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Factors Influencing the Abundance and Metabolic Capacities of Microorganisms in Eastern Coastal Plain Sediments

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Abstract. The abundance and metabolic capacities of microorganisms residing in 49 sediment samples from 4 boreholes in Atlantic Coastal Plain sediments were examined. Radiolabeled time-course experiments assessing in situ mirobial capacities were initiated within 30 min of core recovery. Acetate ($1\text{-}^{14}C$- and ^{3}H-) incorporation into lipids, microbial colony forming units, and nutrient limitations were examined in aliquots of subsurface sediments. Water-saturated sands exhibited activity and numbers of viable microorganisms that were orders of magnitude greater than those of the low permeability dense clays. Increased radioisotope utilization rates were observed after 6–24-h incubation times when sediments were amended with additional water and/or nutrients. Supplements of water, phosphate, nitrate, sulfate, glucose, or minerals resulted in the stimulation of microbial activities, as evidenced by the rate of acetate incorporation into microbial lipids. Additions of water or phosphate resulted in the greatest stimulation of microbial activities. Regardless of depth, sediments that contained >20% clay particles exhibited lower activities and biomass densities, and greater stimulation with abundant water supplementation than did sediments containing >66% sands and hydraulic conductivities > 200 μm sec.$^{-1}$.

Introduction

Diverse microbial communities are often present in subsurface environments. Microbial trophic groups commonly observed in subsurface sediments include aerobic

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heterotrophs [1, 6, 8, 10, 13, 19, 39], methanotrophs [15, 31], nitrifiers and denitrifiers [9, 11, 13, 19], fungi or protozoa [2, 34], anaerobic heterotrophs [17, 18, 21, 30, 36], sulfur, or metal reducers [17, 18, 20, 35, 36], and methanogens [17, 18, 35, 36]. The microorganisms residing in subsurface sediments can impact groundwater quality by producing carbon dioxide [4, 5, 25]; reducing nitrogen, sulfur, and iron species [20, 25]; producing methane [3, 36]; or excreting volatile fatty acids [24]. Several studies have reported evidence of subsurface microbial activities by demonstrating radioisotope transformations corresponding to rates greater than micromolar quantities per day in test-tube experiments [16, 21, 22, 37]. In contrast, geochemical evidence suggests that in situ biological transformations may often occur at rates below micromolar quantities per year [4, 24, 25]. Discrepancies observed between biological and geochemical estimates of in situ microbial activities may result from stimulation caused by experimental procedures or disturbance artifacts [7]. Elucidation of factors that constrain the activities of subsurface microbes may enable better bioremediation monitoring or manipulative procedures for increasing in situ metabolism.

The diverse microbial communities residing in subsurface environments may prove to be a resource for the bioremediation of groundwater pollutants. Microorganisms capable of degrading a variety of contaminants including chlorinated ethenes, petroleum, and polyaromatic hydrocarbons [12, 15, 22, 23] have been enriched from subsurface environments. Little is known about the activities of microorganisms residing in subsurface sediments, the rate at which they can impact groundwater chemistry, or the physical and chemical factors limiting their metabolism and growth. Examinations of the ecology, physiological potentials, in situ carbon and electron flow, and growth and nutrient status are likely prerequisites to satisfactory bioremediation of many subsurface environments. The rates of degradative activity may be limited by intrinsic properties of the subsurface environment such as pH, Eh, temperature, and available electron donors and electron acceptors.

This study examined factors influencing the activity of subsurface microorganisms, using radioisotope time-course incorporation or transformation experiments. The effects of particle size, population size, and abundance of sulfate, nitrate, phosphate, glucose, trace minerals, and water were examined in time-course experiments to evaluate short-term stimulation resulting from sediment manipulations.

Materials and Methods

Description of Experimental Site

Four coreholes used by the U. S. Department of Energy (DOE) Subsurface Science Program were located within the Upper Atlantic Coastal Plain. The study site was on the Aiken Plateau, within and adjacent to the DOE Savannah River Site (SRS). Unconsolidated sediments of Tertiary and Cretaceous age extend to depths approaching 530 m and are underlaid by crystalline metamorphic and igneous rock. Hydrologic and geologic conditions beneath the SRS are discussed in detail elsewhere [8, 25, 32, 33]. Major confining clays of regional extent exhibiting low hydraulic conductivities are found within the Ellenton, Pee Dee, and Middendorf formations. Groundwater recharge for the formations considered in this study occurs approximately 40–100 km north and northwest. Lithological and geological descriptions of the subsurface sediments were provided under contract to DOE and SRS [25, 33]. Groundwater chemistry was provided by Pacific Northwest Laboratories (Richland, Wash.) or Oak Ridge National Laboratory (Oak Ridge, Tenn.).

