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Microbial community structure within a weathered waste-rock pile overlain by a monolayer soil cover

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Abstract

A historic waste-rock stockpile (WRS) at the Detour Lake Mine (DLM), covered with a thin layer (< 1 m) of local overburden, was studied to determine the potential for microbially-mediated generation of acid rock drainage (ARD). The sulfur content of the waste rock ranged from 0 - 2.2 wt. %, with pyrite and pyrrhotite identified as the principal sulfide minerals. Acidity released through sulfide oxidation in the WRS has been neutralized through carbonate mineral dissolution, and has resulted in the generation of neutral mine drainage (pH 6 – 8). However, the WRS is heterogeneous, and localized water samples collected from discrete zones within the stockpile were more highly oxidized and acidic (*i.e.*, pH ≥ 2.5). Enumerations of acidophilic sulfur- (aSOM) and iron-oxidizing microorganisms (aIOM) were performed, yielding mean abundances of 1.2×10^3 and 9.0×10^5 cells g^{-1}, respectively. The mean abundance of neutrophilic sulfur-oxidizing microorganisms (nSOM) was 5.5×10^5 cells g^{-1}. Fungi and bacteria present in the waste rock were identified using high-throughput amplicon sequencing of 18S and 16S rRNA genes, respectively. Sequencing confirmed the presence of *Thiobacillus* and *Acidithiobacillus* species. Bacterial diversity was greatest in samples from the cover material. Unoxidized waste rock samples were characterized by neutrophilic iron- or sulfur-oxidizing genera (*i.e., Thiobacillus*), whereas samples collected from oxidized and acidic zones in the WRS showed greater abundances of acidophilic taxa (*i.e., Acidithiobacillus*). None of the fungal genera identified in this study have been shown to oxidize sulfide minerals directly, other than indirectly through creation of a suitable environment for the prokaryotes involved in the processes. Although installation of a simple, non-engineered cover is anticipated to have slowed the generation of ARD, evidence of ongoing sulfide oxidation within the covered WRS was observed. High abundances and activities of sulfur- and iron-oxidizing microorganisms indicate that the soil cover has not prevented the growth of microorganisms that catalyze sulfide-mineral oxidation.

Keywords: waste rock, mine waste, soil cover, sulfur-oxidizing microorganisms, iron-oxidizing microorganisms, cell enumerations, high-throughput sequencing.

1. Introduction

The environmental challenges associated with mining activities are well understood. The production and storage of mine-waste materials (*i.e.*, mill tailings and waste rock) can result in the release of contaminants to surface water and groundwater. Waste rock consists of host rock or overburden material that is uneconomical to process, and comprises the primary waste stream at most open-pit mining operations. Sulfide-bearing waste rock is retained on mine sites in waste-rock stockpiles. After deposition, sulfide minerals present in the waste rock are often subjected to oxidation via direct or indirect mechanisms, with the oxidant being atmospheric O_2 or Fe^{3+}, respectively (Nordstrom and Alpers, 1999). Under circumneutral pH conditions, direct oxidation of sulfide minerals generates acid and liberates metal(loid)s (Blowes *et al.*, 2003). The liberated Fe^{2+} is oxidized under acidic conditions, and the Fe^{3+} contributes to continued sulfide oxidation. Under low pH conditions, Fe^{3+} likely overtakes dissolved O_2 as the primary oxidant (Moses *et al.*, 1987).

The rate of oxidative dissolution of sulfide minerals exposed to oxygen and water can be greatly enhanced by microbial catalysis. A range of lithoautotrophic microorganisms impact the rate of sulfide and iron oxidation. Among the many bacterial species capable of catalyzing dissimilatory Fe^{2+} oxidation, which is the rate limiting step in sulfide oxidation (Williamson and Rimstidt, 1994), the *Acidithiobacillus, Acidimicrobium, Alicyclobacillus, Leptospirillum,* and *Sulfobacillus* spp. are most commonly found in mine-waste environments, such as waste-rock stockpiles or tailings impoundments (Schippers *et al.,* 2010). Sulfur-oxidizing bacteria may oxidize sulfide minerals directly (Nordstrom and Southam, 1997), or may contribute indirectly *via* the oxidation of sulfur intermediates (such as H_2S, $S_2O_3^{2-}$, $S_3O_6^{2-}$ and $S_4O_6^{2-}$; Baker and Banfield, 2003). *Thiobacillus* (*T.*) *thioparus* is a neutrophilic sulfur-oxidizing microorganism (nSOM; Kuenen *et al.*, 1992; Gould *et al.*, 1994). Many acidophilic iron oxidizers also facilitate sulfur oxidation under low pH (Dopson and Johnson, 2012), such as *Sulfobacillus* and some of the *Acidithiobacillus* spp. (*e.g.*, *Acidithiobacillus* (*At.*) *ferrooxidans*, *At. ferridurans*); other species oxidize sulfur but not iron (*e.g.*, *At. caldus, At. thiooxidans*). Common acidophilic iron-oxidizing (aIOM) and sulfur-oxidizing microorganisms (aSOM) have been cultured in low numbers from mine-waste environments characterized by neutral mine drainage (Southam and Beveridge, 1992; Blowes *et al.*, 1998; Lindsay *et al.*, 2009).

Although the role of prokaryotic catalysis in sulfide oxidation and ARD generation is well established, there is a relative scarcity of studies which incorporates the eukaryotic ecology of mine wastes. Eukaryotes are generally more sensitive to acidity and metals than prokaryotes, and are typically found in lower abundances in mine-waste environments. However, eukaryotic microorganisms, including algae, amoeba, rotifers, fungi, and yeast, inhabit ARD-impacted sites. The diversity and important role of eukaryotes in acidic environments have been reviewed by Aguilera *et al.* (2016). Algae and protozoa have received more attention than fungi and yeast, although acid-tolerant fungi have long been identified to play important roles in ARD-impacted environments by forming biofilms on the surface of lithic fragments; these biofilms provide a suitable environment, or act as adsorption agents, for the prokaryotes involved in sulfide mineral oxidation. Gross and Robbins (2000) reviewed morphological and chemical characteristics of acidophilic and acid-tolerant fungi and yeast. Burford *et al.* (2003) reviewed the contribution of fungi to mineral weathering and contaminant sequestration through secondary mineral precipitation and/or bioaccumulation. A number of fungal genera have been noted for their ability to solubilize silicates and liberate contaminants, including iron (Burford *et al.*, 2003).

