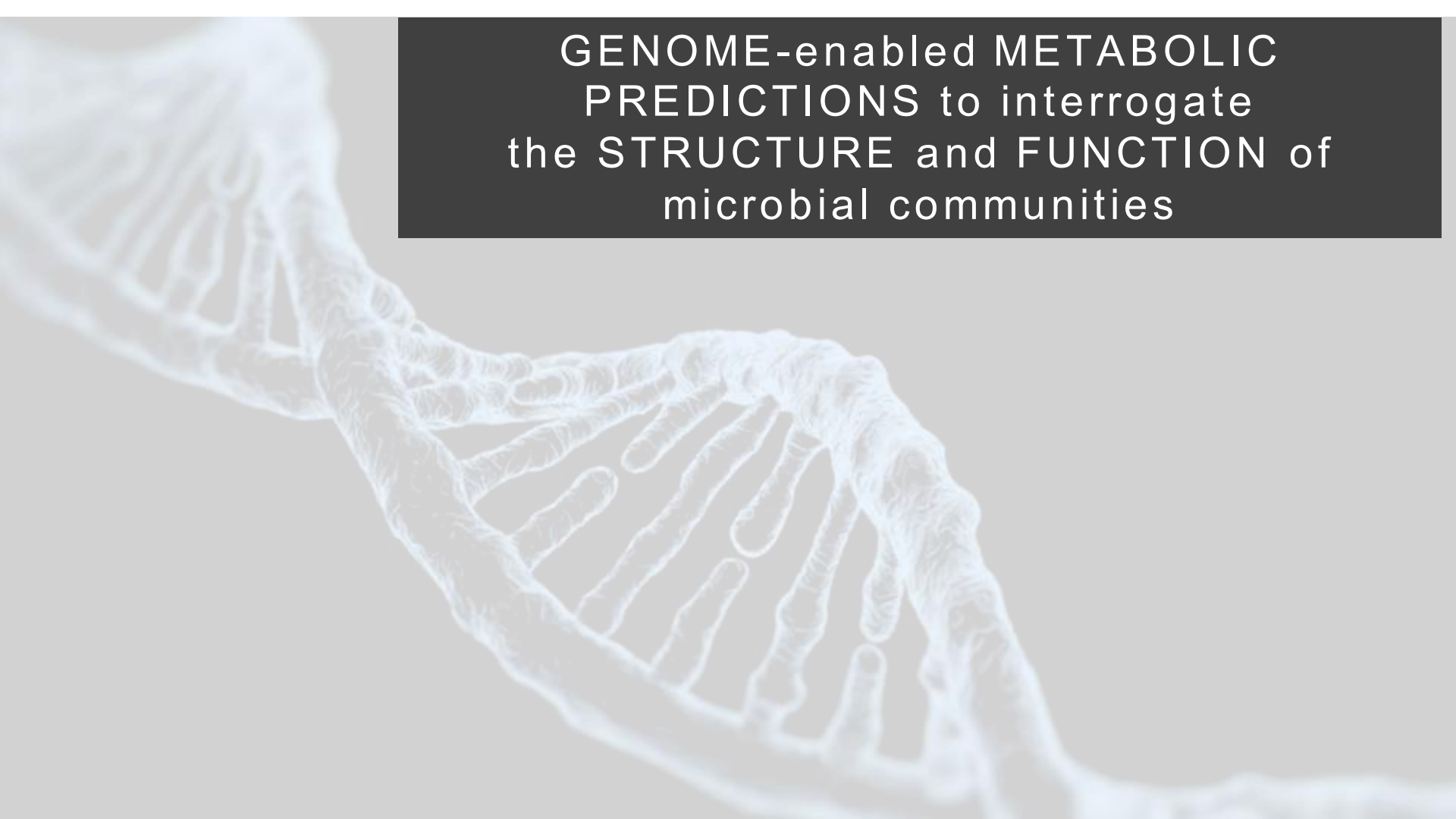
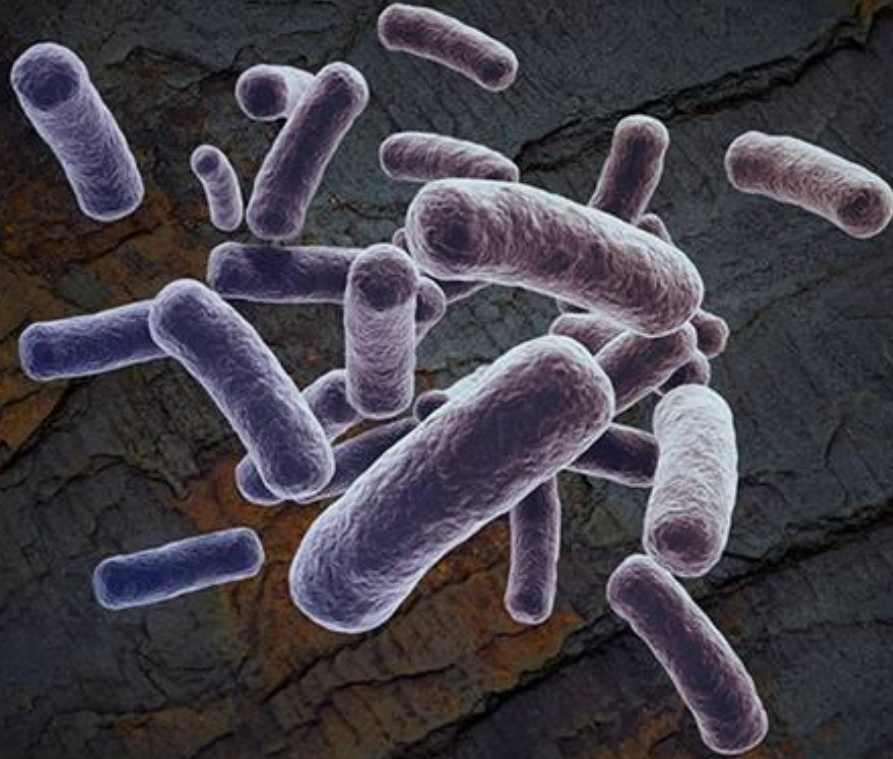




GENOME-enabled METABOLIC
PREDICTIONS to interrogate
the STRUCTURE and FUNCTION of
microbial communities

Case study from my group: Survivor Fractured Shale



How meta-omics can provide new mechanistic insights in an engineered ecosystem

Acknowledgments



Mike Wilkins
PI



Rebecca Daly
Research scientist



Mikayla Borton
grad student



Kaela Amundson
grad student



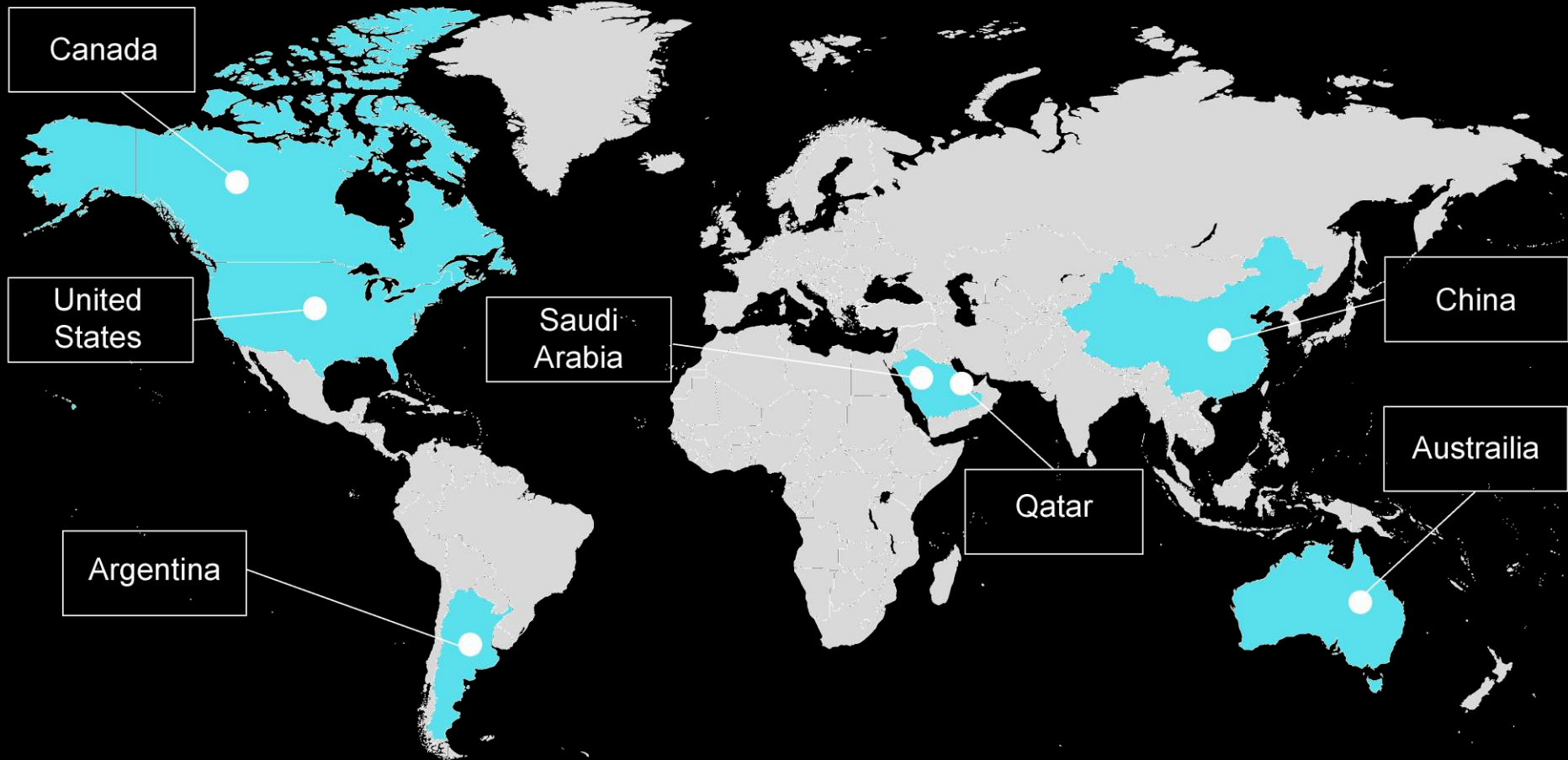
Anne Booker
grad student, alumni



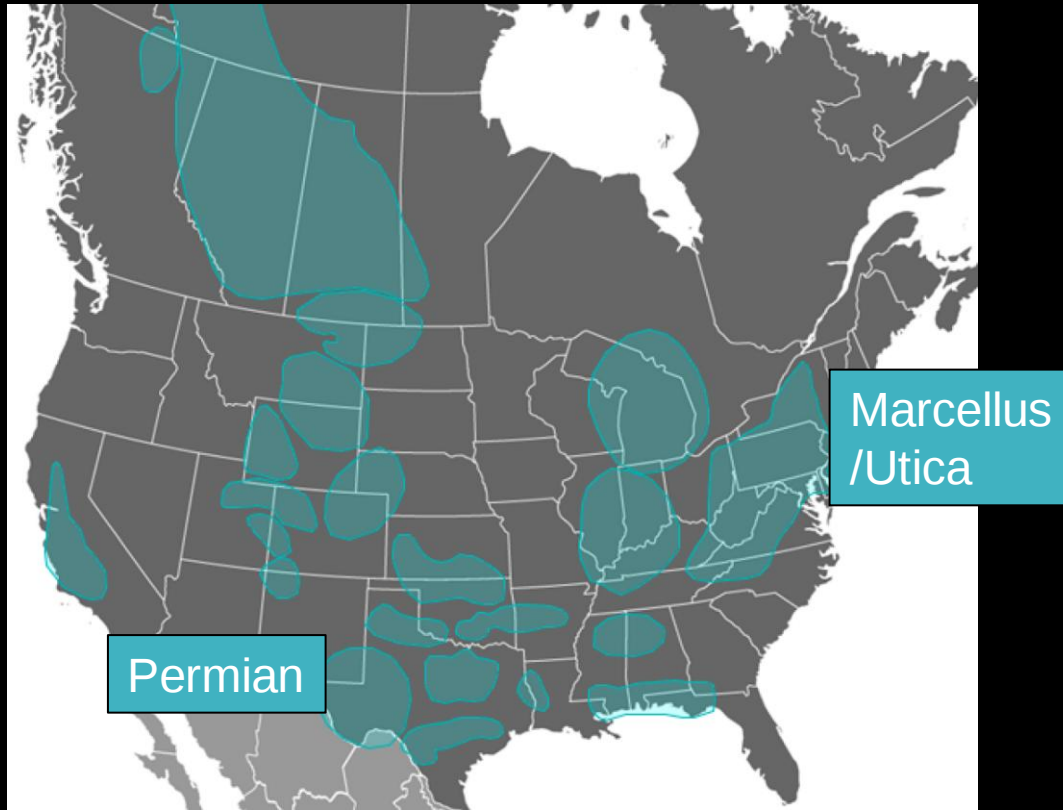
Robert Danczak
grad student, alumni



Countries with commercial shale energy



Hydraulically fractured shales and US energy portfolio



16 States in the US

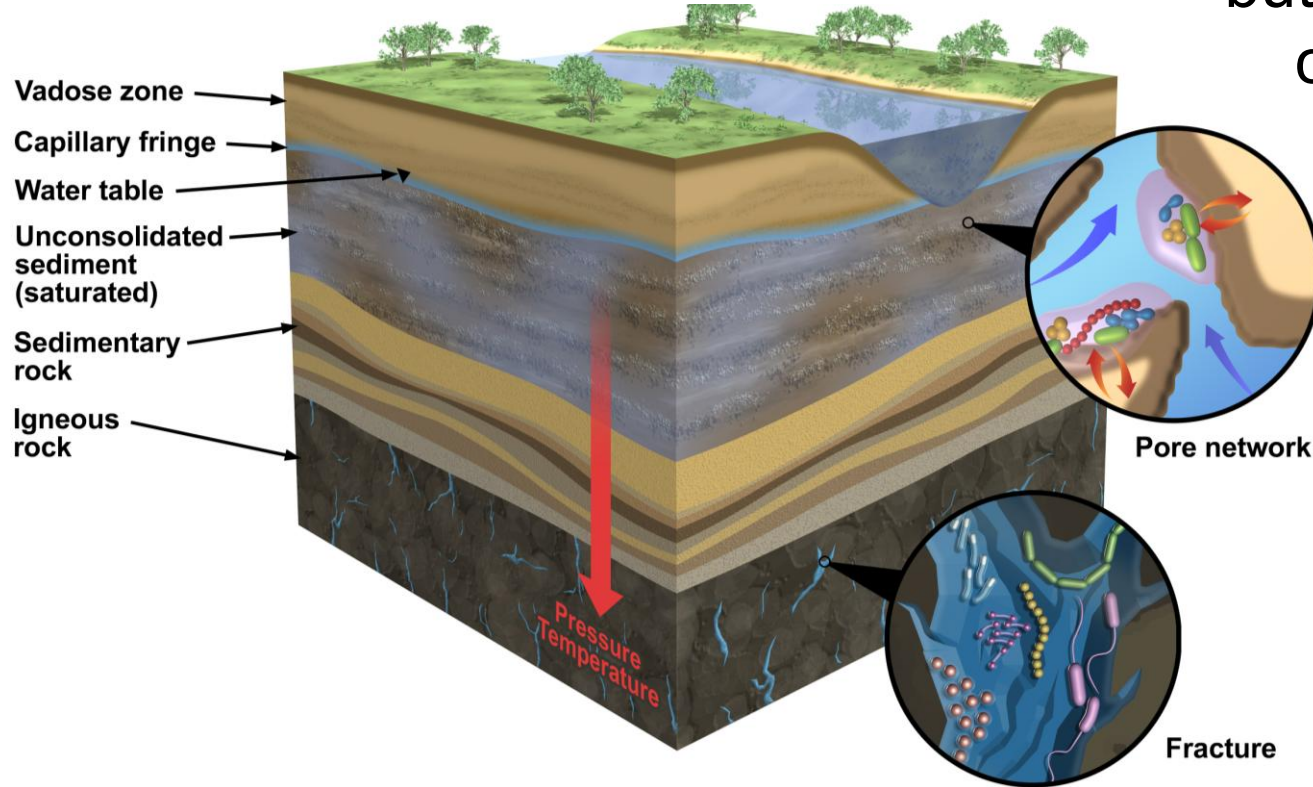
Marcellus contributes 30% of gas for the eastern seaboard

Permian contributes 35% of U.S. crude production and 17% of the natural gas supply

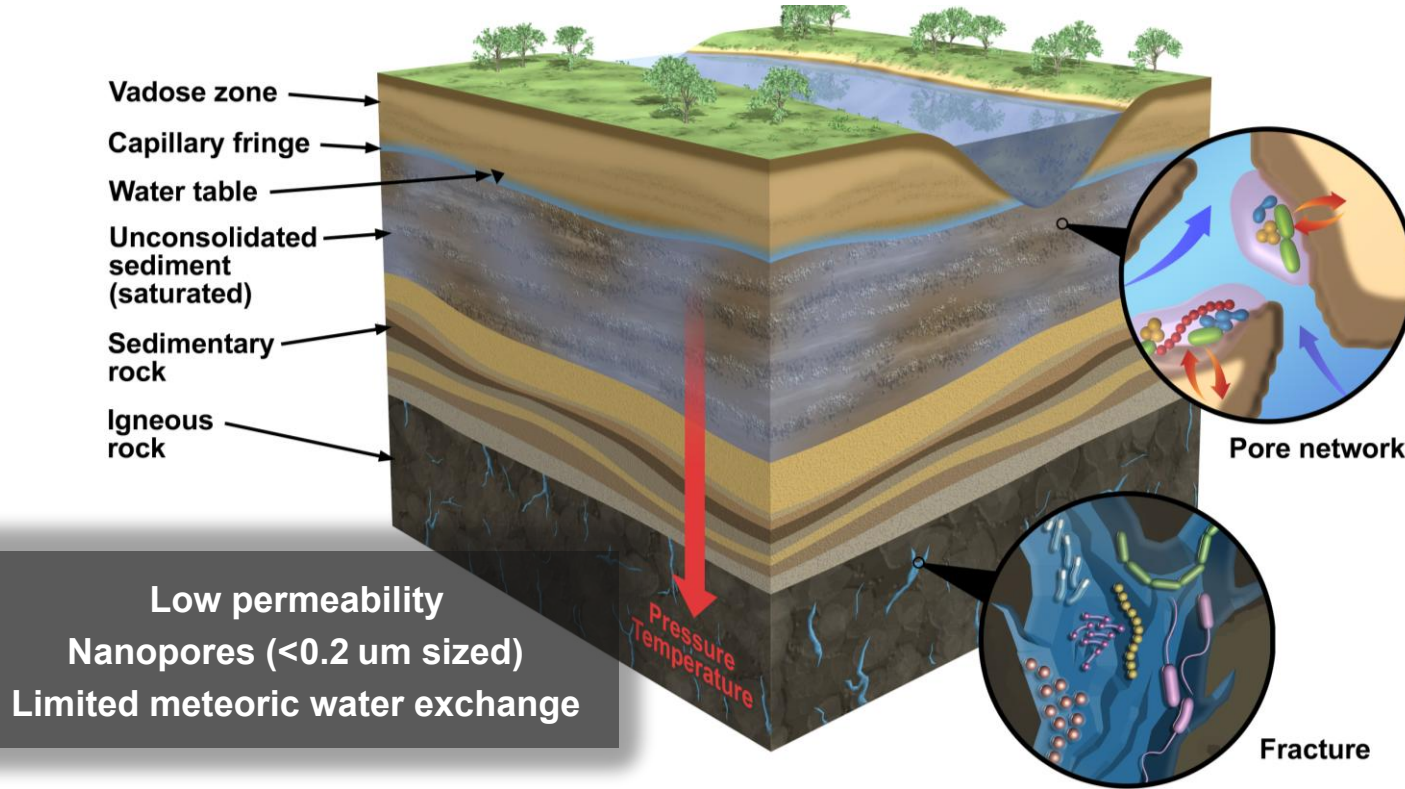
First time in US history natural gas replaces coal as main source for electricity

