THE STRUCTURAL DEVELOPMENT OF SOIL MICROBIAL COMMUNITIES IN RECLAIMED SITES OF A METAL MINE; IMPLICATIONS TO THE RESTORATION OF ANTHROPOGENIC DISTURBANCES.

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Abstract

Anthropogenic activities affect the structure of terrestrial systems, leading to diminished ecosystem functions. With the cumulative area of disturbance caused by mining in British Columbia approximating 45,412 ha, it is necessary to ensure that reclamation projects are directed by ecologically relevant objectives. In this study, phospholipid fatty acid analysis was used to compare the biomass of soil microbial communities in undisturbed reference and reclaimed sites. Similar to the development of microbial communities following natural disturbance events, microbial biomass within reclaimed sites increased through time, but was significantly lower than undisturbed forests until 30 to 45 years post reclamation. Canonical correspondence analysis identified that the differences in microbial community biomass was affected by changes in soil pH and copper. Due to the long-term requirements for soil development, the assessment of reclamation projects should exceed at least 30 years post completion to properly evaluate the recovery of soil ecosystem composition and function.

Keywords

Disturbed soils, soil chemistry, microbial community development, Belowground-aboveground linkages, restoration success

Introduction

Microbial species are the most diverse and abundant organisms within terrestrial and aquatic systems, and are fundamental to the regulation of biogeochemical cycles and the plant communities that depend on nutrient cycling (Jeffries et al. 2003). Soil microorganisms are a required component for proper ecosystem functioning, stimulating decomposition of organic matter, and soil development (Nannipieri et al. 2003). Cumulatively, the major groups in soil microbial communities (e.g., Bacteria, Fungi, and Protozoa) are important features in soil systems, regulating physical soil characteristics and the availability, uptake, and allocation of nutrients and metals in plant species (Song et al. 2004, Asmelash et al. 2016, Bhatti et al. 2017). Consequently, if microbial functional groups are reduced due to natural or anthropogenic disturbance ecosystem level impacts may be observed, such as the reduction in nutrient cycling and decline in the establishment of native plant species (Steenwerth et al. 2002, Moore et al. 2010, Araújo et al. 2013).

Disturbances caused by anthropogenic activities (e.g., open-pit mining) results in the modification of physical, chemical, and biological soil characteristics (Poncelet et al. 2014). Following poor mining practices, the effects of modified physical and chemical soil properties have considerable implications on the structure and function biological soil characteristics (e.g., soil microbial communities) (Harris and Birch 1989). During open-pit mining soil is removed to access valuable minerals and metals. Following the removal of soil layers, organic materials and mineral soils are stockpiled in overburden dumps and stored until needed as growing material in reclamation (Williamson and Johnson 1991). After the removal and storage of soil, microbial functional groups inhabiting upper soil horizons are mixed within subsoil and parent materials leading to the dilution and reduction of microbial communities and nutrients (Li et al. 2014). In addition to the dilution of soil microbial functional groups in overburden dumps, microbial communities are negatively impacted by nutrient leaching, soil compaction, and lack of organic matter in stored soils (Harris et al. 1993). Creating a stressful environment, long-term storage of overburden leads to the reduction of various microbial functional groups and significant decrease in the community biomass when compared to undisturbed soil (Harris and Birch 1989, Harris et al. 1993, Poncelet et al. 2014).

Although the mechanisms of disturbance are different between natural and anthropogenic events, the development of soil microbial communities, and the influence that physical and chemical soil proprieties have on the establishment of soil microbial communities are similar regardless of the disturbance type (Williamson and Johnson 1991, Jangid et al. 2013, Cutler et al. 2014, Quadros et al. 2016). Since physical and chemical properties regulate the structure and function of soil microbial communities after natural and anthropogenic disturbances, it is expected that observations obtained through studying the succession of microbial communities after natural disturbances can be applied to restore microbial communities after anthropogenic disturbances (Banning et al. 2011).

Defined as the process of assisting the recovery of a damaged, degraded, or destroyed systems to a state of ecological, recreational, or economic importance, mine reclamation may overlook key ecological features due to the emphasis on anthropogenic values (Rooney and Bayley 2011, Powter et al. 2012, Poncelet et al. 2014). Criteria for assessing the success of mine reclamation projects in British Columbia is usually based on short-time events (e.g., one or more years), and largely encompass the use of generic visually distinguishable aboveground indicators, such as the presence of soil erosion and extent of vegetation cover (Poncelet et al. 2014). While reclamation and restoration of mined lands are often viewed as vastly different activities, the processes required to assist the recovery of ecosystem structure and function are similar due to the importance of underlining physical, chemical, and biological system features (Ecological Restoration International Science & Policy Working Group 2004). For example, the Society for Ecological Restoration (SER) has set criteria to address the issue of what is restoration, in general, deeming projects as restoration if sufficient biotic and abiotic resources are present within a site to create a self-sustaining system (Ecological Restoration International Science & Policy Working Group 2004). Additionally, the SER has provided nine attributes to determine when restoration has been accomplished, with emphasis on the use of native vegetation species, and representation of all necessary functional groups within an ecosystem (Ecological Restoration International Science & Policy Working Group 2004). With the use of ecologically relevant metrics-of-success to design and assess projects, the recovery of mined lands can be transitioned from a focus on reclamation to ecological restoration.

In the field of mine reclamation, ecologists have neglected to assess the entire impact of mining activities on the structure and function of ecosystems (Dimitriu et al. 2010). Historically, research evaluating the success of reclamation projects has been focused on rudimentary abiotic and biotic parameters such as the status of above ground ecosystem characteristics (e.g., percent cover of vegetation) (Dimitriu et al. 2010). Although above ground ecosystem indicators are important metrics of success, practitioners must continue to assess the impact of mining activities on below ground ecosystem characteristics (e.g., the presence and structure of soil microbial communities), and continue to research the abiotic and biotic drivers of microbial community development (Mummey et al. 2002, Dimitriu et al. 2010). The recovery of native microbial communities is a necessity to restore biochemical processes and successfully establish native plant species within disturbed sites (Jeffries et al. 2003, Dimitriu et al. 2010, Banning et al. 2011). Consequently, observing the structural and functional changes of microbial communities through time may be an effective means to evaluate the recovery of an ecosystem to an anthropogenic stressor (e.g., surface mining) (Li et al. 2014).

In this study, I examined the development of soil microbial communities through time within reclaimed sites, the effects of physical and chemical soil properties on the presence and biomass of microbial functional groups, and the composition of native vegetation communities within reclaimed sites. The primary goal of my research was to identify and introduce ecologically relevant metrics-of-success within the field of mine reclamation, and improve our knowledge on the recovery of soil microbial communities after mining disturbance. Specifically, to identify the time required for the recovery of seven different microbial functional groups (e.g., Saprophytic fungi, and Rhizobacteria), of which have a diversity of functions within soil ecosystems. The information gained may be applied to the field of ecological restoration to assist the recovery of sites damaged, degraded, or destroyed by natural or anthropogenic stressors (e.g., agriculture, forestry, and surface mining).

Methods

Study Area and experimental design

Research was conducted within the permit boundaries of the Gibraltar Mine site, located in the Caribou-Chilcotin region of interior British Columbia. The study area is located at elevations between 914-1231m, and receives on average 618.3mm of total precipitation annually. The mean annual temperature for the study area is 5.6 °C, with the lowest minimum temperatures occurring in January (i.e., -21.3 °C), and highest maximum temperatures occurring in July (i.e., 31.5°C). The study area is within the Sub-Boreal Spruce biogeoclimatic zone, specifically, ecosystems are characterized as moist cold (i.e., SBSmc1) and dry warm (i.e., SBSdw2). Soils in the study area contain basal moraine deposits, and the dominant soil type in undisturbed areas are classified as brunisolic gray luvisols.

