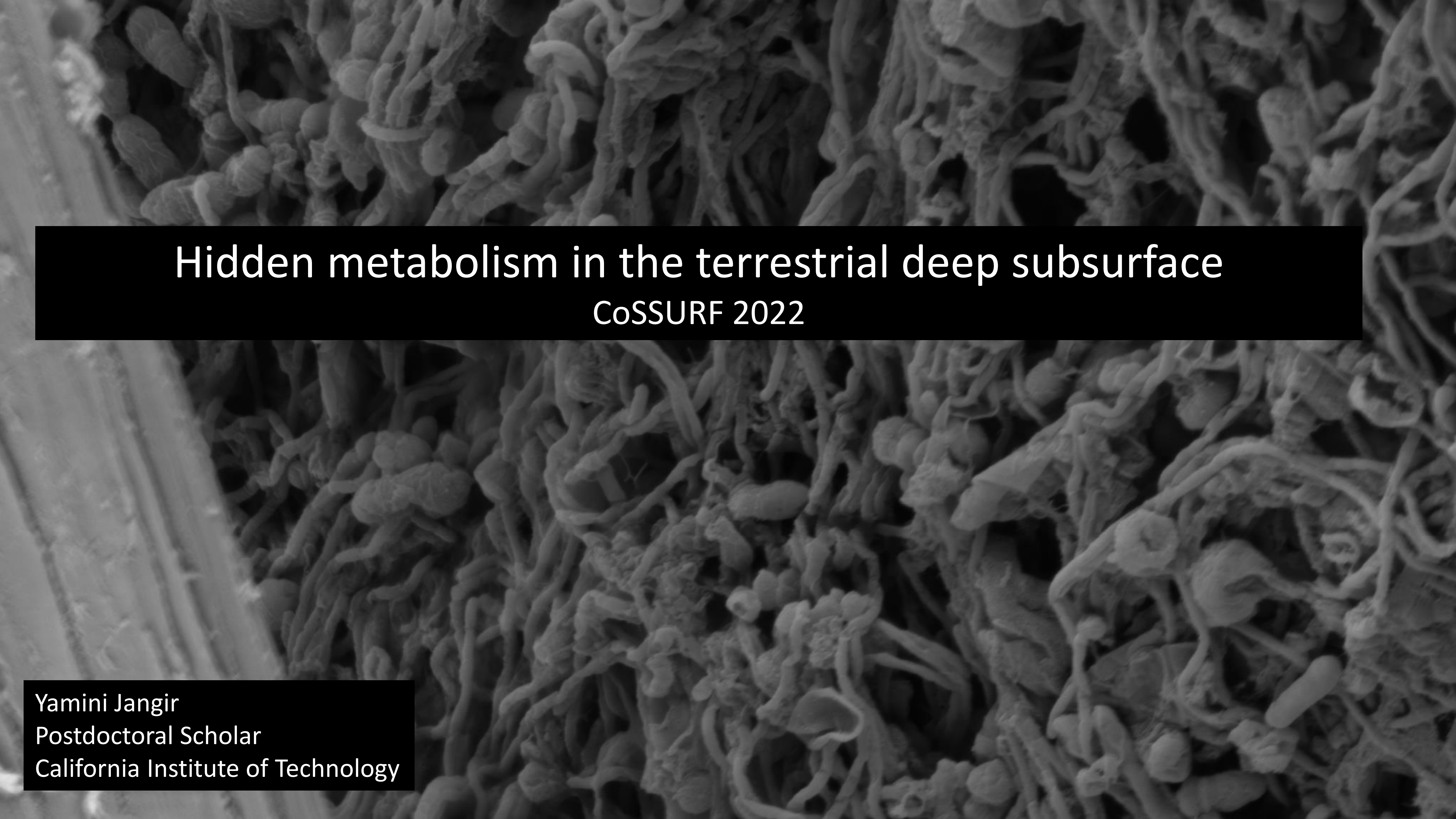
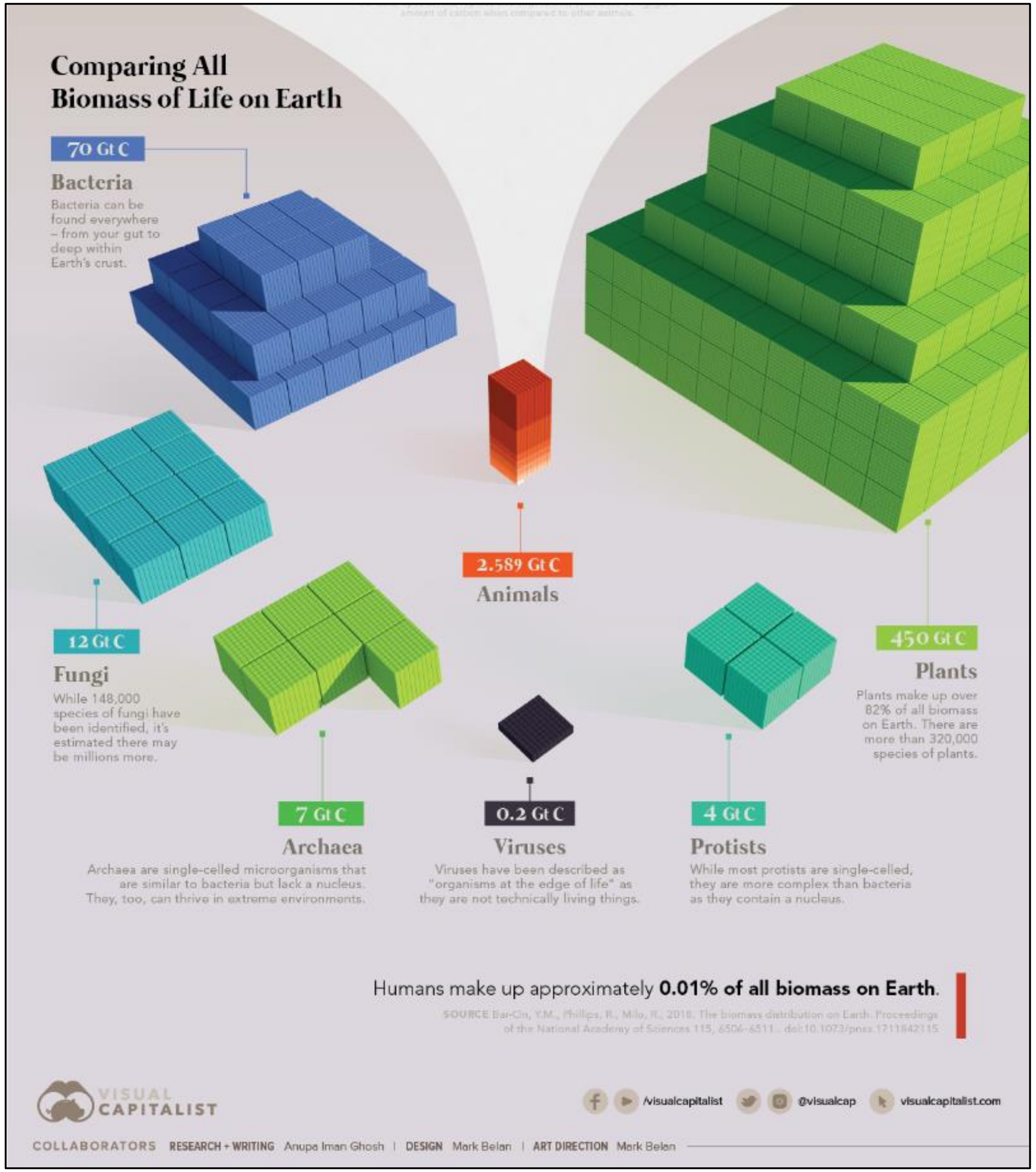