The deep terrestrial subsurface: The microbial frontier

1/3 earth's biomass
is below soil layer,
but remains poorly
characterized



Pristine Appalachian shales do not appear a conducive habitat for life



Andrea Hanson Paula Mouser Kai Hendricks Julius Lipp

Negative Impacts



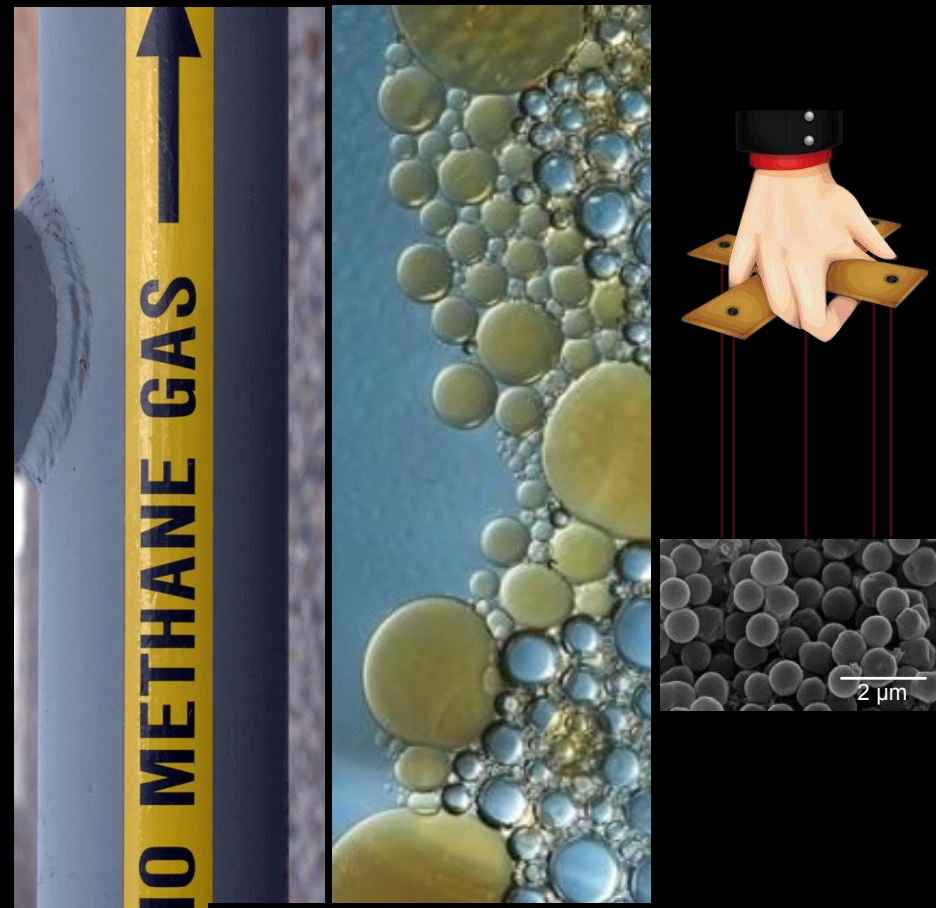
Booker et al, 2016, *AEM* & Booker et al, 2019, *Msphere*

Negative Impacts



Booker et al, 2016, *AEM* & Booker et al, 2019,

Positive Impacts

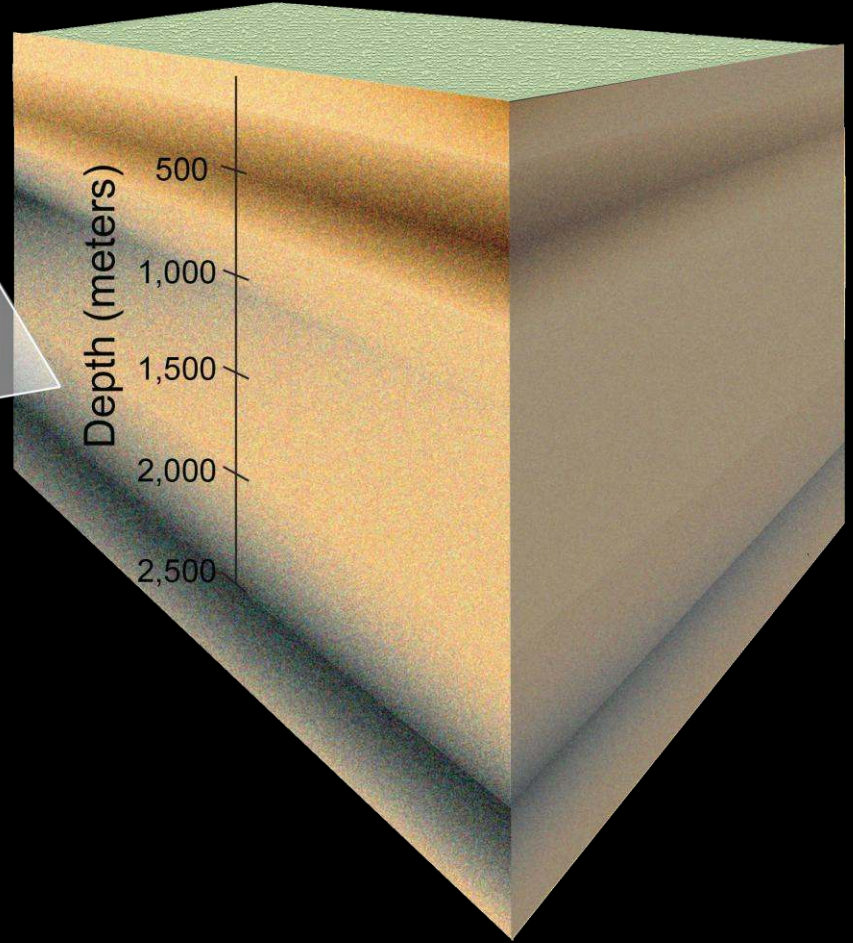


Borton et al, 2018 *Env Micro* & Park et al ,2013

What the frack?

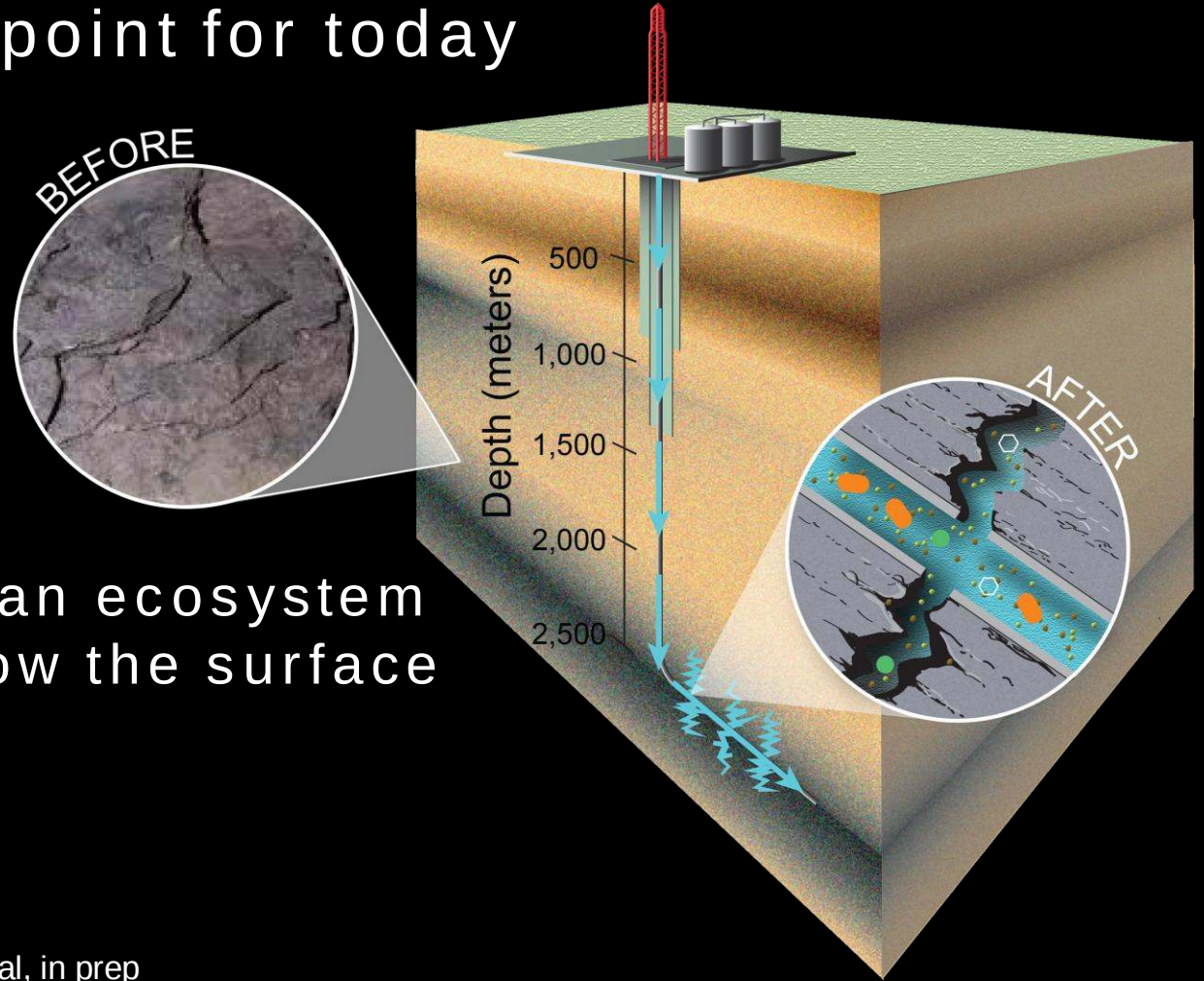


Prior to energy extraction,
most deep shales lack
requirements for life

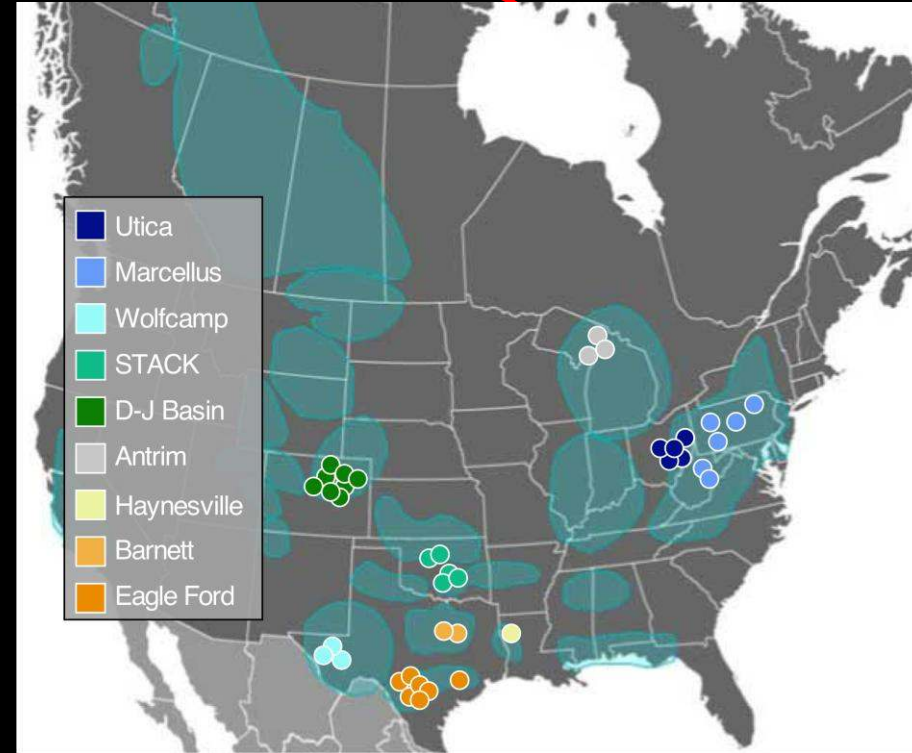
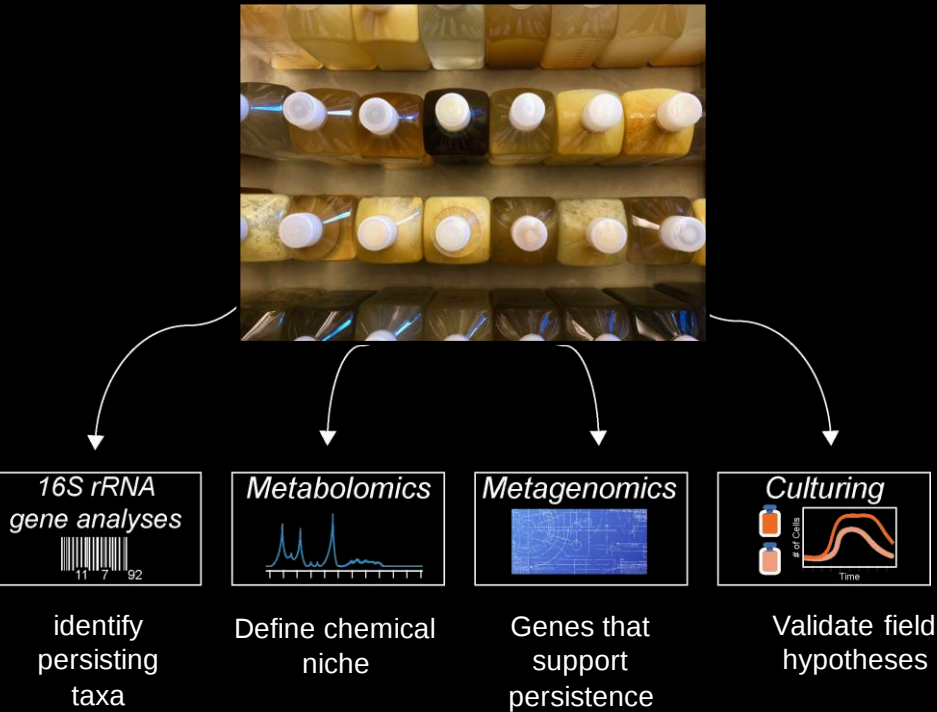


One take away point for today

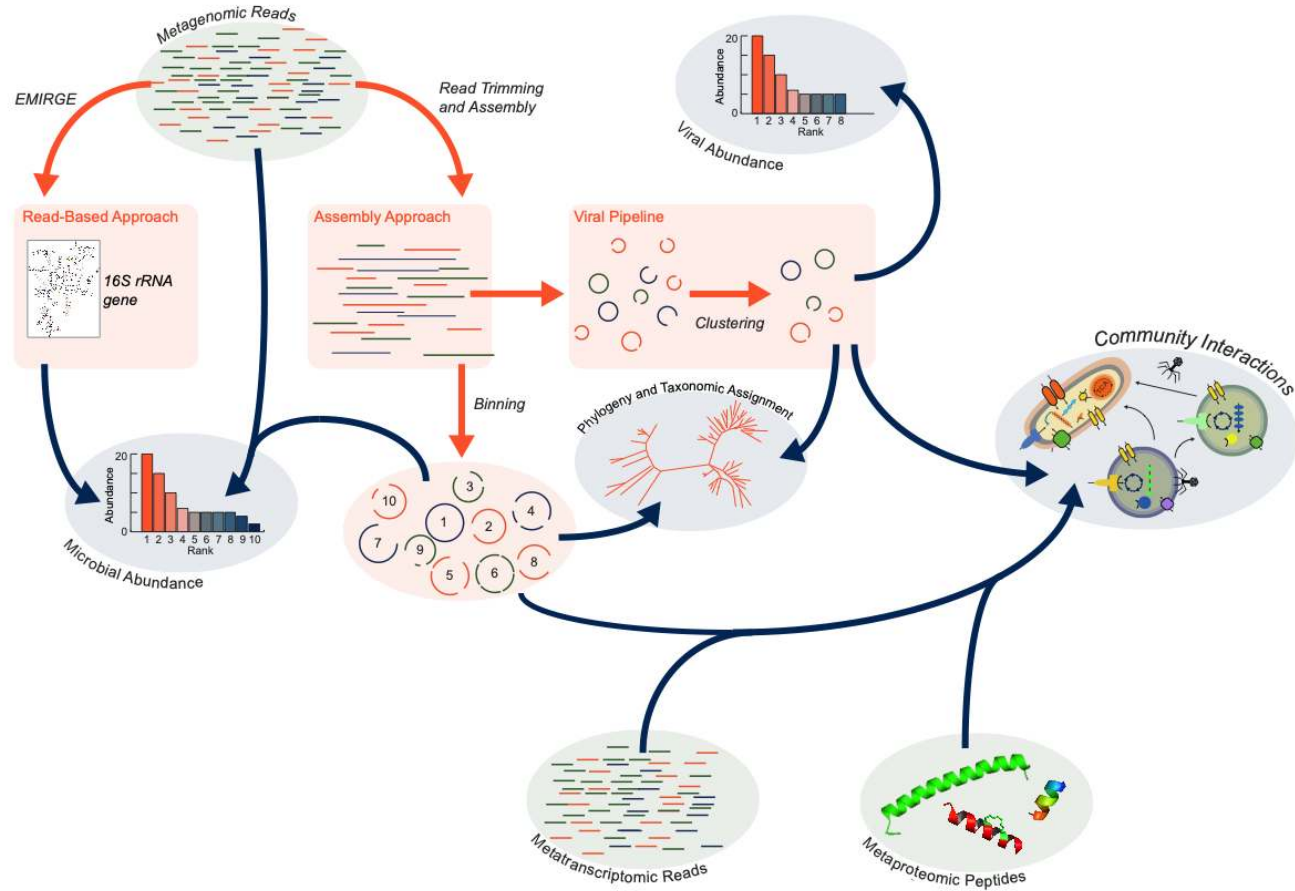
Fracking creates an ecosystem
2,500 meters below the surface



Development of a multi-omic spatial, temporal database



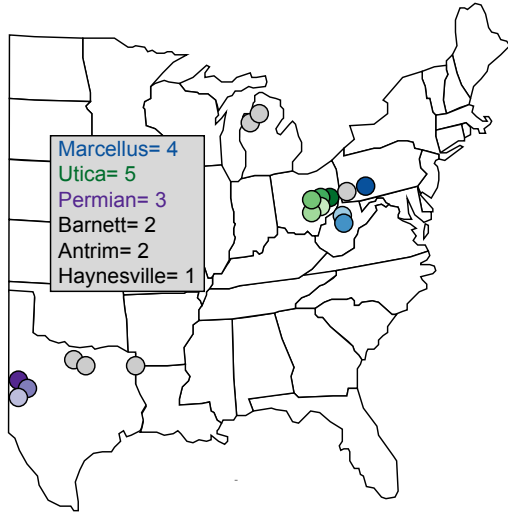
Computational pipeline used in this case study



1

What is the shale environment like?
What type of microorganisms persist in this system?

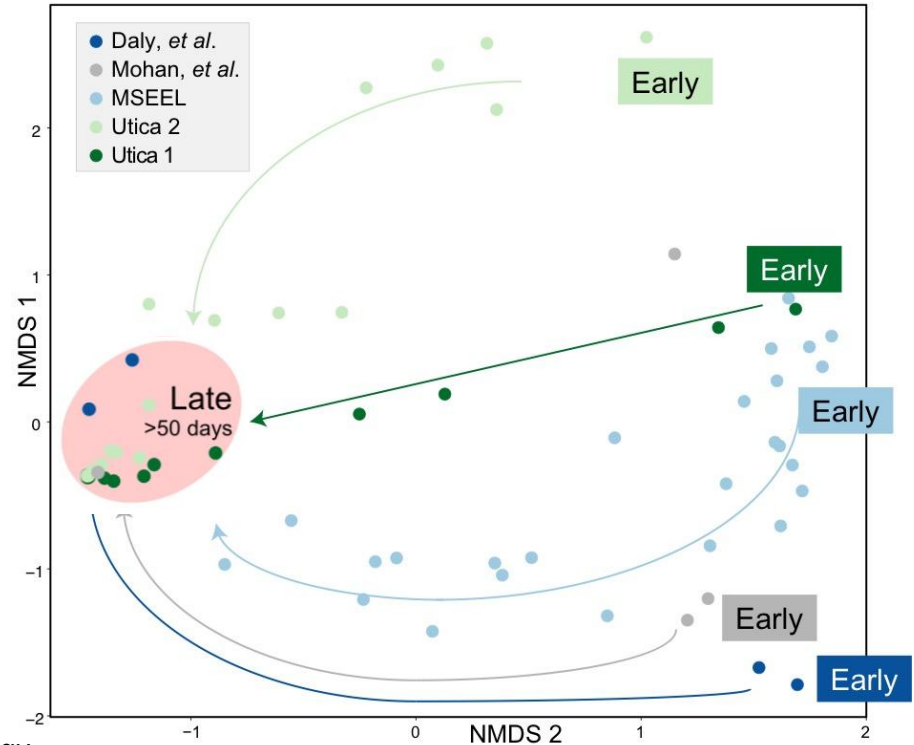
16S rRNA analyses of persisting microbial communities

