Diversity and complexity of microbial communities from a chlor-alkali tailings dump

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A B S T R A C T
Revegetation of the tailings dumps produced by various industrial activities is necessary to prevent dust storms and erosion and represents a great challenge for ecological restoration. Little is known about the microbial colonisation and community structure of revegetated tailings following site exploitation. Here, we report the sequencing of 16S rRNA and internal transcribed spacer (ITS) fungal RNA gene amplicons from chlor-alkali residue and from an adjacent undisturbed soil to define the composition and assembly of the rhizosphere microbial communities. After quality filtering, a total of 72,373 and 89,929 bacterial sequences and 122,618 and 111,209 fungal sequences remained for community analysis from undisturbed soil and tailings dump samples, respectively. These reads were affiliated with 45 bacterial and 9 fungal phyla and 113 bacterial and 35 fungal classes. We observed a clear dominance of Gammaproteobacteria at our study site (24% of total sequences), especially of the Pseudomonas genera (72% of Gammaproteobacteria sequences), together with the dominance of a few fungal taxa, such as Hebeloma and Geopora. However, we also noticed that the core microbiome comprised 64.4% and 62.4% of the bacterial and fungal genera, respectively, despite marked differences in soil physico-chemical properties. A heatmap of correlations between soil parameters and taxa confirmed that approximately 50% of the 33 dominant taxa colonised both types of soil. We further demonstrated that the global bacterial-fungal network topology of the dump approximated that of the undisturbed soil. Our approach illuminates the importance of studying more than just a single component of the microbial community and represents a step forward in uncovering the microbial ecology of disturbed environments beyond what is generally found in conventional studies. Our study also provides novel global community proxies that have led us to conclude that environmental filtering is more likely to occur through the activity of tree roots rather than as a result of specific soil characteristics and could be an important force in the assembly of at least some microbial communities.

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1. Introduction

More than 300 million tons of mining and quarrying waste is estimated to be generated annually in the European Union according to a reference document produced by the European Commission (European Commission, 2009) as a follow-up to the tailings dam bursts that occurred at Baia Mare in Romania and Aznalcollar in
Spain. Large dumps that are used to store industrial tailings can generate significant unvegetated surfaces after they are abandoned, suggesting that these regions become biologically infertile. Microorganisms play crucial roles in energy transfer, the mobilisation and cycling of nutrients, the establishment of plants, and ecosystem perennity (Paul, 2007). They can act as a selective force behind plant installation success and growth (Morris et al., 2007) because of the high level of interaction that occurs between plants and microorganisms in the rhizosphere region.

Successful revegetation of tailings has been observed following site exploitation, and insights into the microbial communities associated have been provided in previous papers (Epelde et al., 2010; Sulovicz et al., 2011). In addition to estimating the microbial fertility of contaminated tailings, knowledge of the associated microbial compartment could be used to help predicting the potential recovery of disturbed lands (Kozdrić, 2000). Most of the existing data on the microbial populations that are associated with unvegetated soils have described these populations using molecular tools such as terminal reduction fragment length polymorphism (T-RFLP) and targeted sequencing of major operational taxonomic units (OTU). However, these methods provide an approximated estimate of OTU diversity and only identify the dominant and rare taxa (Rieder and Frey, 2013). Thus, a greater understanding of the composition of the whole community (including both dominant and rare taxa) is necessary for identifying both resistant and sensitive organisms in contaminated and disturbed soils. Interactions between bacteria, fungi and plants have been shown to contribute to microbial community stability (Bonfante and Anca, 2009; Bell et al., 2014) and some, although scarce, studies have investigated the bacterial and fungal communities that arise in unvegetated environments (Li et al., 2015).

However, the nature of microbial community structure is greatly determined by various characteristics of the soil, including pH, moisture levels (Ansola et al., 2014) and/or contamination (Müller et al., 2001; Rasmussen and Sørensen, 2001; Turpeinen et al., 2004; Lorenz et al., 2006; Yergeau et al., 2014), each of which can impose constraints on the establishment of plant–microbe interactions. It may therefore be hypothesized that the composition and structure of microbial community will be dependent upon the soil characteristics and presence of contaminants. In order to check this hypothesis, we compared fungal and bacterial communities from a chlor-alkali tailings dump to an adjacent undisturbed forest soil. We obtained amplicon sequencing data to provide information about these two major microbial compartments to determine how they were associated with plant establishment and soil characteristics. The data were then analysed in terms of diversity, composition, and co-occurrence network structure to quantify various properties of the communities, an approach that has never been applied to anthropogenic soils.

