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Characterization of the Sediment Bacterial Community in Groundwater Discharge Zones of an Alkaline Fen: a Seasonal Study

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The cell density, activity, and community structure of the bacterial community in wetland sediments were monitored over a 13-month period. The study was performed at Cedar Bog, an alkaline fen. The objective was to characterize the relationship between the sediment bacterial community in groundwater upwelling zones and the physical and chemical factors which might influence the community structure and activity. DNA, protein, and lipid synthesis were measured at three different upwelling zones by using [3H]thymidine, [14C]leucine, and [14C]glucose incorporation, respectively. The physiological status (apparent stress) of the consortium was assessed by comparing [14C]glucose incorporation into membrane and that into storage lipids. Bacterial cell density was determined by acridine orange direct counts, and gross bacterial community structure was determined by bisbenzimidazole-cesium chloride gradient analysis of total bacterial community DNA. Both seasonal and site-related covariation were observed in all estimates of bacterial biomass and activity. Growth rate estimates and cell density peaked in late July at 2.5 × 10^8 cells/g/day and 2.7 × 10^9 cells/g, respectively, and decreased in December to 2.0 × 10^7 cells/g/day and 1.5 × 10^8 cells/g, respectively. Across sites, membrane-to-storage-lipid ratios were generally highest in late spring and peaked in September for one site. Overall, the data indicate dynamic seasonal differences in sediment bacterial community activity and physiology, possibly in response to changing physical and chemical environmental factors which included the C/N/P ratios of the perfusing groundwater. By contrast, total cell numbers were rather constant, and community structure analysis indicated that the overall community structure was similar throughout the study.

Microbial biomass and activity are fundamental variables in determining the importance of microorganisms in a particular environment (4, 22, 28). By a variety of radiotracer methods, bacterial activity, or production, has been assessed in freshwater aquatic systems (21, 23, 24, 28), marine water (7, 11, 12), aquatic sediments (8, 10, 25), and soils (2, 3, 37). This work has provided evidence that bacteria are essential for the cycling of nutrients such as nitrogen, phosphorus, and sulfur and as food for other trophic levels in these environments (28). However, little is known about bacterial communities residing in sediments through which groundwater flows (37). Freshwater wetlands in general, and fens in particular, are not well studied with regard to their bacterial communities. Knowledge of factors regulating bacterial activity and production would help us to better understand nutrient flux and carbon flow in wetland systems.

The objective of this study was to characterize the relationships between the prevailing physical and chemical parameters and the bacterial community in groundwater upwelling zones in Cedar Bog, an alkaline fen located near Urbana, Ohio. The specific objectives were to describe the seasonal patterns of activity, cell density, physiological status, and community structure of the heterotrophic bacterial communities near the surface of several groundwater discharge zones over a 1-year time span. These unique sites, where groundwater emerges from the subsurface to feed a primary stream in an alkaline fen, represent the interface between two vastly different environments. Key physical and chemical parameters were monitored for comparison and correlation with cell activity and numbers.

The activity of bacterial communities at these sites was determined by three different radiotracer methods, specifically, the incorporation of [3H]thymidine into DNA and [14C]leucine into protein in a dual-label experiment and incorporation of [14C]glucose into membrane lipids. In addition, the physiological status, or apparent stress, of the microbial communities was assessed by analysis of 14C allocation patterns between membrane and storage lipids. The cell density of the bacterial communities was determined by direct microscopic enumeration. The overall structure of bacterial communities was determined by analysis of DNA isolated and purified directly from the environmental samples. For this purpose, the approach, first described by Holben and Harris (18), which generates a profile of the bacterial community based on relative abundance of DNA versus percent G+C content was employed.

Statistical comparison of the data by Spearman’s rank correlations indicated several apparent relationships between bacterial community status and the physical and chemical parameters measured. The data support the conclusion that, in this system, the overall bacterial community structure does not change significantly but the bacterial activity and physiological status are subject to large directional shifts seasonally.

