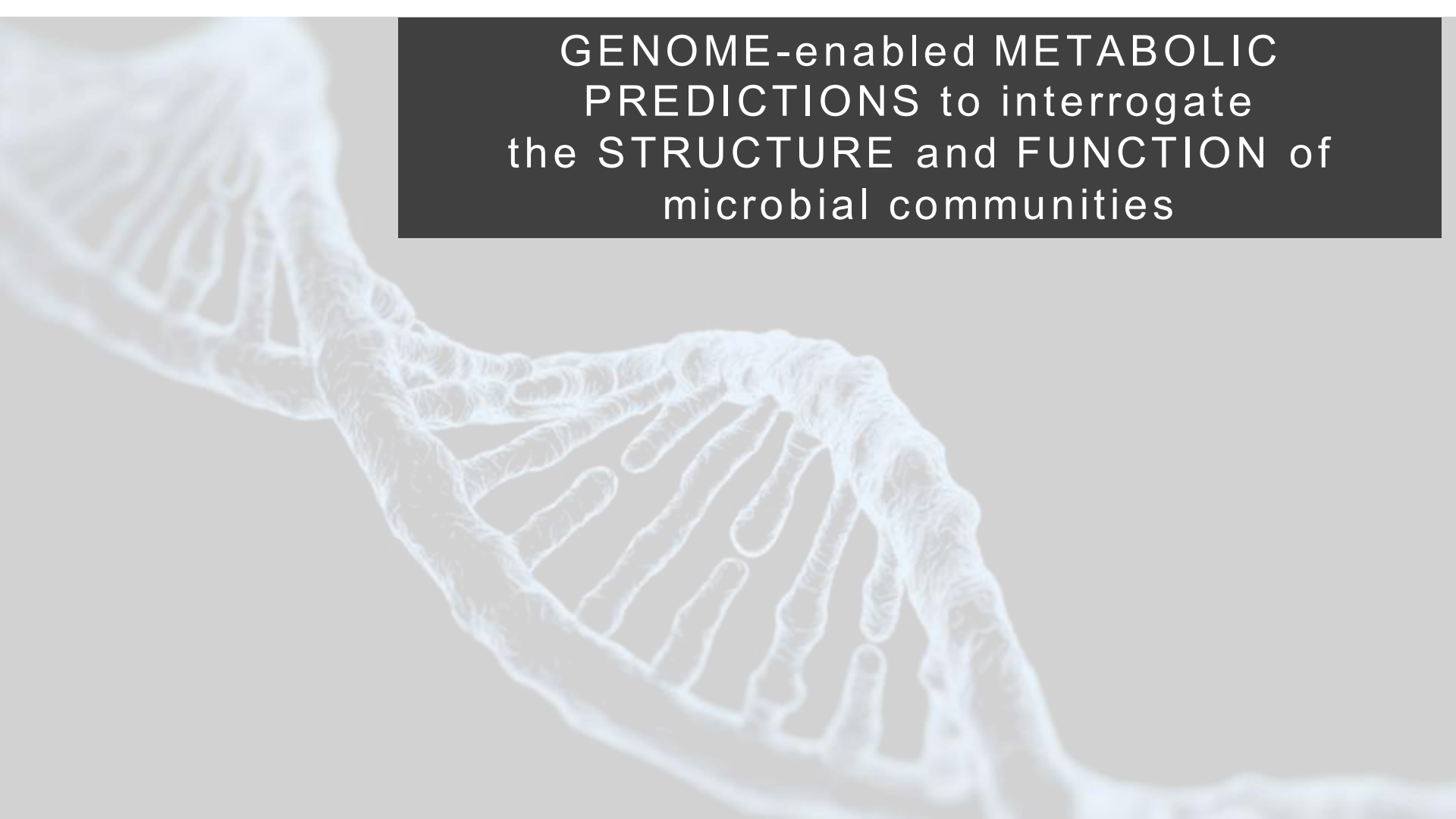
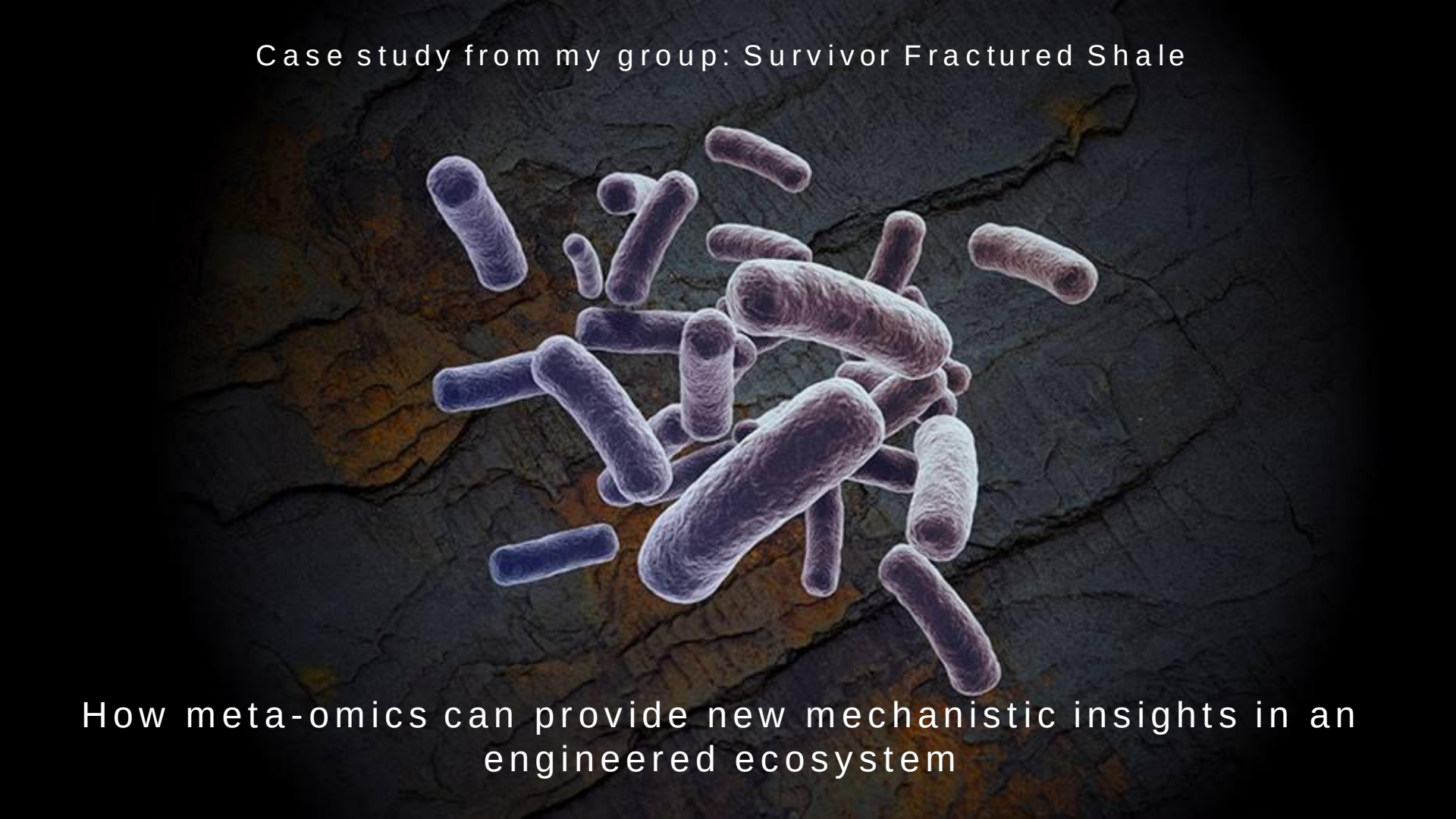
GENOME-enabled METABOLIC PREDICTIONS to interrogate the STRUCTURE and FUNCTION of microbial communities



Case study from my group: Survivor Fractured Shale



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

Acknowledgments

Mike Wilkins
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Rebecca Daly
Research scientist

Mikayla Borton
grad student

Kaela Amundson
grad student

Anne Booker
grad student, alumni

Robert Danczak
grad student, alumni

NSF

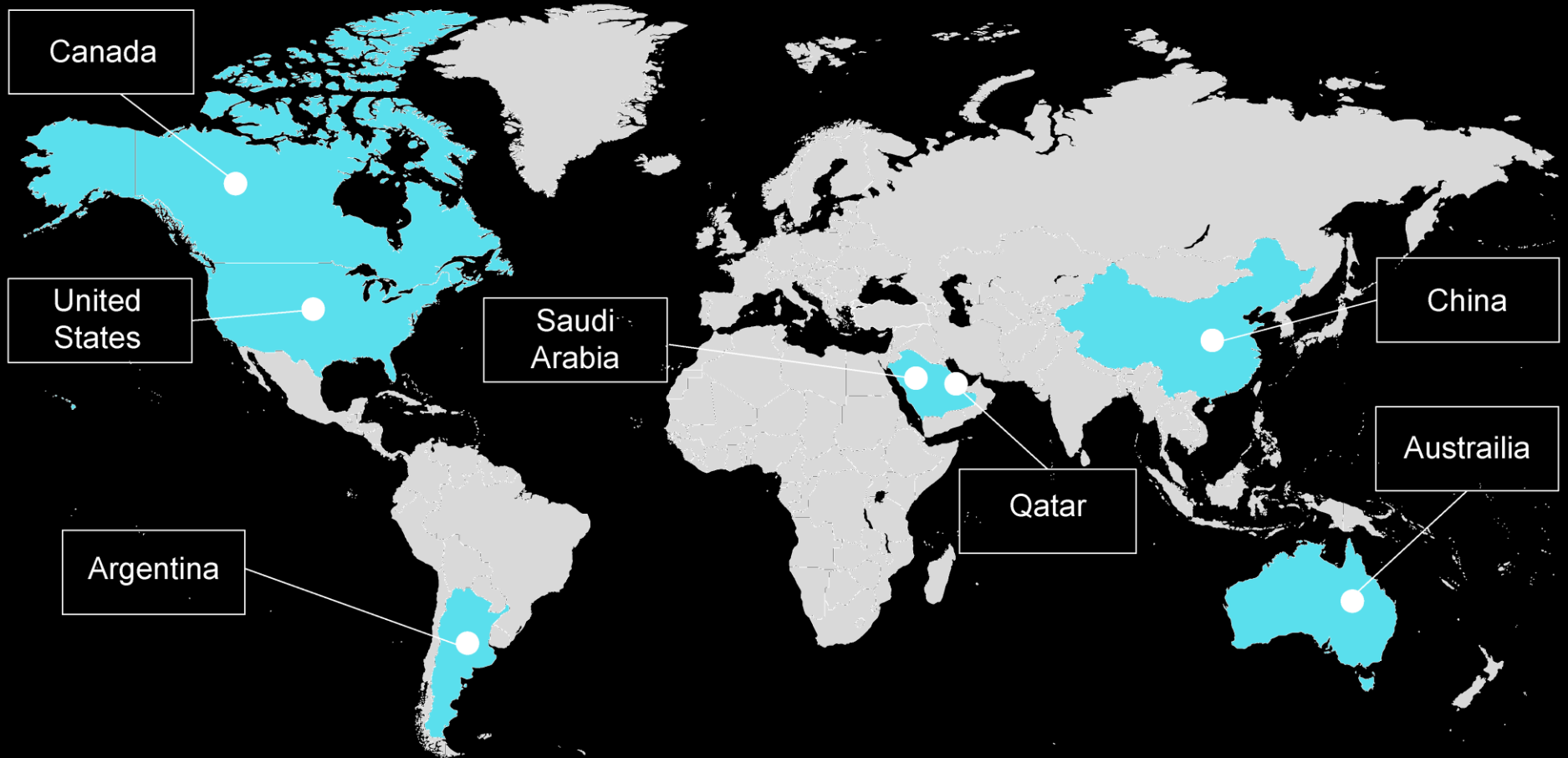
NATIONAL ENERGY TECHNOLOGY LABORATORY

EMSL
SCIENTIFIC INNOVATION THROUGH INTEGRATION

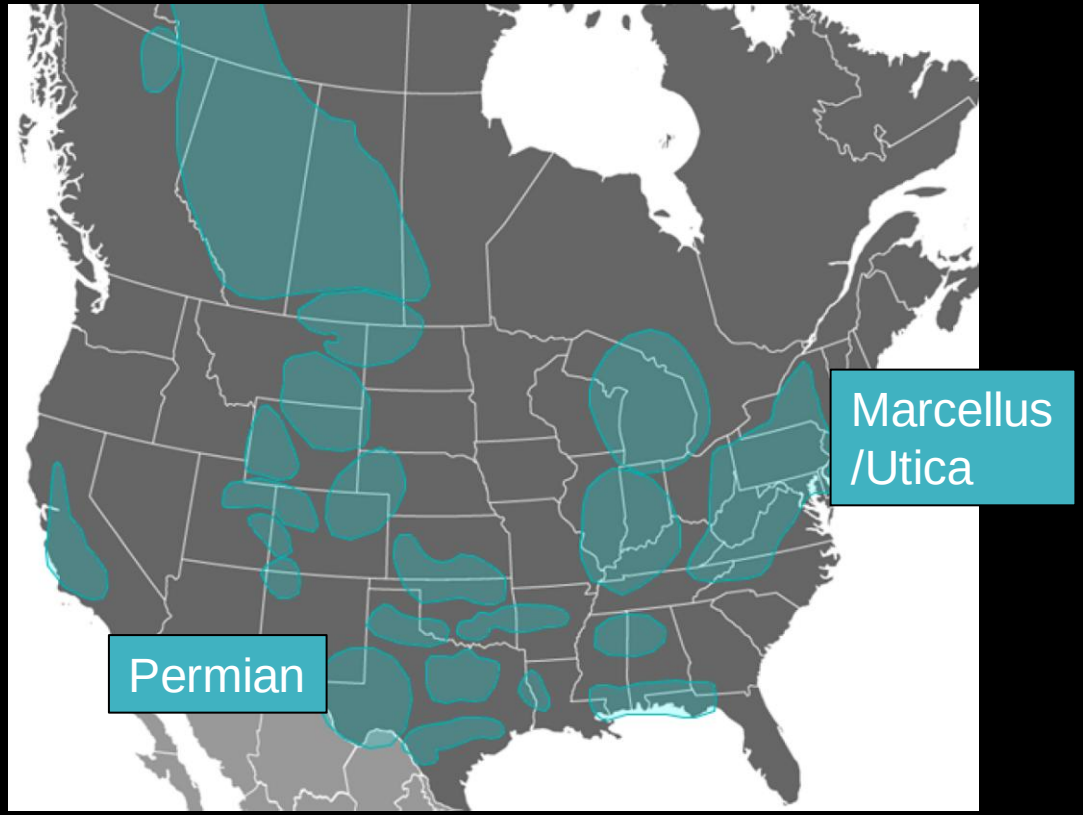
JGI
JOINT GENOME INSTITUTE
DEPARTMENT OF ENERGY

DOW DUPONT™

Countries with commercial shale energy



Hydraulically fractured shales and US energy portfolio



16 States in the US

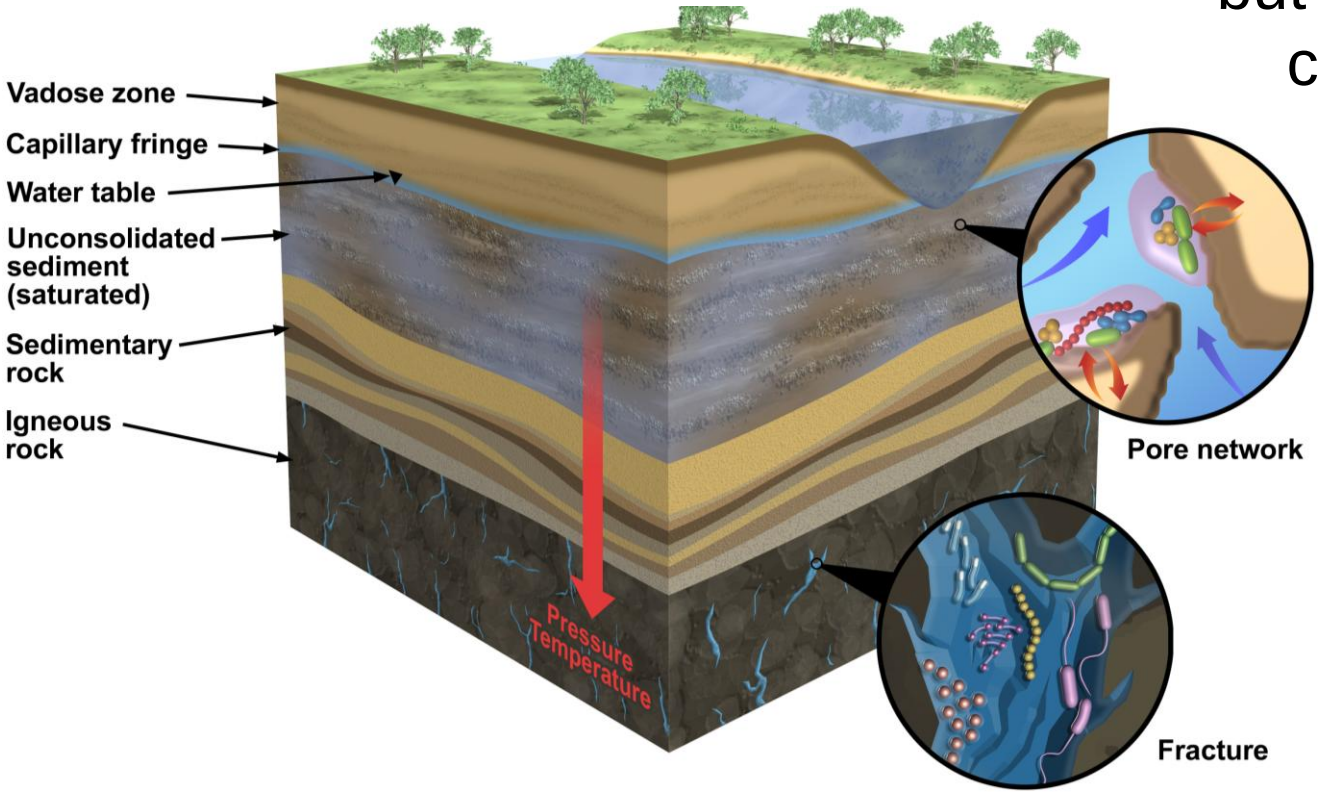
Marcellus contributes 30% of gas for the eastern seaboard

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

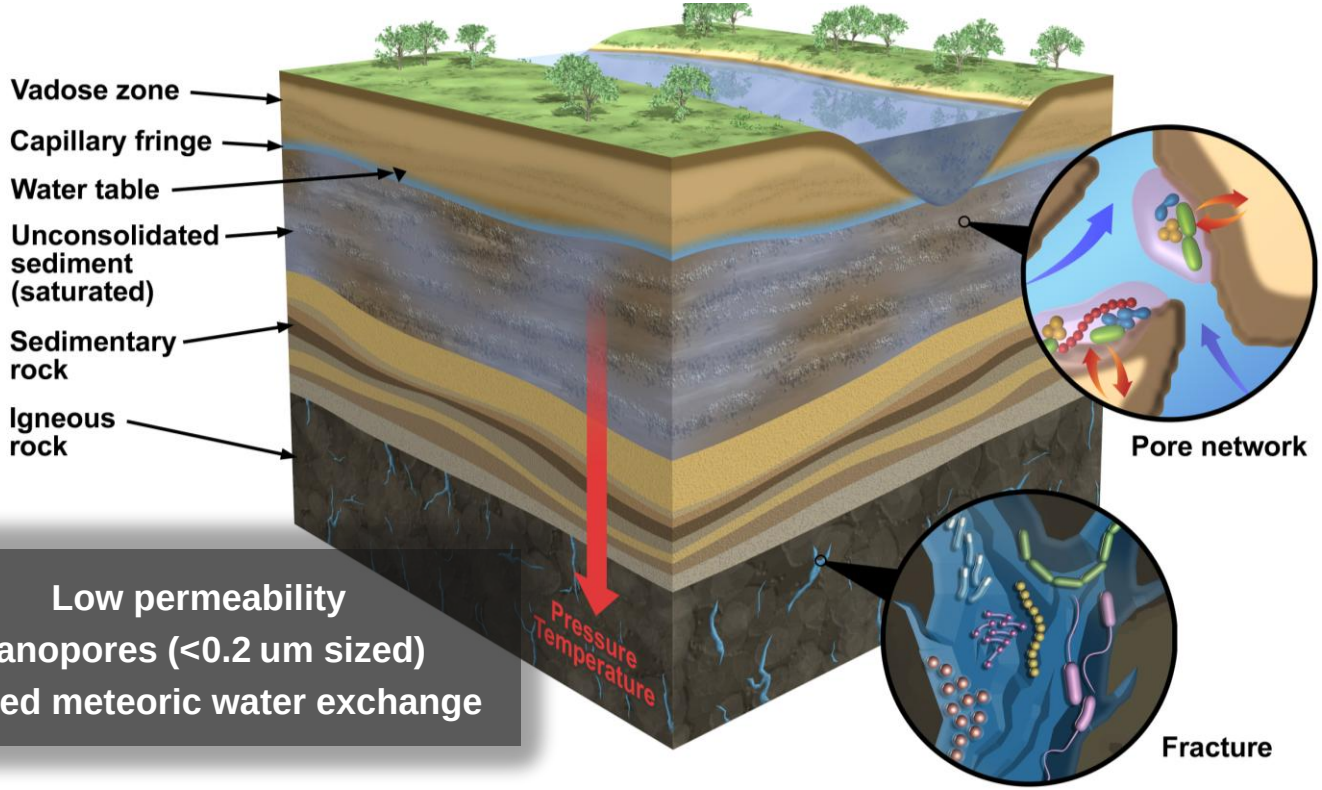
First time in US history natural gas replaces coal as main source for electricity

