

Microbial Biosensors in Through-Cover Mineral Exploration

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Introduction

Mineral exploration in Canada—and other areas across the globe—is becoming increasingly difficult with the likelihood of future mineral-resource discoveries being buried beneath appreciable glacial overburden and/or bedrock. The development of innovative exploration approaches to detect mineralization through overburden is vital for continued success in the discovery of new resources (Winterburn et al., 2017). One such technique, microbial-community fingerprinting, shows great potential when exploring for mineral targets that are hidden by thick (>2 m), complex and transported surficial materials. With continued development, it may transform how exploration is carried out for buried natural resources (Iulianella Phillips, 2020; Simister et al., 2020).

Micro-organisms kinetically enhance geochemical reactions, including the dissolution and formation of diverse minerals, and harness energy from these reactions to support their metabolism and growth in nearly every low-temperature geological setting (Newman and Banfield, 2002; Falkowski et al., 2008). They are acutely sensitive, often responding rapidly to the dynamics of chemical and physical properties in their surrounding environments. Subtle changes in mineral bioavailability, for example, can be reflected in dramatic shifts in the composition and activity of microbial communities (Reith and Rogers, 2008; Wakelin et al., 2012; Leslie et al., 2014; Fierer, 2017). Analyses of microbial-community composition and structure thus have a strong potential to resolve chemical and physical differences between environments that are not readily discernible through conventional geochemical and geophysical surveys.

The advent of high-throughput sequencing platforms over the last decade has transformed the capacity to interrogate complex microbial communities across a wide range of environmental matrices (Binladen et al., 2007; Zhou et al., 2015). The application of these technologies enables high-throughput profiling of the taxonomic compositions and metabolic potential of soil-microbial communities across defined survey areas. Given that every individual soil sample contains thousands of microbial taxa, each containing hundreds to thousands of genes sensing and interacting with the surrounding soil environment (Fierer, 2017), the statistical power of this approach to identify anomalies is unprecedented.

Previous Research

Two British Columbia (BC) porphyry-copper deposits, the Highland Valley Highmont South Cu-Mo deposit (HVC) of Teck Resources Ltd. and the Consolidated Woodjam Copper Corp. Deerhorn Cu-Au deposit, were used to evaluate microbiological techniques for sulphide exploration in BC (Figure 1). B-horizon soil samples were analyzed for inorganic geochemistry (aqua-regia digestion with inductively coupled plasma–mass spectrometry [ICP-MS] finish) and microbial-DNA sequencing (16S rRNA marker gene; Iulianella Phillips, 2020; Simister et al., 2020). In both cases, mineralization is covered by transported glacial overburden (2–10 m at HVC and 25–60 m at Deerhorn), with compositional variation in surface materials (e.g., till blanket, organic deposits, glaciolacustrine sediments; Iulianella Phillips, 2020).

The authors have shown that microbial-community fingerprinting can detect anomalies in bacterial populations in the surface environment that correlate with the surface projection of sulphide mineralization (Iulianella Phillips, 2020; Simister et al., 2020). Deposit-scale investigations of HVC and Deerhorn revealed suites of micro-organisms that have

