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Applicability of API ZYM to capture seasonal and spatial variabilities in lake and river sediments

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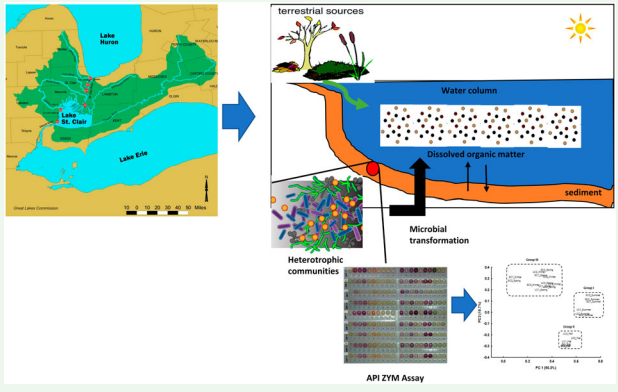
ABSTRACT

Waters draining into a lake carry with them much of the suspended sediment that is transported by rivers and streams from the local drainage basin. The organic matter processing in the sediments is executed by heterotrophic microbial communities, whose activities may vary spatially and temporally. Thus, to capture and evaluate some of these variabilities in the sediments, we sampled six sites: three from the St. Clair River and three from Lake St. Clair in spring, summer, fall, and winter of 2016. At all sites and dates, we investigated the spatial and temporal variations in 19 extracellular enzyme activities using API ZYM. Our results indicated that a broad range of enzymes were found to be active in the sediments. Phosphatases, lipases, and esterases were synthesized most intensively by the sediment microbial communities. No consistent difference was found between the lake and sediment samples. Differences were more obvious between sites and seasons. Sites with the highest metabolic (enzyme) diversity reflected the capacity of the sediment microbial communities to breakdown a broader range of substrates and may be linked to differences in river and lake water quality. The seasonal variability of the enzymes activities was governed by the variations of environmental factors caused by anthropogenic and terrestrial inputs, and provides information for a better understanding of the dynamics of sediment organic matter of the river and lake ecosystems. The experimental results suggest that API ZYM is a simple and rapid enzyme assay procedure to evaluate natural processes in ecosystems and their changes.



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Introduction

The Saint Clair River and Lake Saint Clair are vital parts of the Great Lakes system not only for commercial navigation but also for fish and wildlife that reside in or pass through the area [1]. Lake St. Clair serves as a recreational port, an international shipping channel, as well as a source of drinking water for much of southeastern Michigan. The vast majority of the watershed's original landscape has been altered by human uses and replaced by residential, commercial, and agricultural developments [2]. These developments made drinking water from the St. Clair River and Lake St. Clair potentially vulnerable to chemicals (i.e. agricultural runoff) and have posed emerging issues related to microbial and chemical contaminants [3,4]. Thus, the St. Clair River and Lake St. Clair have been, and continue to be, the subject of considerable efforts from government agencies, the scientific community, and concern citizens. Accordingly, it is crucial to protect and improve river and lake systems.

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Sediment microbial communities may be relevant in polluted aquatic ecosystems. They play a significant role in the interchange of energy and matter between sediment and the water column, as well as in the degradation of a broad range of organic matter (OM) sources in aquatic systems [5]. However, it is not clear how sediment microbial communities and ecological functions respond to pollution stress in aquatic ecosystems [6]. Microbial communities are sensitive to anthropogenic disturbances such as changes in oxygen concentrations and exposure to toxicants [7,8]. For example, heavy metals such as copper have been shown to alter the structure and physiology of microbial communities [9]. Microbial communities are useful biological indicators as they respond rapidly to the environmental variations [8]. Hence, their activities can assist in understanding the level of disturbance in river and lake ecosystems, which may be helpful in mitigating the cost and time for effective watershed management.

Sediments are one of the most diverse microbial habitats with distinct redox gradients and represent an open system in which physical, chemical, and biological reactions take place [10–13]. Much of the organic matter input in the sediments is in the form of chemically complex macromolecules. Bacteria and other heterotrophic microorganisms gain access to this pool of organic matter by producing extracellular enzymes degrading high-molecular-weight organic matter [14] into smaller ones, so that the carbon previously unavailable for uptake can be assimilated by the microorganisms [15–17]. Because extracellular enzymes catalyse the limiting steps of the degradation process [18,19], any factors affecting their activity or disrupting their production or availability will influence the entire degradation pathway [20]. Thus, extracellular enzyme activity is often used as a useful and informative indicator of microbial activity, nutrient status, ecosystem health and quality due to environmental changes or anthropogenic impacts in natural environments [21]. Since extracellular enzymes are substrate specific, the measurements of their activities can also provide insights into specific functional profile of the microbial communities of Saint Clair River and Lake Saint Clair sediments.

The present study addresses the study of an array of enzymes in Lake Saint Clair and Saint Clair River sediments using the API ZYM^{TM} kit for the systematic and rapid study of 19 extracellular enzymes. The API ZYM^{TM} kit has been successfully used for the study of enzyme activities of microorganisms and cell suspensions [22]. It has also been used to study the extracellular enzyme activities in environmental samples such as compost extracts [23–25], soil extracts [26,27], landfill refuse extracts [28], municipal solid waste extracts [29], river waters [30], groundwater [30], and industrial wastewaters [31].

The main goal of this study is to use API ZYM to assess the extracellular enzyme activities of the river and lake sediment heterotrophic communities on temporal and spatial scales and determine the implications of the enzyme activity fingerprints on their physico-chemical properties of the sediments. Six sampling sites (three lake sites and three river sites) were chosen to assess spatial variabilities. Samples were collected during spring, summer, fall, and winter seasons to assess seasonal patterns.

Materials and methods

Sites description, sediment sampling, and field data

Six sites were selected: three from the St. Clair River (SC1, SC2, and SC3) and three from Lake St. Clair (LC1, LC2, and LC3) (Figure 1). These locations are near known point sources (e.g. City of Saint Clair permitted wastewater discharges, marina, major river follow, and public parks) to adequately represent source areas upstream and downstream. Site SC1 (N 42° 58.393; W 82° 25.141) is located upstream where the water from Lake Huron drains into Lake St Clair; site SC2 (N 42° 54.323; W 82° 28.036) is close to the City of Marysville Wastewater Treatment Plant, City Public Park and public boat launch; and site SC3 (N 42° 49.2′9′; W 82° 29.2′0′) is situated at the mouth of Pine River, which empties into the Saint Clair River. Site LC1 (N 42° 37.8′8′; W 82° 30.9′0′) is located downstream of the Saint Clair River where it empties its water into Lake Saint Clair and is close to the residential private boating docks; site LC2 (N 42° 31.6′0′; W 82° 52.2′8′) is close to the Veterans Memorial Park located south of Clinton River; and site LC3 (N 42° 21.560; W 82° 55.5′7′) is situated downstream where Lake Saint Clair empties its water into Detroit River at the City of Grosse Pointe Park.

