

Continuous Soil Redox Sensing and Microbiome Characterization for Monitoring NSZD and Enhanced NSZD

Energy lives here™

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Agenda

- Overview
- Experimental Design
- Data & Results
- Summary

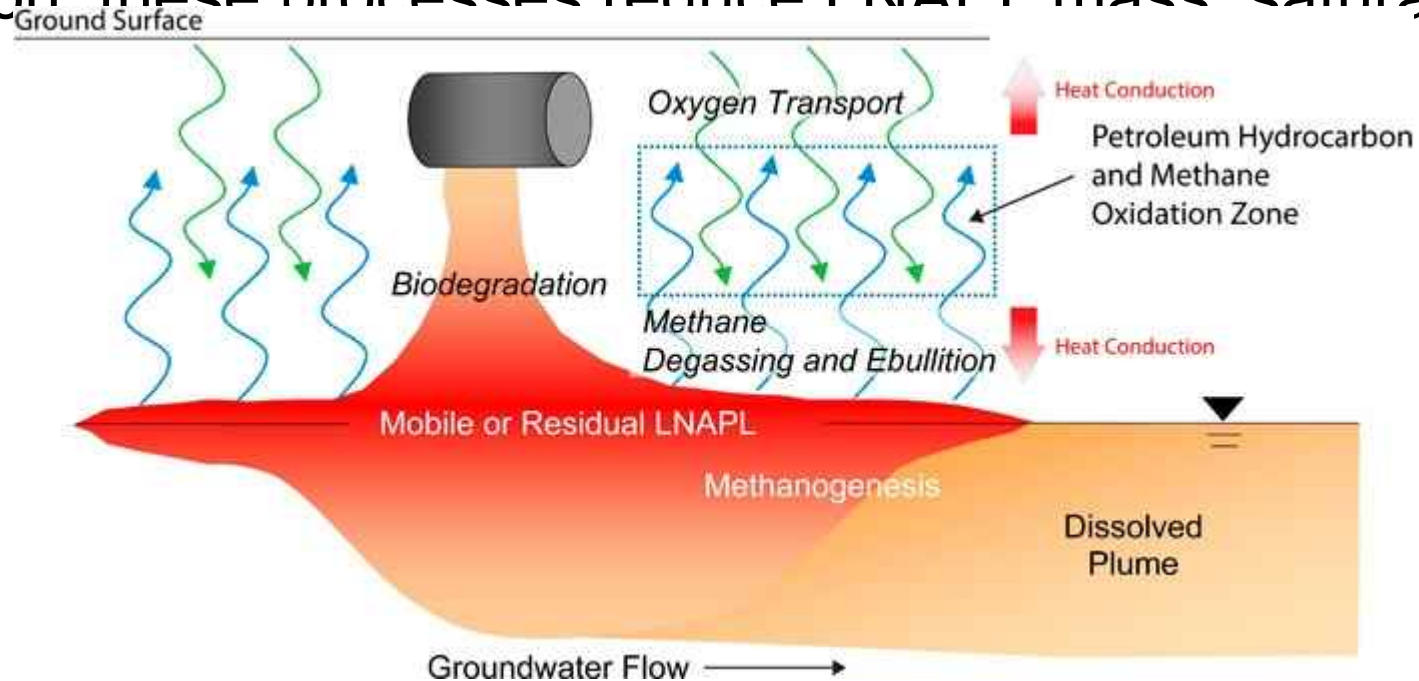
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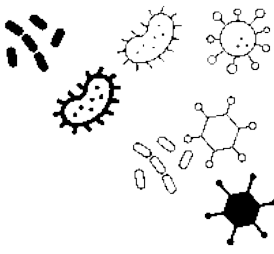
Overview

Natural Source Zone Depletion

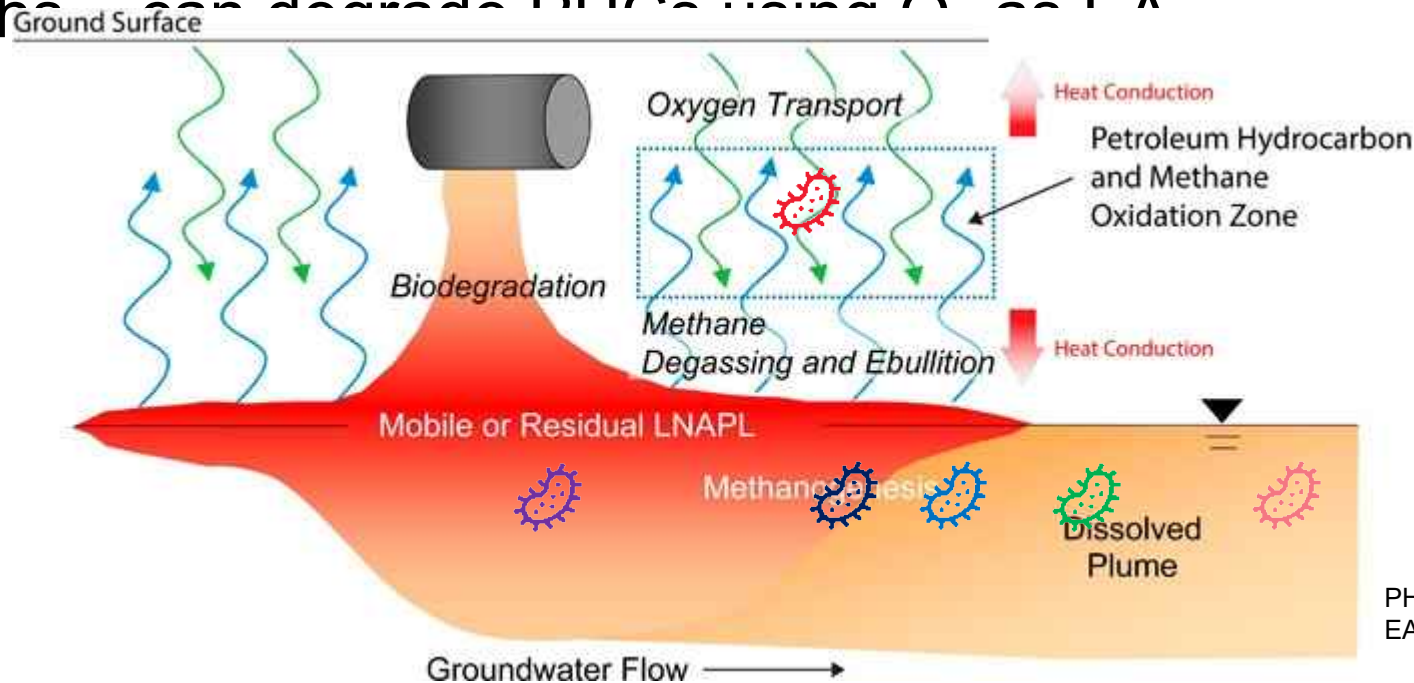
- “LNAPL Natural Source Zone Depletion (NSZD) occurs through the combined action of natural processes that reduce the mass of LNAPL in the subsurface.”
- “Key NSZD processes include volatilization, dissolution, and biodegradation; these processes reduce LNAPL mass saturation, and mobility over



