Estimation of Nutrient Limitation of Bacterial Activity in Temperate Alkaline Fen Sediments from Cedar Bog[1]

TIMOTHY C. GSELL[2] AND ROY M. VENTULLO[3], Department of Biology, The University of Dayton, Dayton, OH 45469-2320

ABSTRACT: Cedar Bog Nature Preserve, located near Urbana, OH, encompasses several wetland types including an alkaline fen. In this fen, groundwater emerges in quicksand-like discharge zones consisting of porous Ca/Mg carbonates mixed with organic detritus. This study evaluates seasonal changes in the heterotrophic sediment microbial communities, their response to nutrient amendment, and in the groundwater chemistry from a fen discharge zone at Cedar Bog. The hypothesis that the microbial community in this fen upwelling is nutrient limited throughout the year, particularly by C and P, was tested. The activity of the heterotrophic bacterial community in the sediment compartment was measured. A series of single factor experiments were conducted to study organic and inorganic nutrient regulation of these communities and to determine what nutrients, if any, were limiting. Activities were based on ^{3}H-thymidine incorporation into DNA by control and nutrient amended sediment slurries and verified with ^{14}C-leucine incorporation into protein. Bacterial cell abundance was determined using Acridine Orange direct counts. Samples amended with carbon showed significant increases in activity in three of four seasons tested. Bog extract also stimulated activity above that of the control for the winter microcosm. The site bacterial activity also appears to be limited by inorganic nitrogen and possibly phosphorus in summer.

OHIO J SCI 104 (3):43–50, 2004

INTRODUCTION

It has long been recognized that bacteria play an important role in nutrient cycling and secondary production in aquatic systems (Bell and others 1983; Elser and others 1995; Fuhrman and Azam 1980; Riemann and Bell 1990; Taylor and Joint 1990). While extensive research has been conducted on the microbiology and nutrient response of bacteria found in aquatic environments (Chrzanowski and others 1995; Ducklow and others 1985; Heinanen and Kuparinen 1992; Hessen 1992; Kuparinen and Heinanen 1993; Meyer and Tate 1983) and in groundwaters (White and others 1982), little is known about the heterotrophic bacterial communities in alkaline fens (Groffman and others 1996: Kang and others 1998). This research was conducted to see if the carbon, nitrogen, and phosphorus levels in this wetland impact the microbial numbers, growth rates, and physiological status in an Ohio fen.

In the Cedar Bog Nature Preserve, alkaline fen upwellings occur where groundwater emerges in discharge zones consisting of porous Ca/Mg carbonates mixed with organic detritus. Since these upwellings are the major source of water and nutrients for the wetland, bacterial growth in the upwellings may represent a significant portion of the system's bacterial secondary production. It is not clear to what extent nutrients limit bacterial activity in these groundwater-saturated sediments. Laboratory microcosms were used to test the hypothesis that nutrients limit the activity and possibly regulate the physiological status of the bacteria in the fen sediments. This was accomplished using bacterial growth rates for control and nutrient amended sediment slurries and total bacterial cell abundance. Specifically, it was hypothesized that dissolved organic matter (DOM) and phosphate limits the growth of bacteria in the alkaline fen discharge zone. Nitrogen levels were known to be relatively high due to local agricultural seepage of anhydrous ammonia and its microbially converted products, and were therefore hypothesized to not be limiting to the sediment microorganisms. It was also recognized that nitrification would not be carbon limited and therefore components of the microbial communities could be impacted by nitrogen shortages, but not by organic carbon levels.

The activity of bacterial communities from various ecosystems has been determined using radiotracer incorporation techniques. ^{3}H-thymidine incorporation is ubiquitous for heterotrophic bacterial DNA and not taken up by cyanobacteria, eucaryotic microalgae, autotrophic bacteria, or fungi (Baath and Johansson 1990; Thorn and Ventullo 1988). ^{3}H-thymidine labeling of DNA has been used extensively to measure bacterial activity, however there has been concern over a lack of specificity of incorporation in addition to extraction variability observed in freshwater (Chin-Leo and Kirchman 1988; Robarts and others 1986) and sediment (Brittain and Karl 1990; Ducklow and others 1985; Moriarty and Pollard 1981). These potential limitations were overcome by using two measurements of microbial communities activity and therefore provide more reliable estimates of bacterial cell growth in this system. Data obtained using this approach and other bacterial activity estimates were compared. Incorporation of ^{14}C-leucine into protein (Baath 1994; Chin-Leo and Kirchman 1988;

[1]Manuscript received 16 October 2002 and in revised form 9 June 2003 (#02-21).

