

Profiling of microbial community structure with depth and across physico-chemical gradients in the uranium-contaminated subsurface

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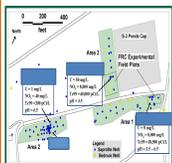
Abstract

The subsurface at the Oak Ridge Integrated Field Research Center (OR-IFRC, Oak Ridge, TN) is contaminated with a mixture of organic and inorganic materials as a legacy of cold war era uranium enrichment. The contaminants of greatest concentration and mobility, and therefore concern, are uranium and nitrate. Tracking and modeling the distribution and fate of contaminants in the subsurface has been impeded by the highly complex subsurface hydrogeology, geochemistry and microbiology. To better understand the relationship between subsurface geochemistry and microbiology, we analyzed multiple depth intervals in OR-IFRC sediment cores from the most highly contaminated zone (Area 3) and from a downgradient and less contaminated location (Area 2). In Area 2, the sediment core was collected from the saturated zone at 5.5 -6.5 m below the surface and subsampled into 6 depths. In Area 3, a larger depth range was analyzed – from depths of 1-15 meters (at 1-1.5 meter intervals) corresponding to roughly 9 meters of fill (shale/saprolite or gravel/saprolite) and fractured saprolite below. Six to eight subsections of each core were analyzed by geochemical methods for pH, sulfate, nitrate, Fe(II), and total Fe and by molecular analyses of the microbial community. The bacterial community composition was also assayed by terminal restriction fragment length polymorphism (TRFLP). Less variation in geochemical parameters and a higher pH of 5-6 was observed in the Area 2 subsurface sediments sampled farther from the contaminant source zone. Approximately 38,000 short sequences (205 bp) were recovered from six depth intervals using a deep sequencing approach. Analysis of these data revealed the dominant presence of bacteria from the phylum *Acidobacteria*, while *Proteobacteria*, *Verrucomicrobia*, *Firmicutes*, *Chloroflexi*, and *Actinobacteria*, were also common. Distinct geochemical and contaminant gradients were observed with depth close to the contaminant source zone in the Area 3 borehole sequence that paralleled with changes in microbial community structure. Nitrate and uranium concentrations increased dramatically with depth, while the pH was highly acidic (<4.0) at all depths except for the top and lowermost depths. Clone libraries of bacterial 16S rRNA genes were generated for 7 depth intervals from Area 3 cores, and diversity and phylogenetic analyses revealed a strong effect of pH on microbial community structure. Acidic samples contained a less diverse microbial community dominated largely by *Rhodanobacter* or *Bradyrhizobium* spp. The limited bacterial diversity in the deeper subsurface of Area 3 selected almost exclusively for key taxa, resulting in limited overlap in microbial community structure between Area 2 and Area 3 sediments. The abundant presence of these unique sequences suggests adaptation of bacterial populations to pH and contaminant (nitrate and uranium) conditions. Conversely, pyrosequencing of Area 2 sediment reveals a highly diverse microbial community with roughly 4,000 putative species (operational taxonomic units, OTU) detected in 1 meter of sediment.

Objectives

- To comprehensively interrogate the microbial community structure of OR-IFRC subsurface sediments across key physico-chemical gradients using a combination of deep sequencing and community fingerprinting.
- To understand the role of sediment geochemistry in defining structure-function relationships of the subsurface microbial community.
- Develop a predictive model based on microbial community structure and geochemical conditions to track the fate of nitrate in the contaminated subsurface.

Subsurface Characteristics

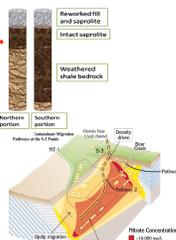


Area 2

- Shallow pathway for contaminant migration.
- Depth of groundwater – 4.5m.
- Geology: 6m of reworked fill and saprolite underlain by 2m of intact saprolite with weathered bedrock.

Area 3

- Located west of 5-3 ponds.
- Depth of ground water – 3.5m.
- Geology: 1m of reworked fill and saprolite and ~ 2.16m of intact saprolite with weathered shale bedrock. Thickness of saprolite in the southern portion is about 15.6 m thick but only about 2-3m in the northern portion. Hydraulic conductivity of the shallow saprolite is fairly low. However, the permeability of the deeper saprolite (9-15.6m) in the southern portion is higher. This deep saprolite zone is probably a preferential pathway for contaminants.

