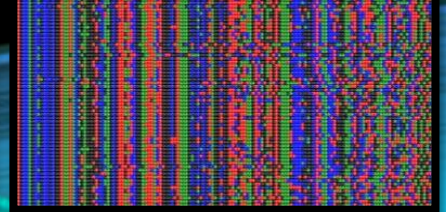
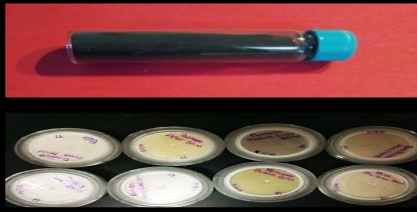
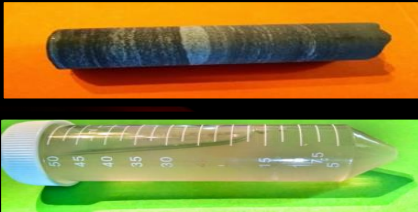




Extreme Microbes in Shales; A Potential Source for Enhanced Production

Yael Tucker, Ph.D.



PRODUCED WATER SOCIETY
SEMINAR 2018

February 13, 2018

The Biological Part of the Equation

Microbial composition of water is considered when adding biocides to hydrofracture fluids, but often the consideration ends there.

However, several studies have found that produced water still contains living sulfur reducing bacteria, acid producing bacteria and other organisms.

Active Ingredient	SRB Efficacy	APB Efficacy	Aerobe Efficacy	Kill Speed	Length of Bacterial Control
Glutaraldehyde	★★★★★	★★★	★★★★★	★★★★★	★★★★★
Glut-Quat Combo	★★★★★	★★★★★	★★★★★	★★★★★	★★★★★
DBNPA	★★★★★	★★★★★	★★★★★	★★★★★	★
THPS	★★★★★	★★★★★	★★★★★	★★★★★	★★★★★
Bronopol	★★★	★★★	★★★★★	★★★	★★★★★
Dazomet	★	★	★	★	★★★
ADBAC	★★★★★	★★★★★	★★★★★	★★★★★	★★
DDAC	★★★★★	★★★★★	★★★★★	★★★★★	★★
TTPC	★★★★★	★★★★★	★★★★★	★★★★★	★★
Halogenated Oxidizers	★★★★★	★★★★★	★★★★★	★★★★★	★

Biocide Selection Guide – The DOW Chemical Company

http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_08b6/0901b803808b64bc.pdf?filepath=microbial/pdfs/noreg/253-02697.pdf&fromPage=GetDoc

Initial Study

Is something down there?

Different Samples Tested

Marcellus shale (17):

- Average depth- 5,000-8,000 ft.
- Generally dry gas with high thermal maturity

Utica Shale (1):

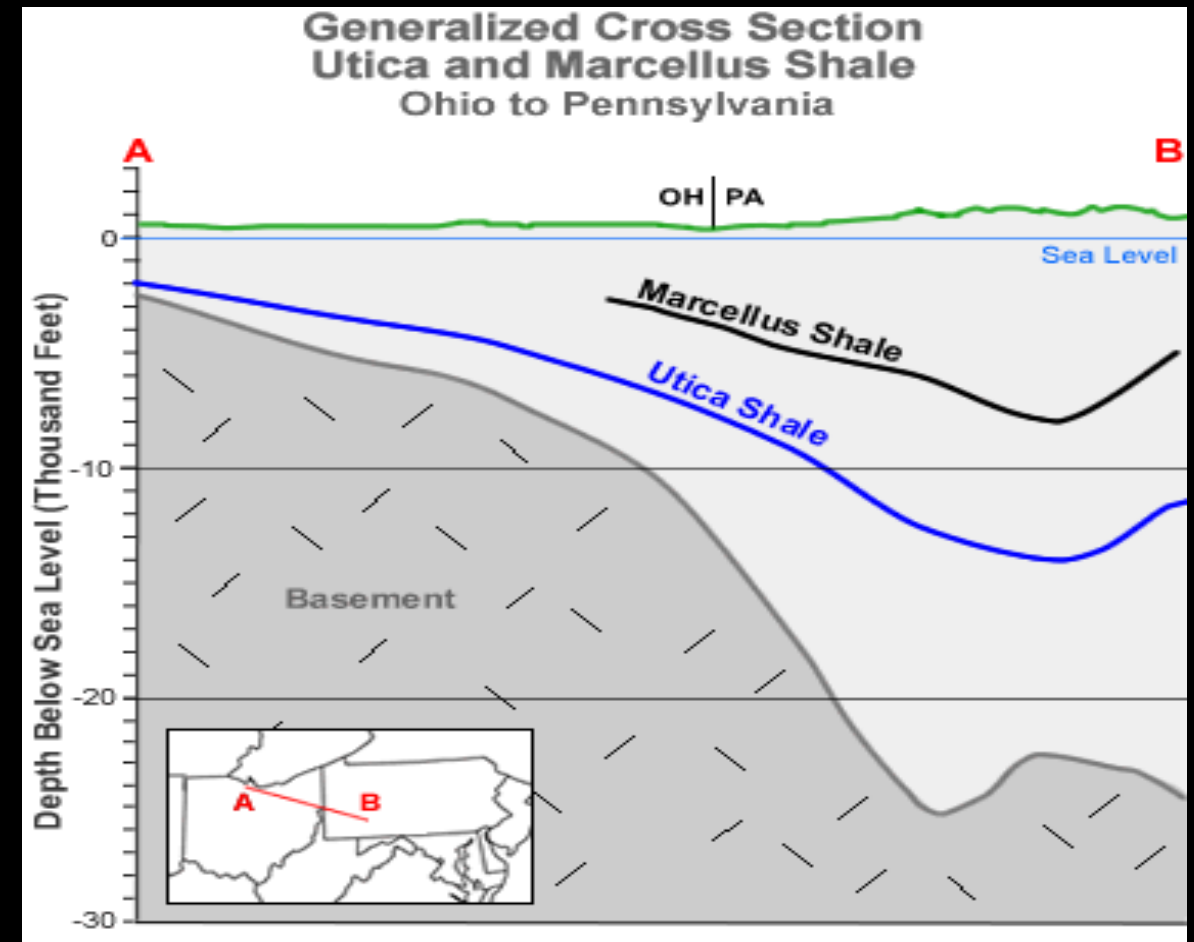
- 3-7,000 ft. below Marcellus
- Contains both Oil and Gas (low conodont alteration index)

Lower Huron Shale (3):

- 3,000 ft. below surface
- Some oil but mostly just natural gas

Coal Samples (43):

- Depths ranged from 300-3,000 ft.
- Higher permeability



Initial Study



Marcellus Shale

Microbiological Marcellus Produced Water Studies

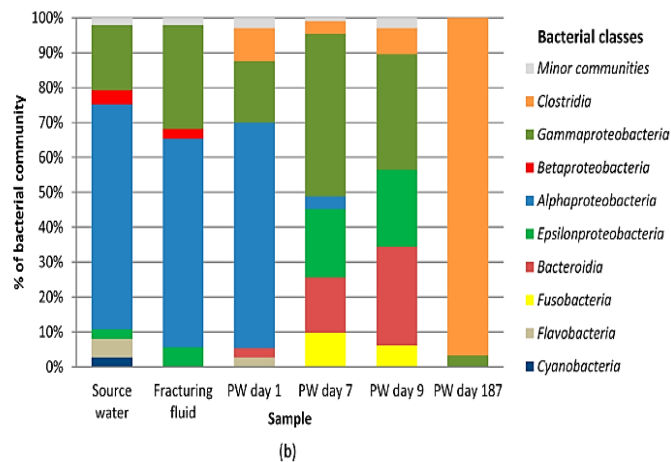
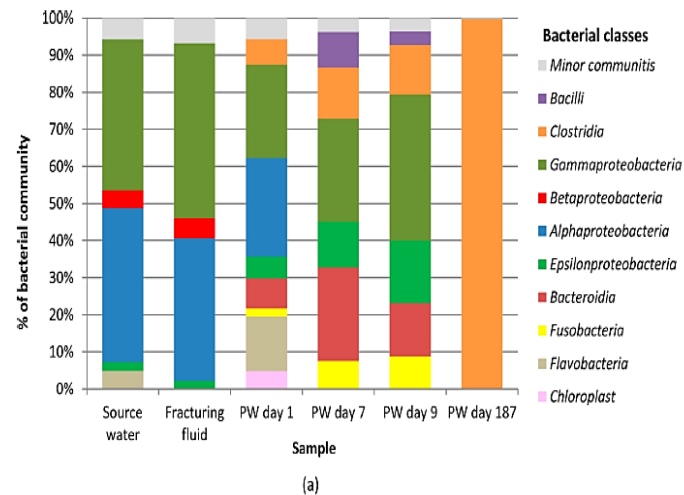


Figure 1. Relative abundance of bacterial 16S rRNA gene sequences in source water, fracturing fluid, and produced water (PW) day 1, 7, 9, and 187 samples from (a) tag-encoded pyrosequencing (b) clone libraries. The RDP Classifier tool was used to assign sequences to taxonomical classes at a cutoff of 80. For both techniques, classes that constituted <2% of the bacterial community in the sample are termed minor communities. We were unable to amplify DNA from synthetic oil-based drilling mud.

Mohan et al. (2013). Microbial community changes in hydraulic fracturing fluids and produced water from shale gas extraction. *Environ Sci. Technology*, 47(22), 13141-13150.

