# BIOLOGICAL COMMUNITIES IN RECLAIMED POST-MINING LANDSCAPES OF BRITISH COLUMBIA: FROM MONITORING TO MANAGEMENT

by

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## ABSTRACT

This thesis explores the use of DNA-based tools to monitor ecological recovery in post-mining landscapes, with studies at two mines in British Columbia: Mount Milligan and Teck Highland Valley Copper. Biological monitoring in mine reclamation traditionally relies on vegetation and soil development, which occur slowly and may delay adaptive management. In contrast, microbial and invertebrate communities respond more rapidly to environmental change, offering potential as early indicators of reclamation success. This research uses DNA sequencing techniques to examine microbial and invertebrate communities across various reclamation strategies and reference conditions, with the aim of assessing biological community responses and informing future frameworks.

Research at Teck Highland Valley Copper Mine focused on the long-term effects of biosolids amendments on microbial communities in reclaimed tailings storage facilities. Soil samples collected in 2015 from plots that received different one-time biosolids applications were analyzed for bacterial and fungal composition using DNA metabarcode sequencing. Diversity metrics and community composition were compared across treatment types, and results demonstrated that biosolids amendments had significant effects on microbial communities. Community composition analyses revealed distinct assemblages associated with biosolids treatments, suggesting that they strongly influence microbial abundance and community structure. Furthermore, the detection of increased abundances of antimicrobial resistance genes in biosolids-treated plots highlights important considerations for their use in mining reclamation.

The second study, at Mount Milligan Mine, assessed microbial and invertebrate communities from 2022 to 2024 across reclaimed plots, bare ground, as well as naturally and anthropogenically disturbed reference ecosystems. Soil and invertebrate samples were collected and processed for DNA metabarcode sequencing to analyze bacterial, fungal, and invertebrate community diversity and composition. Results showed that communities differed significantly between

reclamation and reference sites, revealing distinct microbial and invertebrate assemblages between site types. Certain bacterial, fungal, and invertebrate taxa were consistently associated with reclamation or reference site types, highlighting their value as potential bioindicators. Additionally, protocols for extracting and size-selecting high-molecular-weight DNA from soil were tested and refined to support future functional analyses using long-read sequencing.

Together, this research demonstrates that DNA-based monitoring can detect meaningful differences in microbial and invertebrate communities across treatments and disturbance histories. These methods provide sensitive, high-resolution data that complement traditional monitoring approaches and could accelerate early detection of reclamation trajectories. By identifying specific indicator taxa linked to reclamation and evaluating the effects of varying treatments, this thesis contributes to the development of more adaptive and informative reclamation frameworks. The findings support the inclusion of microbial and invertebrate indicators in reclamation practices, and the thesis provides practical recommendations for future reclamation frameworks in British Columbia.

**Keywords:** reclamation, microbial community metabarcoding, bacterial community metabarcoding, fungal community metabarcoding, invertebrate metabarcoding, bioindicators, biosolids, antimicrobial resistant genes

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# Chapter 1. Introduction

## MINING RECLAMATION

Mining activities are integral to Canada's economic development, contributing significantly to the country's GDP and supplying essential resources for industries and infrastructure (Government of Canada, 2024). In British Columbia (BC), mining plays a vital role in the regional economy but has resulted in numerous disturbed landscapes requiring reclamation (Government of British Columbia, 2024).

Mining significantly disturbs the environment and is associated with persistent environmental issues, such as soil erosion, heavy metal contamination, and the overall disruption of natural ecosystems (Hutchinson & Whitby, 1974; Redmann, 1996; Slingerland et al., 2020). Post-mining landscapes often exhibit degraded soils and a loss of biodiversity, which, in addition to threatening local flora and fauna, has consequences for water quality, climate regulation, and public health (Winterhalder, 1996; Worlanyo & Jiangfeng, 2021;). Mining reclamation—the practice of rehabilitating post-mining landscapes to enhance their physical, chemical, and biological stability—is therefore essential to sustainable land use, as it mitigates the impact of environmental issues (Kwak et al., 2017; Lima et al., 2016).

In BC, a province host to 78 major mines profiled on the provincial government's website, the Mines Act (2025) stipulates that mined lands must be reclaimed to an end land use approved by the chief inspector. Variability in soil type, climatic conditions, and overall disturbance necessitates reclamation strategies tailored to each mine to ensure long-term success. Diverse ecosystems and unique environmental conditions present opportunities and challenges for reclamation efforts. Most strategies are further complicated by nutrient deficiencies and residual contamination, which impair the recovery of microbial, invertebrate, and plant communities, limiting ecosystem resilience (Favas et al., 2018; Price, 2005). In regions like BC, where post-mining landscapes represent environmental concerns, understanding the effectiveness and impact of reclamation strategies on soil physicochemical properties and biological communities is critical.

This thesis contributes to the understanding of reclamation by investigating how different strategies influence soil health, as well as microbial and invertebrate communities through the use of DNA-based ecological monitoring tools. It provides insights into reclamation practices that restore land capacity, promote biodiversity, and minimize risks while also ensuring sustainable end land use and the mitigation of environmental issues.

**Post-mining consequences**

Although mining is a cornerstone of Canada's economy, its aftermath across ecologically sensitive areas, such as boreal forests and freshwater ecosystems, poses serious negative consequences.

The physical impact of mining typically begins with the removal of vegetation and topsoil, allowing access to mineral deposits. This process, known as overburden removal (OBR), strips landscapes of their natural protective layers, altering topography and exposing them to erosion (Sinha & Pathak, 2017). After OBR, mineral mining practices result in waste rock and tailings; waste rock does not contain desired minerals, whereas tailings come from milled ore-containing rock. Tailings have small particle sizes and usually lack soil structure, resulting in a low water-holding capacity (Norland & Veith, 1995; Tordoff et al., 2000). In Canada, open pit mines are notorious for creating vast stretches of disturbed, barren landscapes, full of waste products.

In terms of chemical impacts, soil degradation in mining areas is a major issue, with soils often becoming nutrient-deficient and contaminated (Winterhalder, 1996). Tailings, for example, are often nutrient-poor, lack organic matter, and contain heavy metals (Norland & Veith, 1995; Tordoff et al., 2000). Acid mine drainage (AMD) can also be a concern, specifically in regions with sulfide-bearing rock formations. AMD occurs when sulfide is exposed to atmospheric oxygen and water, producing sulfuric acid, which can exacerbate ecological harm by mobilizing heavy metals into soils and surrounding water bodies (Moncur, 2006).

Finally, the destruction of habitats leads to distinct biological impacts, including the displacement or extinction of species, especially those dependent on

specific ecosystems (Antwi et al., 2008; Jacobi et al., 2011). Fragmentation, caused by mining infrastructure and access roads, can prevent species from migrating or interacting across their natural ranges (Scanes et al., 2018). Additionally, post-mining landscapes often struggle to support the same level of biodiversity due to altered soil conditions and reduced vegetation cover (Antwi et al., 2008).

Overall, mining operations and their waste products severely disturb the vegetation, soil, and natural hydrology, hindering natural ecosystem recovery without substantial intervention (Bradshaw, 2000).

**Environmental and health impacts of post-mining landscapes**

Inadequately reclaimed post-mining landscapes can become long-term sources of pollution and ecological disruption. Specific consequences may include persistent pollution, the degradation of surrounding ecosystems, and health risks for nearby communities.

Persistent pollution, mainly from heavy metals and toxic runoff, is one of the most severe consequences of post-mining landscapes. For example, AMD can mobilize heavy metals, such as arsenic, lead, and cadmium, into nearby water systems (Luo et al., 2020). This contamination affects aquatic ecosystems and can render water sources unsafe for consumption or agricultural use. In BC, AMD has been a notable issue at sites like the Britannia Mine, which discharged acidic runoff into Howe Sound for decades before reclamation efforts began (Wilson et al., 2005). Mineral mining practices also leave behind tailings that can leak contaminants into soil and groundwater; some toxic contaminants can persist for decades, rendering land unusable for agriculture or habitation, and posing ongoing risks to water quality for downstream communities (García-Giménez & Jiménez-Ballesta, 2017).

Mining degrades ecosystems through habitat destruction, soil degradation, and water contamination, and its effects last long beyond its active practice. Post-mining landscapes typically lack vegetation cover, leading to erosion and sedimentation in nearby rivers and streams (Slingerland et al., 2020; Wantzen & Mol, 2013). This sedimentation can smother aquatic habitats, affecting fish and other aquatic species in BC's ecosystems, such as salmon (Sergeant et al., 2022).

Contamination from heavy metals and AMD disrupts food chains as well. For example, metal accumulation in plants can harm herbivores, while toxic contaminants in bodies of water can impact aquatic populations and their predators (Roberts & Johnson, 1978; Sonone et al., 2021). Long-term ecological imbalances create cascading effects, and in the context of mining, this may mean reduced biodiversity and compromised ecosystem resilience.

It is necessary to point out that in BC, many mines are located near Indigenous communities and ecologically significant areas. Communities near unreclaimed mining sites may face health risks from exposure to harmful substances. If contaminated water sources are used for drinking or irrigation, it could lead to chronic health conditions, including heavy metal poisoning or developmental issues in children (Mitra et al., 2022). For instance, some populations near water contaminated by post-mining landscapes have been linked with higher rates of skin lesions and cancer, due to arsenic exposure (Cheung et al., 2020). Furthermore, dust from tailings, if left uncovered, can become airborne, carrying toxic particles into nearby communities (Csavina et al., 2012). Prolonged exposure to such dust increases the risk of respiratory disease and a plethora of other health issues (Witten et al., 2019). This is concerning, given BC's arid mining regions, where wind erosion can spread contaminants over large areas.

A lack of reclamation in BC impacts both long-term human and environmental health as well as undermines efforts toward reconciliation and sustainable land management. Effective strategies to reclaim post-mining landscapes that support microbial, invertebrate, and plant community recovery are critical to protecting BC's natural ecosystems and ensuring safe living conditions for all.

**Bacterial communities in post-mining landscapes**

Bacterial communities are key drivers of ecological recovery in post-mining landscapes because of their unique abilities to mediate nutrient cycling, detoxify pollutants, and support ecosystem reclamation (Peddle et al., 2022; Rawat et al., 2022). Due to their rapid response to environmental changes, bacterial communities could be used as biological indicators to reflect reclamation trajectory. In BC, where

mining operations are extensive and damaging, understanding the role of bacteria in the context of post-mining environments is required for developing effective reclamation strategies that mitigate long-term environmental impacts and promote sustainability.

In soils depleted by mining activities, often lacking essential nutrients required for plant growth and ecosystem function, such as nitrogen, phosphorus, and organic carbon, bacteria are central to restoring them through their involvement in biogeochemical cycles (Huang et al., 2011). For example, species from the genera *Nitrosomonas* and *Nitrobacter* facilitate nitrogen cycling by converting ammonia into nitrite, then nitrate, a form that can be used readily by plants (Norton et al., 2002). Nitrogen-fixing bacteria, including species of *Rhizobium* and *Azotobacter*, can also further enrich soil by converting atmospheric nitrogen into bioavailable forms (Aasfar et al., 2021). And phosphorus in insoluble forms can be converted into soluble forms by phosphate-solubilizing bacteria, such as some *Bacillus* and *Pseudomonas* species (Soares et al., 2023). These processes are critical, as revegetation efforts rely on improved nitrogen and phosphate availability to support plant establishment. Bacteria also decompose organic matter, mitigating the effects of OBR and mining by releasing nutrients like phosphorus and potassium back into the soil, further supporting ecosystem recovery (Sheoran & Sheoran, 2009). Microbial inoculants are being increasingly used in reclamation projects to accelerate the recovery of soil fertility; specific species, like some in the Proteobacteria, may accelerate the decomposition process, sooner enriching post-mining soils with essential nutrients that stabilize them and enable the re-establishment of vegetation (Jia et al., 2022).

As mining activities often leave behind toxic substances, including AMD and heavy metals, certain bacteria have evolved mechanisms, such as biotransformation, bioaccumulation and biosorption, to detoxify these pollutants, making them valuable allies in mitigating contamination (Fashola et al., 2016). In some post-mining landscapes, bacterial communities have been shown to reduce the mobility and bioavailability of some heavy metals, effectively minimizing their impact on downstream water and soil systems (Li & Wong, 2010). Furthermore, some bacteria, including Deltaproteobacteria species, have been shown to degrade

hydrocarbons and other organic pollutants resulting from oil sands mining operations in Alberta and BC, into less toxic compounds (An et al., 2013).

In addition to the effects bacterial communities can have on nutrient cycling and toxic contaminants, they are pivotal to restoring ecological balance in post-mining landscapes by promoting healthy soils and thus vegetation growth. An example of this is when bacteria contribute to the formation of soil aggregates by producing extracellular polymeric substances, which bind soil particles together, improving soil aeration, water retention, and resistance to erosion (Costa et al., 2018). This process can be critical for plant growth in degraded soils, especially in arid landscapes where erosion may pose a significant challenge to reclamation. Rhizosphere bacteria also form symbiotic relationships with plants, providing nitrogen in exchange for carbon, while improving soil structure and producing plant growth-promoting hormones (Thavamani et al., 2017). Previous studies have highlighted how bacterial communities contributed to the success of revegetation in post-mining environments, like the reclamation of the Teck Highland Valley Copper mine in south-central British Columbia (Gardner et al., 2010; Gardner et al., 2012).

Mining severely alters soil properties, under which conditions bacterial diversity is typically reduced and skewed toward stress-tolerant taxa, but research highlights the transformative potential of bacterial communities in restoring post-mining landscapes to functional ecosystems as reclamation progresses. As natural microbiomes and bacterial inoculants are increasingly used to enhance soil health, neutralize contaminants, and accelerate ecological recovery, efforts must be made to monitor and understand microbial dynamics in ways that allow bacterial capabilities to be harnessed for effective site-specific reclamation strategies.

*Antimicrobial resistance genes*

To explore bacterial communities in the context of reclaiming post-mining landscapes without touching on antimicrobial resistance genes (ARGs) would be remiss. ARGs enable bacteria to survive exposure to antibiotics, and they can be naturally present in microbial communities; however, their prevalence can increase in disturbed soils due to several factors. Each year, countless reclamation strategies

are tested, with many including the use of biosolids—treated sewage sludge derived from municipal wastewater (CCME, 2012). Biosolids have garnered attention for their potential to improve soil properties and support ecosystem recovery, but not without ecological and public health concerns that arise from the potential increased presence of ARGs that could accumulate in soils and affect ecosystem health over time (CCME, 2012; Pepper et al., 2013; Wallace et al., 2016). Understanding how ARGs may arise and persist is necessary for evaluating risks and ensuring sustainable reclamation practices.

ARGs may increase in prevalence due to several factors, including the harsh conditions of post-mining soils. Low nutrient availability, heavy metal contamination, and altered microbial interactions can promote the selection of resistant bacterial strains; heavy metals co-select for ARGs because resistance mechanisms, such as efflux pumps that export both metals and antibiotics, often overlap (Baker-Austin et al., 2006; Zou et al., 2021). Additionally, and as previously noted, biosolids amendments can increase the presence of ARGs if used as a reclamation strategy (Pepper et al., 2013; Wallace et al., 2016). This is because biosolids may contain residual antibiotics and broad-host-range plasmid groups, and can act as reservoirs and vectors for ARGs when introduced to post-mining soils, amplifying their presence in microbial communities (Law et al., 2021; Qin et al., 2022). High microbial diversity can act as a barrier that resists the spread of ARGs, but often microbial diversity in post-mining environments is low (Chen et al., 2019; Quadros et al., 2016).

The proliferation of ARGs could alter ecosystems; since ARGs can co-occur with metal resistance genes, the resulting dual resistance could exacerbate the persistence of resistant bacteria in the environment, complicating efforts to manage contamination and restore soil health (Baker-Austin et al., 2006; Thomas et al., 2020). ARGs can also spread among bacteria, and even to pathogens, through horizontal gene transfer, facilitated by mobile genetic elements like plasmids and transposons, further propagating resistance in the ecosystem (Law et al., 2021).

The presence of ARGs in post-mining soils raises public health concerns, including contamination and exposure through water sources, dust, and aerosols, as

well as pathogen evolution (Zou et al., 2021). For example, runoff from reclaimed sites containing ARGs and resistant bacteria can contaminate nearby water bodies (Zou et al., 2021). This is concerning in BC, where mining sites often intersect with watersheds that provide drinking water to local communities and Indigenous populations. Furthermore, in arid locations where wind exacerbates erosion, dust from post-mining soils could carry ARG-harbouring bacteria, increasing the risk of exposure to humans and animals (Csavina et al., 2012). Finally, ARGs in the environment can also be acquired by pathogenic bacteria, potentially creating multidrug-resistant strains (Law et al., 2021). This poses a direct threat to public health, particularly in rural areas with limited access to advanced healthcare facilities.

While bacterial communities are key to the recovery of post-mining soils, their potential to harbour and disseminate ARGs also introduces a challenge. In BC, where biosolids amendments are commonly employed, careful management and monitoring are needed to balance the benefits of soil restoration with the risks to ecological and public health. By integrating advanced and targeted reclamation strategies, the ARG burden in reclaimed soils can be minimized, ensuring sustainable land use.

**Fungal communities in post-mining landscapes**

Fungal communities are also important for the recovery of post-mining landscapes through their contributions to decomposing organic matter, facilitating plant growth, and stabilizing soil ecosystems (Liu et al., 2022; Wang et al., 2021). These functions complement the roles of bacterial communities, and certain fungal taxa could also be used as biological indicators.

Fungi, especially saprotrophic fungi, are effective decomposers of organic matter (Burns & Dick, 2002). By breaking down organic matter, fungi release bioavailable nutrients into the soil, replenishing nutrients in post-mining soils (Rashid et al., 2016). Fungi excel at breaking down lignin, cellulose, and other complex organic compounds that bacteria are less equipped to process (Baldrian et al., 2012; Floudas, 2021). This is relevant in reclamation strategies where fungal inoculants,

including native decomposer mycorrhizal fungi, have previously been applied in tandem with organic amendments, such as lignite-derived humic substances, and enhanced plant biomass (Zhao & Naeth, 2022).

One critical role fungi play in ecosystem recovery is through their symbiotic relationships with plants. For example, mycorrhizal fungi form mutualistic associations with plant roots, enhancing overall plant health in nutrient-deficient environments (Bonfante & Genre, 2010). Additionally, arbuscular mycorrhizal fungi penetrate plant root cells and form extensive hyphal networks that increase surface areas for nutrient absorption (Thavamani et al., 2017). These networks enable plants to access phosphorus, nitrogen, and other essential nutrients that may otherwise be unavailable. In addition to their aid in nutrient uptake, mycorrhizal fungi help improve plant resilience to drought and pathogens by enhancing water absorption and producing bioactive compounds that can suppress soil-borne pathogens (Smith & Read, 2008). Ectomycorrhizal fungi, commonly associated with trees, form external sheaths around plant roots and improve nutrient and water uptake; these fungi could be important in reclaiming BC's forested mining areas, where re-establishing tree cover may be a key goal (Anderson & Cairney, 2007). Overall, these symbiotic relationships are critical to stabilizing soils and initiating the re-establishment and ecological succession of native vegetation (Owiny & Dusengemungu, 2024; Smith & Read, 2008).

Fungal communities may exhibit distinct resilience and adaptability in comparison to bacterial communities, which could influence their roles in post-mining reclamation. Fungi are generally more tolerant of acidic and nutrient-poor conditions than bacteria, making them useful for reclaiming areas affected by mining activities (Ou et al., 2021; Yin et al., 2023). Additionally, while bacterial populations may recover rapidly due to their short generation times, fungi can sometimes persist through mining activities and fluctuating environmental conditions by forming spores and durable hyphal structures (Ainsworth & Sussman, 1968). This adaptability may allow them to establish networks in challenging environments, where bacterial activity can be limited, maintaining ecological functions over extended periods. Further, some previous research has highlighted the cooperative role of fungal and

bacterial communities in promoting soil aggregation and nutrient availability (Chen et al., 2022; Hu et al., 2024). For instance, some bacteria may decompose simpler organic compounds, while fungi specialize in breaking down more complex materials—together, these microbial communities create a more balanced and functional soil ecosystem.

Fungal communities are vital to restoring post-mining landscapes. Although their community shifts may differ from those of bacteria, their importance is being progressively recognized in the context of mining reclamation for their effective decomposition of organic matter, facilitation of plant growth through symbiosis, resilience to harsh conditions, and synergistic interactions with bacterial communities. The integration of underutilized fungal-based approaches and monitoring into reclamation strategies offers a promising pathway to sustainability.

**Invertebrate communities in post-mining landscapes**

Invertebrate communities are critical components of ecosystems and play crucial roles in soil formation, organic matter decomposition, and food web stability (Bagyaraj et al., 2016; Griffiths et al., 2021). In post-mining landscapes, where abiotic conditions are often harsh, invertebrates can serve as ecological engineers and sensitive indicators of ecosystem recovery (Neher et al., 2012; Perry & Herms, 2019; Silva-Monteiro et al., 2022).

Invertebrates influence soil health through several mechanisms. Detritivores, such as earthworms and certain beetle larvae, contribute to the breakdown of organic matter, thereby promoting nutrient mineralization and improving soil structure (Lavelle et al., 2006). Furthermore, their burrowing activities enhance porosity and aeration, which are essential for plant root development and microbial activity (Brown et al., 2000). Predatory invertebrates, including spiders and some insect taxa, regulate prey populations and contribute to balance within soil food webs (Coleman et al., 2004). Nematodes and collembolans, depending on their trophic group, participate in microbial grazing, nutrient mineralization, and even pathogen suppression (Coleman et al., 2004). Collectively, these roles make invertebrates integral to ecosystem restoration.

Invertebrate community structure is sensitive to soil physicochemical conditions, making these organisms reliable indicators of disturbance and recovery (Perry & Herms, 2019; Silva-Monteiro et al., 2022). Mining activities, particularly OBR, lead to the loss of habitat and the disruption of organic inputs. These changes, coupled with soil compaction and heavy metal contamination, could suppress invertebrate abundance and diversity. For example, earthworm populations may be absent in post-mining soils due to metal toxicity and poor organic matter availability (Loureiro et al., 2005).

However, as reclamation progresses and vegetation is re-established, invertebrate populations can begin to recolonize, signalling improvements in soil function. This recolonization is influenced by factors such as organic matter availability, vegetation type, the presence of soil amendments like biosolids, and dispersal limitations (Contos et al., 2021; Gervan, 2023; Silva-Monteiro et al., 2022). As seen in microbial communities, invertebrate assemblages may also vary in response to reclamation strategies; their sensitivity to changes in soil chemistry and habitat structure makes them useful as bioindicators, complementing bacterial and fungal assessments.

Despite their ecological significance, invertebrates remain underrepresented in reclamation strategies and monitoring programs. This is especially relevant for assessing the biological integrity of sites where microbial and plant indicators alone may not fully capture functional recovery. Understanding the role of invertebrates in ecosystem processes could enhance the development of holistic, multi-trophic reclamation strategies tailored to site-specific goals and constraints, while incorporating DNA-based tools would offer opportunities to characterize invertebrate assemblages with high resolution.

**Gaps in the knowledge of post-mining landscapes in British Columbia**

Despite major advances in mining reclamation practices, there remain knowledge gaps regarding the ecological recovery of post-mining landscapes in BC. Previous applications of DNA metabarcoding in mining reclamation has enabled comprehensive surveys of microbial (Ma et al., 2017; Rosenfeld et al., 2018; Singh

et al., 2024) and invertebrate communities (Gervan, 2023; Lynggard et al., 2020; Wardell-Johnson, 2019), providing quantitative biodiversity metrics that can complement or enhance traditional monitoring approaches. But the composition, function, and interactions of microbial and invertebrate communities are still not yet well understood. These gaps hinder further progress, delaying the development of sustainable, evidence-based reclamation strategies that restore ecosystems while mitigating environmental and public health risks.

Microbial and invertebrate communities play a role central to the recovery of post-mining landscapes, but the understanding of their diversity, function, and relationships in this context is incomplete. Although there are data that demonstrate shifting community compositions and reductions in diversity in response to mining activities (Lefcort et al., 2010; Quadros et al., 2016; Singh et al., 2024; Xiao et al., 2021), there is little information on how these communities recover naturally or in response to reclamation strategies; identifying taxa critical to ecosystem restoration and understanding their relationships would be pertinent (Bhatia et al., 2023). Furthermore, while it is known that these communities cycle nutrients, detoxify soil contaminants, and improve overall soil health, their specific functional contributions are often inferred rather than directly measured, hindering abilities to effectively harness them for reclamation strategies.

Additionally, given that the success of reclamation strategies may strongly depend on the interactions between microbial and invertebrate communities and specific treatments, such as soil amendments or vegetation establishment, the lack of studies examining these interactions in terms of response, the impact of physicochemical conditions, and temporal effects is inadequate. Limited data exist on how these communities respond to varying reclamation strategies, how variability in abiotic factors, including those as a result of different reclamation strategies, affects them, and how they and their functions evolve; these data may be key to the development of sustainable reclamation strategies.

Finally, because some reclamation strategies may introduce a new dimension of complexity to post-mining landscapes in terms of environmental and public health risks, it would be apposite to understand what the risks truly are. For example,

biosolids amendments may disseminate ARGs, but there is minimal research about their abundance and persistence.

Addressing these knowledge gaps requires multidisciplinary approaches that combine molecular, ecological, and geochemical methods. Enhanced monitoring of microbial and invertebrate communities using DNA-based tools, along with assessments of soil physicochemical and vegetation properties, could yield more integrated indicators of reclamation progress. Ultimately, a deeper understanding of the biological, functional, and risk-related considerations of reclamation is needed to advance sustainable and effective reclamation practices. Bridging these gaps will support the development of adaptive frameworks, capable of restoring both land capacity and ecological integrity in BC's post-mining landscapes.

**Research objectives**

The primary objective of this thesis is to assess how varying reclamation strategies impact the ecological recovery of post-mining landscapes in BC, with a particular focus on soil microbial and invertebrate communities. Given the lasting degradation associated with mining activities, the overarching goal of this research is to contribute to a more integrated, biologically informed understanding of reclamation effectiveness. Specifically, this thesis aims to:

1. Characterize bacterial and fungal community composition and diversity in reclaimed soils using high-throughput DNA sequencing to evaluate how these microbial communities respond to varying treatments (Chapters 2 and 3).
    a. Characterize invertebrate community composition and diversity in reclaimed post-disturbance environments, using high-throughput DNA sequencing to evaluate how this community responds to varying treatments (Chapter 3).
2. Assess the effects of biosolids amendments on soil physicochemical properties, including pH, nutrient levels, and trace metal concentrations, as well as vegetation properties (Chapter 2).
3. Quantify the abundance of ARGs in biosolids-treated soils and consider potential environmental and public health implications (Chapter 2).

A better understanding of these objectives would help contribute to ecosystem recovery in post-mining environments. By focusing on the biological communities within reclaimed post-mining landscapes and investigating how they respond to different treatments, the work within this thesis aims to provide insights into the potential of DNA-based ecological monitoring to inform adaptive, evidence-based reclamation practices. Additionally, by analyzing soil physicochemical properties, this thesis seeks to further the current understanding of how reclamation strategies influence soil health. Finally, by quantifying the abundance of ARGs in soil microbial communities across treatments, this thesis aspires to add to the knowledge of potential risks associated with reclamation strategies, such as biosolids applications. Understanding these dynamics may be essential to optimizing sustainable reclamation efforts that maximize land capacity while maintaining balanced and functional ecosystems.

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# Chapter 2. Biosolids amendments at different concentrations variably altered the microbial communities of four postmining experimental sites

## INTRODUCTION

Mining operations leave behind disturbed landscapes requiring rehabilitation and reclamation. These landscapes include tailings storage facilities, which store fine residues from the mining process and are often barren and lacking essential nutrients, resulting in inhospitable conditions for vegetation (Lacy, 2005; Vega et al., 2005). Interventions to restore ecosystem functions and prevent environmental hazards, such as erosion and water contamination, are required to reclaim such landscapes effectively (Engels et al., 2004; Gardner et al., 2012; Lacy, 2005). Traditional reclamation approaches, like the application of inorganic chemical fertilizers, have shown limited effectiveness in enhancing soil quality and promoting long-term vegetation growth (Antonelli et al., 2018; Gardner et al., 2010; Harris et al., 2021). This is likely because the addition of organic material is necessary for soil development and structure, both essential to preventing the leaching of nutrients and promoting the growth of vegetation (Borgegård and Rydin, 1989; Ye et al., 2002).

