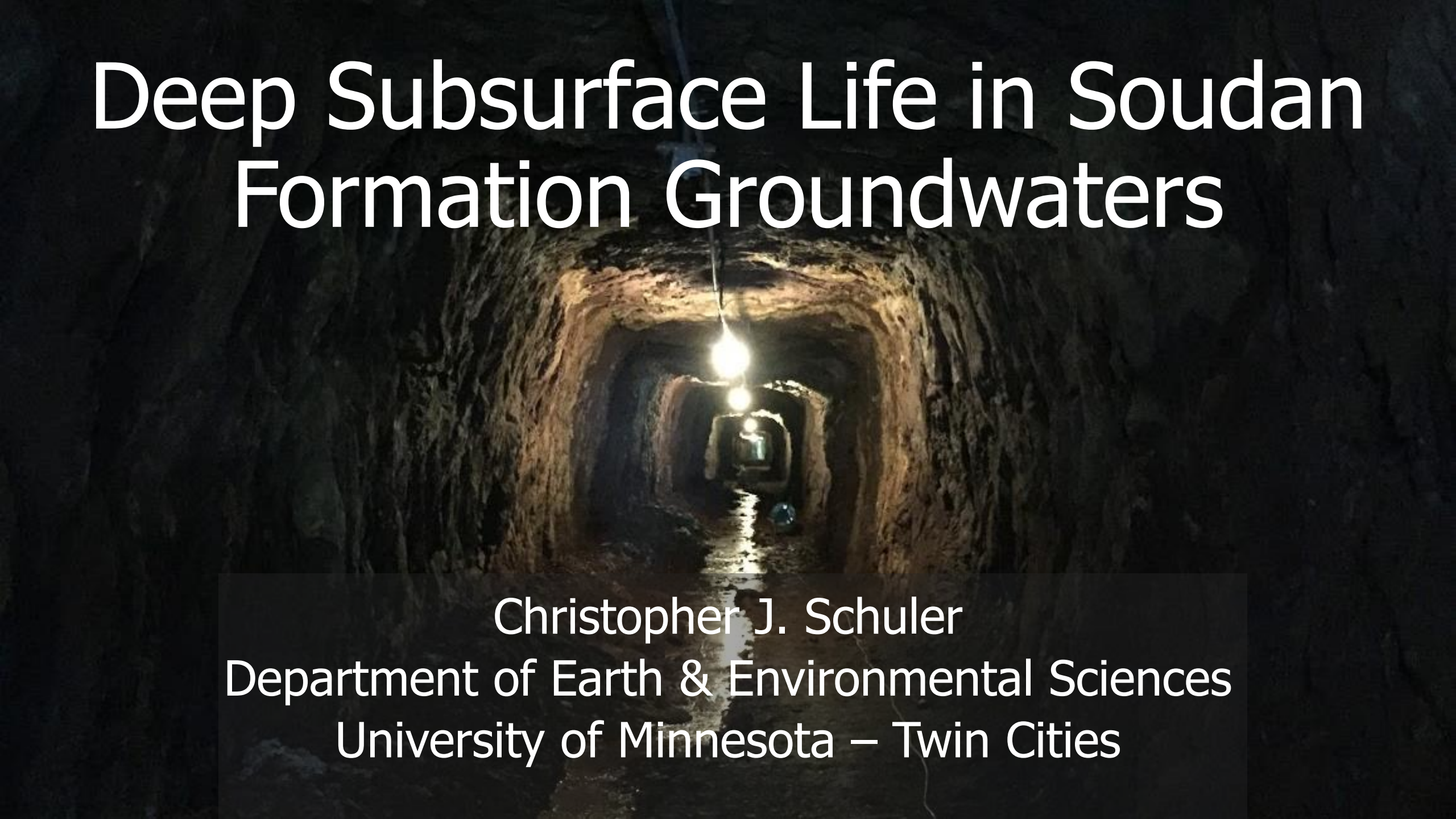
Deep Subsurface Life in Soudan Formation Groundwaters



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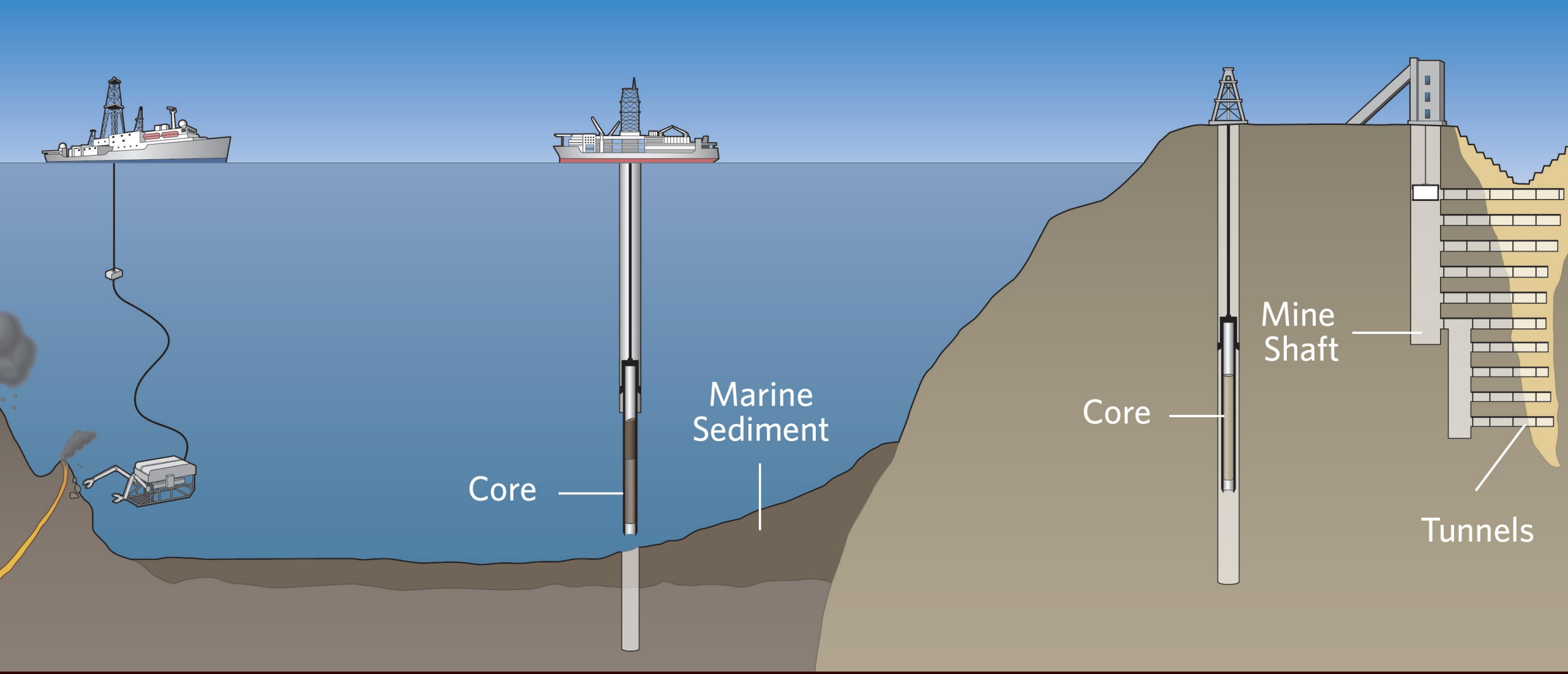
Thanks & Acknowledgements

- **Advisors**
  - Cara Santelli & Brandy Toner
- **First round of Soudan researchers**
  - Lindsey Briscoe, Scott Alexander, Calvin Alexander, Jeff Gralnick
- **Current Soudan team**
  - Billy Dowd, David Hsu, Woonghee Lee, Amanda Patsis, Daniel Bond, Peter Kang, Jill McDermott, Cody Sheik

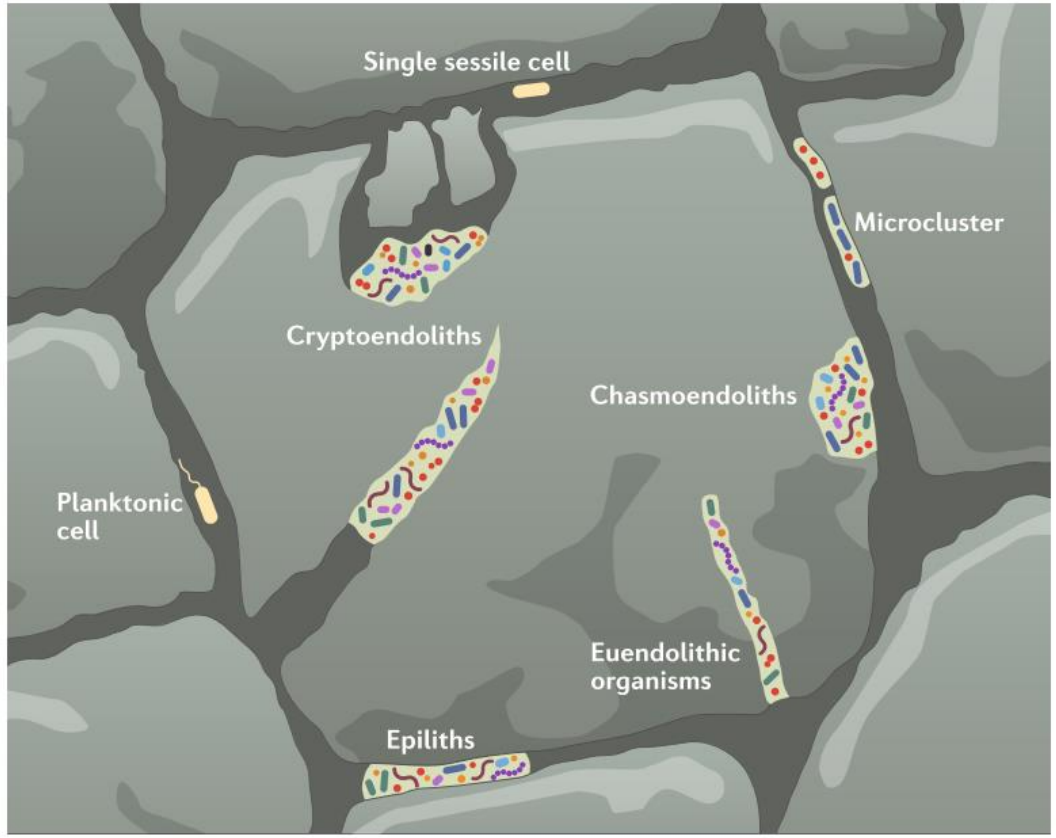


The Deep Biosphere

The Scientist, "Life Thrives within the Earth's Crust"

Life in the Continental Subsurface

- Inorganic substrates are essential to microbial life in the deep biosphere.
- Form fundamental energetic backbone much like sunlight on surface.
- Example processes:
  - Reduction of Fe^{3+} and SO_4^{2-}
  - Oxidation of H_2, H_2S, S^0, CH_4
- Often sourced directly from surrounding rock or result from rock-water interactions.



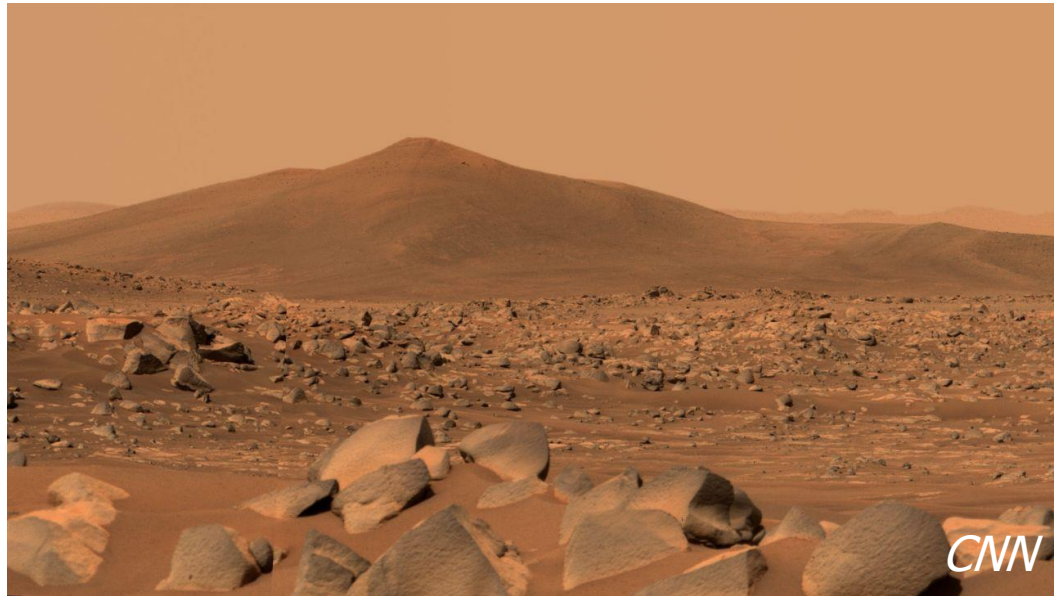
Flemming & Wuertz, *Nature Reviews Microbiology*, 2019

Subsurface Chemistry Impacts Stability of Waste Sites

- Subsurface environments have been suggested as potential storage sites for CO_2 sequestered from the atmosphere and for toxic heavy metals, e.g. nuclear waste.
- Microbes can affect these stability of these arrangements through direct or indirect means.
- Possible changes in pH and redox potential of the environment important to know when planning storage on the order of thousands of years

Deep Crust as Astrobiological Analogue

- Though the surfaces of many worlds without atmospheres are thought to be too harsh for life, the same may not be true of the subsurface.
- Even planets without water today may have had some in the past – studying these environments on Earth can help us understand the signs to look for.
- Can also be seen as an analog for early Earth, before the oxygenation of the atmosphere



