FACILITATING NATIVE PLANT RECOVERY ON COPPER MINE TAILINGS IN THE SEMIARID GRASSLANDS OF SOUTHERN INTERIOR BRITISH COLUMBIA

by

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ABSTRACT

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The semiarid grasslands of interior British Columbia (B.C.) are a unique ecosystem which provides an array of economic and ecological resources, but historic and current human and environmental pressures have led to their decline. Surface mining involves stripping the natural vegetation and topsoil, altering of natural topography, and deposition of waste materials (e.g. tailings and waste rock) at the landscape level. Restoration of natural soil processes and native vegetation communities on such sites is challenging because 1) the mine soils are often characterized by having adverse physiochemical properties such as high metals content, extreme pH, and low organic matter, 2) there are barriers to native seed acquisition and native species do not perform well on nutrient poor sites, and 3) seedling germination and establishment is limited in semiarid environments because of prevailing harsh climatic conditions. The primary objectives at the onset of mine reclamation are to ameliorate the physical and chemical properties of the soils (usually with an organic amendment) and establish a sustainable vegetative cover to prevent wind and water erosion of metals and other harmful contaminants (a process known as phytostabilization). Facilitation by nurse plants and cover crops has recently come to the forefront as a potentially promising practice for restoring natural communities on degraded sites in stressful environments. The objectives of this thesis were to 1) investigate the suitability of locally available organic soil amendments and native bunchgrasses (*Pseudoroegneria spicata* and *Festuca campestris*) for reclamation at the Historic Afton Tailings Storage Facility (TSF), near Kamloops, B.C., in a greenhouse study and 2) assess the facilitative effects of soil amendments, *Artemesia tridentata* nurse plants, and agronomic cover crops on various abiotic and biotic parameters relating to native grassland species establishment at the TSF. In the greenhouse, plants were grown in various ash-compost-wood chip combinations and were evaluated using a randomized complete block design with 13 treatments and 10 replicates. In the field, native plants were seeded in small (1.23 m^2) study plots with cover crops and/or planted with nurse plants in amended tailings and assessed in a randomized complete block design with 8 treatments and 3 replicates. The results of the greenhouse study indicated that compost was the most effective amendment, as it effectively ameliorated tailings physiochemical properties and promoted significantly greater seedling production. Analysis of shoot tissue elemental

concentrations after 90 d growth determined that both species were not suitable candidates for phytostabilization because they accumulated high amounts of molybdenum. *Artemisia tridentata* nurse plants appeared to exert some facilitative effects including shading which resulted in lower soil temperatures early in the growing season, but there was also some evidence that they competed for soil moisture. Nonetheless, plant species diversity was higher under nurse plants compared to in the open, which indicated that some facilitative mechanisms may be at play. Cover crops appeared to have a negative effect on native plant establishment which was likely because of their aggressive growth characteristics and increased competition for soil moisture. The findings of this study provide important considerations for mine restoration practices in B.C.'s semiarid grasslands.

Keywords: Facilitation, nurse plants, cover crops, soil amendments, semiarid grassland, native species

DEDICATION

I hereby dedicate this thesis to my grandfather, Peter L. Antonelli, and my grandmother, Marylu Walters, who have both provided a great amount of support and encouragement throughout my academic pursuit. Thank you.

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CHAPTER 1 – INTRODUCTION

Human activities such as urban development and conversion of land for agriculture and industrialization have been ongoing for over a century in order to meet humankind's current societal and economic needs, but these activities have resulted in alteration to our natural habitats and ecosystems. These land use changes are also contributing to global climate change by disrupting the terrestrial carbon cycle (IPCC, 2014). Globally, extinction rates are estimated to be 100 to 1000 times higher than natural background rates (Ceballos et al., 2015; Thuiller, 2007) and habitat alteration is suspected to be the leading cause (Barnosky et al., 2011). Such human-induced rapid losses in biodiversity and natural habitats are threatening the global ecosystems and the valuable ecosystem services which they provide (Dirzo and Raven, 2003; Mace et al., 2012). By focusing on repairing natural habitats and ecosystems at the site level, the practice of ecological restoration offers mitigation of these environmental problems.

THE IMPORTANCE OF GRASSLANDS

Grasslands are one of many of the Earth's ecosystems experiencing environmental pressures from land use change and global climate change. Conversion of grasslands to agricultural crops, livestock pastures, urban areas, and industrial land continues to be problematic as the human population rises and societies continue to develop. These unique biomes – which cover almost 4 billion hectares (27%) of the earth's surface (Costanza et al., 1997) – are an important natural resource because they provide an array of important ecosystem services such as wildlife habitat, animal forage, pollination, erosion protection and carbon sequestration (Wilson, 2009). Though it is difficult to put a price on these precious benefits, a recent study estimated the value of these ecosystem services to be over $900 billion (USD) per year (Costanza et al., 1997). When grassland areas are converted to alternate land uses, not only is the natural capital lost, but so are these important ecological functions – which play a vital role in maintaining the health and well-being of the global ecosystem. Ecological restoration offers hope in returning these degraded ecosystems to their pre-disturbed condition (Bradshaw, 1987a).

The semiarid grasslands of British Columbia (B.C.) cover less than 1% of the province's total land area yet are home to over 30% of its species at risk (B.C. Conservation Data Centre, 2017), making them one of Canada's most valued hotspots for biodiversity (Grassland

Conservation Council of BC, 2017). The grasslands of southern interior B.C. are considered a northern extension of the Great Basin region that spans from central Oregon through Washington, Idaho and Montana, referred to as Palouse Prairie (Shorthouse, 2010). They are currently in decline due to factors such as urban sprawl, industrial development, agriculture, over-grazing, tree encroachment and invasive plants. The grasslands across the region have experienced an extensive history of livestock grazing which has altered natural plant communities (Huber-Sannwald and Pyke, 2005; van Ryswyk et al., 1966; Wilson, 2009). These areas also coincide with valuable underground mineral resources thus are attractive areas for mineral exploration and mining. Generally, semiarid grasslands are extremely sensitive to disturbances because seedling establishment and soil development are limited by the hot and dry climate characteristic of grasslands (Jing et al., 2014; Munson and Lauenroth, 2012). As alteration of these fragile grassland habitats continues, efforts of conservation and ecological restoration are gaining immense importance.

THE MINING INDUSTRY AND ITS IMPACTS ON THE ENVIRONMENT

With the exponential rise in human population, industrialization, economic development and technological advancements, the global demand for minerals is ever-increasing. Canada is a global leader in mineral and metals production. The mining industry is a significant component of Canada's society and economy, employing over 350,000 people across the country and retaining the largest proportion of aboriginal workers out of any private industry (Mining Association of Canada, 2016). In 2015, Canada's mining industry contributed $56 billion (3.4%) to the total GDP (Mining Association of Canada, 2016). With an abundance of underground mineral resources, British Columbia is one of Canada's top four metal producers and the industry was recently valued at $5.9 billion per year (Mining Association of Canada, 2016). There are currently a total of 14 major metal and coal mines operating in BC, with several others proposed or undergoing development (Mining Association of British Columbia, 2017).

As a resource-driven economy, it is important for Canada to encourage environmental protection and sustainability in the mining industry if it is to maintain its status as a global mining leader. Although the mining industry is both economically and socially beneficial, the activities involved in mineral extraction, particularly during open pit and surface mining, can cause enormous environmental damage to terrestrial ecosystems (Shrestha and Lal, 2011; Ussiri

and Lal, 2005). This is because during the mining process 1) removal of natural vegetation, topsoil, overburden (unconsolidated material), and bedrock is required prior to accessing the desired below-ground mineral resources and 2) the waste materials (e.g. tailings, waste rock) are often deposited at the surface, in immense quantities, over large areas of the landscape, which damages or destroys pre-existing vegetation and soils. In many cases, these landscape level disturbances result in long term impairment of the pre-existing ecosystem functions (Bradshaw, 1997). The ecological consequences of mining include habitat degradation, losses in biodiversity, alteration of natural landscapes, and changes in hydrologic patterns (Sheoran et al., 2010; Shrestha and Lal, 2011). Further, the loss in vegetation and soils leads to a reduction in the natural capacity of terrestrial ecosystems to sequester atmospheric carbon and regulate the global climate (Shrestha and Lal, 2006).

The disturbances caused by mining generally include tailings storage facilities, waste rock dumps, barren stripped areas, roadsides and degraded land used for industrial facilities. It is estimated that over 0.4 million hectares of land has been disturbed from mining in Canada (Gardner et al., 2010). In BC, approximately 0.05% (~45,000 ha) of the land base has been altered by mining (BC Technical and Research Committee on Reclamation, 2017). British Columbia's mining industry has committed to prioritizing environmental protection and sustainability throughout all phases of the mine cycle. Reclamation is the final phase of mining and involves returning the mined land into a useful and productive state. The BC Mines Act provides legislation regarding how mining activities are carried out and includes guidelines for reclamation (Government of British Columbia, Ministry of Energy, 2008).

MINE RECLAMATION

If left to natural processes, mine sites can take hundreds of years to recover from the environmental damage inflicted during the mining process (Bradshaw, 1987a). Ecological restoration is the process of assisting the recovery of disturbed ecosystems to their original state (Bradshaw, 1997). Restoration practitioners strive to achieve similar structure and functions as the pre-existing ecosystems (Palmer et al., 2006). Reclamation is more concerned with achieving a socially acceptable new use for the land that does not necessarily coincide with its prior ecological state (Bradshaw, 1997), a common example being the conversion of mined land to livestock pasture, agricultural crops or wildlife habitat (Akala and Lal, 2001; Tian et al., 2009;

Wood et al., 1995). This type of conventional mine reclamation is becoming less desirable as these 'surface-mine grasslands' tend to lack the diversity, structure and function of natural ecosystems (Brothers, 1990; Viall et al., 2014; Wu et al., 2011). More recently, restoration of mine sites to their previous, natural habitat in order to enhance biodiversity and reinstate natural ecosystem services is becoming the standard (Lesica and Allendorf, 1999). It is now widely recognized that re-establishing biodiversity is the key to achieving restoration success, as diverse ecological communities are more resilient to environmental disturbances (Ives and Cardinale, 2004). To accomplish this, practitioners are urged to use ecological theory and principles as restoration tools to mimic natural processes of ecosystem development during restoration efforts (Bradshaw, 1997).

For mine restoration to be successful, natural soil processes need to be initiated and a self-sustainable, native vegetative cover needs to be established. The starting point for any land restoration project is the soil, or the degraded parent material left behind that has potential to develop into a soil over time (Bradshaw, 1987a). Plants and soils interact to create a positive feedback loop that is the primary driving force of soil development (Brady, 1990). Plants assist in soil development by taking up nutrients from deep soil layers and redistributing them at the surface as organic matter. The organic carbon of which they are comprised was fixed from the atmosphere and is the food source for soil microbes and microfauna which facilitate decomposition, nutrient cycling and the development of soil aggregates (Bradshaw, 1997). Plants also protect soils from erosion and allow for the accumulation of windborne particles (Bradshaw, 1997). This constant cycle leads to the maturing of soils and the development of distinct soil horizons (Brady, 1990). The initiation of these soil development processes on degraded mine sites depends on the initial capacity of the soils to support plant life (Shrestha and Lal, 2006).

The major problems of mine soils are their poor physical and chemical properties that limit plant establishment and growth (Bradshaw, 1997; Sheoran et al., 2010). For example, tailings, which are the waste by-product of ore processing, are low in organic matter and nutrients (Gardner et al., 2010) and have elevated levels of certain heavy metals (Hayes et al., 2009). These materials are often prone to compaction from heavy equipment (Ussiri and Lal, 2005) and have poor water retaining capacity (Cele and Maboeta, 2016). It is not uncommon for pH (Solís-Dominguez et al., 2012) and salinity (Bai et al., 2017) levels to be out of the ordinary which can exuberate issues with metal toxicity (Bolan et al., 2014). Also, microbial populations

in tailings are either severely altered or virtually non-existent (Pepper et al., 2012). In arid and semiarid environments, additional challenges arise from tailings being prone to wind and water erosion (Mendez and Maier, 2007). The immediate goal of tailings reclamation is to establish a vegetative cover to stabilize the loose material in place in a process known as phytostabilization (Mendez and Maier, 2007; Neuman and Ford, 2006). To enhance reclamation success on mine sites, practitioners can use a variety of site preparation techniques derived from ecological theory and principles (Bradshaw, 1997).

Soil Amendments

In mine restoration, there are four fundamental challenges of site preparation to overcome: 1) restoring soil physical structure, 2) retaining soil moisture, 3) providing plant nutrients, and 4) reducing phytotoxicity (Bradshaw, 1987a; Piorkowski et al., 2015). Soil amendments are commonly used in revegetation projects to mitigate these ecological shortcomings. Traditional reclamation involved the application of the topsoil stockpiled during mining operations, but this material is often limiting, so soil amendments need to be imported from external sources.

Commonly used soil amendments include inorganic fertilizers, organic amendments and liming amendments. Long term success with chemical fertilizers is low due to issues with leaching and the need for constant re-application to sustain plant nutrient needs (Gardner et al., 2010). Since the mine soils are low in organic matter, the key is to incorporate nutrient-rich organic materials with enough of a carbon source to initiate microbial activity and nutrient cycling. These materials are called organic amendments and include municipal compost, wood chips, straw mulch, paper/pulp and municipal sewage sludge. Liming amendments are primarily used to reduce soil toxicity by neutralizing acidic soils and immobilizing heavy metals (Brown et al., 2007; Piorkowski et al., 2015). Since each soil amendment possesses its own unique characteristics, plant performance is often optimized when a blend of amendments is utilized rather than a single amendment (Piorkowski et al., 2015). Revegetation success is often greater when soil amendments are used prior to seeding. For this practice to be economical, amendments must be imported from local sources because the cost of transportation is high and most projects require large volumes to cover the vast area being revegetated. Since soil amendments can be waste by-products of various industries (e.g. pulp and saw mills, sewage treatment plants,

composting facilities), the use of these products for mine reclamation can be mutually beneficial for both the waste generator and the mining operation.

Seeding

Since dispersal distance of native grassland species from surrounding areas is limited (Ejrnæs et al., 2006), the establishment of a vegetative cover on mine sites is achieved by seeding and/or transplanting. The techniques for seeding include broadcast seeding, hydro-seeding, drill seeding and hand seeding. Seeding gives restoration practitioners the benefit of choosing which species are introduced to a site. A long-standing ecological theory suggests that initial floristic composition determines the trajectory of which plant communities develop and change over time, and the type of inter-specific interactions that will take place (Egler, 1954). Furthermore, there is evidence that early-growing species can exert "priority effects" (competitive advantages) that prevent the establishment of slower-growing perennial species (Grman and Suding, 2010; Plückers et al., 2013). When taking this theory into consideration, careful selection of appropriate species is important because the composition of the initial seed mix can determine the outcome of restoration (Larson et al., 2011; Munson and Lauenroth, 2012).

Conventional reclamation often involves planting non-native or agronomic crops to achieve erosion control and forage value in the short-term. From an economic standpoint, agronomic species are desirable because they are inexpensive and available in large quantities from several suppliers. They also tend to establish very rapidly on poor sites, allowing for less intensive site preparation and quick return of environmental and economic benefits (Skousen and Venable, 2008; Wu et al., 2011). However, research has shown that these species are extremely competitive and can prevent native species from establishing (Dormaar et al., 1995; Hagen et al., 2014). Recently, it has been argued that agronomics can alter the trajectory of natural succession by preventing the colonization of native species (Davis et al., 2005). These agronomic systems are also known for their low species diversity (Dormaar et al., 1995). In this sense, seeding with agronomics can prevent a site from reaching its full ecological potential. However, some work has shown that given adequate time, native species can, in fact, colonize a site that was initially seeded with agronomics (Skousen and Venable, 2008). This occurred when sites were seeded with annual or biennial 'cover crops' which are often used to provide quick cover and temporary

erosion control while facilitating the establishment of native species. This discrepancy in the literature merits further investigation into the use of agronomic species for mine restoration.

