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Bacterial Biomass, Metabolic State, and Activity in Stream Sediments: Relation to Environmental Variables and Multiple Assay Comparisons

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Bacterial biomass, metabolic condition, and activity were measured over a 16-month period in the surface sediments of the following four field sites with differing dissolved organic matter regimes: a woodlot spring seep, a meadow spring seep, a second-order stream, and a third-order stream. Total bacterial biomass was measured by lipid phosphate and epifluorescence microscopic counts (EMC), and viable biomass was measured by ^{14}C most probable number, EMC with 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride reduction, and ATP. Bacterial metabolic condition was determined from the percentage of respiring cells, poly-β-hydroxybutyrate concentrations, and adenylate energy charge. Activity measures included ^{14}C-lipid synthesis, ^{32}P-phospholipid synthesis, the rate of uptake of algal lysate dissolved organic carbon, and respiration, from which biosynthesis was calculated (dissolved organic carbon uptake corrected for respiration). Total bacterial biomass (from EMC) ranged from 0.012 to 0.354 μg of C/mg of dry sediment and was usually lowest in the third-order stream. The percentage of cells respiring was less than 25% at all sites, indicating that most bacteria were dormant or dead. Adenylate energy charge was measured only in the third-order stream and was uniformly low. Poly-β-hydroxybutyrate concentrations were greater in the woodlot spring seep than in the second- and third-order streams. Uptake of algal lysate dissolved organic carbon ranged from undetectable levels to 166 mg of $C \cdot m^{-2} \cdot h^{-1}$. Little community respiration could be attributed to algal lysate metabolism. Phospholipid synthesis ranged from 0.006 to 0.354 pmol · mg of dry $sediment^{-1} \cdot h^{-1}$. Phospholipid synthesis rates were used to estimate bacterial turnover at the study sites. An estimated 375 bacterial generations per year were produced in the woodlot spring seep, and 67 per year were produced in the third-order stream.

Dissolved organic matter (DOM) is recognized as an important source of energy in aquatic ecosystems. For stream systems, some of the sources and transformations of DOM have been documented (9, 30, 46, 64). While physical and chemical alterations account for minor losses of DOM from solution, the utilization of DOM occurs primarily through heterotrophic microbial activity (4, 10, 31, 51). Bacteria are capable of outcompeting other organisms for organic compounds at low concentrations since they possess high substrate affinities, surface-to-volume ratios, and metabolic rates. Natural DOM sources are heterogenous mixtures of compounds, and bacteria can utilize several molecules simultaneously. Nevertheless, DOM uptake has usually been measured with individual radiolabeled substrates (49, 68), and the use of natural DOM has received much less attention. While some measures exist for the uptake of heterogenous DOM in microcosms (4, 8, 10, 31, 39, 63) and in situ (27, 36, 39, 41), simultaneous rigorous estimation of bacterial parameters usually has not been done.

Our study objectives were to characterize both quantitatively and qualitatively the relationship between benthic bacterial communities and DOM in streams. Accordingly, we did the following: (i) determined the bacterial biomass in selected microhabitats in different stream reaches at different times of year, (ii) estimated the proportion of active and inactive cells in the communities, and (iii) determined lipid synthesis rates and DOM uptake, respiration, and biosynthesis by bacteria on a naturally occurring, heterogeneous dissolved nutrient source, and analyzed our results with respect to the physical and dissolved organic and inorganic chemical characteristics of the study sites. Algal lysate was used as a DOM source because algae were present at all study sites, and products of algal excretion and decomposition were likely to be important sources of DOM for the heterotrophic microflora. Several techniques were used to measure biomass, metabolic state, and activity, thereby permitting methods comparisons.

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MATERIALS AND METHODS

Study sites. Field sites were selected by considering the dissolved organic carbon (DOC) concentration and dominant land use. Saw Mill Spring (SMS) is a permanently flowing woodland seep in which major DOM inputs include leachate from deciduous leaves, throughfall, and exudates of algae, wetland plants, and the aquatic fauna. A meadow seep (HARRIS) receives runoff from a cultivated field and is subject to septic influence, including drainage from a large dairy barn. This seep stopped flowing during the study, and a site referred to as WEST was substituted. WEST, a second-order stream with inputs from a farm pond, transverses a pasture with cattle present from November through April. The last site, a third-order reach of White Clay Creek (WCC), has a protected riparian zone and receives mixed inputs from woodlots, meadows, pastures, and cultivated fields. All sites were within 10 km of the laboratory.

Sampling design. Sites were sampled at base flow over 16 months during 1980 and 1981 at periods chosen to coincide with spring (S), midsummer of each year (SU_1 and SU_2, respectively), autumn (A), winter (W), and spring thaw (ST).

Site water chemistry and sediment characteristics were determined with three to five water and sediment samples collected each season.

Bacterial biomass and activity measures were made on streambed sediments that were sieved through a 4-mm screen to reduce their heterogeneity and transferred to plastic trays (23 by 15 by 5 cm). The bottom of each tray had been removed and replaced with 400-μm mesh nylon screening to permit exchange of water and micro- and meiofauna with surrounding sediments. Ten trays were placed at each site, with the surfaces contiguous with that of the streambed, and incubated for 1 to 2 weeks. Black plastic was placed over incubation areas to retard algal growth on the sediments.

For biological measurements, the trays were brought to the laboratory and nested into solid-bottom trays containing site water. The sides were caulked where the trays joined to prevent seepage of interstitial water. Trays were placed into 22-liter microcosms with site water previously filtered through 5-μm cartridge filters to remove suspended material. Flow rates in microcosms were matched to those at field sites, and the water temperatures were maintained near those of ambient stream water with a water bath supplied with stream water. After an overnight period of equilibration, the water level in the microcosms was lowered, and the trays were removed. Cores (1 cm deep) were taken from two trays with sterile glass tubing (6-mm inner diameter) for biomass, metabolic state, and lipid synthesis assays ("before" samples).

The remaining trays were transferred in pairs to four microcosms (2) containing ca. 10.5 liters of filtered unamended site water (control) or filtered site water plus algal lysate. The lysate was well mixed in site water to elevate DOC concentrations by ca. 4 mg/liter, yielding values within the normal range of DOC concentrations at field sites. Triplicate samples for initial DOC and dissolved oxygen determinations were take ca. 5 min after trays were introduced, and the microcosms were sealed. After 5 h in the dark at ambient stream water temperature and flow rate, DOC and dissolved oxygen determinations and microbial assays were repeated ("after" samples). Measures of abiotic changes were made with sediments presterilized with 20% formaldehyde or mercuric chloride (200 mg of Hg^{2+} per liter, followed by 200 mg/liter additions 24 and 48 h later with no water change). Hg-sterilized sediments were exposed to 10 mg of Hg^{2+} per liter during the uptake measure.

Algal lysate preparation. Lysate was prepared from the following algae that occurred at the study sites: *Vaucheria* sp. and mixed diatoms collected from the field, and cultured *Ulothrix* sp. Harvested algae were frozen, macerated in a blender, transferred to a beaker, and stirred for 4 h to extract cold-water-soluble substances. Particulates were removed by centrifugation and glass fiber filtration. The filtrate was freeze-dried and stored frozen until use. For experiments, equal quantities of the three types were rehydrated, and after the insoluble constituents were removed, the filtrate was analyzed for DOC content. The chemical composition of the mixed algal lysate is presented in Table 1.

Water chemistry determinations. All glassware was acid washed, and for organic analyses, glassware and glass fiber filters were precombusted at 550°C for 6 h. Samples were filtered through Gelman A/E glass fiber filters, and DOC was determined with a model DC 54 DOC analyzer (Dohrmann). Other samples were filtered and frozen for later analyses of monosaccharides (28) and total carbohydrates (5); polysaccharides were determined by difference. Samples used for determination of total carbohydrates by the phenol-sulfuric acid method (12), amino acids plus peptides by the ninhydrin method (54), and hydroxylated aromatic compounds ("phenolics") with the tannin-lignin reagent (1) were preconcentrated by freeze-drying. Dissolved organic nitrogen (DON) was determined by hydrogen peroxide-promoted oxidation with UV radiation (43) followed by assay of NH_3 N (56) and of NO_2 N and NO_3 N, which were determined by the cadmium reduction technique. The difference between total inorganic nitrogen in an oxidized and a companion unoxidized sample was the calculated DON.

TABLE 1. Chemical characterization of concentrated algal lysate

Class of compounds	Mean concn (mg/liter) ± SD (n = 3)	Amt (mg) of C/liter	Amt (%) of total C
Phenolics	67.8 ± 35.4	36.6[c]	4.9
Total carbohydrates[a]	873.4 ± 14.2	349.4[d]	46.5
Total carbohydrates[b]	910.8 ± 86.4	364.3[d]	48.5
Monosaccharides	469.5 ± 0.9	187.8	25.0
Polysaccharides	441.2	176.5	23.5
Amino acids	153.6 ± 9.0	49.2[e]	6.5
Total DOC		750.80 ± 26.69	
Total DON	75.0		

[a] Phenol-sulfuric assay.
[b] 3-Methyl-2-benzothiazolinone hydrazone hydrochloride assay.
[c] Carbon conversion based on gallic acid.
[d] Carbon conversion based on glucose.
[e] Carbon conversion based on glutamate.

