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Philips O. Akinwole
DePauw University

Madeline C. Draper

Amerti Guta

Mahaila J. Martin

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Evaluation of sedimentary bacterial community dynamics and contamination assessment of lower Des Plaines River

Philips O. Akinwole^{a,*}, Madeline C. Draper^a, Amerti Guta^a, Mahaila J. Martin^a, Kenneth L. Brown^b

^a Biology Department, DePauw University, Greencastle, IN 46135, United States

^b Department of Geosciences, DePauw University, Greencastle, IN 46135, United States

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ABSTRACT

Urban rivers often contain elevated concentrations of contaminants such as organic pollutants and heavy metals which can be amplified in lotic ecosystems receiving effluents from wastewater treatment plants (WWTP). However, the impact of WWTPs on the microbial parameters of the urban river sediments has not been well documented compared to urban surface water. Collecting sediment samples at five different locations over a 9,000 m transect during four sampling periods, we investigated spatiotemporal variations of microbial parameters in sediments of lower Des Plaines River; the largest effluent-dominated stream in the United States and the effects of sediment physicochemical properties on the variations were explored. We reported reduced microbial biomass, CFUs, and distinct bacterial communities at the WWTP outfall compared to other sites, indicating that WWTP effluents have the potential to moderate bacterial community structure. Seasonal variations in the sedimentary bacterial community structure were evident regardless of the spatial variations imposed by the effluents. Our community-level physiological profiling of the sedimentary bacterial community structure indicated that temperature was more important than water chemistry, whereas total microbial biomass by phospholipid phosphate analysis responded to the influences of both temperature and water chemistry. Metal concentrations showed values that fall within the “fair” to “very poor” range of biological conditions outlined by the Midwest Biodiversity Institute. We posit that the increased sediment metal loads select for metal-tolerant microorganisms that help to maintain microbial biomass. In the spring, sequencing of bacterial 16S rRNA genes revealed significant effects of effluent on bacterial community composition at the WWTP outfall, showing increases in abundances of *Caldilinea*, *Candidatus*, *Allochromatium*, *Sulfuritalea*, and *Nitrospira* sequences, linked to anthropogenic inputs from WWTP effluents. Given that human dependence on effluent-dominated ecosystems for water resources will increase with rapidly increasing urbanization, studies focusing on remediation and policy changes are dire to develop effective management of existing urban rivers.

1. Introduction

Rivers that flow through urban areas often serve as a major sink for stormwater runoff and wastewater effluents, which may contain large amounts of heavy metals, organic pollutants, and nutrients that alter the physico-chemical and biological parameters of the surface water and sediments (Wang et al., 2018). Wastewater effluents-laden rivers are amongst the most severe examples of ecosystems disturbed by anthropogenic activities and have the potential to contribute to biotic homogenization (Drury et al., 2013). Many urban rivers that flow through larger cities often receive effluents from wastewater treatment plants (WWTP), and generally, WWTP effluents make up a significant proportion of the flow of such urban rivers (Brooks et al., 2006; Drury et al.,

2013). For instance, the lower Des Plaines River and accompanying upstream Chicago waterway is the largest effluent-dominated stream in the United States, and the WWTP effluents represent a significant component (more than 90% during low-flow conditions) of the entire flow (Novotny et al., 2007). Therefore, water quality and microbial parameters of effluent-dominated urban streams might comparatively be significantly different from regular stream conditions upstream of the WWTP outfall or nearby reference sites.

Several studies have documented the potential effects of WWTP effluents on the receiving lotic ecosystems such as increased nutrient loading and eutrophication (Gucker et al., 2006), increased heavy metal contents and pharmaceuticals (Cébron et al., 2004; Sekabira et al., 2010; Sabri et al., 2020), and shown to moderate microbial community

* Corresponding author.

E-mail address: philipsakinwole@depauw.edu (P.O. Akinwole).

structure and metabolic profiles (Wakelin et al., 2008; Tiquia 2010; Akinwale et al., 2021). Effluent discharge can also reduce nutrient-retention efficiencies of streams (Haggard et al., 2001), and deposit sand and grit into the receiving lotic ecosystems (Wakelin et al., 2008). More studies have examined the effects of wastewater effluent on microbial parameters within the water column (Goñi-Urriza et al., 1999; Cébron et al., 2004; Lofton et al., 2007; Tiquia 2010). However, studies that have examined the potential effects of WWTP effluent on sedimentary microbial communities of urban rivers are limited (Wakelin et al., 2008; Drury et al., 2013) despite the economic uses of urban rivers (Novotny et al., 2007) and even though sedimentary microbial communities are ubiquitous essential components of lotic ecosystems.

Microbial communities are essential components of ecosystems and play vital roles in global biogeochemical cycling, (Fierer et al., 2007; Kent et al., 2007; Wang et al., 2018; Akinwale et al., 2021) thereby contributing to the maintenance of the health and balance of ecosystems (Kent et al., 2007), thus, analysis of microbial community structure and biomass can be used to study the effects of pollutants and disturbances. For instance, Lofton et al. (2007) documented a significant increase in bacterial-mediated denitrification rates in samples collected downstream of a WWTP in an urban river. Akinwale et al. (2021) reported that microbial biomass was significantly higher at the WWTP effluents discharge site compared to upstream and downstream sites, and Wakelin et al. (2008) reported the impacts of the WWTP effluents on microbial parameters for more than 1 km downstream. Thus, the sensitivity of microbial parameters to perturbations ensures its usefulness in the assessment of ecosystem integrity (Kent et al., 2007). Besides, an urban stream is a major sink for discharged wastewater, which may contain high heavy metals contents and other organic pollutants, altering the water chemistry and sediments and further reshaping the microbial community structures (Wang et al., 2018). For example, studies have reported a high concentration of heavy metals such as Zn, Cu, Pb, and Cd in urban rivers globally (Xu et al., 2017; Xia et al., 2020). Some heavy metals are required nutrients and essential, thus, play an integral role in the life processes of microorganisms. For example, copper, iron, nickel, and zinc are involved in redox processes or as metalloenzymes. However, silver, aluminum, cadmium, gold, lead, mercury, and titanium have no biological role and are nonessential (Bruins et al., 2000). Heavy metals at high concentrations are toxic to microorganisms (Nies, 1999), and as a consequence of rapid industrialization, urbanization, and poor land management, heavy metals represent a major source of pollution for the aquatic environment (Ge et al., 2021). Hence, it is critical to evaluate the effects of heavy metals on microbial community structures and to what extent microorganisms adapt to polluted urban rivers which may facilitate choosing suitable remediation and eco-friendly management strategies.

We hypothesized that the addition of anthropogenic point source pollutants associated with urbanization is a major driving force altering the microbial communities and adaptation in the sediment of urban rivers. In this study, the Des Plaines River (the lower section) which is the largest effluent-dominated stream in the United States impacted by urbanization and several wastewater treatment plants, was selected as an example to illustrate the spatiotemporal variations of bacterial communities in urban rivers. Bacterial communities in sediments of the lower Des Plaines River were evaluated by community-level physiological profiling (CLPP) and 16S rRNA gene-based high-throughput sequencing. Total microbial biomass was measured by phospholipid phosphate determination. The objectives of the study were to (i) evaluate the spatial and temporal variations in sedimentary bacterial communities along the lower Des Plaines River; (ii) clarify the effects of heavy metals and major environmental factors on the dynamics of microbial communities and biomass; and (iii) assess the response of microbial communities to anthropogenic and natural factors. Five sites along the lower Des Plaines River were sampled to reflect spatiotemporal differences in the context of chlorinated wastewater effluents discharged into the receiving water.

2. Materials and methods

2.1. Site description and sediment sampling

As the largest effluent-dominated river in the United States, the Des Plaines River (Chicago, IL) receives wastewater effluents from a large number of wastewater treatment and other industrial treatment facilities (Novotny et al., 2007). The lower Des Plaines River receives treated effluents from the Metropolitan Water Reclamation District of Greater Chicago (9.5 million inhabitants) via the Chicago Sanitary and Ship Canal. Additionally, at the sites of sampling, the lower Des Plaines River receives chlorinated effluents from the DuPage County Knollwood Wastewater Treatment Plant (WWTP). As a busy navigable waterway, the lower Des Plaines River supports considerable commercial uses, landscape irrigation, and recreational boating traffic (Novotny et al., 2007). The river runs primarily south for 110 miles in Illinois (USA) where it joins the Kankakee River to form the Illinois River.

Sites were chosen above and below Knollwood WWTP to identify spatial patterns in contaminant impact; additionally, sites were analyzed seasonally to evaluate temporal trends. Sediment samples were collected on July 21, 2020 (Summer), October 30, 2020 (Fall), January 22, 2021 (Winter), and June 12, 2021 (Spring), at five stations along an approximately 9100 m-transect of the lower Des Plaines River. Triplicate sediment samples were collected at two locations upstream of the WWTP outfall, one at the WWTP outfall, and two locations downstream of the WWTP outfall, as described below. Samples were collected at (1) Columbia Woods Canoe landing (CWCL), within the Columbia Woods forest preserve, which was located approximately 4500 m upstream of the outfall (inferred as an undisturbed reference site with respect to the outfall); (2) a location approximately 150 m upstream of the outfall referred to as KWWU; (3) a location downstream at the WWTP outlet pipe (i.e. ~100 m near the meeting of the WWTP effluents and the Des Plaines River) referred to as KWWD; (4) a location about 1500 m downstream of WWTP referred to as Bird Viewing (BV) located within the Waterfall Glen forest preserve; and (5) a location approximately 4800 m downstream of the WWTP at Canal Bank Road (CBR) by the Lakes & Rivers Contracting company (Lemont, IL), which specializes in marine and heavy construction projects (Supplementary File Fig. S1). Along our sampling transect, the dominant tree species are silver maple (*Acer saccharinum*), common buckthorn (*Rhamnus cathartica*), quaking aspen (*Populus tremuloides*), Ash trees (*Fraxinus*), and shrubs and herbs including late boneset (*Eupatorium serotinum*), Virginia creeper (*Parthenocissus quinquefolia*), pale smartweed (*Persicaria lapathifolia*), reed canary grass (*Phalaris arundinacea*), etc. The river channel was largely clear of riparian tree shade, however, abundant, dense filamentous algal streamers were observed along the sampling transect during the Spring sampling period. In addition, Des Plaines was ice-covered along our sampling transect in winter when the temperature was at or near the freezing point.

