Chapter 6

MICROBIAL DISTRIBUTIONS AND THEIR POTENTIAL CONTROLLING FACTORS IN TERRESTRIAL SUBSURFACE ENVIRONMENTS

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Abstract: Terrestrial subsurface environments (below the plow layer) contain an enormous amount of the earth's biomass, yet are relatively undersampled compared to topsoil, aquatic, and marine environments. Depth emerges as a primary axis for relating distributions of microorganisms and the factors controlling their distribution. There is generally a sharp drop in microbial biomass, diversity, and activity as organic-rich topsoils deepen to mineral-dominated subsoils. Progressively deeper samples from the vadose zone to the capillary fringe and into saturated zones often reveal increases in biomass and changes in dominant microbial populations. Biomass appears to slowly decline with depth, and cell viability is limited by temperature between 4.5 and 6 km. In many subsurface environments, spatial distributions of microorganisms are extremely variable, frequently defying prediction. In a few highly structured saturated environments, such as confined or contaminated shallow aquifers, predominant terminal electron accepting activities are arranged in a spatially ordered manner that is consistent with selected geochemical measurements. Sampling issues specific to subsurface environments still require substantial added effort and expense to achieve a reasonable sample density in comparison to most other environments. Technological advances in microbial assay methodologies are easing some of the methodological boundaries that are often exceeded by subsurface samples.

Keywords: aquifer, distribution, microorganism, spatial, subsurface, vadose

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1. INTRODUCTION

It has become apparent that terrestrial subsurface microorganisms are distributed in an extremely patchy manner and that the patches are quite variable in magnitude (Brockman and Murray, 1997). Neither the factors that control these distributions nor the distributions themselves have been rigorously quantified. To quantify relationships between microbial spatial distributions and their controlling factors, the relevant scales and most probable controlling factors need to be identified. To that end, published data on subsurface microbial properties from spatially arranged sample sets are reviewed and used to support conceptual models of microbial distributions in terrestial subsurface environments.

1.1. Historical expectations regarding subsurface microbial distributions

It has been estimated that the amount of microbial biomass in subsurface environments approximates the amount of *all* aboveground biomass (Gold, 1992; Onstott et al., 1999; Pedersen, 2000; Whitman et al., 1998), yet knowledge of subsurface microbial distributions rests on a sparse array of analyzed samples. Historical expectations were that biomass declined rapidly with depth and soil carbon content, and that the subsurface was largely devoid of life (Federle et al., 1986; Ghiorse and Wilson, 1988), despite occasional reports of microorganisms in produced waters from petroleum exploration and development (Bastin, 1926; Davis, 1967). Interest in subsurface microbiology grew in association with accelerated groundwater consumption and deterioration, and microbiologically oriented sampling of the subsurface largely commenced in the 1970s (Keswick, 1984). Based primarily on findings by the USEPA, USGS, and university researchers, it was generally acknowledged by the mid-1980s that shallow aquifer sediments were colonized by substantial numbers of microorganisms, largely bacteria (Ghiorse and Wilson, 1988). In the late 1980s, the United States Department of Energy (USDOE) initiated a systematic program to investigate the microbiology of deeper subsurface environments. Other countries, notably Canada, Sweden, Finland, Germany, England, Russia, and Japan, established similar programs, stimulated by groundwater contamination issues or by the challenge of isolating high-level radioactive waste over long duration (>10,000 years) in deep, geologic repositories. Overwhelming, subsurface biomass is prokaryotic (Ghiorse and Wilson, 1988; Sinclair et al., 1990; Whitman et al., 1998), and it appears that bacteria are resident in all areas of the terrestrial subsurface that have been sampled, including cores collected from 2,800 m depth

(Boone et al., 1995; Onstott et al., 1998), and groundwater thought to originate from at least 5,300 m depth (Szewzky et al., 1994).

2. BUILDING CONCEPTUAL MODELS OF DISTRIBUTION: DEPTH AS A PRIMARY AXIS

An inherent and dominating consideration of the terrestrial subsurface habitat is the depth axis. At any location, gradients in the subsurface habitat will be anchored by their depth from the surface, where organic matter primary production is sustained by light energy and dissolved or free gas concentrations approach equilibrium with atmospheric levels. Throughout the depth profile, the subsurface is strongly structured by the vertical (generally) arrangement of geologic units and their weathering profiles. Due to variations in soil profiles and underlying geology, some discussion arises as to what is "subsurface" as opposed to soils. The depth of soils is often considered synonymous with the rooting zone, although the depth of root penetration may vary greatly with vegetation, climate, etc. One convention is to define soil inclusive of the A, E, and B horizons, but not the weathered parent material or regolith composing the C horizon (Hunt, 1986). Alternative definitions of soils include the C horizon, respecting deep-rooted plants (Richter and Markewitz, 1995), or use a 2 m depth criterion (Soil Survey Staff, 1975). The "subsurface" includes everything below the "soils" or alternatively, everything below 1 m (Onstott et al., 1999) or 8 m (Whitman et al., 1998). For the current discussion of microbiology, "subsurface" will be defined as the region beneath the intensively studied plow layer soils that chiefly comprise the A and O horizons. The less frequently sampled "subsoils" (B horizon to bedrock) usually contrast sharply with the overlying topsoils in organic matter and mineral content.

2.1. Soil-unsaturated subsoil transition: the first few meters

Microbial biomass, diversity, and activity often exhibit parallel declines with depth during the transition from the organic-rich topsoils to the mineral-dominated subsoils , although at some point in the C horizon or bedrock, biomass levels become relatively constant with further depth increases as long as unsaturated conditions prevail (Fig. 6-1). A decline in biomass (by lipid analyses), activity (by FDA hydrolysis), and diversity (PLFA profile complexity) was observed >2 m deep profiles sampled within three unimproved pastures and a minimally managed soybean field in Alabama (Federle et al., 1986). Wood et al. (1993) observed 3–4 orders magnitude

decrease in culturable aerobic heterotrophs >7 m depth profiles in silty loam soils at two sites in western North America. Bone and Balkwill (1988) reported decreasing values in total cells, culturable heterotrophs, and heterotrophic diversity over a 3 m depth profile containing loamy sand topsoil underlain by fine, silty clay at an Oklahoma site. Numerically dominant phenotypes differed between surface soils and subsoils. Dodds et al. (1996) showed that biomass, respiration, number of CTC-respiring cells, and numbers of culturable aerobic heterotrophs decreased >4 m of unsaturated clayey soils at a grassland site in northeast Kansas, mirroring the sharp drop in total soil organic matter. However, total numbers of cells remained relatively constant over the same interval, as did the amount of soluble organic carbon. In cropped soil at the same prairie site, microbial properties remained relatively constant over the same depth interval, suggesting that tilling may have resulted in increased homogenization, even below the actual depth tilled. In several coniferous forest soils in Finland, microbial biomass (total PLFA) was observed to decrease roughly tenfold over a 0.4 m depth profile (Fritze et al., 2000). These authors also found changes in community structure (PLFA profiles) between horizons such that O ≠ E ≠ B = BC and that actinomycetes were relatively enriched with depth. Roughly 100-fold decreases in bacteria and fungal biomass were observed in forests soils at two sites in Denmark (Ekelund et al., 2001). Over a 1.5 m depth profile in two Indiana agricultural soils, Blume et al. (2002) observed sharp decreases in bacterial biomass and found that the community structure (PLFA) in the subsurface soils was relatively enriched in gram-positive bacteria compared to the topsoils. In 4 m depth profiles from two mid-western US agricultural soils of differing textures, Taylor et al. (2002) found sharp decreases in microbial abundance (various measures) and activity (enzymes) with depth that were well-correlated with declines in soil organic matter content. At two California loamy grassland sites, Fierer et al. (2003), found that microbial biomass and diversity declined, and community structure was relatively enriched in both actinomycetes and gram-positive bacteria with increasing depth over a 2 m vertical interval.

An exception to the general decline in microbiological parameters with depth in subsoils may be in deep, organic-rich soils that have been recently drained, such as observed in Florida Histosols, where oxygen concentrations regulate activity (Tate, 1979). Similarly, in the partially waterlogged soils of a Danish bog, higher microbial numbers observed at the depth have been attributed to preservation of organic matter and exclusion of protozoan predators by the low oxygen tensions (Ekelund et al., 2001).

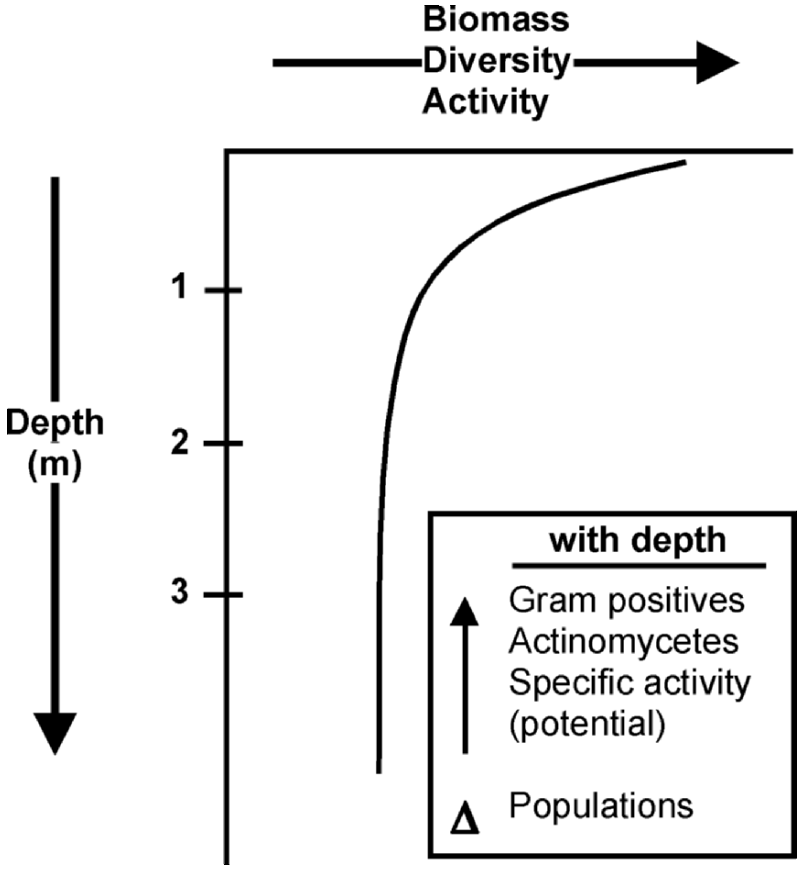

Figure 6-1. Generalized depth distribution of microbial properties from the surface through the first several meters of unsaturated subsoil.

