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Seasonal Variations in Bacterial Communities in Adirondack Streams Exhibiting pH Gradients

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Abstract. Measurements of microbial biomass, bacterial numbers, and microbial production were determined for three small woodland streams located in the Adirondack Mountain region of New York State, USA. These streams exhibited spatial and temporal gradients in water pH ranging from a high of 7.0 to a low of 4.5. Twelve sites along these streams were used for comparative analyses of the effects of pH and related water chemistry parameters on the planktonic, sedimentary, and epilithic bacterial communities. The planktonic bacterial communities were not influenced by water pH or related water chemistry parameters. For sedimentary populations, the organic content of the sediment was more important than the chemistry of the overlying water. The epilithic bacterial communities, however, were influenced significantly by the pH of the water column, showing decreased bacterial production at lower pH.

Introduction

Small woodland stream ecosystems are largely dependent on externally produced organic matter [6, 12, 31]. These flowing-water environments can provide a continuous, albeit uneven, supply of nutrients to stream organisms. Nutrients do not cycle in place but are displaced downstream in what has been described as a spiral, through sediments, organisms, and water. Spiraling can be influenced significantly by physical, chemical, and biological factors. Stream flow can alter the spiraling length, and different sediment sorption properties and biological community activities can interrupt the spiral [26, 33].

Stream water chemistry and nutrient status can change as a result of short-term and long-term effects, including storms, daily temperature variations, light rhythms, inputs of leaves or other detritus, ice cover, snow melt, low-water conditions, algal blooms, beaver impoundments, and anthropogenic inputs. Small low-order streams in the temperate deciduous forests of the Adirondack Mountains of New York State are influenced by all these factors. Because they are low ionic strength waters and thus have low buffering capacities, these streams are susceptible to pronounced changes in chemistry due to atmospheric

deposition of strong acids upon them and their surrounding drainage basins [9].

Neutralization of acid deposition in and near streams has been studied in the Hubbard Brook Experimental Forest in New Hampshire, an area that is considered to be acid sensitive [22]. Here, strong mineral acids are reported to be initially neutralized by dissolution of aluminum in the thin acid soils, resulting in the transport of acidic cations such as hydrogen (H^+) and aluminum (Al^{n+}) to the streams at the top of the watershed. Further down the watershed, increases in soil depth and drainage area allow greater ion retention times within the soil, resulting in neutralization of acidic cations by release of basic cations such as calcium, magnesium, potassium, and sodium [9, 10, 22]. Other processes, such as the biologically-mediated reactions of sulfate and nitrate reduction [24] and anion adsorption [9], contribute to the neutralization of acidic inputs.

Seasonal changes in stream water chemistry due to acid deposition are also seen. During periods of high discharge, such as snow melt or storms, increases in acidic cation concentrations due to increased inputs of nitrate (NO_3^-) and/or dilution of basic cation concentrations occur [8, 9, 16]. In beaver impoundments or marshes, which can retain sulfate by reductive processes, the oxidation of reduced sulfur during the low-flow summer months followed by rain in the fall can result in pulses of sulfuric acid to downstream water [9].

The Adirondack streams in this study have water pH values that are often much lower than the optimum pH range for many aquatic microorganisms. Each of the various microbial communities in streams is necessary in terms of nutrient regeneration and assimilation. Therefore it is important to gather information on the effects of low pH on these communities.

Microbial communities in aquatic systems can be classified into four categories: the planktonic population, which floats freely in the water column; the particle-associated population, also in the water column; epilithic and epiphytic organisms, attached to submerged rock and plant surfaces; and the sediment population, which inhabits the water-filled spaces of the sediment [7]. Planktonic and particle-associated bacteria in small mountain streams are less numerous than either epilithic or sediment organisms. In shallow waters, geometric factors can make substrate-bound bacterial populations as much as 1,000 times more numerous than planktonic bacteria [27]. Sediment microorganisms develop in response to the conditions present in a given portion of the stream, in contrast to the transient planktonic populations [30]. Bedrock type, gradient, sediment supply, and water-flow rate all influence the distribution of substrate in the stream, which determines whether the sediment-associated or the epilithic community is more important to overall microbial production [7].

The physiological effects of low pH on microorganisms in extreme habitats have been described [28, 43]. Extrapolating from these and other studies, one would expect pH changes resulting from acidic deposition to bring about varied consequences [1, 37]. Although most investigations of microbial populations in lakes and streams affected by acidic deposition have examined decomposition processes, more recent studies have focused on populations of planktonic and sediment bacteria [4, 5, 13, 21, 35, 41] and of epilithic bacteria [35, 36].

We followed bacterial numbers, total viable microbial biomass, and bacterial

production spatially as well as temporally in three low-order Adirondack streams. Results of the two-year study reported here focus on seasonal patterns of acid-impacted bacterial communities and the effect on these populations of water chemistry parameters, especially those related to pH.

Methods

Site Selection and Description

All Adirondack study sites were chosen on the basis of the criteria established by the ALSS program [10], which required: 1) forested watersheds with little to no disturbance; 2) drainage water with low acid-neutralizing capacity (ANC < 100 μeq/liter); 3) longitudinal gradient in water chemistry; 4) accessibility; and 5) low concentrations of dissolved organic carbon.

Three low-order woodland streams in the Adirondack Park were chosen: Pancake Creek, Moss Inlet, and Beaver Brook (Fig. 1). All three streams drain watersheds dominated by deciduous species (maple, beech, birch) containing some coniferous species [10]. The stream characteristics are described in Table 1.

Sampling Schedule and Protocol

All twelve sites were sampled for microbial populations in water and sediment and on rocks quarterly from July 1984 through April 1986. Samples were collected concurrently with the water chemistry sampling, taken monthly by the Department of Civil Engineering of Syracuse University. The results of the analyses [9, 10] of these samples were used in the statistical analyses of the microbiological portion of this project. All stream sites were sampled on the same day whenever possible.

Direct Microscopic Counts of Bacteria in Water and Sediments

Water samples (3 per site) were preserved in the field with an equal volume of 10% formalin (all solutions were filter-sterilized by passage through a 0.2 μm Nuclepore filter). Three sediment samples per site were preserved in 5% formalin (1:10, vol : vol). In the laboratory, measured volumes of water samples (2 to 10 ml) were stained with 0.01% acridine orange [20] and filtered through irgalan black-stained Nuclepore filters for examination of total bacterial numbers. Water on the filters did not lead to background fluorescence. From each 10 ml sediment slurry, diluted to 50 ml and homogenized in a blender at high speed for 3 min, a 0.2 ml sample was transferred to a measured amount of filter-sterilized water (1.8 to 4.8 ml) and stained as for water samples. Damp filters were cleared by adding a drop of immersion oil beneath a cover slip, and slides were counted as described previously [4].

