76/981

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MAR 02 1999

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Microbial Transport, Survival, and Succession in a Sequence of Buried Sediments

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Submitted to: *Microbial Ecology*

In Press

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ABSTRACT

Two chronosequence of unsaturated buried loess sediments ranging in age from <10,000 years to >1 million years were investigated to reconstruct patterns of microbial ecological succession that have occurred since sediment burial. The relative importance of microbial transport and survival to succession were inferred from sediment ages, porewater ages, patterns of abundance (measured by direct counts, counts of culturable cells, and total phospholipid fatty acids), activities (measured by radiotracer and enzyme assays), and community composition (measured by phospholipid fatty acid patterns and Biolog substrate usage). Samples were collected by coring at two sites 40 km apart in the Palouse region of eastern Washington State near the towns of Washtucna and Winona. The Washtucna site was flooded multiple times during the Pleistocene by glacial outburst floods; the elevation of the Winona site is above flood stage. Sediments at the Washtucna site were collected from near surface to 14.9 m depth, where the sediment age was ~250 ka and the porewater age was 3700 years; sample intervals at the Winona site ranged from near surface to 38 m (sediment age: ~1 Ma; porewater age: 1200 years). Microbial abundance and activities declined with depth at both sites; however, even the deepest, oldest sediments showed evidence of viable microorganisms. Sediments of equivalent age had equal quantities of microorganisms, but differing community types. Differences in community make-up between the two sites can be attributed to differences in groundwater recharge and paleoflooding. Estimates of the ages of the microbial communities can be constrained by porewater and sediment ages. In the shallower sediments (< 9 m at Washtucna, < 12 m at Winona), the microbial communities are likely similar in age to the groundwater; thus, microbial succession has been influenced by recent transport of microorganisms from the surface. In the deeper sediments, the populations may be considerably older than the porewater ages, since microbial transport is severely restricted in unsaturated sediments. This is particularly true at the Winona site, which was never flooded.

Introduction

The biosphere is now known to extend to depths of tens to thousands of meters below land surface, and microorganisms have been found in a wide variety of subsurface environments [6, 11, 14, 22, 26-28, 33]. Microbial life in subsurface environments raises the question of how they arrived there. Two explanations, not mutually exclusive, can be posited: (1) microbes were deposited at the time of geologic deposition, and (2) microbes have been transported to the subsurface since geologic deposition [33, 38]. In saturated zones with high groundwater flow rates, transport may dominate, as has been noted in Atlantic coastal plain aquifers of the southeastern United States [38]. In subsurface environments with low groundwater flow rates, e.g., in clay-rich aquitards and in thick unsaturated zones of arid and semiarid regions with low rates of moisture recharge, physical filtration inhibits the transport of surface organisms [7]. In these relatively static subsurface environments, the microbial communities are most likely derived from populations of microorganisms that have been buried for hundreds to thousands of years, in some cases since the time of geologic deposition. The age of the microbial community can be constrained by the age of the geologic material (oldest possible) and the porewater age (youngest possible) [37]. In some cases, microbiological and geological data lead one to conclude that a particular microbial community is derived from populations that existed at the time of geologic deposition, e.g., in deep Cretaceous shale sediments of the San Juan Basin in New Mexico, where at least a portion of the community appears to date from the original marine sediment community [17]. Similarly, microbes in clay-rich lacustrine sediments of the Ringold Formation in the Columbia Basin are thought to be derived from Miocene bacteria that were originally present in lake sediments [18].

The changes that occur in microbial communities over geologic time can be considered to form an ecological succession. In relatively static, diffusion-dominated subsurface systems, where colonization by immigrant bacteria is negligible, succession occurs primarily through selection of populations capable of reproduction and/or long-term starvation survival. Reconstruction of such a microbial succession through geologic time requires combined use of the tools of microbiology and geology. We investigated two chronosequences of buried soils (paleosols). A chronosequence is a set of soils that are comparable in climate, organisms,

topography, and parent material, but that differ with respect to time [24, 42, 50]. In our study, the time factor is represented by a range of times since sediment deposition; this type of chronosequence has been defined as a fully time-transgressive chronosequence without historical overlap [50]. The sediment profiles in this research are loess sediments consisting of mostly silt, the Palouse, in eastern Washington State. They were deposited during similar climatic periods that promoted aeolian processes. Once deposited, however, the exposed soils may have been subjected to climatic variations. Times of deposition of these sediments range from modern to >1 million years before present. Soils developed in these sediments and were subsequently buried by further aeolian deposition. The purposes of this study were (1) to determine the relative importance of microbial survival and transport to the succession of microbial communities that has occurred in these sediments since the time of burial and (2) to determine patterns of microbial abundance, activities, and community composition in chronosequences at two sites that have had different moisture inputs.

Materials and Methods

Sites

Two sites located in the Channeled Scabland region of eastern Washington State were chosen for study (Fig. 1). This is an area of silt loess deposits that overlie basalt bedrock. Portions of the loess were eroded and channeled by brief, cataclysmic floods during the Pleistocene when the ice dams that formed Glacial Lake Missoula were breached. Approximately 10-50 of these floods occurred during the last glaciation [51]; although most of these floods were not large enough to impact the Washtucna site. Our sites were near the towns of Washtucna and Winona. Each is located near a road cut where the sediments have been described previously by McDonald and Busacca [34-36]. The Washtucna site of our study was adjacent to the WA-9 site of McDonald and Busacca [34]; the Winona site was adjacent to the WIN-1 site of McDonald and Busacca [36]. The exposed road cuts provided stratigraphic controls for the adjacent drilling sites of this study. The Washtucna site was first cultivated (dry-land wheat) between 1890 and 1900; dry-land wheat

farming at the Winona site has been continuous since approximately 1940. The native plant community in this semi-arid region is shrub-steppe [43].

A moisture gradient exists in the region of these sites: annual precipitation means are 28 cm at Washtucna and 33 cm at Winona. Surface elevations are 469 m at the Washtucna site and 503 m at the Winona site. This difference in elevation is the cause of differences in Pleistocene flooding effects. The Washtucna site underwent multiple catastrophic floods during the last glaciation; whereas, the Winona site lies above the maximum flood level, as indicated by geomorphology and the projected water surface profile of Baker and Numendal [4]. Thus, the Winona site was not flooded and probably remained unsaturated throughout that period. Both sites are at the crests of hills and so runoff and ponding is unlikely, precluding saturated water recharge since the last Pleistocene flood events. Properties of the buried soils indicate that the climate has remained arid to semiarid throughout the >1 million year period of deposition [12].

