ARTHROPOD RECOVERY IN POST-MINE RECLAIMED SITES: THE EFFECTS OF RECLAMATION AGE AND BIOSOLIDS AS A SOIL AMENDMENT ON ARTHROPODS

by

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Abstract

Mining is a significant disturbance on natural ecosystems and mining companies are required to reclaim disturbed lands post mine-closure. This observational study addressed three research questions based on the foundation of using DNA barcoding of arthropods as a new tool for assessing reclamation. First, this study evaluated if differences in arthropod assemblage and biodiversity are visible between sites representative of reclamation ages ('new,' 'old' and 'reference') and soil amendments ('biosolids,' 'no biosolids' and 'reference'). Second, this study assessed species richness in relation to reclamation age and soil amendment. Third, this study assessed if any taxa can be used as indicators of reclamation age and soil amendments. Arthropod samples were obtained in 2018 from Teck Resources Highland Valley and New Gold Inc. New Afton. Arthropods from pitfall traps were processed by extracting DNA and identifying taxa through DNA metabarcoding. Based on the results, the dissimilarity of arthropod assemblage between the reclamation age and amendment sites implied another external factor is a stronger driver. Second, despite treatment correlations with order-level taxa, there was not a statistically significant relationship of the overall richness between the sites. Third, indicator species analyses identified several taxa uniquely associated with age and amendment sites. It is also interesting that there were no invasive taxa representative of the study sites. Using novel methods (high-throughput DNA metabarcoding), this project contributes to the improvement of planning and management practices, leading to more effective post-mining ecosystem-recovery outcomes, as they relate to the sustainable health of ecosystems, which are vital to the continued growth of BC's communities and economy.

Keywords: biodiversity; ecosystem reclamation; arthropods; environmental DNA barcoding

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Dedication

In memory of my father, whose many weekends spent outdoors with me encouraged my love of nature and inspired me to pursue my interest in environmental science.

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Chapter 1 Introduction

Anthropogenically influenced ventures such as industry and urban development are in place to satisfy societal and economic demands. However, these activities alter natural habitats and ecosystems. Habitat alteration is the suspected leading cause of global extinction (Barnosky et al., 2011; Cardoso et al., 2020). Increasing extinction rates contribute to losses in species abundance and biomass, phylogenetic diversity, functional diversity, ecological networks, as well as differences in space and time (e.g., phenology and distribution) (Cardoso et al., 2020). Reclamation and restoration of altered habitats could reduce impacts of ecological consequences from industry and urban development.

Mining, a natural resource industry, is the act of extracting mineral resources from the planet. These extracted resources are used in the making of the technological items used in our everyday lives and have significant economic benefits. For example, in 2017, mining in BC was responsible for generating $11.7 billion in gross revenue and creating over 10,000 jobs for British Columbians (Mining Association of BC, 2019). The mining industry employs more Indigenous peoples proportionally than any other private sector in Canada (Mining Association of Canada, 2019a), which demonstrates responsible industry practice involving stakeholders and landowners. Mining activity significantly alters the landscape and native ecosystems through processes such as heavy metal contamination, acid rock drainage, erosion, sedimentation, hydrology modification, habitat loss, and rare species loss (Canadian Mining Innovation Council, 2013).

Mineral mining involves rock being removed from the ground and stored in stockpiles as waste rock or milled to separate the desired mineral from rock as ore. Waste rock is can vary in size from boulder size to gravel. Comparatively, tailings are typically similar to sand in size. Both of these products are uneconomic waste (waste rock and tailings, respectively) that need to undergo reclamation. To that end, the Canadian mining industry must adhere to federal, provincial, and territorial acts and regulations regarding reclamation (Mining Association of Canada, 2019b). Of note, it is mandatory that planning for mine closure takes place before mining companies begin production (Mining Association of Canada, 2019c). Mine closure includes ecosystem reclamation, returning the altered lands to a functioning, self-sustaining ecosystem. Reclamation in BC addresses terrestrial areas, water bodies, and cultural resources (Government of British Columbia, 2019). Natural ecological succession, the process of ecosystem change over time, occurs without human intervention; however, in the context of mine closure, the time scales may be too long to be acceptable for the public, industry, or Indigenous groups; therefore, active reclamation practices are required.

Reclamation and restoration are commonly confused. Reclamation is the return of a functioning and self-sustaining ecosystem. In contrast, restoration is to return the disturbed land to its initial state (e.g., pre-mining). Post-mining restoration can be difficult, if not impossible, to accomplish as a result of altered habitat, hydrology, and potential for metal contamination (Lima et al., 2015). In British Columbia, planning towards post-mining end-land use objectives is required. The identified end land use objectives can then inform reclamation planning and practices. End Land Use Plans can enhance a community-based approach to reclamation. Specifically, End Land Use Plans can incorporate input from local Indigenous peoples in terms of reclamation goals, as well as establish possible post-closure land uses that are feasible and important to local communities (Melaschenko et al., 2018).

1.1 Soil Amendments

During the mining process, topsoil is removed, reducing the amount of organic matter on site (Larney and Angers, 2012). Organic matter loss negatively alters soil productivity via physical, chemical, and biological processes, thus generating a need for soil amendments during reclamation (Larney and Angers, 2012). Tailings pose reclamation challenges given their lack of organic materials, their physical structure, the presence of toxic metals, and their tendency to be nutrient poor (Hossner and Hons, 1992). Organic amendments, such as biosolids, woodchips, compost, and manure, may address these limitations by adding organic matter vital to soil productivity (Larney and Angers, 2012). Organic amendments may also provide nutrients and structure that improve physical, chemical, and biological soil characteristics (Larney and Angers, 2012).

Biosolids (treated municipal wastewater solids) as a soil amendment have multiple positive effects during mine reclamation because they are made up, largely, of organic matter (up to 50%) and are high in nutrients (Lu et al., 2012). As a result, biosolids promote soil stabilization, porosity, drainage, water and cation exchange, aeration, and support diverse communities of microbes and soil fauna (Lu et al., 2012; Larney and Angers, 2012). The ultimate effect of the amendment will depend on the individual site and amendment characteristics (Larney and Angers, 2012).

British Columbia regulates biosolids under the Organic Matter Recycling Regulations (OMRR). Specifically, biosolids are classified under either class A biosolids, biosolids growing medium, and class B biosolids, as determined by the upper limit of elements within the biosolids (Table 1.1). The upper limits of class A biosolids are not included in the below table, as they are calculated under the Canadian Food Inspection Agency based on predicted accumulation over 45 years (Government of Canada, 2022).

Table 1.1 Upper limits of substances (µg/g dry weight) allowable in class biosolids growing medium and class B biosolids (OMRR, 2019).

Substance (µg/g dry weight)	**Biosolids growing medium**	**Class B biosolids/ Class B Compost**
Arsenic	13	75
Cadmium	1.5	20
Chromium	100	1 060
Cobalt	34	150
Copper	150	2 200
Lead	150	500
Mercury	0.8	15
Molybdenum	5	20
Nickel	62	180
Selenium	2	14
Zinc	150	1 850

1.2 Ecological Succession and chronosequence

In the context of mine reclamation, ecological succession, as first modelled by Clements (1916), is important to understand as post-mining disturbed sites move towards a functioning ecosystem. Clements' model described ecological succession as change in a community where an inhabiting group of organisms modifies an area, leaving it more hospitable so that the group of organisms is replaced by another group of organisms. More recently, Connell and Slatyer (1977) developed the tolerance and inhibition succession models. The tolerance model is based on success of later species, regardless of the presence of earlier species before them. In the tolerance model, plants species are able to establish and mature at lower levels of nutrients. The inhibition model is based on the theory that later species and earlier species do not co-exist, with the later species replacing the early species following local disturbance.

No matter what the model, two types of succession can be broadly defined: primary and secondary. Primary succession occurs in areas where vegetation has not occurred, such as deglaciated areas (Wali, 1999). Secondary succession occurs in areas that have previously been colonized by vegetation but have been disturbed by events such as forest fires, heavy grazing, or logging (Wali, 1999).

As disturbed areas undergo vegetative succession wildlife habitats, plant-animal, and plant-animal-microbe interactions will develop (Wali, 1999; McKelvey, 2015). An interesting experiment assessing arthropod recovery in a given area is Simberloff and Wilson’s study (1969) which monitored the recovery of arthropods on mangrove islands after defaunating the islands. Simberloff and Wilson (1969) found that within a year, arthropod assemblages and diversity on treated islands were comparable to arthropod assemblages and diversity on islands that were not defaunated. It is also interesting to note that strong flying arthropods and non/weak flying arthropods were initial immigrants on the islands and that ants were one of the last species to recover on the defaunated islands (Simberloff and Wilson, 1969). Moreover, oscillations in the number of arthropod species present on the islands indicated a dynamic equilibrium (Simberloff and Wilson, 1969). Understanding of arthropod recovery overtime in disturbed areas can further our knowledge of reclaiming post-mined ecosystems.

The long-term trajectory of reclaimed areas can be studied using a chronosequence. A chronosequence is an approach to a study where multiple sites are sampled to assess the effects of treatment over time, as opposed to sampling the same sites over time (which could take decades). The duration of time since a site has been reclaimed affects soil development (Adeli et al., 2013). Chronosequencing exemplifies varying degrees of ecological succession consisting of varying ecological condition factors (Walker et al, 2010). Abiotic and biotic conditions can impact animal habitats, including arthropods. For example, Li et al., (2018) studied arthropod response in a reclaimed poplar (*Populus deltoides*) plantation over a chronosequence. They found that soil arthropod assemblages varied along the chronosequence. In order to achieve a functioning ecosystem, the ecosystem must be comprised of biotic and abiotic components that interact as a system and operate as a whole through the transfer of energy and cycle of nutrients. An important measure of the biotic component is biodiversity, particularly functional biodiversity.

1.3 Biodiversity

Biodiversity is often described in terms of genetic diversity and relationships in a given area (Gaston and Spicer, 2004). Furthermore, ecological factors such as soil microbes, vegetation, and animal diversity can influence each other (Bennett, 2010). These relationships can have both top-down and bottom-up impacts to a reclaimed ecosystem (Bennett, 2010). It is critical to understand what species are present and how they relate to specific ecosystem functions (Prach and Tolvanen, 2016). For example, despite an area of high biodiversity being perceived as positive for ecosystem functioning, it may be made up of specialist, generalist, and/or invasive species (Prach and Tolvanen 2016). Therefore, measuring biodiversity indicators can assist in manageable ecosystem factors (Prach and Tolvanen 2016). Alpha diversity is a common measure of biodiversity; it is the number of unique taxa in a given area. Alpha diversity, or species richness, is an accessible biodiversity measurement, informs of taxa in an area and can be monitored over time or compared to another area.

1.4 Arthropods

Arthropods make up a significant portion of species biodiversity, are a key factor in ecological succession, and provide important ecosystem services (McGeoch et al., 2011) including soil formation in reclaimed areas, nutrient turnover, decomposition, litter breakup, herbivory, pollination, acting as dispersal agents, and serving as food resources for wildlife (Majer, 2002).

Alternatively, arthropods can be perceived to play negative ecological roles, such as being vectors for disease or as being a nuisance for agriculture and forestry. Many arthropods are herbivorous, and therefore, they may contribute to plant species composition by changing competitive dynamics within the plant community (Yu et al., 2012; Barnett and Facey, 2016). On the other hand, arthropod composition is affected by plant composition through a bottom-up effect whereby vegetation structure and species impact arthropod habitat (Barnett and Facey, 2016). Generally, a high level of arthropod diversity is optimal so that a complete range of ecosystem functions is achieved (Majer et al., 2002).

Arthropods are sensitive to environmental change (Buchori et al., 2018), and changes in their geographic distribution, fecundity and diversity are good indicators of change (Samways et al., 2010). As such, arthropod diversity metrics can be used to draw comparisons between different landscapes to evaluate, for example, land reclamation efforts (Gerlach et al., 2013).

However, because of the complexities of identifying arthropods taxonomically, they have not historically been used to monitor or assess reclamation strategies. Recent progress in DNA metabarcoding, a molecular species identification technique, has helped overcome challenges in the taxonomic identification of arthropod (Fernandes et al., 2018; Beng et al., 2016). In this study, I will assess arthropod assemblage response to mine reclamation using DNA metabarcoding.

1.5 DNA metabarcoding

High-throughput DNA metabarcoding is an identification tool that relies on amplification and sequencing of DNA barcodes (short nucleotide sequences) from whole communities rather than relying on identification of individual specimens from a community. Briefly, DNA is extracted from a sample containing many homogenized arthropods collected in a trap prior to using polymerase chain reaction (PCR) to amplify a barcode region that can be used for taxonomic purposes, such as the mitochondrial cytochrome c oxidase subunit 1 gene (CO1) (Ji et al., 2013). Next, clustering algorithms can be used to group sequences into Operational Taxonomic Units (OTUs) prior to taxonomy assignment against public sequence data collections, such as the Barcode of Life Database (BOLD) (Palmer et al., 2018; https://www.boldsystems.org/). BOLD is a curated database that captures plant, fungal, bacterial, and animal biodiversity and includes phylogenetically relevant barcode sequences alongside traditional taxonomic information. In this study, arthropods captured in pitfall at 19 sites were characterized using COI metabarcoding. Additionally, 16 individual samples were submitted for curation and inclusion in the BOLD database in an effort to improve taxonomy assignment for samples collected at Highland Valley Copper and New Afton. Specifically, this study will use DNA metabarcoding as a foundation to assess arthropod response to reclamation age and biosolids as a soil amendment in two mines in the interior of British Columbia.

1.6 Significance

The study outlined above will work towards reducing knowledge gaps regarding post-mining reclamation outcomes by examining arthropod assemblage composition as an indicator of reclamation trajectory. Additionally, this work will evaluate high throughput DNA metabarcoding of arthropod communities as a tool for planning, managing, and improving post-mining ecosystem reclamation, an activity that is vital to the sustainability of BC's natural ecosystems, communities, and economy.

Researchers have pointed out the lack of information addressing the outcomes of mine reclamation (Buchori et al., 2018; Fernandes et al., 2019), especially regarding arthropod recovery. More research is needed to fully understand mine reclamation success towards re-creating a functioning ecosystem.

1.7 Research questions

This study will address three research questions, based on the foundation of using DNA metabarcoding of arthropods as a new tool for assessing mine reclamation. Firstly, this study will assess whether we can identify changes in arthropod assemblages from sites with different reclamation ages ('new,' (10 years and newer) 'old,' (14 years and older) and reference) and soil amendment ('biosolids,' 'no biosolids,' and 'reference). Secondly, this study will assess the effects of reclamation age and soil amendment on arthropod alpha diversity. Thirdly, this study will examine if any arthropod taxa are indicators of reclamation age or soil amendment. This thesis is made up of three chapters (including this chapter). Chapter two includes data analyses that will answer the above research questions. Chapter three addresses management implications and recommendations for mine reclamation, such as environmental monitoring, based on the findings.

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Chapter 2 Investigating the Effects of Reclamation Age and Biosolids Amendment on arthropods Using DNA Metabarcoding

2.1 Introduction

A global decrease in ecosystem function and biodiversity highlights a need for ecosystem reclamation (Bullock et al., 2011). In particular, post-mining reclamation aims to return altered lands to self-sustaining and functioning ecosystems, inclusive of waterbodies, terrestrial areas, and cultural resources. As a result of resource extraction processes, post-mine reclamation is faced with unique physical challenges, such as altered topography and hydrology (Shrestha and Lal, 2006), and unique uneconomic by-products, including waste rock and tailings.

Tailings and waste rock can result in negative environmental impacts through the release of trace heavy metals, and the introduction of substrate lacking organic material, structure, and nutrients to healthy soils (Hossner and Hons, 1992). Additionally, tailings and waste rock dust (particulate matter) can pose a risk to human respiratory health (EPA, 2020). Therefore, the Canadian mining industry must adhere to federal, provincial, and territorial acts and regulations regarding reclamation (Mining Association of Canada, 2019).

While natural succession occurs in post-mining areas without human intervention, it requires decades to restore (Bradshaw, 1997). The timescales for post-mining areas to return to functioning ecosystems are unacceptable for industry and the public. The duration of time since an area was reclaimed (reclamation age) influences soil development (Adeli et al., 2013). Soil biotic and abiotic conditions such as nutrient availability, texture, structure, and microbes will develop over time (Walker et al., 2010). These below-ground changes are typically correlated with above-ground changes as a result of ecological succession. These temporal changes can be monitored using a chronosequence (Walker et al., 2010). In this case, a chronosequence is the study of multiple sites that differ in time since they were reclaimed.

Currently, long-term post-mining reclamation outcomes and trajectories are not fully understood and there are knowledge gaps regarding community recovery of arthropods. It is currently understood that arthropod communities can be impacted by soil properties as well as vegetation diversity and structure (Buchori et al., 2018; Joern and Laws, 2013). Due to feedback loops between soil, plants, and arthropods, development in one of these three types of organisms can prompt change in another (Bennett, 2010). A meta-analysis conducted by Bennett (2010) outlines the various ways that plant-soil-arthropod dynamics can influence each other. For example, plant diversity influences arthropod diversity through mechanisms such as resource availability, limitation, and variation as well as through volatiles released by vegetation when herbivores consume or attack it. Likewise, soil microbial diversity can influence above ground arthropod diversity through indirect effects via plants, such as plant phenotypic variation and plant quality. Soil microbial diversity and plant diversity can influence each other through decomposition, mineralization, organic matter, moisture transportation, and allelopathy (Bennett, 2010; Walker and del Moral, 2003).

In addition to soil-plant-arthropod relationships, the stage of succession can influence plant composition and structure (Clements, 1916; Davy, 2002), as well as soil microbial and chemical composition (Allen et al., 2002; Ardeshir et al., 2013). For example, Pietrzykowski (2008) identified a greater level of plant diversity in sites as they aged over 20 years since reclamation. Soil nutrients, such as carbon, have also been found to increase since time of reclamation (Ardeshir et al., 2013). Therefore, it stands to reason that as the succession of reclaimed sites progresses (soil and vegetation), arthropod composition will also progress. Furthermore, given that arthropods are mobile and have rapid generational times they can quickly respond to environmental disturbance or recovery (Samways et al., 2010; Gerlach et al., 2013).

Arthropod response to their surrounding environmental factors is nuanced because different arthropod taxa have unique sensitivities to their surrounding environments (Sylvain et al., 2019; Buchori et al., 2018). For example, arthropod habitat requirements can be unique for different arthropods; herbivores require plants, and predators and parasites are dependant on prey and hosts, respectively (Buchori et al., 2018). Some arthropods colonize early succession areas and can be drivers of soil development. In particular, Formicidae and Coleoptera taxa have been identified in studies as early colonizers in reclaimed areas (Varela and Garcia, 2017).

Furthermore, the presence of these taxa before the presence of additional taxa can be interpreted as indicators of environmental conditions or reclamation stage (Buchori et al., 2018).

To return a post-mining reclaimed area to a functional ecosystem, both biotic and abiotic factors must be considered. Biodiversity, the variety of biota in an area accounting for phylogenetic diversity, trophic structure, and genetic diversity (Gaston and Spicer, 2004), can be used as a measure of ecosystem (biotic) health (Hector and Bagchi, 2007). Historically, for mine reclamation, focus has been given to vegetation cover and diversity (Fraser et al., 2015), with less attention given to arthropods such as assemblage composition, diversity, and indicator taxa.

In terms of biotic factors, soil health is an important variable to address (Bradshaw, 1997), with exogenous organic amendments often being used to address soil health limitations by providing nutrients and improving physical characteristics (Larney and Angers, 2012). Compared to organic amendments such as biosolids, inorganic fertilisers do not provide long-term benefits to soil physical and chemical characteristics, and does not benefit vegetation establishment (Gardner et al., 2010).

An example of an organic soil amendment are biosolids made from treated municipal wastewater solids, with treatment and stabilization to reduce pathogens (Government of British Columbia, 2019). Unless otherwise used, biosolids may be disposed of in landfills, or incinerated (BC Ministry of Environment and Climate Change Strategy, 2020). Biosolids are often used as a soil amendment, applied in reclaimed areas, such as mines, in countries including the United States, Australia, New Zealand, the United Kingdom, member states of the European Union, and Canada (Larney and Angers, 2012; Christodoulou and Stamatelatou, 2016).

Biosolids contribute to soil stabilization; improve porosity, drainage, aeration, water and cation exchange; and improve microbial communities by being a source of food for soil microbes (Lu et al., 2012). Furthermore, biosolids release nutrients into the ground more slowly and for a longer duration than chemical fertilisers (Lu et al., 2012). The application of biosolids as a soil amendment at post-mine reclaimed sites typically only requires a one-time application, opposed to alternative amendments (such as fertiliser) requiring annual applications. However, hormones and heavy metals can also be found in biosolids (Lu et al., 2012) and, for this reason, biosolids are provincially regulated to limit the concentration of specific substances according to the British Columbia OMRR policy. Despite government regulations, there can be public concern regarding the movement of heavy metals within the nutrient cycle between trophic levels.

However, hypotheses such as the plateau hypothesis aim to answer these concerns (Lu et al., 2012). The plateau hypothesis states that trace metals are not available for uptake by plants as a result of being retained or 'tightly held' by the soil and biosolids (Lu et al., 2012).

Biosolids, have been shown to enhance vegetation biomass (Gardner et al., 2012). For example, Gardner et al. (2012) found that, in a reclaimed mine environment, vegetation establishment was substantially reduced on sites amended with fertilizer and sites with no added soil amendment. This result was theorized to be caused by the addition of organic matter and nutrients from biosolids.

Gaudreault et al. (2019) found that, in a grassland ecosystem, grasshoppers were more abundant (higher in numbers) on sites amended with biosolids. This scenario illustrates a bottom-up controlled ecosystem, as biosolids act as a source of nutrients where nutrients are limited (Larney and Angers, 2012), and are associated with increased vegetation biomass (Gardner et al., 2012), ultimately impacting arthropod habitat (Gaudreault et al., 2019). That being said, I found research focused on understanding the relationships between fauna and biosolids, used as a soil amendment, is relatively limited.

Globally, there are 1.5 million described arthropod species, representing up to 80% of global animal biodiversity (Zhang, 2013). Furthermore, arthropods benefit their surrounding environment by providing ecosystem services (McGeoch et al., 2011) such as nutrient cycling, pollination, seed dispersal, soil aeration, organic matter decomposition, and as food for wildlife (Majer et al., 2002). Arthropods are good environmental indicators due to their relatively short generation times, high fecundity, mobility, and sensitivity to environmental change (Samways et al., 2010).

To better understand post-mine reclamation trajectories, holistic monitoring of flora, fauna and microbial communities is needed (Fraser et al., 2015). Previous post-mining reclamation techniques focussed on vegetation (Holl, 2002; Cavender et al., 2014), while more recent explorations using DNA metabarcoding technologies have targeted microbial (bacteria, fungi, and protists) communities to monitor reclamation (Francioli et al., 2021; Rosenfeld et al., 2018). Newer studies have approached reclamation by examining whole ecosystems, specifically addressing biodiversity and functional services (Fraser et al., 2015). That being said, arthropods have infrequently been used as a terrestrial biomonitoring tool because of the complexities of identifying them.

Morphologically identifying arthropods is time consuming, costly, and requires specific scientific expertise (Ji et al., 2013). Additionally, larval specimens may not be identifiable using traditional morphological methods (Yu et al., 2012). Studies that do use arthropods as indicator species often focus on a few indicator species, rather than capturing overall arthropod community diversity (Hammond et al., 2018). The assessment of an indicator species does not provide insight into arthropod assemblage composition and community dynamics (Siddig et al., 2016; Fernandes et al. 2019). Recent progress in molecular identification techniques such as deoxyribonucleic acid (DNA) metabarcoding has helped to overcome challenges in the taxonomic identification of arthropods and other invertebrates when founded on morphologically identified reference specimens (Hebert et al., 2003). DNA barcoding is conducted by extracting DNA from a specimen prior to using the polymerase chain reaction (PCR) to amplify a phylogenetically distinctive genetic marker by using specific primers. For individual specimens, Sanger sequencing is used, while if DNA is extracted from homogenized mixtures of arthropods, high throughput metabarcode sequencing is used (Ji et al., 2013).

Studies that have sequenced animals, including arthropod, barcodes often target the cytochrome c oxidase subunit 1 (CO1) gene (Hebert et al., 2003; Ji et al., 2013; Beng et al., 2016). The CO1 gene is a good target gene because it is present in all animals (Hebert et al., 2003), has a relatively short sequence length making amplification through PCR easy, and contains ample nucleotide variation to differentiate taxa (Hebert et al., 2004; Wang et al., 2018).

DNA barcoding and metabarcoding relies on databases such as the Barcode of Life Database (BOLD) to assign taxonomies to the sequenced target genes. This approach can be used to assign taxonomies to a wide range of biota, such as vegetation, fungi, bacteria, and animals (Hebert et a., 2003). The international Barcode of Life Database is evolving based on public submissions and has the potential to identify all multicellular species, creating a ‘library of life,’ which can be used to establish ‘global biosurvalliance program’ (International Barcode of Life, 2021).

For metabarcoding efforts, sequences are first clustered into operational taxonomic units (OTUs) prior to taxonomy assignment. Reclamation trajectory can be monitored by comparing OTU assemblages between reclaimed sites and undisturbed sites (Fernandes et al., 2019; Ji et al., 2013). Furthermore, patterns in reclamation can be identified by categorizing and analyzing arthropod taxonomies (e.g., family, order) to identify indicator taxa. For example, Biaggini et al.

(2007) found that higher taxa, such as order, can inform on primary agricultural land uses (grazed, cultivated, undisturbed) based on diversity and assemblage composition. Specifically, arthropod order assemblage characterized land use in sites even when the sites were located near each other and were small (30 m x 10 m) (Biaggini et al., 2007).

The aim of research on post-mining ecosystem reclamation is to reduce knowledge gaps and to further understanding of reclamation trajectories, thus leading to improved reclamation practices. This study will address three research questions based on the foundation of using DNA metabarcoding of arthropods as a tool for assessing reclamation. First, this study will assess whether we can identify (dis)similarities in arthropod assemblage with different reclamation ages (‘new,’ ‘old,’ and reference) and a soil amendment (‘biosolids,’ ‘no biosolids,’ and ‘reference). Second, this study will assess arthropod alpha diversity between sites with different reclamation ages and amendments. Thirdly, this study will examine if the presence of specific arthropod taxa are indicators of reclamation approach and duration (i.e., soil amendments, reclamation age).

2.2 Methods

Data Collection

Arthropod samples were collected during July and August 2018 at Teck Resources Highland Valley Copper and New Gold Inc. New Afton mine sites (Figure 2.1) when the average temperature in Kamloops, BC was 22.1°C, 20.2°C and the total precipitation was 14.5 mm and 7.8 mm, respectively (Government of Canada, 2022).