Consequently, alternative strategies, including the use of biosolids, are being explored. The application of biosolids—treated sewage sludge derived from municipal wastewater—has garnered attention for its potential to improve soil properties and support ecosystem recovery (CCME, 2012). Biosolids, rich in organic matter and essential nutrients like carbon, nitrogen, and phosphorus, can be valuable soil amendments for disturbed landscapes. They offer a gradual release of essential nutrients, and their application can significantly improve soil structure by increasing organic matter content (Cuevas et al., 2000; Gardner et al., 2010). These factors contribute to better water retention, soil fertility, and microbial activity, all of which can boost vegetation establishment in disturbed environments, such as tailings storage facilities (CCME, 2012; Gagnon et al., 2021; Wallace et al., 2016).

The effectiveness of biosolids in the context of mine reclamation has been demonstrated in various studies, including studies that focused on the Teck-Highland Valley Copper (HVC) Cu–Mo mine tailings storage facility in British

Columbia. At Teck-HVC, biosolids application significantly enhanced plant cover and diversity, especially where traditional methods failed, providing an example of effective biosolids use as a reclamation tool (Gagnon et al., 2021; Gardner et al., 2010; Harris et al., 2021). Specifically, 17 years after a one-time application of biosolids at various concentrations across multiple sites, observations revealed significantly enhanced plant community response characterized by increased plant cover, richness, and diversity (Gagnon et al., 2021).

Despite the ecological benefits, the use of biosolids in reclamation is not without controversy. In addition to demonstrating significantly enhanced plant community responses in the 2017 study by Gagnon et al., biosolids also facilitated the establishment of non-native grasses; while this contributed to landscape stabilization, it also raised concerns about the dominance of non-native over native species. This supports what some critics of biosolids argue: high nutrient concentrations from biosolids may favour invasive species, disrupting native plant communities and leading to unintended ecological consequences (Carpenter et al., 1990; DiTommaso and Aarssen, 1989). This is only one of the complexities of using biosolids in reclamation efforts—the ecological trade-offs must be carefully considered.

There are other concerns about the risks associated with biosolids, arising from the potential presence of heavy metals, pathogens, persistent organic pollutants, and antimicrobial resistance genes (ARGs) that could accumulate in soils and affect ecosystem health over time (Pepper et al., 2013; Wallace et al., 2016). While biosolids offer a promising tool for improving soil conditions and facilitating vegetation establishment, their immediate benefits in reclaiming disturbed lands must be weighed against their potential long-term impacts on biodiversity and ecosystem health. Although stringent regulations ensure that biosolids are treated to reduce these risks, the potential long-term ecological impacts remain a point of contention and balancing these impacts with the need for effective reclamation strategies remains a key challenge for resource managers and regulators. Additional research is needed to assess the impact of biosolids

application on community compositions, soil quality, and broader ecosystem functions in post-mining landscapes.

The primary aim of this study is to assess the impact of varying concentrations of biosolids treatments on both the physicochemical properties and biological communities at the Teck-HVC Cu–Mo mine tailings storage facility in British Columbia. By analyzing soil physicochemical characteristics, such as pH, nutrient concentrations, and trace element concentrations, this study seeks to further the current understanding of how biosolids influence soil health. By focusing on the bacterial and fungal communities within treated plots and investigating how these communities respond to different concentrations of biosolids treatment, this study aims to provide insights into the underlying biological processes and species that contribute to ecosystem functions and long-term reclamation success. This study also quantifies the levels of 23 ARGs in the soil microbial communities across all treatments to enhance knowledge of potential risks associated with biosolids applications. Understanding these dynamics is essential for optimizing reclamation efforts that maximize land capacity while maintaining balanced and functional ecosystems.

## METHODOLOGY

### Site description

This study was completed on the tailings storage facilities (TSFs) of the Teck-Highland Valley Copper (HVC) Cu–Mo mine, an open pit mine located in the southern interior of British Columbia, Canada (50°28′23.22″ N, 121°01′18.50″ W). Part of the Thompson Plateau, the mine is located on granite rock, containing porphyry copper and copper-molybdenum, as well as calc-alkaline deposits with ore grades of approximately 0.40 to 0.45% copper (Bergey, 2009). As described in Gagnon et al. (2021), field experiments were conducted on two TSFs: Trojan, a sand texture deposit (sites A and B), and Bethlehem, a silt loam texture deposit (sites C and D), approximately 1 km apart. Trojan and Bethlehem have similar soil pHs (8.33 and 8.09, respectively) and elevations (1,442 m and 1,481 m, respectively), and both are surrounded by natural vegetation (Gardner et al., 2010).

**Experimental design**

Experimental treatment plots (7 x 3 m) were established on both TSFs in July 1998 in a randomized complete block design, first described by Gardner et al. (2010). Each block, separated by 0.5 m buffers, consisted of a row of randomized treatments, separated by 1 m buffers; the design ensured eight treatment replicates on each TSF.

In August 1998, class B biosolids recovered from a Metro Vancouver wastewater treatment process were applied in a one-time application to the 7 x 3 m plots to achieve concentrations of 0, 50, 100, 150, 200, and 250 dry Mg/ha. Biosolids were applied with the use of an all-terrain vehicle, shovels, and rakes. Two weeks post-application, the biosolids treatments were rototilled into the top 15 cm of the plots; plots that did not receive a biosolids application were also rotovated. In June 1999, the four sites were broadcast seeded with an agronomic seed mix consisting of 33.2% pubescent wheatgrass (*Agropyron trichophorum*), 7.5% orchard grass (*Dactylis glomerata*), 4.0% creeping red fescue (*Festuca rubra* subsp. *rubra)*, 14.7% Russian wild ryegrass (*Elymus junceus*), 34.6% alfalfa (*Medicago sativa*), and 5.9% alsike clover (*Trifolium hybridum*) at a rate of 36 kg/ha; all non-native agronomics introduced to North America (Gardner et al. 2010). At the time of seeding, one-time inorganic fertilizer treatments (F) were also manually broadcasted to their respective 7 x 3 m plots but not incorporated. Fertilizer application rates were based on total nitrogen, phosphorus, potassium, zinc, and boron concentrations found in the 150 Mg/ha biosolids treatments in September 1998, and thus the resulting fertilizer amendment was 87 kg/ha ammonium nitrate (34.5-0-0), 111 kg/ha triple superphosphate (0-45-0), 83 kg/ha potassium chloride (0-0-60), and a mineral mix containing 0.5 kg/ha zinc chloride (99.9%) and 21 kg/ha granular boron (14%) (Gardner et al. 2010). No amendments were applied in the following years but in 2015 treatment plots were reduced to 5 x 2 m to minimize the edge effects of vegetation encroachment and blown-in sediment on treatment plot edges.

**Sampling and laboratory analysis**

As noted by Harris et al. (2021), soil sampling done in September 2015 reflected the methods Gardner et al. used (2010): a composite soil sample, including 10 random subsamples collected using a soil probe to a depth of 30 cm, was taken from each plot. These subsamples were then split into 0–15 cm (depth 1) and 15–30 cm (depth 2), homogenized, air-dried, and sieved to 2 mm. This study used the September 2015 depth 1 soil samples.

Vegetation biomass was collected by randomly placing ten 20 x 50 cm frames within each plot, removing the soil and dead litter from the previous years' growth, and clipping all vegetation at the soil surface to create a sub-sample. Each subsample was dried at 65°C for 24 h and weighed.

The details of the laboratory analysis of historical soil samples are given in Gardner et al. (2010). Soil samples collected in 2015 were analyzed by an accredited laboratory that used the same methodologies (The Standards Council of Canada, The Canadian Association for Laboratory Accreditation and SAI Global) (Austin, 2020). Nitric–hydrochloric acid digestion was used to extract total metals, and total P was extracted using HCl and nitric acids. Total metals and nutrients were analyzed using inductively coupled plasma optical emission spectrometry (ICP/OES), or if concentrations were low, inductively coupled plasma mass spectrometry (ICP/MS). Total C and N were determined using a combustion method (gas chromatography with a flame ionization detector).

**DNA extraction and 16S rRNA gene and ITS amplicon sequencing**

DNA was extracted from approximately 0.25 g of each soil sample using an E.Z.N.A Soil DNA Kit (Omega Bio-tek) following manufacturer's instructions. DNA concentrations were determined in 2 μL of each DNA extract using a Qubit dsDNA High Sensitivity Assay kit and Qubit 2.0 fluorometer (Thermo Fisher Scientific), and then stored at -20 ºC.

The V4 hypervariable of the bacterial 16S rRNA gene was targeted for community amplicon sequencing as previously described (Stephens et al., 2021), with minor modifications. PCR reactions were prepared containing 1x GoTaq Green

Master Mix (Promega), 0.5 μM of 341F (5'-TACGGGAGGCAGCAG-3') and 806R (5'-GGACTACVSGGGTATCTAAT-3') primers, and 3 μL of template DNA in a final reaction volume of 20 μL. Thermocycling conditions consisted of an initial denaturation step of 95°C for 4 minutes, twenty cycles of 95°C for 30 seconds, 49°C for 45 seconds, and 72°C for 2 minutes, and a final extension at 72°C for 2 minutes. PCR products were cleaned using AMPure XP beads (Beckman-Coulter), according to manufacturer's instructions. Cleaned products were used as template for a second round of PCR with adaptor and Ion Xpress barcoded primers. Reaction conditions and thermocycling was the same as for first round PCR, but the annealing temperature was increased to 55°C.

The fungal ITS region was targeted for community amplicon sequencing. PCR reactions were prepared containing 1x GoTaq Green Master Mix (Promega), 0.5 μM of ITS86F (5'- TTCAAAGATTCGATGATTCAG -3') (Vancov and Keen, 2009) and ITS4R (5'- TCCTCCGCTTATTGATATGC-3') primers (Innis et al., 2012), and 3 μL of template DNA in a final reaction volume of 20 μL. Thermocycling conditions consisted of an initial denaturation step of 95°C for 4 minutes, twenty-five cycles of 95°C for 30 seconds, 53°C for 45 seconds, and 72°C for 2 minutes, and a final extension at 72°C for 2 minutes. PCR products were cleaned using AMPure XP beads (Beckman-Coulter), according to manufacturer's instructions. Cleaned products were used as template for a second round of PCR with adaptor and Ion Xpress barcoded primers. Reaction conditions and thermocycling was the same as for first round PCR, but the annealing temperature was increased to 65°C.

PCR products from second-round Bacterial 16S rRNA gene and fungal ITS PCR reactions were cleaned using AMPure XP beads (Beckman-Coulter), according to manufacturer's instructions. Libraries were quantified using an Ion Library Quantitation Kit on a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific), pooled at equimolar concentrations, and sequenced on an Ion S5 XL with 400 base pair chemistry.

**Antimicrobial resistance gene testing**

Quantitative polymerase chain reaction (qPCR) was conducted to determine if antimicrobial resistance gene (ARG) abundances varied between treatments. DNA concentrations of all samples were determined using a fluorometer and adjusted to 1 ng/μL or less in sterile 10 mM Tris buffer. An assay to evaluate potential PCR inhibition was completed by spiking each sample with *Escherichia coli* DH10B Control 600 Library (Thermo Fisher Scientific) and using a Thermo Fisher Ion Library TaqMan Quantitation Kit (Thermo Fisher Scientific) qPCR specific for this library. Reactions contained 1 x PCR Master Mix, 1 x Quantitation Assay, and 1.25 μL of 1:10 diluted control library and 1.25 μL of sample template DNA in a final volume of 10 μL. Positive controls were run with 1.25 μL of 1:10 diluted control library as the template, and no template controls were run as negative controls. qPCR was performed on a QuantStudio 2 Real Time qPCR instrument (Thermo Fisher Scientific) and thermocycling conditions consisted of one cycle of 50ºC for two minutes, one cycle of 95ºC for 20 seconds, and 40 cycles of 95ºC for 1 second and 60ºC for 20 seconds. Ct values for spiked samples were compared to those of the positive and negative controls to determine any PCR inhibition.

To test for ARGs, qPCRs were carried out using Microbial DNA qPCR Assays (Qiagen) to target 23 different ARGs (Table 1.2). Assays were run following the manufacturer's instructions (Qiagen). Each reaction contained 1 x qPCR master mix, 1 x of primer probe assay mixture, and 2 μL of either a sample, the positive control, or the negative control, in a final volume of 25 μL. Positive controls consisted of Epitect Control DNA (Qiagen) and no template negative controls had deionized water instead of template. The QuantStudio™ 3 Real-Time qPCR (Thermo Fisher Scientific) thermocycler conditions consisted of 1 cycle at 95.0ºC for 10 minutes in the initial PCR activation step and 40 cycles at 95ºC for 15 seconds, followed by 60.0ºC for 20 seconds in the denaturation, annealing, and extension steps.

Table 1.2. The 23 ARGs targeted in this study, organized by antimicrobial resistance class.

| **Antibiotic Resistance Class** | **Gene** |
|---|---|
| Aminoglycosides | *aadA1* |
| Class A β-lactams | *sfc1* |
| | *tla1* |
| | *VEB* |
| Class B β-lactams | *imp5* |
| Class C β-lactams | *acc3* |
| | *fox* |
| Class D β-lactams | i*mp12* |
| | *oxa10* |
| | *oxa18* |
| | *oxa23* |
| | *oxa45* |
| | *oxa51* |
| | *oxa54* |
| | *oxa60* |
| Fluoroquinolone | qepA |
| | *qnrb5* |
| | *qnrb8* |
| Macrolides | *ermB* |
| | *ermC* |
| | *mefA* |
| | *tetA* |
| | *vanB* |

**Data processing and statistical analyses**

Statistical analyses were completed using R version 4.4.0, and Qiime 2 2024.5 (Bolyen et al., 2019; R Core Team, 2024).

A principal component analysis (PCA) was completed on soil physicochemical data to compare the properties of the sites after treatments. In RStudio, the measurements were scaled and a PCA was run using base R. The PCA and scree plots were created using the "ggplot2," (Wickham, 2016) and "viridis" packages (Garnier et al., 2024).

Before other analyses were completed, raw sequence data were demultiplexed in AMPtk 1.5.5 using the amptk ion script with default settings and --trim-len set to 350 bases. Demultiplexed data were imported into Qiime 2 with the qiime tools import script set with --input-format SingleEndFastqManifestPhred33V2 (Bolyen et al., 2019). Denoising was done with DADA2 denoise-single with max-ee set to 1.0 (p-trunc-len set to 0 because the reads were previously trimmed in AMPtk) (Callahan et al., 2016). The q2-feature-classifer classify-sklearn script was used to assign taxonomy with a database trained using the qiime feature-classifier with the Greengenes2 database (version 2022.10) extracted with the 341f and 806r primer sequences listed above (Bokulich et al. 2018; McDonald et al., 2024; McDonald et al., 2012). For fungi, the version 9 Qiime2-compatible UNITE reference database was downloaded and the dynamic "developer" version (sh_refs_qiime_ver9_dynamic_25.07.2023_dev.fasta and sh_taxonomy_qiime_ver9_dynamic_25.07.2023_dev.txt) was used to train the classifier using the qiime feature-classifier tool without extracting or trimming reads to the primer sites (Abarenkov et al., 2023). The resulting amplicon sequence variant (ASV) tables were then imported into RStudio and rarefied (to 2040 for bacteria and 6693 for fungi) using the "phyloseq" ("BiocManager") package (Bolyen et al., 2019; McMurdie and Holmes, 2013).

Shannon and Simpson diversity indices based on the rarified ASV tables were calculated using the "vegan" (Oksanen et al., 2024) package in RStudio. The indices were then used to conduct analyses of variance (ANOVAs) for parametric data or Kruskal-Wallis tests for non-parametric data, as well as any necessary post-

hoc tests to analyze microbial diversity within the sites. Non-metric multidimensional scaling (NMDS) plots based on Bray-Curtis dissimilarity, computed using the "vegdist" function, of log-transformed values from the rarified ASV tables were prepared using the "vegan" (Oksanen et al., 2024), "ggplot2" (Wickham, 2016), and "viridis" packages (Garnier et al., 2024) to compare the microbial communities between treatments.

To select microbial communities for composition comparisons, multiple permutational multivariate analyses of variance (PERMANOVAs) were performed in RStudio on Bray-Curtis dissimilarity matrices calculated from the rarified data. One-way PERMANOVAs were conducted with the "adonis2" function from the vegan package (Oksanen et al., 2024), using the model formula: vegdist(rarefied_table, method = "bray") ~ Treatment, where "Treatment" indicated the experimental grouping variable relevant to each comparison. PERMANOVAs were based on 999 permutations, and the "betadisper" function was employed to ensure homogeneous group dispersion. If the p-value of a PERMANOVA was <0.05, an analysis of composition of microbiomes with bias correction (ANCOM-BC) in Qiime 2 was used to identify and quantify differentially abundant ASVs across sites and treatments (Lin and Peddada, 2020). A significance threshold of 0.01 was applied to identify the top enriched and depleted ASVs for each comparison. Log fold changes (LFCs) for each comparison were recorded and visualized in RStudio, using the "ggplot2" (Wickham, 2016) and "viridis" (Garnier et al., 2024) packages. Representative sequences associated with these ASVs, and their unique Qiime 2 Feature IDs, were then used as query sequences to search the nucleotide BLAST database, using BLAST+ 2.16.0, while optimizing for a maximum of 10 highly similar target sequences, to identify where the ASVs had been found previously and how they were classified (Camacho et al., 2009).

Two linear mixed-effects models (LMMs) were employed in RStudio, using the "lme4" (Bates et al., 2015), "lmerTest" (Kuznetsova et al., 2017), and "lattice" (Sarkar, 2008) packages, to test if different treatments significantly affected the input ARG 1/Ct values. The models were both specified as: 1/Ct = Treatment + (1 | ARG), where "Treatment" was included as a fixed effect and "ARG" as a random intercept

to account for baseline variability in gene expression; the “Treatment” factor was coded with unamended plots as the reference level. Model diagnostics included plotting the residuals versus the fitted values to check for homoscedasticity, and Q-Q plots of residuals as well as histograms of residuals to check for normality.

## RESULTS

This study aimed to assess the impact of varying concentrations of biosolids treatments on postmining landscapes by comparing soil physicochemical properties, and fungal and bacterial community diversity and compositions within and between unamended, fertilizer-treated, and biosolids-treated plots. It also quantified the levels of 23 ARGs in the soil microbial communities to examine the potential risks associated with biosolids applications. Through this investigation, an understanding of the underlying risks, processes, and microorganisms that contribute to ecosystem functions and long-term reclamation success can be gained.

To characterize unamended, fertilizer-treated, and biosolids-treated soils at four postmining experimental reclamation sites at the Teck-Highland Valley Copper (HVC) Cu–Mo mine, a PCA of soil physicochemical properties, including pH, electrical conductivity, element concentrations and percentages, and vegetation biomass and cover was carried out (Figure 1.2). This demonstrates that soil pH is lower in the unamended and fertilizer-treated plots, influencing both principal components negatively and indicating that the pH vector is associated with treatment effects. Alternatively, Cu concentrations are generally higher at sites A and B with no obvious treatment effects, instead demonstrating site effects. Also appearing to be associated with site effects, the cluster of vectors in the lower-right corner of Figure 1.2 prevail in site D. Finally, the cluster of vectors in the upper-right corner of Figure 1.2 is mainly associated with higher concentrations of biosolids treatments, demonstrating treatment effects; high vegetation biomass and cover, as well as high percent C and N are evident within biosolids-treated plots. Based on overall clustering, it is apparent that there are treatment effects, as well as site-specific physicochemical characteristics.

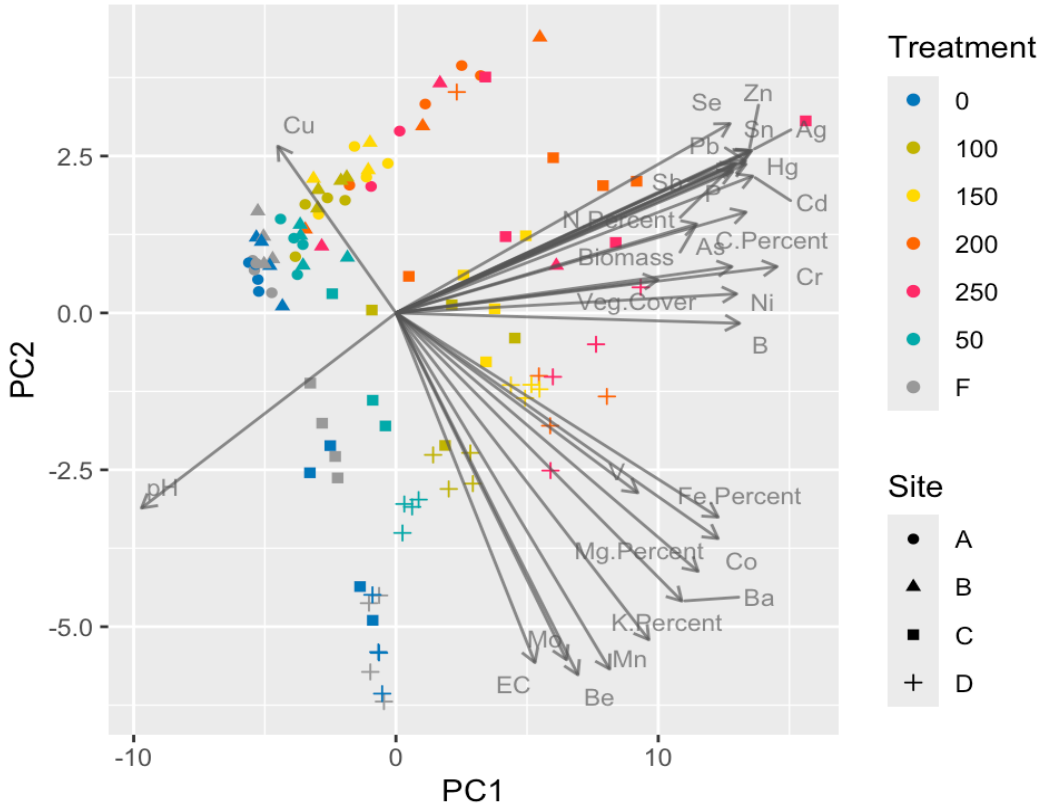


Figure 1.2. A plot of the PCA scores of soil physiochemical properties (depth of 0–15 cm) and vegetation data of reclaimed mining sites A and B (Trojan) and C and D (Bethlehem) treated with fertilizer (F) or different concentrations of biosolids (dry Mg/ha) with the vector loadings overlaid. The PCA accounts for ~82% of the variance across the samples (Figure A2).

To examine microbial diversity within the post-treatment communities of the four experimental reclamation sites, the species richness, and the Shannon and Simpson diversity indices were calculated (Figure 2.2). Each boxplot displays the distribution across treatments, with the central line representing the median value, the box encompassing the interquartile range (IQR), and the whiskers extending to the minimum and maximum values within 1.5x the IQR; points outside the whiskers indicate outliers. Lowercase letters indicate significant differences. Within the bacterial communities, there was not a significant difference in species richness across treatments, as supported by a Kruskal-Wallis test ($p = 0.98$). Shannon and Simpson bacterial diversity appear to decrease with increasing concentrations of biosolids treatment (Figures 2.2C and 2.2E). A Kruskal-Wallis test indicated a significant difference between the Simpson diversities of the treatments ($p < 0.001$), and a post-hoc Dunn's test revealed significant differences between the 0 Mg/ha and the 150 Mg/ha and 250 Mg/ha biosolids treatments ($p = 0.0053$ and $p = 0.0021$, respectively), as well as the fertilizer and the 150 Mg/ha, 200 Mg/ha, and 250 Mg/ha treatments ($p = 0.0021$, $p = 0.018$, and $p < 0.001$) (Figure 2.2E).

Within the fungal communities, there was also not a significant difference in species richness across treatments, as indicated by the results of a Kruskal-Wallis test ($p = 0.055$). Shannon and Simpson fungal diversity appeared to decrease with increasing concentrations of biosolids treatment as well, but both a one-way ANOVA on the Shannon diversities of the treatments and a Kruskal-Wallis test on the Simpson diversities demonstrated no significant differences ($p = 0.43$ and $p = 0.18$, respectively, in Figures 2.2B and 2.2D).

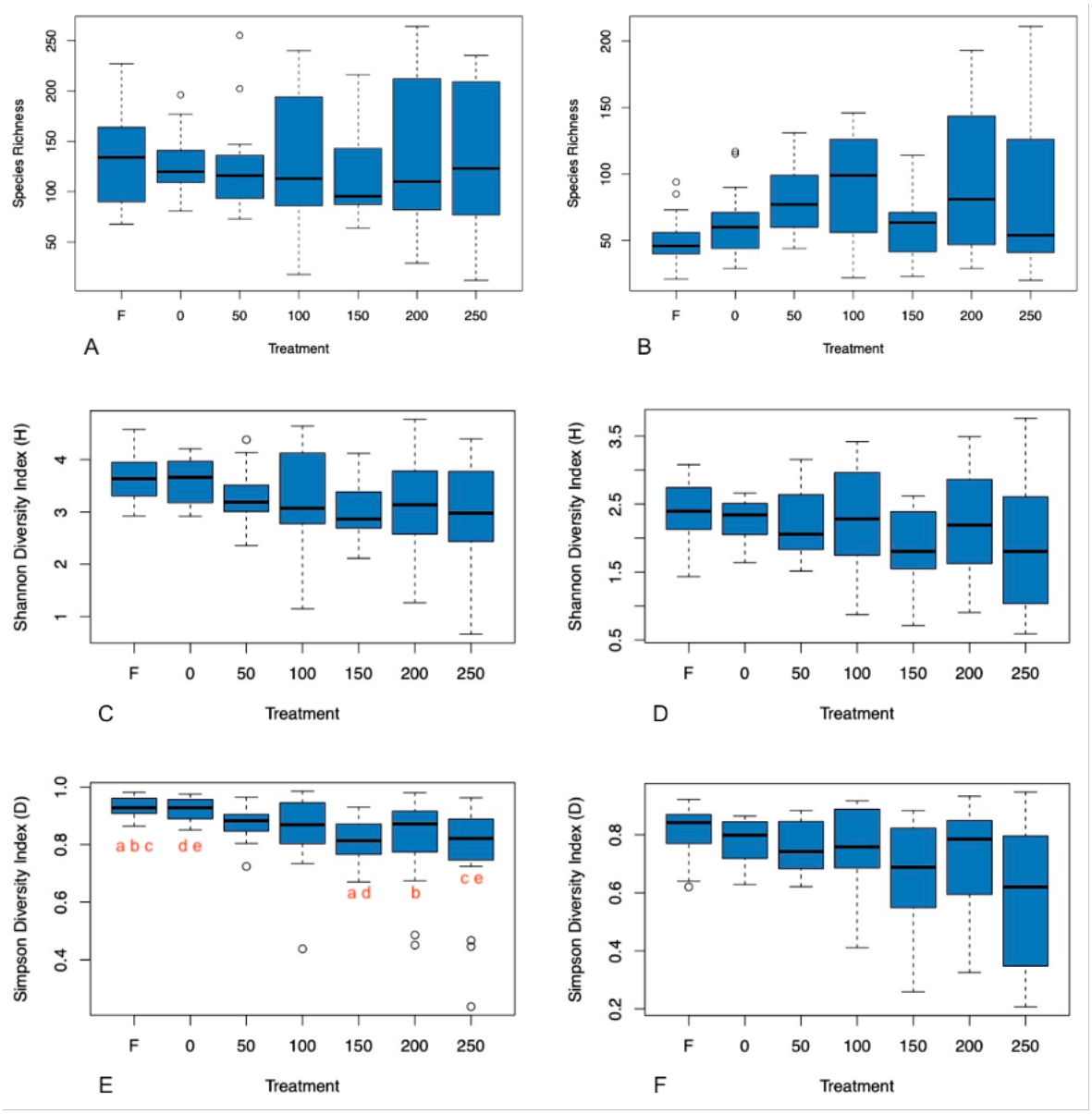


Figure 2.2. Microbial diversity across four reclaimed mining sites treated with fertilizer (F) or different concentrations of biosolids (dry Mg/ha). (2.2A) Bacterial species richness. (2.2B) Fungal species richness. (2.2C) Bacterial Shannon diversity. (2.2D) Fungal Shannon diversity. (2.2E) Bacterial Simpson diversity. (2.2F) Fungal Simpson diversity.

