Life in the Deep Subsurface

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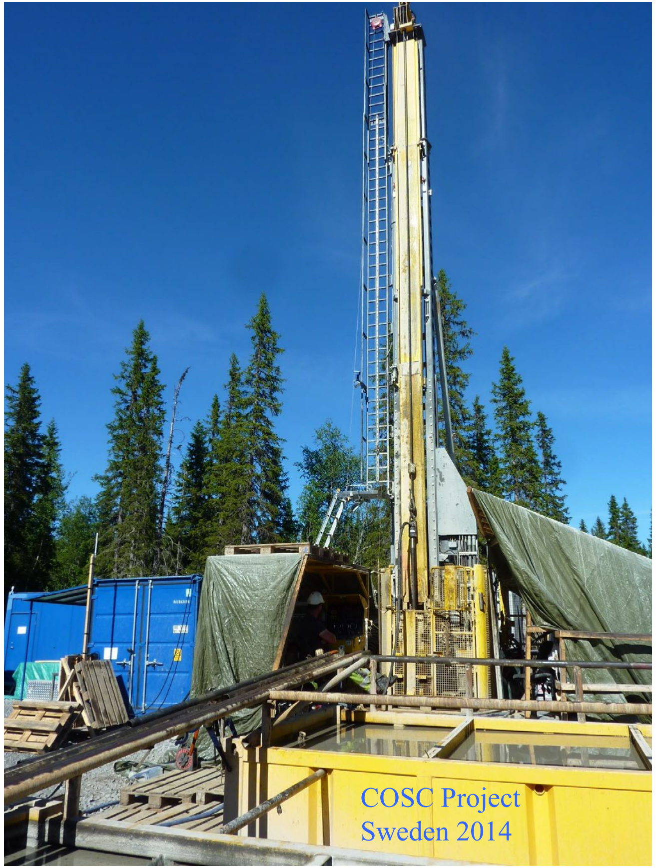
Life in the Deep Subsurface

Subsurface life is <u>microbial</u>:

- ***bacteria***
- ***archaea***
- *fungi?*
- *protozoa*
- *viruses*

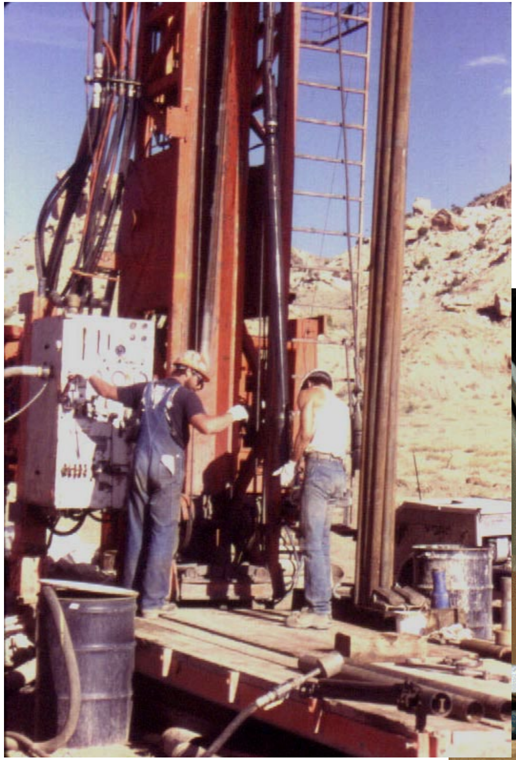
Sampling the deep biosphere

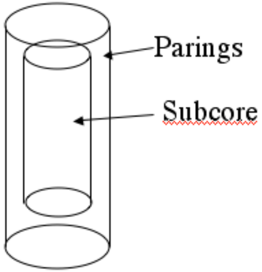


- Drilling/coring from the surface

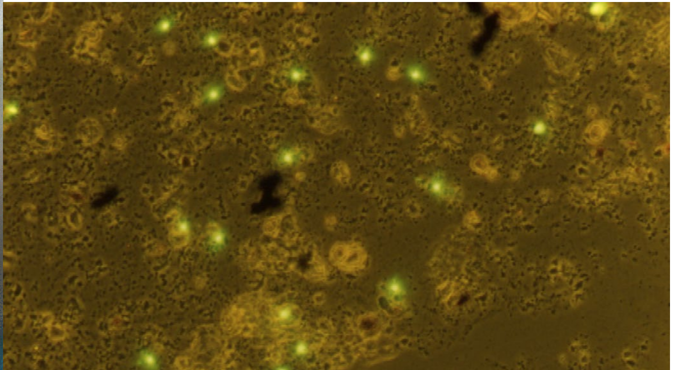
- Access via deep mines or underground labs

