

High Density Oligonucleotide Array Monitoring of Bacterial Community Dynamics During Carbon Stimulated Uranium Bioremediation

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<http://greengenes.llnl.gov/16S>



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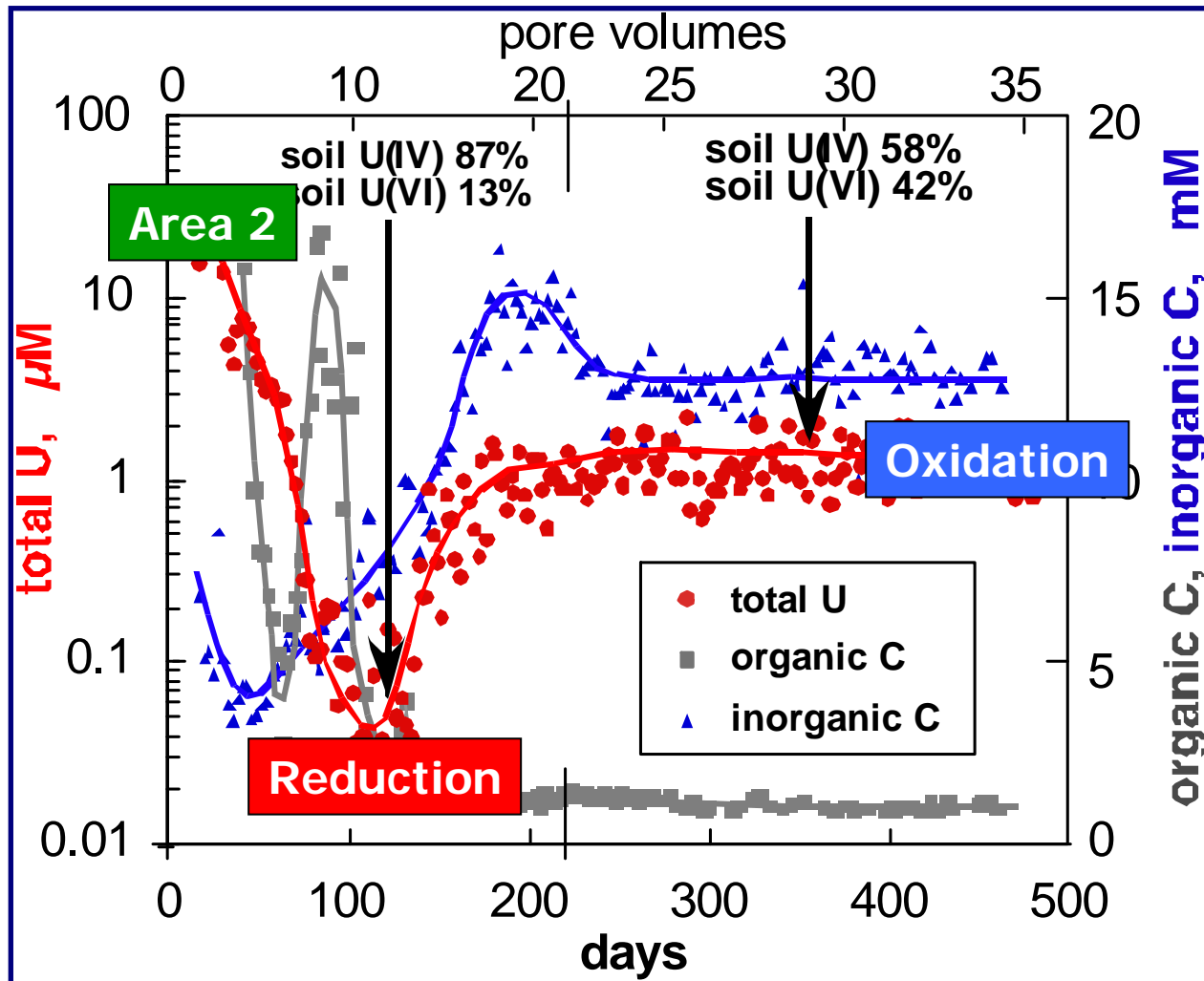


Laboratory flow through column simulation

- Long term stability of bio-reduced U(IV) – 500 day flow through columns
- Area 2 sediment Oak Ridge
- 206 ppm U
- 200 mm x 32 mm columns
- 32 mM Lactate-C supplied
 - @ 20 mm d⁻¹ for 215 days
 - 10 mm d⁻¹ for remainder
- U, TOC, TIC, pH
- Redox potential
- U(VI):U(IV) – μ XANES
- Community analysis
- Microarray analysis