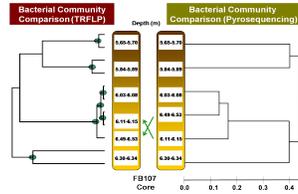


Subsurface Area 2 Sediment Chemistry (FB107)

Sample	Depth (m)	pH	Nitrate (mM)	Sulfate (mM)	Fe(II) (μmol/g)	total Fe (μmol/g)	Fe(III) (μmol/g)
02-19A	5.65 - 5.70	6.39	0	0.447	3.37	22.65	19.28
02-19B	5.84 - 5.89	6.26	0	0.569	18.37	21.55	3.18
02-19C	6.03 - 6.08	6.15	0	0.649	1.58	60.08	58.50
03-00A	6.11 - 6.15	5.70	0	0.775	0.82	21.20	20.38
03-00B	6.30 - 6.34	5.56	0	0.758	0.34	8.72	8.38
03-00C	6.49 - 6.53	5.31	0.26	0.770	0.91	34.06	33.15

Variation in the geochemistry was observed across this span of the subsurface. Extractable Fe(II) concentrations are higher in upper depths.

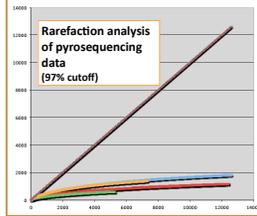
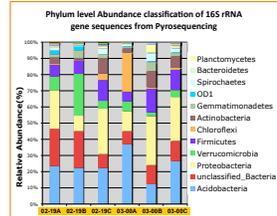
Depth Profile of Microbial Community Structure



Comparison of depth-related shifts in bacterial community structure as assayed using two independent molecular analyses. On the left, a dendrogram of the relationship of bacterial community structure in the Area 2 sediment core using sequence-independent (terminal restriction fragment length polymorphism, TRFLP). On the right, a dendrogram based on relative abundance of operational taxonomic units (OTU, 97% cutoff) identified from pyrosequencing data recovered from the same samples (see below).

Characterization of Sediment Bacterial Communities

Sequences affiliated with the phylum *Acidobacteria* were the most abundant taxon detected in this study (25%), and for each depth represented >10% of the library. *Proteobacteria*, *Firmicutes*, and *Actinobacteria* were also abundant, as were *Verrucomicrobia* (particularly at a depth of 5.85-5.89 m). Rarefaction analysis and statistical analyses (see Diversity, below) indicate that the sediment was well sampled, but that additional sequencing would be required to acquire rare OTUs.



Bacterial Diversity in Contaminated Subsurface Sediment Samples

	Total	02-19A	02-19B	02-19C	03-00A	03-00B	03-00C
Number of Sequences	39634	12607	12411	1439	5292	568	7317
Number of OTU	3871	1800	1163	382	596	142	1375
Shannon-Weiner Index	6.98	6.26	4.93	5.43	4.00	4.77	5.94
Chao1 richness estimate	5536	2536	1918	672	1027	407	1902
Chao1 Percent Coverage	70%	71%	61%	57%	58%	35%	72%
Percent Coverage (97%; Good's Method)	96%	94%	96%	77%	94%	64%	92%

All statistical analyses were conducted using 97% identity as a threshold for OTU determination.
 * Value in parentheses represents upper and lower 95% confidence intervals.
 † Values in parentheses represent lower and upper 95% confidence intervals, respectively.

These data indicate that tremendous bacterial diversity is present in a very short sediment column, and that it varies significantly from depth to depth. These analyses also reveal a strong sensitivity of sampling size on calculated and estimated diversity, and indicate that even 12,000 sequences is insufficient to fully characterize the bacterial community in such samples. Much of this diversity represents organisms of unknown function.

Summary

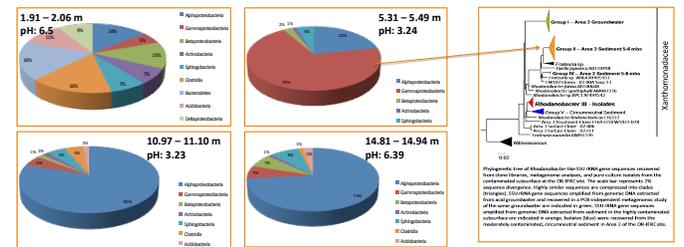
- Significant variation in the geochemistry, particularly with respect to the pH and extractable Fe(II).
- Approximately 38,000 short sequences (205 bp) recovered from six depths (5-6 mbs).
- Dominant phylum was *Acidobacteria* (12.3-36.9%), while *Proteobacteria* (9.6-30.2%), *Verrucomicrobia* (4.9-25.8%), *Firmicutes* (5.9-14.6%), *Chloroflexi* (0.5-24.1%), and *Actinobacteria* (0.7-9.9%) were also common.
- Data indicates a high overall diversity (~ 4,000 operational taxonomic units (OTUs) at a level of 97% sequence identity), with the most diverse sample containing 1,800 OTUs.
- Deep sequencing results support and expand upon community fingerprinting and analysis.
- To our knowledge, this is the first pyrosequencing study to examine microbial community structure across a geochemical gradient, and represents an important step towards characterizing the complex subsurface at the OR-IFRC.

