Polyphasic characterization of microbial communities under the stressful conditions of nitrate, heavy metals, radionuclides, and acidic pH in contaminated groundwater

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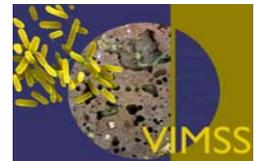
S-3 Source Ponds at the NABIR-FRC

Background at the NABIR-FRC
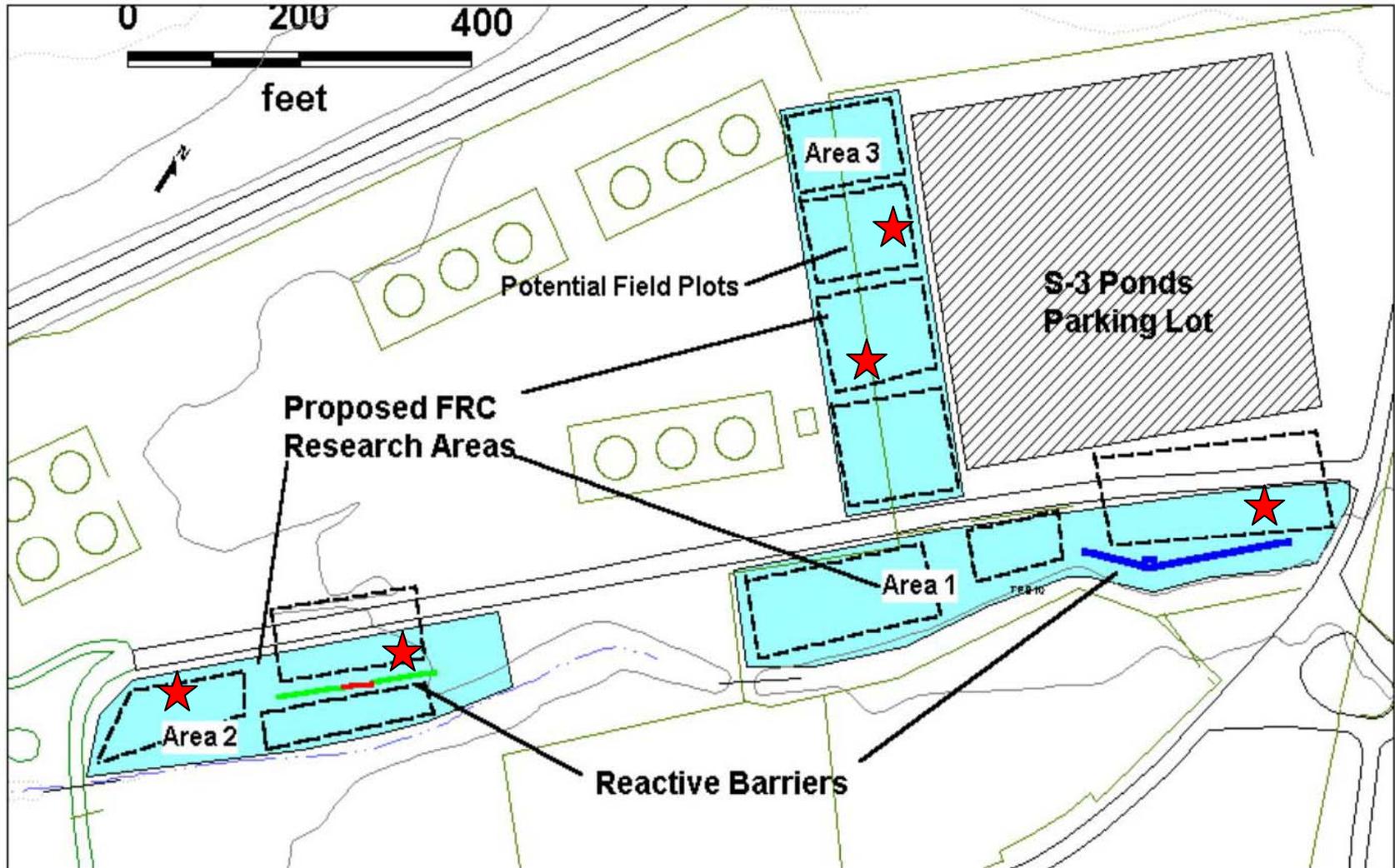
NABIR-Field Research Center
http://www.esd.ornl.gov/nabirfrc/)

