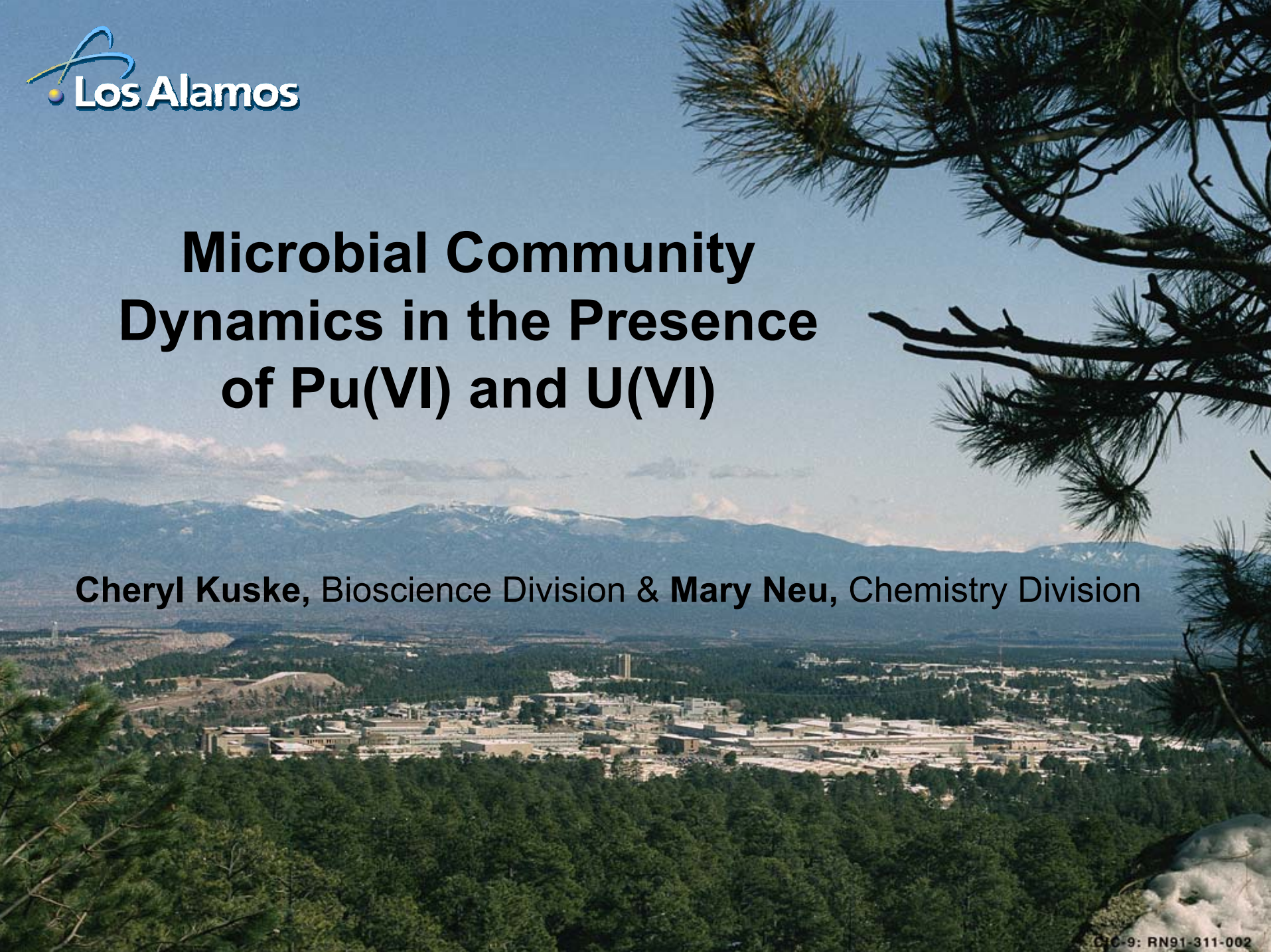
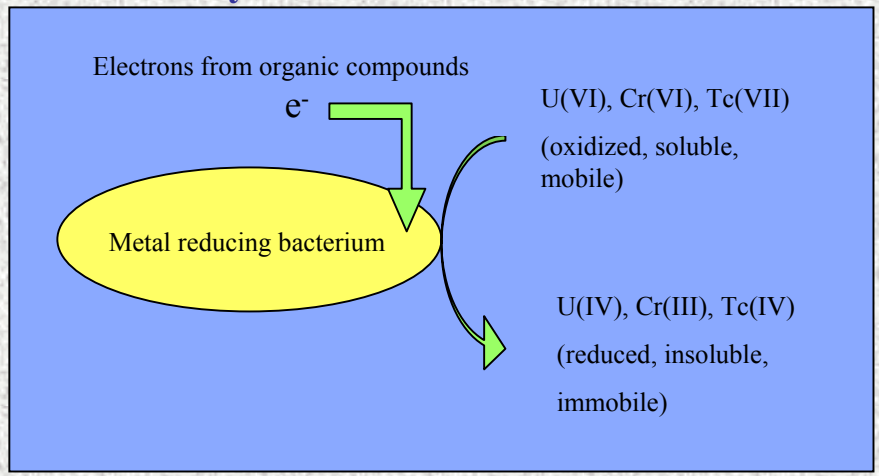

Los Alamos
Microbial Community Dynamics in the Presence of Pu(VI) and U(VI)
Cheryl Kuske, Bioscience Division & Mary Neu, Chemistry Division
CIC-9: RN91-311-002

Diverse Bacterial Species Reduce U(VI)

