Microbial composition and function in reclaimed mine sites along a reclamation chronosequence become increasingly similar to undisturbed reference sites

Jay Prakash Singh [a,*], Eric M. Bottos [b], Jonathan D. Van Hamme [b], Lauchlan H. Fraser [a]

[a] Department of Natural Resource Sciences, Thompson Rivers University, 805 TRU Way, Kamloops, BC V2C 0C8, Canada
[b] Department of Biological Sciences, Thompson Rivers University, 805 TRU Way, Kamloops, BC V2C 0C8, Canada

HIGHLIGHTS

- Microbial communities of reclaimed mines become similar to unmined reference sites along a reclamation chronosequence.
- Functional potential also appears to become similar to unmined reference sites.
- Bacterial communities are increasingly assembled through stochastic processes as the reclamation age increases.
- Microbial genomics is an essential assessment tool to track the trajectory of reclamation activities.

GRAPHICAL ABSTRACT
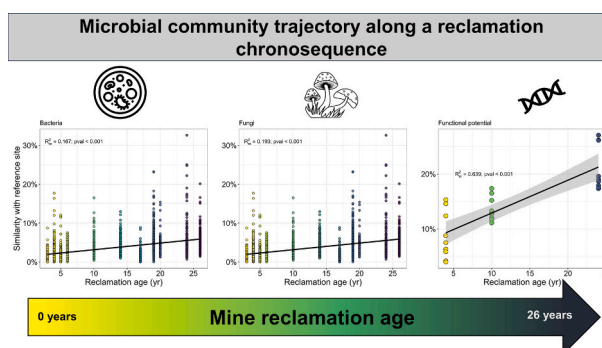
ARTICLE INFO

ABSTRACT

Mine reclamation historically focuses on enhancing plant coverage to improve below and aboveground ecology. However, there is a great need to study the role of soil microorganisms in mine reclamation, particularly long-term studies that track the succession of microbial communities. Here, we investigate the trajectory of microbial communities of mining sites reclaimed between three and 26 years. We used high-throughput amplicon sequencing to characterize the bacterial and fungal communities. We quantified how similar the reclaimed sites were to unmined, undisturbed reference sites and explored the trajectory of microbial communities along the reclamation chronosequence. We also examined the ecological processes that shape the assembly of bacterial communities. Finally, we investigated the functional potential of the microbial communities through meta-genomic sequencing. Our results reveal that the reclamation age significantly impacted the community compositions of bacterial and fungal communities. As the reclamation age increases, bacterial and fungal communities become similar to the unmined, undisturbed reference site, suggesting a favorable succession in microbial communities. The bacterial community assembly was also significantly impacted by reclamation age and was primarily driven by stochastic processes, indicating a lesser influence of environmental properties on the bacterial community. Furthermore, our read-based metagenomic analysis showed that the microbial communities' functional potential increasingly became similar to the reference sites. Additionally, we found that the

* Corresponding author.
  E-mail address: jsingh@tru.ca (J.P. Singh).

plant richness increased with the reclamation age. Overall, our study shows that both above- and belowground ecological properties of reclaimed mine sites trend towards undisturbed sites with increasing reclamation age. Further, it demonstrates the importance of microbial genomics in tracking the trajectory of ecosystem reclamation.

1. Introduction

Mining is a major contributor to worldwide GDP, but it is also a significant disruptor of the environment across trophic levels. The effects of mining on the environment (Bell and Donnelly, 2006; Down and Stocks, 1977; Rybicka, 1996; Sengupta, 2021; Thornton, 1996) and human health (Entwistle et al., 2019; Farjana et al., 2019; Hadzi et al., 2019; Kahhat et al., 2019) are well documented. The impacts of mining on soil ecology are particularly apparent as they fundamentally change the physical, chemical, and biological properties of soils. Therefore, large-scale reclamation efforts are undertaken worldwide to return mining-influenced landscapes to productive, self-sustaining ecosystems.

Historically, reclamation efforts have focused on enhancing plant cover on reclaimed sites to improve the aboveground ecology (Fraser et al., 2015). In contrast, belowground reclamation efforts aim to improve physicochemical properties such as soil organic matter and aggregate stability (Malik and Scullion, 1998). More recently, there is a growing appreciation for the need to study the role of microorganisms in restoration ecology (Harris, 2009). Microorganisms play a crucial role in nutrient cycling (Fierer, 2017), decomposition of organic matter (Sokol et al., 2022), sequestering organic carbon (Plaza et al., 2013), alleviating metal stress (Rajkumar et al., 2012), stabilizing metals in soil (Rajkumar et al., 2012), and maintaining fertility in soils (Fierer, 2017). Associations of microorganisms with plants greatly influence nutrient acquisition, subsequently impacting aboveground primary productivity (Pii et al., 2015). Therefore, incorporating considerations for soil microorganisms can help to improve reclamation strategies considerably.

A growing body of literature highlights the importance of microbial communities in mine reclamation activities (Garris et al., 2016). For example, Guo et al. (2019) showed a positive relationship between soil nutrients (such as organic matter, nitrogen and available phosphorus) and the dominant bacterial species in an open pit mine. Zhu et al. (2022) describe how *Stenotrophomonas maltophilia* increases the solubilization of nutrients (phosphorus, potassium and iron) in mine soils, potentially paving the way for soil reclamation. Li et al. (2014) surveyed the microbial community structure along a reclamation chronosequence. They demonstrated that microbial abundance and diversity increased with age, subsequently improving soil enzymatic activities and overall soil function in reclaimed mines. Ma et al. (2023) showed the microbial community assembly was sensitive to mining activity and that the bacterial community assembly process was dictated by dispersal limitations. Roy et al. (2023) showed that fungal guilds in mining-impacted sites recover substantially to pre-mining levels. Several other studies have characterized microbial diversity in response to mining (Xiao et al., 2021; Zhong et al., 2023) and mine reclamation (Hottenstein et al., 2019; Mitter et al., 2017; Moreira-Grez et al., 2019). Additionally, very few studies have explored the impact of amendments such as biosolids on the composition and assembly of microbial communities. Studies that provide crucial information about microbial community composition and diversity within the reclamation domain are essential for developing remedial strategies for mine reclamation. However, most studies provide a snapshot of microbial communities (Gorzelak et al., 2020) in response to reclamation (Asemaninejad et al., 2021) without including a reference (undisturbed) site. Comparisons with reference sites provide a metric against which a reclaimed site can be benchmarked.

While previous studies have attempted to examine the succession of microbial communities along a reclamation chronosequence (Dangi et al., 2012; Li et al., 2014; Li et al., 2018; Ngugi et al., 2018), most provide only qualitative evidence of microbial trajectory. Recently,

Liddicoat et al. (2022) quantified the succession of bacterial communities by measuring the similarity of microbial communities between reference and reclaimed sites. The similarity metric provides a quantitative measure of how different the microbial community of reclaimed sites is from undisturbed areas. Similar studies are needed to explore compositional (bacterial and fungal) and functional succession along a reclamation chronosequence. Such studies would provide a better understanding of the progression of microbial communities at reclaimed sites and enable tracking of reclamation trajectories along a chronosequence, which will provide insight into the projection of reclamation timelines.

Our study attempts to fill this research gap by examining how reclamation age and strategy impact bacterial and fungal community composition and functional potential along a reclamation chronosequence. We characterized bacterial and fungal community composition using amplicon sequencing and functional potential using metagenomic sequencing. We evaluated the trends of microbial communities with reclamation age and reclamation strategy. For simplification, we broadly classified the reclamation strategy into two groups based on the amendment used during reclamation – i) biosolids (when biosolids were used as an amendment for reclamation); ii) non-biosolids (when biosolids are not used as an amendment for reclamation). Moreover, we investigated how reclamation age and reclamation strategy impacted bacterial community assembly, fungal trophic guilds, and plant diversity. We hypothesize that the reclamation age will favorably impact microbial community structure and functional potential. The overarching goal of the study is to examine the trajectory of microbial composition and potential function along a reclamation chronosequence.

