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Relationship between sediment phosphorus release rates and characteristics of the benthic microbial community in a hypereutrophic marsh

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Cootes Paradise Marsh is a hypereutrophic coastal wetland of Lake Ontario that has received sewage from the town of Dundas, Ontario for over eight decades. As such, sediments are nutrient rich and phosphorus release from the sediments is substantial. Release rates of soluble reactive phosphorus from frozen sediments collected at eleven representative sites in the marsh were highly variable, ranging from 0.96 to 28.28 mg m^2 d^{-1}*. We wanted to evaluate spatial variance of the benthic microbial community and determine if this variation could be correlated to phosphorus release rates from corresponding sediments. Fresh sediment samples were collected from the same sites and characterized on the basis of sole-carbon-source utilization patterns through a Principal Components Analysis. Microbial communities located closest to the sewage outfall, had a high affinity for phosphorylated substrates, and used mainly carbohydrates, and were separated from communities located distal to the sewage source, which readily used polymers and simple sugars. Subsequently, sediment samples were collected from two sources and kept frozen for later phosphorus-release experiments while comparable samples were also collected to characterize the benthic microbial community from these sites. Phosphorus-release rates and utilization of specific substrates for the frozen sediment samples were significantly correlated (Spearman's Rank Correlation Analysis;* $P = 0.041$*), indicating a direct link between release and patterns of carbon utilization. Microbial communities of freshly collected sediments differed significantly from those of frozen sediments, and these differences were also observed for corresponding phosphorus-release rates. We conclude that the microbial community structure likely plays a major and direct role in the release and uptake of phosphorus from the sediment in Cootes Paradise Marsh.*

Keywords: degraded, nutrient-rich, spatial variation, substrate utilization patterns

Introduction

Lake restoration efforts are often focused on the reduction of external phosphorus inputs, even though in highly eutrophic systems, the release of P from sediments may be comparable to, and may even exceed external sources (Premazzi and Provini, 1985; Nürnberg, 1988; Auer et al., 1993), a phenomenon that may explain the delayed recovery in past lake restoration projects (Jacoby et al., 1982; Lijklema, 1993; Sondergaard et al., 1993; Phillips et al., 1994). Although several physical (e.g., wind, bioturbation), chemical (e.g., concentration gradients, pH, redox conditions) and biological (e.g., microbial activity, macrophytes) factors can affect P exchange, by and large research has focused on the traditional model

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of Mortimer (1941, 1942) which is based on redox-driven desorption. Ferric iron (Fe^{3+}) adsorbs phosphate to form solid FeOOH-PO_4 complexes (Gächter et al., 1988), and when redox potentials drop below +120 mV, Fe^{3+} is reduced to Fe^{2+}, releasing PO_4^{3-} into the overlying water (Richardson, 1999; Sinke et al., 1993). Reduced/anoxic conditions can be permanent in the case of deeper stratified lakes, or can be temporary in shallow systems where episodic bacterial or epipelic algal respiration intensifies at the sediment/water interface at night or when algal bloom conditions persist (Carlton and Wetzel, 1988). An increase in the ambient water temperature may also stimulate bacterial metabolism and the associated increase in oxygen consumption may lower the redox potential and stimulate the release of Fe-bound P (Carlton and Wetzel, 1988; Jensen and Andersen, 1992).

Previous models of P-release have been based almost exclusively on abiotic factors; relatively little attention has been paid to biological processes. For example, microbes are assumed to play only an indirect role in P-release by producing reduced conditions through respiration (Gächter et al., 1988). However, Wetzel (1999) demonstrated that bacteria can also mineralize organic phosphorus (OP) to inorganic forms or release P upon cell death and thus directly contribute to the P-release process. It is well known that different bacterial species can sequester P under both aerobic and anaerobic conditions (deMontigny and Prairie, 1993). In addition, bacteria may act as a sink for P through uptake (Gächter et al., 1988; Sinke et al., 1993), and may be able to store P when it is in excess (Gächter and Meyer, 1993). In fact, sediments of nutrient-enriched areas can yield 10^3 to 10^4 times more anaerobes than non-impacted sediments within the same system (Reddy et al., 1999). Therefore, differences in abundances and taxonomic composition of the microbial community may affect P-release rates of sediments, and may be indicative of differences in environmental conditions (Reddy et al., 1999).

