Microbial indicators and detection of Cu-sulfide ore mineralization

Bianca P. Iulianella Phillips[1,2], Rachel L. Simister[3], Shane D. Rich[1,2], Craig J.R. Hart[1,2], Peter A. Winterburn[1,2]†, and Sean A. Crowe[2,3*]

[1]MDRU-Mineral Deposit Research Unit, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4

[2]Department of Earth, Ocean, and Atmospheric Sciences, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4

[3]Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada, V6T 1Z3

† Deceased
* Corresponding author: sean.crowe@ubc.ca

ABSTRACT

Rapid electrification of society is placing unprecedented demand on critical mineral and metal resources. New strategies and technologies are thus needed to promote mineral discovery in regions where deposits are likely buried deep under soil and glacial till. We show that microbial communities from different soil types change in composition in response to amendment with copper. We also show that soil microbial community fingerprinting can detect buried sulfide mineralization through thick successions of Quaternary surface cover. Indicator species abundances, indeed, better resolve the known surface projection of mineralization than geochemical analyses. Therefore, our results indicate that porphyry-style mineralization is discernible in covered terrains through soil microbial community fingerprints.

INTRODUCTION

Global demand for mineral resources – in particular, growth in green energy alternatives, including electric vehicles and batteries – is placing increasing pressure on the mining industry to supply metals such as copper, nickel, and lithium. At the same time, existing mineral deposits are being exhausted, while the frequency of new deposit discovery is declining (Schodde, 2017). This is forcing mineral exploration efforts to increasingly focus on concealed targets (Winterburn et al., 2020), requiring development of tools that detect unexposed mineralization. Whereas biological approaches to exploration have received minimal attention and uptake in the past, emerging DNA sequencing technologies offer new opportunities to harness the power of genomics across the resource sector with strong potential to improve mineral resource discovery (Simister et al., 2023).

Through their growth and metabolism, microorganisms catalyze reactions that drive fluxes of matter and energy from nano- to global-scales (Newman and Banfield, 2002; Falkowski et al., 2008; Fierer, 2017). These reactions can promote precipitation and dissolution of minerals and thus microbial metabolism influences, and is influenced by, the distribution of minerals in the surface and sub-surface. Soil microorganisms are highly sensitive, detecting and responding to variability in the physicochemical properties of their habitats. In a single gram of soil there can be up to tens of thousands of microbial species, each with hundreds to thousands of genes, that collectively sense and interact with their surroundings (Torsvik and Øvreås, 2002; Fierer, 2017). Mineral deposits in the subsurface likely represent an acute example of this, whereby ore minerals and constituent elements can be elevated orders of magnitude above background concentrations (Kelley et al., 2006). As such, microbial community compositions likely reflect

variability in the mineralogical composition of soils based on their proximity to, and interaction with, buried ore minerals (Simister et al., 2023). Furthermore, given the sensitivity of microorganisms to their surroundings, anomalies in microbial community compositions may develop, even if differences in physicochemical properties of the host soils are not resolvable through geochemical analyses.

Microbial community fingerprinting effectively delineates lithologically controlled mineralization, but capacity for resolving disseminated and structural ores remains untested. For example, diamondiferous kimberlites concealed by glacial till can be detected through soil microbial community anomalies that develop over kimberlite pipes, even where geochemical signals have been glacially dispersed (Simister et al., 2023). It remains unknown, however, whether this phenomenon is more broadly extensible, and if other ore types can be detected through similar applications of DNA sequencing. In this study, we thus tested the application of soil microbial community fingerprinting to the discovery of porphyry copper deposits (PCDs) which, when buried, are difficult to locate by traditional methods (Heberlein and Samson, 2010; Winterburn et al., 2020). We show that PCDs concealed by 10s of meters of soil and glacial overburden can be detected through soil microbial community analyses.

METHODS

Incubation Experiments

We conducted two incubation experiments with background soils from the Deerhorn PCD (British Columbia (BC)), and the sub-arctic tundra (Northwest Territories (NWT)). Amendments of copper sulfate ($CuSO_4$) or chalcopyrite ($CuFeS_2$) were made to aseptically dispensed soils at

200 ppm (ambient) or 600 ppm (high) (final concentration in soil), depending on the amendment. Such concentrations of copper and other ore-related elements reflect values typically detected (ambient) or those considered anomalous (high) in soil geochemical surveys above PCDs. The soils were incubated for 35 days (BC) and 85 days (NWT).

Field Area and Survey Design

The Deerhorn Cu-Au calc-alkaline PCD, BC, Canada (Figure 1b), hosts chalcopyrite ($CuFeS_2$), enargite (Cu_3AsS_4), and minimal bornite (Cu_5FeS_4) mineralization within monzonite intrusions, and country rock consists of andesite breccias and thick beds of volcanic derived sandstones (del Real et al., 2017). The mineral resource is delineated by a 0.2 g/t Au equivalent grade shell, including most rocks that are >0.22% Cu, with a surface area expression diameter of ~500 m at the bedrock-till interface (Figure 1a) (Sherlock, R., Blackwell, J., and Skinner, T., 2013). The Deerhorn area was glaciated; the PCD is variably covered by soils, organic deposits, and glacial materials (mapped based on classification schemes: (Howes et al., 1988; Taylor and Eggleton, 2001)), which cover mineralization (10 – 60 m) and host rocks (up to 169 m). 150 soil samples (B-horizon) were collected in three 2.5 km transects (50 m spacing; 200 m fence-lines) across mineralization and background, aligned with recent glaciation (Figure 1a).

Geochemical and Geobiological Analyses

Element concentrations were determined through inductively coupled plasma mass spectrometry (ICP-MS) following aqua-regia digests (Table S1 in the Supplemental Material[1]), with physicochemical variables (e.g., pH) measured *in situ*. Microbial community fingerprints of the same Deerhorn soils and soils from incubation experiments were determined via amplicon

(515f and 806r) (Caporaso et al., 2011; Apprill et al., 2015) sequencing of the 16S rRNA gene (V4) using the Illumina MiSeq. Sequences were analyzed – including richness (Table S2 in the Supplemental Material[1)]) – using QIIME2 (Bolyen et al., 2019) and the SILVA taxonomic reference database (release 138). Differential abundance algorithms, ANCOM (analysis of composition of microbes) (Mandal et al., 2015) and LEfSe (linear discriminant analysis effect size) (Segata et al., 2011), were implemented in QIIME2 and Mothur (Schloss et al., 2009), respectively (Table S3 in the Supplemental Material[1]).

