THE USE OF BIOSOLIDS TO RESTORE NATIVE PLANT COMMUNITIES IN SEMI-ARID GRASSLANDS

by

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Abstract

The grasslands of British Columbia are ecologically and economically valuable, but human activities, climate change and environmental disturbances are major pressures causing grassland degradation. Re-establishing native plant communities is critical for restoring disturbed grasslands or post-mine influenced sites. One of the difficulties is that disturbed grasslands, particularly those disturbed by mining activity, have poor quality soil that is not conducive to plant growth. Soil amendments can provide nutrients that help plants grow. As a soil amendment, biosolids, treated solids recovered from municipal wastewater, can improve soil capacities of degraded land and provide the nutrients for plant growth. The objectives of this study were 1) to determine an appropriate rate of biosolids application to promote the colonization of native plants, 2) to test whether additional seeding of cover crops and successional plants in the following year was effective in promoting colonization of plants, and 3) to determine the effects of the biosolids and sowing treatments on soil properties. In 2021, a field study was devised that tested four biosolids application rates (0, 125, 250, and 375 dry Mg/ha) with a seed mix in all test plots in the first year and only half of the test plots in the second year, at two sites selected from the southern interior grasslands of British Columbia. One site was located in a suburban area with four replicates per experimental combination; the other site was located in a mining area with six replicates per experimental combination. Due to differences in environmental conditions between the two sites, the seed mix involved a cover crop and five to four native successional species respectively. This study demonstrated that biosolids significantly increased the productivity and diversity of the plant community, as well as the soil properties. However, higher rates of biosolids did not lead to significantly higher plant productivity and diversity than the lower rates. Results of this study suggest that biosolids may significantly help in restoring sustainable grassland ecosystems in disturbed grasslands and mine sites.

Keywords: cover crops, soil amendments, biosolids, semi-arid grassland, successional species, invasive plant, reseed, mine

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Acknowledgements

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Chapter 1: Introduction

The Importance of British Columbia Grassland

As one of Canada's most endangered ecosystems, grasslands are relatively scarce in British Columbia (BC) and make up less than 1% of the total land coverage in the province (Tisdale, 1947; Gayton, 2004; Grasslands Conservation Council of BC, 2017). Grasslands of the southern interior BC are semi-arid ecosystems with dry and hot summers (Iverson, 2004). Grasslands provide habitats for many rare plants and endangered wildlife; grasslands also provide important ecological services (ES) such as carbon sequestration and carbon storage, hydrological control, and cultural significance (Bengtsson et al., 2019; Iverson, 2004; Hanisch et al., 2020). Human activities such as livestock overgrazing, and urbanization are some of the major causes of loss to the extent and biodiversity of grasslands (Gayton, 2004; Iverson, 2004; Macdougall, 2008). Degradation of grasslands is the reduction in land and/or soil productivity, which is normally due to human activities such as mining (Fayiah et al., 2020; Gibbs & Salmon, 2015). Degraded grasslands can be susceptible to plant invasions, causing a conversion or alteration of ecosystem functions and states (Macdougall, 2008; Mbaabu et al., 2020).

Conserving grasslands can help maintain the biodiversity and ES that are in high demand by society (Bengtsson et al., 2019). With a relatively lower soil erosion rate and higher productivity, well-managed grasslands can be recognized for high species richness and abundance of plants and animals (Habel et al., 2013; Bengtsson et al., 2019). One important ES of grasslands is the genetic library (Hallikma et al., 2023), and semi-arid grasslands have particular biological diversity and endemic species (Sala et al., 2017). The stability, function, and sustainability of ecosystems are linked to the diversity of plant and animal species, and the genetic library is one of the key components of agricultural and medical evolution (Sala et al., 2017; Sunderlin et al., 2005). High biodiversity can contribute to the development and stability of ecosystems, by improving ES as regulation of water and climate, and pollination (Hanisch et al., 2020). Therefore, the extent and biodiversity of grassland ecosystems should be protected and restored for vital ES.

According to a provincial report by Wilson in 2009, 1.03 billion CAD per year is the estimated total value of grassland ecosystem goods and services based on the coverage in

BC. Major ecosystem services of grasslands are water regulation, erosion control, soil formation, waste treatment, biological control, recreation, pollination, and carbon storage (Wilson, 2009; Grasslands Conservation Council of BC, 2017). Based on an estimation of global grassland carbon storage potential (White et al., 2000), there is the suggested potential for 74 million to 222 million tonnes of carbon to be stored in BC's grasslands (Wilson, 2009). Based on the avoided carbon emissions into the atmosphere, the Ontario green belt study estimated the value of carbon storage per hectare to be $28.46 per year per hectare (Wilson, 2009). If this estimate is used in the context of BC, BC grasslands could be worth 28 million CAD per year for carbon storage (Wilson, 2009). The value estimations of grasslands emphasize the negative impact of grassland degradation on the economy. Economic losses regarding grassland ecosystems may be continuously enlarged in case of not maintaining and restoring grasslands.

Mine Reclamation & Restoration

Canada is one of the top mining countries in the world and one of the largest producers of minerals and metals (Mining Association of Canada, 2020). In 2019, the minerals sector contributed 5% to Canada's total nominal gross domestic product (GDP) and employed more than 39,200 people, as well as provided employment for more than 16,500 Indigenous people (Mining Association of Canada, 2020). Although economically beneficial, mining can be destructive to the environmental, and surrounding terrestrial and aquatic ecosystems. Towards Sustainable Mining (TSM) assists mining companies to minimize ecological impacts (Mining Association of Canada, 2020). The industry association has established environmental standards to minimize the environmental impact of mining activities. One of the fundamental guiding principles of TSM is to minimize the environmental impact after closure, which involves reclamation of impacted sites (Mining Association of Canada, 2020). Mining reclamation requires a thoughtful and feasible environmental strategy, in coordination with the affected Indigenous peoples and local communities.

The focus of reclamation is to achieve a self-sustaining vegetative cover that protects the site from erosion, and revegetation is a component of reclamation and may require the establishment of only one or a few species (SER, 2004; Gerwing et al., 2022; Xiu et al., 2020). Agronomic monocultures are often vulnerable to disturbance associated with climate change, and often provide limited ecosystem services (Niether et al., 2020; Erskine et al., 2006). Furthermore, loss of species (through monoculture) leads to reductions in functional redundancy (Rosenfeld, 2002), which makes reclamations prone to failure. Such failures in the restoration of mines can lead to the appropriation of expensive insurance (bonds) paid upfront by the mining industry (Faure & Grimeaud, 2000). Historically, the Crown is risk-averse and has been reluctant to grant relinquishment to mining companies, such that bonds tend to be retained but in a diminished amount (Faure & Grimeaud, 2000). The government may consider placing additional charges on responsible parties in order for them to achieve appropriate environmental responsibility, thereby mitigating environmental impacts (Faure & Grimeaud, 2000; Smart et al., 2016).

Ecosystem restoration is the process of reversing degradation and returning ecosystems to their pre-disturbed stage for improved ES and a recovered biodiversity (SER, 2004; Gerwing et al., 2022). Unlike the reclamation, ecological restoration is not just about re-establishing vegetation (Cao, 2008; Fagan, 2008; Simmers, 2010; Fraser et al., 2015; SER, 2004). Some common practices are restoring biodiversity and protecting flora and fauna, such as native species, that benefit ecological restoration (Gann et al., 2022). Recreating the exact same physical conditions and the same species assemblages as before is extremely difficult because of large-scale disturbances, such as mining, that alter topography and soil physicochemical properties and disrupt ecosystem integrity (Albertson et al., 2011). Artificial seeding is necessary to stimulate germination and colonization of plants because natural spreading of speeds has a small spatial extent (Kraft & Ackerly, 2014). On post-mine sites with poor soil conditions and a large disturbed area, even if a few seeds enter from outside, the infrequent germination and low growth rate may result in a relatively slow establishment of native plant species (Eriksson & Ehrlen, 1992). Another difficulty is that the landscape has poor soil-forming materials, such as geological materials and nutrients for subsequent ecosystem development (Herath et al., 2009; Smart et al., 2016).

Restoring an Ecosystem

Ecological restoration is a process of guiding an ecosystem to a target ecosystem that is expected to be mature and stable, and accelerating or skipping one or more successional stages is one way of assisting the process (Bossuyt & Hermy, 2003). A traditional restoration goal is to have more growth of native species, while limiting the growth of invasive species (Hess et al., 2019). Having a rapid establishment of plants through seeding is beneficial to rebuild soil, control erosion, and to improve a degraded site's visual appearance (Burton et al., 2006). But at the same time, it is important to prevent the establishment of non-native and invasive plant species. Invasive species can rapidly adapt to new environments, sequester nutrients, grow, and reproduce (Montesinos, 2022).

Revegetation of mine tailings through natural succession is usually extremely slow (Tardif et al., 2019; Gagnon et al., 2020; Santini et al., 2018), because building soil structure, accumulating and improving soil nutrients can take decades and centuries in natural recovery (Hossner & Hons, 1992). Theoretically, these processes can be accelerated by artificial means such as soil amendments and seeding of selected plant species. Selecting plant species based on local climate and soil conditions and sowing seeds of selected species can help speed revegetation. The advantages of using grasses for soil restoration are rapid growth, high biomass (Cestone et al., 2010), better ecological restoration functions, and soil conservation (Rezvani et al., 2012).

Impacts of climate change need to be considered in ecological restoration (Maco et al., 2018; Herrick et al., 2013), because ecology is about the distribution and abundance of organisms in a certain space over a period of time, usually long-term (Ehrlén & Morris, 2015). Extreme climate events can cause abrupt changes in ecological systems, and even these changes can be irreversible (Malhi et al., 2020). For example, drought-induced wildfires can cause ecosystem shifts (from forest to grassland), and possibly endanger or exterminate endemic species (Hill & Field, 2021; Malhi et al., 2020). This has been proven to cause adverse effects on ecosystems that would be detrimental to native plant communities, and accelerate invasions (Malhi et al., 2020; Suarez et al., 2004). In addition, some native species that are more sensitive to environmental changes are more vulnerable to extreme climates, which may lead to extinction or recolonization (Jackson et al., 2009). Thus, resistance to climate change should be a consideration in the selection of plant species for ecological restoration.

Native Successional Species & Reseeding Operation

Native early-successional plant species can rapidly colonize on disturbed soils, thereby reducing erosion and increasing biomass (Tilley et al., 2022). Early successional plants have traits such as higher photosynthetic and growth rates, nutrient uptake and use efficiency, and tolerance to nutrient deficit (Favaretto et al., 2011; Yang & Kim, 2017; Joshi & Garkoti, 2023). Ecosystems at early successional stages tend to have shorter plant lifetimes, higher net primary productivity, lower stability and diversity compared to ecosystems in late successional stages (Favaretto et al., 2011; Pollastrini et al., 2022). Therefore, seeding a mix of early and late successional species together may help establish and improve the stability of the plant community in the short term.

Environmental restoration needs to consider the impacts of climate change, which can lead to low plant survival rate and diversity (Harrison, 2020). Extreme weather conditions, likely caused by climate change, such as high rainfall events and droughts, occur more frequently today (Gupta et al., 2022), and such changes are a challenge to environmental restoration efforts and native ecosystems. As a traditional practice, reseeding is practiced to increase plant establishment success while reducing the negative consequences of extreme climate events (Mocanu & Hermenean, 2009; Runólfsson, 1987; Hubbard, 1975; Beukes & Cowling, 2003; Haussmann et al., 2019). The seeding density of native successional species should be determined based on the nature of the site and targets of the ecological restoration (Dobb & Burton, 2013; Burton et al., 2006). The colonization of native plants may take time due to site conditions (Burton et al., 2006), therefore the reseeding practice can be a remedy, by promoting a higher rate of survival and coverage of native plant species (Kumawat et al., 2019; Abdelsalam et al., 2017). Reseeding can rejuvenate pastures and other degraded sites by increasing plant productivity and restoring ecosystem services (Eastburn et al., 2018; Kumawat et al., 2019; Dobb & Burton, 2013). When the productivity or diversity of the established plant community is lower than expected, reseeding beneficial plants may be effective in increasing the plant productivity and the richness of seeded plant species in the plant community.

Soil Amendments Effects on Plant Growth

Soil amendments can support plant growth and development on semi-arid disturbed lands by adding organic and inorganic nutrients to the soil, and improving soil organic matter content, and water holding capacity (Soria et al., 2021; Clements & Bihn, 2019; Ohsowski et al., 2012). But soil amendments also promote the growth of non-native species due to their ability to sequester nutrients and take advantage of increased nutrient levels (Suding et al., 2005). Biosolids are treated municipal sewage solids that are often used as a soil amendment (Canadian Council of the Ministers of Environment, 2012; McCarthy & Loyo-Rosales, 2015; Ploughe et al., 2021), because biosolids can improve carbon storage capacity and plant productivity of disturbed lands (Robinson et al., 2012, Antonelli et al., 2018, Gardner et al., 2012). Biosolids can provide major plant nutrients and mineralizable nitrogen to degraded lands (Rigby et al., 2016; Sullivan 2022; Ippolito et al., 2021). Biosolids enrich the soil substrate with phosphorus and zinc, and also increase infiltration rate and water-holding capacity of the soil by increasing soil organic matter content, which reduces the soil bulk density (Brown & Chaney, 2016; Khaleel et al., 1981; Larney & Angers, 2012; Wallace et al.,2009).

Biosolids contain organic matter and are therefore considered an important soil amendment to replenish or maintain soil organic matter content (Nicholson et al. 2018; Lu et al, 2012). Soil organic matter (SOM) consists of plant residues, animal manure, and microorganisms in various stages of decomposition, and SOM improves soil quality by improving soil structure and water-holding capacity, and increasing microbial activity (Lehmann & Kleber, 2015; Nicholson et al. 2018; Celestina et al. 2019). In addition, SOM helps to bind mineral particles in the soil into aggregates, thereby improving soil aggregate stability and erosion resistance (Nicholson et al., 2018; He et al., 2021).

Biosolids are safe for environments and people as long as biosolids are properly treated and managed in accordance with regulations and standards (Boczek et al., 2023). In BC, land application of biosolids is regulated by the BC Ministry of Environment and Climate Change Strategy (MOECCS) under the Organic Matter Recycling Regulation (OMRR). The OMRR governs the production, distribution, storage and land application of biosolids and compost in the province. It requires biosolids to meet strict quality standards; biosolids can be classified as Class A and Class B based on treatment processes, pathogens levels and metals concentrations (MOECCS, 2002 & 2022; Canadian Food Inspection Agency, 1997), as listed in Table 1-1.

Table 1-1. The limits for metals and pathogen (expressed in µg/g dry weight) in class A and class B biosolids in British Columbia as defined by the BC Organic Matter Recycling Regulation, and class A biosolids limits for trace elements were determined as the 'maximum acceptable concentration of a metal based on application rate of 4400 kg/ha per year' by the Trade Memorandum T-4-93 Standards for Metals in Fertilizers and Supplements (MOECCS, 2002; Canadian Food Inspection Agency, 1997).

Parameter	Class A Biosolids	Class B biosolids
Pathogens (MNP per gram of total dry solids)	< 1 000	< 2 000 000
Arsenic (As)	75	75
Cadmium (Cd)	20	20
Chromium (Cr)	1060 [a]	1060
Cobalt (Co)	151	150
Copper (Cu)	757	2200
Lead (Pb)	500 [b]	500
Mercury (Hg)	5	15
Molybdenum (Mo)	20	20
Nickel (Ni)	181	180
Selenium (Se)	14	14
Zinc (Zn)	1868	1850

[a] In Table 1 of Appendix A of Organic Matter Recycling Regulation Project Update (MOECCS, 2022), the current OMRR standard of Cr is defined as '-'.