FRC Groundwater Geochemistry

Well	pH	nitrate[a] (mM)	uranium[b] (μM)	nickel[c] (μM)	aluminum[c] (mM)	sulfate[c] (mM)
FW-300	6.1	0.02	ND	0.85	0.01	0.06
FW-005	3.9	6.27	27.0	84.3	1.74	0.15
FW-010	3.5	713	0.71	322	41.5	2.24
FW-015	3.4	173	32.4	147	22.9	1.02
TPB-16	6.3	0.48	4.62	ND	0.01	8.03
FW-003	6.0	17.1	0.04	0.26	0.02	0.17

[a] nitrate was determined via ion chromatography

[b] uranium was determined via ICP-mass spectroscopy

[c] nickel and aluminum were determined via ICP

Geochemistry

PCA of Major Geochemical Measures (n=6)
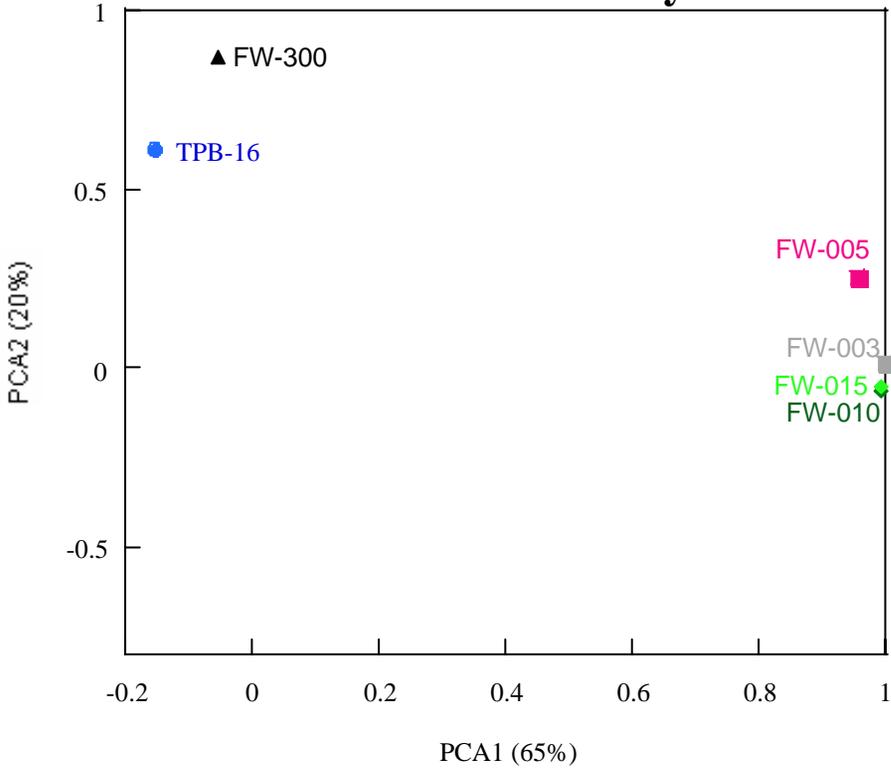
PCA of Geochemical Measures (n=107)

