

Microbial Sensing of Sulphide Mineralization, Southern British Columbia and Vancouver Island (NTS 092F/14, 092I/06, 093A/06)

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Introduction

Mineral exploration in Canada is becoming increasingly difficult because the majority of deposits exposed at the surface have already been discovered, leaving undiscovered commodities buried beneath appreciable glacial overburden and/or bedrock. The effectiveness of many existing exploration tools is diminished and therefore the development of innovative exploration approaches is vital for continued success in the discovery of new resources (Winterburn, 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.

Rationale for Microbial-Community Fingerprinting

Two British Columbia (BC) porphyry-copper deposits, the Highland Valley Highmont South Cu-Mo deposit (HVC) of Teck Resources Ltd. (NTS 092I/06) and the Consolidated Woodjam Copper Corp. Deerhorn Cu-Au deposit (NTS 093A/06), 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

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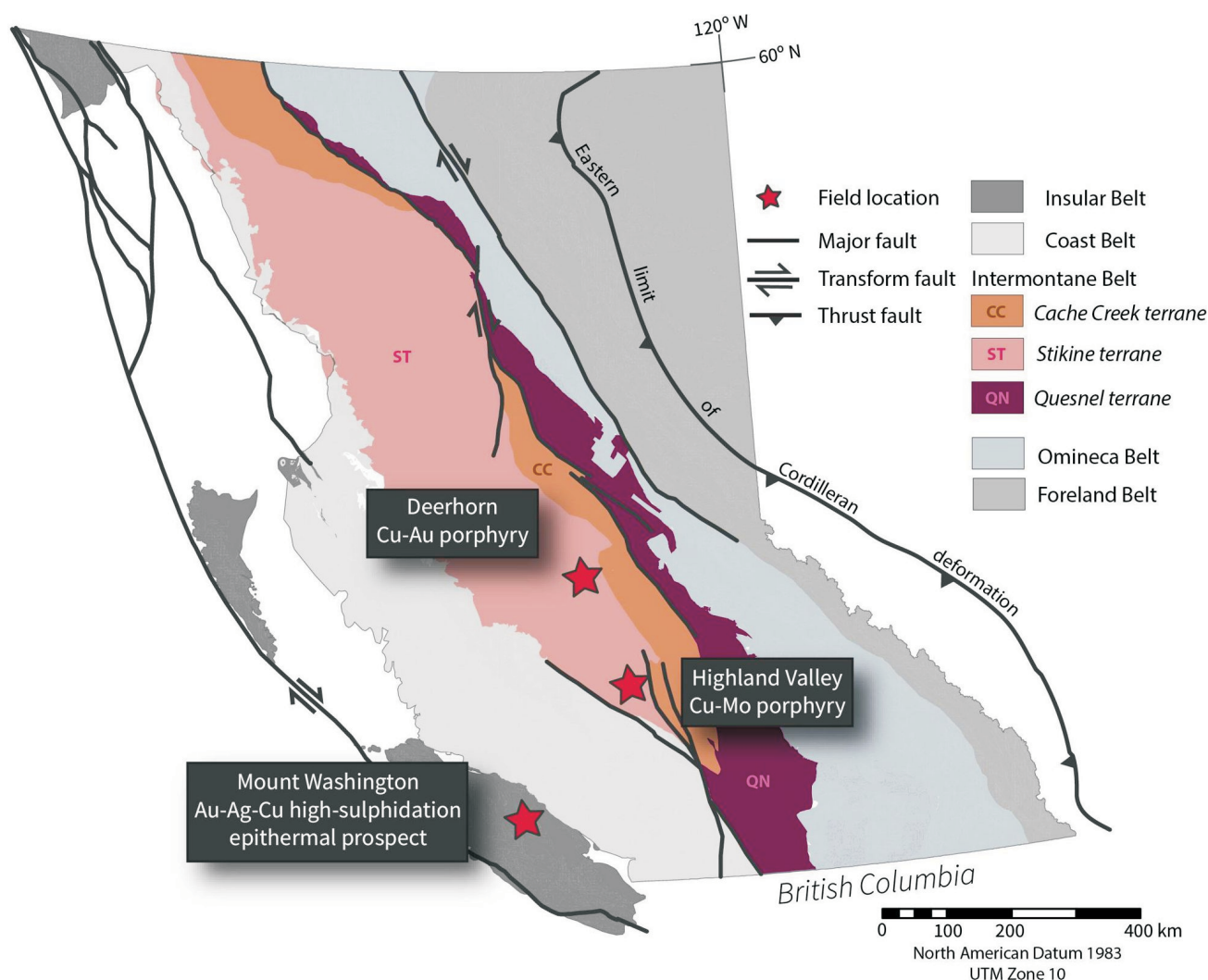


Figure 1. Locations of porphyry-Cu research sites (Highland Valley Highmont South Cu-Mo deposit and Deerhorn Cu-Au deposit) and the Mount Washington high-sulphidation Au-Ag-Cu epithermal prospect. Thick black lines indicate major faults. Terranes and geological belts are characterized based on bedrock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

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 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, 3). Specifically, microbial anomalies at Deerhorn discriminate mineralization at the surface where no detectable geochem-

ical 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 mineralization.