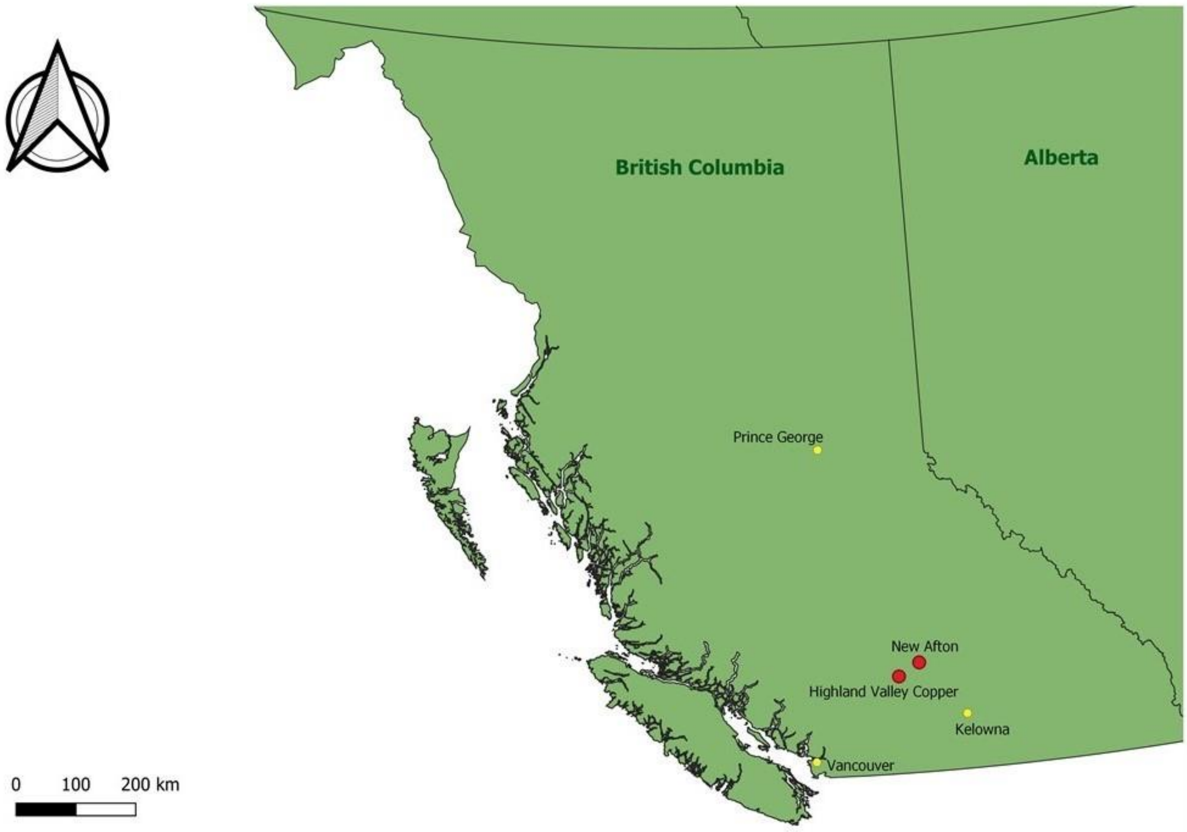

Figure 2.1. Location of mines sampled in July and August 2018 for arthropods in BC. Map created in QGIS using Statistics Canada boundary file projected using NAD83/BC Albers (Statistics Canada, 2021).

At each mine, two treatment areas were sampled based on reclamation age and amendment use (Table 2.1). The sample sites were categorized as 'new' (10 years and newer) or 'old' (14 years and older), as described in Table 2.1. Sites were also categorized into sites amended with 'biosolids' and 'no biosolids'. Two reference sites were sampled near both Highland Valley Copper (forest sites) and New Afton (grassland sites). Maps were created in QGIS to illustrate the sample locations. A total of 19 sites were sampled at Teck Resources Highland Valley Copper (Figure 2.2, Figure 2.3, Figure 2.4) and New Gold Inc. New Afton (Figure 2.5, Figure 2.6). The Highland Valley Copper mine and New Afton mine differ by biogeoclimatic (BEC) zone. BEC zones are used to characterize unique ecosystems (climate and vegetation) in British Columbia (Mackinnon et al., 1992). New Afton mine is located in both the very dry warm Bunchgrass variant (BGx1) and the very dry hot ponderosa pine variant (PPxh2). Comparatively, the sites sampled Highland Valley Copper mine are located in the very dry Montane Spruce (MSxk2) variant. In general, the Bunchgrass zone occurs between 700 meters and 1000 meters above sea level and is characterized by some of the hottest and driest conditions in BC and the absence of trees (Alldritt-McDowell and Coupé, 1998). The Ponderosa Pine zone, often found just above the bunchgrass zone, is the driest forested zone in BC (Alldritt-McDowell, 1998). The Montane spruce zone, typically occurring between 1250-1650 meters, is typified by cold winters and short, dry summers (Alldritt-McDowell and Lloyd, 1999).

Table 2.1 Teck Resources Highland Valley Copper and New Gold Inc. New Afton site descriptions outlining the material reclaimed, reclamation age category, biosolids application, and subsequent seeding, for 2018 sampled sites.

Mine	Site	Reclaimed materials	Reclamation age category	Year seeded	Biosolids application
Teck Resources Highland Valley Copper	Treatment site 1	Tailings, overburden	old	2004	No biosolids
	Treatment site 2	Tailings, overburden	new	2013	Biosolids (2013)
	Treatment site 3	Tailings	new	2012	Biosolids (2011)
	Treatment site 4	Tailings, overburden	old	2004	Biosolids (1998)
	Treatment site 5	Tailings, overburden	new	2008	Biosolids (2000, 2007)
	Treatment site 6	Waste rock, overburden	old	1999	Biosolids (1999)
	Treatment site 7	Waste rock, overburden	old	1999	Biosolids (1999)
	Treatment site 8	Waste rock, overburden	new	2015	Biosolids (2014)
	Treatment site 9	Waste rock, overburden	new	2015	Biosolids (2014)
	Treatment site 10	Waste rock, overburden	old	1999	No biosolids
	Treatment site 11	Waste rock, overburden	old	1992	No biosolids
	Treatment site 12	Waste rock, overburden	old	1994	No biosolids
	Treatment site 13	Waste rock, overburden	old	1998	No biosolids
	Reference site 1	n/a	n/a	n/a	n/a
	Reference site 2	n/a	n/a	n/a	n/a
New Gold Inc. New Afton	Treatment site 14	Tailings	old	2001	No biosolids
	Treatment site 15	Tailings	new	n/a	No biosolids
	Reference site 3	n/a	n/a	n/a	n/a
	Reference site 4	n/a	n/a	n/a	n/a

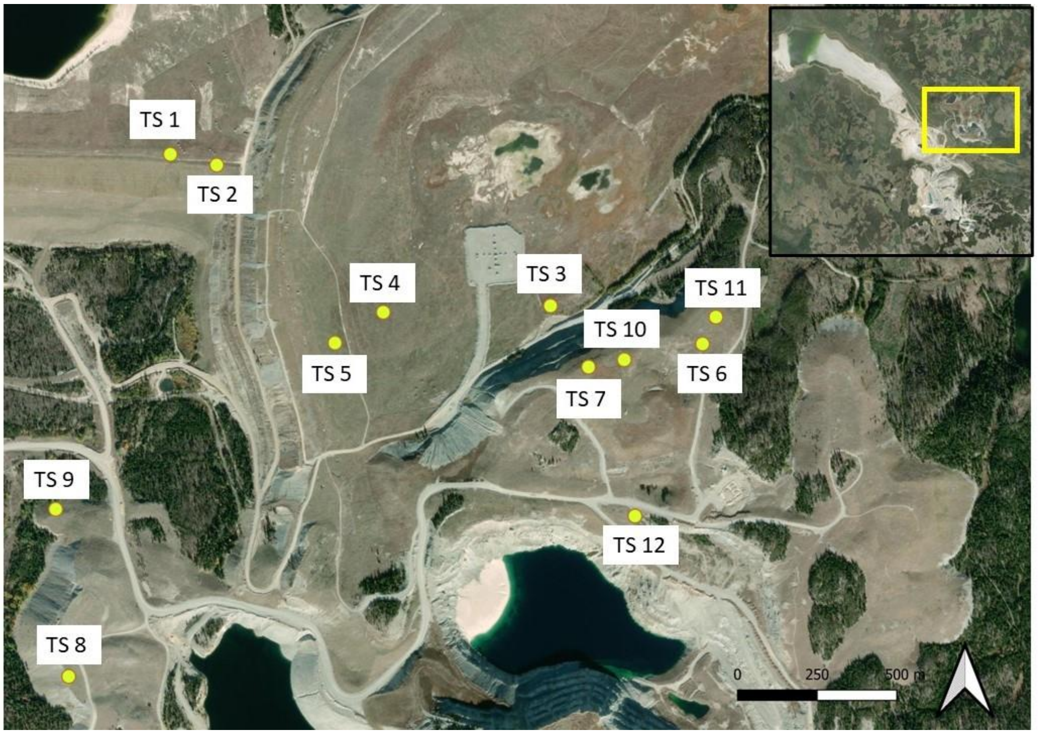

Figure 2.2. Map of Teck Resources Highland Valley Copper sites sampled for arthropods (Treatment Sites (TS) 1-12) in August 2018 created in QGIS using ESRI Satellite base map and projected using NAD83/BC Albers) (ESRI, 2017).

Figure 2.3. Map of Teck Resources Highland Valley Copper sites sampled for arthropods in August 2018 (Treatment site (TS) 13) created in QGIS using ESRI Satellite base map and projected using NAD83/BC Albers (ESRI, 2017).

Figure 2.4. Map of references sites near Teck Resources Highland Valley Copper sampled for arthropods in August 2018 (Reference Sites (RS) 1-2) created in QGIS using ESRI Satellite base map and projected using NAD83/BC Albers (ESRI, 2017).

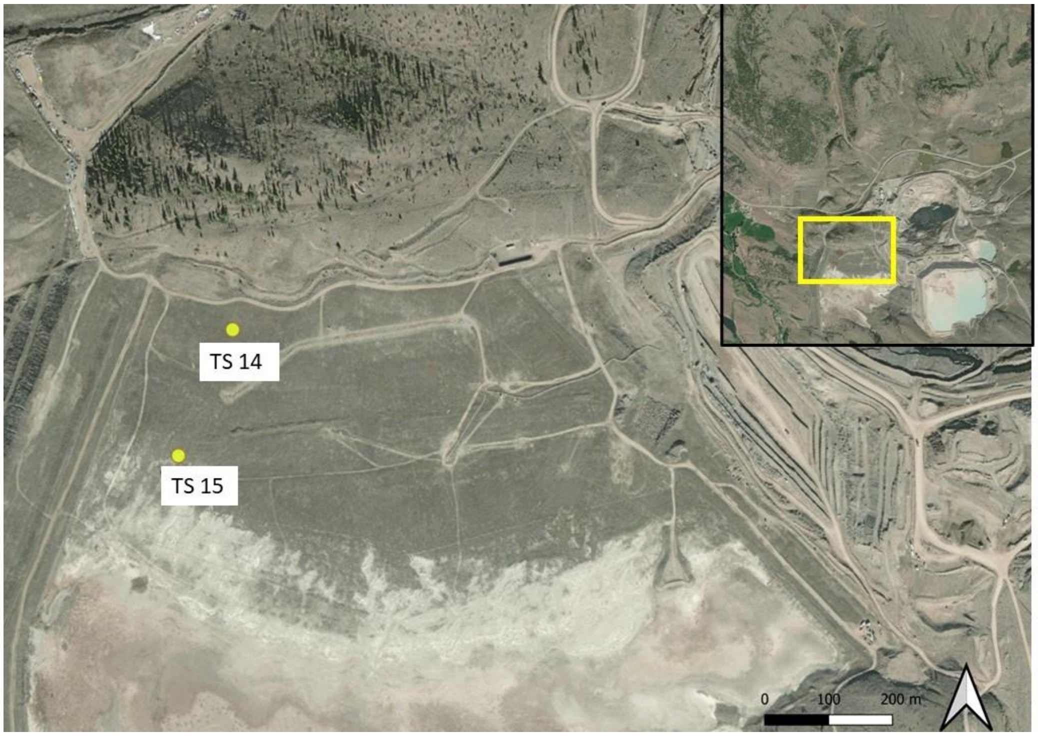

Figure 2.5. Map of New Gold Inc. New Afton July 2018 sampled for arthropods in July 2018 (Treatment Sites (TS) 14-15)) created in QGIS using ESRI Satellite base map and projected using NAD83/BC Albers (ESRI, 2017).

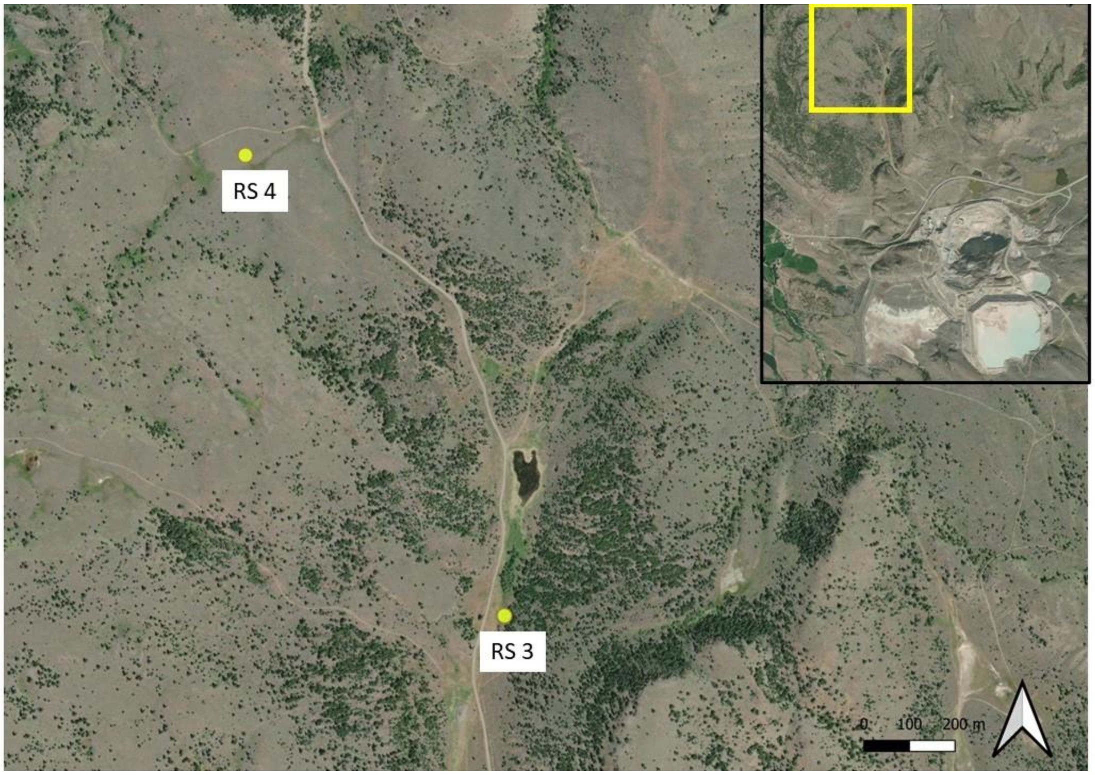

Figure 2.6 Map of reference sites near New Gold Inc. New Afton sampled for arthropods in July 2018 (Reference Sites (RS) 3-4) created in QGIS using ESRI Satellite base map and projected using NAD83/BC Albers (ESRI, 2017).

At each site, traps were set up to collect both flying and ground-dwelling arthropods. To collect flying arthropods, tent-like structures called Malaise traps (Figure 2.7) were used (Thomas, 2016; Lynggaard et al., 2020). One Malaise trap was constructed at each site.

Figure 2.7. Malaise trap set up to capture flying arthropods. Photo taken by Lauchlan Fraser.

Epigeal arthropod samples were collected in a 40 m x 40 m grid layout (

Figure *2.8*). Nine pitfall trap samples were collected in three rows of three within the 40 m x 40 m grid .At each of the nine sample areas, a pitfall trap was set up. Pitfall traps were used to collect ground-dwelling arthropods (Bassett and Fraser, 2015) (Figure 2.9). Pitfall traps were assembled by inserting a 450-g container (Solo® cup) in the soil, flush to the ground. The cups were filled with an 87% denatured ethanol solution. A wooden board was placed approximately 5 cm above the ground, held by nails, to reduce ethanol evaporation as well as reduce the potential for any wildlife from falling into the pitfall trap, or removing sample specimens from the pitfall trap.

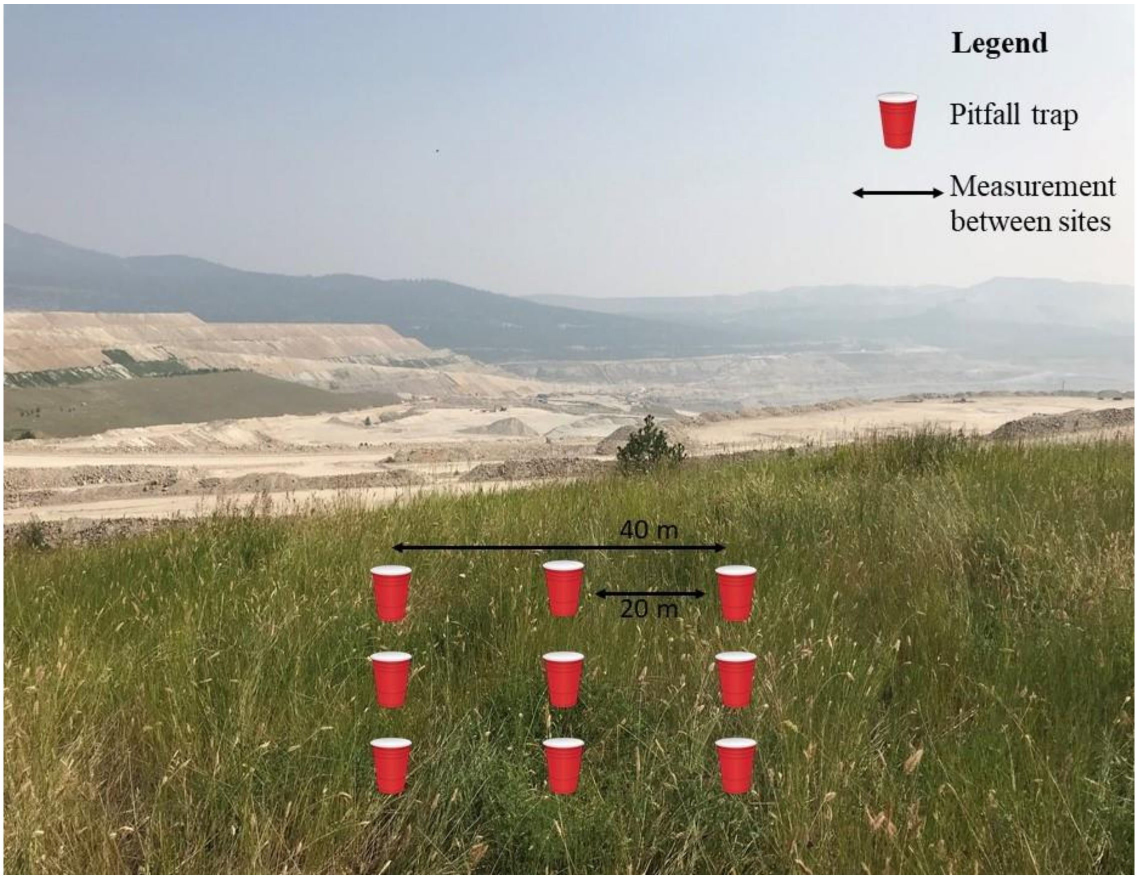

Figure 2.8 2018 Pitfall trap layout that was used to collect epigeal arthropods at Highland Valley Copper. Photo taken by Chantalle Gervan.

Figure 2.9. Sampling of epigeal arthropods using a pitfall trap consisting of A) a 450 g container placed flush with the ground , and B) a plastic plate over the top to reduce ethanol evaporation. The pictured plastic plates were used instead of wooden boards in 2017. Photos taken by Chantalle Gervan.

Malaise traps and pitfall traps remained in the ground for five days in the summer (Lynggaard et al., 2020; Foster et al., 2020). After five days, the trap contents (ethanol and arthropods) were collected and stored in a freezer -20°C until laboratory processing. Due to access constraints at Highland Valley Copper, arthropod traps at some sites were collected after six days (TDA12A1, BMJ11A, BM98B, BM98A) or seven days (BND95e, BN92A, BN99A1, BN99B).

Two pitfall traps were removed from each site with the intention of sending them to the Centre for Biodiversity Genomics at Guelph University to be Sanger-sequenced and added to the Barcode of Life Database (BOLD). Sanger-sequenced specimen are individually morphologically identified by an expert then individually sequenced to determine a portion of that individual specimen’s nucleotide sequence of their genome. Separating the samples to submit to the Centre for Biodiversity Genomics was done to ensure that arthropods collected in this region were available in the barcode of Life database for identification. However, due to complications caused by COVID-19, the samples were not sent.

In 2017 four mines were sampled (Teck Resources Highland Valley Copper, New Gold Inc. New Afton, Avino Silver and Gold Mine Ltd. at Bralorne, and Imperial Metals Corporation Mount Polley) but are not included in this study due to differences in sampling methodologies.

The contents of twelve pitfall traps and four malaise traps from the 2017 samples were sent to the Centre for Biodiversity Genomics at Guelph University to be Sanger sequenced and added to the Barcode of Life database. This was to ensure that arthropods collected in this region were available in the Barcode of Life database for identification.

DNA extraction, PCR amplification, and sequencing

Identification of the collected arthropods was conducted using high-throughput DNA metabarcoding targeting a 402 base-pair region of the mitochondrial cytochrome c oxidase subunit one gene (CO1 gene). The CO1 gene has approximately 650 base-pairs.

Individual arthropod specimens that measured >5 mm were subsampled by removing the body below the head and retaining the head for DNA extractions (Foster et al., 2020; modified from Beng et al., 2016), while for those that were <5 mm, whole specimens were used. The tissues were homogenized in liquid nitrogen with a sterilized mortar and pestle (Beng et al., 2016). DNA was extracted from the homogenized tissue using a Mag Bind® Blood and Tissue Kit (Omega Bio-tek, Inc., Norcross, GA), according to the manufacturer’s instructions (Figure 2.10).

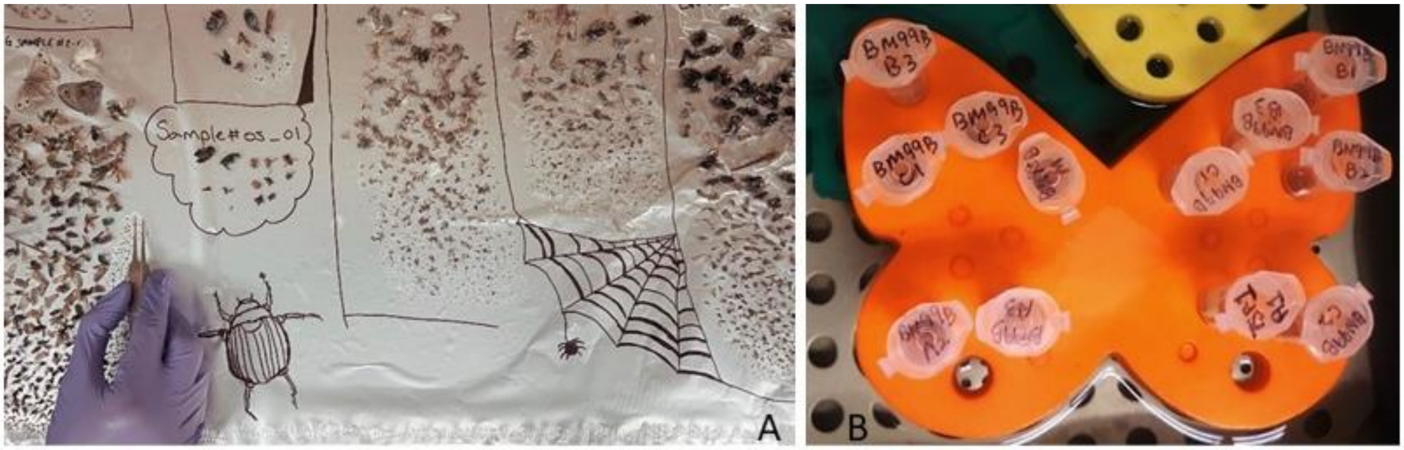

Figure 2.10 Collected arthropods being prepared for sequencing: A) Sorting specimens, previously stored in ethanol, to be homogenized in liquid nitrogen B) Samples in a hot water bath during DNA extraction process using Mag Bind® Blood and Tissue Kit (Omega Bio-tek, Inc., Norcross, GA) according to manufacturer’s instructions. Photos taken by Chantalle Gervan.

A 402 base-pair region of the mitochondrial cytochrome c oxidase subunit one gene (CO1 gene) was amplified via polymerase chain reaction (PCR) using the universal PCR primer pair MHemF and DgHCO-2198 (Table 2.2) (Park et al., 2011; Meyer, 2003). Each 25 μL PCR solution included 12.5 µL 2X GoTaq DNA polymerase (Promega Corporation, Madison, Wisconsin, USA), 1.0 µL of forward primer, 1.0 µL reverse primer, 10 ng DNA extract, and nuclease-free water.

Table 2.2 PCR primer name and sequence targeting the 402 base-pair region of the mitochondrial cytochrome c oxidase subunit.

Primer name	**Sequence (5'-3')**
MhemF	GCA TTY CCA CGA ATA AAT AAY ATA AG
DgHCO-2198	TAA ACT TCA GGG TGA CCA AAR AAY CA

PCR cycling was conducted in a SimpliAmp$^{\text{TM}}$ Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, Massachusetts, USA) using the following temperature program: 94 °C for one minute, seven cycles of 94 °C for 30 seconds, 43 °C for 30 seconds, 72 °C for 40 seconds, then 30 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 40 seconds and 72 °C for five minutes (Foster et al., 2020; modified from Beng et al., 2016).

PCR products (amplicons) were purified to remove DNA shorter than 100 base pairs using AgenCourt AMPure (Beckman Coulter Inc., Brea, California) beads according to the manufacture's protocol. Amplicons were visualized following separation on a 1.5% agarose gel (70 V for 35 minutes in TAE buffer), and their sizes estimated by comparing to a molecular weight standard. Purified DNA was quantified using a Quant-iT dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, USA) and Qubit 2.0.

A second round of PCR was used to add IonXpress barcodes and P1 adapters for subsequent sequencing on an IonS5 system. For example, in the below sample primer sequence the underlined text represents the A adaptor sequence and red text represents the IonXpress barcode in the forward primer; the bold text represents P1 adaptor sequence in the reverse primer.

CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGGTGGTTCGATGCATTYCCACGAATAAATAAYATAAG

CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGATTAAACTTCAGGGTGACCAAARAAYCA

After the second-round PCR, barcoded amplicons were purified and quantified as described above, and then pooled into sub-pools in equimolar amounts to remove non-target DNA, pooled amplicons were separated via gel electrophoresis, and target amplicons were excised from the gel and purified using a MicroElute® Gel Extraction Kit (Omega Bio-tek, Georgia, USA).