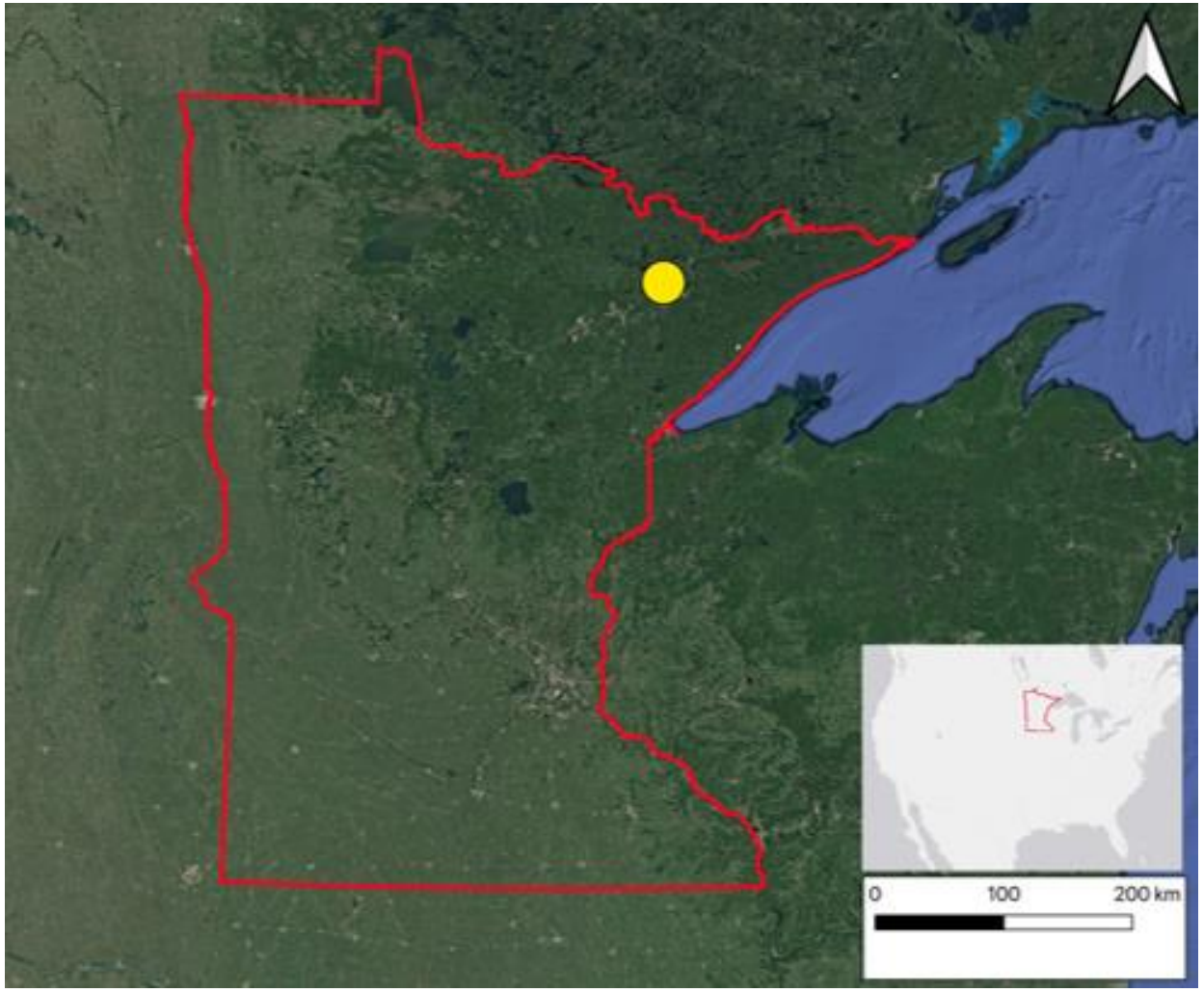
Where has this research been done?

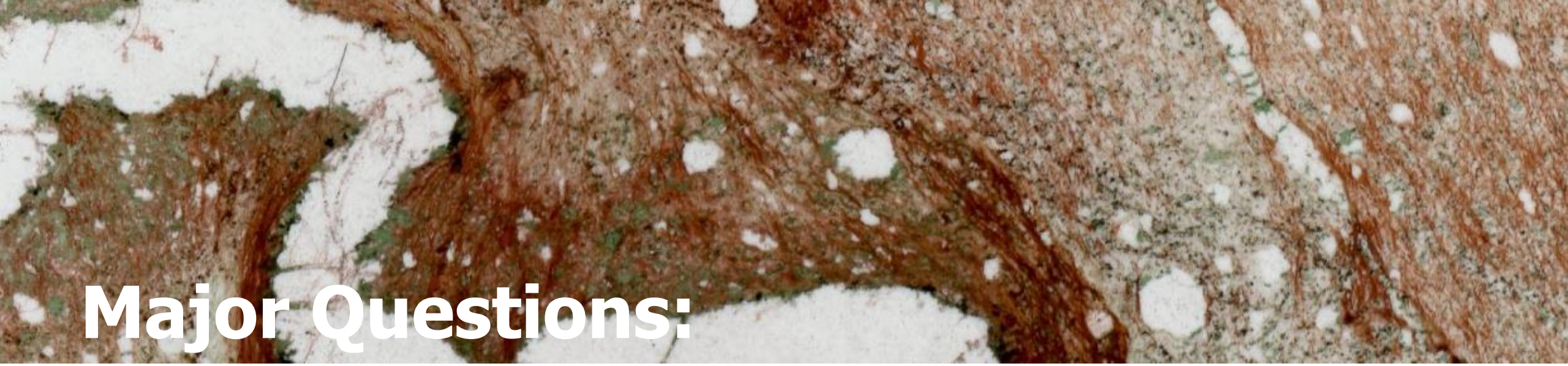
Special interest in regions with old crust


Greenland
Iceland
Norway
Russia
Poland
Germany
Ukraine
France
Italy
Turkey
Kazakhstan
Mongolia
China
South Korea
Afghanistan
Iraq
Iran
Pakistan
India
Thailand
Algeria
Libya
Egypt
Saudi Arabia
Mali
Niger
Chad
Sudan
Nigeria
Ethiopia
Kenya
DRC
Tanzania
Angola
Namibia
Madagascar
South Africa
Mexico
Venezuela
Colombia
Peru
Brazil
Bolivia
Chile
Argentina
Indonesia
Canadian Shield has not yet received the same interest

Our research helps fill in that gap!

Soudan Underground Mine State Park



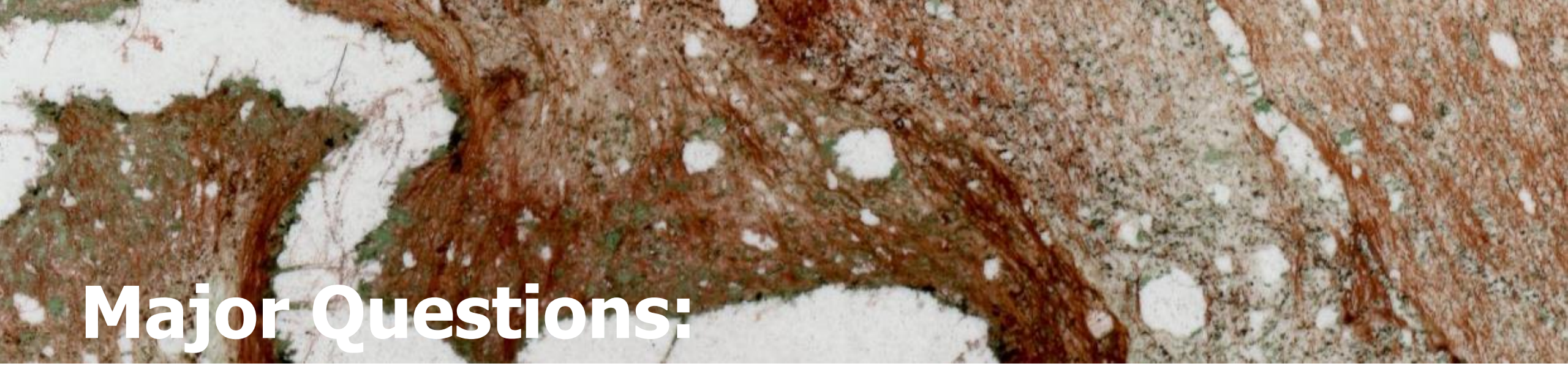


Major Questions:

How can geochemistry help us better understand microbial life in the subsurface?

Two examples:

1. Can water chemistry help guide our site selection and help us better understand where to focus research efforts?
2. How much do microbes in the subsurface really interact with the minerals surrounding them?



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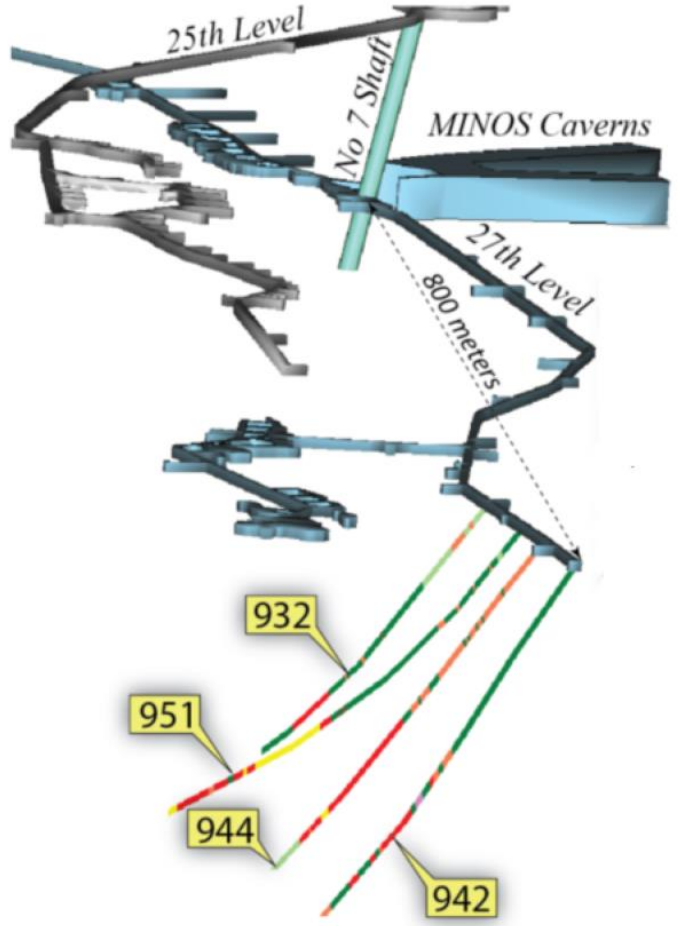
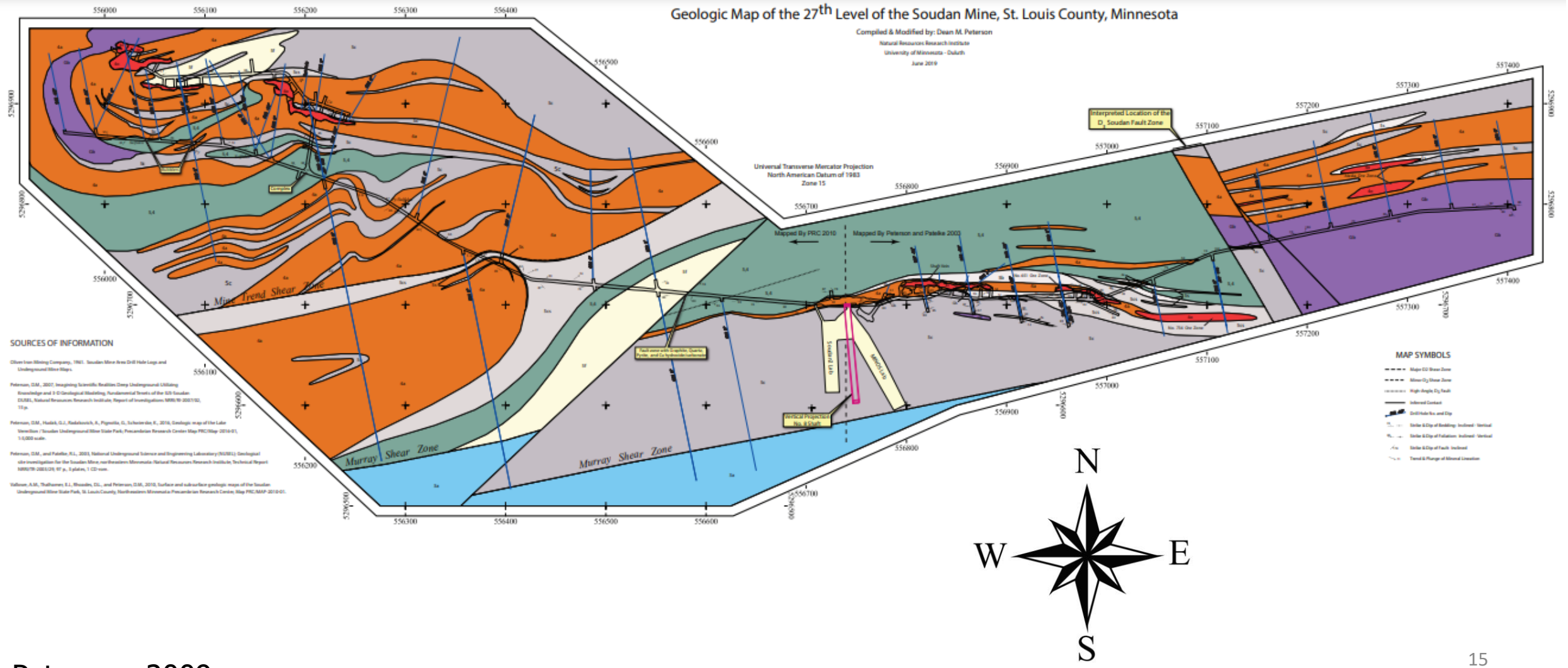
Access to deep fractures through boreholes



