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# Microbial Functional Gene Diversity with a Shift of Subsurface Redox Condition during *in situ* Uranium Reduction

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**Abstract**

To better understand the microbial functional diversity changes with subsurface redox conditions during *in situ* uranium bioremediation, key functional genes were studied with GeoChip, a comprehensive functional gene microarray, in field experiments at a uranium mill tailings remedial action (UMTRA) site (Rifle, CO). The results indicated that functional microbial communities altered with a shift in the dominant metabolic process as documented by hierarchical cluster and ordination analyses of all detected functional genes. The abundance of *dsrAB* genes (dissimilatory sulfite reductase genes) and methane generation related *mcr* genes (methyl coenzyme M reductase coding genes) increased when redox conditions shifted from Fe-reducing to sulfate-reducing conditions. The cytochrome genes detected were primarily from *Geobacter* sp. and decreased with lower subsurface redox condtions. Statistical analysis of environmental parameters and functional genes indicated that acetate, U(VI), and redox potential ($E_h$) were the most significant geochemical variables linked to microbial functional gene structures, and changes of microbial functional diversity were strongly related to the dominant terminal electron accepting process following acetate addition. The study indicates that the microbial functional genes clearly reflect the *in situ* redox conditions and the dominant microbial processes, which in turn influence uranium bioreduction. Microbial functional genes thus could be very useful for tracking microbial community structure and dynamics during bioremediation.

**Key words**

Microbial communities, GeoChip, Uranium, Sulfate-reduction, Fe-reduction, Redox condition

## Introduction

Uranium contamination of groundwater, sediment and soil, initiated from uranium mining, processing, storage and nuclear weapon production is a potential threat to human health and the natural environment. Uranium is present in oxic to sub-oxic waters and soils primarily as soluble uranyl species with high toxicity due to its bioavailability as a heavy metal and radiation source. A proposed method to decrease the risk of uranium contamination is to reduce highly soluble U(VI) to sparingly soluble U(IV) (17). The stimulation of microbial enzymatic reduction of U(VI) has shown a substantial promise for *in situ* bioremediation of uranium contaminated groundwater, where organic compounds such as acetate, ethanol or glucose were injected to the subsurface environment as electron donors (1, 20, 27, 33). Multiple electron acceptors such as Mn(IV), Fe(III), $NO_3^-$, U(VI), $SO_4^{2-}$ in natural subsurface environments are used by microbes typically in sequence of energy yield. For example, *Desulfovibrio vulgaris* showed utilization of Fe(III) first, followed by U(VI) and finally sulfate in a competition experiment (6). In the field, nitrate has been shown to be reduced prior to U(VI) and U(VI) reduction that often occurrs simultaneously with Fe(III) reduction (1, 12). However, relatively few studies have focused on functional diversity of microbial communities with changes of subsurface redox conditions under *in situ* field conditions.

The Old Rifle site is located at a former uranium ore processing facility in Rifle, CO, where the subsurface aquifer was contaminated by uranium. The site is part of the uranium mill tailings remedial action (UMTRA) program of the U.S. Department of Energy. Field experiments conducted at the Old Rifle site demonstrate a decrease in soluble U(VI) from groundwater upon the addition of acetate to the subsurface and stimulation of endogenous microorganisms (1). Loss of soluble U(VI) correlated with the stimulation of Fe-reducing conditions in the subsurface and the enrichment of *Geobacter* spp., microorganisms known to reduce both Fe(III) and soluble U(VI) in the subsurface (1, 10, 14, 21, 28, 30). With continuous injection of acetate, sulfate was then used by microorganisms as the dominant electron acceptor. However, in some cases, an increase in U(VI) concentration was observed to be associated with a shift from Fe-reducing to sulfate-reducing conditions (1, 4). Thus several questions were raised regarding factors that controlled the bioreduction of U(VI) and the specific microbial populations that were stimulated with shift of redox conditions in the field experiments.

However, due to temporal and spatial changes in microbial diversity and the heterogeneity of environmental conditions, characterizing the microbial communities in an accurate and comprehensive way remains a challenge. The development and application of genomic tools has greatly advanced characterization and profiling of the microbial communities in complex environments. One such development, GeoChip 2.0 (9), is a comprehensive functional gene array. The GeoChip 2.0 contains 24,243 oligonucleotide probes and covers >10,000 genes in >150 functional groups involved in carbon, nitrogen, phosphorus and sulfur cycling, metal reduction and resistance and organic contaminant degradation, and has been demonstrated to be a robust tool for investigating biogeochemical, ecological and environmental processes from different habitats (16, 29, 31, 36).