2. Materials and methods

2.1. Site and sampling

Tailings dumps are facilities designed to receive and store tailings, which is the refuse material produced from the sedimentation of minerals. The site used in the current study was exploited as a storage area from the 1950s to 2003 to contain sediments from the adjacent sedimentation basin and contained a total surface area of 12 ha. This region is characterised by an annual average temperature of 11 °C and an average 75% humidity. The sediments originated from effluents produced during electrolytic processes that were associated with a mercury cell chlor-alkali process that was used to produce chlorine until 2012. The tailings dump was confined by 5 m high dikes to preserve the surrounding environment and contained a multi-contaminated calcareous and alkaline anthropogenic soil. The soil from an adjacent undisturbed forest was not affected by anthropogenic activity and was therefore used as the undisturbed soil control in this study. Each region was dominated by slightly different plant covers. In the tailings dump, the plant cover was primarily comprised of woody species such as Salicaceae (poplar and willow), Fabaceae (black locust), and Caprifoliaceae (black elder), in addition to herbaceous genera such as Poaceae (phragmites). The undisturbed area was also covered by Salicaceae and Fabaceae species, and comprised additional tree species from the Fagaceae and Betulaceae families. Phragmites were absent at the control location, whereas other species from i.e. the Rubus, Senecio, Sambucus, Eupatorium, Urtica genera constituted the forest floor vegetation. Both areas experienced similar climatic conditions.

Rhizosphere soil, the thin layer of soil where roots and soil organisms interact in myriad ways (Richter et al., 2007), was sampled from seven Salicaceous trees in each area (tailings dumps and undisturbed soil) in April of 2013. All samples were obtained over a one-day period to reduce any heterogeneity imparted by climatic conditions. After the removal of litter, soils that were attached to roots were collected from the upper 20 cm layer of soil from under the crown projection of the trees. A total of 3 pseudo-replicates were sampled from each tree and mixed to obtain a soil composite. Samples were dried for 24 h at 24 °C under airflow and hand-crushed at 2 mm for homogenisation. Subsamples were stored at either 4 °C for molecular analysis or at ambient temperature (24 °C ± 1) for physical and chemical analyses.

2.2. Soil physico-chemical properties

The extraction of metal trace elements (TE) was performed on a 0.5 g aliquot of each soil sample via mineralisation in 2 ml HCl, 5 ml HNO3 and 33 ml deionised water (modified from the ISO 11466 French Norm) for 260 min at 100 °C in a Digiprep unit (SCP Science Corporate Headquarters, USA). TE (Al, As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Zn) concentrations were determined using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, Thermo Fischer Scientific, Inc., USA) and all samples were analysed in triplicate and run together with certified reference material (a loamy clay soil).

The physico-chemical characteristics that were measured included particle size (French Norm X 31–107), pH (ISO 10 390 French Norm), total carbonates (ISO 10 693 French Norm), total organic carbon and organic matter (OM, ISO 14 235 French Norm), total nitrogen (Ntot, DUMAS method ISO 13 878 French Norm), C/N ratios (ISO 13 878 French Norm), total available phosphorus (Joret Hebert method French Norm X 31–161), cationic exchangeable capacity (CEC, French Norm X 31–130) and several oligo-elements (French Norm X 31–108).

2.3. DNA extraction and amplification

DNA was extracted from a 10 g soil sample using the PowerMax® Soil DNA Isolation Kit (MO-BIO Laboratories, Inc., Carlsbad, CA USA; Yergeau et al., 2014). DNA quality was assessed using a 1% (w/v) agarose gel and by measuring 260/280 nm and 260/230 nm ratios using a BioPhotometer (Eppendorf, AG, Hamburg). DNA concentrations were quantified by measuring the absorbance at 260 nm using the BioPhotometer.