Sequencing adapted amplicons in the sub-pools were quantified using an Ion Library Quantitation Kit via quantitative real-time PCR (qPCR). These were then pooled again prior to sequencing on an Ion S5 XL™ sequencing platform (Thermo Fisher Scientific, Waltham, MA) using an Ion 530™ Chip Kit.

Data processing

The bioinformatic pipeline AMPtk (version 1.5.1) was used to cluster sequences into operational taxonomic units (OTUs) at an identity threshold of 97% (Palmer et al., 2018. Taxonomies were assigned using the (Yu et al., 2012) BOLD database (http://v4.boldsystems.org) downloaded on November 9th, 2020. The original dataset (2017 and 2018) contained 1787 OTUs. OTUs with fewer than 5000 reads were pruned and rarefied using Phyloseq v1.32.0 (McMurdie and Holmes, 2013). Data were rarefied to an even depth (McKnight et al., 2019; Beng et al., 2016). OTUs that were not classified to the domain level were removed. The subsequent data set produced for statistical analysis included a total of 524 OTUs. OTU data were converted to presence-absence to adjust for bias in PCR amplification (Beng et al., 2016; Yu et al., 2021; Lynggaard et al., 2020).

Statistical analysis

Five statistical analysis methods were used to analyze the Teck Resource Highland Valley and New Gold Inc. New Afton data. The analyses included principal coordinate analysis (PCoA), permutational multivariate analysis of variance using distance matrices (adonis), analysis of similarities (ANOSIM), indicator species analysis, and the Kruskal-Wallis test.

All of the statistical analyses were conducted in R (R Core Team, 2019) using RStudio 4.0.0, “Arbor Day “(RStudio Team, 2019). All images were created using ‘ggplot2’ (Wickham, 2016) and ‘wesanderson’ (Ram et al., 2018). The ‘tidyverse’ package (Wickham, 2017) was used for data manipulation and visualization.

PCoA plots were calculated based on the Jaccard distance using the ape package (Paradis and Schliep, 2018). PCoA plots were used to visualize arthropod assemblage data based on treatments. The adonis test partitions sums of squares using dissimilarities and was used to assess whether arthropod assemblages among groups were similar. The ANOSIM analyses were used to test for a difference between groups of OTU assemblages. Specifically, ANOSIM tests if the similarity between the OTU groups is greater than or equal to the similarity within the OTU groups. Both adonis and ANOSIM analyses were calculated based on Jaccard dissimilarity, using the ‘vegan’ function (Oksanen, 2018). The Kruskal-Wallis test was used to compare OTU richness between treatments. OTU richness is defined by the number of operational taxonomic units between each of the study sites. Pairwise comparisons using the Dunn’s test were conducted to identify the significance between sample sites. Indicator species analyses were calculated using the ‘indicspecies’ package (De Caceras and Legendre, 2009), with 999 permutations. The functions ‘multipatt’ and ‘indVal.g’ were used. Multipatt identifies taxa that are associated with sites and a combination of sites (De Caceras and Legendre, 2009). The indicator value ranges from 0-1, 1 being maximum association. The indicator value is based on two factors: positive predictive value and fidelity (De Caceras and Legendre, 2009).

The data were also separated by taxonomic order to better understand the assemblage structure. Presence-absence OTUs were characterized and summed into taxonomic order. OTUs were sorted into 17 taxonomic orders, including Diptera, Coleoptera, Entomobryomorpha, Orthoptera, Hemiptera, Hymenoptera, Archaeognatha, Lepidoptera, Opiliones, Araneae, Psocodea, Thysanoptera, Neuroptera, Poduromorpha, Mesostigmata, Sarcoptiformes, Trombidiformes, and family Formicidae. Although order-level organization was primarily used to categorize the OTUs, Hymenoptera was additionally separated into two groups (family Formicidae and not family Formicidae). Formicidae were separated to family as they have been previously identified as an indicator family (Buchori et al., 2018). Because the order data is not presence-absence, PCoA was calculated using Bray-Curtis dissimilarity and was percent transformed (relative abundance).

2.3 Results

To explore the arthropod assemblage recovery on reclaimed mine sites, all arthropod metabarcoding data from 2018, along with associated metadata, including reclamation age (‘new’, ‘old’), soil amendment (‘biosolids’, ‘not biosolids’), and reference were analyzed using the following groupings: full dataset, separated by mine (Highland Valley Copper and New Afton), separated by taxonomic order. The results implied that the dissimilarity of arthropod assemblage is primarily driven by an external factor, opposed to reclamation age and amendment. The results also found patterns between several order level taxa and reclamation age and amendment. Indicator species analyses identified several taxa uniquely associated with age and amendment sites.

Arthropod assemblage (dis)similarity characterising reclamation sites

In order to examine the effects of reclamation age, amendment, material, and mine location on arthropod assemblages, PCoA was carried out using the full 2018 dataset. Neither reclamation age (Figure 2.11) nor biosolids treatment (Figure 2.12) appeared to influence arthropod assemblages. Indeed, for both of these comparisons, arthropod assemblages on reclaimed sites did not appear to be different than natural reference sites. In contrast, when examining arthropod assemblages in the context of mine location and type of material reclaimed (waste rock and tailings), it is clear that sites located closer to each other in ordination space are more similar to each other (Figure 2.13). Specifically, waste rock sites located at Highland Valley Copper are near the top left of the plot, and tailings sites located at New Afton are near the bottom center of the plot. This separation between sites with waste rock at Highland Valley Copper and tailings at New Afton implies that arthropod assemblages at waste rock sites at Highland Valley Copper are more similar to each other, and arthropod assemblages at tailings sites at New Afton are more similar to each other.

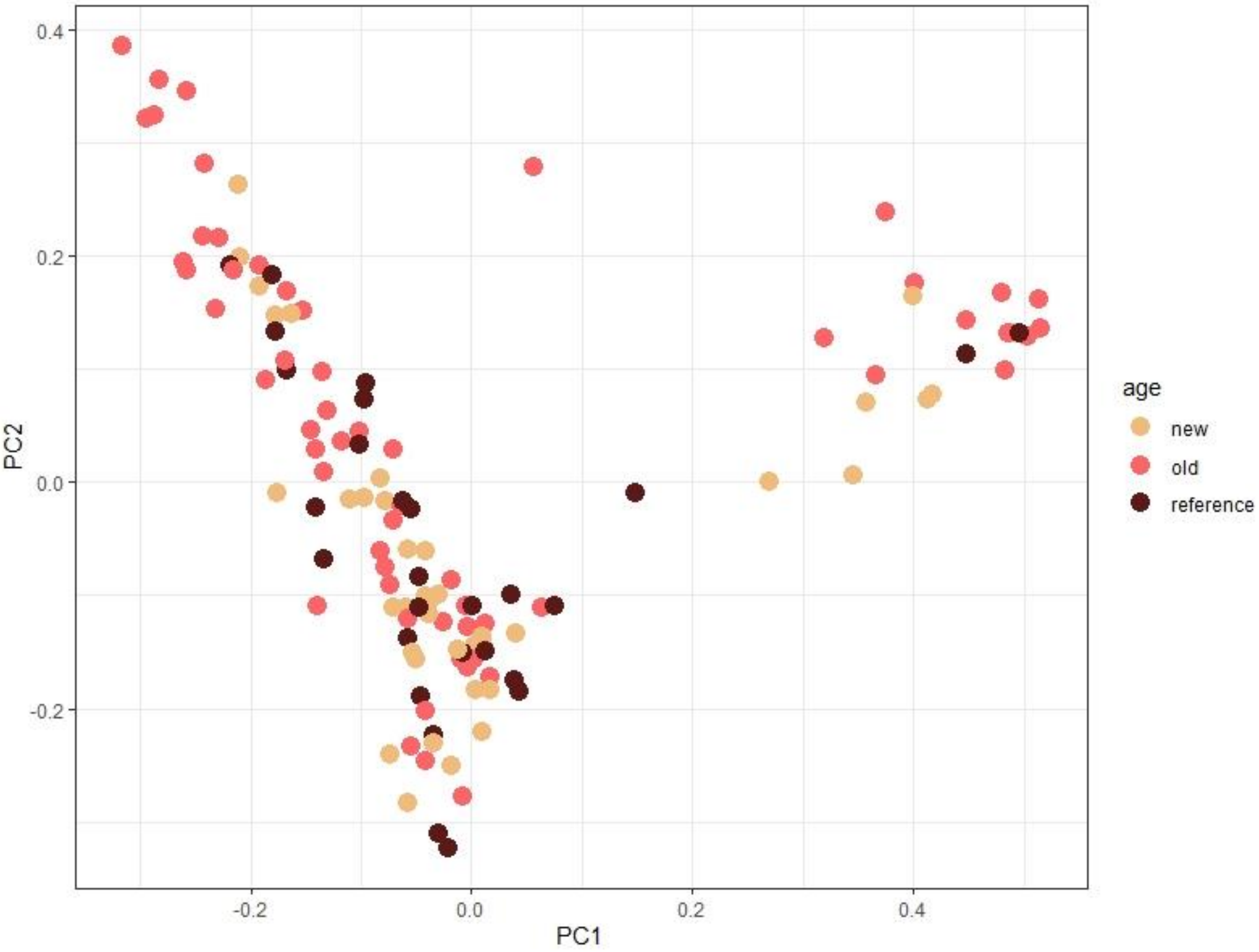

Figure 2.11. PCoA diagram created using Jaccard distance, illustrating arthropod assemblages of different reclamation ages (age, old) and reference collected in 2018 from sample sites at the Highland Valley Copper (Teck Resources) and New Afton (New Gold Inc.) mines.

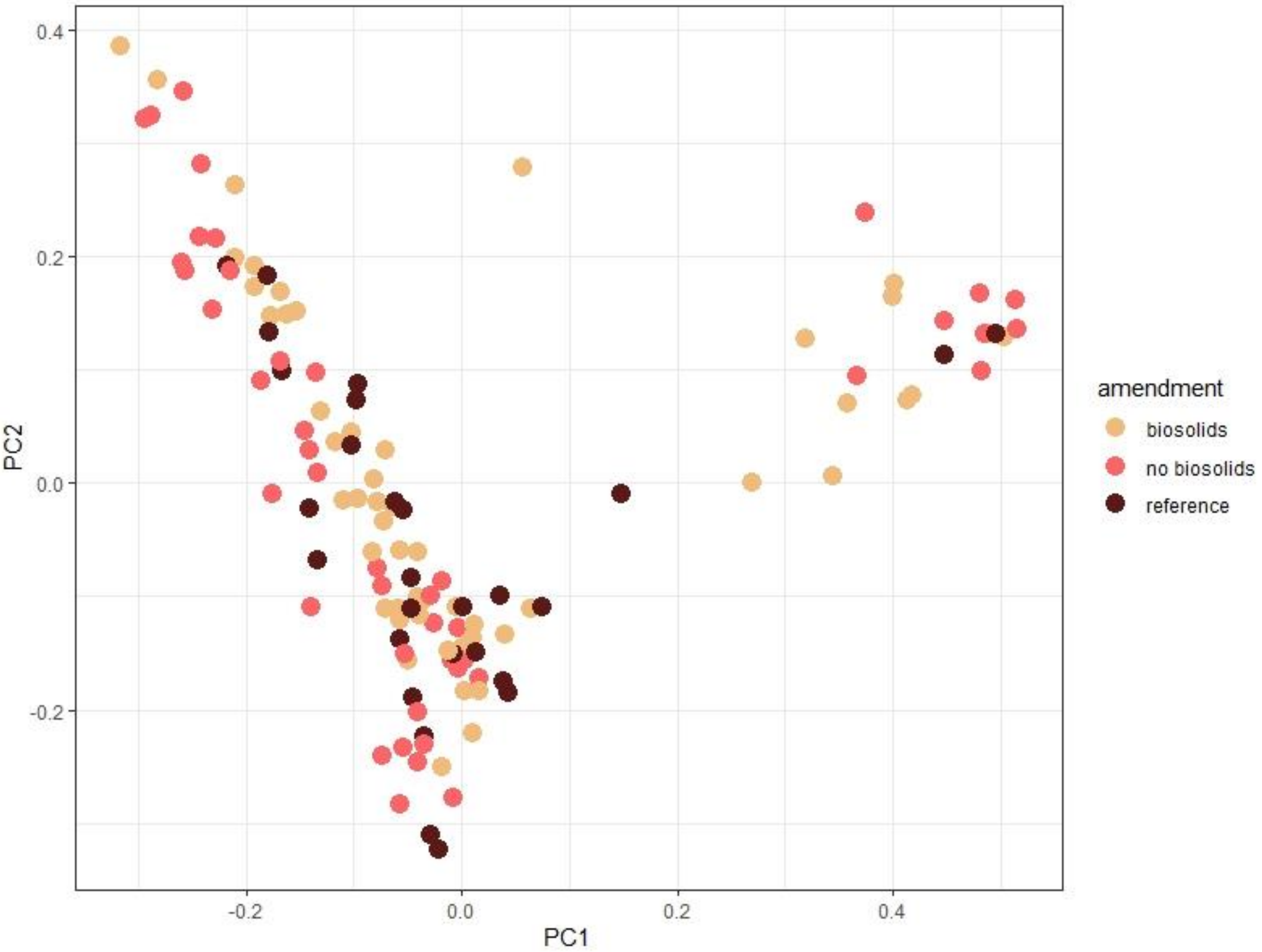

Figure 2.12 PCoA diagram created using Jaccard distance, illustrating arthropod assemblages in sites amended with(out) biosolid collected at sample sites at the Highland Valley Copper (Teck Resources) and New Afton (New Gold Inc.) mines in 2018. No sites were amended with Biosolids at New Afton (New Gold Inc.).

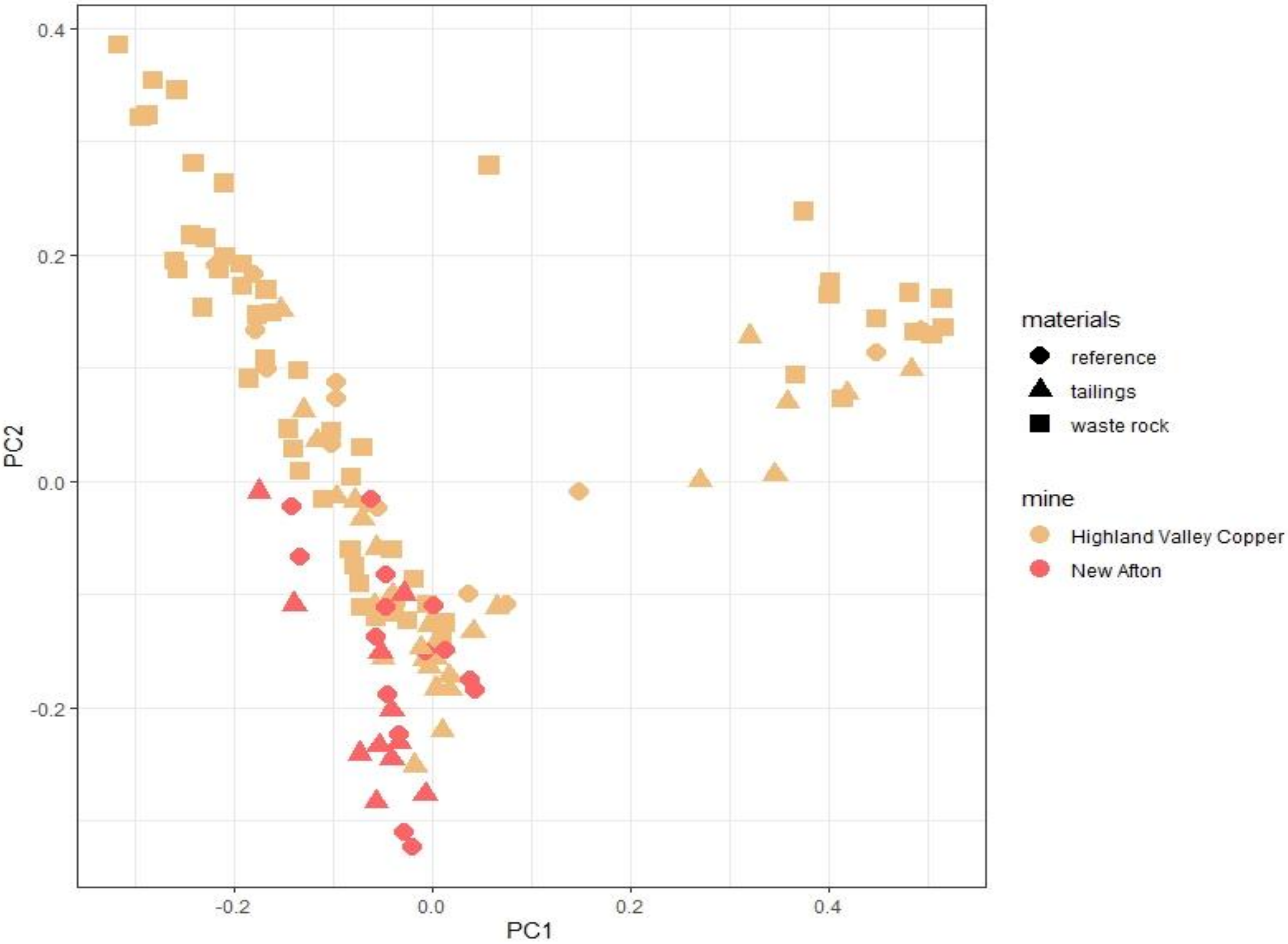

Figure 2.13. PCoA diagram created using Jaccard distance, illustrating arthropod assemblages in sites with different reclaimed materials (waste rock, tailings) at 2018 sample sites at the Highland Valley Copper (Teck Resources) and New Afton (New Gold Inc.) mines.

To explore the sources of arthropod assemblage differences between sites amended with and with(out) biosolids and sites with different reclamation ages, permutational analysis of variance using the Jaccard distance (Adonis) was carried out. Table 2.3, where the R^2 value represents the correlation between the treatments and arthropod assemblages. Here, the reclamation ‘age’ explains 3.0% (p=0.001) of the observed variation, the soil ‘amendment’ explains 1.4% (p=0.005) of the variation, and reclamation age combined with soil amendment (‘Age: Amendment’) explained 1.5% (p=0.003).

Table 2.3 Permutational analysis of variance calculated using Jaccard distance (adonis) addressing reclamation age and amendment, and reference site. Significant values are listed in bold font. R^2 values were considered significant if the p-values were below 0.05. No sites were amended with Biosolids at New Afton (New Gold Inc.).

Factor	Df	Sum sq	Mean sq	F model	R^2	P-value
Age	2	1.703	0.851	1.934	0.030	**0.001**
Amendment	1	0.795	0.795	1.805	0.014	**0.005**
Age: Amendment	1	0.862	0.862	1.957	0.015	**0.003**
Residuals	121	53.261	0.440		0.941	

Two ANOSIM tests were conducted to assess whether the similarity between the groups (reclamation ‘age’ and biosolids as a soil ‘amendment’) is greater than or equal to the similarity within the groups. An examination of the effect of reclamation age (new, old) as compared to natural reference sites, suggests an even ranking of assemblage (dis)similarity given the R-value (R = 0.086; p=0.001) (Figure 2.14). Similarly, an examination of the effect of biosolids as a soil amendment (biosolids, no biosolids) as compared to natural reference sites, suggests an even ranking of assemblage (dis)similarity given the R-value (R = 0.062; p=0.002) (Figure 2.15).

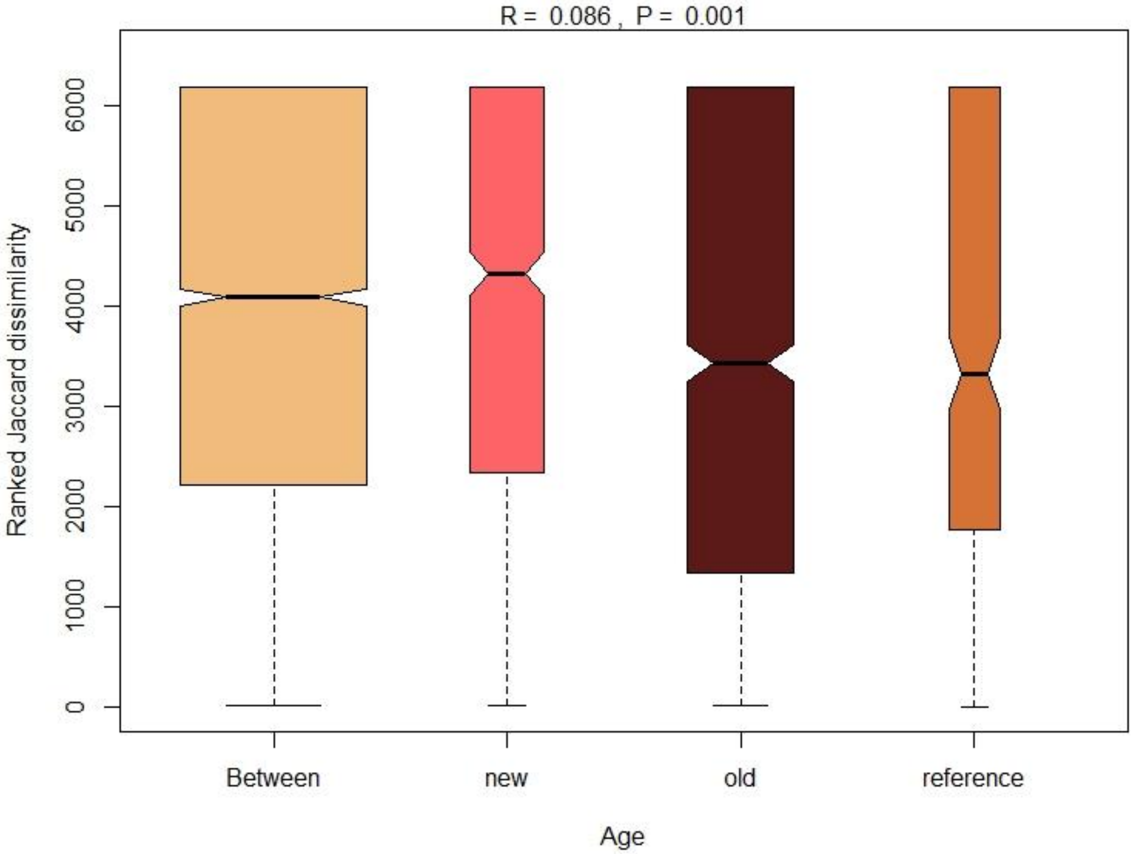

Figure 2.14. Analysis of similarity, testing reclamation age, calculated using Jaccard distance. Variation within groups is calculated by how much the samples differ from the group mean. Comparatively, variation between groups is calculated by how much the samples differ from the overall mean. In these figures, the horizontal line in the box illustrates the median, the top and bottom of the box illustrate the 25th and 75th percentile, respectively, and the whiskers extend to the furthest data points. The width of the boxes represents the sample size within that treatment.

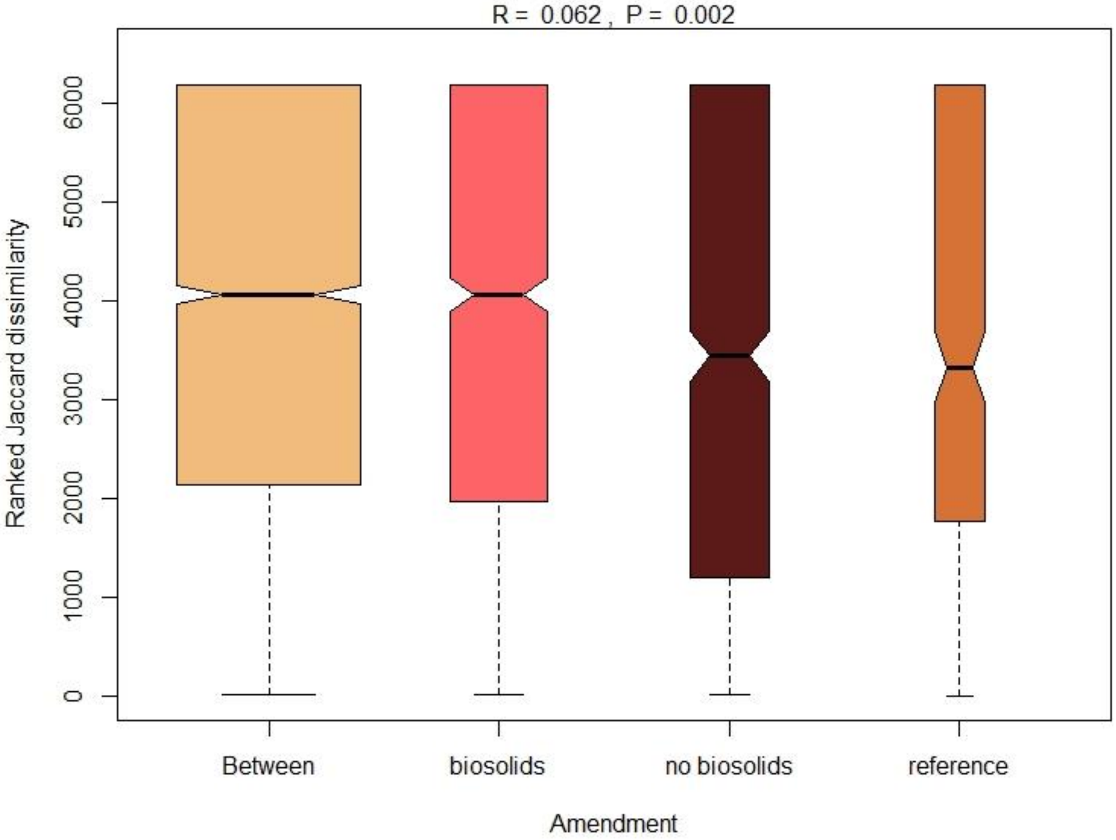

Figure 2.15 Analysis of similarity, testing reclamation amendment, calculated using Jaccard distance. Variation within groups is calculated by how much the samples differ from the group mean. Comparatively, variation between groups is calculated by how much the samples differ from the overall mean. In these figures, the horizontal line in the box illustrates the median, the top and bottom of the box illustrate the 25th and 75th percentile, respectively, and the whiskers extend to the furthest data points. The width of the boxes represents the sample size within that treatment. No sites were amended with Biosolids at New Afton (New Gold Inc.).

The PCoA, exploring the impact of reclamation and reclamation strategy, based on taxa separated by order, did not reveal a pattern distinguishing reclamation age (Figure 2.16) and soil amendment variables (Figure 2.17).