MATERIALS AND METHODS

Site description and sampling. The study site was the Cedar Bog Nature Preserve located on Woodburn Road near Urbana, Ohio. The preserve covers 427 acres mapped out in quadrants. Contrary to its name, Cedar Bog is an alkaline fen and is located above several hundred feet of limestone gravel which was deposited over the last 2 million years by the three great glaciers. Ground-
water flowing from the hills to the east comes to the surface here in tiny springs associated with the limestone gravel below is deposited. This calcium carbonate deposit, called the limestone gravel, forms the gray soil in the bog meadow, which is 0.5 to 1 m deep. In the bog meadow, several upwelling zones can be found and adjacent to the east branch of Cedar Run, a primary stream located in the preserve which flows from north to south.

Groundwater and associated sediments were taken from three upwelling zones along the stream edge in quadrants D5 and D6 in the bog meadow. These sites are referred to here as (i) south pool, (ii) 60 N (60 m north of the south pool), and (iii) 100 N (100 m north of the south pool). Groundwater samples were collected by placing a hollow Plexiglas cylinder (30 cm in length by 15 cm in diameter) vertically over and into a groundwater discharge zone and allowing the aquatic properties of the site to fill and overflow the tube (10 cm above the water surface) for at least 10 min. After this flushing period, groundwater was pumped aseptically through Teflon tubing into sterile, acid-washed 500-ml glass bottles (Corning Glass Works, Corning, N.Y.) with a Nalgene hand-pump (Nalge Corp., Rochester, N.Y.). Sediment samples were collected aseptically in sterile mason jars from the sediment surface (0 to 10 cm) of the discharge zone. All samples were transported to the laboratory at is temperature (−10°C).

Air and groundwater temperature were measured with a minipresil-filled thermometer following a 15-min equilibration period. The pH of the groundwater was measured with a combination electrode (EM 95 of Orion Research Inc., Boston, Mass.) upon arrival in the laboratory. Water chemistry was determined on-site with Hach (Loveland, Colo.) and CHEMetrics (Calverton, Va.) chemical field kits. Total phosphorus levels were determined in triplicate by a colorimetric method using a spectrophotometer by an ammonium molybdate assay (36). The levels of inorganic nutrients in water from the discharge zone were determined by high-pressure ion chromatography ( Dionex Corporation, Sunnyvale, Calif.). Nonpurgeable organic carbon was determined with a TOC 5000 analyzer (Shimadzu, Columbia, Md.). Annual precipitation data for North Springfield and Urbana, Ohio, which are adjacent to the study site, were obtained from the Miami Conservancy District (Dayton, Ohio).

Chemicals All radiotopes ([methyl-3H]thymidine, 50 Ci/mmole; [U-14C]glucose, 310 mCi/mmole; and [U-14C]leucine, 280 mCi/mmole) were obtained from ICN Pharmaceuticals, Inc. (Irvine, Calif.). Chlorofrom, acetone, and methanol were gas chromatography–gas chromatography-mass spectrometry grade (Burdick & Jackson, Muskegon, Mich.). All other chemicals were reagent grade or better.

Enumeration of bacteria. Sediment samples (1 g [wet weight]) from each site were fixed with 2.5% (vol/vol) glutaraldehyde dissolved in 0.1% (wt/vol) sodium pyrophosphate and stored at 10°C prior to counting. Bacterial population densities were determined by the acridine orange direct count (AOCD) method of Hobbie et al. (16) with modifications. Briefly, sediment slurries were homogenized with Colorplast (Stratford, N.J.) in the field and was confirmed with a pH meter (Orion Research Inc., Boston, Mass.) upon arrival in the laboratory. Water chemistry was determined on-site with Hach (Loveland, Colo.) and CHEMetrics (Calverton, Va.) chemical field kits. Total phosphorus levels were determined in triplicate by a colorimetric method using an ammonium molybdate assay (36). The levels of inorganic nutrients in water from the discharge zone were determined by high-pressure ion chromatography ( Dionex Corporation, Sunnyvale, Calif.). Nonpurgeable organic carbon was determined with a TOC 5000 analyzer (Shimadzu, Columbia, Md.). Annual precipitation data for North Springfield and Urbana, Ohio, which are adjacent to the study site, were obtained from the Miami Conservancy District (Dayton, Ohio).