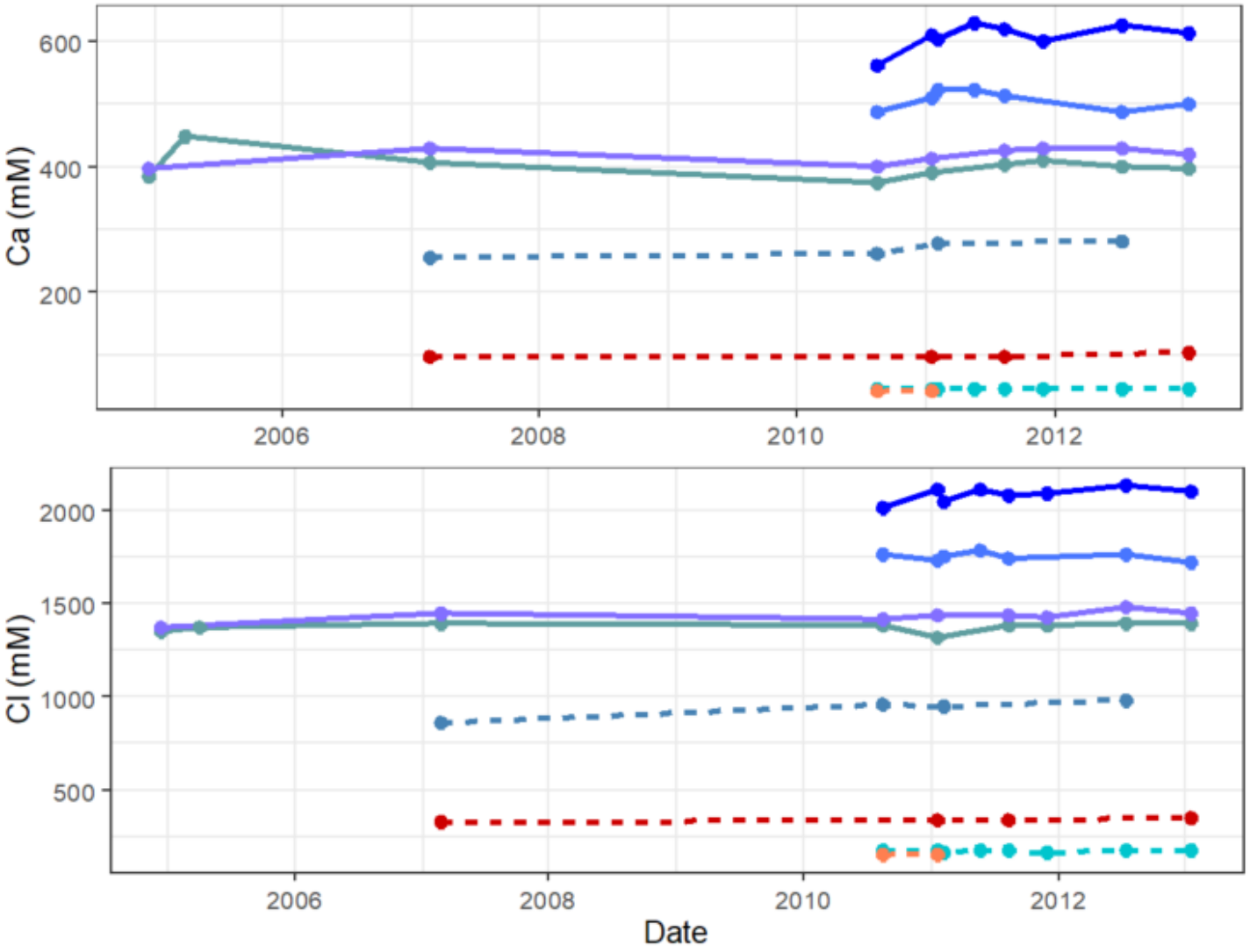
Toner et al.

Mine divided into East and West Drifts



Peterson, 2009

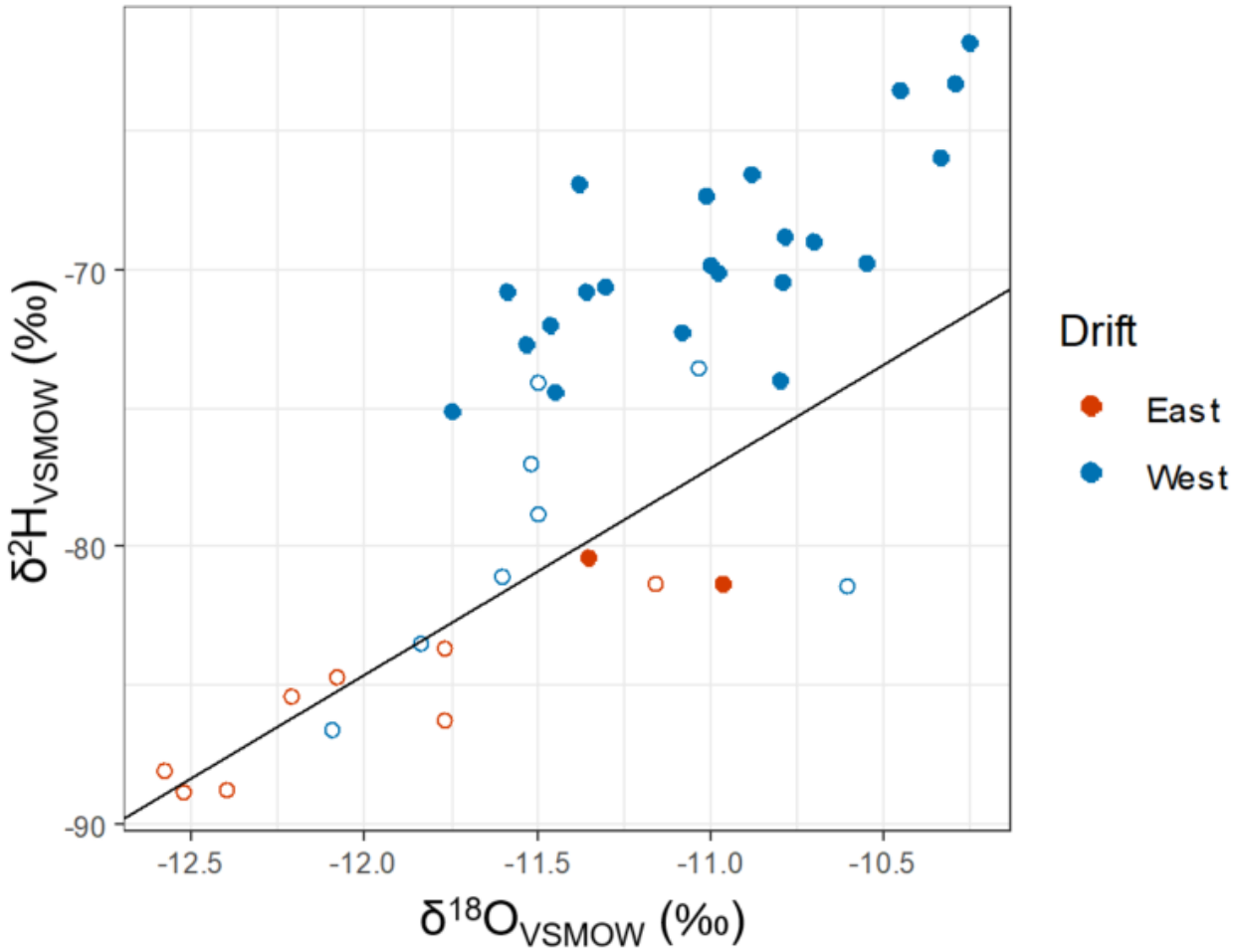
Stable Water Chemistry, but Definite Differences Between Boreholes



- Red lines indicate East Drift boreholes, while blue lines indicate West drift boreholes
- Solid lines indicate boreholes drilled at a downward angle, while dashed lines indicate boreholes drilled at a horizontal angle
- West drift, downward boreholes more saline!

Schuler et al., *Frontiers in Earth Science*, 2022

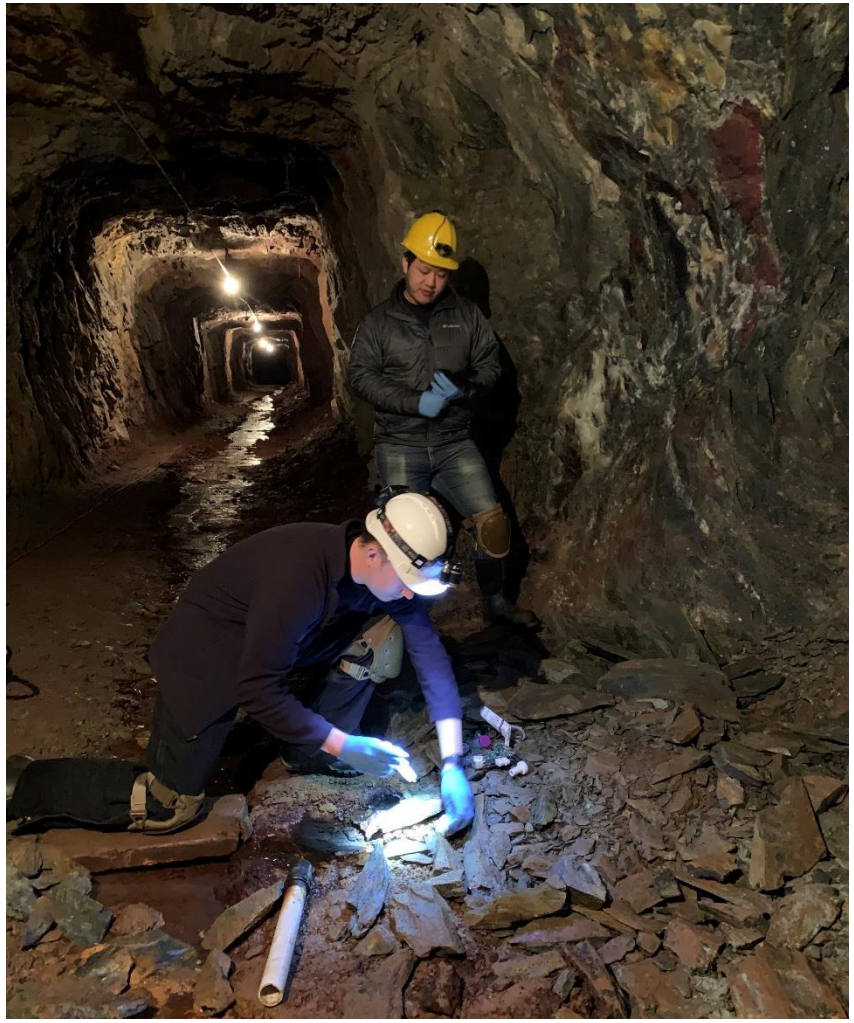
Water isotopes indicate isolated fluids

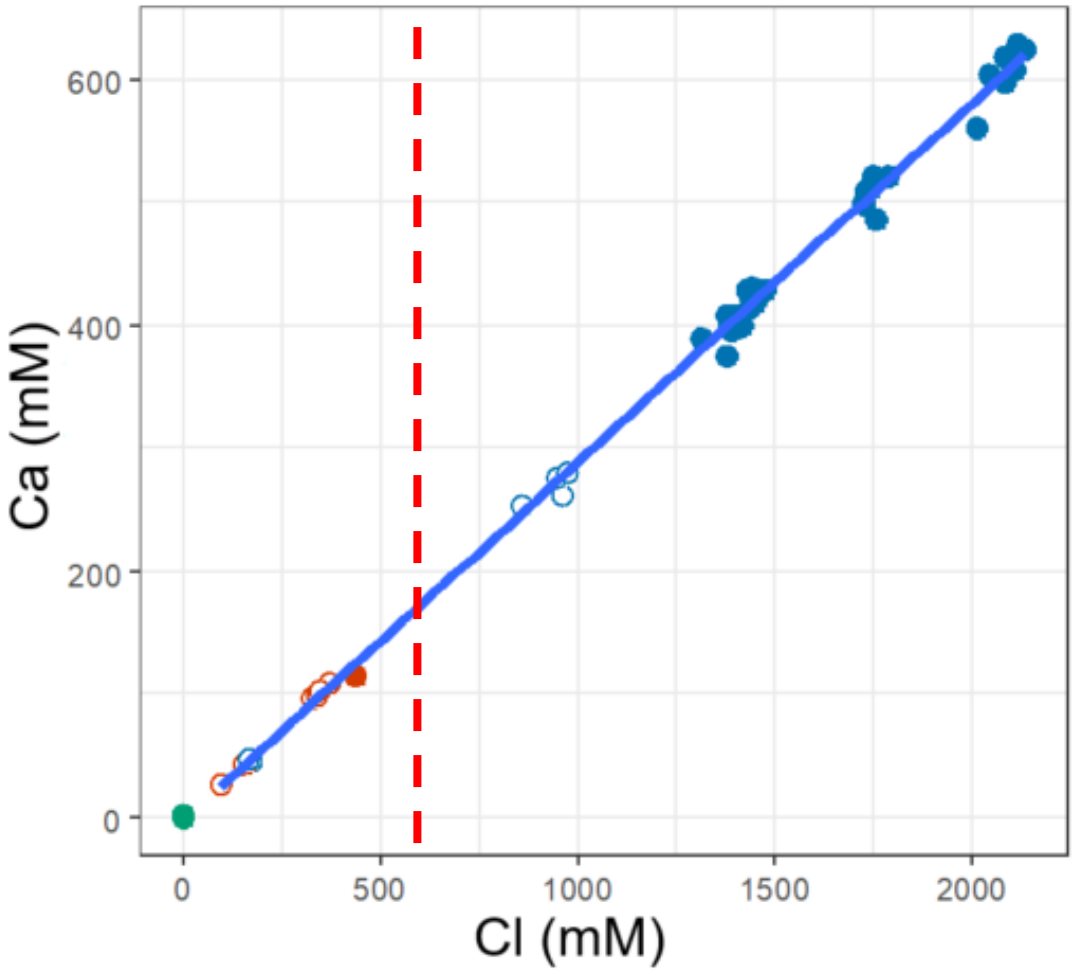


- Waters with a significant meteoric contribution/low residence time plot along the local meteoric water line.
- Long term, low-temperature interactions with silicate rocks causes enrichment in ^2H relative to ^{18}O
- West Drift waters have longer residence time in subsurface, less contribution from meteoric surface inputs

