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Spatial and Temporal Variations of Microbial Properties at Different Scales in Shallow Subsurface Sediments

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ABSTRACT

Microbial abundance, activity, and community-level physiological profiles (CLPP) were examined at centimeter and meter scales in the subsurface environment at a site near Oyster, VA. At the centimeter scale, variations in aerobic culturable heterotrophs (ACH) and glucose mineralization rates (GMR) were highest in the water table zone, indicating that water availability has a major effect on variations in microbial abundance and activity. At the meter scale, ACH and microaerophiles decreased significantly with depth, whereas anaerobic GMR often increased with depth; this may indicate low redox potentials at depth caused by microbial consumption of oxygen. Data of CLPP indicated that the microbial community (MC) in the soybean field exhibited greater capability to utilize multiple carbon sources than MC in the corn field. This difference may reflect nutrient availability associated with different crops (soybean vs corn). By using a regression model, significant spatial and temporal variations were observed for ACH, microaerophiles, anaerobic GMR, and CLPP. Results of this study indicated that water and nutrient availability as well as land use could have a dominant effect on spatial and temporal variations in microbial properties in shallow subsurface environments.

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Index Entries: Aerobic culturable heterotrophs; glucose mineralization rates; community-level physiological profile; seasonal variation; sampling scale effects; water availability.

INTRODUCTION

Release of organic contaminants into aquifers disturbs the *in situ* physical and chemical conditions and the ecology within the subsurface environment. As a result, significant variations in chemical and microbial properties have been observed in contaminated systems. For example, exposure to petroleum contamination altered a microbial community structure by enriching some specific degraders, while suppressing other microbial populations *(1)*. Significant spatial variations in microbial abundance and degradation rates have been observed for contaminated aquifers *(2–5)*. Seasonal changes have also been reported to impact significantly anaerobic microbial activities, such as methanogenesis, sulfate reduction, and Fe(III) reduction in shallow contaminated aquifers *(5–7)*, likely because of changes in temperature, recharge volumes, and chemistry.

Subsurface microbial communities (MCs) in pristine aquifers can often adapt to chemical pollution *8(–9)*. However, because sampling at contaminated sites is usually conducted after the spill or leak has been detected, evaluating the magnitude of MC changes in response to contamination is difficult without knowing the MC status before contamination. To provide background information on microbial changes in uncontaminated shallow aquifers, studies at an Atlantic coastal plain site in Virginia were conducted to investigate spatial and temporal variations in microbial properties and to determine environmental factors controlling microbial variability in uncontaminated subsurface sediments. In this study, we examined microbial variations at the centimeter and meter scales and at varying points in time at the Oyster, VA site. Results showed that temporal changes in water availability and land use properties controlled the observed spatial variability in subsurface sediments within a single field site.

MATERIALS AND METHODS

Site Description and Field Sampling

The field area for this study is located near Oyster, VA (Fig. 1A). Subsurface sediments at the site consist of unconsolidated, fine-to-coarse beach sands and gravels that are clean and well sorted *(10)*. Ground water flow rates at the site are estimated to be approx 20 m/yr with a regional gradient of approx 60 m/km (A. Mills, University of Virginia, personal communication).

Samples were collected during June and August 1994, and during July 1995. Figure 1B shows corehole locations in a soybean field and a corn field. Split-spoon coring tools and a hollow-stem auger system were used during