Throughout the study area, reclamation activities have been completed on sites that have been disturbed by copper mining practices. 147 reclaimed sites were categorized based on reclamation techniques and date of completion. Sites were reclaimed using a diversity of methods, including placement of soil material to provide an adequate growing medium, deep ripping to reduce soil compaction, and revegetation with native and non-native herbaceous grass species such as *Agropyron cristatum* (Crested

wheatgrass), *Dactylis glomerate* (Orchard grass), *Festuca rubra rubra* (Red fescue), and *Phleum pretense* (Timothy) to provide vegetation cover.

The 147 reclaimed sites were placed in categories based on year's post reclamation (i.e., 0-10, 10-20, 20-30, and 30-45 year's post reclamation). The five categories based on year's post reclamation (i.e., 0-10, 10-20, 20-30, and 30-45 year's post reclamation) formed the experimental units. Two sample units were randomly selected from the 147 sites and used to create each of the five experimental units (i.e., 2 (2), 17, 18, 25(2), 43, and 45 year's post reclamation. Two reference sites were used to compare the microbial community structure in mature forests unaltered by direct mining activities to reclaimed sites. The reference sites were selected using terrestrial ecosystem mapping targeting SBSdw2 classified sites. Reference sites were predominated by a mixture of lodgepole pine and/or Douglas-fir within drier sites and hybrid white spruce within wetter sites, and are representative of the expected biological communities for the age and ecosystem type surrounding the mine site.

Soil sampling

12 soil samples were collected within each of the five experimental units, with a total of 60 soil samples: thirty were for physical and chemical soil parameters (e.g., copper (µg/g), and pH), and thirty for assessing the presence and biomass of microbial functional groups. Soil samples were collected in July and August 2017 from reclaimed sites and reference sites. Soil samples were collected along three equally spaced 100 meter transects at a depth of 10 centimeters. Along each transect, eight subsamples were taken at random points and combined to create 1 mixed-sample (approximately 500 grams of soil material) to analyze physical and chemical soil properties, and one mixed-sample (approximately 500 grams of soil material) to analyze the biomass of soil microbial functional groups. Following collection, soil samples were frozen at -20°C until shipment to designated laboratories.

Vegetation community sampling

At each soil sampling location, a 1x1m quadrat and 3.99m circular plot were used to visually assess relative understory percent cover, percentage of litter, percentage of woody debris, percentage of moss, percentage of bare ground, percentage of over story species, and species abundance.

Physical and chemical soil laboratory analysis

The physical and chemical properties of soil samples were determined by AGAT Laboratories , Vancouver BC, following procedures described within the British Columbia Ministry of Environment Laboratory Manual and the Environmental Protection Agency manual for inductively coupled plasma mass spectrometry (ICP-MS) (US EPA 2015). Soil pH was determined by samples drying samples, 60 $\pm$ 5°C, sieving (2mm) and adding de-ionized water at a 2:1 water to soil ratio. Soils to be analyzed for metals were digested using a mixture of nitric acid, hydrochloric acid, and de-ionized water. The concentration of Antimony(Sb), Arsenic (As), Barium (Ba), Beryllium (Be), Bismuth(Bi), Cadmium (Cd), Calcium (Ca), Chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Lead (Pb), Lithium (Li), Magnesium (Mg), Manganese (Mn), Mercury (Hg), Molybdenum (Mo), Nickel (Ni), Selenium (Se),Silver (Ag), Strontium (Sr), Thallium (Tl), Tin (Sn), Vanadium (V), Zinc (Zn), and Zirconium (Zr)

was measured using ICP-MS. The percent of particle size distribution was determined using sieve and hydrometer analysis (J. B. Jones 2001).

Soil microbial laboratory analysis

Phospholipid fatty acid analysis (PLFA) was completed according to methods of Macdonald et al 2015 (Clapperton et al. 2005). Total soil lipids were extracted in test tubes by shaking approximately 2 g (dry weight equivalent) of frozen soil in a 9.5 ml dichloromethane (DMC): methanol: citrate buffer. Phospholipids were separated from neutral and glycolipids using sequential leaching with DCM, acetone, and methanol. Following the creation of fatty acid methyl esters through mild acid methanolysis, samples were analyzed using an Agilent 7890A GC equipped with a 7693 autosampler. Identification of peaks was based on comparison of retention times to known standards (Supelco Bacterial Acid Methyl Esters #47080-U, plus MJS Biolynx #MT1208 for 16:1ω 5). The biomass of individual PLFAs was expressed as ng/g dry soil. Amounts were derived from the relative area under specific peaks, as compared to the 19:0 peak value, which was calibrated according to a standard curve made from a range of concentrations of the 19:0 FAME standard dissolved in hexane. Fatty acids were classified according to total number of carbon atoms, the number of double bonds, the position of the first double bond from the methyl end of the molecule, and the position and characteristics of cyclopropyl rings (DeGrood et al. 2005). PLFA analysis was completed by Ward laboratories Inc.

Data analysis

One-way ANOVA's were applied to determine the differences in soil microbial functional group biomass between sites varying in time post reclamation (years). Differences between all experimental units was verified using Tukey's honest significance test (HSD) using a level of significance of p-value < 0.05. Canonical Correspondence Analysis (CCA) was applied to assess the influence of physical and chemical soil parameters on the biomass of soil microbial functional groups. To examine the relationships between soil microbial functional group biomass and the number of native plants established within reclaimed sites Pearson correlation was used. All statistical tests were conducted using R Language for Windows software package (3.4.1) within R Studio.

Results

Development of microbial communities

The total soil microbial biomass in sites with ages 0-10, 10-20, and 20-30 years post reclamation were not significantly different from each other ($p>0.05$). Sites 30-45 years post reclamation showed no significant differences in total microbial biomass when compared to reference sites ($p>0.05$). By contrast, reference sites had significantly greater biomass as compared to sites 0-10 years 10-20 and sites 20-30 years post reclamation (ANOVA $F_{4, 25} = 4.532$; $p< 0.05$ (Table *1*).

Table 1: Soil microbial functional group biomass (ng/g) (mean ± SE) in reference sites and sites with various ages post reclamation

	Years post reclamation				
Microbial Community	0-10 years	10-20 years	20-30 years	30-45 years	Reference
Total microbial biomass (ng/g)	2627.9 ± 544.0*	2243.4 ± 471.3*	2355.1 ± 714.2*	4602.6 ± 767.3	6332.9 ± 1383.4
Total bacterial biomass (ng/g)	1053.0 ± 249.5*	1008.2 ± 205.3*	976.3 ± 260.0*	2199.3 ± 379.9	2951.9 ± 781.0
Actinomycete biomass (ng/g)	154.6 ± 49.8*	114.7 ± 25.1*	130.3 ± 29.7*	341.1 ± 62.9	436.1 ± 108.3
Rhizobacteria biomass (ng/g)	0*	13.1 ± 7.1*	34.5 ± 11.4*	86.5 ± 30.1*	228.8 ± 49.2
Gram (-) biomass (ng/g)	400.2 ± 91.1*	529.8 ± 108.7*	561.2 ± 161.6	1017.3 ± 176.5	1526.00 ± 443.4
Gram (+) biomass (ng/g)	653.8 ± 164.5	478.4 ± 113.8	415.1 ± 103.1	1182.1 ± 238.3	1424.9 ± 344.9
Total fungal biomass (ng/g)	113.4 ± 33.2*	378.9 ± 85.3*	457.5 ± 111.5	618.1 ± 140.4	893.5 ± 169.9
Arbuscular mycorrhizal biomass (ng/g)	8.4 ± 6.3*	113.7 ± 30.3	103.1 ± 21.5	128.7 ± 20.2	140.6 ± 36.2
Saprophytic fungal biomass (ng/g)	105.0 ± 28.6*	265.2 ± 58.3*	354.4 ± 90.6*	489.4 ± 124.3	752.9 ± 135.5
Protozoa biomass (ng/g)	0*	17.5 ± 4.1	23.5 ± 7.2	19.3 ± 7.5	34.3 ± 9.8

Values followed by an asterix (*) are significantly different ($p<0.05$) when compared to microbial biomass in reference sites according to Tukey's HSD Test.