Direct Enzymatic Reduction of Contaminant

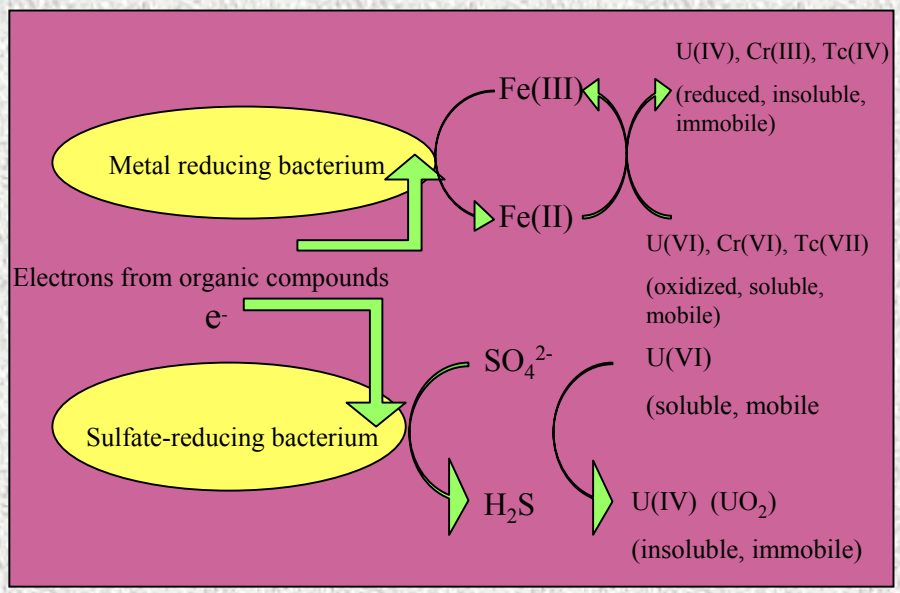


Proteobacteria Division

γ-subdivision: ***Shewanella***
Pseudomonas

δ-subdivision: ***Geobacter***
Anaeromyxobacter
Desulfovibrio
Desulfotomaculum
Desulfosporosinus