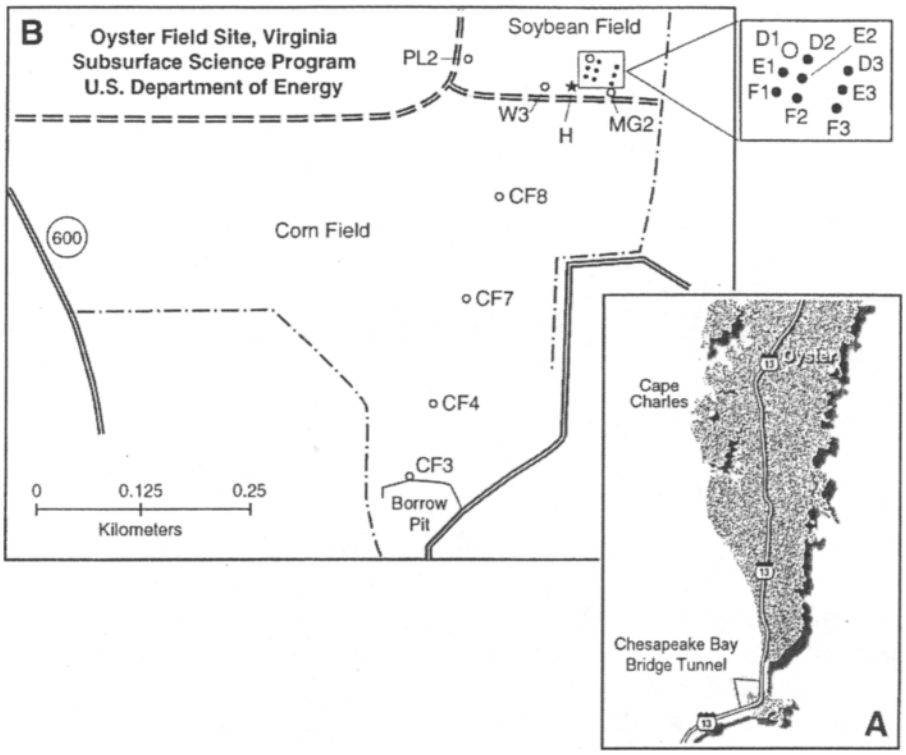

Fig. 1. Geographic location of the study area **(A)** and corehole locations **(B)** at the Oyster site in Virginia. Open circles represent coreholes drilled in June 1994; solid circles represent coreholes drilled in August 1994; the star represents the corehole drilled in July 1995.

the 1994 sampling period, and a sonic drilling system was used during the July 1995 sampling period. Quality assurance and quality-control steps were taken to minimize contamination from drilling operations *(11)*.

To collect undisturbed sediments from the cores, a flame-sterilized handsaw was used to section the core material. The newly exposed core face was pared away with a sterile spatula. The center material was then collected into sterile Whirl-pak bags, and shipped on ice for biological and chemical analyses. At selected depths, sediments were collected 3–5 cm apart for examining small-scale changes in aerobic culturable heterotrophs (ACH), microearophiles, aerobic and anaerobic glucose mineralization rate (GMR), and community-level physiological profiles (CLPP).

Enumeration of Viable Microorganisms

A procedure described by Balkwill *(12)* was used to determine the abundance of ACH. Briefly, sediment samples were blended with sterile 0.1% sodium pyrophosphate (pH 7.0) in the ratio of 1:10 (w/v). Diluted sediment slurries were made with sterile distilled water. An aliquot of 0.1

mL from each dilution (10^{-2}–10^{-5}) was spread onto agar plates in triplicates. After 7 and 14 d of incubation at room temperature (22–25°C), colony-forming units (CFU) were counted by using an automatic bacterial colony counter (Spiral System Instruments, Gaithersburg, MD, Model 500A, countable colony size ≥ 0.25 mm).

Microaerophiles were enumerated on dilute-substrate mineral salts in semisolid medium tubes using single-series dilution techniques *(13)*. Tubes were incubated at room temperature, and observations to detect microbial growth in the tube were performed weekly. Microaerophilic growth was judged on the basis of characteristic color bands in the semisolid medium.

Activity Assays

Experiments examining aerobic and anaerobic GMR were conducted within 30 h of sample collection using anaerobic crimp-top tubes (Bellco Glass, Vineland, NJ). Incubations were at room temperature (22–25°C) for up to 7 d. At t_0 and other appropriate points in time, glucose mineralization was inhibited with 0.5 mL of 2.0*M* NaOH, and tubes were immediately frozen until analysis. One hour before analysis, tubes were acidified with 0.5 mL of 6*M* HCl solution, and the CO_2 plus $^{14}CO_2$ that evolved during mineralization was analyzed by gas chromatography and gas proportional counting according to Phelps et al. *(11)*.

