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Structure of microbial communities in amended and unamended acid-generating mine wastes along gradients of soil amelioration and revegetation

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ABSTRACT

Understanding the structure of microbial communities in acidic, metal contaminated tailings and waste rock in cool, continental northern temperate climates is important for identifying their potential for use in phytostabilization and bioremediation programs. In this study, microbial community compositions across two large mine waste sites in the Sudbury basin, Ontario Canada were analyzed along gradients of barren-to-vegetated tailings and waste rock. Microbial communities were less diverse, more homogenous, and mainly chemolithoautotrophic in barren sites, transitioning to more diverse communities of organoheterotrophs, nitrogen-fixing/plant-growth promoting bacteria, and ectomycorrhizal fungi in the vegetated areas. Co-occurrence analyses of microbial communities demonstrated higher levels of association among microbial groups in the barren tailings, which were related to the extreme environmental and chemical conditions and restricted anabolic nutrient availability of the substrates. We also show that vegetation and plant-derived organic matter in the tailings are associated with the shifts observed in the structure of microbial communities towards more soil-like communities. These changes in microbial communities can improve soil fertility through increased rates of heterotrophic decomposition of plant-derived organic matter, and sequentially further aid in the establishment of vegetation. These findings will help develop phytostabilization approaches in mine tailings and waste rock in cool, continental northern temperate and boreal climates.

1. Introduction

Metal mining produces large quantities of waste material, ranging from waste rock to fine tailings (Franks et al., 2011). These wastes are usually disposed in extensive containment facilities that can cover hundreds of hectares (e.g. Bagatto and Shorthouse, 1999; Forsberg and Ledin, 2003) and can hold residual concentrations of inorganic contaminants including metals. Without the careful rehabilitation of these containment facilities, mine wastes pose significant and long-term environmental risks (Dudka and Adriano, 1997; Ledin and Pedersen, 1996). Common approaches to their rehabilitation include geomembranes and covers of clean fill (O'Kane Consultants, 2012), chemical treatment of surface substrates or downstream waters to neutralize pH and immobilize metals (Peters, 1995), fertilization, and organic amendments (Brown et al., 2004; Campbell et al., 2017). Together, these approaches are effective in producing technosols and a vegetative cover, but they are expensive.

Phytostabilization is a complementary and more cost-effective approach to reduce environmental risks associated with mine wastes. It involves establishing a cover of tolerant vegetation directly in contaminated substrates, sometimes with minor amendments, in order to reduce contaminant mobility and transform them to less bioavailable forms (Kidd et al., 2009; Shackira and Puthur, 2019). Phytostabilization consequently increases soil fertility and subsequent plant

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establishment. The buildup of plant-derived organic matter over long time scales may also help to promote chemically reducing conditions and mitigate acidity and metal solubility associated with mine waste lithotrophy (Rieuwerts et al., 1998; Shen et al., 2014). In remote mine waste sites, phytostabilization may be one of the few feasible rehabilitation tools available (Mendez and Maier, 2007; Sheoran et al., 2010). However, stressful conditions in mine wastes, such as the high concentrations of phytotoxic metals, low nutrient levels and the harsh physical environments, may initially inhibit the establishment of self-sustaining vegetation required for phytostabilization (Chibuike and Obiora, 2014; Dudka and Adriano, 1997).

Recent evidence suggests that the problem could be circumvented by pairing phytostabilization with other bioremediation strategies, such as the inoculation of tailings and seeds or seedlings with targeted rhizosphere microorganisms, such as plant growth-promoting bacteria, endophytes, and mycorrhizal fungi (Grandlic et al., 2008; Hayat et al., 2010; Yamaji et al., 2016; Yang et al., 2016). They can support plant establishment by fostering plant growth and increasing plant metal tolerance. However, the effectiveness of phytostabilization combined with microbial inoculation is strongly dependent on the optimized function of the plants and microorganisms at play (Thavamani et al., 2017). This can be challenging for two main reasons. First, the chemical and mineralogical composition of mine wastes often exhibits unique properties, which differ across mine sites, even with respect to the same target metal (Bosecker, 1997). Second, although the approach of phytostabilization with microbial inoculation has proven to be effective in temperate or tropical climate zones (De-Bashan et al., 2010; Johnson, 2014; Juwarkar et al., 2010), these results may not readily translate to north temperate, boreal and subarctic zones, which have shorter growing seasons and colder temperatures. Thus, additional adaptation strategies may be needed to cope with low temperatures as well as chemical extremes.

The use of microbial co-occurrence structure and network association among microbial groups holds some promise. Co-occurrence of highly correlated guilds in complex microbial communities has been linked to community-level associations and their adaptations to environmental stresses across a range of sediment, agricultural soil, and human gut environments (Duran-Pinedo et al., 2011; Li et al., 2017; Yin et al., 2015). In extremely harsh anthropogenic environments such as acid-generating mine wastes, physiological ecology of bacterial strains leading to both pollution generation and remediation have been characterized and operationalized for both biomining and acid mine drainage mitigation (De Carvalho, 2012; Mykytczuk et al., 2010). However, community-level adaptations to environmental stresses have not been investigated. Identifying co-occurrence mechanisms among microorganisms in mining-impacted sites within the north temperate region is vital to understanding their adaptation strategies to stresses. This in turn would help to characterize the optimal conditions for their growth and function (Shi et al., 2016a), including dynamic microbe-plant interactions that promote phytostabilization.

The main objectives of this study were to identify microorganisms across mine waste environments that have the potential to promote plant growth and to help stabilize mine wastes, and potentially unique community-level co-occurrence mechanisms at nickel-copper (Ni-Cu) tailings and waste rock sites in Sudbury, Ontario. The Sudbury region has large, world-class deposits of Ni and Cu and a long mining history. The mine waste sites here have undergone different management strategies, ranging from no treatment to liming, fertilization and planting, and consequently present a broad range of substrate conditions and vegetation. We aimed to answer two main questions: (1) How do microbial communities (bacteria, archaea, and fungi) differ in diversity, composition, and functional traits between barren and vegetated mine wastes in Sudbury tailings?; and (2) Does the variation in the geochemical properties of the mining wastes between barren and vegetated mine wastes affect the co-occurrence structure and the extent of the associations among microorganisms within the communities? We expected that our findings would contribute to understanding the mechanistic responses of microbial communities across tailings with different biogeochemical properties. The knowledge of microbial community structure is also helpful in developing general reclamation strategies for mining-impacted environments within the cooler continental climates using plant-supporting microbial consortia.