Indirect Reduction of Contaminant



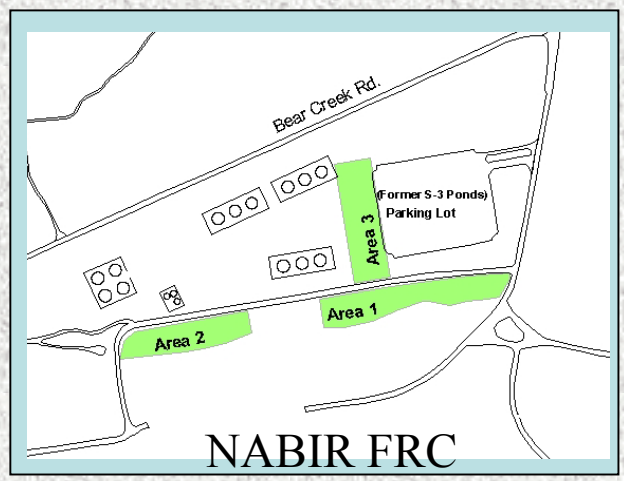
Firmicutes Division

Class I: ***Clostridium***
Class III: ***Bacillus***
Enterococcus

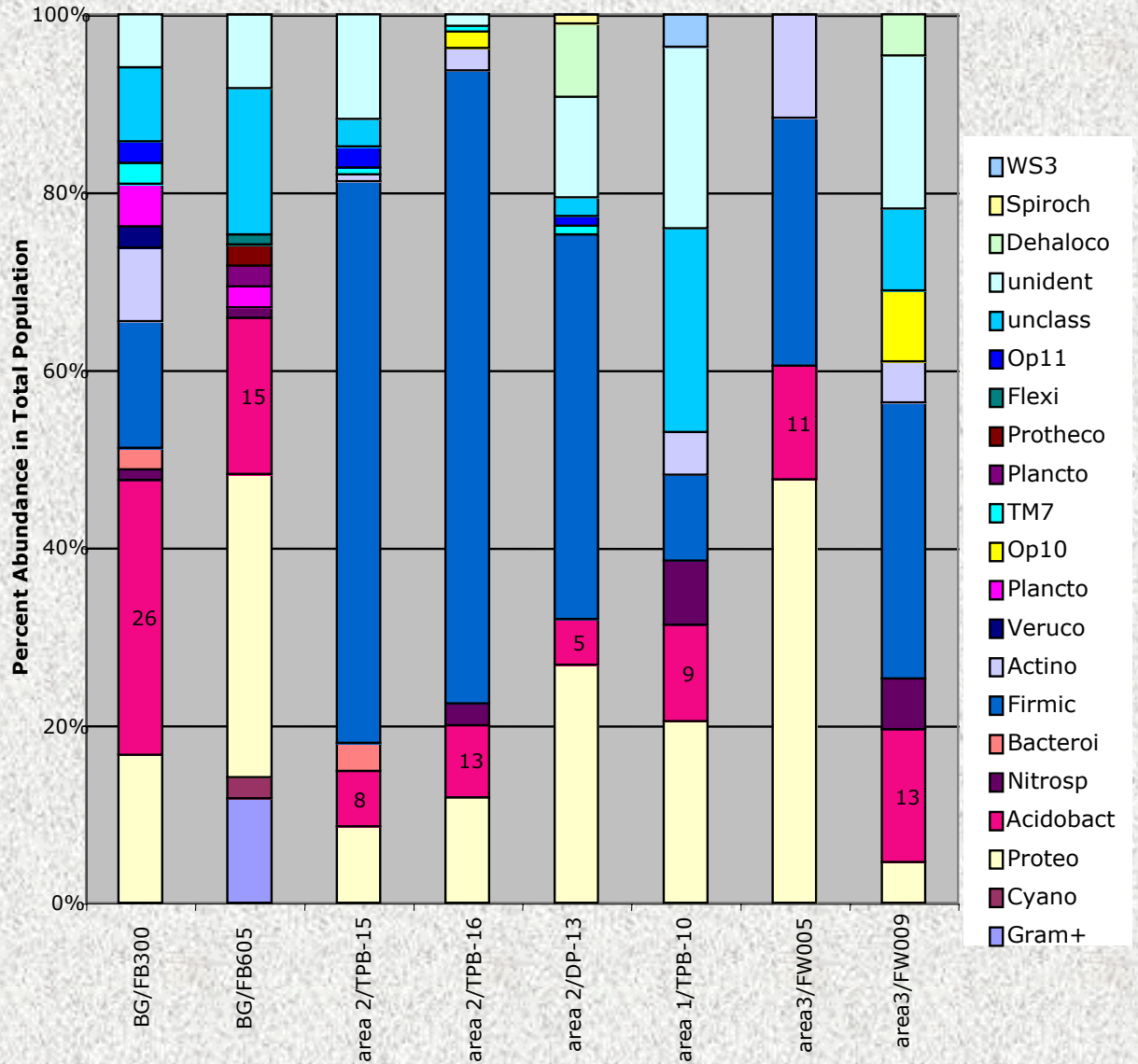
Acidobacteria Division

Subgroup 8: ***Geothrix***

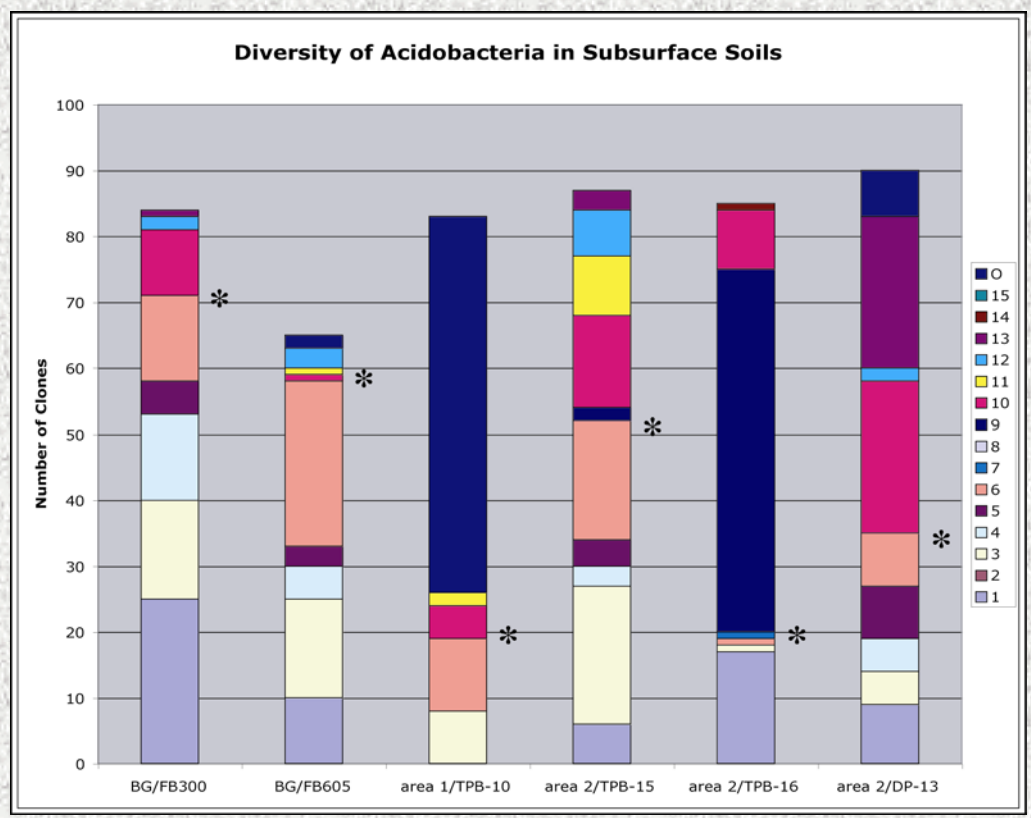
Diverse Bacteria are Present in Contaminated Sites