Hidden metabolism in the terrestrial deep subsurface

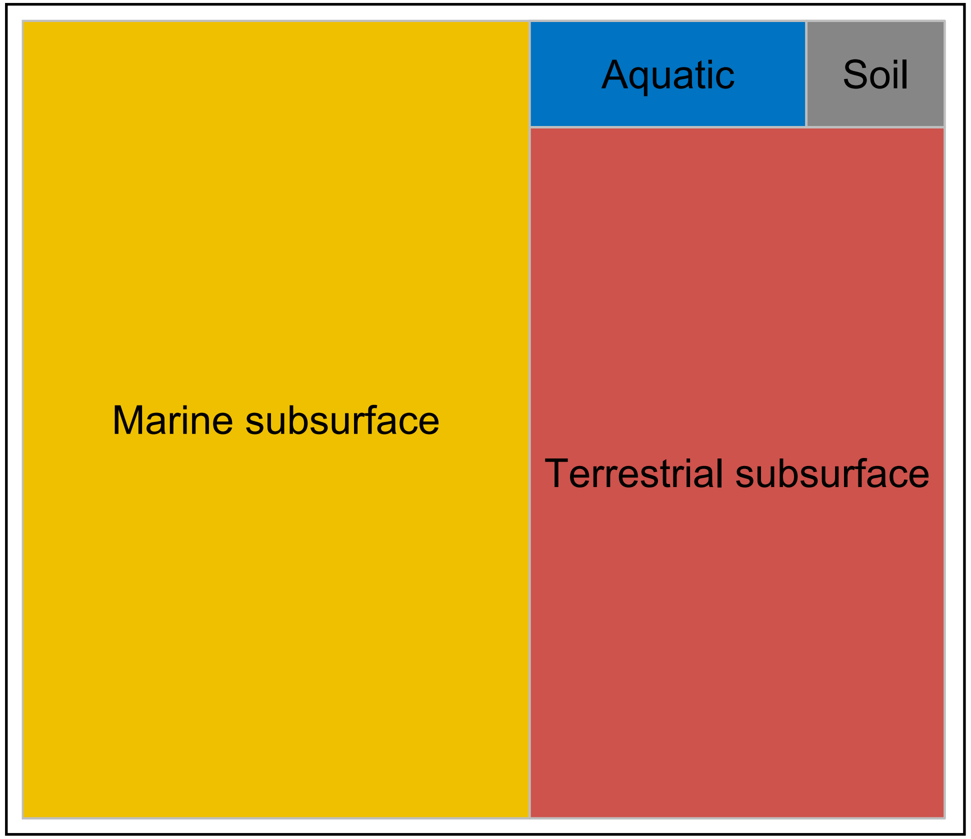
CoSSURF 2022

Yamini Jangir
Postdoctoral Scholar
California Institute of Technology

Biomass of Life



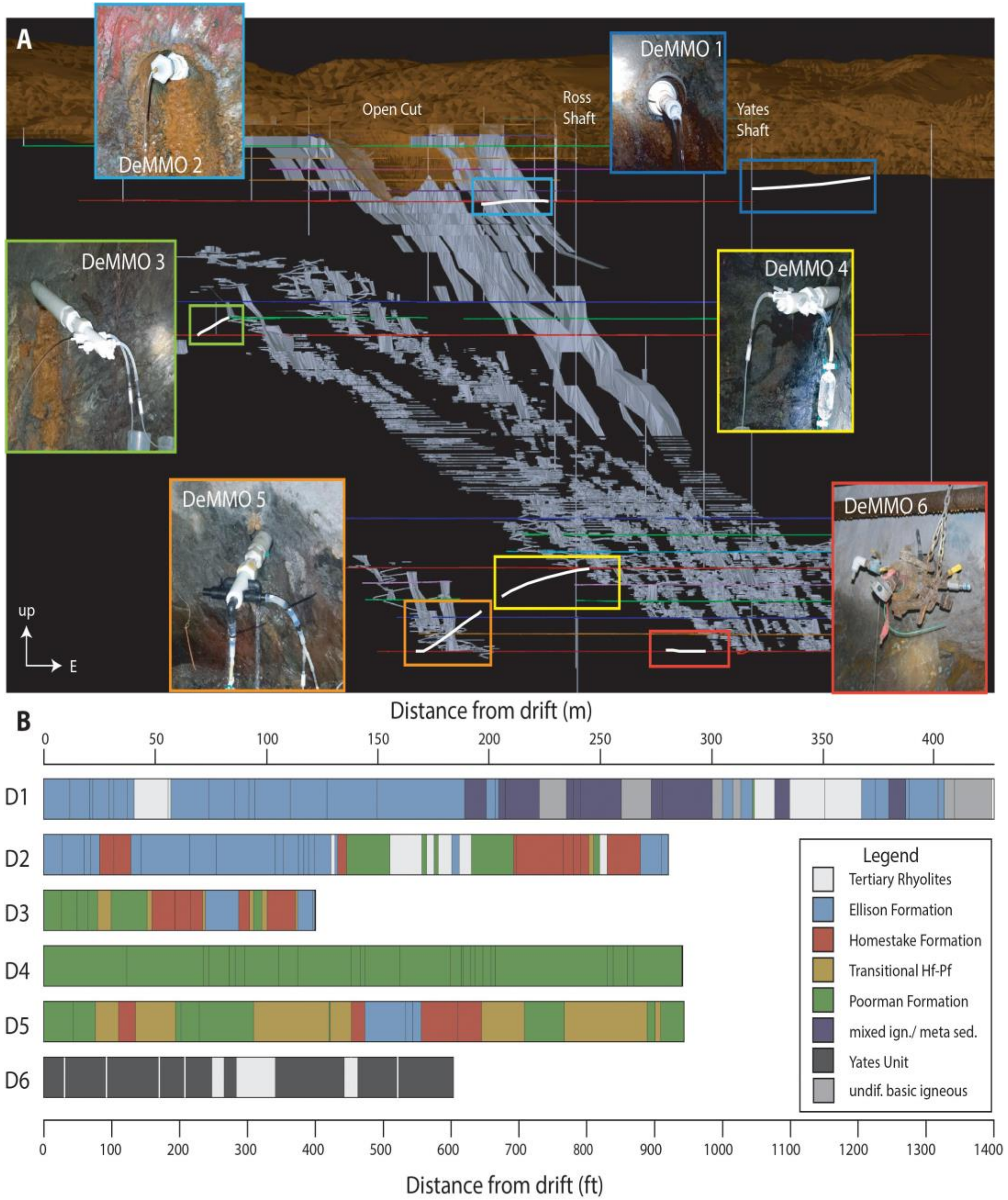
- The unseen majority (Bacteria, Fungi, Archaea, Virus)
- Majority of the microbes are distributed in the subsurface.



| All the Biomass of Earth, in One Graphic | Ghosh and Belan| Visual Capitalist | 2021 |
| The biomass distribution on Earth | Bar-On et al | PNAS | 2018 |
| The Deep, Dark Energy Biosphere: Intraterrestrial Life on Earth| Edwards, Becker, and Colwell | Annual review of earth and planetary sciences | 2012 | c

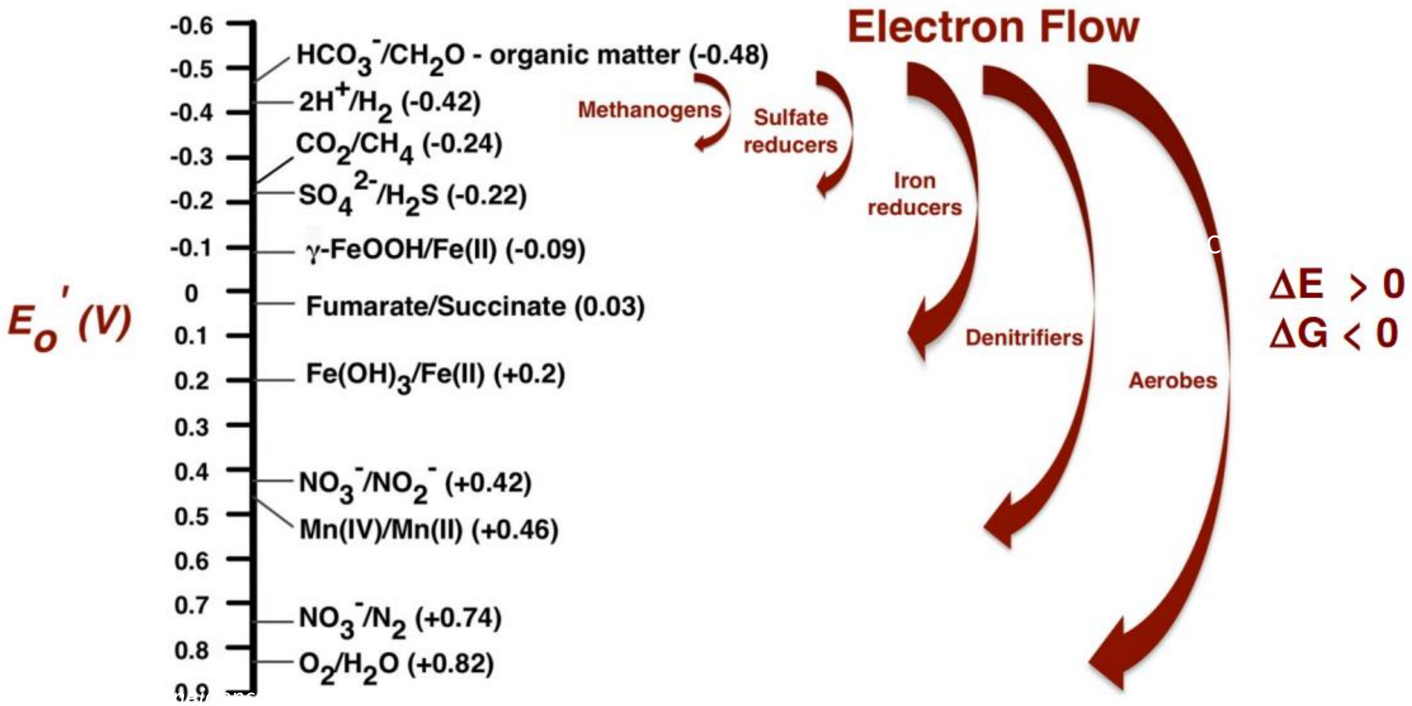
❖ SURF is hosted in heavily deformed, iron-rich, Paleoproterozoic metasediments with a complex geological history.

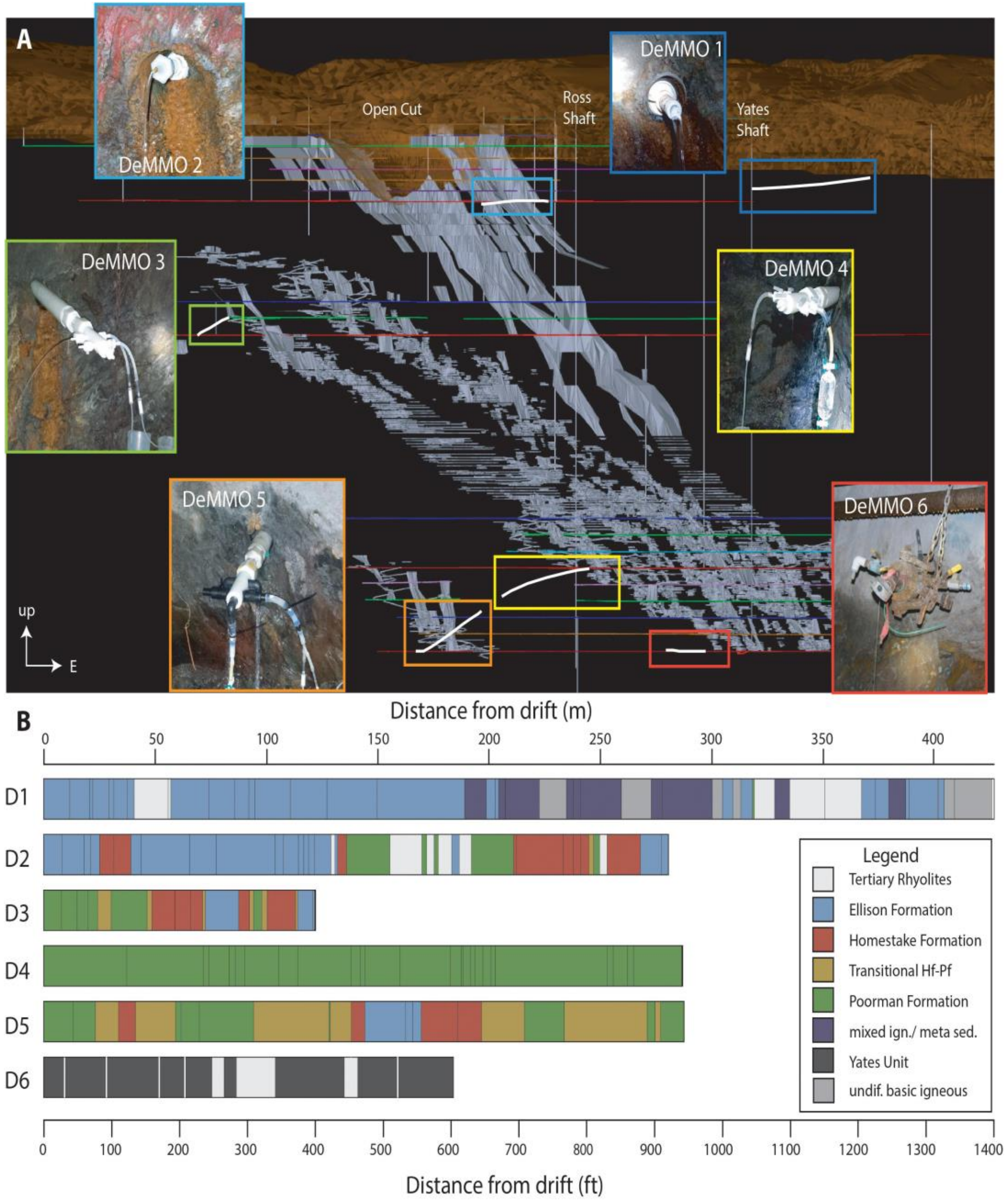
❖ It provides *in situ* study of the early Earth (2 – 3 billion years old)