2. Materials and methods

2.1. Study area and sampling

Two mine waste sites (Sites A and B) were sampled in August and September 2017 within the city of Greater Sudbury, Ontario, Canada. The region has a mean annual temperature of 4.1 °C and mean annual precipitation of 903 mm (Environment Canada, 2019). Both sites were historic Ni-Cu tailings/waste rock storage facilities, containing Cu and Ni-bearing minerals such as chalcopyrite and pentlandite, hosted within norite and co-occurring with an abundance of pyrrhotite, and pyrite (Dudka et al., 1996; Johnson et al., 2000). Both sites ceased operation in 1972 but have different histories and vegetation covers. The materials at both sites were already acid-generating at that time, with paste pH values ranging from 1.5 to 3.0. Site A had pyrrhotite concentrate tailings and were not amended or covered. The site was barren or vegetated with very sparse distribution of naturally colonizing plants mainly composed of white birch (*Betula papyrifera*), balsam poplar (*Populus balsamifera*), grasses/sedges (e.g. *Deschampsia* spp., *Agrostis* spp.), and some mosses (e.g. *Pohlia nutans*) and lichens (e.g. *Cladonia* spp.). Site B had a substrate dominated by crushed waste rock (~1–20% sulfides including pyrrhotite and pyrite) mixed with soil (up to 30%) and variable amounts of historic ore concentrate and smelting residues buried among the shallow soils ($<$ 1.5 m depth). Much of Site B was barren, but approximately 20% had been actively amended (i.e. limed and fertilized) and planted between 1978 and 2005 (Peters, 1995). This vegetated section could be divided into two areas, as identified by vegetation succession. The edge of the amendment, here, referred to as "partially amended" area, was mainly covered by white birch (*Betula papyrifera*), trembling aspen (*Populus tremuloides*), white spruce (*Picea glauca*), hawkweed (Hieracium), some grasses (e.g. *Deschampsia* spp.), mosses (e.g. *Pohlia nutans*) and lichens (e.g. *Cladonia* spp.). The areas central to the amendment, referred to as "fully amended" area, had similar vegetation to the partially amended area, but with higher plant density.

At Site A, 36 samples (each ~20 g wet weight) were collected from the top 15 cm of the barren tailings, with a minimum distance of 5 m between samples. Twenty quadrats (1 m^2) were randomly selected from the sparse vegetated areas, again with a minimum distance of 5 m between quadrats. Soil samples (each ~20 g wet weight) were taken from the root zone (soil closely bound to roots) of each plant type present in each quadrat at a depth of 0–15 cm, where the majority of plant roots existed, resulting in 40 individual samples from the sparsely vegetated area (Table S1). A similar approach was used for Site B to collect 18 samples from the barren area. In the fully and partially amended areas, respectively, a total of 72 and 46 samples (each approximately 20 g wet weight) were collected from the root zone of different plant types at the depth of 0–15 cm from 20 quadrats (each 1 m^2), with a minimum distance of 3 m between quadrats. All samples were transferred to sterile air-tight plastic bags, temporarily stored on ice in a cooler in the field, then stored at −20 °C until they were processed for geochemical measurements, and microbial community analyses.

2.2. Geochemical properties of the tailings

Subsamples of each waste substrate (each approximately 5 g) were soaked in 20 mL 0.01 M $CaCl_2$, and pH was measured using an OAKTON probe (OAKTON WD-35805-06) and an Oakton pH meter (OAKTON

510 series) (Hendershot et al., 2008). Percent organic matter in each sample was quantified by loss-on-ignition in a muffle furnace at a maximum temperature of 400 °C. Total elements (S, Fe, Cu, Ni) concentrations in the tailings were analyzed by inductively coupled plasma - optical emission spectrometry (ICP-OES) on a PerkinElmer Optima 7000 DV Optical Emission Spectrometer following digestion with concentrated trace-grade nitric acid (Aristar® Plus CAS 7697-37-2) and the digestates were analyzed by ICP-OES (Souter and Watmough, 2017). The HCl-extractable elemental concentrations were quantified by a 1:10 ratio of substrate to 0.1 M HCl extractant using ACS-grade HCl (VWR Chemicals, CAS 7647-01-0). Tubes were shaken for 2 h on a reciprocating shaker and the supernatants were filtered through 0.45 µm syringe filters (ChemScience, cat no. CS-GLN2545) and analyzed by ICP-OES on a PerkinElmer Optima 7000 DV Optical Emission Spectrometer (Souter and Watmough, 2017).

2.3. DNA extraction, PCR and sequencing

The waste substrate samples were homogenized, and the total genomic DNA was extracted from 0.25 g of each homogenized soil sample using the Qiagen DNeasy Power Soil kit, following the manufacturer's instructions (Qiagen GmbH, Germany). The total genomic DNA from each sample was amplified using two sets of primers: the universal prokaryote primers, Pro341-F (5′-CCTACGGGNBGCASCAG-3′) (Muyzer et al., 1993) and Pro805-R (5′-GACTACNVGGGTATCTAATCC-3′) (Caporaso et al., 2011), targeting the V3-V4 region of the bacterial 16S rDNA gene, and the universal fungal primers, ITS1-F (5’-ACCTGCGGARGGATCA-3′) and ITS1-R (5′-GAGATCCRTTGYTRAAAGTT-3′) (Bokulich and Mills, 2013), targeting the ITS1 region. The 5′ end of each of forward and reverse primers was modified by adding Illumina MiSeq adaptors and unique 6 nt sequence identifiers to each primer to be able to pool multiple samples within a single sequencing run. (For full description of PCR conditions see Asemaninejad et al., 2018). The normalized PCR amplicons were used to prepare the library for paired-end MiSeq Illumina sequencing (2 × 250 bp with V2 chemistry) at Metagenome Bio Inc. (Toronto, Canada).

2.4. Bioinformatics and statistical analyses

The data from each primer set was subjected to bioinformatics analyses using the QIIME pipeline (Caporaso et al., 2010). The raw MiSeq reads were trimmed to remove primer and barcode sequences and then quality filtered (Q score = 0.9). The filtered reads were pairwise aligned with a minimum overlap length of 80 nucleotides using PANDAseq (Masella et al., 2012). The identical sequences were merged to create ISUs (identical sequence units), and subsequently, singletons and chimeras were discarded using USEARCH 10.0.240_i86linux32, and UCHIME algorithm (Edgar et al., 2011). Operational taxonomic unit (OTU) clustering was performed with a de novo approach and at 97% similarity using the UCLUST algorithm (Edgar, 2010). Subsequently, the OTU-table was filtered to remove the sequence reads with abundance of < 0.1% of the total reads in any sample. The general taxonomic identification of the OTUs was performed using Green Gene database (gg_13_8_99) (DeSantis et al., 2006) and online RDP classifier (16S rRNA training set 16) for 16S rDNA sequences (DeSantis et al., 2006), and RDP classifier (Warcup Fungal ITS Trainset 1) for fungal ITS sequences (https://rdp.cme.msu.edu/classifier/classifier.jsp). To increase the confidence in taxonomic classifications, the indicator OTUs were re-identified using BLAST matching with 100% coverage and at least 80% sequence identity, confirming that the best matching reference sequences were correctly classified relative to other matching sequences.

