U reduction and Hg methylation

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Two Projects

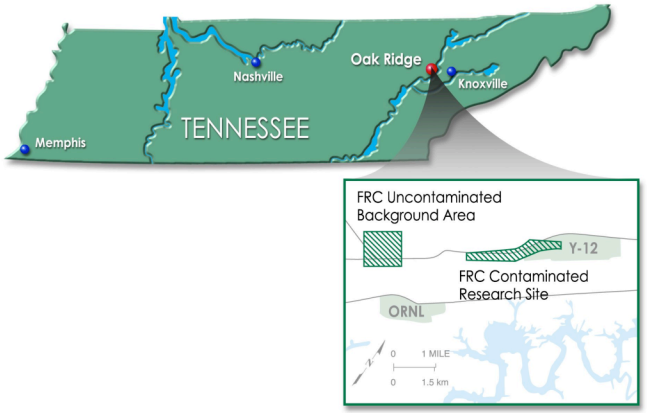
- Final year of
  - An Integrated Assessment of Geochemical and Community Structure Determinants of Metal Reduction Rates in Subsurface Sediments
- First year of
  - Geochemical, Genetic, and Community Controls on Mercury Methylation

U Reduction Background

- The relationships among microbial community structure, geochemistry, and metal reduction rates in subsurface sediments may be critical in remediation of metal contaminated environments.
- Many microorganisms can change the geochemical conditions so metal reduction becomes an energetically favored reaction while some microbes can directly catalyze the necessary reactions.
- In the second case the composition of the community is important but in the first it is not.

Research Questions

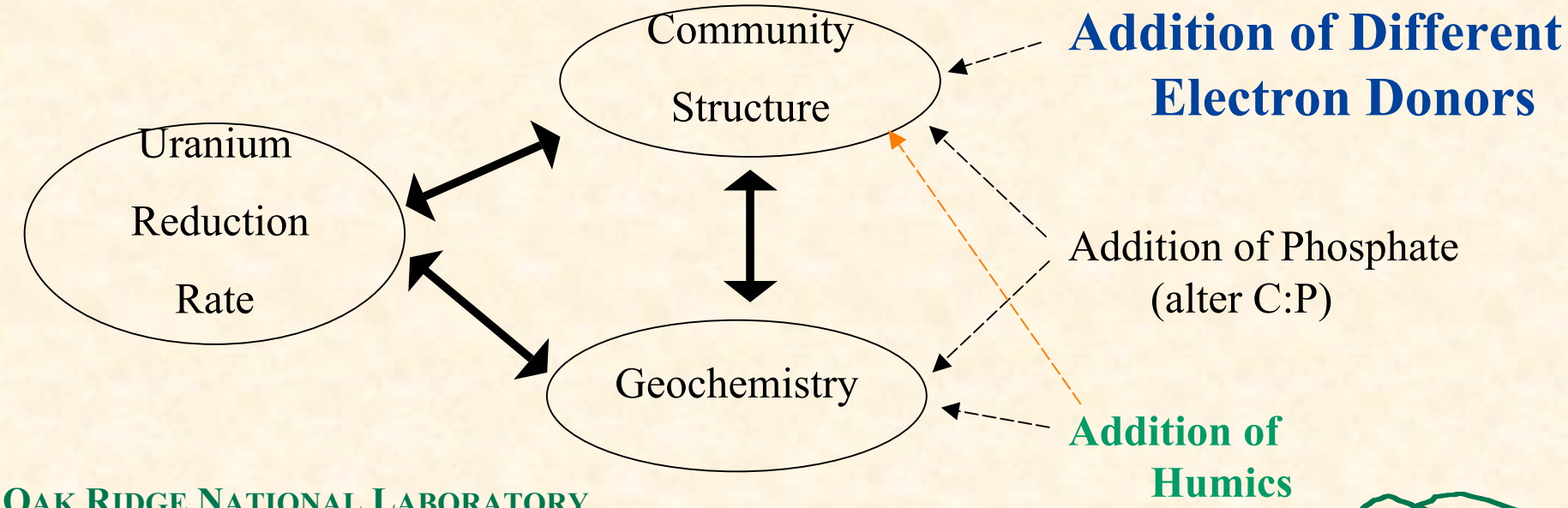
- **Does Microbial Community Structure Effect Uranium Reduction Rates?**
  - Are there donor specific effects that lead to enrichment of specific community members that then impose limits on the functional capabilities of the system?
  - Is the metabolic diversity of the in situ microbial community sufficiently large and redundant that bioimmobilization of uranium will occur regardless of the type of electron donor added to the system?
- **To address these questions, we are using sediment and groundwater from the DOE Field Research Center (FRC) located at ORNL.**



Goal

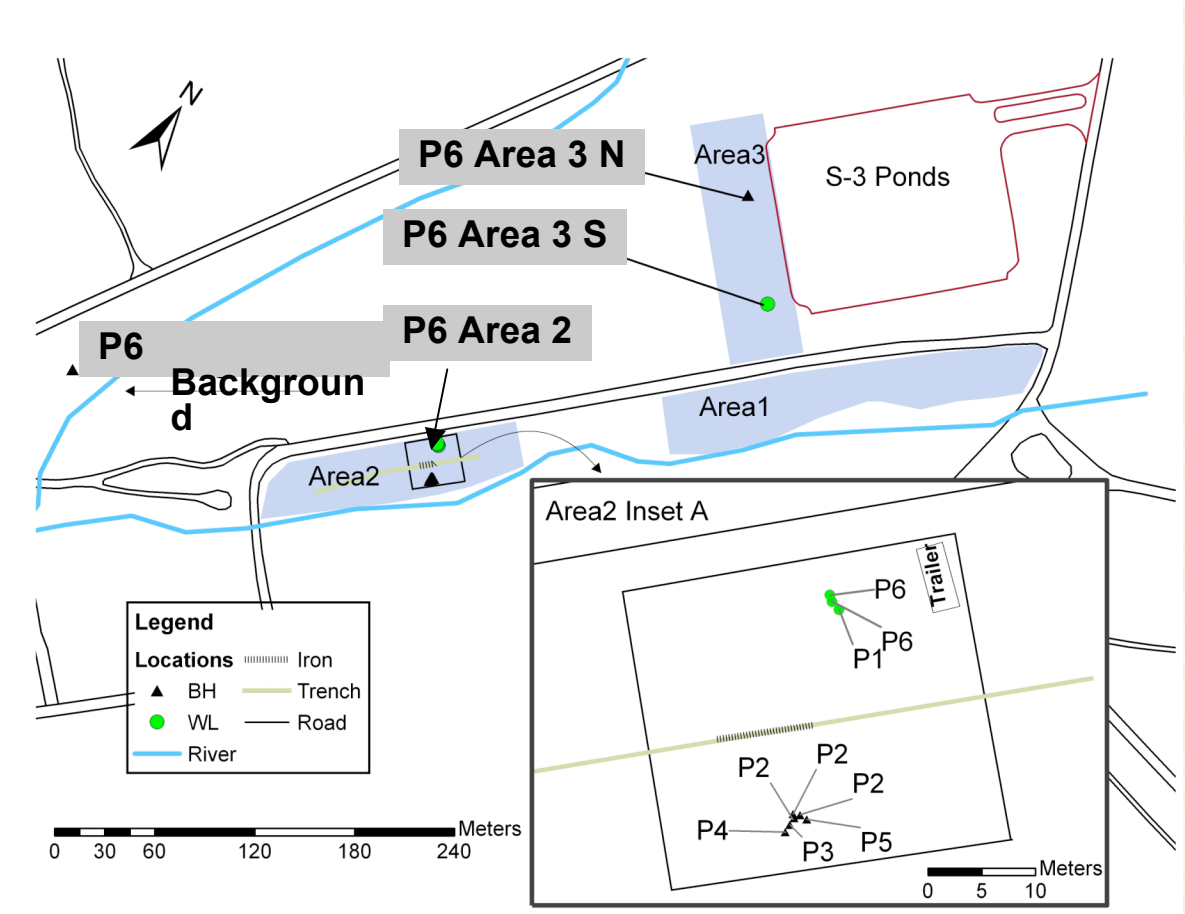
The overall goal of our project is to provide an improved understanding of the relationships between microbial community structure, geochemistry, and metal (uranium) reduction rates.

- Is uranium reduction more like hydrocarbon degradation or chlorinated solvent degradation?



Sampled Sites

- Symbols P1 - P6 indicate sampling locations for Experiments 1 – 6.
- Sediment samples were homogenized under anaerobic conditions prior to use in the microcosms.