| Osburn et al | Front. In Earth Sci. | 2019 |

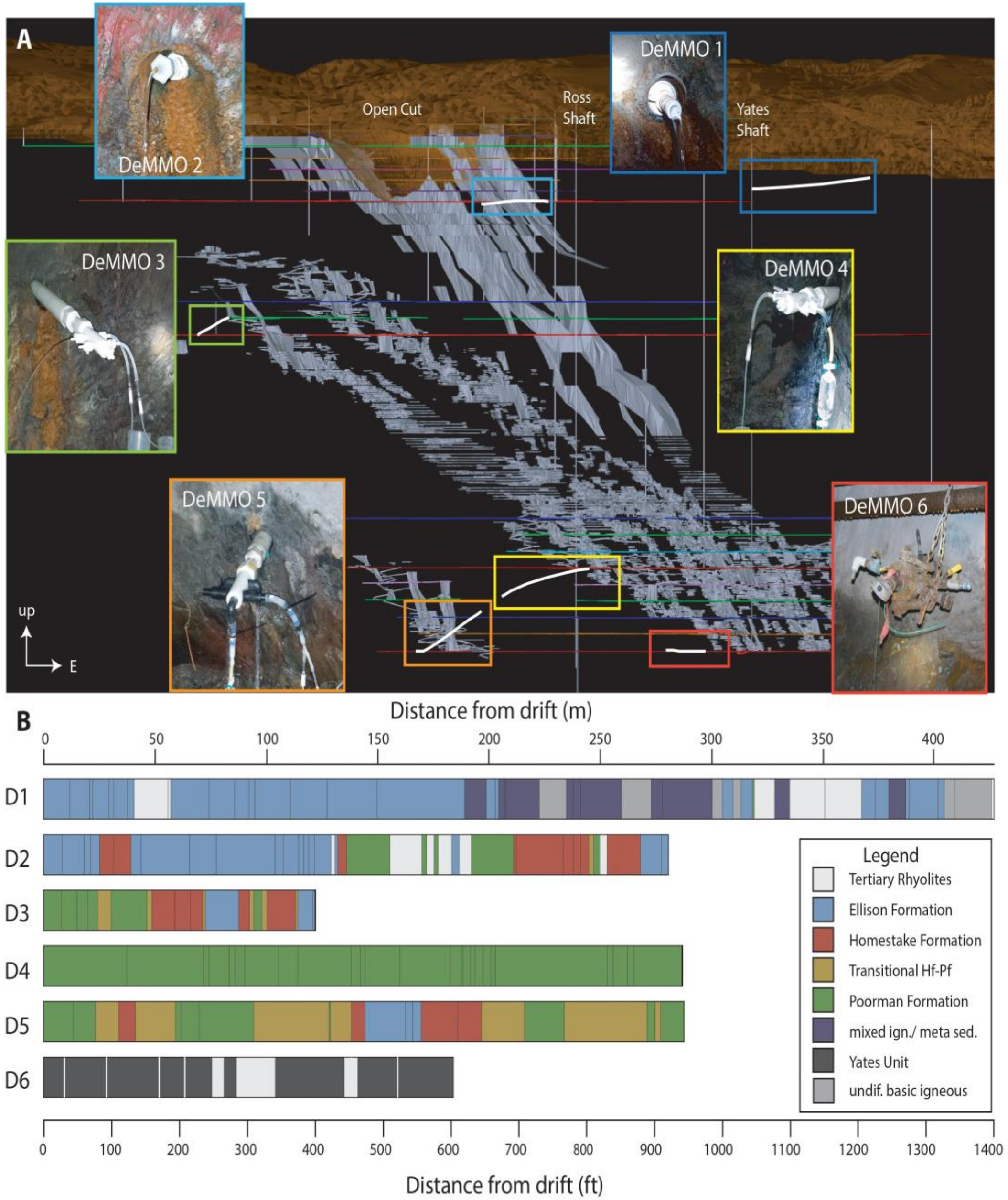
- SURF is hosted in heavily deformed, iron-rich, Paleoproterozoic metasediments with a complex geological history.
- It provides *in situ* study of the early Earth (2 – 3 billion years old)
- Microbial electron transfer to/from minerals





| Osburn et al | Front. In Earth Sci. | 2019 |

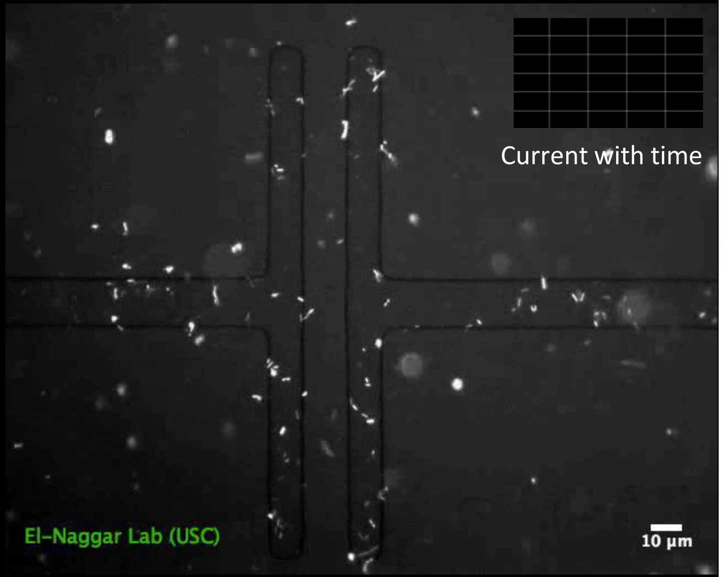
- SURF is hosted in heavily deformed, iron-rich, Paleoproterozoic metasediments with a complex geological history.
- It provides *in situ* study of the early earth (2 – 3 billion years old)
- Microbial electron transfer to/from minerals



| Osburn et al | Front. In Earth Sci. | 2019 |

'Life is nothing but an electron looking for a place to rest'
Albert Szent-Györgyi (Nobel Prize in Physiology, 1937)

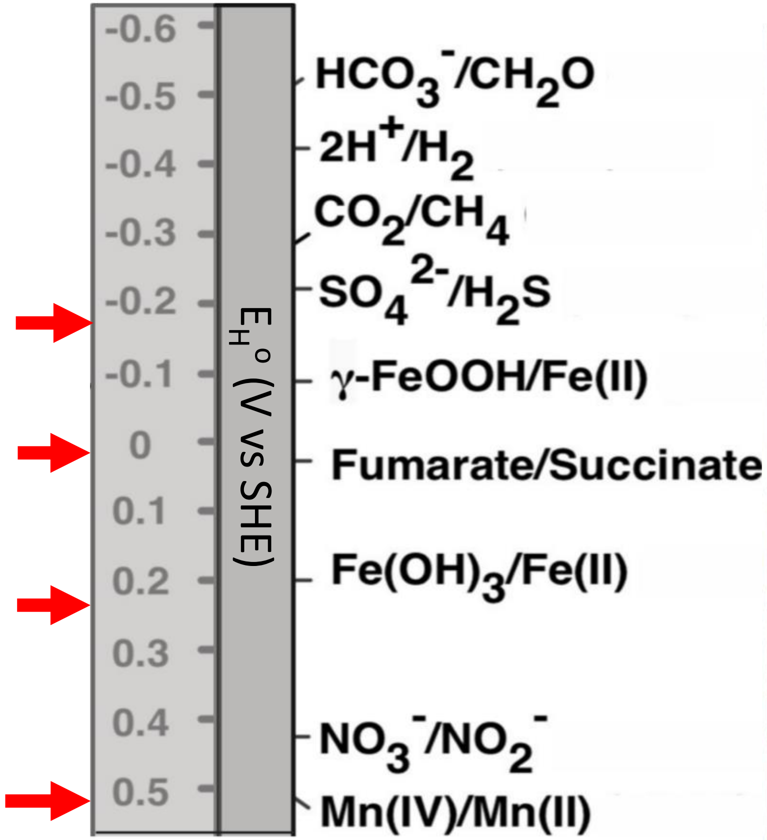
Shewanella oneidensis MR-1 respiring on insoluble MnO_2 (aka reducing insoluble Mn(IV) to soluble Mn(II).
| Harris et al | PNAS | 2009 |

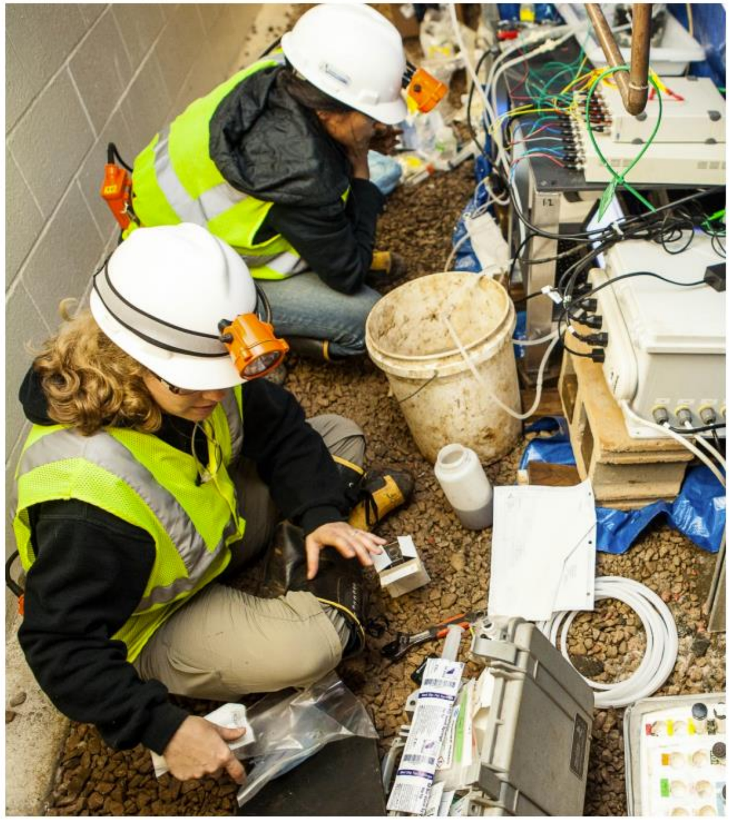


Shewanella oneidensis MR-1 reducing T-shaped indium tin oxide (ITO) electrodes poised at + 440 mV vs. SHE.

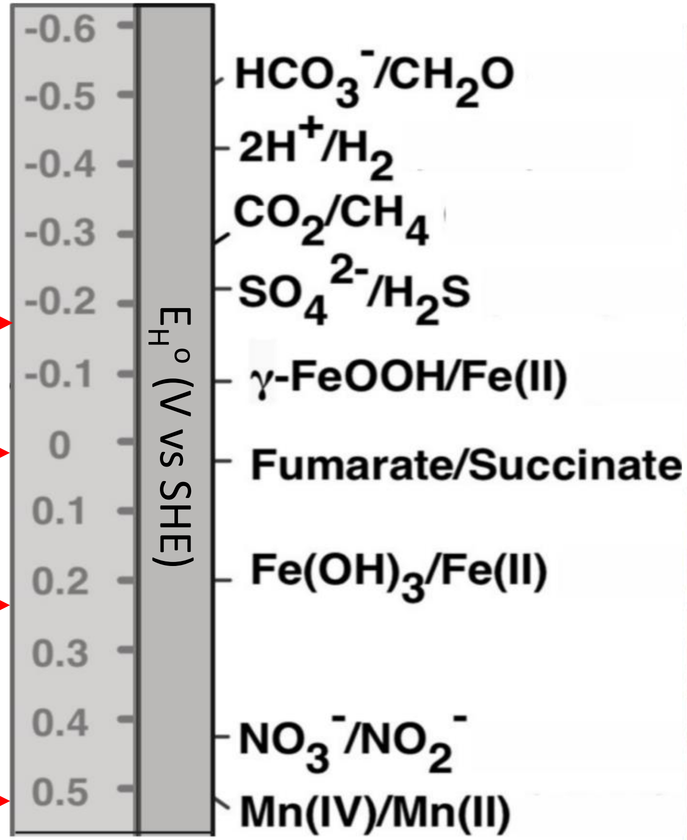
- Microbial electron transfer to/from minerals