Traditionally, culture-dependent methods have been used to analyze diversity and dynamics of microbial communities; however, these are time-consuming, and tend to exclude many species through the use of selective media (van Heerden et al., 2002; Woese, 2002). The recent resurgence of interest in microbial ecology has resulted in development of new methods and conceptual approaches to evaluate the structure of soil microbial communities. Garland and Mills (1991) introduced a relatively rapid methodology to characterize microbial communities based on a technique developed by Biology, Inc. (Hayward, CA) that identifies bacterial isolates from sediment samples. This approach is centred on sole-carbon-source utilization, which uses Biolog gram negative (GN) microplates containing 95 separate carbon sources and a control well (no carbon source), each of which includes the redox dye tetrazolium violet. Plates are inoculated with environmental samples, such as sediment solutions, and are incubated. The formation of colour indicates that microbes present can utilize the particular substrate, and utilization patterns are used to characterize the microbial communities. Even though this approach is culture-based, and therefore only a small proportion of the total community diversity may be represented, Garland and Mills' method has been extensively used to characterize microbial communities and determine diversity (e.g., Folman et al., 2001; Grayston et al., 2001; Müller et al., 2001; Rogers and Tate, 2001; Schutter et al., 2001; Sinsabaugh and Foreman, 2001; Westergaard et al., 2001; Litchfield and Gillevet, 2002).

The aim of this research is to develop a rapid multifunction test (using Biolog microtiter plates) to evaluate spatial variance of the benthic microbial community in a heterogeneous marsh ecosystem, Cootes Paradise Marsh, which has been severely degraded by sewage effluent at the west end of the marsh for over 80 years (Chow-Fraser et al., 1998). We will correlate taxonomic variability associated with sediment samples collected at variable distances from the sewage outfall to corresponding differences in P-release rates. We hope that classification of the benthic microbial community on the basis of sole-carbon-source utilization may become a useful tool for bioassessment of historic P-enrichment in freshwater wetlands.

Study site

Cootes Paradise is an 850-ha wildlife sanctuary owned and managed by the Royal Botanical Gardens (RBG) and is located at the western tip of Hamilton Harbour and Lake Ontario. Within this sanctuary lies a 250-ha wetland, a receiving basin of four major hydrologic inputs: Borer's Creek, Chedoke Creek, Spencer's Creek and the Dundas Wastewater Treatment Plant (WWTP). Spencer's Creek drains 79% of the Cootes Paradise watershed (Chow-Fraser et al., 1998) and flows through mainly agricultural and forested land. This creek is considered a major source of organic and inorganic materials to the marsh. Chedoke and Borer's Creek are much smaller tributaries. Chedoke is an urban creek, which passes through substantial residential and industrial developments and consists mostly of underground channels. The Dundas WWTP discharges

effluent into the western end of the marsh. Prior to construction of the WWTP in 1919, raw sewage was discharged directly into the marsh and although the facility now provides tertiary treatment, historically it has been the major contributor of nutrients and was primarily responsible for the eutrophication of Cootes Paradise (Remedial Action Plan for Hamilton Harbour Stage 1 Report, 1992; Chow-Fraser et al., 1998). Despite the restoration efforts and tremendous declines in external loading to the marsh, P concentrations remain high (Chow-Fraser et al., 1998).

Methods

Sediment phosphorus release rates

Replicate sediment samples were collected in June 1999 with an Ekman Grab from eleven stations along a west-to-east gradient in Cootes Paradise Marsh (Figure 1). These sites were selected to evaluate spatial variance. A basic description of these stations is listed in Table 1. Excess water was drained and samples were stored in Freezer Ziploc^{TM} bags and frozen for up to 4 mo until experimentation. Phosphorus release experiments were conducted in the manner of Chow-Fraser et al. (1996): wet sediment was spread over the bottom of acid washed glass jars, deionized water was added. Jars were covered with foil and were incubated in a dark

Table 1. Characteristics of Cootes Paradise Marsh sampling stations. Total phosphorus (TP) concentrations correspond to ambient concentrations in the water column for the various sediment-sampling stations.

Station	Depth (cm)	TP (ug l^{-1})	Description
CP5*	53.5	312	Sewage lagoon
CP3*	69.5	94.8	Spencer's Creek outfall
CP23	40.0	NA	Surrounded by *Typha* sp., relatively sheltered
CP8*	36.3	217.4	Remnant marsh site, *Typha* sp., *Potemogeton pectinatus*
CP10*	65.5	148.9	Close to shoreline, relatively sheltered
CP24	40.0	NA	Embayment area
CP17*	30.6	377.2	Sheltered cut
CP1*	95.6	162.1	Open water
CP20	80.0	NA	Open water
CP21	70.0	NA	Open water
CP22	230	NA	Outlet to Hamilton Harbour

*Depth and TP values averaged from 1993–2000; all other station correspond to 1999 values only (Chow-Fraser, unpubl. data); NA = not available.