Schuler et al., *Frontiers in Earth Science*, 2022

Salinity Seems to Be Derived from a Single Source



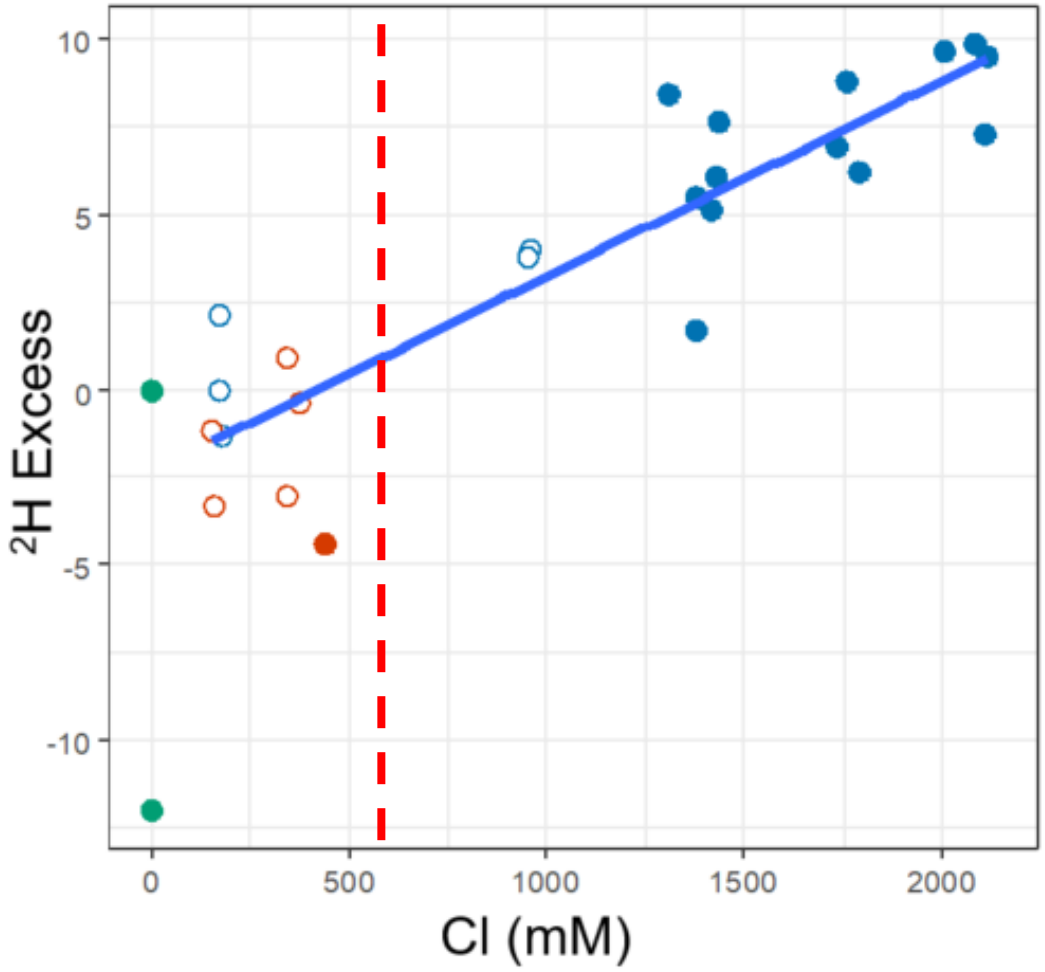


Sample Location
- East Drift
- Surface
- West Drift

Schuler et al. *Frontiers in Earth Science*, 2022

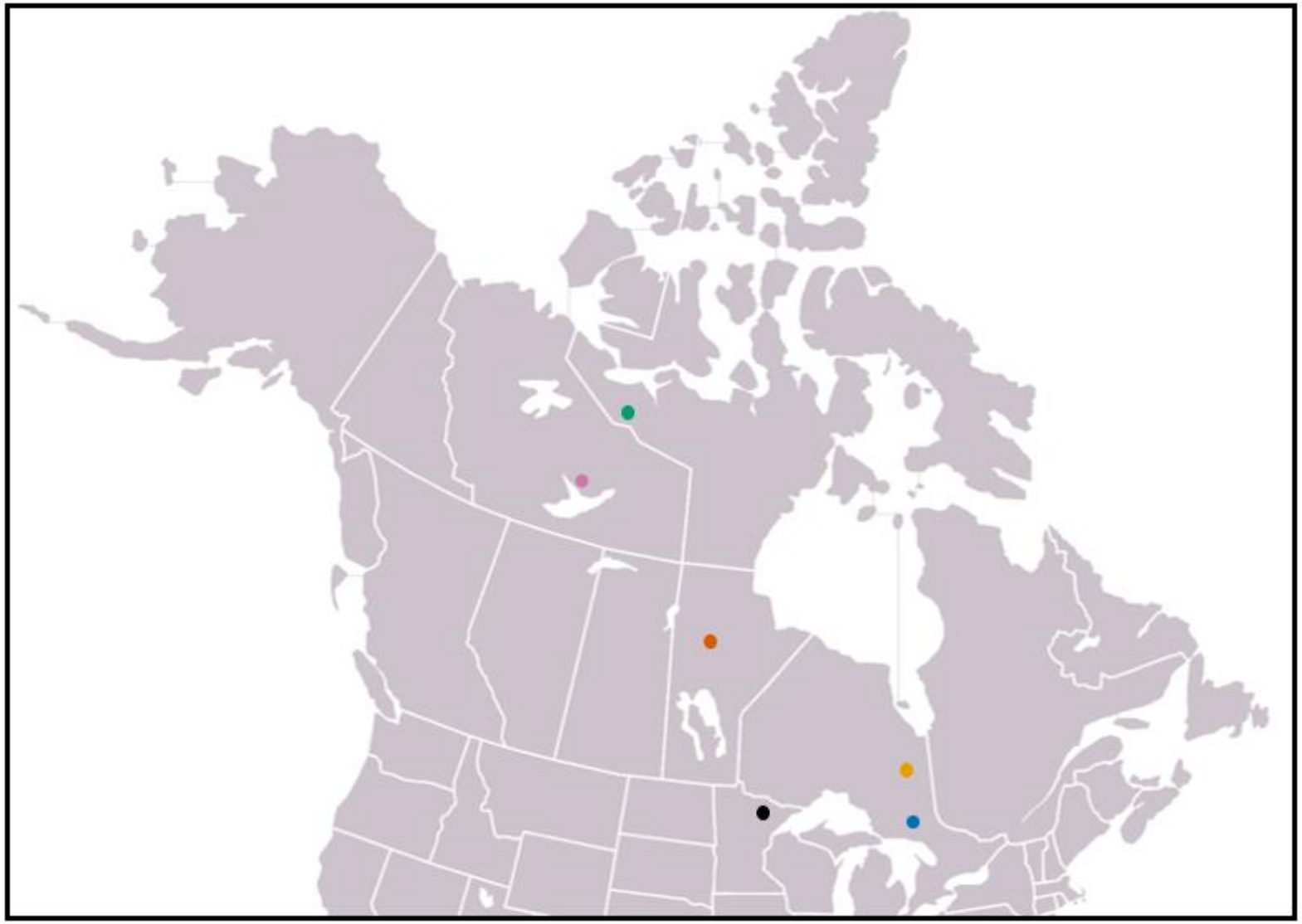
Saline Waters Show Longest Isolation From Surface

- ^{2}H Excess measures deuterium abundance relative to ^{18}O
- Meteoric water has ^{2}H Excess of 0
- Low-temp interactions between water and rock elevates ^{2}H excess
- Values above 5 indicate long-term isolation



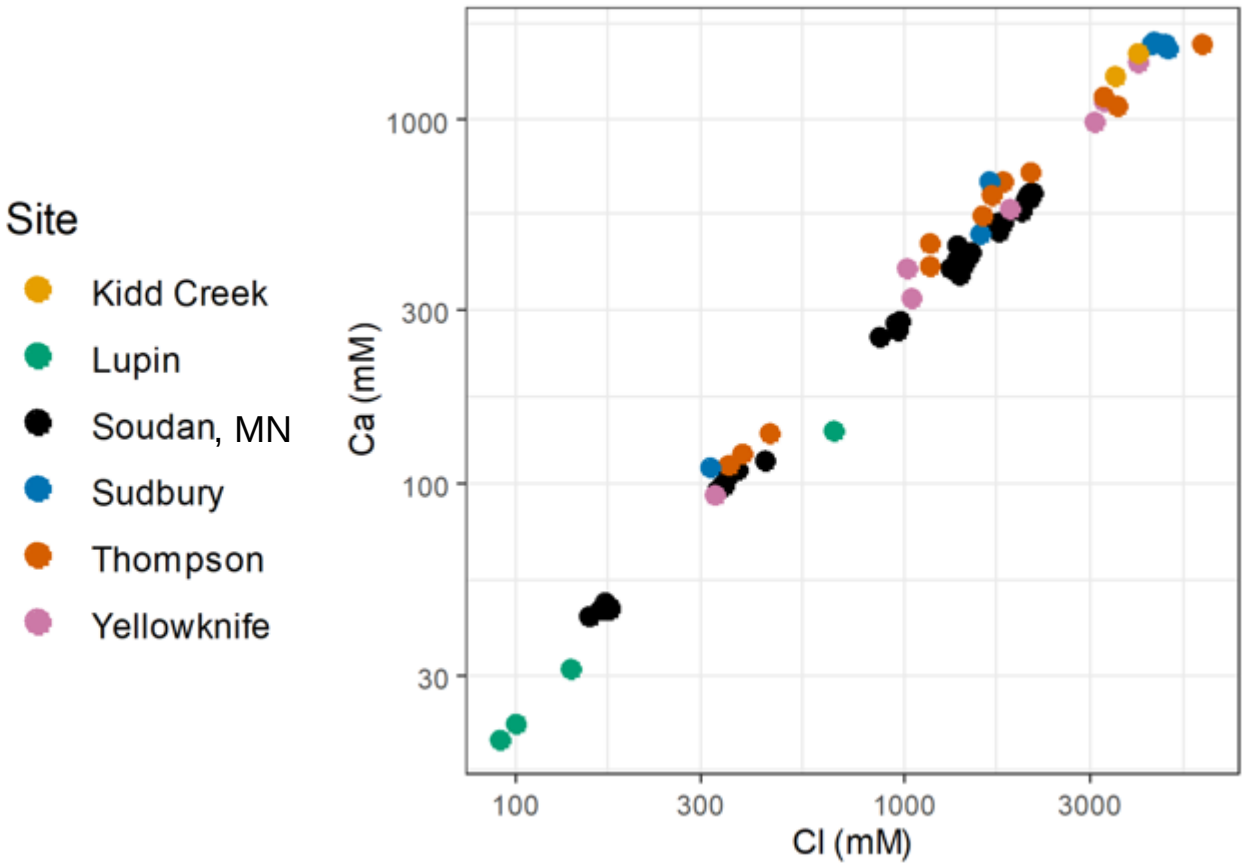
Schuler et al. *Frontiers in Earth Science*, 2022

Soudan and the Broader Canadian Shield



Schuler et al. *Frontiers in Earth Science*, 2022

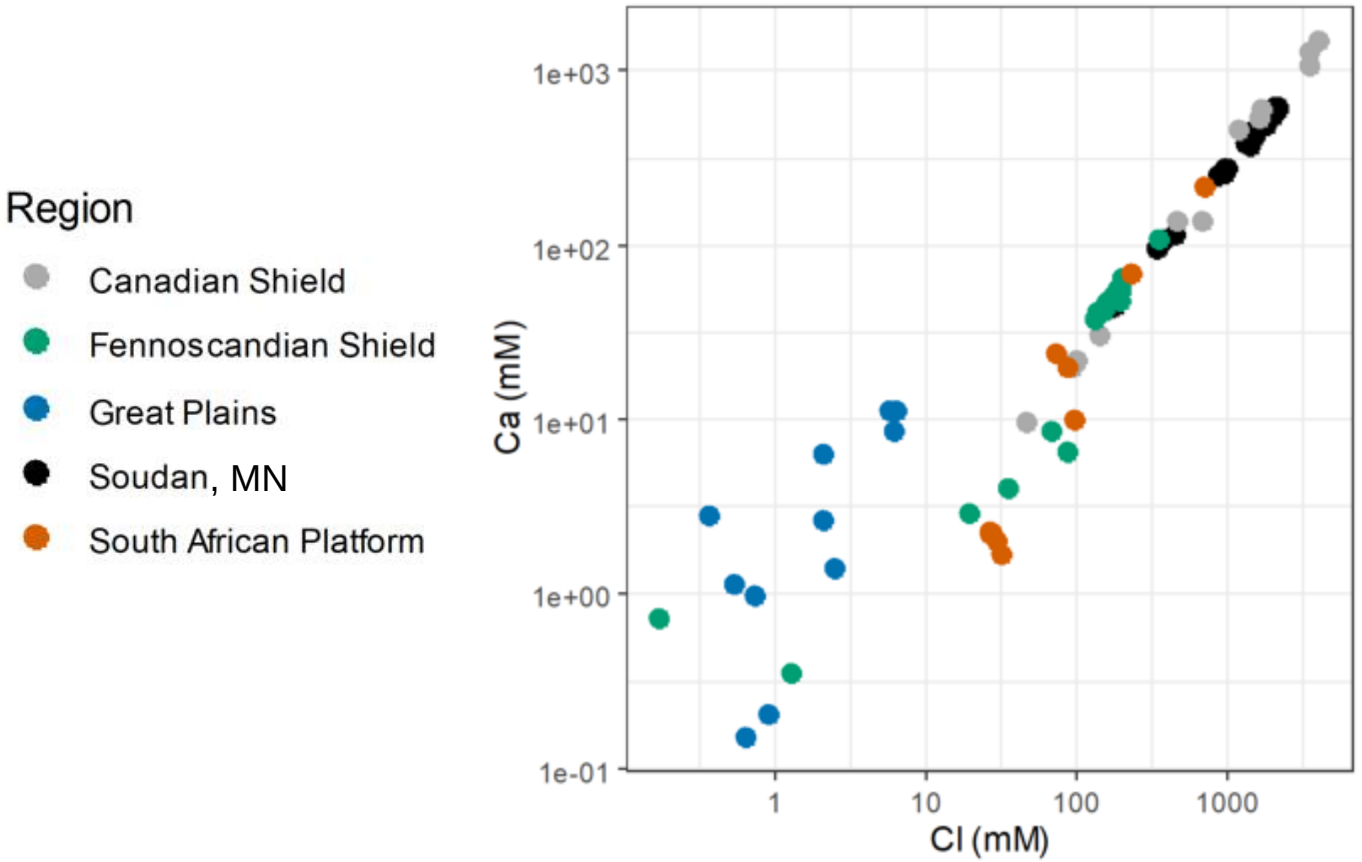
Characteristic Canadian Shield Groundwaters



- Soudan fluids resemble those found in other mines across Canadian shield
- Soudan well poised to provide insight into deep microbiomes across shield
- BIF provides unique look at rock-powered life

Schuler et al. *Frontiers in Earth Science*, 2022

Highest Salinity Deep Biosphere Sites Studied



- Canadian shield contains uniquely saline groundwaters
- Waters from a mine in Ontario have estimated residence time of 1 Ga (Holland et al. *Nature, 2013)*
- Yet understudied relative to Scandinavia & South Africa

