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Life in mine tailings: microbial population structure across the bulk soil, rhizosphere, and roots of boreal species colonizing mine tailings in northwestern Québec

Vanessa Gagnon[1,2], Michaël Rodrigue-Morin[1], Julien Tremblay[2], Jessica Wasserscheid[2], Julie Champagne[2], Jean-Philippe Bellenger[3], Charles W. Greer[2] and Sébastien Roy[1*]

Abstract

Purpose: Mining activities have negative effects on soil characteristics and can result in low pH, high heavy metal content, and limited levels of essential nutrients. A tailings storage area located in northwestern Québec showed natural colonization by plants from the adjacent natural environment. The objective of the study was to determine the main edaphic parameters that structured microbial populations associated with the indigenous woody plants that had naturally colonized the site.

Methods: Microbial populations were studied in the bulk soil, the rhizosphere, and inside plant roots using Illumina sequencing, ordination analysis (i.e., redundancy analysis (RDA) and principal coordinates analysis (PCoA)), ternary plotting, and statistical analysis (MANOVA).

Results: The main variables that drove the microbial community patterns were plant species and the tailings pH. Indeed, the main bacterial classes were Gammaproteobacteria and Deltaproteobacteria in both the rhizosphere and root endosphere. Analysis revealed that some dominant operational taxonomic units (e.g., *Pseudomonas* sp., *Acinetobacter* sp., and *Delftia* sp.) were present in increased proportions in roots for each plant species under study. This study also revealed that many of the most abundant fungal genera (e.g., *Claussenomyces*, *Eupenicillium*, and *Trichoderma*) were more abundant in the rhizosphere than in the root endosphere.

Conclusions: This comprehensive study of the microbial community dynamics in the bulk soil, rhizosphere, and root endosphere of boreal trees and shrubs could be beneficial in facilitating the rehabilitation of disturbed ecosystems.

Keywords: Mine tailings, Contamination, Heavy metal, Bacterial endophyte, Fungal endophyte, Microbial dynamics

Introduction

In Québec, more than 100 years of mining activities have etched into the province. Since 1890, many metals have been mined in this province, most notably iron, nickel, zinc, copper, and gold, leaving hundreds of abandoned mines sites to be restored with public funds (Ministère de l'Énergie et des Ressources Naturelles (MERN) 2015, Ministère de l'Énergie et des Ressources Naturelles (MERN) 2017, Ministère de l'Énergie et des ressources Naturelles (MERN) 2018). Unfortunately, once exploited, mine sites have many common features that hinder primary succession and slow down the autonomous recovery of disturbed ecosystems. Extreme soil characteristics such as acidic pH, high concentrations of heavy metals,

* Correspondence: Sebastien.Roy@USherbrooke.ca
[1]Centre SÈVE, Département de Biologie, Faculté des Sciences, Université de Sherbrooke, 2500 boulevard de l'Université, Sherbrooke, Québec J1K 2R1, Canada
Full list of author information is available at the end of the article

and little or no organic content make it difficult for the establishment of primary successional plants (Sheoran et al. 2010). In this context, it is essential for the plants to associate with soil microorganisms that can promote the growth of the plant, such as PGPR (i.e., plant growth-promoting rhizobacteria), which provide several benefits to plants (Van Der Heijen et al. 2008; Ma et al. 2011). Microbial communities (e.g., bacteria, ectomycorrhizal (ECM) fungi, arbuscular fungi (AM), and dark septate endophytes (DSEs)) living in the plant rhizosphere can also alleviate the phytotoxicity of metals through various mechanisms. They can exclude some metals via restricted membrane permeability or by actively transporting metals outside the cell. They can sequester metals inside the cell and detoxify metals by modifying their speciation (Ma et al. 2011). These microbial communities may also enhance the mobilization and uptake of nutrients by plants (Courty et al. 2010). For their part, plants are able to recruit specific microorganisms to populate the rhizosphere and roots by modulating the secretion of molecules in their root exudates (i.e., sugars, hormones, amino acids, organic acids, etc.). This modulation is in turn influenced by many plant-specific factors, such as species, age and developmental stage, and environmental conditions (Turner et al. 2013). In the context of mine site reclamation, PGPR microorganisms associated with pioneer plants could improve phytoremediation success (Zhuang et al. 2007; Hrynkiewicz and Baum 2011).

The studied mine site, located in the gold-rich region of northwestern Québec, has been naturally colonized by native boreal species (such as alder, birch, and spruce) for over five decades, and it has heterogeneous edaphic characteristics (i.e., plant species, pH, metals). This site is a natural laboratory that enabled us to address a variety of biological inquiries, notably identification of the main drivers for microbial population composition in the bulk soil, rhizosphere, and root endosphere of boreal plant species colonizing the mine tailings.

The main objective of this study was to improve our understanding of plant-microbe systems on acidogenic tailings to assist in restoration efforts. We aimed (1) to determine which environmental factors best explain the microbial communities found in soil, the rhizosphere, and roots; (2) to determine if the microbial population distribution patterns differed between soil, the rhizosphere, and roots for the same plant species; and (3) to determine which microorganisms are significantly more present in soil, the rhizosphere, and roots for a given plant species. Based on the known importance of plant species, pH, and water content which condition the structure of microbial communities in soil, our first hypothesis is that these parameters are the principal drivers of microbial communities in soil in the tailings deposit despite high metal contents (1) (Lauber et al. 2009; Brockett et al. 2012; Hodge and Fitter 2013). Our second hypothesis is that differences in microbial community composition would be observed between plants (this is well-established), but that greater differences in microbial community composition would be observed between root endophytes and the external environments (bulk soil and rhizosphere) of individual plant species due to selective pressure (low pH and metal content) on plants to recruit specific root endophytes to help attenuate environmental stress (2) (Lundberg et al. 2012). Our third hypothesis is that the roots will have the highest relative abundance for the most abundant operational taxonomic units (OTUs) but that the highest diversity (Shannon index) for bacteria and fungi will be found in the rhizosphere (3) (Lundberg et al. 2012; Edwards et al. 2015). A better understanding of the microbial community dynamics between soil, rhizosphere, and roots in a mining context could help us to identify key microorganisms in these recovering environments.

Materials and methods

Study site and experimental design

The mining site is located in northwestern region of Québec (Canada), known for its gold deposits (Fig. 1a). In this region, the forest is mainly composed of balsam fir (*Abies balsamea*), black spruce (*Picea marinara*), white spruce (*Picea glauca*), paper birch (*Betula papyrifera*), and Jack pine (*Pinus banksiana*) (Kneeshaw and Bergeron 1998; Bergeron et al. 2004). This mining site has been less active for the last five decades than it was previously, and natural and heterogeneous vegetation can be observed on all of this tailings deposit, despite the presence of acid mine drainage (generated by mineral sulfur reacting to atmospheric oxygen) and subsequent release of heavy metals from the mineral matrix. The patterns of its plant community development and structure were recently published, describing heavy metal and pH distribution patterns on the site and their influence on plant communities (Tardif et al. 2019). Speckled alder (*Alnus incana* ssp. *rugosa*), paper birch, and spruce (*Picea* sp.) were present in this vegetation (Fig. 1b). For our study, approximately 100 root and soil samples for each species were collected randomly over the mine site at the beginning of September 2015.

Bulk soil, rhizosphere, and root sampling and preparation for DNA extraction

Plants (approximately 0.5 m in height) were dug with a shovel to harvest the soil around the roots. Soil (50 g) was taken from the root ball (bulk soil). The aboveground and underground parts of the plant were separated into two different Whirl-Pak® bags and kept at 4 °C until they arrived at the laboratory. Most of the soil surrounding the roots was shaken free to preserve only the

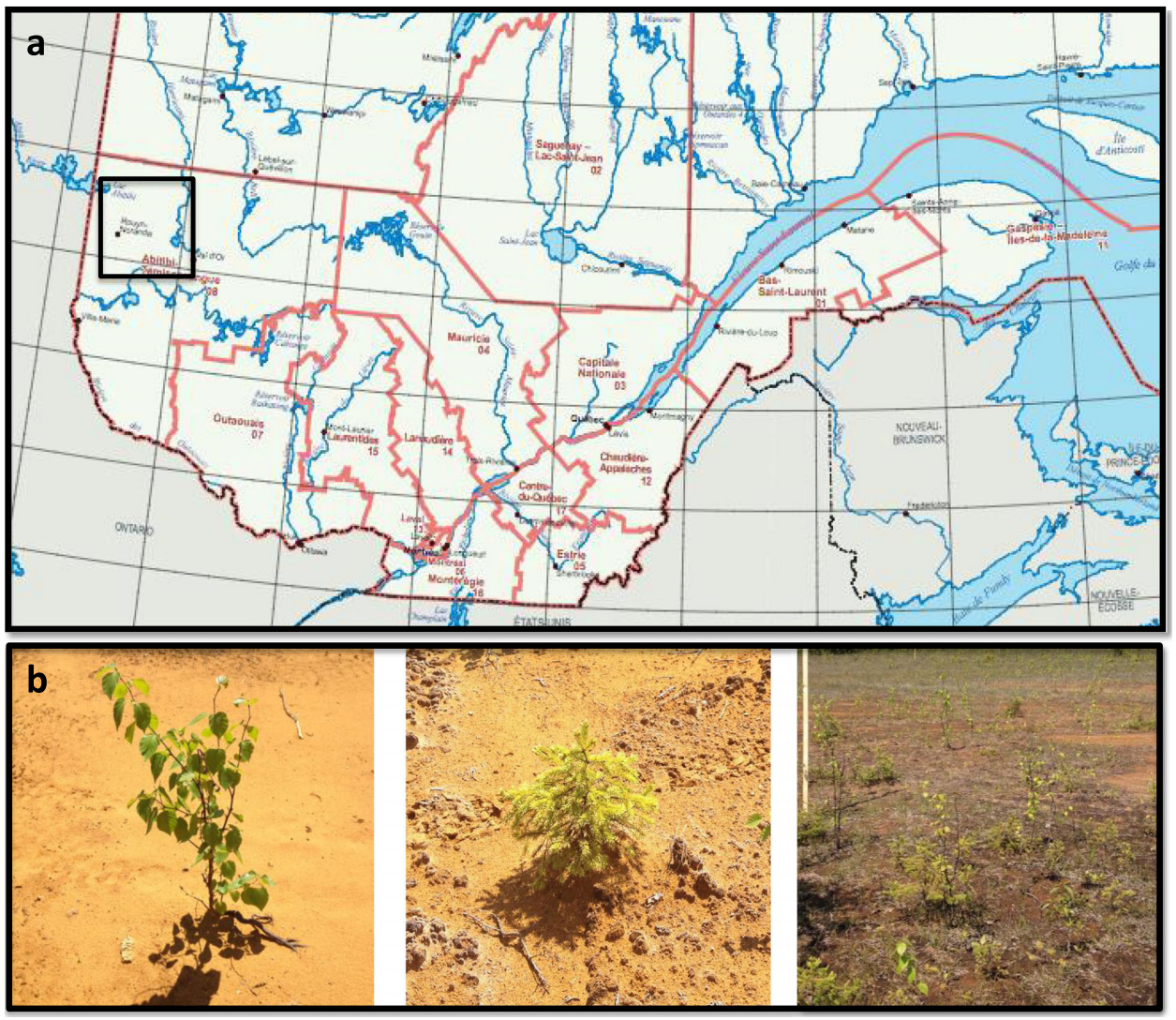

Fig. 1 **a** Maps of meridional Québec with mine site region (black box) (Ministère de l'Énergie et des Ressources Naturelles (MERN), 2013). **b** Alder, spruce, birch, and mix of species on the mine site

rhizosphere and the roots, which were shaken in a beaker to collect the attached (i.e., rhizosphere) soil. Before freezing, roots were washed and surface-sterilized for endophyte DNA extraction. The roots were washed for 1 min in 100% ethanol, 1 min in 2.5% bleach, 30 min in fresh 2.5% bleach, and 1 min in 100% ethanol. Roots were then aseptically transferred to sterile Erlenmeyer flasks and rinsed four times with sterile distilled water. An aliquot of water from the final wash was preserved to perform 16S rRNA amplification to confirm sterility (results not shown). Bulk soil, the rhizosphere, and roots were frozen at − 20 °C until DNA extraction.