MICROBIAL COMMUNITY RESPONSES TO COPPER AMENDMENTS

Soil amendments with copper sulfate and chalcopyrite elicit changes in both microbial community structure and composition. Differences in species richness are more apparent in copper amended tundra soils (Figure 3) than Deerhorn soils (Figure 2). Richness metrics, such as the number of observed species (defined by amplicon sequence variants (ASVs)), are similar in Deerhorn baseline soils (1090 ± 403) and those amended with copper sulfate or chalcopyrite (918 ± 137) (Figure 2b). Conversely, there is a noticeable reduction in species richness in tundra soils amended with chalcopyrite (513 ± 40 and 290 ± 113, respectively; Figure 3b). A richness reduction in tundra soils may reflect growth and/or decay of taxa depending on sensitivity to the amendments. The dominant phyla in each soil differed in abundance, with Acidobacteriota, Proteobacteria, and Verrucomicrobiota dominating in Deerhorn soils (68% avg.), and Proteobacteria and Actinobacteriota in tundra soils (74% avg.). Both soil compositions are typical of soils globally (Fierer, 2017), with tundra and Deerhorn soils exhibiting similar composition to other northern (Johnston et al., 2019; Hale et al., 2019; Frank-Fahle et al., 2014) and temperate soils (Yang et al., 2022; Kaiser et al., 2016), respectively. The tundra soils had

more pronounced shifts in community composition with copper amendment that could be resolved at the phylum-level, with an increase in relative abundance of Proteobacteria (46% to 69%) and a decrease in Acidobacteriota (5% to 3%), Bacteroidota (6% to 2%), and Verrucimicrobiota (6% to 2%) (Figure 3a), relative to baseline controls. Deerhorn soils remained largely unchanged at the phylum level—with the notable exception of minor shifts in Acidobacteriota (31% baseline; 24% copper amendment) and Actinobacteriota (5% baseline; 8% copper amendment) (Figure 2a). Tundra soils are more sensitive to copper amendment than the Deerhorn soils possibly due to lower overall species richness in the baseline soils, and thus differences in structure and composition induced by copper amendments are more readily resolved. Tundra soils also contain lower baseline concentrations of copper than Deerhorn soils (Table S4 in the Supplemental Material[1]), and the tundra soil microbial community may thus be more sensitive to copper amendment. Microbial community responses to copper amendment were further assessed through hierarchical clustering analyses. Baseline control soils and copper amended soils largely clustered independently (Figure 2d and Figure 3d). The coherent clustering of copper amended soils reveals a consistent response to copper amendment with changes in composition that are statistically well-resolved.

Multiple species-level differences in relative abundance develop in response to copper amendments. For example, both the Deerhorn and tundra soil incubations experienced enrichments in taxa belonging to the candidate phylum AD3 and the orders Xanthomonadales, Ktedonobacterales, and Micrococcoles, and depletion in members of the candidate phylum WPS-2, and orders Acidobacteriales and Diplorickettsiales (Figure 2c and Figure 3c). In each experiment, members of the genus *Rhodanobacter* are particularly sensitive to elevated copper

concentrations (Figure 2c and Figure 3c). Members of this genus indeed have conspicuously high abundances in environments with elevated copper and other metals (Green et al., 2012; Cho et al., 2017; Carlson et al., 2018). Despite greater changes to the sub-arctic tundra soils in response to copper amendment than Deerhorn soils, species-level differences were detectable in both. These species thus represent naturally occurring indicators that respond to copper exposure.

DELINEATION OF A CONCEALED PCD

Microbial community composition in soils that overlie mineralization at the Deerhorn PCD are indistinguishable, at the phylum-level, from those in background soils. These phylum-level compositions are also similar overall, to those used in the incubation experiments (Figure 2a and Figure 4a). Across all Deerhorn soils, Acidobacteriota (28%), Proteobacteria (25%), and Verrucomicrobiota (16%) were the most abundant (Figure 3a)—typical of temperate soils around the world (Janssen, 2006; Fierer, 2017; Delgado-Baquerizo et al., 2018). Observed species richness (1034 ± 454; Figure 4b) is also typical of similar such soils (Carini et al., 2016; Thompson et al., 2017), with no appreciable differences between soils that overlie the surface expression of mineralization and background (Figure 4b). It thus appears that species richness and phylum-level community compositions are largely insensitive to the buried Deerhorn PCD.

Differential abundance – or indicator species – analyses reveal a number of species-level enrichments in soils above mineralization. Given the gradational and disseminated nature of porphyry copper mineralization and grade thresholds, we conducted indicator species analyses of soils at Deerhorn in multiple tiers, with soils that are considered in the mineralized zone varying at 5-10 m increments from the known surface projection of mineralization (Figure 4g). In this

range – with minimum and maximum distances from mineralization in rock set at 0 m and 60 m, respectively – indicator species can be defined in the 0 m to 15 m distances from the surface expression, with the number of indicator species declining between 20 m and 60 m (Figure 4d). Indeed, there are no indicator species found through ANCOM beyond 15 m and the number of LEfSe indicator species drops by an average of 42% (Figure 4d). Only the indicator species in 0 m – 15 m distances from the surface projection of mineralization were further considered in this work.

Microbial community fingerprinting delineates the surface expression of mineralization through the spatial distribution of anomalies that emerge at the species level. These indicator species were derived from both differential abundance analyses at Deerhorn (0 m – 15 m) and copper amendment incubation experiments. These indicator species exhibit significantly (LDA score > 2 (LEfSe); W score rejecting null hypothesis (ANCOM)) higher relative abundances in soils above mineralization, and together (normalized sum), reveal strong anomalies at the surface (Figure 4d). Of the indicator species that delineate mineralization, 29 originate from the incubation experiments and 66 originate from the field-based observations supporting the idea that indicators identified through laboratory experiments are effective vectors in the field. Compared to indicator/pathfinder elements associated with sulfide mineralization, the microbial anomalies more accurately resolve the buried PCD, even where the glacial till cover is thick (> 25 m) (Figures 4c and 4e).