ARD derived from sulfide-bearing WRSs is typically characterized by low pH, elevated concentrations of sulfate, and dissolved metal(loid)s (Akcil and Koldas, 2006; Blowes *et al.*, 2014). The acidic pH can be neutralized via dissolution of carbonates, Al- and Fe(III)-hydroxide phases, and aluminosilicate minerals, leading to neutral mine drainage. The large volumes of waste rock typically generated at open-pit mines, combined with the potential for ARD generation, highlight the necessity for appropriate management strategies to minimize sulfide oxidation in waste rock. The environmental liability and economic cost associated with ARD and waste-rock storage are significant. Recent integrated studies investigating the hydrogeological, geochemical, mineralogical, microbiological, and physical controls of sulfide oxidation and metal leaching (*e.g.*, Wilson *et al.*, 2018; Smith *et al.*, 2013; Beckie *et al.*, 2011; Andrina *et al.*, 2006) have evaluated field-scale and multi-scale investigations of WRS behaviour to provide a framework for prediction, mitigation, and management of ARD.

Detour Lake Mine (DLM) is located approximately 185 kilometers northeast of Cochrane (ON, Canada). DLM is situated on the Detour Lake property, which occupies an area of approximately 646 km^2. Reports on local geology and lithology at the Detour Lake deposit have been published

previously (AMEC Earth & Environmental, 2010a,b; Robertson *et al.*, 2012; Oliver *et al.*, 2012). The overburden consists of glacial tills and glaciofluvial materials (depth 0 to 40 m; AMEC Earth & Environmental, 2010a). The bedrock is part of the Abitibi Greenstone Belt (the Superior Province of the Canadian Shield), and consists of volcanic massive and pillow basalts with shear zones associating hydrothermal alteration with mineralization (AMEC Earth & Environmental, 2010b; Robertson *et al.*, 2012). The waste rock is comprised predominantly of plagioclase and horneblende, with lesser amounts of clinochlore, vermiculite, biotite, and quartz. Variable quantities of sulfides (*i.e.*, primarily pyrrhotite, with accessory pyrite and chalcopyrite) and carbonates (*i.e.*, calcite, dolomite) are present throughout the piles (Oliver *et al.*, 2012; Robertson *et al.*, 2012; McNeill, 2016). The Detour Lake deposit contains 17.3 million ounces of Au, hosted within 445.9 million tonnes (Mt) of rock (Oliver *et al.*, 2012).

The original mine DLM was operated by Placer Dome Inc. from 1983 to 1999, and generated several Mt of waste rock which was placed in five stockpiles (WRS1-5; Fig. 1), each covering several hectares at a thickness of 15-20 m. Previous operations also generated approximately 10 Mt of tailings, which are contained in a tailings storage facility. Mine reclamation activities conducted by the former operator in 2000 included covering the WRSs with ~1 m of locally-derived soil. The covers are, however, thinner in most areas, and often discontinuous. Lower permeability (*i.e.*, fine grained) material is often used to cover waste dumps to reduce air permeability and O_2 fluxes into the waste dumps (Yanful *et al.*, 1993; Swanson *et al.*, 2003). In 2006, the site was reassessed and has subsequently been redeveloped by Detour Gold Corporation. Production for the new open pit at DLM resumed in early 2013.

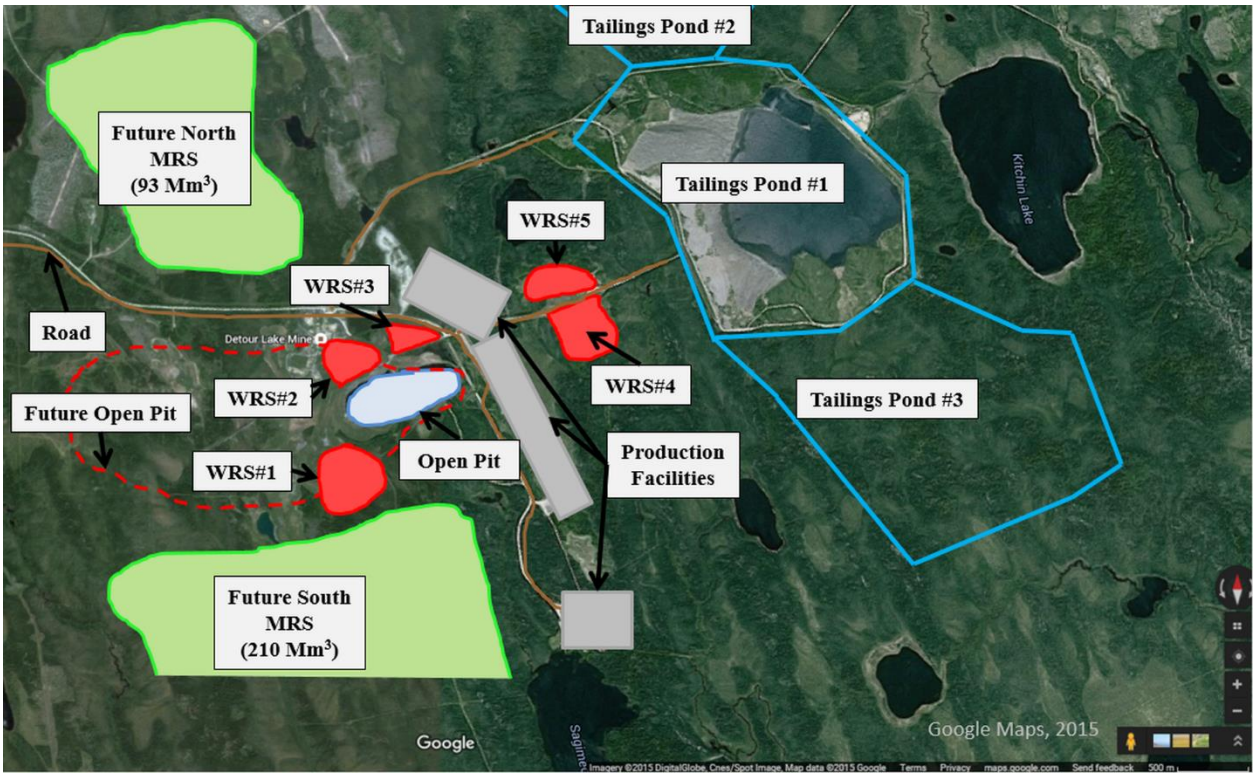

Fig. 1. Site diagram of the Detour Lake Gold Mine showing the location of historic waste-rock stockpiles (WRSs) and future mine-rock stockpiles (MRSs), the extent of planned excavations, production facilities and tailings storage facilities (TSFs). Site image current as of 2015.

