Microbial Ecology of the Vadose Zone

T.L. Kieft

Department of Biology, New Mexico Tech, Socorro, New Mexico 87801

ABSTRACT

Studies in the last decade have demonstrated the presence of microorganisms in a variety of subsurface terrestrial environments, including the unsaturated layer (vadose zone) lying between surface soil and underlying groundwater. Viable microorganisms have been detected in vadose zone sediments and rocks. Populations of microorganisms are generally larger in relatively thin unsaturated zones of mesic environments; vadose zones in arid regions can be tens to hundreds of meters thick and generally have very low microbial abundance and activities. Buried soils (paleosols) can have larger microbial populations than sediments that have not undergone soil development. Although vadose zone environments are generally moist, the thin water films limit the transport of nutrients. Recharge rates can be as low as a few micrometers per year or less, resulting in minuscule nutrient fluxes. Opportunities for transport of microbes into deep, low-recharge unsaturated layers are minimal; thus, microbes in these environments may be descendants of populations that existed when the sediments or rocks were buried. The thin water films and scattered distribution of microorganisms limit cell-cell interactions, and thus vadose zone microbes may not function as true microbial communities. Vadose zone microbial activities may influence the fate and transport of subsurface contaminants and should be considered in the design and performance of hazardous waste repositories.

Introduction

It is now firmly established that microorganisms exist in subsurface environments, including vadose zone sediments and rocks. The vadose zone is defined as the region of the subsurface that extends from land surface to the underlying water table. It is also known as the unsaturated zone, which distinguishes it from the water-saturated zone that extends below the water table. Another designation is the zone of aeration, which describes the presence of the gaseous air phase that occurs in this zone along with the solid phase and liquid water phase. Vadose water has been estimated to comprise 0.005% of the earth's total volume of water [2]. Considering this low percentage, one might conclude that the vadose zone is inconsequential. However, the vadose zone is important for several reasons: (1) It is the zone of recharge to underlying groundwater; (2) it is often the portion of the subsurface in which contamination first occurs; and (3) vadose zone rocks and sediments are frequently chosen as sites for repositories of hazardous wastes.

Vadose zone rocks and sediments of diverse geological origins have been shown to contain viable microorganisms [18, 3, 7, 11, 1, 14]. Populations of microorganisms are generally larger in relatively thin unsaturated zones of mesic environments; vadose zones in arid and semiarid regions generally have lower microbial abundance and activities. Higher

Microbial Biosystems: New Frontiers
Proceedings of the 8th International Symposium on Microbial Ecology
Bell CR, Brylinsky M, Johnson-Green P (ed)
Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 1999.

numbers of organisms have been found in strata with higher amounts of residual organic matter, e.g., some paleosols (buried soils) [3, 11], in vadose zone environments that have received organic contaminants [6], and vadose zone environments that have been subjected to artificial recharge [6, 3]. A diversity of microorganisms has been cultured from unsaturated subsurface environments; however, gram positive microorganisms tend to dominate (Table 1). Despite the low numbers and slow *in situ* activities of unsaturated zone microorganisms, they can play important roles in remediation of subsurface contamination [18], mobilization or immobilization of spilled organic and inorganic contaminants [8], and biogeochemical cycling. This report will focus on the microbiology of the relatively thick vadose zones in arid and semiarid environments.

Table 1. Identities of bacteria cultured from vadose zone samples collected at three sites in arid and semiarid regions of the western United States

Hanford Site [1]	Nevada Test Site [7]	White Bluffs, Eastern WA [3]
Streptomycetes	*Arthrobacter*	**Arthrobacter**
Bacillus	*Micrococcus*	*Micrococcus*
Arthrobacter	*Bacillus*	*Clavibacter*
Azospirillum	Corynebacterium	*Nocardioides*
Bradyrhizobium	*Gordona*	*Planococcus*
Xanthomonas	*Acineteobacter*	*Streptomyces*
Pseudomonas	*Acidovorax*	*Bacillus*
Telluria	*Hydrogenophaga*	*Blastobacter*
	Pseudomonas	*Paracoccus*
		Methylobacterium
		Sphingomonas