At each location, four sediment samples (approx. 10 g each) were collected using a stainless-steel scoop. The four samples were amalgamated to form a single composite sample that reflects the average sediment at that location. Sediments were collected anywhere from 1.5 to 2 m distance from the riverbank and approximately at 0.3 m sampling depth. Sediments were collected by scraping the top 2 mm that were not washed down as they were lifted from the stream with minimum disturbance. Samples were then transferred with a sterile spatula to pre-labeled Whirl-Pak sampling bags and stored on ice until delivery to the laboratory. Within five hours of sampling, sediments were transferred to a clean plastic weighing boat, thoroughly homogenized, and subsampled for microbial biomass, community-level physiological profile, DNA extraction, and elemental analyses.

2.2. Physico-chemical parameters

Water temperature and pH were taken directly in the field with a Direct Soil Measurement pH Portable Meter (HANNA Instruments, USA).

Samples for the total organic carbon, total percent carbon, and nitrogen contents determination by direct combustion were sent to the Fermentation Science Institute, Southern Illinois University, for analysis. A 96-hr HCl-fumigation method was performed to remove inorganic C from soil (Harris et al., 2001).

2.3. Metal analysis

X-ray fluorescence analysis was conducted on each sediment sample using a Bruker Tracer 5 g handheld XRF unit fitted with a rhodium (Rh) source, a graphene window silicon drift detector (SDD), and an 8 mm collimator. Staged in a laboratory configuration, bulk sediment samples were analyzed under atmospheric conditions for 60 s using a Fundamental Parameters (FP) two-phase soil nutrient/contaminant calibration (30 s at 50 kV; 30 s at 15 kV). The accuracy and precision of the analytical session was monitored by analyzing reference materials JSd-2 (Geochemical Survey of Japan Reference Materials; Imai et al., 1996). Unknown samples and reference materials were prepared by placing approximately 4–5 g of material into a Chemplex XRF sample container and analyzed through a 4.0 μm thick Prolene thin-film (Laperche and Lemi re, 2021). Samples were analyzed in replicates of five. Average bulk analyses for each sample and the reference material are reported in Supplementary File Table S1a and 1b.

2.4. Microbial biomass analysis

Total microbial biomass was determined in triplicates using the phospholipid phosphate (PLP) method (Smoot and Findlay, 2001; Akinwale et al., 2021). The determination of total microbial biomass is based on a strong relationship between the PLP and carbon contents of microbial cells (Findlay, 2004). Briefly, frozen sediment subsamples (1.6–6.6 g dry wt) were extracted in 50-ml screw-cap tubes with 27 ml of a 1:2:0.6 (v/v/v) dichloromethane–methanol–50 mM phosphate buffer (pH 7.4) extraction solution. The extraction mixture was allowed to stand overnight in dark at 4 °C. The solution was partitioned into organic and aqueous phases with 7.5 ml dichloromethane and 7.5 ml deionized water, the organic phase (containing total lipid) was collected across a pre-dried 2 V filter (Whatman, Thomas Scientific), and the solvents dried under nitrogen at 37 °C. After lipid extraction, sediment was collected in the filter, dried at 100 °C, and weighed. Total lipid was dissolved in 3000 μl chloroform and duplicate 100- μl aliquots per sample were each oxidized with potassium persulfate at 100 °C overnight in sealed glass ampoules to release orthophosphate. Phosphate was reacted with ammonium molybdate and malachite green, and the phosphomolybdate–malachite green complex was determined spectrophotometrically (610 nm). Concentrations of phosphate were calculated by using the regression line from a standard curve prepared by digesting glycerol phosphate.

2.5. Community-level physiological profiling

Soil microbial C utilization profiles were estimated using 96-well Biolog® EcoPlates™ (Biolog Inc., Hayward, CA) (Garland and Mills, 1991). The EcoPlates contained 31 different carbon compounds and a control in three replicates. Each well contains a carbon substrate and a redox dye (tetrazolium violet). We vortexed homogenized 2 g of soil in 18 ml of Remel™ Butterfield's Phosphate Buffer. The resulting suspension was diluted at 10^{-3} and 150 μl of this latter was inoculated to each well in the EcoPlate. All plates were placed in polyethylene bags to avoid desiccation and incubated aerobically at 27 °C. Optical Density (OD) at 590 nm was measured every 24 hr using a plate reader μQuant (BIO-TEK Instruments, Inc) for 7 days. The OD values of EcoPlates at 120 h were used to analyze bacterial carbon source utilization in the absence of fungal growth biases (Zhang et al., 2013). The metabolic activity of the sediment microbial community was calculated according to the average well color development (AWCD) defined as the arithmetic

average of the absorbance values for each substrate (Harch et al., 1997),

$$AWCD = \sum (C_i - R) / n,$$

where C_i is the absorbance of the carbon source, R is the absorbance of the control well, and n is the number of carbon substrates (31 for EcoPlates). When $C_i - R < 0$, the values were set to 0 to minimize bias. AWCD indicates the total metabolic capacity of sediment microbial communities in terms of carbon-source utilization (Akinwale et al., 2021).

Shannon diversity index (H) and Evenness (E) were calculated using an OD of 0.25 as the threshold for a positive response. Shannon diversity index (H) and Evenness (E) are defined as $H = -\sum P_i \ln(P_i)$ and $E = H/H_{\text{max}} = H/\ln S$, respectively, where $P_i = \text{OD}_i/\sum \text{OD}_i$, which is the proportional color development of the well over total color development of all wells of a plate and S is the number of oxidized C substrates (Garland and Mills, 1991; Mu niz et al., 2014).

2.6. DNA extraction and sequencing

Samples from Spring 2021 were used for metagenomic analysis to compare the spatial variations of the sedimentary bacterial community. Genomic DNA was extracted from 0.3 g frozen subsamples using the Quick-DNA™ Fecal/Soil Microbe MiniPrep Kit (ZYMO Research Corp, Irvine, CA, USA) following the instructions from the manufacturer. The extracted DNA from the triplicate samples of each sample were pooled together for each sample site. DNA was quantified by spectrophotometric absorption at 260 nm, and the purity was assessed from absorbance ratios at 260/280 and 260/230 nm using an ND-2000 Nanodrop spectrometer (Thermo Scientific, Wilmington, DE) (Custodio et al., 2022). The extracted DNA normalized to 20 ng/ μl was sent to GENEWIZ, Inc., New Jersey, USA for sequencing. Briefly, the DNA was used to generate amplicons that cover V3 and V4 hypervariable regions of bacteria and archaea 16S rDNA. Indexed adapters were added to the ends of the 16S rDNA amplicons by limited cycle PCR. DNA libraries were validated and quantified before loading. The pooled DNA libraries were loaded on an Illumina MiSeq instrument according to manufacturer's instructions (Illumina, San Diego, CA, USA). The samples were sequenced using a 2 × 250 paired-end (PE) configuration. Image analysis and base calling were conducted by the Illumina Control Software on the Illumina instrument.

2.7. Statistical analysis

Two-way analysis of variance (ANOVA) with sampling time (season) and the site as factors, followed by Tukey's highly significant difference (HSD) or Fisher LSD as a post hoc test for comparison of means was performed for physico-chemical parameters, heavy metals, H-index, CFUs, and total microbial biomass. Our metal analysis was strengthened by the availability of comprehensive and regionally derived stressor effect thresholds from Northeast Illinois via the Integrated Prioritization System - NE IL IPS- (MBI, 2020). Based on analyses against the most sensitive species to each sediment metal, NE IL IPS classified stressor levels into excellent, good, fair, poor, and very poor biological conditions (MBI, 2020). IPS values are color-coded in accordance with meeting the five narrative categories for each sediment metal (see key at bottom of Fig. 1).

