# Metagenomic insights into microbial processes in Permian Basin produced waters

Djuna Gulliver, Presenter

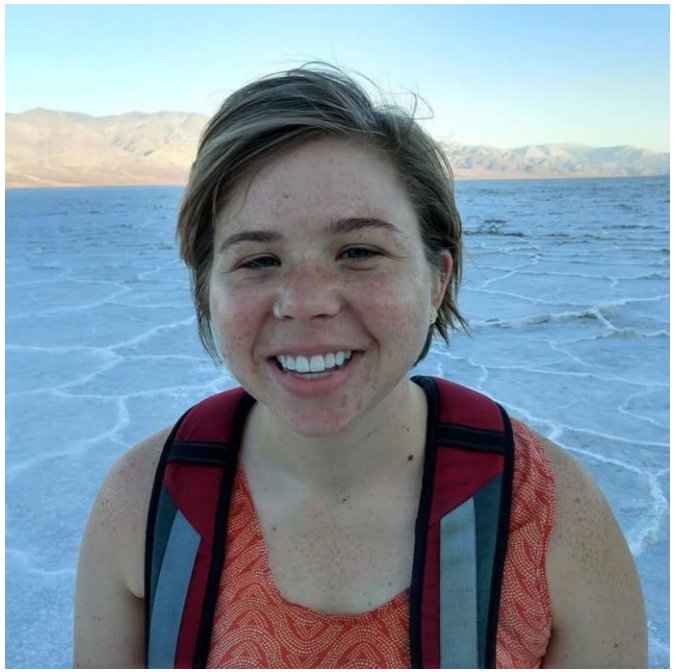
NATIONAL ENERGY TECHNOLOGY LABORATORY

U.S. DEPARTMENT OF ENERGY

# Research Team

NATIONAL ENERGY TECHNOLOGY LABORATORY

Kara Tinker, Geomicrobiology

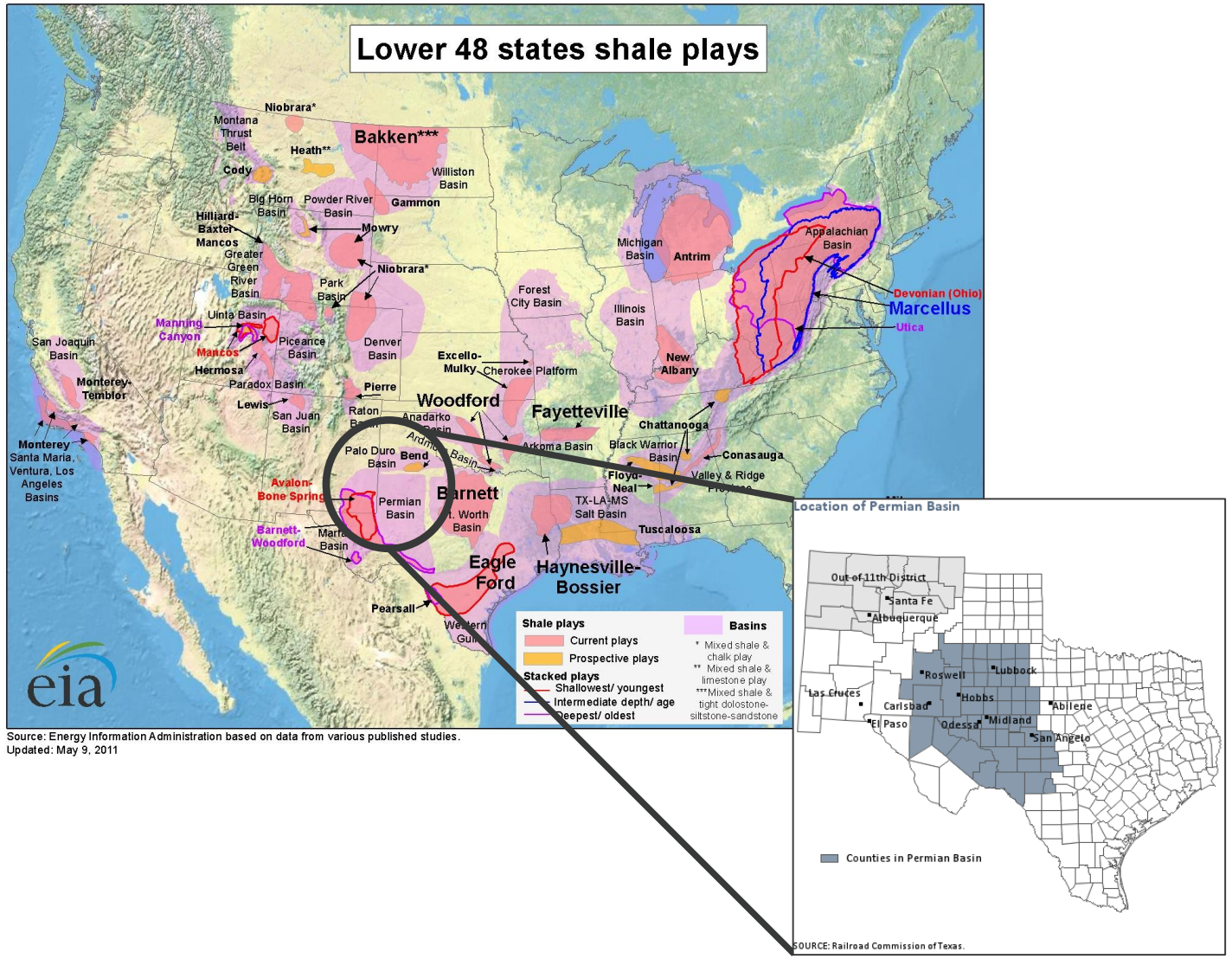


Daniel Lipus, Geomicrobiology

James Gardiner, Geochemistry

Burt Thomas, Biogeochemistry

# Permian Region



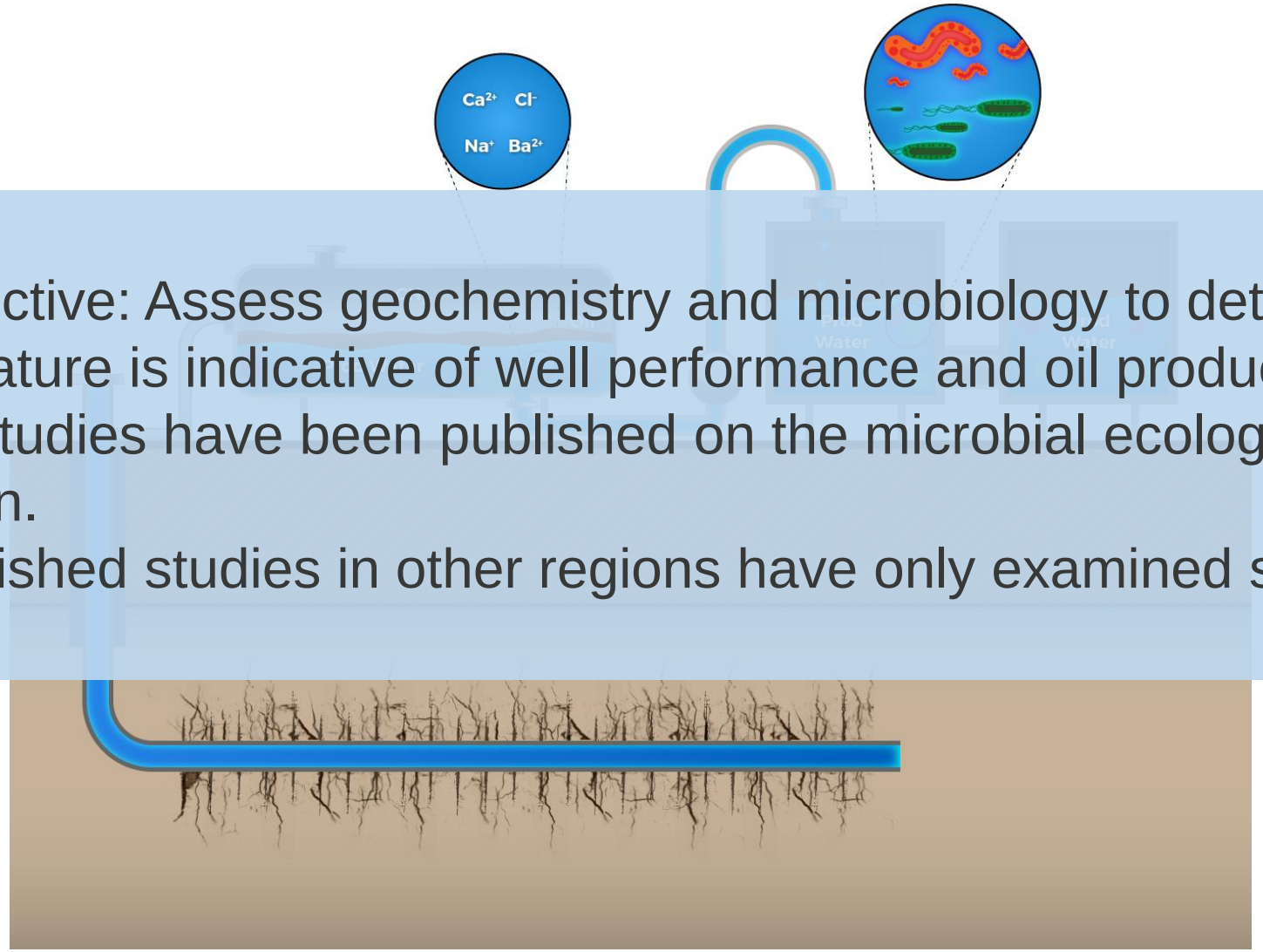

Source: Energy Information Administration based on data from various published studies.
Updated: May 9, 2011

**Oil production**

thousand barrels/day

| Region | February 2019 | March 2019 | change |
|---|---|---|---|
| Anadarko | 587 | 587 | - |
| Appalachia | 138 | 141 | 3 |
| Bakken | 1,439 | 1,452 | 13 |
| Eagle Ford | 1,429 | 1,438 | 9 |
| Haynesville | 43 | 43 | - |
| Niobrara | 697 | 713 | 16 |
| Permian | 3,981 | 4,024 | 43 |
| Total | 8,314 | 8,398 | 84 |

# Hydraulic Fracturing




- Objective: Assess geochemistry and microbiology to determine if signature is indicative of well performance and oil productivity
- No studies have been published on the microbial ecology of the Permian Basin.
- Published studies in other regions have only examined separator data

# 2018 Sampling Trip: Permian Region

## 14 Produced Water Samples:

- 9 Separators, 5 Wellheads
- 4 wells with both separator + wellheads
- 1 Groundwater Sample