PCR amplification of the partial 16S rRNA and ITS genes was performed using the bacterial 520F (5'-AGC AGC CGC GGT GAT-3') and 798R (5'-CAG CCG ATT TAA TCC TGT T-3') primers and the fungal ITS1F (5’-CTT GGT CAT TTA GAA GAA CTA A-3’) and 58A2R (5’-CTG CTT TCA TCG AT-3’) primers, respectively. The
amplification of 16S rRNA was carried out using 100 ng of template DNA, 0.5 mM of each primer, 200 mM dNTPs (Euromedex, France), 1 μl of Phire Hot Start II DNA Polymerase II, and 10 μl of 5X Phire reaction buffer (Thermo Fisher Scientific, Inc., USA) in a final volume of 50 μl. The cycling conditions involved an initial 3 min denaturing step at 98 °C followed by 25 cycles of 5 s at 98 °C, 5 s at 55 °C, and 5 s at 72 °C, with a final elongation step of 1 min at 72 °C. The same protocol was performed to amplify ITS genes, using a 15 ng of template DNA in this case. The cycling conditions for these genes included an initial 3 min denaturing step at 98 °C followed by 30 cycles of 5 s at 98 °C, 5 s at 60 °C, and 15 s at 72 °C, with a final elongation step of 1 min at 72 °C (Yergeau et al., 2012).

2.4. Amplicon sequencing

Both primer pairs contained the 10 bp multiplex identifiers and adaptor sequences necessary for Ion Torrent sequencing, as previously described (Yergeau et al., 2012). Sequencing of the pooled library was performed using the Ion Torrent Personal Genome Machine system (Life Technologies, Burlington, ON, Canada). High-throughput sequencing of the taxonomically informative 16S rRNA gene provides a powerful approach for exploring microbial diversity (Salipante et al., 2014). The sequence data were processed using Mothur (Schloss et al., 2009). The fastq file was transformed into fasta and qual files using the fastq.info() command, after which the sequences were trimmed using the trim.seqs() command, with the following parameters: minimum length = 100 bp, quality window size = 10, quality window average = 20 (Liu et al., 2007, 2008). The quality-filtered data were then classified according to the GreenGenes taxonomy using the classify.seqs() command with the method = wang and cutoff = 50 options. The quality-filtered sequences were also clustered in CD-HIT (Li and Godzik, 2006) with a cutoff of 97%. The groups were then imported into Mothur and the diversity indices were calculated. The weighted, normalised UniFrac distances between each sample pair were calculated using the FastUniFrac website (Hamady et al., 2010) and based on the August 2013 release of the GreenGenes core data set. The procedure described above can be considered comparable to 454 pyrosequencing and has been fully validated (Yergeau et al., 2012).

2.5. Real-time PCR quantification

The quantity of total bacteria and the quantity of bacteria belonging to the Pseudomonas genus were both quantified by targeting 16S rDNA (bacteria) via real-time PCR; gene copy numbers were quantified with the primer sets 968F/1401R (Felske et al., 1998), Pse435F/Pse686R (Bergmark et al., 2012) and FF390R/Fung5F (Lu�ers et al., 2004), respectively. The real-time PCR experiments were conducted using an iCycler iQ (Bio-Rad) outfitted with iCycler Optical System Interface software (version 2.3). The final volume used (20 μl) contained 10 μl of 2X iQ SYBR Green SuperMix (Biorad), 0.4 μl of each primer, 0.06% (w/v) of bovine serum albumin (BSA), 0.2 μl of DMSO, 40 ng of T4 gp32 (MP Biologicals) and 1 μl of DNA. The qPCR program consisted of 5 min at 95 °C followed by four steps of 40 cycles of 20 s at 95 °C, 20 s at the primer-specific annealing temperature (56 °C, 60 °C and 50 °C for the 968F/1401R, Pse435F/Pse686R and FF390R/Fung5F primer sets, respectively), 30 s at 72 °C, and 10 s at 80 °C to dissociate primer dimers and capture the fluorescence intensity of the SYBR green. The procedure concluded with a final elongation step of 5 min at 72 °C. After the procedure was complete a melting curve analysis was performed from 60 °C to 95 °C with a temperature increase of 0.5 °C every 5 s. A negative control was included in each of the qPCR assays and all were performed in triplicate. The linearized plasmids were serially diluted (from 10^8 to 10^1 target gene copies/μl) and the relevant target gene inserts were used to create the standard curves. The presence of PCR inhibitors was evaluated by mixing 1 μl of environmental DNA with 1 μl of 10^6 copies of lambda standard plasmid and compared to the lambda standard curve (Cébron et al., 2008); no inhibitory effect was observed. Real-time PCR quantification was also used to provide information about molecular biomass, which was expressed as copy number per nanogram of DNA.

