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Bacterial and fungal community structure and diversity in a mining region under long-term metal exposure revealed by metagenomics sequencing

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ABSTRACT

Metals are known to alter soil ecosystem diversity, structure and function. The main objective of the present study is to determine the effects of soil metal contamination on bacterial and fungal biomass, abundance, and diversity based on phospholipid fatty acid (PLFA) and metagenomics (pyrosequencing) analyses. Soil samples from six sites from Northern Ontario (Canada) were analyzed. Chemical analysis showed significant difference between metal contaminated and reference sites for pH, cations exchange capacity (CEC), soil organic carbon (C) and nitrogen (N). Significant differences between metal contaminated and uncontaminated reference sites were observed for total bacteria, arbuscular fungi (AM fungi), other fungi and eukaryotes. *Acidobacteria* and *Proteobacteria* were the most dominant bacterial taxonomic groups in all the sites. For fungi, *Ascomycota* were more prevalent in metal contaminated soils (35.07%) while *Basidiomycota* represented 59.26% of all fungi in reference areas. Site-specific bacterial and fungal families and genera were identified and characterized. Analysis of bacterial communities revealed Chao1 index values of 232 and 273 for metal contaminated and reference soils, respectively. For fungi, the Chao index values were 23 for metal contaminated and 45 for reference sites. OTUs followed the same trend for both bacteria and fungi. No significant differences were observed for Simpson index, Shannon index and species evenness between two soil groups for bacteria and fungi. Overall, PLFA and metagenomic analyses revealed significant reductions of microbial biomass and abundance in contaminated sites compared to reference soil type. No significant variations in microbial diversity were observed when all the sites were compared.

1. Introduction

While the effects of many factors such as invasive species, habitat diversification, climate change, pollution and temperature on the biodiversity of plants and animals are well established and studied, little attention has been paid to soil microbial communities. Soil contains enormous microbial diversity, with an estimated 10^7–10^9 distinct bacterial species [1] and 1.5 million fungi taxa [2] worldwide. Nowadays, an ever increasing rate of species extinction is resulting in destructive consequences for ecosystem functions and will also limit the potential economic benefits of biodiversity [3–6].

Mining related activities have resulted in severe land disturbances throughout the world. For nearly a century, logging, smelting and mining activities have caused severe negative effects to the environment in the Greater Sudbury Region (GSR) in Northern Ontario, Canada [5,7–11]. This region was reported to be the greatest single source for sulfur dioxide (SO_2) emissions in Canada [7–10]. In addition, Sudbury smelters released into the atmosphere large quantities of metals including iron (Fe), nickel (Ni) and copper (Cu). In fact, Falkowski [12] reported that, over 14,000 tonnes of Fe, 2000 tonnes of Ni and 1800 tonnes of Cu were released annually into the atmosphere.

These metals and SO_2 emissions have caused damage to plants, animals and soil microorganisms. Severe contamination and acidification of soils and water at sites around smelters have been documented [5,7,10,11]. In the last 40 years, SO_2 and metal emissions have been reduced drastically through a combination of

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developments in industrial technologies (safer extraction methods and better filtration) and legislated controls in the GSR [5,10,11]. This has resulted in an improvement in atmospheric quality and natural recovery of damaged ecosystems. In addition, land reclamation is being implemented by a regional regreening program. It consists of limestone application to soils, seed distribution and tree planting (with over 12 million trees planted so far in many areas of the GSR) [5,10]. This has led to an increase of plant species diversity, soil organic matter, and microbial biomass [5,10,13].

Although some metals are required for life's physiological processes, excessive accumulation in living organisms is always detrimental. A sustainable ecosystem depends on functional microbial communities, which play a significant role in organic matter decomposition, degradation of toxic substances, nitrogen fixation, nutrient cycling, production of phytohormones as well as plant health and growth [4–6,14]. Knowledge of the interaction between metals and soil microorganisms is very limited.

There is no consensus on the effects of metals on microbial diversity and abundance. Guo et al. [15] reported no impact on bacterial diversity in soils from two abandoned copper mines contaminated with metals using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis. Other studies have shown that both short-term and long-term exposure to metals results in the reductions of soil microbial biomass, diversity (based on PCR-DGGE) and its activity [16–18]. It is also established that metals at high concentrations cause enzyme inactivation and damage cells by acting as antimetabolites or by forming precipitates or chelates with essential metabolites in microorganisms [19]. Analyses of microbial diversity in metal contaminated samples based on a sensitive and detailed high throughout sequencing analysis are sketchy. Chodak et al. [20] analyzed bacterial diversity in forest soil with different degrees of heavy metal pollution and found no effects of heavy metals on the soil bacteria structure measured by pyrosequencing. Golebiewski et al. [21] found that zinc (Zn) decreased bacterial diversity and species richness based on 16S rDNA pyrosequencing. Data on damages caused by nickel (Ni) and copper (Cu) to soil microbial communities are sketchy. Likewise, studies on the effects of metals on fungi diversity and structure are lacking.

