APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1995, p. 749–757 Vol. 61, No. 2
0099-2240/95/$04.00+0

Microbiological Comparisons within and across Contiguous Lacustrine, Paleosol, and Fluvial Subsurface Sediments

THOMAS L. KIEFT,[1]* JAMES K. FREDRICKSON,[2] JAMES P. McKINLEY,[2] BRUCE N. BJORNSTAD,[2] SHIRLEY A. RAWSON,[2] TOMMY J. PHELPS,[3] FRED J. BROCKMAN,[2] AND SUSAN M. PFIFFNER[3]

Department of Biology, New Mexico Institute of Mining and Technology, Socorro, New Mexico 87801[1]; Pacific Northwest Laboratory, Richland, Washington 99352[2]; and Environmental Science Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831-6036[3]

Received 13 July 1994/Accepted 14 November 1994

Twenty-six subsurface samples were collected from a borehole at depths of 173.3 to 196.8 m in the saturated zone at the Hanford Site in south-central Washington State. The sampling was performed throughout strata that included fine-grained lacustrine (lake) sediments, a paleosol (buried soil) sequence, and coarse-grained fluvial (river) sediments. A subcoring method and tracers were used to minimize and quantify contamination to obtain samples that were representative of subsurface strata. Sediment samples were tested for total organic carbon, inorganic carbon, total microorganisms by direct microscopic counts, culturable aerobic heterotrophs by plate counts, culturable anaerobes by most-probable-number enumeration, basal respiration rates, and mineralization of ^{14}C-labeled glucose and acetate. Total direct microscopic counts of microorganisms were low, ranging from below detection to 1.9×10^5 cells g (dry weight)$^{-1}$. Culturable aerobes and anaerobes were below minimum levels of detection in most samples. Direct microscopic counts, basal respiration rates, and ^{14}C-glucose mineralization were all positively correlated with total organic carbon and were highest in the lacustrine sediments. In contrast to previous subsurface studies, these saturated-zone samples did not have higher microbial abundance and activities than unsaturated sediments sampled from the same borehole, the fine-textured lacustrine sediment had higher microbial numbers and activities than the coarse-textured fluvial sands, and the paleosol samples did not have higher biomass and activities relative to the other sediments. The results of this study expand the subsurface microbiology database to include information from an environment very different from those previously studied.

It is now evident from numerous studies that microorganisms are present in many subsurface environments (1–3, 5, 6, 8, 9, 14, 21, 22, 25, 30, 37). Now that the existence of subsurface microorganisms has been established, a major research goal is to determine the physical and chemical conditions that control the abundance and the degree of metabolic activity of subsurface microorganisms. In studies of Atlantic coastal plain sediments in South Carolina, it was found that sandy productive aquifers contained higher numbers of bacteria (as high as 10^7 CFU g of sediment^{-1}) than did the finer-grained aquitard sediments (<log 2 CFU g of sediment^{-1}) (1, 9, 33, 37). The potential for mineralization of organic substrates was also greater in highly permeable, coarse-grained aquifers than in low-permeability, fine-grained aquitards (4, 16, 31, 32). Thus, microbial numbers and activities are generally thought to be negatively correlated with clay content in subsurface sediments (26). A second generalization is that saturated zones potentially harbor higher numbers of microbes than unsaturated zones. Microbes have been detected in unsaturated subsurface environments (vadose zones); however, their numbers are generally lower than in saturated sediments (3, 6, 14, 18, 21, 23). Studies of vadose zone strata in the arid and semiarid western United States have shown that the concentrations of microorganisms are generally orders of magnitude lower than in the Atlantic coastal plain aquifers (3, 6, 14, 21). Another finding was that paleosols (buried soils) located in the vadose zone of an arid region in Washington State had higher concentrations of both organic carbon and microorganisms than the nearby sediments that had not undergone soil development (3). It appears that residual organic carbon can provide an energy source to fuel the survival of microbes that either remain from the time of soil development or have been transported to these sediments.

This study was part of a larger project in which a heterogeneous subsurface environment made up of several different lithologies over a relatively short depth interval was targeted for detailed physical, chemical, hydrological, and microbiological investigation. A sampling site was chosen in an undisturbed area of the Hanford Site in south-central Washington State. The specific aim of the research described here was to quantify microbial abundance and activities in relation to both sediment texture and organic carbon concentration.

MATERIALS AND METHODS

Site and sampling. The sampling site was located near the western edge of the U.S. Department of Energy's Hanford Site in south-central Washington State (Fig. 1). This site, termed the Yakima Barricade borehole, had previously been used for collecting sediment samples from the unsaturated (vadose) zone within the subsurface Hanford formation and the Ringold Formation (21). The water table is 100 m below land surface at this site. All samples for this study were collected in the saturated suprabasalt sediments of the Ringold Formation that overlie the Columbia River basalts. The sampling interval is approximately 6 to 8 million years in age and is sandwiched between two highly transmissive zones of fluvial gravels. Three distinct lithologies exist in the sampling interval (Fig. 2). The topmost (youngest) lithologic unit is a sequence of fine-grained lacustrine (lake) sediments extending from 173 to 185 m in depth. The second, underlying unit is a paleosol sequence, extending from 185 to 193 m. A thin (approximately 5 cm) layer of volcanic tuff was present between the lacustrine and paleosol sediments, but insufficient sample volume precluded its inclusion in this particular study. The deepest and oldest of the three lithologic units is a sequence of coarser-grained fluvial (river) sands extending from 193 to 197 m. Initial hydrologic data suggest that the lacustrine sediments and the paleosol sequence func-

* Corresponding author. Mailing address: Department of Biology, New Mexico Institute of Mining and Technology, Socorro, NM 87801. Phone: (505) 835-5321. Fax: (505) 835-6329. Electronic mail address: tkieft@nmt.edu.

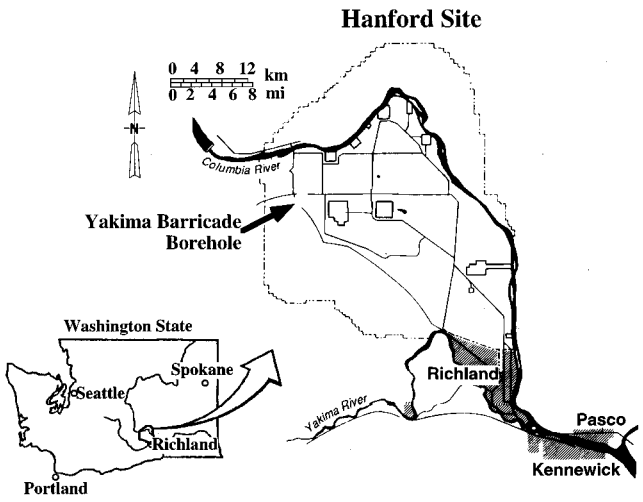

FIG. 1. Map of the Hanford Site in south-central Washington State, showing the location of the Yakima Barricade borehole.

tion as an aquitard with little mixing of groundwaters across the sampling interval. The approximate depths of contacts between these sediments were estimated prior to drilling on the basis of data from a previously drilled nearby borehole.