2.6. Statistical procedures

All statistical analyses were performed using R software v. 3.0.2 (R Development Core Team, 2013). Physico-chemical characterisation and TE data (Al, As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Zn) were compared between undisturbed soil and tailings dump samples using Principal Component Analyses and an intergroup comparison test validated by a Monte Carlo permutation test (ade4 R package). The Shapiro test and the Bartlett test were used to respectively verify the normality and homoscedasticity of the data, and we compared each trace element using either ANOVA or Kruskal–Wallis tests.

The numerical analysis of the biological component of the soil was based on the number of sequences that were obtained for each genus of bacterial or fungal lineage. Various aspects of the microbial communities were compared between the undisturbed soil and tailings dump samples. Four diversity indices were initially calculated (pgirmess R package; see Supplementary Table 1 for detailed calculation methods), including the following: i) the genus richness, which represented the total number of the observed genera; ii) the Shannon and inverse Simpson diversities; iii) the genus evenness computed as Hill’s ratios between the Shannon or inverse Simpson indices and the richness, wherein the inverse Simpson evenness index represents the proportion of the dominant genus within the community irrespective of genus richness and the Shannon evenness index highlights the abundance of genera; and iv) the Pielou evenness, which is a ratio between the Shannon entropy and the maximal entropy that can be used to measure equitability independently of richness, thereby indicating potential dominance. All indices were compared between the tailings dump and the undisturbed soil via the Kruskal–Wallis test for non-parametric data. The detailed structures of the communities that were present in each of the areas were then compared using a Kruskal–Wallis test, with a Bonferroni correction of the p-value for each genus. Finally, community composition was determined via Correspondence Analysis, followed by an intergroup comparison test that was validated by a Monte Carlo permutation test (ade4 R package). Redundancy analysis of the physico-chemical characteristics and TE was conducted using the vegan R package (Dray et al., 2007) to link TE contaminants to other soil parameters according to a method that has been previously described (Braak and Smilauer, 1998).

The number of fungal and bacterial sequences was correlated to soil parameters using Spearman correlation for the dominant taxa (>2% of total sequences). The sensitivity to soil parameters of major fungal and bacterial taxa was compared using the UPGMA clustering. The resulting clustering trees were paired a heatmap of correlation data created with the heatmap2 function from the gplots R package.

Semi-quantitative data obtained from the amplicon sequencing of the bacterial and fungal communities that were present in the soil samples were used to model the microbial co-occurrence networks of the undisturbed soil and tailings dump. A total of seven replicates per area were used to calculate the Spearman coefficient correlations for each pair of genera (Hmisc R package) (Barberán et al., 2012). The matrix of statistically significant
coefficients (p-value ≤ 5%, r > 0.80) was then transformed into an adjacency matrix based on the presence or absence of links between pairs. This new matrix was used to build the network (statnet R package) and to calculate the following network indices (as defined in Supplementary Table 1): node number (N), link number (L), average degree (aD), maximal degree (mD), average betweenness (aB), maximal betweenness (mB), connectance (C), connectedness (Cd), transitivity (T) and clusters (G). To compare the microbial communities of the two areas a total of seven network replicates were created using only six of seven field replicates for each; indices were calculated for all network replicates. The difference in the calculated distributions (n = 7) of the indices was quantified using the Kruskal–Wallis test for non-parametric data for the few replicates that required such analysis or ANOVA when the data followed a normal distribution.