NSZD Biodegradation: Microbiology



- 🦠 Fermenting microorganisms/fermenters – can degrade PHCs without exogenous EA
- 🦠 Methanogens – can produce CH_4 from acetate, H_2 , CO_2 , formate, etc.
- 🦠 Sulfate reducers – can degrade PHCs and use SO_4^{2-} as EA
- 🦠 Iron reducers - can degrade PHCs and use Fe(III) species as EA
- 🦠 Methanotrophs – can consume CH_4 and O_2 to produce CO_2
- 🦠 Aerobic heterotrophs – can degrade PHCs using O_2 as EA



PHCs: petroleum hydrocarbons
EA(s): electron acceptor(s)

Study Approach

- Objective: to explore and enhance understanding of microbial community structure and redox associated with NSZD and enhanced NSZD (air sparging and sulfate addition).
- Microbial community structure measured through molecular biological tools, specifically DNA and RNA sequencing of 16S rRNA gene
- Redox measured via continuous oxidation-reduction potential (ORP) sensors
- Conditions evaluated:
 - NSZD: methanogenic
 - Enhanced NSZD: sulfate amended and air sparged

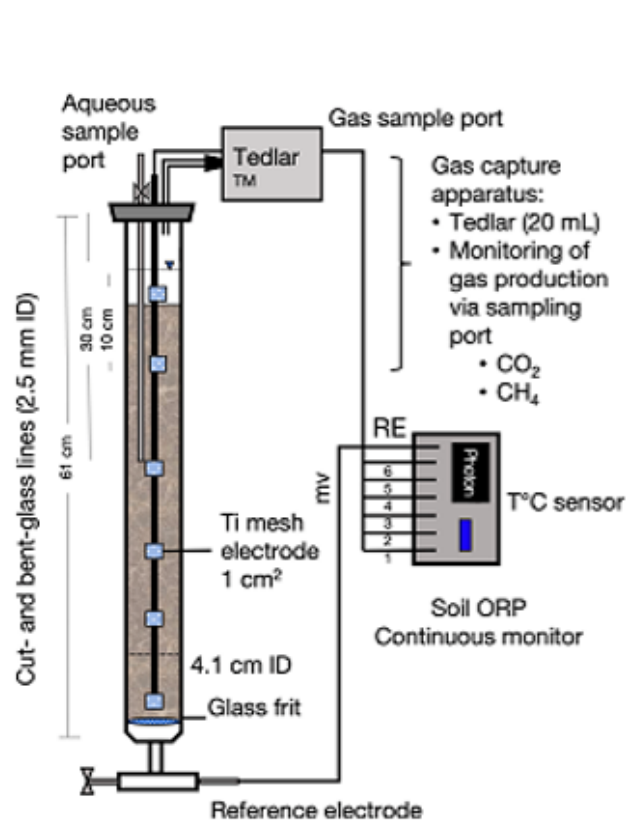
The background features a white space with three large, overlapping triangular shapes in shades of green. One triangle is a medium green, another is a darker forest green, and the third is a lime green. They are arranged in a way that creates a dynamic, abstract composition.

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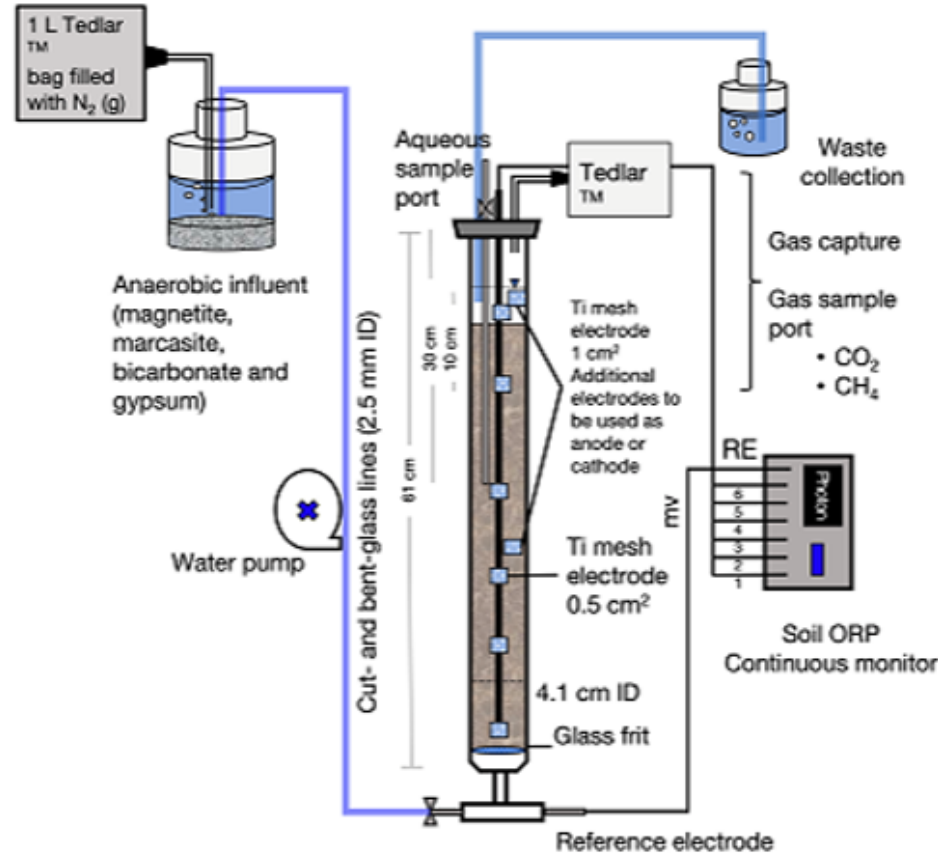
Experimental Design