Mount Washington Au-Ag-Cu Epithermal System

In October 2018, the Mount Washington high-sulphidation, epithermal Au-Ag-Cu prospect (NTS 092F/14) was sampled for microbial-community fingerprinting and inorganic and organic soil geochemistry. The Mount Washington prospect is located on Vancouver Island (Figure 1). The area is underlain by the Mesozoic Karmutsen Group basalts and hosted in the Nanaimo Group sedimentary rocks and intrusive rocks of the Paleogene Mount Washington Plutonic Suite. Several north- and northwest-trending

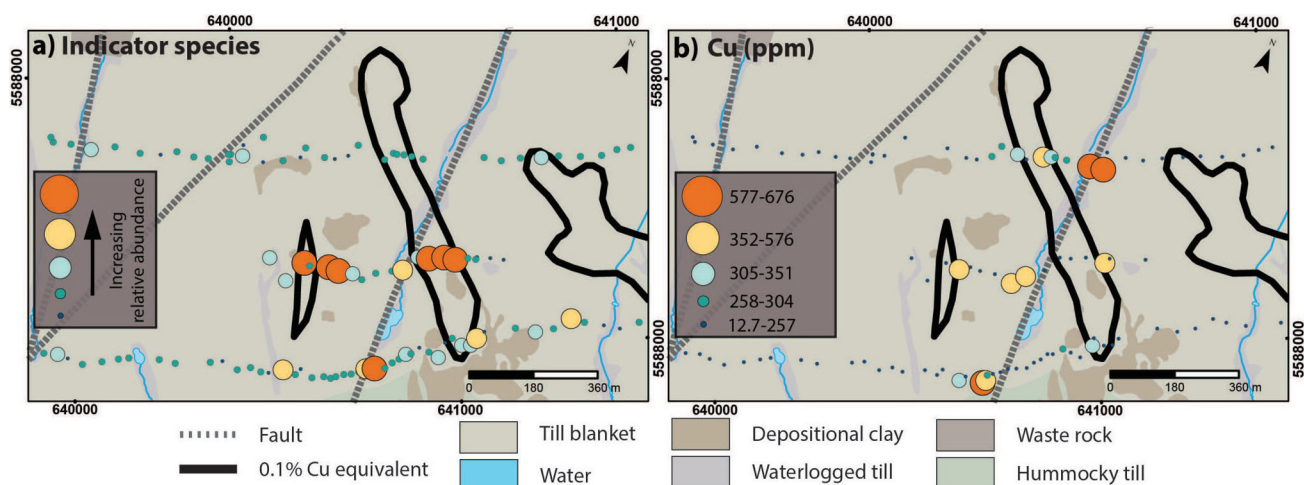


Figure 2. Example of an indicator micro-organism (a) and the Cu-anomaly map of the same area (b) from the Highland Valley Highmont South Cu-Mo deposit. Geochemical data derived from aqua-regia digestion of B-horizon soils with ICP-MS finish. Microbiological data derived from 16S rRNA gene DNA sequencing.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

extensional faults crosscut the area and appear to localize mineralization (Muller, 1989; Massey et al., 2005; Figure 4b). Mineralization is characterized by polymetallic sulphide minerals in a breccia zone and the Domineer vein, which together make up the Lakeview-Domineer resource (Houle, 2013). Surficial materials are dominated by colluvium, glacial till and organic deposits (Figure 4a), with the direction of ice flow at 058° (Fyles, 1960).

The survey was carried out in conjunction with a hydrocarbon survey (Luck, 2021) and comprised five survey lines with a total of 64 samples taken at 50 m spacing perpendicular to the strike of the mineralized breccia (Figure 4b). 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