Geochemistry
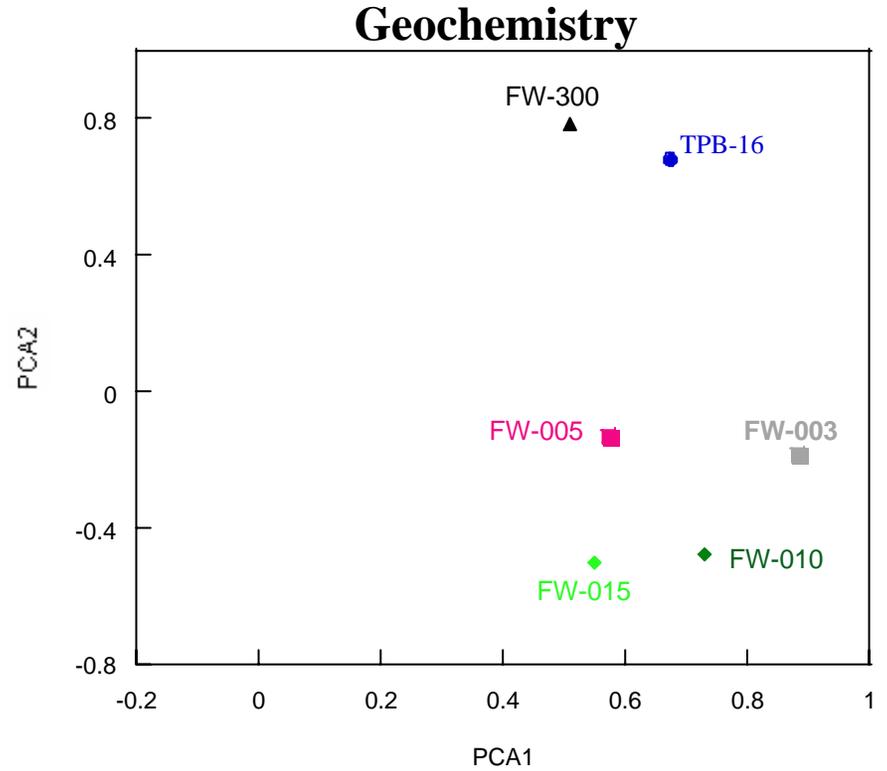
Diversity based on SSU 16S rDNA clonal library and partial sequences

	FW-300	FW-003	FW-005	FW-010	FW-015	TPB-16
Clones screened	320	320	210	115	230	435
Unique OTUs	95	35	30	30	50	205
H'	5.3	3.0	3.1	3.1	3.8	6.7
1/Simpson's	21	3.9	4.5	4.3	7.1	48
Evenness	0.8	0.6	0.6	0.7	0.6	0.8
$\triangle C_{xy}{}^{*}$	-	4.81	8.61	3.52	5.18	13.6

(* $p = 0.001$)