Electron donors used in microcosms to influence community structure

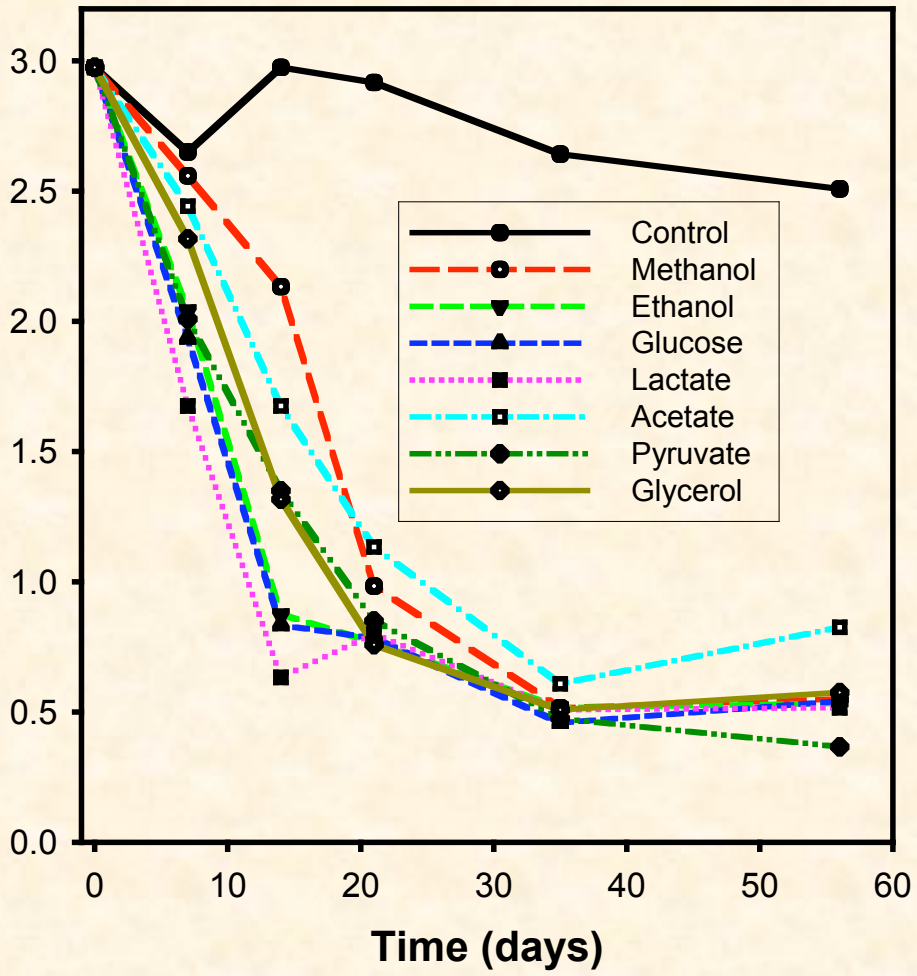
- Carbon substrate concentrations are adjusted to give equivalent electron donor potential.

Donor	Formula	e^-	Predominant Utilization	Exp
Acetate	$C_2H_3O_2$	8	FeRB/acetogenic methanogens	3
Lactate	$C_3H_6O_3$	12	SRB/FeRB	3
Pyruvate	C_3H_4O	10	SRB/FeRB	3
Methanol	CH_4O_3	6	Acetogens/methanogens	1,2,3,4, 5a and b , 6
Ethanol	C_2H_6O	12	SRB/FeRB	1,2,3,4, 5a and b , 6
Glycerol	$C_3H_8O_3$	14	Clostridia/gram positive anaerobes	3
Glucose	$C_6H_{12}O_6$	24	Clostridia/other heterotrophs	1,2,3,4

Typical Nitrate Results

Results consistent across studies

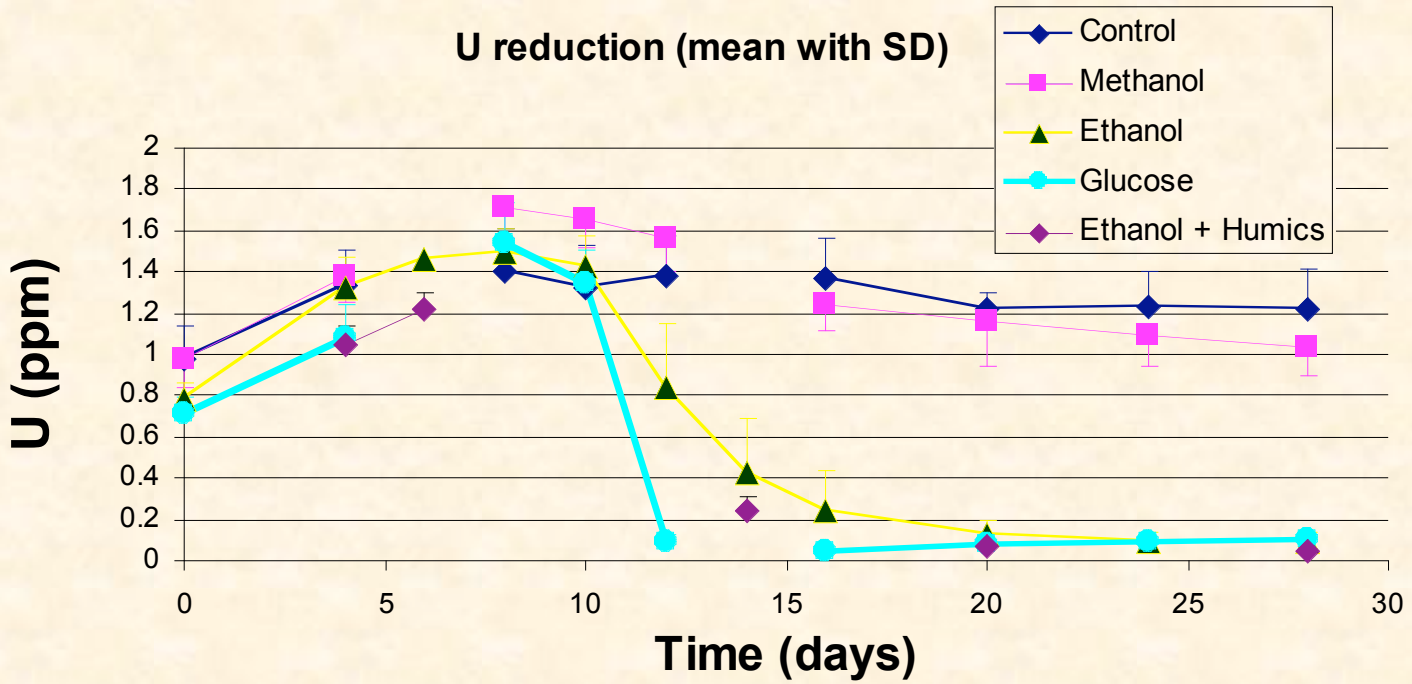
- Nitrate reduction is rapid.
- Differences among substrates are small.
  - Methanol lags.
  - Glucose, ethanol, lactate rapid.
- Minimal to no effect of pH (data not shown).



Exp 3 (data averaged over pH)

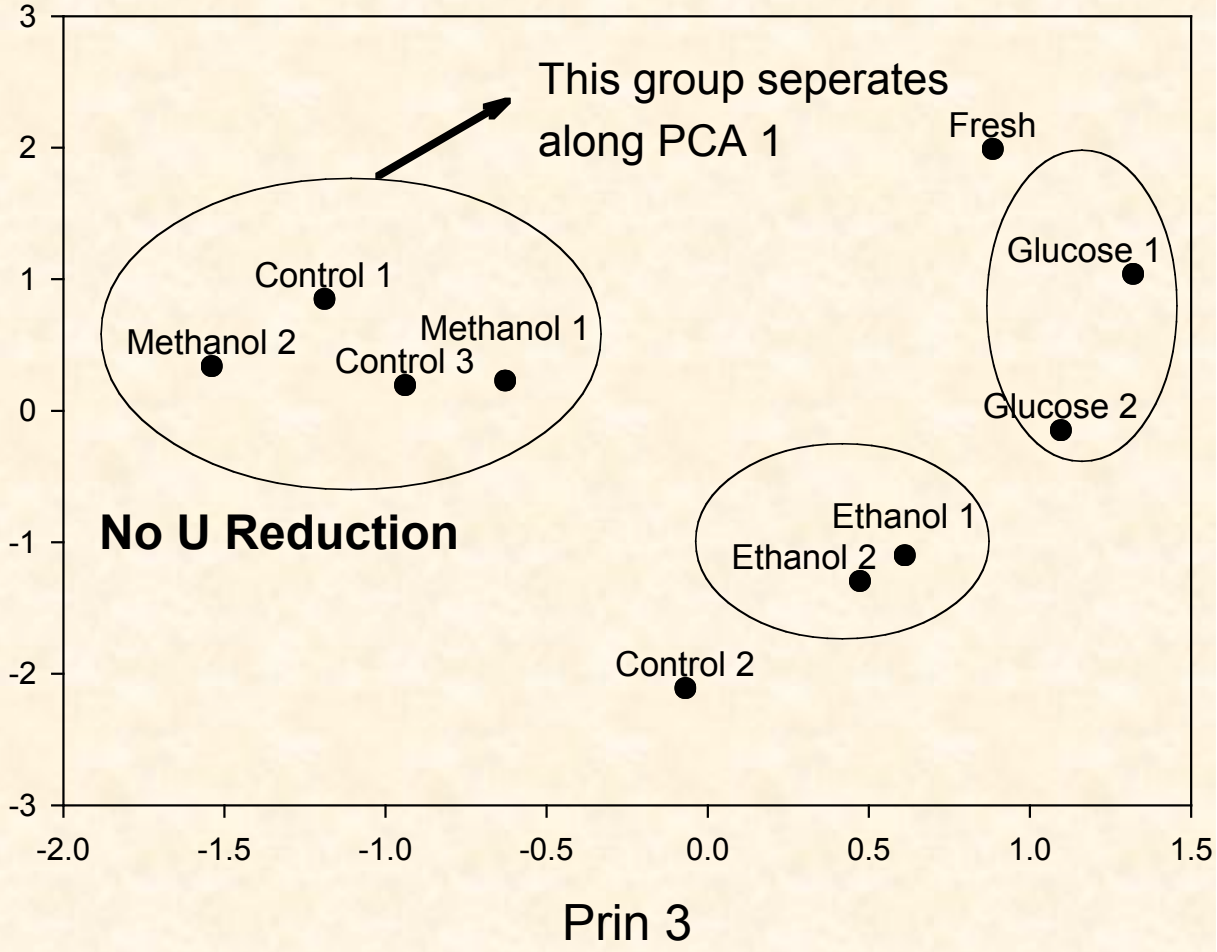
Typical U changes Exp 4 (basically the same for 1 to 3)