More recently, attention is shifting towards the use of native species in restoration projects for many reasons (Burton and Burton, 2002; Godefroid et al., 2011; Kiehl et al., 2010). Firstly, the introduction of native species is essential if pre-existing ecosystems are to be reconstructed and the process of natural succession is to be initiated (Godefroid et al., 2011). Secondly, restoration with native species is often a more desirable land-use objective for First Nations groups, who are often primary stakeholders of lands disturbed by mining operations. Finally, native plant communities tend to be more diverse and offer greater ecosystem services compared to non-native communities (Bradshaw, 1987a; Dormaar et al., 1995), although this is not always the case in restoration (Ross, 2004). Unlike agronomics, establishment success of native species is often limited on degraded sites (Burton et al., 2006; Drozdowski et al., 2012). Additionally, native seed is difficult to obtain in bulk quantities due to a lack of suppliers, and so the market prices can be high (Burton and Burton, 2002). Also, native species do not establish well in hydro-seeding mixtures which are often used in reclamation as an economical means of applying seed to challenging terrain (Oliveira et al., 2013, 2012).

Phytostabilization

Controlling the dispersion of dust is a central challenge for mines operating in arid and semiarid regions (Mendez and Maier, 2007). Airborne dust from mine lands such as tailings impoundments and waste rock dumps can contain heavy metal and metalloid contaminants that cause negative impacts to human health and surrounding ecosystems (EPA, 2016). As such, mining companies are required by law to implement on-site dust control measures to mitigate these negative effects. Industrial chemical tackifiers are an effective solution for controlling dust in the short term, but do not provide a sustainable long term solution because they are expensive and tend to degrade with time without repeated application (Mendez and Maier, 2007). Phytostabilization is a remediation technique aimed at restoring vegetative cover on barren land as a means to control dust and stabilize contaminants belowground (Mendez and Maier, 2007; Neuman and Ford, 2006). Appropriate species for phytostabilization are native species that 1) are adapted the regional climate, 2) tolerate increased levels of heavy metals and metalloids, and 3) minimize shoot uptake while maximizing root uptake of contaminants (Mendez and Maier,

2007). Site specific research of species suitability for phytostabilization is needed to add to the growing body of literature (Solís-Dominguez et al., 2012).

Facilitation and Nurse Plants

For ecological restoration to be successful, it is essential to understand what natural processes take place, what components make up a given ecosystem in its early successional phase, and what limits these processes and components from developing over time (Bradshaw, 1987b). Species interactions are a widely researched topic in terrestrial and aquatic ecology. Species interactions include negative (competition) and positive (facilitation) inter-species interactions, and interacting species often concurrently exert both negative and positive effects on one another (Callaway and Walker, 1997). Facilitation results when the net effect of such interactions is positive. Until recently, ecologists have focussed their research efforts on negative interactions, and theorized that competition is the driving force of ecological succession (Cavieres and Badano, 2009; Grime, 1973; Tilman, 1982). However, research conducted within the past two decades suggests that facilitation also plays an important role in shaping population and community dynamics in both terrestrial and marine environments (Bertness and Callaway, 1994; Bruno et al., 2003). The concept of facilitation encompasses mutualistic interspecies relationships that are both facultative and obligate, and occurs when there is either a direct or indirect positive interaction between associated organisms that are present within the same space and time (Bruno et al., 2003). One example of a positive species interaction occurs during primary succession of glacial recession zones, where edaphic conditions are enhanced by pioneer plants and trees, which, in turn, enables late successional species to successfully colonize the area (Crocker and Major, 1955). Another well-documented example of facilitation in terrestrial environments is the symbiosis between arbuscular mycorrhizal fungi and vascular plants (Quilambo, 2003), a mutualistic interaction involving nutrient and moisture exchange that empowers plant beneficiaries to exist beyond their fundamental niche (Bruno et al., 2003). The practice of revegetating former mine lands with native species requires expanding the fundamental niche of the target native plant community, thus, applying the theory of facilitation into reclamation practices may be beneficial in establishing native plant communities on former mine sites.

It is theorized that the degree of facilitation increases with abiotic stress (Bertness and Callaway, 1994), which explains why several examples of facilitation have been documented in harsh environments, such as those which experience arid or semiarid climates (Padilla and Pugnaire, 2006). Numerous studies have demonstrated that 'nurse plants' play an important role in such environments (Claus Holzapfel and Mahall, 1999; Maestre et al., 2003; Pugnaire et al., 1996). Nurse plants facilitate the growth of neighboring plants by modifying the local abiotic and biotic environment, and the degree of such beneficial effects depends on several factors, including nurse plant life history stage and root physiology (Callaway and Walker, 1997). In arid and semiarid environments, certain shrubs exert a 'nurse effect' on associated grasses and other plants, by shading out solar radiation, which in turn reduces local soil evaporation rates, soil temperature, and plant tissue damage, leading to noticeably enhanced growing conditions for the emerging understory plant community (Padilla and Pugnaire, 2006). Some studies have shown that plant community diversity is greater under nurse plants than in the open (Cavieres and Badano, 2009; Franco and Nobel, 1989). For example Pugnaire et al. (1996) reported improved understory production and plant species diversity under the canopies of a leguminous shrub in a semiarid region in Spain, and that the facilitative effects increased with shrub age and size. Nurse plants can also augment local soil moisture by drawing water from deep soil layers to the surface through a process known as 'hydraulic lifting' (Padilla and Pugnaire, 2006). This phenomenon has been observed for *Artemesia tridentata* (big sagebrush) shrubs (Cardon et al., 2013; Richards and Caldwell, 1987), which are common in B.C.'s semiarid grasslands. Some studies have also reported enhanced soil nitrogen levels under *A. tridentata* shrubs (Burke et al., 1989; Cardon et al., 2013). Although one study by Huber-Sannwald and Pyke (2005), conducted in a semiarid rangeland, showed minimal success of a target bunchgrass community under adult *A. tridentata* shrubs, there is potential for these shrubs to act as a nurse plant in mine reclamation settings, especially considering the establishment success of exhibited by Booth et al., (2003) on reclaimed mine lands.

Cover Crops

Another method of facilitating target plant community establishment is through the use of cover crops. Cover crops are generally used in agricultural settings and in various restoration projects to mitigate soil erosion and prevent the establishment of weedy species, but can also be

used to enhance abiotic and biotic conditions for target plant communities (Espeland and Perkins, 2013). A typical cover crop is made up of agronomic species and often includes annual or short-lived perennial grasses and legumes. Together these plants effectively provide a variety of functions including soil stabilization and improved soil fertility through soil organic matter inputs and atmospheric N-fixation (Espeland and Perkins, 2013; Moro et al., 1997). One would suspect that competition from agronomic cover crop species may inhibit the development of the target community, but research shows that this is less likely in limiting or stressful environments (Espeland and Perkins, 2013). In stressful environments, cover crops can ameliorate harsh abiotic conditions, which allows for increased success of neighboring target species (Maestre et al., 2009, 2003). Based on these results, there is potential to utilize agronomic cover crops in mine reclamation projects to facilitate the establishment of target native plant communities.

THESIS RESEARCH OBJECTIVES

This study delves into contemporary ecological theory in order to address the question of whether facilitation – using nurse plants and cover crops – can be applied in a mine reclamation setting to improve native grassland recovery at the Historic Afton Tailings Storage Facility (TSF) near Kamloops, B.C. In doing so, this study will also look at the suitability of locally available soil amendments for reclamation at the TSF. To address these questions, I have conducted a two-part study involving both a greenhouse (Chapter 2) and a field (Chapter 3) component. The objectives of the greenhouse study are twofold: 1) to investigate the suitability of two native, semiarid bunchgrasses (*Pseudoroegneria spicata* and *Festuca campestris*) for phytostabilization of mine tailings, and 2) to assess the optimum ratio of soil amendments for establishment of these species. The objectives of the field study are threefold: 1) to assess the effects of *Artemisia tridentata* nurse plants on the abiotic environment and plant community establishment during early restoration, 2) to examine whether agronomic cover crops facilitate or impede native grassland species establishment, and 3) to test the suitability of soil amendments for mine tailings rehabilitation. Also included is an Appendix with additional results from a seed germination trial conducted in the greenhouse (Appendix A) and laboratory analytical chemistry results for the experimental materials (tailings and soil amendments) (Appendix B). The results of this thesis will contribute to the body of knowledge regarding facilitation in semiarid

environments and will be of benefit to restoration ecology scientists and practitioners conducting reclamation work within B.C.'s semiarid grasslands and similar environments.

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CHAPTER 2 – GROWTH RESPONSE AND METALS UPTAKE OF NATIVE BUNCHGRASSES DURING ORGANIC AMENDMENT-ASSISTED PHYTOSTABILIZATION OF ALKALINE MINE TAILINGS

INTRODUCTION

As of 2017, there were 14 major metal and coal mines operating in British Columbia (B.C.), with several more either undergoing care and maintenance or awaiting approval from the environmental assessment process (Mining Association of British Columbia, 2017). Some of these projects are located within the interior semi-arid grasslands, which are a unique ecoregion characterized by hot, dry summers and minimal annual precipitation (Shorthouse, 2010). Tailings management is one of several challenges faced by mines operating within these grasslands and other dry environments. If left barren, dust from mine waste sites can spread over long distances through eolian dispersion and water erosion, posing a risk to human and environmental health (Mendez and Maier, 2007). Fine particulate waste materials (e.g. tailings, waste rock) that are stored on mine sites are often high in toxic metals and other contaminants which can cause adverse human health effects including respiratory disease, heart failure, and lung cancer, and also impact the surrounding environment by altering water chemistry and causing soil contamination (EPA, 2016). Conventional remediation methods for controlling tailings dust include chemical (e.g. industrial tackifiers) and physical (e.g. waste rock, gravel, or clay capping) stabilization, however, these methods are costly and do not provide a long term solution (Mendez and Maier, 2007). Phytostabilization is an emerging technology which involves promoting vegetation growth on barren mine lands to control erosion and stabilize metals belowground, and may be a more sustainable alternative compared to conventional remediation techniques.

The goal of phytostabilization is to create a long-term vegetative cap in order to limit the movement of harmful metal contaminants from mine sites. Once established, the aboveground portion of the vegetation (canopy) acts to reduce wind erosion, whereas the belowground portion (roots) limits water erosion and immobilizes metals in the rhizosphere. The belowground processes involved in phytostabilization include precipitation of metals by bacterial and root surfaces, precipitation of metals by bacterial and root secretions, bacterial uptake of metals, and root uptake of metals (Mendez and Maier, 2007). Phytostabilization differs from phytoextraction

(another phytoremediation technology) in that the aim is to reduce metal bioavailability and the risk of metals entering the food chain by minimizing shoot uptake and sequestrating metals belowground via plant roots and exudates. Contrarily, phytoextraction involves remediating contaminated materials by promoting hyperaccumulation of metals in shoots and requires removal of the toxic plant biomass from site which can be laborious and costly (Bolan et al., 2014). As mandated by federal and provincial regulations, reclamation of land disturbed from mining is the responsibility of the mining company (Government of British Columbia, Ministry of Energy, 2008); if successfully implemented, phytostabilization of mine sites within B.C.'s semiarid grasslands can help mining companies meet reclamation targets, while initiating ecological restoration and providing long term environmental and socioeconomic benefits (Costanza et al., 1997; Wilson, 2009).

The starting point for any revegetation project is the soil, or the degraded material left over from disturbance that has potential to develop into a soil over time (Bradshaw 1987, 1997). Mine tailings are the by-product of ore processing and consist of fine particulate matter which often lacks the physical, chemical, and biological properties of a productive soil (Gardner et al., 2012, 2010; Pepper et al., 2013), and therefore, are not a suitable growth medium for most terrestrial plants. In general, tailings are high in toxic metals such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), and zinc (Zn), which, contrary to organic contaminants, can persist in soils for long periods of time (Bolan et al., 2014). The mobility and bioavailability of most metals (e.g., Zn, Cu) increases with soil acidity, with a few exceptions such as molybdenum (Mo) and aluminum (Al) which can become available in alkaline conditions (Delhaize and Ryan, 1995; EPA, 2007). In addition to metal toxicity, extreme pH levels, altered soil microbial communities, limited pore space, low amounts of plant nutrients, and poor water retention make up the factors limiting vegetation establishment success on mine tailings (Brown et al., 2003; Pepper et al., 2012; Sheoran et al., 2010). Soil amendments are commonly used in revegetation projects to mitigate these ecological shortcomings and have been proven to be successful in several scenarios (Brown et al., 2007; Drozdowski et al., 2012; Gardner et al., 2010; Shrestha et al., 2009).

Soil amendments include organic amendments (e.g. municipal compost, sewage sludge, wood chips) and liming agents (e.g. wood ash, fly ash). Generally, organic amendments are used to enhance plant growth by providing nutrients and improving soil physical properties, whereas

liming amendments are used to reduce phytotoxicity by neutralizing acidic soils (EPA, 2007), although, these effects can overlap. Manipulating soil properties such as pH and organic matter with soil amendments can influence the bioavailability of soil-borne metals and potentially mitigate any environmental or health risks caused by toxic metals (Bolan et al., 2014). In a greenhouse investigation, Solís-Dominguez et al. (2012) used compost to increase the pH of acidic iron mine tailings, which reduced metal bioavailability and made the tailings substrate more suitable for plant growth. In a review study, Ussiri and Lal (2005) reported improved physical (bulk density, soil aggregation, and water-holding capacity) and chemical (pH and electrical conductivity) properties when coal mine soils were amended with fly ash (a by-product of coal combustion). Furthermore, in a greenhouse experiment, Piorkowski et al. (2015) found a positive synergistic effect on plant performance when a blend of biosolids and compost was utilized. Although soil amendments can be the answer to poor productivity on mine sites, questions remain regarding the economic cost, particularly relating to the availability and the transport of large volumes required for reclamation at the landscape level. As such, investigation into sourcing economically and ecologically viable, locally available materials can be beneficial for mine operations conducting phytostabilization and other reclamation projects.

Revegetation can begin once site preparation and soil amelioration is complete. Revegetation in arid and semiarid environments is exceptionally challenging due to a variety of environmental factors, such as reduced moisture availability and high temperatures, which can limit seed germination and establishment success (Munson and Lauenroth, 2012; Padilla and Pugnaire, 2006; Simmers and Galatowitsch, 2010). Traditionally, non-native species have been utilized for mine reclamation because of their tendency to successfully germinate and establish in harsh environments, and also because of their low cost and ease of availability in large quantities (Burton and Burton, 2002; Oliveira et al., 2012; Skousen and Venable, 2008). Although, more recently, the disadvantages of using non-native species are becoming increasingly recognized. For example, evidence suggests that, due to their competitive nature, non-native species can alter the trajectory of ecological succession by preventing colonization and establishment of native species (Davis et al., 2005; Grman and Suding, 2010). In recent decades, attention has shifted towards using native plant species for reclamation because of their potential to enhance ecosystem health and function, as well as provide socioeconomic benefits (Burton and Burton, 2002; Kiehl et al., 2010; Skousen and Venable, 2008). Researchers have explored the suitability

of several plant species for phytostabilization across a wide range of environments, but we still remain in the information gathering stage regarding species-specific responses to mine tailings (Solís-Dominguez et al., 2012). It is known that candidate species must minimize accumulation of metals in their shoots and tolerate elevated metals, high salinity, and abnormal pH levels (Mendez and Maier, 2007; Solís-Dominguez et al., 2012). The suitability of plant species can be determined by calculating the "translocation factor (TF)" which is the ratio of elemental concentration of shoots versus roots (Mendez and Maier, 2008). Species with TF values of < 1 are optimal for phytostabilization while those which have values of >1 are more suitable for phytoextraction. Species that are adapted to the local climate are ideal. and so native species are preferred over introduced species (Mendez and Maier, 2008; Neuman and Ford, 2006). Tremendous merit can be derived from investigating the suitability of native species for phytostabilization.

Both *Pseudoroegneria spicata* (bluebunch wheatgrass) and *Festuca campestris* (rough fescue) are bunchgrass species with high forage value, and are native to B.C.'s interior semiarid grasslands (Government of British Columbia, 1991; USDA, 2016). *Pseudoroegneria spicata* tends to occupy low elevation areas and can generally tolerate drier environments compared to *F. campestris* which is predominant at higher elevations and is less suited to drought conditions (Dobb and Burton, 2013; Shorthouse, 2010). Both of these grasses are potential candidates for phytostabilization within the interior semiarid grasslands, but little is known regarding their tolerance to soil metal contaminants and their ability to grow on amended mine spoils (e.g., Thorne et al., 1998). The tailings discharged from the historic Afton copper and gold mine (near Kamloops, B.C.) are currently undergoing reclamation, and dust mitigation was one of the primary objectives of the mining company (KGHM International Ltd.) that held the mineral title at the time of this study. The historic Afton tailings are moderately alkaline (pH >8.5), high in copper (600 mg kg^{-1}) and molybdenum (10.5 mg kg^{-1}), and influenced by a semiarid climate, which provides us with a unique opportunity to conduct phytostabilization research using locally available soil amendments and native grassland species.