Inorganic analyses were performed on other unfiltered samples; pH was determined with a meter and glass electrode (Corning Glass Works, Corning, N.Y.), and total alkalinity was determined by titration (1). Filtered samples were assayed for the following parameters by the indicated procedure: NH_3 (phenol-hypochlorite; 56), soluble reactive phosphorus (SRP; molybdate blue; 45), Cl^- (argentometric), NO_3^- (chromotropic acid), NO_2^- (sulfanilic acid-naphthylamine hydrochloride), SO_4^{2-} (turbidometric), silicate (molybdosilicate), and Fe, Cu, Mg, Ca, Na, K, and Mn (atomic absorption spectrophotometry). All procedures were as previously described (1).

Sediment analyses. The mean particle size of sediments was determined on dried samples with a U.S. Standard sieve series (W. S. Tyler Co., Menton, Ohio). The organic matter content of sediments was determined by weight differences after combustion of samples at 550°C for 4 h.

Microbial biomass assays. Epifluorescence microscopic counts (EMC) of total and respiring (EMC-INT) cells were made (69). Individual sediment cores were transferred to test tubes, and 0.5 ml of 0.2% 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) were added. The volume was brought to 5 ml with 0.1 M phosphate buffer (pH 7.0). Samples were incubated in the dark at the same temperature as the water in the microcosms for 45 min, after which 0.1 ml of 37% formaldehyde was added. Samples were diluted (usually 1:100), stained with 5 ml of a 0.5 mg/liter 4',6-diamidino-2-phenylindole solution for 10 min (50), and filtered onto 0.2-μm (pore size) Nuclepore filters prestained with Irgalan black (23). Filters were mounted in Cargille immersion oil and examined by epifluorescence microscopy with a Zeiss universal microscope with a UG1 excitation filter and no. 41 emission filter. Numbers of bacteria were based on counts of 20 microscopic fields, and then counts were doubled, assuming an equal number of cells on the unviewed surfaces of particles. The dry weight of the sediment was obtained.

We have since combined a sample preparation procedure involving sonication in 0.1 M $NaPP_i$ to release attached and embedded cells (I. Velji and L. J. Albright, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, N4, p. 224), with centrifugation in 30% glycerol to separate bacteria from sediment and debris. This indicated that our counts underestimated bacterial numbers by a factor of 2.35, and our data reported here have been adjusted accordingly.

Bacterial cell volumes were calculated by using samples from each site taken during two or more sampling periods. Cell dimensions were measured by using freeze-dried, rehydrated samples after determining that such treatments did not significantly affect cell dimensions. Differences in cell size between sites were not statistically significant, and carbon estimates were made by using a mean cell volume of 0.13 μm^3 (standard deviation = 0.01, n = 11 samples of 60 cells each), an assumed cell density of 1.1×10^{-12} g/μm^3, and an 80% water and 50% carbon content (40). Lipid phosphate was determined on freeze-dried cores (66) and converted to bacterial carbon by using the following relationship: 10 mg of bacterial C per μmol of lipid phosphate. The ^{14}C most-probable-number (^{14}C MPN) procedure (38) was performed with uniformly labeled [^{14}C]glucose (specific activity, 14 to 329 mCi/mmol; New England Nuclear Corp., Boston, Mass.). $^{14}CO_2$ was trapped in phenethylamine-saturated cotton swabs, and radioactivity was determined in an Omnifluor cocktail in a liquid scintillation counter (model 133, Beckman Instruments, Inc., Fullerton, Calif.). MPN estimates were converted to bacterial carbon by using the assumptions listed for EMC. ATP determinations were performed by the luciferin-luciferase method (26) on neutralized extracts of cores extracted in cold 1.0 M H_3PO_4 (33). Internal standards were used to determine the efficiency of the light reaction which was measured by integrating the light flux from 30 to 36 s in a liquid scintillation counter placed in the noncoincidence mode (57). ATP recovery from sediments was checked by adding [^{14}C]ATP to the acid before adding the core and averaged 77%. ATP and crude luciferin-luciferase were purchased from Sigma Chemical Co., St. Louis, Mo. Conversion to bacterial carbon was based on the following relationship: 1 μg of ATP per 250 μg of bacterial C (19). Muramic acid was determined by gas chromatography (14), and the data converted to bacterial carbon by the following relationship: 12 μg of muramic acid per mg of bacterial C.

Microbial metabolic state assays. Epifluorescence count data were expressed as the percentage of INT-positive cells to evaluate the proportion of actively respiring cells. Poly-β-hydroxybutyrate (PHB) was extracted from freeze-dried sediments and purified (22) before being assayed spectrophotometrically (37). The extracts were corrected for recovery efficiency by adding purified PHB from *Bacillus megaterium* to a portion of each sample as an internal standard. Adenylate energy charge (EC_A) was measured by a modification of the procedure of Karl and Holm-Hansen (34). Pyruvate kinase and myokinase in $(NH_4)_2SO_4$ (Sigma Chemical Co.) were dialyzed against phosphate buffer (100 mM, pH 7.4) before use, and 1.0 ml of neutralized acid extract was incubated for 60 min with 250 μl of the reaction mixtures. AMP and ADP standards were added to acid blanks and sediment extracts to determine the efficiency of conversion to ATP, which was then assayed as described above. AMP and ADP (sodium salts) were obtained from Sigma Chemical Co.

Microbial activity assays. DOC uptake was calculated from concentrations measured before and after algal lysate exposure. Community respiration was determined from Winkler titrations of DO (1) in samples taken before and after exposure to algal lysate. Respiration data were converted to carbon equivalents, assuming a respiratory quotient of 0.85 (21). Biosynthesis was calculated as follows: biosynthesis = DOC uptake − community respiration. Data for the last four sampling periods were corrected for simultaneous changes in control microcosms.

Bacterial lipid synthesis (65) was measured by transferring sediment cores to presterilized aluminum foil cups made the same size as the core to maintain core integrity and reduce disturbance artifacts (16). Single or double label experiments were performed by injecting either 2.5 μCi of $CH_3{}^{14}COONa$ (specific activity, 56 mCi/mmol, New England Nuclear) or both acetate and 12.5 μCi of $Na_2H^{32}PO_4$ (specific activity, 200 Ci/mmol, New England Nuclear) onto the surface of the core and transferring the cup to a presterilized shell vial containing 25 ml of microcosm water. Cores were incubated in the dark with gentle agitation for 2 h at ambient streamwater temperatures. Formalin-treated samples were used as controls for the adsorption of label to sediments. Incubation was terminated by the transfer of the core to a chloroform-methanol-hydrochloric acid lipid extractant. The incubation water was membrane filtered (0.45-μm pore size), and the filter was extracted with the core. After extraction, 5 to 7 ml of chloroform was removed and filtered (Whatman no. 2V), and 1 ml was transferred to a liquid scintillation vial, evaporated at 40°C under an air stream, and counted in 10 ml of OCS cocktail (Amersham Corp., Arlington Heights, Ill.). The sediment was dried at 60°C and weighed. Double label experiments were counted in the full ^{14}C and ^{32}P windows immediately after processing and again after 3 to 4 months, during which time nearly all of the ^{32}P decayed. Selected samples were internally standardized to determine counting efficiency. ^{32}P uptake was converted to phospholipid synthesis by using the P_i content of the isotope and the SRP concentration of the incubation water or water plus lysate after determining that SRP eluted with $^{32}PO_4$ on a Sephadex G 10-120 column. Biosynthesis was calculated from the following relationship: 10 mg of bacterial C per μmol of lipid phosphate (66).

Statistical analyses. Correlation analyses and Scheffe multiple range tests (MRT) were performed with SPSS programs. Multiple linear regression (MLR) analyses were performed by SAS routines. Initial models were derived by the stepwise procedure and included the variables of SRP and those in Table 2 except for Fe, Mn, Na, K, Ca, and Cl^-. Either DOC or the measured DOC constituents were used in separate analyses. Models with five variables were accepted from each set of analyses. Standardized partial regression coefficients for these variables were obtained by entering them into the REG routine (SAS), and the contribution of each variable to the overall r^2 was determined by performing the MLR in the forward mode with those five variables. All of the variables included in the stepwise regressions were usually included in the forward model, but if not, the r^2 values for the equations were very similar.

RESULTS

Site characterization. The physical and chemical characteristics at the study sites that were significantly ($\alpha \leq 0.05$) correlated with measures of biomass, metabolic state or activity are shown in Table 2. The data for SMS and WCC are the means for all sampling periods, but Scheffe MRT comparing these sites with either HARRIS or WEST were

TABLE 2. Water chemistry and sediment characteristics at field sites that were correlated significantly ($\alpha \leq 0.05$) with biological parameters[a]

Parameter	Mean concn (mg/liter) ± SD at following site			
	WCC	SMS	HARRIS	WEST
DOC	2.01 ± 0.38	2.19 ± 0.92	9.38 ± 4.78	4.81 ± 2.13
Phenolics	0.23 ± 0.14	0.33 ± 0.22	0.73 ± 0.54	0.37 ± 0.09
Carbohydrates	1.09 ± 0.34	1.10 ± 0.47	2.35 ± 1.88	2.41 ± 1.12
Monosaccharides	0.36 ± 0.21	0.41 ± 0.23	0.90 ± 0.50	0.48 ± 0.11
Amino acids and peptides	0.05 ± 0.03	0.05 ± 0.02	0.12 ± 0.10	0.10 ± 0.08
DON[b]	0.062 ± 0.038	0.043 ± 0.031	0.437 ± 0.068	0.261 ± 0.028
Nitrate N[b]	3.37 ± 0.63	4.93 ± 0.63	1.13 ± 3.97	1.03 ± 0.34
Iron[b]	0.11 ± 0.09	0.17 ± 0.12	1.64 ± 0.50	0.50 ± 0.02
Manganese	0.02 ± 0.01	0.02 ± 0.01	0.49 ± 0.32	0.16 ± 0.12
Sodium[b]	5.97 ± 1.29	8.45 ± 1.35	11.58 ± 1.25	7.63 ± 0.13
Potassium	1.22 ± 1.66	1.04 ± 0.84	3.02 ± 4.35	2.67 ± 2.66
Calcium	14.04 ± 5.04	13.71 ± 6.62	17.54 ± 9.15	16.38 ± 0.47
Sulfate[b]	18.0 ± 2.1	41.5 ± 2.5	38.1 ± 44.3	41.1 ± 5.1
Chloride[b]	13.1 ± 3.4	20.0 ± 6.1	38.0 ± 25.7	12.0 ± 1.0
Sediment (% organic matter)[b]	1.56 ± 0.55	4.39 ± 0.88	8.07 ± 2.48	0.87 ± 0.05
Sediment (mean particle size [mm])[b]	0.58 ± 0.20	0.39 ± 0.10	0.10 ± 0.03	0.52 ± 0.20

[a] $n = 6$ at SMS and WCC, $n = 3$ at HARRIS, and $n = 2$ at WEST for all parameters except monosaccharides and DON, for which $n = 5$ at SMS and WCC and $n = 2$ at HARRIS.