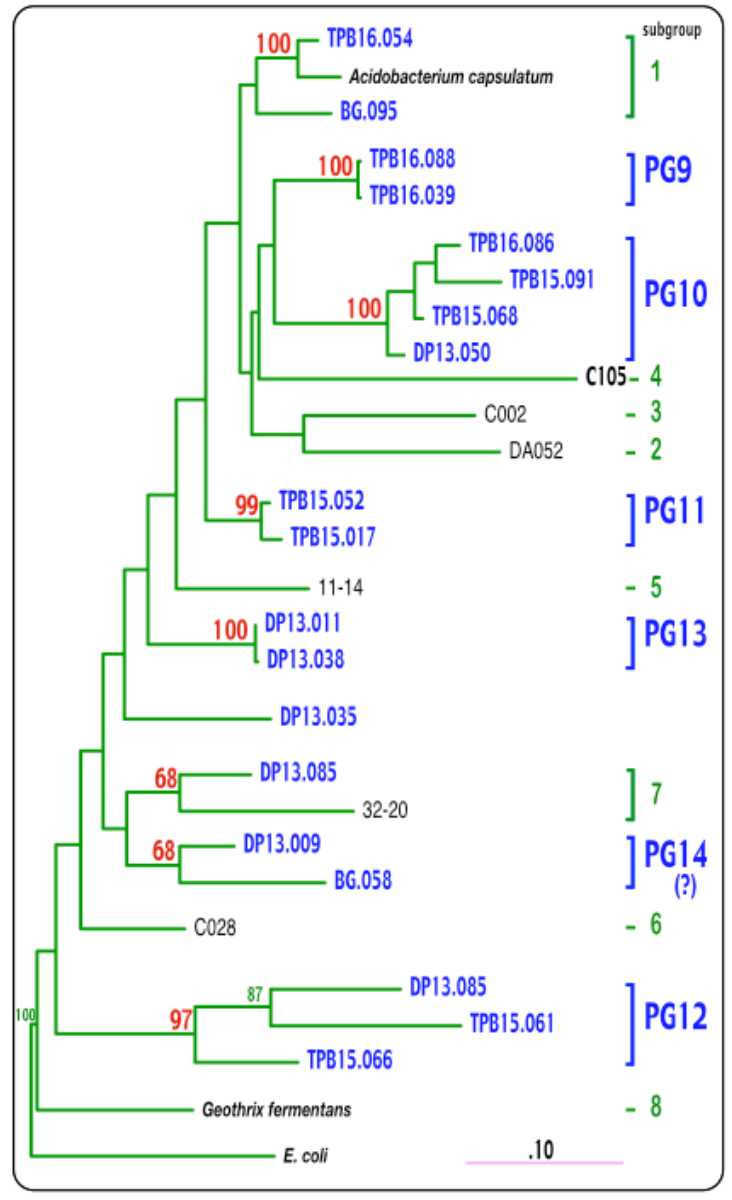


Clostridium
Desulfotomaculum
Desulfobacterium
Desulfuromonales



Novel Bacterial Species in Contaminated Sites





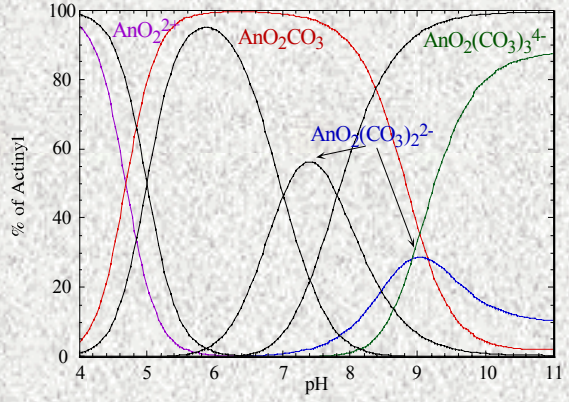
Project Objectives

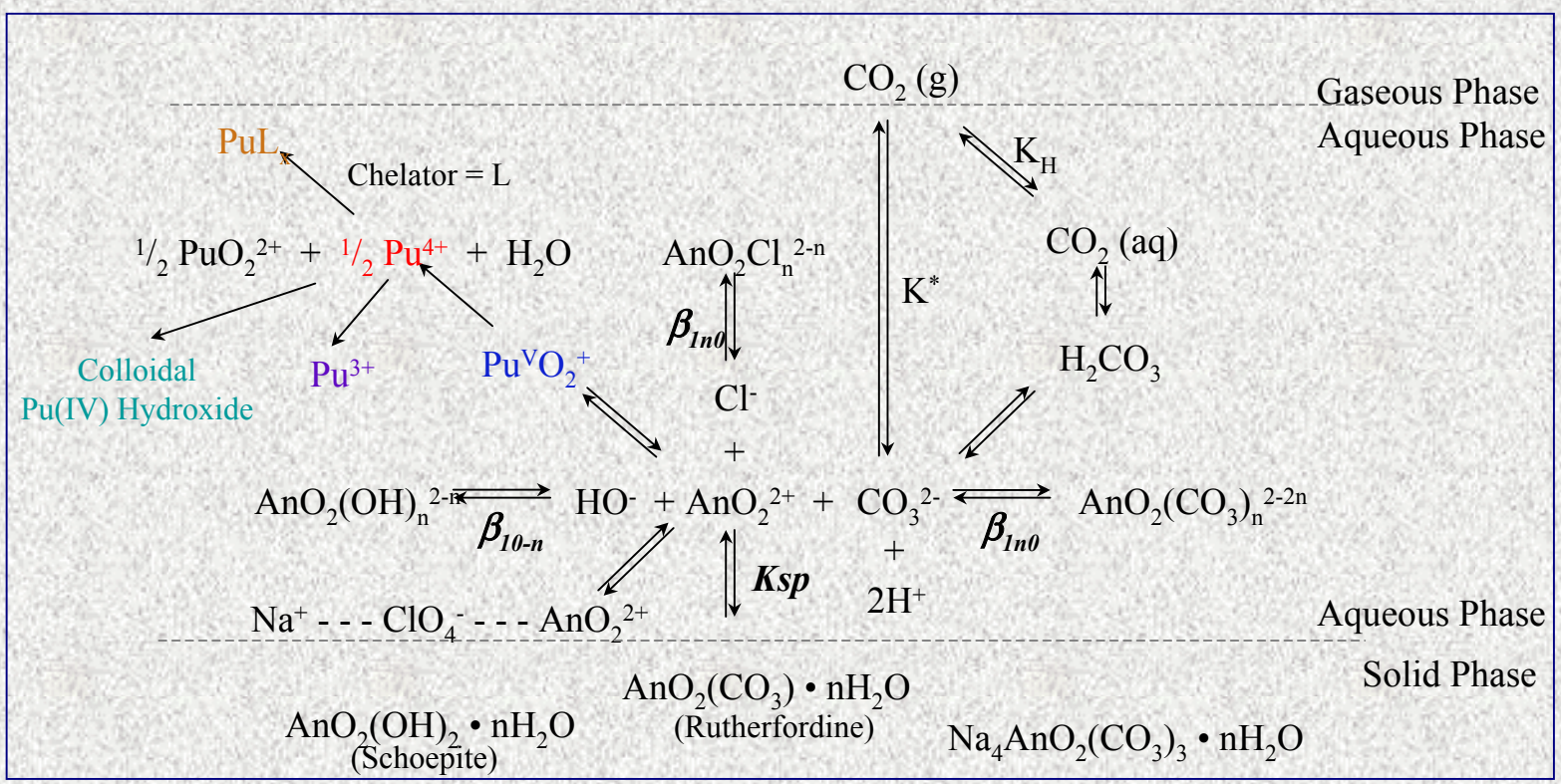
- **Determine the effects of Pu(VI) on natural sediment bacterial communities**
- **Identify bacterial species that are active in the presence of Pu(VI)**
- **Compare bacterial community dynamics exposed to Pu(VI) or U(VI)**