This study summarizes the results of a greenhouse study which was designed to 1) evaluate three locally available organic amendments (municipal compost, wood ash and wood chips) in terms of plant growth response on the historic Afton mine tailings, and 2) assess whether *Pseudoroegneria spicata* and *Festuca campestris* are suitable candidates for

phytostabilization of these tailings in terms of growth response and metals uptake. Here the aim is to couple phytostabilzation techniques with native grassland restoration practices in order to achieve both short and long-term benefits from revegetation of the historic Afton tailings and similar mine sites.

MATERIALS & METHODS

Mine Tailings and Amendment Analysis

Bulk tailings samples were collected from the Historic Afton Tailings Storage Facility (TSF), approximately 15 km west of Kamloops, British Columbia (50° 39' N, 120° 32' W; elevation 700 m) (Figure 2.1). The compost amendment was produced from municipal yard waste at the City of Kamloops Cinnamon Ridge compost facility, the ash was sourced from the Domtar pulp mill (Kamloops, BC) and is a byproduct of waste wood (commonly referred to as 'hog fuel' and derived from softwood) incineration, and the wood chips were waste produced from a local veneer/plywood factory. The amendments were available within a 30 km radius of the TSF (Figure 2.1), making the materials economically viable options for reclamation.

Samples of tailings and amendments (3 of each) were passed through a 2 mm sieve and analyzed for pH and electrical conductivity (EC) using a handheld electrode device (Hanna Combo HI-98130, Hanna Instruments Inc., Woonsocket, RI, USA) in a 2:1 (soil: deionized water, by mass) solution reacted for 1 h (modified from Hayes et al. 2009). Soil texture was classified for tailings samples only, using the pipet sedimentation method (Hayes et al. 2009). Particle size distribution of the amendments was determined using sieves with mesh sizes ranging from 0.1 to 16 mm. Organic matter content was determined for all samples by loss on ignition (550 °C for 6 h) (Hagen et al., 2014). Gravimetric water holding capacity (WHC) was determined using the methods outlined by Haney and Haney (2010). Subsamples of the tailings and amendments were sent to the British Columbia Ministry of Environment Analytical Laboratory (BCMEAL) (Victoria, BC) for analysis of total carbon (C), total nitrogen (N) and metal concentration including the elements aluminum (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), molybdenum (Mo), nickel (Ni), lead (Pb), and zinc (Zn).

Figure 2.1. Map of study site at the Historic Afton Tailings Storage Facility, 15 km west of Kamloops, British Columbia. The red marker indicates the location of the tailings site. Green pins indicate the locations from which the soil amendments were obtained. Compost was from the City of Kamloops composting facility, wood ash was from the Domtar Pulp Mill, and wood chips were from a local veneer/plywood factory.

Greenhouse Experiment

The greenhouse experiment was conducted from January to March 2016 at the Thompson Rivers University Research Greenhouse in Kamloops, B.C. The experiment was designed to investigate the effects of compost and ash amendments on native bunchgrass growth, and to evaluate the suitability of the selected plant species for phytostabilization of the TSF. Two representative forage bunchgrass species of the interior semiarid grasslands were selected using the 'species objective' filters in the British Columbia Rangeland Seeding Manual (Dobb and Burton, 2013). *Pseudoroegneria spicata* was chosen primarily for its drought tolerance while *Festuca campestris* was selected for its tendency to occur naturally at similar elevations to the study site. A total of 13 ash-compost combinations ranging from 0-100% (w/w) of compost and wood ash, and 0-10% (w/w) of wood chips were evaluated using a randomized complete block design with 10 replicates (Figure 2.3). Three subcategories of treatments were selected for further analysis: 'ash' (100% ash), 'compost' (100% compost), and blend (40% ash, 50%

compost, 10% wood chips). A separate germination trial was conducted to determine seed viability and germination rates (see Appendix A).

The growth experiment was conducted under controlled conditions (natural and artificial light: day/night 18 h/6 h; temperature: day/night 21 °C /15 °C; humidity 50-60%) in the research greenhouse. Two-litre nursery pots with drainage (15 cm top diameter × 14 cm height × 14 cm bottom diameter) were filled with 500 g of tailings and amended with 150 g (a field equivalent to 150 Mg ha^{-1}) of ash-compost-wood chip mixtures (Table 2.1). The tailings-amendment mixtures were combined in bulk batches and mixed by hand. *Pseudoroegneria spicata* and *Festuca campestris* seeds (obtained from Pickseed Canada Inc., Abbottsford, B.C.) were sown at a density of 15 seeds per pot at a depth of approximately 0.5 cm. Pots were watered evenly on every second day using a garden hose fitted with a perforated spout. Plant root and shoot tissues were harvested 90 d after seeds were sown. Prior to harvesting, final levels of germination (i.e. seedling emergence) were determined and shoot heights were measured (in natural repose).

Table 2.1. Amendment composition for all treatments used for growth experiment

Treatment	Wood Ash (%)	Compost (%)	Wood chips (%)	Field application rate (Mg/ha)
1 (ash)	100%	0%	0%	150
2	90%	0%	10%	150
3	80%	10%	10%	150
4	70%	20%	10%	150
5	60%	30%	10%	150
6	50%	40%	10%	150
7 (blend)	40%	50%	10%	150
8	30%	60%	10%	150
9	20%	70%	10%	150
10	10%	80%	10%	150
11	0%	90%	10%	150
12 (compost)	0%	100%	0%	150
13 (control)	0%	0%	0%	0

Figure 2.2. View of randomized complete block design used for greenhouse growth trial.

Bunchgrass shoots were clipped at the soil surface and roots were retrieved from the amended tailings substrate. Plant tissue samples were washed and dried (70 °C for 48 h), then weighed on an analytical scale to determine root and shoot biomass. Three composite biomass samples (roots and shoots) were prepared from the amendment treatment subcategories ('ash', 'compost', and 'blend') for analysis of plant tissue elemental concentration by the BCMEAL.

Seedling emergence rates, plant biomass, and tissue metal content data were analyzed in R version 3.2.3 "Wooden Christmas-Tree" (The R Foundation for Statistical Computing). All data were checked for normality using boxplots and residual plots. Homogeneity of variance was assessed using the Fligner-Killeen test, and when necessary, data were transformed using a natural logarithm or a square root function. Significant differences between species were determined using the Welch's two sample t-test. One-way and two-way analysis of variance (ANOVA) tests were employed to find significant differences between treatment means. Analysis of covariance (ANCOVA) was employed to control for seedling density when assessing plant productivity metrics. Treatments were grouped and ranked using Tukey's HSD test ($P < 0.05$).

RESULTS

Mine Tailings and Amendment Characteristics

Soil texture analysis revealed that the historic Afton tailings had a sandy clay loam texture (52.9% sand, 26.5% silt, and 20.6% clay). The gravimetric WHC of the unamended tailings was relatively high compared to the soil amendments, and decreased when amendments

were added (Table 2.2). The tailings were also characterized by a moderately alkaline pH and low amounts of organic matter, total carbon, total nitrogen and phosphorus. Electrical conductivity remained below the threshold of 4 dS m^{-1} at which plant growth is inhibited (Drozdowski et al. 2012). Analysis of tailings for metals revealed high amounts of Cr, Cu, Mo, and Ni (Table 2.3). Of these metals, Cu, Cr, and Ni exceeded the CCME guidelines for industrial land use, while Mo exceeded the less stringent guideline for agricultural land use (Canadian Council of Ministers of the Environment, 2014).

Table 2.2. Select chemical and physical parameters of mine tailings, organic amendments, and amended tailings treatments.

Substrate/ Treatment	pH	OM[a] (%)	C (%)	N (%)	C:N	P (%)	K (%)	EC[b] (dS m^{-1})	WHC[c] (%)
Experimental materials									
Tailings	8.7±0.07	0.1±0.02	1.12	0.01	112:1	0.11	1.09	2.1±0.02	69.5±0.66
Compost	7.8±0.05	23.9±1.45	24.3	1.18	21:1	0.30	1.32	3.5±0.23	50.2±3.44
Ash	10.3±0.02	26.8±1.23	22.5	0.05	450:1	0.47	2.49	2.0±0.02	31.0±0.72
Wood chips	7.5±0.10	97.7±0.90	56.7	0.12	473:1	-	-	0.5±0.04	22.9±1.36
Amended tailings									
100% ash	9.3±0.04	3.9	2.3	-	-	-	-	2.4±0.01	63.6±1.01
100% comp	8.1±0.09	4.6	2.7	-	-	-	-	3.0±0.17	69.0±0.54
Blend	8.7±0.04	4.3	2.5	-	-	-	-	2.0±0.05	65.4±0.26

Values are means ± standard error (n=3). Values without standard errors represent a single sample.
[a] OM, organic matter; [b] EC, electrical conductivity; [c] WHC, gravimetric water holding capacity.

The municipal yard waste compost was mostly made up of organic material and sands ranging from 0.1 to 4 mm, but also contained some large woody debris and coarse rocks (≤ 16 mm diameter). The compost was characterized by a slightly alkaline pH, adequate total nitrogen, and a well-balanced C: N ratio (Table 2.2). Of the substrates studied, the compost had the highest electrical conductivity (EC). Metal content of the investigated compost met the CCME guidelines for both agricultural and industrial land uses (Table 2.3). The wood ash amendment was primarily composed of fine-to-medium particles ranging from 2 to 4 mm and had a considerably high pH and C: N ratio (Table 2.2). Concentrations of Al were alarmingly high and

Zn was above the CCME guideline for agricultural use (Table 2.3). In spite of these physiochemical limitations, we assessed the ash in this study because of its potential to enhance plant performance due to other potentially favourable characteristics such as high levels of phosphorus (P) and potassium (K) (which are also important plant nutrients) relative to the other amendments investigated. The wood chips (used for the "blend" treatment) ranged from 1 to 16 mm in size and were primarily composed of organic matter (Table 2.2). Because of these properties, wood chips are a useful tool for adjusting the C:N ratio of reclamation materials and also for preventing leaching of N from the rooting zone (Piorkowski et al., 2015).

Table 2.3. Select metal and metalloid concentrations (mg kg^{-1}) of mine tailings and amendments used for this study. Values are compared to the CCME guidelines for agricultural and industrial uses.

Element	Ash	Compost	Tailings	CCME* (agricultural)	CCME (industrial)
Al	1967	828	74.6	-	-
As	< 3.0	< 3.0	< 3.0	12	12
Cd	< 1.0	< 1.0	< 1.0	1.4	22
Co	25.7	16.9	30.8	40.0	300
Cr	51.8	52.2	**138**	64	87
Cu	**70.7**	**77.9**	**600**	63	91
Fe	486	545	525	-	-
Hg	< 3	< 3	< 3	6.6	60
Mo	3.15	3.81	**10.5**	5	40
Ni	30.9	26.1	**90.7**	45.0	89
Pb	< 1.0	6.6	< 1.0	70	600
Zn	**216**	106	19.6	200	360

*CCME, Canadian Council of Ministers of the Environment soil quality guidelines (2014). Bolded values are in exceedance of at least one of the referenced guidelines

Growth Response to Soil Amendments

Germination occurred on the amended tailings within four to ten days depending on the species and growth medium used. Seedling emergence after 90 d growth was low on the unamended tailings for both grass species (Figure 2.3). Soil amendments, regardless of composition, had a significant effect ($P < 0.05$) on *Pseudoroegneria spicata* and *Festuca campestris* emergence compared to the unamended tailings, with the exception of *F. campestris* growing on the compost amended tailings which was similar to the unamended tailings (Figure 2.4a). When comparing seedling emergence between species, *P. spicata* outperformed *F.*

campestris on all treatments, but statistical significances were detected for the ash treatment only. No clear relationship was observed between amendment relative composition and seedling emergence rates. Despite successful germination and growth, neither one of the grass species developed seed heads in any of the treated soils during the 90 d growth period.

  Pseudoroegneria spicata shoots were, on average, taller than *Festuca campestris* shoots on all treatments, but statistical significances were only detected on the amended tailings (Figure 2.4b). The compost-amended tailings yielded the tallest shoots for both species, however, shoot heights were not statistically significant compared the blend treatment. There was evidence of a positive correlation (R^2=0.42, $P < 0.0001$) between compost concentration and *P. spicata* seedling height (data not shown). A similar pattern was observed for *F. campestris*, however, the association was less prominent (R^2=0.25, $P < 0.0001$).

  Plant productivity was sparse on the unamended tailings (Figure 2.3); despite some of the seeds germinating, final seedling numbers were low. Shoot and root biomass of *Pseudoroegneria spicata* were significantly greater ($P < 0.05$) than *Festuca campestris* on all treatments (Figure 2.5). On the amended tailings, there was at least a twofold difference in root and shoot biomass between species. For both species, the compost treatment yielded the highest shoot and root biomass (up to 1.4 g total dry biomass per pot) while the unamended tailings yielded the lowest. With regard to total biomass, ash was the least productive amendment while the blend was intermediate. Plants growing in the ash-amended tailings were stunted and showed signs of nutrient deficiency (e.g. discolouration of shoots). Statistically, there were no differences in root biomass between the compost and the blend amendments. There were also no significant differences in root biomass between the ash and the blend treatments. Root-to-shoot ratios were similar for both species and ranged from <1:1 on the unamended tailings to up to 3:1 in the ash treatment (data not shown).

Figure 2.3. *Pseudoroegneria spicata* (left column) and *Festuca campestris* (right column) seedling growth after 90 days. From top to bottom: treatments are control, ash, blend and compost.
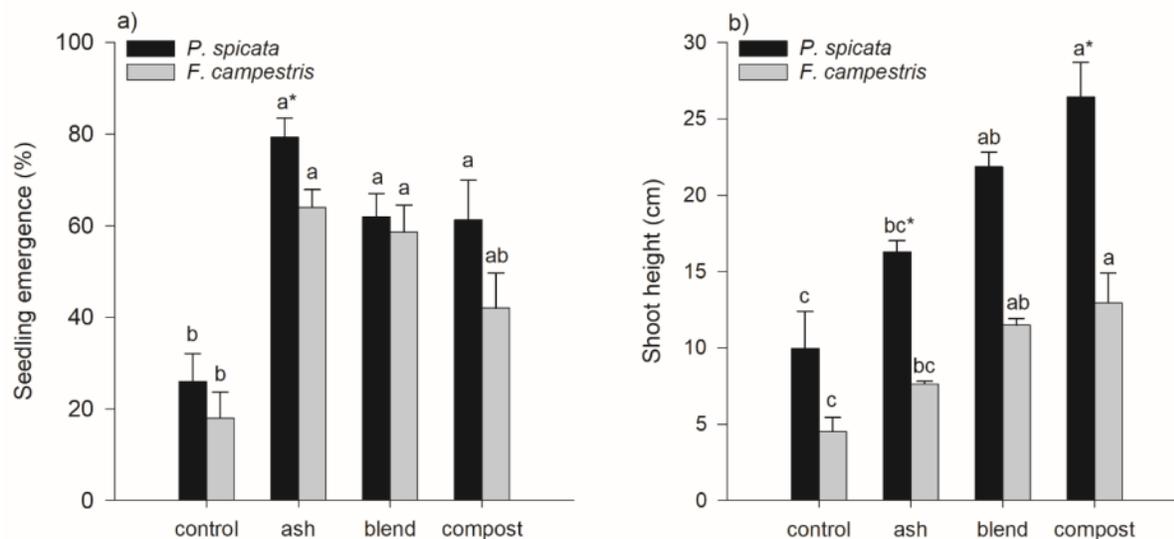
Figure 2.4. Mean *Pseudoroegneria spicata* and *Festuca campestris* a) seedling emergence and b) shoot heights by treatment after 90 d growth in amended mine tailings. Error bars are standard errors of the mean. Treatments with different letters are statistically significant at $P < 0.05$ (one-way ANOVA, Tukey's HSD). * represents a statistical significance between species (determined by Welch's t-test) for that treatment.
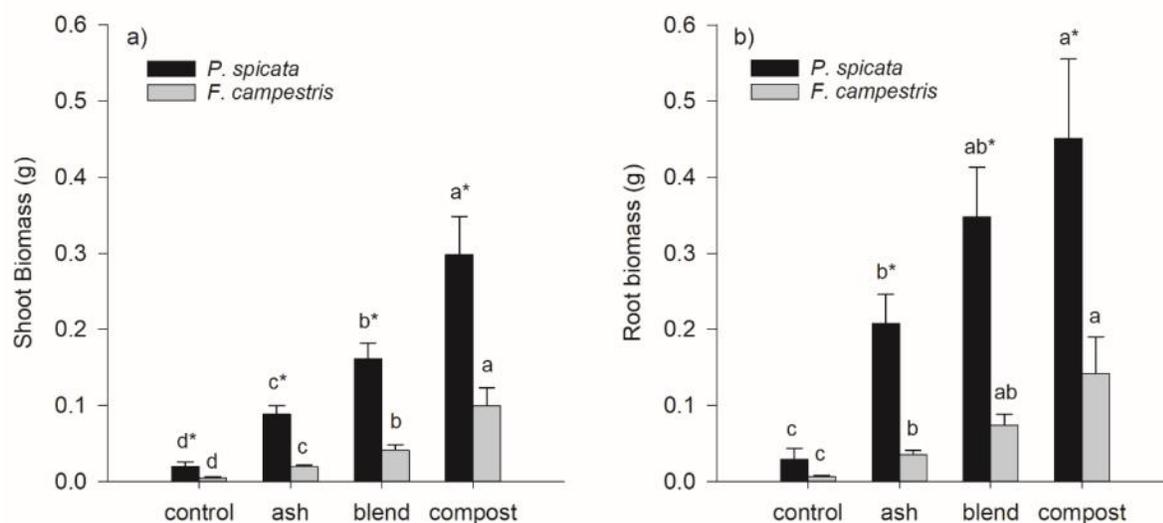
Figure 2.5. Mean *Pseudoroegneria spicata* and *Festuca campestris* a) shoot biomass and b) root biomass per pot by treatment after 90 d growth in amended mine tailings. Error bars are standard errors of the mean. Treatments with different letters are statistically significant at $P < 0.05$ (one-way ANOVA, Tukey's HSD). * represents a statistical significance between species (determined by Welch's t-test) for that treatment.

