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MICROBIAL LIFE IN THE DEEP TERRESTRIAL SUBSURFACE

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ABSTRACT

The distribution and function of microorganisms is a vital issue in microbial ecology. The U. S. Department of Energy's Program, "Microbiology of the Deep Subsurface", concentrates on establishing fundamental scientific information about organisms at depth, and the use of these organisms for remediation of contaminants in deep vadose zone and groundwater environments. This investigation effectively extends the Biosphere hundreds of meters into the Geosphere and has implications to a variety of subsurface activities.

INTRODUCTION

Groundwater constitutes 95% of the freshwater reserves in the United States, and nearly half of the nation's population depends on such water for drinking (1). The withdrawal of freshwater in 1980 was estimated at 88.5 billion gallons per day (2) from a total storage capacity of between 33-100 quadrillion gallons (3, 4), and the contamination of these resources is increasing. Every state has reported the presence of groundwater contamination (5). Moreover, Lehr (4) and EPA (6) have estimated that between 1-2% of the groundwater is contaminated with heavy metals, microbial pathogens, and/or inorganic and synthetic organic chemicals. Pye and Patrick (5) pointed out that such estimates were very conservative because only a limited number of point-source contaminants and no nonpoint-source contaminants were considered.

There are only two remedial action options for deep groundwater and subsurface contamination: (i) *in situ* remediation and (ii) conventional withdrawal, treatment, and disposal. Prevention of groundwater contamination is clearly more cost-effective than the present cure, but the fact remains that vast quantities of our nation's groundwater resources are contaminated and require remediation. The presence of a significant microflora in the deep subsurface has enormous implications for *in situ* remediation or biotransformations of toxicants in contaminated aquifers and deep unsaturated sediments.

The Microbiology of the Deep Subsurface is a focus of the above investigators in conjunction with the Department of Energy's Office of Health and Environmental Research programs (7, 8). It is designed to answer basic fundamental microbial ecology questions about microbiological life hundreds of meters below the earth's crust. We have chosen to concentrate on the deep terrestrial subsurface environments and its biosphere for the following reasons: a) fundamental scientific information about microorganisms at depths greater than 30 meters is virtually nonexistent; b) many of the nations largest aquifers are deep systems and are important for population centers and future energy production needs; and c) deep, inaccessible subsurface ecosystems pose serious long-term restoration challenges because of contamination associated with energy and defense production activities (8).

The distribution and function of microorganisms is a vital issue in microbial ecology. Traditionally, shallow and deep subsurface strata were considered to be devoid of microbial life (9). Waksman (10, 11) and Starc (12) demonstrated by classical plate count techniques that few, if any, bacteria occurred below the root zone of plants. Studies by Leenheer et al. (13) indicated that the concentration of dissolved organic carbon in subsurface aquifers was typically around 1 mg/L. Thus, microbiologists reasoned that such concentrations were too low to support a significant bacterial community. Because of these observations and because of the difficulty and expense of sampling at depth, the microbiology of the deep terrestrial subsurface was essentially ignored for 50 years. Although much work was done on

deep marine sediments during this period, almost nothing was learned about the microbiology of the aquifer and vadose zone materials.

Before the early 1980's, extensive microbiological studies were confined to the upper 10 meters of the earth's crust, although bacteria had been detected in deep marine sediments and stratal water samples often associated with sulfur and oil deposits. Much of this work has been recently been adequately reviewed (14).

SITE AND HYDROLOGICAL CONSIDERATIONS

Subsurface sediment samples were collected from the Savannah River Plant (SRP), a 768-km^2 restricted-access facility near Aiken, SC, which is operated for the Department of Energy by the DuPont Co. SRP is located in the Upper Atlantic Coastal Plain, nearly 32 km southeast of the Fall Line, which separates the Piedmont and the Coastal Plain provinces. It is on the Aiken Plateau, a comparatively flat surface that slopes southeastward, which is dissected by several tributaries of the Savannah River. The surface is underlain by about 400 m of unconsolidated sands, clayey sands, and sandy clays that in turn, are underlain by dense crystalline metamorphic rock or consolidated red mudstone.

Microbiological samples were collected from similar stratigraphic horizons at three different sites; P24, P28 and P29 (Figure 1). The currently accepted names of the stratigraphic formations are given in the cross-section diagram in Figure 1. The Cretaceous-age aquifers

below the Ellenton confining unit (Pee Dee, Black Creek and Middendorf Formations) yield >4000 L/min in properly designed and constructed wells, whereas the Congaree Formation yields 400-1000 L/min in many locations. Apart from these aquifers, the remainder of the Coastal Plain sediments transmit water but not in sufficient quantity to be classified as major aquifers. Confining clay beds are present in the strata and retard, but do not totally prevent, the interchange of water between formations.

The direction of groundwater movement is governed largely by the depth of incision of the creeks that dissect the Aiken Plateau, while the flow in the deep sediments is governed by the Savannah River. In the vicinity of the microbiologically sampled formations, the water levels in the Congaree are drawn down by natural discharge to a greater extent than those in the Cretaceous-age aquifers. Thus, there is a groundwater head reversal at the Congaree Formation, and the vertical groundwater gradients below this formation are upward. Such hydrological data are important since they affect the various hypotheses available to explain the presence of the isolated microorganisms in these deep terrestrial habitats.

There are three hypotheses as to how the microorganisms became associated with the sediments at SRP. The organisms could have been displaced downward during weathering until they reached the depths at which they were isolated. The problem with such a hypothesis is that the groundwater hydrology for the deeper aquifers in the Middendorf formation has a hydrological head reversal so that the

downward percolation of the organisms is resisted by an upward hydrological gradient (15). The second hypothesis is that the organisms may have been transported into the deep sediments from the recharge area located 60-80 km from some of the site locations (15). Horizontal velocities average 20 m/year, so that if the microorganisms moved with the groundwater in the Middendorf aquifer, simple calculations suggest that the organisms could arrive at the deposition location in 3000 to 4000 years. Recent studies by De Victoria et al. (16) from these same aquifers suggest that the bacteria are highly attached to the sediment particles in the formations. Thus, the movement of microorganisms over such distances would appear difficult. The third hypothesis states that the microorganisms were laid down when the sediments were deposited. The hypothesis remains to be tested, but studies on the age of the water in the aquifer and the relational age of the microorganisms in the strata are being investigated.

COLLECTION, HANDLING, AND DISTRIBUTION OF SEDIMENT SAMPLES

Surface soil and 15 subsurface sediment samples were collected from each of the three sampling sites using the protocol of Phelps et al. (17). Much attention was given to the collection of microbiologically intact and uncompromised samples. All samples were collected in stainless steel core-liners which were steam-cleaned before use. Each sample was collected in a way that minimized the influence of drilling

mud. This was done by removing the muds from circulation, by sampling without muds, or by using a drill bit with a shoe that extended beyond the muds. Additional details of the drilling operation have been described elsewhere (17).

Retrieved materials were immediately removed from the sampler and transported to a mobile laboratory adjacent to the drill site. Samples were extruded from the coring tubes into a glove bag continually flushed with nitrogen. The outer third to two-thirds of each 7.5 cm core was aseptically pared away, so that only the center most portion of the core was used for microbiological investigations. Pared samples were quartered, mixed, dispensed into sterile nitrogen-flushed Whirl-Pak bags, stored in nitrogen-flushed canning jars, sealed, iced, and air-expressed to the investigators within 16 hours of sampling.