The deep terrestrial subsurface: The microbial frontier

1/3 earths biomass is below soil layer, but remains poorly characterized



Pristine Appalachian shales do not appear a conducive habitat for life



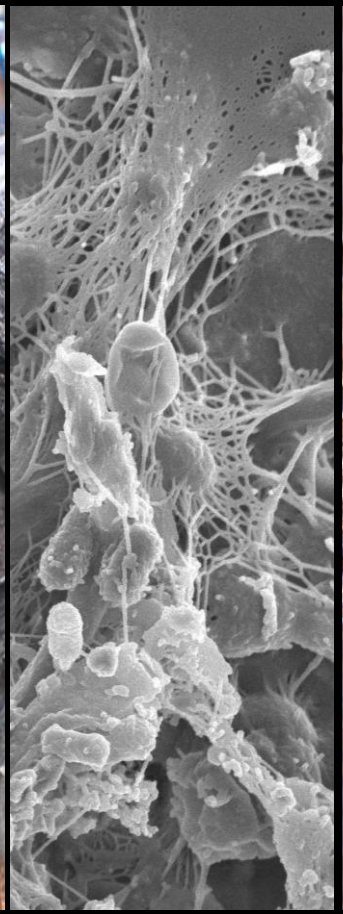
Low permeability
Nanopores (<0.2 um sized)
Limited meteoric water exchange

Andrea Hanson | Paula Mouser | Kai Hendricks | Julius Lipp

Negative Impacts

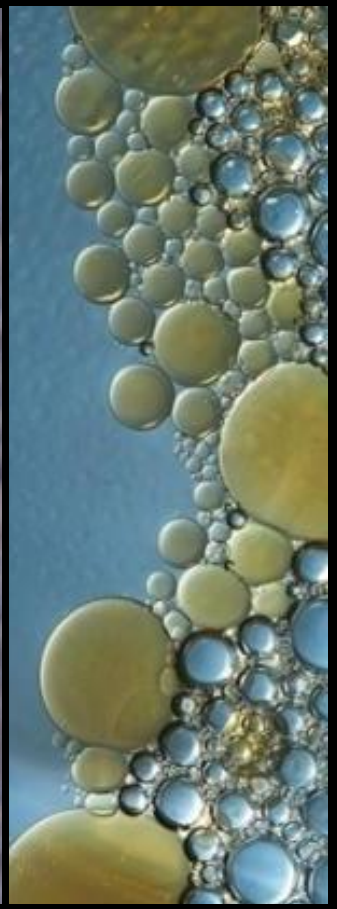
Booker et al, 2016, *AEM* & Booker et al, 2019, *Msphere*

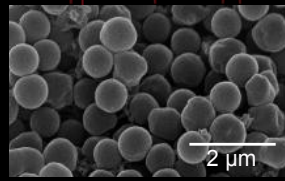
Negative Impacts



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

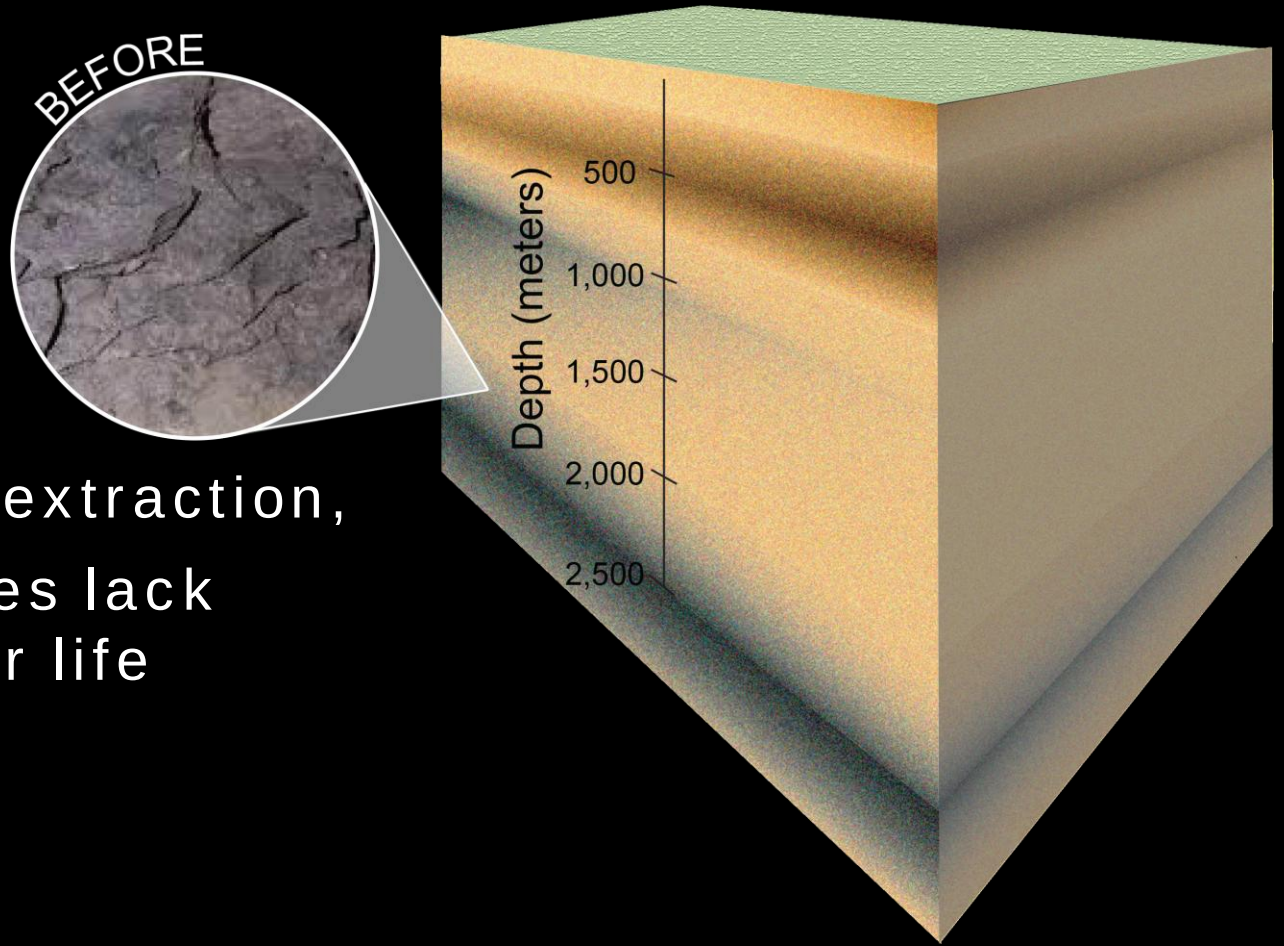
Positive Impacts





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

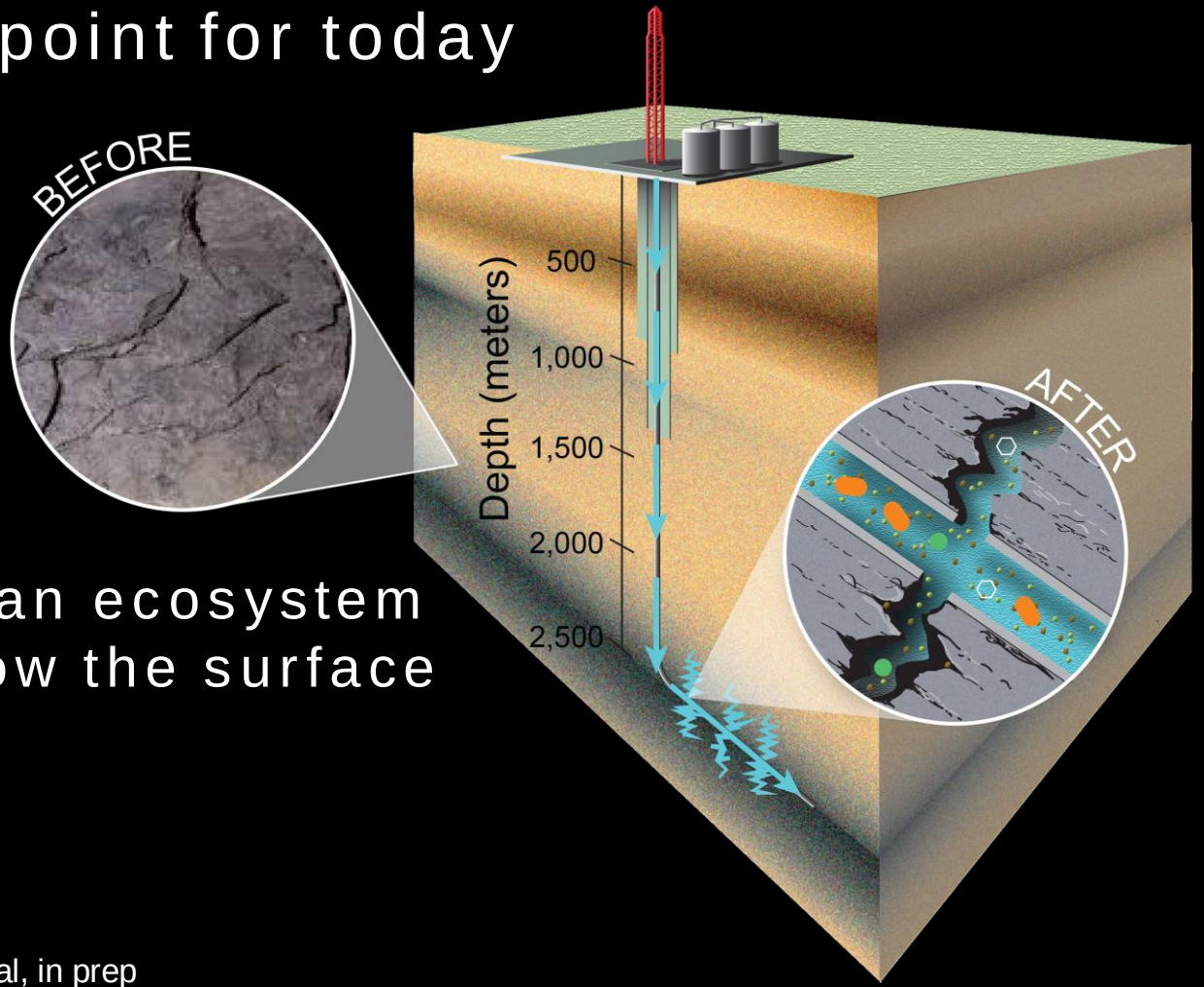
What the frack?



Prior to energy extraction,

most deep shales lack requirements for life

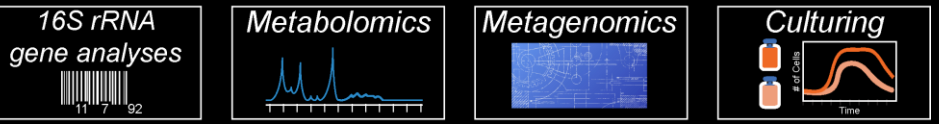
One take away point for today



Fracking creates an ecosystem 2,500 meters below the surface

Daly, 2016, Nature Micro; Hanssen et al, in prep

Development of a multi-omic spatial, temporal database

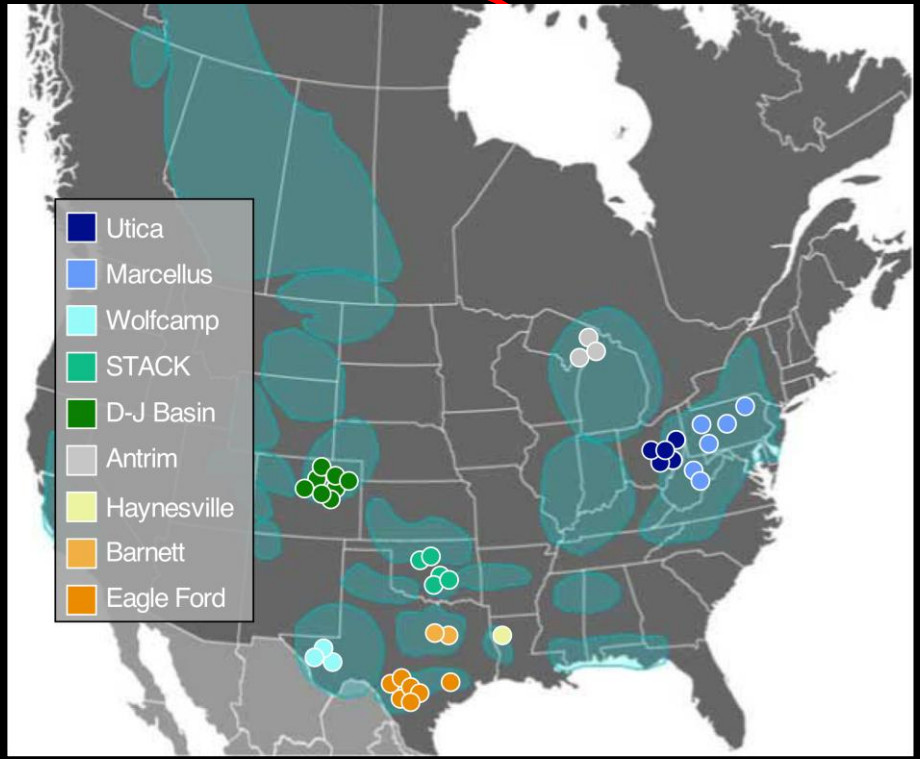


identify persisting taxa

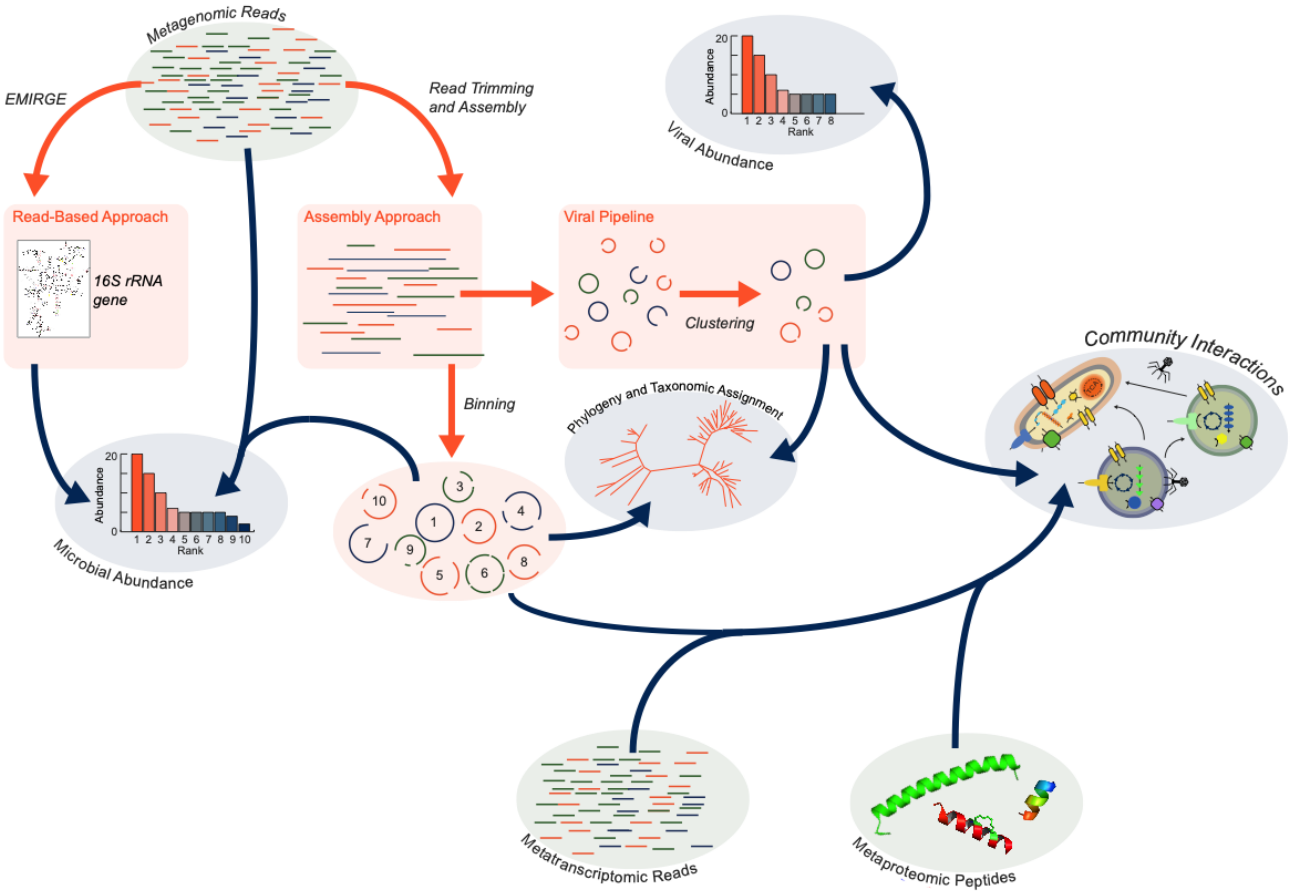
Define chemical niche

Genes that support persistence

Validate field hypotheses



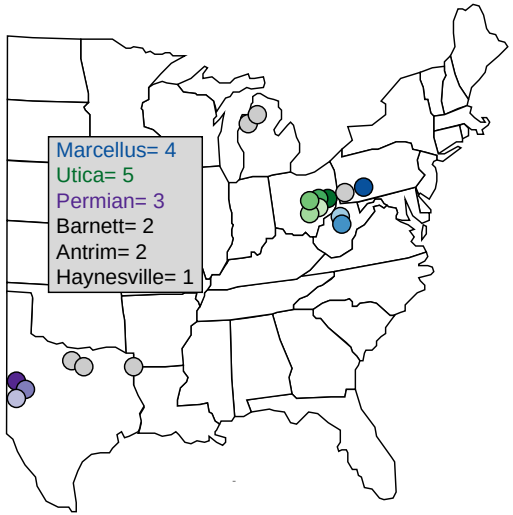
Computational pipeline used in this case study