Table 1. Physical and chemical properties of subsurface sediments and groundwaters[a]

Property (unit)	Formation (depth)				
	Ellenton (194 m)	Pee Dee (239 m)	Pee Dee (256 m)	Middendorf (406 m)	Cape Fear (463 m)
Particle size (%) sand/silt + clay	76/24	27/73	86/14	86/14	87/13
Moisture (%)	34	18	25	16	18
Hydraulic conductivity ($\mu m\ sec^{-1}$)	<0.1	<0.1	213	543	362
Permeability k(D)	<0.1	<0.001	22	56	38
Redox (mV)	250	307	250	370	150
TOC ($mg\ g^{-1}$)[b]	2.0	3.5[c]	0.14	0.57	0.08
DOC ($\mu g\ ml^{-1}$)[d]	6.6	NA[e]	NA	4.5	5.2
DIC ($\mu g\ ml^{-1}$)	12.3	0.4	19	0.2	18
$NO_3 + NH_4$ ($ng\ ml^{-1}$)[f]	50	20	<10	20	32
PO_4 ($ng\ ml^{-1}$)	<10	250	70	<10	<10
SO_4 ($\mu g\ ml^{-1}$)	82	1.4	31	NA	6.5
Iron ($ng\ ml^{-1}$)	14	6	20	29000	50
Sodium ($\mu g\ ml^{-1}$)	4	1	34	98	78

[a]Analyses performed by Pacific Northwest Laboratories under contract to DOE unless stated otherwise
[b]Analyses performed by Palumbo et al. [26]
[c]Results for the Pee Dee sample are from a similar lithology within the same formation. Drill logs also indicated lignaceous materials in the silt and clay samples (authors' unpublished data)
[d]Dissolved organic carbon as measured from extracted pore waters
[e]NA = not available
[f]Exclusively nitrate at 194 and 239 m. Both detected in many deep sediments. Ammonia was detected in only 1 of 15 subsurface sediments <300 m deep but in all samples below 303 m

Field Studies

The sediment cores were retrieved with conventional tools (Christensen Diamin Tools Inc., Salt Lake City, Utah) which were modified by increasing the distance from the drill bit to the sample inner barrel; this resulted in less drilling fluid contamination of the samples [29, 32]. The boreholes were continuously flushed with recirculated sodium bentonite viscosifying drilling fluid (Quick-gel, NL Baroid/NL Industries, Houston, Tex.). More detailed descriptions of sampling techniques, use of conservative drilling fluid tracers, and quality assurance are published elsewhere [29, 32]. Only those samples which exhibited more than 3 orders of magnitude reduction in the concentration of conservative drilling fluid tracers were distributed for analysis. Subsequent off-site analyses revealed that most samples exhibited more than 5 orders of magnitude reduction in conservative tracers as compared to their concentrations in drilling fluids [29, 32]. The retrieved sediments contained within the core liners were removed from the sampler and immediately carried into a mobile laboratory. Sediments were extracted and automatically entered a N_2-flushed glove bag (Coy, Ann Arbor, Mich.) for processing. Experiments were initiated within 30 min of sample recovery.

Geochemical Setting

All sediments >30 m in depth were saturated with respect to water. The sediments consisted of clays, sands, clayey silts, and clayey sands. The Ellenton silt sample at 194 m depth and the Pee Dee clay at 239 m depth (Table 1) are representative of confining layers that do not transmit large volumes of

water. The hydraulic conductivity (K), and the permeability in darcies [k(D)] were estimated based on the measured grain-size distributions of the samples. All sediments that contained >20% clays behaved as confining layers in that they exhibited low hydraulic conductivities ($K < 0.1$ μm sec^{-1}) and low permeabilities [k(D) <0.1]. Confining layers typically contained more total organic carbon (TOC) in sediments and greater concentrations of dissolved organic carbon (DOC) in extruded pore waters than did highly permeable aquifer sediments (Table 1). Aquifer sediments, particularly those that could sustain developed wells, typically exhibited hydraulic conductivities >100 μm sec^{-1} and permeabilities with k(D) >10. The dissolved oxygen content of groundwaters was generally between 0 and 5 mg $liter^{-1}$ (data not shown) [25], and the redox potential was >250 mV in shallower sediments. Deeper and downgradient formations exhibited lower redox values (463 m, Table 1) and were anaerobic [4, 20, 25]. The DOC content of extracted pore waters was <7 mg $liter^{-1}$ (Table 1), and most of the DOC likely represented hydrophobic compounds [26]. Nitrogen, sulfur, iron, and sodium were typically >10 μg $liter^{-1}$. Phosphate concentrations in pore waters extracted from sandy sediments or from pumped groundwaters were typically <10 μg $liter^{-1}$.