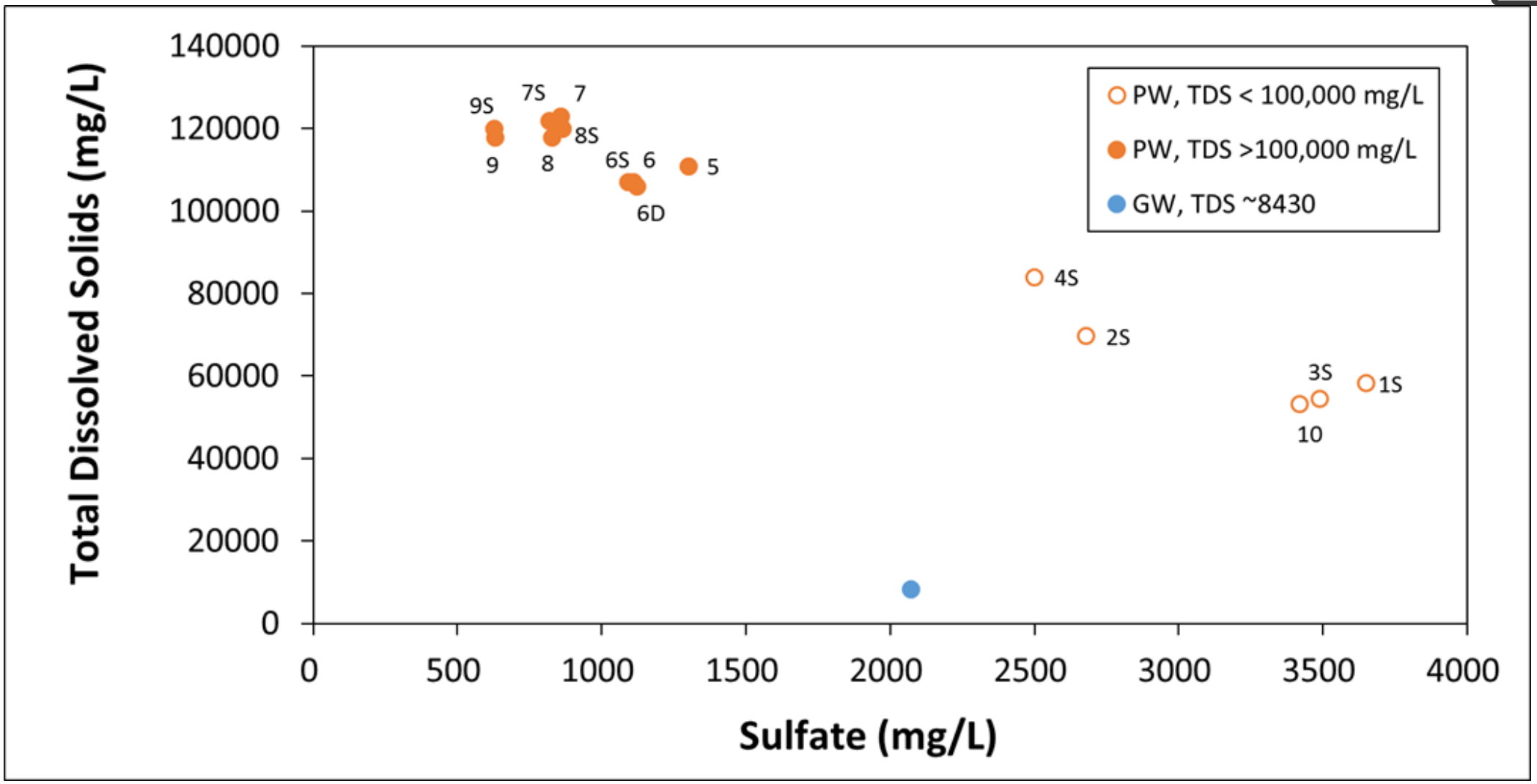
# 2018 Sampling Trip: Permian Region


- **Take onsite measurements**
  - pH, conductivity, temperature
  - $O_2$, $CO_2$, $CH_4$, $H_2S$
  - Alkalinity
- **Prep samples onsite**
  - Ion Chromatography (16 analytes)
  - ICP-OES (17 analytes)
  - Metagenomics