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Enumeration of bacteria. Sediment samples (1 g [wet weight]) from each site were fixed with 2.5% (vol/vol) glutaraldehyde dissolved in 0.1% (wt/vol) sodium pyrophosphate and stored at 10°C prior to counting. Bacterial population densities were determined by the acridine orange direct count (AOCD) method of Hobbie et al. (16) with modifications. Briefly, sediment slurries were homogenized and diluted (generally 1:10) into 10 mM phosphate buffer (pH 7.5). One-milliliter aliquots were dispensed onto EM 95 filters (EM Research Ltd., England) and air dried. The filters were placed onto microscope slides spotted with 1 to 2 drops of low-fluorescence immersion oil. At least 10 randomly stained fields were counted on each slide for each replicate. The Olympus BH5 microscope equipped with epifluorescence (Valencia, Pa.). With this microscope at ×1,000 magnification, there are 2,684 × 104 fields per stained area on each 25-mm filter. The average number of bacterial cells per gram of sediment was determined by the following calculation: number of cells per gram = (average number of cells per field) × (number of fields per filter) × (2,684 × 104) (dilution factor) (sample volume). While direct microscopic counting of bacteria in soils and sediments can be problematic, the high bacterial biomass present and the modifications developed for these samples enabled its use.

Bacterial activity determinations: dual-label incorporation of [3H]thymidine into DNA and [14C]leucine into protein. The dual-label procedure employed involved incubation with 125 mM [3H]thymidine and 160 mM [14C]leucine for 1 h at room temperature. The incubation was followed by cold trichloroacetic acid (TCA) precipitation and extraction of macromolecules and fractionation following the TCA precipitation procedure described by Driscoll and Wackett (17) and used to evaluate community structure in general, based on changes in membrane-lipid-to-storage-lipid ratios. Higher ratios indicate increased stress.

Analysis of community structure. Bacterial community DNA from the south pool was isolated by direct lysis (17) and used to evaluate community structure from four seasonal samples by the bisbenzimidazole-CsCl gradient method described by Holben and Harris (18). The resulting profiles indicate the relative abundance of DNA of specific percent G+C content in the sample. Since percent G+C content of DNA is characteristic for bacterial populations at about the genus level, this analysis can be used to indicate the general structure of the bacterial community in an environmental DNA sample (18).

Statistical significance. Analysis of variance tests were performed to compare the mean values of the data and assess whether there were differences among groups. All statistical analyses were performed with Statistix software, version 3.1 (Analytical Software, Tallahassee, Fla.). Significance levels were set for these analyses as 0.05. All statistical tests are referred to as tests for the significance of differences. The values of the data were transformed by using a logarithmic transformation for all analyses unless otherwise specified.

RESULTS

Seasonal patterns of physicochemical parameters. Seasonal variations in groundwater temperature, groundwater pH, and air temperature at all three sites were determined. While air temperature varied seasonally as expected at this temperate latitude, the groundwater temperature was fairly constant throughout the year, ranging from 9.5°C in January to 13°C in late July (data not shown). The pH of the groundwater from the south pool ranged between 7.06 and 8.08 throughout the year and was similar at all three sites (data not shown).

Nutrient levels in south, 60 N, and 100 N pool groundwaters were measured (Fig. 1). Peak nutrient levels occurred in late summer and generally preceded increases in bacterial activity and carbon. As indicated by the data shown in Fig. 1, nutrient levels (N/P) from the south, 60 N, and 100 N pool groundwaters were calculated from these data. A C/N/P ratio of 100:10:1 is considered to be optimal for microbial activity (34). Generally, the closer the calculated groundwater nutrient ratio was to this optimal value, the greater the measured microbial activity dur-
ing or immediately following that sampling time. The C/N/P ratios observed during June and July for the south pool (100:55:2 and 100:35:14.5, respectively) were, by far, the closest to the optimal levels for maximum growth rates of the seasonal sampling times. Closest-to-optimal ratios for the 60 N pool groundwater were also observed in June and July, being 100:100:5.5 and 100:52:12.8, respectively. Similarly, the most optimal nutrient ratios at the 100 N pool were 100:58:3.4 and 100:98:32 for June and July, respectively. By contrast, the least optimal nutrient ratios for the south pool and the 60 N and 100 N sites were observed in August and were 100:99:5.1, 100:247:36.5, and 100:217:62, respectively. It was at this sampling time that a dramatic drop in organic carbon and an increase in nitrate levels were observed (Fig. 1).