Sediment samples (5 cm depth) were collected in spring (April 18), summer (June 20), fall (September 16), and winter (December 12) of 2016. A ponar grab sampler was used to collect sediment samples. The sampling equipment was decontaminated after each sample was processed. The decontamination procedure included rinsing the sampling pans and spoons with site water and using new sampling tube and gloves for each sample location. All samples were collected and stored in ziploc bags, cooled to 4°C in a cooler containing ice, and stored in darkness. The samples were kept in a cooler and transported to the lab. Upon arrival, the samples were stored in a refrigerator for 24 h before any assay began. Table 1 reports the weather history at the

Figure 1. Map showing the three river sites (SC1, SC2, and SC3) and three lake sites (LC1, LC2, and LC3). The surrounding rivers and cities. The red circles represent the sites in which the samples were taken.

time of sampling. The highest mean air temperature was recorded in the summer (27°C), and the lowest was in winter (10°C). Mean ambient temperature in the fall was 5°C lower than that of the summer (22°C), while the average ambient temperature in the winter was similar to that in spring (11°C). The highest mean humidity was recorded in the summer (84%), and the lowest was in spring (65%). None of the sampling days had any precipitation. Pressure was highest in the winter (771 mmHg) and lowest in the summer (760 mmHg). The visibility was lowest in the fall at 11.27 km, and in summer and winter, the visibility was at its highest at 16.09 km.

Table 1. Weather history at the time of sampling.

	Spring	Summer	Fall	Winter
Temperature				
Mean temperature (°C)	11.11	26.67	22.22	10.0
Maximum temperature (°C)	17.78	23.89	30.0	13.89
Min temperature (°C)	3.89	8.89	15.0	5.56
Moisture				
Dew point (°C)	2.78	15.0	19.44	5.00
Average humidity (%)	65	84	81	72
Maximum humidity (%)	100	100	100	93
Minimum humidity (%)	36	61	55	54
Precipitation (mm)	0	0	0	0
Sea level pressure (mmHg)	764.29	759.97	764.54	770.89
Wind				
Wind speed (kph)	9.66	3.22	4.83	19.31
Minimum wind speed (kph)	32.19	14.48	20.92	32.19
Maximum wind speed (kph)	40.23	nd	25.75	45.06
Visibility (km)	14.48	16.09	11.27	16.09

Note: nd = no data available.

Sediment physico-chemical properties and microbial numbers

Water pH and water temperature at the sites were measured using Horiba's U-10 Water Quality Checker. Water depth and turbidity were measured by using a Secchi disk. The sediments collected from each site were analysed for moisture content (105°C for 24 h); ash content and organic matter (OM) using loss on

Table 2. Substrate composition, pH and expected results from the test.

Enzyme assayed for	Enzyme group	Substrate	pH	Result	
				Positive	Negative
1. Control	–	–	–	Pale yellow	Pale yellow
2. Alkaline phosphatase	Phosphatase	2 naphthyl-phosphate	8.5	Violet	Pale yellow
3. Acid phosphatase	Phosphatase	2 naphthyl-phosphate	5.4	Violet	Pale yellow
4. Phosphohydrolase	Phosphatase	Naphthyl-AS-BI-phosphate	8.5	Blue	Pale yellow
5. Lipase	Esterase	2 naphthyl-myristate	7.5	Violet	Pale yellow
6. Lipase esterase	Esterase	2 naphthyl-caprylate	7.5	Violet	Pale yellow
7. Esterase	Esterase	2 naphthyl-butyrate	6.5	Violet	Pale yellow
8. Leucine aminopeptidase	Aminopeptidase	L-leucyl-2-naphthylamide	7.5	Orange	Pale yellow
9. Valine aminopeptidase	Aminopeptidase	L-valyl-2-naphthylamide	7.5	Orange	Pale yellow
10. Cystine aminopeptidase	Aminopeptidase	L-cystyl-2-naphthylamide	7.5	Orange	Pale yellow
11. Chymotrypsin	Protease	*N*-glutaryl-phenylalanine-2-naphthylamine	7.5	Orange	Pale yellow
12. Trypsin	Protease	*N*-benzol-DL-arginine-2-naphthylamide	8.5	Orange	Pale yellow
13. α-galactosidase	Glycosyl hydrolase	6-Br-2-naphthyl-α-D-galactopyranoside	5.4	Violet	Pale yellow
14. β-glucosidase	Glycosyl hydrolase	6-bromo-2-naphthol-α-D-galactopyranoside	5.4	Violet	Pale yellow
15. *N*-acetyl-β-glucosaminidase	Glycosyl hydrolase	1 naphthyl-*N*-acetyl-βD-glucosaminide	5.4	Brown	Pale yellow
16. α-glucosidase	Glycosyl hydrolase	2 naphthyl-2-D-glucopyranoside	5.4	Violet	Pale yellow
17. β-galactosidase	Glycosyl hydrolase	2 naphthyl-βD-galactopyranoside	5.4	Violet	Pale yellow
18. β-glucuronidase	Glycosyl hydrolase	Naphthyl-AS-BI-βD-glucuronide	5.4	Blue	Pale yellow
19. α-mannosidase	Glycosyl hydrolase	6-bromo-2-naphthyl-2-D-mannopyranoside	5.4	Violet	Pale yellow
20. α-fucosidase	Glycosyl hydrolase	2 naphthyl-αL-fucopyranoside	5.4	Violet	Pale yellow

ignition technique (550°C for 5 h); pH (1:10 water extract; Oakton 500 Series pH meter, Oakton Instruments, Vernon Hills, IL); and electric conductivity (1:10 water extract; Hanna H19810 pH meter, Hanna Instruments, Woonsocket, RI). Quantitative estimations of the populations of total aerobic heterotrophs and fungi in the sediment samples were determined by direct plating on appropriate media [32–34].

API ZYM assay

Each API ZYM strip (BioMerieux, Marcy l'Etoile, France) includes three phosphatases, three esterases, three aminopeptidases, two proteases, and eight glycosyl hydrolases (Table 2). Triplicate 1:10 extracts were prepared for each sediment sample by weighing 5 g of the sediments into falcon tubes with 0.9% saline solution filled up to 50 ml mark. The falcon tubes were shaken at 250 rpm in a shaking incubator for 1 h at room temperature. After shaking, the extracts were filtered using Whatman filter paper # 1 and 65 µl of the sediment extracts was dispensed into each of the microcupules. API ZYM™ strips were then covered and incubated at room temperature for 48 h. After incubation, ZYM A and ZYM B reagents were added to each microcupule as suggested by the manufacturer. The strips were then allowed to sit at room temperature for 10 min before collecting the data. Each microcupule was scored positive (colour development) or negative (no colour development). For microcupule that scored positive, a numerical value of 1–5 was assigned according to the colour chart provided by the manufacturer. The results were then ranked as reactions of low intensity (1), moderate intensity (2–3), and high intensity (4–5) [23–25].