ATP Measurements in Sediments and on Rocks

At each sampling date, 5 samples of both sediment and rocks from each site were assayed. One milliliter aliquots of sediment slurry were added to 4 ml of 1 M phosphoric acid. At each site, rocks of 20 to 50 cm^2 surface area were chosen for uniformity of material and smoothness of surface and were placed directly into wide-mouthed sterile Nalgene bottles containing 1 M phosphoric acid. All samples were placed on ice and transported to the laboratory within 12 hours of collection. After 3 to 12 hours of extraction with cold phosphoric acid, 0.5 ml subsamples from

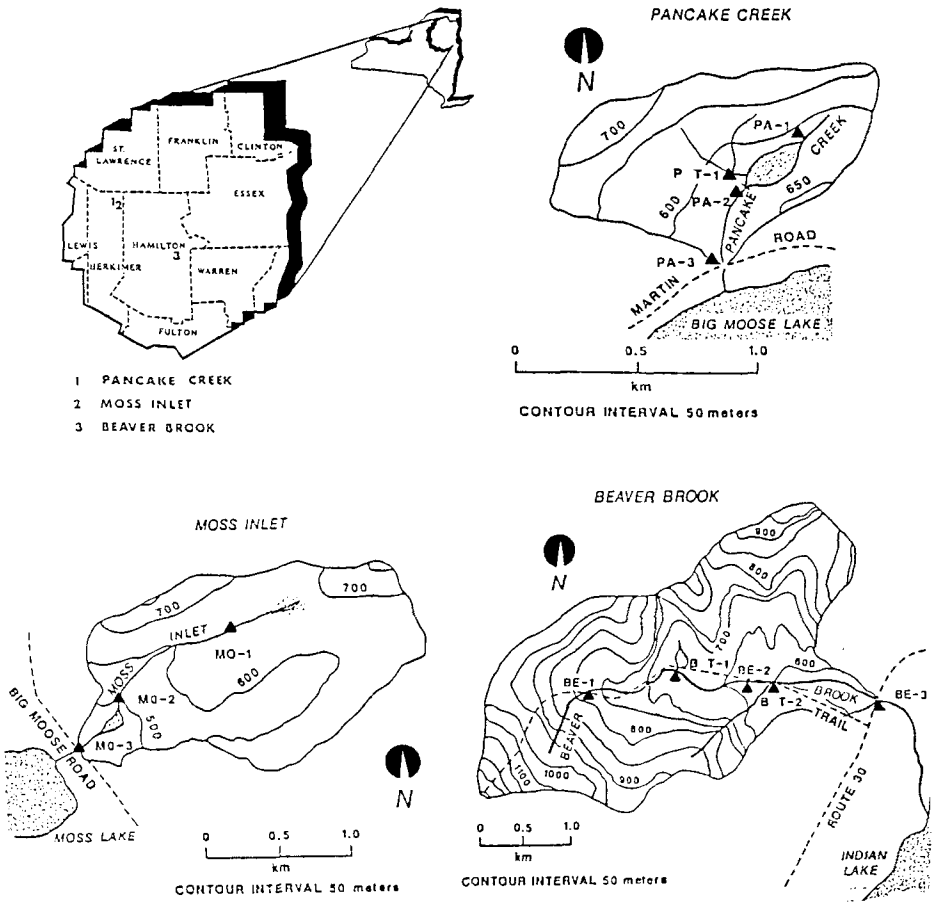

Fig. 1. Maps of the three study streams. Insert on the top left shows their location in the Adirondack Park of New York State. Map of Pancake Creek on the top right shows sampling sites PA1, PA2, PA3, and PT1. The stippled area in the watershed is a beaver impoundment. Map of Moss Inlet on bottom left shows sampling sites MO1, MO2, and MO3. Map of Beaver Brook on bottom right shows sampling sites BE1, BE2, BE3, BT1, and BT2.

extracts of both sediment and rock surfaces were removed for analysis. Extracts were diluted to 8 ml with 0.02 M Tris buffer (pH 7.5), adjusted to pH 7.5 with 1 M NaOH, and brought to a final volume of 10 ml. Subsamples of 100 μl of the diluted extracts were assayed for ATP using an integrating photometer (Lumac/3M Biocounter M2010, 3M Company) and Lumac/3M reagents, measuring integrated light emissions for 10 sec. Internal standardization for each sample was used in calculating the amount of ATP per g dry weight of sediment or per cm^2 of rock surface.

Bacterial Uptake of [3H]-Thymidine in Sediments and on Rocks

Bacterial production by sediment and epilithic populations was determined by measuring incorporation of tritiated thymidine into DNA [11, 14]. The method used for both sediment and rocks was that of Findlay et al. [11] modified for use on rock surfaces [35]. Tritiated thymidine (0.064 nM, New England Nuclear, specific activity = 78 Ci·mM^{-1} in sterile distilled water) was added to

Table 1. Study streams in the Adirondack Park, NY

Name	Number of sites	Site designations	Stream order[a]	Drainage area (km^2)	Elevations (m)
Pancake Creek	4	PA1	1	0.74	556–720
		PT1	1		
		PA2	2		
		PA3	2		
Moss Inlet	3	MO1	1	2.40	543–726
		MO2	1		
		MO3	1		
Beaver Brook	5	BE1	1	7.70	520–1,215
		BT1	2		
		BE2	2		
		BT2	2		
		BE3	2		

[a] 1 represents a higher elevation than 2

2 ml of sediment slurry (3 samples per site) and each reaction mixture was brought to a total volume of 5 ml with sterile water and incubated for 2 hours at ambient stream temperature with shaking. Earlier studies of sediment indicated that the time course for [^{3}H]-thymidine incorporation was linear from 45 min to 3 hours. Extraction of labeled DNA was begun by adding 5 ml of a 0.3 N NaOH, 1% sodium dodecyl sulfate (SDS), and 25 mM ethylenediaminetetraacetic acid (EDTA) solution to each sample. After extraction (with shaking at 25°C overnight) and centrifugation (6 min at 2,500 × *g*), the supernatant was chilled to 4°C and neutralized to pH 7.0–7.5 with 3 N HCl. A sufficient amount of 50% trichloroacetic acid (TCA) to achieve a final concentration of 5% was then added, and unlabeled carrier DNA was added to aid precipitation. The sample was centrifuged at 25,000 × *g* for 10 min at 4°C to collect the TCA-insoluble fraction, after which the pellet was washed once with 5% TCA and centrifuged again. The DNA in the pellet was then hydrolyzed in 3 ml 5% TCA at 95°C for 30 min. Particles were removed by centrifugation at 2,500 × *g* for 6 min and 1 ml of the supernatant was added to 9 ml Scintiverse II (Fisher Scientific Co.). This mixture was then radioassayed, and corrections to DPM were made using an internal standardization.