Total bacterial biomass

The total bacterial biomass within reclaimed sites showed similar trends as total soil microbial community biomass. There were statistically significant differences in total bacterial biomass between reclaimed sites with varying ages post reclamation and reference sites determined (ANOVA $F_{4,25} = 4.34$, $p = 0.0084$). Reference sites were significantly different from sites 0-10 years post reclamation and 10-20 years post reclamation and reference sites ($p<0.05$). No other significant differences were detected (Figure 2).

Actinomycete biomass

Actinomycete biomass within reclaimed sites showed similar trends in comparison to total soil microbial community biomass and bacterial community biomass. There were statistically significant differences between reclaimed sites and reference sites (ANOVA $F_{4, 25} = 5.3$; $p< 0.05$). There were statistically significant differences between sites 0-10 years post reclamation and reference sites ($p=0.030$), sites 10-20 years post reclamation and reference sites ($p=0.010$), and sites 20-30 years post reclamation and reference sites ($p=0.01$) (Figure 2).

Rhizobacterial biomass

Rhizobacterial biomass within reclaimed sites showed different trends in comparison to total soil microbial community biomass and bacterial community biomass. There were statistically significant differences between mean total Rhizobacterial biomass between reclaimed sites with varying ages post reclamation (ANOVA $F_{4,25} = 12.41$, $p < 0.001$).There were statistically significant differences in Rhizobacterial biomass in sites 0-10 years post reclamation and reference sites ($p<0.001$), 10-20 years post reclamation and reference sites ($p=0.00005$), 20-30 years post reclamation and references sites ($P=0.00021$), and 30-45 years post reclamation and reference sites ($p=0.007$) (Figure 2).

Gram (-) and (+) biomass

Gram (-) and (+) biomass within reclaimed sites showed similar trends in comparison to total soil microbial community biomass and bacterial community biomass. There was a significant difference in gram (-) biomass between sites 0-10 years post reclamation and reference sites ($p=0.017$), and 10-20 years post reclamation and reference site ($p=0.042$). Finally, no statistically significant differences were observed between sites 20-30 and 30-45 years post reclamation and reference sites ($p>0.05$) (Figure 2).

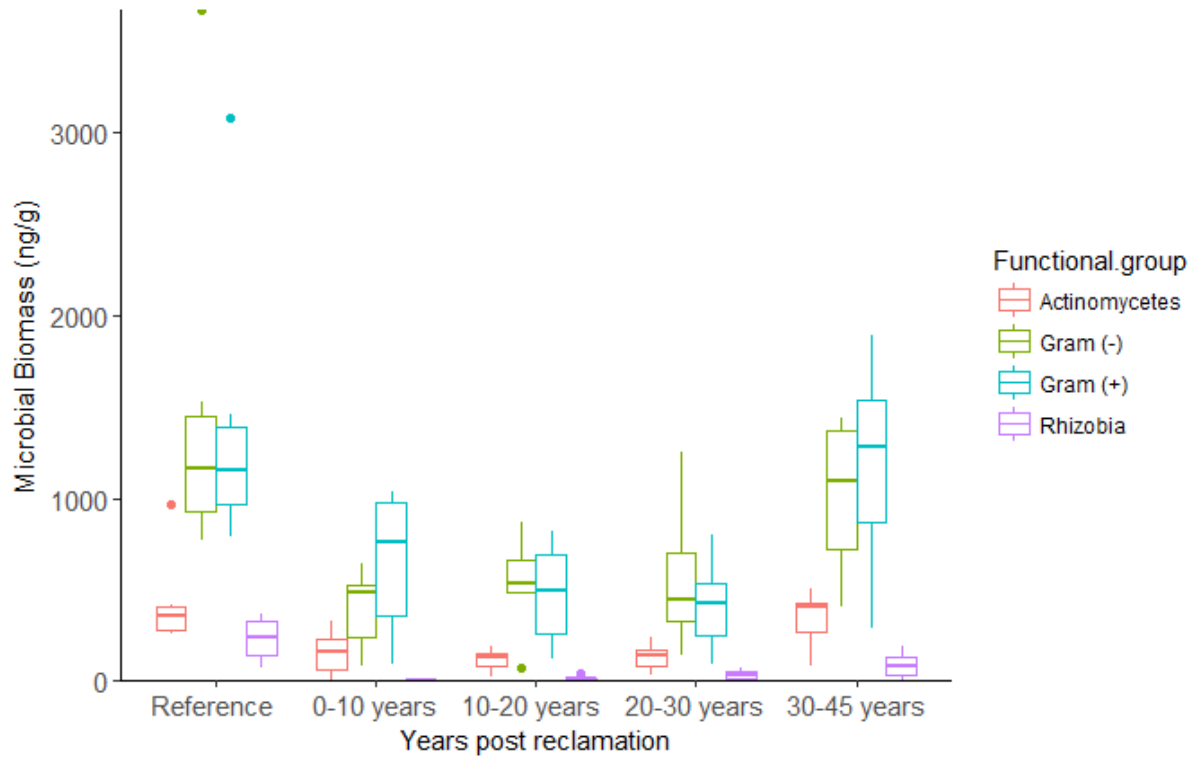

Figure 1: Biomass of total bacterial functional groups, Actinomycetes, Rhizobacteria, and Gram (-) and (+) bacteria in reclaimed sites with various ages post reclamation.

Total fungal biomass

Total fungal biomass (i.e., arbuscular mycorrhizal fungi and saprophytic fungal biomass) within reclaimed sites showed similar trends in comparison to total soil microbial community biomass and bacterial community biomass. There were statistically significant differences in total soil fungal biomass between sites with varying ages post reclamation and reference sites (ANOVA $F_{4,25}$ = 6.03, p = 0.002). There was a statistically significant difference in total fungal biomass between sites 0-10 years post reclamation and reference sites (p=0.017), and sites 10-20 years post reclamation and reference sites (p=0.042). There were no statistically significant differences in total fungal biomass between sites 20-30 and 30-45 years post reclamation and reference sites (p>0.05) (Figure 3).

Arbuscular mycorrhizae fungal biomass

Arbuscular mycorrhizae fungal biomass within reclaimed sites showed different trends in comparison to total soil microbial community and bacterial community biomass. There were statistically significant differences arbuscular mycorrhizae fungal biomass between sites with varying ages post reclamation and reference sites (ANOVA $F_{4,25}$ = 4.41, p = 0.0078). There was a statistically significant difference in sites 0-10 years post reclamation and reference sites (p=0.0079). Sites ranging from 10-20 years, 20-30, 30-45 years post reclamation and reference sites did not differ significantly in arbuscular mycorrhizal biomass (p>0.05) (Figure 3).

Saprophytic fungal biomass

Saprophytic fungal biomass within reclaimed sites showed different trends in comparison to total soil microbial community and bacterial community biomass. There were statistically significant differences in Saprophytic fungal biomass between sites with varying ages post reclamation and reference sites (ANOVA $F_{4,25}$ = 6.48, p = 0.001). There was a significant difference in saprophytic fungal biomass in sites 0-10 years post reclamation and reference sites (p=0.0006). There was significant difference in saprophytic fungal biomass between sites 10-20 years post reclamation and reference sites (p=0.011). In sites 20-30 and 30-45 years post reclamation there was a difference in saprophytic fungal biomass in reference sites (p>0.05) (Figure 3).