Cluff, et al (2014). Temporal Changes in Microbial Ecology and Geochemistry in Produced Water from Hydraulically Fractured Marcellus Shale Gas Wells. *Environ Sci Technology*

Akob et al. (2015) Organic and inorganic composition and microbiology of produced waters from Pennsylvania shale gas wells. *Applied Geochemistry*, 60, 116-125

Found:

- *H₂S* producers - *Halanerobium*, *Vibrio*, *Shewanella*, and *Thermoanaerobacter*.
- Acid producers- *Halanerobium*, *Alphaproteobacteria*

Utica and Lower Huron Shale Microbiological Studies:

NONE

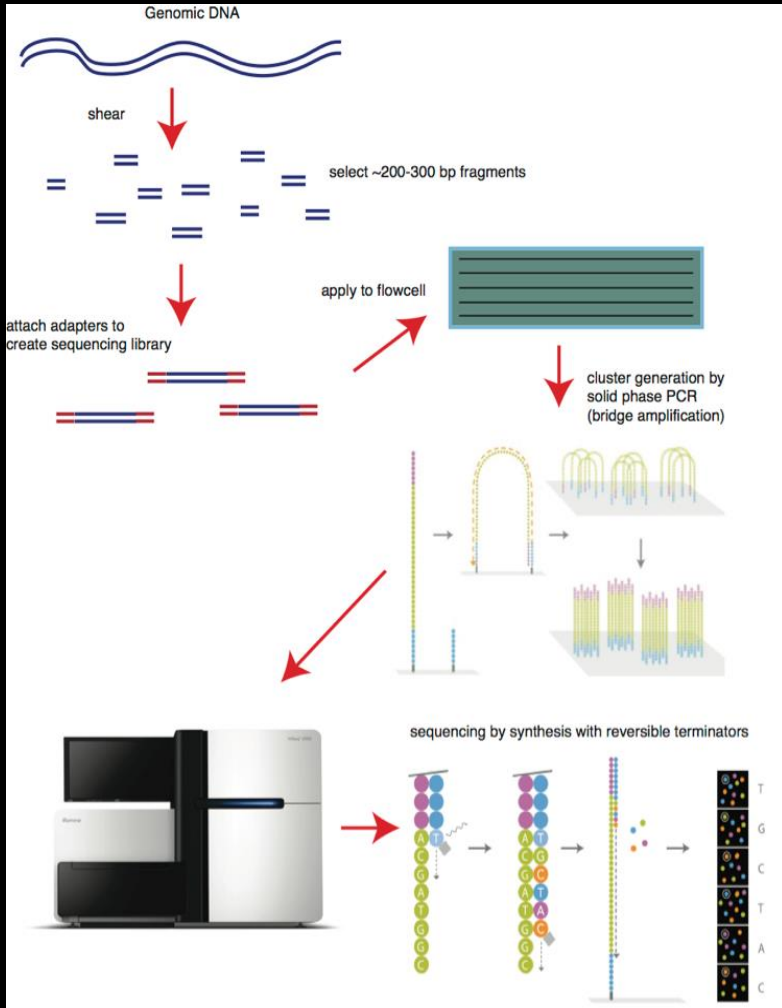
Detection Options

Culture Methods

- Only captures 1% population
- Time consuming and biased
- Getting live cells out of rock samples is challenging...

Next Generation Sequencing

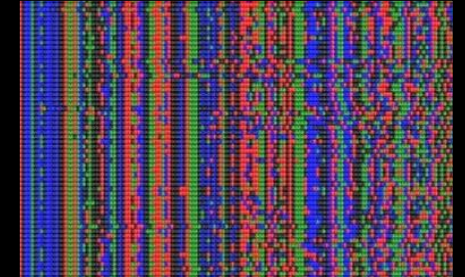
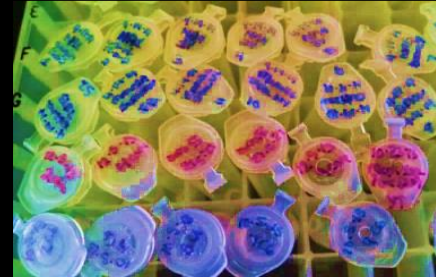
- Faster results than plating
- Captures entire population



DNA Extraction

Use a kit... *but it's not that easy...*

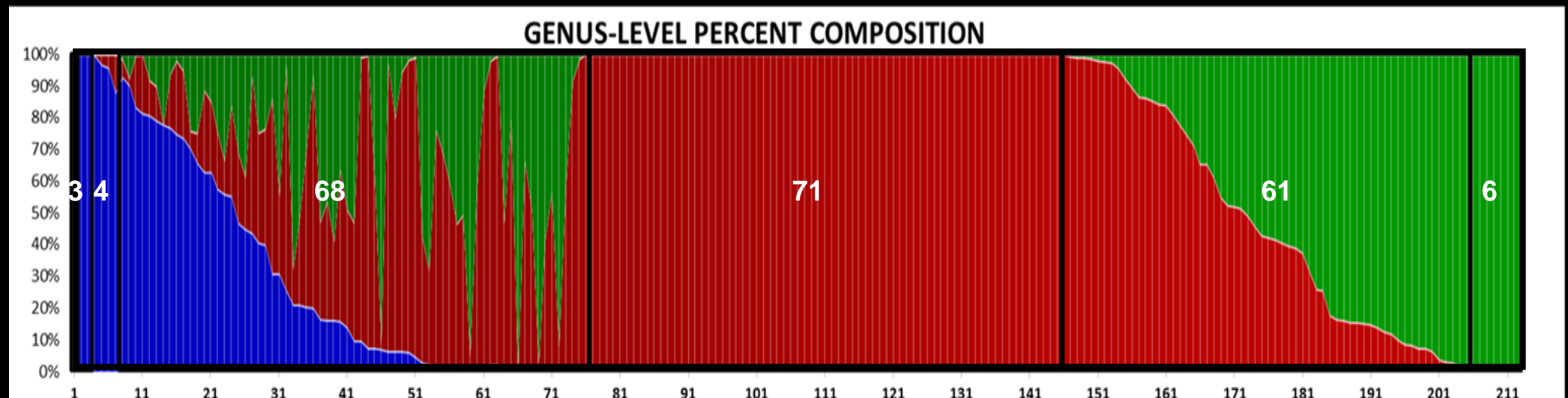
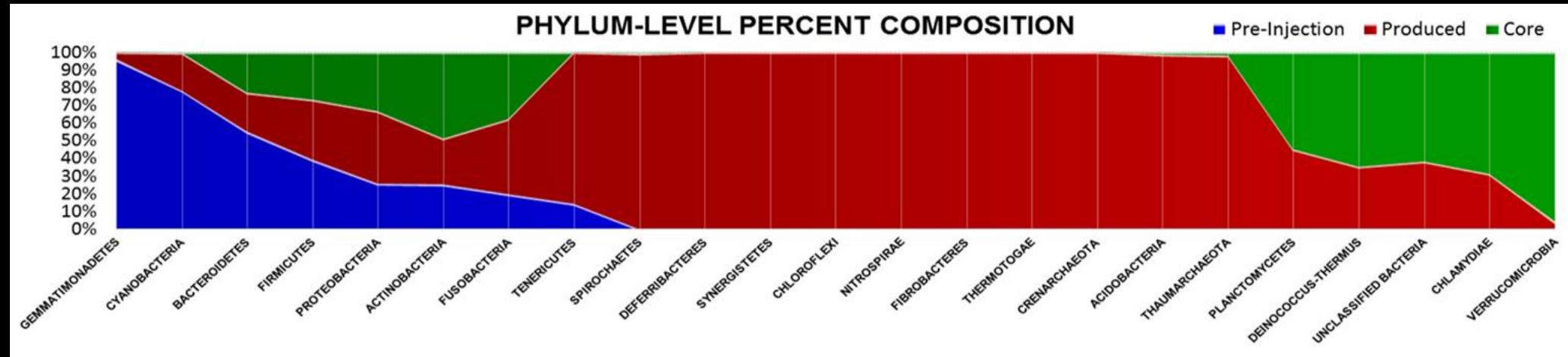
- Which kit?
- How much water?
- How much rock?
- Is there such a thing as too much?