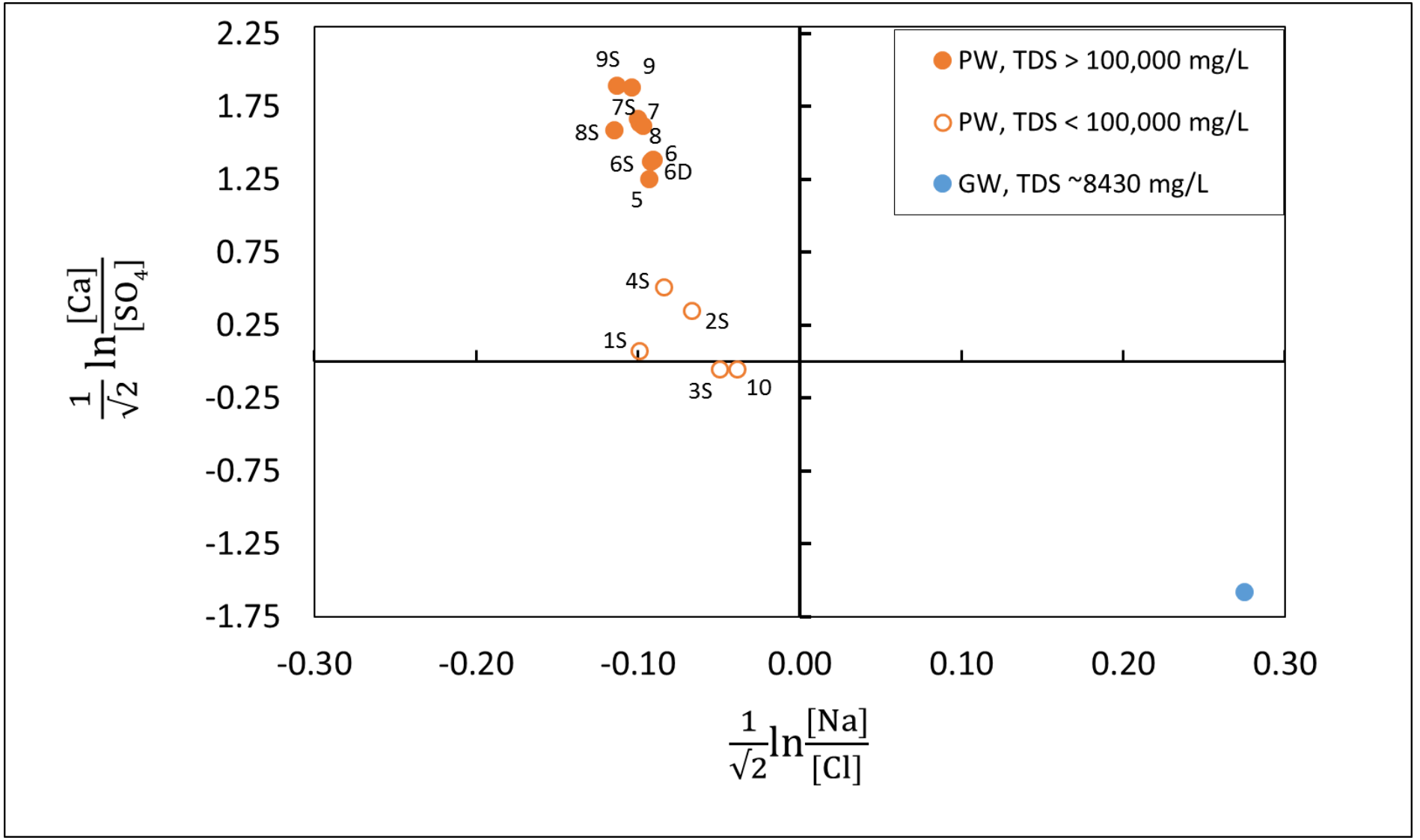
# Produced Water Chemistry

| ID | pH | $HCO_3$ (mg/L) | Ca (mg/L) | Mg (mg/L) | Na (mg/L) | K (mg/L) | Fe (mg/L) | $SO_4$ (mg/L) | Cl (mg/L) | Br (mg/L) | TDS (mg/L) | Water type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Produced Waters, likely Wolfcamp origin*** | | | | | | | | | | | | |
| 1S | 6.6 | 522 | 1680 | 601 | 18500 | 338 | 42.6 | 3650 | 32800 | 229 | 58300 | Na-Cl |
| 2S | 6.9 | 737 | 1830 | 506 | 23500 | 383 | 14.7 | 2680 | 39800 | 308 | 69700 | Na-Cl |
| 3S | 6.4 | 366 | 1350 | 517 | 18200 | 299 | 24.4 | 3490 | 30100 | 238 | 54500 | Na-Cl |
| 4S | 6.7 | 337 | 2150 | 509 | 28400 | 436 | 52.8 | 2500 | 49300 | 388 | 84000 | Na-Cl |
| 5 | 7.3 | 378 | 3210 | 593 | 37900 | 520 | 48.7 | 1300 | 66700 | 524 | 111000 | Na-Cl |
| 6 | 7.2 | 512 | 3300 | 578 | 36400 | 410 | 66.9 | 1110 | 63800 | 469 | 107000 | Na-Cl |
| 6D | 7.2 | 512 | 3320 | 584 | 36200 | 408 | 67.0 | 1120 | 63500 | 471 | 106000 | Na-Cl |
| 6S | 7.0 | 368 | 3180 | 565 | 36600 | 415 | BDL (<1.00) | 1090 | 64300 | 469 | 107000 | Na-Cl |
| 7 | 6.7 | 388 | 3610 | 615 | 42000 | 613 | 52.1 | 856 | 74500 | 604 | 123000 | Na-Cl |
| 7S | 6.6 | 290 | 3600 | 611 | 41700 | 610 | 57.6 | 817 | 74100 | 614 | 122000 | Na-Cl |
| 8 | 6.7 | 381 | 3420 | 599 | 40300 | 540 | 52.1 | 827 | 71300 | 554 | 118000 | Na-Cl |
| 8S | 6.5 | 425 | 3430 | 602 | 40400 | 542 | 64.2 | 864 | 73300 | 555 | 120000 | Na-Cl |
| 9 | 6.6 | 464 | 3790 | 617 | 39900 | 423 | 79.8 | 630 | 71300 | 524 | 118000 | Na-Cl |
| 9S | 6.6 | 398 | 3830 | 623 | 40400 | 428 | 86.8 | 626 | 73100 | 530 | 120000 | Na-Cl |
| 10 | 6.9 | 95.2 | 1320 | 504 | 18000 | 302 | 24.4 | 3420 | 29300 | 229 | 53200 | Na-Cl |
| **min** | 6.4 | 95.2 | 1320 | 504 | 18000 | 299 | 14.7 | 626 | 29300 | 229 | 53200 | |
| **max** | 7.3 | 737 | 3830 | 623 | 42000 | 613 | 86.8 | 3650 | 74500 | 614 | 123000 | |
| **median** | 6.7 | 388 | 3300 | 593 | 36600 | 423 | 52.5 | 1110 | 64300 | 471 | 107000 | |
| **average** | 6.8 | 412 | 2868 | 575 | 33227 | 444 | 52.4 | 1665 | 58480 | 447 | 98113 | |
| ***Groundwater used for mixing frac water*** | | | | | | | | | | | | |
| GW | 7.1 | 427 | 92.9 | 48.5 | 2830 | 11.0 | ND | 2070 | 2960 | 6.23 | 8432 | Na-Cl |