Column Setup and Monitoring

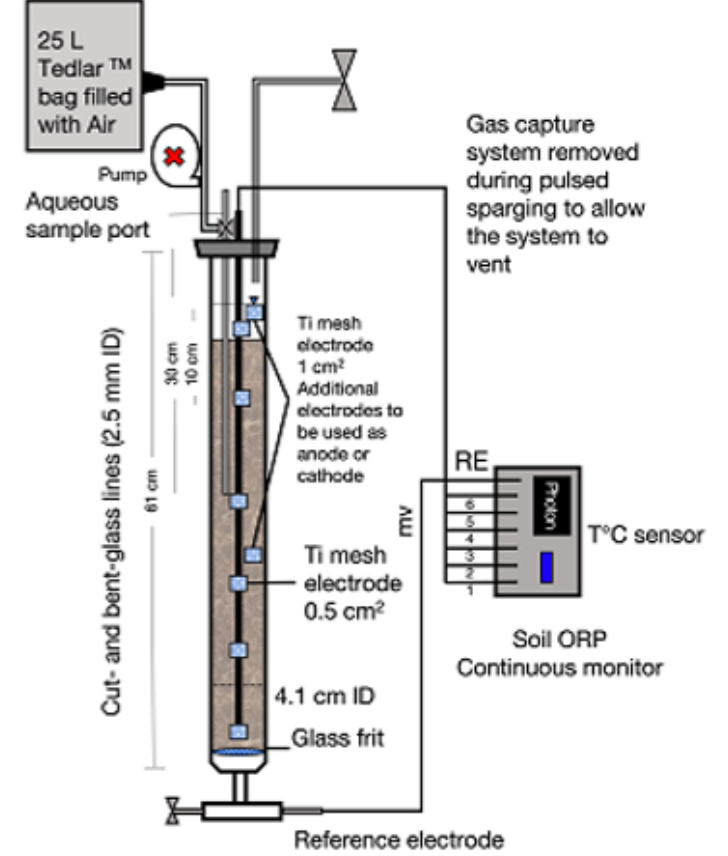
Methanogenic



SO₄²⁻ Enhancement



Pulsed Air Sparging



Column Soils Preservation and Subsampling

Froze columns and stored them -80°C



For subsampling hammered and peeled tape and glass and placed soil in acetate sleeves



Cut 1' hockey pucks every 10 cm with a circular saw and quartered the hockey pucks



Soil Analyses

- Methanol extraction for GC-MS AND GC-FID analysis for quantification and characterization of hydrocarbons.
- Water extraction for quantification of total iron, sulfate, methane, acetate and formate. pH and alkalinity measurements were collected from the water extracts.
- Wrapped in aluminum foil and placed in liquid nitrogen for preservation. This sample was analyzed for present and active microbial community characterization in soil.
- Sample was wrapped in aluminum foil, vacuumed sealed in a freezer bag and stored at -80 °C as archive.



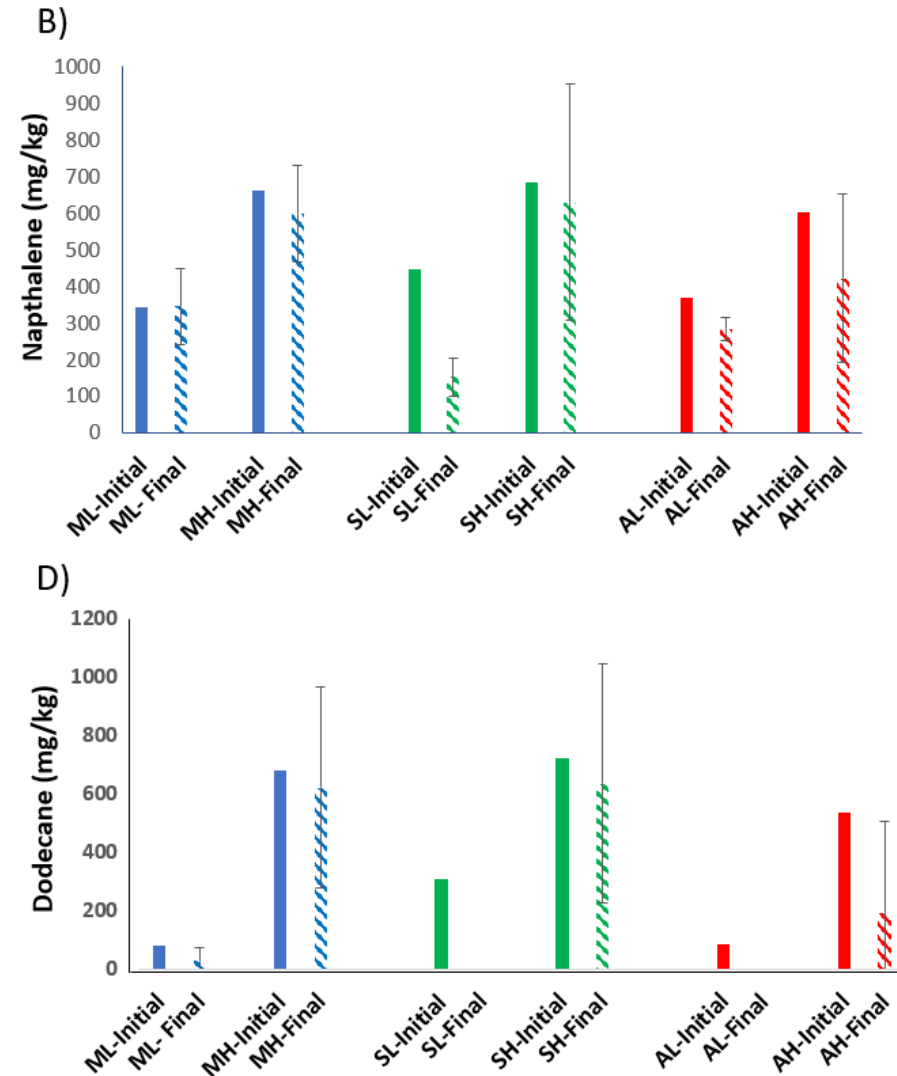
The background features a large green triangle on the left side, which is divided into three distinct shades of green: a dark forest green at the bottom, a medium sea green in the middle, and a light sage green at the top. The right side of the slide is plain white.

