Microbial-Community Fingerprints as Indicators for Buried Mineralization, Southern British Columbia

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Introduction

As global population grows and modernizes, demand for mineral resources is expanding (Kesler, 2007). At the same time, existing orebodies are being exhausted, while the frequency of new discoveries of exposed or partially exposed deposits diminishes. Demand for mineral resources must therefore be met through the discovery and development of buried or concealed mineral deposits. Although mineral resource extraction supported the core of the Canadian economy for more than a century—currently contributing $56 billion to Canada's GDP and providing 19% of its goods exports (The Mining Association of Canada, 2018)—its ability to do so relies on continued discovery of mineral deposits that may be concealed by overburden. Finding these mineral deposits beneath exotic overburden consisting of glacial and preglacial sediments remains a fundamental and widespread challenge to mineral exploration in Canada (Anderson et al., 2012; Ferbey et al., 2014).

New and innovative techniques that complement, enhance or even surpass traditional techniques to define the surface expression of buried mineralization could minimize the cost of exploration and help in targeting drilling activities (Kelley et al., 2006). Several recent studies in British Columbia (BC) have demonstrated the potential for new surface geochemical techniques to lead to the discovery of concealed orebodies. These include indicator minerals (Plouffe et al., 2013), soil partial-leach and selective-extraction geochemistry on multiple soil horizons (e.g., Bissig and Riquelme, 2010), halogen-element detection (e.g., Heberlein and Dunn, 2017), till geochemistry (Reid et al., 2009; Reid and Hill, 2010) and biogeochemistry (Reid et al., 2009; Reid and Hill, 2010). Each geochemical technique and media type has both strengths and weaknesses in identifying buried mineralization.

In particular, geochemical signatures generated from orientation surveys over known deposits are typically noisy with poor anomaly to background resolution (Stanley, 2003), show poor reproducibility and have element patterns that are sometimes difficult to reconcile with mineral-deposit chemistry and known trace-element mobility patterns (Heberlein and Samson, 2010). The limited mechanistic understanding of these techniques has led to compromised utility by the mineral exploration industry, resulting in less return on investment than possible. Shortfalls in many of these commercial techniques to repeat performance shown in orientation surveys over known mineralization has, in large part, resulted in questioned use and reliability by major exploration companies. Despite these issues, there is sufficient empirical evidence to indicate causative links between mineralization beneath transported cover and the presence of geochemical gradients in the surface environment (Hamilton, 1998; Kelley et al., 2006; Nordstrom, 2011).

Although much less explored, biological anomalies may be robust indicators of buried mineralization, and such anomalies may be detectable through low-cost, high-throughput geobiological surveys (Kelley et al., 2006). Micro-organisms kinetically enhance and exploit thermodynamically favourable geochemical reactions, including the dissolution and formation of diverse minerals, to support their metabolism and growth in nearly every low-temperature geological setting (Falkowski et al., 2008). They are acutely sensitive, often rapidly responding to the dynamics of chemical and physical gradients in the environment. Subtle

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changes in mineral bioavailability, for example, can be reflected in dramatic shifts in composition and activity of microbial communities (Fierer, 2017). This can be seen at the global scale as marine-phytoplankton communities respond to traces of iron in seawater, a process that can be viewed as chlorophyll plumes via remote sensing (Fuhrman, 2009). Application of modern sequencing technologies allows high-throughput profiling of the taxonomic diversity and metabolic potential of soil microbial communities across subtle, and often poorly resolved, geochemical gradients. Microbial-community profiles thus have a strong potential to resolve chemical and physical differences in sample suites that are not readily discernible through conventional geochemical and geophysical surveys. In residual terrains, for example, where chemical gradients are high, changes in bacterial population have been clearly demonstrated (e.g., Southam and Saunders, 2005; Reith and Rogers, 2008).

Even outdated techniques with low throughput and resolution, such as Denaturing Gradient Gel Electrophoresis (DGGE), that can produce a crude microbial-community 'fingerprint' (Wakelin et al., 2012) reveal changes in bacterial communities in soils over buried volcanogenic massive-sulphide (VMS) deposits. The advent of high-throughput next-generation sequencing (NGS) platforms over the last decade has transformed the capacity to interrogate the molecular fingerprints of microbial communities (Binladen et al., 2007; Zhou et al., 2015). Application of NGS technologies thus allows profiling of the taxonomic diversity and metabolic potential of soil microbial communities across defined survey areas. Given that each soil sample comprises 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.

The authors therefore propose that high-throughput sequencing technologies may enable use of soil microbial-community profiling as a robust, efficient and cost-effective tool to identify and locate buried mineral deposits in BC. In a pilot study, the use of soil microbial-community fingerprinting with modern DNA sequencing technologies was employed to find buried mineral deposits (Simister et al., 2017). This pilot study has shown that exposure of soil microbial communities to constituents commonly associated with mineral deposits elicits a response detectable on laboratory time scales of several weeks. The strong microbial responses observed are encouraging signs for the use of microbial-community fingerprinting in mineral-deposit exploration.