Principal components analysis (PCA) (IBM SPSS Statistic, version 26) was used to determine differences between community-level physiological profiles across sites and seasons. PCA to analyze CLPPs was performed on normalized and log-transformed OD data for each well. Sediment samples from different seasons and sites were considered independent because samples were collected at least one-month intervals, never collected from the same spot, and reflected ambient conditions in time and space. Assessing the utilization profiles involves grouping the 31 carbon sources into six guilds (Supplementary Table S2) (Sala et al., 2010), and then examining changes in the percent guild utilizations over

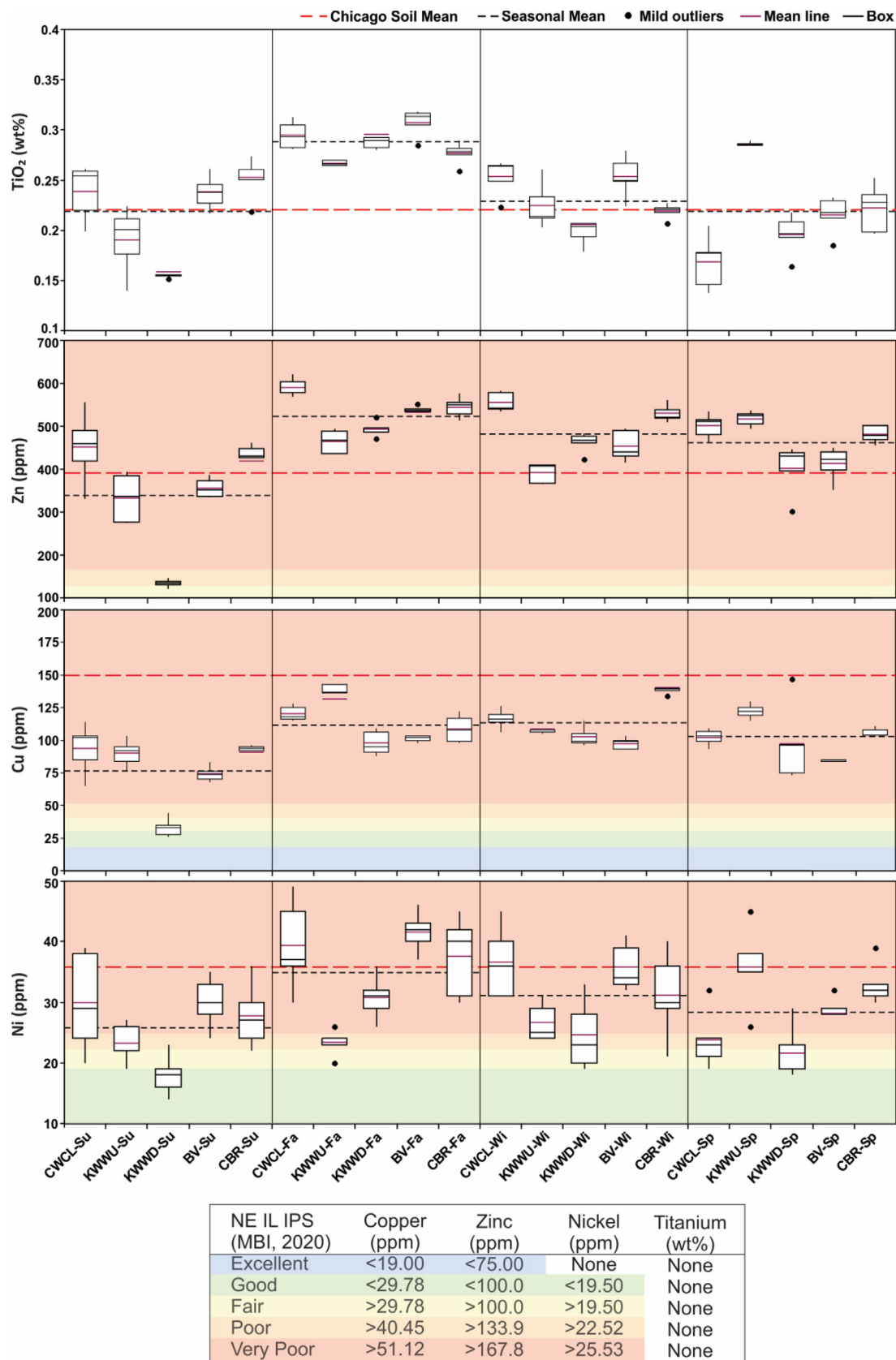


Fig. 1. Box plots of seasonal changes in heavy metal concentrations [Titanium (% weight), Copper, Zinc and Nickel (ppm)] in sediment at different sites along the lower Des Plaines River. Color codes are IPS stressor thresholds in accordance with meeting five narrative categories for each sediment metal: excellent (blue), good (green), fair (yellow), poor (orange), and very poor (red) biological conditions (MBI, 2020). Locations: CWCL Columbia Woods Canoe landing, KWWU Knollwood Wastewater Treatment Plant Upstream, KWWD Knollwood Wastewater Treatment Plant Downstream, BV Bird Viewing and CBR Canal Bank Road. Sampling Season: Su Summer 2020, Fa Fall 2020, Wi Winter 2021 and Sp Spring 2021. n = 5 (p < 0.001). The purple line inside the rectangle indicates the mean of the sample distribution. The upper and lower boundaries of each rectangle indicate the upper quartile and lower quartile respectively. The mean concentrations of Chicago soils are from Cannon and Horton (2009).

Table 1

Seasonal means (\pm se) of the total microbial biomass, colony forming units (CFUs), temperature, percentages of organic carbon (%C) and organic nitrogen (%N) contents, C/N mass ratio, and total organic carbon of the lower Des Plaines River across sampling seasons.

Sampling Date	Site	Biomass (nmol PO4 gdw)	CFUs/g	Temperature (°C)	%N	%C	C/N ratio	TOC (%)	pH
Summer-2020	CWCL	280.87 \pm 21.23	199.76 \pm 5.57	25.67 \pm 0.33	0.42 \pm 0.02	8.30	19.10	5.56	7.51
	KWWU	128.11 \pm 26.29	217 \pm 7.55	23.67 \pm 1.33	0.24 \pm 0.03	8.45	32.36	3.02	7.41
	KWWD	19.24 \pm 3.08	97.67 \pm 10.93	25.33 \pm 0.33	0.08 \pm 0.00	4.79	58.92	1.76	7.37
	BV	348.70 \pm 50.77	128 \pm 8.89	26.00 \pm 0.0	0.47 \pm 0.01	7.44	15.67	6.83	7.31
Fall-2020	CBR	260.35 \pm 13.81	184.83 \pm 4.34	26.00 \pm 0.0	0.43 \pm 0.00	8.07	18.44	6.24	7.37
	CWCL	396.02 \pm 4.4	92.41 \pm 11.54	4.33 \pm 0.33	0.45 \pm 0.01	8.69	19.07	6.60	7.97
	KWWU	239.58 \pm 41.3	735 \pm 118.19	4.33 \pm 0.33	0.26 \pm 0.01	7.75	30.91	4.34	7.72
	KWWD	280.86 \pm 24.0	98.67 \pm 6.77	5.67 \pm 0.33	0.37 \pm 0.02	9.15	23.64	5.02	7.89
Winter-2021	BV	299.10 \pm 16.1	34.33 \pm 2.60	3.33 \pm 0.67	0.45 \pm 0.01	7.82	17.13	6.52	7.76
	CBR	419.43 \pm 39.3	351 \pm 20.50	4.17 \pm 0.17	0.55 \pm 0.01	10.67	19.08	8.83	8.00
	CWCL	167.89 \pm 6.24	3.83 \pm 0.82	1.00 \pm 0.00	0.40 \pm 0.01	7.94	19.30	5.89	8.16
	KWWU	182.48 \pm 32.44	99.92 \pm 16.56	1.17 \pm 0.17	0.22 \pm 0.00	7.04	31.52	3.60	8.47
Spring- 2021	KWWD	135.88 \pm 21.66	36.50 \pm 1.01	3.17 \pm 0.17	0.47 \pm 0.02	12.99	26.20	7.89	8.26
	BV	209.67 \pm 29.18	17.50 \pm 2.50	0.00 \pm 0.00	0.49 \pm 0.02	9.82	19.22	8.21	8.07
	CBR	158.55 \pm 59.79	41.33 \pm 10.10	1.00 \pm 0.00	0.68 \pm 0.06	13.27	18.12	10.53	8.60
	CWCL	247.98 \pm 10.18	247.67 \pm 19.19	33.23 \pm 0.46	0.35 \pm 0.01	7.81	22.62	5.27	7.70
Spring- 2021	KWWU	290.96 \pm 14.51	54.00 \pm 5.69	31.70 \pm 0.61	0.49 \pm 0.05	9.23	17.27	6.50	8.13
	KWWD	403.97 \pm 27.01	67.67 \pm 27.67	28.97 \pm 0.23	0.48 \pm 0.03	9.19	18.29	6.72	8.36
	BV	418.63 \pm 29.21	110.33 \pm 8.74	28.64 \pm 0.14	0.45 \pm 0.00	7.74	17.34	6.89	7.80
	CBR	243.20 \pm 9.19	185.33 \pm 11.05	26.40 \pm 0.12	0.55 \pm 0.05	11.39	18.75	7.81	7.21

the study period. This method compresses the 31-dimensional space of any well plate into six dimensions to facilitate simple plotting and interpretation. PCA was used to analyze substrate utilization assay data after substrates were divided into six groups and the average absorbance per category was calculated. Shannon diversity and evenness indexes were compared by two-way ANOVA. Best subsets regression analysis and ANOVA were performed using the StatPlus for Mac program (AnalystSoft Inc.). Tests of significance were considered statistically significant at p values of < 0.05 or < 0.01.

For the metagenomic analysis, to identify the microbial community of the five sampling sites, data were analyzed and visualized using different software and packages as outlined below. Briefly, the quality of the data was checked, and quality (Q > 30) and length trimming were conducted to obtain higher quality and more accurate bioinformatics analysis results using Cutadapt(v1.9.1), Vsearch(1.9.6), Qiime(1.9.1). Operational Taxonomic Units (OTU) with a percent similarity threshold of 97% were obtained based on the Greengene database (DeSantis et al., 2006). The Ribosomal Database Program (RDP) classifier was used to assign taxonomic category to all OTUs at confidence threshold of 0.8. The RDP classifier uses the UNITE ITS database which has taxonomic categories predicted to the species level. Sequences were rarefied prior to calculation of alpha and beta diversity statistics. OTU heatmap to show the abundance information of selected OTU and the Venn/petal diagram was done using Qiime(1.9.1), Vsearch(1.9.6). PCoA (Principal Coordinates Analysis) was also applied to characterize and visualize the similarities and differences of samples (Beta diversity). PCoA analysis was performed and plotted in R based on the Bray-Curtis distance matrix.