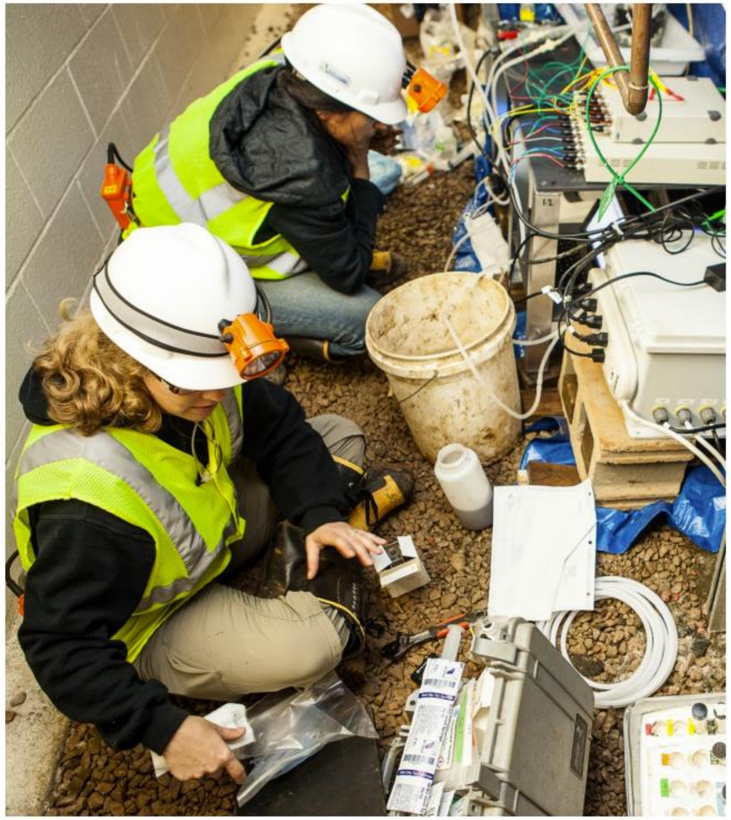
- Targeted 4 metabolisms: S oxidation, Fe (II) Oxidation, Fe (III) reduction, and Mn (IV) reduction

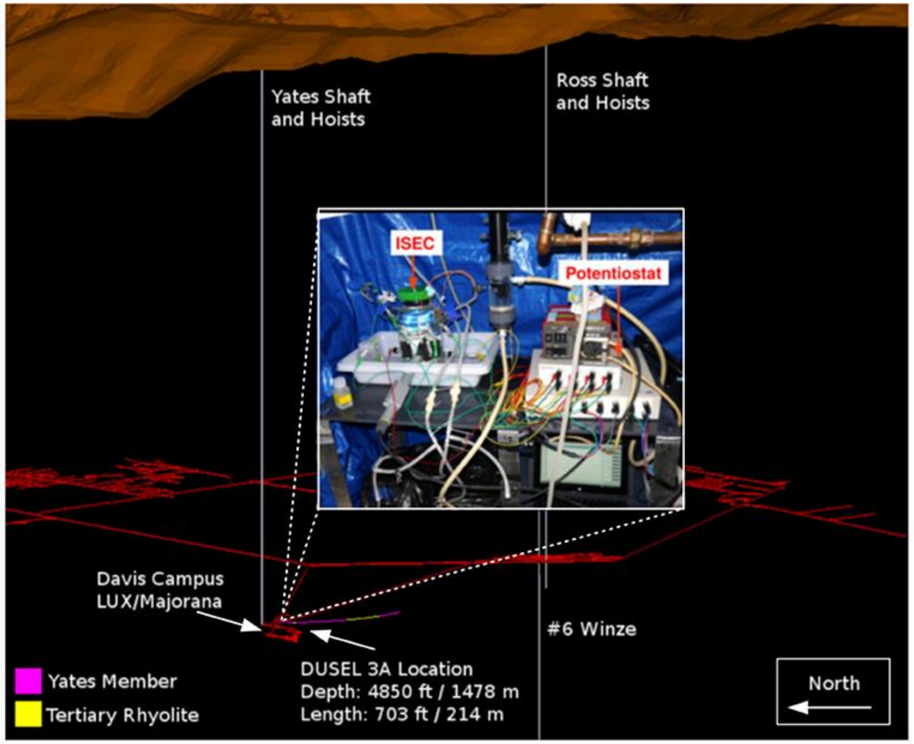


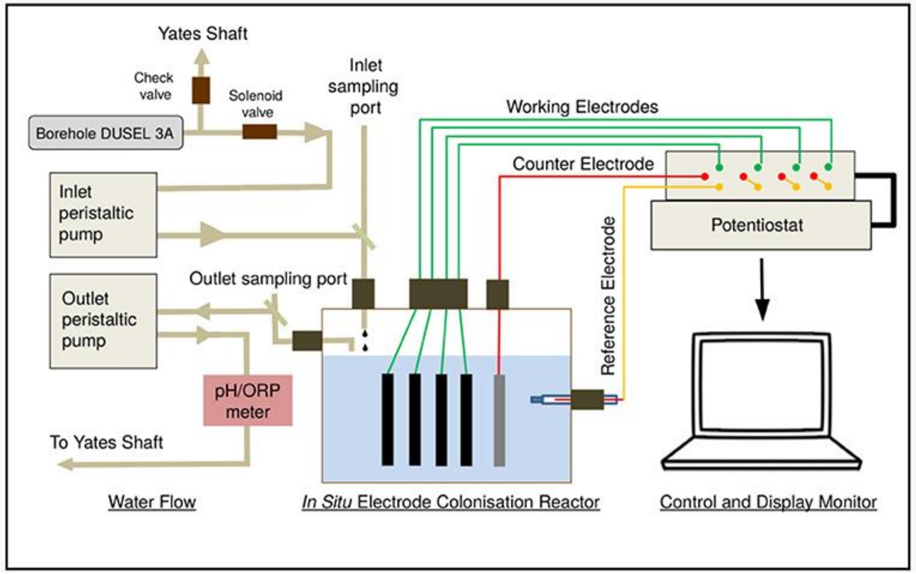


- Microbial electron transfer to/from minerals
- Targeted 4 metabolisms: S oxidation, Iron (II) Oxidation, Iron (III) reduction, and Manganese (IV) reduction
- Electrochemical reactor: Two cathodic and two anodic potentials



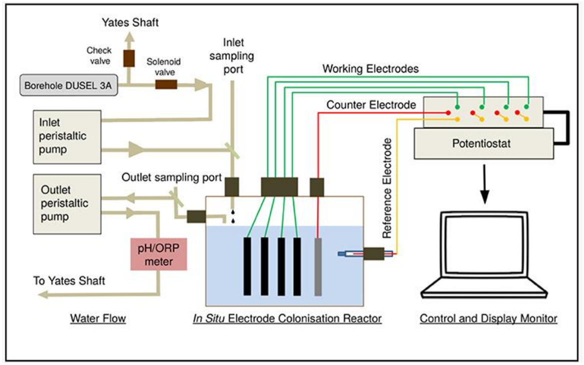






| Jangir et al | Front. Energy Res. | 2019 |

How does the *in-situ* reactor compare to the DUSEL 3A fluid?



	Dec 2014	May 2015
pH	7.08	7.75
Temperature (°C)	18.7	19.31
Conductance (µS/cm)	6514	7869
TDS (ppm)	3257	3929
ORP (mV)	59.2	−50

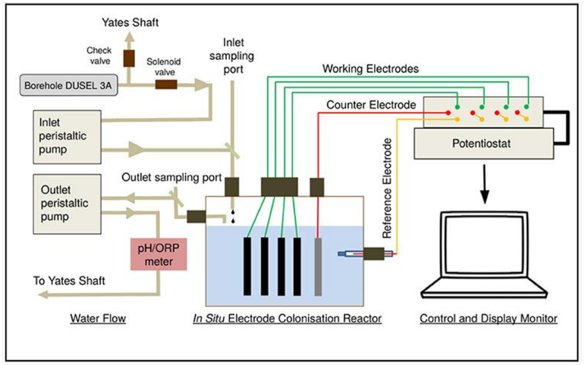
Applied voltage	Average current (µA) ± standard deviation
−0.19 V vs. SHE	−276.68 ± 43.80
+0.01 V vs. SHE	−168.76 ± 26.82
+0.26 V vs. SHE	−0.15 ± 0.09
+0.53 V vs. SHE	−1.47 ± 0.11
ISEC reactor effluent aqueous chemistry	
pH	7.5
Temperature (°C)	19
Conductance (µS/cm)	11000
ORP (mV)	+200

- The observed current is a combination of abiotic and biotic currents
- Possible leakage + no replication!

Active microbial community on each electrode-attached biofilm versus DUSEL 3A

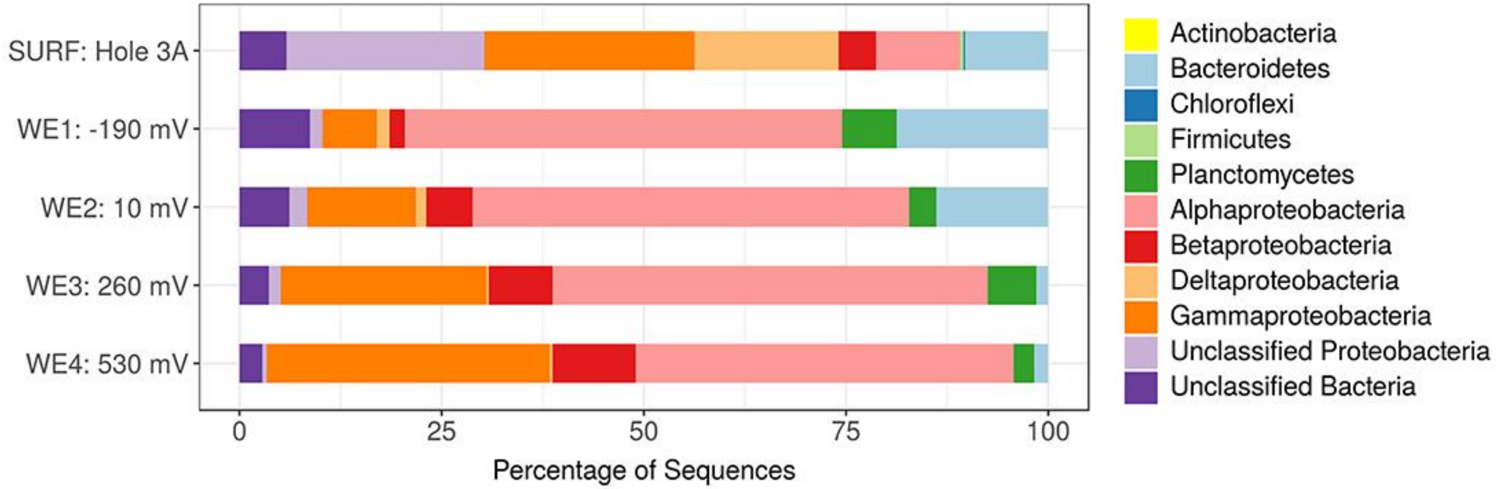
| Osburn et al | Front. Micro. Biol. | 2014 |
| Jangir et al | Front. Energy Res. | 2019 |

How does the *in-situ* reactor compare to the DUSEL 3A fluid?