2.2. Unsaturated–saturated transition in the shallow subsurface: meters to dekameters

As the depth of sampling increases in the unsaturated or vadose zone past several meters, and often into a "C" horizon, microbial properties tend to level off, without further predictable decreases with depth through the "shallow" subsurface. Several scenarios generally present themselves: (i) a "shallow" aquifer is encountered, or (ii) a large vadose zone overlies a "deep" water table aquifer; (iii) less permeable layers overlay a shallow or deep confined aquifer. Discussion again arises as to what is "shallow" and what is "deep" subsurface. The "deep subsurface" has been arbitrarily defined by

depths >10–20 m (Fliermans and Balkwill, 1989), >50 m (Sinclair and Ghiorse, 1989), >50–100 m (Pedersen, 1993), or other depths within these ranges. Often, an aquifer is the target of subsurface microbiological investigations. Although it may be convenient to consider water table aquifers shallow, in areas of the arid western USA, the water table may lie at depths exceeding 200 m. Due to regional geologic distinctions, it has been proposed that the classification of the subsurface be based on aquifer recharge characteristics and that the term "deep subsurface" refer to intermediate and deep aquifer systems, but not local aquifers (Lovley and Chapelle, 1995). However, not all relatively deep locations that have been sampled are aquifers. For the current purposes, the shallow subsurface will be loosely defined as within 20 m of the surface, generally accessible by direct push drilling technologies, and if an aquifer is present, it will be a water table aquifer usually consisting of weakly or unconsolidated clastic material.

Depth-related profiles of solid-associated microbial properties as a shallow aquifer is encountered have been largely performed in sedimentary systems where there is a capillary fringe. Wilson et al. (1983) reported that bacterial cells and culturable heterotrophs decreased from 1.2 (B horizon) to 3.0 m (C horizon) and then increased or remained constant at 5.0 m in the saturated zone of a sandy Oklahoma aquifer. A second sampling at this Lula, Oklahoma location also demonstrated an increase in culturable aerobic heterotrophs at the capillary fringe and then relatively constant numbers through the saturated zone to 6 m (Balkwill and Ghiorse, 1985). Other reports from Lula with more intensive depth-stratified sampling demonstrated the progressive decrease in culturable numbers and diversity from topsoil through 3 m of subsoil and then an increase to relatively constant numbers through 3 m of the saturated zone (Beloin et al., 1988; Bone and Balkwill, 1988). Subsurface samples collected at this location contained a predominance of gram-positive cells and small cellular morphologies (Balkwill and Ghiorse, 1985; Bone and Balkwill, 1988; Wilson et al., 1983), although deeper (7.5 m) aquifer samples produced relatively more gram-negative bacteria (Bone and Balkwill, 1988). Lula subsurface isolates tended to be nutritionally flexible with respect to substrate concentrations and were often affiliated with taxonomic groups with broad substrate ranges (Balkwill and Ghiorse, 1985; Bone and Balkwill, 1988). Predominant populations varied with depth and were distinct from surface populations (Bone and Balkwill, 1988).

At two sites in western North America, Wood et al. (1993) presented data that suggested that microbes were most active, producing CO_2, at the interface between the unsaturated zone and a sandy aquifer. At both the cropped and grassland Kansas prairie soil profiles studied by Dodds et al.

(1996), microbial parameters indicating biomass and activity exhibited transient increases as the capillary fringe was encountered and then leveled off or rose though the saturated zone to 10 m depth. At several sites in agricultural fields overlying a sandy aquifer on Virginia's Eastern Shore, Zhang et al. (1997), witnessed a 100-fold decrease in culturable aerobic heterotrophs with depth over about 3 m, and then numbers increased as the water table was encountered. At a nearby location on the Virginia Eastern Shore, Zhou et al. (2004) produced 16S rDNA clone libraries for three depth intervals, the deepest was saturated sands at 4 m. These authors found the subsurface communities to be less diverse and harboring populations distinct from the surface. Moreover, the composition of subsurface communities varied dramatically (>90% difference in operational taxonomic units (OTUs)) between samples vertically separated by meters in an apparently homogeneous sandy subsoil. In a related report that included samples from this Virginia site, 16S rDNA clone libraries from several saturated subsurface samples had fewer OTUs, but more dominant (high frequency) OTUs compared to corresponding low carbon surface samples. Unsaturated subsoils exhibited intermediate OTU richness and evenness between the surface and saturated subsurface samples. At sites where surface carbon levels were higher, surface soil communities were still very diverse, but exhibited more dominance, similar to the subsoils. These authors hypothesized that increased access to carbon, either by higher concentrations in the unsaturated surface soils or by increasing homogenization in saturated aquifer sediments, leads to decreased competetion and decreased evenness in population distributions.

So, after the first decline in microbial numbers and diversity from topsoil to subsoil, there appears to be no further decrease with depth in the unsaturated zone. Further, once saturated systems are encountered in shallow systems, there is a generally a rise in solid-associated microbial activities and numbers and no further depth-related decreases as depth in the saturated zone is increased (Fig. 6-2). Several additional themes emerge from the current body of work that may apply to shallow subsurface environments: (i) subsurface communities are less diverse than surface communities; (ii) subsurface communities appear to have different populations than surface communities, (iii) gram-positive bacteria and actinomycetes become relatively more abundant in unsaturated subsoils with increasing depth; (iv) gram-negative bacteria become relatively more abundant in the saturated zone; (v) community composition varies considerably among subsurface samples collected from the same site; and, (vi) saturated subsurface communities tend to have dominant populations.

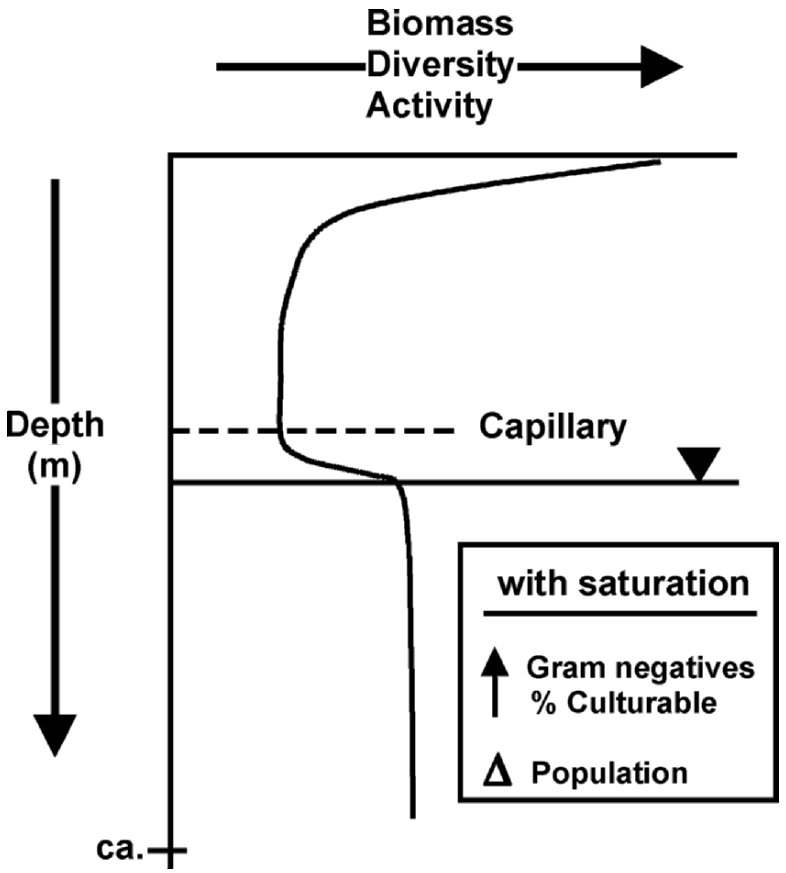

Figure 6-2. Generalized depth distribution of microbial properties though deepening unsaturated subsoils and through an encounter with a shallow water table aquifer.

2.3. Deeper depth distribution: dekameters to kilometers

The initial decline in microbial parameter from the surface to the subsurface followed by little or no discernable depth-related trend has also been witnessed in sediment or rock samples collected over wider depth intervals from deeper profiles. Sampling campaigns comparing surface samples to depth-stratified samples from deeper regions have been collected from (a) deeper sedimentary aquifers, (b) cross sections that contain both aquifer and aquitard formations, (c) sedimentary vadose zones of the western USA, and (d) fractured rock aquifers and highly consolidated deeper formations.

2.3.1. Deeper sedimentary aquifers

In an extensive study of isolates cultured from five coreholes penetrating saturated sedimentary formations in the Germany (Kolbel-Boelke et al., 1988), the number of viable cells in sediment samples was 10- to 1,000-fold lower than surface sample, yet there was no systematic decrease with depth >40 m. The numbers and types of culturable organisms strongly differed among samples of different depths. Coefficients of variation (CV) for aerobic colony forming unit (CFU) ranged from 123% to 226% in the five transects. Within a sand–gravel aquifer in northeastern Kansas, total cell numbers and culture-able heterotrophs in sediment samples were 100- to 1,000-fold lower than surface samples, but did not decline even up to a depth of 90 m (Sinclair et al., 1990). Microbiological properties of a 30 m sequence of sedimentary formations of varying lithology that contained two aquifers was studied in southeast Texas (Martino et al., 1998; Ulrich et al., 1998). The shallow aquifer was primarily oxidizing and the deeper primarily reducing. There was an initial 2–3 log decrease from surface values of total cells and viable aerobic heterotrophs in vadose zone sediments, and then values did not decline fur-ther. Anaerobic heterotrophs and sulfate-reducing bacteria (SRB) decreased from surface values of 10^4–10^5 cells per gram to zero in unsaturated zone sediments. Once the saturated zone was encountered (8 m), total cells and viable aerobes remained relatively constant for the remainder of the inter-vals, while viable anaerobes and SRB increased to 10^2 and peaked at 10^4–10^5 in the lower, reducing aquifer (Martino et al., 1998). No systemic decrease in SRB or iron- and sulfur-oxidizing bacteria with depth was observed across the entire profile (Ulrich et al., 1998).