3. Results

3.1. Physico-chemical parameters

Differences in seasons and among sites in the lower Des Plaines sediment samples were clearly reflected in stream water temperature, pH, sediment %C, %N, and TOC (Table 1)

Temperature varied significantly with sampling sites ($F = 12.60$) and season ($F = 5908.17$) ($p < 0.001$) (Table 2). Mean temperatures were 29.8°C, 25.3°C, 4.4°C, and 1.3°C in Spring 2021, Summer 2020, Fall 2020, and Winter 2021, respectively. Tukey HSD post hoc comparison of mean temperature amongst sites showed a general order of BV = CBR < KWWU = KWWD < CWCL. Water pH showed

Table 2

Results of Two-way analysis of variance of the effects of the season (sampling dates) vs. site on total microbial biomass (log-transformed), CFUs, H - Index, E -Index and some physico-chemical parameters of the lower Des Plaines River.

Parameter	Factor	Df	F	p
Biomass (nmol PO ₄ gdw)	Site	4	13.02	< 0.001
	Season	3	40.15	< 0.001
	Site x Season	12	11.34	< 0.001
CFUs/g	Site	4	35.42	< 0.001
	Season	3	50.67	< 0.001
	Site x Season	12	26.43	< 0.001
E - Index	Site	4	1.91	0.128 ns
	Season	3	4.04	0.013
H-Index	Site	4	5.35	0.002
	Season	3	4.81	0.006
Titanium (weight%)	Site	4	10.09	< 0.001
	Season	3	70.58	< 0.001
	Site x Season	12	13.25	< 0.001
Zinc (ppm)	Site	4	53.17	< 0.001
	Season	3	120.30	< 0.001
	Site x Season	12	13.47	< 0.001
Copper (ppm)	Site	4	42.75	< 0.001
	Season	3	74.47	< 0.001
	Site x Season	12	8.61	< 0.001
Nickel (ppm)	Site	4	13.73	< 0.001
	Season	3	12.95	< 0.001
	Site x Season	12	4.53	< 0.001
Temperature (°C)	Site	4	12.60	< 0.001
	Season	3	5908.17	< 0.001
	Site x Season	12	15.09	< 0.001
%N	Site	4	70.04	< 0.001
	Season	3	34.28	< 0.001
	Site x Season	12	19.11	< 0.001
%TOC	Site	4	5.078	0.013
	Season	3	3.092	0.068 ns
	Site x Season	12	0.51	0.732 ns
pH	Site	4	0.51	0.732 ns
	Season	3	9.38	0.002

ns = no significant difference.

strong seasonality (Table 1) ($p < 0.002$) but no significant differences among sites (Table 2). Sediment %C and %N were significantly different seasonally and spatially ($p < 0.001$) (Table 2). The seasonal variation for both %C and %N showed a general order of Summer < Fall < Winter = Spring. Spatially, Tukey HSD post hoc comparison of %N among sites showed KWWD = KWWU < CWCL < BV < CBR. TOC

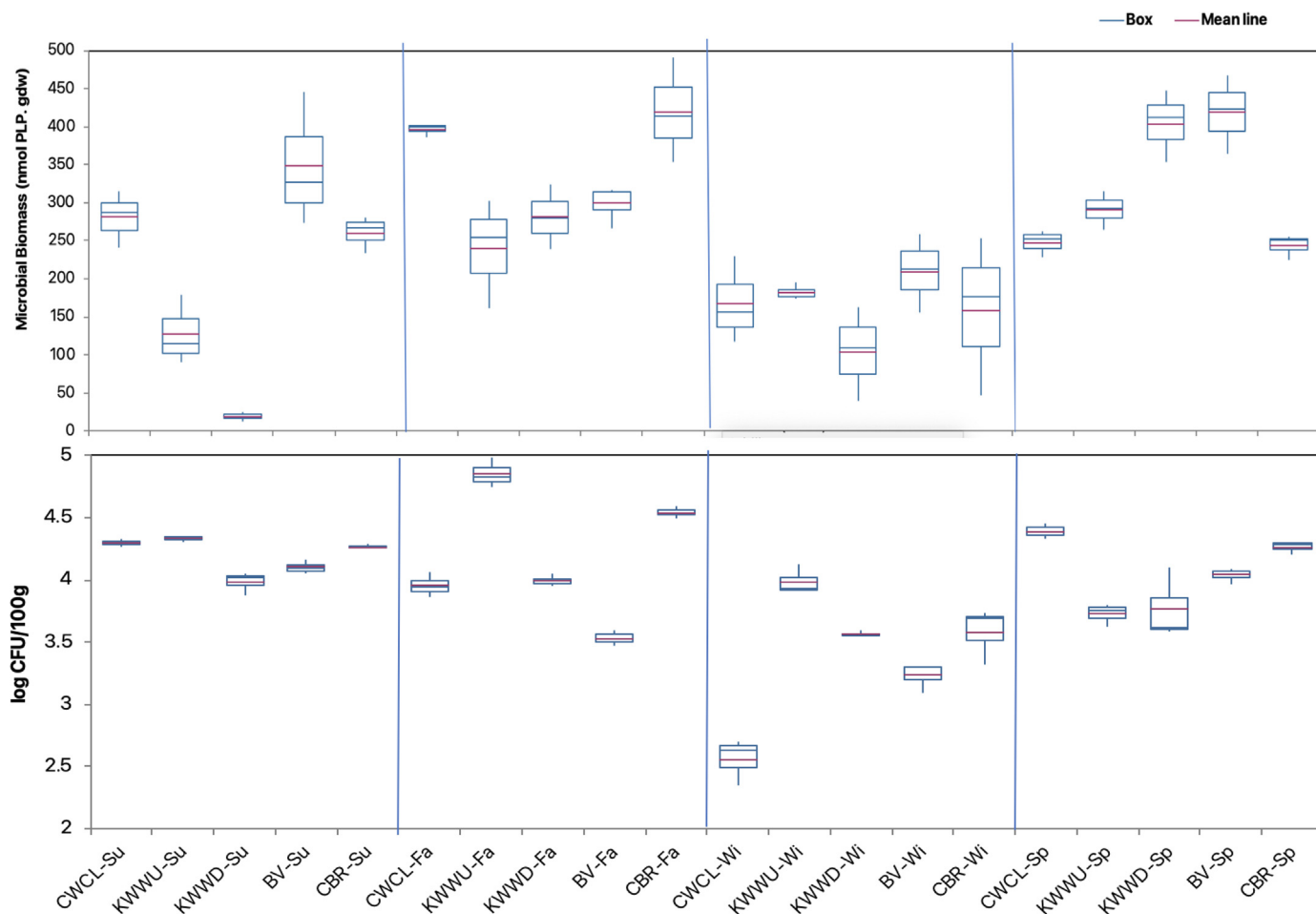


Fig. 2. Box plots of seasonal changes in sedimentary total microbial biomass and CFUs/100 g of sediment at different sites along the lower Des Plaines River. Locations and seasons as shown in Fig. 2. $n=3$ ($p < 0.001$). The red line inside the rectangle indicates the mean of the sample distribution. The upper and lower boundaries of each rectangle indicate the upper quartile and lower quartile respectively.

was seasonally significant ($p < 0.013$) but not significant among sites (Table 2).

3.2. Metal analysis

The concentrations of the heavy metals varied among sites and seasons as presented in Fig. 1. The seasonal variations in average concentrations of the heavy metals were significant ($p < 0.001$) (Table 2) e.g., 0.22 wt% (summer) to 0.29 wt% (fall) for TiO_2 ; 339 ppm (summer) to 525 ppm (fall) for zinc; 76 ppm (summer) to 113 ppm (winter/fall) for copper; and 25 ppm (summer) to 35 ppm (fall) for nickel (Fig. 1). We also documented significant differences ($p < 0.001$) between sample sites (Table 2). Notably, the patterns for TiO_2 , Zn, Cu, and Ni for summer 2020 are strikingly similar, showing consistently lower concentrations at KWWD (Fig. 1; i.e. the further away from the outfall, the higher the heavy metal concentrations measured). We also observed a relatively consistent decrease of heavy metals at KWWD in other seasons compared to adjoining sites with the exceptions of CWCL for Ti in spring, and KWWU for Ni in Fall.

We used the NE IL IPS (MBI, 2020) to assess the sediment quality and risk of heavy metal contamination. IPS values are color-coded in accordance with the established biological conditions or thresholds as shown in Fig. 1. Only the KWWD site was in the “good” range for Ni (18 ppm) in summer, and in the “fair” range (22 ppm) in Spring, however, other sites and/in other seasons had average concentrations with numerous exceedances of “poor” and “very poor” threshold average val-

ues (Fig. 1). Exceedances of the IPS thresholds were extensive for Cu and Zn concentrations in the range of “poor” and “very poor” threshold average values, except for the KWWD site for Cu (33 ppm) in the “fair” level in Summer 2020. The NE IL IPS did not provide any criteria for an evaluation of Ti.

To place the metal data in this study in the context of historical regional surveys, we compared the concentrations of the elements in ambient surface soils of the city of Chicago (Cannon and Horton, 2009) to the concentrations in our sediment samples (Fig. 1). A comparison of titanium concentrations in our study to Chicago soils indicates the median Ti does not vary greatly from Chicago’s mean (0.22 wt%) except for fall samples averaging 0.29 wt% (Fig. 1). In general, zinc concentrations in lower Des Plaines River sediment samples were much greater than the Chicago’s mean of 397 ppm, with a few exceptions that did not vary greatly from the mean, however, concentration (134 ppm) was greatly reduced at the KWWD site in summer. Compared to Chicago’s soil, nickel concentrations were lower generally in our study, however, sediments samples in fall sediments gave somewhat similar results to Chicago’s mean of 36 ppm. In contrast to other elements, copper concentrations in our study were in every season below Chicago’s mean of 150 ppm. Table 3 shows that there was a significant positive correlation ($P < 0.001$) between microbial biomass as measured with the PLP and Zinc ($r^2 = 0.34$). The zinc concentrations revealed patterns like those observed for microbial biomass (Fig. 1 and 2). The best subsets regression analysis showed that combined, titanium, nickel, copper, and zinc explained 41% of the variation observed in the sedimentary microbial

Table 3

The results of the Best Subsets regression analysis looking at the effects of independent variables: TiO₂ (wt%), Ni (ppm), Cu (ppm) and Zn (ppm) on sedimentary (log) microbial biomass.

