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Denitrification and Methane production in Sediment of Hamilton Harbour (Canada)

By:

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Sommaire

Ce rapport est le résultat d'une partie d'une thèse de doctorat par R. Roy (Université McGill). Le travail fut financé par une subvention du Fond pour la recherche sur les Grands Lacs d'Environnement Canada octroyée à R. Knowles (Université McGill) et à M.N. Charlton (INRE) de même que par une bourse du CRSNG à R. Roy.

Les sédiments du port de Hamilton contiennent des niveaux élevés en métaux et en polluants organiques tels les hydrocarbures polyaromatiques (HPA) et les biphényl polychlorés (BPC). De fortes décharges en eaux usées traitées affectent la qualité de l'eau en causant de fortes croissances d'algues. La décomposition des algues de même que l'oxidation de l'ammoniaque (NH_4^+) cause la diminution en oxygène dans les eaux usées et les sédiments. Le devenir des composés azotés tel l'ammoniaque dans le port de Hamilton est peu connu et pourtant le couteux traitement de l'ammoniaque est recommandé dans le plan de restoration.

Les processus microbiens de la dénitrification et de la fixation d'azote moléculaire, de même que la production du méthane et la production du CO_2, furent mesurés dans les sédiments du port de Hamilton à 21 sites. Les expériences visaient à mesurer les taux de différents processus métaboliques bactériens dans les sédiments.

Le résultat le plus important de cette étude est que 70%-90% des différences entre les stations pouvaient être expliquées par les variations logiques des facteurs environnementaux tel que l'ammoniaque dans l'eau interstitielle, le carbone particulaire dans les sédiments, la température et la profondeur. Ainsi, peu de la variance entre les stations quant aux taux métaboliques pouvait s'expliquer par le niveau de contamination ou d'autres facteurs non mesurés.

Plutôt, le métabolisme était largement relié à l'eutrophisation par les usines de traitement des eaux usées. Une station située près de la décharge de l'usine de Burlington était exceptionnelle à cause de la présence de particules en décomposition provenant des eaux traitées.

Le concept de "toxicité des sédiments" est habituellement étudié en exposant des organismes supérieurs à l'eau interstitielle ou aux sédiments entiers dans des conditions grandement artificielles. Ceci est le premier rapport décrivant les variations spatiales et temporelles dans le métabolisme des microorganismes endogènes dans les sédiments. La variation du métabolisme microbien semble être relié aux problèmes d'eutrophisation et aux facteurs physiques plutôt qu'à la contamination.

Bien qu'une compréhension complète du métabolisme de l'azote requière plusieurs autres études semblables, des informations pertinentes quant à la toxicité des sédiments et au besoin de mesures correctives ont été acquises par cette collaboration INRE-Université McGill.

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Abstract

A preliminary factorial experiment was designed to test the importance of each proximal regulator: O_2, NO_3^-, or organic carbon, on potential denitrification in sediment slurries from two contrasted sites in Hamilton Harbour. We found that NO_3^- was the most important limiting factor of denitrification in Hamilton Harbour sediment slurry, followed by the absence of O_2. Potential rates of denitrification and CH_4 production were much higher in Hamilton Harbour sediment slurries when compared to Lake Ontario sediment slurries. Then systematic sampling of 21 sites covering Hamilton Harbour was carried out during the summer in 1990 and 1991 in order to study how well 1) environmental factors, such as O_2, NO_3^-, and organic carbon, measured in Hamilton Harbour, and 2) the spatial structure can explain observed variation of potential denitrification, CH_4 and CO_2 production, as well as N_2 fixation in sediment slurries. Using canonical redundancy analysis and an extension of this method to partial out the variance into spatial and environmental components, we found that most of the explained fraction of potential microbial activities (70-90%) was accounted by the significant environmental variables (NH_4^+, particulate carbon, dissolved organic carbon, dissolved O_2, depth, and temperature) and not much by the spatial locations. We found significant path coefficients (O.53 and 0.57 in 1990 and 1991) between CO_2 production and potential denitrification, which suggest that denitrifiers are dependent on a heterotrophic bacterial population for directly assimilable carbon sources. We found also significant path coefficients between particulate carbon and both CH_4 production (0.67 and 0.33) and CO_2 production (0.50 and 0.38), while significant path coefficients were also found between dissolved organic carbon and CO_2 production (0.34 and 0.47). We conclude that beside well-known abiotic factors such as O_2, NO_3^-, and organic carbon, a biotic factor involved in carbon metabolism may be important in explaining spatial variation of denitrification capacity in sediment of Hamilton Harbour.

Résumé

Une expérience factorielle préliminaire fut élaborée afin de déterminer l'importance relative de chaque régulateur proximal: O_2, NO_3^-, ou carbone organique, sur la dénitrification potentielle dans les sédiments liquéfiés de deux sites du port de Hamilton. Nous avons trouvé que le NO_3^- était le plus important facteur limitant de la dénitrification dans les sédiments du port de Hamilton, suivi par l'O_2. Les taux potentiels de dénitrification et de production du CH_4 étaient bien supérieurs dans les sédiments liquéfiés du port de Hamilton que dans ceux du lac Ontario Par la suite vingt-et-un (21) sites couvrant l'ensemble du port de Hamilton furent systématiquement échantillonnés durant la période estivale de 1990 et 1991 afin d'étudier jusqu'à quel point 1) les facteurs environnementaux, tels l'O_2, le NO_3^-, et le carbone organique, mesurés dans le port de Hamilton, et 2) la structure spatiale pouvaient expliqués les variations observées dans les taux potentiels de dénitrification, de production du CH_4 et du CO_2, et de fixation de N_2 dans les sédiments liquéfiés. En utilisant l'analyse canonique de redondance et une extension de cette méthode afin de réaliser une partition de la variance en ses composantes spatiales et environnementales, nous avons trouvé que la majeure partie de la fraction expliquée de la variation des activités microbiennes anaérobiques (70-90%) était due aux facteurs environnementaux (NH_4^+, carbone particulaire, carbone organique dissout, O_2 dissout, profondeur et température) et très peu à la structure spatiale. Nous avons trouvé des coefficients de direction significatifs (0.53 et 0.57 en 1990 et 1991) entre la production de CO_2 et la dénitrification potentielle, ce qui suggère que les bactéries dénitrifiantes sont dépendantes d'une population de bactéries hétérotrophes leur fournissant une source de carbone pouvant être directement assimilée. Nous avons aussi trouvé des coefficients de direction significatifs entre le carbone particulaire et d'une part la production de CH_4 (0.67 et 0.33) et d'autre part la production de CO_2 (0.50 et 0.38), de même qu'entre le carbone organique dissout et la production potentielle de CO_2 (0.34 et 0.47). Nous concluons que, mise à part les facteurs abiotiques bien connus tels l'O_2, le NO_3^-, et le carbone organique, un facteur biotique impliqué dans le métabolisme du carbone pourrait être important dans l'explication de la variabilité spatiale de la capacité dénitrifiante des sédiments du port de Hamilton.

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Denitrification and Methane Production in Sediment of Hamilton Harbour (Canada)

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Abstract. Systematic sampling of 21 sites covering Hamilton Harbour (Lake Ontario, Canada) was carried out during the summer in 1990 and 1991 in order to study how well environmental factors, such as O_2, NO_3^-, and organic carbon, and the spatial structure can explain observed variation of potential denitrification, CH_4 and CO_2 production, as well as N_2 fixation in sediment slurries. Using canonical redundancy analysis and an extension of this method to partial out the variance into spatial and environmental components, we found that most of the explained fraction of potential microbial activities (70–90%) was accounted for by the significant environmental variables (NH_4^+, particulate carbon, dissolved organic carbon, dissolved O_2, depth, and temperature) and not much by the spatial polynomial trend surface. We found significant path coefficients (0.53 and 0.57 in 1990 and 1991) between CO_2 production and potential denitrification, which suggests that denitrifiers are dependent upon a heterotrophic bacterial population for directly assimilable carbon sources. We also found significant path coefficients between particulate carbon and both CH_4 production (0.67 and 0.33) and CO_2 production (0.50 and 0.38), while significant path coefficients were also found between dissolved organic carbon and CO_2 production (0.34 and 0.47). We conclude that beside well-known abiotic factors such as O_2, NO_3^-, and organic carbon, a biotic factor involved in carbon metabolism may be important in explaining the spatial variation of denitrification capacity in the sediment of Hamilton Harbour.