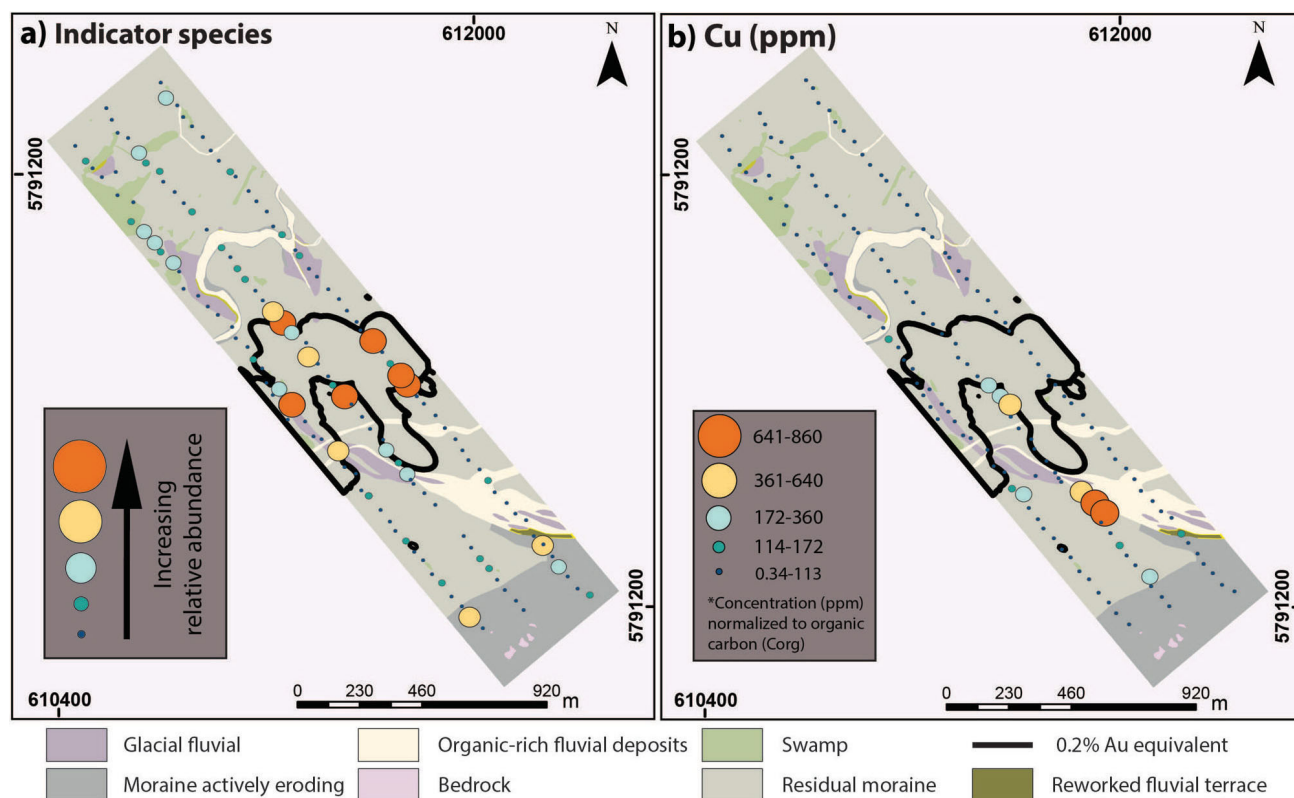


Figure 3. Example of an indicator micro-organism (a) and the Cu-anomaly map (normalized to organic carbon) of the same area (b) from the Deerhorn Cu-Au deposit. Geochemical data derived from aqua-regia digestion of B-horizon soils with ICP-MS finish. Microbiological data derived from 16S rRNA gene DNA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