To compare the microbial communities between treatments at the Teck-HVC Cu–Mo mine, Bray-Curtis dissimilarity matrices of the rarefied amplicon sequencing data were generated, and the NMDS scores were plotted (Figure 3.2). An NMDS score represents the relative position of a sample in a reduced-dimensional ordination space. Shifts in both bacterial and fungal community composition can be observed with variations in biosolids treatment concentration across all four sites. In both Figures 3.2A and 3.2B, samples treated with the same concentrations of biosolids treatment cluster together, suggesting similar microbial communities. Furthermore, points representing communities treated with 0 Mg/ha of biosolids and fertilizer are distinctly separate from points representing communities treated with 250 Mg/ha of biosolids; this aligns with alpha diversity results (Figure 2.2). In addition to the effects of varying biosolids treatment concentrations, bacterial and fungal community composition shifts are discernably related to site variation.

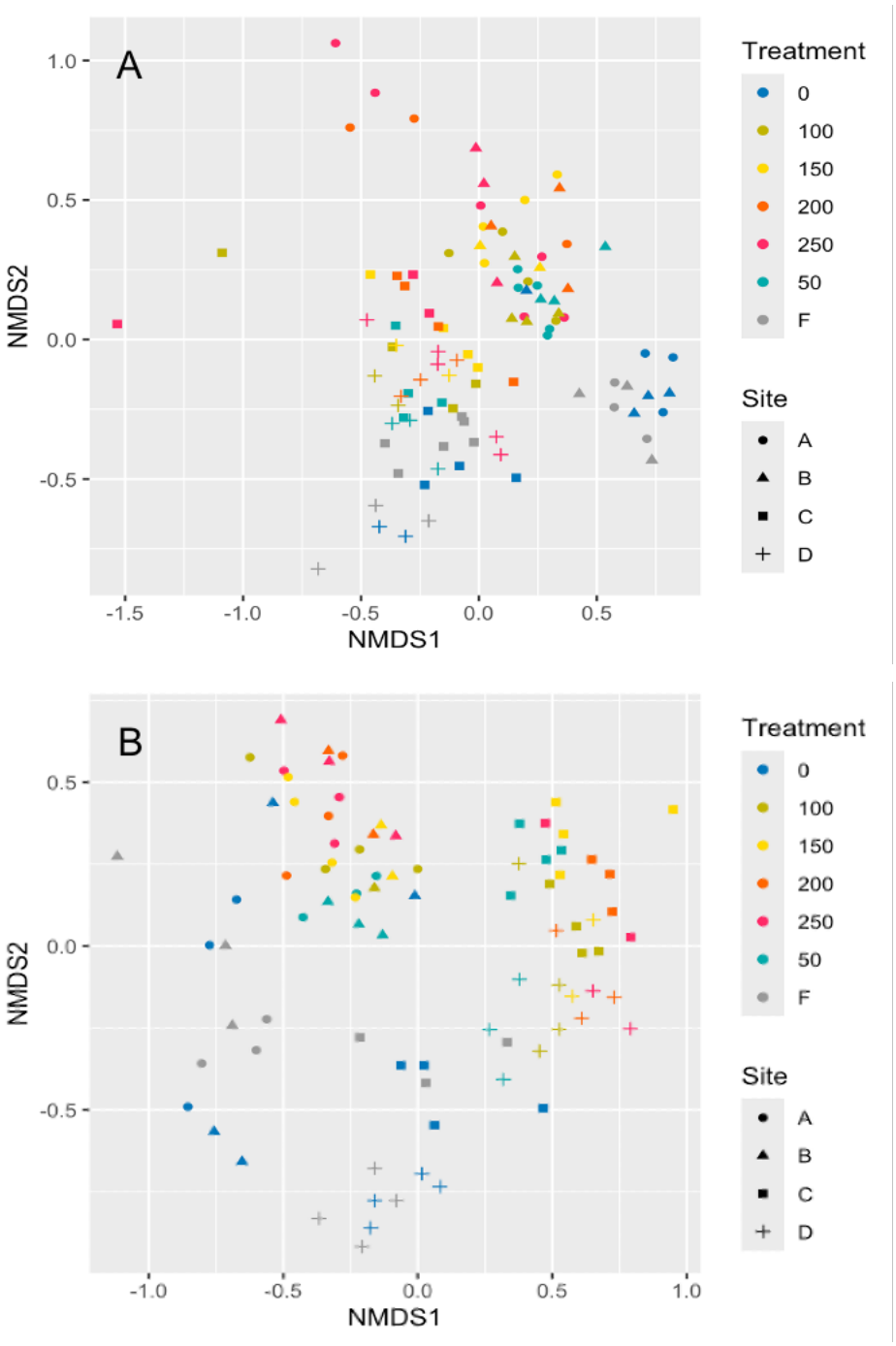


Figure 3.2. Plots of the NMDS scores of the microbial communities, based on Bray-Curtis dissimilarity matrices of rarefied amplicon sequencing data across four reclaimed mining sites treated with fertilizer (F) or different concentrations of biosolids (dry Mg/ha). (3.2A) Bacterial communities, and (3.2B) Fungal communities.

To compare bacterial community composition across treatments at the four Teck-HVC sites, ANCOM-BCs were performed. For the control plots (0 and F), unamended plots (0), and fertilizer plots (F), each with reference to plots treated with biosolids, and for the plots treated with the minimum biosolids concentration (50 Mg/ha) with reference to the plots treated with the maximum biosolids concentration (250 Mg/ha), the p-values of the PERMANOVAs were all < 0.001 (Figure A3). The ANCOM-BCs revealed notable patterns of enrichment and depletion in bacterial community composition across treatments (Figure 4.2). ASVs belonging to the phylum Acidobacteriota were consistently enriched across control plots, with one ASV enriched in the minimum biosolids concentration plots, whereas an ASV of the phylum Nitrospirota exhibited marked depletions across control plots. Additionally, ASVs of the phyla Bacteroidota, Chloroflexota, and Firmicutes were found to be enriched in at least one comparison with reference to plots treated with biosolids or the maximum biosolids concentration. Alternatively, ASVs of some phyla, such as Actinobacteriota and Proteobacteria, demonstrated mixed responses. Within the phylum Actinobacteriota, ASVs belonging to the classes Acidimicrobiia and Thermoleophilia were depleted across control plots, and ASVs belonging to the class Actinomycetia were both depleted and enriched across control plots and minimum biosolids concentration plots. Within the phylum Proteobacteria, three ASVs were depleted across minimum biosolids concentration plots, and one was enriched in fertilizer plots and minimum biosolids concentration plots.

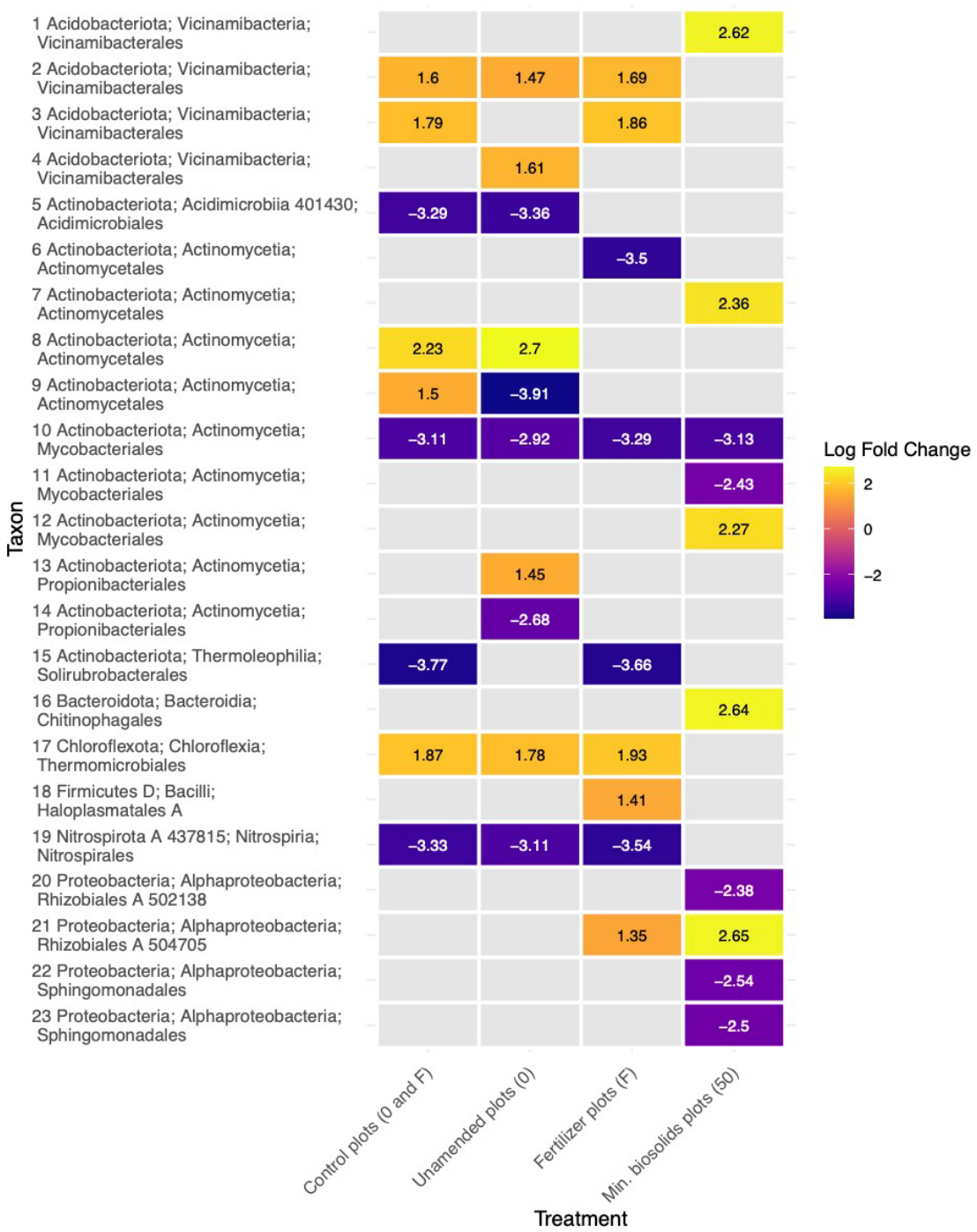


Figure 4.2. ANCOM-BC results, presented as log fold changes, from comparisons of the bacterial communities of the control plots (0 and F), unamended plots (0), and fertilizer sites (F) to plots treated with biosolids, and the plots treated with the minimum biosolids concentration (50 Mg/ha) to the plots treated with the maximum biosolids concentration (250 Mg/ha). Taxa are reported in Phylum; Class; Order format, and repeated taxa have unique Feature IDs in Qiime 2.

ANCOM-BCs were also performed to compare fungal community composition across treatments at the experimental reclamation sites. For the control plots (0 and F), unamended plots (0), and fertilizer plots (F), each with reference to plots treated with biosolids, the p-values of the PERMANOVAs were < 0.001, < 0.002, and < 0.002, respectively (Figure A4). Several fungal ASVs also exhibited notable patterns of enrichment and depletion, revealing differences in community composition across experimental treatments (Figure 5.2). The class Eurotiomycetes and phylum Mortierellomycota were consistently enriched across control plots. Conversely, two ASVs, one of the class Dothideomycetes, and one of the phylum Basidiomycota, were depleted in the fertilizer plots. And, like some bacterial ASVs, fungal ASVs of the classes Leotiomycetes and Sordariomycetes demonstrated mixed responses. Within the class Leotiomycetes, an ASV of the order Erysiphales was enriched across control plots, and an ASV of the order Helotiales was depleted. Within the class Sordariomycetes, ASVs belonging to the orders Microascales and Xylariales were enriched across control plots, whereas an ASV belonging to the order Myrmecridiales was depleted.

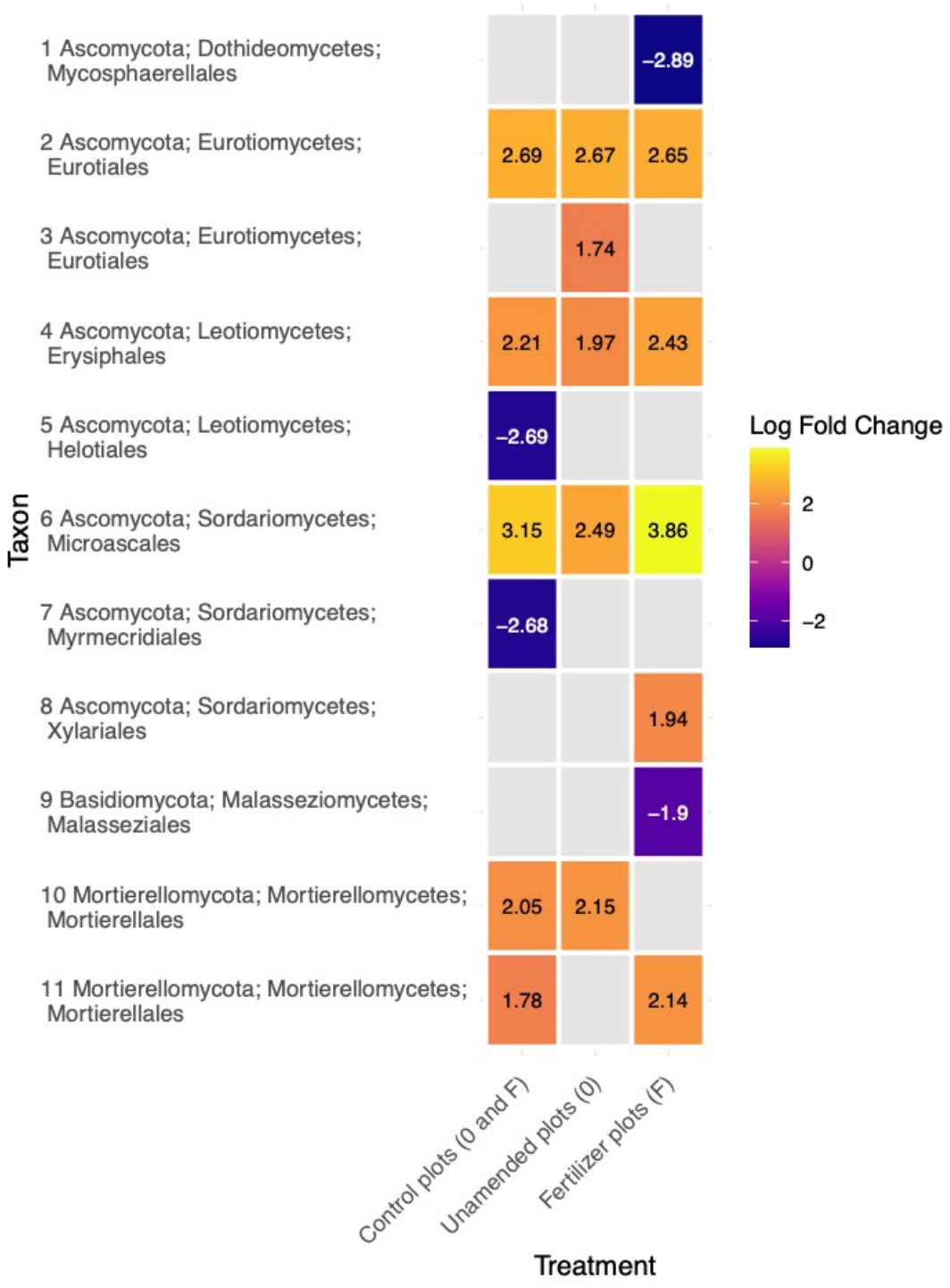


Figure 5.2. The ANCOM-BC results, presented as log fold changes, from comparisons of the fungal communities of the control plots (0 and F), unamended plots (0), and fertilizer plots (F) to plots treated with biosolids. Taxa are reported in Phylum; Class; Order format, and repeated taxa have unique Feature IDs in Qiime 2.

To quantify the levels of different ARGs within the microbial communities across samples of the unamended plots (0), fertilizer plots (F), 100 Mg/ha biosolids plots, and 250 Mg/ha biosolids plots, 23 targeted qPCR assays were completed. Seven different samples (1, 2, 5, 6, 8, 22, and 56) showed significant PCR inhibition and were therefore dropped from further experimentation and analysis (Table A1). Eleven ARGs, including *aadA1*, *ermB*, *ermC*, *imp5*, *mefA*, *oxa51*, *oxa54*, *oxa60*, *sfc1*, *tetA*, and *VEB*, spanning multiple antibiotic resistance classes, were detected via qPCR amplification in least one sample treated with biosolids (Figure 6.2). Of the eleven ARGs detected, only seven (*aadA1*, *ermB*, *ermC*, *mefA*, *oxa54*, *sfc1*, and *VEB*) were found in three or more samples, and seven (*aadA1*, *ermB*, *ermC*, *mefA*, *oxa51*, *oxa54*, and *VEB*) were found in 100 Mg/ha biosolids plots and 250 Mg/ha biosolids plots. Furthermore, only five ARGs (*ermC*, *imp5*, *oxa51*, *oxa60*, and *tetA*) were found in biosolids-treated plots but not unamended (0) or fertilizer plots (F).

To test if different treatments significantly affected ARG presence, while accounting for ARG-specific variability, two linear mixed-effects models (LMMs) were employed. LMM 1 analyzed the 1/Ct values of all 23 ARGs assayed, and the LMM 2 analyzed the 1/Ct values of the 11 ARGs that were detected via qPCR amplification in at least one sample treated with biosolids. Both models demonstrate that the 250 Mg/ha biosolids treatment had a significant effect on ARG presence (LMM 1, estimate = 0.0022, t = 2.52, p = 0.014; LMM 2, estimate = 0.0050, t = 2.92, p = 0.007), while neither model indicates that fertilizer plots (F) or 100 Mg/ha biosolids plots had significant effects compared to unamended plots (Table 2.2). LMM 1's random effects included ARG-specific variability with a variance of 7.65e-06 ± 0.0028 and the residuals with a variance of 8.82e-06 ± 0.0030, and LMM 2's included ARG-specific variability with a variance of 1.05e-05 ± 0.0032 and the residuals with a variance of 1.59e-05 ± 0.0040. The LMM 1 and 2 residuals were also examined, ranging from -2.36–3.63 and -1.49–2.22, respectively, and their diagnostics indicated no major deviations from normality or homoscedasticity.

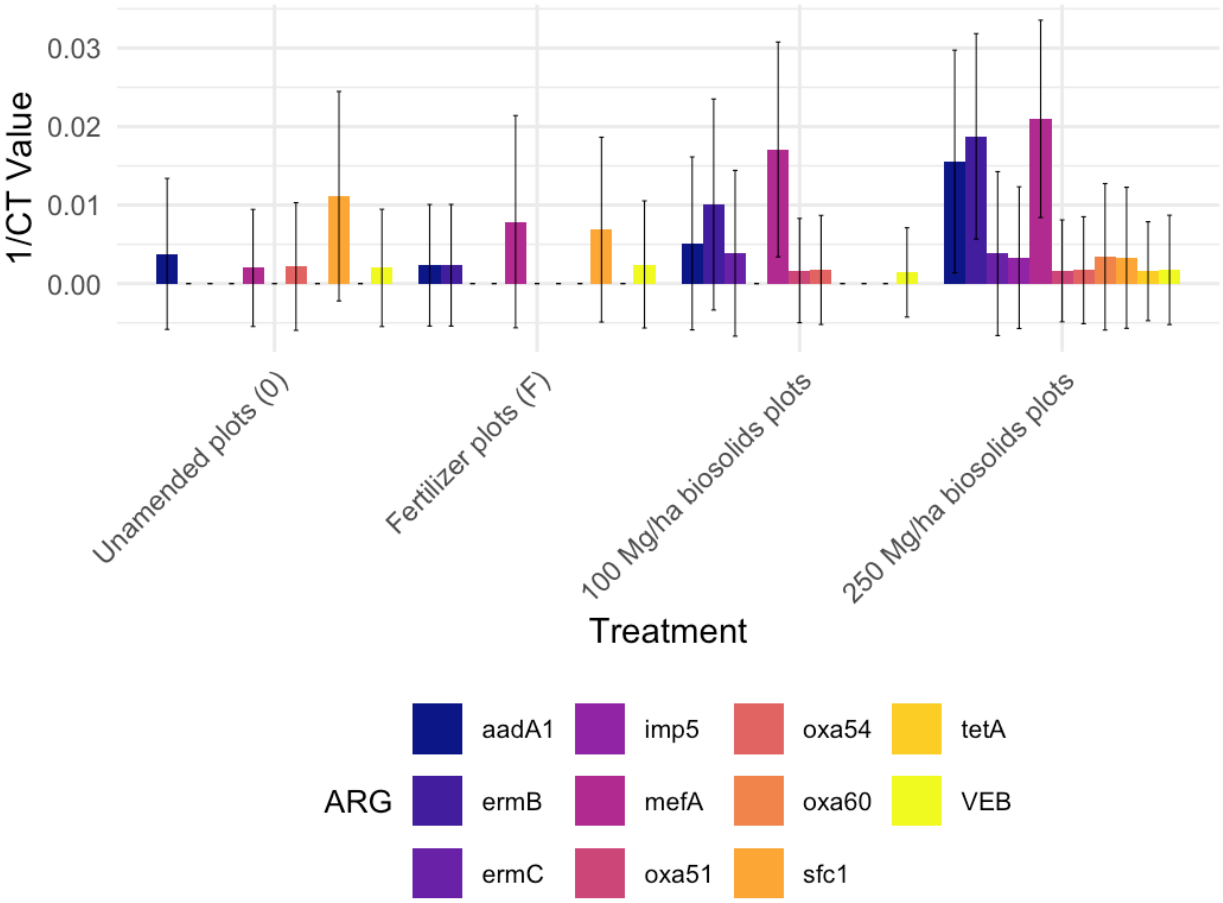


Figure 6.2. The average 1/Ct value across samples of the unamended plots (0), fertilizer plots (F), 100 Mg/ha biosolids plots, or 250 Mg/ha biosolids plots for the eleven ARGs with qPCR amplification detected in at least one sample treated with biosolids.

Table 2.2. The fixed effects of LMM 1, which analyzed the 1/Ct values of 23 ARGs, and LMM 2, which analyzed the 1/Ct values of an 11-ARG subset, including estimate, standard error (SE), t-value, and p-value.

| Fixed Effects | LMM | Estimate | SE | t-value | p-value |
|---|---|---|---|---|---|
| **Intercept** | 1 | 0.0010835 | 0.000846 | 1.280 | 0.206 |
| | 2 | 0.001916 | 0.001548 | 1.238 | 0.226 |
| **Fertilizer plots (F)** | 1 | -0.0001328 | 0.000876 | -0.152 | 0.880 |
| | 2 | 0.00007176 | 0.001701 | 0.042 | 0.967 |
| **100 Mg/ha biosolids** | 1 | 0.0006972 | 0.000876 | 0.796 | 0.429 |
| | 2 | 0.001807 | 0.001701 | 1.062 | 0.297 |
| **250 Mg/ha biosolids** | 1 | 0.0022104 | 0.000876 | 2.524 | 0.014 |
| | 2 | 0.004971 | 0.001701 | 2.923 | 0.007 |

## DISCUSSION

The primary aim of this study was to assess the impact of varying concentrations of biosolids treatments on the soil physicochemical properties, microbial communities, and the presence of 23 ARGs in four postmining experimental reclamation sites at the Teck-HVC Cu–Mo mine in British Columbia. The results demonstrated both benefits and complex ecological implications of biosolids amendments at different concentrations; to optimize reclamation efforts that maximize land capacity while maintaining balanced and functional ecosystems, understanding these dynamics is critical.

### Impacts of biosolids on soil physicochemical properties and microbial diversity

This study's assessment of the impact of varying concentrations of a one-time biosolids amendment on soil physicochemical properties and microbial diversity indicates that biosolids exert a strong influence on soil ecosystem function and recovery in post-mining landscapes.

The PCA analysis of soil physicochemical properties revealed clear shifts in soil composition with increasing biosolids concentrations, marking a strong treatment effect. The clustering of samples by biosolids concentration supports the notion that these amendments may lead to more homogeneous soil conditions, particularly at higher application rates. Key variables, such as pH and a cluster that included selenium (Se), zinc (Zn), silver (Ag), mercury (Hg), vegetation biomass and cover, and percent carbon (C) and nitrogen (N) were positively influenced by biosolids treatments. The associations of Se, Zn, Ag, and Hg with higher concentrations of biosolids treatments indicate that these treatments enrich the soil with metals, but not to a level above the national regulatory limits for agriculture (Harris et al., 2021). They may still, however, have critical implications for soil chemistry and microbial activity (Mossa et al., 2017; Popoola et al., 2023; Smith, 2009). Additionally, the biosolids amendments notably enhanced soil quality, increasing soil carbon and nitrogen content, as well as vegetation biomass and cover, underscoring their role in enhancing soil fertility and vegetation growth (Singh & Agrawal, 2008). This aligns

with prior research by Gardner et al. (2010), further corroborating the role of biosolids in soil organic content and plant growth. In contrast, the unamended and fertilizer-treated plots were linked with lower soil pH and distinct physicochemical profiles, which supports earlier observations by Gardner et al. (2012), that traditional chemical fertilizers may not provide the organic matter required for sustained soil quality improvements.

Alpha diversity analyses demonstrated nuanced and differential changes within bacterial and fungal communities as a result of biosolids treatments. For example, while bacterial species richness remained relatively unaffected by varying concentrations of biosolids treatments, fungal species richness approached significant variation. This contrasts with the findings of Hartmann et al. (2015) and Mossa et al. (2017), who reported that organic amendments often promote soil microbiota diversity due to their nutrient content. The discrepancy could be attributed to differences in amendment concentrations or an inherent variability in microbial community resilience across plots. Alternatively, high biosolids concentrations (150, 200, and 250 Mg/ha) were associated with decreased Simpson diversity in bacterial communities, suggesting a shift toward dominance by fewer, potentially more biosolids-tolerant microorganisms. These findings are consistent with observations by Wallace et al. (2016), who reported that microbial community responses can be dependent on nutrient availability and treatment type, and Gagnon et al. (2021), who indicated that while biosolids foster microbial activity they may also create conditions that favour some taxa at the expense of overall diversity.

The differences in microbial community composition between treatments, highlighted by the NMDS plots of the bacterial and fungal communities, demonstrate that beta diversities are also influenced by biosolids treatments. The bacterial and fungal NMDS plots both show clustering of samples treated with the same biosolids concentrations, and distinct separations between communities treated with 0 Mg/ha of biosolids or fertilizer and communities treated with 250 Mg/ha of biosolids. This is likely due to varying concentrations of nutrients, outlined by Harris et al. (2021), causing treatment-induced shifts, which highlights how nutrient additions can reshape microbial ecosystems. Where there are inconsistencies in microbial

responses, however, it is plausible that other environmental factors, such as site-specific soil conditions, may be playing more significant roles in shaping community structure. The NMDS plots corroborate the results of the alpha diversity analyses, as well as findings from Wang et al. (2017), who emphasize that biosolids, while enhancing nutrient levels, can also create selective pressures that lead to the dominance of specific microbial groups. In particular, clustering observed at higher concentrations of biosolids treatment could reflect the dominance of bacteria or fungi that benefit from increased availability of organic substrates—that are well-adapted to the high organic matter content and enhanced nutrient availability of biosolids amendments—leading to distinct community assemblages (Elgarahy et al., 2024; Schlatter et al., 2017). Interestingly, although the diversity analyses suggest that biosolids treatments create a strong selective pressure, the results of Singh et al. (2024), who used metagenomic sequencing to investigate the bacterial community compositions of 18 Teck-HVC sites, demonstrated that the likelihood of deterministic assembly was lower in biosolids-treated plots.