Seasonal patterns of bacterial activity. Time course experiments for substrate incorporation determinations were performed with sediment samples from all three study sites. For these experiments, 1-h incubation times were used based on observed linear uptake of the three substrates for more than 90 min under experimental conditions (data not shown). Growth rate determinations based on \[^{3}H\]thymidine and \[^{14}C\]leucine incorporation were always within the same order of magnitude and covaried temporally (Fig. 2). Activity values derived from DNA synthesis rates by the \[^{3}H\]thymidine incorporation method were generally higher than values derived from \[^{14}C\]leucine incorporation for each site but were within a factor of 2 for most of the study (Fig. 2). Bacterial activity was highest at the south pool site and peaked in July. Phospholipid synthesis rates (based on incorporation of \(^{14}C\) from glucose into lipid), which can also be used as an estimate of heterotrophic bacterial activity, covaried with the dual-label activity measurements (Fig. 2 and 3A).

Comparative evaluation of the three sites. Overall, the physicochemical and biological parameters measured at the 60 N and 100 N sites were more similar than those measured for the south pool. Further, the observed carbon allocation patterns for membrane and storage lipids indicate that the north pool bacterial communities were more physiologically similar to each other and somewhat different from the community in the south pool (Fig. 3B). The south pool exhibited a large increase in apparent stress following the dramatic drop in activity in early fall (Fig. 2 and 3).

Bacterial growth rate determinations indicated that increases in bacterial activity (Fig. 2 and 3A) preceded increases in cell numbers (Fig. 4A) for all sites throughout the study. Population turnover estimates were derived by dividing the
bacterial biomass values by the corresponding growth rate (determined by $^{3}$H]thymidine incorporation) and are given in terms of the number of days required to replace the current bacterial biomass at that growth rate (Fig. 4B). Turnover values were highest in winter and early spring and lowest during the summer when bacterial activity and nutrient concentration peak and the C/N/P ratios are closest to 100:10:1. Despite the higher growth rates at the south pool (Fig. 3), the turnover times were similar to those obtained for the two north pools (Fig. 4B).

**Spearman’s rank correlations $(r_s)$**. Spearman’s rank correlations were used to assess the relationships among the various physical, chemical, and biological parameters measured. With this approach, correlation values greater than the significance levels set for $n = 16$ for these two-tailed comparisons $(P = 0.05$ for $r_s$ values $\geq 0.503, P = 0.01$ for $r_s$ values $\geq 0.635, P = 0.002$ for $r_s$ values $\geq 0.732$, and $P = 0.001$ for $r_s$ values $> 0.765$) were generally accepted as being significant (45). The results of these analyses are presented in Tables 1 and 2 and are highlighted below.

Turnover time values co-vary inversely with DNA synthesis rates from all sites and provided the strongest correlations in this study ($= 0.874, = 0.948$, and $= 0.877$ for the south, 60 N, and 100 N pools, respectively [Table 1]). Less strong correlations were observed for turnover times and protein synthesis from all three sites and for phospholipid synthesis at the 100 N pool (Table 1). 100 N pool physiological status values also correlated with the turnover times $(0.721)$ that were generated by using activity and biomass data from that site. Other significant correlations observed include south pool DNA and protein synthesis (0.538), south pool phospholipid synthesis and physiological status (0.512), 60 N pool DNA and protein synthesis (0.606), 60 N pool DNA synthesis and physiological status (0.677), and 100 N pool DNA synthesis and phospholipid synthesis (0.729).

Trends in thymidine incorporation values observed for the south pool during the 13-month study period correlated well with the values obtained for the 60 N (0.850) and 100 N (0.590) pools although the south pool was generally two to three times higher in activity (Fig. 2). DNA synthesis rates for the 60 N pool and the 100 N pool also correlated well (0.764). The protein synthesis rates were also correlated for the south pool and 60 N pool (0.564) and the 60 N pool and 100 N pool (0.765) but not between the south pool and 100 N pool (0.291). Phospholipid synthesis rates appeared to be correlated only between the 60 N and 100 N pools (0.592). Biomass measurements by AODC indicated a significant correlation only between the south pool and 60 N sites (0.594). In general, the data patterns yielded the strongest correlations between the two north pools, followed by the south and 60 N pools, and were weakest between the most spatially separated sites, the south and 100 N pools (some data not shown).