PCA of SSU rDNA Gene OTU Distribution

* *Acidovorax* sp.
* *Arthrobacter* sp.
* Uncultivated α-*Proteobacterium*

Groundwater Based Upon SSU rDNA
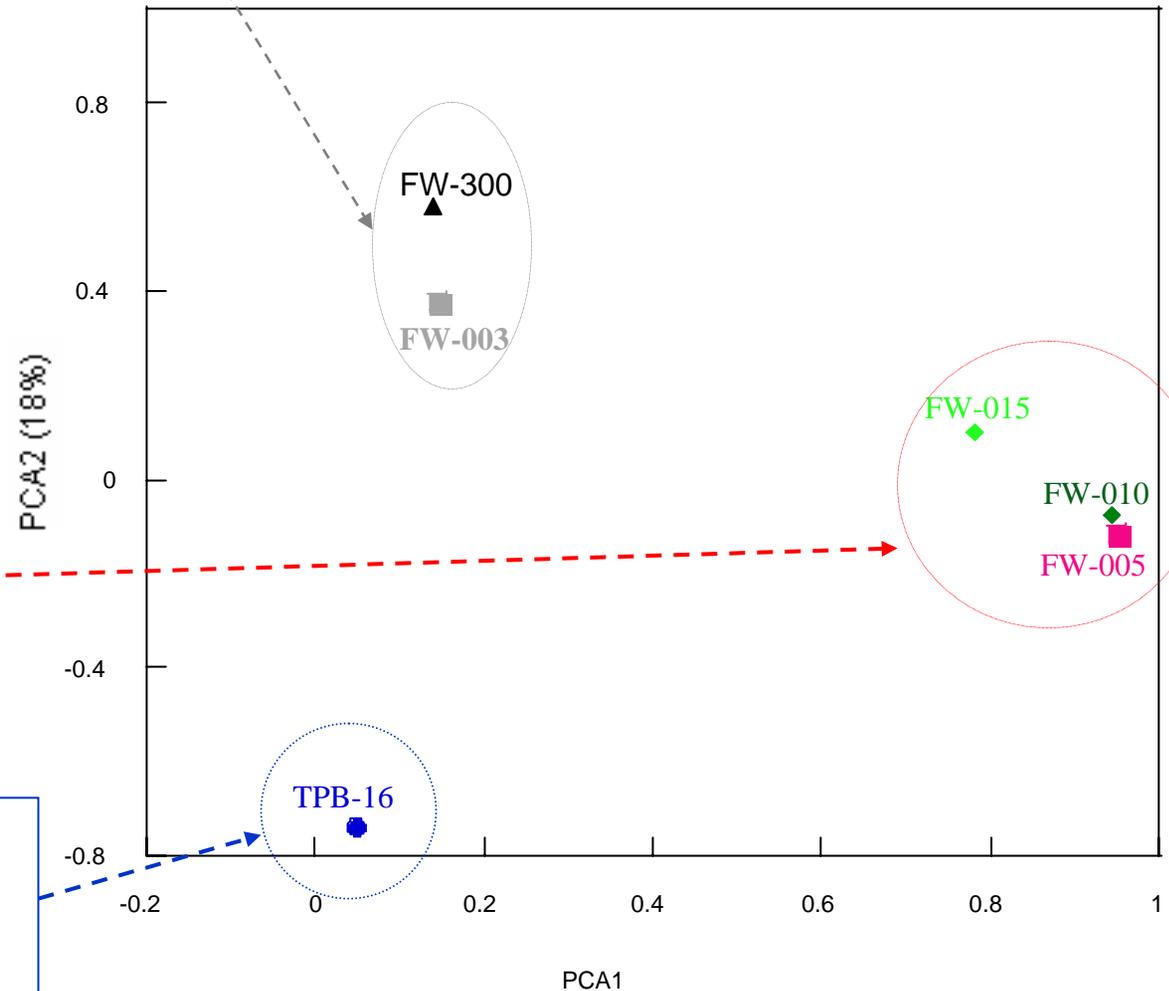
* *Azoarcus* sp.
* *Pseudomonas* sp.
* *Ralstonia* sp.

* *Methylobacter* sp.
* Uncultivated clone
* Uncultivated clone
* Uncultivated clone

FW-300

FW-003

FW-015

FW-010

FW-005

TPB-16

PCA2 (18%)

PCA1

0.8

0.4

0

-0.4

-0.8

-0.2   0   0.2   0.4   0.6   0.8   1

Sequence libraries from FRC groundwater along contaminant plume

	Screened Clones	Unique OTUs
SSU rRNA gene[a]	1630	353
*nir*K[b]	958	48
*nir*S[b]	1162	144
*amo*A[c]	539	63
*pmo*A[c]	26	14
*dsr*AB[d]	1812	163

[a] Fields et al. (in review); [b] Yan et al., 2003;
[c] Yan et al., (in preparation); [d] Bagwell et al., (in review)

