

# Microbial Ecology of Hydraulic Shale Environments

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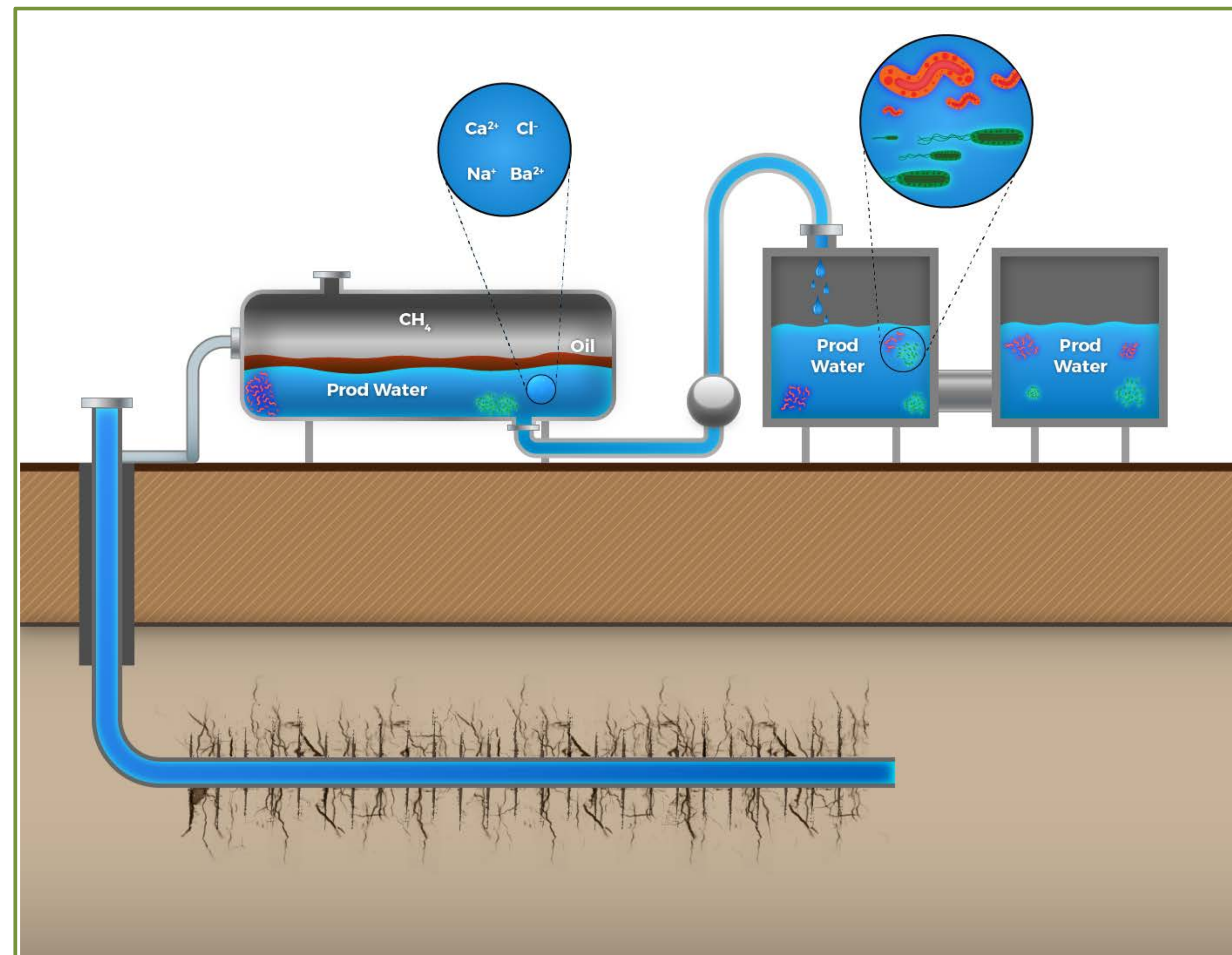