Sampling

Samples were collected by coring using a hollow-stem auger, split-spoon sampler technique. Cores (8.9 cm diam x 76 cm length) were collected in sterile Lexan liners. Fluorescent carboxylated microbeads (1 μ m diameter) were added to the open borehole after collection of the first core and thereafter at 7.6 m intervals (i.e., every 10th core). These microbeads served as tracers for potential microbiological contamination of subcores [16, 40]. Cores were sealed in the Lexan liners with sterile plastic end caps in the field and returned to Pacific Northwest National Laboratories (PNNL) for further processing. Lexan liners were opened and the cores were processed in a sterile transfer hood in the flow of HEPA-filtered air. Samples for chloride measurements were collected at approximately 10-cm intervals. Samples for microbiological analyses were collected from subcores after the outer 0.5 cm had been aseptically pared away. Microbiology samples were collected from subcore intervals of 20 to 50 cm; each was designated by the depth at the top of the subsample interval. These samples, approximately 1000 g each, were mixed and subsampled into sterile plastic bags (NASCO Whirl-Pak, Ft. Atkinson, WI). Subsamples were sent on ice by overnight delivery to other labs for microbiological analyses. Samples intended for lipid analysis were frozen immediately and sent on dry ice to the University

of Tennessee. Samples intended for fluorescent microbead tracers were obtained from the tops of cores that were collected immediately after adding beads to the borehole and from subcore material approximately 10 cm below the tops of these cores. Tracer beads were quantified by epifluorescence microscopy. The concentration of beads at the tops of cores collected immediately following tracer bead addition ranged from 2.4×10^7 to 5.8×10^7 beads g^{-1} ; concentrations of beads in the subcores were all below detection ($< 3 \times 10^4$ beads g^{-1}).

Stratigraphy

The sediments at both sites are relatively well sorted, largely consisting of silt and very-fine sands (Figs. 2). The loess stratigraphy at each site appears to be vertically continuous without major unconformities (large breaks in the time record or sediment deposition resulting from erosion or truncation by catastrophic loss to erosion or flooding). The presence of buried soils between loess layers indicates variable rates of sediment deposition (soil formation during periods of slow deposition), but that the vertical succession of loess deposition is largely uninterrupted. The surface soils have been plowed to a depth of approximately 1 m. The underlying loess contains multiple buried soils, each representing variable lengths of soil formation at the land surface before burial by younger loess. Typical features of soil development include: pedogenic calcium carbonate in disseminated and segregated forms, soil structure, reddish brown accumulation of iron oxides, and evidence of burrowing by soil invertebrates (e.g., earthworms, cicadas). The shallowest of the well developed buried soils is the Washtucna Soil, previously characterized by McDonald and Busacca [35, 36]. Formation of the Washtucna Soil occurred approximately 15,000 to 50,000 years ago and is bracketed between two volcanic ash layers (tephra), erupted from Mt. St. Helens (approximately 300 km to the west). The shallower tephra is the Mt. St. Helens set S, which was deposited 13,000 to 15,000 years ago; the deeper tephra is the Mt. St. Helens set C, deposited 40,000 to 50,000 years ago [8, 36]. Individual loess layers at the Washtucna site are generally thicker than those at the Winona site, reflecting the fact that Washtucna is closer to the source of sediments in the vicinity of the Pasco Basin to the southwest [13, 36]. The deepest sediments sampled at the Winona site are fluvial sediments, containing

basaltic clasts and thinly stratified sands and silts, deposited by giant glacial outburst flooding in the Channeled Scabland. The underlying basalt layer was encountered at a depth of 39 m at Winona, which was consistent with estimated depths from the geologic map.

Ages of the sediments were estimated using (1) radiocarbon dating of tephra layers, (2) estimated rates of soil development, and (3) paleomagnetic orientation. Estimated soil development rates were based on the degree of soil development within well dated sections of loess (e.g., the Washtucna soil). These rate estimates were compared with the degree of soil development for each buried soil at the Washtucna and Winona sites. Cumulative ages were determined for the top of each buried soil, yielding age estimates for the entire thickness of loess sediment. A paleomagnetic reversal was detected at 29.4 m in the Winona borehole, corresponding to an age of about 770 ± 20 ka [25].

Chemical and Physical Analyses

The profiles of anion, dissolved inorganic carbon, and dissolved organic carbon concentrations in the soil water were determined at 0.1-m intervals over the depth of the borehole at each site. Bulk density and gravimetric water contents were determined on each sample. Anions were leached from the sediment by mixing approximately 10 g sediment with 20 g of purified water (Milli-Q, Millipore, Bedford, MA) in a glass centrifuge tube, shaking overnight, and removing the sediment by centrifugation. The supernatant was filtered through a 0.22- μ m MF-Millipore filter (Millipore, Bedford, MA) and major anions were measured using ion chromatography (Dionex, Sunnyvale, CA). Dissolved organic and inorganic carbon was quantified in the same leachates using a Dohrman carbon analyzer (Dohrman, Rosemount Analytical, Santa Clara, CA). The concentration of the anions in the soil water was estimated using the gravimetric water content [37]. Very little natural chloride, nitrate, sulfate, or phosphate are present in these predominantly siliceous sediments; however, some of these species, such as phosphate, may exist in precipitated or complexed forms in the natural environment. Leachable organic and inorganic carbon does not necessarily reflect the total carbon in these sediments, but rather the concentrations of carbon available to the microorganisms if sufficient water is present. Sediment size distributions were determined using standard sieving and hydrometer methods [20].

Determination of Recharge Rates

Atmospheric tracers provide site-specific recharge rates and are most successful in arid and semi-arid environments [1, 21, 37]. The chloride mass balance method provides long-term average recharge rates by applying a mass-balance argument on the chloride ion, which is deposited naturally from the atmosphere. The difference between the chloride concentration in the soil water and the atmospheric input concentration is due to evapotranspirative enrichment. Stated differently, the chloride concentration in the pore water is inversely proportional to the flux of water through the sediments. Recharge is determined by the relationship

$$J_R = (Cl_o / Cl_{sw}) * p \quad (1)$$

where J_R is the net downward residual flux (e.g., recharge in cm y^{-1}), Cl_o is the average atmospheric chloride concentration in local precipitation and dry fallout (mg l^{-1} or equivalent units of g m^{-3}), Cl_{sw} is the average chloride concentration in the soil water (mg l^{-1}), and p is the average annual precipitation (cm y^{-1}). Cl_o can be expressed as the total chloride mass deposited at ground surface, q_{Cl} , divided by precipitation, p . The fundamental mass-balance relation (mass input = mass present) allows determination of the pore water age, t , at a given depth interval:

$$tq_{Cl} = \sum_i (Cl_i z_i \rho_b) \quad (2)$$

where Cl_i is the chloride concentration in the interval i (g Cl/g soil), z_i is the thickness of the interval i (m), ρ_b is the bulk density (g m^{-3}). The term $(Cl_i z_i \rho_b)$ represents the total chloride mass of the peak in g m^{-2} over depth interval z_i .