Protozoa biomass

Protozoa biomass within reclaimed sites showed similar trends in comparison to the biomass of Arbuscular mycorrhiza fungal biomass. There were statistically significant differences between mean Protozoa biomass between reclaimed sites with varying ages post reclamation (ANOVA $F_{4,25}$ = 3.48, p = 0.022). There were statistically significant differences in protozoa biomass in sites 0-10 years post reclamation compared to reference sites (p=0.0099). There was no significant difference in protozoa biomass in sites 10-20, 20-30, 30-45 years post reclamation in comparison to reference sites (p>0.05) (Figure 3).

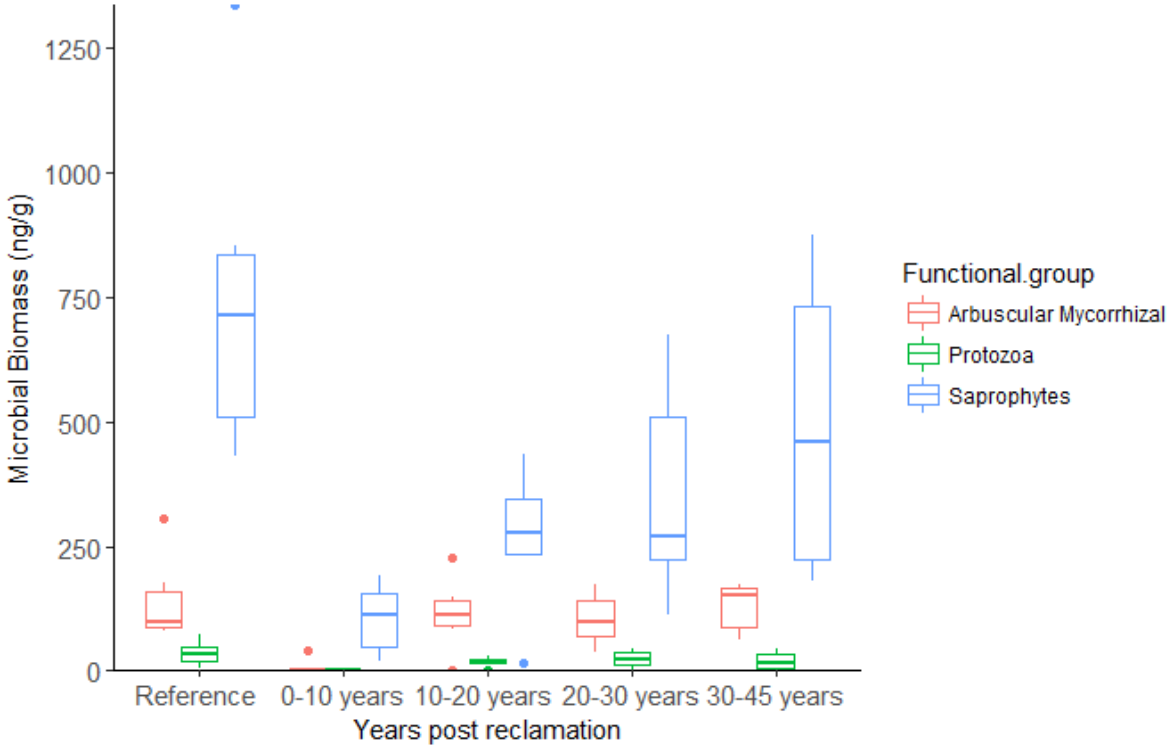

Figure 2: Biomass of total fungal functional groups, Arbuscular mycorrhizal fungi, Saprophytic fungi, and protozoa (ng/g) in reclaimed sites with varying in time post reclamation.

Soil Microbial Biomass – Physical and chemical soil properties

CCA indicated that the 30 chemical and physical soil parameters explained 89% of observed microbial community variation, leading to 11 % of the variation being unexplained by the chemical soil parameters observed. Of the 30 physical and chemical soil parameters observed, soil pH and the concentration of copper exerted the greatest influence on soil microbial biomass, accounting for 29% of the total explained variance of the 30 physical and chemical soil properties (Figure 4).

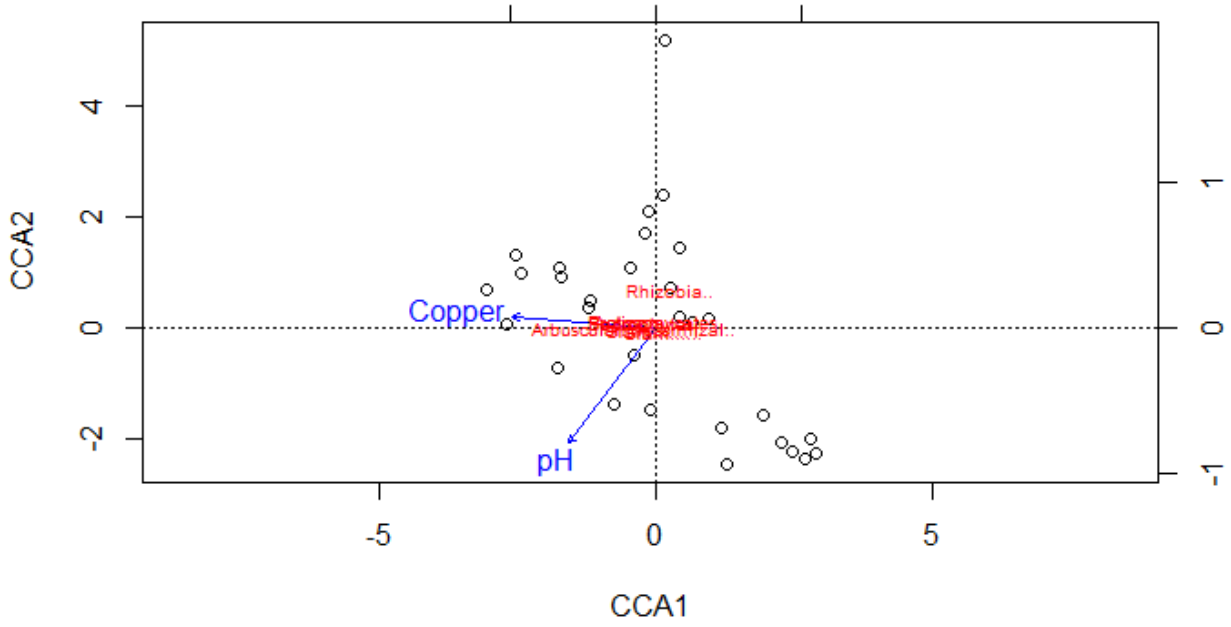

Figure 3: The influence of soil pH and the concentration soil of copper on biomass of soil microbial communities.

Increased soil acidity had a significant influence on the biomass of bacterial functional groups such as Actinomycetes (rho = -0.75, and p-value = 0.0062) and Rhizobacteria (rho= -0.51, p-value = 0.0039) (Figure 4) and fungal functional groups such as Saprophytic fungi (rho = -0.45, p-value = 0.01) (Figure 5). Changes in the concentration of soil copper was observed to significantly influence fungal functional groups. For example, changes in soil copper concentrations significantly influenced the biomass of Arbuscular mycorrhizal fungi (rho = 0.63, p-value = 0.0001), but did not significantly influence the biomass and presence of Saprophytic fungi and Protozoa (Figure 6). It was observed that fluctuations of soil copper concentrations did not have significant influences on the presence and biomass of Actinomycetes (rho = -0.47, p-value = 0.0087), Gram (-) (rho = -0.45, p-value = 0.012) and Gram (+) bacteria (rho = 0.36, p-value = 0.05), and Rhizobacteria (rho = -0.019, p-value = 0.92) (Figure 7).