British Columbia is host to numerous mineral deposits of economic value, including a wealth of Cu-porphyry mineralization. Successful exploration for these deposits is dependent on technologies that can detect mineralization through thick sequences of Quaternary glacial sediments. The province is an ideal region to evaluate microbial-community sequencing as an exploration methodology for 'seeing' through overburden, as multiple field sites can be tested. Data have been collected from three different Cu-porphyry systems (Figure 1): 1) the Deerhorn Cu-Au porphyry of Consolidated Woodjam Copper Corp. in central BC, 2) the Highland Valley Copper Highmont South Cu-Mo porphyry of Teck Resources Ltd. in south-central BC, and 3) the Mount Washington Cu-Au porphyry on Vancouver Island.

Methodology

Field Locations

Sampling for geochemical and microbiological analysis was completed in July 2017 for both the Deerhorn Cu-Au porphyry and the Highland Valley Copper (HVC) Highmont South Cu-Mo porphyry. An additional Cu-Au porphyry at Mount Washington on Vancouver Island has recently been sampled, with fieldwork concluding in mid-October 2018.

The Deerhorn survey consisted of three transects across subsurface mineralization (Figure 2), with overlying glacial sediments ranging from 10 to 60 m in thickness and an extremely variable surficial environment with respect to regolith and vegetation (Rich, 2016). Mineralization is hosted primarily in monzonite intrusions as disseminated and vein-hosted Cu and Au, with the main zone of mineralization located beneath the thick glacial overburden (Rich, 2016).

The Highland Valley sampling program, consisting of three transects perpendicular to the main mineralized cores in the

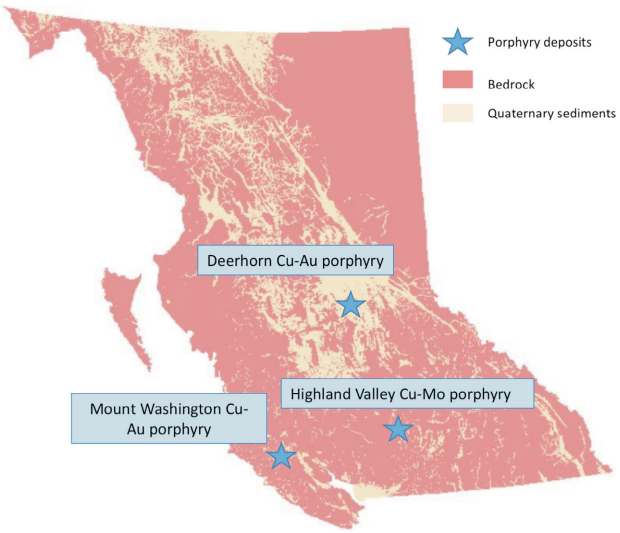

Figure 1: Locations of deposits from which data have been collected for this study.

Highmont South region (Figure 3), was carried out over two separate field surveys. The mineralization is expressed in a gradational change from Cu-sulphide–rich minerals in the centre (bornite, chalcopyrite) to a primarily Fe-sulphide (pyrite only)–rich outer zone (Chouinard, 2018), with an average till thickness above mineralization of 5 m. Sample sites were selective, as the till in the region was variable, with changes in vegetation, anthropogenic influences and areas that have been appreciably waterlogged (Chouinard, 2018).