Microorganisms in Marcellus Shale

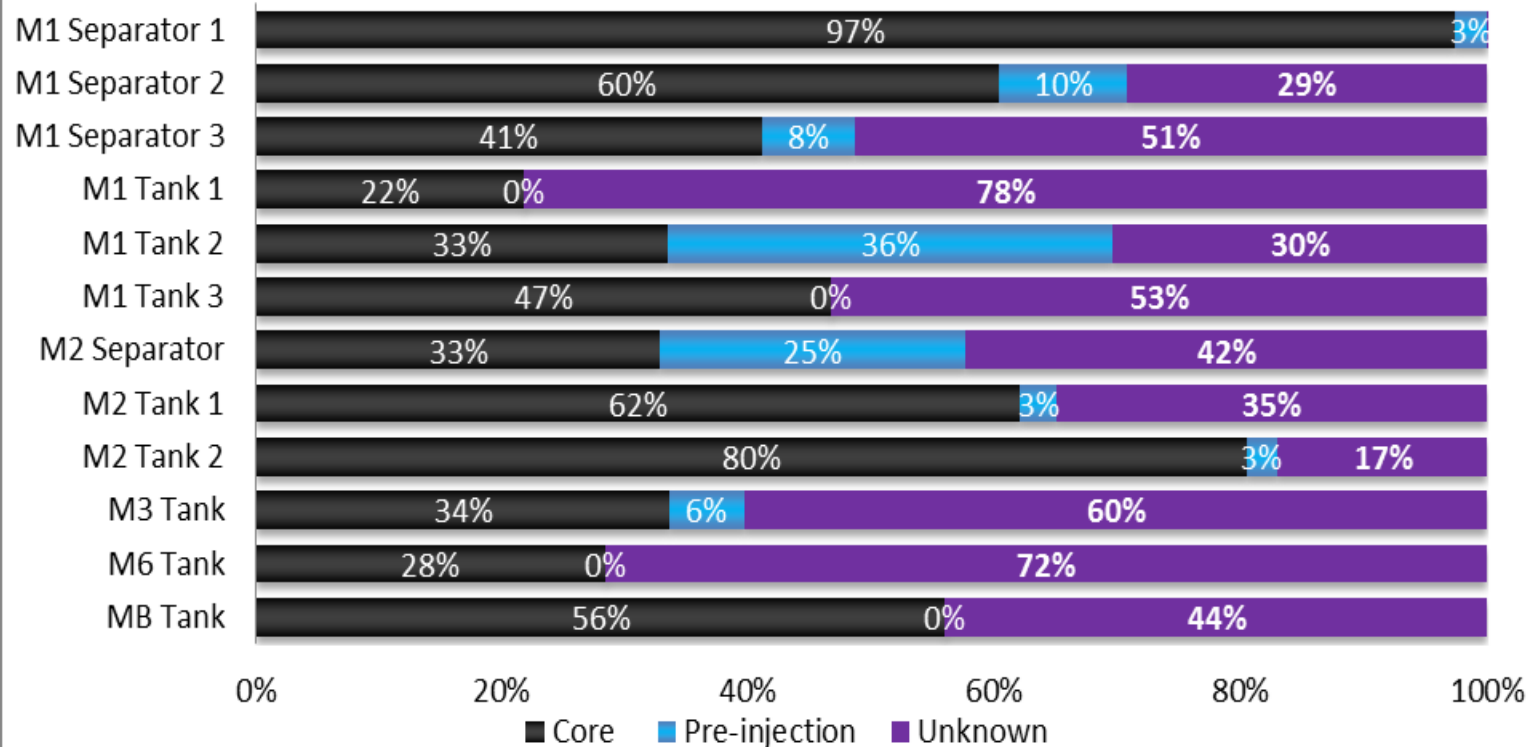
Well	Age of Well	Sample Type	OTU	Total Sequences	Total Methanogens
Pre- Injection					
MB	-	Before Injection	212	25040	0%
Produced Fluid Tank					
MB	139	Produced	221	32621	0.02%
	210	Produced	231	40739	0.01%
M1	689	Produced	198	36897	0%
	787	Produced	196	38186	0%
M2	220	Produced	233	22747	0.14%
	787	Produced	191	74234	0.15%
M3	230	Produced	159	21145	0.01%
M6	1825	Produced	221	23151	0%
Gas-Water Separator					
M1	661	Produced	250	317056	17%
	689	Produced	160	78568	0%
	787	Produced	204	18937	0%
M2	787	Produced	158	23905	0%
Shale Cores					
Core 1	-	Core	289	23943	0.01%

Microorganisms Native to Marcellus Shale?



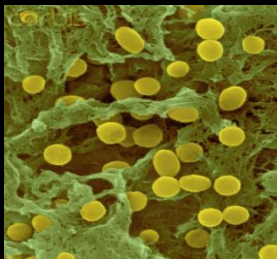
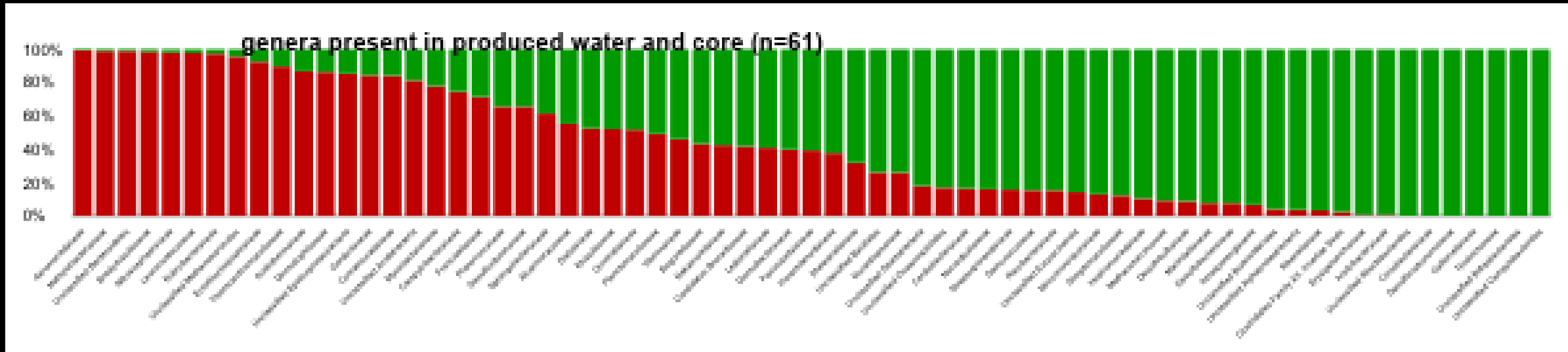
Predicted Source of Microorganisms

SourceTracker % Composition of Produced Fluids



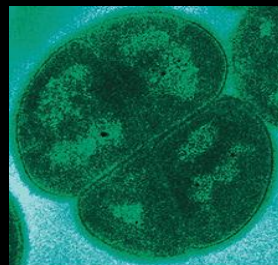
Knights, Dan, et al. "Bayesian community-wide culture-independent microbial source tracking." *Nature methods* 8.9 (2011): 761-763.

Microorganisms Native to Marcellus Shale?



Acidobacterium capsulatum

- Often found in coal mines
- Tolerate low pH found in produced waters (possibly subsurface)
- Utilize iron metabolism that produces ferric iron



Deinococcus radiodurans

- Most notable for high tolerance to radioactivity
- Tolerate high levels of acid, cold and even vacuum



Deinococcus geothermalis

- Thermophilic radiophile
- Thrives at temperatures between 45-50° C
- Engineered to consume radioactive waste



Methanogens

Methanosarcina Acetivorans

- Methane producing Microbe
- Can convert CO₂, acetate, methanol and CO into methane
- Thrives in iron sulfide.

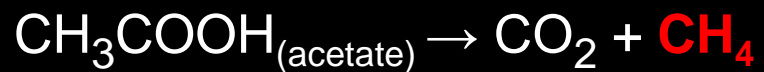
What is a Methanogen?

Methanogens microorganisms from the kingdom Archaea.

Why do we care about them?

Methanogens produce methane which is a large part of natural gas.

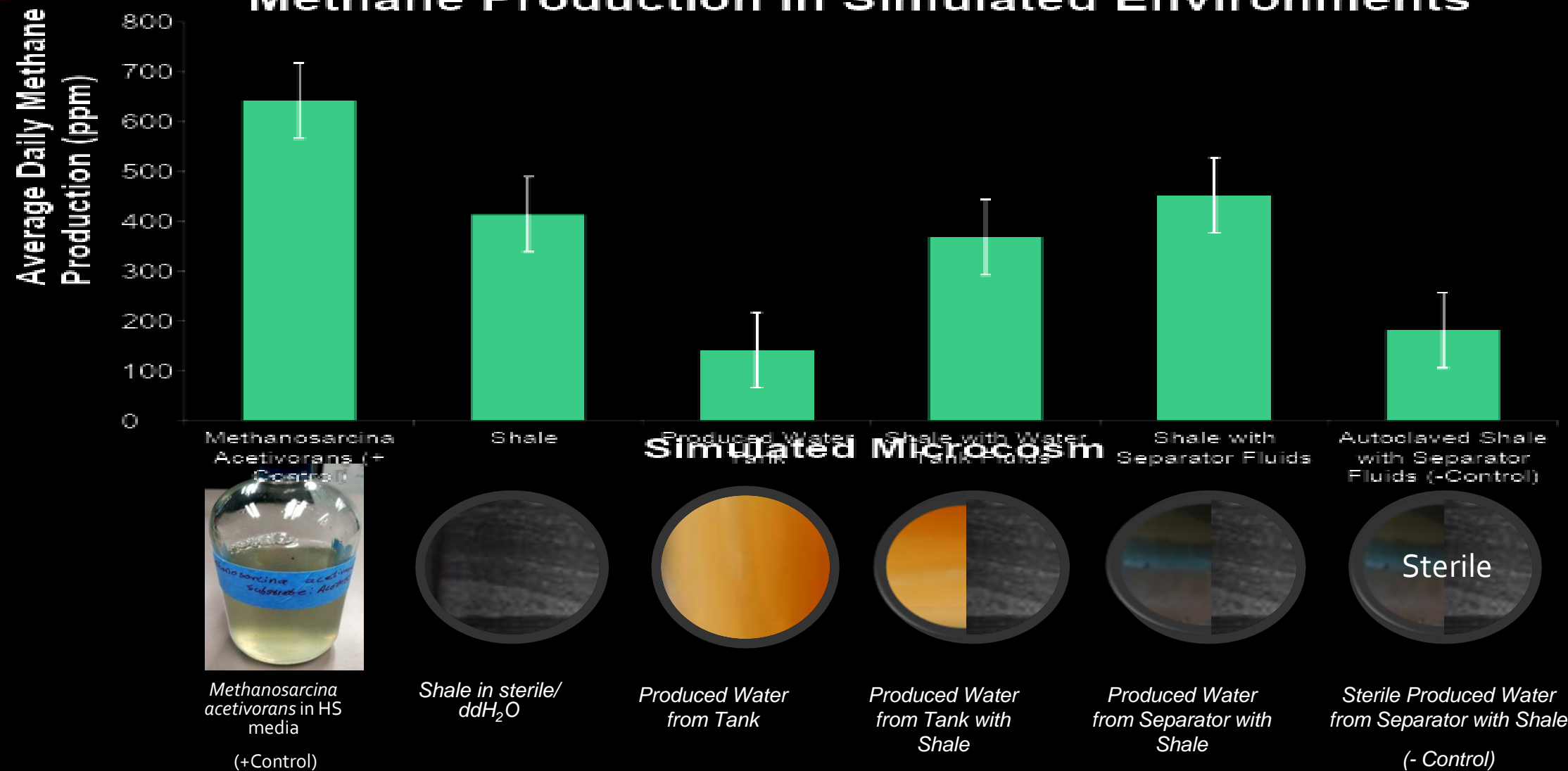
Work faster than geologic processes.