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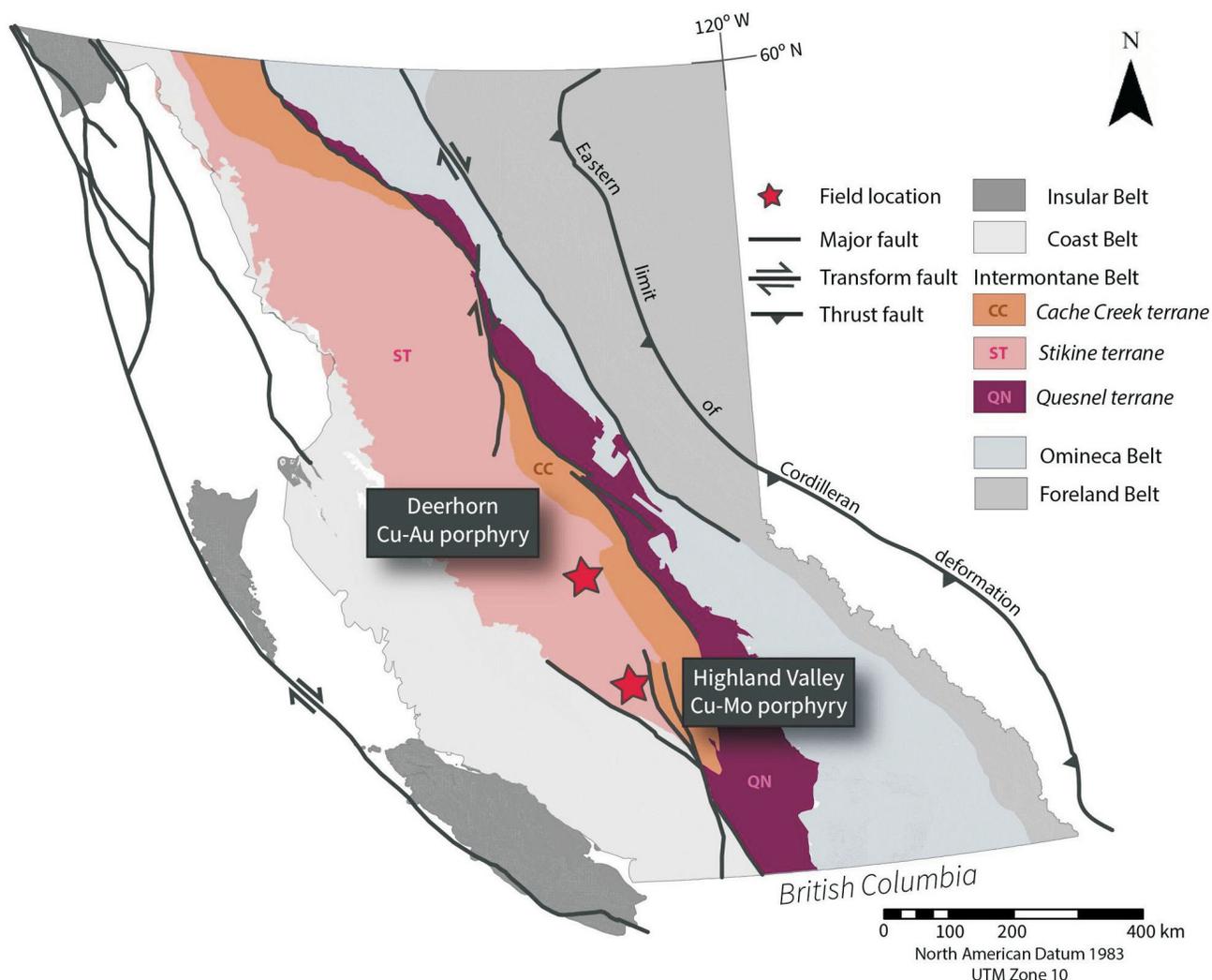


Figure 1. Locations of porphyry-Cu research sites (Highland Valley Highmont South Cu-Mo deposit and Deerhorn Cu-Au deposit) and the Mount Washington high-sulphidation Au-Ag-Cu epithermal prospect. Thick black lines indicate major faults. Terranes and geological belts are characterized based on bedrock mapping carried out by the British Columbia Geological Survey (BCGS; Cui et al., 2017).

statistically significant ($p < 0.05$) shifts in relative abundance occurring directly above the surface projection of mineralization (0.1% Cu equivalent and 0.2% Au equivalent at HVC and Deerhorn, respectively; Figures 2 and 3). Specifically, microbial anomalies at Deerhorn discriminate mineralization at the surface where no detectable geochemical signal has been generated (Figure 3). These results signify the efficacy of using modern DNA sequencing to elucidate buried mineralization and provide the support for further investigations into the use of microbial communities to sense chemical and physical changes in their environment, with respect to ore mineralization.