[b] In Table 1 of Appendix A of Organic Matter Recycling Regulation Project Update (MOECCS, 2022), the current OMRR standard of Pb is 505 µg/g.

Appropriate soil amendments may contribute to a rapid establishment of vegetation. For example, woodchips (chipped/ground woody material) are a soil amendment relatively low in readily available plant nutrients, but provide organic material that decomposes slowly and is less likely to blow away in windy, dry conditions (Throop & Belnap, 2019; Cheng, 1987). Adding wood chips to the soil lowers the pH of highly alkaline soils, helps add hard-to-degrade carbon, and increases water infiltration and aeration (Yuan et al., 2020).

Research Goals

Biosolids are used in small quantities for multiple applications or years in agricultural uses (Pierce et al., 1998; Johnson et al., 2023; US Environmental Protection Agency, 2000), but are often used in large quantities at one time for long-term land restoration or reclamation purposes (Pepper et al., 2013; Lu et al., 2012; Valdecantos & Fuentes, 2018). For instance, a meta-analysis mentioned that the most field studies had biosolids application at levels below 100 Mg/ha (megagrams per hectare), and relatively less studies applied biosolids at the levels over 100 and did not exceed 404 Mg/ha (Ploughe et al., 2021). In this study, biosolids, the main soil amendment, was applied at different rates between 125 to 375 Mg/ha to disturbed grasslands and mining soils to determine if biosolids can effectively promote the establishment of native plant communities and its impact on plant community diversity, productivity, and soil properties. Therefore, this study includes field experiments to quantify the effects of one-time non-agronomic application of biosolids on plant communities in natural settings on an overburden and a disturbed land in semi-arid grasslands.

The research project applies ecological theory and restoration practices to test if a reseed practice may increase plant diversity and productivity on mine-influenced subsoil. More specifically, the study examined the effects of biosolids and their different application rates on plant community productivity and diversity. The study also compared the differential effects of single and multiple seeding of native successional plants on plant communities. By analyzing the effects of different treatments and time on plant communities, we expect to gain insight into the effects of these two treatments on plant community structure and species diversity, and to reveal the influence of the time factor on experimental results. In addition, the study examined the effects of interactions between the biosolids application rates and sowing treatments on plant communities, as well as the effects on soil properties. This study was to determine the potential effects of biosolids application and seeding with native successional plants on plant communities and soils.

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Chapter 2: Testing the Efficacy of Biosolids and Repeat Seeding Practice to Promote Plant Species Community Restoration

Introduction

Biosolids are organic materials that have been treated from municipal wastewater and can be used to improve soil properties and vegetative cover on disturbed grasslands and mine sites (Gardner et al., 2012; Wijesekara et al., 2016; Brown & Henry, 2001). Biosolids also contain macronutrients and micronutrients that can improve soil fertility and physical properties (Gardner et al., 2012; Kim & Owens, 2010). Using biosolids as a fertilizer in mine sites is particularly effective since such soils usually have nutrient deficiencies and imbalances (Brown & Henry, 2001). Biosolids enhance soil quality and fertility by increasing soil organic matter and nutrients such as carbon and nitrogen, making them effective for building soil (Gardner et al., 2012; Kim & Owens, 2010). Biosolids in most field studies were applied below 100 Mg/ha, and biosolids in less field studies were applied from 100 to 404 Mg/ha (Ploughe et al., 2021), as the biosolids are often used in large quantities at one time for long-term land restoration and reclamation purposes (Pepper et al., 2013; Lu et al., 2012; Valdecantos & Fuentes, 2018).

Plant diversity is critical to plant communities, enhancing primary productivity, invasion resistance and resilience (Nighswander et al., 2021). Therefore, seed mixes containing diverse plants are key to restoring diverse communities (Barr et al., 2017). Early successional species, often referred to as pioneer species, are the first species to colonize disturbed or degraded ecosystems (Favaretto et al., 2011; Tilley et al., 2022). Often hardy and fast-growing, these species can thrive in harsh climates where other species cannot, helping to stabilize the soil and creating conditions for other species to grow and live (Tilley et al., 2022; Déri et al., 2011). Early successional species can alter soil biology and nutrient cycling in ways that benefit later successional species (Tilley et al., 2022). The use of seeds of early successional species is particularly effective in mine sites restoration (Jean & Khasa, 2022). Restoring plant communities on disturbed soils may require extra support, such as cover crops that can quickly cover bare ground. Cover crops protect and enhance the soil, and they are mainly planted to enhance soil health, control pests and diseases, and decrease weed growth (Sheldon et al., 2021).

Late successional species are species that appear late in the successional process, are usually slower-growing and longer-lived, and often require the improved soil conditions created by earlier successional species or cover crops to survive (Law et al., 2023). Late successional plants can also contribute to vegetation succession by improving soil conditions, maintaining native biodiversity, and maintaining some important ecosystem services such as carbon storage (Osorio-Salomón et al., 2021; Caspersen & Pacala, 2001). Successful establishment of long-lived, late-successional plant species can suppress invasive plants (Middleton et al., 2010).

This two-year-study examines the effects of one-time non-agronomic biosolids application on plant community productivity (biomass) and diversity (species richness) of semi-arid grasslands in south interior of BC.

Objective 1 - To investigate the effects of soil and sowing treatments and their interactions on total plant productivity and diversity of the plant community, native plants and other plant functional groups, where the soil treatments are the application of biosolids at different rates and the sowing treatments are sowing native successional species with and without a reseeding practice.

Objective 2 - To examine the effects of the biosolids application rate and sowing treatments on soil properties, including soil pH, organic matter, carbon and nitrogen content, and macronutrients, micronutrients, and metallic elements.

Materials and Methods

Site Description

Sites are located in south-central BC. One field study is conducted at a mine site (49°19'25"N; 120°33'08"W; New Ingerbelle South Waste Rock Storage Area) at Copper Mountain Mine (CMM), which is an open pit mine about 15 km south of Princeton. The second field study is located next to the Kamloops Wastewater Treatment Plant (KAM) (50°41'29"N; 120°27'17"W), which is on the south bank of the Thompson River in Kamloops, BC. Satellite maps of the two study sites are shown in Figure 2-1. The CMM site has a loam to sandy loam soil substrate and the KAM site has a silt to silty loam soil substrate, as shown in Figure A-1.

The KAM site is located in the Thompson Very Dry Hot Bunchgrass (BG) Zone Variant BGxh2, and the CMM site is in the Cascade Dry Cool Interior Douglas-fir (IDF) Zone Variant IDFdk2 (Hope et al., 1991; BC Ministry of Forests, 2018). Characterized by warm to hot, dry summers and moderately cold winters with little snowfall, the BG Zone is located at low elevations in the southern interior of BC, and the region is semi-arid due to evaporation exceeding precipitation (Chourmouzis et al., 2009; Lloyd et al., 1990; Nicholson et al., 1991). The region experiences a mean annual temperature of 5.9 °C, mean annual precipitation of 337 mm, and mean summer precipitation of 163 mm (Chourmouzis et al., 2009). The IDF is generally located above the BG and has a cool temperate climate with a mean annual temperature of 4.2°C, mean annual precipitation of 503 mm and mean summer precipitation of 203 mm (Chourmouzis et al., 2009). The growing season of the IDF zone is warm, dry, and relatively long (3–5 months), and winters are cool with little snow (Chourmouzis et al., 2009; Hope et al., 1991).

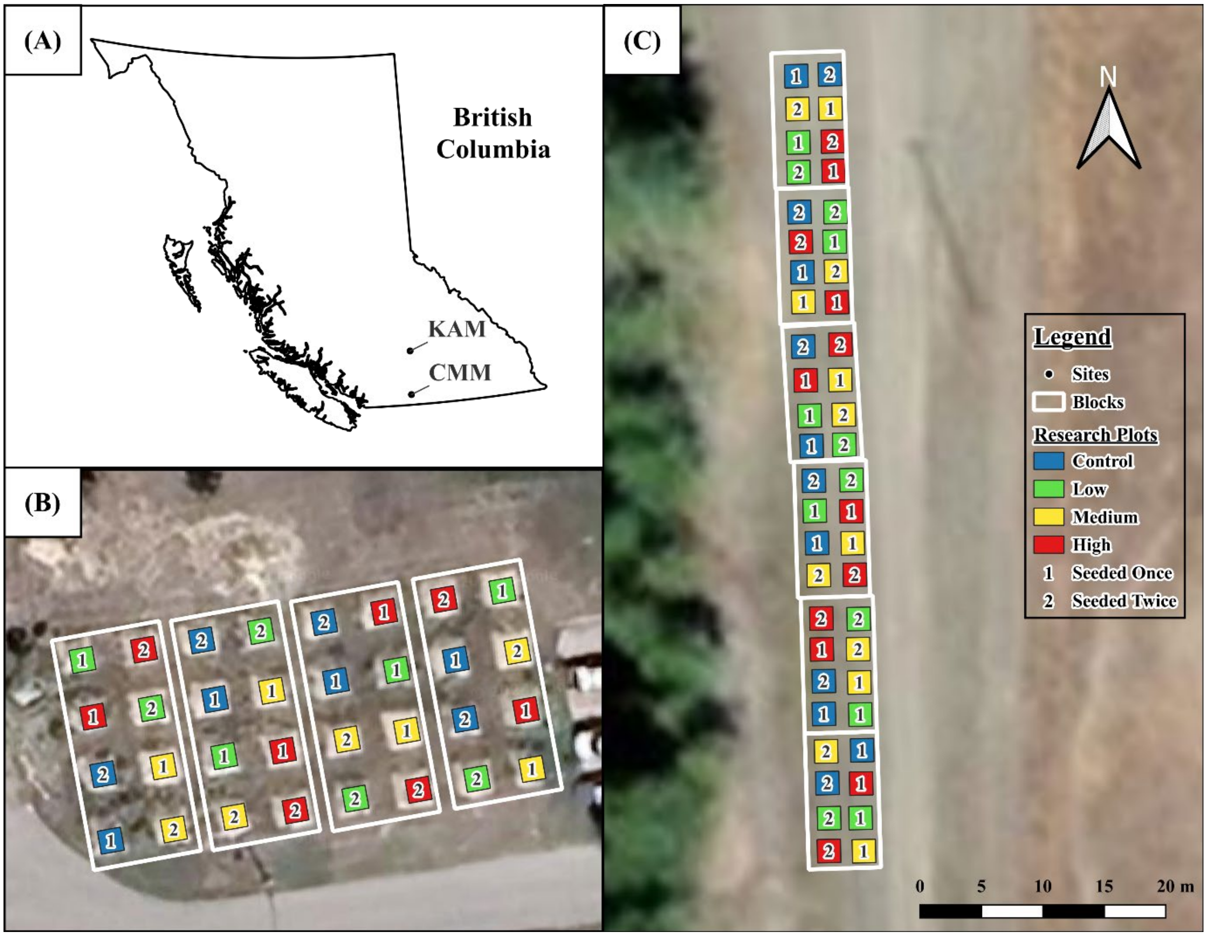

Figure 2-1. Map depicting the blocks and plots of two study sites in British Columbia (A) at the Kamloops Wastewater Treatment Plant (B) and the Copper Mountain Mine (C).

Experimental Design - Field Study

Each site consisted of a split plot design with four soil treatments and two sowing treatments for a total of eight unique treatment types. The treatment type was replicated four times at the KAM site and six times at the CMM site for a total of 80 experimental plots. Each plot was 3 m * 3 m (9 m^2), and there was a one-meter-buffer between plots. The CMM site had 665 m^2 effective seeding area, and the KAM site had 465 m^2 effective seeding area.

The four types of soil amendment in this study were: 1) 'Control' soil group receiving no biosolids, only 30 cm of subsoil and 10 cm woodchips; 2) 'Low' soil group receiving 30 cm of subsoil, 10 cm woodchips and 5 cm biosolids; 3) 'Medium' soil group receiving 30 cm of subsoil, 10 cm woodchips and 10 cm biosolids; and 4) 'High' soil group receiving 30 cm of subsoil, 10 cm woodchips and 15 cm biosolids. So the depth of each plot is about 40 cm for the 'Control' soil group, about 45 cm for the 'Low' group, about 50 cm for the 'Medium' group, and about 55 cm for the 'High' group. The application rates of biosolids in 'Low', 'Medium' and 'High' soil groups are 125, 250 and 375 dry Mg/ha, respectively (Table 2-1).

Table 2-1. Soil medium composition breakdown by percentage by volume.

Soil Medium	**Soil treatment groups**			
	Control	**Low**	**Medium**	**High**
Biosolids	0 %	11 % [a]	20 % [b]	27 % [c]
Woodchips	25 %	22 %	20 %	18 %
Substrate	75 %	67 %	60 %	55 %

Assumptions: One cubic meter of biosolids weighs 1000 kg and is about 25 % total dry solids.
[a] approximately 125 dry Mg/ha for 0.05 meter of biosolids application.
[b] approximately 250 dry Mg/ha for 0.10 meter of biosolids application.
[c] approximately 375 dry Mg/ha for 0.15 meter of biosolids application.
Formula of the application rate calculation:

$$\sigma = h * \rho * S_T * \cdot \frac{10000\ m^2}{1\ ha} * \frac{1\ Mg}{1000\ kg}$$

Where σ is the application rate (megagrams per hectare, Mg/ha), h is the height/depth (meters), ρ is the density (kilograms per cubic meter, kg/m3), and S_T is the total dry solid (percent, %).

Each of the four soil treatments were prepared on site within four separate treatment piles, containing the appropriate percentage of soil medium. Each pile was then mixed thoroughly using heavy machinery, and distributed into pre-assigned test plots. The source for biosolids was 'Class A' biosolids from the Annacis Island Wastewater Treatment Facility in Delta for the CMM site, and 'Class B' from the Kamloops Wastewater Treatment Plant for the KAM site. Woodchips were sourced by Arrow Transportation Systems Inc (Kamloops, BC, Canada). Both study sites were on flat ground. The '3 m × 3 m' plots at the CMM site were excavated and refilled in-situ, whereas the plots at the KAM site were mounded. The subsoil used at the KAM site came from a surrounding area, and the subsoil at the CMM site is in-situ. All site preparation was undertaken by Arrow Environmental Services.

In addition to the four amendment types, there were two sowing treatments to test the short-term effectiveness of one-year and two-year (repeat) seeding. The sowing method was direct broadcast seeding and no tillage. Although both sites are in the grassland phase, their biogeoclimatic ecosystem classifications are BG and IDF, meaning that climate, geography, and vegetation conditions differ between the two sites. For this reason, the native successional plants selected for each site were different (Table 2-2). The eight treatments with four replicates at the KAM site and six replicates at the CMM site were randomly designed in blocks, as shown in Table 2-3.

Annual ryegrass (*Lolium multiflorum*) is a commonly used agronomic grass in BC, and extensively used for quick ground cover in post-wildfire and other rehabilitation contexts (Dobb & Burton, 2013). Sandberg bluegrass (*Poa secunda*), fireweed (*Chamaenerion angustifolium*) and yarrow (*Achillea millefolium*) are good selections as native early successional species. Bluebunch wheatgrass (*Pseudoroegneria spicat*a), Arrowleaf balsamroot (*Balsamorhiza sagittata*), and blanket flower (*Gaillardia aristata*) are native late successional species.