- U reduction lags behind Nitrate reduction
- No U reduction seen for methanol
- No detectable difference with Ethanol + Humic
- In some experiments Ethanol is the same or faster than Glucose



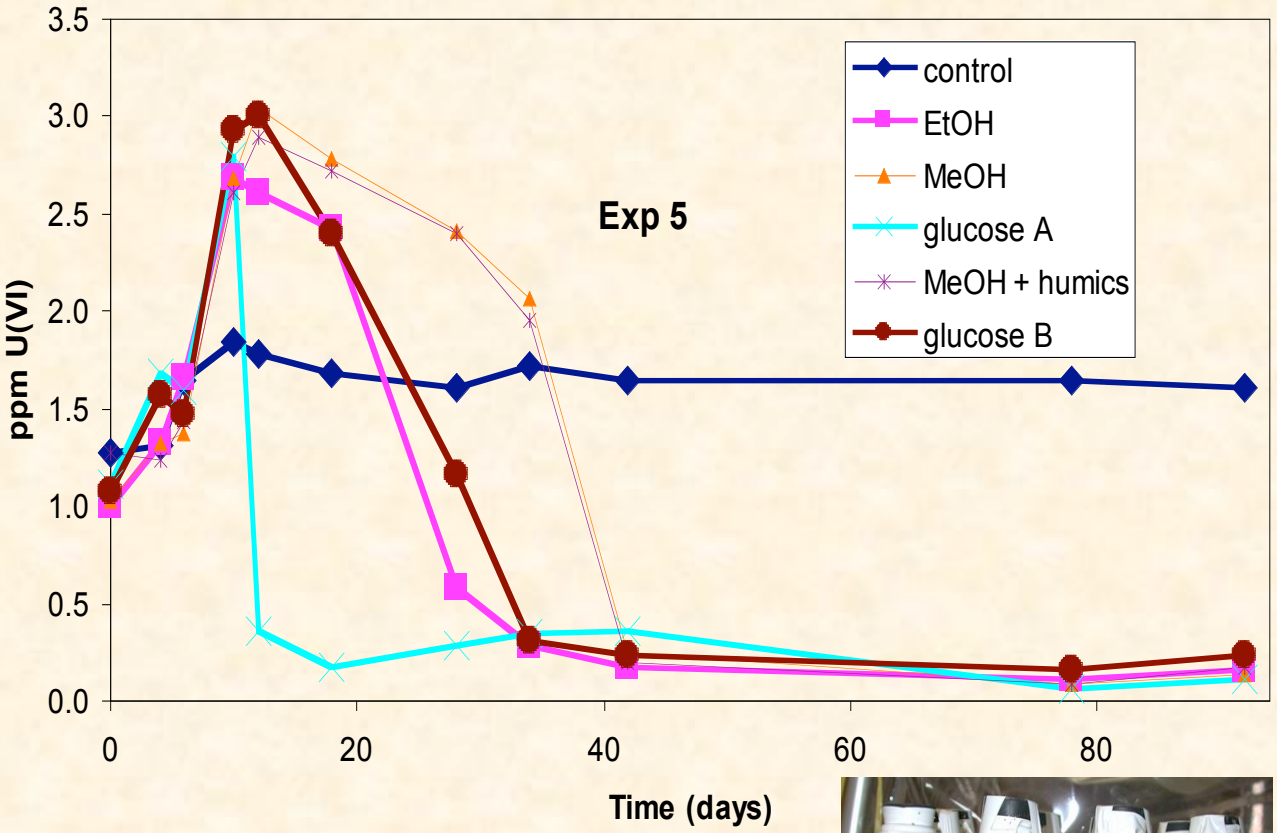
PLFA Community Analyses by Principal Component Analysis (PCA)

- **Treatments tend to be similar using PCA on the PLFA data.**
- **High U reduction treatments (ethanol and glucose) separate from control and methanol (also by cluster analysis).**
  - **One control (2) is consistently different than the other two controls.**
- **There was also higher stress indicated in the control and methanol treatments.**



Atypical U Results in Exp 5

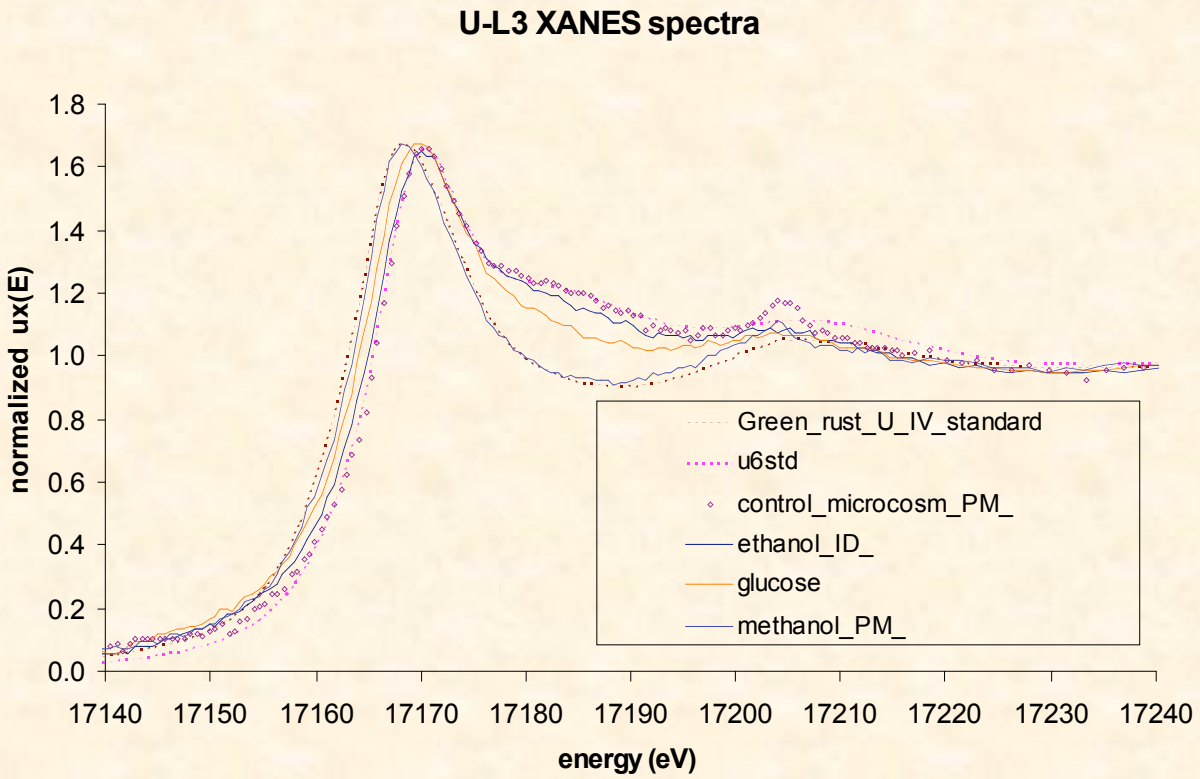
- Monitoring loss of U from Solution as indicator of U reduction
- In Exp 5 there was evidence for U reduction with methanol
- Repeated & confirmed with stored samples
- For methanol iron reduction goes with U reduction and can be visually assessed by color changes in the sediments



- Exp 6 samples on map to investigate possible community heterogeneity leading to different outcomes with methanol additions

U Valence by X-ray Absorption Spectroscopy Confirms U reduction

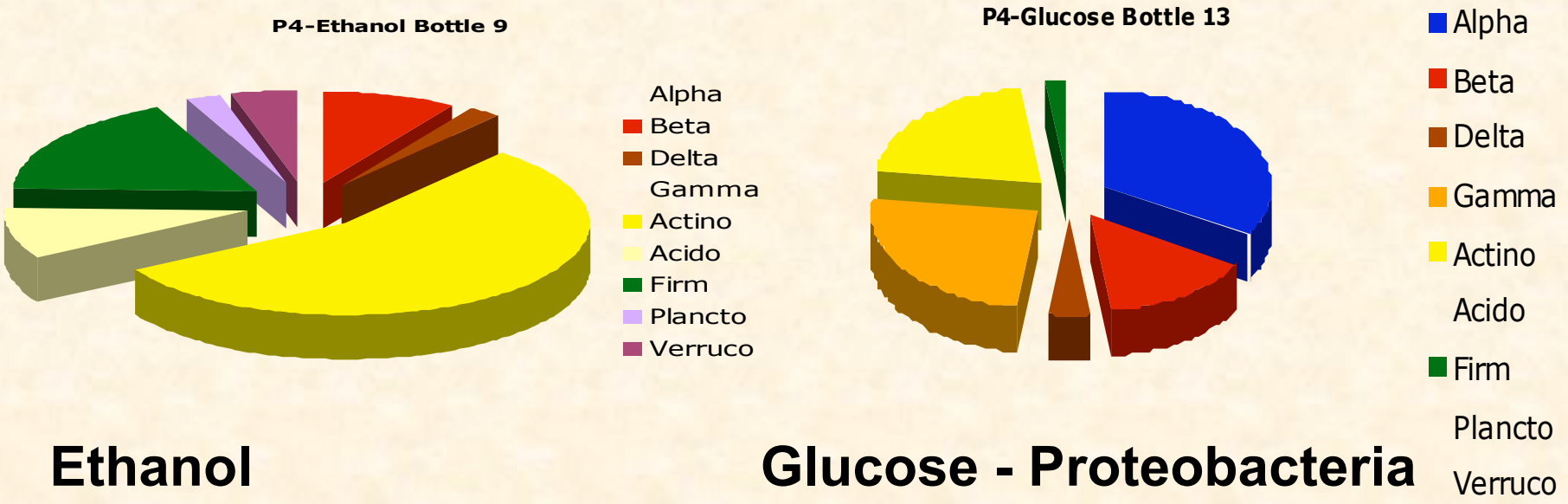
- Glucose Exp 4
  - 57 % U(VI)
  - 43 % U(IV)
- Ethanol Exp 4
  - 87 % U(VI)
  - 13 % U(IV)
- Methanol Exp 5
  - 4 % U(VI)
  - 93 % U(IV)