Two of the waste-rock stockpiles from the previous operations (WRS1 and WRS2; Fig. 1) were relocated into a mine-rock stockpile (MRS), which contains waste rock from current operations. Excavation and relocation of waste rock from WRS1 occurred from late 2011 to mid-2012. The WRS relocation provided a rare opportunity to sample the interior of a weathered WRS through a joint research program involving personnel from the University of Waterloo and University of Alberta (Steinepreis *et al.*, 2018; Steinepreis, 2017; McNeill, 2016; Cash, 2014, Cash *et al.*, 2014). The objective of the research initiative was to assess the extent and controls of sulfide oxidation by describing the physical structure of the weathered WRS, and the waste-rock hydrogeology, geochemistry, mineralogy, and microbiology. Microbiological data can be used to inform rates of sulfide oxidation and predictions of water quality, and to evaluate the efficacy of remediation efforts. Consolidated findings of the research program will assist in developing long-term predictions of drainage-water quality, and can be used to inform mine-waste management scenarios, including closure-cover designs.

Although generally low seepage volumes and low concentrations of metals have been observed at DLM (Robertson *et al.*, 2012), zones of highly-weathered waste rock were observed throughout the WRSs, indicating that sulfide oxidation had occurred prior to placement of the soil cover, or beneath the cover following installation (McNeill, 2016; Cash, 2014; Cash *et al.*, 2014). The current study focuses on the microbiology of a historic waste-rock stockpile (WRS1 in Fig. 1), and the role of microorganisms in the release, transport, and attenuation of acidity and dissolved metals at the DLM. Detailed geological and mineralogical characterization was outside of the scope of this study; however, concise description of the DLM waste rock is included. Detailed compositional and structural characteristics of the stockpiles at DLM have been reported previously (Steinepreis *et al.*, 2018; Steinepreis, 2017; McNeill, 2016; Cash, 2014, Cash *et al.*, 2014; Oliver, 2012). This study is part of a broader initiative, the overall objective of which is to improve waste-rock management practices by providing a better understanding of the physical, chemical, and microbiological processes which govern pore-water geochemistry in sulfide-bearing waste rock.

2. Materials and methods

2.1. Microbiological analyses

Microbiological analyses were performed on waste rock and overburden samples recovered from two depths at 12 separate sample sites in WRS1 in July of 2013 (Fig. 2, Table 1).

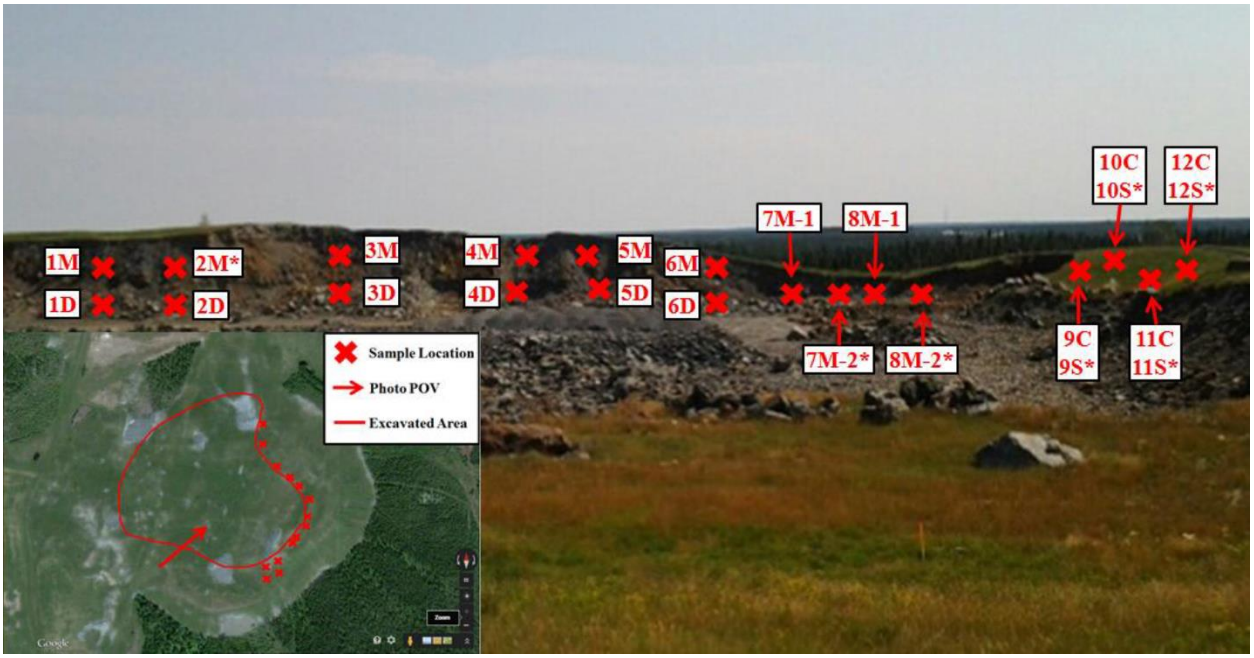

Fig. 2. Approximate locations of samples used for microbiological analysis at WRS1. Photo taken in July, 2013 (inset modified from Google Maps, 2015).

An excavator was used to dig approximately 1.0 m into the exposed flank of the stockpile to access waste rock. Waste-rock material was retrieved from the upper flank of the 25 m tall stockpile at sites 1 to 6 at the base of the stockpile (samples D, Table 1) and 10 m below the crest of the pile (samples M, Table 1) at each site. Samples were collected at sites 7 and 8 from the edge of the stockpile approximately 10 m above the base of the pile (samples M), and were comprised of more fine-grained material. At sites 7 and 8, samples were collected from adjacent zones of oxidized, orange material (denoted by an asterisk in Table 1) and light grey, unoxidized material. Sites 9 to 12 were located in a covered section of the WRS, beneath an intact soil cover; samples of the cover material (< 1 m depth; samples C) and of the underlying waste rock (1 – 2 m depth, samples S) were collected.

Table 1. Qualitative depth designation of WRS1 samples used for microbiological analyses. Due to spatial variations within the stockpile, stated depths are indicative. Legend: S = shallow, M = mid, D = deep depths, and C = cover. Oxidized or partially oxidized samples are denoted by an asterisk.