A weak positive correlation between amendment compost concentration and total biomass was detected (Figure 2.6). Total biomass responded negatively to ash concentration.

When controlling for seedling density (using ANCOVA), this relationship was strengthened (R^2=0.48 and 0.45 for *Pseudoroegneria spicata* and *Festuca campestris*, respectively).
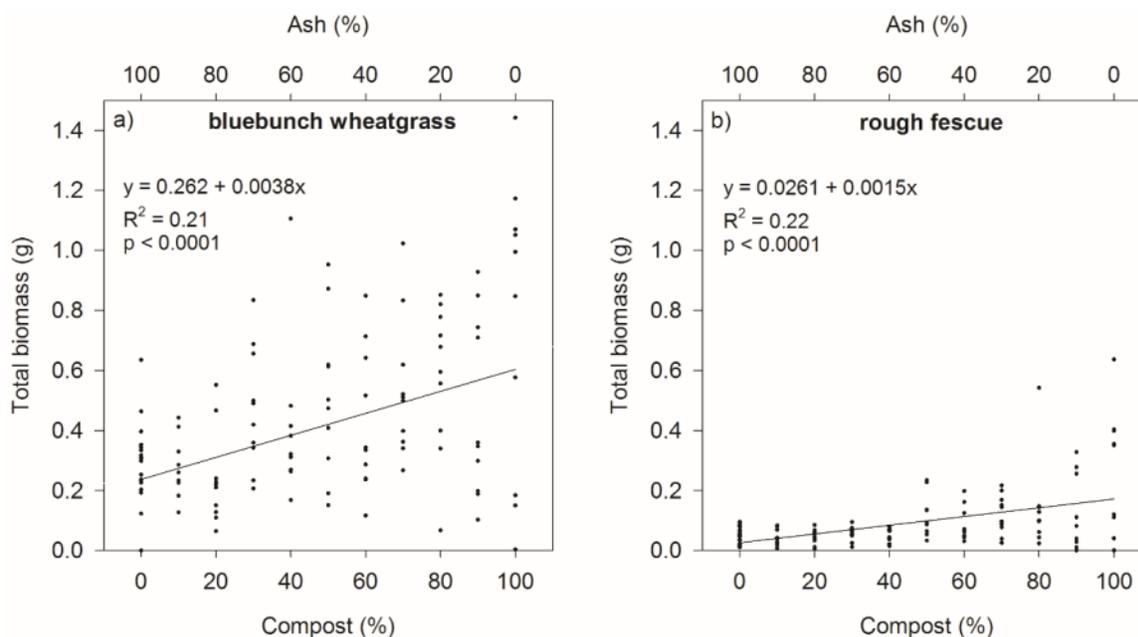
Figure 2.6. Relationship between a) *Pseudoroegneria spicata* total biomass (roots + shoots) and b) *Festuca campestris* total biomass per pot and relative concentrations of compost and ash in the soil amendment mixtures. Data points are untransformed raw data.

Plant Metals Uptake

Shoot concentrations of select metals were determined for both plant species growing on the three amendment mixtures (Table 2.4). Analysis indicated that Fe and Mo concentrations were greater than the domestic animal tolerance limit for several of the treatments. The only significant treatment effect was for Mo; both grass species accumulated a substantially greater amount of Mo when growing in the ash-amended tailings compared to the other treatments. Molybdenum concentration was significantly greater (nearly twofold) in *Pseudoroegneria. spicata* tissue compared to *Festuca campestris* when grown on compost-amended tailings. In examining the results more closely, Fe exceeded the tolerance limit in *F. campestris* growing on the ash-amended tailings but the exceedance was negligible if sampling error is considered. Despite the aluminum content of the wood ash being considerably high, shoot tissue aluminum

concentration remained below the domestic animal tolerance limit on the ash-amended tailings. Translocation factors were >1 for Mo and Zn and <1 for the remaining metals.

Table 2.4. *Pseudoroegneria spicata* and *Festuca campestris* shoot accumulation (mg kg^{-1}) of select metals and metalloids after 90 d growth in amended mine tailings.

Element	Total[a]	MTL[b]	Amendment treatment	shoot tissue metal accumulation			TF[e] (PS)	TF (FC)
				P. spicata	*F. campestris*	PS vs. FC (t-test)[d]		
Al	75	1000	100% compost	361 ± 93.0 a	245 ± 108 a	NS	0.08 ± 0.02	0.07 ± 0.04
			100% ash	526 ± 99.4 a	697 ± 291 a	NS	0.15 ± 0.06	0.3 ± 0.11
			blend	323 ± 46.6 a	442 ± 202 a	NS	0.07 ± 0.02	0.15 ± 0.04
Cu	600	40	100% compost	26.4 ± 1.69 a	14.39 ± 1.87 a	NS	0.50 ± 0.04	0.12 ± 0.02
			100% ash	27.1 ± 2.25 a	24.0 ± 7.53 a	NS	0.33 ± 0.14	0.28 ± 0.09
			blend	22.7 ± 0.68 a	22.3 ± 8.54 a	NS	0.24 ± 0.03	0.21 ± 0.07
Fe	525	500	100% compost	331 ± 65.1 a	273 ± 71.4 a	NS	0.08 ± 0.02	0.08 ± 0.03
			100% ash	457 ± 72.8 a	**576 ± 216** a	NS	0.18 ± 0.08	0.33 ± 0.11
			blend	308 ± 32.1 a	343 ± 147 a	NS	0.08 ± 0.02	0.12 ± 0.03
Mo	21.9	5	100% compost	**37.4 ± 1.32** b	19.7 ± 3.28 b	*	2.44 ± 0.16	0.41 ± 0.02
			100% ash	**183 ± 46.5** a	**202 ± 14.4** a	NS	12.5 ± 4.62	2.53 ± 0.30
			blend	**58.3 ± 15.7** b	**48.4 ± 5.41** b	NS	3.43 ± 0.47	1.01 ± 0.18
Zn	< 3.0	500	100% compost	34.9 ± 1.18 a	85.9 ± 27.7 a	NS	0.89 ± 0.04	1.60 ± 0.47
			100% ash	29.3 ± 1.0 a	28.1 ± 3.78 a	NS	1.46 ± 0.34	1.02 ± 0.28
			blend	32.1 ± 1.95 a	38.8 ± 6.96 a	NS	0.87 ± 0.08	0.90 ± 0.22

[a]Total elemental concentration of mine tailings prior to planting. Values are means ± standard error of the mean. [b]MTL = maximum tolerable levels of metals in the shoots; values are for cattle (National Research Council, 2005) and bolded values indicate an exceedance. [c]Treatment means with different letters are statistically significant at $P < 0.05$ (one-way ANOVA, Tukey's HSD) for each species corresponding to each element. [d]Welch's two sample t-tests were performed for each row (NS = non-significant; * = significant difference); PS = *Pseudoroegneria spicata*, FC = *Festuca campestris*. [e]TF = translocation factor; the shoot:root ratio of the concentration of the corresponding element.

DISCUSSION

Effect of Organic Amendments on Tailings Characteristics

The establishment of vegetation on mine tailings is often facilitated by the addition of organic soil amendments which are used to enhance soil physiochemical conditions for plant growth (Brown et al., 2003; Solís-Dominguez et al., 2012). The efficacy of three locally available soil amendments for reclamation and phytostabilization of the TSF were assessed in a greenhouse trial. Assessment of tailings characteristics before and after amendment addition revealed increased organic matter and total C content in the amended tailings, which is consistent with other mine tailings studies (Cele and Maboeta, 2016; Shrestha et al., 2009). The compost-treated tailings had the highest organic matter content and the ash-treated tailings had the lowest. Organic matter is important for soil rehabilitation and reclamation for several reasons: 1) the organic C provides an energy source for soil microorganisms which accelerates decomposition and nutrient cycling, 2) long-term plant nutrient availability is enhanced because nitrogen is in an organic form and is slowly released over time, and 3) the organic matter improves soil physical conditions such as water retention and bulk density (Drozdowski et al., 2012; Gardner et al., 2010; Larney and Angers, 2012; Shrestha et al., 2009). Because of these properties, the longevity of positive effects is often greater when using organic amendments compared to traditional reclamation methods such as inorganic fertilizers (Gardner et al., 2012; Tian et al., 2009).

The addition of organic amendments also altered tailings pH levels. The compost and the blended amendment lowered tailings pH, but the effect was suboptimal as the amended tailings remained moderately alkaline (pH >8). The ash amendment increased the tailings pH level from moderately alkaline to strongly alkaline (pH >9), which is above what is deemed normal in most soils (normal range is 5.5 to 8.5). In a recent review, Sheoran et al. (2010) reported that mine soil pH range of 6 to 7.5 is adequate for agronomic or horticultural uses of mine sites. Although, in arid environments, it is normal for pH to be slightly to moderately alkaline (pH between 7 and 9) (Brady, 1990). Abnormally high soil pH can lead to mobility of As, Mo, and Se as well as reduced availability of P and certain micronutrients (e.g., B, Mn, Fe) (Bolan et al., 2014b; EPA, 2007). In general, the addition of organic amendments increased the EC of the tailings, with the exception of the blend which had little or no effect. In all treatments, EC remained below the critical level of 4 dS m^{-1} at which plant growth is negatively affected (Drozdowski et al. 2012).

Based on the soil parameters assessed, the compost treatment appeared to provide the most suitable soil conditions for revegetation of the historic Afton tailings.

The tailings investigated in this study had a relatively high gravimetric WHC, and so water retention was not considered a limitation. Although, it is important to note that high WHC (>80%) can lead to poor drainage and anoxic conditions which can affect root productivity (Brady, 1990) and reduce overall revegetation success. The addition of organic amendments reduced the WHC of the tailings, likely because the addition of larger organic particles reduced bulk density and improved drainage of the substrate.

Growth Response to Organic Amendments

The results of the experiment indicate that the addition of organic amendments, regardless of treatment, improved seedling germination and growth of native bunchgrass species *Pseudoroegneria spicata* and *Festuca campestris* on the historic Afton tailings. The positive influence of soil amendments on plant productivity was likely a result of increased organic matter content in the amended tailings which improved tailings physiochemical conditions (Rivard and Woodard, 1989; Shrestha et al., 2009). In a greenhouse study, Solís-Dominguez et al. (2012) reported improvements in tailings pH, EC, organic carbon and total nitrogen as a result of compost addition which led to improved plant growth. Of the treatments investigated in my study, the 100% compost amendment was the most effective at promoting germination and growth of both species. Plants growing in the ash-amended tailings were stunted and showed signs of nutrient deficiency; even though the C content of the ash amendment was comparable to the compost treatment, the lack of nitrogen coupled with increased pH levels may have created less favourable conditions for plant growth. Under extreme soil pH conditions (<5.5 to >8.5) certain plant macronutrients (e.g. N, P, K) can become immobilized and microbial activity can decline (EPA, 2007). Because of these properties, it is possible that certain nutrients that were contained in the ash, such as P and K, were not available for plant uptake. The data suggests that incorporating the very strong alkaline ash material into the alkaline tailings was not an effective method for optimizing plant growth. Assessment of post-amendment nutrient concentrations would have revealed more insight as to the limitations of the ash material as a soil amendment.

Root-to-shoot ratios were around 1 for the compost treatment which indicates balanced biomass allocation and adequate nutrient availability in the amended substrate (Wilsey and

Wayne Polley, 2006). Generally, when nutrients are limiting, plants will allocate more resources to their roots which increases the root-to-shoot ratio (Ågren and Franklin, 2003). In this study, the high root-to-shoot ratios of plants growing in the ash-amended tailings can be explained by the lack of nitrogen in the growing medium which may have forced plants to allocate more effort into root production at the cost of shoot production.

With regard to plant growth and overall productivity *Pseudoroegneria spicata* outperformed *Festuca campestris* on all treatments. This can be partially attributed to the ability of *P. spicata* to germinate under a wider range of conditions compared to other grassland species (Young et al., 1981). The results were consistent with a recent field study where Carlyle (2012) reported higher relative growth rates and shoot and root biomass for *P. spicata* compared to *F. campestris* at the Lac du Bois Grassland Provincial Park (near Kamloops, BC). In the current study, both grass species responded positively to increases in compost concentration which suggests that nitrogen may have been a limiting factor for plant growth on these tailings. Several studies have underscored the importance of soil nitrogen in mine reclamation because it is an essential plant nutrient, yet it is often limiting in mine soil ecosystems (Bradshaw, 1997; Shrestha et al., 2009; Shrestha and Lal, 2011).

Effect of Amendments on Metals Uptake

Shoot and root concentrations of select metals were assessed for both species after 90 d of growing in the amended tailings. The results indicated high concentrations of Mo for both plant species which exceeded toxicity limits in all treatments, but most notably when the ash amendment was used. Elevated Mo levels can lead to molybdenosis (induced Cu deficiency) when ingested by cattle or other ruminants (Drozdowski et al., 2012; Gardner et al., 2012). This condition is influenced by relative concentrations of copper, molybdenum, and sulfur. In general, the risk of molybdenosis increases when the Cu: Mo ratio is <2:1 (Mason 1971; cited by Gardner et al., 2012). In this study, Cu: Mo ratios for all treatments were well below this threshold, with the highest ratio being for the wheatgrass growing in the compost treatment (0.7:1). The enhanced Mo uptake by grasses growing on the ash-amended tailings was likely the result of elevated soil pH levels. Doran and Martens (1972) found similar effects of soil pH on metals uptake when growing alfalfa in a fly-ash amendment. Under abnormally high pH conditions (pH >8.5) molybdenum is soluble and readily available for plant uptake (EPA, 2007). This

information suggests that *Pseudoroegneria spicata* and *Festuca campestris* may not be suitable for phytostabilization of these tailings due to their tendency to uptake molybdenum under alkaline conditions. However, further additions of compost and/or wood chips may be worth investigating because doing so may further neutralize tailings pH, thereby reducing the potential for Mo uptake and lowering the risk of molybdenosis.

Aluminum concentration of the ash amendment was notably high (Table 2.3). When coupled with its high pH (Table 2.2) this created ideal conditions for the formation of soluble Al in the form of aluminate $(Al(OH)_4^{-1})$ which can cause soil toxicity and inhibit plant growth (Fuller and Richardson, 1986). According to Hodson (2012), some plants are able to tolerate excessive levels of aluminum and other metals by avoiding shoot uptake and concentrating them in their roots. Both plant species used in this study accumulated substantially more aluminum in their roots (up to seven times, data not shown) compared to their shoots which provides some indication of their tolerance to aluminum. These results suggest that these species may be useful for remediation of tailings and other mine wastes high in aluminum.