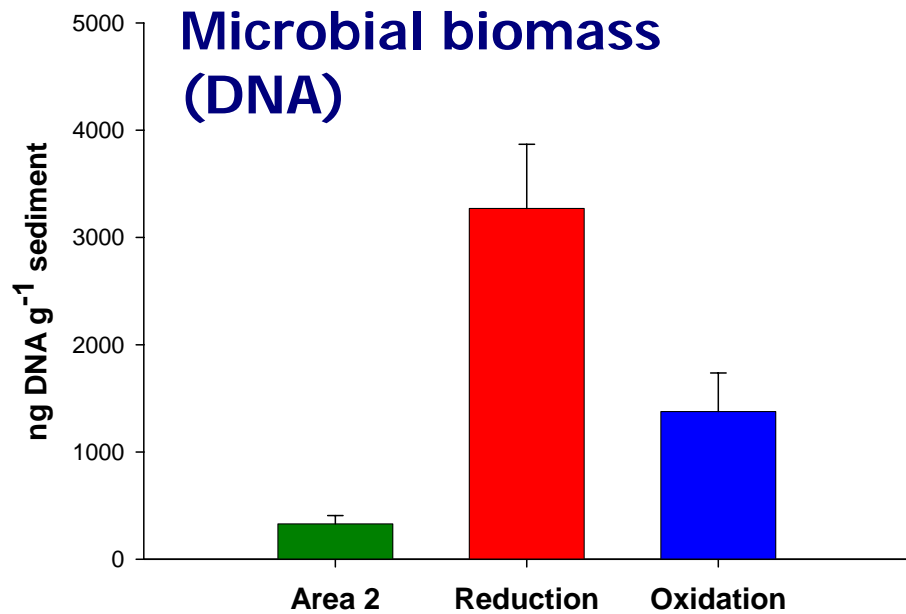


Re-mobilization of U(IV) under reducing conditions

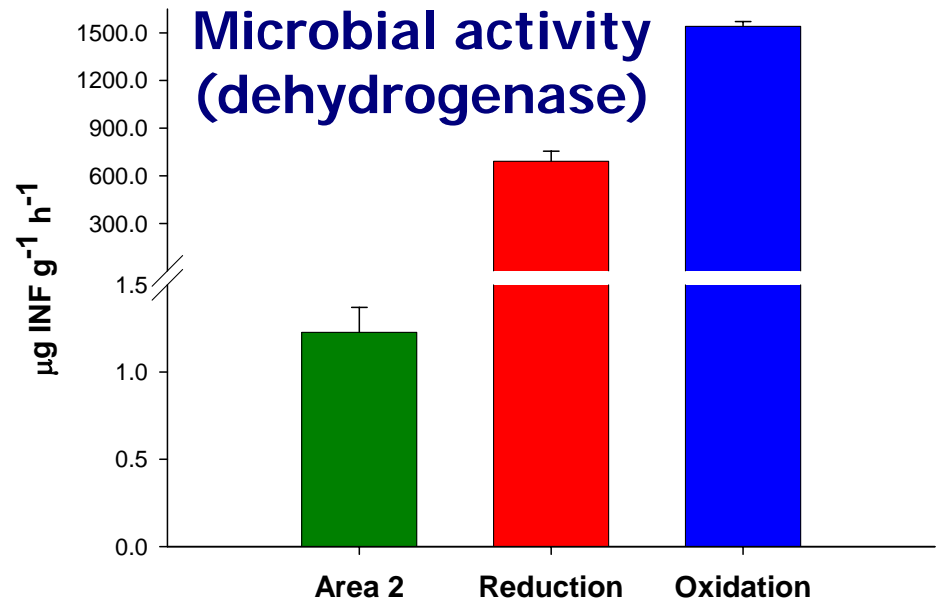


So what's going on?

- U(IV) being re-mobilized under reducing (methanogenic) conditions!
- Alteration in microbial communities
 - Decline of functional groups capable U(VI) reduction?
 - Increase in species capable of U(IV) oxidation?
 - leading to net U(IV) re-oxidation