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Introduction

Denitrification, the stepwise reduction of NO_3^- to N_2 [27, 39], plays a key role not only in the oxidation of organic matter but especially in the nitrogen cycling of freshwater ecosystems. It leads to the permanent loss of fixed nitrogen, balancing the fixation of N_2 in the global nitrogen cycle [27]. Facultative anaerobic heterotrophic bacteria such as *Pseudomonas* and *Alcaligenes* are the most abundant and important denitrifying organisms [15]. In the absence of O_2, these organisms can use NO_3^- as a terminal electron acceptor during the oxidation of organic carbon [27]. In his conceptual model of environmental regulation of denitrification in soil, Tiedje [51] recognized the direct effect of O_2, NO_3^-, and organic carbon on denitrification by defining them as proximal regulators. By contrast, distal regulators affect denitrification indirectly by acting on the proximal regulators in natural environments.

Various reports have already shown the importance of dissolved oxygen as an inhibitor of denitrification in sediments [1, 36], as well as the importance of NO_3^-, which is often the limiting factor of denitrification in aquatic sediments [25, 26, 43]. Because they are related to NO_3^- availability, benthic mineralization [9, 16, 17] and nitrification [21, 25] are likely to play key roles as distal regulators of denitrification in aquatic systems. So far, few field studies, besides those of Dodds and Jones [12] and Sweerts and DeBeer [47], have measured denitrification rates in relation to organic carbon [5]. In addition to its direct role, the metabolism of organic carbon may also affect denitrification by other means. Methane, which is one end product of carbon mineralization, is a competitive inhibitor of nitrification [4]. Therefore production of methane may indirectly affect denitrification by reducing the availability of NO_3^- from nitrification.

Hamilton Harbour, the most important natural embayment in Lake Ontario, Canada, is one of the most polluted bodies of water in North America [3, 35]. Denitrification capacity was previously investigated by slurry experiments [24], but no attention was given to O_2 or organic carbon as possible limiting factors of denitrification. Spatial variation, especially between epilimnetic and hypolimnetic sites, was not considered, even though such variations can be important in sediment [13, 22]. Space may often act as a hidden variable and cause the appearance of spurious correlations, as demonstrated by Legendre and Troussellier [31] for planktonic heterotrophic and marine bacteria. To our knowledge, no other study has tried to evaluate the importance of space in the sedimentary environment of a lake.

This work investigated how well (1) the proximate regulators (O_2, NO_3^-, and carbon) measured in Hamilton Harbour and (2) the spatial structure can explain the observed variation of potential sediment denitrification, CH_4 production, CO_2 production (as an indicator of fermentative activities), and benthic N_2 fixation measured in sediment slurries. These four microbial activities are considered together to represent the "anaerobic community."

Materials and Methods

Study Area and Sediment Sampling

Hamilton Harbour (43°16'–43°18'N, 79°47'–79°53'W), located at the western end of Lake Ontario, is about 8 km east-west and 4.8 km north-south, covering an area of 22 km^2 [35]. The thermocline during

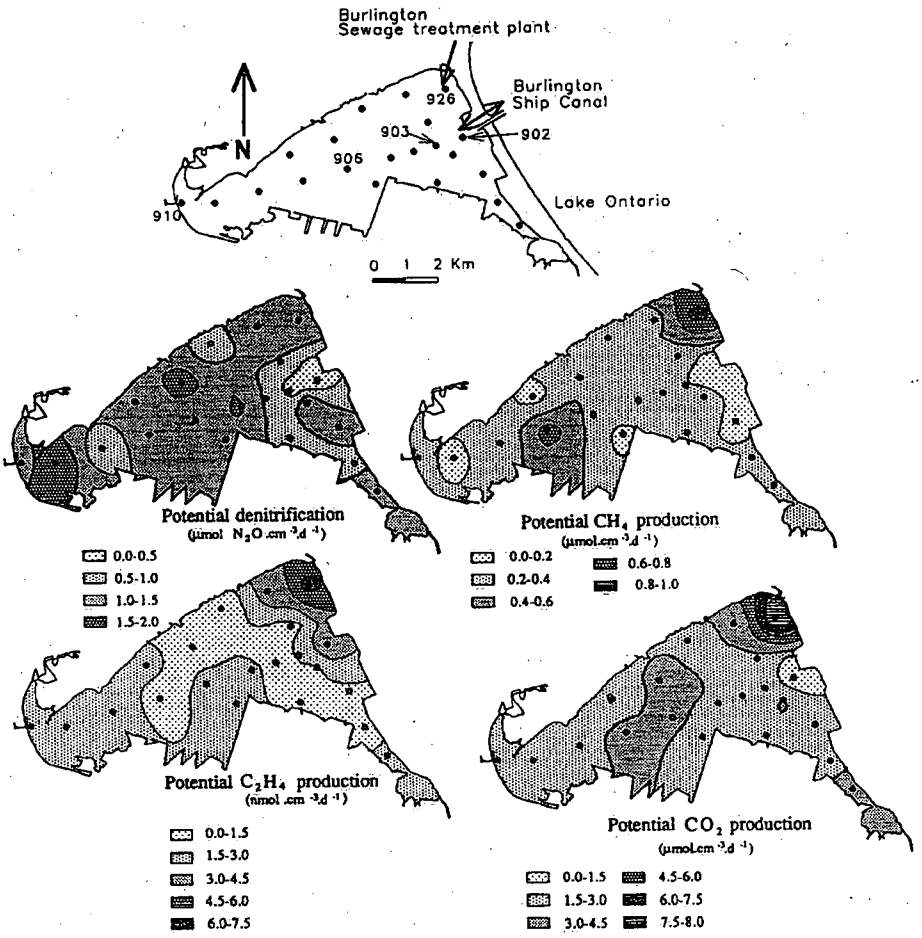

Fig. 1. Location of the sampling sites in Hamilton Harbour and spatial distribution of potential anaerobic microbial activities under study on 26 June 1990. Each site datum is the average of triplicate observations. Each interval is equivalent to one standard deviation of the plotted activity.

summer stratification is located at 10 m from the surface (unpubl. data) while the mean depth is 13 m. The Harbour, a recipient of municipal and industrial waste waters [38, 41], is connected to Lake Ontario by the Burlington Ship Canal (732 m long, 90 m wide, and about 10 m deep). The water exchange between the two bodies of water contributes dissolved O_2 (DO) to Hamilton Harbour [38], which becomes severely depleted in O_2 in the hypolimnetic sites during summer stratification [40]. The net load of NH_4^+ to Lake Ontario is of the order of 1.610×10^6 kg NH_4^+–N yr^{-1} [3].

Sampling was performed monthly on Hamilton Harbour from June to October 1990 and June to September 1991. Using an Ekman dredge (30 cm^2), the top 5 cm of sediment was collected at 21 sites covering the whole Harbour (Fig. 1). Sites were located by triangulation using a mini-ranger system. At each site, triplicate samples of sediment were put in 220-ml sterile containers, kept on ice during the cruise, and stored in a cold room (4°C) upon arrival at the laboratory. All sites were sampled within a single day. For microbial assays, sediments were processed within three days after collection. Pore water extraction from sediments by centrifugation took place within a week after collection. No

significant variation of nitrogen ions (NH_4^+, NO_2^-, and NO_3^-) in the pore water was observed over a 3-week period when sediments were stored at 4°C (unpubl. data). Physical and chemical factors in the water column (depth, temperature, dissolved oxygen (DO), pH, conductivity, and transmissivity) were measured at the time of sampling by a continuous in-situ profiling apparatus designed at the National Water Research Institute (NWRI, Burlington, Canada). The apparatus was calibrated during the night prior to sampling, as described by Ford and Charlton [14].