Drilling and coring were performed by a cable tool, split-spoon sampling method as previously used in the unsaturated zone in the same borehole (21). The overall scheme for collecting and handling the samples was a modification of the subcoring procedure described by Phelps et al. (31), Russell et al. (36), and Colwell et al. (7). Cores were collected beginning at a depth of 173 m and extending to 197 m. Two different lengths of core barrels were used: 2 and 3 feet (0.6 and 0.9 m, respectively). Samples were collected in sterilized Lexan liners fitted inside the stainless-steel, split-spoon core barrels.

Multiple tracers were used to quantify chemical and microbiological contamination of the subcore samples during the coring procedures. Carboxylated fluorescent microspheres (approximately 1 μm in diameter; Polysciences, Inc., Warrington, Pa.) were used as microbiological tracers. Carboxylated fluorescent microspheres have been used previously as surrogate microorganisms for microbial transport experiments (15) and as tracers in subsurface sampling (7). Yellow-green fluorescent microspheres were placed in suspension in sterile deionized water in zip-lock bags just below the prongs of the core catcher at the bottom of the core barrel. Red fluorescent microspheres were suspended in sterile sodium perchlorate tracer (200 mg liter^{-1}) in the Lexan liner itself (retained within the core barrel by a cylindrical core marker). The tracer solution prevented intrusion of borehole slurry into the liner/split-spoon sampler during transit through the open well bore and was released through the check valve at the top of the split spoon during coring. When the core barrel encountered the formation, the bags broke, exposing the outside of the core to tracer. Bromide (added as KBr to borehole fluids) was used as a solute tracer and was maintained at approximately 200 mg liter^{-1} in the borehole slurry. Immediately upon recovery from the borehole, the cores were transferred to an on-site argon-filled glove bag to preserve the viability of strict anaerobes. The Lexan barrels were opened by scoring them lengthwise with a hand-held battery-powered circular saw. The outer portion of the cores was pared away by using sterile implements. Intervals of subcore material of approximately 12 to 50 cm each were removed and homogenized. Samples were designated by the mean depth (meters) of the homogenized subcore interval. Microbiological and chemical analyses were performed on subsamples of these homogenized samples. The fluorescent microspheres were quantified in samples collected from the exposed ends of cores and from the homogenized subcore samples by epifluorescence microscopy. Bromide was quantified by ion-exchange chromatography, using a Dionex 2020i ion chromatograph (Dionex, Sunnyvale, Calif.). The concentrations of microspheres and solute tracers in the homogenized subcores indicated that the subcores were not significantly contaminated with particles or solutes from the borehole or from the drilling and coring tools. Bromide concentrations in subcore pore waters were ≤4 mg liter^{-1}; background bromide concentrations in the groundwater of overlying formations were approximately 2 mg liter^{-1}. Fluorescent microspheres were generally not detected in the homogenized subcores (minimum level of detection, approximately 10^3 microspheres g of sediment^{-1}). The concentrations of microspheres in the subcored material were at least 1,000-fold lower than those in the exposed core ends. The logic underlying this tracer technology is that the presence of high numbers of microspheres in the center of the core would indicate a contaminated sample, i.e., one that may be contaminated with microbes that are not indigenous to the formation; such a sample would be rejected for analysis. Likewise, any samples showing high concentrations of solute tracers in the center of the cores would be rejected as contaminated. Portions of the homogenized subcores were sent to the New Mexico Institute of Mining and Technology and to Oak Ridge National Laboratory on ice by overnight delivery for microbiological analyses. Microbiological analyses were initiated within 24 h of receipt of samples; prompt processing is necessary to minimize postsampling changes in microbial populations (3).

Physical analyses. Moisture contents of all samples were measured by weighing samples before and after 24-h dessication at 105°C. All microbiological and chemical data were corrected for moisture content and expressed on a per-gram-of-dry-weight basis. Lithologic descriptions of the cores were made at the time samples were pared in the glove bag. Grain size distributions were determined by the method of Gee and Bauder (10). Cumulative grain size distribution plots were used to establish the proportions of sand (0.5- to 2.0-mm diameter), silt (0.002- to 0.5-mm diameter), and clay (<0.002-mm diameter).

Chemical analyses. Total organic carbon (TOC) and total inorganic carbon were analyzed by Huffman Laboratories, Golden, Colo. Total carbon (organic and inorganic) was quantified with a Leco CR-12 carbon analyzer (Leco, St. Joseph, Mich.) by combusting the sediments at 1,100°C and measuring the release of CO_2 by infrared gas analysis. Inorganic carbon was quantified by acidification, using a Coulometric System 140 (U.I.C., Joliet, Ill.). Total organic content was quantified by subtraction of inorganic content from the total carbon content.

Microbiological analyses. Direct microscopic counts were performed by using a modification of the acridine orange method (12, 37), as previously described (3, 21). Samples were fixed in glutaraldehyde within 24 h of receipt in preparation for analyses. Cells were counted in 20 microscopic fields on each of four microscope slides (previously combusted at 450°C for 24 h), by using a Zeiss Axioskop epifluorescence microscope at ×630 total magnification. This method resulted in a minimum detection limit of log 5.2 cells g (dry weight) of sediment^{-1} for the 20 fields counted on each slide and a final minimum level of detection of log 4.6 cells g (dry weight)$^{-1}$ for the four slides combined. Means and standard deviations of data from the four slides were calculated. For statistical analysis, slides on which no cells were observed were assigned the value of one-half the minimum level of detection for a slide (13). Sterile controls consisting of sediment samples combusted at 550°C for 3 h were prepared in parallel with the samples. The analyses were rejected and repeated if any of the control slides from a particular batch showed any cells.

Aerobic heterotrophic plate counts were performed by blending 10.0-g (wet weight) samples into 100 ml of 0.1 M $Na_4P_2O_7$ (pH 7.0) in a Waring blender. Subsequent 10-fold dilutions were made in a phosphate-buffered saline solution consisting of 1.18 g of Na_2HPO_4, 0.223 g of $NaHPO_4 \cdot H_2O$, and 8.5 g of NaCl liter of deionized water^{-1}. Diluted sediment was spread plated in triplicate onto 1% PTYG agar, dilute actinomycete agar (Difco, Detroit, Mich.), and dilute potato dextrose agar (Difco). The 1% PTYG agar consisted of 0.1 g of glucose, 0.1 g of yeast extract, 0.05 g of peptone, 0.05 g of tryptone, 0.6 g of $MgSO_4 \cdot 7H_2O$, 0.07 g of $CaCl_2 \cdot H_2O$, and 15 g of agar liter of deionized water^{-1} (1). The dilute actinomycete and potato dextrose agars were both modified from standard recipes to contain 10% of the concentrations of organic nutrients but the same concentration of agar. All dried medium components were obtained from Difco. The plates were incubated at 22°C for a minimum of 2 months.