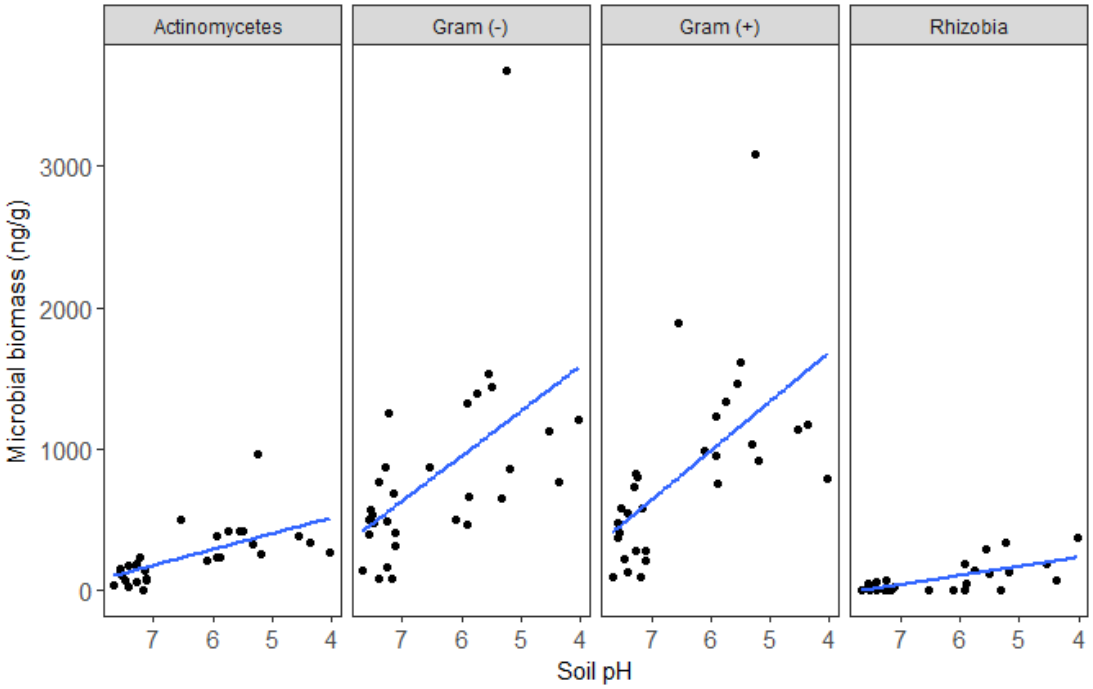

Figure 4: Effect of decreasing soil pH on the biomass of Actinomycetes Gram (-) and (+) bacteria, and Rhizobacteria (ng/g).

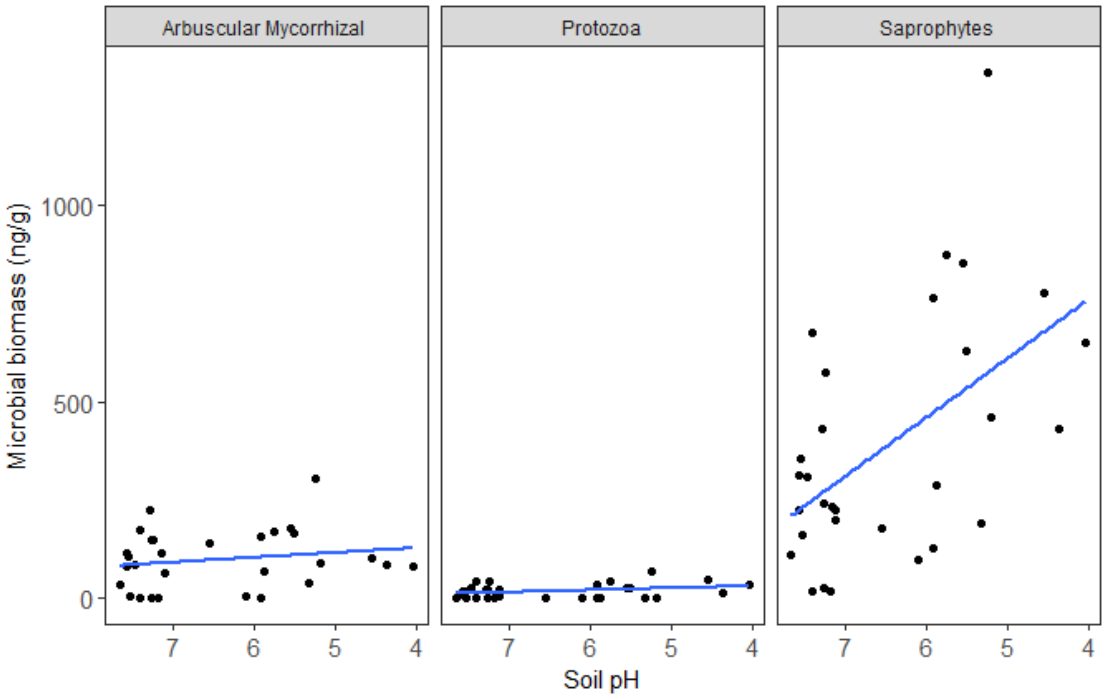

Figure 5: Effect of decreasing soil pH on the biomass of Arbuscular mycorrhizal fungi, Saprophytic fungi, and Protozoa (ng/g).

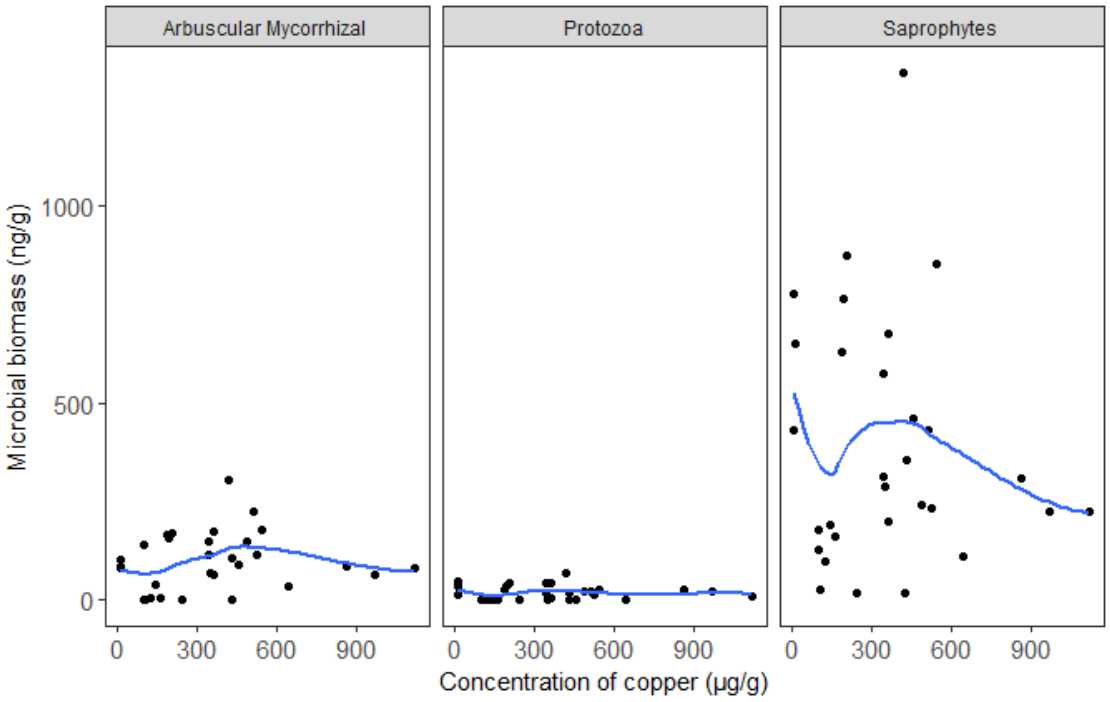

Figure 6: The influence of increasing soil copper concentrations (µg/g) on the biomass of Arbuscular mycorrhizal fungi, Saprophytic fungi, and Protozoa (ng/g).