Environmental parameters analysis

The environmental parameters studied in soils were pH, water content, total N, organic matter, sodium (^{23}Na), magnesium (^{24}Mg), aluminum (^{27}Al), phosphorus (^{31}P), potassium (^{39}K), calcium (^{44}Ca), titanium (^{47}Ti), vanadium (^{51}V), chromium (^{52}Cr), manganese (^{55}Mn), iron (^{57}Fe), cobalt (^{59}Co), nickel (^{60}Ni), copper (^{65}Cu), zinc (^{66}Zn), arsenic (^{75}As), selenium (^{82}Se), molybdenum (^{95}Mo), silver (^{107}Ag), cadmium (^{111}Cd), antimony (^{121}Sb), barium (^{137}Ba), tungsten (^{182}W), thallium (^{205}Tl), and lead (^{206}Pb). The results for alder, birch, and spruce bulk soil samples are presented in Supplementary Table 1.

DNA extraction, library preparation, and sequencing

Two grams of frozen bulk soil or the rhizosphere were weighed in tubes containing 1.5 g of silica beads. DNA was extracted, purified, and eluted as described in the PowerSoil® Total RNA Isolation Kit (MO BIO Laboratories, Inc., #12866-25, Carlsbad, CA, USA) and RNA

PowerSoil® DNA Elution Accessory Kit (MO BIO Laboratories, Inc., #12867-25, Carlsbad, CA, USA). Root DNA extraction was performed according to the manufacturer's instructions (PowerLyzer® UltraClean® Tissue & Cells RNA Isolation Kit, MO BIO Laboratories, Inc., #15055-50, Carlsbad, CA, USA). The V4 variable region of the 16S rRNA ribosomal DNA gene sequences (eubacteria and archaea) and the fungal nuclear ribosomal internal transcribed spacer (ITS) region were first amplified. The specific primer sequences (Integrated DNA Technologies, Canada) for these regions are described in Supplementary Table 2. For each region (16S rRNA gene and ITS), four pairs of primers with additional bases inserted between the sequence overhang and the specific sequence (staggered pad) were used proportionally between the samples to create diversity in the sequence reads, thereby improving the quality of sequencing results. For amplification of the 16S rRNA region, a peptide nucleic acid (PNA) clamp was used to prevent amplification of eukaryotic RNA. PNA chloroplast and PNA mitochondrial blockers were used as proposed in Hong et al. (2016). Reverse transcriptase PCR was carried out using the OneStep RT-PCR Kit (Qiagen, #220211, CA, USA).

Amplicons were visualized on a 2% agarose gel. The samples were purified with AMPure XP magnetic beads (Beckman Coulter Genomics, #A63881, CA, USA), and a second short 8-cycle amplification step was carried out using the Nextera XT index kit (Illumina® #FC-131-1001, set A-B-C and D, CA, USA) to barcode the amplicons according to the protocol in the Illumina "16S Metagenomic Sequencing Library Preparation" guide (part #15044223 Rev. B). Unique codes were added to each sample by amplifying 5 μl of the purified PCR product with 25 μl of KAPA HIFI HotStart Ready Mix, 300 nM each Nextera XT Index Primer (Illumina® Inc., CA, USA), and 10 μl of UltraPure™ DNase/RNase-Free Distilled Water for a total volume of 50 μl. Thermal cycling conditions were as follows: 3 min at 98 °C, 8 cycles of 30 s at 98 °C, 30 s at 55 °C, 30 s at 72 °C, and a final elongation step of 5 min at 72 °C. Indexed amplicons were purified with magnetic beads as described above. Amplicon products were quantified with a Quant-iT™ PicoGreen™ dsDNA Assay Kit following the manufacturer's instructions (Thermo Fisher Scientific, MA, USA) and then pooled in equimolar amounts. The amplicon pool was verified with a Bioanalyzer 2100 (Agilent Technologies, CA, USA) to confirm amplicon size and to visualize the potential presence of primer or adapter dimers, in which case an additional purification step on the pool with SPRI Select (Beckman Coulter, #B23319, CA, USA) beads was performed. Finally, DNA in the pool was denatured with 0.2 N NaOH, and an internal PhiX DNA control was added at a 5% ratio (Illumina® Inc., #FC-110-3001, CA, USA). Samples were sequenced on a MiSeq instrument (Illumina® Inc., #SY-410-1003, CA, USA) using the MiSeq v2 500 cycle kit (Illumina® Inc., #MS-102-2003, CA, USA) at the National Research Council Canada in Montréal.

Bioinformatics analysis

Sequencing results were analyzed using a previously described methodology (Tremblay et al. 2015; Yergeau et al. 2015). Briefly, reads associated with both the 16S rRNA gene and ITS were separately filtered, assembled, trimmed, and quality controlled.

OTU tables were generated using a three-round clustering strategy. Quality-controlled sequences were dereplicated at 100% identity. These 100% identity clusters were denoised at 99% identity using dnaclust v3 (Ghodsi et al. 2011). Clusters presenting an abundance of three or more reads were scanned for chimeras with UCHIME de novo and reference-based algorithms using the Broad Institute 16S rRNA gene Gold reference (http://microbiomeutil.sourceforge.net). The remaining clusters were clustered at 97% identity (dnaclust) to generate OTUs.

The taxonomic assignment of bacterial and fungal OTU results was performed with the RDP classifier (Bayesian classifier) with a training model constructed from the Greengenes database (version 13.5) (DeSantis et al. 2006; Wang et al. 2007). Fungal OTUs were classified using a training model constructed with the United Database (Kõljalg et al. 2013). The OTU tables were filtered for archaea or fungi and normalized using the edgeR R package/archaea or fungi and normalized using the edgeR R package (Robinson et al. 2010). Sequencing data are available on the NCBI Sequence Read Archive (SRA) portal under accession number PRJNA517646.

Statistical analysis

Redundancy analysis (RDA) was performed to simplify the relationship between an explanatory matrix (x, edaphic parameter data) and a matrix to be explained (y, species data matrix). For the explicative matrix (x), extreme values were determined using the "grubbs.test" function, and each of these values was removed from the dataset. Then, to remove variables that were highly collinear, we calculated the variance inflection factor (VIF) for each and removed those variables with VIF > 5 (Borcard et al. 2011). Subsequently, the "imputeBDLs" function was used for the parametric replacement of rounded zeros. All the compositional data (metals) in matrix (x) were transformed by a centered log ratio by the function "cenLR" to allow their interpretation. We first calculated the log ratio of X to Y and then centered them around the grand mean of these values (Aitchison 2002). Each of the values was scaled to unit variance by dividing by the standard deviation of each. The species data matrix (y) was transformed by a centered log ratio since OTU data are considered compositional data (Fernandes

et al. 2014). Subsequently, the 30 most abundant classes and genera were kept for RDA, which was performed using the "rda" function with the significant explanatory variables selected by the "ordiR2step" function. A second RDA was performed on the significant explanatory variables. The significance of the explanatory variable (red arrows) was verified using the "anova.cca" function. The RDAs are presented in type II scaling to allow for a better interpretation of the correlation between matrices (x) and (y) (Legendre and Legendre 2009).

Principal coordinate analysis (PCoA) was performed on normalized OTU tables. A distance matrix was created via the "vedgist" function, and PCoA was performed using the "pcoa" function. Then, multivariate analysis (MANOVA) was carried out with the function "pairwise.perm.manova" (nperm = 999) to determine if microbial and fungal communities were significantly different between bulk soil, the rhizosphere, and roots associated with alder, birch, and spruce. The microbial community profiles of the 20 most abundant bacterial and fungal classes and genera were determined using Shiny software (In-house R script).

Ternary plots were prepared using the "ggtern" function. Each of the OTUs associated with class is presented in Fig. 5, where *x*, *y*, and *z* represent the mean relative abundance of one OTU in bulk soil (*x*), the rhizosphere (*y*) and roots (*z*). Each corner of the triangle represents an environment (bulk soil, the rhizosphere or roots). The colored point nearest to the corner indicates an increased relative abundance of this OTU in this particular environment. For results expressed as a percentage, the absolute value corresponds to 100%.

Results

The complete dataset used for this study included 1906 OTUs for the 16S rRNA gene region and 889 OTUs for the ITS region. The number of replicates (i.e., individual plants of a given species and type of environment) varied according to availability on site and are listed in Supplementary Table 3. The RDAs were performed using the 30 most abundant taxa composing the bacterial community (fraction representing 37.1%) and the fungal community (fraction representing 81.3%). Canonical RDAs showed that the key environmental parameters driving the presence of the 30 most abundant bacterial and fungal taxa in the three environments (bulk soil, the rhizosphere and root endosphere) are plant species (p_s) and pH, which partly confirms our first hypothesis stating that plant species, pH, and water content would be the key drivers of the microbial community structure (Fig. 2 and Table 1). The ANOVA tests made it possible to determine that the axes "p_s" and "pH" have *p* values varying between 0.001 and 0.050 (p_s) and between 0.001 and 0.003 (pH) (Table 1). The "p_s" axis partly explains the distribution of microbial communities (bacteria and fungi) in all environments studied except for bacterial communities in the rhizosphere and roots. The "pH" axis partly explains the distribution of bacterial and fungal communities present in all environments studied except bacterial communities in the roots. The bacterial community structure in roots was more closely related to magnesium (Mg) levels in adjacent soil (Fig. 2c and Table 1a).

The results shown in Fig. 3 and Table 2 demonstrate that plant species were the main driver of microbial and fungal community structures. Bacterial communities associated with alders differed when root endosphere communities were compared with the communities of the other two environments (the rhizosphere and bulk soil), while in birch, differences were noted between microbial population structure in the bulk soil and the roots. For microbial populations associated with spruce, the population structure differed between bulk soil and the rhizosphere (Table 2c). In addition, fungal populations in alder differed between bulk soil and the root endosphere. Fungal populations associated with birch revealed differences between bulk soil and the two other environments, while in spruce, changes were observed in root endosphere versus bulk soil and the rhizosphere (Table 2d–f).

We also observed that bulk soil, rhizosphere, and roots on this site were dominated by the bacterial classes Gammaproteobacteria (~ 11%), Deltaproteobacteria (~ 10%), Alphaproteobacteria (~ 9%), Planctomycetacia (~ 8%), and Acidobacteria (~ 4%) and the fungal classes "Others" (~ 29%), Agaricomycetes (~ 19%), Leotiomycetes (~ 19%), "Others" (Ascomycota) (~ 12%), and *Incertae sedis* (~ 4%) (Fig. 4 and Supplementary Table 4).