Model/Number of variables	Independent variables:	R	R ²
1	Zn	0.59	0.34**
2	Zn, TiO ₂	0.61	0.38**
3	Zn, TiO ₂ , Cu	0.63	0.40**
4	Zn, TiO ₂ , Cu, Ni	0.64	0.41**

** significant at $P < 0.001$.

biomass ($r^2 = 0.41$, Table 3). No significant correlation between metal and coliform concentrations was found (data not shown).

3.3. Total microbial biomass, coliform concentrations, and physico-chemical parameters

Microbial biomass values varied between 19.24 at KWWD in summer and 419.43 nmol PLP g⁻¹ dry wt sediment at CBR in fall. Two-way ANOVA indicated microbial biomass displayed both significant seasonal ($F = 40.15$, $p < 0.001$) and spatial ($F = 13.02$, $p < 0.001$) differences (Fig. 2a, Table 2). The mean values of microbial biomass across seasons show a general order of Fall = Spring > Summer = Winter (Fisher LSD $\alpha = 0.05$; Table 1). Across sites, microbial biomass was significantly lower at the outfall location (KWWD) in all seasons and ranged from 19.24 to 280.27 nmol g⁻¹, except in Spring when the biomass was the highest (403.97 nmol g⁻¹). Log CFUs varied significantly both seasonally ($F = 50.67$; $p < 0.001$) and spatially ($F = 35.42$; $p < 0.001$) (Fig. 2; Table 2) with the lowest mean value in the Winter (Table 1). The mean values of log CFUs across seasons show a general order of Fall > Spring = Summer > Winter (Fisher LSD $\alpha = 0.05$; Table 1). The best single variable, C:N ratio, explained approximately half of the variation observed in the sedimentary microbial biomass ($r^2 = 0.46$, Table 4). The best subsets regression analysis showed that combined, percent nitrogen content, carbon content, total organic carbon, C:N ratio, temperature, and pH explained 68% of the variation observed in the microbial biomass ($r^2 = 0.68$, Table 4). pH was the most influential factor for the concentration of coliform in this study explaining approximately 20% of the variation ($r^2 = 0.21$; $p = 0.04$) as seen in Table 4. Though the best subsets regression analysis in Table 4 showed that the combination of pH and percent nitrogen content explained 25% of the observed variation in coliform concentration, it was not significant statistically ($r^2 = 0.25$; $p = 0.07$).

Table 4

The results of the Best Subsets regression analysis looking at the effects of independent variables: Temperature, pH,%C,% N, C:N ratio and TOC (%) on sedimentary (log) microbial biomass, CFUs and bacterial community structure (PC1).

Model/Number of variables	Independent variables:	R	R ²
Microbial Biomass			
1	C:N ratio	0.68	0.46**
2	%C, C:N ratio	0.76	0.58**
3	%C, C:N ratio, pH	0.80	0.64**
4	%N,%C, C:N ratio, pH	0.82	0.68**
5	%N,%C, C:N ratio, pH, Temperature	0.83	0.68*
6	%N,%C, C:N ratio, pH, Temperature, TOC (%)	0.83	0.68*
CFUs			
1	pH	0.46	0.21 ($p = 0.041$)
2	pH,%N	0.50	0.25 ($p = 0.084$)
Community Structure (PC1)			
1	Temperature	0.46	0.21 ($p = 0.043$)
2	Temperature, TOC	0.49	0.24 ($p = 0.096$)

** significant at $P \leq 0.001$.

* Significant at $P \leq 0.01$.

3.4. Community-level physiological profiling (CLPP)

The principal component (PC) analysis of blank adjusted and log transformed AWCD was used to determine the similarities and differences among the sites and seasons. As presented in Fig. 3a, PC 1 explained 21% of the variation in the samples, while PC 2 further explained 11% of the variation, showing two distinct clusters of fall (negative component scores) and spring (positive component scores) bacterial communities. However, we did not observe clear patterns in winter and summer samples. An additional 9% of the variance is found within the PC 3 (Fig. 3a). PC loadings (Table S3) showed that microorganisms that utilize intensely Glycogen (Polymer), L-Threonine, L-Phenylalanine (Amino acids), β -Methyl-D-Glucoside, Pyruvic Acid Methyl Ester (Carbohydrates), and D-Glucosaminic Acid, Itaconic Acid (Carboxylic and Acetic Acids) influenced the bacterial community along PC1 axis. Tween 40, Tween 80 (Polymer), L-Asparagine (Amino acid), and Pyruvic Acid Methyl Ester (Carbohydrates) were utilized disproportionately on the PC 2 by the dominant bacterial community. To further investigate spatial variation in bacterial community structure and exclude the effect of temperature variability ($p < 0.001$) (Table 2), PC analysis was conducted on bacterial physiological profiles during the period of warmer water (edited to remove samples collected in Fall and Winter) and during the period of cold water (edited to remove samples collected in Summer and Spring) (Fig. 3b and c). For the period of warmer water, the samples formed four clusters (labeled I - IV) of bacterial metabolic communities showing site-season specific variations (Fig. 3b). Clusters I (BV), II (KWWU), and III (KWWD, CBR, and CWCL) are all from summer samples, however, are distinct from one another on PC 1. Notably, cluster IV consists of all the sites from the spring samples showing overall similarity in the bacterial community. Similarly, there are four distinct clusters of bacterial community structure for the period of cold water along the PC 1 presented in Fig. 3c. Clusters I (CWCL), II (BV), and III (CBR, KWWU, and KWWD) are all from winter samples, however, are distinct from one another on the negative component scores of PC 1. Cluster IV consisting of all fall samples on the positive component scores of PC 1 showed high similarity among sites but significantly different from the winter samples revealing a strong seasonal pattern in sedimentary bacterial community structure (Fig. 3c). The best subsets regression analysis presented in Table 4 showed that temperature explained 21% of the variation observed in the bacterial community structure (PC 1). Though, the combination of temperature and TOC explained about a quarter of the observed variation in the bacterial community structure, but not significant statistically ($r^2 = 0.24$; $p = 0.096$).

Sedimentary bacterial functional diversity index (Shannon–Wiener diversity index) was significantly affected by both seasons ($F = 4.81$,

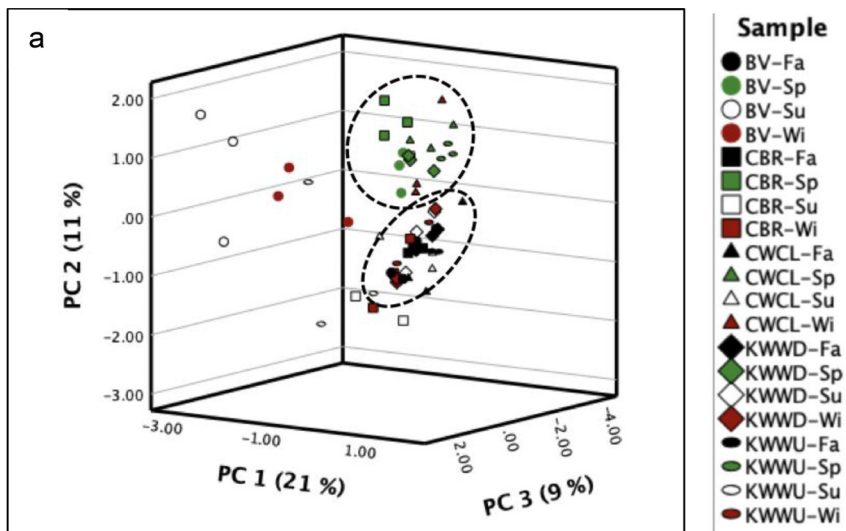
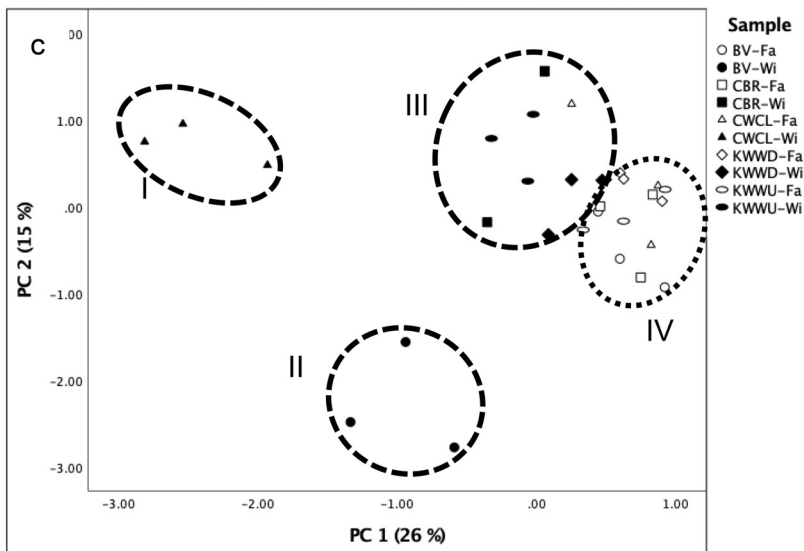
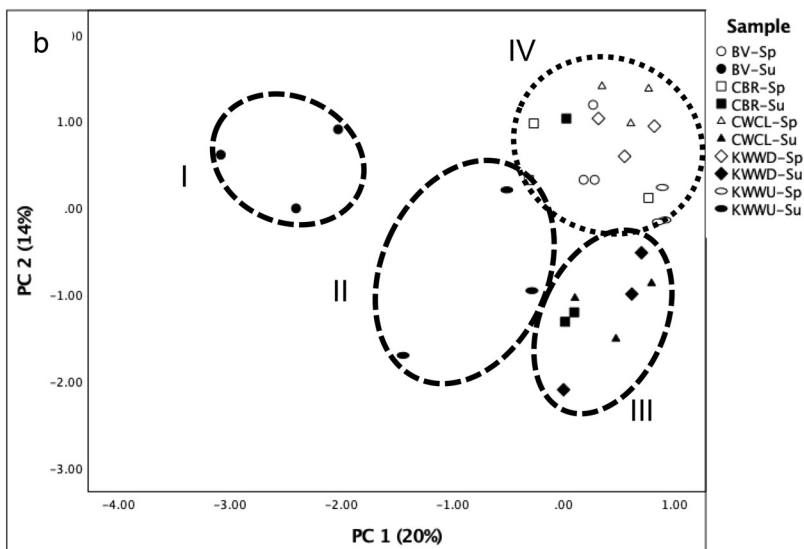


