

Functional Gene Array-Based Analysis of Microbial Community Structure in Groundwaters with a Gradient of Contaminant Levels

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To understand how contaminants affect microbial community diversity, heterogeneity, and functional structure, six groundwater monitoring wells from the Field Research Center of the U.S. Department of Energy Environmental Remediation Science Program (ERSP; Oak Ridge, TN), with a wide range of pH, nitrate, and heavy metal contamination were investigated. DNA from the groundwater community was analyzed with a functional gene array containing 2006 probes to detect genes involved in metal resistance, sulfate reduction, organic contaminant degradation, and carbon and nitrogen cycling. Microbial diversity decreased in relation to the contamination levels of the wells. Highly contaminated wells had lower gene diversity but greater signal intensity than the pristine well. The microbial composition was heterogeneous, with 17–70% overlap between different wells. Metal-resistant and metal-reducing microorganisms were detected in both contaminated and pristine wells, suggesting the potential for successful bioremediation of metal-contaminated groundwaters. In addition, results of Mantel tests and canonical correspondence analysis indicate that nitrate, sulfate, pH, uranium, and technetium have a significant ($p < 0.05$) effect on microbial community structure. This study provides an overall picture of microbial community structure in contaminated environments with functional gene arrays by showing that diversity and heterogeneity can vary greatly in relation to contamination.

Introduction

Environmental contaminants can have large and complex impacts on microbial community structure, and under-

standing these impacts will facilitate better management of communities for bioremediation. Detection, characterization, and quantification of microorganisms in natural settings, however, are very challenging endeavors. Establishing links between microbial diversity and ecosystem functions presents even more challenges. Functional gene arrays (FGAs), or GeoChips, allow simultaneous detection of thousands of populations of bacteria (1–5). It is expected that, by correlating gene diversity with geochemistry, we can better understand which variables are most important in determining microbial community structure and how that community changes along an environmental gradient.

The Oak Ridge, TN, Field Research Center (FRC) established by the Department of Energy has been extensively studied for bioremediation of heavy metals due to legacy contamination from Cold War-era uranium enrichment (6). The former S-3 waste ponds located at the Y-12 Security Complex received large quantities of waste containing radionuclides, nitric acid, and various organic solvents, until their closure in 1983. These unlined ponds are the source of a contaminant plume traveling through the groundwater that has resulted in gradients of U, technetium, nitrate, and pH throughout the area. Several heavy metals, as well as high levels of sulfate, calcium, and chloride, are also present.

Knowledge of microbial diversity and the effects of contaminants on community structure are critical for successful bioremediation. Contaminants can alter some local microbial populations by exerting selective pressure (7) but leave other populations intact (8). Many studies of this site have used culturing techniques (9, 10) and molecular biomarker analysis (7, 8, 10–12) to examine the microbial diversity and functional capabilities of microorganisms in the area, but none of these studies have simultaneously examined the diversity of multiple functional genes. Thus, the objective of this study is to examine the microbial community structure in wells of varying contamination by addressing the following questions: (i) How does microbial community diversity and functional structure vary across gradients of contamination and pH? (ii) How heterogeneous is the microbial community in this environment? (iii) How do the contaminants and geochemical parameters shape community functional structure? To answer these questions, the GeoChip, with probes from 2000 functional genes, was used to analyze microbial community structure (13). Our results indicate that metals (Tc, U), nitrate, and pH have significant effects on microbial community composition. Regardless of the variations in contaminant levels among different wells, the microbial communities are quite heterogeneous. Metal-resistant and metal-reducing microorganisms were also detected in both contaminated and pristine wells, indicating great potential for successful bioremediation of the heavy metal-contaminated groundwaters through biostimulation.

Experimental Procedures

Site Description. The Oak Ridge FRC (Oak Ridge, TN) is divided into five contaminated areas, flanking the former waste ponds, and an uncontaminated background site, located approximately 6 km away. For a complete description of the extent of contamination, geochemistry, and hydrogeology of the area, see <http://www.esd.ornl.gov/orifrc/>. Six groundwater monitoring wells at the FRC, varying in distance from the former waste ponds, were selected for sampling (Table 1). The initial analysis of microbial diversity of some samples was previously reported for evaluation of whole community genome amplification (WCGA) technology (4).