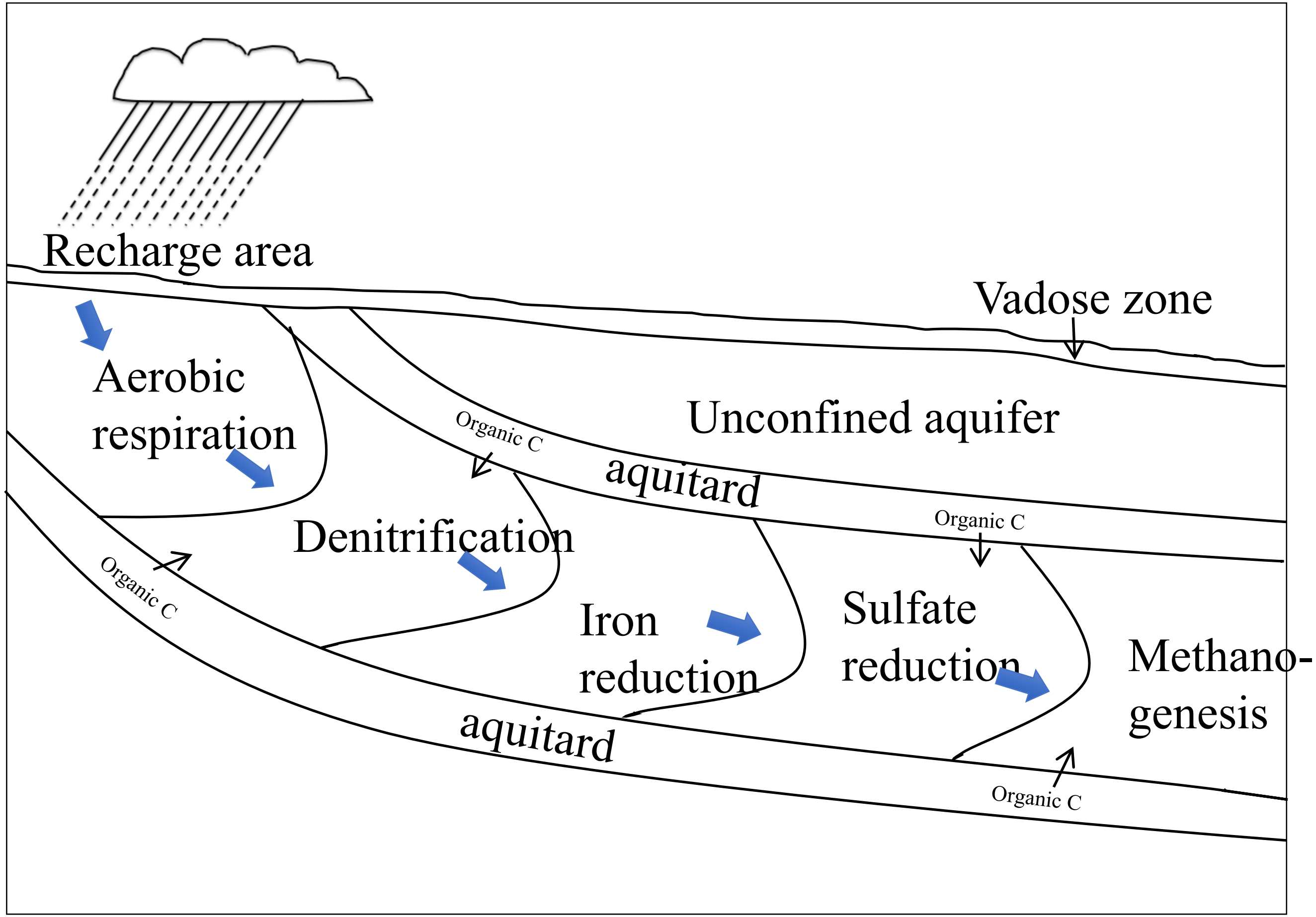
Drilling/coring needs



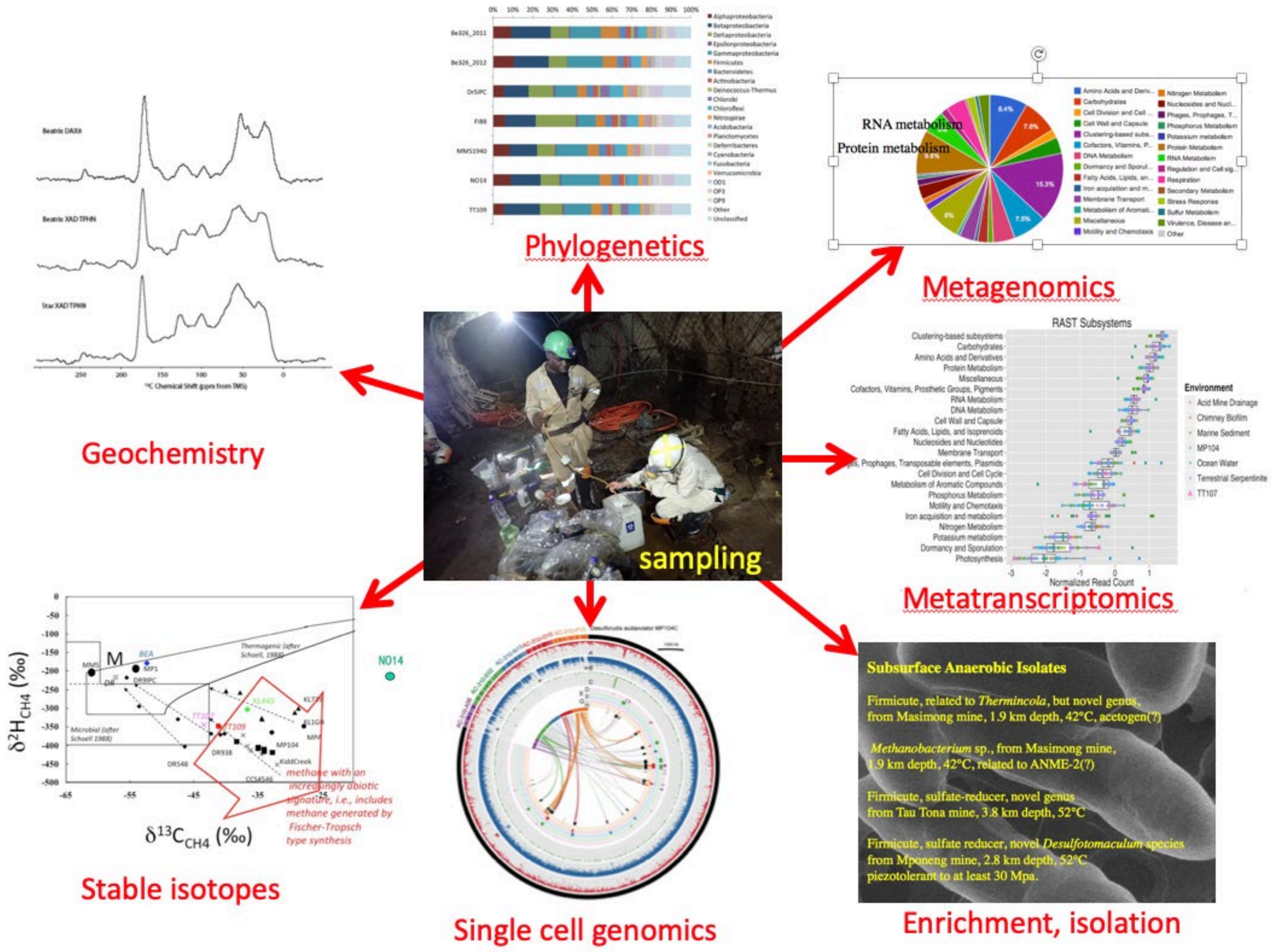


- tracers
  - Solute: Br^-, fluorochromes (e.g., rhodamine), perfluorinated hydrocarbons
  - Particulate: fluorescent carboxylated 1-µm microbeads
  - Microbial community comparisons
- core diameters $\geq$2 inches preferred
- drilling methods are highly site specific.
- anaerobic glove bag
- core barrels should be steam cleaned, core barrel liners





Terminal electron-accepting processes along a flow-path, e.g., Atlantic coastal plain aquifers.
After Smith and Harris (2007)

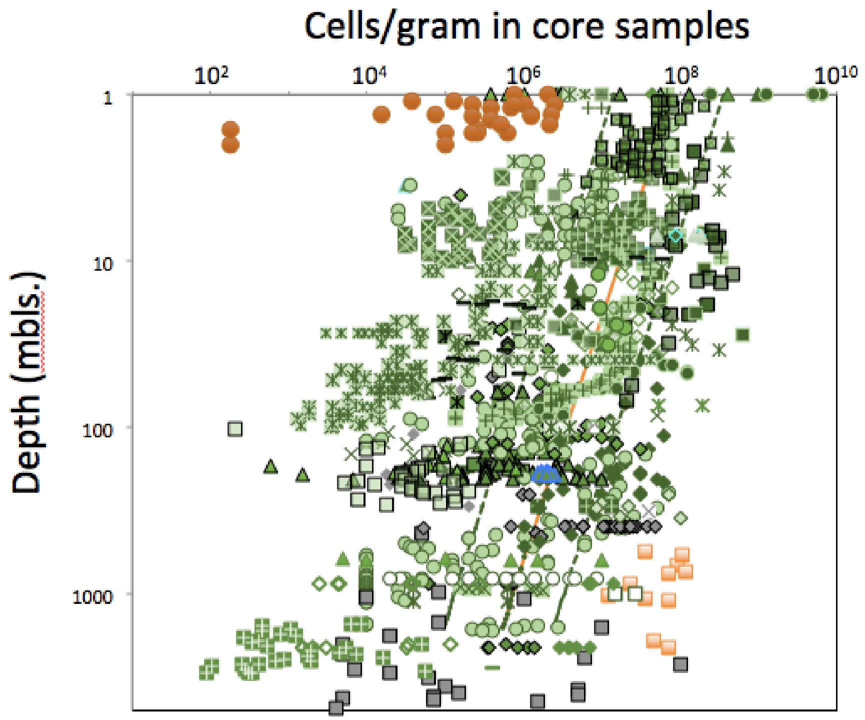


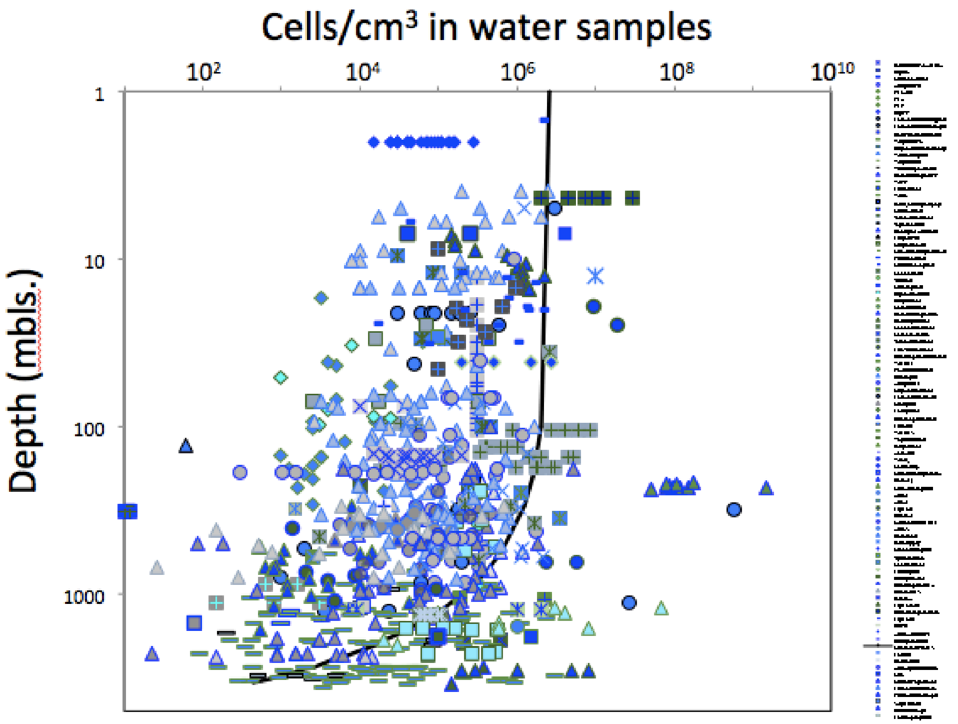
Geochemistry
Phylogenetics
Metagenomics
RNA metabolism
Protein metabolism
RAST Subsystems
Environment
Acid Mine Drainage
Chimney Biofilm
Marine Sediment
MP104
Ocean Water
Terrestrial Serpentinite
TT107
Normalized Read Count
Metatranscriptomics
sampling
Stable isotopes
Single cell genomics
Subsurface Anaerobic Isolates
Firmicute, related to Thermincola, but novel genus, from Masimong mine, 1.9 km depth, 42°C, acetogen(?)
Methanobacterium sp., from Masimong mine, 1.9 km depth, 42°C, related to ANME-2(?)
Firmicute, sulfate-reducer, novel genus from Tau Tona mine, 3.8 km depth, 52°C
Firmicute, sulfate reducer, novel Desulfotomaculum species from Mponeng mine, 2.8 km depth, 52°C piezotolerant to at least 30 Mpa.
Enrichment, isolation

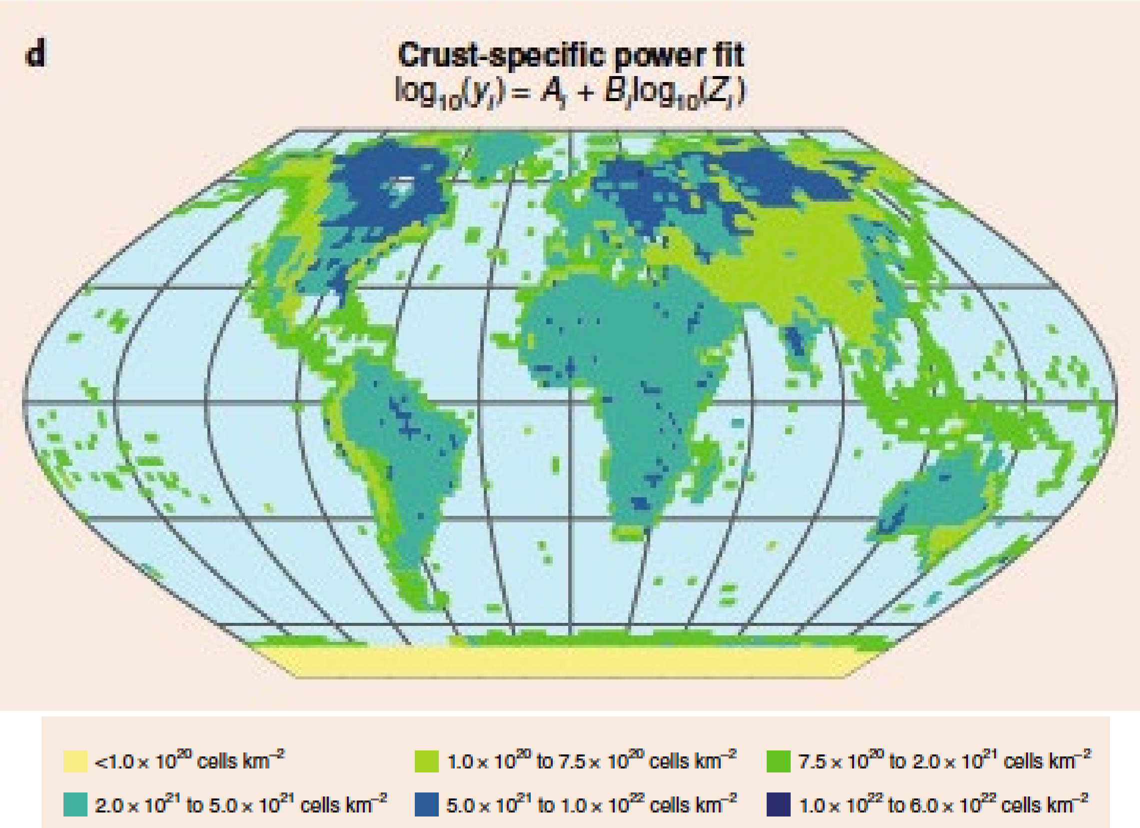
Big Objectives/Questions

- Global characterization of subsurface microbial abundance, diversity, and activities.
- What adaptations enable persistence for geologic time periods under:
  - Low nutrient flux, high temperature, extreme pH, high pressure, etc.
- How do subsurface microbes maintain/repair macromolecules?
- Do subsurface microbes represent early life on earth?
  - Deep branching phylogenies
  - Primitive metabolism, e.g. H_2 utilization