[2]Corresponding Author: Governors State University, University Park, IL 60466-0975 USA. Ph: 708/534-4921; Fax: 708/534-1641; Email: t-gsell@govst.edu

[3]Present Address: Department of Biology, Wartburg College, Waverly, IA 50677.

Riemann and others 1990) was used as a supplemental measurement for estimating bacterial growth rates by evaluating the amount of label found in total cellular protein. Radiotracer incorporation into the DNA and protein pools in these assembleges has been shown to be proportional to the growth rate of the bacterial communities (Baath 1994; Tibbles and others 1992) and, therefore, of value to this study. Lipid incorporation of ^{14}C-leucine was also examined as an indicator of environmental stress (Guckert and others 1992).

MATERIALS AND METHODS

Description of Site

The study site was in the Cedar Bog Nature Preserve near Urbana, OH. The preserve covers 1.73 million hectares and is located above approximately 100 m of limestone gravel, which was deposited over the last two million years by the three great glaciers. Groundwater flowing from hills to the east comes to the surface in springs or discharge zones. There is a constant flow of cool (~10° C), slightly alkaline water that keeps the water table high.

In the fen meadow, several upwelling discharge zones can be found in and adjacent to the east branch of Cedar Run. In fens, as the cool alkaline spring water moves to the surface of the meadow, the excess calcium carbonate dissolved from the limestone gravel below is deposited. The calcium carbonate deposit, called marl, forms the gray soil in the fen meadow, which is 0.5 to 1.0 m deep. The groundwater upwelling zones never freeze, have not been known to dry up, and constantly churn the sediments near the surface without displacing much of the material downstream. The groundwater that feeds these systems has been found to be low in organic and, in many cases, inorganic nutrients (Gsell and others 1997). The hydraulic conductivity for the sand and gravel aquifer below the fen ranges between 24.4-152.4 m/day.

Sampling and Site Description

Samples were taken from a groundwater discharge zone in the fen meadow referred to as the South Pool. Sediments were collected aseptically in Mason® jars from the surface of the discharge zone at each sampling period. Groundwater was collected by placing a 30 cm long, 15 cm diameter hollow Plexiglas™ cylinder vertically over and into a groundwater discharge zone and allowing the artesian properties of the site to fill and overflow the tube (10 cm above the water surface) for at least 10 minutes. After allowing groundwater to displace any remaining surface water in the tube, groundwater was pumped aseptically through Teflon™ tubing into sterile acid-washed Corning glass bottles (Corning, NY). The groundwater nutrient concentrations (nitrate-N, ammonia-N, phosphate-P, and dissolved oxygen) were determined on site using Hach (Loveland, CO) or CHEMetrics (Calverton, VA) chemical field kits (Table 1). Trace phosphate levels were also measured in the laboratory spectrophotometrically using an ammonium molybdate titration method for the summer study only (Strickland and Parsons 1977). The samples were transported to laboratory facilities at *in situ* temperature (~10° C). The levels of inorganic nutrients (N and P) were confirmed using high-pressure ion chromatography (Dionex Corp., Sunnyvale, CA). Nonpurgable organic carbon (NPOC) was determined (1 hour after sample collection) using a TOC 5000 Carbon Analyzer (Shimadzu Scientific Instruments Inc., Columbia, MD).

Chemicals

All radioisotopes ([methyl-^{3}H]thymidine; 50 Ci/mmol, [U-^{14}C]L-leucine; 310 mCi/mmol, and [U-^{14}C]D-glucose; 280 mCi/mmol) were obtained from ICN Pharmaceuticals Inc., Irvine, CA. The chloroform, acetone, and methanol were GC/GC-MS grade (Baxter). All other chemicals were reagent grade or better. All amendments added to sediments were prepared with sterile Norganic® cartridge treated Milli-Q™ water (Millipore Corp., Bedford, MA).

Enumeration of Bacteria

Biomass of the sediment-associated bacteria was determined by epifluorescence microscopy of Acridine

TABLE 1

Nutrient concentration of upwelling zone groundwater from the South Pool.