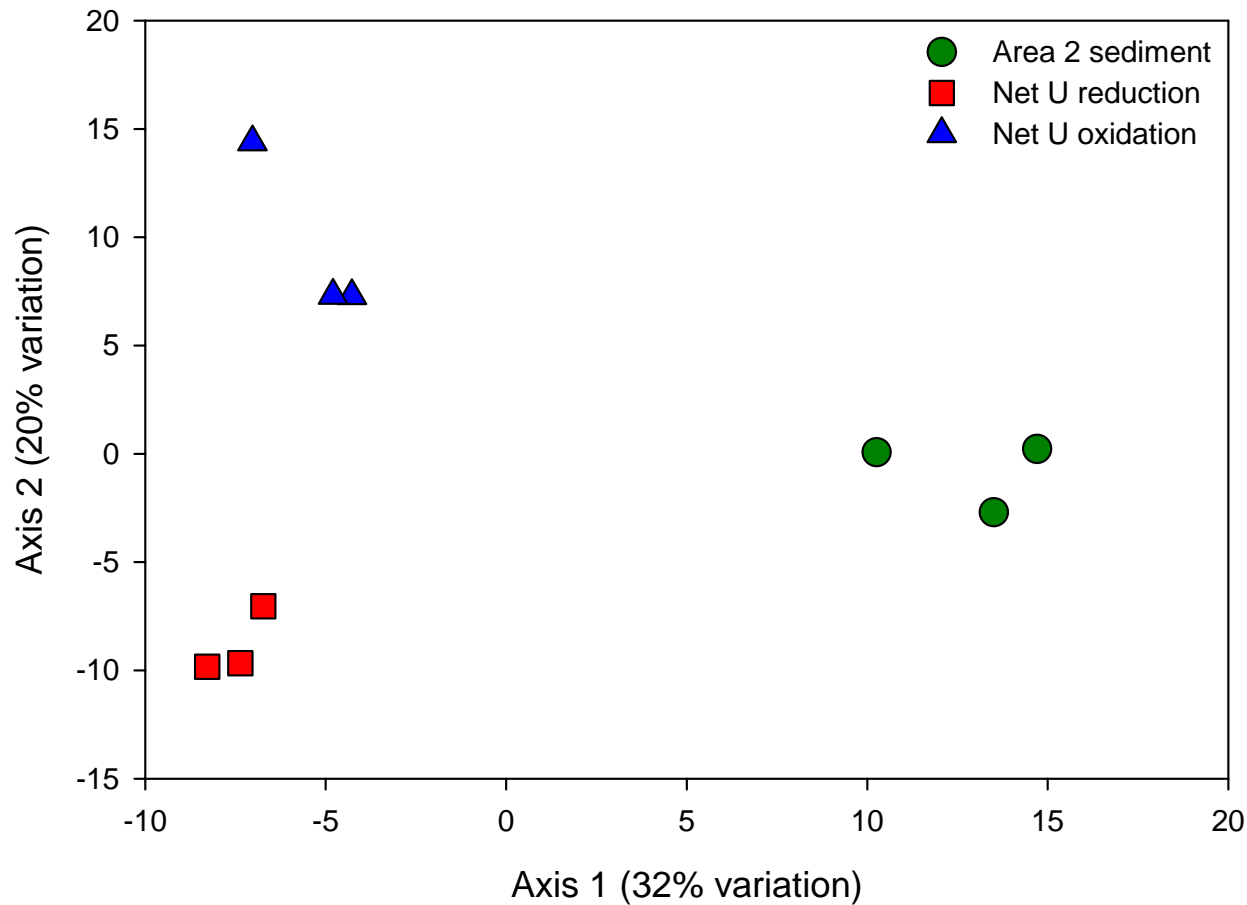


- decrease in biomass
 - less suitable TEAs
- activity not adversely affected



Do decreases in biomass result in community shifts?

- PCA of 16S T-RFLP profiles



Are there changes in functional groups of relevance?

High Density Oligonucleotide Array

- Probes based on largest available 16S rDNA alignment
>86,000 sequences

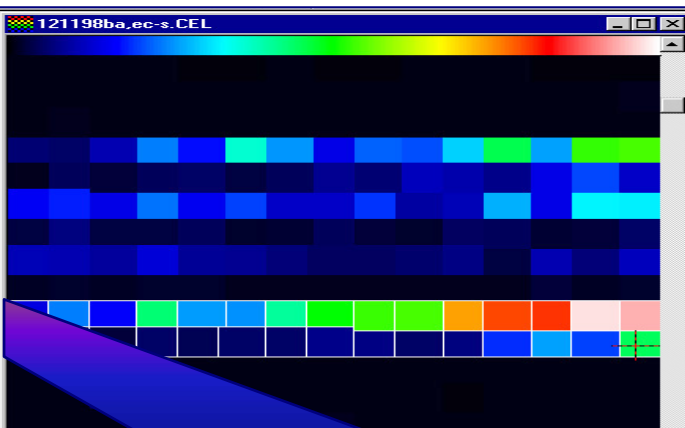
<http://greengenes.llnl.gov/16S/>

(currently being moved to LBL)