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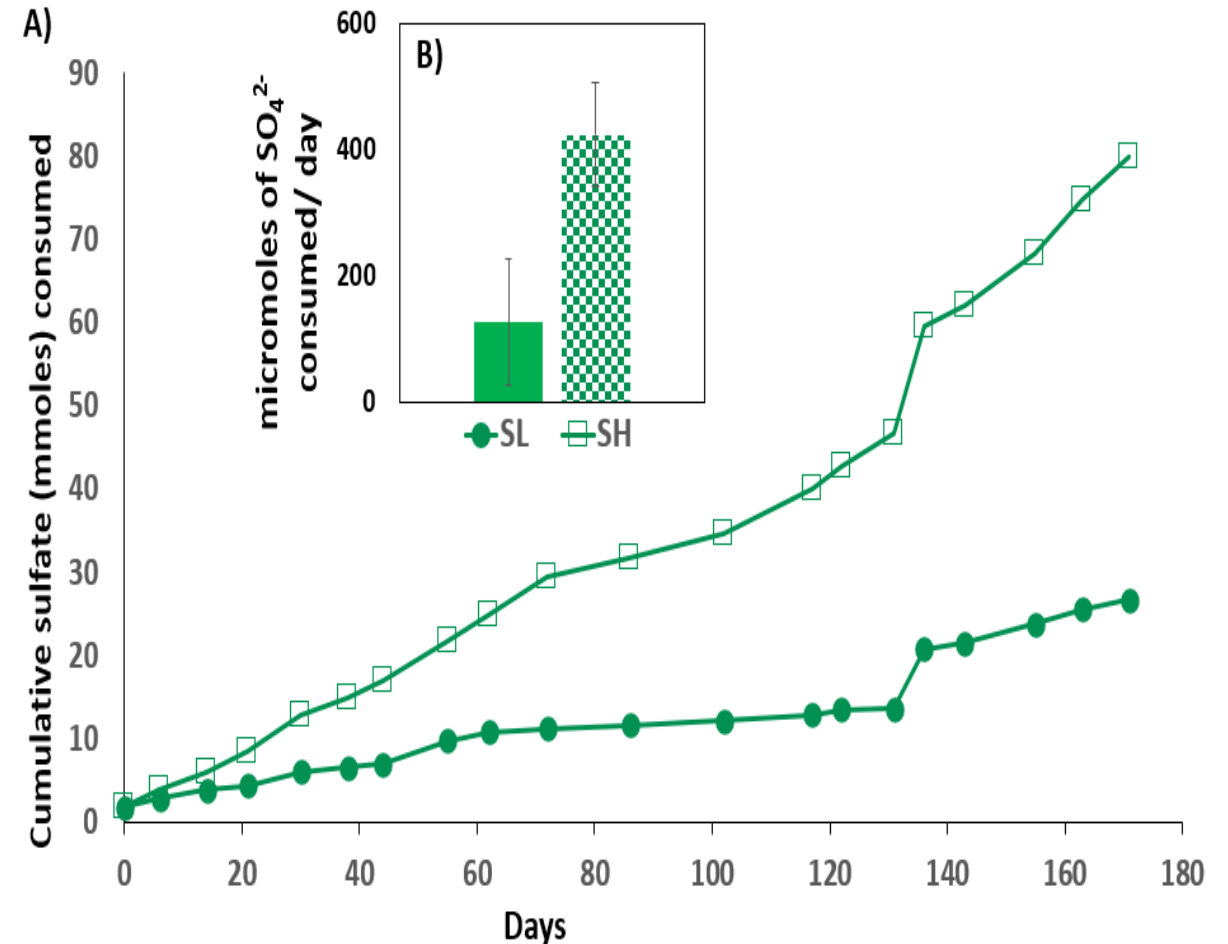
Data & Results