First seed sowing consisted of an annual agronomic grass—Annual ryegrass (*Lolium mutliflorum*) as a cover crop, with a mix of five native species (two grasses and three forbs species), and all of them were planted in the spring of first year (2021). For the second season, the trials of each type of soil amendment were randomly divided in half. In early May of the second year (2022), half of total plots were seeded by the same species with the same density of the first year (2021). And to avoid changes in carbon stocks due to removal of biomass, no vegetative tissue was removed before the end of the two growing seasons in this experiment.

Table 2-2. 2021 field design of grasses and forbs species selection and the application seeds.

Mix of cover crop and native species		Site	Density (seeds/m²)	Weight of seeds needed per square meter (g) with deviation (g)		% by Count KAM	% by Count CMM
Grasses	Annual Ryegrass[a] (*Lolium multiflorum*)	CMM[b] & KAM[c]	400	1.613	-	30.77	33.33
	Bluebunch wheatgrass (*Pseudoroegneria spicata*)	CMM & KAM	400	1.588	-	30.77	33.33
	Sandberg Bluegrass (*Poa secunda*)	CMM & KAM	200	0.102	(±)0.002	15.38	16.67
Forbs	Fireweed (*Chamaenerion angustifolium*)	CMM	100	0.0046	-	-	8.33
	Arrowleaf Balsamroot *(Balsamorhiza sagittata*)	CMM & KAM	100	0.96	(±)0.002	7.69	8.33
	Yarrow (*Achillea millefolium*)	KAM	100	0.015	-	7.69	-
	Blanket flower (*Gaillardia aristata*)	KAM	100	0.189	-	7.69	-

[a] Annual Ryegrass is exotic species, but used as a cover crop.
[b] 48 plots at Copper Mountain Mine (CMM); each plot is 9 m^2.
[c] 32 plot at Kamloops (KAM) Wastewater Treatment Plant; area of each plot was 9 m^2.

Table 2-3. A list of the 8 treatments, including year 1 (2021) and 2 (2022) sowing treatments and soil amendments (rate of biosolid addition).

#	Year 1 sowing treatment	Year 2 sowing treatment	Soil treatment
1	Seed mix of grasses & herbs + cover crop	Same as year 1	Control
2	Seed mix of grasses & herbs + cover crop	Same as year 1	Low
3	Seed mix of grasses & herbs + cover crop	Same as year 1	Medium
4	Seed mix of grasses & herbs + cover crop	Same as year 1	High
5	Seed mix of grasses & herbs + cover crop	No treatment	Control
6	Seed mix of grasses & herbs + cover crop	No treatment	Low
7	Seed mix of grasses & herbs + cover crop	No treatment	Medium
8	Seed mix of grasses & herbs + cover crop	No treatment	High

Experimental Design - Greenhouse

The potential seed bank of each study site may have an effect on field experiments, so this research included a greenhouse experiment to test seed bank existence. Soil substrates were collected from each site. Perforated germination trays (28 x 56 x 5.5 cm) were covered with landscaping fabric at bottom then filled with a 2 cm layer of sterile sand. Each composite soil substrate was sieved with 4 mm mesh to remove rocks and plant materials, then spread out into a germination tray; soil substrate was allowed to air dry for two days prior to sieving. Soil substrates from each study site were assigned to four germination trays and covered with plastic wrap to keep in moisture, then exposed to a 16-hour day and 8-hour night regime, and a temperature setting of 20-25°C, as recommended by Plue and Hermy

(2012). Excessive or insufficient moisture can affect seed germination and growth, so the germination trays were checked once a day to make sure the soil was always moist.

Emerging seedlings were identified, counted, then removed. Unknown seedlings were transplanted into potting soil and left to grow until identification was possible. After 6 weeks, a 1000 ppm (mg/kg) gibberellic acid solution was applied in an attempt to stimulate the growth of remaining, dormant seeds (Finkelstein et al., 2008). The greenhouse pod was monitored and maintained to prevent contaminations on the germination trays. The experiment was terminated on the 16^{th} week. Eventually, all identified plant species that grew from the seed bank of the soil substrate were recorded.

Data Collection and Analysis

Plant Productivity and Diversity

At the end of each growing season (August), plant cover percentage for each species was collected at the KAM and CMM sites using the Daubenmire canopy cover method and a half-square-meter-quadrat (Daubenmire, 1959).

In addition to the plant coverage measurements, plant productivity was measured at the end of the second growing season (2022) by collecting all aboveground biomass at the two sites, and sorting them by species. The CMM test site had a third growing season (2023), at the end of which plant cover and aboveground biomass were collected and recorded again.

Biomass & Soil Physicochemical Tests

Within a 2 m × 2 m area at the center of every plot, three 50 cm × 50 cm quadrats were randomly selected for a plant coverage survey followed by the collection of plant and soil. All the aboveground vegetation in the surveyed quadrat of both sites were clipped and stored in bags individually according to species at the end of the second growing season, 2022. The field experiment at the CMM site continued through the end of summer 2023 and aboveground biomass data were available at the CMM site for both 2022 and 2023. At the end of the 2023 growing season, sampling quadrats were randomly selected for each plot at the CMM site. These selected quadrats were different from those measured in 2022, since the biomass of those quadrats sampled in 2022 was collected. All aboveground biomass in the selected quadrat were collected in one sampling paper bag after the plant coverage survey for each species in the quadrat, in 2023.

Soil samples were collected from each plot before any seed application (May, 2021) and at the end of the second growing season (Mid August, 2022). From every quadrat of each plot, three soil samples were collected within the 0-10 cm depth and below the litter by using a soil probe having about 2.5 cm diameter. All soil samples were air-dried and sieved (2 mm) before any test. Soil testing included:

A. 20 grams of every soil sample was used to measure soil pH using EPA method 9045D (USEPA, 2004).
B. A gravimetric loss on ignition (LOI) method was used to estimate soil organic matter (SOM) (Hoogsteen et al., 2015). Soil samples taken from the quadrats were homogenized according to each plot, SOM of every homogenized soil sample was determined by placing 2 to 5 grams of dry soil in the oven and evaporating the organic matter at 540 °C for at least four hours. The weight difference from before to after drying in the oven was calculated and recorded as the SOM.
C. Total carbon and nitrogen of every dried and homogenized sample (approximately 10 mg) were determined using a CHNS elemental analyzer (Thermo Fisher Scientific™ FlashSmart™), then the % carbon and nitrogen of each plot were calculated and recorded.
D. Approximately 0.5 grams of each soil sample were processed by hot acid digestion process as USEPA 3051A (da Silva et al., 2014). Briefly, pulverized soil samples were placed in a Teflon tube with 9 milliliters of nitric acid (HNO_3) and 3 milliliters of hydrogen chloride (HCl), then the samples were heated to 175 °C and held at that temperature for 4.5 minutes in a microwave oven. Inductively coupled plasma mass spectrometry (ICP-MS) was then used to measure concentrations of a suite of trace elements for all digested and diluted soil samples. Tested macronutrients were potassium (K), calcium (Ca), magnesium (Mg); tested micronutrients were iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), molybdenum (Mo); and some potentially toxic elements sodium (Na), aluminum (Al), lead (Pb), chromium (Cr), cadmium (Cd), arsenic (As) (Liu et al., 2022; Tarar et al., 2022; Zhao & Shen 2018; Kan et al., 2021; Horie & Schroeder 2004).

Statistic Analysis

Since the environmental conditions (Table 2-4) and the native successional species sown at the two study sites were different (Table 2-2), analyzing the data separately for each site can avoid the data bias caused by spatial factors, such as different climate and soil quality.

Biomass in this experiment was defined as the dry weight of aboveground plant tissue of non-litter plants within the sampling quadrat, and the biomass data analysis had unit transformation to kilograms per hectare (kg/ha). Species richness was defined as the number of plant species within the sampling quadrat (0.25 m^2). Biomass and species richness of each species within every quadrat were summed for the entire plant community and plant functional groups for multiple data analysis. The plant functional groups were further grouped in the growth form as grasses and forbs; and in the status as natives, exotics, invasives, early successional species, and the cover crop (annual ryegrass). Groups of natives and early successional species includes nonsown native species. Details of functional groups are listed in Table 2-4, and the plant species characterization are refer to E-flora BC (2021), BC Rangeland Seeding Manual (Dobb & Burton, 2013), Provincial Priority Invasive Species (IMISWG, 2023) and Grasses of the Columbia Basin of British Columbia (Stewart & Hebda, 2000).

Table 2-4. All species found at the Kamloops site in 2022 and at the Copper Mountain Mine site in 2022 and 2023. Asterisks on ‘common name’ indicate species found in a seed bank experiment of the Copper Mountain Mine site.

Common Name	Scientific Name	Growth Form	Status	Biogeoclimatic Zone	
				BEC-BG	BEC-IDF
Goosefoot	*Chenopodium album*	forb	invasive	recorded	recorded
Summer cypress	*Kochia scoparia*	forb	invasive	recorded	not recorded
Canada thistle	*Cirsium arvense*	forb	invasive	recorded	recorded
Hairy nightshade	*Solanum physalifolium*	forb	invasive	not recorded	not recorded
Annual ryegrass	*Lolium multiflorum*	grass	agronomic	not recorded	not recorded
Knapweed*	*Centaurea* spp.	forb	invasive	not recorded	not recorded
Black medic	*Medicago lupulina*	forb	exotic	recorded	recorded
Cheatgrass	*Bromus tectorum* L.	grass	invasive	recorded	recorded
Alfalfa*	*Medicago sativa* L.	forb	agronomic	recorded	recorded
Loesel's tumble-mustard	*Sisymbrium loeselii* L.	forb	exotic	recorded	recorded
Bushy knotweed	*Reynoutria* spp.	forb	invasive	not recorded	not recorded
Aster douglasii	*Symphyotrichum subspicatum* (Nees) G.L. Nesom	forb	late successional	recorded	recorded
Bluebunch wheatgrass*	*Pseudoroegneria spicata* (Pursh) Á. Löve	grass	late successional	recorded	recorded
Blanket flower	*Gaillardia aristata*	forb	late successional	recorded	recorded
Nodding brome	*Bromus anomalus*	grass	early successional	not recorded	not recorded
Slender hawksbeard	*Crepis atribarba*	forb	early successional	recorded	recorded
Shepherd's purse	*Capsella bursa-pastoris*	forb	invasive	recorded	recorded

Common Name	Scientific Name	Growth Form	Status	Biogeoclimatic Zone	
				BEC-BG	BEC-IDF
Rough fescue	*Festuca campestris* Rydb.	grass	late successional	recorded	recorded
Great mullein	*Verbascum thapsus* L.	forb	invasive	recorded	recorded
Kentucky bluegrass*	*Poa pratensis* L.	grass	agronomic	recorded	recorded
Pinegrass	*Calamagrostis rubescens* Buckley	grass	early successional	recorded	recorded
Crested wheatgrass	*Agropyron cristatum* (L.) Gaertn.	grass	agronomic	recorded	recorded
Peppergrass	*Lepidium densiflorum* Schrad.	forb	early successional	recorded	recorded
Common sow-thistle	*Sonchus oleraceus* L.	forb	invasive	recorded	recorded
Red clover	*Trifolium pratense* L.	forb	agronomic	recorded	recorded
Slender wheatgrass	*Elymus trachycaulus*	grass	early successional	recorded	recorded
Salsifies	*Tragopogon* spp.	forb	exotic	recorded	recorded
Prickly lettuce*	*Lactuca serriola* L.	forb	exotic	recorded	recorded
Dandelion*	*Asteraceae* spp.	forb	unknown	recorded	recorded
Timber oatgrass	*Danthonia intermedia*	grass	late successional	not recorded	recorded

Statistical analyses were conducted using R for Statistical Computing (R Core Team, 2020). The R package Tidyverse was used for data sorting and visualization (Wickman et al., 2019). All field experiment data were tested for significance at the 5% probability level. Normality was checked for all field data by plotting residuals, histograms, using the Shapiro-Wilk test. Using a ‘vegan’ package, the species richness was calculated for each quadrat based on plant canopy coverage data (Jari et al., 2022). However, original data did not pass the assumption of a normal distribution of parametric analysis, so the non-parametric aligned rank transformed (ART) models of align-and-rank data (species richness and biomass) were developed and analyzed with the analysis of variance (ANOVA) (Wobbrock et al., 2011). The ‘ARTool’ R package allows for non-parametric testing of interactions and main effects using standard ANOVA techniques (Durner, 2019). The ART method ranks the data and aligns the ranks across tested factors, preserving the structure of the data and allowing valid statistical inference (Durner, 2019). Then, two-way ANOVA on ARTool Linear Mixed-effect models of non-2021 plant biomass and species richness were conducted separately for the analysis of plant productivity and diversity, with ‘soil treatment’ and ‘sowing treatments’ as fixed effects, and ‘year’ and ‘block’ as random effects, which is for accounting the variation in the data due to the sampling year and block positioning. Because the reseeding practice occurred in 2022, the two-way ANOVA did not include species richness in 2021. Since the KAM site does not have data of biomass from 2023, the models of the KAM site have only ‘block’ as random effects.

There are three hypotheses for the two-way ANOVA of the field plant data: (1) application of biosolids has a positive impact on biomass and species richness; (2) reseeding practice has a positive impact on biomass and species richness; (3) biosolids and reseeding practice interact in positively affecting the plant biomass and species richness.

Biomass

Effects of soil and sowing treatments on total biomass of the quadrat (0.25 m^2) plant community and the biomass of quadrat native plants were analyzed using the ART model and ANOVA, while the model had ‘year’ and ‘block’ as random effects on total living biomass and the biomass of native plant groups. Post hoc tests for pairwise comparisons between groups were conducted using the Tukey honestly significant difference (HSD) method.

In addition to the native plant group, there were other functional groups. The total biomass was grouped and analyzed by plant functional groups based on the growth form and the status of the plants, respectively. Therefore, Spearman's correlation analysis was conducted for each experimental year to investigate the trend of biomass changes among plant functional groups under different growth forms and different states, for example, an analysis of plant growth form, and an analysis of plant status. Details of plant functional groups are listed in Table 2-4.

Plant Canopy Coverage

Species richness, the number of unique species (Mahaut et al., 2019), was calculated from the canopy cover data for each quadrat. Species richness of each quadrat (0.25 m^2) was used as an index of the community alpha diversity level (Lira-Noriega et al., 2007; Mahaut et al., 2019). The intragroup (alpha) and intergroup (beta) diversity of plant communities were analyzed using ART species richness data (Maddah et al., 2023).

The beta-diversity between plant communities of different soil groups/treatments were analyzed by using Principal Coordinates Analysis (PCoA) and total coverage of each species within every quadrat. Data were first standardized using the 'decostand' function and the 'presence-absence' method. Then, a distance matrix of Bray-Curtis dissimilarity was calculated using the 'vegdist' function from the standardized data. A PCoA was conducted using the 'pcoa' function from the package 'ape' (Paradis et al., 2023). The other two functions come from the 'vegan' package (Jari et al., 2022).

The distribution of data for plant communities at the KAM site was extremely skewed. Therefore, PCoA of plant communities, PCA of soil elements, and time effects on plant species richness were not performed for the KAM site.

Soil Characterization & Elemental Analysis

Data analyses for various soil parameters included two-way ANOVAs for ART soil pH, SOM, total carbon, total nitrogen, and C/N ratios, with 'soil treatments' and 'sowing treatments' as fixed effects. The two-way ANOVA with soil treatments and sowing treatments as fixed effects and block as a random effect was used. Tukey's post-hoc tests were used to test the effect of the significant fixed effects on the soil parameters with the 'art.con' function of the 'ARTool' package (Durner, 2019).

Principal component analysis (PCA) was used to analyze the elemental composition between soil treatments. A PERMANOVA analysis was conducted to test the differences between soil treatments (biosolids application versus control) with the 'adonis' function of the 'vegan' package (Jari et al., 2022).