Principal Components Analysis for Functional Gene Distribution
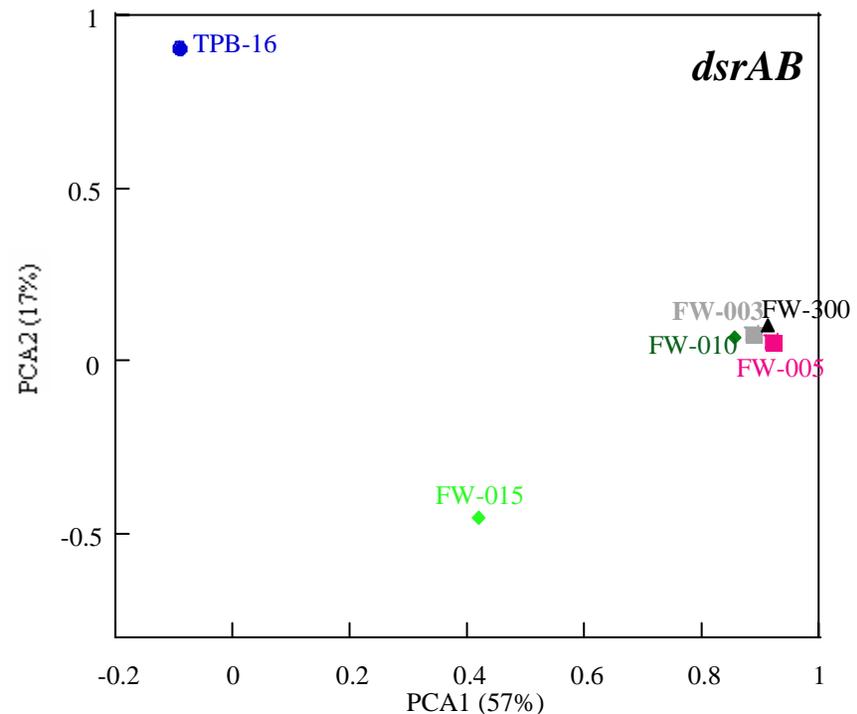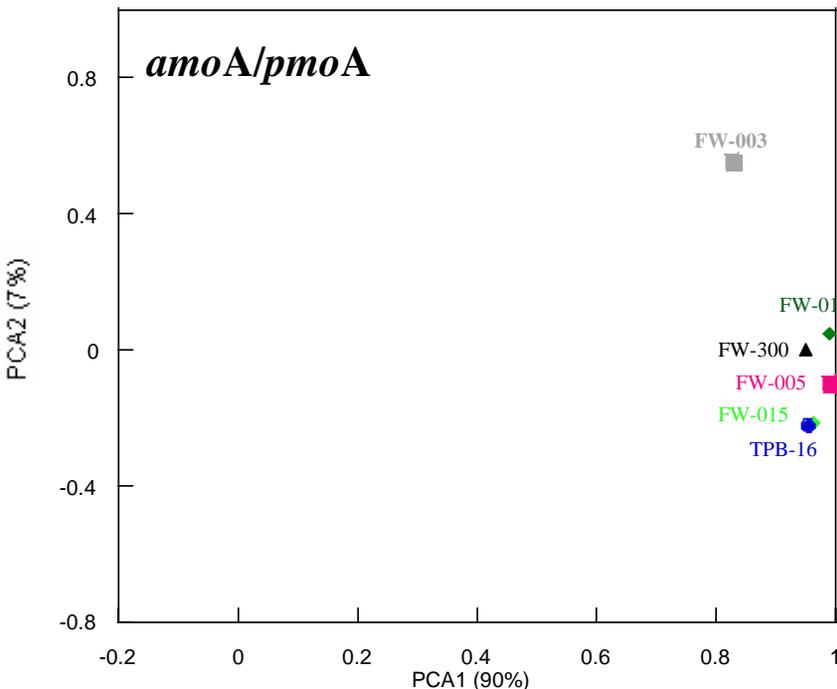