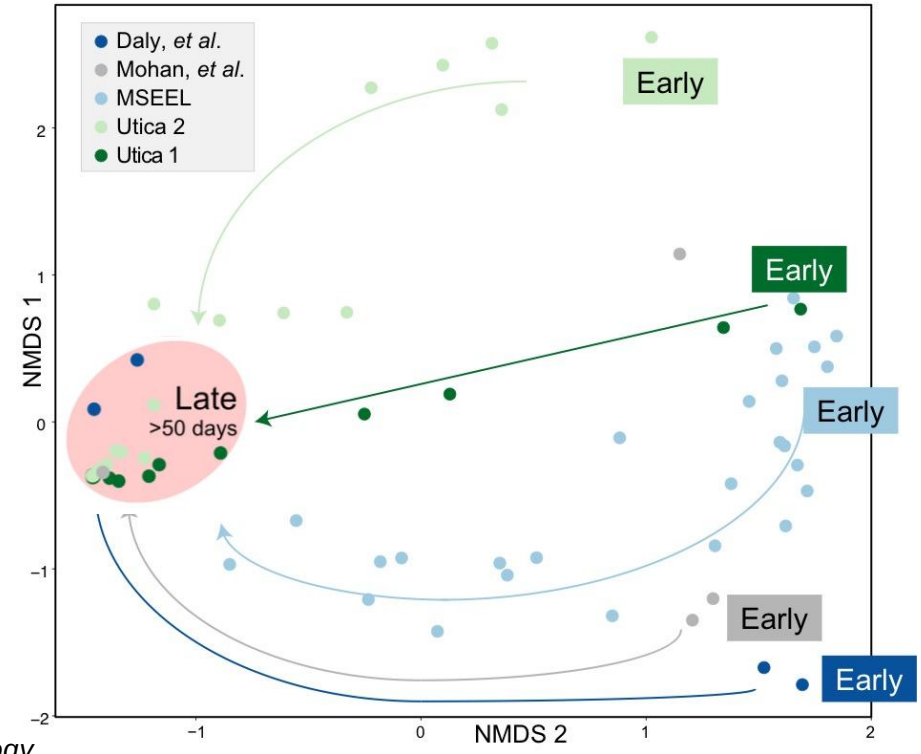
1

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

16S rRNA analyses of persisting microbial communities



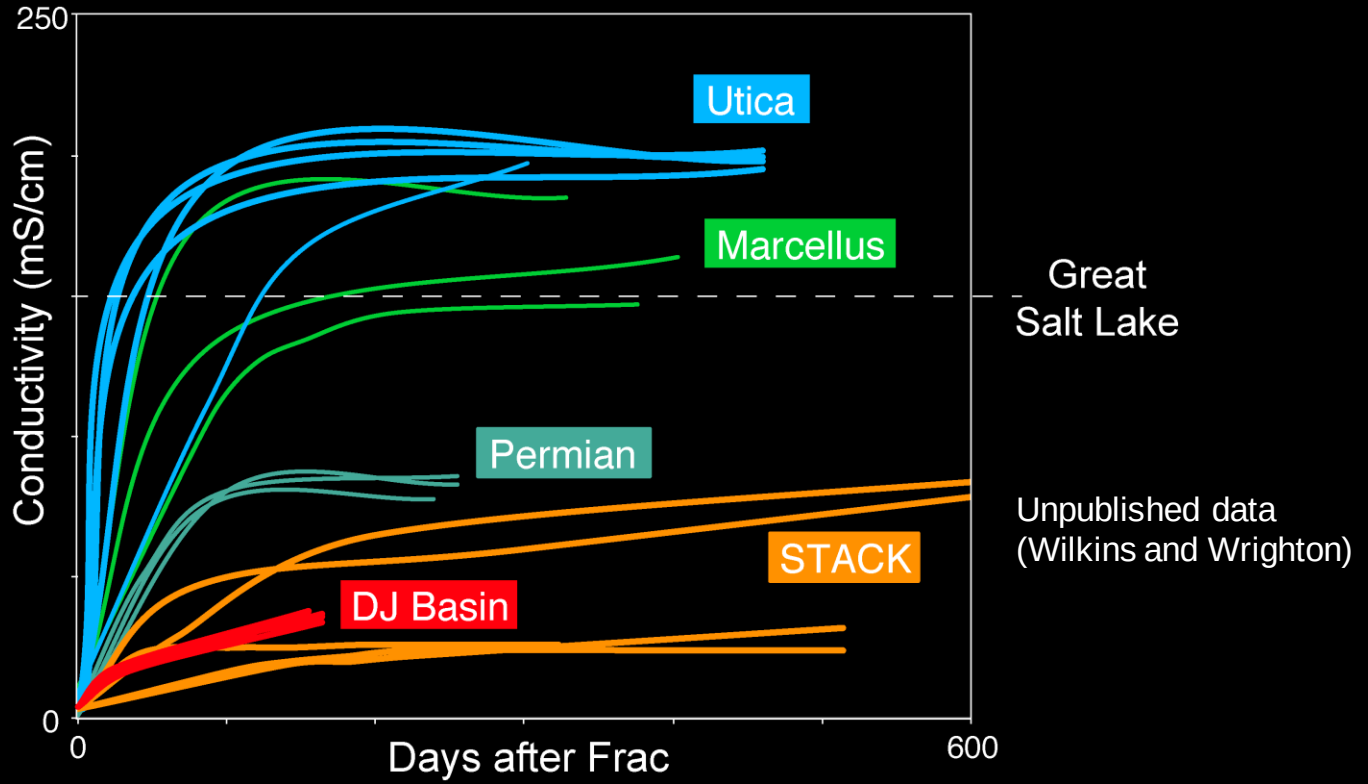
Cell counts increased by ~2 log fold in late samples



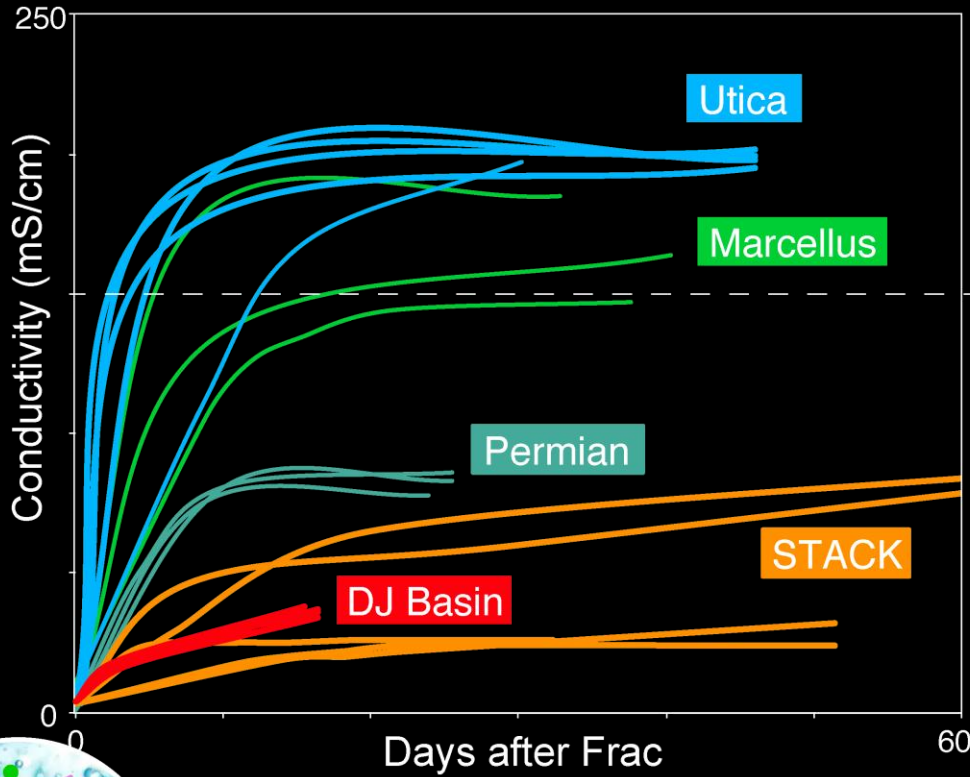
16S rRNA gene analyses

11 7 92

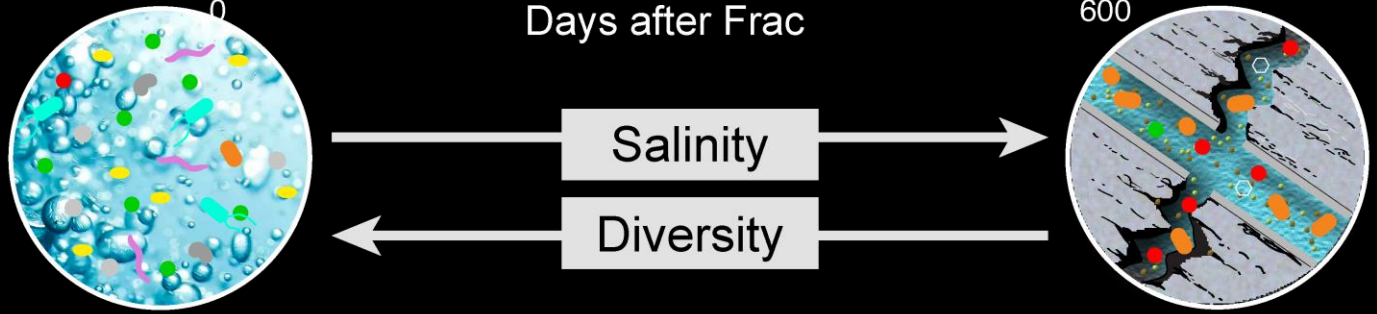
Data from Borton, reviewed in part in Mouser et al 2016, *FEMS Microbiology*



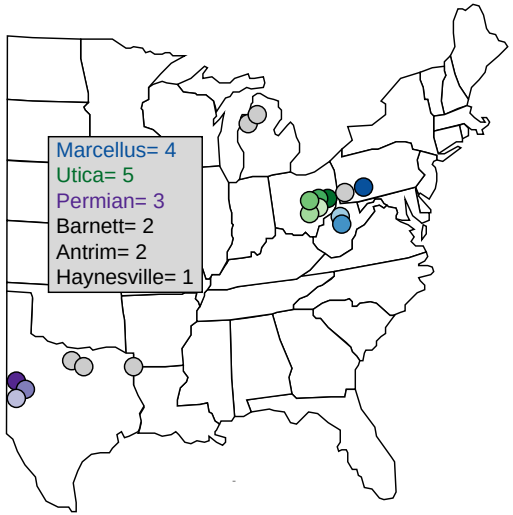
Fractured shale communities must Adapt to increasing salinity

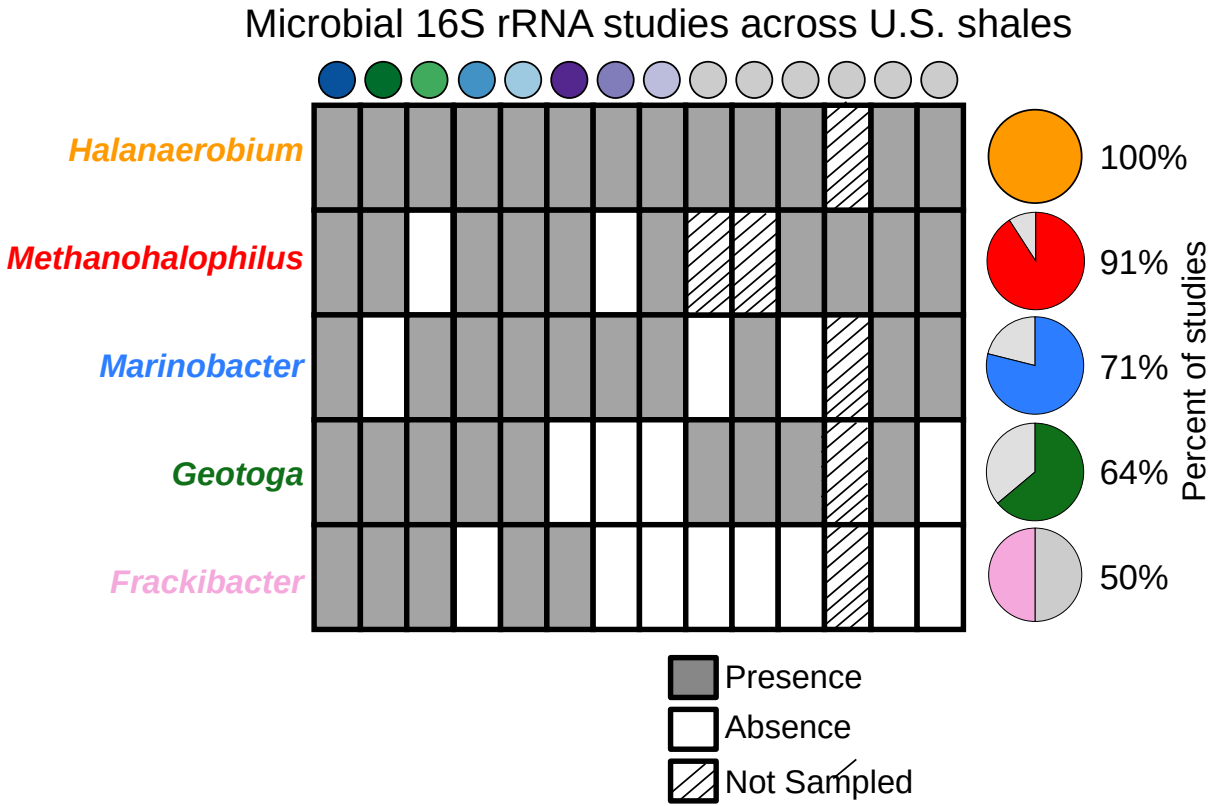


Mouser, FEMS 2016



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





16S rRNA gene analyses

11 7 92

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*?

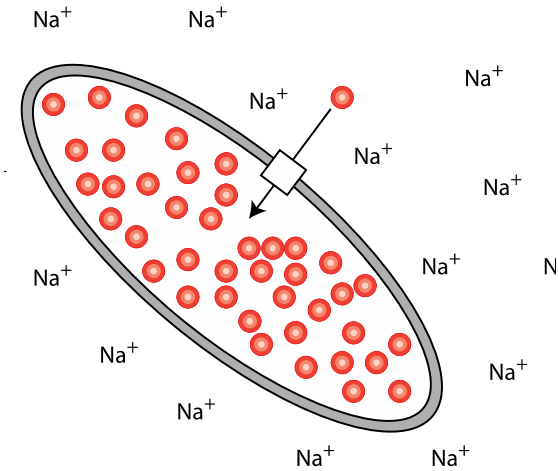
Osmoprotection

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

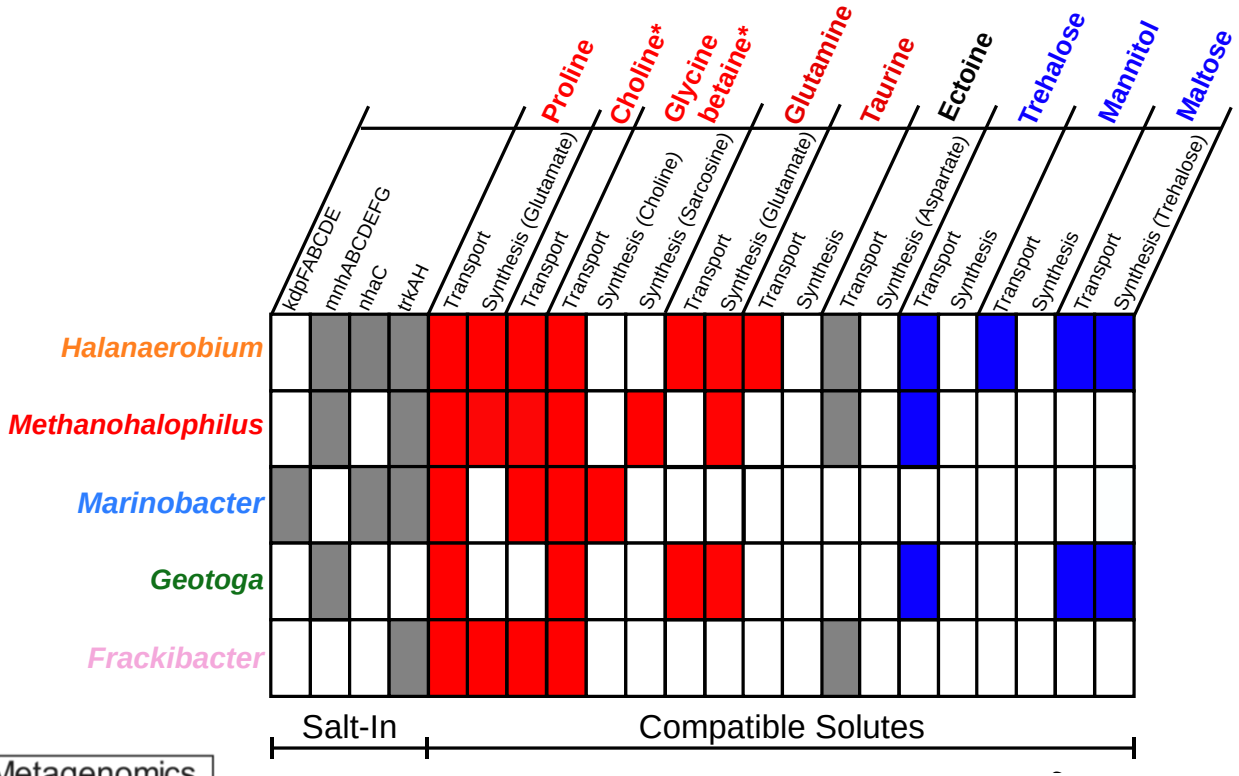
Other microbes **utilize metabolites**

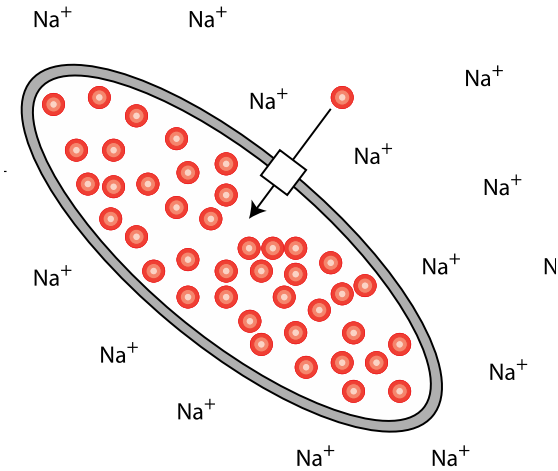
Energetically costly to produce small, organic acid or amino acid products