Schuler et al. *Frontiers in Earth Science*, 2022

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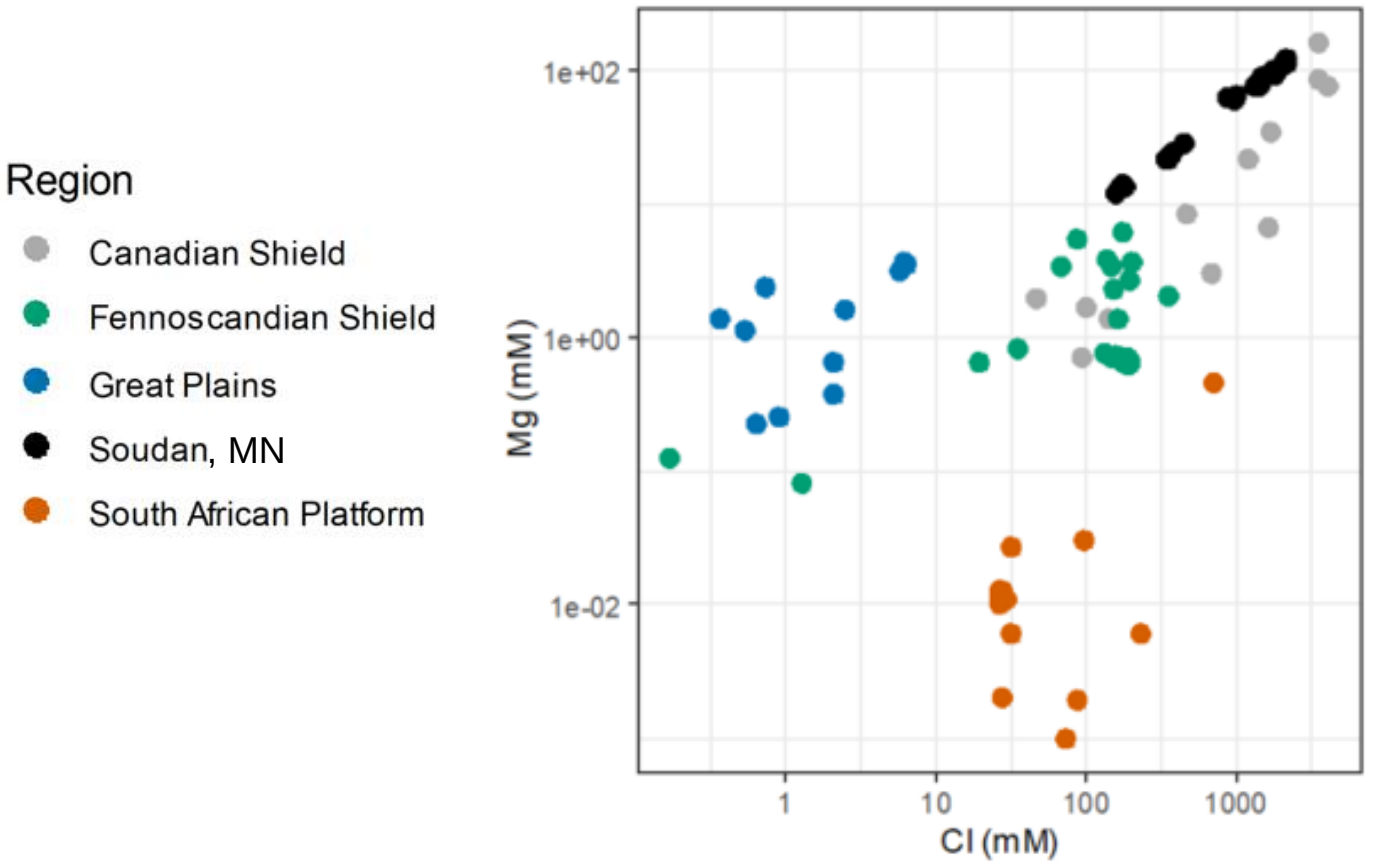


Schuler et al. *Frontiers in Earth Science*, 2022

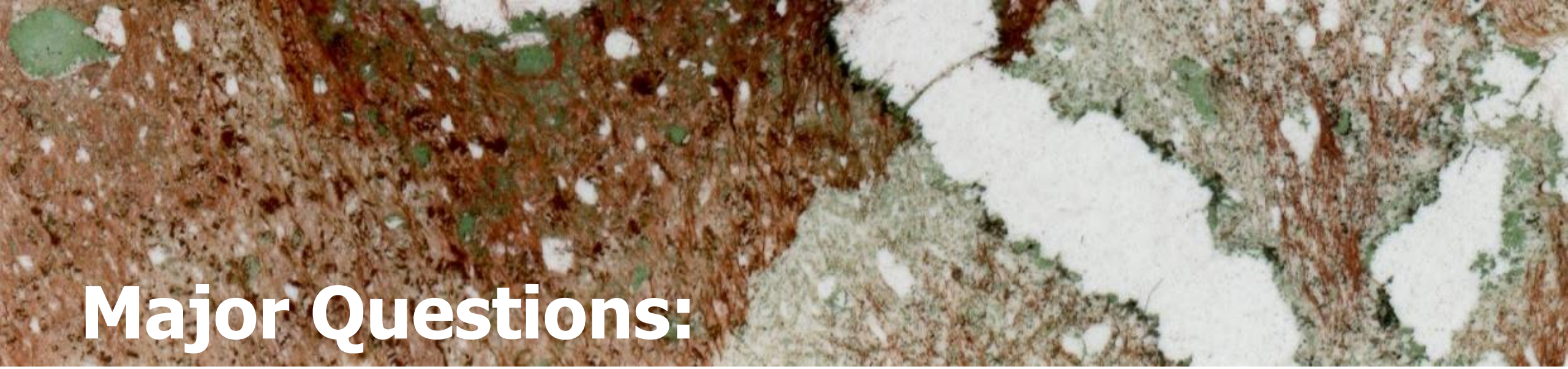
- Soudan has uniquely high Mg concentrations relative to overall salinity
- Mg groundwater concentrations diverse across deep biosphere sites
- Ca-Mg ratios (as well as –Na ratios) point to differences in rock chemistry and subsurface processes.

Major Insights from Water Chemistry

- Some boreholes in Soudan access highly saline groundwaters, but not all do. (But we do see influence of these brines across all Soudan samples.)

- Soudan is representative of broader Canadian Shield with respect to water chemistry. (Though it also has some unique aspects – high Mg and Fe!)

- Canadian shield has potential to host some of the most isolated waters in the world, and therefore potentially the most distinctive microbial communities. (If we take salinity as a barometer for "subsurface character".)



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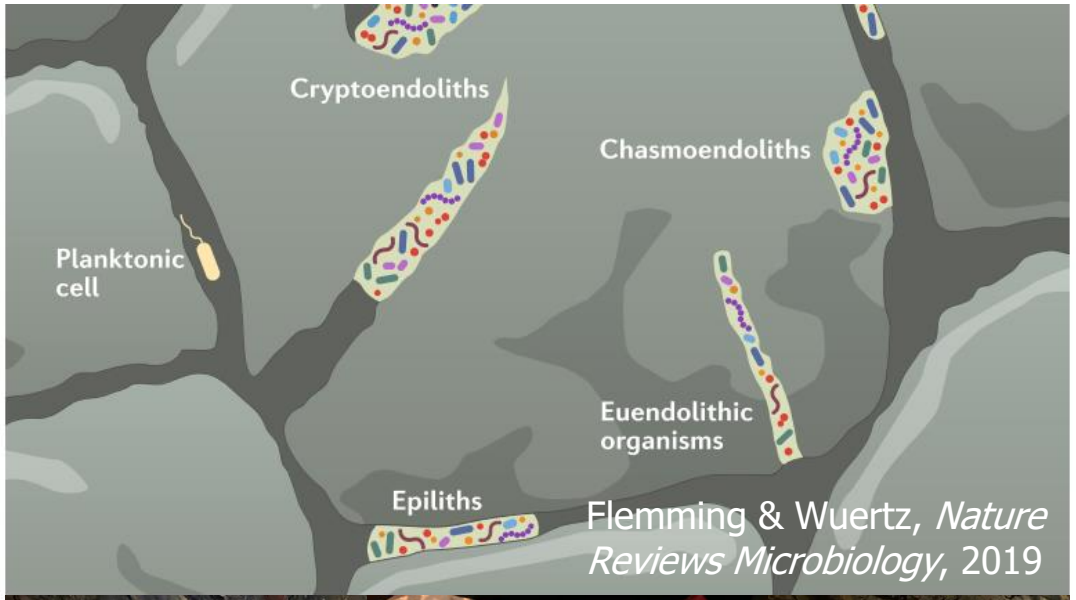
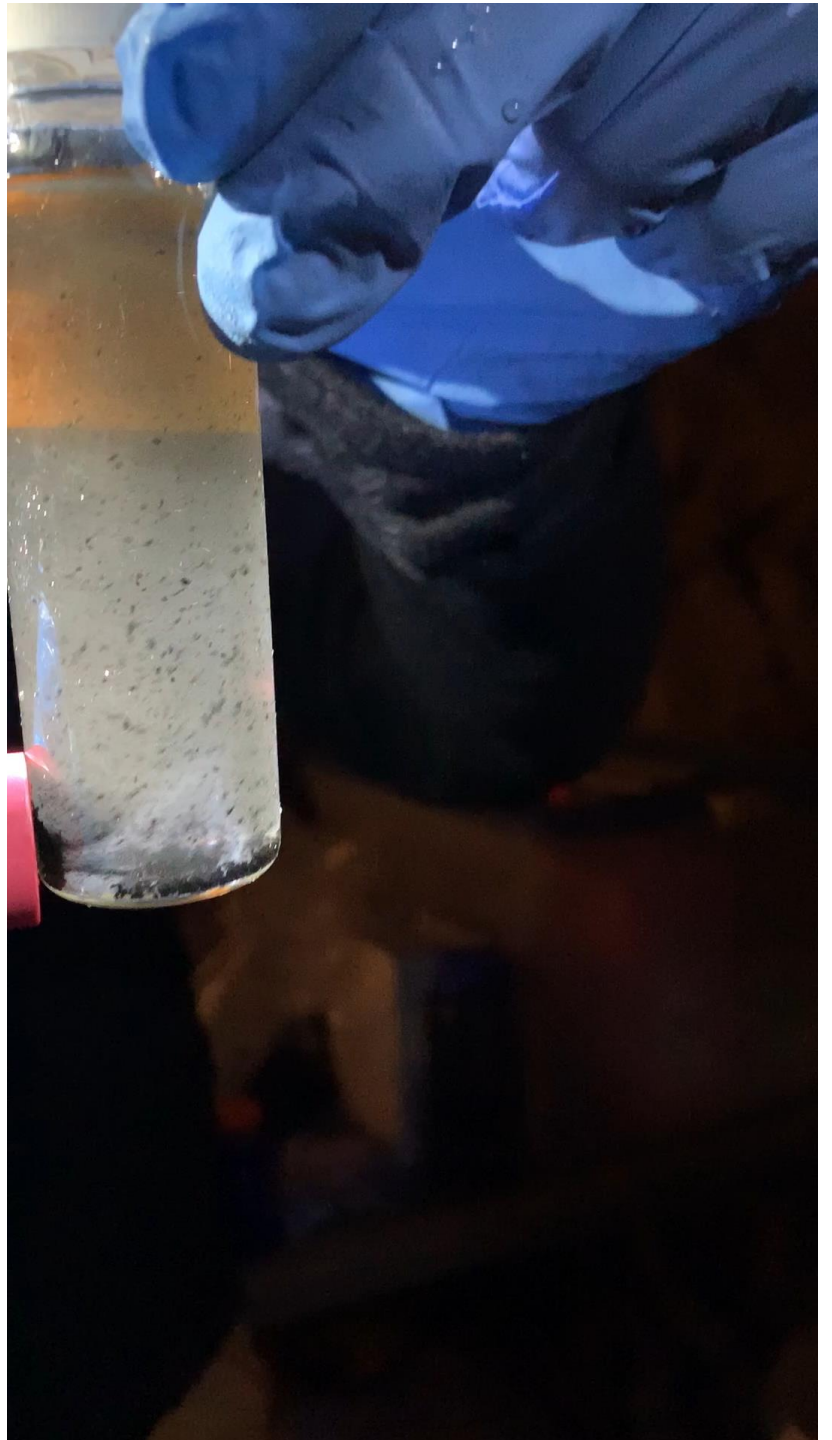
Where is Subsurface Life Really Hosted?

- Vast majority of what we know about these environments comes from water samples
- Distribution of life in the crust still unknown – low cell counts in water, but hard to access actual biofilms
- Fundamental questions remain:
  - **How much of this life resides in biofilms?**
  - **How does biofilm-hosted life differ from planktonic life?**