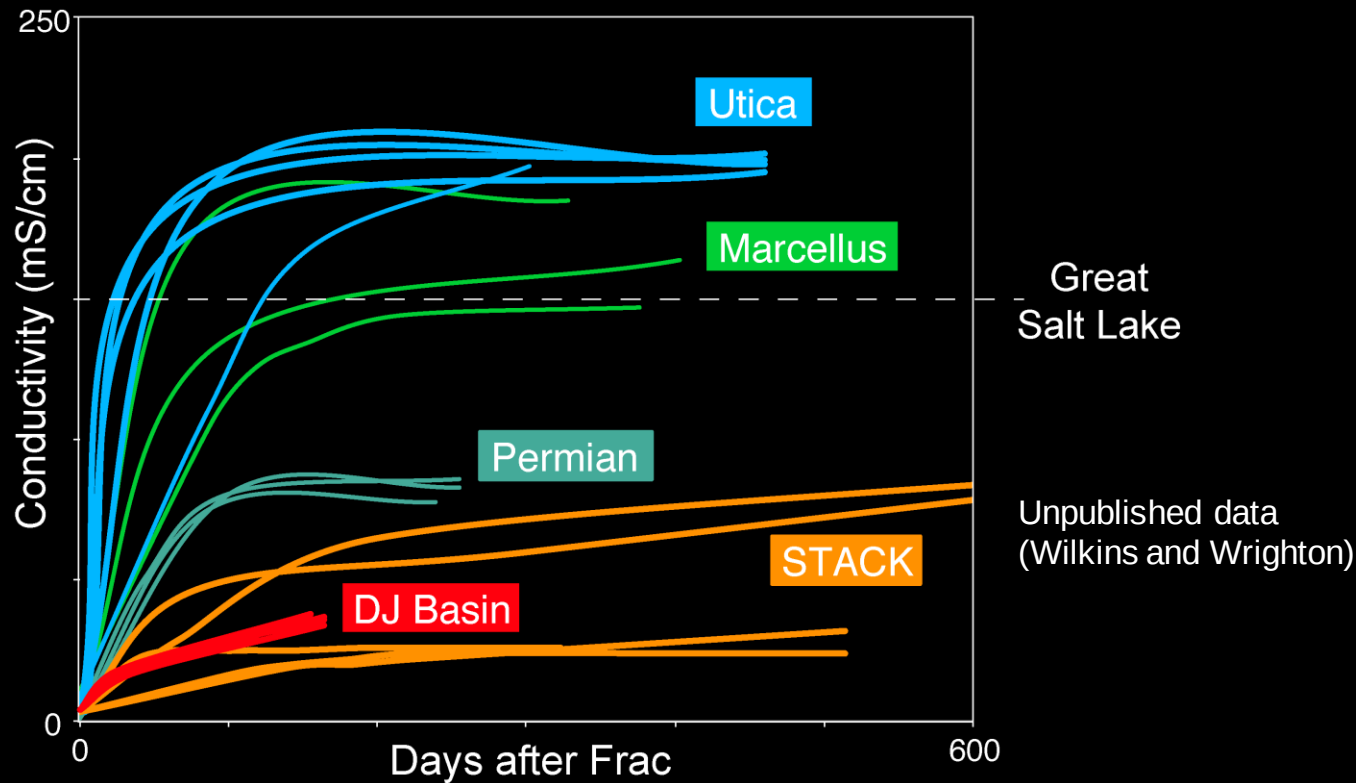


16S rRNA
gene analyses

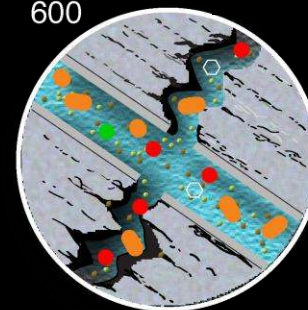
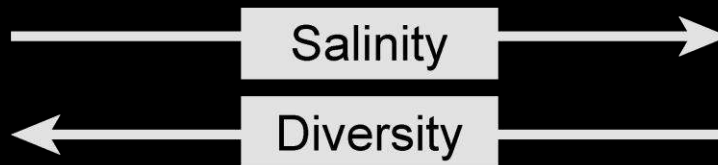
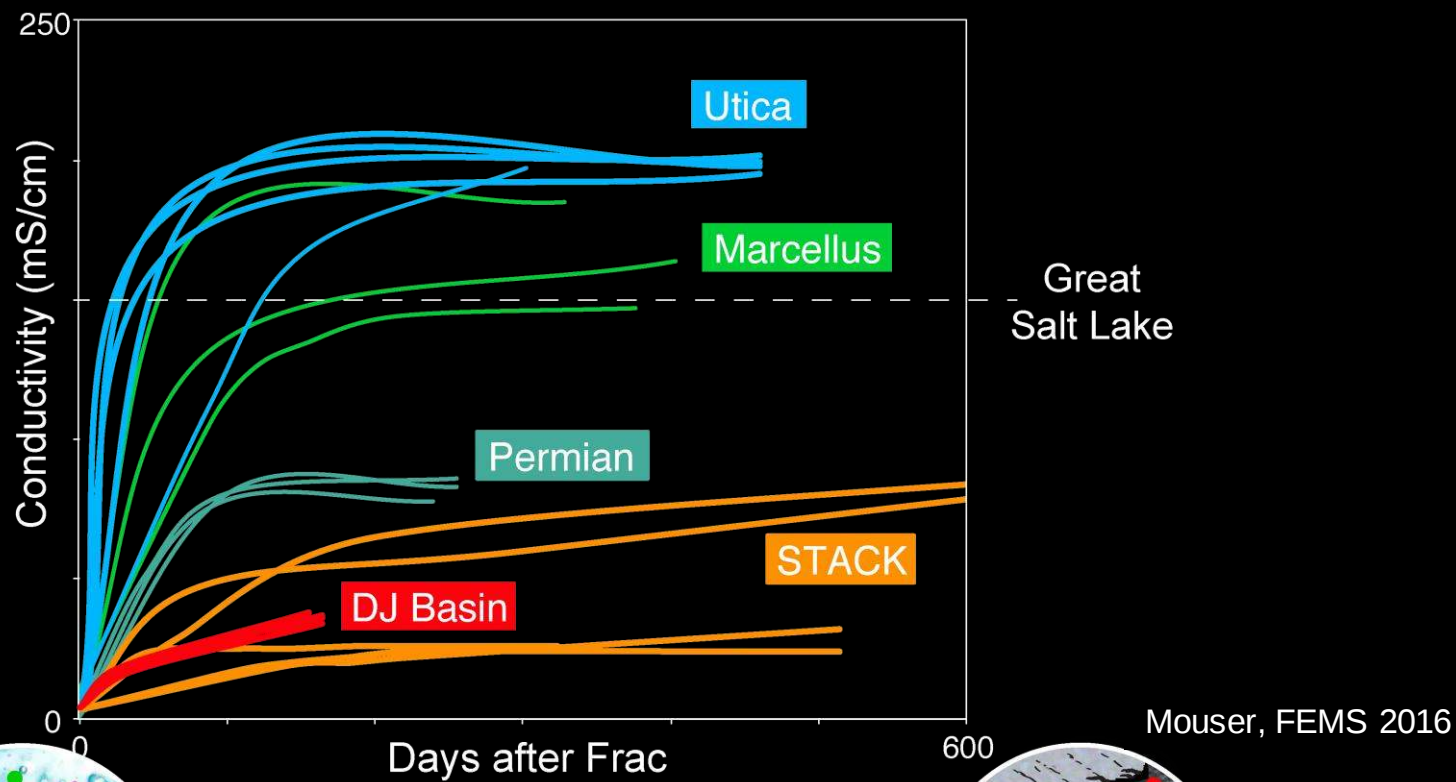
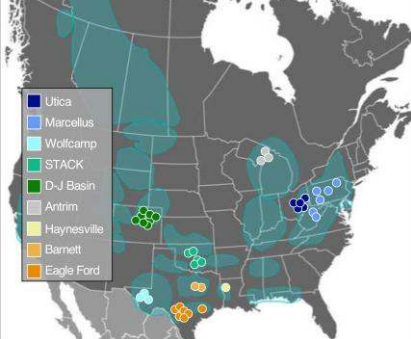


Cell counts increased by ~2 log fold
in late samples

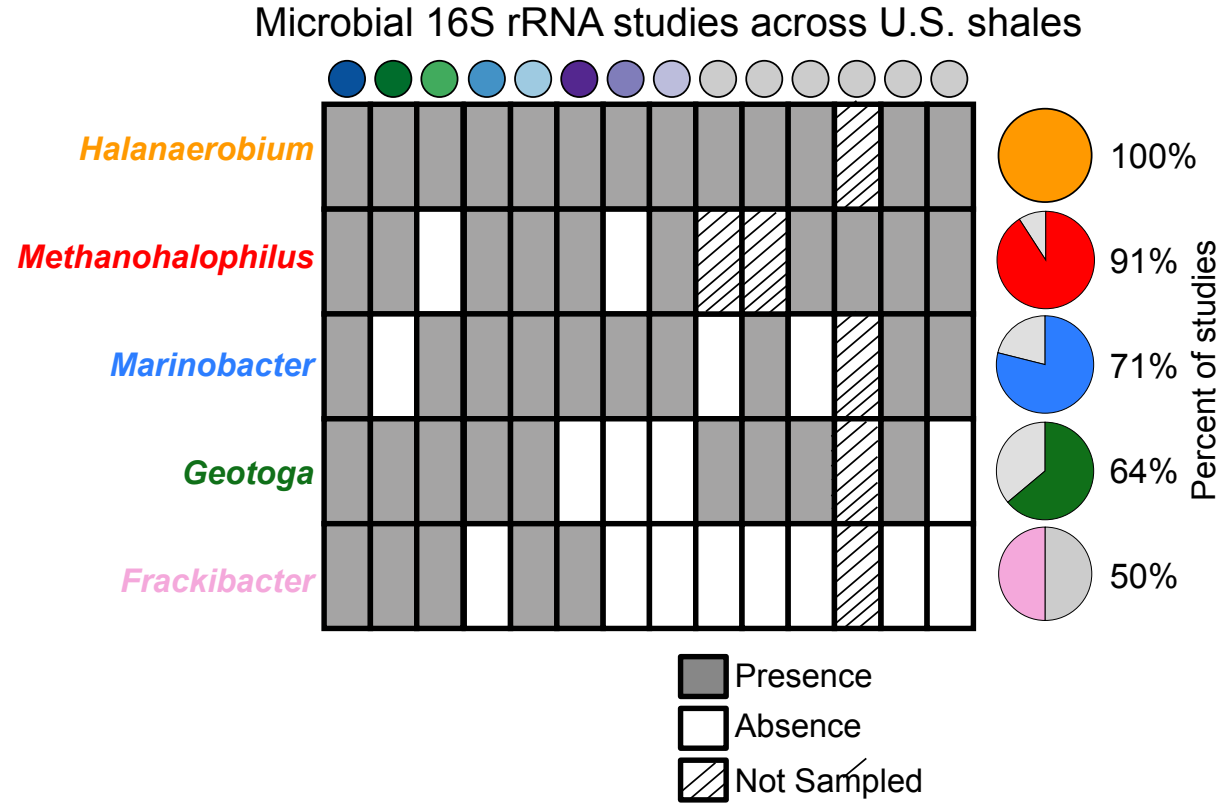
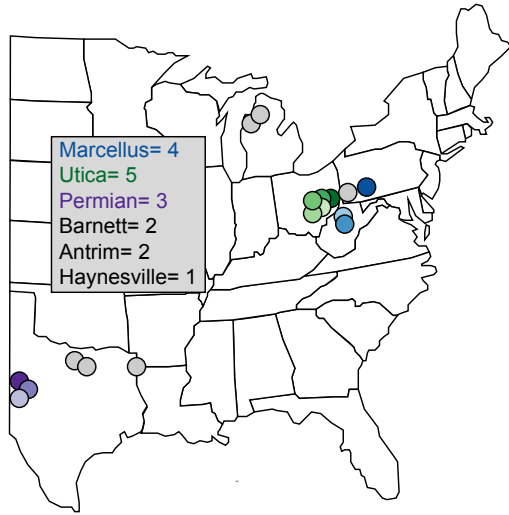




Fractured shale communities must
Adapt to increasing salinity



16S rRNA Fracking “core” members persist after 50 days (saline adapted)



16S rRNA
gene analyses





Microbes compete, cooperate, and ward off elimination

1

Core community composed of several genera

How do surface organisms adapt to deep subsurface?

To address persistence: What microbial metabolites are produced *in situ*?

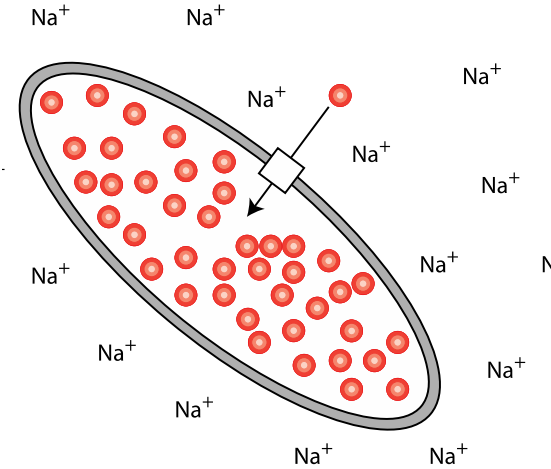
Osmoprotection

Some microbes **synthesize metabolites** that maintain osmotic balance with environment

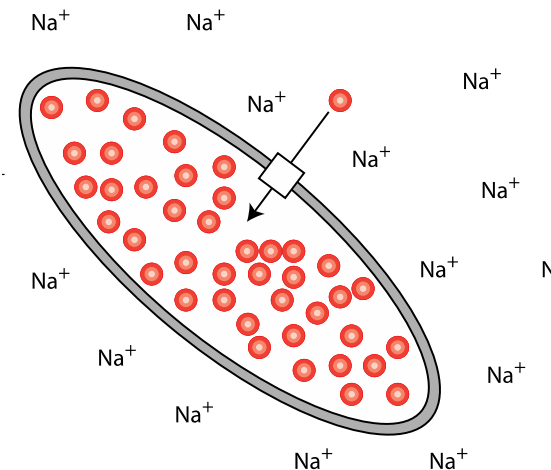
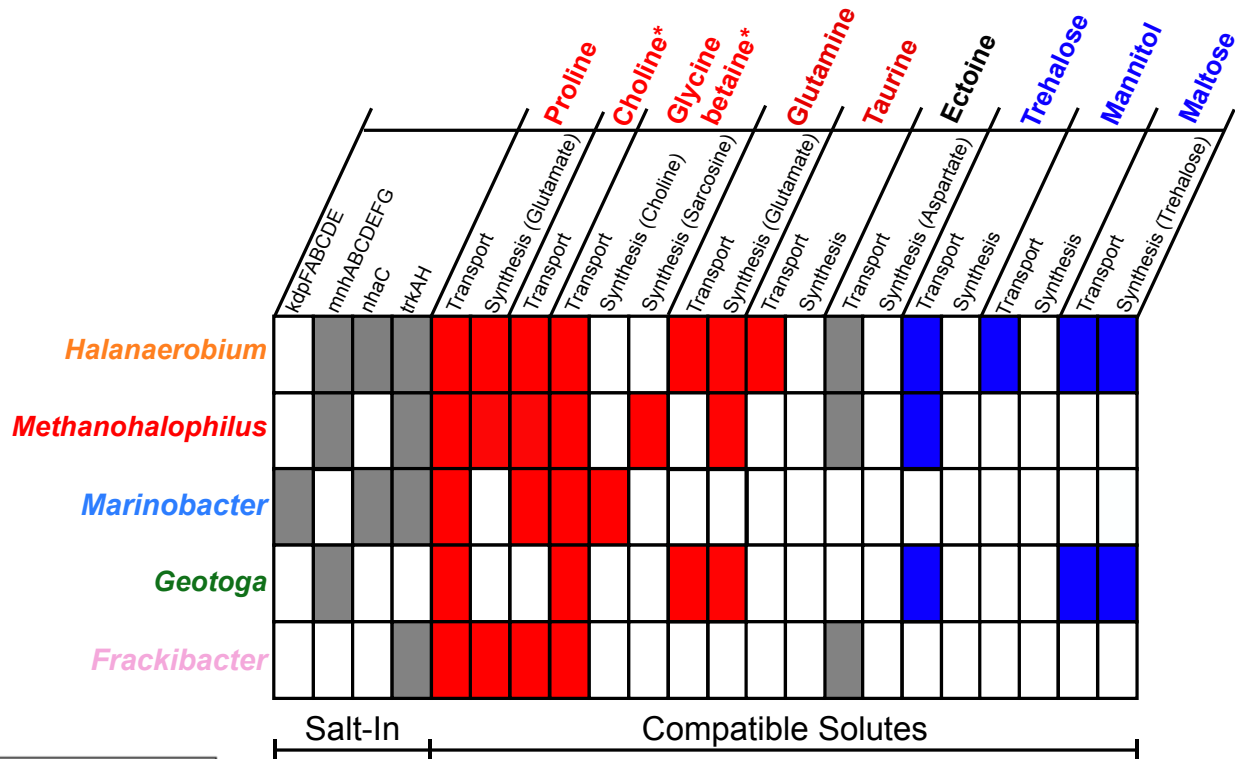
Other microbes **utilize metabolites**

Energetically costly to produce small, organic acid or amino acid products

Enable flexibility to salt ranges

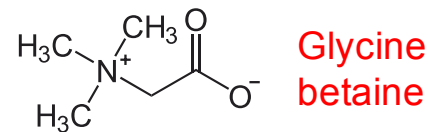
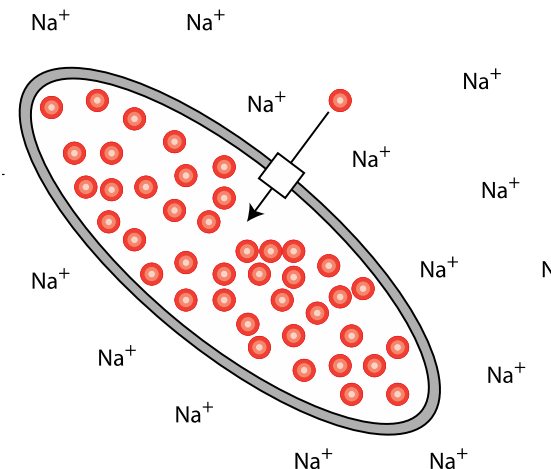
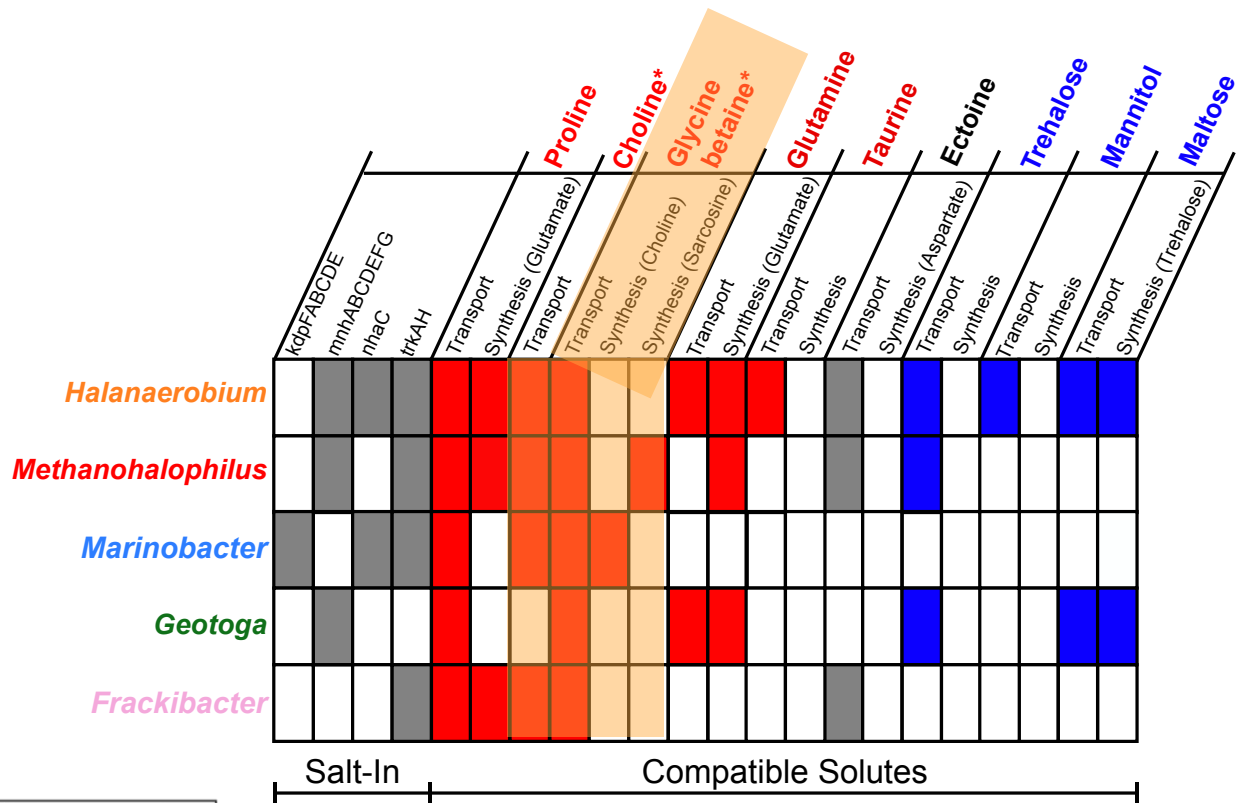


Genome resolved: no single signal in our MAGs

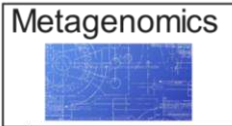


2

Genome resolved: Glycine betaine was interesting...