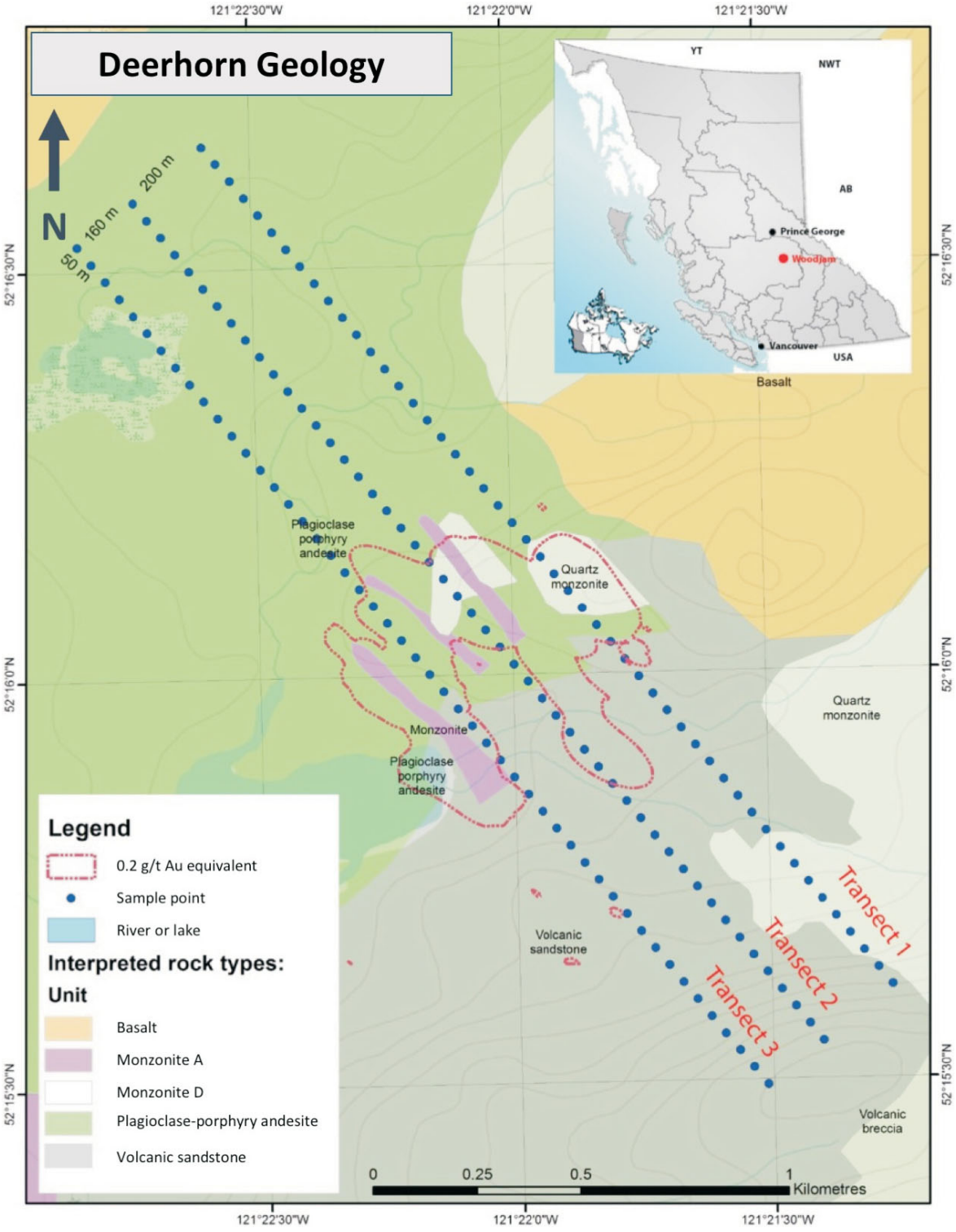

Figure 2: Bedrock geology of the Deerhorn Cu-Au–porphyry deposit of Consolidated Woodjam Copper Corp. (Rich and Winterburn, 2016). Microbial analysis was performed on every sample point.

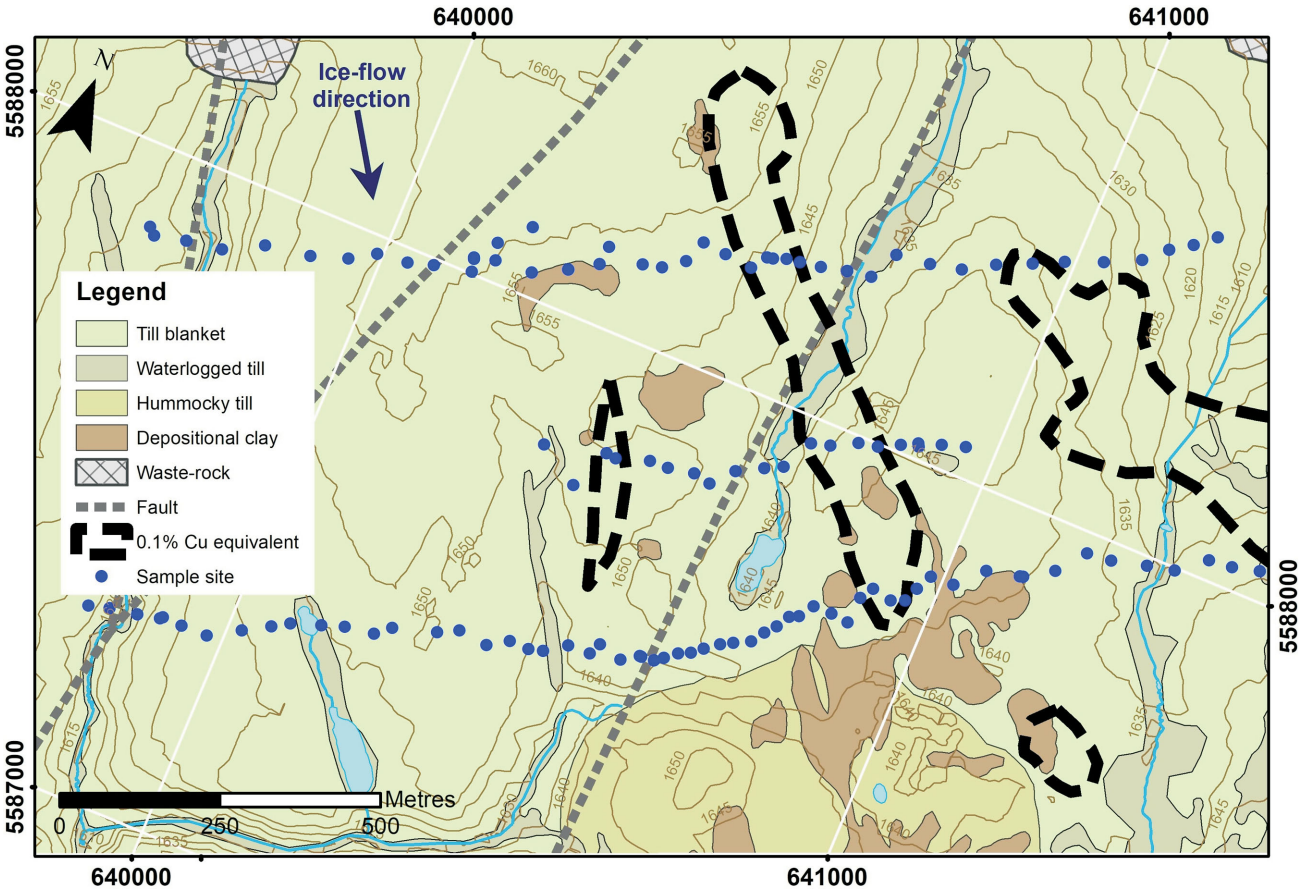

Figure 3: Surficial deposits in the Highmont South region of the Highland Valley Copper Cu-Mo–porphyry deposit of Teck Resources Ltd. (modified from Chouinard, 2018). Microbial analysis was performed on every site.