PHYSICAL AND CHEMICAL CHARACTERISTICS OF SAMPLES

Pore water from each sample was obtained as previously described (18) and assayed for E_h, pH, conductivity, ammonia, and fluoride (selective probe analyses); dissolved organic and inorganic carbon (normal NDIR detection of CO_2); dissolved major and minor elements (Inductively Coupled Argon Plasma Emission Spectrometry); and major and minor anions (ion chromatography). Specific analysis of the Fe^{+2}/Fe^{+3} was made on a modified Dionex 2020 ion chromatography; NO_2^-/NO_3^- analysis was determined by ion chromatography and verified by comparison with colorimetric determinations (19).

Selected pore water chemical and sediment physical properties from one of the sample sites, P28, are shown in relation to sample depth and geological stratum in Table 1. Anion/cation balances were calculated to identify deficiencies in analyses of major components, potential interferences in analyses, presence of major species not detected in the analysis scheme, or errors in data handling and recording. Anion/cation balance deficits in excess of 10% occurred in near-surface samples and where pore waters contained very low total ionic content. In near-surface samples, an anion deficit is not unusual since organic acids can account for missing anions (19) and can interfere with inorganic anion analyses (20). A reasonable anion/cation balance (< 6% difference) was observed for all other core segments suggesting that the analyses detected the majority of the ionic species present.

Considerable heterogeneity in particle size and total ionic content was evident within major geological formations when the data from the three sampling sites were grouped and expressed as ranges (Table 2). The concentrations of organic carbon, ammonia, nitrate, and phosphate were variable within the major formations, indicating the presence of distinct subformations in the Cretaceous-age sediments.

With a few exceptions, pH increased at each sample site when clay content increased. The pH was relatively high in the Congaree but relatively low in the Black Creek and Ellenton Formations (Table 2).

Pore water ionic composition in the formations is dominated by $CaSO_4$ in the Upland, NaCl in the Dry Branch, Na^+/Ca^{+2} and Cl^-/HCO_3^- in the McBean, $NaHCO_3^-$ in the Congaree, Na^+/Ca^{+2} sulfate in the Ellenton,

Na^{+}/Ca^{+2} sulfate in the highest segments of the Pee Dee, and primarily NaCl in the remaining segments of the Black Creek and Middendorf Formation. Total ionic content in the pore waters was low in the Dry Branch, McBean, and Pee Dee, but generally increased with increasing depth in the Black Creek and Middendorf.

With the domination of the sulfate ion in the pore water and the presence of dissolved organic carbon, the isolation of sulfate-reducing bacteria with depth is not surprising (Table 8). Although the oxygen concentrations were not measured in the sediment pore water, samples of bulk water collected from well clusters screened at various depths at the three sites indicated that the levels of oxygen ranged from 0.16-7.40 mg/L (P29); 0.02-8.26 mg/L (P24); 0.03-4.23mg/L (P28). The deepest wells in the Middendorf formation generally had the highest concentration of dissolved oxygen. Thus the levels of dissolved organic carbon, oxygen, nitrogen, and phosphate were sufficient to support a diverse heterotrophic microbial community. The concentration of inorganic nutrients that can serve as energy sources for a variety of chemoautotrophic microorganisms may be restrictive in these environments as demonstrated by the infrequent isolation of chemoautotrophic bacteria.

DENSITY AND DIVERSITY OF CHEMOHETEROTROPHIC BACTERIA

The density of culturable aerobic (and/or facultatively anaerobic) chemoheterotrophic bacteria in each subsurface sediment sample was determined with the plate-count procedure described by Balkwill and Ghiorse (39). Several plating media (ranging from nutrient-rich to extremely dilute, and/or nutrient poor) were used, including peptone-tryptone-yeast-extract-glucose (PTYG) medium, 1% PTYG medium (1:100 dilution of PTYG), and Difco Brain Heart Infusion (BHI). Plates were incubated at several temperatures for 2 weeks to 6 months, depending on the medium and the temperature.

The plate count data indicated that a substantial number of viable aerobic (and/or facultatively anaerobic), chemoheterotrophic bacteria were present in many of the deep subsurface sediments (Table 3). The bacterial densities varied widely from one stratum to another, ranging from no culturable organisms to 3.3×10^7 CFU/gdw (gram dry weight) of sediment. Despite the variations from one stratum to another, there was no obvious overall decrease in viable bacterial numbers with increasing depth. The lowest viable counts were observed for poorly transmissive sediments that contained more than 40% clay (R=-.917 for P24; R=-.299 for P28; R=-.688 for P29). Although there were some exceptions, the variations in viable counts were related to the formations and were similar at all three drilling sites.

Viable counts were consistently higher on dilute, nutrient-poor media (i.e. 1% PTYG), than on the richer media (PTYG, BHI). Over 90% of the bacteria isolated from the SRP subsurface sediments were able to grow under low-nutrient conditions, but a significant minority,

approximately 10%-40%, grew rapidly (18-24h) on rich medium. Most Probable Number (MPN) analyses, using a 5-tube assay with equivalent media, agreed closely with the plating results (data not shown).

The highest viable counts were obtained at an incubation temperature of 23°C, the *in situ* temperature of the sediments, although counts at 37°C were similar. Plate counts at 4°C were generally lower than those observed at 23° or 37°C. Plate counts at 55°C were also very low (generally less than 10^3 CFU/gram), indicating that very few thermophiles were present in these sediments. Very low numbers of bacterial spores (generally less than 10^3 CFU/gdw, as determined by plating of heat-shocked samples) were present in the SRP sites. These results indicate that most of the microorganisms were mesophilic, and that heat-resistant bacterial spores were not a prominent component of the deep subsurface microbial community at the SRP sites.

Morphologically distinct colonies were isolated from all of the enumeration plates, and the occurrence and relative frequency of each colony type was noted. A considerable variety of colony types was observed on the plates for many sediments as illustrated in Figure 2 ; in fact, 62 distinct colony morphologies were isolated from a single sediment sample (178 m). The greatest colony diversity was observed on full strength PTYG medium. Such diversity was not related to depth but rather fluctuated from one strata to another (Figure 2). Vertical distribution analysis of the colony types observed on PTYG plates at 23°C revealed that 74-89% of the colony types were detected only at one depth (i.e., in a single stratum), while an additional 8-16% were

detected at only 2 depths, and only 2-3% of the total colony types appeared at four or more depths. These results indicate that the heterotrophic bacterial composition of the deep terrestrial subsurface was diverse and varied markedly from one stratum to the next.

Each colony type was subjected to 22 biochemical tests using API Rapid NFT strips (Analytab Products, Plainview, NY) for physiological characterization (Balkwill submitted). The results of the physiological tests supported the colony diversity observations in that there were very few isolates with identical physiological characteristics between or among strata. Based on the API physiological testing, over 85% of the 1100 isolates examined were unique to a single depth, while only 3% of the tested isolates were present in sediments from four or more depths.

