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The Distribution of Microbial Communities in Anaerobic and Aerobic Zones of a Shallow Coastal Plain Aquifer

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ABSTRACT

Randomly amplified polymorphic DNA (RAPD) fingerprinting was used to determine the genetic similarity of whole-community DNA extracts from unattached microorganisms in several groundwater wells. The study site was a shallow coastal plain aquifer on the Eastern Shore of Virginia that contains distinct regions of anaerobic and aerobic groundwater. Several wells in each region were sampled, and principal component and cluster analyses showed a clear separation of the microbial communities from the two chemical zones of the aquifer. Within these zones, there was no relationship between the genetic relatedness of a pair of communities and their spatial separation. Two additional sets of samples were taken at later times, and the same clear separation between communities in the different zones of the aquifer was observed. The specific relationships between wells within each zone changed over time, however, and the magnitude and direction of these changes corresponded to concurrent changes in the groundwater chemistry at each well. Together, these results suggest that local variation in groundwater chemistry can support genetically distinct microbial communities, and that the composition of the microbial communities can follow seasonal fluctuations in groundwater chemistry.

Introduction

The microbiology of aquifers and subsurface sediments is becoming a subject of expanding interest, in part because aquifers are a major source of freshwater in many countries. These underground waters represent 97% of all global freshwater and, though they are commonly exploited for drinking water, agriculture, and industry, they are poorly understood ecosystems [16]. Studies of shallow subsurface waters have found substantial numbers of microorganisms, predominantly prokaryotic, and have shown that these communities may perform a number of significant functions that may dramatically affect the chemical composition of the groundwater (see review in [17]). Much of this research has been stimulated by concern over contamination of groundwater

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supplies, particularly organic contamination from industrial operations, and the potential for microbial degradation of such pollutants as a means of restoring and purifying these waters [1, 2, 23, 27].

Numerous studies have detailed the groundwater habitat, focusing on the abundance (see references in [15] and [23]), morphology [3, 6], physiological state [4, 5, 35], and genomic diversity [18, 32] of its microbial residents. Research emphasis has shifted from isolate and culture-based approaches toward community-level analyses, where entire microbial communities are used as the functional units of study. Such investigations have considered rates of metabolism of specific substrates by entire communities [4, 12, 22], performance of specific activities (e.g., denitrification [29]), and expression of certain genes [38]. Overall community structure in groundwater has also been compared using community-level physiological profiling (CLPP) [9, 21], phospholipid fatty acid profiling [19], and 16S ribosomal RNA gene sequencing [26].

Microbial community structure in groundwater systems may be influenced by a number of factors, including site history, biological interactions (e.g., synergistic/mutualistic relationships [7], competition [8], and predation [28]), and physical habitat variation. The distribution of community members has also been correlated with changes in the groundwater chemical environment (e.g., availability of organic and inorganic carbon, dissolved oxygen, sulfur, nitrogen, phosphorus, and iron [3, 5, 7, 24]). However, when such studies evaluate how these different types of variables influence microbial community structure, they usually do so on a very broad scale and rarely consider how the spatial separation of organisms within a system may influence population interactions and community dynamics. There likely exists a coupling of distance with community relatedness (a microbial community patch size) at spatial scales below that of the aforementioned physical, chemical, and biological variables. Studies in other environments suggest that the scale of bacterial patchiness can be quite small; for example, the patch size of a microbial community in agricultural soils was estimated to be approximately 1 m [Balser TC, Firestone MK (1996) Meeting Abstracts, Substrate Utilization for Characterization of Microbial Communities in Terrestrial Ecosystems, Innsbruck, Austria], and marine bacterioplankton communities exhibit strong patchiness at the centimeter scale [11].

The present study examined two chemically distinct zones of a shallow aquifer (well-defined regions of low-oxygen and aerobic groundwater) to evaluate the extent to which differences in aquifer redox chemistry may influence the genetic structure of the resident microbial communities. At the research site, the proximity of the two zones of groundwater flow, and the fact that the water percolates through sediment from a similar depositional environment, eliminated the need to consider differences in macroenvironment (e.g., rainfall and climate or variation in sediment properties) that may also influence microbial community development in the subsurface. Within these two zones, a fundamental question motivating the research was whether the scale of microbial community relatedness occurred on a scale similar to that of the major chemical differences, i.e., aerobic vs anaerobic conditions.

Several samples were collected within each chemical zone of the aquifer, and randomly amplified polymorphic DNA (RAPD) was used to compare genetic community structure and estimate percent similarity among the different communities [14]. RAPD analysis showed that the communities in the anaerobic and aerobic regions of the field were quite different. Some temporal variation in community structure was observed, and those changes paralleled fluctuations in the groundwater chemistry of the wells over the same time period. There was little correlation between community relatedness (percent similarity between wells) and spatial separation of the sampling locations, either throughout the entire field, or within each region. Although the communities were distinctly different in the two regions of the field, community patch size was smaller than the smallest sample separation distance, viz. 10 m.