Chemistry and Geochemistry Trends

Hydrocarbon Degradation

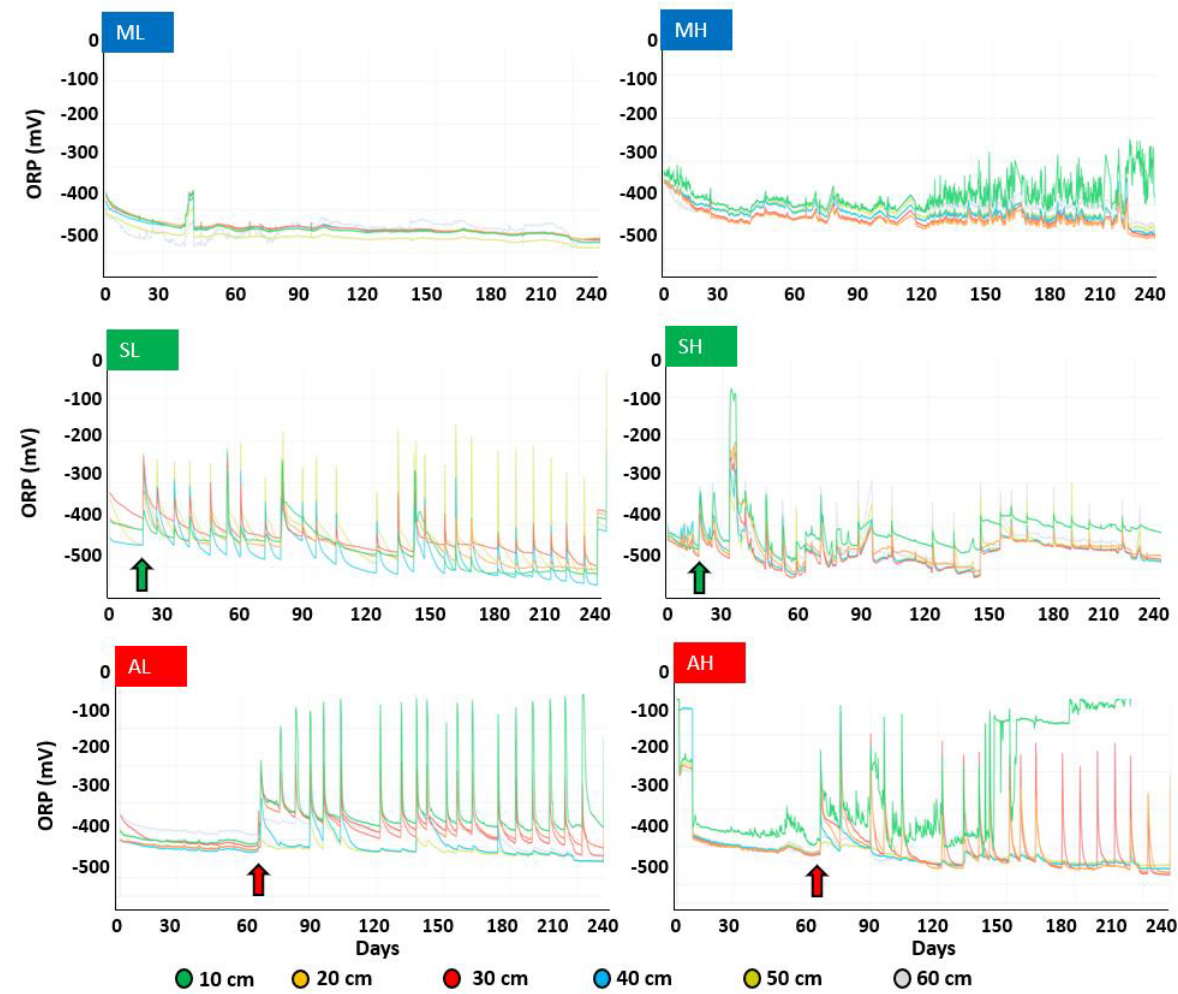


SO₄²⁻ Reduction

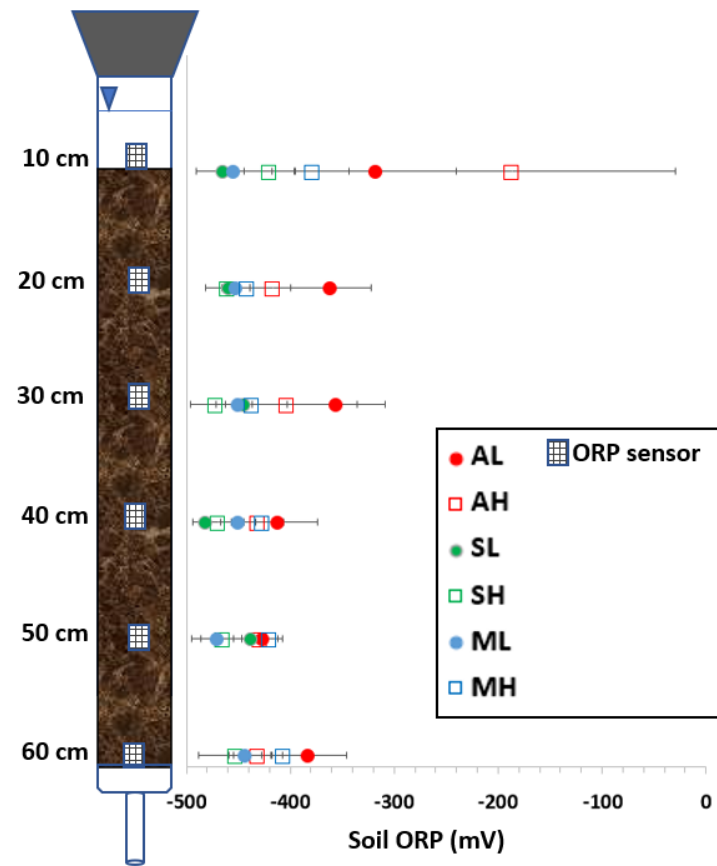


Oxidation-Reduction Potential Trends

Continuous Soil ORP



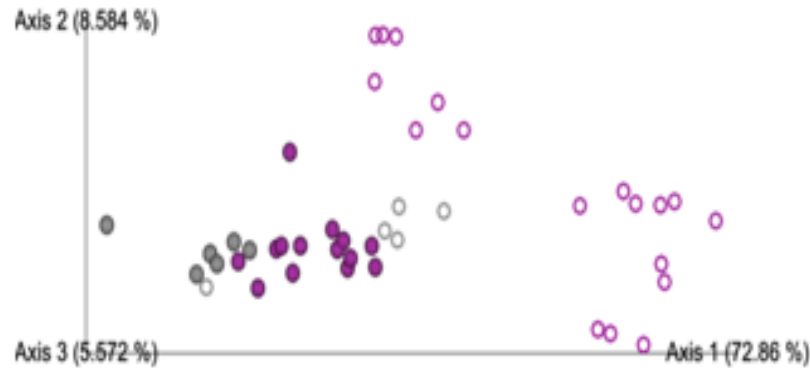
Average ORP (mV)



