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Microbiology of a multi-layer biosolid/desulfurized tailings cover on a mill tailings impoundment

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ABSTRACT

The Strathcona Waste Water Treatment System (SWWTS; Sudbury, ON, Canada) has received mill tailings from Ni/Cu ore processing from 1970 to present. Demonstration-scale, multi-layer cover systems were installed on selected tailings deposition cells at the SWWTS. The cover systems are comprised of an upper layer of organic carbon-rich material, composed of a layer biosolids fertilizer along with composted municipal food and yard waste, then a layer of desulfurized, fine-grained tailings. Organic carbon components used in these covers promote microbial communities that consume O_2, thus decreasing sulfide oxidation rates in the underlying tailings. The aim of this study was to investigate the microbiology of the cover systems and the underlying tailings, using a combination of culture-dependent (most probable number) and culture-independent (16S rRNA gene amplicon sequencing) techniques, and assess the impact of the organic component of the cover system four to six years after implementation. Most tailings samples were characterized by circumneutral bulk pH and low concentrations of dissolved metals. The presence of the organic cover resulted in elevated counts of sulfate-reducers (by two orders of magnitude, compared to control samples) immediately below the organic cover, as well as an increased abundance of heterotrophic species (~10^8 cells g^{-1}) at greater depth (~4 m) in the tailings profile. Mineral-oxidizing microorganisms were also present in the tailings, with neutrophilic sulfur-oxidizers dominating the samples (mean ~10^6 cells g^{-1}). Relative abundances of sulfur- and/or iron-oxidizers determined by sequencing ranged from 0.5 to 18.3% of total reads (mean ~5.6% in amended tailings) and indicated the presence of local microenvironments with ongoing sulfide oxidation. This work provides a detailed characterization of the microbiology of a tailings impoundment with an organic cover, highlighting the opportunities associated with monitoring microbial processes in such remediation systems.

1. Introduction

Metal mining and mineral processing generate large quantities of fine-grained gangue residue (mill tailings) that is commonly rich in iron sulfides. Typically, mill tailings are disposed of in impoundments at mine sites. Oxidation of sulfide minerals can lead to the production of acid mine drainage (AMD), which contains high concentrations of dissolved sulfate, iron, and other metal(loid)s (e.g., Skousen et al., 2019). Direct oxidation of sulfide minerals by O_2 and indirect oxidation by Fe^{3+} is greatly accelerated in the presence of iron- and sulfur-oxidizing microorganisms (Hedrich and Schippers, 2020; Johnson and Hallberg, 2003). Lithotrophic sulfur (Ghosh and Dam, 2009) and iron oxidation (Singh et al., 2018; Hedrich et al., 2011) by taxonomically and ecologically diverse bacteria and archaea are reviewed elsewhere. Neutrophilic mineral-oxidizers are often detected in neutral-pH mine wastes (e.g., McNeill et al., 2020), while acidophilic species dominate in acidic mine wastes (Hedrich and Schippers, 2020; McNeill et al., 2020; Johnson and Hallberg, 2003).

Physical, chemical, phytoremediation, and bioremediation approaches can be used to restore mill tailings impoundments. The time

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required, economic factors, and physicochemical properties are considered when selecting a suitable approach for a mine site reclamation. The advantages and disadvantages of each technology type are described in detail elsewhere (Skousen et al., 2019; Sun et al., 2018). AMD formation can be prevented or mitigated by different cover systems that limit contact between the mill tailings and O_2 (e.g., Pabst et al., 2018). Dry covers, serving as barriers to O_2 or H_2O transport, are commonly used for *in situ* remediation of heavy metal-contaminated mine wastes (e.g., Anawar et al., 2015; Pepper et al., 2012; Cousins et al., 2009; Hallberg et al., 2005; Peppas et al., 2000; Haering et al., 2000). The incorporation of organic carbon components in cover systems promotes O_2 consumption by microbial communities before it reaches the tailings, thus decreasing sulfide oxidation rates. Accelerated microbial activity can improve physicochemical properties of soils disturbed by surface mining and mine wastes (Campbell et al., 2017; Alvarenga et al., 2013; Gardner et al., 2010; Brown et al., 2003). Several studies have investigated enzymatic activity (Touceda-Gonzalez et al., 2017; Zornoza et al., 2012; de Vareness et al., 2010; Perez de Mora et al., 2005, 2006), microbial counts (Gardner et al., 2010), phospholipid fatty acids (Baker et al., 2011), or microbial community structure (Touceda-Gonzalez et al., 2017; Pepper et al., 2012; Perez de Mora et al., 2006) in heavy metal-contaminated mine wastes. However, most studies investigating the effects of biosolids applications at mine tailings sites have been short term (<5 years; Touceda-Gonzalez et al., 2017; Gardner et al., 2010, 2012; Brown et al., 2003), and few studies have focused on the longer-term (>5 years) effects of organic covers on reclaimed mine wastes (Antonelli et al., 2018; Nason et al., 2014; Trlica and Teshima, 2011).

Sudbury Integrated Nickel Operations (Sudbury INO) encompasses a currently operational mining and milling site owned by Glencore that is located adjacent to the community of Onaping (City of Greater Sudbury, ON, Canada). The Strathcona Waste Water Treatment System (SWWTS) began receiving tailings from ore processing in 1968 (Blowes et al., 1996; Bain et al., 1998; Bain and Blowes, 2013) and is one of the largest tailings containment areas in the Sudbury region (MEND, 1997). During the initial years of milling, unsegregated tailings (~15 wt% S, mainly in the form of pyrrhotite ($Fe_{(1-x)}S$)) were deposited in the impoundment. Active deposition of tailings concluded in 2012, and demonstration-scale, multi-layer cover systems were installed in selected cells. These covers are composed of an upper 0.5 m layer of organic material over a thick layer (1–3 m) of lime-stabilized, fine-grained desulfurized mill tailings (containing 0.4 to 1 wt% S) deposited from 1996 until 2012 (EcoMetrix Incorporated, 2014). The Strathcona covers have been in place for up to 7 years, providing an opportunity to evaluate the impacts of the cover systems on the hydrology, water quality, and microbiology within the cover and underlying tailings.

Although mine waste remediation depends on the presence and activity of microorganisms, data describing the long-term effects of organic matter on microbial communities that affect the performance of cover systems in mitigating sulfide oxidation are lacking. The aim of this study was to assess the composition, diversity, and function of bacterial and archaeal communities (BACs) in samples recovered from two cells that include multilayer covers composed of organic carbon and desulfurized tailings components, then compare these observations to a control location with a desulfurized tailings cover without an organic carbon component.

High-throughput amplicon sequencing of the 16S rRNA genes was used to analyze entire BACs, as well as assess relative abundances of microorganisms catalyzing dissimilatory oxidation-reduction of sulfur and iron. Culturable acidophilic and neutrophilic microorganisms were enumerated using a culture-dependent technique, including: (i) acidophilic iron- (aIOM) and sulfur-oxidizing microorganisms (aSOM); (ii) neutrophilic sulfur-oxidizing microroganisms (nSOM), heterotrophs (nHET), iron-reducing microroganisms (nIRM), and sulfate-reducing bacteria (nSRB). Functions of each group within the cover systems and underlying tailings are illustrated in a schematic diagram (Supplementary Fig. S1). The detailed microbiological data were compared with the results of geochemical analyses performed within an ongoing broader study.

The novelty of this work lies in bringing together two complementary microbiology approaches (culture-dependent and culture-independent) to thoroughly describe the microbial community structure within the multilayer cover system and underlying tailings. Moreover, the current study provides an unprecedented, detailed evaluation of the performance of the cover system at the SWWTS using microbiological investigations. The data obtained contribute to knowledge regarding microbial processes within a tailings management system several years after installation of a dry cover system incorporating a biosolids component. The results of this research also demonstrate that microbiological analyses can provide a useful and sensitive tool for assessing the performance of cover technologies to remediate mine wastes.