Materials and Methods

Site Descriptions and Sampling Schedule

The research site is a shallow coastal plain aquifer on the lagoonal shoreline of Virginia's Eastern Shore, located in a small (1.7 ha), abandoned agricultural field. This surfacial aquifer is approximately 24–30 m thick, and the depth to the water table across the sloping field varies from 1 m to about 6 m. A distinct zone of anaerobic groundwater surrounds a mass of buried vegetable waste, from a tomato cannery, on the far north side of the field (Fig. 1). The anoxic conditions (below 0.5 mg liter^{-1} dissolved oxygen) extend down-gradient, while the water in the rest of the field is aerobic, consistent with the regional groundwater. Dissolved oxygen concentration in the aerobic region varies seasonally between 5 and 11 mg liter^{-1} [Knapp EP (1997) PhD Dissertation, University of Virginia, Charlottesville, VA]. In the zone we have termed anaerobic, dissolved oxygen concentrations are always at or near 0 (nearly always below 1 mg liter^{-1}), except for occasions when large storms bring oxygenated water downward to the surface of the aquifer. Other chemical analyses have shown that these two regions of the

Table 1. Concentrations of a number of groundwater constituents in the aerobic and anaerobic portions of the aquifer[a]

	Aerobic zone[b]	Anaerobic zone[c]
pH	5.3–6.5	5.9–6.6
Alkalinity (mg liter^{-1} HCO_3^-)	18.3–39.7	150–384
DOC (mg liter^{-1})	1.72–4.47	2.22–5.86
Dissolved oxygen (mg liter^1)	5.0–10.6	<0.9
Total dissolved iron (mg liter^{-1})[d]	0.0001–0.01	12–42
Sulfate (mg liter^{-1})	28.8–36.5	26.2–44.2
Nitrate (mg liter^{-1})	40–50	0–1
NH_4^+ (mg liter^{-1} N)	0	0.434–2.42

[a] The range presented represents observed values from 16 sampling efforts between June 1994 and November 1996. Data from Knapp (Ph.D. Dissertation, University of Virginia, Charlottesville, VA, 1997).
[b] These values for groundwater chemistry are from well PL2, an aerobic well ~55 m directly up-gradient from wells C3 and B3 that were sampled in this project.
[c] Values from well D1.
[d] Samples were filtered and acidified in the field, returned to the laboratory, and passed through a cadmium column to reduce Fe^{3+} to Fe^{2+} followed by colorimetric analysis by the Ferrozine assay [33].

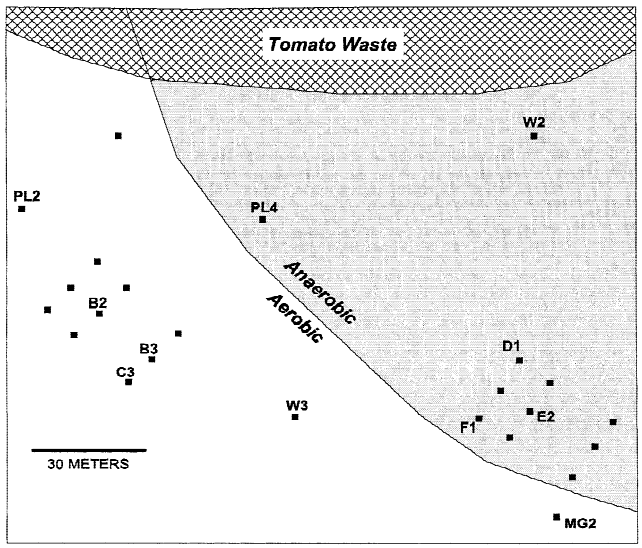

Fig. 1. Map of the field site displaying sampling wells and regions of aerobic and anaerobic groundwater. The hydrologic gradient at the site points roughly eastward; to the north lies the organic contamination, buried vegetable waste from a tomato cannery.

field differ in the amount of nitrate, alkalinity, ammonia, and dissolved iron present (Table 1) in a manner consistent with oxygen depletion and reducing conditions. Moreover, average microbial abundance, measured by acridine orange direct counts, differs between the two zones, and higher concentrations of cells are found in the anaerobic zone (well D1: 5×10^6 cells ml^{-1}; well W2: 3×10^7; wells C3 and B3: 3×10^5) [Lancaster LL, Mills AL (1995) Abstracts of the General Meeting of the American Society for Microbiology].

Several groundwater wells, constructed of 2-inch diameter PVC, have been installed throughout the field (Fig. 1), and nine of them were chosen for use in this study. Wells B3, C3, W3, and MG2 (in the aerobic zone), and wells F1, E2, D1, W2, and PL4 (in the anaerobic zone) were sampled in August 1997. Four of these wells, two aerobic (B3 and C3) and two anoxic (D1 and W2), were also sampled in June 1997 and January 1998.