Prior to statistical analyses, the samples with < 5000 reads in total were removed to minimize the potential sequencing errors usually introduced by samples with low sequencing depth and increase the accuracy of the estimate of diversity within samples (Smith and Peay, 2014). In order to reduce the technical noise generated by OTUs with low frequencies and very low abundances (Aitchison and Egozcue, 2005), the datasets were further filtered to contain only OTUs that occurred in at least 25% of the tailing samples with a proportional abundance of at least 3% in any of these samples. In each fungal and prokaryotic dataset, the non-target OTUs (i.e. not assigned to prokaryotes or the kingdom fungi) were filtered, and subsequently OTU richness (the number of observed OTUs) and diversity (Shannon Index) in each sample were calculated by rarefaction with 100 permutations without sample replacement, and with minimum rarefaction depth of 10400 and 5100 reads for prokaryotic and fungal datasets, respectively (Kenneth et al., 1975). The differences in OTU richness and diversity as well as geochemical properties across different areas (vegetated versus barren) in each site were assessed using a one-way analysis of variance (ANOVA) with a Tukey post hoc test in STATISTICA 7 (StatSoft Inc. 2004).

At the community level, the data were transformed by a centered log-ratio transformation approach as suggested for compositional data to meet the assumption of linearity and normality of the distribution of data points prior to parametric statistical analyses (Aitchison, 1982; Lovell et al., 2015). The significance of the variation in the structure of bacterial and fungal communities across different areas (vegetated versus barren) in each site was assessed through the Microbiome Regression-based Kernel Association Test (MiRKAT) using the “ALDEx2” and “MiRKAT” packages in R 3.3.3. The environmental variables best explaining the variations observed in microbial communities across different sections of each site were identified through redundancy analysis (RDA) using “vegan” package in R 3.3.3. The significant taxa driving the variation in the structure of bacterial and fungal communities between different areas within each site were detected with ALDEx2 tool (Fernandes et al., 2013). Then the functional assignments of the significant taxa were performed based on the known function of their closest relative type strains via PICRUSt and FungGuild tools as well as based on literature/sources describing these strains.

To understand the co-occurrence structure and association mechanism of microbial communities under harsh environmental conditions, the strength of the correlation between indicator OTUs in different areas of the sites was measured through phi analysis using CoDa Microbiome Tutorial (https://github.com/ggloor/CoDa_microbiome_tutorial/wiki/Part-3%3A-OTU-Correlations-with-Phi) in R 3.3.3. Phi analysis incorporates the association of direction of variance in the distribution of OTU abundance in the log ratio transformed data and the association of the amount of variance into a value, called “phi value” (Erb and Notredame, 2016). A phi value is a strength of association measure (similar to an effect size). In the co-occurrence analysis of microbiome metagenomics data, OTUs with low phi values (typically phi of 0.2 or lower) are those with significant positive correlations. The extent of the association in positively correlated taxa is inferred by the number of ecological links attached to each OTU, and among OTUs (Raes and Bork, 2008). Phi analysis conducted through “propr” functions in this study looks at features that share a common ratio across all samples, and reports the associations that show positive co-abundance relations with lower variations within treatment than between treatments (i.e. associations that are stable to sample variations). Coupled to phi analysis, a covariance PCA biplot was created to highlight the positively correlated taxa by clustering them in different colours and to show the environmental conditions where these groups of OTUs took place. To investigate the driver of the variation explained by the first two PCA components, the first two PC scores (Eigenvalues) were analyzed through a multiple linear regression for the effects of geochemical properties on the distribution of the taxa in each site. Due to negative correlation bias in compositional microbiome data (Egozcue et al., 2018; Gloor et al., 2017; Lovell et al., 2015), we only focused on positive associations among taxa in this study.

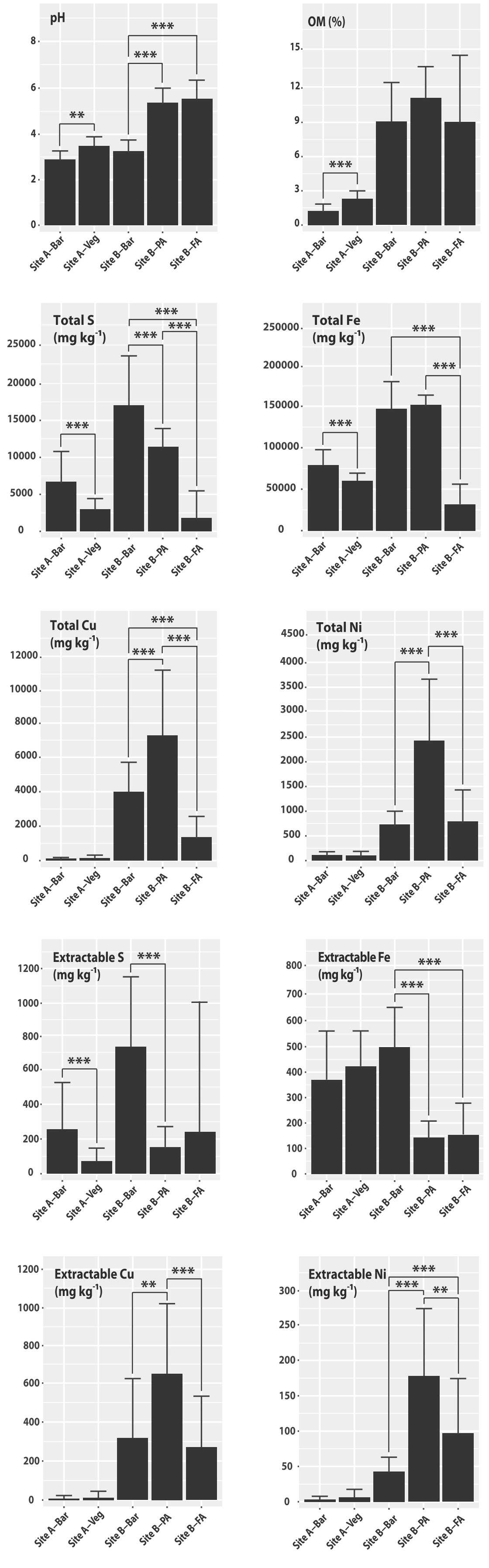

Fig. 1. pH, OM content, total and HCl-extractable concentrations of selected elements (mean ± SD) in the barren and vegetated tailings of site A shown by "Site A-Bar" and "Site A-Veg" respectively, and barren, partially and fully amended areas of Site B shown, in order, by "Site B-Bar", "Site B-PA" and "Site B-FA". The (*), (**), and (***) indicate significant differences at $P < 0.05$, 0.01, and 0.001.