Are They Alive and Active?

Methane Production in Simulated Environments

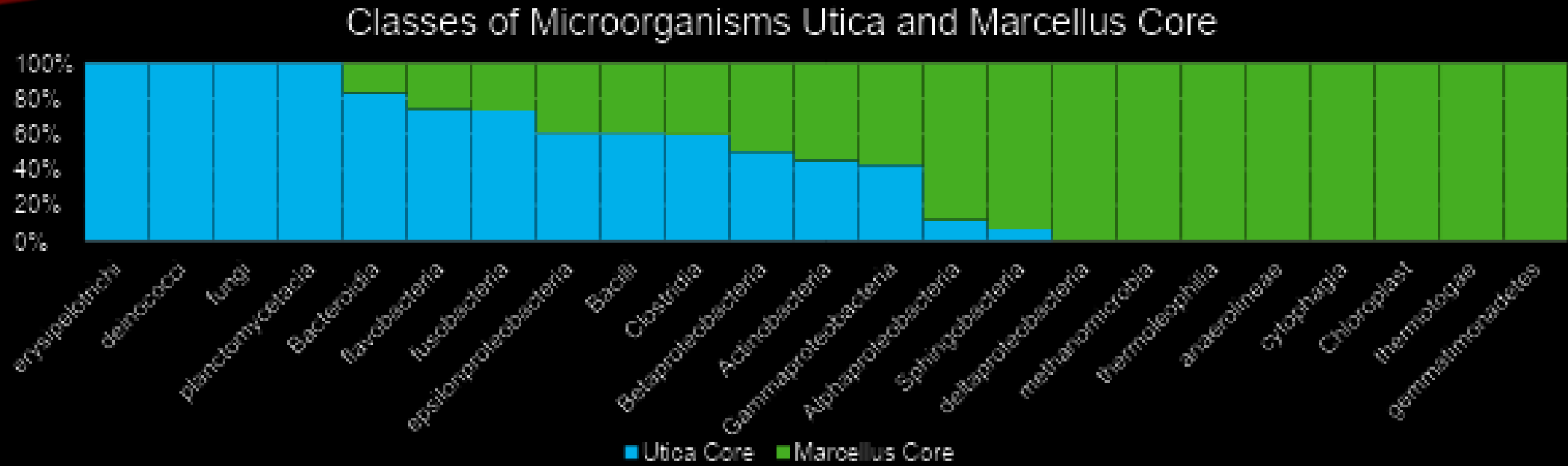


Brief Study



Utica Shale

Microorganisms in Utica and Marcellus Cores



* Preliminary data comparing several Marcellus cores to one Utica shale core.

Notable organisms found in Marcellus cores:

Deltaproteobacteria - includes sulfate-reducing bacteria (*Desulfobacterales*, *Desulfovibrionales* and *Syntrophobacterales*.)

Alphaproteobacteria - Acid producer

Pseudomonas and *Acinetobacter* - Biofouler

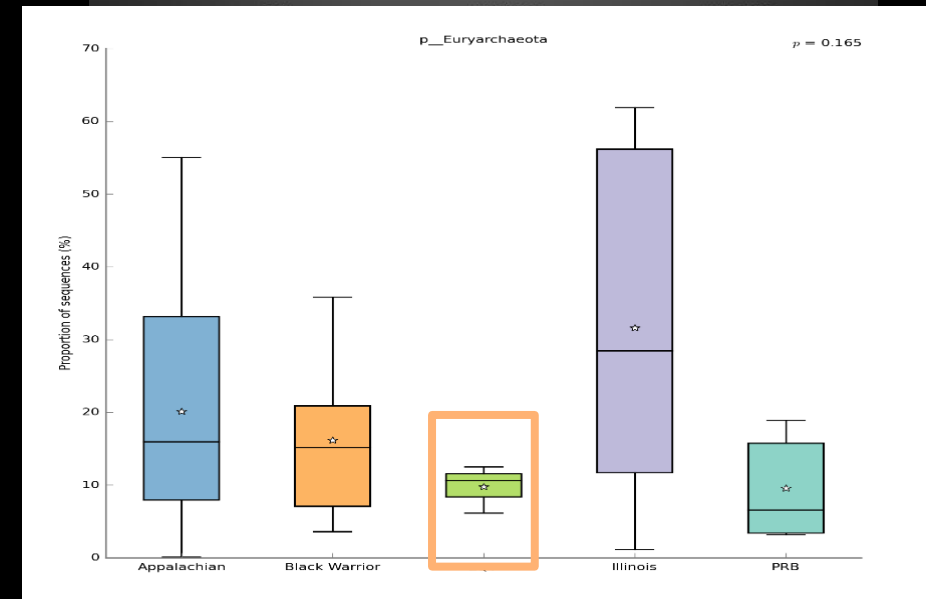
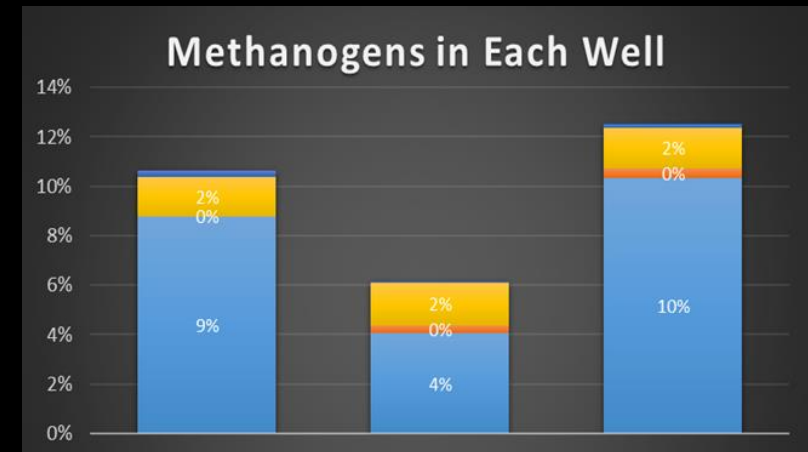
Methanomicrobia - can produce methane.

Brief Study

Lower Huron Shale

Lower Huron Shale

Phylum	1	2	3	Description
Firmicutes	6%	21%	22%	Many produce spores that can survive extreme conditions.
Proteobacteria	78%	33%	21%	Broad group with a wide range of genera and metabolisms (from Nitrogen to Oil)
Actinobacteria	1%	28%	16%	Soil dwelling organisms that can decompose organic matter and fix nitrogen.
Euryarchaeota	11%	6%	12%	Methane producing microorganisms.
Fusobacteria	0%	2%	11%	Gain energy by fermentation of carbohydrates and amino acids. Can be key for biofilm production.
Bacteroidetes	1%	4%	9%	Widely distributed organisms in the environment, sediments and soil that can be crucial for organic matter breakdown.
Chloroflexi	0%	1%	3%	Include microbes that use halogenated organics as energy sources.
Tm7	0%	0%	2%	Uncultivated phylum found in soil samples, can take up a wide variety of carbon sources.
Synergistetes	1%	2%	2%	Can produce biogas in anaerobic digesters.
Thermotogae	0%	0%	1%	Produce hydrogen. Required for methane production with CO ₂ .
Op9	0%	0%	1%	Uncultivated microorganisms often found in hydrothermal vents. Also, commonly found in the deep subsurface.
Acidobacteria	0%	0%	0%	Only phylum found in all caves.
Spirochaetes	0%	0%	0%	Anaerobic organisms that can digest cellulose and other plant polysaccharides.