CONCLUSIONS AND IMPLICATIONS FOR MINERAL EXPLORATION

We've shown that anomalies in soil microbial community composition develop in proximity to the surface expression of a PCD. Indicator species derived from incubation experiments and field-based analyses of soils above a PCD resolve the surface expression of the buried mineral deposit even where inorganic geochemical signals are weak. Given that 60% of global copper is extracted from PCDs (Tabelin et al., 2021), microbial community fingerprinting may help in the discovery of new copper deposits improving copper and associated critical metal (e.g., Bi, Te) supply chains. Furthermore, our results extend the potential power of DNA sequencing and microbial community fingerprinting of surface soils to a wider range of mineral resources, suggesting the approach may be extensible to other commodities (e.g., Ni, Zn, Pb) that are critical to the energy transition.

ACKNOWLEDGMENTS

Support for this work came from the NSERC/AcmeLabs/Bureau Veritas Minerals Industrial Research Chair in Exploration Geochemistry held by PAW, a Geoscience BC grant held by PAW and SAC, and a Tier II Canada Research Chair in Geomicrobiology and NSERC Discovery grant held by SAC. Gold Fields Canada Exploration and Consolidated Woodjam Copper provided access to the Deerhorn field site. Ramon Vitto helped mapping and collecting soils.

[1]Supplemental Material. Supplemental methods, Tables S1-S4. Please visit https://doi.org/10.1130/XXXX to access the supplemental material, and contact editing@geosociety.org with any questions.

REFERENCES CITED

Apprill, A., McNally, S., Parsons, R., and Weber, L., 2015, Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton: Aquatic microbial ecology: international journal, v. 75, p. 129–137.

Bolyen, E. et al., 2019, Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2: Nature biotechnology, v. 37, p. 852–857.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., and Knight, R., 2011, Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample: Proceedings of the National Academy of Sciences of the United States of America, v. 108 Suppl 1, p. 4516–4522.

Carini, P., Marsden, P.J., Leff, J.W., Morgan, E.E., Strickland, M.S., and Fierer, N., 2016, Relic DNA is abundant in soil and obscures estimates of soil microbial diversity: Nature microbiology, v. 2, p. 16242.

Carlson, H.K., Price, M.N., Callaghan, M., Aaring, A., Chakraborty, R., Liu, H., Kuehl, J.V., Arkin, A.P., and Deutschbauer, A.M., 2018, The selective pressures on the microbial community in a metal-contaminated aquifer: The ISME journal, v. 13, p. 937–949.

Cho, G.-Y., Lee, J.-C., and Whang, K.-S., 2017, Rhodanobacter rhizosphaerae sp. nov., isolated from soil of ginseng rhizosphere: International journal of systematic and evolutionary microbiology, v. 67, p. 1387–1392.

Cui, Y., Miller, D., Schiarizza, P., and Diakow, L.J., 2017, British Columbia digital geology: British Columbia Gelogical Survey, Ministry of Energy, Mines, and Petroleum Resources.

Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge, D.J., Bardgett, R.D., Maestre, F.T., Singh, B.K., and Fierer, N., 2018, A global atlas of the dominant bacteria found in soil: Science, v. 359, p. 320–325.

Falkowski, P.G., Fenchel, T., and Delong, E.F., 2008, The microbial engines that drive Earth's biogeochemical cycles: Science, v. 320, p. 1034–1039.

Fierer, N., 2017, Embracing the unknown: disentangling the complexities of the soil microbiome: Nature reviews. Microbiology, v. 15, p. 579–590.

Green, S.J. et al., 2012, Denitrifying bacteria from the genus Rhodanobacter dominate bacterial communities in the highly contaminated subsurface of a nuclear legacy waste site: Applied and environmental microbiology, v. 78, p. 1039–1047.

Heberlein, D.R., and Samson, H., 2010, An Assessment of Soil Geochemical Methods for Detecting Copper-Gold Porphyry Mineralization through Quaternary Glaciofluvial Sediments at the Kwanika Central Zone , North-Central British Columbia:,

https://www.semanticscholar.org/paper/0cfb6c6cf113b125437d6fd011acad7087a70aa1 (accessed September 2023).

Howes, D.E., British Columbia. Recreational Fisheries Branch, Kenk, E., and British Columbia. Surveys and Resource Mapping Branch, 1988, Terrain Classification System for British Columbia: A System for the Classification of Surficial Materials, Landforms and Geological Processes of British Columbia: Recreational Fisheries Branch, Ministry of Environment, 90 p.

Janssen, P.H., 2006, Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes: Applied and environmental microbiology, v. 72, p. 1719–1728.

Kelley, D.L., Kelley, K.D., Coker, W.B., Caughlin, B., and Doherty, M.E., 2006, Beyond the Obvious Limits of Ore Deposits: The Use of Mineralogical, Geochemical, and Biological Features for the Remote Detection of Mineralization: Economic geology and the bulletin of the Society of Economic Geologists, v. 101, p. 729–752.

Mandal, S., Van Treuren, W., White, R.A., Eggesbø, M., Knight, R., and Peddada, S.D., 2015, Analysis of composition of microbiomes: a novel method for studying microbial composition: Microbial ecology in health and disease, v. 26, p. 27663.

Newman, D.K., and Banfield, J.F., 2002, Geomicrobiology: how molecular-scale interactions underpin biogeochemical systems: Science, v. 296, p. 1071–1077.

del Real, I., Bouzari, F., Rainbow, A., Bissig, T., Blackwell, J., Sherlock, R., Thompson, J.F.H., and Hart, C.J.R., 2017, Spatially and Temporally Associated Porphyry Deposits with Distinct Cu/Au/Mo Ratios, Woodjam District, Central British Columbia: Economic geology and the bulletin of the Society of Economic Geologists, v. 112, p. 1673–1717.

Schloss, P.D. et al., 2009, Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities: Applied and environmental microbiology, v. 75, p. 7537–7541.

Schodde, R.C., 2017, Long-term forecast of Australia's mineral production and revenue: The outlook for gold, v. 2057, http://minexconsulting.com/wp-content/uploads/2019/04/Long-Term-Gold-Prodn-Study-Prestn-to-CET-5-Dec-2017.pdf.

Segata, N. et al., 2011, Microbial community function and biomarker discovery in the human microbiome: Genome biology, v. 12, p. P47.

Sherlock, R., Blackwell, J., and Skinner, T., 2013, Techncical report for 2012 activities on the Woodjam North property; prepared for Gold Fields Horsefly Exploration Corp. and Consolidated Woodjam Copper Corporation: NI 43-101.