[b] Paired sample t test ($\alpha = 0.05$) was used to compare data for WCC and SMS. Scheffe MRT ($\alpha = 0.05$) was used to compare (i) WCC, SMS, and HARRIS and (ii) WCC, SMS, and WEST. Significant results were as follows: DON (H > WCC, SMS; WE > WCC, SMS), NO_3 (SMS > WCC > H; SMS > WCC; SMS > WE), Fe (H > SMS, WCC; WE > WCC), Na (H > SMS, WCC; SMS > WCC), SO_4 (SMS > WCC; SMS, WE > WCC), Cl^- (SMS > WCC; SMS > WE), percentage of organic matter (H > WCC; SMS > WCC; SMS > WE, WCC) and sediment particle size (WCC, SMS > H; WCC > SMS).

performed in a balanced design with only the data for the periods in which the latter sites were sampled. Only the following eight parameters differed significantly between sites (Scheffe MRT, $\alpha \leq 0.05$): DON, Fe, Na, SO_4, Cl^-, NO_3, and the organic matter content and particle size of sediments. DOC concentrations were high at HARRIS, low in SMS and WCC, and intermediate at WEST, but differences were statistically significant only at the $\alpha = 0.1$ level. DON data varied similarly, but the DOC/DON ratio was high in SMS (50:1), intermediate in WCC (32:1), and low in both HARRIS (21:1) and WEST (19:1), where fecal derived inputs were greatest. The concentrations of measured constituents of the DOC pool were also higher, although not significantly so, at HARRIS or WEST than at SMS or WCC. The concentration of phenolic substances at SMS, while not particularly high overall, increased to 0.67 mg/liter during A from levels of 0.09 to 0.47 mg/liter at other seasons, probably in response to leaf litter inputs. Highest concentrations of the cations that we monitored usually occurred at HARRIS, with intermediate levels at WEST and lowest levels at either WCC or SMS. Of the anions measured, SO_4^{2-} was lower in WCC compared with the other sites, Cl^- was higher at HARRIS, and NO_3^- was higher at SMS and WCC than at HARRIS or WEST. The organic matter content of the sediment in HARRIS was significantly greater than elsewhere and greater in SMS than in WCC or WEST. The mean particle size of sediments was least at HARRIS and greatest at WCC; only the difference between HARRIS and WCC was statistically significant.

Bacterial biomass. Total bacterial biomass estimates from lipid phosphate were higher than from EMC data by a factor of 6.1 at HARRIS, 7.9 at WCC, 16.6 at WEST, and 31.0 at SMS, differences that were statistically significant at all sites but HARRIS (paired sample t test, $\alpha = 0.05$, 0.05, and 0.01, respectively [Table 3]). Bacterial C estimates based on muramic acid assays were obtained for only a few sampling periods (Table 4). Values were significantly higher by factors ranging from 2 to 22 over estimates from lipid phosphate and

TABLE 3. Bacterial biomass estimates in surface sediments at field sites[a]

Site (no. of samples)	Mean ± SD				
	Total biomass (μg of C/mg of dry sediment)		Viable biomass (μg of C/mg of dry sediment)		
	Lipid PO_4[b]	EMC[c,d]	EMC-INT[c,e]	^{14}C MPN[c,d]	ATP[f]
SMS (6)	2.173 ± 1.159	0.070 ± 0.046	0.026 ± 0.014	0.043 ± 0.032	
SMS (4)			0.025 ± 0.016	0.056 ± 0.029	0.075 ± 0.019
HARRIS (3)	1.646 ± 0.144	0.270 ± 0.118	0.114 ± 0.035	0.070 ± 0.034	
WEST (2)	0.480 ± 0.038	0.029 ± 0.002	0.010 ± 0.002	0.036 ± 0.020	0.036 ± 0.006
WCC (6)	0.554 ± 0.467	0.070 ± 0.103	0.027 ± 0.038	0.016 ± 0.013	
WCC (4)			0.012 ± 0.006	0.012 ± 0.006	0.043 ± 0.009

[a] All data for a site were combined. Statistical tests of data from WCC and SMS with HARRIS or WEST were performed with only the appropriate sampling periods. Paired sample t test was used to compare SMS and WCC, and the Scheffe MRT was used when more than two sites were compared ($\alpha = 0.05$).

[b] Converted to micrograms of C by using the relationship 10 mg of bacterial C per μmol of lipid phosphate. SMS > WCC; H = WCC = SMS; SMS > WCC, WE.

[c] Converted to micrograms of C by assuming a cell volume of 0.13 μm^3, weight of 2.9×10^{-14} g (dry weight), and 50% C content.

[d] No significant differences between sites.

[e] No significant differences between SMS and WCC or among H, WCC, and SMS; but WE, SMS > WCC.

[f] Converted to micrograms of C by assuming an ATP/C ratio of 1:250. SMS and WCC not significantly different, but SMS > WE, WCC.

TABLE 4. Bacterial biomass estimates from muramic acid analyses

Site	Mean bacterial biomass (μg of bacterial carbon/mg of dry sediment) ± SD (*n*) for indicated season		
	S	ST	SU_2
SMS	6.98 ± 1.33 (2)		15.29 ± 2.39 (3)
HARRIS	11.50 ± 2.40 (3)		
WEST		11.08 ± 0.33 (3)	1.62 ± 0.49 (3)
WCC		1.45 ± 0.18 (3)	0.65 ± 0.21 (3)

from 31 to 567 (although usually 31 to 64) over EMC-based estimates.

Between sites, estimates of total biomass from lipid phosphate were higher in SMS than elsewhere during every sampling period but SU_1 and lowest in WCC (Fig. 1). Overall means were significantly higher in SMS than in WCC and WEST when corresponding sampling periods were compared (Table 3). EMC-based estimates were greatest at HARRIS during two of the three seasons in which that site was sampled and usually least at WCC (Fig. 1). While significant differences between sites occurred in most sampling periods, overall means did not differ between sites (Table 3).

EMC-based biomass estimates differed maximally during the study by a factor of 5.5 at SMS and 23.3 at WCC, the sites that were sampled every period. In SMS, high values occurred during S and A and lipid phosphate was also higher during S than some other periods (Fig. 1). Both assay procedures indicated significantly higher biomass in WCC during SU_1 than any other time. In HARRIS, EMC-based

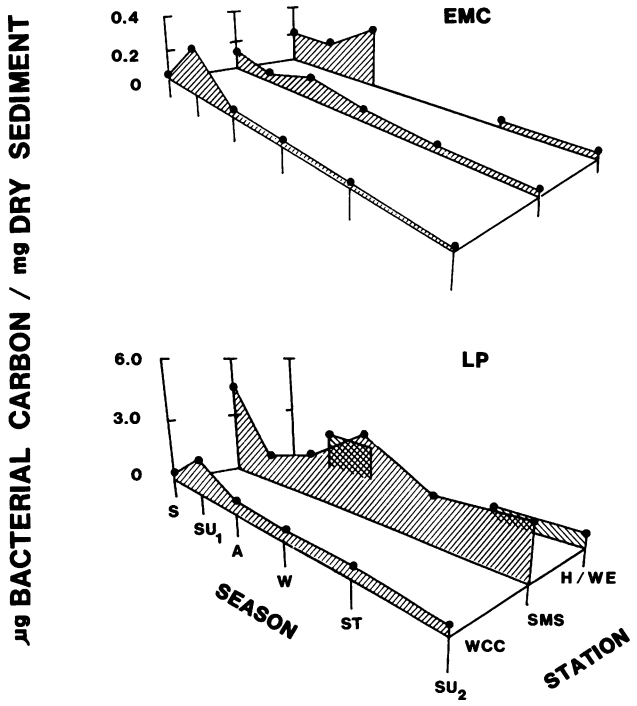

FIG. 1. Total bacterial biomass estimated from EMC and lipid phosphate in sediments from WCC, SMS, HARRIS (H), and WEST (WE) at each sampling period (S, SU_1, A, W, ST, SU_2). Significant differences between sites at the indicated sampling period (Scheffe MRT, $\alpha = 0.05$; or *t* test when $n = 2$, $\alpha = 0.05$) from EMC: S (H > SMS > WCC), SU_1 (WCC > H > SMS), A (H > SMS > WCC), W (SMS > WCC), ST (NSD [no significant difference]), and SU_2 (WE, SMS > WCC). Significant differences between sites at each sampling period from lipid phosphate: S (SMS > WCC), SU_1 (NSD), A (SMS, H > WCC), W (SMS > WCC), ST (SMS > WE, WCC), and SU_2 (SMS > WE, WCC). Significant differences between sampling periods at a site (Scheffe MRT, $\alpha = 0.05$) from EMC: at WCC (SU_1 > all other periods), at SMS (S, A > SU_1, ST, SU_2), at H (A > S, SU_1), and at WE (NSD). Significant differences under the same conditions from lipid phosphate at WCC (SU_1 > all other periods), at SMS (S > SU_1, A, ST, SU_2), and at H and WE (NSD).