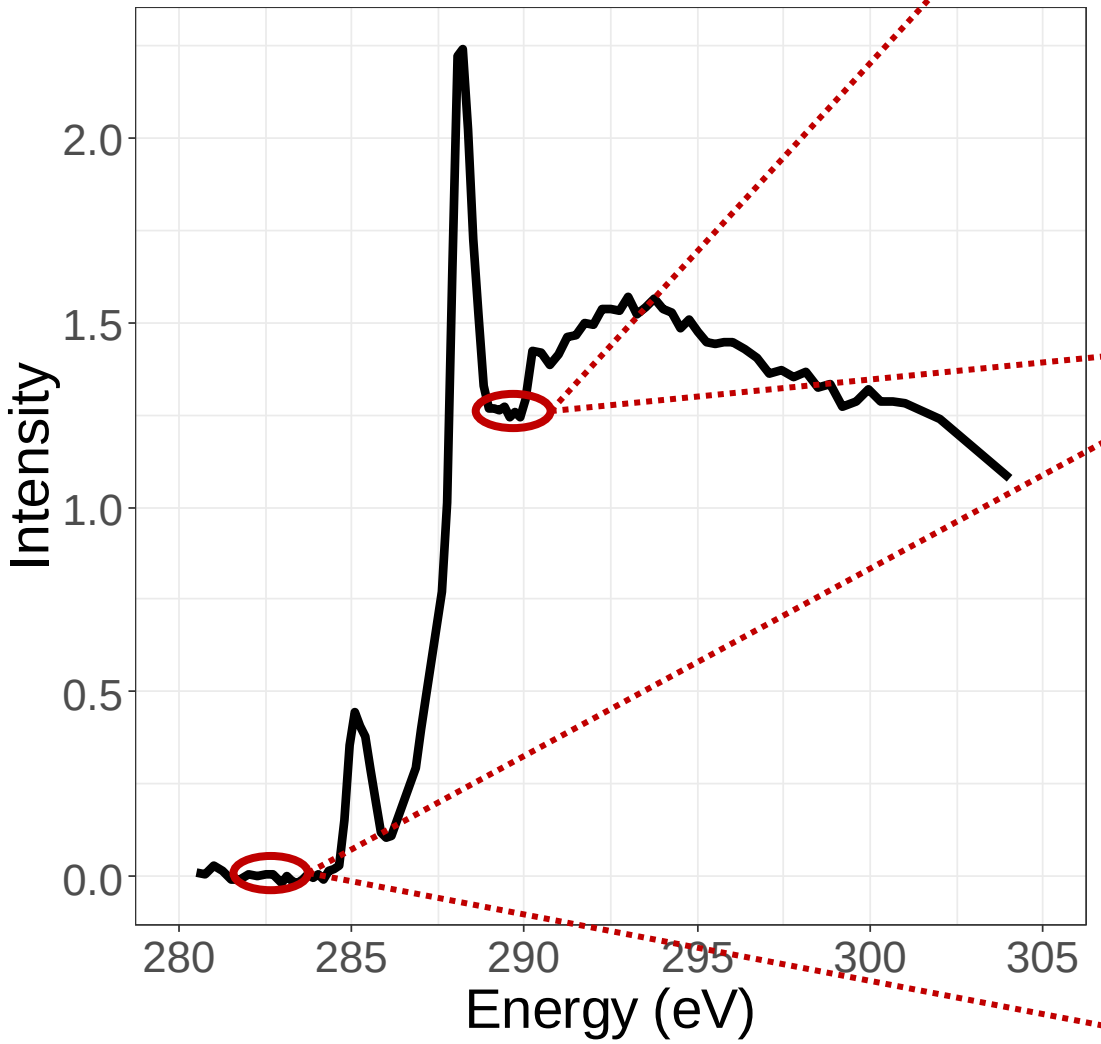


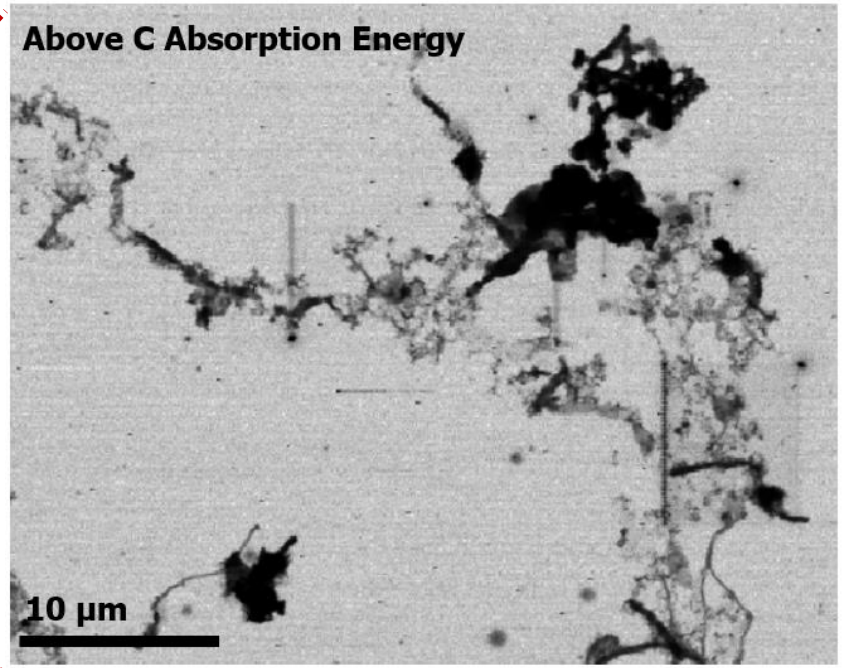
Flemming & Wuertz, *Nature Reviews Microbiology*, 2019