**Impacts of biosolids on bacterial community composition**

ANCOM-BCs exhibited distinct patterns of bacterial enrichment and depletion in treatment plots, demonstrating how biosolids amendments influence soil bacterial communities in post-mining landscapes. Notably, across all plots treated with biosolids, the enriched taxa were not pathogens but rather common soil microorganisms (Delgado-Baquerizo et al., 2018).

One finding was the enriched presence of Acidobacteriota in control plots, and plots treated with 50 Mg/ha of biosolids, despite generally basic soil conditions. Although Acidobacteriota are typically associated with acidic environments, their persistence here suggests broader ecological adaptability (Jones et al., 2009; Kielak et al., 2016). This aligns with studies indicating that Acidobacteriota in subdivision 4 can be prevalent and even thrive in more neutral or basic conditions (Kalam et al., 2020; Kielak et al., 2016). The enrichment of Acidobacteriota in unamended and fertilizer-treated plots specifically highlights their roles in soils where organic input is limited, echoing findings by Fierer et al. (2007), who noted that this phylum tends to

diminish in abundance when organic nutrient sources are introduced. Furthermore, the concurrent depletion of Nitrospirota in control plots, parallels the expected pattern observed by Li et al. (2020). Nitrospirota, with vital roles in nitrogen cycling, appear particularly sensitive to the lack of organic amendments that likely reduced the availability of substrates necessary for their metabolic activities (Li et al., 2020).

Additionally, ASVs of the phyla Chloroflexota, Bacteroidota, and Firmicutes were found to be enriched in at least one comparison with reference to plots treated with biosolids or the maximum biosolids concentration. The enrichment of Chloroflexota, known for their resilience under harsh conditions and ability to thrive in diverse ecological niches, may reflect their roles in early recovery processes in soils with minimal organic input or where chemical fertilizers have been applied (Delgado-Baquerizo et al., 2018; Freches & Fradinho, 2024; Janssen, 2006). Alternatively, Bacteroidota, known for their roles in the decomposition of organic material, were expected to be enriched in biosolids-treated plots due to the higher levels of organic materials, and per the results of Li et al. (2020). There may, however, be a dose-dependent response, as suggested by Mossa et al. (2017), for which optimizing biosolids treatment concentrations could enhance the effects. Like Bacteroidota, the abundance of the phylum Firmicutes, which includes species adapted to high-nutrient environments, was also expected to reflect nutrient-driven changes (Gupta et al., 2018). The ANCOM-BC results, however, found an ASV of the phylum Firmicutes to be enriched in fertilizer plots. This instead corroborates the hypothesis by Li et al. (2020) that Firmicutes may have a lower potential for utilizing manure-derived carbohydrates, potentially leading to decreased abundance after biosolids amendments.

Responses among Actinobacteriota and Proteobacteria varied, with several ASVs demonstrating depletion in control plots. The depletion of Actinobacteriota ASVs in control plots with respect to biosolids-treated plots, especially ASVs that would be involved in organic matter decomposition and carbon cycling, further indicates that without biosolids treatments these soils may struggle to support microbial processes essential to nutrient turnover and soil fertility (Barka et al., 2016; Zhang et al., 2019). While it should be noted that some ASVs of the phylum

Actinobacteria were enriched, more were depleted. And, aside from one ASV, Proteobacteria were also found to be depleted across the minimum biosolids concentration plots. This aligns with previous findings by Li et al. (2020), that as Proteobacteria are typically linked to environments enriched by organic inputs, manure increases their abundance. These responses reinforce the complex relationship between biosolids amendments and bacterial community composition, underscoring the importance of maintaining a diverse and functional bacterial community that supports ecosystem resilience and soil health.

**Impacts of biosolids on fungal community composition**

ANCOM-BCs of fungal communities across treatment plots also revealed patterns of enrichment and depletion, indicating how biosolids amendments influence soil fungal communities in post-mining landscapes.

In control plots, the consistent enrichment of the class Eurotiomycetes and the phylum Mortierellomycetes suggests that these fungal taxa are well-adapted to unamended or fertilizer-treated soils. Eurotiomycetes, known to thrive in stressed environments with minimal intervention, play crucial roles in decomposing organic material and cycling nutrients, maintaining soil health (Ciss et al., 2023; Liang et al., 2021). The persistent enrichment of Eurotiomycetes in unamended plots supports findings by Liang et al. (2021) that highlight the resilience of the class. Similarly, enrichments of Mortierellomycota across control plots are consistent with their known roles in nutrient cycling and soil health (Xu et al., 2021). This phylum, associated with early stages of organic matter decomposition and phosphorus dissolution, may dominate in control plots as organic matter accumulates naturally, contributing to soil recovery (Liang et al., 2021; Wang et al., 2020).

Additionally, the depletion of specific fungal taxa in fertilizer-treated plots, particularly within the class Dothideomycetes and the phylum Basidiomycota, suggests that the application of inorganic fertilizer may negatively impact their populations. Dothideomycetes, which include species that form symbiotic relationships with plants, may be sensitive to changes in soil chemistry and nutrient availability introduced by fertilizers, leading to their reduced abundance (Hyde et al.,

2013). Likewise, the decline in Basidiomycota, a diverse class encompassing pathogens to mutualists, might indicate that fertilizers alter soil conditions in ways that are less favourable to these fungi, potentially disrupting their ecological roles (Taylor et al., 2015b).

Fungal taxa within the classes Leotiomycetes and Sordariomycetes exhibited mixed responses to biosolids treatments, reflecting the complexity of fungal community dynamics in response to organic amendments. Within these classes, the enrichment of ASVs from the orders Erysiphales, Microascales, and Xylariales across control plots suggests that these fungi may be linked to low soil pH and are active in decomposing complex organic materials under natural conditions (Johnston et al., 2019; Liang et al., 2021; Samaradiwakara et al., 2023; Taylor et al., 2015a). On the other hand, the depletion of ASVs of the orders Helotiales and Myrmecridiales in control plots may indicate that these fungi are more specialized and less adaptable to stable environments with few disturbances. These nuanced responses suggest that while biosolids can selectively enrich or deplete certain fungal taxa, the impact on community structure could depend on the functional attributes of the fungi involved. Similar patterns of selective influence by organic amendments have been noted by Harris et al. (2021), emphasizing the need for balanced strategies that support diverse fungal functions in post-mining reclamation sites.

**Impacts of biosolids on antimicrobial resistance gene prevalence**

The detection of ARGs in biosolids-treated plots in this study highlights important considerations for biosolids use in mining reclamation, as it may raise concerns about the potential for spreading antibiotic resistance (AR) in the environment. It should be noted, however, that some of the ARGs detected are also prevalent in unamended soils, possibly due to their association with naturally occurring or pre-existing AR microbial populations in the soil (Davies and Davies, 2010; Evans and Amyes, 2014; Poirel et al., 1999; Zhu et al., 2019).

Among 23 targeted ARGs, 11 (*aadA1*, *ermB*, *ermC*, *imp5*, *mefA*, *oxa51*, *oxa54*, *oxa60*, *sfc1*, *tetA*, and *VEB*) were detected in at least one biosolids-treated

plot (100 Mg/ha or 250 Mg/ha), and 7 were detected in both biosolids treatments (*aadA1*, *ermB*, *ermC*, *mefA*, *oxa51*, *oxa54*, and *VEB*). These results align with research by Chen et al. (2016) and Qin et al. (2022), which highlights that biosolids treatments increase the abundance of ARGs in soil ecosystems, particularly genes conferring multidrug resistance and resistance to aminoglycosides, β-lactams, and tetracyclines. The persistence of ARGs in soils, even years after biosolid application, as seen in this study, also underscores their durability in soil environments.

Five ARGs (*ermC*, *imp5*, *oxa51*, *oxa60*, and *tetA*) were uniquely detected in biosolid-treated plots but absent in unamended (0) or fertilizer-treated soils (F), indicating the role of biosolids as a vector for introducing resistance genes. This observation is consistent with work by Ondon et al. (2021) and Vikesland et al. (2017), who report that biosolids amendments contribute novel ARGs to soil microbiomes, and increase the abundance of ARGs in soil microbial communities. It is also consistent with the findings of Law et al. (2021), who hypothesized that biosolids amendments could be a pathway that leads to the spread of ARGs, via horizontal gene transfer. In their 2021 study, Law et al. identified six distinct resistance plasmids, with two including *tetA*, that could be successfully transferred to pathogenic or commensal bacteria.

Furthermore, the significant impact of biosolids concentration on ARG prevalence, specifically in the 250 Mg/ha plots, parallels findings by Qin et al. (2022), who observed a significant positive correlation between the dose of biosolids applied and ARG abundance in agricultural fields. Exposure to biosolids may also be correlated with the emergence and proliferation of ARGs in indigenous microbiota, per Udikovic-Kolic et al. (2014) and Berendonk et al. (2015), again demonstrating the complex ecological implications of biosolids amendments. These findings highlight a potential threshold effect, where higher concentrations of biosolids amplify the dissemination and persistence of ARGs within an environment's soil microbiome.

While biosolids provide numerous benefits for soil fertility and microbial activity, the organic amendments may also promote conditions conducive to

spreading AR by increasing microbial biomass and activity (Heuer et al., 2011). The detection and differential prevalence of ARGs in soils treated with biosolids underscores the need for careful management and monitoring of their applications. Future reclamation strategies should consider the potential for ARG dissemination and explore mitigation strategies, such as the use of ARG monitoring, to minimize their impact on reclaimed ecosystems.

## CONCLUSION

This study provides valuable insights into the profound impacts of biosolids on soil physicochemical properties, microbial communities, and the prevalence of ARGs in the context of postmining experimental reclamation sites. The findings demonstrate that biosolids are effective in enhancing soil ecosystems, with regard to vegetation properties and carbon and nitrogen percent, and that while biosolids can also enrich specific microbial taxa, the responses are varied and context dependent. Thus, the strategic use of biosolids, with attention to dosage, could optimize their benefits and potential to help maintain a diverse and functional microbial community that supports ecosystem resilience and soil health while mitigating potential negative impacts. Future research should continue to explore optimal biosolids application rates, as well as strategies to mitigate the spread of ARGs and reduce other potential risks, as their detection in this study highlights important considerations for biosolids' use in mining reclamation. Optimization would ensure that biosolids can be used safely, sustainably, and effectively in the restoration of disturbed landscapes post-mining.

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# Chapter 3. Integration of genomic tools in mine reclamation at Mount Milligan Mine: Soil microbial community potential and invertebrate community characterization

## INTRODUCTION

Mine reclamation is a critical phase in the life cycle of extractive operations, with growing expectations from stakeholders, such as governments, First Nations, and the public, that post-closure landscapes support functional, self-sustaining ecosystems. In British Columbia, these expectations often include the re-establishment of native plant communities, habitats for wildlife, and conditions that provide opportunities for traditional land uses, such as hunting, gathering, and recreation. To meet these goals, mining companies must meet technical closure criteria and demonstrate that their reclamation efforts are achieving ecological recovery in a meaningful and measurable way (Mines Act, 2025).

Unfortunately, traditional monitoring programs are often limited by the slow pace at which vegetation communities and soil properties develop post-mining. While essential, these indicators may take years or decades to demonstrate conclusive trends, delaying interventions and adaptive management. In contrast, microbial and invertebrate communities respond more rapidly to changes in their surrounding environments and have the potential to serve as early indicators of reclamation success. Despite this, microbes and invertebrates remain underutilized in reclamation assessment frameworks.

Given their roles in nutrient cycling, organic matter decomposition, and soil structure development, soil microbial communities are among the first to respond to reclamation efforts (Wang et al., 2021; Liu et al., 2022; Peddle et al., 2022; Rawat et al., 2022). As such, they may be ideal to serve as both drivers and indicators of ecosystem development. Numerous studies have shown that bacterial diversity and composition change predictably with reclamation age, gradually converging toward reference ecosystem profiles (Ji et al., 2022; Li et al., 2022; Singh et al., 2024). Fungal communities also exhibit sensitivity to reclamation processes, though their dynamics differ from those of bacteria; recent research suggests that while fungal

richness may recover quickly under certain treatments, community composition may remain distinct from reference sites for decades (Gorzelak et al., 2020; Ji et al., 2022; Singh et al., 2024).

In addition to microbial indicators, invertebrates provide a complementary, and sometimes overlooked, window into ecosystem recovery. Invertebrate communities play essential roles in soil aeration, organic matter decomposition, and food web support (Bagyaraj et al., 2016; Griffiths et al., 2021). Their abundance and diversity are sensitive to substrate availability, vegetation development, and organic matter content, making them valuable indicators of ecological function limitations (Majer et al., 2002; Neher et al., 2012; Perry & Herms, 2019; Silva-Monteiro et al., 2022). While invertebrates have long been used in monitoring programs, their inclusion in reclamation assessment remains limited in North America (Andersen, 2002).

**Progressive reclamation at Mount Milligan Mine**

Centerra Gold Inc.'s Mount Milligan Mine (MtM), located between Fort St. James and Mackenzie, British Columbia, Canada, is implementing progressive reclamation of the mine site during operations and, as part of this program, is engaged in reclamation research trials to identify the site preparations (i.e., soil placement) and native vegetation species suitable for large-scale reclamation of the mine post-closure. Guided by the values and priorities of the local First Nations, McLeod Lake Indian Band and Nak'azdli Whut'en, this innovative reclamation research uses lessons learned from reclamation at the Kemess and Endako Mines to develop and implement a Proof of Concept (POC) reclamation prescription on the Tailings Storage Facility (TSF) South Berm at MtM. Specifically, this POC reclamation area is informed by the 5-Year Conceptual Reclamation Plan for MtM (Mount Milligan, 2019) and incorporates site preparation treatments from Kemess and Endako Mines, with ongoing monitoring to track the success of the reclamation through time occurring in years 1, 2, 5, 10, and 20 post-treatment; the monitoring consists of soil, vegetation, and wildlife use monitoring, in addition to the invertebrate and soil bacterial and fungal community monitoring that are the focus of this paper.

Site preparation activities were completed for the POC reclamation area in fall 2020, and native vegetation species were planted in 2021.

Reclamation research at MtM focuses on restoring lands impacted by mining to a productive biological condition and in a way that protects downstream aquatic resources and adjacent wildlife habitat, and to create a landscape that requires minimal post-closure monitoring and maintenance (Mount Milligan Mine, 2019). Reclamation efforts are underway across five treatment units within the POC reclamation area, which provides a unique opportunity to evaluate the potential of microbial and invertebrate communities as early indicators of reclamation success. This study aims to use metabarcode sequencing to profile bacterial, fungal, and invertebrate communities in an effort to compare diversity, and community composition across reclamation and reference site types. In addition, this research will attempt to identify specific bioindicator taxa, using linear discriminant analysis effect size (LEfSe). Finally, DNA extraction and size-selection protocols are being evaluated with the goal of obtaining a high concentration of high molecular weight DNA for long-read Oxford Nanopore metagenome sequencing; the most up-to-date protocols, which support modelling the functional profiles of the metagenomes and identifying genes of interest, are included in this thesis.

This work contributes to the evidence base supporting the inclusion of microbial and invertebrate indicators in reclamation frameworks, ensuring that post-mining landscapes are both stable and ecologically functional. Ultimately, by integrating DNA-based data with ecological recovery, this research aims to provide actionable insights for monitoring mine reclamation.

## METHODOLOGY

### Site description

The Mount Milligan Mine (MtM), operated by Centerra Gold Inc., is a conventional truck-shovel open pit copper and gold mine located 155 km northwest of Prince George in British Columbia, Canada. As part of the ongoing reclamation research at MtM, being implemented by Chu Cho Environmental (CCE), monitoring data from reclamation units, reference ecosystems, and bare ground (i.e., soil

placed for reclamation but not yet planted) sites are used to examine whether microbial and invertebrate communities can be used as early indicators of ecological recovery.

The majority of monitoring for this project was completed within the Tailings Storage Facility (TSF) South Berm Proof of Concept (POC) reclamation area, which consists of five treatments: Waterbars – Low, Waterbars – Medium, Waterbars – High, Hydroseed, and Rough and Loose (Figure 1.3). For Waterbars treatments, the water bar channels were installed horizontal to the slope to a depth of 20–30 cm, every 10 m, vertically, through back blading with a dozer as it worked up to the toe of the slope. Hydroseeding was carried over a 1.19 ha area using a 4,500 L hydroseeder; the application rate was 3038 kg/ha mulch, 39 kg/ha fall rye, and 14 kg/ha of the native seed mix. Finally, the Rough and Loose treatment implemented an excavator with a digging bucket to excavate holes to a depth of 0.4–0.8 meters below ground surface, depending on the depth of overburden. The excavated holes were 1.2 m wide and ranged from 2–2.5 m in length, with 1.2 m spaces between holes. Excavated material was mounded downslope of the hole with the front lightly packed and the back material, on the downslope side of the mound, loose. Year 1 post-treatment monitoring of the POC reclamation area occurred in 2022, with systematic monitoring of the soil, vegetation, and wildlife use of the area, and a pilot project to monitor invertebrate communities initiated. Year 2 post-treatment monitoring occurred in 2022, focused on the same elements as above with the addition of soil microbial and fungal community monitoring.

Within each POC treatment unit, three 400 $m^2$ long-term Ecological Monitoring and Assessment Network (EMAN) plots have been established. The EMAN protocol is a methodology developed for the long-term monitoring of vegetation, including species richness, abundance, and diversity (Roberts-Pichette & Gillespie, 1999). Plots 1–3 are in the in the Waterbars – Low treatment unit, plots 4-6 in the Waterbars – Medium treatment unit, plots 7-9 in the Waterbars – High treatment unit, plots 10-12 in the Hydroseed treatment unit, and plots 13-15 in the Rough and Loose treatment unit.


Figure 1.3. The Tailings Storage Facility (TSF) South Berm Proof of Concept (POC) reclamation area, consisting of Waterbars – Low, Waterbars – Medium, Waterbars – High, Hydroseed, and Rough and Loose treatment units.

In addition to the TSF South Berm POC reclamation area, monitoring was also undertaken in reference ecosystems, selected to represent both naturally and anthropogenically disturbed conditions within comparable ecological zones. Specifically, in 2022, these included two regenerating forestry disturbed sites (referred to as “Cut” sites), with one in the Engelmann Spruce – Subalpine Fir Moist Very Cold (ESSFmv3) Biogeoclimatic Ecosystem Classification (BEC) Zone (6-years post-disturbance) and the other in the Sub-boreal Spruce Moist Cool (SBSmk1) BEC Zone (4-years post disturbance), and two regenerating wildfire-disturbed sites (referred to as “Burn” sites) both in the SBSmk1 BEC Zone (both 5-years post-disturbance). Furthermore, in 2024, monitoring was conducted at an additional eight reference sites in the SBSmk1 ecosystem type, at four regenerating forestry disturbed sites and four regenerating wildfire disturbed sites (Figure 2.3); all eight of these sites were 1-year post-disturbance at the time of monitoring.


Figure 2.3. The four regenerating forestry disturbed and four regenerating wildfire disturbed reference sites monitored in 2024, in relation to Mount Milligan Mine. All reference sites monitored in 2024 were 1-year post-disturbance.

Finally, invertebrate monitoring also occurred at one bare ground control location in 2022—an area on the TSF Southeast Berm, where earthworks had been completed but no planting had yet occurred.

**Monitoring timelines**

Invertebrate monitoring was conducted over a three-year period, from 2022 to 2024. During 2022, the pilot project invertebrate monitoring included two intervals, from June 20–25 and July 18–31, to refine the timing for monitoring efforts, and occurred on both the TSF South Berm POC (1-year post-treatment at the time of monitoring) and in the ESSF Cut 1, SBS Cut 1, SBS Burn 1 and SBS Burn 2 reference sites. The malaise trap and pitfall traps deployed in June 2022 on the TSF South Berm POC Waterbars – Medium treatment unit, ESSF Cut 1 reference site, and SBS Burn 1 reference site had low capture rates and thus were not submitted for further analyses. In contrast, the July 2022 invertebrate monitoring was completed in the TSF South Berm POC reclamation area, as well as in four reference sites (ESSF Cut 1, SBS Cut 1, and SBS Burn 1 and 2) and one bare ground control site and yielded sufficient data. Based on the 2022 results, in 2023, invertebrate monitoring only occurred from July 14–21 to better coincide with peak invertebrate abundance. That year, monitoring was completed on the TSF South Berm POC reclamation area. Finally, in 2024, invertebrate monitoring took place from July 16–24 in eight reference sites—SBS Cut 3, 4, 5, and 6, and SBS Burn 3, 4, 5, and 6; all reference sites monitored in 2024 were 1-year post-disturbance.

Soil microbial community monitoring spans two years, from 2023 to 2024, coinciding with the dates and locations of invertebrate monitoring in those years. In 2023, soil monitoring was conducted at all fifteen EMAN plots established on the TSF South Berm POC, and in 2024, soil monitoring occurred at the same eight reference sites in the SBSmk1 ecosystem type.

**Flying invertebrate monitoring**

Malaise traps (MegaView Science Co. Ltd.) were deployed for the collection of flying invertebrates (Foster et al. 2020; Gervan et al. 2020; Lynggaard et al.

2020), with a collection bottle of 87% (v/v) denatured ethanol as the preservation medium. Each year, crews deployed one malaise trap per reclamation unit, reference ecosystem, or bare ground site, in the approximate center to minimize potential edge effects. Malaise traps were deployed for 4-day periods. After each period, the collection vessel was removed, sealed, and stored in a freezer until they were transported on dry ice to TRU for genetic analysis.

**Terrestrial invertebrate monitoring**

Pitfall traps for ground-dwelling arthropod collection were constructed using 450 g containers (Solo® cups), buried such that the rims were flush with the ground. For each trap, a hole approximately 12 cm deep was dug, and a plastic Solo® cup was inserted and capped with a paper plate staked in place, leaving approximately 1 cm clearance between the ground surface and the plate. The plates acted as lids to exclude debris and minimize the risk of bycatch (Bassett and Fraser 2014).

On steeply sloped sites, such as those along the TSF reclamation units, transects were placed diagonally across the slope so that pitfall traps were on lower, middle, and upper slopes. Each year, crews deployed ten pitfall traps per reclamation unit, reference ecosystem, or bare ground site. Pitfall traps were deployed for 4-day periods, and when the Solo® cup was removed, any captured invertebrates were transferred into 25 mL test tubes. The tubes contained ethanol and were stored in a freezer until they were transported on dry ice to TRU for genetic analysis. Upon removal of the pitfall traps, all holes were backfilled.

**Soil microbial community monitoring**

At each site, a hand trowel was sanitized with an ethanol wipe and then used to dig a sampling hole, taking care to avoid material, such as the desert crust, falling into the hole. With gloved hands, a sanitized sampling spoon was used to sample soil into an aseptically opened 7.5x15 cm Whirl-Pak bag for each depth point. Each Whirl-Pak bag was filled, taking care to avoid rocks and larger organic components, before it was sealed and massaged to homogenize the sampled soil. Using a sterilized spoon, the homogenized sample was used to fill a 50 mL sterile

polypropylene tube for future DNA extractions. Once collected, the 50 mL tubes, as well as the remaining soil in the Whirl-Pak bags, were immediately stored in a cooler in the field. Each hole was filled and made to resemble the surrounding area to minimize disturbance and prevent a potential safety hazard for other crew members working in the area. Between sites, the tools were sterilized, and gloves were changed to avoid contamination between samples. Samples from each field day were stored in a chest freezer on site at Mount Milligan, then transferred to a deep freeze with dry ice, before they were packed with dry ice and transported to TRU for genetic analysis.

**Monitoring data analysis**

*Invertebrate sample preparation*

Upon arrival at TRU, samples were transferred to a -20ºC laboratory freezer. To standardize the amount of tissue analyzed from a single trap, specimens larger than 5 mm were decapitated, and the heads, along with specimens <5 mm, were homogenized in liquid nitrogen using sterilized mortar and pestles (Beng et al. 2016; Gervan et al. 2020). Following preparation, the homogenized invertebrate mixtures were transferred to labelled 2 mL or 15 mL sterile tubes, depending on the volume of material present, and stored at -80°C until DNA extraction.

*CO1 library preparation and sequencing*

To extract total genomic DNA from the homogenates, a KingFisher Duo Prime DNA extraction robot was used in combination with reagents from the MagMAX DNA Multi-Sample Ultra 2.0 Kit (Applied Biosystems) and MagMAX DNA Cell and Tissue Extraction Buffer (Applied Biosystems). These methods were used previously for invertebrate DNA extractions (Foster et al. 2020; Gervan et al. 2020). Following extraction, quality control (QC) was performed by measuring the DNA concentration and quality of each sample via a fluorometric assay (Quant-iT dsDNA HS Assay Kit; Thermo Fisher Scientific), gel electrophoresis, or an Agilent Bioanalyzer. The DNA was stored at -20°C until further processing.

Polymerase chain reaction (PCR) was used to amplify the DNA region of interest for metabarcode sequencing—a 402-base-pair region of the mitochondrial cytochrome c oxidase subunit one gene (CO1 gene)—from each extracted sample, using 10 µM primers MHemF (5-GCATTYCCACGAATAAATAAYATAAG) and dgHCO-2198 (5-TAAACTTCAGGGTGACCAAARAAYCA) (Meyer 2003; Park et al. 2011). PCR mixtures included 12.5 µL GoTaq MasterMix, 1 µL MHemF, 1 µL dgHCO-2198, 10 ng DNA sample, and PCR-grade water to bring the total reaction volume to 25 µL. Thermocycling conditions were 94°C for one minute, seven cycles of 94°C for 30 seconds, 43°C for 30 seconds, and 72°C for 40 seconds, followed by 30 cycles of 94°C for 30 seconds, and 55°C for 30 seconds. Low molecular weight DNA (fragments < 100 base-pairs) was removed using AMPure XP Reagent (Beckman Coulter) and a DynaMag 96 Side Magnet (Invitrogen), following the manufacturer's instructions. The DNA concentrations of each purified sample were measured using fluorometry, and the DNA amplicons were visualized on an agarose gel.

A second round of PCR was used to add IonXpress barcodes and P1 adapters for sequencing on an IonS5 system (Beng et al. 2016; Foster et al. 2020; Gervan et al. 2020). The reaction mixtures included 12.5 µL of GoTaq MasterMix, 1 µL of MHemF XP, 1 µL dgHCO-2198 P1adapt, 10 ng of DNA sample, and PCR-grade water to bring the total reaction volume to 25 µL. Thermocycling conditions were 94°C for one minute, followed by 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 40 seconds, then finished with 72°C for 5 minutes. Again, low molecular weight DNA was removed, DNA concentrations were measured, and DNA amplicons were visualized by gel electrophoresis. Purified samples were stored at -20°C.

Barcoded amplicons were pooled based on relative DNA concentrations, separated by gel electrophoresis, and gel-purified using a MicroElute Gel Extraction Kit (Omega Bio-Tek), following the manufacturer's instructions. The concentration of barcoded DNA in each pool was determined by quantitative PCR using an Ion Library TaqMan Quantitation Kit (Applied Biosciences) on a QuantStudio 3 qPCR instrument (Applied Biosciences), after which each pool of samples was diluted

accordingly, to represent samples equally in terms of total mass, and combined into one tube. Sequencing libraries were templated onto sequencing beads and loaded onto Ion 530 sequencing chips using an Ion Chef and an Ion 510 & Ion 520 & Ion 530 Chef Reagents kit and sequenced with an Ion S5 XL system using 400 base pair Ion Semiconductor sequencing chemistry. Samples collected in 2022 were re-sequenced in 2023.

*16S rRNA gene and ITS region library preparation and sequencing*

A workflow similar to what is described for CO1 library preparation, quality control, and sequencing was followed for 16S rRNA gene region and ITS region data. Soil samples were collected aseptically from the top 10 cm, stored on dry ice in the field, and shipped frozen to TRU for nucleic acid extraction. Upon arrival, samples were transferred to the laboratory freezer and stored at -80°C. 16S rRNA gene and ITS region metabarcode libraries were prepared using 341F/806R and gITS86F/ITS4r primer pairs, respectively (Gorzelak et al. 2020).

