Spatial Analysis of the Microbial Community in Mining Waste Rock: Activities & Signatures

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INTRODUCTION

Waste rock from mining activities represents a source of constituents of interest (CI's) including selenium (Se), and nitrate. During and after mining, rocks with sulphide minerals are exposed to air and water, and the co-occurrence of Se with sulphide minerals causes the mobilization and release of Se. While microbial mediated control of Se and other CI's is known to occur in mine waste, surprisingly little information is currently available

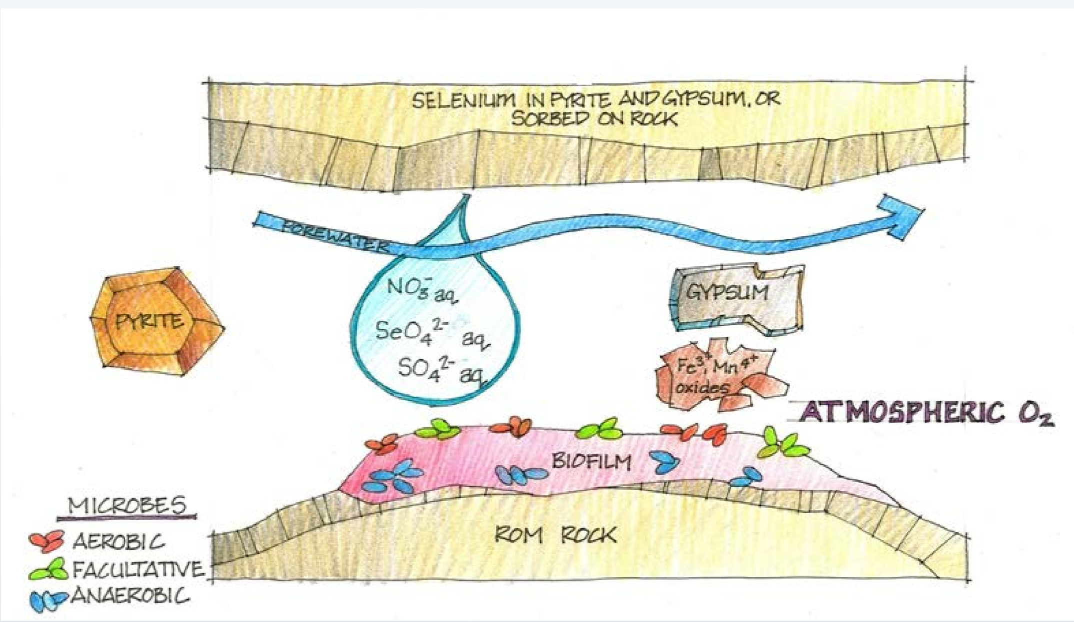

Figure 1. Microbes living in mine waste rock are known to directly influence Se, NO_3, Fe, and SO_4 geochemical cycling.

concerning the distribution and diversity of the microorganisms involved. In this study, the baseline microbial community structure and richness were assessed from samples obtained from selected mine sites by pyrosequencing of 16S rRNA gene amplicons. The following questions were then posed:

- **Are Se, NO_3, Fe, SO_4 reducing microbes present?**
- **What is the character of the community?**
- **What conditions influence community development and function?**
- **Do these communities stabilize CI's in the field conditions observed at the mine sites being studied?**

