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The Factors Controlling Microbial Distribution and Activity in the Shallow Subsurface

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The relationships among sedimentological, geochemical, and microbiological parameters in the vadose zone of a barrier sediment from the eastern shore of Virginia were examined. Pairs of samples were taken 10 cm apart in the vertical direction and 2 cm apart in the horizontal direction along three transects with one sample from each depth being processed aerobically and the other being processed anaerobically. Little variation was observed in the sedimentological and microbiological parameters tested. The sediment of all samples was fine-to-coarse sand, and the grain sizes ranged from 0.19–1.16 mm. Sediment moisture was low for all samples, but increased near the top and bottom of each sampling transect. These were regions where bioavailable Fe(III) concentrations were high. Rates of H_2 uptake ranged from below detection limit to 0.064 μmol $H_2 \cdot day^{-1} \cdot g^{-1}$ of sediment with a median rate of 0.01 μmol $H_2 \cdot day^{-1} \cdot g^{-1}$ of sediment. The variation in bacterial numbers was slightly more than an order of magnitude range over the entire sampling face. Phospholipid fatty acid analysis showed a diverse but fairly uniform microbial community from sample to sample. We found that the quartile of aerobically processed samples with the highest H_2 uptake rates had statistically higher moisture content and bioavailable iron content than did the rest of the samples. The quartile of aerobically processed samples with the lowest H_2 uptake rates had significantly more gravel, less moisture, and less bioavailable Fe(III) than did the rest of the samples. Similar trends were observed for anaerobically processed samples, but the differences were not significant. Our data indicate that the spatial variation in microbial parameters is low within strata with uniform grain sizes.

Keywords distribution, grain size, microbial activity, moisture, scale, subsurface, vadose, variation

The ability to predict the microbial metabolic potential at a contaminated site is critical for the development of successful bioremediation strategies. However, extensive drilling and testing is both expensive and time-consuming. The use of noninvasive geophysical approaches combined with a limited number of sediment cores offers a cost-effective approach to describe the geophysical properties of the aquifer. However, it is not clear how variation in the physical structure of the aquifer relates to variation in microbial activity and abundance in subsurface environments. Although early studies found little variability in total cell numbers (Ghiorse and Wilson 1988), numerous later studies have shown that microorganisms are heterogeneously distributed in subsurface environments (Hicks and Fredrickson 1989; Albrechtsen and Winding 1992; Brockman and Murray 1997; Colwell et al. 1997; Ernstsen, Binnerup, and Sorensen 1998). In addition to abundance, diversity indices, growth rates, and metabolic activity also are highly variable in subsurface samples (Fredrickson et al. 1989; Jones, Beeman, and Suflita 1989; Phelps, Raione, White, and Fliermans 1989; Chapelle and Lovley 1990; Kieft et al. 1993; Stevens and

Holbert 1995; Ringelberg, Sutton, and White 1997; Ulrich et al. 1998; Vreeland, Piselli, McDonnough, and Meyers 1998). The great majority of the studies have focused on microbial variability between geological strata in deep saturated sediments (Brockman and Murray 1997).

Typically, microbial properties vary by two to four orders of magnitude between strata, although much larger differences have been observed (Hicks and Fredrickson 1989; Chapelle and Lovley 1990; Albrechtsen and Winding 1992; Zhang et al. 1998). This variability appears to be related to variations in physical properties, e.g., grain size, although other untested factors may also contribute. Several studies found, for example, that microbial activity is lower in clays than in sandy sediments (Thorn and Ventullo 1988; Phelps, Murphy, Pfiffner, and White 1994a). Similarly, poorly permeable shales had little or no sulfate-reducing activity, whereas the more permeable adjacent sandstones exhibited high levels of sulfate-reducing activity (Krumholz, McKinley, Ulrich, and Suflita 1997). It is clear that large variations in microbial activity and abundance will be observed between strata with markedly different sedimentological features. However, the relationship between microbial activity and abundance and sedimentological properties within a single geological unit have not been quantified. If the sedimentological features between different strata are the cause of the variability seen in microbial activity and abundance between strata, then much less variability in microbial activity and abundance should occur within a single geological stratum.

Whereas the relationship between sedimentological and microbial properties in deep, saturated sediments has been fairly well documented (Brockman and Murray 1997), much less research has been done on unsaturated or vadose zone sediments. The numbers of culturable bacteria as well as microbial phospholipid fatty acids (PLFA) decreased with depth in a variety of vadose zone environments (Balkwill, Murphy, Fair, Ringelberg, and White 1998), including deep vadose zone sediments from a semiarid high desert (Colwell 1989). Many studies implicate moisture as the most important factor in controlling microbial activity and numbers in the vadose zone (Kieft et al. 1993; Phelps, Pfiffner, Sargent, and White 1994b; Wilson and Griffin 1975). The addition of moisture to deep vadose zone samples from Nevada, Idaho, and Washington stimulated microbial activity (Kieft et al. 1993). However, not all the samples were stimulated by the addition of water, suggesting that other nutrients may also have been limiting. After fractionating vadose zone sediment into different grain sizes, Nacro, Benest, and Abbadie (1997) found that most of the sediment's organic carbon and organic nitrogen were located in the smaller grain size fractions while the larger grain size fractions contained the most metabolic activity.

