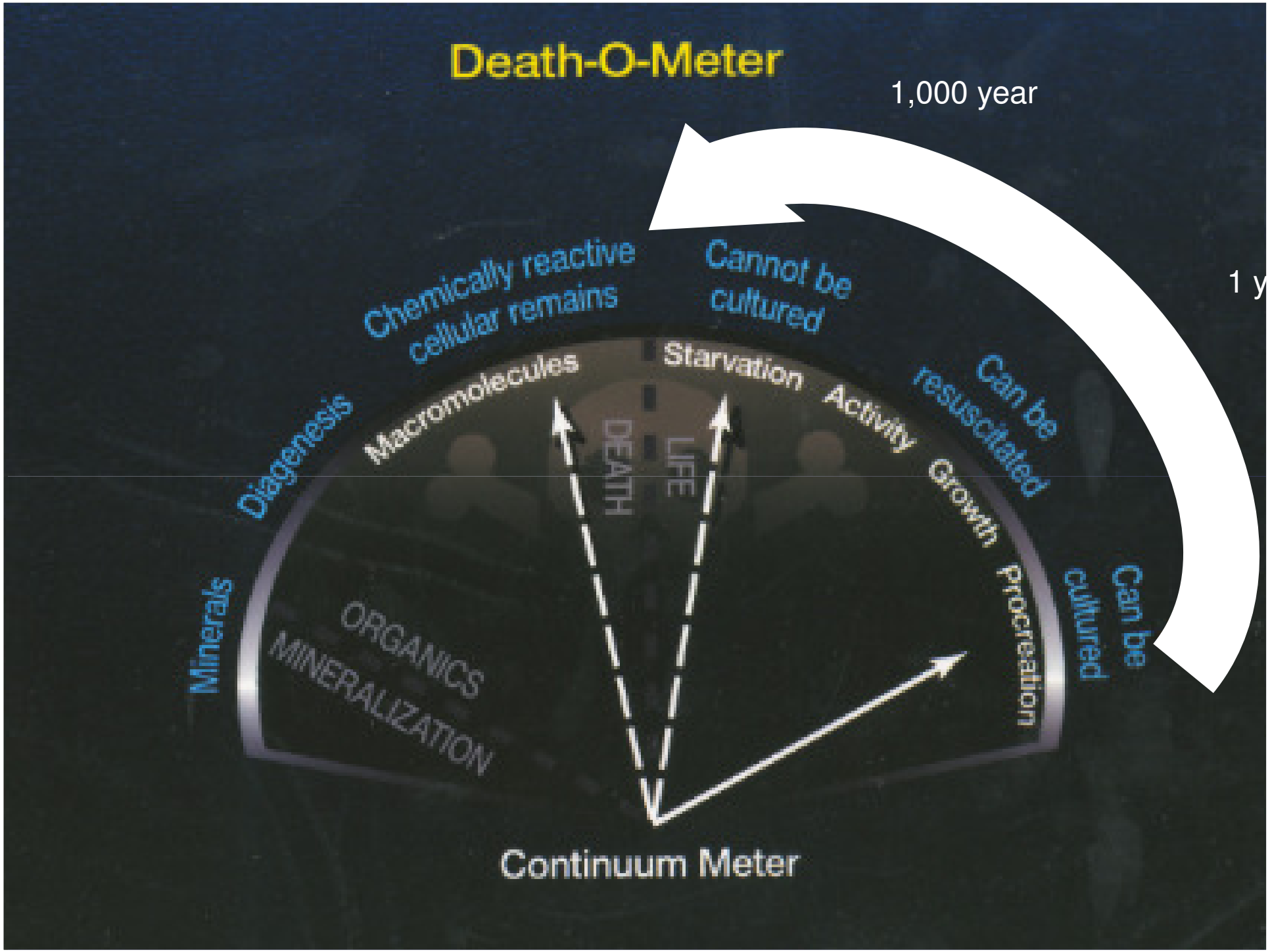
DUSEL Biology

- Key scientific questions have emerged from the exploration of life underground:
- How deep does life go?
- How do biology and geology interact to shape the world underground?
- Did life on earth originate beneath the surface?
- What can we learn from subsurface genomes?
- Is there life underground as we don't know it?

*from **DEEP SCIENCE**
A DEEP UNDERGROUND SCIENCE AND ENGINEERING INITIATIVE


Death-O-Meter
1,000 year
1 y
Chemically reactive cellular remains
Cannot be cultured
Can be resuscitated
Can be cultured
Diagenesis
Macromolecules
Starvation
Activity
Growth
Procreation
Minerals
ORGANICS
MINERALIZATION
DEATH
LIFE
Continuum Meter

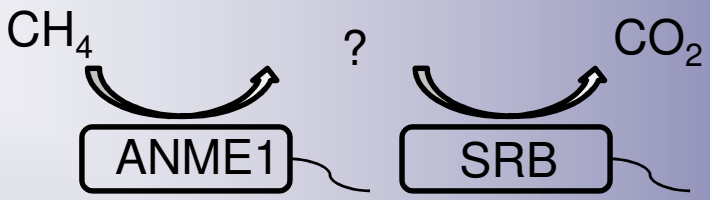
Deciphering the structure and function of complex microbial communities is a central theme in microbial ecology. This addresses the two major questions:

- How do biology and geology interact to shape the world underground?
- What can we learn from subsurface genomes?