Community-Level Physiological Profiles

CLPP integrates the metabolic potential of the heterotrophic community by using the Biolog microplate method (Biolog, Hayward, CA), which tests the utilization of preselected substrates and characterizes environmental isolates. Experiments examining subsurface CLPP were performed according to methods in Garland and Mills *(14)* and Lehman et al. *(15)*. Briefly, sample slurries, made from blending 10–25 g of sediments with 100 mL of 0.1% sodium pyrophosphate, were flocculated with 0.5 g of a calcium chloride and magnesium carbonate mixture ($CaCl_2 \cdot 2H_2O$:$MgCO_3$ = 8:5). Then 150 µL of the supernatant were inoculated into each of the 95 wells in Biolog GN microplates (well 96 served as a control). Each well contained lyophilized nutrients and a tetrazolium redox dye as well as one sole carbon source *(16)*. Incubation of these plates was done in the dark at 22°C under a locally humidified atmosphere. Oxidation of the carbon source by the mixed community is indicated by colorimetric reduction of the redox dye. The sum of positive tests for a given sample equals the total number of carbon sources respired.

Statistical Methods

All statistical calculations were performed with SAS *(17)* on log-transformed values because the majority of microbiological and chemical

properties at the Oyster site followed a log-normal distribution (data not shown). Analyses of spatial and temporal effects on ACH and aerobic GMR were performed using a logistic regression analysis with the transformed response regressed on depth. Because more than one corehole was drilled during the June and August 1994 sampling periods, the ACH and GMR data were first analyzed for slope variations between coreholes within a single sampling data. If slopes for the individual coreholes did not differ significantly, these coreholes were grouped to derive a composite slope value for that date for comparison with the slope from a different sampling date. However, if the slopes of a variable in different coreholes differed significantly within a single sampling date, the regression model could not be used for further evaluation of the temporal effect on parameter variation. In this case, the temporal effect on the variable was evaluated on the basis of other information. We excluded coreholes that had samples only below a 1-m depth because of the restricted depth range for the coreholes.

The replicate-to-replicate variability in ACH was estimated from the pooled variance from replicate samples across all coreholes for a given date. This pooled variance estimate was then compared with the error mean square (EMS) from the regression of ACH on depth for the same data set. The EMS provided an estimate of the variability of the ACH observations around the regression line.

RESULTS AND DISCUSSION

Spatial and Temporal Variations at the Centimeter Scale

At the centimeter scale, ACH varied much less, in all three depth zones during the July 1995 sampling period, than during the June and August 1994 sampling periods (Table 1). On the other hand, the Max:Min CFU was highest in the water table zone that transited between the capillary fringe and water-saturated depths for all three sampling periods (Table 1). This indicated a major effect of water table fluctuation on microbial abundance at the centimeter scale. The Max:Min ratio for available aerobic and anaerobic GMR ranged from 1.2 in the zone below the water table to 15.6 in the water table zone, suggesting that greater variability in microbial activity was also because of water level fluctuations.

Variations in microbial abundance and activity at the centimeter scales have been reported for soil environments, aquifer systems, and marine sediments *(4,18–20)*. Microbial abundance and activity reported by Beloin et al. *(18)* for subsurface sediments appeared to vary by a factor of 3 to >220 at intervals spaced 10 cm apart. However, less variability of microbial abundance at centimeter intervals was reported for a marine sediment study *(20)*. In this study, microbial abundance (ACH) at close intervals appeared to be most variable in the zone transiting between the unsaturated capillary fringe to the water-saturated depth, reflecting the

Table 1
Spatial and Temporal Variations in Aerobic Culturable Heterotrophs at Close Intervals (3–5 cm Apart)[a]

	Max:min CFU		
Depth zone	June 1994	August 1994	July 1995
Above WT[b]	8 ± 2	10 ± 3	3
At WT	10 ± 2	14 ± 2	4
Below WT	7 ± 8	4 ± 2	1

[a]values are ratios of Max:Min CFU (i.e., $10^4/10^3 = 10$) between sampled intervals. For June and August 1994, each value is a mean +1SD for two to three coreholes in each depth zone where close intervals were selected. The June 1994 data included a cord field and a soybean field; August 1994 and July 1994 data were from the soybean field only.