The variation in microbial activity, abundance, and community structure was examined on a centimeter-level scale in a vadose zone sediment near Oyster, Virginia. The central hypothesis driving this investigation was that the major controls of microbial activity in the subsurface are strongly correlated with a statistical description of the variation in the physical and chemical properties of the formation. Our prediction was that within strata, the variation in the physical parameters would be low, as would the variability in microbial parameters. Hydrogen was chosen as an electron donor to assay microbial activity for several reasons: (1) it is a widely utilized substrate by both aerobic and anaerobic bacteria (Bowien and Schlegel 1981; Kluber, Lechner, and Conrad 1995), (2) it may be added without altering the sediment's physical properties, and (3) it is possible to monitor its microbial uptake without sample destruction. We found that the sediment at the test site had relatively uniform geological features over scales of tens of meters. The measured microbial properties within this sediment were also relatively uniform. Local variations in microbial activity did occur, which could be explained by local changes in sediment chemistry and/or sediment moisture content.

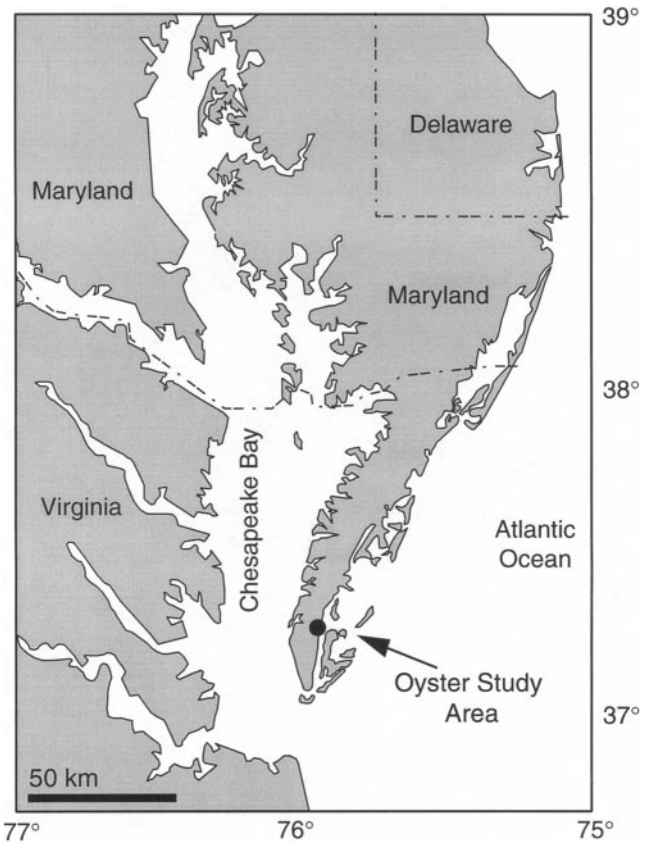


FIGURE 1 Location of Oyster, Virginia, study site.

Materials and Methods

Sampling Site Description and Sample Processing

The sampling site is located near the small fishing village of Oyster, Virginia (Figure 1), on the eastern coast of the United States on the southern Delmarva peninsula about 19 km north of the Chesapeake Bay Bridge Tunnel. The sampling site is located in a barrier complex that is underlain by the Late Pleistocene Wachapreague Formation, composed of repetitive deposits of prograding shoreface sand. The sediment consisted of unconsolidated to weakly cemented, well-sorted, medium- to fine-grained sand and pebbly sand (Green et al. 2000). On the edge of an agricultural field, an excavator was used to dig a pit approximately 2 m deep. A 1.5-m high sampling face was established and smoothed utilizing hand tools, with the top of the sampling face located below the root zone, approximately 0.5 m below ground surface (bgs). Samples were taken along three vertical transects located about 5.5 m apart in the horizontal direction at 3.3 m, 8.7 m, and 14.3 m from the left (south) side of the sampling face (subsequently referred to as transects A, B, and C, respectively).

Because of the sloping surface topography, vertical sample position was designated relative to the floor of the trench. All samples were taken aseptically within 3 h after the excavation was completed, using an ethanol-rinsed trowel to remove the sediment on the surface of the sampling face after which a sterile polycarbonate tube (10 cm in length, 4.5 cm inner diameter) was driven into the sampling face. After the sediment was removed,

both ends of the polycarbonate tube were sealed with sterile rubber stoppers. Samples were taken 2 cm from each other in the horizontal direction and 10 cm from each other in the vertical direction. At each sampling depth, one sample was processed aerobically while the other sample was processed anaerobically. Aerobically processed samples were immediately placed into a cooler at 4°C, and the anaerobically processed samples were placed in a nitrogen-filled portable anaerobic chamber. The rubber stoppers were removed from the ends of the anaerobically processed samples and the samples were left in the chamber for several minutes before the stoppers were placed back on the ends of the tube. After removal from the anaerobic chamber, the anaerobically processed samples were placed into a cooler at 4°C. Samples were stored at 4°C for no longer than 48 h before being shipped to the University of Oklahoma. Samples were processed within 48 h of arrival in Oklahoma. Additionally, samples were taken as described here from throughout the sampled face for phospholipid fatty acid (PLFA) analysis. The PLFA samples were placed on dry ice immediately after removal from the sampling face and shipped to the University of Tennessee the same day where they were stored at −80°C until processed.