ENUMERATION OF EUKARYOTIC MICROORGANISMS

Protozoa populations were determined using a modified version of the MPN technique as described by Sinclair and Ghiorse (22), which was able to detect as few as 2 protozoa per 10g of sediment sample. Fungi were enumerated on Sabouraud's Dextrose Agar containing penicillin and streptomycin (to inhibit bacterial growth), or 5% Potato Dextrose Medium (Difco) at pH 5.6. Algae were grown on mineral salts agar in the presence of direct sunlight. In general, algae, fungi, and protozoa were either absent or present at low densities throughout the core profile (Table 4). As was the case with heterotrophic bacteria (Table 3), the lower eukaryotic densities were generally associated with

sediments that had greater than 20% clay. Although the greatest densities of algae and protozoa were found in the surface soils, protozoa were usually found in the substantial numbers where the platable bacteria approached 10^7/gdw in the subsurface. The data for site P28 indicated that one of the geological formations, the Congaree, had protozoan densities high enough to play a role in the regulation of the bacterial communities at that depth (Table 4). Fungal densities were highest at the surface, immediately declined at 15 m, with none of the subsurface sediments containing greater than 35 CFU/gdw sediment.

MEASUREMENTS OF MICROBIAL BIOMASS

Bacteria in each sediment sample were enumerated by direct epifluorescence microscopy using acridine orange direct counting (AODC) techniques to estimate the total bacterial density (23). The AODC was compared with the numbers of iodonitrophenyl tetrazolium dye reducing cells (AOINT) to determine the density of actively respiring cells in the total bacterial population (24). The results shown in Table 5 for P28 are similar to the other sites in that, a number of the samples collected from the 3 sites (19 of the 49) had platable bacterial counts greater than 90% of the total AODC estimates. The AOINT measurements reflect a bacterial population (up to 46%) that reduced the tetrazolium dye and indicated electron transport activity. The data in Table 5 show that the microbial community in these samples was viable, and that a surprisingly high percentage of the bacterial

population was cultured. This is most unusual since it is a general observation in soil microbiology that less than 10 % of the total bacterial population as observed by AODC is culturable (9).

Bacterial biomass in subsurface sediment samples was also determined by phospholipid analyses (25). Sediment samples were immediately frozen at the drill site, transported to the laboratory, lyophilized, and stored until analyzed. Lipids were extracted from 40g of sediment, using a modification of the Bligh and Dyer extraction procedures (26). Surface soils contained 6.6 ± 0.3 nmoles phospholipid ester-linked fatty acids (PLFA)/gdw, equivalent to the AODC counts of 10^9/gdw, while subsurface materials contained 3 to 5 orders of magnitude fewer PLFA. Clay zones contained less PLFA than the water-bearing sands.

ENUMERATION OF SPECIFIC BACTERIAL GROUPS

Chemolithotrophic bacteria were enumerated with the techniques outlined in Table 7. While detected in many of the core samples, the populations of chemolithotrophic microorganisms were generally sparse throughout the profile (Table 5). Nitrifying bacteria, while present at low population densities, were most frequently encountered in cores from P24 and P28. The total nitrifying bacterial populations correlated significantly with pore-water ammonium concentrations ($r=.83$, $p=.001$), and pore-water nitrate in P28 ($r=.67$, $p=.006$). Ammonium-oxidizers

made up the greatest proportion of total nitrifiers in all of the core samples.

Sulfur-oxidizing bacteria were the most numerous and frequently encountered group of chemolithotrophs, and their presence or absence corresponded well between the same formations in P24 and P28. The population size of sulfur-oxidizing bacteria did not correlate with pore-water pH, Eh, % clay, NH_4^+, NO_3^-, or SO_4^{-2} in any of the cores, but correlated significantly (R=.64, p=0.1) with the population of sulfate-reducing bacteria in the P28 samples. The data suggest a dependency of the chemolithotrophic sulfur-oxidizers on the reduced sulfur compounds produced by the sulfate-reducing bacteria (SRB). Most-probable-number assays for sulfate-reducing bacteria (27) demonstrated the presence of these organisms in many strata in all three boreholes (Table 8). The number of SRB varied from undetectable to greater than 10^5/gdw and were not correlated with sulfate levels at any of the sites. Generally, higher numbers of sulfate-reducing bacteria were found in the more transmissive strata, but viable SRB populations were detected in dense clays.

Enrichments for microaerophilic microorganisms indicated that this physiological group occurred repeatedly throughout the subsurface profile. Subsequent transfers onto nitrogen-free media indicated that many of these bacteria fixed nitrogen (data not shown). In nitrogen-limited environments, nitrogen fixation provides nitrogen-fixers with a competitive edge as well as an important nutrient input to the deep subsurface environment upon cell death and subsequent mineralization.

Methane-oxidizing bacteria were present in a majority of the core samples that supported significant populations of heterotrophic bacteria, as indicated by pellicle formation in methane enrichment cultures. Pellicle formation was never observed in the controls, which consisted of inoculated carbon-free minimal media incubated without a methane atmosphere.

Hydrogen-oxidizing bacteria were observed most frequently in samples from site P28. These bacteria are generally facultative heterotrophs that use H_2 as an electron donor and grow chemoautotrophically (28). These bacteria frequent occurrence in P28 sediment samples suggests that past geological conditions (in conjunction with the hydrology at this site) are influencing the microbiological composition.

Isolates collected from the sediments of the three sites were tested for their sensitivity to six antibiotics (Penicillin, Ampicillin, Carbenicillin, Kanamycin, Streptomycin, and Tetracycline). Bacteria isolated from the drilling muds were also subjected to the same set of antibiotics to determine their sensitivity. Based on the mean antibiotic inhibition zone diameters for all the antibiotics, the bacteria in the return drilling muds were from a different population than all the subsurface isolates ($p=0.01$) as determined by the Wilcoxin Signed Rank Test (29; Fredrickson, et al., in press).

Total coliform bacteria were also enumerated in samples from the three sites using standard techniques (30) except that incubation periods (10 days) and temperatures (24°-26°C) for presumptive,

confirmed, and completed tests were altered. The data in Table 5 show the population of coliforms with depth at P28. Coliforms were enumerated from the surface and seven strata taken from P28 and P24, while four strata at P29 were also positive in this assay. The more shallow water-bearing zones of the McBean/Congaree formations consistently harbored coliforms. Coliforms were also detected in the deeper Middendorf formations and elevated temperature testing (44°C) of isolates from these samples indicated that these organisms were not from direct fecal contamination. Assays of the drilling muds and the water used to make the drilling muds were negative for coliforms. These results strongly suggest that coliforms are part of the indigenous subsurface microflora.

MICROBIOLOGICAL ACTIVITY MEASUREMENTS

Activity measurements based on ^{14}C-acetate incorporation into lipids and ^{3}H-thymidine incorporation into DNA (31,32) indicated that the subsurface microbial populations were metabolically active (Table 6). The lipid analysis data indicated that the surface soil samples were generally 100 to 1000 times more active than the subsurface sediment samples, although substantial activity was noted in the sandy samples from the Black Creek Formation. The microbial activity in the nontransmissive boundary clays was consistently low or at background, while the coarser textured transmissive sediments always had significant activity. The incorporation of acetate into lipids was quite

pronounced in many P28 samples and demonstrated levels of incorporation substantially greater than the corresponding strata from the P24 or P29 sites. Uptake of ^{3}H-thymidine supported the ^{14}C-acetate incorporation data in that the clay zones were significantly lower in activity than the more sandy zones. The samples from the P28 site had a greater number of active strata than did either of the other two sampling sites. These data suggest that microorganisms were present that readily metabolized indigenous substrates.

Metabolic activity, as measured by the reduction of INT, has been used in a number of terrestrial (33) and aquatic systems (34) to enumerate the active microbial populations as well as selected bacteria in autecological studies (35). This method was used to determine the proportion of the total microbial population, as measured by AODC, which was metabolically active. In some of the samples, greater than 40% of the microbial population was active for the reduction of the INT (Table 5).