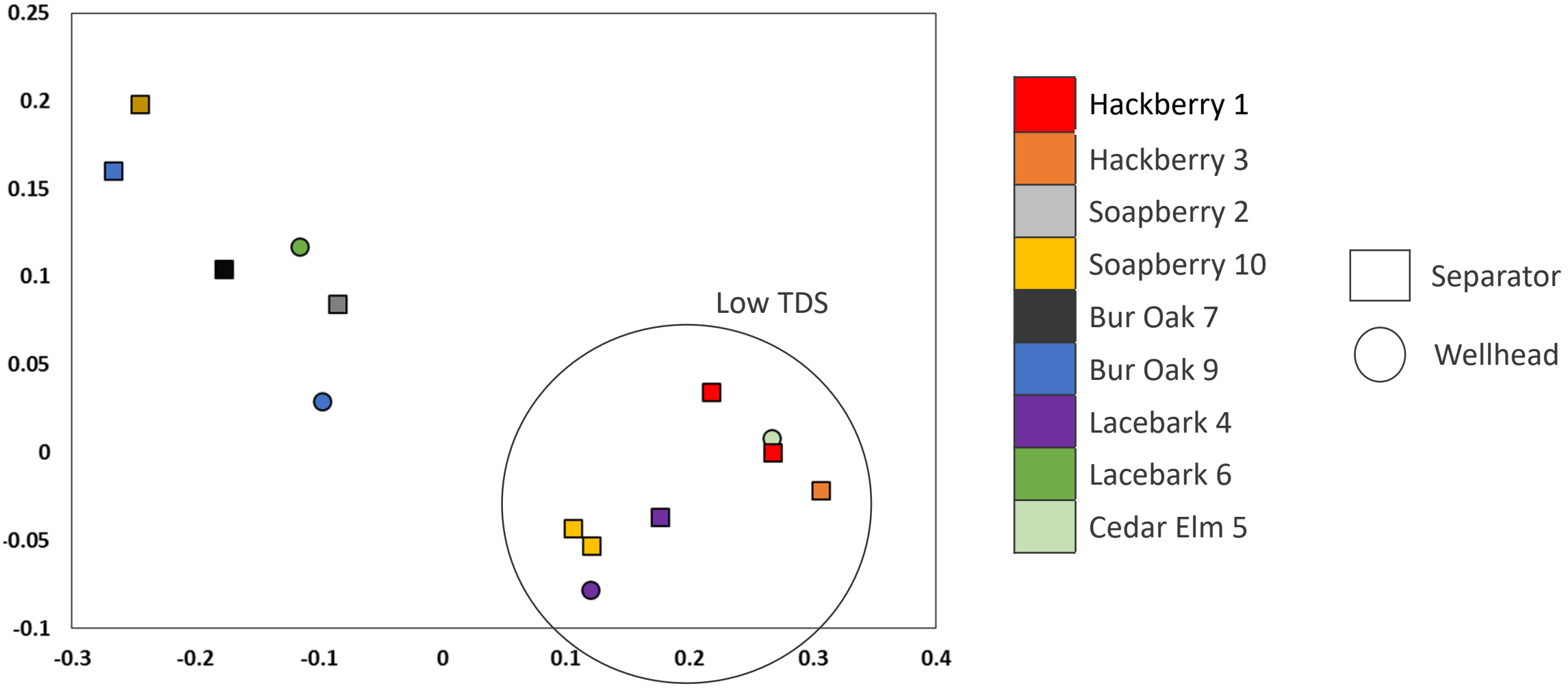
# Two distinct groups of produced water



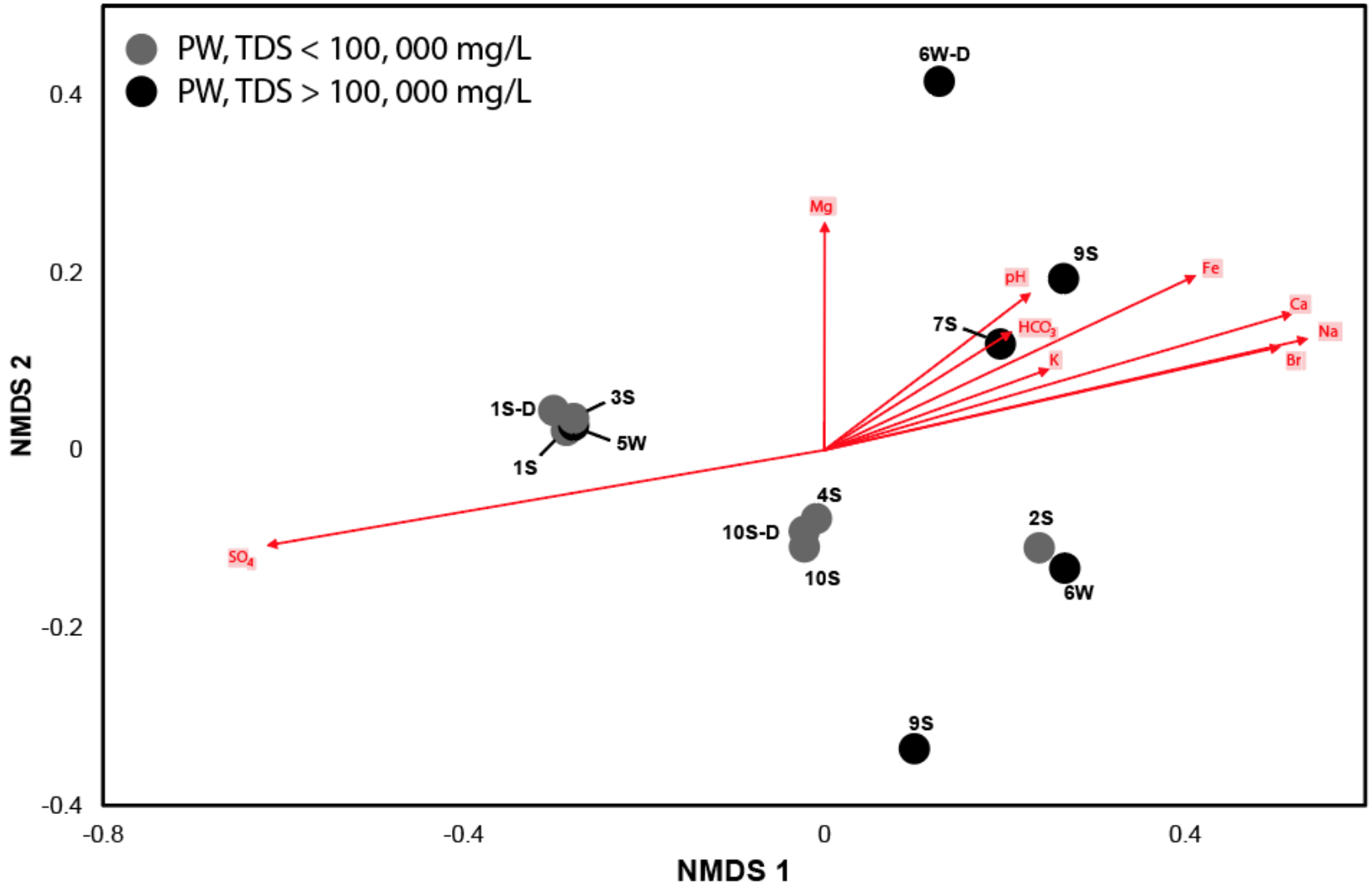
# Two distinct groups of produced water



# Weighted Unifrac



# Microbiota cluster by produced water group



# Are some sites affected by nearby injections?

# 16S RNA Taxonomy

| Taxon | Hackberry 1 | Hackberry 1 Duplicate | Hackberry 3 | Hackberry 3 Duplicate | Soapberry 2 | Soapberry 10 | Soapberry 10 Duplicate | Lacebark 4 Wellhead | Lacebark 4 Separator | Lacebark 6 | Lacebark 6 Duplicate* | Cedar Elm 5 Wellhead | Cedar Elm 5 WH Duplicate | Bur Oak 9 Wellhead | Bur Oak 9 Separator | Bur Oak 7 Separator |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Methanobacteriales | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.6% | 0.0% | 0.7% | 3.1% |
| Methanococcales | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.5% | 0.7% | 2.3% | 0.7% | 0.1% | 0.0% | 0.0% | 0.3% | 0.5% | 0.1% | 0.0% |
| Methanosarcinales | 0.0% | 0.0% | 0.0% | 0.0% | 0.3% | 0.0% | 0.1% | 0.9% | 0.5% | 0.0% | 0.0% | 0.0% | 0.1% | 0.3% | 0.0% | 1.6% |
| Actinomycetales | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 0.1% | 0.0% | 1.9% | 0.0% | 2.4% | 3.6% | 1.2% | 0.0% |
| Bacteroidales | 0.1% | 0.1% | 0.3% | 0.3% | 2.1% | 2.1% | 1.6% | 0.9% | 0.5% | 0.1% | 0.0% | 0.2% | 0.0% | 0.3% | 0.0% | 1.6% |
| Deferribacterales | 0.0% | 0.0% | 0.2% | 0.3% | 1.8% | 12.4% | 9.4% | 0.1% | 0.1% | 20.3% | 0.0% | 0.1% | 0.0% | 65.3% | 4.8% | 0.0% |
| Bacillales | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 4.6% | 1.0% | 0.0% |
| Clostridiales | 0.0% | 0.0% | 0.2% | 0.2% | 1.9% | 3.0% | 3.8% | 0.9% | 1.5% | 0.0% | 34.0% | 0.1% | 15.8% | 0.7% | 26.8% | 9.4% |
| Halanaerobiales | 66.7% | 81.2% | 38.7% | 65.1% | 33.8% | 10.6% | 12.8% | 23.6% | 27.1% | 43.1% | 2.9% | 69.1% | 50.9% | 8.6% | 6.2% | 31.3% |