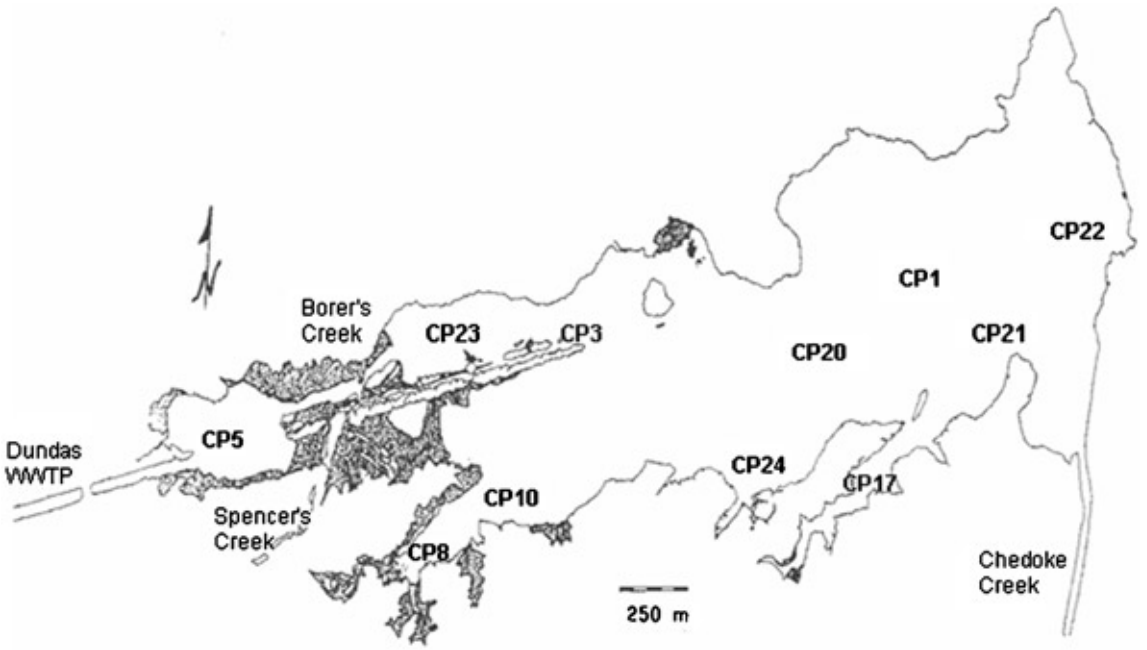

Figure 1. Map of Cootes Paradise Marsh. Approximate locations of sampling stations are indicated in bold.

growth chamber for up to 8 d at 25°C. Three jars from each site were removed from the growth chamber daily. Samples were analyzed for soluble reactive phosphorus (SRP) using the molybdate blue method (Murphy and Riley, 1962) to measure inorganic P in the extracts. The slope of the best-fit line relating P-release (mg m^{-2}) to time (d) was used to estimate release rate (mg m^{-2} d^{-1}).

In one set of experiments, parallel sediment samples were collected from CP5 in 2000; one set was used immediately to determine P-release rates, while the other was frozen for up to 4 mo prior to experimentation.

Characterization of the microbial community

Replicate sediment samples were collected in June 2000 with an Ekman Grab from the eleven representative sites to characterize the microbial community. Samples were stored at 4°C for 24 h until further analysis. A 1:10 soil solution for each site was prepared using a 1% saline solution. The suspension was shaken, allowed to settle and the supernatant was decanted into a sterile petri plate. A multimicropipettor was used to inoculate the 96 wells of the Biolog GN microtiter plates with 150 μl of solution (Biolog plates inoculated in triplicate). The plates were incubated at room temperature in the dark. Each well contained the redox dye tetrazolium chloride in addition to a carbon substrate (see Garland and Mills, 1991), with the exception of the control well. Oxidation of the substrate resulted in reduction of the dye producing a purple colour. Colour development (measured as optical density at 590 nm and related to the rate of substrate use) was determined after 24 h with a Biolog MicroStation plate reader using MicroLog 3N Software (Release 3.50 Version DE, ©Biolog Inc. 1994). This time was chosen in order to obtain a 'snapshot' of which substrates could readily be used by the community and to reduce the confounding effects of cell death and lysis on the redox dye. Raw difference data sets were obtained by subtracting the absorbance of the control well from each substrate well. Average well colour development (AWCD) was used an indicator of overall rate of substrate use and the number of positive tests (>0.10 abs units) was used as an indicator of substrate diversity. Differences between sites were determined by an ANOVA using SAS Jmp (SAS Institute Inc. 1982). We divided the corrected absorbances by the AWCD for each microtiter plate. This procedure reduces the influence of rate of colour development on the classification of samples (see Garland and Mills, 1991). Relationships among different samples were then determined using the transformed data by a Principal Components Analysis (PCA; SAS Jmp 4.0, Cary, N.C.).