Vadose Zone Physical and Chemical Conditions

Water exists in the vadose zone as thin, discontinuous water films that form concave menisci in crevises between particles. The average thickness of the water film is dependent on the matric water potential. The thin, discontinous nature of these water films restricts the movement of dissolved nutrients and also microorganisms. Advective flow is minimal and so transport of nutrients and microorganisms is controlled primarily by diffusion. Conditions in vadose zones are generally moist (water potentials are typically > -0.1 MPa) and so microbes are not subjected to desiccation. However, because the thin water films severely restrict solute diffusion and microbial movement, the indirect effect of reduced water potential in vadose zones on microorganisms is a low nutrient flux. This is especially true in arid and semiarid regions where rates of recharge may be extremely slow, e.g., 10 μm year^{-1} [1].

Sources of energy and nutrients in the unsaturated zone include those that are dissolved in the bulk water, solid-phase organic and inorganic nutrients, gaseous and vapor-phase organic and inorganic nutrients, and cellular reserves of energy. Given the generally low concentrations of organic matter in uncontaminated vadose zone environments combined with the slow rates of solute diffusion, microorganisms are often in a state of severe nutrient

limitation that may extend through time periods that are best expressed on a geologic time scale. Pristine subsurface environments, both saturated and unsaturated, have generally been characterized as nutrient limited. As a result, rates of microbiological activity in subsurface environments have been estimated by geochemical modeling techniques to be orders of magnitude slower than in surface environments [5, 21, 15, 20]. These slow rates of metabolism in the subsurface correspond to average microbial generation times of hundreds to thousands of years [21]. These estimates have been made for saturated subsurface communities; the average time interval between cell divisions in the unsaturated subsurface are likely even longer. Vadose zone microorganisms are thus subjected to periods of long-term starvation.

Starvation Survival in the Vadose Zone

Laboratory studies

Traditionally, starvation responses of microorganisms have been studied in pure culture experiments in which bacteria are cultured in a rich medium, washed and resuspended in a non-nutrient buffer, and tested at intervals for culturability and for changes in intracellular components. This approach has been especially useful for studying starvation responses of marine bacteria. We have modified this approach in order to study starvation responses of subsurface bacteria under vadose zone conditions by incubating cells for over a year in unsaturated sediments [17]. We studied starvation responses of two different genera: *Pseudomonas* and *Arthrobacter*. Within each of these genera, two closely related strains were compared: one isolated from a surface environment and one isolated from the subsurface. Two different sediments were tested: one a paleosol and the other a fluvial sediment that had not undergone significant soil development. Sediment microcosms were incubated under saturated and unsaturated conditions. Microcosms were sacrificed at intervals and tested for total cell numbers, culturable cells, and cell sizes. Membrane lipid phospholipid fatty acid profiles were quantified to determine temporal patterns of fatty acid stress signatures.

All strains of bacteria survived reasonably well in the microcosms; however, there were significant differences between treatments (Fig. 1). The *Arthrobacter* strains survived better than the *Pseudomonas* strains. Arthrobacters have previously been reported to remain viable despite severe desiccation and nutrient stress; they may be especially well adapted for long-term starvation in unsaturated environments. The surface strains of both genera survived equally as well as their subsurface counterparts, refuting our hypothesis that subsurface strains would be better adapted for long-term survival. Survival was better under saturated conditions than in unsaturated sediments, and bacteria survived at higher population densities in the paleosol than in the non-paleosol sediment. These findings reflect patterns of nutrient availability. Greater solute diffusion under saturated conditions favors starvation survival and residual organic carbon in buried soils favors long-term starvation survival. Both the *Pseudomonas* and *Arthrobacter* strains diminished in cell size during long-term nutrient stress. This is similar to patterns observed in other bacteria subjected to starvation and is also consistent with the generalization that when bacteria are observed in situ, they are of the dwarf cell type [10].