A ternary plot representing the four most abundant bacterial classes showed that the majority of class-associated OTUs were present in greater proportion in roots than in bulk soil and the rhizosphere (Fig. 5). In contrast, some of the most abundant fungal classes had OTU proportions that were different in different plants for the same fungal class and had more even partitioning of OTU proportions among the three types of environments (bulk soil, the rhizosphere, and roots) (Fig. 6).

The OTUs associated with *Acinetobacter*, *Delftia*, and *Pseudomonas* were relatively more abundant in the roots of the plant species studied (Fig. 7a–c). In addition, *Actinoplanes*, *Bryobacter*, *Candidatus Solibacter*, H16, *Rhizomicrobium*, and *Singulisphaera* OTUs show a higher relative abundance in birch roots than in bulk soil and the rhizosphere (results not shown). Furthermore, Fig. 7d–f illustrate that *Claussenomyces*, *Eupenicillium*, and *Trichoderma* were less abundant in the roots than in the bulk soil and rhizosphere of all plant species studied. Other species,

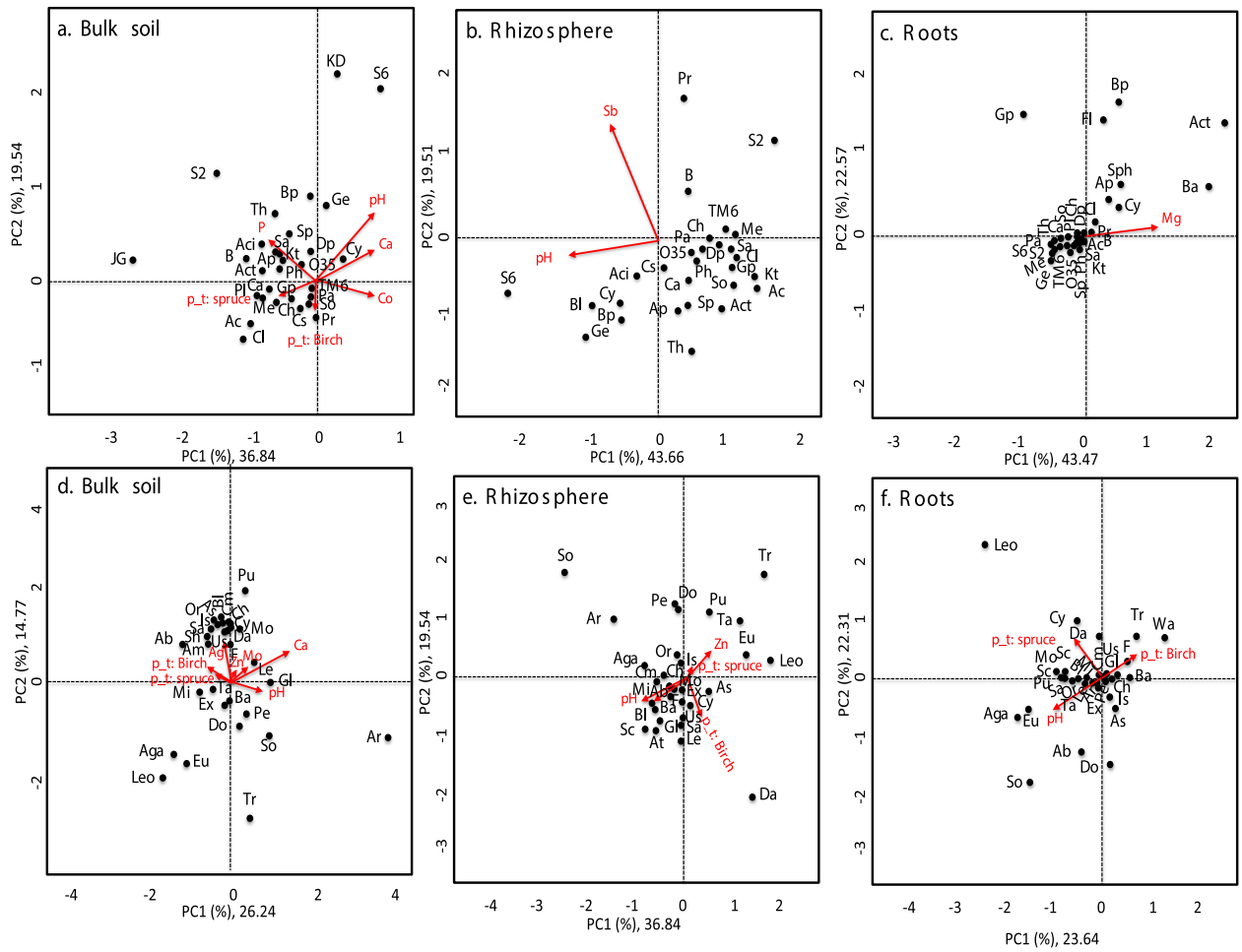

Fig. 2 **a**–**c** Redundancy analysis (RDA) of the effect of soil environmental variables on the composition of bacterial classes in bulk soil (**a**), the rhizosphere (**b**), and roots (**c**). **d**–**f** RDA of the effect of soil environmental variables on the composition of fungal classes in bulk soil (**d**), the rhizosphere (**e**), and roots (**f**). Black points represent the 30 most abundant bacterial and fungal taxa. Red arrows indicate soil significant variables. **a**–**c** Legend: Ac, Acidobacteria; Aci, Acidimicrobiia; Act, Actinobacteria; Ap, Alphaproteobacteria; B, k_Bacteria; Ba, Bacilli; Bl, Blastocatellia; Bp, Betaproteobacteria; Ca, Candidatus Azambacteria; Ch, Chlamydiae; Cl, Clostridia; Cy, Cytophagia; Dp, Deltaproteobacteria; Fl, Flavobacteria; Ge, Gemmatimonadetes; Gp, Gammaproteobacteria; JG, JG37.AG.4; KD, KD4.96; Kt, Ktedonobacterales; Me, Melainobacteria; O35, OPB35 soil group; Pa, Parcubacteria; Ph, Phycisphaerae; Pl, Planctomycetacia; Pr, Proteobacteria; S2, Acidobacteriaceae_Subgroup 2; S6, Acidobacteriaceae_Subgroup 6; Sa, Saccharibacteria; So, Solibacter; Sp, Spartobacteria; Sp, Sphingobacteriia; TM6, TM6; and Th, Thermophilia. **d**–**f** Legend: Ab, Agaricostilbomycetes; Ag, Agaricales; Aga, Agaricomycetes; Am, Arthoniomycetes; Ar, Archaeorhizomycetes; As, Ascomycota; Ba, Basidiomycota; Bl, Blastocladiomycetes; Ch, Chytridiomycetes; Cm, p_Chytridiomycota; Cy, Cystobasidiomycetes; Da, Dacrymycetes; Do, Dothideomycetes; Eu, Eurotiomycetes; Ex, Exobasidiomycetes; F, k_Fungi; Gl, Glomeromycetes; Is, *Incertea sedis*; Le, Lecanoromycetes; Leo, Leotiomycetes; Mi, Microbotryomycetes; Mo, Monoblepharidomycetes; Or, Orbiliomycetes; Pe, Pezizomycetes; Pu, Pucciniomycetes; Sa, Saccharomycetes; Sc, Schizosaccharomycetes; So, Sordariomycetes; Ta, Taphrinomycetes; Tr, Tremellomycetes; Us, Ustilaginomycetes; and Wa, Wallemiomycetes

such as *Amphinema* sp., *Capronia* sp., *Cladophialophora* sp., *Cryptotrichosporon* sp., *Epicoccum* sp., *Inocybe* sp., *Mortierella* sp., *Oidiodendron* sp., *Schizangiella* sp., and *Tomentella* sp. were present in lower relative abundance in the roots than in the rhizosphere of some plant species (results not shown and Table 3). Finally, *Cistella* sp. (alder), *Hyaloscypha* sp. (alder), and *Trichocladium* sp. (alder and birch) are equally represented in the rhizosphere and roots of different species (shown in parenthesis) (results not shown). Graphical representation of the Shannon index highlighted that the rhizosphere environment presented the highest diversity for bacterial and fungal communities (Fig. 8).

Discussion

Canonical analyses showed that plant species, pH, and Mg were the main drivers of bacterial and fungal communities associated with alder, birch, and spruce in most of the studied environments (bulk soil, the rhizosphere,

Table 1 Statistically significant RDA axes and their *p* values for bacterial communities (a) and fungal communities (b)

a

Bulk soil		**Rhizosphere**		**Roots**	
Variable	p-value	Variable	p-value	Variable	p-value
Co	0.001	pH	0.001	Mg	0.003
P	0.003	Sb	0.032		
pH	0.003				
Ca	0.011				
p_t	0.050				

b

Bulk soil		**Rhizosphere**		**Roots**	
Variable	p-value	Variable	p-value	Variable	p-value
pH	0.001	pH	0.001	pH	0.001
Ag	0.001	p_t	0.001	p_t	0.008
Mg	0.005	P	0.007		
p_t	0.026	Zn	0.038		
P	0.020				
Zn	0.015				
Mo	0.037				

Blue indicates *p* value ≤ 0.001; light blue, *p* value ≤ 0.01; and light gray, *p* value ≤ 0.05

and roots) (Fig. 2). These results partly confirmed our hypothesis that plant species, pH, and water content might be the key drivers of microbial community structure. Microbial communities are subject to many changes due to biotic factors (such as the presence of plants or other organisms) and many abiotic factors (such as pH, metal concentration, and water content) (Berg and Smalla 2009). Numerous studies have shown that the number of root exudate compounds that can be released by plants depends on factors such as plant type and age, nutritional status, and general homeostatic state (Cavaglieri et al. 2009; Huang et al. 2014). The diversity in root exudates supports a pool of microorganisms with increased biodiversity, contributing to a plant's adaptability to recruit rhizospheric and endophytic microbial populations according to its needs (Rosenblueth and Martínez-Romero 2006; Cavaglieri et al. 2009). Our findings corroborate that plant species partly explain the 30 most abundant bacterial and fungal taxa (Fig. 2).