Good correlations between activity measurements and chemical and physical parameters were obtained for the $^{3}$H]thymidine incorporation experiments (Table 2). For the south pool, DNA synthesis data correlated with C/N ratios (0.533). The south pool physiological status values showed

![FIG. 3. (A) Lipid synthesis as measured by $^{14}$C]glucose incorporation into phospholipid. (B) Ratio of $^{14}$C]glucose incorporation into membrane to that into storage lipid for physiological status (stress) assessment. Data represent the means $\pm$ standard errors of the means ($n = 4$). DPM, disintegrations per minute; PL/GL, phospholipid-to-glycolipid ratio.](http://aem.asm.org/)

![FIG. 4. (A) Seasonal variation in bacterial cell numbers as determined by the AODC method; (B) population turnover times based on AODC and thymidine incorporation data. Data represent the means $\pm$ standard errors of the means ($n = 10$ fields/sample).](http://aem.asm.org/)
similar trends compared to local precipitation ($r_s$ of 0.520). Overall, bacterial activity values covaried most consistently with increasing organic carbon, water temperature, and precipitation (Table 2 and Fig. 2C). South pool phospholipid data also correlated well with the organic carbon (0.727), ammonia (0.601), and C/N ratios (0.545) obtained at that site, indicating that, as nutrient ratios at the site approached optimum, bacterial biomass and activity increased (Table 2).

Bacterial activity estimates at the 60 N and 100 N pool sites were also strongly correlated with groundwater chemistry and physical parameters measured at each site. For example, bacterial activities ($[^3]$H thymidine incorporation) throughout the year in the two north pools appear to be most strongly influenced by phosphate levels with $r_s$ values of 0.558 and 0.524 for the 60 N and 100 N sites, respectively (Table 2). 100 N pool protein synthesis had an $r_s$ value of 0.509 compared to the Urbana precipitation patterns. Groundwater temperature had an $r_s$ of 0.529 for 60 N pool DNA synthesis and 0.567 for phospholipid synthesis (Table 2). Positive correlations of 0.567 and 0.767 were also obtained between groundwater temperature and 100 N pool DNA and phospholipid synthesis estimates, respectively. The pattern of 100 N $[^3]$H thymidine incorporation into DNA was also related to organic carbon levels (0.667).

**Community structure.** To further characterize the bacterial communities, DNA was isolated from south pool samples and the bacterial community structures were compared for spring, summer, fall, and winter samples. Although individual bacterial populations are not detected by this broad-resolution approach, the data indicate that no significant alterations in total bacterial community structure occurred in response to seasonal changes since nearly identical community structure profiles were obtained at each time point (Fig. 5). The lipid-based stress determinations were highest in the fall, but no comparable community shift was detected at that time by the percent G+C method (Fig. 3B and 5).

**DISCUSSION**

While seasonal fluctuations in air temperature were evident, due to the continuous upward flow of groundwater little change in groundwater temperature was observed. In fact, in January when the marl meadow was frozen, Cedar Run had algae growing in it and there was no indication of ice formation. Rainfall appears to initiate the primary peaks in activity

### TABLE 1. Correlations ($r_s$) among activity measurements, biomass, and metabolic state from the south, 60 N, and 100 N pools