Sample Collection

To isolate the microbial community for analysis, water samples were concentrated by filtration onto 0.22-μm pore-size polycarbonate membranes after pre-filtration through AE glass-fiber filters. Prior to sample collection, each well was purged for 10–15 min. For the June 1997 sampling, approximately 20 liters of water were filtered onto a single filter. However, preliminary analysis of these samples indicated that the amount of DNA obtained was far in excess of that needed for RAPD community profiling, so further sampling efforts focused on replication rather than collecting large volumes of water. In August 1997 and January 1998, three replicate samples of approximately 5 liters each were filtered at each well.

After each sampling, filters were quickly frozen in dry ice and ethanol (within 5 h of collection), transported to the lab on dry ice, and stored at −80°C. Filters were later processed and whole-community DNA was extracted as described elsewhere [14]. An additional purification step, using the High Pure PCR Template Preparation Kit (Boehringer Mannheim, Indianapolis, IN), was added to the original procedure and the isolated DNA was resuspended in 100 μl of 10 mM Tris buffer (pH 8.5).

RAPD

After extraction of whole-community DNA, RAPD amplification reactions were carried out in a volume of 25 μl using the procedure suggested by Williams et al. [37] with slight modification. A 5 μl portion of a DNA solution was added to a 20 μl reaction mixture containing 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 100 μM each of dATP, dCTP, dGTP, and dTTP, 0.2 μM primer, and 1 unit of *Taq* DNA polymerase (Perkin Elmer, Norwalk, CT). Reaction mixtures were covered with one drop of mineral oil and amplifications were performed in a Hybaid PCR Express Thermal Cycler programmed for 45 cycles of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C. PCR products were separated by electrophoresis in 1% agarose gels, stained with ethidium bromide, and photographed under UV light.

The amount of DNA entering each PCR reaction was standardized so that differences in the concentration of cells at each groundwater well would not artificially bias the RAPD results. For the June samples, the concentration of DNA was estimated using PicoGreen dsDNA quantification reagent (Molecular Probes, Eugene, OR) and approximately 400 pg of DNA were used in each PCR reaction. DNA yields from August and January were expected to be much lower, based on the smaller volume of water sampled, and, because of the desire not to sacrifice sample for quantification purposes,

standard spectrophometric/spectrofluorometric quantification procedures were not used. Instead, the DNA concentration was normalized across all August and January samples based upon the number of bacterial cells entering the extraction procedure [14]. Extractions were performed on approximately 10^8 cells.

Several arbitrary primers, purchased from Operon Technologies (Alameda, CA), were used to profile all three sets of samples: C4 (CCGCATCTAC), D5 (TGAGCGGACA), F4 (GGTGATCAGG), F1 (ACGGATCCTG), F14 (TGCTGCAGGT), S10 (ACCGTTCCAG), and T7 (GGCAGGCTGT). Additionally, primer F3 (CCTGATCACC) was used for the June and August samples, and primers F7 (CCGATATCCC) and S14 (AAAGGGGTCC) were also used for the June samples. This resulted in an overall comparison of 70 bands in June, 97 bands in August, and 76 bands in January. Within a set of samples, nearly all of the bands were variable (8% were present in all samples screened), and an individual well sample contained between 18 and 42 bands.

Data Analysis

For each primer, each band observed on the agarose gel was treated as a unit character and scored as present or absent in each sample. The data sets from each primer were then consolidated and a distance matrix was calculated using the Jaccard coefficient [30]. Dendrograms were then constructed using UPGMA clustering, and a bootstrapping procedure was used to assess the significance of the groupings and subgroupings in each dendrogram [31, 34].

In this research, the bootstrapping was accomplished by first using "SEQBOOT" in PHYLIP (Version 3.5c) to bootstrap the presence/absence (1/0) data sets 100 times [13]. Each of the resultant data sets was then fed into the clustering program of SPSS (Version 8) and similarity matrixes were determined using Jaccard's coefficient. Next, distance matrices (one minus similarity) were computed and the "NEIGHBOR" subroutine of PHYLIP was used generate 100 different recomputed trees. The "bootstrap value," the proportion of recomputed trees that contain a given node, was then determined by feeding the tree file from "NEIGHBOR" into the "CONSENSE" subroutine of PHYLIP.

Principal component analyses (SPSS, Version 8) of the original data were also performed, and diagrams of the first two principal components were constructed. Though PCA is not usually recommended for use with binary data such as these, it is often used as an alternate means of visualizing the relationships among the different RAPD profiles [10, 14, 36].