Results

Species Found on Fields & the Greenhouse Test

In the greenhouse test, germination trays made from soil from the KAM site showed no sprouting during the 16-week seed bank test. Germination trays made from soil from the CMM site showed sprouting during the 16-week seed bank test, but only six species survived and grew to a recognizable state within the 16-week period. The six species recognized from the seed bank test of CMM were signed with asterisks and listed in Table 2-4.

Species that established in the two plant surveys (2021 & 2022) at KAM field site were annual ryegrass, summer cypress (*Kochia scoparia*), goosefoot (*Chenopodium album*), Canada thistle (*Cirsium arvense*), salsify (*Tragopogon* spp.), hairy nightshade (*Solanum physalifolium*), Loesel's tumble-mustard (*Sisymbrium loeselii* L.), bushy knotweed (*Reynoutria* spp.), and aster douglasii (*Symphyotrichum subspicatum* (Nees) G.L. Nesom).

Species that established in the three plant surveys (2021 to 2023) at CMM field site were annual ryegrass, bluebunch wheatgrass, blanket flower, knapweed (*Centaurea* spp.), black medic (*Medicago lupulina*), cheatgrass (*Bromus tectorum* L.), alfalfa (*Medicago sativa* L.), goosefoot (*Chenopodium album*), red clover (*Trifolium pratense* L.), slender wheatgrass (*Elymus trachycaulus*), nodding brome (*Bromus anomalus*), Loesel's tumble-mustard (*Sisymbrium loeselii* L.), slender hawksbeard (*Crepis atribarba*), shepherd's purse (*Capsella bursa-pastoris*), rough fescue (*Festuca campestris* Rydb.), great mullein (*Verbascum thapsus* L.), Kentucky bluegrass (*Poa pratensis* L.), Canada thistle (*Cirsium arvense*), pinegrass (*Calamagrostis rubescens* Buckley), crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), peppergrass (*Lepidium densiflorum* Schrad.), common sow-thistle (*Sonchus oleraceus* L.), bushy knotweed (*Reynoutria* spp.), dandelion (*Asteraceae* spp.) and timber oatgrass (*Danthonia intermedia*).

Site at Copper Mountain Mine

Vegetation Response

The ART model showed that the biomass (F statistics = 169.5; $p < 0.001$) and the species richness ($F = 113$; $p < 0.001$) were significantly impacted by biosolids treatment. The post hoc analysis showed that the plots with control treatment had the lowest biomass (Figure 2-2-A) and observed species richness (Figure 2-2-B). As shown in Figure 2-2, there was no significant difference in the biomass of total plant communities among the three soil groups amended with biosolids, but the species richness of the 'Low' soil group was significantly different from that of the 'Medium' and 'High' soil groups at the CMM site. The soil group 'Low' consistently had the highest species richness (Figure 2-2-B).

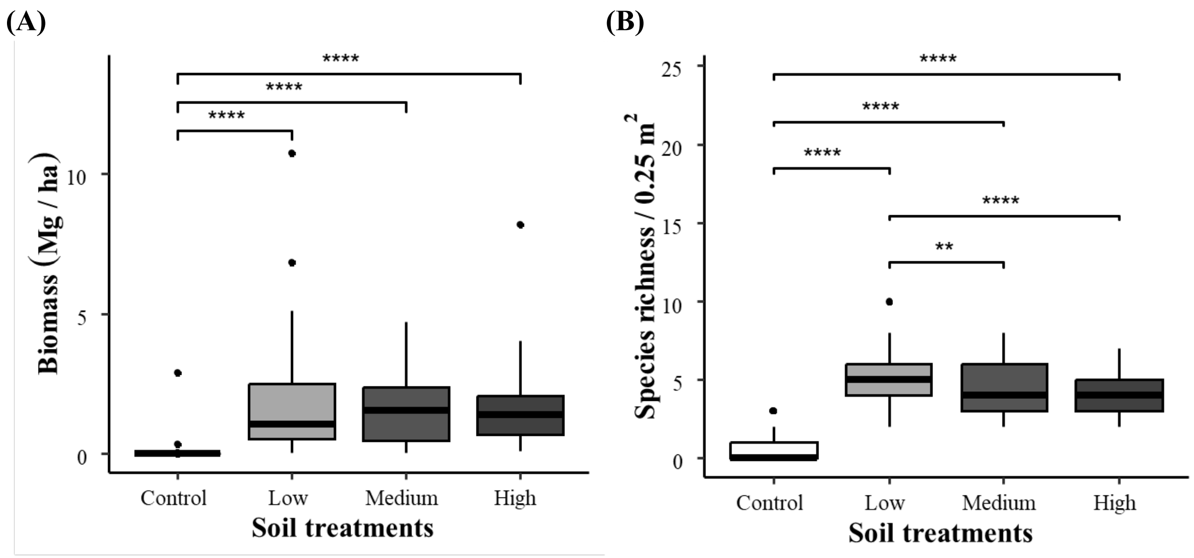

Figure 2-2. Biomass (A) and species richness (B) of plant communities in different soil treatments at Copper Mountain Mine site. Asterisks above brackets denote significant pairwise comparisons between soil treatments as determined by Tukey HSD with aligned rank transformed data,p<0.01, ****p<0.0001.**

The interaction between soil treatment and seeding treatment had a significant effect on the species richness of plant communities at CMM site (F statistics = 4.2; $p < 0.01$). Within the 'Control' group, both the reseeded plots and the single seeded plots had a significantly ($p < 0.0001$) lower species richness than the other six treatments. The mean species richness measure for the group 'Low, Seed_Once' was significantly larger than the mean for the group 'Medium, Reseed' ($p < 0.05$) and 'High, Seed_Once' ($p < 0.01$). Detailed comparison results can be found in Table A-4 (Appendix A).

The biomass (F statistics = 49; $p < 0.001$) and species richness (F statistics = 208.7, $p<0.001$) of plant communities have temporal variations as shown in Figure 2-3. There were significant differences between years, indicating notable changes in the biomass. Among them, 2022 always had the highest biomass and species richness.

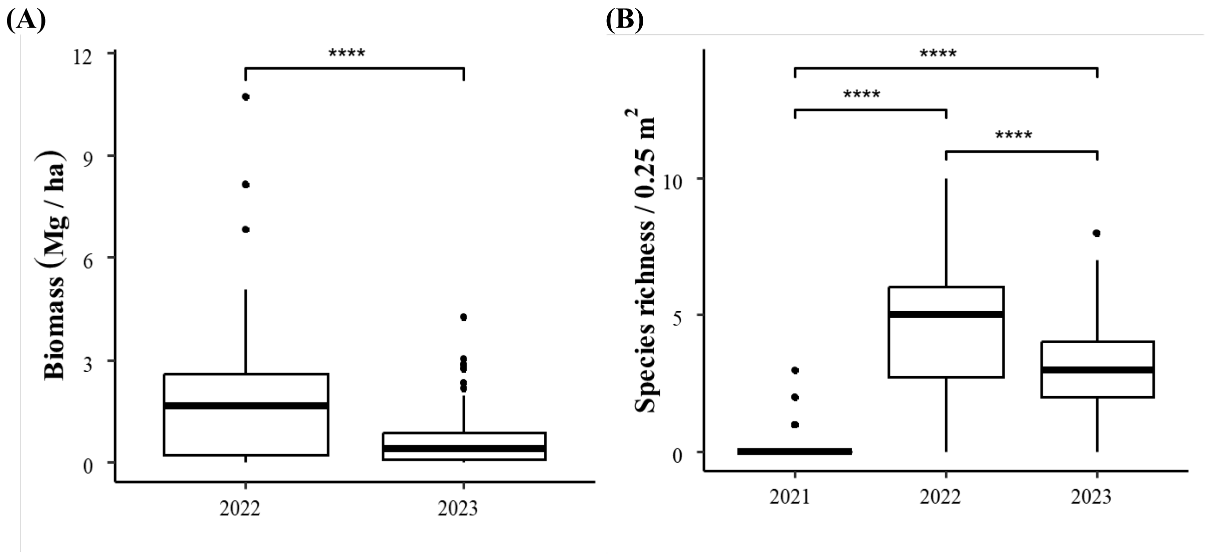

Figure 2-3. Biomass (A) and species richness (B) of plant communities at Copper Mountain Mine site in every experimental year. Asterisk(s) above brackets denote significant pairwise comparisons between experimental years by Tukey HSD with aligned rank transformed data, ****p<0.0001.

The ART model showed that the biomass (F statistics = 94.4; $p < 0.001$) and the species richness (F statistics = 127.5; $p < 0.001$) of native species were significantly affected by biosolids treatment. Post hoc analysis showed that the plots with control treatment had the lowest biomass (Figure 2-4-A) and observed species richness (Figure 2-4-B). There were also significant differences in the biomass and species richness among the other three soil groups in the native plant communities at the CMM test site. The 'Low' group had a significantly higher biomass and species richness than the 'Medium' group (Figure 2-4), and the 'High' group had a significantly higher biomass than the 'Medium' group (Figure 2-4-A).

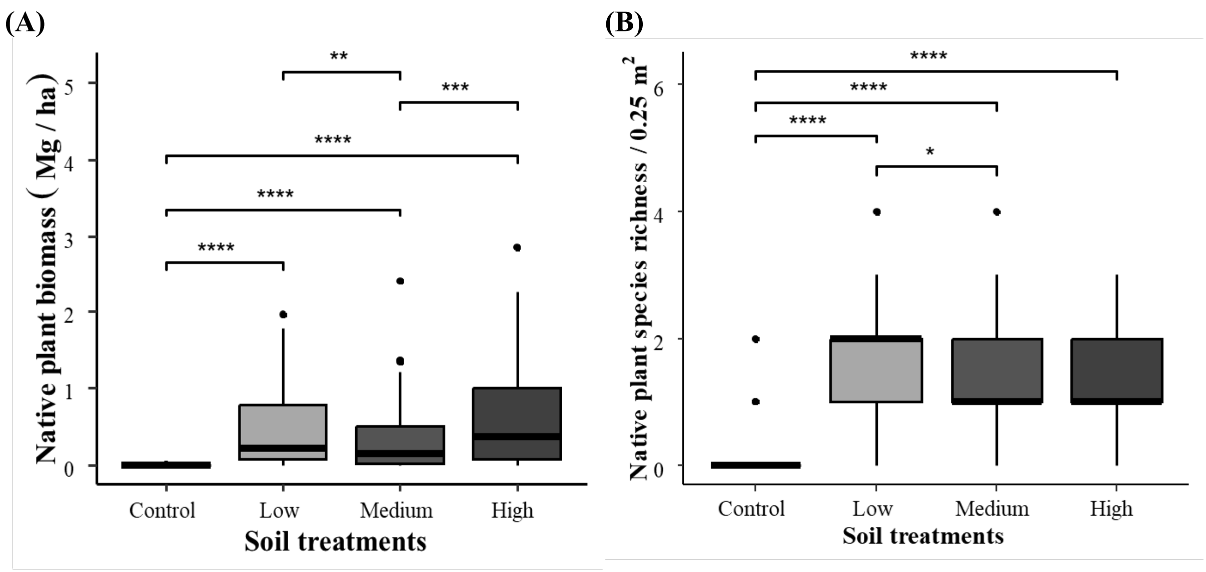

Figure 2-4. Biomass (A) and species richness (B) of native plant groups in different soil treatments at the Copper Mountain Mine site. Asterisk(s) above brackets denote significant pairwise comparisons between soil treatments as determined by Tukey HSD with aligned rank transformed data, **p<0.01, ***p<0.001, ****p<0.0001.

As shown in Figure 2-5, correlations between biomass of all plant functional groups were 'positive' and significant for all plant groups at CMM site in 2022. Based on the biomass of another set of functional groups of grasses and forbs in 2022, there is a positive trend (correlation coefficient = 0.51; $p < 0.001$) between the biomass of grass group and forb group at the CMM site.

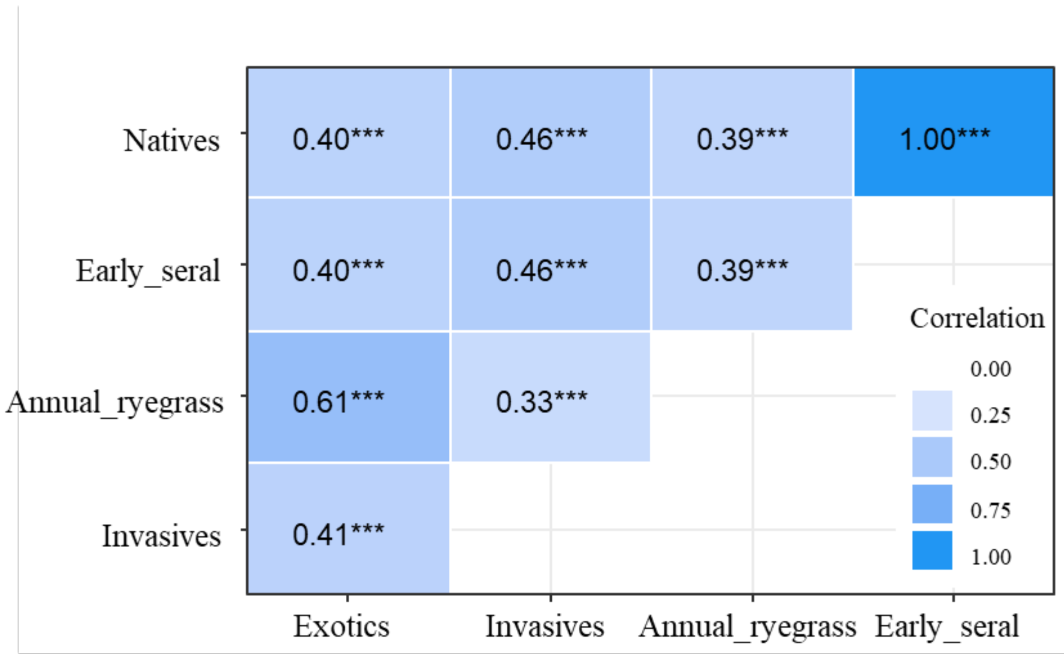

Figure 2-5. Correlation matrix showing spearman correlation statistics for relationships between the biomass of plant functional groups in all plots of the Copper Mountain Mine site in 2022. 'Annual ryegrass' denotes the cover crop group. 'Early_seral' denotes a group of early successional plants. 'Natives' plant group includes early successional species. Asterisks on correlation coefficients denote significance level, *p<0.05, ***p<0.001. Colors show the direction of the correlation, blue shows a positive correlation.

PCoA captured over 55 % of the total variation in plant community composition (Figure 2-6). Furthermore, the PCoA revealed that the biosolids treated sites and control sites had distinct clusters. PERMANOVA analysis showed that the plant community composition in the 'Control' plots was significantly different from the biosolids treated plots (Figure 2-6).