2. Material and methods

2.1. Site and sampling

The vertical profile at the SWWTS includes a layer of desulfurized tailings overlying older (partially oxidized) sulfide-rich tailings. In Tailings Cell 1 (Fig. 1), the desulfurized tailings were covered with a ~0.5 m organic cover (~3.5 ha ML33 in 2014 and ~0.9 ha ML34 in 2012), composed of compost mixed with a locally produced biosolids fertilizer. The compost is a product of composting 10% source-separated organics (municipal food waste), 30% leaf waste, 30% yard waste, 10% aged bark, and 10% small sticks (approximately 1.5 cm × 5 cm) (Tisch et al., 2008). The biosolids fertilizer is a soil amendment produced from wastewater treatment residuals (biosolids) mixed with cement kiln dust and/or quicklime. Two locations within Tailings Cell 1 (ML33 and ML34; Fig. 1), as well as a control location without an organic cover (ML5 within Tailings Cell 2a; Fig. 1) were sampled for microbiological and geochemical characterization.

Core samples of the tailings were collected in September 2018 to a depth of approximately 4.5 m using the method described by Starr and Ingleton (1992). The core samples were stored at 4 °C and transported to the laboratory for further processing. Subsamples (Table 1) were collected from opened cores into sterile 50-mL tubes, stored at 4 °C, and processed for the most probable number (MPN) within 7 days. Another set of samples was frozen (20 °C) and later used for total DNA extractions.

2.2. Enumerations of culturable microorganisms

Groups of culturable microorganisms were enumerated using the most probable number (MPN) technique (Cochran, 1950; Garthright and Blodgett, 2003). Anaerobic cultivations were performed (in triplicate) as described by Pakostova et al. (2020a). In short, SRB were cultivated in a modified Postgate C medium (pH ~7.5; Postgate, 1963), containing 2.92 g L^{-1} Na lactate (60%) and 1.28 g L^{-1} Na acetate, and supplemented with an anaerobic indicator (resazurin). IRM were enumerated in a liquid basal medium supplemented with 1.5 g L^{-1} peptone as a carbon source (pH ~7.0; Gould et al., 2003). After 5 weeks of anaerobic incubation, SRB cultures were inspected for precipitation indicating biogenic H_2S production by sulfate reduction, while iron reduction was determined by Fe^{2+} formation detected by the ferrozine reagent (Stookey, 1970).

MPN enumerations of nSOM were performed in five replicates under aerobic conditions in a filter-sterilized basal salt medium (amended ATCC medium 450 T2; pH ~7.0) containing (in g L^{-1}): NH_4Cl (0.1), KH_2PO_4 (3.0), $MgCl_2{\cdot}6H_2O$ (0.2), $CaCl_2{\cdot}2H_2O$ (0.13), and $Na_2S_2O_3{\cdot}5H_2O$ (5.0). The samples were incubated for 27 days in the dark. A pH decline of ≥0.5 unit was considered a threshold to determine nSOM activity (Orion Combination pH Electrode, Orion Star A321 pH

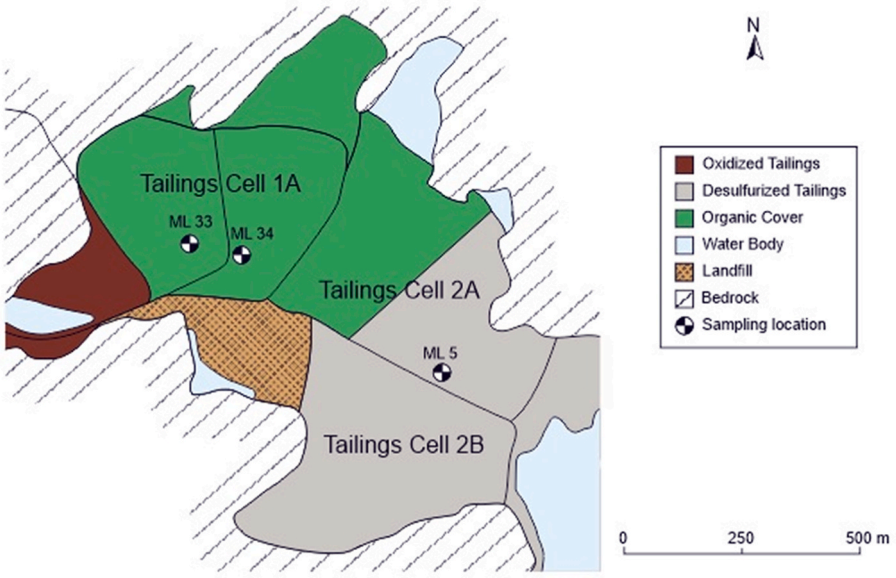

Fig. 1. Site map of the Strathcona Tailings area within the Strathcona Waste Water Treatment System (SWWTS) area showing the three locations (ML33, ML34, and ML5) sampled for detailed microbiological characterization.

Table 1
Summary of environmental samples collected from three locations within the SWWTS; the ML33 and ML34 sites were covered with an organic cover while the ML5 site served as a control site (i.e., no organic cover). The microbial diversity was analyzed using high-throughput amplicon sequencing of the 16S rRNA gene. Samples in which metabolically active groups of microorganisms were also enumerated using MPN are in bold. † m below ground surface. * cored, sub-sampled, and analyzed in duplicate.

Sample type	Depth (mbgs†)		
	ML33 site	ML34 site	ML5 site
Organic cover	0.15	**0.21**	n.a.
	0.30*		
	0.40		
Oxidized desulfurized tailings	**0.73***	0.40	**0.21**
		0.62	
Unoxidized desulfurized tailings	**1.15***	2.00	**1.10**
	2.20*	**2.71**	2.20
Historical tailings	**2.80***	3.20	3.20
	3.20*	**4.37**	**4.28**
	3.80	n.a.	n.a.

Portable Meter; Thermo Scientific, USA). Acidophilic oxidizers were enumerated by plating serially diluted samples onto selective solid overlay media (Johnson and Hallberg, 2007): aIOM onto the iFeo medium and aSOM onto a modified FeSo medium (containing 500 μM Fe^{2+} in the overlay). To enumerate heterotrophs, serially diluted samples were plated onto R2A agar (Sigma Aldrich, USA). Plates were incubated for 20 days for acidophiles and 5 days for heterotrophs, after which colonies were counted. All (aerobic and anaerobic) cultivations were performed at laboratory temperature (~23 °C) without agitation.

2.3. DNA extraction and Illumina MiSeq sequencing

Prokaryotic populations were analyzed using high-throughput amplicon sequencing of 16S rRNA genes (details provided in Pakostova et al., 2020b). In short, DNeasy PowerSoil Kit (Qiagen Inc., Germany) was used to extract total genomic DNA in duplicate from solid-phase samples, following the manufacturer's protocol. The V4 region of 16S rRNA genes was amplified using the modified universal primers 515F/806R (Walters et al., 2015), after which Illumina MiSeq sequencing of the amplicons was performed, both by Metagenom Bio Inc. (Toronto, Canada).

