation and biological phosphorus removal (Wang et al., 2020). The overall composition of bacteria in Kreiser Pond is similar when comparing influent and effluent water. However, an enrichment is seen in certain bacterial orders in KP3 compared to KP1 or KP2. For example, there is an enrichment of *Verrucomicrobiae* in KP3. *Verrucomicrobiae* are ubiquitous in soil and may demonstrate effluent bacteria emerging from the Kreiser Pond

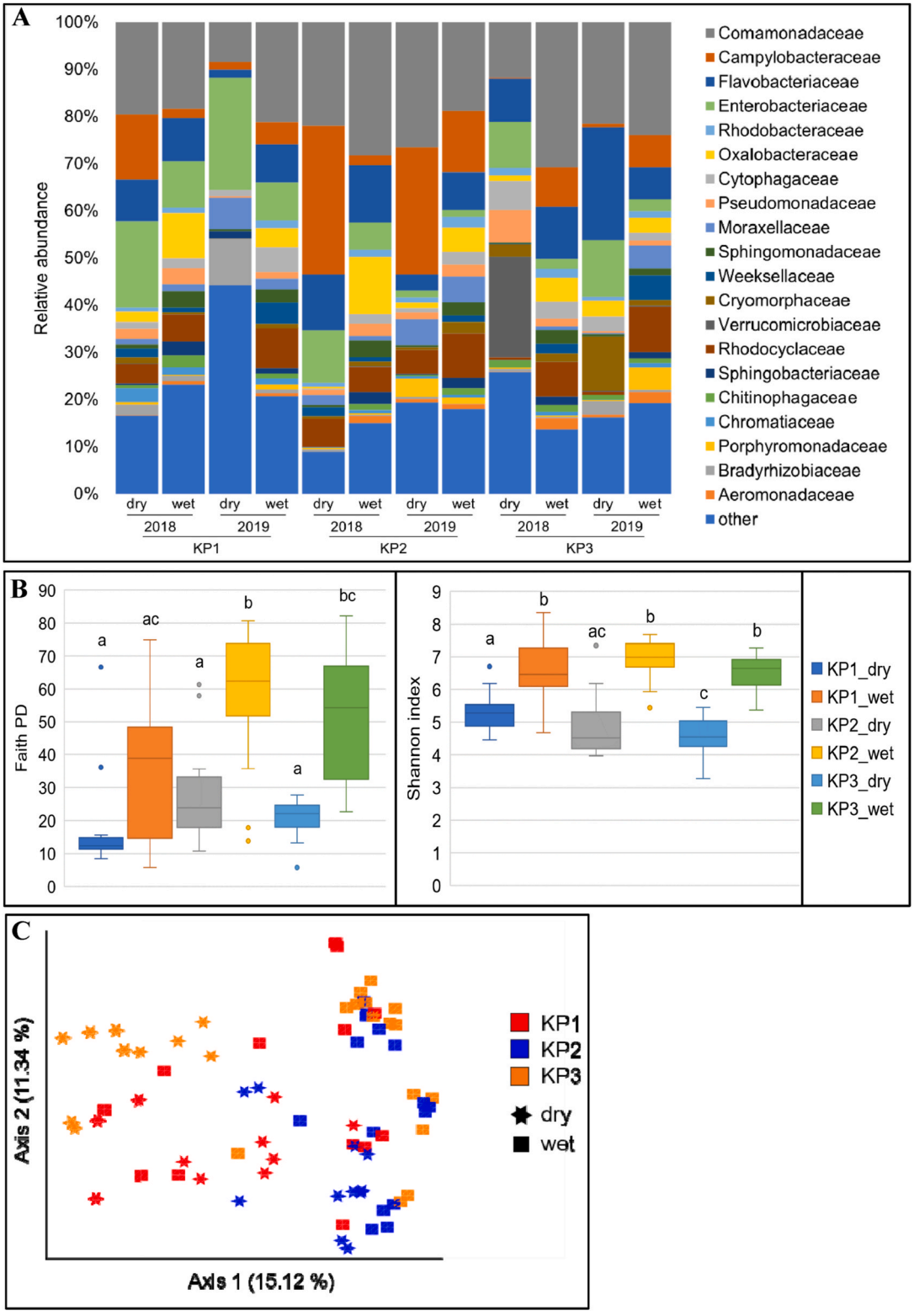

Fig. 3. 16S rRNA gene-based metagenomic Analysis of Kreiser pond Samples. Genomic DNA was isolated from Kreiser pond water samples and was used for metagenomic analysis of the V3 16s RNA genomic region. **(A) The relative abundance of bacteria at the family level in influent and effluent water samples collected at Kreiser pond.** Top 20 families are displayed. A Taxonomic bar plot of the bacteria present in Kreiser pond suggests that sampling environment (dry/wet) influences the microbial community to a greater extent than sampling location. **(B) Shannon Diversity and Faith's Phylogenetic Diversity indices were calculated for Kreiser Pond water samples.** Center line represents the median and box edges show the first and third quartiles. Whiskers extend to 1.5x IQR from the quartiles. Kruskal-Wallis test, different letters indicate significant difference ($p < 0.05$). **(C) Principle coordinate analysis generated using Bray-Curtis dissimilarity distances.** Samples are color coded based on location within Kreiser Pond. Shapes differentiate wet and dry samples. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Antibiotic resistance in culturable *E. coli* from Kreiser Pond. *E. coli* colonies isolated from 2019 water samples were cultured and tested for antibiotic resistance using the Kirby-Bauer method. More than 86 % of cultured *E. coli* were resistant to ampicillin. Resistance to tetracycline, streptomycin, ciprofloxacin, and doxycycline was less prevalent but still present.