<table>
<thead>
<tr>
<th>Activity, biomass, and metabolic state for pool</th>
<th>Cellular turnover time</th>
<th>Thy</th>
<th>Leu</th>
<th>Glu</th>
<th>AODC</th>
<th>PL/GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>South</td>
<td>Thy</td>
<td>$0.874^{****}$</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leu</td>
<td>$-0.556^{*}$</td>
<td>0.538</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glu</td>
<td>$-0.467$</td>
<td>0.482</td>
<td>0.324</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AODC</td>
<td>$-0.003$</td>
<td>0.451</td>
<td>0.201</td>
<td>0.038</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>PL/GL</td>
<td>$-0.397$</td>
<td>0.424</td>
<td>0.076</td>
<td>0.512</td>
<td>0.202</td>
</tr>
<tr>
<td>60 N</td>
<td>Thy</td>
<td>$-0.948^{****}$</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leu</td>
<td>$-0.703^{**}$</td>
<td>0.606</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glu</td>
<td>$-0.544^{*}$</td>
<td>0.729</td>
<td>0.409</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AODC</td>
<td>$0.341$</td>
<td>$-0.139$</td>
<td>$-0.381$</td>
<td>$-0.181$</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>PL/GL</td>
<td>$0.662^{*}$</td>
<td>$0.677^{**}$</td>
<td>$0.336$</td>
<td>0.021</td>
<td>$-0.055$</td>
</tr>
<tr>
<td>100 N</td>
<td>Thy</td>
<td>$-0.877^{****}$</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leu</td>
<td>$-0.615^{*}$</td>
<td>0.478</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glu</td>
<td>$-0.544^{*}$</td>
<td>0.729</td>
<td>0.409</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AODC</td>
<td>$0.240$</td>
<td>$-0.128$</td>
<td>$-0.314$</td>
<td>$-0.181$</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>PL/GL</td>
<td>$0.721^{**}$</td>
<td>$0.490$</td>
<td>$0.468$</td>
<td>$0.024$</td>
<td>$-0.055$</td>
</tr>
</tbody>
</table>

* Activity measurements were determined by $[^3]$H thymidine, $[^5]$C leucine, and $[^14]$C glucose incorporation into DNA, protein, and phospholipids, respectively. AODC represents biomass estimates, and PL/GL represents the phospholipid-to-glycolipid ratio (stress). Cellular turnover time was based on thymidine incorporation into DNA, protein, and phospholipids, respectively. The pattern of 100 N $[^3]$H thymidine incorporation into DNA was also related to organic carbon levels (0.667).

### TABLE 2. Correlations ($r_s$) between measured community parameters and physical and chemical parameters from the groundwater

<table>
<thead>
<tr>
<th>Activity and parameter for pool</th>
<th>Organic carbon</th>
<th>NO$_3$-N</th>
<th>NH$_4$-N</th>
<th>PO$_4$-P</th>
<th>C/N</th>
<th>Groundwater temp</th>
<th>Precipitation for Urbana, Ohio</th>
</tr>
</thead>
<tbody>
<tr>
<td>South</td>
<td>Thy</td>
<td>0.497</td>
<td>$-0.141$</td>
<td>0.062</td>
<td>0.227</td>
<td>0.533$^*$</td>
<td>0.334</td>
</tr>
<tr>
<td></td>
<td>Leu</td>
<td>0.488</td>
<td>$-0.346$</td>
<td>0.061</td>
<td>$-0.047$</td>
<td>0.212</td>
<td>0.467</td>
</tr>
<tr>
<td></td>
<td>Glu</td>
<td>0.727$^{**}$</td>
<td>$-0.044$</td>
<td>0.601$^{*}$</td>
<td>0.221</td>
<td>0.545$^{*}$</td>
<td>0.475</td>
</tr>
<tr>
<td></td>
<td>AODC</td>
<td>0.202</td>
<td>$-0.584$</td>
<td>0.010</td>
<td>$-0.208$</td>
<td>0.231</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>PL/GL</td>
<td>0.468</td>
<td>0.423</td>
<td>0.442</td>
<td>0.422</td>
<td>0.309</td>
<td>0.090</td>
</tr>
<tr>
<td>60 N</td>
<td>Thy</td>
<td>0.356</td>
<td>$-0.170$</td>
<td>0.068</td>
<td>0.558$^{*}$</td>
<td>0.215</td>
<td>0.529$^{*}$</td>
</tr>
<tr>
<td></td>
<td>Leu</td>
<td>0.452</td>
<td>0.111</td>
<td>0.310</td>
<td>0.517$^{*}$</td>
<td>0.309</td>
<td>0.358</td>
</tr>
<tr>
<td></td>
<td>Glu</td>
<td>0.302</td>
<td>$-0.111$</td>
<td>$-0.308$</td>
<td>0.313</td>
<td>0.386</td>
<td>0.567$^{*}$</td>
</tr>
<tr>
<td></td>
<td>AODC</td>
<td>$-0.209$</td>
<td>0.382</td>
<td>0.420</td>
<td>$-0.414$</td>
<td>$-0.419$</td>
<td>$-0.177$</td>
</tr>
<tr>
<td></td>
<td>PL/GL</td>
<td>$-0.082$</td>
<td>$-0.274$</td>
<td>0.240</td>
<td>0.240</td>
<td>$-0.106$</td>
<td>0.312</td>
</tr>
<tr>
<td>100 N</td>
<td>Thy</td>
<td>0.667$^{**}$</td>
<td>0.167</td>
<td>$-0.363$</td>
<td>0.524$^{*}$</td>
<td>0.477</td>
<td>0.757$^{***}$</td>
</tr>
<tr>
<td></td>
<td>Leu</td>
<td>0.385</td>
<td>$-0.198$</td>
<td>0.261</td>
<td>0.390</td>
<td>0.464</td>
<td>0.389</td>
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<tr>
<td></td>
<td>Glu</td>
<td>0.253</td>
<td>0.338</td>
<td>$-0.424$</td>
<td>0.287</td>
<td>0.162</td>
<td>0.767$^{***}$</td>
</tr>
<tr>
<td></td>
<td>AODC</td>
<td>$-0.002$</td>
<td>0.385</td>
<td>$-0.116$</td>
<td>$-0.108$</td>
<td>0.220</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>PL/GL</td>
<td>0.235</td>
<td>0.044</td>
<td>$-0.100$</td>
<td>0.458</td>
<td>0.202</td>
<td>0.492</td>
</tr>
</tbody>
</table>