PLFA patterns of the *Pseudomonas* strains changed in accordance with patterns typically observed for other nutrient-stressed gram negative bacteria. These changes included

increased ratios of saturated to unsaturated fatty acids, increased ratios of *trans* to *cis* monoenoic fatty acids, and increased ratios of cyclopropyl fatty acids to their monoenoic precursors. Changes were greatest during the first two weeks of incubation. In many cases, these stress ratios returned to initial, pre-stress levels after extended incubation (32-64 weeks). This suggests that the classical PLFA stress signatures are transitory, short-term responses and that during long-term adjustment to nutrient stress, the membrane lipid PLFAs (and membrane fluidity) of gram negative bacteria are similar to those of unstressed cells. This finding is consistent with the fact that gram negative PLFA stress signatures are rarely, if ever, detected in subsurface microbial communities, despite the generally low nutrient availability. The *Arthrobacter* strains showed little if any change in membrane lipid PLFA ratios, as previously observed in a shorter term starvation experiment [16]. Evidently, *Arthrobacter* does not alter its membrane fluidity in response to nutrient stress and thus does not reveal its physiological status in convenient PLFA stress ratios, at least not during a one-year incubation.

Field study

Although laboratory studies of bacterial survival under vadose zone conditions are useful for elucidating initial physiological responses to nutrient stress, this approach falls far short of answering the question of whether vadose zone microbes are capable of survival for time intervals that may extend to thousands or even millions of years. To answer this question, it is necessary to go to the field. Field samples from a variety of environments have been used to test the hypothesis that ancient microorganisms have persisted in a viable state over geologic time periods [9, 4]. In this approach, environments that have been sequestered for thousands or even millions of years (e.g., insects in amber) are sampled aseptically and attempts are made to cultivate microbes or to extract, amplify, and sequence intact DNA.

Deep, unsaturated sediments in arid and semiarid regions are ideal candidates for examining ancient bacteria in that they are typically influenced very little by surface environments. Average rates of recharge by precipitation to deep vadose zones can be extremely slow. This means that nutrient fluxes are extremely slow and that the opportunities for transport of surface microbes to depth are negligible. Therefore, it is highly probable that microbes encountered in samples from deep vadose zones are relatively close descendents of the microbes that were present in the sediments at the time of burial, rather than populations of microbes that have been transported from the surface more recently.

We examined a series of buried loess sediments at 2 sites in eastern Washington State near the towns of Washtucna and Winona [10]. Ages of the sediments ranged from modern at the surface to 250 thousand years at 15 m depth at the Washtucna site and to 1 million years at 37 m depth at the Winona site. Porewater ages (determined by the chloride mass balance method, Murphy et al, 1996) ranged from modern to 3,700 years at Winona and to 1,200 years at Winona. These sediment and porewater ages broadly constrain the ages of the microbial communities, in that they cannot be younger than the porewater nor older than the sediments.