3. Results

3.1. Two contrasting environments were depicted following physico-chemical analysis

To provide geochemical and mineralogical context for our microbial community analysis we compared the physico-chemical characteristics of topsoil (0–20 cm layer) taken from the tailings dump to the adjacent undisturbed soil. Physical analysis (see Supplementary Table 2 for detailed soil analyses) showed that tailings dumps contained a significantly greater proportion of thin silt, whereas undisturbed soils contained more clay and coarse sand (N = 14; p-values = 0.045, 0.003 and 0.020, respectively, for clay, thin silt and coarse sand). The above detailed physical parameters were further investigated using multivariate analysis (Fig. 1a). Chemical analysis revealed that pH, calcium carbonate (CaCO₃) concentration and exchangeable calcium oxide (CaOex) were all significantly higher in the soil of the tailings dump, whereas CEC was significantly enhanced in undisturbed soil (ANOVA or Kruskal–Wallis for non-parametric data, p-value < 0.0001; Supplementary Table 2). Interestingly, no significant differences were found between the two soils with respect to the total quantities of organic carbon and nitrogen, the total organic matter (OM), or the C/N ratios; therefore, these variables could not be used to discriminate between samples taken from the tailings dump versus the adjacent soil (Fig. 1a). ICP-AES analyses revealed that the quantities of total Hg and total As (p-value < 0.0001) were significantly increased (see Supplementary Table 3 for detailed TE soil analyses) in the tailings dump. Other TE were weakly represented in the tailings dump compared to the adjacent soil (Fig. 1b). A redundancy analysis showed that silt, CaCO₃, pH and exchangeable CaO, and total Hg and As were all positively correlated in the tailings dump (p-value = 0.04, adjusted R² = 0.88; Supplementary Fig. 1).

3.2. Analysis of bacterial and fungi community diversity

A total of 186,558 16S bacterial and 398,578 ITS fungal raw Ion Torrent reads were obtained from 14 DNA soil samples. After quality filtering and target extraction (demultiplexing, trimming and denoising) a total of 72,373 and 89,929 bacterial sequences and 122,618 and 111,209 fungal sequences remained for community analysis of undisturbed soil and tailings dump samples, respectively. These reads were found to be associated with 45 bacterial and 9 fungal phyla and 113 bacterial and 35 fungal classes (Fig. 2). To further compare the bacterial and fungal communities residing within the tailings dump and undisturbed soil samples we calculated richness and diversity indices (Supplementary Table 1) and found that there was a significant site-specific effect on the bacterial variables that were examined, particularly with respect to richness (P = 0.035) and inverse Simpson evenness (P = 0.048; Fig. 3a), including a trend of increasing soil bacteria richness in the tailings dump of approximately 20%. In contrast, neither the calculations of soil bacteria Pielou evenness nor those representing Shannon evenness were found to be significantly different between the soils. There were also no remarkable differences found between

![Fig. 1](image-url) Principal component analysis showing the positions of undisturbed soil (U) and tailings dump (T) samples according to site environmental characteristics. a. Results from the between test followed by the Monte Carlo permutation test showed that samples taken from undisturbed soil and an adjacent tailings dump had significantly different physico-chemical characteristics (33.2% variance explained, P = 0.001). The detailed results of soil parameter analyses are provided in Supplementary Table 2. b. Results from the between test followed by the Monte Carlo permutation test demonstrated that samples taken from undisturbed soil and an adjacent tailings dump had significantly different chemical characteristics (35.4% variance explained, P = 0.001). The detailed results of ICP-AES analyses are provided in Supplementary Table 3.
**Fig. 2.** Microbial community composition at the phylum/class level averaged for each site (N = 7), based on 16S rRNA and ITS gene sequencing of samples taken from undisturbed soil and an adjacent tailings dump.

**Fig. 3.** Diversity indices and composition of bacterial and fungal communities found within samples taken from undisturbed soil and an adjacent tailings dump. **a,** Diversity indices of the bacterial community. **b,** Diversity indices of the fungal community. The mean values and standard deviations of the diversity indices (as defined in Supplementary Table 1) are provided in addition to the P-value of the Kruskal–Wallis comparison test. **c,** Weighted Venn diagram of the percentage of bacterial genera that were either shared between (not significantly different at P = 0.05) or specific (significantly different at P = 0.05) to the two sites. **d,** Weighted Venn diagram of the percentage of fungal genera that were either shared between (not significantly different at P = 0.05) or specific (significantly different at P = 0.05) to the two sites.
the fungal communities that were residing in the two study areas for any of the four calculated diversity indices that were used in our study (Fig. 3b). We found that 599 bacterial and 500 fungal genera, and 386 bacterial genera (i.e., 64.4%, Fig. 3c) and 312 fungal genera (i.e., 62.4%, Fig. 3d) were shared between the tailings dump and undisturbed soil samples, respectively. Thus, on a global scale 63.4% genera were shared. We used qPCR to further quantify 16S and 18S to lend additional support to the conclusions derived from these data, which allowed us to estimate bacterial/fungal molecular biomass ratios. The ratios were 43.3 in the tailings dump and 40.3 in the undisturbed soil, respectively, and the difference between the two was not found to be significant.