3. Results

3.1. Geochemical properties of the tailings

The tailings in Site A were acidic, with barren areas being significantly more acidic (pH = 2.8) with lower organic matter content (OM = 1.3%) than the vegetated areas (pH = 3.4, OM = 2.3%; Fig. 1). Site B demonstrated a similar pattern between barren and vegetated areas, with barren areas being similarly acidic (pH = 3.2), but vegetated areas being much less so (pH = 5), and with both barren and vegetated areas having four to seven times more OM content (≈9–11% on average).

Site A had significantly higher total sulfur (S), total iron (Fe), and HCl-extractable S in the barren sections than in the vegetated areas, while HCl-extractable Fe, total and HCl-extractable Cu and Ni did not show significant differences between barren and vegetated areas. In contrast, Site B had strikingly higher element concentrations, particularly total and HCl-extractable Cu and Ni, than at Site A. The barren and partially amended areas of Site B had substantially higher total S, Fe and Cu concentrations than in the fully amended areas. The concentration of HCl-extractable Fe was also significantly lower in both the vegetated areas of Site B. However, total Cu and Ni and HCl-extractable Cu and Ni showed the highest concentrations in partially amended areas (Fig. 1).

3.2. Microbial community composition

Prokaryotic OTU richness and diversity at both Sites A and B were significantly lower in the barren areas compared with the vegetated areas (Fig. 2a, b; Fig. S1, and Table S2). At Site B, prokaryotic OTU richness and diversity in the partially amended areas was intermediate between the barren and fully amended areas.

Fungal OTU richness and diversity at Site A did not show any significant differences between barren and vegetated areas, but at site B, fungal OTU richness was lowest in the barren areas and highest in the fully amended vegetated areas, with partially amended areas again being intermediate (Fig. 2c). However, there was no significant differences in fungal diversity among the barren, partially, and fully amended areas (Fig. 2d).

MiRKAT results revealed significantly different prokaryotic communities between the barren and vegetated areas at both sites A and B (all *P*-values ≤ 0.001). Site A had no significant differences in fungal communities between the barren and vegetated areas ($P = 0.42$), but fungal communities did differ at Site B ($P = 0.007$).

Redundancy analysis of prokaryotic communities in Sites A and B showed high amount of variations between the two sites with axes 1 and 2, respectively, explaining 56.3% and 17.5% of total variations observed that were mainly driven by differences in pH ($F = 12.1$, $P = 0.001$), OM content of tailings ($F = 2.31$, $P = 0.048$), total S ($F = 3.79$, $P = 0.009$), and HCl-extractable Fe ($F = 3.17$, $P = 0.023$) between these two sites (Fig. 3a). Within site A, 39.9% and 17% of variance in overall prokaryotic communities was explained by RDA axes 1 and 2, respectively (Fig. 3b), and the major geochemical properties shaping the community structure between barren and vegetated areas were pH ($F = 5.74$, $P = 0.001$), and OM content of the tailings ($F = 2.44$, $P = 0.045$). However, none of the geochemical properties were identified as key drivers of fungal community composition between barren and vegetated areas in Site A ($P = 0.71$). In Site B, 42.1%

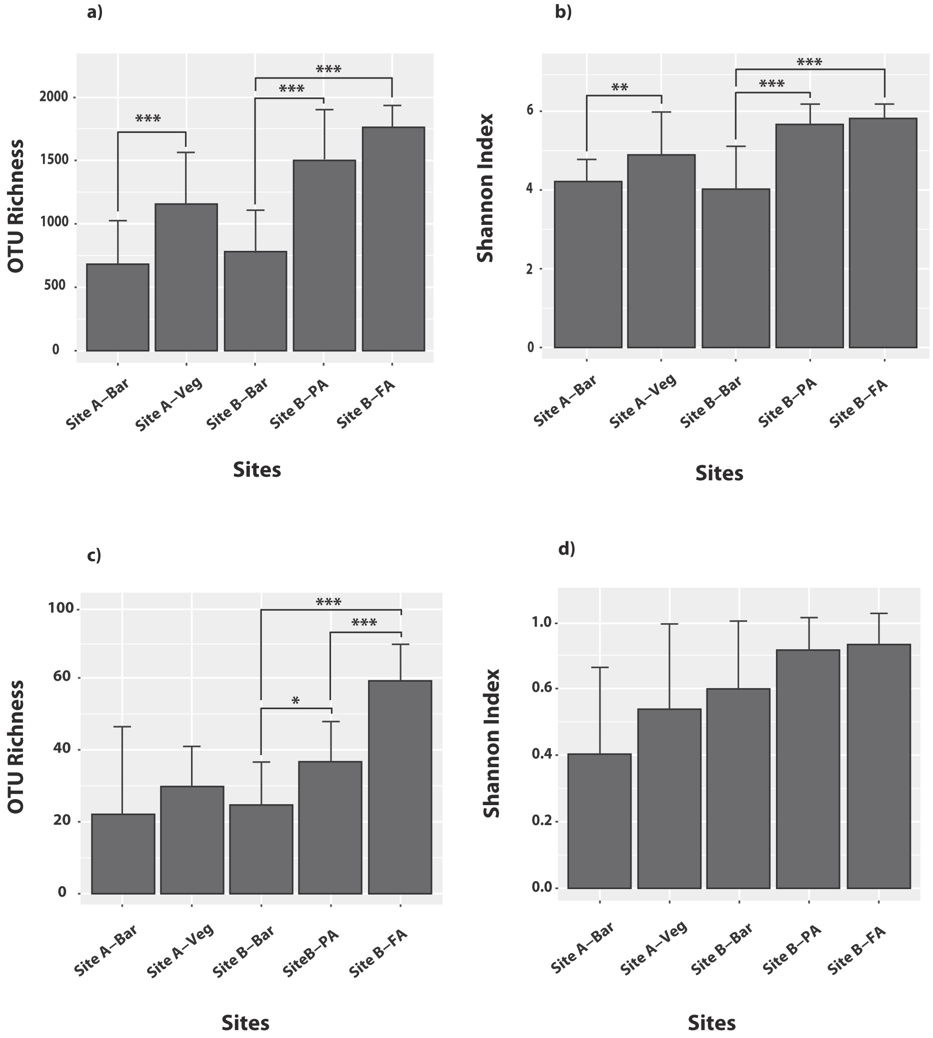

Fig. 2. OTU richness of (a) prokaryotic community and (c) fungal community and Shannon diversity of (b) prokaryotic community and (d) fungal community across barren and vegetated areas of Site A and Site B (mean ± SD). See Fig. 1 for abbreviations and significance levels.

and 19.3% of variance in overall prokaryotic communities was explained by RDA axes 1 and 2, respectively (Fig. 3c), and the major geochemical properties shaping the community structure were pH ($F = 9.7$, $P = 0.001$), HCl-extractable Fe ($F = 4.4$, $P = 0.001$), and total S ($F = 3.3$, $P = 0.01$). Again, none of the geochemical properties were identified as key drivers of fungal community composition between barren and vegetated areas in Site B ($P = 0.95$).