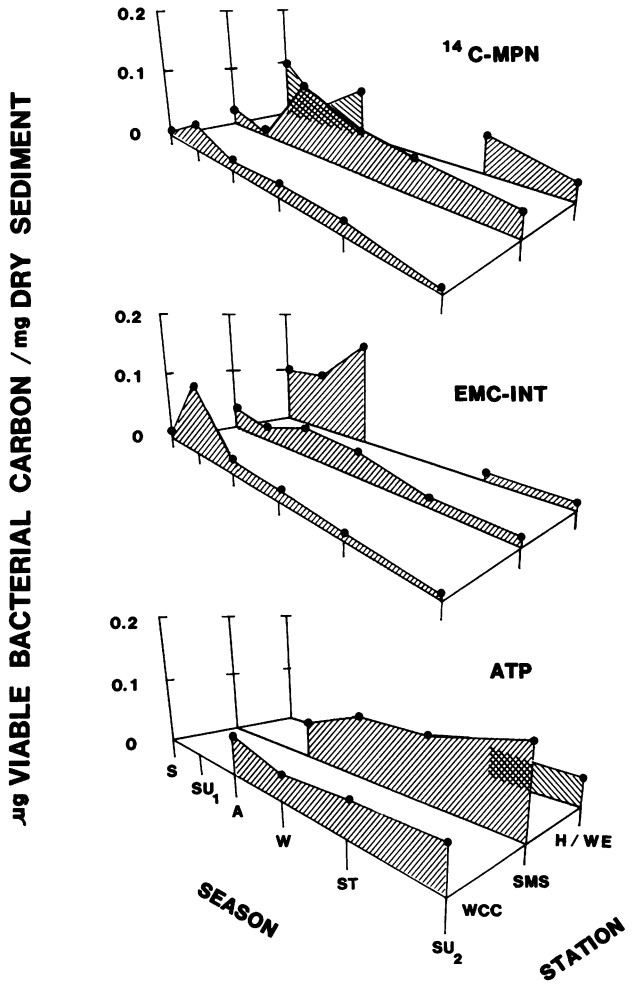

FIG. 2. Viable bacterial biomass estimates (from ^{14}C-MPN, EMC-INT, and ATP) in sediments at the study sites at the indicated sampling period. Significant differences between sites at each sampling period (Scheffe MRT, $\alpha = 0.05$; or *t* test, $\alpha = 0.05$ [when $n = 2$]) from ^{14}C-MPN: SU_1 (WCC > SMS) and other periods (NSD). Significant differences between sites at each sampling period from EMC-INT: S (H > SMS > WCC), SU_1 (WCC, H > SMS), A (H > SMS > WCC), W (SMS > WCC), ST (NSD), and SU_2 (SMS > WCC). Significant differences between sites at each sampling period from ATP: A (NSD), W (SMS > WCC), ST (SMS > WCC, WE), and SU_2 (SMS > WCC, WE). Significant differences between sampling periods at a site (Scheffe MRT, $\alpha = 0.05$) from ^{14}C-MPN: at WCC (SU_1 > S, SU_2) and other sites (NSD). Significant differences under the same conditions from EMC-INT: at SMS (S, A > ST, SU_2), at WCC (SU_1 > all other periods), and at H (A > S, SU_1). Significant differences under the same conditions from ATP: NSD at any site; NSD at WE with any method.

TABLE 5. Correlation coefficients among biomass measures and between biomass measures and independent variables[a]

Biomass measures and environmental variables	Lipid PO_4	EMC	ATP	EMC-INT	^{14}C MPN
Lipid PO_4	1				
EMC	0.358	1			
ATP	0.758**	0.064	1		
EMC-INT	0.310	0.983***	0.183	1	
^{14}C MPN	0.389	0.572**	0.222	0.579**	1
DOC	0.011	0.457	−0.243	0.561*	0.262
Phenolics	0.076	0.546*	0.017	0.591**	0.513*
Monosaccharides	0.265	0.501	−0.075	0.550*	−0.012
DON	0.019	0.519	−0.502	0.592*	0.167
Sediment organic matter	0.665**	0.572*	0.630*	0.614*	0.516
Sediment particle size	−0.469*	−0.687**	−0.297	−0.706***	−0.455
Fe	0.081	0.576**	−0.027	0.682**	0.399
Mn	0.041	0.689**	−0.215	0.761***	0.401
Na	0.363	0.317	0.782**	0.433	0.558*
Chloride	0.207	0.585**	0.904***	0.632**	0.536*

[a] Statistical significance was as follows: $\alpha = 0.05$ (*), $\alpha = 0.01$ (**), and $\alpha = 0.001$ (***). No statistically significant correlations between biomass measures and the following: temperature, total carbohydrates, polysaccharides, NH_4 N, NO_3 N, amino acids, total alkalinity, sulfate, silicate, Mg, Ca, or K.

estimates were higher during A, but differences between seasons were not significant at WEST.

In contrast to these findings for total biomass, EMC-INT and ^{14}C-MPN-based estimates of viable biomass never differed significantly (paired sample t test, $\alpha \leq 0.05$ [Table 3]). ATP-based biomass estimates were obtained only for the last four sampling periods. They were usually higher than the corresponding estimates from the EMC-INT and ^{14}C MPN assays but were significantly different from both of the other methods only at WCC and from the EMC-INT-based estimates at SMS (Scheffe MRT, $\alpha = 0.05$ [Table 3]).

Overall estimates of viable biomass in SMS and WCC did not differ by any procedure (Table 3). However, seasonal estimates from ATP were significantly higher in SMS than in WCC or WEST, except in A (Fig. 2). Corresponding EMC-INT- and ^{14}C MPN-based estimates were also higher in SMS than in WCC, although only the EMC-INT estimates during W and the SU_2 were significantly different. Differences between SMS and WEST, however, were not significant.

ATP-based biomass estimates did not differ significantly with season at any site, and ^{14}C MPN-based estimates differed only in WCC. Significant seasonal differences in EMC-INT-based estimates occurred at each site but WEST. However, the only difference corroborated by more than one method occurred in WCC, in which viable biomass during SU_1 was significantly higher than during S and SU_2.

Biomass estimates from lipid phosphate were correlated with those from ATP, and estimates from ^{14}C MPN assays were correlated with those from epifluorescence counts which were also intercorrelated (Table 5). The greatest

TABLE 6. The first five variables included in MLR analysis of bacterial biomass with either DOC or DOC constituents and physical and inorganic chemical parameters, and their relative importance in the models[a]

MLR analysis	Parameters included in MLR equations for indicated dependent variable								
	EMC			EMC-INT			^{14}C MPN		
	Variable	Relative β	r^2 Contribution	Variable	Relative β	r^2 Contribution	Variable	Relative β	r^2 Contribution
With DOC	Temp	0.10	—[b]	Temp	0.20	—	Temp	0.53	0.178
	DOC	0.47	0.037	DOC	0.79	0.054	DOC	0.29	0.021
	SRP	0.66	0.033	SRP	0.74	0.042	NO_3	0.36	0.018
	SO_4	0.61	0.062	SO_4	0.63	0.080	SO_4	0.19	0.032
	SPS	1.00	0.472	SPS	1.00	0.498	S%O	1.00	0.482
Model r^2	0.606		0.604	0.679		0.674	0.731		0.731
df	16		16	16		16	16		16
With DOC Constituents	Temp	0.27	0.043	Temp	0.32	0.042	Phenolics	1.00	0.263
	Phenolics	1.00	0.298	Phenolics	0.57	0.373	AA	0.60	0.038
	NO_3	0.31	—	Monosac	1.00	0.252	NO_3	0.73	0.055
	NH_4	0.09	—	SRP	0.42	0.029	NH_4	0.32	—
	SO_4	0.08	—	SO_4	0.32	0.010	SRP	0.31	—
Model r^2	0.353		0.341	0.706		0.706	0.383		0.356
df	16		16	13		13	16		16

[a] Determined by the ratio of the standardized partial regression coefficient, β to the highest β value. Abbreviations: AA, amino acids; Monosac, monosaccharides; S%O, sediment percent organic matter; SPS, mean sediment particle size; and df, degrees of freedom.

[b] —, Negligible r^2 contribution compared with other variables.