Individual rock samples (5 samples per site) with a minimum volume of stream water (50 ml), enough to totally immerse the rock, were inoculated with 0.64 nM of [^{3}H]-thymidine. Each reaction mixture was incubated with shaking at ambient stream temperature for 20 min. Since time-course experiments with rock samples had indicated a much shorter period of linear uptake of the thymidine than that measured for the sediment samples, the incubation solution was poured off the rock sample after 20 min, after which the rock was then rinsed three times with distilled water. Fifty milliliters of the NaOH-EDTA-SDS mixture was then added to the rock sample, which was extracted overnight at 25°C. One half of the extractant was chilled, neutralized, and treated as in the sediment procedure except that the precipitated material was collected on membrane filters (0.45 μm) to avoid losing any precipitate. The filter and precipitate were then hydrolyzed and radioassayed as described above. Isotope dilution measurements on the rock samples were made on one sampling date.

Recovery Efficiencies and Isotope Dilution Measurements

DNA recovery was estimated during several of the sediment and rock extractions by adding known amounts of [^{3}H]-DNA ([thymine-Me-^{3}H] DNA) from *E. coli* (New England Nuclear). Recovery

ranged from 66% to 79% for sediment samples and was 75% for rock samples. Tritiated thymidine incorporation with different concentrations of unlabeled thymidine added to the sediment and rock samples (0 to 10 nM) was measured.

The results of tritiated thymidine uptake experiments using varying concentrations of added unlabeled thymidine were transformed and analyzed to determine if any isotope dilution was occurring. The regression of the inverse of DPM incorporated into DNA vs the concentration of added thymidine was significant at least at the 95% level. In this analysis, the internal plus external pools of thymidine are estimated by the negative of the x-intercept of the regression line. If the y-intercept is zero, then the x-intercept is also zero, and there is no evidence for dilution of the isotope. In the five isotope dilution experiments done with the stream sediments, the y-intercept was within 1 SE of 0. Because of this result, thymidine incorporation was determined using a specific activity that was calculated from the amount of thymidine added in the laboratory, assuming no exogenous dilution of the thymidine. Tritiated thymidine uptake was expressed per g dry weight of sediment or per cm^2 of rock surface area.

Sediment Dry Weight and Organic Content Determinations

Dry weight was determined by drying the sediments to constant weight at 80°C for at least 72 hours. The organic fraction of dried samples was determined by combustion at 550°C for 1 hour in a muffle furnace.

Rock Surface-Area Determinations

The surface area of rocks used in the ATP and [^{3}H]-thymidine uptake analyses was determined by carefully wrapping the rocks with aluminum foil so that the foil approximated the surface area of the rock sample. This foil was then weighed, and that value was divided by the weight of 1 cm^2 of the same foil.

Statistical Analyses

Analysis of variance (ANOVA) and Tukey pair-wise comparison tests were used to determine differences between the twelve sites in terms of each of the microbiological analyses. The Pearson product-moment correlation was used to measure the closeness of a linear relationship between biological and chemical parameters. All statistical analyses were done using the SAS statistical package [39].

Results

Of the three streams studied, Beaver Brook had the largest range of pH values (4.9 to 7.0) and acid-neutralizing capacity (ANC) (−16 to 130 μeq/liter). Moss Inlet had pH and ANC ranges of 5.1 to 6.6 and −1 to 142 μeq/liter, respectively, and Pancake Creek generally exhibited the lowest pH and ANC ranges (4.5 to 6.4 and −35 to 70 μeq/liter, respectively). The water chemistry of all three streams varied spatially, though variations were most obvious for Pancake Creek and Beaver Brook, with headwater sites of both streams exhibiting lower pH and ANC values than downstream sites [9, 10].

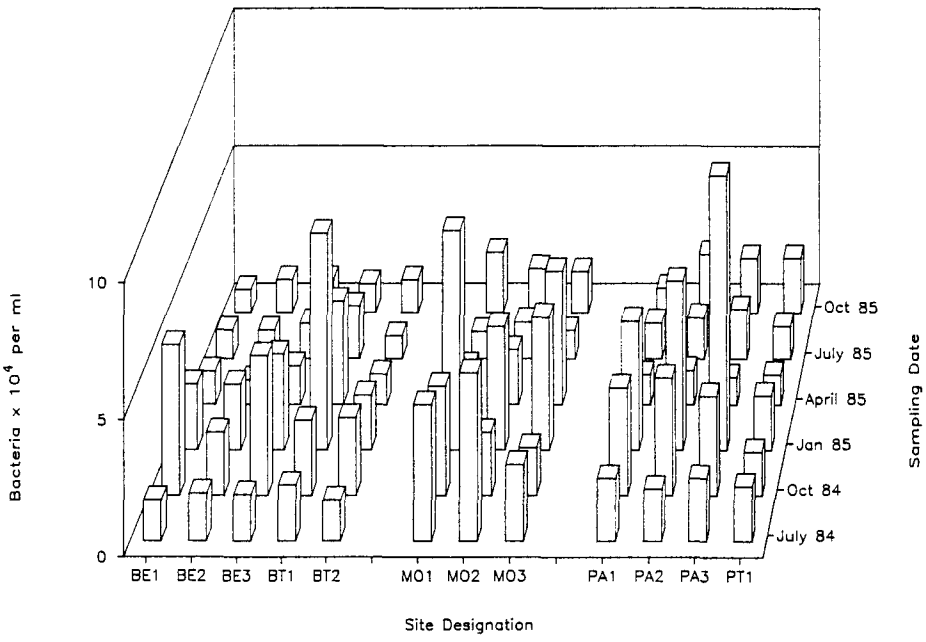

Fig. 2. Planktonic bacterial numbers in three Adirondack streams (n = 3). Beaver Brook sites BE1, BE2, BE3, BT1, and BT2. Moss Inlet sites MO1, MO2, and MO3. Pancake Creek sites PA1, PA2, PA3, and PT1.