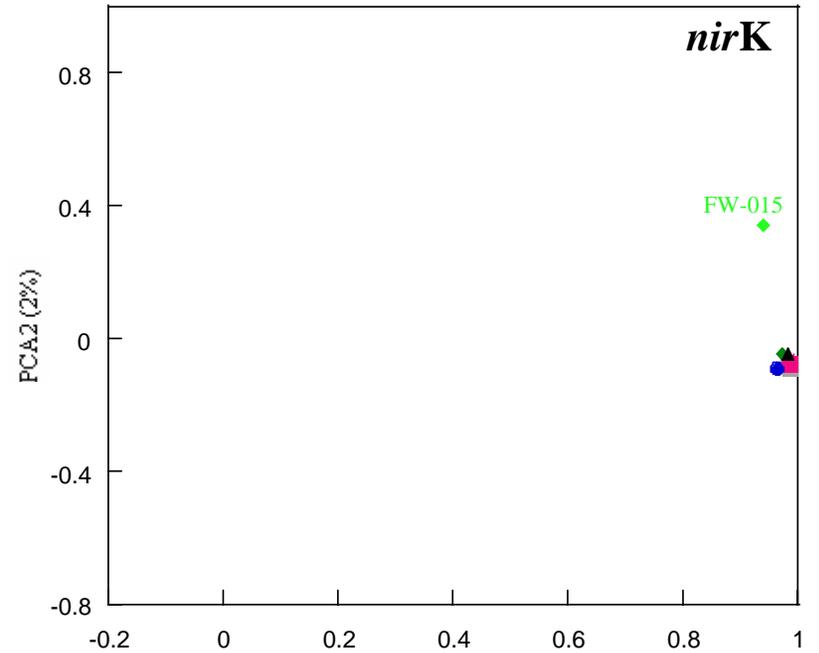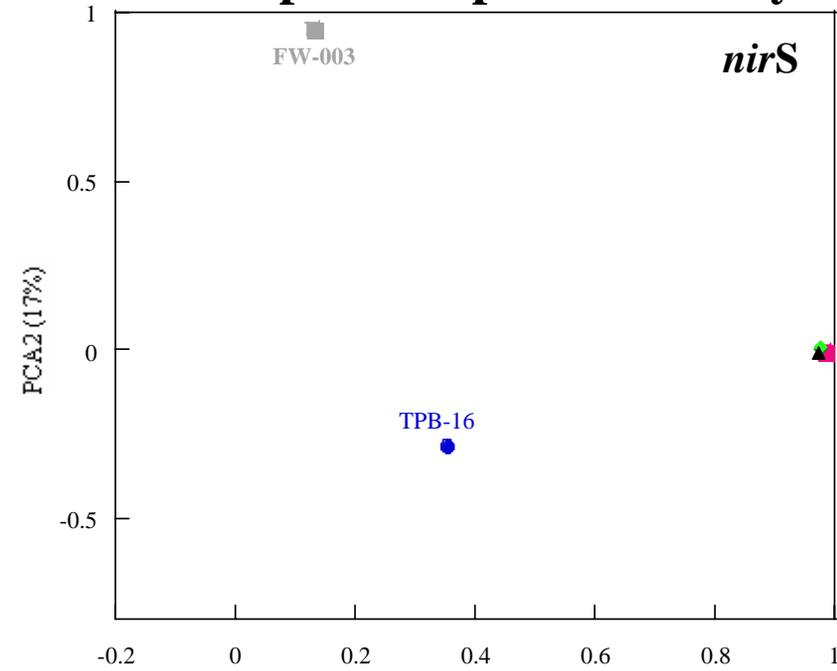
Principal Components Analysis
87% variance
107 compounds