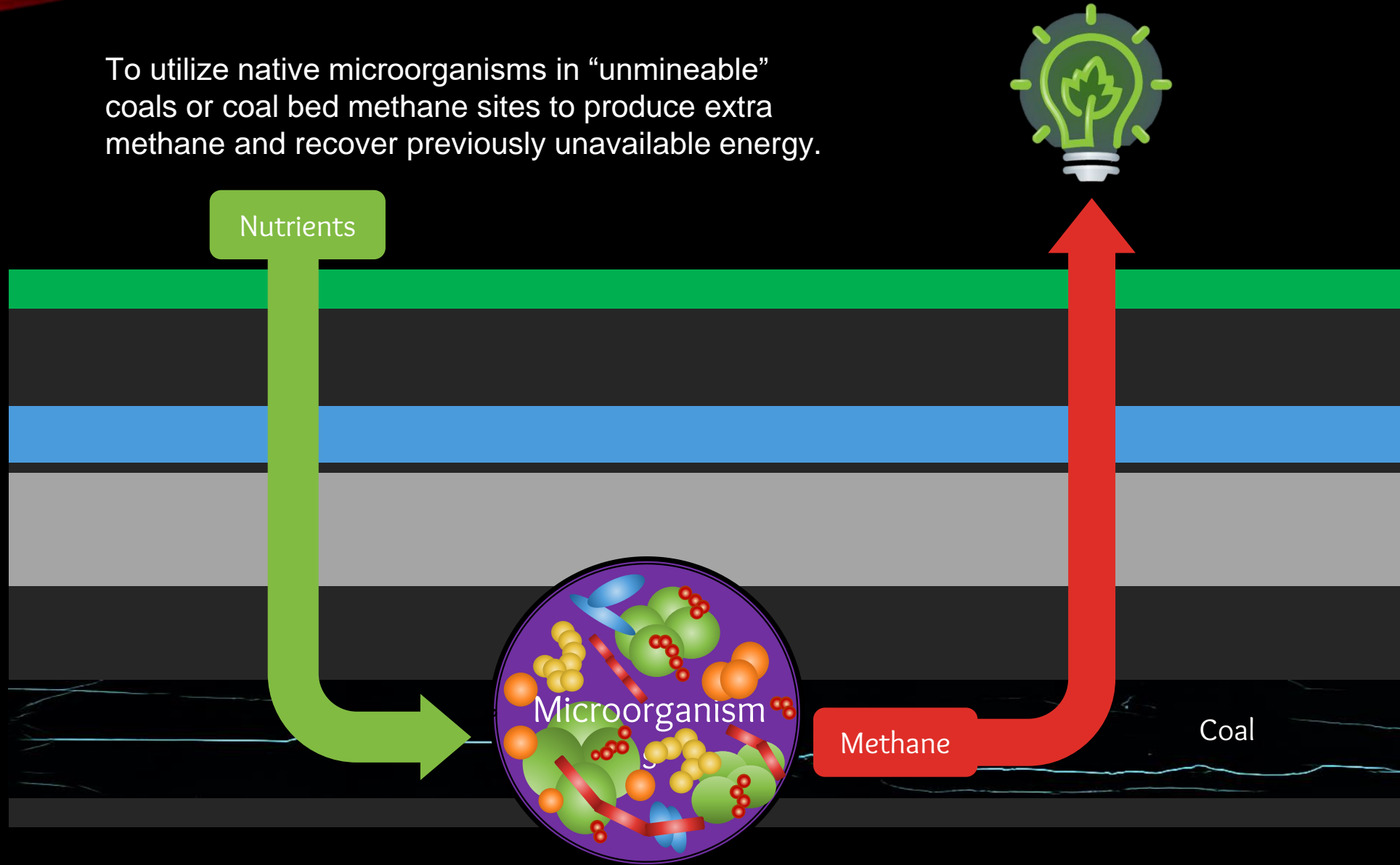


Next Study

US Coalbed Methane

Coal Research Purpose

To utilize native microorganisms in “unmineable” coals or coal bed methane sites to produce extra methane and recover previously unavailable energy.



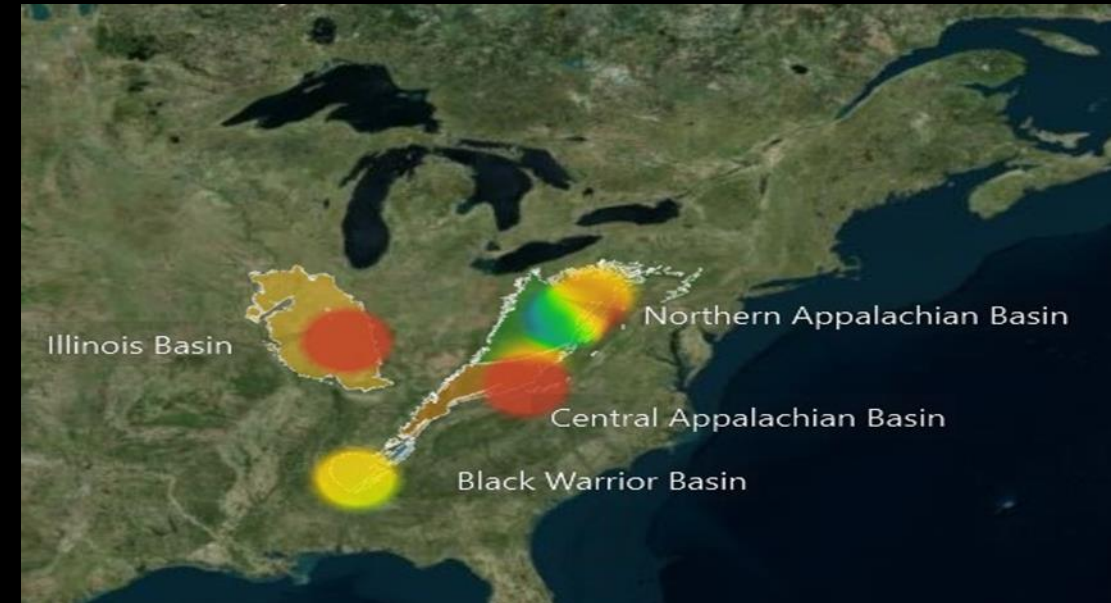
Summary of Results

Wide variation was seen between samples of the same basin even when they were very close together.

Between 0-61% methanogens were found in the samples assessed.

This could suggest faster rates of production possible than previously expected.

No significant numbers of methanotrophs (methane consumers) found in any of the wells.



Production Correlations Across Basins									
Location	Appalachian			Black Warrior			Illinois		
Production (MCF)	<i>Cumm</i>	<i>Avg</i>	<i>Max</i>	<i>Cumm</i>	<i>Avg</i>	<i>Max</i>	<i>Cumm</i>	<i>Avg</i>	<i>Max</i>
Euryarchaeota	0.17	0.29	0.12	0.93	0.87	0.92	-0.48	-0.33	-0.06
MCG	-0.02	-0.06	0.24	0.17	0.02	0.32	0.66	0.85	0.98
Methanobacteriales	0.60	0.67	0.46	0.96	0.91	0.94	-0.23	0.02	0.35
Methanococcales	0.72	0.70	0.54	0.23	0.08	0.34	-0.37	-0.46	-0.49
Methanomicrobiales	-0.41	-0.33	-0.36	0.56	0.59	0.45	0.01	-0.09	-0.19
Methanosarcinales	-0.31	-0.06	-0.12	-0.05	-0.20	0.11	-0.07	-0.10	-0.18
E2	0.32	0.54	0.45	0.04	-0.10	0.18	-0.15	-0.15	-0.16

Production Correlations

Production Correlations On Phylum Level									
Location	Appalachian			Black Warrior			Illinois		
Production	Cumulative	Average	Maximum	Cumulative	Average	Maximum	Cumulative	Average	Maximum
[Thermi]	0.45	0.58	0.42	-0.48	-0.34	-0.63	-0.28	-0.38	-0.46
Acidobacteria	0.79	0.81	0.68	0.93	0.98	0.81	-0.07	0.22	0.61
Actinobacteria	0.01	0.11	-0.15	-0.67	-0.60	-0.73	-0.08	-0.17	-0.28
Bacteroidetes	-0.07	0.11	0.22	-0.34	-0.43	-0.19	-0.02	0.01	0.07
Chlorobi	-0.36	-0.50	-0.34	0.22	0.23	0.23	0.29	0.35	0.33
Chloroflexi	0.45	0.55	0.31	-0.38	-0.40	-0.29	0.49	0.64	0.72
Crenarchaeota	-0.09	-0.04	0.14	0.91	0.94	0.81	0.37	0.42	0.39
Cyanobacteria	-0.39	-0.13	-0.06	0.00	-0.25	0.26	-0.13	-0.20	-0.29
Elusimicrobia	-0.24	-0.11	-0.20	-0.12	-0.10	-0.11	-0.14	-0.18	-0.25
Euryarchaeota	0.18	0.29	0.13	0.93	0.87	0.92	-0.48	-0.33	-0.06
Firmicutes	-0.35	-0.50	-0.52	0.34	0.12	0.57	0.29	0.06	-0.28
Fusobacteria	-0.20	-0.30	-0.42	-0.13	-0.33	0.09	0.07	-0.02	-0.09
Gemmatimonadetes	0.62	0.74	0.61	-0.48	-0.34	-0.63	0.17	0.05	-0.09
Hyd24-12	0.48	0.36	0.28	0.99	0.98	0.92	0.61	0.68	0.67
Lentisphaerae	-0.01	0.02	0.23	0.93	0.99	0.81	-0.16	-0.24	-0.28
Nitrospirae	-0.53	-0.54	-0.50	-0.15	-0.30	0.03	-0.26	-0.32	-0.39
OD1	-0.36	-0.48	-0.43	-0.15	-0.32	0.05	-0.03	-0.12	-0.25
OP1	0.58	0.74	0.55	-0.06	-0.21	0.11	-0.16	-0.09	0.00
OP11	-0.13	-0.05	-0.23	-0.15	-0.30	0.03	-0.31	-0.33	-0.33
OP3	-0.16	-0.06	-0.22	-0.15	-0.30	0.03	0.95	0.89	0.65
OP9	0.83	0.82	0.68	0.94	0.92	0.90	-0.06	0.24	0.63
Planctomycetes	0.02	-0.19	-0.05	0.93	0.99	0.81	0.79	0.79	0.64
Proteobacteria	-0.02	-0.03	0.13	-0.65	-0.54	-0.67	0.42	0.27	-0.03
SAR406	-0.18	-0.39	-0.40	0.93	0.99	0.81	0.04	0.23	0.50
Spirochaetes	0.29	0.44	0.23	0.07	0.15	0.02	0.56	0.60	0.59
Synergistetes	0.28	0.43	0.23	0.45	0.57	0.26	0.03	-0.05	-0.16
Tenericutes	0.06	-0.03	0.27	-0.35	-0.25	-0.40	0.04	-0.02	-0.13
Thermotogae	0.70	0.66	0.59	0.39	0.55	0.17	0.17	0.45	0.77
TM7	-0.49	-0.31	-0.36	0.71	0.63	0.74	0.06	-0.02	-0.09
Verrucomicrobia	0.07	0.08	-0.01	0.93	0.95	0.83	-0.08	0.21	0.60
WPS-2	-0.45	-0.19	-0.22	-0.15	-0.30	0.03	-0.14	-0.18	-0.25
WS1	-0.32	-0.46	-0.40	0.02	-0.12	0.18	-0.06	0.23	0.62
WWE1	0.84	0.76	0.62	0.94	0.97	0.84	0.93	0.87	0.63