Statistical analyses

To achieve normal distribution, the resulting data were transformed by natural logarithm. Paired *t*-test was used to analyse differences between the river sediments and lake sediments. To calculate the general correlation between enzymes and biological parameters (bacterial and fungal densities), the Pearson-product moment correlation was used. Analysis of variance (ANOVA) tests were used to compare among three lake sites (LC1, LC2, and LC3) and three river sites (SC1, SC2, and SC3). A separate ANOVA test was also carried out to compare the carbon utilization patterns of the microbial communities at different seasons (spring, summer, fall, and winter) for the lake and river sediments. When ANOVA showed a significant difference between sites ($P \leq .05$), means were separated using Tukey's *t*-test. Principal component analysis (PCA) was used to determine differences among enzymatic profiles [35] from the sediment samples. The interpretation of the principal components was based on significant factor loading of the individual substrates on each of the PCs. All statistical analyses were performed using SYSTAT version 13.1 (SPSS Inc., Chicago, IL, USA).

Results

Physico-chemical properties

Water temperature at the sites (Table 3) correlated with the ambient temperature (Table 1), with the highest temperature observed in the summer and the lowest in the winter sampling. The average water temperature for spring was 12°C, summer was 25°C, fall was 19°C,

Table 3. Water temperature, water depth, turbidity and sediment pH.

Sites	Water temperature (°C)	Water depth (m)	Sediment height (m)	Turbidity (m)	Water pH
Spring					
Saint Clair River sites					
SC1	11.5	3.05	172.2	nd	8.7
SC2	10.2	091	174.3	nd	8.2
SC3	12.9	1.22	170.0	nd	7.9
Lake Saint Clair sites					
LC1	12.4	1.07	174.2	nd	7.9
LC2	15.5	0.76	174.2	nd	7.9
LC3	12.3	0.52	174.2	nd	8.0
Summer					
Saint Clair River sites					
SC1	25.0	3.05	172.2	031	7.9
SC2	23.9	1.19	172.2	1.19	8.9
SC3	22.2	1.31	172.2	0.31	8.2
Lake Saint Clair sites1.49					
LC1	22.8	0.76	174.8	0.76	7.3
LC2	25.6	0.61	174.9	0.61	7.1
LC3	28.9	1.31	174.2	1.22	8.0
Fall					
Saint Clair River sites					
SC1	22.9	2.35	173.2	0.31	7.7
SC2	21.3	1.49	174.0	1.49	8.7
SC3	20.6	1.41	173.1	0.31	7.7
Lake Saint Clair sites					
LC1	20.6	0.91	174.6	0.91	8.7
LC2	15.6	0.31	175.2	1.19	8.7
LC3	12.3	1.80	173.7	0.31	8.8
Winter					
Saint Clair River sites					
SC1	5.00	2.68	172.4	0.31	8.6
SC2	6.67	0.91	174.2	1.19	8.1
SC3	8.89	1.95	173.1	0.31	7.9
Lake Saint Clair sites					
LC1	7.22	0.91	174.2	0.76	8.0
LC2	7.78	0.61	174.5	0.61	7.7
LC3	7.22	1.37	173.7	1.22	7.8

Note: nd = no data available.

and winter was 7°C (Table 3). The water temperature between the river and lake sites was similar at each sampling season. Throughout the sampling period, the water depth remained fairly consistent with the shallowest in site LC2 (0.31–0.76 m) and the deepest in site SC1 (2.31–3.05 m). Sediment height varied between 172 and 174 m. The turbidity data were measured in the summer, fall, and winter (Table 3). Turbidity readings were higher in sites SC2 (1.19–1.49 m) and LC3 (1.22 m) than those of sites SC1 (0.31 m), SC3 (0.31 m), LC1 (0.76–0.91 m), and LC2 (0.61 m). Water turbidity remained consistent at each site at different seasons. Water pH was relatively consistent across all seasons (Table 3), with the lowest pH being 7.1 (LC2 summer) and the highest being 8.9 (SC2 summer) (Table 3).

Sediment samples from the Saint Clair River had relatively higher moisture contents than those from Lake Saint Clair (19–68%), particularly those from sites SC1 (72–102%) and SC3 (48–95%) (Table 4). Moisture contents of the sediments from the lake sites (LC1, LC2, and LC3) were stable in the spring (22–68%), summer (31–56%), and fall (38–45%) but dropped significantly during the winter sampling (19–20%). Moisture contents of the sediments from the river sites were highest in the spring, with site SC1 reaching as high as 102%. Summer and winter moisture content ranges for the three sites (SC1, SC2, and SC3) were similar (24–72% and 19–78%, respectively). The moisture contents between the lake sites (19–68%) and the river sites (22–102%) also varied considerably. The pH ranges of the sediment extracts (1:10 sediment–water ratio) from the river and lake sites were very similar (6.29–7.32 for river sites; 6.52–7.23 for lake sites), and varied little between seasons. The average sediment pH of all sites for spring, summer, fall, and winter was 6.77, 7.00, 6.99, and 6.81, respectively (Table 4). These pH values were lower than the water pH of the sites at the time of sampling (Table 3). The seasonal averages for all sites were 8.10 in spring; 7.90 in summer; 8.26 in fall; and 8.01 in winter (Table 4). Regardless of the lower sediment pH values, the extracts had similar pH between the seasons. The electrical conductivity of the extracts (1:10 sediment–water ratio) varied significantly between sites (Table 4). The electrical conductivity reading of the extracts from the river sites varied between 20 and 258 $\mu S\ cm^{-1}$, while that of the lake sites fluctuated between 25 and 225 $\mu S\ cm^{-1}$. The highest electrical conductivity reading (258 $\mu S\ cm^{-1}$) was from sediment extracts in the summer at site SC1, while the lowest (20 $\mu S\ cm^{-1}$) was in the winter at site SC2. The average conductivity for spring across all sites was 152 $\mu S\ cm^{-1}$; 119 $\mu S\ cm^{-1}$ for summer; 131 $\mu S\ cm^{-1}$ for fall; and 89 $\mu S\ cm^{-1}$ for winter (Table 4).

Sediment samples from the river sites tended to have higher OM contents than the lake sites, particularly from sites SC1 and SC3, which ranged from 4.94% to 67.5% (Table 2). In general, the OM contents were found to be highest in the spring, especially in the sediment samples from sites SC1 (40.5%), SC3 (67.5%), and LC3 (24.63%). As the seasons went on, the OM contents of the sediments declined in all sites from an average reading of 31.59% in spring to 2.68% by winter sampling. This decline in OM contents coincided with increases in ash contents, which reached their peaks in the winter (Table 4).