Simister, R.L., Iulianella Phillips, B.P., Wickham, A.P., Cayer, E.M., Hart, C.J.R., Winterburn, P.A., and Crowe, S.A., 2023, DNA sequencing, microbial indicators, and the discovery of buried kimberlites: Communications Earth & Environment, v. 4, p. 1–10.

Tabelin, C.B., Park, I., Phengsaart, T., Jeon, S., Villacorte-Tabelin, M., Alonzo, D., Yoo, K., Ito, M., and Hiroyoshi, N., 2021, Copper and critical metals production from porphyry ores and E-wastes: A review of resource availability, processing/recycling challenges, socio-environmental aspects, and sustainability issues: Resources, Conservation and Recycling, v. 170, p. 105610.

Taylor, G., and Eggleton, R., 2001, Regolith geology and geomorphology:

Thompson, L.R. et al., 2017, A communal catalogue reveals Earth's multiscale microbial diversity: Nature, v. 551, p. 457–463.

Torsvik, V., and Øvreås, L., 2002, Microbial diversity and function in soil: from genes to ecosystems: Current opinion in microbiology, v. 5, p. 240–245.

Winterburn, P.A., Noble, R.R.P., and Lawie, D., 2020, Advances in exploration geochemistry, 2007 to 2017 and beyond: Geochemistry: Exploration, Environment, Analysis, v. 20, p. 157–166.

Figure 1. Deerhorn location and survey design. (A) Soil sample locations (pink bubbles) and surficial materials map. Mineralization defined by >0.2 g/t Au equivalent ore (black line). **(B)** Deerhorn Cu-Au PCD location and BC geology (BCGS Open File 2017-8, 9p, access 2019-12-19 (Cui et al., 2017)).

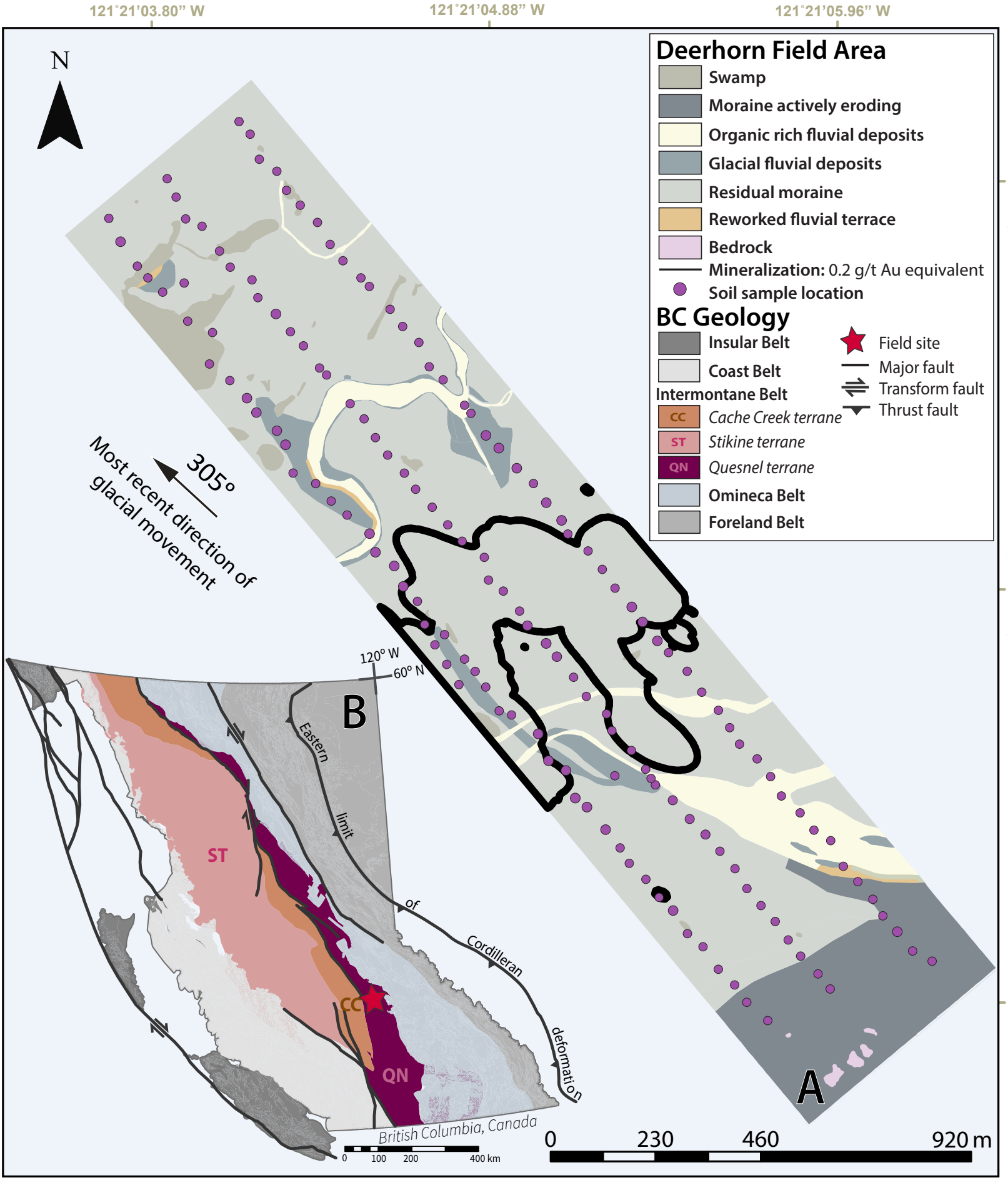
Figure 2. Microbial community composition and richness in Deerhorn soil incubations. (A) Phylum-level microbial community composition (percentage of total 16S rRNA gene sequence reads) for each soil sample. **(B)** Number of observed species (ASVs), grouped as control (beige), or copper amended soils (blue). **(C)** Species-level (ASV) shifts in relative abundance across treatments over time. **(D)** Hierarchical relationships among soils based on Euclidean distance of ASV abundances, obtained with the unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm. Node labels indicate the timepoint and treatment.

Figure 3. Microbial community composition and richness in tundra soil incubations. (A) Phylum-level microbial community composition (percentage of total 16S rRNA gene sequence reads) for each soil sample. **(B)** Number of observed species (ASVs), grouped as control (beige), or copper amended soils (blue). **(C)** Species-level (ASV) shifts in relative abundance across treatments over time (error bars = standard deviation). **(D)** Hierarchical relationships among soils based on Euclidean distance of ASV abundances, obtained with the unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm. Node labels indicate the timepoint and treatment.