Carbon mineralization studies for glucose and indole indicated that microbial activity was present in nearly all of the samples, but this activity varied greatly with depth. Mineralization of indole and glucose was generally greater in the surface sediments than in the subsurface samples, and glucose was more readily metabolized than indole. All mineralizations were stimulated by the injection of sterile air into the headspace of the serum bottles, including sediments previously shown to be anaerobically inactive. Such data suggest a predominance of microbial aerobic metabolism.

Denitrification (reduction of nitrate to nitrogen) was measured by accumulation of nitrous oxide after incubation of the sediments for 72hours in the presence of acetylene. Duplicate sediment samples (2g) were incubated with 1 mL of prereduced deionized water. Indigenous denitrification (without carbon amendments) was detected in numerous sediments from P24, P28, and P29. The data in Table 5 show that the surface sediments of P28 had the highest activity (25.8 nmoles/gdw), while sediments from the McBean Formation exhibited the highest activity of all the subsurface formations. Denitrification was more pronounced in the sandy zones than in the clay sediments. Addition of nitrate enhanced denitrification, whereas the addition of a carbon source, succinate, had no stimulatory effect (data not shown). These data suggest that denitrification in these sediments is limited by the availability of nitrate and not by a particular carbon substrate.

Dissolution of iron and manganese from sediment samples occurred in 20% slurries, with and without carbon and nitrogen additions under anaerobic conditions, as determined by an increase in Fe and Mn concentration after 0.22μm filtration. Significant anaerobic dissolution of Fe and Mn was observed in almost all subsurface sediments, but dry clays exhibited the least activity. The addition of carbon and nitrogen enhanced Fe and Mn dissolution, indicating that dissolution of metals was catalyzed either directly or indirectly through microbial activity (data not shown).

Additional investigations were undertaken to determine the indigenous rates of anaerobic microbial activities including

methanogenesis, sulfate reduction, $^{14}CO_2$ evolution, and the microbial response to added electron acceptors and donors. Strict anaerobic techniques as summarized by Costilow (36) were used throughout these investigations. Short-term assays for methane production and sulfate reduction were conducted with sediment samples (3.0g) amended with 0.5 mL of sterile distilled water, with and without the following ^{14}C-amendments: formate + acetate, bicarbonate, lactate, glucose, indole, and $^{35}SO_4$. Samples were incubated under $5\%H_2/95\%N_2$ in 10 mL serum bottles, and the bottle headspace was assayed for acid-volatile ^{35}S, $^{14}CO_2$, and $^{14}CH_4$. The data were analyzed with respect to negative controls and those receiving neither sediments nor amendments. The short-term assays showed that anaerobic microbial activities were relatively minor in that methanogenesis was not observed in any of the samples from P24, P28, and P29 after 5 days of incubation. This was true even in the presence of added formate, acetate, and bicarbonate. Further, an MPN assay for methanogens indicated that these organisms were below detectable levels (0.3/gdw) in all the samples except for two strata in P29 and one in P24. Long-term assays (6 months) for methanogenesis with larger samples (10-25g) were positive in only a few samples from P24 and P29, while P28 samples showed methane production in 10 of the highly transmissive strata.

Long-term anaerobic activity assays indicated the potential for acetogenic fermentation in most samples of P28 (13) and P29 (10) and the shallow, transmissive strata of P24 (5). At each site, lactate and formate were anaerobically metabolized in all strata except the

consolidated clay layers. In 10 of the transmissive samples from P28, mass balances indicated that anaerobic carbon mineralization was mostly by methanogenesis. Methanogenesis was also evident in anaerobically incubated surface samples but was detectable in only trace amounts in one other stratum from the Pee Dee formation.

In nine of the transmissive samples from P28, anaerobic incubations indicated that up to 300 μgC/gdw of indigenous, fermentable carbon was present. Acetate accumulations and methane production in samples without exogenous carbon amendments indicated that the potential for anaerobic fermentations in these strata were not likely to be carbon limited. In P24 and P29, the factors limiting fermentation were less obvious since acetogenesis and methanogenesis were only infrequently measured even in carbon-amended samples.

The data from the diversity and activity measurements indicate that the terrestrial deep subsurface is a functioning biological ecosystem. The microbiological structure of the ecosystem may vary, but the functional aspects of the ecosystem include many if not all of the important microbiological components.

PHYSIOLOGICAL CHARACTERISTICS OF THE SUBSURFACE MICROFLORA

The subsurface microflora of the SRP site was very diverse in terms of its specific physiological characteristics and metabolic activities. The sediment samples contained microorganisms that metabolized glucose, indole, benzoate, lactate, and formate both anaerobically and aerobically. Of the 1100 isolates that have been tested for specific metabolic characteristics (with API Rapid NFT strips), 33% assimilated all the carbon sources tested (i.e., arginine, lysine, ornithine, sodium citrate, urea, tryptophane, glucose, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose), while most of the isolates assimilated over 50% of the tested compounds.

The ability to assimilate individual organic compounds as a sole carbon source was common among subsurface bacterial isolates from different depths. Of the 1100 isolates tested, 91% assimilated malate, 87% D-gluconate, 74% glucose, and 73% citrate. Thus, as a group, these isolates were versatile with respect to the range of compounds they metabolized. Some of the tested physiological characteristics occurred commonly among subsurface microbial isolates from different depths.

None of the tested isolates demonstrated tryptophanase activity, and only 6% were able to reduce nitrate to N_2. Only 8% of the isolates obtained on the PTYG plates fermented glucose, even though 74% of the isolates were able to assimilate glucose aerobically. Thus, most of the

bacteria isolated on aerobically incubated PTYG plates have an oxidative rather than fermentative metabolism.

DISCUSSION

The reported investigation has demonstrated that the terrestrial deep subsurface is a habitat of great biological diversity and activity. The study has shown that microorganisms are present at depth, and that the traditional scientific concept of an abiological terrestrial deep subsurface is not valid. The presence of microorganisms at depth has been shown by a variety of direct and indirect techniques and by a number of different investigators at various laboratories. The data indicate an extension of the Biosphere to depths of nearly 300 m into the Geosphere, and that unique microbiological niches have been established and colonized by a variety of bacteria in the terrestrial deep subsurface. Microbial populations in these deep sediments are more active than had been expected from the scientific literature, and thus an enhanced role might be expected for microorganisms that influence the groundwater chemistry (37, 38) and the geological processes.

The data demonstrated that the density of microorganisms does not decrease significantly with depth. Such information suggests that the Biosphere may extend a substantial distance into the Geosphere and only cease to exist where temperature and pressure become the limiting factors for life.

One of the problems associated with terrestrial subsurface microbiological research is that of obtaining and assuring noncontamination of the microbiological populations of the subsurface strata during the sampling effort. The investigators were convinced that contamination from the drilling muds was nonexistent, or at the most, very minimal and was not a problem. The data that support this conclusion include: stratification of distinct physiological, biochemical and genetic types of microorganisms; distinct stratification of densities and metabolic activities of the microorganisms; distinctive pore-water chemistry of the drilling muds as compared to the sediments; stratification of the geophysical and geochemical data; stratification of antibiotic and metal resistance and initial plasmid patterns of the isolated microorganisms. A more extensive discussion of the lines of evidence are developed by Phelps et al., (17).