	Dec 2014	May 2015
pH	7.08	7.75
Temperature (°C)	18.7	19.31
Conductance (μS/cm)	6514	7869
TDS (ppm)	3257	3929
ORP (mV)	59.2	−50

Applied voltage	Average current (μA) ± standard deviation
−0.19 V vs. SHE	−276.68 ± 43.80
+0.01 V vs. SHE	−168.76 ± 26.82
+0.26 V vs. SHE	−0.15 ± 0.09
+0.53 V vs. SHE	−1.47 ± 0.11
ISEC reactor effluent aqueous chemistry	
pH	7.5
Temperature (°C)	19
Conductance (μS/cm)	11000
ORP (mV)	+200



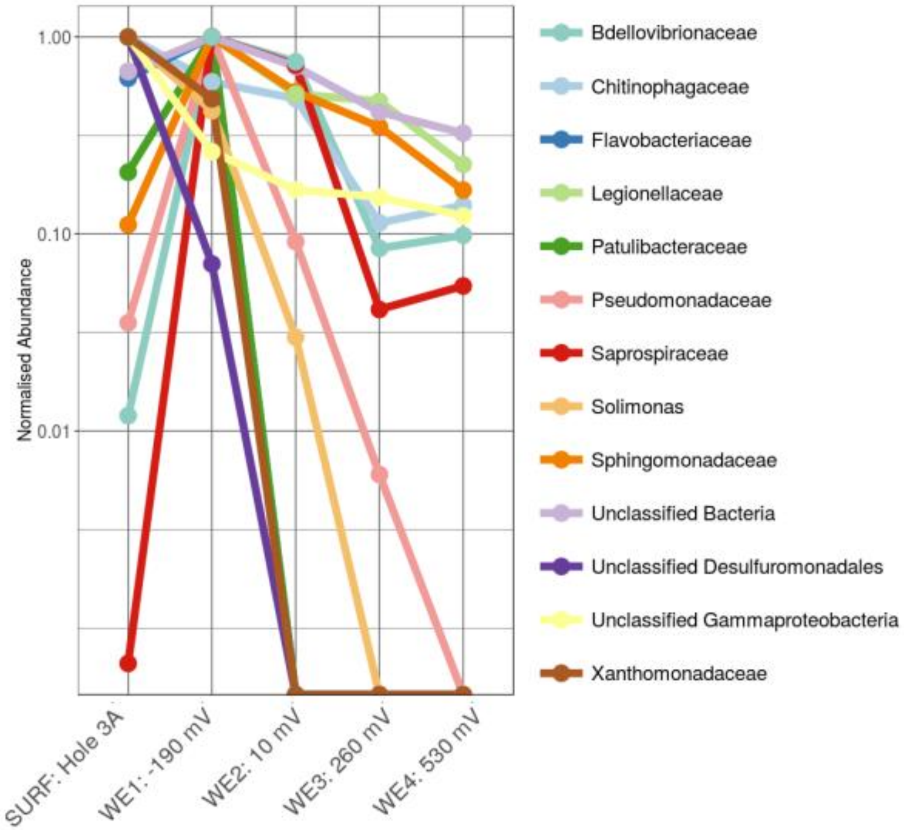
- The observed current is a combination of abiotic and biotic currents
- Possible leakage + no replication!
- The archaeal community (0.3%) in DUSEL 3A fluid was dominated by sequences related to Crenarchaeota, Euryarchaeota, and Thaumarchaeota.
- Osburn et al. (2014) observed a higher abundance of *Firmicutes* in the borehole fluids
  - RNA versus DNA studies
  - Higher copy number of *Firmicutes*

Active microbial community on each electrode-attached biofilm versus DUSEL 3A

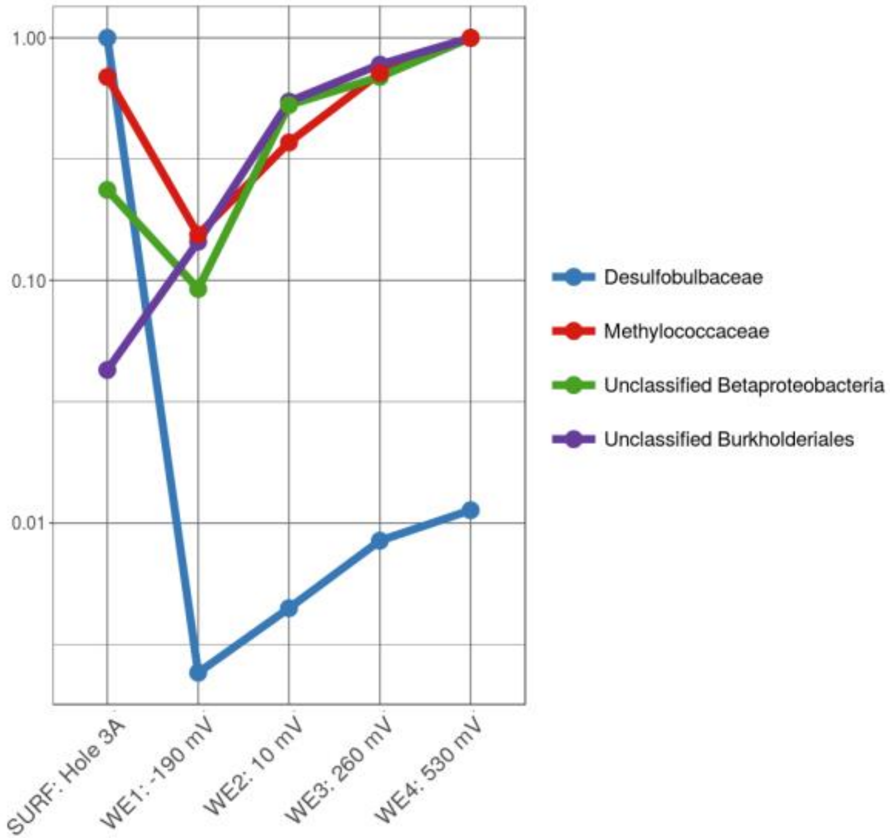
| Osburn et al | Front. Micro. Biol. | 2014 |
| Jangir et al | Front. Energy Res. | 2019 |

Trends observed in the electrode-attached community of the *in situ* electrochemical reactor (ISEC)

Decreasing abundance



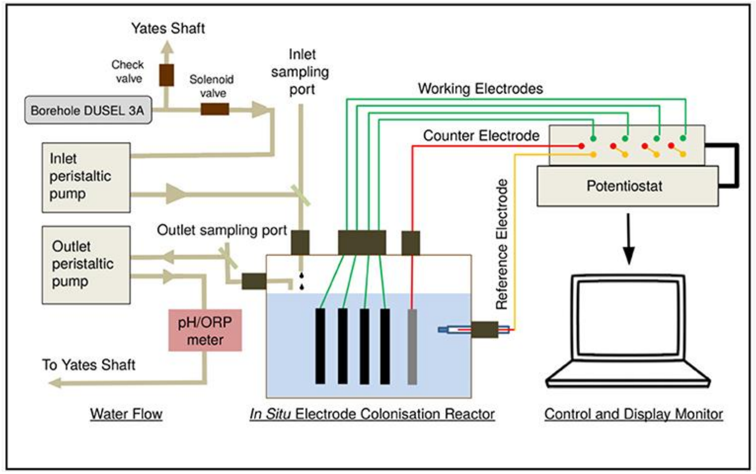
Increasing abundance



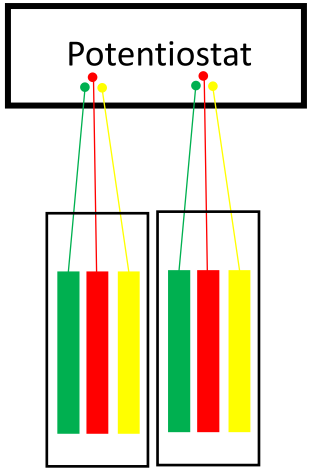
- Some members of *Flavobacteriaceae* have shown to oxidize manganese and colonize surface of sulfur minerals.
- Members of *Pseudomonadaceae* family performs EET to poised electrodes.
- *Desulfobulbaceae* (Sulfate reducer) colonized more at higher potentials. Members include filamentous cable bacteria which couples sulfide oxidation to oxygen reduction along cm scales.
- *Methylococcaceae* (Methylotroph) – possibly using methane as carbon source and electrode as electron acceptor

| Jangir et al | Front. Energy Res. | 2019 |

From field to the lab



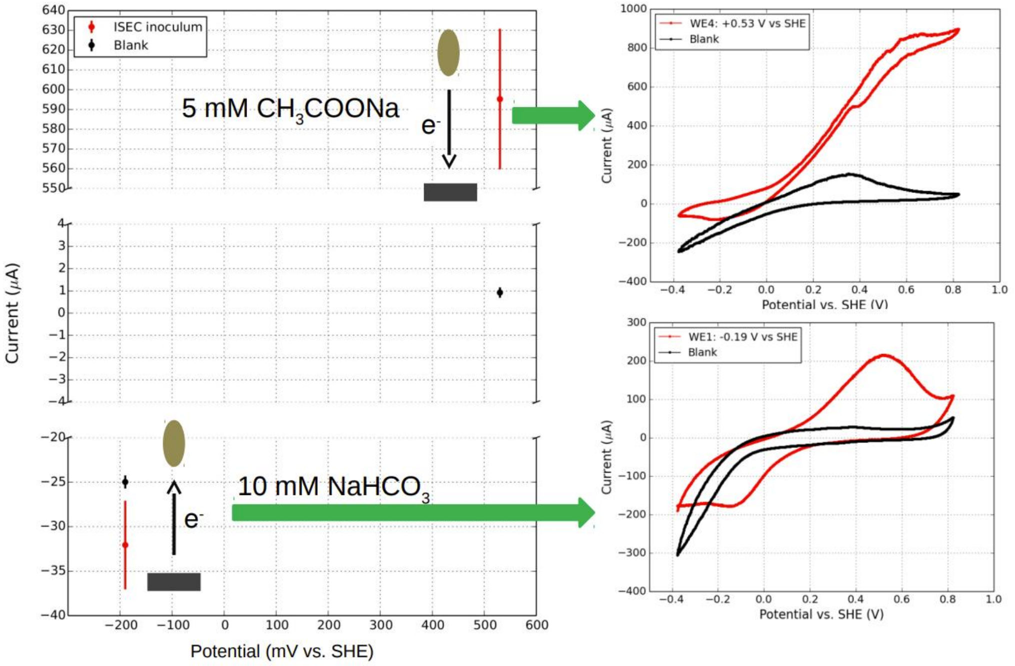
In situ electrochemical reactor

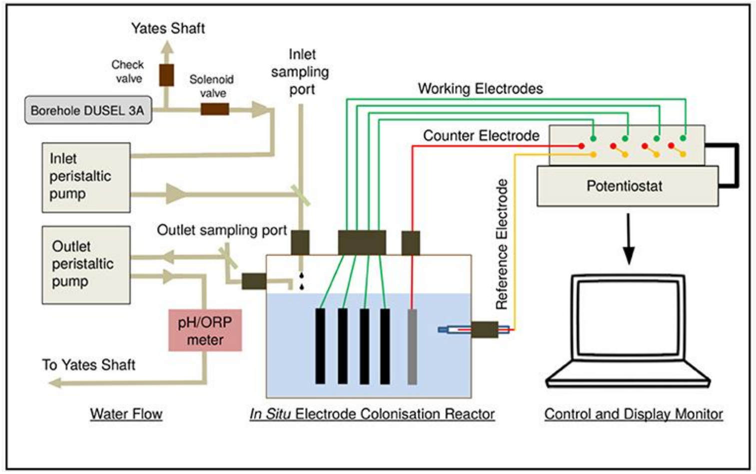


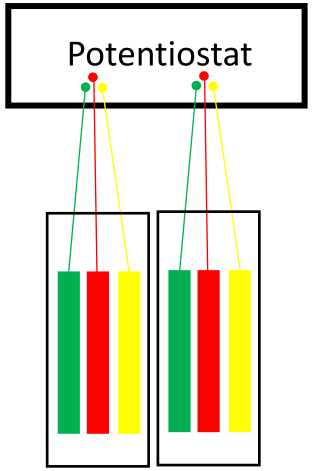
Ex situ electrochemical reactor

- ISEC : continuous flow reactor + interaction between various potentials
- ESEC : Batch-fed reactor + no interaction between potentials.