Hydrogen Uptake Activity

Standard anaerobic and aseptic techniques were used throughout this study (Balch and Wolfe 1976). All samples were processed in an anaerobic chamber (Coy Laboratory Products Grass Lake, MI) filled with a 100% N_2 atmosphere. In the chamber, 3 g of sediment from each sample was added to a sterile 30-ml serum bottle. The serum bottle was sealed with a sterile butyl rubber stopper before being removed from the chamber. Each serum bottle was then aseptically evacuated with vacuum and repressurized with an O_2-free, 100% N_2 gas phase five times using sterile gassing probes. For the aerobically collected samples, O_2 was added into the headspace of the serum bottle by transferring air from a sterile bottle using a sterile syringe and needle. The final O_2 concentration was 13% (14.42 kPa). Selected samples from both the aerobically and anaerobically processed samples were used as sterile controls by autoclaving them at 121°C for 20 min. At the start of the experiment, H_2 was transferred from a sterile bottle filled with 100% H_2 (110.66 kPa) using a sterile, 1-ml syringe into the headspace of each serum bottle to give a final concentration of 0.026% (2.877×10^{-2} kPa) H_2 in the gas phase (Conrad, Aragno, and Seiler 1983). The disappearance of H_2 over time was measured by using a gas chromatograph (GC) equipped with a 100-μl sample loop and a mercury vapor reduction detector (Conrad et al. 1983). The H_2 concentration of each sample was checked every 24 h until the rapid disappearance of H_2 was detected, after which H_2 concentrations were checked every 8–12 h until the H_2 levels became undetectable. The H_2 concentration of samples showing no detectable H_2 uptake activity was checked every 24 h for 30 days. At each sampling time, 0.3 ml of gas was aseptically removed from the headspace of each sample and injected into the sample loop of the GC. The linear portion of the change in H_2 concentration with time was then used to calculate the rate of H_2 uptake. A minimum of three data points were used to calculate each rate, whereas five or more data points were used to calculate most rates. Linear regression was performed on all samples, and samples with correlation coefficients below 0.9 were not used in subsequent analysis. To ensure that mass transfer did not limit the rate of H_2 uptake, the concentration of H_2 was varied in the serum bottle while the amount of sediment was held constant. No change in the rate of H_2 uptake was observed in bottles with varying H_2 concentrations suggesting the system was biomass limited.

Moisture and Nutrient Additions

To determine the effect of moisture and electron acceptors on H_2 uptake, a bulk sample of sediment was collected by digging below the root zone and aseptically filling sterile mason

jars with sediment. Bottles for H_2 uptake measurements were prepared as described above with each bottle receiving 3 g of sediment. Sterile water was added to duplicate bottles to give a final moisture content of 10%, 20%, or 100% (of saturation), while another set of duplicate bottles was left at their original moisture content (approximately 5%). Another set of duplicate bottles was heated at 105°C for 48 h to serve as an abiotic control. Different electron acceptors were added to other sets of bottles. Nitrate, sulfate, and Fe(III) were added in concentrations of 0 mM, 1 mM, 5 mM, and 10 mM with NaCl added in the appropriate amount to ensure that the ionic strength was equal under all conditions tested. The amount of moisture in each bottle was also held constant.

Phospholipid Fatty Acid Analysis

Each sample taken for PLFA analysis was homogenized then split into triplicate subsamples. Lipids were extracted using the modified Bligh/Dyer extraction (White, Davis, Nickels, King, and Bobbie 1979), with fractionation of polar, neutral, and glycolipids and methylation of the polar fraction performed as described by Guckert, Antworth, Nichols, and White (1985). Fatty acid methyl esters (FAMES) were separated and quantified by GC with peak identification by mass spectrometry (MS). PLFA analysis was used to estimate biomass and community structure over the sampling face.

Bacterial Enumeration

Estimations of bacterial numbers were performed via two methods. Biomass content was estimated from each sample's PLFA using a conversion factor described by Balkwill et al. (1988) to convert the total amount of PLFA in each sample to cell abundance per gram dry weight of the sediment. Three tube most probable number (MPN) analyses were also performed on selected samples to enumerate aerobic heterotrophic bacteria, anaerobic heterotrophic bacteria, and sulfate-reducing bacteria (SRB). One gram of sediment from the selected samples was placed into 9 ml of sterile 0.1% sodium pyrophosphate at pH 7 (Balkwill and Ghiorse 1985). The liquid-sediment solution was subjected to two 30-s mixing cycles on a test tube mixer. One milliliter was removed from the resulting suspension and used to inoculate the first set of tubes. After mixing, 1 ml was aseptically withdrawn with a sterile syringe and needle and inoculated into the next tube in the series. This procedure was repeated until all of the tubes were inoculated. Degassed syringes and needles were used for the anaerobic heterotrophic and sulfate-reducing cultures (Balch and Wolfe 1976). The medium used for both the aerobic and anaerobic heterotrophic bacteria was a 1:10 dilution of the peptone, trypticase, yeast extract, and glucose (PTYG) medium described by Balkwill and Ghiorse (1985). Standard anaerobic techniques were used to prepare the anaerobic heterotrophic medium (Balch and Wolfe 1976). The SRB medium was API-RST medium and was prepared as described by Tanner (1989). For each medium, an organism known to grow in the medium was added to additional MPN tubes that had been inoculated with sediment to ensure that the medium was capable of supporting bacterial growth and inhibitory compounds were not present. The positive control for the aerobic heterotrophic medium was *Bacillus subtilis*, for the anaerobic heterotrophic medium was *Clostridium difficile*, and for the SRB medium was *Desulfovibrio vulgaris*. All of the positive controls grew to 10^8 to 10^9 cells/ml. Uninoculated media served as the negative controls, and none of the negative controls showed any signs of growth after 6 months of incubation.

All tubes were incubated at room temperature without shaking and were checked periodically for growth. For the aerobic and anaerobic heterotrophic cultures, tubes that had an increase in absorbance of 0.1 units at 600 nm above the uninoculated medium and

contained viable cells as determined by plating on agar medium of the same composition were scored as positive. For sulfate reducers, tubes with blackening indicative of iron sulfide production were scored as positive.

Geochemical Analysis

The total C, N, and S were determined on selected samples by dry combustion using an EA 1110 Elemental Analyzer (CE Elantech, Inc., Lakewood, NJ, USA). Aliquots of the sediment samples were placed in Sn capsules and combusted at 1800°C with O_2. The oxidized gases are then reduced, separated chromatographically, and detected with a thermal conductivity detector. The volumes of CO_2, N_2, and SO_2 released were then converted into weight percent of C, N, and S by calibration with standards (Page, Miller, and Keeney 1986).