We conducted a separate study to determine differences in microbial communities corresponding to fresh versus frozen sediments. Parallel samples were collected from three sites (CP1, CP5, and CP8) on a monthly basis from May to August 2001. One set of the samples were immediately inoculated into microplates, while the other set was frozen for up to 4 mo prior to inoculation. Other details are essentially as described above.

Relationship of the microbial community to sediment phosphorus release rates

To determine if the observed variation in the microbial community among sites is related to P-release rates from respective sediments, microbial characterizations and P-release experiments were carried out (using the same sediments collected above). For logistical reasons, we restricted this portion of the study to only two sites, CP1 and CP5, which were associated with lowest and highest PC1 scores, respectively. Sediment samples were collected and processed as previously described. Because of the small sample size, a non-parametric correlation analysis (Spearman's rank correlation) was used to test the predictive power of the PC scores with respect to SRP release rates.

Results

A significant difference among sediment samples from the eleven sites within Cootes Paradise was observed with respect to SRP release rates (Table 2; $P < 0.0001$) and these release rates were correlated with the overlying water TP concentrations ($P = 0.0082$). Release rates ranged from 0.96 to 28.3 mg P m^{-2} d^{-1} and are consistent with those reported by Nürnberg (1988) for lakes. A significant difference was also observed in terms of AWCD (Table 2; $P < 0.0001$). CP5 had the largest AWCD (and was presumably associated with highest microbial activity) while CP1 had the lowest. This was as expected since an increase in eutrophication is generally associated with an increase in numbers of microorganisms (Tilman, 1982). A similar pattern was observed for the number of positive well tests (Table 2; $P = 0.0005$). The community in CP1, which is from a less eutrophic part of the marsh than CP5 (see Table 1), therefore, used fewer substrates than that in CP5.

The PCA separated sites along the first axis based on utilization of polymers versus carbohydrates and

Table 2. Soluble reactive phosphorus (SRP) release rates and microbial community indices: average well colour development (AWCD) and number of positive tests in Biolog GN microtiter plates, from sediment collected at Cootes Paradise Marsh sampling stations. SRP releases were calculated from sediment collected in June 1999, while diversity indices were determined from sediment collected in June of 2000. Values in brackets are standard errors.

Site	SRP Release Rate (mg m^{-2} d^{-1})	Mean AWCD	Mean # of Positive Tests
CP5	17.18 (1.56)	0.563 (0.025)	80.3 (0.67)
CP3	5.91 (0.69)	0.494 (0.031)	73.3 (1.20)
CP23	5.81 (0.89)	0.432 (0.016)	72.0 (1.53)
CP8	15.18 (1.05)	0.429 (0.016)	72.0 (3.06)
CP10	1.67 (0.66)	0.290 (0.018)	65.3 (3.53)
CP24	0.96 (0.12)	0.310 (0.021)	63.0 (2.00)
CP17	28.28 (6.58)	0.395 (0.033)	72.3 (3.18)
CP1	4.56 (0.46)	0.206 (0.033)	51.3 (6.98)
CP20	4.30 (0.21)	0.293 (0.047)	56.7 (6.89)
CP21	4.49 (0.81)	0.451 (0.026)	64.0 (3.00)
CP22	9.31 (0.83)	0.452 (0.012)	65.3 (2.73)

phosphorylated substrates, and explained 19% of the variation (Figure 2). The PC1 scores for CP1 and CP5 were the lowest and highest, respectively; benthic microbes at CP1 which were furthest from the external loading source tended to use polymers, whereas those proximal to the sewage outfall at CP5 used only carbohydrates and phosphorylated substrates (Table 3). Separation along the second PC, which explained 11% of the variance, was based on the use of carboxylic acids and amino acids for stations on the positive end of the axis and carbohydrates on the negative end; however, there was no obvious pattern that could explain separation among sites. The third and fourth PCs explained 8.4 and 6.5% of the variance, respectively. Very few substrates were correlated with these axes and no site-type pattern was observed for either axis.

As expected, we observed significant differences in AWCD and number of positive tests between fresh and frozen sediment samples (Figure 3; $P < 0.0001$), suggesting that fresh sediment is more diverse and contains more viable bacteria. There was no effect of site on either AWCD or number of positive tests; however, significant differences in time of sampling (month) on AWCD were observed when the effect of sediment condition was removed ($P = 0.0002$ and 0.0063 for fresh and frozen sediment, respectively).