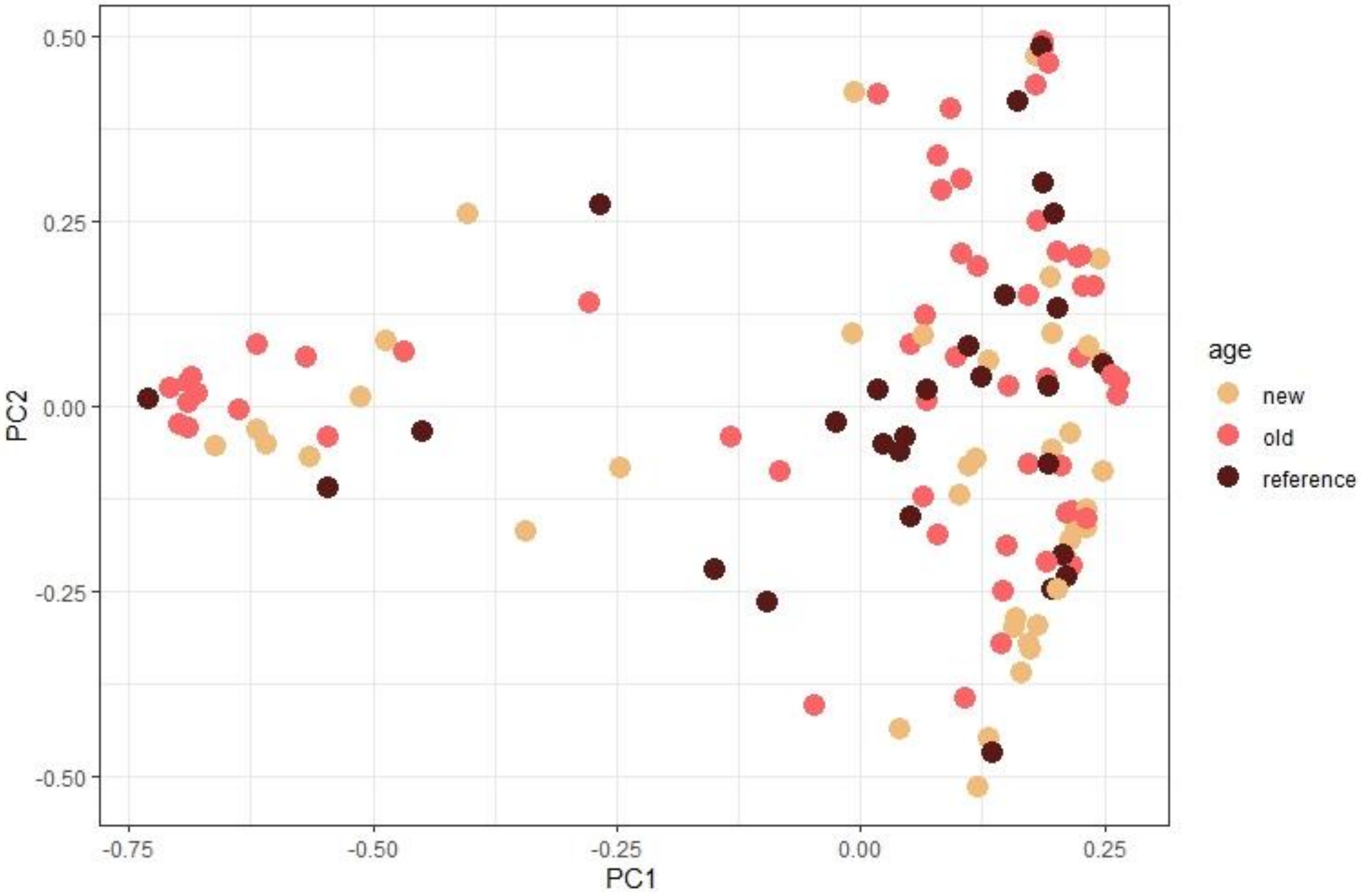

Figure 2.16 PCoA diagram based on the number of taxa characterizing each order, using Bray-Curtis percentage to calculate distance, illustrating arthropod assemblages in sites with different reclamation ages (new, old) at 2018 sample sites from Highland Valley Copper (Teck Resources) and New Afton (New Gold Inc.) mine

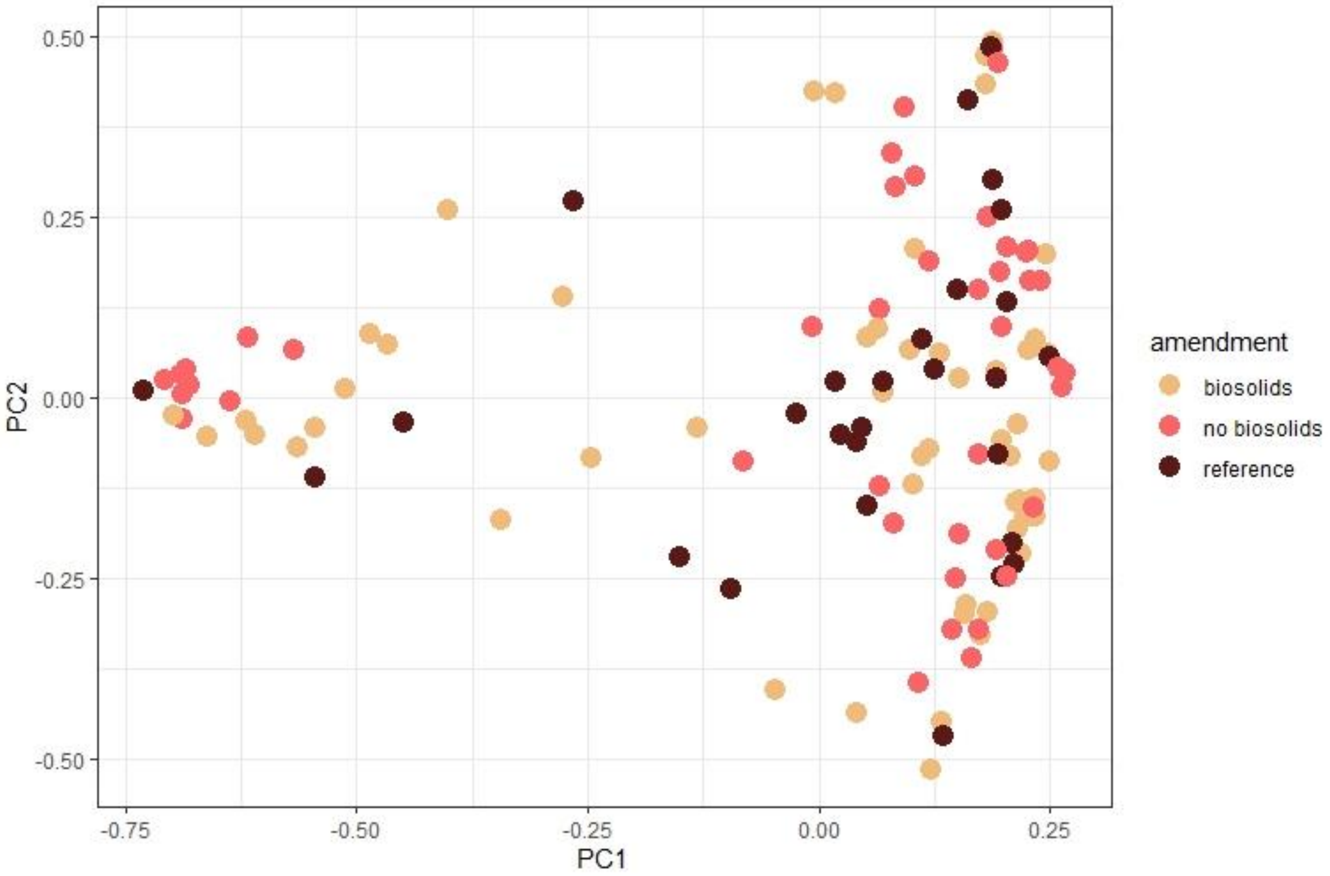

Figure 2.17 PCoA diagram based on the number of taxa characterizing each order, using Bray-Curtis percentage to calculate distance, illustrating arthropod assemblages in sites amended with(out) biosolids at 2018 sample sites from Highland Valley Copper (Teck Resources) and New Afton (New Gold Inc.) mines. No sites were amended with Biosolids at New Afton (New Gold Inc.).

Alpha diversity characterising reclamation sites

From the pitfall traps collected at Highland Valley Copper and New Afton, the average pitfall trap identified 17 unique taxa. The results of Kruskal-Wallis tests comparing species richness between ‘new,’ ‘old,’ and ‘reference’ sites revealed that there was no significant difference in taxa richness between reclamation age sites at New Afton and Highland Valley Copper (p = 0.783; Table 2.4).

Data organized by taxonomic order were also analyzed for richness between treatments. The results of Kruskal-Wallis tests comparing the number of unique arthropod taxa in 18 arthropod taxonomic groups between sites revealed that there were significant differences in the richness of Diptera (p=0.021), Entomobryomorpha (p=0.006), Psocodea (p=0.025), and Hemiptera (p=0.042) taxa between reclamation age sites (Table 2.4).

Table 2.4 Alpha diversity defined by the number of operational taxonomic units assigned to the taxonomic group, between sites with different reclamation age sites ('new,' 'old,' and 'reference') at Highland Valley Copper (Teck Resources), and New Afton (New Gold Inc.). Significance is based on Kruskal-Wallis test. Bold values represent significant p-values.

Taxonomic group	**X^2**	**P**
All taxa	0.490	0.783
Diptera	7.691	**0.021**
Entomobryomorpha	10.302	**0.006**
Psocodea	7.359	**0.025**
Hemiptera	6.325	**0.042**
Poduromorpha	4.998	0.082
Mesostigmata	3.5	0.174
Sarcoptiformes	3.5	0.174
Trombidiformes	1.419	0.492
Neuroptera	1.955	0.376
Thysanoptera	5.356	0.069
Araneae	2.81	0.245
Coleoptera	0.424	0.809
Orthoptera	0.807	0.786
Opiliones	3.516	0.172
Lepidoptera	1.486	0.476
Archaeognatha	3.185	0.204
Hymenoptera (Non -Formiciade)	1.972	0.373
Formicidae	3.270	0.195

A post hoc analysis was done to identify the significance between the age sites (Table 2.5). The Dunn's test found that Diptera OTU richness was significantly greater on 'new' sites compared to 'old' sites (p=0.017). The 'old' site has significantly less Entomobryomorpha OTU richness than the 'new' (p=0.041) site and the 'reference' site (p=0.015). Psocodea OTU richness was greater at the 'old' sites compared to the 'reference' sites (p=0.039). Despite the Kruskal-Wallis displaying significance for the order Hemiptera, there was not a significant difference between age sites and Hemiptera richness (p>0.05). Comparisons between order (Diptera, Entomobryomorpha, and Psocodea) richness and reclamation age sites are illustrated in Figure 2.18.

Table 2.5 Dunn test comparing alpha diversity defined by the number of operational taxonomic units assigned to the taxonomic group, between sites with different reclamation age sites ('new,' 'old,' and 'reference') at Highland Valley Copper (Teck Resources), and New Afton (New Gold Inc.). Bold values represent significant p-values.

Taxonomic group	**Pairwise comparisons**	**Z**	**P**
Diptera	New vs. Old	-2.77	**0.017**
	New vs. Reference	-1.36	0.525
	Old vs. Reference	1.02	0.916
Entomobryomorpha	New vs. Old	-2.46	**0.041**
	New vs. Reference	0.58	1
	Old vs. Reference	2.81	**0.015**
Psocodea	New vs. Old	1.91	0.167
	New vs. Reference	-0.73	1
	Old vs. Reference	-2.49	**0.039**
Hemiptera	New vs. Old	0.18	1
	New vs. Reference	2.27	0.069
	Old vs. Reference	2.27	0.070

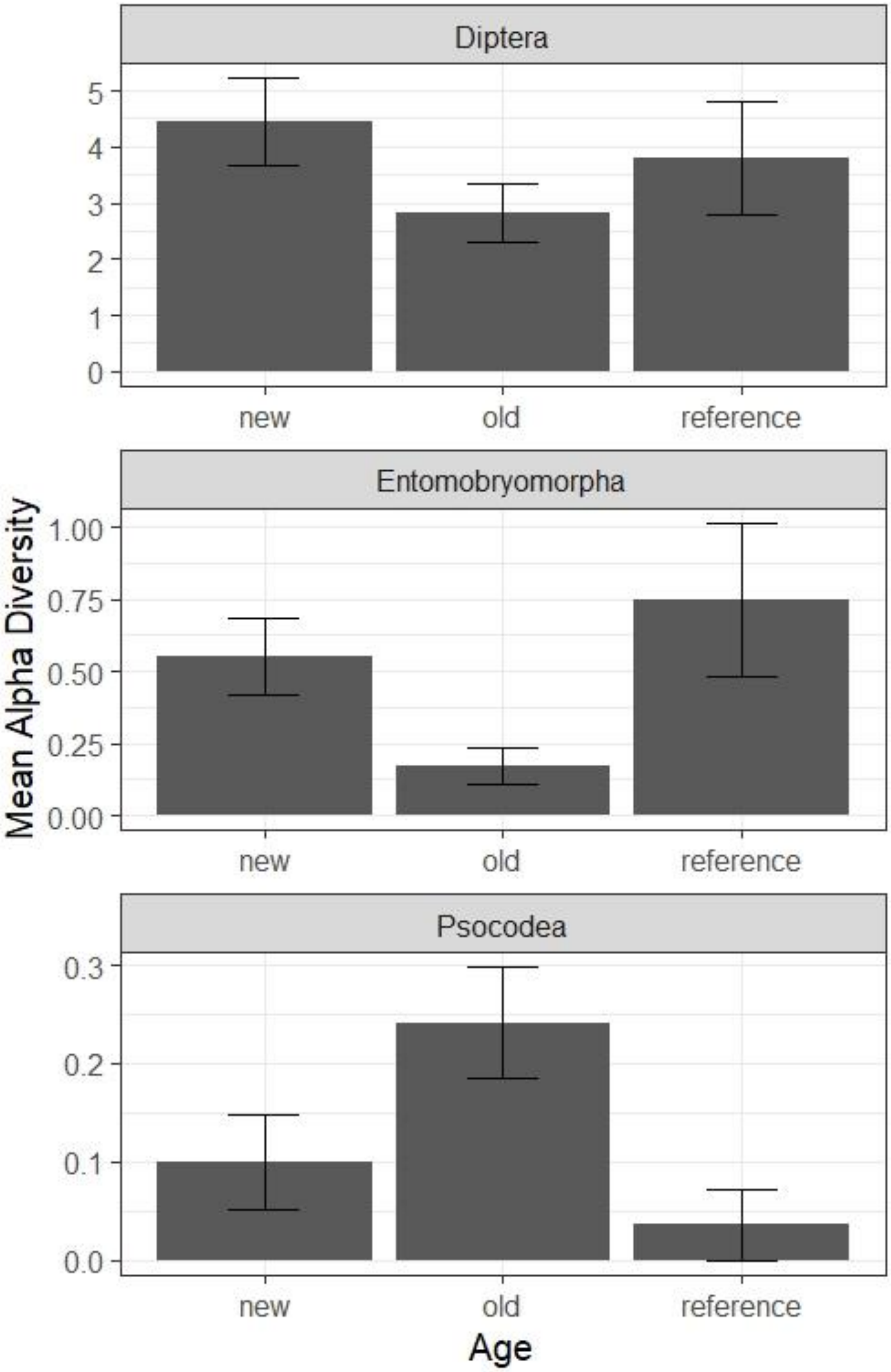

Figure 2.18 Bar plot with standard error illustrating the mean distribution of Diptera, Entomobryomorpha, and Psocodea richness defined by the number of operational taxonomic units assigned to the order, between sites with different reclamation ages ('new,' 'old,' and 'reference') at Highland Valley Copper (Teck Resources), and New Afton (New Gold Inc.).

The results of Kruskal-Wallis tests comparing species richness between ‘biosolids,’ ‘no biosolids,’ and ‘reference’ sites revealed that there was no significant difference in species richness between reclamation soil amendment sites at New Afton and Highland Valley Copper (p = 0.318; Table 2.6).

Data organized by taxonomic order were also analyzed for richness between treatments. The results of Kruskal-Wallis tests comparing the number of unique arthropod taxa in 18 arthropod taxonomic groups between sites revealed that there were significant differences in the richness of Entomobryomorpha (p=0.013), Formicidae (p=0.003), Thysanoptera (p=0.005), Araneae (p=0.049), and Hemiptera (p=0.042) and taxa between sites with and without biosolids (Table 2.6).

Table 2.6 Alpha diversity defined by the number of operational taxonomic units assigned to the taxonomic group, between sites with different reclamation amendment treatments (‘biosolids,’ ‘no biosolids,’ and ‘reference’) at Highland Valley Copper (Teck Resources), and New Afton (New Gold Inc.). Significance is based on Kruskal-Wallis test. Bold values represent significant p-values. No sites were amended with Biosolids at New Afton (New Gold Inc.).

Taxonomic group	**X^2**	**P**
All taxa	2.293	0.318
Entomobryomorpha	8.641	**0.013**
Formicidae	11.488	**0.003**
Thysanoptera	10.702	**0.005**
Araneae	6.026	**0.049**
Hemiptera	6.320	**0.042**
Poduromorpha	5.569	0.062
Mesostigmata	3.5	0.174
Sarcoptiformes	3.5	0.174
Trombidiformes	0.236	0.889
Neuroptera	1.622	0.444
Psocodea	3.966	0.158
Coleoptera	0.1352	0.935
Orthoptera	0.289	0.865
Diptera	1.378	0.502
Opiliones	5.413	0.067
Lepidoptera	1.177	0.555
Archaeognatha	3.373	0.185
Hymenoptera (Non -Formicidae)	3.325	0.190

A post hoc analysis was done to identify the significance between the age sites (Table 2.7). The Dunn’s test found that Entomobryomorpha demonstrated a significant difference in richness between the ‘reference’ sites and the ‘biosolids’ sites (p=0.020). The family Formicidae had greater richness on sites with no biosolids compared to sites amended with biosolids (p=0.002). There was greater Thysanoptera richness on sites without biosolids compared to sites amended with biosolids (p=0.003). Despite the orders Aranea and Hemiptera being statistically significant in the Kruskal-Wallis test, the Dunn’s test did not uncover a significant relationship between the sites (p>0.05). Comparisons between Thysanoptera, Entomobryomorpha, and Formicidae and soil amendment sites are illustrated in Figure 2.19.

Table 2.7 Dunn’s test comparing alpha diversity defined by the number of operational taxonomic units assigned to the taxonomic group, between sites with different reclamation amendment treatments (‘biosolids,’ ‘no biosolids,’ and ‘reference’) at Highland Valley Copper (Teck Resources), and New Afton (New Gold Inc.). Bold values represent significant p-values. No sites were amended with Biosolids at New Afton (New Gold Inc.).

Taxonomic group	**Pairwise comparisons**	**Z**	**P**
Entomobryomorpha	Biosolids vs. No biosolids	2.10	0.108
	Biosolids vs. Reference	2.72	**0.020**
	No biosolids vs. Reference	0.85	1
Formicidae	Biosolids vs. No biosolids	3.37	**0.002**
	Biosolids vs. Reference	1.64	0.300
	No biosolids vs. Reference	-1.25	0.636
Thysanoptera	Biosolids vs. No biosolids	3.26	**0.003**
	Biosolids vs. Reference	0.98	0.984
	No biosolids vs. Reference	-1.79	0.219
Araneae	Biosolids vs. No biosolids	-1.81	0.211
	Biosolids vs. Reference	-2.24	0.08
	No biosolids vs. Reference	-0.63	1
Hemiptera	Biosolids vs. No biosolids	-0.17	1
	Biosolids vs. Reference	2.24	0.074
	No biosolids vs. Reference	2.30	0.064

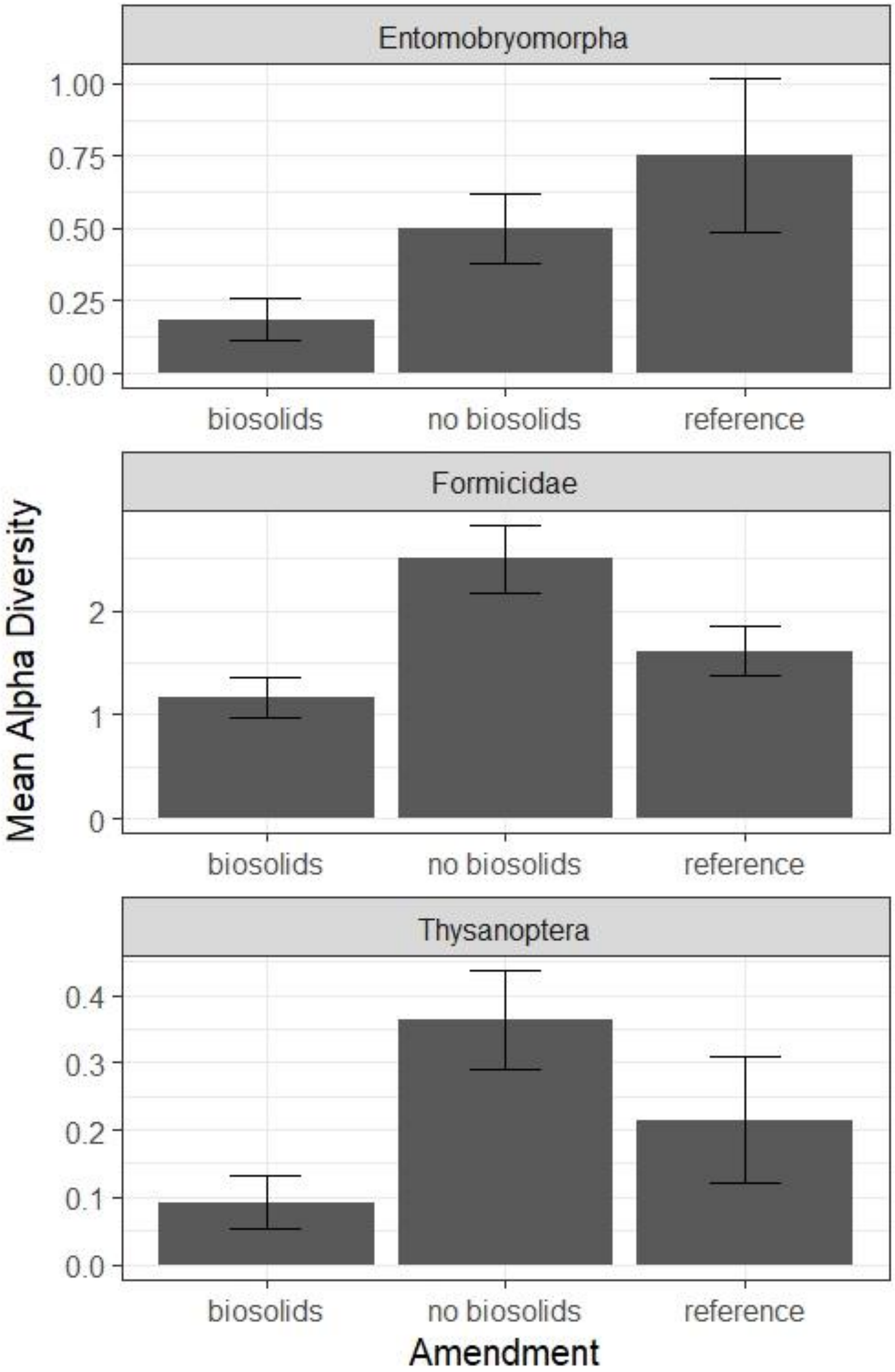

Figure 2.19 Bar plots with standard error illustrating the distribution of mean Entomobryomorpha, Formicidae, and Thysanoptera richness defined by the number of operational taxonomic units assigned to the order, between sites amended with different soil amendment ('biosolids', 'no biosolids', and 'reference) at Highland Valley Copper (Teck Resources), and New Afton (New Gold Inc.). No sites were amended with Biosolids at New Afton (New Gold Inc.).

Indicator taxa characterising reclamation sites

To assess if specific taxa were associated with reclamation age and amendment treatments, indicator species analyses were used. The association between the OTU and the reclamation variable increases with the measure of the statistic, from 0-1. The below tables describe the taxa with the strongest affinity for different site types. It should be noted that these tables are condensed for efficiency, and that the complete indicator tables are available in Appendix C.

Arthropod taxa were more associated with different reclamation ages (‘new’, ‘old’, and ‘reference’) and a combination of sites at Highland Valley Copper mine and New Afton mine (Table 2.8). The taxon that was most correlated with the reference site was *Latalus misseullus*. The taxon that was most associated with the ‘new’ sites was *Leia spp*. In this analysis, there was not a taxon that was strongly tied to the ‘old’ site. However, *Formica lasioides* (potential genetic variations of ant species) were most associated with a combination of the ‘old’ and ‘new’ sites. Finally, *Haplothrips tenuipennis* was the taxon most identified with the ‘old’ and ‘reference’ sites.

Table 2.8. Indicspecies analysis outlining the top operational units (OTU) associated with different reclamation ages (‘new,’ ‘old,’ ‘reference’) of the 2018 sample sites at the Highland Valley Copper (Teck Resources) and New Afton (New Gold Inc.) mines, where and ‘p’ is the probability of finding the obtained results given that the null hypothesis is true, and ‘indicator stat’ is an indicator value that ranges from 0 to1.

OTU	Taxon	Indicator stat	p-value
	Reference		
0181	*Latalus missellus*	0.463	0.001
0124	*Formica neorufibarbis*	0.438	0.001
0050	Sciaridae	0.404	0.003
	New		
0930	*Leia spp*	0.392	0.036
0030	*Fannia canicularis*	0.373	0.031
0206	*Delia extensa*	0.316	0.012
	New and Old		
1367	*Formica lasioides*	0.440	0.021
1552	*Formica lasioides*	0.404	0.050
	Reference and Old		
0205	*Haplothrips tenuipennis*	0.469	0.025

Data were divided into unique mines (Highland Valley Copper and New Afton) to further understand and identify patterns within the data set. To assess if specific taxa were associated with reclamation age and amendment treatment, indicator species analyses were used.

Arthropod taxa were more associated with different reclamation ages (‘new’, ‘old’, and ‘reference’) and a combination of sites at Highland Valley Copper mine (Table 2.9). The ‘reference’ site was most associated with *Formica neorufibarbis*, *Latalus missellus* taxon, and *Tachinus spp*. The ‘new’ site was correlated with the taxa *Fannia canicularis*, Staphylinidae taxon, and Machilidae taxon. Two potential genetic variations of the same taxa were associated with the ‘old’ site; *Otiorhynchus ovatus*. The combination of the ‘reference’ and ‘old’ sites were correlated with *Phloeostiba lapponica,* and *Haplothrips tenuipennis.* Only one taxon, *Formica lasioides*, was tied to the ‘new’ and ‘old’ site combination.

Table 2.9. Indicspecies analysis outlining the top operational units (OTU) associated with different reclamation age (‘new,’ ‘old,’ ‘reference’) of the 2018 sample sites at the Highland Valley Copper (Teck Resources) mine, where and ‘p’ is the probability of finding the obtained results given that the null hypothesis is true, and ‘indicator stat’ is an indicator value that ranges from 0 to1.

OTU	Taxon	Indicator stat	p-value
	Reference		
0124	*Formica neorufibarbis*	0.659	0.001
0181	*Latalus missellus*	0.620	0.001
0011	*Tachinus spp*	0.566	0.002
	New		
0030	*Fannia canicularis*	0.450	0.024
0040	Staphylinidae	0.442	0.028
0111	Machilidae	0.343	0.044
	Old		
0012	*Otiorhynchus ovatus*	0.484	0.019
0344	*Otiorhynchus ovatus*	0.412	0.025
	Reference and Old		
0035	*Phloeostiba lapponica*	0.623	0.015
0205	*Haplothrips tenuipennis*	0.477	0.040
	New and old		
0002	*Formica lasioides*	0.614	0.038

Arthropod taxa were more associated with different reclamation ages (‘new’, ‘old’, and ‘reference’) and a combination of sites at New Afton mine (Table 2.10). The ‘new’ site was strongly associated with a Heleomyzidae taxon as well as *Tapinoma sessile*. The ‘old’ site was correlated with Meinertellidae taxon, *Myrmica fracticornis*, and Archaeognatha taxon. Both the 'new' and 'old' age sites were most affiliated with *Entomobrya unostrigata*.