Direct Microscopic Counts

Direct counts were performed using 4',6-diamidino-2-phenylindole (DAPI) as described by Schallenberg et al. [47]. Sediment (4.0 g) was homogenized in 45 ml filter-sterilized 0.1% sodium pyrophosphate buffer (pH 7.5) and then allowed to settle for 1 minute. The supernatant was filtered onto 0.2 μm pore-size black polycarbonate filters (Nucleopore, Pleasanton, CA). Filters were then treated for 1 hour with a filter-sterilized solution containing 0.5% formaldehyde and 10

$\mu\text{g ml}^{-1}$ DAPI. Stained slides were placed onto microscope slides and examined using epifluorescence microscopy. Cells were counted on 10 randomly chosen fields, unless fewer than 10 cells per field were detected, in which case 30 fields were examined.

Plate Counts

Counts of culturable heterotrophic microorganisms were made on R2A agar (Difco) as previously described [22].

Anaerobe enumerations

Culturable anaerobic bacteria were quantified by a three-tube most-probable number method using medium MS [9] amended for enumerating fermentative bacteria and iron reducers. For fermentative bacteria, the medium was amended as described by Boone et al. [9]; for iron-reducers, the medium of Boone et al. [10] was used. Iron-reduction was considered to be a potential terminal electron accepting process, given the presence of iron oxide coatings on sediment particles [12]. At least three dilutions containing 0.001, 0.01, and 0.1 g sediment were tested for each sediment; for the shallower sediments, dilutions containing 0.0001 and 0.00001 g sediment were also tested. Each MPN assay was run in triplicate. Tubes were incubated at 30°C for 85 days. Growth was detected microscopically. Microscopy is more reliable than iron analysis for detecting iron reducers in MPN tubes because abiotic iron reduction can occur during long incubations, leading to false positives (D. R. Boone, unpublished data).

Phospholipid fatty acid (PLFA) and diglyceride fatty acid (DGFA) analyses

Samples for PLFA and DGFA analyses were removed and frozen at -70°C immediately after subcoreing. Extraction and quantification of PLFA and DGFA were performed essentially as described by Kieft et al. [30]. Fatty acid designations are as described by Ringelberg et al. [44].

^{14}C -glucose mineralization

Sediment was added in 10-g quantities to sterile 60 ml serum vials containing a stopper with

attached alkaline trap. To each vial, 10 μ l of [U- 14 C] D-glucose (1.28 mM, 3.7 kBq, >99% radiopure, Sigma Chemical Co., St. Louis, MO) was added. Sterile water was mixed into the sediment to a water potential of approximately -0.03 MPa, as previously described [27]. Vials were sealed with a sterile stopper with attached trap (Kontes 882320-0000, Vineland, NJ) containing 300 μ l of 0.3 N NaOH plus an approximately 2.5 x 0.635 cm piece of rolled Whatman #1 chromatography paper as a wick [27]. Triplicate mineralization microcosms for each sample were also prepared. Three poisoned control vials in which the sediment was moistened with a 3.7% formaldehyde solution instead of water were set up for each sample. Vials were incubated at 22°C. At intervals of 1, 3, 5, 7, 11, and 14 days, the chromatography paper and alkaline solution in each vial were removed and replaced. The chromatography paper and alkaline solutions were added to scintillation vials containing 10 ml Scintiverse BD liquid scintillation cocktail (Fisher Scientific, Pittsburgh, PA). Radioactivity was measured in a liquid scintillation counter (Packard Tri-Carb 460 CD), and the sample CPM was compared to input CPM for each substrate to determine the percent mineralization. Percent mineralization in poisoned controls was subtracted from percent mineralization in live samples, yielding the amount of biological mineralization. Lag times and rates of 14 C-glucose mineralization were estimated using non-linear curve fitting, as described by Brockman et al. [10]. Non-linear curve-fitting was performed using Systat 5.2 for Macintosh (Systat, Inc., Evanston, Ill.).

3 H-glucose uptake

In preparation for 3 H-glucose uptake assays (and enzyme assays), sediment samples were crushed in a sterile mortar and pestle and then sieved through a sterile stainless steel 500- μ m pore-size sieve. Crushed and sieved sediment (0.1 g each) was placed into sterile 2.0 ml microcentrifuge tubes. For each sediment sample, 3 replicate vials were used. Additionally, sediment in one control vial was treated with 0.1 ml 0.1 M HgCl_2 . To each vial, 0.1 ml [5, 6- 3 H] D-glucose (27 nM, 7.3 kBq, >95% radiopure, DuPont NEN, Boston) solution in artificial pore water was added. The artificial porewater contained 20 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.19 mg $\text{Al}_2(\text{SO}_4)_3$, 0.55 mg

$\text{Na}_2\text{BO}_4\text{O}_7$, 14 mg CaSO_4 , 28 mg $\text{Na}_2\text{SiO}_3 \cdot \text{H}_2\text{O}$, 13.5 mg KNO_3 , 33 mg $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 170 mg NaHCO_3 , and 0.05 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per liter of deionized water. Vials were incubated at 24°C for 24 h, after which 1.5 ml of a 1 g l^{-1} unlabeled glucose solution was added. The unlabeled glucose solution diluted the radioactive glucose solution such that further uptake of labeled glucose was negligible. The sediment was vortex mixed and then centrifuged (4 min, $13,000 \times g$). The pellet was washed twice in artificial pore water and then treated with 0.2 ml tissue solubilizer (Solvable, DuPont NEN, Boston) at 50°C for 1 h. Scintillation cocktail (1.5 ml, Atomlight, DuPont NEN, Boston) was added to the mixture, which was then centrifuged. Radioactivity was quantified using a Beckman model LS 100 liquid scintillation counter. Results are reported with the poisoned control values subtracted.