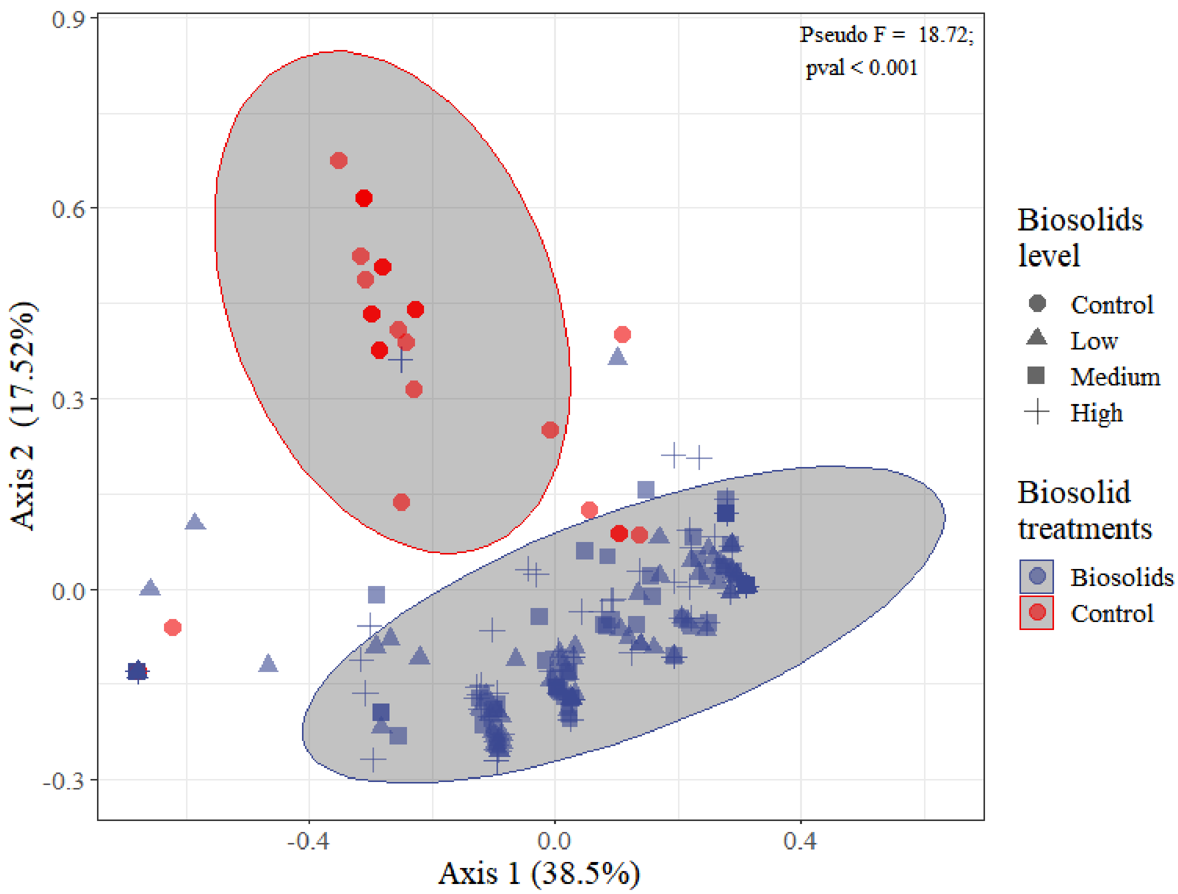

Figure 2-6. PCoA representing the differences in composition of plant communities among the quadrats at Copper Mountain Mine site in 2022 and 2023. Principal axes 1 and 2 (Axis 1 and Axis 2) explained 38.5% and 17.52% of the variation associated with the data, respectively.

Soil Properties

The ART two-way ANOVA revealed that sowing treatments significantly affected C/N ratios (F statistics = 5.6; $p < 0.05$), while soil treatments significantly affected all soil property parameters at the CMM site: pH (F statistics = 145.1; $p < 0.001$), SOM (F statistics = 12.3; $p < 0.001$), carbon (F statistics = 21.2; $p < 0.001$), nitrogen (F statistics = 40.6; $p < 0.001$) and C/N ratio (F statistics = 28.3; $p < 0.001$). Soil parameters of the 'Control' group significantly differ from the other three soil groups (Figure 2-7 & Figure 2-8), and the 'High' and 'Medium' groups had lowest soil pH (Figure 2-7-A), and highest SOM (Figure 2-7-B), carbon (Figure 2-8-A) and nitrogen (Figure 2-8-B) concentrations. The 'High' soil group and the 'Reseed' (Seeded 2021 & 2022) sowing group had the lowest C/N ratio (Figure 2-8-D). Similar to the vegetation responses, the 'Control' soil group at the CMM site was always significantly different from the other three groups, for example, higher soil pH and C/N ratio, and lower SOM and C/N content (Figure 2-7, Figure 2-8).

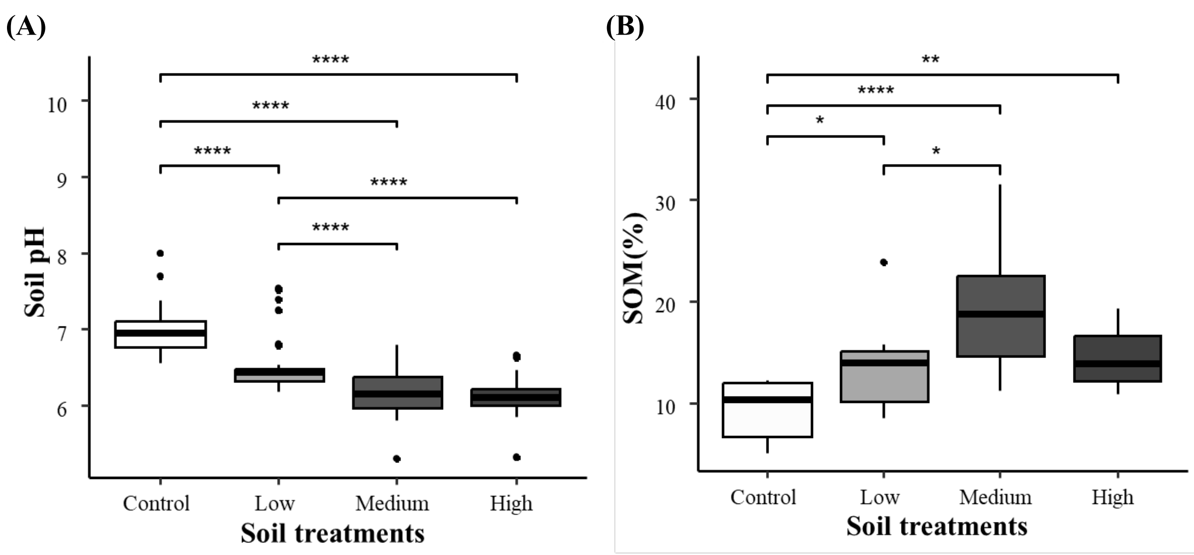

Figure 2-7. Soil pH (A) of soils within 0-10 cm depth and soil organic matter (SOM) (B) in different soil treatments at Copper Mountain Mine site. Asterisk above brackets denote significant pairwise comparisons between soil treatments as determined by Tukey HSD with aligned rank transformed data, *p<0.05, **p<0.01, ****p<0.0001.

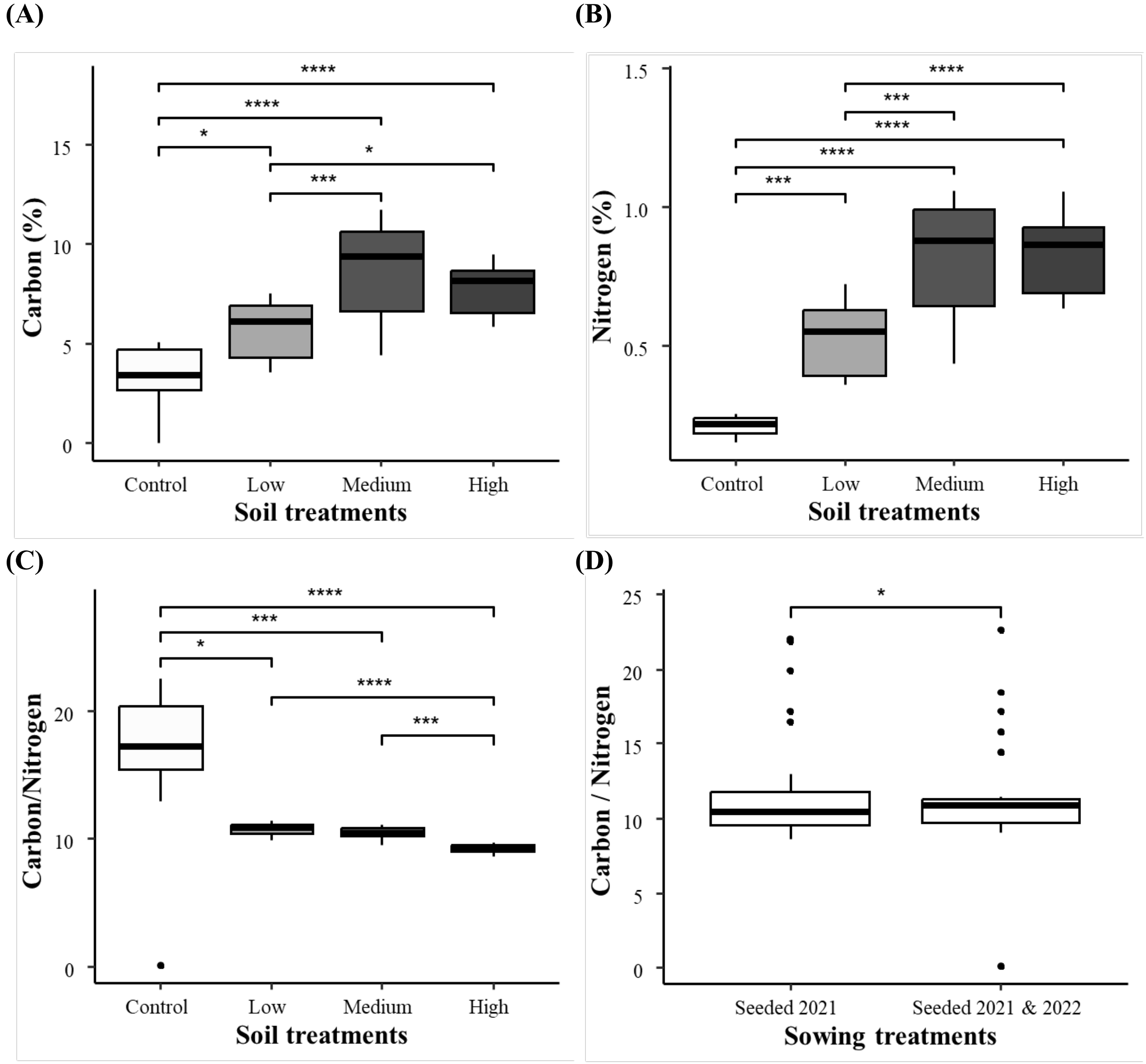

Figure 2-8. Total carbon (A), nitrogen (B), and C/N ratios (C) of soils within 0-10 cm depth in different soil treatments, and C/N ratios (D) of the soils in different sowing treatments at Copper Mountain Mine site in 2022. Asterisk(s) above brackets denote significant pairwise comparisons between soil treatments as determined by Tukey HSD with aligned rank transformed data, *p<0.05, ***p<0.001, ****p<0.0001.

As shown in Figure 2-9, there were significant differences in soil elemental composition between the 'Control' soil group and soil groups with biosolids applied (pseudo F statistics = 6.4; $p < 0.001$). As shown in Table 2-5, the soil content of As, Cu, Mo, and Zn in each soil group and seeding group exceeded the limits of agricultural land use of Canadian Council of Ministers of the Environment (CCME) soil quality guidelines for the Protection of Environmental and Human Health, and the soil content of As and Cu also exceeded the CCME guidelines for industrial land use, except for As in the 'High' soil group and Zn in the 'Control' (CCME, 1999).

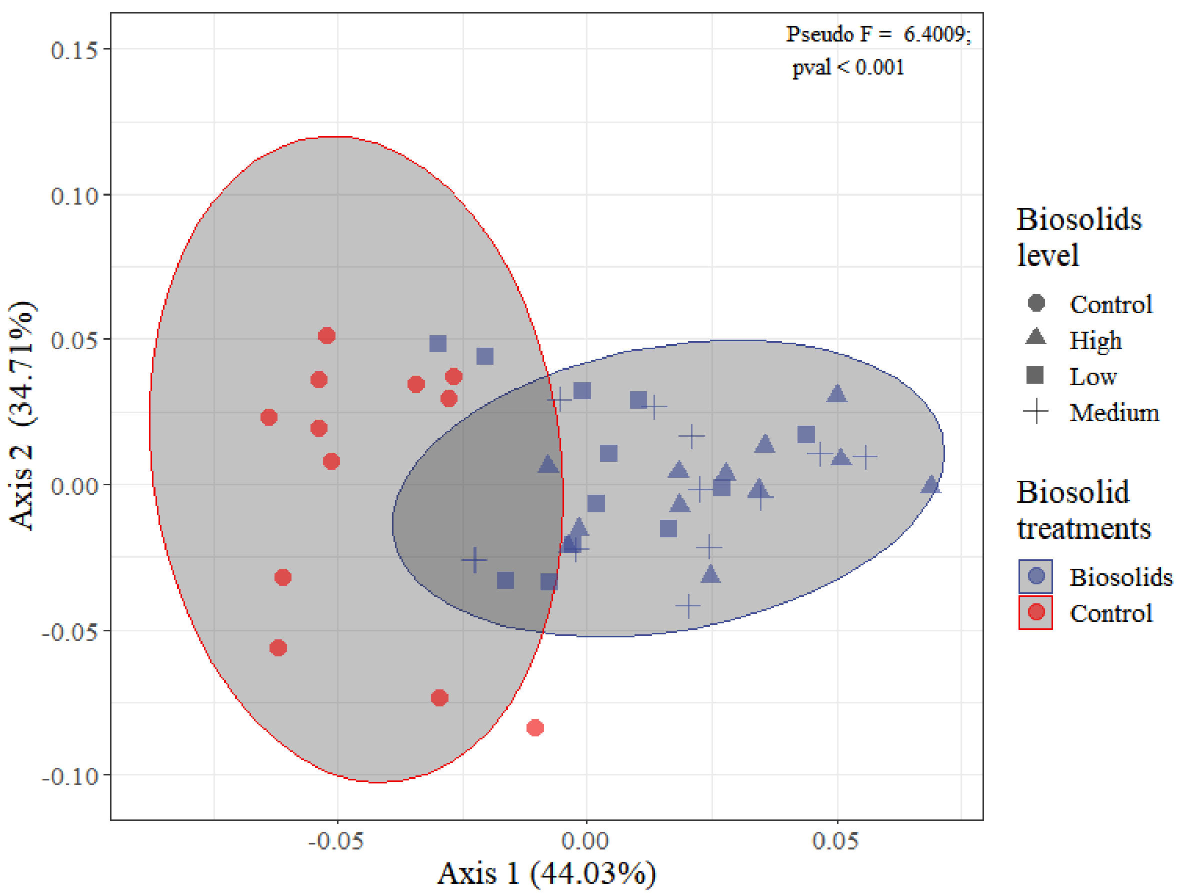

Figure 2-9. Principal component analysis (PCA) of 14 elements in soils at Copper Mountain Mine site in 2022. The first and second axes explained 44.03% and 34.71% of the variance, respectively.

Table 2-5. Concentrations (mg/kg dry weight) of metals and trace elements in soils of different soil and sowing treatments at Copper Mountain Mine site. Values are compared to the Canadian Council of Ministers of the Environment soil quality guidelines (CCME, 1999) guidelines for agricultural and industrial uses. Bolded values are in exceedance of at least one of the referenced guidelines.