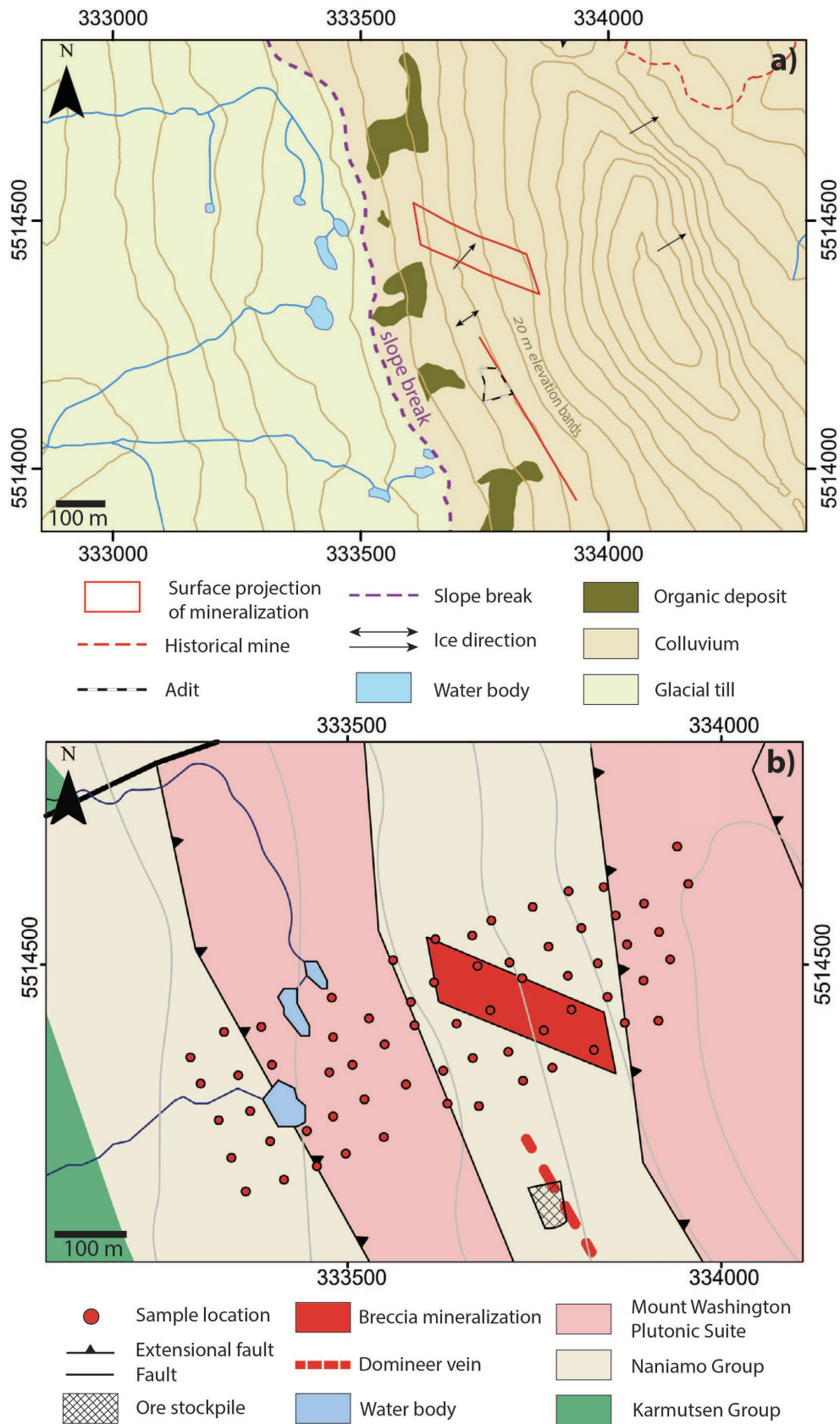


Figure 4. Mount Washington prospect **a)** surficial materials with field-survey transects (Luck, 2021), and **b)** bedrock geology (Luck, 2021; after Muller [1989] and Massey et al. [2005]). Mineralization denoted on both maps from McDougall (1987), Houle (2014) and Heberlein and Dunn (2017).

variables at each sample site for every observed soil horizon in the profile. The B-horizon soils 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 ALS Chemex (Vancouver, BC) for fire assay and four-acid digestion, and subsequent analysis by ICP-MS (results can be found in Luck, 2021). The microbial samples were frozen at -4°C 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 extractions have been carried out for the Mount Washington soil samples and results are pending. Once data are received, they will be investigated for microbiological anomalies associated with the epithermal mineralization. Results from Mount Washington will be the first step in incorporating high-sulphidation, epithermal-style mineral systems into the project databases of indicator micro-organisms for use in future mineral exploration.

Bog Wetlands

A substantial challenge in the mineral-exploration industry is the lack of geochemical methodologies when exploring in saturated surficial environments (i.e., bog wetlands). The surface can be inconsistent in these environments, and traditional acid-digestion methods coupled with ICP-MS are not always possible because the dominant surface materials are composed of waterlogged organic matter. High degrees of saturation change the oxidation-reduction potential (ORP) of the local environment, thus exerting a control on the mobility of Eh-sensitive elements (e.g., Fe, Mn, S) that may obscure geochemical signals derived from subsurface mineralization. Furthermore, a high abundance of organic matter in soils may attenuate indicator and/or pathfinder elements (e.g., Mo) and generate false anomalies. One of the very few techniques marketed for application in these environments is the Spatiotemporal Geochemical Hydrocarbons (SGH) analysis of Activation Laboratories Ltd. However, a drawback to this technique is that the compound class or compound concentrations themselves are not released to the client, so the interpreted anomaly heat map is the only data product provided. Clearly, new tools are required to meet industry demand when exploring in complex low-relief and saturated terrains.

Bog wetlands also provide a unique opportunity to study the feedback relationships between land/soil type and

microbial communities. Not only does this generate knowledge about microbial-community composition and function in saturated surface environments to support microbial-community fingerprinting as an exploration tool, but it informs the role micro-organisms play in biogeochemical cycling and the fate of carbon in the environment. Peatlands are extremely important carbon sinks globally (Dise, 2009). With changing temperatures, the investigation into microbial-community controls on carbon cycling of greenhouse gases such as CO_2 and CH_4 in these environments is vital (Belyea et al., 2008; Limpens et al., 2008; Dise, 2009).

Burns bog, located in Delta, BC, is a field location for studying microbial communities: their composition, structure and function across different land types (Figure 5). It is a raised-bog ecosystem with acidic, nutrient-poor waters and maintains a reducing environment due to its low oxygenation (Hebda et al., 2000). Plans are being made to sample across different land types in the bog along saturation gradients; characterize the composition of soil-microbial communities and its functional potential; and measure rates of microbial population turnover. The accessibility of Burns Bog also provides seasonal opportunities for sampling should it become relevant. Outcomes from these efforts may inform the applicability of microbial-community fingerprinting in saturated surface materials and shed light on the functional relationship between micro-organisms and peatland carbon storage.