Onstott database of worldwide subsurface microbial abundance







Magnabosco et al. 2018. *Nature Geoscience*

Table 2 | Biomass estimates derived from this study

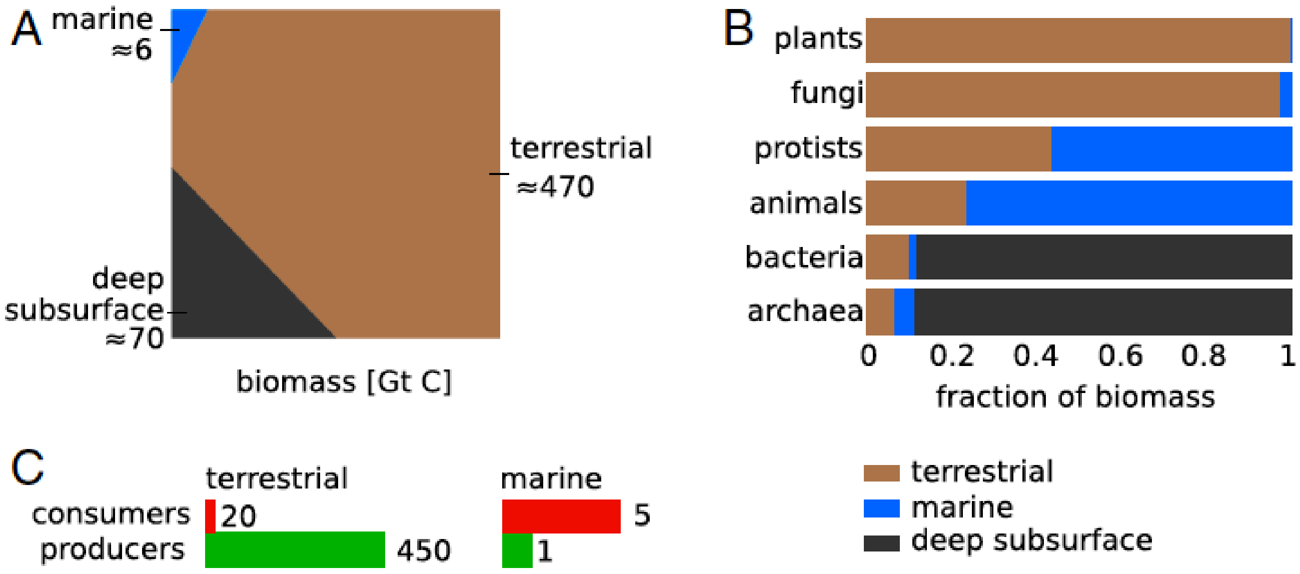
Model	10^{29} cells (±95% CI)		Mean squared error[a]
	85 °C isotherm	122 °C isotherm	
Depth power fit	2.0(+0.3/−0.4)	2.3(+0.3/−0.3)	1.33
Temperature fit	10.0(+1.6/−1.2)	10.2(+1.0/−1.3)	1.13
GLM (temperature and depth)	2.6(+0.2/−0.4)	3.7(+0.6/−0.8)	1.29
Crust-specific depth power fit	5.2(+1.0/−1.1)	5.5(+1.0/−1.1)	0.27

These estimates are based on the integration of each model to the designated isotherm. The parameters identified for each fit can be found in Supplementary Table 7. [a]Mean squared error = $\frac{1}{n}\sum_{i=1}^{n}(Y_i-\hat{Y}_i)^2$ where n is the number of data points in the test set, Y_i is the $\log_{10}$ concentration of cells per cm^3 of data point i in the test set, and $\hat{Y}_i$ is the $\log_{10}$ concentration of cells per cm^3 prediction of data point i.

Magnabosco et al. 2018. Nature Geoscience

~60 Gt C in deep continental subsurface, ~10% of Earth's biomass

~2 x 10^{30} prokaryotes



Bar-on et al. 2018 PNAS

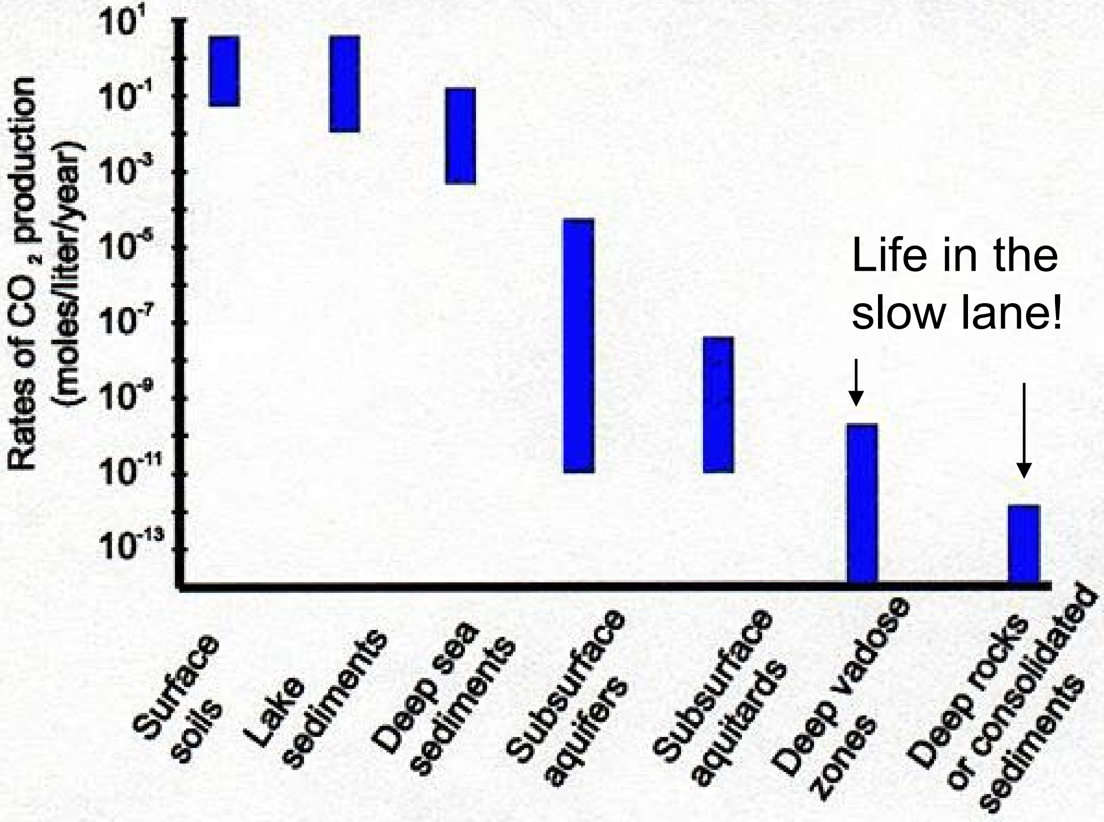
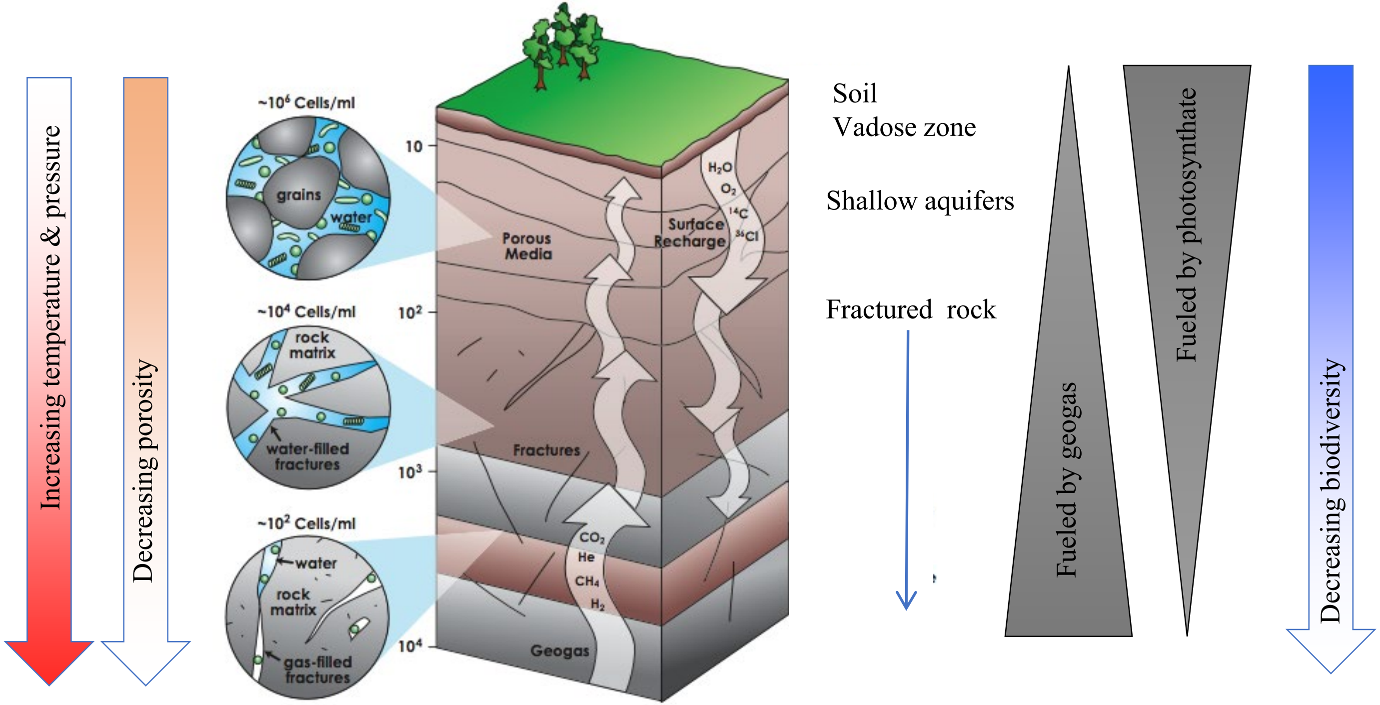
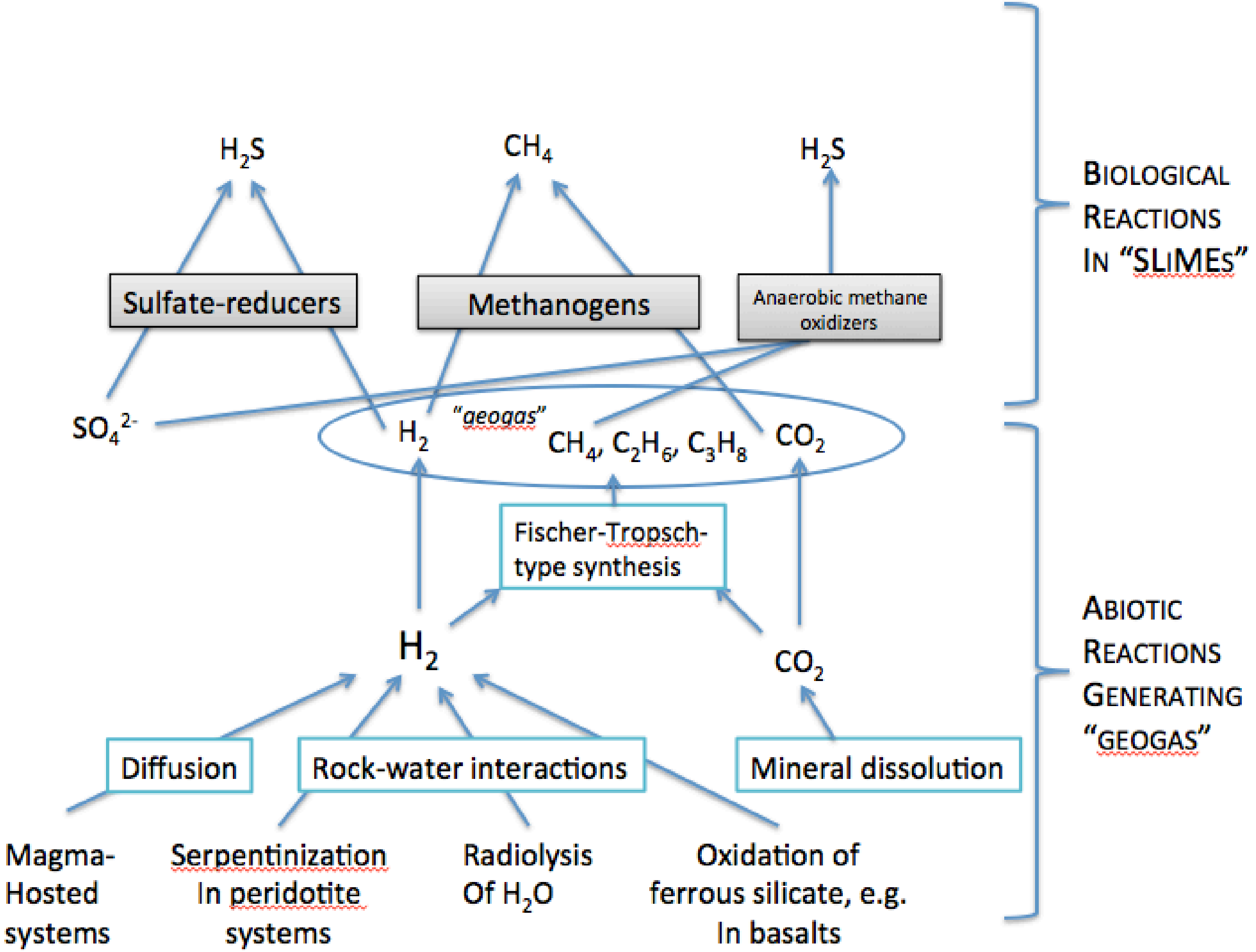