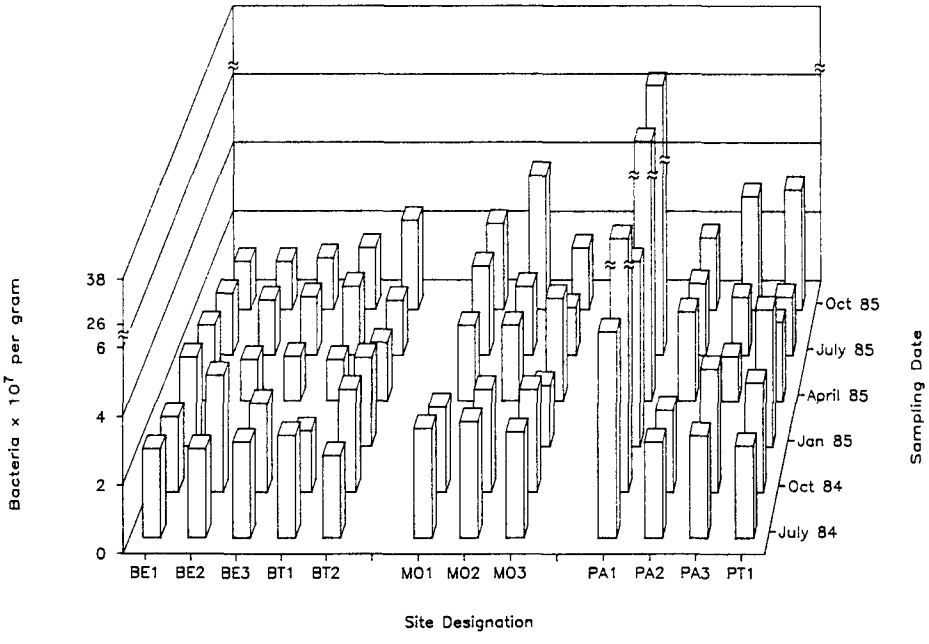

Fig. 3. Sediment bacterial numbers (n = 3). Beaver Brook sites BE1, BE2, BE3, BT1, and BT2. Moss Inlet sites MO1, MO2, and MO3. Pancake Creek sites PA1, PA2, PA3, and PT1.

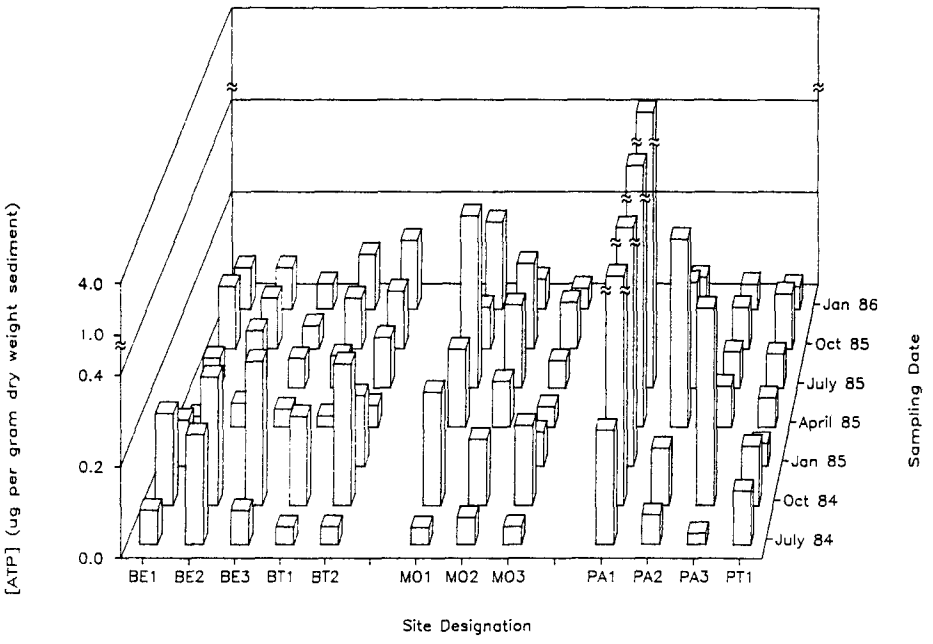

Fig. 4. ATP in sediments (n = 4). Beaver Brook sites BE1, BE2, BE3, BT1, and BT2. Moss Inlet sites MO1, MO2, and MO3. Pancake Creek sites PA1, PA2, PA3, and PT1.

Bacterial Numbers in the Water Column

Bacterial counts, reported as numbers per milliliter, are shown in Fig. 2. Concentrations of planktonic bacteria always appeared low, with no significant difference between the sites irrespective of season, stream, or location in a given stream ($P < 0.05$). We observed a general seasonal trend of higher numbers in the fall and winter months, with five sites showing a peak in January 1985. Counts in April 1985 were the lowest over the course of the study, coinciding with the highest seasonal stream flow.

Bacterial Numbers in Sediments

Figure 3 shows the bacterial numbers in sediments (reported as numbers per g dry weight). A significantly greater number of bacteria was always found in the sediments from site PA1 than from all the other sites ($P < 0.0001$). With this one exception, sediment bacterial numbers showed no consistent pattern among sites or seasons.

ATP Levels in Sediments

Sediment microbial biomass, as measured by the levels of ATP per g dry weight of sediment, is shown in Fig. 4. The most noticeable difference among sites

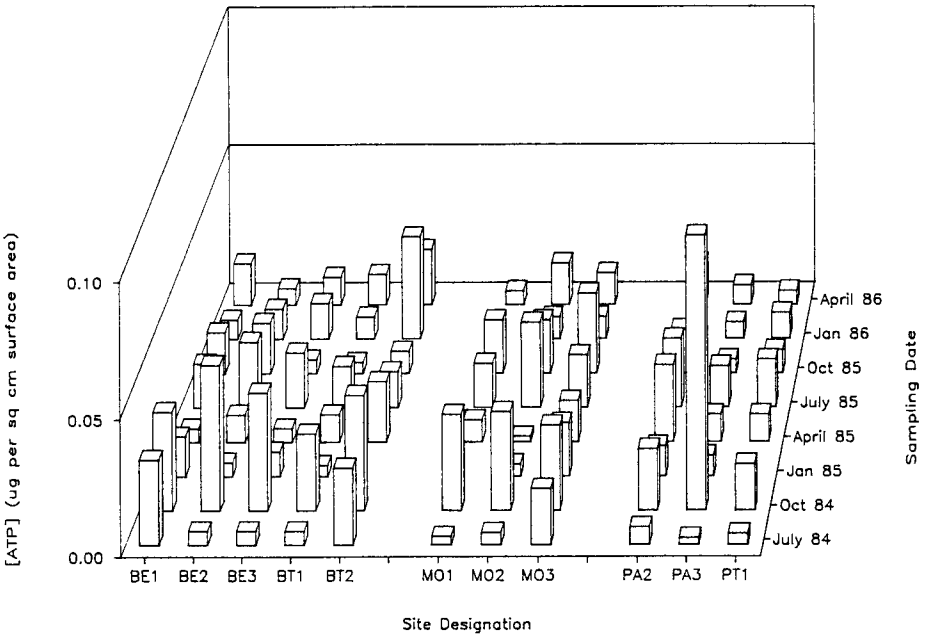

Fig. 5. ATP on rock surfaces (n = 5). Beaver Brook sites BE1, BE2, BE3, BT1, and BT2. Moss Inlet sites MO1, MO2, and MO3. Pancake Creek sites PA2, PA3 and PT1. Rocks were not collected at PA1.

was that site PA1 had higher levels of sediment ATP than all other sites on almost all sampling dates. Post-hoc analysis of variance done for each sampling date showed that on all dates when PA1 was sampled (except for the July 1984 sampling), the ATP content of the sediment at this site was significantly greater ($P < 0.0001$) than at all other sites. We could find no other discernible patterns of ATP levels, either when we considered all twelve sites together or when we examined the three streams separately. Although a seasonal pattern was seen in the Beaver Brook system, with all five sites showing the highest levels of ATP in October of 1984 and lowest levels in April of 1985, no consistent seasonal patterns were discernible in the Moss Inlet or Pancake Creek systems.