Gases, Chemicals, and Isotopes

Nitrogen, N_2H_2 (95:5), and N_2:CO_2 (90:10) gases were >99.9% pure. The following were purchased from New England Nuclear Corp. (Boston, Mass.): 1-^{14}C-acetate (56 mCi $mmol^{-1}$), 1,2,3-^{3}H-acetate (3.3 Ci $mmol^{-1}$), and ^{35}S-sulfate (481 mCi $mmol^{-1}$). The following were obtained from Amersham (Arlington Heights, Ill.): U-^{14}C-glucose (2.8 mCi $mmol^{-1}$) and 2-^{14}C-acetate (56 mCi $mmol^{-1}$). All chemicals used were of reagent grade and were obtained from Mallinckrodt (Paris, Ky.) or Sigma Chemical Co. (St. Louis, Mo.). Reagents and solvents were purchased from J. T. Baker Chemical Co. (Phillipsburg, Pa.) or Burdick and Jackson (Muskegon, Mich.).

Time-Course Experiments

Sediment aliquots were inoculated for aerobic and anaerobic activity experiments on-site within 30 min of core extrusion. All isotope solutions (1–50 μCi) were frozen prior to use, thawed, and transferred with gas-tight syringes (Hamilton Co., Reno, Nev.) All time-course experiments consisted of more than 5 time points and were performed in duplicate or triplicate. Sterile polypropylene centrifuge tubes (15 ml) were used to perform aerobic incorporation experiments. Anaerobic crimp-top tubes (Bellco Glass Co., Vineland, N.J.) were used for mineralization and anaerobic incorporation experiments. All incubations were at ambient temperature which varied between 21 and 25°C, which was similar to the in situ temperature of 20–25°C. Typical time points included 0, 2 h, 6 h, 12 h, 24 h, 3 days and 10 days. At each time point, sets of tubes were inhibited and frozen until analyzed in the laboratory.

Acetate incorporation experiments used 2.0 g sediment, 5.0 μCi of radiolabeled 1-^{14}C-acetate, or 1,2,3-^{3}H-acetate plus 1.0 ml sterile distilled water. At t_0 and appropriate time points, duplicate incorporation tubes were inhibited with 3.0 ml of a phosphate-buffered chloroform-methanol solution. Sulfate reduction experiments used 2.0 g sediment plus 2.0 μCi of $^{35}SO_4$ and were inhibited with 0.5 ml of 2.0 M anaerobic sodium hydroxide. Mineralization time-course experiments contained 2.0 μCi of isotopes and were inhibited with sodium hydroxide. The same design as that of the tritiated acetate incorporation experiments was used to perform nutritional studies, except that specific nutrients were added. Polypropylene centrifuge tubes contained a total of 2.0 g sediment plus 1.0 ml of sterile nanopure water and 5.0 μCi of ^{3}H-acetate. In some experiments the water content was varied among 0.25, 0.5, 1.0, and 2.0 ml. Nutritional supplements included sodium nitrate, sodium phosphate, sodium sulfate, and glucose at final concentrations of 10 and 500 μM at pH 6.8–7.0. An additional nutritional supplement was a mineral solution which contained the following final concentrations in micrograms per milliliter: $FeCl_2 \cdot 4H_2O$, 1.0; $MgCl_2 \cdot 6H_2O$, 0.5; $NaWO_4$, 0.1; $MnCl_2 \cdot 4H_2O$, 0.5; $CoCl_2$,$6H_2O$, 0.5; $CaCl_2 \cdot 2H_2O$, 0.5; $ZnCl_2$, 0.3; $CuCl_2 \cdot H_2O$, 0.01; H_3BO_3, 0.03; $Na_2MoO_4 \cdot 2H_2O$, 0.05; NaCl, 5; Na_2SeO_3, 0.08; $NiCl_2 \cdot 6H_2O$, 0.1.

Analytical Procedures

In the laboratory, the tubes from the acetate incorporation experiments were thawed and sediments extracted by the single-phase chloroform-methanol method [38]. The lipid extract was evaporated to dryness under a stream of nitrogen gas, and aliquots were prepared for scintillation counting to determine the amount of radioactivity incorporated into microbial lipids. The earliest time points yielding measurable results were used to calculate a linear rate as disintegrations per minute per day. Radioactive $^{14}CO_2$ and $^{14}CH_4$ from mineralization experiments were examined by gas chromatography-gas proportional counting [30]. A Packard 417 gas chromatograph (GC), equipped with a thermal conductivity detector, was connnected to a Packard 894 gas proportional counter. One hour before analysis, tubes were acidified with 0.5 ml of 6 M hydrochloric acid. Radioactive sulfide from sulfate reduction experiments was trapped in acidified zinc acetate and assayed by scintillation counting.