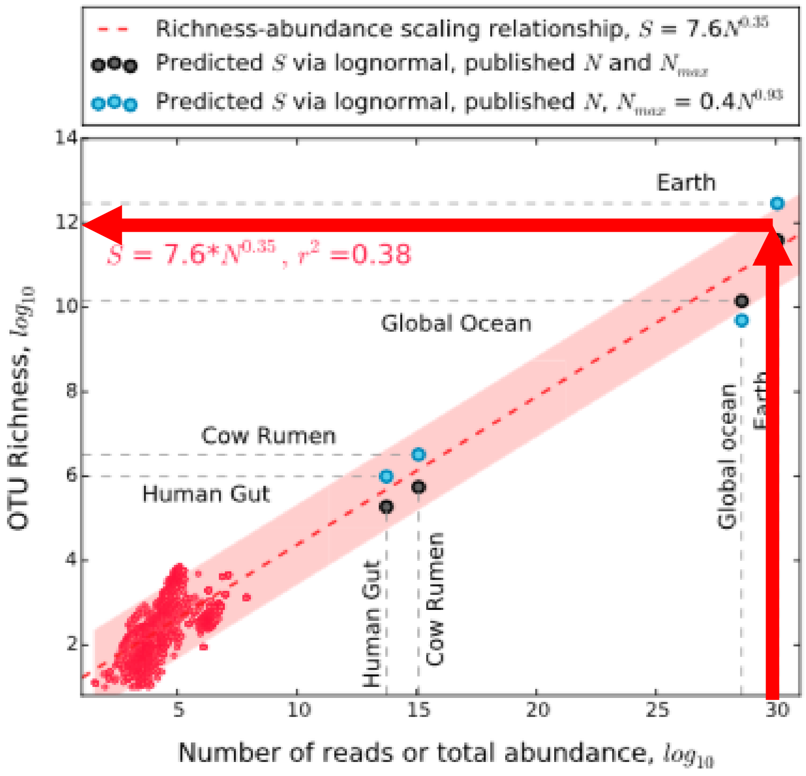
FIGURE 4.22 Ranges of rates of *in situ* CO_2 production for various surface and subsurface environments, as estimated by groundwater chemical analyses and geochemical modeling. (Adapted from Kieft and Phelps, 1997.)



Kieft 2016. Microbiology of the deep continental biosphere



Kieft 2016. Microbiology of the deep continental biosphere

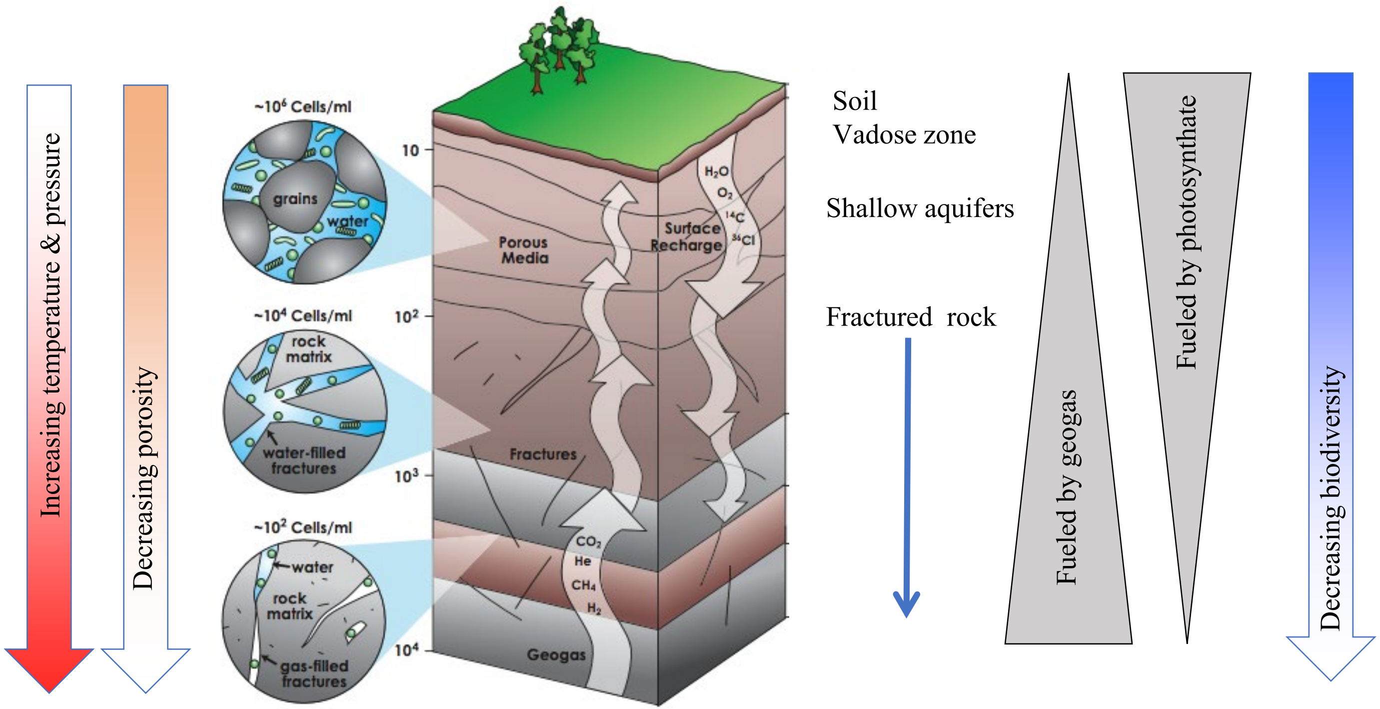


~10^{12} microbial species
in the continental subsurface

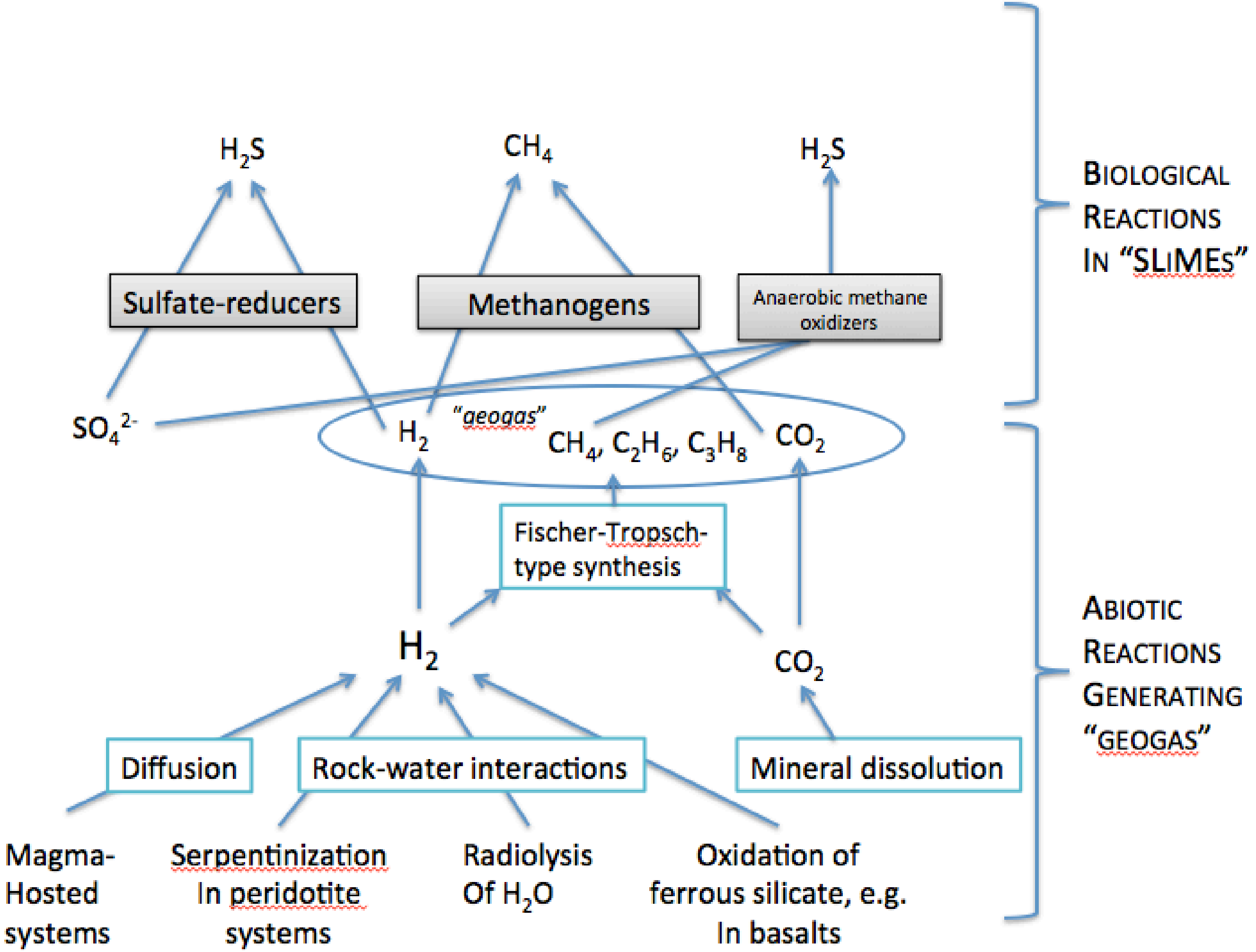
~1/3 of all Earth species!