In this study, GeoChip 2.0 was used to characterize microbial communities under Fe-reducing conditions and shift from Fe-reducing to sulfate-reducing conditions during *in situ* uranium bioreduction. Two experimental plots were amended with acetate for stimulating microbial reduction of uranium. One was maintained mainly in Fe-reducing conditions and the other intentionally driven to conditions in which sulfate reduction dominated. The objectives of this study were to: (i) determine the microbial functional diversity under Fe-reducing conditions and transition from Fe-reducing to sulfate-reducing conditions and (ii) link geochemical changes to microbial functional diversity. Our results demonstrate a shift in the functional structure of microbial communities from Fe-reducing to sulfate-reducing conditions. The microbial community structure and functional dynamics changed in a manner consistent with geochemical differences associated with different redox conditions.

## Materials and Methods

### *Site description and plot design*

The Old Rifle UMTRA site is a flood plain of the Colorado River consisting of recent alluvium overlying the Eocene Wasatch Formation. The flood plain is approximately 2 km long, and virtually the entire site was contaminated as a result of a long-term vanadium and uranium milling operation. The groundwater flows at 0.46 to >0.61 m/day in a direction that normally parallels the Colorado River. The geology and hydrogeology of the site were described previously (1, 3, 4, 20, 28). In this experiment, two adjacent experimental plots of background, injection and

down-gradient wells, one installed in 2004 and the other in 2005, were run simultaneously. The experimental plots had similar layouts with the 2005 experimental plot, 3.8 m to the southeast. Each experimental plot had five injection wells perpendicular to groundwater flow, four monitoring wells down-gradient of acetate injection, and one monitoring well positioned up-gradient of the injection wells (4) (Figure 1). The 2004 experimental plot was amended with acetate for about three-weeks to introduce Fe-reducing conditions. Amendment to the 2005 experimental plot was started earlier and the subsurface was driven to sulfate-reducing conditions. Injections to both experimental plots were stopped simultaneously on September 19, 2006.

*Sampling*

Groundwater were sampled and analyzed from July 17 to October 31 in the two experimental plots (4). Groundwater (2 liters) was collected in sterile glass bottles using a peristaltic pump and kept on ice until it was delivered to the laboratory and then filtered (0.2 μm) to collect biomass. Filters were stored at -80 °C until DNA extraction. To better understand the microbial functional structure with subsurface redox changes, 8 groundwater samples, B05 (7/27, 9/16), M16 (9/5, 9/19) M21 (7/27, 8/10) and M24 (7/27, 8/10) were selected for microbial functional structure analysis with GeoChip. The 9/5 sample and 9/19 sample (M16) corresponded to the early days and the end of acetate injection in the 2004 experimental plot, where there was a continuous decrease of redox potential ($E_h$) under Fe-reducing condition. The 7/27 and 8/10 sample set (M21, and M24) corresponded to the shift from iron-reducing to sulfate-reducing conditions in the 2005 experimental plot. The samples of B05 were used as background control. Groundwater was pumped from the designated depth(s) in the monitoring wells using a portable peristaltic pump (ColePalmer Instrument Co.). The geochemical analysis of U(VI), Fe(II), bromide (potassium bromide as tracer), acetate, sulfate and molecular analysis was described previously (4). The pH, dissolved oxygen (DO), sulfide, conductivity and the redox potential of groundwater were determined in the field (4). Since only trace level of nitrate was detected previously (1, 3), it was not considered in this experiment.

*DNA isolation and purification*

Community DNA was extracted from groundwater filters by combining grinding and SDS for cell lysis and purified as detailed in Zhou *et al* (35). Purification was modified by elution of DNA from

the resin column two times with 30 µl of hot water (80 °C). The purified DNA was quantified with an ND-1000 spectrophotometer (Nanodrop Inc.) and Quant-It™ PicoGreen® (Invitrogen, Carlsbad, CA).