Subsurface Area 3 Sediment Chemistry (FWB124)

Sample	Depth (m)	pH	Nitrate (mM)	Sulfate (mM)	Fe(II) (μmol/g)	total Fe (μmol/g)	Fe(III) (μmol/g)	U (ppm)	Nucleic Acid Content (ng/g)
02-27	1.91 - 2.06	6.50	0.802	1.227	0.47	11.05	10.57	4.76	2776
06-00	4.57 - 4.70	2.94	0.000	0.507	0.33	25.17	24.84	20.7	124
06-45	5.31 - 5.49	3.24	0.144	0.593	0.46	16.54	16.08	31.86	30
10-26	7.32 - 7.44	3.28	5.863	2.357	0.58	21.62	21.04	16.92	44
14-00	9.75 - 9.88	4.00	7.769	1.416	2.48	184.20	181.72	18.69	52
16-30	10.97 - 11.10	3.23	8.289	2.339	0.57	8.98	8.41	16.88	346
19-22	13.28 - 13.41	3.21	3.922	1.543	0.41	11.05	10.64	196.64	88
23-07	14.81 - 14.94	6.39	32.038	4.799	0.43	10.99	10.56	171.98	106

The Area 3 core represents a much more dramatic geochemical gradient across a larger spatial scale. The core was taken from the most highly contaminated zone, and for much of the core the pH is highly acidic. In general, contaminant concentrations (i.e. nitrate and uranium) increase with depth, while the nucleic acid content decreases with depth.

Depth Distribution of Dominant Taxa (16S rRNA gene clone libraries)



As a prelude to pyrosequencing, clone libraries were generated from a number of depths in the FW124 borehole sequence. These libraries show a stark transition in microbial community structure, much more salient than in the Area 2 core. The surface sediment, with a circumneutral pH, relatively low contaminant presence, and likely access to terrestrial organic carbon, contains the greatest nucleic acid content and microbial diversity. Below this depth, the microbial communities are predominantly composed of a single taxon representing 70% of more of the total clone library. At a depth of 5.4m, the clone library is dominated by sequences affiliated with the genus *Rhodanobacter* within the subphylum *Gammaproteobacteria*. In the lowest two depths, the libraries are dominated by sequences affiliated with the genus *Bradyrhizobium* within the subphylum *Alphaproteobacteria*. We have isolated *Rhodanobacter* from circumneutral sediments and demonstrate that these organisms are denitrifiers. See the Kostka et al. poster (board #26, on wed) for further information on subsurface isolates. We are currently working to isolate the acidophilic representatives of this subsurface clade. Members of the genus *Bradyrhizobium* have also been shown to denitrify, suggesting the importance of this process in the acidic subsurface of the OR-IFRC.

Bacterial Diversity in Contaminated Subsurface Sediment Samples

	Total	02-27	06-45	16-30	23-07
Number of Sequences	325	57	73	57	54
Number of OTU	138	49	10	13	17
Shannon-Weiner Index	4.17 (0.16)	3.80 (0.43)	1.35 (0.62)	2.00 (0.54)	2.37 (0.54)
Chao1 richness estimate	526 (342/877)	238 (123/533)	10 (10/16)	21 (14/56)	62 (31/165)
Chao1 Percent Coverage	26%	21%	100%	62%	27%
Percent Coverage (97%; Good's Method)	69%	23%	97%	89%	81%

All statistical analyses were conducted using 97% identity as a threshold for OTU determination.
 * Value in parentheses represents difference between upper and lower 95% confidence intervals.
 † Values in parentheses represent lower and upper 95% confidence intervals, respectively.

Summary

- Distinct geochemical and contaminant gradients as a function of depth.
- Phylogenetic analyses revealed a strong effect of pH on microbial community structure, but the shift from *Rhodanobacter* to *Bradyrhizobium* indicates that other factors are involved in determining microbial community composition. Nitrate levels are much lower in the depth dominated by *Rhodanobacter*.
- Low pH samples are dominated by single taxa. The low diversity and biomass of these contaminated, acidic depths indicate the extreme hostility of the subsurface environment.
- The dominant presence of putative denitrifying bacteria in the acidic subsurface further indicates the utility of studying this critical functional group in the OR-IFRC subsurface. The isolation of acidophilic or acidotolerant denitrifying bacteria from these samples is a major aspect of our continued investigation of the subsurface.

Acknowledgements

This research is supported by the Office of Science (BER), U.S. DOE, Grant No. DE-FG02-07ER64373. We thank Phil Jardine, David Watson, and the staff of the Integrated Field-Scale Subsurface Research Challenge at Oak Ridge for their collaboration.