The translocation factor (TF) is a useful metric for measuring metal accumulation in plant tissues (Mendez and Maier, 2007). Suitable candidate plant species for phytostabilization are those which minimize shoot accumulation without limiting root uptake, thus TF values of <1 are preferred (Mendez and Maier, 2008, 2007). While, in the current study, TF generally remained below this threshold in both species for most of the metals investigated, values for Mo and Zn exceeded (or were close to) this threshold for all treatments (Table 2.4). The results indicate that the species investigated may not be suitable for phytostabilization of the TSF because of their tendency to accumulate high amounts of Mo in their shoots.

CONCLUSION

Of the organic amendments investigated in this study, the City of Kamloops municipal compost was the most effective at promoting native bunchgrass growth on the amended tailings. Further investigation using higher rates of compost would be meritable because I suspect that this will result in enhanced plant performance and reduced Mo uptake. Due to its high pH and elevated aluminum content, the Domtar pulp mill wood ash was not suitable for amelioration of alkaline mine tailings, as plants growing in the ash amended tailings were subjected to the ideal

conditions for aluminate toxicity. However, there may be potential to use this amendment for remediation of acidic mine tailings such as those investigated by Solís-Dominguez et al. (2012).

Festuca campestris growth on the amended tailings was sparse in comparison to *Pseudoroegneria spicata*. Although the latter exhibited good germination and growth, it also accumulated elevated levels of Mo in its shoots which counted against its candidacy for phytostabilization and use as a forage species at the TSF. However, there may be potential to use these species in other technologies such as phytoremediation, where shoot accumulation is encouraged and aboveground biomass is subsequently removed from site (Best et al., 2008). Despite this verdict, further investigation of these grass species is required because it is likely that under optimal soil pH conditions (pH range of 6 to 7.5), Mo uptake will decrease. Both species minimized shoot uptake of aluminum when present in high quantities by concentrating it in their roots, which prompts investigation of these species' performance on aluminum rich mine wastes.

The results of this study indicate that the 100% compost amendment is best suited to ameliorate the mine tailings investigated, and that *Pseudoroegneria spicata* is the most suitable candidate for revegetaion and phytostabilization at the TSF. In summary, this study provides practical information regarding the suitability of soil amendments available in the Kamloops region and the performance of native grassland species during restoration and phytostabilization of alkaline mine tailings. In addition to this information being directly applicable to reclamation at the TSF, it may also be useful for remediation planning and implementation at other sites located in similar environments. Further research is needed to investigate native bunchgrass performance on the compost amendment more closely, and to test the greenhouse results in the field.

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CHAPTER 3 – FACILITATING MINE RESTORATION IN A SEMIARID ENVIRONMENT WITH NURSE PLANTS, COVER CROPS AND SOIL AMENDMENTS

INTRODUCTION

Within the past few decades, the importance of conservation and restoration of native ecosystems and their biological diversity has gained the attention of scientists and practitioners (Barnosky et al., 2011; Ceballos et al., 2015; Menninger and Palmer, 2006). Globally, grasslands are an important natural resource because they provide an array of critical ecosystem services such as wildlife habitat, water filtration and climate regulation, which are beneficial for the environment and the economy (Costanza et al., 1997). The semiarid grasslands of interior British Columbia (B.C.), Canada make up only less than 1% of the province's land area, and are known for their biological diversity and abundance of endemic plant and animal species (Government of British Columbia, 1991; Wilson, 2009). These grasslands contain over 30% of the province's species at risk as well as several culturally important plant species (BC Conservation Data Centre, 2017). Human activities such as extensive livestock grazing, urban development, agriculture, and mining have led to the degradation of these unique ecosystems (Huber-Sannwald and Pyke, 2005; van Ryswyk et al., 1966; Wilson, 2009). As such, these areas are the focus of many conservation and restoration efforts (e.g. Grassland Conservation Council of BC, 2017; Lysne, 2005).

The mining industry is an important component of the Canadian economy (Mining Association of Canada, 2016), but it is environmentally destructive. The activities throughout the mine cycle create disturbances on the landscape that leave pre-existing ecosystems in an altered state (Bradshaw, 1992). For example, during the development of tailings storage facilities, natural soils and vegetation are removed and replaced with fine-textured waste material generated from ore processing (known as tailings). These anthropogenic materials typically lack the physiochemical and biological capacity to support plant growth (Cele and Maboeta, 2016; Gardner et al., 2010; Pepper et al., 2012); therefore, natural processes such as soil development and plant community succession are severely impaired unless there is human intervention (Bradshaw, 1997; Sheoran et al., 2010).

Mine reclamation is a type of ecological restoration concerned with returning mined land to a productive land use. In B.C., mining companies are required to fulfill reclamation targets of "equivalent land capability" under the B.C. Mines Act (Government of British Columbia, Ministry of Energy, 2008). A conventional end land use for tailings storage facilities has been the development of pasture-based ecosystems using non-native agronomic species (Brothers, 1990). Agronomic species were used because of their low cost and availability in large quantities, and because they tend to establish rapidly on nutrient-poor, environmentally-stressed sites allowing for quick economic returns and improvements in aesthetic qualities (Skousen and Venable, 2008; Wu et al., 2011). These reclamation plans are limited and becoming out-dated for a variety of ecological and social reasons. Firstly, research is showing that agronomic species can alter the path of succession by preventing the establishment of native species (Davis et al., 2005; Hagen et al., 2014) which results in plant communities with low genetic diversity (Dormaar et al., 1995) and ecological resiliency (Menninger and Palmer, 2006). Secondly, non-native species can rapidly disperse from the site of introduction and invade nearby natural communities (Richardson et al., 2000). Finally, restoration of natural communities with native species and biological diversity is a preferred end land use for mine sites from the perspective of First Nations groups – who hold traditional rights to the land disturbed by mining activities – and other primary stakeholders of the land.

Establishing native plant communities on mine sites is challenging because native seed is expensive and difficult to obtain (Burton and Burton, 2002), and seeds do not germinate and establish well on degraded sites (Drozdowski et al., 2012; Skousen and Venable 2008). If the mine site being restored is located within a semiarid environment the challenges are exacerbated because, not only are there physiochemical limitations associated with the mine soils (Mendez and Maier, 2008), there are also climatic stressors such as intense temperatures, high evapotranspiration rates, low precipitation, and strong winds (Munson and Lauenroth, 2012; Pueyo et al., 2009). In BC's semiarid sagebrush-steppe plant communities, the lack of rainfall and high evaporation rates during the summer lead to prolonged periods of drought which reduce germination and growth (Shorthouse, 2010). In such ecosystems, restoration practices can help ameliorate these harsh abiotic conditions and improve revegetation success (Huber-Sannwald and Pyke, 2005; Munson and Lauenroth, 2012; Pueyo et al., 2009).

Restoration begins with addressing the physiochemical and biological soil limitations so that a vegetative cover can be established (Bradshaw, 1987; Sheoran et al., 2010). The use of organic soil amendments is a common practice for reclamation of mine sites (Drozdowski et al., 2012; Orman and Kaplan, 2007; Pepper et al., 2013). The addition of organic matter leads to improved soil conditions because it increases plant nutrients and microbial activity, enhances water retention, and reduces soil bulk density (i.e. compaction) (Brown et al., 2003; Cele and Maboeta, 2016; Gardner et al., 2010). The enhanced soil conditions increase plant productivity (Gardner et al., 2010; Waterhouse et al., 2014) which, in turn, supports the positive feedback loop between plants and soils (Shrestha et al., 2009), leading to the development of self-sustainable plant and microbial communities (Palmer et al., 2006; Pepper et al., 2012), the latter of which is the ultimate goal of restoration (Bradshaw, 1997; Palmer et al., 2006). During mining operations, natural topsoil is usually conserved for reclamation (Sheoran et al., 2010), but is less available in arid and semiarid regions because these areas typically have low levels of biomass productivity and organic matter accumulation (Burke et al., 1989), and so soil amendments often need to be imported from external sources. From an economic standpoint, locally available materials are preferred by mining companies because the cost of hauling is reduced. Many commonly used soil amendments, including municipal sewage sludge, compost, biochar and wood ash, are waste by-products of various industries; therefore the practice of using these soil amendments for mine reclamation is mutually beneficial for both the source and the mining company.

Successful restoration can be achieved when natural ecological processes are mimicked during the restoration process (Bradshaw, 1997, 1992, 1987). Species interactions among plants are a well-researched topic in contemporary ecology and should be considered during restoration (Bertness and Callaway, 1994; Bruno et al., 2003; Stachowicz, 2001). In natural plant communities, it is understood that neighboring plants simultaneously exert both positive and negative effects on one another, and it is the net balance that determines the outcome of the relationship (Callaway and Walker, 1997). Until recently, ecologists have focused on negative interactions (i.e. competition) as a driving force for ecological succession (Grime, 1973; Tilman, 1982), but the role of positive interactions (facilitation) is rapidly gaining importance in restoration theory (Bruno et al., 2003) and practice (Gómez-Aparicio, 2009; Padilla and Pugnaire, 2006). Positive interactions occur when one species (the benefactor) benefits from the

presence of another species (the facilitator) that ameliorates abiotic and/or biotic conditions in some way, making the environment within their vicinity more favorable for survival and growth (Bertness and Callaway, 1994). For there to be a facilitative effect, the net balance between the positive (e.g. habitat amelioration, resource enhancement, protection from herbivory) and negative interactions (e.g. root competition, excess shade, allelopathy) must be positive (Callaway, 1995). Research has supported the theory that facilitation increases along an abiotic stress gradient (Bertness and Callaway, 1994; Cavieres and Badano, 2009; Maestre et al., 2009), therefore it is believed to be an important process during plant community assembly in harsh environments such as deserts and alpine areas (Padilla and Pugnaire, 2006). However, when ecological conditions become more favorable, the outcome of the relationship between associated species may shift from facilitative to competitive (Padilla and Pugnaire, 2009). The use of facilitation as a restoration tool in stressful environments has gained increased attention in recent years (Gómez-Aparicio, 2009; Padilla and Pugnaire, 2006; Ren et al., 2008).

In stressful environments, such as deserts and alpine areas, seedling establishment is enhanced near adult shrubs or other large plants (known as "nurse plants") (Padilla and Pugnaire, 2006), resulting in noticeably visible "islands of fertility" (Moro et al., 1997; Walker et al., 2001) around the nurse plants. This phenomenon is a result of the nurse plants facilitating neighboring individuals by ameliorating extreme environmental conditions through canopy protection and variety of other mechanisms including resource enhancement and protection from herbivory (Padilla and Pugnaire, 2009, 2006). There are several advantages to growing close to a nurse plant, such as 1) the shade provided by nurse plant canopies can buffer extreme air temperatures and solar radiation which leads to lower soil temperatures and reduced soil water evaporation (Franco and Nobel, 1989; Padilla and Pugnaire, 2009), 2) nurse plants can improve soil moisture at the surface through a process known as "hydraulic lift", where deep soil is accessed by the taproot and re-distributed at the surface (Cardon et al., 2013; Richards and Caldwell, 1987), 3) the enhanced conditions under nurse canopies improves productivity and subsequently increases nutrient cycling through the accumulation of organic matter (Cardon et al., 2013; Claus Holzapfel and Mahall, 1999; Pugnaire et al., 1996), and 4) canopy protection by nurse plants prevents understory plants from being grazed (Padilla and Pugnaire, 2006). The facilitative effects of nurse plants on neighbors (referred to as the "nurse effect"), can be beneficial for

establishing desirable species during restoration of degraded ecosystems (Padilla and Pugnaire, 2006).

Studies of nurse effects have been conducted in several biomes throughout the world. In the Mediterranean semiarid-steppe regions of Spain, the legumous shrub *Retama sphaerocarpa* facilitates a diverse understory plant community by reducing air temperature and solar radiation levels, and by improving soil water and nutrient status (Moro et al., 1997; Padilla and Pugnaire, 2009; Pugnaire et al., 1996). In the extremely arid Sonoran Desert of California, adult *Ambrosia deltoidea* and *Cercidium microphyllum* shrubs facilitate the establishment of the rare cacti species *Carnegia gigantea* by providing refuge from high soil temperatures and enhancing soil nitrogen levels (Franco and Nobel, 1989). A meta-analysis of a wide range of nurse species in the alpine regions of the Andes Mountains in Chile and Argentina revealed enhanced plant community diversity under nurse plants compared to exposed areas. As such, facilitation with nurse plants can be used as a tool for improving the overall health and resiliency of ecological communities during restoration (Bertness and Callaway, 1994; Callaway, 1995; Cavieres and Badano, 2009).

Artemesia tridentata (big sagebrush) is a keystone species in the shrub-steppe ecosystems of B.C.'s semiarid grasslands (Lysne, 2005). In addition to being an important food source for ungulates and critical habitat for small mammals and birds (McArthur, 2008), *A. tridentata* is known for its various facilitative effects on neighboring plants (Cardon et al., 2013; Schlesinger, 1990). Adult shrubs have dense canopies with spread-out branches, and can grow to a height of 4 m (USDA, 2016). The large canopy can act to protect understory herbaceous plants from extreme temperatures, solar radiation and herbivory, while accumulating moisture (from snow) in the winter, which facilitates spring growth (West, 2000). The root morphology of *A. tridentata* is a dual root system that includes a shallow, diffuse root mass and a deep taproot that can carry out the process of hydraulic lift (Richards and Caldwell, 1987). Several studies have attributed enhanced moisture and nutrient availability under of *A. tridentata* shrubs (Burke et al., 1989; Cardon et al., 2013) to this phenomenon. Most of the research investigating sagebrush nurse effects was conducted in degraded rangelands where the shrubs were already well-established adults (Cardon et al., 2013; Drivas and Everett, 1988; Huber-Sannwald and Pyke, 2005). However, in mine reclamation settings, there are no mature shrubs because pre-existing plant communities have been removed by the mining process, therefore seeding, or transplanting

seedlings obtained from nurseries are the only feasible options for establishing nurse plants (Booth, 2005). To date, there has been no research on the facilitative effects of transplanted *A. tridentata* seedlings during mine tailings reclamation (but see Schuman et al., 1998).

Another restoration practice widely used in agricultural, rangeland and forested systems is the use of cover crops. Cover crops are annual or short-lived perennial species which can be sown together with the target species to achieve short-term ecological benefits, such as forage production, erosion control, and exclusion of weedy species while the desired plant community establishes (Espeland and Perkins, 2013). Similar to nurse plants, cover crops can also ameliorate harsh abiotic conditions by creating favourable microclimates that facilitate the establishment of desired species (Gómez-Aparicio, 2009; Maestre et al., 2009, 2003). In arid or semiarid environments, cover crops are capable of reducing soil temperature and evaporation by shading the soil surface, resulting in improved soil water availability (Choi and Mohan, 1995; Krueger-Mangold et al., 2006). Cover crops can also improve soil fertility by adding organic matter (or "green manure") and fixing nitrogen (depending on whether the species is a nitrogen fixer) (Bai et al., 2017; Tribouillois et al., 2014). Since most cover crop species tend to be extremely competitive, introduced species (Tribouillois et al., 2014), there is debate on whether they impede or facilitate the establishment of long-term, more desirable perennial species (Espeland and Perkins, 2013). Some studies have shown that sowing with agronomics results in competitive exclusion of native species, thus halting the progress of natural succession (Davis et al., 2005; Hagen et al., 2014), while others have reported minimal impacts of cover crops on early establishment and growth of desired species (Espeland and Perkins, 2013; Skousen and Venable, 2008). Generally, it seems that the facilitative effects of cover crops are species specific (Choi and Mohan, 1995; Davis et al., 2005; Espeland and Perkins, 2013; Tribouillois et al., 2014), and are more pronounced under stressful conditions (Espeland and Perkins, 2013; Maestre et al., 2009, 2003), therefore, there is potential to use cover crops to facilitate native grassland plant community development during reclamation of mine sites.

This study investigates the use of nurse plants, cover crops and soil amendments as restoration tools for re-establishing semiarid grassland communities at the Historic Afton Tailings Storage Facility. I assessed 1) whether nurse plants influence the abiotic environment and facilitate the establishment of native species during early restoration, 2) whether agronomic cover crops facilitate or impede native plant community development, and 3) the efficacy of

locally available soil amendments under field conditions. Firstly, I expected plant community establishment to be more successful under *Artemesia tridentata* canopies compared to in the open because of the reported abilities of semiarid shrubs to ameliorate harsh environmental conditions and improve soil resources. Secondly, I expected annual cover crops to reduce native plant community diversity in the short-term because of their competitive nature. Finally, I expected soil amendments to have a positive effect on plant performance and overall restoration success.