* Tetrachloroethene
* ^{99}Tc, SO$_4$, Na
* 1,1,2-trichloro-1,2,2-trifluoroethane

* **Absence of 1,1,2-TC-1,2,2-TFE**
* **Absence of ^{99}Tc, NO$_3$, Al, Ca**

* **Ca**
* **NO$_3$**
* **Total organic carbon**

Component 2

* **Tetrachloroethene**
* **^{99}Tc, NO$_3$, Al, Nb, Zn, Sb, V, Th**
* **1,1,2-trichloro-1,2,2-trifluoroethane**

1.0

.5

0.0

-.5

fw300
tpb16

fw005

fw003

fw015

fw010

1.0

.5

0.0

-.5

Component 1

.5

0.0

-.5

0.0

.5

1.0

Component 3

SSU rRNA gene
76% of variance

* *Diaphorobacter* sp.
* *Rhizobium* sp.

* *Pseudomonas stutzeri*
* *Pseudomonas marginalis*
* *α-Proteobacterium*

* *Acidovorax* sp.
* *Gallionella* sp.
* Distribution of *Pseudomonas*

* *Methylobacter* sp.
* Uncultivated *Acidobacteria*
* Uncultivated *δ-Proteobacterium*
* Uncultivated clone

Component 2

Component 1

Component 3

fw300
fw015
fw010
fw005
fw003
tpb16
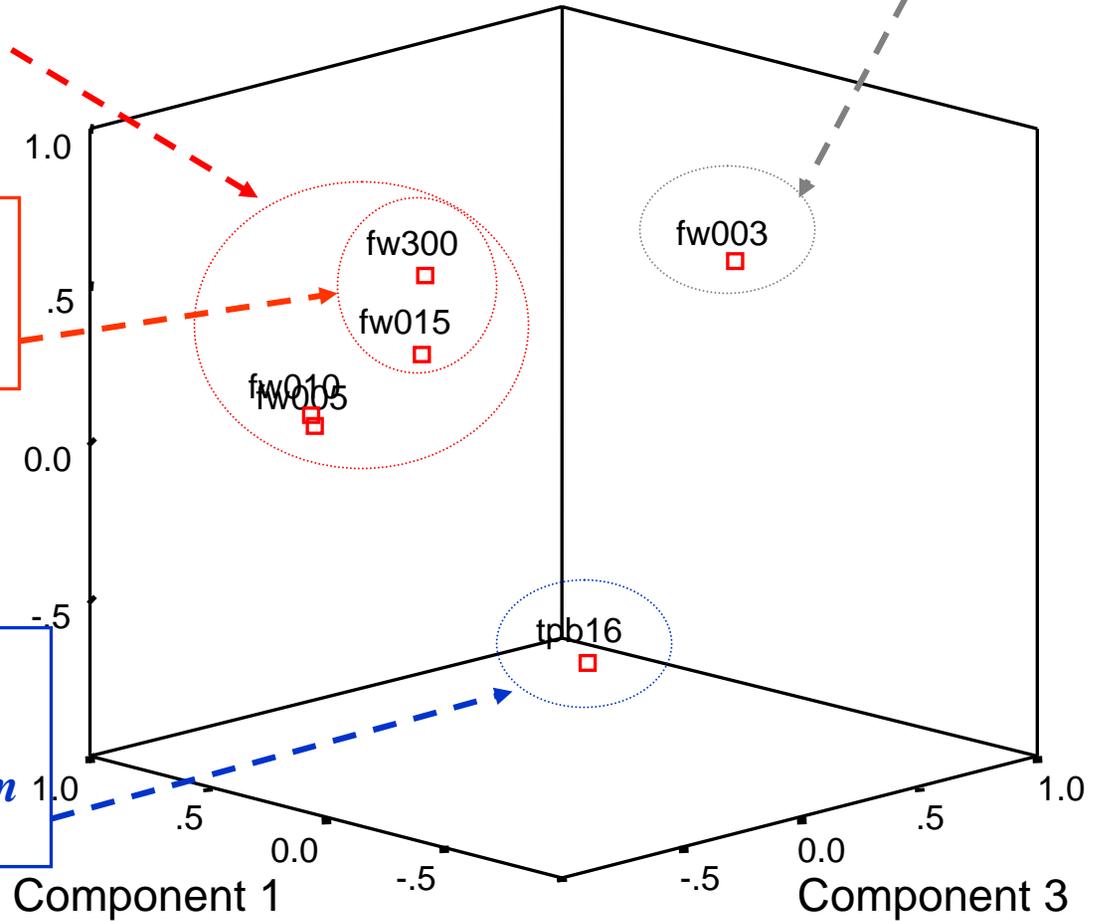
SSU rDNA, *nir*S, *nir*K, *amo*A, *pmo*A, *dsr*
94% of variance

* ***Methylobacter* sp.**
* ***Aquicella* sp.**
* **uncultivated *Acidobacteria***
* **uncultivated δ-*Proteobacterium***
* **predominant *pmo*A, *amo*A, *nir*S**

Component 2

* ***Diaphorobacter* sp.**
* ***Rhizobium* sp.**
* **predominant *dsr, amo*A, *nir*S**