Sequence data were analyzed using mothur software (v.1.39.5, updated: 3/20/2017; Schloss et al., 2009), and the mothur MiSeq Standard Operating Procedure (Kozich et al., 2013). One out of a total of 55 samples that contained <12,000 sequences was removed prior to duplicate merging. A detailed description of sequence data processing procedures can be found in Pakostova et al. (2020b). The updated release 132 (for mothur; downloaded March 18, 2019) of the Silva database was used in this study. The statistics tools applied are as follows. Good's coverage (calculated for an operational taxonomic unit (OTU) definition of 0.03) was generated to estimate the portion of total species represented in each sample. The Gini-Simpson diversity index and Chao1 richness estimator were chosen to assess α-diversity for each library (sites with and without organic cover components). Significance of differences in α-diversity between libraries was determined using the Wilcoxon rank sum test. Weighted UniFrac (Lozupone and Knight, 2005) was applied to investigate β-diversity, and three-dimensional non-metric multidimensional scaling (3D-NMDS) was used for visualization. Diversity within groups (sites or layers) was compared using AMOVA (analysis of molecular variance; Martin, 2002). Relative abundances of sulfur- and/or iron-metabolizing prokaryotes were obtained by screening the taxonomy file for genera containing at least one species with the investigated metabolic trait. Student's t-test was applied to test for statistical significance between relative abundance means as well as between MPN means.

2.4. Geochemical analyses

pH measurements of pore-water samples were taken using an Orion Ross Ultra combination pH electrode coupled to an Orion 3 Star pH/mV meter. Aqueous samples were filtered (0.22 μm, polyvinylidene fluoride (PVDF)) and stored at 4 °C prior to chemical analyses. Inductively coupled plasma-optical emission spectrometry (ICP-OES ICAP 6000, Thermo Scientific; U.S. EPA Method 6010C, 2000) and inductively coupled plasma-mass spectrometry (ICP-MS X Series II, Thermo Scientific; U.S. EPA Method 6020A, 1998) were used to determine cation concentrations (in samples preserved in HNO_3; pH < 2.0). Anions were determined by ion chromatography (Dionex IC-CO3 system; U.S. EPA Method 300.0, 1993). Aqueous samples (filtered through 0.22-μm PVDF membranes, acidified with H_2SO_4 to pH ~2.0, and stored in glass) were analyzed for dissolved organic carbon (DOC) with an Aurora 1030W

TOC Analyzer (O.I Analytical - College Station, TX) using wet oxidation with heated sodium persulfate. Inorganic carbon was removed from the sample by the instrument with the addition of 5% H_3PO_4. Levels of gaseous oxygen were determined in the field in gaseous samples collected at regular depths using a QUANTEK 902P O_2/CO_2 analyzer. Solid-phase samples were analyzed for total carbon and total sulfur contents (ELTRA CS-2000 carbon/sulfur analyzer coupled with an induction furnace CS800).

3. Results

3.1. Overview of geochemistry data

Samples consisting of organic cover material and tailings collected from the SWWTS have been characterized in detail within a broader initiative. Selected geochemical parameters of extracted pore-water and gaseous- and solid-phase samples (corresponding to samples used for microbiological analysis) are listed in Table S1. Most samples used for microbiological analyses (Tables 1, S1) had a pH that was circumneutral (6.4–7.8) or moderately acidic (lowest recorded value ~4.8; Table S1). Dissolved sulfate concentrations ranged from 1.0 to 3.5 g L^{-1} (2.3 ± 0.6 g L^{-1}, mean ± s.d.). Total dissolved iron concentrations were low (<5.5 mg L^{-1} in most samples, with the exception of 10 samples all located within the historical tailings at these locations (maximum concentration 51.2 mg L^{-1}). Most transition metals (e.g., Ag, Al, As, Cd, Co, Cr, Cu, Mo, Zn) were detected with mean concentrations <2.0 mg L^{-1}, except at a depth of 0.65 mbgs at ML33 where concentrations as high as 11.7 mg L^{-1} were recorded (McAlary, 2021). Only Ni and Mn were detected at elevated (>2.0 mg L^{-1}) levels, with mean concentrations of 10.5 and 2.5 mg L^{-1}, respectively. DOC values varied greatly across samples, ranging from 5.5 to 225 mg L^{-1}.

Total solid-phase sulfur content of the samples collected at ML33 and ML34 ranged from 0.4 wt% in the shallow tailings, which had undergone extensive sulfide oxidation, to 1.9 wt% in the historical tailings (Table S1). At ML5, the sulfur content ranged from 2.1 wt% at the tailings surface to 11.6 wt% at a depth of 4.3 m, reflecting previous deposition of a high-sulfur material within Tailings Cell 2. Total solid-phase carbon content in the tailings samples ranged from 0.05 to 0.3 wt% (0.14 ± 0.09 wt%). Dissolved oxygen (DO) data for most of the SWWTS depth profiles were not obtained. However, the available DO values, determined from measured gaseous O_2 concentrations, decreased with depth (at ML33 and ML34 where more values were obtained, compared to ML5), with values ranging from 9.52 mg L^{-1} near the tailings surface to 0.05 mg L^{-1} at a depth of 1.1 mbgs at ML5 (corresponding to gaseous O_2 concentrations decreasing from 19.7% near the tailings surface to 0.1% O_2 at depth).

3.2. Viable cell counts

Fig. 2 shows viable counts of selected metabolic groups of microorganisms at two locations with organic carbon layers in the cover system (M33 and M34) and the control site (ML5), where the cover consists of desulfurized tailings only. Differences between the locations with covers and the control site were not significant ($p > 0.05$; *t*-test), and culturable neutrophilic groups of microorganisms dominated over acidophiles in all tailings samples. High numbers of nSOM (mean ~10^6 MPN g^{-1}) indicated high rates of sulfur oxidation in tailings collected at all three locations. Iron-reducers were observed in greater numbers at the sites with organic carbon components when compared to the control site, however, the differences between the means (ML33 vs. ML5; ML34 vs.