To extract total DNA from all soil samples, a MagAttract PowerSoil DNA KF Kit (Qiagen) was used in combination with a FastPrep-24 bead beater and KingFisher Duo Prime extraction robot, following the manufacturer's instructions. DNA was quantified using fluorometry before storage at -20°C.

To amplify the 16S rRNA gene fragment, PCR was carried out using primers 341F (5'-TACGGGAGGCAGCAG) and 806R (5'-GGACTACVSGGGTATCTAAT). To amplify the fungal DNA region of interest, PCR was carried out using primers gITS86F (5'-GTGARTCATCGARTCTTTGAA) and ITS4r (5'-TCCTCCGCTTATTGATATGC). PCRs consisted of 10μL GoTaq MasterMix, 0.5 μM primers, 10 ng DNA sample, and PCR-grade water to bring the total reaction volume to 20 μL. Thermocycling conditions were 95°C for four minutes, 25 cycles of 95°C for 30 seconds, 53.4°C for 45 seconds, and 72°C for two minutes, followed by 72°C for five minutes. Non-specific low molecular weight DNA was removed after the first round of PCR, as in the CO1 methodology, and quality control was performed before storage in labelled 2 mL tubes at -20°C.

During a second round of PCR, unique sequencing barcodes were added to each sample. Reactions consisted of 10 µL GoTaq MasterMix, 0.5 µM primers, 10 ng DNA sample, and PCR-grade water to bring the total reaction volume to 20 µL. Thermocycling conditions were 95°C for four minutes, 20 cycles of 95°C for 30 seconds, 65°C for 45 seconds, and 72°C for two minutes, followed by 72°C for five minutes. Again, non-specific low molecular weight DNA was removed, and quality control was performed before storage at -20°C.

Barcoded amplicons were pooled based on relative DNA concentrations, separated via gel electrophoresis, and amplicons were purified with a GeneJET Gel Extraction Kit (ThermoFisher), following the manufacturer's instructions. Purified pool concentrations were quantified, diluted, and then combined before the libraries were sequenced, all as in the CO1 methodology.

**Metagenomic library preparation and sequencing**

*DNA extraction*

To extract high molecular weight DNA from homogenized soil samples, a MagAttract® PowerSoil® DNA KF Kit (Qiagen) is being used. To begin, 0.5 g from each sample is weighed into separate, labelled bead tubes using flame-sterilized scoopulas. Next, 750 µl of PowerBead Solution and 4 µl of RNase A (1 mg/mL) is added. Then, 60 µl of SL Solution is added to each sample tube before vortexing for 60 seconds using a FastPrep-24™ classic bead beating grinder and lysis system (MP Biomedicals). Once vortexed, the manufacturer's kit instructions are followed for the remainder of the protocol. Upon completion of the KingFisher MO BIO PowerMag® Soil robotic program, the sample DNA is transferred out of the elution strip and placed in new labelled tubes to be stored at -20ºC.

*DNA size-selection*

To size-select DNA extracted from homogenized soil samples, a HighPrep PCR PB Kit (MagBio Genomics) is being used. Each sample is mixed with 35% (v/v) HighPrep PCR PB at a ratio of 3.1X to remove DNA <5 kb. Samples are mixed thoroughly 10–15 times using wide-bore pipette tips and then incubated at room

temperature for 15 minutes. After incubation, the manufacturer's kit instructions are followed, and 10 mM Tris-HCl (pH 8.5-9.0) is used as an elution buffer.

**Data processing and statistical analyses**

Before analyses were completed, sequencing data were quality filtered to Q20 using onboard Torrent Suite software on the IonS5 XL system. Then, raw sequence data were demultiplexed in AMPtk 1.5.5 using the amptk ion script with default settings and --trim-len set to 350 bases. Demultiplexed data were imported into Qiime 2 with the qiime tools import script set with --input-format SingleEndFastqManifestPhred33V2 (Bolyen et al., 2019). Denoising was done with DADA2 denoise-single with max-ee set to 1.0 (p-trunc-len set to 0 because the reads were previously trimmed in AMPtk) (Callahan et al., 2016). For bacteria, the q2-feature-classifer classify-sklearn script was used to assign taxonomy with a database trained using the qiime feature-classifier with the Greengenes2 database (version 2022.10) extracted with the 341f and 806r primer sequences listed above (Bokulich et al. 2018; McDonald et al., 2024; McDonald et al., 2012). For fungi, the version 9 Qiime2-compatible UNITE reference database was downloaded and the dynamic "developer" version (sh_refs_qiime_ver9_dynamic_25.07.2023_dev.fasta and sh_taxonomy_qiime_ver9_dynamic_25.07.2023_dev.txt) was used to train the classifier using the qiime feature-classifier tool without extracting or trimming reads to the primer sites (Abarenkov et al., 2023). Finally, for CO1 data, SklearnClassifier_COins_QIIME2_v2023.5.qza, a database developed from all CO1 sequences available in the Barcode of Life Data System for insects and validated on previously published DNA-metabarcoding sequences data, was downloaded from https://figshare.com/articles/dataset/COins_database/19130465 and used for taxonomy assignment (Magoga et al., 2022).

Statistical analyses were completed using R version 4.5.0, and Qiime 2 2025.7 (Bolyen et al., 2019; R Core Team, 2025). Amplicon sequence variant (ASV) tables were imported into RStudio, and the 16S rRNA gene and ITS region data were rarefied (to 35183 for bacteria and 47119 for fungi) using the "phyloseq" ("BiocManager") package (McMurdie and Holmes, 2013; Bolyen et al., 2019), but the

CO1 data were not. Instead, the CO1 data were normalized using upper quartile normalization to reduce library size effects; raw sampling depths ranged from 106 to 442,599 reads per sample. For the rarified ASV tables, Shannon and Simpson diversity indices were calculated using the “vegan” (Oksanen et al., 2025) package in RStudio. Species richness was calculated for all three data types. The diversity indices and species richness values were then used to conduct analyses of variance (ANOVAs) for parametric data or Kruskal-Wallis tests for non-parametric data, as well as any necessary post-hoc tests to analyze diversity within the sites. Non-metric multidimensional scaling (NMDS) plots based on Bray-Curtis or Sørensen dissimilarity of the communities were prepared using the “vegan” (Oksanen et al., 2025), “ggplot2” (Wickham, 2016), and “viridis” packages (Garnier et al., 2024). Permutational multivariate analyses of variance (PERMANOVA) were performed in RStudio on the Bray-Curtis or Sørensen dissimilarity matrices. PERMANOVAs were conducted with the “adonis2” function from the vegan package (Oksanen et al., 2024), with separate one-way models specified as vegdist(rarefied_table, method = "bray") ~ Treatment and ~ Site Type, where “Treatment” and “Site Type” represented categorical variables from the experimental metadata. Each PERMANOVA was based on 999 permutations, and the “betadisper” function was employed to ensure homogeneous group dispersion. If the p-value of a PERMANOVA was < 0.05, a pairwise PERMANOVA using “pairwiseAdonis” (Arbizu, 2017) was run. Linear discriminant analysis effect size (LEfSe) was performed on raw counts transformed to relative abundance using the “lefser” (Khleborodova et al., 2024) and “SummarizedExperiment” (Morgan et al., 2025) packages to compare microbial and invertebrate taxa across reclamation and reference site types. Finally, for one partial metagenomic dataset that was obtained during method development, the taxonomy of the most abundant phyla and families were visualized in R, using the “SQMtools” package (Puente-Sánchez et al., 2020).

## RESULTS

Microbial community diversity was assessed across reclamation and reference site types using species richness, as well as Shannon and Simpson

diversity indices (Figure 3.3). Each boxplot displays the distribution across sites, with the central line representing the median value, the box encompassing the interquartile range (IQR), and the whiskers extending to the minimum and maximum values within 1.5x the IQR; points outside the whiskers indicate outliers. Lowercase letters indicate significant differences. In the bacterial communities, there were contrasting significant differences in Simpson diversity and species richness, as supported by the Wilcoxon rank-sum result for Simpson diversity ($p = 0.0031$) and the one-way ANOVA for species richness ($p = 0.011$), with reclamation sites having higher species richness but lower Simpson diversity (Figures 3.3A and 3.3E). There was no significant difference in Shannon diversity between the bacterial communities of the reclamation and reference site types (Figure 3.3C). Within the fungal communities, there were consistent significant differences in Simpson diversity, species richness, and Shannon diversity, indicated by Wilcoxon rank sum tests for the first two and one-way ANOVA for the last ($p = 0.0074$, $p < 0.001$, and $p < 0.001$, respectively); reclamation sites had higher species richness and diversity indices (Figures 3.3B, 3.3D, and 3.3F).

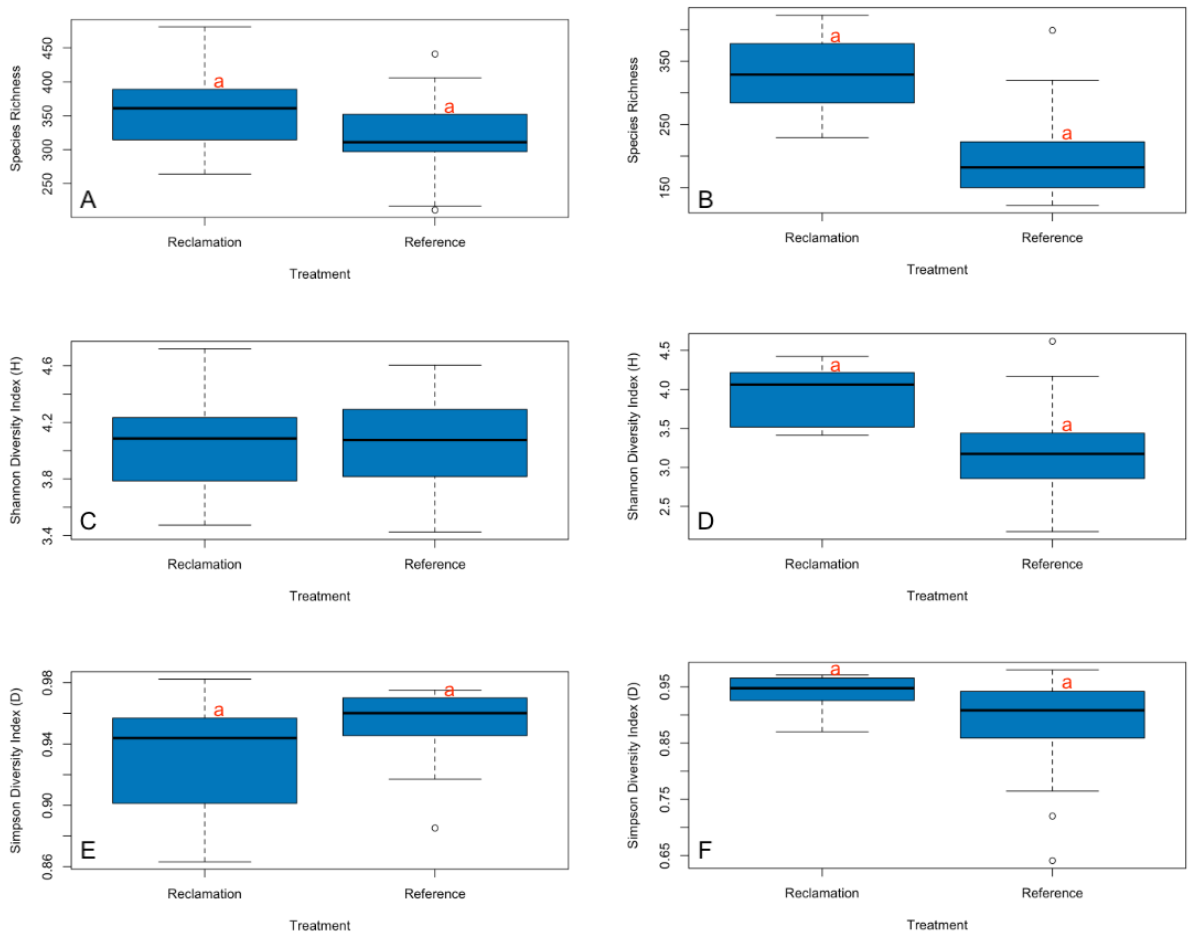


Figure 3.3. Microbial diversity across reclamation and reference sites in post-disturbance communities: (3.3A) Bacterial species richness; (3.3B) Fungal species richness; (3.3C) Bacterial Shannon diversity; (3.3D) Fungal Shannon diversity; (3.3E) Bacterial Simpson diversity; (3.3F) Fungal Simpson diversity. Lowercase letters denote significant differences of at least 0.05.

Microbial diversity was further analyzed using species richness, and Shannon and Simpson diversity indices across seven post-disturbance sites: Burn, Cut, Hydroseed, Rough and Loose, Waterbars – Low, Waterbars – Med, and Waterbars – High (Figure 4.3). Again, each boxplot displays the distribution across treatments, with the central line representing the median value, the box encompassing the interquartile range (IQR), and the whiskers extending to the minimum and maximum values within 1.5x the IQR; points outside the whiskers indicate outliers. Lowercase letters indicate significant differences.

For the bacterial communities, significant differences were observed in Simpson diversity via Kruskal-Wallis test ($p = 0.0031$), as well as Shannon diversity and species richness via one-way ANOVA ($p = 0.0049$ and $p = 0.0025$, respectively). A post-hoc Tukey's HSD tests revealed that Hydroseed sites had significantly higher bacterial species richness compared to Burn, Cut, and Rough and Loose sites (Figure 4.3A). There were also significant differences between the Burn sites and the Cut and Waterbars – Low sites, given the high Simpson diversity of the Burn sites (Figure 4.3E). There were, however, no specific significant differences in Shannon diversity found between any sites (Figure 4.3C).

Fungal communities also showed significant differences in Shannon diversity ($p < 0.001$) and species richness ($p < 0.001$) between sites, as demonstrated by one-way ANOVA and a Kruskal-Wallis test, respectively. A post-hoc Dunn's test indicated lower fungal species richness at Burn sites compared to every other kind of site, except Waterbars – Low (Figure 4.3B). A Tukey's HSD test revealed that Shannon diversity was significantly lower at Burn sites compared to Hydroseed, Rough and Loose, and Waterbars – Med sites (Figure 4.3D). There were no significant differences in Simpson diversity between the fungal communities of different site treatments (Figure 4.3E).

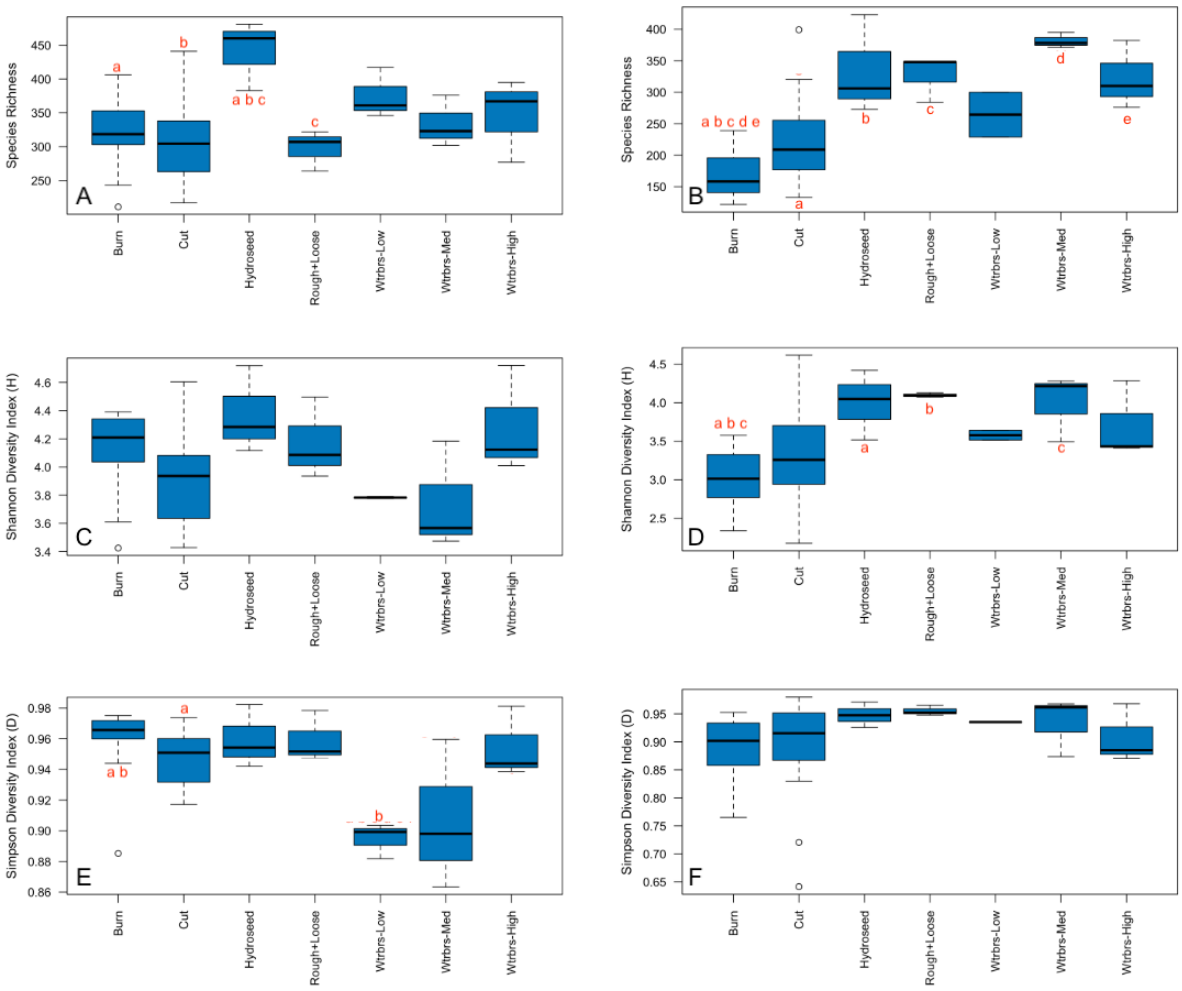


Figure 4.3. Microbial diversity across seven post-disturbance site treatments: (4.3A) Bacterial species richness; (4.3B) Fungal species richness; (4.3C) Bacterial Shannon diversity; (4.3D) Fungal Shannon diversity; (4.3E) Bacterial Simpson diversity; (4.3F) Fungal Simpson diversity. Lowercase letters denote significant differences of at least 0.05 between treatments.

Invertebrate diversity was examined by calculating species richness across control, reclamation, and reference site types' post-disturbance communities, as well as by site (Figure 5.3). Each boxplot displays the species richness across sites, with the central line representing the median value, the box encompassing the interquartile range (IQR), and the whiskers extending to the minimum and maximum values within 1.5x the IQR; points outside the whiskers indicate outliers. Lowercase letters indicate significant differences. There were no significant differences between the eight different site treatments, including control, but a Kruskal-Wallis test indicated a significant difference between the control, reclamation, and reference site types ($p = 0.038$); however, the post-hoc Dunn's test revealed no significant differences between specific site types.

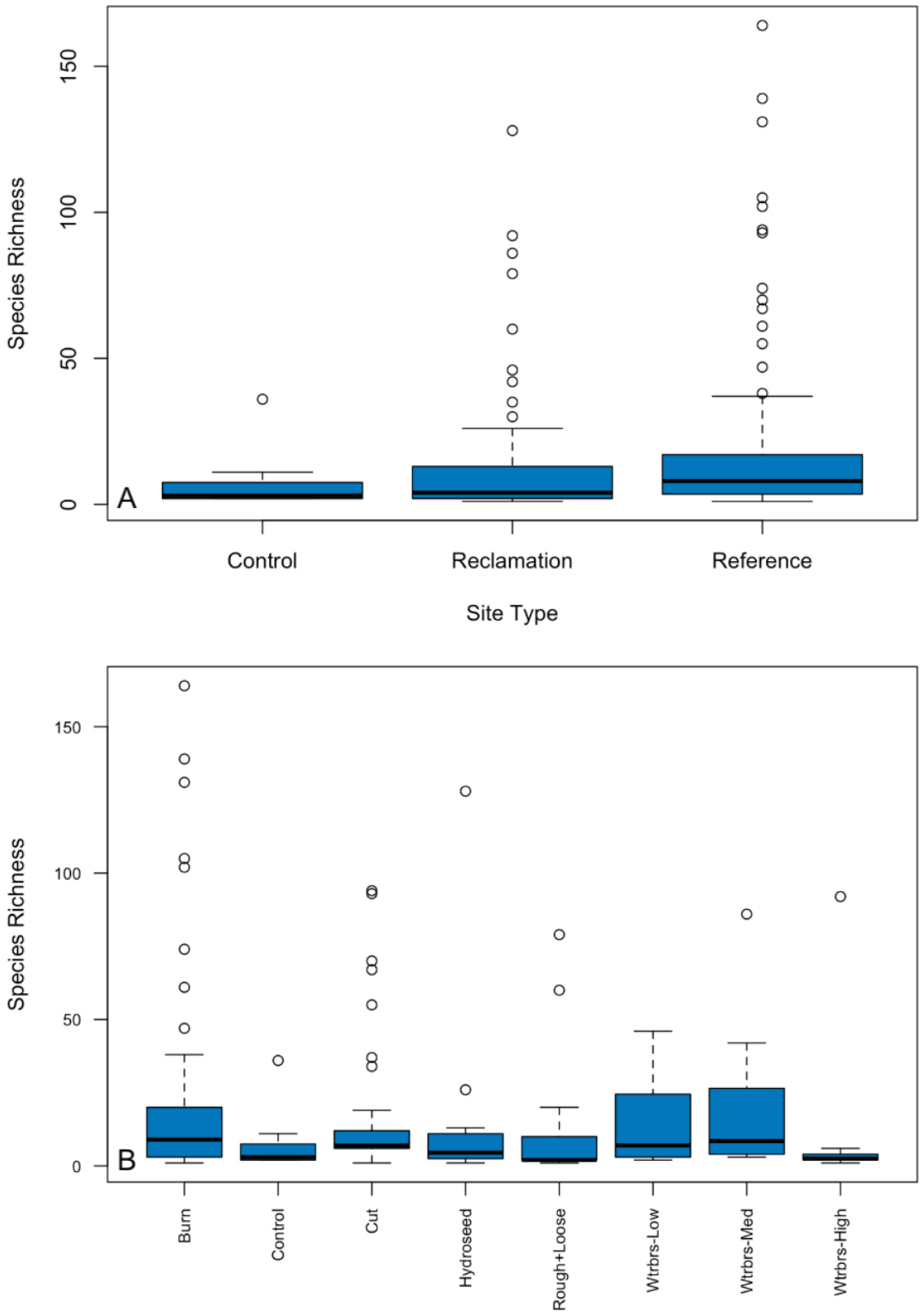


Figure 5.3. Invertebrate diversity across post-disturbance communities: (5.3A) Control, reclamation, and reference sites; (5.3B) Treatment, including control.

To compare the microbial communities at the Mount Milligan Mine, Bray-Curtis dissimilarity matrices were generated from rarefied amplicon sequencing data, and the NMDS scores were plotted (Figures 6.3 and 7.3). An NMDS score represents the relative position of a sample in a reduced-dimensional ordination space. Sites treated in similar ways can be found in close proximity, with clustering between reclamation and reference site types in both the bacterial and fungal plots. Pairwise PERMANOVAs (Tables B1 and B2) indicated that bacterial and fungal communities in Burn and Cut sites were distinct from each other ($p = 0.021$ for both comparisons), and that Burn and Cut sites were distinct from reclamation site types across 16/20 comparisons (Tables B1 and B2). A test for homogeneity of dispersion indicated a significant difference in group variance for fungi ($p < 0.001$). Site-level variation explained ~49.1% of overall variation in bacterial communities, and ~27.8% in fungal communities, suggesting strong site-driven differences. These patterns are consistent with the alpha diversity results, highlighting distinct microbial communities between reclamation and reference site types. Shrub percentage cover was typically highest across Cut sites and lowest across Burn sites, with reclamation sites falling in between. Vegetation scores follow a similar trend but exhibit broader variability in Burn and Cut sites. Overall, sampling depth does not appear to affect microbial communities with any discernible pattern.

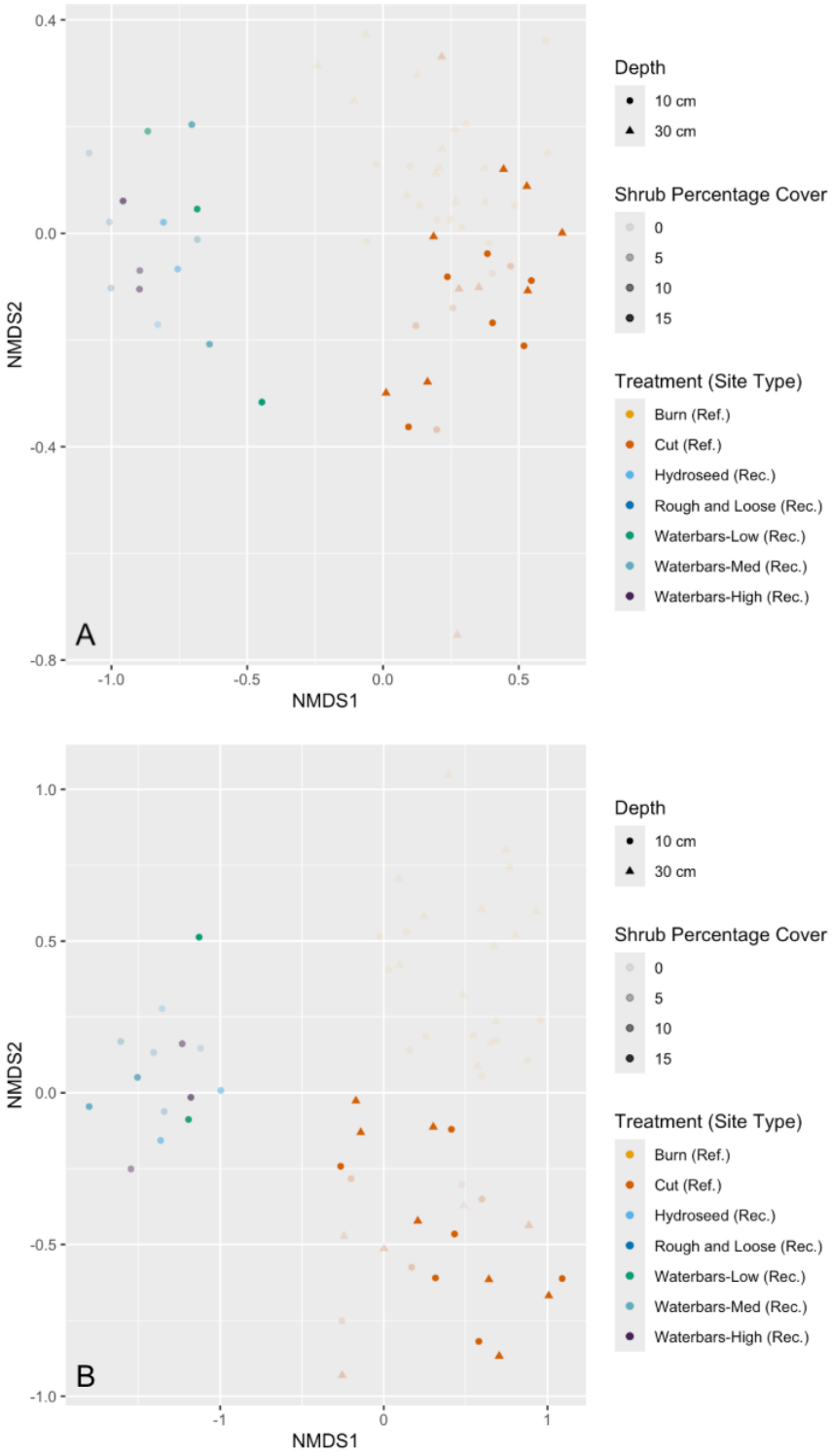


Figure 6.3. NMDS plots of microbial communities based on Bray-Curtis dissimilarity matrices from rarefied sequencing data: (6.3A) Bacterial communities with depth and shrub percentage cover; (6.3B) Fungal communities with depth and shrub percentage cover.