Microbial Assays

As an indication of active bacterial population sizes, the potential activities were determined by the following microbial assays. Sediment (5 ml) from each site replicate was dispensed into a 50-ml Erlenmeyer flask and capped with a serum stopper (Suba-Seal Works, William Freeman and Company, England) filled with silicone rubber to reduce gas leakage following repeated injections. Sterile distilled water was added by disposable syringe to obtain a final slurry of 10 ml. For potential denitrification assays, 50 μmol l^{-1} of $NaNO_3$ was added to a final slurry concentration of 5 mM. All flasks were shaken, evacuated 3 times (15 min each), and backfilled with ultra-pure helium (Linde, Union Carbide, Montreal, Canada) in order to eliminate dissolved gases in the pore water. Acetylene (C_2H_2) was added to a final concentration of 10 kPa to flasks used for denitrification [2, 28, 55] and N_2 fixation determination [26]. Since C_2H_2 is an inhibitor of methanogens and some heterotrophs [37], it was not added to slurries for the determination of CH_4 and CO_2 production. All flasks were wrapped with aluminum foil to avoid light exposure. They were incubated statically in the dark at 20°C. After 24 h and/or 5 days of incubation, the four potential activities were determined by their gas production: N_2O for denitrification, C_2H_4 for nitrogen fixation, CH_4 for methanogenesis, and CO_2 as an indicator of fermentative processes.

Analytical Procedures

Gases were analyzed by gas chromatography. N_2O and CO_2 were analyzed on a GC (Fisher 1200, Fisher, Canada) equipped with a thermal conductivity detector (TCD) and a Porapak Q column. N_2O was also measured on a GC (Perkin-Elmer 3920, Perkin Elmer, Canada) with an electron capture detector (ECD) and a Porapak Q column (Supelco, Canada) for some experiments. Because CH_4 concentrations were usually high, it was possible to measure them on the TCD.

When CH_4 was not detectable on the TCD, it was determined with a GC (HP 5700A, Hewlett-Packard) equipped with a flame ionization detector (FID) and a Poraplot U column (Chrompak, The Netherlands). C_2H_4 concentrations were also determined on this GC. A gas standard with the following concentrations (v/v): CH_4 (0.514%), C_2H_4 (0.544%), N_2O (1.04%), C_2H_2 (11.1%), and CO_2 (14.9%), from Canadian Liquid Air Ltd. (Montreal, Canada) or dilutions of this mixture (5%, 10%, 15%) were used as standards.

Gas concentrations were calculated taking into account the dissolved gas using the Ostwald solubility coefficient [54]. Calculations of the production rates of the gases are based on actual time elapsed between the beginning of the incubation and the time of gas analysis. Since the 3-cycle evacuation was efficient enough to eliminate any dissolved gases, we could assume a complete helium gas phase for routine assays. Data presented are averages of triplicates.

Pore water for each sediment sample was extracted by centrifuging (6000 *g*, 20 min, 4°C) approximately 150 ml of sediment. Then the supernatant was membrane-filtered (0.45 μm) and stored at 4°C. Chemical analyses of the pore water were performed at the National Water Quality Laboratory of Environment Canada, Burlington. NH_4^+ was determined by the Berthelot reaction [45] on an automated system (TRAACS-800, Mandel, Guelph, Ont., Canada). NO_2^- and NO_3^- concentrations were determined by an automated colorimetric method, based on the Griess reaction [46], after reduction of the NO_3^- by cadmium on a TRAACS-800. Dissolved inorganic carbon (DIC) was determined by infrared detection of CO_2 released from acidification. Dissolved organic carbon (DOC) was measured in a similar fashion after further acidic digestion of the water sample.

From the centrifugation, the pellet was kept and dried at 70°C overnight, and then manually ground with a mortar. Particulate carbon (PC) and particulate sulfur (PS) were measured by a combustion method on a LECO automated carbon-sulfur analyzer. Carbon and sulfur standards from Leco Instruments Ltd. (Mississauga, Ontario), as well as a carbon standard from sediment of Lake Ontario (3.04% C) (kindly provided by Dr. P.G. Manning, NWRI), were run every 15–20 samples. Particulate organic carbon (POC) was measured as just described, except that the dried sediment was acidified with 10% phosphoric acid and dried again to eliminate carbonates. Each replicate was run twice on the LECO. Averages of analytical duplicates were used to compute a site average of triplicates.

Numerical Analyses

Each sampling campaign generated a matrix of 21 objects (sites) by 15 variables (4 dependent and 11 independent). Preliminary statistics (position and dispersion) were calculated using the Statview software (Abacus Corporation, Berkeley, USA). Normality of the distributions was tested using the Kolmogorov-Smirnov test of normality [33] included in the R-package [32]. Using the same package, the Box-Cox method [44] was used to find the best normalizing transformation for the variables to improve the symmetry of the distribution. Spearman and Pearson correlation coefficients were computed on the raw and transformed data using the R-package and Statview.

In order to know how a set of environmental factors can explain the observed variation of microbial activities among localities, we used an eigenvector technique recently applied to the field of ecology: canonical redundancy analysis (RDA) [53; see also 7, 49]. The matrix of dependent variables contained the 4 microbial activities measured during this study, while the matrix of independent variables contained the 11 environmental variables. Since the microbial activities have different units (nmol $cm^{-3}\ d^{-1}$, $\mu mol\ cm^{-3}\ d^{-1}$), the RDA was performed on a correlation matrix of microbial activities. The CANOCO program that we used for this analysis [50], standardizes the independent variables to avoid problems of interpretation of the canonical coefficients arising from dimensional differences. Redundancy analyses were performed separately for each sampling campaign. We also included the time of sampling among the independent variables and performed a RDA on the combined data of 1990 and 1991. Because the sampling of August 1991 was executed with a different sampling device, these data have been excluded from the analysis.

Analysis of the spatial structure started with interpolated maps generated with the help of the MacGridzo software (RockWare Inc., Wheat Ridge, Colorado, USA). Gridding was done by using 6-neighbor points and the inverse distances with a cell spacing of 30 units, to produce high resolution maps. Contour intervals were equivalent to one standard deviation of the variable being mapped. Significant spatial autocorrelation was not detected (R-package). To partial out the spatial and temporal fractions of the variation of the anaerobic microbial activities, we applied the method described by Borcard et al. [7]. For this analysis three matrices were used. The first one was the matrix of dependent variables which contained the four microbial activities under study. The second matrix contained the significant environmental variables identified by a forward selection procedure, which is readily available in the CANOCO program: PC, DOC, NH_4^+, DO, temperature, and depth were selected. The third matrix contained the spatial variables which included the geographical coordinates of each sampling station. The geographical coordinates of each sampling station were completed, as suggested by Legendre [29], by including only the most significant terms (underlined) for a cubic trend surface regression of the form

$$z = b_1\underline{x} + b_2\underline{y} + b_3x^2 + b_4\underline{xy} + b_5\underline{y}^2 + b_6x^3 + b_7\underline{x}^2\underline{y} + b_8\underline{x}\underline{y}^2 + b_9y^3$$

Selection of the significant terms was done again by the forward selection procedure of the CANOCO program. As an additional analysis, time (months) of sampling, represented by binary variables, was also added as a variable into the space matrix. For a detailed description of the method see Borcard et al. [7]. The partition of the variance was performed by a program written by P. Legendre.