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TABLE 1. Geochemical Variables Measured in Groundwater from Each FRC Monitoring Well

well	FRC area location	pH	Al (mg/L)	Cl (mg/L)	Ni (mg/L)	nitrate (mg/L)	sulfate (mg/L)	U (mg/L)	Tc (pCi/L)
FW300	B ^a	6.7	0.2	2.4	0.0	2.6	6.4	0.0	0.0
FW003	2	6.0	0.4	124.7	0.0	1015.0	16.3	0.1	141.0
FW021	1	3.4	398.0	220.2	11.9	8823.0	122.1	12.2	30 974.0
FW010	3	3.5	1120.0	686.4	15.7	43 019.0	8.3	0.2	7190.0
FW024	3	3.6	527.4	281.4	12.5	8481.0	987.1	44.8	36 956.0
TPB16	2	6.3	0.0	78.3	0.0	25.4	116.1	1.2	886.0

^a B = background.

Functional Gene Array Description. To examine microbial communities in the selected wells, a functional gene array was employed. The GeoChip is a 50-mer oligonucleotide array consisting of 2,006 probes for a variety of genes involved in carbon, nitrogen, sulfur and phosphorus cycling, metal resistance and organic contaminant degradation (2–4, 13). The array contains 16S rRNA gene positive control spots, and human and plant gene probes for negative controls.

DNA Extraction, Amplification, Labeling, and Hybridization. Microbial cells were collected from groundwater (2 L) by centrifugation. High molecular weight DNA was extracted from the pellets by a previously described method (14). The DNA was dissolved in 20 μ L of water. Nanogram amounts of community DNA (1 μ L) were amplified by WCGA (Templiphi kit, Amersham Biosciences, Piscataway, NJ) (4) and labeled with a Cy5 fluorescent dye (GE Healthcare, Piscataway, NJ) by a random priming method (2, 3). GeoChip slides were sealed inside individual flow cells (Telechem International) and the labeled DNA was hybridized at 50 °C overnight. Arrays were washed in buffer and air-dried. Amplification, labeling, and hybridization for each sample were performed in triplicate. Technical instead of biological replicates were done because there was insufficient biomass in the groundwater for three separate DNA extractions.

Microarray Scanning, Data Processing, and Analysis. Arrays were scanned with a ScanArray 5000 analysis system (Perkin-Elmer, Wellesley, MA), digitally analyzed by use of ImaGene (Biodiscovery Inc., Los Angeles, CA), and processed as described previously (4). Normalization was performed using the mean for the spiked internal positive control genes. The normalized microarray data were then used for further analysis. Spots with a signal-to-noise ratio [SNR = (signal intensity – background intensity)/standard deviation of the background] (15) greater than 2 were used for further analysis. Gene diversity analysis was performed by use of the hierarchical clustering algorithm in CLUSTER and was visualized with TREEVIEW (16). Geochemical analysis of the groundwater was provided by the FRC, and data were normalized by square root transformation prior to analysis. A BioEnv analysis was performed on the geochemical and gene diversity data by use of the vegan package in R (17). BioEnv identifies the best Spearman rank correlation of the Euclidean distances of scaled environmental factors, with the observed community dissimilarities. Mantel tests (18) for linking geochemistry and diversity were performed with PC-ORD v. 5.0 (19). Canonical correspondence analysis (CCA) (20) and partial CCA (pCCA) were performed with CANOCO (21). Of all the variables measured, four geochemical variables—U combined with Tc, pH, and sulfate—were selected for CCA with microarray hybridization signal intensities based on automatic forward selection of the variables in CANOCO and their inflation factors. pCCA was used to partition variation in community composition to these geochemical variables. All microarray hybridization data are available at the Institute for Environmental Genomics, University of Oklahoma (<http://ieg.ou.edu>).

Results and Discussion

Site Geochemistry. Six wells were selected from the Oak Ridge FRC that composed a gradient of heavy metals, nitrate, and pH. The water geochemistry of each well is described in Table 1. Three of the wells, FW010, FW024, and FW021 (Areas 1 and 3), have extensive metal contamination. TPB16 and FW003 (Area 2) have lower metal and nitrate levels. FW300 is a pristine well located approximately 6 km from the S-3 waste ponds with no detectable U or Tc.