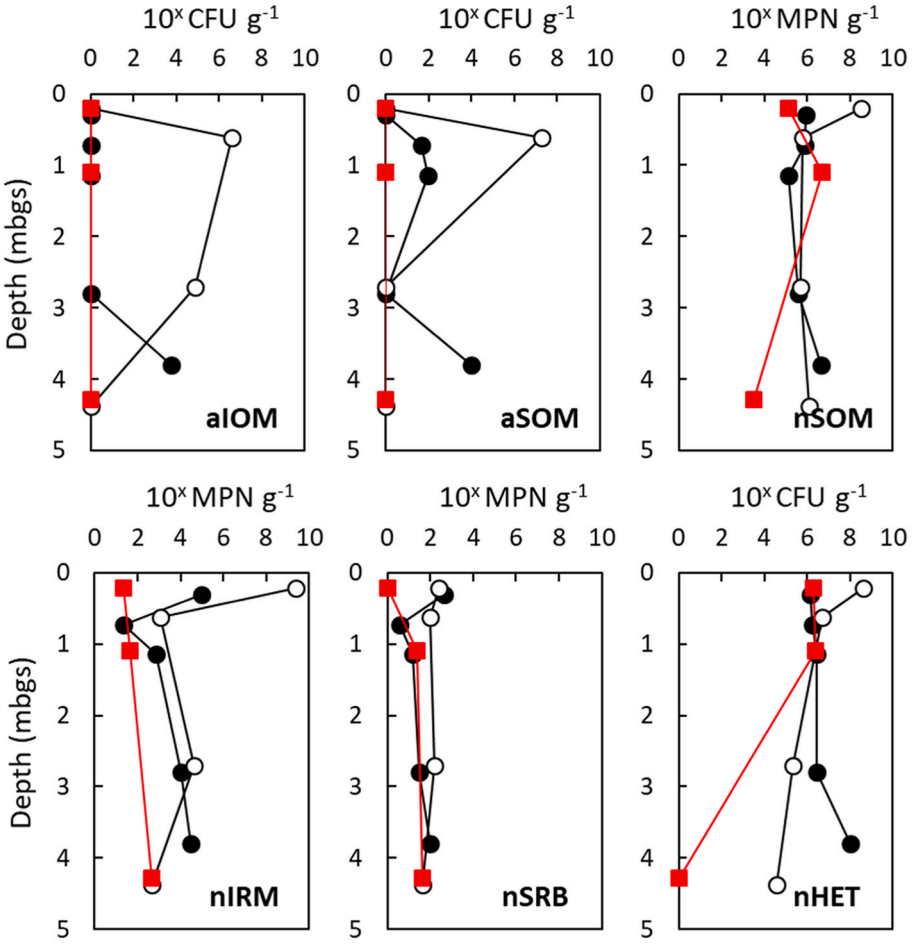

Fig. 2. Vertical profiles for microbial counts in solid-phase samples collected at SWWTS, determined by most probable number (MPN) in liquid media and as CFU (colony forming units) on solid media, at the (●) ML33 (averaged duplicate values) and (○) ML34 sites with covers including an organic carbon component, and (■) at the control site, ML5. Legend: mbgs = m below ground surface, aIOM = acidophilic iron-oxidizers, a/nSOM = acidophilic/neutrophilic sulfur-oxidizers, nIRM = neutrophilic iron-reducers, nSRB = neutrophilic sulfate-reducers, and nHET = neutrophilic heterotrophs.

ML5) were insignificant ($p > 0.05$; *t*-test). SRB enumerations indicated low abundances of sulfate reducers. Except for a complete absence of SRB in the topmost sample at ML5, numbers of SRB ranged from 2.3×10^1 to 4.6×10^2 MPN g^{-1}. However, the comparison between the SRB profiles for covered and control sites indicated the SRB numbers increased (by two orders of magnitude) immediately beneath the organic cover. The organic carbon present in the cover did not seem to affect the SRB population at greater depths, although greater counts of other heterotrophs were observed deeper in the tailings profiles at ML33 and ML34 compared to ML5.

3.3. 16S rRNA gene sequence data statistics

A total of 2,205,142 raw sequences were obtained, with a mean of 81,672 ± 39,906 reads per pooled duplicate sample. About 15.8% of sequences were flagged as chimeric, and another 9.4% of reads were lost to quality trimming. The total effective sequence number was 1,648,468, with 61,054 ± 32,702 reads per sample and a mean of 1,197 ± 1,118 OTUs (97% sequence similarity) per library (Supplementary Table S2). The Good's coverage ranged from 89.3 to 99.6% (Supplementary Table S2).

3.4. Entire BACs

Proteobacteria were the most abundant phylum in most samples (with the mean accounting for 48.9% of the total amplicons), followed by *Actinobacteria* (9.7%), *Firmicutes* (6.7%), *Chloroflexi* (6.0%), *Bacteroidetes* (4.4%), and *Acidobacteria* (4.1%; Fig. 3). The phyla *Proteobacteria* and *Firmicutes* contain SOM and IOM, and their abundances were slightly elevated in the ML5 samples compared to samples collected at locations with organic carbon components. However, the differences between abundances of both *Proteobacteria* and *Firmicutes* in samples from locations with and without organic carbon layers were not significant ($p > 0.05$; *t*-test). The mean relative proportion of unclassified bacteria was relatively low, accounting for 5.8% of total reads.

BACs were also assessed on the genus level and a total of 1,539 genera were detected. Major genera (or higher taxa when identification to the genus level was not possible) are shown in Supplemental Table S3. Minor genera (<0.5% of total amplicons) were grouped together, and their sum accounted for 53.9% of total reads. Prokaryotes that were dominant in the samples use many different types of metabolic strategies and have been observed in a variety of different habitats, including plant symbionts (*Rhizobia,* 4.8%), decomposers found in soil (e.g., *Actinobacteria,* 1.7%), or human and animal pathogens (e.g., *Legionella,* 0.6%; *Clostridia,* 0.6%). *Alpha-* (3.1%), *Beta-* (6.4%), and *Gamma-proteobacteria* (5.4%) include species that are diverse in terms of their phylogenetic, ecological, and physiological properties. Among the most abundant genera were those containing species known to metabolize iron and/or sulfur (described in more detail in §3.5. Iron- and sulfur-metabolizing genera).

To determine whether the presence of the organic cover affected microbial richness (number of OTUs), Chao1 index was determined for two libraries representing treated and untreated samples. A pairwise comparison revealed no significant differences ($p > 0.05$) between the two values. A similar result was obtained for the Gini-Simpson index (a metric considering OTU abundances). Fig. 4 shows an NMDS plot of weighted UniFrac comparing entire BACs (on the genus level) at different SWWTS locations. Significant differences ($p < 0.05$; AMOVA) were determined between BACs in samples collected at ML34 and ML5 (but not between ML33 and ML5). However, different results were

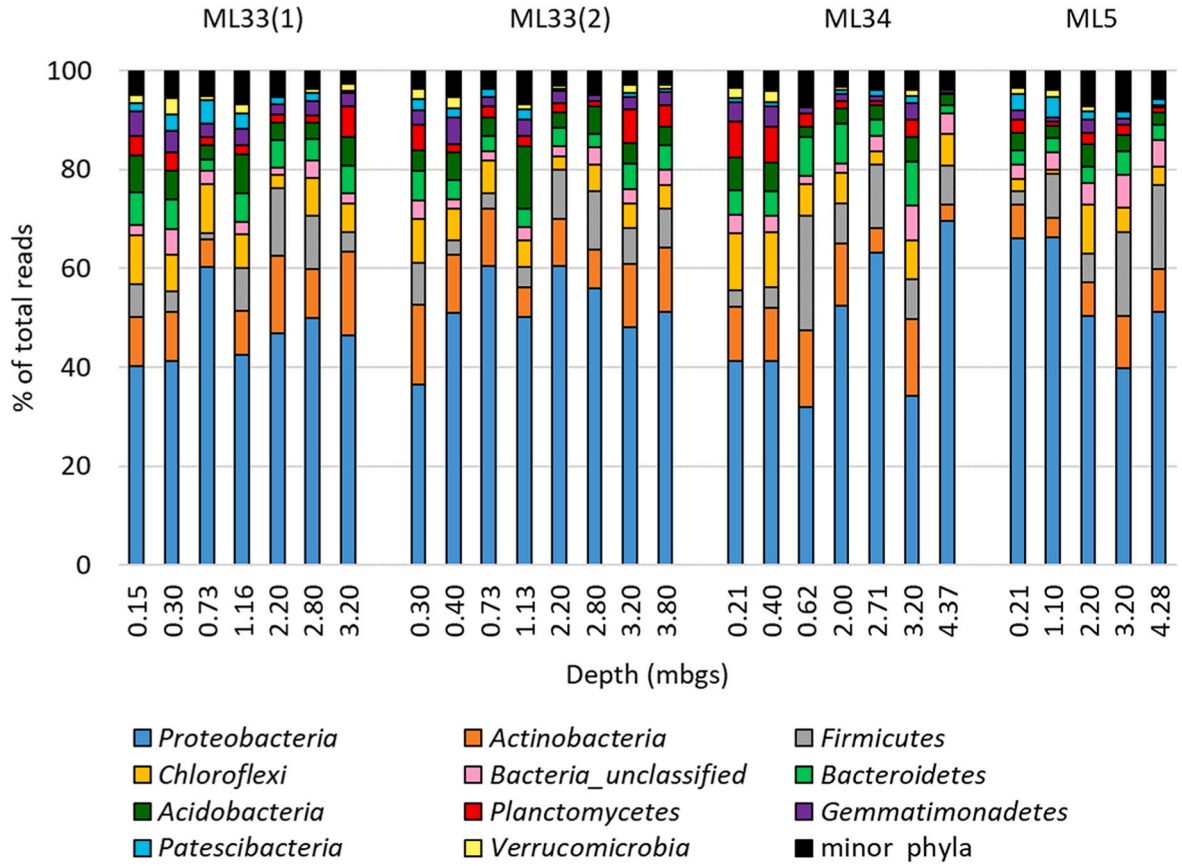

Fig. 3. Proportions of total reads of dominant major phyla (with mean relative abundance >1% of total amplicons) at three locations (ML33 was analyzed in duplicate) at the Strathcona Waste Water Treatment System (SWWTS). Minor phyla with mean relative abundance <1% of total reads were grouped together. Legend: mbgs = m below ground surface.