From field to the lab

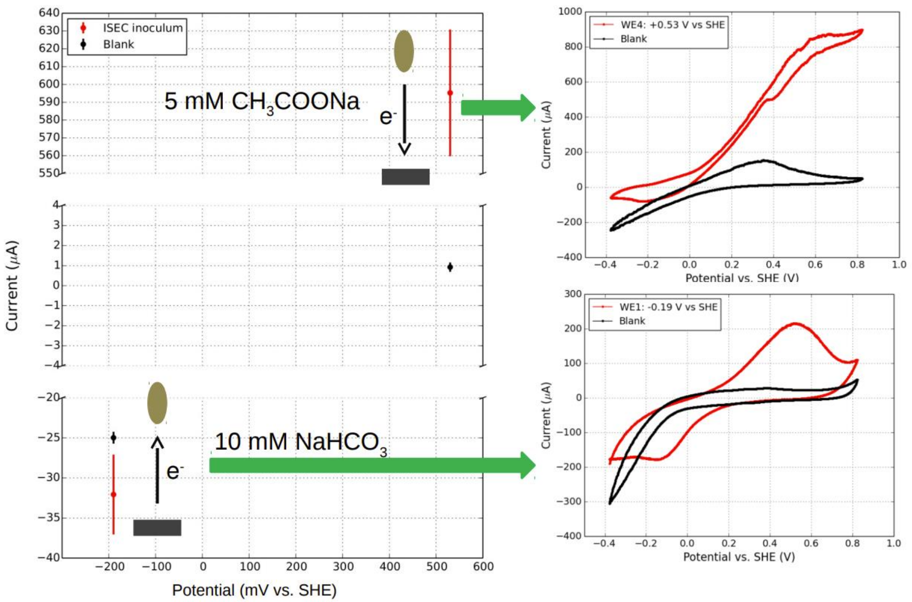


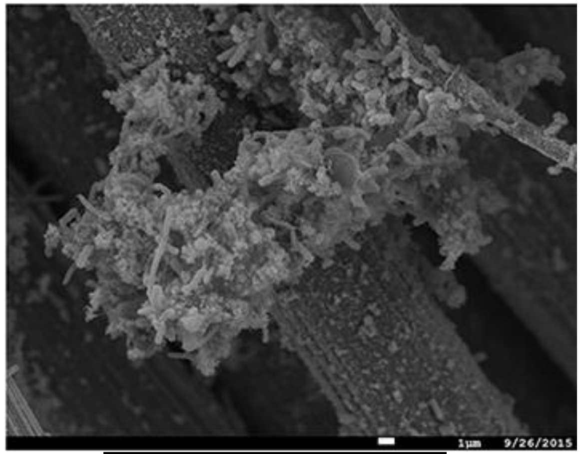




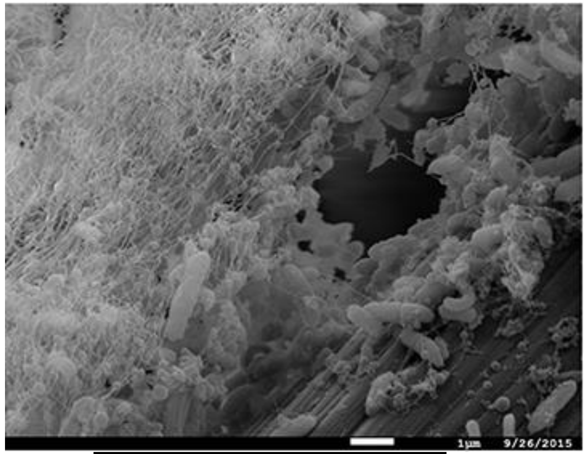
- ESEC :
  - Able to provide targeted nutrients required for different metabolisms
- Observed :
  - Cathodic current (electron source for microbes)
  - Anodic current (terminal electron acceptor for microbes)
- Cyclic voltammetry used to identify redox reactions at the electrode surface shows sigmoidal curve indicating presence non diffusing enzymes capable of acetate oxidization.
- ISEC : continuous flow reactor + interaction between various potentials
- ESEC : Batch-fed reactor + no interaction between potentials.