*Sample amplification, labeling, microarray hybridization and data processing*

An aliquot of DNA (25 ng) of each sample was amplified in triplicate using the TempliPhi kit (Amersham Biosciences, Piscataway, NJ) and labeled as described previously (31, 32). Hybridizations were performed with GeoChip 2.0 (9) on an HS4800 Pro Hybridization Station (TECAN US, Durham, NC, USA) in triplicate at 45 °C for 10 hrs. Microarrays were scanned on a ScanArray5000® Microarray Analysis System (PerkinElmer, Wellesley, MA) at 95% laser power and 68% PMT (photomultiplier tube gain). Signal intensities were measured with ImaGene 6.0 (Biodiscovery Inc., El Segundo, CA, USA). Background was subtracted from all intensity data used for further analysis. Intensities of three replicates for each set of experiments were normalized with total intensities of all spots with signal-to-noise ratio (SNR; SNR = (signal intensity - background intensity)/background standard deviation) greater than 1.0. Spots with SNR <2.0 and outliers of replicates (>2 standard deviation) were removed. A gene was included in the analysis when a positive hybridization signal was obtained from >34% of the spots (generally, 9 spots for each gene) on the arrays in triplicate hybridizations. The microarray data presented are available at http://ieg.ou.edu/4download/.

*Statistical analysis*

Cluster analysis was performed using the pairwise average-linkage hierarchical clustering algorithm (5) in CLUSTER (http://rana.stanford.edu), and the results of hierarchical clustering were visualized using TREEVIEW (http://rana.stanford.edu/). Bio-Env procedure was used to select environmental variables to find the best subset of environmental variables with maximum (rank) correlation with community dissimilarities (15) in R version 2.11.1 with vegan package. Canonical correspondence analysis (CCA) was performed to identify the relationship between geochemical parameters and microbial functional genes using CANOCO for Windows Version 4.5 (25). Monte Carlo tests were used to assess the significance of the environmental variables with 999 permutations. Mantel test was performed to infer the correlation between geochemistry and

functional genes based on Euclidean distance measurement with PC-ORD (MjM Software, Gleneden Beach, Oregon, USA). The *P*-value of the standardized Mantel statistic (r) was calculated from 999 Monte Carlo randomizations. All analyses of variances (13) were performed with SPSS 13.0 (SPSS Inc. Chicago, IL) with Kruskal-Wallis test and variance homogeneity by Levene's test first.

## Results and Discussion

### *Field geochemical changes*

Geochemical analysis of the eight samples from four wells with different time points were selected for this study (acetate, U(VI), sulfate, sulfide, Fe(II), DO, pH, conductivity and redox potential) (Table 1). U(VI) concentrations in the background well and the two experimental plots were as high as 1.0 ~ 1.5 μM. There was a continuing decrease of U(VI) with acetate injection in the 2004 experimental plot, and the concentration was lowered to 0.19 μM on September 19. In the 2005 experimental plot (M21 and M24), the U(VI) concentration decreased firstly, while there was a rebound of U(VI) with a shift of subsurface redox condition from Fe-reducing to sulfate-reducing conditions (4). In the same wells, there was a loss of sulfate and an accumulation of sulfide when they were dominated by sulfate-reducing conditions.

Acetate additions were used to stimulate U(VI) reduction at the Rifle site, CO (1, 4, 28, 34). At that site, continuous U(VI) reduction was observed until dominant microbial communities shifted from Fe-reducing bacteria to sulfate-reducing bacteria, at which point U(VI) reduction slowed, ceased or rebound (1, 4). Furthermore, the greatest rate of reduction of uranium was observed under Fe-reducing conditions, consistent with previous work showing that Fe-reducing conditions were more favorable for uranium reduction in a laboratory bioreduction experiment (2).

### *Overall functional gene diversity pattern*

To track the microbial community dynamics during biostimulation, functional genes from selected samples in the unstimulated background (B05), Fe-reducing dominance (M16) and a transition from Fe-reducing to sulfate-reducing condition (M21 and M24) wells were analyzed with GeoChip 2.0. More than 1300 genes showed positive hybridization signals. Hierarchical cluster analysis of all detected functional genes was performed (Figure 2). The cluster analysis indicated that samples

in treatment wells (M16, M21, M24) and background well (B05) grouped separately, except the sample in the early days of acetate injection in well M21 (Figure 2(a)). Samples in treatment wells grouped together mainly by Fe- or sulfate-reducing conditions, consistent with the expected correlation of the dominant terminal electron accepting process with microbial functional structure (Figure 2(a)).

From the functional gene cluster results, a total of six major groups were observed (Figure 2(b)). Groups 1 and 5 represented genes in high abundance under the beginning and the end of Fe-reducing conditions, respectively. These groups mainly contained metal resistance and reduction genes such as chromium-, arsenic-, and tellurium- related genes as well as cytochrome genes. Groups 2 and 3 represented genes in high abundance in M21 7/27, which was closer to the injection well and referred to the transition from Fe-reducing to sulfate-reducing conditions, of which many sulfate-reducing genes (*dsrA* and *dsrB*) were observed. Group 3, 4, and 6 represented genes in high abundance in the background wells. These groups of genes were mainly involved in carbon degradation, nitrogen cycling and metal resistance. These results suggest that the overall functional structure of microbial communities is different with subsurface redox changes, and that they were also different from those in the background well.