To measure organic C, a separate aliquot of each sample was acidified with 1 M HCl in an Ag capsule to convert any carbonates to CO_2. The sediment and capsule were then dried at 80° to 100°C overnight, and then dry combusted at 1000°C and analyzed. The volume of CO_2 released was then converted into weight percent of organic C by calibration with standards. The difference between the total C and the organic C represents the weight percent of inorganic C.

The bioavailable (surface reducible) Fe(III) was estimated by a modified version of the hydroxylamine-HCl procedure (Lovley and Phillips 1987). Aliquots of the sediments, 0.1 to 0.5 g, were immersed in 5 ml of 0.5 M HCl, shaken for 30 s, and then heated at 50°C for 30 min in the dark. The mixture was allowed to cool to room temperature, centrifuged at 3,000 rpm for 8 min, and the supernatant decanted into a beaker. The Fe in the extract was measured by ferrozine (Lovley and Phillips 1986) and by ICP-AES. A split of the same sample was acidified with 5 ml of 0.25 M HCl and 0.25 M hydroxylamine-HCl. The mixture was shaken for 30 s, and then equilibrated at 50°C for 30 min in the dark, followed by centrifugation and removal of the supernate. The concentration of Fe in the supernate was determined by ferrozine and ICP-AES. The difference between the Fe concentration of the 0.25 M HCl + 0.25 M hydroxylamine extract and that of the 0.5 M HCl extract was converted into mg kg^{-1} sediment of Fe. According to Lovley and Phillips (1987) this Fe concentration is equivalent to the amount of amorphous Fe(III)oxide or bioavailable Fe(III). Of the 78 samples analyzed, only 2 samples yielded higher Fe concentrations in the 0.5 M HCl extraction than in the hydroxylamine-HCl extraction. These two discrepancies are attributed to the release of Fe from clay in the samples. The Al content associated with the 0.5 M HCl extraction was also quantified on the same extracts by ICP-AES.

Sediment Grain Size and Moisture Content Analyses

The moisture content of each sample was determined gravimetrically by drying 5 g of wet sediment for 48 h at 105°C (Klute 1986). The grain sizes of the samples were determined by the Sedimentary Dynamics Laboratory at Old Dominion University. Samples were sieved using standard methods of sieve analysis with Endecott sieves ranging from 4 mm to 0.15 mm and spaced at an interval of 0.84 mm (Folk 1974). Sediments that were finer than 0.15 mm were subsequently analyzed using an Elzone 280PC Particle Size Analyzer (Micromeritics, Norcross, GA, USA). Additionally, percent gravel and percent silt were determined for all samples.

Statistical Analysis

Standard statistical methods were used to analyze the H_2 uptake rate data and to compare it to the geological and geochemical properties of the samples. Histograms of H_2 uptake rates and

grain size were prepared to examine the shape and the degree of spread of their frequency distributions. Bivariate scatterplots and Pearson product moment correlation coefficients (Zar 1996) were used to compare the H_2 uptake rates to the geological and geochemical properties of the samples. The H_2 uptake rate samples were divided into four classes using the quartiles of the data (i.e., the 25th, 50th, and 75th percentiles). Notched box plots (McGill, Tukey, and Larsen 1978) were then used to graphically compare 95% confidence intervals for the medians of the geological and geochemical properties of the samples for the four classes of H_2 uptake data. Analysis of variance was used to determine if there was a significant difference in H_2 uptake rates in samples with different amounts of total carbon, nitrogen, sulfur, and organic carbon.

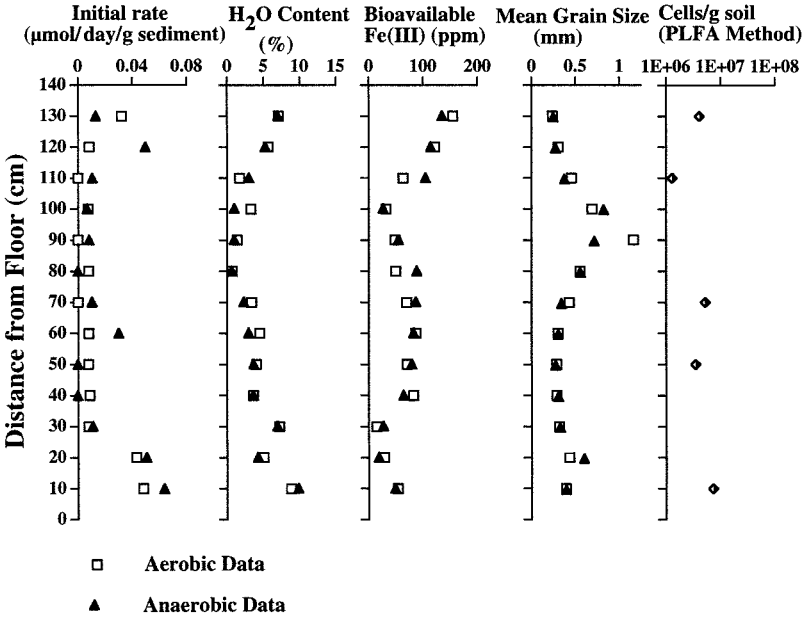
Results

Relationship Between Initial Rates of H_2 Uptake and Sediment Properties with Depth