Bacterial and fungal abundance

The heterotrophic bacterial counts of the river sediments were higher (4.53–7.07 $\log_{10}$ CFU g^{-1}) than the lake sediments (3.22–5.53 $\log_{10}$ CFU g^{-1}) (Table 4). The total average CFU g^{-1} for the lake across all sites was 5.12 $\log_{10}$ CFU g^{-1}, whereas the average for the river across all seasons was 2.58 $\log_{10}$ CFU g^{-1}. Average bacterial counts between seasons ranged between 4.67 and 5.65

Table 4. Physico-chemical and microbial properties of the sediments.[a]

Sites	Moisture content (%)	pH (1:10 water extract)	Electrical conductivity (μS cm^{-1})	Ash content (%)	Organic matter (%)	Bacterial Counts (log_{10} CFU g^{-1} dry wt.)	Fungal Counts (Log_{10} CFU g^{-1} dry wt)
Spring							
Saint Clair River sites							
SC1	102 ± 16.3	6.64 ± 0.02	228 ± 17.1	36.67 ± 40.57	64.33 ± 40.46	6,19 ± 1.07	4.43 ± 0.43
SC2	22 ± 2.2	6.29 ± 0.14	33 ± 5.0	86.40 ± 16.84	13.60 ± 16.84	4.53 ± 0.94	4.23 ± 0.27
SC3	95 ± 15.0	6.88 ± 0.07	255 ± 20.8	32.54 ± 11.17	67.50 ± 11.17	5.97 ± 0.08	2.04 ± 0.48
Lake Saint Clair sites							
LC1	26 ± 0.3	6.53 ± 0.35	90 ± 11.6	96.95 ± 1.568	3.052 ± 1.568	3.22 ± 0.46	1.98 ± 0.31
LC2	32 ± 6.2	7.08 ± 0.20	80 ± 0.0	78.65 ± 37.12	21.35 ± 37.12	3.72 ± 0.06	2.53 ± 0.15
LC3	68 ± 16.1	7.17 ± 0.29	225 ± 5.8	80.28 ± 24.63	19.72 ± 24.63	5.23 ± 0.85	2.90 ± 0.21
Summer							
Saint Clair River sites							
SC1	72 ± 81.46	6.67 ± 0.02	258 ± 17.1	67.37 ± 32.58	32.63 ± 32.58	6.48 ± 0.42	4.61 ± 0.08
SC2	24 ± 6.50	7.13 ± 0.13	45 ± 5.8	99.05 ± 0.36	0.95 ± 0.36	5.03 ± 0.03	3.14 ± 0.02
SC3	48 ± 5.80	7.32 ± 0.01	168 ± 5.0	95.21 ± 0.88	4.79 ± 0.88	7.07 ± 0.29	5.18 ± 0.12
Lake Saint Clair sites							
LC1	31 ± 0.90	6.68 ± 0.21	73 ± 20.6	98.26 ± 0.99	1.75 ± 0.98	4.68 ± 0.45	3.08 ± 0.12
LC2	28 ± 0.54	6.96 ± 0.15	55 ± 5.8	98.34 ± 0.10	1.66 ± 0.10	4.04 ± 0.27	2.58 ± 0.20
LC3	56 ± 7.41	7.23 ± 0.05	115 ± 5.8	92.46 ± 0.67	7.54 ± 0.67	4.68 ± 0.40	3.36 ± 0.04
Fall							
Saint Clair River sites							
SC1	81 ± 22.50	6.80 ± 0.05	193 ± 9.6	91.34 ± 1.80	8.66 ± 1.80	6.06 ± 0.09	3.78 ± 0.04
SC2	37 ± 7.96	7.10 ± 0.10	63 ± 5.0	98.16 ± 0.09	1.84 ± 0.09	4.07 ± 0.29	2.89 ± 0.22
SC3	81 ± 27.34	6.86 ± 0.03	150 ± 0.0	90.89 ± 0.91	9.11 ± 0.91	6.24 ± 0.03	4.46 ± 0.05
Lake Saint Clair sites							
LC1	38 ± 10.06	6.78 ± 0.12	160 ± 58.8	97.18 ± 0.80	2.82 ± 0.80	4.71 ± 0.20	1.74 ± 0.30
LC2	42 ± 15.89	7.18 ± 0.04	113 ± 9.6	98.27 ± 0.97	1.74 ± 0.97	5.29 ± 0.28	3.14 ± 0.11
LC3	45 ± 14.50	7.24 ± 0.10	105 ± 5.8	95.76 ± 1.16	4.24 ± 1.16	5.29 ± 0.13	2.76 ± 0.46
Winter							
Saint Clair River sites							
SC1	74 ± 23.93	6.70 ± 0.43	185 ± 12.9	95.06 ± 2.05	4.94 ± 2.05	5.69 ± 0.06	4.43 ± 0.43
SC2	19 ± 0.84	6.75 ± 0.14	20 ± 0.0	98.81 ± 0.38	1.19 ± 0.38	3.38 ± 0.02	4.23 ± 0.27
SC3	78 ± 5.38	6.73 ± 0.06	165 ± 17.3	92.82 ± 1.42	7.18 ± 1.42	5.76 ± 0.05	2.04 ± 0.48
Lake Saint Clair sites							
LC1	21 ± 1.85	6.75 ± 0.04	33 ± 5.0	99.36 ± 0.31	0.87 ± 0.29	3.63 ± 0.26	1.98 ± 0.31
LC2	19 ± 0.86	7.17 ± 0.27	103 ± 59.67	98.76 ± 0.33	1.24 ± 0.33	4.02 ± 0.09	2.53 ± 0.15
LC3	20 ± 1.28	6.77 ± 0.10	25 ± 5.8	99.13 ± 0.27	0.64 ± 0.32	5.53 ± 0.34	2.90 ± 0.21

[a]Mean and standard deviation of three replicates are shown.

log_{10} CFU g^{-1}, with the highest average bacterial count observed in the fall. The river sediments had higher average bacterial counts (5.55 log_{10} CFU g^{-1}) than the lake sediments (4.51 log_{10} CFU g^{-1}) (Figure 3(B)). Sediments with higher clay contents (SC1 and SC3) had the highest bacterial numbers (6.10–6.26 log_{10} CFU g^{-1}) (Table 4).