Actinyl(VI) Chemical Equilibria, An = U or Pu

Pu(VI) and U(VI) are chemically similar; however

- **Speciation/complexation constants differ**
- **U(VI) is generally more stable than Pu(VI)**
- **Pu(VI) can be reduced to Pu(V, IV, III)**
- **Pu(V) can disproportionate**
- **Pu(IV) and U(IV) have very different chemistries**





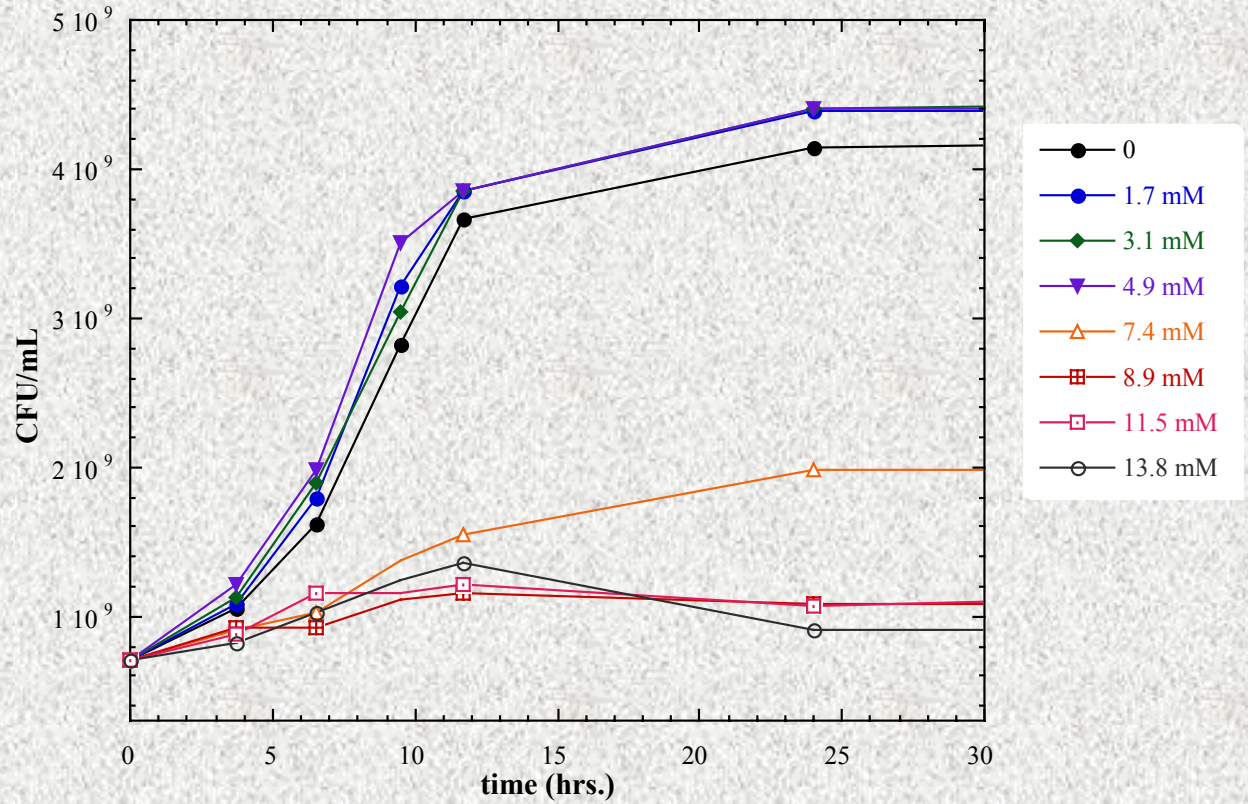
Toxicity of Pu(VI) to *Shewanella putrifaciens* CN32

Growth with 50 mM Fe as electron acceptor, 10 mM lactate, and varying [Pu(VI)]

< 5 mM Growth

6-8 mM Toxic, dec. growth

>8 mM No growth



Toxicity depends on chemical form

Actinides tend to be toxic to bacteria in the concentration range of mM to mM

10^{-3} and 10^{-7} M chosen to study effects of actinide stress

Experimental Strategy

Laboratory time course experiments with sediments exposed to Pu(VI) or U(VI)

- actinide concentration
- incubation time
- incubation conditions (anaerobic, aerobic)

Monitor bacterial community

- total biomass, composition and relative abundance of the total bacterial community (16S rRNA-based, PCR/RT-PCR, T-RFLP, clone/sequence libraries)

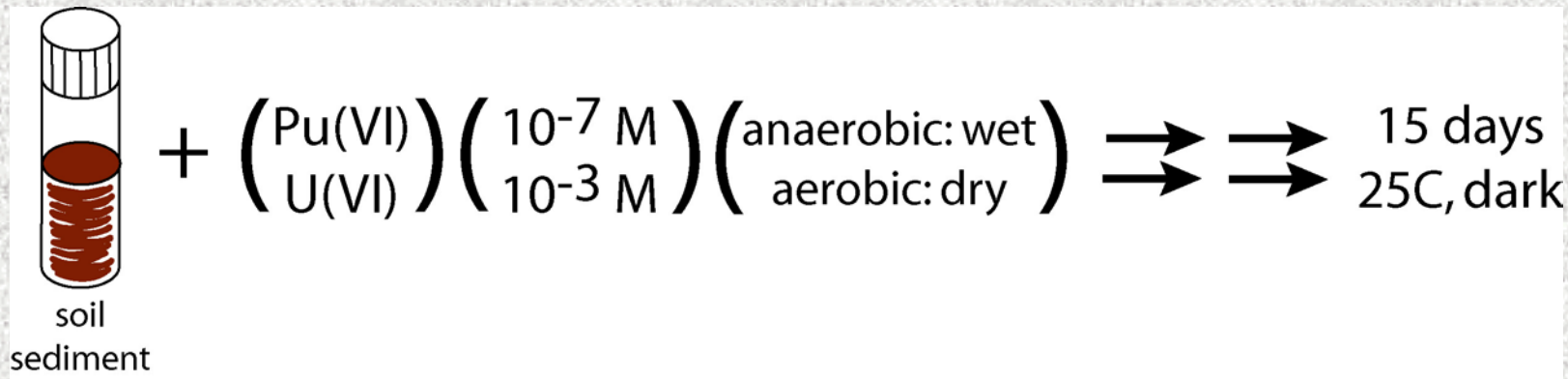
- composition and relative abundance of target bacterial groups (quantitative PCR, clone/sequence ID, culture)