Enumeration Studies

Enumeration of microorganisms was performed with two types of media. A dilute peptone, tryptone, yeast extract, and glucose (PTYG) medium contained, in grams per liter, the following: glucose, 0.1; yeast extract, 0.1; peptone, 0.05; tryptone, 0.05; $MgSO_4 \cdot 7H_2O$, 0.6; and $CaCl_2 \cdot 2H_2O$, 0.01. The second medium contained 10 mg each of tryptone, yeast extract, peptone, and glucose with 2 mM phosphate buffer, and trace minerals. Media used for spread plate, colony forming unit (CFU) determinations also contained 20 g purified agar per liter. These media incubated at ambient temperature for one month were observed to provide the greatest recovery of microorganisms from Eastern Coastal Plain sediments [1], and in several sandy formations CFU values approached 100% of the acridine orange direct counts [34]. Five-tube, most probable number (MPN) broths using the above media were incubated aerobically and anaerobically to assess heterotrophic populations. Culturable spores were enumerated in the more concentrated heterotrophic medium after heat treating the initial slurry for 10 min at 80°C. All anaerobic media were reduced with 0.3 g cysteine-HCl liter^{-1} and contained a trace mineral solution, vitamins, and a 2.0 mM bicarbonate plus a 2.0 mM phosphate buffer. Acetogens and methanogens were quantified in a single medium containing 10 mg yeast extract and 10 mmol methanol liter^{-1} with a 95:5 (V/V) N_2H_2 headspace. Sulfate reducers were enumerated in a medium that contained 10 mg yeast extract and 10 mmol lactate liter^{-1}, plus a N_2H_2 headspace. Cultures were incubated 1 month at 20–24°C.

Results

Microbial Biomass

Silt and clay samples typically exhibited MPN or CFU values of $<10^4$ g^{-1} sediment (Table 2). In contrast, $>10^6$ g^{-1} CFU or MPN values were typical of sandy sediments. Microflora were dominated by obligately aerobic heterotrophs. Anaerobic MPNs revealed that viable microorganisms were approximately 2 orders of magnitude fewer than those of the aerobic MPN or CFU producers (Table 2). Facultative anaerobes were minor constitutents of the microbial community. Microaerophiles constituted 10–25% of the total microbial community observed in enumeration studies (data not shown).

Sediments from greater depths, which were also further removed in time and space from recharge and surface waters, typically revealed smaller microbial populations. Middendorf and Cape Fear sediments exhibited about 10% or less of the population densities of the shallower Pee Dee sediments (Table 2). Despite the lower redox of the Cape Fear sediments (Table 1), few anaerobes or facultative

Table 2. Microbiological properties of subsurface sediments[a]

	log Microorganisms per gram sediment (depth)				
	Ellenton silt (194 m)	Pee Dee clay (239 m)	Pee Dee sand (256 m)	Middendorf sand (406 m)	Cap Fear sand (463 m)
Heterotrophic (CFU)	3.8	<2	6.7	5.8	5.2
Heterotrophic (MPN)	4.0	3.0	6.5	6.0	6.3
Facultative heterotrophs	1.7	1.1	4.6	3.7	3.0
Obligate anaerobes	1.5	0.5	2.2	1.0	0.3

[a]Experimental procedures as described in text

anaerobes were detected. In general, anaerobes represented a greater fraction of the cultured biomass in confining layers (Table 2).

Use of Radioisotopes

Deeper sediments evidenced a pattern of stimulated activity with longer incubation times (Fig. 1). Aquifer sediments from the 176-m depth exhibited no significant stimulation in the rate of acetate incorporation over time, i.e., the rate was linear over time. In contrast, aquifer sediments from 178 m and 416 m revealed negligible radiolabel incorporation within the first 2 h, an increased rate after 2 h, and further increases after 8 h of incubation. Based on initial slopes, the 1-^{14}C-acetate incorporation rate was 24×10^3 dpm day^{-1} and 6×10^3 dpm day^{-1} for the 178-m and 416-m sediments, respectively, versus 155×10^3 and 140×10^3 dpm day^{-1} after the 24-h incubation. Values based on the longer incubation time represented stimulation of more than fivefold and over 20-fold, respectively. Stimulation of >100-fold was evidenced in samples from the 213-m depth between 8 and 24 h of incubation (Fig. 1).