New Research

Several new avenues of research are currently being explored to reduce knowledge and technology barriers in the application of DNA sequencing for the exploration of con-

cealed mineral deposits. Field-based approaches include new orientation studies across glacial till-covered Pb-Zn sulphide mineralization and saturation-gradient soil sampling in a bog-wetland setting. Experimental approaches focus on assessing bacterial responses to changes in mineralogy and temperature. These objectives support the progressive development of microbial-community fingerprinting into a tool that may be employed by the mineral exploration and mining industry.

Pine Point Mississippi Valley–Type Deposit

In September 2022, a geochemical and geobiological soil survey was completed across mineralized collapse structures at the Pine Point Mississippi Valley–type (MVT) Pb-Zn deposit in the Northwest Territories (NWT). The Pine Point district is located on the southern shore of Great Slave Lake (Figure 4), on the eastern edge of the Western Cana-

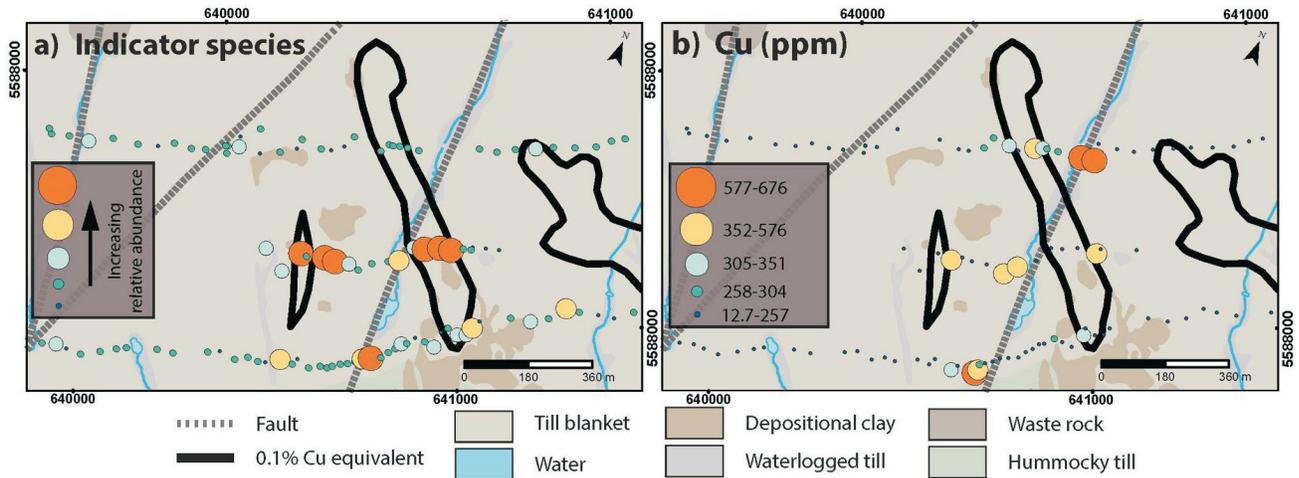


Figure 2. Example of an indicator micro-organism (a) and the Cu-anomaly map of the same area (b) from the Highland Valley Highmont South Cu-Mo deposit. Geochemical data derived from aqua-regia digestion of B-horizon soils with ICP-MS finish. Microbiological data derived from 16S rRNA gene DNA sequencing. Co-ordinates in UTM Zone 10, NAD 83.

dian Sedimentary Basin (WCSB). Sulphide mineralization (galena+sphalerite±marcasite±pyrite) is hosted predominantly in Middle Devonian carbonate units within or proximal to the dolomitized Presqu'ile barrier-reef complex (Hannigan, 2007). Mineralization is largely structurally controlled along the McDonald–Great Slave Lake fault system (Hannigan, 2007), exhibiting either prismatic or tabular geometries (Krebs and Macqueen, 1984; Cumming

et al., 1990). Generally, the area is covered with Quaternary sediments that are dominated by organic deposits, lacustrine and glaciolacustrine sediments, glacial-till blanket, and lesser alluvial and eolian deposits (Oviatt et al., 2013). The sampled field area is characterized by prismatic mineralization and covered by glaciolacustrine beach sediments, with overburden at its thickest where it infills the collapse structures (~40 m).