Soil Sampling

Soils for microbial-community analysis were sampled with sterilized equipment 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. Soils at the field sites are derived from the breakdown of till by surface-weathering processes in situ, so the soils are considered residual weathering products of the till blanket. The B-horizon soils (Figure 4) were targeted for microbial soil samples, although multiple horizons (including O, Ah, Ae, and C) were taken where possible. Soil samples were also collected for geochemical analysis. Field measurements consisted of slurry tests for pH and oxidation-reduction potential (ORP) after field sieving through a 6 mm screen. Geochemical samples for each site were sent to either Bureau Veritas Minerals (Vancouver, BC) or ALS Chemex (Vancouver, BC) for acid-digestion and subsequent analysis via ICP-MS, and the microbial samples were frozen at –4ºC upon return to the laboratory at The University of British Columbia (UBC) prior to DNA extraction. A subset of the Mount Washington microbial samples has been preserved to perform a cell-count analysis. A small amount of each soil sample was transferred with aseptic instruments into smaller vials containing an RNA preservative.

DNA Extraction

Microbial-community DNA was extracted from samples using a MO BIO Laboratories Inc. PowerMax® Soil DNA Isolation Kit; as per manufacturer's instructions, approximately 0.25 g of soil was used. Resulting DNA was stored at –20°C. The quality and quantity of genomic DNA were measured on a ThermoFisher Scientific NanoDrop® ND-1000 spectrophotometer and by using Invitrogen™ PicoGreen™ (Quant-iT™ dsDNA Assay Kit) dye.

Small Subunit Ribosomal RNA (SSU rRNA) Gene Amplification and iTag Sequencing

Bacterial and archaeal 16S rRNA gene fragments from the extracted genomic DNA were amplified using primers 515f and 806r (Apprill et al., 2015). Sample preparation for amplicon sequencing was performed as described by Kozich et al. (2013). In brief, the aforementioned 16S rRNA gene-targeting primers, complete with Illumina adapter, an 8-nucleotide index sequence, a 10-nucleotide pad sequence, a 2-nucleotide linker and the gene-specific primer, were used in equimolar concentrations together with

Figure 4. Typical soil profiles for porphyry deposits: **a)** horizons at the Deerhorn Cu-Au porphyry deposit of Consolidated Woodjam Copper Corp., and **b)** Highmont South property at the Highland Valley Copper Cu-Mo–porphyry deposit of Teck Resources Ltd. (Chouinard, 2018; Rich, 2016).

Deoxynucleotide triphosphate (dNTPs), Polymerase chain reaction (PCR) buffer, $MgSo_4$, 2U/µl ThermoFisher high-fidelity platinum Taq DNA polymerase and PCR-certified water to a final volume of 50 µL. PCR amplification was performed with an initial denaturing step of 95°C for 2 min, followed by 30 cycles of denaturation (95°C for 20 s), annealing (55°C for 15 s) and elongation (72°C for 5 min), with a final elongation step at 72°C for 10 min. Equimolar concentrations of amplicons were pooled into a single library. The amplicon library was analyzed on an Agilent Bioanalyzer using the High-Sensitivity DS DNA Assay to determine approximate library fragment size, and to verify library integrity. Library pools were diluted to 4 nM and denatured into single strands using fresh 0.2 N NaOH, as recommended by Illumina. The final library was loaded at a concentration of 8 pM, with an additional PhiX spike-in of 5–20%. Sequencing was conducted on the MiSeq platform at the Sequencing + Bioinformatics Consortium, The University of British Columbia, Vancouver, BC (The University of British Columbia, 2018).

Informatics

Sequences were processed using the mothur (Schloss et al., 2009) MiSeq protocol (Kozich et al., 2013). Briefly, sequences were removed from the analysis if they contained ambiguous characters, had homopolymers longer than 8 base pairs and did not align to a reference alignment of the correct sequencing region. Unique sequences, and their frequency in each sample, were identified and then a pre-clustering algorithm was used to further de-noise sequences within each sample (Schloss et al., 2011). Unique sequences were identified and aligned against a SILVA alignment (mothur Project, 2018a). Sequences were chimera checked using VSEARCH (Rognes et al., 2016) and reads were then clustered into 97% operational taxonomic units (OTUs) using the Matthews correlation coefficient (MCC; Westcott and Schloss, 2017). Operational taxonomic units were classified using the SILVA reference taxonomy database (release 132; mothur Project, 2018b).