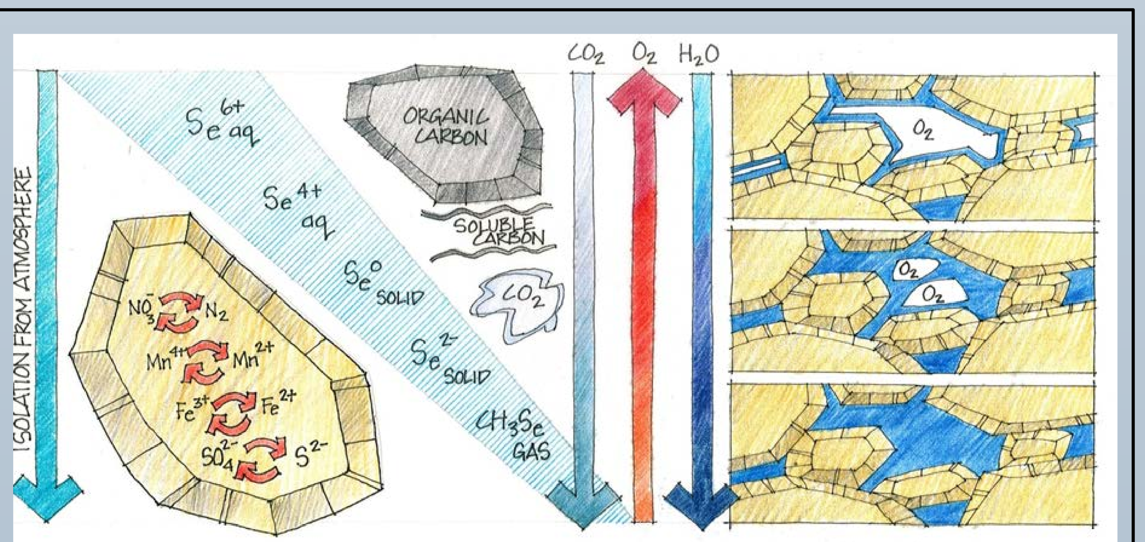

Figure 2. During the course of sulphide oxidation in mined waste rock, selenate ($Se^{6+}O_4$) is released. Released selenate is mobile due to its low tendency to absorb to reactive surfaces. Reduced conditions are known to develop in biofilm micro-environments, allowing selenate (Se^{6+}) to be converted to selenite (Se^{4+}), and possibly also elemental selenium (Se^0) and selenide (Se^{2-}). The resulting selenium forms all have decreased mobility in the environment.

INDUSTRIAL RELEVANCE

- **Can microbial communities in waste dumps be managed through dump design and dump management approaches to help influence the biogeochemical cycling and the release of CI's?**

METHODS

Drill Hole Sampling

- Sample mine waste rock from a variety of conditions:
  - Ex-pit waste rock
    1. Saturated
    2. Unsaturated
  - Saturated fills in pits
- Sample at varying depths/lithologies

Aseptic Sampling

- Appropriate aseptic handling for biological collection
- Proper preservation at -80C for molecular analysis

Extraction of Genomic DNA

- Fast DNA Spin Kit for Soil
- 500 mg max. biomass per DNA extraction
  - Purpose: Lower the prevalence of humic contaminants, known to inhibit DNA amplification
- Post extraction cleanup with PCR inhibitor removal kit (Zymo)

Amplification of Targeted DNA

- SSU rRNA gene sequence
  - V1V2 and V3 regions
  - Barcoded 8F-529R primers
- 1 ng DNA/reaction (25 cycles)

Pyrosequencing & Post-run Processing

- Amplicon processing
- Sequence trimming
  - 1 SD from the mean length
  - Short sequence removal
- Q score cutoffs: 25, with 15% allowance below cutoff
- Removal of primer and ambiguous nucleotide errors
- Complete linkage clustering at 97% ID to generate sample OTU's