- DeSantis *et al.* (2003) *Bioinformatics*, **19**:1461

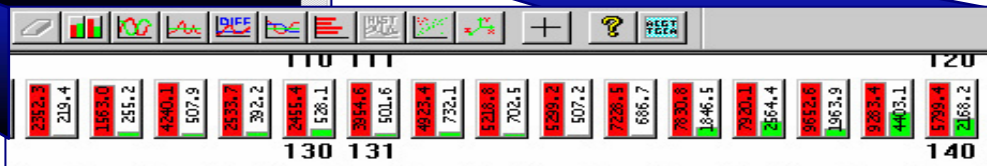
DOE 16S rDNA microarray

- Rapidly detect the composition and diversity of microbes in an environmental sample
- Massive parallelism - 550,000 probes in a 1.28 cm² array
- all 9,900 species in 16S rDNA database \geq 11 probes pairs per OTU
- Single nucleotide mismatch resolution



cctagcatgCattctgcata
cctagcatgGattctgcata

MATCH
MISMATCH

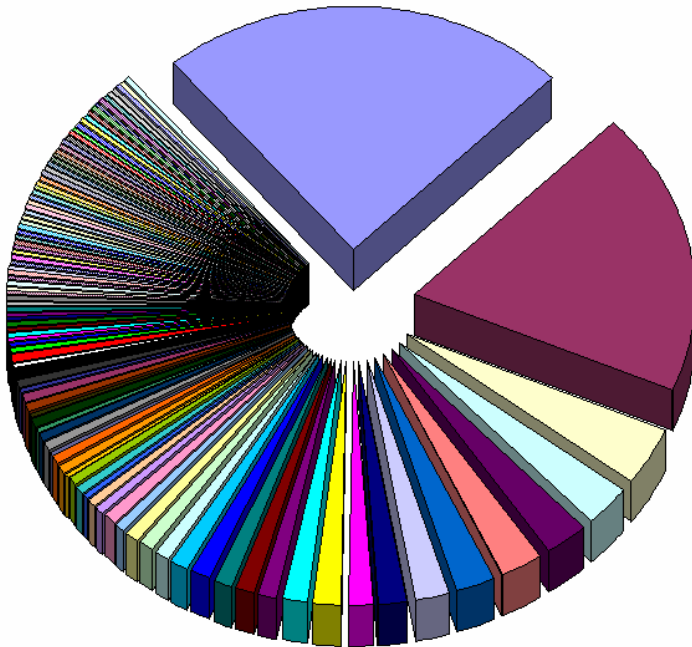
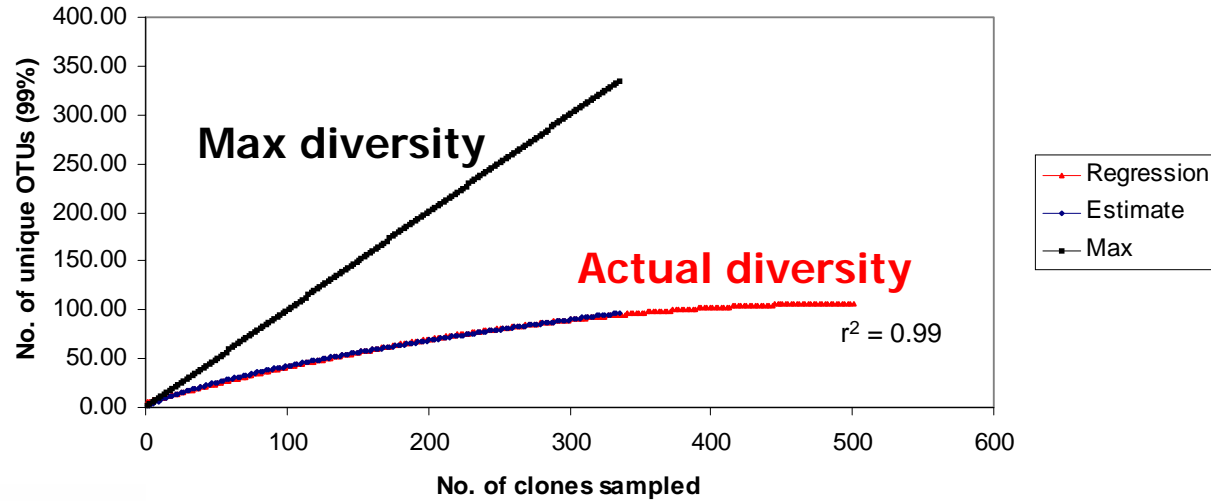


Testing array utility - Area 2 Oak Ridge FRC – Clone library

1484 reads; 742 clones

Quality filtered

- Trimmed Phred20 (641)
- F&R assembled into contigs
- <4 gaps, >1300bp (606)
- Chimeras (167, ~28%) (439)



115 OTUs @ 97% similarity

%	OTU
23.7	<i>Arthrobacter oxydans</i>
18.7	<i>Cellulomonas fermentans</i>
4.1	<i>Chloroflexi</i>
2.7	<i>Blastochloris viridis</i>
2.5	<i>Rathayibacter rathayi</i>
2.5	<i>Streptococcus salivarius</i>
2.1	<i>Fulvimonas soli</i>
2.1	<i>Streptomyces mashuensis</i>
1.6	<i>Micrococcus lylae</i>
1.6	<i>Mycobacterium brisbanense</i>