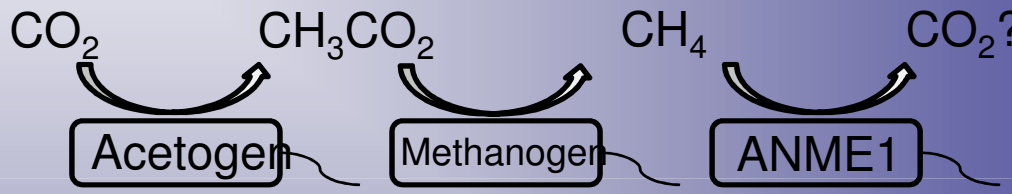
Follow the Carbon – Trophic Cascades

Ecosystem

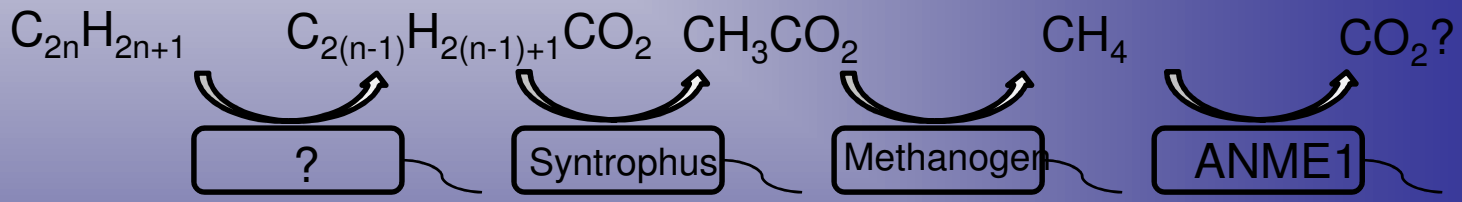
Anaerobic CH_4 Oxidation



Subsurface Lithoautotrophic Microbial Ecosystems



Anaerobic Alkane Degradation

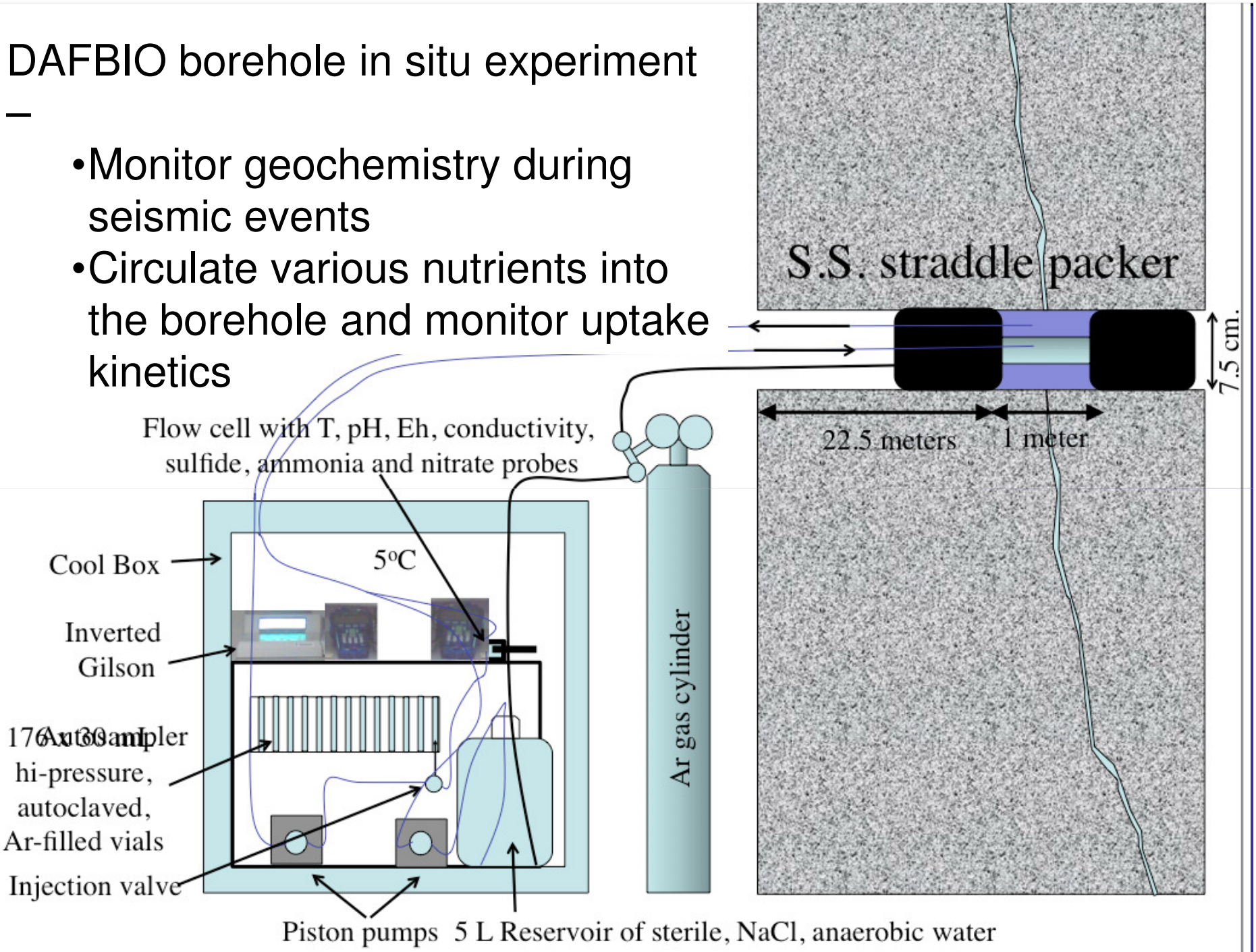


RNA @ Time Points

Trophic Level	T_1	T_2	T_3	T_4
1	+	+	+	+
2	-	+	+	+
3	-	-	+	+
4	-	-	-	+

DAFBIO borehole in situ experiment –

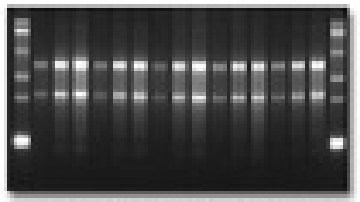
- Monitor geochemistry during seismic events
- Circulate various nutrients into the borehole and monitor uptake kinetics