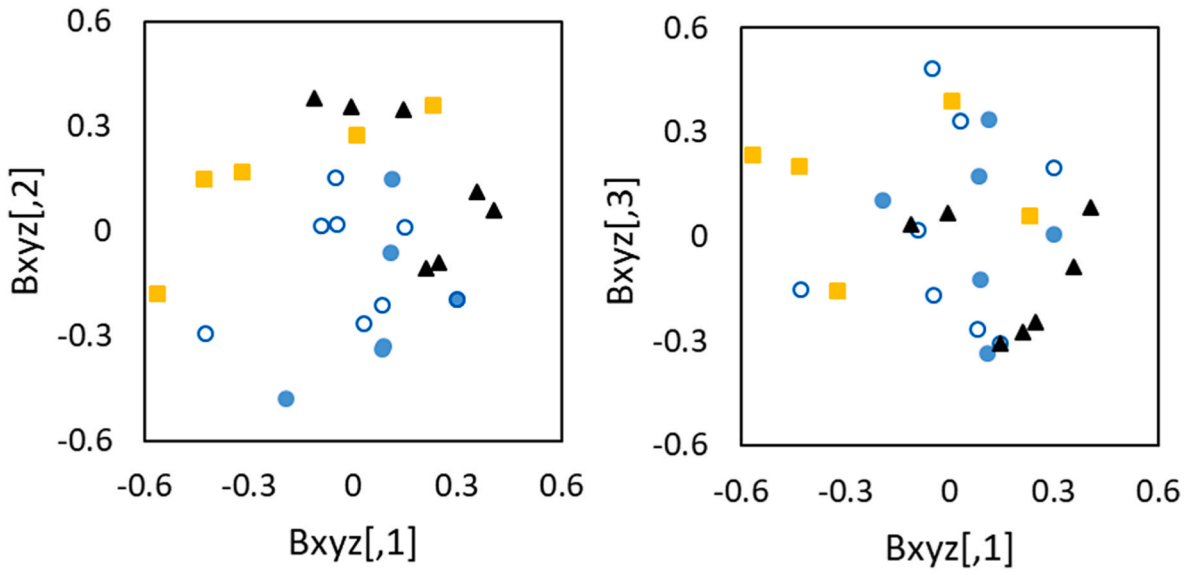

Fig. 4. Three dimensional non-metric multidimensional scaling plot (3D-NMDS, stress = 0.220) of weighted UniFrac used to investigate bacterial and archaeal communities (BACs) at (○ ●) covered ML33 (analyzed in duplicate), (▲) covered ML34, and (■) control ML5 locations within SWWTS. The distance between any two points represents the difference between those two microbial communities.

obtained for unweighted UniFrac (data not shown); a significant difference ($p < 0.05$; AMOVA) between BACs in ML33 and ML5 samples was observed, indicating that abundance of each OTU affected the overall β-diversity.

β-diversity in layers within the SWWTS vertical profile was compared using weighted UniFrac (Fig. 5). Significant differences ($p < 0.05$; AMOVA) were observed between BACs in organic cover samples and unoxidized desulfurized tailings samples, and also between BACs in organic cover and historical tailings.

3.5. Iron- and sulfur-metabolizing genera

Relative abundances (not considering numbers of individual genera in each sample) and physiological traits of sulfur- and/or iron-metabolizing genera detected in samples collected from the SWWTS are summarized in Table 2. In contrast, the numbers in the following paragraphs refer to weighted relative abundances (considering different numbers of genera in the samples). Abundances of IOM and/or SOM (Fig. 6A) ranged from 0.5 to 18.3% of total reads. No significant differences ($p > 0.05$; *t*-test) were observed between relative abundances of IOM and/or SOM in tailings samples collected at locations with (ML33, ML 34; 5.5 ± 5.6% of total reads) and without (ML5; 6.6 ± 4.0%) organic carbon cover components. Also, the higher sulfur content in the historical tailings compared to the desulfurized tailings did not result in significantly elevated proportions of SOM and/or IOM ($p > 0.05$; *t*-test), yielding 6.1 ± 6.2% vs. 6.7 ± 4.6% of total reads in desulfurized tailings.

SOM were far more abundant in the tailings samples than IOM. The mean abundance of sulfur-oxidizing (but not iron-oxidizing) genera reached 4.75% of total reads in the samples (ranging from 0.1 to 16.5%), with the neutrophilic *Thiobacillus* genus identified as the most abundant SOB (mean abundance of 2.8%). Microorganisms that oxidize both sulfur and iron (SOM/IOM) accounted for 0.5% of total reads, out of

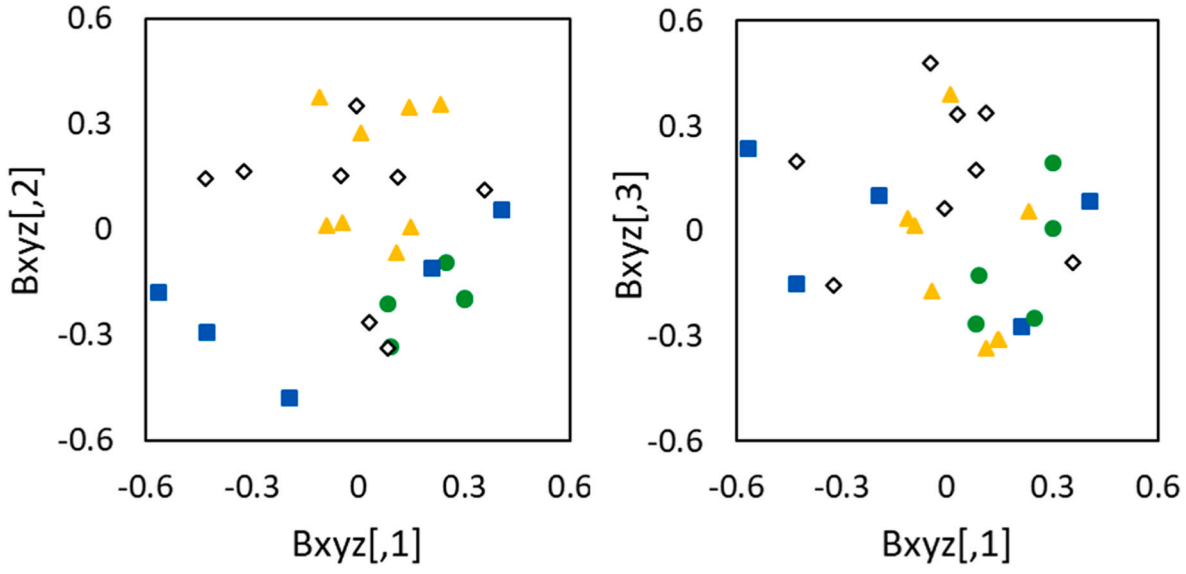

Fig. 5. 3D-NMDS plot (stress = 0.220) of weighted UniFrac used to investigate BACs in (●) organic cover, (■) oxidized desulfurized tailings, (◊) unoxidized desulfurized tailings, and (▲) historical tailings at the Strathcona Waste Water Treatment System (SWWTS). More details about each layer can be found in Table 1.