| Thermoanaerobacterales | 0.0% | 0.0% | 0.3% | 0.3% | 0.0% | 8.3% | 5.3% | 0.2% | 1.1% | 0.0% | 0.0% | 0.2% | 0.1% | 0.0% | 0.1% | 0.0% |
| Caulobacterales | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.2% | 1.1% | 0.0% |
| Rhizobiales | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 0.3% | 0.0% | 0.0% | 11.7% | 0.0% | 8.2% | 0.2% | 17.1% | 10.9% |
| Rhodobacterales | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.5% | 1.2% | 0.4% | 0.0% |
| Burkholderiales | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.4% | 0.2% | 1.3% | 0.0% |
| Desulfobacterales | 0.0% | 0.0% | 0.0% | 0.0% | 2.5% | 0.3% | 0.4% | 0.5% | 0.4% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% |
| Desulfovibrionales | 1.1% | 0.5% | 0.2% | 0.3% | 38.3% | 4.3% | 4.3% | 6.2% | 4.4% | 32.1% | 42.7% | 0.8% | 12.2% | 3.6% | 32.8% | 32.8% |
| Desulfuromonadales | 0.5% | 0.1% | 0.1% | 0.3% | 1.5% | 11.7% | 9.6% | 0.0% | 0.0% | 0.2% | 0.0% | 0.3% | 0.0% | 0.0% | 0.0% | 0.0% |
| Campylobacterales | 28.8% | 16.9% | 59.2% | 32.4% | 6.6% | 34.1% | 37.8% | 31.7% | 43.1% | 0.5% | 4.9% | 28.3% | 2.7% | 4.5% | 2.2% | 6.3% |
| Alteromonadales | 2.1% | 0.7% | 0.7% | 0.7% | 7.3% | 10.9% | 12.7% | 16.2% | 7.0% | 0.2% | 1.9% | 0.6% | 0.1% | 0.2% | 0.1% | 0.0% |
| Methylococcales | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.1% | 0.9% | 3.3% | 1.3% | 0.0% |
| Synergistales | 0.0% | 0.0% | 0.0% | 0.0% | 1.2% | 0.5% | 0.4% | 0.5% | 0.3% | 0.1% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% |
| Pseudomonadales | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 1.6% | 0.8% | 1.6% | 1.6% |
| Thermotogales | 0.0% | 0.0% | 0.0% | 0.0% | 1.8% | 0.7% | 0.7% | 15.1% | 13.1% | 3.0% | 0.0% | 0.0% | 1.3% | 0.4% | 0.1% | 1.6% |
| Minor | 0.6% | 0.2% | 0.0% | 0.0% | 0.8% | 0.4% | 0.2% | 0.4% | 0.1% | 0.2% | 0.0% | 0.0% | 1.4% | 1.5% | 1.0% | 0.0% |