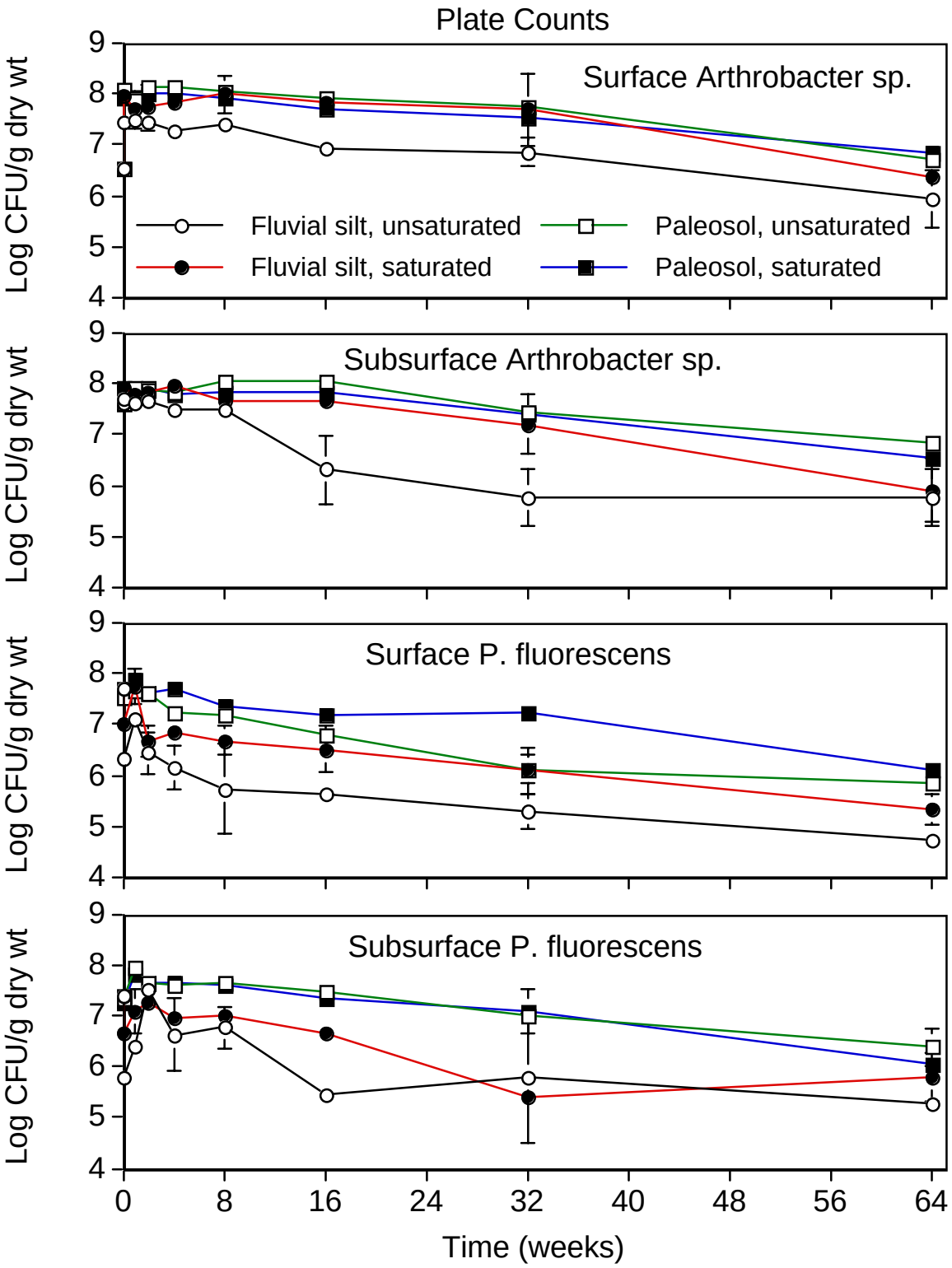

Fig. 1. Survival of surface and subsurface strains of *Pseudomonas fluorescens* and an *Arthrobacter* sp. [17].