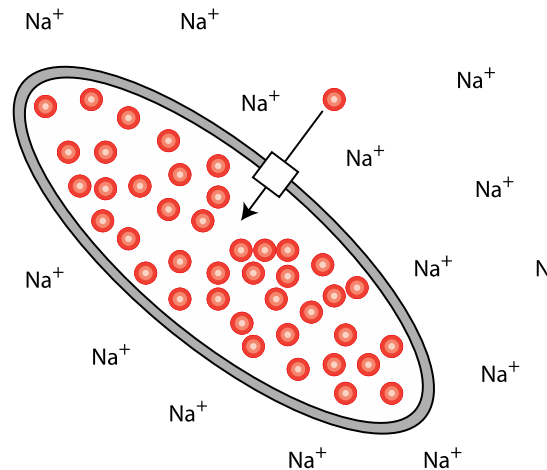
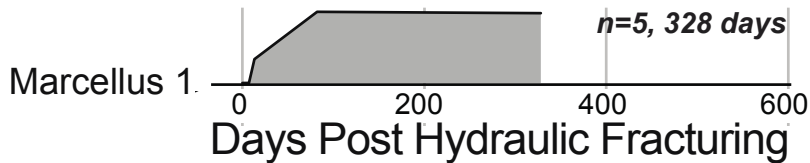
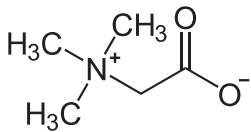


2

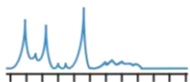


Extracellular metabolites:
Glycine betaine is a core, persisting metabolite

Glycine betaine



Metabolomics



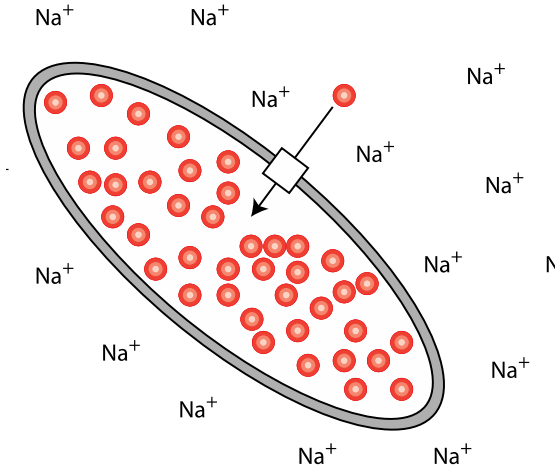
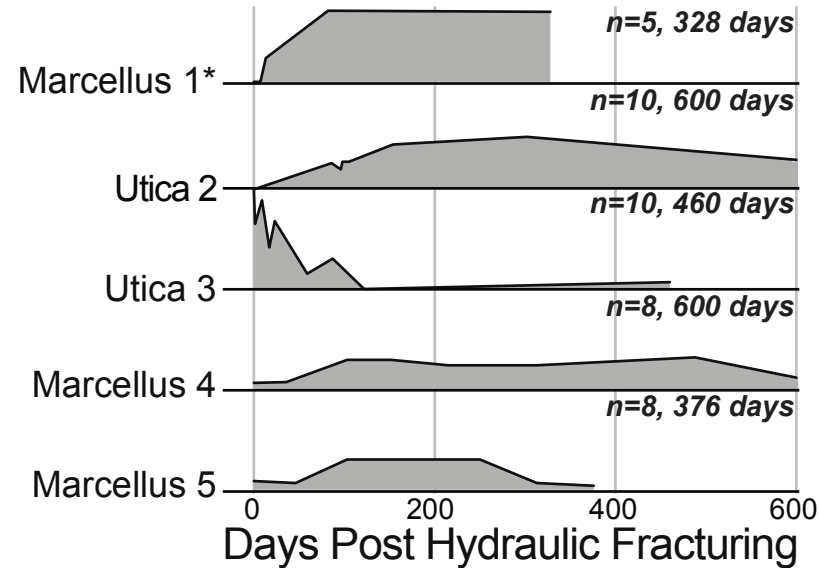
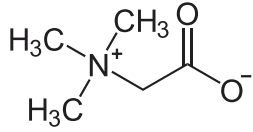
David
Hoyt



EMSL

Glycine betaine is a core, persisting metabolite in fractured shale fluids

Glycine betaine



1 Certain microbes and osmolyte metabolites persist across shales in response to salinity

2 Can glycine betaine serve as an energy source for microbes persisting in shales

Could glycine betaine also serve as energy source?



1

Stickland reaction

amino acid
electron donor
?

Reductase

glycine betaine

trimethylamine

2

Demethylation reaction

Methyl
transferase

glycine betaine

dimethylglycine

Could glycine betaine also serve as energy source?



1

Stickland reaction

amino acid
electron donor
?

Reductase

glycine betaine

trimethylamine

2

Demethylation reaction

Methyl
transferase

glycine betaine

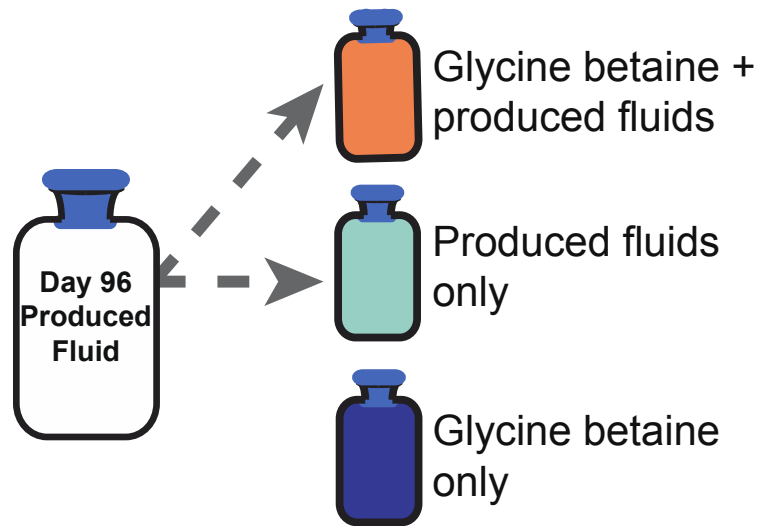
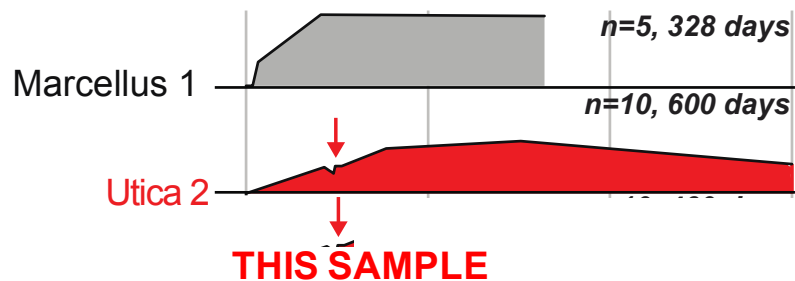
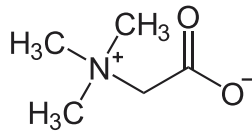
dimethylglycine

Isn't metagenomics enough?

- Genes are poorly annotated in genomes
- Electron donor cannot be identified from existing data alone
- Expression is challenging due to sample collection

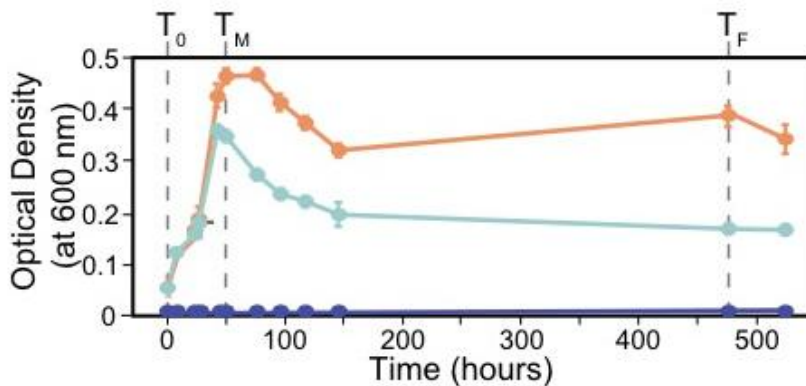
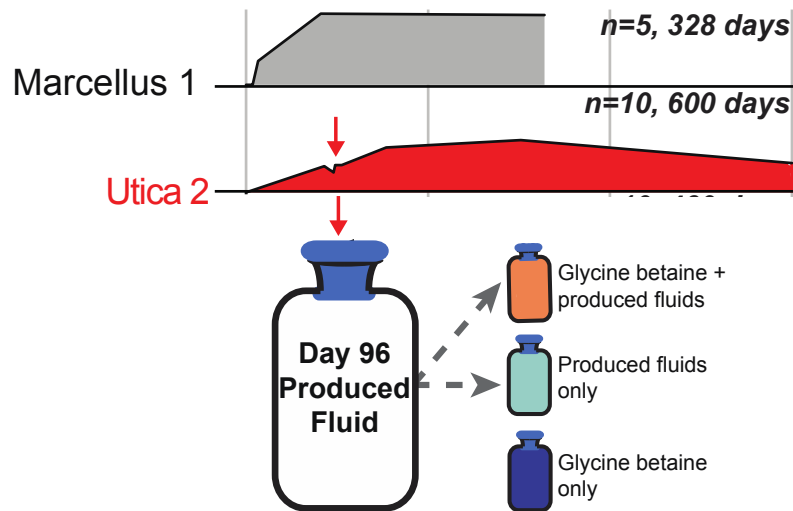
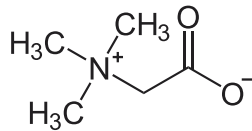
Validating a meta-omics hypothesis from the field in the lab

Glycine betaine



Co-expression patterns from shale reactors could identify

Glycine betaine



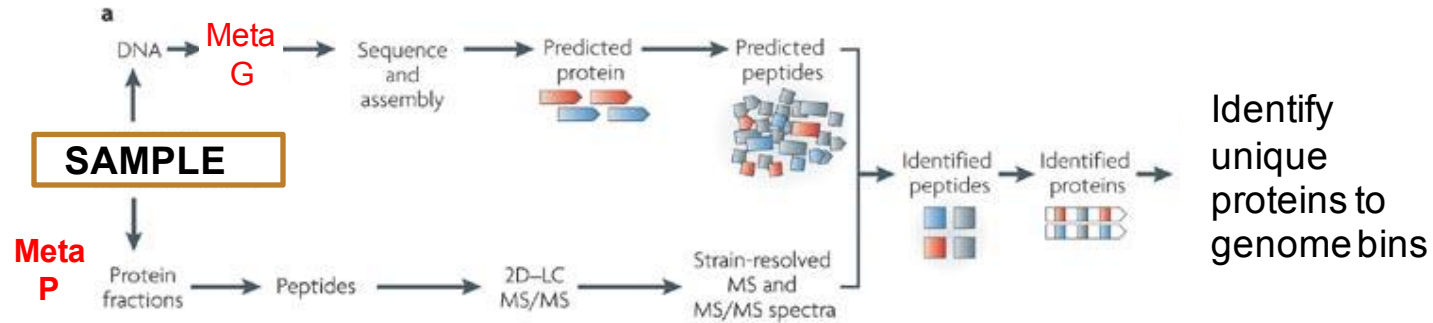
$T_0 \rightarrow T_M \rightarrow T_F$

Metagenomics
Proteomics
NMR Metabolites
Gas Chromatography

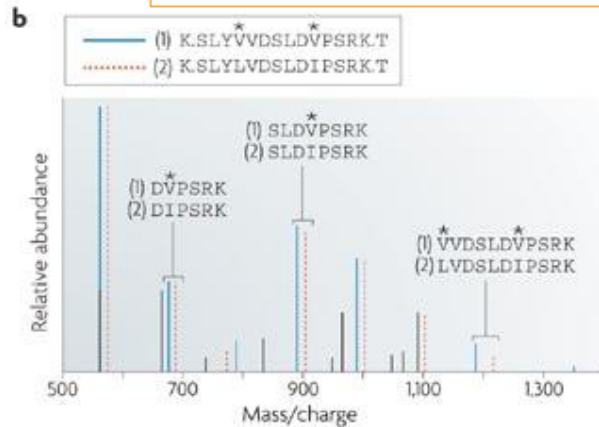
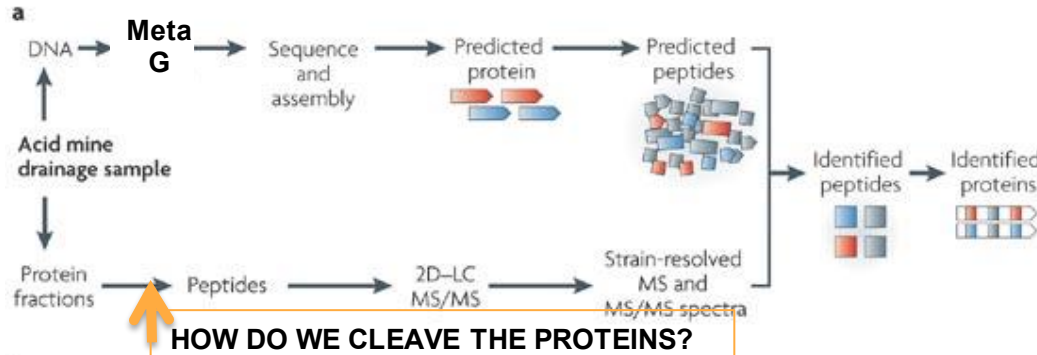
EMSL



Metaproteomics crash course- paired to metagenomes



Now that we know who is there,
proteome can give us how they can function

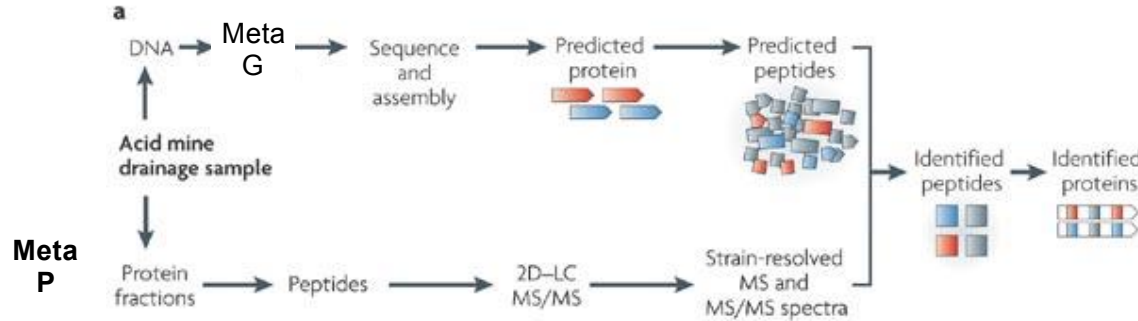


Create peptide database by *in silico* trypsin digest
(cleave proteins at the carboxyl aide of the amino acid)
lysine and arginine

Lysine=K
Arginine=R

(1) K.SLYVVDSDLVPSRK.T
(2) K.SLYLVDSLDIPSRK.T

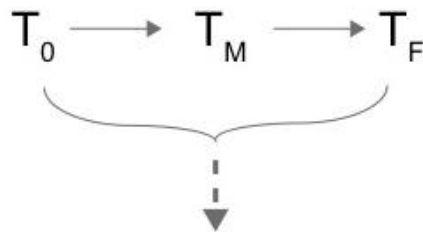
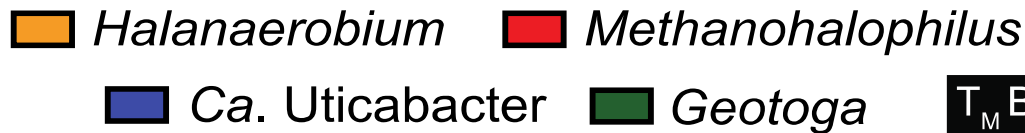
Proteomics- uses and challenges



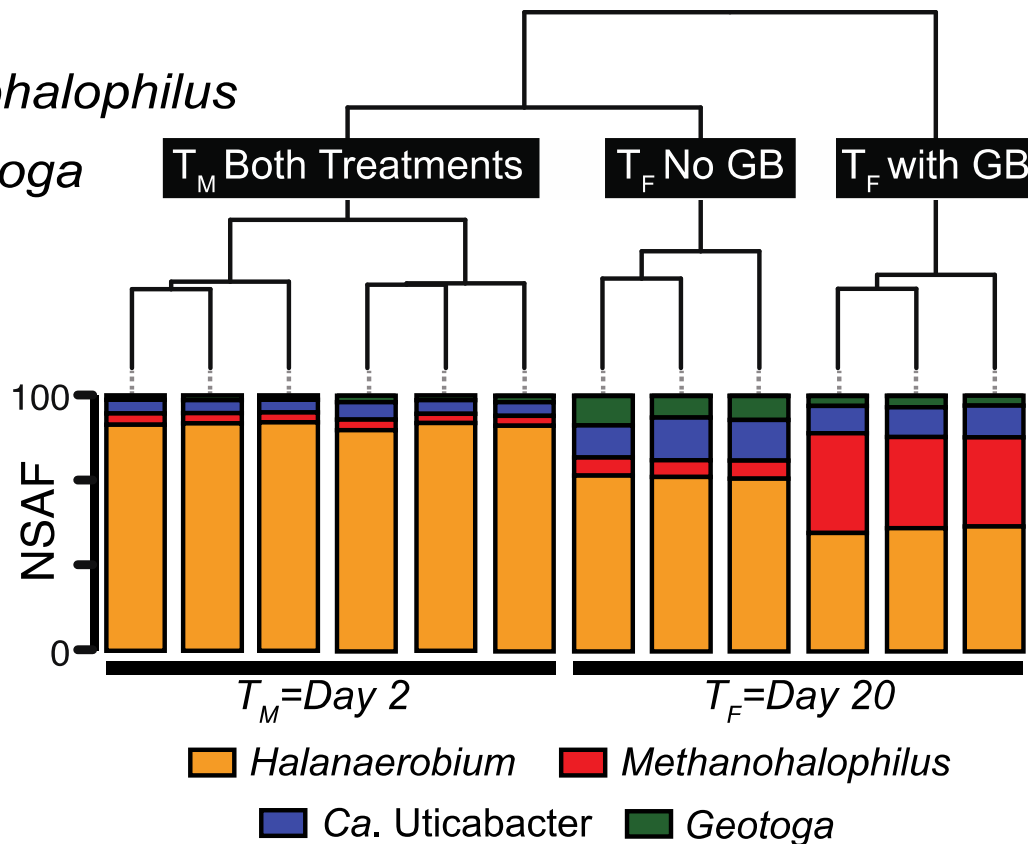
● Proteomics

- Demonstrates expression of genes in metagenomes
- Correlate to metagenomic data
- No amplification: need high signal, doesn't do well for low abundance organisms
- Outer membrane proteins not as well resolved unless separate fractionation done

4 core, persisting members from field detected in metaproteomics from laboratory microcosms

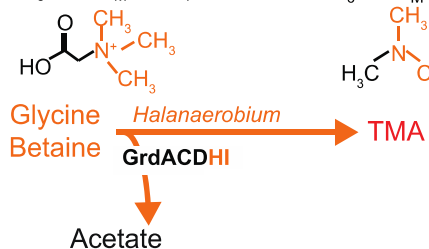
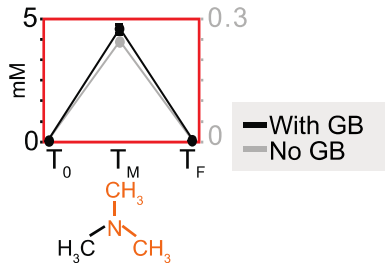
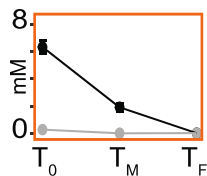


Metagenomics
Proteomics
NMR Metabolites
Gas Chromatography



Combining time series meta- omics

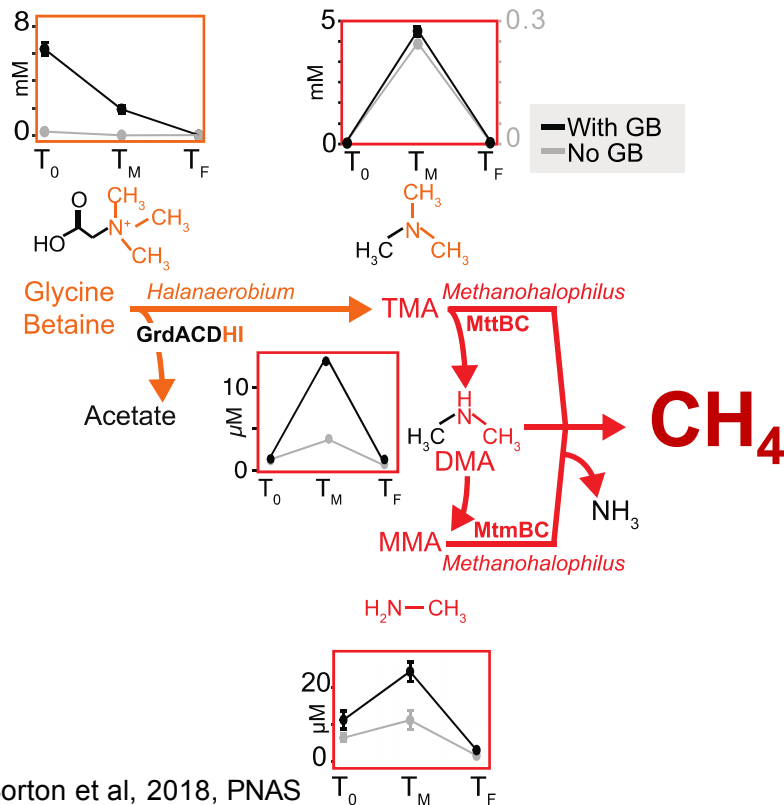
Halanaerobium reduced GB to yield TMA, a methanogenic substrate