[b]WT = water table.

effects of water level fluctuation on MC dynamics. On the other hand, the relatively small variation in ACH at close intervals in the other two depth zones, especially the zone below the water table (Table 1), may indicate the uniform texture of the sediments. At the Oyster site, grain sizes changed little with depth; they mainly consisted of fine sands that were clear and well sorted. Physical heterogeneity of subsurface sediments often increases with increasing grain sizes *(21)*. At a different site of the same geological formation, it was shown that significant increases in microbial abundance and activity occurred in a coarse grain zone, and grain size is a major factor controlling microbial abundance and activity *(22)*.

Spatial and Temporal Variations at the Meter Scale

ACH, Aerobic GMR, and Microaerophiles

When ACH data of the soybean field samples were plotted against depth, significant correlations ($P < 0.05$) were found between log-transformed ACH and depth for all three sampling dates (Fig. 2). Using a likelihood ratio test, the slopes of estimated regression lines for log-transformed ACH from three sampling dates were significantly ($P < 0.001$) different (Table 2). The two dates with the greatest difference in slope were June 1994 (S = –1.81) and August 1994 (S = –2.96); thus, ACH decreased with depth more dramatically in August than in June. When we examined the pooled replicate-to-replicate ACH (transformed) variability and compared this with the EMS from the regression of ACH (transformed) on depth, the ratio was >20. This implies that the replicate-to-replicate variability was a trivial component of the overall variability (EMS) noted in the regression analysis.

Abundances of microaerophiles also decreased significantly ($P < 0.05$) with depth from $>10^4$ cells/g above a 1-m depth to <10 cells/g below a

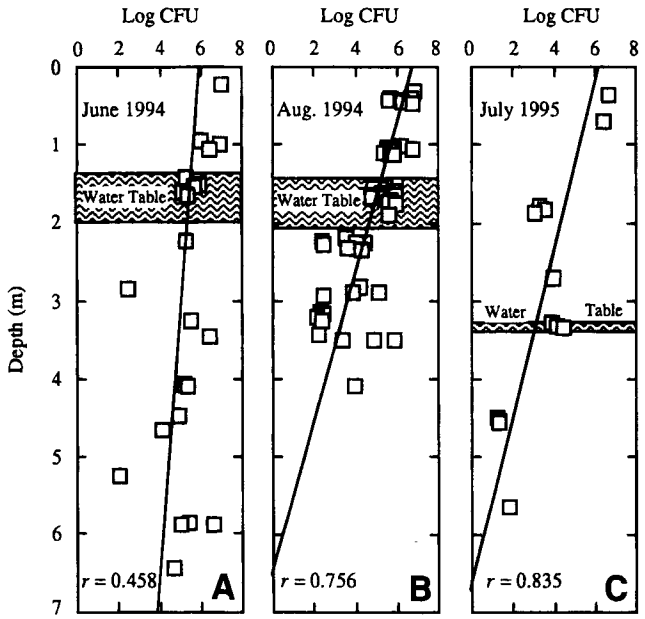

Fig. 2. Depth profiles of aerobic culturable heterotrophs (log CFU) in the soybean field for the June 1994 **(A)**, August 1994 **(B)**, and July 1995 **(C)** sampling dates at the Oyster site in Virginia. r = correlation coefficient. The water table ranges from 1.4–2.0 m in A, from 1.5–2.2 m in B, and is about 3.3 m in C.