Group	Depth (m)	Sample ID
Cover	< 1	9C, 10C, 11C, 12C
Shallow	1 - 2	9S*, 10S*, 11S*, 12S*
Mid	~ 10	1M, 2M*, 3M, 4M, 5M, 6M, 7M-1, 7M-2*, 8M-1, 8M-2*
Deep	~ 20 - 25	1D, 2D, 3D, 4D, 5D, 6D

Fine-grained waste rock (< 2 mm) and soil were retained in sterile 250 mL glass containers. The samples were kept at 4 °C for enumerations of aIOM, aSOM, and nSOM, and were frozen at -20 °C for subsequent DNA extraction. The most probable number enumeration technique (MPN; Cochran, 1950; Garthright and Blodgett, 2003) was used to enumerate metabolically-active microorganisms, following the methods described by Blowes *et al.* (1995), and modified by Hulshof *et al.* (2006). Briefly, aIOM were incubated at laboratory temperature (~23 °C) for 4 weeks in a pH 2.3 medium (containing 33.4 g L^{-1} $FeSO_4 \cdot 7H_2O$) described by Tuovinen and Kelly

(1973); the development of a rusty color in the medium indicated a positive result. A second medium containing 5 g L^{-1} $Na_2S_2O_3 \cdot 5H_2O$, (ATCC medium 23; Gherna *et al.*, 1989) was used to grow nSOM and aSOM, adjusted to final pH values of 7.0 and 4.2, respectively; positive results were indicated by a decrease of 0.2 pH units at the end of the 4-week incubation period at laboratory temperature.

DNA for high-throughput amplicon sequencing was isolated using a PowerSoil DNA Extraction Kit (Qiagen, formerly MoBio Laboratories, Carlsbad, California). DNA yields were determined by nano-drop spectrophotometer, and ranged from 2.2 to 15 ng μL^{-1}. PCR amplification (using 515F/806R primers for 18S rRNA, and 530F/1100R for 16S rRNA genes) and pyrosequencing (using a proprietary barcoded amplicon sequencing process under the trademark bTEFAP; Dowd *et al.*, 2008) were performed by MR DNA (Shallowater, Texas).

2.2. Solid-phase geochemistry and mineralogy

Waste-rock samples for mineralogical and geochemical analyses were recovered from test pits excavated from the surface of WRS1 in July and August of 2011 (Cash *et al.*, 2014; Cash, 2014). The locations of 13 test pits were spaced to ensure that representative areas of WRS1 were sampled. A total of 38 samples (Table 2) were collected, and stored at 4 °C before analysis. Vertical profile samples were also collected from the excavated face of the stockpile (Cash *et al.*, 2014; Cash, 2014); 21 samples (depths not recorded) from 11 locations on WRS1 were recovered in June 2012.

Table 2. Samples recovered from test pits excavated from the surface of WRS1 used for geochemical and mineralogical characterization. Legend: C = cover, WR = waste rock, S = sand.

Sample	Material	Mean depth (m)	Sample	Material	Mean depth (m)
S1-S3	C	0.25	**S8-S3**	WR	2.775
S1-S2	WR	1.5	**S9-S1**	WR	1.35
S1-S1	WR	3.625	**S9-S2**	WR	1.35
S2-S3	WR	0.65	**S9-S3**	WR	2.85
S2-S2	WR	2.6	**S10-S1**	C	0.15
S2-S1	WR	2.6	**S10-S2**	S	1.425
S3-S2	WR	2.2	**S10-S3**	WR	0.975
S3-S1	WR	2.2	**S11-S1**	C	0.15

S4-S1	C	0.175	**S11-S2**	WR	1.2
S4-S2	WR	1.5	**S11-S3**	WR	1.2
S4-S3	WR	3.35	**S11-S4**	WR	2.725
S5-S1	C	0.15	**S14-S1**	C	0.25
S5-S2	WR	1.275	**S14-S2**	WR	1.2
S5-S3	WR	3.125	**S14-S3**	WR	2.725
S6-S1	C	0.15	**S15-S1**	WR	1.375
S6-S2	WR	2	**S15-S2**	WR	2.75
S6-S3	WR	3.5	**S17-S1**	WR	1.65
S8-S1	C	0.275	**S17-S2**	WR	1.65
S8-S2	WR	0.85	**S17-S3**	WR	3.4

Test pit and vertical profile samples were analyzed for total carbon and total sulfur content using an ELTRA CS-2000 carbon/sulfur analyzer, and paste-pH determinations were conducted according to ASTM standard D4972 (2007). The elemental composition of the samples was determined by energy dispersive X-ray fluorescence (ED-XRF) using a Panalytical Minipal 4 desktop ED-XRF analyzer. Mineral composition was studied using X-ray diffraction (XRD; using a PANanalytical Model Empyrean II diffractometer with a copper anode ($\lambda = 1.543$ Å)), optical microscopy (Nikon Eclipse LV100N-POL polarizing microscope with epi-illumination attachment), and scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS). Acid-base accounting (ABA) was performed using a modified Sobek method, which determined acid-producing potential (APP) using the total-S content and neutralizing potential (NP) using the total-C carbonate mineral content (Skousen *et al.*, 2002; Brady and Cravotta, 1992). The total-S in the samples was assumed to be sulfide minerals, and total-C was assumed to be carbonate minerals. Previously-established, site specific criteria were used to classify any waste material with neutralization potential ratio (NPR) < 1.5 as potentially acid generating (PAG) material. Waste rock with NRP > 1.5 was considered non-acid generating.

3. Results

3.1. Mineralogical and geochemical characterization of a deconstructed historic WRS

The whole-rock composition of WRS1 samples (Table 3) was consistent with the local host rock composition. Silica was the single largest component in the waste rock (average mass fraction = 56 %), with SiO_2 and various metal oxides (*i.e.*, Al_2O_3, CaO, Fe_2O_3, K_2O, MgO, MnO, Na_2O) accounting for > 99 wt. % of the samples. Trace metals cumulatively accounted for < 1 wt. % of the sample in most cases. The cover material, which was analyzed only at WRS3 by McNeill (2016), contained elevated concentrations of K and Na, slightly higher concentrations of SiO_2 (63 wt. %), and was generally deficient in Fe, S, and trace metals. The principal sulfide minerals identified at WRS1 were pyrite and pyrrhotite, with lesser amounts of chalcopyrite and covellite. Variable quantities (mostly < 5%) of sulfides (*i.e.*, pyrite, pyrrhotite) and carbonates (*i.e.*, calcite, dolomite) were present throughout the pile.

Table 3. Solid-phase mineralogy and concentrations of trace elements for WRS1 samples. Minimum, maximum, and (mean) values are shown for each parameter.