From field to the lab

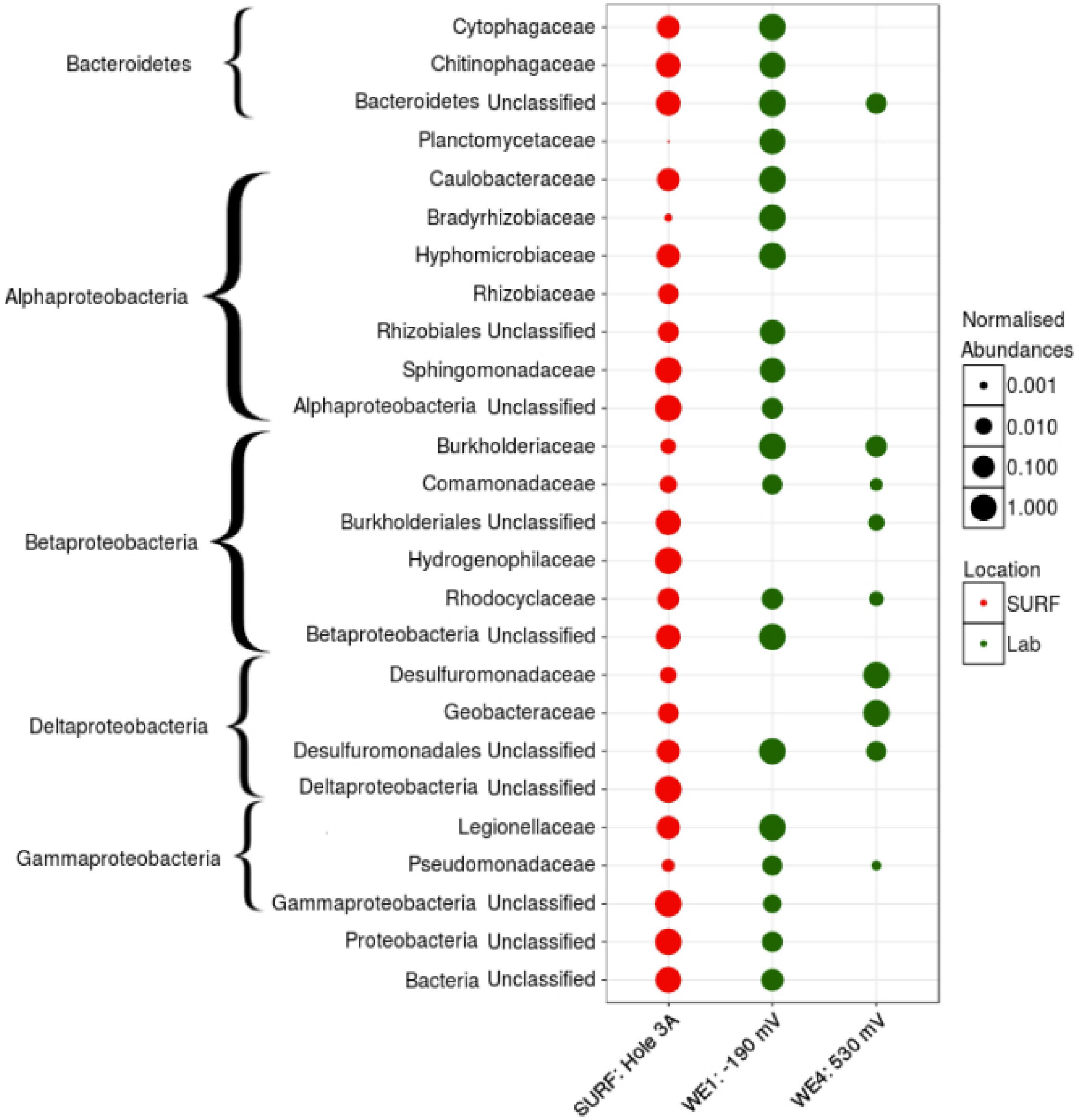




-190 mV vs SHE

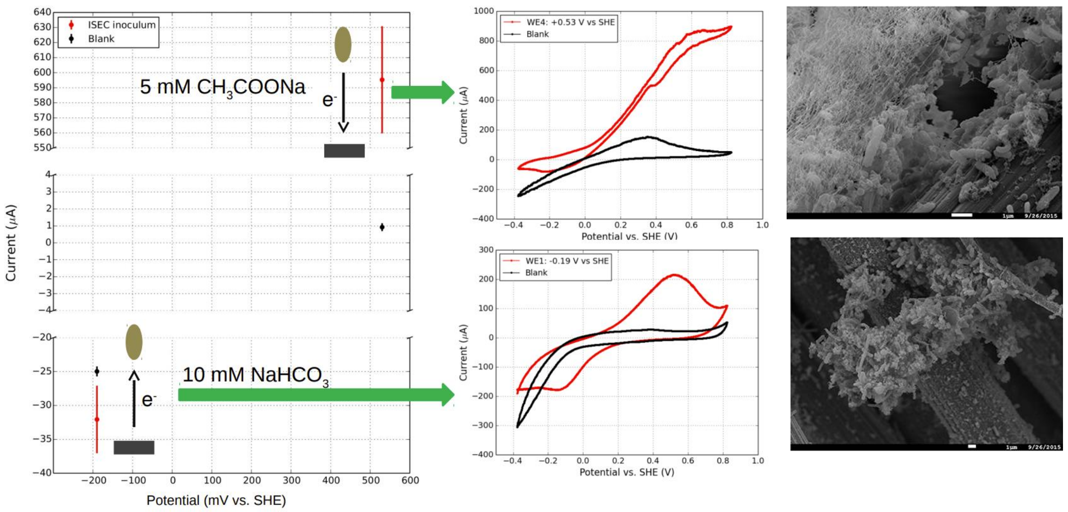


+530 mV vs SHE

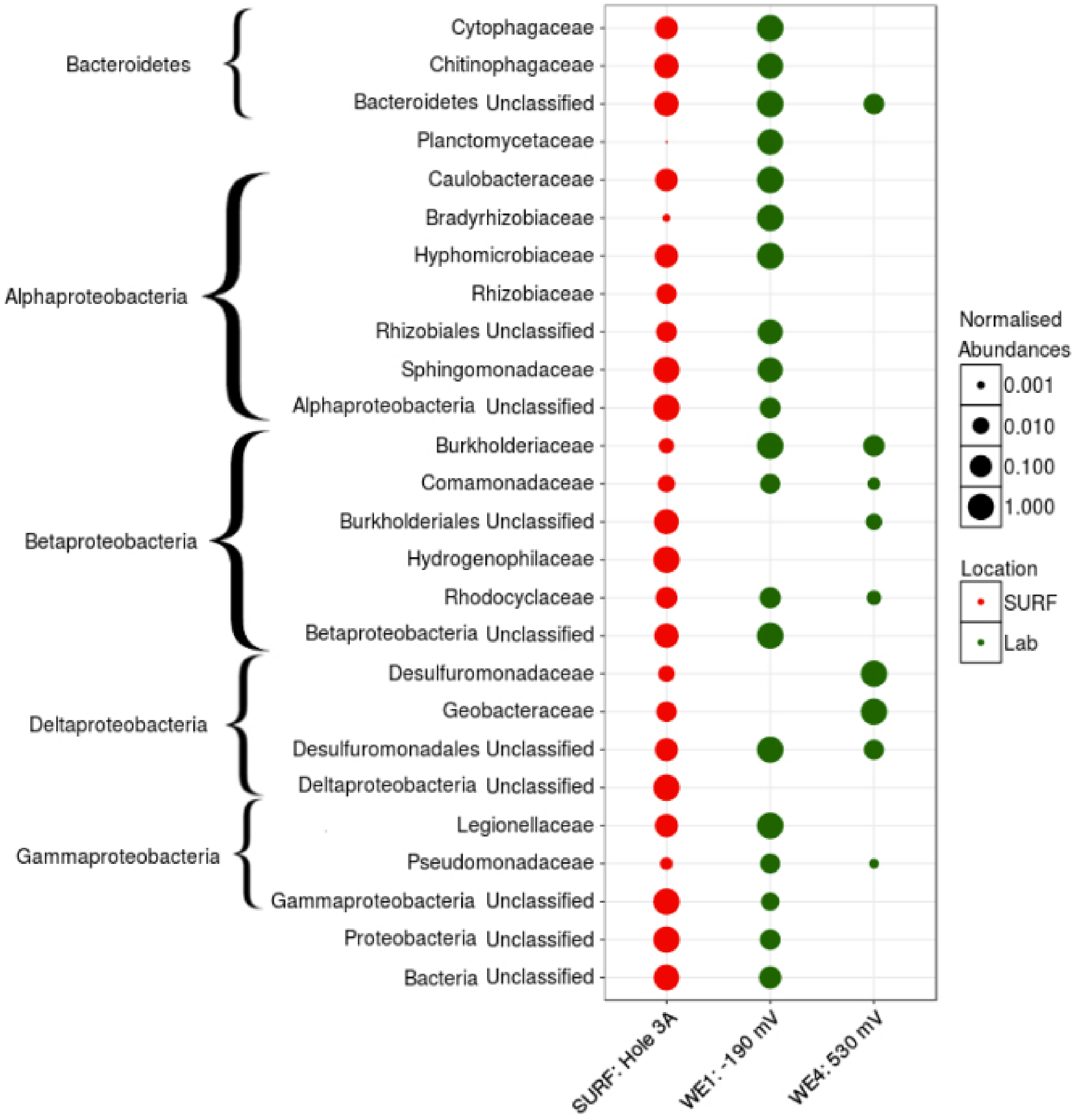


| Jangir et al | Front. Energy Res. | 2019 |

From field to the lab



- ESEC active microbial community:
  - Higher/lesser diversity at cathodic/anodic potentials
  - Unexplored metabolism of possible electron uptake mechanisms in the subsurface biosphere
  - *Geobacteraceae* and *Desulfuromonadaceae* – well studied model organisms for microbe-mineral interactions.

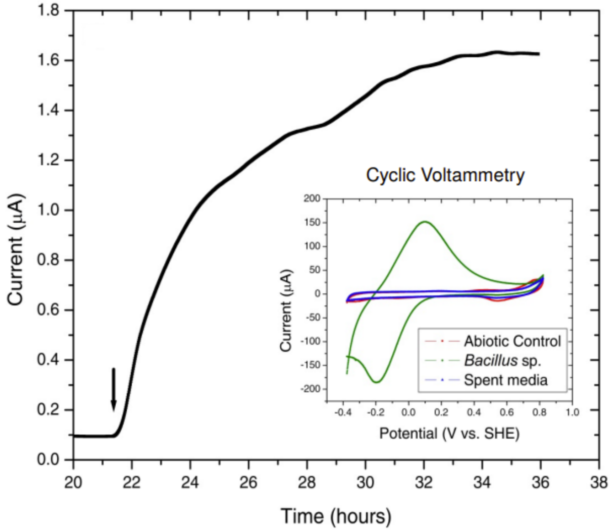


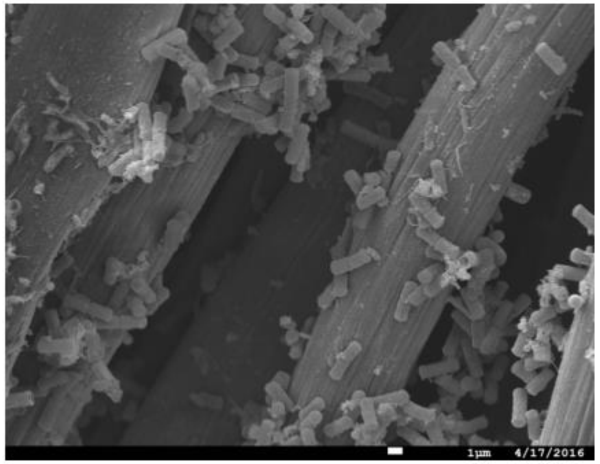
Firmicute Bacillus sp. (strain WE4-1A1-BC)

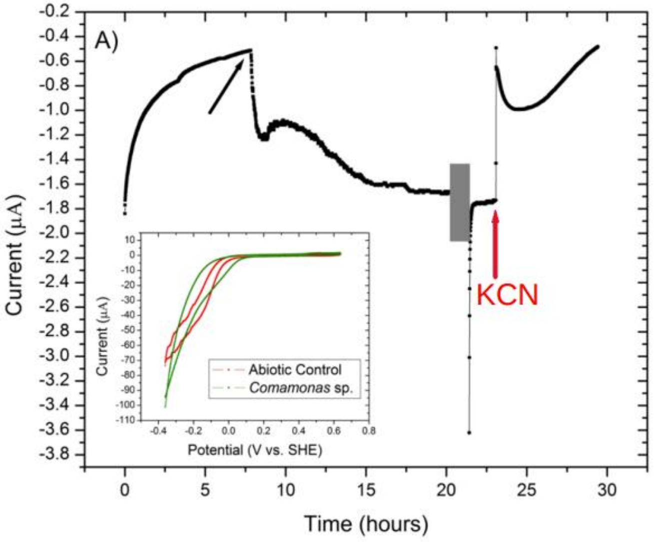
- Electrode poised at +0.530 V vs. SHE acts as an electron source
- Anodic current increases 10 folds from the abiotic baseline
- Reversible peak did not arise form any soluble mediators.
- Coulombic efficiency was calculated as 1.81%

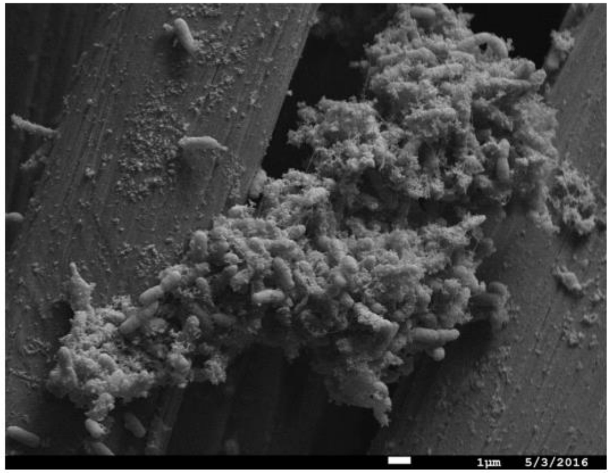
Burkholderiales Comamonas sp. (strain WE1-1D1)

- Electrode poised at −0.012 V vs. SHE acts as an electron source
- Cathodic current increases 3 folds from the abiotic baseline
- Upon addition of K-CN, where CN binds with Cytochrome c oxidase inhibits the electron transfer to oxygen, collapses the electron uptake





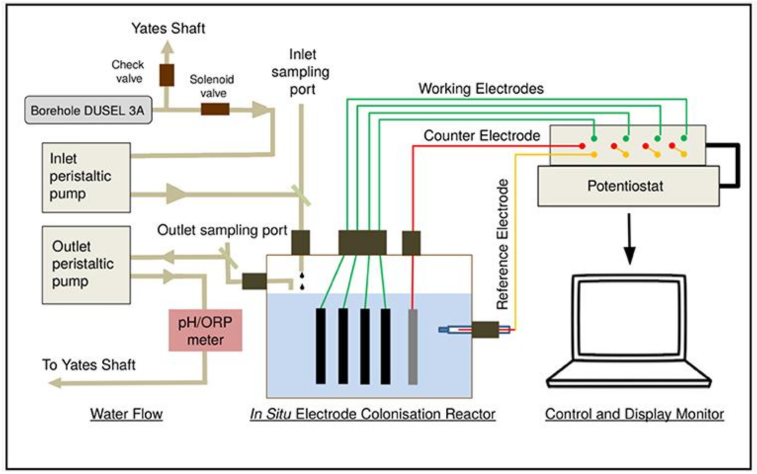




Take aways:

- Poised electrodes serve as *in situ* observatories to capture deep terrestrial biosphere.

- ISEC reactor electrodes were poised to mimic certain metabolisms, the microbial community structure pointed to sequences matching families capable of diverse metabolisms, indicating microbe – microbe interaction active in the subsurface

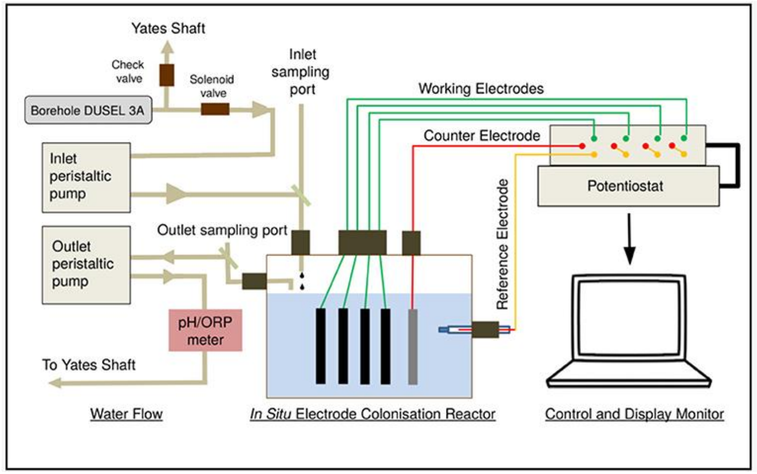


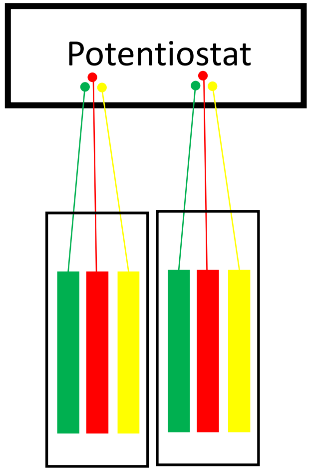
Take aways:

- Poised electrodes serve as *in situ* observatories to capture deep terrestrial biosphere.