A PCA indicated a clear distinction between fresh and frozen sediments based on separation along the first PC axis (Figure 4). The first PC explained 21% of the

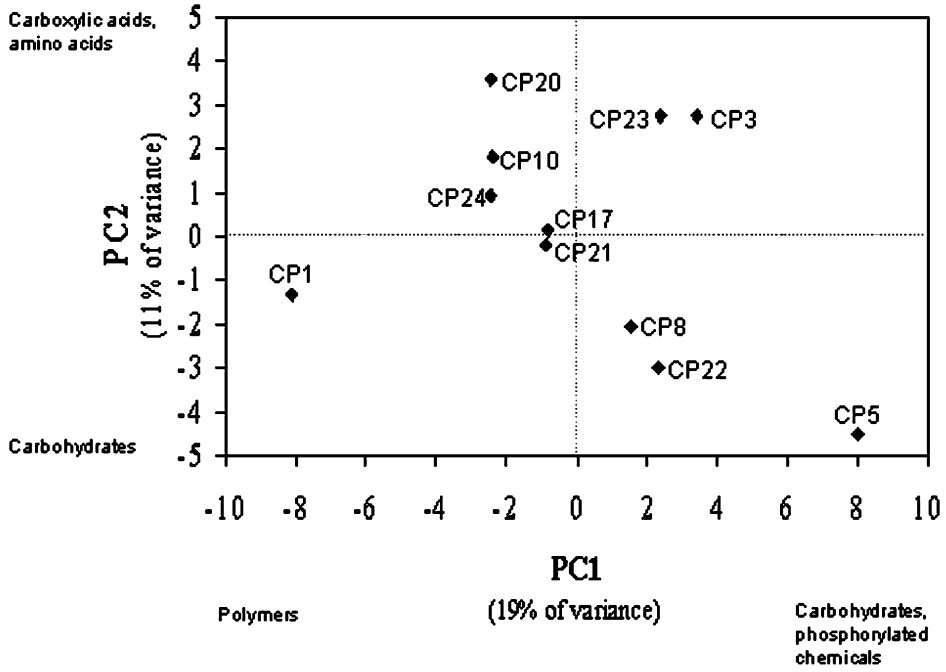

Figure 2. Principal component analysis ordination plot of Cootes Paradise Marsh sampling stations based on differences in microbial community structure in June 2000. Scores for the first two principal components are plotted.

Table 3. Results of principal component analysis correlations of sole carbon sources to principal component (PC) axes 1 and 2 as indicators of microbial community structure (based on sediments of 11 Cootes Paradise Marsh sampling sites collected in June 2000).

PC1		PC2	
Carbon source	r*	Carbon source	R
Polymers		Carbohydrates	
Dextrin	−0.843	D-melibiose	−0.618
Glycogen	−0.857	Carboxylic acids	
Tween 40	−0.852	α-hydroxybutyric acid	0.656
Carbohydrates		β-hydroxybutyric acid	0.629
N-acetyl-D-galactosamine	0.808	Sebacic acid	0.638
N-acetyl-D-glucosamine	−0.817	Amino acids	
D-fructose	−0.793	D,L-carnitine	0.655
D-galactose	−0.729		
D-mannose	−0.844		
D-mannitol	−0.77		
D-raffinose	0.615		
L-rhamnose	0.661		
D-sorbitol	0.620		
Carboxylic acids			
Citric acid	−0.787		
D-galactonic acid lactone	0.689		
D-galacturonic acid	0.700		
D-gluconic acid	−0.839		
D-glucuronic acid	0.768		
Phosphorylated chemicals			
Glucose-1-phosphate	0.613		

*Regression coefficient.

variance in the data set and was based on the use of polymers, carbohydrates, aromatic chemicals and phosphorylated chemicals for fresh sediments, whereas frozen sediments relied on carboxylic acids, amino acids and amines (Table 4). The second PC accounted for 12% of the variance and no pattern was observed for month or site.

Since freezing had an obvious effect on the microbial community, it was essential to relate P-release rates from frozen sediment with corresponding characteristics of the microbial community from frozen sediment samples. Data were available from CP1 and CP5, collected monthly from June to August 2000 for this purpose ($n = 6$). We found a significant correlation (Spearman's rank correlation analysis; $P = 0.041$) between release rates and PC1 scores, indicating a direct link between microbial activity in terms of P-release rates and patterns of C utilization. We further determined that mean release rates obtained from fresh sediment (3.21 $mg{\cdot}m^{-2}{\cdot}d^{-1}$) collected at CP5 were five-fold lower than those for corresponding samples that had been frozen for 4 mo prior to experimentation (17.18 $mg{\cdot}m^{-2}{\cdot}d^{-1}$).

Discussion

Many factors influence microbial diversity in sediments such as nutrient availability and the presence of organic matter (Buckley and Schmidt, 2002). As such, there is great potential for both spatial and temporal variation in microbial structure and/or function. Growth-limiting resources control the composition and diversity of biotic communities (Myers et al., 2001), and hence, the composition and structure of the benthic microbial community will differ according to the degree of nutrient availability or quality.