The composition of microbial communities was further analyzed in nine functional categories: carbon degradation, carbon fixation, sulfate reduction, metal reduction and resistance, nitrogen fixation, nitrification, nitrogen reduction, organic contaminant remediation, and methane generation (Figure 3). Microbial functional gene patterns at the beginning and at the end of the study period in the background well were quite similar (B05 7/27, B05 9/19). Metal reduction genes increased under Fe-reducing conditions (M16) over time. A previous study also showed that metal-reducing δ-Proteobacteria increased from 5% to nearly 40% by analyzing 16S rRNA gene clone libraries in contaminated subsurface sediments (22). The stimulation of both Fe-reducing bacteria and their metal reducing related genes plays an important role in enzymatic uranium reduction.

When redox condition shifted from Fe- to sulfate-reducing conditions, an increase in the abundance of sulfate reduction genes (*dsrA* and *dsrB*) was observed in M21 and M24, respectively (Figure 3). Simultaneously, methane generation genes increased during redox transition period in both M21 and M24 (Figure 3). This transition was also observed that microbial communities shifted from predominance by metal-reducing *Geobacteraceae* populations to sulfate-reducing

*Desulfovibrionaceae* (11). It was hypothesized that the decrease in U(VI) removal efficiency was due to a loss in the metal-reducing population (i.e., *Geobacter*) with a depletion of the bioavailable Fe(III) concentration, suggesting that redox conditions should be optimized for continued growth and survival of *Geobacter* species for long-term *in situ* uranium reduction (1).

***Representatives of functional genes detected under Fe- and sulfate-reducing conditions***

*(i) Cytochrome genes for metal reduction*

Biogeochemical and genetic studies both indicate that c-type cytochromes are required for U(VI) reduction by a range of microorganisms including *Desulfovibrio* sp. (18), *Geobacter* sp. (23), and *Shewanella oneidensis* MR-1 (19). Here, we examine the cytochrome genes detected during uranium reduction under Fe-reducing conditions and transition from Fe-reducing to sulfate-reducing conditions, as visualized by clustering analysis (Figure 4). The cytochrome genes showed significant correlations ($r = 0.71$, $P = 0.016$) with uranium concentrations based on the Mantel test. And cytochrome genes decreased during subsurface redox condtions shifted from Fe-reducing to sulfate-reducing conditions, consistent with the hypotheses that c-type cytochromes are directly involved in U(VI) reduction and that a decrease of U(VI) in groundwater is greatest under Fe-reducing conditions. The c-type cytochrome genes appear to be derived mainly from *Geobacter* sp. and *Desulfovibrio* sp., and major groups of cytochrome genes across all samples were from *Geobacter sulfurreducens* (Figure 4), whose c-type cytochromes have been shown to be involved in extracellular uranium reduction (23).

*(ii) dsrA/B for sulfate reduction*

To examine the shift of a microbial community composition dominated by sulfate reducers in more detail, total *dsr* genes were analyzed. In well M16, the number of *dsr* genes decreased when it was driven to Fe-reducing conditions, while in wells M21 and M24, it increased with shift to sulfate-reducing conditions (Table S1). The diversity increased in well M21 during sulfate reduction, most of the genes (95%) could be found in samples collected before and after acetate injection (Table S2). Also, *dsr* genes in M21 showed a high similarity with those in the background well (92%-97%). Hierarchical clustering was performed to assess the *dsr* gene patterns (Figure S1). Compared to the Fe-reducing conditions, an obvious increase of *dsr* genes was detected when the

subsurface was dominated by sulfate reduction. The *dsr* genes came from both cultured bacteria (e.g., *Desulfomonile* sp., *Desulfosporosinus* sp., *Desulfotomaculum* sp., *Desulfovibrio* sp., *Desulfovirga* sp. and *Chlorobium* sp.) and uncultured sulfate-reducing bacteria in well M21 at the late sampling date (8/10/06), when the sulfide concentration was highest. Several *dsr* genes from uncultured sulfate-reducing bacteria similar to *Desulfosporosinus* sp. and *Pelotomaculum* sp. were abundant in all samples.