Table 2.10. Indicspecies analysis outlining the top operational units (OTU) associated with different reclamation age ('new,' 'old,' 'reference') of the 2018 sample sites at the New Afton (New Gold Inc.) mine, where and 'p' is the probability of finding the obtained results given that the null hypothesis is true, and 'indicator stat' is an indicator value that ranges from 0 to1.

OTU	Taxon	Indicator stat	p-value
	New		
0771	Heleomyzidae	0.82	0.005
1504	*Tapinoma sessile*	0.707	0.016
	Old		
0017	Meinertellidae	0.685	0.018
0267	*Myrmica fracticornis*	0.632	0.034
0300	Archaeognatha	0.632	0.032
	New and Old		
1499	*Entomobrya unostrigata*	0.798	0.018

Arthropod taxa were more associated with different amendment treatments ('biosolids,' 'no biosolids,' and 'reference') and a combination of sites at Highland Valley Copper mine and New Afton mine (Table 2.11). Staphylinidae taxon, *Amara fortis*, and Lepidoptera taxon were the taxa most associated with the 'biosolids' variable. *Latalus missellus* was the taxon most strongly correlated with the 'reference' site. The reference site was most strongly tied to *Latalus missellus, Formica neorufibarbis,* and Sciaridae taxon. 'No biosolids' shared the strongest relationship with *Leptothorax spp*, Heleomyzidae taxon, and *Camnula pellucida.*

Table 2.11. Indicspecies analysis outlining the top operational units (OTU) associated with different reclamation amendments ('biosolids,' 'no biosolids,' 'reference) of the 2018 sample sites at the Highland Valley Copper (Teck Resources) and New Afton (New Gold Inc.) mines, where and 'p' is the probability of finding the obtained results given that the null hypothesis is true, and 'indicator stat' is an indicator value that ranges from 0 to1. No sites were amended with Biosolids at New Afton (New Gold Inc.)

OTU	Taxon	Indicator stat	p-value
	Biosolids		
0040	Staphylinidae	0.416	0.024
0076	*Amara fortis*	0.332	0.025
0948	Lepidoptera	0.304	0.028
	Reference		
0181	*Latalus missellus*	0.463	0.001
0124	*Formica neorufibarbis*	0.444	0.001
0050	Sciaridae	0.398	0.004

No biosolids			
0064	*Leptothorax sp.*	0.403	0.012
0771	Heleomyzidae	0.327	0.043
0590	*Camnula pellucida*	0.313	0.042
No biosolids and reference			
0015	*Formica subaenescens*	0.557	0.025
0570	*Entomobrya spp*	0.545	0.001
0205	*Haplothrips tenuipennis*	0.523	0.002
No biosolids and biosolids			
1367	*Formica lasioides*	0.440	0.025
1552	*Formica lasioides*	0.404	0.039

Arthropod OTUs were more associated with different amendment treatments (‘biosolids,’ ‘no biosolids,’ and ‘reference’) and a combination of sites at Highland Valley Copper mine (Table 2.12). *Formica neorufibarbis, Latalus missellus,* and *Tachinus spp* were most associated with the reference site. Sites without biosolids were linked to *Haplothrips tenuipennis*, *Otiorhynchus ovatus*, and *Leptothorax spp*. The ‘biosolids’ and ‘no biosolids’ were most associated with *Formica lasioides.* Both the ‘reference’ and ‘no biosolids’ sites were correlated with *Formica subaenescens (formerly Formica fusca var complex).*

Table 2.12. Indicspecies analysis outlining the top operational units (OTU) associated with different reclamation amendment (‘biosolids,’ ‘no biosolids,’ ‘reference’) of the 2018 sample sites at the Highland Valley Copper (Teck Resources) mine, where and ‘p’ is the probability of finding the obtained results given that the null hypothesis is true, and ‘indicator stat’ is an indicator value that ranges from 0 to1.

OTU	Taxon	Indicator stat	p-value
Reference			
0124	*Formica neorufibarbis*	0.666	0.001
0181	*Latalus missellus*	0.620	0.001
0011	*Tachinus spp*	0.572	0.001
No biosolids			
0205	*Haplothrips tenuipennis*	0.526	0.006
0012	*Otiorhynchus ovatus*	0.500	0.018
0064	*Leptothorax spp*	0.459	0.032
Biosolids and No biosolids			
0002	*Formica lasioides*	0.617	0.026
Reference and No biosolids			
0015	*Formica subaenescens*	0.677	0.001

2.4 Discussion

The effects of postmining reclamation on recovering arthropod assemblages were examined at two mines in BC. DNA metabarcoding techniques were used, and few differences in arthropod assemblages between mine sites and different reclamation treatments groups were detected. In this section, arthropod assemblage similarity, arthropod alpha diversity, and arthropod indicator species are used to interpret patterns in reclamation against soil amendments and reclamation age. Analyses discussed in this section used OTUs categorized to order-level, as well as to the lowest identified taxonomic rank i.e., species.

Arthropod assemblage (dis)similarity distinguishing reclamation sites

Over time, arthropod communities recover in reclaimed areas following disturbance (Steed et al. 2018; Watts and Mason, 2015; Fernandes et al. 2019). In this study, arthropod assemblage similarity was assessed between different age plots at Highland Valley Copper and New Afton mines to better understand post mine reclamation trajectory. Arthropod assemblages did not show a strong relationship with reclamation age, as shown in Figure 2.11 and Table 2.3. Figure 2.14 illustrates that the ‘old’ and ‘reference’ sites are slightly more similar than ‘new’, indicating that over time the arthropod assemblage becomes more similar to the reference sites. Despite this pattern being significant, it only explains a small percentage (8.6%) of the variation within the dataset. Order-level arthropod assemblage similarity of different age plots (‘new,’ ‘old,’ and ‘reference) at Highland Valley Copper and New Afton mines were also analyzed. Arthropod assemblage composition did not show a strong relationship with reclamation age, as shown in Figure 2.11. This indicates that no trajectory of order-level arthropod assemblages that becomes more similar to the corresponding reference site with age was detected with the methods used here. Fernandes et al. (2019) carried out a similar experiment, examining terrestrial arthropod community response to mine reclamation age, using DNA metabarcoding. Their study identified that ‘older’ sites were more analogous to ‘reference’ sites than recently reclaimed sites.

The arthropod assemblage similarity of sites without biosolids and with biosolids at Highland Valley Copper and New Afton mines were also compared in an effort to understand the long-term ecological response to a soil amendment known to impact nutrient content and soil properties (Gardner et al., 2010). In this study, soil amendments (‘biosolids,’ ‘no biosolids’) did not explain the variation in arthropod assemblages (Figure 2.12). Likewise, Figure 2.15 shows that sites with ‘no biosolids’ are slightly more similar to the ‘reference’ sites; however, the pattern only accounts for 6.2% of the variation. Order-level arthropod assemblage similarity of soil amendment (‘biosolids,’ ‘no biosolids’) at Highland Valley Copper and New Afton mines were also assessed (Figure 2.12) and, once again, no pattern was detected. A similar study in New Zealand compared arthropod assemblage to sites before and after biosolids application (Denholm, 2003), and no additional taxa (family-level taxa and individual species taxa) were detected post biosolids treatment.

Understanding the reclamation trajectory of arthropod assemblage post-mining reclamation is an important objective on the road to achieving successful end land use. Below sections in this discussion address the patterns between reclamation age and amendment to order level taxa and indicator taxa. Analysing arthropod assemblages did not detect a successional trajectory between reclamation and soil amendment. Other factors than reclamation age and biosolids application may be responsible for the separation seen in Figure 2.11 and Figure 2.12, such as elevation, soil chemistry and biology, moisture, vegetation, solar radiation and climate of the sampled sites; and they should be included in future analyses. For example, Buchori et al. (2018) conducted a study looking at arthropod diversity in post-mine reclaimed areas. The most notable variables associated with insect diversity recovery were vegetation diversity and total nitrogen content in the soil.

To further understand potential patterns within the data set, data were analyzed using ‘mine’ location and ‘reclaimed material’ as factors (Figure 2.13). The clear separation in Figure 2.13 suggests that the arthropod assemblages are correlated with the ‘mine’ and ‘reclaimed material’ factors. The Highland Valley Copper sites are located on a combination of waste rock and tailings, whereas the ‘reference’ sites are located on a naturally forested site. New Afton sites occur on a historical tailings facility, and the reference sites are located in a natural grassland.

The two mines (Highland Valley Copper and New Afton) have a variety of differences between each other. For example, the elevation between the two sampled mines differs by 300-1050 meters above sea level (New Afton 700 meters, Highland Valley Copper 1000-1750 meters). The distribution and range of plant and arthropod species are affected along elevation gradients (Parmesan and Hanley, 2015; Gonzalez-Reyes et al., 2017). Change in insect taxa along elevational gradients can be a direct or indirect response to other biota, including insect host flora, as well as a response to predation, competition, and parasitoids (Hodkinson, 2005).

Altitude can affect morphology, phenology, nutrient concentrations, and reproduction in insect host vegetation (Hodkinson, 2005) and these variations in vegetation can result in differences in insect nutrient intake, growth rate, survival, and fecundity (Hodkinson, 2005).

Corresponding with the elevation change, the Highland Valley Copper mine and New Afton mine also differ by their aforementioned BEC zone. Specifically, Highland Valley Copper is located in the Montane Spruce zone, whereas New Afton is located in the Bunchgrass and Ponderosa Pine zone. Using BEC zones to classify ecosystems allows for the uncovering of community relationships within and between ecosystems, which can be applied to research and management (MacKinnon et al., 1992). In this study, understanding the biotic and abiotic environmental characteristics of the BEC zones associated with the two mines could aid in explaining the difference in arthropod assemblage community.

The data point separation, seen in Figure 2.13, between sites that were reclaimed on mining material by-products, tailings and waste rock, implies that the materials the sites were built on affect arthropod assemblage composition. Waste rock is bedrock removed prior to the mining process due to a lack of marketable materials (minerals, metal, bitumen). Comparatively, tailings are the fine residuals after the marketable materials have been isolated from the ore. These two mining by-products differ in moisture-retaining potential. In particular, tailings fine size has slow draining, compared to waste rocks large porosity, resulting in tailing having a greater potential for retaining moisture (Blowes, 2003). Though the by-product material that is amended through the reclamation process is not a practice or material that is able to be changed, it is interesting to note other environmental variables that can impact arthropod assemblage compositions, post-mine reclamation.

Alpha diversity distinguishing reclamation sites

As a measure of species richness, alpha diversity (to the point of functional redundancy) is associated with ecological stability and resilience (Naeem and Li, 1997), and can be influenced by environmental and biological factors, including immigration, competitive exclusion, predation, pathogens, herbivory, facilitation, resource availability, temporal variation, and habitat disturbance (Brown et al., 2007). For example, increased immigration, impacted by population size and distance between areas, can positively affect species richness (Brown et al., 2007). Additionally, variance in population can be influenced by whether an arthropod is a strong and weak disperser. Specifically, active dispersers (e.g., winged arthropods), compared to passive dispersers (e.g., transported via wind or other animals), can colonize new areas more effectively (Vannette and Fukami, 2017). It could then be anticipated that a greater amount of active dispersing arthropods taxa would be identified in the new site than arthropods that are passive dispersers.

The intermediate disturbance hypothesis, proposed by Connell (1978), could also be relevant to the analyses in this study examining arthropod alpha diversity. The intermediate disturbance hypothesis predicts that areas with both 'high' and 'low' levels of disturbance determined by intensity, frequency, scale, and duration, will have less species richness than areas with intermediate levels of disturbance. The rationale for the theory is that in areas with 'low' disturbance levels, species are excluded as a result of competition. In areas with 'high' disturbance levels, failure to survive of disturbance reduces species richness. Based on this hypothesis, we would expect to see higher alpha diversity in reclaimed areas with intermediate disturbance. However, in this study, the Kruskal-Wallis test (Table 2.4; Table 2.6) demonstrated no significant differences in species richness between the sampled sites (reclamation age and soil amendment, and reference); this is potentially a result of large variation between the replicates, which could be addressed in future studies by increasing the number of samples collected representing each treatment.

To further understand arthropod species richness response to reclamation, arthropods were categorized to taxonomic order prior to analysis. At this taxonomic level, the order Diptera OTUs (true flies) had greater diversity in 'new' sites compared to 'old' sites. Currently, having 160,000 species globally, the order Diptera is diverse both phylogenetically and functionally (Courtney et al., 2017). Dipteran taxa can occupy a range of functional feeding guilds including herbivores, predators, omnivores, detritivores, and parasites (Sarwar, 2020). Diptera can also provide various ecosystem services, depending on species, such as, pollination, decomposition, prey for other species, mobilizing nutrients, and regulating populations of prey species (Adler and Courtney, 2019).

Diptera are commonly used as indicators of environmental characteristics, however, they are typically identified to lower taxonomic levels (e.g., species, genus, family) opposed to order level (De Souza et al., 2014; Lynggaard et al., 2020). For example, Lynggaard et al. (2020) conducted a study examining the arthropod response to reclamation succession in post-mining sites. They found evidence that different Diptera families were more associated with initial stages (Family Micropeziidae), intermediate stages (Family Muscidae), and advanced stages (Family Chironomidae) of succession (Lynggaard et al., 2020). This finding could illustrate that different families of Diptera have varying environmental sensitivities, making it difficult to infer the environmental relationships based on Diptera diversity and highlighting the need for analyzing lower-level taxa, such as conducted in the indicator section of this study.

Similar to the order Diptera, Entomobryomorpha OTUs, springtails, were more diverse in 'new' sites than in 'old' sites. Likewise, the 'reference' sites had a greater richness of Entomobryomorpha than the 'old' sites (Table 2.5; Figure 2.18). Fernandes et al. (2019) also found that post-reclamation mine sites that were more recently reclaimed were typified by Entomobryomorpha presence. Site factors including, pH, organic matter and nutrient availability can affect Collembola diversity (Cassagne et al., 2003), and vegetation species richness has been correlated with increased Collembolan diversity (Sabais et al., 2011). The reference sites in this study could also be expected to have greater vegetation richness than reclaimed sites as supported by a study assessing vegetation species-based diversity Highland Valley Copper (Smyth et al., 2016). Additionally, a study conducted by Ji et al (2022) measured microbial diversity in reclaimed mine sites over five unique reclamation ages and a reference site. They found that more recently reclaimed sites had lower fungal diversity than the 'reference' site (Ji et al., 2022). Studies have linked Collembola and soil fungal communities (biomass and diversity) through grazing, dissemination of spores, soil mixing, and modifying available nutrients (Klironomos and Kendrick, 1995; Coulibaly et al., 2019).

Moreover, Collembolans positively affect plant production by mobilising nutrients through their consumption of fungi (Harris and Boerner, 1990). Similar to other decomposer species, interspecific interactions between Collembola taxa may play a role in environmental function (Eisenhauer et al., 2011). The presence of Collembola taxa in early phases of reclamation help advance the return of soil function and play a meaningful part in soil restoration (Rusek, 1998; Langmaack et al., 2001). Collembola taxa colonize specific soil depths (between 0 cm and 15 cm) and affect soil processes at these depths uniquely (Ponge, 2000); Eisenhauer et al. (2011) theorized that the taxonomic diversity of Collembola affects litter decomposition and vegetation success. Because samples were retrieved at the ground surface level, Collembola taxa that colonize deeper soil depths were not analyzed in this study.

Contrary to the orders Diptera and Entomobryomorpha, the order Psocodea had greater OTU richness on ‘old’ sites compared to ‘reference’ sites (Table 2.5). Psocodea, formerly Psocoptera, are bark lice often found on trees or shrubs (Hollier, 2008). Simberloff and Wilson (1969) examined the recovery of arthropods on sites that had been defaunated. They collected arthropod recovery data over time and compared it to pre-defaunation arthropod communities. They found that Psocodea taxa were one of first arthropod taxa to return to sites and produce large populations (Simberloff and Wilson, 1969). Moreover, Psocodea were found in greater abundance on the re-faunating site than generally found on undisturbed sites, potentially a result of a lack of Psocodea predators on the re-faunating islands (Simberloff and Wilson, 1969). Increased predation could contribute to reduced diversity in the ‘reference’ sites compared to the ‘old’ reclaimed sites, seen in the results of this thesis. However, if predation was the primary the cause of decreased Psocodea diversity in the reference sites compared to the ‘old’ sites, it would stand to reason that the ‘new’ sites would also be statistically more diverse than the reference sites. Moreover, Gerlach et al. (2013) conducted a review of terrestrial arthropods as bioindicators and noted that Psocodea most likely had little potential for inference, as they are often generalists. It would be interesting for future studies to assess trophic interactions on post-mining reclaimed sites compared to reference sites.

In addition to reclamation age, the effects of soil amendments (‘biosolids’, ‘no biosolids’, ‘reference’) on arthropod alpha diversity were examined. No significant difference in species richness was detected between the sampled soil amendment sites (Table 2.6). Denholm (2003) conducted a study examining biosolids affect on arthropods and also found that biosolid applications did not significantly affect arthropod species diversity. However, this study did identify patterns between biosolids as a soil amendment and Entobryomorpha, Formicidae, and Thysanoptera. Similar to the pattern seen typifying reclamation age, differences in Entomobryomorpha richness were statistically significant between ‘biosolids’ sites and ‘reference’ sites (Table 2.7; Figure 2.19).

In addition to a significant difference in Entomobryomorpha richness between sites, Formicidae (ant) richness varied between sites with ‘biosolids’ and ‘no biosolids.’ Sites with ‘no biosolids’ had significantly more Formicidae taxa richness (Table 2.7; Figure 2.19). Formicidae taxa are expected, given that they are the most dominant insect in terrestrial ecosystems globally (Wilson, 1990) and are paramount to environmental functioning (Higgins and Lindgren, 2009).

Buchari et al. (2018) explored the role of insects as bioindicators for reclamation success. In that study, Formicidae were the most notable insect group in terms of species richness and abundance. Indeed, it was concluded that the successes of post-reclaimed areas was best evaluated by using ants as bioindicators (Buchari et al. 2018).

In some instances, Formicidae species richness has been more strongly associated with soil properties than plant communities (Boulton et al., 2005). In particular, soil chemical properties (Mg and Cu concentration), were negatively correlated with Formicidae richness (Boulton et al., 2005). Interestingly, a study conducted by Gardner et al. (2012) found that sites located at Highland Valley Copper mine had a positively correlated relationship between biosolids and Mg (supported by Griebel et al.,1979; Hinesly et al., 1982). This relationship between biosolids and increased Mg could potentially contribute to the decrease of Formicidae richness on sites amended with biosolids, however, other factors are likely affecting the relationship between sites with and without biosolids.

In addition to abiotic soil characteristics, Formicidae can be correlated with soil biota. Anderson and Sparling (1997) found that Formicidae richness was positively correlated with microbial biomass in sites subject to disturbance. Their findings point to the complementary relationship between decomposition within the soil and biotic activity on the surface. Furthermore, their study illustrates that Formicidae can indicate the status of environmental functions, such as nutrient cycling (Anderson and Sparling, 1997), which highlights the potential for Formicidae to act as bioindicators of reclamation. Despite not being included in this study, soil microbial data were collected at the same time as arthropod samples at Highland Valley Copper mine and New Afton mine. It would be interesting to analyze the relationships between Formicidae richness and soil microbial community composition and biomass in the future.

Thysanoptera (thrip) was the final order to have a statistically significant correlation between soil amendment sites and alpha diversity. A greater diversity of Thysanoptera OTUs occurred on sites with ‘no biosolids’ compared to sites with ‘biosolids’. This result is complemented by the indicator species analysis results that associate *Haplothrips tenuipennis* (a Thysanoptera species) with sites amended without biosolids at Highland Valley Copper. Thysanoptera taxa are known to be associated with vegetation, and many species breed specifically in flowers (Mound et al., 2018). For example, *Haplothrips spp.* can be linked to Asteraceae plants (Mound et al., 2018). That being said, Thysanoptera’s close association to vegetation and flowers has resulted in them being considered a pest species. Thysanoptera, as an order, appear to be understudied outside the context of pest management, making it difficult to identify patterns between Thysanoptera diversity and sites with and without biosolids. Future studies linking amendments, vegetation composition, and Thysanoptera biodiversity could potentially uncover further links in bottom-up trophic interactions in a reclamation setting.

Overall, my results did not show a difference in alpha diversity between sites, when analysed using the full data set. There was, however, a significant difference in the richness of the orders Diptera, Entomobryomorpha, Psocodea, Thysanoptera, and the family Formicidae. Researchers have noted that differences in alpha diversity may be difficult to detect or be negligible due to the substitution of taxa (Dornelas et al., 2014). Furthermore, categorizing the OTUs into higher taxonomic classifications (order/family) was helpful for making inferences about the study sites for Entomobryomorpha and Formicidae. However, lesser studied orders including Psocodea and Thysanoptera, as well as diverse orders such as Diptera provided less insight into patterns between the study sites. Therefore, biodiversity composition between sites could be a more informative measure of post-reclamation arthropod return than species richness (Lynggaard et al., 2020).

Indicator arthropod taxa distinguishing reclamation sites

Understanding arthropod assemblage (dis)similarities and richness can illustrate an overall picture of tested variables, in this case ‘reclamation age’ and ‘soil amendment’. However, policy decisions and land management have previously been motived by indicator species (Ji et al., 2013). Therefore, identifying (arthropod) indicator species for reclamation age and soil amendments is also constructive. The presence of indicator taxa was measured using the ‘Indicspecies’ analyses, which is based on the indicator value index and identifies the association of taxa with grouping variables.

Indicator analysis showed that despite statistically significant associations between several taxa and site age, they did not demonstrate strong sensitivity and specificity values (near 1), indicating that they were not ideal indicator taxa (Table 2.8). To better understand indicator taxa between sites, data were separated by mine. Because the mine locations were separated using ordination (Figure 2.13), implying that there are differences in taxa composition, additional analyses addressed the arthropod assemblage at each mine individually. This section will only discuss indicator values greater than 0.65 for efficiency (Hammond et al., 2018).

The ‘new’ site at New Afton mine had the strongest arthropod indicator species associations. In particular, OTU 0771 (family Heleomyzidae taxon) and OTU 1504 (*Tapinoma sessile*) showed a strong affiliation with the ‘new’ sites (Table 2.10). Heleomyzidae (a true fly taxon) was the taxon with the highest association with a treatment. The Heleomyzidae OTU was associated with ‘new’ sites at the New Afton mine. Studies have noted the members of the Heleomyzida*e* family can be found in various adverse locations, including caves (Kocot-Zalewska and Woźnica, 2021), high elevation areas (Lee et al., 2015), and in arctic conditions (Danks, 2004). Heleomyzidae’s presence in adverse environments indicates that the taxa has the ability to survive in harsh environmental conditions, which may explain our observation that they were primarily associated with the ‘new’, most recently disturbed, sites in this study.

The second taxon strongly associated with the ‘new’ sites at the New Afton mine was *Tapinoma sessile*, an ant species. *Tapinoma sessile* is a small, potentially aggressive ant that is common in North America (Higgins and Lindgren, 2009). Their nests are typically correlated with wood but are also often located in moss or soil. Often undetected due to their small size (Higgins and Lindgren, 2009), Salyer et al. (2014) found that *Tapinoma sessile* adapts quickly in urban (disturbed) environments by taking advantage of unfamiliar resources. They also identified the reduced ant species richness and consequent, interspecific competition, allowed *Tapinoma sessile* to capitalize on the available resources (Salyer et al., 2014). Specifically, Tapinoma does not compete effectively, and instead are more likely to be a colonizer taxon (Buczkowski and Bennett, 2008). Given their adaptability to harsh environments, it is understandable that they were the dominant taxa in the ‘new’ sites in this study.

Entomobrya unostrigata (cotton springtail) was strongly associated with the ‘new’ and ‘old’ reclaimed sites. A review of *Entomobrya unostrigata* noted that this Collembolan species was an early colonizer in reclaimed mine sites (Greenslade, 1995). More specifically, *Entomobrya unostrigata* was primarily detected in disturbed sites and was not commonly found in areas with native vegetation (Greenslade, 1995). Greensade’s (1995) findings is aligned with my results, that *Entomobrya unostrigata* was associated with both the ‘new’ and ‘old’ sites, but not the ‘reference’ sites.

It is also interesting that ‘reference’ sites were significantly affiliated with substantially more indicator taxa (36) than the ‘new’ sites (7), while the ‘old’ sites were not associated with any indicator species. When data were separated by mine, the indicator species analysis for the

Highland Valley Copper ‘reference’ was associated with 36 taxa, but the New Afton ‘reference’ was not represented by any indicator taxa.

Similar to what was seen with reclamation age, the results of the complete soil amendment dataset produced a variety of statistically significant indicator taxa without high indicator values meaning that the arthropods did not demonstrate strong sensitivity and specificity values, indicating that they were not ideal indicator taxa. Data were separated by mines to identify patterns between indicator taxa and biosolids as a soil amendment; however, biosolids were only applied at Highland Valley Copper mine. As seen in Table 2.12, two Formicidae taxa are significantly associated with the ‘reference’ sites and a combination of the ‘reference’ sites and ‘no biosolids’ sites. This result is complemented by the results found in the alpha diversity statistics from this study, where a greater Formicidae richness was associated with the ‘no biosolids’ sites than the ‘biosolids’ sites.

Both strong indicator species associated with soil amendments belong to the genus *Formica*. Ant species that belong to this genus have nests housing tens of thousands of worker ants. Moreover, worker ants in the genus *Formica* are often observed collecting food (prey) for their colony (Higgins and Lindgren, 2009). It stands to reason that the taxa belonging to the genus *Formica* can make a notable impression of the functioning on a site, specifically through predation on local prey species (Higgins and Lindgren, 2009).

Indeed, the ant species *Formica neorufibarbis* was predominantly associated with the Highland Valley Copper mine ‘reference’ sites that were visually rich in woody materials. Indeed, *Formica neorufibarbis* is frequently located in woody materials within clear-cuts (Higgins and Lindgren, 2009). Concerning my findings, it could make sense that the species was correlated to these sites because the ‘reference’ sites were visually rich in woody materials.

Formica subaenescens was associated with a combination of the soil amendment variables ‘reference’ and ‘no biosolids’. *Formica subaenescens* are often found at disturbed sites, nesting in the soil (Higgins and Lindgren, 2009). Similar to *Formica neorufibarbis, Formica subaenescens* has an affinity for woody materials, more specifically for structurally intact wood (Higgins and Lindgren, 2009). The strong association of this taxon with the combination of sites could potentially be explained in part by the disturbance in the ‘no biosolids’ site and the visually observed woody materials at the ‘reference’ sites. Like the relationship between indicator taxa and reclamation age, there was a noticeable difference in the number of indicator taxa associated with the treatment sites and the reference sites at Highland Valley Copper mine. Again, the ‘reference’ sites were associated with a greater number of indicator taxa (34) than with the ‘biosolids’ treatment (1) and the ‘no biosolids’ treatment (4).