Counts of anaerobic culturable bacteria were performed by using a most-probable-number method, as previously described by Phelps et al. (33). Total culturable anaerobes were quantified in TYEG medium (33) containing 1.0 g of tryptone, 1.0 g of yeast extract, 1.0 g of glucose, and 0.3 g of cysteine-HCl liter of deionized H_2O^{-1}, along with trace minerals, vitamins, and a carbonate-plus-phosphate buffer. Headspace gas was 9:1 (vol/vol) N_2/CO_2. Culturable spores were enumerated in the same medium after heat treating the initial slurry. Acetogens and methanogens were quantified in a single medium containing 10 mg of yeast extract and 10 mmol of methanol liter of deionized H_2O^{-1}, along with trace minerals, vitamins, and a carbonate-plus-phosphate buffer. All dried medium components were obtained from Difco. The headspace gas for methanogens and acetogens was 95:5 (vol/vol) N_2/H_2. Cultures were incubated for 30 days at 21 to 24°C. Positive results were indicated by turbidity.

Respirometry. Basal respiration of sediment samples was measured by quantifying the changes in CO_2 concentration in the headspace gas above sediment within sealed vials. The concept of basal respiration is adapted from soil microbiology (17, 20, 22) and provides a relative measure of microbial activity (but not an estimate of in situ respiration rates). Sediment samples (5.0 g [wet weight] each) were placed into sterile 70-ml serum vials, and the vials were then sealed with sterile rubber septa. Triplicate vials were set up for each sediment type. The sediments in the vials were incubated at 22°C. CO_2 concentrations in the headspace gas were measured by gas chromatography after 3, 7, and 14 days of incubation. The samples were vortex mixed for 5 s immediately prior to sampling of the headspace gas. The rate of basal respiration was calculated as the slope of the linear regression of the CO_2 concentrations versus time and was expressed as milligrams of CO_2 per gram (dry weight) of sediment per hour. The minimum level of detection was approximately 0.001 mg of CO_2 g (dry weight) of sediment^{-1} h^{-1}. Typically, basal respiration rates are measured after a period of equilibration of 1 or more days (17). Our calculations did not include CO_2 evolution during the first 3 days of incubation and thus minimized the influence of CO_2 production that may have been caused by the initial disturbance of subsampling and placing the subsamples into the vials. This initial disturbance could also have generated CO_2 abiologically by dissolving carbonates. Previous

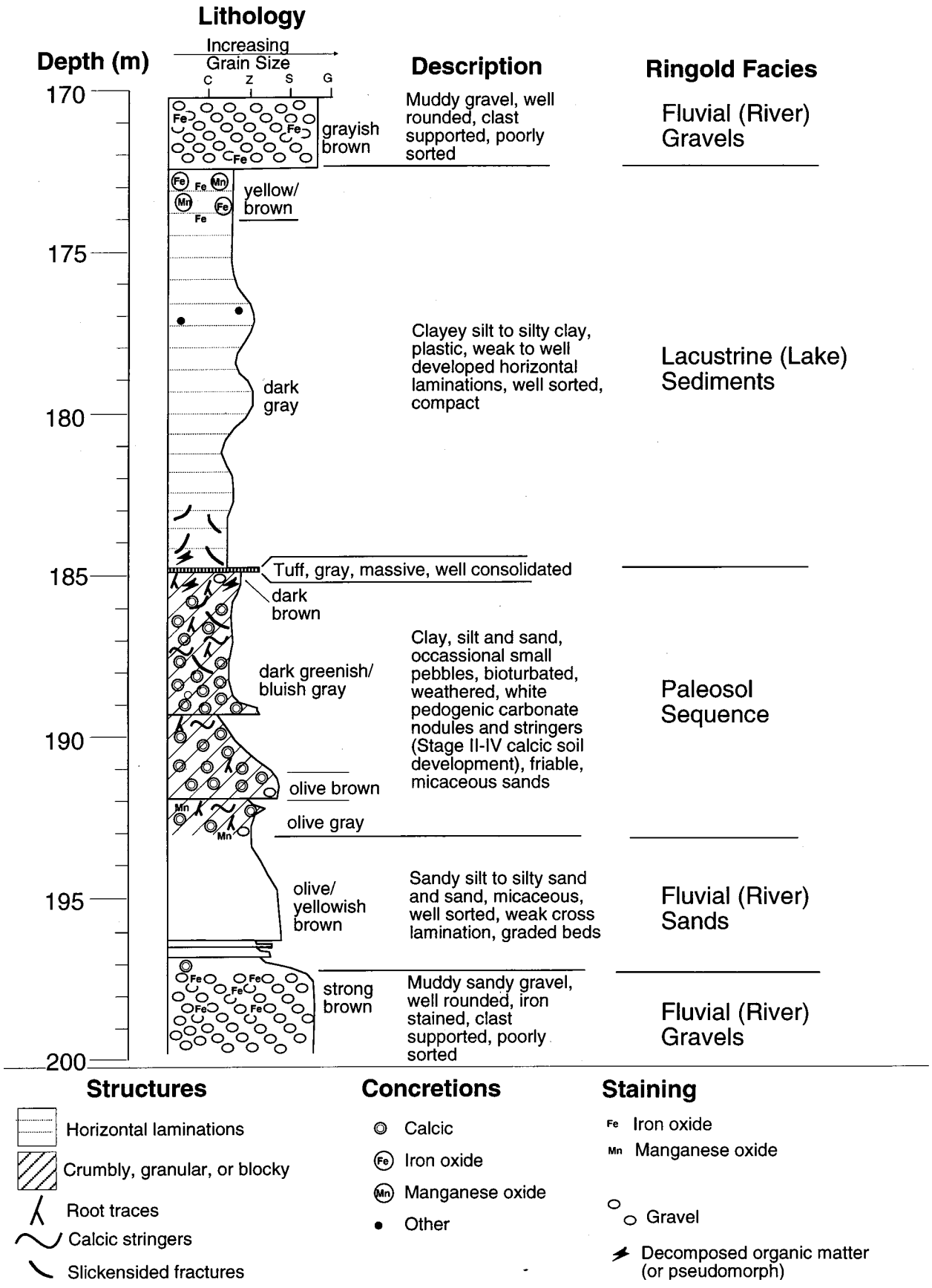

FIG. 2. Lithologic profile of the sampling interval within the saturated zone of the Ringold Formation, showing the locations and textural descriptions of the lacustrine sediments, paleosol sequence, and fluvial sediments (as well as overlying and underlying fluvial gravels).

experience with highly calcareous soils has indicated that the majority of abiotic CO_2 production occurs within the first few hours following addition of water (20, 35). It was assumed that carbonate dissolution after the first 3 days of incubation was negligible. Data from poisoned controls used to measure abiotic CO_2 production support this assumption. Poisoned controls were run on the 178.0-m lacustrine sediment and the 185.5-m paleosol sample by adding $HgCl_2$ to achieve a concentration of 250 μg ml^{-1} in the sediment solution.