	Mean ± SD						CCME	CCME
Element	**Control**	**Low**	**Medium**	**High**	**Seed_Once**	**Reseed**	(agricultural)	(industrial)
Al	17,758 ± 1,697	17,329 ± 2,923	16,067 ± 1,912	15,670 ± 3,391	16,421 ± 2,343	16,991 ± 2,934	-	-
As	**11 ± 3**	**10 ± 3**	**9 ± 3**	8 ± 2	**9 ± 3**	**10 ± 3**	12	12
Cd	0 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1.4	22
Ca	17,383 ± 2,074	20,093 ± 4,355	18,588 ± 2,901	19,109 ± 5,799	18,354 ± 3,417	19,232 ± 4,599	-	-
Cr	39 ± 5	40 ± 8	40 ± 7	39 ± 12	39 ± 7	40 ± 9	64	87
Cu	**134 ± 38**	**238 ± 65**	**317 ± 84**	**262 ± 81**	**240 ± 101**	**236 ± 91**	63	91
Fe	36,428 ± 3,856	38,905 ± 7,024	38,413 ± 4,953	38,285 ± 8,595	37,554 ± 5,383	38,461 ± 7,086	-	-
Pb	4 ± 3	6 ± 3	8 ± 3	7 ± 3	6 ± 3	6 ± 4	70	600
Mn	751 ± 84	721 ± 121	697 ± 98	681±156	709 ± 109	716 ± 127	-	-
Mo	**5 ± 8**	**4 ± 3**	**4 ± 5**	**6 ± 5**	**4 ± 4**	**6 ± 6**	5	40
Mg	9,630 ± 2,172	9,292 ± 2,016	8,585 ± 1,301	8,309 ± 1,640	8,804 ± 1,701	9,104 ± 1,985	-	-
K	2,126 ± 394	2,287 ± 612	2,232 ± 551	2,542 ± 929	2,224 ± 529	2,369 ± 754	-	-
Na	772 ± 394	852 ± 269	935 ± 411	1,011 ± 409	891 ± 377	893 ± 380	-	-
Zn	135 ± 33	**234 ± 34**	**311 ± 55**	**290 ± 61**	**242 ± 79**	**243 ± 88**	250	410

Site at Kamloops Wastewater Treatment Plant

Vegetation Response

Soil treatments had a significant effect on biomass (F statistics = 14.9; $p < 0.001$) and species richness (F statistics = 14.8; $p < 0.001$) of plant communities at the KAM site, and there was a significant difference in plant community biomass between the 'Reseed' (Seeded 2021 & 2022) and 'Seed_Once' (Seeded 2021) sowing groups (F statistics = 6.2; $p < 0.05$) (Figure 2-10). As shown in Figure 2-10, 'Low' soil group was significantly higher than the 'High' and 'Medium' soil groups for both biomass and species richness at the KAM site.

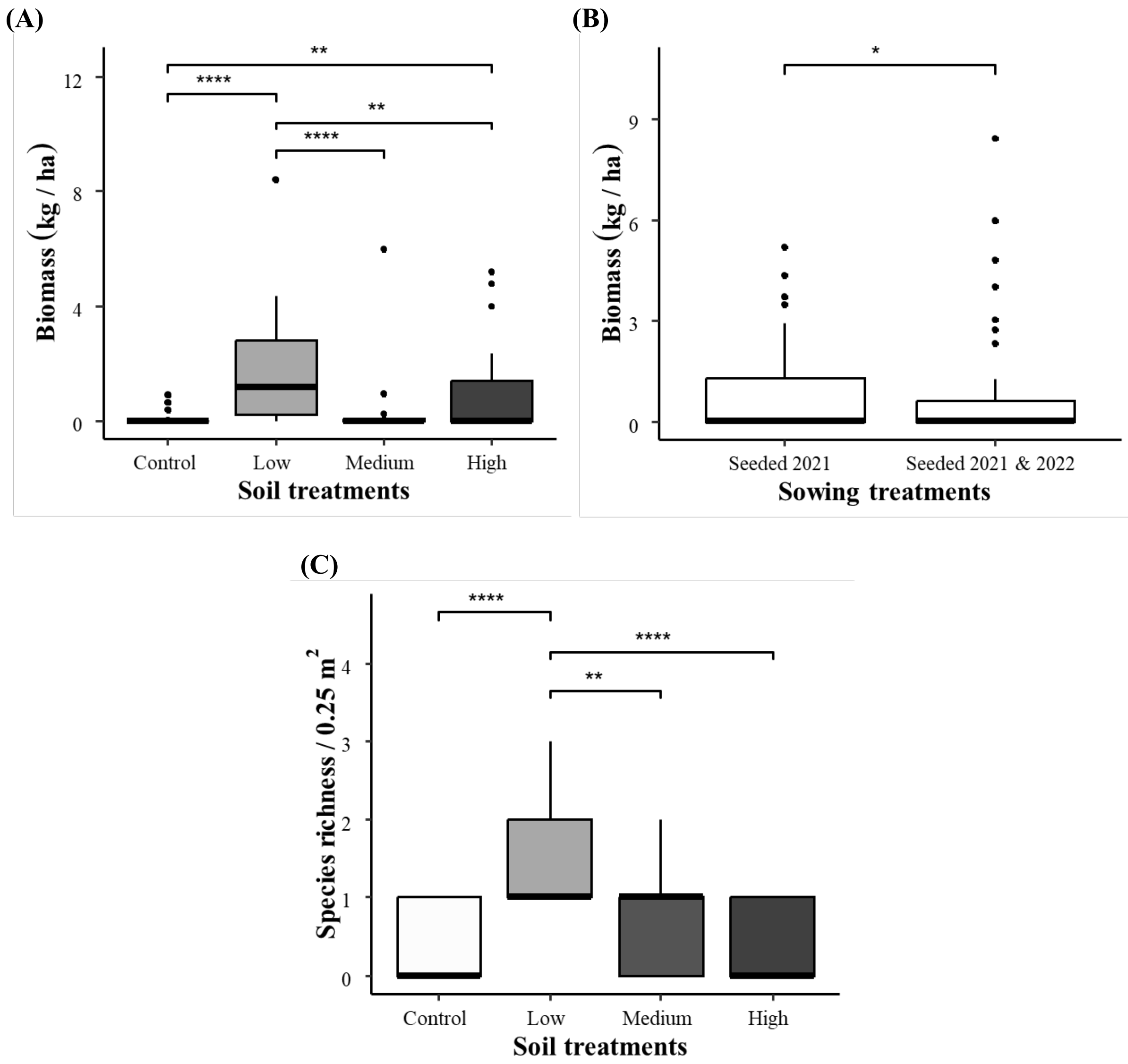

Figure 2-10. Biomass of plant communities in different soil treatments (A) and sowing treatments (B) in 2022, and species richness (C) of plant communities in different soil treatments in 2022 at Kamloops Wastewater Treatment Plant site. Asterisk(s) above brackets denote significant pairwise comparisons between soil treatments as determined by Tukey HSD with aligned rank transformed data, *p<0.05,p<0.01, ****p<0.0001.**

An ANOVA performed on the species richness of the plant communities from the two growing seasons at the KAM site showed no significant differences.

None of the early successional plant species had analyzable biomass at the KAM site, and there was no significant correlation between other functional groups (Figure 2-11). Of the plant species selected for anthropogenic seeding in this experiment (Table 2-2), only annual ryegrass had aboveground biomass collected and recorded at the KAM site after two anthropogenic seeding events and two growing seasons.

Based on the biomass of another set of functional groups of grasses and forbs in 2022, there is a positive trend (correlation coefficient = -0.24; $p < 0.018$) between the biomass of grass group and forb group at the KAM site.

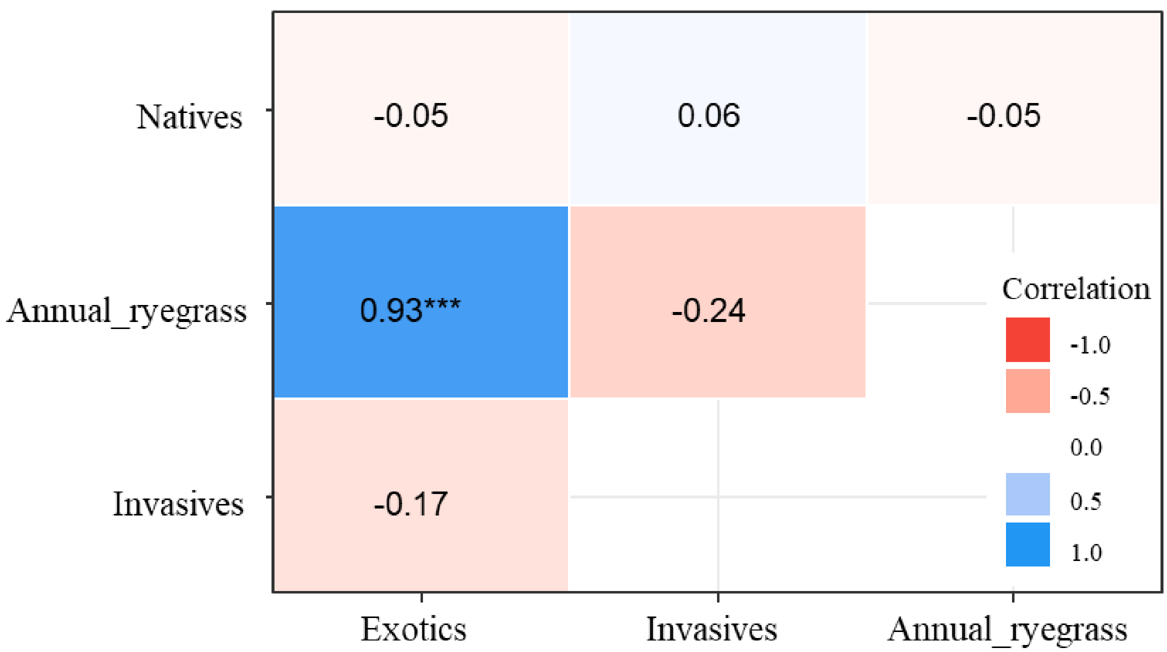

Figure 2-11. Correlation matrix showing spearman correlation statistics for relationships between the biomass of plant functional groups in all plots of the Kamloops Wastewater Treatment Plant site in 2022. 'Annual ryegrass' denotes the cover crop group. Asterisks on correlation coefficients denote significance level, ***p<0.001. Colors show the direction of the correlation, blue being positively correlated while red shows a negative correlation.

Soil Properties

The ART two-way ANOVA results demonstrated that at the KAM site, sowing treatments significantly affected SOM (F statistics = 5.1; $p < 0.05$), while soil treatments significantly affected all soil property parameters: pH (F statistics = 127.4; $p < 0.001$), SOM (F statistics = 6.2; $p < 0.01$), carbon (F statistics = 9.4; $p < 0.001$), nitrogen (F statistics = 36.4; $p < 0.001$), and C/N ratio (F statistics = 7.7; $p < 0.001$). Soil parameters of the 'Control' group significantly differ from the other three soil groups (Figures 2-12 & Figure 2-13). The 'High' group had lowest soil pH (Figures 2-12-A) and C/N ratio (Figure 2-13-C). For the SOM, 'Medium' and 'High' groups were significantly higher than the 'Control' group (Figures 2-12-B), while the 'Reseed' sowing group had a significantly higher SOM (Figures 2-12-C). The 'Low' group had a significantly lower nitrogen concentration than other two soil groups with biosolids additions (Figure 2-13-B).

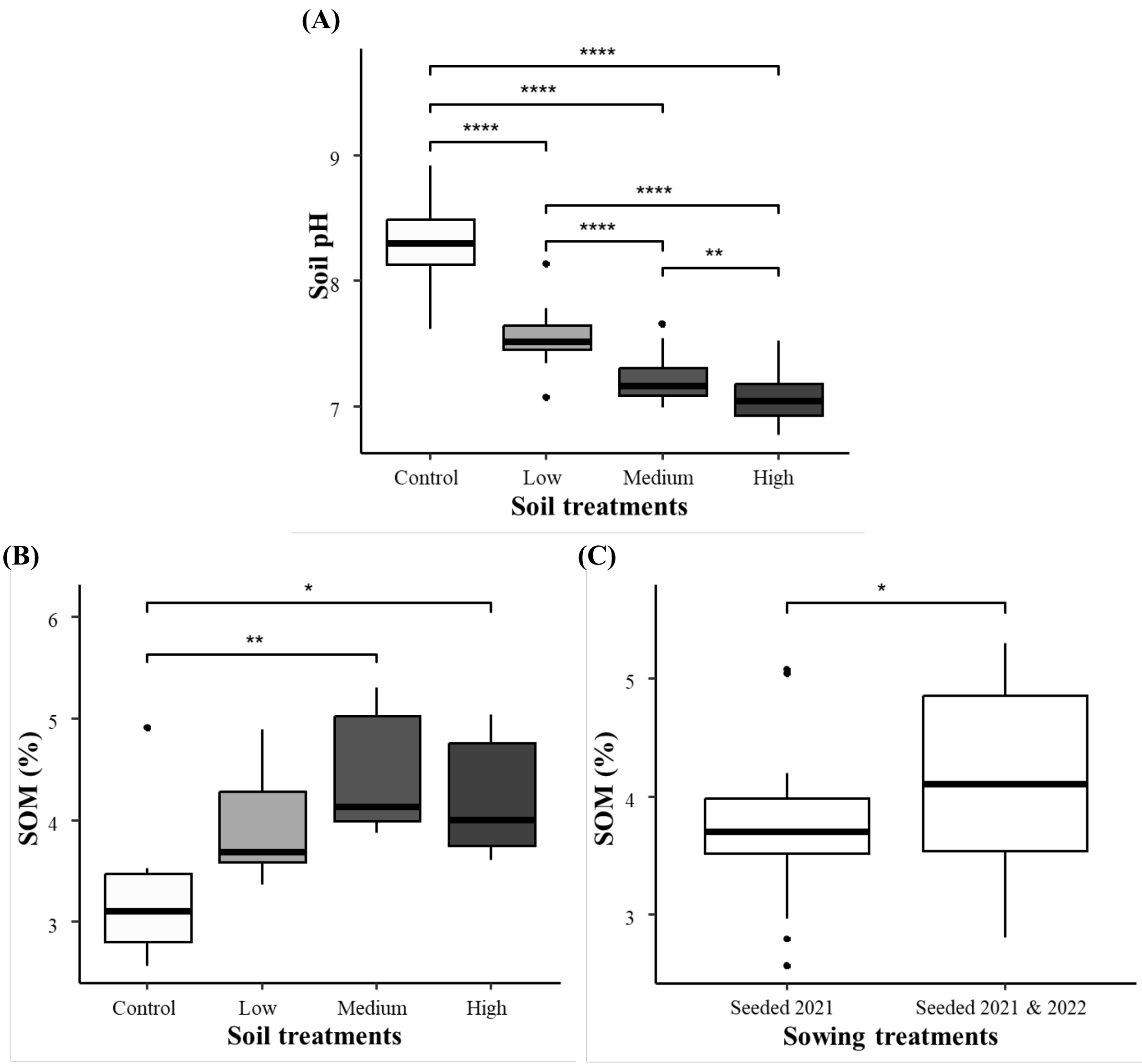

Figure 2-12. Soil pH of soils within 0-10 cm depth in different soil treatments (A), and soil organic matter (SOM) in different soil (B) and sowing treatments (C) at Kamloops Wastewater Treatment Plant site in 2022. Asterisk above brackets denote significant pairwise comparisons between soil treatments as determined by Tukey HSD with aligned rank transformed data, *p<0.05, **p<0.01, ****p<0.0001.