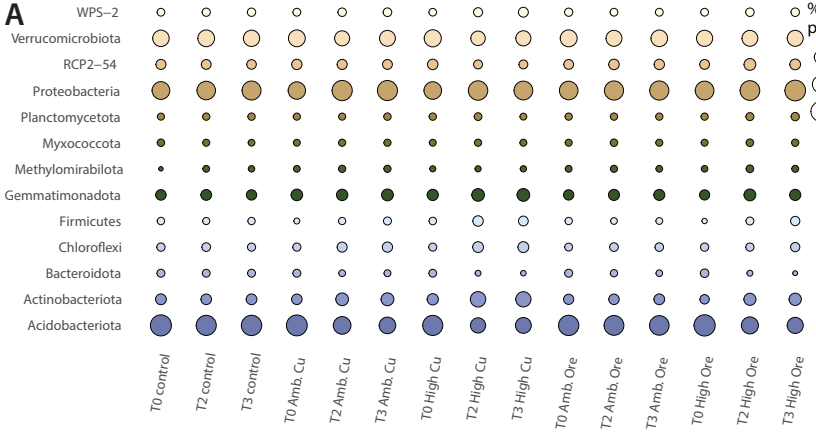
Figure 4. Soil microbial community composition, richness, and microbial and geochemical anomalies above the Deerhorn PCD. (A) Phylum-level microbial community composition (expressed as a percentage of total reads) for each soil sample. **(B)** Number of observed species (ASVs), grouped by origin: "above background" (beige) or "above mineralization" (blue). **(C)** Response ratio of indicator/pathfinder elements vs. indicator species from copper amendment incubation experiments and Deerhorn soil analyses (average "above mineralization" over the average "above background" relative to an equivalent ratio of 1, expressed in %). **(D)** The number of indicator species from multi-tiered differential abundance analyses (ANCOM and LEfSe) – soils "above mineralization" or "above background" for each tier are determined by soil sample proximity to projected mineralization. Anomaly maps at the Deerhorn PCD, showing the normalized sum of all indicator species **(E)** and Cu, Mo, K, and As, **(F)** with each element normalized to $C_{organic}$ (abundant in some areas of the field site). Individual indicator species **(E)** or elements **(F)** are normalized to the mean prior to summation, with anomaly intervals based on

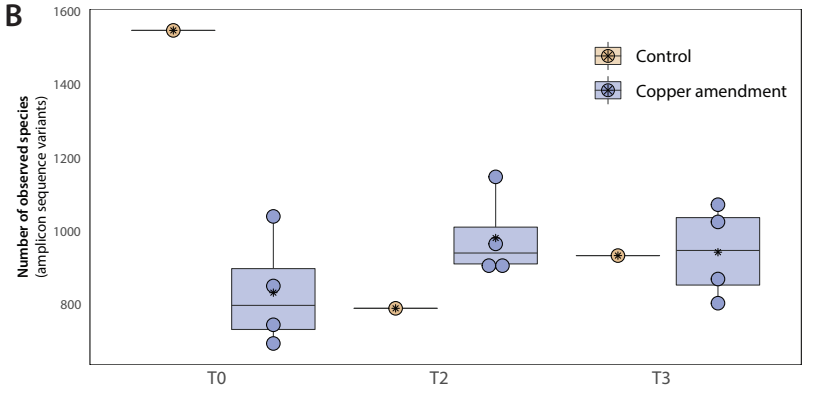
probability plots. **(G)** Gradational distances from mineralization used for differential abundance analyses.

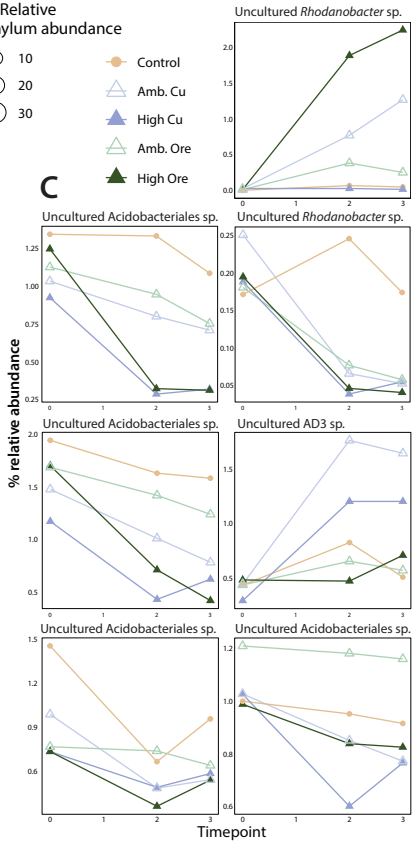
Iulianella Phillips et al. **Figure 1** - MS#