Table 2
Maximum Likelihood Estimates of Slopes for Log ACH vs Depth at Different Sampling Dates in the Soybean Field

Date	Number of samples	Number of coreholes	Estimates of slopes, SE
June 1994	13	1[b]	–1.81 (0.69)
August 1994	49	7[c]	–2.96 (0.27)
July 1995	13	1[d]	–2.21 (0.39)

[a]See Fig. 1 for corehole locations. SE = standard error.
[b]Corehole MG2.
[c]Coreholes D3, E1, E2, E3, F1, F2, and F3.
[d]Corehole H.

6-m depth. Large differences in slopes were observed between the soybean field and corn field from the June 1994 sampling date. However, because a significant slope difference was observed between individual coreholes, slopes for microaerophiles could not be pooled for a given site, and thus, the difference in the microaerophile slopes of these two fields could not be evaluated by using the likelihood ratio test.

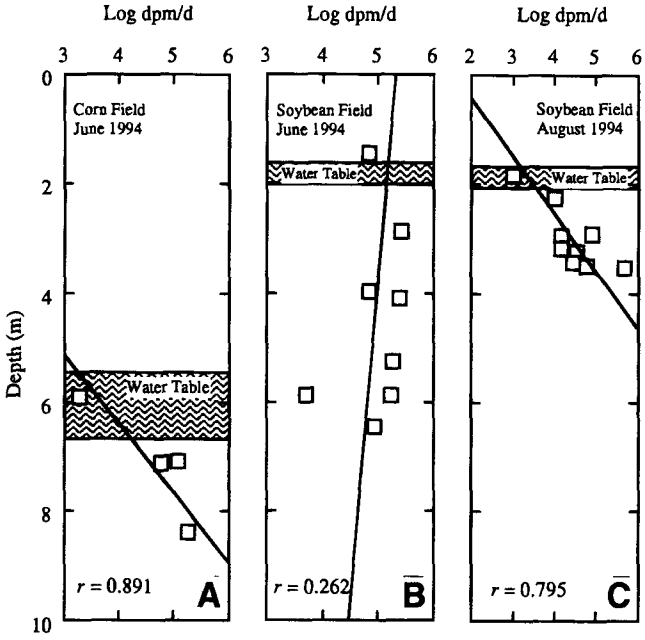

Fig. 3. Depth profiles of anaerobic glucose mineralization rates (log dpm/d) in the corn field (**A**) and soybean field (**B** and **C**) for the June and August 1994 sampling dates. r = correlation coefficient. The water table ranges from 5.3–6.5 m in A, from 1.4–2.0 m in B, and is about 3.3 m in C.

When the likelihood test was applied to aerobic GMR, the slopes did not vary significantly between the two fields or between different sampling dates (data not shown). This finding suggests that aerobic GMR was not sensitive to spatial and temporal variations at this site.

Anaerobic GMR

Trends for anaerobic GMR differed between fields and between two sampling dates (Fig. 3A, B, C). Although the individual slopes for the June 1994 corn field and soybean field data did not significantly differ from 0 ($P > 0.13$ for both), the two slopes did differ significantly ($P = 0.01$) from each other. Anaerobic GMR profiles in the soybean field appeared to be distinct between the June and August 1994 sampling dates: the latter showed a significant ($P < 0.05$) increase in anaerobic GMR with depth (Fig. 3C). The increase in anaerobic GMR with depth in the corn field samples of June 1994 and in the soybean field samples of August 1994 may indicate lower redox conditions with depth. Such conditions are possible, because at lower depths oxygen can be gradually consumed by aerobic microorganisms and result in anoxic conditions, at least in some microenvironments *(23–25)*. Interestingly, bacterial enumeration experiments failed to detect any heterotrophic anaerobes from subsurface sediments collected during the August 1994 sampling period (data not shown). This

result suggests that anaerobic glucose mineralization may have been more sensitive in characterizing potential anaerobic activities than other microbiological methods.