Ultimately, the goal of indicator species analyses is to identify a relatively short list of species that can be used as reclamation bioindicators (Denholm, 2003). For example, in this study *Formica neorufibarbis* is a strong indicator species of the ‘reference’ variable at Highland Valley Copper mine, for both reclamation age and soil amendment. The presence of this species on treatment sites could suggest a trajectory towards natural forested sites free of previous mining activity. Other environmental and situational factors, such as vegetation mix seeded, can affect the trajectory of succession, relating to the available habitat and recovery of arthropod taxa (Swab et al., 2017).

It is notable that no known invasive species, as listed by Invasive species of BC (n.d.) and Government of BC (n.d.), were detected at any of the sites sampled in this study. In general, invasive species success can be attributed to adverse conditions, such as disturbance regime, anthropogenic influences, altered weather patterns (Dix et al., 2010). That is, invasive insect species have better success adapting to altered environmental conditions than native species (Dix et al., 2010). Invasive species may alter ecosystem structure and function by changing environmental processes and food chains (Dix et al., 2010). These ecosystem alterations can cause both environmental and economic challenges. Continued monitoring will best inform on invasive species presence and management.

DNA metabarcoding as a research technique

Historically, arthropod monitoring methods primarily relied on morphological methods to identify taxa. However, using morphological methods to identify arthropod taxa requires significant time, labour, and expertise (Fernandes et al., 2018). Additionally, morphological identification is particularly challenging for taxa with variable phenotypes over life stages. DNA metabarcoding provides potential solutions for some of these concerns but does come with important limitations.

PCR amplification bias can skew sequence abundance for specific taxa and may result in missing some taxa altogether (Elbrecht and Leese, 2015). Additionally, the number of sequencing reads can be affected by total biomass for an individual group, as well as the physical size of individual arthropods (Elbrecht and Leese, 2015). For these reasons, neither absolute nor relative abundance were used in this study. Instead, presence-absence was used to measure arthropod biodiversity and assemblage.

Species level taxonomic assignment of OTUs in can be negatively impacted by information gaps in biological databases, such as BOLD. Biological refence databases are relatively new, and therefore are still acquiring taxonomic data (Beng et al., 2016; Yu et al., 2012). Additionally, taxonomic resolution can face challenges due to short sequences, which can complicate capture overall genetic diversity (Porter and Hajibabaei, 2018). These factors could contribute to some (39) OTUs only being identified to order level, or higher, taxonomies.

DNA metabarcoding can fail to detect certain taxa; for example, Fernandes et al. (2019) experienced difficulties detecting Hymenoptera in their study that used DNA metabarcoding to identify terrestrial invertebrates that were collected on reclaimed mine sites. However, regardless of current limitations, DNA metabarcoding identifies a greater diversity of taxa than alternative identification methodologies. Specifically, Elbrecht et al. (2017) compared molecular OTU identification methods, using a range of primer combinations, against morphological identification methods and found that DNA metabarcoding ultimately detected a greater diversity of macroinvertebrates than morphological methods. The overall benefits of DNA metabarcoding held true despite the fact that morphological identification methods found taxa that were not detected through DNA metabarcoding.

Overall, DNA metabarcoding offers an efficient, accessible, and accurate method for environmental monitoring. Using the current reference databases, this study found differences related to environmental variables and detected indicator taxa. Taxonomic identification and precision continue to improve as more samples are identified and added to reference databases.

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Chapter 3 Research Conclusions

3.1 Research Synthesis

The results of this study aid in reducing the knowledge gap for postmining reclamation outcomes. Using novel methods (high-throughput DNA metabarcoding), this project contributes to the improvement of planning and management practices, leading to more effective post-mining ecosystem-recovery outcomes. In turn, the outcomes and methodologies of this project can assist in the development of further advancements in mine reclamation as it relates to the sustainable health of ecosystems, which are vital to the continued growth of BC's communities and economy.

In particular, this study investigated the effects different reclamation ages and soil amendments have on arthropod assemblages, indicator taxa, and alpha diversity. In this study, differences in arthropods between sampled areas were detected. First, the (dis)similarity of arthropod assemblages between the reclamation age and amendment sites implied that neither factor is the primary driver of arthropod composition; rather an external environmental factor is a stronger driver of arthropod composition. Second, despite treatment correlations with higher-level taxa, there was not a statistically significant relationship of overall species richness between sites. Thirdly, indicator taxa analyses detected several taxa associated with study sites. Examining these unique research questions, and comparing their outputs with each other, helped navigate the story of reclamation trajectory. Individually, each analysis is limited in the scope of the information that it can provide about the sites. For example, alpha diversity alone can determine how many OTUs are present on study sites, however, it can not determine what species are present on the sites. By examining both alpha diversity and indicator species, we see that there is greater Formicidae richness in sites amended without biosolids, and we can also see that none of the Formicidae species were the invasive European fire ant, *Myrmica rubra*, which could negatively impact the ecosystem by displacing native arthropods.

3.2 Limitations

The purpose of this study was to shed light on arthropod recovery in a post-mine environment. In general, field studies, compared to laboratory studies, are less able to control additional environmental variables. Moreover, this study was descriptive in nature, as it was based on observation and did not involve a manipulative study. For example, species-specific relationships with soil amendments in a manipulative trial setting would expand the current knowledge base.

Arthropod data used in this research were collected once per site during the summer of 2018. This study thus represented a 'snapshot' of arthropod assemblage and biodiversity. A one-time sampling methodology is associated with several limitations. Specifically, the idiosyncratic behaviour of species (seasonality and life span) can impact the likelihood of capturing them at a single collection time (Danks, 1996). Furthermore, passively detecting arthropods can also be affected by their abundance in the given area, which could lead to reduced recognition of rare taxa. Other studies have recommended collecting arthropod samples over different seasons to capture a more complete view of species composition (Lynggaard et al., 2020; Danks, 1996).

Data were gathered from mines at a time that satisfied industry partner schedules. As a result, the two mines were sampled four weeks apart from each other. Foster et al. (2020) found that there were seasonal differences (early and late summer) in arthropod assemblages in the Kamloops' Lac du Bois grasslands. Their findings suggest that seasonal influences, such as temperature and precipitation, impact arthropod assemblage composition (Foster et al., 2020; Liu et al., 2013). Seasonality can also have indirect effects on arthropods communities through availability of vegetation, litter, and soil moisture (Liu et al., 2013). For example, Denholm (2003) highlighted the importance of understanding arthropod life cycles and behaviour to explain biodiversity. In their experiment, they found craneflies (Tipulidae) were a negative indicator of biosolids application; however, if sampling occurred before or after their approximate 14-day temporal window of emergence, the relationship would go undetected (Denholm, 2003).

In this study, sites reclaimed in different years were sampled to provide insight to how arthropod assemblages change over time. Continual sampling at the same sites would deepen insight to unique site-specific characteristics and development of arthropod assemblages. Two 'reference' sites were sampled near both mines for the purpose of comparing arthropod assemblage and diversity at reclaimed mine sites to undisturbed sites. Ideally, arthropod assemblages and biodiversity could be compared to pre-disturbance conditions, as each site may have unique microsite conditions. However, opportunities to collect baseline data pre-disturbance can be limited; but are possible in some situations, as exemplified by Kega (2021).

3.3 Management Implications

This study intended to identify patterns between mine reclamation and arthropod assemblage composition, ultimately aiming to better understand reclamation practices to reach end land-use goals. As previously mentioned, reclamation efforts are commonly focused on achieving end land use objectives, opposed to restoring the disturbed area to its original state (Lima et al., 2015). End Land Use Plans, such as the ones developed by Highland Valley Copper With engagement and consultation from Nlaka'pamux communities, and the ones developed by New Afton with engagement and consultation from Stk'emlupsemc te Secwepemc Nation and Tk'emlúps te Secwépemc can enhance a community-based approach to reclamation. Specifically, end land use at both Highland Valley Copper and New Afton focuses on returning post-mined areas to natural ecosystems, with potential uses including hunting and trapping (Melaschenko et al., 2018; New Gold Inc., 2017). Studies such as this one aim to reduce the knowledge gap and provide management recommendations, which ultimately strive to achieve a functional ecosystem for end land uses. In particular, future explorations could conduct an ecological assessment or multi-year monitoring, determining arthropod assemblage and biodiversity, prior to mining an area. This would provide baseline data about the pre-disturbance natural ecosystem which could be used as a comparison and/or target for reclaimed ecosystems. Additionally, continued monitoring arthropod assemblage and biodiversity can inform on potential ecological concerns, such as the presence of invasive species.

Historically, reclamation has been focused on vegetation health, but recently the focus has shifted to include additional environmental characteristics such as overall biodiversity, structure, and ecosystem function (Fraser et al., 2015). However, methods to measure whole ecosystem reclamation trajectories are poorly understood and can be difficult for monitors to select, as they are unfamiliar. This study aimed to reduce the knowledge gap of arthropod recovery trajectories in a post-mining reclaimed area and demonstrate efficient DNA methodologies for arthropod monitoring.

The use of DNA metabarcoding technology to characterize arthropod assemblages reduces reliance on indicator taxa (Lindenmayer and Likens, 2011). Despite the practical benefits of indicator taxa, outlined in chapter 2, using indicator taxa exclusively to monitor biodiversity has unique limitations and challenges. Specifically, indicator taxa may be incorrectly used as a surrogate of environmental conditions, causing confusion for policymakers (Lindenmayer and Likens, 2011).

3.4 Future Research

Moving forward, research should work to inform what treatments or environmental factors are correlated with arthropod recovery in mine sites through a whole ecosystem approach. This research can be used by policymakers and land managers to aid post-mining reclamation by creating optimal recovery conditions (Fernandes et al., 2018). Additionally, as climate change progresses and temperatures increase, arthropods will have varied reactions, such as increasing or decreasing ranges across altitudes (Hodkinson, 2005) and latitudes (Wilson and Fox, 2020). A deeper understanding of insect response to climate conditions, such as temperature, will prepare reclamation scientists with optimal practices to face upcoming challenges. Ultimately, this study provides a benchmark for future research to build on, to understand optimal environmental conditions to aid post-mining ecosystem recovery. Moreover, this study was descriptive in nature, and identified multiple small patterns between arthropods and the conditions of the study environments in an effort to interpret the results and provide recommendations. Based on these results, future studies could look at correlations between vegetation (community and structure), soil conditions (microbial and chemical), water availability, elevation, and arthropod biodiversity and assemblages. For example, different plant communities and structures can affect predatory success; despite greater food availability, some taxa favor sparce plant cover (Gaudreault et al., 2019). Additionally, this study assessed the effects of biosolids as a soil amendment on arthropod assemblages in a binary sense (biosolids and no biosolids). It would be interesting to conduct a study comparing the effects of compost, biosolids, and manure on arthropods.

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Appendix A OTUs used in statistical analyses

Table A.1. Operation taxonomic units of arthropods, collected from Highland Valley Copper and New Gold Inc. New Afton used in data analyses

OTU	**Taxonomy**	**Kingdom**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	***Species***
0001	GS|99.3|BOLD:AAL2821_KR504455	Animalia	Arthropoda	Insecta	Diptera	Muscidae	Phaonia	*Phaonia apicalis*
0010	GS|96.3|BOLD:AAG8962_KR042869	Animalia	Arthropoda	Insecta	Hemiptera	Nabidae	Nabicula	*Nabicula nigrovittata*
0100	GS|90.7|BOLD:AAC6116_KR039436	Animalia	Arthropoda	Insecta	Hemiptera	Scutelleridae	Homaemus	*Homaemus bijugis*
1004	GS|91.0|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
1006	GS|86.7|BOLD:ACA5986_NA	Animalia	Arthropoda	Insecta	Coleoptera			
0101	GS|97.7|BOLD:AAN6555_MF606846	Animalia	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Lepidocyrtus	*Lepidocyrtus cyaneus*
1010	GS|92.0|BOLD:ADC9253_NA	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Ceratagallia	
0102	GS|91.0|BOLD:ACK5012_KM909774	Animalia	Arthropoda	Insecta	Diptera	Sciaridae		
1027	GS|94.7|BOLD:AAG2875_MF932157	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Ceratagallia	
1039	GS|99.3|BOLD:AAH0413_NA	Animalia	Arthropoda	Insecta	Coleoptera			
0104	GS|100.0|BOLD:AAL2821_MF889772	Animalia	Arthropoda	Insecta	Diptera	Muscidae	Phaonia	*Phaonia apicalis*
1040	GS|95.7|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
1042	GS|100.0|BOLD:AAP7809_KM843868	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Atheta	*Atheta capsularis*
1048	GS|97.9|BOLD:AAF2735_NA	Animalia	Arthropoda	Insecta	Coleoptera			
1055	GS|85.7|BOLD:ACZ1106_KU916435	Animalia	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Aphodius	*Aphodius zenkeri*
0106	GS|90.3|BOLD:AAP8157_KM952442	Animalia	Arthropoda	Insecta	Diptera	Mycetophilidae	Katatopygia	
1060	GS|98.0|BOLD:AAA2674_KR971587	Animalia	Arthropoda	Insecta	Diptera	Drosophilidae	Drosophila	*Drosophila subquinaria*
1068	GS|90.5|BOLD:AAM7579_NA	Animalia	Arthropoda	Insecta	Coleoptera	Dermestidae	Dermestes	*Dermestes marmoratus*

1069	GS|90.7|BOLD:AAN6562_JN290616	Animalia	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae		
1072	GS|96.3|BOLD:AAE0406_KR896490	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	*Formica lasioides*
1076	GS|78.7|BOLD:ACO5516_NA	Animalia	Arthropoda	Insecta	Diptera	Dolichopodidae	Thinophilus	
0109	GS|81.4|BOLD:AEB3573_NA	Animalia	Arthropoda	Insecta	Archaeognatha			
0011	GS|88.4|BOLD:AAP7095_KM844723	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Tachinus	
0110	GS|100.0|BOLD:AAL5087_MF635391	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Philonthus	*Philonthus cognatus*
1104	GS|92.3|BOLD:AAU6111_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*
0111	GS|75.3|BOLD:ADA7549_NA	Animalia	Arthropoda	Insecta	Archaeognatha	Machilidae		
1112	GSL|94.3|BOLD:AAB3450_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
1125	GS|96.7|BOLD:AAU6111_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*
1127	GS|92.8|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0113	GS|100.0|BOLD:ACE3663_NA	Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	Amara	*Amara farcta*
1132	GS|93.3|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0114	GS|100.0|BOLD:AAD6543_KR934988	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Aphaenogaster	*Aphaenogaster occidentalis*
1141	GS|82.7|BOLD:AAP7796_JF888053	Animalia	Arthropoda	Insecta	Coleoptera	Curculionidae	Cossonus	*Cossonus piniphilus*
1144	GS|98.4|BOLD:AAV1530_JN309491	Animalia	Arthropoda	Insecta	Archaeognatha	Machilidae		
0115	GS|99.6|BOLD:AAF7755_MG477257	Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	Cymindis	*Cymindis cribricollis*
1152	GS|97.0|BOLD:AAU6111_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*
0116	GS|81.3|BOLD:AEB3573_NA	Animalia	Arthropoda	Insecta	Archaeognatha			
1162	GS|94.7|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
1163	GS|95.3|BOLD:AAU6111_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*
1165	GS|82.3|BOLD:AEB3573_NA	Animalia	Arthropoda	Insecta	Archaeognatha			

1166	GS|77.6|BOLD:AAG2875_KR569482	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Ceratagallia	
1167	GS|96.0|BOLD:ACJ0553_NA	Animalia	Arthropoda	Arachnida	Opiliones			
1174	GS|96.7|BOLD:AAB3450_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Circotettix	*Circotettix carlinianus*
0119	GS|98.0|BOLD:ACF5385_KJ964115	Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	Amara	*Amara alpina*
0012	GS|100.0|BOLD:AAG5198_KM843557	Animalia	Arthropoda	Insecta	Coleoptera	Curculionidae	Otiorhynchus	*Otiorhynchus ovatus*
1205	GS|93.4|BOLD:AAA8764_KM533615	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
1207	GS|73.3|BOLD:AAW9344_NA	Animalia	Arthropoda	Insecta	Diptera	Asilidae	Ospriocerus	*Ospriocerus aeacus*
1219	GS|95.3|BOLD:AAA8764_JN294649	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0122	GS|100.0|BOLD:AAA8914_KM824705	Animalia	Arthropoda	Arachnida	Araneae	Gnaphosidae	Zelotes	*Zelotes fratris*
1223	GS|87.4|BOLD:ACP5629_NA	Animalia	Arthropoda	Insecta	Diptera			
1224	GS|100.0|BOLD:ACC6491_KM848552	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Earota	*Earota dentata*
0123	GS|100.0|BOLD:AAD0461_KP653242	Animalia	Arthropoda	Arachnida	Araneae	Gnaphosidae	Haplodrassus	
1230	GS|93.0|BOLD:AAY6676_KM838130	Animalia	Arthropoda	Arachnida	Opiliones	Sclerosomatidae	Togwoteeus	*Togwoteeus biceps*
1235	GS|88.7|BOLD:ACP5629_NA	Animalia	Arthropoda	Insecta	Diptera			
1236	GS|96.3|BOLD:AAN6561_JN290615	Animalia	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae		
0124	GS|100.0|BOLD:ACF2871_KR923535	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	*Formica neorufibarbis*
1247	GS|100.0|BOLD:ACM2411_KR683544	Animalia	Arthropoda	Insecta	Diptera	Cecidomyiidae		
0127	GS|99.7|BOLD:AAL7523_HM860450	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae	Eutrichota	*Eutrichota tarsata*
1270	GSL|91.0|BOLD:AAB3450_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
1274	GSL|89.3|BOLD:ACF3208_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0128	GS|99.7|BOLD:ACF3749_KR688854	Animalia	Arthropoda	Insecta	Diptera	Phoridae	Megaselia	
1280	GS|98.5|BOLD:AAG8804_NA	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Psammotettix	*Psammotettix confinis*

1287	GS|76.3|BOLD:ACG9299_NA	Animalia	Arthropoda	Insecta	Diptera			
0129	GS|90.6|BOLD:AAP9950_KR987592	Animalia	Arthropoda	Insecta	Diptera	Mycetophilidae	Cordyla	
1293	GS|100.0|BOLD:ACI8307_MF936428	Animalia	Arthropoda	Insecta	Hymenoptera	Braconidae		
0013	GS|100.0|BOLD:AAV1530_JN309491	Animalia	Arthropoda	Insecta	Archaeognatha	Machilidae		
1300	GS|93.3|BOLD:ACJ0553_NA	Animalia	Arthropoda	Arachnida	Opiliones			
1303	GS|89.2|BOLD:AAC8406_MF937863	Animalia	Arthropoda	Insecta	Hemiptera	Rhyparochromidae	Sphragisticus	*Sphragisticus nebulosus*
1310	GS|79.0|BOLD:ACA5986_NA	Animalia	Arthropoda	Insecta	Coleoptera			
1314	GS|99.7|BOLD:AAC6116_KR032723	Animalia	Arthropoda	Insecta	Hemiptera	Scutelleridae	Homaemus	*Homaemus bijugis*
0132	GS|100.0|BOLD:ABA5839_MG403656	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Euscelis	
1339	GS|87.3|BOLD:ACP5629_NA	Animalia	Arthropoda	Insecta	Diptera			
0134	GS|100.0|BOLD:ABY7010_NA	Animalia	Arthropoda	Insecta	Diptera	Fanniidae	Fannia	
1343	GS|87.3|BOLD:AAP8157_KM952442	Animalia	Arthropoda	Insecta	Diptera	Mycetophilidae	Katatopygia	
1344	GSL|90.3|BOLD:ACF3208_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
1346	GS|100.0|BOLD:ABZ3849_MF831862	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Diplocolenus	*Diplocolenus evansi*
0135	GS|96.3|BOLD:ACJ8525_KM841491	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Quedius	
1350	GS|89.7|BOLD:ABX4044_NA	Animalia	Arthropoda	Insecta	Coleoptera	Nitidulidae	Urophorus	*Urophorus humeralis*
0136	GS|83.3|BOLD:ACM2566_KR147632	Animalia	Arthropoda	Insecta	Archaeognatha	Meinertellidae	Machilinus	
1363	GS|89.1|BOLD:ACP5629_NA	Animalia	Arthropoda	Insecta	Diptera			
1367	GS|97.6|BOLD:AAE0406_KR879680	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	*Formica lasioides*
1370	GS|100.0|BOLD:AAA9476_MF813717	Animalia	Arthropoda	Arachnida	Araneae	Linyphiidae	Grammonota	*Grammonota gentilis*
1380	GS|89.3|BOLD:ACG3239_KM955768	Animalia	Arthropoda	Insecta	Diptera	Sciaridae	Claustropyga	
0014	GS|99.3|BOLD:ACI5842_KM642700	Animalia	Arthropoda	Insecta	Diptera	Hybotidae		

0140	GS|100.0|BOLD:ACC5897_KM841972	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae		
1400	GS|98.0|BOLD:AAG2472_KR689987	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae	Delia	
1401	GS|100.0|BOLD:AAV1530_JN309491	Animalia	Arthropoda	Insecta	Archaeognatha	Machilidae		
1418	GS|96.0|BOLD:AAU6111_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*
0142	GS|100.0|BOLD:AAG2341_KR693011	Animalia	Arthropoda	Insecta	Diptera	Tachinidae	Paradidyma	
1423	GS|90.3|BOLD:ACF3208_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Conozoa	*Conozoa sulcifrons*
1424	GS|87.7|BOLD:ACP5629_NA	Animalia	Arthropoda	Insecta	Diptera			
1430	GS|98.0|BOLD:AAA8764_JN294532	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
1431	GS|97.3|BOLD:ACX6073_MG035016	Animalia	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae		
1455	GS|90.9|BOLD:AAG2875_MF938947	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Ceratagallia	
1457	GS|90.7|BOLD:AAH4207_HM412662	Animalia	Arthropoda	Insecta	Diptera	Chloropidae	Meromyza	
0146	GS|97.0|BOLD:AAP9080_KM945424	Animalia	Arthropoda	Insecta	Diptera	Mycetophilidae	Leia	
1464	GS|99.7|BOLD:AAH4153_KM631260	Animalia	Arthropoda	Insecta	Diptera	Chloropidae		
0147	GS|100.0|BOLD:ACU2723_MF760195	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae	Delia	
1477	GS|96.7|BOLD:AAA8764_JN294649	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
1479	GS|92.5|BOLD:AAG2875_MG509309	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Ceratagallia	
1481	GS|83.5|BOLD:ACP5629_NA	Animalia	Arthropoda	Insecta	Diptera			
1486	GS|100.0|BOLD:ACM2385_MF891107	Animalia	Arthropoda	Insecta	Diptera	Muscidae	Coenosia	
1488	GS|83.2|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0149	GS|82.0|BOLD:AEB3573_NA	Animalia	Arthropoda	Insecta	Archaeognatha			
1490	GS|98.3|BOLD:ACG1460_NA	Animalia	Arthropoda	Insecta	Coleoptera			
1492	GS|88.3|BOLD:ADR1193_NA	Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	Amara	*Amara fortis*

1495	GS|92|BOLD:AAA3898_KF605178	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Tapinoma	*Tapinoma sessile*
1499	GS|97.0|BOLD:AAH6630_KJ445452	Animalia	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	*Entomobrya unostrigata*
0015	GS|100.0|BOLD:AAA1468_KR801242	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	*Formica subaenescens (formerly Formica fusca var. complex)*
1500	GS|83.9|BOLD:AAA8764_JN294649	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
1502	GSL|84.4|BOLD:AAI9028_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
1504	GS|99.0|BOLD:AAA3898_KR791510	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Tapinoma	*Tapinoma sessile*
0152	GS|99.3|BOLD:ACE2096_KM844015	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Atheta	
1522	GS|90.3|BOLD:AAG2875_KR037999	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Ceratagallia	*Ceratagallia sanguinolenta*
1528	GS|98.3|BOLD:ACF6995_KR577795	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Ceratagallia	*Ceratagallia sanguinolenta*
0153	GS|81.7|BOLD:AEB3573_NA	Animalia	Arthropoda	Insecta	Archaeognatha			
0154	GS|100.0|BOLD:AAA7374_JN285899	Animalia	Arthropoda	Insecta	Diptera	Syrphidae	Sphaerophoria	*Sphaerophoria philanthus*
1542	GS|94.3|BOLD:AAY3979_KR457459	Animalia	Arthropoda	Insecta	Diptera	Tachinidae	Freraea	*Freraea gagatea*
1545	GS|94.3|BOLD:ACC5897_KM849278	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae		
1548	GS|96.3|BOLD:ACK7799_KM846655	Animalia	Arthropoda	Insecta	Coleoptera	Leiodidae	Neoeocatops	*Neoeocatops decipiens*
1550	GS|88.6|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
1551	GS|93.7|BOLD:ACJ0553_NA	Animalia	Arthropoda	Arachnida	Opiliones			
1552	GS|96.7|BOLD:AAE0406_KR897079	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	*Formica lasioides*
1554	GS|96.7|BOLD:AAZ1683_MG170293	Animalia	Arthropoda	Insecta	Hemiptera	Miridae	Psallovius	*Psallovius piceicola*
1560	GS|99.1|BOLD:AAP7044_KM848665	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Phloeostiba	*Phloeostiba lapponica*
1561	GS|75.7|BOLD:AAC9088_KM570098	Animalia	Arthropoda	Insecta	Diptera	Tipulidae	Tipula	*Tipula angulata*

1568	GS|99.7|BOLD:AAI5560_KR044828	Animalia	Arthropoda	Insecta	Hemiptera	Scutelleridae	Eurygaster	*Eurygaster amerinda*
1574	GS|95.7|BOLD:AAA8764_MG468655	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
1576	GSL|94.0|BOLD:ACF3208_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
1579	GS|96.0|BOLD:AAG5198_KJ963586	Animalia	Arthropoda	Insecta	Coleoptera	Curculionidae	Otiorhynchus	*Otiorhynchus ovatus*
1580	GS|100.0|BOLD:AAA4977_KR934170	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	*Formica neorufibarbis*
1585	GS|98.9|BOLD:ABA5839_KR583463	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Euscelis	
1586	GS|99.3|BOLD:AAG2877_MF832746	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Cuerna	*Cuerna cuesta*
0159	GS|97.0|BOLD:ACF9170_KR722833	Animalia	Arthropoda	Insecta	Diptera	Sphaeroceridae	Pullimosina	*Pullimosina moesta*
0016	GS|92.0|BOLD:ABA2351_NA	Animalia	Arthropoda	Insecta	Diptera	Fanniidae	Fannia	
0160	GS|99.7|BOLD:AAD5009_KP651027	Animalia	Arthropoda	Arachnida	Araneae	Gnaphosidae	Gnaphosa	*Gnaphosa muscorum*
1601	GS|97.5|BOLD:AAP7044_KR481778	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Phloeostiba	*Phloeostiba lapponica*
1623	GS|83.0|BOLD:AAI9028_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Cratypedes	*Cratypedes neglectus*
0163	GS|100.0|BOLD:ACA9180_KR482485	Animalia	Arthropoda	Insecta	Coleoptera	Melyridae	Hypebaeus	*Hypebaeus bicolor*
1645	GS|99.3|BOLD:AAG8681_KR918070	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Psammotettix	*Psammotettix lividellus*
0166	GS|97.0|BOLD:ACJ0553_NA	Animalia	Arthropoda	Arachnida	Opiliones			
1673	GS|92.3|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
1678	GS|92.7|BOLD:AAM7579_NA	Animalia	Arthropoda	Insecta	Coleoptera	Dermestidae	Dermestes	*Dermestes marmoratus*
1679	GS|99.7|BOLD:AAE1002_NA	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	*Formica subpolita*
1697	GS|83.4|BOLD:ABW2776_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0017	GS|81.3|BOLD:AAG6186_HM416864	Animalia	Arthropoda	Insecta	Archaeognatha	Meinertellidae		
0170	GS|100.0|BOLD:AAW1617_KR486565	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Aleochara	*Aleochara rubricalis*
1700	GS|88.8|BOLD:AAG2442_KM864353	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae	Zaphne	*Zaphne implicata*