Overall Functional Structure of the Microbial Communities. GeoChip detected numerous genes with high signal intensity from each well (130–302 genes/sample) (4). Compared to the other wells reported previously (4), well TPB16 had relatively low gene diversity, with 133 genes detected (S1). TPB16 is located near a zerovalent iron-reactive barrier that was installed for the purpose of passively remediating the contaminated groundwater (8). Its proximity to this installation may affect the diversity and heterogeneity of the microbial community. Likely due to its lower diversity, TPB16 had higher overlap of its community with some other wells (24–70%). TPB16 and FW003 are both mildly contaminated and located within the same area but had only 26% overlap in their community composition (Table S1 in Supporting Information).

Gene overlap varied between other wells (20–37%) and most had a small percentage of unique genes (0–20%), except for TPB16, indicating that there is moderately high heterogeneity, even in the most contaminated areas (S1). FW024 and FW010 are very close to each other in highly contaminated Area 3 and had 37% overlap. The pristine site, FW300, had the greatest diversity and the greatest percentage of unique genes (20%). Gene diversity was lower in more contaminated wells than in pristine and low-contamination wells, and the Simpson's diversity index reflects this trend (4). This trend has also been observed in 16S clone libraries from FRC groundwater (9) and is likely the result of contaminant stress reducing the numbers of species that can survive in this polluted environment.

General differences in community structure existed, which can be seen in Figure S1 in Supporting Information. FW024, FW010, and TPB16 had higher percentages of genes related to sulfate reduction than other wells at 16%, 18%, and 16%, respectively. FW021, FW003, and FW300 have higher percentages of contaminant degradation genes at 33%, 30%, and 32%. The abundance of nitrogen-cycling genes was similar across all wells, ranging from 6% to 10% of the total genes detected. Carbon fixation and nitrogen cycling had the lowest numbers of genes detected on average (6–12%), while contaminant degradation (21–33%) and metal-related genes (14–23%) had the highest numbers. Though the relative abundances of the gene categories were similar, differences in total diversity and community composition between the wells were observed.

Functional Gene Populations. To visualize how the functional gene composition differs across the gradient of contaminants and pH, heatmaps of all detected genes were constructed via cluster analysis (Figure 1). The wells divided