Monitor actinide concentration and species

16S rRNA Quantitative PCR Assays for Target Groups

Target Group	Amplicon Length	Reference
Desulfotomaculum spp. (lineage 1)	1066 bp 700 bp	Stubner (2002) Daly *et. al.* (2000)
Desulfovibrio spp.	610 bp	Daly *et al*. (2000)
Geobacteraceae family	~ 330 bp	Holmes *et. al.* (2002)
Shewanella putrefaciens subgroup	540 – 570 bp	Barns and Kuske (in progress)

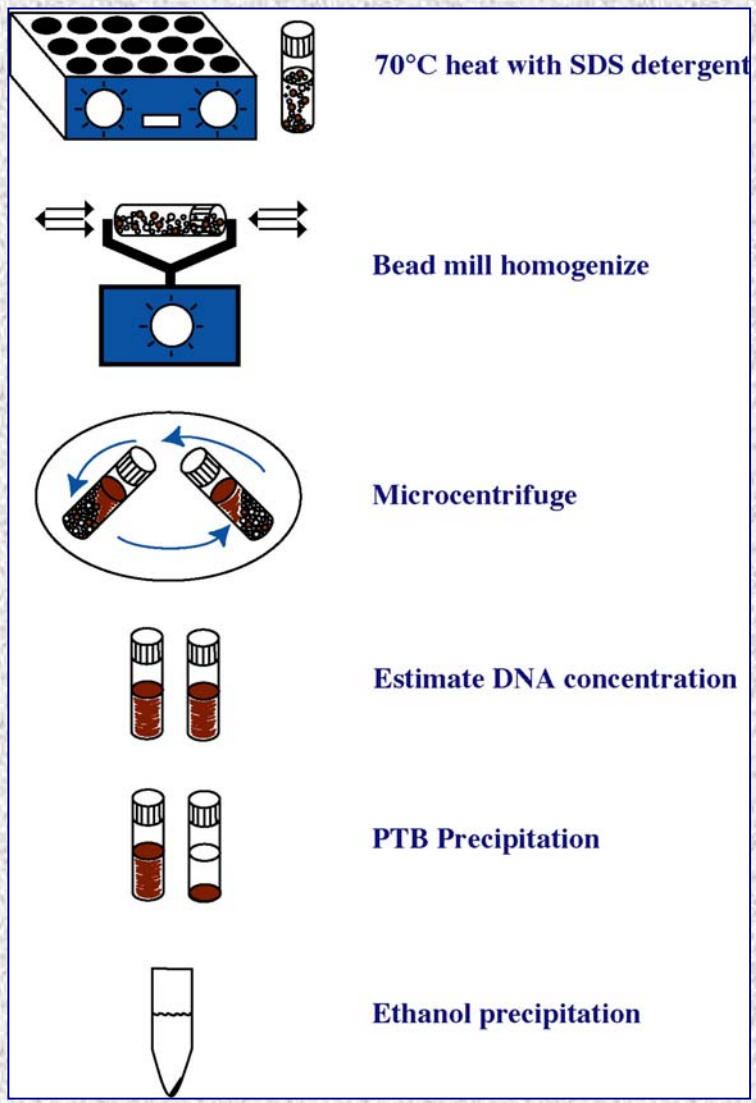
Preliminary Experiment



Actinide Concentration
DNA Concentration
16S rDNA T-RFLP Profiles
Clone/Sequence Libraries

Method Modification for the Actinide Lab

DNA Preparation



Challenges

Time

Multiple centrifugation steps

RNA extraction adds steps

Mixed waste (EtBr, chloroform)

Removing actinide

Controls, Solution pH, and Final Actinide Concentration

Treatment	Initial [An] µg/g soil	Soln pH	Final [An] µg/mL
Original Soil		~ 7.0	-----
NO_3^- control WET		5.0	-----
NO_3^- control DRY		5.0	-----
U 10^{-7} M WET	0.0238	3.4	4.08×10^{-4}
U 10^{-7} M DRY	0.0238	3.4	3.79×10^{-4}
U 10^{-3} M WET	238	3.8	8.99×10^{-4}
U 10^{-3} M DRY	238	3.8	1.51×10^{-3}
Cl^- control WET		5.0	-----
Cl^- control DRY		5.0	-----
Pu 10^{-7} M WET	0.0239	4.5	2.17×10^{-7}
Pu 10^{-7} M DRY	0.0239	4.5	4.17×10^{-9}
Pu 10^{-3} M WET	239	5.0	1.80×10^{-1}
Pu 10^{-3} M DRY	239	5.0	6.15×10^{-2}