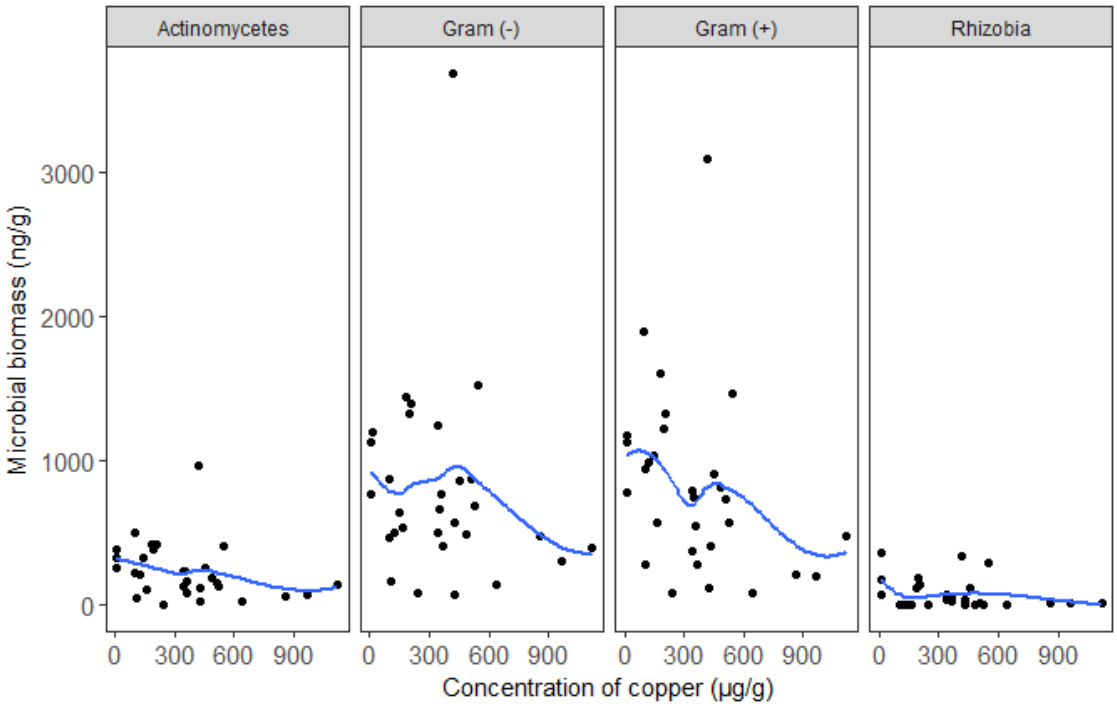

Figure 7: The influence of increasing soil copper concentrations (µg/g) on the biomass of Actinomycetes, Gram (-) and (+) bacteria, and Rhizobacteria (ng/g).

Vegetation community – influence of microbial functional groups

The vegetation community structure differed significantly between sites with varying age post reclamation (ANOVA $F_{4,25} = 27.48$, $p < 0.001$). Vegetation cover in sites 0-10 years post reclamation were significant lower than vegetation cover in sites 10-20, 20-30, 30-45 years post reclamation, and reference sites ($p<0.05$). Sites 10-20 years post reclamation, and reference sites did not differ significantly in vegetation cover ($p>0.05$). There was a difference in vegetation cover in sites 20-30 years post reclamation in comparison to reference sites ($p=0.009$). There were no significant differences in vegetation cover in sites 30-45 years post reclamation in comparison to reference sites ($p>0.05$). There was a positive correlation observed between the increasing biomass of Arbuscular mycorrhizal fungi and increase in vegetation cover ($p=0.027$, $rho=0.53$).

Discussion

With the cumulative area of disturbance caused by mining in British Columbia approximating 45,412 ha (TRCR 2011), it is necessary to ensure that the regulatory requirements and project objectives to reclaim and restore mine sites are directed by quantitative parameters and ecologically relevant metrics-of-success. Mining activities degrade the structure and dynamics of ecosystems, leading to diminished ecosystem functions (e.g., nutrient availability and cycling) (Howell and MacKenzie 2017). Industrial companies completing reclamation attempt to mitigate the effects of mining (e.g., soil compaction, soil erosion, and nutrient leaching) through management of soil material, and placement of overburden as a growing medium for native plant communities(Harris et al. 1993, Macdonald et al. 2015b, 2015a). Currently in British Columbia, regulatory requirements specifying the quality of soil material used in reclamation are vague, stating that material shall satisfy land use, capability, and water quality objectives set by the mining company. Due to broad regulatory requirements and lack of focus on below ground ecosystem characteristics, the quantitative assessment of soil using ecologically relevant indices is deficient in the field of mine reclamation and restoration within British Columbia.

Using ecologically relevant indices to quantify the status of soil as a growing medium, I compared soil microbial functional group biomass in reference sites that were not mechanically disturbed by mining activities with reclaimed sites that varied in years post-reclamation. Consistent with studies completed previously (Li et al. 2014, Quadros et al. 2016), I observed a significant difference in soil microbial community structure and biomass in recently reclaimed sites compared to reference sites. Total microbial biomass within reclamation sites was significantly different than reference sites until 30 years post reclamation, indicating the use of current mine reclamation practices do not significantly increase the recovery of soil microbial communities when compared to the development of microbial communities within ecosystems disturbed by forestry practices (Banning et al. 2011). Although taking at least 30 years to have a similar composition and total microbial biomass, sites previously disturbed by mining have biological soil properties that are similar to undisturbed Sub-Boreal Spruce forests located in the Cariboo-Chilcotin region of British Columbia.

The biomass of microbial functional groups (e.g., Actinomycetes, Rhizobacteria, Arbuscular mycorrhizae, Saprophytic fungi, and protozoa) differed with time post reclamation and were significantly different in

comparison to the functional group biomass within reference sites. The biomass of Actinomycetes in reclaimed sites was considerably lower than reference sites until 20 to 30 years post reclamation, while the presence and biomass of other bacterial functional groups such as Rhizobacteria show significantly lower levels of establishment in reclaimed sites, even after 45 years post reclamation. Similar trends were observed in the establishment and increase in the biomass of Saprophytic fungi, it was observed that after 45 years post reclamation the biomass of Saprophytic fungi was significantly lower than undisturbed reference sites. The delayed response of Rhizobacteria and Saprophytic fungi within reclaimed sites shows that additional measures are required to accelerate the development of specific microbial functional groups to mimic the conditions within undisturbed reference sites.

By contrast, the biomass of functional groups such as Arbuscular mycorrhizal fungi and Protozoa were significantly lower in sites less than 10 years post reclamation when compared to reference sites, showing that the biomass and development of specific fungal groups may resemble the conditions observed in reference sites sooner than expected. The observed differences in community structure, and biomass of various microbial functional groups in reclaimed sites was affected by changes in soil pH, and the concentration of micronutrients (e.g., copper) through time. As in research focused on the succession of ecosystems following anthropogenic (Poncelet et al. 2014, Chao et al. 2016) and natural disturbance events(Moore et al. 2010, Jangid et al. 2013), the natural process of pedogenic development influences environmental variables (e.g., soil pH) through time, having significant influence on the presence of various microbial species(Moore et al. 2010, Jangid et al. 2013, Poncelet et al. 2014, Chao et al. 2016). For example, the increase in biomass of Saprophytic fungi within reclaimed sites was observed to significantly correlated to decreases in soil pH. Similarly, weathering of soils and tailings material lead to the alteration of pH (Li et al. 2015a). The associated changes in pH due to weathering had significant influences on the structure and function of bacterial functional groups (Li et al. 2015a). Specifically, changes in soil pH altered the availability of nutrients and metals used for biochemical processes of fungal and bacterial functional groups increasing in development of microbial communities (Li et al. 2015a).