Microbiome Characterization: DNA & RNA Extraction

DNA and RNA

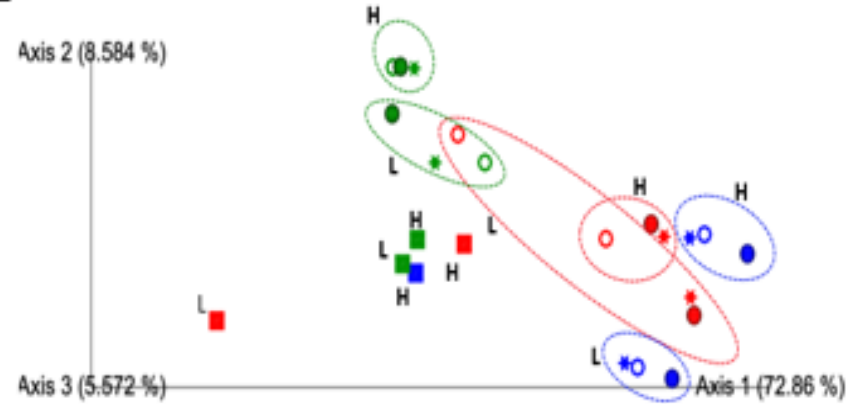
A



- DNA Initial
- DNA Final
- RNA Initial
- RNA Final

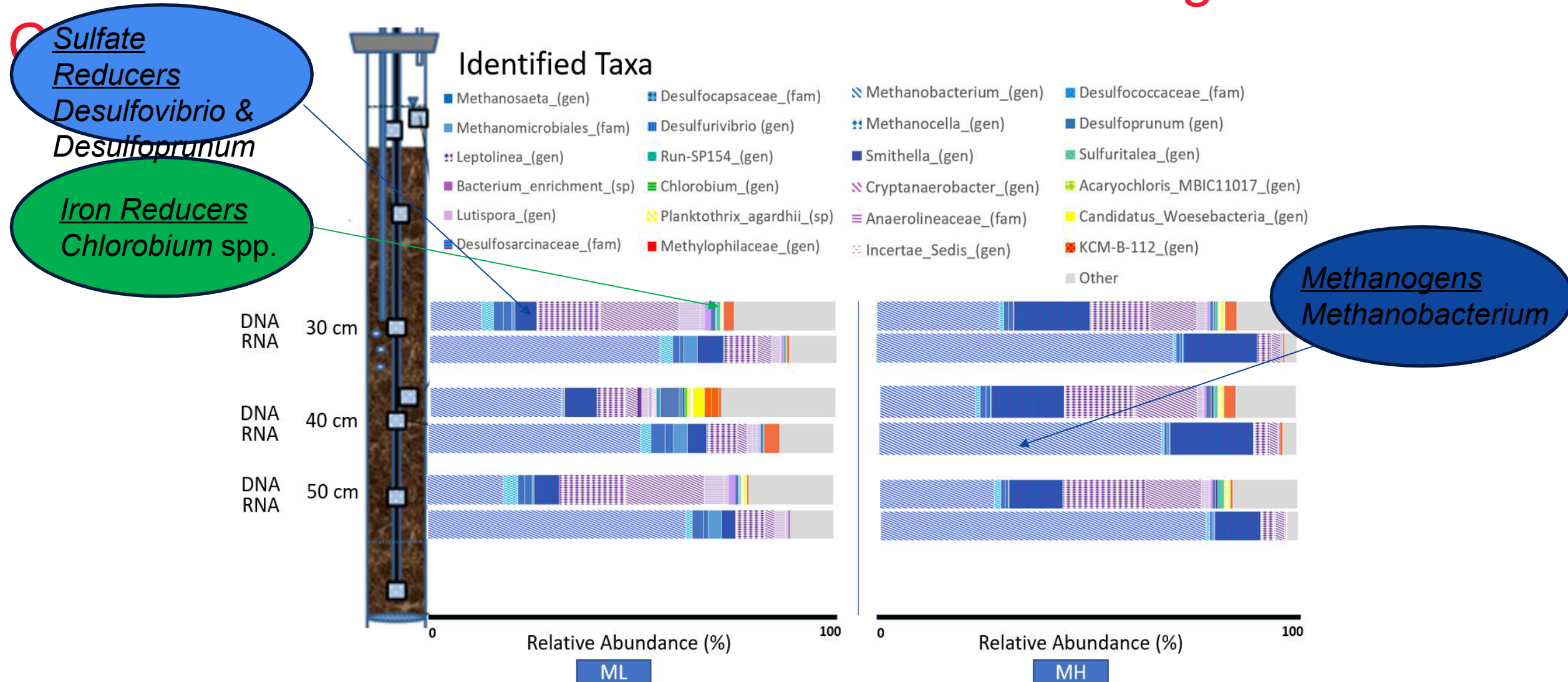
RNA Only

B



- RNA Methanogenic – initial
- RNA Methanogenic – 30 cm
- ★ RNA Methanogenic – 40 cm
- RNA Methanogenic – 50 cm
- RNA Sulfate addition – initial
- RNA Sulfate addition – 30 cm
- ★ RNA Sulfate addition – 40 cm
- RNA Sulfate addition – 50 cm
- RNA Pulsed air sparging – initial
- RNA Pulsed air sparging – 30 cm
- ★ RNA Pulsed air sparging – 40 cm
- RNA Pulsed air sparging – 50 cm

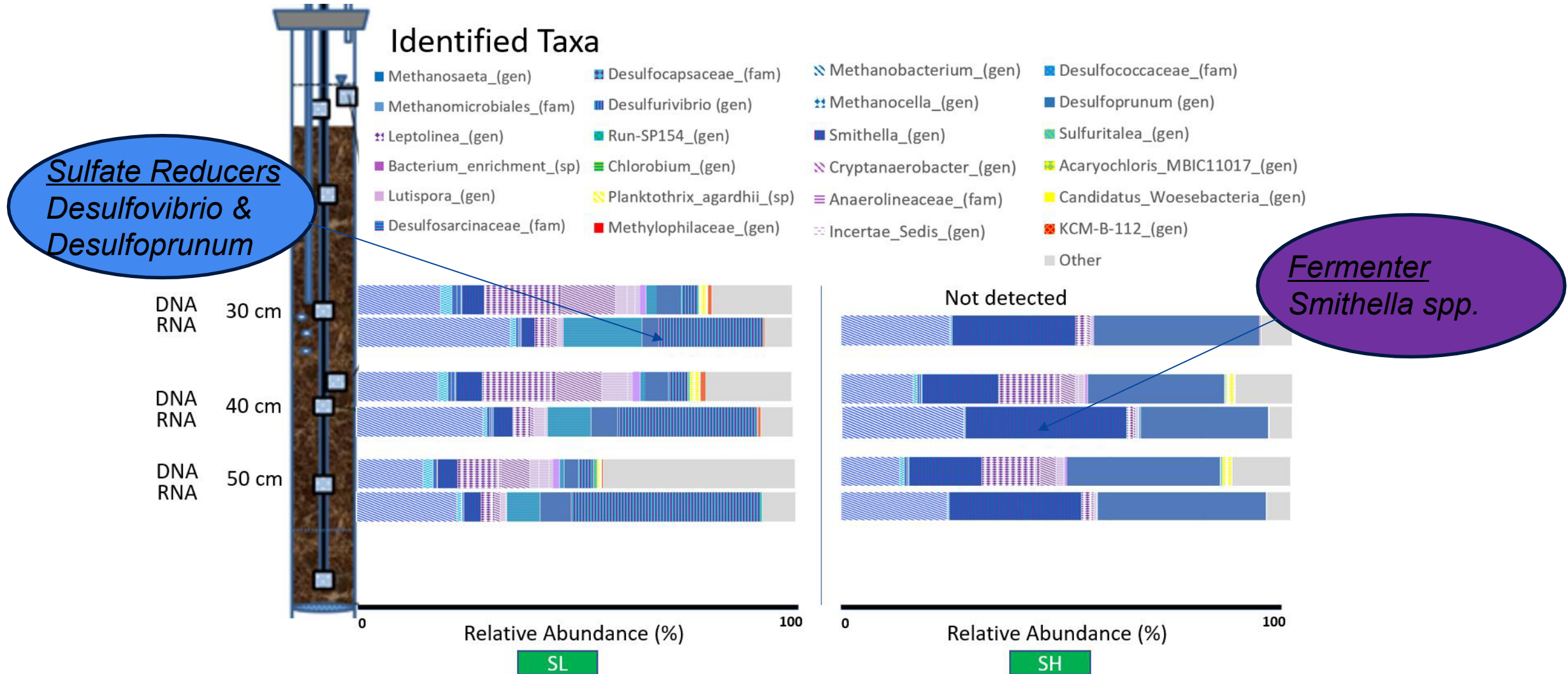
Microbiome Characterization: NSZD/Methanogenic