3.3. Changes in the compositions of the bacterial and fungal communities

We used correspondence analysis followed by an intergroup comparison to establish that the bacterial and fungal communities residing within the tailings dump and the undisturbed soil samples each had significantly different compositions (Monte Carlo permutation test, P = 0.001; Supplementary Fig. 2), which were primarily influenced by the presence of a few specific taxa within each soil. The majority of the emergent bacterial genera that were found within the tailings dump represented less than 2% of the total sequences; a noticeable exception was the *Pseudomonas* genus, which represented 17.0% of the sequences in the tailings dump and only 0.9% of the sequences in undisturbed soil (Fig. 4). We further confirmed the large-scale *Pseudomonas* colonisation of the tailings dump by using qPCR. The results indicated an average molecular biomass of 26,818 copies/ng DNA in the tailings dump compared with 1820 copies/ng DNA in undisturbed soil. In contrast, the bacterial genus MC18 was dominant in the undisturbed soil (2.3% of total DNA sequences) and was not significantly represented in the tailings dump (0.007% of total DNA sequences; Fig. 4). The bacterial genus *Acidobacteria* g.1 was also highly present in the undisturbed soil (3.1% of total DNA sequences) and barely represented in the tailings dump (0.0004% of total DNA sequences). Most of the fungal genera that were present in the undisturbed soil were also detected in significant proportions in the tailings dump (Fig. 3d); however, fungi of the Agaricomycete family represented 6.6% of the DNA sequences that were found in the tailings dump and only 1.5% of the sequences found in the undisturbed soil (Fig. 4).

![Fig. 4. Soil-microbe relationships. Left. Heatmap of the Spearman correlation coefficient values computed for bacterial or fungal genera and soil parameters using relative microbial abundance data >2%. The colour key for the correlation values is shown on the top right of the figure; non-significant correlations are in grey, positive correlations are in green, negative correlations are in red. Dendrograms of hierarchical cluster analysis grouping genera and soil parameters are also shown on the left and at the top, respectively. b. Relative abundance of dominant microbial genera (>2%) in tailings dump and undisturbed soil samples. The colour key for the relative abundance is shown on the top right of the figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)](image-url)
3.4. Microbial co-occurrence networks

We built co-occurrence networks to further assess links both within and between the bacterial and fungal communities in addition to the community complexity. To maintain consistency across the analyses we used sequencing data at the genus taxonomic level and calculated indicators of the network topology (Table 1). However, network visualization was found to be more appreciable at the class level (Fig. 5) and was highlighted with phylum details (Supplementary Fig. 3). The node that was calculated to have the greatest maximal betweenness, which represented the bacterial genera with the most connections (8105 connections), was an unidentified environmental bacterium (GN07) found to be residing in the tailings dump. The most highly represented bacteria (Pseudomonas, Fig. 4) were found to be poorly connected to other bacteria, as they did not exhibit the highest number of either direct or indirect links (Betweenness = 1291 connections). The key bacterial genus discovered within the undisturbed soil network was an unclassified clostridia (with 8106 total connections). Both the bacterial and the fungal correlation networks exhibited less complexity in the tailings dump compared to undisturbed soil, as illustrated by the values calculated for the connectance (i.e., the ratio between the number of realised links and the number of potential links) and the degree, which were approximately 25% and 24% lower for bacteria and fungi, respectively (P < 0.0001, Fig. 5, Table 1, Supplementary Fig.3). A corresponding 20% reduction in the complexity of the overall network (merging bacteria and fungi) was calculated. In parallel, calculation of the connectedness and betweenness indices of the tailings dump revealed that bacterial connectedness and betweenness increased by 23% and 21%, respectively, whereas fungal connectedness and betweenness decreased by 17% and 24%, respectively. Most importantly, no differences were observed in either connectedness or betweenness between the two areas with regard to the merged bacterial and fungal communities.