To compare the spatial distribution of the microbial communities in the field, plots were made of relative community similarity (as determined by the Jaccard coefficient) versus distance between sampling locations. Additional graphs were made to evaluate this relationship separately in the aerobic and anaerobic zones. An analysis of covariance (ANCOVA) was then used to determine whether the relationship between a pair of communities was more influenced by chemical zone (are the two samples from the same or different chemical regions of the field?) or by spatial separation. Average genetic similarity between each pair of communities was used as the dependent measure and the distance between each pair of wells was the covariate. Chemical "zone" was coded into three groups: 1, both communities sampled from aerobic wells; 2, both communities sampled from anaerobic wells; and 3, samples compared from different chemical zones of the field.

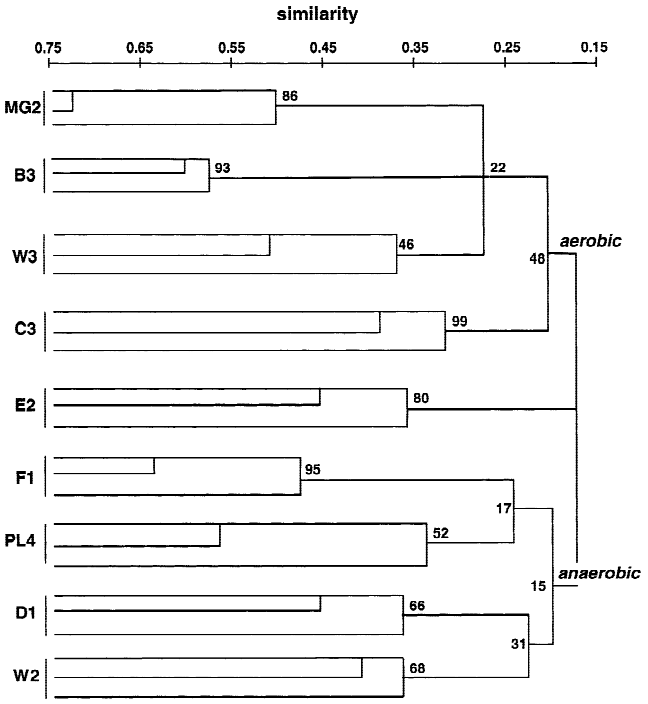

Fig. 2. Dendrogram displaying the results of a cluster analysis of all nine wells sampled in August 1997. The scale along the top represents similarity, the three prongs for each well represent independent replicates (separate 5-liter fractions of water collected from each well), and the numbers at each node are bootstrap values (bootstrap performed using 100 replications).

Results

Spatial Distribution of Communities within the Field

Principal Components and Cluster Analyses. Cluster analysis of all nine wells sampled in August showed a clear separation of the microbial communities in the anaerobic and aerobic zones of the aquifer; however, well E2 did not fall within either group (Fig. 2). Although high bootstrap values were calculated for the internal nodes of the dendrogram, the larger groupings of "aerobic" and "anaerobic" were not well supported. This is because, in the different bootstrapping runs, well E2 moved between these two clusters, and the fluctuation of this single well accounted for the low bootstrap values associated with the two major clusters [aerobic/anaerobic (Fig. 2)]. When the E2 data were excluded from the cluster analysis, the same overall pattern of separation of

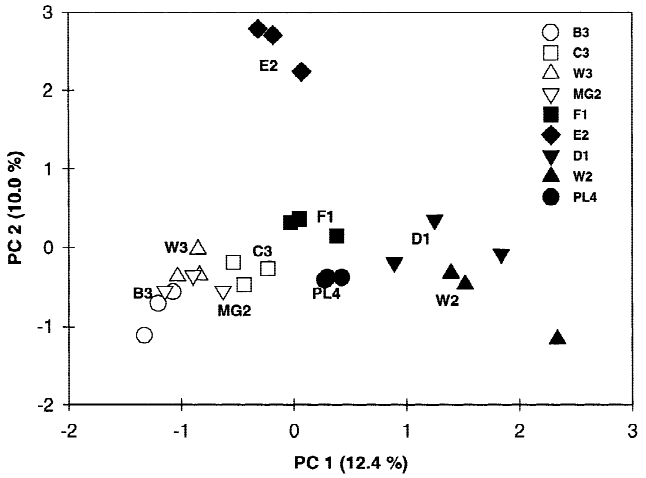

Fig. 3. Principal components diagram of all nine wells sampled in August 1997. The three points for each well represent independent replicates (separate 5-liter fractions of water collected from each well). The black symbols are samples collected from the anaerobic zone of the field; the white symbols represent samples from aerobic wells. The percent of variance explained by each principal component is also listed.

the communities was seen, but with higher bootstrap values (complete results not shown; see Fig. 5b for example).