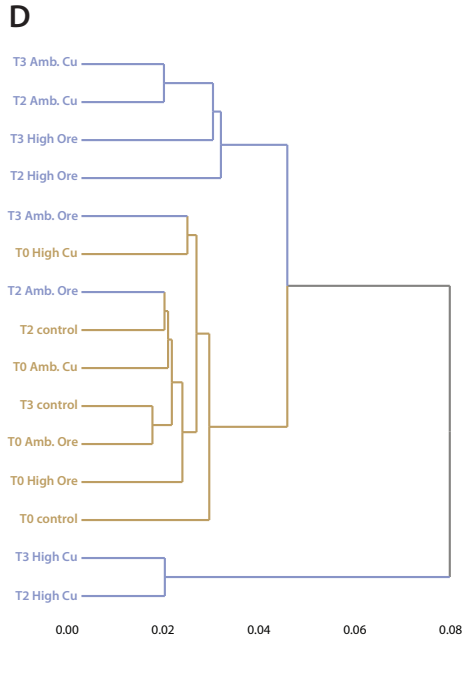


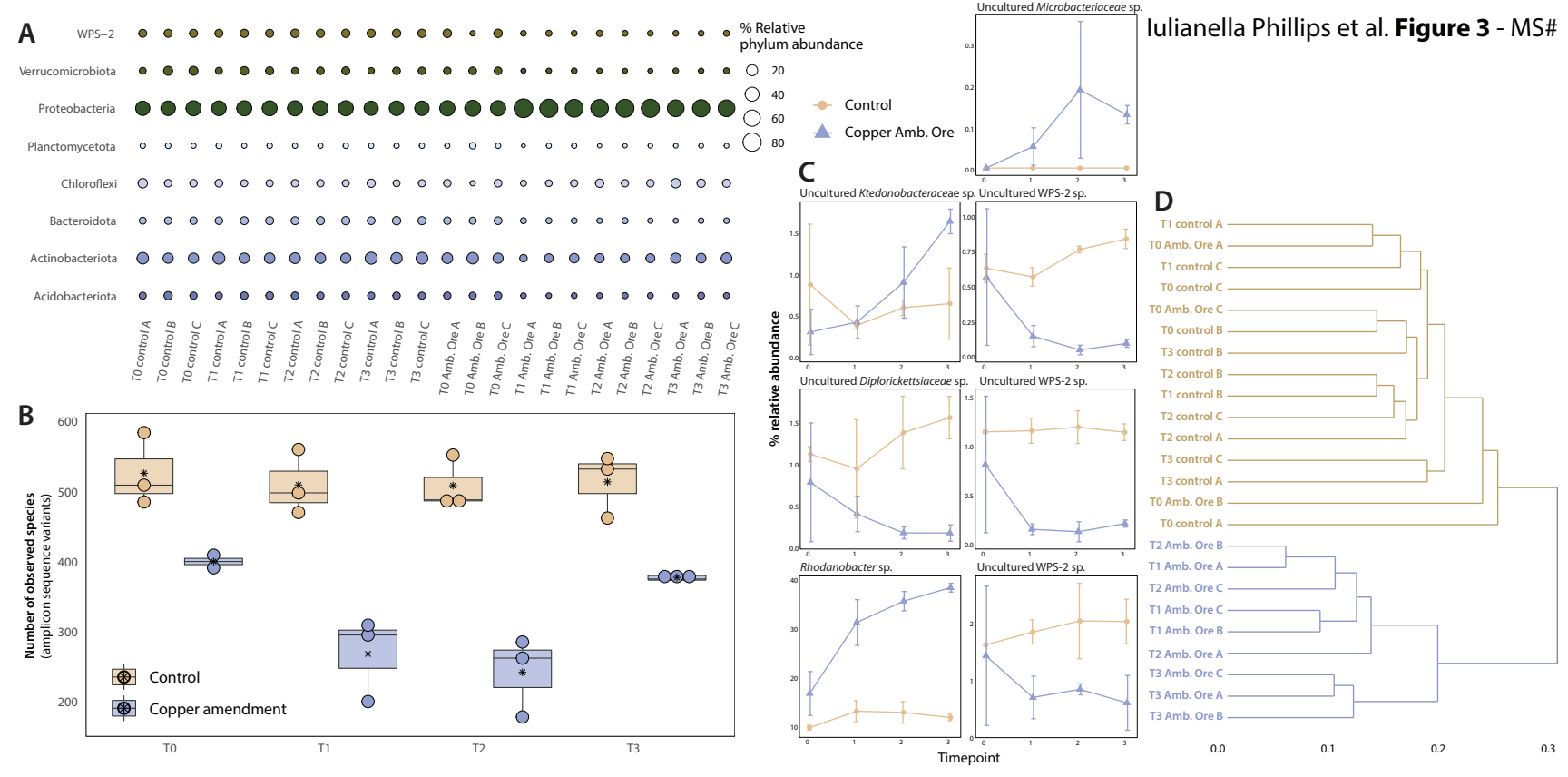
Iulianella Phillips et al. **Figure 2** - MS#