Sampling Season [a]	Nutrient concentration in groundwater (mg l^{-1}) NPOC-C [b]	NO_3-N	NH-N	PO_4-P	Water Temperature	mM; C:N:P [c]
Fall	3.50	4.50	0.50	0.06	12° C	0.29:0.36:0.002
Winter	4.02	4.50	0.20	<0.001	9.5° C	0.34:0.34:-0.00
Spring	2.90	4.50	0.20	0.10	12° C	0.25:0.34:0.003
Summer	10.80	1.60	3.50	0.60	15° C	0.90:0.29:0.05

[a] 13 October 1992 (fall), 3 March 1993 (winter), 20 May 1993 (spring), and 28 July 1993 (summer).
[b] Nonpurgable organic carbon.
[c] C:N:P ratios considered optimal for microbial growth is approximately 100:10:1 (Sims and others 1984).

Orange stained cells (Hobbie and others 1977). Sediment samples were removed and preserved in 2.5% (v/v) glutaraldehyde dissolved in 0.1% (w/v) sodium pyrophosphate and refrigerated until counted. Prior to counting, sediment samples were homogenized using a tissue homogenizer and diluted with the phosphate buffered glutaraldehyde. At least 10 randomly selected fields were counted on each slide, using an Olympus BHS microscope (Valencia, PA).

^{3}H-Thymidine Incorporation into DNA alone and with ^{14}C-Leucine Incorporation into Protein: Bacterial Activity

The ^{3}H-thymidine method was used to determine the activity of sediment associated bacteria (Carman and others 1988; Moriarty and Pollard 1981). The dual-label procedure involves incubation with both labels, 125 nM ^{3}H-thymidine and 160 nM ^{14}C-leucine for one hour followed by cold trichloroacetic acid (TCA) precipitation of macromolecules and subsequent extraction and macromolecular fractionation procedure according to the Type 3 procedure of Craven and Karl for carbonate sands (1984) and modifications by Carman and others (1988). Briefly, replicate 2.0 g wet weight samples (n = 4-6) were incubated and extracted for each treatment. These sediments were treated with ice cold TCA, then washed in TCA, followed by ice-cold ethanol and dried, to remove exogenous label. RNA and DNA were then solubilized in base, centrifuged, and the supernatant treated with 10% TCA (cold) to hydrolyze the RNA. DNA was recovered from the supernatant of subsequent hot 5% TCA (90° C for 30 min) treatment. Protein is insoluble in the base treatment mentioned above. The pellet (see above) was washed and treated with base (1N NaOH) for 18 h at 37° C to solubilize the protein, which was collected from the supernatant. Aliquots (250 µl) of DNA and protein fractions were placed in 5.0 ml of Ready Safe™ cocktail (Beckman Inc., Palo Alto, CA). Radioactivity was determined using an LS 3801 liquid scintillation counter (Beckman Inc.). Dual label was used for the summer microcosms only. Growth rates (Fig. 1) were calculated by determining the radioactivity in the DNA fraction, converting this to nmoles of thymidine incorporated, and employing the conversion factor of 1×10^{18} cells per mole of thymidine incorporated into DNA (Riemann and others 1987; Reimann and others 1990).

Lipid Analysis and Physiological Stress Determinations

For the summer samples only sediments (20-30 g wet weight) was added to a 125 ml flask and shaken for one hour at 50 rpm at 15° C with 10 µCi of 160nM ^{14}C-glucose. One g wet weight sediment sub-samples were extracted for lipids using a chloroform methanol buffer (Guckert and others 1985). After phase separation, an aliquot from the aqueous phase was added to liquid scintillation cocktail. The organic phase containing the lipids (bottom layer) was drained into a round bottom flask (for removal of solvents). Half of the total lipid was removed for liquid scintillation counting and used