Locey and Lennon, 2016. *PNAS*

Magnabosco et al., 2018, *Nature Geoscience*



Kieft 2016. Microbiology of the deep continental biosphere



Kieft 2016. Microbiology of the deep continental biosphere

More Big Objectives/Questions

- Relationship(s) between geophysics and microbes?
  - Do earthquakes stimulate microbial activities?
  - Do microbial activities increase the likelihood of seismic events.
  - Do microbial activities lead to fracture formation?
- What's the importance of viruses?
- Determine the involvements of transport, long-term survival, energy flux, and evolution in the distribution of microbes in the deep subsurface
  - As a specific example, how can nearly identical strains of *Desulforudis audaxviator* be distributed at sites thousands of km apart in deep, ancient groundwater?

The ISME Journal (2021) 15:2830–2842
https://doi.org/10.1038/s41396-021-00965-3

ARTICLE

Evolutionary stasis of a deep subsurface microbial lineage

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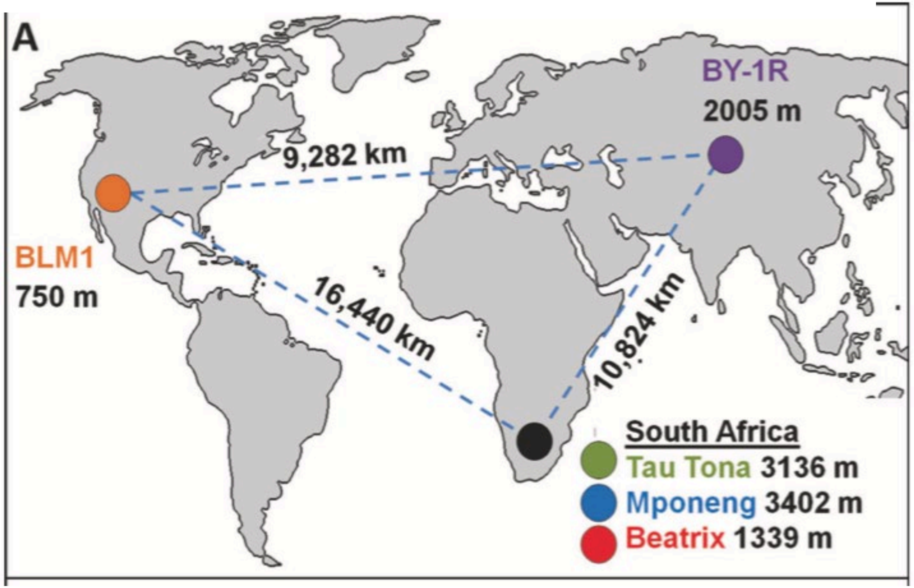
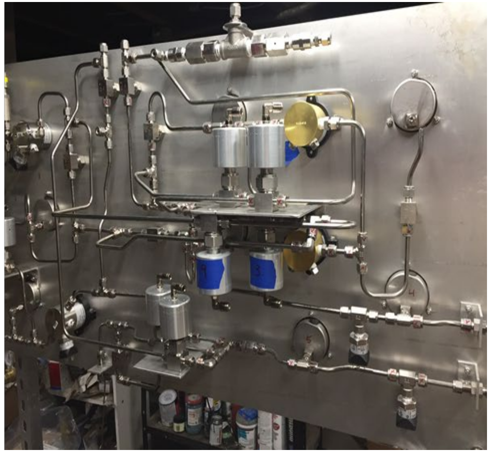
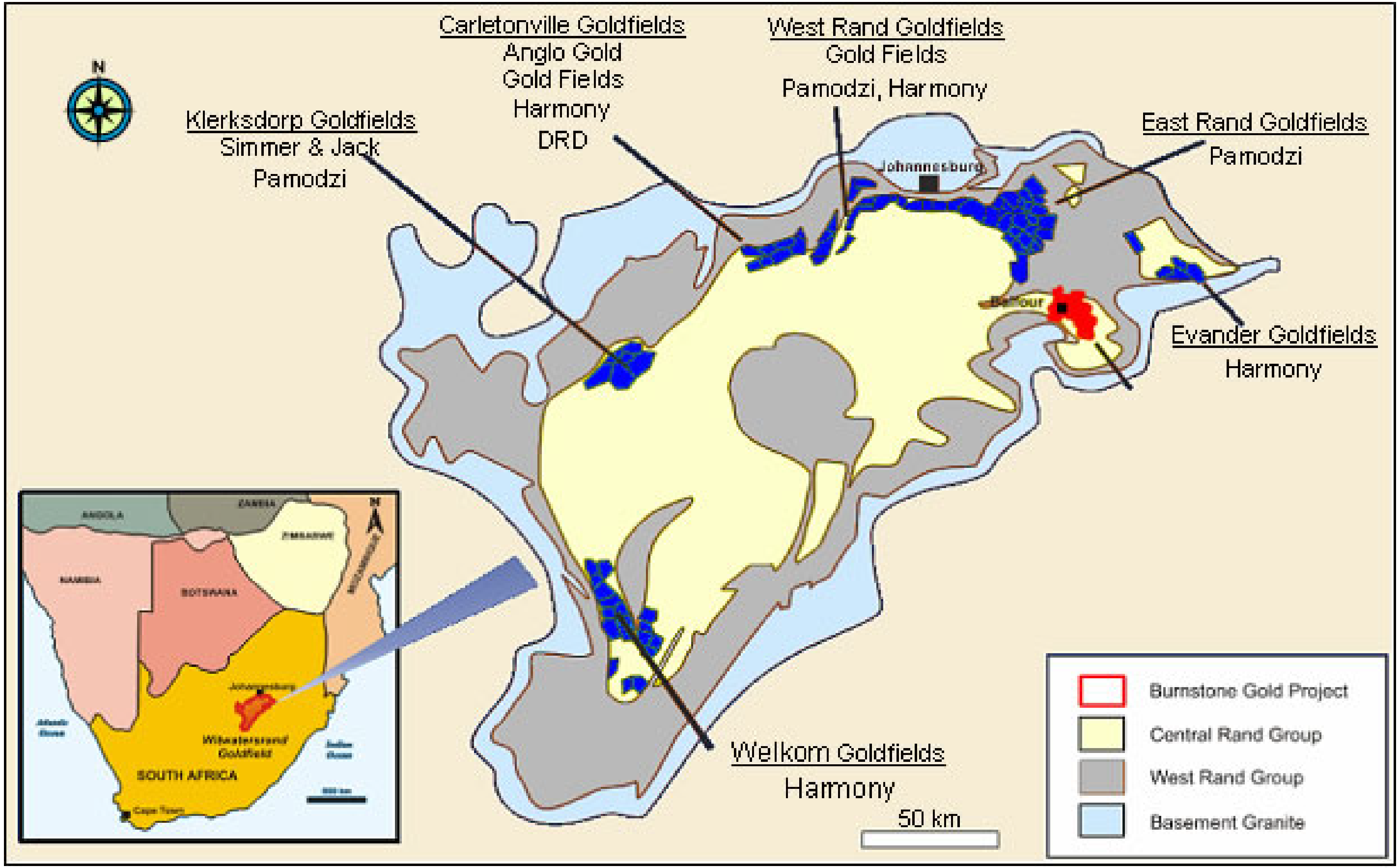


Hypothesis: Earthquakes stimulate microbial activity
Test with KASMS (Kinetically Activated Subsurface Microbial Sampler)

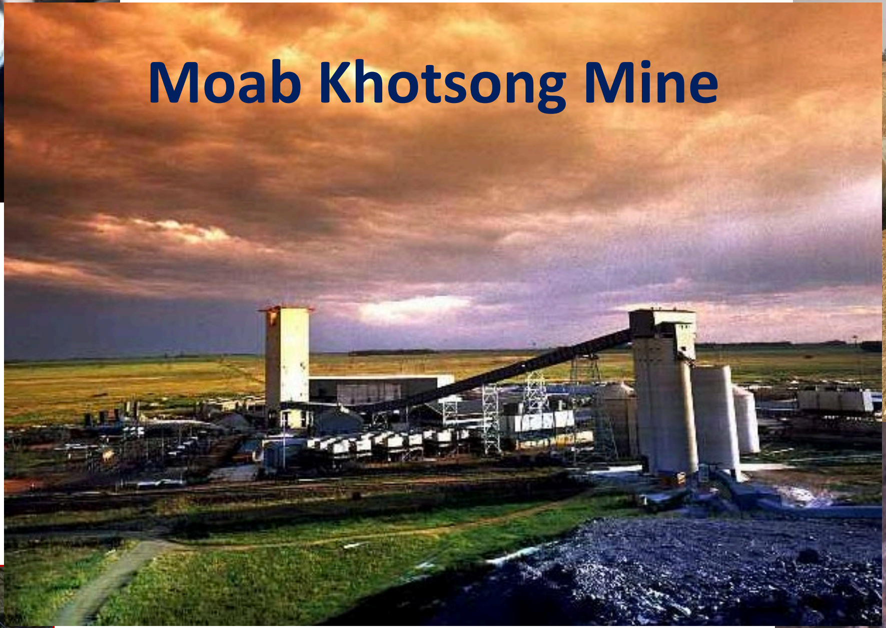
- KASMS is autonomous, triggered by seismic activity to collect formation and preserve geofluids for microbial analysis.
- Electronic control module, U-tube sampler, sample collection units, preservative pump, solenoid valve control systems.