Resistance profile	ANTIBIOTIC				
	Ampicillin	Tetracycline	Streptomycin	Ciprofloxacin	Doxycycline
RESISTANT	86.05 %	11.63 %	4.65 %	2.33 %	4.65 %
INTERMEDIATE	4.65 %	44.19 %	25.58 %	9.30 %	4.65 %
SUSCEPTIBLE	9.30 %	44.19 %	69.77 %	88.37 %	90.70 %

environment itself rather than from influent water (Bergmann et al., 2011). Precipitation prior to sample collection had a strong effect on bacterial diversity at all three collection sites within Kreiser Pond. Whether the sample was grabbed in wet or dry conditions influenced the microbial composition to a greater extent than sampling location (Fig. 3b). Comparing within wet or dry sample types, there was no significant difference in the Shannon diversity index across sample locations. Faith's phylogenetic diversity also demonstrated similarity among sampling locations when similar sample types are compared, suggesting that Kreiser Pond does not significantly influence the composition of the bacterial community present (Fig. 3b). Wet samples, on the other hand, have increased diversity compared to dry samples across all locations, the increase in bacterial diversity seen in wet samples could be the result of unique bacterial populations entering Kreiser Pond during precipitation events (Fig. 3b). Analysis of dissimilarity using a Bray-Curtis plot demonstrates that some groups do cluster by sampling site (Fig. 3c). This suggests that there are some unique bacterial phylogenies that differentiate the sampling sites.

3.6. Antibiotic resistance in the E. Coli population of Kreiser Pond

Widespread antibiotic resistance has been noted in bacterial communities of urban waterways (Garner et al., 2017; Baral et al., 2018a, 2018b). We sought to determine if the bacteria present in influent and effluent water from Kreiser Pond contained antibiotic resistant *E. coli*. We focused on *E. coli* because of its relevance for public health (Marathe et al., 2013; Park et al., 2018). We cultured isolated *E. coli* and used a Kirby Bauer assay to analyze susceptibility to common broad-spectrum antibiotics (Table 4). We found widespread resistance to ampicillin and at least some resistance to each of the antibiotics analyzed. 38 % of isolated *E. coli* showed resistance to multiple antibiotics and another 38 % that were resistant to one antibiotic showed an intermediate resistance phenotype in the presence of another. The occurrence of multidrug resistant *E. coli* in retention ponds increases the risk of infection for both animals and humans that contact contaminated surface water and may also contribute to the spread of multidrug resistance in the environmental microbial community (Barguigua et al., 2019; Leonard et al., 2018; Liu et al., 2013). While the number of isolates analyzed in this study is limited (n = 7 from each inlet and outlet), the high degree of antibiotic resistance observed indicates extensive prevalence of multidrug resistant pathogenic bacteria in this urban watershed. Further avenues for investigation include a genetic analysis of ARGs present in coliform isolates from Kreiser Pond. Genetic analysis has been used to elucidate the genetic basis for antibiotic resistance in surface water microbes as well as the likelihood of microbial transfer of ARGs within the microbial community (Baral et al., 2018a, 2018b, 2018b; Liu et al., 2013; Park et al., 2018). A more thorough analysis is needed in order to understand if antibiotic-resistant bacteria are entering Kreiser Pond via human or animal fecal contamination, urban water runoff, stormwater surges or other unidentified sources, and whether ARGs are being transferred among the microbial populations of Kreiser Pond. Increases in bacterial load seen during wet weather conditions have been correlated with increases in the prevalence of ARGs present in the bacterial population (Baral et al., 2018a, 2018b, 2018b; Garner et al., 2017), suggesting that wet weather conditions, in which we have demonstrated a significant increase in coliform bacteria in Kreiser Pond, may contribute to increased prevalence of antibiotic resistance. The presence of environmental multi-drug resistant *E. coli* can have negative effects on the health of nearby human populations (Zhang et al., 2020). It would be beneficial to better understand the impact of the presence of multidrug-resistant *E. coli* on the health of the human population that interacts with surface waters in the Plaster Creek watershed.

4. Conclusions

- Runoff from residential land that forms the drainage basin of Kreiser Pond contains high levels of indicator microbes. The sources of this contamination are currently unknown, although one of the inlet sites consistently contributed higher concentrations of microbes than the other.
- The ability of detention ponds to affect indicator microbe levels is variable. Kreiser Pond detention basin does not significantly impact the concentration of indicator microbes when comparing influent water to effluent water.
- Kreiser Pond decreases the flow of water from influent to effluent locations. Combining flow rate data with measured indicator microbe concentrations demonstrated a significant reduction in *E. coli*, FIB, F + RNA coliphage, and somatic coliphage in dry conditions. We advise that both the flow rate and microbe concentration should be measured to have an accurate assessment of the performance of a detention pond with respect to indicator microbe removal.
- Samples collected in wet conditions have a different microbial profile and increased microbial diversity compared to samples collected under dry conditions. Sampling conditions have a greater influence on the microbial populations than sampling location based on 16 S rRNA gene-based metagenomic analysis.
- Residential runoff flowing into Kreiser Pond contains *E. coli* that are resistant to several common antibiotics. This degree of resistance may pose a serious public health threat to surrounding communities. Sources of antibiotic resistant *E. coli* should be elucidated.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2021.113503.

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