2.3.2. Cross sections that contain both aquifer and aquitard formations

In samples collected across multiple horizons of eastern US coastal plain saturated sediments (Maryland), the number of cells and viable bacteria ini-tially declined from the uppermost 21 m deep sample to about 100 m deep, but then no further declines were observed to a total depth of 168 m (Chapelle et al., 1987). In the late 1980s, several 250 m deep profiles of microbiological properties were established across at least seven geologic formations, including several aquifers, in southeastern US coastal plain sediments (South Carolina). Microbial populations generally decreased from surface values through the vadose zone, and then increased under saturated conditions. While numbers of culturable aerobic heterotrophs were about 100-fold less numerous and species richness was less in subsurface samples compared to surface samples

at this site, no depth-related decreases were observed in numbers or diversity once saturated sediments were encountered (Sinclair and Ghiorse, 1989). Even at depths to 265 m, about 10^7 CFU g^{-1} were recovered and relatively high numbers of other oxygen-utilizing organisms were maintained (Fredrickson et al., 1989); other methods for enumerating aerobic heterotrophs yield similar results (Balkwill, 1989). Gram-positive bacteria tended to have highest relative abundances in the vadose zone, while gram-negative bacteria increased in the saturated zone. From sample to sample throughout the depth profile, bacterial populations varied considerably evidenced by both physiology (Balkwill et al., 1989; Fredrickson et al., 1991) and colony morphology (Balkwill, 1989). Aerobic CFU had CVs of 128–178% in each of the vertical transects. Morphological, physiological, and molecular evidence suggests that the aerobic heterotrophic isolates from the subsurface were primarily different populations from those isolated from surface soils at this site (Balkwill et al., 1989; Jimenez, 1990). Several studies indicated that subsurface isolates tended to be capable of existing under low-nutrient conditions and were affiliated with taxonomic groups that are nutritionally versatile and often capable of degrading complex compounds including aromatics (Balkwill et al., 1989; Jimenez, 1990). Frederickson et al. (1988) reported that isolates from progressively deeper horizons had a higher occurrence of plasmids and larger plasmids.

2.3.3. Sedimentary vadose zones of the western USA

In areas of the Western USA, where the water table may be at great depths, several studies have examined microbiological properties in unsaturated sediments over vertical transects. Deep vadose zones in arid climates tend to have very low levels of biomass compared to surface soils and extremely patchy distributions (Colwell, 1989; Hersman, 1997; Kieft et al., 1993). At five sites with contrasting recharge characteristics in Washington, Balkwill et al. (1998) observed that the numbers of culturable organisms and biomass (PLFA) declined sharply in the first 1–2 m and then remained relatively constant, but often very low, to total depths examined between 10 and 15 m. At several of the five sites, the percentage of actinomycetes increased with depth. Kieft et al. (1998) reported on microbial depth distributions across chronosequences of unsaturated buried loess at two sites in Washington. The authors found that at both sites biomass (PLFA), total and viable cells (plate counts), and activities (various methods) declined sharply in the first several meters, but then remained relatively constant from 10 to >30 m total depth. Community composition (PLFA profiles) shifted and appeared to become less complex with depth. At an uncontaminated location in south central

Washington, viable counts and activity (heterotrophic potential) in vadose sediments from a depth range of 30–90 m were compared with sandy surface soils (Fredrickson et al., 1993). At this site, viable counts and activity were much lower in the subsurface (frequently at or below detection) than the surface, although the highest subsurface values occurred in the deepest samples collected.

2.3.4. Fractured rock aquifers and highly consolidated deeper formations

Microbial properties have been determined from sediment and rock samples collected from additional sites at varying depths. Often, vertical sampling transects were concentrated at certain depths and/or comparative analyses were not performed on surface samples. Nonetheless, total cell counts on subsurface sample from multiple studies, including those with limited vertical distributions, can be plotted on a depth axis to provide a rough view of cell number with depth (Fig. 6-3). Although Fig. 6-3 does present total cells (for which there is the most data), the majority of samples where cells were observed also yielded evidence of cell viability. The primary exceptions are some deep, dry vadose zone sediments (Colwell, 1989; Fredrickson et al., 1993; Kieft et al., 1993), igneous rock samples (massive basalt) (Kieft et al., 1993; Lehman et al., 2004), and saturated sediments of low permeability (Colwell et al., 1997; Fredrickson et al., 1997; Kieft et al., 1995) including massive clays (Boivin-Jahns et al., 1996; Lawrence et al., 2000), where the limited number of tests performed did not reveal cell viability. Over a depth range of several kilometers, cell numbers are lower in the subsurface than surface soils (~10^8–10^9 cells g^{-1}), however, there remains great variability in cell numbers that does not appear associated with depth. So, what are the ultimate limiting factors for microbes in the subsurface? Is there an ultimate depth past which they will not be found? If 110°C is assumed to be the maximum for survival and the average geothermal gradient is approximately 25°C km^{-1}, then about 4.5 km may be an absolute depth for microbial life to persist. The estimated *in situ* temperature at 2,800 m where viable organisms were cultivated was approximately 75°C (Onstott et al., 1998). Alternatively, if 150°C (with pressure) is assumed to be the limit of microbial survival, then the absolute depth would be closer to 6 km. Other potentially limiting factors such as the presence of toxic substances, extreme pH, or radioactive fields will only exclude microorganisms from very limited portions of the subsurface. Ionic strength is not likely to be an absolute limitation as microorganisms have been shown to tolerate saturated (32% NaCl) solutions, and while pressure will rise about 100 atm per 1,000 m, many microbes can

endure 100s of atm and some can grow at 600–1,000 atm (Madigan et al., 1997). At deeper depths, physical space may become a factor – if pore volumes decrease to <0.1 μm^3 – then there will simply be no room for microbial cells. Oxygen generally decreases with depth from its source at the surface as it is consumed during organic matter oxidation, although many relatively deep aquifers remain well-oxygenated (Winograd and Robertson, 1982). The presence and concentrations of dissolved or free gases will vary with depth, distance from recharge, nature of the overlying vadose zone, and other location-specific characteristics, yet the most likely impact of local atmosphere will be a selection for certain physiologies, not uniform elimination. The availability of water or water activity will influence the distribution and activities of microorganisms in the unsaturated subsurface, but will probably not decrease to levels that cause cell dessication and death (Kieft et al., 1993).

Microorganisms need to conduct energy yielding reactions and assimilate carbon to maintain cell integrity. The distribution of physiological types and the magnitude of activity in subsurface environments will reflect amounts, and perhaps quality of available carbon, as well as terminal electron acceptors and other nutrients that are sustained by advective and diffusional fluxes at a given location. These geochemical parameters will be constrained on a larger scale by the broader geological history and regional climate of each site, but will also be expressed on the centimeter–meter scale by the immediate geochemical and hydrological conditions imposed by the specific location.

3. PATTERNS AMONG SPECIFIC GEOLOGIC FORMATIONS AND DEPOSITIONAL ENVIRONMENTS

Up to this point, the microbiological attributes of core samples, sediment or rock, have been discussed with respect to depth. Obviously, a lot of knowledge concerning terrestrial subsurface microbiology has been gathered by analysis of groundwater samples. At some sites, it has been shown that free-living groundwater communities may differ significantly in structure and potential function from those attached to the geologic media (Bekins et al., 1999; Godsy et al., 1992; Kolbel-Boelke et al., 1988; Lehman et al., 2004; Lehman et al., 2001) and this represents another dimension of spatial variability. However, it is important to recognize the limitations inherent in spatially locating the origin of a particular sample of groundwater. Many groundwater samples have been collected in bulk from wells that are completely open,

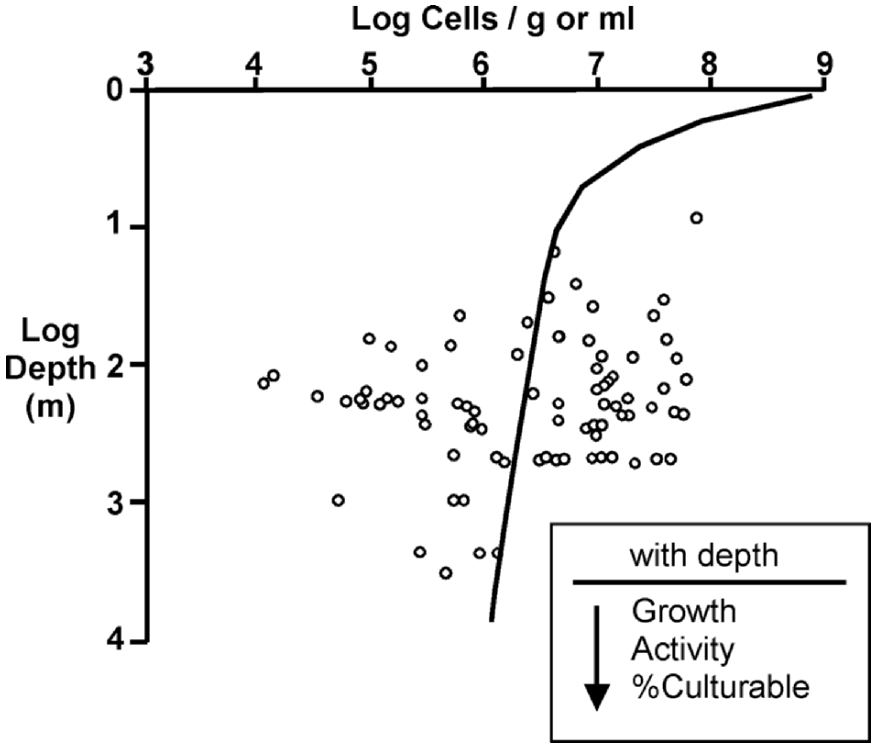

Figure 6-3. Microbial cell numbers over a >3 km depth in terrestrial subsurface environments. (Adapted from Onstott et al., 1999; Courtesy R. Colwell.) Hand-drawn trend line superimposed on data to suggest a conceptual model for cell distributions across this broadscale.

open at the bottom, or screened across some length. In other cases, multi-level wells have been installed wherein individual inlet ports occur at certain intervals (Smith et al., 1991). Straddle-packers have been used to isolate sections of an open hole or screened interval to pump water specific for a given interval (Lehman et al., 2001). Lastly, diffusion cells separated by baffles have been used to collect depth-discrete water samples (Lehman et al., 2004; Takai et al., 2003). In many of the studies of vertically stratified groundwater samples, decimeter–meter variations in microbial properties have been observed. But, regardless of the methods used to acquire the groundwater samples, there is uncertainty regarding the water's previous location. Even when using multilevel wells, straddle-packers, or diffusion cells separated by baffles, there is the chance that the groundwater originated from locations above or below the desired sampling point by traveling vertically through the formation by natural or induced gradients. The well itself presents

an intrusion that may lead to short-term artifacts associated with well construction, or long-term artifacts due to colonization of the foreign well environment. For those reasons, only selected examples of groundwater analyses that were intentionally conducted on a number of vertically or horizontally arrayed samples will be included in the following discussion.