Enzyme activity

Enzyme activity was measured using a 4-methylumbelliferone- (4-MUF) labeled substrate. The 4-MUF is linked to the substrate by an ester bond that is enzymatically cleaved releasing fluorescent 4-MUF [15, 23]. For our assays, β -glucosidase activity was measured using 4-methylumbelliferyl glucoside (Sigma). Crushed and sieved sediment (prepared as for ^3H -glucose uptake) was placed in 1.0 g aliquots into scintillation vials. Ten ml 0.25 mM β -glucoside in artificial porewater was added and the vials were incubated with shaking for 5 days at 24°C . Fluorescent 4-MUF was quantified in a fluorimeter with an excitation wavelength of 355 nm and an emission wavelength of 455 nm. Triplicate subsamples plus one poisoned control were run for each sediment sample. Poisoned controls received 0.1 ml 0.1 M HgCl_2 . Results are reported with the poisoned control values subtracted.

Metabolic diversity

Microbial metabolic diversity in sediments was quantified using Biolog GN plates (Biolog, Inc., Hayward, CA) according to the method devised by Garland and Mills [19] for analysis of environmental samples, including subsurface sediments. Biolog plates were inoculated with the same cell suspensions used for the direct microscopic counts. Plates were incubated aerobically at

24° C for 7 days. Color development, indicating use of a particular carbon source, was quantified using a Vmax Kinetic Microplate Reader (Molecular Devices, Sunnyvale, CA).

Statistical analyses

Spearman rank correlations were performed using Systat version 5.2 software (Systat Inc., Evanston, IL) to determine significance of microbiological trends with depth and to determine correlations among microbiological data.

Results

The sand, silt, clay analyses (Fig 2) show a relatively uniform distribution of particle sizes with depth. The Winona Site has a somewhat smaller percentage of sand and greater percent silt than the Washtucna Site. This site is farther from the source of the sediment (the Pasco Basin) and therefore is expected to have a greater proportion of fine particles. Sediment size distribution is a good indicator of the structural heterogeneity of the profiles. For example, large shifts in the grain size distribution may indicate regions of preferential flow in the sediment profile, or at the other extreme, hydraulic barriers to flow that often occur at caliche layers in arid regions. The consistency in the sediment sizes with depth at the sites promotes greater uniformity in the water flux through the profile. Therefore, when the chloride profile is superimposed over the sediment size distribution (Fig. 2a, b), it is evident that the accumulation of chloride at depth is not an artifact of the physical structure of the sediments.

Sediment ages increased with depth to ~250 ka at 14.9 m at the Washtucna site and to ~1 million years at 38 m depth at the Winona site (Fig. 3a). The porewater ages are considerably younger: 3000 y at 14.9 m at the Washtucna site and 1300 years at 38 m depth at the Winona site (Fig. 3b). The long-term average recharge rates at the Washtucna and Winona sites are 0.1 cm y^{-1} and 0.8 cm y^{-1} , respectively, calculated using the chloride mass balance method. It is important to note, however, that agricultural practices have greatly altered the unsaturated flow rates at these sites [21]. The chloride profile accumulates at the bottom of the root zone due to evapotranspirative enrichment. The chloride profiles at Washtucna (peak at ~12 m) and Winona

(peak at ~15 m) are too deep to be considered part of the root zone. A more logical explanation is that the change in land use (from natural undisturbed vegetation to yearly cultivation) over the last century has increased the flux of water through the system, by eliminating root extraction of water during the wet winter months, thereby convecting the chloride peak downward. This results in relatively young porewater ages from the surface soil to 9 m depth (Washtucna) or 12 m depth (Winona).

Patterns of porewater chemistry differed between the two sites. Leachable inorganic carbon peaked at 5 m depth (3500 mg l^{-1}) and declined to approximately 200 mg l^{-1} at 15 m in the Washtucna borehole; whereas leachable inorganic carbon ranged from 100 to 2000 mg l^{-1} without a pattern with respect to depth at the Winona site. Leachable organic carbon was highest near the surface of both sites (approximately 3000 mg/l) and showed a second peak near 30 m (2000 mg/l) in the Winona borehole. Leachable sulfate was low at both sites ($<100 \text{ mg l}^{-1}$), except for high values ($\sim 4,000 \text{ mg l}^{-1}$) at about 10 m depth in the Washtucna borehole. Leachable phosphate concentrations were low ($<100 \text{ mg l}^{-1}$) except in a depth interval of 3-4 m in the Washtucna borehole, where they peaked at $\sim 300 \text{ mg l}^{-1}$. Leachable nitrate concentrations were relatively high ($50\text{-}200 \text{ mg l}^{-1}$) in the top 5 m in the Winona borehole, but were low at greater depth at this site and were low ($<50 \text{ mg l}^{-1}$) throughout the Washtucna borehole.

Microbial biomass, as indicated by total PLFA and direct microscopic counts, declined by several orders of magnitude with depth (and also with sediment age and porewater ages) at both sites (Figs. 4a and 5). Similar patterns were observed for counts of culturable aerobic heterotrophs (Fig. 5) and for percents of ^{14}C glucose mineralization and ^3H -labeled glucose uptake during 24 h sediment incubation (Fig. 6). The abundance of culturable anaerobes was also highest in the shallowest sediments: $2,100$ fermenters g^{-1} and 240 iron-reducers g^{-1} were cultured from 0.3 m depth at Washtucna; $>240,000$ fermenters g^{-1} and 460 iron-reducers g^{-1}

were cultured from the 0.5-m sample at Winona. Fewer than 10 fermenters g^{-1} and <3 iron-reducers g^{-1} were cultured from deeper sediments at both sites. The ratio of dead cells (indicated by DGFA) [30, 53] to live cells (PLFA) generally increased with depth and age at both sites, though it declined with depth in the 12- to 15-m interval at the Washtucna site (Fig. 4b). The percentage of cells that were culturable in heterotrophic plate counts declined from 1.35% at the shallowest sample to 0% in the deepest sediment at Washtucna; the percent culturable cells declined from 1.5% near the surface to 0.0003% in the 38 m sample at the Winona site. The rates of ^{14}C -glucose mineralization to CO_2 , calculated based on time course measurements over three weeks incubation, declined with sediment depth and ages; whereas the lag times until ^{14}C -glucose mineralization commenced increased with depth (Fig. 7). β -glucosidase activity, measured in sediments incubated 5 days declined with depth over a 2 to 3 order of magnitude range (Fig. 8). When incubated in the presence of chloramphenicol, β -glucosidase activity declined with depth even more sharply.