Considering well E2's groundwater chemistry, and the fact that it is physically positioned between wells F1 and D1, the expectation was that the community would closely resemble those from the other anaerobic wells. Instead, the communities in E2 were equally similar to the communities from the aerobic or the anaerobic zones of the field. It is possible that a sampling error occurred at E2 and insufficient purging of the well prior to collection may have caused these puzzling results. If the well was not adequately purged, the microbes sampled may have been part of a different community, associated with the stagnant water in the well. Apart from this one well, the PC plot (Fig. 3) shows a clear separation of the communities that correlates with the large change in dissolved oxygen availability. Communities from the aerobic wells have very low scores (negative) on PC1, wells near the aerobic/anaerobic boundary have scores close to zero, and the wells in the anaerobic zone have positive values. When the PCA was rerun without the E2 data, tighter clusters formed and greater separation occurred between groups in the principal components plot.

In the cluster analysis, the communities from the aerobic wells were further divided into two subgroups (MG2, B3, and W3 separated from C3). However, this separation was poorly supported by the data (bootstrap value of 22) indicating that community structure did not differ greatly among the aerobic wells. This conclusion is further supported by the principal components analysis (Fig. 3), which shows little separation of the communities from the different aerobic sampling locations.

Within the cluster of anaerobic wells (F1, PL4, D1, W2), the communities in wells F1 and PL4 grouped together, as did D1 and W2 (Fig. 2). Though neither of these subgroups was particularly well supported in the cluster analysis (bootstrap values of 17 and 31, respectively), they did separate on the first axis of the principal components plot, suggesting that these subdivisions may be relevant. Both F1 and PL4 lie near the boundary of the anaerobic/aerobic regions, where fluctuations in the water table could cause these wells to experience a wide range of environmental conditions. On the other hand, wells D1 and W2 lie deeper in the anaerobic zone, closer to the source of the organic contamination, and are most similar to one another.

Comparison of Community Relatedness and Spatial Separation. The spatial distribution of communities within the well field was compared by plotting average relative similarity between each community (as determined by the Jaccard coefficient) vs spatial separation (distance in meters). Because of the increased hydrological linkage and more similar water chemistry of spatial proximal wells, it was expected that genetic similarity would be high when the distance between communities was small, and would decrease when more distant pairs of wells were compared. In fact, no such relationship was found (Fig. 4). Exclusion of the E2 data, as its comparison with other wells may result in a calculation of percent similarity that is unusually low, had no effect. Furthermore, graphs made of the anaerobic and aerobic zones separately showed no relationship between community similarity and distance (results not shown).

To examine the relative importance of spatial separation vs groundwater chemistry on the observed patterns of community structure, an analysis of covariance (ANCOVA) was calculated comparing average similarity between communities. Each pair of communities was grouped as being from the same chemical zone, either both aerobic wells or both anaerobic wells, or as being from different zones. The average similarity for any two communities was greater if they were from the same chemical zone (average similarity for aerobic pairs: 0.27, anaerobic pairs: 0.21, different zone pairs: 0.18, $p = 0.0035$) but the influence of spatial separation on this relationship was not significant ($p = 0.621$). As with the cluster and PC analyses, these results suggest a more

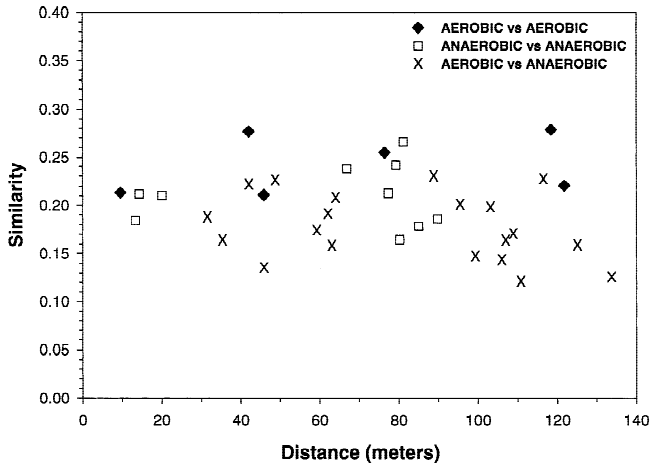

Fig. 4. Plot of relative similarity vs distance between sampling locations for all samples from August 1997. The closed symbols represent distances/similarities calculated between aerobic wells, the open symbols represent distances/similarities calculated between anaerobic wells, and the crosses represent distances/similarities calculated between aerobic and anaerobic wells.

homogeneous distribution of communities in the aerobic zone of the aquifer.

Temporal Consistency of Anaerobic/Aerobic Patterns in Community Structure

Temporal variability of the microbial communities in these two zones of the aquifer was assessed by comparing four wells [two anaerobic (D1 and W2) and two aerobic (C3 and B3)] at three different times: June 1997, August 1997, and January 1998 (Fig. 5). In all three cases, the communities from the aerobic and anaerobic zones separated, though the specific relationships among wells changed. In June and August, two distinct clusters formed, one aerobic (wells B3 and C3) and the other anaerobic (D1 and W2), and these were supported by high bootstrap values (Figs. 5a and 5b). In January, B3 and C3 formed a well supported aerobic cluster (bootstrap value of 82) but the relationship of the anaerobic wells changed—W2 moved within the clade containing the aerobic wells, and D1 remained distinct (Fig. 5c).