Table 2
Metabolic traits of genera detected in tailings at the SWWTS that are known to catalyze the dissimilatory oxido-reduction of iron and sulfur (Quatrini and Johnson, 2016). '+' indicates that at least one species of the genus has been reported to catalyze the dissimilatory reaction referred to in that column. EA = extremely acidophilic, MA = moderately acidophilic, N = neutrophilic, and A = alkaliphilic. * higher taxa that could not be identified at the genus level.

Genus	Mean % of total reads	pH preference	Sulfur oxidation	Iron oxidation	Sulfate reduction	Iron reduction[a]
Thiobacillus	1.32	N	+			
Sulfurifustis	0.81	N	+			
*Desulfuromonadales**	0.77	N			+	+
Desulfurivibrio	0.76	A			+	+
Sulfuriferula	0.37	MA (& N)	+			
*Acidimicrobiia**	0.37	EA		+		+
Acidithiobacillus	0.24	EA	+	+		+
*Desulfobulbaceae**	0.18	N			+	+
*Acidiferrobacteraceae**	0.15	EA	+	+		+
Desulfurispora	0.12	N			+	+
Dethiobacter	0.12	A			+	+
Desulfosporosinus	0.11	N & MA			+	+
*Desulfobacteraceae**	0.10	N & MA			+	+
Geobacter	0.07	N				+
*Gallionellaceae**	0.06	N (& MA)		+		
Acidibacillus	0.04	EA	+	+		+
Sulfuricurvum	0.04	N	+			
Desulfitobacterium	0.04	N & MA			+	+
Ferrovibrio	0.04	N		+		
Desulfuromonas	0.04	N			+	+
Desulfobacca	0.04	N			+	+
*Sulfuricellaceae**	0.04	N	+			
Sulfuricella	0.03	N	+			
Desulfatiglans	0.03	N			+	+
Halothiobacillus	0.03	N (& MA)	+			
Desulfotomaculum	0.02	N & A			+	+
Thiovirga	0.02	N	+			
Acidithrix	0.02	EA & MA		+		+
Ferrithrix	0.02	EA		+		+
Sulfurimonas	0.02	N	+			
Acidiphilium	0.02	EA	+			+
*Desulfobacterales**	0.02	N & MA			+	+
Desulfobulbus	0.02	N			+	+
Desulfovibrio	0.02	N & MA			+	+
Thiomonas	0.02	MA	+			
Desulfitibacter	0.01	N & A			+	+
Desulfomonile	0.01	N & MA			+	+
Acidiferrobacter	0.01	EA	+	+		+
Gallionella	0.01	N (& MA)		+		
Leptospirillum	0.01	EA		+		
Desulfatirhabdium	0.01	N			+	+
*Desulfobulbaceae**	0.01	N			+	+
*Desulfuromonadales**	0.01	N			+	+
*Halothiobacillaceae**	0.01	N (& MA)	+			
Sulfurovum	<0.01	N	+			
Dethiosulfatibacter	<0.01	N			+	+
Desulfitispora	<0.01	A			+	+
Acidicaldus	<0.01	EA	+			+
Desulfatiferula	<0.01	N			+	+
Desulfatitalea	<0.01	N			+	+
Desulfobacula	<0.01	N			+	+
Desulfococcus	<0.01	MA & N			+	+
Desulfocapsa	<0.01	N			+	+
Desulfoprunum	<0.01	N			+	+
*Desulfuromonadaceae**	<0.01	N			+	+
*Acidiferrobacteraceae**	<0.01	EA	+	+		+
Ferrovum	<0.01	EA		+		+
Sulfurisoma	<0.01	N	+			
Sulfuritalea	<0.01	N	+			
Ferritrophicum	<0.01	N (& MA)		+		
Sulfurirhabdus	<0.01	N	+			
Acidihalobacter	<0.01	EA	+	+		

[a] Both direct and indirect.

which 0.3% was *Acidithiobacillus* spp. Genera that oxidize iron (but not sulfur) were detected with a mean abundance of 0.5%.

Fig. 6B shows relative abundances of SRB in the SWWTS samples. A slightly greater relative abundance of SRB (8.3 ± 7.2% of total reads) was observed in ML5 samples, compared to samples from the locations with organic carbon cover components (2.4 ± 3.9%). The difference was, however, insignificant ($p > 0.05$; *t*-test). SRB in desulfurized tailings accounted for 3.2 ± 3.8% and in historical tailings 5.4 ± 6.8% of total reads, the difference between which was again insignificant ($p > 0.05$; *t*-test). The overall mean abundance of SRB constituted 3.5% of the total reads, with *Desulfuromonas* (and higher taxa) detected as the most abundant SRB (reaching 1.1% of total amplicons).

