Invertebrate Response to Mine Reclamation in South-Central British Columbia (NTS 092I): Effects of Reclamation Age on Arthropod Assemblages at the Highland Valley Copper and New Afton Mines

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Introduction

Resources extracted from mines are used in the making of many items used in everyday life; clearly, there are vast social and economic benefits associated with mining operations. In 2017, for example, mining in British Columbia (BC) was responsible for generating $11.7 billion in gross revenue and creating more than 10 000 jobs for British Columbians (Mining Association of British Columbia, 2019). However, mining activity significantly alters the landscape and surrounding native ecosystems.

In Canada, planning for mine closure must occur before mining operations can begin (Mining Association of Canada, 2019). Therefore, areas (terrestrial, aquatic and cultural resources) altered by mining activities must be returned to self-sustaining ecosystems (Government of British Columbia, 2019) through a process called ecosystem reclamation. The aim of research on postmining ecosystem reclamation is to reduce knowledge gaps and further understanding of the reclamation trajectory, thus leading to improved reclamation practices.

Postmining areas naturally undergo a process of secondary succession. It should be noted that natural succession occurs without human intervention; however, since the timescale needed for natural succession to occur may not be acceptable for the public or industry, use of reclamation practices is required. A knowledge gap currently exists concerning long-term postmining reclamation outcomes and trajectory (Buchori et al., 2018); this knowledge gap is particularly notable with regard to invertebrate-community recovery. To be fully functional, an ecosystem must comprise both biotic and abiotic components. One measure of biotic health is biodiversity. Biodiversity refers to the variety of life in a given area with regard to taxonomy, trophic levels and genetic variance (Gaston and Spicer, 2004). Therefore, biodiversity can be used as a measure of ecological health.

Invertebrates work well as indicators of environmental change because they have short generation times and produce large numbers of offspring; they also have mobility, which allows them to efficiently relocate in response to change (Samways et al., 2010). Similarly, invertebrates act as a good subject for species richness (alpha diversity). Invertebrates such as arthropods make up a significant portion of species biodiversity and support vital ecosystem services (McGeoch et al., 2011). Invertebrates encourage nutrient turnover, litter breakup and decomposition (Majer et al., 2002). In addition, many invertebrates are herbivorous, thus potentially contributing to plant-species composition by changing competitive dynamics within the plant community (Yu et al., 2012; Barnett and Facey, 2016). Invertebrates are affected by plant-community composition through a bottom-up effect (Barnett and Facey, 2016). Re-establishment of diverse invertebrate assemblages in postmining reclamation areas, particularly species

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correlated with ecosystem functions, is encouraged (Majer et al., 2002).

Objectives

Arthropods have seldom been used as a measure of examining reclamation success because of the difficulties associated with identifying them morphologically. Recent progress in molecular identification techniques (deoxyribonucleic acid or DNA metabarcoding) has helped to overcome challenges in taxonomic identification of arthropods and other invertebrates. In this study, invertebrate-assemblage response to mine reclamation is assessed, using DNA metabarcoding to identify collected invertebrates. The research questions this study aims to address are based on the use of parallel sequencing of invertebrate DNA metabarcodes as a novel method for assessing reclamation trajectory. The first step entails examining whether changes in invertebrate assemblages can be identified across reclamation sites of different age as a result of ongoing succession characterized by changes in biotic assemblages. Secondly, careful examination will reveal whether specific taxa indicate the age of reclamation. Thirdly, species richness (alpha diversity, i.e., average species diversity within a site) among different age sites will be measured.