0171	GS|96.0|BOLD:ABX3986_NA	Animalia	Arthropoda	Insecta	Coleoptera	Anthicidae	Anthicus	*Anthicus punctulatus*
1717	GS|99.7|BOLD:ACD0897_MF762743	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae		
0172	GS|100.0|BOLD:ABZ3849_MF831862	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Diplocolenus	*Diplocolenus evansi*
1720	GS|93.3|BOLD:ACW8722_KR805644	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Myrmica	
1728	GS|95.0|BOLD:ACF3208_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Conozoa	*Conozoa sulcifrons*
0173	GS|79.3|BOLD:AAZ7477_NA	Animalia	Arthropoda	Arachnida	Opiliones			
1733	GS|99.3|BOLD:AAP6497_MG091533	Animalia	Arthropoda	Insecta	Diptera	Mycetophilidae	Phronia	
1742	GS|96.3|BOLD:AAH4273_KM545000	Animalia	Arthropoda	Insecta	Lepidoptera	Gelechiidae	Chionodes	
1749	GS|98.0|BOLD:ACF3950_KM633224	Animalia	Arthropoda	Insecta	Diptera	Phoridae	Megaselia	
0175	GS|100.0|BOLD:AAN4465_KM623929	Animalia	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Lepidocyrtus	*Lepidocyrtus cyaneus*
1751	GS|100.0|BOLD:ACX5751_KT104856	Animalia	Arthropoda	Insecta	Diptera	Sciaridae		
1758	GS|96.6|BOLD:AAG5331_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Chorthippus	*Chorthippus montanus*
1769	GS|96.3|BOLD:AAN6561_JN290615	Animalia	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae		
1775	GS|95.2|BOLD:AAP7044_KR487949	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Phloeostiba	*Phloeostiba lapponica*
1776	GS|89.1|BOLD:AAE2480_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Spharagemon	*Spharagemon campestris*
1778	GS|94.1|BOLD:AAU6111_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*
0179	GS|86.0|BOLD:ABY0171_NA	Animalia	Arthropoda	Insecta	Hemiptera	Rhyparochromidae		
0018	GS|99.7|BOLD:AAG8804_NA	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Psammotettix	*Psammotettix confinis*
0180	GS|79.0|BOLD:ACI5842_KM642700	Animalia	Arthropoda	Insecta	Diptera	Hybotidae		
0181	GS|98.7|BOLD:ACE4532_KR044858	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Latalus	*Latalus missellus*
0182	GS|88.7|BOLD:AAN6145_KR489904	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Philonthus	*Philonthus varians*
0183	GS|100.0|BOLD:AAF2735_NA	Animalia	Arthropoda	Insecta	Coleoptera			

0184	GS|85.7|BOLD:ACB8951_MF747714	Animalia	Arthropoda	Insecta	Psocodea	Lachesillidae	Lachesilla	
0185	GS|99.3|BOLD:ACG1315_KF549892	Animalia	Arthropoda	Insecta	Coleoptera	Elateridae	Selatosomus	*Selatosomus aeripennis*
0189	GS|100.0|BOLD:ACZ4231_KM532906	Animalia	Arthropoda	Insecta	Thysanoptera	Thripidae	Frankliniella	*Frankliniella occidentalis*
0019	GS|100.0|BOLD:ACB0775_KR694678	Animalia	Arthropoda	Insecta	Diptera	Hybotidae		
0191	GS|100.0|BOLD:ACL6983_KM645139	Animalia	Arthropoda	Insecta	Diptera	Chloropidae	Olcella	
0194	GS|100.0|BOLD:ACG1894_KM844736	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Phloeostiba	*Phloeostiba lapponica*
0197	GS|83.3|BOLD:AEB3573_NA	Animalia	Arthropoda	Insecta	Archaeognatha			
0198	GS|92.4|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0199	GS|100.0|BOLD:ABA1213_KR633872	Animalia	Arthropoda	Insecta	Diptera	Phoridae	Megaselia	
0002	GS|100.0|BOLD:AAE0406_KR897079	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	*Formica lasioides*
0200	GS|100.0|BOLD:ABZ2976_KR970957	Animalia	Arthropoda	Insecta	Diptera	Drosophilidae	Drosophila	*Drosophila munda*
0202	GS|100.0|BOLD:AAG7279_JN301730	Animalia	Arthropoda	Insecta	Diptera	Sphaeroceridae	Eulimosina	*Eulimosina ochripes*
0204	GS|100.0|BOLD:AAG1503_KP046668	Animalia	Arthropoda	Insecta	Diptera	Chloropidae	Tricimba	*Tricimba melancholica*
0205	GS|89.7|BOLD:AAN4488_KP845857	Animalia	Arthropoda	Insecta	Thysanoptera	Phlaeothripidae	Haplothrips	*Haplothrips tenuipennis*
0206	GS|100.0|BOLD:AAA6915_KM840627	Animalia	Arthropoda	Arachnida	Araneae	Linyphiidae	Islandiana	*Islandiana holmi*
0207	GS|90.1|BOLD:ACI5842_KM625260	Animalia	Arthropoda	Insecta	Diptera	Hybotidae		
0208	GS|98.3|BOLD:AAN6561_JN290615	Animalia	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae		
0021	GS|100.0|BOLD:AAI2023_NA	Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	Harpalus	*Harpalus fraternus*
0210	GS|98.3|BOLD:ACS0312_KR459682	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae	Delia	*Delia extensa*
0211	GS|100.0|BOLD:AAE2976_GQ373468	Animalia	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobryidae	
0213	GS|91.0|BOLD:AAW9040_KR579838	Animalia	Arthropoda	Insecta	Hemiptera	Anthocoridae	Orius	*Orius tristicolor*

0214	GS|99.7|BOLD:AAD0484_KR588113	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	Psectrocladius	*Psectrocladius barbimanus*
0215	GS|94.7|BOLD:ADA3514_NA	Animalia	Arthropoda	Insecta	Diptera	Heleomyzidae		
0219	GSL|79.3|BOLD:ADA7549_NA	Animalia	Arthropoda	Insecta				
0022	GS|99.0|BOLD:ACR9309_KM943654	Animalia	Arthropoda	Insecta	Diptera	Drosophilidae	Drosophila	
0220	GS|100.0|BOLD:AAD2901_KR571882	Animalia	Arthropoda	Insecta	Hemiptera	Rhyparochromidae	Emblethis	*Emblethis vicarius*
0222	GS|98.7|BOLD:ACB0946_KM990445	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	Smittia	
0223	GS|91.7|BOLD:ACM2566_KR147632	Animalia	Arthropoda	Insecta	Archaeognatha	Meinertellidae	Machilinus	
0224	GS|97.3|BOLD:AAA4555_NA	Animalia	Arthropoda	Insecta	Orthoptera			
0227	GS|85.6|BOLD:ACP5629_NA	Animalia	Arthropoda	Insecta	Diptera			
0228	GS|100.0|BOLD:AAP8834_MF636045	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae	Delia	*Delia albula*
0023	GS|100.0|BOLD:AAU6111_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*
0230	GS|100.0|BOLD:AAC9614_KR944318	Animalia	Arthropoda	Insecta	Diptera	Calliphoridae	Protophormia	*Protophormia terraenovae*
0231	GS|73.0|BOLD:ACB0901_KX844319	Animalia	Arthropoda	Insecta	Diptera	Tachinidae	Cylindromyia	*Cylindromyia rufifrons*
0232	GS|88.0|BOLD:AAU3560_JN299334	Animalia	Arthropoda	Insecta	Neuroptera	Hemerobiidae	Hemerobius	*Hemerobius lutescens*
0233	GS|100.0|BOLD:ACX4703_KR694949	Animalia	Arthropoda	Insecta	Diptera	Ceratopogonidae		
0234	GS|89.9|BOLD:AAN5901_MF634185	Animalia	Arthropoda	Insecta	Coleoptera	Chrysomelidae	Phyllotreta	*Phyllotreta pusilla*
0237	GS|85.0|BOLD:ABA1267_NA	Animalia	Arthropoda	Collembola	Poduromorpha			
0242	GS|100.0|BOLD:AAG3286_MG104438	Animalia	Arthropoda	Insecta	Diptera	Phoridae	Megaselia	*Megaselia lombardorum*
0244	GSL|76.6|BOLD:ADL1711_NA	Animalia	Arthropoda	Insecta	Diptera			
0245	GS|76.3|BOLD:ACK3624_NA	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	Heterotrissocladius	*Heterotrissocladius subpilosus*
0246	GS|99.7|BOLD:ACF4680_KR890066	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	*Formica planipilis*

0249	GS|95.7|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0025	GS|99.7|BOLD:AAG2472_JF876932	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae	Delia	
0250	GS|90.3|BOLD:ACA5986_NA	Animalia	Arthropoda	Insecta	Coleoptera			
0251	GS|98.4|BOLD:AAV1530_JN309491	Animalia	Arthropoda	Insecta	Archaeognatha	Machilidae		
0253	GS|86.7|BOLD:AAZ4548_NA	Animalia	Arthropoda	Insecta	Diptera			
0256	GS|100.0|BOLD:ABX3926_KR039655	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Auridius	*Auridius auratus*
0258	GS|79.7|BOLD:ACO5516_NA	Animalia	Arthropoda	Insecta	Diptera	Dolichopodidae	Thinophilus	
0259	GS|99.7|BOLD:ACE1079_MG088886	Animalia	Arthropoda	Insecta	Diptera	Muscidae	Spilogona	*Spilogona pacifica*
026	GS|99.7|BOLD:AAP9080_KM945424	Animalia	Arthropoda	Insecta	Diptera	Mycetophilidae	Leia	
0260	GS|98.4|BOLD:AAV1530_JN309491	Animalia	Arthropoda	Insecta	Archaeognatha	Machilidae		
0263	GS|100.0|BOLD:ACY7534_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Melanoplus	*Melanoplus rugglesi*
0264	GS|94.0|BOLD:AAF7101_NA	Animalia	Arthropoda	Insecta	Diptera	Fanniidae	Fannia	*Fannia canicularis*
0266	GS|97.3|BOLD:ACJ0553_NA	Animalia	Arthropoda	Arachnida	Opiliones			
0267	GS|93.3|BOLD:ACJ7984_KR930486	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Myrmica	
027	GS|99.0|BOLD:AAF6788_NA	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	*Formica neogagates*
0271	GS|100.0|BOLD:ACE8833_MF892636	Animalia	Arthropoda	Insecta	Diptera	Muscidae	Helina	
0272	GS|97.7|BOLD:ABY5198_MG170315	Animalia	Arthropoda	Insecta	Diptera	Syrphidae	Melangyna	*Melangyna umbellatarum*
0273	GS|98.7|BOLD:AAQ0769_JF885347	Animalia	Arthropoda	Arachnida	Araneae	Linyphiidae	Scotinotylus	*Scotinotylus exsectoides*
0274	GS|99.3|BOLD:ACD1662_KM638892	Animalia	Arthropoda	Insecta	Diptera	Cecidomyiidae		
0275	GS|78.3|BOLD:ABY3348_NA	Animalia	Arthropoda	Insecta	Coleoptera	Cerambycidae	Placosternus	*Placosternus guttatus*
0276	GS|99.7|BOLD:AAA3453_KM634911	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae	Delia	*Delia platura*
0277	GS|95.7|BOLD:AAU6111_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*

0280	GS|100.0|BOLD:ACX5072_KR679990	Animalia	Arthropoda	Insecta	Diptera	Hybotidae		
0281	GS|80.7|BOLD:AEB3573_NA	Animalia	Arthropoda	Insecta	Archaeognatha			
0282	GS|83.3|BOLD:ACM2566_KR147632	Animalia	Arthropoda	Insecta	Archaeognatha	Meinertellidae	Machilinus	
0284	GS|76.7|BOLD:ACU6811_NA	Animalia	Arthropoda	Insecta	Diptera	Micropezidae		
0285	GS|97.3|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0291	GS|79.3|BOLD:AAU6693_NA	Animalia	Arthropoda	Insecta	Diptera	Asilidae		
0292	GS|100.0|BOLD:AAG4872_KR983531	Animalia	Arthropoda	Insecta	Diptera	Mycetophilidae	Mycetophila	*Mycetophila perpallida*
0294	GS|100.0|BOLD:AAL8938_KM826455	Animalia	Arthropoda	Arachnida	Araneae	Linyphiidae	Collinsia	*Collinsia ksenia*
0295	GS|79.7|BOLD:ACO5516_NA	Animalia	Arthropoda	Insecta	Diptera	Dolichopodidae	Thinophilus	
0297	GS|95.3|BOLD:ACL0992_MG118623	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae		
0298	GS|99.7|BOLD:AAG8838_KF919844	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Helochara	*Helochara communis*
0003	GS|100.0|BOLD:AAA8764_MG468655	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0030	GS|99.7|BOLD:AAF7101_NA	Animalia	Arthropoda	Insecta	Diptera	Fanniidae	Fannia	*Fannia canicularis*
0300	GS|80.3|BOLD:AEB3573_NA	Animalia	Arthropoda	Insecta	Archaeognatha			
0304	GS|77.6|BOLD:ACO5516_NA	Animalia	Arthropoda	Insecta	Diptera	Dolichopodidae	Thinophilus	
0307	GSL|86.7|BOLD:AAH3537_HM417301	Animalia	Arthropoda	Insecta	Diptera			
0308	GS|84.0|BOLD:ACF9803_NA	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae		
0031	GS|86.0|BOLD:ADP0308_NA	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Neoaliturus	*Neoaliturus fenestratus*
0311	GS|94.0|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0313	GS|97.3|BOLD:AEC6585_KP657062	Animalia	Arthropoda	Arachnida	Araneae	Linyphiidae	Islandiana	*Islandiana holmi*
0316	GS|96.7|BOLD:AAA8764_KM533615	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0319	GS|100.0|BOLD:ABZ3849_MF831862	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Diplocolenus	*Diplocolenus evansi*
0032	GS|100.0|BOLD:ACL0992_MG118623	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae		

0321	GS|100.0|BOLD:AAY7951_KY269962	Animalia	Arthropoda	Arachnida	Araneae	Linyphiidae	Silometopus	*Silometopus reussi*
0322	GS|89.0|BOLD:AAC6413_NA	Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	Amara	*Amara obesa*
0326	GS|100.0|BOLD:AAE6488_KU875052	Animalia	Arthropoda	Insecta	Hemiptera	Miridae	Europiella	*Europiella decolor*
0330	GS|100.0|BOLD:AAF9976_NA	Animalia	Arthropoda	Insecta	Lepidoptera	Gelechiidae	Aroga	*Aroga websteri*
0331	GS|94.7|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0332	GS|99.7|BOLD:AAG2875_KR038524	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Ceratagallia	*Ceratagallia siccifolia*
0333	GS|99.7|BOLD:AAP8985_JF875965	Animalia	Arthropoda	Insecta	Diptera	Milichiidae	Leptometopa	
0334	GS|100.0|BOLD:AAC2393_MF829622	Animalia	Arthropoda	Insecta	Hemiptera	Berytidae	Neoneides	*Neoneides muticus*
0337	GS|99.3|BOLD:ACL3102_KR346406	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Diplocolenus	
0338	GS|90.7|BOLD:AAC6413_NA	Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	Amara	*Amara obesa*
0034	GSL|99.7|BOLD:AAB3450_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0340	GS|100.0|BOLD:AAE4456_KM628530	Animalia	Arthropoda	Insecta	Diptera	Syrphidae	Platycheirus	*Platycheirus pictipes*
0344	GS|97.0|BOLD:AAG5198_KJ963586	Animalia	Arthropoda	Insecta	Coleoptera	Curculionidae	Otiorhynchus	*Otiorhynchus ovatus*
0347	GS|90.7|BOLD:ADC9253_NA	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Ceratagallia	
0035	GS|100.0|BOLD:AAG4317_KM842658	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Phloeostiba	*Phloeostiba lapponica*
0351	GS|94.7|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0356	GS|93.4|BOLD:AAM7579_NA	Animalia	Arthropoda	Insecta	Coleoptera	Dermestidae	Dermestes	*Dermestes marmoratus*
0358	GS|80.7|BOLD:ACM2566_KR147632	Animalia	Arthropoda	Insecta	Archaeognatha	Meinertellidae	Machilinus	
0036	GS|95.3|BOLD:AAG3766_NA	Animalia	Arthropoda	Insecta	Diptera			
0360	GS|70.6|BOLD:ADK0436_KX072071	Animalia	Arthropoda	Insecta	Lepidoptera	Geometridae	Elophos	*Elophos caelibaria*
0361	GS|90.6|BOLD:AAN6555_MF602326	Animalia	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Lepidocyrtus	*Lepidocyrtus cyaneus*
0368	GS|90.7|BOLD:ADB1949_NA	Animalia	Arthropoda	Insecta	Coleoptera	Cleridae		

0369	GS|99.7|BOLD:ABA6488_KM967845	Animalia	Arthropoda	Insecta	Diptera	Sciaridae	Bradysia	*Bradysia trivittata*
0037	GS|100.0|BOLD:AAA1831_KR654855	Animalia	Arthropoda	Insecta	Diptera	Drosophilidae	Drosophila	*Drosophila barbarae*
0373	GSL|90.1|BOLD:ACF3208_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0375	GS|96.4|BOLD:AAA8764_KM533615	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0380	GS|96.3|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0381	GS|95.0|BOLD:AAA8764_KM536285	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0382	GSL|92.3|BOLD:ACF3208_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0384	GS|98.7|BOLD:AAG2454_KR514726	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae		
0385	GS|99.7|BOLD:ACT5834_KR950814	Animalia	Arthropoda	Insecta	Diptera	Cecidomyiidae		
0386	GS|97.3|BOLD:ABX3928_KR036427	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Athysanella	*Athysanella obesa*
0388	GS|100.0|BOLD:ACJ8319_KM630123	Animalia	Arthropoda	Insecta	Diptera	Chloropidae	Meromyza	
0389	GS|100.0|BOLD:ACY9729_NA	Animalia	Arthropoda	Insecta	Coleoptera	Tenebrionidae	Eleodes	*Eleodes vandykei*
0392	GS|98.0|BOLD:ACI3068_KR576576	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Ceratagallia	*Ceratagallia siccifolia*
0393	GS|95.7|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0395	GS|84.0|BOLD:ACF9803_NA	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae		
0397	GS|100.0|BOLD:AAG6953_KM938527	Animalia	Arthropoda	Insecta	Diptera	Hybotidae		
0004	GS|100.0|BOLD:AAP7044_KM850147	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Phloeostiba	*Phloeostiba lapponica*
0040	GS|87.7|BOLD:ADF5560_NA	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae		
0400	GSL|79.0|BOLD:AAI1266_GU689776	Animalia	Arthropoda	Insecta	Diptera			
0407	GS|97.0|BOLD:AAG5198_KJ963586	Animalia	Arthropoda	Insecta	Coleoptera	Curculionidae	Otiorhynchus	*Otiorhynchus ovatus*
0408	GS|76.0|BOLD:AAV4709_NA	Animalia	Arthropoda	Insecta	Diptera	Asilidae		
0411	GS|96.0|BOLD:AAE0406_NA	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	*Formica lasioides*

0412	GS|94.0|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0413	GS|100.0|BOLD:AAL7755_KR691547	Animalia	Arthropoda	Insecta	Diptera	Sphaeroceridae	Spelobia	*Spelobia tufta*
0414	GS|90.0|BOLD:ACP5629_NA	Animalia	Arthropoda	Insecta	Diptera			
0415	GS|89.0|BOLD:AAZ4548_NA	Animalia	Arthropoda	Insecta	Diptera			
0416	GS|82.9|BOLD:ACB8951_NA	Animalia	Arthropoda	Insecta	Psocodea	Lachesillidae	Lachesilla	
0417	GS|84.0|BOLD:ADP0308_NA	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Neoaliturus	*Neoaliturus fenestratus*
0418	GS|89.9|BOLD:ABX9412_KR804190	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Leptothorax	
0419	GS|99.3|BOLD:AAA5308_JF876496	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus	
0042	GS|71.8|BOLD:AAA8764_MG468655	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0420	GS|100.0|BOLD:ACF0670_KM955493	Animalia	Arthropoda	Insecta	Diptera	Cecidomyiidae		
0426	GS|97.5|BOLD:AAG4317_KM848028	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Phloeostiba	*Phloeostiba lapponica*
0043	GS|100.0|BOLD:AAP6259_MG048117	Animalia	Arthropoda	Arachnida	Araneae	Linyphiidae	Agyneta	*Agyneta ordinaria*
0432	GS|98.7|BOLD:AAB1385_KR899940	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Leptothorax	
0436	GS|100.0|BOLD:ACE1891_MF911832	Animalia	Arthropoda	Arachnida	Mesostigmata	Parasitidae		
0437	GS|96.7|BOLD:AAM7650_KU914062	Animalia	Arthropoda	Insecta	Coleoptera	Chrysomelidae	Chaetocnema	*Chaetocnema hortensis*
0439	GS|89.9|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0044	GS|99.3|BOLD:ACI4294_KM628173	Animalia	Arthropoda	Insecta	Diptera	Anthomyzidae	Stiphrosoma	*Stiphrosoma hirtum*
0447	GS|86.4|BOLD:ABU8876_NA	Animalia	Arthropoda	Insecta	Lepidoptera			
0045	GS|100.0|BOLD:AAB0377_KM627814	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	Smittia	
0452	GSL|92.7|BOLD:ACA5984_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0454	GS|98.7|BOLD:AAP7807_JF888079	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Phloeostiba	*Phloeostibala pponica*
0046	GS|94.6|BOLD:AAC7186_KT085784	Animalia	Arthropoda	Insecta	Diptera	Muscidae	Helina	*Helina flavisquama*

0460	GS|100.0|BOLD:AAA1834_KR879274	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Myrmica	*Myrmica detritinodis*
0463	GS|83.7|BOLD:ACM2566_KR147632	Animalia	Arthropoda	Insecta	Archaeognatha	Meinertellidae	Machilinus	
0047	GS|92.0|BOLD:AAG2442_KJ444803	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae	Zaphne	*Zaphne implicata*
0472	GS|96.3|BOLD:AAU6111_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*
0473	GS|93.7|BOLD:ACA5984_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Trimerotropis	*Trimerotropis fontana*
0475	GS|99.7|BOLD:ACC3017_NA	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Phloeonomus	
0476	GS|100.0|BOLD:ACF9614_MG150830	Animalia	Arthropoda	Insecta	Diptera	Sciaridae	Bradysia	*Bradysia strenua*
0478	GS|100.0|BOLD:ACR5480_KR696819	Animalia	Arthropoda	Insecta	Diptera	Carnidae	Meoneura	
0479	GS|96.2|BOLD:AAG5198_KU911307	Animalia	Arthropoda	Insecta	Coleoptera	Curculionidae	Otiorhynchus	*Otiorhynchus ovatus*
0048	GS|100.0|BOLD:ACG4478_KT113373	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae	Delia	
0484	GS|100.0|BOLD:AAG2474_KM638129	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae	Delia	
0485	GS|84.0|BOLD:ACF9803_NA	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae		
0487	GSL|90.1|BOLD:AAE2480_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0489	GS|97.0|BOLD:ACL3102_KR345756	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Diplocolenus	
049	GS|99.7|BOLD:ABX3899_KR035906	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Athysanella	*Athysanella acuticauda*
0490	GS|99.7|BOLD:ABY5760_KU875076	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	*Formica aserva*
0491	GS|92.0|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0496	GS|100.0|BOLD:ADO7114_KM645444	Animalia	Arthropoda	Insecta	Diptera	Sphaeroceridae	Leptocera	*Leptocera erythrocera*
0497	GS|97.3|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0005	GS|100.0|BOLD:AAG8681_KR577666	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Psammotettix	*Psammotettix lividellus*

0050	GS|94.0|BOLD:AAM9235_JF866971	Animalia	Arthropoda	Insecta	Diptera	Sciaridae		
0502	GS|92.0|BOLD:ACM2566_KR147632	Animalia	Arthropoda	Insecta	Archaeognatha	Meinertellidae	Machilinus	
0508	GS|94.7|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0051	GS|83.0|BOLD:AAP7796_JF888053	Animalia	Arthropoda	Insecta	Coleoptera	Curculionidae	Cossonus	*Cossonus piniphilus*
0513	GS|97.3|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0514	GSL|98.0|BOLD:AAB3450_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0517	GS|95.0|BOLD:AAH1663_MG504469	Animalia	Arthropoda	Insecta	Hymenoptera	Ichneumonidae		
0519	GS|93.3|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0052	GS|99.3|BOLD:AAF6787_NA	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	
0521	GS|99.7|BOLD:AAG3311_MG105542	Animalia	Arthropoda	Insecta	Diptera	Phoridae	Megaselia	*Megaselia tecticauda*
0526	GS|99.7|BOLD:ACG2756_KR481284	Animalia	Arthropoda	Insecta	Coleoptera	Monotomidae	Monotoma	*Monotoma longicollis*
0053	GS|91.7|BOLD:ACV8058_MF712440	Animalia	Arthropoda	Insecta	Diptera	Cecidomyiidae		
0531	GS|99.7|BOLD:AAC5850_NA	Animalia	Arthropoda	Insecta	Lepidoptera	Gelechiidae	Bryotropha	
0532	GS|95.0|BOLD:AAG6437_KR498973	Animalia	Arthropoda	Insecta	Diptera	Ceratopogonidae	Dasyhelea	
0535	GS|97.7|BOLD:ACE4532_KR036390	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Latalus	*Latalus curtus*
0536	GS|97.3|BOLD:ACG1315_KF549892	Animalia	Arthropoda	Insecta	Coleoptera	Elateridae	Selatosomus	*Selatosomus aeripennis*
0537	GS|79.7|BOLD:ACP2164_NA	Animalia	Arthropoda	Insecta	Diptera	Dolichopodidae		
0054	GS|100.0|BOLD:ACX5303_MF873972	Animalia	Arthropoda	Insecta	Diptera	Heleomyzidae	Suillia	*Suillia nemorum*
0540	GS|92.7|BOLD:ACG1460_KF549893	Animalia	Arthropoda	Insecta	Coleoptera	Elateridae	Selatosomus	*Selatosomus aeripennis*
0541	GS|78.7|BOLD:ABW2464_NA	Animalia	Arthropoda	Insecta	Diptera	Asilidae		
0542	GS|85.3|BOLD:ACM2566_KR147632	Animalia	Arthropoda	Insecta	Archaeognatha	Meinertellidae	Machilinus	
0543	GS|100.0|BOLD:AAM9013_MG094039	Animalia	Arthropoda	Insecta	Diptera	Keroplatidae	Macrocera	*Macrocera pusilla*