Stimulation of microbial activities upon longer incubation times was not unique to acetate incorporation experiments (Table 3). The lowest rates of activities for six assays were noted from the Pee Dee clay sample, and the greatest activities were from the Ellenton aquifer sand sample. The rate of radiolabeled acetate incorporation into microbial lipids was below detectable limits in Ellenton silts at the 194-m depth and in Pee Dee clay sediments at the 239-m depth. Stimulation of the acetate incorporation rate was observed in the 213-m-depth Ellenton and sample. For all three sediments, increased minearlization rates were observed with aerobic and anaerobic acetate and glucose experiments when 1-day time points were compared to 3-day time points (Table 3). Increases in rates ranged from less than twofold for aerobic glucose mineralization from the 213-m-deep sediments to nearly 1000-fold for anaerobic mineralizations. Stimulation in the sulfate reduction rate was noted after incubating the 213-m-deep sediments for 3–10 days.

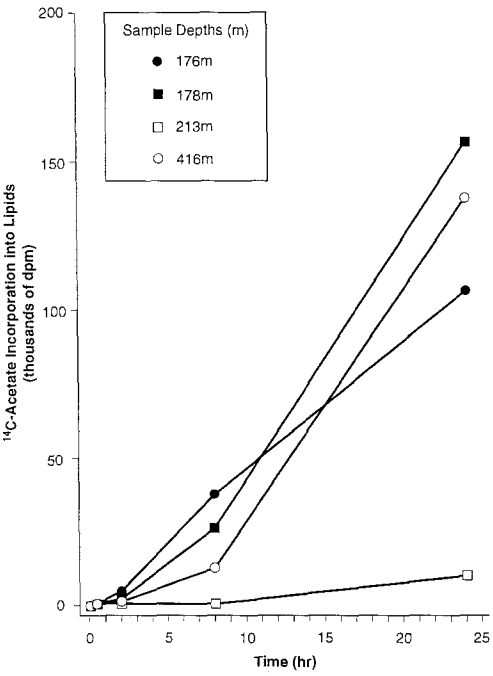

Fig. 1. Effects of experimental incubation times on estimates of subsurface microbial activities. Incorporation of 1-^{14}C-acetate into microbial lipids over time are shown for four aquifer sediment samples: (●) 176 m, (■) 178 m, (□) 213 m, and (○) 416 m.

Nutrient Amendment Studies

Table 4 shows the effects of nutrient amendments on microorganisms residing in subsurface aquifer sands. Rates of acetate incorporation from amended treatments are compared with those of the 1.0-ml water treatment. Reproducibility of the results is indicated by the upward trend in acetate incorporation with increased amendment concentration in those treatments that exhibited stimulation, and a similarity in the amount of acetate incorporated at differing amendment concentrations in treatments that did not exhibit stimulatory effects. In contrast, results from different formations varied by 2 orders of magnitude. In the upper Pee Dee sand samples, all treatments resulted in stimulation of activities within 6 h of incubation. The addition of 2.0 ml of water resulted in a greater acetate incorporation rate than the <1.0-ml water treatments. Lower Pee Dee sand samples showed stimulation after additions of 10 μM phosphate or 2.0 ml of water.

The deeper Middendorf sand samples exhibited less than 10% of the activity of the Pee Dee sands (Tables 4 and 5), whereas the Cape Fear sediments exhibited acetate incorporation rates another order of magnitude lower. Phosphate, sulfate, and water supplements resulted in stimulation within the Middendorf sands, whereas most treatments resulted in increased acetate incorporation in the Cape Fear sediments. The greatest extent of stimulation in both deeper aquifer sands were observed with the addition of 2.0 ml of water. The addition of glucose did not stimulate acetate assimilation in either of the sands from depths >400 m, which suggests that those microorganisms may be more severely limited by nutrients other

Table 3. Stimulation of microbial activities from subsurface sediment samples[a]

Activity measured (Time points compared)	Formation (depth)		
	Ellenton silt (194 m)	Ellenton sand (213 m)	Pee Dee clay (239 m)
Aerobic incorporation of 1-^{14}C-acetate into lipids (2–24 h)	<0.1–<0.1	0.1–10	<0.1–<0.1
Aerobic mineralization of U-^{14}C-glucose (day 1–3)	220–570	160–300	6–280
Aerobic mineralization of 2-^{14}C-acetate (day 1–3)	70–670	<4–590	<2–310
Anaerobic mineralization of 2-^{14}C-acetate (day 1–3)	4–1100	<2–1300	<2–13
Anaerobic mineralization of U-^{14}C-glucose (day 1–3)	170–550	<2–1000	<2–100
Anaerobic ^{35}S-SO_4 reduction (day 3–10)	<1–<1	<1–8	<1–<1