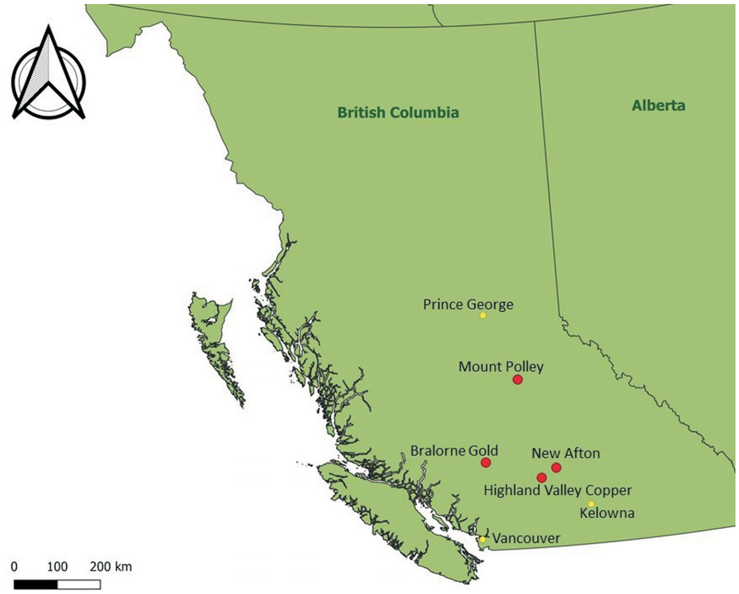

Figure 1. Location of mines in the study area sampled in July and August of 2017 and 2018 for vegetation and invertebrate data. Data from Highland Valley Copper (Teck Resource) and New Afton (New Gold Inc.) mines are the focus of this paper.

Methodology

In 2017, sampling was conducted at four sites: the Teck Resource Highland Valley Copper mine, the New Gold Inc. New Afton mine, the Imperial Metals Corporation Mount Polley mine and the Avino Silver & Gold Mines Ltd. Bralorne Gold mine (Figure 1). However, the Highland Valley Copper and New Afton mines are the focus of this paper. At each mine, two reclamation treatments were sampled: one site reclaimed within the last eight years ('new') and one site that was reclaimed more than eighteen years ago ('old'). A grassland 'control' site was also sampled at the Highland Valley Copper mine. 'Control' sites were also sampled at the New Afton mine but were not processed for this paper. Site descriptions of the sampled areas (including the year of reclamation, reclaimed materials and the methods used to conduct reclamation) can be seen in Table 1.

Both vegetation data and invertebrate samples were collected from the Highland Valley Copper and New Afton sites in 2017. Vegetation was measured using canopy cover by species with 0.5 by 0.5 m quadrats. Invertebrate samples were collected using two types of traps: pitfall traps (Figure 2) and Malaise traps (Figure 3). Pitfall traps were used to collect primarily ground-dwelling invertebrates (Bassett and Fraser, 2015). At each site, a 100 m transect was laid

Table 1. Site description (years since reclamation from 2020, reclaimed materials and reclamation methods) of two mines (Teck Resource's Highland Valley Copper and New Gold Inc.'s New Afton) sampled for invertebrates and vegetation data in the summer of 2017.

Mine	Site	Year reclaimed	Years since reclamation	Reclaimed material	Reclamation method
Highland Valley Copper	New	2014	6	Waste rock and overburden	Biosolids, seeded
	Old	2000	20	Waste rock and overburden	Seeded (crested wheatgrass)
	Control	n/a	n/a	n/a	n/a
New Afton	New	n/a	n/a	Tailings	n/a
	Old	2001	19	Tailings	Fertilizer and cattle manure, seeded

Abbreviation: n/a, not available

Figure 2. Sampling of epigeal invertebrates at the Imperial Metals Corporation Mount Polley mine in 2017 using a pitfall trap consisting of **a)** a 450 g container placed flush with ground level; and **b)** a plastic plate over the top to reduce ethanol evaporation.

out and pitfall traps were placed every 10 m. Pitfall traps were constructed using a 450 g container (Solo® cup) placed flush with ground level. The pitfall traps were filled with ethanol and a plastic plate, held up by nails, was placed over them to reduce the amount of ethanol evaporation. Malaise traps are tent-like structures with bottles of ethanol attached to them and are used to collect primarily flying invertebrates (Thomas, 2016). One Malaise trap was placed at each site. The invertebrate traps were left on site for five days once over the summer. After collection, the samples were stored at -20 °C until laboratory processing.

Laboratory Methods

Identification of the collected invertebrates was conducted using high-throughput DNA metabarcoding. This was done by homogenizing invertebrate tissue in liquid nitrogen using mortar and pestle (Beng et al., 2016). The DNA was extracted from the homogenized tissue using a Mag Bind® Blood and Tissue Kit (Omega Bio-tek, Inc., Norcross, GA). A 450 base-pair region of the mitochondrial cytochrome c oxidase sub-unit 1 gene was amplified in two rounds of polymerase chain reaction (PCR) using the universal PCR primer pair MHemF and dghCO2198 (Beng et al., 2016). The amplicons were sequenced on an Ion S5™ sequencing platform (Thermo Fisher Scientific, Waltham, MA) using an Ion 520™ and Ion 530™ Chip Kit. The bioinformatic pipeline AMPtk was used to cluster sequences into operational taxonomic units (OTUs) at an identity threshold of 97% (Palmer et al., 2018); one OTU represented the sequence of one species (Ji et al. 2013). Taxonomies were assigned to each OTU using the Barcode of Life Data system (BOLD) downloaded at the time of analysis (Yu et al., 2012).