Fig. 3. (a). Principal component (PC) analysis of 120-h OD₅₉₀ readings taken from Biolog EcoPlates of the sedimentary bacterial community structure of the lower Des Plaines River seasonal sampling site. The percentage in parentheses after the PCs indicate the percentage of total variance explained by each PC. Scores are plotted by site and season as shown in Fig. 1, where colors represent seasons and shapes represent sites. (b) PCA analysis of bacterial physiological profiles during the period of warm water (Summer 2020 and Spring 2021). (c) PCA analysis of bacterial physiological profiles during the period of cold water (Fall 2020 and Winter 2021). Scores are plotted by site and season.



$p = 0.006$) and sites ($F = 5.35$, $p = 0.002$). Differences were observed between sites in summer and winter (Fig. S2). For the Evenness index, there was a significant difference between seasons ($F = 4.04$, $p = 0.013$), and Tukey HSD further revealed significant differences between sites in winter (Fig. S2).

3.5. Bacterial community composition and diversity of sampled sites in spring

Sequencing of the 16S rRNA gene resulted in a total of 4575,114 sequences, out of which 2287,557 sequences passed quality trimming. OTUs were determined for calculating the richness, diversity, and rarefaction curves of the microbial communities. The number of predicted OTUs in each sample and across all samples based on the Greengene database are reported in Fig. S3. 6368 OTUs were common across all samples. Among the five sites, KWWD (1365 OTUs) and CBR (1086) had the highest number of unique OTUs respectively. In contrast, CWCL (558 OTUs), BV (814 OTUs), and KWWU (993) harbored the lowest number of unique OTUs respectively (Fig. S3). To assess the similarity threshold among the sites, a principal coordinate analysis (PCoA) plot was generated based on Bray-Curtis distance metrics. PCoA results (Fig. 4a) showed a clear separation among three clusters: location downstream at the WWTP outlet pipe (KWWD), upstream of the outfall (KWWU), and other sites (BV, CWCL, CBR) along the first axis, which explained 49.4% of the variation, while PC2 explained additional 21.2% variation. Fig. 4a clearly demonstrates the PCoA of samples collected in Spring revealed KWWD and KWWU samples to be most distant. The three sampling location samples >1500 m away from the outfall clustered together and showed similarities in bacterial composition.

Fig. 4b presenting bacterial diversity on phylum level showed Proteobacteria as dominant in all samples (average 52%), followed by Actinobacteria (13%), Acidobacteria (10%), Chloroflexi (8%), Bacteroidetes (4.5%), and Firmicutes (3%). On the phylum level, KWWD exhibited higher and lowest abundances of Chloroflexi (10%) and Acidobacteria (7.5%), respectively. To further elucidate the spatial bacterial diversity among sites studied in Spring, reads from all the samples were resolved into classes (Fig. 4c). Among the Proteobacteria, Betaproteobacteria (18%) predominated followed by Gammaproteobacteria (13%), Deltaproteobacteria (11%), and Alphaproteobacteria (10%). However, KWWD exhibited higher and lowest abundances of Gammaproteobacteria (15%) and Deltaproteobacteria (7%), respectively. Other notable classes include Acidobacteria-6 (7%) and Actinobacteria (6%), however, KWWD exhibited a higher abundance of Actinobacteria (8%) compared to other sites (Fig. 4c).

Furthermore, the genera of bacterial communities were compared between samples with a hierarchical cluster analysis based on the Bray-Curtis index (Fig. 5). Bacteria communities were separated into two main clusters: one contained samples taken at KWWD, and the other contained samples taken at KWWU, BV, CBR, and CWCL. However, samples taken at CBR and CWCL grouped together showed similar community composition compared to BV and KWWU. In KWWD samples, seven bacterial genera displayed elevated levels but were found to be either absent or in low abundance in other locations. These genera are *Caldilinea*, *Dok 59*, *Sulfuritalea*, *Candidatus*, *Allochromatium*, *SMB53*, *Nitrospira*. Moreover, *GOUTA19*, *Desulfococcus*, *Desulfobacca*, and *Snytrophobacter* were found to be either absent or in low abundance in KWWD compared to other sites. However, *Thiobacillus*, *Dechloromonas*, *Crenothrix*, *Rhodobacter*, *Hyphomicrobium*, and unclassified genera are equally represented in all sites (Fig. 5).

4. Discussion

4.1. Microbial biomass, CFUs concentrations, and environmental variables

Results presented in this study showed that the sedimentary microbial biomass, CFUs concentrations, and bacterial community structure

at lower Des Plaines River exhibited significant spatial variability in the context of effluent perturbation and temporary variation associated with seasonality. Differences in microbial biomass were strongly linked with the physicochemical characteristics of the sediment. Best subsets regression analysis indicated that percent N, percent C, C:N ratio, pH, temperature, and total organic carbon explained nearly 70% of the variance in sediment microbial biomass. These environmental factors are known to be important environmental constraints of sedimentary microbial biomass (Sutton and Findlay, 2003; Fierer et al., 2007; Akinwale et al., 2021). For instance, the C:N ratio alone in this study directly contributed about 50% of the biomass variation observed, which studies (Findlay et al., 2002; Fierer et al., 2007; Wang et al., 2007) have also shown to influence microbial biomass through its quantity, quality, or both. Site-specific variance showed overall lower microbial biomass at the outfall (KWWD) which matched with a high C:N ratio (i.e. lower N-availability) at this site compared to other sites, except in Spring when microbial biomasses were generally higher across sites (Fig. 2). The abundant dense filamentous algal streamers attached to the streambed in Spring are known to contribute to increased microbial biomass (Artigas et al., 2009) and might have compensated for higher biomass observed at the outfall in Spring. Our findings suggest that the decrease in microbial biomass at the outfall of the chlorinated effluents may indicate changes in the receiving sediments that are detrimental to the growth of the microbial populations since toxic compounds may be present in WWTP effluent. Agnelo et al. (2020) showed a decrease in microbial biomass with increasing calcium hypochlorite concentrations applied to the soil. While this study did not directly measure chlorination byproducts such as trihalomethanes and haloacetic acids it is reasonable to imply that the input of chlorinated effluents reduced the microbial biomass at the KWWD compared to higher biomass measured at other sites away from the WWTP outfall. This finding is consistent with Wakelin et al. (2008) study that documented the lowest microbial biomass just below a WWTP outfall, and the highest microbial biomass at the most-downstream location from the WWTP outfall of chlorinated effluent. In contrast to chlorinated effluent in this study, streams that received UV radiation treated effluents exhibited higher microbial biomass at the outfall (Akinwale et al., 2021).

The overall increase in microbial biomass in fall and spring seasons compared to winter might be due to abundant sunlight, warmer water temperatures, lower pH, increased allochthonous inputs primarily through deposition of leaf litter (in fall), increased algal productivity (in spring) and elevated nutrient concentrations in those seasons. These environmental factors are key drivers controlling temporal variation in total microbial biomass in stream ecosystems (Suberkropp 1997; Findlay et al., 2002; Fierer et al., 2007; Artigas et al., 2009; Tank et al., 2010; Akinwale et al., 2021).

Concentrations of CFUs showed seasonality in our study varying from 2.6 (in winter) to 4.9 (in fall) log₁₀ units. The concentration of CFUs at a given time may vary depending on various factors such as the occurrence of rain, fecal contributions to the sewage and the epidemiology status of the population, temperature, and the time of day of sampling (Mackowiak et al., 2018; Jofre et al., 2021). In addition, environmental factors affecting sedimentary microbial biomass (discussed above) are important environmental constraints of sedimentary coliform bacteria.