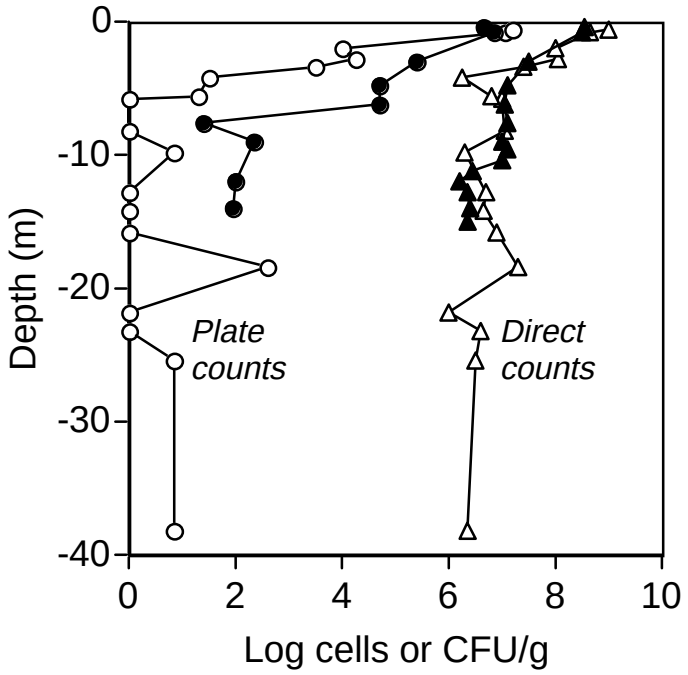

Fig. 2. Plate counts and direct microscopic counts of microorganisms in loess sediment samples from Wastucna (solid symbols) and Winona (open symbols) sites [14].

Patterns of microbial abundance at the Washtucna and Winona sites declined with depth and age (Fig. 2), resembling the patterns observed when bacteria are starved in laboratory microcosms. Other measurements of abundance (total PLFAs) and activities (glucose uptake and mineralization to CO_2, ß-glucosidase activities) were positively correlated with total and viable populations [10]. The identities of the microorganisms can be inferred from their PLFA profiles (Fig. 3). The pattern of steady decline with depth could be explained by death during long-term sequestration and/or retardation of bacteria during transportation from the surface. The ratio of diglyceride fatty acids (indicative of dead cells) to PLFA (live cells) increased exponentially with depth at both sites, indicating that many microbes have died since sediment burial. Because transport of microbes through unsaturated environments leads to their sorption at solid-liquid and air-liquid interfaces [23], it can be argued that most of the survivors are probably much older than the porewater. Thus, the majority of the microorganisms in the deepest sediments were likely derived from the communities that existed at or near the time of burial of these sediments.

Vadose Zone Waste Repositories

Vadose zone environments have frequently been targeted as relatively safe locations for hazardous waste repositories. A site for the United States' high-level nuclear waste repository has been selected at Yucca Mountain on the Nevada Test Site. The unsaturated zone at Yucca Mountain is several hundred meters thick and consists of multiple layers of volcanic tuff. Recent examination of pristine tuff in the tunnel at Yucca Mountain revealed populations of viable microorganisms [13]. These microbes were evidenced by