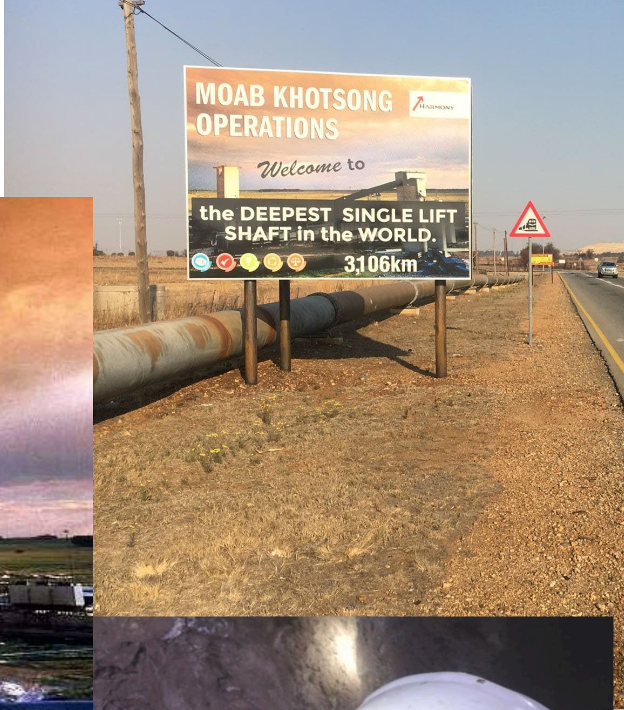


Witwatersrand Deep Microbiology Project

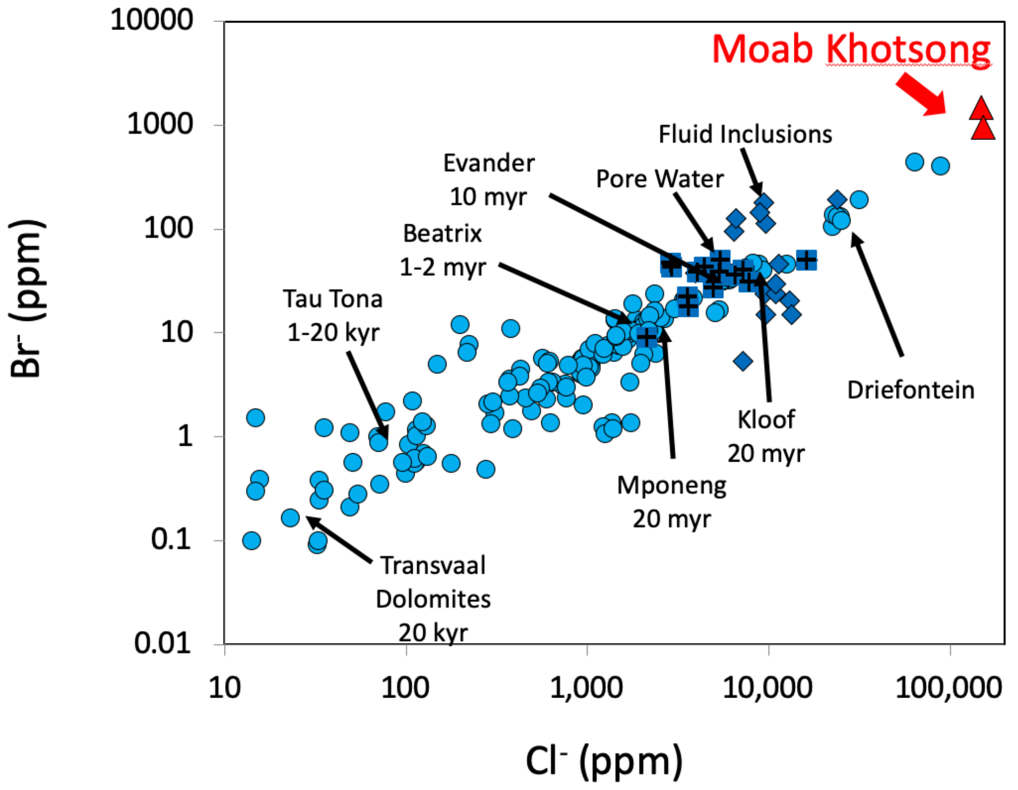


Moab Khotsong Mine





Deep, ancient (1.2 Ga) brine: ~24% Na-Ca-Cl_2

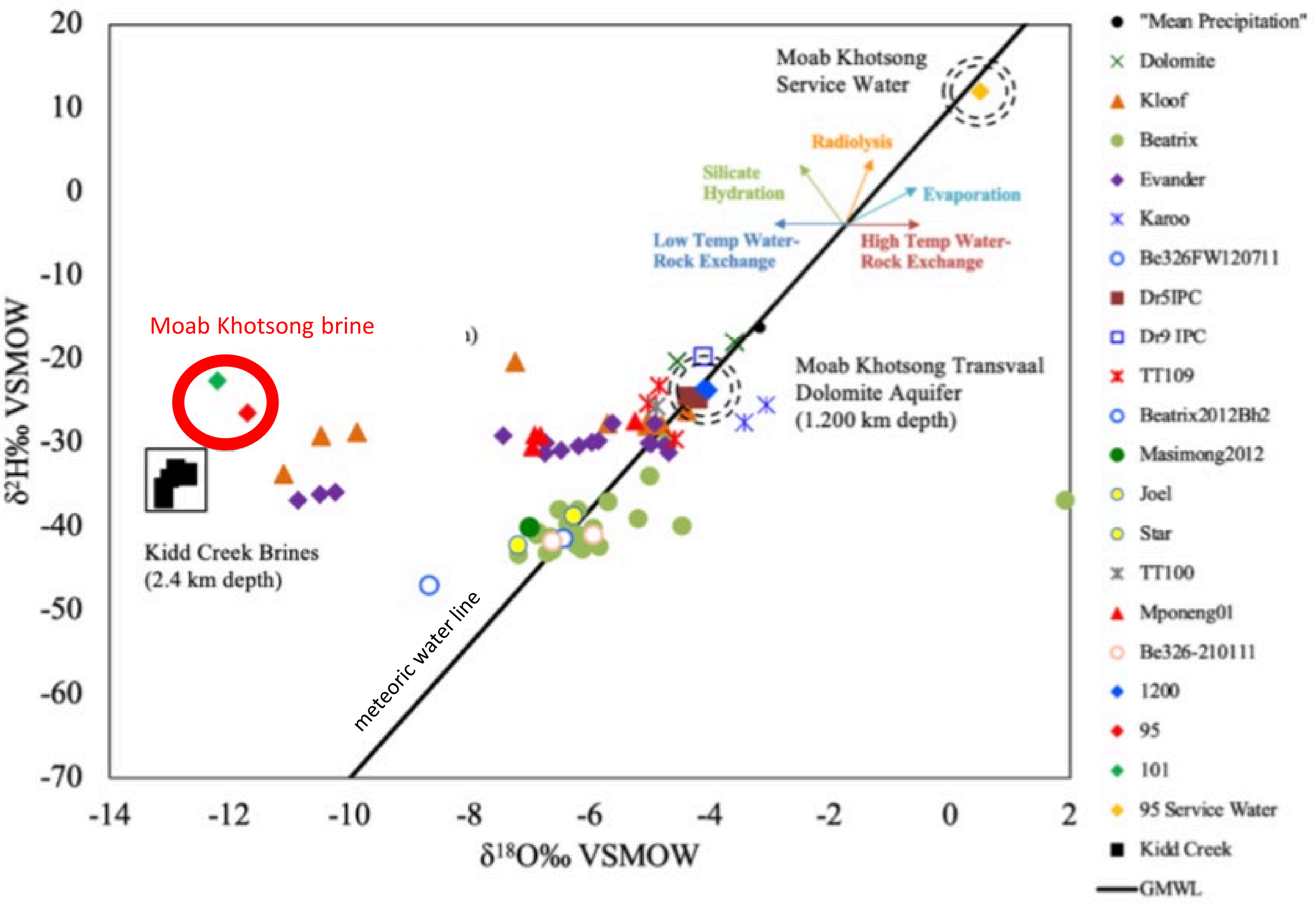


Origin of the brine:

2.9 Ga ocean water trapped and altered during metamorphosis.

Primarily radiolytic removal of water

Nisson et al., 2023 *Geochimica et Cosmochimica Acta*. 340:65-84



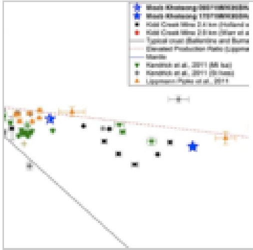
Nisson et al., 2023 *Geochimica et Cosmochimica Acta*. 340:65-84

Groundwater dating by Nobel gas isotope analyses:
>1 billion year average residence time for Moab Khotsong brine

Sample	^{4}He (Ma)	^{21}Ne (Ma)	^{40}Ar (Ma)	^{136}Xe (Ma)
MK95BHA (July 9th, 2019)	316 ± 143	458 ±207	602 ±272	3181 ±1443
MK95BHA (July 17th, 2019)	1028 ±466	1361 ±616	**1196 ±542**	4113± 1867

Noble Gas Residence Times in Ma for Moab Khotsong Brine 95 level (3.2km)

Most radiogenic groundwaters ever described!