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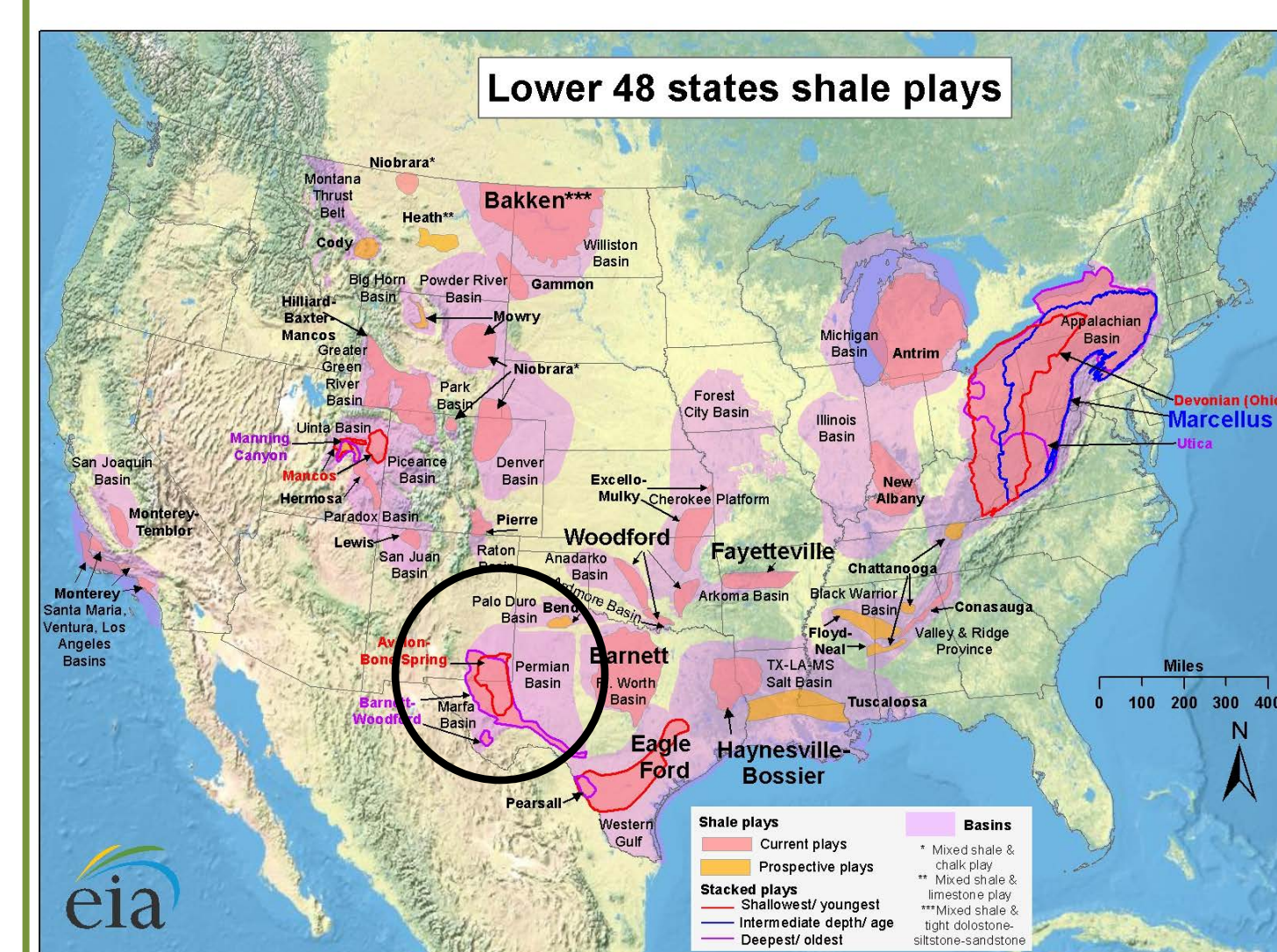
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## Background