The microbiology of a variety of sedimentary sequences has been studied. The individual sedimentary units differ in their lithology, exhibiting characteristic mineralogy, grain sizes, carbon content, etc. according to the origin of the particles, their original depositional environment and subsequent history, including metamorphosis. If saturated, the microbiology in a given horizon will be influenced by the local geochemistry of the water. Consideration of subsurface sedimentary formations as habitats compels us to assess how the subsurface environment differs from that observed at the surface. For instance, some locations were originally the sediments of an inland sea, others were the floodplain of a meandering river, and some were soils in their own right, at various stages of development. All were initially subjected to processes that occur at or near the earth's surface. Yet, over staggering time spans, these sediments have been relocated and physically transformed so that current conditions do not resemble historic conditions. In comparing one sedimentary unit with another, it must be recognized that these units also span large time intervals and that deeper is older (usually). The timescale becomes important when considering spatial scales of microbes in the subsurface. Current evidence suggest that bacteria in some sedimentary rocks may be progeny of those that were associated with original deposition, while others were subsequently transported to that location at uncertain intervals over a long time period (Fredrickson et al., 1995, 1997; Kieft et al., 1998). This evidence is produced by examining the ages of the sediments or rock, the pore water or groundwater ages, the pore structure of the formation and neighboring formations, the overall history of geologic formation (e.g., temperature history), current and historical recharge, and other hydrogeological conditions. Therefore, when trying to ascertain distributions, do we expect organisms that might have thrived in the originally depositional environment? Or, do we confine the search to organisms that have physiologies consistent with the current circumstances? There is fairly strong evidence that microorganisms can persist isolated in geologic formations for very long periods of time (Fredrickson et al., 1995; Lawrence et al., 2000; Onstott et al., 1998), with estimated growth rates that appear static (Chapelle and Lovley, 1990; Lawrence et al., 2000; Onstott et al., 1998, 1999; Phelps et al., 1994). If that is the case, then inactive, but viable cells (original progeny or transported) may compose much of the biomass and account for

the low percentage of cultivable cells observed in many deeper locations. Because of these issues that are unique to or exaggerated in subsurface environments and the necessarily selected (and selective) methods used to analyze the samples, it might be expected that relatively few of the inhabitants have actually been evaluated. The origin of subsurface microbes in a particular formation, their degree of adaptation to the current conditions, and their ability to respond to given assays may have strong consequences for our perception of subsurface microbial distributions beyond total cells.

3.1. Vertically distributed patterns within the subsurface: centimeters, meters, dekameters

Microbiological analyses of depth transects through stacked sedimentary layers of differing lithologies have produced several trends concerning the distribution of microorganisms and their activities in relation to their physicochemical location. These trends can be broken into two groups: (i) those that relate to the characteristics of the individual sedimentary unit examined; and (ii) those that relate to the juxtaposition of two units with differing characteristics.

3.1.1. Vertical trends according to lithology, meters to dekameters

A primary trend in the analysis of unconsolidated sedimentary units is a relationship between the microbiology and sediment texture. Sediment texture directly influences key characteristics including porosity, permeability, water potential, and frequently is related to organic carbon and particle mineralogy. Chappelle et al. (1987) observed increased numbers of total cells in clay layers compared to coarser-grained layers, but the number of viable cells was not correlated with sediment texture in their study of Miocene-Lower Cretaceous age northern Atlantic coastal plain sediments. Albrechtson and Winding (1992) found that sediment texture was correlated with microbiological properties in a series of Quarternary glacio-fluvial sediments in Denmark. They reported that clay content was associated with higher numbers of total cells, although there was a high degree of correlation between clay content and organic matter content in these samples. Conversely, activity (aerobic C-14 substrate mineralization) was associated with sediment sand content and higher permeability (calculated from grain size distributions). In size-fractionated aquifer sediments, Albrechtsen (1994) found that the number of viable cells and the amount of activity (aerobic C-14 substrate mineralization) was highest in the silt-sized particle fraction. In an earlier study

of size-fractionated sediments collected from a coastal aquifer, Harvey et al. (1984) reported that the majority of bacterial cells were associated with fine-grained (<20 μm) sediments. In analyses of samples collected from four coreholes penetrating a glacial till aquifer (Kansas), total cells and viable cells were negatively correlated with sediment clay content, and positively correlated with sand content (Sinclair et al., 1990). A number of papers on samples collected from Tertiary and Cretaceous age coastal plain sediments of the southeastern USA indicate that sediment clay content was negatively correlated (and sand content positively correlated) with total cell numbers (Sinclair and Ghiorse, 1989), culturable aerobic bacteria (Balkwill, 1989; Fredrickson et al., 1989; Phelps et al., 1994; Sinclair and Ghiorse, 1989) and anaerobic bacteria (Jones et al., 1989; Phelps et al., 1989, 1994), eukaryotes (Sinclair and Ghiorse, 1989), chemoheterotrophic diversity (Balkwill et al., 1989), denitrifying activity (Francis et al., 1989), aerobic activity (C-14 substrate mineralization) (Hicks and Fredrickson, 1989; Phelps et al., 1994), and anaerobic activity (C-14 substrate mineralization) (Hicks and Fredrickson, 1989; Phelps et al., 1994, 1989), and growth (C-14 acetate incorporation into lipids) (Phelps et al., 1994). Samples with high clay content were associated with relatively higher proportions of gram-positive bacteria (Balkwill, 1989; Sinclair and Ghiorse, 1989). Despite the overall relationships of microbial biomass and activities with sediment texture, there appeared to be considerable variation in chemoheterotrophic community composition in samples within a single formation and from different formations (Balkwill, 1989; Fredrickson et al., 1991). Even given the distance from the surface (>100 m) and the apparent ages of the groundwater (up to 1,000s of years in some samples (Phelps et al., 1994)), the higher nutrient fluxes enabled by the relative high hydraulic conductivities (20–60 kD) of the sandy formations should support higher microbial activities in the sandy aquifer layers than the clay aquitards (Phelps et al., 1994). In support of this conclusion, the addition of water to clay sediments dramatically increased their activity, presumably by increasing the availability of nutrients in the low-water potential clays (Phelps et al., 1994).

A contrasting relationship of microbial properties with sediment texture was observed during analysis of depth-stratified saturated samples (~1 m intervals >172–197 m depth) from a Miocene age profile consisting of fine-grained lacustrine sediments, a paleosol sequence, and coarse-grained fluvial sediments located in south-central Washington. Biomass (PLFA) (Fredrickson et al., 1995), number of bacterial cells, aerobic basal respiration (CO_2 production), and aerobic activity (C-14 glucose and acetate substrate mineralization) were significantly higher in the fine-grained lacustrine sediments compared to the other layers (Kieft et al., 1995). Very little anaerobic

activity (C-14 substrate mineralization) was apparent in any of the samples, although multiple lines of evidence suggest that the presence and activity of dissimilatory iron reducing bacteria and SRB were also highest in the lacustrine sediments (Fredrickson et al., 1995; McKinley et al., 1997). There was a high degree of correlation of total cells and aerobic activities (basal respiration and glucose mineralization) with total organic carbon (TOC) content of the sediments, which was markedly high in the lacustrine sediments (~1%). Unexpectedly, the paleosol sequence did not have high amounts of organic carbon (~0.1%), although the fluvial sands were even lower (~0.03%). Glucose mineralization exhibited an 85% CV over the vertical series of samples. It was also noted that the cell numbers observed in these saturated zone samples were lower than cell numbers previously reported from the shallow unsaturated zone samples collected from the same borehole (Kieft et al., 1993). This arid site has much lower rates of surface primary production and almost negligible annual recharge rates compared to the southeastern coastal plain site. Groundwater ages are estimated to exceed 13,000 years and permeabilities of all sediments ranged from 1 to $<10^{-3}$ mD. Thus, the fluvial sediments also had low permeability, although relatively higher than the lacustrine sediments, and may not have received sufficient fluxes of exogenous carbon to maintain any level of activity. Therefore, it may be the higher amounts of carbon originally deposited in the sediment that provides for the higher microbial numbers and activities in the lacustrine sediments, although their *in situ* activities may be severely limited by electron acceptor flux (Fredrickson et al., 1995; Kieft et al., 1995).

3.1.2. Juxtaposed sedimentary formations: centimeters to meters

A second recognizable trend in the distributions of microbes and activities in stacked sedimentary layers has also been observed at several sites. When a low-permeability, higher carbon, fine-grained sedimentary formation lies adjacent to a more permeable, coarser-grained formation, diffusion of organic substrates from higher concentrations in the low permeability sediments into the higher permeability formation where electron acceptor flux may be higher, results in elevated respiratory activities (McMahon and Chapelle, 1991) (Fig. 6-4). The organic substrates can be solubilized detrital carbon or fermentation products thereof. Respiration in the coarse grained sediments is limited by the low flux of exogenous carbon in the groundwater. This phenomenon was initially proposed following study of pore water organic acid concentrations in a clay-rich aquitard overlaying a sandy aquifer in the southeastern USA (McMahon and Chapelle, 1991). It was concluded that fermentation predominated in the organic-rich aquitard

producing low-molecular weight fatty acids that diffused into the aquifer where they supported anaerobic respiration of advectively supplied electron acceptors. Peaks in respiratory activity in the coarser-grained sediments may be particularly pronounced at the interface between two layers. Such a pattern was reported in a Cretaceous sandstone–shale sequence cored to a total depth of 230 m in northwestern New Mexico. In these anaerobic consolidated sediments, peaks in sulfate-reducing activity potentials were observed in sandstones adjacent to the interface with high-carbon shales (~1% TOC) (Krumholz et al., 1997). The autoradiograph technique used indicated that SRB activities varied considerably within samples at millimeter scales. No evidence was found of active fermentation in the shales, but it was proposed that acetogens co-occurring at the interface converted the diffusing detrital carbon to fuel the SRBs (Krumholz et al., 1999). General anaerobic activities (C-14 substrate mineralization) were recorded in sandstones with pore throat diameters >0.2 μm, but not shales with pore-throat diameters <0.2 μm, although overall detectable microbes and activities were extremely patchy thoughout the profile (Fredrickson et al., 1997). Over the entire vertical sequence, microbial biomass (PLFA) exhibited 185% CV. Permeability is directly related to pore throat diameter, and therefore the shales would experience low groundwater fluxes and are probably electron acceptor limited. Small pore diameters (<0.2 μm) that reduce diffusive fluxes (less connectivity, more tortuosity) and represent a critical boundary for cell movement may explain the carbon preserved in the shales. A later study (Walvoord et al., 1999) indicated an upwards groundwater gradient at this location and that microfractures in the shale may have also contributed to the observed microbial distributions. Vertical distributions of groundwater Archaea were studied at this site using dialysis chambers incubated in the open corehole over an 8 m interval spanning a shale–sandstone interface (Takai et al., 2003). rDNA profiles (tRFLP) of Archaeal communities shifted considerably at scales of 10s of cm across the interface of the two formations. In conjunction with groundwater geochemistry, methanogens were found to be present and active in these groundwater samples collected adjacent to the shale. No evidence of methanogens or their activity had been previously observed during study of the core samples (Fredrickson et al., 1997; Krumholz et al., 1997).