Soil Preservation

Little is known about the stability of soil-microbial communities during transport and prolonged storage. It is widely accepted that soils should be frozen as soon as possible post sampling to preserve DNA (Delavaux et al., 2020). However, it is unknown how fluctuations in temperature and moisture over reasonable transport and storage time scales impact the preservation of soil-microbial communities, specifically in the microbial anomalies generated above mineral deposits and the relative abundance of indicator micro-organisms. To test this, a soil-preservation experiment has been designed that utilizes readily accessible soil material.

The UBC Totem Plant Science Field Station provides an excellent on-campus soil environment to conduct, and collect materials, for soil-related experiments. A bulk soil from the field station was sampled and the initial microbial-community composition characterized with 16S sequencing (Figure 6). The current phase of the experiment involves exposing the soil (within Poly Ore sample bags) to a range of temperature and moisture perturbations. These perturbations include freezing samples at -20°C , leaving samples at room temperature, and allowing the samples to dry out. The soil-microbial communities will be assessed at weeks- to

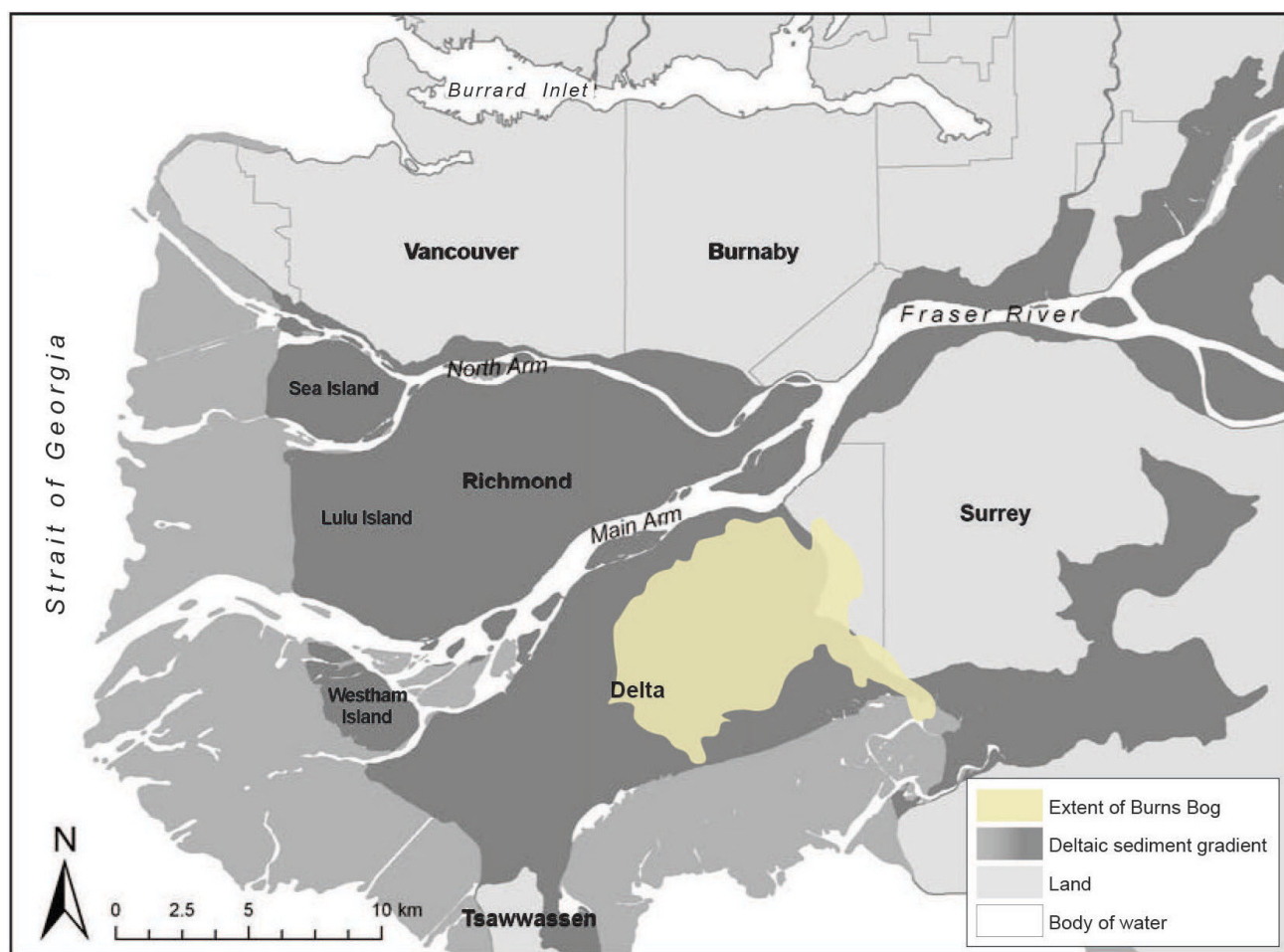


Figure 5. Burns Bog location (indicated by yellow shading) in the context of the Metro Vancouver deltaic system. Modified after Clague (1998).

months-long time intervals for at least one year and sampled in triplicate to examine the effects of temperature and moisture fluctuations on the microbial-community composition, diversity, structure and metabolic activity. This experiment will inform the development of robust sampling protocols for the mineral-exploration industry and serve as a laboratory test of the relationships between warming and drying soil conditions and microbial populations.