The dissimilarities among the microbial communities between the barren and vegetated areas in each site were further clarified by ANOVA-like differential expression procedure (ALDEx) analyses. These analyses demonstrated higher abundances of chemolithotrophs as well as some chemoorganotrophs such as *Acidisoma* and *Conexibacter* in the

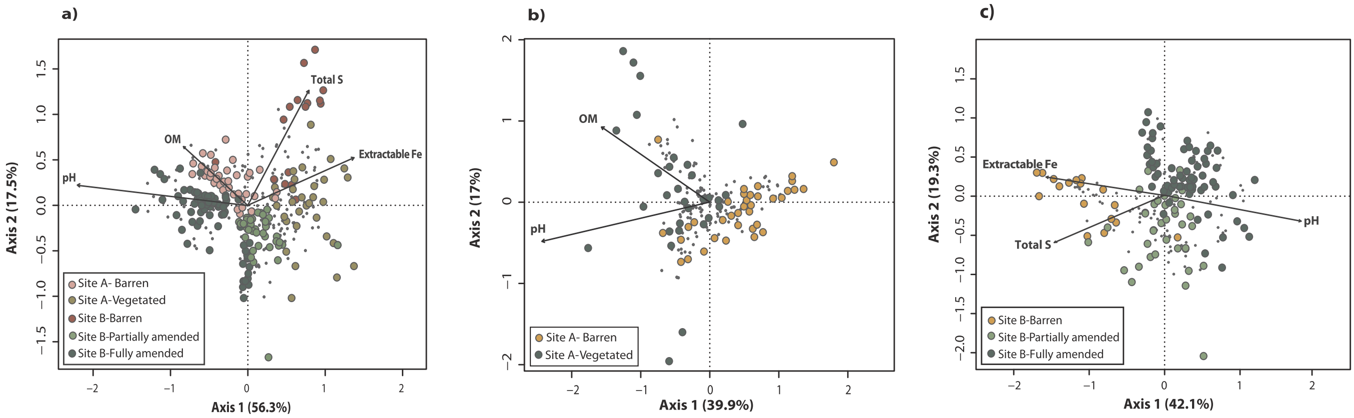

Fig. 3. Redundancy analysis of prokaryotic communities and their associations with geochemical properties across Sites A and B (a), within Site A (b), and within Site B (c). Coloured circles represent samples, while the black dots represent the OTUs present in the samples. Significant environmental factors driving variation in overall community structure are shown ($P < 0.05$).

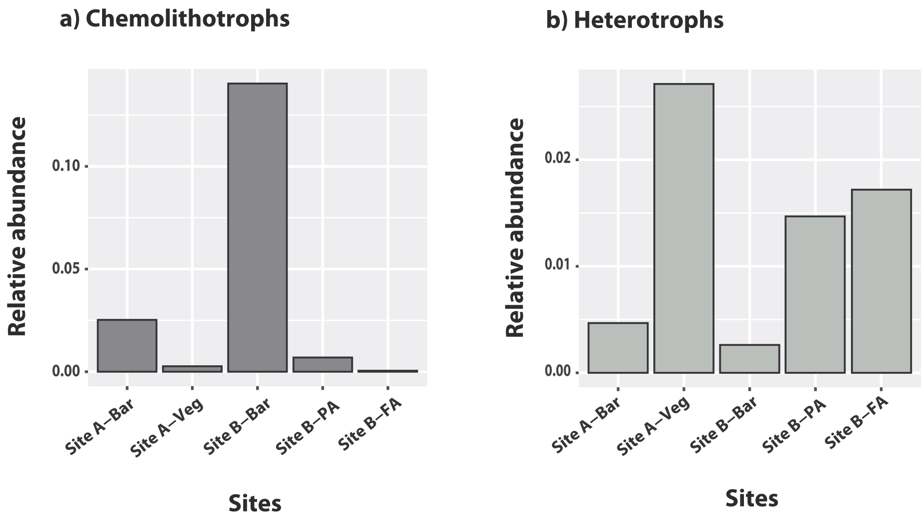

Fig. 4. Average relative abundance of (a) chemolithotrophic and (b) heterotrophic microbial groups across the barren and vegetated tailings of Site A, and barren, partially and fully amended areas of Site B. See Fig. 1 for abbreviations.

barren areas. While, a larger prevalence of heterotrophic groups in general, including decomposers, nitrogen-fixing bacteria, plant-growth promoting bacteria, and ectomycorrhizal fungi was found in the vegetated areas in both sites (Fig. 4; Tables S3, and S4).

3.3. Microbial co-occurrence structure

Co-occurrence analysis of prokaryotic indicator OTUs showed four sets of nodes with high associations (with phi value < 0.2) in Site A, two of which were found in the barren areas and two in the vegetated areas (Fig. 5a; Tables S3, S4; Fig. S2). In the barren tailings, OTUs closely related to *Acidiferrobacter*, a Fe and S oxidizer (OTUs 3, 11, 5952), OTUs closely related to *Metallibacterium* and *Desulfotomaculum*, known genera of Fe and sulfate reducers (OTUs 7, 560), *Acidisoma*, a chemoorganotroph (OTU 95), and some unclassified bacteria (e.g. OTUs 10, 55) had the highest number of links in their network of co-occurrence. The other network in barren tailings was composed of OTUs with high affinities to *Acidiphilium*, a mixotroph (OTU 35), and the thermophilic heterotrophic bacterium *Thermobaculum terrenum* (OTU 52), and some unclassified bacteria (OTUs 2, 25, 435). In the vegetated section of site A, one network association contained an OTU closely related to an ammonia oxidizer (OTU 72), paired with an OTU closely related to *Rudaea cellulosilytica* that is known to hydrolyze cellulose and urea (OTU 32); the second network was a larger group composed of OTUs closely related to *Burkholderia*, a plant-growth-promoting bacterium (OTUs 106, 15), some purported chemoorganotrophs (OTUs 31, 61), OTUs with high affinity to *Bradyrhizobium* and *Mesorhizobium* nitrogen-fixing bacteria (OTUs 5, 124), and some OTUs belonging to the class Acidobacteria (e.g. OTUs 166, 186). For Site B, co-occurrence analysis of prokaryotic indicator OTUs again showed four sets of nodes (with phi value < 0.2), but only one appeared in the barren areas (Fig. 5c; Tables S3, S4; Fig. S2). As with Site A, this network association in the barren areas contained OTUs with high similarity to *Acidiferrobacter*, a Fe and S oxidizer (OTUs 1, 3), *Metallibacterium*, an iron reducer (OTU 7), and some unclassified bacteria (e.g. OTUs 2, 435). However, unlike Site A, none of the potential chemoorganotrophs detected in high abundances in the barren area of Site B were involved in this network association. A large network in both vegetated areas of Site B was composed of potential chemoorganotrophs (e.g. OTUs 14, 217 closely related to *Kineosporia* and *Solirubrobacter*), OTUs with high affinity to *Microvirga* a nitrogen-fixing bacterium (OTU 19), *Streptomyces* a genus with known plant growth-promoting species (OTU 103), and some unclassified bacteria (OTUs 210, 66). The other two smaller groups were in the fully amended vegetated areas and were composed of OTUs closely related to *Geobacter* that are known to couple oxidation of acetate to the reduction of FeIII (OTUs 81, 42), and OTU 191 and OTU 37, both classified as Acidobacteria. Comparing the network associations in Site A with very low OM content particularly in the barren areas, and Site B with higher OM in general, revealed a higher number of taxa involved in the set of nodes, and a higher number of links attached to each OTU, and among taxa in the barren areas of Site A than in the vegetated areas, or in the prokaryote networks seen in Site B (Fig. 5; Table S5). However, no fungal OTUs were found to be significantly correlated in either Site A or B.