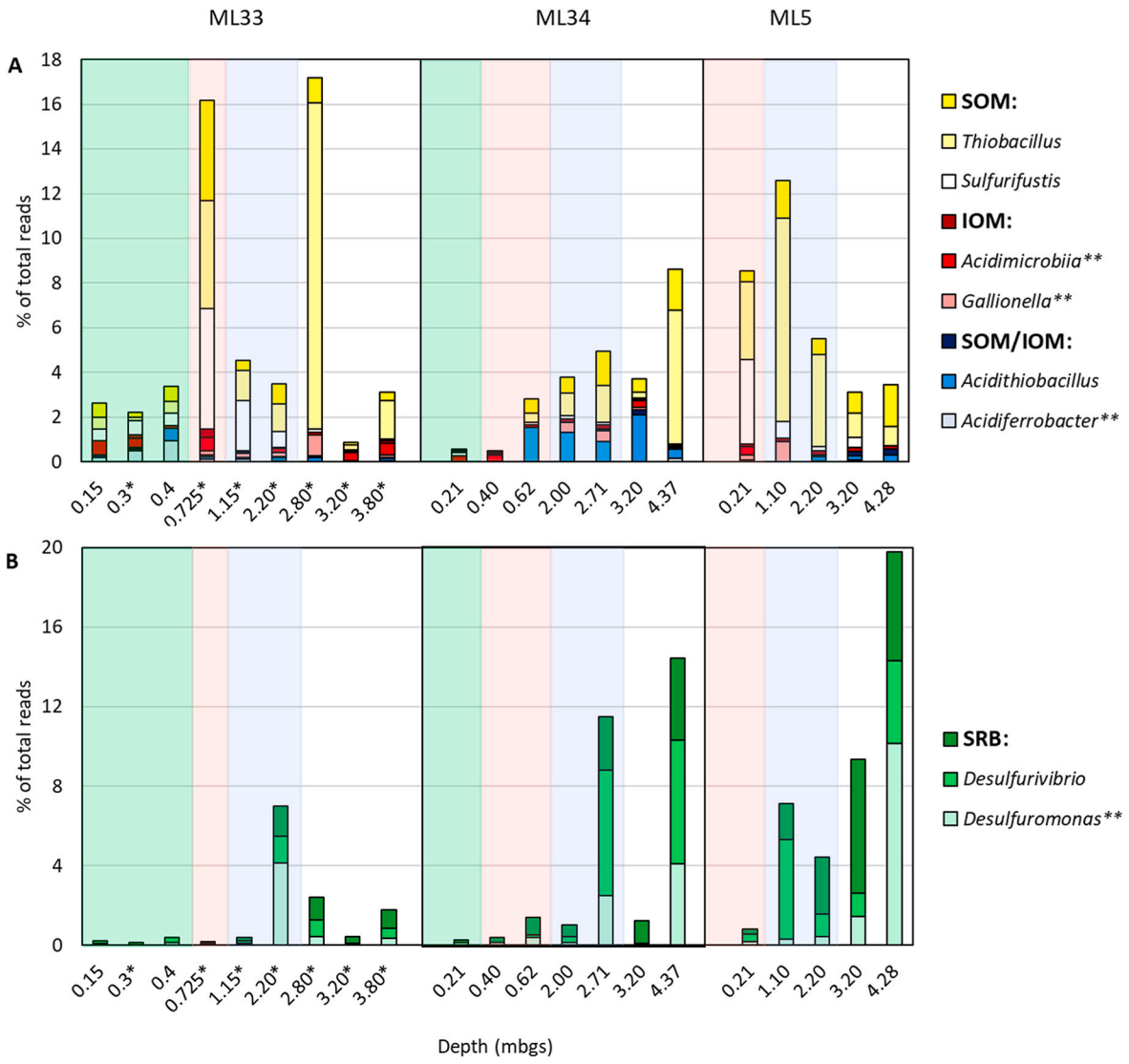

Fig. 6. Proportions of total reads of (A) sulfur- (SOM) and/or iron- (IOM) oxidizing microorganisms, and (B) sulfate- and/or sulfur-reducing bacteria (SRB) at three locations at the Strathcona Waste Water Treatment System (SWWTS); ML33 and ML34 had been covered with an organic cover, while the untreated ML5 location served as a control site. Vertical profile layers are delineated in colored fields: organic cover in green, oxidized desulfurized tailings in pink, unoxidized desulfurized tailings in blue, and historical tailings in white. Legend: * duplicate means, ** higher taxa that could not be identified at the genus level, mbgs = m below ground surface.

4. Discussion

Initially, the tailings at the Strathcona impoundment were deposited subaqueously and remained continuously saturated, minimizing sulfide oxidation. However, as tailings deposition continued, parts of the impoundment became progressively exposed and extensive sulfide oxidation was observed in the tailings, generating high concentrations of oxidation products (up to 13 g L^{-1} Fe, 21 g L^{-1} SO_4^{2-}, 125 mg L^{-1} Ni, 1 mg L^{-1} Co, 17 mg L^{-1} Zn, and 0.8 mg L^{-1} Pb) and low pH water (<4) (Blowes et al., 1996). Between 1994 and 1996, the tailings surface was buried beneath 3–4 m of fresh tailings. To reduce the rate of sulfide oxidation, 2–3 m of fine-grained, high-moisture content, desulfurized and neutralized tailings were deposited over the beached tailings in 1996, after which basic pH (~7.2–9.5) conditions dominated in the shallow layers (Bain et al., 1998). Bain and Blowes (2013) evaluated the changes in pore-water quality at the SWWTS between 1996 and 2012, reporting that the layer of desulfurized tailings limited the ingress of O_2, and therefore sulfide oxidation, to shallow depths. Elevated concentrations of dissolved Al (25 mg L^{-1}), Co (5 mg L^{-1}), Cu (10 mg L^{-1}), Ni (184 mg L^{-1}), and SO_4^{2-} (5.4 g L^{-1}) as well as low pH (4.25) were found at ~0.9 m depth in the desulfurized tailings, likely originating from the time when the desulfurized tailings were still exposed to the atmosphere. In the areas that had not been covered, acidic pH (3–4) and elevated concentrations of dissolved SO_4^{2-}, Al, Co, Cu, Fe, Ni, and Zn were detected in the vadose zone, due to more extensive sulfide oxidation.

Concentrations of dissolved metals in the cover system determined in the current study were generally low (with the exception of Mn and Ni). However, the mobility of metals (including Fe, which plays a particularly important role in mine-impacted environments) is pH dependent, and also for some (e.g., Co, Cu and Ni) potentially limited by adsorption onto ferric oxyhydroxide and clay-mineral surfaces. Despite improved pore-water quality, dissolved SO_4^{2-} concentrations (1.0–3.5 g L^{-1}) and low pH values (min. ~4.8) indicated that localized sulfide oxidation occurred in the tailings since the deposition of the organic cover, although at generally low rates. Other authors have also reported that the installation of organic covers over metal-contaminated mining areas suppressed sulfide oxidation. In a study by Brown et al. (2003), a surface

application of biosolids mixed with wood ash resulted in a significant decrease in subsoil acidity and aqueous metal concentrations. Many studies have also demonstrated that even a single application of biosolids is sufficient to restore plant cover to the contaminated areas (Trlica and Teshima, 2011; Brown et al., 2003).

Mine wastes generally have very low soil organic matter content. The aims of organic cover application over tailings impoundments are to support plant growth, increase biodiversity, improve water retention, and limit erosion of the underlying tailings (Campbell et al., 2017). Importantly, organic covers promote heterotrophic microbial communities that compete for O_2 with mineral-oxidizers and facilitate reductive physicochemical processes (such as sulfate reduction), resulting in positive changes in the mine wastes. Increased enzymatic activities in tailings as a result of a treatment with municipal waste compost and biosolids have been reported shortly after amendments (de Vareness et al., 2010; Alvarenga et al., 2008; Perez de Mora et al., 2005) as well as several years after the treatment (Touceda-Gonzalez et al., 2017; Zornoza et al., 2012; Baker et al., 2011; Perez de Mora et al., 2006). Some studies detected an increase in microbial biomass (Zornoza et al., 2012; Perez de Mora et al., 2005, 2006) or in total phospholipid fatty acids (Baker et al., 2011) in mine-waste soils amended with organic matter, compared to unamended controls. Pepper et al. (2012) and Seaker and Sopper (1988) observed increases in aerobic heterotrophic microbial populations at biosolid-amended mine sites, compared with those receiving chemical fertilizers. In a 3-year field study, Gardner et al. (2010) reported that biosolids enhanced the numbers of total aerobic, total anaerobic, IRM, SRB, and denitrifying microorganisms near the surface of an open-pit Cu/Mo mine, but the total aerobic heterotroph counts declined two years after the biosolids application. A similar observation was reported in another study (Harris and Megharaj, 2001), where a significant increase in numbers of culturable aerobic heterotrophs was detected even 4–6 years after an organic amendment. Large populations (10^5 to 10^8 cells g^{-1}), similar to values reported for undisturbed soils (1–34 $\times$ 10^6 cells g^{-1}; Sopper, 1993), were detected in samples from the sites with organic carbon cover components, whereas no heterotrophic growth was observed in (high-sulfide) historical tailings samples collected at ~4 mbgs depth at the control site.

Application of organic matter over tailings impoundments can effectively promote sulfate reduction mediated by strictly anaerobic, heterotrophic SRB (Gardner et al., 2010). However, these microorganisms cannot utilize complex organic compounds and are therefore dependent on other microorganisms (e.g., fermentative bacteria) to produce short-chain organic molecules. Sulfate reduction facilitates depletion of sulfate anions along with metal reduction and immobilization. Due to the availability of oxygen in the surficial part of the impoundment, most organic carbon supplied to the SWWTS system seemed to be utilized by aerobic species. Oxygen levels in the system decreased with depth; measurements from August and October 2019 showed O_2 was depleted below 1 mbgs at the control site and between 1 and 2 mbgs at the two locations with organic carbon cover components (McAlary, 2021). The sequence data indicated a substantially higher relative abundance of SRB below historical desulfurized tailings (with maxima as high as 14.5 and 19.8% of total reads at the greatest depths at ML34 and ML5, respectively). The counts of culturable SRB, however, did not follow this trend, and stayed constant throughout the tailings vertical profile. The low numbers of culturable SRB (and to a lesser extent IRM) suggest a large proportion of SRB detected by sequencing might have been inactive. However, MPN numbers might also have been underestimated, in which case the sequence data might provide a more realistic representation. Nevertheless, the IRM cultivation data in this study were most likely overestimated due to the capacity of many iron-metabolizing species to both oxidize and reduce iron, depending on oxygen availability.