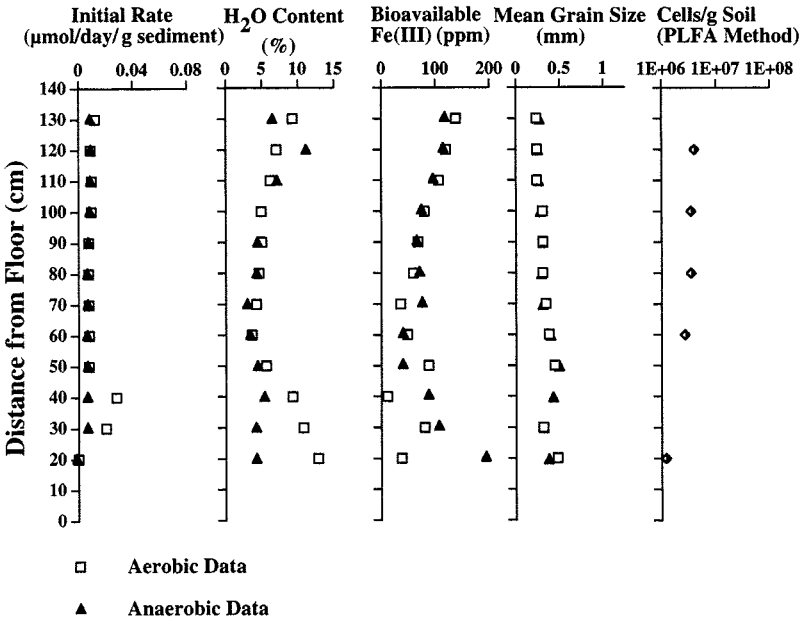
The change in H_2 uptake rate, moisture content, surface reducible (bioavailable) Fe(III), grain size, and biomass for all samples with depth is shown in Figure 2a–c. In general, H_2 uptake rates varied little from sample to sample, regardless of either depth, transect location, or whether the sample was processed aerobically or anaerobically. Moisture content was highest at the top and bottom of all three transects, as was the amount of bioavailable Fe(III). Grain size varied little from sample to sample, both with depth and horizontally across the sampling face. The PLFA biomass measurements exhibited a range of slightly more than an order of magnitude for all samples tested, with 16 of 18 samples falling within the same order of magnitude. No significant correlation was found by linear regression between PLFA biomass concentrations and H_2 uptake rates. The areas that exhibited higher H_2 uptake rates were located at the top and bottom of each transect, where moisture contents and bioavailable Fe(III) concentrations were highest.

Most of the samples tested had low H_2 uptake rates, varying from 0.005 to 0.05 μmol H_2 consumed/day/g sediment, with a median H_2 uptake rate of 0.01 μmol H_2 consumed/day/g sediment (Figure 3). No autoclaved control showed any loss of H_2 after 60 days of incubation. The values exhibited by the live samples were orders of magnitude lower than H_2 oxidation rates measured for different soils (Schuler and Conrad 1991) or sediments (Robinson and Tiedje 1982). The coefficient of variation for all H_2 uptake rate measurements was 101.7%. The mean grain sizes tended to be uniform both throughout each sampling transect and between transects. The range of mean grain sizes for all samples spanned about an order of magnitude, with all samples falling into the fine-to-coarse sand classification (Figure 3).

To assess whether significant relationships between H_2 uptake rates of the aerobically and anaerobically processed samples and the geological and geochemical properties of the sediment existed, the samples were separated into four classes, based on quartiles of the H_2 uptake rates. Notched box plots were then used to graphically compare 95% confidence intervals for the medians of the geological and geochemical properties of the samples for the four classes of H_2 uptake data. Notched box plots provide graphical displays of the central tendency and variability of groups of data (McGill et al. 1978) and can be used to perform the graphical equivalent of a *t*-test or analysis of variance. Notched box plots showed that, for the case of the aerobically processed samples, the class with the lowest rate of H_2 uptake had significantly lower moisture content, lower bioavailable iron, and significantly higher gravel content compared to the other classes (Figure 4). Additionally, the class with the highest H_2 uptake rate had significantly higher moisture content and bioavailable iron than the other classes. For anaerobically processed samples, the class with the highest H_2 uptake

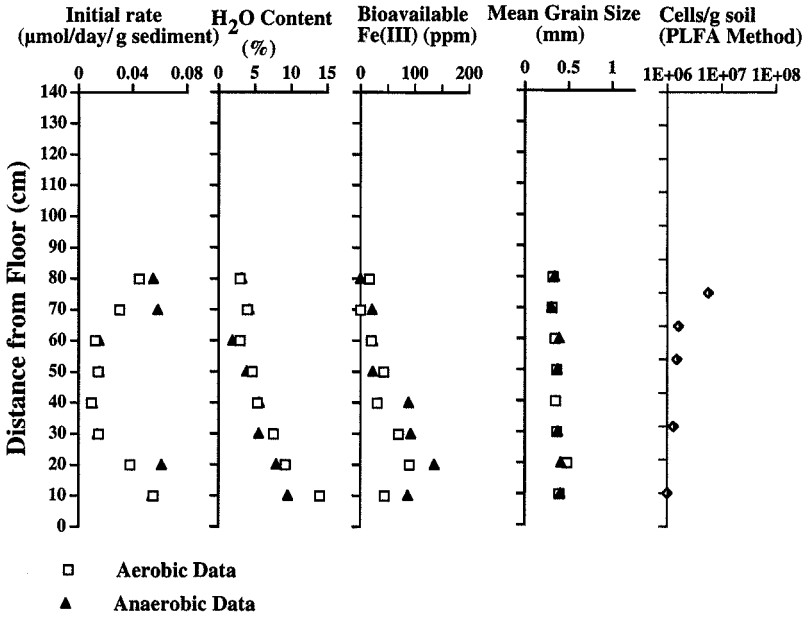


(a)



(b)

FIGURE 2 Initial rates of hydrogen uptake, moisture contents, bioavailable iron concentrations, mean grain size, and estimates of total bacterial cell numbers for all samples from a) transect A, b) transect B, and c) transect C. Note: Distance from floor is the distance from the bottom of the sampling face to the point where a sample was taken. *(Continued)*



(c)

FIGURE 2 (Continued)

rate had less gravel and greater moisture content than the other classes, but the differences were not significant (data not shown).