Parameter	Test pits	Profile excavation
SiO_2 (wt. %)	33 – 65 (56)	49 – 60 (55)
MgO (wt. %)	2 – 25 (9)	7 – 25 (17)
Fe_2O_3 (wt. %)	2 – 16 (10)	9 – 13 (11)
Al_2O_3 (wt. %)	2 – 11 (9)	7 – 11 (9)
CaO (wt. %)	3 – 11 (6)	4 – 8 (6)
Na_2O (wt. %)	0 - 3.1 (1.5)	0 - 2.1 (1)
K_2O (wt. %)	0.3 - 2.1 (1.1)	0.2 - 1.1 (0.7)
MnO (wt. %)	0 - 0.2 (0.1)	0.1 - 0.1 (0.1)
Cr (ppm)	112 – 1780 (592)	280 – 1977 (1143)
Cu (ppm)	52 – 1642 (480)	141 – 782 (391)
Ni (ppm)	23 – 504 (144)	104 – 688 (372)
Zn (ppm)	21 – 125 (71)	54 – 99 (80)
Pb (ppm)	1 – 13 (9)	7 – 10 (9)

Characterization of the stockpile material (Table 4) indicated that the S content ranged from 0.05 – 2.2 wt. % (*i.e.*, acid production potential (APP) of 1.4 – 69.3 kg $CaCO_3$ $tonne^{-1}$) with an average of 0.9 wt. % (*i.e.*, APP = 30.4 kg $CaCO_3$ $tonne^{-1}$). Total carbon content, assumed to be present as carbonate, ranged from 0.03 – 1.3 wt. %, with a mean of 0.4 wt. %. Neutralization potential (NP), assuming all carbon was present as carbonate, ranged from 0.3 – 111 kg $CaCO_3$ $tonne^{-1}$ and averaged 39.1 kg $CaCO_3$ $tonne^{-1}$. The NPR of samples ranged from 0.04 – 42. Test-pit samples were largely comprised of the cover material; greater NPR values were measured in the test-pit samples relative to profile excavation samples. Mean test pit NPR values were 8.1, whereas the mean of the profile excavation samples were lower with a value of 1.6 (Table 4). The NPR values indicated that approximately 62% of samples were considered PAG based on the NPR cut off value of 1.5. Acid-base accounting results were consistent with the results of paste-pH analyses that were conducted on the samples. The paste pH measurements were generally circum-neutral (*i.e.*, 6 to 8), with oxidized or partially oxidized samples (as indicated by red colouration) typically characterized by lower paste pH (*i.e.*, $\geq$ 2.5).

Table 4. Sulfur and carbon concentrations, and ABA results for WRS1 samples. Minimum, maximum, and (mean) values are shown for each parameter. Legend: APP = acid production potential; NNP = net neutralization potential; NP = neutralization potential; NPR = neutralization potential ratio.

Parameter	Test pits	Profile excavation
C (wt. %)	0.03 - 1.3 (0.4)	0.005 - 1.3 (0.4)
S (wt. %)	0.05 - 2.2 (0.8)	0.5 - 1.6 (1)
NP (kg $CaCO_3$ $tonne^{-1}$)	2.4 - 108 (34)	0.3 - 111 (39.1)
APP (kg $CaCO_3$ $tonne^{-1}$)	1.4 - 69 (26.6)	16.1 - 51 (31.5)
NNP (NNP=NP-APP)	-49.9 - 105 (7.3)	-37.5 - 94 (7.5)
NPR (NPR=NP/APP)	0.04 - 42 (8.1)	0 - 6.8 (1.6)

3.2. Microbiological characterization of a deconstructed historic WRS

Measurements of carbon and sulfur were conducted on samples for microbiological characterization to ensure that the samples contained carbon and sulfur contents that were representative of the bulk sample set. Carbon and sulfur contents of the microbiological samples ranged from 0.1 to 1.3 wt. % (mean = 0.6 wt. %) and 0.1 to 2.4 wt. % (mean = 1.0 wt. %),

respectively. Viable populations of nSOM, aSOM, and aIOM were detected by MPN throughout WRS1 (Fig. 3). The most numerous culturable populations were those of nSOM, which ranged from 3.9 x 10^2 (paste pH = 5.72) to 4.0 × 10^6 bacteria g^{-1} of waste rock (paste pH = 2.48). Excluding the results from cover samples, the mean and median density of nSOM in the waste-rock samples were 5.5 x 10^5 and 8.4 x 10^4 bacteria g^{-1}, respectively. Acidophilic IOM had moderate to low bacterial abundances (*i.e.*, < 1.0 x 10^4 bacteria g^{-1}) at most locations, but had extremely high bacterial density in many of the samples recovered from highly oxidized zones (maximum = 1.6 x 10^7 bacteria g^{-1}, paste pH = 3.65). The mean and median aIOM densities were 9.0 x 10^5 and 5.3 x 10^2 bacteria g^{-1}, respectively; mean and median densities decreased to 2.7 x 10^3 and 1.0 x 10^2 bacteria g^{-1}, respectively, if oxidized samples were excluded. Acidophilic SOM had the lowest population density of cultivated species across the sample set, with the mean and median densities of 6.6 x 10^2 and 1.6 x 10^2 bacteria g^{-1}, respectively. The maximum recorded abundance of aSOM was 4.0 x 10^3 bacteria g^{-1}, detected in the most acidic sample (paste pH = 2.48).