RESULTS

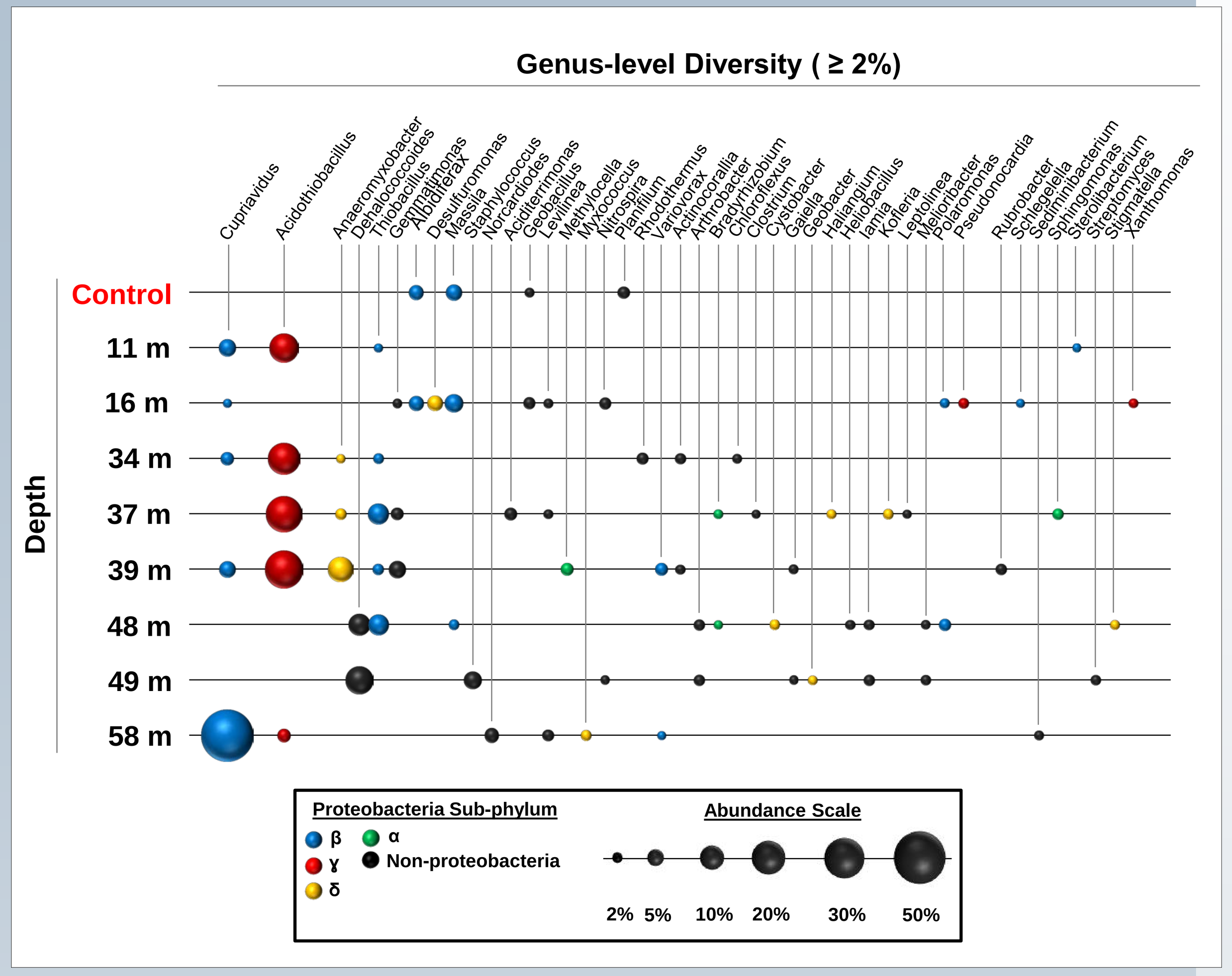

Figure 3. Subsurface microorganisms detected from ex-pit waste rock and relative abundances of each genera-level clade. A background control sample from undisturbed native ground is also included for comparison. The relative abundance of a genera is proportional to the circular area, as illustrated by the scale bar on the bottom. Circular color coding represents the major proteobacterial sub-phylum each genera is a member of.