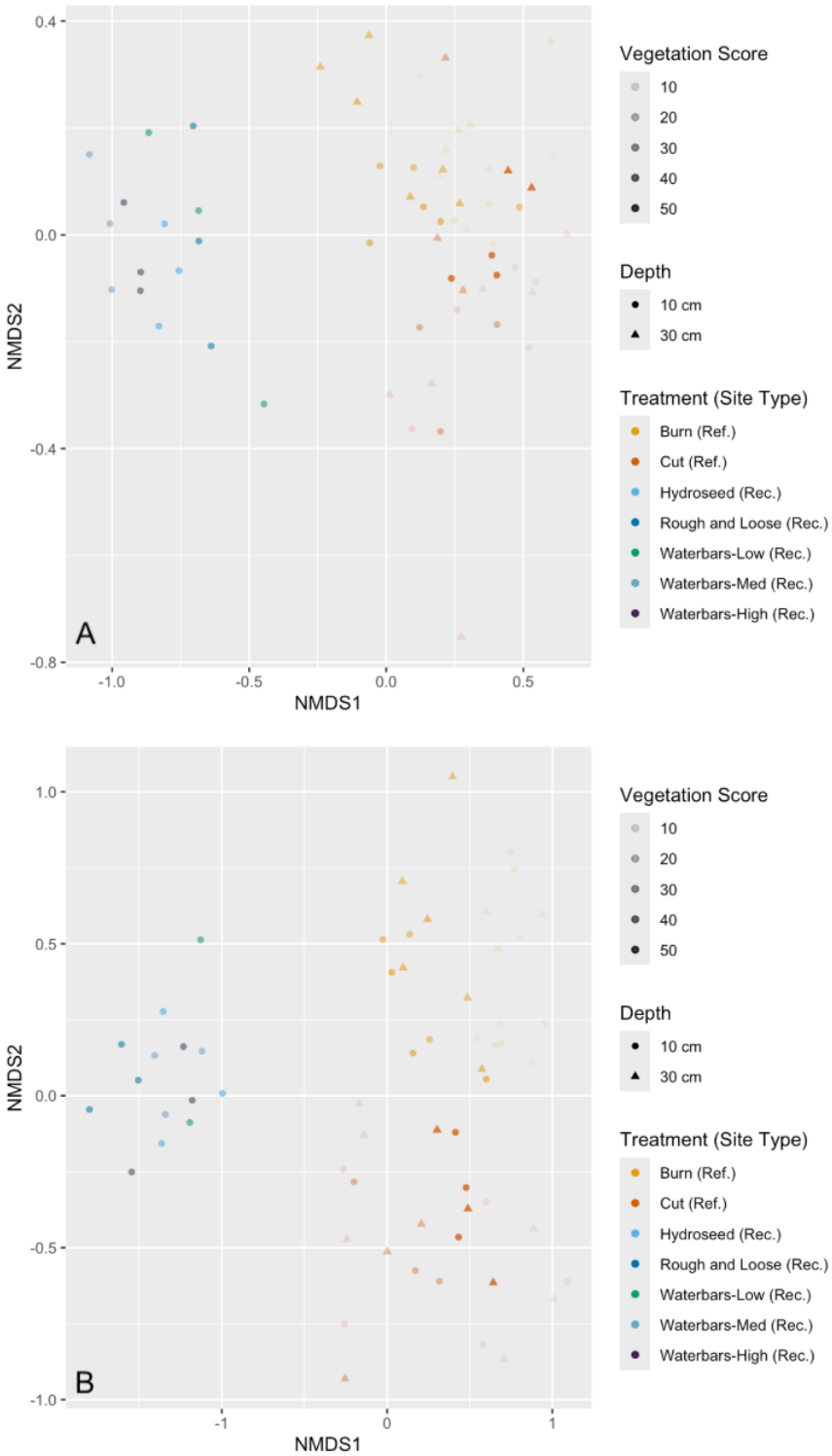


Figure 7.3. NMDS plots of microbial communities based on Bray-Curtis dissimilarity matrices from rarefied sequencing data: (7.3A) Bacterial communities with depth and vegetation score; (7.3B) Fungal communities with depth and vegetation score.

Invertebrate communities were also compared via NMDS scores, using Sørensen dissimilarity matrices of the CO1 sequencing data (Figures 8.3 and 9.3). An NMDS score represents the relative position of a sample in a reduced-dimensional ordination space. Again, there appears to be clustering between reclamation and reference site types. A pairwise PERMANOVA (Table B3) indicated that invertebrate communities in Burn and Cut sites were not quite distinct from each other ($p = 0.084$), or from Control sites, but that Burn and Cut sites were distinct from reclamation site types across 5/10 comparisons (Table B3). Site-level variation explained ~9.4% of overall variation, suggesting modest site-driven differences. The figures do not indicate any discernible patterns in shrub percentage cover, vegetation score, or herb percentage cover across site types. An additional PERMANOVA highlighted significant variation in invertebrate communities between trap types ($p < 0.001$), accounting for ~8.9% of overall variation.

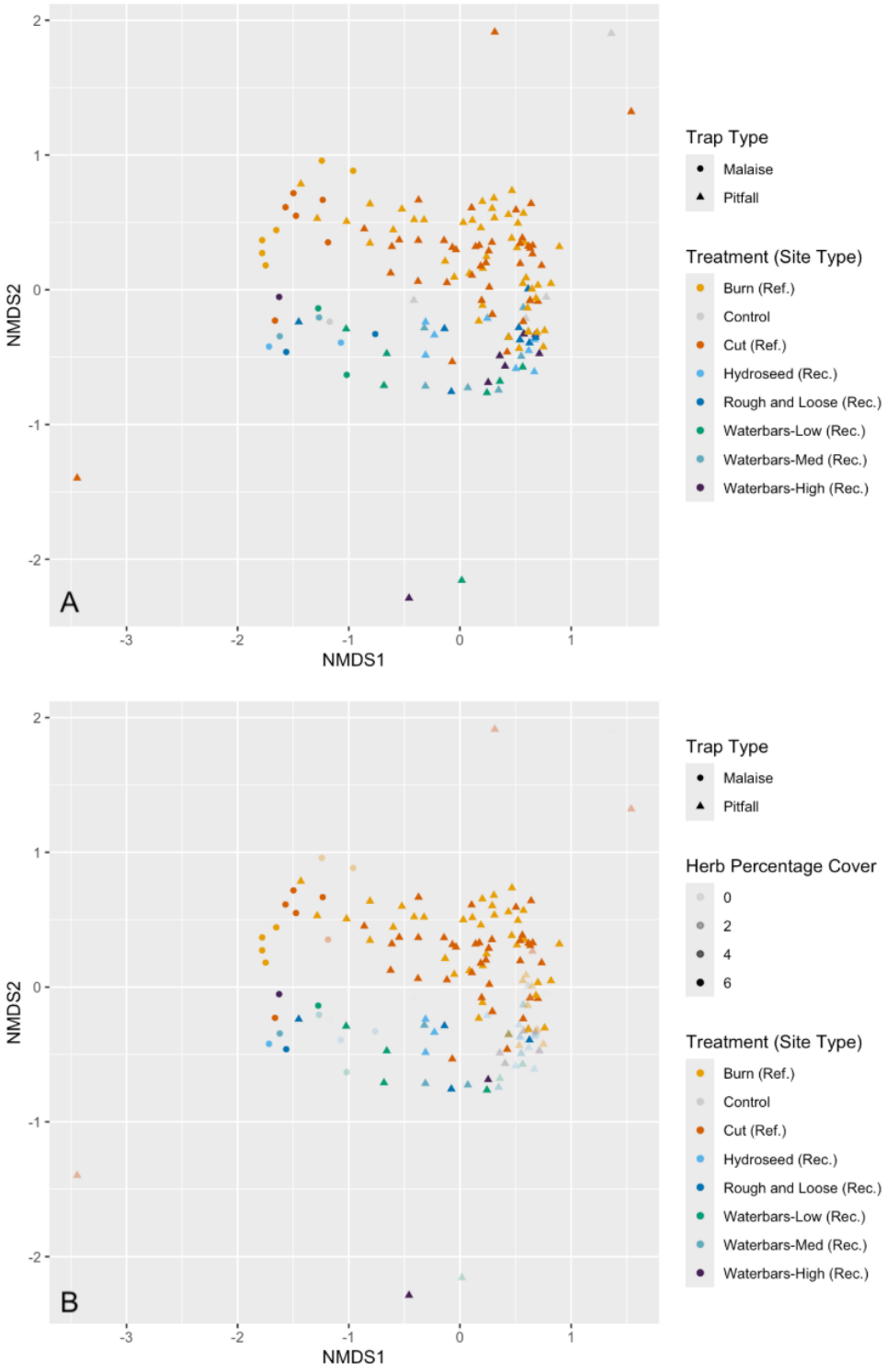


Figure 8.3. NMDS plots of invertebrate communities based on Sørensen dissimilarity matrices of CO1 sequencing data: (8.3A) Communities with trap type; (8.3B) Communities with trap type and herb percentage cover.

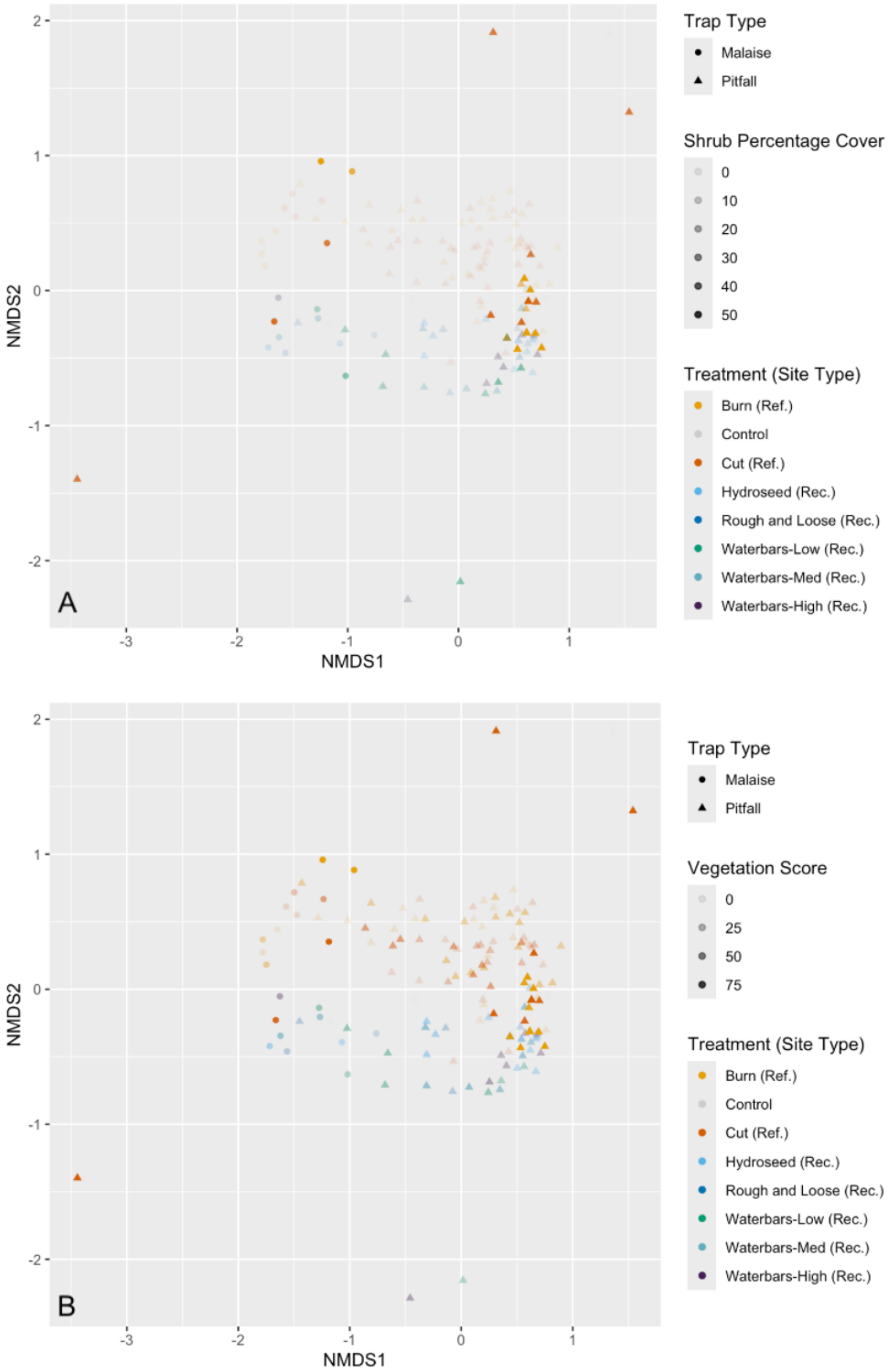


Figure 9.3. NMDS plots of invertebrate communities based on Sørensen dissimilarity matrices of CO1 sequencing data: (9.3A) Communities with trap type and shrub percentage cover; (9.3B) Communities with trap type and vegetation score.

To compare bacterial community composition by taxa across reclamation and reference site types, linear discriminant analysis effect size (LEfSe) was performed. The LEfSe analysis revealed several taxa that were significantly enriched in each group, with positive linear discriminant analysis (LDA) scores indicating features associated with reference (orange) and negative LDA scores indicating features associated with reclamation (green) site types (Figure 10.3). Notably, features such as *Bradyrhizobium* (family Xanthobacteraceae, order Rhizobiales, class Alphaproteobacteria, phylum Proteobacteria), *Dormibacter* (family Dormibacteraceae, order Dormibacterales, class Dormibacteria, phylum Dormibacterota), and Xanthobacteraceae (order Rhizobiales, class Alphaproteobacteria, phylum Proteobacteria) were strongly associated with reference site types, while features like Chloroflexota (phylum Bacteria), UBA2999 (order Vicinamibacterales, class Vicinamibacteria, phylum Acidobacteriota), and *Aestuariivirga litoralis* (family Aestuariivirgaceae, order Rhizobiales, class Alphaproteobacteria, phylum Proteobacteria) were strongly associated with reclamation site types. These results suggest distinct microbes characterize each site type.

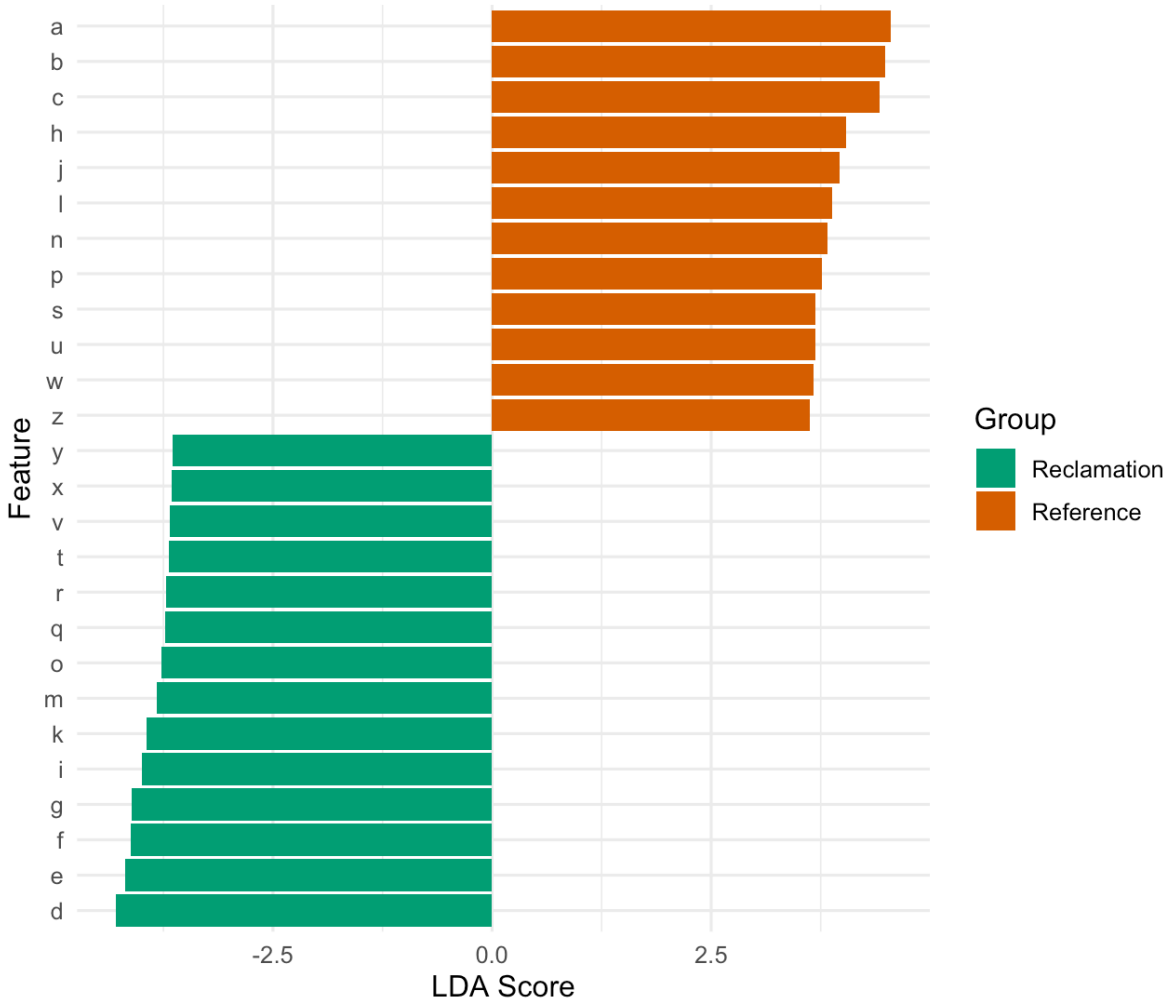


Figure 10.3. LEfSe results, presented as LDA score, from comparisons of the bacterial communities between the reclamation and reference site types. Labelled LEfSe results are presented in Table 1.3.

Table 1.3. Labelled LEfSe results from comparisons of the bacterial communities between the reclamation and reference site types.

| Label | Taxon | LDA Score |
|---|---|---|
| a | d__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales_A_504705.f__Xanthobacteraceae_503485.g__Bradyrhizobium.__ | 4.552335434 |
| b | d__Bacteria.p__Dormibacterota.c__Dormibacteria.o__Dormibacterales.f__Dormibacteraceae.g__Dormibacter.s__ | 4.486534994 |
| c | d__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales_A_504705.f__Xanthobacteraceae_503485.g__.s__ | 4.426105682 |
| d | d__Bacteria.p__Chloroflexota.__.__.__.__.__ | -4.294025532 |
| e | d__Bacteria.p__Acidobacteriota.c__Vicinamibacteria.o__Vicinamibacterales.f__UBA2999.g__.s__ | -4.191986315 |
| f | d__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales_A_504723.f__Aestuariivirgaceae.g__Aestuariivirga.s__Aestuariivirga.litoralis | -4.123670434 |
| g | d__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Sphingomonadales.f__Sphingomonadaceae.g__Sphingomicrobium_483265.__ | -4.113525405 |
| h | d__Bacteria.p__Acidobacteriota.c__Acidobacteriae.o__Acidoferrales.f__UBA7541.g__Acidoferrum.s__ | 4.037341122 |
| i | d__Bacteria.p__Acidobacteriota.c__Vicinamibacteria.o__Vicinamibacterales.f__UBA2999.__.__ | -3.990700667 |
| j | d__Bacteria.p__Actinobacteriota.c__Actinomycetia.o__Mycobacteriales.f__Mycobacteriaceae.g__Mycobacterium.__ | 3.969278151 |
| k | d__Bacteria.p__Actinobacteriota.c__Actinomycetia.o__Actinomycetales.f__Micrococcaceae.__.__ | -3.945644672 |
| l | d__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Dongiales.f__Dongiaceae.g__Hypericibacter.s__ | 3.879348621 |
| m | d__Bacteria.p__Chloroflexota.c__Chloroflexia.__.__.__.__ | -3.829548213 |
| n | d__Bacteria.p__Acidobacteriota.c__Acidobacteriae.o__Bryobacterales.f__Bryobacteraceae.g__Palsa.187.s__Palsa.187.sp902826605 | 3.825556578 |
| o | d__Bacteria.p__Acidobacteriota.c__Vicinamibacteria.o__Vicinamibacterales.f__UBA2999.g__WHSN01.s__WHSN01.sp902826465 | -3.768064028 |
| p | d__Bacteria.p__Acidobacteriota.c__Acidobacteriae.o__Acidoferrales.f__UBA7541.g__Acidoferrum.__ | 3.764653593 |
| q | d__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales_A_504721.f__Hyphomicrobiaceae.g__.s__ | -3.733428636 |
| r | d__Bacteria.p__Gemmatimonadota.c__Gemmatimonadetes.o__Gemmatimonadales.f__GWC2.71.9.g__JABFSM01.s__JABFSM01.sp009692115 | -3.715498791 |
| s | d__Bacteria.p__Actinobacteriota.c__Thermoleophilia.o__Gaiellales.f__Gaiellaceae.g__Gaiella.s__Gaiella.occulta | 3.69175294 |
| t | d__Bacteria.p__Chloroflexota.c__Dehalococcoidia.__.__.__.__ | -3.687481734 |
| u | d__Bacteria.p__Acidobacteriota.c__Acidobacteriae.o__Bryobacterales.f__Bryobacteraceae.g__.s__ | 3.684770922 |
| v | d__Bacteria.p__Actinobacteriota.c__Acidimicrobiia_401430.o__Acidimicrobiales.f__Ilumatobacteraceae.g__.s__ | -3.676676612 |
| w | d__Bacteria.p__Dormibacterota.c__Dormibacteria.o__CF.121.f__CF.121.g__CF.13.s__ | 3.670641956 |
| x | d__Bacteria.p__Actinobacteriota.c__Acidimicrobiia_401430.o__Acidimicrobiales.__.__.__ | -3.657738799 |
| y | d__Bacteria.p__Acidobacteriota.c__Vicinamibacteria.o__Vicinamibacterales.f__UBA2999.g__Gp6.AA45.s__ | -3.650267283 |
| z | d__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales_A_504705.f__Beijerinckiaceae.__.__ | 3.622676168 |

Fungal community composition by taxa across reclamation and reference site types was also analyzed by LEfSe, revealing several taxa that were significantly enriched in each group (Figure 11.3). Positive LDA scores indicate features like *Umbelopsis dimorpha* (family Umbelopsidaceae, order Umbelopsidales, class Umbelopsidomycetes, phylum Mucoromycota), with UNITE sequence ID SH0898191.09FU, *Inocybe* sp. (family Inocybaceae, order Agaricales, class Agaricomycetes, phylum Basidiomycota), with UNITE sequence ID SH1332890.09FU, and *Tricholoma portentosum* (family Tricholomataceae, order Agaricales, class Agaricomycetes, phylum Basidiomycota), with UNITE sequence ID SH1086723.09FU, were strongly associated with reference (orange) site types. While negative LDA scores indicate features like Ascomycota (class, order, family, and genus all *Incertae sedis*), with UNITE sequence ID SH0956787.09FU, *Pseudogymnoascus roseus* (family Pseudeurotiaceae, order Thelebolales, class Leotiomycetes, phylum Ascomycota), with UNITE sequence ID SH1477560.09FU, and *Pholiota terrestris* (family Strophariaceae, order Agaricales, class Agaricomycetes, phylum Basidiomycota), with UNITE sequence ID SH1243425.09FU were strongly associated with reclamation (green) site types. These results also suggest that distinct fungi inhabit each site type.

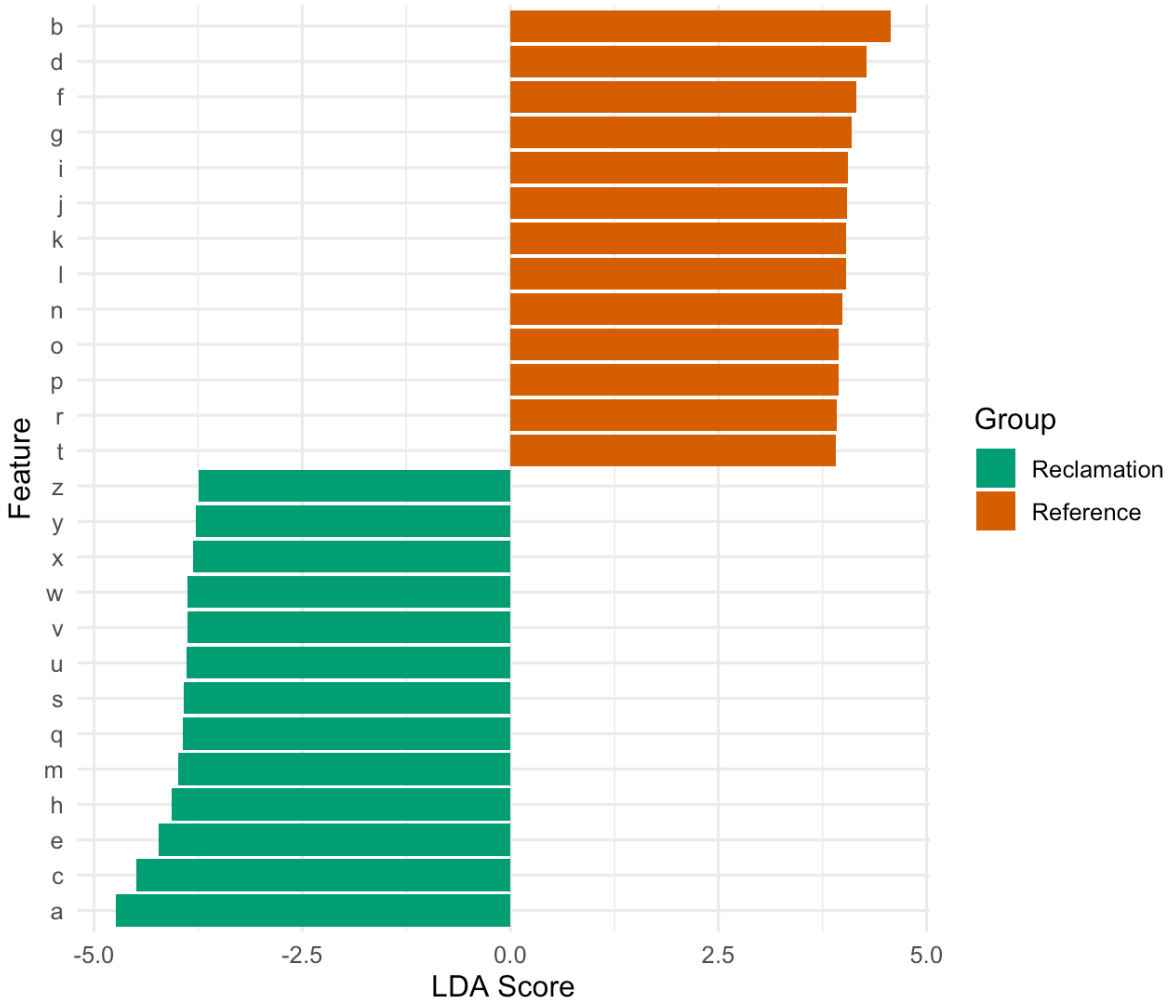


Figure 11.3. LEfSe results, presented as LDA score, from comparisons of the fungal communities between the reclamation and reference site types. Labelled LEfSe results are presented in Table 2.3.