The fungal counts of the river sediments (SC1, SC2, and SC3) were generally higher than the lake sediments (LC1, LC2, and LC3) (Table 4). The fungal numbers of the river sediments over time had an average of 4.27 log_{10} CFU g^{-1}, while the lake sediments showed an average of 2.65 log_{10} CFU g^{-1}. These fungal numbers fluctuated for both ecosystems across seasons, with both reaching their peak growth in the summer (lake sediments, 4.69 log_{10} CFU g^{-1}; river sediments, 5.99 log_{10} CFU g^{-1}). The average fungal counts between seasons ranged between 3.0 and 3.6 log_{10} CFUg^{-1}. Sediments from sites SC1 and SC3 had higher fungal numbers (3.62–4.31 log_{10} CFU g^{-1}) than SC2, LC1, LC2, and LC3 (2.19–3.43 log_{10} CFU g^{-1}) (Table 4).

Extracellular enzyme profiles in the sediments

Extracellular enzyme profiles from different sediment samples showed that the measured activities were varied among enzymes, and revealed differences and similarities among sediment samples. Of the 19 enzymes examined, 4–19 showed evidence of activity (Figure 1). These enzymes were detected in river and lake sediments collected at different sites and seasons. Four enzymes including, α-galactosidase, β-glucoronisase, α-mannosidase, and α-fucosidase showed no evidence of activity in the lake sediment samples (LC1, LC2, and LC3). These enzymes were active in river sediments, particularly from site SC1. In general, the numbers of active enzymes from the lake sediment samples were similar (6–14 enzymes) to the river sediment samples (4–19 enzymes). Both river and lake sediment samples displayed low to moderate activity with only 1–4 enzymes exhibiting high enzyme activities for each site (Figure 1). Paired *t*-test results showed that activities of the 19 extracellular enzymes were similar

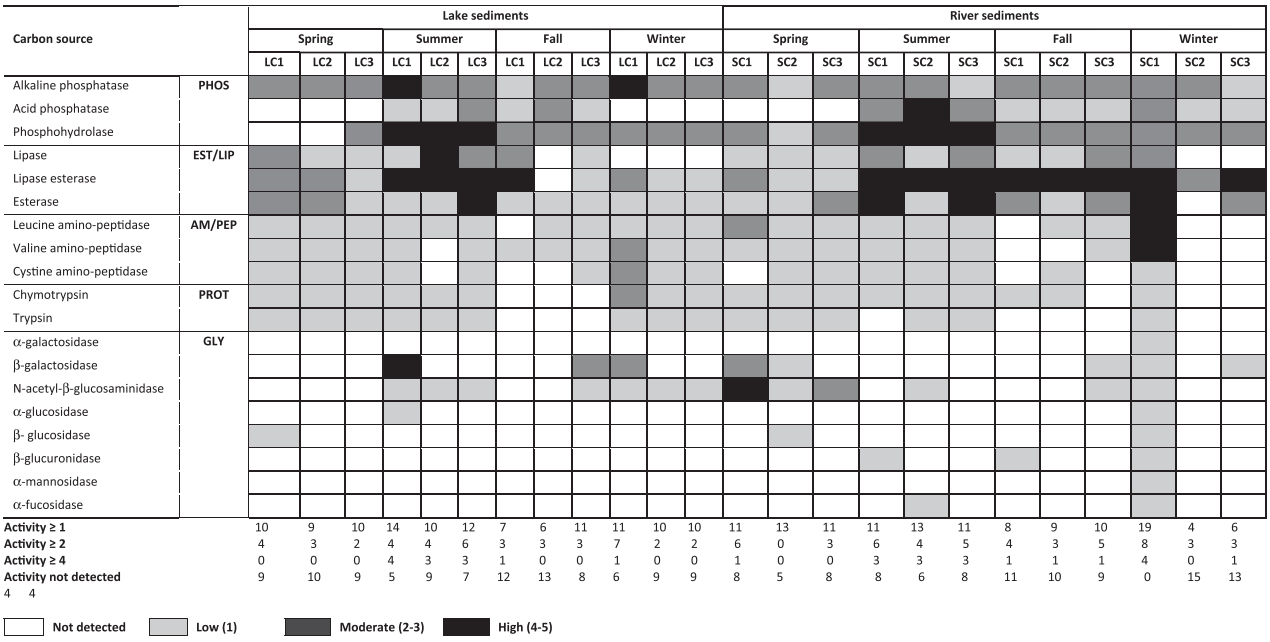

Figure 2. Profiles of the 19 extracellular enzymes from the lake (LC1, LC2, and LC3) and river sediment samples (SC1, SC2, and SC3) taken during spring, summer, fall, and winter. Shading in the boxes indicates the relative abundance of the enzyme. Values are as follows: White, not detected; light grey, reactions of low intensity (value of 1); dark grey, reactions of moderate intensity (values of 2–3); black, reactions of high intensity (value of 5). The number of enzymes showing reactions ≥1, ≥2, ≥4, and the total number of enzymes that did not display activity are indicated below each column.

and varied only in three extracellular enzymes (esterase, leucine aminopeptidase, and β-galactosidase). The river sediments had significantly higher esterase ($P = .007$) and leucine aminopeptidase activities ($P = .019$) than the lake sediments. The lake sediments, on the other hand, had significantly higher β-galactosidase activity ($P = .047$) than the river sediments.

Spatial variabilities

The number of positive enzyme activities was higher in SC1 (8–19 enzymes) and LC3 (10–12) than the other four sites (4–10 enzymes). Site SC1 is located upstream where the water from Lake Huron drains into the Saint Clair River and site LC3 is situated downstream where Lake Saint Clair empties its water into Detroit River at the City of Grosse Pointe Park (Figure 1). The number of enzymes showing moderate to high activities was higher in sites LC1 and LC3 than site LC2 (Figure 2). The lake sites LC1 and LC3 had significantly higher leucine aminopeptidase (ANOVA, $F = 4.40$, $P = .031$), cysteine aminopeptidase (ANOVA, $F = 3.17$, $P = .041$), and *N*-acetyl-P-glucosaminidase activities (ANOVA, $F = 1.46$, $P = .042$) than site LC2. A higher number of enzymes demonstrating moderate to high activities was found in the river sites SC1 and SC3 than SC2 (Figure 2). Results of ANOVA revealed significantly higher activities of lipase (ANOVA, $F = 2.65$, $P = .014$), leucine aminopeptidase (ANOVA, $F = 7.27$, $P = .0062$), valine aminopeptidase (ANOVA, $F = 9.26$, $P = .0024$), and trypsin (ANOVA, $F = 0.51$, $P = .041$) enzymes in sites SC1 and SC3 than in site SC2.