TABLE 7. PHB concentrations in sediments at SMS, WCC, and WEST

Site	Season	Mean PHB concn (μg/mg of dry sediment) ± SD (n = 6)	Amt (μg) of PHB/mg of C[a]
SMS	W	0.079 ± 0.020	1.241
	SU_2	0.038 ± 0.011	1.674
WCC	W	0.005 ± 0.000	0.170
	SU_2	0.002 ± 0.000	0.192
WEST	ST	0.005 ± 0.001	0.167
	SU_2	0.004 ± 0.000	0.138

[a] From EMC determinations.

number of statistically significant correlations between biomass measures and environmental variables were obtained with epifluorescence counts. Environmental factors which correlated positively with one or more of the biomass estimates ($\alpha \leq 0.05$) were concentrations of DOC, DON, monosaccharides, phenolics, Fe, Mn, Na, and Cl^- and the organic matter content of sediments. Epifluorescence counts were better correlated with DOC than were other biomass measures, and these counts usually correlated even better with constituent classes of the DOC. On the other hand, all biomass assays were correlated with sediment organic matter. Biomass was negatively correlated with the mean particle size of sediments.

MLR analyses were performed to see which variables accounted for most variance in EMC, EMC-INT, and ^{14}C MPN data. Sediment particle size accounted for most variance in the EMC and EMC-INT data and was the most important variable in these models (Table 6). Sediment organic matter was the most important variable in the ^{14}C MPN model. DOC ranked second to fourth in order of importance in these models, and some anions and temperature explained minor amounts of variance in the data. Replacing DOC with the measured DOC constituents never substantially increased the variance accounted for in the models and greatly lowered the variance explained by EMC and ^{14}C MPN models. Phenolics and monosaccharides were the DOC constituents most important in the equations.

Metabolic state. The percentage of respiring cells initially appeared to range from 35 to 46%. However, as many as 31, 22, and 32% of the cells in control samples from WCC, WEST, and SMS, respectively, had "formazan-like" deposits associated with them during one sampling period, although they were never exposed to INT. When our data were corrected for false-positives, the percentage of respiring cells ranged from 11.5 to 22.4% at SMS, 8.9 to 15.8% at WCC, 13.7 to 21.4% at HARRIS, and 12.9 to 14.8% at WEST. Thus, we conclude that more than half of the cells at the study sites were dormant or dead, and the figure was probably greater than 75%. The percentage of respiring cells showed no statistically significant correlations with biomass or other metabolic states and activity parameters. Positive correlations were noted with Mg (r = 0.480, α = 0.05) and Na (r = 0.592, α = 0.01).

PHB concentrations were from 15- to 19-fold higher in SMS than in WCC or WEST; when normalized for biomass, they were from 7- to 12-fold higher, all significant differences

TABLE 8. DOC uptake, respiration, and biosynthesis of communities exposed to algal lysate

Sampling period and site	Temp (°C)	Mean ± SD (n)[a]		
		Control-corrected DOC uptake	Control-corrected respiration	Biosynthesis
S (4/23/80–5/8/80)				
SMS	12.0–19.7	21.10 ± 21.29 (4)[b]	7.08 ± 0.74 (3)[b]	
HARRIS	12.0–21.0	29.20 ± 10.91 (4)[b]	10.47 ± 0.77 (2)[b]	
WCC	13.0–18.0	52.89 ± 6.75 (4)[b]	12.93 ± 6.89 (4)[b]	
SU_1 (7/15/80–7/31/80)				
SMS	20.0–22.5	56.66 ± 13.22 (4)[b]	18.23 ± 2.06 (4)[b]	
HARRIS	19.4–22.1	110.15 ± 9.88 (4)[b]	37.80 ± 3.87 (4)[b]	
WCC	17.0–21.0	46.77 ± 11.11 (4)[b]	11.25 ± 2.66 (4)[b]	
A (10/30/80–11/25/80)				
SMS	7.0–10.2	14.83 ± 7.36 (3)	5.67 ± 2.27 (3)	9.17 ± 7.55 (3)
HARRIS	8.0–10.0	165.50 ± 12.63 (4)	0 (4)	165.50 ± 12.63 (4)
WCC	7.9–10.7	48.32 ± 4.89 (6)	1.27 ± 1.73 (6)	47.05 ± 4.31 (6)
W (1/21/81–2/5/81)				
SMS	1.5–4.7	— (4)	0 (4)	— (4)
WCC	2.8–3.5	7.93 ± 15.86 (4)	1.59 ± 0.44 (4)	6.34 ± 15.31 (4)
ST (3/5/81–3/25/81)				
SMS	7.0–8.7	14.64 ± 0.38 (3)	1.92 ± 0.99 (3)	12.72 ± 0.62 (3)
WEST	3.3–5.1	27.82 ± 4.10 (3)	0 (3)	27.82 ± 4.10 (3)
WCC	5.3–10.3	19.18 ± 4.43 (4)	0 (4)	19.18 ± 4.43 (4)
SU_2 (7/15/81–7/31/81)				
SMS	18.3–21.9	39.70 ± 26.13 (4)	5.79 ± 3.70 (4)	33.91 ± 22.76 (4)
WEST	19.3–23.2	37.69 ± 9.23 (4)	4.54 ± 2.10 (4)	33.15 ± 10.17 (4)
WCC	18.2–22.2	52.15 ± 10.23 (4)	3.65 ± 3.02 (4)	48.50 ± 7.99 (4)

[a] All data reported as milligrams of C per square meter per hour. —, High DOC release in control microcosm; data not reported.
[b] No control correction for these periods.

TABLE 9. Correlations (r) between activity measures and between activity measures and biomass, metabolic state, and environmental parameters[a]

Activity measures and parameters	DOC uptake	Respiration	Biosynthesis	^{14}C-lipid synthesis	^{14}C-lipid synthesis/ EMC	Phospholipid synthesis	Phospholipid synthesis/ EMC
DOC uptake	1						
Respiration	0.500*	1					
Biosynthesis	0.982***	0.104	1				
^{14}C-lipid synthesis	−0.204	−0.033	−0.296	1			
^{14}C-lipid synthesis/EMC	−0.424	0.084	−0.495	0.558*	1		
Phospholipid synthesis	0.211	0.637*	0.034	0.227	0.713*	1	
Phospholipid synthesis/EMC	0.287	0.682*	0.193	0.062	0.668	0.980*	1
EMC	0.677**	0.092	0.834**	0.134	−0.496*	−0.065	−0.246
Lipid PO_4	−0.012	−0.013	−0.090	0.391	0.065	0.329	0.245
ATP	0.709***	0.313	−0.282	0.424	0.650*	0.749**	0.690*
EMC-INT	−0.097	0.180	0.852**	0.149	−0.459	−0.064	−0.244
^{14}C MPN	0.093	−0.220	0.164	0.639**	0.021	0.084	−0.103
PHB	−0.474	0.090	−0.847	0.958**	0.787	0.339	0.233
PHB/EMC	−0.120	0.447	−0.439	0.658	0.909**	0.828*	0.763
% Respiring cells	−0.077	0.213	−0.068	0.356	0.318	0.146	0.138
Temperature	0.407	0.663**	0.108	−0.291	−0.604*	0.439	0.517
DOC	0.641**	0.685**	0.754**	−0.015	−0.220	0.122	0.123
Phenolics	0.612**	0.090	0.830**	0.286	−0.091	0.293	0.196
Monosaccharides	0.605**	0.759**	0.535	−0.047	−0.291	0.276	0.266
Amino acids and peptides	0.459*	0.336	0.321	−0.135	−0.200	−0.099	−0.118
Carbohydrates	0.416	0.709**	0.287	0.076	0.052	0.058	0.125
Sediment organic matter	0.556*	0.416	0.345	0.223	−0.257	0.444	0.294
Sediment particle size	−0.513*	−0.354	0.449	−0.039	0.499*	−0.296	−0.162
NO_3	−0.612**	−0.405	−0.601	0.331	0.418	0.174	0.065
DON	0.756**	0.574*	0.717*	−0.152	−0.314	−0.211	−0.162
SO_4	0.325	−0.252	0.649*	0.338	0.222	0.372	0.238
Cl^-	0.607**	−0.023	0.846**	0.202	−0.039	0.751**	0.659*
Fe	0.618**	0.608**	0.831**	0.015	−0.270	0.358	0.360
Ca	0.069	−0.195	0.730*	0.264	0.398	0.460	0.474
K	0.383	−0.248	0.738*	0.036	0.014	−0.219	−0.196
Mn	0.818***	0.283	0.917***	0.008	−0.249	0.077	0.098
Na	0.247	0.425	0.281	0.345	0.175	0.746**	0.653*

[a] Statistical significance was as follows: $\alpha = 0.05$ (*), $\alpha = 0.01$ (**), and $\alpha = 0.001$ (***). Correlations for lipid synthesis measures are based on measures made before algal lysate exposure.

(Scheffe MRT, $\alpha = 0.05$ [Table 7]). Because data are available for only two seasons per site, correlations with other variables must be viewed cautiously. PHB was significantly correlated (r) at $\alpha = 0.001$ with lipid phosphate (0.998) and ^{14}C-lipid synthesis (0.958) and at $\alpha = 0.01$ with EMC-INT (0.862). PHB per EMC-carbon was correlated (r) at $\alpha = 0.001$ with ATP (0.992), at $\alpha = 0.01$ with ^{14}C-lipid synthesis per cell (0.909), percent organic matter of sediment (0.966), Na (0.985), and Cl^- (0.965), and at $\alpha = 0.05$ with lipid phosphate (0.822), Ca (0.861), and phospholipid synthesis (0.828). Seasonal differences in PHB/EMC ratios were also determined for each site and regressed against the ratios of DOC to the following: DON, SRP, K, Cl^-, and the sum of NO_3, NH_4, and SRP. Strongest correlations were obtained with the ratios of DOC/DON and DOC/K ($r = 0.830$ and −0.870, respectively).