*(iii) mcr for methane generation*

In the background well, *mcr* genes were mainly derived from uncultured archaeon and *Methanothermobacter* sp. (Figure S2). An increase of total signal intensity of methane generation genes (*mcrA*, *mcrG*, *mcrC, mcrD*) were observed in the well M21 7/27 in comparison to the background well (B05). Methane concentrations would need to be measured during biostimulation to confirm this. The increasing *mcr*-containing populations detected included primarily *Methanoculleus* sp., *Methanocorpusculum* sp., *Methanocaldococcus* sp., *Methanothermobacter* sp., *Methanopyrus* sp., and uncultured archaea.

*(iv) nirS/K, and nifH for nitrogen cycling*

Functional genes *nirS*, *nirK* and *nifH* coding key enzymes involved in denitrification and nitrogen fixation, respectively were examined. The nitrogen reducing genes, *nirS* and *nirK* genes, were present under both redox conditions as well as in the background well (Figure S3). It has been recognized that nitrogen reducing bacteria are essential for the removal of nitrate to creat the low-redox conditions favorable for U(VI) reduction (26, 7). Since only trace level of nitrate was detected in the Old Rifle site (1, 3), no clear relationship between nitrogen reducing genes and the redox conditions was observed in this study. These genes were likely derived from populations of *Pseudomonas* sp., *Sinorhizobium* sp., *Nitrosomonas* sp., *Ochrobactrum* sp., and *Paracoccus* sp., yet the majority of the nitrogen cycling genes was from uncultured bacteria. Similarly, most of the *nifH* genes observed were from uncultured bacteria (Figure S4).

*(v) Carbon degradation related genes*

A variety of carbon degradation genes were detected in the samples (Figure S5). The functional genes for cellulase from *Leuconostoc* sp., chitinase from *Salinivibrio* sp., *Burkholderia* sp., and

laccase from *Trametes* sp., were detected across all samples. Additionally, genes for cellulase from *Clostridium* sp. and *Gluconacetobacter* sp., chitinase from *Serratia* sp., *Xanthomonas* sp., and *Burkholderia* sp., and polygalacturonase gene (*pgl*) from *Penicillium* sp., were highly abundant in the background well. In the Fe-reducing well, genes for cellulase from *Neurospora* sp., *Reticulitermes* sp., *Clostridium* sp., *Xanthomonas* sp., and *Fusarium* sp., chitinase from *Aeromonas* sp., laccase from *Trametes* sp., *Basidiomycete* sp., and *pgl* from *Xanthomonas* sp., were also highly abundant. In the sulfate-reducing well, the cellulase genes from *Clostridium* sp., *Fusarium* sp., *Halobacterium* sp., and *Gluconacetobacter* sp., chitinase genes from *Serratia* sp., *Xanthomonas* sp., and *Microbulbifer* sp., mannanase gene from *Cellvibrio* sp., and *pgl* from *Xanthomonas* sp., were in a high abundance. Several different species of *Clostridium* have been shown to reduce U(VI) to U(IV) to various degrees (2, 24) and the hypothesis was proposed that U(VI) reduction occured through hydrogenases and other enzymes (8). However, hydrogenase genes derived from *Clostridium* sp.were not contained on the GeoChip and further work is required to confirm the mechanism of bioreduction of U(VI) by *Clostridium* sp. Detrital organic matter that is locally abundant at the Rifle site is probably the principal source of carbon compounds sustaining this part of the microbial community.

***Linking geochemistry and microbial community structure***

The responses of the microbial community to subsurface redox conditions can be very different based on the local environmental conditions and the biostimulation operations (i.e., exogenous substrate injection) (26). Bio-Env procedure was used to find the best subset of environmental variables with maximum (rank) correlation with community dissimilarities and the result indicated that acetate, U(VI) and $E_h$ had the highest correlation coefficient (r = 0.579). Canonical correspondence analysis (CCA) was performed to discern possible linkages between geochemical parameters and microbial community functional structure (Figure 5). Only geochemical parameters that were significant were included in the CCA biplot (acetate, U(VI), sulfate, sulfide, Fe(II), $E_h$), based on a forward selection procedure and variance inflation factors with 999 Monte Carlo permutations. For all functional genes, 37.1% of the total variance could be explained by the first two constrained axes with the first axis explaining 19.5%. The specified model was significant (first axis, $P = 0.019$; all axes, $P = 0.040$). The CCA results reflected the microbial functional

distributions along the environmental variables such as U(VI), acetate, $E_h$, sulfate,sulfide and Fe(II) (Figure 5). B05 samples distributed in the area with highest U(VI). In the Fe-reducing well (M16), the change of microbial structure was correlated with $E_h$. With continuous decrease of $E_h$ and accumulation of sulfide, a clear shift of microbial structure was observed with transition from Fe-reducing to sulfate-reducing conditions (wells M21 and M24, from 7/27 to 8/10). The results here indicated that changes of microbial functional diversity were strongly related to the dominant terminal electron accepting process following acetate addition.