Soil pH may directly and indirectly modulate the microbial population in the soil. A high concentration of H^+ associated with an acidic pH could directly disrupt intracellular pH in a microbial population without any "acidic pH" adaptations (i.e., reverse of membrane

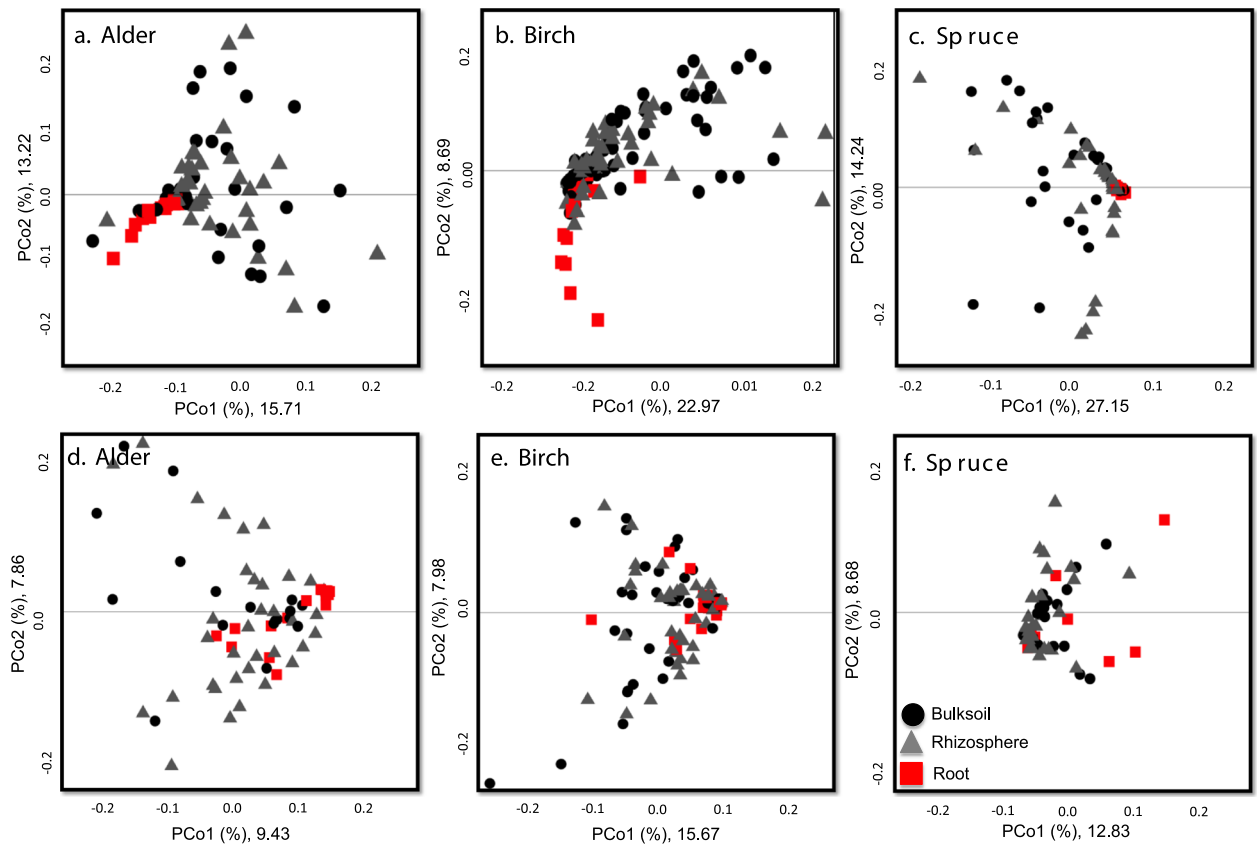

Fig. 3 **a**–**c** Principal coordinates analysis of bacterial communities associated with alder (**a**), birch (**b**), and spruce (**c**) in bulk soil, the rhizosphere, and roots. **d**–**f** Principal coordinates analysis of fungal communities associated with alder (**d**), birch (**e**), and spruce (**f**) in bulk soil, the rhizosphere, and roots

potential, cell impermeability to protons, or enzymes or chemicals capable of binding or using protons) (Booth 1985; Baker-Austin and Dopson 2007; Krulwich et al. 2011). In addition, an acidic pH can indirectly influence microbial populations through a modification of the solubility and therefore bioavailability of certain metals (Rieuwerts et al. 1998; Bolan et al. 2014). Solubility modification of certain elements, whether essential (e.g., Ca, Co, Cr, Cu, Fe, K, Mg, Mn) or nonessential (e.g., Al, As, Cd, Pb) to microbial metabolism, can be toxic for organisms that do not possess mechanisms to alleviate the oxidative stress caused by metals (Hall 2002; Hossain et al. 2012). Microbial populations will, however, adapt to survive in environments with harsh conditions such as fluctuations in pH and high metal concentrations (Bruins et al. 2000; Nwuche and Ugoji 2008). In the present study, the results shown in Supplementary Table 1 indicate a wide range of pH values in the tailings deposit (2.84 to 8.90). Rousk et al. (2010) and Liu et al. (2014) also reported that pH could greatly affect bacterial species abundance and diversity in soil. In this context, it is logical to hypothesize that depending on individual plant location, the role of pH was paramount in structuring microbial communities via its influence on the bioavailability of specific metals and metalloids.

The tailings deposit we studied showed high spatial variability in Mg concentrations (44.62 to 5247.96 $\mu g_{metal}.g_{dryweight}^{-1}$) (Supplementary Table 1). Shaul (2002) stated that optimal Mg concentrations in the leaves of healthy plants should range from 83 to 415 $\mu g_{metal}.g_{dryweight}^{-1}$. However, our previous study of the mine site recorded Mg foliar levels that spanned a much wider range (< 1 to 5399 $\mu g_{metal}.g_{dryweight}^{-1}$) for alder, birch, and spruce (unpublished results). This is noteworthy since Mg is an essential element for plant photosynthesis and acts as a cofactor and modulator for more than 300 enzymes (e.g., RNA polymerase and ATPase) (Shaul 2002; Verbruggen and Hermans 2013). In addition, Mg^{2+} is the most prevalent cation found in prokaryotic cells, with a concentration of approximately 15–25 mM (Moomaw and Maguire 2008). In these cells, Mg^{2+} is a cofactor of ATP and numerous enzymes, promoting the stability of the membrane and representing a structural element of the ribosome (Moomaw and Maguire 2008; Ramesh and Winkler 2010). However, how the Mg^{2+} concentration in soil could lead to Mg^{2+} imbalance in

Table 2 MANOVA test result (nperm = 999) performed on data presented in Fig. 3. Bacterial community comparisons between bulk soil, the rhizosphere, and roots associated with alder (a), birch (b), and spruce (c). Fungal community comparisons between bulk soil, the rhizosphere, and roots of alder (d), birch (e), and spruce (f)

a

Alder	Bulk soil	Rhizosphere
rhizosphere	0.803	-
roots	0.004	0.003

b

Birch	Bulk soil	Rhizosphere
rhizosphere	0.146	-
roots	0.003	0.027

c

Spruce	Bulk soil	Rhizosphere
rhizosphere	0.051	-
roots	0.257	0.464

d

Alder	Bulk soil	Rhizosphere
rhizosphere	0.045	-
roots	0.008	0.008

e

Birch	Bulk soil	Rhizosphere
rhizosphere	0.030	-
roots	0.018	0.089

f

Spruce	Bulk soil	Rhizosphere
rhizosphere	0.054	-
roots	0.030	0.092

Light blue indicates p value ≤ 0.01, and light gray, p value ≤ 0.05

microbial cells remains poorly known. Therefore, the spatial variability in Mg concentrations and heterogeneous pH values affect the bioavailability of Mg in the tailings deposit and could lead to deficiency or/and toxicity in plant communities, leading to physiological stress for plants (i.e., a decrease in photosynthetic rates) (Huber and Jones 2013; Verbruggen and Hermans 2013; Guo et al. 2016). As previously stated, the physiological state of plants is one of the factors governing the selection of rhizosphere and root endophytic organisms (Rosenblueth and Martínez-Romero 2006; Hartmann et al. 2009). The great variations in Mg concentration at the mine tailings site could explain why this parameter was found to be partly responsible for the bacterial community structure in roots (Fig. 2c and Supplementary Table 1).

The rhizosphere is defined as the thin layer of soil firmly attached to plant roots. It is considered a different

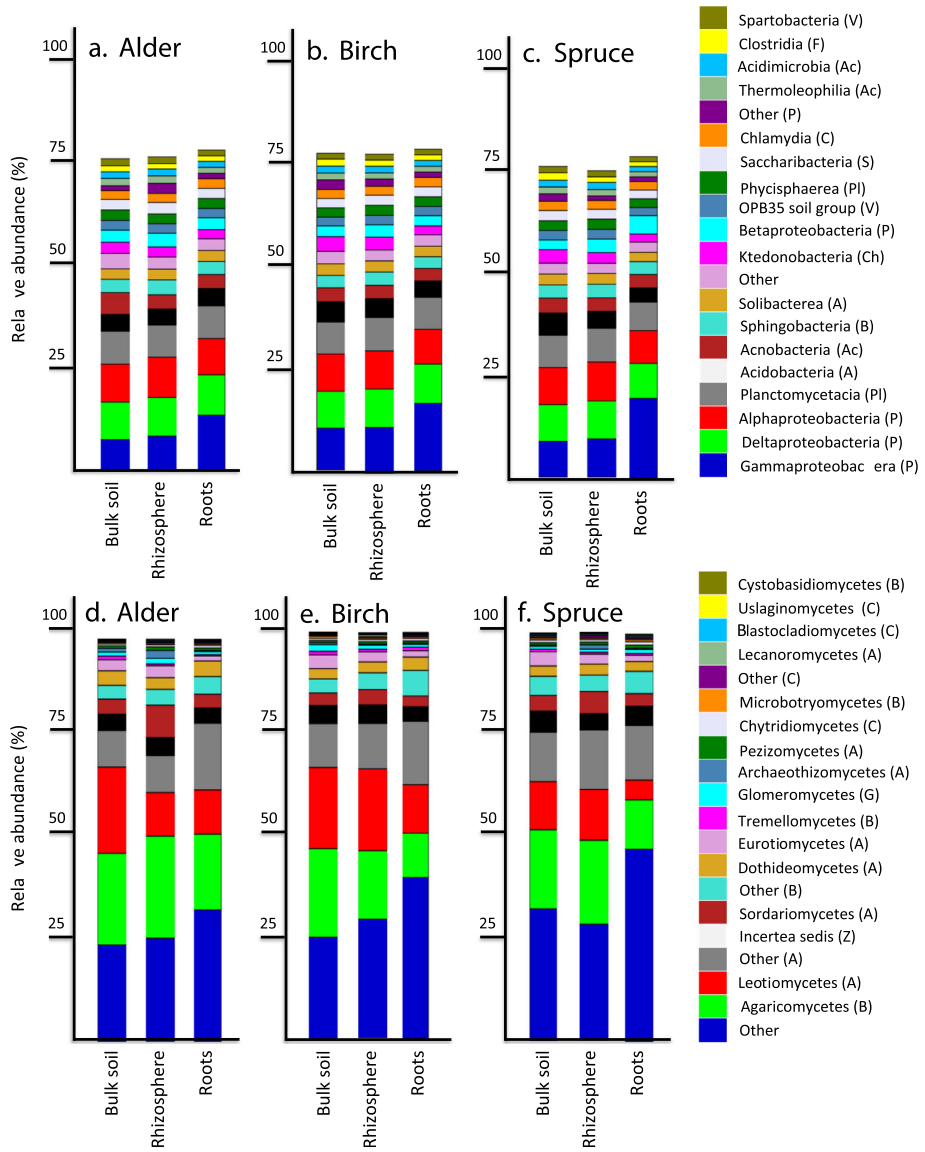

Fig. 4 **a**–**c** Microbial community profiles of the 20 most abundant bacterial classes in bulk soil, the rhizosphere, and roots of alder (**a**), birch (**b**), and spruce (**c**). Letters in parentheses indicate phyla (A, Acidobacteria; Ac, Actinobacteria; B, Bacteroidetes; C, Chlamydiae; Ch, Chloroflexi; F, Firmicutes; P, Proteobacteria; Pl, Planctomycetes; V, Verrucomicrobia). **d**–**f** Microbial community profiles of the 20 most abundant fungal classes in bulk soil, the rhizosphere, and roots of alder (**d**), birch (**e**), and spruce (**f**). Letters in parentheses indicate phyla (A, Ascomycota; B, Basidiomycota; C, Chytridiomycota; G, Glomeromycota; Z, Zygomycota)

area from the bulk soil because the roots, present only in the rhizosphere, have a biological, chemical, and physical influence on the soil through root growth, nutrient and water uptake, respiration, and rhizodeposition (via root exudates). The rhizosphere is rich in root exudates composed of sugars, organic acids, proteins, and hormones and can attract a plethora of diverse microorganisms to meet the needs of the plant (Broeckling et al. 2008, Huang et al. 2014; York et al. 2016). Indeed, Berendsen et al. (2012) mentioned that plants could shape their rhizomicrobiome by secreting specific root exudates to draw microorganisms to their rhizosphere. The attracted microorganisms could be beneficial for the plant by facilitating nutrient uptake (i.e., phosphorus and nitrogen) and preventing pathogen colonization (Berendsen et al. 2012), among other functions. The abundance and diversity of the molecules found in the rhizosphere could explain why the highest alpha diversity (Shannon index) was observed in the rhizosphere compared to the two other environments, bulk soil and the root endosphere (Fig. 8). In addition, PCoA showed that most of the major differences in bacterial and fungal