Adenylate energy charge values were obtained only for WCC during A, W, and S. Data were low and constant, equalling 0.22 ± 0.10 ($n = 12$), 0.32 ± 0.15 ($n = 12$), and 0.28 ± 0.08 ($n = 6$), respectively ($\bar{x} \pm$ standard deviation).

Bacterial activity. Control-corrected uptake rates of algal lysate DOC varied ca. 20-fold (Table 8). DOC utilization was significantly higher in HARRIS than elsewhere during SU_1 and A and in WEST during ST (Scheffe MRT, $\alpha = 0.05$) and usually lowest in SMS. DOC uptake rates at each site varied seasonally less than 10-fold, with high values usually during SU_2 and A, but significant differences occurred only in WCC (A, SU_2 > W, ST; Scheffe MRT, $\alpha = 0.05$). Uptake rates (uncorrected for controls) did not differ significantly between summers in either SMS or WCC (t test, $\alpha = 0.05$). DOC concentrations at these sites did not differ significantly between summers, although biomass was greater during SU_1 than SU_2 in WCC.

While DOC uptake rates tended to be greatest at warmer temperatures, the correlation with temperature was not significant (Table 9). Positive correlations of uptake with DOC concentration and some biomass measures were obtained. Significant positive correlations also occurred with concentrations of phenolics, monosaccharides, amino acids plus peptides, DON, Cl^-, Fe, and Mn as well as the organic content of sediments. DOC uptake was negatively correlated with NO_3 and the mean particle size of sediments.

Respiration rates of lysate-exposed communities varied approximately ninefold (Table 8). However, only a small percentage of community respiration could be attributed to the metabolism of algal lysate. Respiration rates of controls were greater than 90% of the rate of lysate-exposed communities in nearly half of the 44 measures made (and in a few instances exceeded 100%) and were less than 50% of the rate of lysate-exposed communities only 4 times.

TABLE 10. ^{14}C-lipid synthesis by sediment microbes before and after exposure to algal lysate or site water (controls)

Sampling period and site	Mean ^{14}C-lipid synthesis ± SD[a]			dpm of ^{14}C-/μg of EMC-C per h before lysate exposure
	Before lysate exposure (n = 12)[b]	After lysate exposure (n = 9)	Control (n = 3)[c]	
S (4/23/80–5/8/80)				
SMS	114 ± 56	222 ± 110**		898
HARRIS	322 ± 151	421 ± 260		1,586
WCC	67 ± 38	109 ± 76		3,738
SU_1 (7/15/80–7/31/80)				
SMS	219 ± 109	193 ± 59		4,297
HARRIS	203 ± 83	475 ± 140***		1,086
WCC	263 ± 147	313 ± 115		940
A (10/30/80–11/25/80)				
SMS	674 ± 312	136 ± 69**	231 ± 146	5,349
HARRIS	258 ± 47	150 ± 64***		729
WCC	65 ± 24	90 ± 59		1,512
W (1/21/81–2/5/81)				
SMS	872 ± 327	683 ± 366	165 ± 121	13,625
WCC	93 ± 30	55 ± 28**	53 ± 4	2,906
ST (3/5/81–3/25/81)				
SMS	146 ± 48	113 ± 54	144 ± 14	4,563
WEST	271 ± 68	169 ± 42***	276 ± 77*	8,742
WCC	91 ± 20	31 ± 16***	20 ± 4*	3,956
SU_2 (7/15/81–7/30/81)				
SMS	344 ± 240	407 ± 97	454 ± 297	14,957
WEST	182 ± 34	242 ± 53	257 ± 100	6,500
WCC	73 ± 21	114 ± 38	54 ± 1*	6,083

[a] Data reported as disintegrations per minute (dpm) per milligram (dry weight) per hour. Statistical significance was as follows: t test at $\alpha = 0.05$ (*), $\alpha = 0.01$ (**), and $\alpha = 0.001$ (***). Comparison on data obtained before and after lysate exposure.

[b] Between-site differences (Scheffe MRT, $\alpha = 0.05$) were as follows: S (H > SMS, WCC), SU_1 (no significant difference), A (SMS > H, WCC), W (SMS > WCC), ST (WE > SMS > WCC), and SU_2 (SMS > WE, WCC).

[c] Statistical significance (t test at $\alpha = 0.05$, 0.01, and 0.001) between control and lysate exposed communities.

Respiration rates (control corrected) ranged from 0 to 6 mg of $C \cdot m^{-2} \cdot h^{-1}$. Significant differences between sites occurred during A (SMS > HARRIS, WCC), W (WCC > SMS), and ST (SMS > WCC, WEST), but not SU_2 (Scheffe MRT; t test, $\alpha = 0.05$). The uncorrected respiration rates differed during SU_1 (HARRIS > SMS, WCC) but not S. Uncorrected respiration rates were significantly positively correlated with DOC uptake, phospholipid synthesis, temperature, and concentrations of DOC, DON, total carbohydrates, monosaccharides, and Fe (Table 9).

Bacterial biosynthesis resulting from algal lysate utilization ranged from undetectable levels to 166 mg of $C \cdot m^{-2} \cdot h^{-1}$, but most values were between 8 and 50 mg of $C \cdot m^{-2} \cdot h^{-1}$ (Table 8). Between sites, significant differences (Scheffe MRT, $\alpha = 0.05$) occurred during A (HARRIS > WCC > SMS) and ST (WEST > WCC, SMS). Biosynthesis on algal lysate was greater in WCC than elsewhere at other times, but not with statistical significance.

Seasonal differences in biosynthesis were not significant in SMS, but in WCC, productivity during ST and W was less than during A and SU_2 (Scheffe MRT, $\alpha = 0.05$). Biosynthesis was highly correlated with some biomass measures and concentrations of DOC, DON, phenolics, SO_4, Cl^-, Fe, Mn, Ca, and K (Table 9).

Measures of lipid synthesis were also used to assess microbial activity at the field sites and responses to algal lysate. ^{14}C-lipid synthesis before lysate exposure was nearly always significantly lower in WCC than in one or more of the other sites and was highest in SMS for half of the sampling periods (Table 10). Likewise, phospholipid synthesis was always lowest in WCC and highest in SMS, with significant differences at all seasons (Table 11).

Between seasons, ^{14}C-lipid synthesis before lysate exposure was significantly greater in WCC during SU_1 than at other times (Scheffe MRT, $\alpha = 0.05$ [Table 10]). On the other hand, in SMS ^{14}C-lipid synthesis was greater in A and W than during the other periods. Phospholipid synthesis before exposure was significantly greater during SU_2 than other periods in both WCC and SMS, and in WCC the activity during ST was also significantly greater than during A (Scheffe MRT, $\alpha = 0.05$ [Table 11]). Cell-specific rates of lipid synthesis were greatest during SU_2 in both SMS and WCC, although ^{14}C-lipid synthesis was nearly as great during W in SMS (Tables 10 and 11). Cell-specific rates showed less seasonal variation elsewhere.

Algal lysate exposure significantly depressed phospholipid synthesis rates of the SMS community during A but elevated rates for communities from WEST and WCC during SU_2 (Table 11). At other times, rates either did not change significantly or controls changed similarly, so that the response could not be attributed to algal lysate exposure. Algal lysate exposure decreased ^{14}C-lipid synthesis significantly only once (ST at WEST [Table 10]). While the trend was for ^{14}C-lipid synthesis to increase on lysate exposure during the

TABLE 11. Phospholipid synthesis by sediment microbes before and after exposure to algal lysate or site water (controls)

Sampling period and site	Mean phospholipid synthesis ± SD[a]			Amt (pmol)/mg of EMC-C per h before lysate exposure
	Before lysate exposure (n = 12)[b]	After lysate exposure (n = 9)	Control (n = 3)[c]	
A (10/30/80–11/6/80)				
SMS	0.069 ± 0.037	0.020 ± 0.020*	0.163 ± 0.194*	0.548
WCC	0.006 ± 0.004	0.012 ± 0.008*		0.139
W (1/21/81–2/15/81)				
SMS	0.056 ± 0.028	0.040 ± 0.037	0.013 ± 0.004	0.875
WCC	0.009 ± 0.004	0.008 ± 0.008	0.007 ± 0.004	0.281
ST (3/5/81–3/25/81)				
SMS	0.059 ± 0.027	0.038 ± 0.014*	0.047 ± 0.010	1.844
WEST	0.029 ± 0.015	0.018 ± 0.004	0.063 ± 0.030**	0.935
WCC	0.015 ± 0.005	0.013 ± 0.008	0.014 ± 0.001	0.652
SU_2 (7/14/81–7/30/81)				
SMS	0.354 ± 0.315	1.012 ± 0.333***	1.018 ± 0.768	15.826
WEST	0.034 ± 0.008	0.317 ± 0.078***	0.168 ± 0.050**	1.274
WCC	0.024 ± 0.008	0.196 ± 0.037***	0.091 ± 0.016***	2.000

[a] Data reported as picomoles per milligram (dry weight) per hour. Statistical significance was as follows: t test at $\alpha = 0.05$ (*), $\alpha = 0.01$ (**), and $\alpha = 0.001$ (***). Comparison of data obtained before and after lysate exposure.

[b] Between-site differences (Scheffe MRT, $\alpha = 0.05$) were as follows: A (SMS > WCC), W (SMS > WCC), ST (SMS > WE, WCC), and SU_2 (SMS > WE, WCC).

[c] Statistical significance (t test at $\alpha = 0.05$, 0.01, and 0.001) between control and lysate exposed communities.

S and both summers and decrease at other times, again the controls often changed similarly.