**74% of GB
converted to TMA
In amended or unamended!**

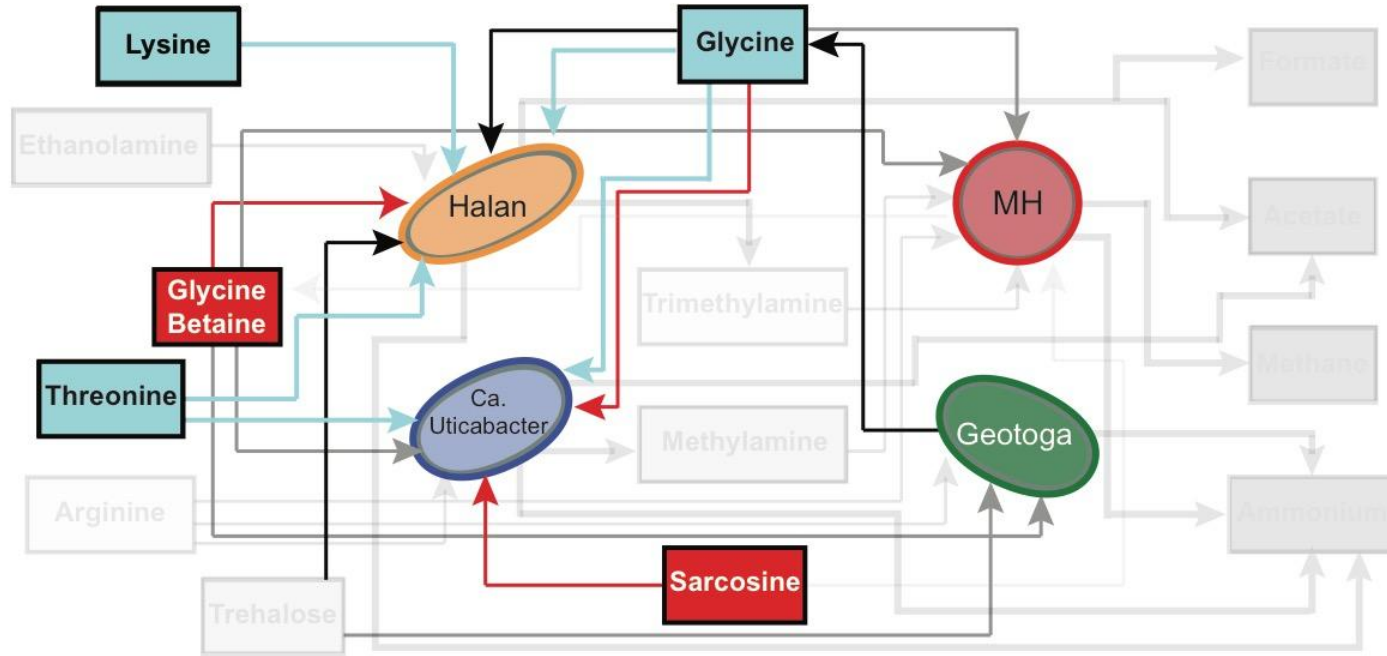
Laboratory Proteomics and Metabolomics:

Methanohalophilus preferentially utilizes *Halanaerobium* metabolites

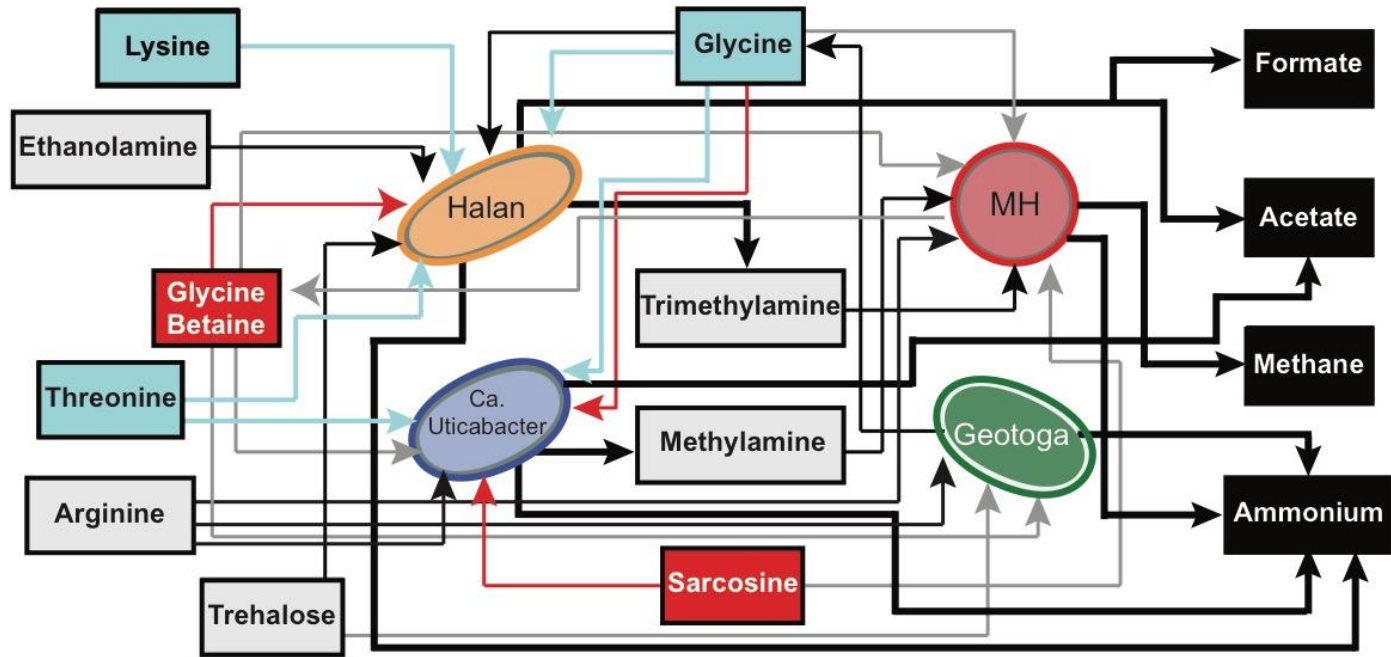


Expression and metabolite correlation analyses

Glycine betaine, glycine, and sarcosine are **Stickland acceptors**
glycine and lysine are possible **Stickland donors**



Even very “simple” microbial communities,
have complicated mutualistic and competitive metabolic interactions

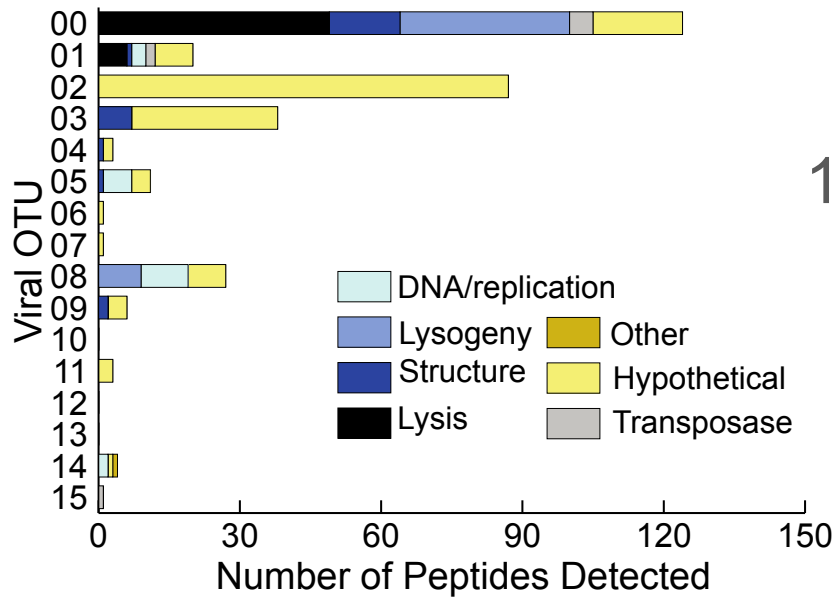


1 What type of microorganisms persist in this system?

2 Can glycine betaine metabolism sustain microbes in shales long after fracking

3 What roll do viruses play in controlling population dynamics?

Laboratory proteome studies hint at active viruses in fluids

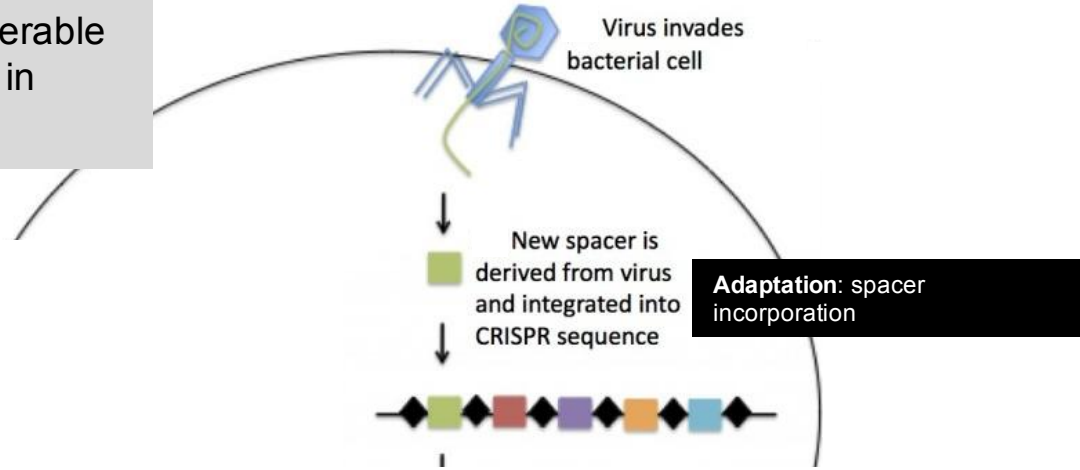


16 unique viral genomic contigs
*viral genes expressed in lab

*Clustergenomes-54 contigs with 95%ANI, 80% shortest contig

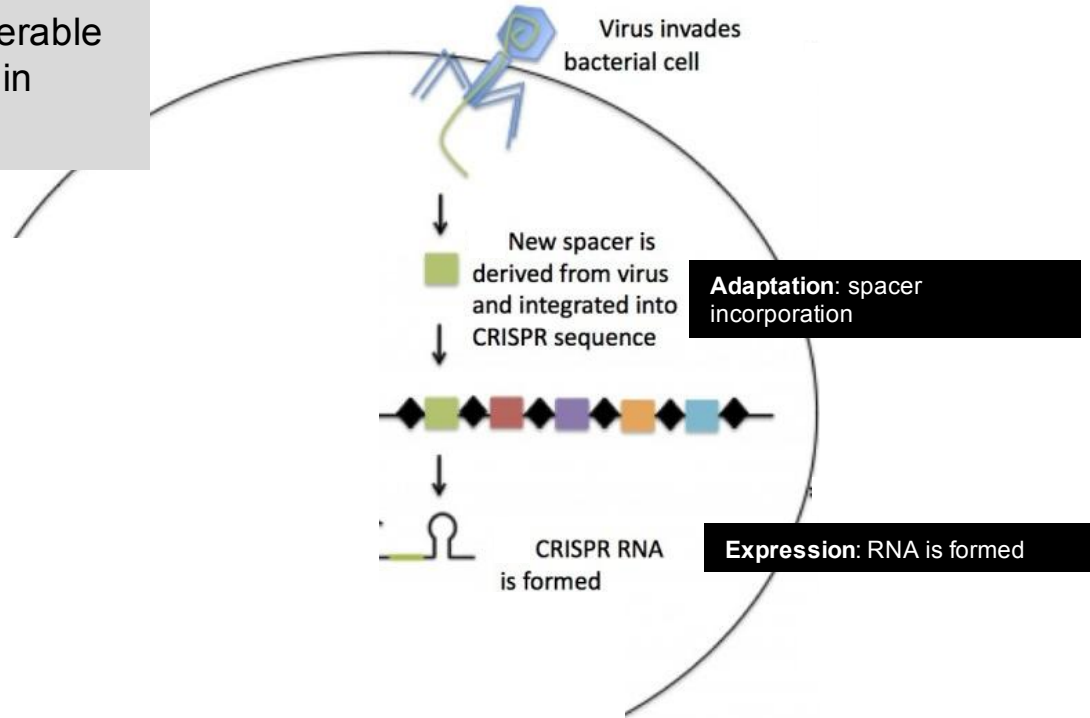
CRISPR-Cas systems in genomes are a mechanism for tracking viral-microbial encounters

CRISPR stores genomically recoverable timelines of virus-host coevolution in natural organisms



CRISPR-Cas systems in genomes are a mechanism for tracking viral-microbial encounters

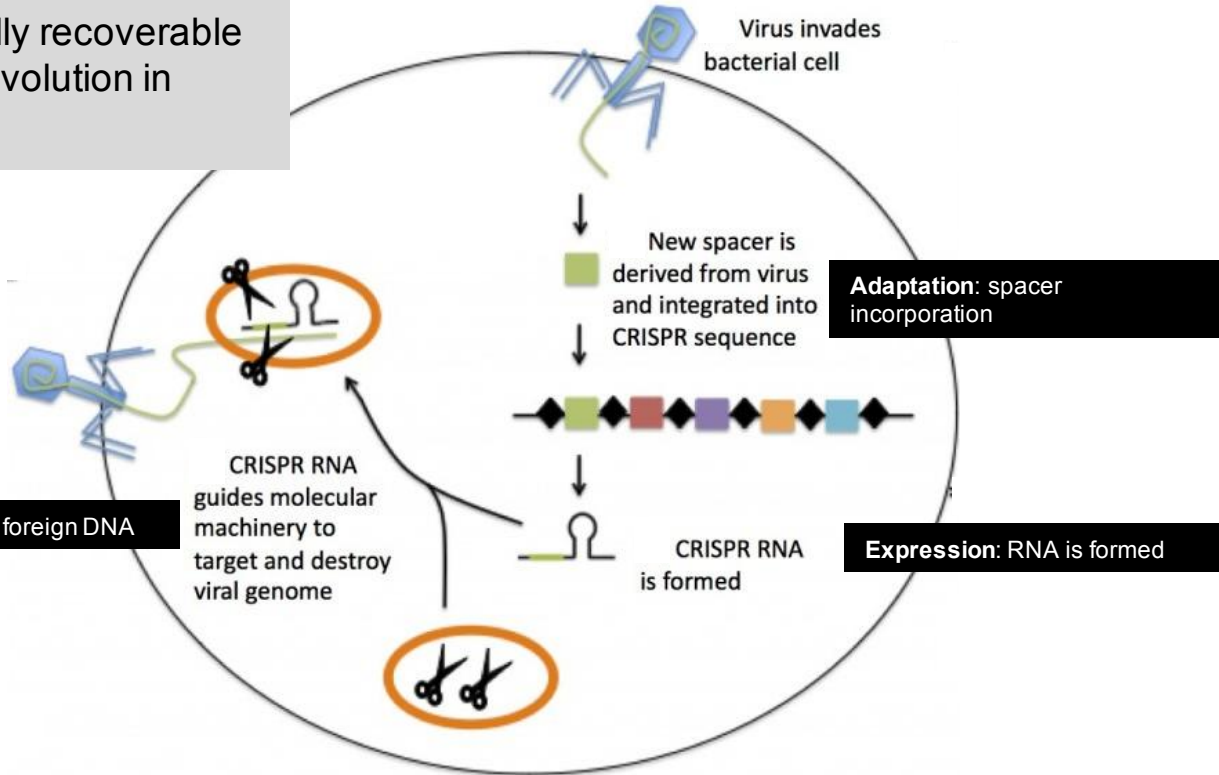
CRISPR stores genomically recoverable timelines of virus-host coevolution in natural organisms



CRISPR-Cas systems in genomes

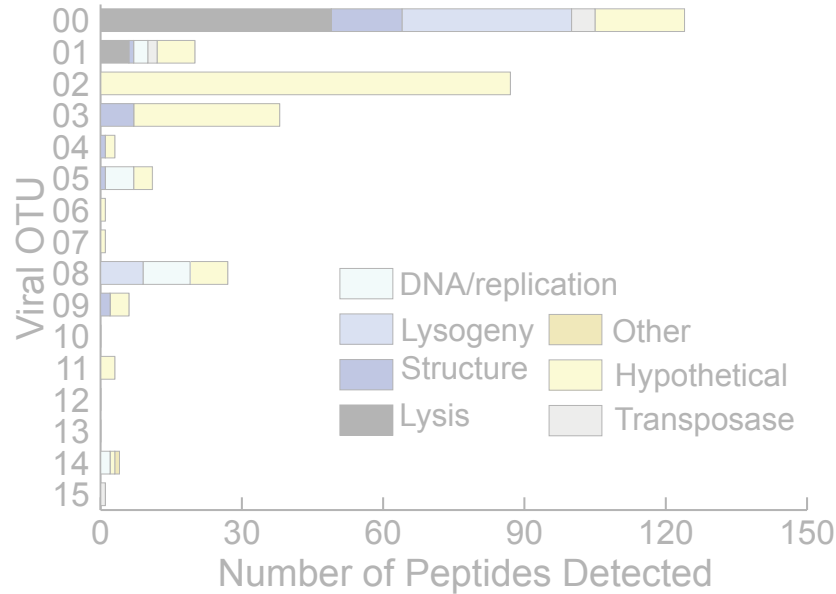
are a mechanism for tracking viral-microbial encounters

CRISPR stores genomically recoverable timelines of virus-host coevolution in natural organisms

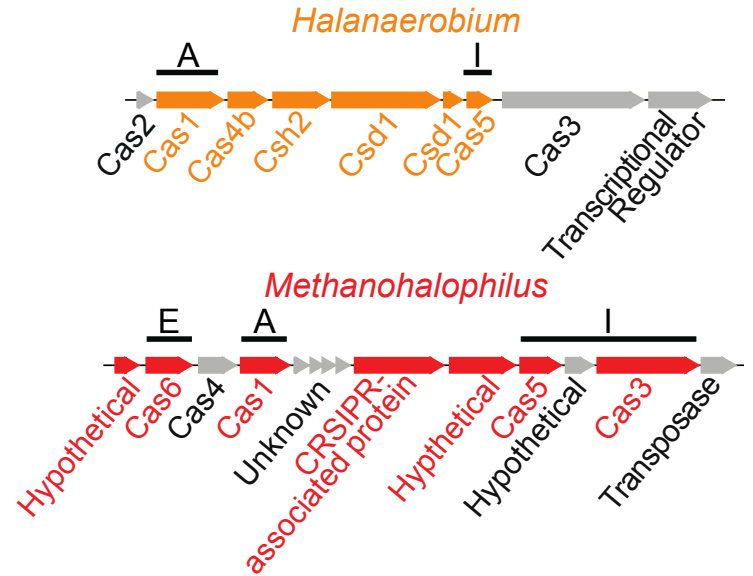


Host expression data highlights viral predation in fractured shales

15 unique viral populations
viral genes expressed in lab



2 bacterial hosts
express CRISPR genes in lab

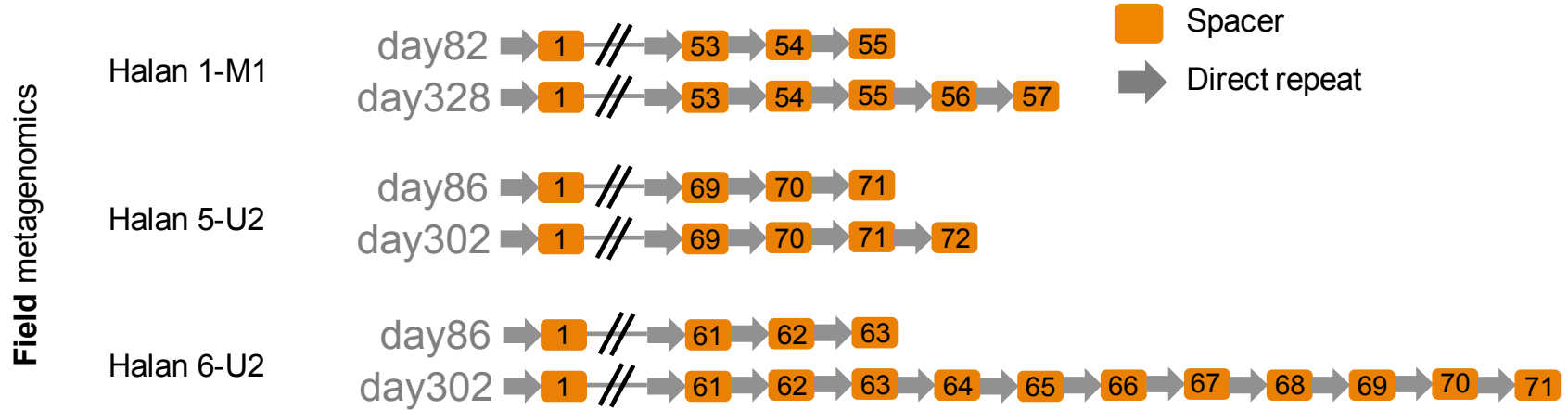


(A) Adaptation: spacer incorporation

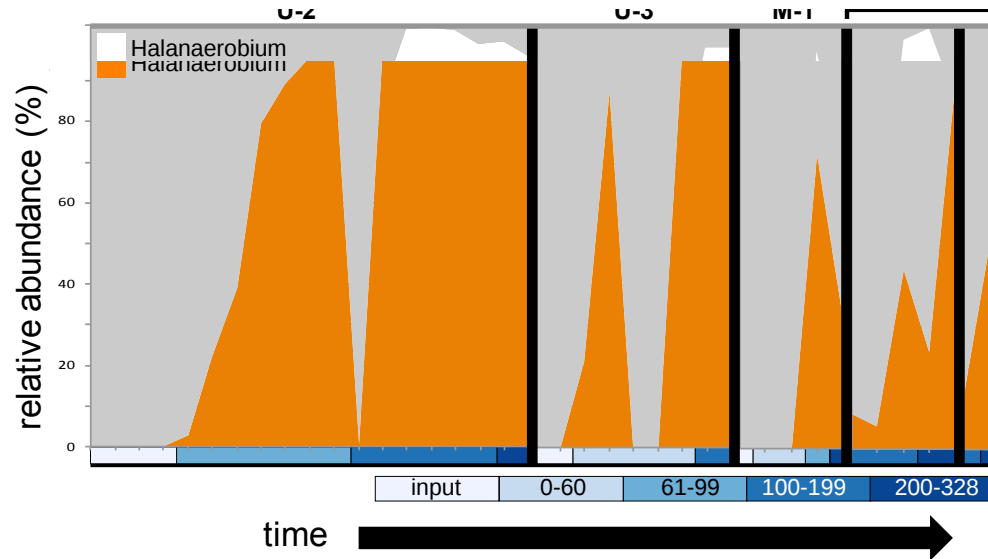
(E) Expression: cognate RNA synthesis

(I) Interference: cleavage-complex to destroy foreign DNA

Time-series genome sampling provides evidence that NEW spacer incorporation occurs in the field