After having established the importance of the environmental factors versus space in explaining the observed variation of the dependent variables, the relative significance of each environmental factor in explaining each microbial activity under study, and especially denitrification, was assessed. This

Table 1. Basic statistics for the variables under study (21 sites monthly from June to October 1990; $n = 103$)

Variable[a]		Min	Mean	Max	CV[b] (%)
Depth(m)		1.5	15	26	38
Temperature (°C)	wc-1	4.5	14.0	21.0	22
pH	wc-1	6.8	7.7	10.1	5
Dissolved oxygen (mg liter^{-1})	wc-1	0.12	5.06	12.2	69
Particulate carbon (% dw)	sed	0.93	6.9	13.1	31
Particulate organic carbon (% dw)	sed	0.29	5.4	12.4	40
Particulate sulfur (% dw)	sed	0.019	0.369	0.682	41
$NO_3^- + NO_2^-$-N (μM)	pw	0.21	1.5	11.3	105
NH_4^+-N (mM)	pw	0.02	1.5	14.3	137
Dissolved organic carbon (mM)	pw	0.36	1.2	3.1	35
Dissolved inorganic carbon (mM)	pw	0.01	5.1	7.6	30
CH_4 production (μmol cm^{-3} day^{-1})	sed	0	0.30	0.85	58
CO_2 production (μmol cm^{-3} day^{-1})	sed	0.16	3.6	10.7	46
Denitrification[c] (μmol N_2O cm^{-3} day^{-1})	sed	0	1.60	4.87	50
N_2 fixation (nmol C_2H_4 cm^{-3} day^{-1})	sed	0	2.29	6.66	57

[a] wc-1, water column 1m above the sediment; pw, pore water; sed, sediment
[b] CV, coefficient of variation
[c] Slurries amended with 50 μmol fl^{-1} $NaNO_3$

assessment was carried out by path analysis [30, 44, 52] to test the significance of known causal relationships in three different models including, respectively, denitrification, CH_4 production, and CO_2 production as dependent variables. Independent variables were depth, temperature, PC, DOC, DO, $NO_2^- + NO_3^-$, and NH_4^+. For denitrification and CH_4 production models, CO_2 production was also included as an independent variable. In these cases it is considered as a biotic factor acting as a potential source of directly assimilable carbon compounds for denitrifiers and/or for methanogens. Path analyses were executed with a program written by P. Legendre and A. Vaudor for the Macintosh.

Results

Time courses of microbial activities, examples of which are reported by Roy et al. [42], were similar to those obtained by Knowles [26] for Lake St. George sediments. Table 1 reports the basic statistics for the physical, chemical, and microbial variables measured from June to October 1990. The sampling effort was a little higher for the hypolimnetic environment, since the average depth of the sampled sites (15 m) was below the thermocline (10 m). Hamilton Harbour water is circumneutral. Pore water concentration of mineral N had the highest coefficient of variation (CV). $NO_2^- + NO_3^-$ concentration was very low (1.5 μM) while NH_4^+ concentration was about 3 orders of magnitude higher (1.5 mM). Concentrations of DOC (1.2 mM) were in the same order of magnitude as NH_4^+, but without the extreme values noted for NH_4^+ as shown by the lower CV (35%). As indicated by their CV (46–58%), potential anaerobic microbial rates demonstrated important spatial variation.

Interpolated maps of the microbial activities suggest a patchy pattern in the sediments of Hamilton Harbour, as illustrated for 26 June 1990 (Fig. 1). Rates are lower than average in the vicinity of the Burlington Ship Canal (902, 903). They are

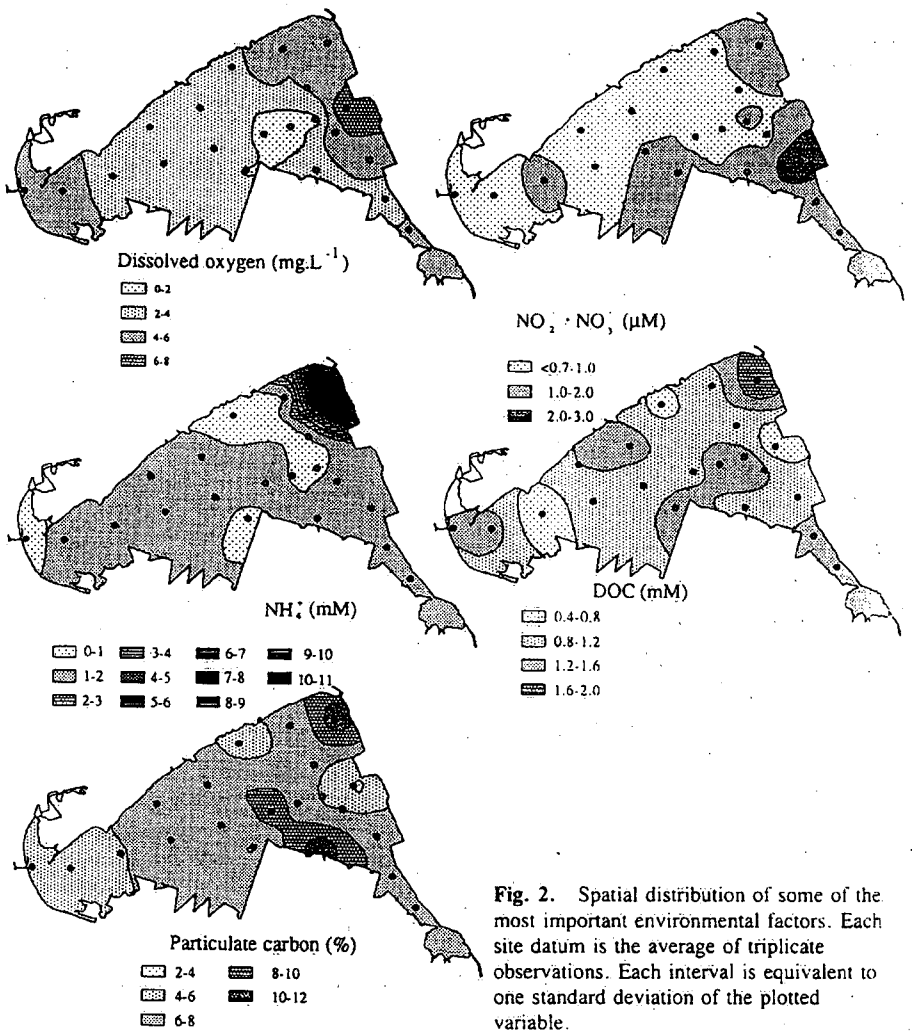

Fig. 2. Spatial distribution of some of the most important environmental factors. Each site datum is the average of triplicate observations. Each interval is equivalent to one standard deviation of the plotted variable.

higher in the northern section of the Harbour at the discharge of the Burlington sewage treatment plant (926). Interpolated maps of the environmental factors suggest also a patchy distribution (Fig. 2). Site 926 (Fig. 1) seems to contribute largely to the heterogeneity of the system, with concentrations of carbon (dissolved and particulate) and NH_4^+ higher than the average. Sites near the Burlington Ship Canal also contribute to the heterogeneity of the system by having lower carbon and NH_4^+ concentrations, and higher O_2 concentrations. As a preliminary step in the multivariate interpretation of these maps, we used spatially constrained clustering based upon the Gower similarity coefficient [30] calculated for the 21 sites, using all 15 variables as descriptors. This analysis, performed with the BIOGEO program (R-package), led to the identification of 6 possible clusters of stations at a similarity