Based on RNA sequencing, redox processes higher than methanogenesis is less significant compared to observations based on DNA sequencing alone. Also aligned with RT-qPCR results targeting *mcrA*

ExxonMobil
transcripts

Microbiome Characterization: ENSZD/Sulfated Columns

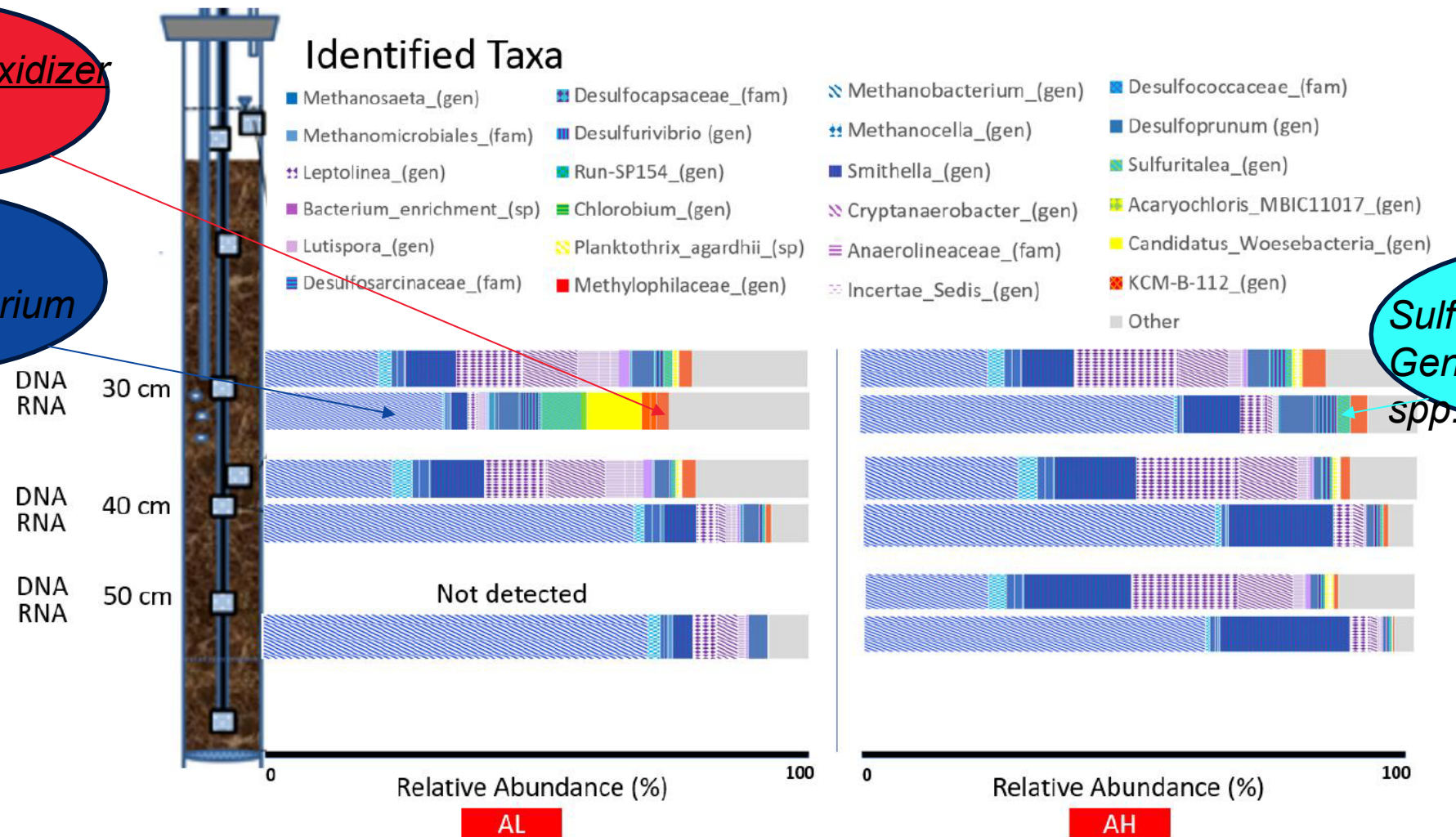


Sulfate reducer composed ~50-60% of the active microbial community dominated by *Desulfovibrio* and *Desulfoprunum*. *Smithella* (fermenter, syntroph n-alkane degrader) was present in SH at 30% and 5% in SL. *mcrA* transcripts were non-detect in sulfate amended columns.

Microbiome Characterization: ENSZD/Air Sparged

Aerobic PHC Oxidizer
KCM-B-112

Methanogens
Methanobacterium



Array of microbial processes were active near the point of aeration, ranging from aerobic PHC oxidation to methanogenesis. Aeration does not inherently indicate halting of methanogenesis, dependent on mass and deliverability of air.