Radiorespirometry. Mineralization of ^{14}C-labeled glucose under aerobic conditions was measured in sterile 160-ml wide-mouthed milk dilution bottles as described by Brockman et al. (3). Sediment samples (5.0 g, wet weight) and 0.1 ml of filter-sterilized labeled glucose solution (0.83 mM; 9.3 kBq of [U-^{14}C]glucose [New England Nuclear, Boston, Mass.]) were placed into each dilution bottle. The radiochemical purity of the glucose was 99% (measured by New England Nuclear by paper chromatography, reverse isotopic dilution analysis, and paper electrophoresis). The bottles were sealed with rubber stoppers. A 7.0-ml liquid scintillation vial containing 1.0 ml of 0.3 N NaOH was suspended by a string in each bottle to act as an alkaline trap for the evolved CO_2. The traps were removed at intervals and replaced with fresh ones. After the vials were removed from the bottles, liquid scintillation cocktail (Ultima Gold XRTM; Packard Instrument Co., Meriden, Conn.) was added directly to the NaOH solution, and the radioactivity was counted in a liquid scintillation counter. Duplicate bottles were set up for each sample. Data were reported without subtraction of sterile controls.

Mineralization of ^{14}C-glucose under anaerobic conditions was measured in duplicate in sterile 25-ml crimp-top tubes (Bellco Glass Co., Vineland, N.J.), by the method described by Phelps et al. (33). Sediment samples (2.0 g, wet weight) and 1.0 ml of sterile labeled glucose solution (0.36 mM; 37 kBq of [U-^{14}C]glucose [New England Nuclear]; >98% radiochemical purity) were placed into each dilution bottle. The headspace gas was a 9:1 (vol/vol) mixture of N_2 and CO_2. Tubes were incubated at 21 to 24°C. At intervals of 12 h, 1 day, and 7 days, one pair of tubes was sacrificed and mineralization was halted by the addition of 0.5 ml of 2 N NaOH. Samples were then stored at −20°C. At the time of analysis, the slurries in the tubes were thawed and then acidified with 0.5 ml of 6 N HCl, and $^{14}CO_2$ and $^{14}CH_4$ were then quantified in the headspace gas by using gas chromatography-gas proportional counting. The minimum level of detection for the gas chromatography-gas proportional counting was 0.13% day^{-1}. The earliest time points yielding measurable results were used to calculate a linear rate of glucose mineralization.

Mineralization of ^{14}C-labeled acetate under aerobic conditions was measured by a slight modification of the method described by Kieft et al. (21). Sediment samples (1.5 g, wet weight) were placed into sterile 60-ml serum vials. Radioactively labeled substrate in the form of 10 μl of [1-^{14}C]acetate (1.33 mM; 3.7 kBq [Sigma Chemical Co., St. Louis, Mo.]) was added to the sediment in each vial. The radiochemical purity of the acetate was 100% (measured by Sigma, using high-performance liquid chromatography with radiochemical detection). The vials were sealed with sterile rubber septa equipped with sterile plastic cups (Centerwells [Kontes, Vineland, N.J.]). Each cup contained a strip of sterile Whatman no. 1 chromatography paper (approximately 1 by 3 cm) as a wick and 0.3 ml of 0.4 N NaOH as a trap for CO_2. The vials were incubated for 7 days at 22°C. The paper strips and NaOH solutions were removed and placed into 10 ml of scintillation cocktail (Universol; ICN Radiochemicals, Irvine, Calif.), and the radioactivity was measured by liquid scintillation counting. Sterile controls consisted of sediment samples that had been combusted at 550°C for 24 h; these were treated the same way as the other samples. Samples from 176.6 to 182.1 m were not tested for acetate mineralization. Samples were tested in triplicate. Data were reported without subtraction of sterile controls.

Statistical analyses. Pearson product-moment correlation analyses were performed with Systat 5.2 (Evanston, Ill.).

RESULTS

Lithologic descriptions of each unit are shown in Fig. 2. Lacustrine sediments were weakly to strongly laminated, dark gray clayey silts and silty clays, with the exception of the interval from 173 to 175 m, which consisted of yellow-brown clayey silts with orange-red concretions assumed to be ferric oxides. Paleosols were olive to dark greenish blue-gray silts and sands, with minor clays. Pedogenic features included white nodules and stringers that appeared to be carbonate as indicated by reaction with dilute HCl in the field. Manganese oxides were present in the deepest portion of the paleosol sequence. The fluvial sands were predominantly olive to yellowish brown silty sands to sandy silts.

Grain size distribution data supported the lithologic descriptions (Table 1). Sediment grain size generally increased with depth, as evidenced by the decreased proportions of clay- and silt-sized material with increasing depth. The finest-grain sediments (lowest percentage of sand) occurred in the middle of the lacustrine sequence. Sand contents were generally highest in the fluvial sands. The 191.8-m paleosol sample also showed a relatively high sand content, consistent with its occurrence near the transition between the underlying fluvial sands and the overlying paleosol. Clay contents were higher in the lacustrine and paleosol samples than in the fluvial sediments.

TABLE 1. Physical and chemical characteristics of sediments from the Yakima Barricade borehole at the Hanford Site, south-central Washington State

Lithology and sample depth (m)	Clay (%)	Silt (%)	Sand (%)	TOC (mg/kg [dry wt])	Total inorganic C (mg/kg [dry wt])
Lacustrine sediments					
173.3	38	58	4	379	0
174.1	41	57	2	388	0
174.8	24	76	0	11,067	51
176.7	38	53	9	13,506	1,572
177.2	33	56	11	14,719	1,272
178.0	41	57	2	14,164	1,356
178.9	34	64	2	10,946	2,672
179.8	45	55	0	12,499	1,675
180.6	28	71	1	10,098	1,744
181.4	28	72	2	10,755	2,542
182.1	30	70	0	8,584	2,087
182.7	29	71	0	8,147	2,303
183.5	36	64	0	7,040	2,648
184.7	35	63	2	16,493	162
Paleosol sequence					
184.9	15	52	33	1,360	188
185.5	35	58	7	1,348	2,737
186.5	45	52	3	2,121	873
188.0	51	34	15	2,808	2,795
189.1	10	75	15	9,410	813
191.8	36	29	35	1,611	62
192.7	22	61	17	786	226
Fluvial sand	0				
193.4	36	29	35	288	0
194.1	10	52	38	302	0
195.3	8	33	59	272	0
196.1	10	16	74	210	0
196.8	10	40	50	551	84

The TOC contents of the sediments were, in general, highest in the lacustrine sediments and lowest in the fluvial sands (Table 1). Within the lacustrine interval, there was an abrupt change in the organic C contents between the 174.1- and 174.8-m samples, with the topmost samples (173.3 and 174.1 m) having particularly low values (379 and 388 mg kg^{-1}, respectively). These two upper lacustrine sediment samples were in the oxidized portion of the lacustrine sediments. Lacustrine sediment samples below the 174.1-m depth had the highest TOC contents of the entire cored interval, and the lacustrine sediment samples taken as a group had the highest average TOC: 9,913 mg kg^{-1} (standard deviation [SD] = 4,820 mg kg^{-1}). The TOC contents of the paleosol sequence were generally low (average = 2,778 mg kg^{-1}; SD = 2,994 mg kg^{-1}), and the fluvial sand samples had consistently low values (average = 325 mg kg^{-1}; SD = 131 mg kg^{-1}). The inorganic carbon contents were lower than the TOC values. Inorganic carbon values were highest in the lacustrine and paleosol samples.