Statistical Analysis

Four methods of statistical analysis were used to analyze the 2017 Highland Valley Copper and New Afton data: principal co-ordinate analysis (PCoA), permutational multivariate analysis of variance using distance matrices (adonis), and random-forest regression and linear mixed-effects models. Data used in the PCoA, adonis and random-forest model analyses were rarified to 10 000 reads and converted into presence-absence data. The PCoA plot was calculated using Euclidean distances and compared five sites (Highland Valley Copper 'control', 'old' and 'new', and New Afton 'old' and 'new'). Calculations car-

Figure 3. Malaise trap set up at the Imperial Metals Corporation Mount Polley mine in 2017 to capture flying invertebrates.

ried out using adonis were also based on Euclidean distance. The random-forest regression model uses the OTU as a predictor value to describe the most change relative to reclamation treatment. The linear mixed-effects model was used to compare species richness between the sites. Data residuals from the linear mixed model were tested for normality using the Shapiro-Wilk test. A log transformation was performed on the data. There was a gap in the New Afton 'old' data as a result of samples still being processed for sequencing; therefore, the mean of five sites was calculated and applied to the sixth sample to create the linear mixed-effects model measuring species richness. All the statistical analyses were conducted in RStudio, a free, open-source integrated development environment for the R software system for statistical computing (RStudio, 2015).

Results

The multivariate analysis methods PCoA and adonis were used to examine invertebrate assemblages by reclamation age group ('old', 'new', 'control') and mine location. Figure 4 illustrates that the difference in mine locations and their respective reclamation ages explain 35.1% of the variation in the invertebrate assemblages collected. The PCoA diagram shows that sites (classified by reclamation age and mine location) located closer to each other in ordination space are more similar. There are three distinct groupings, depicted in the top right, bottom right and bottom left (Figure 4). The group in the top right consists exclusively of 'old' sites', the small group into the left consists of 'old' and 'control' sites, and the largest grouping is made up of a combination of reclamation ages. It should be noted that PCoA works to preserve the calculated distances.

The statistical analysis using the adonis function, highlighted in Table 2, gives an R^2 value that represents the correlation between the treatments and invertebrate assemblages. The 'mine' (location) factor explains 13.8% of the variation, the (reclamation) 'age' factor explains 8.6% of variation and mine location combined with reclamation age ('mine: age') explains 4.4%. The p-value shows if the R^2 value is likely a result of chance; in this case, the p-values for mine location and reclamation age are 0.002, respec-