Slow groundwater flow was previously observed in the SWWTS tailings (Blowes et al., 1996) and because elevated counts of heterotrophs have been reported in tailings systems even 10 years after biosolids application (Pepper et al., 2012), the surface organic matter supply at the SWWTS can likely sustain heterotrophic metabolism for years. However, multiple long-term applications and field studies demonstrate the organic matter used in cover designs for mine tailings remediation decomposes over time and eventually becomes depleted (e.g., Jia et al., 2015). Re-applications of the amendments are required to sustain the systems. Another drawback of organic covers is that the dissolved organic material can act as a complexing agent for heavy metals, thus increasing concentrations of these elements of environmental concern in tailings (from where they could be mobilized) as well as in plant tissue (Gardner et al., 2012; McBride, 2003; Sopper, 1993). Additionally, some bacterial strains might use organic matter to reduce Fe^{3+} to Fe^{2+} (Johnson and McGinness, 1991), increasing metal mobilization. Finally, sufficient hydration of the cover layers is important. According to Mbonimpa et al. (2003), O_2 diffusion significantly decreases when saturation is >70%, while Peppas et al. (2000) determined in a laboratory experiment with municipal sewage sludge that the cover usually fails when the water content in the organic layer drops to approximately 50%. McAlary (2021) measured the variation in volumetric water content (saturation) at the SWWTS over the 2019 field season. The desulfurized tailings (measured at 0.75 and 1.05 mbgs) maintained a higher degree of saturation than the organic cover (0.2 and 0.45 mbgs), in which saturation was generally <60% (v/v). Desiccation cracks developed within the SWWTS cover systems (both at locations with and without organic cover component) during the summer months of 2018 and 2019. However, measurements of gas-phase O_2 concentrations indicated O_2 was depleted within the desulfurized tailings.

Oxygen availability generally supports the growth of mineral-oxidizing microorganisms. Neutrophilic SOM were more numerous than acidophilic SOM in the SWWTS samples, determined by both MPN (means ~10^2 vs. 10^6 cells g^{-1}, respectively) and high-throughput sequencing (4.4 vs. 1.3% of total reads). IOM were detected with a much lower relative abundance (0.5% of total reads). The MPN counts of acidophilic IOM could be overestimated, as several SOM (e.g., *Acidithiobacillus ferrooxidans*) also metabolize iron.

Important shifts in the entire microbial structure have been detected by molecular techniques in metal-rich tailings amended with organic materials. Touceda-Gonzalez et al. (2017) reported differences between BACs in untreated and compost-amended soils during a three-year field trial using a denaturing gradient gel electrophoresis (DGGE) analysis. Through amplified ribosomal DNA restriction analyses (ARDRA), Perez de Mora et al. (2006) observed changes in structural diversity in both bacterial and fungal communities under seven different treatments (representing four different organic amendments, one inorganic treatment, and two controls). Pepper et al. (2012) investigated 16S rRNA clone libraries obtained from community DNA in Cu mine tailings, reporting significant shifts in dominant bacterial populations 10 years after biosolids application. However, no consistent, significant differences between entire BACs or proportions of SOM/IOM in samples from the sites amended with organic carbon and the control site were observed in the present study. Nevertheless, elevated numbers of heterotrophs detected at greater depth at locations with organic carbon layers indicate a potential benefit in using organic covers for mine waste remediation. Increased amendments with the biosolids mixture (and/or the frequency of application) might, however, be considered for the Strathcona site, due to organic matter decomposition over time (discussed above).

In summary, previous studies showed the application of desulfurized tailings at the SWWTS has mitigated sulfide mineral oxidation and improved pore water quality (Bain and Blowes, 2013; Bain et al., 1998). The additional amendment with organic-rich material further strengthened the remediation efforts at the SWWTS. Most of the samples investigated in the current study had a circumneutral pH and contained low concentrations of dissolved metals. However, the presence of mineral-oxidizing prokaryotes, which were detected by both culture-dependent and culture-independent microbiological techniques,

indicated the presence of sulfide oxidation hotspots (as postulated by Pakostova et al., 2020b) might not always be possible to detect using geochemical analyses but can be easily determined by extremely sensitive microbiological techniques.

5. Conclusion

The combination of subaqueous disposal of high-sulfide tailings and covering exposed tailings with a layer of partly saturated, fine-grained, desulfurized tailings has been previously reported to reduce rates of sulfide oxidation. The addition of organic materials is an effective method for amending closed tailings storage facilities that are no longer active and providing the nutrients to establish a vegetative cover over the impacted area. A combination of complementary culture-dependent and culture-independent techniques was used to characterize the microbial populations in a SWWTS tailings deposition cell covered with a demonstration-scale, multi-layer cover. The data were compared with results obtained at a control site without the organic cover. The application of an organic cover consisting of a mixture of biosolids and composted municipal waste in conjunction with the desulfurized tailings layer can be beneficial for the reclamation of sulfide-rich tailings. The numbers of SRB, which mediate bio-immobilization of dissolved metals in mine wastes, increased by two orders of magnitude four to six years after application of biosolids. Additionally, greater counts of other heterotrophs were observed deeper in the tailings profiles at locations that had received the organic carbon amendment, indicating the surface deposition of organic carbon sustains heterotrophic metabolism at the SWWTS for several years. However, the presence of mineral-oxidizing prokaryotes was detected, indicating the presence of sulfide oxidation hotspots within the tailings, which were detected by extremely sensitive microbiological (but not other) techniques.

Sensitive microbiological analyses have potential to provide a novel tool to monitor microbial processes (particularly sulfide oxidation, sulfate reduction, and heterotrophy) at the SWWTS and other mine-waste sites. This microbiological characterization could, in combination with data from geochemical, mineralogical, and hydrogeological analyses, provide deeper insight into the extent of microbial processes and development of prokaryotic populations at mine waste sites, thus helping to assess the long-term performance of remediation strategies and guide future developments of passive remediation systems. Future research will further the integration of these complementary approaches.

Author contributions

Eva Pakostova: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. Mason McAlary: Methodology, Investigation, Formal analysis, Visualization. Stephanie Marshall: Resources, Supervision, Writing – review & editing. Samantha McGarry: Resources, Supervision, Writing – review & editing. Carol J. Ptacek: Resources, Supervision, Funding acquisition. David W. Blowes: Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Data availability

Illumina sequence data used in this study are openly available in the European Nucleotide Archive (ENA) (accession no. PRJEB44733).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

(a)IOM	(acidophilic) iron-oxidizing microorganisms
(a/n)SOM	(acidophilic/neutrophilic) sulfur-oxidizing microorganisms
AMD	acid mine drainage
AMOVA	analysis of molecular variance
ARDRA	amplified ribosomal DNA restriction analysis
BAC	bacterial and archaeal community
DGGE	denaturing gradient gel electrophoresis
DO	dissolved oxygen
DOC	dissolved organic carbon
HT	historical tailings
ICP-OES	inductively coupled plasma-optical emission spectrometry
ICP-MS	inductively coupled plasma-mass spectrometry
INO	Integrated Nickel Operations
mbgs	meters below ground surface
MPN	most probable number
(n)HET	(neutrophilic) heterotrophs
(n)IRM	(neutrophilic) iron-reducing microorganisms
(3D-)NMDS	(three-dimensional)- non-metric multidimensional scaling
(n)SRB	(neutrophilic) sulfate-reducing bacteria
OC	organic cover
ODT	oxidized desulfurized tailings
OTU	operational taxonomic unit
PVDF	polyvinylidene fluoride
SWWTS	Strathcona Waste Water Treatment System
UDT	unoxidized desulfurized tailings

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2021.114030.

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