Direct microscopic counts of bacteria were generally low in all of the samples, ranging from below detection (<log 4.6 cells

TABLE 2. Direct counts, basal respiration rates, and percents mineralization (7-day incubations) of ^{14}C-labeled glucose and acetate in sediments from the Yakima Barricade borehole at the Hanford Site[a]

Lithology and sample depth (m)	Mean (SD):			
	Direct counts (log cells/g [dry wt])	Basal respiration rate (μg of CO_2/g [dry wt]/h)	Glucose mineralized (%)	Acetate mineralized (%)
Lacustrine sediments				
173.3	5.21 (0.35)	ND[b]	0.14 (0.03)	0.86 (0.35)
174.1	<4.6	<0.001	1.19 (0.21)	1.23 (0.10)
174.8	5.21 (0.35)	0.402 (0.008)	9.10 (0.49)	1.60 (0.26)
176.7	<4.6	0.652 (0.015)	8.89 (0.34)	ND
177.2	<4.6	0.664 (0.011)	8.91 (0.61)	ND
178.0	<4.6	0.609 (0.021)	9.61 (0.52)	ND
178.9	5.07 (0.30)	0.555 (0.048)	9.19 (0.18)	ND
179.8	4.99 (0.15)	0.540 (0.009)	9.14 (0.23)	ND
180.6	4.99 (0.15)	0.550 (0.013)	7.90 (0.21)	ND
181.4	4.99 (0.15)	0.254 (0.014)	9.50 (0.25)	ND
182.1	5.27 (0.32)	0.179 (0.012)	7.93 (0.34)	ND
182.7	5.15 (0.29)	0.141 (0.032)	8.45 (0.84)	0.70 (0.16)
183.5	5.00 (0.15)	0.098 (0.007)	7.14 (0.19)	0.10 (0.01)
184.7	5.09 (0.030)	0.051 (0.015)	3.65 (0.31)	0.81 (0.12)
Paleosol sequence				
184.9	5.22 (0.29)	0.035 (0.004)	4.95 (0.26)	1.03 (0.23)
185.5	<4.6	<0.001	1.54 (0.05)	0.33 (0.07)
186.5	<4.6	<0.001	0.86 (0.03)	0.27 (0.08)
188.0	<4.6	<0.001	1.08 (0.04)	0.17 (0.02)
189.1	5.06 (0.30)	0.017 (0.030)	2.75 (0.04)	0.48 (0.20)
191.8	<4.6	0.010 (0.013)	3.40 (0.10)	0.51 (0.12)
192.7	<4.6	0.027 (0.028)	2.48 (0.70)	0.30 (0.03)
Fluvial sand				
193.4	<4.6	0.010 (0.001)	0.35 (0.08)	1.73 (0.06)
194.1	<4.6	0.001 (0.021)	0.30 (0.00)	1.71 (0.06)
195.3	<4.6	0.034 (0.030)	0.17 (0.01)	0.99 (0.03)
196.1	<4.6	0.028 (0.017)	0.23 (0.04)	23.1 (7.62)
196.8	<4.6	0.032 (0.008)	0.34 (0.15)	1.22 (0.42)

[a] Percents mineralization of ^{14}C-labeled substrates are not corrected for sterile controls, which consistently had values of <1%.
[b] ND, not determined.

g [dry weight] of sediment^{-1}) to log 5.27 cells g (dry weight) of sediment^{-1} (Table 2). Ten of 14 (71%) of the lacustrine samples had direct microscopic counts above detection, 2 of 7 (29%) of the paleosol samples showed detectable total cells, and none of the fluvial sediments had direct counts above detection. Aerobic heterotrophic plate counts on 1% PTYG agar were below detection (<100 CFU g [dry weight] of sediment^{-1}) in all of the samples. Anaerobic most-probable-number counts of culturable microorganisms were generally low in all of the sediment samples (Table 3). Numbers of culturable anaerobes were ≤10 cells g (dry weight) of sediment^{-1} in the growth media tested for all samples.

The highest basal respiration rates were also measured in the lacustrine sediments (Table 2). Within the lacustrine sediments, the highest respiration rates increased with depth to the 177.2-m sample and thereafter declined with depth to the bottom of the lacustrine sediment interval. The paleosol sequence and fluvial sand sediments had low basal respiration rates ranging from <0.001 to 0.035 μg of CO_2 g (dry weight) of sediment^{-1} h^{-1}. The amounts of organic carbon respired between days 3 and 14 during the basal respiration assay ranged from 0 to approximately 3% of the TOC. The amounts of CO_2 generated in the poisoned 178.0- and 185.5-m samples between days 3 and 14 were negligible.

The average percent glucose mineralized in the aerobic radiorespirometry study was highest in the lacustrine sediments (mean = 7.20%; SD = 3.15%), lower in the paleosol samples (mean = 2.44%; SD = 1.44%), and lowest in the fluvial sand samples (mean = 0.278%; SD = 0.077%) (Table 2). Although the overall mineralization values for glucose were low during the 3-week incubations (<10%), nearly all of the samples tested showed mineralization values that were greater than those of the sterilized controls (<1%). Mineralization of glucose under anaerobic conditions was also low (Table 3). Anaerobic rates of glucose mineralization were below detection for 16 of 26 samples and no greater than 4.3% day^{-1} for any of the samples. Radioactively labeled CH_4 was not detected in the headspace gas of any of the anaerobically incubated samples. The ^{14}C-labeled acetate mineralization patterns were very different from the ^{14}C-glucose data (Table 2). Only one of the sediment samples mineralized a significantly higher percentage of ^{14}C-acetate than the sterile control (≤1%): the 196.1-m fluvial sand sample averaged 23.1% mineralization.