2. Methodology

We selected the reclaimed sites of an active mine, Highland Valley Copper (HVC) mine, near the town of Logan Lake in the interior of British Columbia, Canada. HVC is Canada's largest open-pit copper mine, with reclaimed sites covering a wide range of reclamation chronosequence. We chose 18 sites for this study, of which 15 varied by reclamation age and three were undisturbed reference sites. The reference sites were selected so that they were near the reclaimed mines. Therefore, they experienced similar climatic conditions. Both the reference sites and the reclaimed mines are located in the interior British Columbia region, near Logan Lake, and are classified as the Interior Douglas-fir (IDF) biogeoclimatic zone of British Columbia. This biogeoclimatic zone is described as relatively dry, with annual precipitation of 393.2 mm and a mean daily temperature of 4.4 °C (Government of Canada, 2019). The study area is part of the Thompson Plateau in an open-ended valley where the predominant soils are cambisols and luvisols (FAO-UNESCO, 1975). The representative plant species in this biogeoclimatic zones are as follows;Tree species - Douglas fir, Ponderosa pine, Lodgepole pine juniper, etc. Understory species - Kinnikinnik, Snowberry, Bluebunch wheatgrass, Yarrow, Birch leaved spirea, Prickly rose, Twinberry, Pinegrass, etc. The reclamation age of the reclaimed sites ranged between three and 26 years. These sites were either amended with biosolids or not amended with biosolids. Biosolids are waste products from wastewater treatment plants that are treated to stabilize for application on disturbed land. They are rich in carbon and other nutrients and serve as a fertilizer in nutrient-poor soils (Antonelli et al., 2018). The biosolids that were used at HVC for mine reclamations were classified as class A and B (Antonelli et al., 2018), which are

suitable for application on barren lands. After extracting precious elements, the leftover product from mining is extremely poor in soil carbon and other nutrients. Therefore, biosolids, as a sustainable amendment, provide nutrients for plant colonization and growth. At HVC, the biosolids were applied on top of the tailings and overburden using heavy machinery. Over 380,000 wet tonnes of biosolids have been applied to >700 ha of disturbed land at HVC (Teck, 2013). The experimental design allowed us to examine the impact of the reclamation age and strategy (amended with biosolids or not) on the composition and structure of microbial communities.

2.1. Plant survey

For the plant community survey, we laid down a transect of 90 m at each site and conducted plant surveys every 10 m using a 0.5 × 0.5-m quadrat starting at the 0 m mark. The plant surveys included measuring the coverage of every plant species found in a quadrat.

2.2. Soil sampling

We collected soil samples from the same plots as the plant surveys. We collected soil samples from the top 10 cm of the soil profile in sterile Whirl-Pak bags and kept them on dry ice in the field before processing them at the lab. All apparatus, including trowels used for collecting soil samples, were cleaned with 70 % ethanol between samples to prevent carryover contamination. We stored the samples at −80 °C before extracting genomic DNA for further analysis.

2.3. DNA extraction and sequencing

We extracted the genomic DNA from stored soil samples using the MagAttract PowerSoil DNA Kit (Qiagen, Germany) following the manufacturer's protocol and quantified the extracted DNA using Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific, USA). We amplified the bacterial 16S rRNA gene and fungal ITS2 gene using the primers 341R and 806F, and ITS86F and ITS4R, respectively. We then added sequencing barcodes in a second round of PCR. Amplicons were purified using AgenCourt AMPure XP reagent before sequencing on an Ion S5 XL system using 400-bp chemistry.

We selected 12 samples between four and 24 years old for metagenomic sequencing using 2 × 150 bp paired-end chemistry on an Illumina NextSeq 550 sequencing platform at the University of British Columbia sequencing center to study the functional potential of microbial communities at reclaimed sites. Three samples from reference sites were randomly chosen, while the other nine were selected based on DNA yield and reclamation chronosequences. The ages of the three samples chosen for metagenome sequences were four, 10 and 24 years.

2.4. Sequence processing

We analyzed the amplicon sequences using the Dada2 package (Callahan et al., 2016) in R (version 4.2.1, R Core Team (2022)). First, we filtered out all sequences with a maximum expected error rate of more than two and a sequence length of <50 bp. We truncated the length of the bacterial sequences to 210 bp after trimming lower-quality reads from both ends. For fungal sequence analyses, we did not use any truncation as recommended by the package developers. We then used the filtered sequence to learn error rates and fed the sequences and error rates model to the Dada2 algorithm. After running the Dada2 algorithm, we removed the chimeras and generated amplicon sequence variants (ASVs) tables. We then assigned taxonomy to the ASVs using the naive Bayesian classifier method with bootstrap confidence of 50. We used the SILVA (version 138.1) and the UNITE (version 9.0) databases for the taxonomic assignment of bacterial and fungal sequences, respectively. Finally, we generated ASVs count data, a FASTA formatted sequence of the ASV sequences, and taxonomy tables.

We employed read-based analysis of the metagenomic sequences to investigate the functional potential of the microbial community. We used Humann3 (Beghini et al., 2021) and the EC-filtered UniRef90 database for gene calling. After generating the gene abundance table using Humann3 from the metagenomic reads, we normalized the data to get the relative abundance of genes. We then merged all samples to generate a sample vs. gene relative abundance table.

2.5. Data analysis

We examined how reclamation age and reclamation strategy impacted plant diversity by measuring observed species richness. We analyzed plant species richness and plant litter cover using a polynomial mixed-effect model to investigate the influence of reclamation age and reclamation strategy (biosolids vs. non-biosolids). We included plant species richness and litter cover as the response variable, reclamation age and method as fixed effects, and replicates within sites as random variables.

We used count data generated from the sequence processing in DADA2 to investigate the trends in community compositions. We removed samples with fewer than 2000 reads and all ASVs with a relative abundance of <0.001 % for both fungal and bacterial datasets. We then used Hellinger transformed count data to calculate Bray-Curtis dissimilarities (Okansen et al., 2020) and ran a principal coordinate analysis (PCoA) (Paradis and Schliep, 2019) on bacterial and fungal counts. To examine the impact of the age of reclamation and the strategy of reclamation on bacterial and fungal community structures, we used a permanova analysis. While ordination and permanova results provide context about factors that impact beta diversity, they do not give the exact relationship between the explanatory variables and beta diversity. Therefore, we used pairwise similarity to study the trajectory of the microbial communities along the reclamation chronosequence (Liddicoat et al., 2022) and how the reclamation strategy impacted the communities overall. We filtered the pairwise Bray-Curtis dissimilarity between the reference plots and the reclaimed sites to investigate the trends in reclamation trajectory along a reclamation chronosequence. We converted dissimilarities into similarities using the formula described by Liddicoat et al. (2022), which was adapted from Legendre and Legendre (2012).

$$\%Similarity\ to\ the\ reference = 100 \times \left(1 - Dissimilarity_{Bray\ Curtis}\right) \quad (1)$$

The Bray-Curtis dissimilarity ranges between '0' and '1', with '0' indicating no difference between the samples and '1' indicating no similarity (i.e., no species overlap). High similarity values indicate more similarity of the microbial community of reclaimed sites with the reference sites. We analyzed percentage similarities using linear mixed effect models (Bates et al., 2015) with the similarity values as the response variable and the age of reclamation and reclamation strategy as fixed effects. We used the replicates within the sites as random variables. We analyzed both fungal and bacterial community similarities using the same model structure.

We calculated Bray-Curtis dissimilarities from the annotated gene count data to examine the functional potential from our metagenomic sequences and used a PCoA ordination to visualize it. We also used a permanova to test if the reclamation age impacted functional potential. Further, we used the pairwise Bray-Curtis dissimilarities of functional potential between the reference site and the reclaimed sites. We then converted the dissimilarities values to similarities percentage using Eq. (1) and used a linear mixed-effect model with similarity as a response, reclamation age as a fixed effect, and the replicates within the sites as random variables.

We also calculated the β nearest taxon index (βNTI) to understand the underlying ecological process that shapes the assembly of microbial communities along the reclamation trajectory. For calculating βNTI, we generated a phylogenetic tree by aligning the bacterial ASVs using

Muscle (v5) and then inferred the approximate maximum-likelihood trees from the aligned sequences using FastTree2.1 (Price et al., 2010). We utilized the phylogenetic tree and the bacterial Bray-Curtis dissimilarity to calculate pairwise βNTI using the iCAMP R package (Ning et al., 2020). βNTI measures how taxonomic dissimilarity deviates from the null expectation. When βNTI ranges between −2 and +2, the community is considered to be assembled through stochastic processes; deviations from the null expectation (<−2 or >+2) indicate that deterministic processes drive community assembly (Stegen et al., 2012). We re-coded the βNTI values as binary variables - '0' when the community assembly was stochastic and '1' when deterministic. We then used a mixed-effect logistic model with the community assembly (0 = stochastic; 1 = deterministic) as binary responses. We included the reclamation age and method as the fixed effects with the plots within sites coded as random variables. We did not use the fungal sequences for βNTI as the sequences do not align well, which results in highly uncertain phylogeny.