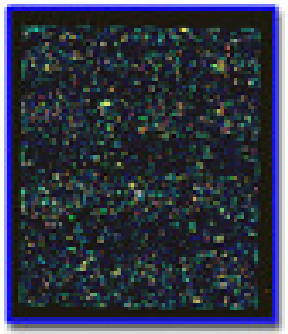
PhyloChip Procedure

- All procedures can be completed within the lab.
- Greatly reduces time needed to get results
- Detects a broader spectrum in microbial community

DNA/RNA extracted



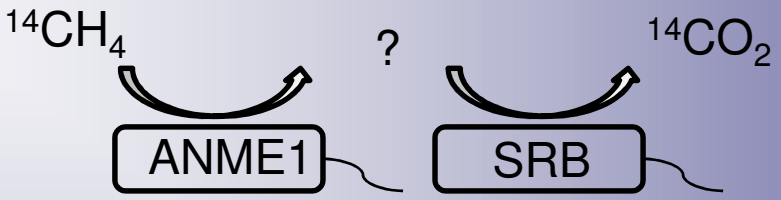
16S rRNA gene amplified



Data analyzed & microbes identified

Ecosystem

Anaerobic CH_4 Oxidation



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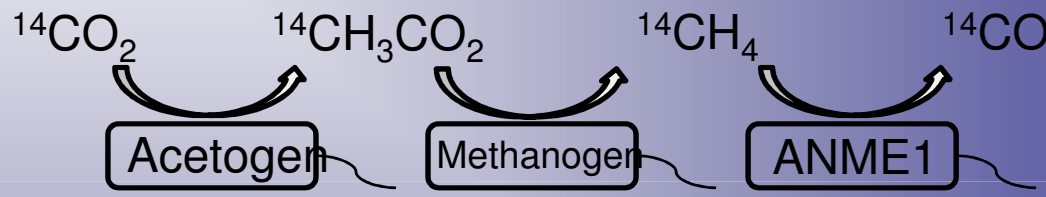

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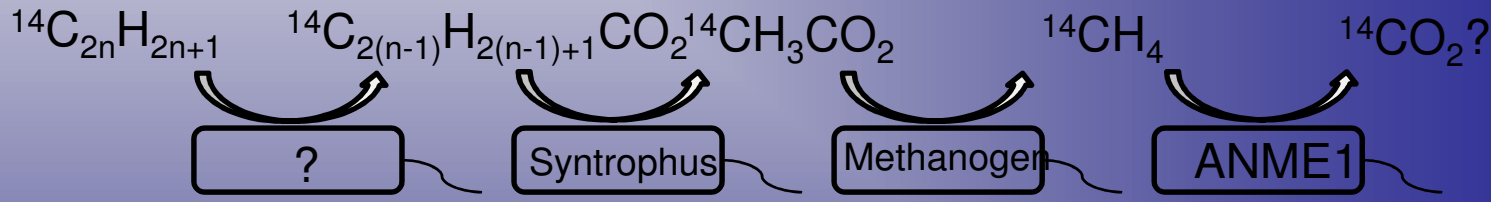
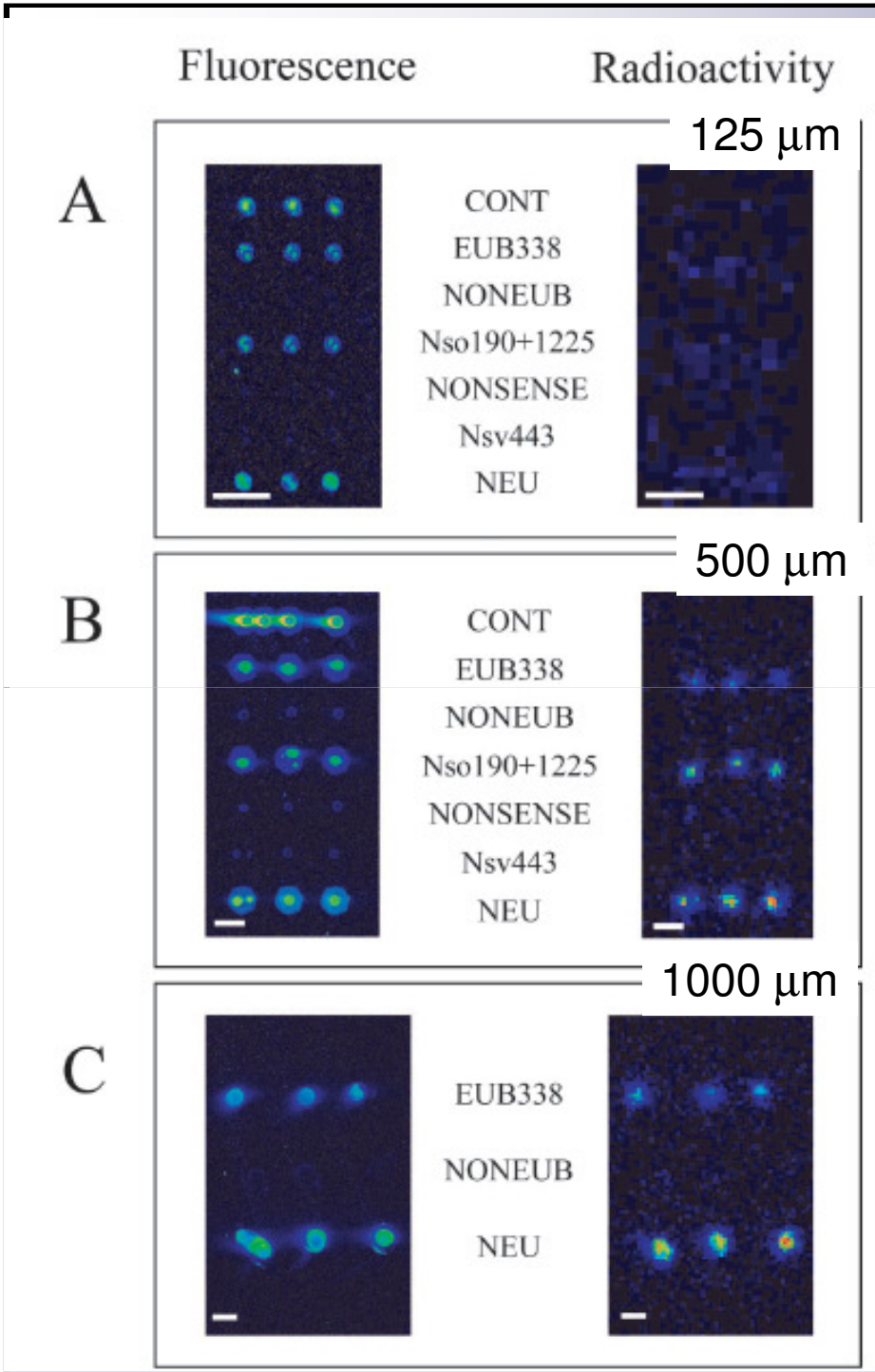