Conversely, while fermentation may be occurring in the low permeability, higher carbon sediments, respiration in the same would be controlled by diffusion of electron acceptors from the higher permeability sediments. This situation was hypothesized to occur in the Miocene lacustrine sediments by Fredrickson et al. (1995) based on dissolved pore-water sulfate profiles and by McKinley et al. (1997) with additional data on fermentor and SRB distributions surrounding the contact interval. It appeared as if fermentation was ongoing within the low permeability lacustrine sediments and

SRB activity peaked near the edges of the fine-grained sediments where SO_4^{2-} was supplied. So, peaks in respiratory activity should occur at the interface, but could exist on either side. Samples collected from 30 m of unconsolidated Eocene sediments in east central Texas are another location where the diffusion of organic acids (presumably fermentation products) from lower permeability high-carbon clays or lignites stimulated SRB activity in the adjacent higher permeability sands, particularly at the interface (Ulrich et al., 1998). Sulfur and iron-oxidizing bacteria were also preferentially present in the sandy sediments at these interfaces, even under reducing conditions. Similarly, SRB activity was observed in areas that were considered oxidizing. Over the 30 m profile, SRB activity varied several orders of magnitude, even when samples were taken <10 cm apart. Finer scale studies performed with an autoradiograph technique demonstrated that SRB activity in cores also varied at the millimeter scale. It was proposed that pyrite oxidation preferentially occurring in the more shallow areas supplied SO_4^{2-} for SRB activity in the deeper aquifer.

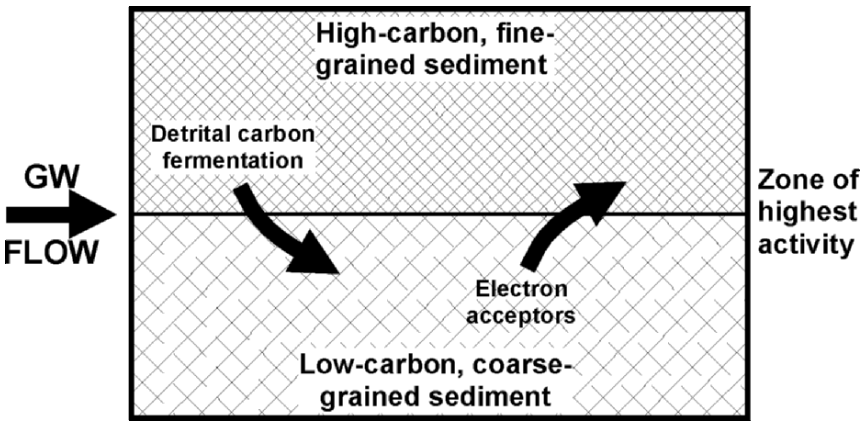

Figure 6-4. Diffusion gradients of organic matter from high-carbon, fine-grained sediments encounter higher electron acceptor concentrations in low-carbon, coarser-grained sediments, and vice versa.

3.2. Trends within shallow sandy formations: centimeters to meters

Barbaro et al. (1994) examined microbial properties at 10 cm intervals over nine 1.5 m vertical transects though a shallow sandy aquifer near Borden, Ontario. At these sites dissolved oxygen declined sharply over this depth

interval from values of 5–6 mg L^{-1} near the top of the aquifer to 1–3 mg L^{-1} near the bottom. They found that aerobic numbers and activities (ETS) generally declined in parallel with depth and oxygen, although occasional peaks were exhibited. No assessment of anaerobic physiologies was performed. Samples collected at 10 cm intervals from three 1.5 m vertical transects in unsaturated sandy sediments from Virginia's Eastern Shore were analyzed for a variety of microbial properties (Musslewhite et al., 2003). The transects were established at 1–3 m depth and were confined to a sedimentologically homogenous stratum. No evidence was provided that indicated redox changes over the profile. Microbial properties (biomass, aerobic and anaerobic H_2 oxidation) varied little over the vertical direction, with slight increases near the bottom of the profile where moisture was higher (possibly capillary fringe, no groundwater table depth given).

3.3. Deep vadose zones in arid regions: meters

In the deep vadose zones of arid regions, numbers and activities are very low and very sparsely distributed. It appears that water potentials exert an overriding control on the activity, and perhaps persistence of microbes in these dry sediments. Even at the low (–0.1 to –1.0 MPa) matric water potentials commonly observed in arid vadose zones sediments; however, it is unlikely that that microbes will be killed by dessication (Kieft et al., 1993). In comparison, surface soil water potentials in these same arid regions can fall below –10 MPa (Kieft et al., 1993), and some microorganisms can tolerate water potentials as low as –400 MPa (Potts, 1994). The effect of low water potential is likely to be indirect (Fredrickson et al., 1993; Kieft et al., 1993). There are infrequent (if any) saturated episodes to advectively supply or redistribute nutrients. Decreasing water potentials (<–0.1 MPa) in the unsaturated sediments further restrict diffusional fluxes of carbon and nutrients required for maintenance activities or growth. Further, the movement of bacteria would be expected to decrease as water films become thinner than 1 μm below –0.1 MPa (Kieft et al., 1993). Kieft et al. (1998) assessed the microbiological properties of two vertical vadose zone sediment profiles with contrasting porewater ages. Samples from profiles were composed of relatively uniform silty-fine sand sediments that primarily differed with age as a function of depth (chronosequence). A unified and steady decrease with depth (15 and 35 m) was observed in all microbiological parameters measured on samples collected at approximately 1–2 m intervals. In a similar study of arid vadose zone sediments in southeastern Washington, Ballkwill et al. (1998) analyzed samples from ~7 m depth profiles from five sites that differed with respect to recharge characteristics. The authors concluded that

at the higher recharge sites, episodes of preferential flow (at or near saturating conditions) via vertical fissures allow for periodic recolonization of spore-forming bacteria (i.e., Streptomyces) from the surface. The result of more frequent recolonization is a more uniform depth profile of populations prone to transport that can also thrive under the subsurface conditions.

3.4. Deeper, low permeability saturated formations

In samples collected via mining from a massive clay at 224 m depth, very low activities and viable cells (aerobic and anaerobic), frequently below detection, have been reported (Boivin-Jahns et al., 1996). These authors conclude that the small pore throat dimensions (<0.1 μm) of this formation preclude sufficient fluxes to sustain microorganisms, despite the high carbon content (3%) of the clay. In a series of massive clay aquitard and surrounding clay-rich sediments, Lawrence et al. (2000) used a variety of culture-dependent and -independent methods to characterize the microbial communities to a total depth of 122 m. In the upper range of these sequences, numbers and diversity of physiological types were high in the less dense, more weathered saturated sediments overlying the aquitard. Progressing downward through the profile into a more undisturbed, massive clay aquitard, distributions of microorganisms became extremely patchy, with occasional peaks of uncertain trend. These authors conclude that the low permeability ($<10^{-3}$ mD) generally limits fluxes of nutrients, and only occasional localized areas (less than millimeter scale) possess favorable conditions.

Core segments from deep (~860, 2,000, and 2,100 m) Cretaceous and Tertiary sandstones interbedded with shales were analyzed for a variety of microbiological properties with considerable emphasis on anaerobic bacteria (Colwell et al., 1997). These strata had previously experienced sterilizing temperatures (>120°C) and present temperatures ranged from 43°C in the 860 m samples to 85°C in the 2,100 m samples. Primarily fermentors and iron-reducing bacteria were recovered from some of the 860 m samples, while the deeper samples yield little evidence of microorganisms. The positive enrichments were from 860 m deep sandstone cores that had relatively higher permeability (up to 1 mD), higher porosity (up to 12%), and higher average pore-throat dimensions (up to 1 μm) compared to adjacent shale cores with low permeabilities ($<10^{-3}$ mD), lower porosity (<5%), and smaller pore-throat diameters (<0.2 μm). According to this report, recolonization of these sandstones was hypothesized to occur via vertical fractures due to the relatively young groundwater age. The absence of organisms in the deeper cores containing more ancient groundwater was attributed to those formations being hydrologically closed since the period of sterilization.

3.5. Fractured, crystalline rock

Fractured igneous rocks such as granite and basalt have also been sampled intensively at a limited number of locations to characterize their microbiology. The origin of these habitats strongly contrasts with sedimentary systems as they were formed en mass, either as a plug emplaced from below, or as flow extruded onto the earth's surface. Igneous rocks exposed to the earth's surface will have encountered various periods of weathering, sometimes being covered with sedimentary deposition. Intrusives may undergo further changes via metamorphosis or secondary mineralization. Regardless, the temperature of the originally molten rock would have rendered them initially sterile.

The microbiological distributions within the ash-fall volcanic tuff of the Ranier Mesa (Nevada Test Site) near the proposed Yucca Mountain (Nevada) geologic repository for high-level nuclear waste have been studied in several papers. Detectable numbers of cells, biomass (PLFA) viable heterotrophs, and diversity of heterotrophs were observed in seven samples arrayed over a 50–450 m depth range from three locations (within 10 km) in unsaturated tuff (Haldeman and Amy, 1993). Microbiological properties strongly varied and samples separated by several meters were as different as those separated by >1 km, despite the geophysical similarity of the tuffs. There were no reported correlations between microbiological and physical properties (including moisture and temperature) of the rock samples. An intensive study of the heterogeneity of chemoheterophic bacteria was conducted on a three-dimensional array of samples collected from a 21 m^3 section of the same tuff (Haldeman et al., 1993). Again, a great deal of variation was observed in the numbers and populations of isolates among the rock samples, and there were no apparent correlations with rock physical properties. A thorough statistical analysis was performed on the relationships between biological and abiological properties of these same tuff samples (Russell et al., 1994). The magnitude of culturable counts exhibited a coefficient of variation of 276% (3D) and it was concluded that the chemoheterotrophic populations were distributed randomly thoughout the 21 m^3 tuff section.