However, we investigated the fungal trophic guilds to better understand fungal functional roles in the reclaimed sites. We used the FunguildR package (version 0.2.0.9; an R package to query the FUN-Guild database) to classify the sequences into their trophic guilds as pathotrophs, saprotrophs, and symbiotrophs (Nguyen et al., 2016). We selected the guilds that were either "probable" or "highly probable". We then calculated the species richness for each of the three guilds. To analyze the species richness of pathotrophs and saprotrophs, we used a general polynomial regression model using the Poisson distribution with richness as the response and age as the polynomial term. We noticed that the symbiotroph had several zero values, so we used a hurdle model with age as the polynomial term, using a Poisson distribution for the non-zero count model and binomial distribution for the zero-inflated model.

We built our models using different model structures and selected the ones with the lowest Akaike information criterion (AIC). We conducted all analyses with R (version 4.2.1, R Core Team (2022)) in Rstudio (version 0.14) (RStudio Team (2020)). We used the "tidyverse" meta package for data processing and plotting (Wickham et al., 2019). We used "vegan" (Okansen et al., 2020) for diversity estimation and Hellinger transformation. We used "lme4" (Bates et al., 2015) and "lmerTest" (Kuznetsova et al., 2017) packages for mixed-effect modelling. The marginal R-squared values were extracted from the mixed models using the "Mumin" (Barton, 2009) package. We used the "pscl"

(Jackman et al., 2015) package to run the hurdle model and extracted R square values from the polynomial regressions and hurdle model using the performance package.

3. Results

3.1. Plant community

To understand the effect of reclamation age and biosolids addition on plant diversity, we examined how plant richness changed with reclamation age and biosolids addition. Our analyses indicate that reclamation age and reclamation strategy significantly impacted the observed plant richness, with richness increasing significantly with reclamation age ($\chi^2 = 6.96$; $p < 0.05$, Fig. 1A) and biosolid reclamation ($\chi^2 = 4.01$; $p < 0.05$, Fig. 1B). Despite the significantly higher richness in biosolid-treated sites, the difference in mean species richness between the reclamation strategies was minimal (Fig. 1B).

3.2. Bacterial community

We investigated how the reclamation age and biosolids application impacted bacterial alpha diversities (Shannon-Weiner index, Simpson's Index and Observed species richness). We found that none of the aforementioned indexes were significantly impacted (p-value > 0.05) by reclamation age or biosolids application (Figs. SI 5 and 6).

We examined how the reclamation age and biosolids application impacted bacterial composition using PCoA ordinations. The first two axes from the bacterial PCoA ordination captured 26.4 % of the total variance in bacterial community composition (Fig. 2A). Permanova analysis showed that the reclamation strategy (pseudoF = 2.88, $p < 0.002$) and reclamation age (pseudoF = 5.45, $p < 0.001$) explained significant variation in bacterial communities, with reclamation age (marginal $R^2 = 0.29$) explaining more variation than the reclamation strategy (marginal $R^2 = 0.02$). Results from the mixed-effect model revealed that the similarity between the undisturbed references site and reclaimed sites increased with reclamation age (F value = 44.41; $p < 0.001$). The biosolids application also significantly impacted community similarity (F value = 10.27; $p < 0.005$). The Bray-Curtis similarity between reference and reclaimed sites was marginally higher in biosolids-treated plots (estimated marginal means = 12.77) than in non-biosolid-
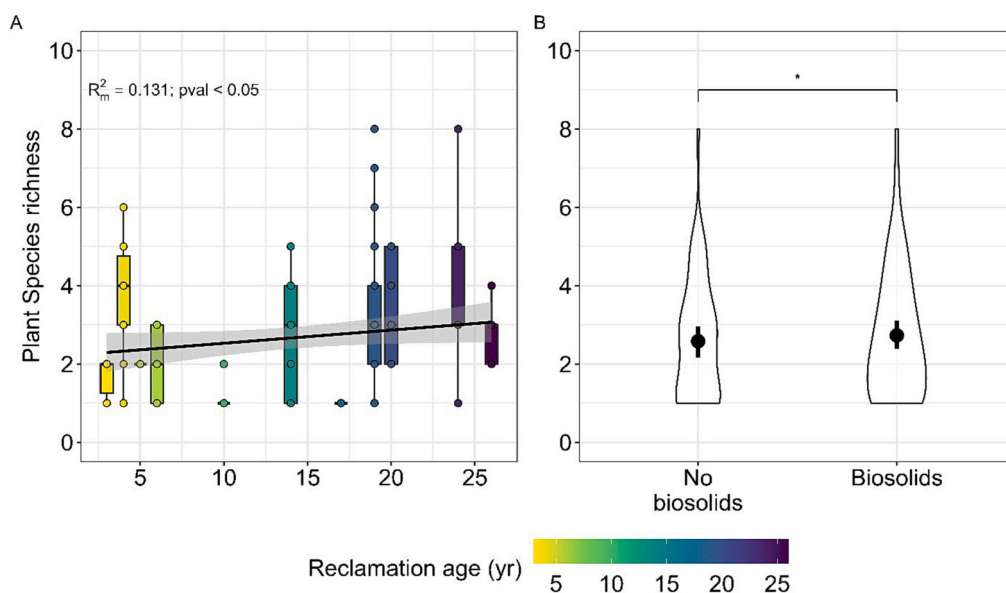

Fig. 1. (A) Reclamation age impacts plant richness, with the number of observed species increasing with age ($\chi^2 = 6.96$; $p < 0.05$). (B) Sites reclaimed with biosolids had slightly higher species richness ($\chi^2 = 4.01$; $p < 0.05$) when compared to sites reclaimed with other materials. The marginal R^2 of the model is indicated in the figure. "*" denotes significance at $p < 0.05$. The error bars represent the lower and upper Gaussian confidence limits based on the t-distribution.
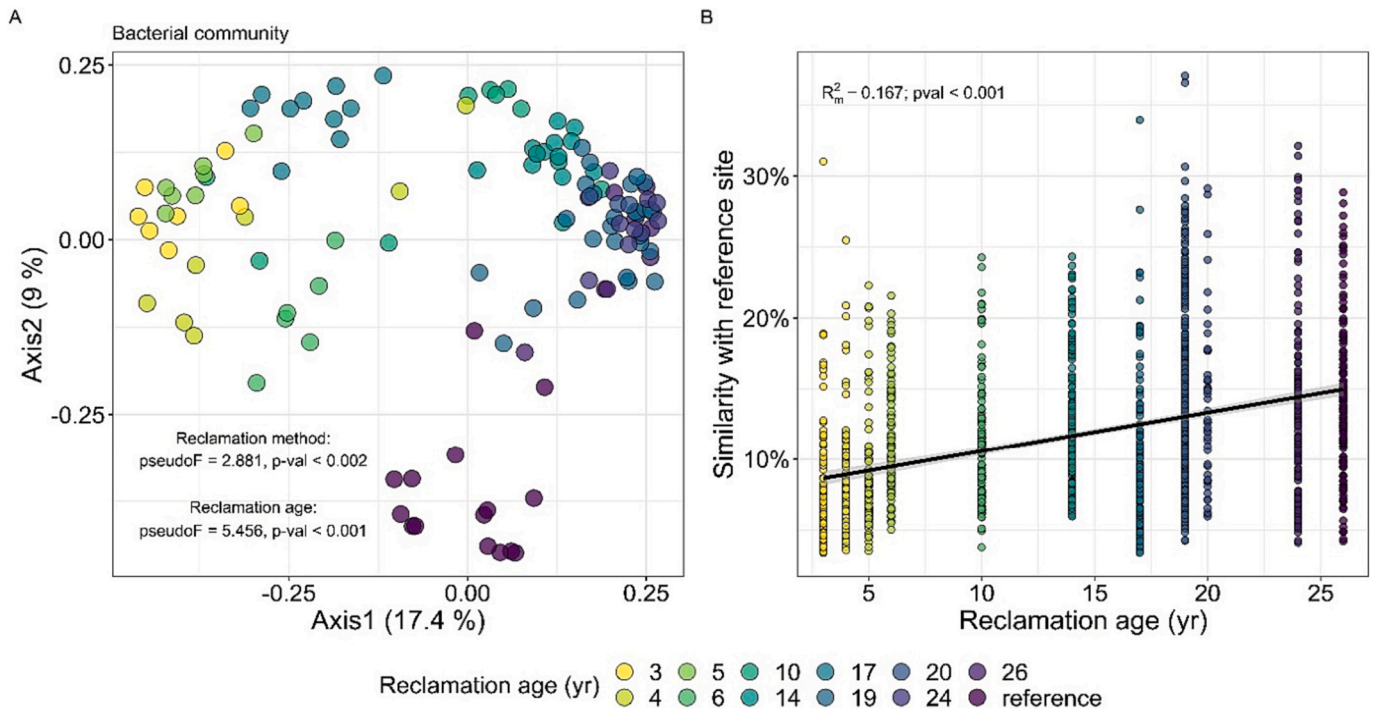
Fig. 2. (A) Principal coordinate analysis of the bacterial community with the first two PCoA axes explains ~26 % of the total variance. Permanova results show that the reclamation method and age are significant predictors of the variance associated with the bacterial community (B) Bray-Curtis similarity between reclaimed and reference plots increases linearly with reclamation age with the fixed effects explaining a significant amount of the variation. Each point represents the percentage similarity between a reference and a reclaimed site.

treated plots (estimated marginal means = 9.85).