Results and Discussion

Soil is one of the most complex and diverse microbial habitats, with merely 1 g containing up to 10^{10} cells and 10^4 bacterial species (Torsvik and Øvreås, 2002; Roesch et al., 2007). The current study's approach relies on the ability to capture this diversity through next-generation sequencing technologies. In microbiology, the assessment of diversity often involves calculation of species richness (number of species present in a sample; Magurran, 2013). The most common approach is to assign 16S rRNA sequences into OTUs and represent these as rarefaction curves, which plot the cumulative number of OTUs captured as a function of sampling effort, and therefore indicate the OTU richness in a given set of samples. Other common methods include

nonparametric analysis, such as Chao1, which estimates the overall sample diversity (also known as alpha diversity; Hughes et al., 2001).

The current study extracted microbial-community DNA from soil transects at Deerhorn and Highland Valley Copper (Figures 2, 3) and sequenced the 16S rRNA gene. Samples taken from Mount Washington (Figure 1) are currently undergoing DNA extraction. Analysis of these sequences reveals that the number of observed OTUs (hereafter referred to as species) is 2417 ±344 (range 1041–3044), with an alpha diversity (Chao1 index) of 4015 ±814 (range 1784–5666; Figure 5a, b), at Deerhorn and 2671 ±445 (range 1068–3768), with an alpha diversity (Chao1 index) of 4066 ±710 (range 1663–5763) (Figure 4a, b), at Highland Valley Copper, indicating that the sequencing coverage was sufficient to capture 65% of the microbial-community diversity. These levels of diversity are well in line with diversity commonly observed in soils (Thompson et al., 2017). These measurements dispel the dogma that extremely high diversity in soil microbial communities renders them intractable to molecular-based microbial-community analysis. There was no pronounced difference in

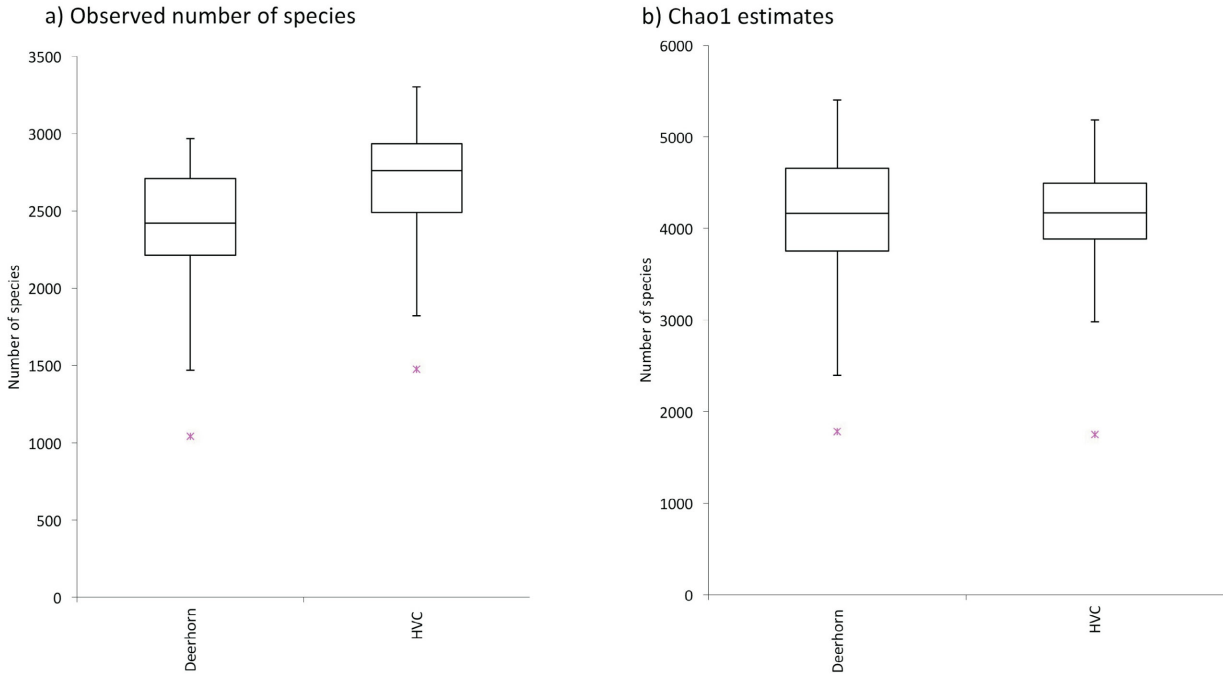

Figure 5. Boxplots for Deerhorn and Highland Valley Copper sites of **a)** observed number of species, and **b)** Chao1 richness estimates. Outliers indicated by pink 'x' symbols.