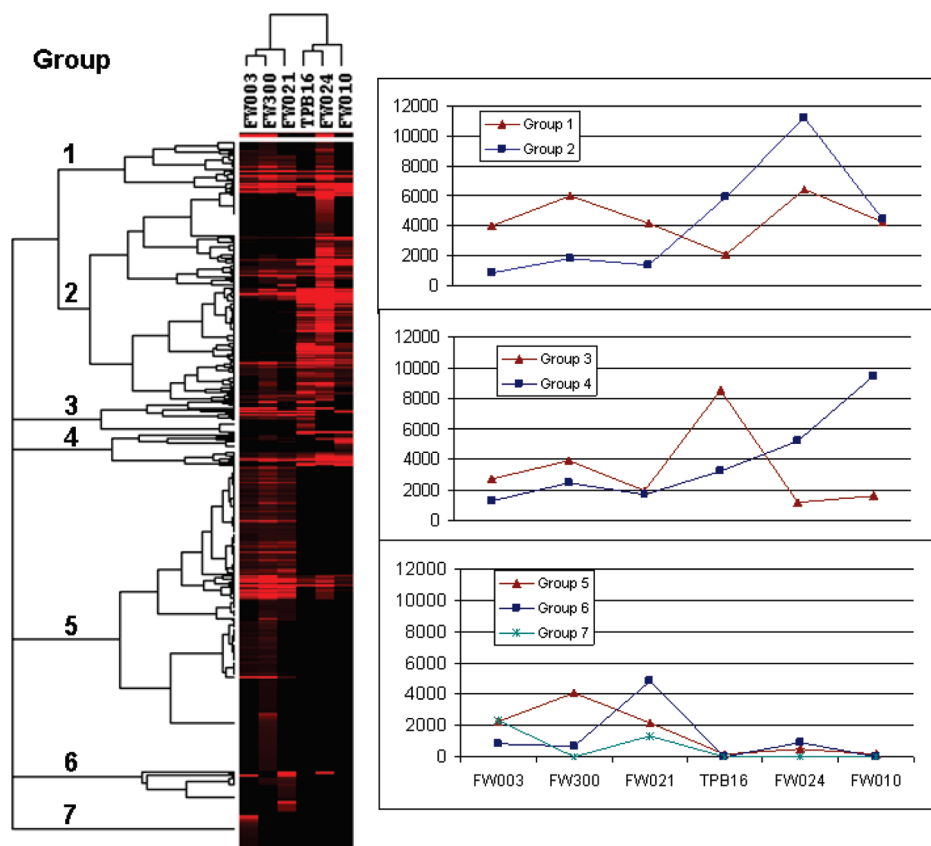


FIGURE 1. Hierarchical cluster analysis of gene diversity based on hybridization signal intensities for all wells. The color red indicates the level of signal intensity; black indicates no hybridization above the background level. Seven different hybridization patterns were detected, as indicated by the group number on the branches of the tree. Average signal intensities of these groups for each well are shown on the right.

into two clusters. Surprisingly, a highly contaminated well, FW021, clustered with the less contaminated wells FW003 and FW300. TPB16 clustered with FW024 and FW010, both of which are highly contaminated. These clusters were also observed with individual functional gene groups.

Seven major patterns were detected based on hierarchical clustering of all detected genes, as indicated by the group numbers in Figure 1. Groups 2 and 4 have genes that are particularly abundant in TPB16, FW024, and FW010. Many of these genes are from metal-resistant genera, such as *Rhodococcus*, *Pseudomonas*, *Ralstonia*, and *Burkholderia*. These microorganisms likely thrive in highly contaminated wells. The genes detected in FW003, FW300, and FW021 had a more even distribution of abundance throughout the groups. These wells also had a higher number of genes, and FW300 and FW003 had higher diversity. Group 1 contains genes present in most wells, and the majority of genes are for carbon and organic contaminant degradation, with some genes involved in sulfur and nitrogen cycling. Groups 5, 6, and 7 had a greater abundance of genes in FW300, FW003, and FW021, with organic contaminant degradation genes predominating. Overall, FW300, FW003, and FW021 have a greater number of genes with more even abundance, while FW010, FW024, and TPB16 have fewer genes but with greater abundance.

Nitrogen Cycling. Since nitrate is one of the most abundant contaminants in this environment, the presence of nitrate-reducing organisms may be important in remediating the area. The nitrite reductase genes detected (*nirS* and *nirK*) grouped the wells into clusters similar to the heatmap of all genes (Figure S2A in Supporting Information). Generally, TPB16, FW010, and FW024 had lower diversity than the other wells, but the individual genes detected had higher signal intensities. This would be expected in environ-

ments where contaminant concentrations select for a few resistant, denitrifying bacteria, thus lowering the total diversity and evenness, as indicated by a previous study at the FRC (9). Most of the *nirS* and *nirK* sequences detected were from uncultured bacteria from environmental samples. Small numbers of other nitrogen-cycling genes were also detected, including a nitric oxide reductase (*norB*) similar to the sequences from *Paracoccus pantotrophus* and *Sinorhizobium meliloti* and an ammonia monooxygenase (*amoA*) from uncultured organisms (data not shown).

Other studies have found differences in *nirS* and *nirK* diversity in FRC wells. Higher *nirK* diversity has been detected in wells with extremely high nitrate concentrations, while higher *nirS* diversity has been detected in low-nitrate wells (12). Greater *nirS* diversity was also detected in clone libraries from wells stimulated with ethanol in Area 2 (10). Our results show approximately equal numbers of *nirS* and *nirK* genes in each well (Figure S2A in Supporting Information). In some wells, the *nirS* signal intensities were severalfold greater than the intensity of *nirK*, indicating a greater abundance of *nirS* in the sample (data not shown); however, the differences do not correlate with nitrate concentration.