The first two axes from the PCoA analysis of functional potential captured over 55 % of the variation (Fig. 3A). Additionally, permanova analysis showed that the reclamation age significantly impacted functional potential (pseudoF = 3.373; p < 0.003, Fig. 3A) and accounted for substantial variation (marginal R^2 = 0.56). Further, analysis of pairwise Bray-Curtis similarity revealed a significant relationship between

reclamation age and functional similarity (F value 70.313; p < 0.001, Fig. 3A), which increased linearly (Fig. 3B). The marginal R^2 from the pairwise similarity analysis was >63 %, demonstrating that the reclamation age explained substantial variation in functional potential (Fig. 3B).

Furthermore, we found that the bacterial community assembly process was impacted by reclamation age (χ^2 = 17.31; p < 0.001) and
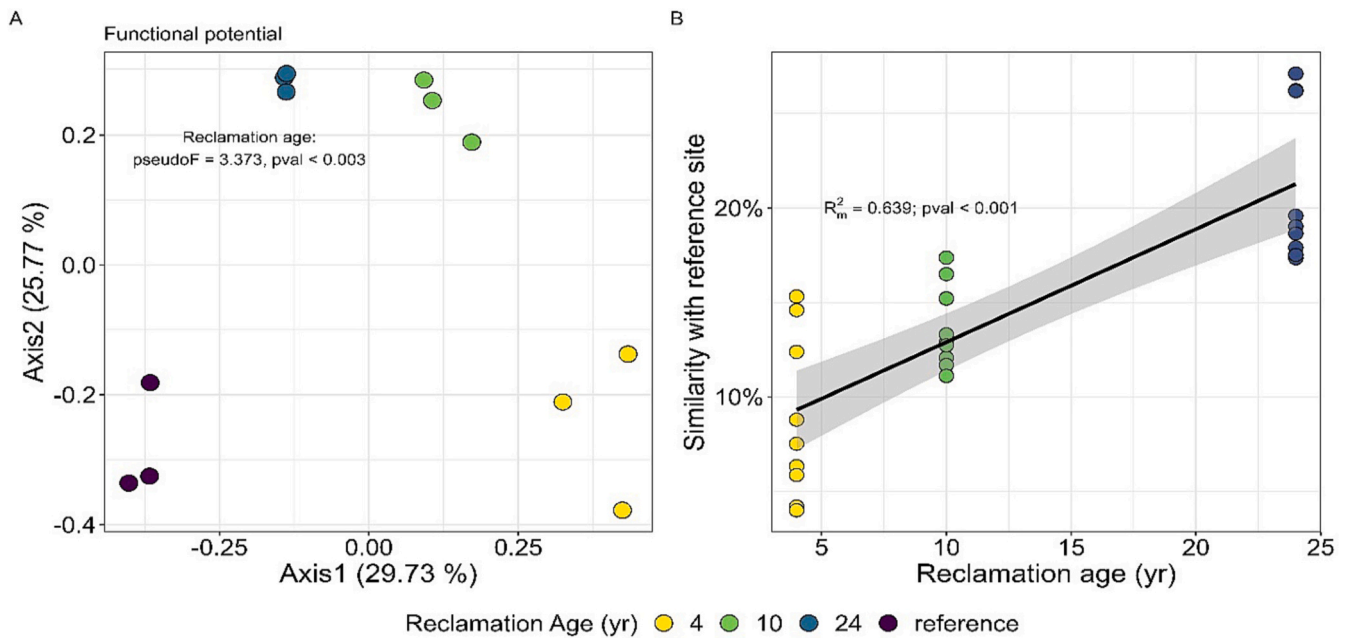

Fig. 3. (A) Functional potential from read-based analysis of the microbial metagenomic sequences indicates that reclamation age significantly impacts the functional beta diversity. (B) Pairwise Bray-Curtis similarity shows a significant increase with reclamation age. Each point represents the percentage similarity between a reference and a reclaimed site.

biosolids reclamation ($\chi^2 = 9.96$; $p < 0.005$). Despite the high variance with biosolids reclamation, we observed that the odds of deterministic assembly were lower in biosolids-treated plots (odd ratio = 0.41 ± 0.116) (Fig. 4, Fig. SI 1B). Similarly, reclamation age impacted the bacterial community assembly process and significantly lowered the odds of deterministic assembly (odd ratio = 0.924 ± 0.0175) with reclamation age (Fig. SI 1A).

3.3. Fungal community

We examined the impact of reclamation age and biosolids application on the fungal alpha diversities (Shannon-Weiner index, Simpson's Index and Observed species richness). Like the bacterial alpha diversity, we found no significant impact of reclamation age and biosolids application ($p > 0.5$) on any of the fungal alpha diversity metrics (Figs. SI 3 and 4).

To examine how reclamation age and biosolids application impacted fungal community composition, we used PCoA ordination. The first two PCoA axes explained over 18 % of the total variation in the fungal community composition (Fig. 5A). Permanova analyses indicate that both reclamation age (psuedoF = 4.69; $p < 0.001$) and biosolids application (psuedoF = 3.45; $p < 0.001$) significantly impacted the fungal community composition. Reclamation age (marginal $R^2 = 0.25$) explained more variation in fungal community composition than the reclamation method (marginal $R^2 = 0.01$) (Fig. 5A). Our analyses of similarity between reclaimed and reference sites indicate that reclamation age (F value = 107.77; $p < 0.001$) and reclamation strategy (F value = 10.313; $p < 0.005$) significantly impacted the Bray-Curtis similarity (Fig. 5B). We found that the fungal Bray-Curtis similarity between reference and reclaimed sites increased with reclamation age. We also noticed that the Bray-Curtis similarity of biosolids reclaimed plots with reference (estimated marginal means = 4.18) was higher than the non-biosolids treated plots (estimated marginal means = 3.16).

To better understand the changes in fungal guild diversity, we examined the impact of reclamation age on the species richness of trophic guilds in fungal communities. Reclamation age impacted the richness of the three guilds in different ways. Specifically, we observed a significant negative linear relationship between pathotroph richness and reclamation age (F value = 6.38; $p < 0.005$, Fig. 6A), a negative non-linear relationship between saprotroph richness and reclamation age (F value = 5.41; $p < 0.01$, Fig. 6B) and a positive non-linear relationship between symbiotroph richness and reclamation age (F value = 32.75; p

< 0.001, Fig. 6C). We also found that reclamation age significantly impacted the litter density (F value = 11.17; $p < 0.001$, Fig. SI 2) and closely followed the trend of saprotrophs. R^2 (Fig. 6) values for all three models indicate that the reclamation age explains a considerable portion of the variation in guild richness. We do not find any significant relationship between any fungal guild richness and the reclamation method.