The extracellular enzyme profiles in the sediments at different sites were compared based on the five enzyme categories including various phosphatases, esterases/lipases, aminopeptidases, proteases, and glycosyl hydrolases (Figure 3). The sediment microbial communities varied in their ability to synthesize hydrolytic extracellular enzymes. Phosphatases and esterases/lipases were synthesized most actively in sediment samples from lake sites LC1 and LC3, and it was phosphatases in lake site LC2 (Figure 3(A)). Phosphatases, esterases/lipases, and glycosyl hydrolases were the most active enzymes in river site SC1 (Figure 3(B)). Phosphatases and esterases/lipases enzymes were also synthesized most actively in sediment samples from SC2 and SC3, while of glycosyl hydrolases were synthesized least actively (Figure 3(B)).

Seasonal variabilities

To determine the seasonal effects on enzyme activities, sediment samples were collected in spring, summer, fall, and winter. Analysis of variance results only showed significant difference between seasons for six enzymes: alkaline phosphatase (*F*-value = 3.40, $P = .026$), phosphohydrolase

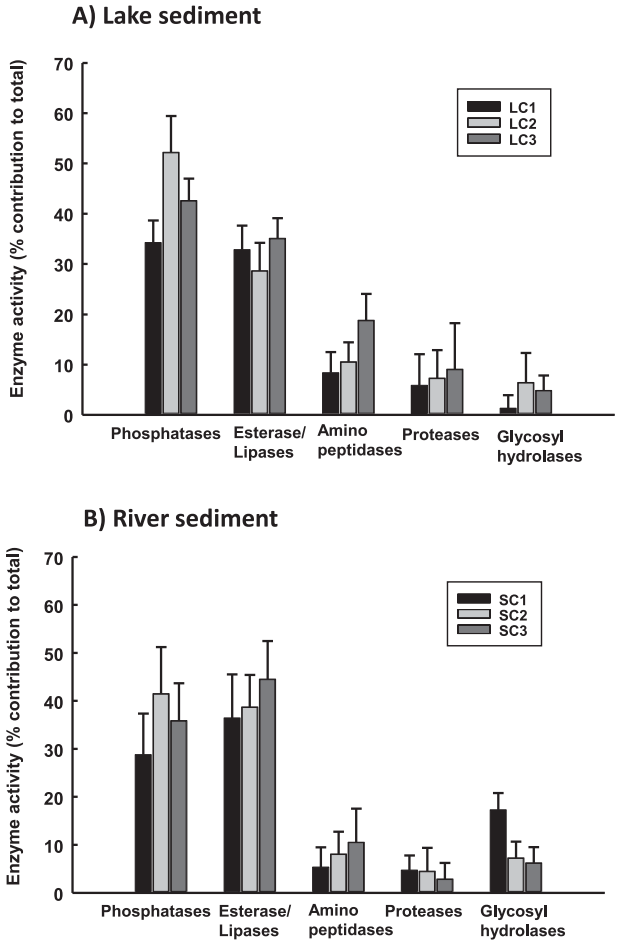

Figure 3. Percentage contributions of the extracellular enzyme activities separated into substrate categories from the (A) lake sites (LC1, LC2, and LC3) and (B) river sites (SC1, SC2, and SC3) different sites.

(*F*-value = 10.91, *P* =< .0001), lipase esterase (*F*-value = 8.23, *P* = < .0001), and cysteine aminopeptidase (*F*-value = 3.08, *P* = .037), chymotrypsin (*F*-value = 2.99, *P* = .046), and trypsin (*F*-value = 2.60, *P* = .044). That is, alkaline

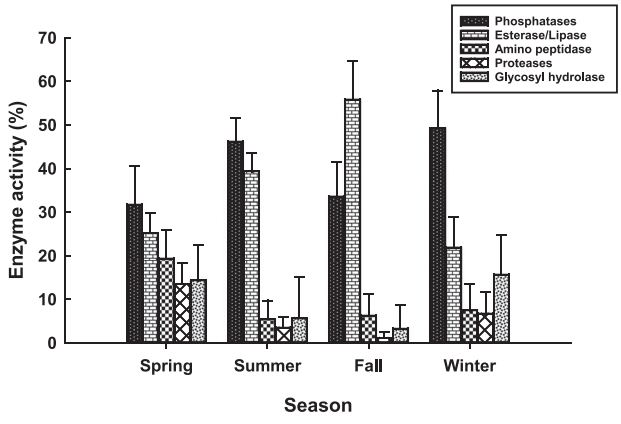

Figure 4. Percentage contributions of the extracellular enzyme activities separated into substrate categories from different seasons.

Table 5. Pearson-product moment correlation coefficients between biological parameters, TOC concentration, and enzymes among across the river and lake sediment samples.

Lake sediment	Correlation coefficient (r^2)
Bacterial cell count	
Enzyme	
Alkaline phosphatase	0.585* (*P* = .042)
Acid phosphatase	0.619* (*P* = .035)
Esterase	0.741** (*P* = .007)
Esterase lipase	0.552 * (*P* = .048)
Leucine aminopeptidase	0.750** (*P* = .005)
Valine aminopeptidase	0.543* (*P* = .049)
α-chymotrypsin	−0.610* (*P* = .035)
Fungal cell count	
Enzyme	
Acid phosphatase	0.675* (*P* = .015)
Phosphohydrolase	0.656* (*P* = .020)
Esterase-lipase	0.543* (*P* = .049)
Esterase	0.649 (*P* = .049)
Leucine aminopeptidase	0.759** (*P* = .005)
Organic matter	
Enzyme	
Alkaline phosphatase	−0.591* (*P* = .040)
Phosphohydrolase	−0.545* (*P* = .049)
Valine aminopeptidase	−0.682* (*P* = .012)
N-acetyl-β-glucosaminidase	−0.570* (*P* = .046)
β-glucosidase	−0.542* (*P* = .049)

Note: Only significant correlations are shown. Data are inclusive of all samples (river sediments and lake sediments) collected at different sites and seasons. Single, double, triple asterisks indicate *P*-values less than .05, .01, and .001. *n* = 72.

phosphatase, phosphorylase, and lipase esterase activities were highest in the summer, but lowest in the winter; and the cysteine aminopeptidase, chymotrypsin, and trypsin activities were highest in the winter but lowest in the fall. Although there were no significant trends between seasons for most enzymes assayed, more enzymes exhibited high activities during summer (3–4 enzymes) than in spring (1 enzyme), fall (1 enzyme), and winter (1–3 enzymes) (Figure 2). Extracellular activities of microbial communities based on enzyme categories were also compared in the sediments collected in spring, summer, fall, and winter seasons (Figure 4). In general, phosphatases were produced the most actively by the microbial communities in spring summer and winter, while esterases/lipases were produced most intensively in the fall samples (Figure 4).