^{14}C RNA @ Time Points

Trophic Level	T_1	T_2	T_3	T_4
1	+	+	+	+
2	-	+	+	+
3	-	-	+	+
4	-	-	-	+



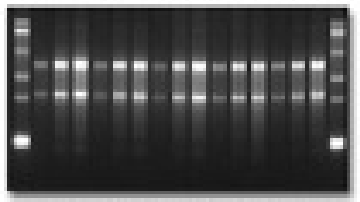
rRNA + ^{14}C microarray

- CONT – no ^{14}C
- Eubacterialprobe for bacteria
- Noneubacterialprobe or Archaea probe
- Nso190+1225 - mRNA
- NONSENSE – nonsense probe
- NSV443 – specific species
- NEU – specific species
- <1% incorporation of ^{14}C into the rRNA
- Detection limit ~19,000 CPM per µg of rRNA

Isotope RNA microarray

- Utilizes same format as standard microarrays with 25 μm spots and thousands of spots
- Combines fluorescent and ^{14}C imaging into a single viewer
- Manufactured from low background materials and is operated in a low background environment.

DNA/RNA extracted



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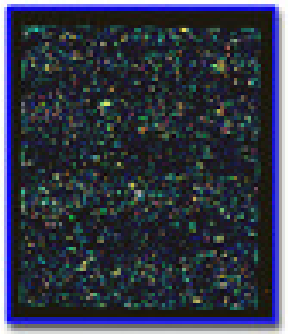


Data analyzed & microbes identified

rRNA Isotope Microarrays and Subsurface Environments

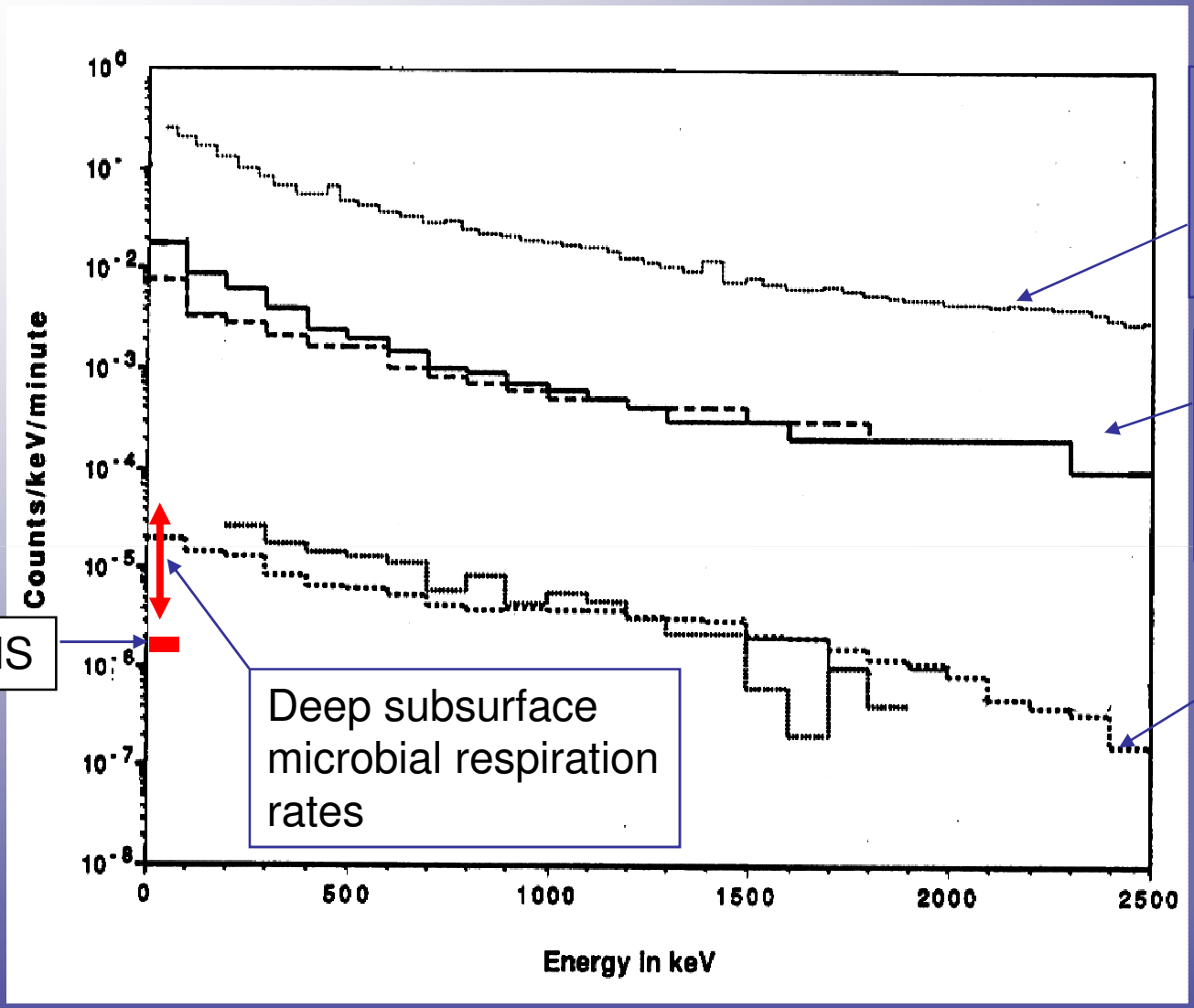
- Response time shorter for RNA than DNA
- RNA changes do not require growth
- Transfer of ^{14}C from one organism to another can be monitored over time