Testing array utility

Same amplicon pool analyzed by cloning and 16S microarray

Only 8 clones out of 439 not physically detected by array

- all single occurrence clones

Many OTUs (>300) detected by array not detected in clone library

- Sampling issues - Array (entire amplicon mixture)
 - Clone library (serial sampling by plasmid)
- False positives? - PCR with specific primers has confirmed many OTUs present

Triplicate arrays

e.g. ~740 clones necessary per sample
740 x 3 reps x 3 samples = 6660 clones

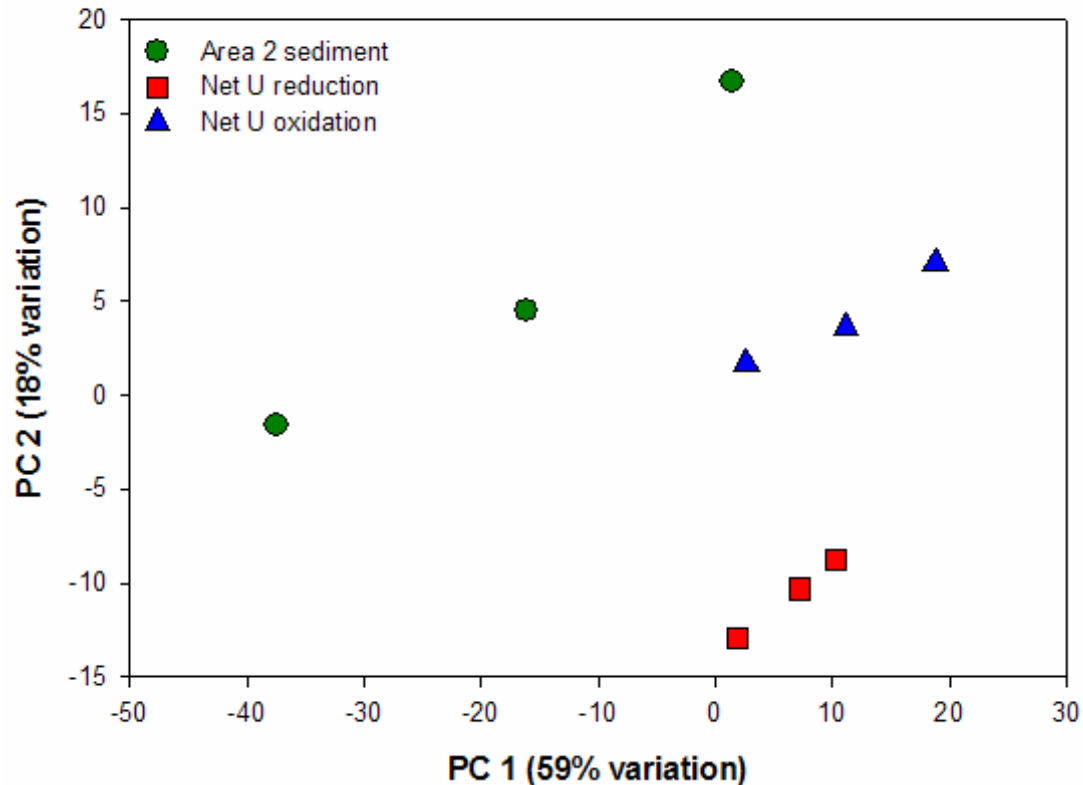
What OTUs have changed in abundance?

- Using Average Difference as a proxy of abundance
 - Average Difference is:
Mean (Perfect Match Probes – Mismatch Probes) for each OTU

hybridization intensity corrected for non-specific hybridization

- Interested in most dynamic organisms
 - Analysis limited to OTUs with greatest standard deviation (top 5%)
- Principal Component Analysis
 - Shows divergence between microbial communities
 - Similarly responding/covarying OTUs cluster together

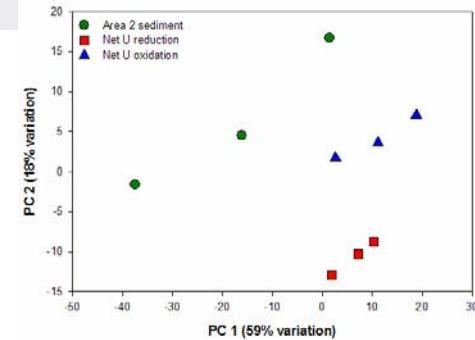
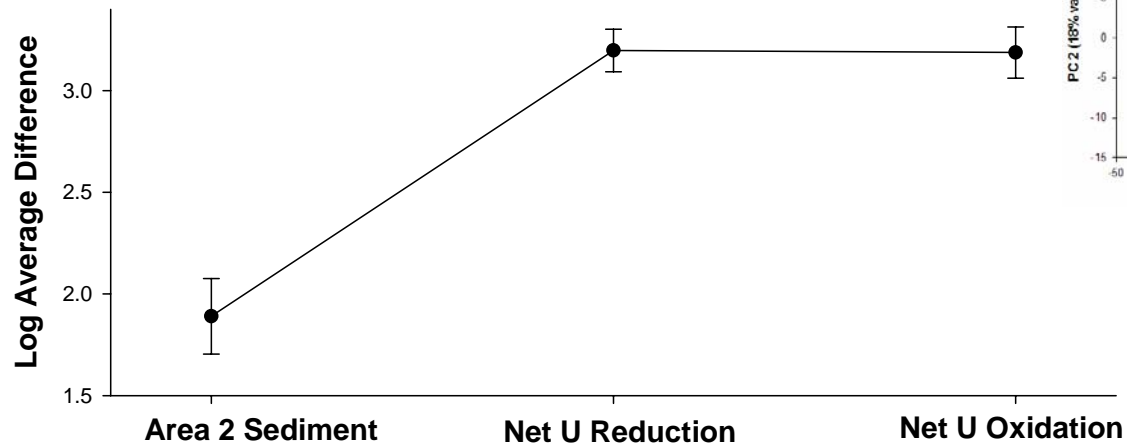
PCA analysis of positive matches