Although the total PLFA data indicate that the magnitudes of the biomasses were approximately equal in samples of equivalent sediment age at the two sites (Fig. 4a), the PLFA profiles indicated that the microbial communities at the two sites differed markedly from each other in their compositions (Fig. 9). In the Washtucna borehole, membrane lipids representative of a several functional groups, including high G + C gram positive bacteria, e.g., *Arthrobacter* (terminally branched saturated PLFA), actinomycetes (midbranched saturated PLFA), gram-negative bacteria (monounsaturated PLFA), and microeukaryotes (polyunsaturated PLFA), were found throughout the depth profile. The Winona sediments showed a decline in the percentage of each of these functional groups over the same depth interval. The most striking difference between the two sites was in the percentage of polyunsaturated PLFA. These microeukaryotic lipid biomarkers were present throughout the Washtucna profile and nearly absent from the Winona profile. Percentages of terminally branched saturated PLFA (gram-positive bacterial biomarkers) also differed considerably sites, with the greater percentages occurring in the Washtucna sediments.

Microbial metabolic diversity, indicated by the number of carbon substrates that could be oxidized in the Biolog assay, declined with depth at both sites (Fig. 10). Nearly all of the 95 substrates were utilized by microbes in the shallowest sediments. In the deeper sediments, the few substrates that were utilized were a subset of those used by microbes in the shallower sediments.

The following microbiological data were negatively correlated with depth at both sites ($P < 0.05$): PLFA, direct microscopic counts of total cells, plate counts, ^3H -glucose uptake, and percent ^{14}C -glucose mineralization (24-h). Significant positive correlations ($P < 0.05$) were found between all possible pairs of these microbiological measurements at both sites.

Discussion

Characterizing the abundance, activities, and physiological states of subsurface microbes requires a suite of microbiological assays, as noted by Chapelle [14] with regard specifically to vadose zones. Although the results of different assays are frequently in apparent conflict [14], the data of our study show remarkably consistent patterns, as indicated by correlation analysis. Microbial biomasses and activities declined sharply with depth at both sites. This simple pattern of monotonic, continuous decrease in microbial abundance with depth reflects the uniform nature of these sediments. They are alike in texture and mineralogy, with differences occurring primarily in sediment and porewater ages. Many of the correlations between microbiological assays are to be expected. For example, agreement between measures of total biomass and total cells (e.g., PLFA concentrations and direct microscopic counts: $r = 0.916$, $P < 0.001$ and $r = 0.541$, $0.02 < P < 0.05$ at Washtucna and Winona, respectively) has been observed previously [5]. Direct counts and culturable counts were also correlated, although they differed by several orders of magnitude, a common occurrence in natural environments, including those in the subsurface [26-28, 32, 45]. The decline in the ratio of cultured to total cells with depth corresponded to an increase in the ratio of DGFA to PLFA. The DGFA/PLFA data suggest that at least a portion of the discrepancy between total and cultured cells is attributable to dead cells. Viable but nonculturable cells are likely also important; this is supported by the detection of the potential for metabolic activity in samples from which cells were not cultured.

Microbial activity (glucose uptake, glucose mineralization, and enzyme) data closely follow the microbial abundance (PLFA, direct count, and plate count) patterns in these sediments, as indicated by significant correlations between assays. Results from short-term, 24-h, radiorespirometry incubations demonstrate the potential for metabolic activity by microbes present in the sediment samples at the time of collection. The longer-term incubations demonstrate a potential for growth as well as for transforming a substrate. The increase in lag times with sediment depth and age shows the increased time required for microbial growth and/or enzyme induction in the older sediments. Smaller populations of cells that have been dormant for longer intervals require correspondingly longer times to respond to substrate addition [3]. This is also evident in the slower rates of substrate mineralization in the older sediments. While glucose is not expected to be an important source of available energy in these sediments, it does provide a useful, sensitive means of detecting the presence of viable microorganisms and for quantifying potential metabolic activities in subsurface environments [11, 39, 26-28, 32]. The 5-day β -glucosidase activity data demonstrate the importance of growth and/or enzyme induction in metabolic response to substrate addition. The differences in response between the samples incubated without chloramphenicol and those incubated with chloramphenicol show the amount of *de novo* enzyme synthesis. In the deeper, older sediments, i.e., those buried for longer than approximately 100 ka, the extant β -glucosidase activity was negligible.

The pattern of declining microbial biomass and activity with depth can be explained by transport of microorganisms from the surface and/or by long-term microbial survival *in situ*. Transport from the surface combined with attenuation over distance and time due to sorption, filtration, and cell death would lead to just such a curve. Likewise, starvation survival of indigenous soil microorganisms following soil burial would yield the same pattern. The pattern of microbial biomass and activities with depth resembles the pattern one obtains over time when bacteria are starved in the laboratory [2, 31]. The relative importance of transport and survival can be assessed with respect to porewater ages and sediment ages as well as geohydrological characteristics of the vadose zone.

The minimum time required for transport of microorganisms from the surface to a particular depth interval is the porewater age of the sediment at that depth interval. The relatively

young ages (approximately 100 years or less) of the groundwater to depths of 9 m at the Washtucna site and 12 m at the Winona site suggest a strong transport component. Dryland farming, wherein the land is kept fallow for extended periods to minimize evapotranspiration, may have accelerated recharge in these shallow sediments during the last 100 years [21]. Thus, downward transport of bacteria may have made immigration the dominant influence on microbial succession in the shallow sediments of these sites.

In the deeper sediments, long-term survival of indigenous populations may have been a greater influence on microbial succession than transport. Microorganisms tend to move more slowly than water in a porous medium, particularly under unsaturated conditions [7]. Vadose zone bacteria are thought to be transported in water films that move by capillary forces in unsaturated flow systems. As water contents decrease, capillary forces retain water in pores of diminishing size. Under these conditions, bacterial transport is severely limited by physical filtration and attachment to particle surfaces [7], as well as attachment to gas-water interfaces [52]. The rate of microbial transport by unsaturated flow processes has been reported to be negligible at similar sites in southeastern Washington State [7]. Thus, the microbial communities at depth may be much older than the porewater ages. The living bacteria detected in the deepest sediments may even be close descendants of original surface soil populations, i.e., to be closer in age to the sediments than the porewaters. It is unlikely that individual cells have survived in the oldest sediments since sediment deposition; however, cells may have replicated relatively few times since sediment burial. Physical constraints on nutrient availability likely limited cell proliferation. Generation times of several centuries, which have been estimated for subsurface sediments [29, 41], may be adequate to enable long-term persistence of a population.