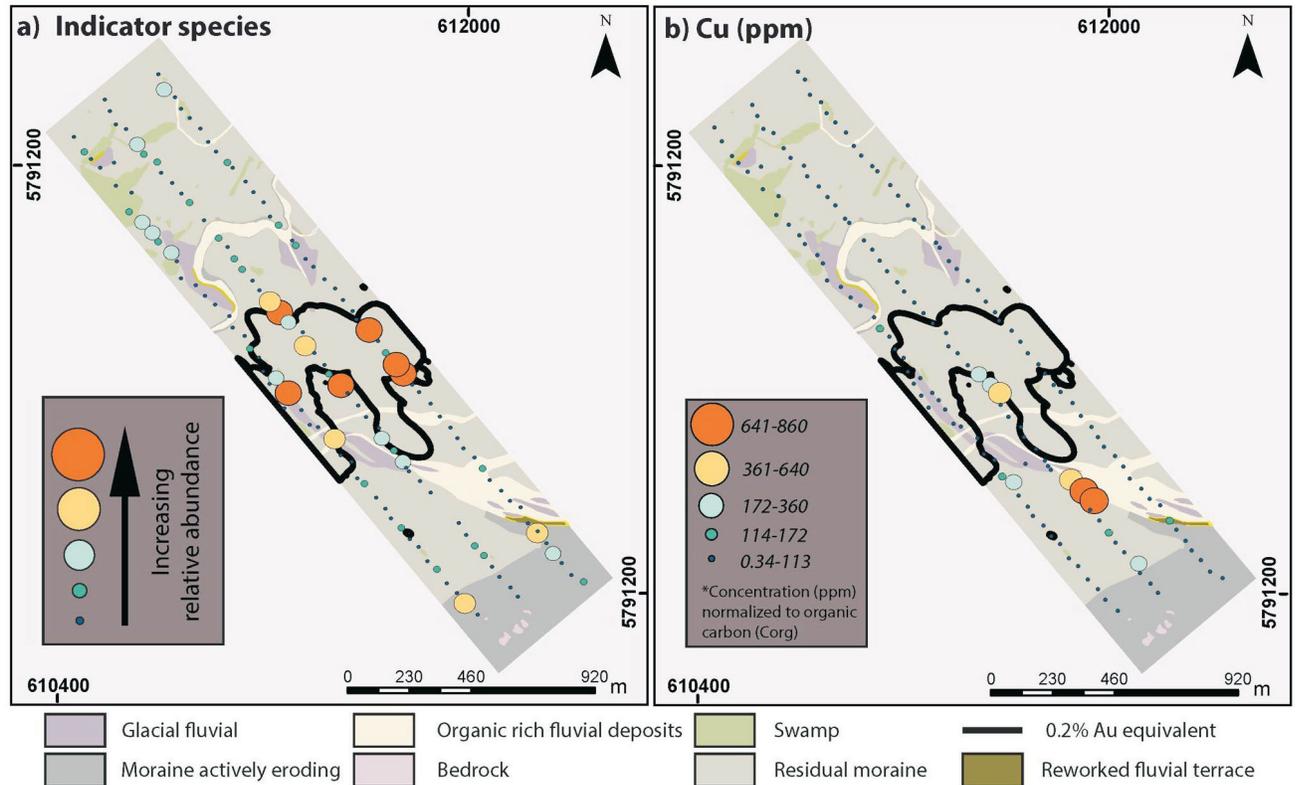


Figure 3. Example of an indicator micro-organism (a) and the Cu-anomaly map (normalized to organic carbon) of the same area (b) from the Deerhorn Cu-Au deposit. Geochemical data derived from aqua-regia digestion of B-horizon soils with ICP-MS finish. Microbiological data derived from 16S rRNA gene DNA sequencing. Co-ordinates in UTM Zone 10, NAD 83.