# Metagenome Taxonomy

| Taxon | Bur Oak 7 Separator | Bur Oak 9 Separator | Hackberry 3 Separator | Hackberry 3 D Separator | Hackberry 1 Separator | Soapberry 2 Separator | Soapberry 10 Separator | Lacebark 6 Separator |
|---|---|---|---|---|---|---|---|---|
| Halanaerobiales | 20.0% | 0.4% | 48.0% | 45.0% | 53.0% | 18.0% | 14.0% | 15.0% |
| Clostridiales | 1.0% | 4.0% | 8.0% | 8.0% | 4.0% | 2.0% | 14.0% | 8.0% |
| Bacillales | 0.6% | 9.0% | 3.0% | 3.0% | 3.0% | 0.8% | 2.0% | 7.0% |
| Lactobacillales | 0.0% | 7.0% | 0.4% | 0.4% | 0.0% | 0.0% | 0.0% | 6.0% |
| Minor Firmicutes | 0.0% | 2.0% | 1.6% | 3.0% | 1.0% | 2.0% | 6.0% | 0.0% |
| Micrococcales | 0.3% | 3.0% | 0.0% | 0.1% | 0.1% | 0.1% | 0.1% | 0.5% |
| Corynebacterales | 0.3% | 7.0% | 0.0% | 0.1% | 0.1% | 0.1% | 0.1% | 4.0% |
| Propionibacterales | 0.6% | 5.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 1.0% |
| Minor Actinobacteria | 1.0% | 11.0% | 0.6% | 0.8% | 0.4% | 1.8% | 0.3% | 3.0% |
| Campylobacterales | 0.0% | 0.3% | 13.0% | 6.0% | 7.0% | 0.0% | 2.0% | 2.0% |
| Minor Epsilonproteobacteria | 0.0% | 0.0% | 1.0% | 0.0% | 1.0% | 0.0% | 0.0% | 1.0% |
| Desulfovibrionales | 23.0% | 0.2% | 2.0% | 3.0% | 4.0% | 32.0% | 4.0% | 1.0% |
| Desulfuromonadales | 0.8% | 0.1% | 1.0% | 2.0% | 4.0% | 2.0% | 5.0% | 0.0% |
| Desulfurobacterales | 1.0% | 0.1% | 0.5% | 0.8% | 0.4% | 3.0% | 1.0% | 0.0% |
| Minor Deltaproteobacteria | 1.2% | 0.0% | 3.0% | 2.0% | 1.0% | 1.0% | 1.0% | 2.0% |
| Alteromonadales | 0.1% | 0.0% | 6.0% | 10.0% | 8.0% | 9.0% | 14.0% | 0.8% |
| Enterobacterales | 0.2% | 6.0% | 0.4% | 0.0% | 0.5% | 0.3% | 0.5% | 0.0% |
| Pseudomonadales | 0.2% | 2.0% | 0.0% | 0.0% | 0.0% | 0.2% | 0.4% | 1.0% |
| Minor Gammaproteobacteria | 1.0% | 5.0% | 2.0% | 2.0% | 4.0% | 1.5% | 3.0% | 4.0% |
| Deferribacterales | 0.2% | 4.0% | 0.0% | 0.9% | 0.0% | 1.0% | 5.0% | 2.0% |
| PVC group | 1.0% | 4.0% | 0.4% | 0.0% | 1.0% | 1.0% | 0.5% | 0.0% |
| Marinilabiales | 3.0% | 0.0% | 1.0% | 1.0% | 0.6% | 3.0% | 3.0% | 0.0% |
| Methanosarcinales | 40.0% | 0.3% | 2.0% | 2.0% | 0.5% | 12.0% | 4.0% | 4.0% |
| Methanococcales | 0.6% | 0.0% | 0.7% | 1.0% | 0.0% | 0.7% | 6.0% | 0.0% |
| Archaeoglobales | 0.0% | 0.0% | 0.0% | 0.8% | 0.0% | 0.7% | 3.0% | 6.0% |
| Thermotogales | 0.0% | 0.0% | 0.0% | 0.6% | 0.0% | 1.0% | 1.0% | 2.0% |
| Minor Archaea | 1.4% | 2.0% | 1.3% | 0.2% | 0.1% | 1.0% | 1.0% | 3.0% |
| Unassigned Bacteria | 1.0% | 16.0% | 0.4% | 2.0% | 2.0% | 0.0% | 2.0% | 21.0% |

# 19 Recovered MAGs

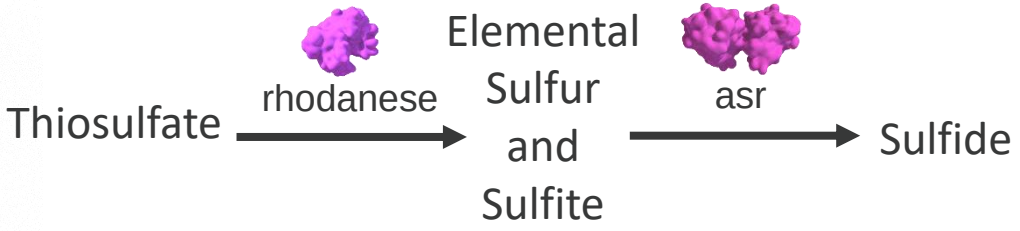
| | Organisms | Sample ID | Completeness | Contamination | Quality |
|---|---|---|---|---|---|
| 1 | Orenia | 3S-D | 98.33 | 9.83 | high |
| 2 | Geoglobus | 4S | 97.39 | 6.87 | high |
| 3 | Orenia | 1S | 96.03 | 5.42 | high |
| 4 | Methanohalophilus | 4S | 95.22 | 8.47 | high |
| 5 | Methanothermococcus | 4S | 95.1 | 2.68 | high |
| 6 | Desulfohalobium | 2S | 95.03 | 1.97 | high |
| 7 | Methanohalophilus | 7S | 93.95 | 4.3 | high |
| 8 | Desulfohalobium | 7S-D | 90.67 | 1.45 | high |
| 9 | Marinobacter | 4S | 83.82 | 9.4 | high |

| | Organisms | Sample ID | Completeness | Contamination | Quality |
|---|---|---|---|---|---|
| 10 | Archaeoglobus | 4S | 97.71 | 14.71 | medium |
| 11 | Halomonas | 3S-D | 83.65 | 9.45 | medium |
| 12 | Methanohalophilus | 2S | 76.2 | 5.89 | medium |
| 13 | Pseudomonas | 3S | 72.57 | 5.56 | medium |
| 14 | Pelobacter | 3S | 67.54 | 10.17 | medium |
| 15 | Clostridium | 3S | 65.22 | 9.25 | medium |
| 16 | Marinobacter | 1S | 76.67 | 22.21 | low |
| 17 | Halanaerobium | 3S | 75.5 | 15.2 | low |
| 18 | Pelobacter | 3S-D | 69.33 | 9.69 | low |
| 19 | Alcanivorax | 10S-D | 51.03 | 4.89 | low |
| 20 | Archaeoglobus | 3S-D | 50.25 | 6.66 | low |
| 21 | Ralstonia | 1S | 41.08 | 6.78 | low |