Enable flexibility to salt ranges



Genome resolved: no single signal in our MAGs

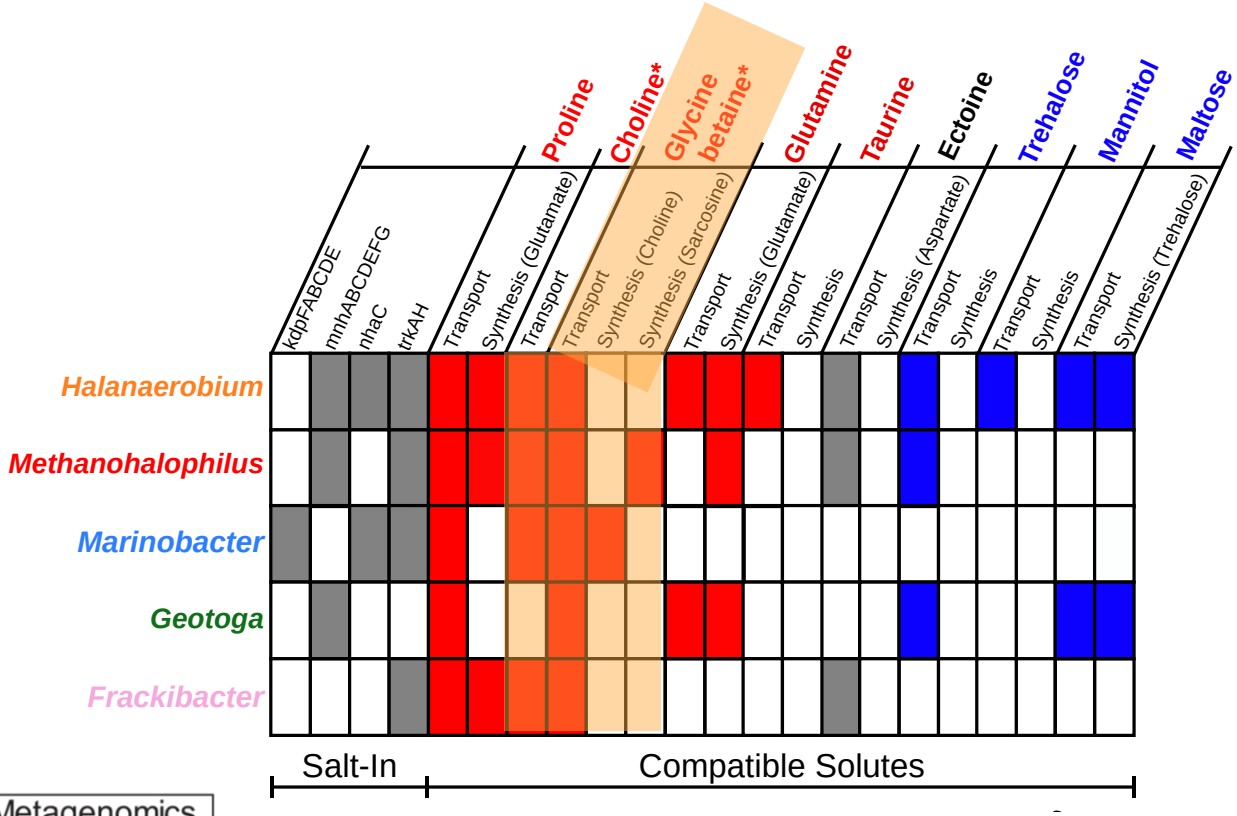


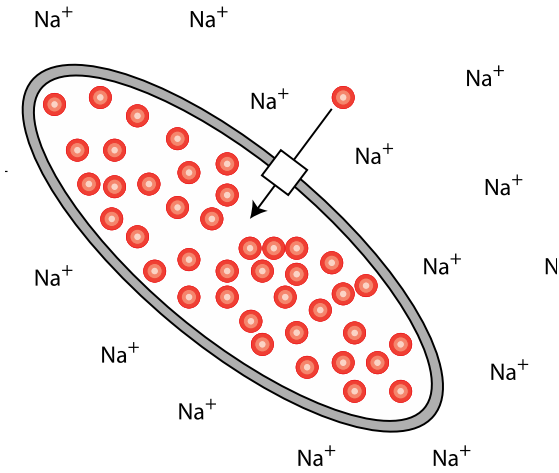


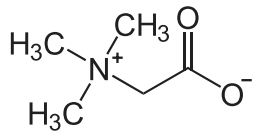
Metagenomics

Daly et al, 2016, *Nature Microbiology*

Genome resolved: Glycine betaine was interesting…







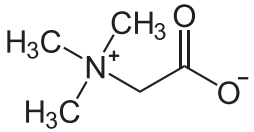
Glycine betaine

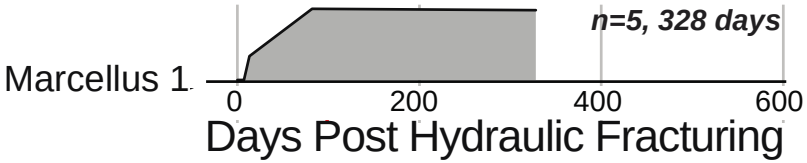
Metagenomics

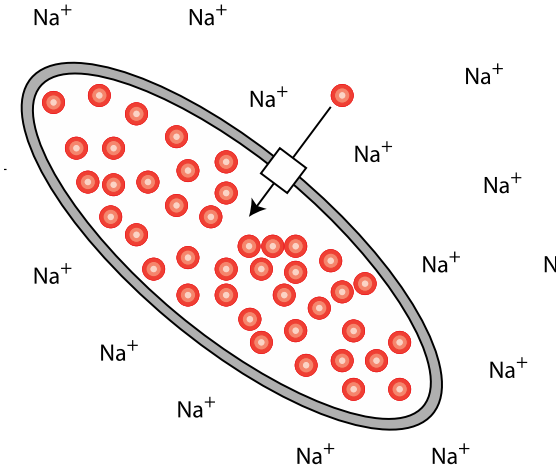
Daly et al, 2016, *Nature Microbiology*

Extracellular metabolites:
Glycine betaine is a core, persisting metabolite

Glycine betaine







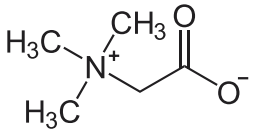
Metabolomics

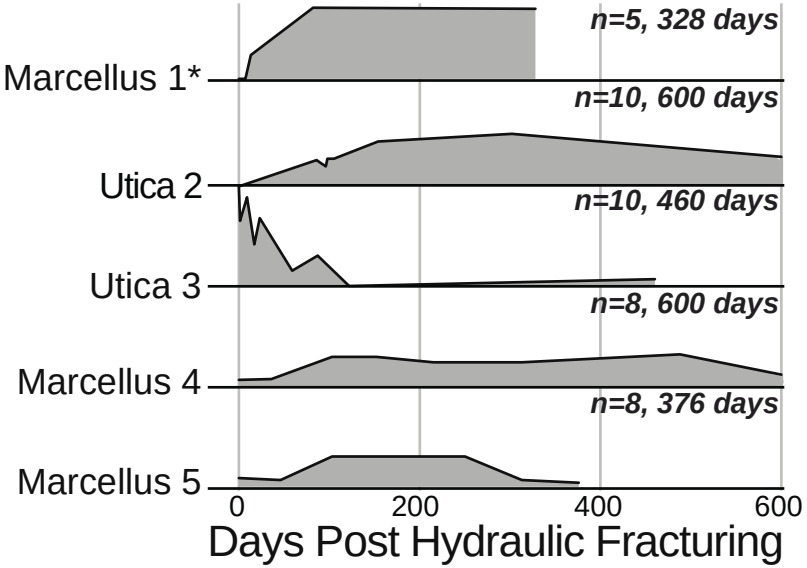
David Hoyt

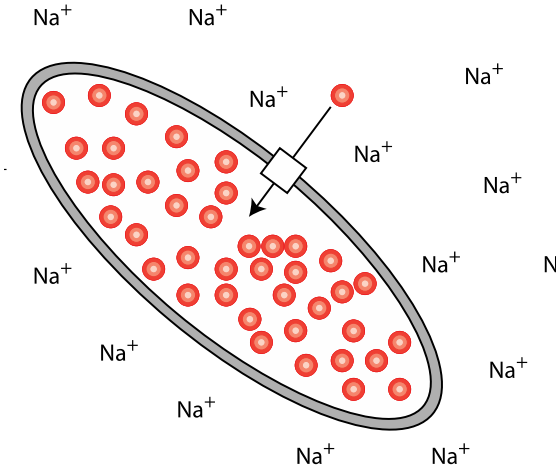
EMSL

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

Glycine betaine





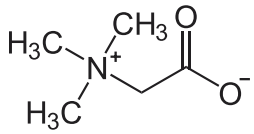


1 Certain microbes and osomolyte metabolites persist across shales in response to salinity

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

Could glycine betaine also serve as energy source?

Glycine betaine



1 ***Stickland reaction***

amino acid electron donor **?**

Reductase

glycine betaine

trimethylamine

2 ***Demethylation reaction***

Methyl transferase

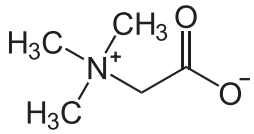
glycine betaine

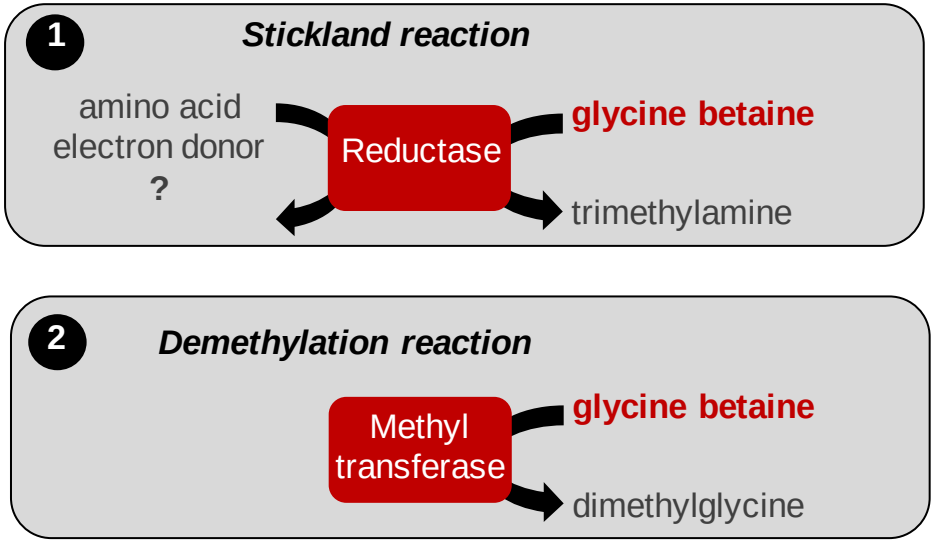
dimethylglycine

Metabolism Ref: Ticak et al, 2014 + Andreesen et al, 2004

Could glycine betaine also serve as energy source?

Glycine betaine



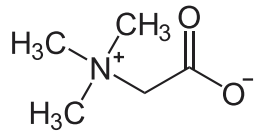


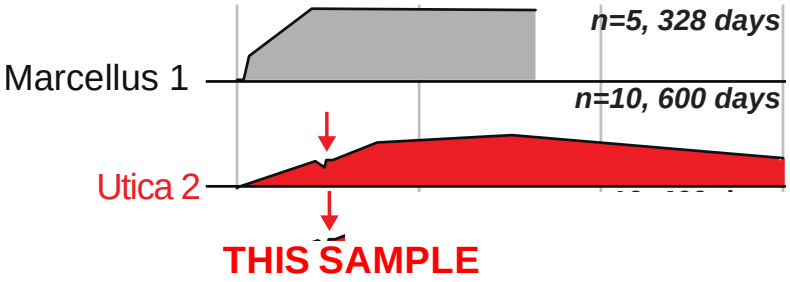
Isn't metagenomics enough?

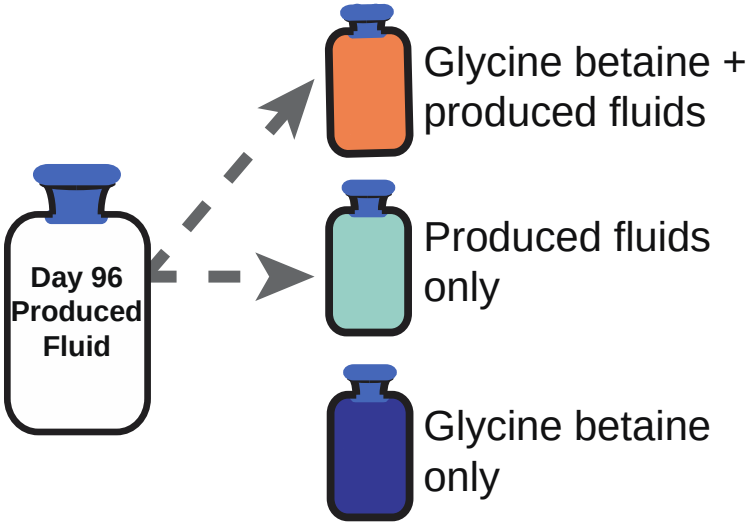
- Genes are poorly annotated in genomes
- Electron donor cannot be identified from existing data alone
- Expression is challenging due to sample collection

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

Glycine betaine

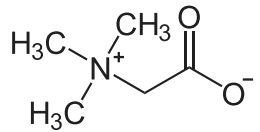


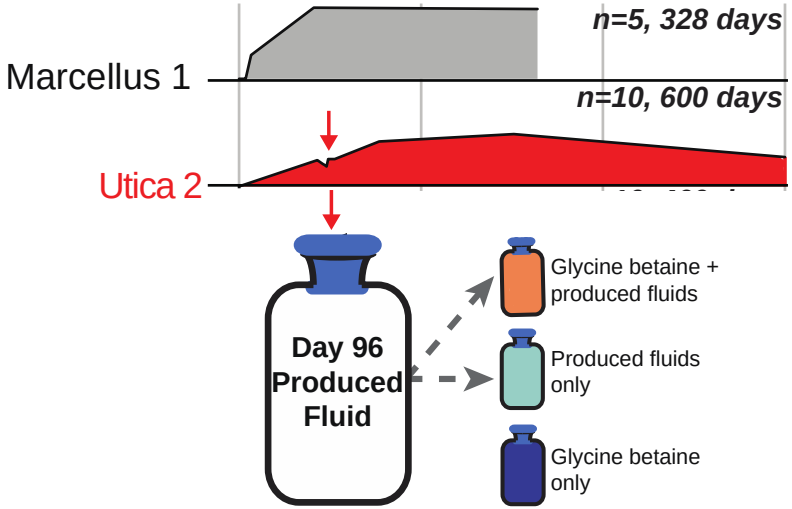


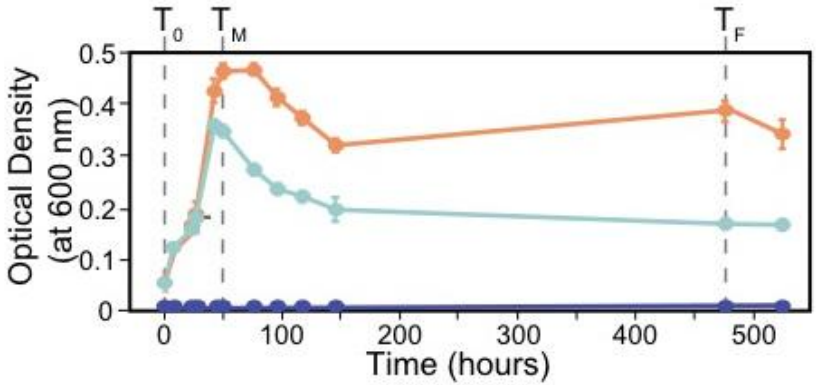


Co-expression patterns from shale reactors could identify

Glycine betaine





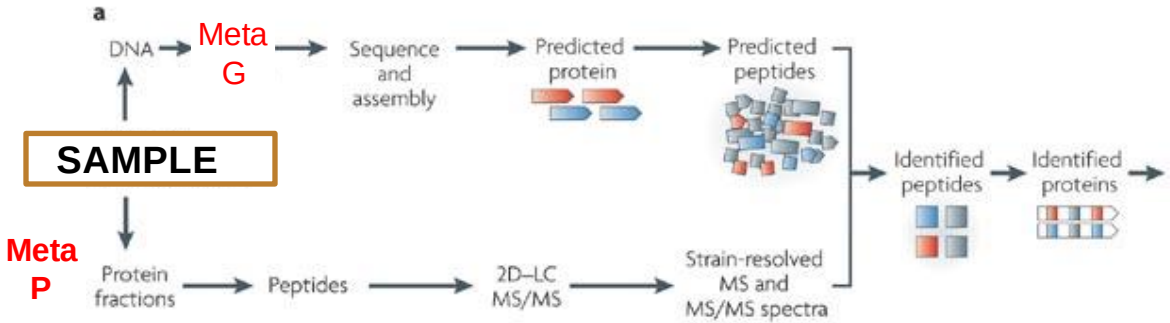


$T_0 \rightarrow T_M \rightarrow T_F$

Metagenomics
Proteomics
NMR Metabolites
Gas Chromatography

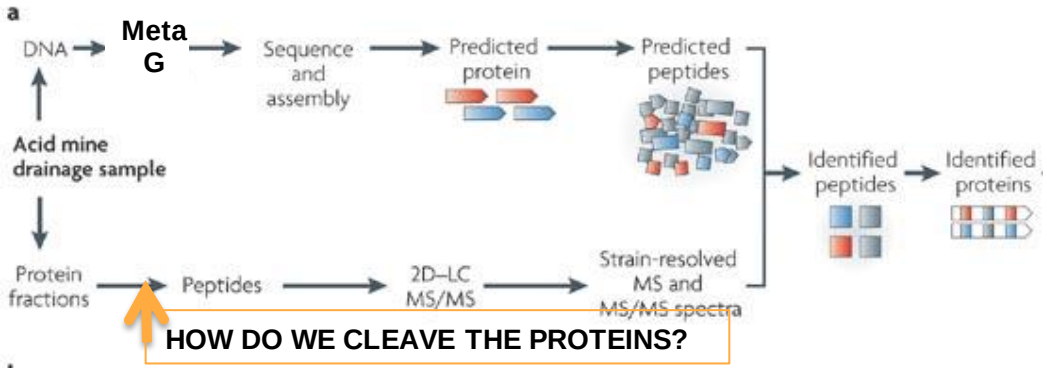
EMSL

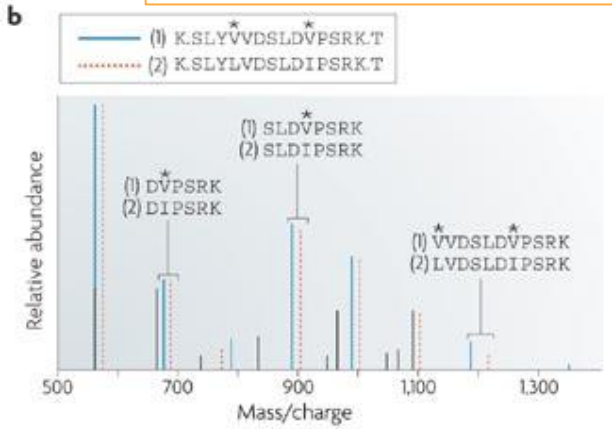
Metaproteomics crash course- paired to metagenomes



Identify unique proteins to genome bins

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





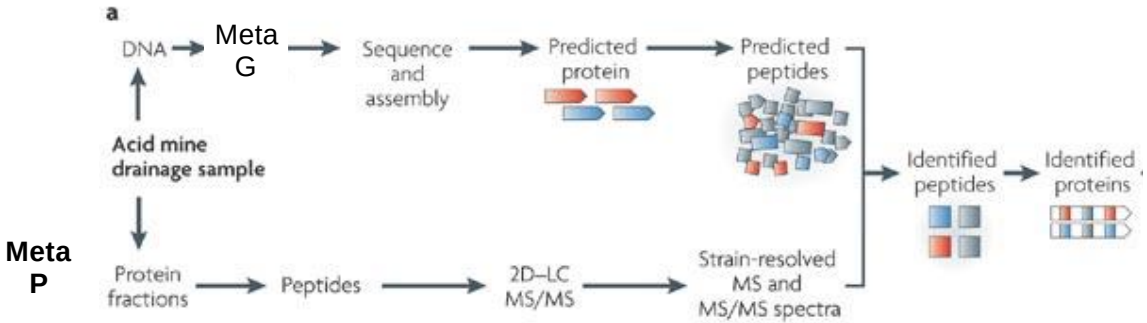
(1) K.SLYVVDSLDVPSRK.T
(2) K.SLYLVDSLDIPSRK.T

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

Lysine=K
Arginine= R

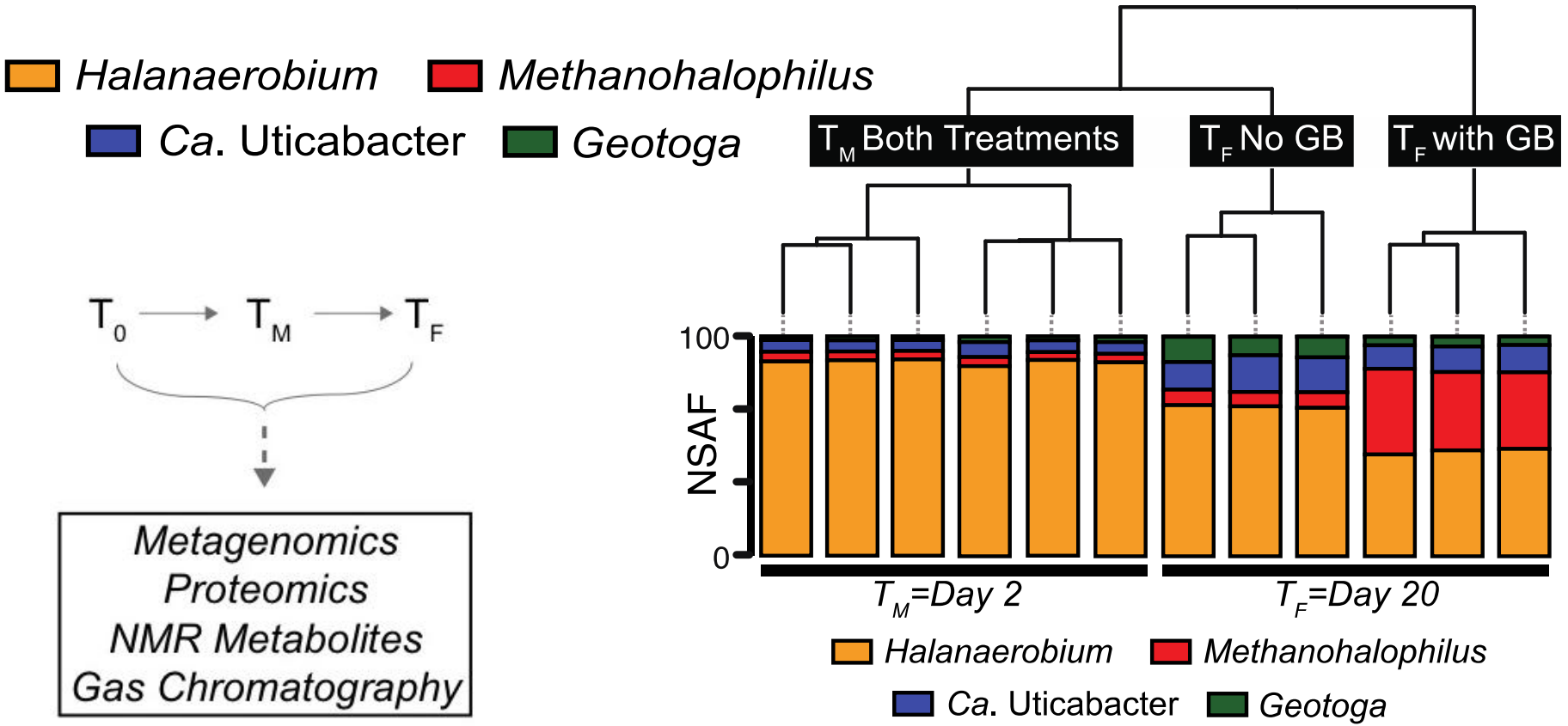
Nature Re

Proteomics- uses and challenges

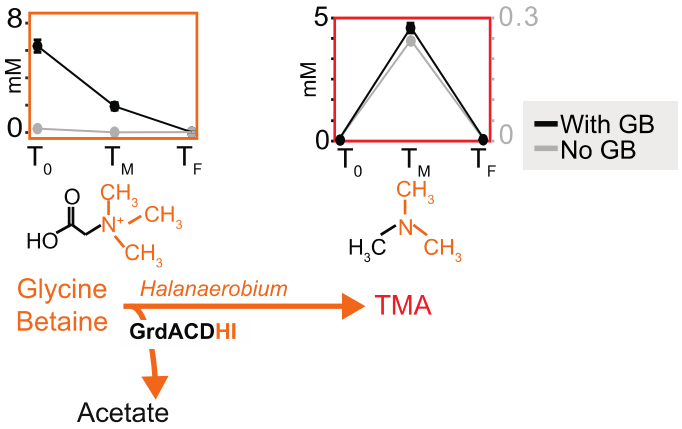


- **Proteomics**
  - Demonstrates expression of genes in metagenomes
  - Correlate to metagenomic data
  - No amplification: need high signal, doesn't do well for low abundance organisms
  - Outer membrane proteins not as well resolved unless separate fractionation done