To examine how the communities changed over time, the summer and winter samples were compared using a principal component analysis performed on the combined August and January datasets (Fig. 6). Because a different concentration of DNA was used in the RAPD profiling of the June samples, a direct analysis comparing those results with the later samplings could not be made; however, August and January profiles were generated using the same starting concentration of DNA in the PCR reaction. Analysis of the pooled data showed that the profiles for the aerobic wells were consistent over time, but that the communities in both W2 and D1 changed between the two sampling dates. The communities in these two wells became more like the communities from the aerobic zone [W2 moved toward the aerobic communities on PC1; D1 moved through them on PC2 (Fig. 6)].

Quarterly monitoring of several groundwater chemical parameters (including pH, alkalinity, dissolved oxygen, ammonium, phosphate, dissolved iron, calcium, magnesium, potassium, and manganese) has been taking place at this site for several years, and Fig. 7 shows a principal components plot comparing the summer 1997 and winter 1998 chemical samplings for wells D1, W2, W3, and B2 (L. Lancaster, unpublished data). Unfortunately, data were not collected for either B3 or C3, the two aerobic wells compared with RAPD profiling, so B2 and W3 were used as proxy measures of their chemical patterns (a reasonable substitution given the relative homogeneity of the water chemistry in the aerobic zone). The principal components plot shows that the chemical patterns in the anaerobic wells change over time, becoming more like what is seen in the aerobic wells—the same shift observed for the genetic composition of the community (Fig. 6). The chemical parameters that loaded highly in the principal components analysis were for PC1, pH, alkalinity, dissolved oxygen, iron, and soluble cations, and for PC2, ammonium.

Discussion

In this aquifer, the distinct regions of aerobic and anaerobic groundwater supported different microbial communities. Within the aerobic zone, where the groundwater chemistry is fairly homogeneous, the communities from the various wells were quite similar. The degree of similarity among the wells in the anaerobic region was less than in the aerobic zone and reflected the higher level of dissimilarity in the chemical conditions found in the anaerobic area. Beyond this, the expectation was that spatially proximal communities would be more similar than spatially distant ones, based on the notion that nearby wells would be more hydrologically linked and have more similar water chemistries. Failure to find such a relationship indicates that the distance between sampling locations was greater than the scale at which the microbial communities organize. Although it is clear that