We hypothesized that metal contamination (as due to mining activities such in the GSR) imposes distinct impacts on the microenvironment (specifically bacteria and fungi) in which microorganisms exist and the variation in the microbial community structures would be associated with the changes in the physiochemical properties of the soils. The main objective of the present study is to determine the effects of long-term soil metal contamination on bacterial and fungal diversity and abundance using PFLA and metagenomics (pyrosequencing) analyses.

2. Materials and methods

2.1. Study site and soil sample collection

The study was carried at six locations close to mining sites in Northern Ontario, Canada. This region has been exposed to abundant deposition of metals, notably Ni and Cu, emitted through active smelting for over a century. The soils of the Sudbury area are mostly podzols or brunisols and formed in sandy fluvial and glaciofluvial deposits [22]. Six sites were selected from the Northern Ontario (Fig. 1, [23]). Sites were classified as metal contaminated and reference based on data from previous soil physico-chemical analyses [5,11,24]. Metal contaminated sites include Laurentian, Kelly Lake, and Kingsway. Reference sites include Onaping Falls, Capreol, and Killarney. No specific permissions were required for soil sampling at these locations since the sites are part of Laurentian University research areas and Crown (public) lands that are not within a park or a conservation reserve.

Major environmental conditions such as temperature, and rainfalls were presumed similar among the sites based on literature [22,25,26] while metal, organic matter contents, and cation exchange capacity (CEC) may vary [5,11,24]. Metal contaminated sites were characterized as sandy/clay soil rich in total Ni and Cu. Each site was approximately 5 km^2 in size. At each site, 20 samples were collected randomly from the organic layer (0–5 cm in depth). Plant material, stones and residues were removed; and soil samples from each site were bulked and mixed. They were sieved using a 2 mm mesh and stored for a short period prior to analyses (in an incubator for soil chemical analyses and in a freezer for PLFA analysis). DNA from the soil samples were extracted the same day (within hours) after sampling.

2.2. Soil chemical analysis

Soil pH was determined on air dried subsamples in deionized water and in neutral salt solution (0.1 M $CaCl_2$) [27]. CEC was measured using an ammonium acetate extraction method at pH 7 developed by Lavkulich [28]. CEC is a measure of the quantity of readily exchangeable cations neutralizing negative charge in the soil. The exchangeable cations aluminum, Al^{3+}; calcium, Ca^{2+}; iron, Fe^{3+}, potassium, K^{+}; magnesium, Mg^{2+}, manganese, Mn^{2+} and sodium, Na^{+} were quantified by inductively coupled plasma mass spectrometry (ICP-MS). The total exchange capacity was estimated as the sum of the exchangeable cations [29]. Total concentration of metals were measured after digestion of 0.5 g of soil samples with 10 ml of 10:1 ratio of HF/HCl at 150 °C [24]. Bioavailable metals were assessed after shaking 5 g of soil samples with 20 ml of 0.01 M $LiNO_3$ for 24 h at 20 °C following by filtration of extracts [24]. Total and bioavailable metals were detected using ICP-MS.

2.3. Phospholipid fatty acid analysis

PLFA analysis was performed at FAME Lab, Microbial ID. Inc, Newark, Delaware (USA) as described by Buyer and Sasser [30]. Mole percentage of each PLFA was used to indicate the relative abundance of bacteria (gram positive and gram negative bacteria), arbuscular mycorrhizal (AM) fungi, other fungi and actinomycetes in soil. Total PLFA extracted from soil was used as an index of living microbial biomass [5,30].

2.4. Microbial DNA extraction and purification

Microbial DNA was extracted from 10 g of fresh soil per site by using the PowerMax® Soil DNA Isolation Kit for soil (MO BIO Laboratories, Inc., Carlsbad, CA, USA, cat # 12,988–10), according to the manufacturer's instructions. The concentration and purity of the extracted DNA were determined with a fluorescent DNA quantification kit (Bio-Rad Laboratories, Hercules, CA, USA, cat # 170-2480) following the procedure described by the manufacturer. Finally, extracted DNAs were diluted to concentration of 2 ng/μl for all samples prior to metagenomic analysis that was performed at Molecular Research DNA laboratory (MR DNA, Shallowater, Texas, USA).

2.5. PCR amplification and pyrosequencing

Bacterial and fungal microbiotas were assessed using high throughout sequencing of 16S and internal transcribed spacer (ITS) genes. Tag-encoded FLX-titanium 16S rDNA gene amplicon pyrosequencing (bTEFAP) was performed using 16S universal eubacterial primers 530F (5′ GTG CCA GCM GCN GCG G) and 1100R (5′ GGG