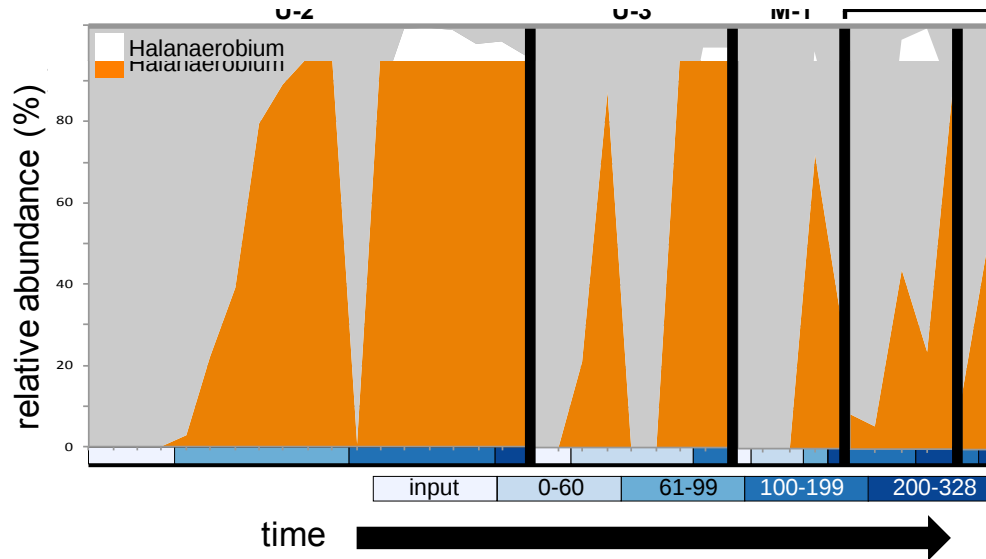
Can viral predation explain *Halanaerobium* 16S rRNA relative abundance changes?

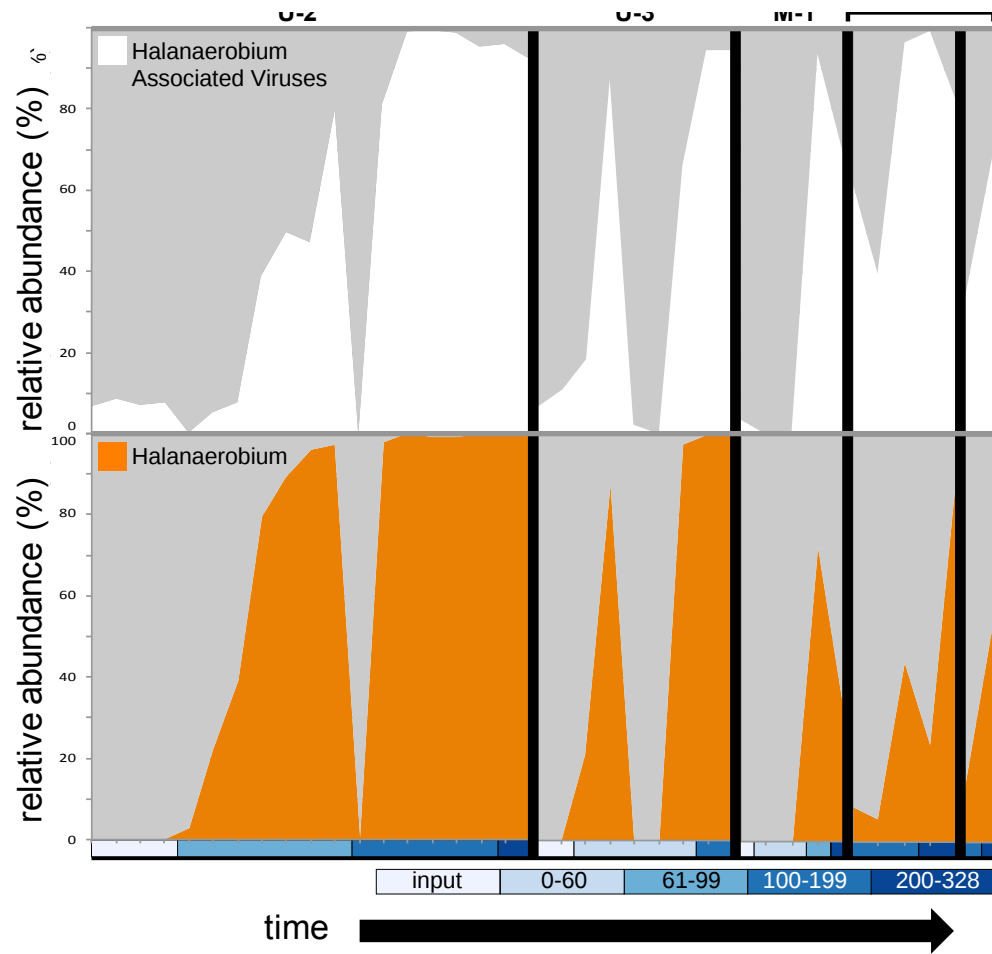


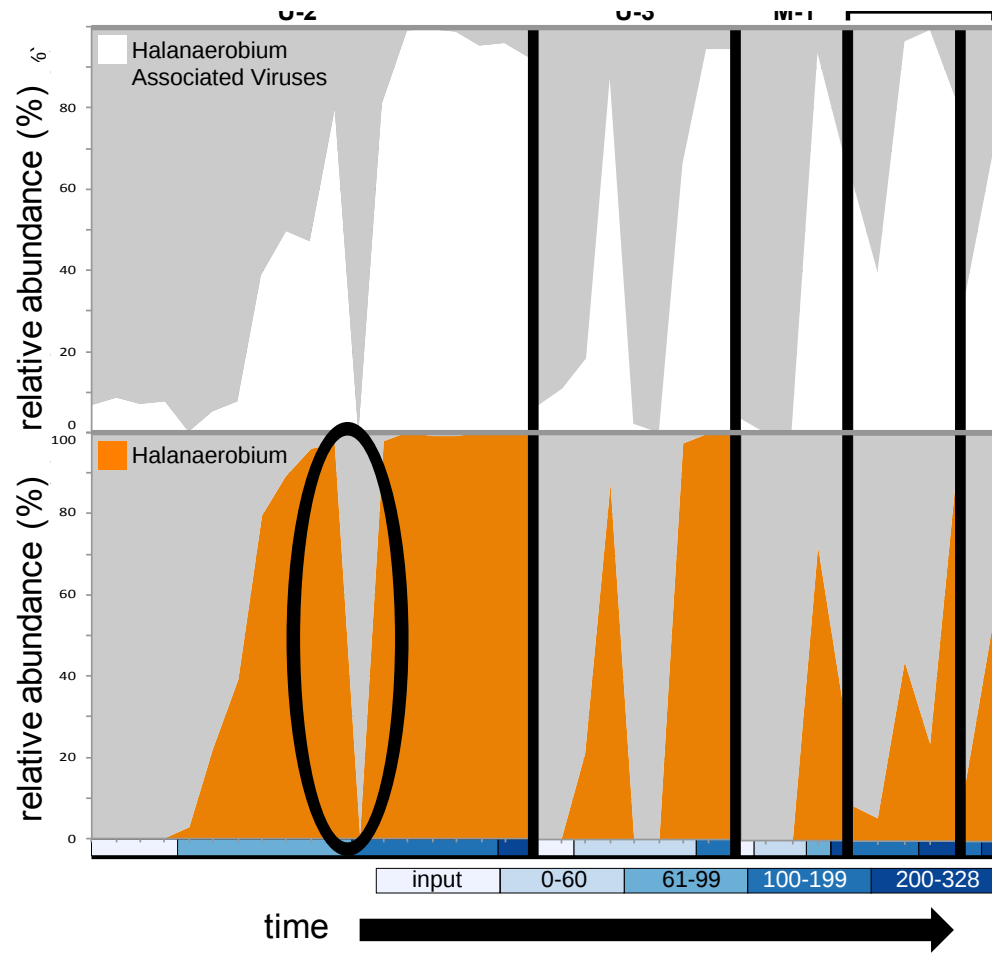
Halanaerobium viruses are prevalent in metagenomes

1,838 viral genome populations (vOTUs) identified
representing 156 new viral genera from fractured shales

68.2% of viruses are associated with *Halanaerobium*

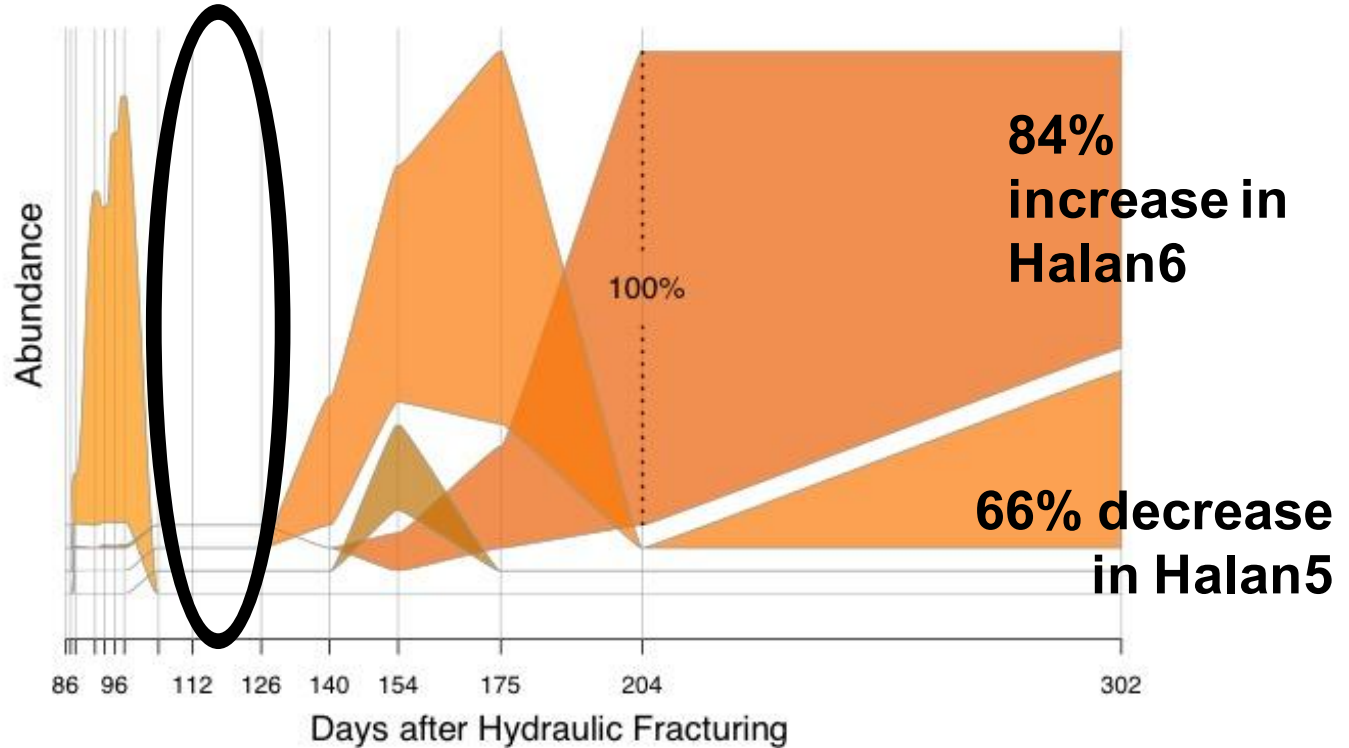
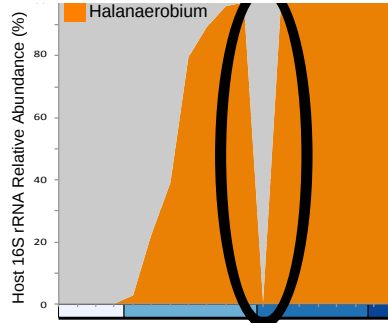




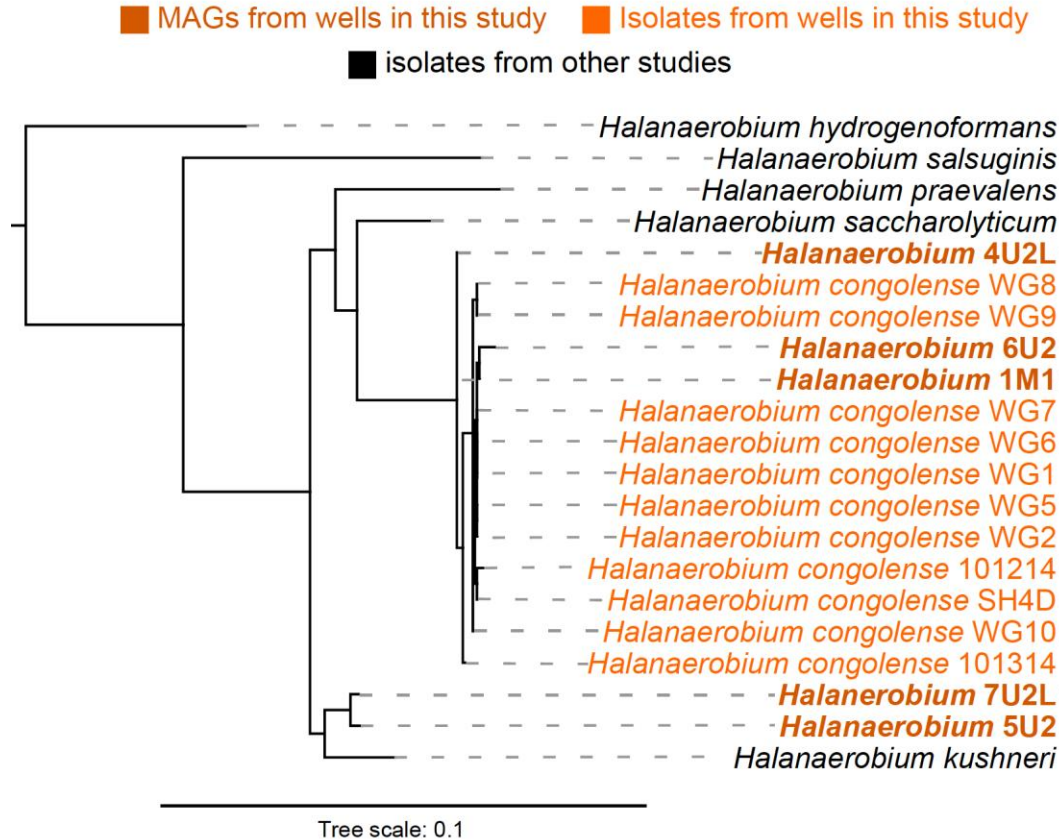


Viral predation may cause changes in *Halanerobium* strain dominance

4 most dominant strains shown

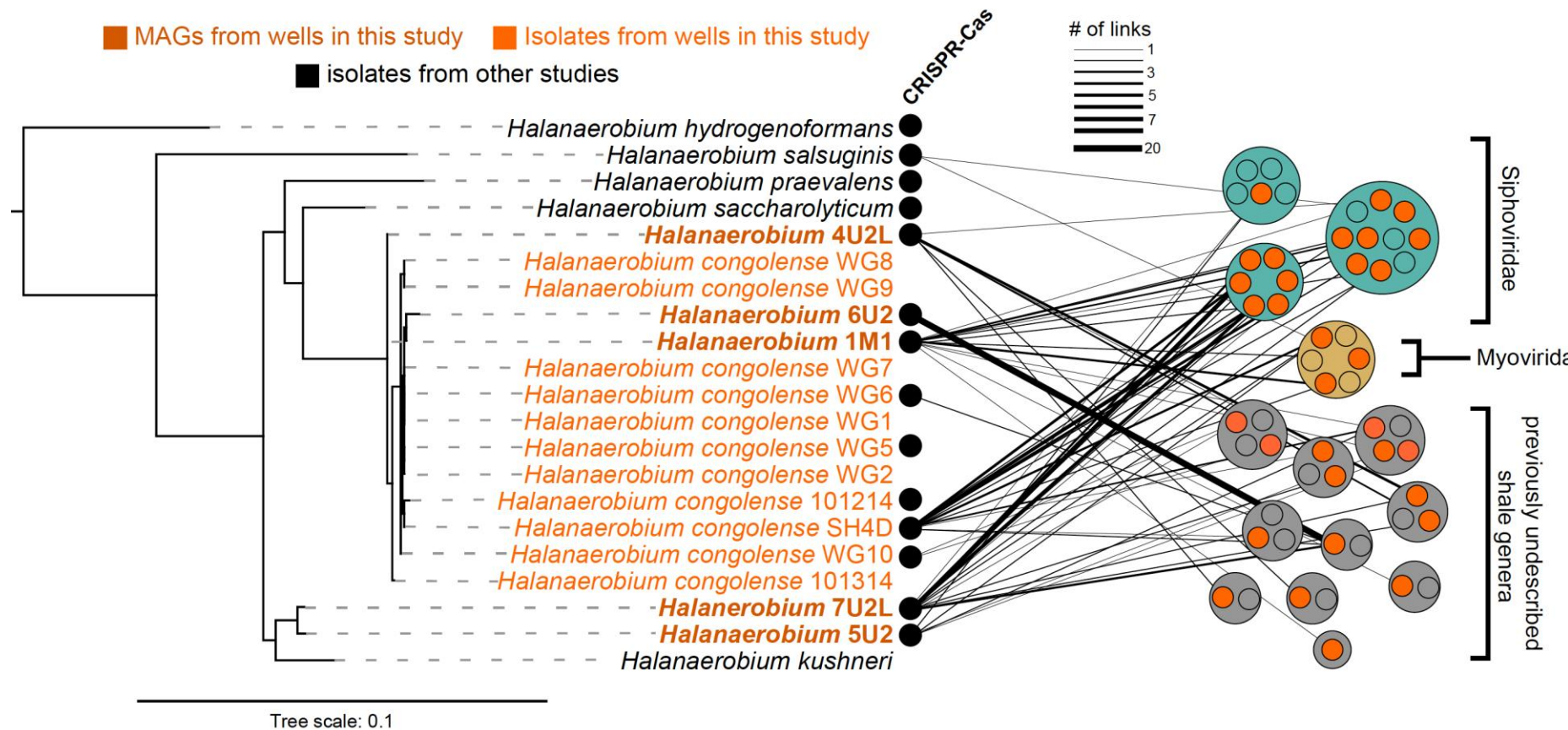


Halanaerobium strains recovered from a single well



Link Halanaerobium spacers to viral genome database

host have multiple link to same virus



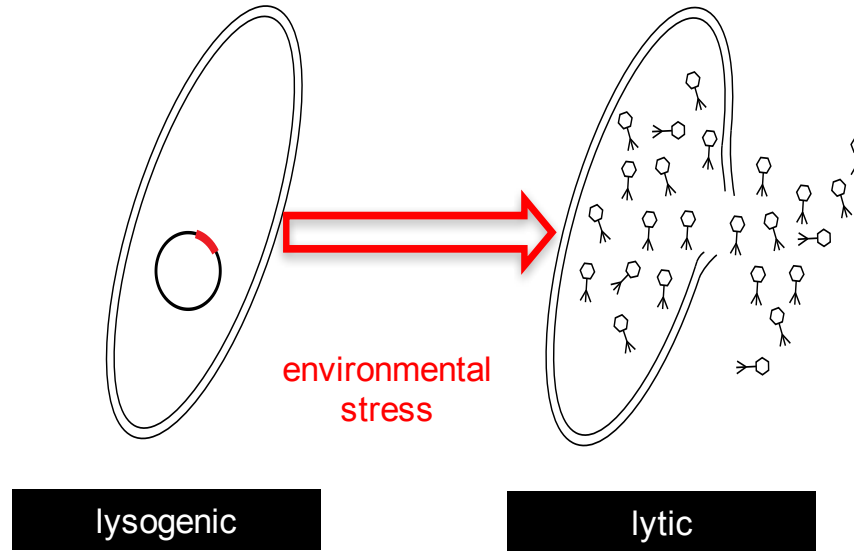
1 What type of microorganisms persist in this system?