Carbon Cycling. Carbon utilization is important at the FRC because carbon is severely limited in this environment. To detect the presence of carbon-fixation genes, probes for ribulose 1,5-bisphosphate carboxylase (Rubisco, specifically, *cbbL* and *cbbM*), which is a biomarker for the Calvin-Benson-Bassham CO₂ fixation pathway (22), are present on the array. Though Rubisco is commonly associated with phototrophy, a variety of subsurface chemolithotrophs and mixotrophs also use Rubisco for CO₂ fixation (23). Probes for formyltetrahydrofolate synthetase are also present to detect acetogenic, fermentative bacteria (24). The heatmap of Rubisco and formyltetrahydrofolate synthetase genes show

distinct groups of genes present throughout all the wells, such as a sequence similar to the Rubisco gene from *Methylococcus capsulatus*, and some that are present in only one branch of the tree (Figure S2B in Supporting Information). A gene similar to the *S. meliloti* Rubisco gene was detected in FW300, FW003, and FW021, while Rubisco genes similar to those of several uncultured environmental species were detected in TPB16, FW010, and FW024. Though formyltetrahydrofolate synthetase and Rubisco are present in each well, more Rubisco genes and with higher signal intensities were detected than fermentation genes, despite a greater number of formyltetrahydrofolate synthetase probes on the array. Due to the limited carbon availability, the use of Rubisco by chemolithotrophic microorganisms may be a more important process than fermentation in this environment, but activity measurements must be done to confirm this.

Sulfur Reduction. Sulfite reductase genes (*dsrAB*) were most prevalent in the well with the highest sulfate concentration, FW024 (987.1 mg/L) (Figure S2C in Supporting Information). FW300 (6.4 mg/L) has a surprisingly high diversity of *dsrAB* genes, considering its low sulfate concentration. Notably, a gene similar to the sulfite reductase from *Desulfovibrio desulfuricans*, a bacterium capable of Tc reduction (25), was detected in FW003, which has a moderately low level of Tc (141.0 pCi/L). The presence of this type of bacteria may be important for successful bioremediation of the Tc-contaminated groundwaters.

Despite differences in geochemistry, TPB16, FW024, and FW010 have very similar *dsrAB* diversity and high signal intensity (Figure S2C in Supporting Information). TPB16 has a moderate contaminant load and sulfate concentration (116.1 mg/L) and, as mentioned earlier, is near a zerovalent reactive iron barrier. Increased sulfate reduction has been observed close to the barrier at this site and in other Fe⁰ barriers (26–28). FW010 (8.3 mg/L) also clusters within this group despite its low sulfate concentration. Certain *dsr* genes from uncultured organisms are prevalent in well FW010, as well as a *Desulfobacterium*-like *dsr* gene and one sequenced from the contaminated Shiprock, NM Uranium Mill Tailings Remedial Action (UMTRA) site (29).

The presence of sulfate-reducing bacteria (SRB) in these wells is significant because they may play an important role in reducing and stabilizing heavy metals during bioremediation. Several sulfate-reducing bacteria use U(VI) as an electron acceptor (30, 31), transforming it into insoluble U(IV), and they can generate uranium sulfides through the production of H₂S (32). A study of SRB diversity in U-contaminated groundwater found significant relationships between the abundance of *Desulfotomaculum* and *Desulfotomaculum*-like organisms and the concentration of sulfate and U (29). The researchers speculated that the increase in *Desulfotomaculum* with increasing U concentrations was indicative of a competitive advantage conferred by the U, either through *Desulfotomaculum*'s U-resistance or its use of U(VI) as a terminal electron acceptor. Though *dsr* genes similar to *D. desulfuricans*, a known U-reducing organism (30, 33), and *Desulfotomaculum*, a group that is prevalent at the Shiprock, NM UMTRA site [U(IV) ≤ 2.85 mg/L] (29), were detected in this study; the highest signal intensities were from *dsr* sequences from uncultured laboratory clones (Figure S2C in Supporting Information). Several of these *dsrAB* sequences were from previous studies of *dsrAB* diversity in FRC wells FW300, FW010, FW005, and FW003. These results indicate that the GeoChip is capable of detecting genes that are expected to be present at the FRC but suggests that many of the SRB at this site have not been cultured, indicating a need for further study of these organisms.