Vertical transects (63–134 m) of microbiological properties were studied in two sets of saturated cores collected from the layered basalt flows of the Snake River Plain Aquifer in southeast Idaho (Lehman et al., 2004). Twenty-three basalt cores were analyzed from a well (TAN-37) that was influenced by an organic-rich, mixed waste plume and 19 cores from another well (TAN-33) that was minimally influenced by this plume. The basalt core samples were compositionally similar; however, the physical structure of the individual basalt cores at the centimeter–meter scale varied from extremely

dense to highly vesicular and each core possessed varying occurrences of small fractures (generally infilled with clays or calcite). At the larger scale spanning meters to 10s of meters, the core samples varied with respect to their proximity to vertically zoned areas of high-lateral hydraulic conductivity. Although groundwater is contained in the basalt matrix, groundwater flow primarily occurs via formation level features such as large, open fractures and weathered, rubble zones existing at basalt flow interfaces. The basalt is considered a dual permeability geologic media wherein high formation permeabilities result from the large fractures and rubble zones, while the bulk of the basalt matrix possesses low permeability (ca. <1 mD). There may be relatively high pore space (~15%) represented by vesicles in the basalt matrix, yet these vesicules may be only minimally connected by small apertures, microfractures, or mineral grain boundaries that greatly restrict flow. It was originally hypothesized that the occurrence of the highly conductive fracture and rubble zones would control microbiological properties through the vertical transect. In basalt cores collected from the relatively pristine TAN-33 location where oxic conditions predominate, over a dozen assays (total cells, viable heterotrophs, enumerations of various aerobic and anaerobic physiologies, aerobic and anaerobic C-14 substrate mineralization) demonstrated little evidence of viable or active microorganisms (Lehman et al., 2004). For the limited number of assays that had a positive response, CVs ranged from 100% to 200% over the entire vertical transect (Lehman et al., 1999). At the suboxic, contaminated TAN-37 site, much higher values were obtained from all assays, however individual parameter magnitudes varied considerably over the vertical transect, often exceeding 200% CV. No significant positive correlations were observed between measurable microbiological properties and the occurrence of highly conductive flow zones (identified by multiple geophysical measures) in either well.

At the Mineral Park Mine (Arizona), 16 cores collected at regular intervals between 11 and 103 m deep within an igneous intrusive (biotite-quartz-monzonite porphyry) were subjected to intensive analysis of microbiological properties (Lehman et al., 2001). The rock itself had very low permeability and porosity, and groundwater was largely conducted via larger, open macrofractures. Groundwater in the upper 25 m had detectable dissolved oxygen (1–3 mg L^{-1}) compared to the lower anoxic portion of the well. Cores often contained smaller fractures of various sizes which were heavily mineralized with quartz, pyrite, chalcopyrite, and molybdenite. No neutrophilic aerobic or anerobic chemoheterotrophic bacteria were cultured from any of the cores. Using solid media plating approaches, acidophilic chemolithotrophic bacteria were also universally absent, while acidophilic chemoheterotrophs were enumerated in about half the cores spanning the

entire depth. No fracture surfaces or fracture infills were examined as these were presumed to be contaminated during sample collection. Liquid enrichments using various donors for iron- and sulfur-oxidizing bacteria were largely negative, except $FeSO_4$ enrichments on samples from the lower half of the vertical sequence. Considering the data in sum, there was little evidence of vertical stratification of microbial properties in this relatively uniform crystalline rock.

The microbiology of deep granitic aquifers in Finland and Sweden have been the focus of numerous studies (Pedersen, 1997). This work has been primarily conducted on anoxic groundwater collected from boreholes and excavations or on biofilms from substrata incubated in the flowing groundwater. Total cells range from 10^3 to 10^7 mL^{-1} and a variety of physiologies have been documented including facultative anaerobic chemoheterotrophs, DIRB, SRB, autotrophic and heterotrophic acetogens, and methanogens. Analysis of 16 groundwater samples collected from a 65 to 350 m range of depths from multiple wells showed total cells did not decrease with depth. Multivariate statistical analyses showed that the distributions of physiologically distinct populations were largely independent of a range of groundwater geochemistries (Haveman and Pedersen, 2002). One exception is that DIRB and SRB were not cultured from deeper, older, more saline groundwater. Anaerobic physiologically distinct populations were enumerated from groundwaters spanning 200–950 m depth across four sites (Haveman et al., 1999). Total cells did not decrease with depth. This study concluded that the nature of fracture infills in the vicinity of the groundwaters predicted the relative population distributions. Specifically SRB numbers were highest where iron sulfide minerals filled fractures and DIRB were more abundant where fractures were filled with iron hydroxides. In contrast, groundwater geochemistry did not predict these anaerobic populations. In a previous study of granitic groundwaters, it was found that groundwater TOC correlated with total cell numbers (Pedersen and Ekendahl, 1990). A similar correlation was observed between TOC and total cell numbers in groundwaters collected from up to 105 m deep from sedimentary formations in Gabon, Africa (Pedersen et al., 1996).

3.6. Hot spots – vertical gradients stimulated from below

The possibility exists for deep subsurface communities to exist independent of photosynthetically fixed carbon. It has been proposed that autotrophic microorganisms form the base of these ecosystems and that they are fueled by either hydrogen generated by low-temperature water–rock interactions (Stevens and McKinley, 1995) or by H_2 and CO_2 generated from thermal

processes in deeper, hotter regions in the crust or mantle (Chapelle et al., 2002; Pedersen, 2000). Alternatively, inverted vertical gradients of microorganisms may be sustained by thermal decomposition of buried, photosynthetically fixed carbon that produces hydrogen, methane, and short chain hydrocarbons. In either case, microbial populations would be distributed according to the presence of these abiotic reactions that supply the electron donors and the availability of CO_2.

4. HORIZONTALLY DISTRIBUTED MICROBIAL PATTERNS IN THE SUBSURFACE

There are few studies that report on horizontally distributed samples of subsurface sediments or rocks, primarily due to the expense and effort in coring at multiple locations. In order to interpret lateral continuity in microbiological properties, not only would depth have to be comparative, but also relative sample position within a given geologic formation. Shallow unconsolidated sediments that are accessible by push probe technology could be sampled at reasonable expense and effort, but there are few instances where such data has been reported. These few exceptions concerning samples within a single formation will be discussed below. Several studies report on samples that progress laterally from a contaminant point source that strongly influences and structures the subsurface communities. Other studies have examined groundwater microbiology in association with spatial distributed differences in groundwater geochemistry, frequently due to the occurrence of contamination. Some of these will be included because of the lack of appropriate comparisons for core samples.

4.1. Horizontally distributed patterns within a sedimentary unit

Information on horizontal distributions can be gained from study of cores collected from different locations within laterally contiguous formations. Mineralization patterns (aerobic C-14 substrates) (Hicks and Fredrickson, 1989) and cell growth (acetate and thymidine incorporation) (Phelps et al., 1989) according to sediment textural characteristics were observed within vertical transects through Tertiary and Cretaceous formations cored in the southeastern coastal plain. These same mineralization patterns were observed in all three boreholes separated by distances up to 16 km. The distribution of physiological distinct chemoheterotrophs isolated showed less variability in samples collected from the same formation in different boreholes than from

samples collected at different depths or formations within a single borehole (Balkwill et al., 1989). These results indicate that where formations exhibit sharp contrast, there is a degree of lateral continuity in these associated microbiological properties with the formation. Although at this same site, even closely spaced vertical samples confined to a single formation showed substantial heterogeneity in their chemoheterotrophic populations (Fredrickson et al., 1991). Together, these observations indicate that the observed lateral continuity in formation microbial properties was a relative consequence of the coarse differences between formations.

In the study of fluvial sediments of varying texture from eight boreholes (20–30 m deep) within a ~6 km^2 (Germany) area, community structure (based on phenotypic testing of chemoheterotrophic isolates) varied greatly among the boreholes (Kolbel-Boelke et al., 1988). In nine cores collected within an 20 m^2 area from a shallow (<3 m), sandy aquifer (Borden Ontario), depth profiles of activity (ETS) and viable cells were consistent in five of the nine boreholes, but exhibited various departures from this pattern in the other four boreholes (Barbaro et al., 1994). The rather low numbers and activities observed in these sediments with relatively high dissolved organic carbon (3–29 mg L^{-1}) led investigators to propose that microbial activity may be regulated by the quality of available carbon.

In a study of excavated samples from three vertical transects each separated by 5.5 m in a shallow (2 m), sandy, vadose zone on Virginia's Eastern Shore, depth profiles of microbial properties were strongly consistent (Musslewhite et al., 2003). In this sedimentological uniform subsurface environment, little lateral variability in microbiological properties was thus observed, although most values were generally low. In nearby shallow (<7 m), sandy aquifer, Franklin et al. (2000), examined the distribution of microbial community profiles (DNA–RAPD) in groundwater samples with respect to groundwater geochemistry as it varied in distinct oxic and anoxic zones occurring within a 0.02 km^2 area. Groundwater communities from oxic and anoxic locations were easily distinguished, but geostatistical analysis demonstrated no relationship between well location and community profiles within each location (oxic or anoxic). The results suggest that scales of spatial correlation in groundwater communities in this relatively homogeneous sandy aquifer must be smaller than the distance between wells in this study (10 m).

4.2. Horizontally distributed patterns within a fractured rocks

Within uncontaminated, unsaturated volcanic tuffs of the Nevada Test Site, there was no evidence of lateral continuity in the microbiology of geophysically similar tuff samples within a 21 m^3 volume (Haldeman et al., 1993; Russell et al., 1994).

In basalt flows of the Snake River Plain Aquifer (southeastern Idaho), microbiological properties (inclusive of aerobes and anaerobes) were measured in cores from three boreholes arranged along the local hydrological gradient at varying distance from a point source of a mixed-waste contaminant plume (injection well). Mean property values for vertically distributed cores were plotted against distance from the injection well (Fig. 6-5). In this case, the eutrophication of an oligotrophic aquifer can easily be detected. While there is substantial variation in the magnitude of microbial properties in the lateral direction, this variation was associated with contaminant plume as all of the detected quantities had correlations >0.93 with TCE concentration (used as a relatively conservative tracer of the plume).

In a spatially broader study of Snake River Plain Aquifer microbial communities, groundwater samples were analyzed from 85 wells variably distributed over a roughly 1,500 km^2 section of the aquifer. Groundwater was pumped from the layered basalt aquifer from depths of 60–250 m (depth to water table increases in a southerly direction across the region). A total of 132 groundwater samples were analyzed in triplicate by community-level physiological profiling with Biolog plates (O'Connell and Lehman, unpublished data) in an effort to determine a baseline against which perturbations could be compared. The resulting responses were so variable that multivariate comparisons were not required, even when comparing samples collected from a single well at different dates. Using the number (of 95) carbon sources respired as the response, numbers ranged from 2 to 95 (mean = 56) positive carbon source utilization tests by the groundwater communities. The CV for the number of carbon sources (a very coarse measure) used by these 132 samples was 466%. Despite the physical heterogeneity of the basalt flows that comprise this aquifer, the mineralogical make-up of the basalt is very uniform and with very few exceptions, the groundwater is oligotrophic with DOC <1 mg L^{-1}. The results indicate a very unpredictable pattern in the distribution of groundwater microbial communities in the absence of a strong selective gradient (e.g., contaminant plume) or sharply contrasting lithological features.