0544	GS|88.7|BOLD:ACP5629_NA	Animalia	Arthropoda	Insecta	Diptera			
0545	GS|96.7|BOLD:AAL2821_MF889772	Animalia	Arthropoda	Insecta	Diptera	Muscidae	Phaonia	*Phaonia apicalis*
0546	GS|100.0|BOLD:ACE1420_KM843710	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Oxypoda	*Oxypoda irrasa*
0547	GS|83.7|BOLD:AAE1524_KF549248	Animalia	Arthropoda	Insecta	Diptera	Tachinidae	Houghia	*Houghia romeroae*
0549	GS|99.2|BOLD:AAB8583_KF920426	Animalia	Arthropoda	Insecta	Hemiptera	Aphrophoridae	Philaenarcys	*Philaenarcys spartina*
0055	GS|99.3|BOLD:AAV0264_KR044992	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Latalus	*Latalus mundus*
0552	GS|90.7|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0557	GS|91.3|BOLD:AAN6561_JN290615	Animalia	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae		
0559	GS|80.3|BOLD:ACO5516_NA	Animalia	Arthropoda	Insecta	Diptera	Dolichopodidae	Thinophilus	
0056	GS|100.0|BOLD:AAI1608_NA	Animalia	Arthropoda	Insecta	Hymenoptera			
0560	GS|100.0|BOLD:AAP6246_KM646447	Animalia	Arthropoda	Insecta	Diptera	Piophilidae	Mycetaulus	*Mycetaulus bipunctatus*
0569	GS|99.7|BOLD:AAH3941_KM629859	Animalia	Arthropoda	Insecta	Diptera	Sciaridae	Lycoriella	
0057	GS|100.0|BOLD:AAG8842_KR576758	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Scaphytopius	
0570	GS|99.7|BOLD:AAH6630_NA	Animalia	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Entomobrya	
0572	GS|77.4|BOLD:ACO5516_NA	Animalia	Arthropoda	Insecta	Diptera	Dolichopodidae	Thinophilus	
0576	GS|100.0|BOLD:ACE5391_KR678446	Animalia	Arthropoda	Insecta	Diptera	Tephritidae	Chaetorellia	
0577	GS|86.3|BOLD:ACP5629_NA	Animalia	Arthropoda	Insecta	Diptera			
0579	GS|98.0|BOLD:AAB3450_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Trimerotropis	
0585	GS|89.7|BOLD:ACP5629_NA	Animalia	Arthropoda	Insecta	Diptera			
0588	GS|94.7|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0590	GS|92.0|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0593	GS|78.4|BOLD:AEB3573_NA	Animalia	Arthropoda	Insecta	Archaeognatha			
0594	GS|91.0|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*

0596	GS|79.3|BOLD:ACA8144_KR385033	Animalia	Arthropoda	Insecta	Diptera	Dolichopodidae		
0006	GS|87.7|BOLD:ACD9132_MG398954	Animalia	Arthropoda	Insecta	Hemiptera	Rhyparochromidae	Perigenes	*Perigenes constrictus*
0060	GS|97.3|BOLD:AAH3943_KM639609	Animalia	Arthropoda	Insecta	Diptera	Sciaridae		
0600	GS|77.0|BOLD:ADA7549_NA	Animalia	Arthropoda	Insecta	Archaeognatha	Machilidae		
0601	GS|94.3|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0603	GS|96.7|BOLD:AAA8764_KM533615	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0606	GS|99.7|BOLD:ACK7799_KM846655	Animalia	Arthropoda	Insecta	Coleoptera	Leiodidae	Neoeocatops	*Neoeocatops decipiens*
0607	GS|91.0|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0609	GSL|90.3|BOLD:ACF3208_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0610	GS|96.6|BOLD:AAV0237_MF830974	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Latalus	*Latalus personatus*
0616	GS|95.0|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0618	GSL|87.3|BOLD:AAH3915_MG151438	Animalia	Arthropoda	Insecta	Diptera	Sciaridae		
0619	GS|94.7|BOLD:ACB0775_KR694678	Animalia	Arthropoda	Insecta	Diptera	Hybotidae		
0062	GS|77.3|BOLD:ACV4172_KY831905	Animalia	Arthropoda	Insecta	Diptera	Tachinidae		
0621	GS|90.3|BOLD:AAF9959_KR042773	Animalia	Arthropoda	Insecta	Hemiptera	Miridae	Lygidea	*Lygidea annexa*
0623	GS|100.0|BOLD:AAZ1768_KR033451	Animalia	Arthropoda	Insecta	Hemiptera	Miridae	Litomiris	
0630	GS|88.5|BOLD:AAU6111_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*
0632	GS|95.3|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0633	GS|91.7|BOLD:ACM2566_KR147632	Animalia	Arthropoda	Insecta	Archaeognatha	Meinertellidae	Machilinus	
0637	GS|93.4|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0064	GS|98.3|BOLD:AAB7203_KR890199	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Leptothorax	

0643	GS|92.4|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0645	GS|100.0|BOLD:ACG3736_KM628503	Animalia	Arthropoda	Insecta	Diptera	Mycetophilidae	Exechiopsis	
0065	GS|100.0|BOLD:AAC2498_HM860486	Animalia	Arthropoda	Insecta	Diptera	Muscidae	Helina	*Helina evecta*
0653	GS|99.7|BOLD:AAA2372_KR785178	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Camponotus	*Camponotus herculeanus*
0066	GS|84.4|BOLD:AAV6192_NA	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae		
0660	GS|91.3|BOLD:AAA8764_KM533615	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0663	GS|84.0|BOLD:ACM2566_KR147632	Animalia	Arthropoda	Insecta	Archaeognatha	Meinertellidae	Machilinus	
0664	GS|86.0|BOLD:ADL7370_NA	Animalia	Arthropoda	Arachnida	Sarcoptiformes	Gymnodamaeidae		
0067	GS|100.0|BOLD:ACI3968_KM624882	Animalia	Arthropoda	Insecta	Diptera	Anthomyiidae	Hylemya	
0674	GSL|95.3|BOLD:AAB3450_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0675	GS|94.7|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0677	GS|70.2|BOLD:AAM6781_JF871903	Animalia	Arthropoda	Insecta	Diptera	Dolichopodidae	Neurigona	*Neurigona tenuis*
0068	GS|99.3|BOLD:AAU6757_KM631883	Animalia	Arthropoda	Insecta	Diptera	Cecidomyiidae		
0681	GS|94.4|BOLD:ACI3068_KR576576	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Ceratagallia	*Ceratagallia siccifolia*
0682	GSL|97.7|BOLD:AAB3450_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0683	GS|92.3|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0685	GS|75.7|BOLD:ACU6811_NA	Animalia	Arthropoda	Insecta	Diptera	Micropezidae		
0687	GS|92.0|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0691	GS|87.4|BOLD:ACP5629_NA	Animalia	Arthropoda	Insecta	Diptera			
0693	GS|91.2|BOLD:AAA3075_GU694503	Animalia	Arthropoda	Insecta	Lepidoptera	Geometridae	Pasiphila	*Pasiphila rectangulata*
0696	GS|88.0|BOLD:AAI2112_NA	Animalia	Arthropoda	Insecta	Diptera	Pipunculidae	Jassidophaga	*Jassidophaga villosa*
0698	GS|82.7|BOLD:AEB3573_NA	Animalia	Arthropoda	Insecta	Archaeognatha			

0007	GS|90.3|BOLD:ACM2566_KR147632	Animalia	Arthropoda	Insecta	Archaeognatha	Meinertellidae	Machilinus	
0070	GS|99.0|BOLD:AAN6462_KR691637	Animalia	Arthropoda	Insecta	Diptera	Tachinidae	Linnaemya	
0700	GS|99.0|BOLD:ACA5986_NA	Animalia	Arthropoda	Insecta	Coleoptera			
0702	GS|100.0|BOLD:AAP7048_KU875710	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Oxypoda	*Oxypoda irrasa*
0706	GS|91.4|BOLD:AAU6111_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*
0709	GS|100.0|BOLD:ACW5117_MF734954	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	Smittia	
0071	GS|82.0|BOLD:ADF4779_NA	Animalia	Arthropoda	Insecta	Psocodea	Psocidae		
0714	GS|100.0|BOLD:ACA2681_KR950089	Animalia	Arthropoda	Insecta	Diptera	Cecidomyiidae		
0715	GS|85.0|BOLD:ACA6706_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*
0721	GS|100.0|BOLD:AAA5953_NA	Animalia	Arthropoda	Insecta	Lepidoptera	Gelechiidae	Coleotechnites	*Coleotechnites piceaella*
0731	GSL|97.7|BOLD:AAB3450_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0734	GS|94.3|BOLD:AAE2480_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Spharagemon	*Spharagemon campestris*
0738	GS|80.3|BOLD:ACS7235_NA	Animalia	Arthropoda	Insecta	Diptera	Tabanidae	Chrysops	*Chrysops madagascarensis*
0074	GS|100.0|BOLD:AAG2186_KM636151	Animalia	Arthropoda	Insecta	Diptera	Tachinidae	Peleteria	*Peleteria iterans*
0740	GS|97.7|BOLD:AAG7279_KR662822	Animalia	Arthropoda	Insecta	Diptera	Sphaeroceridae	Eulimosina	*Eulimosina ochripes*
0745	GS|99.0|BOLD:ABA6352_NA	Animalia	Arthropoda	Insecta	Coleoptera	Mycetophagidae	Typhaea	*Typhaea stercorea*
0746	GS|97.0|BOLD:AAU6111_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*
0749	GS|78.7|BOLD:ACY5086_NA	Animalia	Arthropoda	Insecta	Diptera			
0075	GS|100.0|BOLD:AAG1725_KM634700	Animalia	Arthropoda	Insecta	Diptera	Muscidae	Coenosia	
0754	GS|70.7|BOLD:AAI9028_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Cratypedes	*Cratypedes neglectus*
0076	GS|90.7|BOLD:ADR1193_NA	Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	Amara	*Amara fortis*
0762	GS|91.0|BOLD:AAA8764_JN294649	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*

0763	GS|75.7|BOLD:AAC4201_NA	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	Paraphaenocladius	*Paraphaenocladius impensus*
0771	GS|94.3|BOLD:ADA3514_NA	Animalia	Arthropoda	Insecta	Diptera	Heleomyzidae		
0774	GS|83.4|BOLD:ABW2776_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0776	GS|93.3|BOLD:AAY6676_KM838130	Animalia	Arthropoda	Arachnida	Opiliones	Sclerosomatidae	Togwoteeus	*Togwoteeus biceps*
0777	GS|84.3|BOLD:AAV6192_NA	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae		
0778	GS|98.3|BOLD:ACI2871_KR569600	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae		
0078	GS|100.0|BOLD:ABX3085_NA	Animalia	Arthropoda	Insecta	Diptera	Phoridae	Megaselia	
0781	GS|84.0|BOLD:AAA8764_JN294649	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0787	GS|99.3|BOLD:ABX3968_KR036090	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Psammotettix	*Psammotettix lividellus*
0079	GS|100.0|BOLD:AAG8821_MF829417	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Doratura	*Doratura stylata*
0790	GS|90.3|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0798	GS|96.0|BOLD:ACI5842_KM642700	Animalia	Arthropoda	Insecta	Diptera	Hybotidae		
0008	GS|92.7|BOLD:AAM7579_NA	Animalia	Arthropoda	Insecta	Coleoptera	Dermestidae	Dermestes	*Dermestes marmoratus*
0080	GS|100.0|BOLD:AAA1858_JN292000	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Myrmica	*Myrmica incompleta*
0800	GS|94.7|BOLD:ACI4795_KR790290	Animalia	Arthropoda	Insecta	Hymenoptera	Ichneumonidae		
0081	GS|93.0|BOLD:AAL7874_NA	Animalia	Arthropoda	Insecta	Diptera	Sciaridae	Lycoriella	
0810	GS|95.0|BOLD:AAF6546_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Trimerotropis	*Trimerotropis saxatilits*
0812	GS|91.3|BOLD:AAN6561_JN290615	Animalia	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae		
0822	GSL|91.0|BOLD:ACF3208_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0828	GS|100.0|BOLD:AAP7560_KM625819	Animalia	Arthropoda	Insecta	Diptera	Chloropidae	Meromyza	
0832	GS|90.0|BOLD:ACM2566_KR147632	Animalia	Arthropoda	Insecta	Archaeognatha	Meinertellidae	Machilinus	
0084	GS|100.0|BOLD:AAA4555_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Melanoplus	

0846	GS|97.7|BOLD:AAI4346_KY268967	Animalia	Arthropoda	Arachnida	Opiliones	Phalangiidae	Phalangium	*Phalangium opilio*
0847	GS|87.1|BOLD:AAA8764_JN294649	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0849	GSL|91.3|BOLD:ACF3208_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0085	GS|100.0|BOLD:AAH6662_KP040076	Animalia	Arthropoda	Insecta	Diptera	Tachinidae	Tachina	
0854	GS|93.7|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0855	GS|99.7|BOLD:ACH2123_KR578876	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae		
0086	GS|99.7|BOLD:AAG6835_KR686798	Animalia	Arthropoda	Insecta	Diptera	Therevidae		
0860	GS|99.3|BOLD:AAF4462_HQ105942	Animalia	Arthropoda	Insecta	Hemiptera	Rhyparochromidae	Megalonotus	*Megalonotus sabulicola*
0865	GSL|79.0|BOLD:ACW9757_NA	Animalia	Arthropoda	Insecta	Diptera			
0869	GS|96.3|BOLD:AAA8764_JN294535	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0871	GS|92.7|BOLD:AAY6676_KM838130	Animalia	Arthropoda	Arachnida	Opiliones	Sclerosomatidae	Togwoteeus	*Togwoteeus biceps*
0875	GS|96.3|BOLD:ABX3968_KR566017	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Psammotettix	
0877	GS|99.0|BOLD:AAM7650_HQ551565	Animalia	Arthropoda	Insecta	Coleoptera	Chrysomelidae	Chaetocnema	*Chaetocnema hortensis*
0088	GS|99.7|BOLD:ACB0918_KM641668	Animalia	Arthropoda	Insecta	Diptera	Sphaeroceridae	Spelobia	
0885	GS|97.3|BOLD:AAA8764_JN294532	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0888	GS|92.7|BOLD:ABX3926_KR039655	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Auridius	*Auridius auratus*
0889	GS|88.0|BOLD:ACU8123_MF937387	Animalia	Arthropoda	Insecta	Hymenoptera	Braconidae	Elasmosoma	
0089	GS|100.0|BOLD:AAD0642_HQ962013	Animalia	Arthropoda	Insecta	Diptera	Muscidae	Helina	*Helina reversio*
0890	GS|100.0|BOLD:AAB0973_NA	Animalia	Arthropoda	Insecta	Hymenoptera	Halictidae	Agapostemon	
0090	GS|100.0|BOLD:AAA4659_KT148359	Animalia	Arthropoda	Insecta	Lepidoptera	Gelechiidae	Chionodes	
0901	GS|99.7|BOLD:AEF1036_NA	Animalia	Arthropoda	Insecta	Diptera	Acroceridae	Ogcodes	

0903	GSL|96.1|BOLD:AAB3450_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0905	GS|99.7|BOLD:AAN6196_KM846085	Animalia	Arthropoda	Insecta	Coleoptera	Eucinetidae	Eucinetus	*Eucinetus terminalis*
0907	GS|93.0|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0908	GS|100.0|BOLD:AAG7284_KR398833	Animalia	Arthropoda	Insecta	Diptera	Sphaeroceridae	Pullimosina	*Pullimosina pullula*
0091	GS|100.0|BOLD:AAC4680_KT127902	Animalia	Arthropoda	Insecta	Lepidoptera	Nymphalidae	Cercyonis	*Cercyonis sthenele*
0910	GS|93.3|BOLD:AAA8764_JN294644	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Camnula	*Camnula pellucida*
0913	GS|88.7|BOLD:ACM2566_KR147632	Animalia	Arthropoda	Insecta	Archaeognatha	Meinertellidae	Machilinus	
0917	GS|97.0|BOLD:AAU6111_NA	Animalia	Arthropoda	Insecta	Orthoptera	Rhaphidophoridae	Ceuthophilus	*Ceuthophilus agassizii*
0926	GS|99.7|BOLD:ACN9284_NA	Animalia	Arthropoda	Insecta	Hemiptera	Miridae		
0930	GS|98.7|BOLD:AAP9080_KM945424	Animalia	Arthropoda	Insecta	Diptera	Mycetophilidae	Leia	
0934	GSL|92.7|BOLD:AAD3251_KR142810	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0094	GS|99.3|BOLD:AAP6481_KR688517	Animalia	Arthropoda	Insecta	Diptera	Muscidae	Coenosia	
0943	GS|87.4|BOLD:ACP5629_NA	Animalia	Arthropoda	Insecta	Diptera			
0944	GS|81.2|BOLD:AAI9028_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae	Cratypedes	*Cratypedes neglectus*
0948	GS|83.2|BOLD:ABU8876_NA	Animalia	Arthropoda	Insecta	Lepidoptera			
0950	GS|92.0|BOLD:AAG2875_KR041070	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Ceratagallia	*Ceratagallia cinerea*
0955	GS|98.7|BOLD:AAA1468_KR876306	Animalia	Arthropoda	Insecta	Hymenoptera	Formicidae	Formica	*Formica glacialis*
0970	GS|92.1|BOLD:ABA5839_KR034424	Animalia	Arthropoda	Insecta	Hemiptera	Cicadellidae	Euscelis	*Euscelis confinis*
0973	GS|95.3|BOLD:AAH0190_KM850258	Animalia	Arthropoda	Insecta	Coleoptera	Melyridae	Hoppingiana	*Hoppingiana hudsonica*
0975	GS|83.4|BOLD:ABW2776_NA	Animalia	Arthropoda	Insecta	Orthoptera	Acrididae		
0098	GSL|93.4|BOLD:AAA3920_KJ207446	Animalia	Arthropoda	Insecta	Lepidoptera	Crambidae		
0984	GS|100.0|BOLD:AAE3210_NA	Animalia	Arthropoda	Insecta	Diptera	Culicidae	Culiseta	*Culiseta morsitans*

0989	GS|90.1|BOLD:AAM7579_NA	Animalia	Arthropoda	Insecta	Coleoptera	Dermestidae	Dermestes	*Dermestes marmoratus*
0099	GS|88.7|BOLD:ACG4154_KM443320	Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	Atheta	*Atheta cribrata*
0992	GS|100.0|BOLD:AAP6790_KR692867	Animalia	Arthropoda	Insecta	Diptera	Chloropidae	Incertella	*Incertella incerta*
0996	GS|95.7|BOLD:AAA2674_MF873013	Animalia	Arthropoda	Insecta	Diptera	Drosophilidae	Drosophila	*Drosophila subquinaria*
0997	GS|99.3|BOLD:AAM7983_KM825699	Animalia	Arthropoda	Arachnida	Trombidiformes	Erythraeidae		
0998	GS|99.7|BOLD:ACX2014_KT106334	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	Tanytarsus	*Tanytarsus mendax*

Appendix B GPS coordinates of sample sites

Table B.1 GPS coordinates (decimal degrees) of 2018 sample sites at Teck Resources Highland Valley Copper mine and New Gold Inc. New Afton mine based on NAD 83/ BC Albers projection.

Site	GPS Coordinates
Treatment site 1	50.511519, -121.002735
Treatment site 2	50.510847, -120.999348
Treatment site 3	50.506219, -120.985045
Treatment site 4	50.506376, -120.992450
Treatment site 5	50.505604, -120.994691
Treatment site 6	50.504845, -120.978449
Treatment site 7	50.504424, -120.983555
Treatment site 8	50.496785, -121.007454
Treatment site 9	50.501484, -121.007532
Treatment site 10	50.504564, -120.981955
Treatment site 11	50.505577, -120.977773
Treatment site 12	50.500136, -120.981978
Treatment site 13	50.491549, -121.066168
Reference site 1	50.518789, -121.026010
Reference site 2	50.536450, -121.006242
Treatment site 14	50.655090, -120.534504
Treatment site 15	50.653393, -120.535907
Reference site 3	50.681645, -120.531749
Reference site 4	50.690958, -120.539467

Appendix C Complete 'Indicspecies' analysis tables

Table C.1 complete age

OTU	Indicator statistic	p-value
Reference (number of taxa=36)		
0181	0.463	0.001
0124	0.438	0.001
0050	0.404	0.003
0011	0.360	0.017
0136	0.357	0.006
0045	0.349	0.011
0114	0.348	0.036
0055	0.339	0.014
0219	0.327	0.011
0244	0.327	0.011
0251	0.327	0.011
0258	0.327	0.011
0291	0.327	0.011
0358	0.327	0.007
0460	0.327	0.014
0502	0.327	0.007
0832	0.327	0.007
0063	0.310	0.048
0013	0.304	0.035
0140	0.304	0.031
1076	0.267	0.046
1144	0.267	0.046
1401	0.267	0.046
0214	0.267	0.046
0260	0.267	0.046
0275	0.267	0.046
0295	0.267	0.046
0304	0.267	0.046
0385	0.267	0.041
0400	0.267	0.046
0053	0.267	0.037
0546	0.267	0.050
0572	0.267	0.046
0606	0.267	0.048
0610	0.267	0.046
0865	0.267	0.046
New (number of taxa=7)		
0930	0.392	0.036
0030	0.979	0.031

0206	0.316	0.012
1504	0.274	0.035
0234	0.274	0.040
0245	0.274	0.037
0067	0.274	0.043
Reference and Old (number of taxa=1)		
0205	0.469	0.025
New and Old (number of taxa=2)		
1367	0.440	0.021
1552	0.404	0.050

Table C.4 HVC Age

OTU	Indicator statistic	p-value
Reference (number of taxa=37)		
0124	0.659	0.001
0181	0.620	0.001
0011	0.566	0.002
0045	0.555	0.003
1601	0.553	0.017
0050	0.538	0.002
0219	0.480	0.003
0244	0.480	0.003
0251	0.480	0.003
0258	0.480	0.003
0291	0.480	0.003
0460	0.480	0.005
1560	0.468	0.007
0013	0.462	0.006
0140	0.462	0.004
0063	0.417	0.011
0700	0.417	0.011
0426	0.410	0.02
1076	0.392	0.021
1144	0.392	0.021
1401	0.392	0.021
1586	0.392	0.021
0260	0.392	0.021
0275	0.392	0.021
0295	0.392	0.021
0304	0.392	0.021
0385	0.392	0.009
0400	0.392	0.021
0546	0.392	0.017
0572	0.392	0.021
0606	0.392	0.014
0610	0.392	0.017
0865	0.392	0.021
0088	0.388	0.046
0128	0.370	0.030
0454	0.359	0.041
0055	0.351	0.041
New (number of taxa=4)		
0030	0.450	0.024
0040	0.442	0.028
0111	0.343	0.044
0081	0.343	0.043

Old (number of taxa=2)		
0012	0.484	0.019
0344	0.412	0.025
Reference and Old (number of taxa=2)		
0035	0.623	0.015
0205	0.477	0.040
New and Old (number of taxa=1)		
0002	0.614	0.038

Table C.5 New Afton age

OTU	Indicator statistic	p-value
New (number of taxa=2)		
0771	0.820	0.005
1504	0.707	0.016
Old (number of taxa=3)		
0017	0.685	0.018
0267	0.632	0.034
0300	0.632	0.032
New and Old (number of taxa=1)		
1499	0.798	0.018

Table C.2 all data amendment

OTU	Indicator statistic	p-value
Biosolids (number of taxa=3)		
0040	0.416	0.024
0076	0.332	0.025
0948	0.304	0.028
Reference (number of taxa=33)		
0181	0.463	0.001
0124	0.444	0.001
0050	0.398	0.004
0011	0.366	0.009
0114	0.352	0.032
0136	0.351	0.008
0045	0.351	0.008
0055	0.329	0.028
0219	0.327	0.011
0244	0.327	0.011
0251	0.327	0.011
0258	0.327	0.011
0291	0.327	0.011
0358	0.327	0.009
0460	0.327	0.010
0502	0.327	0.009
0832	0.327	0.009
1076	0.267	0.049
1144	0.267	0.049
1401	0.267	0.049
1586	0.267	0.049
0214	0.267	0.037
0260	0.267	0.049
0275	0.267	0.049
0295	0.267	0.049
0304	0.267	0.049
0385	0.267	0.049
0400	0.267	0.049
0053	0.267	0.044
0572	0.267	0.049
0606	0.267	0.043
0610	0.267	0.041
00865	0.267	0.049
No biosolids (number of taxa=7)		
0064	0.403	0.012
0771	0.327	0.043
0590	0.313	0.042

0526	0.302	0.038
0116	0.261	0.049
0215	0.261	0.042
0389	0.261	0.047
Biosolids and no biosolids (number of taxa=2)		
1367	0.440	0.025
1552	0.404	0.039
Reference and no biosolids (number of taxa=7)		
0015	0.557	0.025
0570	0.545	0.001
0205	0.523	0.002
0027	0.505	0.002
1499	0.391	0.008
0007	0.342	0.021
0063	0.333	0.034

Table C.3 HVC amendments

OTU	Indicator statistic	p-value
Biosolids (number of taxa= 1)		
0040	0.462	0.034
Reference (number of taxa= 34)		
0124	0.666	0.001
0181	0.620	0.001
0011	0.572	0.001
0045	0.555	0.001
1601	0.546	0.049
0050	0.529	0.002
0219	0.480	0.003
0244	0.480	0.003
0251	0.480	0.003
0258	0.480	0.003
0291	0.480	0.003
0460	0.480	0.003
1560	0.463	0.010
0013	0.452	0.008
0140	0.452	0.006
0426	0.418	0.10
0700	0.396	0.043
1076	0.392	0.014
1144	0.392	0.014
1401	0.392	0.014
1586	0.392	0.029
0260	0.392	0.014
0275	0.392	0.014
0295	0.392	0.014
0304	0.392	0.014
0385	0.392	0.023
0400	0.392	0.014
0546	0.392	0.018
0572	0.392	0.014
0606	0.392	0.018
0610	0.392	0.021
0865	0.392	0.014
0454	0.371	0.023
0152	0.352	0.025
No Biosolids (number of taxa=4)		
0205	0.526	0.006
012	0.500	0.018
064	0.459	0.032
0590	0.367	0.042

Biosolids and No Biosolids (number of taxa=1)		
0002	0.617	0.026
Reference and No Biosolids (number of taxa =1)		
0015	0.677	0.001