* Activity measurements were determined by $[^3]$H thymidine, $[^5]$C leucine, and $[^14]$C glucose incorporation into DNA, protein, and phospholipids, respectively. AODC represents biomass estimates, and PL/GL represents the phospholipid-to-glycolipid ratio (stress). $^{*}$ to $^{***}$, weakest to strongest correlation, respectively.
and nutrient levels in August and September. Secondary peaks in nitrogen and phosphorus in the south pool in the September-to-October time frame may have resulted from nutrient release due to disturbance of topsoil and cedar root mycorrhizal zone caused by cedar tree removal adjacent to this site. The groundwater was typically low in carbon and phosphate, particularly phosphate, with values generally less than 5 ppb. However, nitrate levels were much higher than expected for groundwaters, approaching 5 mg/liter, probably due to agricultural runoff and seepage. This is probably the case since the surrounding fields were fertilized twice a year, in both the spring and the fall. Nonpurgeable organic carbon levels varied but were much higher in summer (10.8 mg/liter) than during the other seasons (3 to 4 mg/liter). In groundwater-fed wetlands (fens), carbon levels generally range between 1 and 20 mg of C/liter.

It was expected that carbon, nitrogen, and phosphorus levels would affect the bacterial communities in the sediments from the three sites sampled. It is not only the absolute supply of nutrients that regulates growth but also the stoichiometry of the dissolved nutrient pool (15, 35). While the C/N/P ratio at these sites was never optimal, the N/P ratios do approach the optimal ratio. The C/N ratios are consistently low at all three sites, suggesting carbon limitation. Recent studies in freshwater lakes and streams have shown that N and P levels have profound effects on community growth rates and that the ratio of these important nutrients influences bacterial growth (19, 26, 27, 39–41). It has also been shown that low phosphate concentrations (below 5.0 μg/liter) and high N/P ratios can result in phosphorus limitation (1). Thus, it is possible that the C/N/P ratio in the waters perfusing Cedar Bog sediments is the key parameter regulating bacterial activity and growth.

Spearman’s rank correlations ($r_s$) were made by using entire data sets from each site to compare the patterns of growth rates with measured chemical and physical parameters throughout the study. Overall, bacterial activity values correlated well between sites, with the sites that are closest in proximity having the best correlation. It is not clear whether this is due to differences in groundwater flow (hydrology), nutrients, or other parameters. Correlations between activity and physical and chemical parameters, although indicative of a regulatory role, did not consistently yield complementary results among activity measurements. For example, groundwater temperature alone displayed a significant correlation with both thymidine and glucose incorporation activity measurements at the 100 N pool. However, leucine incorporation activity data at this site does not show a correlation with groundwater temperature, and yet it does correlate with the thymidine incorporation pattern (Table 1). It should be noted that responses in the activity, cell density, and physiological status of the bacterial communities to patterns in physicochemical parameters may be lagging, which would affect the strength of the correlation because the patterns would be slightly offset.