Relevant to S4 Projects

- Ecohydrology Observatory
- DUSEL CO_2 Facility
- Thermal-Hydrological-Mechanical-Chemical and Biological Coupling Facility

Microbial Respirometry

- Because they are incorporated into biomass they can be directly used to relate physiology to phylogeny and genomes to transcription (identifying new genes).
- Radiotracers, e.g. ^{3}H, ^{14}C and ^{35}S, also quantify rates for enzymatic processes (i.e. proteins), which for subsurface environments are extremely slow. This requires measurement of metabolites by counting.



Surface counters LBL Bldg 72, PNNL, and many other places etc.

Shielded on surface PNNL 17-A LBNL Oroville

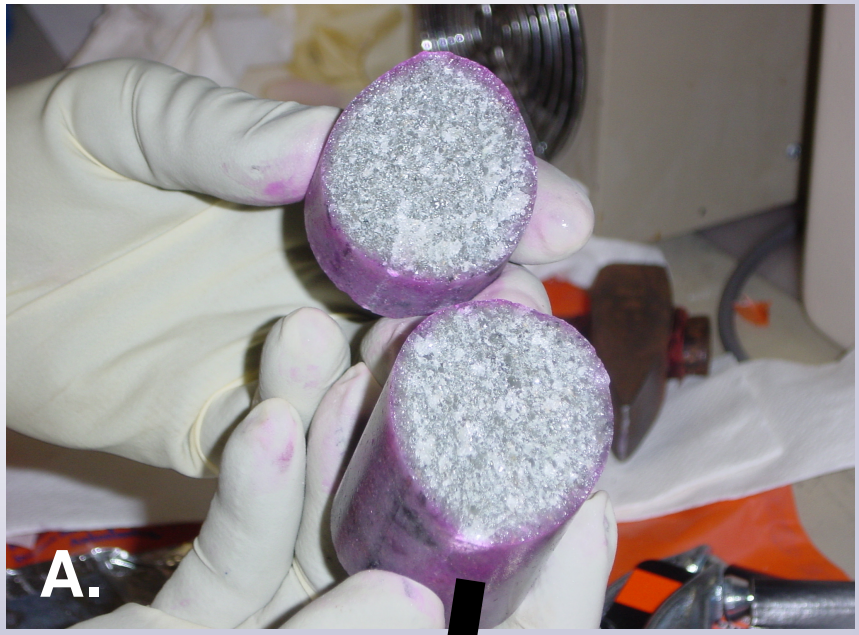