Correlation analysis indicated significant correlations between several parameters measured in this study (Table 4). Depth was significantly and negatively correlated with percent clay, percent silt, TOC, direct counts, basal respiration, and glucose mineralization. Textural data were significantly correlated with several other factors, reflecting the higher TOC values and generally higher microbial abundance and activities in the fine-grained lacustrine sediments. The basal respiration

TABLE 3. Anaerobic enumeration of culturable microbes by the most probable number method and percent mineralization of glucose under anaerobic conditions

Lithology and sample depth (m)	Anaerobic enumeration (log cells g [dry wt] of sediment^{-1})			Anaerobic mineralization of ^{14}C-glucose (% day^{-1})
	TYEG medium	TYEG medium spores	Methanogen-acetogen medium	
Lacustrine sediments				
173.4	<0	0	0	1.4
174.1	<0	0	<0	<0.1
174.8	<0	<0	0	<0.1
176.6	<0	<0	<0	<0.1
177.2	<0	<0	0	<0.1
178.0	<0	<0	<0	<0.1
178.9	<0	<0	<0	<0.1
179.9	<0	<0	<0	<0.1
180.6	<0	<0	0	1.4
181.4	<0	<0	0	<0.1
182.1	1	<0	<0	<0.1
182.6	0	<0	<0	0.5
183.5	<0	<0	0	0.6
184.7	0	<0	<0	<0.1
Paleosol sequence				
184.9	1	0	0	1.1
185.5	<0	<0	<0	<0.1
186.5	<0	<0	<0	<0.1
188.0	<0	<0	<0	<0.1
189.1	<0	<0	<0	1.4
191.9	<0	<0	1	4.3
192.6	0	<0	0	1.4
Fluvial sand				
193.1	<0	<0	<0	<0.1
194.2	<0	<0	<0	<0.1
195.4	<0	<0	1	3.1
196.0	<0	<0	0	2.3
196.6	<0	<0	<0	2.7

rates were significantly correlated with the TOC values (r = 0.677; $P < 0.001$) but were not correlated with inorganic carbon contents (r = 0.207; P = 0.310). TOC values were also significantly correlated with the log of the direct counts (r = 0.451; P = 0.021) and ^{14}C-glucose mineralization (r = 0.829; $P < 0.001$).

DISCUSSION

The patterns of microbial abundance and activities observed in these subsurface sediments differ markedly in a number of respects from those of previously studied subsurface environments. First, the numbers and activities of microorganisms

TABLE 4. Pearson product-moment correlation matrix for physical, chemical, and microbiological data[a]

Parameter	Depth	Clay	Silt	Sand	TOC	TIC[b]	Log AODC[c]	Basal respiration rate	Glucose (aerobic)[d]	Glucose (anaerobic)[e]
Clay	−0.480*									
Silt	−0.663***	0.138								
Sand	0.767***	−0.655***	−0.838***							
TOC	−0.575**	0.353	0.500**	−0.581**						
TIC	−0.329	0.584**	0.303	−0.556**	0.414*					
Log AODC	−0.444*	0.061	0.609***	−0.503**	0.451*	0.285				
Respiration	−0.687***	0.270	0.368	−0.409*	0.677***	0.207	0.088			
Glucose (aerobic)	−0.634***	0.236	0.518**	−0.551**	0.829***	0.549**	0.509**	0.727***		
Glucose (anaerobic)	0.548**	−0.642***	−0.573**	0.789***	−0.454*	−0.451*	−0.195	−0.405*	−0.388	
Acetate (aerobic)[f]	0.317	−0.302	−0.429	0.502*	−0.179	−0.222	−0.207	−0.028	−0.207	0.226

[a] Pairwise comparisons were used to allow for missing ^{14}C-acetate mineralization data. Values of zero were used for the log of acridine orange direct counts that were below detection. Asterisks following the correlation coefficients indicate significant correlations: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[b] TIC, total inorganic carbon.
[c] AODC, acridine orange direct counts.
[d] ^{14}C-glucose mineralization (aerobic conditions).
[e] ^{14}C-glucose mineralization (anaerobic conditions).
[f] ^{14}C-acetate mineralization (aerobic conditions).

were higher in the fine-textured lacustrine sediments than in the coarse-textured fluvial sands. Second, the numbers and activities of microorganisms were not higher in the saturated zone (this study) than in the unsaturated zone (21). In a previous study of the unsaturated zone in the same borehole at this site (21), the total numbers of cells ranged from log 6.3 to log 7.4 cells g (dry weight)$^{-1}$ and the extents of glucose and acetate mineralization were generally less than 10%. Culturable counts of aerobes were below detection by dilution plate counts in nearly all of the unsaturated-zone samples (21) and in all of the saturated-zone samples (this study). However, differences other than moisture (e.g., texture, TOC, mineralogy, and depositional history) also exist between the saturated and unsaturated zones at this site. Third, in contrast to previously studied vadose zone paleosols (3), the saturated paleosol samples in this study had neither higher organic carbon contents nor higher microbial numbers and activities than the sediments that had not undergone soil development. The findings of this study emphasize the need to consider site-specific environmental factors controlling the distribution and activities of subsurface microbiota.

The lower numbers and activities of microorganisms in the saturated zone at the Hanford Site in Washington State than in the Atlantic coastal plain aquifers of South Carolina can be explained, at least in part, by the lower annual precipitation, lower recharge rate, and lower nutrient flux in the sediments at the semiarid Hanford Site. The mean annual precipitation at the Hanford Site is 162 mm (11). The vertical recharge rate at the borehole site of this study has been estimated to be 1 μm year^{-1} (24). Groundwater ages (i.e., the time since infiltration) have not been directly measured but can be constrained in part by the timing of the last major vertical recharge event: cataclysmic flooding that occurred 13,000 years ago following the last glaciation (24). The groundwater age in the sampling interval at this site depends on whether recharge is dominantly vertical (local) or lateral (regional). In either case, the age appears to be at least 13,000 years. Groundwater of comparable age (11,500 years) was sampled from the C-10 borehole in the Middendorf aquifer in South Carolina (29), but significantly higher numbers and activities of microorganisms were observed (1, 2, 8). Nutrient flux in subsurface environments is a function of groundwater flow rates and nutrient concentrations. Subsurface organic nutrients are derived from primary production at the surface and organic matter that was present in the sediment at the time of burial. Semiarid lands have lower rates of primary productivity, lower soil organic carbon contents, and fewer microorganisms compared with more mesic environments (19). Lower biological activity at the surface coupled with lower recharge rates results in lower inputs of new organic matter and microorganisms to the subsurface than in the Atlantic coastal plain aquifers. Thus, the microbes in the Hanford Site sediments of this study are dependent primarily on the organic matter remaining since geologic deposition for their energy needs.

The larger amounts of organic carbon and the greater abundance of microorganisms in the lacustrine sediments than in the paleosols or fluvial sands may be explained by the depositional history and fine-grained texture of the sediments. As lake sediments, the initial deposits likely had high concentrations of organic matter, high microbial numbers, and anoxic conditions. The organic matter in these sediments is probably a portion of the original lake sediment organic matter. The microbes may also be derived from original lake sediment populations; however, their numbers were considerably lower than those of modern lake sediments. Neither aerobes nor anaerobes were abundant. The fine-grained nature of these sediments can be expected to limit both the mobility of microorganisms and the transport of dissolved nutrients, including both electron donors and electron acceptors. The low numbers of microbes suggest a physical separation of microbes from nutrients. Thus, diffusion-controlled transport of nutrients and the relatively low number of viable microbes may be responsible for a portion of the original lake sediment organic matter persisting for millions of years.