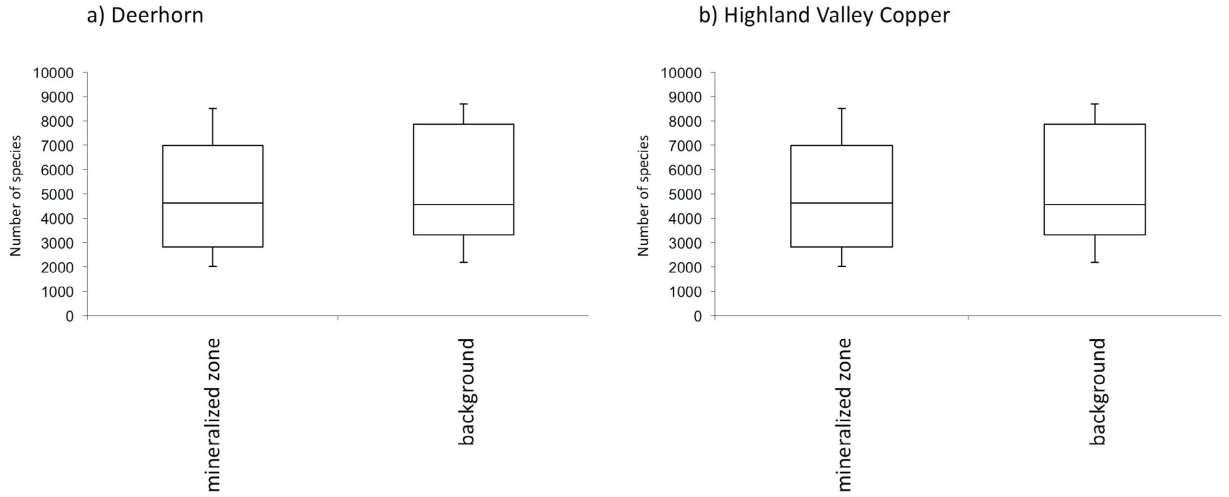

Figure 6. Boxplots of observed number of species in the mineralized zone compared to the rest of the site for **a)** Deerhorn, and **b)** Highland Valley Copper.

species richness (i.e., the number of species in a given sample) across the mineralized zone at both Deerhorn and Highland Valley Copper (Figure 6). The study's measurements demonstrate that soil diversity can be captured through next-generation sequencing technologies, which bodes well for the approach and imparts enormous and unprecedented statistical power to community profiles as anomaly indicators.

The number of reads per microbial phylum was normalized to total read number for a given sample and expressed as a percentage of the total reads from that sample (Figure 7). Most microbial-community members belong to the Proteobacteria, Acidobacteria and Verrucomicrobia phyla at both sites (Figure 7). The relative proportions are consistent with previous studies on soil ecosystems (Choi et al., 2017, Fierer, 2017). This high-level taxonomic analysis reveals strong similarities across all samples, thus giving confidence that the analyses are not overwhelmed by intersample variability arising because of the very high levels of microbial diversity and chemical and physical heterogeneity commonly found in soils. As found with the incubation experiments (Simister et al., 2017); however, the similarity across the samples suggests that discrimination between background and anomalous soils may be more sensitive with analyses at the genus or species level rather than at the phylum level.

Ongoing Work and Conclusions

The same soil microbial-community members that responded to the presence of mineralization in the field samples will be identified using the profiled microbiome compositions from the incubation experiments. For example, the species that increased in response to chalcopyrite ore and copper amendment relative to controls included *Rhodanobacteria* sp., SC-I-84 sp. and *Acidimicrobiales* sp. Therefore, particular attention will be paid to any anomalous abundances of these species over the mineralized zone at the field sites. Furthermore, work is progressing on standard hierarchical-clustering analysis of the samples to discriminate between background and anomalous values. Specifically, anomalous versus background communities will be identified using the multivariate statistical analyses (UniFrac, ANOSIM) commonly employed in microbial ecology (Lozupone and Knight, 2005; Ramette, 2007). Indicator analyses will be used to identify microbial taxa responsible for these community anomalies, an example of which is shown in Figure 8.

Application of modern sequencing technologies enabled profiling of the taxonomic diversity of soil microbiomes across subtle, and often poorly resolved, geochemical gradients that can develop in soils in response to mineralization beneath cover. Given that each soil sample comprises thousands of microbial taxa, each containing hundreds to thousands of genes, the statistical power of this approach to identifying anomalies is unprecedented.