In summary, this study characterized the functional structure and dynamics of microbial communities under Fe-reducing and sulfate-reducing conditions during *in situ* U(VI) bioreduction. Analysis of key functional genes indicated a shift of functional potential of microbial communities in response to the transition from Fe-reduction to sulfate reduction as the dominant terminal electron accepting process. As a result, an increase of *dsr* and *mcr* genes was detected when the subsurface was dominated by sulfate-reducing conditions. Also, the c-type cytochrome genes detected were primarily from *Geobacter* sp. and *Desulfovibrio* sp. and decreased during subsurface redox condtions shifted from Fe-reducing to sulfate-reducing conditions. Overall, microbial functional structure is sensitive to changes in subsurface redox conditions, indicating that tracking microbial functional genes could be very useful for tracking microbial community structure and dynamics during bioremediation.

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## TABLE AND FIGURE LEGENDS

Table1 Groundwater geochemical data of samples selected for GeoChip analysis

Figure 1 Layout of the experimental plots at the Old Rifle uranium mill tailings site. Each plot had 5 injection wells (open circles) perpendicular to groundwater flow, 4 monitoring wells (filled circles) down-gradient of acetate injection, and 1 monitoring well positioned upgradient of the injection wells (filled triangle). The 2004 experimental plot was maintained in Fe-reducing conditions and the 2005 experimental plot was driven to sulfate-reducing conditions by using different durations of biostimulation.

458 Figure 2 Hierarchical cluster analysis of all functional genes detected (a). Genes that were present
459 in at leat three time points were used for cluster analysis. Results were generated in CLUSTER and
460 visualized using TREEVIEW. Red indicates signal intensities above background while black
461 indicates signal intensities below background. Brigher red colourng indicates higher signal
462 intensities. A total of 6 major groups were observed (b). The numbers equal groupings found
463 among the hybridization patterns.

464 Figure 3 Functional gene abundance in background well (B05), Fe-reducing well (M16) and shift
465 from Fe-reducing to sulfate-reducing wells (M21 and M24). The abbreviations are as follows: Org,
466 Organic contaminant degradation; Nred, Nitrogen reduction; Nit, Nitrification; Nfix, Nitrogen
467 fixation; Methane, Methane generation; Met, Metal reduction and resistance; DSR, Sulfate
468 reduction; Cfix, Carbon fixation; Cdeg, Carbon degradation.

469 Figure 4 Hierarchical clustering of c-type cytochrome genes. Red indicates signal intensities above
470 background while black indicates signal intensities below background. Brigher red colourng
471 indicates higher signal intensities. The bar of colors below the sample names indicates the average
472 signal intensities of each sample. Mantel test results indicated significant correlations between the
473 functional gene patterns and the U(VI) concentration ($P < 0.05$).

474 Figure 5 Canonical correspondence analysis (CCA) of total functional genes and geochemical data.

Table 1 Groundwater geochemical data of samples selected for GeoChip analysis

| | Acetate (mM) | U(VI) (μM) | Sulfate (mM) | Sufide (mM) | Fe(II) (mM) | DO (mg/L) | pH | Conductivity (us/cm) | $E_h$ (mV) |
|---|---|---|---|---|---|---|---|---|---|
| B05 7/27/06 | 0.00 | 0.91 | 6.62 | 0.000 | 0.063 | 0.33 | 7.04 | 2047 | 148 |
| B05 9/19/06 | 0.00 | 1.00 | 7.52 | 0.000 | 0.018 | 0.32 | 6.92 | 2190 | 94 |
| M16 9/5/06 | 2.34 | 0.52 | 7.38 | 0.003 | 0.0246 | 0.36 | 6.87 | 2431 | 84 |
| M16 9/19/06 | 4.44 | 0.19 | 7.34 | 0.010 | 0.034 | 0.33 | 6.92 | 2659 | 11 |
| M21 7/27/06 | 6.87 | 0.44 | 7.21 | 0.009 | 0.124 | 0.16 | 7.08 | 2936 | 33 |
| M21 8/10/06 | 2.30 | 0.36 | 6.54 | 0.114 | 0.038 | 0.11 | 7.21 | 2465 | -129 |
| M24 7/27/06 | 2.10 | 0.24 | 7.05 | 0.004 | 0.127 | 0.1 | 7.02 | 2383 | 40 |
| M24 8/10/06 | 0.83 | 0.50 | 5.80 | 0.096 | 0.013 | 0.2 | 7.19 | 2429 | -141 |