3.5. Soil microbe relationships

We calculated the Spearman correlation coefficient for each pairwise combination of parameters (i.e., taxa versus physico-chemical parameters) and the results were displayed as heatmaps (Fig. 4). Calculations were made on the dominant genera (>2% of
sequences). A total of nine fungal and bacteria taxa (i.e., *Hebeloma*, *Pyrenochaeta*, *Pseudomonas* and *Geopora*) grouped together into a single cluster and were highly correlated with As, Hg, exchangeable CaO, total CaCO3 and pH conditions in the tailings dump. In contrast, a total of eight microbial taxa (primarily *Basidiomycota* fungal class and MC18 bacteria) clustered together and were primarily correlated with CEC, Co and Pb. When considering the bacterial communities residing within the soil samples we found that the *Proteobacteria* dominance was primarily *Alphaproteobacteria* in the undisturbed soil (*Rhodoplanes* for example) and *Gammaproteobacteria* in the tailings dump (primarily *Pseudomonas* and *Proteobacteria g1*). In addition to these taxa specific features we noticed that approximately 50% of the 33 dominant taxa were equally represented in both soils and that few were sensitive to soil parameters (Fig. 4).

### 4. Discussion

With respect to diversity indices, major microbial taxa, and soil-taxa relationships the tailings dump might be considered to represent a habitat for a relatively small number of taxa (e.g., *Pseudomonas* or *Hebeloma*). In agreement with our work, previous studies have indicated that among structuring factors, anthropogenic Hg has a broad-spectrum effect and can alter the structure of both the culturable and total bacterial communities (*Müller et al.*, 2001). For example, some studies have shown anthropogenic Hg to impart a negative effect on the culturable diazotrophic community (*Lauber et al.*, 2008; *Boer et al.*, 2012) and a predominance of *Gammaproteobacteria Pseudomonas* isolates among Hg tolerant bacteria (*Oliveira et al.*, 2010). We observed a dominance of *Gammaproteobacteria* in our study site (24% of total number of sequences) and found that *Pseudomonas* genera (72% of *Gammaproteobacteria* sequences) were especially well represented within the tailings dump community. These findings were confirmed by quantitative PCR and revealed the specialization of the bacterial community. Mercury conditions within the dump had likely facilitated the dominance of *Pseudomonas* within the associated bacteria community; this genus may have rapidly adapted to its environment, for instance by acquiring Hg resistant genes via horizontal gene transfer (*Barkay et al.*, 2003). The other metallic contaminant that was detected at the dumpsite was arsenic. Previous work has revealed that dominant As-resistant isolates within As-contaminated soils can be identified by their fatty acid methyl ester (FAME) profiles and include *Pseudomonas* (*Turpeinen et al.*, 2004) in addition to other bacterial species. Similarly, fungi and *Proteobacteria* were shown to be able to evolve tolerance to As contamination, whereas all other groups of bacteria were reduced (*Lorenz et al.*, 2006). Thus, the cumulative presence of Hg and As could explain the abundance of *Pseudomonas* species that were found within the tailings dump.

In interpreting our data it is important to take into account the potential effects that can be imparted by other confounding factors, as has been recently highlighted in the literature (*Azarbad et al.*, 2013; *Chodak et al.*, 2013). Among the edaphic characteristics that
were measured here, pH is often regarded as capable of shaping the bacterial community (Lauber et al., 2008; Boer et al., 2012) and the presence of Acidobacteria can be indicative of acidic soils. Here, we found the abundance of Acidobacteria to be consistently and significantly lower in tailings dump samples, wherein pH was the highest (pH 8.1 versus 5.7 in undisturbed soil). High levels of CaCO₃ and exchangeable CaO within the dumpsite may also have been a key determinant in shaping the associated bacterial community. Indeed, calcite-rich sediments have often been found to be dominated by Gammaproteobacteria (Nercessian et al., 2005), as was observed in our study.