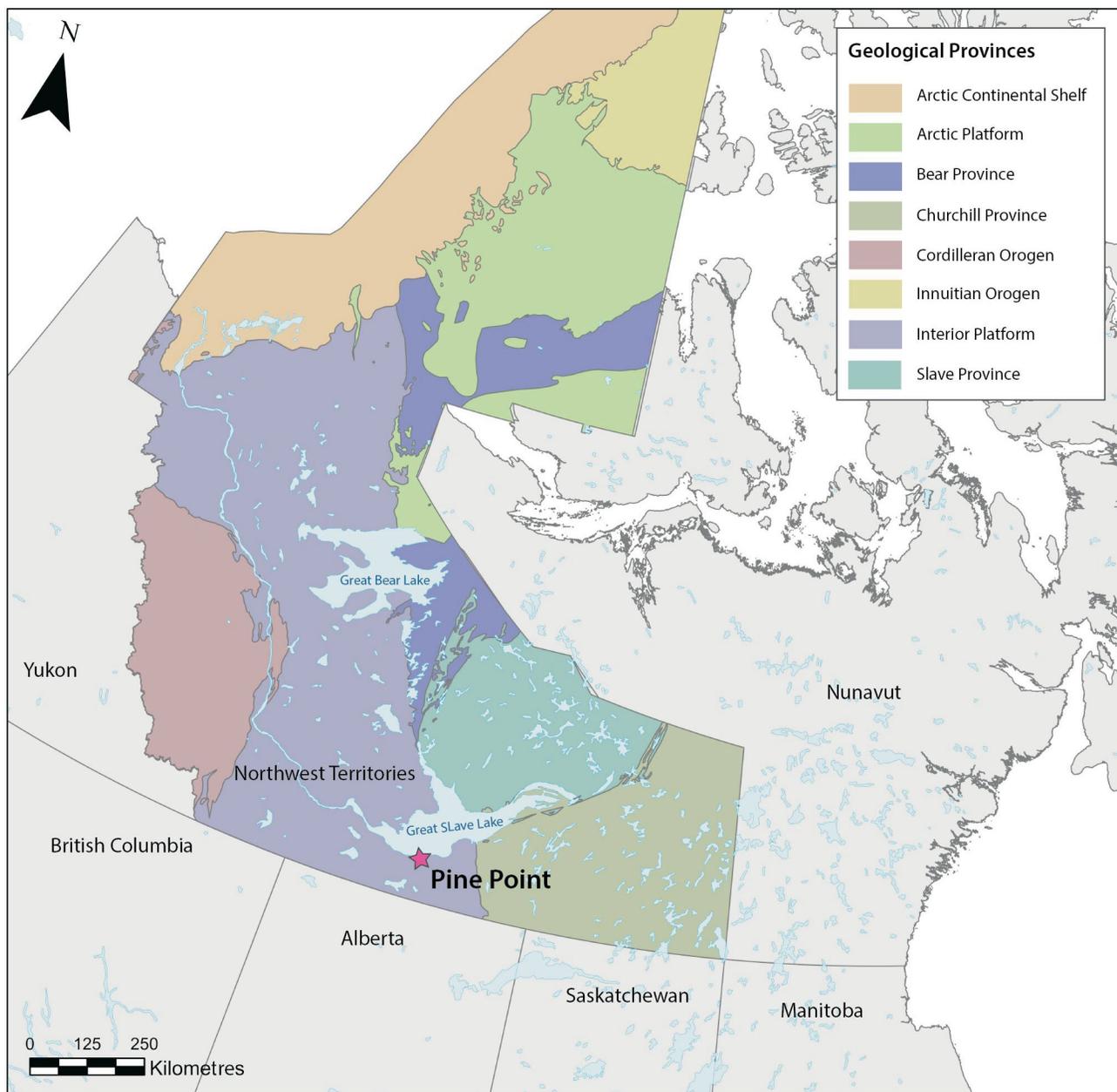


Figure 4. Location of the Pine Point Pb-Zn district, Northwest Territories. Geological provinces are compiled from publicly available data provided by the Northwest Territories Geological Survey (Wheeler et al., 1997).