MATERIALS & METHODS

Study Site

In summer 2014, a research site was established at the Historic Afton Tailings Storage Facility (TSF), which is located approximately 15 km west of Kamloops, B.C. (50° 39' N, 120° 32' W; elevation 700 m) (Figure 3.1). The TSF is located within the BGxw1, the Nicola variant of the very dry warm subzone of the bunchgrass biogeoclimatic zone (Government of British Columbia Ministry of Forests, 1991). The region experiences a semiarid climate with minimal annual precipitation (typically <350 mm) and hot, dry summer months with the highest average daily maximum temperatures (29 °C) occurring in July (Table 3.1). Eighty-one percent of the precipitation is comprised of rainfall and the remaining 19 % is snowfall. The growing season typically spans from April to September with average temperatures ranging from 9.9 to 21.5 °C. Winter mean temperatures range from -2.8 to 5.2 °C (Government of Canada, 2015). The study year (2016) was hotter and wetter than the long-term average. Daily average and maximum temperatures during the 2016 growing season were 0.8 and 1.1 °C higher than the normal, respectively, and mean precipitation was 111% of the long-term average.

The tailings material is fine textured and originated from rock mined from the Afton Pit and the East and West Ajax Pits (currently owned by New Gold Ltd.) during previous mining operations which spanned from 1977 to 1997. A series of reclamation activities were conducted at the ~75 hectare tailings storage facility between 1978 and 1992 in an effort to enhance wildlife forage and domestic rangeland, and to stabilize soils and prevent erosion. The previous reclamation involved seeding with agronomic species. At the time of this study, the existing plant community was sparse and comprised primarily of non-native grasses such as *Agropyron*

cristatum (crested wheatgrass). The tailings facility was dewatered in 2015 and is currently undergoing reclamation.

Table 3.1. Climate data from Kamloops A weather station (345 m in elevation and 8 km northeast of the study site) including study years and long-term normals.

Climate Parameter	2015	2016	1981-2010 long-term normals
Mean annual precipitation (mm)	313.1	335.1	277.6
Mean precipitation Apr. to Oct. (mm)	142.4	181.6	163.4
Mean annual temperature (°C)	10.8	10.4	9.3
Mean temperature Apr. to Oct. (°C)	18.0	17.6	16.8
Mean maximum temperature (°C)	16.6	16.0	14.8
Mean maximum temperature Apr. to Oct. (°C)	25.6	24.9	23.8
Growing degree days Apr. to Oct.*	2376	2301	2175
Frost free period (days)	182	195	169

*Reference temperature = 5 °C

Figure 3.1. Map of study site at the Historic Afton Tailings Storage Facility adjacent to the New Gold mine, 15 km west of Kamloops, British Columbia. The red marker indicates the location of the research site. Green pins indicate the locations from which the soil amendments were obtained. Compost was from the City of Kamloops composting facility and wood ash was from the Domtar Pulp Mill.

Experimental Design

The TSF research site was located at the northwest end of the tailings pond and was comprised of 24 experimental plots arranged in a 3 x 8 grid (Figure 3.2). Each plot was a 180-gallon cattle tank buried into the tailings such that the upper rim was situated evenly with the ground surface. The dimensions of the plots measured 102 cm wide $\times$ 147 cm long $\times$ 61 cm deep. The surface area of plots measured 1.23 m^2 and the distance between plots was 1.5 m within columns and 3 m within rows. In fall 2014, stockpiled tailings material was added to each tank, leaving 15-20 cm of vertical space for the soil amendments to occupy.

Figure 3.2. View of Historic Afton Tailings Storage Facility research plots (left) and individual plot containing unamended tailings (right).

A randomized complete block design with three factors and three replicates was implemented at the TSF research site for the 2016 field season (Figure 3.3). The first factor was soil amendment type (compost/compost + ash), the second factor was nurse plant (sagebrush/no sagebrush) and the third factor was cover crop (cover crop/no over crop) for a total of eight different treatment combinations (2 $\times$ 2 $\times$ 2 = 8). Treatments were randomly assigned to each of the three blocks using "The Random Number Generator" iPhone iOS application (Nicholas Dean, 2013).

	A	B	C
1	NP		NP + CC
2		NP + CC	NP
3	NP + CC		NP + CC
4	CC	NP	
5	NP	NP	CC
6		NP + CC	NP
7	CC	CC	
8	NP + CC	CC	CC

Soil Amendments

Compost
Compost + Ash

Figure 3.3. Experimental plot layout for 2016 Historic Afton Tailings Storage Facility field study where NP = nurse plant and CC = cover crop. Soil amendments are indicated by shaded cells and include a 'compost' treatment and a 'compost + ash' treatment. All plots were seeded with a custom native seed mix. Each combination of treatment factors is represented once within each column (block), and therefore is replicated three times (n=3).

Soil Amendments

Two locally available soil amendments: compost from the City of Kamloops Cinnamon Ridge composting facility and wood ash from the Domtar pulp mill (Kamloops, B.C.) were selected for the field experiment. The compost was produced from municipal yard waste and the ash was a byproduct of waste wood (commonly referred to as 'hog fuel') incineration (refer to Chapter 2 for chemical and physical attributes of the soil amendments). Both amendments were available within a 15 km radius of the TSF. The amendments were stockpiled at the TSF in September 2014 and covered with tarps until needed for the experiment. The soil amendment treatments were a 'compost' and a 'compost + ash' treatment applied at 325 and 340 Mg ha^{-1}, respectively. The 'compost' treatment was a 2:1 mixture of compost and tailings and the 'compost + ash' treatment was a 2:1:1 mixture of compost, ash and tailings. In September 2015, the amendments were mixed in the field and applied to the upper surface of each plot (Figure 3.4).

Figure 3.4. View of soil amendments and tailings prior to mixing (left) and amended study plot (right).

Nurse Plants

The nurse plants were one-year old *Artemisia tridentata* (big sagebrush) seedlings (15-35 cm in height) purchased from Splitrock Environmental (Lillooet, B.C.) in April 2015. Prior to transplanting, seedling heights were measured and the tallest 36 seedlings were categorized into three size classes: small (15-17.5 cm), medium (18-22 cm), and large (23-35 cm) and stored in the greenhouse until needed for the experiment. Nurse seedlings were transplanted to the study plots in September 2015; one seedling from each size class was randomly selected and planted on each plot, equating to three seedlings per experimental plot. Care was taken to arrange the seedlings systematically and consistently throughout study plots (Figure 3.5). The plots – at this time containing the soil amendments and nurse plants – were overwintered. Four out of thirty-six (11%) seedlings did not survive the winter. These seedlings were replaced with new seedlings of the same size class in March 2016.
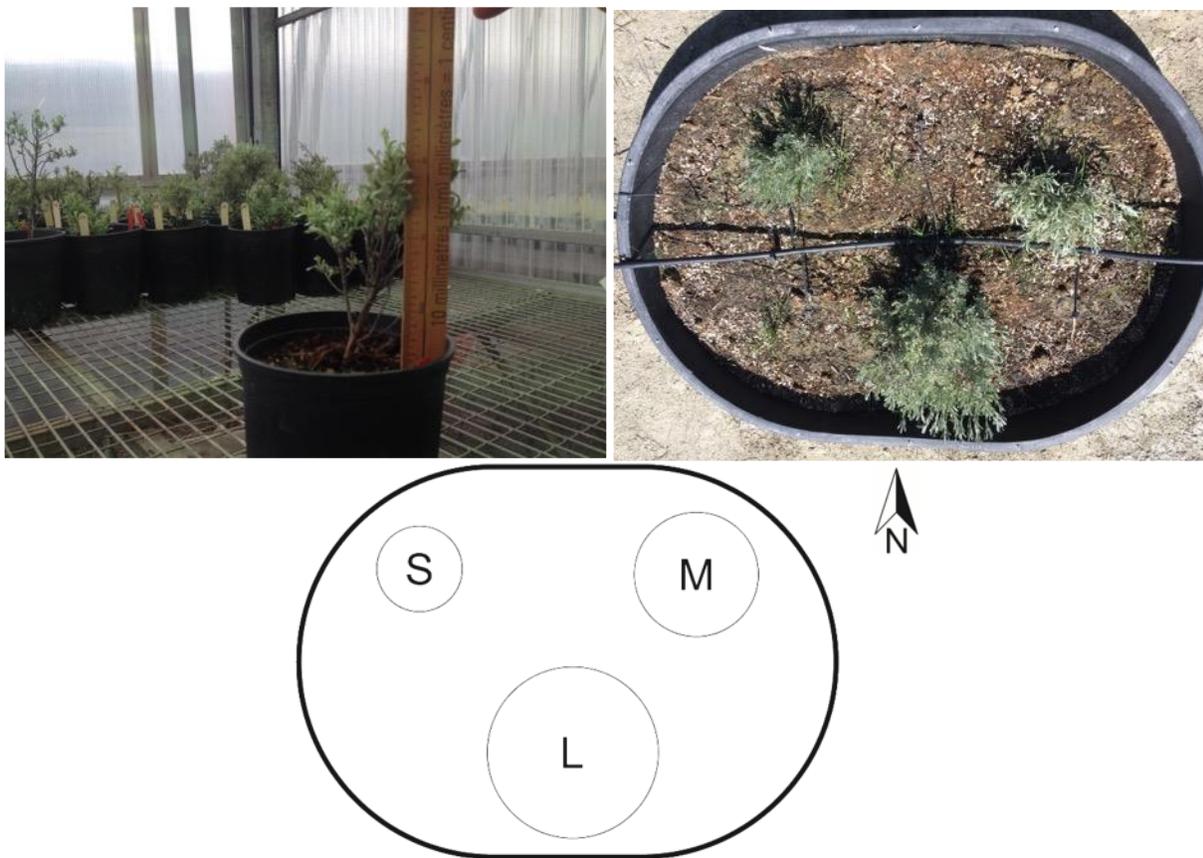
Figure 3.5. Measuring sagebrush seedlings in the greenhouse prior to transplanting (top left), transplanted nurse plants at the Historic Afton Tailings Storage Facility (top right) and arrangement of nurse plants (S=small, M=medium, L=large) (bottom). The arrangement of nurse plant size classes was systematic and consistent throughout study plots.

Seed Mix Selection and Seeding

A total of six native grasses, five native forbs and two agronomic species were selected for the field experiment (Table 3.2). The native forb species were chosen for their cultural significance (medicinal and food) and the remaining species were selected based on their precipitation and elevation range, forage value, erosion control potential, drought tolerance, and ease of establishment (Dobb and Burton, 2013). The native species chosen for this study are representative of B.C.'s interior grassland communities. Six of the seven grass species used in this study are contained in the operational seed mixes used for reclamation at the adjacent New Gold New Afton Mine.

Twenty-four seed packets (1 per study plot) were filled with 200 seeds of each of the native grass and legume species. For the 'cover crop' treatment, an additional 200 seeds of the

agronomic species *Lolium multiflorum* and *Medicago sativa* were added to half (12) of the packets (Table 3.2). All packets received 25 mg of sand to help achieve even dispersal when sowing. Based on the results of the germination trial (see Appendix A), the seeding rates equated to ~1200 and ~1500 pure live seeds (PLS) per m^2 for the 'native seed' and 'cover crop' treatments, respectively. These seeding rates are near the uppermost limit of the recommended sowing density of 750-1500 PLS/m^2 for revegetation of degraded grasslands (Burton et al., 2006). At the end of April 2015, the overwintered study plots were raked to achieve a heterogeneous soil surface and then seeded by hand. This technique was used because, in a recent study at the adjacent New Gold mine, raking increased seedling establishment on stockpiled topsoil (Baethke, 2015). Each study plot received 3 gallons of water immediately after seeds were sown.

Table 3.2. List of plant species chosen for field experiment.

Common Name	Scientific Name	Type	Source	Uses
Bluebunch wheatgrass	*Pseudoroegneria spicata*	Native grass	Pickseed	
Rough fescue	*Festuca campestris*	Native grass	Pickseed	
Sandberg bluegrass	*Poa secunda*	Native grass	Pickseed	
Junegrass	*Koeleria macrantha*	Native grass	Pickseed	
Slender wheatgrass	*Elymus trachycaulus*	Native grass	Pickseed	
Alkali Bluegrass	*Poa juncifolia*	Native grass	Pickseed	
American vetch	*Vicia americana*	Native legume	GPEC	Medicinal/food
Yarrow	*Achillea millefolium*	Native forb	Quality Seed	Medicinal
Arrow-leaved balsamroot	*Balsamorhiza sagittata*	Native forb	Quality Seed	Food/medicinal
Brown-eyed susan	*Gaillardia aristata*	Native forb	Quality Seed	Medicinal
Nodding onion	*Allium cernuum*	Native forb	Quality Seed	Food
Annual ryegrass[a]	*Lolium multiflorum*	Agronomic grass	Pickseed	
Alfalfa[a]	*Medicago sativa*	Agronomic legume	Pickseed	
Big sagebrush[b]	*Artemisia tridentata*	Native shrub	Splitrock	

[a]Agronomic seed was added to half of the plots to represent the 'cover crop' treatment.
[b]*Artemisia tridentata* seedlings were planted in half of the plots as to represent the 'nurse plant' treatment.

Irrigation System Set-up

In April 2015, an automatic gravity fed drip irrigation system was constructed at the TSF (Figure 3.6). The system utilized a 2000-gallon water tank and consisted of three zones (one per block), each controlled by a battery powered timer. The irrigation timers were equipped with rain sensors that prevented the system from operating during rain events. The irrigation system was

turned on immediately after the plots were seeded and was programmed to deliver 8-10 mm of water per week during the seedling establishment period (from the seeding date until mid-May). The irrigation system was turned off in mid-May and plots became dependent on natural precipitation for the remainder of the growing season. The reason for limiting the moisture supply was to reduce the potential for competitive interactions between nurse plant and target species, thus making any facilitative effects more apparent (Padilla and Pugnaire, 2009). Plots were monitored several times a week throughout the field season.

Figure 3.6. View of automatic gravity feed drip irrigation system installed at the Historic Afton Tailings Storage Facility research site in 2015. A 2000-gallon water tank elevated on the tailings dam (top left), 3-way independent water delivery system (top right), low pressure automatic irrigation timer (bottom left) and ½ gallon/hr. drip nozzle (bottom right).

Data Logger Set-up

In March 2016, prior to seeding, each plot was instrumented with Hobo[®] data loggers (Onset Computer Corporation, Bourne, MA) to monitor soil moisture and soil temperature levels throughout the duration of the study. Two additional probes were deployed in an open location, directly into the tailings. Soil probes were installed at the ~5 cm soil depth (Padilla and Pugnaire, 2009) and data were recorded in hourly intervals from April 1 to August 15 (Figure 3.7).
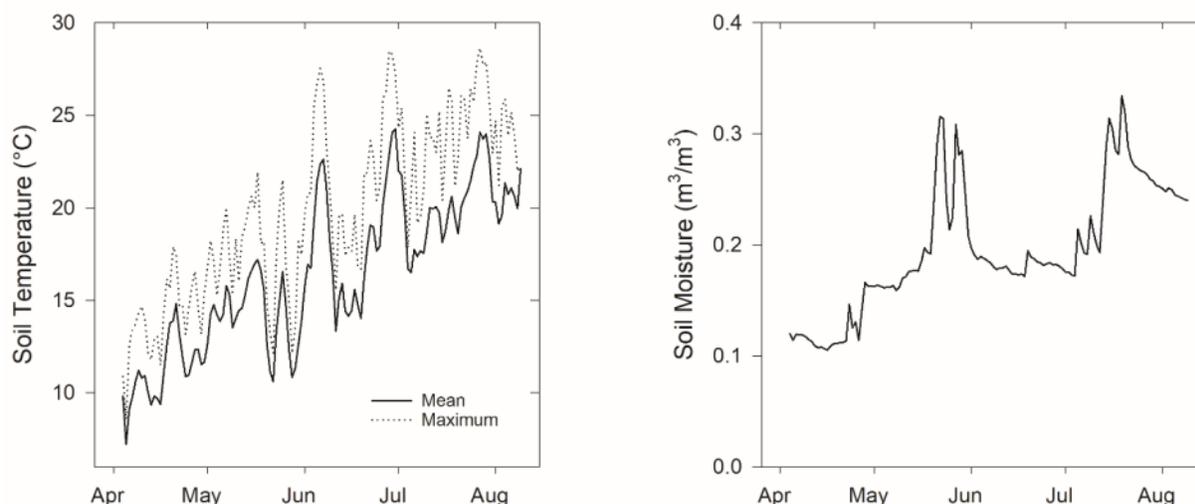
Figure 3.7. Tailings mean and maximum temperature (left) and moisture (right) at the Historic Afton Tailings Storage Facility over the 2016 study season (April–August). Soil temperature and moisture were measured at the 5 cm depth (Hobo[®], Onset Computer Corporation, Bourne, MA) in an exposed location.