In the covariance PCA biplot for Site A, a total of 51% of variance in the distribution of prokaryotic taxa across different samples was explained by PC1 (39%) and PC2 (12%). PC1 was significantly negatively related to pH and OM content, and positively correlated with total Fe while PC2 was significantly negatively related to OM content and HCl-extractable Fe (Fig. 5b; Table 1). Similarly, in Site B, PC1 explained 38% of the variation in microbial communities and PC2 explained 19% (Fig. 5d). PC1 was negatively correlated with pH, total S, total Ni, and HCl-extractable S, and positively associated with total Fe, total Cu, and HCl-extractable Fe. PC2 was mainly driven by OM content, and HCl-extractable Fe (Table 1).

4. Discussion

We demonstrate significant compositional differences that indicate unique associations among microbial communities of barren and vegetated areas of mine waste soils. Barren areas contained less diverse microbial communities, which were mainly composed of taxa affiliated with known chemolithotrophic lineages. Microbial communities in the vegetated areas were more heterogeneous, with higher abundances of potential decomposers, plant growth-promoting bacteria, and fungi. This was a consistent pattern in both sampling locations. Similar to other studies (Honeker et al., 2017; Jones et al., 2017; Nannipieri et al., 2008), much of the variability in overall microbial communities was associated with the variability in vegetation cover and geochemical properties of the mine waste soils. In addition, a higher extent of association was observed among the microbial groups in the barren tailings, which was related to the highly acidic pH, high metal concentrations, and very low OM content of the substrates in barren areas of the sites.

The barren tailings/mine wastes in our study were acidic, with concentrations of HCl-extractable Fe, Cu, and Ni that exceeded thresholds for plant growth (Li et al., 2016a; Theriault and Nkongolo,

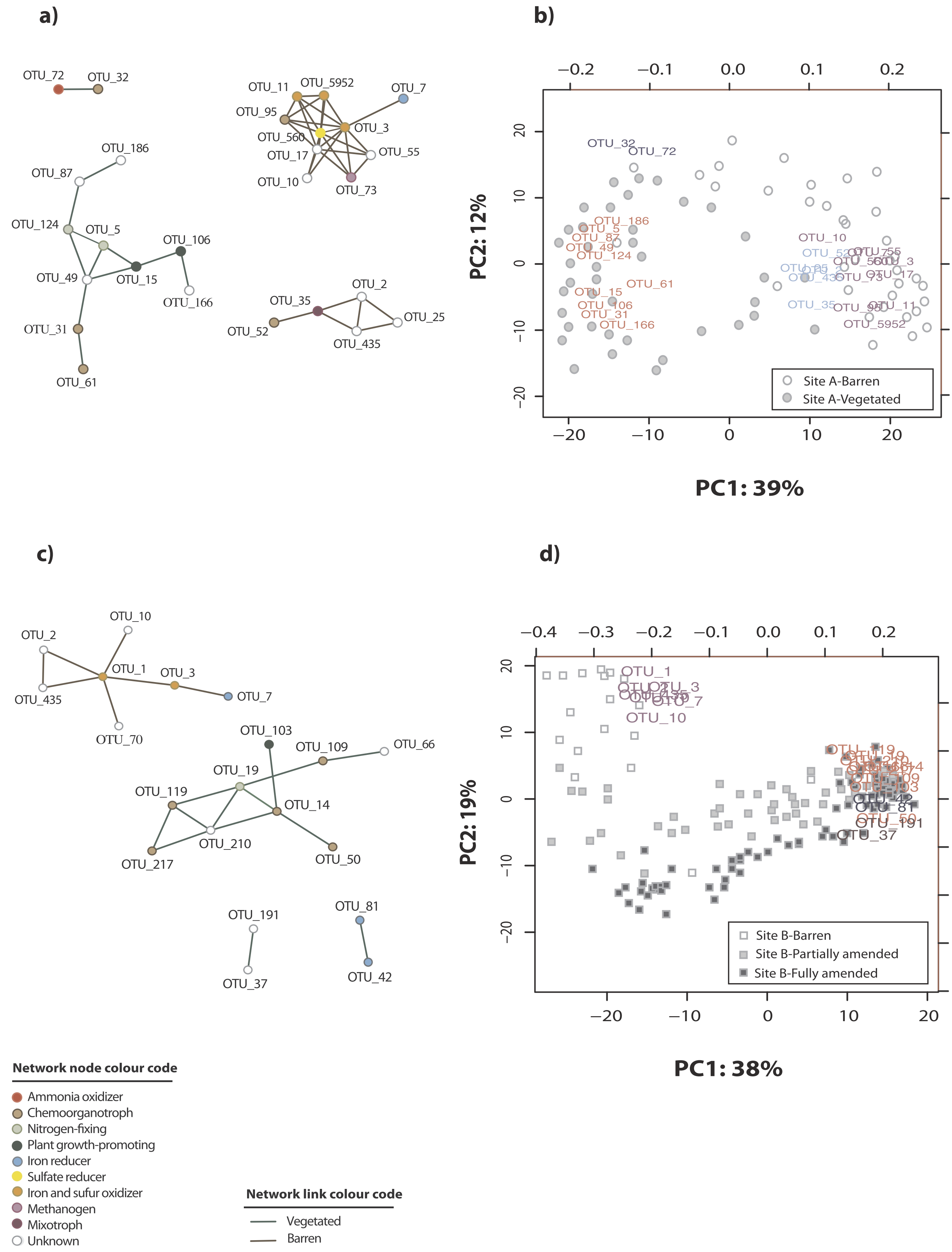

Fig. 5. Co-occurrence structure and network association of highly correlated prokaryotic taxa as shown in (a) ordinal diagrams and (b) covariance biplot for Site A, and (c) ordinal diagrams and (d) covariance biplot for Site B. Nodes in each network were determined with phi < 0.2 and are coloured by the purported function of the taxa, while the links among OTUs are coloured by the location where these networks were detected (i.e. barren vs vegetated areas of the sites).