What organisms are responsible for shift in community composition?

Are they functionally relevant?

PCA Group 1 - Negative correlation with PC 1



Representative organism	Phylocode	Group	Log ₁₀ Average Difference		
			Area 2	Reduction	Oxidation
<i>Geothrix fermentans</i>	2.13.8.386	Acidobacteria	1.65a	3.37b	3.36b
<i>Pseudomonas spinosa</i>	2.28.2.9.4.3.7937	β-proteobacteria	1.93a	2.92b	2.82b
<i>Geobacter metallireducens</i>	2.28.4.7.4.10207	δ-proteobacteria	2.4a	3.35b	3.34b
<i>Geobacter arculus</i>	2.28.4.7.4.10209	δ-proteobacteria	1.58a	3.15b	3.23b

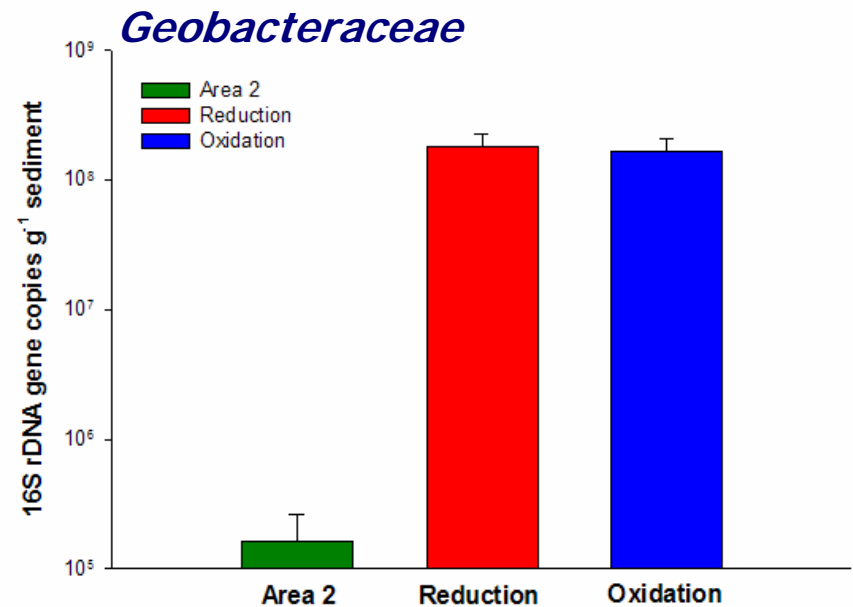
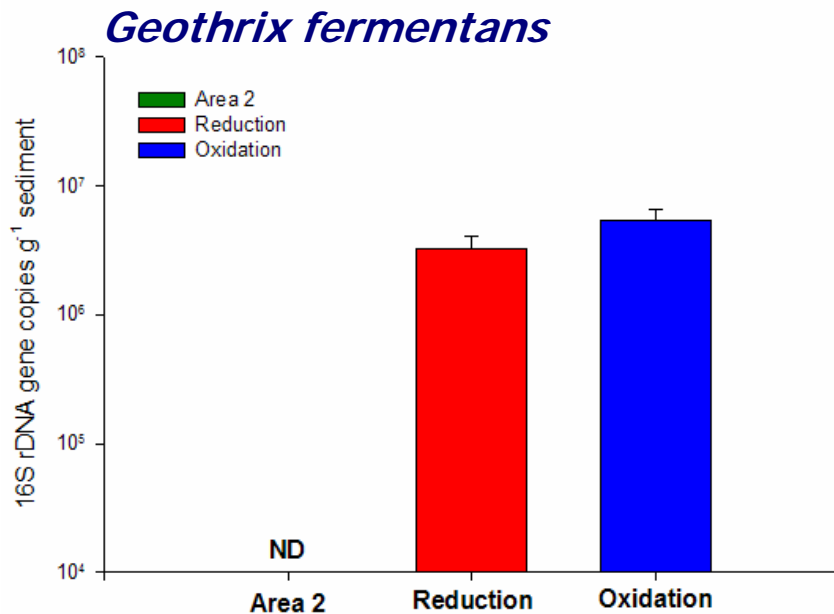
Same letter denotes no significant difference (p>0.05)