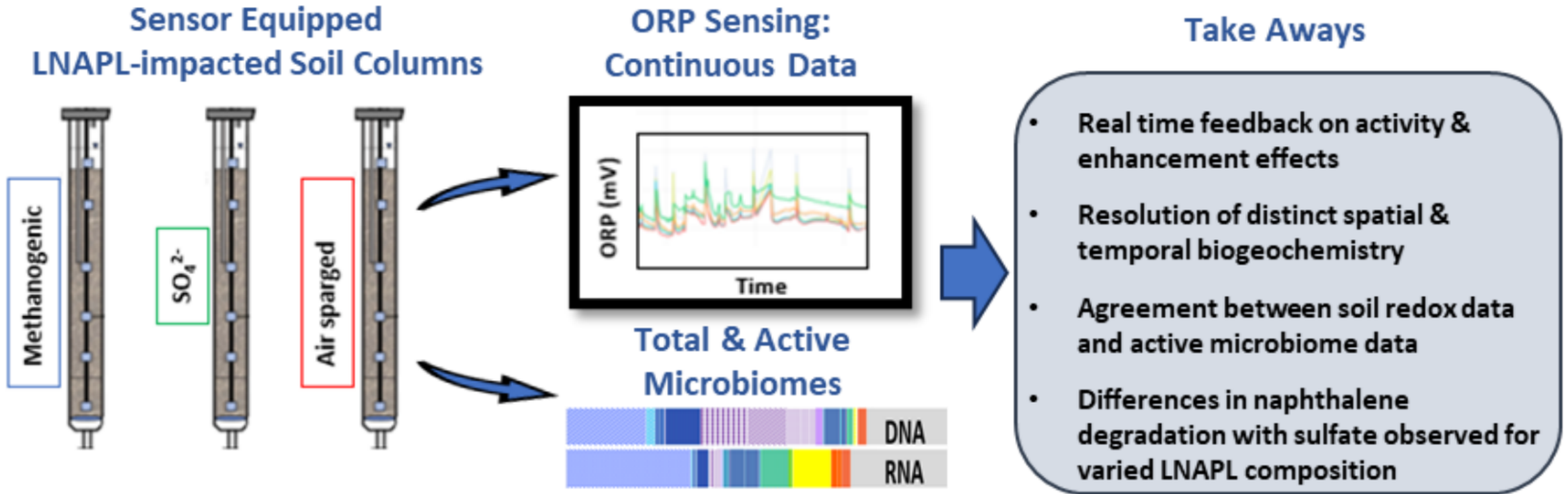
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Summary

Summary



Manuscript currently under peer-review

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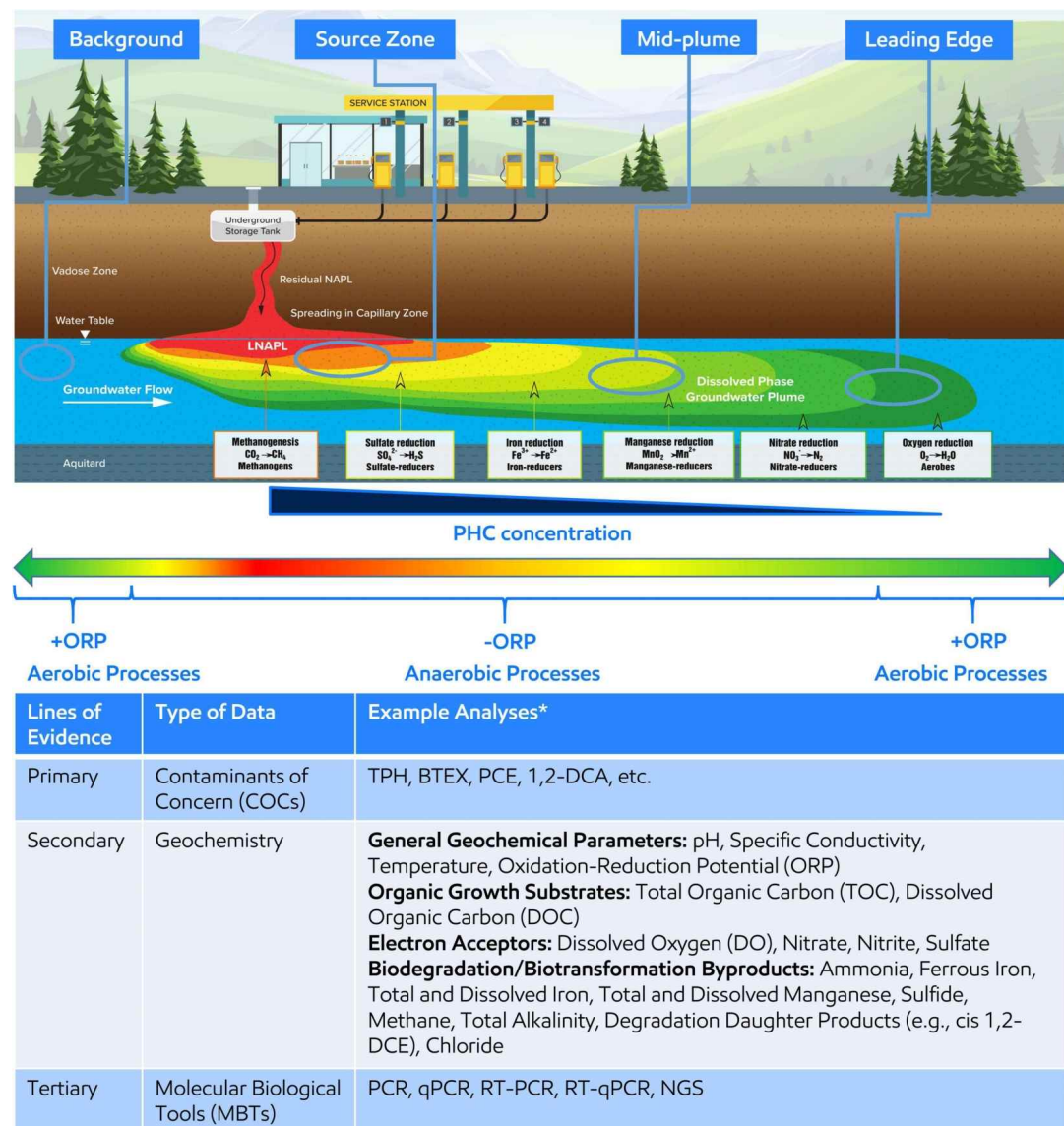
Q&R

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Back Up

MBT Framework



*Example analyses may differ depending on site-specific scenarios or goals