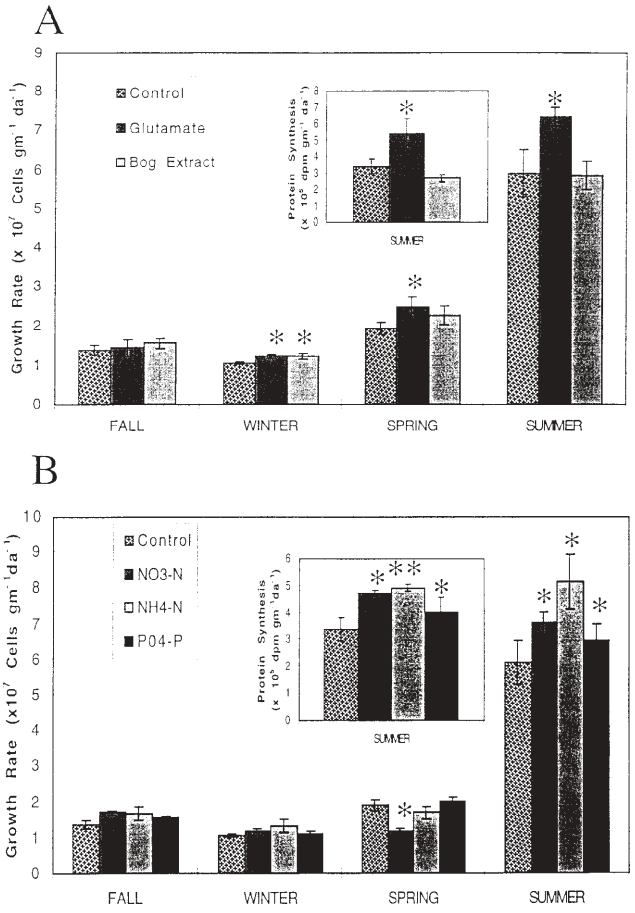

FIGURE 1. Effect of organic (A) and inorganic (B) amendments on the bacterial growth rate in microcosm South Pool sediments based on incorporation of ^{3}H-thymidine into DNA. Inset on both A and B is the incorporation of ^{14}C-leucine into protein. In the summer study only, nutrient levels were 5× the measured field level, and glucose replaced glutamate as a simple organic amendment. Data represents the mean ± SEM of amended and control samples, (n = 4). *Statistical difference ($P \leq 0.10$) compared to control. **Statistical difference ($P \leq 0.05$) compared to control.

to describe phospholipid synthesis (activity), expressed in DPM/g sediment. The other half of the total lipids was separated into three general lipid classes by silicic acid column chromatography (Unisil®, 0.5 g of 100-200 mesh) using a series of mobile phases of increasing polarity: neutral lipids, 10 ml of chloroform; glycolipids, 10 ml of acetone; and polar lipids, 10 ml of methanol (Guckert and others 1992). Solvents were removed under nitrogen purge and a 10% aliquot from each of the lipid classes were removed for LSC. For bacteria, membrane lipid (polar phospholipids) to storage lipid (glycolipid) ratios were used as indicators of physiological status as described by Guckert and others (1985). Radioactivity in phospholipid was used to determine microbial (heterotrophic) activity (Guckert and others 1992; Vestal and White 1989).

Experimental Design: Organic and Inorganic Nutrient Amendments for Microcosms

A series of single factor experiments were conducted to measure their response to addition of nutrient (C, N, and P). The four seasonal sampling dates were 13 October

1992 (fall), 3 March 1993 (winter), 10 May 1993 (spring) and 28 July 1993 (summer). Laboratory microcosms consisted of 120 ml Teflon™ screw-cap jars. Each jar received 60 g wet weight sediment, was loosely capped, and placed on an orbital shaker (Thermolyne, Dubuque, IA) at 50-100 rpm at the measured groundwater temperature for each sampling period (Table 1). The orbital shaker was used because sediments in the upwelling zones were continuously mixed by the ground-water flow to the surface. Amendments contained enough dissolved nutrient to give a final concentration of twice the measured field concentration from Table 1 in a volume of ~50 μl except for the summer where 5× concentrations were used to see if a larger increase in carbon would have an effect on activity and biomass (Table 2). These solutions were administered by amending sediment samples that were at groundwater holding capacity (approximately 3.0 ml per 1 gram dry weight of sediment). All stock solutions were adjusted to the pH of the bog water at the time of sediment sampling (mean pH = 7.5) and sterilized prior to addition to sediment. Sediments were amended with one of the two groups of organic substrates or one of the three inorganic nutrients.