Until now, detailed investigations of the subsurface microflora have been confined to comparatively shallow (i.e., < 10-20 m) environments (14). The present study has shown that the deeper subsurface microflora differs from that seen in the shallow subsurface in several ways. The deep subsurface microflora (specifically, the platable microflora) were considerably more diverse (see below); exhibited a larger proportion of oxidative, Gram-negative rods; and contained a much larger proportion of forms that grew readily in the laboratory even on nutrient-rich media as compared with the microflora of a shallow aquifer in Oklahoma (39, 40).

The diversity of the microbiological communities in deep terrestrial sediments is one of the most striking findings in this study. Both structurally and functionally, a wide and diverse variety of metabolically active microorganisms were present and capable of transforming a variety of organic and inorganic compounds. These sediments contained many types of aerobic chemoheterotrophic bacteria (colony analysis and API data), as well as a wide assemblage of other forms (albeit in somewhat lower numbers). These results are in distinct contrast to those from shallow aquifers, in which it has been suggested that only a limited number of strains (probably those that are specially adapted for growth and survival under low-nutrient conditions) are present as numerically predominant forms (39, 40). The diversity of platable forms also decreased sharply with depth in shallow aquifers, but that was not the case in the deeper sediments studied here. Such diversity is surprising for a presumably nutrient-limited environment and contrary to traditional thinking in soil microbiology. In this study, the diversity was not limited by depth. Diversity limitation was observed only in the nontransmissive zones where the concentration of clays was greater than 20%. Such zones may not be less diverse but, because they contain less microorganisms than the more transmissive sand zones, they were not readily evaluated for diversity.

The documentation of active bacterial communities at depth establishes the potential for *in situ* restoration of deep terrestrial vadose zones and aquifers. The ability of deep subsurface bacteria to

grow on rich media and to utilize a wide variety of carbon sources suggests the possibility that the organisms could be stimulated *in situ* and their degradative capacities enhanced for the complete degradation or detoxification of some hazardous wastes. The microflora of deep unconsolidated sediments appear to vary considerably in structure but not in function from one stratum to another. This variability occurs even in the same stratum between different boreholes. Such an observation is important with respect to the possible use of microbial systems or consortia to mitigate groundwater contaminants, since the microbial populations at different depths may selectively respond to contaminants or nutrient amendments in different ways. It may be necessary to use very distinct strategies when dealing with contamination problems in different geological strata. The interactions among the varied microbial types and the manner in which they interact within their environment are issues that require investigation.

Additionally, this investigation has isolated an extensive number of bacteria which may be new to the scientific community and, at the very least, provide investigators with a new source of genetic material from organisms adapted to living and metabolizing hundreds of meters below the earth's surface. Such genetic information may be of importance in genetically engineered systems that will detoxify harmful material under real world conditions.

The Microbiology of the Deep Subsurface Program is an initiative that has demonstrated the presence of numerous, active and diverse microorganisms associated with the sediments of the terrestrial deep

subsurface. The understanding of the microbiology of the deep terrestrial environment is not only an important advance for the sciences of microbial ecology, geomicrobiology and geology, but has great applicability to a variety of industrial and governmental concerns, (e.g., fossil fuel recovery and storage, deep waste repositories, groundwater storage and retrieval, biologically produced products and transformations, as well as transport and fate of groundwater contaminants). This investigation opens new avenues for research and fundamental investigations into the interaction between the Biosphere and the Geosphere as well as the possibilities of *in situ* remediation strategies of deep groundwater and vadose zones.

Future deep borings for microbiological investigations are scheduled for 1988 and 1989 and will provide increased knowledge of life at greater depths in association with other depositional environments. The presence of diverse microbiological life with depth provides basic microbial ecology, geological, chemical, and physical information about unique terrestrial habitats where microorganisms live.

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Figure and Table Legends

Figure 1. Location of sampling sites at the Savannah River Plant for the Microbiology of the Deep Subsurface Program.

Figure 2. Distribution of bacterial colony types in sediments collected from P28, and grown on PTYG agar.

Table 1. Pore water chemistry and physical composition of samples from Site P28.

Table 2. Concentration ranges of selected parameters in formations at SRP.

Table 3. Numbers of viable, aerobic, chemoheterotrophic microorganisms in deep subsurface environments.

Table 4. Biomass estimates for protozoa, algae, and fungi in P28.

Table 5. Biomass estimates (gdw^{-1}) for selected microbiological parameters in P28.

Table 6. Phospholipids and isotopic measurements with depth for P28 sediment cores.

Table 7. Procedures used for the characterization of chemolithotrophic and other physiological types of bacteria from the SRP site.

Table 8. Most probable numbers of sulfate-reducing bacteria from sediments collected from the SRP sites.

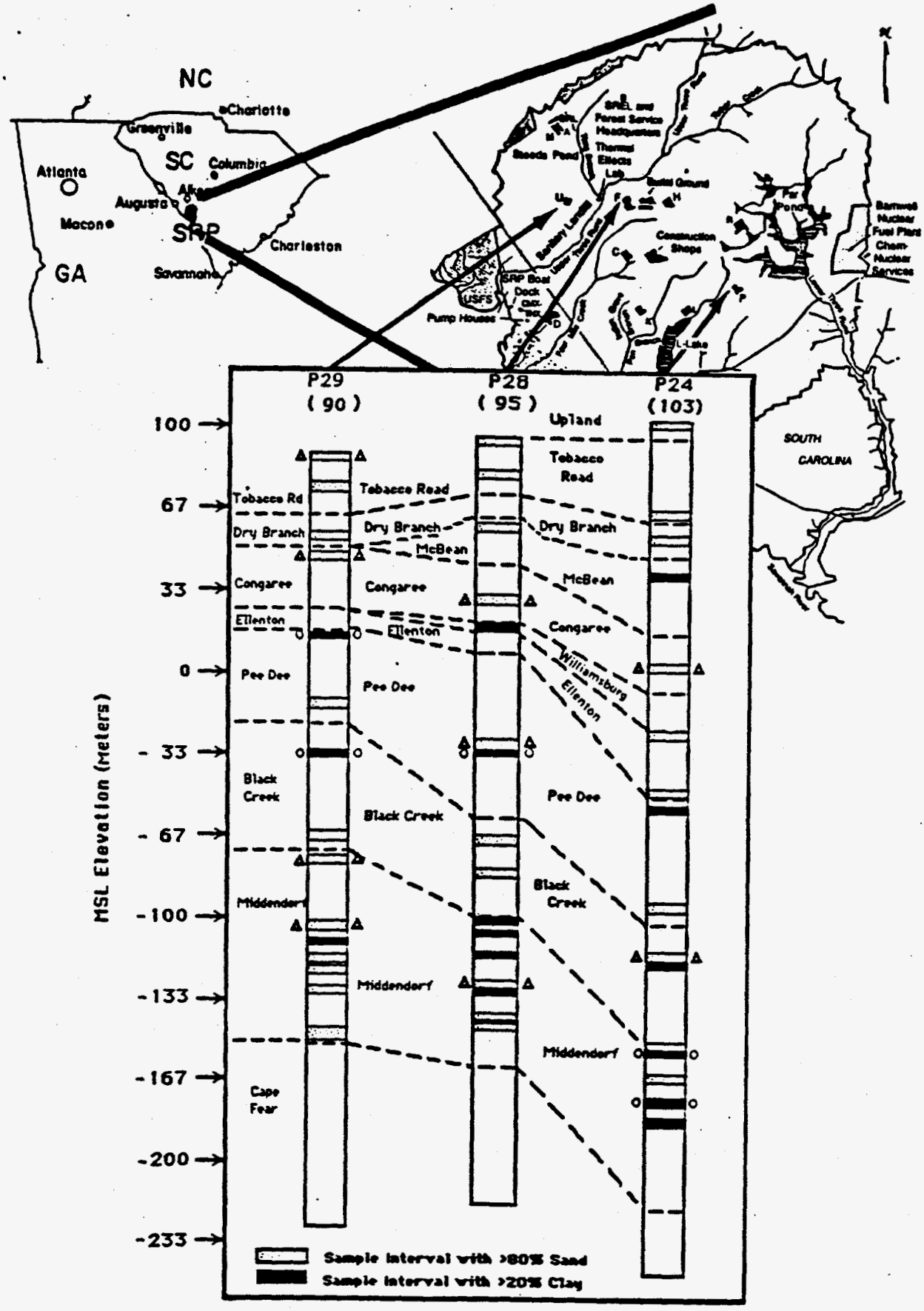
NC
Charlotte
Greenville
SC
Columbia
Atlanta
Augusta
Aiken
Macon
SRP
Charleston
GA
Savannah
SRNL and Forest Service Headquarters
Thermal Effects Lab
Reeds Pond
Burial Ground
Fire Pond
Construction Shops
Barnwell Nuclear Fuel Plant
Chem-Nuclear Services
SRP Boat Dock
USFS
Pump Houses
L-Lake
SOUTH CAROLINA
P29
(90)
P28
(95)
P24
(103)
MSL Elevation (meters)
100
67
33
0
- 33
- 67
-100
-133
-167
-200
-233
Upland
Tobacco Road
Tobacco Rd
Dry Branch
McBean
Congaree
Ellenton
Pee Dee
Williamsburg
Black Creek
Middendorf
Cape Fear
Sample interval with >80% Sand
Sample interval with >20% Clay