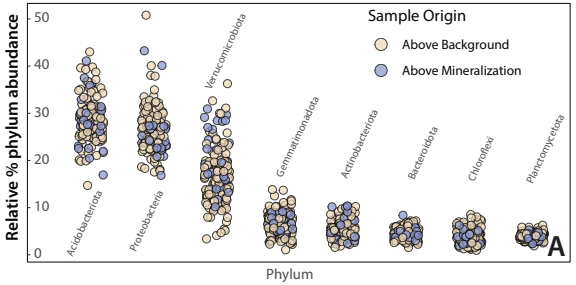


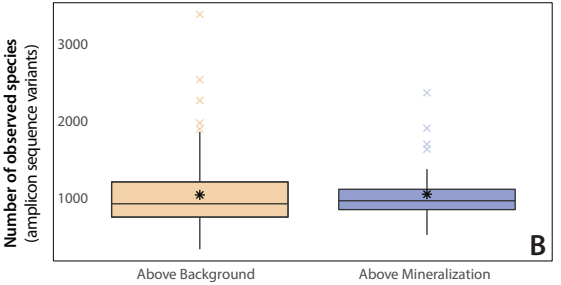


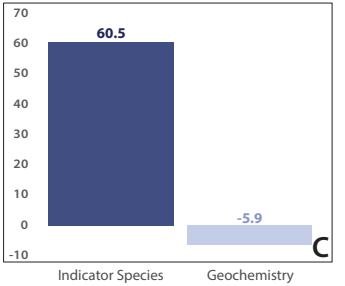


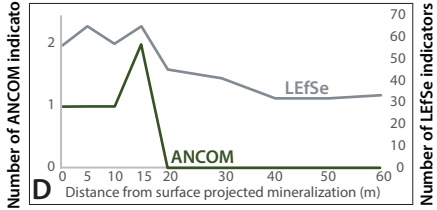


Iulianella Phillips et al. **Figure 3** - MS#

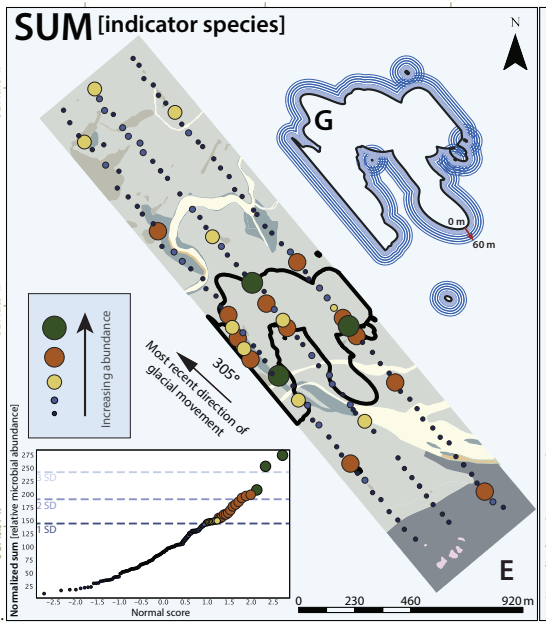


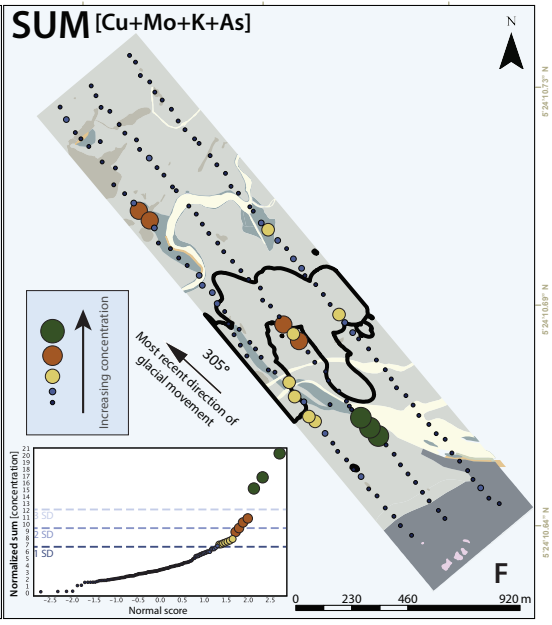






- **Swamp:** thick organic horizons; well-sorted fine sand, clast supported organic-rich material; low lying areas.
- **Moraine actively eroding:** erosional component of the glacially deposited residual moraine.
- **Organic rich fluvial deposits:** slow, but active fluvial deposition with accumulation of organic-rich materials.
- **Glacial fluvial deposits:** fine-medium to coarse sand, moderate to well-sorted, clast supported; depositional and erosional materials; abundant kame structures.
- **Residual moraine:** dominant material blanketing bedrock; typically planer, but can be undulating; poorly-sorted till, matrix supported (clay), rounded-angular clasts.
- **Reworked fluvial terrace**
- **Bedrock:** Nicola Group volcanic sandstone outcrops.
- **Mineralization:** 0.2 g/t Au equivalent (incl. >0.22% Cu).





Iulianella Phillips, B.P., et al., 202X, Microbial indicators and detection of Cu-sulfide ore mineralization: Geology, https://doi.org/10.1130/XXXX

Supplemental Material

Supplemental Methods.
Figure captions for data Tables S1-S4.

Microbial indicators and detection of Cu-sulfide ore mineralization

Bianca P. Iulianella Phillips[1,2], Rachel L. Simister[3], Shane D. Rich[1,2], Craig J.R. Hart[1,2], Peter A. Winterburn[1,2]†, and Sean A. Crowe[2,3]

[1]MDRU – Mineral Deposit Research Unit, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4

[2]Department of Earth, Ocean, and Atmospheric Sciences, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4

[3]Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada, V6T 1Z3

SUPPLEMENTAL MATERIAL

Field sampling and QA/QC

Three soil sampling transects (2.5 km long with 50 m sample spacing and 200 m fence-lines) were established over a porphyry-style Cu-Au mineral deposit (Deerhorn) buried beneath glacial till (Figure 1a). Mineralization is well-defined by drilling with a resource grade of 0.2 g/t Au equivalent ore, which includes most rocks >0.22% Cu. The survey was designed such that it captured up-ice background materials, down-ice background materials and materials that directly overly the surface expression of mineralization. Soils for DNA sequencing and microbial community fingerprinting at Deerhorn were sampled with sanitized 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 (Rich, 2016). 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. B-horizon soils were targeted for each soil sample, although multiple horizons (including O, Ah, Ae, and C) were taken, where possible, for future analyses. Soils for microbial community fingerprinting were frozen at –20° C upon return to the laboratory at The University of British Columbia (UBC) after 1-2 weeks in field storage (-5 – 0 □C) and transit, prior to DNA extraction. Sub-samples of the soils used in microbial community profiling were also collected for geochemical analysis. Field measurements (methods described in: (Rich, 2016)) consisted of slurry tests for pH and oxidation-reduction potential (ORP) after field sieving to below 180 µm (80 mesh). Soil samples for elemental geochemical analysis (~1 kg) were sent to ALS Global (North Vancouver, BC) for aqua-regia acid digestion and deionized water digestion prior to inductively coupled mass spectrometry (ICP-MS). Field duplicates, CRMs certified reference materials (CRMs), and blanks were inserted into the analytical stream every 20 samples.

Incubation experiments

Bulk background soil samples from the Deerhorn field area (Sample ID 154414; 52.274, -121.379) and from the Canadian sub-arctic tundra (63.484, -109.08) (Slave Craton, Northwest Territories) were used as baseline soils for the copper incubation experiments. These areas contained background-level metal concentrations and were collected from the upper B-horizon under aseptic conditions. Samples are considered as representative of background soil if its geochemical indicator and pathfinder elements are below the anomalous threshold determined statistically for the surveyed area. The soils were packed into a sealed Poly Ore sample bag and stored at ambient temperature.

The Deerhorn (BC) soil was digested using a multi-acid near total digestion and the digestate analyzed by inductively coupled plasma–mass spectrometry (ICP-MS) ALS Global (North Vancouver, BC) to determine that the soil contained 9 ppm Cu, 1 ppm As, and 0.32 ppm Mo. The bulk soil was not dried prior to the start of the experiment. The soil was dispensed aseptically into sterile containers for each treatment and thoroughly mixed with copper-bearing materials. Amendment concentrations were chosen to represent either concentrations of copper that are routinely detected in geochemical surveys over buried mineral deposits (ambient or '(Am)') or very high levels of copper that might be expected in highly anomalous soils (high or '(Hi)'). The amendments were as follows: 1) 'Hi-ore' soil was amended with chalcopyrite ($CuFeS_2$) ore at 600 ppm Cu; 2) 'Am-ore' soil was amended with chalcopyrite ore at 200 ppm Cu; 3) 'Hi-Cu' soil was amended with copper in the form of $CuSO_4$ (dissolved in Milli-Q®

filtered water) at 600 ppm Cu; and 4) 'Am-Cu' soil was amended with copper in the form of $CuSO_4$ to 200 ppm Cu. Soil was sampled at T = 0, T = 1 (14 days), and T = 2 (35 days).