2000+ mwe Soudan, MEGA

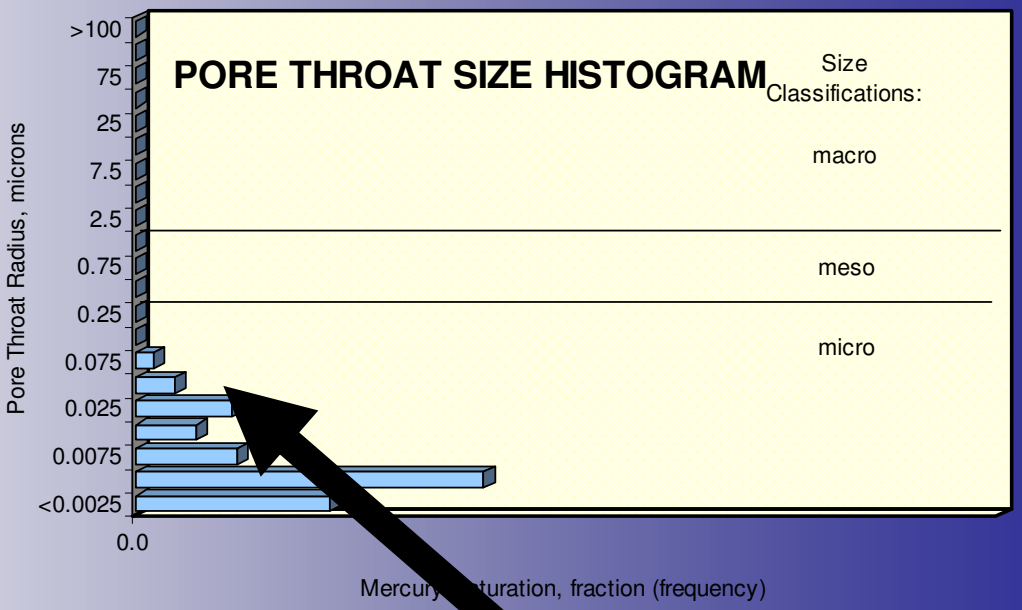
^{14}C AMS

Deep subsurface microbial respiration rates

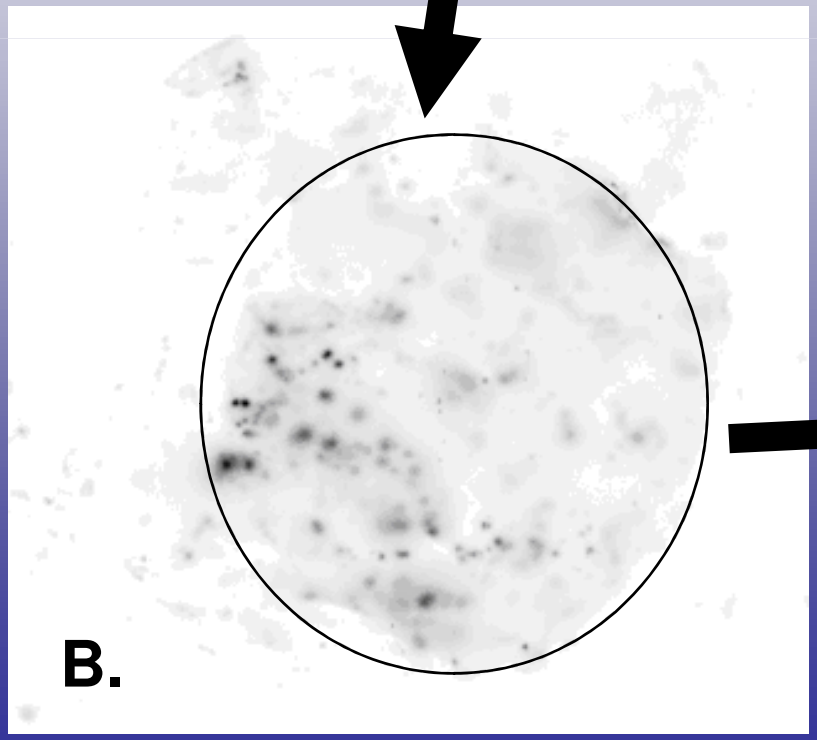
Sessile (rock bound) microbial communities are likely more diversified and metabolically active than the planktonic (suspended) communities

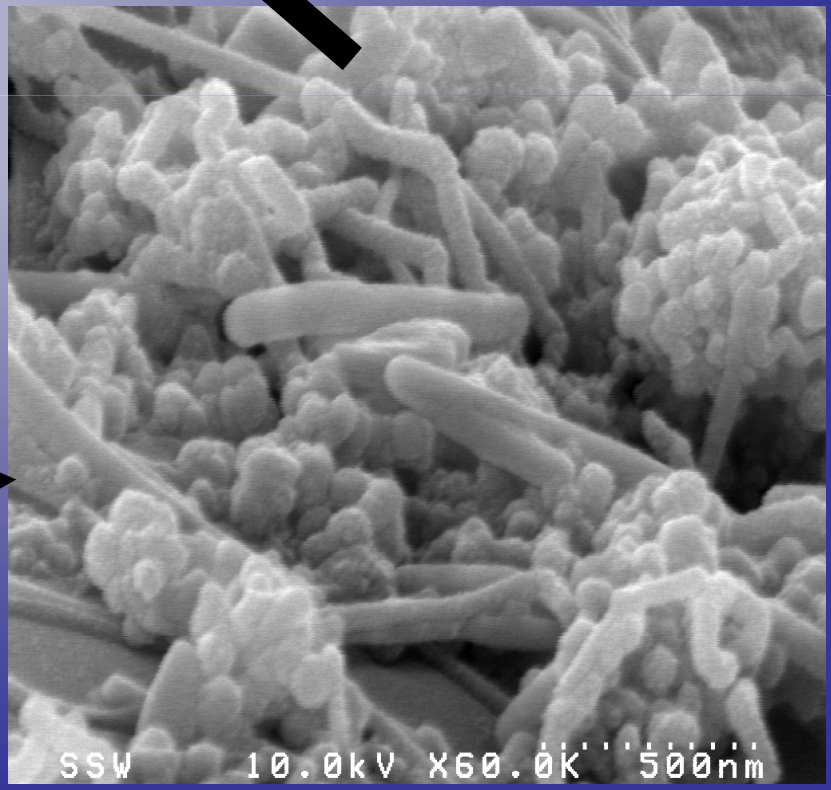
- How do biology and geology interact to shape the world underground?
- Is there life underground as we don't know it?


A.


PORE THROAT SIZE HISTOGRAM
Size Classifications:
macro
meso
micro
Pore Throat Radius, microns
>100
75
25
7.5
2.5
0.75
0.25
0.075
0.025
0.0075
<0.0025
0.0
Mercury Saturation, fraction (frequency)

D.