Kelly, Kemner, & Ravel (Adv. Photon Source at ANL) working with A. Madden (ORNL)

Exp 4 – Communities

Both communities reduce U but there are large differences in composition (data from final time point)



Ethanol

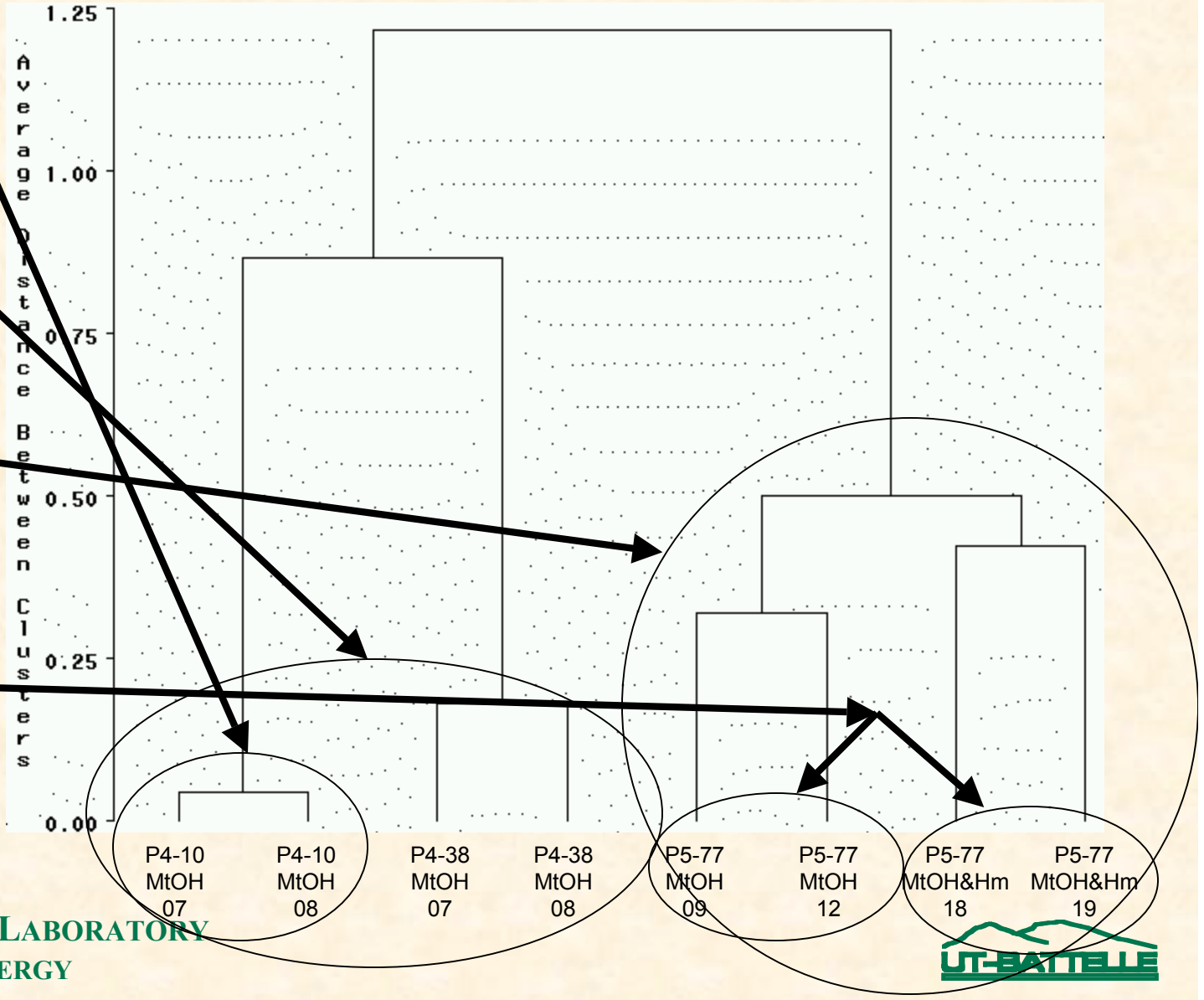
Actinos dominate

Glucose - Proteobacteria

Tend to be more Alpha & B

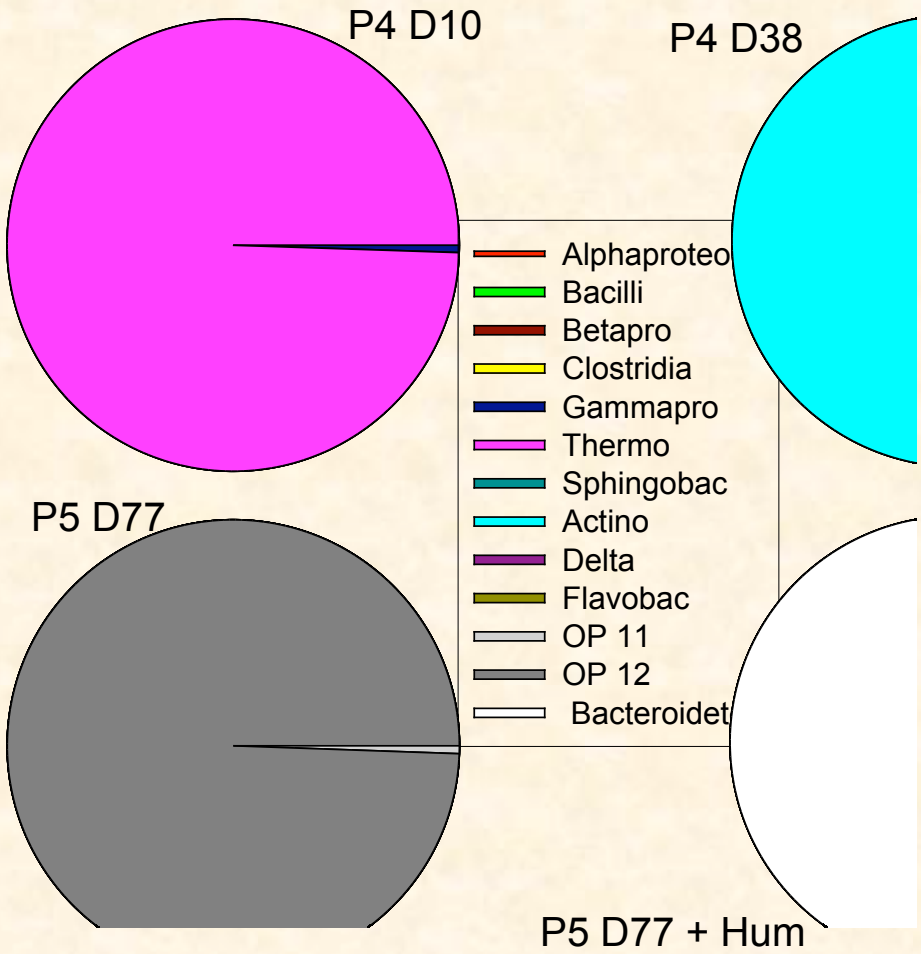
Hierarchical Cluster Analysis of Clone libraries from Exp 4 (P4) & Exp 5 (P5) Communities Grown on Methanol

- Replicates at the same time point cluster together (e.g, day 10)
- Non U reducing samples (P4) at two time points (day 10 & 38) cluster together
- U reducing samples (P5) with and without humics (day 77) cluster together
- Our hypothesis that humics would not change the community composition was not correct



Clone Distribution In P4 methanol – no U reduction and p5 methanol – U reduction

- High level of taxonomy averaged over reps
- No clones in common between P4 and P5
- More diversity in P5
- Many clones in P5 were associated with organics (e.g. solvent, toluene) degradation – e.g. Trichlor-obacter (delta) & Polaromonas *(beta)*
- Different beta-proteobacter dominate (e.g., Rhodoferax in P5, Laribacter in P4)
- P4 Has more gamma-proteobacter (e.g., Escherichia)
- P5 has more deltaproteobacter (e.g., Geobacter)
- P5 has more Sphingo-bacteria (e.g., Roseivirga)



Heterogeneity in Response to Methanol Addition

- In previous sampling there were two types of response
  - iron and U reduction or no iron or U reduction
  - beta-proteobacter dominate both conditions with some shifts with U reduction
- Major questions
  - Do differences in community structured resulting from methanol addition correlate with iron and U reduction
  - How common is each response
- Soils from different areas dosed with methanol
  - Area 3 (2 cores –FW116 and FB104)
    - Area 3 North
    - Area 3 South
  - Area 2
  - Background Area
- In these samples we saw iron reduction in all methanol enriched samples except those from background area
- Clone libraries produced from control and treated samples