In general, microbial activity and diversity of substrate use was highest at locations of high productivity within Cootes Paradise Marsh and declined as sites

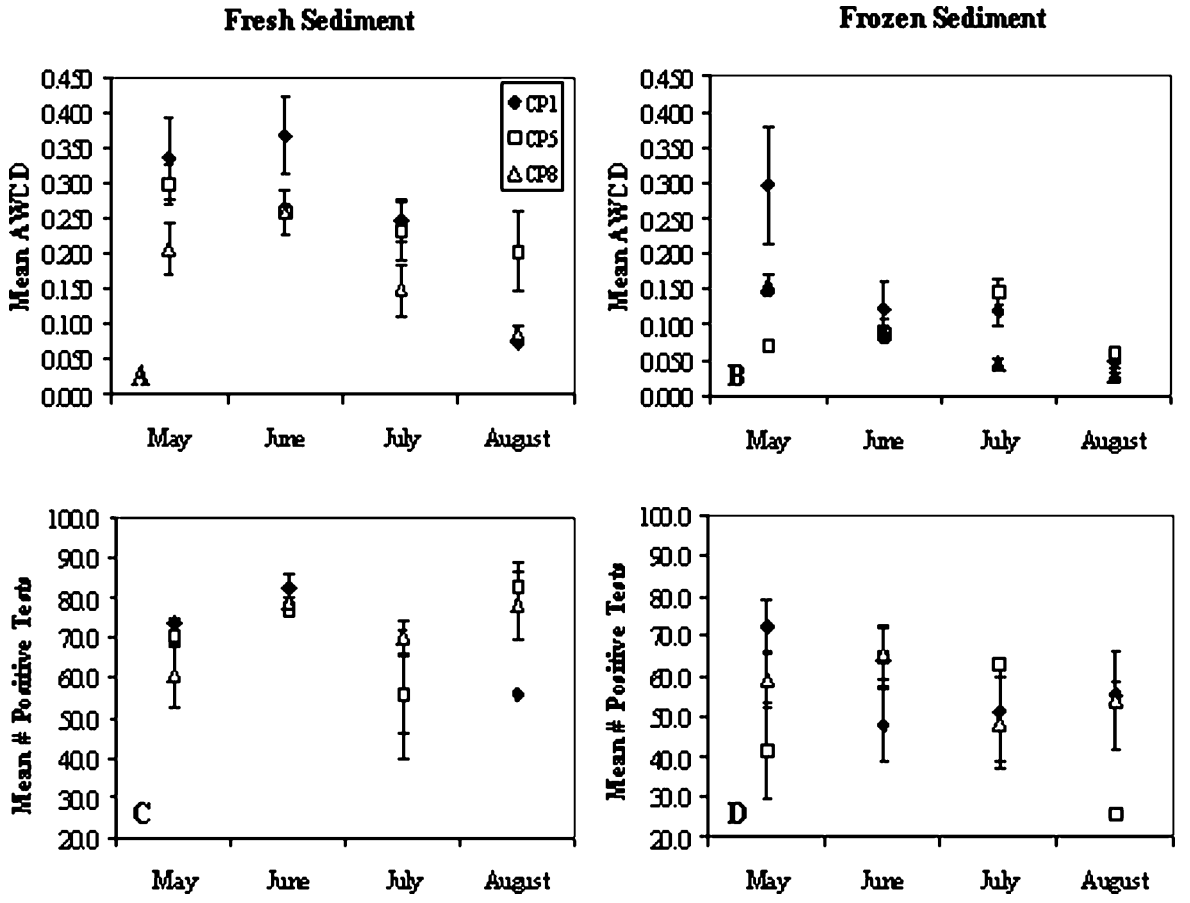

Figure 3. Microbial community diversity indices: average well colour development (AWCD) and number of positive tests in Biolog GN microtiter plates from fresh (A, C) and frozen (B, D) sediment collected at Cootes Paradise Marsh sampling stations: CP1, CP5 and CP8 over a 4-month period in 2001. Vertical bars are standard errors.