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



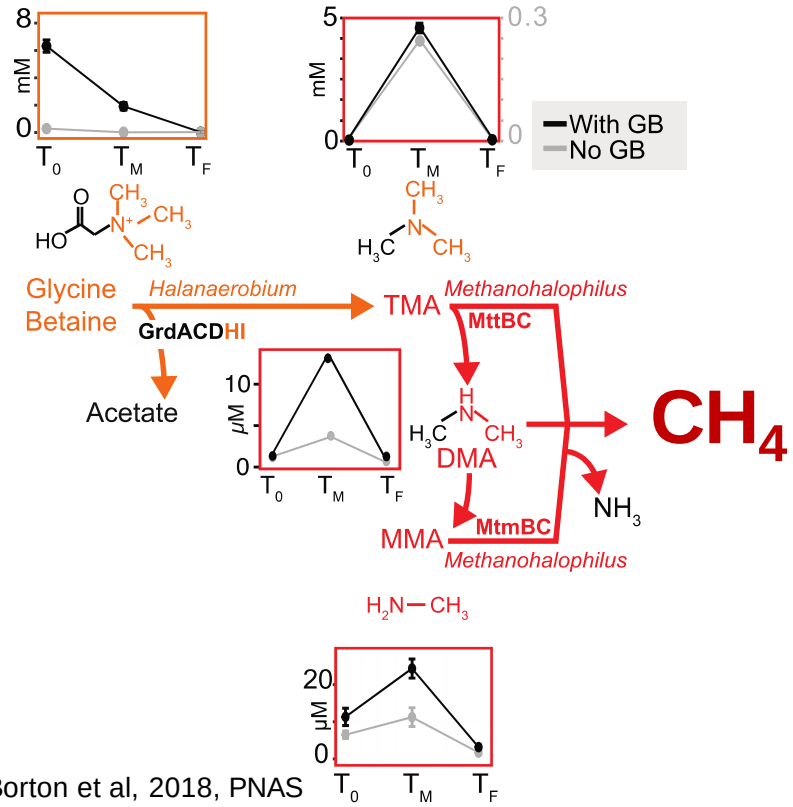
Combining time series meta- omics
Halanaerobium reduced GB to yield TMA, a methanogenic substrate



74% of GB converted to TMA

In amended or unamended!

Laboratory Proteomics and Metabolomics: *Methanohalophilus* preferentially utilizes *Halanaerobium* metabolites

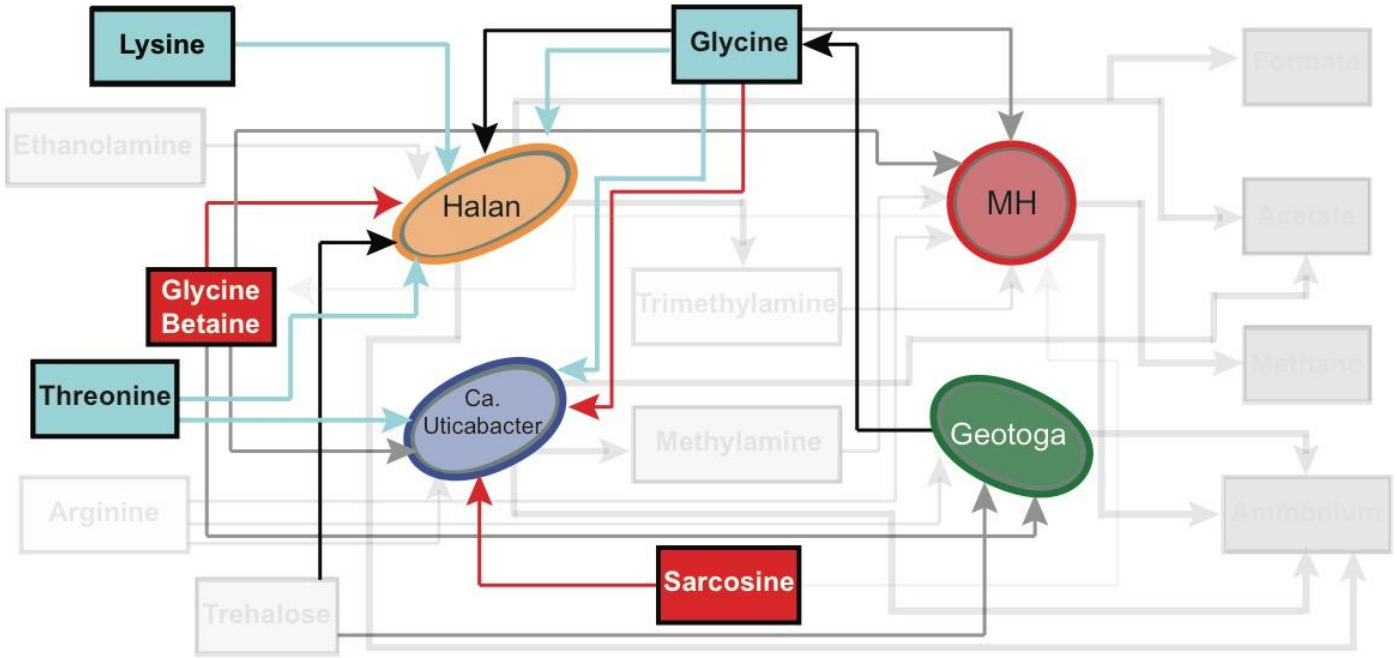


Borton et al, 2018, PNAS

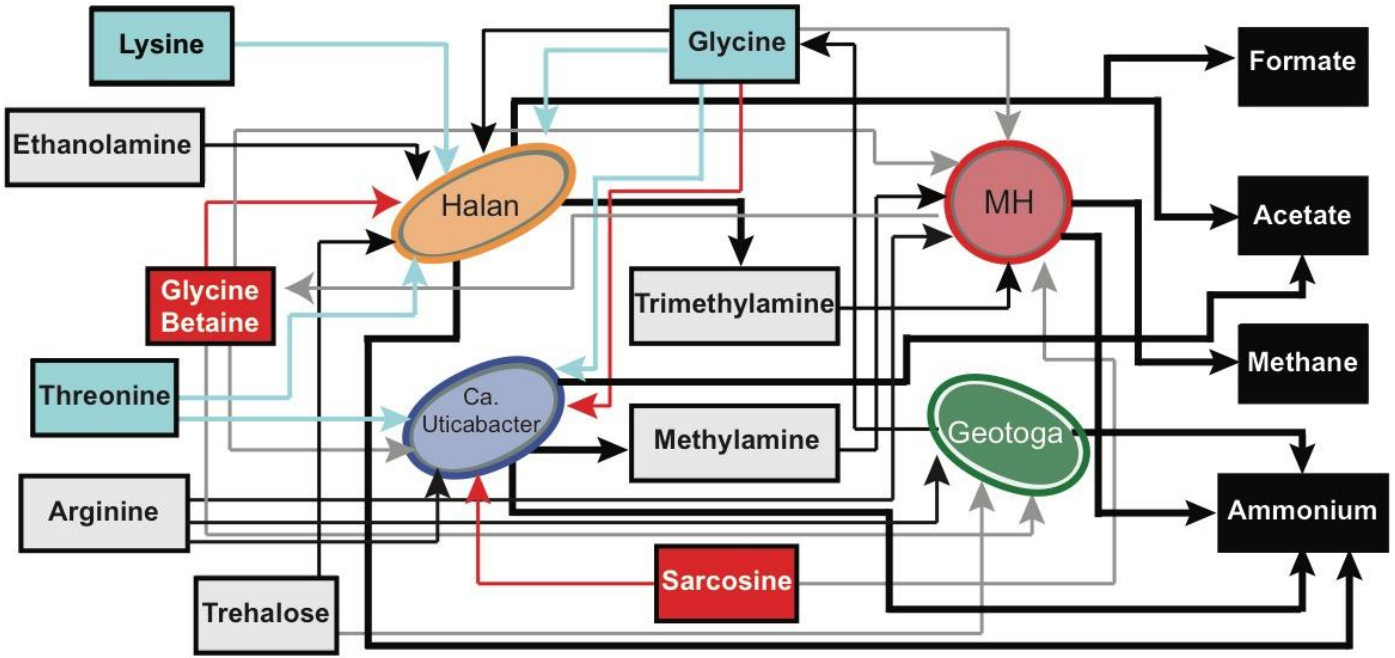
Expression and metabolite correlation analyses

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



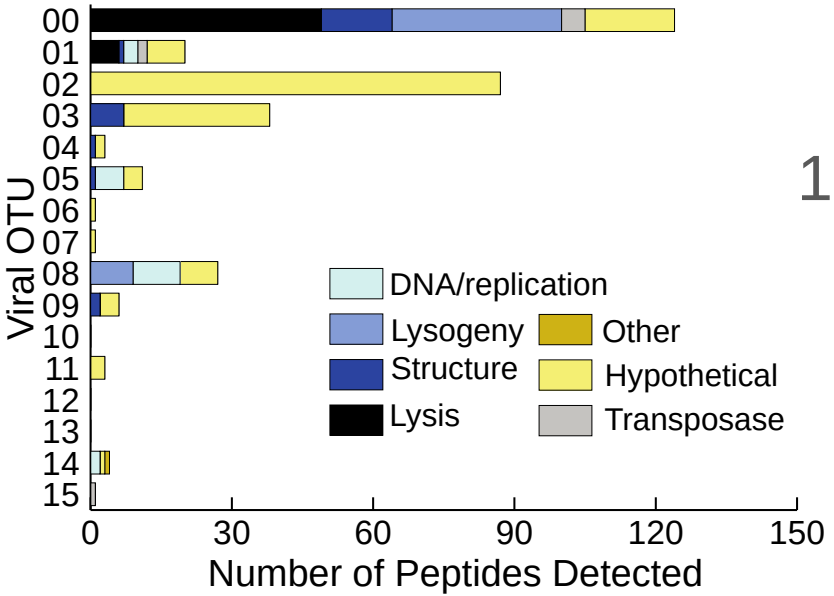
Borton et al, PNAS

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

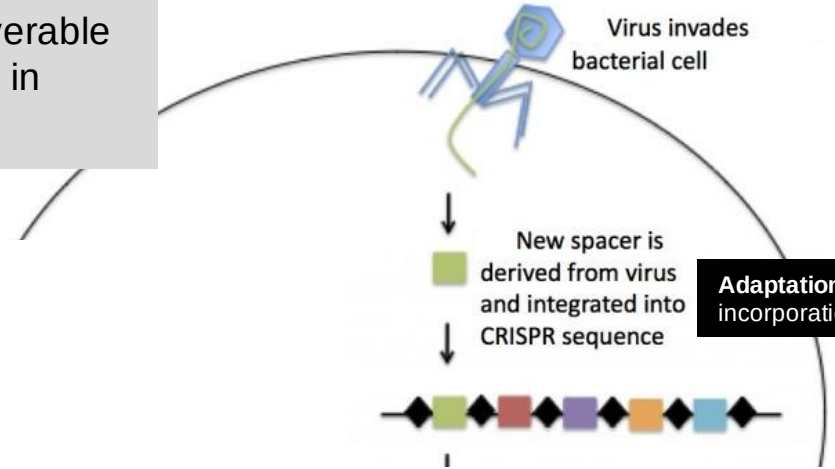


16 unique viral genomic contigs
*viral genes expressed in lab

*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



Adaptation: spacer incorporation

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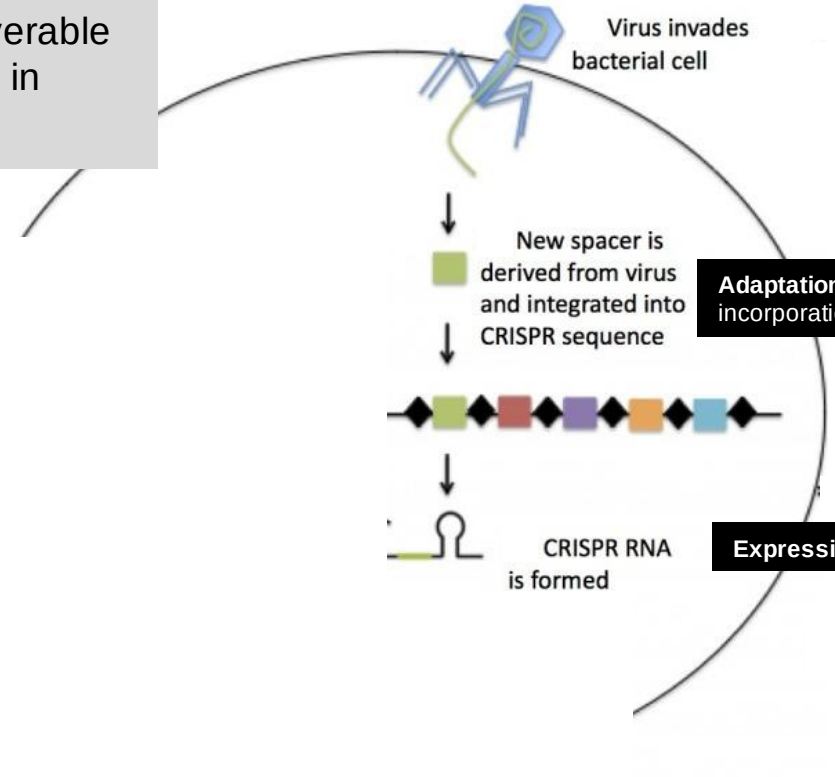

Adaptation: spacer incorporation

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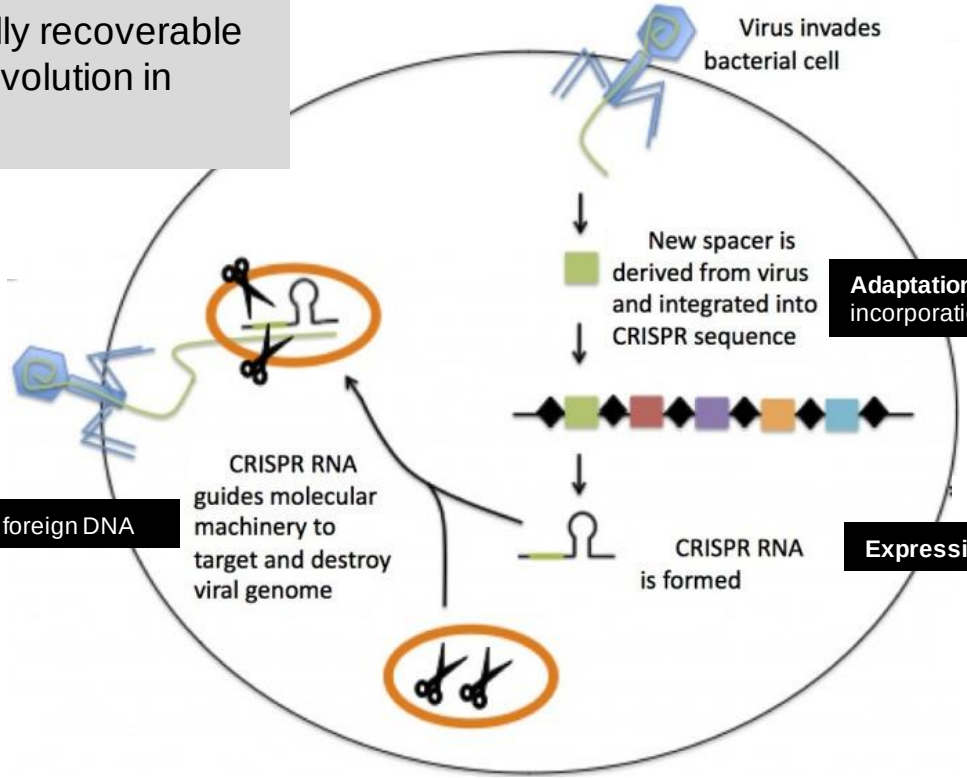


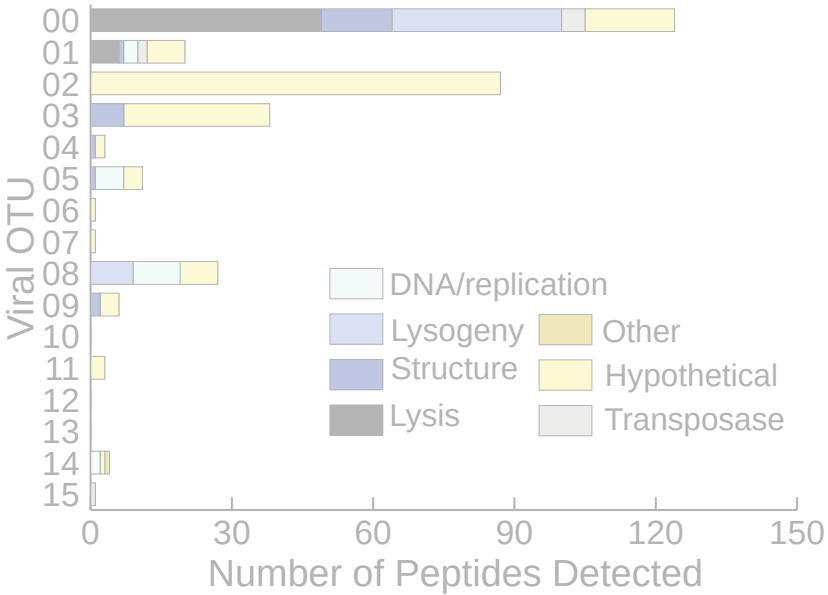
Adaptation: spacer incorporation

Expression: RNA is formed

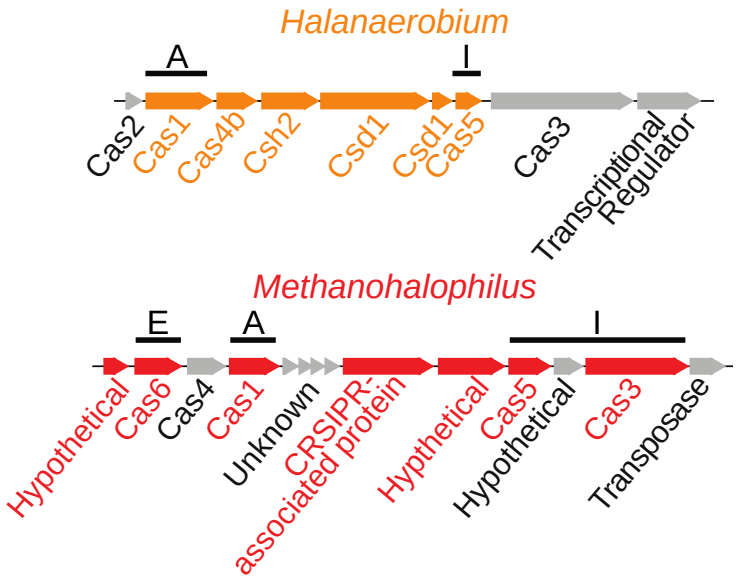
Interference: cleavage complex destroys foreign DNA