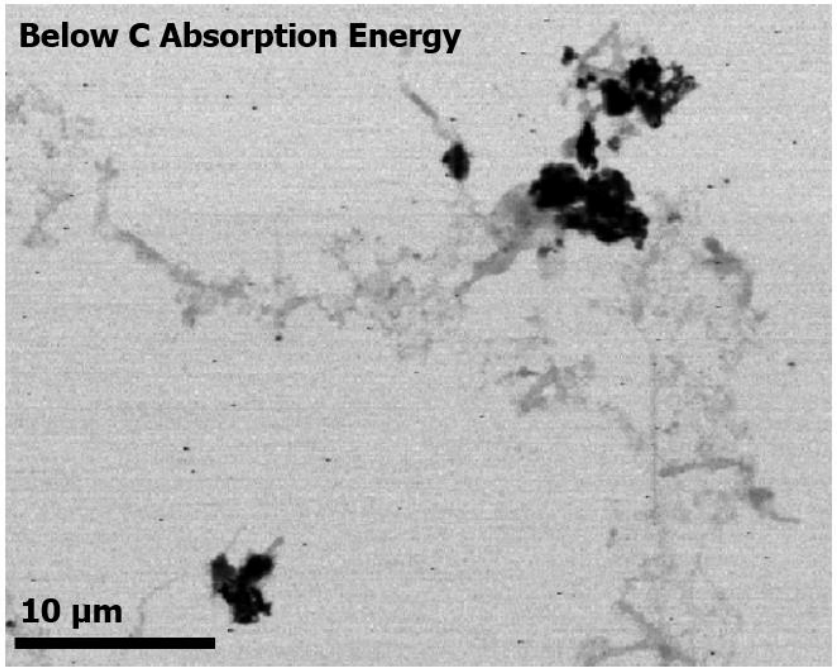
Magnetic Sediments found in Borehole



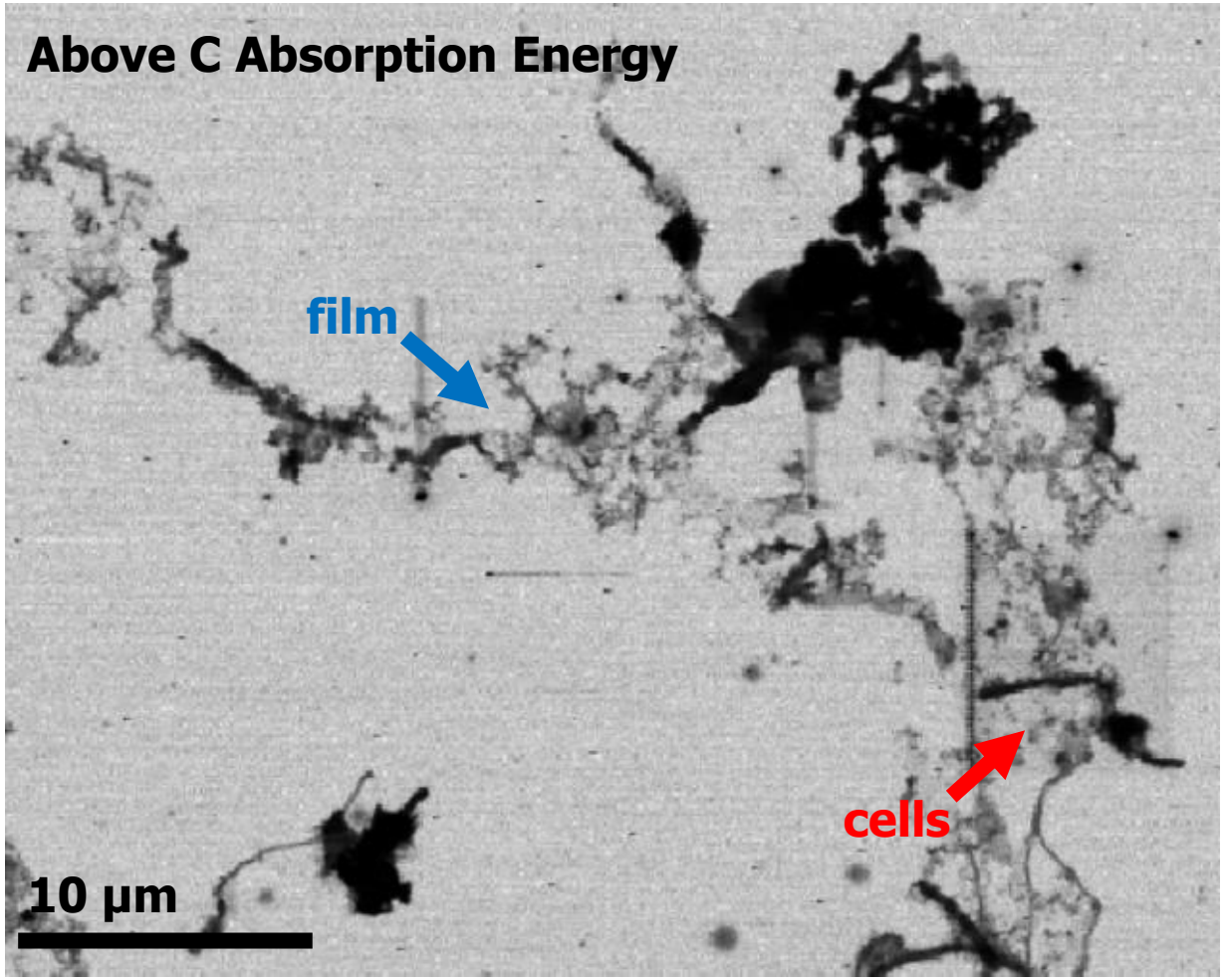
X-ray Absorption Edges

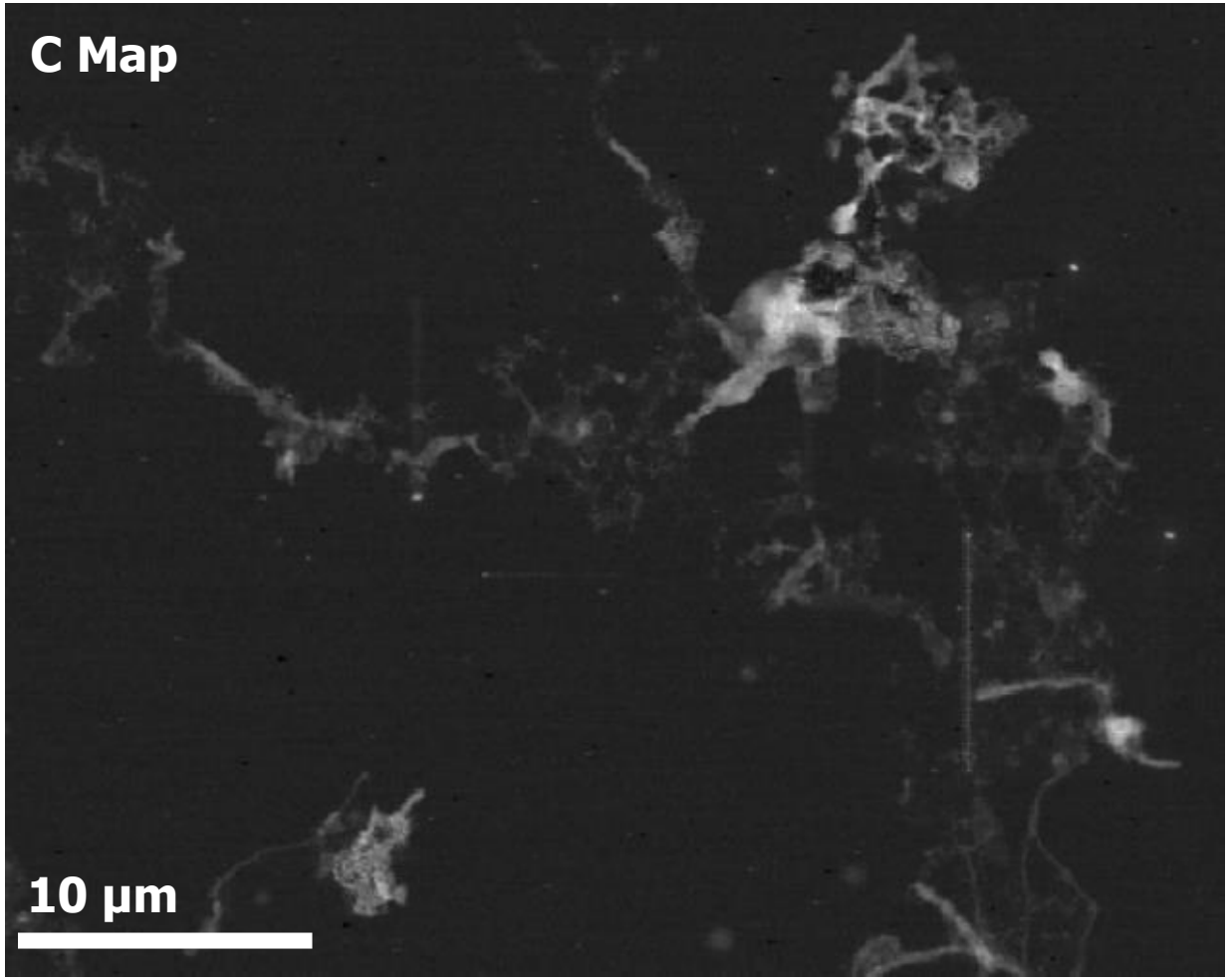




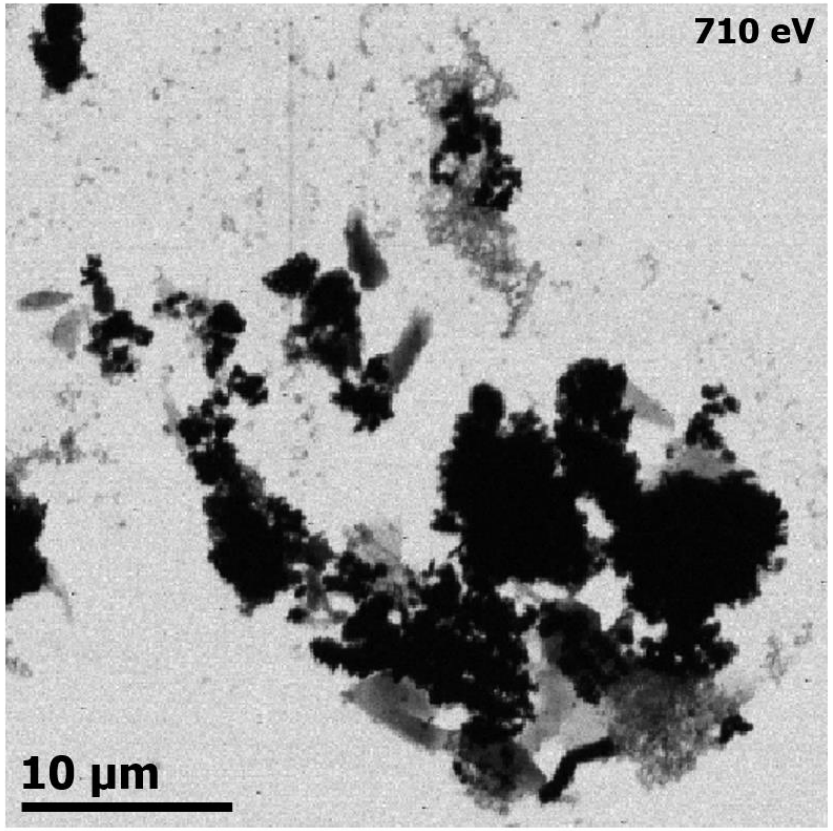


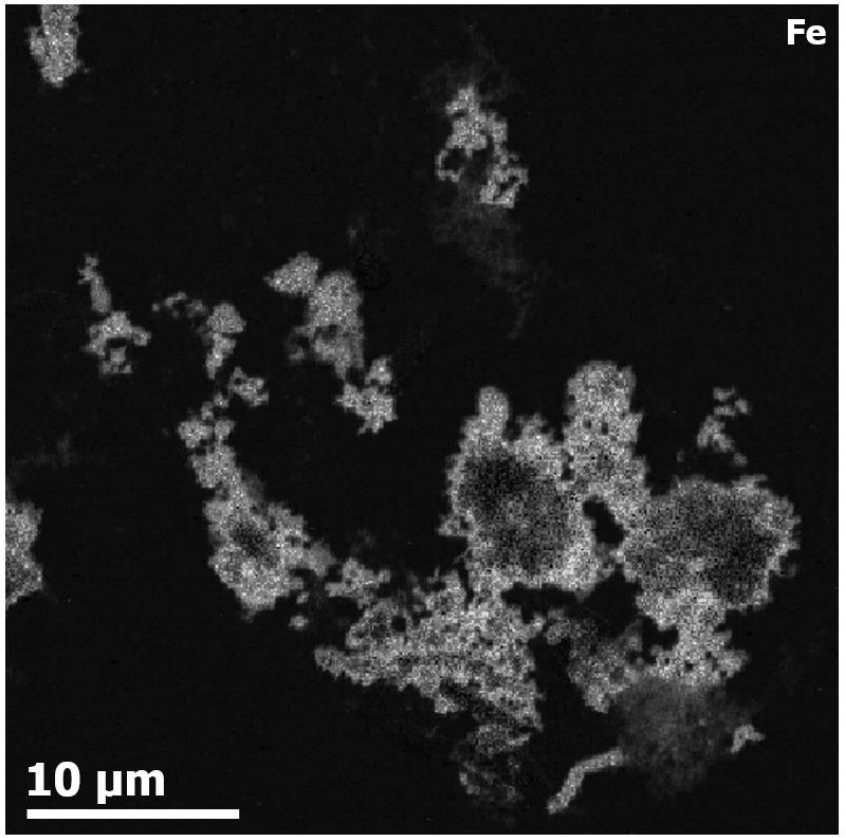
More carbon than expected – but what form?

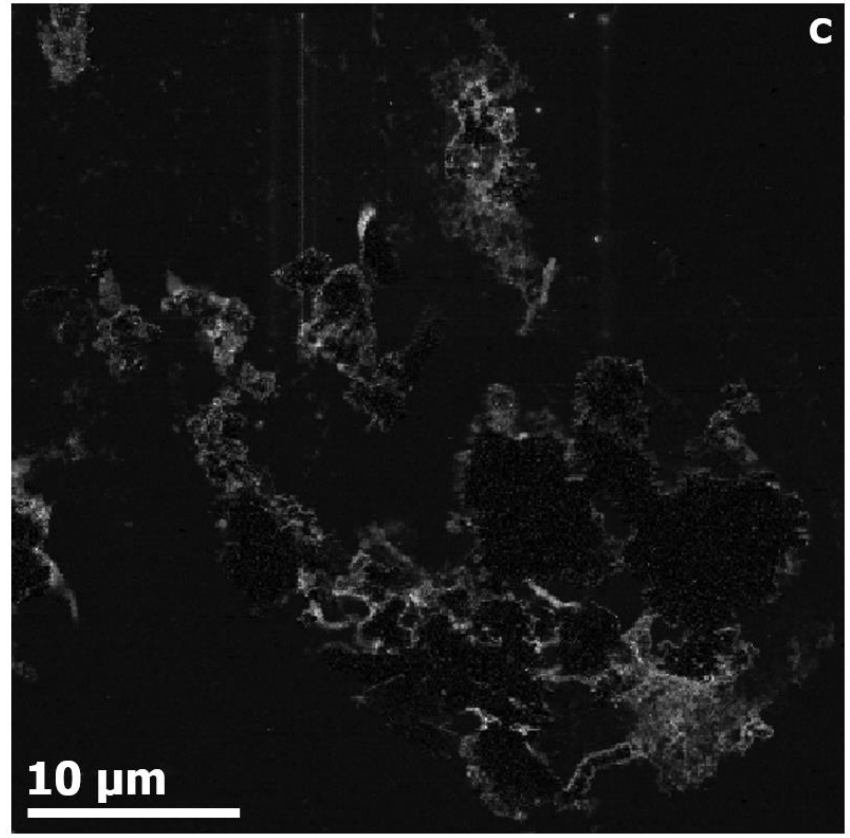




Sediment Samples: Large Particles, Mat-like Carbon



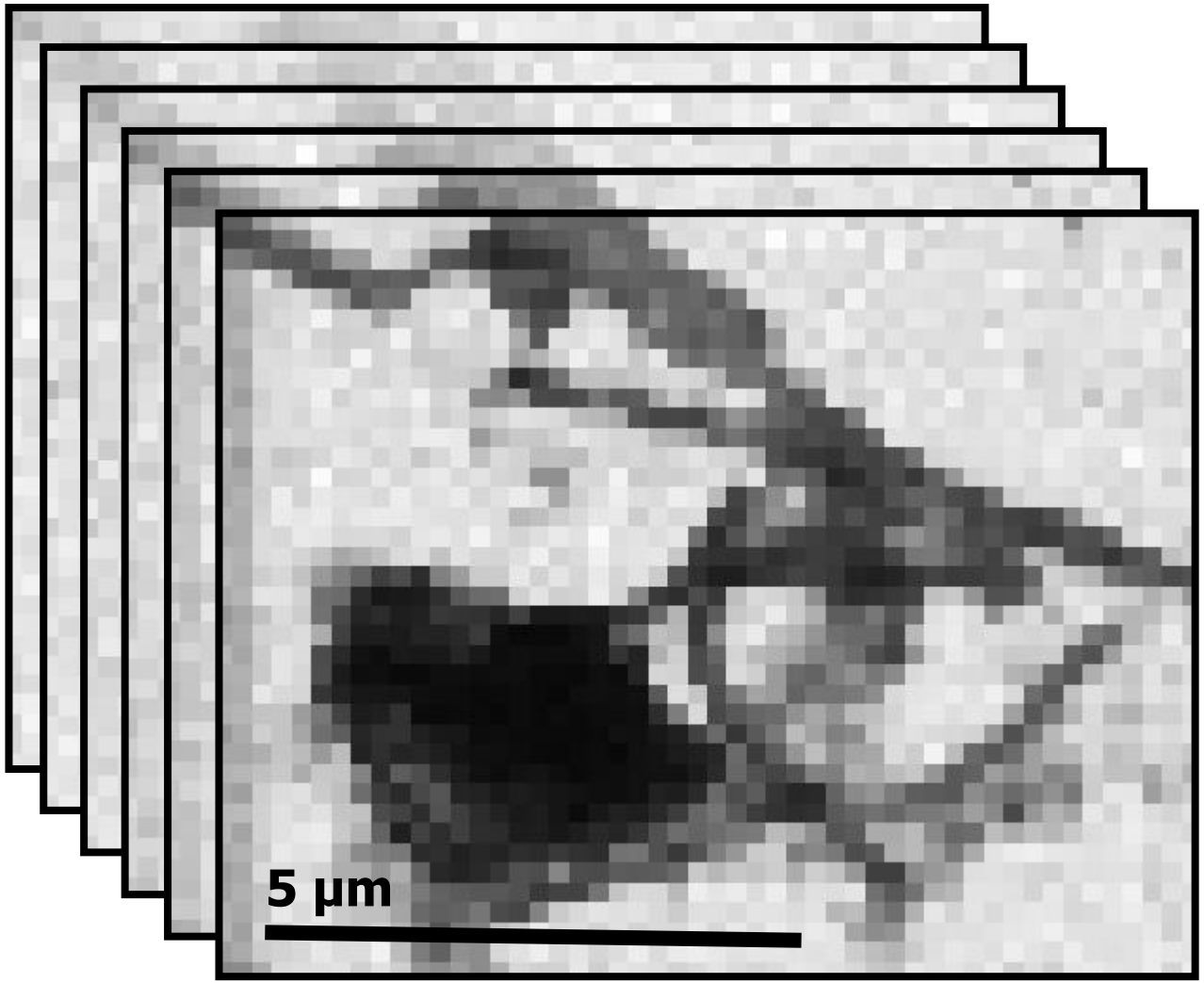


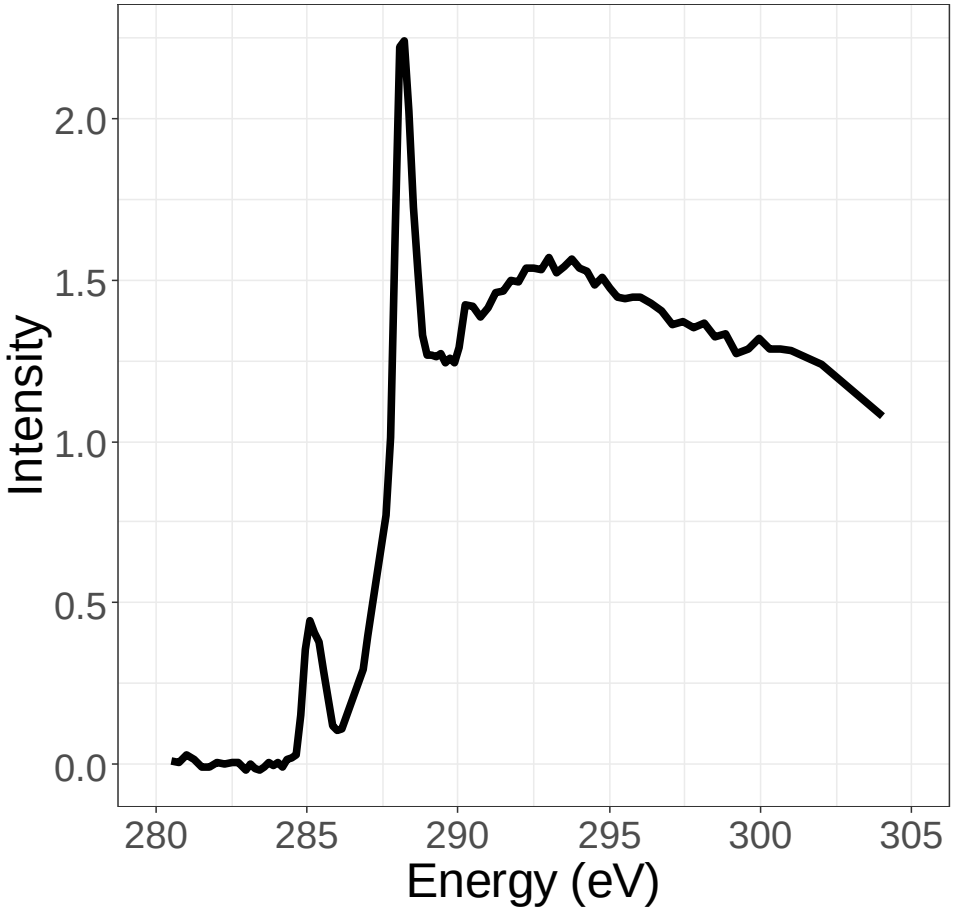


Sediments particles were uniformly composed of iron.

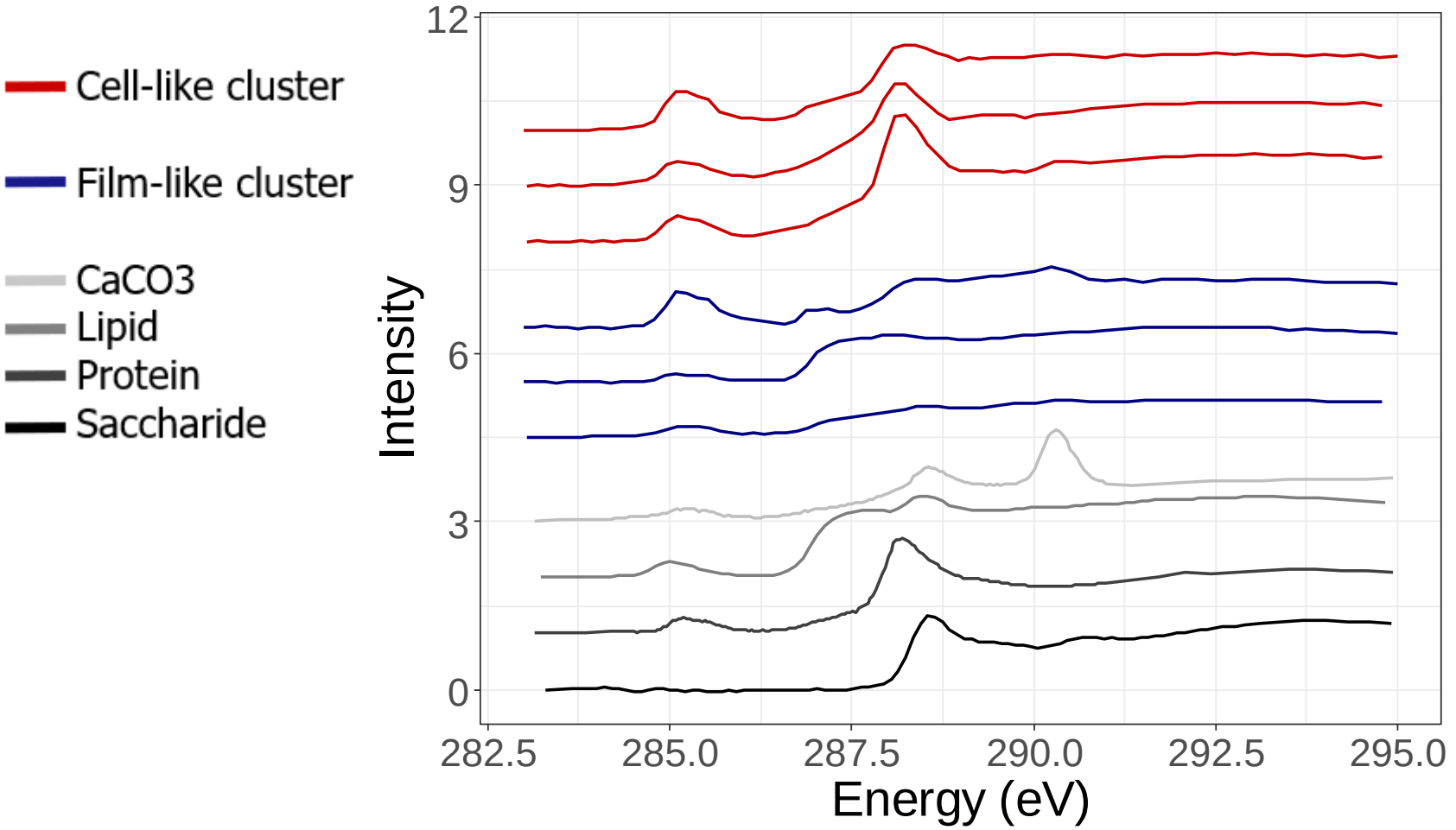
Carbon was ubiquitous in the samples, spread between mat and cell like forms.