[a]Rates expressed as 10^3 dpm day^{-1} are presented for results after 2- and 24-h incubations, or rates observed after day 1 and day 3. Results were extrapolated to dpm day^{-1} for the time points considered. Samples that produced increases in the rate of dpm recovered with longer incubation suggest stimulation of microbial activities over time

Table 4. Evaluation of nutrient limitations in water-producing subsurface aquifer sediments

Formation (depth m)	*methyl*-^{3}H-Acetate incorporation into lipids (10^3 dpm day^{-1})[a]									
	Nutrient	Concentration (μM) 0	10	500	Stimulation[b]	Nutrient	Concentration (μM) 0	10	500		Stimulation
Pee Dee sands (256 m)	NO_3	27	140	85	++	glucose	27	110	88		++
	PO_4	27	37	54	+	minerals[c]	27	–	84		++
	SO_4	27	56	103	++	H_2O[d]	23	41	27	60	+
Pee Dee sands (290 m)	NO_3	150	186	165	–	glucose	150	186	212		–
	PO_4	150	253	243	+	minerals	150	–	156		–
	SO_4	150	99	161	–	H_2O	188	159	150	351	+

[a]An inhibited control and pairs of duplicate tubes containing 2.0 g of sediments and 1.0 of aqueous phase (except H_2O tubes) were inhibited after 2- or 6-h incubations, and radioactive lipids were extracted and counted. Aqueous phases contained 5.0 μCi of *methyl*-^{3}H-acetate and appropriate additions. Results were compared to the 1.0-ml H_2O experiment. Additions were sodium nitrate, sodium phosphate, sodium sulfate, glucose, and a stock mineral solution

[b]Stimulation of 1.5–3 times over control = +, >3 = +. Tests were: no additions, 10 μM, or 500 μM

[c]The complex mineral solution was at concentrations used in media

[d]H_2O was total water added to sediments, which was 0.25, 0.5, 1.0, and 2.0 ml, respectively

than carbohydrates. In all of the sands examined, the addition of 10 μM phosphate resulted in anabolic stimulation, as did the addition of 2.0 ml of water.

As was observed with the sands, additions of water followed by the addition of phosphate had the greatest effect in stimulating activity in clay layers. In three of the six sediments examined (Tables 4–6) sulfate additions and mineral additions

Table 5. Evaluation of nutrient limitations in deeper aquifer sediments

Formation (depth m)	*methyl*-^{3}H-Acetate incorporation into lipids (10^3 dpm day^{-1})[a]										
	Concentration (μM)					Concentration (μM)				
	Nutrient	0	10	500	Stimulation[b]	Nutrient	0	10	500		Stimulation
Middendorf	NO_3	2	2.6	2.2	−	glucose	2	2.8	0.5		−
sands	PO_4	2	3.3	5.5	+	minerals[c]	2	−	2.7		−
(406 m)	SO_4	2	3.2	3.2	+	H_2O[d]	1.7	1.5	2.0	7.9	++
Cape Fear	NO_3	0.24	1.3	0.1	+	glucose	0.24	0.54	0.1		−
sands	PO_4	0.24	0.1	0.6	+	minerals	0.24	−	2.1		+++
(463 m)	SO_4	0.24	0.2	0.8	++	H_2O	0.14	1.1	0.24	3.2	+++

[a]Experimental procedures as described in Table 4

[b]Stimulation of 1.5–3 times over control = +, >3 = ++, >6 = +++. Tests were: no additions, 10 μM, or 500 μM

[c]The complex mineral solution was at concentrations used in media

[d]H_2O was total water added to sediments, which was 0.25, 0.5, 1.0, and 2.0 ml, respectively

Table 6. Evaluation of nutrient limitations within subsurface confining clay layers

Formation (depth m)	*methyl*-^{3}H-Acetate incorporation into lipids (10^3 dpm day^{-1})[a]										
	Concentration (μM)					Concentration (μM)				
	Nutrient	0	10	500	Stimulation[b]	Nutrient	0	10	500		Stimulation
Ellenton	NO_3	1.5	1.4	2.0	−	glucose	1.5	6.0	7.0		+
clay	PO_4	1.5	25	30	++++	minerals[c]	1.5	−	2.4		+
(194 m)	SO_4	1.5	2.6	1.3	−	H_2O[d]	0.7	1.1	1.5	17	++++
Pee Dee	NO_3	0.1	0.1	0.1	−	glucose	0.1	0.02	0.03		−
clay	PO_4	0.1	.08	.04	−	minerals	0.1	−	0.13		−
(239 m)	SO_4	0.1	0.1	0.1	−	H_2O	0.07	0.07	0.01	1.1	++++