4. Discussion

This study examined the impact of reclamation age and biosolids application on diversity and ecology at different trophic levels along a reclamation chronosequence. Our results revealed that plant species richness increased with reclamation age, which is consistent with previous studies (Brown and Naeth, 2014; Gagnon et al., 2021; Melnik et al., 2018). Biosolids reclaimed plots showed marginally higher plant richness, but the mean richness along the chronosequence was still lower than nearby natural grasslands (Cumming et al., 2016). The low species richness could be attributed to the seeding of agronomic plant species used for reclamation. Agronomic plants proliferate quickly and hinder the establishment of native species (Hierro et al., 2022). Initial colonization by agronomic species may be beneficial when reclaiming a disturbed site, as it may accelerate soil carbon sequestration (Dignac et al., 2017). However, higher plant diversity is needed for functional redundancy (Eisenhauer et al., 2023), which promotes ecosystem stability and improves community resilience (Biggs et al., 2020). Therefore, management strategies such as prescribed burns could be employed to increase plant diversity (Fynn et al., 2004; Valkó et al., 2016) and improve ecosystem functionality (Huerta et al., 2022) at these reclaimed sites. The prescribed burn will reduce the dominance of the agronomic species and potentially enable colonization and growth of native species (Sample et al., 2022; Williams, 2022).

Results from our bacterial and fungal alpha diversity analyses suggest that the reclamation age and biosolids application did not impact the alpha diversity. We also found that the alpha diversities found at our sites were similar to other studies conducted in different mines (Van Rossum et al., 2016; Vieira et al., 2018). As such, the results indicate that much of the compositional changes in the microbial community (bacterial and fungal communities) are driven by species turnover. Therefore, we take a close look at our beta diversity analyses. Our results demonstrate that reclamation age and reclamation strategy impacted bacterial and fungal beta diversity. We observed that the reference sites clustered separately from the reclaimed sites in PCoA plots; however,
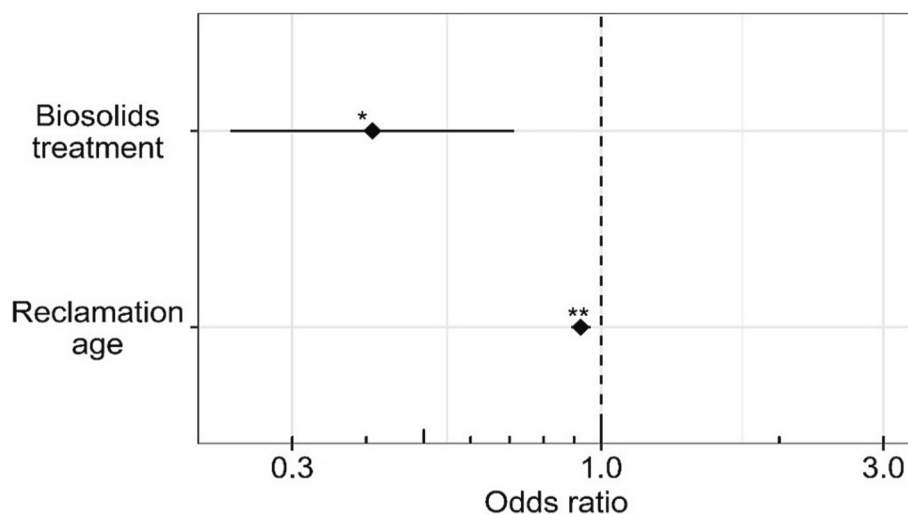

Fig. 4. Odds of the bacterial community assembly (deterministic vs. stochastic) are significantly impacted by reclamation age ($\chi^2 = 17.31$; $p < 0.05$) and biosolids reclamation ($\chi^2 = 9.96$; $p < 0.005$). The odds ratio of <1 indicates that the likelihood of deterministic assembly is lower with biosolids application and reclamation age.
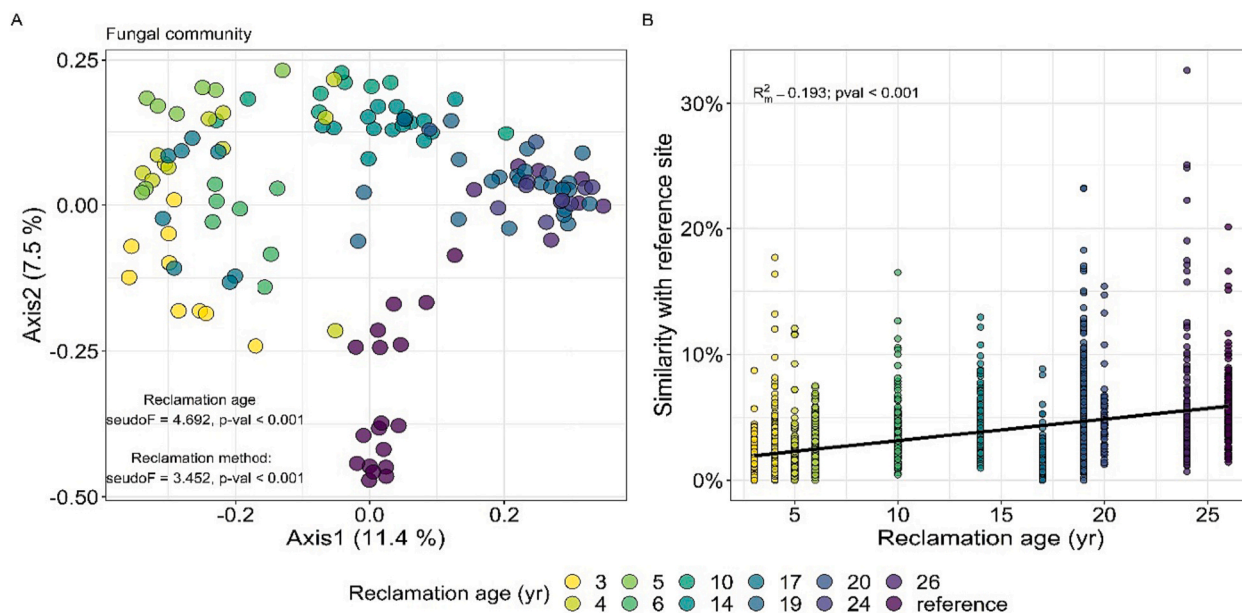
Fig. 5. (A) Principal coordinate analysis of fungal community composition with the first two PCoA axes explains over 18 % of the total variance. Permanova shows that reclamation age and method are significant predictors of the variance associated with the fungal community diversity (B) Bray-Curtis similarity between reclaimed and reference plots increases linearly with reclamation age with the fixed effects explaining a significant amount of the variation.
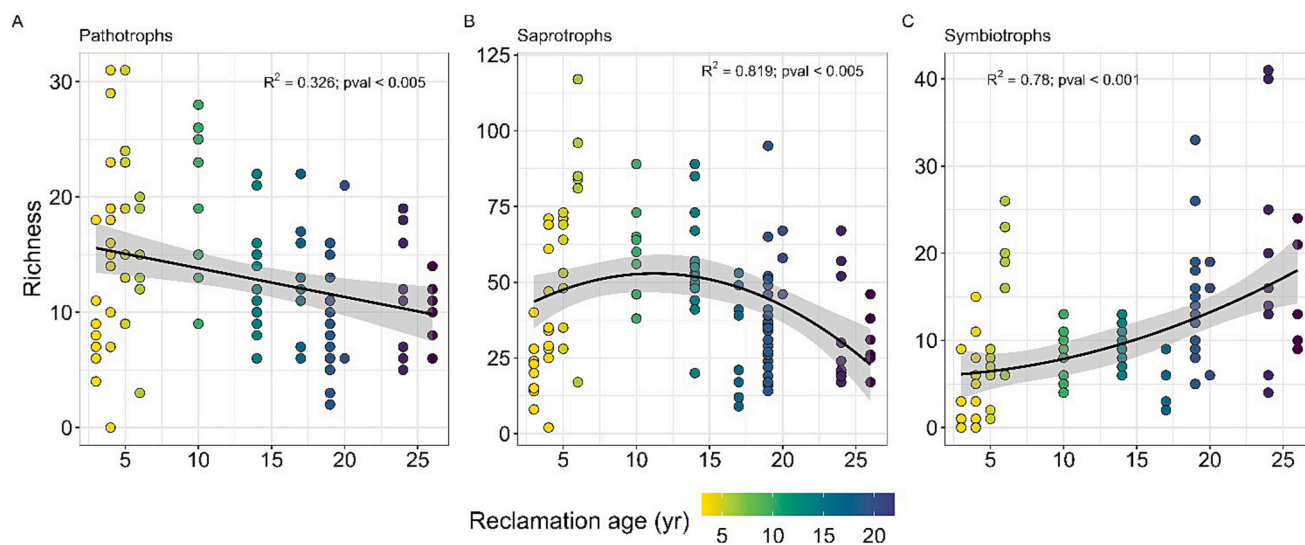