ATP Levels on Rock Surfaces

Epilithic microbial biomass, as measured by the level of ATP per square centimeter of rock surface area is shown in Fig. 5. Overall, levels of ATP attest to the low microbial biomass found in these streams. Only the biomass at site BT2 was shown to be different than that at other sites, being greater than that at sites BT1 and PT1 (rocks were never collected at PA1). Post-hoc analyses of variance for each sampling date showed no pattern of any site having consistently greater or lower levels of epilithic ATP ($P < 0.05$).

When we separated and analyzed the sites by stream systems, we found differences between the Beaver Brook sites for January 1986, when site BT2

had higher levels of ATP than all other Beaver Brook sites ($P < 0.05$). In the Pancake Creek system, site PA3 had greater levels than the other sites in October 1984 ($P < 0.002$). Site PA2 had the highest mean level of epilithic ATP among the Pancake Creek sites for six of the eight sampling dates, but values were never significantly greater than those at PA3 and PT1. Differences in epilithic ATP levels were found between the Moss Inlet sites only in January 1985, when MO3 was significantly greater ($P < 0.05$) than MO2.

When we examined seasonal variations in epilithic ATP levels for individual sites, we found levels in October 1984 at all the Beaver Brook sites to be the highest, with sites BT1, BE2, and BE3 having greater levels compared to all other sampling dates ($P < 0.05$). For the Moss Inlet system, the samples obtained in October 1984 were again the ones consistently showing the highest levels of ATP, with differences often being significant ($P < 0.05$). In the Pancake Creek system, sites PT1 and PA3 showed highest levels of ATP in October 1984 with the level on this sampling date being significantly higher ($P < 0.0001$) only at PA3. Site PA2's highest level was measured in April of 1985, but this level was not significantly different from other sampling dates. No other patterns were discernible among the other sampling dates.

Bacterial Thymidine Uptake in Sediments

We determined thymidine incorporation using a specific activity calculated from the amount of thymidine added in the laboratory, assuming no exogenous dilution of the thymidine (on the basis of isotope dilution determinations done on the sediments). Uptake of tritiated thymidine by bacteria in the sediment is reported per cm^3 of sediment per hour and is shown in Fig. 6. No significant differences ($P < 0.05$) were found between the individual sites.

We investigated seasonal trends for each site. All but BE2 of the Beaver Brook sites showed the highest uptake in April of 1985, those levels being significantly higher than for January and July of 1985 at BT2 and July 1985 at BE3 ($P < 0.05$). In October of 1984, the highest level of uptake was measured at BE2, but this was not significantly different than the other two sampling dates. The uptake levels measured at MO2 and MO3 were highest in April 1985 ($P < 0.05$). MO1 showed a peak of uptake in July 1985 no greater than that measured on the other two sampling dates. Three of the Pancake Creek sites, PA1, PA2, and PA3, had their highest levels of sedimentary thymidine uptake in April 1985. The highest level at PT1 was measured in January 1985. However, none of these levels differed significantly from those on the other sampling dates.

Bacterial Thymidine Uptake on Rock Surfaces

Uptake of tritiated thymidine by epilithic bacteria is reported per cm^2 of rock surface area per hour (Fig. 7). Although no significant difference was found between the three sampling dates ($P < 0.05$), there were differences between sites. Sites MO1 and MO2 had higher levels of thymidine uptake than sites BE1 and BT1, all three Pancake Creek sites, and site MO3 ($P < 0.05$). Sites

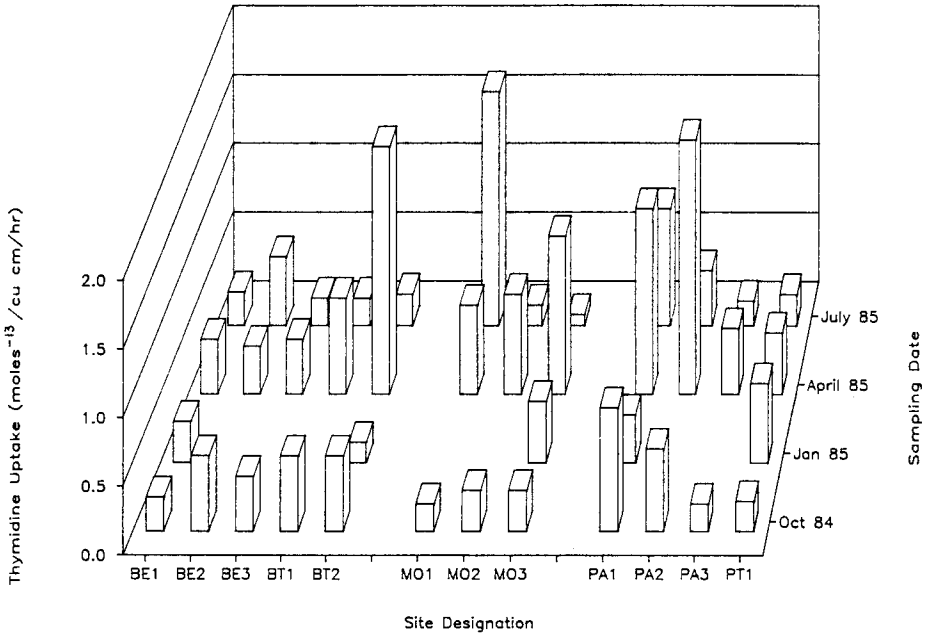

Fig. 6. Tritiated thymidine uptake by bacteria in sediments (n = 3). Beaver Brook sites BE1, BE2, BE3, BT1, and BT2. Moss Inlet sites MO1, MO2, and MO3. Pancake Creek sites PA1, PA2, PA3, and PT1.