Data Collection

Baseline soil sampling was conducted in April 2016, prior to seeding the plots. A total of six composite soil samples (three from each amendment treatment) were taken from the top 15 cm layer of randomly selected plots. The field samples along with samples of pure tailings material and soil amendments were sent to the British Columbia Ministry of Environment Analytical Laboratory (BCMEAL) (Victoria, BC) for analysis of soil pH, nutrient and metals content. Laboratory methodology and results of soil analysis are discussed in Chapter 2 (also see Appendix B for a list of tailings total elemental concentrations).

Field data collection was conducted during the second week of August 2016. Prior to sampling, the data loggers were retrieved from the plots and the irrigation system was disassembled. The data were downloaded from the Hobo[®] devices using the provided

HOBOware software (Onset Computer Corporation, Bourne, MA) and summarized into the variables 'mean soil temperature', 'mean daily maximum soil temperature' and 'mean soil moisture'. Three soil cores (approx. 53 cm^3 each) were taken from the surface of plots representing the "ash" and "compost" treatments, and from the unamended tailings on the ground in order to calculate bulk density. Vegetation cover of each plot was documented with high resolution photographs taken using a Nikon SLR Camera. Plant species cover was assessed using a 0.25 m^2 quadrat (Coulloudon et al., 1999) placed in a representative location within each study plot (Figure 3.8). The Shannon-Weiner index of plant community diversity was calculated with the species cover data for each plot using the equation:

$$Shannon - Weiner\ Index\ (H) = -\sum_{i=1}^{s} p_i\ ln\ p_i \qquad [1]$$

and a Simpson diversity index was calculated using the same data with the equation:

$$Simpson\ Index\ (D) = \frac{1}{\sum_{i=1}^{s} p_i^2} \qquad [2]$$

where *p* is the proportion (n/N) of individuals of one particular species (n) divided by the total number of individuals found (N) and *s* is the number of species. Both equations are from Colwell (1988). Four parameters were selected to represent sagebrush/nurse plant abundance. They were 'cover', 'horizontal canopy area', 'canopy volume' and 'dry biomass'. When measuring the horizontal canopy area of the sagebrush nurse plants in the field, plants were removed from the plots, repotted then placed (one at a time) in front of a custom made checkerboard with 2-inch squares, then photographed from a standardized distance of about 2 m (modified from Collins and Becker, 2001) (Figure 3.8). The photographs were imported into ImageJ 1.50i software (National Institutes of Health, USA) and analyzed by setting the scale (using the known distance of the checkers) and drawing a polygon around the perimeter of the shrubs. Sagebrush cover was measured by considering the entire plot as one quadrat. Sagebrush canopy volume was measured by taking three measurements of the shrub canopy (major axis, minor axis and vertical axis) (modified from Franco and Nobel, 1989) and inputting the variables into the equation:

$$V = abc\frac{\pi}{6} \qquad\qquad [3]$$

which is used to calculate the elliptical volume (V) of an object; *a, b* and *c* are the lengths of the major axis, minor axis the vertical axis, respectively. Sagebrush seedlings were placed in paper bags and oven-dried for 24 h at 70 °C to determine dry biomass. Aboveground plant biomass (excluding sagebrush) within each 0.25 m^2 quadrat was clipped as close to the soil surface as possible. Samples were placed in paper bags and oven-dried for 24 h at 60 °C. Dry weights were measured and extrapolated to a per hectare yield basis.

Figure 3.8. Assessing plant cover with a 0.25 m^2 quadrat (left) and measuring horizontal sagebrush canopy area with a custom 2-inch checkerboard (R) during 2016 data collection.

Statistical Analyses

  Data for the field experiment were analyzed statistically using R version 3.2.3 "Wooden Christmas-Tree" (The R Foundation for Statistical Computing). All data were checked for normality using boxplots and residual plots. Homogeneity of variance was assessed using the Fligner-Killeen test, and when necessary, the data were transformed using a natural logarithm or a square root function. To determine if any of the three factors (soil amendment, nurse treatment and cover crop) influenced reclamation success, the measured field parameters were analyzed using a three-factor analysis of variance (ANOVA) followed by a Tukey-HSD post-hoc test. All data were tested for significance at the 5% probability level. The mean ± standard error was reported for the significances detected. A correlation matrix was developed to investigate for relationships between sagebrush abundance and the abiotic and biotic parameters collected in the

field. Significant correlations (at *P* < 0.05) were investigated further using linear regression analysis.

RESULTS

Soil Temperature

Mean daily soil temperatures of the study plots during the growing season were 11.0 ± 0.1 °C in April, 12.7 ± 0.1 °C in May, 16.3 ± 0.1 °C in June and 18.3 ± 0.1 °C in July (data not shown). *Artemisia* nurse plants had a significant effect on mean daily soil temperature in the early months of the growing season (April-May) and when averaged across the entire study period (April-July) (Table 1). Mean daily temperatures in both April and May were 0.3 °C cooler with nurse plants compared to without (Figure 3.9). When averaged over the entire study period, soil temperature was 0.2 °C lower with nurse plants compared to in the open (Figure 3.9). There were no significant correlations between sage abundance parameters (canopy volume, aboveground biomass, horizontal canopy area and canopy cover) and mean soil temperature (Table 3.3).

Mean daily maximum soil temperatures of the study plots during the growing season were 14.1 ± 0.12 °C in April, 15.7 ± 0.18 °C in May, 19.3 ± 0.2 °C in June, and 20.8 ± 0.19 °C in July (data not shown). When analyzing by month, there was only some evidence of *Artemesia* canopies ameliorating extreme soil temperatures and this occurred in April, prior to seeding the study plots (Table 3.3). During April, daily maximum soil temperatures were, on average, 0.6 °C lower on nurse plots compared to the control (Figure 3.9). When analyzing soil temperatures over the course of a single day, variations in temperature extremes were more apparent. On the hottest day in May (May 28, 2017), when air temperatures reached 33.2 °C, maximum soil temperatures were 13.8 °C in the open compared to 12.2 °C in plots without shrubs and 11.8 °C in plots with shrubs (Figure 3.10). Overnight temperatures dropped to 7.5 °C, 9.6 °C and 9.5 °C for the open location, and on plots without shrubs and with shrubs, respectively.

There was slight evidence (at the 10% probability level) of cover crops having a moderating effect on soil temperature levels during the month of June (Table 3.3). Mean daily soil surface temperatures of the study plots were, on average, 0.2 °C cooler in the shaded area beneath the agronomic grasses compared to under stands of native grasses (data not shown).

Mean soil temperatures were 16.2 ± 0.1 vs 16.4 ± 0.1 °C for cover crops and no cover crops, respectively.
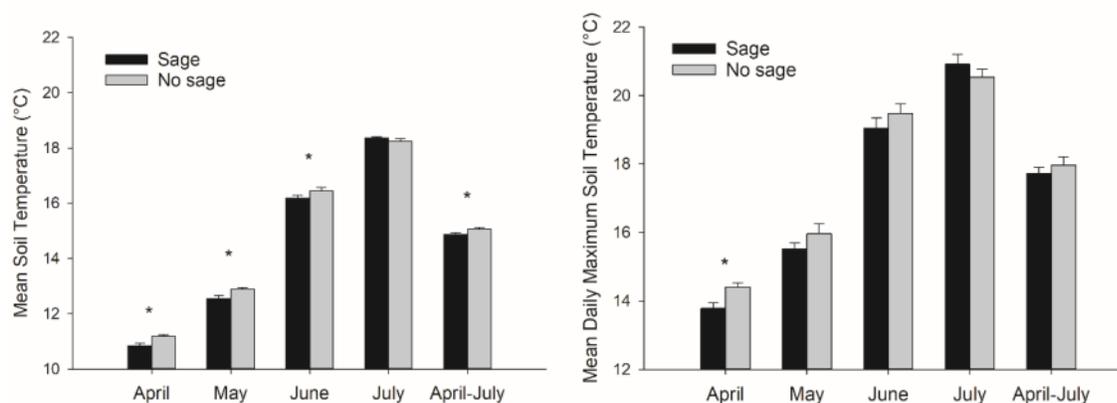
Figure 3.9. Mean daily soil temperature (left) and mean daily maximum soil temperature (right) of study plots with and without *Artemisia tridentata* (big sagebrush) nurse plants. Error bars are standard error of the mean. The * represents a significant treatment effect at the 5% probability level.

Soil Moisture

Volumetric soil moisture content averaged 0.12 ± 0.01 $m^3 m^{-3}$ in April, 0.33 ± 0.02 $m^3 m^{-3}$ in May, 0.27 ± 0.02 $m^3 m^{-3}$ in June, and 0.17 ± 0.02 $m^3 m^{-3}$ in July. There was no direct evidence of the presence of *Artemisia* shrubs exuding a positive (nor negative) effect on soil moisture levels throughout the study period (Table 3.3). However, a slight negative trend (at the 10% probability level) between sagebrush canopy volume and understory soil moisture levels was identified in the months of May and June (Table 3.4). A similar trend was identified between sage biomass and soil moisture in May only.

Cover crops had a negative effect on soil moisture levels over the entire study period and in the months of June and July (Table 3.3). Soil moisture content was, on average, 22.5% lower with cover crops (Figure 3.11). When analysing each month separately, moisture levels on plots seeded with cover crops were 8% lower in May and 39% lower in July. In June, the effect on soil moisture also depended on soil amendment type as indicated by the *Seed × Amendment* interaction (Table 3.3). During June, soil moisture content of the study plots ranged from 19.2 to 30.6%, with the combination of cover crops and ash having the lowest amount of all treatments (Figure 3.11).

Soil Amendments

After one growing season, the bulk density values of the amended tailings were almost half that of the unamended tailings (1.52, 1.56 and 2.32 Mg m^{-3} for the ash, compost and control, respectively) (data not shown). There were no clear direct effects of soil amendments on the other abiotic variables examined (Table 3.3).

Table 3.3. Results of 3-way ANOVA looking at the effects of nurse plants (sage/no sage), seed type (native/native with cover crop) and soil amendments (compost only/compost-ash mix) on physical soil parameters of study plots at the Historic Afton Tailings Storage Facility.

Abiotic Environment	Nurse plant F	Nurse plant P-value	Seed F	Seed P-value	Amendment F	Amendment P-value	Nurse x Seed F	Nurse x Seed P-value	Nurse x Amendment F	Nurse x Amendment P-value	Seed x Amendment F	Seed x Amendment P-value	Nurse x Seed x Amendment F	Nurse x Seed x Amendment P-value	Block F	Block P-value
Mean daily soil temperature																
April-July	6.34	**0.025**	0.70	0.419	0.00	0.957	3.74	0.074	1.00	0.335	3.48	0.083	0.73	0.407	2.75	0.099
April	12.0	**0.003**	-	-	0.63	0.437	-	-	0.22	0.646	-	-	-	-	2.55	0.106
May	16.5	**0.001**	0.15	0.709	0.82	0.382	1.67	0.218	1.26	0.280	4.57	0.051	0.62	0.445	11.4	**0.001**
June	4.40	**0.055**	3.72	*0.074*	0.02	0.889	2.97	0.107	1.85	0.195	4.18	0.060	0.00	0.968	7.58	**0.006**
July	0.62	0.445	0.00	0.988	0.41	0.535	3.08	0.101	0.07	0.795	0.10	0.759	0.07	0.793	0.74	0.494
Max daily soil temperature																
April-July	0.41	0.535	0.02	0.900	0.00	0.966	0.15	0.702	0.01	0.945	0.20	0.664	0.09	0.770	0.11	0.896
April	8.75	**0.008**	-	-	0.03	0.865	-	-	0.93	0.347	-	-	-	-	1.38	0.277
May	1.12	0.308	0.20	0.663	0.25	0.625	0.00	0.979	0.00	0.962	0.18	0.676	0.03	0.865	0.31	0.737
June	0.81	0.382	0.51	0.487	0.09	0.767	0.22	0.650	0.05	0.828	0.03	0.871	0.02	0.889	0.32	0.735
July	0.70	0.418	0.47	0.502	0.03	0.856	0.32	0.580	0.00	0.978	0.36	0.559	0.01	0.929	0.25	0.782
Soil moisture																
April-July	0.38	0.548	5.68	**0.032**	1.99	0.180	0.03	0.862	0.22	0.644	1.20	0.292	1.15	0.301	4.06	**0.041**
April	1.61	0.221	-	-	0.05	0.834	-	-	0.08	0.785	-	-	-	-	7.60	**0.004**
May	0.58	0.460	0.81	0.382	1.30	0.274	0.21	0.651	1.21	0.290	0.01	0.947	0.09	0.769	2.07	0.164
June	0.16	0.697	8.14	**0.013**	3.40	0.086	0.46	0.509	0.05	0.826	4.81	**0.046**	1.73	0.209	8.55	**0.004**
July	0.27	0.610	10.9	**0.005**	2.74	0.120	0.17	0.684	0.00	0.982	2.45	0.140	3.63	0.078	5.08	**0.022**

Bolded values indicate a statistical significance at the 5% probability level (non- italicized) or 10% probability level (italicized).
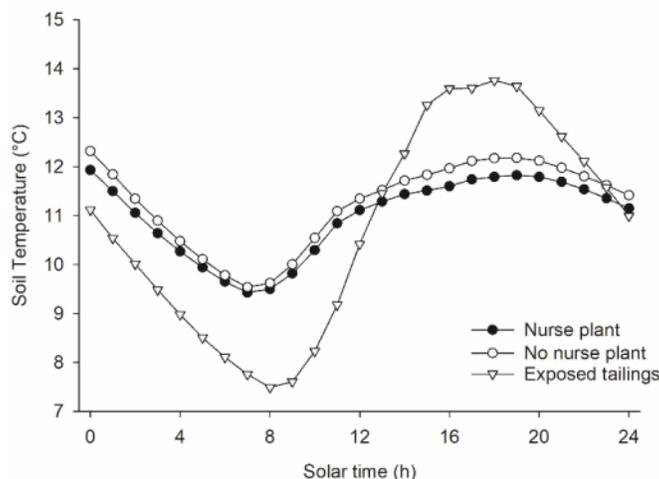
Figure 3.10. Time course of soil surface temperature of plots with and without *Artemisia tridentata* nurse plants and at an exposed location at the Historic Afton Tailings Storage Facility, on a clear, hot day in late spring (May 28, 2017). Data points are an average of twelve study plots except for the exposed tailings which is an average of two similar locations.
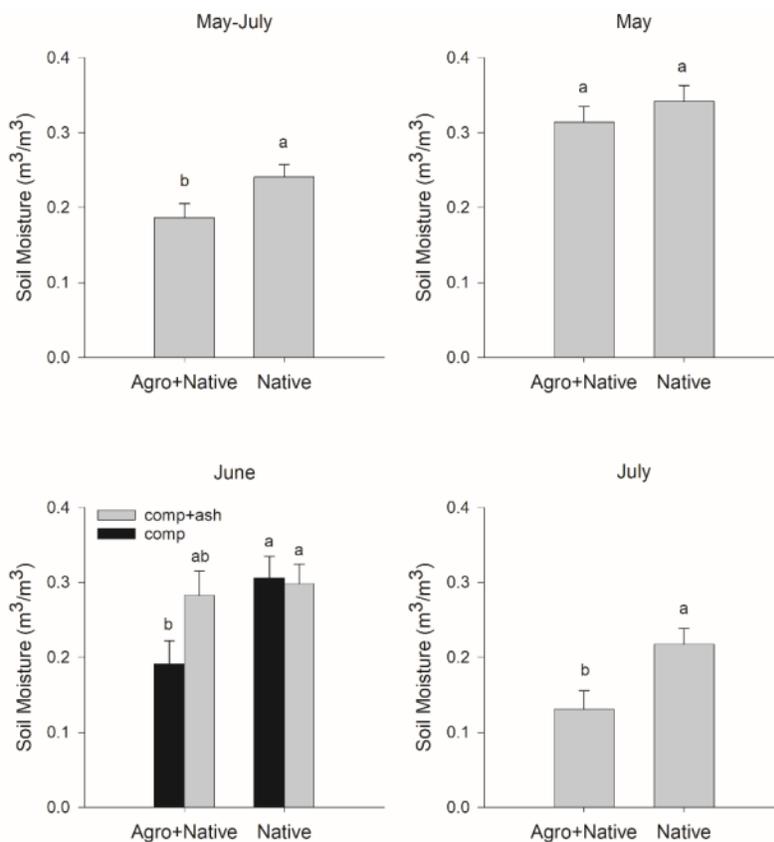
Figure 3.11. Mean volumetric soil moisture content of study plots with and without agronomic cover crop (averaged over the study period and by month). June data includes *Seed × Amendment* interaction (comp = compost). Error bars are standard error of the mean. Treatments with different letters are statistically different at the 5% probability level.