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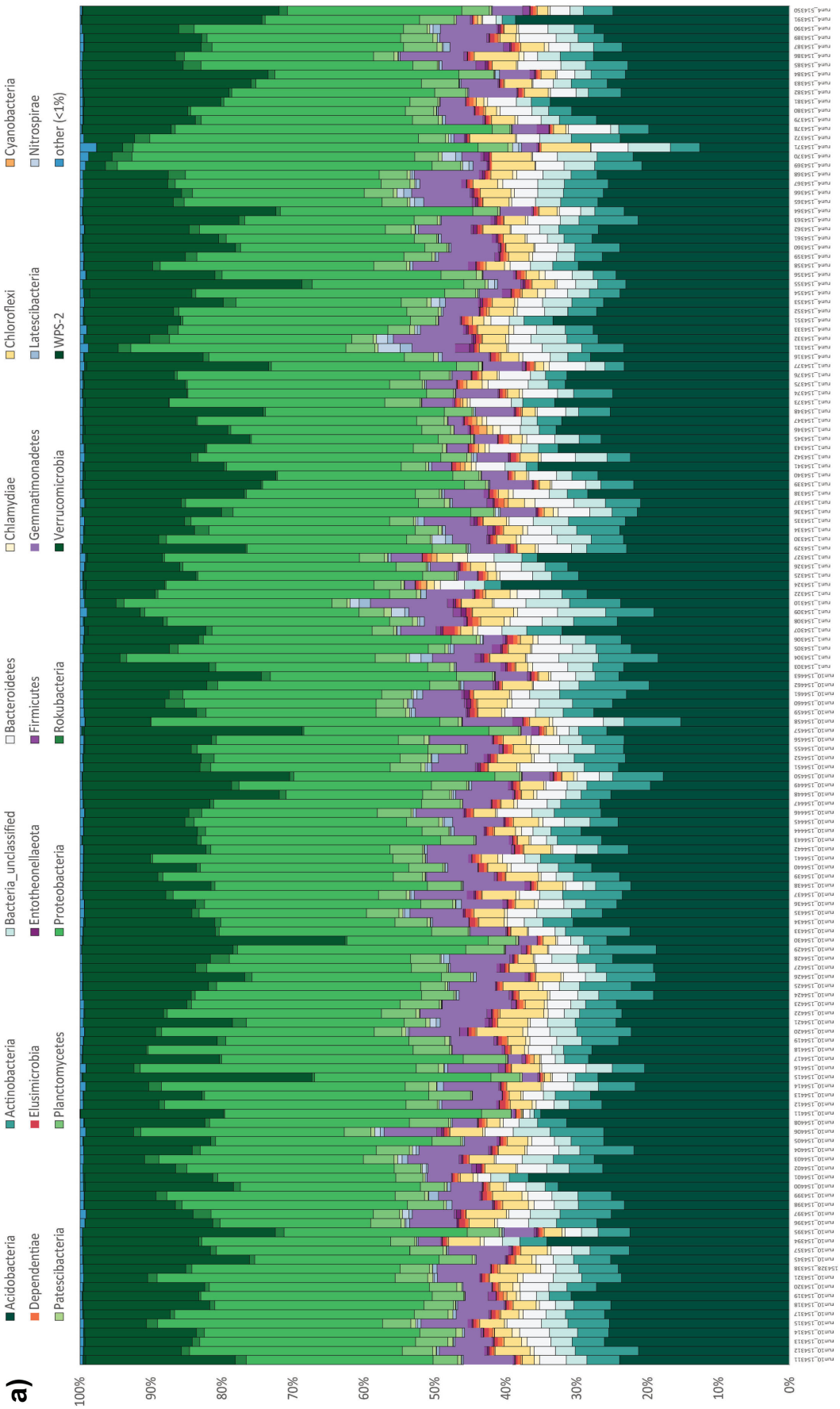
a)
Acidobacteria
Actinobacteria
Bacteria_unclassified
Bacteroidetes
Chlamydiae
Chloroflexi
Cyanobacteria
Dependentiae
Elusimicrobia
Entotheonellaeota
Firmicutes
Gemmatimonadetes
Latescibacteria
Nitrospirae
Patescibacteria
Planctomycetes
Proteobacteria
Rokubacteria
Verrucomicrobia
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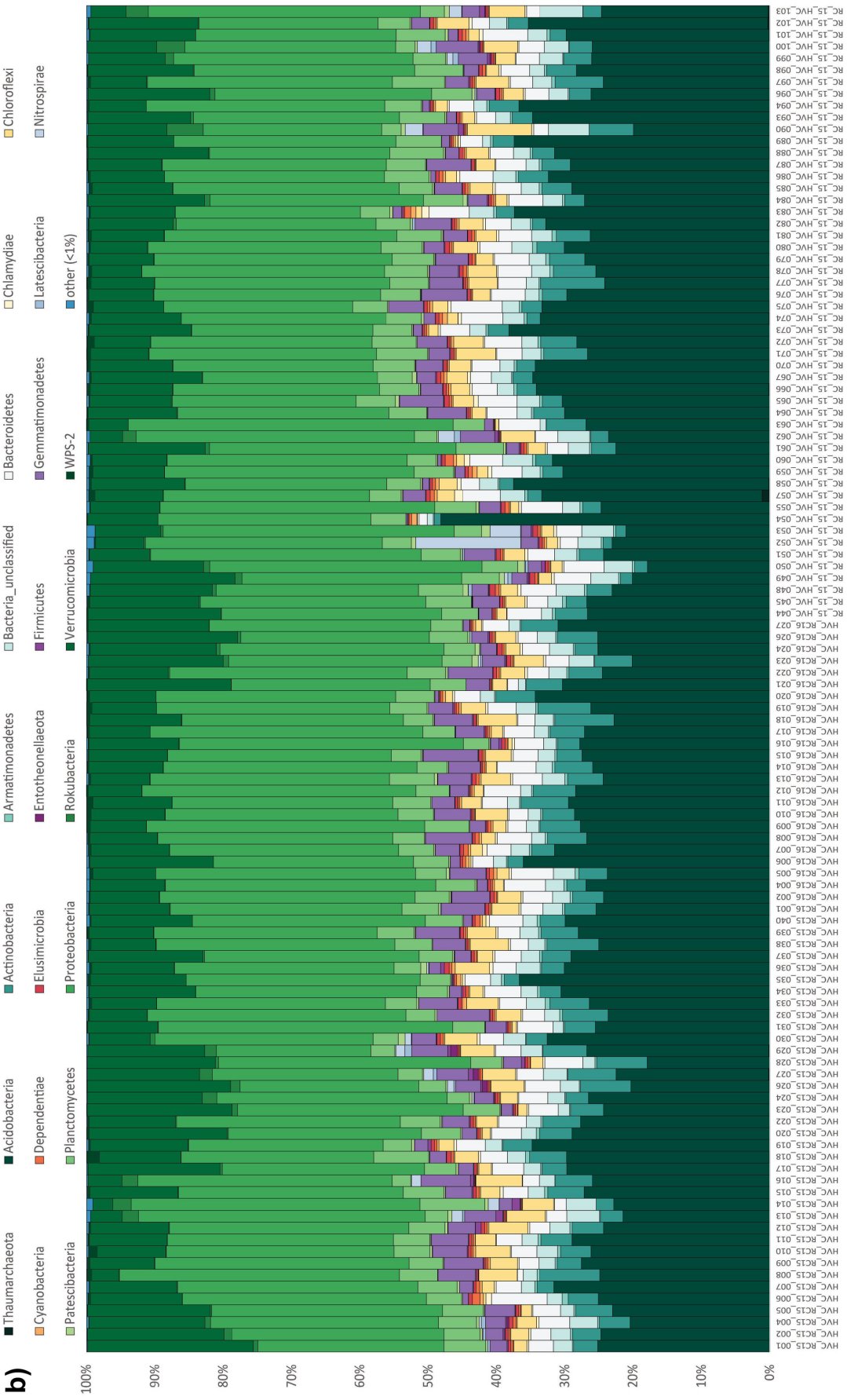