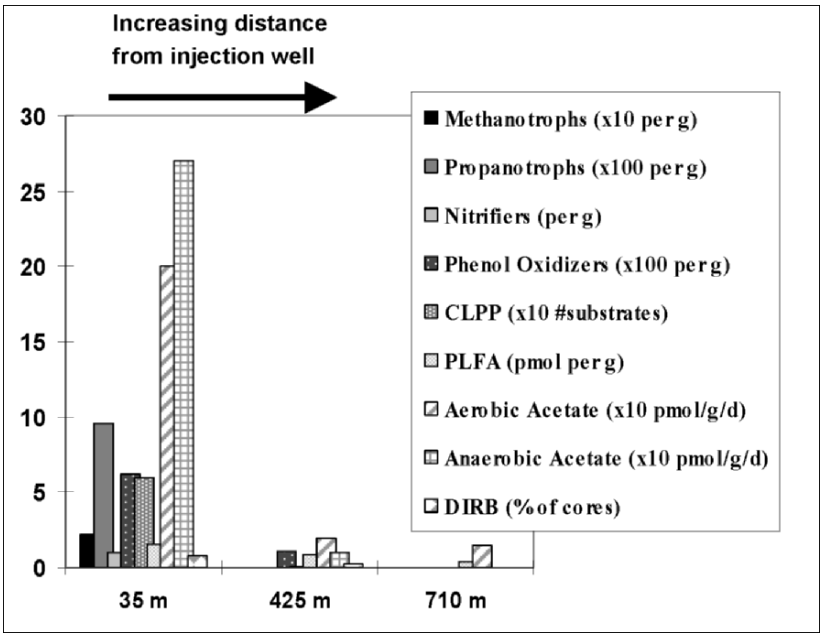

Figure 6-5. Distribution of mean values (all cores) of microbiological properties from three boreholes in the fractured basalt Snake River Plain Aquifer (SE Idaho) arranged with increasing distance (TAN-37, 35 m; TAN-33, 425 m, TAN-48, 710 m) from an injection well used for the disposal of sanitary and mixed industrial wastes. (Modified from Lehman et al., 1999.)

4.3. Horizontally distributed patterns along a groundwater flowpath

A trend that has been observed in the microbiology of aquifers relates to the distribution of predominant terminal electron accepting processes (TEAP) along a groundwater flow path. Redox potentials in systems with restricted oxygen resupply are driven progressively downwards with the oxidation of organic matter. Most subsurface systems have restricted access to atmospheric oxygen, either dependent on free gas diffusing though the overlying vadose zone or by dissolved gas in water infiltrating from the surface or from some horizontally located recharge area. Some of the oxygen is consumed by organic oxidation occurring during recharge/infiltration and the remainder is consumed within the aquifer during oxidation of dissolved carbon entrained in the groundwaters during its passage through soils. In some

instances, detrital carbon deposited in deeper sediments may support respiratory activities, although this is likely to be at slow activity rates. Once oxygen is consumed, a predictable sequence of TEAPs will be observed based on the relative energy yield of these reactions and the availability of such acceptors either dissolved in the incoming water or present in the rocks (Fig. 6-6) (Lovley and Chapelle, 1995). Oxidized iron may be particularly important in the subsurface due to its abundance in solid forms (Lovley, 1991). This progressive sequence with distance from a recharge site, that is similar to that observed with depth in aquatic and marine sediments, will result in physiologically distinct populations being more numerous and active in horizontally distributed aquifer locations. Exceptions to this pattern occur when an aquifer is resupplied with oxygen from the atmosphere, or there is little dissolved carbon entering the aquifer in oxygenated recharge waters. The evolution of the organic carbon, both in quantity and quality, is suspected to govern overall activity rates and the distributions of populations associated with various TEAPs (Murphy et al., 1992). In aquifers that receive little infiltration from meteoric waters, the evolution of dissolved carbon and microbial activities will be associated with progressive age of the groundwater (Murphy et al., 1992). Unfortunately, there are few data concerning the composition or quality of organic carbon in aquifers and relationships with microorganisms.

In samples collected at varying distance from a contaminant point source, a continuum of elevated biomass can be observed and/or a continuum of predominant physiologies consistent with progressive changes in the local redox state (Lovley, 1991). The resulting distributions of biomass are not unlike the increase of biomass in a stream or lake near a sewage outfall and the sequence of predominant TEAP along the flow path is the reverse of uncontaminated aquifers. In this analogous case, it is the overall BOD that decreases with distance from the source. Once the added carbon is consumed, the aquifer microbial communities will resume the pattern described in the previous paragraph. Contaminated sites tend to receive the most study, and this pattern has been commonly observed in groundwater samples.

One of the most thoroughly documented examples of sediment-associated microbial community changes associated with distance from a carbon source is a study of core samples collected from a shallow, sandy aquifer along the flow path at increasing distances from a landfill (Denmark) (Ludvigsen et al., 1999). The top of the aquifer is about 3 m deep and the aquifer is composed of a heterogeneous assortment of Quaternary- to Tertiary-aged sediments of varying texture and origin. Sediment samples from depth profiles of nine boreholes arranged along a horizontal transect, 0 to 305 m from the landfill. Biomass (PLFA) generally decreased with distance

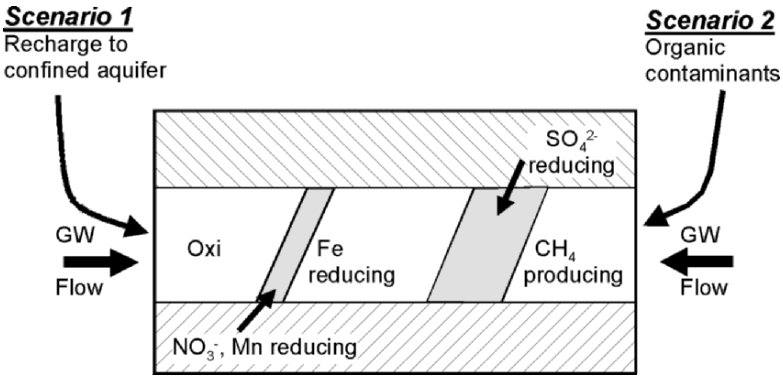

Figure 6-6. Distribution of predominant TEAPs: Scenario 1 – oxidation of groundwater organic matter in a confined or semi-confined aquifer results in decreasing redox potential with distance from recharge; Scenario 2 – high concentrations of organic contaminants drive redox potential to low levels that subsequently increase with distance from the contaminant source as the introduced organic matter is oxidized and dispersed to ambient levels. (Adapted from Lovely and Chapelle, 1995.)

from the landfill while total cells (AODC) show little discernable pattern. With increasing distance from the landfill, numbers (MPN and PLFA) of the following groups reached their peak and then declined in spatially distributed sequences: methanogens, SRB, DIRB, Mn-reducers, and nitrate-reducers.

4.4. Other examples of spatial distributions at contaminated sites

In addition to the study of Franklin et al. (2000) described above, several other studies have examined subsurface microbial communities or activities using a collection of spatially distributed wells. Although these studies do not examine core samples, they are included because they specifically illustrate horizontal distributions of subsurface microbial properties. Adrian et al. (1994) examined methane production rate from well clusters at two geochemically distinct locations impacted by landfill leachate in an otherwise homogeneous, shallow (<2.5 m), sandy aquifer. Over a 9-month period, the headspace methane concentrations at the two sites were measured in sixteen wells arranged in a grid covering 35 m^2. Methane production rates over the entire period at both sites were extremely variable, with CVs exceeding 340%. When individual seasons were examined, CVs of methane production in each of the two sites still ranged between 200% and 300%. It was

concluded that the magnitudes of methane production in the aquifer were log-normally distributed.

The association of the geochemical and microbiological characteristics of groundwater samples has rarely been quantified. Groundwater samples from multiple depth intervals from a series of wells located upstream and at regular intervals downstream from a landfill were used to relate microbial community structure (16S rDNA DGGE profile) to the groundwater geochemistry (Roling et al., 2001). Overall, large variations in very complex community profiles among the groundwater samples were observed. Multivariate statistical analysis of community profiles was able to distinguish groundwater samples from polluted and unpolluted zones and to correlate these communities and their members with contaminant concentrations. This data set was used in subsequent geostatistical analyses (Mouser et al., 2005) that found horizontal correlation distances of 40–50 m in the community profiles. It must be emphasized, that these groundwater samples were taken from a location that was highly structured (horizontally) by the landfill leachate. Nonetheless, the pattern could not have been quantified without the regular intervals of access, horizontal and vertical, that the multiple wells provided in a shallow (<8 m), unconsolidated sediments. Some sediment samples were also studied at this location, but the geochemistry of the groundwater was not reflected in the sediment communities. In another example linking microbiology to geochemistry in wells that were not spatially structured, but varied with respect to the occurrence of mixed contaminants, distributions of genes involved in nitrate and sulfate reduction were studied in groundwater samples (Palumbo et al., 2004). Artificial neural network models were used to establish relationships between the occurrence of two of five groups of dsrAB-related genes with uranium and sulfate concentrations and the occurrences of the other three groups with pH, nickel, and organic carbon concentrations.

4.5. Horizontally distributed patterns across regions

At most study locations, there are insufficient spatially arranged core samples to determine trends, let alone quantify these trends. However, the distribution of the study sites themselves may allow additional trends to be identified. Superimposed on the mosaic of historically evolved and complex geologic formations that comprise the subsurface environments will be patterns associated with modern-day climates. While climate may influence subsurface microorganisms, its influence will be largely a function of annual means (i.e., temperature and precipitation), rather than the diurnal and seasonal fluctuations that may strongly influence terrestrial surface microbes. Regional variations in climate will influence the amount and type of surface primary

production, land use patterns, and water. So, to the extent that these regional trends may result in difference in the delivery of either carbon or water, it would be expected that subsurface communities will respond. An example might be the deep vadose zone of arid regions, where microbial population seem to be considerably less numerous, less diverse, and less active than their counterparts in more humid environments.

5. LIMITATIONS TO UNDERSTANDING TERRESTRIAL SUBSURFACE MICROBIAL DISTRIBUTIONS

There are a number of considerations that are either unique to subsurface environments or elevated in comparison to surface environments. These considerations may significantly alter conclusions regarding subsurface microorganisms.