The tundra soils (NWT) were digested using a near total multi-acid digestion and the digestate was analyzed by ICP-MS at ALS Minerals Laboratories Ltd (North Vancouver, BC) to determine that the soil contains 8 ppm Cu, < 1 ppm As, and 0.73 ppm Mo. The bulk soil was not dried prior to the start of the experiment. Soil was dispensed aseptically into sterile containers for each treatment and amended with the same chalcopyrite ore described above at 200 ppm. Soil was sampled at T = 0, T = 1 (15 days), T = 2 (55 days), and T = 3 (85 days).

DNA extraction and QA/QC
DNA extraction and quantification was performed as described in: (Simister et al., 2023).

SSU rRNA gene amplification and DNA amplicon sequencing
Gene amplification and DNA amplicon sequencing were performed as described in: (Simister et al., 2023).

Bioinformatics
DNA sequences were processed using the QIIME2 amplicon sequence analysis program (Bolyen et al., 2019) with the DADA2 denoising pipeline (Callahan et al., 2016). Sequences were clustered to amplicon sequence variants (ASV) and classified using SILVA reference taxonomy database (release 138). ASVs that had less than 1 read were filtered from analysis. Species richness measures (number of observed features) were calculated in QIIME2. Sequences were deposited into the Sequence read archive (SRA) under accession numbers PRJNA698256 and PRJNA698251.

Anomaly identification and mapping
LEfSe indicator species analyses (Segata et al., 2011) were performed in the Mothur program (subsampled to 11132 sequences) (Schloss et al., 2009) and ANCOM indicator species analyses (Mandal et al., 2015) were performed in the QIIME2 program, where indicator species (ASVs) are considered statistically significant if the LDA score > 2 (LEfSe) or a W score rejects null hypothesis (ANCOM). Sample groups for the copper amendment incubation experiments are based on unamended "control soils" and amended "copper-bearing soils". Sample groups were set for field analyses based on their origin from "background soil" or "soils above mineralization". These groups are defined based on underlying geology whereby "background soils" come from above the andesite breccias and thick beds of volcanic derived sandstones (del Real et al., 2017) and "soils above mineralization" come from above the surface projection of mineralization (0.2 g/t Au equivalent) as defined by drilling.

Differential abundance analyses of Deerhorn soils were multi-tiered with the soil samples considered "above mineralization" and "above background" defined based on the distance from the drilling-defined surface projection of mineralization. Soil samples "above mineralization" in each analysis ranged from 0 m – 60 m (0 m, 5 m, 10 m, 15 m, 20 m, 30 m, 40 m, 50 m, and 60 m). The combined collection of indicator species derived from 0 m – 15 m were used downstream to create the microbial anomaly map.

Incubation-derived indicator species showing an enrichment in copper amended soils were curated to plot at Deerhorn. Indicator species with > 1 average reads per sample in the incubation experiment and positive response ratios at the Deerhorn field site were included. Response ratios for indicator species were calculated as the ratio between the average relative abundance in "soils above mineralization" and the average relative abundance of "background soils". Indicator species predicted from the Deerhorn field site were not curated further, thus each indicator species output was included in the generation of the anomaly maps.

Both map data plots were created using relative abundances of indicator species from 16S rRNA gene sequencing and pathfinder/indicator element concentrations from aqua-regia acid digest ICP-MS results. Individual indicator species and pathfinder/indicator elements were normalized to the mean prior to summation. Response ratio bar plots of the normalized sums of indicator species and pathfinder elements are expressed by the following equation:

$$\left(\left(average\left(\frac{\text{on deposit}}{\text{off deposit}}\right)\right)-1\right)*100$$

Anomaly identification through probability plots was done in the Reflex/Imdex ioGAS software (version 8.0), and mapping of anomalies and surficial geology was performed in the ESRI ArcGIS software. QGIS was used to determined soil sample distance from the surface projection of mineralization.

REFERENCES

Bolyen, E. et al., 2019, Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2: Nature biotechnology, v. 37, p. 852–857.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P., 2016, DADA2: High-resolution sample inference from Illumina amplicon data: Nature methods, v. 13, p. 581–583.

Mandal, S., Van Treuren, W., White, R.A., Eggesbø, M., Knight, R., and Peddada, S.D., 2015, Analysis of composition of microbiomes: a novel method for studying microbial composition: Microbial ecology in health and disease, v. 26, p. 27663.

del Real, I., Bouzari, F., Rainbow, A., Bissig, T., Blackwell, J., Sherlock, R., Thompson, J.F.H., and Hart, C.J.R., 2017, Spatially and Temporally Associated Porphyry Deposits with Distinct Cu/Au/Mo Ratios, Woodjam District, Central British Columbia: Economic geology and the bulletin of the Society of Economic Geologists, v. 112, p. 1673–1717.

Rich, S.D., 2016, Geochemical mapping of porphyry deposits and associated alteration through transported overburden: University of British Columbia, https://open.library.ubc.ca/soa/cIRcle/collections/ubctheses/24/items/1.0307413.

Schloss, P.D. et al., 2009, Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities: Applied and environmental microbiology, v. 75, p. 7537–7541.

Segata, N. et al., 2011, Microbial community function and biomarker discovery in the human microbiome: Genome biology, v. 12, p. P47.

Simister, R.L., Iulianella Phillips, B.P., Wickham, A.P., Cayer, E.M., Hart, C.J.R., Winterburn, P.A., and Crowe, S.A., 2023, DNA sequencing, microbial indicators, and the discovery of buried kimberlites: Communications Earth & Environment, v. 4, p. 1–10.

SUPPLEMENTAL DATA TABLES S1-S4 FIGURE CAPTIONS

Table S1. Soil geochemical data from the Deerhorn field survey. Elemental concentrations from ALS Global (North Vancouver, BC), with digest/analysis codes indicated above the concentration value for each analyte.

Table S2. Species richness data from soil microbial communities (number of observed species/features/ASVs) from the soil incubation experiments and the Deerhorn field survey.

Table S3. List of indicator species and their relative abundances for the Deerhorn porphyry copper deposit.

Table S4. Baseline geochemistry for the starting soils for each incubation experiment (Deerhorn (BC) and tundra (NWT)). Digest/analysis codes for ALS Global (North Vancouver, BC) are indicated above the concentration value for each analyte.