[a]Experimental procedures as described in Table 4

[b]Stimulation of 1.5–3 times over control = +, >3 = ++, >6 = +++, >10 = ++++. Treatments were: no additions, 10 μM, or 500 μM

[c]The complex mineral solution was at concentrations used in media

[d]H_2O was total water added to sediments, which was 0.25, 0.5, 1.0, and 2.0 ml, respectively

resulted in stimulation. Glucose and nitrate additions were stimulatory in two of the formations examined. Phosphate was stimulatory to five of six sediments analyzed, whereas the addition of 2.0 ml of water resulted in stimulation in all of the sediments analyzed. The addition of <1.0 ml of water was not significantly different from the 1.0-ml amount, whereas the addition of 2.0 ml of water resulted in slurry formation and more than sixfold increases in the acetate incorporation rates in three of six sediments, including both confining clays.

Particle size and flux of water are major determinants of subsurface microbial ecology. For comparison purposes, sediments were separated into four groups on the basis of sediment lithology and measures of permeability and hydraulic conductivities (Fig. 2). Surface sediments (8–12 cm depth) that contained moisture, plant materials, and were aerated, were contrasted with sediments from depths of more than 7 m. Sediment lithologies were of two major types of particle distributions, >20% clays or >66% sands. Sandy samples were considered in two groups, with k(D) < 10 and hydraulic conductivity (K) < 200 μm sec^{-1}, or with k(D) > 10 and

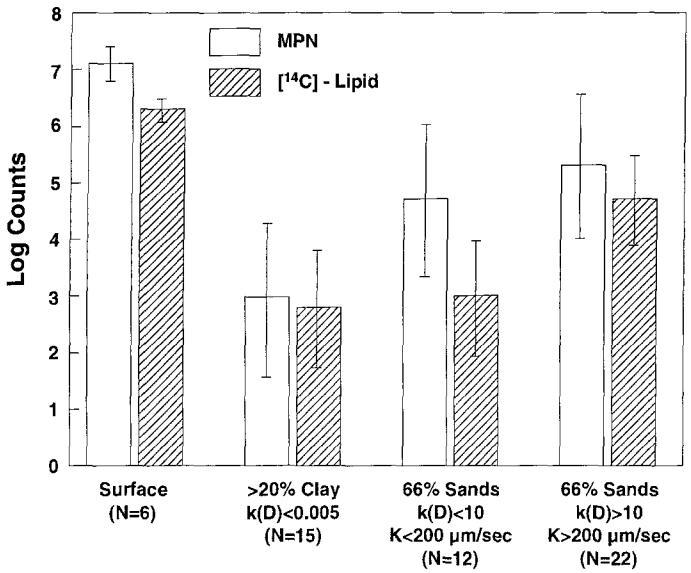

Fig. 2. Influence of geological and hydrological properties on microbial activity and biomass from eastern coastal plain sediments. Samples were segregated into four groups. Surface sediments (8–12 cm in depth) were contrasted with sediments from depths of >7 m. Biomass was estimated by most probable number (MPN) techniques, and incorporation of 1-^{14}C-acetate into microbial lipids (dpm day^{-1}) was used to estimate microbial activities. Results, expressed as log counts, are from 55 samples examined from four core hole sites. N = number of samples examined, D = k(D) or permeability in darcies, and K = hydraulic conductivity estimated from grain-size distributions.

hydraulic conductivity >200 μ sec^{-1}. Clays consistently exhibited lower activities and biomass than did sands, whereas viable biomass in sands was similar regardless of permeability. Microbial activity differed dramatically between the two sand groups. Low permeability sands exhibited activities similar to clay samples; activities in the aquifer sands averaged values that were 100 times greater than those from the low permeability samples. These results suggested that although many Eastern Coastal Plain sandy sediments may contain similar populations, those with abundant water flux may exhibit greater activities.

Discussion

As in previous studies [1, 8, 10, 30, 34, 39], this work observed the presence of large and metabolically diverse communities in subsurface aquifer sediments, and fewer CFUs were observed in confining clays. Although this distribution may be predictable, the factors controlling activities and biomass are less understood. Increased water abundance, as determined by these experimental protocols with Eastern Coastal Plain sediments, resulted in a rapid and significant stimulation of acetate incorporation into lipids, particularly in clayey samples.