Table 2.3. Labelled LEfSe results from comparisons of the fungal communities between the reclamation and reference site types.

| Label | Taxon | LDA Score |
|---|---|---|
| a | k__Fungi.p__Ascomycota.c__Ascomycota_cls_Incertae_sedis.o__Ascomycota_ord_Incertae_sedis.f__Ascomycota_fam_Incertae_sedis.g__Ascomycota_gen_Incertae_sedis.s__Ascomycota_sp.sh__SH0956787.09FU | -4.7396791 |
| b | k__Fungi.p__Mucoromycota.c__Umbelopsidomycetes.o__Umbelopsidales.f__Umbelopsidaceae.g__Umbelopsis.s__Umbelopsis_dimorpha.sh__SH0898191.09FU | 4.5732739 |
| c | k__Fungi.p__Ascomycota.c__Leotiomycetes.o__Thelebolales.f__Pseudeurotiaceae.g__Pseudogymnoascus.s__Pseudogymnoascus_roseus.sh__SH1477560.09FU | -4.4959284 |
| d | k__Fungi.p__Basidiomycota.c__Agaricomycetes.o__Agaricales.f__Inocybaceae.g__Inocybe.s__Inocybe_sp.sh__SH1332890.09FU | 4.27644696 |
| e | k__Fungi.p__Basidiomycota.c__Agaricomycetes.o__Agaricales.f__Strophariaceae.g__Pholiota.s__Pholiota_terrestris.sh__SH1243425.09FU | -4.2250287 |
| f | k__Fungi.p__Basidiomycota.c__Agaricomycetes.o__Agaricales.f__Tricholomataceae.g__Tricholoma.s__Tricholoma_portentosum.sh__SH1086723.09FU | 4.15691158 |
| g | k__Fungi.p__Ascomycota.c__Leotiomycetes.o__Helotiales.f__Myxotrichaceae.g__Oidiodendron.s__Oidiodendron_chlamydosporicum.sh__SH1239495.09FU | 4.10132882 |
| h | k__Fungi.__.__.__.__.__.__ | -4.063297 |
| i | k__Fungi.p__Basidiomycota.c__GS25.o__GS25_ord_Incertae_sedis.f__GS25_ord_Incertae_sedis_fam_Incertae_sedis.g__GS25_ord_Incertae_sedis_gen_Incertae_sedis.s__GS25_ord_Incertae_sedis_sp.sh__SH1268087.09FU | 4.05165966 |
| j | k__Fungi.p__Basidiomycota.c__Tritirachiomycetes.o__Tritirachiales.f__Tritirachiaceae.g__Paratritirachium.s__Paratritirachium_curvibasidium.sh__SH0918204.09FU | 4.03934697 |
| k | k__Fungi.p__Basidiomycota.c__Agaricomycetes.__.__.__.__.__ | 4.03577086 |
| l | k__Fungi.p__Basidiomycota.c__Agaricomycetes.o__Agaricales.f__Inocybaceae.g__Inocybe.s__Inocybe_sp.__ | 4.02960178 |
| m | k__Fungi.p__Basidiomycota.c__Agaricomycetes.o__Polyporales.f__Podoscyphaceae.g__Hypochnicium.s__Hypochnicium_sp.sh__SH1762990.09FU | -3.9923036 |
| n | k__Fungi.p__Ascomycota.c__Eurotiomycetes.o__Sclerococcales.f__Sclerococcaceae.g__Sclerococcum.s__Sclerococcum_sp.__ | 3.98761098 |
| o | k__Fungi.p__Ascomycota.c__Leotiomycetes.o__Leotiomycetes_ord_Incertae_sedis.f__Leotiomycetes_fam_Incertae_sedis.g__Leotiomycetes_gen_Incertae_sedis.s__Leotiomycetes_sp.sh__SH0956769.09FU | 3.94459812 |
| p | k__Fungi.p__Basidiomycota.c__Agaricomycetes.o__Agaricales.f__Inocybaceae.g__Inocybe.__.__ | 3.93842575 |
| q | k__Fungi.p__Basidiomycota.c__Agaricomycetes.o__Polyporales.f__Polyporales_fam_Incertae_sedis.g__Polyporales_gen_Incertae_sedis.s__Polyporales_sp.sh__SH0898878.09FU | -3.9288162 |
| r | k__Fungi.p__Ascomycota.c__Eurotiomycetes.o__Eurotiales.f__Aspergillaceae.g__Penicillium.__.__ | 3.92671933 |
| s | k__Fungi.p__Ascomycota.c__Sordariomycetes.o__Sordariales.f__Chaetomiaceae.g__Chaetomiaceae_gen_Incertae_sedis.s__Chaetomiaceae_sp.sh__SH0971570.09FU | -3.9195218 |
| t | k__Fungi.p__Basidiomycota.c__Agaricomycetes.o__Agaricales.f__Inocybaceae.g__Inocybe.s__Inocybe_lacera.sh__SH0891217.09FU | 3.90499927 |
| u | k__Fungi.p__Basidiomycota.c__Agaricomycetes.o__Hymenochaetales.f__Hymenochaetales_fam_Incertae_sedis.g__Hymenochaetales_gen_Incertae_sedis.s__Hymenochaetales_sp.sh__SH1100020.09FU | -3.892931 |
| v | k__Fungi.p__Ascomycota.c__Dothideomycetes.o__Pleosporales.f__Pleosporales_fam_Incertae_sedis.g__Pleosporales_gen_Incertae_sedis.s__Pleosporales_sp.sh__SH0930145.09FU | -3.8836277 |
| w | k__Fungi.p__Ascomycota.c__Sordariomycetes.o__Hypocreales.f__Hypocreaceae.g__Trichoderma.s__Trichoderma_harzianum.__ | -3.8819271 |
| x | k__Fungi.p__Ascomycota.c__Leotiomycetes.o__Thelebolales.__.__.__.__ | -3.8160509 |
| y | k__Fungi.p__Ascomycota.c__Sordariomycetes.o__Xylariales.f__Bartaliniaceae.g__Truncatella.s__Truncatella_angustata.sh__SH1264483.09FU | -3.777208 |
| z | k__Fungi.p__Rozellomycota.c__Rozellomycotina_cls_Incertae_sedis.o__GS08.f__GS08_fam_Incertae_sedis.g__GS08_gen_Incertae_sedis.s__GS08_sp.sh__SH0901371.09FU | -3.7398471 |

Lastly, invertebrate community composition by taxa across reclamation and reference site types was analyzed by LEfSe as well (Figure 12.3). Positive LDA scores indicate that features like *Synuchus impunctatus* (subfamily Harpalinae, family Carabidae, order Coleoptera, class Insecta, phylum Arthropoda), *Formica aserva* (subfamily Formicinae, family Formicidae, order Hymenoptera, class Insecta, phylum Arthropoda), and *Calathus ingratus* (subfamily Harpalinae, family Carabidae, order Coleoptera, class Insecta, phylum Arthropoda) were strongly associated with reference (orange) site types. While negative LDA scores indicate that *Amara quenseli* (subfamily Harpalinae, family Carabidae, order Coleoptera, class Insecta, phylum Arthropoda*)*, *Procladius culiciformis* (subfamily Tanypodinae, family Chironomidae, order Diptera, class Insecta, phylum Arthropoda), and *Chlorochroa ligata* (subfamily Pentatominae, family Pentatomidae, order Hemiptera, class Insecta, phylum Arthropoda) were features strongly associated with reclamation (green) site types. Findings again indicate that distinct invertebrates inhabit each site type.

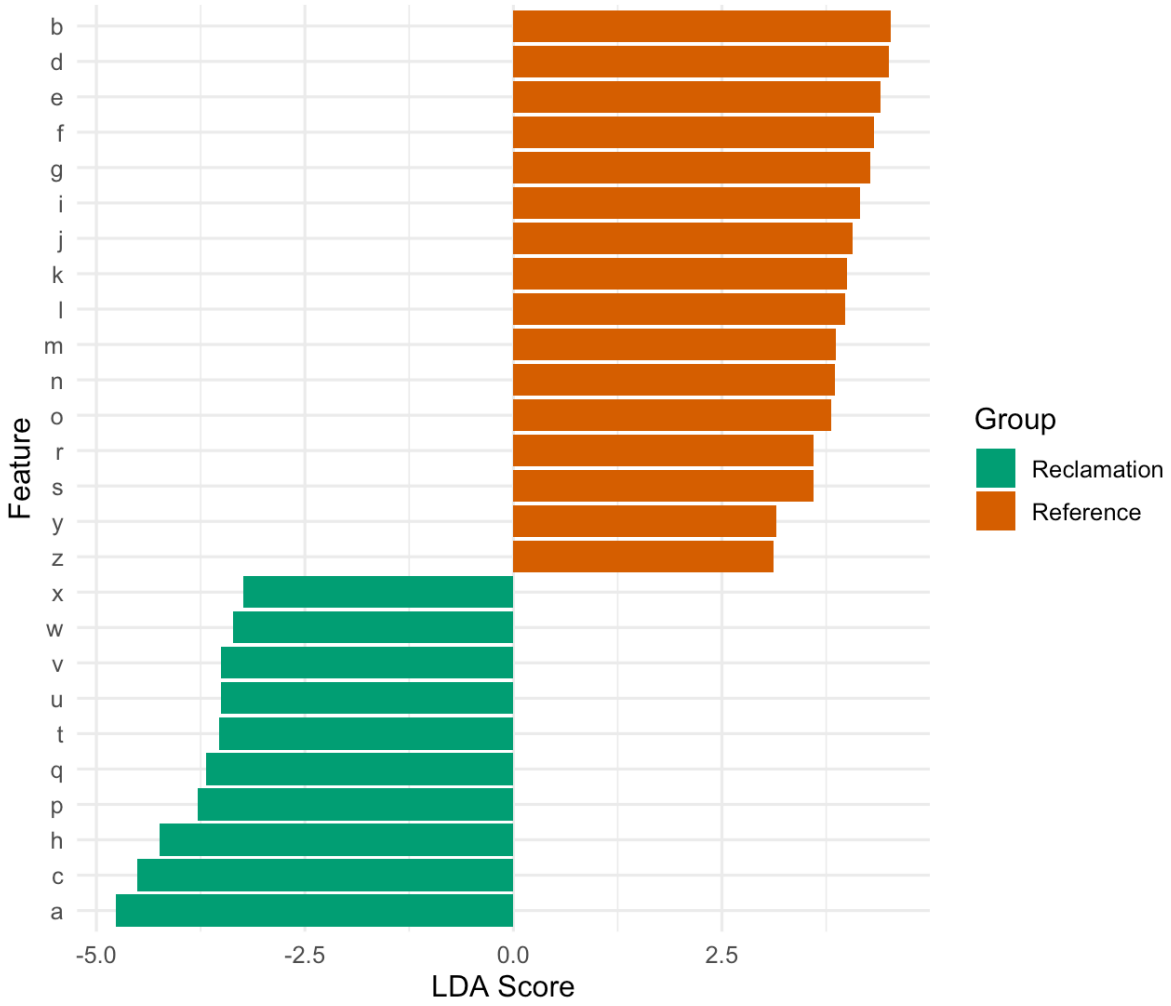


Figure 12.3. LEfSe results, presented as LDA score, from comparisons of the invertebrate communities between the reclamation and reference site types. Labelled LEfSe results are presented in Table 3.3.

Table 3.3. Labelled LEfSe results from comparisons of the invertebrate communities between the reclamation and reference site types.

| Label | Taxon | LDA Score |
|---|---|---|
| a | p__Arthropoda.c__Insecta.o__Coleoptera.f__Carabidae.sf__Harpalinae.g__Amara.s__Amara.quenseli | -4.7680502 |
| b | p__Arthropoda.c__Insecta.o__Coleoptera.f__Carabidae.sf__Harpalinae.g__Synuchus.s__Synuchus.impunctatus | 4.52735811 |
| c | p__Arthropoda.c__Insecta.o__Diptera.f__Chironomidae.sf__Tanypodinae.g__Procladius.s__Procladius.culiciformis | -4.5080994 |
| d | p__Arthropoda.c__Insecta.o__Hymenoptera.f__Formicidae.sf__Formicinae.g__Formica.s__Formica.aserva | 4.50061075 |
| e | p__Arthropoda.c__Insecta.o__Coleoptera.f__Carabidae.sf__Harpalinae.g__Calathus.s__Calathus.ingratus | 4.39594217 |
| f | p__Arthropoda.c__Insecta.o__Diptera.f__Tabanidae.sf__Chrysopsinae.g__Chrysops.s__Chrysops.excitans | 4.32177209 |
| g | p__Arthropoda.c__Insecta.o__Lepidoptera.f__Erebidae.sf__Arctiinae.g__Phragmatobia.s__Phragmatobia.fuliginosa | 4.27895157 |
| h | p__Arthropoda.c__Insecta.o__Hemiptera.f__Pentatomidae.sf__Pentatominae.g__Chlorochroa.s__Chlorochroa.ligata | -4.242734 |
| i | p__Arthropoda.c__Insecta.o__Coleoptera.f__Tenebrionidae.sf__Stenochiinae.g__Iphthiminus.s__Iphthiminus.serratus | 4.15176958 |
| j | p__Arthropoda.c__Insecta.o__Coleoptera.f__Carabidae.sf__Harpalinae.g__Harpalus.__ | 4.0642426 |
| k | p__Arthropoda.c__Insecta.o__Coleoptera.f__Carabidae.sf__Harpalinae.g__Sericoda.s__Sericoda.quadripunctata | 3.9960646 |
| l | p__Arthropoda.c__Insecta.o__Coleoptera.f__Carabidae.sf__Harpalinae.g__Calathus.s__Calathus.advena | 3.98243535 |
| m | p__Arthropoda.c__Insecta.o__Coleoptera.f__Curculionidae.sf__Scolytinae.g__Hylurgops.s__Hylurgops.porosus | 3.86067652 |
| n | p__Arthropoda.c__Insecta.o__Coleoptera.f__Staphylinidae.sf__Aleocharinae.g__Aleochara.s__Aleochara.bilineata | 3.86011327 |
| o | p__Arthropoda.c__Insecta.o__Coleoptera.f__Carabidae.sf__Harpalinae.g__Pterostichus.s__Pterostichus.adstrictus | 3.8077443 |
| p | p__Arthropoda.c__Insecta.o__Coleoptera.f__Chrysomelidae.sf__Eumolpinae.g__Bromius.s__Bromius.obscurus | -3.7826648 |
| q | p__Arthropoda.c__Insecta.o__Coleoptera.f__Carabidae.sf__Harpalinae.g__Amara.__ | -3.6828554 |
| r | p__Arthropoda.c__Insecta.o__Hemiptera.f__Aradidae.sf__Aradinae.g__Aradus.s__Aradus.shermani | 3.60054909 |
| s | p__Arthropoda.c__Insecta.o__Hymenoptera.f__Formicidae.sf__Formicinae.g__Formica.s__Formica.neorufibarbis | 3.59373609 |
| t | p__Arthropoda.c__Insecta.o__Diptera.f__Chloropidae.sf__Oscinellinae.g__Malloewia.s__Malloewia.aequa | -3.534236 |
| u | p__Arthropoda.c__Insecta.o__Diptera.f__Syrphidae.sf__Syrphinae.g__Syrphus.s__Syrphus.attenuatus | -3.5084912 |
| v | p__Arthropoda.c__Insecta.o__Coleoptera.f__Carabidae.sf__Harpalinae.g__Amara.s__Amara.quenseli.quenseli | -3.5024668 |
| w | p__Arthropoda.c__Insecta.o__Diptera.f__Chironomidae.sf__Chironominae.g__Tanytarsus.s__Tanytarsus.volgensis | -3.3603668 |
| x | p__Arthropoda.c__Insecta.o__Diptera.f__Chironomidae.sf__Chironominae.g__Tanytarsus.s__Tanytarsus.heliomesonyctios | -3.2349576 |
| y | p__Arthropoda.c__Insecta.o__Diptera.f__Muscidae.sf__Phaoniinae.g__Helina.s__Helina.evecta | 3.14758573 |
| z | p__Arthropoda.c__Insecta.o__Diptera.f__Tachinidae.sf__Exoristinae.g__Platymya.s__Platymya.confusionis | 3.11493585 |

# DISCUSSION

## Soil microbial communities

### *Bacterial communities*

The composition of bacterial communities at MtM reclamation sites reflects the progression and complexity of ecological recovery in post-mining landscapes. The results demonstrate that reclaimed site types exhibit higher bacterial species richness than reference site types, but show lower Simpson diversity, which indicates a greater number of taxa in communities dominated by a few species. Banning et al. (2011) postulate that changes in community structure are driven by limited resources and the ability of populations to use them. This suggests that although reclaimed soils can support an array of bacterial taxa, community assembly may be shaped by abiotic and biotic factors that select for or against specific species. For example, the observation of increased species richness in treatment units, like Hydroseed, could be attributed to an influx of opportunistic bacteria capitalizing on increased moisture and nutrients in microenvironments created by hydroseeding.

Microbial metrics as measures of reclamation success have a long-standing precedent, and much recent work echoes it (Emmerling et al., 2000; Ezeokoli et al, 2020; Mummey et al., 2002; Garris et al., 2016). A chronosequence study at the Teck-Highland Valley Copper Mine in British Columbia, where bacterial communities were tracked across sites of reclamation age 3–26 years using 16s rRNA gene sequencing (Singh et al., 2024), as well as work by Li et al. (2022), provide direct context for interpreting the diversity trends observed at MtM. Singh et al. (2024) found that as reclamation age increased, bacterial community composition became more similar to undisturbed forest soils, while Li et al. (2022) identified that long-term reclamation processes significantly increased bacterial abundance and diversity. Like the work at hand, both studies support the idea that bacterial succession can be used as a measurable indicator of reclamation progress, reinforcing the need for high-resolution monitoring over multiple years.

Community composition analyses further support the distinction between reclamation and reference site types. NMDS revealed divergence in bacterial

assemblages via clustering. This separation indicates that the communities of reclamation site types have not yet converged toward those of reference ecosystems, which may also reflect differences in soil composition, vegetation cover, organic matter inputs, or microbial succession pathways. Additionally, LEfSe analysis identified indicator taxa specific to site type. *Bradyrhizobium* and Xanthobacteraceae—taxa that contribute to nitrogen cycling and are known to form symbiotic relationships with plants (Jordan, 1982; Oren, 2014)—were prevalent in reference sites. In contrast, taxa like Chloroflexota and Acidobacteriota were enriched in reclamation sites, potentially indicating adaptation to soils with lower organic content and different redox conditions (Freches & Fradino, 2024; Kalam et al., 2020; Kielak et al., 2016). These findings align with previous studies, which show that soil bacterial communities serve as indicators of recovery, but composition is modulated by environmental factors (Ezeokoli et al., 2020; Garris et al., 2018).

Overall, these outcomes suggest that reclamation sites support microbial communities with relevance to ecosystem recovery, but also distinct from those of reference sites. Nevertheless, metabarcode sequencing only reveals taxonomic presence and not activity; while these patterns offer high-resolution insights into community composition and reclamation trajectory, future metatranscriptomic analyses would be necessary to determine metabolically active taxa that are contributing to key biogeochemical functions.

*Fungal communities*

Fungal community composition analyses at MtM revealed assemblages in reclamation site types that were more diverse than reference site types. Unlike bacterial communities, which showed higher richness but lower evenness in reclamation sites, fungal communities at MtM exhibited both higher species richness and greater diversity indices. High alpha diversity is in alignment with prior research outcomes, indicating that early diversity could be correlated with changes in substrate availability (Frankland et al., 1998; Gorzelak et al., 2020; Hart et al., 2019). This was apparent in Hydroseed, Rough and Loose, and Waterbars – Med treatment units, where the fungal taxa may strongly reflect differences in plant community

composition and substrate availability. Fungi are key to soil aggregation, organic matter decomposition, nutrient cycling, and plant symbiosis, and reclaimed sites, particularly those with diverse native seed mixes and soil amendments, may support more rapid development of fungal guilds (Bidartondo et al., 2011; Burke et al., 2011; Frąc et al., 2018; Lehmann et al., 2020).

Similar to bacterial communities, recent ITS region sequencing research has shown that the abundance and diversity of soil fungi in reclamation sites gradually approach those of communities in reference sites (Gorzelak et al., 2020; Ji et al., 2022; Singh et al., 2024). Previous studies have, however, also demonstrated slower succession rates and more consistent composition in contrast to bacteria, indicating strong resilience to environmental changes across fungal taxa (Hart et al., 2019; Ji et al., 2022; Jin et al., 2024). In the study at hand, NMDS analyses showed clear separation between reclamation and reference site types. LEfSe analysis further highlighted that Basidiomycota and Mucoromycota were enriched in reference sites, while reclamation sites were dominated by Basidiomycota and Ascomycota. Basidiomycota is a diverse class, including pathogens and mutualists, and Agariomycetes—filamentous fungi that decompose lignocellulose—are known to cycle nutrients in soils; it is unsurprising that species of the class would be enriched across both site types (Kersten and Cullen, 2013; Taylor et al., 2015). Alternatively, Mucoromycota moulds are known to be abundant in carbon-rich soils specifically, while Ascomycota are known as generalists that dominate in many soil types, which aligns with where each phylum was enriched (Egidi et al., 2019; Tedersoo et al., 2020). The dominance of generalist colonizers in reclamation sites suggests that the fungi are opportunistic but may not be central to long-term community stability.

Multiple studies have concluded that fungi are more sensitive than bacteria to changes in soil, making them valuable indicators of soil health and reclamation success (Bai et al., 2024; Kaisermann et al., 2015; Li et al., 2022). Collectively, these results provide evidence that reclamation efforts at MtM are supporting the establishment of diverse fungal communities. Again, caution is warranted when interpreting taxonomic data in isolation and integrating functional analyses to

research at MtM would be critical to understanding the functional ecological services being provided by the fungal taxa.

**Invertebrate communities**

DNA-based analyses of the ground-dwelling and flying invertebrate community compositions within reclamation and reference site types at MtM highlighted some distinct differences as well. Although the species richness results show that invertebrate alpha diversity did not differ significantly across different treatment sites, they did between the reclamation, reference, and control site types, aligning with the NMDS plots that demonstrated clear clustering between reclamation and reference site types. Invertebrate communities are influenced by a range of factors post-disturbance, including substrate availability, vegetation development, organic matter content, and dispersal limitations (Majer et al., 2002; Neher et al., 2012; Perry & Herms, 2019; Silva-Monteiro et al., 2022). This study adds to the growing body of evidence that has emerged, pointing to meso- and macro-faunal communities as indicators of ecological recovery (Borges et al., 2021; Majer, 1983; Rainio and Niemelä, 2003; Sanchez et al., 2021).

LEfSe analysis revealed taxa characteristic of each site type. For instance, *Formica aserva* and *Synuchus impunctatus* were enriched in reference sites, while *Amara quenseli* and *Procladius culiciformis* were more strongly associated with reclamation sites. Although widely distributed throughout North America, *F. aserva* primarily live in coniferous forests (Naumann et al., 1999 as cited in Scarparo et al., 2024), which aligns with these results. Also, in parallel with this research, Hammond et al. (2018) found that beetle communities of reclaimed Alberta oil sands land differed from those of natural forests, despite comparable species richness; reclaimed sites were dominated by smaller, generalist species, while reference sites supported larger forest species. This is consistent with *A. quenseli*, a smaller species, being more abundant in reclamation sites, and *S. impunctatus*, a larger species, being more abundant in reference sites. Overall, the patterns in the carabid beetle and ant populations of this study corroborate research showing that they are responsive to habitat changes, and that the presence or absence of certain species

can signal reclamation trajectory (Majer, 1983; Rainio and Niemelä, 2003; Saint-Germain et al., 2005; Silva et al., 2025).

These findings underscore the value of using invertebrate communities as bioindicators of reclamation projects, but research has shown that invertebrate community re-establishment may take decades, especially in nutrient-poor substrates (Auclerc et al., 2019; Majer et al., 2007; Zaitsev et al., 2016). This highlights the need for long-term monitoring frameworks that integrate invertebrate data with microbial and plant indicators; integrating invertebrate monitoring into reclamation programs offers a comprehensive multi-level assessment of ecosystem recovery and sustainability. Finally, in addition to surface-active invertebrates, soil nematodes may also provide valuable insights, especially in terms of soil food web structure (Biederman et al., 2008).

## CONCLUSION

This study demonstrates that DNA-based monitoring of microbial and invertebrate communities offers high-resolution insights into reclamation trajectories in post-mining landscapes. At Mount Milligan Mine, DNA metabarcode sequencing revealed distinct microbial and invertebrate assemblages between reclamation and reference sites. These findings align with broader results across British Columbia, showing that microbial and faunal communities respond sensitively to reclamation efforts and could serve as early bioindicators of recovery. Furthermore, they affirm the value of integrating molecular tools, such as metabarcoding, into traditional monitoring frameworks to improve sensitivity and timeliness when evaluating reclamation strategies. As the field moves toward defining clearer benchmarks for success, studies like this will be essential to shaping adaptive, evidence-based reclamation practices that support sustainable post-mining ecosystems. Future research should build on these results through longitudinal studies that incorporate functional analyses, including metagenomics and metatranscriptomics, to link community composition to function, thereby strengthening the ability to evaluate and refine reclamation strategies.

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# Chapter 4. General conclusions

## KEY FINDINGS

This thesis examines microbial and invertebrate responses to post-mining reclamation in British Columbia, drawing from community-level analyses, as well as a unique exploration of biosolids amendments and antimicrobial resistance genes (ARGs) in Chapter 2. Across study sites at the Teck Highland Valley Copper (Teck-HVC) and Mount Milligan (MtM) mines, findings demonstrate the value of applied molecular ecology to inform reclamation strategies and assessment.

Chapter 2 focused on soil physicochemical, vegetation, and microbial responses to biosolids amendments at the Teck-HVC mine. Results showed improvements in soil physicochemical and vegetation properties, alongside clear shifts in microbial community and structure, particularly at higher biosolids treatment concentrations. Importantly, biosolids amendments were also linked to a dose-dependent increase in ARGs, potentially raising environmental and public health concerns (Zhang et al., 2022). These findings are from approximately 20 years post-biosolids amendments, making the long-term dataset a rarity in reclamation literature. Chapter 3 examined microbial and invertebrate communities at MtM, revealing distinct community assemblages between reclaimed and reference sites. Variations in bacterial, fungal, and invertebrate community richness and composition appear to be closely tied to site type. Certain taxa were consistently enriched in reclaimed versus reference ecosystems, indicating both their potential utility as bioindicators and the complexity of post-mining landscapes. The diversity in trajectories across reclaimed sites also suggests that there is no single “endpoint” in reclamation, but rather multiple paths that may lead to ecologically functional landscapes.

While Chapter 2 explored the impacts of biosolids amendments on soil microbial communities and ARG proliferation, and Chapter 3 highlighted how microbial and invertebrate communities may act as bioindicators, together they highlight the value of DNA-based monitoring in reclamation. When used with traditional assessment methods, high-throughput sequencing and community

profiling can capture subtle, early indicators of recovery that would otherwise go undetected (Vallin et al., 2025). Ultimately, both chapters support that reclamation strategies must be tailored and context-specific, considering the type of mine waste, biome, temperature, pH, implications for ground and surface water, as well as local Indigenous perspectives, to balance benefits with potential risks.

## MINING RECLAMATION IMPLICATIONS

### Biosolids

Biosolids have emerged as a promising amendment to restore nutrient-poor post-mining landscapes due to their ability to improve soil fertility and vegetation growth (Cuevas et al., 2000; Gagnon et al., 2021; Gardner et al., 2010). At the Teck-HVC mine, higher biosolids treatment concentrations led to improved soil physicochemical properties, including soil organic carbon and nitrogen content, as well as vegetation properties. However, the same treatments were also associated with decreased microbial diversity and increased ARG abundance, suggesting that while biosolids may catalyze short-term recovery, they could also exert long-term selective pressures on microbial communities. High concentrations, such as 250 Mg/ha, appeared to increase the dominance of particular taxa, like Nitrospirota and Proteobacteria; Acidobacteriota thrived in control plots. In addition to nutrients, biosolids introduce trace levels of heavy metals, which can have implications for soil chemistry and microbial activity (Mossa et al., 2017; Popoola et al., 2023; Smith, 2009). Perhaps more critical, however, is the association between biosolids and ARGs. The observed dose-dependent relationship raises questions about the long-term environmental and human health impacts.

Consequently, these findings suggest the need for reclamation strategies that optimize soil improvement while minimizing risk. Moderate biosolids doses, informed by both baseline assessments and long-term monitoring, may offer a middle ground that balances biosolids' restorative effects with their ecological trade-offs. Future reclamation efforts should incorporate adaptive management, including pre- and regular post-treatment screening for ARGs, heavy metals, and microbial shifts, alongside consideration of runoff and water contamination or airborne vectors.