Real-time quantitative PCR confirmation

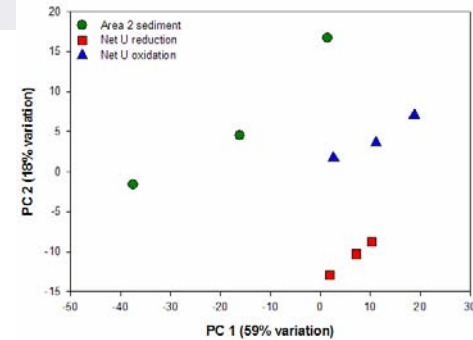
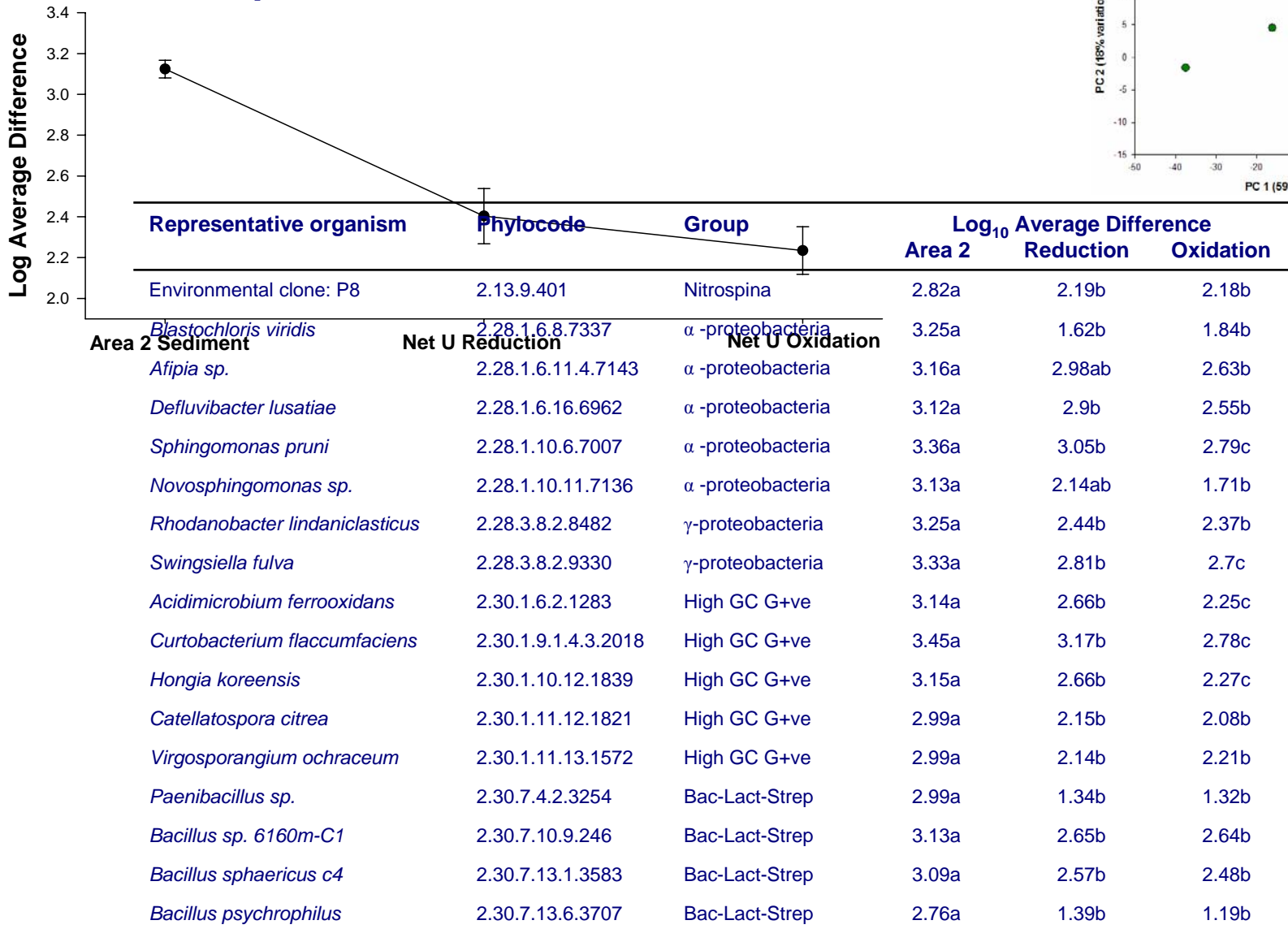
(a) Array quantitation