became less productive. The PCA was able to distinguish the open-water stations from other embayment and lagoon sites in the marsh, and was able to reveal spatial separation of other sites along both axes. This separation was attributable to differences in the patterns of substrate use among the microbial communities. Interpretation of the functional differences in these communities based on utilization of specific carbon sources would require a better understanding of microbial physiology, which is beyond the scope of this study and the relevance of such an undertaking would be questionable. Garland (1997) and Folman et al. (2001) have recommended that differences be interpreted as an indicator of community structure rather than function. However, we speculate that potential differences among stations may be attributable in part to distance from the Dundas WWTP. Sewage is an exceptional medium for bacterial growth; it consists of carbohydrates, lignins, fats and proteins (Bolton and Klein, 1971). The variety of substrates facilitates the development of a wide variety of microorganisms able to break down these various substances (Gainey and Lord, 1952). Prior to 1919, West Pond (CP5) received raw sewage, at which point primary treatment began. We theorize that the raw sewage had already been broken down into smaller carbohydrate molecules by the time it entered West Pond and that the microbial community shifted from one with enzymes capable of mineralizing complex molecules into a community that uses primarily simple sugars. This may explain why the CP5 sample did not grow well on polymers. Alternatively, differences among stations may be attributable to other characteristics of the sediment such as the presence or source of organic matter (OM). Sediment OM was found to be slightly higher at CP5 (11%), which is derived from sewage and autochthonous algae, compared to that at

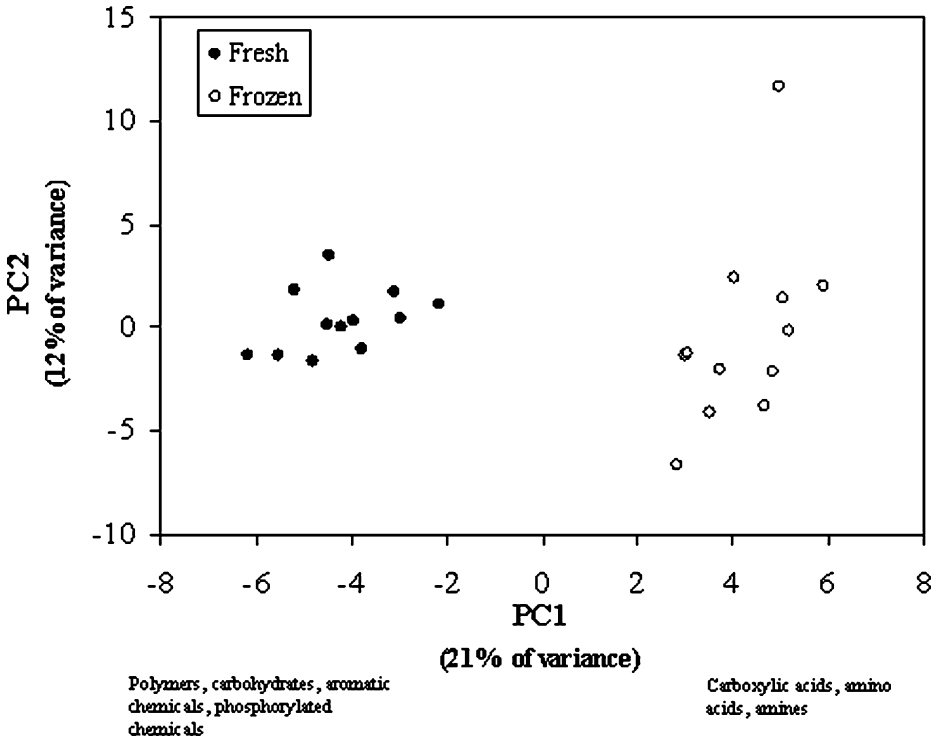

Figure 4. Principal component analysis ordination plot of Cootes Paradise Marsh sampling stations (CP1, CP5 and CP8) based on differences in microbial community structure in fresh and frozen sediment over May to August 2001. Scores for the first two principal components are plotted.

CP1 (6%), which is only derived from autochthonous production.

Of particular interest to us were phosphorylated substrates, which were positively correlated with PC1. Stations CP5, CP3, CP22, CP23 and CP8 were associated with positive values, while CP1, CP20, CP10 and CP24 (open water sites) were associated with negative values, indicating that microbial communities associated with the former were better able to utilize these substrates. Since the open water sites are located far from external P sources, they probably contain few microbes capable of utilizing P-enhanced substrates. In fact, sediment TP concentrations at site CP1 (0.678 mg μg^{-1} d.w.) were significantly lower than those found at CP5 (1.73 mg μg^{-1} d.w.). On the other hand, sites exposed to external P-sources may be expected to have a dominance of microbes capable of utilizing phosphorylated substrates. For example, CP8 is a remnant marsh site which presumably supports an environment favourable for microorganisms that are able to mineralize organic matter (Kairesalo and Matilainen, 1994) and decompose organic material to release phosphate (Wetzel, 1999). Sites CP23 and CP3 support a substantial waterfowl population, which are known to enrich the area with their guano. In addition, CP3 is located at the outfall of the Desjardins Canal, which drains Spencer's Creek, a largely agricultural watershed, responsible for 30% of the annual external total P load into Cootes Paradise (XCG Consultants Ltd., 1997). Site CP22 is located adjacent to Hamilton Harbour, which is another source of P to the marsh, accounting for 22% of the annual external total P load (XCG Consultants Ltd., 1997). Stations CP17 and CP21 were unrelated to PC1 or PC2. Even though CP17 is located at a combined sewer overflow and was expected to have a similar microbial community to the lagoon site, bacteria at this site were only able to grow to a limited extent on P-containing C-substrates, the reason for which requires further study.