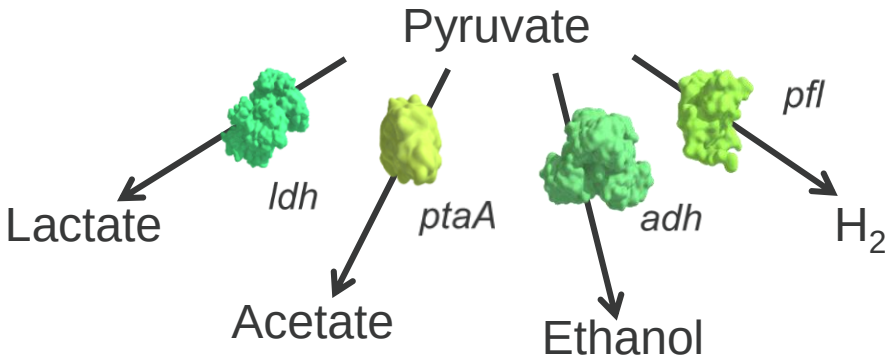
# Preliminary MAG Analysis

## Orenia

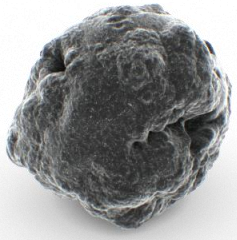
### Thiosulfate Reduction



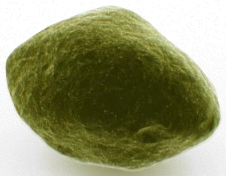

### Fermentation



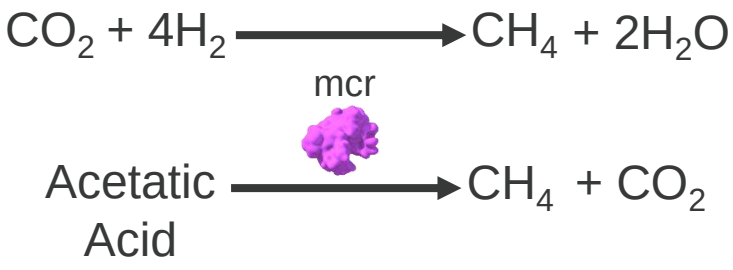

## Methanothermococcus



## Methanohalophilus



### Methanogenesis

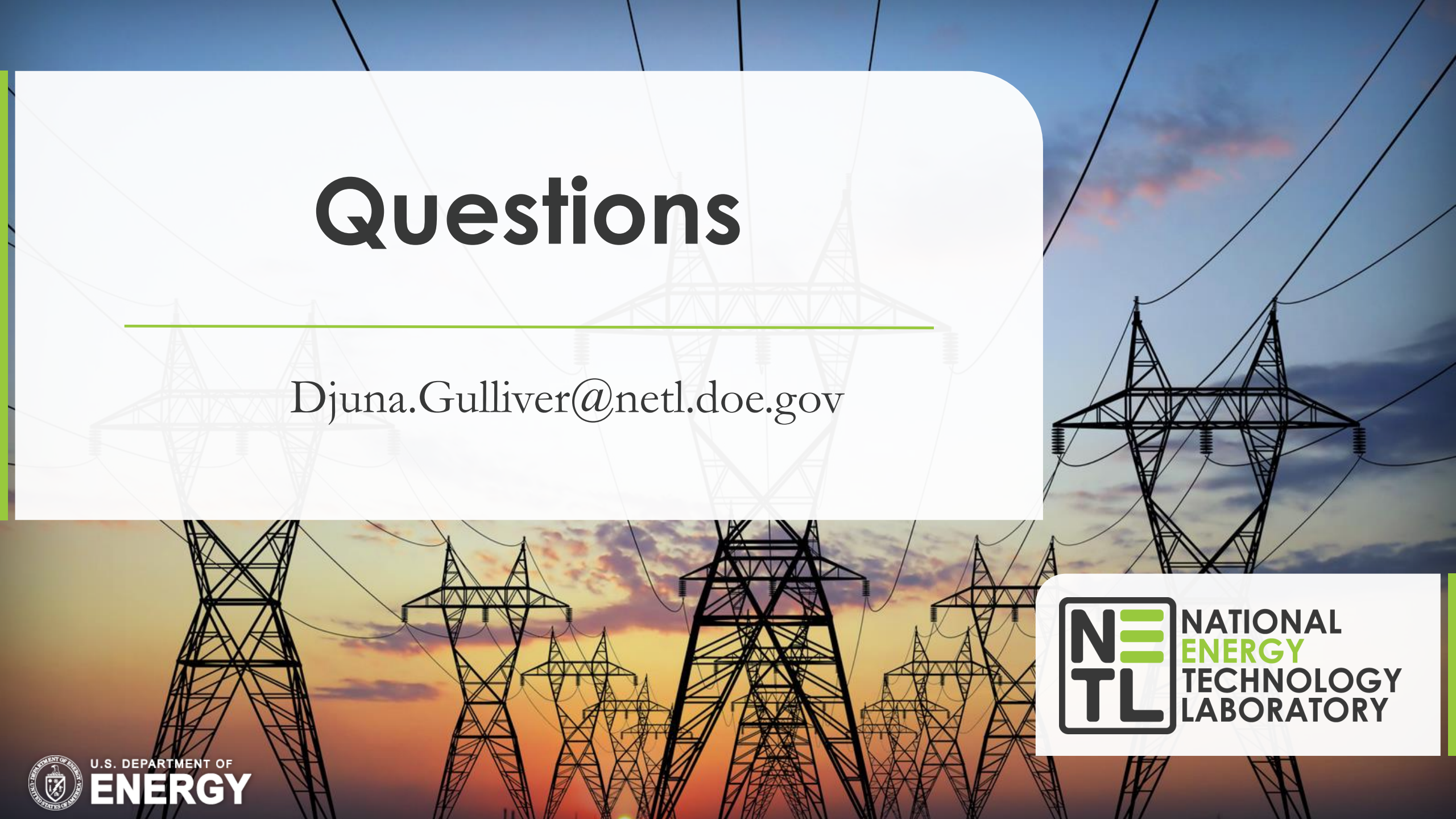


# Conclusions

- Geochemistry demonstrates dilution curve, suggestion well communication
- Taxonomy appears to be impacted by geochemical dilution trends
- *Orenia* dominated the samples
- Traditional sulfate reducers were in high abundance
- Future work
  - Refine and assess MAGs
  - October sampling event

# Questions

Djuna.Gulliver@netl.doe.gov



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