The effect of phosphate concentration is more apparent for the two north pools, suggesting that in this system at least some sites may be regulated by this nutrient. However, for the south pool C/N ratio is suggested as a key regulator of growth based on the statistical comparisons between DNA and phospholipid synthesis and the nutrient ratios.

One aim was to compare three independent activity estimates since the combination of several methods to measure growth should give more reliable estimates (21, 31). Incorporation of [3H]thymidine is specific for heterotrophic bacterial DNA, and [3H]thymidine is reportedly not taken up by cyanobacteria, eucaryotic microalgae, autotrophic bacteria, or fungi (3, 12, 29, 37). While [3H]thymidine labeling of DNA has been used extensively to measure bacterial activity, lack of specificity of incorporation and extraction variability have been observed in freshwater (7, 20, 32) and sediment (5, 9, 25) microbial communities with this approach. To overcome these potential limitations, data obtained by this approach and other bacterial activity estimates were compared. Uptake and incorporation of [14C]leucine into total cellular protein (2, 7, 31) have provided an alternative technique for estimating bacterial growth. For each method, the amount of radiotracer incorporated into the corresponding macromolecules has been shown to be proportional to the growth rate of bacterial communities (10, 22, 30, 38, 44). Because growth determinations generally rely on cellular processes which are independent of one another but are all related to growth or new cell production, covariation during periods of balanced growth is anticipated. Growth rate estimates based on thymidine incorporation were only approximately twofold higher than those based on leucine incorporation, a small difference considering that the conversion factors employed differ by 2 orders of magnitude. Thus, the dual-label approach produced highly similar results based on DNA and protein synthesis rates, allowing greater confidence in the growth estimates obtained.

The rates of [3H]thymidine, [14C]leucine, and [14C]glucose incorporation into DNA, protein, and phospholipids, respectively, by sediment heterotrophic bacteria were found to covary in the two north pools and the south pool. When label incorporation data were converted to growth rates (number of cells per gram per day), similar estimates of growth were obtained. The observed increases in growth rate and cell numbers in the south pool may be explained by higher nutrient levels, higher temperatures, or changing ratios of these nutrients. The different nutrient levels in the south, 60 N, and 100 N pool groundwaters may be due to differences in sediment composition, such as organic detritus or carbonate makeup common to these groundwater upwelling zones. Also, it appears that increases in precipitation influence availability of nutrients at the upwelling zones.

Few studies have addressed the effects of nutrients on activity and biomass of microbial communities in sediments (4). This study provides much-needed information concerning sediment bacterial communities from groundwater-fed ecosystems. The data indicate dynamic seasonal differences in some physical and chemical parameters. The data also indicate significant seasonal variation in growth and activity measurements of the bacterial community. The bacterial community profiles based on the percent G+C content of component
populations in the complex bacterial community provided a profile of the structure of the entire bacterial community at the time of sampling in terms of relative abundance of DNA versus percent G+C content. While limited in resolution for individual bacterial populations, this approach has been successfully employed to demonstrate gross changes in microbial community structure in response to anaerobiosis and carbon amendment (18). While we cannot preclude the possibility that the abundance of specific individual populations varies seasonally, it is apparent that there are no major shifts in overall community structure during the course of the year. Thus, the data support the interpretation that, while the activity and physiological status of the entire community are subject to large directional shifts seasonally, the overall bacterial community structure and biomass do not change significantly.

The data provide new information regarding seasonal variation in the bacterial community in an understudied wetland environment and implicate many factors that may influence activity and biomass of the bacterial consortium in the sediment compartment of groundwater discharge zones in Cedar Bog. Since no protozoa were evident in the bog sediments, it is likely that bacterial biomass and activity in this system are regulated by nutrients or other physicochemical parameters rather than by predation.

ACKNOWLEDGMENTS

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REFERENCES