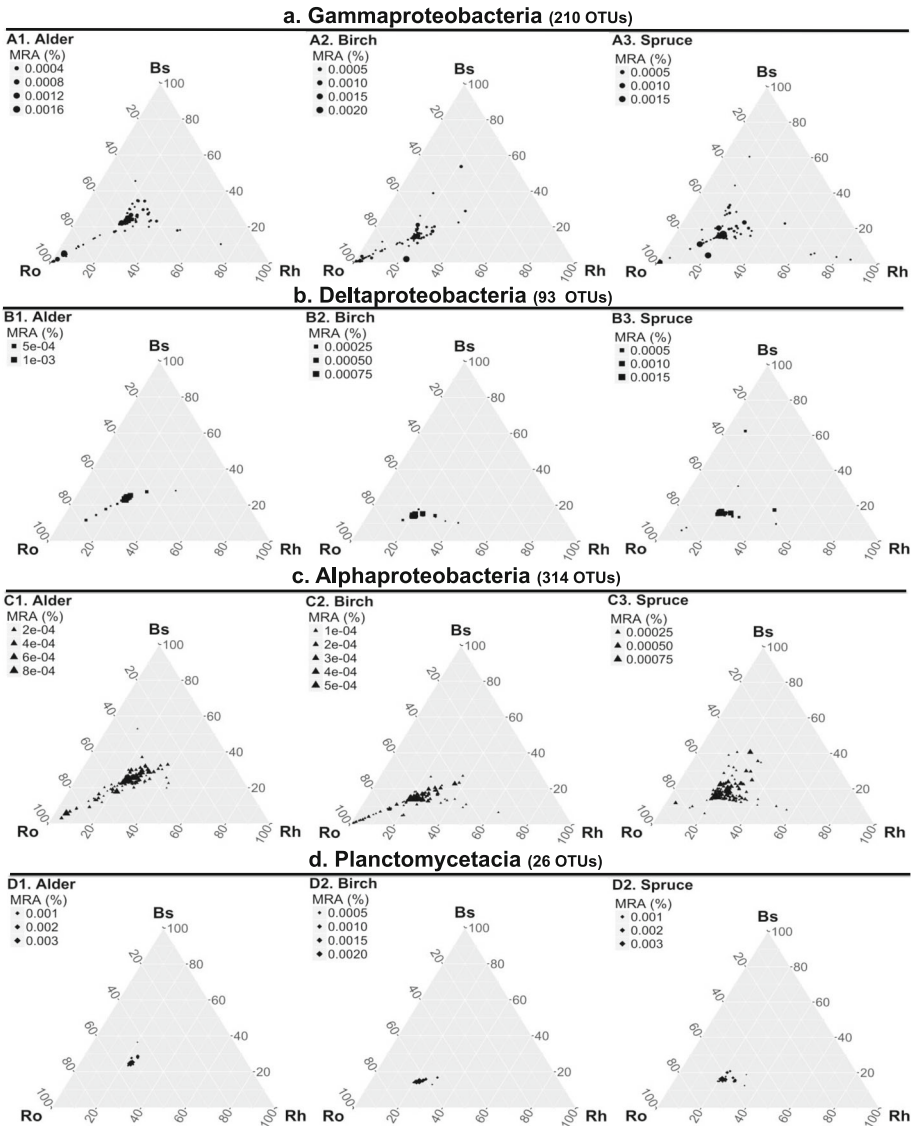

Fig. 5 Ternary plots of mean relative abundances (MRAs (%)) of the 4 most abundant bacterial classes. Each point is an OTU whose position is indicative of its MRA in bulk soil (Bs), the rhizosphere (Rh), and roots (Ro) of alder, birch, and spruce

community structures were observed between bulk soil and the two other studied environments, the rhizosphere and root endosphere. This refutes the second hypothesis, which proposed that the most important differences in microbial communities would be observed between the roots and other two environments (Fig. 3 and Table 2). This contradiction could be explained by the fact that the combination of nutrients found at the plant-soil interface consisted of a much wider variety of molecules excreted by the roots than that available in the bulk soil (Gaiero et al. 2013; Turner et al. 2013).

Moreover, root endosphere acquisition is driven by three main factors: (1) soil parameters, (2) plant factors (root exudates) that allow colonization and compatibility, and (3) microbial factors that influence the viability of microorganisms inside the roots (Gaiero et al. 2013). Once inside the roots, microorganisms could have many functions, such as enhancing the acquisition of mineral nutriments by solubilizing them (e.g., phosphate, potassium and nitrogen), stimulating plant growth through production of growth factors (among these auxins and cytokinin), stimulating root growth through production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase enzymes, and inducing systemic resistance against plant pathogens (Badri et al. 2009; Ma et al. 2016). Our results showed that alder and birch root endospheres had different bacterial populations than bulk soil and the rhizosphere (Fig. 3 and Table 2). Some studies have shown that the root endophytic compartment presents microorganisms distinct from those of the rhizosphere

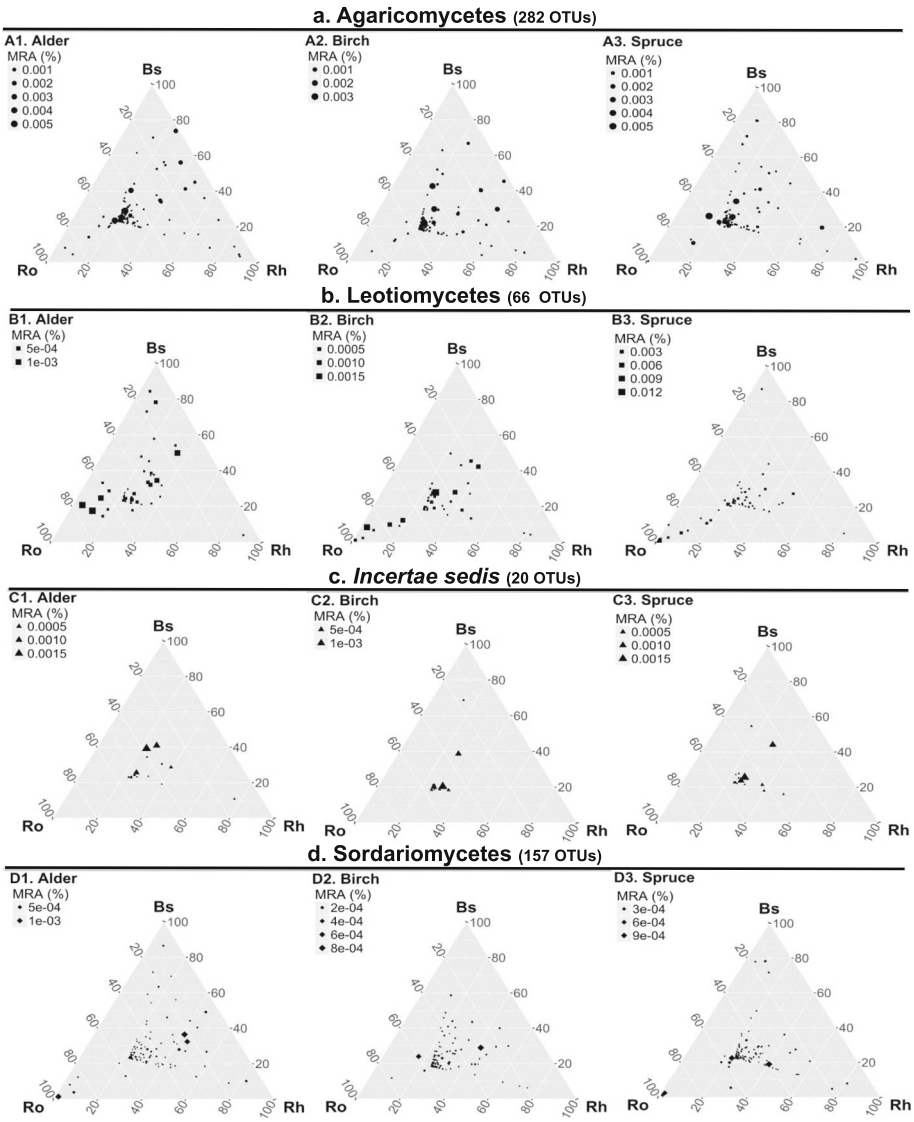

Fig. 6 Ternary plots of mean relative abundances (MRAs (%)) of the 4 most abundant fungal classes (which are not "other"). Each point is an OTU whose position is indicative of its MRA in bulk soil (Bs), the rhizosphere (Rh), and roots (Ro) of alder, birch, and spruce

and bulk soil. Edwards et al. (2015) showed that the phyla inside *Arabidopsis* roots (endophytic compartment) were less diverse than those found in the rhizosphere. Furthermore, Gottel et al. (2011) demonstrated that in *Populus deltoides*, the microorganism profile in the root endophytic compartment was different from that in the rhizosphere. It was also suggested that endophytic microorganisms possess genes that are different from those found in microorganisms present in the rhizosphere, which could explain the former's endophytic behavior (Santoyo et al. 2016). The root endosphere is a highly selective environment and allows the penetration of only specific beneficial microorganisms (i.e., rapid contact with plant tissues) versus microbes found in the rhizosphere (Lundberg et al. 2012; Edwards et al. 2015; Santoyo et al. 2016). These observations could explain the significant difference in microbial populations found in roots and the other soil compartments studied, the rhizosphere and bulk soil (Fig. 3 and Table 2).