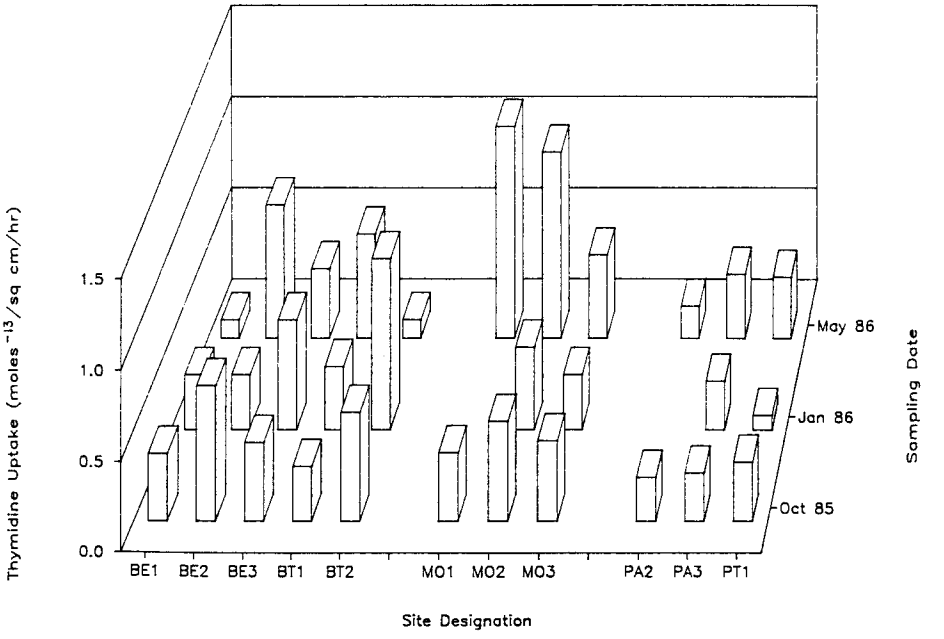

Fig. 7. Tritiated uptake by bacteria on rock surfaces (n = 5). Beaver Brook sites BE1, BE2, BE3, BT1, and BT2. Moss Inlet sites MO1, MO2, and MO3. Pancake Creek sites PA2, PA3, and PT1. Rocks were not collected at PA1.

Table 2. Matrix of Pearson correlation coefficients between the chemical and microbiological variables

Variable	AVSATP	AVRATP	AVRTDR	ASEDTDR	AVORG	AVAOWA	AVAOSG
AVSATP	1.00000						
AVRATP	0.67888**	1.00000					
AVRTDR	0.34090	0.14306	1.00000				
ASEDTDR	0.31872†	−0.11871	ND	1.00000			
AVORG	0.67244**	−0.01791	−0.10094	0.23190	1.00000		
AVAOWA	0.00053	0.03901	−0.46355	−0.08340	0.13112	1.00000	
AVAOSG	0.92883**	0.22729	−0.21392	0.32149†	0.72958**	0.01809	1.00000
PH	−0.18763	0.10044	0.16713	−0.24510	−0.27345†	−0.19369	−0.17218
GAUGE	0.08565	−0.14423	−0.23118	0.21607	0.17846	0.26992	−0.20369
TEMP	0.00587	0.15020	0.22386	−0.29859	0.14872	−0.33046††	−0.00475
ANC	−0.13926	0.19048	0.06925	−0.32610†	−0.20212	−0.19417	−0.16810
DIC	0.14910	−0.09277	−0.21687	0.02394	0.27139†	−0.00891	0.16838
DOC	0.03996	0.23564†	0.05567	−0.16558	0.16571	0.13357	0.02366
NA	0.22838	0.08727	−0.14305	−0.26174	0.06442	−0.11545	0.26196†
K	0.06724	0.27075†	−0.14933	−0.12183	0.12167	0.11023	0.02491
CA	−0.05903	−0.04402	0.10129	−0.14798	−0.24122†	0.03232	−0.07612
MG	−0.04565	0.02624	0.14596	−0.17884	−0.18928	−0.03288	−0.07007
TOTF	0.08701	−0.13497	−0.11837	0.39827††	0.01307	0.12104	0.09333
CL	0.02037	0.53617**	−0.37020†	−0.29269	0.07094	0.05075	−0.01507
NO3	0.19319	−0.25958†	−0.27451	0.34192†	0.05161	0.13853	0.14528
SO4	0.19977	−0.14050	−0.03092	0.28200	0.14325	0.13487	0.22870
NH4	−0.01796	−0.09242	0.08484	0.01797	−0.05012	0.03359	−0.05197
SI	0.16239	0.01595	−0.33988	−0.12108	0.06818	0.04572	0.18426
TOTAL	0.28444†	−0.25850†	−0.18959	0.31221†	0.41294*	0.07935	0.26117†
MONOAL	0.26312†	−0.14837	−0.24202	0.30125	0.32332††	0.09906	0.23959
ORGAL	0.12965	−0.12696	−0.03274	0.12191	0.25904†	0.19217	0.10059
TOTALP	−0.03910	−0.01051	0.12622	0.07919	0.15889	−0.22976	−0.09543
SRP	−0.14342	−0.05202	0.28670	−0.08442	0.17139	−0.05823	0.09136
CONDUCT	0.16021	−0.12517	−0.16969	0.19650	0.15883	0.30346††	0.14984

Table 2. Continued

Variable	SI	TOTAL	MONOAL	ORGAL	TOTALP	SRP	CONDUCT
SI	1.00000						
TOTAL	−0.29480††	1.00000					
MONOAL	−0.21170†	0.93753**	1.00000				
ORGAL	−0.38903**	0.62517**	0.64246**	1.00000			
TOTALP	−0.31747††	0.24550†	0.02617	0.32106††	1.00000		
SRP	−0.22841	0.08123	−0.02307	0.16730	0.48461**	1.00000	
CONDUCT	0.43400**	0.37742*	0.44628**	−0.08468	−0.46403**	−0.21831	1.00000