As expected, microbial activity as measured by AWCD was higher in fresh compared with frozen sediment. Freezing presumably kills many intolerant species and also affects the physical and chemical nature of the sediment. Activity generally declined from May to August, suggesting that there may be a seasonal effect. Schutter et al. (2001) also found that the

Table 4. Results of principal component analysis correlations of sole carbon sources to principal component (PC) axes 1 and 2 as indicators of microbial community structure (based on sediments of CP1, CP5 and CP8 collected in May–August 2001).

PC1		PC2	
Carbon source	R	Carbon source	R
Polymers		Carbohydrates	
Dextrin	−0.868	i-erythritol	0.737
Glycogen	−0.892	L-fucose	0.648
Carbohydrates		D-psicose	0.627
N-acetyl-D-galatosamine	−0.713	Xylitol	0.711
N-acetyl-D-glucosamine	−0.756	Carboxylic acids	
D-mannose	−0.655	p-hydroxyphenylacetic acid	0.689
β-methyl D-glucoside	−0.850	α-ketobutyric acid	0.697
Sucrose	−0.834	α-ketovaleric acid	0.601
D-trehalose	−0.601	Malonic acid	0.643
Carboxylic acids		Amino acids	
β-hydroxybutyric acid	0.619	L-phenylalanine	0.731
Amino acids		L-threonine	0.643
L-proline	0.666		
L-pyroglutamic acid	0.733		
O-serine	0.670		
Aromatic chemicals			
Inosine	−0.854		
Amines			
2-aminoethanol	0.605		
Phosphorylated chemicals			
Glucose-6-phosphate	−0.801		

Biolog assays revealed significant seasonal impacts on microbial communities in agricultural soils. Possible explanations for this observation include increasing temperatures in the marsh, declining external nutrient inputs or possibly year-to-year variation. The PCA also revealed significant structural differences between the fresh and frozen benthic microbial communities. Because the SRP release rates from fresh sediment are substantially lower than from frozen sediment, we conclude that benthic bacteria may be a relatively large P sink, attributable to P release through cell lysis. In addition, the correlation between P-release rates and PC1 scores indicates that the use of Biolog GN microplates could potentially be used as a bioassessment tool to predict release rates from sediments, which are extremely tedious to determine from laboratory and field experiments.

There are some limitations which need to be addressed to warrant further investigation. First, release rates were initially determined from sediment collected in 1999, a year prior to microbial community characterization in 2000, and we have assumed that the monthly variation in the bacterial community overshadows year-to-year variation. Secondly, analyses were performed on plates from incubations after only 24 h. This means that microorganisms with a longer growth period were not included in this analysis. In addition, the characterizations do not necessarily reflect in situ conditions, but only the potential for particular species within the microbial community to utilize the substrates in the wells (Winding and Hendriksen, 1997). In other words, our results only represent the culturable portion of the microbial community and therefore, the associated biodiversity is probably much lower than that found in nature (Garland, 1997). Characterization using several techniques, as well as selection of more relevant substrates, would likely provide more insightful information as to the true nature of the community. Thirdly, we have only related release rates to microbial community characteristics for a small sample of frozen sediments; future experiments should be carried out to incorporate more sites, using fresh sediments, since the extent

to which freezing may have destroyed the existing microbial population and have led to artificially increased release rates is not known.

The microbial community acts as a potentially large sink and source of P to overlying water in lacustrine systems (e.g., Doremus and Clesceri, 1982; Premazzi and Provini, 1985) For example, Gächter et al. (1988) found that bacteria grown on P-limited substrates under aerobic conditions sequestered large concentrations of this nutrient; however, when conditions became anoxic these microorganisms released between 14 to 25% of that stored. Our results confirm that bacterial processes also potentially play a substantial role in regulating P exchange across the sediment/water interface in Cootes Paradise Marsh (unpublished results of Kelton and Chow-Fraser revealed that the contribution of bacterial mineralization could be as high as 23% of the total P-load). We recommend that future mass-balance studies (e.g., Prescott and Tsanis, 1997) of degraded marshes acknowledge the role of the microbial community in the sequestering and release of P be sediments in nutrient enriched waters.

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