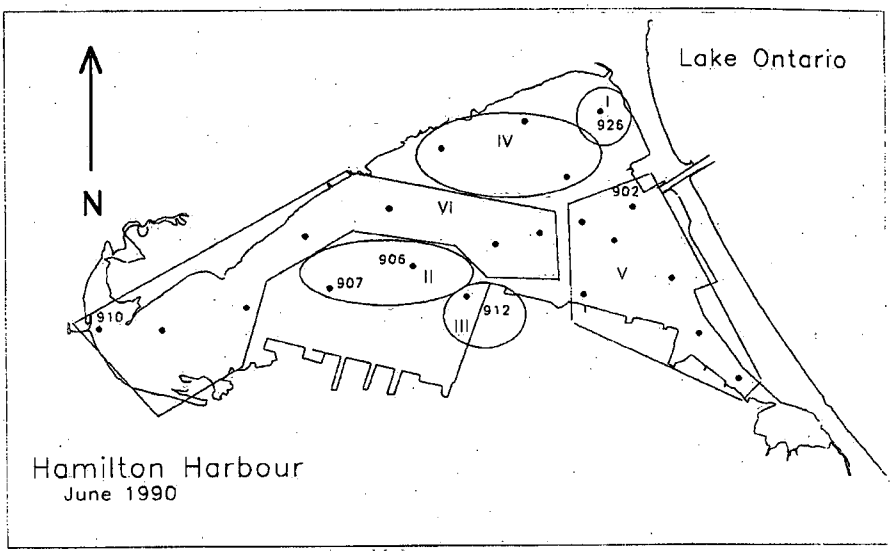

Fig. 3. Clusters of sites as found by the K-means method. This method was performed on the principal coordinates (19) calculated from a distance matrix based upon the Gower similarity coefficient calculated for all the sites (21) using the 15 variables as descriptors. Delaunay triangulation was used to generate a list of spatial links acting as constraints in the clustering.

level of 0.80. Knowing this, we used a K-means partitioning algorithm, also with a spatial contiguity constraint (R-package), in order to obtain the statistically best spatial partition of stations with the lowest value for the sum of within group sum-squares statistic over 100 trials. The six resulting clusters are shown on the map (Fig. 3). To determine their environmental or biological significance, we performed a multiple regression of each cluster, described in a binary fashion, over all 15 variables. The first cluster was a singleton, 926, showing high NH_4^+ and DOC concentrations in the pore water. High potential CH_4 and CO_2 productions were also observed at this site. High CH_4 and CO_2 production are also a feature of the second cluster of two stations (906–907) in the profundal area. The third cluster was singleton 912, with low NH_4^+ concentration and low potential CH_4 production. Three stations, located beside site 926, formed the fourth cluster characterized by low NH_4^+ concentrations in the pore water. The fifth cluster was characterized by slightly higher $NO_2^- + NO_3^-$ concentrations and generally lower potential denitrification, while the sixth cluster in the west end was characterized by lower $NO_2^- + NO_3^-$ concentrations.

The correlation matrix (Table 2), including all variables and all elements from June to September 1990, suggests three groups of environmental variables as schematized in Fig. 4. A first group contained PC, PS, and POC ($r = 0.91$ to 0.94). A second group included temperature, DO, and pH of the water column 1 m above the sediment ($r = 0.85$ to 0.91). These variables were highly negatively correlated to the depth of the water column ($r = -0.81$ to -0.95) as expected. A third group of factors comprised NH_4^+, DOC, and DIC in the pore water ($r = 0.76$

Table 2. Correlation matrix of variables measured from June to September 1990 (n = 84)

	Den.	CO_2 pro.	CH_4 pro.	N_2-fix.	PC	PS	POC	Depth	Temp.	D.O.	pH	No_3^-	NH_4^+	DOC	DIC
Den.	—	**0.53***	**0.35**	**0.43***	**0.33**	**0.23**	**0.27**	**0.04**	**0.20**	**0.24**	**0.12**	**0.23**	**0.29**	**0.30**	**0.33**
CO_2pro.	0.53*	—	**0.61***	**0.30**	**0.51***	**0.58***	**0.50***	**0.20**	**−0.03**	**−0.36**	**−0.15**	**−0.05**	**0.55***	**0.41***	**0.44***
CH_4pro.	0.28	0.58*	—	**0.09**	**0.64***	**0.67***	**0.48***	**0.36**	**−0.27**	**−0.53***	**−0.43***	**0.11**	**0.45***	**0.36**	**0.31**
N_2-fix.	0.40*	0.50*	0.21	—	**0.04**	**0.06**	**−0.04**	**−0.31**	**0.35**	**0.11**	**0.25**	**0.08**	**0.22**	**0.12**	**0.28**
PC	0.40*	0.74*	0.78*	0.39	—	**0.85***	**0.89***	**0.44***	**−0.34**	**−0.57***	**−0.39***	**0.01**	**0.50***	**0.55***	**0.40***
PS	0.27	0.70*	0.82*	0.19	0.91*	—	**0.81***	**0.48***	**−0.39***	**−0.60***	**−0.47***	**−0.01**	**0.37**	**0.39***	**0.25**
POC	0.34	0.73*	0.80*	0.37	0.94*	0.91*	—	**0.50***	**−0.45***	**−0.60***	**−0.53***	**0.06**	**0.55***	**0.58***	**0.35**
Depth	0.18	−0.18	0.53*	−0.15	0.41*	0.59*	0.44*	—	**−0.76***	**−0.81***	**−0.64***	**−0.07**	**0.26**	**0.34**	**0.07**
Temp.	−0.21	−0.18	−0.62*	0.14	−0.44*	−0.60*	−0.48*	−0.88*	—	**0.62***	**0.68***	**0.23**	**−0.09**	**−0.18**	**0.10**
D.O.	−0.25	−0.24	−0.55*	0.12	−0.46*	−0.62*	−0.51*	−0.95*	0.88*	—	**0.76***	**0.01**	**−0.27**	**−0.45***	**−0.20**
pH	−0.11	−0.19	−0.54*	0.22	−0.42*	−0.59*	−0.47*	−0.81*	0.91*	0.85*	—	**0.09**	**−0.23**	**−0.27**	**−0.00**
NO_3^-	−0.24	−0.19	−0.15	−0.22	−0.06	−0.02	−0.04	−0.07	0.03	0.04	−0.08	—	**0.15**	**−0.02**	**−0.15**
NH_4^+	0.52*	0.85*	0.52*	0.64*	0.67*	0.60*	0.66*	0.06	0.10	−0.15	−0.12	−0.28	—	**0.60***	**0.59***
DOC	0.42*	0.69*	0.53*	0.49*	0.69*	0.60*	0.69*	0.28	−0.21	−0.37	−0.25	−0.30	0.79*	—	**0.48***
DIC	0.44*	0.74*	0.43*	0.54*	0.66*	0.56*	0.60*	0.12	−0.08	−0.20	−0.17	−0.34	0.84*	0.76*	—

Spearman non-parametric correlation coefficients calculated on the raw data are shown in bold in the top half of the table. Pearson correlation coefficients calculated on the transformed data are shown in normal font in the bottom part of the table.

*denotes significant coefficients at the Bonferroni-corrected level ($p \leq 0.05/105 = 0.0005$). Den. = denitrification, PC = particulate carbon, PS = particulate sulfur, POC = particulate organic carbon, Temp. = temperature, D.O. = dissolved oxygen, DOC = dissolved organic carbon, DIC = dissolved inorganic carbon. Single line delineates the dependent variables, double lines delineate colinear variables, and dashed line delineates group of significantly correlated variables.