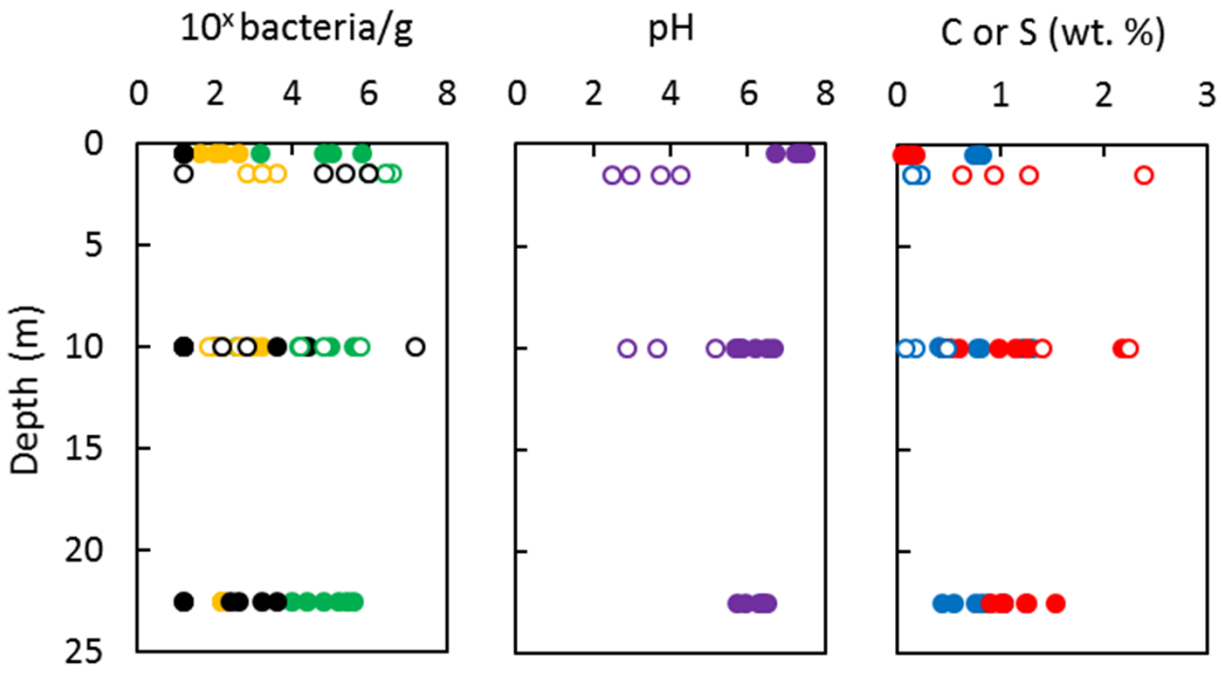

Fig. 3. Most probable number (MPN) counts of (●○) neutrophilic sulfur-oxidizing (nSOM), (●○) acidophilic sulfur-oxidizing (aSOM), and (●○) acidophilic iron-oxidizing microorganisms (aIOM), compared to (●○) pH, (●○) solid-phase carbon and (●○) sulfur contents. Legend:

unfilled symbols indicate samples recovered from highly oxidized zones (based on sample colour). Stated depths are indicative, due to spatial variations within WRS1.

The microbiology samples from WRS1 were characterized by a high diversity of species. 23 genera (Supplementary Table S1) accounted for a significant (> 1%) contribution of genetic material in at least one sample. Only two genera, both of which are sulfur oxidizers, accounted for > 5% of genetic material cumulatively across the data set. *Thiobacillus* was the most significant genus, providing 12% of the total reads among all samples. *Acidithiobacillus* contributed to 7% of the total bacterial amplicons. Several of the most represented genera were common soil microbes regularly found in waste-rock stockpiles (*e.g.*, *Arthrobacter*, *Sphingomonas* or *Dokdonella;* Eschbach *et al.*, 2003, Schippers *et al.*, 2010). The acidophilic iron oxidizer *Leptospirillum* and heterotrophic *Pseudomonas*, which also catalyzes Fe^{2+} oxidation, are often encountered in waste-rock environments (Schippers *et al.*, 2010), and accounted for ~ 3% of total reads in the WRS1 samples. The remaining main genera were assorted soil microorganisms, and accounted for < 3% of the total reads.

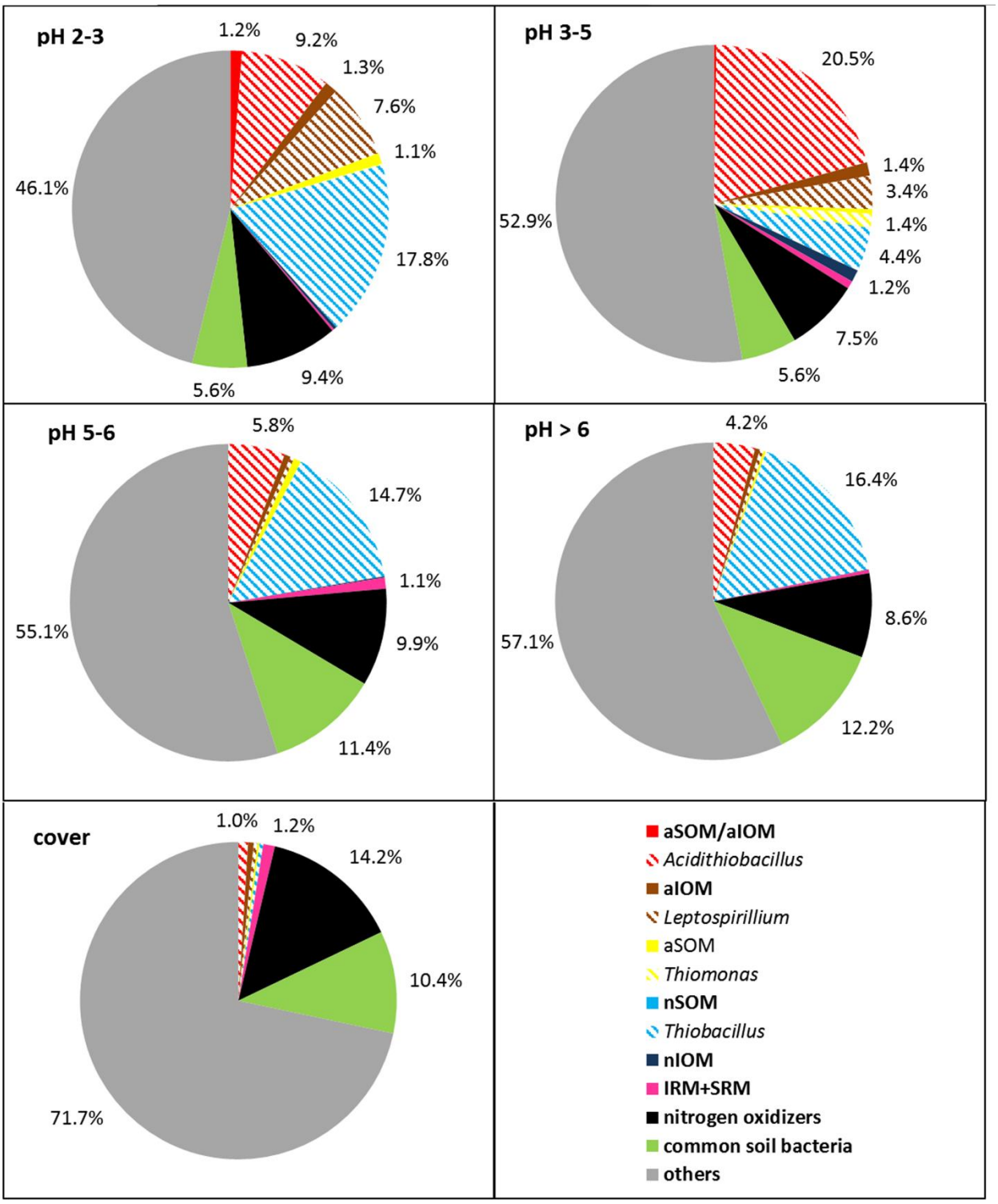

Fig. 4. Bacterial ecology of pH 2-3, pH 3-5, pH 5-6, pH > 6 waste-rock samples, and samples of the cover materials, collected from WRS1 at the Detour Lake Mine, determined by high-throughput amplicon sequencing of the 16S rRNA gene. Legend: aSOM/aIOM = acidophilic