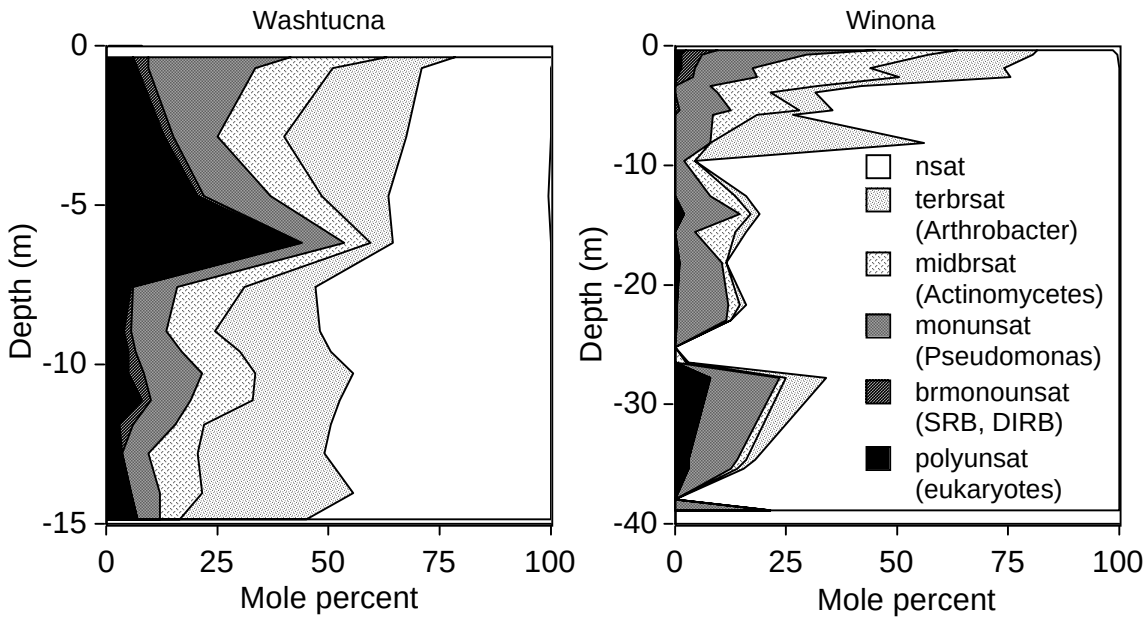

Fig. 3. Phospolipid fatty acid profiles of microorganisms in loess sediments from Wastucna and Winona sites [14].

heterotrophic plate counts (1.0×10^1 to 3.2×10^3 CFU g^{-1}), PLFAs (0.2 to 2.3 pmol g^{-1}), and potential for mineralizing organic substrates (glucose, acetate, and glutamate). Growth and activities of these microorganisms appear to be water-limited. Addition of water increased the numbers of culturable heterotrophs by as much as 10^7 CFU g^{-1} and significantly increased the amounts of glucose, acetate, and glutamate that could be mineralized to CO_2. Addition of N and P along with the water did not result in further stimulation; however, addition of organic C stimulated growth more than water alone. Tunnel-boring activities at Yucca Mountain result in significant addition of water to the tuff. Additionally, some organic C, e.g., straw, is added. As might be expected, this enhances microbial activity. PLFA concentrations as high as 11,000 pmol g^{-1} in straw and 5,700 pmol g^{-1} in sand were found after several months exposure in the tunnel [22].

The significance of microbial activities in the vadose zone surrounding a waste repository could be profound [8]. In the near field, i.e., the area immediately surrounding the waste, microorganisms could accelerate corrosion of the waste containers. If containment is breached, then microorganisms in the far field, i.e., the rock more distant from the repository, could influence transport of radionuclides. Transport could be accelerated by microbial chelates or by microbially mediated redox reactions; alternatively, microbes could retard transport by sorbing contaminants. Without further knowledge of the physical/chemical conditions that would occur in the far field in the event of contamination, it is impossible to predict microbial effects on transport; however, one can predict that there will be an effect. Although the rates of vadose zone activity may be slow and the distances great, microbes could significantly alter performance of the repository during the 10,000-year period that is being considered for risk-assessment.

Summary

In summary, deep vadose zone rocks and sediments in arid and semiarid regions may be among the most extreme on earth in terms of nutrient flux and availability. Despite these challenging conditions, microbes have persisted in deep vadose zones for thousands or even millions of years. Although their abundance is generally quite low and their rates of activity are slow, they have the potential to influence the chemistry of subsurface environments, including the fate and transport of contaminants.