Subsurface microbial communities currently affect energy production, reservoir properties, and wellbore integrity through processes such as biomineralization (scaling), acid formation (corrosion), biofilm formation (biofouling), and metal mobility. However, it is still widely unknown in the energy industry which of these processes will occur during unconventional resource production, how much they impact energy production, and how to properly use or mitigate these biopathways. Additionally, it is still unknown whether the microbial community will act as an indicator of potential well productivity/performance.

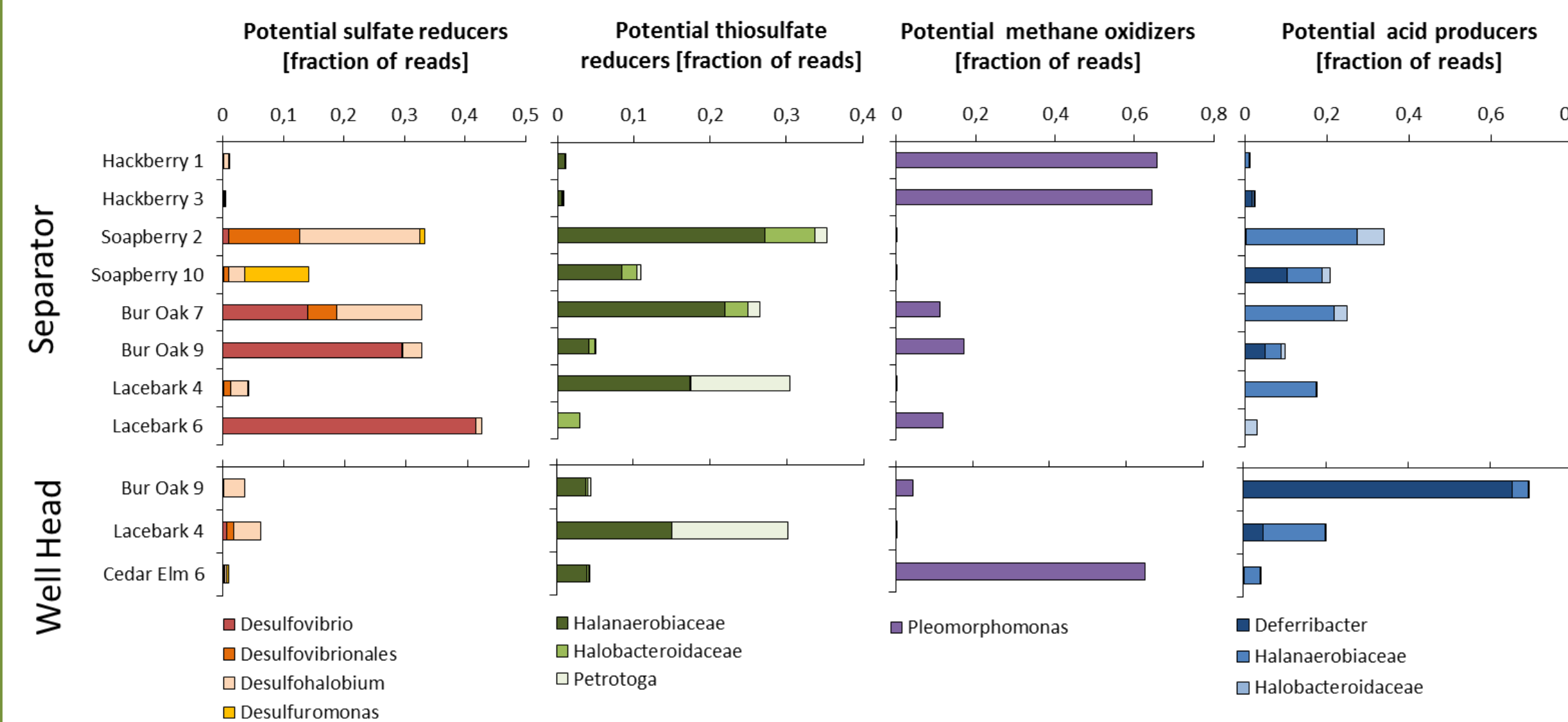


NETL has previously assessed the geochemistry and microbiology of the Marcellus Shale and Bakken Shale. However, no large-scale, in-depth biogeochemistry data currently exists for the Permian Basin, one of the major hydrocarbon producing regions in the country.