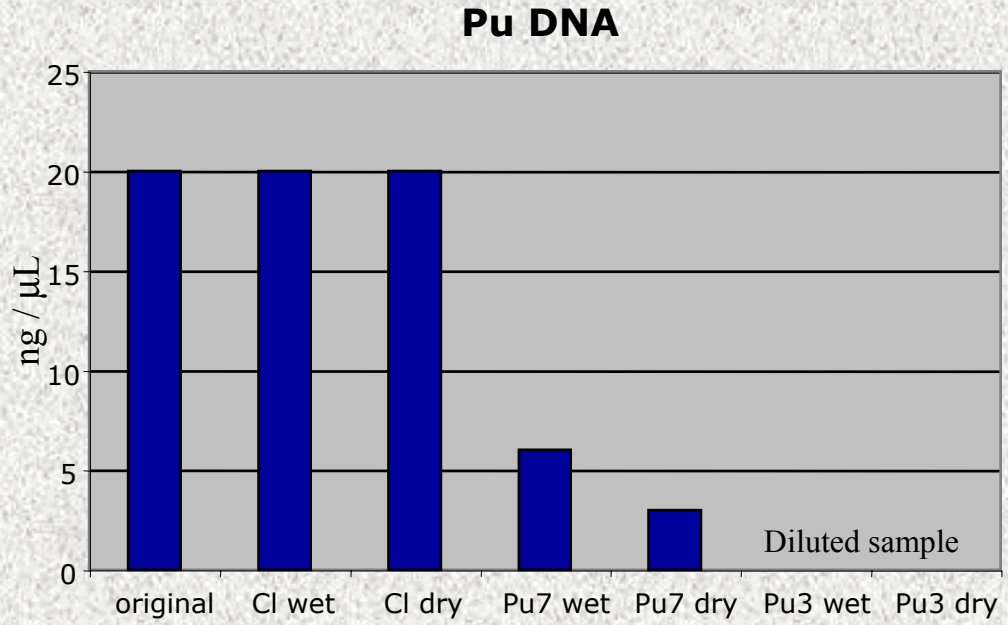
U = ^{233}U(VI) + ^{238}U(VI) in HNO_3
Pu = ^{239}Pu(VI) in HCl

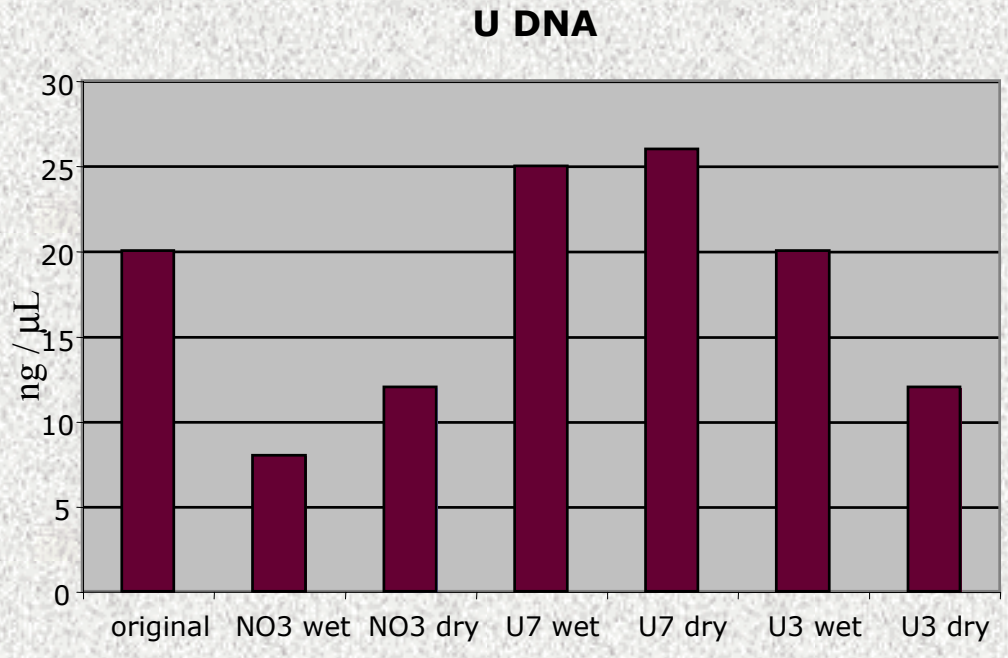
- incubation pH is below soil pH
- extraction procedure removed most of actinide
- high Pu samples had to be diluted 1:100 for transfer