FIGURE 1.

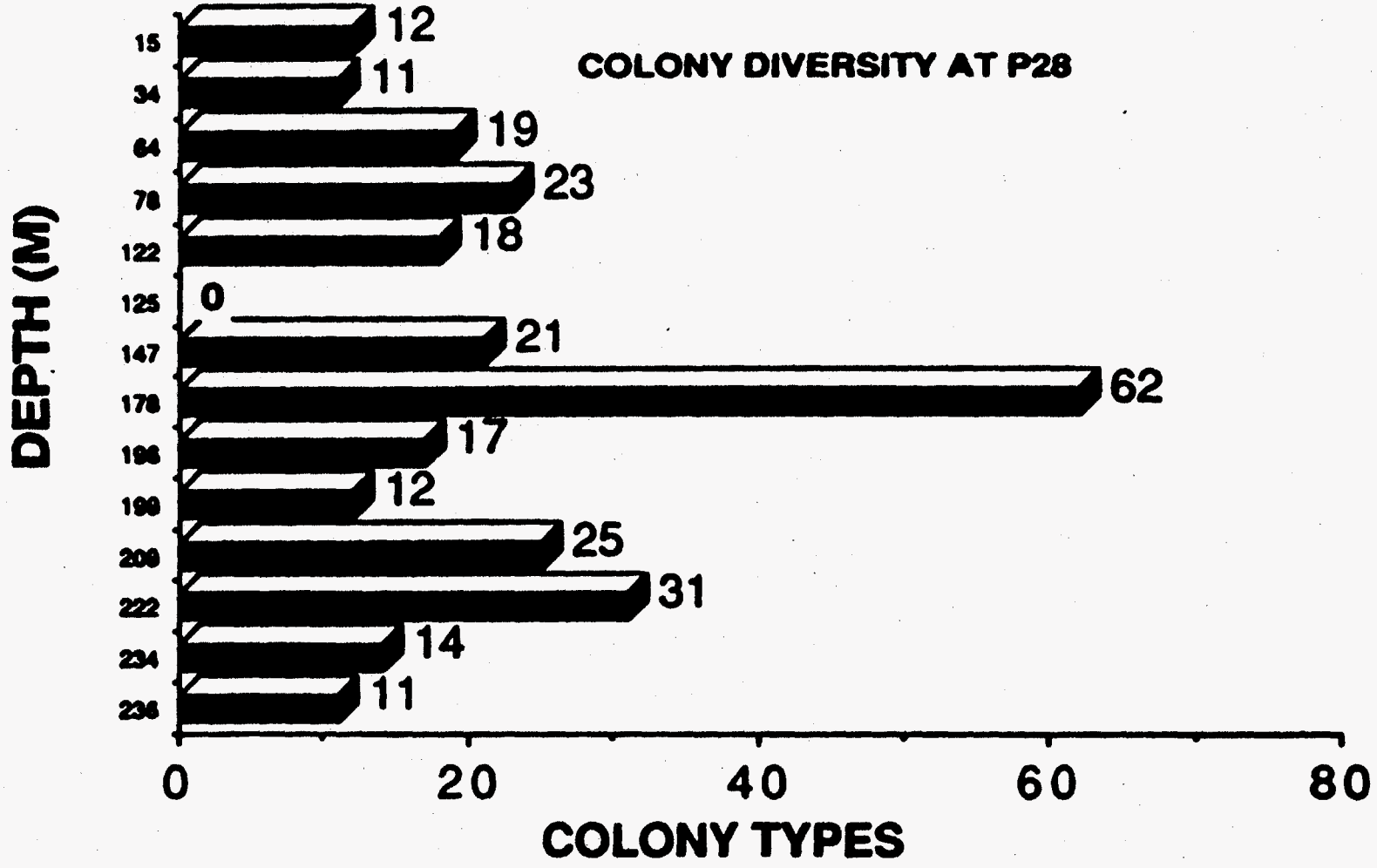
COLONY DIVERSITY AT P28
DEPTH (M)
15
34
64
78
122
125
147
178
186
199
209
222
234
236
12
11
19
23
18
0
21
62
17
12
25
31
14
11
0
20
40
60
80
COLONY TYPES

FIGURE 2.

Table 1. Pore Water Chemistry and Physical Composition of Samples from Site P28.