Radiolabeled acetate incorporation into lipids has been observed to be a sensitive and reproducible measure of microbial activities or potentials [7, 27, 30, 31]. Although reported values have been based on the initial rate of incorporation, longer experimental incubation periods often resulted in increased rates, previously

expressed as a qualitative measure of stimulation [30]. Many aquifer sands exhibited acetate incorporation values $>100 \times 10^3$ dpm day^{-1} [30], whereas clays or low permeable sands typically exhibited $0.1–10 \times 10^3$ dpm day^{-1} ([30], Tables 4–6). Acetate incorporation into lipids is common to many life forms, and the efficient extraction assay provides a low background (<100 dpm day^{-1}) with a range of measurement that is 6 orders of magnitude. Consequently, the assay is useful for comparing general microbial activities [7, 30, 31] and the effects of amendments.

Within 16 h of collection, the addition of water and/or nutrients led to the stimulation of microorganisms residing in the sediments. Only two of six sediments exhibited increased acetate incorporation rates after the addition of glucose. Palumbo et al. [27] reported that particulate organic carbon (POC) $<0.05\%$ or leachate DOC <1 mg $liter^{-1}$ may constrain bacterial biomass and growth in sediments from arid sites. Lithological logs [33] and physical evidence from these coastal plain sites indicated that lignite and wood were present in several of the subsurface formations, which suggests that POC is available. DOC and TOC were also available in these groundwaters (Table 1) [26]. Although addition of glucose did not result in widespread stimulation of microbial activities, the addition of low specific activity acetate (^{14}C-acetate at a concentration >1500 ng g^{-1}) did result in an average sixfold increase in the rate of radiolabeled acetate incorporation when compared to experiments containing higher specific activity ^{3}H-acetate (30 ng g^{-1}) [28]. These results suggest that while organic carbon may be available, the acetate assimilation rate may accelerate as the pool size increases from approximately 30 ng g^{-1} to >1500 ng g^{-1}.

Addition of mineral solutions did not stimulate activity in most sediments. Also, pore-water chemistries indicated that trace elements were present in subsurface sediments and pore waters. Nitrate and sulfate, which are alternative electron acceptors for respiration, resulted in stimulation in two of the samples (Tables 4 and 5). Other evidence suggested that nitrate and sulfate are not major electron acceptors and may not be major participants in carbon and electron flow in these sediments [1, 14, 25, 30, 31]. Phosphate additions of 10 μM led to stimulation of microbial activities in five of six samples examined. Pore-water chemistry data agreed that phosphates were below 1 mg kg^{-1} and a likely limiting nutrient. The only sample that did not exhibit stimulation by phosphate additions was a dense clay layer in the Pee Dee formation, which exhibited few CFUs and negligible activity.

The only addition that caused high levels of stimulation in all sediments examined was the addition of 2.0 ml of water. Water stimulation of clays was expected, as nutrients held within the confining layers were available after slurry formation and vortexing. The addition of >1 ml of water per 2 g of sediment resulted in the formation of a slurry which apparently increased bioavailability of nutrients (Tables 4–6). Reasons for water stimulation of aquifer sands are not understood, but they could be related to disturbance artifacts [7] and/or the formation of slurries resulting form the vigorous vortexing protocols that may have mixed existing nutrients making them more accessible to the resident microflora.

Increased acetate incorporation rates observed after the addition of the various amendments to several of the sediments (Tables 4–6) may not support the idea of a single-nutrient limiting metabolism. These results suggest that either multiple nutrients may simultaneously limit activities, or the subsurface microflora may scav-

enge and store available resources. Storage of nutrients by subsurface microorganisms has been evidenced by the observation of highly granulated cells from aquifer sediments [2]. Nutrient scavenging and sequestering may enable subsurface microorganisms to rapidly assimilate nutrients; be poised for stimulation of activities; simultaneously maintain low concentrations of DOC, phosphorus, and nitrogen species in the groundwater; and maintain population size while consuming little oxygen [25] or producing little carbon dioxide [4, 5, 25].

Depth did not exhibit a primary constraint on the subsurface biomass or radiotracer incorporation rates. Zones deep beneath the surface exhibited large and potentially active microbial communities. However, sediments that were hundreds of meters beneath the surface and tens of kilometers from recharge did exhibit few CFUs per gram and lower activities than similar lithologies that were closer to the surface and to groundwater recharge areas [10, 16, 30]. In Eastern Coastal Plain environments the sediment particle size and the flux of water appeared to dramatically affect the abundance of microorganisms, their anabolic acetate incorporation rates, and their catabolic use of electron donors and acceptors. Increasing the abundance and flux of water increased anabolism and catabolism of the microorganisms in these experiments.

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