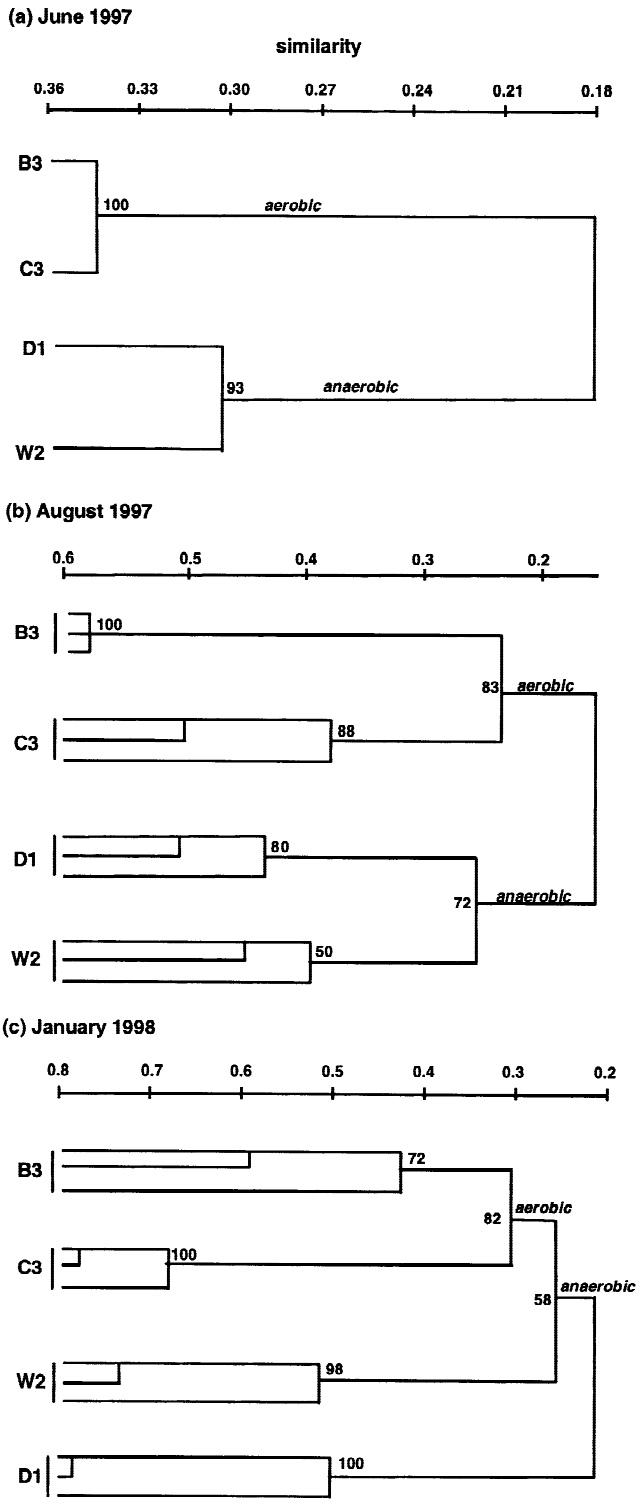

Fig. 5. Dendrograms displaying the results of a cluster analysis of the four wells sampled for the temporal comparison. The scale along the top represents similarity and the numbers at each node are bootstrap values (bootstrap performed using 100 replications). (a) June 1997; (b) August 1997; (c) January 1998. In (a) a single 20-liter sample was collected from each well. For (b) and (c) the three prongs for each well represent independent replicates (separate 5-liter fractions of water collected from each well).

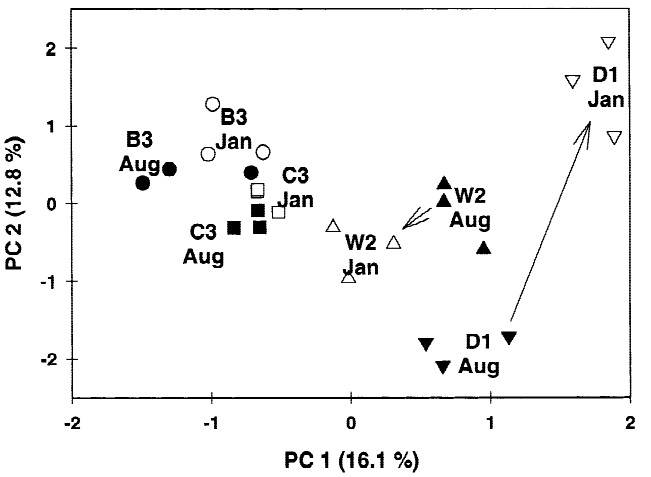

Fig. 6. Principal components diagram of the four wells compared in August 1997 and January 1998. The three points for each well represent independent replicates (separate 5-liter fractions of water collected from each well). The black symbols are from the August sampling; white symbols represent January. The percent of variance explained by each PC is also listed.

the groundwater environment is heterogeneous, there are relatively few data available on the spatial and temporal scales of variance in these systems. The results of this research imply that a great deal of variance in microbial community structure exists below the scale of measurement used (10 m) even within environments thought to be fairly ho-

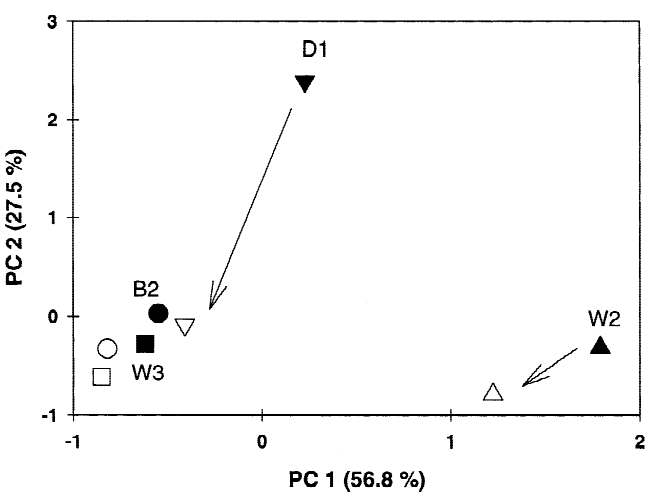

Fig. 7. Principal components diagram of overall groundwater chemistry in summer 1997 and winter 1998. As in Fig. 6, the filled symbols represent the summer samples and the open symbols represent the following winter's samples. The percent of variance explained by each PC is also listed. Data used for the analysis were obtained from Luke Lancaster.

mogeneous (e.g., within the aerobic zone). Indeed, the findings of Balser and Firestone (1996, Meeting Abstracts, Substrate Utilization for Characterization of Microbial Communities in Terrestrial Ecosystems, Innsbruck, Austria) and Duarte and Vaqué [11] suggest that variance scales on the order of 10 to 100 cm may be expected, a distance less than the minimum inter-well distance at the field site. Some studies indicate microbial variance scales on the order of 1 to 3 m in cropped soils [39]. However, other studies indicate a larger range of spatial scales of variability: 10 to 100 m for microbiological activities in seafloor sediments [20], and 30 cm to 150 m in a study of spatial variation in the surface sediment of the Okefenokee swamp [25]. In general, these scales appear to reflect the scales of heterogeneity in distribution of physical and geochemical properties of the environment under examination. The results presented here indicate the need to consider other hydrological, physicochemical, and biological factors, besides those evaluated in this work, that might influence patch size (e.g., particulate and dissolved organic material, permeability, porosity, grain size, or substrate stability) before developing expectations about the patterns of distribution of microbiota in groundwater.