Representative organism	Phylocode	Group	Average Difference		
			Area 2	Reduction	Oxidation
<i>Geothrix fermentans</i>	2.13.8.386	Acidobacteria	45	2344	2290
<i>Geobacter metallireducens</i>	2.28.4.7.4.10207	δ -proteobacteria	251	2238	2188
<i>Geobacter arcus</i>	2.28.4.7.4.10209	δ -proteobacteria	38	1412	1698

(b) qPCR quantitation

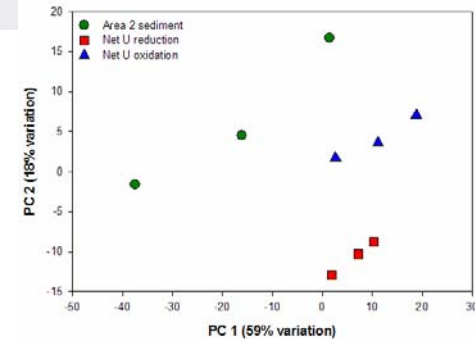
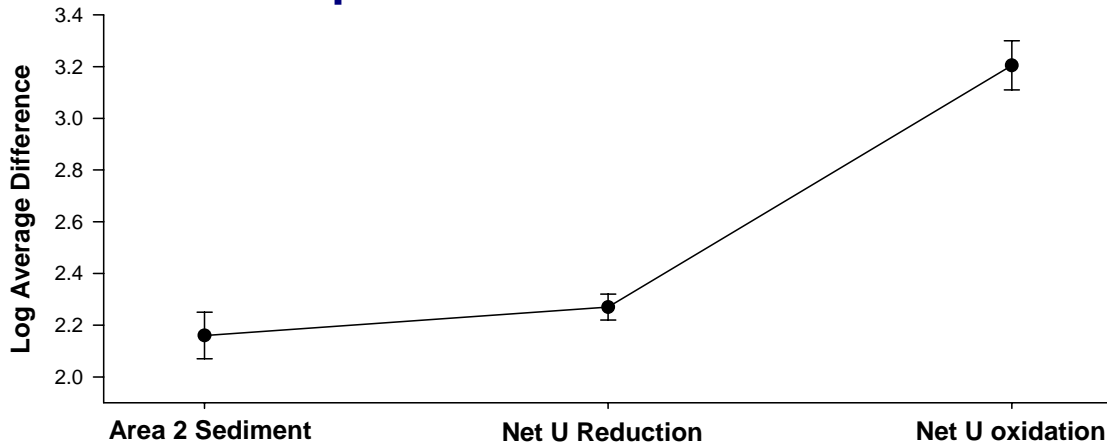


PCA Group 2 - Positive correlation with PC 1



<i>Arthrobacter</i> sp.	2.30.1.9.1.6.1.1579	High GC G+ve	3.25a	3.25a	2.98b
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PCA Group 3 - Positive correlation with PC 2



Representative organism	Phylocode	Group	Log ₁₀ Average Difference		
			Area 2	Reduction	Oxidation
Anaerobic dechlorinating bioreactor clone SHA-18	2.25.3.1.6429	Fibrobacter-Acidobacterium	2.25a	2.32a	3.3b
<i>Desulfovibrio africanus</i>	2.28.4.1.4.6.9689	δ-proteobacteria	2.07a	2.22a	3.11b

- Sulfate reducer co-varying with anaerobic dechlorinator?
 - Sulfate reducer – dechlorinator syntrophy
- Coincidental with Uranium re-oxidation?
 - Chlorinated hydrocarbons as TEA for U(IV) oxidation?

Summary I

High density Affymetrix array capable of accurately tracking community shifts

>9,000 OTUs may be followed simultaneously

- Reliable assay for community composition
- Real-time PCR allows accurate verification of quantitative aspects
- Multivariate statistics allow hypothesis driven data analyses and interpretation
- Microarray data used as a springboard for future laboratory and field experiments

Summary II

- Continual OC supply maintains reducing conditions
- Supports microbial activity
- Supports metal reducing bacteria
- *Arthrobacter* sp. and others decline – release of accumulated U?
- *Desulfovibrio* sp. and dechlorinator increase – associated with U re-ox

Geochemical explanation

- Increase in dissolved inorganic C (from microbial respiration) and presence of calcium results in thermodynamically favorable U(IV) oxidation under reducing (methanogenic) conditions.
- Terminal electron acceptor for U(IV) oxidation not identified as yet

Acknowledgements



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Paul Richardson



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CHICAGO

Stephen Sutton, Matthew Newville



US DOE – Natural and Accelerated Bioremediation Program