The availability of exogenous nutrients affects the ability of microorganisms to persist over geologic time. The leachable DOC data give some measure of the quantity (but not the quality) of carbon sources for heterotrophic metabolism. Patterns of DOC were very different at the two sites; however, these patterns appear not to be reflected in the microbial biomass and activity patterns at both sites. While DOC declined with depth at the Washtucna site, the Winona site showed less variation with depth. Severson et al. [48] reported a decline in both heterotrophic plate counts and total organic carbon with depth at a site in the Palouse near Pullman, WA. They

attributed this pattern to downward transport of surface-soil-derived organic carbon as well as slow depletion of buried organic matter in the paleosols. Given the unsaturated nature of these sediments, downward transport of easily metabolized organic energy sources is likely limited. Microbes are therefore more likely to be surviving and replicating sporadically on relatively recalcitrant organic matter in these paleosols. Dead cells may also be a source of nutrients. The increased ratio of DGFA to PLFA with depth indicates a greater number of dead cells than live cells in the deepest sediments. However, physical access to this and other sources of energy and other nutrients is extremely limited in unsaturated sediments [26]. Patterns of leachable anion concentrations (NO_3^- , SO_4^{2-} , and PO_4^{3-}) generally did not coincide with microbial biomass and activity patterns and thus appear not to be a major factor in overall microbial distribution with depth. However, chemical characterization of the sediments was not exhaustive; these and other chemical features of the sediments are undoubtedly important, especially at the scale of individual pores. The differences between sites in leachable anions likely reflect differing moisture inputs.

PLFA profiles show patterns of change in the community profiles with depth and sediment age. If the deepest subsurface communities represent the survivors from original soil communities, then the patterns with sediment age indicate which microbial groups can persist for at least 1 million years and which groups declined in biomass or disappeared. The finding of polyunsaturated fatty acids indicative of eukaryotes at depth is surprising, since microeukaryotes are thought to occur in the subsurface only in aquifers with relatively young groundwater [6, 49]. At the Washtucna site, the eukaryotic PLFA signature is found in the highest proportions in the shallower, 0-10 m depths, where relatively recent microbial transport is likely. Fungal spores may have been transported to these depths within the last 100 years. Eukaryotic signature PLFA were rare in the Winona samples, except in some very deep samples (34 to 38 m). Although the mole percents of polyunsaturated fatty acids in these deep sediments were in the 3-8% range, the actual quantities were minuscule. PLFA signature biomarkers indicative of gram negative bacteria such as *Pseudomonas* and of gram positive bacteria such as *Arthrobacter* were present in sediments that have been buried for nearly 1 million years. The differences in community profiles within sediments of similar ages observed in the two boreholes may be the result of spatial heterogeneity in microbial communities; i.e., soil microbial communities at sites 40 km apart may have differed

significantly from each other and may remain different as communities in buried sediments. Alternatively, the differences in community structure that are evident between the two boreholes may be due primarily to differences in moisture patterns (higher precipitation and recharge rate at the Winona site, and exclusively unsaturated recharge at the Winona site vs. intermittent saturation by Pleistocene flooding at the Washtucna site).

Rothfuss et al. [46] used an approach similar to ours in investigating microbial survival in a sequence of lake sediments. In their study, microbial transport to deeper strata was limited by the high clay content. Microbial abundance declined exponentially from the shallowest, youngest lake sediments to the deeper, older sediments. Microbes in 6-7 m deep, 8,900 year-old sediments consisted entirely of endospores. In our study, several of the deepest, oldest sediments showed evidence of a greater microbial diversity. They contained PLFA representative of 3 or 4 different major taxonomic groups of microorganisms, including ones that do not form endospores. Vegetative gram positive and gram negative bacterial populations have evidently persisted for a minimum of 1000 years and likely much longer in the deepest, oldest sediments.

Although the sediments sampled in this study include ones that underwent pedogenesis following deposition as well as ones that appear to have undergone less extensive soil development, the microbiological data do not reflect differences between these two categories of sediments. This may be explained by the complexity of these slowly aggrading sediments. Soil development occurred with previously buried soils as underlying parent material, resulting in a sequence of superimposed soil profiles [35]. Sediments at all depths were once exposed at the surface and thus were subjected to some degree of soil development before subsequent burial. Most of the sediment sequence now consists of B horizons, with only subtle differences to distinguish them from A or C horizons. The surface A horizons of these profiles have lost nearly all of their original organic content and now superficially resemble B horizons [12, 35]. Thus, the effects of different soil horizon types on the remanent microbial community are overshadowed by the influence of time since burial.

In summary, microbial communities in the shallower sediments (<9 m at Washtucna, <12 m at Winona) appear to have been strongly influenced by relatively modern transport of microorganisms from the surface, whereas in the deeper sediments, the surviving communities

may be considerably older than the porewater ages. Dryland farming has altered the rates of recharge and has increased the potential for downward transport of bacteria into the shallower sediments. Bacteria in the deepest, oldest sediments may be relatively close descendants of bacteria present in the soil at or near the time of sediment burial. Differences in microbial community composition between the two sites may be attributable to differences in moisture input, including differing rates of precipitation and effects of Pleistocene flooding.

Acknowledgments

This research was supported by the Subsurface Science Program, Office of Energy Research, U. S. Department of Energy [Grant no. DE-FG03-93ER-61683 (TLK and PSA), Grant no. DE-FG05-90ER60988 (DCW), and Grant no. DE-FG03-96ER62154/A002 (WEH)]. Pacific Northwest National Laboratory is operated for the Department of Energy by the Battelle Memorial Institute under contract DE-AC06-76RLO 1830. We thank Frank J. Wobber for support. We thank Bruce Harrison for helpful discussions.