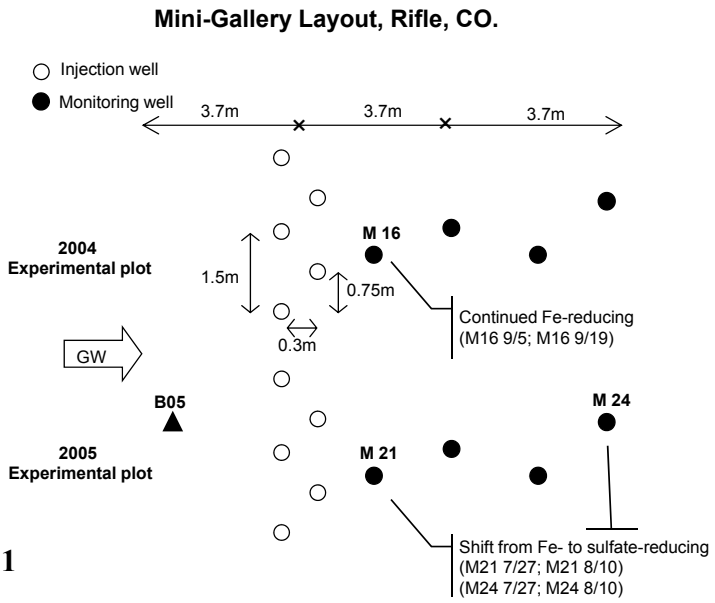


**Figure 1**

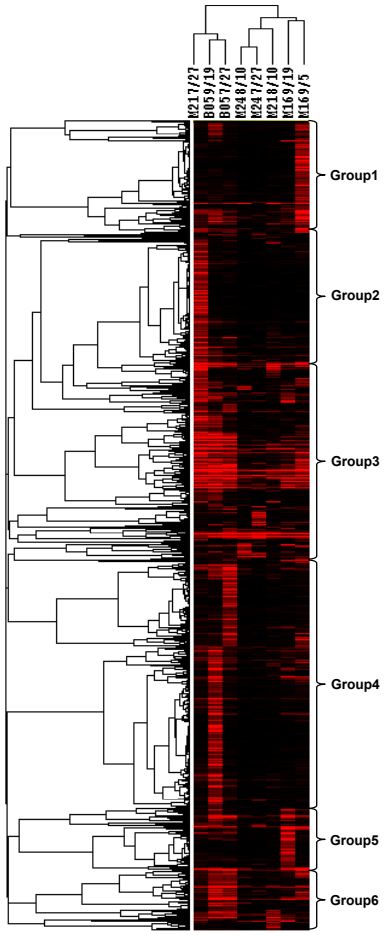


**Figure 2 (a)**

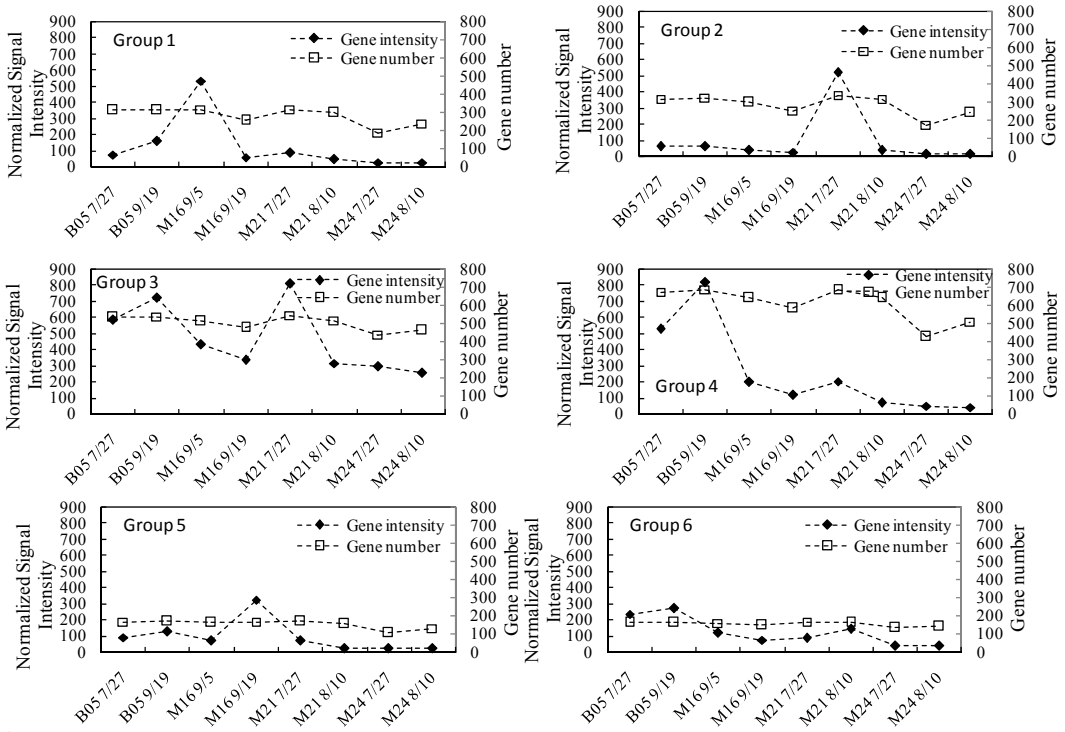