Thirty-eight soil samples were collected on two survey lines that crossed the surface projection of the mineralized collapse structures, with sampling gradationally spaced outward from mineralization into background cover soils. Soils for microbial-community analysis were sampled with sterilized equipment and without field screening to preserve the microbial community as much as possible. Descriptions were documented for in situ physicochemical variables at each sample site for every observed soil horizon in the profile. The B-horizon soils were targeted for

geochemical and microbial soil samples, although multiple horizons were taken where possible. Soils for microbiological analyses were preserved each day in the field for cell-count analysis, RNA profiling, and intracellular and extracellular DNA profiling. Although this work is not focused in BC, the application of soil microbial-community fingerprinting in the Pine Point district has direct implications when exploring for metalliferous sulphide deposits in covered terrain across BC, northern latitudes in North America, and globally.

Microbial Community Response to Soil Moisture

Soil Preservation

Water content is a fundamental control on the composition and activity of microbial communities in soils, globally (Fierer, 2017). Soils in climates with higher moisture also typically contain higher levels of microbial biomass (Serna-Chavez et al., 2013). Soil-moisture contents, furthermore, likely have an important effect on the preservation of soil-microbial communities during prolonged transport and storage. Whereas it is common practice that microbial communities in soils are immediately frozen to preserve the integrity of DNA and the fidelity of community profiles by arresting further growth or decay (Delavaux et al., 2020), soil surveys in mineral-exploration programs are often in remote areas with limited resources for freezing and typically have lengthy transport times to the laboratory. Successful surveys therefore require knowledge of how temporary and extended sample-storage regimes, like cooling and drying, influence soil-microbial communities, specifically with respect to the potential for community change during storage. Here, it is important to know how community-turnover rates respond to storage temperature and moisture contents.

The University of British Columbia (UBC) Totem Plant Science Field Station provides an excellent on-campus soil environment to conduct, and collect materials, for soil-related experiments. The lead author has sampled bulk soil from the UBC Totem Plant Science Field Station and sub-

jected it to a range of temperature and moisture conditions to examine subsequent effects on the microbial community composition, diversity and structure, as well as microbial population turnover rates. These perturbations include freezing samples at -20°C , leaving samples at room temperature, and allowing the samples to dry out (Figure 5). Currently, 9 months of timepoints have been sampled, while preparation for DNA sequencing of the soil-microbial communities is ongoing (initial microbial-community composition of this soil is described in Iulianella Phillips et al. [2022]). These experiments will inform the development of a minimal and robust sample-storage protocol and serve as a test of the relationships between warming and drying of soils and microbial-community turnover.

Bog Wetlands

Bog wetlands represent an important carbon sink globally, with northern peatlands estimated as storing 415 ± 150 Pg (petagrams) of carbon (Beaulne et al., 2021)—approximately 30% of total carbon stores on Earth (Parish et al., 2008). The impact of soil moisture on microbial-community composition and activity is particularly relevant as micro-organisms likely play an important role in controlling carbon release versus storage in response to changing climate (Kitson and Bell, 2020; Carrell et al., 2022). Water-saturated surface materials, such as bogs, also present a substantial challenge to the mineral-exploration industry as there are few methodologies that are effective when exploring in these settings. In microbial fingerprinting, substantial community anomalies are expected to develop in response to water saturation and discriminating between these