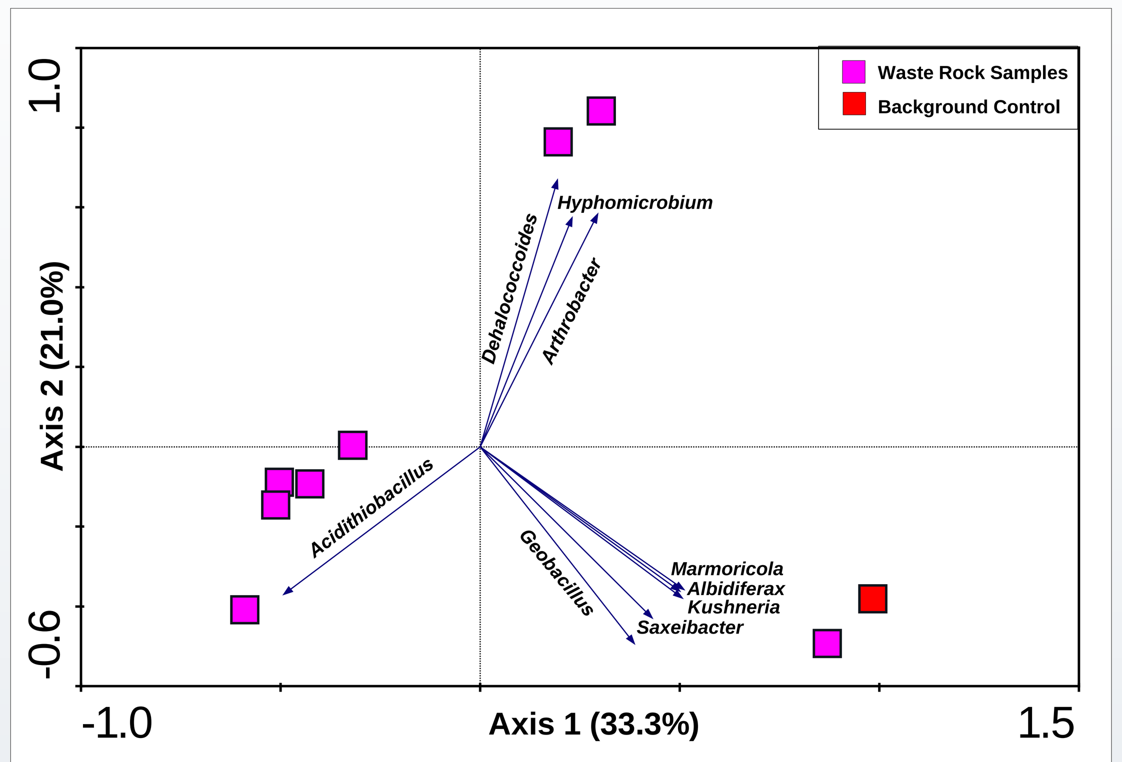

Figure 4. Ordination diagram of axes 1 and 2 of the bacterial genera (≥2% abundance) in ex-pit waste rock. Samples are represented as pink squares, while a background control is depicted as a red square. Arrows illustrate important genera involved in explaining the variance across samples.

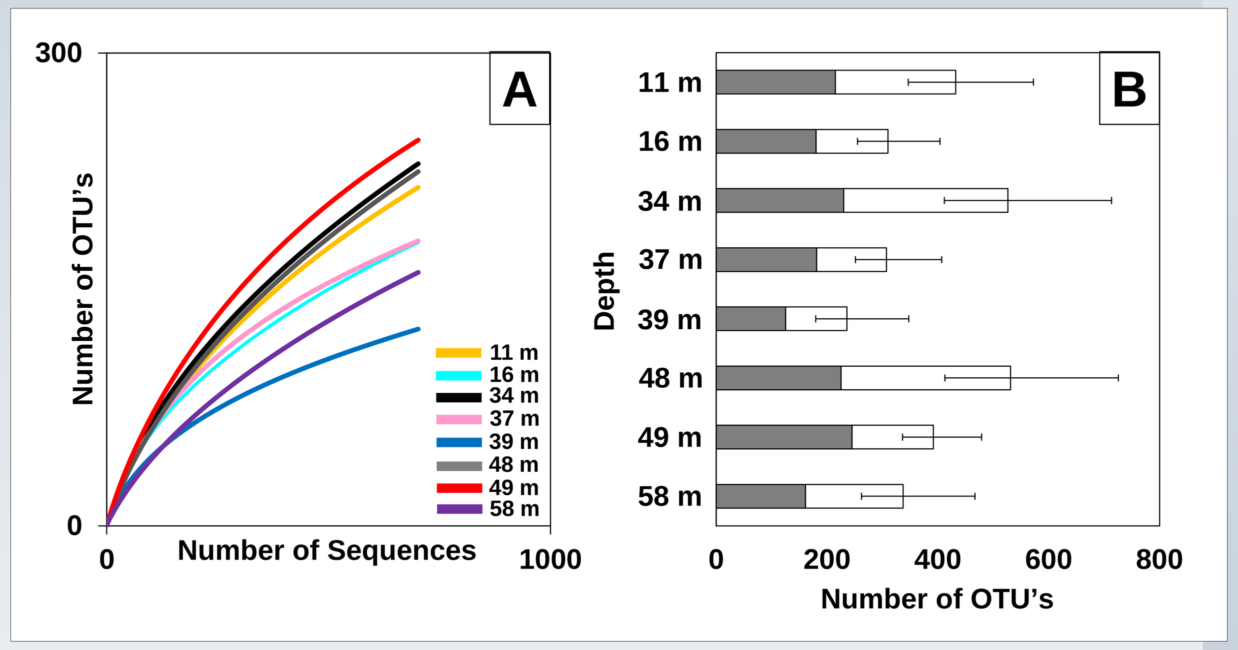

Figure 5. Measurements of bacterial diversity in ex-pit waste rock. (A) Comparison of diversity (97% ID cutoff) across drill hole sampling depths. Calculations were normalized to the same number of reads across samples. (B) Normalized Chao1 estimates of community richness (97% ID cutoff) with error bars depicting the upper and lower confidence interval. Grey bars represent the number of OTU's already targeted in the pyrosequencing analysis, suggesting ≥42% of the bacterial community was covered by current sequencing efforts in all samples.