Given that the study area is characterised as a mineral rich location, microbial communities colonizing reclaimed sites are likely to be relatively adapted to high metal concentrations. Of the trace nutrients observed, the increase in the concentration of soil copper was shown to have significant influences on the presence and biomass of microbial functional groups. Although being relatively resilient to elevated levels of copper in reclaimed sites, soil microbial communities were characteristically found within sites with concentrations of soil copper less than 500 µg/g. For example, the biomass and presence of Arbuscular mycorrhizal fungi was observed to be significantly correlated to increases in the soil copper until concentrations approximated 500 µg/g. The remaining microbial functional groups (e.g., Actinomycetes, Rhizobacteria, and Saprophytic fungi) were observed to be relatively resilient to increased concentrations of copper. Although resilient to high levels of copper, Actinomycetes, Rhizobacteria, and Saprophytic fungi had the highest levels of biomass in soils with concentrations of copper less than 450 µg/g.

Observing the beneficial and detrimental effects of soil copper researchers (Maliszewska et al. 1985) discovered that low doses of copper, approximately 10-100µg/g, lead to detrimental impacts on bacterial communities such as Actinomycetes. My results suggest that microbial functional groups inhabiting

naturally mineral rich soils are more resilient to elevated metal levels when compared to microbial communities within naturally mineral poor sites. Similarly, studies focused in mineral rich areas indicate that native microbial species were resilient to elevated soil metals, accrediting microbial community resilience to the ability for microbial communities to produce organic compounds such as Siderophores (Reith et al. 2015, Chao et al. 2016). Since microbial communities play a significant role in regulating ecological processes (e.g., nutrient cycling), the ability to tolerate and adapt to changing chemical properties will have significant implications on the establishment and development of vegetation communities that have co-evolved with native and local microbial communities (Li et al. 2015a).

Assessment of the underlining abiotic and biotic ecosystem changes that influence the long-term success of reclamation projects is crucial to devise appropriate techniques to mitigate the effects of anthropogenic disturbance (Chao et al. 2016). The establishment and development of native vegetation communities within restored sites is a priority motivated by ecological, social, and economic factors. The composition of native plant communities with reclaimed and restored sites depends on interactions with several abiotic (e.g., available soil nutrients) and biotic factors (e.g., herbivory, plant competition, and symbiotic relationships) (Bever et al. 2010, Zobel and Öpik 2014). Of the biotic and abiotic factors considered, growing evidence is continuing on the significance of soil-microbe-plant interactions for the successful establishment of native vegetation species within reclaimed and restored sites (Zobel and Öpik 2014).

The occurrence and magnitude that plants interact with microorganisms in the soil environment directly influences the uptake and accumulation of macronutrients and micronutrients required for establishment and productivity of plants (Asmelash et al. 2016). Within soil microbial communities, specific microbial groups (e.g., Actinomycetes, Rhizobacteria, Arbuscular mycorrhizae, and Saprophytic fungi) can directly modify the bioavailability of metals through the breakdown of organic matter, release of organic compounds (e.g., chelating agents) forming chemical bonds with soil metals, and stimulating oxidation-reduction reactions modifying the chemical structure of metals present in soil (Rajkumar et al. 2012). For example, through the production and release of hydrolytic enzymes Actinomycetes breakdown complex materials (e.g., cellulose, lignin, and chitin), leading to increased nutrient availability by interacting with elements and compounds previously bound to organic matter (Nair et al. 2007). After organic matter is broken down nutrients are readily available and are released into the soil medium for other microorganisms and plants to use for essential biochemical reactions (Fontaine et al. 2003, Garcia-Pausas and Paterson 2011).

Local and native microbial interactions between functional groups can drive ecosystem function, stimulating nutrient cycling within soil and improving the establishment of a diversity native plant in disturbed areas (Manaut et al. 2015). I observed a positive correlation between the increasing biomass of arbuscular mycorrhizal fungi and increase in native vegetation cover. Additionally, it was noted that the number of native plant species within reclaimed sites was positively correlated with the increase in biomass of Actinomycetes, Rhizobacteria, Arbuscular mycorrhizal fungi, and Saprophytic fungi. The process of selective symbiotic relationships between native plant species and microbial communities has been found to be the driver increasing the capacity for native plant species to outcompete non-native plant species (Shivega and Aldrich-Wolfe 2017). Specifically, native microbial communities that adapted to local soil properties, and that have co-evolved with local native plant communities readily alter the bioavailability of essential nutrients for symbiotic biological partners(Manaut et al. 2015, Shivega and

Aldrich-Wolfe 2017). The selectivity for native symbiotic partnerships between native microbial and plant communities may be the factor controlling the transition of plant communities from being predominantly introduced species (e.g., agronomics) to native plant species in disturbed sites(Shivega and Aldrich-Wolfe 2017). In general, research completed within the study area has shown that nutrient availability within reclaimed sites has been limited, the presence and increase in microbial community biomass has been shown to alter the bioavailability of nutrients and may be the primary factor increasing capacity of native species to establish and compete with non-native plant species.

Implications to ecological restoration

To increase the rate of native soil microbial and plant community development following disturbance events several restoration activities can be completed, including by not limited to, using the combination of passive or active restoration techniques (McIver and Starr 2001, Banning et al. 2011, Strickland et al. 2017). Passive restoration refers to the process of removing stressor affecting the site or landscape followed by no action or treatment, relying on natural processes to stimulate the recovery of ecosystem characteristics or function (McIver and Starr 2001). Passive restoration of disturbed soils may be a sufficient technique to develop microbial communities that are similar to undisturbed reference over a long-time period (Bowker 2007). Although successfully restoring microbial communities, passive treatments require at least 30 years post reclamation to meet project goals and final mine closure objectives. To successfully expedite the development soil microbial communities within disturbed sites of the study area, a minimum of three passive restoration techniques should be completed, including the improvement site connectivity, alteration of soil copper, and modification of soil pH.

It has been observed that several microbial functional groups such as arbuscular mycorrhizal fungi are poor dispersers, relying on soil macroinvertebrates and terrestrial vertebrates as transport vectors (Mangan and Adler 2000). While the colonization of soil fungal groups is dependent on the migration of other soil organisms and mammals, researcher observing the transport vectors of soil microbial functional groups have identified that wind is a likely factor improving the colonization of several bacterial groups (e.g., Ascomycetes) to recently disturbed sites (Egan et al. 2014). Having relatively large spores (0.01–1 mm), wind is not a primary vector influencing the dispersal of Arbuscular mycorrhizal fungi. Instead, invertebrates, birds, small and large mammals are the primary transporters of arbuscular mycorrhizal fungi (Egan et al. 2014). Therefore, improving site connectivity with a focus on ecosystem characteristics required for bird species, small and large mammals may improve the dispersal and colonization of microbial communities into recently disturbed sites. Additionally, to assist the passive recovery of bacterial groups such as Actinomycetes, improvement of site microtopography may allow for the colonization and capture of wind dispersed microorganisms (George et al. 2000, O'Donnell et al. 2007). Although limited by dispersal and requiring transport by abiotic features and other terrestrial organisms, soil conditions within reclaimed sites have significant influences on the successful establishment of microbial communities (O'Donnell et al. 2007). Therefore, modification of soil conditions must be an additional step to improve the long-term success of restoration of soil microbial communities.