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. Current research directions focus specifically on reducing fundamental unknowns about the behaviour and variation of microbial communities in response to chemical and physical changes in the environment. This focus includes assessing DNA sequencing and microbial-community fingerprinting in a colluvium-dominated, Au-Ag-Cu, high-sulphidation, epithermal mineral system; exploring the relationships between micro-organism function and variation in land type; and assessing the

impact of transport and storage on the persistence of microbial-community anomalies in soils. 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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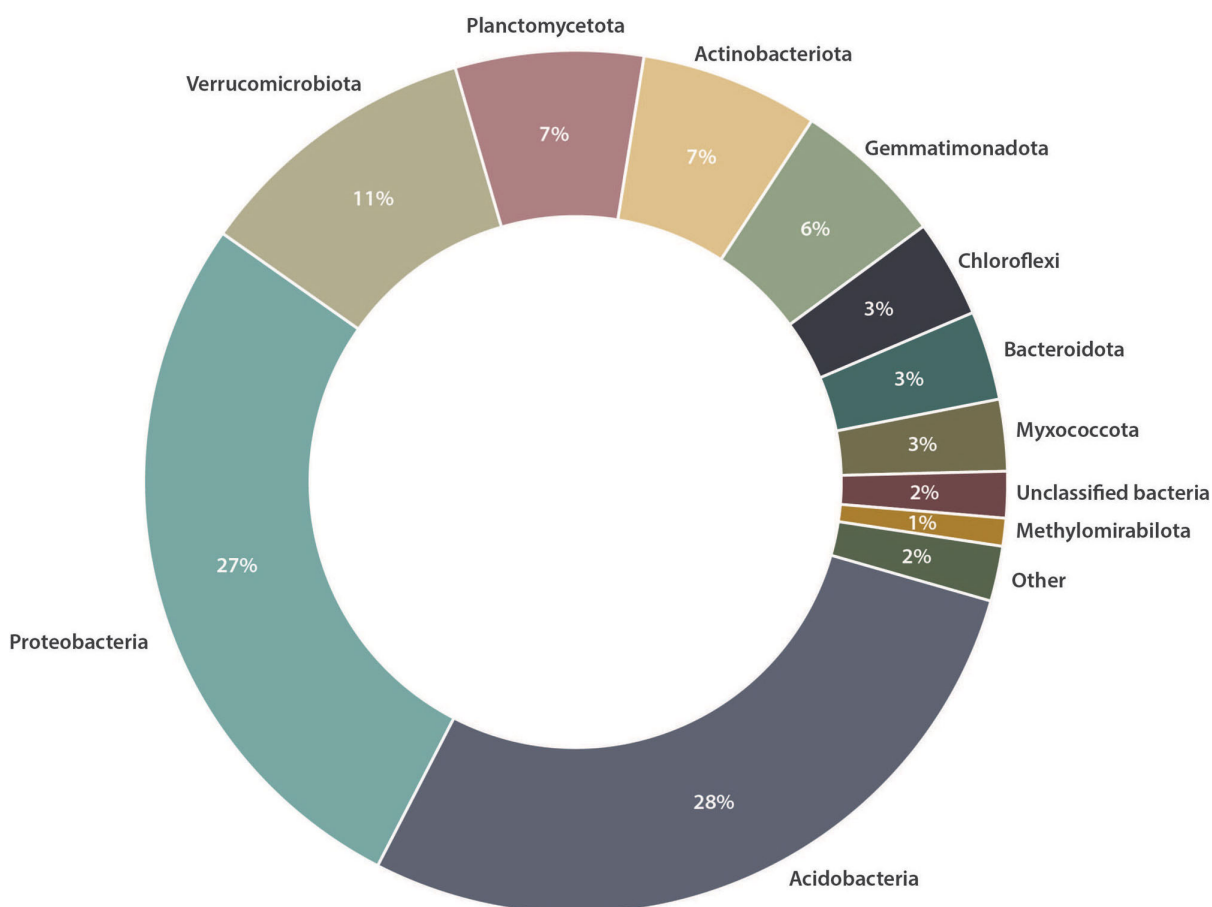


Figure 6. Phyla-level microbial-community composition of the initial soil derived from the UBC Totem Plant Science Research Station, expressed as the distribution of 16S rRNA reads per phylum. The number of reads per phylum is calculated as a percentage of the total reads for the sample. 'Other' is the sum of any phyla with less than 1% representation.