Fig. 6. Reclamation age significantly impacts the richness of (A) Pathotrophs (F value = 6.38; $p < 0.005$); (B) Saprotrophs (F value = 5.41; $p < 0.01$); (C) Symbiotrophs (F value = 32.75; $p < 0.001$).

bacterial and fungal microbial community composition became more similar to the reference sites with increasing reclamation age. The linear mixed-effect model further demonstrates that the older reclaimed sites show higher levels of microbial community similarity to the reference sites than the newly reclaimed sites. This is consistent with previous studies that used phospholipid fatty acid analysis (Dangi et al., 2012), community-level physiological profiles (Chodak et al., 2009), and the older generation pyrosequencing technologies (Li et al., 2014) to investigate microbial community changes in response to mine reclamation. Ngugi et al. (2018) and Liddicoat et al. (2022) also showed that bacterial communities at reclaimed sites become similar to undisturbed sites over time. However, they did not investigate how the fungal communities change. Our results demonstrated that fungal communities also became increasingly similar to adjacent reference sites, but their rate of change was slower than the bacterial communities. A possible

explanation for the slower rate of recovery is that fungal communities from the reclaimed sites can better adapt to harsher conditions (de Vries et al., 2018; Rafiq et al., 2019) through physiological adaptations (Manzoni et al., 2012; Manzoni et al., 2014). As such, they can likely resist changes compared to bacterial communities, thereby displaying a slower reclamation trajectory.

Additionally, we explored how functional potential changes with reclamation age using read-based analysis of metagenomic sequences. Despite the caveats associated with read-based analyses (Quince et al., 2017), they provide a broad-scale understanding of the functional potential of the ecosystem. Therefore, we employ a read-based analysis (Scholz et al., 2012) followed by ordination and quantifying functional similarities using Bray-Curtis similarity (Galand et al., 2018). Our results show that the functional similarity of reclaimed sites with the reference increases with age, implying a gradual recovery of soil functional

potential in reclaimed sites. Given the strong relationship between functional potential and reclamation age (marginal R^2 = 0.639), we suggest that microbial metagenomic analyses may be a powerful tool for predicting and monitoring reclamation progress and guiding management decisions for ecosystem reclamation. For example, functional similarities (Bray-Curtis) can inform where a reclaimed site is along a reclamation trajectory and may allow environmental managers to predict the timeline for ecosystem recovery.

Further, we studied the assembly process of the bacterial community to test how reclamation age and biosolids application impacted bacterial community assembly (stochastic vs deterministic). We found that the likelihood of deterministic bacterial community assembly decreased with reclamation age, implying a weakening of environmental filtering (i.e., environmental factors are no longer the strongest indicator of the bacterial community assembly), and suggesting that stochastic assembly processes such as dispersal and drift become more notable as reclamation age increases (Zhou and Ning, 2017). Importantly, the results show that the environmental properties alone are no longer the dominant factor shaping bacterial community assembly (Zhou and Ning, 2017). We also discovered that biosolid reclamation results in a significantly higher stochastic assembly process. Biosolids are rich in nutrients, and their application possibly decreases the selection pressure exerted by the parent material (mine tailings). Together, these results suggest that improved site characteristics are potentially the outcome of the reduction in selective pressures on the ecosystem. This reduction of selective pressure is significantly associated with reclamation age and biosolids treatment, favoring a more stochastic community assembly process and increasing the colonization of dispersed microorganisms (Doherty et al., 2020).

We did not explore the community assembly process in the fungal community due to uncertainties in the ITS sequence alignment. However, we investigated the impact of the reclamation age on three fungal trophic guilds. Specifically, we examined the species richness of pathotrophs, saprotrophs, and symbiotrophs. While we observed an overall reduction in pathototroph and saprotroph richness, symbiotroph richness increased with reclamation age. The decline in pathotroph richness (Fig. 6A) was associated with a marginal increase in plant species richness (Fig. 1A), potentially indicating improvement in ecosystem-level functioning as phytopathogenic fungi could negatively impact plant performance (Kolaříková et al., 2017). We saw a polynomial relationship between saprotrophs and reclamation age (Fig. 6B), which closely follows the trend in litter cover (Fig. SI.2). We notice that saprotroph richness (Fig. 6B) and litter cover (Fig. SI.2) increases with age and then reaches an inflection point (between 15 and 20 years of reclamation age) followed by a decline. This trend is expected as saprotrophs are essential for litter decomposition (Dighton et al., 1987). Consequently, the high litter cover can likely support the high saprotroph richness, and a decline in litter cover will reduce saprotroph richness. Symbiotroph richness, on the other hand, increased with reclamation age, potentially indicating a more functional ecosystem as arbuscular and ectomycorrhizal fungi constitute the symbiotrophic guild. The importance of arbuscular and ectomycorrhizal association in soil ecosystem is well documented (Liang et al., 2015; Smith et al., 2010; Veresoglou et al., 2012), with positive implications for nutrient cycling (Lin et al., 2017; Averill et al., 2018; Liu et al., 2018) and soil reclamation (Allen and Allen, 1980; Stahl et al., 1988; Zhao and Naeth, 2022).

5. Conclusion

This study demonstrates that the compositional and functional similarity between reclaimed mine sites and undisturbed sites increased with reclamation age. It establishes that diversity metrics such as plant species richness and fungal guilds become favorable with age, indicated by an increase in plant and fungal symbiotrophs richness, and a reduction in pathotrophs diversity. Results from our study revealed that

biosolids-reclaimed sites performed slightly better than non-biosolids reclaimed ones (revealed by higher bacterial and fungal Bray-Curtis similarity), but reclamation age exerted a more substantial and significant influence., indicating time as a critical factor in reclamation. As such, expectations of reclamation timelines need to be tempered accordingly, and efforts to accelerate reclamation through sustainable technologies (e.g., soil amendments and seeding native plant species) must be supported. Furthermore, the strong trend between metagenomics and reclamation age suggests that metagenomics is helpful in monitoring reclamation at the soil microbial level and warrants further analyses. The analysis of community assembly suggests a mechanism for improved reclamation, where reduced selective pressure may improve the ecosystem function.

CRediT authorship contribution statement

Jay Prakash Singh: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Eric M. Bottos:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Jonathan D. Van Hamme:** Writing – review & editing, Writing – original draft, Software, Funding acquisition, Data curation, Conceptualization. **Lauchlan H. Fraser:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could influenced the work reported in this paper.

Data availability

The data and codes available on GitHub "https://github.com/jaymicro/mine_microbiome".

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.170996.

References

Allen, E.B., Allen, M.F., 1980. Natural re-establishment of vesicular-arbuscular mycorrhizae following stripmine reclamation in Wyoming. J. Appl. Ecol. 139–147.
Antonelli, P.M., Fraser, L.H., Gardner, W.C., Broersma, K., Karakatsoulis, J., Phillips, M. E., 2018. Long term carbon sequestration potential of biosolids-amended copper and molybdenum mine tailings following mine site reclamation. Ecol. Eng. 117, 38–49.
Asemaninejad, A., Langley, S., Mackinnon, T., Spiers, G., Beckett, P., Mykytczuk, N., Basiliko, N., 2021. Blended municipal compost and biosolids materials for mine reclamation: long-term field studies to explore metal mobility, soil fertility and microbial communities. Sci. Total Environ. 760, 143393.

Averill, C., Dietze, M.C., Bhatnagar, J.M., 2018. Continental-scale nitrogen pollution is shifting forest mycorrhizal associations and soil carbon stocks. Glob. Chang. Biol. 24, 4544–4553.

Barton, K., 2009. MuMIn: multi-model inference. R package version 1.0.0. http://r-forge.r-project.org/projects/mumin/.

Bates, Douglas, Maechler, Martin, Bolker, Ben, Walker, Steve, 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Beghini, F., McIver, L.J., Blanco-Míguez, A., Dubois, L., Asnicar, F., Maharjan, S., Mailyan, A., Manghi, P., Scholz, M., Thomas, A.M., 2021. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. elife 10, e65088.

Bell, F.G., Donnelly, L.J., 2006. Mining and Its Impact on the Environment. CRC press.

Biggs, C.R., Yeager, L.A., Bolser, D.G., Bonsell, C., Dichiera, A.M., Hou, Z., Keyser, S.R., Khursigara, A.J., Lu, K., Muth, A.F., 2020. Does functional redundancy affect ecological stability and resilience? A review and meta-analysis. Ecosphere 11, e03184.