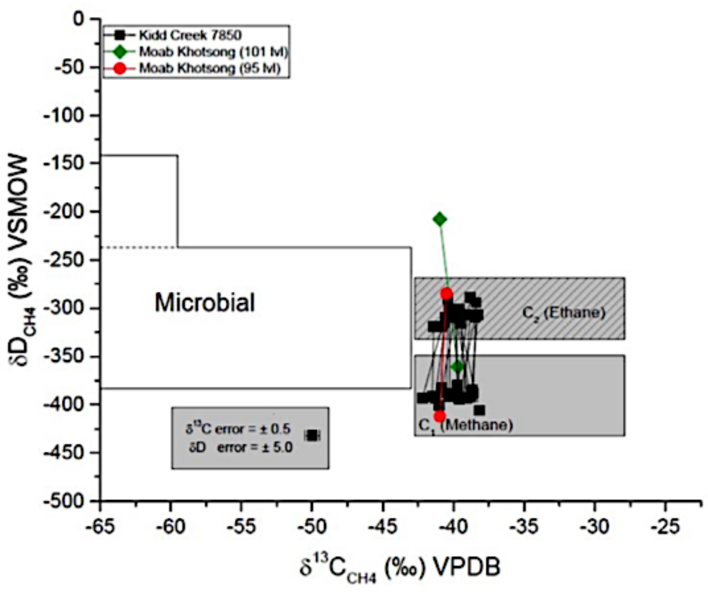
Nature

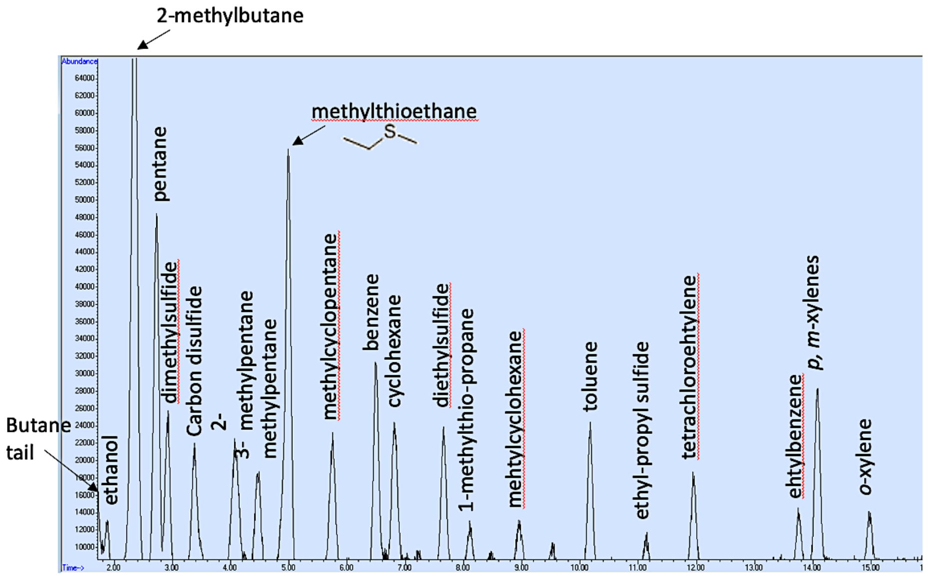
86Kr excess and other noble gases identify a billion-year...

Noble gases confirm billion-year groundwater residence times and external fluxes in deep crustal settings globally with implications for subsurface...

Warr et al., 2022. Nature Communications

Rock-water interactions generate
H_2, methane, and short-chain hydrocarbons

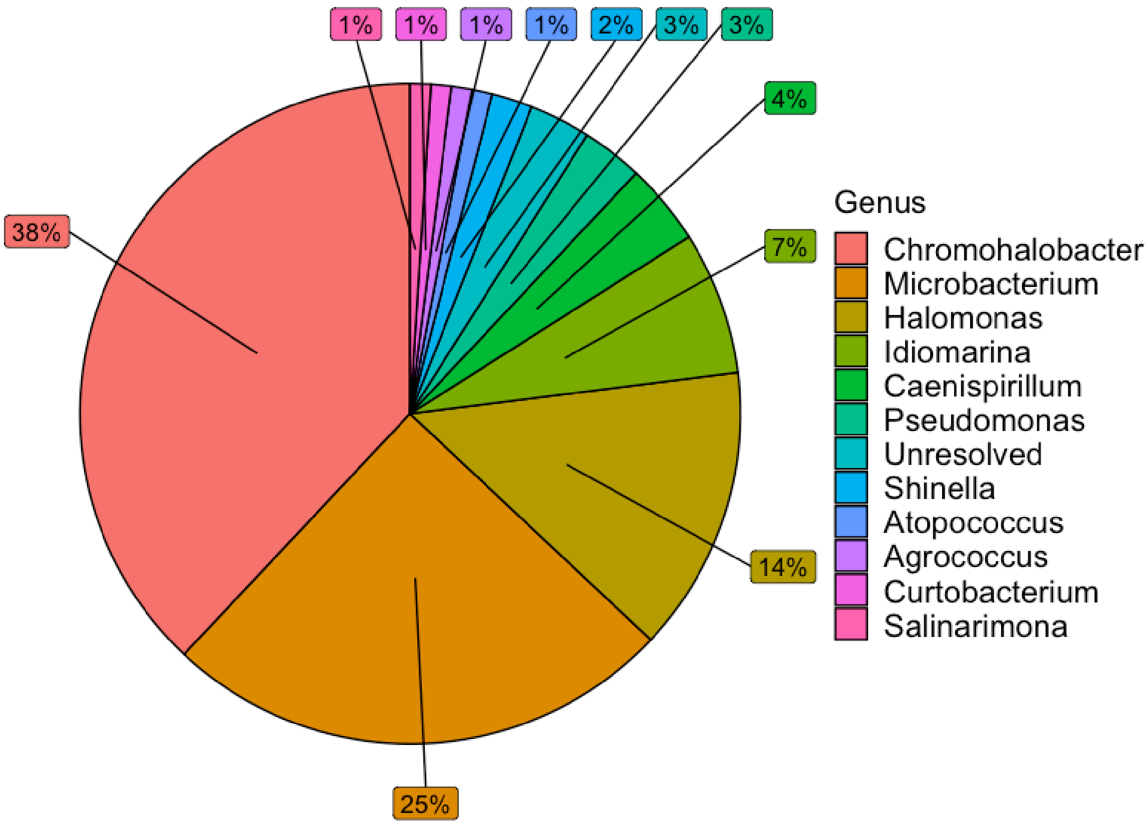




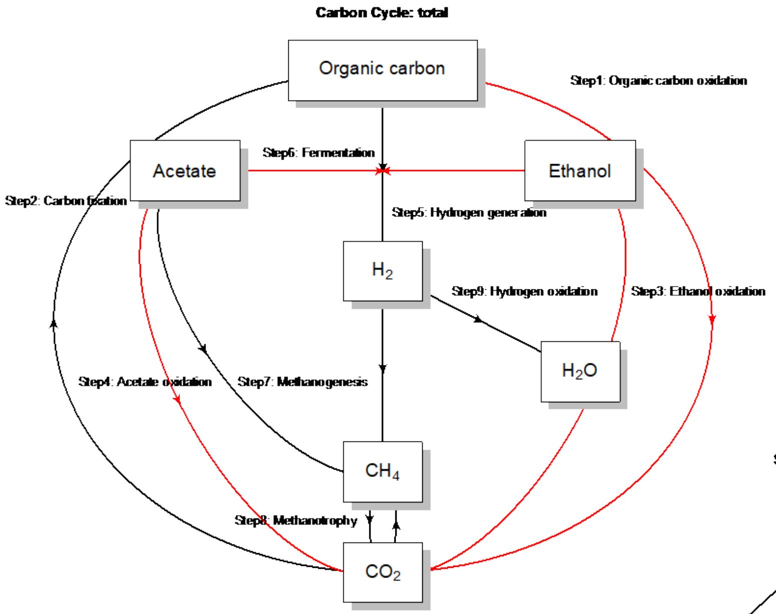
Organic carbon in gas exsolved from
Moab Khotsong brine –
C_1-C_6 compounds, including thiols

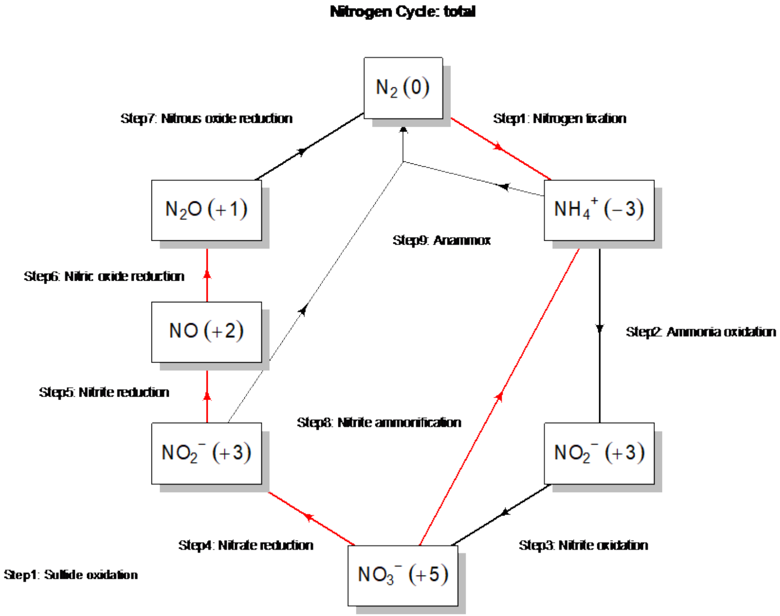
Pre-biotic chemistry related to origin of life?

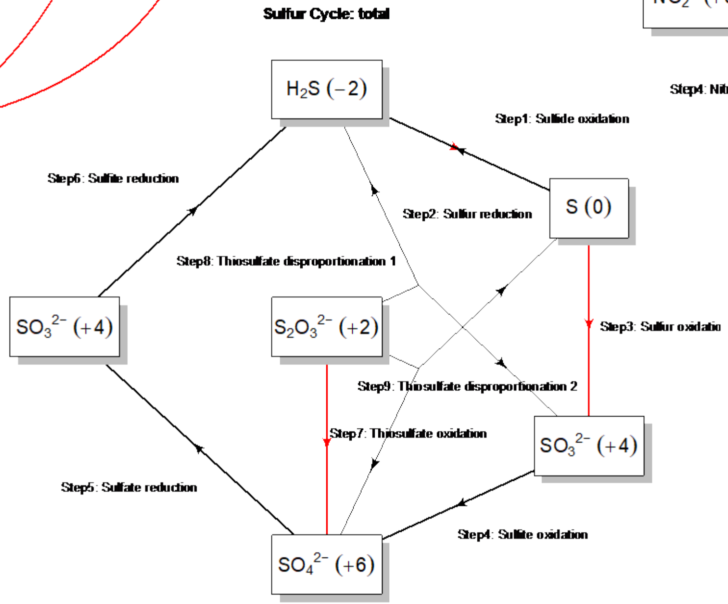
Taxonomic Distribution of MK101 SAGs



Nisson et al. in prep.



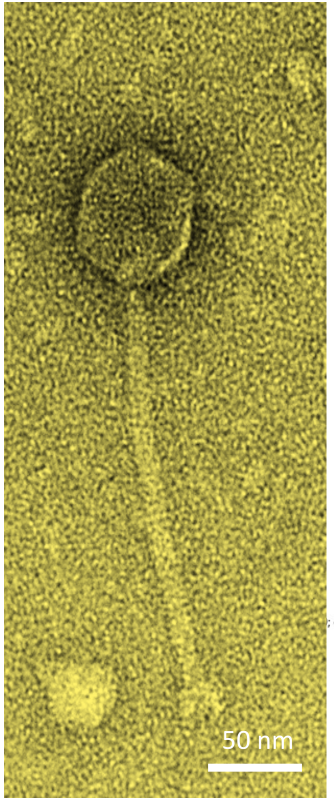


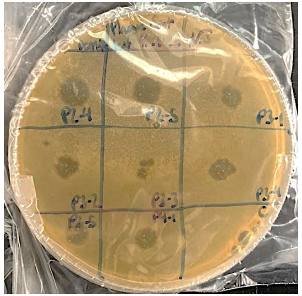


Nisson et al. in prep.