Extracted DNA Concentration

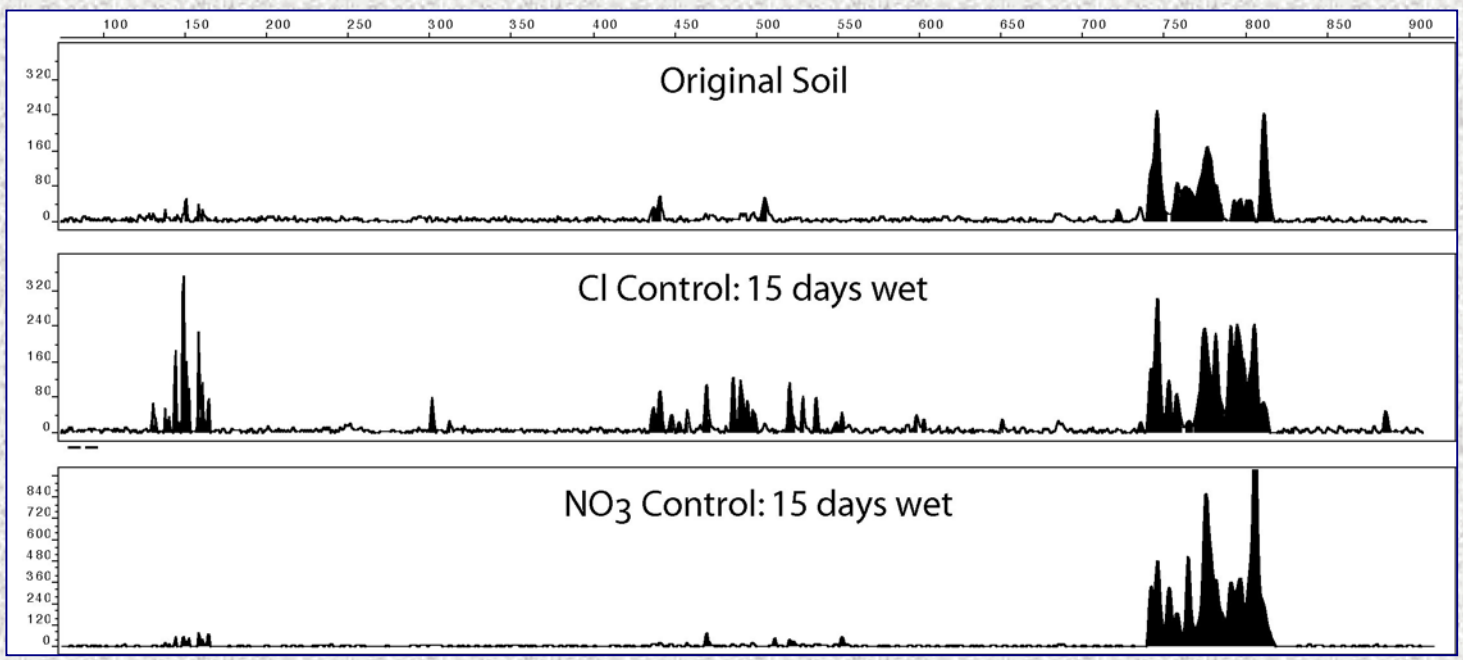
Observations

- DNA yield low in Pu 10^{-7} M treatments
- DNA yield appears reduced in NO_3^- control treatment but not in U 10^{-7} M treatment



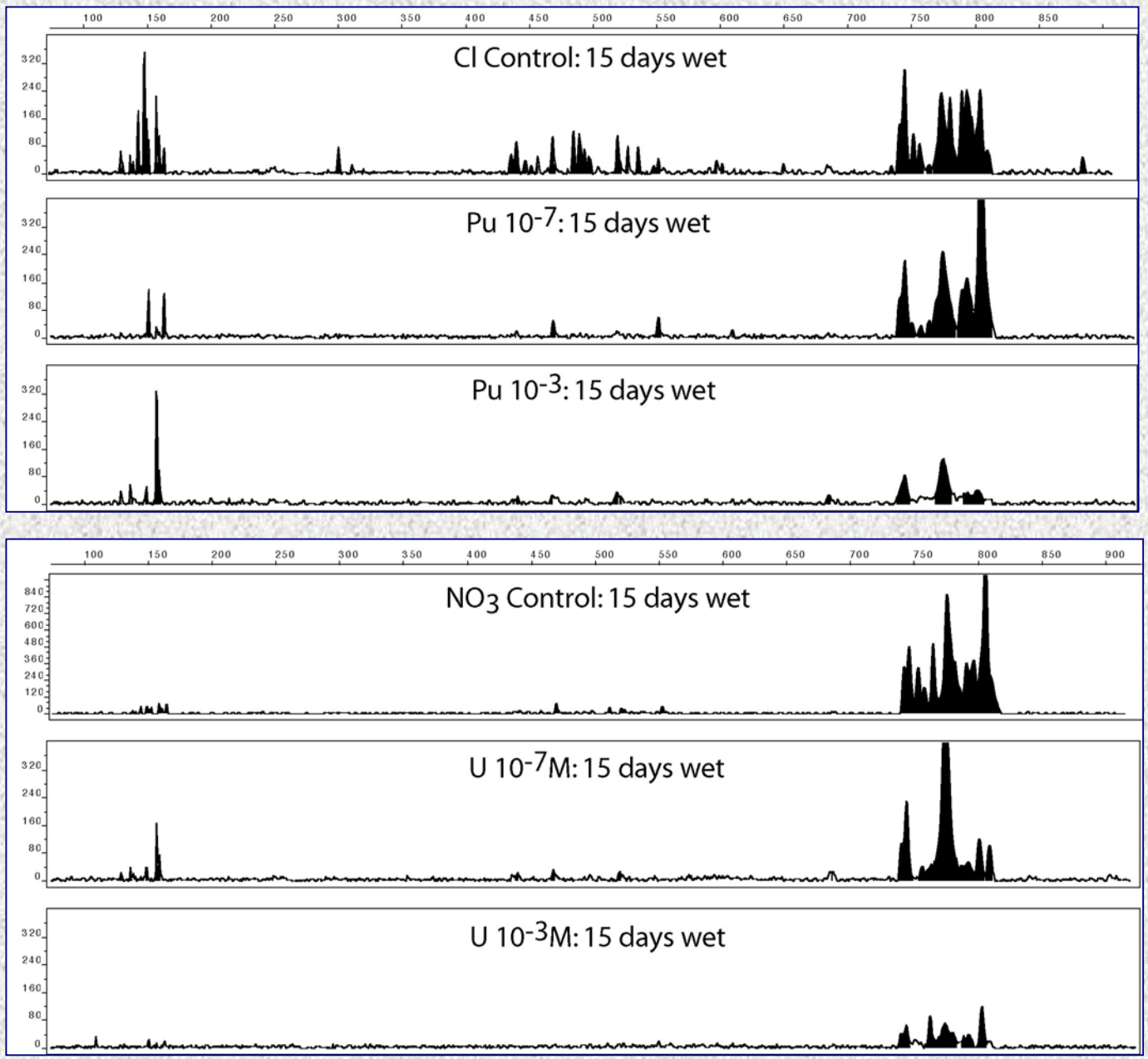


Bacterial Community Shifts in Cl^- and NO_3^- Controls



- effects of 15 day saturated incubation conditions
- Cl^- control is different from NO_3^- control

Bacterial Community Shifts with Pu(VI) or U(VI) Addition

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