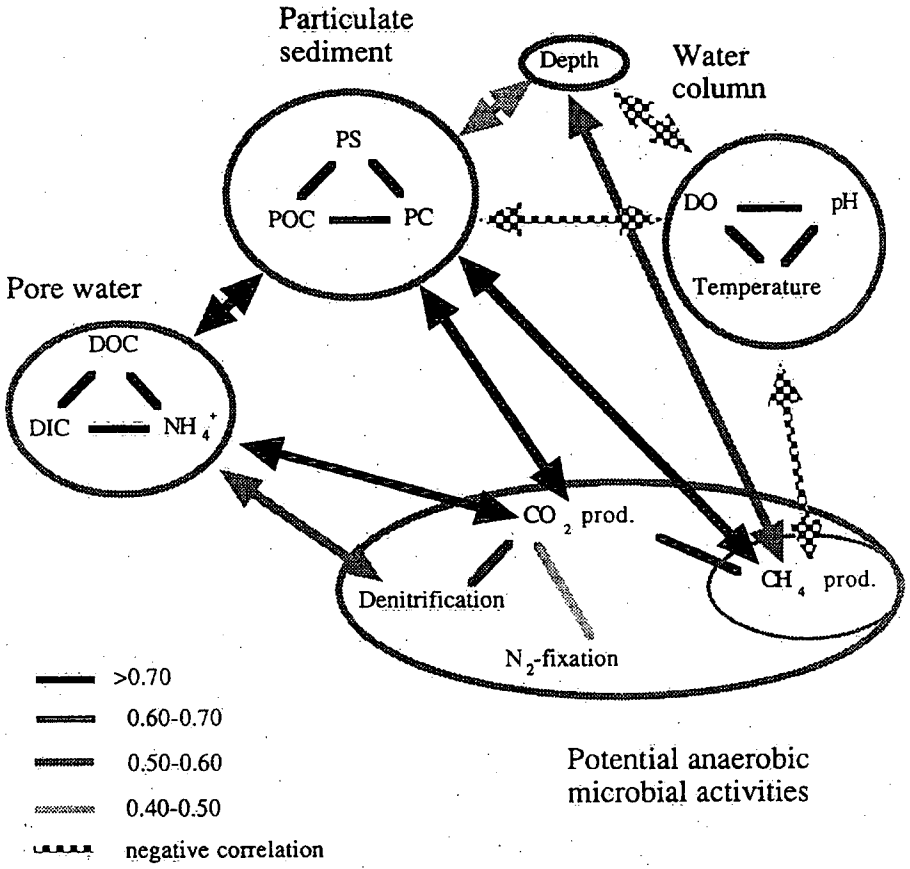

Fig. 4. Schematic representation of the significant Pearson correlations among the physical, chemical, and microbial variables measured from June to September 1990.

to 0.84). Correlations between the pore water and the water column variables were not significant at the Bonferroni-corrected level ($P \leq 0.05/105 = 0.0005$), but both of these groups had significant correlations ($r = 0.40$ to 0.70) with the particulate fraction of the sediment. Microbial activities were all significantly correlated with pore water factors (Table 2). Only CO_2 and CH_4 productions had significant correlations with the particulate fraction. CH_4 production was also the only microbial activity significantly correlated ($r = -0.54$ to -0.62) to water column variables. Within the group of microbial activities, CO_2 production was significantly correlated to the three other microbial activities. CH_4 production did not have any significant correlation with either denitrification ($r = 0.28$) or C_2H_4 production ($r = 0.21$). Interestingly, potential denitrification had its highest correlation with CO_2 production ($r = 0.53$), which suggests a biotic control of potential denitrification in sediment of Hamilton Harbour.

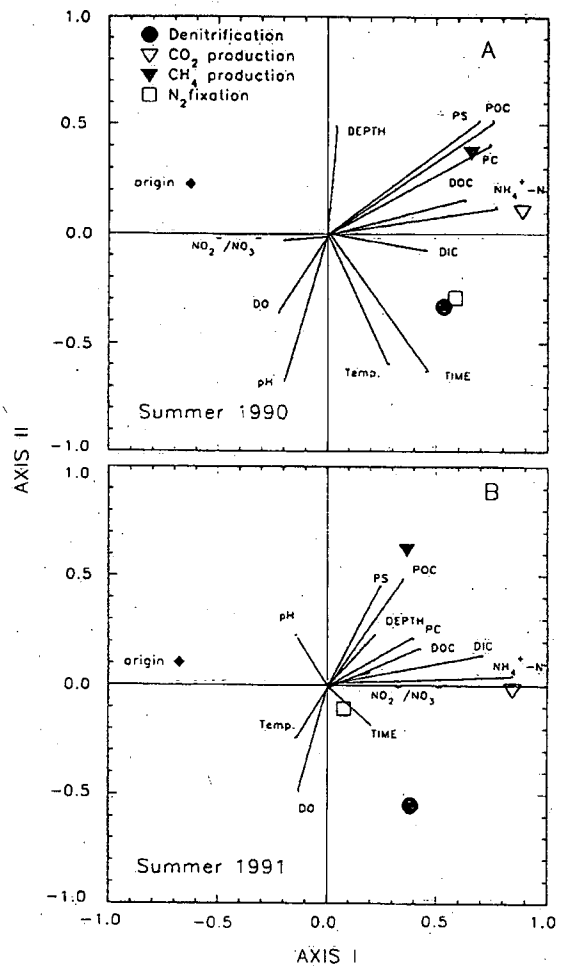

Fig. 5. Ordination diagram of the canonical redundancy analysis performed on the data of June to September 1990 ($n = 84$) and 1991 ($n = 62$). Environmental variables are shown as vectors while the microbial activities are shown as symbols.

The structure of interactions among variables suggested by the correlation matrix is further supported by the ordination resulting from the canonical redundancy analysis (Fig. 5). The sums of the canonical eigenvalues for 1990 (0.581) and 1991 (0.559) indicate that 58.1% and 55.9% of the observed variation in the potential rates of the four microbial activities measured in this study were accounted for by the measured environmental factors (Table 3). The first axis of the 1990 ordination explained 75.1% of the microbial activities–environmental interaction and was highly significant ($P \leq 0.01$), as found by a Monte-Carlo permutation test. The second axis explained only 15.0% of the environmentally constrained variation of microbial activities. The 1991 ordination shows a somewhat different result relative to the axes: the first axis explained 44.9% of the activities–environment variation ($P \leq 0.01$), while the second axis explained 31.3% of the activities–environment variation. Potential CO_2 production was greatly correlated to DOC and NH_4^+ (Fig.

Table 3. Summary of the canonical redundancy analyses performed on data (4 microbial activities and 11 environmental variables) measured from June to September 1990 (n = 84) and 1991 (n = 62). For each analysis is shown the eigenvalues corresponding to each axis of the ordination and the sum of the eigenvalues for the overall analysis. Also shown is the amount of microbial activities variation accounted for by each axis when environmental variables are controlled. Finally the significance of the first axis and the overall analysis are also included

Year		Eigenvalues	Activities–environment (%)	Significance
1990	1st axis	0.437	75.1	0.01
	2nd axis	0.087	90.1	
	3rd axis	0.049	98.6	
	4th axis	0.008	100.0	
	Sum	0.581	100.0	0.01
1991	1st axis	0.251	44.9	0.01
	2nd axis	0.175	76.2	
	3rd axis	0.114	96.6	
	4th axis	0.019	100.0	
	Sum	0.559	100.0	0.01

5). These three variables contributed largely to the first axis of variation in Hamilton Harbour. Potential CH_4 production and denitrification were both correlated to CO_2 production, but they were not correlated with each other (Fig. 5). Variation of CH_4 production rates was well explained by the variation of PC in the sediment. The ordination diagram also suggests that, in general, sites with higher concentrations of PC had lower DO concentrations and pH during summer stratification. CH_4 production and PC did not show any relationship to sampling time, although this affected the variation of potential denitrification, as already mentioned. The potential denitrification rate is not explained well by any of the selected environmental factors except the potential CO_2 production rate. The overall analysis was highly significant ($P \leq 0.01$) (Table 3). The ordination of 1991 data is similar to the 1990 ordination, except for pH which is located differently on the second axis. Again the overall analysis was highly significant ($P \leq 0.01$). The general ordination pattern was conserved throughout the summer of both 1990 and 1991, as found by RDA performed separately on data of each sampling campaign. Sums of canonical eigenvalues varied from 0.633 to 0.881, indicating that the environmental factors measured during this study explained between 63.3% and 88.1% of the variation in potential microbial activities at each sampling time (Table 4). Half of the analyses were highly significant ($P \leq 0.01$), while four of the analyses did not quite meet the criteria for significance.