There were only minor differences found between the fungal communities residing in the tailings dump and undisturbed soil samples; differences between genus richness, diversity and biomass were all insignificant. It was also demonstrated that fungal communities did not robustly respond to changes in environmental conditions (Müller et al., 2001), whereas other studies reported the responses of fungi to specific physico-chemical conditions from a metallurgical slug compared with a natural soil (Kozdroj, 2000). In details, our study did find that the fungal community structure changed with respect to a few genera; for example, increased numbers of Hebeloma and Geopora sequences were found at the tailings dump. Correspondingly, calenbirs of the Hebeloma morpherial genus (notably H. mesophaeum species) have been frequently found within unvegetated soils (Hrynkiewicz et al., 2008; Krpata et al., 2008) and have been known to promote the growth of host trees in soils contaminated with metal (Hrynkiewicz et al., 2012). Our data therefore indicated that the fungal colonization of the Salicaceous rhizosphere within the tailings dump by indigenous taxa of the adjacent undisturbed soil had occurred during the decade after substrate deposition had ceased. The earlier colonisation of deposited substrates by woody plants can substantially increase the input of carbon into the soil through both litter and root exudates, which in turn can induce root colonisation by mycorrhizal symbionts (i.e., Hebeloma species).

The strong evolutionary relationships between plants and microbes that have been previously described might offer an explanation for the weak divergence that was observed amongst the communities that we assayed, as the samples used in this study were collected from rhizospheric environments that were present under similar tree species. Indeed, recent research suggests that plant species in natural ecosystems are likely to be a more important determinant of the rhizosphere microbial community than soil type (Westover et al., 1997; Chaparro et al., 2012; Bell et al., 2014). This viewpoint might well apply to our study, as we found that both Salicaceous trees and a complex associated microbial community had fully colonised the dump. Environmental filtering, as was discussed earlier, is more likely to occur through the activity of tree roots than in response to soil characteristics and could be an important force in the assembly of at least some microbial communities. Overall, plant–microbe interactions are known to influence soil properties through a number of plant and microbial processes (Chaparro et al., 2012).

In addition to some of the more specific community characteristics that were found, we also calculated a core microbiome of 63.4% among the whole community and of 50% among the 33 more dominant genera. A core microbiome consists of a suite of members that are shared among the microbial consortia of similar habitats; the estimation that was calculated for the core microbiome here is reasonable in light of the additional community information that was observed. Regardless of the calculation method that was employed, however, to our knowledge this study represents the first description of a core microbiome that possesses similarities between anthropogenic and unvegetated soils. One explanation for this finding might relate to the fact that previous studies have focused only the groups that were found to be different among dominant taxa (Rieder and Frey, 2013). Specifically, previous studies have described individual microbial populations at the class or genus level using molecular tools such as terminal restriction fragment length polymorphism and targeted sequencing of major OTUs (Rieder and Frey, 2013).

The network dataset generated in this study indicates that detailing the extensive fungal—bacterial relationships that exist within soil provides complementary information on the complexity of the associated community (Rousk et al., 2008). To our knowledge, our work represents the first published field study of a microbial co-occurrence network within samples taken from anthropogenic, contaminated soils. Even if the network topology was more simplistic in the tailings dump than in the undisturbed soil we were still able to demonstrate that a complex structure could be achieved in this highly constrained environment. It has been previously highlighted (Shade and Handelsman, 2012) that the number of interactions within a microbial community may be more informative than the identity of its OTUs for predicting and managing core microbiomes across systems. Indeed, the slightly similar network structures that we observed between the two soils is consistent with the size of the core microbiome and suggests that a large number of interactions may stabilise the overall community of microbes by providing them with greater resistance to harsh conditions (Pimm, 1984; Neutel et al., 2007; Shade et al., 2012).

In conclusion, the multidisciplinary approach (physico-chemical analysis, ion Torrent sequencing and related bioinformatics tools) used in this study facilitated in-depth exploration of the characteristics of the soil microbial communities that were found to reside within a tailings dump and those found within adjacent undisturbed soil and let us to conclude that environmental filtering is more likely to occur through the activity of tree roots rather than as a result of specific soil characteristics. Accordingly, the multidisciplinary approach that was used in this study might offer the most appropriate method of studying the impact of anthropogenic activities on soil biology and ecology.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.08.008.

References