References

1. Balkwill DL, Murphy EM, Fair DM, Ringelberg DB, White. DC 1998. Microbial communities in high and low recharge environments: implications for microbial transport in the vadose zone. Microb Ecol 35:156-171.
2. Bouwer H. 1979. Groundwater Hydrology. McGraw-Hill, New York.
3. Brockman FJ, Kieft TL, Fredrickson JK, Bjornstad BN, Li SW, Spangenburg W, Long PE 1992. Microbiology of vadose zone paleosols in south-central Washington State. Microb Ecol 23:279-301.
4. Cano RJ, Barucki MK 1995. Revival and identification of bacterial spores in 25- to 40-million-year-old Dominican amber. Science 268:1060-1064.
5. Chapelle FH, Lovley DH 1990. Rates of microbial activity in deep coastal plain aquifers. Appl Environ Microbiol 56:1865-1874.
6. Fredrickson JK, Brockman FJ, Bjornstad BN, Long PE, Li SW, McKinley JP, Conca JP, Kieft TL, Balkwill DL. 1994. Microbiological characteristics of pristine and contaminated deep vadose sediments from an arid region. Geomicrobiol J 11:95-107.
7. Haldeman DL, Amy PS 1993. Bacterial heterogeneity in deep subsurface tunnels in Rainier Mesa, Nevada Test Site. Microb Ecol 183-194.
8. Hersman LE 1997. Subsurface microbiology: effects on the transport of radionuclides in the vadose zone, pp. 299-323. In: The Microbiology of the Terrestrial Subsurface. P. S. Amy and D. L. Haldeman, Eds. CRC Press, Boca Raton.
9. Kennedy MJ, Reader SL, Swierczynski LM 1994. Preservation records of micro-organisms: evidence of the tenacity of life. Microbiology 140:2513-2529.
10. Kieft TL 1998. Dwarf cells in soil and subsurface terrestrial environments. Ch. 3. In: Non-culturable Microorganisms in the Environment. R. R. Colwell and D. J. Grimes, Eds. Chapman and Hall, NY. (In press).
11. Kieft TL, Amy PS, Bjornstad BN, Brockman FJ, Fredrickson JK Rosacker LL 1993. Microbial abundance and activities in relation to water potential in the vadose zones of arid and semiarid sites. Microbiol Ecol 26:59-78.
12. Kieft TL, Fredrickson JK, McKinley JP, Bjornstad BN, Rawson SA, Phelps TJ, Brockman FJ, Pfiffner SM 1995. Microbiological comparisons within and across contiguous lacustrine, paleosol, and fluvial subsurface sediments. Appl Environ Microbiol 61:749-757.
13. Kieft TL, Kovacik Jr WP, Ringelberg DP, White DC, Haldeman DL, Amy PS, Hersman LE 1997. Factors limiting to microbial growth and activity at a proposed high-level nuclear repository, Yucca Mountain, Nevada. Appl Environ Microbiol 63:3128-3133.

14. Kieft TL, Murphy EM, Haldeman DL, Amy PS, Bjornstadt BN, McDonald EV, Ringelberg DB, White DC, Stair JO, Griffiths RP, Gsell TC, Holben WE, Boone DR Microbial transport, survival, and succession in a sequence of buried sediments. Microb Ecol (In press).
15. Kieft TL, Phelps TJ 1997. Life in the slow lane: Activities of microorganisms in the subsurface. pp. 137-163. In: The Microbiology of the Terrestrial Subsurface. P. S. Amy and D. L. Haldeman, Eds. CRC Press, Boca Raton.
16. Kieft TL, Ringelberg DB, White DC 1994. Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Appl Environ Microbiol 60:3292-3299.
17. Kieft TL, Wilch E, O'Connor K, Ringelberg DB, White DC. 1997. Survival and phospholipid fatty acid profiles of surface and subsurface bacteria in natural sediment microcosms. Appl Environ Microbiol 63:1531-1542.
18. Konopka A, Turco T 1991. Biodegradation of organic compounds in vadose zone and aquifer sediments. Appl Environ Microbiol 57:2260-2268.
19. Murphy EM, Ginn TR, Phillips JR 1996. Geochemical estimates of paleorecharge in the Pasco Basin: evaluation of the chloride mass balance technique. Water Resource Res 32:2853-2868.
20. Onstott TC, Phelps TJ, Kieft TL, Colwell FS, Balkwill DL, Fredrickson JK, Brockman FJ A global perspective on the microbial abundance and activity in the deep subsurface. In: Enigmatic Microorganisms and Life in Extreme Environments. (J. Seckbach, Editor), Kluwer Publications. In press.
21. Phelps TJ, Murphy EM, Pfiffner SM, White DC 1994. Comparison of geochemical and biological estimates of subsurface microbial activities. Microbiol Ecol 28:335-350.
22. Ringelberg DB Waterways Experiment Station, Vicksburg, MS, Personal communication.
23. Wan J, Wilson JL, Kieft TL 1994. Influence of the gas-water interface on transport of microorganisms through unsaturated porous media. Appl Environ Microbiol 60:509-516.