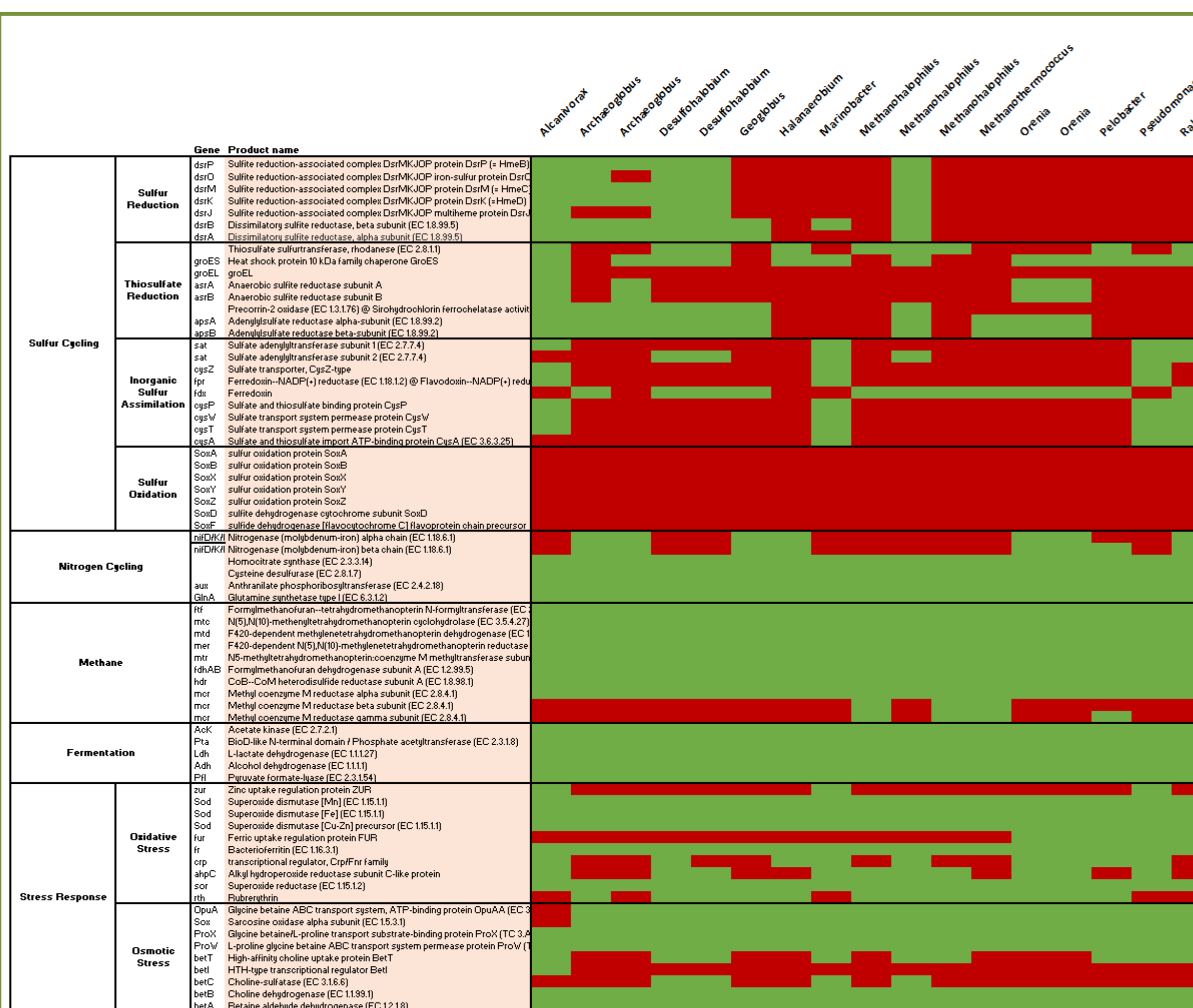
In the spring of 2018, a total of 15 samples were collected from the Permian Basin. Fluid from 9 separators and 5 wellheads were collected, as well as source injection fluid from 1 groundwater well. 10 unique sample sites were represented, with 4 sample sites having paired separator and wellhead samples. Measurements for pH, conductivity, temperature, O<sub>2</sub>, CO<sub>2</sub>, CO, H<sub>2</sub>S, and alkalinity were recorded on site. Samples were preserved so ion chromatography (16 analytes), ICP-OES (17 analytes), transition metal analysis, 16S rRNA gene (taxonomy) sequencing, and metagenomic sequencing could be completed off site.