0 240 m

Analysis Approach and Samples

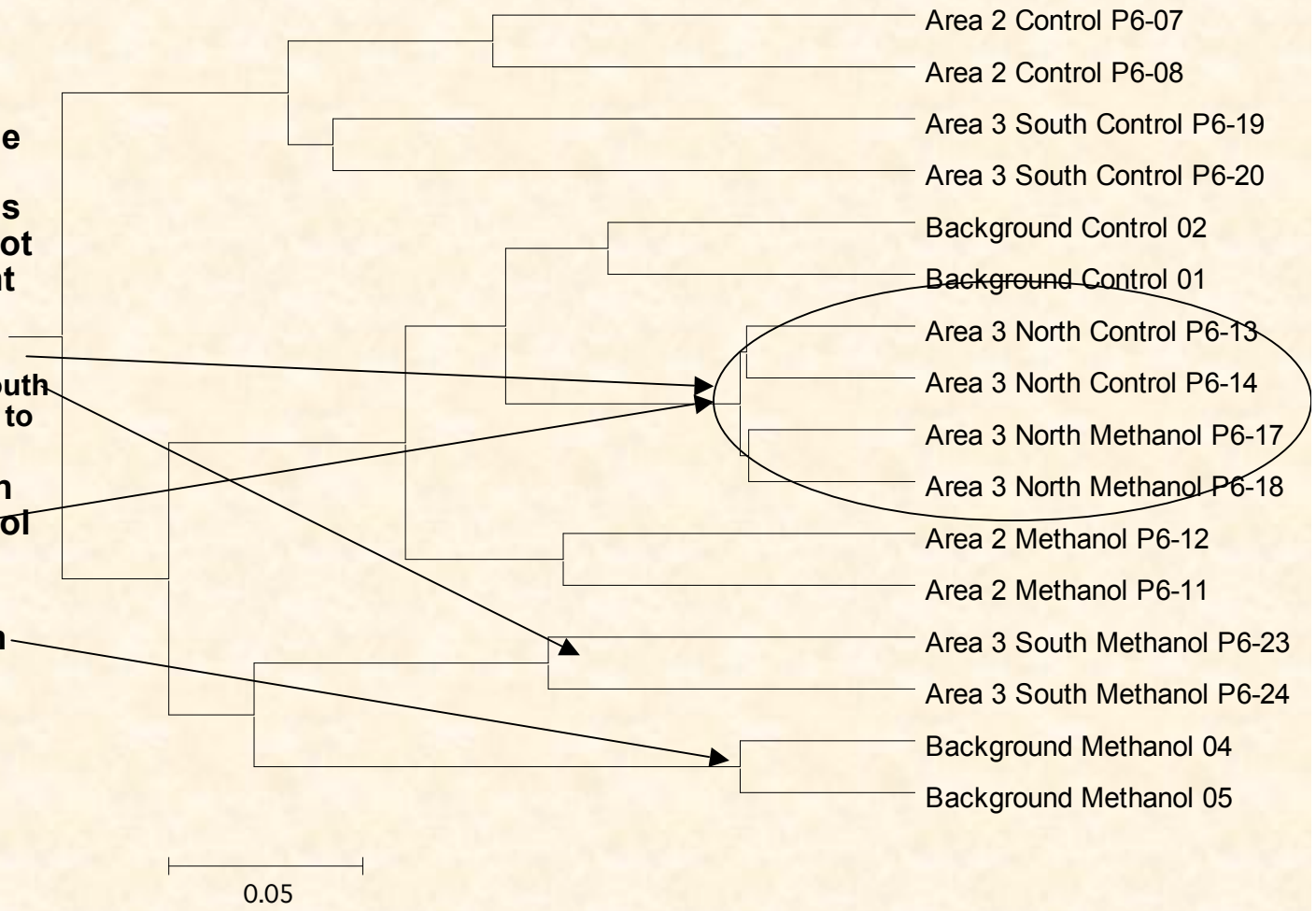
- Primary analysis to date is using UniFrac
  - http://bmf.colorado.edu/unifrac
- Compare microbial communities in a phylogenetic context
- Find differences among communities
- Cluster multiple environments
- Test which environments are significantly different
- These are all from one time point – analysis of second time point is underway

n/n	Environment Name	Count
1	Area 2 Control P6-07	96
2	Area 2 Control P6-08	95
3	Area 2 Methanol P6-11	89
4	Area 2 Methanol P6-12	91
5	Area 3 North Control P6-13	96
6	Area 3 North Control P6-14	95
7	Area 3 North Methanol P6-17	91
8	Area 3 North Methanol P6-18	91
9	Area 3 South Control P6-19	96
10	Area 3 South Control P6-20	91
11	Area 3 South Methanol P6-23	93
12	Area 3 South Methanol P6-24	96
13	Background Control 01	93
14	Background Control 02	95
15	Background Methanol 04	91
16	Background Methanol 05	90
	Total Count	1489

Jackknife Environment Clusters

Cluster analysis reveals Heterogeneity and Consistency

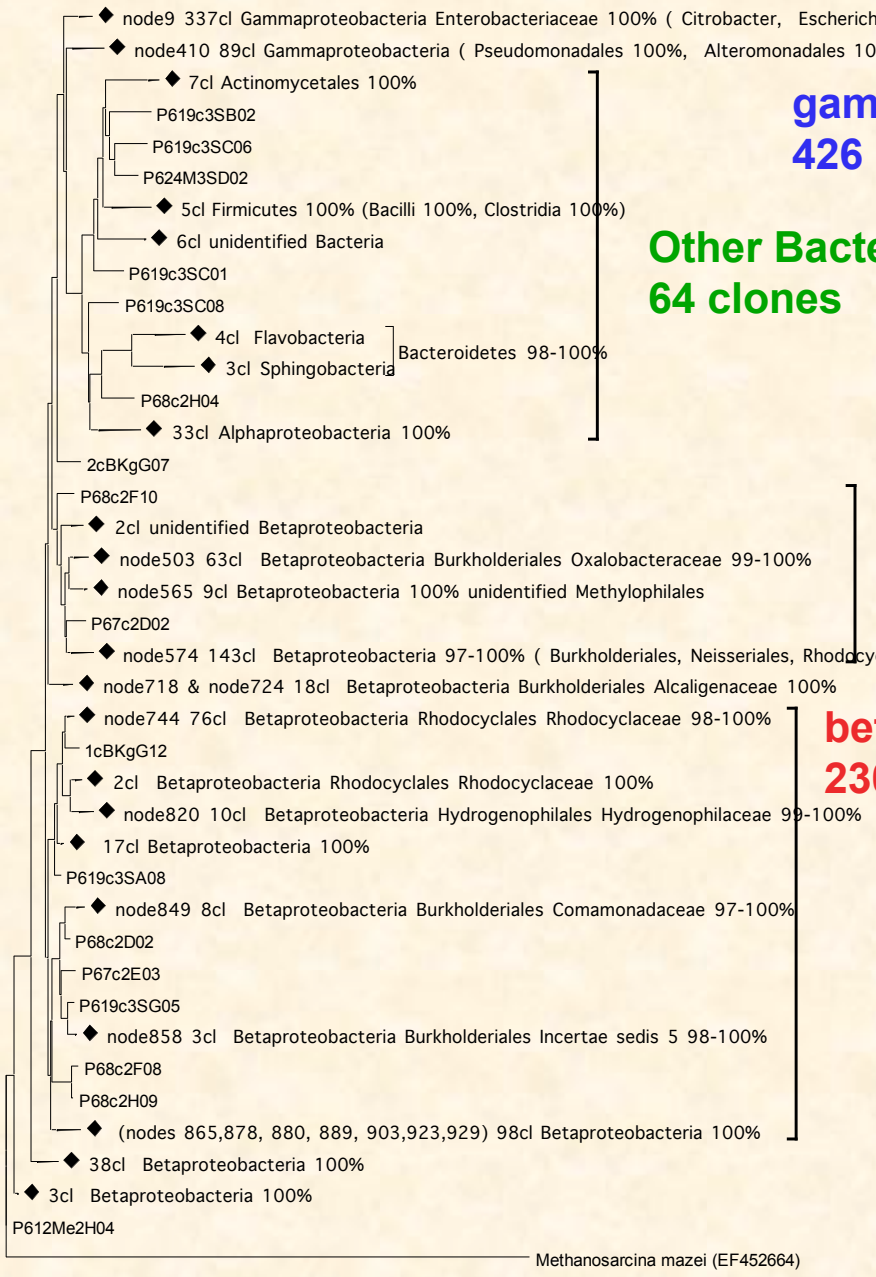
- **Heterogeneity in communities response to methanol addition and untreated samples among samples but not necessarily consistent within regions**
  - **E.g. area 3 north very different than area 3 south – actually more similar to area 2**
- **Area 3 north not much of a shift with methanol addition**
- **Background sample with no iron reduction has very different community**
- **Replicates samples consistently cluster togethe**