X-Ray Microscopy II: XANES





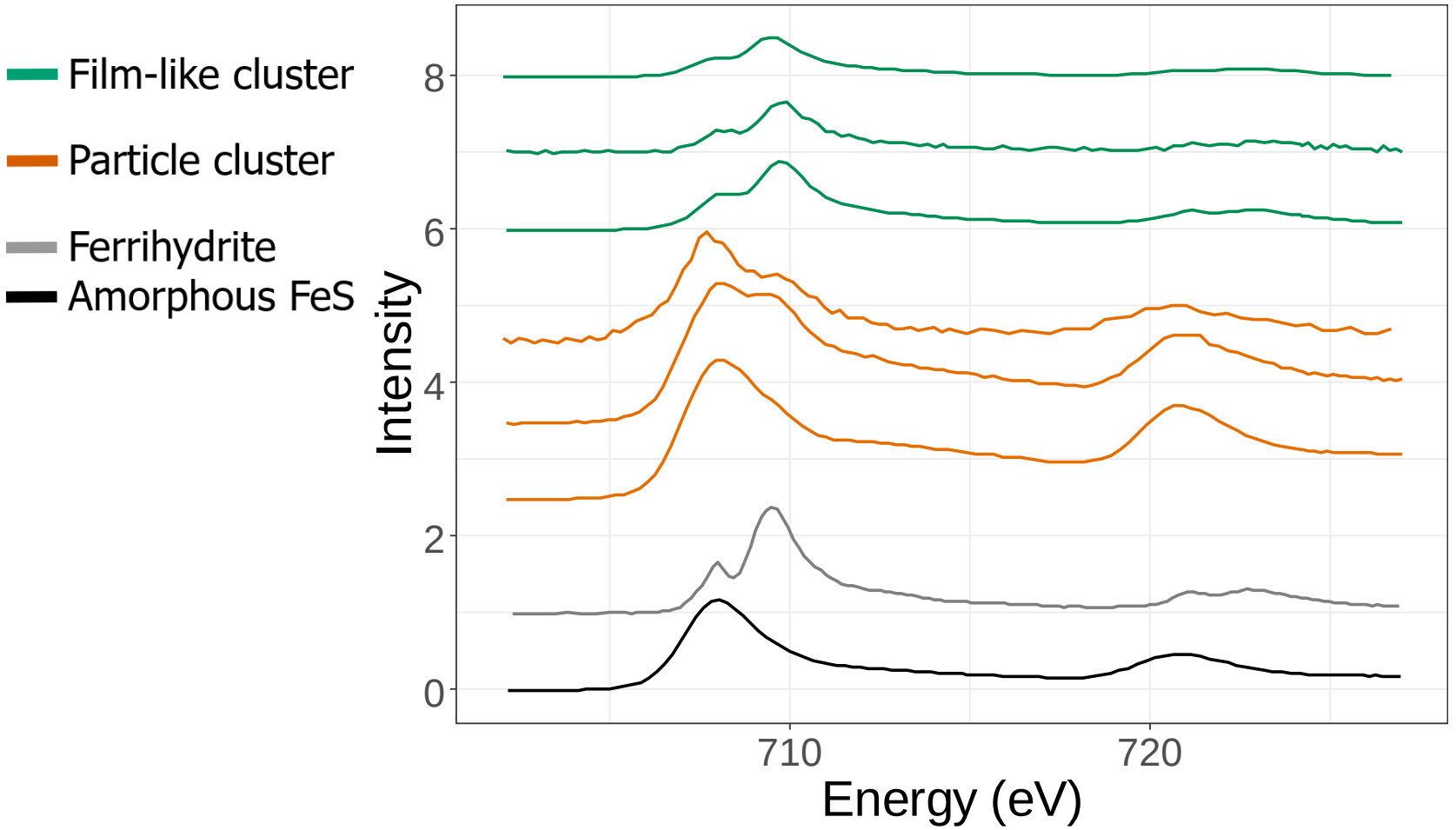
Carbon XANES



- Cell-like spectra resemble protein.
- This is expected for microbial cells – strong protein signal from cell walls.

- Film-like spectra are less uniform.
- They resemble CO_3^{2-}, lipids, or saccharides.

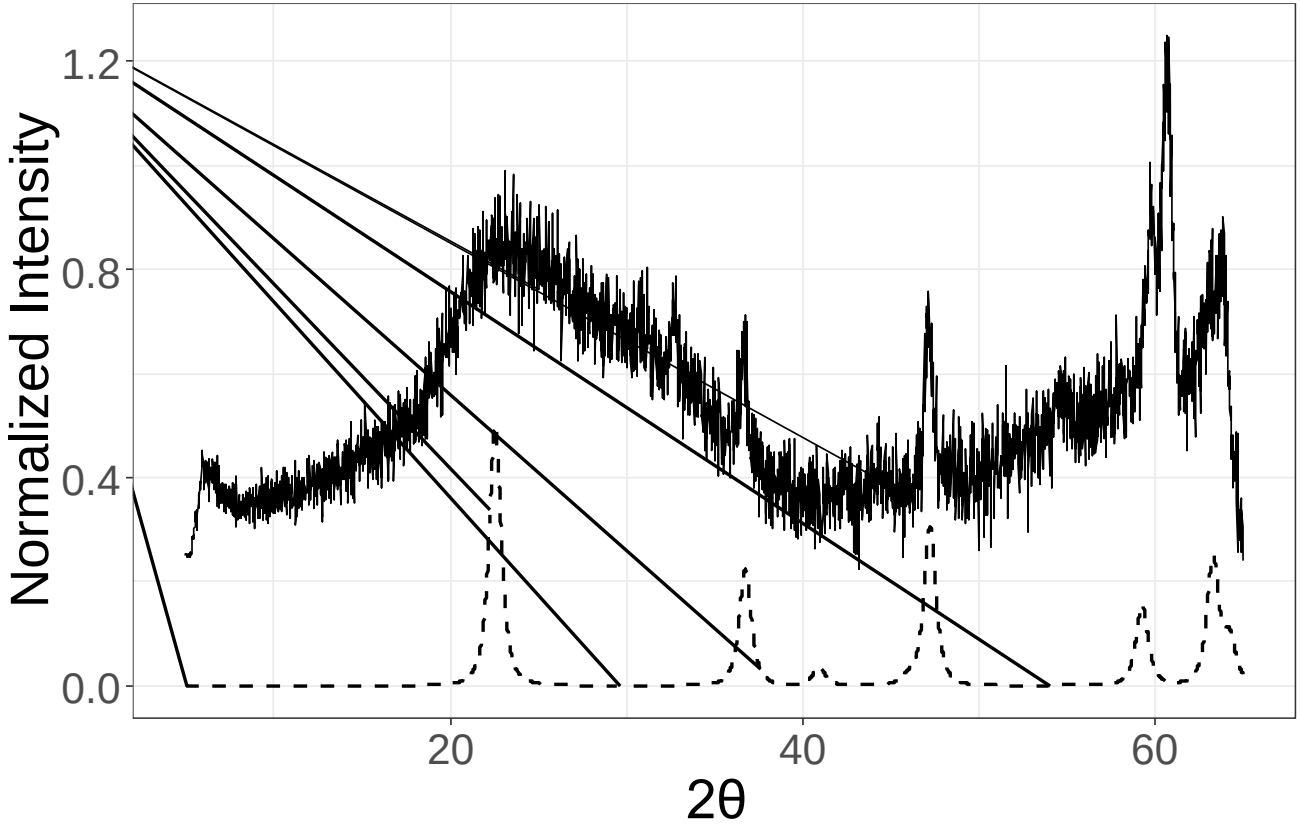
Iron XANES



- Film-like clusters contain ferric iron.
- Their spectra resemble ferrihydrite.

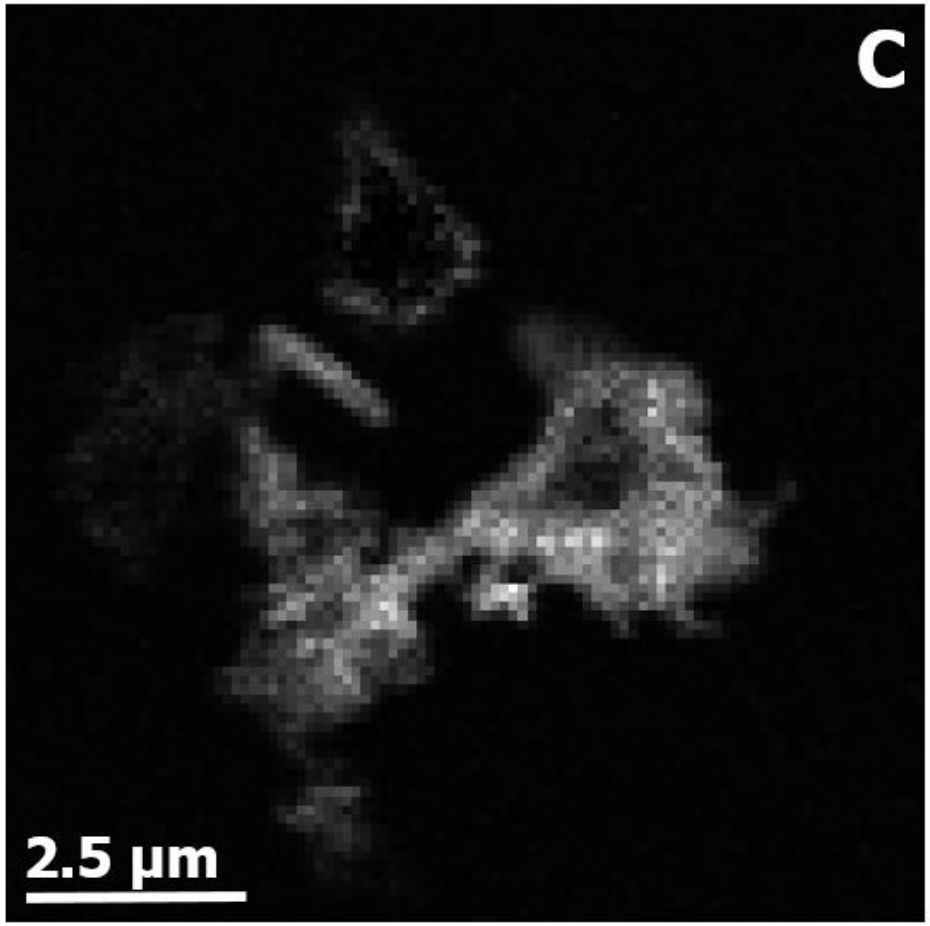
- Particles contain ferrous iron.
- Highest quality spectra are a strong match for FeS, though Fe-sulfides have similar spectra.

Identified by XRD as Mackinawite



- Powder XRD showed that the sample was poorly crystalline mackinawite (FeS)
- This phase commonly forms in anoxic environments due to micorbial Fe and S reduction
- Explains why Soudan fluids have high SO_4^{2-} yet no HS^-

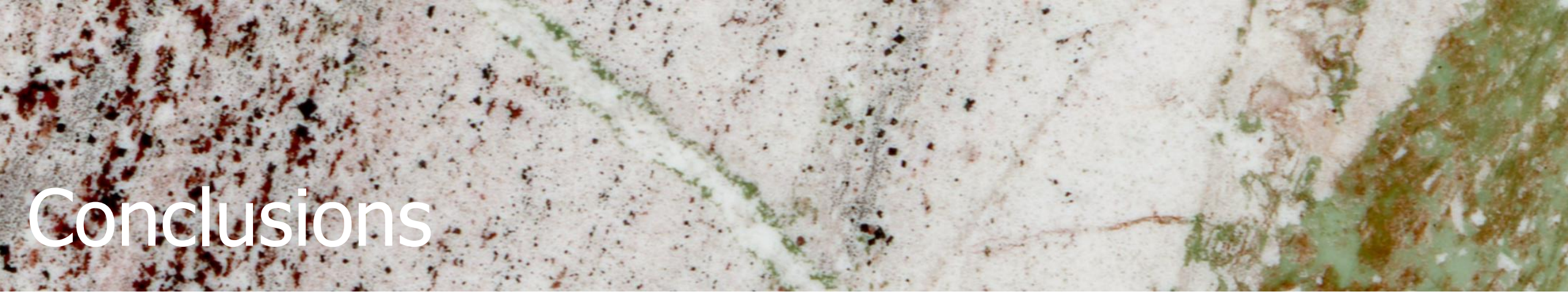
Implications of Microbial Growth in Deep Sediments



1. STXM provides evidence of both cell- and biofilm-like C in Soudan sediments.

2. Carbon was ubiquitous in the sediments, but cell-like carbon was not evenly distributed.

3. Sediments are a promising way to sample film-hosted microbes in the deep biosphere.



Conclusions

- Deep Canadian Shield groundwaters are an opportunity to study the most isolated microbial communities on Earth.
- Sediments formed by iron and sulfur reducing microbes may allow for a unique look at biofilm-hosted life in the subsurface.
- Understanding these communities requires interdisciplinary approaches – geochemistry must guide microbiological sampling (and vice versa!).
- Contact me: schu4222@umn.edu