Variable	PH	GAUGE	TEMP	ANC	DIC	DOC	NA	K
PH	1.00000							
GAUGE	−0.36731	1.00000						
TEMP	0.24176†	−0.07921	1.00000					
ANC	0.75513**	−0.33101	0.22705	1.00000				
DIC	−0.01686	−0.15539	−0.03013	0.19607	1.00000			
DOC	−0.13798	0.52570††	0.35288*	−0.01713	0.05775	1.00000		
NA	0.46120**	0.05242	−0.02343	0.62910**	0.35694*	−0.17617	1.00000	
K	−0.12086	0.55770††	0.17877	0.05881	0.08872	0.76620**	−0.00899	1.00000
CA	0.52892**	−0.42666†	−0.25404†	0.60811**	0.04645	−0.53495**	0.39762**	−0.42139**
MG	0.66177**	−0.42515†	−0.10245	0.79107**	0.13252	−0.38094*	0.61152**	−0.25520†
TOTF	−0.36521*	0.30351	−0.18188	−0.21422†	0.07402	0.00181	−0.18124	0.27534††
CL	0.28735††	−0.05507	0.23983†	0.31639††	−0.00285	0.43403**	0.31389††	0.43658**
NO3	−0.43264**	0.31146	−0.64401**	−0.27947††	0.07307	−0.37851*	−0.04332	−0.12246
SO4	−0.36379*	−0.17935	−0.28395††	−0.38391**	−0.07742	−0.49195**	−0.08426	−0.36936*
NH4	0.05532	−0.04839	0.25053†	−0.12885	0.00141	−0.02396	−0.07306	0.01016
SI	0.47228**	−0.16976	−0.24426†	0.58611**	0.27059††	−0.40915**	0.75276**	−0.25362†
TOTAL	−0.76113**	0.63362*	−0.19242	−0.60867**	0.19186	0.11729	−0.31493††	0.16584
MONOAL	−0.78210**	0.59448††	−0.25890†	−0.61804**	0.14326	0.04859	−0.28922††	0.12511
ORGAL	−0.58253**	0.51359††	0.12118	−0.51099**	0.20981†	0.41331**	−0.30684††	0.25339†
TOTALP	0.01458	0.49936†	0.44705**	0.13556	0.36605††	0.61352**	0.01034	0.41654*
SRP	−0.03490	0.26552	0.34240††	0.08212	0.05420	0.22858	0.02021	0.21191
CONDUCT	−0.31974††	−0.03526	−0.53289	−0.00036	0.12162	−0.41607**	0.14634	−0.15678

Table 2. Continued

Variable	CA	MG	TOTF	CL	NO3	SO4	NH4
CA	1.00000						
MG	0.88679**	1.00000					
TOTF	0.03324	−0.08846	1.00000				
CL	−0.14777	−0.02344	−0.35010*	1.00000			
NO3	0.11788	−0.02453	0.47498**	−0.43784**	1.00000		
SO4	0.18757	0.00125	0.16678	−0.24430†	0.17555	1.00000	
NH4	−0.24672*	−0.19209	−0.06530	−0.12325	−0.14128	−0.06286	1.00000
SI	0.62027**	0.72192**	−0.17251	0.21799†	0.10678	0.14557	−0.24312†
TOTAL	−0.49380**	−0.57016**	0.34754*	−0.22291†	0.48922**	0.30101††	−0.00977
MONOAL	−0.46107**	−0.53308**	0.28657††	−0.16777	0.49069**	0.44803**	−0.00927
ORGAL	−0.59998**	−0.57199**	0.06053	−0.04950	−0.00991	0.09034	0.17286
TOTALP	−0.35099††	−0.18930	0.04525	0.06178	−0.18905	−0.64502**	0.20310
SRP	−0.27140†	−0.09406	−0.01769	−0.14674	−0.11176	−0.31526†	0.42867*
CONDUCT	0.42467**	0.33350*	0.37666*	−0.28791††	0.64379**	0.51660**	−0.21923†

† = $P < 0.05$; †† = $P < 0.01$; * = $P < 0.001$; ** = $P < 0.0001$.

AVSATP = ATP in sediments, AVRATP = ATP on rock surfaces, AVRTDR = thymidine uptake on rock surfaces, ASEDTDR = thymidine uptake in sediments, AVORG = organic content of the sediments, AVAOWA = directly counted bacteria in the water, AVAOSG = directly counted bacteria in sediments, GAUGE = staff gauge reading, TEMP = water temperature, ANC = acid neutralizing capacity, DIC=dissolved inorganic carbon, DOC=dissolved organic carbon, TOTF=total fluoride, TOTAL = total aluminum, MONOAL = monomeric aluminum, ORGAL = organic aluminum, TOTALP = total phosphorus, SRP = soluble reactive phosphorus, CONDUCT = conductivity. All other chemistry variables are equal to the total concentration of the metal or ion

BE2 and BT2 showed uptake levels greater than BE1 and all three Pancake Creek sites ($P < 0.05$). On a date-to-date basis, the sites mentioned above as having higher levels were often higher ($P < 0.05$), but no consistent pattern was seen.

Organic Content of Sediments

Mean organic content of sediments, for all but two sites, was on the order of 1 to 5% (w/w), reflecting the characteristic sandy nature of Adirondack stream bottoms. Site PA2 showed a wide range and a mean of 8%; this was not significantly greater ($P < 0.05$) than the other sites. Site PA1, however, had leaf-derived sediments with mean organic content of 72%, and this was significantly greater ($P < 0.0001$) than all the other sites.

Relationships of Water Chemistry Parameters and Other Site Characteristics to Microbial Parameters

We calculated Pearson correlation coefficients between the water chemistry variables [9, 10] and the quarterly microbiological parameters. The water chemistry parameters of ANC and concentrations of calcium, magnesium, silicon, and sodium were all highly and positively correlated to pH ($P < 0.0001$). Concentrations of nitrate and of three measured forms of aluminum were highly and negatively correlated to pH ($P < 0.0001$). Total chloride, fluoride, sulfate, and conductivity were less highly correlated to pH (Table 2).

Many of the microbiological variables were related to each other and to sediment characteristics. The levels of ATP and the numbers of bacteria in the sediment were highly correlated to the organic content of the sediment and to each other ($P < 0.0001$). The uptake of tritiated thymidine by sediment bacteria was also correlated to sediment ATP levels and sediment bacterial numbers ($P < 0.05$). Another statistical relationship found was between levels of sediment ATP and epilithic ATP ($P < 0.0001$).

However relationships between water chemistry parameters and microbiological parameters were not always consistent. In an attempt to understand these better, we calculated correlations separately for the three stream systems. For the five Beaver Brook sites, we found a positive correlation between water pH and epilithic ATP ($P < 0.05$), a correlation that was also reflected in the negative relationship between epilithic ATP and total and monomeric aluminum ($P < 0.05$) and in the positive relationship between ATP and chloride concentration ($P < 0.001$). Total phosphorus and soluble reactive phosphorus were related to epilithic bacterial thymidine uptake ($P < 0.05$ and $P < 0.005$, respectively). The concentration of DOC in the water was related to sediment ATP levels ($P < 0.0001$), to sediment bacterial numbers ($P < 0.005$), and to epilithic ATP, epilithic thymidine uptake, organic content of the sediment, and planktonic bacterial numbers ($P < 0.05$).

For the Pancake Creek sites, we found no relationships other than the overall relationships for the twelve study sites. The Moss Inlet sites revealed relation-

ships between the planktonic bacterial numbers and several water physical and chemical characteristics, the water level, temperature, conductivity, and nitrate and ammonia concentrations all being positively correlated to the numbers of bacteria ($P < 0.05$).