Brown, R.L., Naeth, M.A., 2014. Woody debris amendment enhances reclamation after oil sands mining in Alberta, Canada. Restor. Ecol. 22, 40–48.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.

Chodak, M., Pietrzykowski, M., Niklińska, M., 2009. Development of microbial properties in a chronosequence of sandy mine soils. Appl. Soil Ecol. 41, 259–268.

Cumming, W., Newman, R., Bradfield, G., Krzic, M., Thompson, D., 2016. Grazing and solar irradiance drive patterns of change in two grassland plant communities in southern British Columbia. J. Arid Environ. 133, 94–101.

Dangi, S.R., Stahl, P.D., Wick, A.F., Ingram, L.J., Buyer, J.S., 2012. Soil microbial community recovery in reclaimed soils on a surface coal mine site. Soil Sci. Soc. Am. J. 76, 915–924.

de Vries, F.T., Griffiths, R.I., Bailey, M., Craig, H., Girlanda, M., Gweon, H.S., Hallin, S., Kaisermann, A., Keith, A.M., Kretzschmar, M., 2018. Soil bacterial networks are less stable under drought than fungal networks. Nat. Commun. 9, 3033.

Dighton, J., Thomas, E., Latter, P., 1987. Interactions between tree roots, mycorrhizas, a saprotrophic fungus and the decomposition of organic substrates in a microcosm. Biol. Fertil. Soils 4, 145–150.

Dignac, M.-F., Derrien, D., Barré, P., Barot, S., Cécillon, L., Chenu, C., Chevallier, T., Freschet, G.T., Garnier, P., Guenet, B., 2017. Increasing soil carbon storage: mechanisms, effects of agricultural practices and proxies. A review. Agron. Sustain. Dev. 37, 1–27.

Doherty, S.J., Barbato, R.A., Grandy, A.S., Thomas, W.K., Monteux, S., Dorrepaal, E., Johansson, M., Ernakovich, J.G., 2020. The transition from stochastic to deterministic bacterial community assembly during permafrost thaw succession. Front. Microbiol. 11, 596589.

Down, C.G., Stocks, J., 1977. Environmental Impact of Mining. John Wiley & Sons, New York.

Eisenhauer, N., Hines, J., Maestre, F.T., Rillig, M.C., 2023. Reconsidering functional redundancy in biodiversity research. npj Biodiversity 2, 9.

Entwistle, J.A., Hursthouse, A.S., Marinho Reis, P.A., Stewart, A.G., 2019. Metalliferous mine dust: human health impacts and the potential determinants of disease in mining communities. Curr. Pollut. Rep. 5, 67–83.

FAO-UNESCO, 1975. FAO-UNESCO. Soil Map of the World, 1:5,000,000, vol I. FAO-UNESCO, Paris.

Farjana, S.H., Huda, N., Mahmud, M.P., Saidur, R., 2019. A review on the impact of mining and mineral processing industries through life cycle assessment. J. Clean. Prod. 231, 1200–1217.

Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15, 579–590.

Fraser, L.H., Harrower, W.L., Garris, H.W., Davidson, S., Hebert, P.D.N., Howie, R., Moody, A., Polster, D., Schmitz, O.J., Sinclair, A.R.E., Starzomski, B.M., Sullivan, T.P., Turkington, R., Wilson, D., 2015. A call for applying trophic structure in ecological restoration. Restor. Ecol. 23 (5), 503–507.

Fynn, R.W., Morris, C.D., Edwards, T.J., 2004. Effect of burning and mowing on grass and forb diversity in a long-term grassland experiment. Appl. Veg. Sci. 7, 1–10.

Gagnon, A., Ploughe, L.W., Harris, M.P., Gardner, W.C., Pypker, T., Fraser, L.H., 2021. Grassland reclamation of a copper mine tailings facility: long-term effects of biosolids on plant community responses. Appl. Veg. Sci. 24, e12612.

Galand, P.E., Pereira, O., Hochart, C., Auguet, J.C., Debroas, D., 2018. A strong link between marine microbial community composition and function challenges the idea of functional redundancy. ISME J. 12, 2470–2478.

Garris, H.W., Baldwin, S.A., Van Hamme, J.D., Gardner, W.C., Fraser, L.H., 2016. Genomics to assist mine reclamation: a review. Restor. Ecol. 24, 165–173.

Gorzelak, M., McAmmond, B.M., Van Hamme, J.D., Birnbaum, C., Thomsen, C., Hart, M., 2020. Soil microbial communities in long-term soil storage for sand mine reclamation. Ecol. Restor. 38, 13–23.

Government of Canada, 2019. Canadian climate normals 1981–2010 station data. Available at: https://climate.weather.gc.ca/climate_normals/results_1981_2010_e.html?searchType=stnName&txtStationName=Lornex&searchMethod=contains&txtCentralLatMin=0&txtCentralLatSec=0&txtCentralLongMin=0&txtCentralLongSec=0&stnID=998&dispBack=1.

Guo, A., Zhao, Z., Zhang, P., Yang, Q., Li, Y., Wang, G., 2019. Linkage between soil nutrient and microbial characteristic in an opencast mine, China. Sci. Total Environ. 671, 905–913.

Hadzi, G.Y., Ayoko, G.A., Essumang, D.K., Osae, S.K., 2019. Contamination impact and human health risk assessment of heavy metals in surface soils from selected major mining areas in Ghana. Environ. Geochem. Health 41, 2821–2843.

Harris, J., 2009. Soil microbial communities and restoration ecology: facilitators or followers? Science 325, 573–574.

Hierro, J.L., Eren, Ö., Čuda, J., Meyerson, L.A., 2022. Evolution of increased competitive ability may explain dominance of introduced species in ruderal communities. Ecol. Monogr. 92, e1524.

Hottenstein, J.D., Neilson, J.W., Gil-Loaiza, J., Root, R.A., White, S.A., Chorover, J., Maier, R.M., 2019. Soil microbiome dynamics during pyritic mine tailing phytostabilization: understanding microbial bioindicators of soil acidification. Front. Microbiol. 10, 1211.

Huerta, S., Marcos, E., Fernández-García, V., Calvo, L., 2022. Short-term effects of burn severity on ecosystem multifunctionality in the northwest Iberian Peninsula. Sci. Total Environ. 844, 157193.

Jackman, S., Tahk, A., Zeileis, A., Maimone, C., Fearon, J., Meers, Z., Jackman, M.S., Imports, M., 2015. Package 'pscl'. Political Science Computational Laboratory, vol. 18.

Kahhat, R., Parodi, E., Larrea-Gallegos, G., Mesta, C., Vázquez-Rowe, I., 2019. Environmental impacts of the life cycle of alluvial gold mining in the Peruvian Amazon rainforest. Sci. Total Environ. 662, 940–951.

Kolaříková, Z., Kohout, P., Krüger, C., Janoušková, M., Mrnka, L., Rydlová, J., 2017. Root-associated fungal communities along a primary succession on a mine spoil: distinct ecological guilds assemble differently. Soil Biol. Biochem. 113, 143–152.

Kuznetsova, A., Brockhoff, P.B., RHB, Christensen, 2017. lmerTest package: tests in linear mixed effects models. J. Stat. Softw. 82 (13), 1–26.

Legendre, P., Legendre, L., 2012. Numerical Ecology. Elsevier.

Li, Y., Wen, H., Chen, L., Yin, T., 2014. Succession of bacterial community structure and diversity in soil along a chronosequence of reclamation and re-vegetation on coal mine spoils in China. PLoS One 9, e115024.

Li, J., Xin, Z., Yan, J., Li, H., Chen, J., Ding, G., 2018. Physicochemical and microbiological assessment of soil quality on a chronosequence of a mine reclamation site. Eur. J. Soil Sci. 69, 1056–1067.

Liang, M., Liu, X., Etienne, R.S., Huang, F., Wang, Y., Yu, S., 2015. Arbuscular mycorrhizal fungi counteract the Janzen-Connell effect of soil pathogens. Ecology 96, 562–574.

Liddicoat, C., Krauss, S.L., Bissett, A., Borrett, R.J., Ducki, L.C., Peddle, S.D., Bullock, P., Dobrowolski, M.P., Grigg, A., Tibbett, M., 2022. Next generation restoration metrics: using soil eDNA bacterial community data to measure trajectories towards rehabilitation targets. J. Environ. Manag. 310, 114748.