Also, concentrations of CFUs displayed spatial differences, but not particularly in the context of WWTP outfall location (KWWD), given that high concentrations were observed above and below the outfall at different sampling seasons. However, our studied river received other pollutants and WWTP effluents elsewhere, thus, CFUs concentrations at a given site might be determined by various factors such as the distance from other outfalls and nonpoint pollution, effluent volumes, the levels of dilution, sedimentation or re-suspension of sediments and inactivation of fecal coliform by treatment type or natural stressors. Consequently, the choice of sampling locations for routine water quality monitoring could have a major impact on the as-

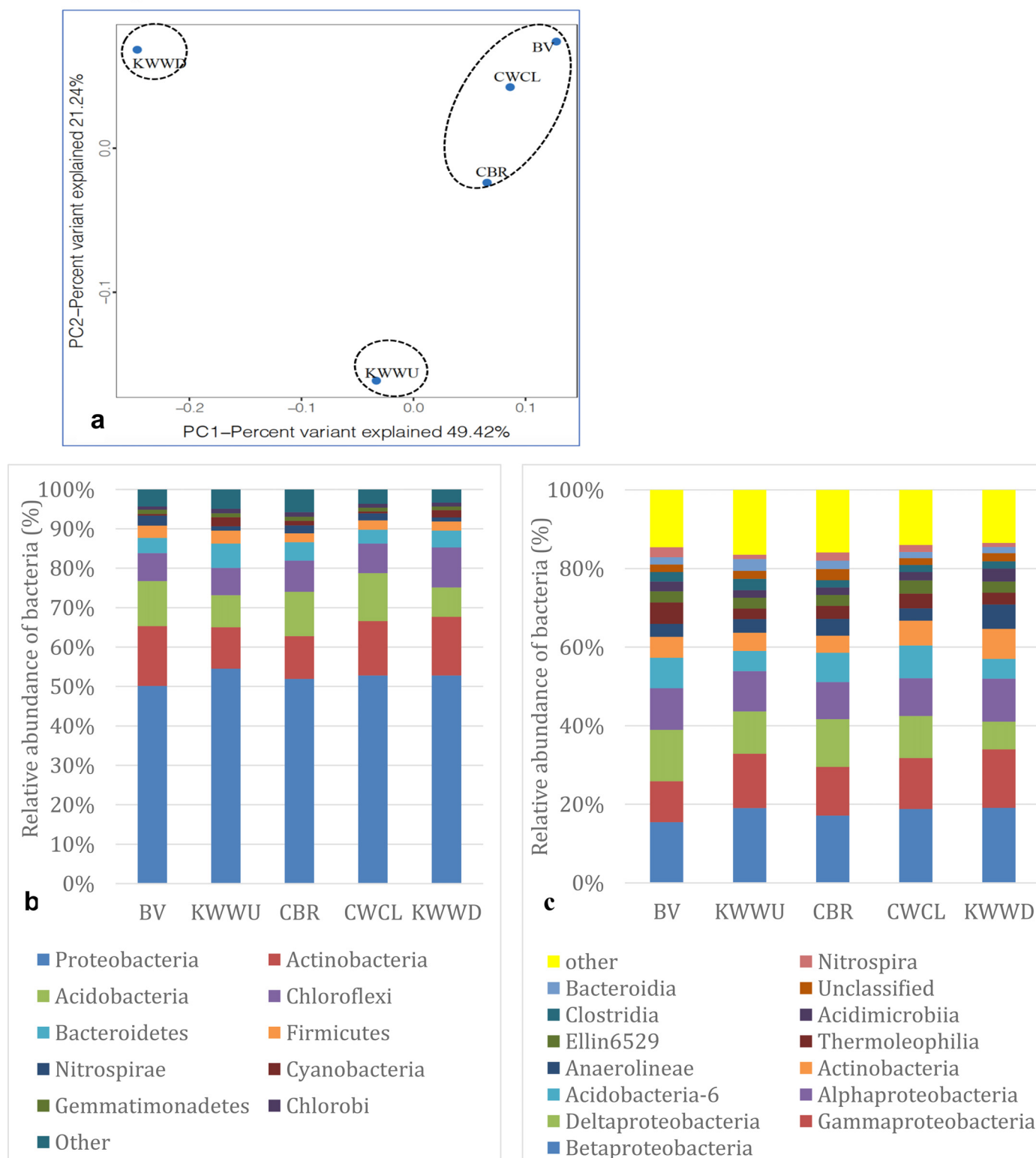


Fig. 4. (a) PCoA (Principal Co-ordinates Analysis) analysis based on Bray-Curtis distance matrix showing correlation of the most abundant OTUs across Spring 2021 samples. (b) Relative abundance (%) of the overall most prevalent phyla (a) and class (c) for the five sampling sites/sample groups in Spring 2021. Bacterial OTUs with an overall abundance of >1% are shown, while the remaining phyla and class were pooled and marked as “other.”.

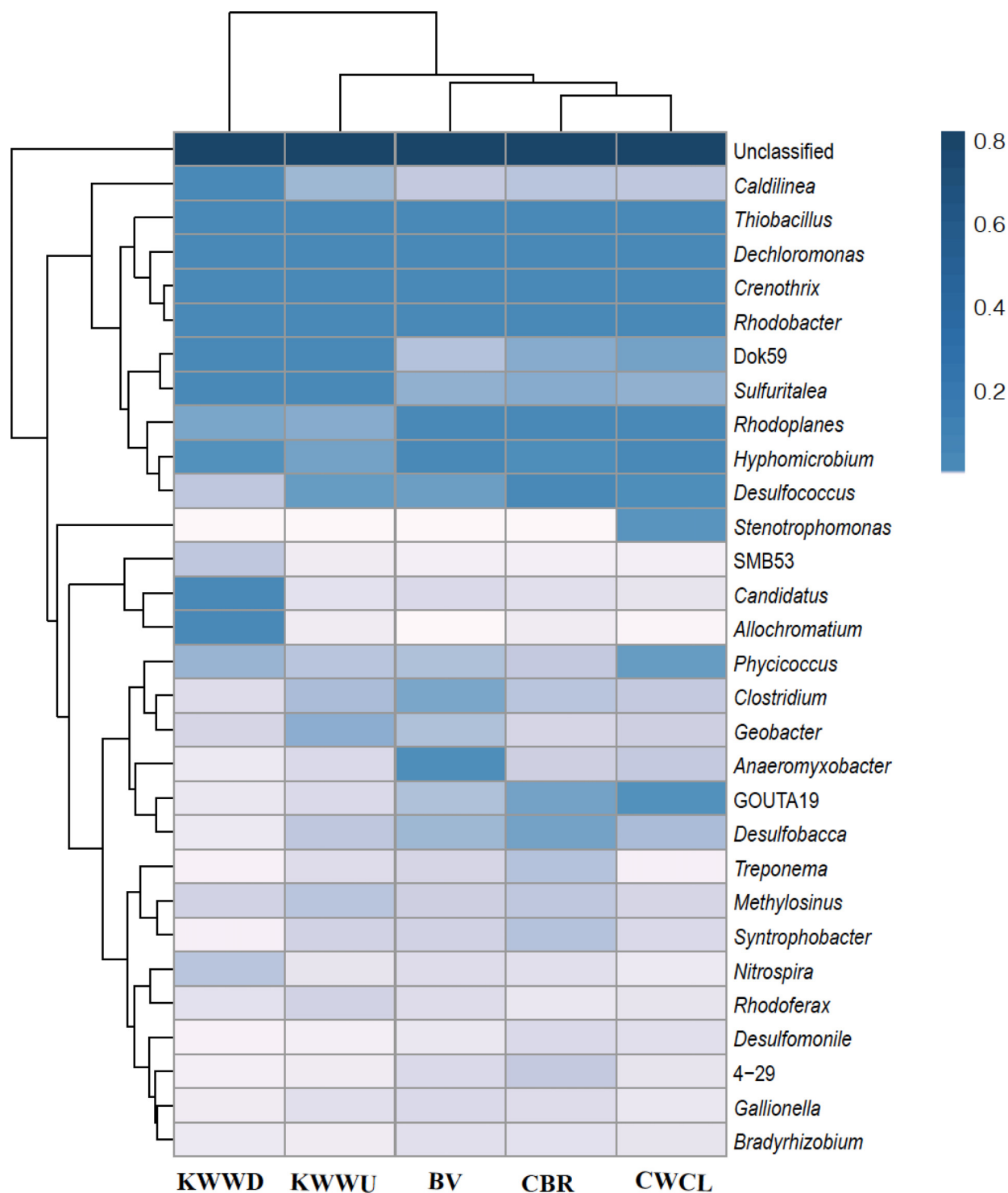


Fig. 5. Clustering of the samples using heatmap illustration of top 30 abundant bacterial genera. The color legend represents the correlation between samples.

assessment of microbiological water quality in the lower Des Plaines River.

Although regulatory standard for sediment-associated fecal coliforms has not been developed *per se*, however, Malin et al. (2007) estimated a useful comparison of suspending 200 CFU cm⁻² of sediment fecal coliform bacteria in a 1-m deep water column which corresponds to the 200 CFU 100 ml⁻¹ standard for human body contact. Also, in wastewater treatment, reported reductions in the concentrations of fecal coliforms when discharged to the surrounding environment ranged

from 0.3 to 3.0 log₁₀ units, that is, from 50% to 99.9%, depending on the treatment type (secondary or tertiary treatment) (Jofre et al., 2021). However, sedimentary CFUs concentrations (17.5 - 735.1 CFUs/g) remain consistently high and variable throughout the year in the lower Des Plaines River as reported in this study, given that fecal indicators concentrations detected in sediments outnumber by several orders of magnitude those in overlying waters (Perkins et al., 2014; Mackowiak et al., 2018).

4.2. Correlations between biological attributes and sedimentary heavy metals

Heavy metal pollution is a global concern due to its latent, long-term, cumulative, and non-biodegradable characteristics (Margesin et al., 2010; Hu et al., 2021). Anthropogenic activities are a major source of these harmful metals, particularly in urban environments (e.g., Mielke et al., 2000; Filippelli et al., 2005; Wakelin et al., 2008; Margesin et al., 2010; Hu et al., 2021). Metal concentrations in Des Plaines River sediments showed significant seasonality and spatial variation, and yield values that fall within the “fair” to “very poor” range of biological conditions outlined by the Midwest Biodiversity Institute (MBI, 2020; Fig. 1). The concentrations of these metals are also close to average values reported for Chicago soils. According to Cannon and Horton (2009), titanium is not strongly enriched relative to regional concentrations and to soils collected from 90 sites across the state of Illinois. This suggests that the TiO_2 contents in the lower Des Plaines River likely reflect natural geological factors such as heterogeneities within the underlying Pleistocene glacial deposits (till, outwash, and lake sediments). However, we cannot rule out the possibility that the observed TiO_2 variations may also reflect local anthropogenic contributions. Furthermore, we also observe elevated concentrations of Zn, Cu, and Ni in Des Plaines River sediments that result in poor biological conditions. The concentrations of these heavy metals are very similar to Chicago soils, suggesting that soil erosion may be a significant source of metal pollution in the Des Plaines River. Atmospherically transported materials sourced from vehicular pollution (e.g. tire wear, brake pads, lubricants, and roadway emissions) and street sediments may also be contributing factors (Cannon and Horton, 2009).