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References

- Belyea, L.R. and Malmer, N. (2008): Carbon sequestration in peatland: patterns and mechanisms of response to climate change; *Global Change Biology*, v. 10, no. 7, p. 1043–1052, URL <<https://onlinelibrary.wiley.com/doi/10.1111/j.1529-8817.2003.00783.x>> [October 2021].
- Binladen, J., Gilbert, M.T.P., Bollback, J.P., Panitz, F., Bendixen, C., Nielsen, R. and Willerslev, E. (2007): The use of coded PCR primers enables high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing; *PLoS ONE*, v. 2, no. 2, p. 1–9, URL <<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0000197>> [October 2021].
- Clague, J.J. (1998): Geological setting of the Fraser River delta; in *Geology and Natural Hazards of the Fraser River Delta*, British Columbia, J.J. Clague, J.L. Luternauer and D.C. Mosher (ed.), Geological Survey of Canada, Bulletin 525, p. 7–16, URL <<https://geoscan.nrcan.gc.ca/starweb/geoscan/servlet.starweb?path=geoscan/fulle.web&search1=R=210033>> [October 2021].
- Cui, Y., Miller, D., Schiarizza, P. and Diakow, L. (2017): British Columbia digital geology; British Columbia Ministry of Energy, Mines, and Low Carbon Innovation, British Columbia Geological Survey, Open File 2017-8, 9 p., URL <<https://www2.gov.bc.ca/gov/content/industry/mineral-exploration-mining/british-columbia-geological-survey/geology/bcdigitalgeology>> [October 2021].
- Delavaux, C.S., Dever, J.D., Karppinen, E.M. and Bainard, L.D. (2020): Keeping it cool: soil sample cold pack storage and DNA shipment up to 1 month does not impact metabarcoding results; *Ecology and Evolution*, v. 10, p. 4652–4664, URL <<https://onlinelibrary.wiley.com/doi/full/10.1002/ece3.6219>> [October 2021].
- Dise, N.B. (2009): Peatland response to global change; *Science*, v. 326, p. 810–811, URL <<https://www.science.org/doi/full/10.1126/science.1174268>> [October 2021].
- Falkowski, P.G., Fenchel, T. and Delong, E.F. (2008): The microbial engines that drive Earth's biogeochemical cycles; *Science*, v. 320, p. 1034–1040, URL <<https://www.science.org/doi/10.1126/science.1153213>> [October 2021].
- Fierer, N. (2017): Embracing the unknown: disentangling the complexities of the soil microbiome; *Nature Reviews Microbiology*, v. 15, no. 10, p. 579–590, URL <<https://www.nature.com/articles/nrmicro.2017.87>> [October 2021].
- Fyles, J.G. (1960): Surficial geology, Oyster River, Comox, Nanaimo and Sayward districts, British Columbia; Geological Survey of Canada, Preliminary Map 49-1959, URL <<https://>>