Table 1

Summary of statistical results for the multiple linear regression modeling the relationship between geochemical properties and the structure of prokaryotic communities through covariance PCA biplot. Adjusted R^2 (partial correlation coefficient) shows the unique contribution of each geochemical property relative to other geochemical properties to the total variance observed in the distribution of prokaryotic taxa and their co-occurrence across different sections of site A and site B. All significant associations between geochemical properties and the structure of prokaryotic communities in each site are shown in bold.

	Site A						Site B					
	PC1			PC2			PC1			PC2		
Variable	*P*-value	F-value	Adjusted R^2	*P*-value	*F*-value	Adjusted R^2	*P*-value	F-value	Adjusted R^2	*P*-value	F-value	Adjusted R^2
pH	**0.007**	7.74	−0.351	0.708	0.141	0.05	< **0.001**	112	−0.71	0.26	1.28	0.107
OM (%)	< **0.001**	14.2	−0.453	**0.001**	11.5	−0.416	0.983	0.005	0.002	**0.031**	4.77	−0.203
Total S	0.870	0.027	−0.022	0.44	0.604	0.104	**0.008**	7.13	−0.246	0.43	0.625	0.075
Total Fe	< **0.001**	14.09	0.462	0.264	1.27	−0.15	**0.022**	5.37	0.215	0.527	0.401	0.06
Total Cu	0.486	0.489	0.093	0.816	0.054	−0.031	< **0.001**	12.4	0.318	0.176	1.85	−0.128
Total Ni	0.254	1.32	−0.153	0.709	0.14	−0.05	**0.001**	10.5	−0.296	0.924	0.009	0.009
Extractable S	0.961	0.002	0.006	0.635	0.227	−0.064	**0.005**	8	−0.26	0.33	0.954	−0.092
Extractable Fe	1.00	0.00	0.004	**0.044**	4.21	−0.266	**0.017**	5.78	0.223	< **0.001**	35.5	0.494
Extractable Cu	0.921	0.009	0.013	0.428	0.636	0.106	0.915	0.011	0.01	0.276	1.19	−0.103
Extractable Ni	0.665	0.188	0.058	0.951	0.003	−0.008	0.812	0.056	0.022	0.086	2.99	0.162

2016). The major microbial groups colonizing the carbon-limited barren areas were purported Fe- and S- oxidizers and reducers. This is consistent with the geochemical properties of the substrate, and the metabolic capabilities of these microorganisms, which drive the weathering of sulfide minerals, and in line with the results of other studies in other mining environments (Chen et al., 2016; Jones et al., 2017; Korehi et al., 2013, 2014).

The ecological network analysis revealed more links attached to each OTU, and among prokaryotic OTUs in the barren areas particularly in Site A. The positive correlations between the concentrations of elements of concern and the number of links among prokaryotic taxa in metal-impacted river sediments and farmlands have been previously reported (Li et al., 2017; Yin et al., 2015). Such positive associations among prokaryotic taxa might reflect their syntrophic relationships, or preferred environmental conditions (Raes and Bork, 2008). In particular, the co-existence of potential Fe and S oxidizers and reducers that have close connections to each other and to purported chemoorganotrophs in the barren areas of Site A might reflect their specific partnership in which the assimilation of the secreted metabolites by the consumer microorganisms can promote the energy metabolism of the producer microorganisms (Kato et al., 2012; Kouzuma et al., 2015; Liu et al., 2011; Shi et al., 2016b). During the oxidation of metal sulfide minerals such as pyrite, the acidophilic Fe and S oxidizing bacteria, or archaea, transform reduced elements to ferric Fe, sulfate and elemental S. These constituents in turn support the growth of Fe and S reducers (Blackmore et al., 2018; Jerez, 2017). However, the high concentration of glucose produced through the Calvin cycle by these lithoautotrophs inhibits microbially-mediated Fe oxidation (Liu et al., 2011; Marchand and Silverstein, 2003). The chemoorganotrophs that consume the organic inhibitory compounds as the sole source of energy under such nutrient limited conditions can potentially diminish the inhibitory effects of the metabolic by-products of the Calvin cycle on iron oxidation. At the same time, they must be able to cope with the nutrient deficiencies inherent to their environment (Liu et al., 2011; Marchand and Silverstein, 2003). Our investigations of microbial communities in the surface of these soils demonstrate the potential for oxidation and reduction reactions to occur in close proximity. The concept of the "thermodynamic ladder", in which redox chemistry in natural environments arranges itself in vertically stratified zones, arguably does not accurately reflect how microbes regulate redox reactions (Bethke et al., 2011). For example, Sobolev and Roden (2002) demonstrated the close spatial relationship between Fe reduction and oxidation. We have shown that microbes that have very different energy and metabolic requirements, including strict anaerobes, can coexist at the soil surface despite apparent oxidizing conditions. Also, these dynamic redox environments favour mixotrophic species that are able to switch between oxidative and reductive metabolism (i.e. some *Acidiphilium* spp.) (Bridge and Johnson, 2000). Given the existence of soil microbes in biofilms (Davey and O'toole, 2000; Redmile-Gordon et al., 2014), it is feasible that biofilm communities create conditions that allow the redox cycling of elements and the exchange of carbon sources to occur at very small spatial scales.

It is surprising that the network associations among similar functional groups contained lower microbial diversity and fewer ecological connections in the barren areas of Site B than Site A, despite higher metal concentrations observed in Site B compared to Site A. For instance, the dominant chemoorganotrophs identified in this section of the site did not seem to have high associations with other microorganisms in the community. The analysis of Eigenvalues showed that geochemical properties, and the OM concentration of the tailings/mine waste were significant factors regulating the topological positions of prokaryotic taxa and their association in different areas of the mine tailings. In barren areas of Site B, the OM concentration of the mine waste was almost seven times higher than the OM concentration in barren areas of Site A. This is likely due to the proximity of these barren areas to the amended and vegetated sections, which would receive more aeolian deposition of OM. The benefits of increased OM in tailings on microbial communities (Asemaninejad et al., 2018), and metabolic activities of Fe and sulfate reducing bacteria have been previously reported (Kumar et al., 2013). The results of our metabarcoding studies suggest that even when the concentrations of elements of concern are relatively high, and in the absence of vegetation, high OM (~8%) in tailings might ease the impact of these environmental stressors and nutrient deficiencies for microbial growth and activities. This may promote the establishment of heterotrophic microbial groups that help to alleviate some of the inhibitory conditions in the mine waste for plant establishment/survival. These effects were found to be similar to the effects of vegetation on the extent of association among microbial communities observed in our study.