Forward selection of the significant independent variables decreased the original eleven environmental variables to six: NH_4^+, PC, temperature, DO, DOC, and depth. These six variables were used to partial out space by the method of Borcard et al. [7]. When sampling time is not considered as a variable of the space matrix, environmental factors explained 33.2% (1990) and 33.3% (1991) of the variation in the microbial activity matrix (Fig. 6) after controlling for the effect of the spatial variables. This fraction is significant at the Bonferroni-corrected level (0.05/4 = 0.0125) for four simultaneous tests. The interaction of space and environment was also significant at the Bonferroni-corrected level (0.05/4 = 0.0125), but explained much less (10.9% for 1990 and 5.9% for 1991) of the variation in the

Table 4. Summary of the canonical redundancy analyses on the 4 microbial activities and the 11 environmental variables measured on each sampling campaign in 1990 and 1991 (n = 21). For each sampling date is shown the sum of eigenvalues and the significance of the overall analysis

Sampling date		Sum of eigenvalues	Significance
1990	June	0.735	0.01
	July	0.633	0.09
	August	0.797	0.01
	September	0.881	0.01
	October	0.685	0.33
1991	June	0.789	0.02
	July	0.739	0.15
	September	0.871	0.01

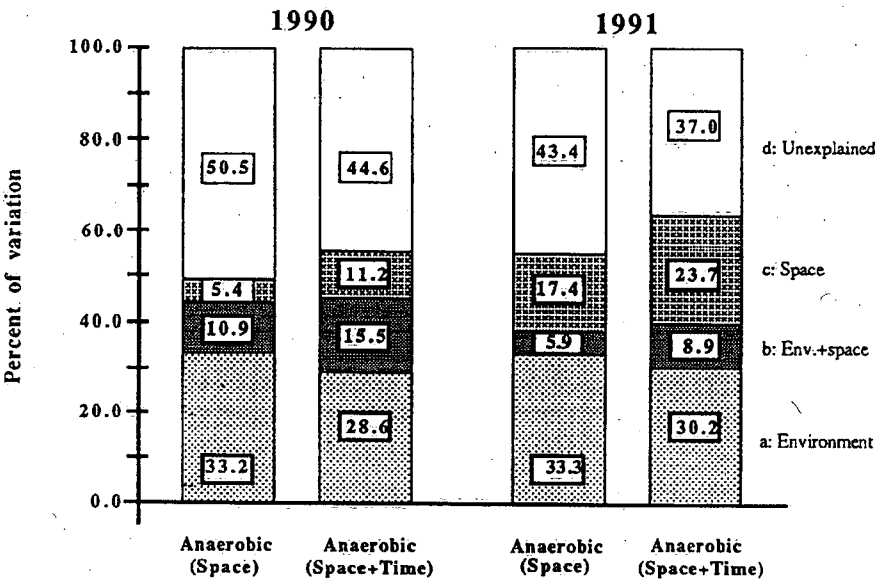

Fig. 6. Partition of the variation of the anaerobic community ascribed to the measured significant environmental variables (PC, DOC, NH_4^+, DO, depth, and temperature) only, the spatial structure (geographical coordinates) only, and the unexplained fractions. Sampling time (month) was added to the SPACE matrix.

matrix of microbial activities. The purely spatial fraction, when controlling for the effect of the environmental variables, was not significant and accounted for only 5.4% of the activities variation in 1990. In 1991, the purely spatial fraction was significant and accounted for 17.4% of the variation of potential microbial activities. The unexplained fraction was slightly higher in 1990 (50.5%) than it was in 1991 (43.4%). Introducing the time of sampling as one variable in the space matrix reduced the unexplained fraction by 5.9% and 6.4% in 1990 and 1991, respec-

tively. When considering the four microbial activities together, site location or sampling time did not account, therefore, for much of the observed variation when the effect of the environmental variables was first extracted from the data.

Knowing that most of the explained variation of the microbial activities is accounted for by the environmental factors, the contribution of each of these environmental variables was established next. For this purpose we used path analysis [30, 44] to test the significance of the causal relationships between the proximal regulators in Tiedje's model [51] and potential denitrification. CH_4 production, and CO_2 production rates. The models that we tested for denitrification (Fig. 7a), CH_4 production (Fig. 7b), and CO_2 production (Fig. 7c) are illustrated. Results are shown as synthetic models resulting from the analyses for 1990 (Fig. 7d) and 1991 (Fig. 7e). They were very similar from year to year. Only the relationships between DO and DOC, and DO and $NO_2^- + NO_3^-$, which did not significantly correlate, differed between 1990 and 1991. As expected in eutrophic lakes during summer stratification, sites under deep water columns had significantly lower temperatures and DO concentrations. At the same time these sites had significantly higher PC concentrations but not necessarily higher levels of DOC in the pore water. PC, DOC, DO, or $NO_2^- + NO_3^-$, had no significant direct effect on potential denitrification. Potential CO_2 production in the sediment is the only good predictor of potential denitrification rates, as shown by the path coefficients 0.53 and 0.57 for 1990 and 1991, respectively. This biotic factor is itself significantly predicted by PC and POC (0.50 and 0.34 in 1990; 0.38 and 0.47 in 1991). On the contrary, CH_4 production rates are not predicted by the potential rate of CO_2 production, but rather by the level of particulate carbon in the sediment (0.67 and 0.33 in 1990 and 1991, respectively). High DO concentrations in the water column significantly predicted low potential of CH_4 production in sediment slurries in 1990 but not in 1991. The smaller sample size ($n = 62$) in 1991 may explain why the path coefficient does not meet the criterion for significance. The significant path coefficients between DOC, CO_2 production, and NH_4^+ support the idea that NH_4^+ concentration is related to anaerobic mineralization of organic matter.

Discussion

Because of its simplicity, low cost, and sensitivity, we selected the C_2H_2 inhibition technique to study denitrification in sediment slurries of Hamilton Harbour. Aerobic as well as anaerobic metabolism of C_2H_2, reported for stream and estuarine sediments [11, 48] may relieve the inhibition of N_2O reductase [28]. We found both types of metabolism occurring in sediment slurries from Hamilton Harbour after an initial 4-day incubation (unpubl. data). This problem was overcome by measuring rates on the basis of a short incubation period (18–24 h). Nitrate was found to be the most important limiting factor of denitrification in sediment slurries of Hamilton Harbour as demonstrated by preliminary laboratory experiments (unpubl. data). The average potential rate we have measured (1.60 μmol cm^{-3} day^{-1} or 3.80 mM NO_3^--N day^{-1}) agrees with a rate previously reported for Hamilton Harbour (85 mg N $liter^{-1}$ day^{-1} or 6.14 mM NO_3^--N day^{-1}) [24] when fourfold differences in $NaNO_3$ concentrations are taken into account.

Rates of C_2H_4 evolution measured in slurries of Hamilton Harbour were generally lower than those reported for Lake Erie (28.6–34.2 nmol C_2H_4 g^{-1} day^{-1}) [18] but higher than those reported for several Wisconsin Lakes (0.5–1.3 nmol C_2H_4