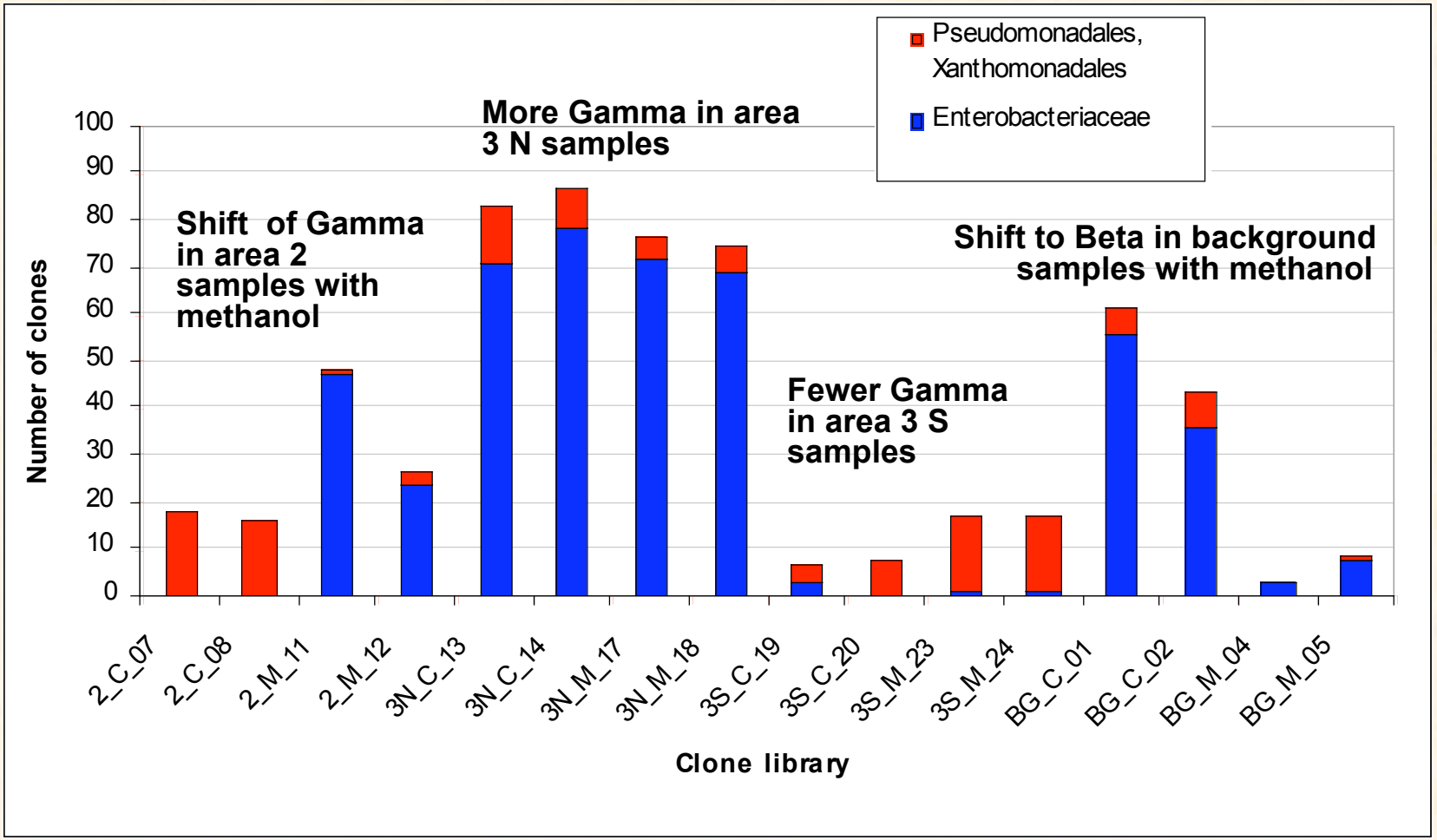
A distance a 0 means that two environments are identical, and a distance of 1 means that two environments contain mutually exclusive lineages.

Types of Bacteria

- Almost all were gamma and beta proteobacteria
- Beta divided in two major groups
- Relatively few represented as single clones

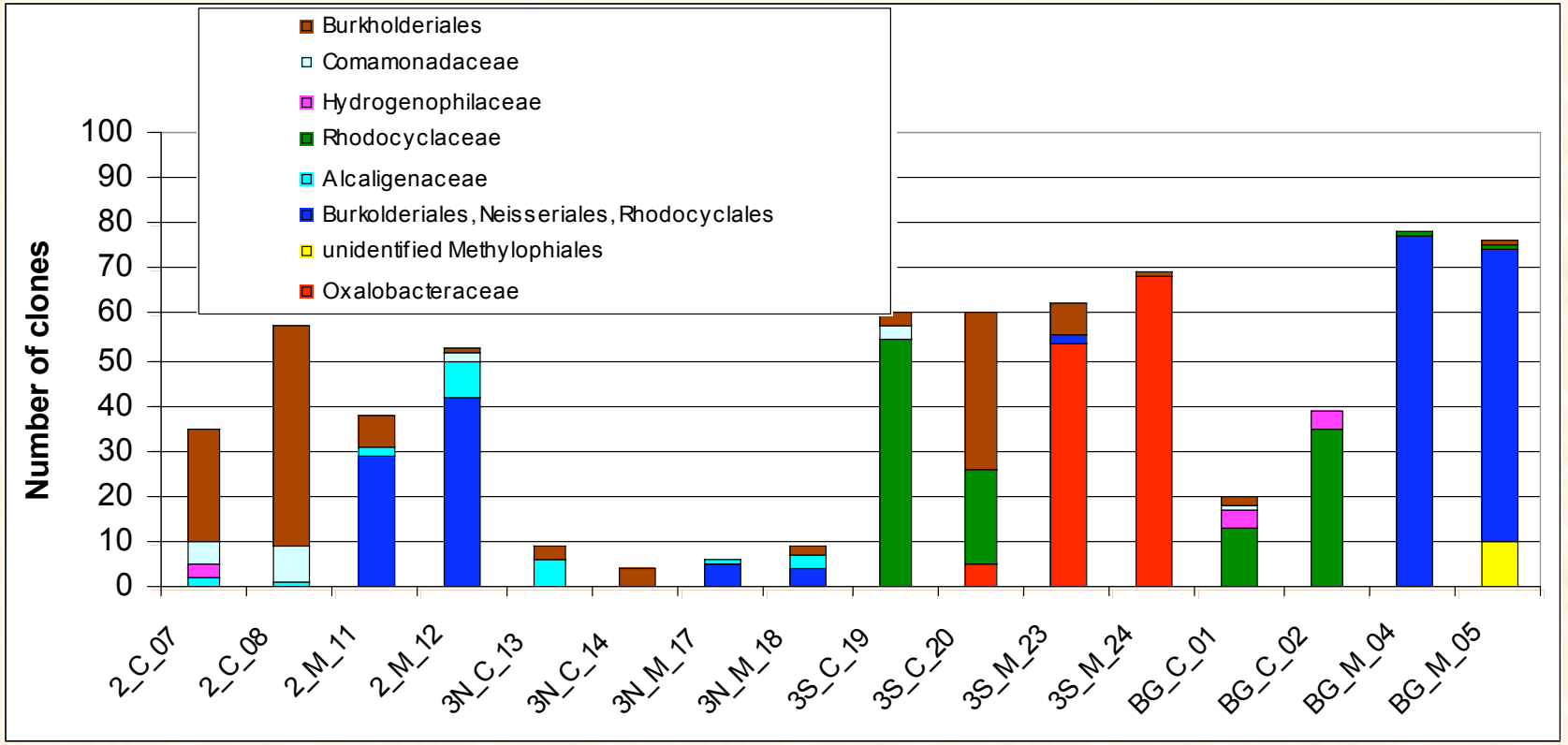


Gama-proteobacteria



- Not a widely diverse set of gamma-proteobacteria – few major groupings
- No consistent shifts in major types with methanol addition

Distribution of Beta-proteobacteria



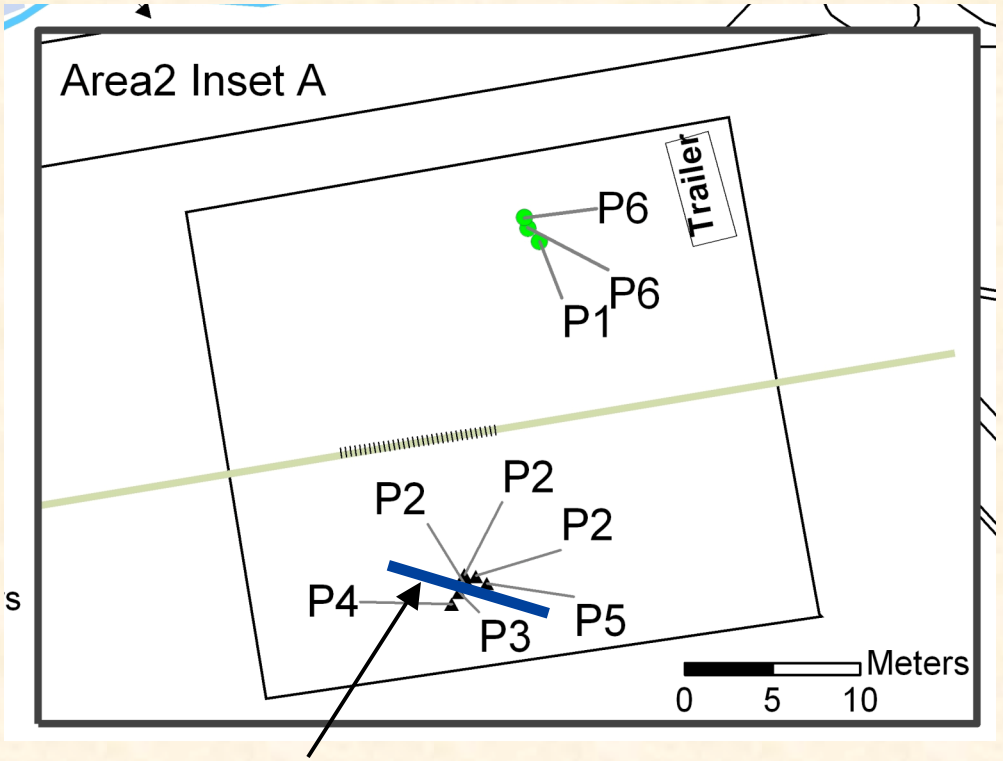
- More diverse set of beta-proteobacteria
- Shifts in types of beta in all samples with methanol addition but not consistent among samples (e.g., Area 3 south to Oxalobacteriace area 2 to Burkolderiales, Neissriales, Rhrodocyclales)

Resource-ratio theory and uranium reduction – ongoing experiment

- Do changes in C:P ratio impact community dynamics and lead to differential rate and extent of metal reduction?
- Treatments (C:P)
  - no added P (donor only)
  - 106:1
  - 106:5
- Methanol or ethanol, P_i

Additional Heterogeneity Experiment – Smaller scale

- Transect across area 2 is planned in high U zone
- 4 to 5 samples across 10 meters