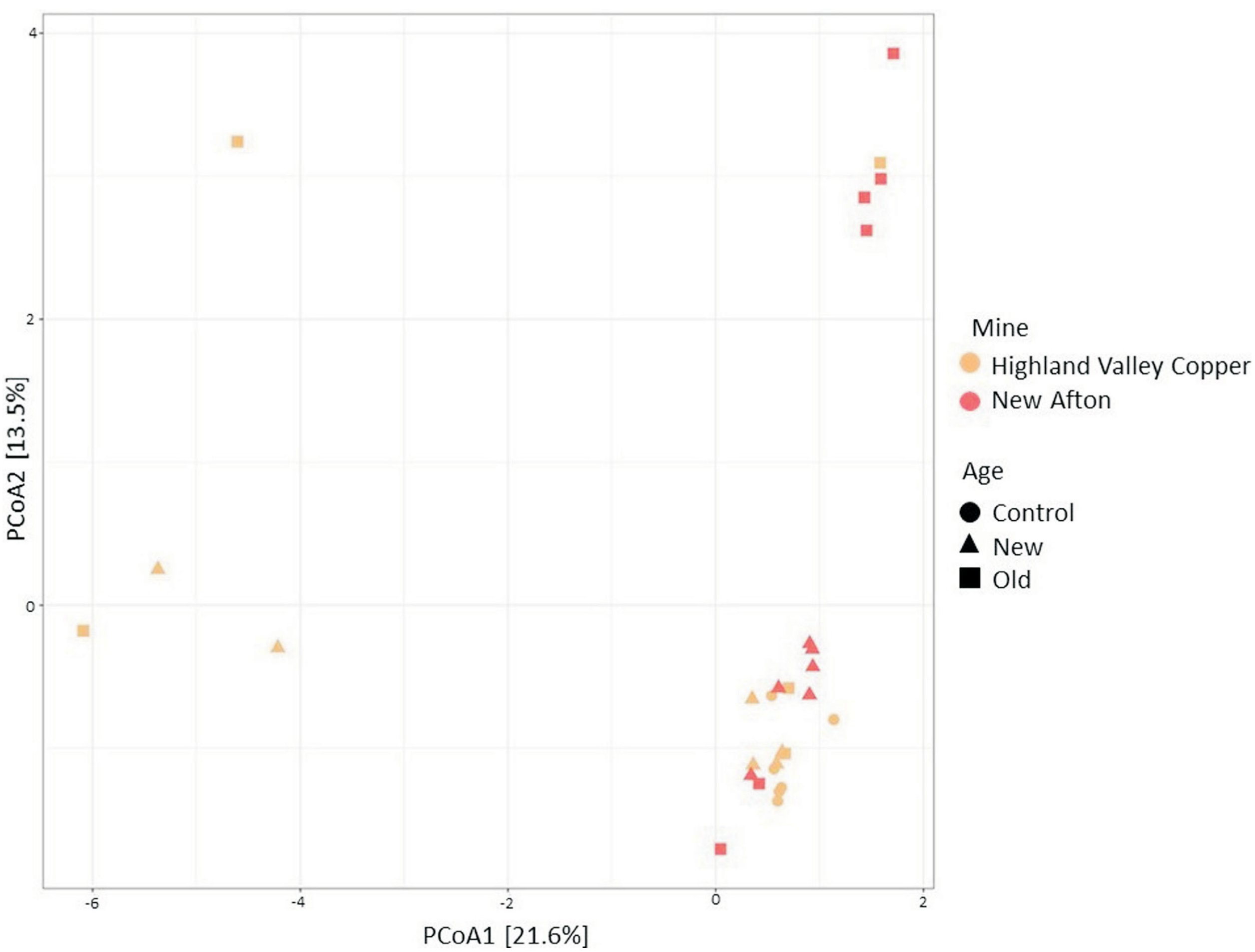

Figure 4. PCoA diagram created using Euclidean distance, illustrating invertebrate assemblages of different reclamation ages ('new', 'old', 'control') between 2017 sample sites at the Highland Valley Copper (Teck Resources) and New Afton (New Gold Inc.) mines. In this method, uncorrelated (orthogonal) axes measure the variability within data and an eigenvalue is determined, which indicates the influence of each axis.

tively, well below a 0.05 significance level, indicating that the results are not likely caused by chance.

To assess if specific taxa indicate the age of reclamation, a random-forest regression model was used to plot the top 20 OTUs that account for the observed change in invertebrate assemblages between reclamation age sites (Figure 5). As the measure of mean decrease of accuracy reduces, the more influential the variable. Thus, OTUs with a larger mean decrease in accuracy are more meaningful for classification of data. In other words, variable accuracy describes how useful the variables are for explaining the change. As a result, OTU 4 (*Nabicula nigrovittata*), OTU 69 (*Phloeostiba lapponica*) and OTU 52 (*Trichoptera*) appear most valuable in classifying this dataset (Figure 5; Table 3).

Table 2. Permutational analysis of variance calculated using Euclidean distance (adonis), where 'f' represents the ratio of the two mean-square values and 'p' is the probability of finding the obtained results given that the null hypothesis is true; the R^2 value represents the correlation between the treatments and invertebrate assemblages, where the degrees of freedom represent the number of ways the values are able to vary and where the sum of squares is the measure of the data points from the mean.

Factor	Degrees of freedom	Sum of squares	Mean square	f-value	R^2	p-value
Mine	2	1.3835	0.69173	2.22823	0.13884	0.002
Age	1	0.8659	0.86589	2.8569	0.0869	0.002
Mine:Age	1	0.4412	0.44124	1.45558	0.04424	0.133
Residuals	24	7.2742	0.30309		0.72999	
Total	28	9.9648			1	

Table 3. Top three operational taxonomic units (OTU), and their corresponding taxonomy, that account for the change in invertebrate presence between the New Afton (New Gold Inc.) 'new' and Highland Valley Copper (Teck Resource) 'control' sites, as determined by a random-forest regression model using Euclidean distance.