Being necessary for life, Zn, Ni, and Cu, along with Fe and Al are abundant in soil and sediments, but are potentially toxic when present in excess and can damage cell membranes (Nies, 1999; Bruins et al., 2000). Zn, Ni, and Cu are essential metals that play an important role in regulating gene expression and the activity of biomolecules. For instance, Zn is part of enzymes and DNA binding proteins for essential biochemical reactions (Nies, 1999). We found a significant correlation between total microbial biomass and the Zn, Cu, and Ni concentrations (Table 3). Though titanium is present in the environment as slightly soluble oxides (e.g. TiO_2), it has never been shown to be essential for organisms, or occur as any metalloenzymes (Buettner and Valentine, 2012). However, studies have demonstrated the toxicity effects of TiO_2 at high concentrations on microbial communities (Binh et al., 2014; Hou et al., 2019).

Although we documented high metal concentrations in the lower Des Plaines River indicating “fair to very poor” biological conditions (MBI, 2020), the relationship between microbial biomass and heavy metal concentrations is remarkable (i.e., generally, the higher the metal concentration, the higher the microbial biomass across seasons).

In general, significant negative relationships were observed between total heavy metal concentrations and the soil microbial parameters (Castaldi et al., 2004; Li et al., 2020), however, contradictory data on microbial parameters in metal-contaminated soils have been reported (Sandaa et al., 1999; Ellis et al., 2003; Castaldi et al., 2004). Long-term stresses caused by heavy metal pollution can significantly change microbial communities, resulting in an increase in the abundance and activity of metal-tolerant species (Guo et al., 2017; Li et al., 2020). Previous studies have found that Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, and Gemmatimonadetes were the dominant phyla in long-term heavy metal contaminated sites (Hu et al., 2021). In our study, we show that these phyla are the most prevalent, with combined relative abundances ≥ 75 –80% (Fig. 4b, c). Additionally, numerous studies have documented freshwater microbes that are co-resistant to heavy metals and antibiotics (Squadrone, 2020; Hu et al., 2021). These bacteria are resistant to elements such as Zn, Cu, Ni, Pb, Hg, As, Cd, and Cr (see Table 1, Squadrone, 2020). Thus, we posit that increased sediment metal loads select for metal-tolerant microbial communities that help

to maintain microbial biomass. When a cell faces a high concentration of any heavy metal in an ecosystem, resistance to such metal could be induced to protect sensitive cellular components. The microbial resistance to heavy metals has been attributed to a variety of detoxifying mechanisms developed by resistant microorganisms such as complexation by exopolysaccharides, binding with bacterial cell envelopes, intra- and extracellular sequestration, metal reduction, and efflux (Nies and Silver, 1995; Nies, 1999; Bruins et al., 2000). The elevated metal concentrations and relatively high microbial biomass and community present in the Des Plaines River sediments suggests that the microbial community has adapted to the polluted urban river conditions.

4.3. Effects of physicochemical variables on the bacterial community structure

The bacterial community-level physiological profiles were seasonally different, supporting other annual cycle studies of seasonal variations in sedimentary bacterial communities (Oest et al., 2018; Akinwale et al., 2021). Temperature was the most important environmental constraint of sedimentary bacterial communities in our study, where bacterial communities were similar to one another in the spring and fall and distinguishable from those in the winter and summer. Our data for the sedimentary bacterial community structure of this study indicated that temperature was more important than water chemistry, whereas total microbial biomass responded to the influences of both temperature and water chemistry.

The overall CLPPs showed that the metabolic diversity (Shannon–Wiener diversity index) was higher in fall and spring, but not significantly different among sites when compared to summer (when water temperatures were high) and winter (when water temperatures were the lowest). The evenness index was also significantly different among sites in winter. Sources of organic matter change remarkably throughout a year and have been shown to influence microbial community structure (Hullar et al., 2006). The fall and spring communities (with higher H-index) have access to allochthonous and pulses of algal productivity, respectively, that likely provide better quality organic matter to sedimentary microorganisms and may be stimulating the observed seasonal shift in the bacterial community. The fall and spring communities significantly utilized carbohydrates, amines and amides, amino acids, carboxylic acids, and polymers guilds. Our work supported previous studies that reported broader metabolic pathways in fall and spring (Oest et al., 2018; Akinwale et al., 2021). It is not immediately clear why metabolic diversity was more variable between sites in winter in our study, however, we posit that winter freezing temperature may have delayed or slowed the decomposition rate of fall allochthonous inputs, allowing the continuous supply of substrate in multiple patches for a variety of microorganisms. Moreover, when favorable temperature returned in summer, a spurt of leaf residue decomposition that resulted in the availability of labile carbon that can be used by a variety of microorganisms caused increased metabolic diversity.

4.4. Bacterial community composition and diversity of sampled sites in spring

Sediment bacterial communities from all sites were dominated by Proteobacteria (Gram-negative bacteria), Actinobacteria (mostly gram-positive), and Acidobacteria (Gram-negative), metabolically diverse groups that are ubiquitous in freshwater sediments (Ge et al., 2021), which accounted for almost 75% of the total bacterial abundance in all sediment samples in our study.

The bacterial community structure formed three main clusters, based on distance from the WWTP outfall, with the bacterial community structures at the upstream and downstream sites being markedly different from those at the outfall.

Although there was no significant difference in the abundance of proteobacterial sequences between the sampling sites, it exhibited distinct

compositional differences at the class and genus levels. The filamentous bacteria of the genus *Candidatus* most frequently linked to filamentous bulking or operational problems in WWTPs with nutrient removal (Nierychlo et al., 2020) dominated the KWWD site. The genus *Allochro-matium*, which is capable of reducing thiosulfate to sulfur compounds as an electron donor for growth (Grimm et al., 2011) are highly represented in KWWD compared to other sites. A core denitrifier and pollution indicator *Sulfuritalea* dominated both KWWU and KWWD locations. A high abundance of *Sulfuritalea* has been observed in the anoxic water columns and nitrate-depleted waters (Watanabe et al., 2017). This finding is supported by the decrease in total microbial biomass at the outfall location and KWWU. *Nitrospira* sequences were more abundant in the KWWD sediments. *Nitrospira* species are the dominant nitrite oxidizers within freshwater sediments that are involved in the process of nitrification (Altmann et al., 2003). Finally, Chloroflexi sequences were significantly more abundant in the KWWD site sediment. The filamentous bacteria belonging to the genus *Caldilinea* within the phylum Chloroflexi commonly found in the activated sludge of municipal WWTP dominated the KWWD location (Fig. 5). Kragelund et al. (2011) showed that filamentous bacteria belonging to phylum Chloroflexi are highly abundant in both municipal and industrial WWTPs. In some estimates, filamentous *Chloroflexi* constitutes up to 30% of the entire biovolume in nutrient removal plants (Nielsen et al., 2010). Consequently, significant differences in the relative abundances of several bacteria taxa at the KWWD site and non-outfall site sediments, including *Caldilinea*, *Candidatus*, *Allochro-matium*, *Sulfuritalea*, and *Nitrospira*, may be linked to anthropogenic inputs from WWTP effluents and the major responses in biomass and bacterial community structure, and function.

5. Conclusions

Our data demonstrated significant spatiotemporal variations in the sedimentary microbial parameters in an urban river, shaped by both natural and anthropogenic factors, and provided an insight into the dynamic nature of sedimentary heavy metals in urbanized rivers. We reported reduced sedimentary microbial biomass, CFUs, and distinct bacterial communities at the WWTP outfall compared to other sites which showed that WWTP effluents have the potential to modulate the composition of bacterial communities, and confirms previous findings that sedimentary bacterial communities are generally sensitive to disturbances. We reported an increase in some bacteria taxa including *Caldilinea*, *Candidatus*, *Allochro-matium*, *Sulfuritalea*, and *Nitrospira*, linked to anthropogenic inputs from WWTP effluents. To better understand patterns of disturbance in urban river ecology, analysis of sediment microbial communities ought to become integrated with regular ecological and water quality assessments involving higher trophic levels. In addition, the reported high heavy metal contents and relatively high microbial biomass and bacterial activities present in lower Des Plaines River sediments suggests that the microbial community has adapted to the polluted urban river conditions. From a health perspective, contamination with fecal coliforms and chronic heavy metal levels as seen in this study poses a risk to human health in urban river use for recreational activities such as swimming or fishing and results in the deterioration of water abstracted for agricultural or drinking purposes. With increasing urbanization and industrialization, human dependence on effluent-dominated ecosystems for water resources will intensify globally over the next few decades, thus, studies focusing on remediation and policy changes are dire to develop effective management of existing urban rivers.

Credit author statement

MC Draper and PO Akinwale designed the experiments. MC Draper, MJ Martin, A Guta and PO Akinwale performed the experiments and analyzed data. KL Brown analyzed heavy metals samples. KL Brown and PO Akinwale wrote the original draft. All authors

edited and revised the manuscript. All authors have approved the final manuscript and agree with submission to the Journal of Hazardous Materials Advances.

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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.

Data Availability

Data will be made available on request.

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Supplementary materials

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