Summary

- There were consistent results in the experiments indicating;
  - All substrates promoted nitrate reduction,
  - Glucose, ethanol, acetate always promoted U reduction
  - Although rare, methanol did occasionally promote extensive U reduction – (community heterogeneity?)
  - there appear to be limitations imposed on the community related to some substrates (e.g. methanol).
  - PLFA indicated different communities with methanol
  - TRFLP and Clone libraries indicated distinct differences among communities even in treatments that promoted U reduction
- Further sampling is taking place (e.g., smaller scale heterogeneity) as is additional analysis of the community structure (e.g., functional gene arrays)

Genetic, Geochemical and Community Controls on the Microbial Methylation of Mercury

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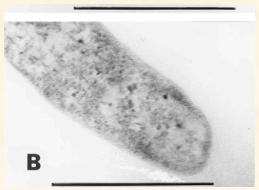
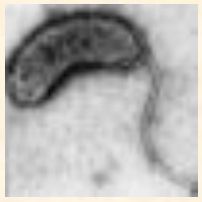


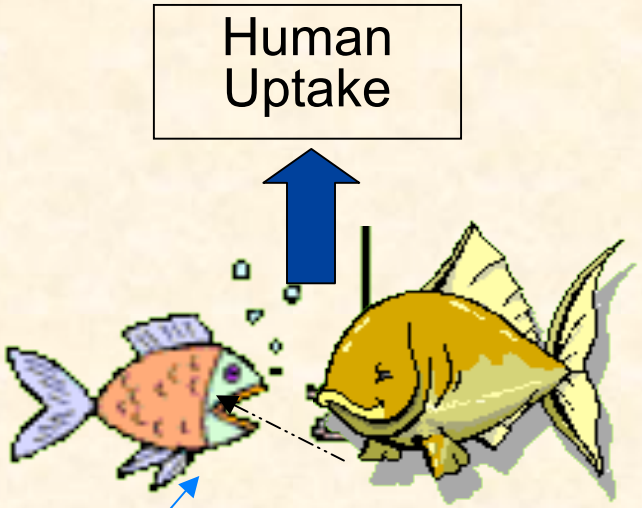
Desulfovibrio desulfuricans

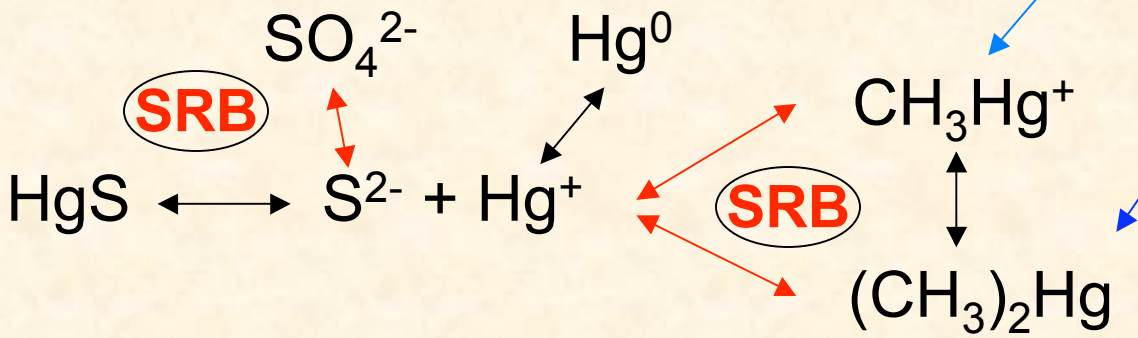


Desulfovibrio vulgaris

Sulfate Reducing Bacteria and the Mercury Bioaccumulation Pathway

- Anaerobic sulfate-reducing bacteria (SRB) produce a significant fraction of monomethylmercury (MMHg).
- MMHg is the compound that enters the food chain, and, when ingested by humans, is a potent neurotoxin.





Research Questions

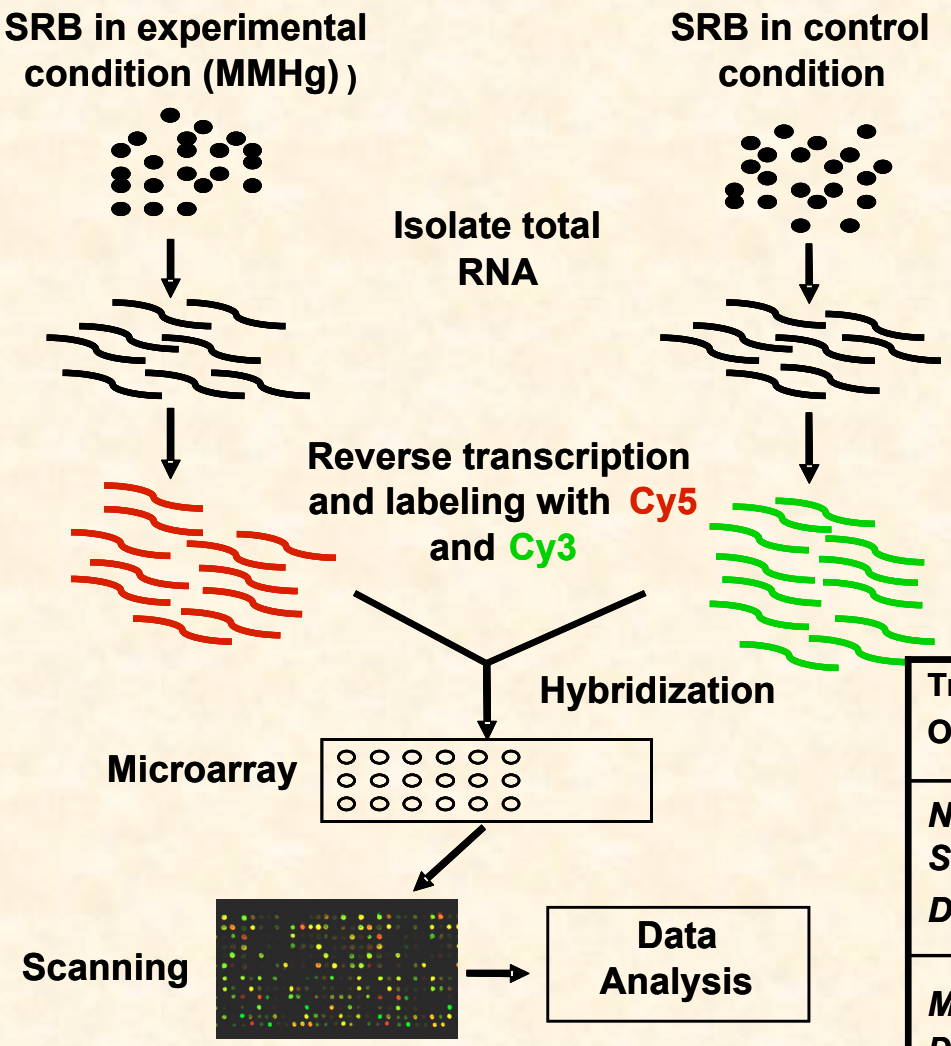
- What is genetic basis of methylation in SRB?
  - The only proposed pathway is based on work in *Desulfovibrio desulfuricans* LS in which a corrinoid-containing protein involved in the acetyl-CoA pathway has been identified as a key component (Compeau and Barth 1985; Berman et al. 1990; Choi and Bartha 1993; Choi et al. 1994a, 1994b).
  - A related species, *D. vulgaris,* does not methylate mercury at detectable rates (Ekstrom et al. 2003).
- What is the effect of geochemistry on the genetic regulation of MMHg production?
  - Possible important factors are pH, redox, [HgS^0], ferric-iron reducing conditions.
- How does the composition of the microbial community affect MMHg production?

OBJECTIVES

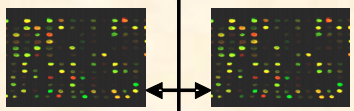
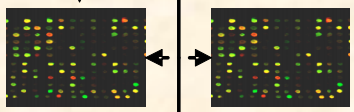
The objectives of our research are to:

- **Delineate the genetic basis for mercury methylation in *Desulfovibrio***
  - **Gene expression – ORNL lead**
  - **Mutagenesis – U of Missouri-Columbia**
  - **Complementation - U of Missouri-Columbia**
- **Examine the biogeochemical controls on mercury methylation – ORNL**
- **Translate the knowledge of the genetic basis and the understanding of the environmental controls (biogeochemical and community) influencing the mobilization and immobilization process to the field. - ALL**