Appalachian Basin

Methanogens: Methanobacteriales (0.60, 0.67, 0.46)
Methanococcales (0.72, 0.70, 0.54)

Acidobacteria: Soil microorganisms with versatile carbohydrate metabolisms

Thermatogae: Produce hydrogen for CO₂ conversion

Black Warrior Basin

Methanogens: Methanobacteriales (.96, .91, .94)

Verrucomicrobium: facultative anaerobic fermenters

Acidobacteria: Soil microorganisms with versatile carbohydrate metabolisms

Illinois Basin

Methanogens: Only MCG (.66, .85, .88) small population)

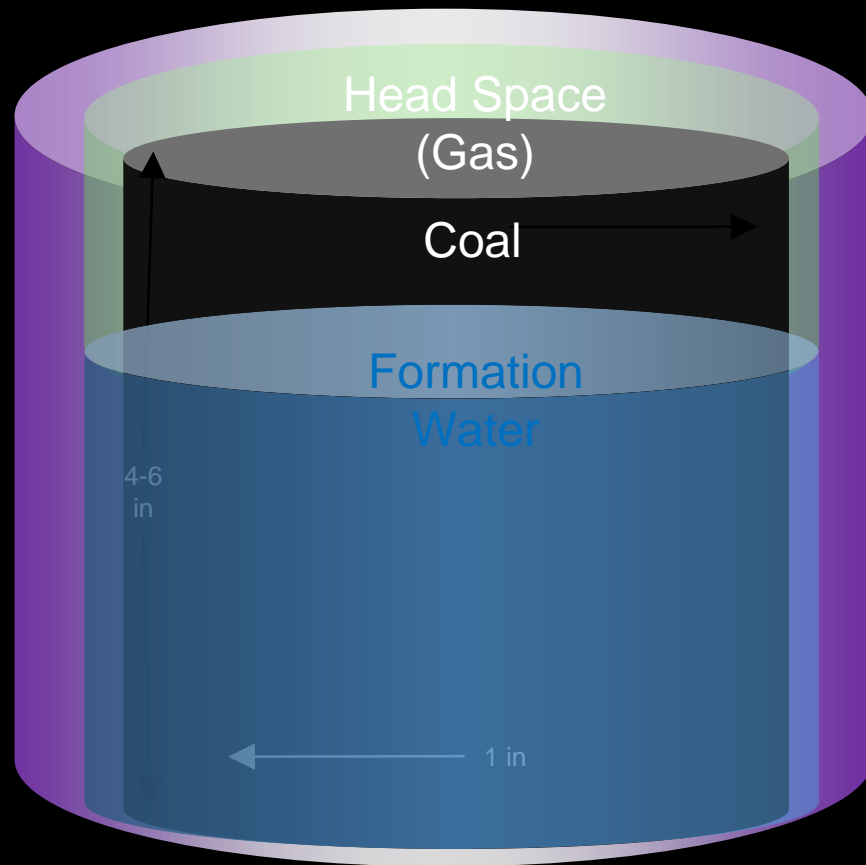
Thermatogae: Produce hydrogen for CO₂ conversion

Spirochaetes: Digest cellulose

WWE1: known to remove CO₂ and H₂ from formate

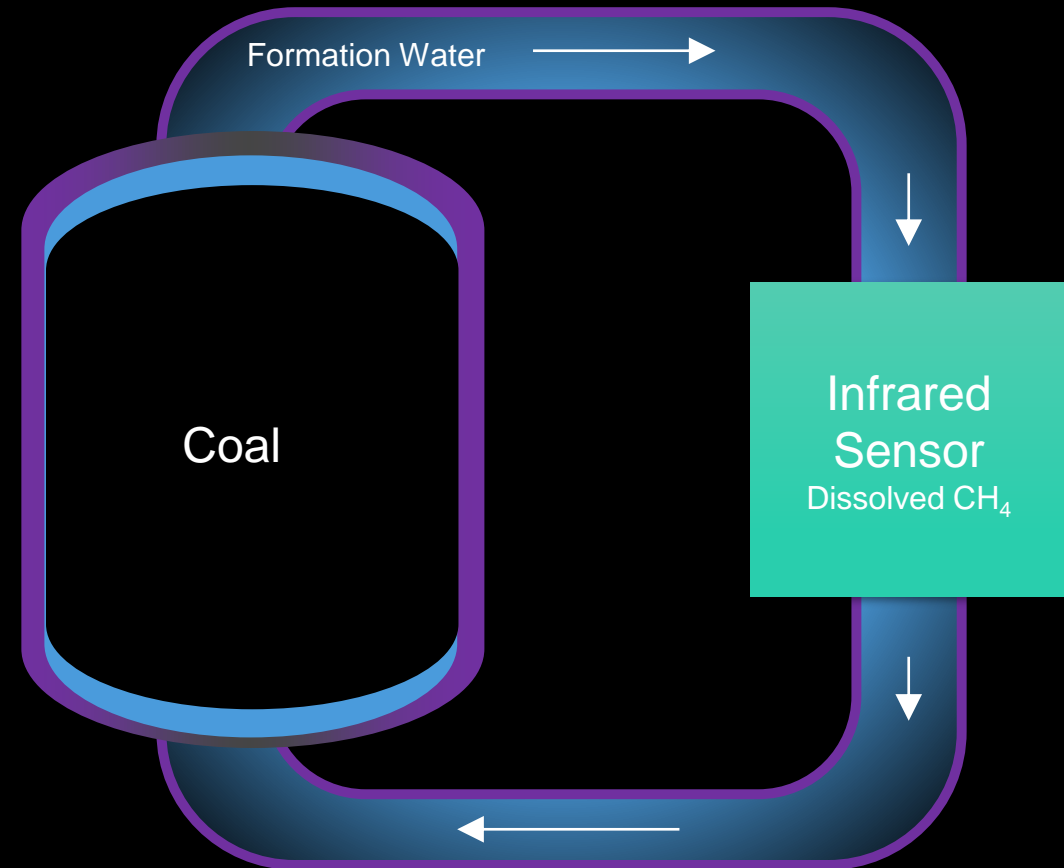
Tests at *In-Situ* Conditions

Static Bioreactors



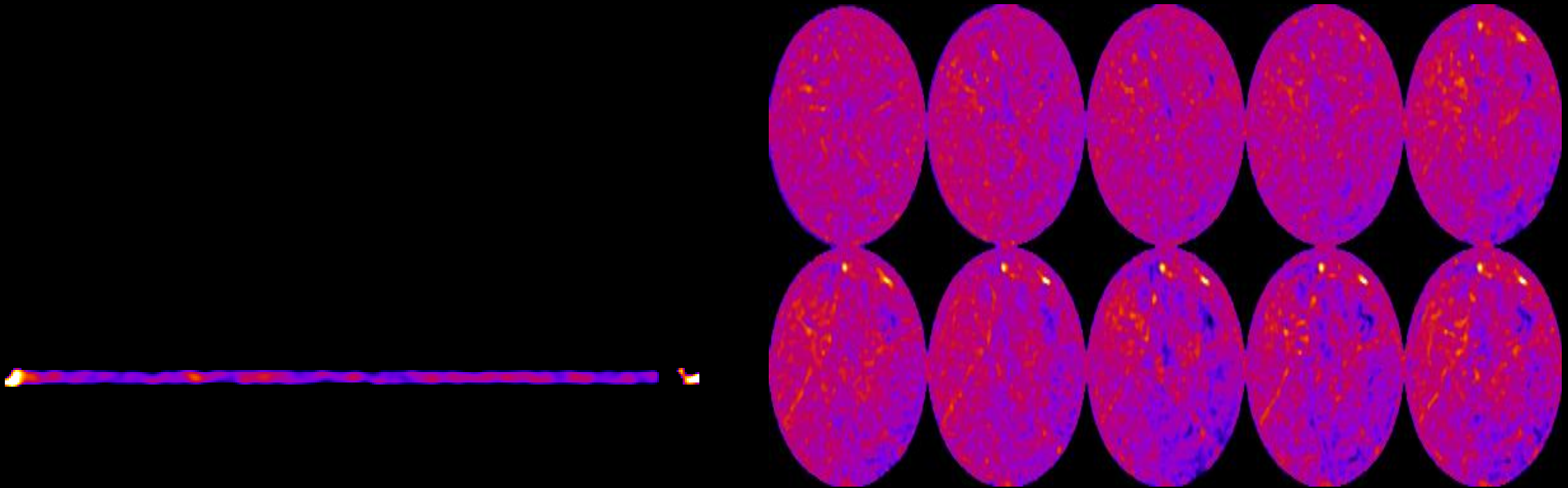
Pressure Range: 0-1000 psi.
Temperature: 40-150 °F