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



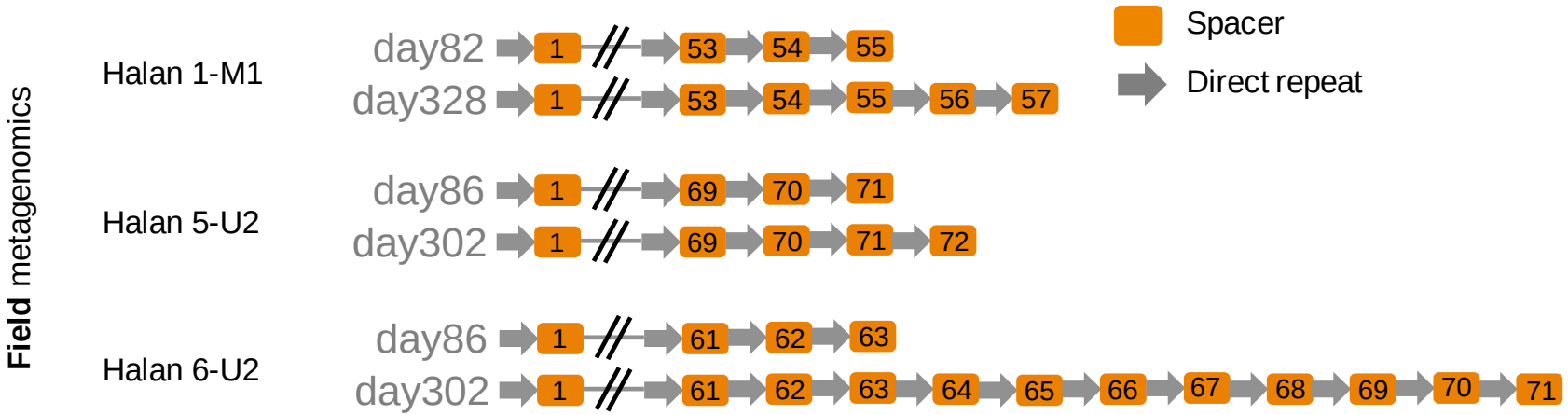
(A) Adaptation: spacer incorporation

(E) Expression: cognate RNA synthesis

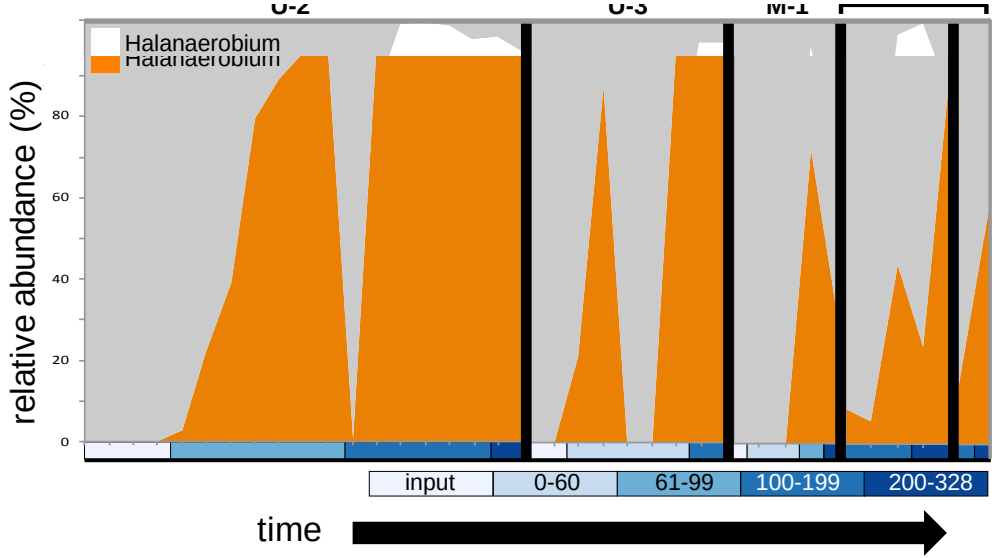
(I) Interference: cleavage-complex to destroy foreign DNA

Interpretation from Makarova et al, 2015

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



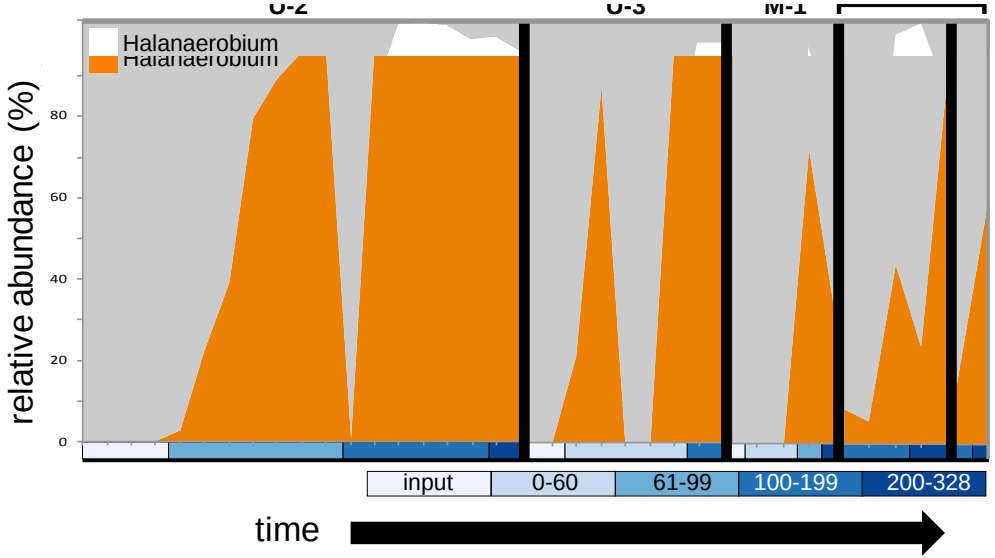
Can viral predation explain *Halanaerobium* 16S rRNA relative abundance changes?

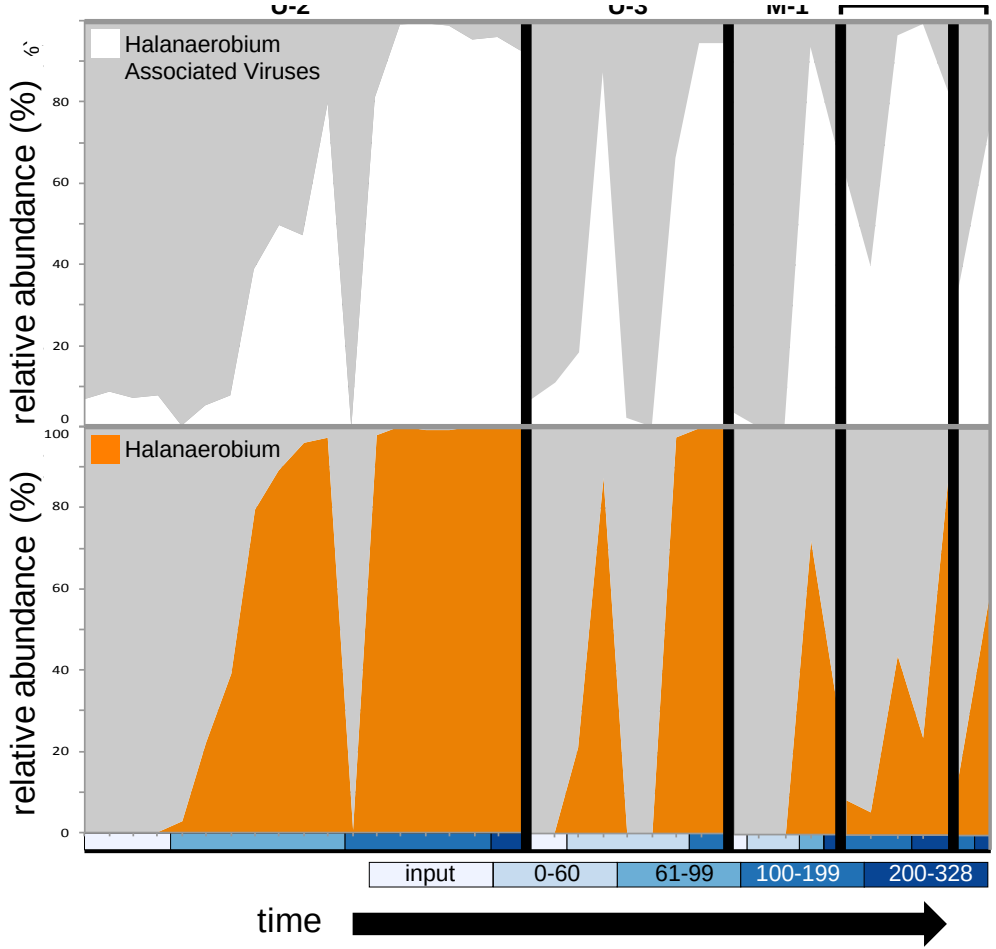


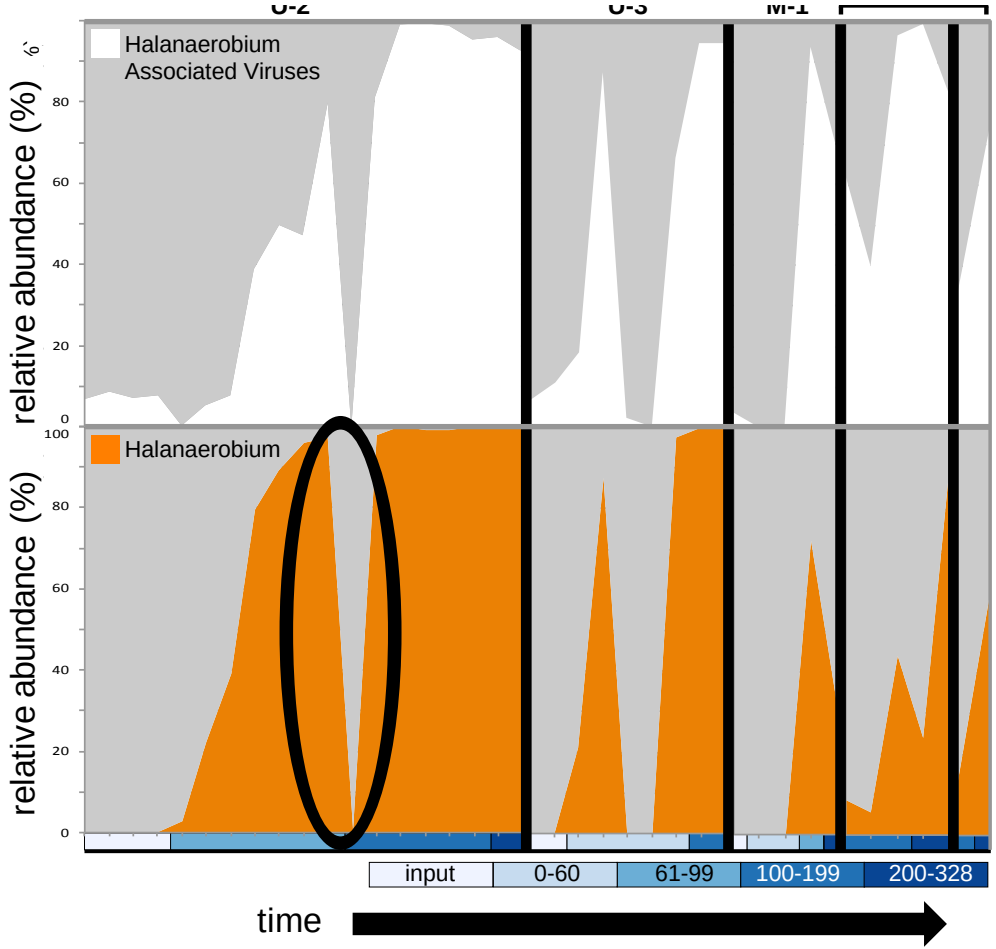
Halanaerobium viruses are prevalent in metagenomes

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

68.2% of viruses are associated with *Halanaerobium*




C-2
C-3
M-1
Halanaerobium
Associated Viruses
relative abundance (%)
0
20
40
60
80
100
Halanaerobium
relative abundance (%)
input
0-60
61-99
100-199
200-328
time


Halanaerobium
Associated Viruses
relative abundance (%)
Halanaerobium
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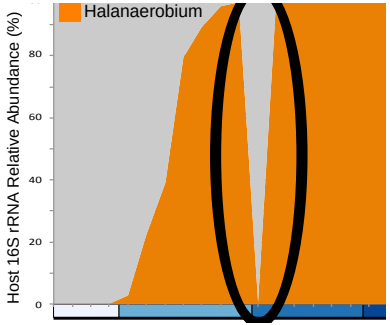
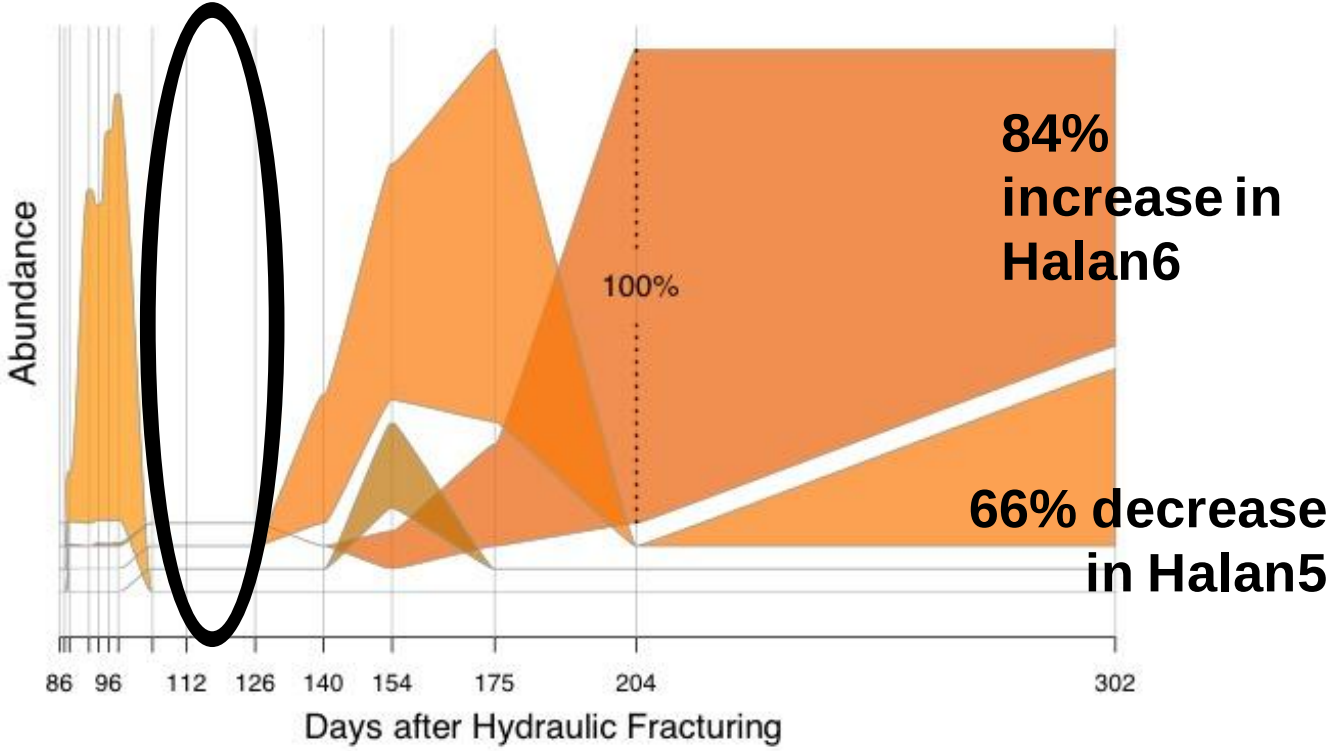
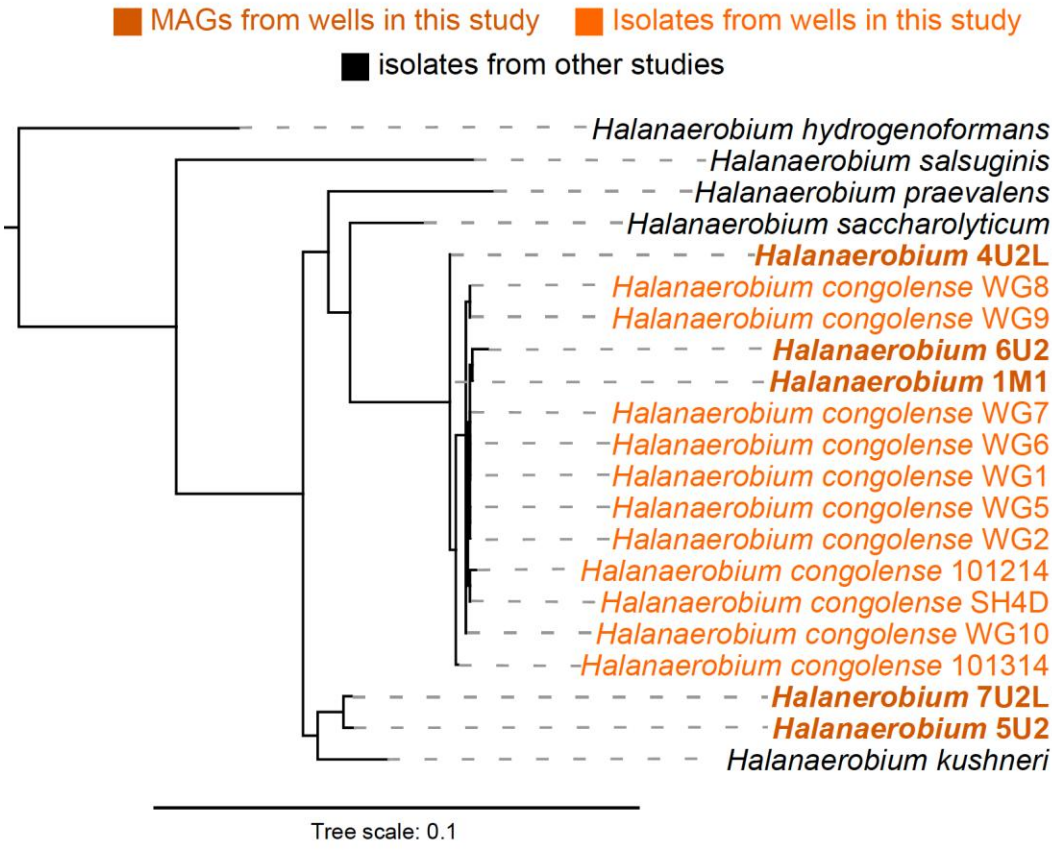
input
0-60
61-99
100-199
200-328
time

Viral predation may cause changes in *Halanerobium* strain dominance 4 most dominant strains shown