The paleosol sequence apparently underwent substantial soil development before burial, as evidenced by root traces, aggregate structure, and secondary clay development and mineralization. However, the organic matter that likely existed when these strata were surface soils has now largely disappeared. This disappearance may be explained by microbial mineralization and/or leaching of organic matter away from the original soil after it was buried. Either of these processes would have been favored in coarse sediments with somewhat higher groundwater flow rates. Thus, the small amounts of TOC and the concomitant low numbers and activities in the paleosol sediments may be the result of the coarser grain size in these sediments. The inorganic carbon in the lower lacustrine sediments and the paleosol sediments may reflect past microbial activity in that mineralization of organic matter can lead to precipitation of carbonates. The bulk of this inorganic carbon precipitation likely occurred during surface soil development. Soil carbonate formation has been associated with soil microbial activity (28, 34). Significant concentrations of $CaCO_3$ were observed in the paleosol sequence in the form of calcic stringers and nodules, which are common products of soil development in arid environments. Heterotrophic microbial activity can also generate dissolved inorganic carbon in buried sediments, thereby increasing the likelihood of calcite precipitation (5, 27, 29). However, the pore waters in the paleosols were undersaturated with respect to calcite (data not shown), suggesting that calcite has partially dissolved since the soils were buried.

River sediments generally have lower organic carbon contents than lake sediments, and so the fluvial sediments in this study likely contained less organic carbon at the time of deposition than either the paleosol or lacustrine sediments at the time they were buried. A relatively low TOC at the time of deposition combined with the low recharge rate and, hence, low nutrient flux at this semiarid site explains the lack of organic matter and microbial activity in the fluvial sands.

As applied to subsurface sediments such as those of this study, the basal respiration rate cannot be considered to estimate the in situ rates of metabolism. The physical disturbance that occurs during sampling and during establishment of microcosms for respiration assays can bring microbes in contact with previously inaccessible nutrients. Although disturbance effects were minimized in this study by not measuring CO_2 evolution during the first 3 days of incubation, they cannot have been eliminated entirely. The basal respiration rate reflects the availability and biodegradability of organic carbon in the sample as well as the availability of microorganisms and their abilities to metabolize the organic carbon. Since precautions were taken to avoid contamination during sampling and preparation of microcosms for the respiration assay, any respiration indicates the presence of viable microorganisms. Although these rates of respiration were relatively low, it is significant that as much as 3% of the TOC was mineralized during the 11-day incubation. This indicates that at least a portion of the organic matter can be readily degraded. The fact that it was not degraded in situ suggests that it was inaccessible to the few microbes present. The fact that more of the TOC was not degraded in the respiration assay suggests that much of the

TOC is relatively recalcitrant. It is also possible that inorganic nutrients limit rates of microbial mineralization of carbon in these sediments.

The radiorespirometry data should also not be considered to represent in situ rates of metabolism but instead to indicate a metabolic potential for degrading a particular substrate. They are primarily useful for comparisons within and among lithological strata. The aerobic ^{14}C-glucose mineralization data follow the same general patterns observed for the direct counts, TOC, and basal respiration data; i.e., the highest average value occurred in the lacustrine sediment, a lower average was observed in the paleosol samples, and the lowest average value occurred in the fluvial sediment samples. The ^{14}C mineralization data are similar to those for the unsaturated sediments at this site in that a higher percentage of the samples was able to mineralize glucose than acetate (21). The extents of mineralization of radiolabeled glucose and acetate were much lower in the sediments of this study than those of mineralization of ^{14}C-labeled glucose, acetate, and other substrates measured in saturated Atlantic coastal plain aquifer sediments in South Carolina (16, 33). Potential explanations for the low mineralization values include the generally low numbers of viable cells, the inability of some of the cells to utilize either of these substrates, and limitation by inorganic nutrients. It is also possible that the glucose and acetate were added in concentrations below threshold values for biodegradation.

The correlations between TOC and three microbiological parameters (direct counts, basal respiration, and aerobic glucose mineralization) suggest that organic carbon is important to the maintenance of viable microorganisms in these sediments. It is likely that the organic carbon has provided energy for long-term survival of microorganisms, particularly in the fine-grained lacustrine sediments.

In summary, microbial abundance and activities were in general not greater in these saturated sediments than in unsaturated sediments sampled in the same borehole. The low populations and activities may reflect the low rates of precipitation, recharge, and primary productivity at this semiarid site. The numbers and activities were generally higher in the fine-grained lacustrine sediments than in either the underlying paleosol or coarse-grained fluvial sediments. This is in marked contrast to patterns observed in Atlantic coastal plain sediments of the southeastern United States. The saturated paleosol samples of this study were much more depauperate in microbial numbers and activities than many unsaturated paleosol samples collected from nearby locations. The results of this study underscore the complexity of microbial ecology in terrestrial subsurface environments. Depositional environment, hydrology, and climatic conditions all appear to influence the composition of subsurface microbial communities and their associated activities.

ACKNOWLEDGMENTS

We thank Kelley DeGroff, Laurie Rosacker, and Tamara Stevens for technical assistance.

This research was supported by the Deep Microbiology Subprogram of the Subsurface Science Program, Office of Energy Research, U.S. Department of Energy, under grants DE-FG04-90ER-60922 (T.L.K.), DE-AC06-76RLO-1830 (B.N.B., F.J.B., J.K.F., J.P.M., and S.A.R.), and ERKP944 (S.M.P. and T.J.P.).