The inorganic amendments, and glucose and glutamate (L-glutamic acid) were prepared from laboratory reagents (KNO_3-N, NH_4Cl-N, Na_2HPO_4-P) in sterile Milli-Q™ water as stated above. "Bog extract" was prepared by adding dried organic detritus from the upwelling to hot (100° C) sterile Milli-Q™ water. The mixture was allowed to cool at room temperature overnight and was subsequently filtered through a GF/C (Whatman) glass fiber filter. Filtrates (0.2 μm) of the resulting solution was used as an organic amendment source (Bog Extract). The TOC of the final stock solution was 300 mg/l. The extracts and prepared nutrient solutions were assayed for traces of other nutrients and TOC, but the individual organic molecules present were not determined. For each amendment tested, one set of microcosms were amended with Norganic® filtered Milli-Q™ water as a no amendment control. The samples were incubated with nutrient amendments for 24 h. After incubation, 2.0 g sediment sub-samples were removed from each container (n = 4) for all amendments and radio-labeled for 1.0 h as described above. Microcosm batch cultures were maintained at the *in situ* temperature for the corresponding sampling period (Table 1) and kept in the dark. In the summer study (Fig. 2), nutrient levels were 5× the measured field level, and glucose replaced glutamate as a simple organic amendment for all three activity measures (Fig. 2). C:N:P ratios were based on the TOC-C, NO_3-N + NH_4-N and PO_4-P levels in groundwater samples from each study period and are expressed in mM amounts (Table 1). Glucose replaced glutamate as the organic amendment in summer because it does not contain N. It was hoped that by using glucose it would be easier to determine if carbon was limiting at this time.

Statistical Analysis

Mann-Whitney U rank sum test was used to determine differences between means where indicated (Sokal and Rohlf 1981). The non-parametric rank correlation was used because we did not assume a linear relationship among any of the data. All statistics were calculated using *Statistix*, Ver. 3.5 (Analytical Software, Tallahassee, FL). Significance levels were set at P = 0.1 - 0.05 for all analyses.

RESULTS AND DISCUSSION

Physical and Chemical Parameters from the Sampling Site

Measured physical and chemical parameters of fen groundwater from the South Pool are given in Table 1. The groundwater was typically low in some measured nutrients. In winter *P* was undetectable (below 0.001 mg l^{-1}). However, nitrate levels were much higher than expected for groundwaters, approaching 5.0 mg l^{-1}. This is likely due to agricultural runoff and seepage from nitrogen containing fertilizer applied to distant and surrounding fields. In groundwater-fed wetlands (fens), carbon levels generally range between 1.0 and 20 mg l^{-1} C (White and others 1982). Groundwater from Cedar Bog commonly yielded 2.0-10 mg l^{-1} C (Table 1). Non-purgeable organic carbon was generally higher in the summer (10.8 mg l^{-1}) than during the other seasons (3.0-4.0 mg l^{-1}). Although this describes the level of

TABLE 2

Nutrient concentration of microcosms based on groundwater chemistry from the South Pool.

Sampling Season [a]	Final nutrient concentration in microcosm (mg l^{-1})				
	NPOC-C Glutamate	"Bog Extract"	NO_3-N	NH_4-N	PO_4-P
Fall	6.95	6.95	9.00	1.00	0.06
Winter	8.044	8.044	9.00	0.40	<0.001
Spring	5.80	5.80	9.00	0.40	0.10
Summer	54.00 [b]	54.00	17.50	3.00	0.60

[a] 13 October 1992 (fall), 3 March 1993 (winter), 20 May 1993 (spring), and 28 July 1993 (summer).
[b] Summer amendments were all 5× the amount measured in the field for all nutrients and glucose replaced glutamate for this season only.