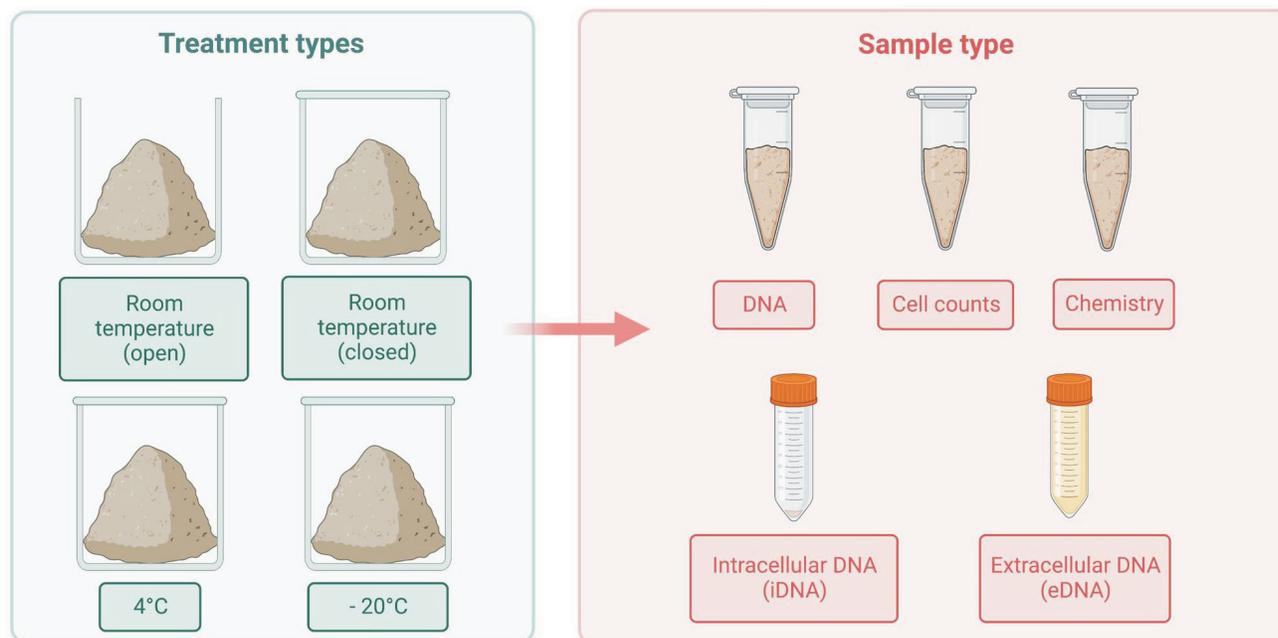


Figure 5. Schematic representation of the soil-preservation experiment, including the treatment types and the different sample products. For each timepoint and treatment type, soil is sampled for DNA, bacterial-cell counts, chemistry, intracellular DNA (DNA found within intact cells) and extracellular DNA (DNA derived from outside the cell in environmental samples).

and bona fide anomalies related to mineral resources will be key to successful surveys. Therefore, bog wetlands provide a unique opportunity to study the effects of soil moisture on microbial communities, both in the context of microbial carbon cycling and in the development of ore-mineral anomalies.

The lead author has sampled organic soils within bog wetlands in northern Canada across a soil-moisture gradient to determine differences in soil-microbial-community composition, structure and activity related to water content. Sampling focused around both the active and permafrost layers of a palsa, as well as the adjacent organic-rich materials from a bog and a fen (Figure 6). Differences in composition and structure will be determined through DNA sequencing, whereas activity will be determined through isotope-labelling experiments.

Mineral Sensing

Micro-organisms are acutely sensitive to chemical and physical differences in their environments. For example, subtle variability in trace Fe concentrations in seawater is reflected in striking differences in phytoplankton distribution and activity in the oceans (O'Reilly et al., 1998; Fuhrman, 2009). They also typically have strong affinities for surfaces (Grinberg et al., 2019), often creating biofilms (Donlan et al., 2002; Dang et al., 2016), and are able to selectively colonize specific solid substrates to meet a broad

diversity of physiological needs (Tuson et al., 2013; Finley et al., 2022). However, despite a large body of evidence showing that micro-organisms preferentially associate with surfaces, the molecular mechanisms through which they sense these surfaces, and select those that confer a specific advantage, remain almost entirely unknown. To test for microbial capacity to sense mineral surfaces, the gene expression of *E. coli*, a well-studied model micro-organism, will be evaluated when exposed to rock- and ore-forming minerals. Specifically, microbial responses to olivine, K-feldspar, plagioclase-group minerals, quartz and pyrite will be assessed. These analyses will enable the identification of genes that are differentially activated in response to mineral exposure. The identities of upregulated genes will provide insight into the metabolic machinery behind microbial mineral sensing. Such novel insight into biological sensing of surfaces will inform conceptual models of microbe-mineral interactions in soils, as well as open up new opportunities for the development of mineral biosensors for a broad range of applications.