CLPP

The CLPP approach has proven to be a rapid, sensitive, and reproducible method for discriminating between microbial communities from a variety of environments, including the subsurface *(14,15,26,27)*. CLPP integrates a measure of the metabolic diversity in heterotrophic communities using a high number of substrate utilization tests to resolve differences in community structure and/or potential function. The CLPP approach is not dependent on isolation. Use of the number of positive tests (sum of binary data) or "community metabolic diversity" has been demonstrated as an effective method of distinguishing communities by Zak et al. *(26)* and Bossio and Scow *(27)*, whereas multivariate analysis of the carbon source utilization profile (continuous data) is a more powerful approach for comparing communities that oxidize a similar number of carbon sources. In either case, the carbon sources may be treated as dimensionless test responses without losing any ability of the CLPP to distinguish communities effectively and reproducibly and assess variability within and among samples.

At the Oyster site, use of the number of positive tests was sufficient to demonstrate community differences. Examination of CLPP data revealed that several features corroborated with microbial abundance and activity analyses. First, CLPP showed decreasing trends with depth for all three sampling dates in the soybean field (Table 3), as did the ACH and microaerophile profiles. Second, large variations in CLPP, as indicated by large standard deviations associated with the mean values of multiple carbon sources utilized by the community, existed for some intervals (1–2, 2–3, and 5–6 m in the soybean field; 6–7 m in the corn field; Table 3). These variations suggest that the MC sometimes changed dramatically within a meter distance at this site. Third, the soybean field appeared to harbor a MC more capable of growth on sole carbon sources than the cornfield within the first 6-m depths. This may be because of the difference in crops between the two fields. Because N_2 fixation occurs more readily with soybeans than with corn, more nutrients may be available in the soil zone in the soybean field than in the corn field. The use of different pesticides or herbicides for soybean and corn crops may also contribute to the difference in CLPP between the two fields. Microbial utilization of multiple carbon sources increased at the water table in the corn field (Table 3). This increase suggests that water availability may be a limiting factor for microbial metabolism at the Oyster site.

Water availability may affect subsurface microbiology in several ways. In arid regions of the US, increased moisture content often stimulates microbial abundance and activity, possibly because of increased bioavailability and transport of sediment-associated nutrients *(28–30)*. Also, deep subsurface sediments containing abundant clays showed

Table 3
Community-Level Physiological Profile for the Soybean Field and Corn Field Samples Collected During Three Sampling Dates Between June 1994 and July 1995

Depth interval, m	Community-level physiological profile			
	Corn field, June 1994	Soybean field, June 1994	Soybean field, August 1994	Soybean field, July 1995
0–1[a]	—[b]	87	93 ± 1	83 ± 8
1–2	0	78[c]	57 ± 50[c]	36 ± 50
2–3	0	35 ± 50	7	0
3–4	0	0.5 ± 0.7	3	5 ± 6[c]
4–5	—	0.5 ± 0.7	—	0
5–6	0	0	—	19 ± 26
6–7	36 ± 51[c]	4	—	—
7–8	60	—	—	—

[a]A value in each depth interval indicates the number of positive tests of a sample to the 95 carbon sources used. Average values + 1 SD indicate multiple samples (2–3) in that depth zone.

[b]Not available.

[c]Interval where water table exists.

greater stimulation of microbial growth with nutrient supplements than did sediments dominated by sands *(31)*. In humid areas where shallow subsurface sediments are composed of loosely compacted sands, increased water availability may adversely affect sediment microbial abundance and activity by either decreasing the bacterial attachment capability to sediment particles *(32)* or transporting nutrients away from the sediments. At the Oyster site, the magnitude of effects of water and phosphate on microbial variability was estimated by using a multiple-constraints model (Palumbo et al., manuscript in preparation).

In summary, significant spatial and temporal variations in microbial abundance (ACH, microaerophiles), activity (anaerobic GMR), and community structures (CLPP) occurred at the Oyster site. Results of this study demonstrated that water abundance, nutrient availability, scale of sampling, and even land use could have dominant effects on microbial variability within a single lithology and field site in shallow subsurface environments.

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