	SEDIMENT COMPOSITION				PORE WATER COMPOSITION						
DEPTH (m)	GEOLOGICAL FORMATION	CLAY %	SAND %	pH	Eh (mv)	DOC[1] (mg/L)	NH_4 (mg/L)	NO_3 (mg/L)	CL (mg/L)	SO_4 (mg/L)	C-A/C+A[2]
0	TOBACCO RD.	2.1	96	5.3	471	15.6	0.93	0.93	3.0	15.2	-0.057
15	TOBACCO RD.	7.1	93	6.5	483	8.8	0.08	0.02	7.7	89.9	0.014
34	McBEAN	6.1	93	6.1	256	3.3	0.07	0.02	9.3	6.2	-0.007
64	CONGAREE	3.3	94	7.6	175	7.9	0.71	0.02	8.4	62	0.009
78	ELLENTON	20.3	64	5.6	417	3.3	0.21	0.01	1.5	102	0.011
122	ELLENTON	9.4	85	6.9	59	3.7	0.06	0.01	3.7	31.5	-0.030
125	PEE DEE	42.8	27	6.0	483	4.4	0.10	0.18	4.0	13.6	-0.037
147	PEE DEE	5.9	91	6.6	285	6.8	0.08	0.01	2.9	67.1	-0.021
178	BLACK CREEK	6.8	88	6.0	390	4.2	0.12	0.01	5.1	141	-0.059
196	BLACK CREEK	47.5	40	5.7	418	5.8	0.31	0.01	4.2	181	-0.023
199	MIDDLE CLAY	62.7	23	4.7	514	2	0.07	0.13	5.4	17.3	-0.090
209	MIDDENDORF	19.9	74	6.6	388	6	0.37	0.01	4.4	164	-0.016
222	MIDDENDORF	15.7	81	4.9	471	1.9	0.05	0.31	1.9	80.6	-0.038
223	MIDDENDORF	37.6	50	4.4	532	2	0.14	1.95	2.9	57	-0.031
234	MIDDENDORF	10.8	84	6.9	222	7.2	0.37	0.01	7.2	119	-0.013
236	MIDDENDORF	13.8	79	6.2	184	5.6	0.15	0.01	6.1	112	-0.006
Return mud		NA[3]	NA	7.5	275	13.3	NA	<0.02	22.6	126	-0.025

1 Dissolved Organic Carbon
2 Anion/Cation Balance used to define deficiencies in analyses
3 Not Applicable

Table 2. Concentration Ranges of Selected Parameters in Formations at SRP.

Formation	Function	% Sand	% Silt	% Clay	pH	Eh (mV)	Cond (µS)	Org C (mg/L)	NH^4 (mg/L)	NO_3 (mg/L)	PO^4 (mg/L)
Tobacco Rd	Max	92.9	5.4	11.7	6.55	491	250	52.80	2.84	3.74	0.10
	Min	84.7	<1	7.1	5.69	409	17	1.92	DL[1]	0.04	DL[3]
	Ave	89.6	4.5	8.6	6.15	449	115	14.84	0.77	1.38	0.07
McBean	Max	92.9	14.1	14.4	7.58	469	170	7.31	0.11	7.21	0.25
	Min	71.6	1.0	6.1	4.22	256	11	1.40	DL	0.01	DL
	Ave	85.0	5.0	10.1	6.20	402	78	3.04	0.09	1.63	0.08
Congaree	Max	94.2	5.8	7.2	7.99	420	630	7.90	0.71	4.18	3.00
	Min	87.0	2.5	3.3	7.42	175	300	2.92	DL	0.02	0.15
	Ave	91.4	3.8	4.8	7.66	279	464	5.46	ND	1.41	1.21
Ellenton	Max	90.4	16.3	71.7	6.93	512	510	4.42	0.21	1.06	0.03
	Min	13.8	1.3	7.4	3.39	59	100	0.92	DL	DL[2]	DL
	Ave	66.8	11.0	22.2	5.76	375	222	2.71	0.12	0.18	0.02
Pee Dee	Max	90.6	30.2	79.7	7.71	508	840	6.78	0.54	4.89	0.19
	Min	8.8	3.4	4.4	4.33	243	19	0.76	DL	DL	DL
	Ave	69.2	9.6	21.3	6.25	425	318	3.21	0.16	0.62	0.06
Black Creek	Max	47.1	16.3	66.4	4.73	546	130	2.58	0.12	2.76	0.01
	Min	17.3	12.8	40.1	4.12	470	65	2.00	0.07	0.28	DL
	Ave	29.1	14.4	56.4	4.50	510	101	2.24	0.09	1.21	0.01
Middendorf	Max	92.5	21.9	51.8	7.13	532	540	7.20	0.37	2.29	0.04
	Min	26.3	1.5	6.0	4.36	184	35	0.54	DL	0.01	DL
	Ave	76.1	6.5	17.5	5.92	402	283	2.87	0.20	0.80	0.03
Return Mud					7.54	274	794	13.3	NM	0.01	<.02

DL = Detection Limits
1=0.05 mg/L
2=0.01 mg/L
3=0.03 mg/L
NM = Not Measured

Table 3. Numbers of Viable, Aerobic, Chemoheterotrophic Microorganisms in Deep Subsurface Sediments

		Plate Counts (log CFU/g[dry wt] sediment) on these media and with these treatments[a]					
		1% PTYG[b]			PTYG		BHI
		N[c]	N	N	N	S	N
Sample Site	Depth (m)	4°[d]	23°	55°	23°	23°	37°
P24	0[e]	3.93	6.49	2.79	6.31	4.72	6.24
P24	38	3.66	4.09	2.57	4.13	3.17	3.77
P24	49	5.17	6.37	NG	5.51	NG	5.01
P24	63	3.93	4.81	NG	4.61	NG	4.56
P24	100	5.08	6.48	NG	5.68	2.98	5.48
P24	129	3.87	5.76	NG	5.06	NG	5.08
P24	152	2.78	3.22	NG	3.12	NG	2.86
P24	159	2.49	3.24	NG	3.32	NG	2.93
P24	197	4.75	6.65	NG	5.96	2.05	5.89
P24	219	n.d.[g]	4.97	NG	5.71	<2	5.52
P24	223	4.30	4.98	NG	4.80	2.72	4.42
P24	259	NG	2.12	NG	NG	NG	NG
P24	267	4.31	4.40	NG	4.46	NG	NG
P24	278	NG	NG	NG	NG	NG	NG
P24	284	4.73	6.46	NG	5.94	2.12	5.69
P24	289	2.20	2.48	NG	2.90	NG	<2
P28	0[e]	n.d.	6.38	3.70	6.48	4.60	6.42
P28	15	4.96	2.88	NG	3.05	NG	2.40
P28	34	5.77	5.58	NG	5.29	2.87	4.90
P28	64	3.70	7.01	n.d.	6.42	2.80	6.30
P28	78	NG	3.84	NG	3.86	NG	3.58
P28	122	3.79	7.29	2.09	6.09	3.35	5.94
P28	125	<2	NG	NG	NG	NG	<2
P28	147	5.40	5.16	NG	4.40	2.38	4.08
P28	178	3.29	3.27	NG	3.88	2.99	3.65
P28	196	6.10	5.72	NG	5.67	3.22	5.60
P28	199	6.15	3.68	NG	3.70	NG	3.51
P28	209	3.86	7.16	NG	5.86	2.64	5.66
P28	222	4.87	7.61	2.39	6.70	3.27	6.59
P28	234	4.06	6.40	2.36	6.10	2.90	5.34
P28	236	NG	3.90	<2	3.74	<2	3.16
P29	0[e]	4.51	6.59	4.12	6.29	>3	6.29
P29	8	4.22	5.67	NG	4.02	NG	3.94
P29	31	4.86	6.20	n.d.	5.46	NG	5.20
P29	43	4.60	6.72	NG	4.84	2.11	5.87
P28	75	NG	NG	NG	NG	NG	<2
P29	103	4.29	4.74	NG	5.08	NG	5.15
P29	122	NG	NG	NG	<2	NG	<2
P29	154	4.01	5.54	NG	5.39	NG	4.76
P29	165	3.86	6.56	NG	5.91	2.68	5.94
P29	192	4.74	6.28	NG	5.79	<2	5.85
P29	198	NG	2.25	NG	<2	NG	NG
P29	204	4.07	5.90	NG	4.67	2.37	3.69
P29	211	3.59	5.20	NG	4.50	NG	3.86
P29	218	3.76	5.93	NG	4.02	<2	4.05
P29	233	4.57	6.89	<2	5.74	2.79	5.51

[a] CFU = colony-forming units; all data reported as the average count from triplicate spread plates.