Limited research has been conducted on microbial communities across bulk soil, the rhizosphere, and tree roots. However, Gottel et al. (2011) presented recent advances in the study of microbial communities across different environments (the rhizosphere and root endosphere) of *Populus deltoides*. They showed that the rhizosphere is dominated by Acidobacteria (31%) and Alphaproteobacteria (30%), while the root endosphere is dominated by Gammaproteobacteria (54%) and Alphaproteobacteria (23%). In addition, the study also pointed

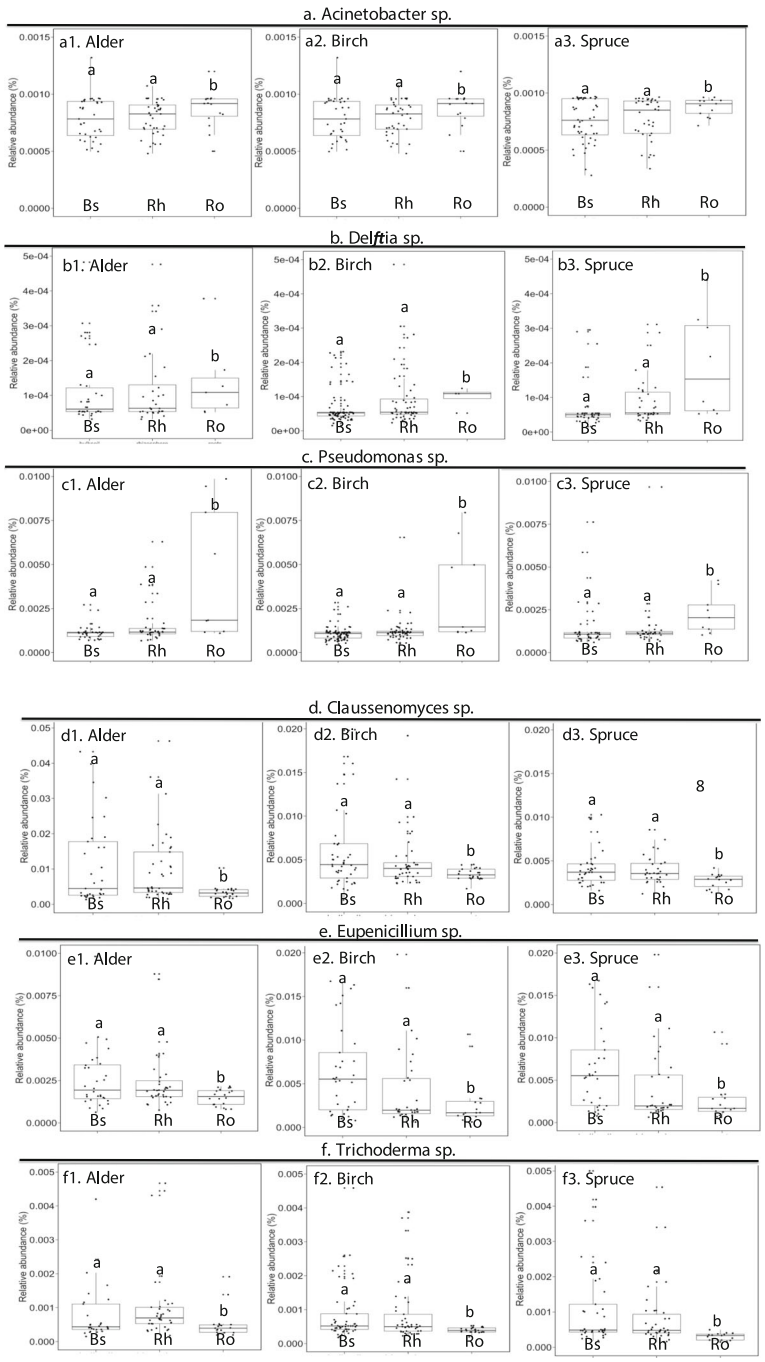

Fig. 7 Relative abundances (%) of various bacterial (**a**–**c**) and fungal species (**d**–**f**) in bulk soil (Bs), the rhizosphere (Rh), and roots (Ro) of alder, birch, and spruce. Different letters represent a significant difference after unbalanced ANOVA followed by Tukey's multiple comparison test. Non-parametric Kruskal-Walis test followed by Dunn multicomparison test was done when data transformation was not possible to meet the normality requirement

out that a single *Pseudomonas*-like OTU represents 34% of root endophyte sequences. Our study demonstrated that the rhizosphere of alder, birch, and roots differ from those of *P. deltoides* reported in Gottel et al. (2011). The rhizospheres were composed of 8.19%, 10.80%, and 9.22% Gammaproteobacteria and 9.07%, 9.09%, and 9.16% Deltaproteobacteria, respectively. These bacterial classes were the most abundant classes found in each of the studied plant species. In addition, the root endospheres of alder, birch, and spruce were also mostly populated by Gammaproteobacteria, with relative abundances of 13.29%, 15.96%, and 19.28%, respectively.

Table 3 Species found in decreased relative abundance in roots of plant species found on the mine site

Root endophyte	Phylum	Class	Plant species in which relative root abundance is decreased
Amphinema sp.	Basidiomycota	Agaricomycetes	Spruce
Capronia sp.	Ascomycota	Eurotiomycetes	Spruce
Cladophialophora sp.	Ascomycota	Eurotiomycetes	Birch
Claussenomyces sp.	Ascomycota	Leotiomycetes	Alder, birch, and spruce
Cryptotrichosporon sp.	Basidiomycota	Tremellomycetes	Birch and spruce
Eupenicillium sp.	Ascomycota	Eurotiomycetes	Alder, birch, and spruce
Epicoccum sp.	Ascomycota	Dothideomycetes	Spruce
Inocybe sp.	Basidiomycota	Agaricomycetes	Alder and spruce
Mortierella sp.	Zygomycota	*Incertae sedis*	Birch and spruce
Oidiodendron sp.	Ascomycota	Dothideomycetes	Spruce
Schizangiella sp.	Zygomycota	*Incertea sedis*	Spruce
Tomentella sp.	Basidiomycota	Agaricomycetes	Spruce
Trichoderma sp.	Ascomycota	Sordariomycetes	Alder, birch, and spruce

Deltaproteobacteria are also the second most abundant bacterial class in alder, birch, and spruce root endospheres, with relative abundances of 9.46%, 9.26%, and 8.45% (Fig. 4a-c and Supplementary Table 4b), respectively. In fact, it was shown in the literature that plant species and environmental parameters are strong drivers of microbial population composition in the rhizosphere and roots, which could explain the differences between our study and those of Gottel et al. (2011) and other scientists (Hartmann et al. 2011; Philippot et al. 2013).

To be more specific, ternary plots showed that OTUs associated with the most abundant bacterial classes seem to have a higher relative abundance in roots than in bulk soil and the rhizosphere (Fig. 6). At the species level, *Acinetobacter* sp., *Delftia* sp., and *Pseudomonas* sp. showed a higher relative abundance in plant roots than

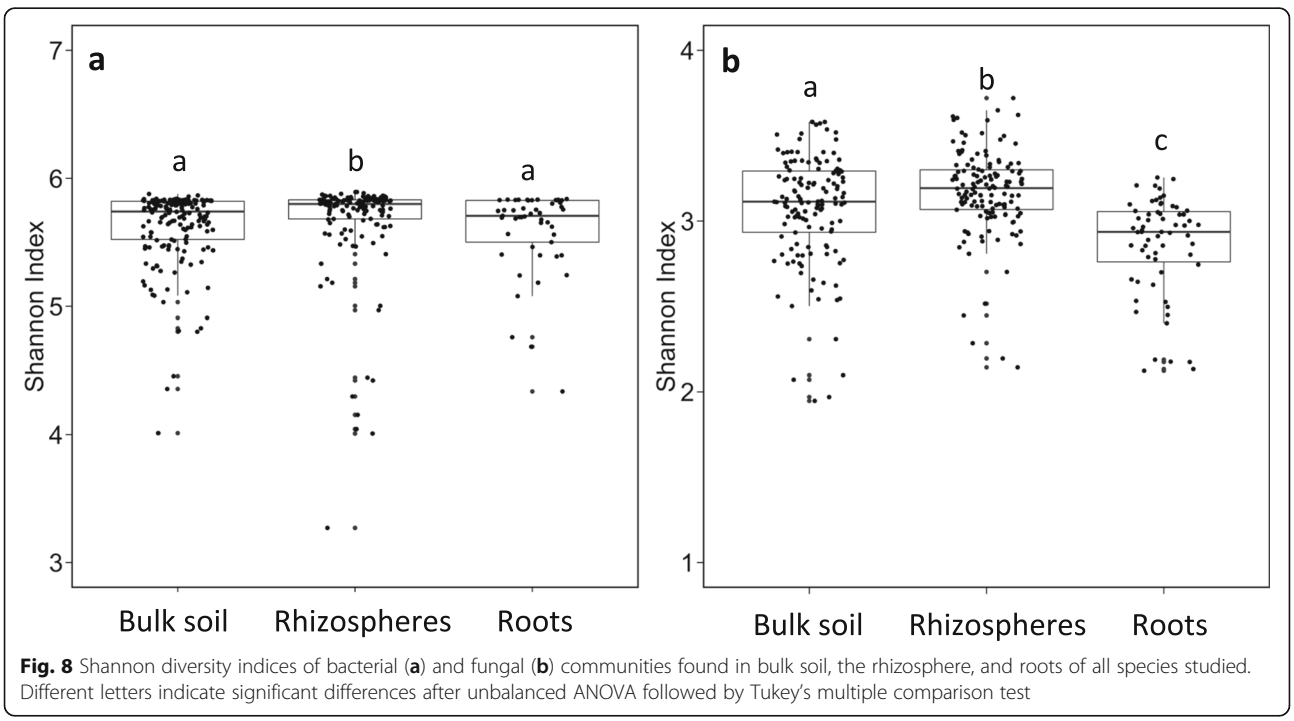

Fig. 8 Shannon diversity indices of bacterial (**a**) and fungal (**b**) communities found in bulk soil, the rhizosphere, and roots of all species studied. Different letters indicate significant differences after unbalanced ANOVA followed by Tukey's multiple comparison test

in the rhizosphere and bulk soil for a given plant species (Fig. 7a, b and c). Izumi (2011) reported that the endophytic bacterium *Acinetobacter* sp. is more represented in forest tree roots than in agricultural crops. Indeed, the genus *Pseudomonas* is one of the best root colonizers and represents one of the major groups (with *Bacillus* sp.) of endophytic communities in trees (Izumi 2011). *Pseudomonas* sp. and *Acinetobacter* sp. have PGPR activity in the rhizosphere of diverse plants, including nonsymbiotic biological fixation of nitrogen (Sivasakthi et al. 2014). More specifically, Patten and Glick (2002) showed that inactivation of the *ipdc* gene encoding a key enzyme involved with indoleacetic acid (IAA) in *Pseudomonas putida* leads to the development of fewer roots than the wild type, which suggests that IAA secreted by *P. putida* plays a major role in host root development. Moreover, the fluorescent microbe *Pseudomonas* could secrete 2,4-DAPG (2,4-diacetylphloroglucinol), which could enhance the defense of plant roots against phytopathogens (Weller et al. 2007). In addition, *Acinetobacter* sp., an organism living in plant tissues, including roots, promotes plant growth through IAA production (Li et al. 2012). Bacteria belonging to the genus *Delftia* have been poorly identified in plant roots; however, Roesch et al. (2007) reported the presence of *Delftia* spp. in roots of field-grown maize. Moreover, Han et al. (2005) described *Delftia tsuruhatensis* HR4 as a diazotroph organism with potential biocontrol activity against phytopathogens. Within the context of mine site reclamation, it has already been shown that endophytic microorganisms are major players in plant adaptation to environmental stress. The increased relative abundance of *Pseudomonas* sp., *Acinetobacter* sp., and *Delftia* sp. observed in roots could stimulate plant growth in a disturbed environment, such as the studied mine site (Rosenblueth and Martínez-Romero 2006; Weyens et al. 2009; Li et al. 2012).