Future Design



Pressure Range: 0-5000 psi.
Temperature: 40-150 °F

Nutrient Delivery Tests



2' by 6' in Coal Core Sample Scanned using a Medical CT Scanner During Impregnation by KI Tracer to investigate potential nutrient transport mechanisms. Maximum resolution is about 400 micron.

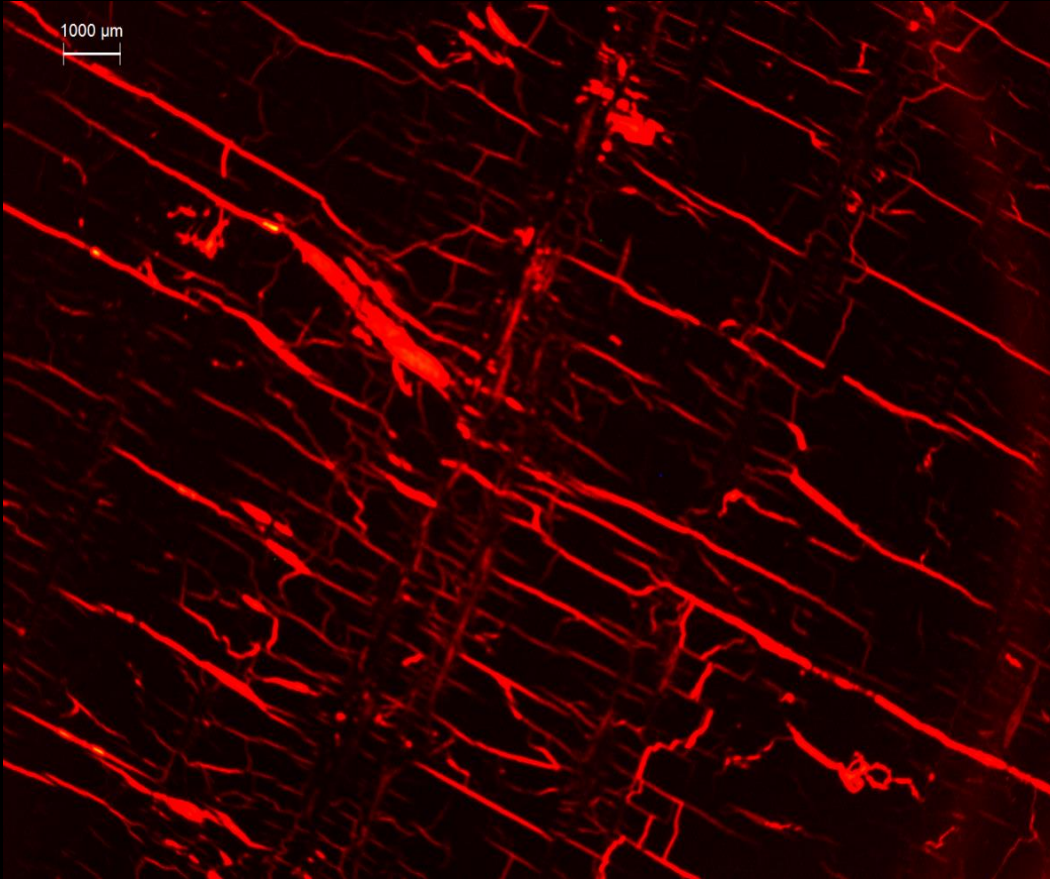
Conclusions

- We have found that nearly all samples (all but the one Utica core) contain methane producing microorganisms that are capable of helping in natural gas recovery.
- Not only are these organisms detectable, but they are viable and able to produce methane even after extraction using only the compounds naturally found after drilling a well.
- As expected, microbial composition varies significantly across samples, locations, and types of sediments.
- However, all samples studied in this research contained populations of microorganisms that may be useful for natural gas recharge.
- Each type of sample contained its own microorganisms with the capability to break down complex hydrocarbons into smaller compounds useful for methanogens.
- In addition, all samples microorganisms capable of producing hydrogen gas and other compounds required for various metabolic pathways of methanogenesis.
- While natural rates may be slow, there is a possibility that we could enhance rates to an economically viable level.

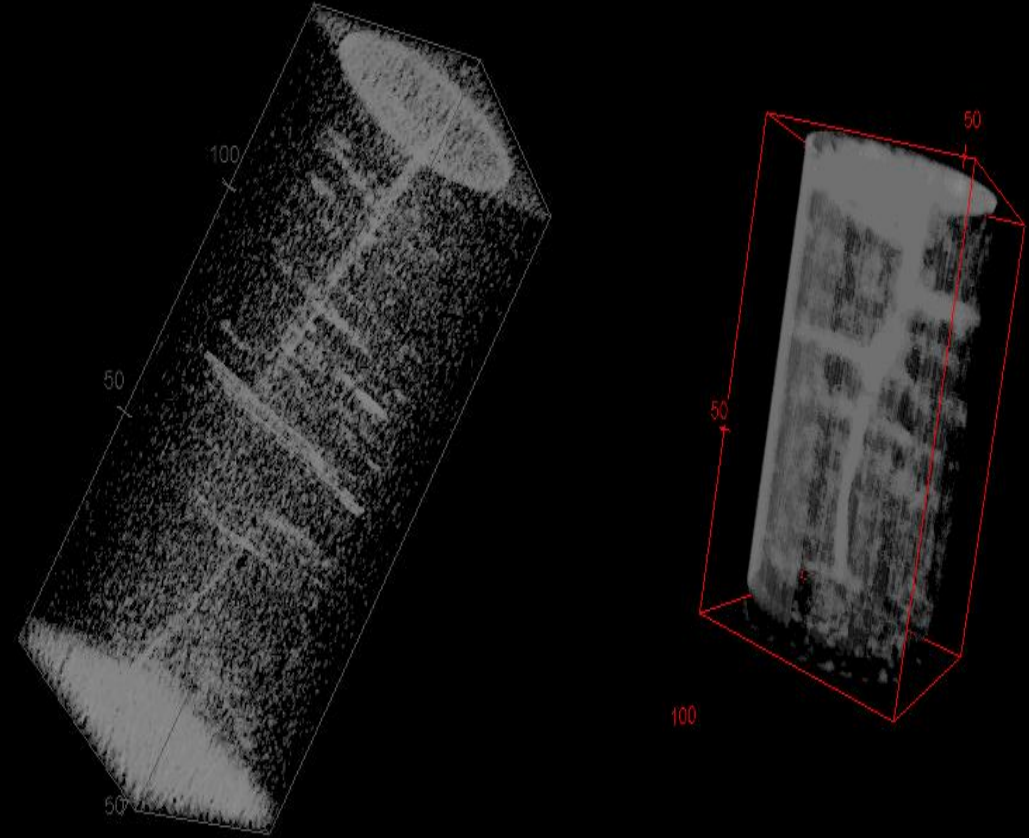
Therefore, it appears that injection of new microorganisms would be unnecessary for biogenic production of methane in any of the sampled wells.

From CT injection tests- It appears that nutrient injection could deliver nutrients across samples.

Questions?



Polished section of coal impregnated with rhodamine-dyed, fluorescent epoxy, which makes macerals, cleats and microcleats visible.



WV Coal Core Samples Scanned with a Medical CT scanner after KI impregnation.