Enumeration of Bacteria

The MPN of aerobic heterotrophs ranged from 10^4 to 10^7 cells/g sediment and that of the anaerobic heterotrophs ranged from 10^3 to 10^6 cells/g sediment (data not shown). Most of the MPN counts were in the 10^5 to the 10^6 range for both analyses. Estimates of total

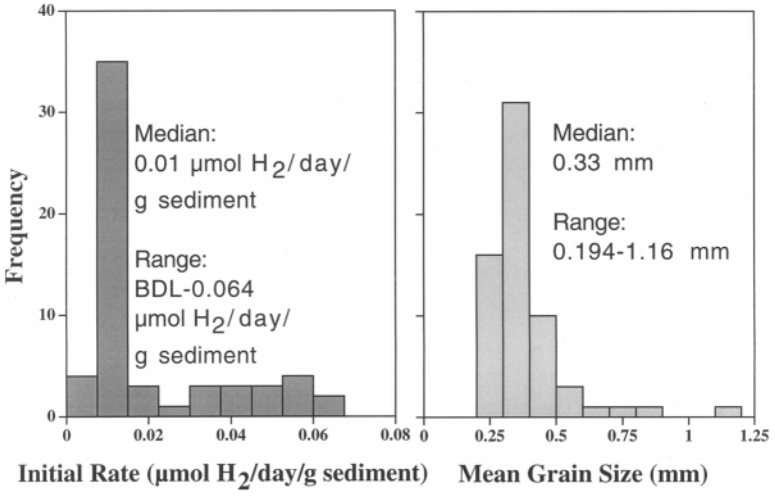


FIGURE 3 Distribution of hydrogen uptake rates and mean grain sizes for all aerobic and anaerobic samples. BDL-Below Detection Limit (0.001 μmol H_2/day/g sediment).

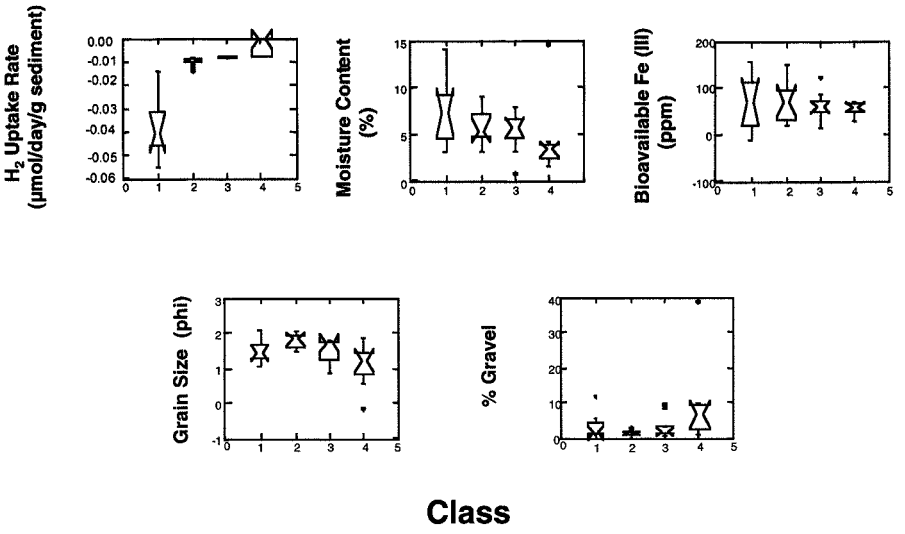


FIGURE 4 Notched box plots of metabolic and geochemical data for the aerobically processed samples. The medians of the distributions are shown as the center of the notches, and the lower and upper quartiles are the hinges (upper and lower ends) of the boxes. Asterisks and circles represent outlier data points at 1.5 and 3 times the interquartile range, respectively. The notches themselves represent an approximate 95% confidence interval around the median, so statistically significant differences exist between groups for which the notches do not overlap. Note: For this figure, a greater negative number for the hydrogen uptake rate represents a faster hydrogen uptake rate.

bacterial cell numbers from the PLFA analysis were in the 10^5 to 10^6 cells/g sediment range (Figure 2a–c). Regression analysis revealed no significant correlation between cell numbers obtained from either the MPN or the PLFA methods and any other variable tested. Most samples tested had no detectable SRB, whereas a few samples had counts in the 10 to 10^2 cells/g sediment range.

Effect of the Addition of Moisture and Electron Donors and Acceptors on Metabolic Activity

The data in Figure 2 indicated that metabolic activity may be related to moisture content. To test this hypothesis, an experiment was performed to determine the effect of varying levels of sediment moisture on H_2 uptake rate. The addition of moisture stimulated the level of microbial metabolic activity within the sediment. Bacteria within sediment that either had no additional moisture added or were heated until all moisture was removed displayed no detectable metabolic activity (<0.001 μmol H_2/day/g sediment). However, samples that had moisture added (the 10%, 20%, and 100% moisture samples) all had detectable metabolic activity (0.004 to 0.005 μmol H_2/day/g sediment). The metabolic activity detected in the samples with added moisture was low, however, suggesting that other nutrients might be limiting.

The addition of 1 to 10 mM sodium nitrate increased the rate of H_2 uptake four-to five-fold, relative to controls with equal ionic strength of sodium chloride. The addition of sodium sulfate or Fe(III) did not affect H_2 uptake rates relative to sodium chloride controls.