[b] BHI = brain heart infusion; PTYG = peptone (5 g/liter), tryptone (5 g/liter), yeast extract (10 g/liter), glucose (10 g/l), $MgSO_4 \cdot 7H_2O$ (0.6 g/liter), and $CaCl_2 \cdot 2H_2O$ (70 mg/liter); 1% PTYG = 1:100 dilution of PTYG, except for the $MgSO_4 \cdot 7H_2O$ and $CaCl_2 \cdot 2H_2O$.

[c] N = no special treatment prior to plating; S = sample heat-shocked at 80°C for 1 h prior to plating.

[d] Incubation temperatures: 4° plates counted after 4 months incubation; other 1% PTYG counted after 2 weeks incubation at the indicated temperature; PTYG and BHI plates counted after 1 week incubation at the indicated temperature.

[e] Surface soil sample, taken from just beneath the rhizosphere of the surface vegetation.

[f] NG = no growth on any of the lowest-dilution (10^{-2}) plates.

[g] n.d. = no data.

Table 4. Biomass Estimates for Protozoa, Algae and Fungi in P28

Formation	Depth (m)	% S/C[1]	Protozoa[2] (MPN/gdw)	Algae[3] (MPN/gdw)	Fungi (CFU/gdw)
Tobacoo Road	0	96/2	18200	ND[4]	63400
Tobacco Road	15	93/7	0	0	2
McBean	34	93/6	30	18	2
Congaree	64	94/3	1190	24	4
Ellenton	78	64/20	0	0	0
Ellenton	122	85/9	42	1	12
Pee Dee	125	27/43	0	0	3
Pee Dee	147	91/6	29	6	1
Black Creek	178	88/7	43	8	6
Black Creek	196	40/48	1	2	18
Middle Clay	199	23/63	0	0	6
Middendorf	209	74/20	120	49	9
Middendorf	222	81/16	338	17	35
Middendorf	223	50/38	0	0	0
Middendorf	234	84/11	28	1	1
Middendorf	236	79/14	3	0	1
Return Mud	NA[5]	NA	920	2	12

1= Sand/Clay (international convention)
2= flagellates and amoebae; no ciliates
3= mostly unicellular, some phytoflagellates and blue-greens
4= not determined
5= not applicable

Table 5. Biomass Estimates (gdw^{-1}) for Selected Microbiological Parameters in P28.

Formation	Depth (m)	LOG_{10} CFU	LOG_{10} AODC	AOINT[1] (%)	LOG_{10}MPN Coliforms	LOG_{10}MPN S-Oxid	LOG_{10}MPN Nitrifiers	Denitrification nmol N_2O/gdw
Tobacco Rd.	0	6	8.4	51	0.5	0	3.13	25.8± 0.76
Tobacco Rd	15	3	6.6	18	NG[2]	0	0	0
McBean	34	6	7.6	30	NG	1.4	1.26	18.6± 1.20
Congaree	64	7.3	7.7	46	2.1	0	1.59	0.42± 0.04
Ellenton	78	<1.7	7.3	14	0.5	1.4	0	0.35± 0.21
Ellenton	122	7.1	7.8	29	0.6	1.2	0	5.62± 0.81
Pee Dee	125	2.3	6.6	6	NG	0	0	0
Pee Dee	147	6	7.6	29	2.7	1.9	0.22	2.50± 0.01
Black Creek	178	7	7.3	17	3.5	1.6	0.57	0.53± 0.18
Black Creek	196	4.8	6.7	12	NG	0.4	0.7	0
Middle Clay	199	3.8	<5.7	13	NG	0	0	0
Middendorf	209	7.3	7.5	20	2.4	1.3	1.17	0.93± 0.05
Middendorf	222	7.6	7.7	22	1.9	3.0	0.94	0.23± 0.12
Middendorf	223	<1.7	<5.8	22	NG	ND	ND	0
Middendorf	234	6.3	7.2	19	NG	ND	ND	0.79± 0.04
Middendorf	236	4.2	6.7	17	NG	ND	ND	1.83± 0.17
Return Mud	ND[3]	7.2	7.7	ND	NG	ND	ND	ND

1=Proportion of AODC that reduced INT
2=No growth
3=Not determined

Table 6. Phospholipid and Isotopic Measurements with Depth for P28 Sediment Cores.

Depth (M)	14 C-Acetate1 Lipids	3H-Thymidine1 in DNA
0	1128000	2920000
15	0	46000***
34	216000	4000***
64	167000	22500***
78	512	182000
122	39400*2	308000
125	0	87500
147	78600**	40500**
178	192000	140000
196	10000	155000
199	980	0
209	101300*	19800*
222	649000	693000
234	104000	248000
236	11340	135000

ND = Not Determined

1 dpm/d/gdw. of radiotracers in samples based on time course exp.

2 * = stimulation of activity with longer incubation greater than or equal to three **10 fold; *** 30 fold.

Table 7. Procedures Used for the Characterization of Chemolithotrophic and Other Physiological Ty of Bacteria from the SRP Site.

Microorganism	Medium	Procedure	Confirmation
Nitrosomonas	NH_4^+ oxidizer	MPN	Presence of NO_2^-, NO_3^-
Nitrobacter	NO_2^- oxidizer	MPN	Disappearance of NO_2
H_2 Oxidizers	N and org.C deficient	MPN	Pellicle formation
Methylotrophs	N and C deficient	MPN	Pellicle formation
T. thiooxidans	Thiobacillus	MPN	Drop in pH
T. ferrooxidans	Thiobacillus	MPN	Yel. oxi. Fe^{3+} ppt
Cellulose degrader	1.2% cellulose noble	Plate Count	Col. surround by clear zone
Mn^{2+} Oxidizer	Sheltons min. + 3ppm Mn^{2+}	Enrichment	Feigl spot test for Mn^{4+}
Fe^{+3} Depositing	Sheltons+soft iron	Enrichment	Yel. slime on tubing
Heterotrophic Iron Ppt. Bacteria	Fe-NH_4 citrate	Plate Count	Colony Forming Unit
Microaerophiles	N-fixing soft agar	Enrichment	Growth as rings, var. depth
$SO_4^=$ Reducers	N-fixing soft agar	enrichment	Black sulfide ppt

Table 8. Most Probable Numbers of Sulfate-Reducing Bacteria

Formation	P29	P28	P24
Tobacco Road	1.6	1.0	3.9
Dry Branch	2.0	0.0	-0.5
McBean	1.4	4.6	NG
McBean	NF	NF	0.1
Congaree	2.4	4.7	2.9
Ellenton	NG	-0.8	NG
Ellenton	NG	4.2	NG
Pee Dee	NG	2.6	NG
Pee Dee	NF	3.3	-0.1
Pee Dee	NF	0.0	NF
Black Creek	2.2	-0.2	-0.1
Black Creek	1.0	5.4	NG
Black Creek	NF	NF	NG
Middendorf	0.4	5.7	2.4
Middendorf	-1.4	3.8	NG
Middendorf	1.4	0.0	1.4
Middendorf	1.4	0.4	NG
Middendorf	5.3	ND	ND
Middendorf	3.9	ND	ND

Numbers represent Log10 MPN
NG = No growth.
ND = Not determined
NF = No formation to sample at this site