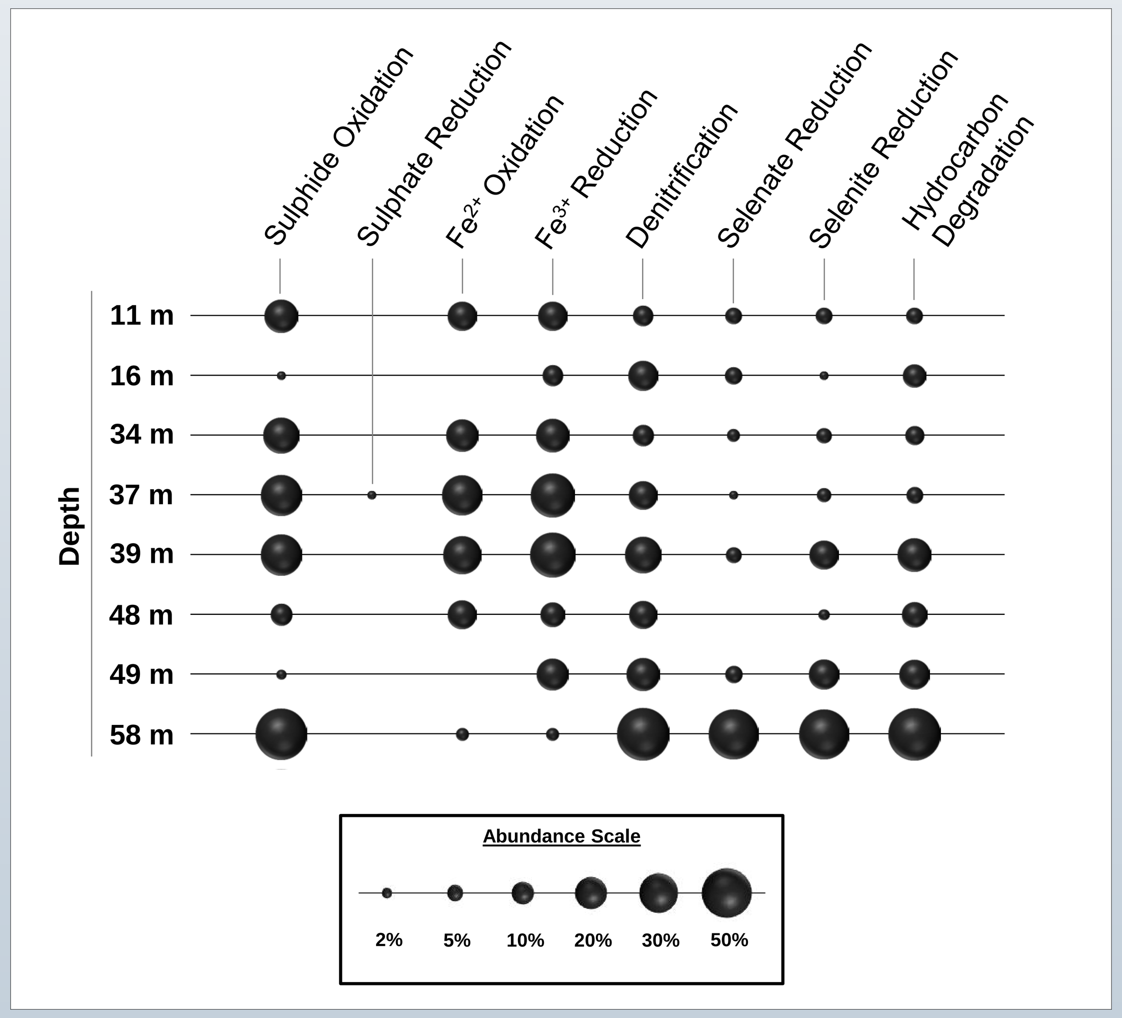

Figure 6. Metabolic potential of the bacterial community detected in ex-pit waste rock. The relative abundance of genera associated with various target microbial activities are proportional to the circular area, as illustrated by the scale bar on the bottom.

CONCLUSIONS

- **Microbes that reduce Se, NO_3, Fe, and that break down hydrocarbons are present in waste rock samples.**
- **Few microbes detected have the metabolic capacity to reduce sulphate.**
- **Initial sequencing efforts appear to be sufficient at capturing ~>50% of the total community diversity.**
- **Principle coordinate analysis reveals the clustering of samples based on important bacterial communities.**

FUTURE WORK

- **Conduct community analysis on ex-pit waste rock samples with corresponding aqueous geochemistry.**
  - Will allow for multivariate analysis and a statistically significant connection to important environmental variables
- **Improve understanding of community function.**
- **Advance research efforts into how to improve nitrate and selenate reduction.**