TABLE 1 Geochemical analysis of selected samples

Sample	Transect	Total organic C (%)	Total N (%)	Total C (%)
8	14.3 m	0.059	0.007	0.066
16	14.3	0.084	0.014	0.123
18	14.3	0.053	0.01	0.083
28	8.7	0.08	0.016	0.113
35	8.7	0.078	0.091	0.073
45	8.7	0.035	0.026	0.045
47	8.7	0.06	0.008	0.091
48	8.7	0.056	0.003	0.005
56	3.3	0.054	0.011	0.078
78	3.3	0.036	0.004	0.046
79	3.3	0.051	0.007	0.066

Site Geochemistry

The amount of total organic carbon, total nitrogen, and total carbon present in select samples is shown in Table 1. The low levels of nitrogen and organic carbon are consistent with the beach sand type of sediment typified by low amounts of clay, silt, and organic matter (DeFlaun, Murray, Holben, Scheibe, Mills, Ginn, Griffin, Majer, and Wilson 1997). An analysis of variance revealed no significant relationship between the levels of the above nutrients and sampling depth, transect location, H_2 uptake rates, or cell numbers.

The acid-extractable Fe(III) concentrations ranged from 10 to 155 ppm, whereas the acid-extractable Al concentrations ranged from 87 to 1037 ppm. These values are a mere fraction of the total Fe and Al concentrations for these sediments, which are 4,300 and 17,400 ppm, respectively. The majority of the Fe and Al resides in smectitic grain coatings identified by extensive SEM analyses of intact cores recovered from the same excavation with a minor portion of the Fe locally residing in goethitic pseudomorphs of pyrite framboids (Dong et al. 2002). Extraction experiments revealed that the Fe and Al associated with these smectite phases could be solubilized by 0.5 M HCl (data not shown) as was found to be the case for Fe by Tuccillo, Cozzarelli, and Herman (1999). The Fe(III) released by the hydroxylamine-HCl treatment is likely to be comprised of the Fe found in Si- and Al-bearing amorphous grain coatings and in Halloysite-like grain coatings by TEM analyses (Penn, Zhu, Xu, and Veblen 2001; Dong et al. 2002).

Phospholipid Fatty Acid Analysis

The microbial community structure of the site was relatively diverse, but uniform between samples (Figure 5). All samples tested contained high relative proportions of terminally branched, saturated PLFA. These PLFA are indicative of the presence of Gram-positive and anaerobic Gram-negative bacteria. The markers indicative of both aerobic and anaerobic Gram-negative bacteria (monoenoic fatty acids) were relatively low. Specific biomarkers for both sulfate-reducing bacteria and for actinomycetes were found in all samples tested. Likewise, PLFA markers for eukaryotic organisms, specifically fungi, algae, plant, and animal matter, were detected throughout the sampling face.

Principal components analysis of the PLFA data showed that the majority of samples clustered together with only two samples as outliers (Figure 6). One of these samples

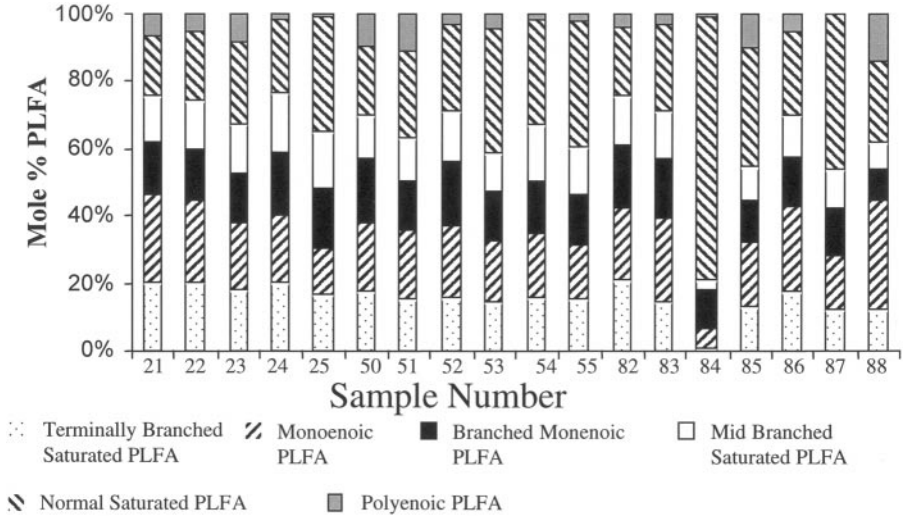


FIGURE 5 Community structure as determined by PLFA analysis.

contained both the highest relative proportion of high molecular weight normal saturated PLFA as well as high amounts of dicarboxylic acids and high molecular weight hydroxy fatty acids. Taken together, these PLFA are indicative of relatively large amounts of higher plant matter in this sample. The other outlying sample, conversely, contained higher relative portions of the biomarkers for Gram-negative bacteria and fungi than did any other sample analyzed. The percent of the total variance explained by factor 1 is 52.4% and the percent explained by factor 2 is 12.6%.