2 Can glycine betaine metabolism sustain microbes in shales long after fracking?

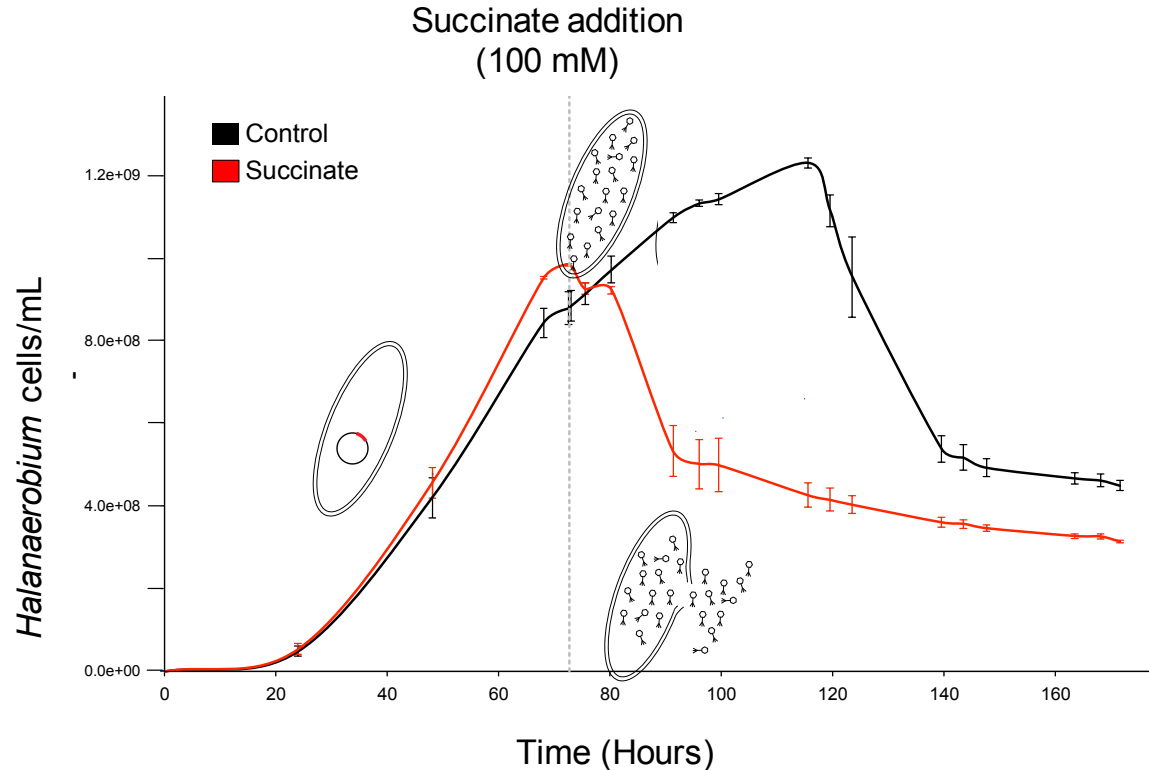
3 What roll do viruses play in controlling population dynamics?

4 Viruses: Friend or foe? Roles in nutrient release

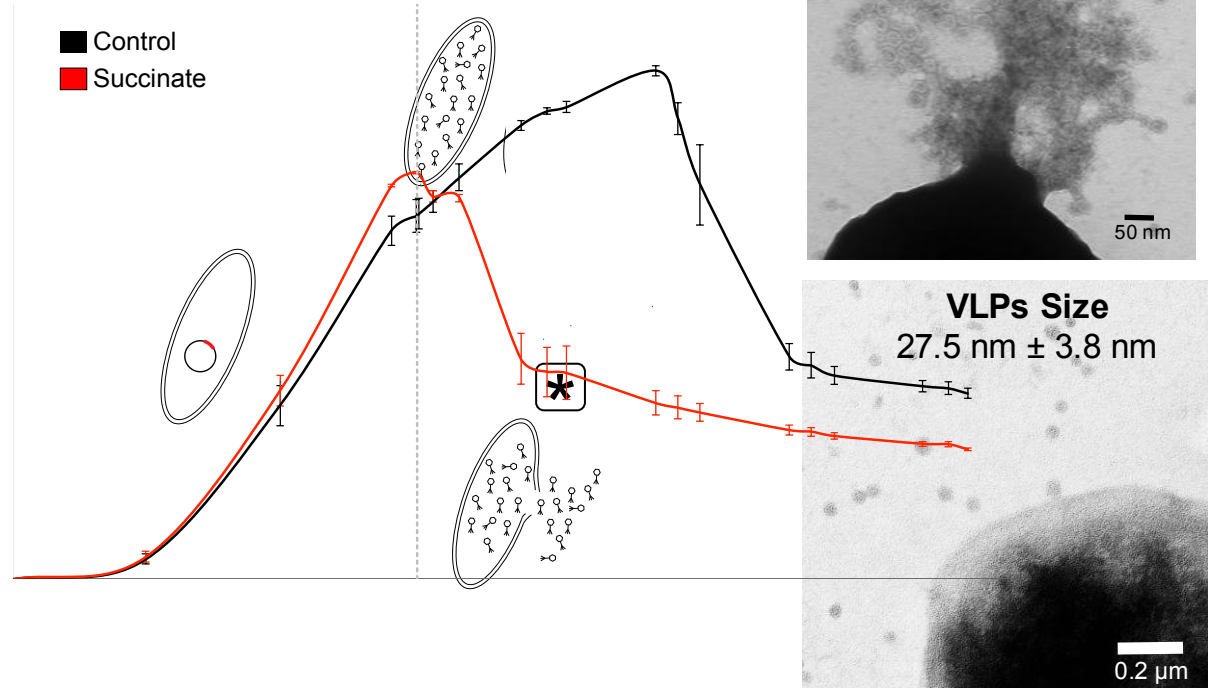
Where are the viruses in fractured shales coming from?



With an isolated strain of *Halanaerobium* that has a prophage
Can we induce the virus and lyse the cell

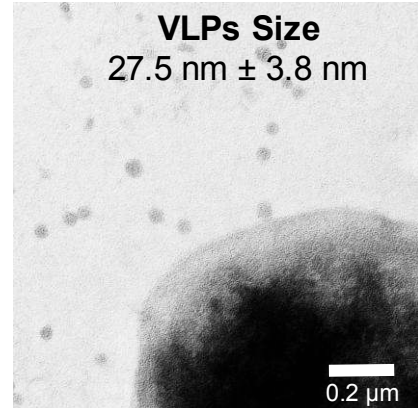
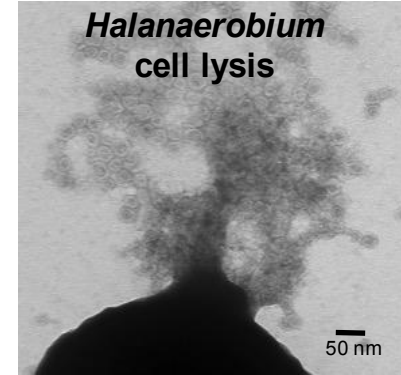
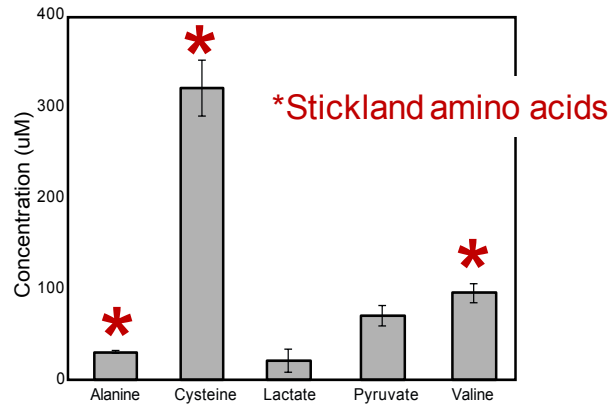


In lab induced prophage into lytic lifestyle



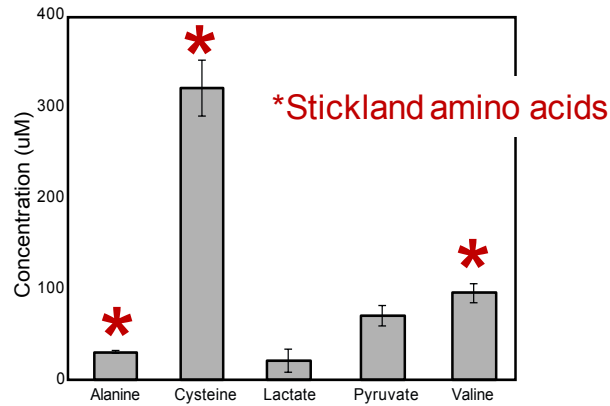
Prophage induced cell lysis causes host metabolite release

metabolites released following viral lysis

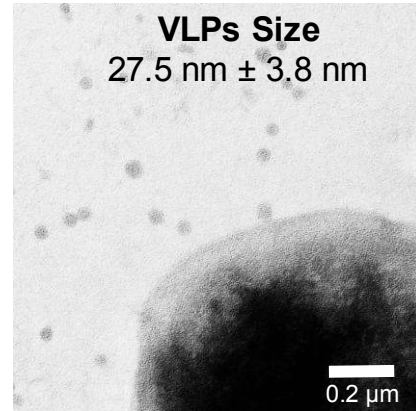
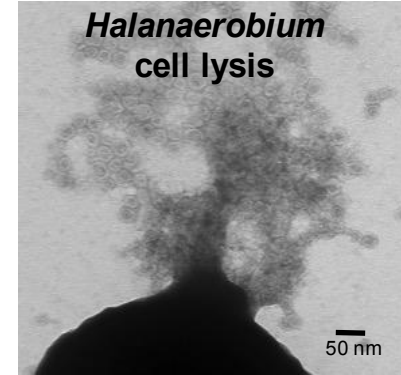
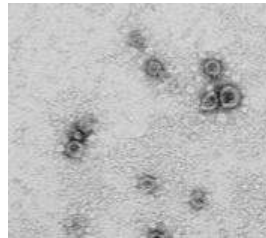
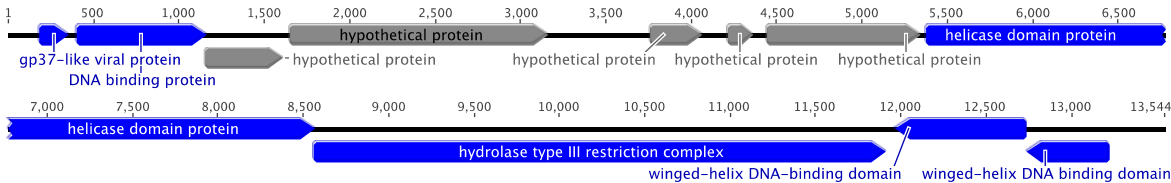


Virome and microscopy suggest tail less, novel virus

metabolites released following viral lysis



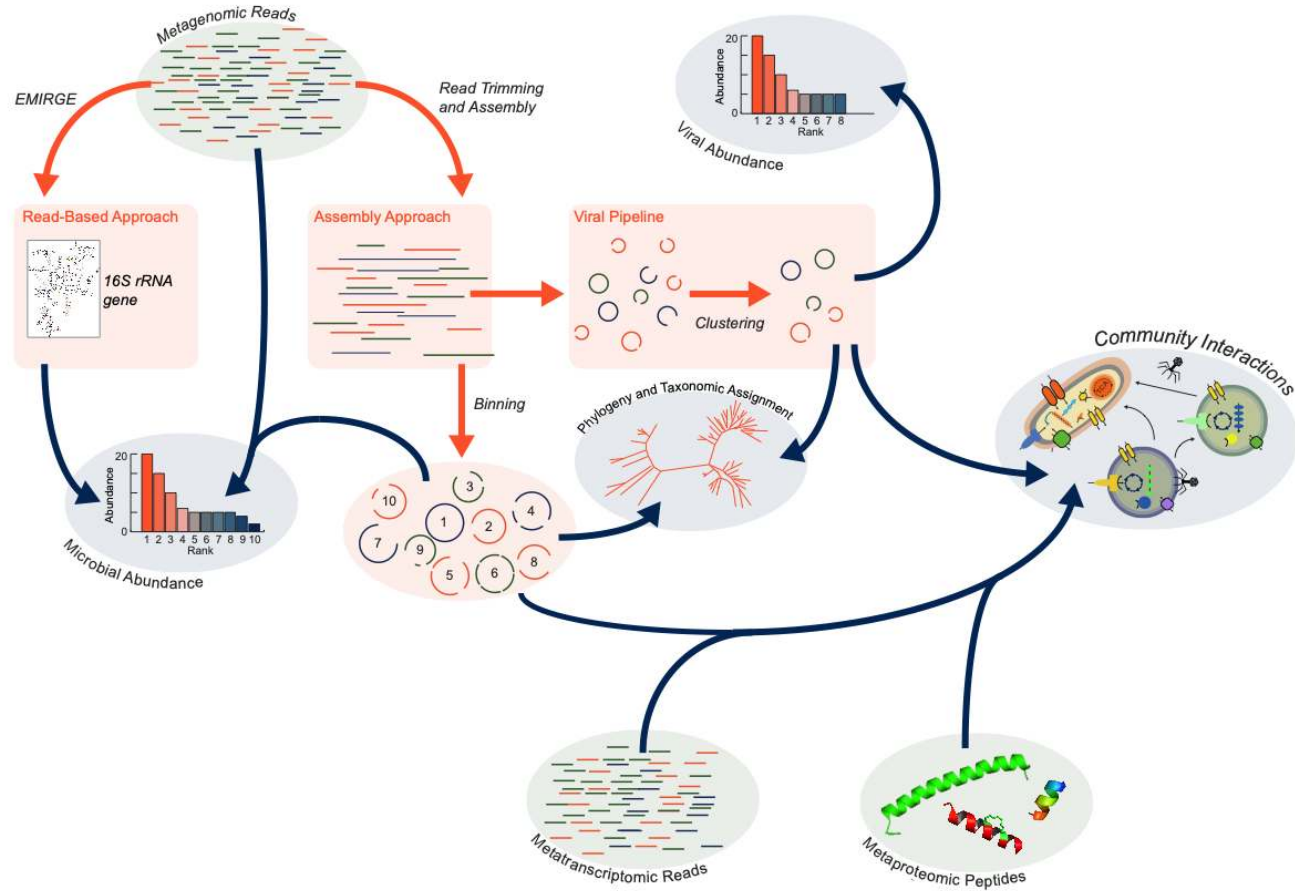
viral genomic fragment 45.8 Kbp, novel taxonomy



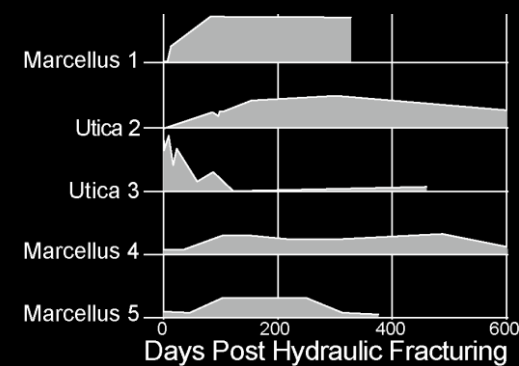
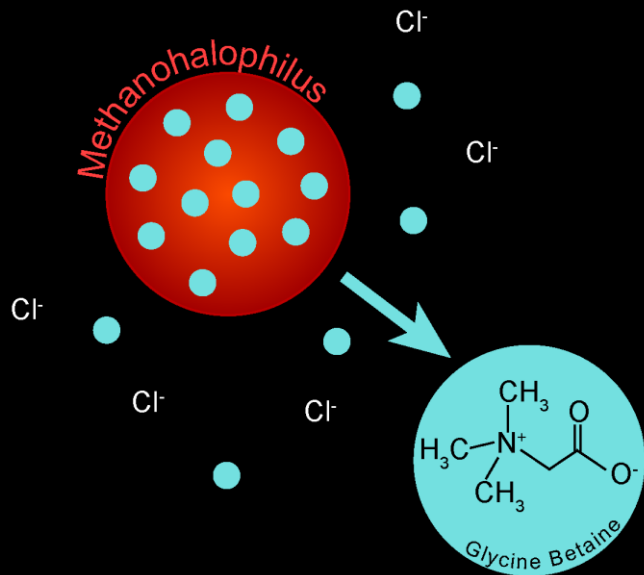


Microbes compete, cooperate, and ward off elimination

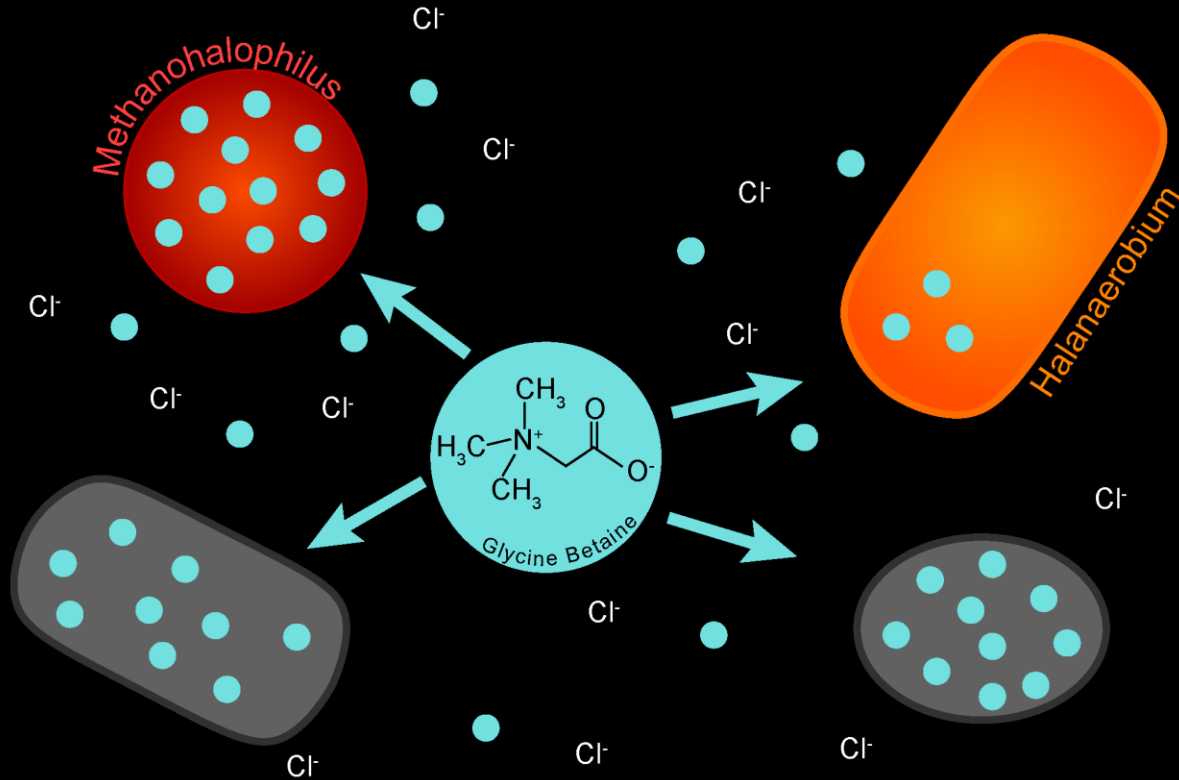
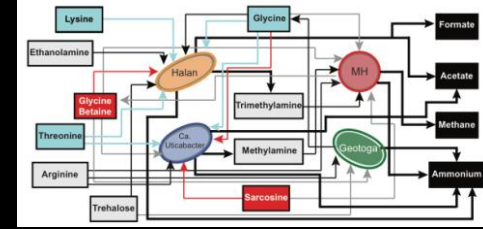
REVIEW: computational pipeline used in this case study