Lin, G., McCormack, M.L., Ma, C., Guo, D., 2017. Similar belowground carbon cycling dynamics but contrasting modes of nitrogen cycling between arbuscular mycorrhizal and ectomycorrhizal forests. New Phytol. 213, 1440–1451.

Liu, X., Burslem, D.F., Taylor, J.D., Taylor, A.F., Khoo, E., Majalap-Lee, N., Helgason, T., Johnson, D., 2018. Partitioning of soil phosphorus among arbuscular and ectomycorrhizal trees in tropical and subtropical forests. Ecol. Lett. 21, 713–723.

Ma, X., Qu, H., Liao, S., Ji, Y., Li, J., Chao, L., Liu, H., Bao, Y., 2023. Changes in assembly processes and differential responses of soil microbial communities during mining disturbance in mining reclamation and surrounding grassland. CATENA 231, 107332.

Malik, A., Scullion, J., 1998. Soil development on restored opencast coal sites with particular reference to organic matter and aggregate stability. Soil Use Manag. 14, 234–239.

Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930–938.

Manzoni, S., Schaeffer, S., Katul, G., Porporato, A., Schimel, J., 2014. A theoretical analysis of microbial eco-physiological and diffusion limitations to carbon cycling in drying soils. Soil Biol. Biochem. 73, 69–83.

Melnik, K., Landhäusser, S.M., Devito, K., 2018. Role of microtopography in the expression of soil propagule banks on reclamation sites. Restor. Ecol. 26, S200–S210.

Mitter, E.K., de Freitas, J.R., Germida, J.J., 2017. Bacterial root microbiome of plants growing in oil sands reclamation covers. Front. Microbiol. 8, 849.

Moreira-Grez, B., Muñoz-Rojas, M., Kariman, K., Storer, P., O'Donnell, A.G., Kumaresan, D., Whiteley, A.S., 2019. Reconditioning degraded mine site soils with exogenous soil microbes: plant fitness and soil microbiome outcomes. Front. Microbiol. 10, 1617.

Ngugi, M.R., Dennis, P.G., Neldner, V.J., Doley, D., Fechner, N., McElnea, A., 2018. Open-cut mining impacts on soil abiotic and bacterial community properties as shown by restoration chronosequence. Restor. Ecol. 26, 839–850.

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248.

Ning, D., Yuan, M., Wu, L., Zhang, Y., Guo, X., Zhou, X., Yang, Y., Arkin, A.P., Firestone, M.K., Zhou, J., 2020. A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. Nat. Commun. 11, 4717.

Okansen, J., Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., 2020. vegan: Community Ecology Package. R Package Version 2.5–7.

Paradis, E., Schliep, K., 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35, 526–528.

Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., Crecchio, C., 2015. Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biol. Fertil. Soils 51, 403–415.

Plaza, C., Courtier-Murias, D., Fernández, J.M., Polo, A., Simpson, A.J., 2013. Physical, chemical, and biochemical mechanisms of soil organic matter stabilization under conservation tillage systems: a central role for microbes and microbial by-products in C sequestration. Soil Biol. Biochem. 57, 124–134.

Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS One 5, e9490.

Quince, C., Walker, A.W., Simpson, J.T., Loman, N.J., Segata, N., 2017. Shotgun metagenomics, from sampling to analysis. Nat. Biotechnol. 35, 833–844.

R Core Team, 2022. R: a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project. org/.

Rafiq, M., Hassan, N., Rehman, M., Hasan, F., 2019. Adaptation mechanisms and applications of psychrophilic fungi. In: Fungi in Extreme Environments: Ecological Role and Biotechnological Significance, pp. 157–174.

Rajkumar, M., Sandhya, S., Prasad, M., Freitas, H., 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol. Adv. 30, 1562–1574.

Roy, J., Reichel, R., Brüggemann, N., Rillig, M.C., 2023. Functional, not taxonomic, composition of soil fungi reestablishes to pre-mining initial state after 52 years of recultivation. Microb. Ecol. 86, 213–223.

RStudio Team, 2020. RStudio: integrated development for R, RStudio, PBC, Boston, MA. URL. http://www.rstudio.com/.

Rybicka, E.H., 1996. Impact of mining and metallurgical industries on the environment in Poland. Appl. Geochem. 11, 3–9.

Sample, M., Thode, A.E., Peterson, C., Gallagher, M.R., Flatley, W., Friggens, M., Evans, A., Loehman, R., Hedwall, S., Brandt, L., Janowiak, M., Swanston, C., 2022. Adaptation strategies and approaches for managing fire in a changing climate. Climate 10 (4), 58.

Scholz, M.B., Lo, C.-C., Chain, P.S., 2012. Next generation sequencing and bioinformatic bottlenecks: the current state of metagenomic data analysis. Curr. Opin. Biotechnol. 23, 9–15.

Sengupta, M., 2021. Environmental Impacts of Mining: Monitoring, Restoration and Control. CRC Press.

Smith, S.E., Facelli, E., Pope, S., Andrew Smith, F., 2010. Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. Plant Soil 326, 3–20.

Sokol, N.W., Slessarev, E., Marschmann, G.L., Nicolas, A., Blazewicz, S.J., Brodie, E.L., Firestone, M.K., Foley, M.M., Hestrin, R., Hungate, B.A., 2022. Life and death in the soil microbiome: how ecological processes influence biogeochemistry. Nat. Rev. Microbiol. 20, 415–430.

Stahl, P.D., Williams, S., Christensen, M., 1988. Efficacy of native vesicular-arbuscular mycorrhizal fungi after severe soil disturbance. New Phytol. 110, 347–354.

Stegen, J.C., Lin, X., Konopka, A.E., Fredrickson, J.K., 2012. Stochastic and deterministic assembly processes in subsurface microbial communities. ISME J. 6, 1653–1664.

Teck, 2013. accessed from. https://www.teck.com/news/stories/2013/biosolids-assist -reclamation-at-highland-valley-copper. on 13th Feb 2024.

Thornton, I., 1996. Impacts of mining on the environment; some local, regional and global issues. Appl. Geochem. 11, 355–361.

Valkó, O., Deák, B., Magura, T., Török, P., Kelemen, A., Tóth, K., Horváth, R., Nagy, D.D., Debnár, Z., Zsigrai, G., 2016. Supporting biodiversity by prescribed burning in grasslands—a multi-taxa approach. Sci. Total Environ. 572, 1377–1384.

Van Rossum, T., Pylatuk, M.M., Osachoff, H.L., Griffiths, E.J., Lo, R., Quach, M., Palmer, R., Lower, N., Brinkman, F.S., Kennedy, C.J., 2016. Microbiome analysis across a natural copper gradient at a proposed northern Canadian mine site. Front. Environ. Sci. 3, 84.

Veresoglou, S.D., Chen, B., Rillig, M.C., 2012. Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biol. Biochem. 46, 53–62.

Vieira, C.K., dos Anjos Borges, L.G., Marconatto, L., Giongo, A., Stürmer, S.L., 2018. Microbiome of a revegetated iron-mining site and pristine ecosystems from the Brazilian Cerrado. Appl. Soil Ecol. 131, 55–65.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D.A., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., 2019. Welcome to the Tidyverse. J. Open Source Softw. 4, 1686.

Williams, B., 2022. Utilizing Indigenous Prescribed Burning Methods to Reclaim a Tailings Storage Facility in the Southern Interior of British Columbia. Thompson Rivers University.

Xiao, E., Ning, Z., Xiao, T., Sun, W., Jiang, S., 2021. Soil bacterial community functions and distribution after mining disturbance. Soil Biol. Biochem. 157, 108232.

Zhao, Y., Naeth, M.A., 2022. Soil amendment with a humic substance and arbuscular mycorrhizal Fungi enhance coal mine reclamation. Sci. Total Environ. 823, 153696.

Zhong, X., Chen, Z., Ding, K., Liu, W.-S., Baker, A.J., Fei, Y.-H., He, H., Wang, Y., Jin, C., Wang, S., 2023. Heavy metal contamination affects the core microbiome and assembly processes in metal mine soils across Eastern China. J. Hazard. Mater. 443, 130241.

Zhou, J., Ning, D., 2017. Stochastic community assembly: does it matter in microbial ecology? Microbiol. Mol. Biol. Rev. 81, e00002-00017.

Zhu, X., Gong, W., Li, W., Bai, X., Zhang, C., 2022. Reclamation of waste coal gangue activated by *Stenotrophomonas maltophilia* for mine soil improvement: solubilizing behavior of bacteria on nutrient elements. J. Environ. Manag. 320, 115865.