Phospholipid synthesis was weakly but significantly correlated with ^{14}C-lipid synthesis per EMC and respiration, but other correlations between lipid synthesis assays and other activity measures were not significant (Table 9). Lipid synthesis measures were significantly correlated with two biomass measures (^{14}C MPN and ATP), and ^{14}C-lipid synthesis was highly correlated with PHB. Except for the correlations of phospholipid synthesis with concentrations of Cl^- and Na, there were only two other significant correlations of lipid synthesis assays with environmental variables (^{14}C-lipid synthesis per EMC-C with temperature and sediment particle size).

MLR analyses showed that sediment particle size was relatively unimportant in models of lipid synthesis, in contrast to the results of MLR analyses of biomass (Table 12). The variables explaining most variance in the lipid synthesis data or having highest β values were an estimation of biomass, NO_3, DOC, and temperature. Replacement of DOC with the constituents of the DOC pool resulted in a slight improvement of the models, and the amino acid or DON and carbohydrate terms were the components with greatest importance in the models.

TABLE 12. The first five variables included in MLR analysis of lipid synthesis with either DOC or DOC constituents, physical and inorganic chemical parameters, and biomass as independent variables and their relative importance in the models[a]

MLR analysis	Parameters included in MLR equations for indicated dependent variable					
	^{14}C-lipid synthesis			Phospholipid synthesis		
	Variable	Relative β	r^2 Contribution	Variable	Relative β	r^2 Contribution
With DOC	Temp	0.32	0.089	Temp	0.51	0.192
	^{14}CMPN	1.00	0.418	EMC-INT	0.49	0.133
	DOC	0.47	0.087	DOC	0.66	0.258
	NO_3	0.70	0.175	NO_3	1.00	0.168
	S%O	0.68	0.044	SPS	0.24	0.100
Model r^2	0.813		0.813	0.851		0.851
df	16		16	9		9
With DOC constituents						
	^{14}CMPN	1.00	0.528	Temp	0.62	0.192
	CHO (BS)	0.83	0.052	CHO (BS)	0.93	0.481
	AA	0.92	0.169	SRP	0.14	0.085
	NO_3	0.42	0.118	DON	1.00	0.063
	SRP	0.44	0.108	SPS	0.14	0.120
Model r^2	0.975		0.975	0.941		0.941
df	10		10	9		9

[a] Determined as described in Table 6, footnote a. Abbreviations: CHO (BS) = Carbohydrates determined as described previously (5). Other abbreviations are defined in Table 6, footnote a, or text.

TABLE 13. Seasonal bacterial carbon turnover in sediments at SMS, WCC, and WEST

Site and sampling period	EMC-based bacterial standing crop[a]	Production of C from phospholipid synthesis[b]	Generation time (days)	Generations/season
SMS				
A (October-November 1980)	126	0.694	7.6	12.0
W (January-February 1981)	63	0.560	4.7	19.4
ST (March 1981)	32	0.590	2.3	39.6
SU_2 (July 1981)	23	3.540	0.3	304.2
WCC				
A (October-November 1980)	43	0.061	29.4	3.1
W (January-February 1981)	32	0.090	14.8	6.2
ST (March 1981)	23	0.150	6.4	14.3
SU_2 (July 1981)	12	0.240	2.1	43.5
WEST				
ST (March 1981)	31	0.290	4.5	20.3
SU_2 (July 1981)	28	0.340	3.4	26.8

[a] Data reported as nanograms of C per milligram of dry sediment.
[b] Data reported as nanograms of C per milligram of dry sediment per hour.

Estimates of bacterial productivity were derived from data for phospholipid synthesis on streamwater before lysate exposure (Table 13). EMC-based estimates of bacterial standing crops were used to calculate generation times which varied seasonally from 2 to 29 days and from 0.3 to 8 days at WCC and SMS, respectively. Only limited data were obtained at WEST, but the generation time was intermediate compared with SMS or WCC during ST and greater than at these other sites during SU_2. Based on phospholipid synthesis, the number of generations produced per year totaled 375 at SMS and 67 at WCC.

DISCUSSION

These studies underscore the influence of multiple environmental factors on bacterial communities in streambed sediments. Biomass was related to sediment properties, DOC concentrations, and several inorganic parameters. MLR analyses implied that bacterial biomass may be related more strongly to sediment properties than to DOC concentrations which ranked second to fourth in importance in the models. Detailed manipulative experimentation is needed to further substantiate this point. However, in one approach, we transferred SMS sediments from a location in the spring seep in which DOC concentrations averaged 1.8 mg/liter to the groundwater source in which the concentrations averaged 0.6 mg/liter and found that streamwater DOC supported approximately half of the bacterial biomass in surface sediments (3). Correlations of biomass estimators with selected DOC constituents, e.g., phenolics or monosaccharides, imply the influence of qualitative differences in DOM on bacterial biomass.

Complex environmental controls on bacterial activity were also demonstrated. Many activity measures were positively correlated with bacterial standing crops as expected. However, DOC uptake, respiration, and biosynthesis showed stronger correlations with environmental variables than did the lipid synthesis assays. MLR analyses of lipid synthesis suggested that sediment properties may have less effect on bacterial activity than on bacterial biomass, since other environmental parameters were more strongly related to lipid synthesis than to bacterial biomass. The lipid synthesis models improved slightly when DOC constituents were substituted for the DOC term, whereas the same substitution reduced the variance explained by two of the three biomass models. We infer that qualitative differences in DOM probably affect microbial activity more directly than biomass.

Estimates of total bacterial numbers at our sites ranged from 9.4×10^8 to 2.35×10^{10} cells per g of dry sediment, data which are in the range of reported cell densities in intertidal, beach, and marsh sediments (1.2×10^8 to 10.0×10^{10} cells per g [dry weight]; 11, 44, 53) and pond and lake sediments (2.1×10^{10} to 12.6×10^{10} cells per g [dry weight]; 24, 29). Most of the bacteria at our sites were coccoid rods, and our reported biomass values are based on a mean measured cell volume of 0.13 μm^3. This cell volume is close to the mean value of 0.2 μm^3 reported for both river (17) and salt marsh sediment bacteria (52), although a wide range of sizes were encountered in our work and these other studies.

Nearly all of the cells in our samples were attached to particles, and our EMC were corrected for cells on unviewed surfaces of particles. After finding that the sonication and centrifugation procedure was 95% effective in recovering cells from sediments for epifluorescence microscopic enumeration, we have used it routinely for the enumeration of sediment bacteria.

The negative correlation with mean sediment particle size is consistent with other reports of greater bacterial biomass in finer grained sediments (11, 20) which present greater surface area per unit mass than do larger grained sediments. The positive correlations of most biomass assays with sediment organic matter suggests the potential importance of detritus as a colonization site or source of nutrients. Cammen (6), in relating bacterial abundances to size fractions within sediments, suggested that organic content may be more important than particle size in determining bacterial abundance. Geesey and Costerton (17) found from scanning electron microscope observations that bacteria suspended in the Athabasca River were concentrated in the crevices of detritus and were absent from the planer surfaces of silt particles. Particle size and organic content were correlated in our study ($r = -0.778$, $\alpha = 0.001$) so that discrimination between the separate effects of these factors on biomass is impossible, but our direct microscope observations have shown attachment to both organic and inorganic surfaces.

Lipid phosphate has been shown to be a useful indicator of

bacterial biomass since phospholipids are major constituents of procaryotic cell membranes (66) and since the assay is relatively simple to perform. The lipid phosphate-based biomass estimates presented here exceeded EMC-based estimates by factors ranging from 6 to 31. The discrepancy is presumably attributable to the presence of lipid phosphate in organisms other than bacteria and in detritus; the greatest difference existed at SMS, in which leaf litter detritus was greatest. This suggests a potential problem in using the lipid phosphate assay as an indicator of bacterial biomass in natural samples unless checks for contributions from other sources are made.

Total bacterial biomass estimates from muramic acid done by gas chromatography were obtained for only a few season-site combinations. Greater use of the assay was prevented by interference from other organic compounds in the sediments, the presence of at least two apparent derivitization products, low recovery, and time constraints. For muramic acid to serve as a measure of biomass, it is necessary that the compound not remain long in the environment after cell death and lysis or fragmentation. Assuming that the conversion factors employed were appropriate, our data suggest the opposite, since estimates from muramic acid always exceeded estimates based on lipid phosphate and EMC. There is little supportive information, but it has recently been shown that both muramic acid and α,ε-diaminopimelic acid in soil may be bound extracellularly, particularly to humic acids (13).

Bacterial carbon as a percentage of total sediment carbon was lower at SMS (0.32%) than elsewhere (0.68% at both HARRIS and WEST and 0.72% at WCC). Bacteria contributed less than 2% of the organic matter in a marine sediment (6), but our percentages are quite low and more like the 0.6% reported for salt marsh surface sediments (52).

ATP provides a measure of total viable community biomass, and carbon estimates from ATP were usually greater than estimates from the ^{14}C MPN and EMC-INT assays which measured only the viable bacterial component. However, we found significant differences between these three methods only at WCC. One reason for this may be that the ^{14}C MPN technique was replicated fewer times, thereby generating estimates with large variance terms. The positive correlations among EMC, EMC-INT, and ^{14}C MPN based estimates are not unexpected, because the microscopic counts were obtained on the same samples and the same constants for size, density, and organic content of cells were applied in all of these approaches. The correlation we observed between lipid phosphate- and ATP-based biomass estimates also was found in estuarine sandy sediments by White et al. (66).