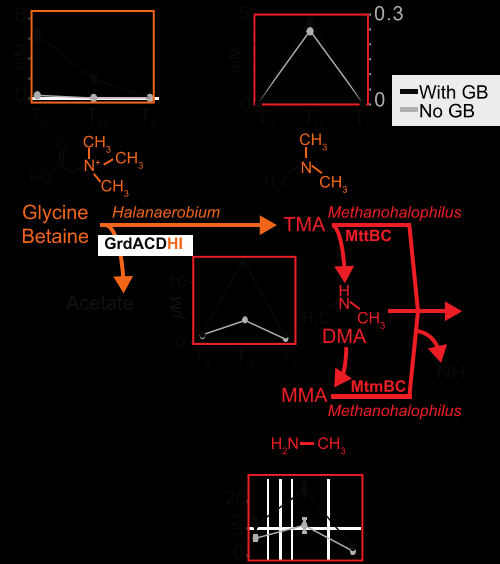
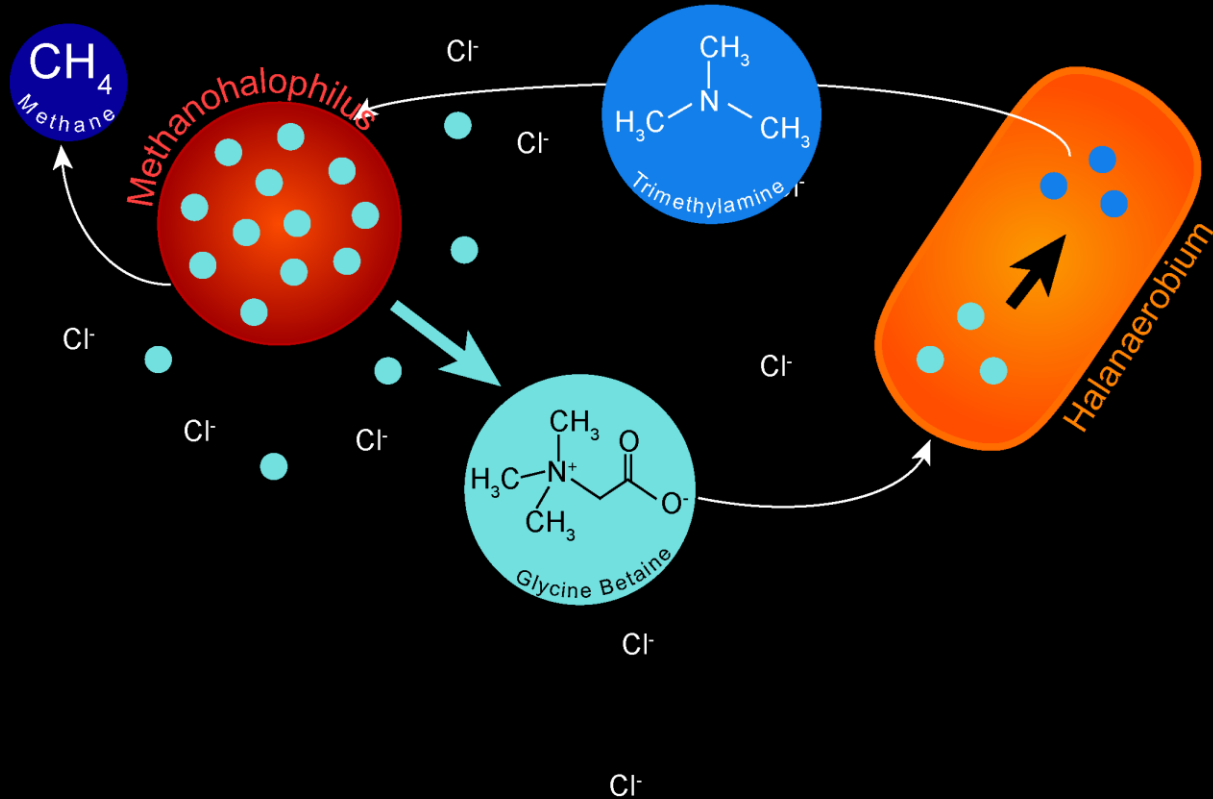
Core microbes adapt to environmental hardships



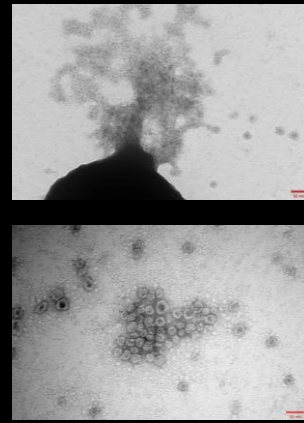
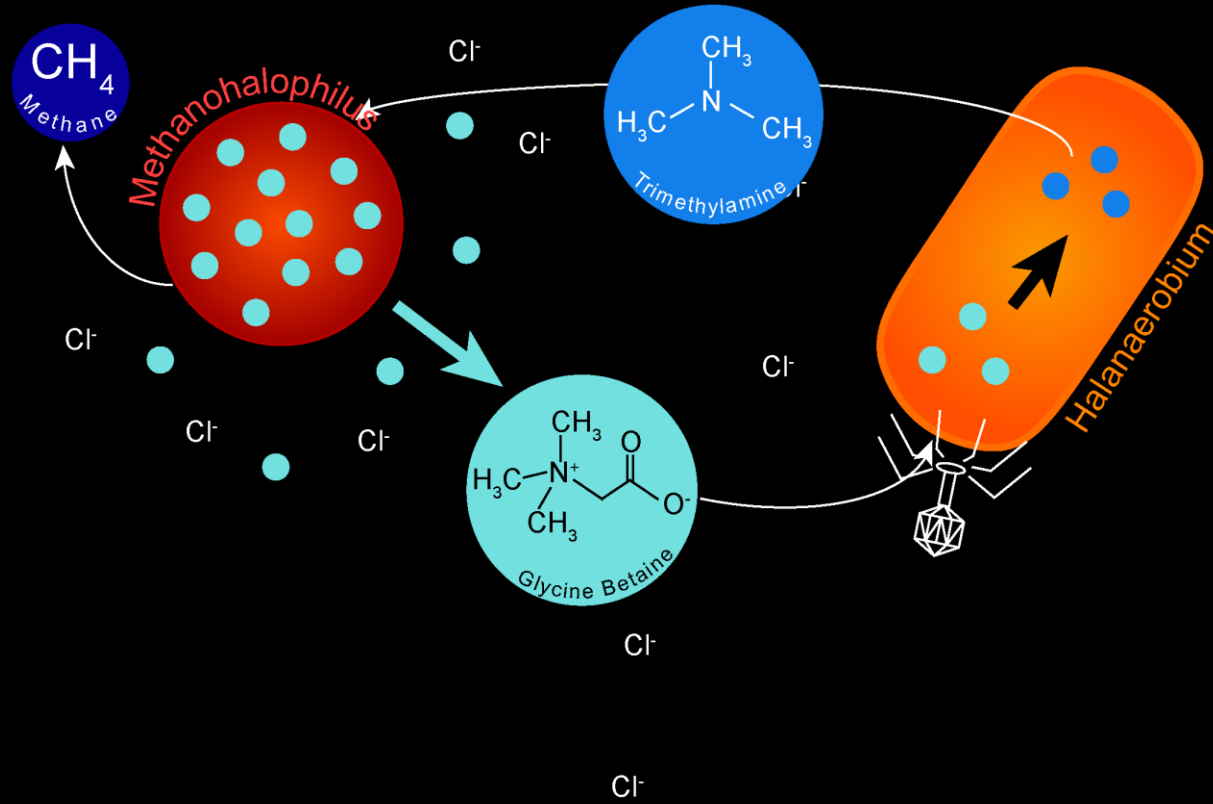
Compete for resources in the tribe



Form alliances within the tribe



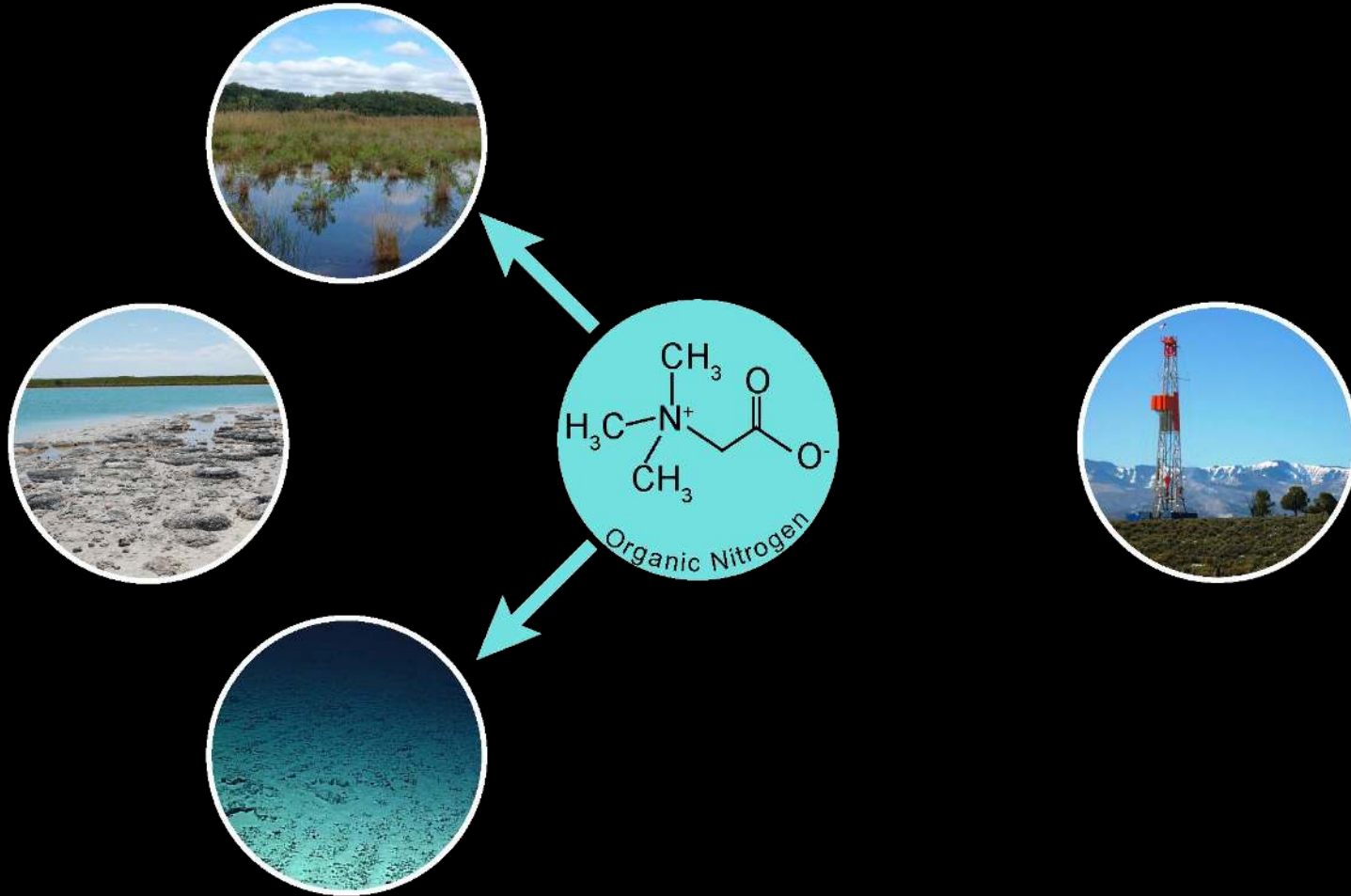
Ward off viral elimination



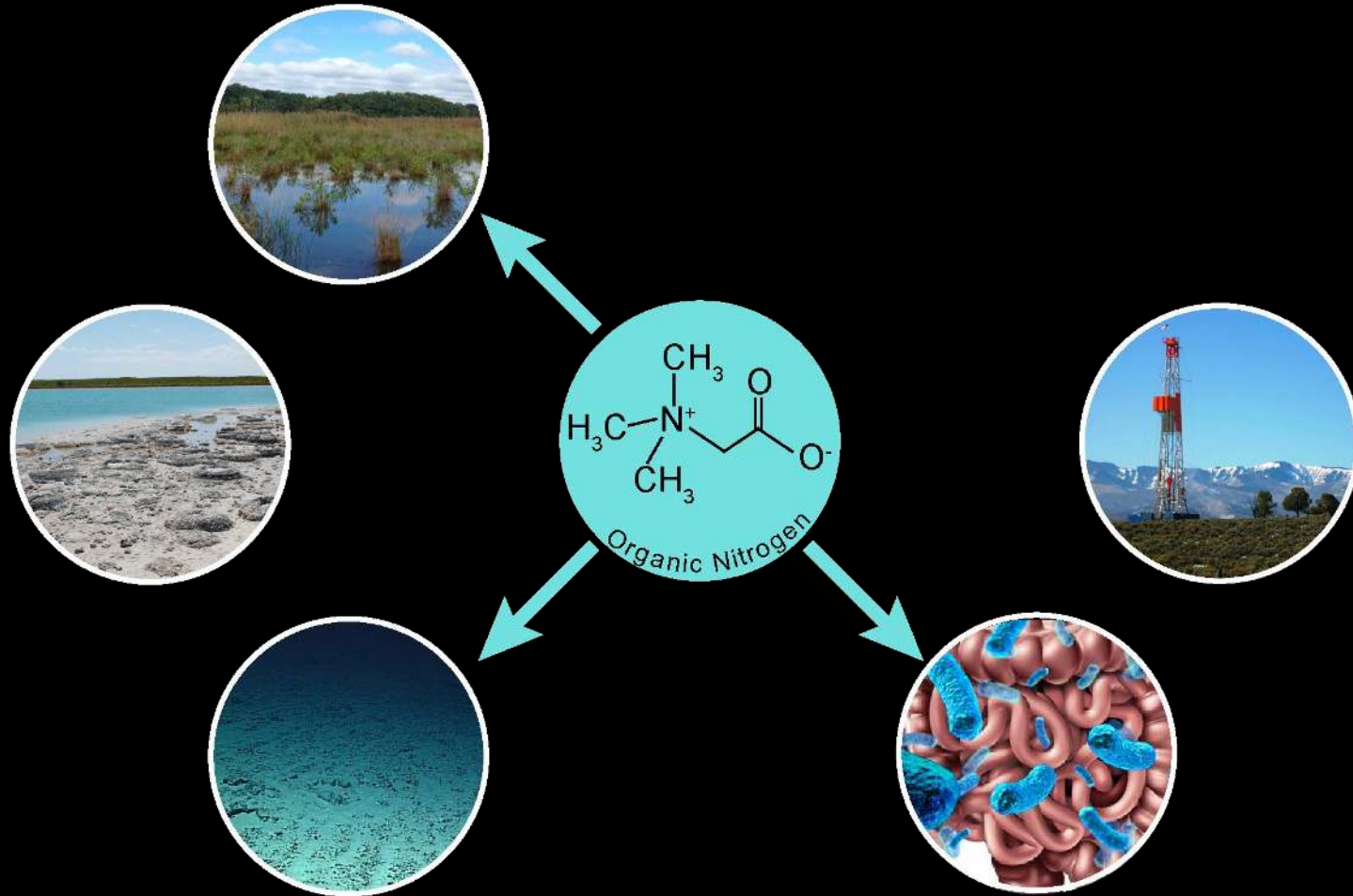
Shale is a model for other ecosystems



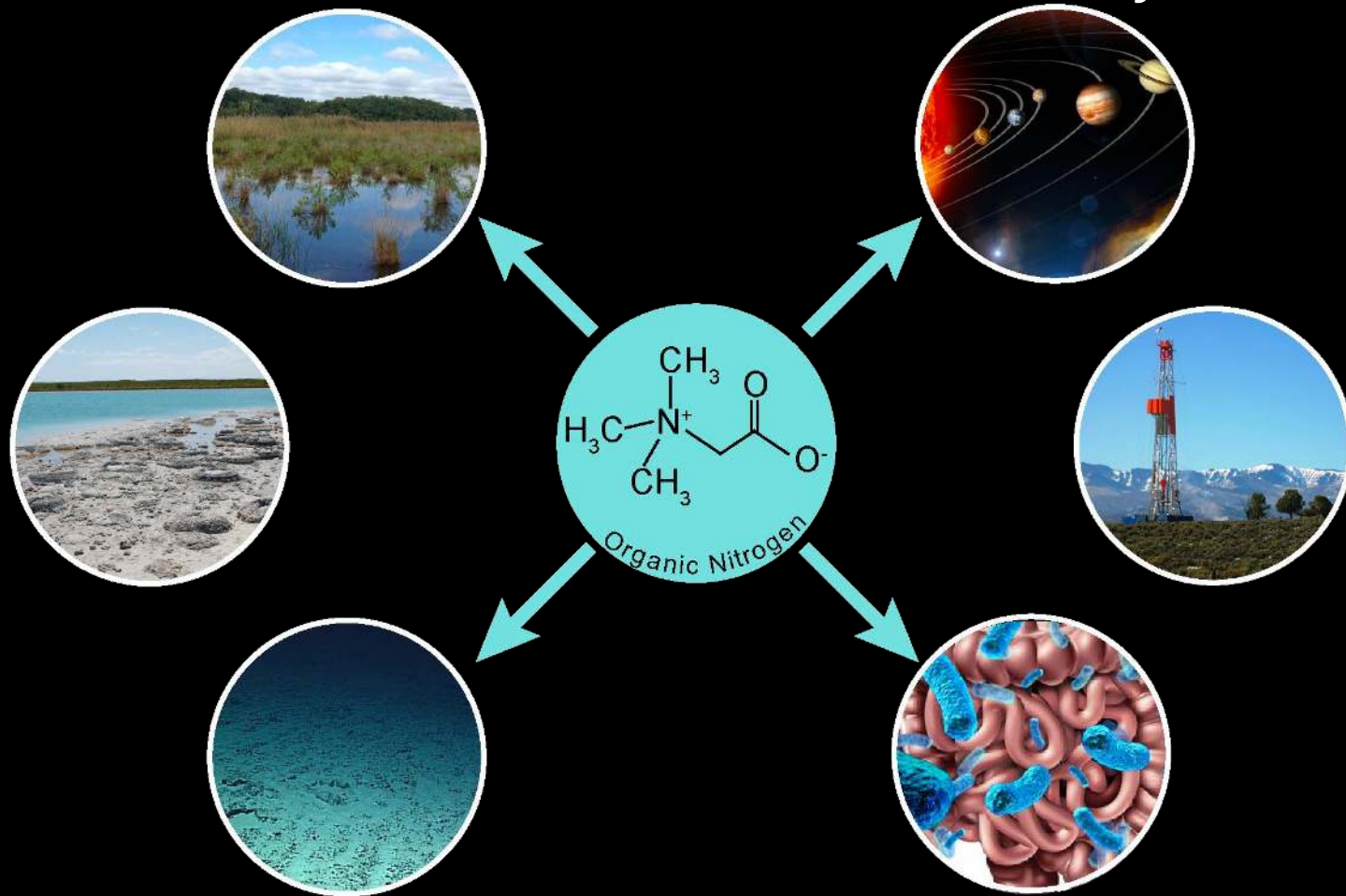
Shale is a model for other ecosystems



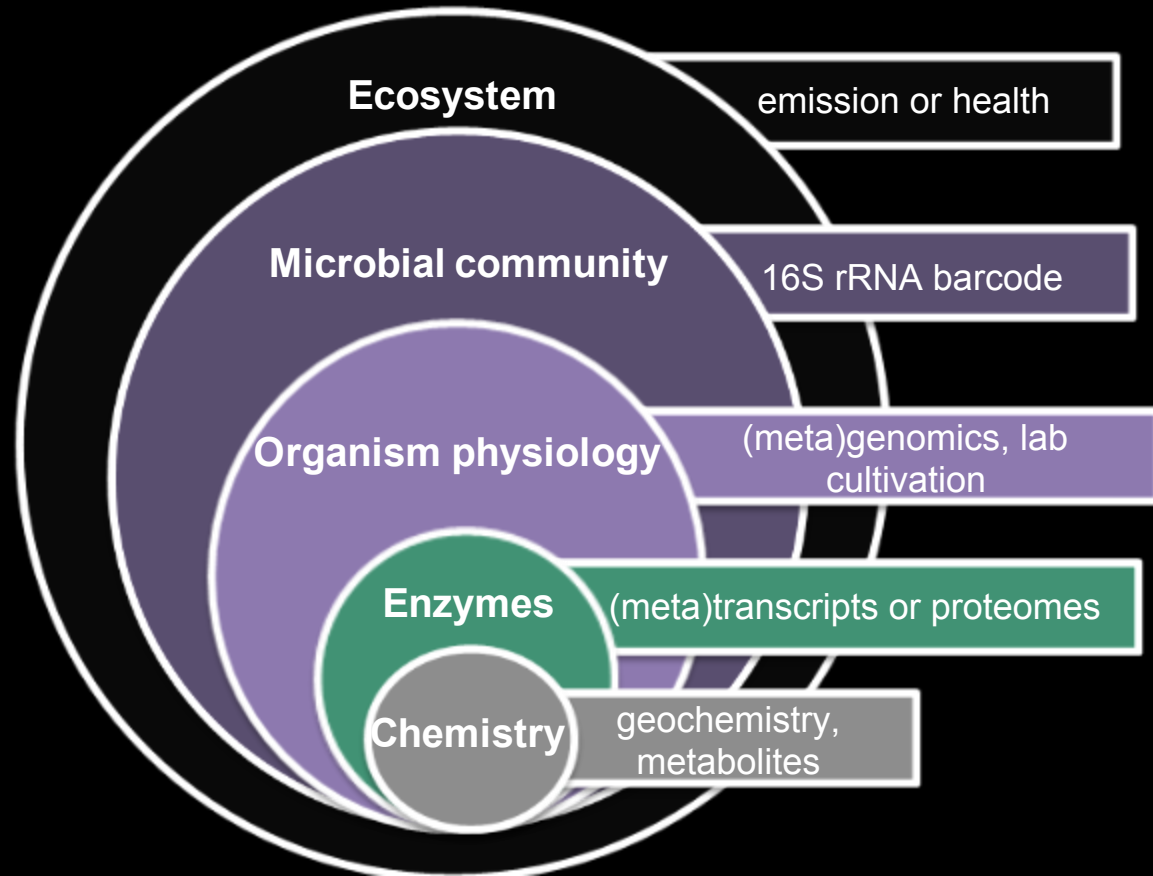
Shale is a model for other ecosystems



Shale is a model for other ecosystems



Summary- Genes to Ecosystem



I welcome your questions