**Figure 2 (b)**

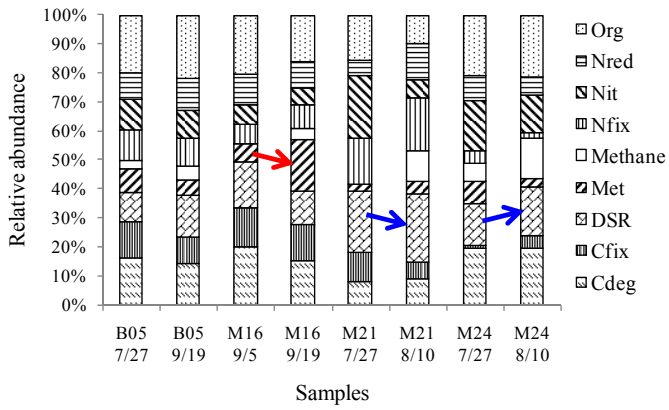


**Figure 3**

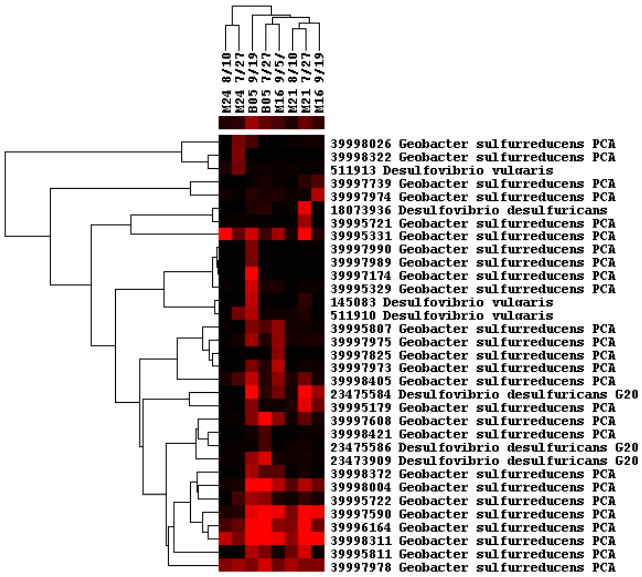


**Figure 4**

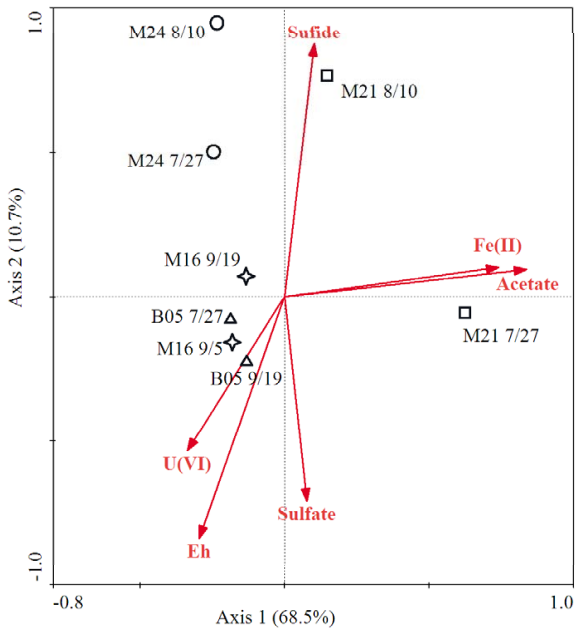


**Figure 5**