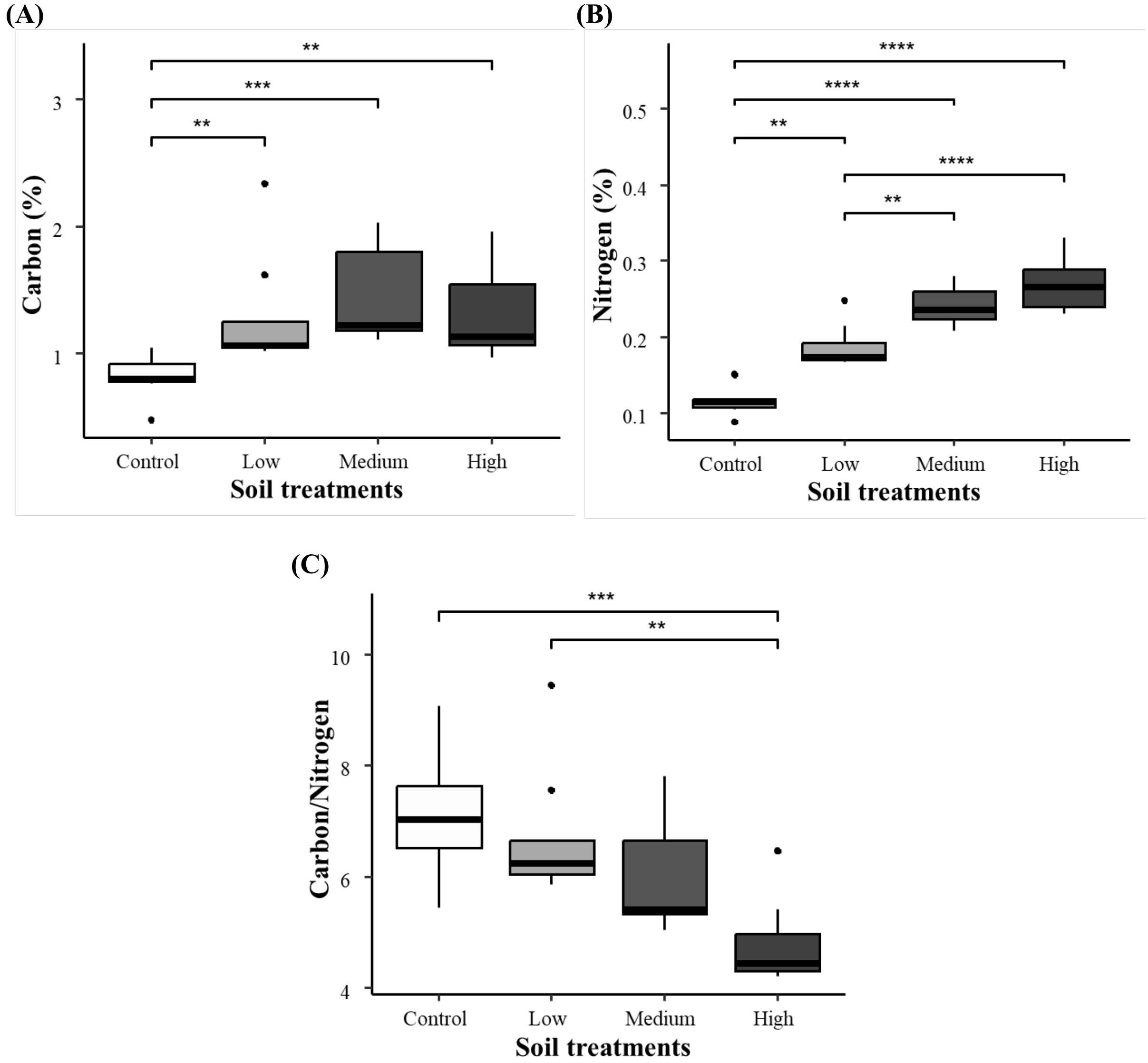

Figure 2-13. Total carbon (A), nitrogen (B), and C/N ratios (C) of soils within 0-10 cm depth in different soil treatments at Kamloops Wastewater Treatment Plant site in 2022. Asterisk(s) above brackets denote significant pairwise comparisons between soil treatments as determined by Tukey HSD with aligned rank transformation data, **p<0.01, ***p<0.001, ****p<0.0001.

As shown in Table 2-6, the soil content of Mo in each soil group and seeding group exceeded CCME guidelines for agricultural land use, and the soil content of Cd in ‘High’ soil group and Cu in ‘Medium’ soil group and ‘Reseed’ sowing group also exceeded the CCME guidelines for agricultural land use (CCME, 1999).

Table 2-6. Concentrations (mg/kg dry weight) of metals and trace elements in soils of different soil and sowing treatments at Kamloops Wastewater Treatment Plant site. Values are compared to the Canadian Council of Ministers of the Environment soil quality guidelines (CCME, 1999) guidelines for agricultural and industrial uses. Bolded values are in exceedance of at least one of the referenced guidelines.

	Mean +/- SD						CCME	CCME
Element	**Control**	**Low**	**Medium**	**High**	**Seed_Once**	**Reseed**	(agricultural)	(industrial)
Al	11,471 ± 1,004	11,513 ± 1,110	11,189 ± 1,163	11,139 ± 1,616	11,326 ± 1,171	11,331 ± 1,258	-	-
As	4 ± 1	5 ± 1	5 ± 2	5 ± 2	5 ± 1	5 ± 1	12	12
Cd	0 ± 0	0 ± 0	0 ± 0	**1 ± 1**	0 ± 1	0 ± 0	1.4	22
Ca	19,144 ± 1,026	18,299 ± 772	17,552 ± 445	17,589 ± 760	18,106 ± 1,205	18,187 ± 757	-	-
Cr	51 ± 3	49 ± 3	48 ± 2	48 ± 4	49 ± 3	49 ± 3	64	87
Cu	40 ± 9	43 ± 5	**56 ± 21**	48 ± 6	44 ± 7	**49 ± 17**	63	91
Fe	30,432 ± 1,463	28,459 ± 1,100	27,802 ± 1,102	28,247 ± 1,655	28,658 ± 1,903	28,813 ± 1,394	-	-
Pb	3 ± 1	5 ± 1	5 ± 2	5 ± 4	4 ± 3	4 ± 2	70	600
Mn	538 ± 24	504 ± 18	476 ± 15	491 ± 26	501 ± 36	503 ± 26	-	-
Mo	**5 ± 3**	**7 ± 7**	**6 ± 6**	**7 ± 7**	**7 ± 5**	**6 ± 6**	5	40
Mg	12,194 ± 491	12,100 ± 533	11,767 ± 437	11,503 ± 733	11,798 ± 727	11,984 ± 446	-	-
K	2,160 ± 136	2,570 ± 173	2,566 ± 92	2,431 ± 168	2,401 ± 171	2,462 ± 260	-	-
Na	1,296 ± 203	1,279 ± 179	1,410 ± 180	1,219 ± 171	1,300 ± 219	1,303 ± 159	-	-
Zn	93 ± 19	93 ± 8	106 ± 20	103 ± 20	97 ± 12	101 ± 22	250	410

Discussion

Vegetation Response

The addition of biosolids appeared to increase the biomass and species richness of plant communities, as the 'Control' group had significantly lower biomass and diversity than the other three groups with biosolids added in the CMM site (Figure 2-2, Figure 2-4), as well as the composition of plant communities of the 'Control' group was different from the other three groups with biosolid addition (Figure 2-6). However, there was no significant difference in biomass between the 'Control' group and the 'Medium' group at the KAM site, and there was no significant difference in species diversity between the 'Control' group and the 'Medium' and 'High' groups at the KAM site (Figure 2-10-A, Figure 2-10-C). Nonetheless, these beneficial effects of biosolids not only promoted the growth of native plants and cover crops, but also other exotic species (Table 2-4), and there were no significant negative associations between different plant groups (Figure 2-5, Figure 2-11). Nutrients from biosolids and the climate are potential causes of this situation. The nutrients that biosolids provide for plant growth are also used by unintended species (Table 2-4), allowing invasive and exotic plants from the surrounding environment and/or soil seed banks to establish mixed plant communities with intended species (selected native successional plants and the cover crop) (Table 2-2). Furthermore, the seeds of native successional plants and cover crops can be affected by climatic factors (e.g., drought), as seed germination and survival rates of native plants may have difficulty establishing a plant community in the early stages of the ecosystem due to climatic effects. The low species richness in 2021 may be due to the drought conditions experienced by both CMM and KAM sites (Figure 2-3, Table A-1, Table A-2). Unlike expectations, the reseeding practice in early spring did not significantly increase the productivity and diversity of native plants in the plant community (Figure 2-10), even though the second growing season had a better climate (Table A-1). As shown in Figure 2-3, the productivity and diversity of plant communities can be affected by differences in climate, soil conditions, or other environmental factors between years. The results of two long-term studies have shown that reseeding is an effective way to improve grassland productivity, as the aboveground biomass of reseeded sites increases after long-term growth (Zhang et al., 2020; Smith et al., 2003). The results of this experiment showed that reseeding did not significantly increase biomass, probably because the effect of reseeding requires a longer period to have a significant effect. However, in one of the long-term studies, reseeding did not significantly affect plant species richness (Zhang et al., 2020), which is consistent with the results of this study.

This study found that biosolids had a positive effect on plant productivity, probably due to the increased organic matter content improving the physicochemical conditions of the tailings (Shrestha et al., 2009). Consistent with the results of a related long-term experiment, this study observed a significant increase in biomass with biosolids application, but no further significant increases in biomass and species richness were observed beyond an application rate of 125 Mg/ha of biosolids ('Low' soil group), and there were no significant differences between the three biosolid-applied soil groups (Harris et al. 2021). This may explain the fact that the 'Low' soil group in this experiment had the best plant biomass and species richness (Figure 2-2, Figure 2-4, Figure 2-10), despite being the soil group with the least amount of biosolids added (Table 2-1). This suggests that higher application rates did not result in greater improvement in soil and vegetation communities compared to the 125 Mg/ha application rate ('Low' soil group). Differences in how these three biosolid-applied soil groups were associated in total and native plant communities suggest that the rates of biosolids application have varying effects on different plant species, for instance, the 'Medium' soil group has significantly lower native plant biomass than other two biosolid-applied soil groups (Figure 2-2, Figure 2-4).

Soil Response

Previous studies have reported that biosolids have positive effects on soil fertility and function (Nicholson et al., 2018; Gilmour et al., 2003; Bhogal et al., 2009; Case & Jensen, 2019). This would be consistent with the results of this study, as the 'Control' group had lowest SOM, carbon, and nitrogen, the C/N ratio was the highest among soil groups (Figure 2-7, Figure 2-8). In addition, the carbon and nitrogen concentrations in the CMM site were always significantly higher in the 'Medium' and 'High' soil groups than in the 'Low' soil group, and the SOM in the 'Medium' soil group was also significantly higher than that in the 'Low' soil group (Figure 2-7, Figure 2-8). Similar trends were observed between carbon and nitrogen concentrations and SOM among soil groups at KAM sites (Figure 2-12, Figure 2-

13). This result concurs with a previous short-term study: soil nutrients and organic matter are positively correlated with the amount of biosolids applied (Humphries et al., 2023). Furthermore, the plots of the 'Control' group at the CMM site had soil elemental compositions different from that of other three soil groups amended with biosolids (Figure 2-9).

The SOM of the 'Seed_Once' sowing group was significantly lower than that of the reseeding group (Figure 2-12), possibly because the significantly higher biomass in the 'Seed_Once' sowing group was partly contributed by SOM (Figure 2-10), and another possibility is that the reseeding group had more seeds, which may have more plant residues as a source of SOM in the 'Reseed' sowing group (Wang et al., 2019). The addition of organic matter to the soil improves soil properties like aeration, water holding capacity, and fertility. Thus, improved soils ensure a more stable and long-lasting productivity while reducing dependence on fertilizer, irrigation, and other external inputs (Oldfield et al., 2018).

Similar to the results of the CMM experiment (Figure 2-7), the soil pH with higher biosolids addition was always significantly higher than that of the soil group with lower biosolids addition in the KAM experiment (Figure 2-12), perhaps because biosolids may contain more organic acids or produce acidic substances during decomposition (Guo et al., 2022; Ning et al., 2021; Zhang et al., 2020). The original soil pH of the KAM site was above 8 (Figure 2-12), so the soil substrate may contain carbonates that act as a pH buffer to moderate soil acidification (Wang et al., 2015). Carbonates in the soil may be a factor in maintaining neutral soil pH in the biosolids-applied soil groups at the KAM site (Figure 2-12), while the soil pH was acidic in the biosolids-applied soil groups at the CMM site (Figure 2-7).

The 'Seed_Once' sowing group at the CMM site had a significantly higher C/N ratio than the 'Reseed' sowing group (Figure 2-8), perhaps because the reseeded group had more seeds than the single-seeded group, resulting in a higher microbial activity in the reseeded group (Wang et al., 2005). As microbial activity increases, soil carbon is depleted faster than nitrogen, resulting in a decrease in C/N ratio, which is also consistent with the similar trend of soil C/N ratio at the two sites (Wang et al., 2005). The soil nitrogen content of both sites was poor (Total Nitrogen $< 0.15\%$) only in the 'Control' soil group (Laekemariam & Kibret, 2020), and the soil C/N ratio was lower than the normal grassland C/N ratio range (13.4-

14.2) for all soil groups except the 'Control' soil group of the CMM site (Cleveland & Liptzin, 2007). The results show that the application of biosolids has made the poor soil not nitrogen deficient, but has added too much nitrogen to the soil, resulting in an imbalance in the soil C/N ratio. Biosolids are known as a source of nitrogen (Cogger et al., 2013), so woodchips were applied as a source of carbon (Gong et al., 2021).

Native plants are often not adapted to excess nitrogen, which may be one of the reasons why exotic plants have higher productivity and diversity as shown in table (Lejeune & Seastedt, 2001; Gaya Shivega & Aldrich-Wolfe, 2017; Lowe et al., 2002). 'Medium' and 'High' soil groups of both sites had the lower soil pH and C/N ratio among the soil treatments, probably they will have better performance in a long-term research (Figure 2-8, Figure 2-13). Reseeding can also lead to significant changes in soil properties, such as the results of a long-term study showing that reseeded soils had significantly higher SOM and nutrient levels than non-reseeded soils, which is consistent with the fact that their reseeded plots had significantly higher biomass (Zhang et al., 2020). In contrast, the results of this experiment showed that reseeding did not significantly increase soil nutrition or SOM, which may be due to the limited time.

The soil concentrations of As, Cu, Mo, Zn and Al at CMM site were relatively high (Table 2-5), and the concentrations of Cu, Zn, As and Pb were higher at the CMM site compared to the KAM site (Table 2-5, Table 2-6). Soil contents of Cu and As at the CMM site exceeded both the recommended agricultural and industrial thresholds, at the same time, Mo and Zn exceeded the recommended agricultural thresholds (CCME, 1999). This is an indication of the potential impact of these elements in the soil during environmental remediation and other land development in the region. Soil content of Mo at the KAM site also exceeded the recommended agricultural thresholds (Table 2-6). If the region of the KAM site will be developed for other uses (e.g., agricultural), the soil content of Mo needs to be considered. It should be noted that the high SOM inherent with the biosolids treatment can have the effect of binding heavy metals such that they are not biologically accessible (Farrell & Jones, 2009).

With reference to data from other studies, the high levels of Na and Fe in the soil at both sites and Mn at the CMM trial site deserve attention (Bibi et al., 2023; Abraham & Orikiriza, 2023; Liu et al., 2022). The availability of these elements with high concentrations in soil must also be taken into account, for example, the bioavailability of each element may be different (Zhao et al., 2020). As one of the major minerals in soils, aluminum (Al) is not essential for plant growth, but excess Al in very acidic soils may negatively affect plants (Haynes & Mokolobate, 2001; Vardar & Unal, 2007). If applying biosolids results in a lower soil pH, soil content of Al should be monitored where biosolids are applied, especially for high application rates of biosolids. However, arid environments rarely have very acidic soil (Dierickx, 2009; Li et al., 2024). Iron and aluminum oxides have good adsorption properties towards heavy metals, so the large amounts of iron and aluminum introduced by biosolid may help mitigate impacts of heavy metals on post-mining soils (Jacukowicz-Sobala et al., 2015).