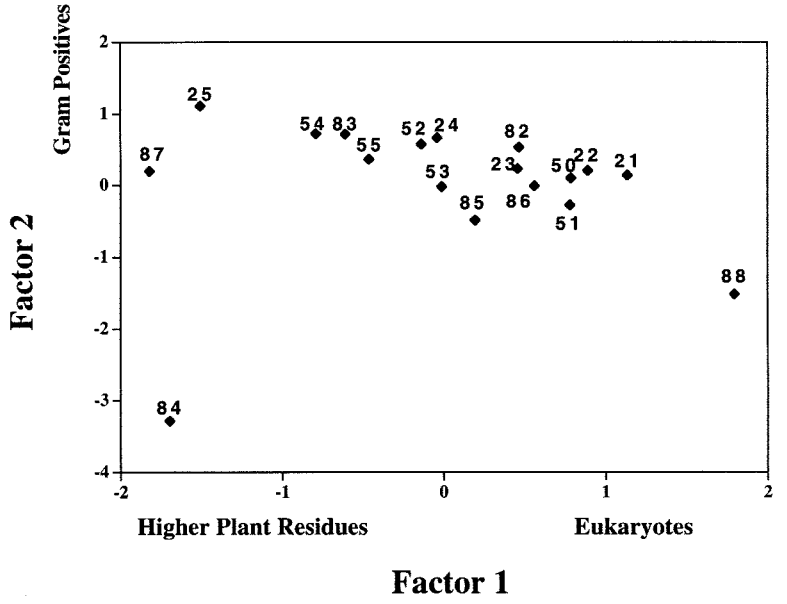


FIGURE 6 Principal component analysis of PLFA. Influential biomass-types were inferred from the presence of specific PLFA and are shown in the appropriate positions.

Discussion

Several studies have attributed the high degree of spatial variability in microbial activity and abundance in subsurface sediments to differences in grain size or pore size between strata (Fredrickson et al. 1989; Hicks and Fredrickson 1989; Jones et al. 1989; Krumholz et al. 1997). If the major controls of microbial activity in the subsurface are strongly correlated with the statistical description of the physical and chemical variation of the formation, then one would predict that sites with little variation in grain size should have little variation in microbial parameters. Our data are consistent with this hypothesis. Not only were microbial activity and abundance fairly uniform throughout the site, but the microbial community structure as inferred from PLFA analysis was also uniform, except for two samples (Figure 6). Other workers have shown similar relationships between grain size and microbial activity. Albrechtsen and Winding (1992) found large variations (orders of magnitude differences) in metabolic activity between different grain size fractions from an unconsolidated, sandy aquifer. Metabolic activity within the same grain-size fraction was within an order of magnitude for samples taken from three different sites, however, indicating less variability in regions with similar grain sizes.

Although some nutrients at the Oyster site varied by up to two orders of magnitude from sample to sample (Table 1), the concentration of all of the measured nutrients was low regardless of the sampling location. Organic carbon was 10-fold and total nitrogen was 10^3- to 10^6-fold lower at the Oyster site than in a typical, Midwestern, agricultural, vadose-zone soil (Konopka and Turco 1991). Konopka and Turco (1991) found that total phosphorous, total nitrogen, exchangeable K^+, and organic C decreased with depth in the vadose zone, whereas an analysis of variance revealed no relationship between sampling depth and the concentration of any nutrient tested at the Oyster site. The low moisture content at the Oyster site would compound the effects of low nutrient content to limit microbial activity. Low moisture in unsaturated sediments would slow cell growth due to increased energy expenditure to maintain turgor pressure, slow diffusion of nutrients to cells, and would inhibit bacterial movement toward nutrients (Wilson and Griffin 1975). Not surprisingly, researchers found that vadose-zone samples with higher moisture have higher metabolic activity than those with low moisture (Wilson and Griffin 1975). The majority of the samples at the Oyster site had very low moisture content, with many samples containing less than 5% moisture (Figure 2a–c). These samples were located in the middle of the sampling transects, where microbial activity was also the lowest. In a subsequent sampling trip, we did not detect metabolic activity in samples taken from the vadose zone with moisture contents less than or equal to the original moisture content, even after extended incubation. However, vadose zone sediment samples to which moisture was added all had measurable metabolic activity. Similarly, others have found that the addition of moisture markedly increases metabolic activity (Kieft et al. 1993; Cattaneo, Masson, and Greer 1997) and bacterial abundance (Cattaneo et al. 1997) in vadose-zone soils and sediments. Given that nutrient diffusion is probably limiting in the vadose zone (Kieft et al. 1993), the combination of low levels of nutrients and moisture may explain, in part, the low levels of metabolic activity observed at the Oyster site. Whereas some nutrients may be higher at one location compared to another, the low concentration of all nutrients along with limiting moisture would result in generally low and uniform rates of microbial activity.

The few samples that did exhibit somewhat higher H_2 uptake rates than the rest of the samples were from areas where moisture and bioavailable Fe(III) were high (Figures 2 and 4). The iron hydroxides at this site coat the sand grains (Dong et al. 1999). The iron hydroxides are positively charged and very fine-grained, on the order of 10–30 μm. Because bacterial cells are negatively charged, bacteria would adhere and accumulate on the

iron-coated sand grains (Gannon, Manilal, and Alexander 1991). This may explain the large number of cells found in fine-grained sediments (Dale 1974; Albrechtsen and Winding 1992) as well as the increased H_2 uptake rates observed in samples with high Fe content at the Oyster site.

We have shown multiple lines of evidence supporting the hypothesis that sediments with little sedimentological variation harbor microbial communities with little spatial variation in activity and abundance. Grain size is often one of the key factors controlling microbial abundance and activity because grain size is a major determinant of the permeability and porosity of the porous matrix. Porosity and permeability are parameters that reflect the amount of water that a porous material can hold and the ease with which water can move through the material. These parameters would control the flux of nutrients and microbial cells. In regions where the grain size is uniform, nutrient content and flux would be fairly uniform, and, as a consequence, the microbiology of such environments should be relatively uniform. This is consistent with our observations at the Oyster site, which include uniform grain size, uniformly low moisture and nutrient levels, and uniformly low microbial activity. The few samples that did show higher levels of microbial activity were from areas that had local variations in grain size and geochemistry. Our data suggest that spatial variability will be low within strata with uniform sedimentological properties. Extensive drilling and sampling may not be necessary within strata with uniform geophysical properties to obtain an adequate assessment of the spatial variability of microbial properties.

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