Q-2348 Effects of nitrate exposure on the functional structure of a microbial community in a uranium-contaminated aquifer

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Ecosystems and Networks Integrated with Genes and Molecular Assemblies

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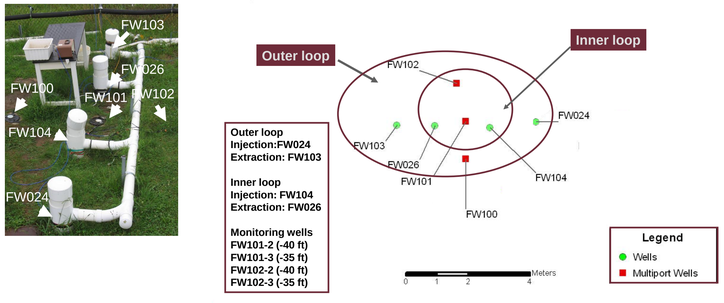
Abstract

A pilot-scale system at the U.S. DOE's Field Research Center in Oak Ridge, TN was established for biostimulation of subsurface U(VI) reduction by injection of ethanol. The system was able to reduce U(VI) to a level below EPA drinking water standards. In this study, the impact of nitrate exposure on the microbial community was examined. Introduction of nitrate to the reduced area resulted in U(IV) oxidation but subsequent removal of the nitrate and injection of ethanol led to a reduction of the oxidized U(VI). Geochip 3.0, a comprehensive 50mer microarray containing probes for genes involved in the geochemical cycling of N, S, C, and P, metal resistance, contaminant degradation, and antibiotic resistance was used to monitor the dynamics of the groundwater microbial community structure and function before, during, and after nitrate exposure. After exposure to nitrate the diversity and richness increased several fold but quickly returned to pre-nitrate levels. Detrended correspondence analysis of all detected genes indicated that some community structure changes occurred during the nitrate exposure, although a larger shift occurred after the communities had not been fed ethanol for 54 days. Ammonification genes (ureC) increased immediately after nitrate exposure and then decreased. Most of the denitrification genes (nirS, narG, norB, and nosZ) showed a similar pattern of increase after nitrate exposure as well. Nitrogen fixation genes (nifH) exhibited the greatest increase in relative abundance, more than doubling after nitrate exposure and then slowly decreasing over the subsequent time points. Nitrogen reduction genes (nasA and nrfA) decreased following exposure to nitrate. Canonical correspondence analysis was used to determine the most significant environmental variables controlling the microbial community structure. The three variable used in the final model were COD, iron, and sulfate (p=0.020; f-ratio=1.601), which explained 66.3% of the variation observed. These results demonstrate that while introduction of nitrate caused a shift in the community structure and composition, the community did recover once nitrate was removed.

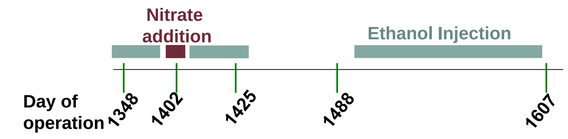
Methods

S-3 Waste Ponds. The four unlined S-3 waste collection ponds were constructed in 1951 (left). Effluent waste, consisting primarily of nitric acid, nitrate, metals, and radionuclides (U, Tc), were discharged into the ponds until 1983. The ponds were neutralized and denitrified in 1984 and then capped in 1988. The site is currently covered with asphalt and serves as a parking lot (right). Waste from the ponds seeped into the groundwater and has contaminated the surrounding area, resulting in a site with low pH (3.4-3.6), high U (50 mg L^{-1}), and high nitrate (8-12 g L^{-1}). (Oak Ridge Field Research Center, 2007).



Groundwater recirculation system. The Stanford-ORNL project, located adjacent to the S-3 ponds, was started to examine the feasibility of *in situ* bioremediation of contaminated groundwater. The system consists of two injection and two extraction wells and several monitoring wells in a nested design. An above ground treatment system was used to reduce nitrate and other contaminants in the groundwater and treated/clean water was reinjected to further reduce the contaminants within this system. Ethanol was injected intermittently to serve as an electron donor and promote reduction of residual nitrate and immobilize U. Concentrations of U were reduced to below EPA drinking water standards (30 µg L^{-1}). This study examined changes in the functional community of FW101-2 and FW102-2 when nitrate was introduced to the system.

Results



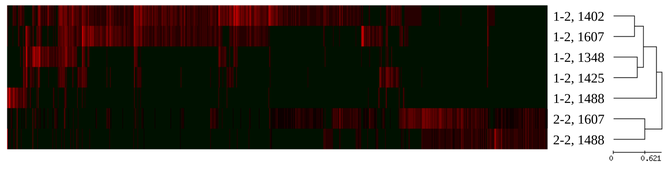
Geochemical Analysis of Groundwater Samples in FW101-2 and [FW102-2] (µM)

Day #	COD[a]	Sulfate	Sulfide	Iron	pH	Nitrate	U(VI)	Nitrite	NH_4-H
1348	101	547	689	14	6.2	1	0.065	0	20
1402	0	1680	0	23	6.1	2042	0.936	56	51
1425	67	1229	0	21	6.6	362	3.602	66	46
1488	0 [0]	1694 [1687]	0 [0]	0 [0]	6.1 [6.4]	13 [22]	0.397 [0.485]	0 [0]	0 [0]
1607	3 [4]	1136 [1007]	41 [38]	14 [30]	6.2 [6.2]	13 [5]	0.259 [0.140]	0 [0]	10 [0]