In both sampling locations, the vegetated tailings/mine wastes demonstrated higher OTU richness and lower level of network association among microbial groups than the barren areas in Site A. This is likely related to the higher pH, higher OM concentration, and much lower total metal and S concentrations in these areas compared with the barren areas as demonstrated in both redundancy and network association analyses in our study. There is an established strong negative correlation between acidity and toxic concentration of many elements, and the phylogenetic richness of microorganisms in both soil and tailings (Bier et al., 2015; Chen et al., 2013; Jones et al., 2017; Xie et al., 2016). In addition, in the root zone of plants, microorganisms are linked to plant communities through diverse associations (i.e. symbiotic to antagonistic), or as decomposers of plant products. These can partly explain the higher richness and heterogeneity of microbial communities in the vegetated areas (Khan et al., 2000; Li et al., 2016b; Shen et al., 2014). Also, both microbial diversity and richness were higher in PA and FA of Site B than vegetated areas of Site A, corresponding to the differences in pH and plant diversity between the vegetated areas of the two sites. Compared with the results reported in other studies of mine tailings (Fan et al., 2018; Hao et al., 2012; Huang et al., 2011; Rastogi et al., 2010), we observed an abundance of OTUs with high affinities to *Herbaspirillum lusitanum*, *Microvirga*, *Mesorhizobium* and *Bradyrhizobium* that are known nitrogen-fixing bacteria and acid tolerant heterotrophic microbial consortia belonging to Acidobacteria and Alphaproteobacteria in the vegetated tailings of both sampling sites.

The assortment of potential nitrogen-fixing bacteria, acid tolerant heterotrophic bacteria, and plant-growth-promoting bacteria such as OTUs closely related to *Burkholderia* and *Streptomyces* in the vegetated tailings might reflect their common niche requirements which are directly or indirectly related to the presence of plants, assembling them into similar environments (Green et al., 2008). Specifically, in Site B, the OTUs closely related to *Geobacter* were also highly abundant in the fully amended sections. *Geobacter* spp. are neutrophilic, but have a wide range of pH tolerance, and are generally prominent among bacteria that drive metal redox transformations in many metal-impacted freshwater ecosystems (Bond et al., 2012; Williams et al., 2011; Wu et al., 2006). They are capable of reducing ferric Fe to ferrous Fe, using acetate or other simple organic compounds as electron donors (Hedrich et al., 2011; Williams et al., 2011), and thereby limiting the impacts of metal contamination on plant growth through immobilization of metals. In particular, for areas with vegetated mine waste materials that have persistent high metal concentrations, such as those we investigated, the appearance of metal reducers such as *Geobacter* in microsites with reducing conditions are beneficial for the bioimmobilization of such metalliferous substrates through adsorption to iron oxides and iron sulfides.

In contrast, fungal richness showed less distinct patterns across both sites. For the majority of samples, and particularly those in Site A, we could not get adequate sequencing depth from fungal ITS1 amplicons. This might have been due to a very low biomass of fungi (below detection level), potentially caused by low OM, high metal content, and a very sparse distribution of plants at this site (Anahid et al., 2011; Malik et al., 2016). As such, no significant differences in fungal richness and community composition were found between barren and vegetated areas of the site. However, the vegetated mine waste at Site B, particularly fully amended section which had higher OM content and denser vegetation than Site A, was dominated by fungal sequences closely related to *Cadophora*, which is a root endophytic fungus that degrades plant cell walls (Knapp et al., 2018). The sequences closely affiliated with *Cladonia* (a lichen) were also in abundance in the vegetated areas of this site, and were more common in the partially amended areas. The lichen mat along with some moss and plant litter accumulation in this section of the site might have contributed to the higher OM content observed in these areas versus the barren and fully amended areas. Greater OM contents could adsorb metal particles from the surrounding soil that may otherwise leach off site (Tipping et al., 2003). Additionally, the *Cladonia* are lichens capable of accumulating heavy metals from wet and dry atmospheric deposition and surface water in their thalli (Sueoka et al., 2016; Tsurykau and Golubkov, 2015). All these factors might have collectively driven the extreme average levels of total Cu and Ni in partially amended areas, and accordingly might have also led to the underestimation of the true concentrations of bioavailable metals in this section of the site (Rieuwerts et al., 1998). The OTUs closely related to *Paxillus* and *Lactarius*, known ectomycorrhizal fungi, were also highly abundant in the vegetated areas of Site B. They form mutualistic associations with conifers and broad-leaved trees (Tedersoo et al., 2010), and similar to *Cladonia*, play an important role in metal accumulation and plant tolerance in impacted soils (Khan et al., 2000; Kuiper et al., 2004). Ectomycorrhizal fungi can also enhance water and nutrient uptake by plants through the extra-matrical fungal hyphae extended into the surrounding soil (Hock, 2012). Additionally, the labile carbon input from the plant root exudates, and fungal extra-matrical hyphae in the tailings substrate, supplies the primary source of energy for saprotrophs and heterotrophic consortia that can stimulate the microbial decomposition of more complex organic compounds. This can potentially lead to an increase in the organic matter content of the tailings (de Vries and Caruso, 2016). All of these features make these members of the microbial community of interest for application in the mine tailings as microbial inoculum or in conjunction with phytoestablishment to relieve the stress of metal toxicity and nutrient deficiencies (Khan et al., 2000; Kuiper et al., 2004).

5. Conclusions

Overall, our study describes taxa that may aid in the succession, establishment, and persistence of vegetation on mine wastes (with and without chemical amendments) in northern temperate zones. Our results also demonstrate higher interspecies microbial associations in the challenging conditions present in sulfidic mine wastes. The transition in the microbial community structure towards more heterotrophic groups, and the extent of the association among microbial populations in the inhospitable environments of mine wastes are significantly correlated to vegetation and OM content of the tailings. Further genetic and biochemical analyses at other sites with established chemical and vegetation gradients are required to confirm our observations. This will also help to identify the particular adaptations of microbes in these environments and the optimal conditions for their growth and function that facilitate plant growth on mine wastes.

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The raw sequences were deposited in the European Nucleotide Archive (ENA) under accession number PRJEB30328. The processed sequence data can be found on figshare with DOI number: https://doi.org/10.6084/m9.figshare.9232610.

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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Authors' contribution

Primary execution of the study, microbial community data analyses and writing of the manuscript: AA. Field support and geochemical data analyses: KM. Support the project design, evaluation of analyses and writing: NB, NM, and SG. Conceptual project design, and evaluation of the data and writing: DC, SW, AA.

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