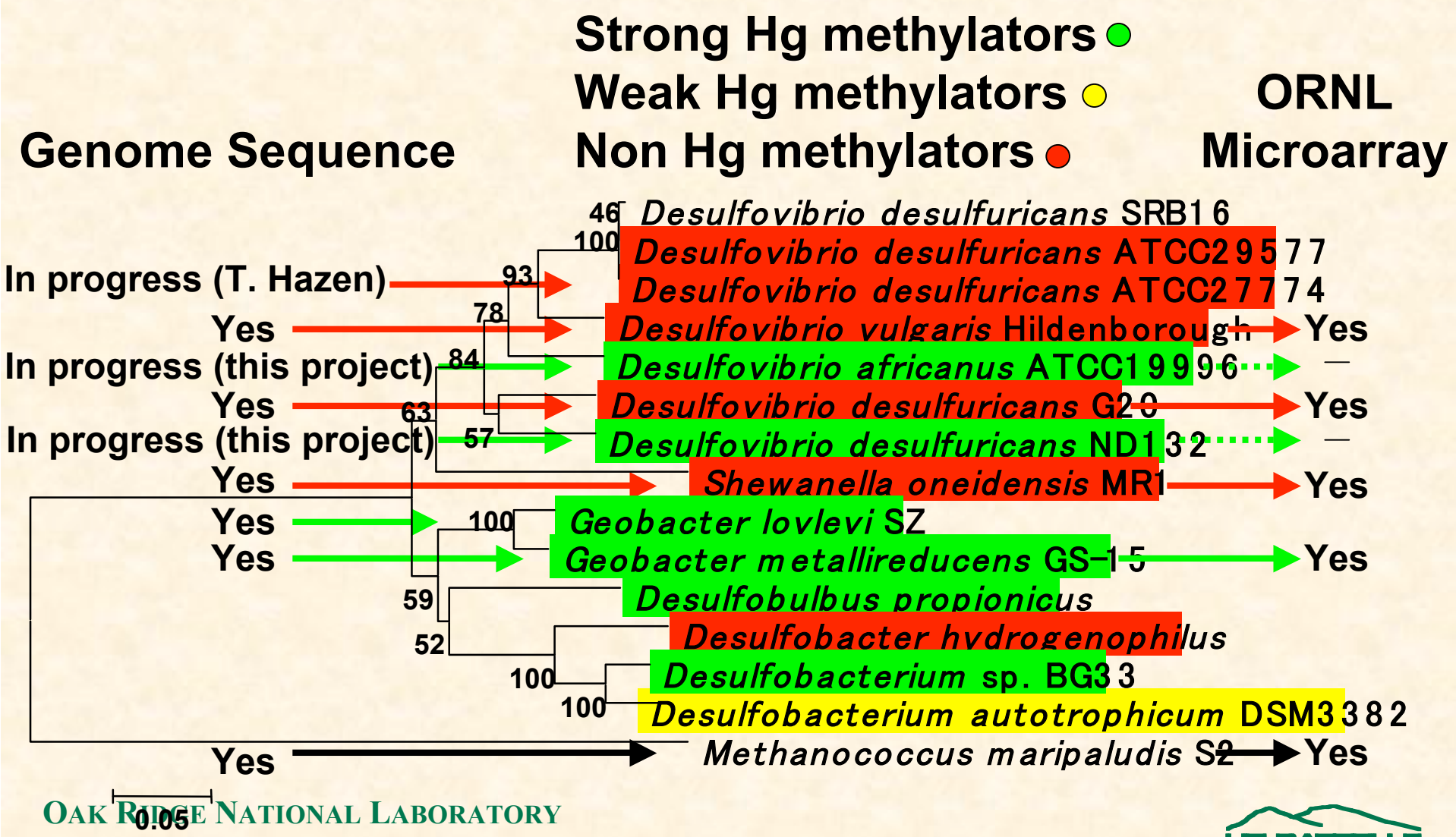
Comparative gene expression studies



- Use whole genome microarrays to compare gene expression in methylating and non methylating strains in response to Hg
- Whole genome arrays will also be used to compare gene expression in non methylating strains with and without Hg

Treatment & Organism	Hg	No Hg
Non-methylating Strains *D. vulgaris & G20*		
Methylating Strains *D. africanus & ND132*		

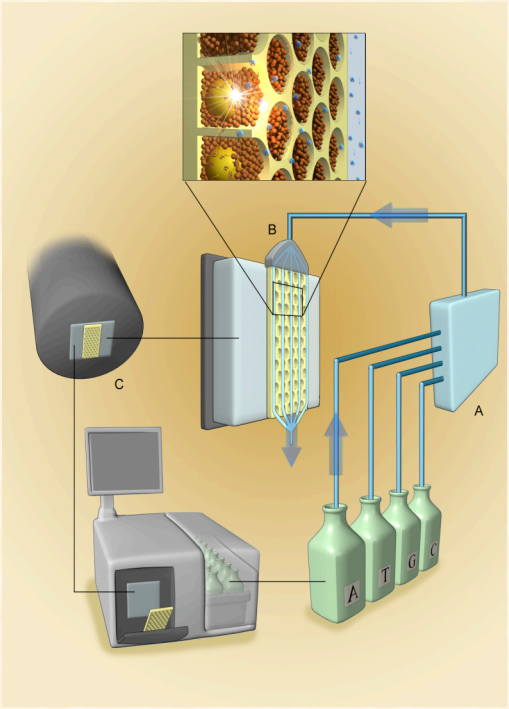
Preliminary Progress



Modified from C. Gilmour, et al (2006)

Sequencing Plan and Methylation

- Sequencing underway with U of Oklahoma and 454
  - *D. africanus*
  - *Desulfovibrio desulfuricans* ND132 also
- LSP prepared to finish these at JGI and do one additional *Desulfovibrio desulfuricans* strain that can methylate



Sequencing by synthesis on a 454 instrument

Ongoing Methylation and Transcriptomics Studies

- Started methylmercury production measurements
- Started mercury sensitivity studies
- Transcriptomics studies about to start for non-methylating strains using
  - G20 array
  - DvH array

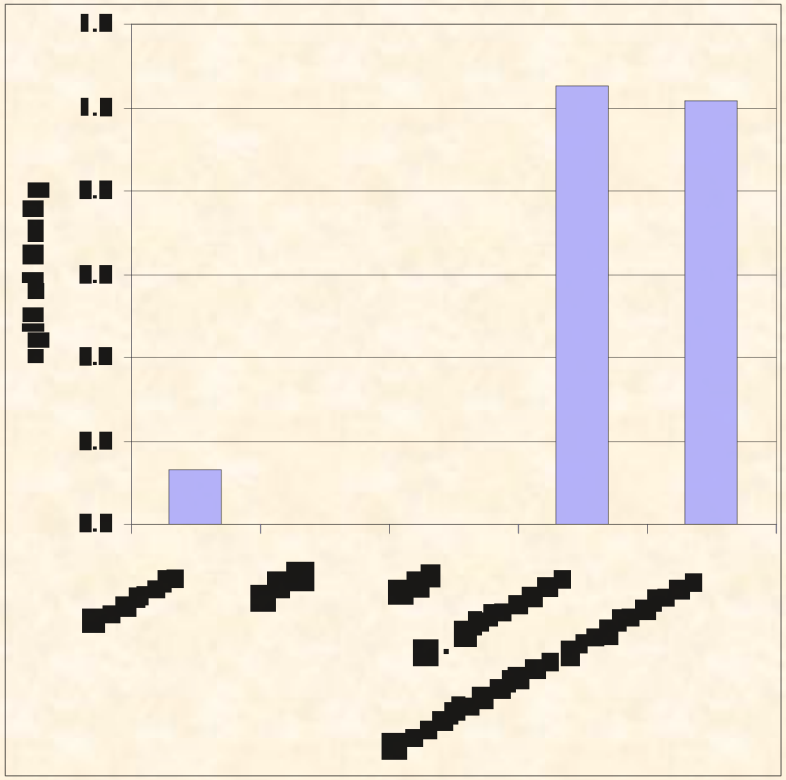


Figure. Methylmercury production in several strains of sulfate reducing bacteria.

ACKNOWLEDGEMENTS

- This research is funded by the Environmental Remediation Science program (ERSP), Biological and Environmental Research (BER), U.S. Department of Energy.
- Oak Ridge National Laboratory is managed by UT-Battelle, LLC for the U.S. Department of Energy under contract DE-AC05-00OR22725.
- We thank the organizers of the meeting for the opportunity to present this ongoing work.

Results from a GS FLX Run

	E. coli
Genome Size:	4,639,675
Number of Runs:	1
Assembly contigs:	105
Assembly Cover:	97.61%
Overall Accuracy:	99.998%
Avg. Contig Size:	43.3 kb
N50 Contig Size:	105.5 kb
Largest Contig:	204.7 kb

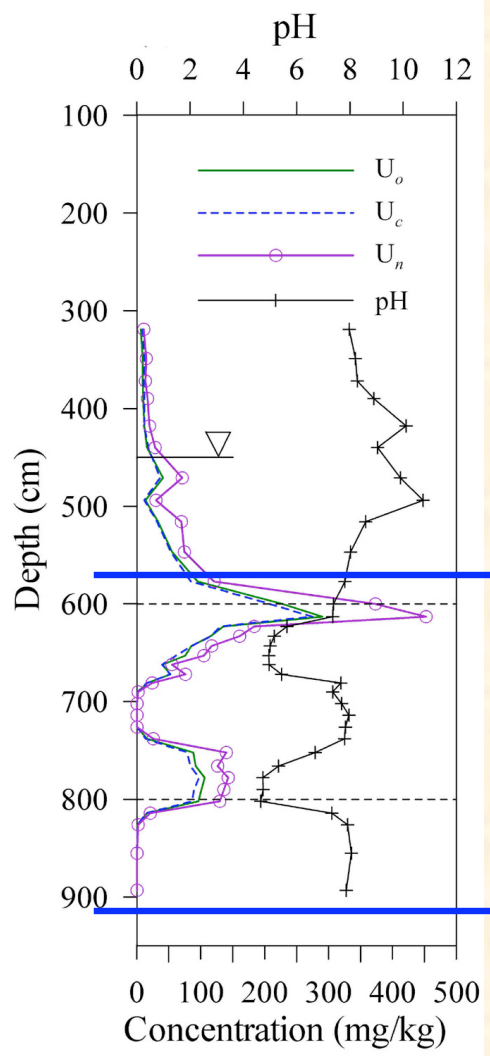
Newer Paired End Approach:

20bp paired "tags" allow for contig orientation and scaffolding

112K pairs gave 20 multi-contig scaffolds

This is an example of what we might expect.

Not all U is equally reducible



Microcosms –
4:1 liquid:solid;
initially ~1.5 ppm
U(aq)

Average total solid phase U ~96 ppm

~6% total U in aqueous phase

17% reduction for glucose

4% for ethanol

Consistent with literature
e.g., Ortiz-Benard et al. (2004) AEM

Sulfate Changes Compared to U Changes in Exp 4

- Sulfate reduction (top) lags behind Nitrate (largely gone before day 4) and U reduction (bottom)
- Very slow response for methanol
- No detectable difference with Ethanol + Humic