- ISEC reactor electrodes were poised to mimic certain metabolisms, the microbial community structure pointed to sequences matching families capable of diverse metabolisms, indicating microbe – microbe interaction active in the subsurface

- Separating the environment in the laboratory incubations resulted in significantly different microbial populations





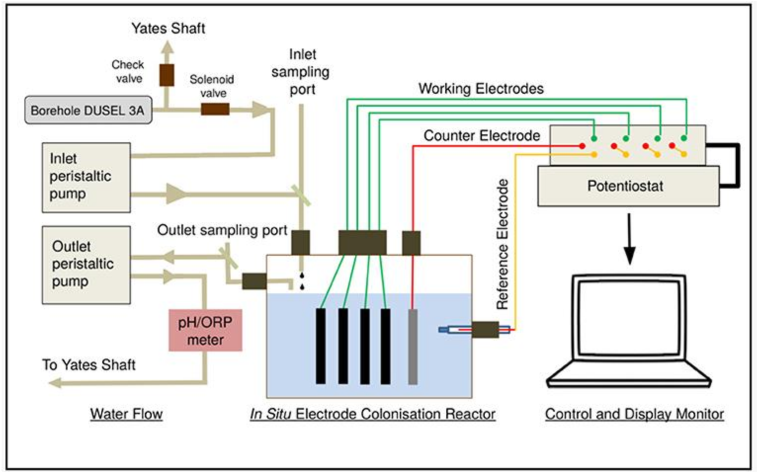
Take aways:

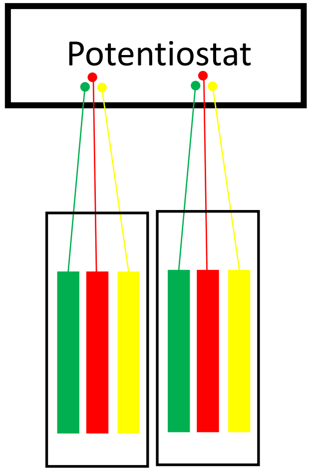
- Poised electrodes serve as *in situ* observatories to capture deep terrestrial biosphere.

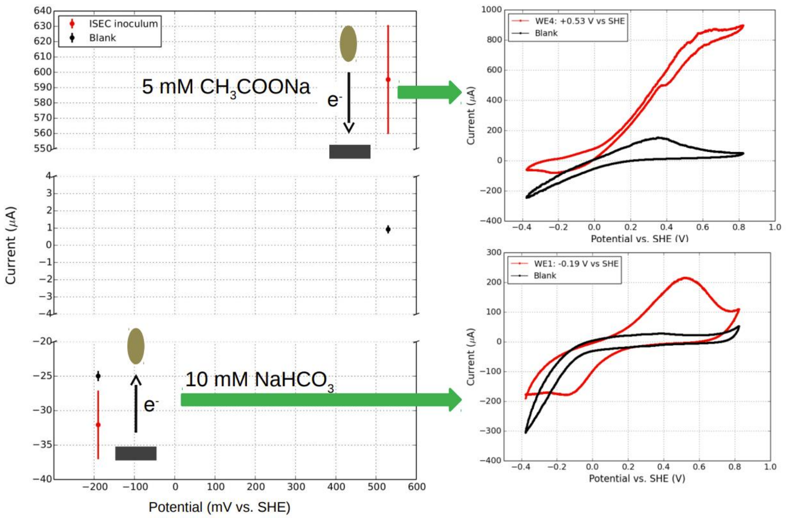
- ISEC reactor electrodes were poised to mimic certain metabolisms, the microbial community structure pointed to sequences matching families capable of diverse metabolisms, indicating microbe – microbe interaction active in the subsurface

- Separating the environment in the laboratory incubations resulted in significantly different microbial populations

- Enriched and isolated cathode-oxidizing and anode-reducing microbes originating from the same environment.







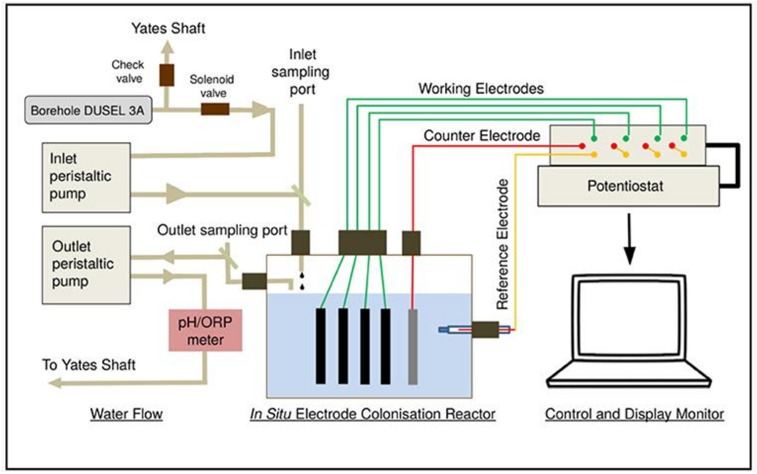
Take aways:

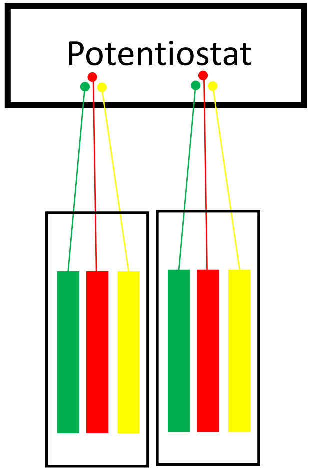
- Poised electrodes serve as *in situ* observatories to capture deep terrestrial biosphere.
- ISEC reactor electrodes were poised to mimic certain metabolisms, the microbial community structure pointed to sequences matching families capable of diverse metabolisms, indicating microbe – microbe interaction active in the subsurface

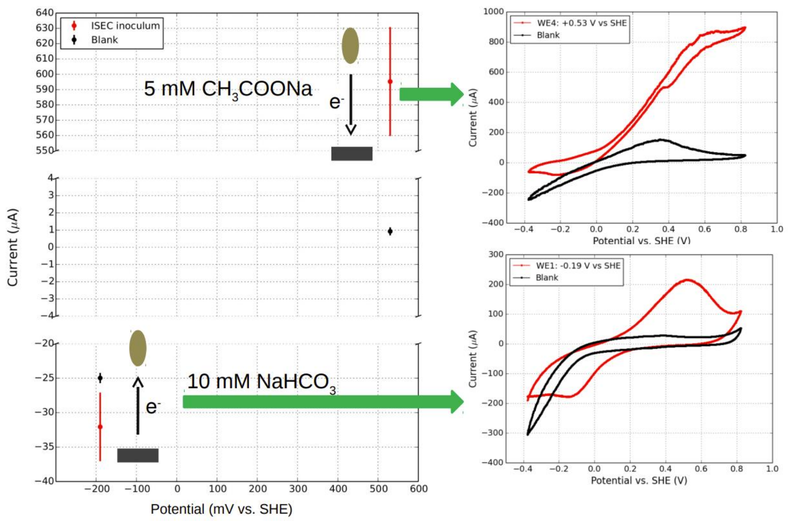
In situ Electrochemical Studies of the Terrestrial Deep Subsurface Biosphere at the Sanford Underground Research Facility, South Dakota, USA

Yamini Jangir[1], Amruta A. Karbelkar[2], Nicole M. Beedle[3], Laura A. Zinke[4], Greg Wanger[4], Cynthia M. Anderson[5], Brandi Kiel Reese[6], Jan P. Amend[3,4] and Mohamed Y. El-Naggar[1,2,3]*

- Separating the environment in the laboratory incubations resulted in significantly different microbial populations
- Enriched and isolated cathode-oxidizing and anode-reducing microbes originating from the same environment.



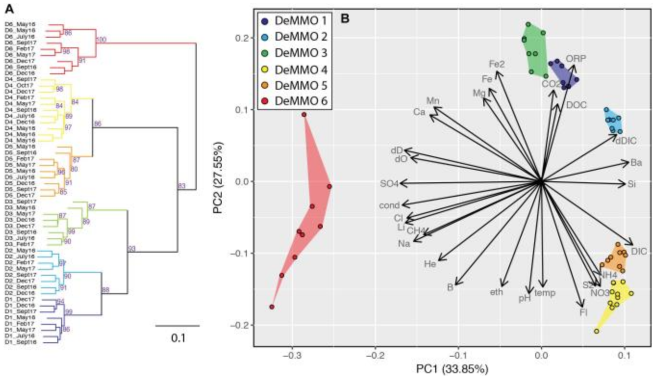




Further interesting work from Sanford Lab

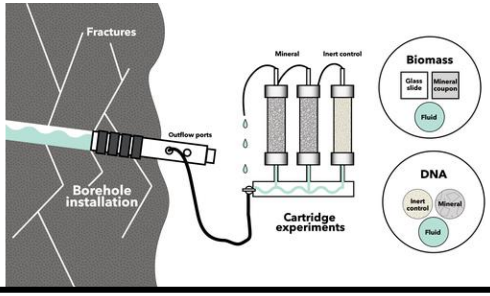
Establishment of the Deep Mine Microbial Observatory (DeMMO), South Dakota, USA, a Geochemically Stable Portal Into the Deep Subsurface

Magdalena R. Osburn[1]*, Brittany Kruger[2], Andrew L. Masterson[1], Caitlin P. Casar[1] and Jan P. Amend[3]



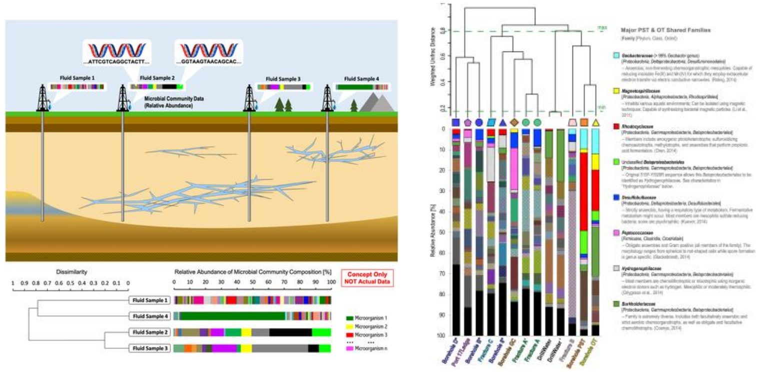
Mineral-hosted biofilm communities in the continental deep subsurface, Deep Mine Microbial Observatory, SD, USA

Caitlin P. Casar, Brittany R. Kruger, Theodore M. Flynn, Andrew L. Masterson, Lily M. Momper, Magdalena R. Osburn



Microbial Community Composition in Deep-Subsurface Reservoir Fluids Reveals Natural Interwell Connectivity

Yuran Zhang, Anne E. Dekas, Adam J. Hawkins, Alma E. Parada, Oxana Gorbatenko, Kewen Li, Roland N. Horne



Electrochemical evidence for in situ microbial activity at the Deep Mine Microbial Observatory (DeMMO), South Dakota, USA

Annette R. Rowe, Karla Abuyen, Bonita R. Lam, Brittany Kruger, Caitlin P. Casar, Magdalena R. Osburn, Mohamed Y. El-Naggar, Jan P. Amend