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Chapter 3: Research Summary and Implications

Natural habitats and ecosystems within the semi-arid grasslands of British Columbia have been altered by a variety of human activities. Conservation and restoration of these grasslands is important because they provide many important ecological and economic benefits (Wilson, 2009; Grasslands Conservation Council of BC, 2017). Establishment of native plant communities and biodiversity is recommended for environmental restorations (Burton et al., 2006; Fraser et al., 2015), however, neither the soils nor the semi-arid environment of a closed mine site are ideal growth media and climatic conditions for native successional species (Gardner et al., 2012; Gupta et al., 2022). The purpose of this dissertation was to examine the effectiveness of various restoration practices, including 1) using biosolids to improve soil conditions, 2) using native successional species and cover plant seeds to establish a primary plant community, and 3) promoting native plant community establishment by a reseeding practice with native successional species and cover plants. This experiment was conducted concurrently in two grasslands with different environmental conditions beginning in the spring of 2021. One of the study sites was at the Thompson Very Dry Hot Bunchgrass Zone which ends in the summer end of 2022, and another study site was at the Cascade Dry Cool Interior Douglas-fir Zone which ended in the summer end of 2023.

Research Summary and Recommendations

All three biosolids addition rates applied in the study had better results on plant productivity and diversity than the control, with the low addition rate (100 dry Mg/ha) having the best results, especially in the Kamloops site. Biosolids had a positive effect on total plant productivity, but the reseeding practice of native successional species did not have significant effects on plant productivity and diversity. Higher application rates of biosolids and reseeding seeds in the second year may have a positive impact in a long-term study (Alvarez-Campos & Evanylo, 2019; McBride, 2022). When considering the use of biosolids for plant community management and ecological restoration, the balance between productivity and biodiversity must be considered. In particular, the proportion of native and invasive plants.

Considering the semi-arid environmental condition, native grassland restoration projects also need to monitor contingencies and develop timely responses to unusual climatic scenarios in order to achieve the restoration goal of accelerating the establishment of native plant communities while controlling invasive plant species.

As a soil amendment, biosolids can affect soil properties (Gutiérrez-Ginés, 2023), such as pH, carbon, nitrogen, SOM and elemental composition. Soil characteristics may be further improved by applying biosolids to promote the establishment of plant communities for a long-term.

Research Limitations & Future Opportunities

The greenhouse seed bank test detected only a fraction of the species present in the seed bank within 10 cm of the soil surface. With deeper and wider seedling trays and a longer experiment time to provide more nutrients and time for the seeds, this seed bank test should provide more information, such as a higher diversity and the density of individual species.

If a project insists on a one-time application of biosolids, the application rate of biosolids having the relatively best short-term effects may not have long-term effects to restore the degraded land for a long-term, sustainable nutrient cycle. When looking for biosolids application rates that are most beneficial to native plant communities, knowing how long these effects will last can help remediation projects consider whether to apply additional biosolids in future years. This study did not analyze the immediate effect of biosolids on the soil properties at the study sites due to pre-treatment sample size limitation, so it is recommended to collect sufficient soil samples at the study sites prior to the addition of biosolids.

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Appendix A

Weather Conditions

Drought is a recurring climatic condition in which there is insufficient precipitation over an extended period of time, resulting in water shortages. Because drought is an important factor that can affect plant growth, the experiment collected historical drought conditions for the test period and earlier at both sites. Historical drought conditions for each watershed in the province can be viewed on BC Drought Information Portal (BCDIP) (BC Ministry of Forests, 2023).

Table A-1. Drought conditions of study sites from 2016 to 2020 and experimental years (2021 to 2023). Median of drought level from 2016 to 2020 is the long-term normal. Experimental years are bold faced. Level of drought: 0 - no adverse impacts to socio-economic or ecosystem values, 1 - adverse impacts rare, 2 - adverse impacts unlikely, 3 - adverse impacts possible, 4 - adverse impacts likely, 5 - adverse impacts almost certain.

Study Site	Basins	Year	Max drought levels recorded			
			May	Jun	Jul	Aug
Kamloops Wastewater Treatment Plant	Middle Fraser	Long-term normal (2016-2020)	1	2	2	1
		2021	1	1	3	3
	Lower Thompson	**2022**	0	0	0	1
		2023	0	2	3	4
Copper Mountain Mine	Similkameen	Long-term normal (2016-2020)	1	2	2	2
		2021	0	1	3	3
		2022	0	0	0	1
		2023	0	3	4	5

Table A-2. Weather conditions of study sites from 1991 to 2020 and experimental years (2021 to 2023), data collected from Climate BC (Centre for Forest Conservation Genetics 2024). Experimental years are bold faced.

Study Site	Year	Mean Precipitation (mm)					Mean Temperature (°C)				
		Annual	May	Jun	Jul	Aug	Annual	May	Jun	Jul	Aug
Kamloops Wastewater Treatment Plant	1991-2020	276	24	34	28	20	9.2	14.7	17.9	21.4	20.7
	2021	240	12	10	11	34	9.7	14.5	21.8	24.5	20.7
	2022	309	23	57	30	20	9	12.7	17.6	23	23.7
	2023	211	21	27	13	18	10.7	18.5	19.9	23.2	22.1
Copper Mountain Mine	1991-2020	515	30	43	28	26	5.9	10	13.1	16.8	16.7
	2021	473	11	11	6	20	6.5	9.9	16.9	20	17
	2022	475	35	61	28	17	6.1	7.8	12.8	18.5	19.5
	2023	366	29	28	16	16	7	13.5	14.7	18.3	18

Drought conditions for the growing season in study years 2021 to 2023 and the past years 2015 to 2020 were obtained from BCDIP, as shown in Table A-1 (BC Ministry of Forests, 2023). The drought in the region belonging to the two study sites during the period from May to mid-August 2021 was maximum level 3, which could have had a significant impact on the establishment of the plant community in the first year of the experiment; the climate in 2022 was more favorable for the growth of the plant community than in other years; and the climate in 2023 was the driest compared to the previous years, reaching even level 5, which should have had a major impact on the plant community at the Copper Mountain Mine (CMM) site. As shown in Table A-2, the two study sites had lower annual precipitation and higher temperature in the experimental years than the long-term normal (1991 to 2020), except the Kamloops Wastewater Treatment Plant (KAM) site in 2022.

From 2015 to 2023, drought levels were typically higher at the CMM site than at the KAM site, and both sites had the highest drought levels in nine years in 2023, with both sites again having wetter climates in 2022 than the six years of record prior to the start of the experiment.

Early spring drought can affect the germination of seeds (Haeussler et al., 1995; Orsenigo et al., 2015), and the KAM site was first seeded in May 2021, when the basin was at the drought level 1 (Table A-1). Level 1 means that weather conditions are starting to become dry and the likelihood for adverse impacts to socio-economic or ecosystem values is rare (BC Ministry of Forests, 2023), but the 2021 Spring was the driest on record in Kamloops (Environment and Climate Change Canada, 2021). Drought conditions of both sites ranged from the level 1 to level 3 during the next three months of the first growing season (Table A-1), which may be a cause for low species richness of plant communities at the end of first growing season for both sites (Figure 2-4) in 2021. The 2022 growing season had the moistest climate among all growing seasons at both sites (Table A-1), and 2022 biomass and species richness were also highest at both sites. The 2023 growing season was the driest, which may be one reason why both biomass and species richness at the CMM sites were significantly lower in that year than in 2022.

Soil Substrates of the Two Sites

Figure A-1. Soil substrates of the study site at Copper Mountain Mine sieved 4 mm (A) and 2 mm (C), and soil substrates of the site at Kamloops Wastewater Treatment Plant sieved 4 mm (B) and 2 mm (D).

Metal and Trace Elements in Biosolids

Table A-3. Concentrations (mg/kg dry weight) of metals and trace elements in the biosolids applied on study sites. Class A biosolid was applied on the study site at Copper Mountain Mine, and class B biosolid was applied on the study site at Kamloops Wastewater Treatment Plant.

Biosolids	Elements (mg/kg)													
	K	Ca	Mg	Fe	Mn	Zn	Cu	Mo	Na	Al	Pb	Cr	Cd	As
Class A	1235	25300	4914	56722	391	1158	601	15	650	4303	27	52	2	7
Class B	7751	8786	5991	8281	125	292	368	8	1381	9569	4	24	1	3

Statistic data

Table A-4: Tukey HSD pairwise comparisons of CMM plant community species richness between each treatment grouping among combinations of soil and sowing treatments. Significant p values are bold faced.

Group 1	Group 2	df	t.ratio	p.value
Control,Reseed	Control,Seed_Once	269.0000	-0.0400	1.0000
Control,Reseed	High,Reseed	269.4213	-13.6524	0.0000
Control,Reseed	High,Seed_Once	269.0000	-10.1763	0.0000
Control,Reseed	Low,Reseed	269.5377	-14.0496	0.0000
Control,Reseed	Low,Seed_Once	269.0000	-15.4863	0.0000
Control,Reseed	Medium,Reseed	269.0000	-11.8599	0.0000
Control,Reseed	Medium,Seed_Once	269.0000	-13.2996	0.0000
Control,Seed_Once	High,Reseed	269.4213	-13.6117	0.0000
Control,Seed_Once	High,Seed_Once	269.0000	-10.1363	0.0000
Control,Seed_Once	Low,Reseed	269.5377	-14.0106	0.0000
Control,Seed_Once	Low,Seed_Once	269.0000	-15.4463	0.0000
Control,Seed_Once	Medium,Reseed	269.0000	-11.8199	0.0000
Control,Seed_Once	Medium,Seed_Once	269.0000	-13.2596	0.0000
High,Reseed	High,Seed_Once	269.4213	3.2976	0.0242
High,Reseed	Low,Reseed	270.7603	-0.9761	0.9775
High,Reseed	Low,Seed_Once	269.4213	-2.1056	0.4140
High,Reseed	Medium,Reseed	269.4213	1.5845	0.7593
High,Reseed	Medium,Seed_Once	269.4213	0.1195	1.0000

Group 1	Group 2	df	t.ratio	p.value
High,Seed_Once	Low,Reseed	269.5377	-4.1253	0.0013
High,Seed_Once	Low,Seed_Once	269.0000	-5.3100	0.0000
High,Seed_Once	Medium,Reseed	269.0000	-1.6836	0.6979
High,Seed_Once	Medium,Seed_Once	269.0000	-3.1233	0.0410
Low,Reseed	Low,Seed_Once	269.5377	-1.0532	0.9656
Low,Reseed	Medium,Reseed	269.5377	2.4834	0.2070
Low,Reseed	Medium,Seed_Once	269.5377	1.0794	0.9606
Low,Seed_Once	Medium,Reseed	269.0000	3.6265	0.0082
Low,Seed_Once	Medium,Seed_Once	269.0000	2.1867	0.3631
Medium,Reseed	Medium,Seed_Once	269.0000	-1.4397	0.8379

Table A-5: Mean plant canopy coverage (%) of every individual species occurred in each soil group (Control, Low, Medium, High) at the Kamloops Wastewater Treatment Plant (KAM) site and Copper Mountain Mine (CMM) site.

Study Sites	Year	Status	Species	Mean Coverage (%)			
				Control	Low	Medium	High
KAM	2021	Cover Crop	Annual ryegrass	0.5	0	0.04	0
		Native	**Total**	0	0	0	0
		Invasive	Goosefoot	0	55.38	28.58	13.83
			Summer Cypress	0	0.67	0	4.67
			Canada thistle	0	0.21	0.04	0
			Hairy nightshade	0	0.33	0	0
			Salsifies	0	0	0.08	0
			Bushy knotweed	0	0	0	0
			Total	0	56.58	28.71	18.5
		Exotic	**Total**	0	0	0	0
	2022	Cover Crop	Annual ryegrass	1.42	0.38	0.42	0.71
		Native	Aster douglasii	0	0	0.13	0
			Total	0	0	0.13	0
		Invasive	Goosefoot	0	0.75	0.04	0.63
			Summer Cypress	0	34.83	5.33	19.38
			Canada thistle	0	0	0.83	0
			Bushy knotweed	0	0.33	0	0
			Total	0	35.92	6.21	20
		Exotic	Loesel's tumble-mustard	0	0.63	0	0
			Total	0	0.63	0	0
CMM	2021	Cover Crop	Annual ryegrass	0.33	1.31	0.58	0.89
		Native	Total	0	0	0	0
		Invasive	Goosefoot	0.14	0	0	0.25
			Knapweed	0	0.14	0	0
			Cheatgrass	0	0.03	0	0
			Total	0.14	0.17	0	0.25
		Exotic	Black medic	0	0.17	0	0
			Alfalfa	0	0.36	0	0
			Total	0	0.53	0	0

Study Sites	Year	Status	Species	Mean Coverage (%)			
				Control	Low	Medium	High
CMM	2022	Cover Crop	Annual ryegrass	0	2.92	1.33	1.92
		Native	Bluebunch wheatgrass	0	0.78	0.06	0.06
			Blanket flower	0.06	0	0	0
			Nodding brome	0.14	6.89	5.47	15.03
			Slender hawksbeard	0.03	7.72	6.5	7.06
			Rough fescue	0.11	0.44	0.89	0.17
			Pinegrass	0	0	0.03	0
			Peppergrass	0	0	0	0.03
			Slender wheatgrass	0	0.44	0.06	0.19
			Total	0.33	16.28	13	22.53
		Invasive	Canada thistle	0	0.06	0	0.06
			Knapweed	0	4.94	1.67	1
			Shepherd's purse	0	0.08	0	0.42
			Cheatgrass	0	17.94	27.97	8.06
			Great mullein	0	0.69	0	0
			Common sow-thistle	0	0.64	0.94	1.86
			Bushy knotweed	0.03	0.14	0.19	0.03
			Total	0.03	25.14	32.08	11.97
		Exotic	Alfalfa	0.03	0.28	0.44	2.14
			Loesel's tumble-mustard	0	4.89	4.86	4.25
			Kentucky bluegrass	0	0.78	1.31	0.17
			Crested wheatgrass	0	0.08	0.03	0.06
			Red clover	0.25	0	0	0
			Total	0.28	6.03	6.64	6.61
	2023	Cover Crop	Annual ryegrass	0.00	0.75	0.31	0.14
		Native	Bluebunch wheatgrass	0	0.83	0.17	0.03
			Nodding brome	0	15	4.97	11.14
			Timber oatgrass	0.08	1.28	0	0
			Total	0.08	17.11	5.14	11.17
		Invasive	Goosefoot	0	1.28	0.58	0.39
			Knapweed	0.03	3.67	0.28	0.19
			Cheatgrass	0.03	22.33	22.5	12.19

Study Sites	Year	Status	Species	Mean Coverage (%)			
				Control	Low	Medium	High
CMM	2023	Invasive	Great mullein	0.06	0	0	0
			Bushy knotweed	0	0.06	0	0.19
			Total	0.11	27.33	23.36	12.97
		Exotic	Black medic	2.58	0	0	0
			Alfalfa	0.44	0.11	0	0.08
			Loesel's tumble-mustard	0.03	10.89	21	32.5
			Kentucky bluegrass	0	0.08	0	0
			Red clover	0.19	0	0	0
			Prickly lettuce	2.44	0.61	0.28	0.56
			Total	5.69	11.69	21.28	33.14

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