Another study performed on bacterial and fungal communities in *P. deltoides* conducted by Shakya et al. (2013) showed that both the rhizosphere and root endosphere are dominated by Ascomycota (52%), Basidiomycota (26.9%), and Chytridiomycota (7.8%). Our results are in agreement with those presented by Shakya et al. (2013), where the rhizosphere of alder, birch, and spruce are dominated by Ascomycota with relative abundances of 33.18%, 34.55%, and 31.96%, respectively, and Basidiomycota with relative abundances in alder, birch, and spruce of 29.41%, 26.48%, and 29.45%. In addition, root endospheres of alder, birch, and spruce were also dominated by Ascomycota, with relative abundances of 37.17%, 38.62%, and 29.68%. Basidiomycota is the second most abundant phylum in the root endospheres of alder, birch, and spruce, with relative abundances of 19.02%, 18.58%, and 18.02% (Fig. 4d–f and Supplementary Table 5a). As observed in bacterial communities, fungal community composition is driven by plant species and environmental parameters (Hartmann et al. 2011; Gaiero et al. 2013). These soil environmental parameters partially drive the global physiological state of the plant, which ultimately results in specific selection of rhizosphere and root endosphere fungi by selective root exudates (Hartmann et al. 2011). The context of the mining site could explain the divergences in fungal communities (Ascomycota) observed in plants growing on the mine site (such as alder, birch, and spruce) versus communities in *P. deltoides* from the study reported by Shakya et al. (2013).

Ternary plots highlighted that fungal OTUs associated with the four most abundant classes identified (Agaricomycetes, Leotiomycetes, *Incertae sedis*, and Sordariomycetes) had relative abundances well distributed across the various environments in all of the plant species represented in the study (Fig. 6). More specifically, OTUs identified as *Cistella* sp. (alder), *Hyaloscypha* sp. (alder), and *Trichocladium* sp. (alder and birch) associated with the Pezizomycotina subdivision were equally represented in the rhizosphere and roots of some of the plant species (in parenthesis), as suggested by Gottel et al. (2011) (results not shown). Indeed, many fungal species, such as *Claussenomyces* sp., *Eupenicillium* sp. and *Trichoderma* sp., presented a decreased relative abundance in roots for all studied species (Fig. 6). In addition, Table 3 presents genera that were less abundant than the previously mentioned genera in the roots of alder, birch, and/or spruce. The colonization of roots by fungal endophytes leads to various benefits for both mutualistic partners, such as induction of defense against pathogens by release of a myriad of metabolites, enhanced resistance to abiotic stress (i.e., drought and heavy metals), and increased capture of essential nutrients (i.e., nitrogen and phosphorus) (Porras-Alfaro and Bayman 2011; Fortin et al. 2015). The promotion of plant growth by mycorrhizal fungi is possible via an extraradical network that develops in the rhizosphere. Among others, this network is responsible for uptake of nutrients and water that will ultimately be transferred to the plant via the intraradical network (hyphae in host root tissues) (Bonfante and Genre 2010). This extraradical network also greatly extends into the rhizosphere and bulk soil to allow high nutrient and water absorption, which could explain why many of the 30 most abundant fungal species studied are present in lower relative abundance in the roots than in the rhizosphere of some tree species (Table 3) (Grünig et al. 2011; Fortin et al. 2015).

Conclusions

This study revealed that in an acidogenic mine tailings deposit, plant species and soil pH were the key environmental drivers that structured plant-associated microbial

communities in the three compartments studied: bulk soil, the rhizosphere, and the root endosphere. The plant species was the most influential parameter in almost all of the compartments studied. We also observed that for a given plant species, microbial community structure varied considerably between bulk soil, rhizosphere, and root endosphere compartments (PCoA). The highest biodiversity indices were found in the rhizosphere, corroborating the rhizosphere effect, whereby the greatest variety and most abundant supply of nutrients supports a larger and more diverse microbial community than that found in other compartments. Our results also revealed that the most abundant bacterial classes were Gammaproteobacteria and Deltaproteobacteria in both the rhizosphere and root endosphere. We highlighted the fact that plant species drive microbial composition in the rhizosphere and root endosphere, which could explain the differences between the results of the study conducted on *P. deltoides* and the results presented in this paper (Gottel et al. 2011).

The results indicated that bacteria such as *Acinetobacter* sp., *Delftia* sp., and *Pseudomonas* sp. consistently dominated the root endosphere (increased relative abundance), although they were not very abundant in the rhizosphere or in bulk soil. In addition, we found that most of the fungal taxa were associated with Ascomycota and Basidiomycota, which is in agreement with results of other published studies on *P. deltoides*. The divergences in the relative abundance of the Ascomycota phylum in this study versus that in the study published by Shakya et al. (2013) could be explained by influences specific to the environment studied on the rhizosphere and root fungal species selection by plants. Thus, many fungal endophytes (such as *Claussenomyces* sp., *Eupenicillium* sp., and *Trichoderma* sp.) are less represented in the roots of species selected for this study than in the roots in their study.

The selectivity in fungal endophyte recruitment by alder, birch, and spruce is supported by the observation of a lower relative abundance for some fungal species (*Claussenomyces* sp., *Eupenicillium* sp., and *Trichoderma* sp.) in root endosphere than in adjacent soil. The potentially important extension of the extraradical network into the rhizosphere compared to intraradical networks in the root endosphere could account for the decreased relative abundance observed for many fungal species. This study sheds light on the biotic and abiotic environmental drivers that shape microbial communities associated with alder, birch, and spruce in the context of an acidogenic mine tailings deposit. To our knowledge, this is the first study on microbial community structures across boreal plant species and compartments (roots, rhizosphere, bulk soil) in a mine setting in Québec. This stepping stone is significant in beginning to elucidate the interactions between plant species and their abiotic environment using approaches such as transcriptomics. Identifying the mechanisms involved in plant-microbe-geosphere interactions will contribute to developing more effective, tailored phytotechnologies for the ecological restoration of disturbed sites.

Supplementary information

The online version of this article (https://doi.org/10.1186/s13213-020-01582-9) contains supplementary material, which is available to authorized users.

Additional file 1: Supplementary Table 1. Bulk soils environmental parameters analysis. Water content and organic content are reported in percentage (%) and the elemental results in $\mu g_{metal} \cdot g_{dryweight}^{-1}$. Min: minimum, Max: maximum, Avg: average, MD: mean deviation, Med: median, NA: non available. **Supplementary Table 2.** Primers used during the first amplification of the 16S rRNA of bacteria and ITS region of fungi. **Supplementary Table 3.** Number of replicate (n=) associated with each figures.

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Competing interests

The authors declare that they have no conflict of interest.

Ethics approval and consent to participate

This work does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

This manuscript is approved by all authors for publication.

Authors' contributions

VG performed the investigation, data analysis, validation, visualization and wrote the original draft. MRM performed the investigation and validation. JT performed data curation and the validation. JW performed data curation and validation. JC performed validation. JPB contributed to the conceptualization, methodology, resources, validation and funding acquisition. CWG contributed to the conceptualization, methodology, resources, validation, supervision and funding acquisition. SR contributed to the conceptualization, methodology, resources, validation, supervision, funding acquisition and project administration. The authors read and approved the final manuscript.

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Author details

[1]Centre SÈVE, Département de Biologie, Faculté des Sciences, Université de Sherbrooke, 2500 boulevard de l'Université, Sherbrooke, Québec J1K 2R1, Canada. [2]National Research Council Canada, Energy, Mining and Environment, 6100 avenue Royalmount, Montréal, Québec H4P 2R2, Canada. [3]Centre SÈVE, Département de Chimie, Faculté des Sciences, Université de Sherbrooke, 2500 boulevard de l'Université, Sherbrooke, Québec J1K 2R1, Canada.

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References

Aitchison J (2002) A concise guide to compositional data analysis. CDA Work. Girona. 24:73–81. https://doi.org/10.1016/j.envexpbot.2014.07.014

Badri DV, Weir TL, van der Lelie D, Vivanco JM (2009) Rhizosphere chemical dialogues: plant-microbe interactions. Curr Opin Biotechnol 20(6):642–650. https://doi.org/10.1016/j.copbio.2009.09.014

Baker-Austin C, Dopson M (2007) Life in acid: pH homoeostasis in acidophiles. Trends Microbiol 15(4):165–171. https://doi.org/10.1016/j.tim.2007.02.005

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486 https://doi.org/10.1016/j.tplants.2012.04.001

Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68(1):1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x

Bergeron Y, Gauthier S, Flannigan M, Kafka V (2004) Fire regimes at the transition between mixed wood and coniferous boreal forest in Northwestern Quebec. Ecology 85(7):1916–1932. https://doi.org/10.1890/02-0716

Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Park J, Makino T, Kirkham MB, Scheckel K (2014) Remediation of heavy metal(loid)s contaminated soil – to mobilize or to immobilize? J Hazard Mater 266:141–166 https://doi.org/10.1016/j.hazmat.2013.12.018

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant-fungus interaction in mycorrhizal symbiosis. Nat Commun 48:1–11. https://doi.org/10.1038/ncomms10

Booth IR (1985) Regulation of cytoplasmic pH in bacteria. Microbiol Rev 49(4):359–381

Borcard D, Gillet F, Legendre P (2011) Numerical ecology with R. Springer: New York. https://doi.org/10.1002/aheh.200390025

Brockett BFT, Prescott CE, Grayston SJ (2012) Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biol Biochem 44(1):9–20. https://doi.org/10.1016/j.soilbio.2011.09.003

Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM (2008) Root exudates regulate soil fungal community composition and diversity. App Env Microbiol 4(3):738–744. https://doi.org/10.1128/AEM.02188-07

Bruins MR, Kapil S, Oehme FW (2000) Microbial resistance to metals in the environment. Ecotoxicol Environ Saf 45(3):198–207. https://doi.org/10.1006/eesa.1999.1860

Cavaglieri L, Orlando J, Etcheverry M (2009) Rhizosphere microbial community structure at different maize plant growth stages and root locations. Microbiol Res 164(4):391–399. https://doi.org/10.1016/j.micres.2007.03.006

Courty P-E, Buée M, Diedhiou AG, Frey-Klett P, Le Tacon F, Rineau F, Turpault M-P, Uroz S, Garbaye J (2010) The role of ectomycorrhizal communities in forest ecosystem process: new perspectives and emerging concepts. Soil Biol Biochem 42(5):679–698. https://doi.org/10.1016/j.soilbio.2009.12.006

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72(7):5069–5072. https://doi.org/10.1128/AEM.03006-05

Edwards J, Johnson C, Santos-Medellin C, Lurie E, Podishetty NK, Bhatnagar S, Eisen JA, Sundaresan V (2015) Structure, variation, and assembly of the root-associated microbiomes of rice. PNAS 112(8):911–920. https://doi.org/10.1073/pnas.1414592112

Fernandes AD, Reid JNS, Macklaim JM, McMurrough TA, Edgell DR, Gloor GB (2014) Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. Microbiome 2(15):1–13. https://doi.org/10.1186/2049-2618-2-15

Fortin JA, Plenchette C, Piché Y (2015) Les Mycorhizes - L'essor de la Nouvelle Révolution Verte. Multimonde, Montréal