**DNA-based strategies**

Molecular tools were fundamental to this thesis, enabling high-resolution insights into microbial and invertebrate community composition. Amplicon sequencing of 16S rRNA gene and ITS regions at both mines and CO1 genes at the MtM mine allowed for detailed characterization of bacterial, fungal, and invertebrate communities across study sites. Statistical techniques, like analysis of compositions of microbiomes with bias correction (ANCOM-BC) and linear discriminant analysis effect size (LEfSe) helped identify specific taxa that reliably differentiated between treatments or site types, demonstrating the power of taxonomic resolution in ecological monitoring (Khleborodova et al., 2024; Lin & Peddada, 2020). For instance, *Bradyrhizobium* was frequently associated with reference ecosystems, while Chloroflexota was enriched in reclaimed plots at the MtM mine. Patterns like this suggest that certain microbes may serve as robust, site-specific bioindicators. And, because molecular data capture changes unnoticed by traditional reclamation frameworks, they hold potential as early warning tools and open doors for predictive modelling in adaptive management frameworks.

Looking ahead, as molecular monitoring becomes more accessible, it could be integrated into long-term reclamation strategies to track progress, guide interventions, and evaluate outcomes with greater precision. Furthermore, by also applying metagenomics-based approaches, and thus enabling strain-level and functional gene resolution, future researchers would be equipped to provide reclamation professionals with a more actionable toolkit.

**LIMITATIONS**

While the findings presented in this thesis offer valuable insights, they are not without limitations. One of the most significant is the cross-sectional nature of the study conducted at the Teck-HVC mine, based on a single time point nearly two decades after biosolids amendments, making it impossible to trace microbial succession or ARG abundance over time. Furthermore, while ARGs were detected and quantified, their activity and potential for horizontal gene transfer were not assessed. Without data on gene expression or mobility, their ecological and public

health relevance remains speculative. Future work should employ metatranscriptomics, plasmidomics, or functional assays to assess whether ARGs pose a risk of dissemination into natural environments.

Another limitation is spatial heterogeneity at both mines. Despite controls, differences in soil type, disturbance history, and microclimate likely introduced variability within and between study sites. Although this reflects real-world reclamation conditions, it complicates comparisons and limits the generalizability of results to other mines or regions. Sampling across a broader range of sites would aid in identifying more universally applicable trends.

With regard to methodology, metabarcoding provided taxonomic data but not direct information on function. Although metabarcoding allows for high-throughput taxonomic profiling, it provides limited insights into microbial activity (Francioli et al., 2021). For example, taxa may be abundant but inactive, or rare yet ecologically important. Functional analyses, such as microbial respiration assays, enzyme measurements, or stable isotope probing, would add depth to community-level interpretations. Additionally, while the thesis focused primarily on microbial and invertebrate communities, it does not explicitly examine how these communities interact with plants. Plant-microbe and plant-invertebrate relationships are central to nutrient cycling and successional dynamics, and while data were collected to contextualize microbial and invertebrate responses, this thesis does not explicitly integrate these interactions; doing so would provide a more holistic view of ecosystem recovery (Bartelt-Ryser et al., 2005; Curry, 1989; Griffiths et al., 2021).

Finally, while this thesis provides support for DNA-based bioindicators, the field still lacks clear benchmarks for reclamation success. It is not yet clear what constitutes a “functionally recovered” microbial or invertebrate community, or how different it can be from a reference ecosystem while still meeting ecological goals. This ambiguity reflects the need for developing standardized, evidence-based criteria to guide reclamation monitoring and evaluation. Without it, interpretations remain partly subjective.

## FUTURE RESEARCH

Building on this thesis, future research should prioritize longitudinal studies to understand the dynamics of ecosystem recovery. Repeated measures over time could reveal patterns of convergence or divergence from reference conditions, identify early signs of success or failure, and inform adaptive management. These studies should incorporate interdisciplinary approaches that unite molecular biology, soil science, ecology, and risk management to ensure that reclamation strategies are effective, sustainable, and responsible.

Another key direction is functional characterization. For example, metagenomic and metatranscriptomic analyses can uncover which taxa are present, as well as their metabolic potential and real-time activity. Paired with tools like microbial respiration assays, enzyme measurements, or stable isotope probing, these techniques could provide a more complete picture, linking identity to ecosystem function, including nutrient cycling and pathogen suppression. There is also a need to explore interactions across trophic levels. Integrating microbial, plant, and faunal data would enhance knowledge of the relationships within and between trophic levels, improving understanding of whole-ecosystem recovery and resilience. This could be crucial to predicting how reclaimed ecosystems may respond to secondary stressors. As climate change heightens, post-mining landscapes may experience new pressures that challenge their resilience, and simulating such disturbances could inform future frameworks (Xie & Zyl, 2022).

To advance this field, collaborative networks between academic, industry, and Indigenous partners should be developed to support long-term monitoring at mines across BC. Moreover, educational programs could be designed to train reclamation professionals in molecular monitoring, ensuring that these useful, emerging tools are appropriately translated into practice. These networks could also integrate molecular, chemical, and ecological data into regional or national repositories, which may aid in the development of benchmarks for reclamation success. By defining more universal metrics, stakeholders could better compare outcomes across sites and jurisdictions. Collaborative efforts will be vital in co-developing these frameworks and ensuring that they reflect local ecological knowledge.

Finally, future research should continue to address the intersection of ecological reclamation and public health. The presence of ARGs in biosolids-treated soils presents a potential risk that must be balanced against reclamation benefits. Incorporating ecological monitoring with public health safeguards would provide a more holistic outlook on reclamation outcomes and could help effectively bridge environmental and health policy.

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# Appendix A

Table A1. DNA template samples and their corresponding plot locations, biosolid rates, and measured concentrations.

| Sample # | Plot Location | Biosolid Rate (Mg/ha) | Concentration (ng/uL) |
|---|---|---|---|
| 1 | A102 | 0 | 0.1924 |
| 2 | A206 | 0 | 0.1912 |
| 3 | A305 | 0 | 0.3628 |
| 4 | A402 | 0 | 0.2644 |
| 5 | A107 | 0 + F | 0.828 |
| 6 | A201 | 0 + F | 0.233 |
| 7 | A302 | 0 + F | 0.0892 |
| 8 | A404 | 0 + F | 0.448 |
| 9 | A106 | 100 | 1.46 |
| 10 | A207 | 100 | 4.4 |
| 11 | A303 | 100 | 6.04 |
| 12 | A405 | 100 | 4.52 |
| 13 | A104 | 250 | 8.48 |
| 14 | A202 | 250 | 3.21 |
| 15 | A306 | 250 | 6.6 |
| 16 | A401 | 250 | 6.36 |
| 17 | B105 | 0 | 0 |
| 18 | B201 | 0 | 0.0376 |
| 19 | B304 | 0 | 0 |
| 20 | B404 | 0 | 0 |
| 21 | B106 | 0 + F | 0 |
| 22 | B203 | 0 + F | 0 |
| 23 | B306 | 0 + F | 0 |
| 24 | B401 | 0 + F | 0.382 |
| 25 | B107 | 100 | 2.27 |
| 26 | B202 | 100 | 2.56 |
| 27 | B303 | 100 | 4.08 |
| 28 | B406 | 100 | 2.8 |
| 29 | B101 | 250 | 1.49 |
| 30 | B205 | 250 | 4.84 |
| 31 | B302 | 250 | 4.68 |
| 32 | B407 | 250 | 4.4 |
| 33 | C101 | 0 | 4.44 |
| 34 | C203 | 0 | 1.26 |
| 35 | C304 | 0 | 1.24 |
| 36 | C406 | 0 | 2.1 |
| 37 | C104 | 0 + F | 0.776 |
| 38 | C207 | 0 + F | 1.91 |
| 39 | C305 | 0 + F | 3.78 |
| 40 | C402 | 0 + F | 4.48 |
| 41 | C107 | 100 | 9.16 |
| 42 | C201 | 100 | 9.48 |
| 43 | C306 | 100 | 16.3 |
| 44 | C403 | 100 | 20.4 |
| 45 | C103 | 250 | 12.3 |
| 46 | C205 | 250 | 21.2 |
| 47 | C301 | 250 | 24 |
| 48 | C407 | 250 | 12.8 |
| 49 | D104 | 0 | 1.31 |
| 50 | D201 | 0 | 3.7 |
| 51 | D307 | 0 | 2.41 |
| 52 | D406 | 0 | 3.96 |
| 53 | D102 | 0 + F | 2.2 |
| 54 | D206 | 0 + F | 12.2 |
| 55 | D304 | 0 + F | 2.16 |
| 56 | D401 | 0 + F | 0.92 |
| 57 | D101 | 100 | 1.78 |
| 58 | D105 | 100 | 8.48 |
| 59 | D306 | 100 | 18.2 |
| 60 | D407 | 100 | 0 |
| 61 | D203 | 250 | 0 |
| 62 | D205 | 250 | 0 |
| 63 | D301 | 250 | 14.1 |
| 64 | D404 | 250 | 19.5 |

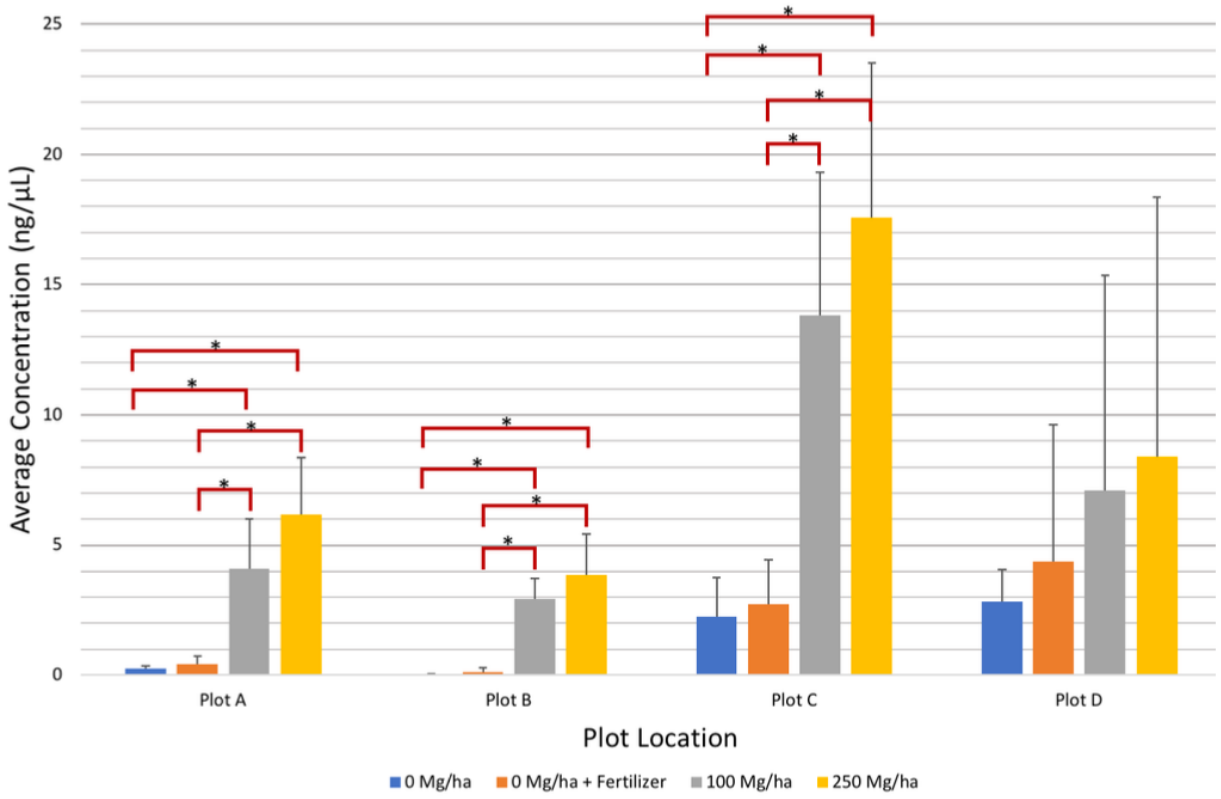


Figure A1. The average concentration of DNA per biosolid treatment in each plot location. Values represent the mean of all samples per treatment, and the error bars represent standard deviations. Each asterisk indicates a p-value < 0.05.

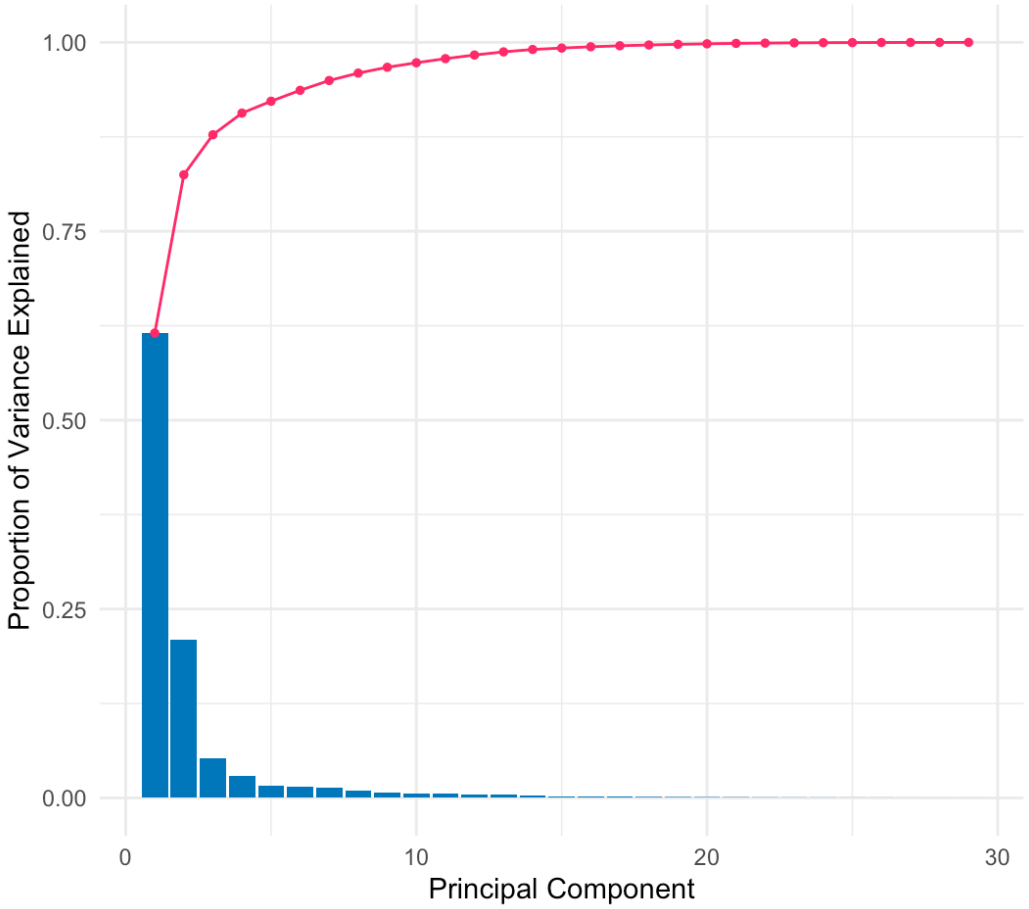


Figure A2. A scree plot demonstrating the proportion of variance explained by the principal components of the soil physiochemical properties and vegetation data of four reclaimed mining sites treated with different concentrations of biosolids.

| | Df | SumOfSqs | R2 | F | Pr(>F) |
|---|---|---|---|---|---|
| Biosolids vs Other | 1 | 2.35738174 | 0.12764421 | 14.1931631 | 0.001 |
| Residual | 97 | 16.1109985 | 0.87235579 | NA | NA |
| Total | 98 | 18.4683802 | 1 | NA | NA |
| | | | | | |
| | Df | SumOfSqs | R2 | F | Pr(>F) |
| Biosolids vs Control | 1 | 1.37812335 | 0.09419943 | 8.52765291 | 0.001 |
| Residual | 82 | 13.2517254 | 0.90580057 | NA | NA |
| Total | 83 | 14.6298488 | 1 | NA | NA |
| | | | | | |
| | Df | SumOfSqs | R2 | F | Pr(>F) |
| Biosolids vs Fertilizer | 1 | 1.48750459 | 0.09900775 | 9.23054693 | 0.001 |
| Residual | 84 | 13.5366177 | 0.90099225 | NA | NA |
| Total | 85 | 15.0241223 | 1 | NA | NA |
| | | | | | |
| | Df | SumOfSqs | R2 | F | Pr(>F) |
| Fertilizer vs Control | 1 | 0.1451685 | 0.02744992 | 0.73384184 | 0.54 |
| Residual | 26 | 5.14331684 | 0.97255008 | NA | NA |
| Total | 27 | 5.28848534 | 1 | NA | NA |
| | | | | | |
| | Df | SumOfSqs | R2 | F | Pr(>F) |
| MaxorMin | 1 | 0.75576716 | 0.15895897 | 5.6700791 | 0.001 |
| Residual | 30 | 3.99871227 | 0.84104103 | NA | NA |
| Total | 31 | 4.75447944 | 1 | NA | NA |

Figure A3. The PERMANOVA results from the comparisons of the bacterial communities across treatments.

| | Df | SumOfSqs | R2 | F | Pr(>F) |
|---|---|---|---|---|---|
| Biosolids vs Other | 1 | 1.37936583 | 0.06417059 | 5.8285192 | 0.001 |
| Residual | 85 | 20.1159319 | 0.93582942 | NA | NA |
| Total | 86 | 21.4952977 | 1 | NA | NA |
| | | | | | |
| | Df | SumOfSqs | R2 | F | Pr(>F) |
| Biosolids vs Control | 1 | 0.91286773 | 0.04940512 | 3.74204467 | 0.002 |
| Residual | 72 | 17.5643217 | 0.95059488 | NA | NA |
| Total | 73 | 18.4771895 | 1 | NA | NA |
| | | | | | |
| | Df | SumOfSqs | R2 | F | Pr(>F) |
| Biosolids vs Fertilizer | 1 | 0.87176645 | 0.05019453 | 3.69930148 | 0.002 |
| Residual | 70 | 16.495993 | 0.94980547 | NA | NA |
| Total | 71 | 17.3677595 | 1 | NA | NA |
| | | | | | |
| | Df | SumOfSqs | R2 | F | Pr(>F) |
| Fertilizer vs Control | 1 | 0.17482321 | 0.02915311 | 0.78074191 | 0.644 |
| Residual | 26 | 5.82190264 | 0.97084689 | NA | NA |
| Total | 27 | 5.99672585 | 1 | NA | NA |
| | | | | | |
| | Df | SumOfSqs | R2 | F | Pr(>F) |
| MaxorMin | 1 | 0.40765195 | 0.07792511 | 1.77472272 | 0.094 |
| Residual | 21 | 4.82367805 | 0.92207489 | NA | NA |
| Total | 22 | 5.23133 | 1 | NA | NA |

Figure A4. The PERMANOVA results from the comparisons of the fungal communities across treatments.

# Appendix B

Table B1. The bacterial pairwise PERMANOVA results.

| Pairs | SumsOfSqs | F.Model | R2 | p.value | p.adjusted |
|---|---|---|---|---|---|
| Waterbars-Low vs Waterbars-Med | 0.100374584 | 0.915981778 | 0.186327334 | 0.4 | 1 |
| Waterbars-Low vs Waterbars-High | 0.147271879 | 1.425205308 | 0.262700714 | 0.3 | 1 |
| Waterbars-Low vs Hydroseed | 0.149972338 | 1.450086858 | 0.266066743 | 0.4 | 1 |
| Waterbars-Low vs Rough and Loose | 0.22262679 | 1.910280976 | 0.323213225 | 0.2 | 1 |
| Waterbars-Low vs Burn | 0.768704464 | 8.645514457 | 0.256958902 | 0.001 | 0.021 |
| Waterbars-Low vs Cut | 0.702592162 | 6.600871696 | 0.222995855 | 0.001 | 0.021 |
| Waterbars-Med vs Waterbars-High | 0.290126811 | 4.474981557 | 0.528022572 | 0.1 | 1 |
| Waterbars-Med vs Hydroseed | 0.14348572 | 2.210115282 | 0.355889574 | 0.1 | 1 |
| Waterbars-Med vs Rough and Loose | 0.298412518 | 3.823808225 | 0.488740025 | 0.1 | 1 |
| Waterbars-Med vs Burn | 0.814139424 | 9.83811974 | 0.282395256 | 0.001 | 0.021 |
| Waterbars-Med vs Cut | 0.899568462 | 9.018815934 | 0.281672375 | 0.001 | 0.021 |
| Waterbars-High vs Hydroseed | 0.147139604 | 2.507719858 | 0.385345392 | 0.1 | 1 |
| Waterbars-High vs Rough and Loose | 0.074663167 | 1.039977799 | 0.20634571 | 0.4 | 1 |
| Waterbars-High vs Burn | 1.13475477 | 13.88012337 | 0.356997925 | 0.001 | 0.021 |
| Waterbars-High vs Cut | 1.05979463 | 10.74221654 | 0.318361318 | 0.002 | 0.042 |
| Hydroseed vs Rough and Loose | 0.163038 | 2.2681262 | 0.361850755 | 0.2 | 1 |
| Hydroseed vs Burn | 0.886618889 | 10.84307534 | 0.302515207 | 0.002 | 0.042 |
| Hydroseed vs Cut | 1.062098152 | 10.76387272 | 0.318798522 | 0.001 | 0.021 |
| Rough and Loose vs Burn | 1.173427658 | 13.99150453 | 0.358834692 | 0.001 | 0.021 |
| Rough and Loose vs Cut | 1.176345754 | 11.65230038 | 0.336263401 | 0.001 | 0.021 |
| Burn vs Cut | 0.59744435 | 6.445211727 | 0.127766571 | 0.001 | 0.021 |

Table B2. The fungal pairwise PERMANOVA results.

| Pairs | SumsOfSqs | F.Model | R2 | p.value | p.adjusted |
|---|---|---|---|---|---|
| Waterbars-Low vs Waterbars-Med | 0.307904247 | 1.277716892 | 0.298691317 | 0.2 | 1 |
| Waterbars-Low vs Waterbars-High | 0.233668667 | 0.844301237 | 0.21962411 | 0.7 | 1 |
| Waterbars-Low vs Hydroseed | 0.249505177 | 1.230109839 | 0.290798557 | 0.3 | 1 |
| Waterbars-Low vs Rough and Loose | 0.324382525 | 1.707667306 | 0.362741714 | 0.1 | 1 |
| Waterbars-Low vs Burn | 0.767186789 | 2.668728839 | 0.100069593 | 0.007 | 0.147 |
| Waterbars-Low vs Cut | 0.692286699 | 2.231936803 | 0.088456816 | 0.004 | 0.084 |
| Waterbars-Med vs Waterbars-High | 0.282092811 | 1.275531713 | 0.241782588 | 0.2 | 1 |
| Waterbars-Med vs Hydroseed | 0.286212456 | 1.727179624 | 0.301575948 | 0.1 | 1 |
| Waterbars-Med vs Rough and Loose | 0.437383652 | 2.802761361 | 0.412003481 | 0.1 | 1 |
| Waterbars-Med vs Burn | 1.124639199 | 4.043315456 | 0.139216732 | 0.001 | 0.021 |
| Waterbars-Med vs Cut | 1.068880022 | 3.568718027 | 0.129448095 | 0.002 | 0.042 |
| Waterbars-High vs Hydroseed | 0.189309423 | 0.983191983 | 0.197301647 | 0.4 | 1 |
| Waterbars-High vs Rough and Loose | 0.236053608 | 1.290690476 | 0.243955015 | 0.1 | 1 |
| Waterbars-High vs Burn | 1.071887761 | 3.795080828 | 0.131796151 | 0.002 | 0.042 |
| Waterbars-High vs Cut | 0.916760212 | 3.01579551 | 0.111630824 | 0.001 | 0.021 |
| Hydroseed vs Rough and Loose | 0.217118929 | 1.703651897 | 0.298694929 | 0.1 | 1 |
| Hydroseed vs Burn | 1.244714164 | 4.549893305 | 0.153973257 | 0.001 | 0.021 |
| Hydroseed vs Cut | 1.100689075 | 3.734375239 | 0.134647895 | 0.001 | 0.021 |
| Rough and Loose vs Burn | 1.197504665 | 4.402186813 | 0.149723109 | 0.003 | 0.063 |
| Rough and Loose vs Cut | 1.065612241 | 3.635217195 | 0.131542921 | 0.003 | 0.063 |
| Burn vs Cut | 1.464020475 | 4.929459607 | 0.098728479 | 0.001 | 0.021 |

Table B3. The invertebrate pairwise PERMANOVA results.

| Pairs | SumsOfSqs | F.Model | R2 | p.value | p.adjusted |
|---|---|---|---|---|---|
| Control vs Cut | 0.428698726 | 1.32369278 | 0.023926327 | 0.17 | 1 |
| Control vs Waterbars-Low | 0.470507961 | 1.361736708 | 0.078433208 | 0.182 | 1 |
| Control vs Waterbars-High | 0.348313451 | 1.096143863 | 0.06809978 | 0.292 | 1 |
| Control vs Waterbars-Med | 0.348529471 | 0.990451165 | 0.070794798 | 0.421 | 1 |
| Control vs Hydroseed | 0.262662917 | 0.812243788 | 0.045600307 | 0.651 | 1 |
| Control vs Rough and Loose | 0.216317761 | 0.704286749 | 0.037653761 | 0.744 | 1 |
| Control vs Burn | 0.306709731 | 0.945617691 | 0.015515762 | 0.458 | 1 |
| Cut vs Waterbars-Low | 1.290847322 | 3.992151641 | 0.064397694 | 0.001 | 0.028 |
| Cut vs Waterbars-High | 1.185390448 | 3.755365161 | 0.061811252 | 0.001 | 0.028 |
| Cut vs Waterbars-Med | 0.791882933 | 2.446785287 | 0.042592206 | 0.005 | 0.14 |
| Cut vs Hydroseed | 1.001146727 | 3.1547834 | 0.050756888 | 0.001 | 0.028 |
| Cut vs Rough and Loose | 1.14987279 | 3.678738445 | 0.057770278 | 0.001 | 0.028 |
| Cut vs Burn | 0.928863192 | 2.89855314 | 0.027631965 | 0.003 | 0.084 |
| Waterbars-Low vs Waterbars-High | 0.4799075 | 1.511702107 | 0.073699496 | 0.144 | 1 |
| Waterbars-Low vs Waterbars-Med | 0.129177854 | 0.376037933 | 0.021641178 | 0.982 | 1 |
| Waterbars-Low vs Hydroseed | 0.451715549 | 1.40268069 | 0.062612181 | 0.167 | 1 |
| Waterbars-Low vs Rough and Loose | 0.529122552 | 1.713395526 | 0.072254331 | 0.09 | 1 |
| Waterbars-Low vs Burn | 1.17759028 | 3.636248124 | 0.053761825 | 0.001 | 0.028 |
| Waterbars-High vs Waterbars-Med | 0.428466334 | 1.350029437 | 0.077811363 | 0.174 | 1 |
| Waterbars-High vs Hydroseed | 0.350350539 | 1.167661178 | 0.055162503 | 0.257 | 1 |
| Waterbars-High vs Rough and Loose | 0.282782848 | 0.9844822 | 0.044780777 | 0.388 | 1 |
| Waterbars-High vs Burn | 0.910000671 | 2.871619748 | 0.043594188 | 0.002 | 0.056 |
| Waterbars-Med vs Hydroseed | 0.305201949 | 0.945707601 | 0.049916721 | 0.469 | 1 |
| Waterbars-Med vs Rough and Loose | 0.384850814 | 1.252046519 | 0.06182321 | 0.211 | 1 |
| Waterbars-Med vs Burn | 0.762787953 | 2.353273619 | 0.037145257 | 0.009 | 0.252 |
| Hydroseed vs Rough and Loose | 0.221728993 | 0.754062508 | 0.03174457 | 0.634 | 1 |
| Hydroseed vs Burn | 0.775846828 | 2.436779143 | 0.036134275 | 0.011 | 0.308 |
| Rough and Loose vs Burn | 0.767870976 | 2.445151745 | 0.03572425 | 0.01 | 0.28 |