- geoscan.nrcan.gc.ca/starweb/geoscan/servlet.starweb?path=geoscan/fulle.web&search1=R=108639> [October 2021].
- Hebda, R.J., Gustavson, K., Golinski, K. and Calder, A.M. (2000): Burns Bog ecosystem review synthesis report for Burns Bog, Fraser River delta, south-western British Columbia, Canada; Environmental Assessment Office, Victoria, BC, URL <<https://www.osti.gov/etdweb/biblio/20205111>> [October 2021].
- Heberlein, D.R. and Dunn, C.E. (2017): Halogens and other volatile compounds in surface sample media as indicators of mineralization, part 2: Mount Washington epithermal Au-Cu-Ag prospect, Vancouver Island, BC (NTS 092F/14); in Geoscience BC Summary of Activities 2016, Geoscience BC, Report 2017-1, p. 151–158, URL <http://www.geosciencebc.com/i/pdf/SummaryofActivities2016/SoA2016_Heberlein-MtWashington.pdf> [October 2021].
- Houle, J. (2013): Geochemical and prospecting technical assessment report on the Mount Washington property for North Bay Resources Inc., Nanaimo Mining Division, BC; BC Ministry of Energy, Mines and Low Carbon Innovation, Assessment Report 34200, 206 p., URL <<https://aris.empr.gov.bc.ca/ArisReports/34200.PDF>> [October 2021].
- Houle, J. (2014): NI 43-101 technical report on the Mount Washington property, Vancouver Island, British Columbia; NI 43-101 technical report prepared for North Bay Resources Inc., 92 p., URL <<http://www.northbayresources.com/2014mw43101.pdf>> [November 2021].
- Iulianella Phillips, B.P. (2020): Microorganisms as sensors for concealed mineral deposits: application and development of microbiological mineral exploration in the Northwest Territories and British Columbia, Canada; M.Sc. thesis, The University of British Columbia, 211 p., URL <<https://open.library.ubc.ca/soa/cIRcle/collections/ubctheses/24/items/1.0390297>> [October 2021].
- Leslie, K., Oates, C.J., Kyser, K.T. and Fowle, D. A. (2014): Biogeochemical controls on metal mobility: modeling a Cu-Zn VMS deposit in column flow-through studies; *Geochemistry: Exploration, Environment, Analysis*, v. 14, p. 59–70, URL <<https://geea.lyellcollection.org/content/14/1/59/figures-data>> [October 2021].
- Limpens, J., Berendse, F., Bloudau, C., Canadell, J.G., Freeman, C., Holden, J., Roulet, N., Rydin, H. and Schaepman-Strub, G. (2008): Peatlands and the carbon cycle: from local processes to global implications – a synthesis; *Biogeosciences*, v. 5, p. 1475–1491, URL <<https://bg.copernicus.org/articles/5/1475/2008/>> [October 2021].
- Luck, P. (2021): Hydrocarbons in soil as pathfinders for base and precious metal exploration: an orientation study at the Mt. Washington epithermal Au-Ag-Cu prospect, British Columbia, Canada; M.Sc. thesis, The University of British Columbia, 271 p., URL <<https://open.library.ubc.ca/soa/cIRcle/collections/ubctheses/24/items/1.0397404>> [October 2021].
- Massey, N.W.D., MacIntyre, D.G., Desjardins, P.J. and Cooney, R.T. (2005): *Geology of British Columbia*; BC Ministry of Energy, Mines and Low Carbon Innovation, Geoscience Map 2005-3, (3 sheets), scale 1:1 000 000, URL <<https://www2.gov.bc.ca/gov/content/industry/mineral-exploration-mining/british-columbia-geological-survey/geology/bcdigitalgeology>> [October 2021].
- McDougall, J.J. (1987): Summary report on the Mt. Washington gold property; Better Resources Limited, February 1987.
- Muller, J.E. (1989): Tertiary low angle faulting and related gold and copper mineralization on Mount Washington, Vancouver Island (92F/11, 14); in *Geological Fieldwork 1988*, BC Ministry of Energy, Mines and Low Carbon Innovation, BC Geological Survey, Paper 1989-1, p. 81–91, URL <<https://www.semanticscholar.org/paper/TERTIARY-LOW-ANGLE-FAULTING-AND-RELATED-GOLD-AND-ON-Muller/53774dc7d9230f510d8e9e33b91e3800ad47c975?p2df>> [October 2021].
- Newman, D.K. and Banfield, J.F. (2002): Geomicrobiology: how molecular-scale interactions underpin biogeochemical systems; *Science*, v. 296, no. 5570, p. 1071–1077, URL <https://www.science.org/doi/abs/10.1126/science.1010716?keytype=tf_ipsecsha&ikey=3b7bf439b72ef152968ec5b99e05a350dd73007> [October 2021].
- Reith, F. and Rogers, S.L. (2008): Assessment of bacterial communities in auriferous and non-auriferous soils using genetic and functional fingerprinting; *Geomicrobiology Journal*, v. 25, no. 34, p. 203–215, URL <<https://www.tandfonline.com/doi/abs/10.1080/01490450802081846>> [October 2021].
- Simister, R.L., Iulianella Phillips, B.P., Winterburn, P.A. and Crowe, S.A. (2020): Microbial-community fingerprints as indicators for buried mineralization, Geoscience BC Report 2020-03 and MDRU Publication 446, 31 p., URL <http://www.geosciencebc.com/i/project_data/GBC%20Report2020-03/GBC%20Report%202020-03%20Microbial-Community%20Fingerprints%20as%20Indicators%20for%20Buried%20Mineralization%20in%20British%20Columbia_revised_BIP.pdf> [October 2021].
- Wakelin, S., Anand, R.R., Macfarlane, C., Reith, F., Noble, R. and Rogers, S. (2012): Assessing microbiological surface expression over an overburden-covered VMS deposit; *Journal of Geochemical Exploration*, v. 112, p. 262–271, URL <<https://www.sciencedirect.com/science/article/abs/pii/S0375674211001853?via%3Dihub>> [October 2021].
- Winterburn, P., Noble, R. and Lawie, D. (2017): Advances in exploration geochemistry, 2007 to 2017 and beyond; *Geochemistry: Exploration, Environment, Analysis*, v. 20, p. 157–166, URL <<https://www.mdru.ubc.ca/wp-content/uploads/2019/01/WinterburnExplore17.34.pdf>> [October 2021].
- Zhou, J., He, Z., Yang, Y., Deng, Y., Tringe, S. and Alvarez-Cohen, L. (2015): High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats; *mBio*, v. 6, no. 1, p. 106–113, URL <<https://pubmed.ncbi.nlm.nih.gov/25626903/>> [October 2021].