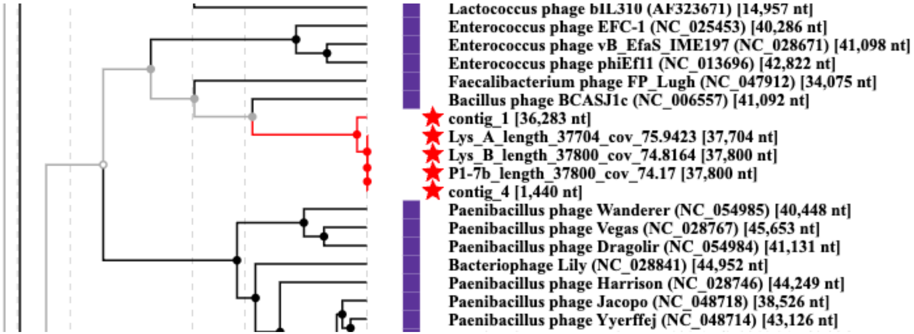
101-level bacterial isolates from ancient brine

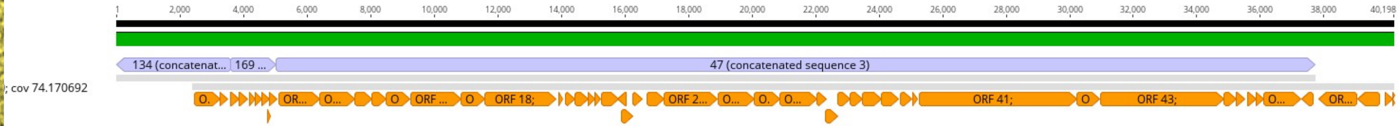
Halalkalibacterium halodurans plus phages





Novel Siphoviridae
temperate phage
37,800 bp
53 ORFs

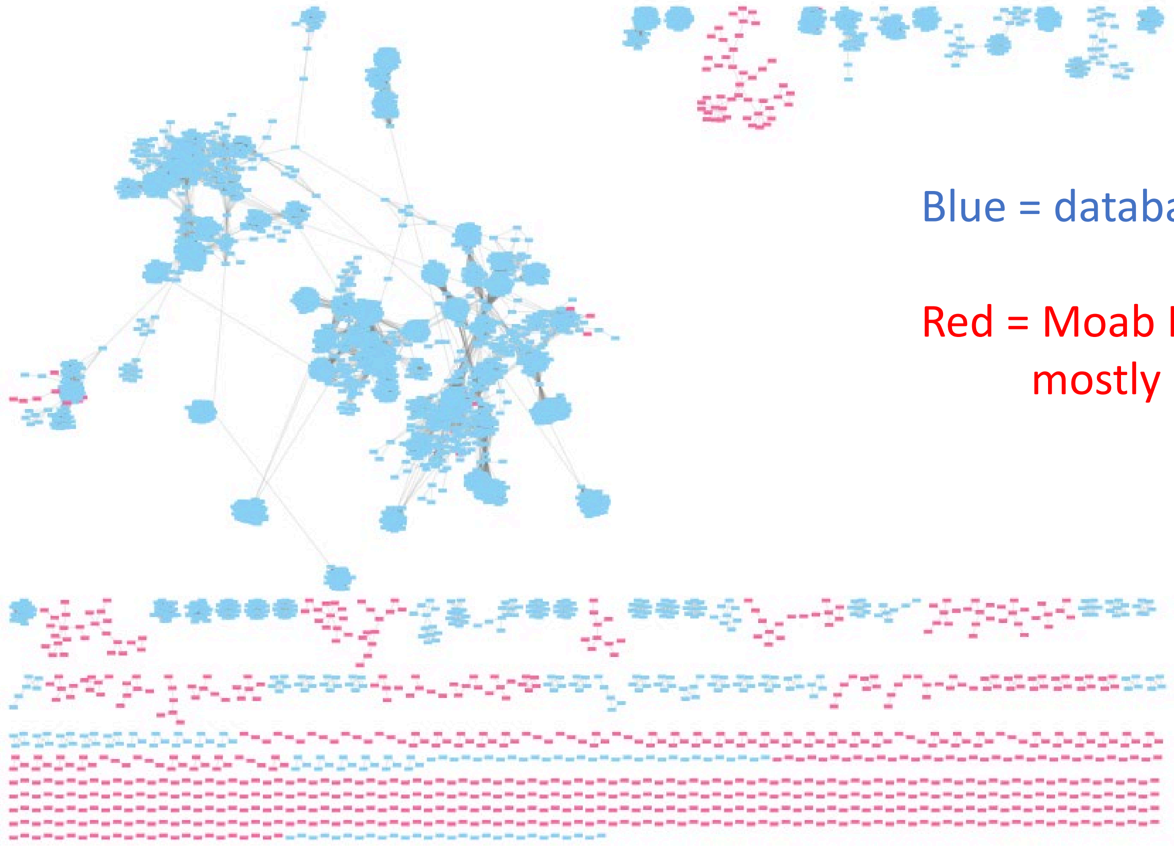




Also: diverse 1200L phages

Nathaniel Jobe
Cassandra Skaar

1200-level dolomite aquifer – **Vcontact2 Cluster Analysis** of viral sequences

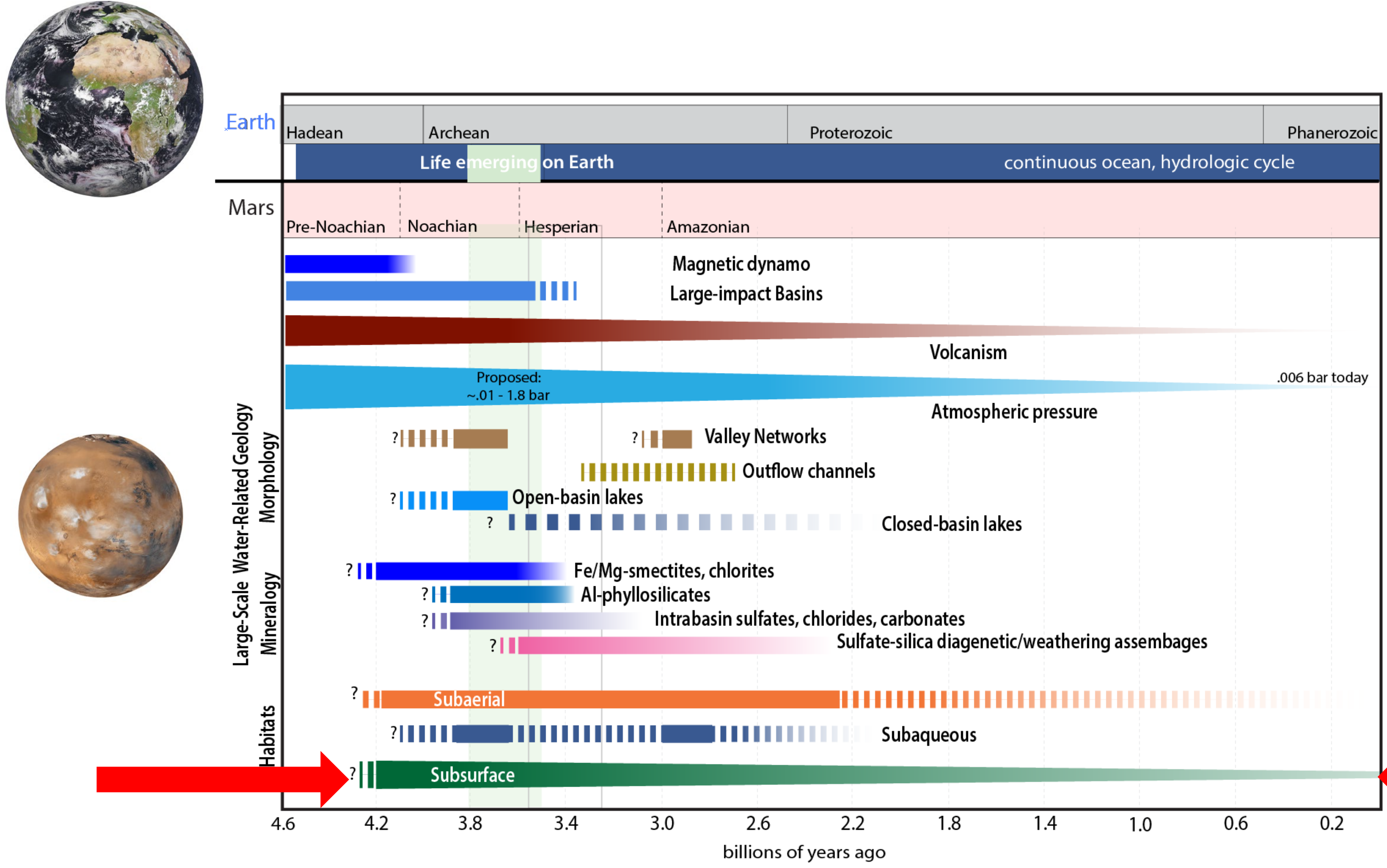


Blue = database sequences

Red = Moab Khotsong sequences
mostly novel viruses!

Moab Khotsong DSEIS Geomicro findings

- Boreholes drilled near a fault zone intercepted a hypersaline brine.
- Noble gas isotopes indicate ~1 Ga residence times and reveal radiolysis as a contributor to brine formation.
- A large complex carbon pool is present, with contribution from both abiotic processes and chemoautotrophic activity.
- Single-cell amplified genomes reveal a low diversity bacterial community with adaptations for compatible solute synthesis and for coupling S/N oxidation to nitrate reduction.
- Moab Khotsong fracture water has yielded a diversity of phages, both through metagenomic sequencing and culture-dependent analysis.



Look for extant Martian life in the subsurface

Amador and Ehlman. In: Planetary Astrobiology, Space Science Series. University of Arizona Press, 2020

Take-home messages:

- Deep *continental* subsurface environments are important and need more love.
- Radioloysis of water generates H_2, CH_4, and a range of electron acceptors
- Radiolysis modifies buried organic C
- Deep *continental* subsurface environments are analogs for habitable subsurface environments on Mars.

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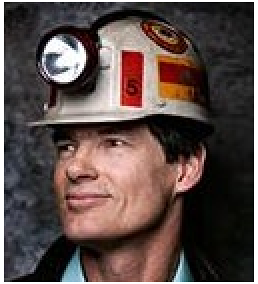
T.C. Onstott →



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Maggie Lau
Cara Magnabosco

HARMONY

Bennie Liebenberg
Moab Khotsong Mine

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Errol Cason

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Oliver Warr

Many, many others!

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