5.1. Sampling constraints

Spatial distributions in subsurface environments have generally resisted quantification because subsurface environments are: (i) highly complex, (ii) not directly observable, (iii) technically and economically difficult to sample, and (iv) not intensively studied over longer periods (>10 year). As a result, few (if any) subsurface environments have been representatively sampled. The overall density of samples is often too low for geostatistical comparisons. Sample replication is not usually achieved, and estimates of variance for replicate samples attempting to characterize a limited volume are rare. At many locations, only a single borehole is investigated, so often $n = 1$. The scale of variability is invariably smaller than the sampling scale, which is constrained by the required material for analyses. In a study to determine the representative elemental volume (REV) for culturable heterotrophs in vadose zone sediments, investigators found that large REVs would be required to account for the extreme patchiness of microbial distributions observed in these samples (and other subsurface samples) (Stevens and Holbert, 1995). Further, a good estimate of REV was not achieved for these sediments due to density-dependent growth during dilution plating, a phenomenon that was observed in other subsurface studies. Even simple correlations between biotic and abiotic variables are difficult as measurements often cannot be made on the same material, especially if a large REV is necessary for microbiological analyses. Given multiple interacting factors controlling microbial distributions, it is certain that the distributions of these factors will be on

differing scales. The exception is when there is a strong overriding selective factor such as organic contamination.

5.2. Methodological constraints

In addition to the patchy distributions of subsurface microorganism, several other characteristics of microorganisms present exaggerated difficulties when subsurface samples are analyzed.

1. The percentage of total cells that are culturable is generally orders of magnitude lower than surface environments.
    a) Enumeration detection limits are confronted.
    b) A small percentage of the community is evaluated.
2. Cells are frequently small and often indistinguishable from sediment particles by direct observation.
3. Activities are generally very low.
    a) Assay detection limits are confronted.
    b) Longer assay durations encourage selection.
    c) Laboratory conditions sharply contrast with *in situ* conditions.
4. *In situ* activities are technically difficult to measure.

The high disparity between laboratory estimates of subsurface microbial activities and their apparent *in situ* activities has been thoroughly documented (Chapelle and Lovley, 1990; Phelps et al., 1994).

6. TENDENCIES AND TRENDS IN TERRESTRIAL SUBSURFACE MICROBIAL DISTRIBUTIONS

Like other environments, microorganisms exhibit heterogeneous distributions at a number of different scales. At the micron scale, microorganisms experience the local geochemical gradients that regulate their metabolism, although this scale remains a challenge. Minerals are often distributed in rocks at the micron and tens of microns scale. Some work has revealed these fine-scale distributions of microorganisms with respect to mineral phases and their boundaries (Edwards et al., 1998; Lawrence et al., 1997; Taunton et al., 2000). At the sediment particle or aggregate scale (microns to millimeters), work has shown substantial gradients in populations, both in number and type (Albrechtsen, 1994; Murphy et al., 1992). A few studies have specifically addressed variations of subsurface microbial properties at the centimeter scale, particularly with respect to REV (Brockman and Murray, 1997; Stevens

and Holbert, 1995). Analyses of frequency distributions of subsurface microbial properties (their magnitude) suggest that they are log-normally distributed (Brockman and Murray, 1997; Zhang et al., 1997), like many other environments. Large CVs for microbiological parameters measured on spatially distributed samples are consistent with log-normally distributions. Conventionally, sample sizes are approximately 100 g (~35 cm^3), which may be a subsample from a larger (~1–2 kg homogenized sample). Yet, the frequency distributions must depend on the sample size in relation to the patch size, which is undetermined for most environments. Subsurface microbial properties clearly vary at the meter scale, both within and between formations, and all higher spatial scales. Considering that the spatial variations in populations and the magnitude of their activities may be as great over 1 mm as they are in 1 km, the scale of interest must be determined apriori, and then the investigation can turn to detecting the scales of the controlling factors. Few reports have even attempted geostatistical analyses of subsurface microbial distributions. Unpublished geostatistical analyses of the centimeter-spaced samples from relatively homogenous, bedded vadose sediments indicated a vertical variogram range of 15 cm and a horizontal range of 250 cm at one site and a vertical range of 1 m at another site for aerobic mineralization (Brockman and Murray, 1997). The other two studies analyzed groundwater samples. The first (Franklin et al., 2000) could not detect a correlated lateral range of microbial community composition at the minimum distance sampled (10 m). The second (Mouser et al., 2005) found a lateral range of 40–50 m at a site strongly structured by landfill contamination.

6.1. Vadose versus saturated zones

The trends identified in Figs. 6-1 and 6-2 appear to be most reproducible – increasing depth beyond some point is not accompanied by decreases in microbial biomass. The initial decrease in biomass from the subsurface is strongly related to changes in TOC. In the subsoil region, depth appears to be associated with a greater predominance of gram-positive bacteria and Actinomycetes. Some observations indicate that cell-specific activities may be higher in subsoils compared to surface soils (Blume et al., 2002; Federle et al., 1986; Fierer et al., 2003), suggesting these organisms may be opportunistic, poised for a fresh supply of carbon. In deeper vadose zones, particularly in arid regions, water seems to be the controlling factor on populations and activities. Distributions are frequently extremely patchy and a low fraction of the total biomass is culturable. The very low activities respond to water as it delivers a flux of nutrients (Fredrickson et al., 1993;

Kieft et al., 1993). Even under the driest subsurface conditions, the lack of water-induced nutrient fluxes will not ultimately limit survival of all bacteria, especially when considering the viability of cells that have been isolated from nutrient fluxes in amber (Cano and Borucki, 1995), fluid inclusions (Vreeland et al., 2000), permafrost (Shi et al., 1997), and highly impermeable formations (Fredrickson et al., 1995; Lawrence et al., 2000; Onstott et al., 1998) for millions of years.

Biomass generally increases when sediments are saturated and it has been frequently reported that gram-negative bacteria become increasingly predominant. The more uniform distribution of nutrients may produce a more dominant community structure due to increased competitive opportunities. Redox potential in the saturated zone will be a function of its isolation from atmosphere, the amount of carbon oxidized near the surface during infiltration or recharge, the amount of carbon that reaches the aquifer (including anthropogenic), and the distance from the recharge source. The ambient redox potential should predict the predominance of physiologically distinct populations and their activities (Fig. 6-6). There are two primary habitats in the saturated, groundwater and sediments (or rock), and different microbes may inhabit these environments and respond to perturbations in different ways.

6.2. Shallow versus deep saturated sediments

The microbiological activity of shallow aquifers is more likely to be controlled by quantity of carbon reaching the aquifer from the surface. In comparison, activities in deeper sedimentary aquifers are more likely to be related to detrital carbon sources. Shallow aquifers are usually unconsolidated sediments and fluxes of electron acceptors should be sufficient, although ambient redox conditions will modulate dominant populations and activities. In deeper aquifers, groundwater flow is generally slower and the flux of electron acceptors may become as important as the flux of carbon in controlling overall levels of activity. Deeper formations have lower porosity and smaller pore-throat dimensions that restrict movement of cells and the diffusion of electron donors and acceptors. Some evidence suggests that bacteria cells in shallow aquifers (Balkwill and Ghiorse, 1985) or deeper transmissive formation (Brockman and Murray, 1997; Sinclair and Ghiorse, 1989) may have relatively high culturability and potential for activity, while most evidence indicates very low culturability in deep, lower permeability and unsaturated formations (Brockman and Murray, 1997; Onstott et al., 1999).

It appears that in many deeper subsurface environments, it is the flux of nutrients (especially electron donors and acceptors) that limits and

distributes microbial populations and their activities. Therefore, the factors that control the hydrology seem paramount. Carbon must be present either associated with sediments at deposition or remaining in the groundwater after its travel from the recharge or infiltration site. In deeper areas with older groundwater, the amount and quality of carbon supplied by groundwater fluxes may be insufficient to support much activity. If detrital carbon in sediments is sustaining microbial activity, it must be physically accessible to cells via sufficiently size pore throats (>0.2 μm) or there must be sufficient water in the small pores to sustain diffusional fluxes to areas where cells exist. Even given the existence of sufficient carbon, fluxes of electron acceptors may limit respiratory activity to higher permeability regions where groundwater provides a supply of electron acceptors (Fredrickson et al., 1995). Ultimately survival will be limited by temperature in formations deeper than 4–6 km.

6.3. Vertical versus horizontal distributions

Microbial biomass and activity initially declines with depth, then remains fairly uniform or slightly decreases over increasing depth (Fig. 6-3). Many potential controlling variables (T, TOC, porosity) covary with depth over varying intervals. The occurrence of different formations varies according to changing depth intervals within a single site and between sites. Subsurface microbial properties in a single borehole have been observed to vary considerably within formations and between formations with CVs of measured properties often exceeding 200%. The microbiology of sedimentary formations often correlates with textural characteristics. The number of cells in fine-grained sediments may be equal or greater than in coarse-grained sediments, but the viable cells and activity are usually higher in the coarse-grained materials. The amount of carbon associated with sediments and the relatively permeability of these formations may alter this relationship (Kieft et al., 1995). Where high-carbon, fine-grained sediments abut lower carbon coarse-grained sediments, areas of higher activity have been observed near the interface (Fig. 6-4). The most common explanation is that organic carbon or fermentation products thereof diffuse from the fine-grained sediments into the coarser-grained sediments where higher fluxes of electron acceptors are present.

Data on horizontal distributions of sediment- (or rock-) associated microorganisms are limited. Reports on US southeast coastal plain sediments indicate a degree of lateral continuity in microbiological properties within a formation (Balkwill et al., 1989; Phelps et al., 1989), although this continuity may be relative to high contrasts observed between formations at this location. Studies of shallow, uniform, unsaturated sands indicate a high

degree of lateral continuity, although little variation was observed vertically at this site, and the magnitude of measured properties was very low overall (Musslewhite et al., 2003). In other instances discussed above, there appears to be high variation (high CVs) in microbial properties measured at laterally separated locations. High horizontal and vertical variation in microbial properties observed at most sites shed doubt on the reliability of using a single control well or borehole for comparative analyses. In locations where there is a strong selective pressure such as organic contamination, the ambient community may react strongly and predictably (higher biomass, TEAP sequence) to the structure imposed on the system. The magnitude of the response of the microbial community under conditions of organic enrichment will make ambient variations appear very small in comparison.

6.4. Sedimentary versus crystalline formations

The microbiology of fractured, crystalline rock environments contrasts strongly with that of sedimentary environments. Fractured, crystalline rock will not possess any detrital carbon and the groundwater in those environments that have been studied is usually low in dissolved carbon. The seemingly amorphous rock often defies attempts to find any horizontal or vertical trends in microbiological properties. Populations and activities are often low or undetectable in the low permeability rock matrix. Fluxes of nutrients will control populations and activities in fractured rock; however, fracture surfaces have not been well-studied because they are presumed contaminated during the sampling process. Groundwater samples often produce much higher populations and activities than samples of the rock. Given the low organic carbon in igneous rock aquifers, their frequent proximity to thermally active regions, and the occurrence of reduced inorganic energy sources (e.g., sulfides), there may be a higher probability of autotrophic populations and processes dominating these regions than many sedimentary formations. This has been observed in deep granite (Pedersen, 2000) and basalt aquifers (Chapelle et al., 2002; Stevens and McKinley, 1995).

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