References

1. Allison, GB, Gee GW, Tyler SW (1994) Vadose zone techniques for estimating groundwater recharge in arid and semiarid regions. *Soil Sci Soc Amer J* 58:6-14
2. Amy, PS, Morita RY (1983) Starvation-survival patterns of sixteen freshly isolated open-ocean bacteria. *Appl Environ Microbiol* 45:1109-1115
3. Amy PS, Pauling EC, Morita RY (1983) Recovery from nutrient starvation by a marine *Vibrio* sp. *Appl Environ Microbiol* 45:1685-1690
4. Baker VR, Numendal D (1978) The channeled scabland. Planetary Geology Program, Office of Space Science, National Aeronautics and Space Administration, Washington, D.C.
5. Balkwill DL, Leach FR, Wilson JT, McNabb JF, White DC (1988) Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface aquifer sediments. *Microb Ecol* 16:73-84
6. Balkwill DL (1989) Numbers, diversity, and morphological characteristics of aerobic, chemoheterotrophic bacteria in deep subsurface sediments from a site in South Carolina. *Geomicrobiol J* 7:33-52
7. Balkwill DL, Murphy EM, Fair DM, Ringelberg DB, White DC. Microbial communities in high and low recharge environments: implications for microbial transport in the vadose zone. *Microb Ecol* (in press)
8. Berger GW, Busacca AJ (1995) Thermoluminescence dating of late Pleistocene loess and tephra from eastern Washington and southern Oregon and implications for the eruptive history of Mount St. Helens. *J Geophys Res* 100:22361-22374
9. Boone DR, Johnson RL, Liu Y (1989) Diffusion of the interspecies electron carriers H_2 and formate in methanogenic ecosystems, and its implications in the measurement of K_m for H_2 or formate uptake. *Appl Environ Microbiol* 55:1735-1741
10. Boone DR, Liu Y, Zhao Z, Balkwill DL, Drake GR, Stevens TO, Aldrich HC (1995) *Bacillus infernus* sp. nov. Fe(III)- and Mn(IV)-reducing anaerobe from the deep terrestrial subsurface. *Int J Syst Bacteriol* 45:441-448
11. Brockman FJ, Kieft TL, Fredrickson JK, Bjornstad BN, Li SW, Spangenburg W, Long PE (1992) Microbiology of vadose zone paleosols in southcentral Washington State. *Microb*

Ecol 23:279-301

12. Busacca, AJ (1989) Long Quaternary record in eastern Washington, U. S. A., interpretation from multiple buried paleosols in loess. *Geoderma* 45:105-122
13. Busacca AJ, McDonald EV (1994) Regional sedimentation of Late Quaternary loess on the Columbia Plateau: source areas and wind distribution patterns. In Cheney ES, Lasmanis R (ed) *Regional geology of Washington*. Washington Division of Geology and Earth Resources Bulletin No. 80, pp. 181-190
14. Chapelle F (1993) *Ground-water microbiology and geochemistry*. Wiley, New York
15. Chrost RJ (1991) Microbial ectoenzymes in aquatic environments. In: Overbeck J, Chrost RJ (ed) *Aquatic microbial ecology: biochemical and molecular approaches*. Springer-Verlag, New York, pp. 47-78
16. Colwell FS, Stormberg GJ, Phelps TJ, Birnbaum SA, McKinley J, Rawson SA, Veverka C, Goodwin S, Long PE, Russell BF, Garland T, Thompson D, Skinner P, Grover S (1992) Innovative techniques for collection of saturated and unsaturated subsurface basalts and sediments for microbiological characterization. *J Microbiol Methods* 15:279-292
17. Fredrickson JK, McKinley JP, Bjornstad BN, Long PE, Ringelberg DB, White DC, Krumholz LR, Suflita JM, Colwell FS, Lehman RM, Phelps TJ (1997) Pore-size constraints on the activity and survival of subsurface bacteria in a late Cretaceous shale-sandstone sequence, northwestern New Mexico. *Geomicrobiol J* 14:183-202
18. Fredrickson JK, McKinley, Nierzwicki-Bauer SA, White DC, Ringelberg DB, Rawson SA, Li S-M, Brockman FJ, Bjornstad BN (1995) Microbial community structure and biogeochemistry of Miocene subsurface sediments: implications for long-term microbial survival. *Molec Ecol* 4:619-626
19. Garland JL, Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Appl Environ Microbiol* 57:2351-2359
20. Gee GW, Bauder JW (1986) Particle size analysis. In: Klute A (ed) *Methods of soil analysis, part 1*. American Society of Agronomy, Madison, Wisconsin, pp 383-409
21. Ginn TR, Murphy EM (1997) A transient flux model for convective infiltration: forward and

- inverse solutions for chloride and mass balance studies. *Water Resour Res* 33:2065-2079
22. Haldeman DL, Amy PS, Ringelberg DB, White DC (1993) Characterization of the microbiology within a 21 m³ section of rock from the deep subsurface. *Microb Ecol* 26:145-159
 23. Hoppe, H.-G. (1990) Microbial extracellular enzyme activity: a new key parameter in aquatic ecology. In: Chrost RJ (ed) *Microbial enzymes in aquatic environments*. Springer-Verlag, New York, pp. 60-83
 24. Jenny H (1980) *The soil resource: origin and behavior*. Springer-Verlag, New York
 25. Johnson RG (1982) Brubhes-Matuyana magnetic reversal dated at 790,000 B.P. by marine-astronomical correlations: *Quaternary Res* 17:135-147
 26. Kieft TL, Amy PS, Brockman FJ, Fredrickson JK, Bjornstad BN, Rosacker LL (1993) Microbial abundance and activities in relation to water potential in the vadose zones of arid and semiarid sites. *Microb Ecol* 26:59-78
 27. 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
 28. Kieft TL, Kovacik WP Jr, Ringelberg DB, White DC, Haldeman DL, Amy PS, Hersman LE (1997) Factors limiting microbial growth and activity at a proposed high-level nuclear repository, Yucca Mountain, Nevada. *Appl Environ Microbiol* 63:3128-3133
 29. Kieft TL, Phelps TJ (1997) Life in the slow lane: activities of microorganisms in the subsurface. In: *The microbiology of the terrestrial subsurface*. Amy PS, Haldeman DL (eds) CRC Press, Boca Raton, pp 137-163
 30. 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
 31. 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
 32. Konopka A, Turco R (1992) Biodegradation of organic compounds in vadose zone and