sulfur and iron oxidizers (plotted in red); aIOM = acidophilic iron oxidizers (in brown); aSOM = acidophilic sulfur oxidizers (in yellow); nSOM = neutrophilic sulfur oxidizers (in blue); nIOM = neutrophilic iron oxidizers (in dark blue); SRM = sulfur and sulfate reducers, and IRM = iron reducers (both in pink); nitrogen oxidizers (in black); common soil bacteria (in green). The genera which do not belong in the previous categories have been grouped together, and are plotted in grey. Genera with highest relative abundances within aSOM/aIOM, aIOM, aSOM, and nSOM are highlighted. Labels are shown only for relative abundances > 1%.

The bacterial taxonomy of the samples (Fig. 4) fell into three general categories correlated to the spatial origin and paste pH of the samples. The first category was comprised of the soil samples recovered from the cover material. The bacterial taxonomy of the cover samples was typical of most soils, and consisted of a large diversity of non-dominant organisms (*i.e.*, comprising < 10% of total amplicons), with very little relative contribution of iron- and sulfur-oxidizing genera. The second category was comprised of waste-rock samples collected from unoxidized zones in the stockpile; samples collected from these locations were characterized by a high diversity of organisms, including significant populations of both acidophilic and neutrophilic SOM and IOM. The third category was comprised of waste-rock samples collected from oxidized zones in the stockpile; these samples were distinguishable from the cover samples by higher proportions of *Thiobacillus* and *Acidithiobacillus*. The microbial communities in the unoxidized zones were dominated by *Acidithiobacillus*, compared to *Thiobacillus* in oxidized zones. Figure 3 illustrates these trends by showing the bacterial taxonomy of the samples recovered from WRS1 based on the pH levels.

The majority of fungal DNA amplicons in each sample was contributed by genera which accounted for < 1% of total reads. The second largest contributor of DNA was from fungal species which have yet to be classified into appropriate genera (10% of total reads). *Pycnopeziza* (9.2%), *Leptosphaeria* (6.1%), *Tetracladium* (5.8%), *Cucurbitaria* (5.3%), *Sclerotinia* (4.7%), *Chaetomium* (4.4%), *Chytridiomycota* (3.6%), and *Anguillospora* (3.2%) were among the most dominant genera at WRS1; each of these genera accounted for > 10% of total amplicons in several samples.

4. Discussion

Zones of highly weathered waste rock were detected throughout the WRSs at DLM (Cash, 2014; McNeill, 2016). The degree of oxidation of the waste rock within test pits and profiles investigated at the WRSs was highly variable, and oxidation was observed more frequently and to a greater extent on WRS1 compared to WRS2 (Cash, 2014). A majority of waste-rock samples recovered from the historic WRSs had a circum-neutral paste pH of 6-8, and approximately 20% of the samples had a paste pH 2.5-4. Cover samples were of neutral pH (Cash, 2014; Cash *et al.*, 2014).

Waste-rock stockpiles are generally highly heterogeneous and anisotropic, and samples collected from the historic WRSs at DLM were heterogeneous with respect to grain size, texture, extent of oxidation, and abundance of sulfide minerals (Cash, 2014). In contrast, the overall microbial diversity in WRS1 (investigated on the family level; data not shown) was relatively homogeneous across the samples analyzed, although a clear division was observed between samples of waste rock versus samples of cover material. The major difference between the two sample groups was a significantly greater abundance ($P > 0.05$) of the family *Hydrogenophilaceae*, which contains solely thermophilic species, in the waste-rock samples. The sums of relative abundances of sulfur and iron oxidizers, determined by high-throughput amplicon sequencing, negatively correlated with paste pH, and accounted for 38.7%, 33.2%, 22.5%, and 21.8% in samples with paste pH values of 2-3, 3-5, 5-6, and > 6, respectively. Abundances of acidophilic sulfur- and iron-oxidizing genera increased with decreasing paste pH, except in samples with paste pH values of 2-3, wherein the ratio of neutrophilic and acidophilic genera was approximately 1:1, due to a great abundance of the neutrophilic *Thiobacillus* spp.). As expected, generally low IOM/SOM abundance (*i.e.*, 2.5% of total amplicons) was detected in the cover.

High-throughput amplicon sequencing has been used in other studies to investigate microbial communities in mine-waste environments; however, most reports focused on mill tailings (*e.g.*, Liu *et al.*, 2014; Bruneel *et al.*, 2017), rather than on waste rock (Blackmore *et al.*, 2018). Many reports which describe microbial populations in mill tailings identified the predominance of typical leaching bacteria, such as *Acidithiobacillus, Leptospirillum, Sulfobacillus*, or *Ferroplasma*, at abundances reaching tens of percent (*e.g.*, Liu *et al.*, 2014; Diaby *et al.*, 2015;

Kwon *et al.*, 2015; Xiao *et al.*, 2016; Bruneel *et al.*, 2017); these findings are consistent with the observations made through this study. The overall viable counts of IOM and SOM at WRS1 were within the range reported at similar sites. Schippers *et al.* (2010) reviewed microbiological studies of mine wastes from eight different countries, and reported maximum nSOM density ranging from $< 10^2$ to 1.2×10^7 bacteria g^{-1}, maximum IOM density ranging from 2.8×10^2 to 3.1×10^8 bacteria g^{-1}, and maximum aSOM densities ranging from 5.2×10^3 to 1.2×10^7 bacteria g^{-1}. The maximum recorded abundance of both nSOM and IOM at WRS1 fell within the range reported by Schippers *et al.* (2010), whereas the maximum counts of aSOM at WRS1 (4.0×10^3 bacteria g^{-1}) was slightly lower than the range reported for other sites. The relative abundances of nSOM, aSOM, and IOM at WRS1 were similar to the distribution of these bacteria at other neutral-pH waste-rock sites. Dockrey (2014) evaluated the distribution of iron- and sulfur-oxidizing bacteria within waste-rock test piles at the Antamina Mine, Peru, and reported that *T. thioparus* (nSOM) was the dominant organism, with an abundance $> 10^6$ bacteria g^{-1} throughout the test piles. The low overall abundances of *At. thiooxidans* (aSOM; usually undetected, one sample $> 10^7$ bacteria g^{-1}) and *At. ferrooxidans* (aSOM/aIOM; usually undetected, one sample $> 10^6$ bacteria g^{-1}) at the Antamina Mine was possibly due to the recent (*i.e.*, < 5 years) deposition of the waste rock; extensive acidic microenvironments, in which the aSOM and aIOM may thrive, had not yet been established due to the recent deposition of waste rock (Dockrey, 2014). The microbiology of WRS1 was also very similar to several neutral-pH tailings ponds in North America. For example, greater relative DNA contribution of nSOM over aSOM observed in WRS1 was similar to the distribution and range of abundance for these species in tailings at the Agnico Eagle Joutel Mine, QC, Canada (Blowes *et al.*, 1998) and the Greens Creek Mine, AK, USA (Lindsay *et al.*, 2009), where nSOM abundances were six and two orders of magnitude larger than aSOM, respectively. IOM were overall more abundant at WRS1 than at most neutral-pH sites, although several studies have shown that aIOM are capable of living in neutral-pH conditions. IOM populations were generally larger in acidic samples at WRS1, as with other sites (*e.g.*, Southam and Beveridge, 1992; Dockrey, 2014; Blowes *et al.*, 1998).