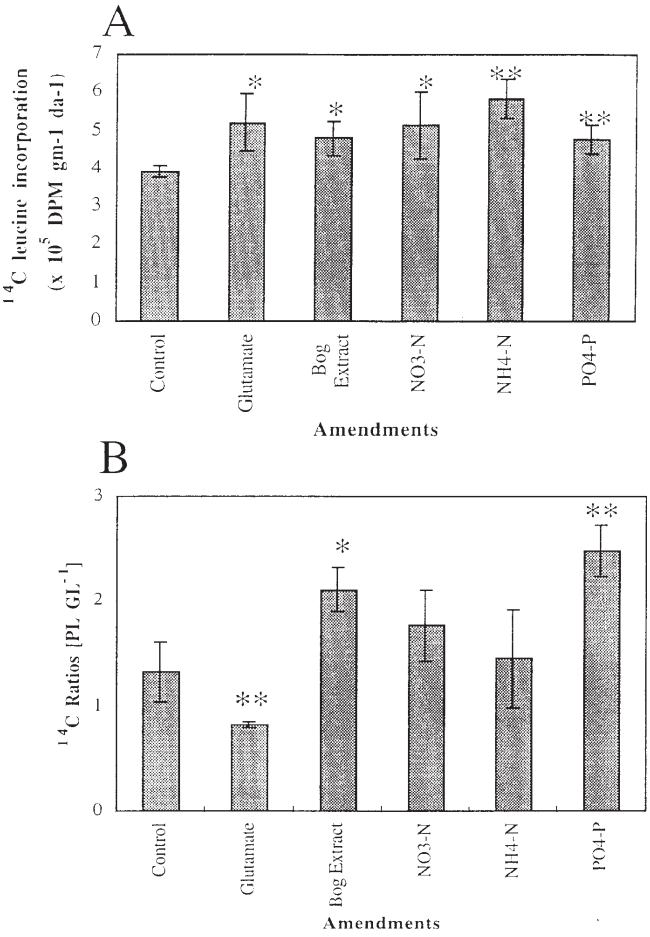

FIGURE 2. Phospholipid synthesis (A) and ^{14}C-glucose labeled membrane/storage lipid ratios (B) for the summer amendment study (higher ratios indicate higher stress). In this summer study amendment nutrient levels were 5× the measured field level, and glucose replaced glutamate as a simple organic amendment. Data represents the mean ± SEM of amended and control samples, (n = 4). *Statistical difference ($P \leq 0.10$) compared to control. **Statistical difference ($P \leq 0.05$) compared to control.

carbon found at the site in relation to *N* and *P*, it does not necessarily describe the presence or absence of nutrient limitation of bacterial growth. It is not clear why nitrogen is lower and carbon is higher in the summer versus other times of the year.

Acridine OrangeCell Abundance from Amended and Control Sediment Microcosm Samples

Cell abundance for unamended and amended samples can be found in Fig. 3. Cell counts typically were in the low to mid 10^9 Cells/g range for all samples. This was an interesting result because fens are known to have low nutrient content and low microbial abundance, due mainly to the oligotrophic conditions which typically prevail in groundwater (Groffman and others 1996). These values were much higher than expected, but this can be explained by the nutrient loading that occurs from agricultural runoff that would not be present in more pristine fen sites. There are several occasions where cell abundance was significantly greater than that of the unamended control. In the spring glutamate and summer glucose addition results were significantly greater than the unamended controls. Nitrate amended samples were significantly higher in fall and spring (Fig. 3B). Ammonia amended samples showed significant positive differences in spring and summer, as did the phosphate-amended samples. These events do not completely correspond to increases in bacterial growth detected after 24 h using thymidine incorporation, but many patterns from the spring and summer time points co-vary (see Figs. 1,3) indicating some similarities between seasons. For any discrepancy it may be that the short incubation time was not long enough to show marked increases in cell abundance following activity increases.

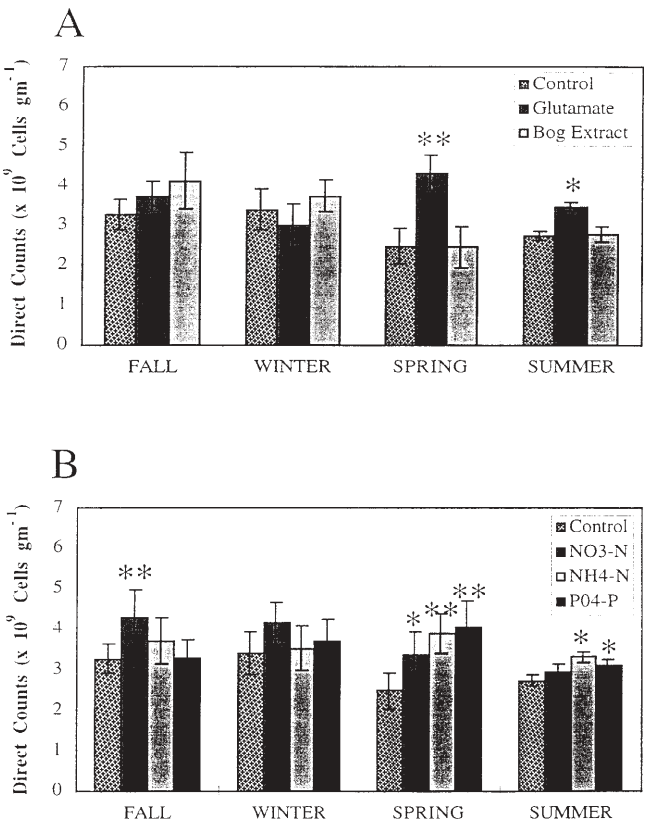

FIGURE 3. Effect of organic (A) and inorganic (B) amendments on direct counts of sediment bacteria from the South Pool. Samples taken in samples included the fall (13 October), winter (3 March), spring (20 May), and summer (28 July), and used in these amendment studies. In the summer study only, nutrient levels were 5× the measured field level, and glucose replaced glutamate as a simple organic amendment. Data represents the mean ± SEM of amended and control samples, (n = 10 fields per filter). *Statistical difference ($P \leq 0.10$) compared to control. **Statistical difference ($P \leq 0.05$) compared to control.