Organic Contaminant Degradation. Numerous organic contaminant degradation genes were detected in the wells

(Figure S3D in Supporting Information). Besides nitrate and heavy metals, the FRC is also contaminated with organic solvents, most notably trichloroethylene (TCE). Several species of bacteria are able to degrade TCE to dichloroethylene (DCE) and vinyl chloride, and these activities have been observed previously at the FRC (34). A heatmap of the most abundant and relevant organic contaminant degradation genes was constructed. The greatest diversity of genes was observed in FW300 and FW003, and the highest total signal intensity was detected in FW300 and FW024. The most numerous degradation genes were for common aromatic compounds and their intermediates, including benzoate, biphenyl, naphthalene, and phenol.

When we looked specifically at TCE degradation genes, there is greater diversity in the wells with low or background-level organic contaminants (FW300, FW003), but higher signal intensity in FW021 and FW024, indicating an abundance of these genes. No TCE degradation genes were detected in FW010, despite significant levels of TCE and other organic compounds (6). No TCE genes were detected in TPB16, which has no TCE contamination. These results suggest that the distribution of the genes involved in TCE degradation is highly heterogeneous at the FRC.

Metal resistance. In the metal-resistance heatmap (Figure S3E in Supporting Information), the genes with the greatest signal intensity were a grouping of arsenate, tellurite, and mercury resistance genes present in both low and high contaminant-level wells: FW024, FW021, FW300, and FW003 (Figure S3E in Supporting Information). Very few metal resistance and transport genes were detected from wells FW010 and TPB16, despite the high levels of aluminum, nickel, and Tc in FW010 and moderate Tc and U in TPB16. A tellurite resistance gene (*telA*) (35) and a cation efflux system gene (*czcA*) that exports the redox-inactive metals Co, Zn, and Cd from several types of bacteria (36) was found to be prevalent in all wells. These results indicate that the heterogeneity of the horizontal gene distribution is also gene-dependent.

Relationship between Microbial Community Structure and Groundwater Geochemistry. BioEnv was used to identify the best correlation between the measured variables and the species composition (37). Sulfate, pH, Al, and Tc were identified as the combination of environmental variables that gives the best correlation ($r = 0.8036$) of environmental factors with the community. The combination of these variables has a strong, positive correlation with the community structure. Mantel tests were also performed to correlate gene composition with measured environmental variables (Table S2 in Supporting Information). Of all the geochemical variables, only nitrate ($p = 0.0270$, $r = -0.255$) and U ($p = 0.0491$, $r = -0.233$) produced significant correlations with the detected genes.

The top five geochemical variables identified by automatic forward selection were examined by CCA for correlation with community composition (Figure 2). Due to the multicollinearity of the variables, U and Tc were combined ($r = 0.922$) and nitrate was removed to reduce inflation. The first axis explained 47.3% of the microbial diversity observed, and the second axis explained 14.3% of the diversity, describing 61.6% of the total variation. FW300 and FW003 clustered together, and FW010 and FW024 grouped closely. TPB16 and FW021 did not cluster with either group. The microbial diversity present in FW010 and FW024 appears to be strongly affected by the sulfate, Tc, and U concentrations in those wells, as indicated by their proximity to those arrows. The community structure in FW300 and FW003 appears to be mainly affected by the circumneutral pH in those wells. FW021 and TPB16 are not close to any of the geochemical arrows, indicating that unmeasured factors are likely to be the important controllers of microbial diversity in these wells. The CCA

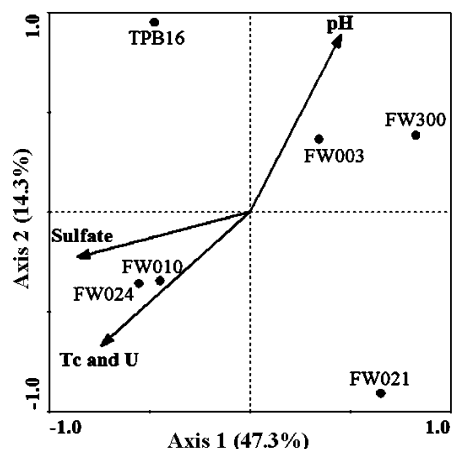


FIGURE 2. Canonical correspondence analysis (CCA) of GeoChip hybridization signal intensities and groundwater geochemistry variables: technetium and uranium, sulfate, and pH. The percent of variation explained by each axis is shown, and the relationship is significant ($p = 0.041$).