In agreement with the sequence data, MPN enumerations arranged in order of increasing paste pH indicate a negative correlation between the densities of each of the three enumerated microbial groups and paste pH. All of the enumerated species had higher population densities in oxidized samples with paste pH < 5.5. Large variations in IOM (*i.e.*, 2-5 orders of magnitude),

and nSOM and aSOM (both groups 1-2 orders of magnitude) densities between samples with similar paste pH values indicated that, although paste pH was not an absolute indicator for the abundance of these species, a weak correlation with bacterial diversity was present.

Several genera of fungi were identified in the waste rock in WRS1, including *Pycnopeziza, Leptosphaeria, Tetracladium*, and *Cucurbitaria*. None of the fungal genera encountered were ubiquitous or have been shown to impact pore-water geochemistry through sulfide oxidation. A member of the genus *Tetracladium* was observed as an early colonizer in a surface-water system impacted by the catastrophic release of high-pH and metal-rich bauxite residue (Vass *et al.*, 2013), indicating potential resistance within this genus to extremes in pH and metal concentrations. Members of the genus *Leptosphaeria* are known to detoxify cyanide compounds to formamide using a cyanide hydratase enzyme (*e.g.*, Sexton and Howlett, 2000). The use of cyanide for gold extraction at the DLM may explain the presence of *Leptosphaeria* in the mine-waste environment at this site. Minimal information regarding the genera *Pycnopeziza* and *Cucurbitaria* in the context of sites impacted by extreme pH or metal concentrations exists in the literature; the occurrence of these genera in mine-waste systems is not believed to have been reported previously.

The presence and abundance of bacterial species involved in dissimilatory sulfur and iron oxidation support the ongoing occurrence of microbially-catalyzed sulfide oxidation within the covered WRS1. Previous studies have identified the establishment of acidic microenvironments and sulfide-mineral surfaces under bulk circum-neutral pH (*e.g.*, Mielke *et al.*, 2003; Dockrey *et al.*, 2014). Acidophilic iron- and sulfur-metabolizing species thrive in these low-pH microniches and catalyze the biogeochemical redox cycling of iron and sulfur, with potential impacts on ARD generation. Detection of sulfide minerals with varying levels of oxidation by McNeill (2016) are consistent with the microbiological observations presented in this study. Observations made by McNeill (2016) emphasized the importance of a more comprehensive and intact cover system to restrict air flow into WRS to minimize recharge of atmospheric O_2 and the generation of ARD.

Two additional WRS at the DLM were investigated, including one with an intact cover (WRS3), and one with an incomplete cover (WRS4). WRS3 was characterized by decreasing pore-gas O_2 concentrations at depth below the cover material, whereas atmospheric O_2 concentrations were

observed throughout the WRS4 (McNeill, 2016). Elevated sulfate concentrations in excess of 1,000 mg L^{-1} were observed in WRS4 (Cash, 2016), indicating that substantial sulfide oxidation has occurred. Numerical simulations, consistent with measurements of pore gas and pore pressure, indicated that the advective flux of O_2 through the intact cover and into the interior of WRS3 was approximately 100 times higher than diffusive fluxes (Steinepreis *et al.*, 2018; Steinepreis, 2017). It is evident that the thin soil covers applied to WRS1 and WRS3 were not sufficient to completely mitigate sulfide oxidation, and may be explained by the existence of defects (*i.e.*, cracks or thin areas) in the cover. Use of a thicker cover system comprised of low-permeability material (*i.e.*, a clay-rich material) is anticipated to reduce the recharge of atmospheric O_2, and the oxidation of sulfide minerals, within WRS1.

5. Conclusion

The biogeochemical evolution of waste-rock piles and the long-term potential for ARD generation has received limited attention in the literature to date. The characterization of waste rock that was emplaced more than two decades prior to sampling activities conducted through the present study indicate that sulfide oxidation is ongoing, as evidenced through the development of zones characterized by acidic paste-pH values and increased abundance of acidophilic taxa. Microbiological analyses indicated abundances of iron and sulfur oxidizers, and confirmed the dominance of nSOB within the WRS. Oxidized and acidic waste-rock samples were characterized by a community shift toward dominance of acidophilic taxa. The broader research program, which integrated hydrogeological, geochemical, microbiological, and physical characterization, demonstrated that the thin and/or discontinuous, monolayer soil covers used for the WRS were not sufficient to completely inhibit sulfide oxidation, but limited the advective transport of atmospheric oxygen into the WRS, thereby slowed acid generation. It is important to note that, despite the presence of localized acid-generating conditions, WRS drainage was neutral overall.

Abbreviations

ABA	acid-base accounting
aIOM	acidophilic iron-oxidizing microorganisms
ARD	acid rock drainage
APP	acid production potential

a/nSOM	acidophilic/neutrophilic sulfur-oxidizing microorganisms
At.	*Acidithiobacillus*
bTEFAP	bacterial tag-encoded FLX amplicon pyrosequencing
DLM	Detour Lake Mine
ED-XRF	energy dispersive X-ray fluorescence
MPN	most probable number
MRS	mine-rock stockpile
NNP	net neutralization potential
NP	neutralization potential
NPR	neutralization potential ratio
PAG	potentially acid generating
SEM-EDS	scanning electron microscopy with energy dispersive spectroscopy
T.	*Thiobacillus*
WRS	waste-rock stockpile
XRD	X-ray diffraction

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