Most of the bacteria at our study sites were inactive; less than 25% were actively respiring. This finding is supported by ongoing work in which both INT reduction and autoradiography reveal low numbers of active cells in WCC. Others have also reported low percentages of active cells in freshwaters (7 to 13% [42] and 5 to 36% [69]) and a wide range for estuarine and marine environments (4 to 61% [35, 59, 61, 69]), but ours are the first such data available for freshwater sediment communities. The condition of inactive cells has not been established. They may be either dead or dormant. Stevenson (58) has suggested that most bacteria in nature are present in a condition of exogenous dormancy.

In studies of INT reduction, the need for blanks to control for the presence of spots that could be interpreted microscopically as formazan crystals has not been generally appreciated, although Newell (47) found false-positive cells associated with floc, but not among bacteria freely suspended in the water column. On the basis of our experience, blanks are imperative for work in sediments. We found that a mounting medium of 30% glycerol instead of Cargille immersion oil reduced the problem of false-positives and that Gelman membrane filters had less background than did Nuclepore filters.

PHB, a storage product formed by some bacteria, accumulates under stressful conditions such as unbalanced growth when an essential nutrient (O_2, N, S, or K) is limiting in the presence of sufficient carbon and energy resources, or exposure to chelating agents, dissolved humic substances, increased salinity (48), anoxia (55), or physical disturbance (15). With our small sample size and lack of experimental manipulations, we cannot establish causative factors. Apart from floristic differences which were beyond the scope of this study, the higher values for SMS were correlated most strongly to the higher DOC/DON ratio of the DOM, to higher concentrations of inorganic ions or sediment organic matter in SMS than in WCC or WEST, or to a combination of these variables. Additional MLR analyses, not detailed here because of the small sample size, suggested that sediment organic matter, Mg, and phenolic concentrations were most closely related to PHB/cell ratios and that Ca, the ratio between monosaccharides and polysaccharides, and K explained most variance in the PHB data. These parameters may be useful to monitor in future studies of PHB formation. Low K concentrations have been implicated in the formation of PHB rather than endotoxin in a study of *Bacillus thuringiensis* mutants (62).

As we began these studies, we were hopeful that EC_A ratios could be used to measure bacterial metabolic status in these habitats. EC_A has been shown to range from 0.8 in rapidly growing cells to 0.5 in senescent cells (7). Our experience with this assay is described in detail in Kaplan and Bott (32). The low EC_A values we found for field populations were possibly the result of extracellular AMP, the existence of which we have documented in a culture study with cells from WCC. We have not been able to isolate extracellular AMP from the interstitial water or surfaces of WCC sediments, but it may be bound with sediment constituents or microbial extracellular products. Other conditions that would yield low EC_A values are the presence of large numbers of dead or dormant cells, compared with the numbers of active cells, and the asynchronous growth of populations comprising natural communities.

During the first two sampling periods, control activity measures were made on each chamber overnight just before lysate exposure, but cooler nighttime temperatures made the application of these rates questionable. Measures of the uptake of algal lysate DOC and respiration during the last four sampling periods were corrected for measures made simultaneously in control microcosms. In all but two of these controls (SMS and HARRIS in A), DOC increased during the 5-h exposure. Abiotic removal of DOC was at most 7% of changes measured with natural sediments, indicating that biotic utilization was primarily responsible for changes in DOC, as others have noted in work cited above.

DOC uptake was greater at warmer temperatures and at sites where DOC concentrations and sometimes biomass were greatest. However, the correlation matrix suggests interacting (or overriding) effects of DOC concentration and biomass with temperature on DOC uptake. Qualitative differences in DOC at the sites undoubtedly influenced DOC uptake rates as well. For example, although DOC concentrations did not differ seasonally at WCC, a bloom of

Ulothrix zonata (Weber and Mohr) Kutz occurred in early spring, and prior exposure to algal DOC may have conditioned the cells for the use of algal lysate at that time. Growths of wetland plants in SMS in the summer may have had a similar stimulatory effect on the community there. Data for S, which showed greatest activity at WCC, may be anomalous because sediments from SMS and HARRIS were disturbed in the respirometers, possibly obscuring true values.

The increases in respiration anticipated as a response to the threefold increase in DOC concentration on algal lysate exposure did not occur. Höfle (25) recently reported similar findings for glucose-limited cells grown in chemostats. An increase in glucose concentration of 2 to 3 orders of magnitude increased the growth rate with no concomitant increase in respiration rate. The small difference between lysate-exposed and control communities could also indicate that only a small portion of the community responded to algal lysate addition.

The high respiration rates for our control communities also suggest the following: (i) a high maintenance respiration for these presumably slow-growing natural communities and (ii) that energy sources other than algal lysate were being respired. High maintenance respiration rates may be a response to pulsed additions of nutrients to streams, which are usually related to storms. Laboratory studies with organisms grown at slow rates in chemostats showed that discontinuous glucose inputs resulted in increased maintenance respiration compared with companion cultures given a continuous supply (60). Sediment-associated detritus and DOC in interstitial and site water could serve as alternative energy sources for the communities.

The biosynthesis data reported here are in general agreement with other data for WCC populations exposed to extracts of jewel weed (*Impatiens capensis* L.) and spice bush (*Lindera benzoin* (L.) Blume; 31). Our low productivity estimates are consistent with our general inability to detect differences in biomass before and after 5-h incubations with algal lysate, since the increase expected would amount to on the average only 11.35 ± 11.60% ($\bar{x}$ ± standard deviation; n = 11) of the initial standing crop. We are confident that sampling to a depth of 1 cm did not obscure biomass changes occurring in the upper 0 to 3 mm of sediment, since exposure to forest leaf litter leachate for 7.5 h in a manner analogous to the algal lysate exposures did not result in the enrichment of biomass and activity in the surface (0- to 3-mm) sediments.

Both measures of lipid synthesis before lysate exposure indicated greater activity in SMS than in WCC (by factors averaging 4.8 for ^{14}C-lipid synthesis and 9.1 for phospholipid synthesis). Overall, the lipid synthesis measures were only weakly correlated with each other. This is not surprising, since ^{14}C-lipid synthesis measures both procaryotic and eucaryotic cell activity and may be affected by storage product formation, whereas phospholipid synthesis is biased toward bacterial activity because of the higher phospholipid content of procaryotic cell membranes and more rapid rates of procaryotic activity (65). Acetate concentrations at the study sites were not determined, and thus our ^{14}C-lipid synthesis assays yielded relative activity measures. We found that ^{14}C-lipid synthesis and PHB were correlated as were these parameters when normalized for biomass. Acetate is a precursor of PHB, and PHB would have been present in the lipid extracts. Thus, at some seasons it is likely that [^{14}C]acetate incorporation was significantly influenced by PHB synthesis.

Lipid synthesis measures were only weakly correlated with biosynthesis estimates overall, although when activity was high in the autumn or summer, the estimates of bacterial C synthesis usually agreed by a factor of 2 or less. Although we anticipated some correspondence between these measures, biosynthesis would not be related to membrane synthesis if, for example, large amounts of storage product were formed. Biosynthesis on algal lysate was always greater for WCC communities than for SMS communities, suggesting that WCC communities were perhaps better adapted for the use of algal lysate than those in SMS. Although algae were present in all study sites, they undoubtedly provided a greater proportion of the energy budget in WCC than in SMS (which was heavily shaded), perhaps priming the communities in WCC for use of lysate. We have demonstrated that the communities in these sites have preferences for DOC sources like those generated in situ or on the surrounding watersheds (L. A. Kaplan and T. L. Bott, Freshwater Biol., in press). Phospholipid synthesis on site waters was always greater in SMS than WCC, which may be related to the greater organic matter content of the sediments in SMS, differences in the DOC in interstitial waters, or unmeasured qualitative differences in the DOC in site water.

The phospholipid synthesis rates of communities exposed to site water were used to estimate annual bacterial production at SMS and WCC, since they have been shown to parallel bacterial growth rates in laboratory experiments (67). Our communities, with 375 and 67 generations annually, are more active than the estuarine community studied by Gerlach (18) which had 21 generations per year. The turnover values in Table 13 incorporate EMC determinations for standing crop estimates. If viable biomass data (averaged ^{14}C MPN and EMC-INT data) were used instead, then the number of generations per year would increase by a factor of 1.5 at SMS and 2.3 at WCC, with the community at SMS remaining the more active. Although many factors may be related to this finding, we suspect that the overriding influence is the stable discharge in SMS, in contrast to periodic storm flows in WCC which disrupt sediment communities.

With the average weight of a core from WCC (631 mg) and a core surface area of 0.283 cm^2, phospholipid synthesis rates translate to a bacterial production rate of 26.4 g of C · m^{-2} · year^{-1} in WCC. This can be compared with estimates of benthic algal primary productivity in WCC, based on 3 years of measurement. Net algal primary productivity was 66 g of C · m^{-2} · year^{-1} in a wooded reach and 305 g of C · m^{-2} · year^{-1} in a meadow reach, yielding an average of 185.5 g of C · m^{-2} · year^{-1} (T. L. Bott, unpublished data). Our bacterial assays were performed only on silt and sand substrates, whereas algal measures include all substrate types. With that caution in mind, we estimate that annual bacterial productivity is only 8.7 and 14.2% of annual algal productivity in a meadow reach and in the stream as a whole, respectively, but that it could be as much as 40% of the annual algal productivity occurring in a wooded reach. Thus, benthic bacterial productivity in some stream habitats may represent a significant source of energy in the food web. Studies of the utilization of this resource are in progress.

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