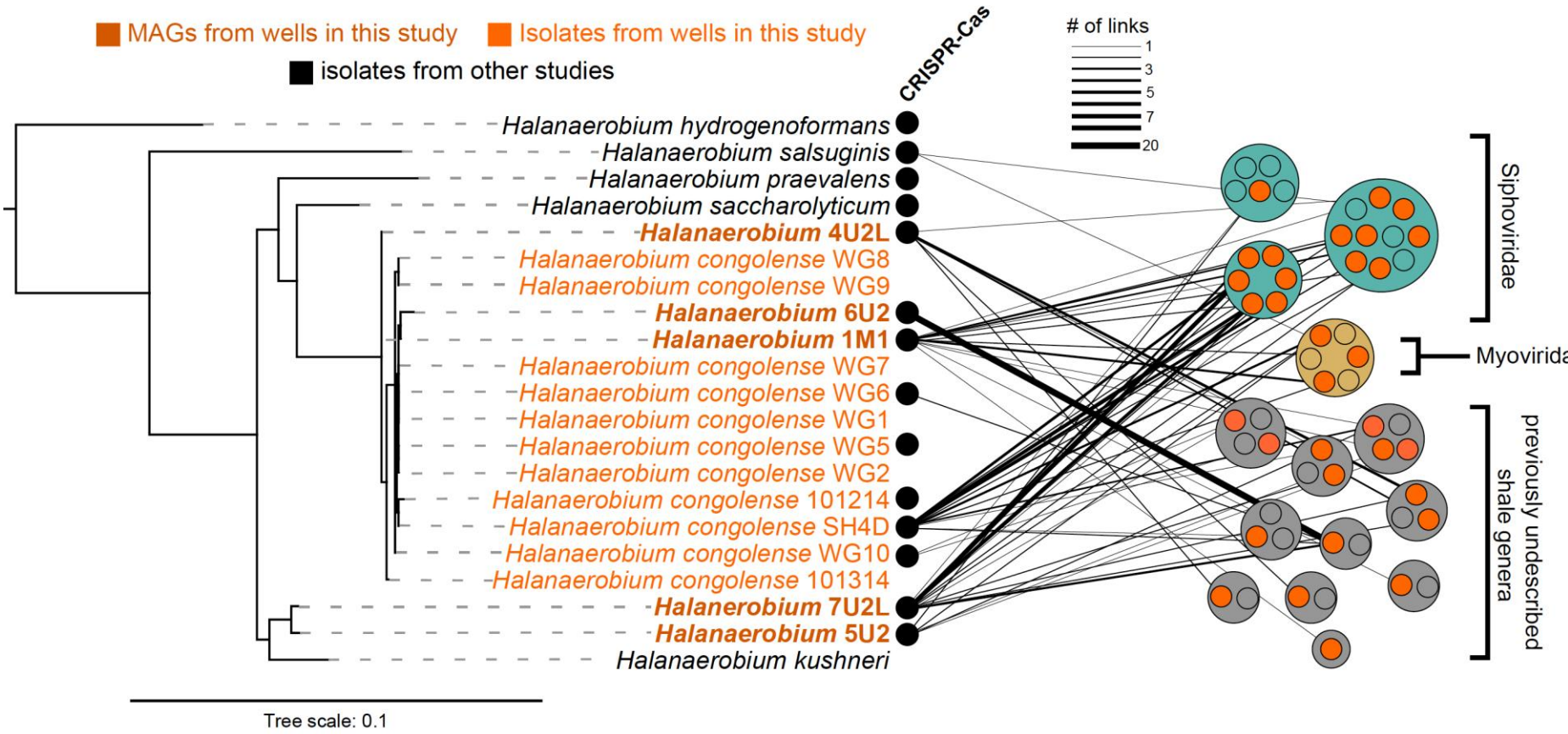




Halanaerobium strains recovered from a single well



Link Halanaerobium spacers to viral genome database host have multiple link to same virus



1 What type of microorganisms persist in this system?

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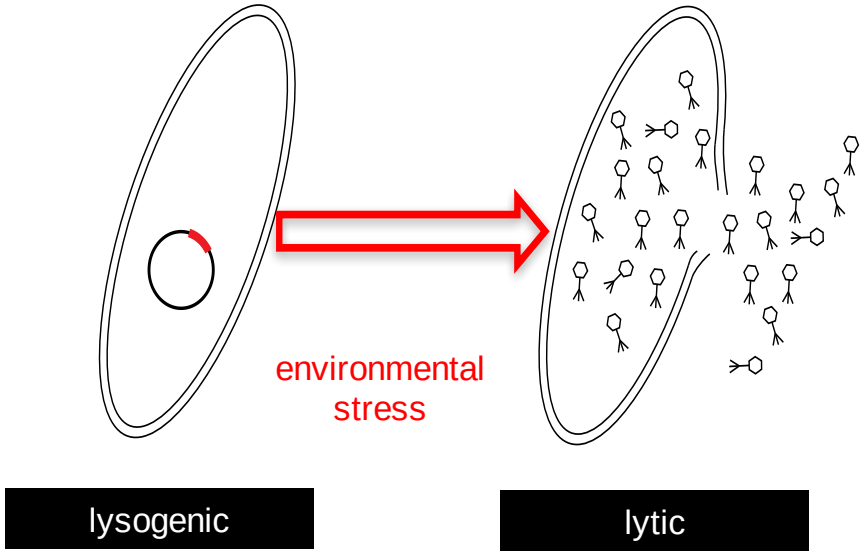
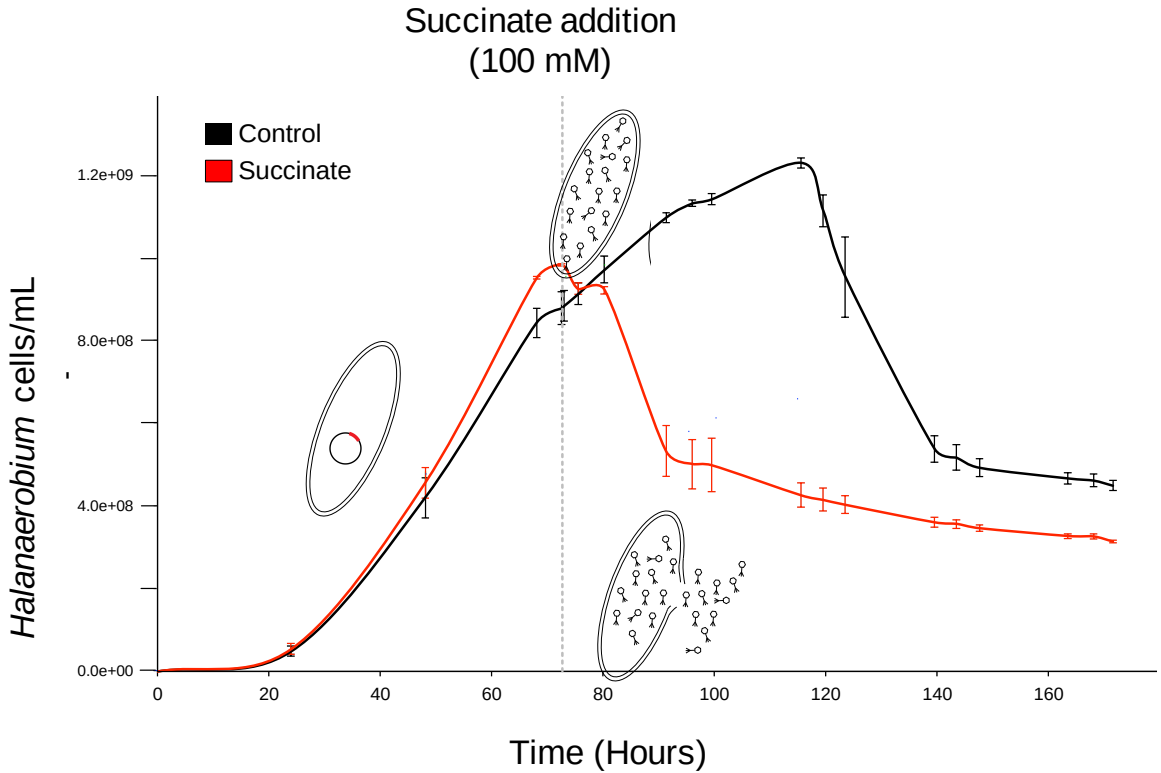
4 Viruses: Friend or foe? Roles in nutrient release

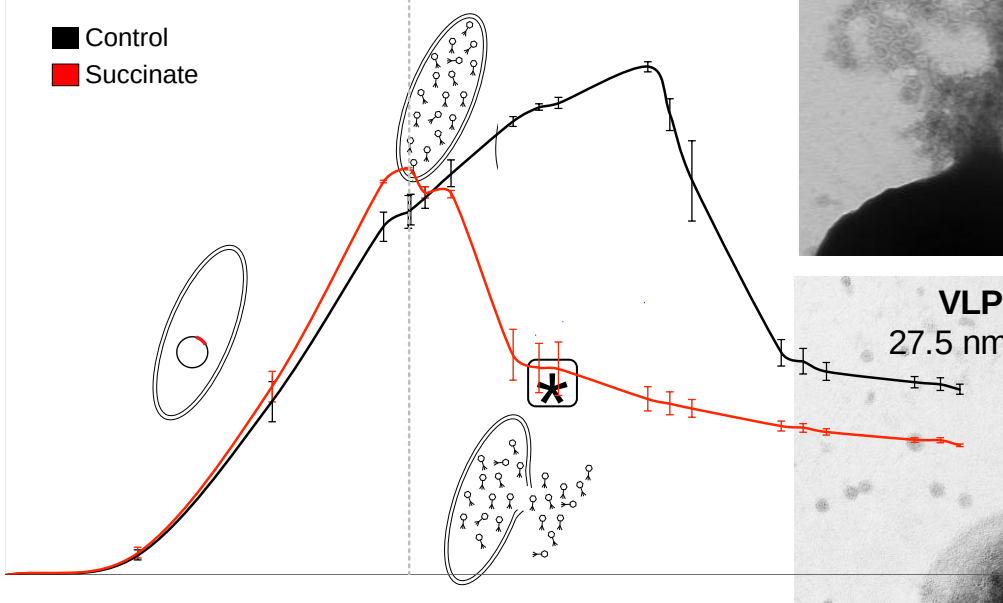
Where are the viruses in fractured shales coming from?

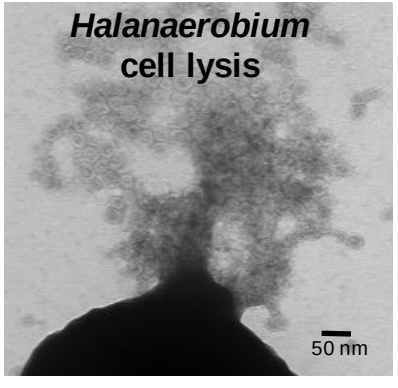


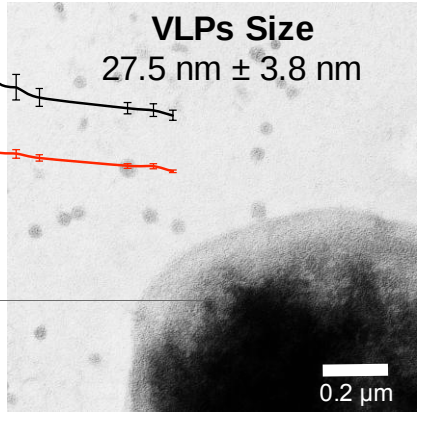
With an isolated strain of Halanaerobium that has a prophage Can we induce the virus and lyse the cell



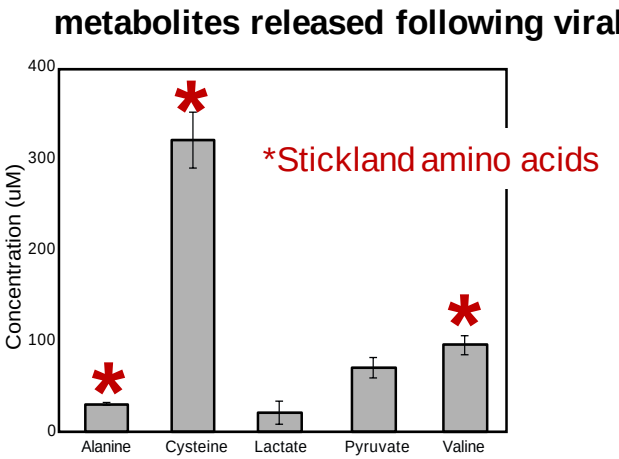
In lab induced prophage into lytic lifestyle

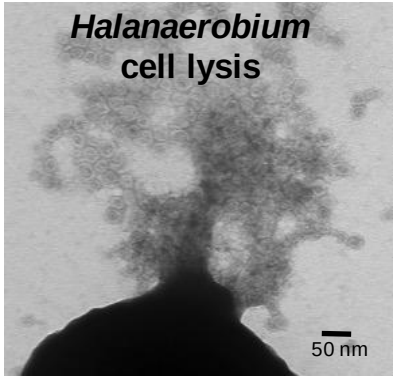


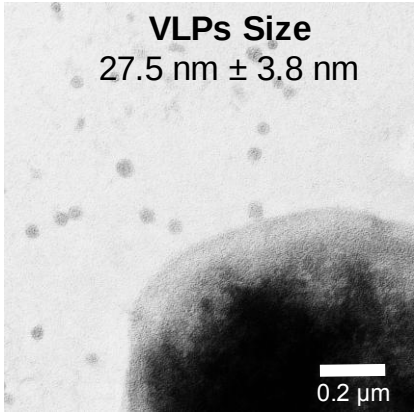




Prophage induced cell lysis causes host metabolite release

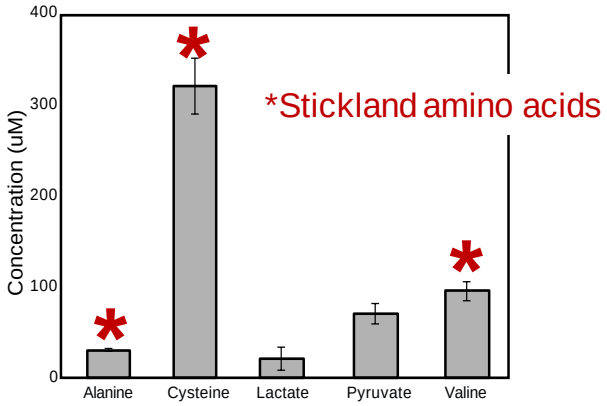


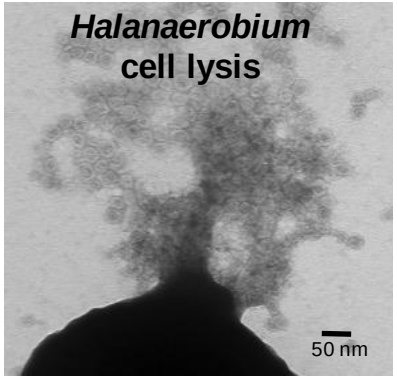


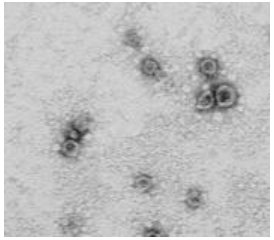


Virome and microscopy suggest tail less, novel virus

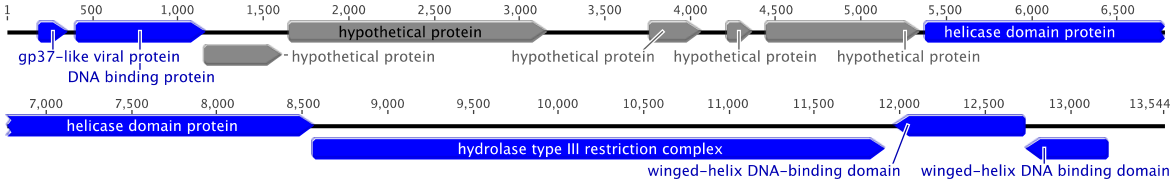
metabolites released following viral lysis

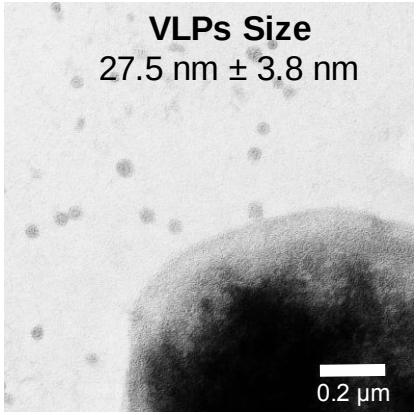






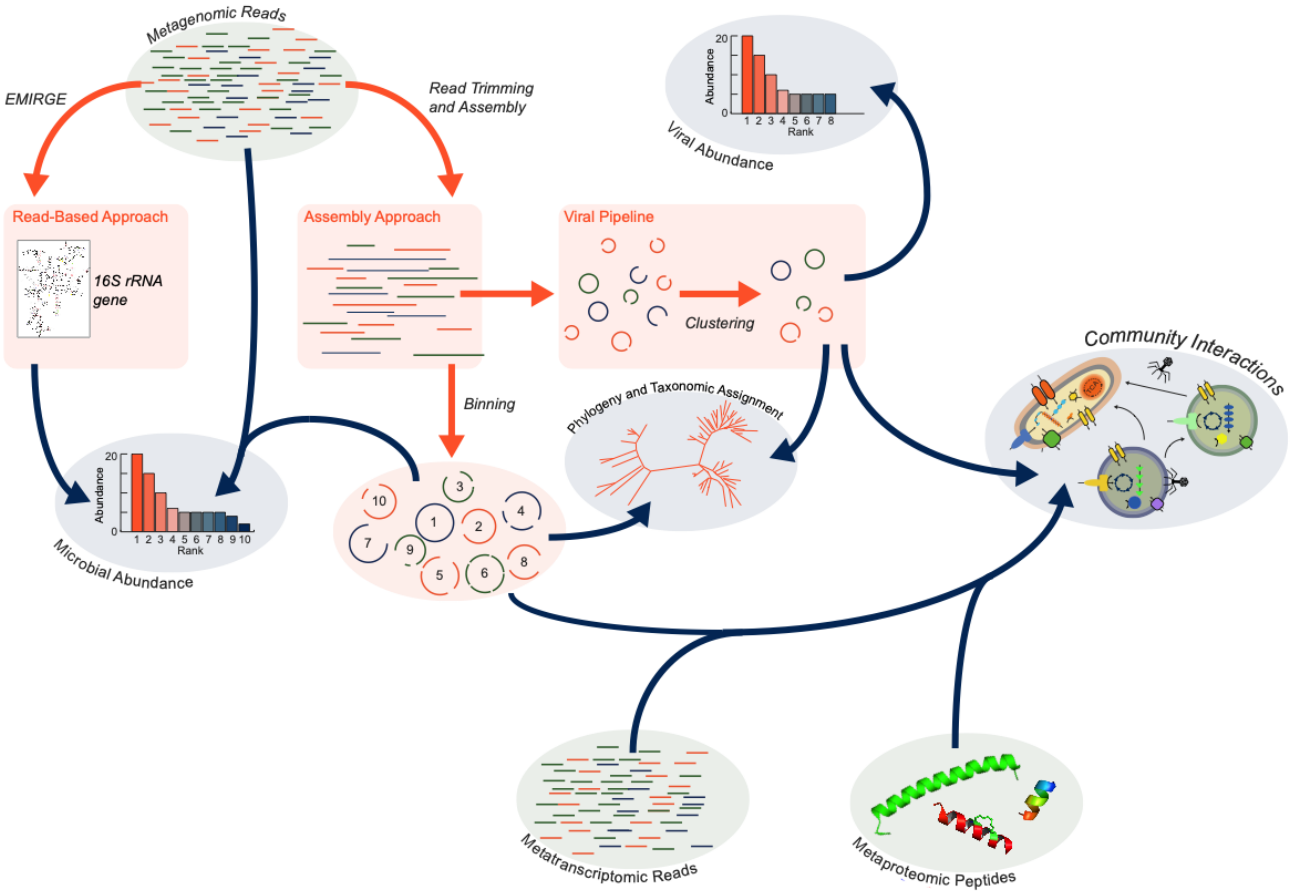
viral genomic fragment
45.8 Kbp, novel taxonomy



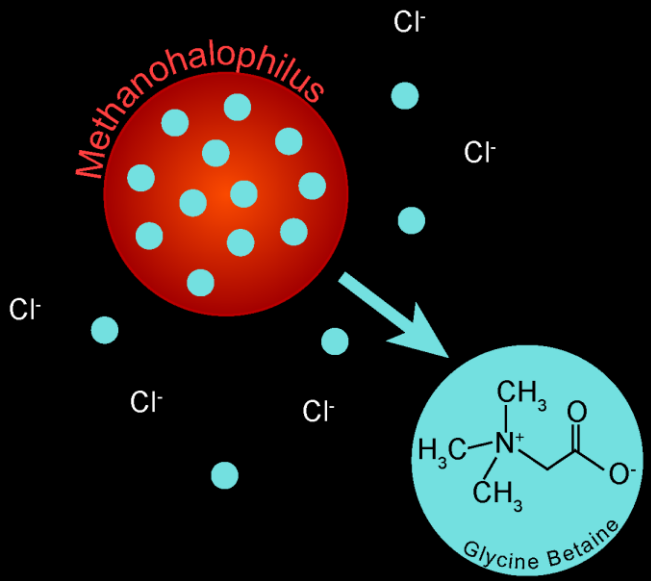


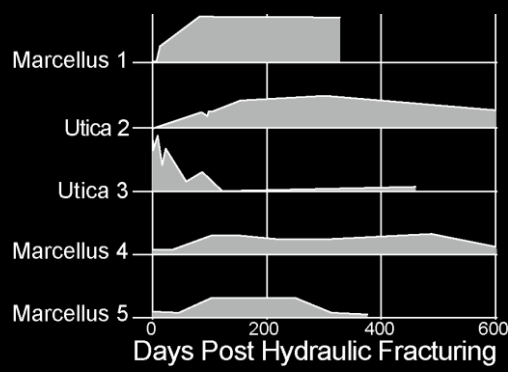
Microbes compete, cooperate, and ward off elimination