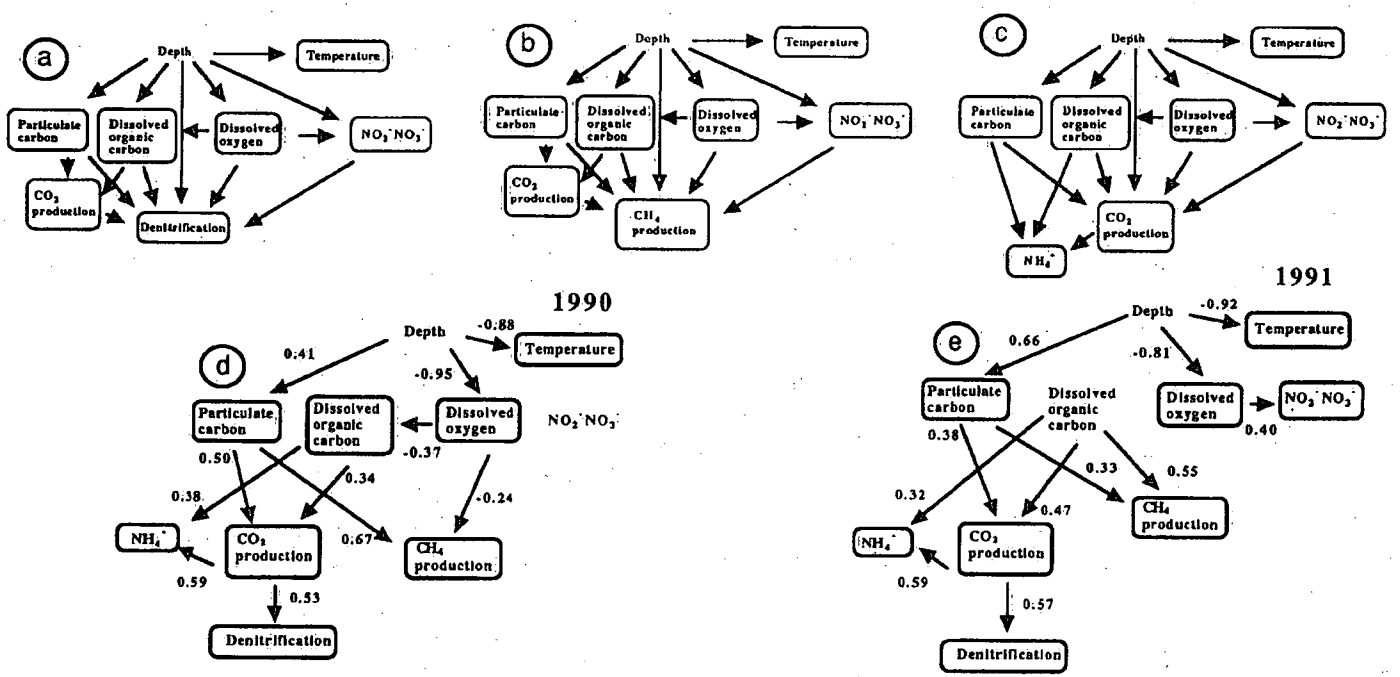

Fig. 7. Schematic representation of the path analyses performed on the data of 1990 and 1991. Hypothetical models tested for potential denitrification (**a**), potential CH_4 production (**b**), and potential CO_2 production (**c**) are shown as well as the results of these analyses for 1990 (**d**) and 1991 (**e**). In the hypothetical models (**a**), (**b**), and (**c**), the arrows are putative causal relationships tested by path analysis with potential denitrification (**a**), potential CH_4 production (**b**), or CO_2 production (**c**), as the dependent variable. In the synthetic models (**d**) and (**e**), boxes indicate significant R^2 ($P \leq 0.05$) and arrows indicate significant path coefficients ($P \leq 0.05$) shown beside each arrow.

g^{-1} day^{-1}) [34]. Because of the relatively high NH_4^+ concentration in the pore water (1.5 mM), N_2 fixation is likely to be repressed [19]. CO_2 production rates found for Hamilton Harbour slurries were lower than those reported by Jones [22] for a eutrophic lake in England. CH_4 production potential in Hamilton Harbour sediment (0.28 μmol cm^{-3} day^{-1} or 11.7 nmol cm^{-3} h^{-1}) was intermediate to rates reported for Lawrence Lake (4.6 nmol cm^{-3} h^{-1}) and Lake Wintergreen (26–40 nmol cm^{-3} h^{-1}) [8].

The average NH_4^+ concentration in the sediment pore water of Hamilton Harbour (1.5 mM in 1990) was found to be in the range of values reported by Klapwijk and Snodgrass [24] (0.71–3.57 mM). However we found concentrations reaching > 10 mM NH_4^+–N for site 926, which is much higher than previously reported for Hamilton Harbour. It is to be noted that the average NH_4^+ concentration in pore water of Hamilton Harbour is twice those reported by Jones [22] for a eutrophic lake in England (0.05–0.65 mM) or for Lake Mendota (0.34–0.96 mM) [10].

Our data (Figs. 1 and 2) suggest that the water exchange between Hamilton Harbour and Lake Ontario through the Burlington Ship Canal has an effect not only on the water column [3] but also on the sediment chemistry and microbiology. The high potential of CH_4 and CO_2 production as well as the high NH_4^+ concentration in the pore water at site 926 are all indicators of high organic matter mineralization. The source of this organic matter is probably the treated effluent of the Burlington sewage treatment plant. However, neighboring sites with low NH_4^+ concentration in the pore water suggest that the spatial extent of the impact of the treated effluent on the Harbour is limited. Slightly higher $NO_2^- + NO_3^-$ concentration in the pore water, as well as generally lower potential of denitrification in the south-east corner, may reflect the contribution of dissolved O_2 from the Lake Ontario water. But a definitive statement on this matter remains outside the scope of this study.

The introduction of spatial locations among the independent variables does not seem to allow an important explanation of the variation of potential anaerobic microbial activities after the environmental variables have been introduced. When compared to the results of Borcard et al. [7], the selected significant environmental variables were found to account for most of the explained fraction of microbial activities (70–90%). The amount of unexplained variation was similar to that reported by these authors. As stated by Borcard and Legendre [6], it is unclear how much of the spatial fraction is caused by population or community dynamics and how much is caused by environmental variables, biotic or abiotic, not measured during this study; these could include, for example, biotic factors such as bioturbation, predation, or parasitism. In any case, this fraction is negligible in 1990 and small in 1991. However, the fact that the selected environmental variables were significant confirms their importance in the spatial structure of benthic microbial activities.

Path analysis shows that depth of the water column does not directly affect potential denitrification, CH_4 production, and CO_2 production, but only indirectly through the level of PC. It is well-known that in eutrophic aquatic environments such as Hamilton Harbour the summer stratification leads to DO depletion in hypolimnetic sites, as it is reflected by the significant negative path coefficients between depth of the water column and DO. Lower DO levels are likely to slow down organic matter mineralization and lead to accumulation of PC, as supported by the significant positive path coefficients. However, depth does not seem to affect directly or indirectly the concentration of DOC in the pore water.

Path analysis demonstrated also a significant putative causal relationship between CO_2 production and potential denitrification. Significant correlations between denitrification and PC or DOC are indeed indirect relationships mediated through CO_2 production. This suggests that PC and DOC need to be transformed by some heterotrophic bacterial populations before being used by denitrifiers. This clearly indicates the possible importance of biotic factors in the control of denitrification, especially in relation to the carbon transfer between members of benthic bacterial communities.

On the other hand, potential CH_4 production rates are directly related to PC. Input of organic matter was already known to be linked to CH_4 flux in lake sediments [23]. The non-significant direct relationship of potential CO_2 production to CH_4 production suggests that the heterotrophic activity measured by CO_2 production is not directly involved in supplying methanogens with substrate. Since we have used initial rates of CH_4 production (24 h), this unexpected result may be a reflection of the pool of carbon directly available to methanogens which is likely to be related to PC levels. Rates of CH_4 production measured over a longer period of time could well lead us to a different conclusion. The relationships between NH_4^+ and DOC, and NH_4^+ and CO_2 production suggest that the NH_4^+ concentration is associated, as expected, with mineralization of organic matter by sediment bacteria [20].

Conclusion

Besides NO_3^- and O_2, we conclude that carbon may limit the capacity of denitrification in Hamilton Harbour sediments. We found that anaerobic CO_2 production best predicted the observed spatial variation of potential denitrification and therefore of denitrifier populations. This result suggests that some heterotrophs may provide the denitrifiers with readily available carbon by acting on PC and DOC, which seem mostly recalcitrant to the dominant denitrifiers.

Spatial variation of anaerobic microbial activities in Hamilton Harbour sediments is mostly explained by variations of the environmental variables (PC, DOC, DO, NH_4^+, depth, and temperature); not much of the spatial or temporal structure remains to be explained after these environmental variables have been partialled out. Inputs of treated waste water from Burlington sewage plant and water exchange between Lake Ontario and Hamilton Harbour explain most of the spatial variation of potential anaerobic microbial activities in Hamilton Harbour sediments, by affecting the levels of the selected environmental factors.

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