OTU	Taxonomy
OTU 4	*Nabicula nigrovittata*
OTU 69	*Phloeostiba lapponica*
OTU 52	*Trichoptera*

Table 4 shows the random effects (mine location) in the linear mixed-effects model. The standard deviation column measures the variability of the random effects. The variance of the 'mine' location variable is smaller than that of the 'residual' variable. A low standard deviation in the random effect indicates the contributing values are near the mean. The 'residual' indicates the variability that is not a result of the 'mine' location factor.

Table 5 displays the fixed effects (reclamation age) analyzed in the linear mixed-effects model. The coefficient 'AgeNew' is the slope (standard error of the estimate) for the effect of species richness between the 'control' and 'new' sites. The standard error of the estimate for 'AgeNew' highlights the change in species richness from Highland Valley Copper to New Afton and indicates that the 'new' sites have fewer species (inverse log 0.003849) accounted for than the 'control' site. The 'AgeOld' stan-

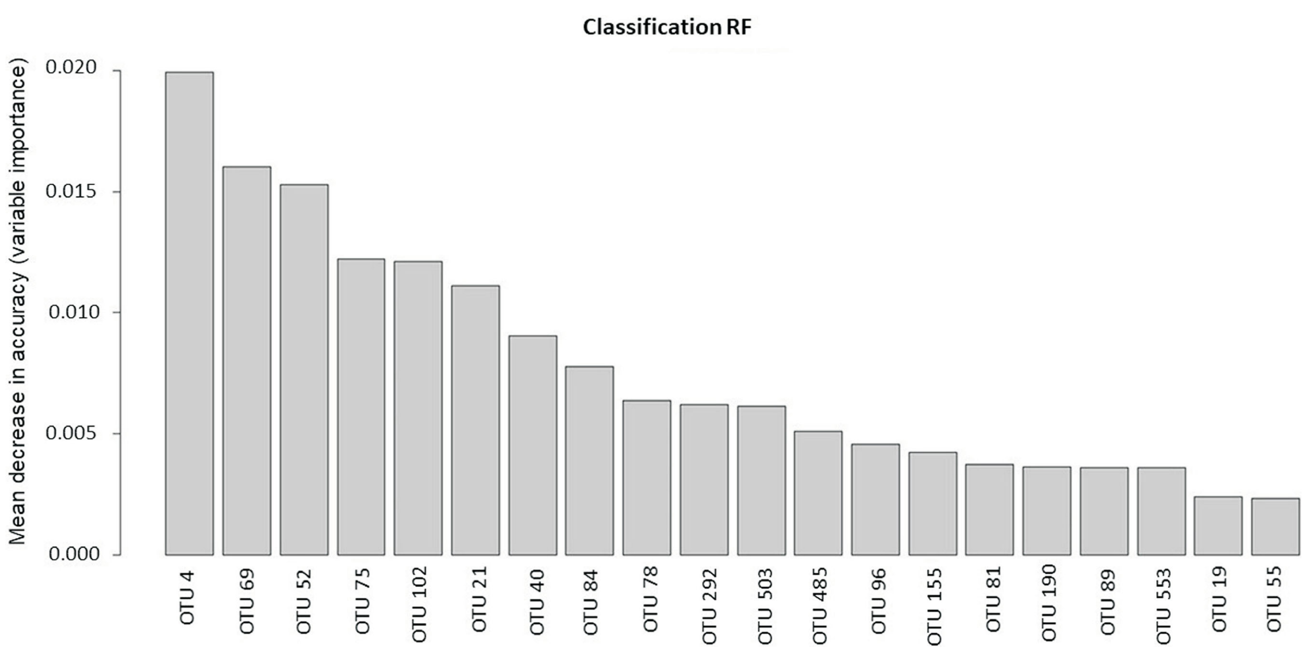

Figure 5. Top 20 operational taxonomic units (OTU) explaining the change in invertebrate presence between different reclamation ages ('new', 'old', 'control') of the 2017 sample sites at the Highland Valley Copper (Teck Resources) and New Afton (New Gold Inc.) mines, where a larger mean decrease in accuracy is the more meaningful variable for classification of data. Abbreviation: RF, random forest.

dard deviation indicates that the 'old' sites have more species (inverse log 0.15214; Table 5) accounted for than the 'control' site. It should be noted that both the 'new and 'old' reclamation age categories are not statistically significant (p>0.05).

Discussion

The effects of postmining reclamation on recovering invertebrate assemblages were examined at four sites in BC using vegetation and invertebrate surveys over two years. For invertebrates, DNA metabarcoding techniques were used and few differences in invertebrate-community composition between mine sites and different reclamation-age plots were detected.