^{3}H-Thymidine Incorporation into DNA and ^{14}C-Leucine Incorporation into Protein: Protein Synthesis: Bacterial Activity from Amendment Studies

Amendment experiments were performed on sediment samples obtained at various seasonal intervals from the South Pool upwelling zone at the Cedar Bog study site.

All amendment solutions showed no trace of other nutrients (C, N, and P), including the "bog extract" (for example, the glutamate had no measurable N or P). However, the concentrations of organic nitrogen and organic phosphorus were not determined either in ground water or in "bog extract," so possible availability of N and/or P from these sources is not known and should not be excluded.

South Pool control sediments showed similar bacterial abundance and activities to other aquatic sediments (Baath 1994; Moriarty and Pollard 1981). Amended samples showed significant increases in activity upon addition of glutamate in the winter, spring, and summer (Fig. 1). Bog extract addition resulted in significant increases in thymidine uptake exclusively in the winter (Fig 1). The response of the discharge zone sediment community to carbon, in general, suggests potential carbon limitation in the spring, summer and winter microcosms (Fig. 1). This data also indicates seasonal variation in sediment community bacterial activity; for example, bacteria are more active in summer, which is in support of previously published findings from a similar seasonal timeframe, during a different year (Gsell and others 1997). Amendment studies revealed that sediment bacterial communities are inorganic (N+P) limited exclusively in summer microcosms (Fig. 1). Although P was undetectable in winter samples, the addition of P resulted in no significant stimulation of activity, thus does not appear to limit activity. Although not limiting in the microcosm experiments, P limiting conditions appear to exist *in situ* based on the levels measured. P being undetectable for one sampling time made it difficult to draw conclusions from the winter microcosm results, although when added at 1.0 mg/l P, no activity increase was noted except in the summer sampling period. It is possible that a steady state P flux may meet the bacterial demand, but is not enough to form a measurable phosphorus level.

In another study at this site it was found that large quantities of P (from 0.3 mg/l to 3.5 mg/l) were measured in water samples taken from the South Pool when adjacent Cedar trees were cut down (Gsell and others 1997). The root systems of these trees ran directly through the upwelling zone studied and it is suspected that the removal caused the release of P from the root system due to the demise of the mycorrhizal association between the White Cedar and fungi. This phenomenon had been reported previously (Likens and others 1970).

Nitrate-N addition had a significant inhibitory effect on activity exclusively in the spring microcosm ($P \leq 0.05$, Mann-Whitney U [Fig. 1]). The reason for the NO_3 inhibition is not clear, but it may be due to cell stress caused by a large decrease in the C:N ratio (Table 1). Activity measurements in all amended samples, except the "bog extract" in summer, were significantly higher than control values. Slight groundwater temperature increases at this time of year may be partially responsible for the 3-6-fold increase in field and control microcosm's activity measurements over that found in the other seasons. This could also be responsible for the faster response of activities in summer-amended microcosms. There is a possibility that winter microcosms may be delayed in response and increases in activity may not be detected in the 24 h incubation period. The C:N:P ratios during the summer are by far the closest to the optimal level for maximum rates of thymidine uptake of the four seasonal sampling times (Table 1 [Sims and others 1984]). Thymidine and leucine incorporation rates in summer show comparable values and identical pattern changes (Fig. 1).

Dual labeling allows for the validity of one method to be determined by comparing calculated growth rates according to both methods, using a single sample (Chin-Leo and Kirchman 1988). The dual label method has yielded concomitant results between DNA and protein synthesis in the present study as it has in other research (Baath 1994; Gsell and others 1997).

Lipid Based Activity Analysis and Physiological Stress Determinations

Lipid ratios indicated that physiological stress decreased significantly compared to control for the glucose amended samples (Fig. 2). The ^{14}C-glucose incorporation into phospholipid results confirms the trends from the other two activity measures (Fig. 2). The membrane to storage lipid ratios also indicated that complex carbon (bog extract) and phosphate addition significantly increased the physiological stress index of the sediment bacterial communities from these microcosms. The results of the summer amendments reinforce previous findings (Gsell and others 1997) and support the contention that this season is distinguished by large shifts in activity. It seems to be the point where DNA synthesis, protein synthesis and lipid synthesis are at their highest for the entire year (Chin-Leo and Kirchman 1988). There may also be a difference in the effect of the various nutrients used in that cells receiving too much C when N and P are unchanged will probably store C material as lipid if concurrently limited in N and P.