Gaiero RJ, McCall AC, Thompson AK, Day NJ, Best AS, Dunfield KE (2013) Inside the root microbiome: bacterial root endophytes and plant growth promotion. Am J Bot 100(9):1738–1750. https://doi.org/10.3732/ajb.1200572

Ghodsi M, Liu B, Pop M (2011) DNACLUST: accurate and efficient clustering of phylogenetic marker genes. BMC Bioinformatics 12(271):1–11. https://doi.org/10.1186/1471-2105-12-271

Gottel NR, Castro HF, Kerley M, Yang Z, Pelletier DA, Podar M, Karpinets T, Uberbacher E, Tuskan GA, Vilgalys R, Doktycz M, Schadt CW (2011) Distinct microbial communities within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil types. App Environ Microbiol 77(17):5934–5944. https://doi.org/10.1128/AEM.05255-11

Grünig CR, Queloz V, Sieber TN (2011) Structure of diversity in dark septate endophytes: from species to genes. In: Pirttilä A, Frank A (eds) Endophytes of forest trees. Springer, Dordrecht, pp 3–30

Guo W, Nazim H, Liang Z, Yang D (2016) Magnesium deficiency in plants: an urgent problem. Crop J 4(2):83–91 https://doi.org/10.1016/j.cj.2015.11.003

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53(366):1–11 https://doi.org/10.1093/jxb/53.366.1

Han J, Sun L, Dong X, Cai Z, Sun X, Yang H, Wang Y, Song W (2005) Characterization of a novel plant growth-promoting bacteria strain *Delftia tsuruhatensis* HR4 both as a diazotroph and a potential biocontrol agent against various plant pathogens. Syst Appl Microbiol 28(1):66–76. https://doi.org/10.1016/j.syapm.2004.09.003

Hartmann A, Schmid M, vab Tuinen D, Berg G (2009) Plant-driven selection of microbes. Plant Soil 321(1-2):235-257. https://doi.org/10.1007/s11104-008-9814-y

Hodge A, Fitter AH (2013) Microbial mediation of plant competition and community structure. Funct Ecol 27(4):865–875. https://doi.org/10.1111/1365-2435.12002

Hong SC, Yang C, Zhuang Z (2016) Application of peptide nucleic acid-based assays toward detection of somatic mosaicism. Mol Ther-Nucl Acids 5:1–5. https://doi.org/10.1038/mtna.2016.22

Hossain MA, Piyatida P, Teixeira da Silva JA, Fujita M (2012) Molecular mechanism of heavy metal toxicity and tolerence in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. J Bot 2012:1–37 https://doi.org/10.1155/2012/872875

Hrynkiewicz K, Baum C (2011) The potential of rhizosphere microorganisms to promote the plant growth in disturbed soil. Springer, Dordrecht

Huang X-F, Chaparro JM, Reardon KF, Zhang R, Shen Q, Vivanco JM (2014) Rhizosphere interactions: root exudates, microbes, and microbial communities. Botany 92(4):267–275. dx.doi.org. https://doi.org/10.1139/cjb-2013-0225

Huber DM, Jones JB (2013) The role of magnesium in plant disease. Plant Soil 368(1-2):73–85. https://doi.org/10.1007/s11104-012-1476-0

Izumi H (2011) Diversity of endophytic bacteria in forest trees. In: Pirttilä A, Frank A (eds) Endophytes of forest trees. Springer, Dordrecht, pp 95–106

Kneeshaw DD, Bergeron Y (1998) Canopy gap characteristics and tree replacement in the southeastern boreal forest. Ecology 79(3):783–794. https://doi.org/10.1890/0012-9658(1998)079[0783:CGCATR]2.0.CO;2

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM et al (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22(21):5271–5277. https://doi.org/10.1111/mec.12481

Krulwich TA, Sachs G, Padan E (2011) Molecular aspects of bacterial pH sensing and homeostasis. Nat Rev Microbiol 9:330–343. https://doi.org/10.1038/nrmicro2549

Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. App Environ Microbiol 75(15):5111–5120. https://doi.org/10.1128/AEM.00335-09

Legendre P, Legendre L (2009) Numerical ecology. Elsevier, Oxford

Li HY, Wei DQ, Shen M, Zhou ZP (2012) Endophytes and their role in phytoremediation. Fungal Divers 54(1):11–18. https://doi.org/10.1007/s13225-012-0165-x

Liu J, Hua Z-S, Chen L-X, Kuang J-L, Li S-J, Shu W-S, Huang L-N (2014) Correlating microbial diversity pattern with geochemistry in an extreme and heterogenous mine tailings environement. Appl Environ Microbiol 80(12):3677–3686. https://doi.org/10.1128/AEM.00294-14

Lundberg DS, Lebeis SL, Parades SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrekston A, Kunin V, Glavina del Rio T et al (2012) Defining the core *Arabidopsis thaliana* root microbiome. Nature 488:86–90. https://doi.org/10.1038/nature11237

Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29(2):248–258. https://doi.org/10.1016/j.biotechadv.2010.12.001

Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Beneficial role of bacterial endophytes in heavy metal phytoremediation. J Environ Manage 174:14–25 https://doi.org/10.1016/j.jenvman.2016.02.047

Ministère de l'Énergie et des Ressources Naturelles (MERN) (2013) Carte du Québec. https://mern.gouv.qc.ca/territoire/portrait/portrait-quebec.jsp.
Ministère de l'Énergie et des Ressources Naturelles (MERN) (2015) Tableau de bord sur les indicateurs de développement durable dans le secteur minier du Québec. https://mern.gouv.qc.ca/publications/mines/Tableau_bord_IDD.pdf Accessed January 14 2020
Ministère de l'Énergie et des Ressources Naturelles (MERN) (2017) La restauration des sites miniers abandonnés au Québec – Bilan 2016. https://mern.gouv.qc.ca/mines/quebec-mines/2017-05/restauration-site-minier.asp Accessed January 14 2020
Ministère de l'Énergie et des Ressources Naturelles (MERN) (2018) History of the mining industry. https://mern.gouv.qc.ca/en/mines/mining-industry/history-mining-industry/ Accessed September 24 2019
Moomaw AS, Maguire ME (2008) The unique nature of Mg^{2+} channels. Am Physiol Soc 23(5):275–285 https://doi.org/10.1152/physiol.00019.2008
Nwuche CO, Ugoji EO (2008) Effects of heavy metal pollution on the soil microbial activity. Int J Environ Sci Tech 5(3):409–414. https://doi.org/10.1007/BF03326036
Patten CL, Glick BR (2002) Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. App Environ Microbiol 68(8):3795–3801. https://doi.org/10.1128/AEM.68.8.3795-3801.2002
Philippot L, Raaijmakers JM, Lemeanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799 https://doi-org.ezproxy.usherbrooke.ca/10.1038/nrmicro3109
Porras-Alfaro A, Bayman P (2011) Hidden fungi, emergent properties: endophytes and microbiomes. Annu Rev Phytopathol 49:291–315. https://doi.org/10.1146/annurev-phyto-080508-081831
Ramesh A, Winkler WC (2010) Magnesium-sensing riboswitches in bacteria. RNA Biol 7(1):77–83 https://doi.org/10.4161/rna.7.1.10490
Rieuwerts JS, Thornton I, Farago ME, Ashmore M (1998) Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. Chem Spec Bioavail 10(2):61–75. https://doi.org/10.3184/095422998782775835
Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26(1):139–140. https://doi.org/10.1093/bioinformatics/btp616
Roesch LF, Camargo FAO, Bento FM, Triplett EW (2007) Biodiversity of diazotrophic bacteria within the soil, roots and stem of field-growth maize. Plant Soil 302(1-2):91–104. https://doi.org/10.1007/s11104-007-9458-3
Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant-Microbe Interact 19(8):827–837. https://doi.org/10.1094/MPMI-19-0827
Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4:1340–1351 https://doi.org/10.1038/ismej.2010.58
Santoyo G, Moreno-Hagelsied G, Orozco-Mosqueda MC, Glick BR (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183:92–99 https://doi.org/10.1016/j.micres.2015.11.008
Shakya M, Gottel N, Castro H, Yang ZK, Gunter L, Labbé J, Muchero W, Bonito G, Vilgalys R, Tuskan G et al (2013) A multifactor analysis of fungal and bacterial community structure in the root microbiome of mature *Populus deltoides* trees. PLOS One 8(10):1–12 https://doi.org/10.1371/journal.pone.0076382
Shaul O (2002) Magnesium transport and function in plants: the tip of the iceberg. Biometals 15(3):307–321. https://doi.org/10.1023/a:1016091118585
Sheoran V, Sheoran AS, Poonia P (2010) Soil reclamation of abandoned mine land by revegetation: a review. Int J Soil Sediment Water 3:1–21
Sivasakthi S, Usharani G, Saranraj P (2014) Biocontrol potential of plant growth promoting bacteria (PGPR)-*Pseudomonas fluorescens* and *Bacillus subtilis*: a review. Afr J Agric Res 9(16):1265–1277 https://doi.org/10.5897/AJAR2013.7914
Tardif A, Rodrigue-Morin M, Gagnon V, Shipley B, Roy S, Bellenger J-P (2019) The relative importance of abiotic conditions and subsequent land use on the boreal primary succession of acidogenic mine tailings. Ecol Eng 127:66–74 https://doi.org/10.1016/j.ecoleng.2018.11.003
Tremblay J, Singh K, Fern A, Kirton ES, He S, Woyke T, Lee J, Chen F, Dangl JL, Tringe SG (2015) Primer and platform effects on 16S rRNA tag sequencing. Front Microbiol 6:1–15. https://doi.org/10.3389/fmicb.2015.00771
Turner TR, James EK, Poole PS (2013) The plant microbiome. Genome Biol 14(6):209. https://doi.org/10.1186/gb-2013-14-6-209
Van Der Heijden MGA, Bardgett RD, Van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11(3):296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x
Verbruggen N, Hermans C (2013) Physiological and molecular responses to magnesium nutritional imbalance in plants. Plant Soil 368(1-2):87–99. https://doi.org/10.1007/s11104-013-1589-0
Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73(16):5261–5267. https://doi.org/10.1128/AEM.00062-07
Weller DM, Landa BB, Mavrodi OV, Schroeder KL, De la Fuente L, Blouin Bankhead S, Allende Molar R, Bonsall RF, Mavrodi DV, Thomashow LS (2007) Role of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. in the defense of plant roots. Plant Biol 9:4–20. https://doi.org/10.1055/s-2006-924473
Weyens N, van der Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant-endophyte partnerships take the challenge. Curr Opin Biotechnol 20(2):248–254. https://doi.org/10.1016/j.copbio.2009.02.012
Yergeau E, Bell TH, Champagne J, Maynard C, Tardif S, Tremblay J, Greer CW (2015) Transplanting soil microbiomes leads to lasting effects on willow growth, but not on the rhizosphere microbiome. Front Microbiol 6:1–14. https://doi.org/10.1128/AEM.00062-07
York LM, Carminati A, Mooney SJ, Bennett MJ (2016) The holistic rhizosphere: integrating zones, processes, and semantics in the soil influenced by roots. J Experiment Bot 67(12):3629–3643. https://doi.org/10.1093/jxb/erw108
Zhuang X, Chen J, Shim H, Bai Z (2007) New advances in plant growth-promoting rhizobacteria for bioremediation. Environ Int 33(3):406–413 https://doi.org/10.1016/j.envint.2006.12.005

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