REFERENCES

1. **Balkwill, D. L.** 1989. Numbers, diversity, and morphological characteristics of aerobic chemoheterotrophic bacteria in deep subsurface sediments from a site in South Carolina. Geomicrobiol. J. **7:**33–52.
2. **Balkwill, D. L., J. K. Fredrickson, and J. M. Thomas.** 1989. Vertical and horizontal variations in the physiological diversity of the aerobic chemoheterotrophic bacterial microflora in deep southeast coastal plain subsurface sediments. Appl. Environ. Microbiol. **55:**1058–1065.
3. **Brockman, F. J., T. L. Kieft, J. K. Fredrickson, B. N. Bjornstad, S. W. Li, W. Spangenburg, and P. E. Long.** 1992. Microbiology of vadose zone paleosols in south-central Washington State. Microb. Ecol. **23:**279–301.
4. **Chapelle, F. H., and D. R. Lovley.** 1990. Rates of microbial metabolism in deep coastal plain aquifers. Appl. Environ. Microbiol. **56:**1865–1874.
5. **Chapelle, F. H., J. L. Zelibor, D. J. Grimes, and L. L. Knobel.** 1987. Bacteria in deep coastal plain sediments of Maryland: a possible source of CO_2 to groundwater. Water Resources Res. **23:**1625–1632.
6. **Colwell, F. S.** 1989. Microbiological comparison of surface soil and unsaturated subsurface soil from a semiarid high desert. Appl. Environ. Microbiol. **55:**2420–2423.
7. **Colwell, F. S., G. J. Stormberg, T. J. Phelps, S. A. Birnbaum, J. McKinley, S. A. Rawson, C. Veverka, S. Goodwin, P. E. Long, B. F. Russell, T. Garland, D. Thompson, P. Skinner, and S. Grover.** 1992. Innovative techniques for collection of saturated and unsaturated subsurface basalts and sediments for microbiological characterization. J. Microbiol. Methods **5:**279–292.
8. **Fredrickson, J. K., D. M. Balkwill, J. M. Zachara, S. W. Li, F. J. Brockman, and M. A. Simmons.** 1991. Physiological diversity and distributions of heterotrophic bacteria in deep cretaceous sediments of the Atlantic coastal plain. Appl. Environ. Microbiol. **57:**402–411.
9. **Fredrickson, J. K., T. R. Garland, R. J. Hicks, J. M. Thomas, S. W. Li, and K. M. McFadden.** 1989. Lithotrophic and heterotrophic bacteria in deep subsurface sediments and their relation to sediment properties. Geomicrobiol. J. **7:**53–66.
10. **Gee, G. W., and J. W. Bauder.** 1986. Particle size analysis, p. 383–412. *In* A. Klute (ed.), Methods of soil analysis, part I. Physical and mineralogical methods, 2nd ed. American Society of Agronomy, Madison, Wis.
11. **Gee, G. W., J. Fayer, M. L. Rockhold, and M. D. Campbell.** 1992. Variations in recharge at the Hanford Site. Northwest Sci. **66:**237–250.
12. **Ghiorse, W. C., and D. L. Balkwill.** 1983. Enumeration and morphological characterization of bacteria indigenous to subsurface sediments. Dev. Ind. Microbiol. **24:**213–224.
13. **Gilbert, R. O.** 1987. Statistical methods for environmental pollution monitoring. Van Nostrand Reinhold, New York.
14. **Haldeman, D. L., and P. S. Amy.** 1993. Bacterial heterogeneity in deep subsurface tunnels at Rainier Mesa, Nevada Test Site. Microb. Ecol. **25:**183–194.
15. **Harvey, R. W., L. H. George, R. L. Smith, and D. R. LeBlanc.** 1989. Transport of microspheres and indigenous bacteria through a sandy aquifer: results of natural- and forced-gradient tracer experiments. Environ. Sci. Technol. **23:**51–56.
16. **Hicks, R. J., and J. K. Fredrickson.** 1989. Aerobic metabolic potential of microbial populations indigenous to deep subsurface environments. Geomicrobiol. J. **7:**67–77.
17. **Insam, H., and K. H. Domsch.** 1988. Relationship between soil organic carbon and microbial biomass on chronosequences of reclamation sites. Microb. Ecol. **15:**177–188.
18. **Johnson, A. C., and M. Wood.** 1992. Microbial potential of sandy aquifer material in the London basin. Geomicrobiol. J. **10:**1–13.
19. **Kieft, T. L.** 1991. Soil microbiology in reclamation of arid and semiarid lands, p. 209–256. *In* J. Skujins (ed.), Semiarid lands and deserts: soil resource and reclamation. Marcel Dekker, New York.
20. **Kieft, T. L.** 1994. Grazing and plant canopy effects on semiarid soil microbial biomass and respiration. Biol. Fertil. Soils **18:**155–162.
21. **Kieft, T. L., P. S. Amy, F. J. Brockman, J. K. Fredrickson, B. N. Bjornstad, and L. L. Rosacker.** 1993. Microbial abundance and activities in relation to water potential in the vadose zones of arid and semiarid sites. Microb. Ecol. **26:**59–78.
22. **Kieft, T. L., and L. L. Rosacker.** 1991. Application of respiration- and adenylate-based soil microbiological assays to deep subsurface terrestrial sediments. Soil Biol. Biochem. **23:**563–568.
23. **Konopka, A., and R. Turco.** 1991. Biodegradation of organic compounds in vadose zone and aquifer sediments. Appl. Environ. Microbiol. **57:**2260–2268.
24. **Long, P. E., S. A. Rawson, E. Murphy, and B. Bjornstad.** 1992. Hydrologic and geochemical controls on microorganisms in subsurface formations, p. 49–71. *In* Pacific Northwest Laboratory annual report for 1991 to the DOE Office of Energy Research, part 2. Environmental sciences (PNL 8000, part 2). Pacific Northwest Laboratory, Richlands, Wash.
25. **Lovley, D. R., F. H. Chapelle, and E. J. P. Phillips.** 1990. Fe(III)-reducing bacteria in deeply buried sediments of the Atlantic Coastal Plain. Geology **18:**954–957.
26. **Madsen, E. L., and W. C. Ghiorse.** 1993. Groundwater microbiology: subsurface ecosystem processes, p. 167–213. *In* T. E. Ford (ed.), Aquatic microbiology: an ecological approach. Blackwell, Oxford.
27. **McMahon, P. B., D. F. Williams, and J. T. Morris.** 1990. Production and carbon isotopic composition of bacterial CO_2 in deep coastal plain sediments of South Carolina. Ground Water **28:**693–702.
28. **Monger, H. C., L. A. Daugherty, W. C. Lindemann, and C. M. Liddell.** 1991. Microbial precipitation of pedogenic calcite. Geology **19:**997–1000.
29. **Murphy, E. M., J. A. Schramke, J. K. Fredrickson, H. W. Bledsoe, A. J.**

Francis, D. S. Slarew, and J. C. Linehan. 1992. The influence of microbial activity and sedimentary organic carbon on the isotope geochemistry of the Middendorf aquifer. Water Resources Res. **28:**723–740.
30. **Pederson, K., and S. Ekendahl.** 1990. Distribution and activity of bacteria in deep granitic groundwaters of southeastern Sweden. Microb. Ecol. **20:**37–52.
31. **Phelps, T. J., C. B. Fliermans, T. R. Garland, S. M. Pfiffner, and D. C. White.** 1989. Methods for recovery of deep terrestrial subsurface sediment for microbiological analyses. J. Microbiol. Methods **9:**15–27.
32. **Phelps, T. J., D. B. Hedrick, D. Ringelberg, C. B. Fliermans, and D. C. White.** 1989. Utility of radiotracer activity measurements for subsurface microbiology studies. J. Microbiol. Methods **9:**15–27.
33. **Phelps, T. J., E. G. Raione, D. C. White, and C. B. Fliermans.** 1989. Microbial activities in deep subsurface sediments. Geomicrobiol. J. **7:**79–91.
34. **Rettalack, G. J.** 1990. Soils of the past. Unwyn-Hyman, Winchester, Mass.
35. **Rosacker, L. L., and T. L. Kieft.** 1990. Biomass and adenylate energy charge of a grassland soil during drying. Soil Biol. Biochem. **8:**1121–1127.
36. **Russell, B. F., T. J. Phelps, W. T. Griffin, and K. A. Sargent.** 1992. Procedures for sampling deep subsurface microbial communities in unconsolidated sediments. Ground Water Monit. Rev. **12:**96–104.
37. **Sinclair, J. L., and W. C. Ghiorse.** 1989. Distribution of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments. Geomicrobiol. J. **7:**15–31.