Nutrient Limitation of Bacterial Activity

There are several possible reasons for the low C:N ratios (Table 1) which are common throughout the year. High N levels throughout the year can be explained by fertilization of nearby and distant agricultural lands. Carbon limitation is apparent in these sediments and is likely due to the low carbon levels in relation to relatively high N levels. Adaptations to low nutrient environments allow aquatic bacteria to exploit the diverse yet dilute available carbon sources to maintain relatively high growth rates of 1×10^7 to 10^8 cells/ml/day on average, with cell numbers exceeding 1×10^9 cells/g in many sediments (Bell and others 1983; Gsell and others 1997; Scavia and others 1986). This is the level of activity found in Cedar Bog when growth rates were calculated by determining the radioactivity in the DNA fraction and employing the conversion factor of 1×10^{18} cells per mole of thymidine incorporated into DNA (Riemann and others 1987; Reimann and others 1990). Also, the biomass that is supported in the sediment compartment of discharge zones in Cedar

Bog were also typical for aquatic sediments.

The question concerning utilizable carbon was not addressed in this communication, but has been discussed in the literature. It is acknowledged that this may be an important factor in determining why carbon may be limiting even when it appears that there is sufficient levels to sustain microbial growth. It was thought until recently that much of this carbon may be in a non-utilizable form. Some studies have reported that large molecular weight carbon sources are utilized by bacteria (Amon and Benner 1996; Kaplan and Newbold 1993). The communities from the sediments of groundwater up-wellings are exposed to allochthonous carbon, which may have originated from a variety of surface sources a long distance away. These sources include leachates from the surrounding crops and carbon associated with plant-life from within the fen.

It has been emphasized that it is not only the DOC available to bacteria but the levels of important nutrients relative to one another that regulate bacterial growth (Chrzanowski and others 1995; Elser and others 1995; Munster 1991; Tulonen 1993). The C:N:P ratio in the waters perfusing through Cedar Bog sediments may be the key element in regulating bacterial growth (Gsell and others 1997). Tulonen and others (1992) found additions of nutrients caused increased availability of allochthonous DOM for bacteria in a highly humic lake. Humic substances are, however, responsible for enzyme complex formation which cause phosphorus limitation in aquatic environments by inhibition of enzyme activities (Tulonen 1993). Also, when phosphate concentrations are low (below 5.0 μg/l) and the N:P ratio very high, phosphorus limitation can occur for both algae and bacteria throughout the year (Arvola 1991). The ratio of N:P may be responsible for the apparent phosphate limitation that was found in Cedar Bog sediments during the summer sampling period. Recent studies on a fen in North Wales by Kang and others (1998) have emphasized availability of important nutrients such as C and N for enzyme activity and its effect on trace gas emission. This report suggests that nitrate concentrations below the 10 mg L^{-1} may be a limiting factor for denitrification, and that levels above that are non-limiting.

Summary and Conclusions

In this study a series of single factor experiments were conducted to examine organic and inorganic nutrient regulation of the bacterial communities in a Cedar Bog upwelling site. The main goal was to determine what nutrients, if any, were limiting activity in laboratory microcosms with samples collected seasonally. Activities were based on ^{3}H-thymidine incorporation into DNA by control and nutrient amended sediment slurries. Amended samples showed significant increases in activity above unamended control values upon addition of a readily utilizable carbon source in samples taken in winter, spring, and summer. Bog extract, a complex carbon source, exclusively in the winter microcosm stimulated activity above that of the control for that time period. This study further describes the microbial community and their response to nutrients in addition to what was found in the previous study (Gsell and others 1997). From the work described here it was confirmed that these sediment communities, in general, appear to be carbon limited throughout the year with the exception of fall, and inorganic nitrogen and possibly phosphorus limited in summer.

ACKNOWLEDGMENTS. This work was partially funded through the Research Institute at the University of Dayton. We are grateful to Terry Jawarski, curator of the Cedar Bog Nature Preserve at the time of this research, for his assistance and knowledge concerning the study site, and to the Ohio Historical Society for permission to access and sample the upwelling site on the East Fork of Cedar Run.

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