- aquifer sediments. *Appl Environ Microbiol* 57:2260-2268
33. Madsen EL, Ghiorse WC (1993) Groundwater microbiology: subsurface ecosystem processes. In: T. E. Ford (ed) *Aquatic microbiology: an ecological approach*. Blackwell, Oxford, pp 167-213
 34. McDonald EV, Busacca AJ (1988) Record of pre-late Wisconsin giant floods in the Channeled Scabland interpreted from loess deposits. *Geol* 16:728-731
 35. McDonald EV, Busacca AJ (1990) Interaction between aggrading geomorphic surfaces and the formation of a late Pleistocene paleosol in the Palouse loess of eastern Washington state. *Geomorphol* 3:449-470
 36. McDonald EV, Busacca AJ (1992) Late Quaternary stratigraphy of loess in the Channeled Scabland and Palouse regions of Washington State. *Quaternary Res* 38:141-156
 37. Murphy EM, Ginn TR, Phillips JR (1996) Geochemical estimates of paleorecharge in the Pasco Basin: evaluation of the chloride mass-balance technique. *Water Resour Res* 32:2853-2868
 38. Murphy EM, Schramke JA, Fredrickson JK, Bledsoe HW, Francis AJ, Sklarew DS, Linehan JC (1992) The influence of microbial activity and sedimentary organic carbon on the isotope geochemistry of the Middendorf aquifer. *Water Resour Res* 28:723-740
 39. Pedersen K, Ekendahl S (1992) Assimilation of CO₂ and introduced organic compounds by bacterial communities in ground water from Southeastern Sweden deep crystalline bedrock. *Microb Ecol* 22:1-14
 40. Phelps TJ, Fliermans CB, Garland TR, Pfiffner SM, White DC (1989) Methods for recovery of deep subsurface sediments for microbiological studies. *J Microbiol Methods* 9:267-279
 41. Phelps TJ, Murphy EM, Pfiffner SM, White DC (1994) Comparison between geochemical and biological estimates of subsurface microbial activities. *Microb Ecol* 28:335-349
 42. Retallack GJ (1990) *Soils of the past*. Unwin Hyman, Winchester, MA
 43. Rickard WH, Rogers LE, Vaughn BE, Liebetrau SF (eds) (1988) *Shrub-steppe, balance and change in a semi-arid terrestrial ecosystem*. Elsevier, New York
 44. Ringelberg DB, Davis JD, Smith GA, Pfiffner SM, Nichols PD, Nickels JS, Henson JM,

- Wilson JT, Yates M, Kampbell DH, Read HW, Stocksdales TT, White DC (1989) Validation of signature polar lipid fatty acid biomarkers for alkane-utilizing bacteria in soils and subsurface aquifer materials. *FEMS Microbiol Ecol* 62:39-50
45. Roszak DB, Colwell RR (1987) Survival strategies of bacteria in the natural environment. *Microbiol Rev* 51:365-379
 46. Rothfuss F, Bender M, Conrad R (1997) Survival and activity of bacteria in a deep, aged lake sediment (Lake Constance). *Microb Ecol* 33:69-77
 47. Schallenberg M, Kalff J, Rasmussen JB (1989) Solutions to problems in enumerations of sediment bacteria by direct counts. *Appl Environ Microbiol* 55:1214-1219
 48. Severson KJ, Johnstone DL, Keller CK, Wood BD (1991) Hydrologic parameters affecting vadose-zone microbial distributions. *Geomicrobiol J* 9:197-216
 49. Sinclair JL, Ghiorse WC (1989) Distribution of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments. *Geomicrobiol J* 7:15-31
 50. Vreeken, WJ (1975) Principal kinds of chronosequences and their significance in soil history. *J Soil Sci* 26:378-394
 51. Waitt RB Jr (1980) About forty last-glacial Lake Missoula Jokulhlaups through southern Washington. *J Geol* 88:653-679
 52. 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
 53. White DC, Ringelberg DB (1997) Utility of the signature lipid biomarker analysis in determining the *in situ* viable biomass, community structure, and nutritional/physiological status of deep subsurface microbiota. In: *The Microbiology of the Terrestrial Subsurface*. Amy PS, Haldeman DL (eds) CRC Press, Boca Raton, pp 119-136

Figure Legends

- Fig. 1. Locations of Washtucna (WA) and Winona (WI) boreholes in eastern Washington State (after McDonald and Busacca, 1992). Stippled areas indicate Palouse loess, arrows indicate flows of Pleistocene floodwaters, and horizontal lines indicate valleys where floodwater sediments were deposited. Annual precipitation contours (250 mm and 500 mm) are also shown.
- Fig. 2. Percents sand, silt, and clay in sediments with chloride profile superimposed as black lines, in mg Cl l^{-1} as a function of depth at the Washtucna (a), Winona (b) sites.
- Fig. 3. Sediment age (a) and porewater age (b) as a function of depth.
- Fig. 4. PLFA (a) and ratio of DGFA to PLFA (b) as a function of depth. Data points represent averages of two analyses.
- Fig. 5. Direct counts (triangles) and plate counts (circles) as a function of depth. Washtucna (closed symbols) Winona (open symbols). Points lying directly on the Y-axis represent plate counts that were below detection ($<3 \text{ CFU g}^{-1}$), i.e., no growth on any plates.
- Fig. 6. Percent ^{14}C -glucose mineralized (a) and amount of ^3H -glucose uptake (b) as a function of depth in 24 h incubations. Data points represent averages of triplicate analyses.
- Fig. 7. C-14 glucose mineralization: rate constant (a) and lag time (b) as a function of depth.
- Fig. 8. Production of fluorescent 4-methylumbelliferone from 4-methylumbelliferyl β -D-glucoside by β -glucosidase in sediments incubated for 5 days with and without chloramphenicol. Washtucna (a), Winona (b). Data points represent averages from triplicate microcosms.

Fig. 9. Mole percents of signature PLFA classes (representing different functional groups of microorganisms) as a function of depth at two sites in the Palouse region of eastern Washington State. Polyunsaturated PLFA (polyunsat) for micro-eukaryotes; branched monounsaturated PLFA (brmonounsat) for sulfate reducing and iron reducing bacteria; normal monounsaturated PLFA (monounsat) for typical gram negative bacteria such as *Pseudomonas*; mid-chain branched saturated PLFA (midbrsat) for actinomycetes; terminally branched saturated PLFA for typical gram positive bacteria such as *Arthrobacter*; normal saturated PLFA (nsat) which are ubiquitous and not indicative of any single group of microorganisms. Washtucna (a), Winona (b). Data points represent averages of duplicate analyses.

Fig. 10. Biolog assays: Number of positive wells (absorbance_{590 nm} > 0.1) as a function of depth. Data points represent the average from two Biolog plates.

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