[a]COD, chemical oxygen demand – a measure of ethanol concentration

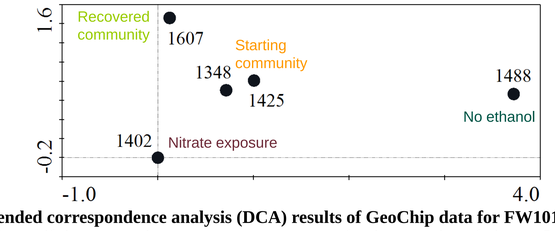
Overlap of Genes Detected by GeoChip Analysis

Sample name	1348	1402	1425	1488	1607	102-2 1488	102-2 1607
1348	X	17.67%	28.03%	14.47%	19.77%	3.22%	5.69%
1402			15.06%	6.12%	57.61%	7.49%	28.39%
1425				16.49%	17.54%	3.04%	4.61%
1488					7.84%	2.39%	3.27%
1607						4.71%	12.14%
102-2 1488							44.18%
102-2 1607							X
Richness	250	1135	225	114	780	487	864
Shannon diversity	5.10	6.60	5.06	4.17	6.22	5.72	6.26



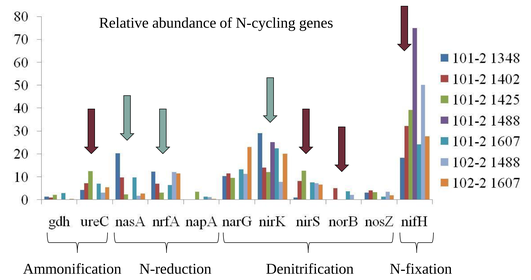
Hierarchical cluster analysis of all genes detected by GeoChip for FW101-2 and FW102-2. Trees were created with CLUSTER and visualized with TREEVIEW. Red indicates a positive hybridization; brightness indicates signal intensity. Black areas represent no hybridization above background level. 1-2, FW101-2; 2-2, FW102-2; numbers indicated operational day.

FW101-2 and FW102-2 show very different community profiles, indicating different community composition in each well.

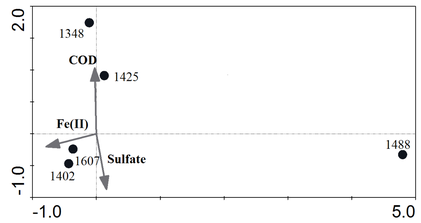


Detrended correspondence analysis (DCA) results of GeoChip data for FW101-2. A transient shift in community structure was observed after introduction of nitrate. Lack of ethanol had a much greater impact on the community structure, but after ethanol was restarted, the community recovered and was more similar to the original community.

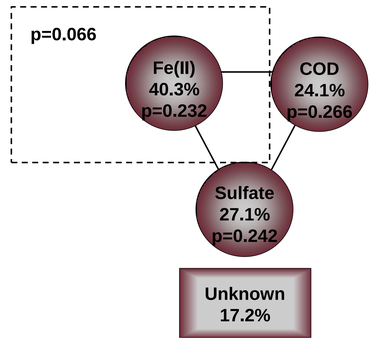
Results



Relative abundance of N-cycling genes. The relative abundance of individual N-cycling genes were calculated by dividing the signal intensity of individual genes by the total signal intensity for all N-cycling genes. Results indicate that nitrate exposure resulted in an enrichment (red arrow) of denitrification and N-fixation genes and a decrease (blue arrow) in genes involved in nitrate reduction to ammonium.



Canonical correspondence analysis (CCA) of GeoChip and geochemistry data. CCA ordination plots indicated that Fe(II), COD, and sulfate were the most important geochemical variables in determining community structure. This model was significant (p=0.020) and explained 66.3% of the total variation observed (41.5% axis 1; 24.8% axis 2). A significant model could not be obtained when data for FW101-2 and FW102-2 were combined.



Variance partitioning of environmental variables analyzed by CCA. The diagram represents the relative effects of each variable upon the functional community in FW101-2. The circles represent the effect of individual variables, by partitioning out the effects of the other variables. The square at the bottom represents the effect that could not be explained by any of the variables tested. The dashed line indicates the significance of the total influence of Fe, including overlapping influence. Variables used in CCA were used for the VPA. p-values shown were generated during partial CCA.

Summary

Introduction of nitrate caused an increase in U(VI) concentrations indicating the reoxidation of the reduced U(IV).

Clustering results indicated that communities in the two sampling wells were quite different.

A transient shift in community structure was observed after nitrate exposure. However, a larger shift was observed in the absence of ethanol addition compared to that observed in the presence of nitrate.

During nitrate exposure and immediately following cessation of nitrate, an increase in abundance of genes involved in denitrification and nitrogen fixation was noted concurrently with a decrease in genes involved in nitrate reduction to ammonium.

COD, Fe(II), and sulfate had the most influence on the community structure with Fe having the greatest influence.

Overall, nitrate did have an effect on the microbial community at this site but lack of ethanol had a much greater effect. Both system permutations were transient and communities returned to structures similar to that of the original community.

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