* ***Pseudomonas stutzeri***
* ***Pseudomonas marginalis***
* **α-*Proteobacterium***
* **predominant *nir*S**
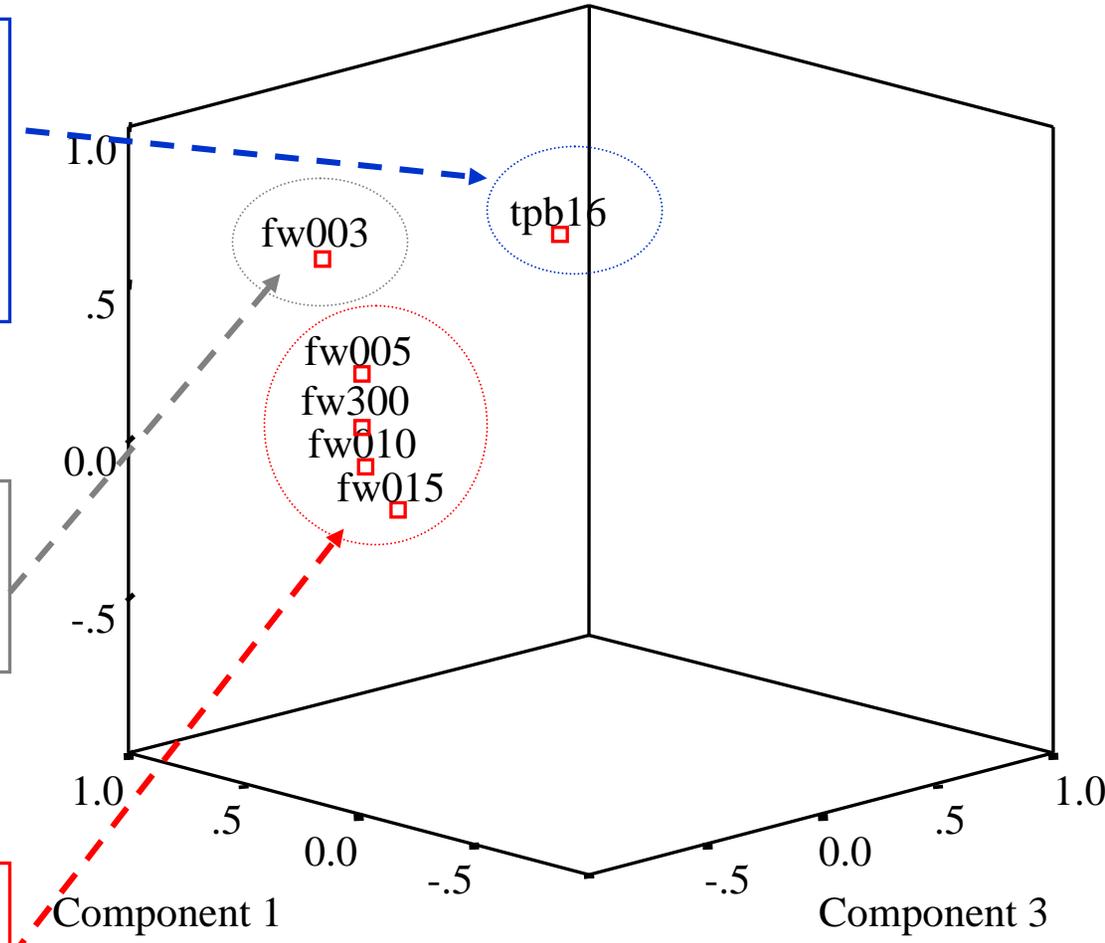
Conclusions

- A subset of geochemical parameters could differentiate the tested sites; however, a larger set of measures was able to differentiate the more heavily contaminated sites

- The distribution patterns for the SSU rRNA gene suggested different groupings compared to geochemistry alone

- Correlations between groundwater chemistry and the recovery and diversity of different functional gene sequences gave different results. However, a theme seemed to be the association of the background site with the heavily contaminated sites

- The distribution of different functional genes and a few SSU rDNA sequences suggested that the background was more similar to the acidic, contaminated sites

Conclusions

- ^{99}Tc, NO_3, Al, Nb, Zn, Sb, V, Th, 1,1,2-trichloro-1,2,2-trifluoroethane were dominant factors for the acidic sites

- TOC, Ca, and NO_3 appeared to drive the association of FW-003 with the acidic sites

- Similar functional genes and occurrence of two *Pseudomonas* populations appeared to drive the association of the background with the acidic sites (e.g., similar *nir*S and *nir*K sequences)

- The distribution of different *Acidovorax* and *Diaphorobacter* populations seemed to differentiate FW-003 from the other high nitrate sites as well as some unique *dsr*, *amo*A, and *nir*S gene sequences

- Overall increased diversity, the occurrence of *Methylobacter* sp., several uncultivated organisms, and unique amoA and pmoA sequences differentiated TPB-16 from the other sites

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