Discussion

Planktonic bacterial numbers (Fig. 2) were within the range of those found by other researchers studying the microbial ecology of diverse streams [10, 17, 19, 35]. The study reported here indicates that the densities of water-column bacteria are not affected by pH or pH-related water chemistry parameters, which is in agreement with the results of other researchers [4, 5, 41]. Francis et al. [13], however, found fewer bacteria in the most acidic of three Adirondack lakes studied. Hoeniger [21] found differences between planktonic bacterial numbers in several lakes of varying pH, but attributed the greater numbers to the humic nature of those lakes. A relationship between planktonic bacterial numbers and concentration of organic carbon has been postulated [13, 29, 35, 41] and results from Beaver Brook are in agreement.

It is not surprising that the planktonic populations were not influenced by localized water chemistry within a site, especially in streams where the water column bacteria are transient. The method used in this study for directly counting the bacteria did not allow us to determine whether they were alive and active, dormant, or dead; these total counts therefore cannot provide an assessment of the health and functioning of the population.

Sediment bacterial numbers directly counted in this study were on the low end of ranges reported by other researchers for freshwater streams [2, 3, 23, 35]. Throughout the study, site PA1, whose sediment organic content was much greater than that at all of the other sites, consistently had the highest numbers of sediment bacteria. The organic content of the sediments was highly correlated not only to numbers of bacteria, but also to ATP and thymidine incorporation in the sediment. Organic carbon appears to be much more important than the pH of the overlying water in influencing sediment microbial populations. This result agrees with work done by other researchers, which show sediment bacterial numbers to be correlated with sediment organic matter [2, 35].

There were no significant relationships between sediment bacterial numbers and water pH or other water chemistry parameters related to pH. This is in agreement with the results of Bott and Kaplan [2], Boylen et al. [4], Boylen et al. [5], and Palumbo et al. [35]. The pH of sediments has often been found to be higher than that of the water column in acid lakes [13, 25], though this was not the case in an acidic Adirondack lake recently studied [38]. Some possible reasons for higher pH in aquatic sediments include increased levels of nutrients and a more "constant" environment [42], higher buffering capacity in the sediment [15], and anaerobic microbial processes that generate alkalinity [24, 25, 40]. This last effect would not be relevant in the Adirondack streams we studied, where sediments were thin, patchy, and except for the sediments at PA1, very low in organic matter. However, even the limited amount of sediment

present in the streams represents an environment in which the microbial communities are somewhat protected from overlying low-pH waters.

Measurement of ATP gives an estimate of total biomass, not simply bacterial biomass. Sediment ATP levels were within the range of those found elsewhere [35]. The Adirondack stream sediments had ATP levels ranging from 20 to 450 $ng \cdot g^{-1}$ dry weight. These measurements were within the range found by Kaplan and Bott [23]. However, there were no significant correlations of sediment ATP with pH or ANC of the overlying water. There was, however, a significant correlation of ATP to organic carbon in the sediment in these streams. Bott and Kaplan [2] also found sediment ATP to be significantly correlated with sediment organic matter in streams in Pennsylvania.

Sediment bacterial incorporation of tritiated thymidine into DNA was low but similar to that found by Palumbo et al. [35] in their southern streams. As in that study, there were significant correlations of thymidine uptake with sediment bacterial numbers, and no correlations with pH or related water chemistry parameters. Once again, the bacterial communities found in the stream sediments seem to be more influenced by the properties of the sediment than by those of the water above.

General information on epilithic microbial communities is limited. They have been studied in terms of total bacterial numbers and activity [27], but this work was done using organisms that had been scraped from the rock surface and physically dispersed, so the results differed from what would be expected in situ. Geesey et al. [18], using phase-contrast and electron microscopy to study the epilithic community in a small subalpine stream, described dense and highly active communities of primarily gram-negative organisms that seemed to be responsible for the slime matrix in which they were enclosed. The bacteria were enumerated by direct counting methods and were found to number between 7.0×10^5 and 1.0×10^8 $cells \cdot cm^{-2}$ of surface area. These numbers were several orders of magnitude greater than those counted per ml of stream water, and in a shallow stream (<1 m deep) this made the epilithic community the dominant bacterial population.

We followed epilithic microbial-ATP levels and bacterial-production estimates and found that despite the protective slime matrix, the epilithic communities were affected by water chemistry parameters. The ATP levels measured were very similar to those found by Palumbo et al. [36]. These researchers found significant correlations of epilithic ATP to water pH and ANC and to total phosphorus and temperature. Seasonal trends illustrated the relationship of rock ATP and pH: ATP levels were low in early spring but increased through summer and into the fall. These authors suggested that the correlation with temperature was probably also related to the seasonal, pH-related pattern. While many of the Adirondack sites had their highest levels of epilithic ATP in the fall, no overall seasonal pattern could be seen. However, the significant correlations between epilithic ATP and several water chemistry parameters related to pH at the Beaver Brook sites agree with other studies [35, 36].

Tritiated thymidine uptake by the bacteria on rock surfaces in our Adirondack streams did not show any seasonal pattern, nor was there a significant relationship between thymidine uptake and any pH-related chemistry mea-

surement. However, the Pancake Creek sites, generally the stream system with the lowest pH, and the two most acidic of the Beaver Brook sites (BE1 and BT1) did have the lowest levels of uptake. This is in agreement with studies done by Palumbo et al. [36] which showed lower levels of thymidine uptake at low pH sites when compared to matched high pH sites.

In order to determine the effects of sudden changes in water chemistry on the epilithic microbial communities, a series of transplant experiments was done at Beaver Brook sites BE1 and BE3. These sites were chosen because they represented the largest difference in pH (4.9 to 7.0) and ANC (−16 to 130 meq/liter) within a single stream. Rocks, with their associated microbial communities, were transplanted from BE1 to BE3 and from BE3 to BE1. ATP levels and tritiated thymidine uptake was measured on control (unmoved) and transplanted rocks after zero, two, four, seven and nine days.

Results from these experiments indicated that epilithic ATP levels were decreased by the change from the higher pH to the lower pH site. Thymidine uptake was also reduced by moving the rocks to the lower pH site, and increased by moving the rocks from the low pH to the higher pH site. This is in agreement with transplant studies done by Palumbo et al. [36]. Reduction in the bacterial thymidine uptake at lower pH could have been due to direct toxicity of either hydrogen ion or aluminum or to an indirect effect such as reduced grazing by invertebrates on the rocks [32].

The immediate response of an organism to adverse conditions is not always detected; the schedule of sampling in this study was not sufficiently intensive to discern short-term (daily) changes in the microbial community. Very short-term changes, on the order of hours or minutes, also could not be measured. It is possible that a detrimental effect of low pH is realized more rapidly than an ameliorative effect of increased pH. However, because of the large percentage of dormant bacterial cells generally found in aquatic ecosystems, the injury to, or recovery of, the whole community will be staggered.

The influence of multiple environmental factors on the microbial communities is evident. Despite extremely thorough water chemistry analyses [9, 10], relatively few significant relationships could be found between biological and chemical measurements.

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