## Taxonomy Results



16S sequencing determined the microbial community to have a high abundance of *Halanaerobium*, a thiosulfate-reducing bacteria that has previously been identified in other unconventional resource basins. Additionally, the microbial community had a high abundance of *Desulfovibrio* and *Desulfuromonas*, sulfate-reducing bacteria that have not been previously recorded in large abundance in other basins.

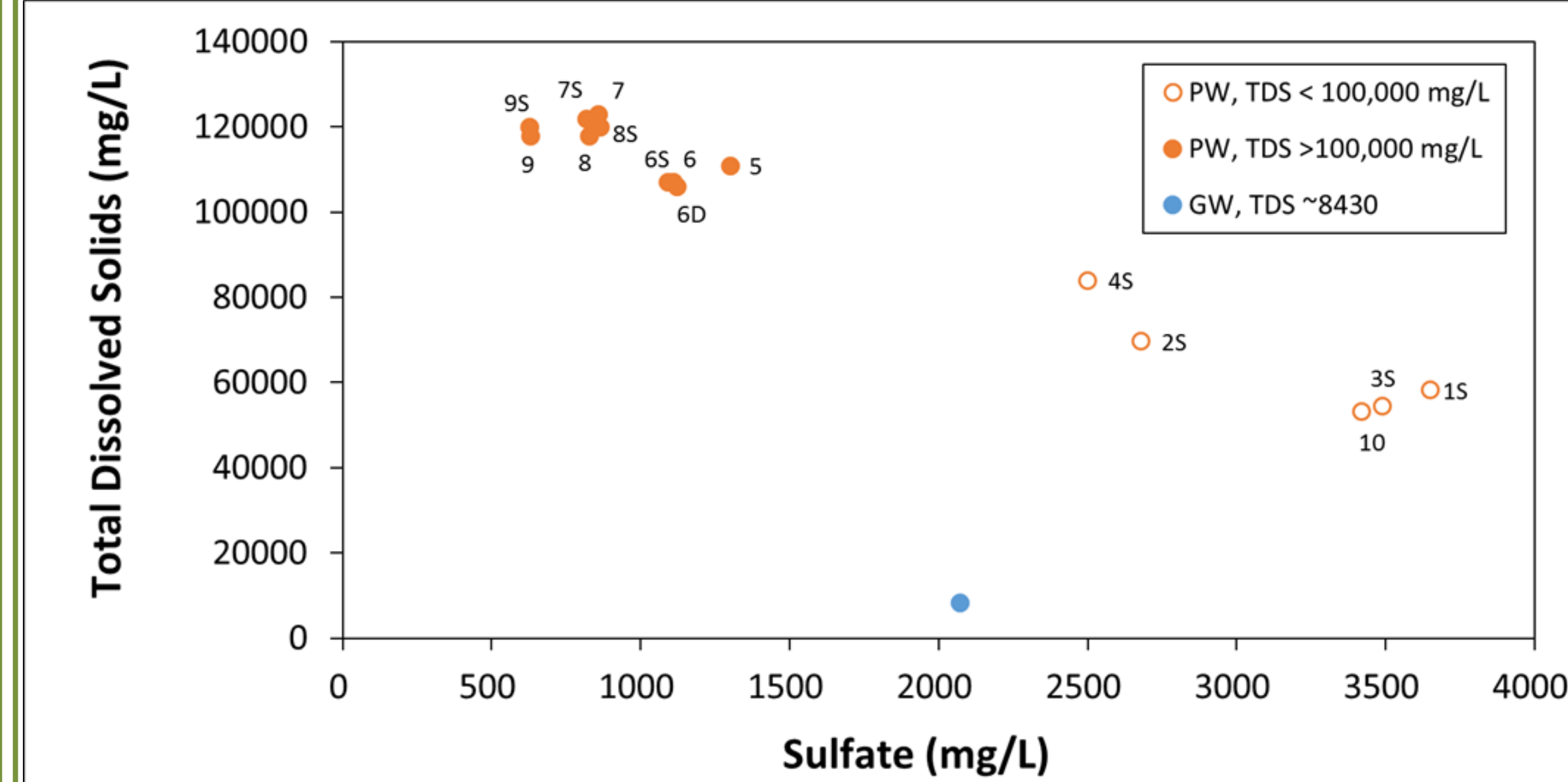
## Metagenomic Results



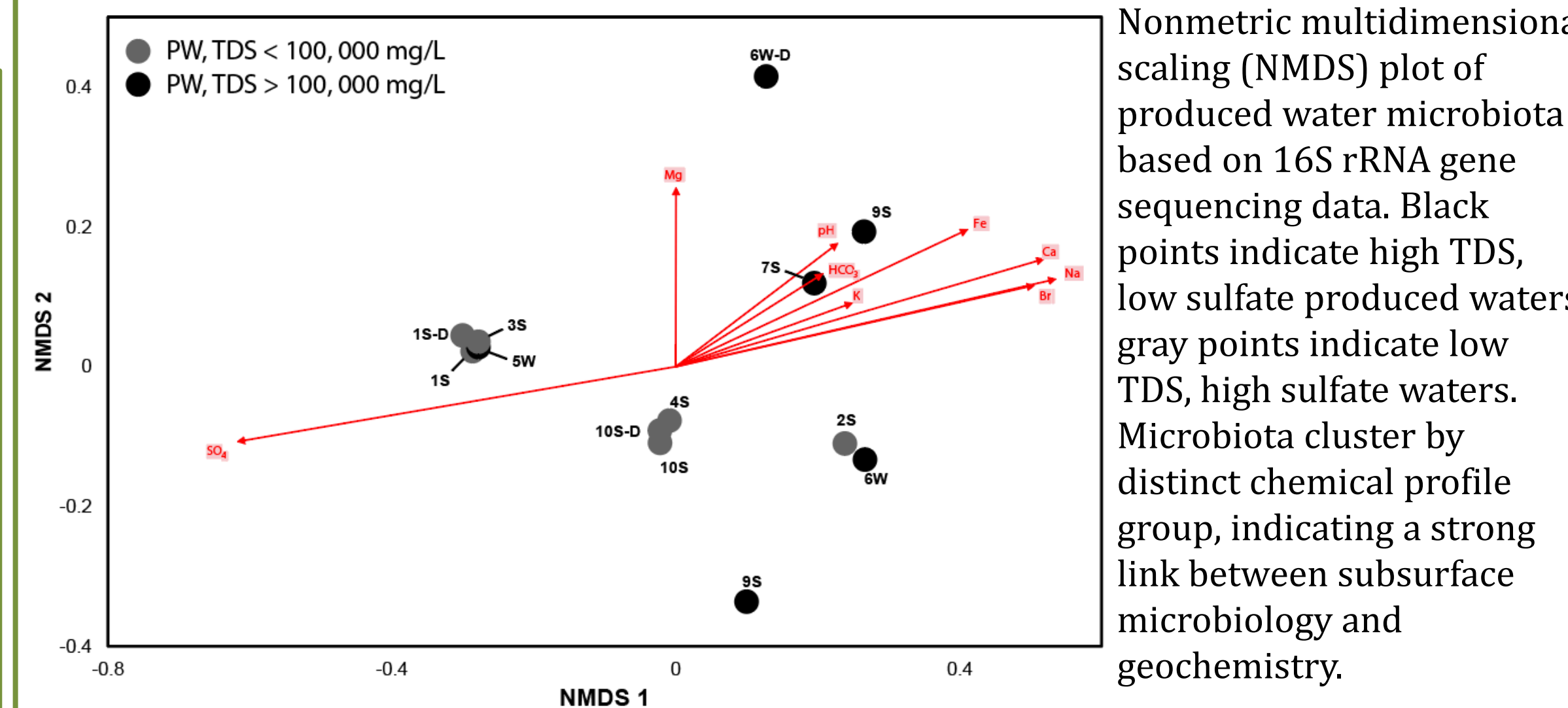
Shotgun sequencing was performed to determine potential microbial processes of the samples. 19 metagenome-assembled genomes (MAGs) have been recovered, 9 high quality, 6 medium quality, and 6 low quality. Recovered MAGs represent halophilic, thermophilic, sulfate reducing, fermentative, and methanogenic bacteria and archaea. Reconstruction of metabolic pathways allowed us to demonstrate that both classical sulfate reduction and thiosulfate reduction may contribute to gas souring in this region. Recovery of multiple methanogenic archaea suggests a high potential for biological methane production. These draft genomes will be further utilized to determine reactions that may occur in the reservoir and infrastructure.