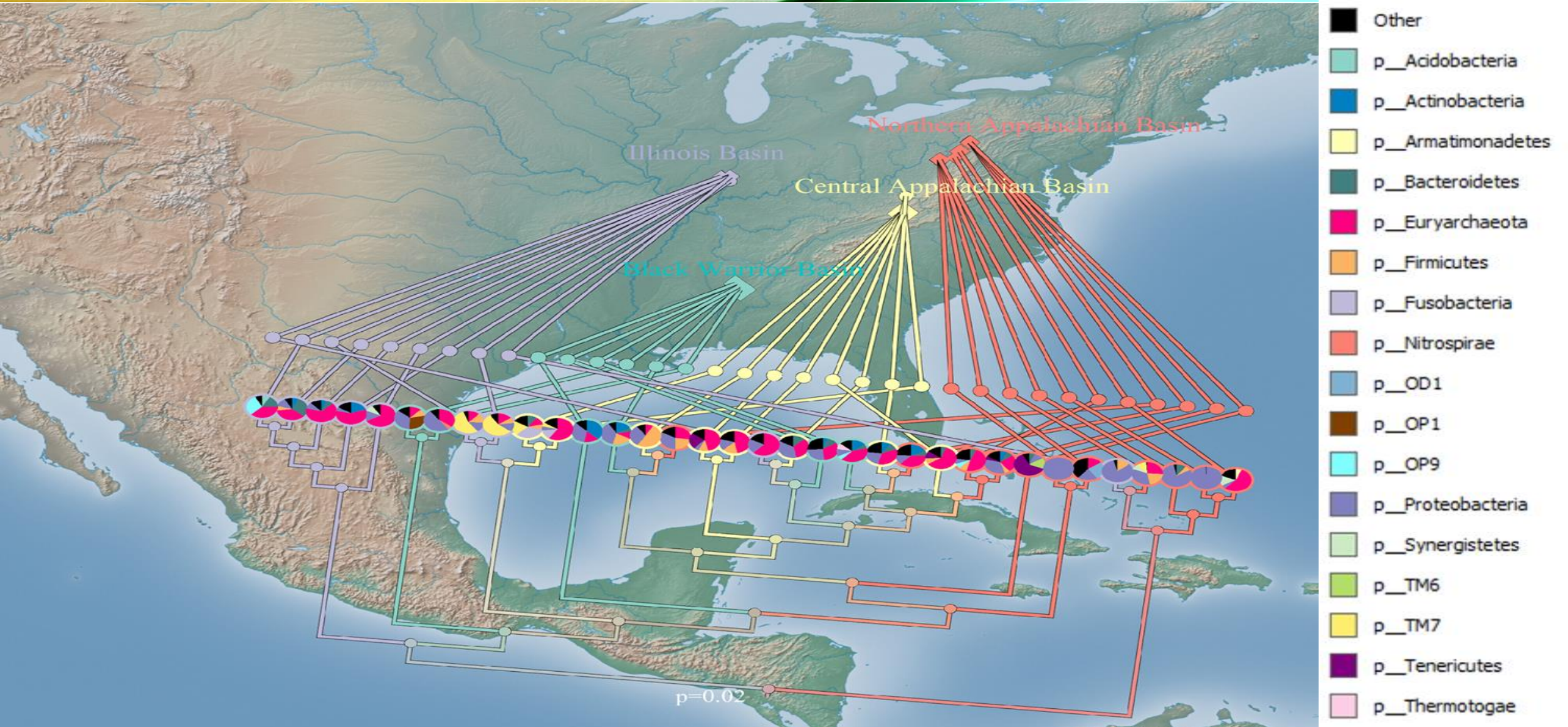
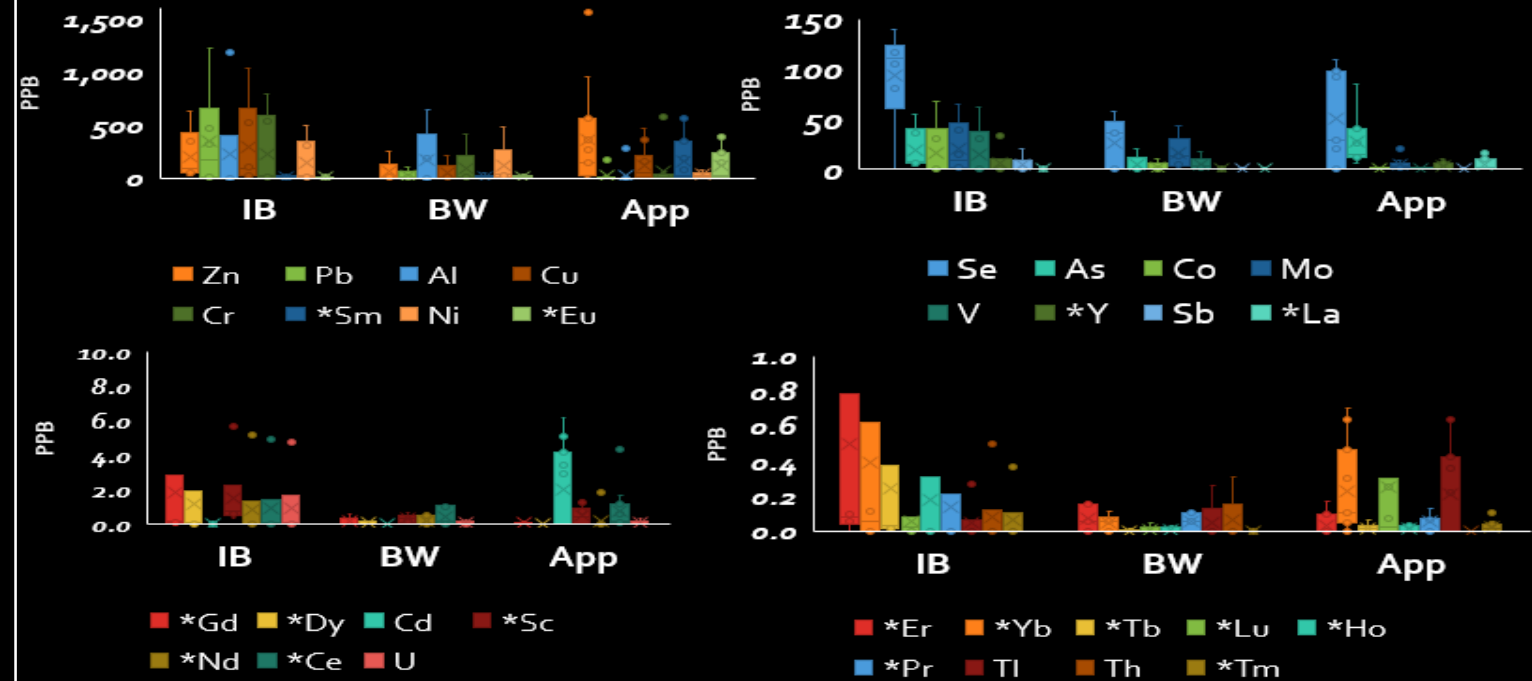
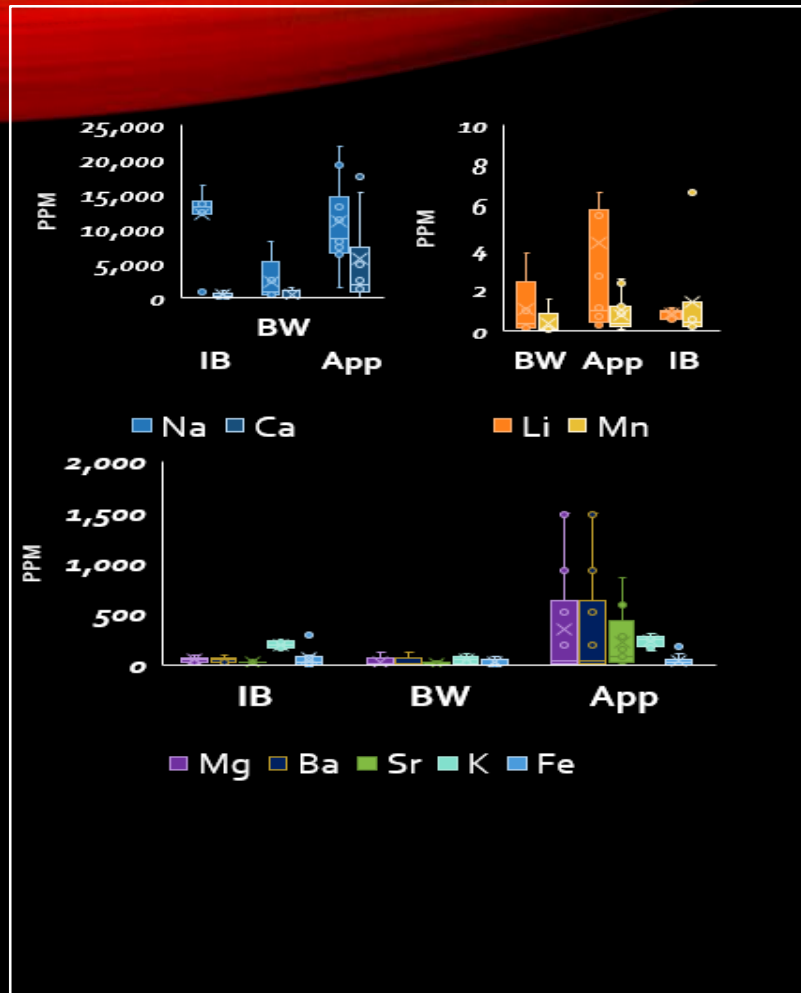


Fig. 1- Sequencing Data of Basins from the Eastern US: Map shows the microbial composition of samples from different locations in the pie graphs on the tree. Colors of circle outlines and lines indicate the basin from which the sample originated. Unweighted UPGMA unifracs tree suggests that changes in microbial communities can be attributed geographic relationships at a ($p < 0.05$).

Elemental Composition Of CBM Water



Chemical Concentrations of Elements In Produced Water Across Basins ICP-MS results for produced water samples across the different basins. Part A has elements with high concentrations in PPM values. Part B has elements with lower concentrations in PPB values. The basins are denoted as Illinois Basin: IB, Black Warrior Basin: BW, and Appalachian Basin: App. Elements with an asterisk (*) are rare earth elements.