Described within the Baas-Becking hypothesis, the presence, establishment, and productivity of soil microbial communities is primarily regulated by local abiotic factors, such as the concentration of soil micronutrients and pH, rather than just landscape geographical features alone (Hazard et al. 2013).

Similar to results found in the research studying the local and regional characteristics of microorganisms establishment and development (Hazard et al. 2013), soil abiotic factors significantly influenced the presence of various microbial functional groups. Based on my findings, restoration techniques focused on the modification of soil copper concentrations and soil pH are likely to stimulate the development and recovery of microbial communities within disturbed sites. To determine the appropriate steps and requirements to assist the recovery of soil microbial communities during restoration projects quantitative assessment of soils should be completed prior to placement. If copper concentrations are above 600 µg/g remediation should be completed. Reduction of soil copper concentrations below 600 µg/g will stimulate Arbuscular mycorrhiza recovery, while the reduction of soil copper below 360 µg/g will assist the recovery of the remaining microbial functional groups such as Saprophytic fungi and Rhizobacteria. Several remediation treatments to reduce the concentration of copper may be completed, including but not limited to, phytoremediation followed by removal and disposal of the roots and shoots of plants containing accumulated copper.

In addition to using remediation techniques to reduce the concentration of copper within soil, activities focused on the reduction of soil pH to approximately 6.0 may also stimulate the recovery of soil microbial communities within reclaimed and restored sites of the sub-boreal spruce forests of the Cariboo-Chilcotin region. Similar to the modification of metal concentrations in soil environments, soil pH should be modified gradually, ensuring that microbial communities can adapt to changes overtime (Girvan et al. 2005). Gradual and spatially heterogenous addition of macronutrient soil amendments such as ammonium sulfate or sulfur-coated urea may be sufficient techniques to reduce pH overtime (van Breemen et al. 1982, Zhao and Xing 2009). For example, addition of nitrogen compounds decreased soil pH which lead to increases in the biomass and productivity of soil microbial communities (Zhou et al. 2017). The observed changes in soil microbial community structure and development was dependent on the decreases in soil pH, leading to increased nutrient bioavailability within soil, and improved the uptake of nutrients by microbial functional groups (Zhou et al. 2017). Additionally, restoration techniques decreasing soil pH may directly benefit phytoremediation efforts through increased bioavailability of metals and accumulation by plant species (Bolan et al. 2003). Following the improvement site connectivity, reduction of soil copper, and modification of soil pH active treatments such as the use of microbial inoculants are expected to expedite the recovery of degraded sites.

To assist the recovery of ecosystems, and to restore the structure and function of microbial communities to representative conditions similar to undisturbed sites, a variety of soil inoculation techniques can be used (Kouadio et al. 2017). Inoculation of soil using non-native microbial communities is common due to the prevalence of commercial stocks, and a generalist view of soil-microbe-plant interactions (Kouadio et al. 2017). Commercial microbial inoculants are usually in granular, powder, or liquid form and applied using broadcast dispersal. The use of non-native or exotic microbial species are frequently implemented when soil conditions within a reclamation or restoration site are significantly different from the natural soil conditions, such as having higher concentrations of trace metals or lack essential nutrients (Kouadio et al. 2017). As noted by (Kouadio et al. 2017), the use of commercial microbial inoculants are continuously being reported to not preform expected functions within soil environments, leading to broad conclusions that microbial functional communities used in reclamation or restoration do not participate in ecosystem functioning as observed in natural systems. Similar to successful implementation of plant

species in restoration and reclamation sites, the ecosystem requirements and origin of microbial communities must be taken into account before inoculation or introduction of microbial functional groups occurs (Kouadio et al. 2017). Therefore, the type of inoculant (e.g., commercial, or native microbial communities), source of inoculant, concentration, location of application (e.g., broadcast, seed coating, or placement of inoculant directly under planting), and timing of application must be considered to ensure that re-introduction of microbial communities into restored sites is successful.

The source of native microbial communities is often selected based on the structure and characteristics of late seral plant species, with practitioners idolizing the conditions of an undisturbed forests (Wubs et al. 2016). Focusing on late seral ecosystems as donor sites, microbial communities associated with early succession plant communities are overlooked (Wubs et al. 2016). Studying the influence of various microbial communities on the development of terrestrial plant communities, the microbial communities associated with various ecosystems lead to the differential effects on early and later seral vegetation species (Wubs et al. 2016). For example, inoculation of microbial communities from grass and shrub predominated ecosystems had significant positive implications on the growth and cover of early seral vegetation species characteristic to the natural ecosystems (Wubs et al. 2016). Whereas restoration sites planted with late seral species did not benefit from the native microbial inoculant from grass and shrub ecosystems. Consequently, the source of soil microbial inoculant should be derived from a system that possesses similar species and ecosystem characteristics that are trying to be reclaimed or restored (Wubs et al. 2016). Although tempting to use soil from late seral forests, it is suggested that inoculation of restoration sites should be completed using donor soils from a diversity of seral stages.

While incorporating donor soils from undisturbed systems to restored sites is an effective method to improve project success, current practices to obtain donor soils from intact system are highly destructive, leading the requirement of additional restoration activities to mitigate the impacts of obtaining donor soils (Wubs et al. 2016). Techniques to inoculate soils using native microbial communities usually involves stripping soils from undisturbed donor sites and incorporation into restored soils (Wubs et al. 2016). The process of soil stripping typically involves the removal the entire top 30 cm of organic matter and soil material from donor sites, followed by the broadcast application of material onto the disturbed site (Wubs et al. 2016).

To limit the disturbance of soil horizons and removal of large amounts of soil in donor sites, the use of soil plugs is a recommended technique that requires further research (Middleton and Bever 2012). Migrating throughout the soil environment, microbial functional groups have the ability for localized dispersion from colonized areas (Middleton and Bever 2012). Due to the local mobility of microorganisms in soil, the application of native soil from plugs in the form of islands or clusters may provide sufficient concentrations of microbial communities while limiting disturbance in donor sites (Middleton and Bever 2012). As observed in the succession of ecosystems following natural disturbance events, spatial patterns for the recovery and establishment of native species commonly show initial colonization of in clusters or islands (Yarranton and Morrison 1974). Known as the nuclei for establishment and ecosystem recovery, the initial clusters of colonizing organisms progress, expanding and migrating throughout the disturbed site (Yarranton and Morrison 1974). Following the nucleation development model, re-introduction of microbial communities through the implementation of soil plugs in large clusters emulates natural patterns of ecosystem development (Yarranton and Morrison 1974,

Middleton and Bever 2012). Therefore, it is recommended that the implementation of soil plugs should be completed in clusters rather than a broadcast application or dense plantation style introduction, allowing for the natural migration of microbial communities to assist the recovery of ecosystem structure and function over large spatial domains.

Conclusion

This study demonstrated that there was a significant difference in soil microbial community structure and biomass in reclaimed sites compared to reference sites. It was observed that total microbial biomass within reclaimed sites was significantly different than undisturbed forests until 30 years post reclamation. Analysis indicated that the observed differences in microbial community structure and biomass was affected by changes in soil pH and the concentration micronutrients through time. Further analysis revealed that the presence of native vegetation species within reclaimed sites was highly correlated to increases in microbial community biomass. Our results indicate that reclamation activities successfully promote the development of microbial communities over a long-time scale, and suggests that the assessment of reclamation projects should exceed at least 30 years to properly evaluate the recovery of ecosystems. To assist the recovery of microbial and vegetation communities resulting from mining disturbance, reclamation activities may include but not limited to modification of chemical soil characteristics (e.g., soil pH), and inoculation of soil with native microbial communities adapted to local conditions.

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