Figure 7. Distribution of 16S rRNA reads per phylum for each sample from **a)** Deerhorn, and **b)** Highland Valley Copper. The number of reads per phylum is calculated as a percentage of the total reads for each sample. The 'Other' grouping represents summed phyla that individually contributed <0.5% of the total number of reads per sample.

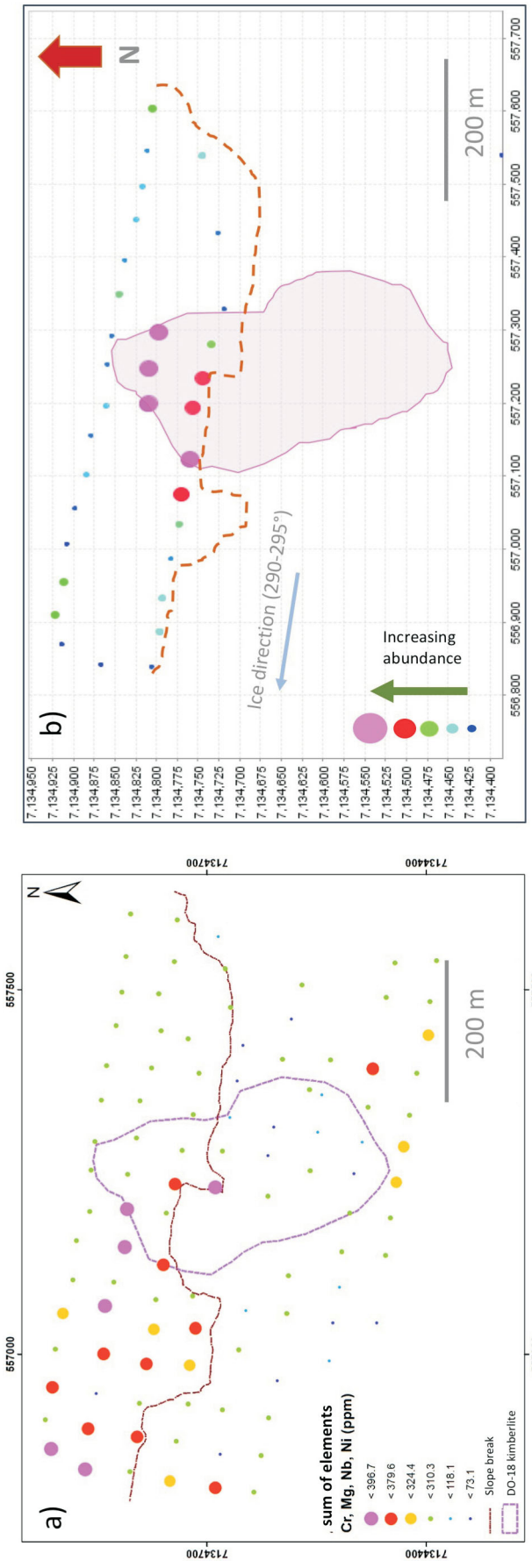

Figure 8. Microbial-community fingerprinting of data from the DO-18 diamondiferous kimberlite at Lac de Gras, Northwest Territories, shown with sample sites overlain on **a)** multi-element anomaly map for anomalous populations of Cr+Mg+Nb+Ni extracted by aqua regia, and **b)** anomaly map based on microbial 16S rRNA sequencing data. The community shift indicative of buried mineralization occurs directly over the kimberlite **(b)**, which contrasts with the traditional multi-element geochemical approach in which the kimberlite is picked up primarily down ice from the kimberlite **(a)**.

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