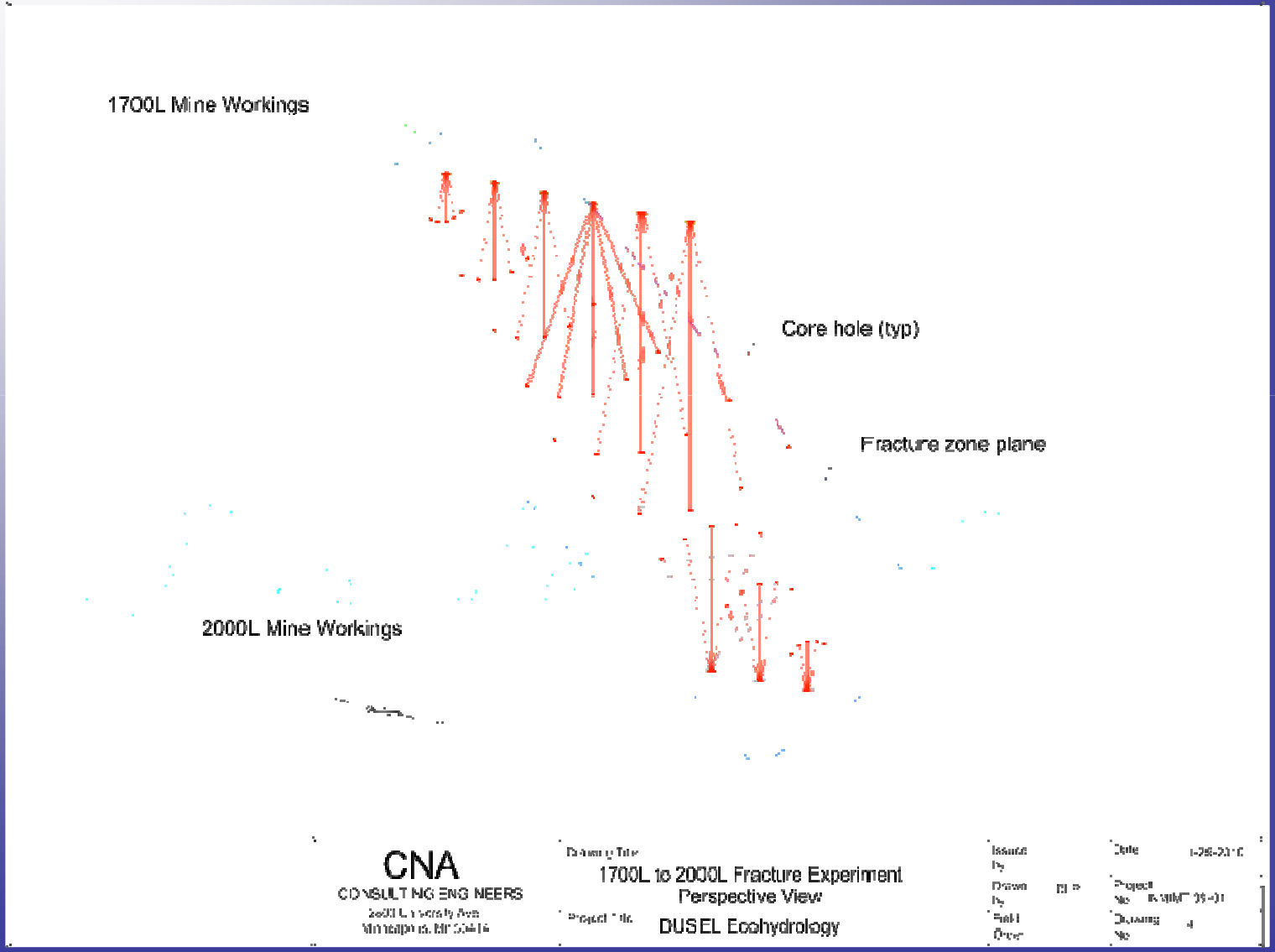

B.


C.
SSW 10.0kV X60.0K 500nm

SEM imaging of ‘hotspots’

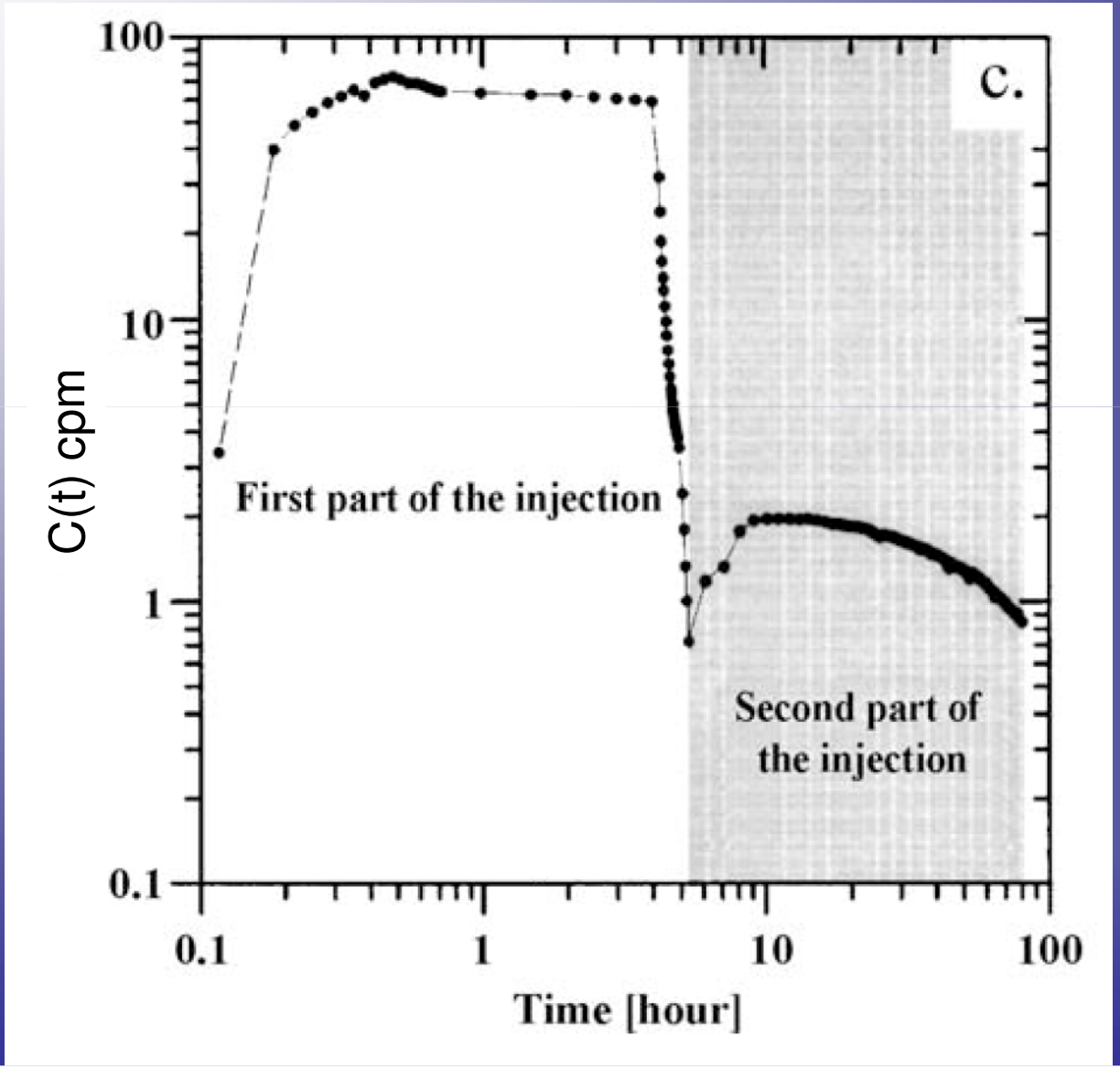
Courtesy of Mark Davidson P.U. and
Greg Wanger and Gordon Southam, Univ. Western Ontario