Conclusions

Outcomes from these deposit-scale orientation studies have highlighted the potential for geomicrobiological tools and techniques for successful application to through-cover mineral exploration in British Columbia and beyond. Current research directions focus specifically on reducing fundamental unknowns about the behaviour and variation of

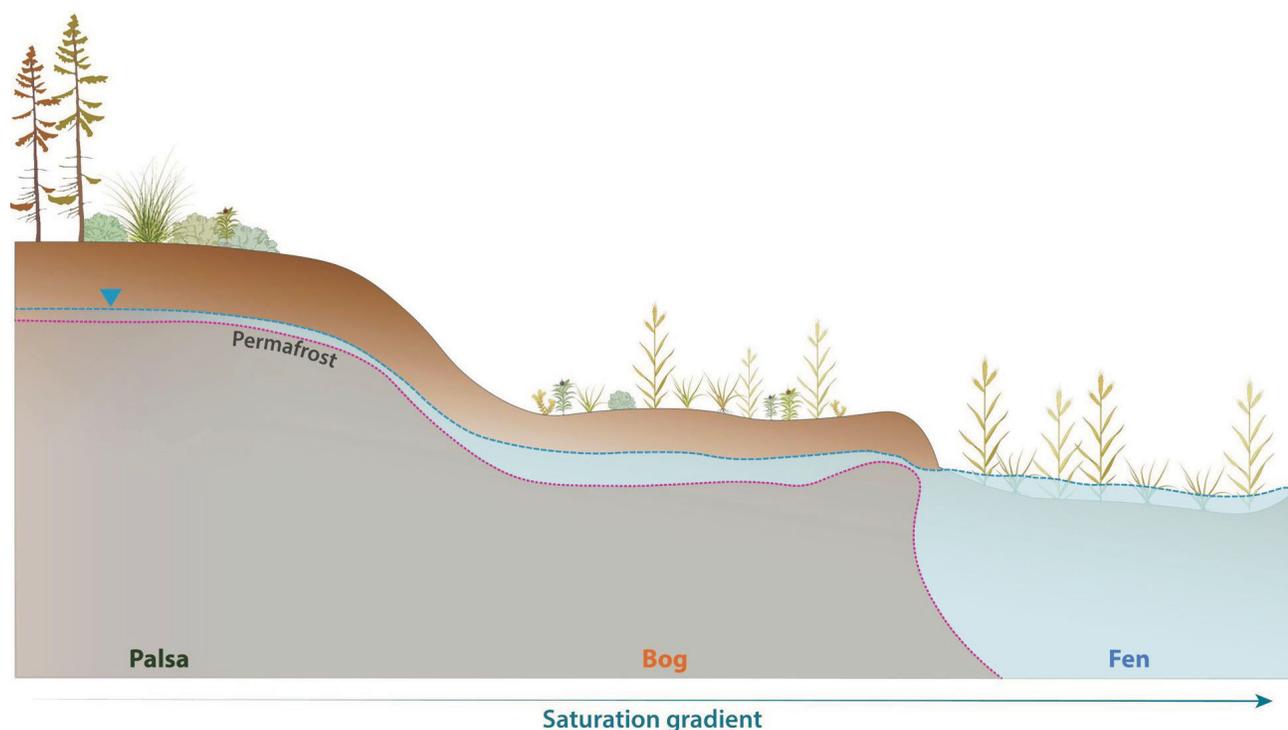


Figure 6. Schematic diagram of a saturation gradient from a dry palsa with an active and a permafrost layer to saturated soil conditions in bogs and fens. Depth of soil saturation is denoted by the dashed blue line and depth of the permafrost layer is indicated by the dashed pink line.

microbial communities in response to chemical and physical changes in the environment. This focus includes assessing DNA sequencing and microbial-community fingerprinting in a carbonate-hosted Pb-Zn-sulphide mineralization district covered by glacial sediments; assessing the impact of transport and storage on the persistence of microbial-community anomalies in soils; exploring the relationships between micro-organism function and variation in land type and water content; and evaluating gene expression of *E. coli* in response to different mineral exposures. Each of these activities serves on a different level to support the use of microbiology-based mineral exploration in different mineral systems, in various terrains and climates, and to develop practical and informed transport and storage protocols for use by industry.

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