In the anaerobic zone, the results suggest that two different types of communities may be present; the communities from the wells near the aerobic/anaerobic boundary were different from those more interior to the anaerobic zone. Given the potential for a change in chemistry as the water moves through the anaerobic zone, and the hydrologic character of the site, this is a reasonable separation. Wells along the boundary experience a wider range of environmental conditions, both hydrological and chemical, compared to the more interior wells. For example, dissolved oxygen concentrations as high as 6.5 mg liter^{-1} have been recorded in the anaerobic boundary well PL4 following major precipitation events. However, the highest recorded concentration of dissolved oxygen for in well W2 (interior of the anaerobic region) was 1.5 mg liter^{-1} [Callaghan, AV (1999) MS Thesis, University of Virginia, Charlottesville, VA].

The microbial communities in the aerobic and anaerobic regions of the aquifer remained genetically distinct over time. Between summer and winter, community structure changed somewhat, and these changes were similar in magnitude and direction to changes in groundwater chemistry during the same period. Communities from the aerobic wells, and the groundwater chemistry in that region, remained relatively constant at the different sampling times. In January, however, the communities in the anaerobic wells had changed significantly from earlier sampling dates; the profiles shifted so that the communities approached what is typically found in the aerobic zone. A similar change was observed in the overall pattern of groundwater chemistry for those wells. For well D1, where the change in groundwater chemistry was greatest, the microbial communities acquired some characteristics of the aerobic communities (moved through them on PC2, Fig. 6), but also some unique characteristics (which separate it from the other communities in Fig. 6).

During the month prior to the January sampling, the research site received an unusually large amount of precipitation, and the input of this water to the aquifer may partly explain the observed changes in community structure and groundwater chemistry in the January/Winter samplings. The average monthly precipitation at the site is 73.5 mm (1997 and 1998 average) and the average winter precipitation is 98.9 mm month^{-1}. However, the total precipitation in January 1998 was 166.9 mm, and nearly half (80.3 mm) of that fell during the week prior to sampling. Water from such precipitation events can be an important source of dissolved oxygen in shallow aquifers [7], and the percolation of oxygenated rainwater into this aquifer just prior to the January sampling could have caused the shift in the water chemistry of the anaerobic wells, making them more like the aerobic wells. Moreover, recharge events such as this may facilitate transport of dissolved nutrients into the flow system, either from the surface or as the water passes through the unsaturated zone, reducing the differences in water chemistry between the two regions of the aquifer. In contrast to the 80.3 mm of rain falling at the site in the week before sampling in January 1998, only 5.1 mm of total precipitation occurred during the 3 months prior to the August 1997 sampling. The sharp differences between the two zones of the aquifer during the summer may have reflected the absence of a recent recharge event.

The potential for precipitation events to have a dramatic impact on this aquifer can be further illustrated by considering fluctuations in the water table levels over this same time period. For example, the water level in well W2 was 2.56 m (above mean sea level) during the August sampling and 3.38 m on the January sampling date [increase of 0.82 m; Callaghan AV (1999) MS Thesis, University of Virginia, Charlottesville, VA]. Seventy percent of this increase (0.57 m) occurred during the three weeks prior to the January sampling, representing a major influx of water to the system over a relatively short time period.

Further research is necessary to determine whether the

sort of community turnover observed in this aquifer is a regular (i.e., seasonal) aspect of the system and to evaluate what level of disturbance (e.g., quantity and rate of precipitation) might be required to elicit a community response. Another factor that must be investigated is the response time—how long after an event do changes in the microbial communities become visible? Do these changes persist and, if so, for how long?

This study of groundwater microbial communities employed a relatively novel procedure (RAPD) for visualizing the overall differences between the microbial consortia at the different sampling locations. Because RAPD uses short primers of an arbitrary sequence to direct the PCR amplification, it may provide a more complete representation of the genetic structure of the entire community, compared to many of the more traditional PCR-based procedures that rely on amplification of sequences from specific organisms, groups of organisms, or genes. Monitoring the entire community as a unit, rather than gathering information on the presence/abundance of individual types of organisms, allowed for a more comprehensive comparison of the overall community dynamics and the physical and chemical conditions of the site. Major differences (both spatial and temporal) were observed within the well field and were qualitatively correlated with changes in the overall groundwater chemistry at each well. This research suggests that microbial communities in aquifers may track spatial and temporal variation in the environment to such an extent that distinct microbial communities tend to converge genetically as their environments become more similar. It remains to be seen how shifts in abundance of different microbial taxa are responsible for these changes and the spatial and temporal scale at which these changes in abundance take place.

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