Enzyme activities, microbial numbers, and OM content

Pearson-product moment correlation showed correlations between enzyme activities and microbial and OM content. Significant correlations occurred for bacterial cell counts. These include alkaline phosphatase, acid phosphatase, esterase, esterase lipase, leucine aminopeptidase, valine aminopeptidase, and α-chymotrypsin (Table 5). These enzymes exhibited higher activities as the bacterial counts increased. Six of these enzymes had significant correlations fungal counts (Table 5). For

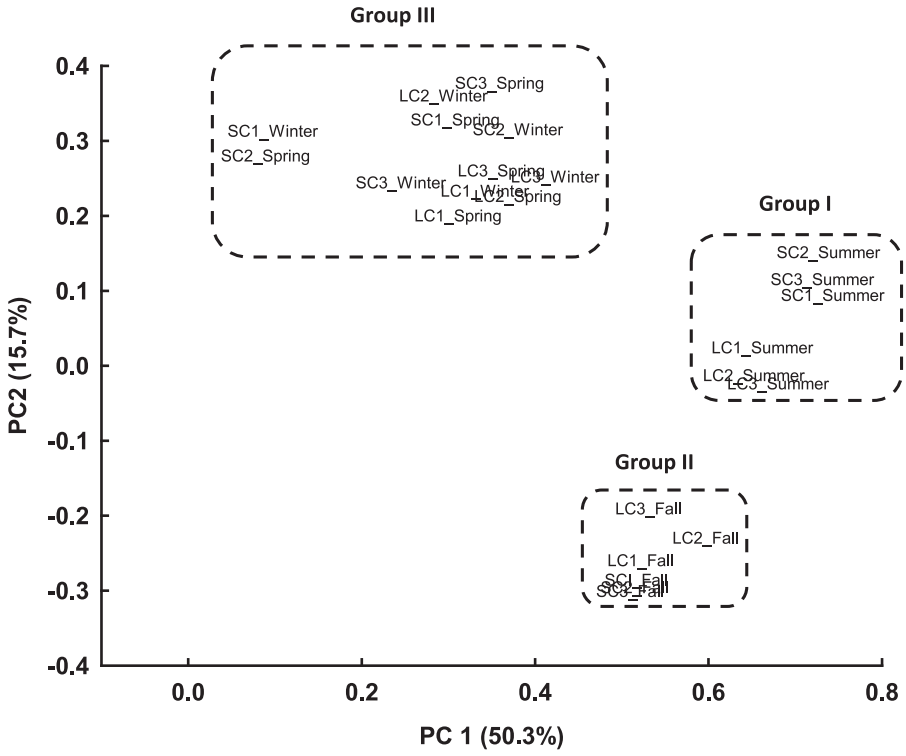

Figure 5. Principal component analysis of extracellular enzyme profiles of lake and river samples from different seasons.

the OM content, three significant correlations occurred for OM content and the correlations were all negative. These included alkaline phosphatase, phosphohydrolase, valine aminopeptidase, *N*-acetyl-β-glucosaminidase and β-glucosidase (Table 5).

Table 6. Correlation between principal components (PC1 and PC2) and the single variables for the PCAs described in the text.

	PC 1	PC 2
I. Phosphatases		
Alkaline phosphatase	0.549	
Acid phosphatase	0.656	
Phosphohydrolase	0.655	
II. Esterases		
Lipase	0.548	
Lipase esterase	0.549	−0.732
Esterase		0.641
III. Aminopeptidase		
Leucine aminopeptidase		
Valine aminopeptidase	0.552	
Cystine aminopeptidase		
IV. Proteases		
Chymotrypsin		
Trypsin		0.640
V. Glycosyl hydrolases		
α-Galactosidase		
β-Galactosidase	xx	
N-acetyl-P-glucosaminidase		
α-Glucosidase		
β-Glucosidase	xx	
β-Glucuronidase		
α-Mannosidase		
α-Fucosidase		

Note: All substrates with an *r*-value of >0.5 are shown with $P < .05$. *r* = Pearson correlation coefficient.

Multivariate analysis

To understand how the different samples were related to each other based on the extracellular enzyme profiles of the sediment samples, the API ZYM scores were analysed using PCA (Figure 5). PC1, which accounts for most of the variance, separated the sediment samples into three groups. Samples with similar activities grouped together. For instance, Group I consisted of samples with the highest enzyme activity (the summer samples); Group II comprised of samples with moderate enzyme activities (fall samples); and Group III included samples marked with lowest enzyme activities (spring and winter samples) (Figure 5). To determine which enzyme accounted for the differences between Group I (summer samples), Group II (fall samples), and Group III (spring and winter samples), the correlation between enzyme variable were determined (Table 6). For PC1, significant loadings were observed for 7 of the 19 extracellular enzymes. These loadings include three phosphatases (alkaline phosphatase, acid phosphatase, and phosphohydrolase), two esterases (lipase and lipase esterase), one aminopeptidase (valine aminopeptidase) and two glycosyl hydrolase (*N*-acetyl-P-glucosaminidase and β-glucosidase). Variances in PC2 were explained principally by the activities of two esterases (lipase and lipase esterase) and one protease (trypsin) (Table 6).

Discussion

The present study attempted to monitor the extracellular enzyme activity in Saint Clair River and Lake Saint Clair sediments using a phenotypical approach (API ZYM), and the potential of this technique in resolving spatial and temporal differences and ecological function attributable to river and lake pollution. A wide range of extracellular enzymes was found to be active in the river and lake sediments samples (Figure 2) suggesting that the extant heterotrophic microbial communities were able to decompose a wide spectrum of organic compounds whose molecules differ in size from monomers to polymers. For the heterotrophic communities, those compounds constitute a very important source of carbon, nitrogen, and energy, and were used for biosynthesis or respiration processes [36]. The presence of phosphatases (indicators of phosphorus acquisition), aminopeptidases (indicators of nitrogen acquisition), and glycosyl hydrolases (indicators of carbon acquisition) confirms previous findings from other river [37–39] and lake sediments [40,41]. However, the occurrence of lipases, lipase esterases, and esterases has not yet been previously reported and broadens the spectrum of the metabolic capabilities of the microbial communities in Saint Clair River and Lake Saint Clair sediments. Esterases are linked to phosphorus and sulfur acquisition. They comprise a diverse group of hydrolases that catalyse the breakdown or formation of ester bonds. These enzymes widely distributed in animals, plants, and microorganisms. Lipases are linked to carbon acquisition. They are mainly active against water-insoluble substrates, such as triglycerides which are composed of long-chain fatty acids, whereas esterases preferentially hydrolyse simple esters and triglycerides composed of fatty acids shorter than C6 [42]. Esterases and lipases are both capable of attacking emulsified mono-, di-, and triglycerides, and of splitting them with the yield of glycerol and fatty acid residues [43].