## Geochemistry Correlation Results



Produced waters plot in two distinct groupings based on TDS and sulfate concentrations: (1) high TDS, low sulfate waters and (2) low TDS, high sulfate waters. The groundwater sample has a significantly lower TDS concentration, but its sulfate concentration is elevated relative to the median produced water concentration. Produced water samples have two distinct chemical profiles, suggesting injection fluid from a nearby well is diluting reservoir fluid in the low TDS, high sulfate producing wells.



Nonmetric multidimensional scaling (NMDS) plot of produced water microbiota based on 16S rRNA gene sequencing data. Black points indicate high TDS, low sulfate produced waters; grey points indicate low TDS, high sulfate waters. Microbiota cluster by distinct chemical profile group, indicating a strong link between subsurface microbiology and geochemistry.

## Future Work

- Continue sampling efforts in Permian Basin with a broader geospatial representation
- Correlate microbiology with metadata and well productivity
- Finish Permian data analysis of 2018 sampling event
- Process & analyze Bakken Data from 2018 sampling event
- Begin investigating microbiology from a new, uncharacterized shale play
- Review protocols for low biomass samples
- Establish internal metatranscriptomic protocols

## References

1. Lipus, D., Vikram, A., Gulliver, D. M., Bibby, K., 2019. Upregulation of peroxide scavenging enzymes and multidrug efflux proteins highlight an active sodium hypochlorite response in *Pseudomonas fluorescens* biofilms, *Biofouling*, 35(3), doi: [10.1080/08927014.2019.1605357](https://doi.org/10.1080/08927014.2019.1605357).
2. Lipus, D., Vikram, A., Hammack, R., Bibby, K., Gulliver, D. M., 2018. The Effects of Sample Storage Conditions on the Microbial Community Composition in Hydraulic Fracturing Produced Water, *Geomicrobiology Journal*, 36(7), doi: [10.1080/01490451.2019.1599470](https://doi.org/10.1080/01490451.2019.1599470).
3. Lipus, D., Roy, D., Khan, E., Vikram, A., Gulliver, D. M., Hammack, R., Bibby, K., 2018. Microbial communities in Bakken region produced water, *FEMS Microbiology Letters*, doi: [10.1093/fems/lny107](https://doi.org/10.1093/fems/lny107).
4. Lipus, D., Vikram, A., Ross, D., Bain, D., Gulliver, D. M., Hammack, R., Bibby, K., 2017. Predominance and metabolic potential of *Halanaerobium* in Marcellus Shale hydraulic fracturing produced water, *Applied Environmental Microbiology*, doi: [10.1128/AEM.02659-16](https://doi.org/10.1128/AEM.02659-16).
5. Lipus, D., Ross, D., Bibby, K., Gulliver, D. M., 2017. Draft genome sequence of *Pseudomonas* sp. BDAL1 reconstructed from a Bakken Shale hydraulic fracturing produced storage tank metagenome, *Genome Announcements*, 5(11): e00033-17.