Fracture Flow Array



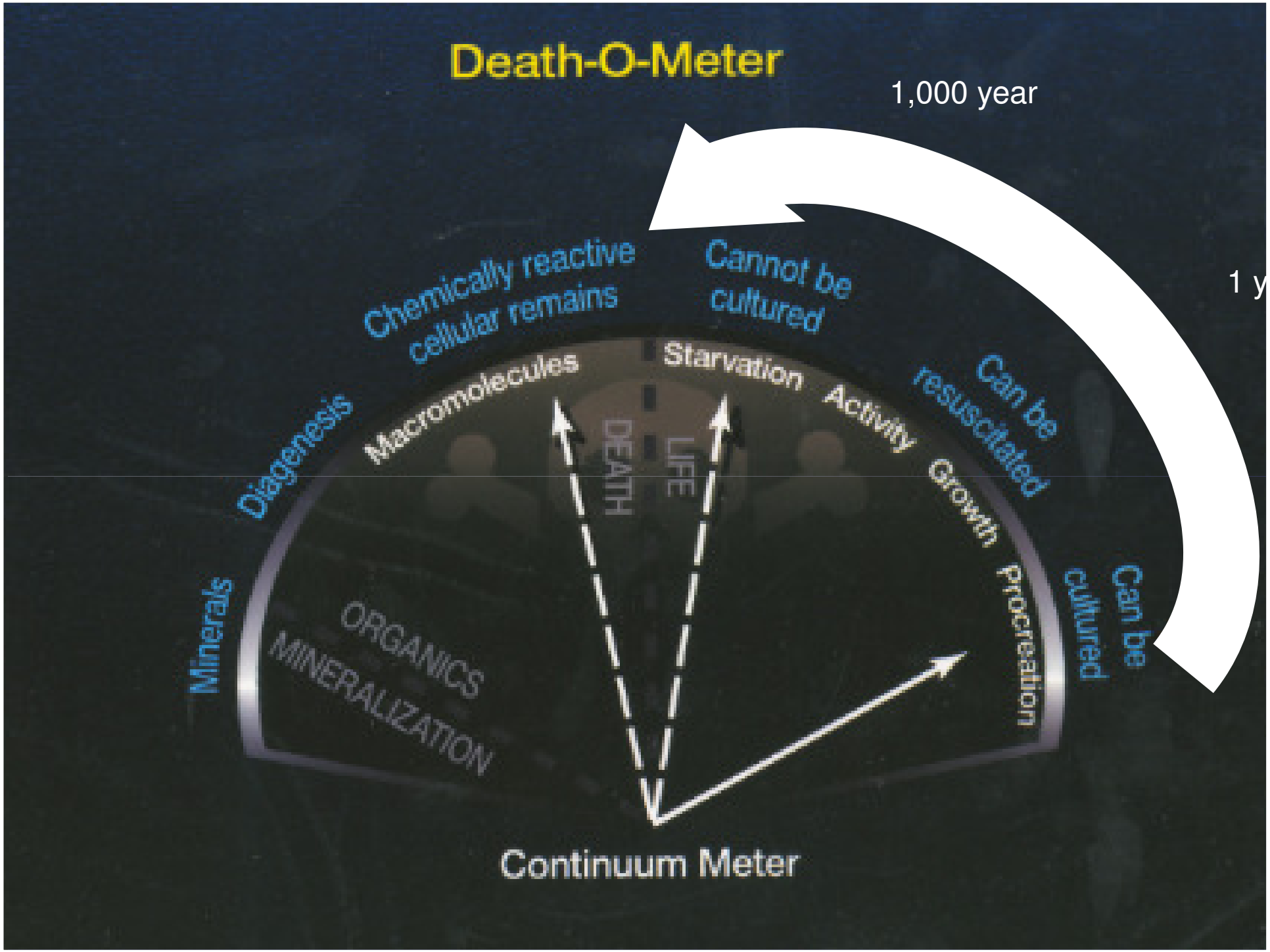
Tracking migration with ^{14}C labeled microorganisms

^{14}C activity = cells/mL



Ecohydrology

- Transport of humic compounds, fulvic acids, etc.
- Death of organisms by measurement of labeled macromolecules
- Growth of organisms by measuring specific activity of living bacteria and dilution during cell division
- Predation by detection of label in protists or viral particles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Diagenesis
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Requirements

- Experiments will take place at the borehole sites or within the MULE's.
- DOE BER has just funded the development of RNA isotope microarray as part of carbon cycle initiative

  β counters for low level liquid samples
- 2D imaging of radioactivity for ~ 1-5 cm diameter samples, foils, filters
- Scintillation counter for gas samples