REVIEW: computational pipeline used in this case study



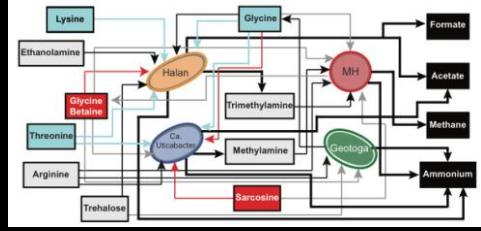
Core microbes adapt to environmental hardships

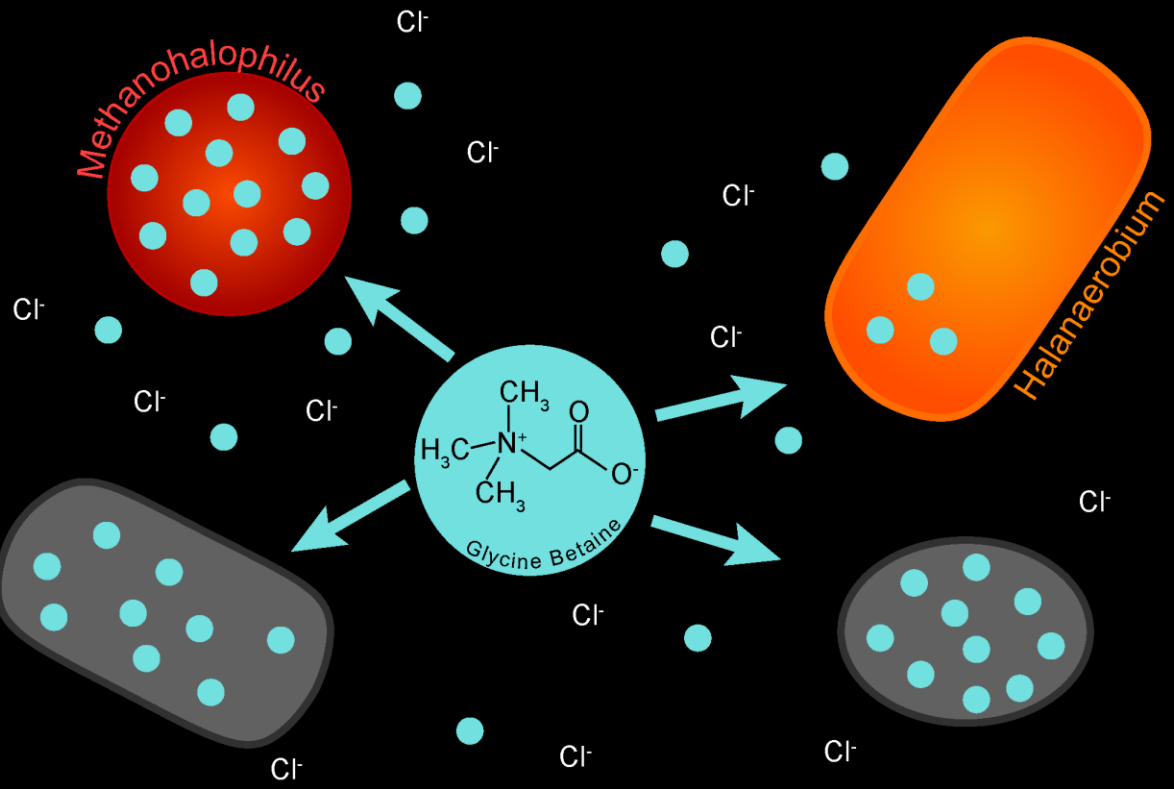




Borton et al, PNAS, 2018

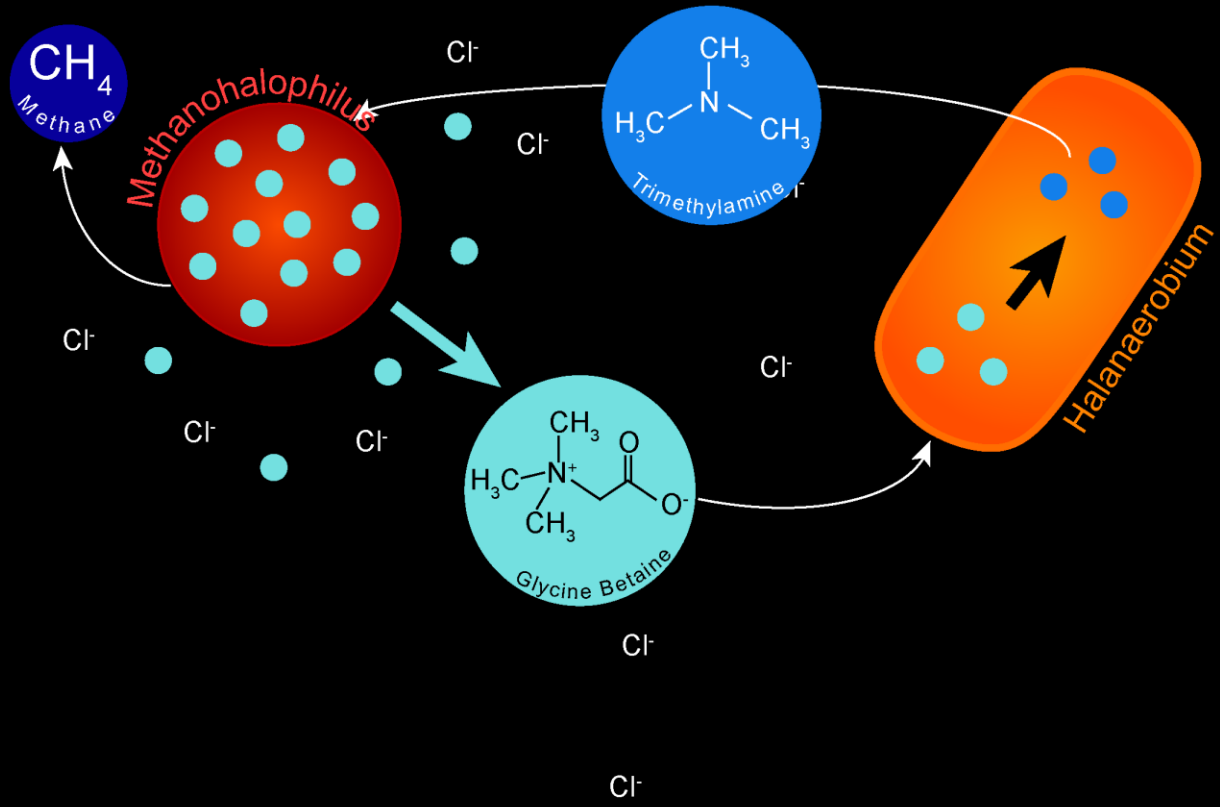
Compete for resources in the tribe

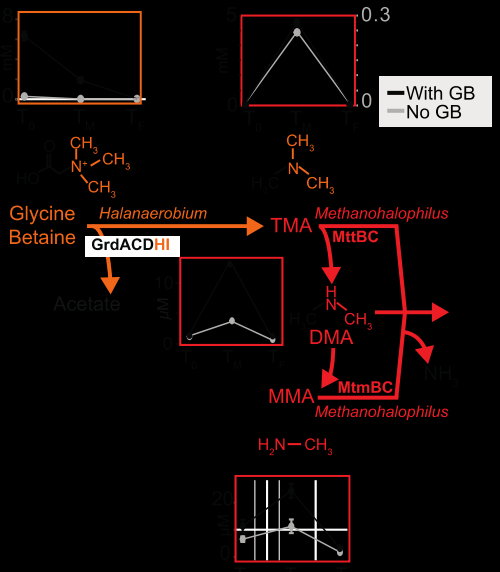




Borton et al, PNAS, 2018

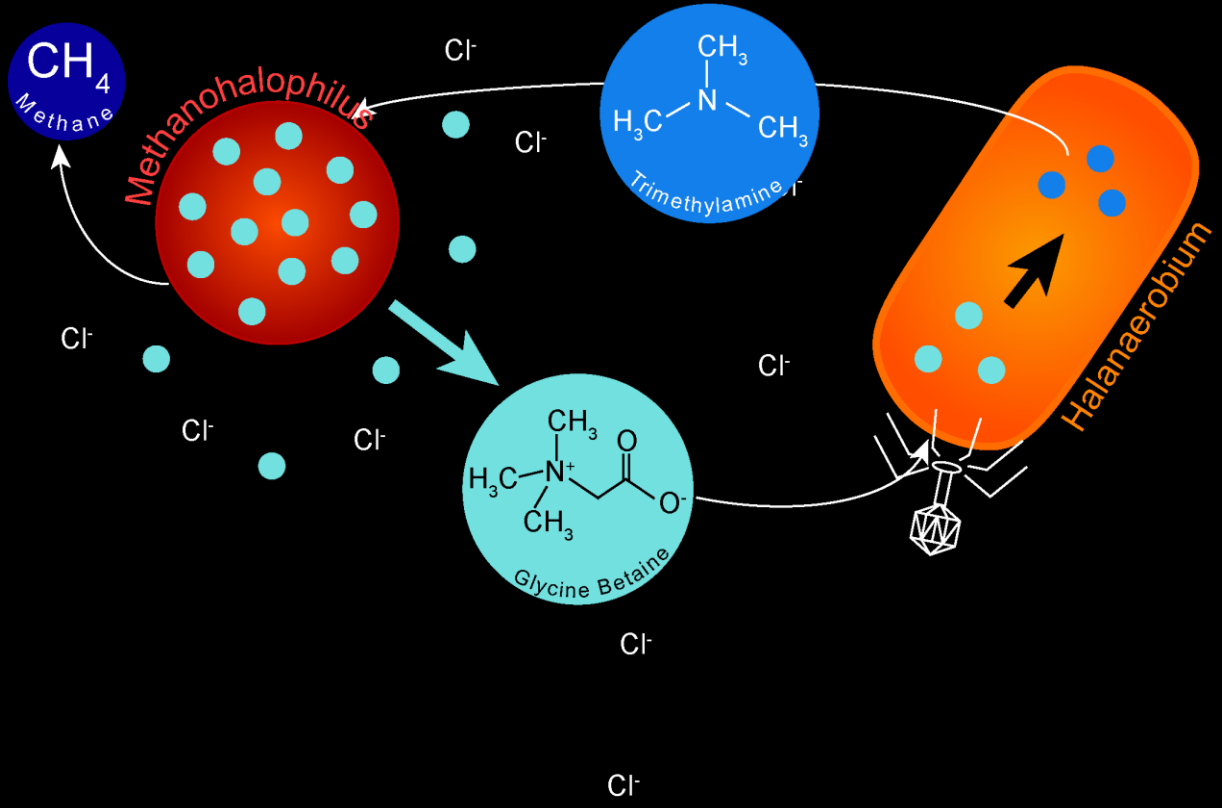
Form alliances within the tribe

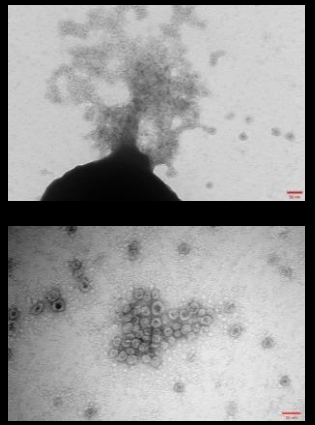




Borton et al, PNAS, 2018

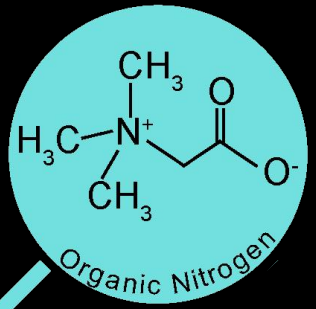
Ward off viral elimination





Daly et al, Nature Micro, 2019

Shale is a model for other ecosystems



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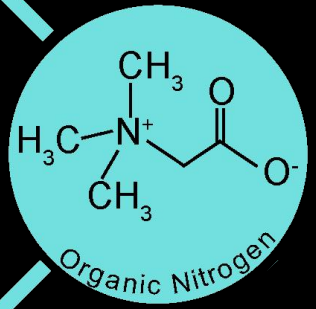

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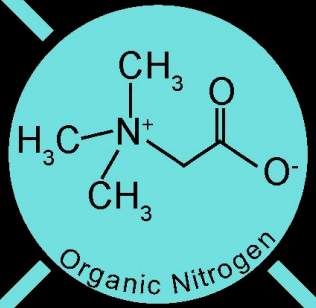

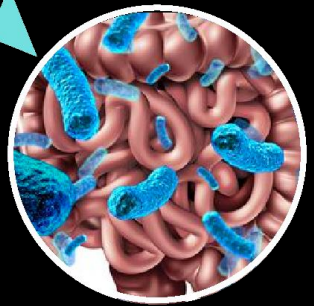

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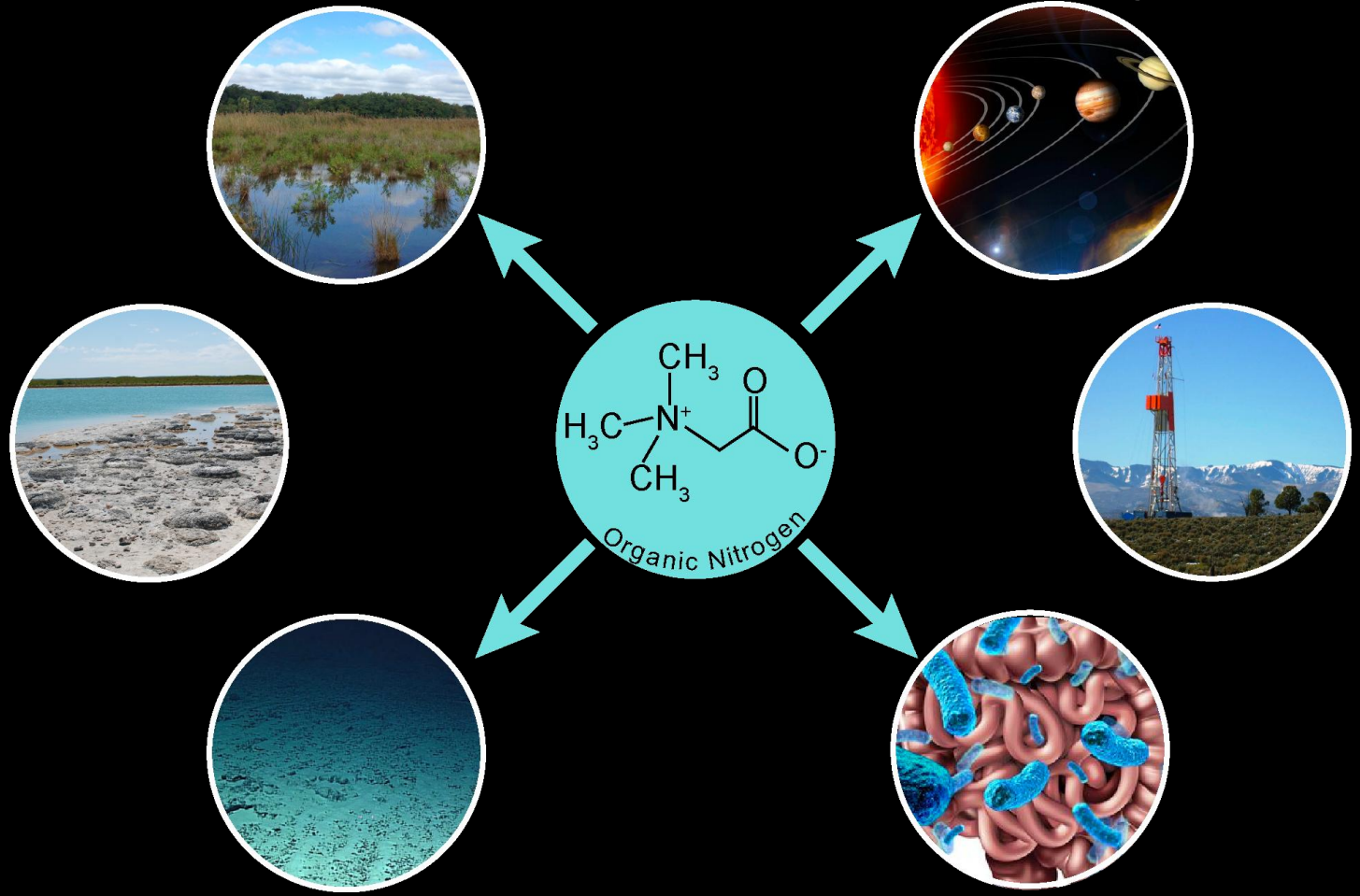
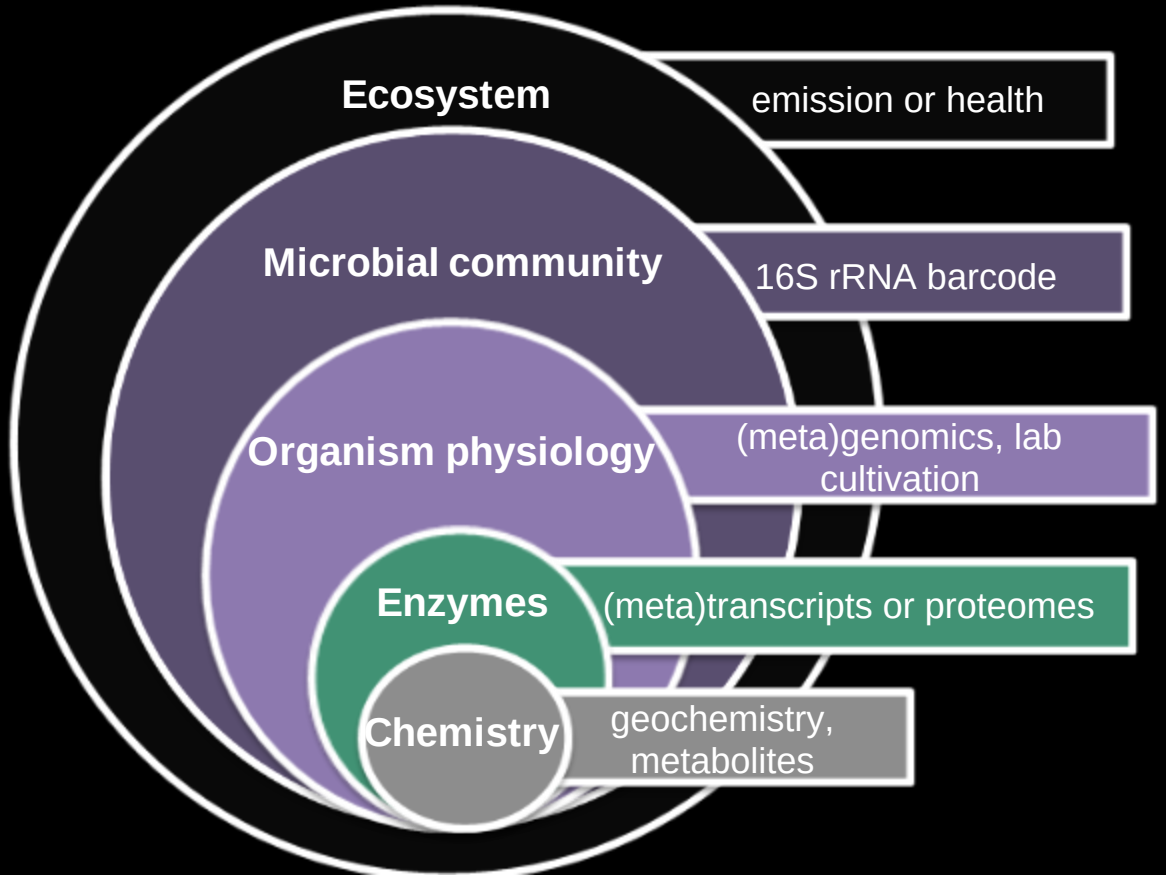


Summary- Genes to Ecosystem



I welcome your questions