As a first step, invertebrate-assemblage similarity of different age plots ('new', 'old' and 'control') between 2017 sample sites at the Highland Valley Copper and New Afton mines was measured using PCoA and adonis. Understanding the reclamation trajectory of invertebrate assemblage postmining reclamation is an important objective on the road to achieving successful end land use. The PCoA used in this study shows visible separation between 'old', 'new' and 'control' age sites (Figure 4). The PCoA explains 35.1% of the variability seen within these assemblages, with three distinct groupings. Most notably, there is a cluster of 'old' sites; however, the largest group consists of a variety of reclamation age treatments and mine locations, indicating that the variables (reclamation age, mine location) analyzed in this paper fail to explain the variation in invertebrate assemblage. Likewise, adonis analysis (Table 2) confirms that the correlation between the reclamation age and mine location variables does not account for the invertebrate variation seen between the plots. This is a result of low R^2 values for mine location (13.8%), reclamation age (8.6%) and a combination of mine location and reclamation age (4.4%). It should be noted that the Highland Valley Copper 'old' and 'new' sites are located on wasterock and overburden, whereas the 'control' site is located within a fenced enclosure near the highway. New Afton 'old' and 'new' sites occur on a historical tailings facility.

Secondly, a measure of influence of invertebrate taxa characterizing reclamation age and mine sites was examined. Site assemblage-species profiles can be generated using DNA metabarcoding. In this study, the focus was put on recognizing the change in invertebrate taxa between sites. The taxon that accounts for the greatest observed change in assemblage was *Nabicula nigrovittata*, a predatory insect that belongs to the order *Hemiptera* (Lattin, 1989). In this case, *Nabicula nigrovittata* was primarily found on the Highland Valley Copper and New Afton 'old' sites, whereas it was absent on the 'new' and 'control' sites. Another species that accounted for the disparity between sites was OTU 69, *Phloeostiba lapponica*, which is a member of the *Staphylinidae* family (rove beetle). In the random-forest model, *Phloeostiba lapponica* was identified at the Highland Valley Copper 'new' and 'old' sites. A final species, OTU 52 (*Trichoptera*) contributed to site differences; this genus belongs to the order that includes caddisflies. Trichopteran taxa were identified in both the Highland Valley Copper and New Afton 'old' sites. The presence of *Trichoptera* can be used as a positive bioindicator for good water quality (Pereira et al., 2012).

Table 4. Linear mixed-effects model residual and random output (mine location), comparing species richness defined by the number of operational taxonomic units between each of the five study sites: Highland Valley Copper (Tech Resource) 'new', 'old' and 'control', and New Afton (New Gold Inc.) 'new' and 'old'.

Factor	Name	Variance	Std
Mine	Intercept	<0.0001	<0.001
Residual		0.07967	0.2823

Abbreviation: Std, standard deviation

Table 5. Linear mixed-effects model fixed output (reclamation age), comparing species richness defined by the number of operational taxonomic units between each of the five study sites: Highland Valley Copper (Tech Resource) 'new', 'old' and 'control', and New Afton (New Gold Inc.) 'new' and 'old'.

Coefficient	Estimate Std	Error	d.f.	t-value	p-value
Intercept	1.25966	0.11523	27	10.932	2.03E-11
AgeNew	-0.003849	0.14113	27	0.273	0.787
AgeOld	0.15214	0.14113	27	1.078	0.291

Abbreviations: d.f., degrees of freedom; Std, standard deviation

Lastly, alpha diversity characterizing reclamation age plots was measured between the sampled sites. The linear mixed-effects model demonstrated no significant difference in species richness between the sampled sites; this is potentially a result of large variation between the replicates.

Conclusions and Ongoing Work

The above results and correlations are based on data from 2017; additional analyses are underway as the remaining 2017 samples are currently being sequenced. The 2018 samples are also being sequenced and future papers will address the effects of biosolids as a soil amendment. However, it should be noted that the COVID-19 pandemic has affected the sequencing timeline of the remaining 2017 and 2018 samples.

The results of this study should aid in reducing the knowledge gap regarding postmining reclamation outcomes. Using novel methods (high-throughput DNA metabarcoding), this project will contribute to the improvement of planning and management practices, leading to more effective postmining ecosystem-reclamation outcomes. This in turn will assist in the development of further advancements in the field of mine reclamation as they relate to the sustainable health of ecosystems, which are vital to the continued growth of BC's communities and economy. Future studies, determining which environmental variables are associated with invertebrate recovery, may help land managers facilitate restoration through simulation of the relevant conditions.

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