Examination of the extracellular enzyme profiles revealed very little differences between lake and river sediment samples (Figure 2) in terms of the numbers of active enzymes. However, the river sediments were characterized by higher activities of esterase and leucine aminopeptidase with values significantly higher than the lake sediments. Generally, the magnitude of activity ranges of these enzymes in river sediments was in the order phosphohydrolases > esterase-lipase > alkaline phosphatase > esterase > leucine aminopeptidase. Conversely, significantly higher β-galactosidase activity was observed in the lake sediments than the river sediments. The magnitude of activity in the lake sediments was in the order phosphohydrolases > alkaline phosphatase > esterase-lipase > acid phosphatase > β-glucosidase. The higher activities of leucine aminopeptidase in the river sediments indicate the presence of many peptides and amino acid amides of the L-configuration (e.g. L-leucyl-peptides and L-leucyl-amides) [44]. The activity of leucine aminopeptidase is exclusively associated with heterotrophic bacteria [44]. In laboratory studies, leucine aminopeptidase activity was seen to be closely related to the content of labile OM released by algae, and this indicates close algal–bacterial coupling [45]. The higher activities of β-galactosidase in the lake sediments indicate the presence of β-linked disaccharides of glucose, cellohexaose, and carboxymethylcellulose [46].

Lipases, esterases, and phosphatases are groups of enzymes that were synthesized most intensively by the sediment microbial communities. The high activity of lipid- and esterase-hydrolysing enzymes in the sediments might be due to the presence of higher lipid compounds in the sediments. Lipid compounds constitute a minor but important fraction of the total organic matter in aquatic sediments [47]. The main sources of lipids in the water bodies include phytoplanktons, zooplanktons, meiobenthos, macrobenthos, and detritus [48]. Other sources include products associated with microbial activity, inputs of terrestrial material from the watershed region, allochthonous pollutant sources such as unleaded and diesel fuels, used engine oils, and engine exhausts [49,50]. Rigo et al. [51] and Aluyor et al. [52] noted that lipases and esterases are capable of hydrolysing fats and oils. Of particular relevance to sediments are organic compounds such as ketones, sterols, fatty acids, hydroxy acids, n-alkanes, and n-alkanols [53,54]. Among the five groups of enzymes surveyed in this study, phosphatases showed the highest potential level of activity in the sediments. Phosphatase activity is prevalent in aquatic bacteria and is closely related to both the phosphorus and carbon cycles [55]. Extracellular phosphatases play important role in supplying phosphorus to heterotrophic microorganisms and to autotrophic algae [56]. Their activities originate from bacterioplankton but also from phytoplankton and zooplankton [57]. Phosphorus acquisition, especially in phosphorus-limited areas, is dependent on the available enzymes to hydrolyse dissolved organic compounds. It is interesting to see that phosphatases increased during summer, which is linked to the decrease in inorganic phosphates in water. Moreover, algae are also producing phosphatases and their variation can be highly modulated by primary production and the factors (e.g. temperature, inorganic phosphates) enhancing it. There are two forms of phosphatases (alkaline and acid phosphatases). While both forms of phosphatases can hydrolyse all phosphoric

esters, the activity of alkaline phosphatase is usually higher than the activity of acid phosphatase [58,59]. Higher activity of alkaline phosphatase was also determined in the present study. The least actively synthesized enzymes in the lake sediments were the glycosyl hydrolases, while it was the aminopeptidases in the river sediments (Figure 3(A)). The activity of these enzymes was either low or non-existent in most sediment samples. The highest glycosyl hydrolase activities were observed from site SC1 (located upstream where the water from Lake Huron drains into Lake St Clair). This site also had the highest bacterial and fungal counts and OM concentration (Table 4).

The sediments in river site SC1 (upstream site where the water from Lake Huron drains into the Saint Clair River) and LC3 (downstream site situated downstream where Lake Saint Clair empties its water into Detroit River at the City of Grosse Pointe Park) had the highest metabolic diversity. The microbial communities in these sites were able to produce more active extracellular enzymes that are able to breakdown a broader range of substrates. Their capacity to use a wide range of enzymes may be linked to differences in river and lake water quality.

The microbial communities in lake and river sediments responded to seasonal shifts in substrate availability by changing the compositional extracellular enzymes released. Alkaline phosphatase, phosphorylase, and lipase/esterase activities were highest in the summer, but lowest in the winter; cysteine aminopeptidase, chymotrypsin, and trypsin activities were highest in the winter but lowest in the fall. This trend corresponded with higher bacterial and fungal densities in the summer and lower counts in the winter and fall (Table 4), which is not surprising as bacteria (and fungi to a lesser extent) are the main producers of extracellular enzymes. Majority of the enzymes showed evidence of activity at various extents during the four seasons; however, four enzymes including α-galactosidase, β-glucoronisase, α-mannosidase, and α-fucosidase showed no evidence of activity in all sediment samples. There was no evidence of activity observed for β-gluconidase in spring, α-glucosidase, and β-gluconidase in both summer and fall, and trypsin in fall. The lack of activity of the enzymes investigated in this study may indicate the abundance of available substrates. Microorganisms normally synthesize enzymes only when simple sources of organic C are insufficient [60–62]. The synthesis of many extracellular enzymes in aquatic environments may also be inhibited by the accumulation of the hydrolysis end-product in the cell or in the surrounding environment [63]. The seasonal dynamics of specific extracellular enzyme activity in this study was not only due to changes in temperature [39] and bacterial and fungal abundance [30,64], but also the varying supply of major substrates [8,65,66].

Conclusions

Although this study did not indicate a clear and consistent difference in the level of potential activity of bacterial enzymes between river and lake sediments, the API ZYM assay has proven to be effective in discriminating seasonal and spatial differences in lake and river sediments. This study provided important information on relevant sediment processes, associated to changes in enzyme activity and functional diversity. The enzyme activities in this study reflected the variations in temperature, the availability, and degradability of organic compounds, as well as differences in the composition of microbial populations. The differences in the extracellular enzyme profiles among the different seasons might suggest that the sediment microbial communities are perhaps forced to adapt to the changing inputs of organic matter and nutrients. Hence, the microbial communities in the sediments demonstrate a higher plasticity in metabolic pathways in order to exploit the shifting and limited carbon sources available for their growth and survival. While a correlation was observed between bacterial/fungal densities and enzyme activities was observed in this study, the estimation of total aerobic heterotrophic bacteria and fungi in sediments by culture methods has some limitations since many microorganisms from the environment will not grow. The low cultivability and the incubation limitations of cultural methods using environmental samples are well known, and the limitations should be considered when the results are interpreted. The results suggest that API ZYM is a simple and rapid enzyme assay procedure to evaluate natural processes in ecosystems and their changes.

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Tiquia-Arashiro conceived and designed the study. Patel performed the experiments and analysed the data with Gismondi. Alsaffar collected the sediment samples and field data. Tiquia-Arashiro and Patel wrote the manuscript, which was completed with input from Gismondi and Alsaffar.

Disclosure statement

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