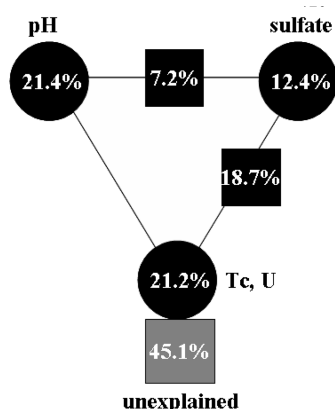


FIGURE 3. Partitioning of microbial diversity variance among important geochemical variables, technetium and uranium ($p = 0.086$), pH ($p = 0.175$), and sulfate ($p = .006$), and their interactions. There was zero variance attributed to the interaction of pH and U and Tc, or to the interaction of all variables.

had high species–environmental correlations (Table S3 in Supporting Information) and the combination of all canonical axes was significant ($p = 0.041$). Similar to results in the present study, Palumbo et al. (11) identified U, sulfate, pH, Tc, and nitrate as important geochemical variables in a principal component analysis of *dsrAB* genes.

Variation partitioning analysis was performed to attribute the variation observed in the microbial community composition and abundance to the environmental variables identified to be most important by CCA (Figure 3). Three variables explained a large portion of the variation observed, leaving 45.1% of the variation unexplained by these factors. pH alone explained 21.4% ($p = 0.175$); 12.4% of the variation was attributed to sulfate ($p = 0.006$); and Tc and U explained the largest amount of variation, 21.2% ($p = 0.086$). Interactions between pairs of variables explained smaller amounts of variation, except for the interaction between sulfate and Tc and U, which accounted for 18.7% of the variation. When subsequent variables were added to the analysis, the amount of variation explained did not increase due to the multicollinearity of the geochemical variables. Since all contaminants in the wells originated from the same source, concentrations tended to vary in the same ways, often in relation to distance from the S3 ponds. Since the contaminants varied in the same way, it was difficult to distinguish their effects statistically.

The amount of unexplained variation in this study (45.1%) is less than the unexplained variation in analyses of more diverse soil communities (up to 80%) (38) but more than in other groundwater studies at the FRC (J.D.V.N., personal communication). In a study of the groundwater recirculation and bioremediation system in area 3, up to 65% of the variation observed was able to be explained. That system, however, is less complex and more controlled, through groundwater recirculation and ethanol stimulation, than the wells in the present study.

Some of the unexplained variation may be a result of other ecological variables that were not explored. There are likely to be synergistic effects between contaminants and natural environmental factors on microbial community structure. Seemingly innocuous natural conditions can have significant effects on the fate of contaminants, such as the effect of carbonate concentrations on U sorption and mobility (39), which may affect toxicity and reduction rates. Other natural variables that might affect microbial community composition include differences in soil type and composition between the different areas sampled, disparities in hydrogeology, and the presence of organic carbon to support microbial growth. Also, the GeoChip was used to detect the relative abundance of multiple functional groups, but these results do not necessarily indicate microbial activity. Hybridizing microbial mRNA instead of amplified DNA to the GeoChip, though preferable, was not possible in this experiment due to the low biomass of the contaminated wells. Quantifying which bacteria are metabolically active may give a stronger correlation with geochemistry.

In summary, this study indicates that metal, sulfate, and nitrate contamination have significant effects on microbial functional gene diversity at the Oak Ridge FRC. Tc, U, nitrate, sulfate, and pH all had a significant correlation with functional gene diversity, as determined by Mantel test and CCA. Microbial diversity varied across the gradients of pH and metals and even varied in adjacent wells with similar contamination levels. The presence of genes from a variety of metal-resistant microbes and genes for metal and nitrate reduction and organic solvent degradation indicate great potential for successful bioremediation of the contaminated groundwaters through biostimulation.

As this study demonstrates, functional gene microarrays are ideal for monitoring changes in microbial functional populations in time and space as they can detect a variety of populations in a high-throughput fashion. The results demonstrate the complicated spatial relationships between environmental variables and community functional structure. Future studies are needed to determine how these communities change with time and how they are affected by bioremediation technologies.

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Supporting Information Available

Detailed functional gene heatmaps, relative abundance of all genes detected, Mantel test results, CCA values provided by CANOCO, and a listing of unique and overlapping genes detected in wells. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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