Table 3.4. Correlation matrix showing Pearson correlation statistics and P-values for relationships between sage abundance parameters and physical soil parameters of study plots at the Historic Afton TSF.

Abiotic Environment	Canopy Volume[a]		Biomass[b]		Horizontal Area[c]		Canopy Cover[d]	
	Pearson	P-value	Pearson	P-value	Pearson	P-value	Pearson	P-value
Mean daily soil temperature								
April-July	-0.07	0.818	0.14	0.655	0.06	0.857	0.22	0.485
April	-0.16	0.626	-0.11	0.730	-0.01	0.968	0.03	0.934
May	-0.12	0.708	0.15	0.646	0.03	0.934	0.15	0.651
June	0.14	0.674	0.32	0.318	0.21	0.519	0.34	0.274
July	-0.05	0.866	0.03	0.931	-0.07	0.823	0.11	0.728
Maximum daily soil temperature								
April-July	-0.20	0.530	-0.19	0.562	-0.15	0.645	-0.10	0.762
April	-0.12	0.720	-0.11	0.733	0.01	0.965	0.10	0.749
May	-0.25	0.425	-0.19	0.559	-0.17	0.597	-0.14	0.672
June	-0.15	0.647	-0.13	0.692	-0.13	0.676	-0.06	0.860
July	-0.19	0.555	-0.22	0.501	-0.18	0.580	-0.15	0.641
Soil moisture								
April-July	-0.45	0.140	-0.36	0.245	-0.41	0.186	-0.17	0.591
April	-0.09	0.782	0.02	0.945	-0.05	0.873	0.12	0.706
May	*-0.52*	*0.081*	*-0.50*	*0.099*	-0.49	0.102	-0.30	0.348
June	*-0.50*	*0.099*	-0.43	0.160	-0.42	0.169	-0.22	0.502
July	-0.49	0.103	-0.40	0.204	-0.48	0.118	-0.26	0.424

[a]sagebrush canopy elliptical volume; [b]sagebrush aboveground biomass; [c] sagebrush horizontal canopy area; [d] sagebrush canopy cover. Bolded values are statistically significant ($P < 0.1$).

Plant Community Establishment

At the end of the growing season, three (*Pseudoroegneria spicata*, *Elymus trachycaulus* and *Poa spp.*) of the six seeded native grasses were observed to have established on the study plots (Table 3.5). Native forb establishment was limited to only one (*Achillea millefolium*) of four species seeded. Three non-seeded, volunteer species (*Hordeum jubatum, Puccinellia nuttaliana* and *Danthonia intermedia*) were also recorded on the study plots.

Plant Productivity

Statistically, there was no evidence of *Artemisia* shrubs facilitating target species establishment (Table 3.6). In fact, total plant cover (excluding nurse plant cover) was lower on plots planted with nurse shrubs compared to those without (76.2 ± 5.3 vs. 93.5 ± 3.2%, respectively) (Figure 3.12). There was also some evidence (at the 10% probability level) of a negative effect of *Artemisia* on total aboveground plant biomass (excluding nurse plant biomass) when the data was transformed using a natural logarithm function (5323 ± 295 Kg ha^{-1} without sage vs. 4658 ± 330 Kg ha^{-1} with) (Figure 3.12).

When analyzed by functional group, native graminoid cover was lower with *Artemisia* than without, but this effect also depended the addition of cover crops, as shown by the *Nurse × Cover Crop* interaction (Table 3.6). Native graminoid cover was highest (69.8 ± 3.6%) when nurse plants and cover crops were excluded and lowest (21.1 ± 7.3%) when both treatments were included (Figure 3.13). A notable positive trend between sagebrush abundance parameters and volunteer species cover was detected, with the strongest determinant of volunteer cover being horizontal canopy area (Table 3.7; Figure 3.15).

When analysing plant cover by species, *Poa spp.* followed a similar pattern where cover was determined by an interactive effect involving nurse plants and cover crops (45.8 ± 5.8% for no sage/no cover crop vs. 14.2 ± 4.5% for sage/cover crop, respectively) (Figure 3.13). The agronomic cover crop had a negative (i.e. competitive) effect on *Pseudoroegneria spicata* cover (16.4 ± 2.6 without agronomics vs. 3.4 ± 0.6% with) (Figure 3.14). The establishment of *Elymus trachycaulus* depended on an interactive effect between cover crops and soil amendments (Table 3.6), with the compost-native seed combination yielding the highest cover (7.5 ± 1.7%) and the compost-cover crop combination yielding the lowest (0.83 ± 0.8) (Figure 3.14).

Plant Community Diversity

There was evidence of nurse plants and cover crops having an effect on plant community diversity. *Artemisia* shrubs and cover crops had an interactive effect on the Simpson's diversity index (D) (Table 3.6); the combination of nurse plants without cover crops had the highest *D* value (4.2 ± 0.3) while nurse plants with cover crops had the lowest (2.8 ± 0.4) (Figure 3.16). Species richness was influenced by the addition of agronomic cover crops; on average, 6.4 ± 0.19 species were counted on plots with cover crops compared to 5.3 ± 0.2 without (Figure 3.16).

Table 3.5. List of species identified and mean relative cover on study plots (by factor) at the Historic Afton Tailings Storage Facility.

Species	Scientific Name	Type	Functional group	Sage	No Sage	Cover crop	No cover crop	Compost	Compost -ash mix
						Relative Cover (%)			
Bluebunch wheatgrass	*Pseudoroeugneria spicata*	Seeded	Native graminoid	9.4	11.6	3.4	18.2	10.6	10.5
Bluegrass species	*Poa spp.*	Seeded	Native graminoid	18.9	34.3	16.6	37.4	24.7	28.3
Slender wheatgrass	*Elymus trachycaulus*	Seeded	Native graminoid	5.6	4.6	3.3	7.1	4.5	5.8
Yarrow	*Achillea millefolium*	Seeded	Native forb	4.8	3.1	2.5	5.5	4.0	3.9
Annual ryegrass[a]	*Lolium multiflorum*	Cover crop	Agronomic graminoid	23.3	29.4	50.5	0.0	30.1	22.7
Alfalfa[a]	*Medicago sativa*	Cover crop	Agronomic legume	1.1	1.6	2.5	0.0	1.6	1.1
Foxtail barley	*Hordeum jubatum*	Volunteer	Native graminoid	8.3	7.4	7.5	8.1	6.1	9.5
Nuttall's alkaligrass	*Puccinellia nuttalliana*	Volunteer	Native graminoid	8.8	8.0	4.8	12.3	8.8	8.1
Timber Oatgrass	*Danthonia Intermedia*	Volunteer	Native graminoid	0.1	0.0	0.1	0.0	0.1	0.0
Big sagebrush[b]	*Artemisia Tridentata*	Nurse plant	Native shrub	19.8	0.0	8.7	11.4	9.7	10.2

[a] agronomic species; [b] nurse plant

Table 3.6. Results of 3-way ANOVA looking at the effects of nurse plants (sage/no sage), seed type (cover crop/no cover crop) and soil amendments (compost only/compost+ash) on vegetation parameters of study plots at the Historic Afton Tailings Storage Facility.

Biotic Environment	Nurse Plant F	P-value	Seed F	P-value	Amendment F	P-value	Nurse x Seed F	P-value	Nurse x Amendment F	P-value	Seed x Amendment F	P-value	Nurse x Seed x Amendment F	P-value	Block F	P-value
Plant Productivity																
Plant biomass[a]	3.30	0.091*	2.49	0.137	0.06	0.804	0.54	0.474	0.42	0.527	0.09	0.772	0.91	0.357	0.26	0.776
Total cover[b]	10.5	**0.006**	3.32	0.090	0.15	0.707	0.04	0.836	0.92	0.353	0.07	0.788	4.16	0.061	3.70	0.051
Native graminoid cover	8.43	**0.012**	42.3	**<0.01**	1.21	0.289	4.89	**0.044**	0.01	0.931	1.59	0.227	1.08	0.317	0.09	0.918
Native forb cover	1.22	0.288	2.79	0.117	0.00	0.978	1.22	0.288	0.35	0.562	0.29	0.599	1.35	0.265	0.79	0.471
Volunteer cover	0.15	0.704	1.52	0.238	0.34	0.567	0.33	0.578	0.00	0.981	0.02	0.877	0.27	0.611	1.69	0.221
P. spicata cover	0.41	0.530	20.1	**0.001**	0.01	0.944	0.81	0.383	0.01	0.944	0.22	0.644	0.06	0.811	1.26	0.313
Poa spp. cover	10.5	**0.006**	16.0	**0.001**	0.91	0.357	5.22	**0.039**	0.14	0.709	0.14	0.709	0.91	0.357	0.24	0.786
E. trachycaulus cover	0.47	0.503	4.74	**0.047**	0.84	0.375	0.64	0.436	0.47	0.503	5.79	**0.031**	0.12	0.736	3.37	0.064
Plant community diversity																
Species richness	1.22	0.288	14.9	**0.002**	0.30	0.590	2.74	0.120	0.30	0.590	0.00	1.000	1.22	0.288	0.30	0.742
Shannon (H)	1.67	0.217	0.20	0.663	1.49	0.242	4.58	0.050	0.48	0.501	0.76	0.397	1.05	0.322	1.57	0.243
Simpson (D)	1.53	0.237	2.18	0.162	1.52	0.238	5.68	**0.032**	1.24	0.284	0.54	0.475	0.65	0.435	1.67	0.224

Bolded values are significant at 5% probability level; [a] Significant at 10% probability level; [b] Excludes *Artemisia tridentata* nurse plant
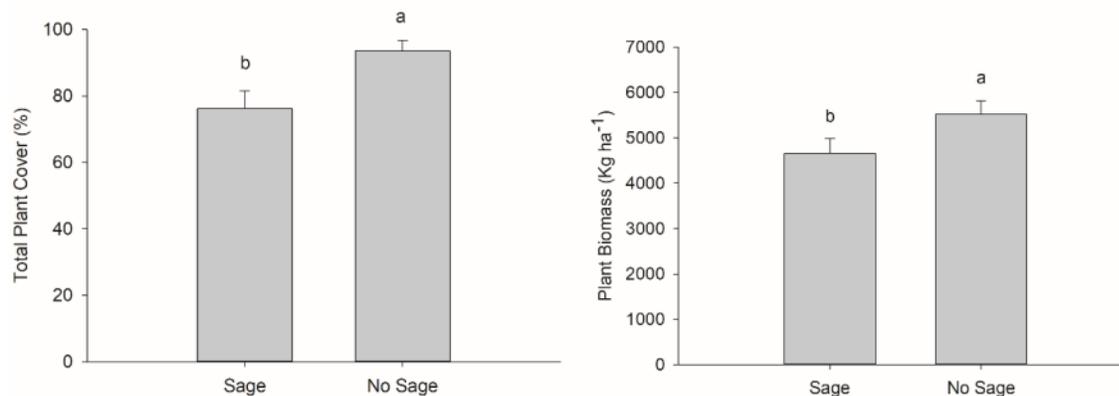
Figure 3.12. Total plant cover (left) and aboveground plant biomass (right) with and without *Artemisia* nurse plants. Error bars are standard error of the mean. Treatments with different letters are statistically different at the 5% probability level.
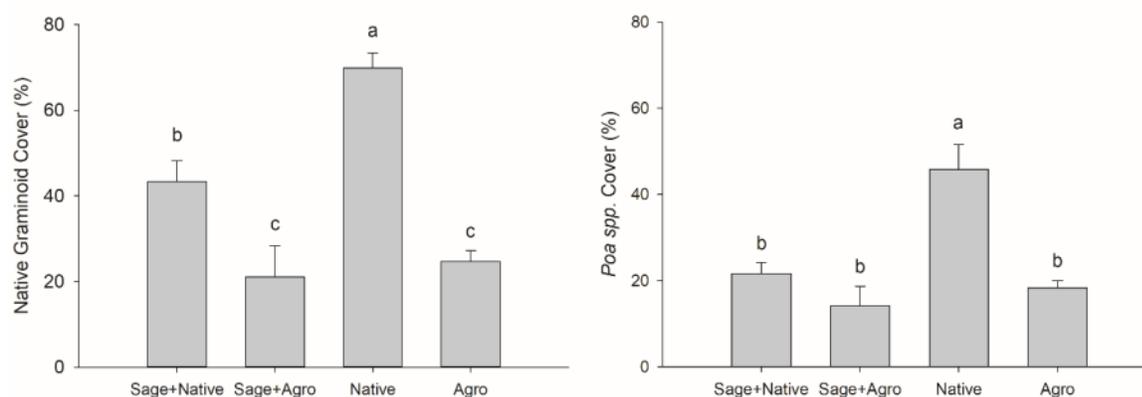
Figure 3.13. Native graminoid cover (left) and *Poa spp.* cover (right) response to nurse plant/cover crop treatments. Error bars represent standard error of the mean. Treatments with different letters are statistically different at the 5% probability level.
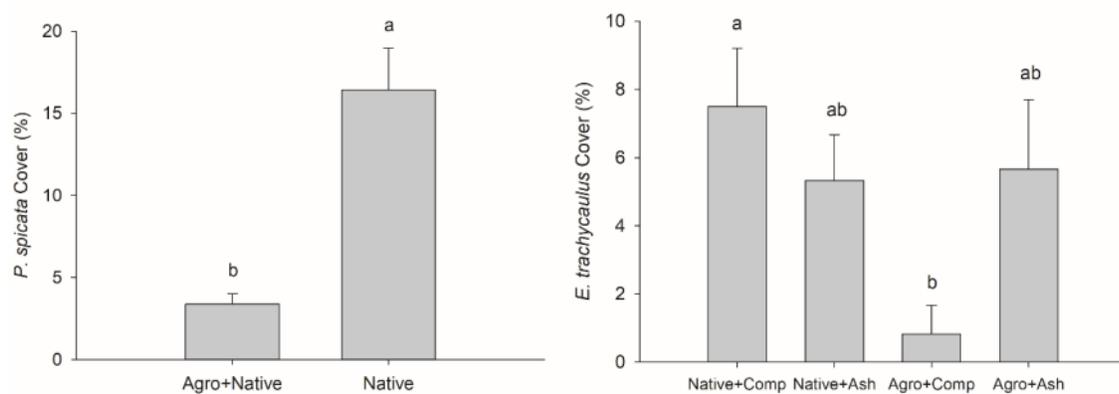
Figure 3.14. Effect of agronomic cover crops on *Pseudoroegneria spicata* cover (left) and effect of cover crops/soil amendments on *Elymus trachycaulus* cover. Error bars represent standard error of the mean. Treatments with different letters are statistically different at the 5% probability level.

Table 3.7. Correlation matrix showing Pearson correlation statistics and P-values for relationships between sagebrush abundance parameters and vegetation parameters of study plots at the Historic Afton Tailings Storage Facility.

Biotic Environment	Canopy Volume[a]		Biomass[b]		Canopy Area[c]		Canopy Cover[d]	
	Pearson	P-value	Pearson	P-value	Pearson	P-value	Pearson	P-value
Plant productivity								
Plant biomass*	-0.52	0.081	-0.53	0.079	-0.44	0.150	-0.45	0.141
Total cover*	-0.09	0.786	-0.05	0.869	0.08	0.796	0.08	0.795
Native gramnoid cover	-0.31	0.323	-0.24	0.454	-0.28	0.386	-0.15	0.647
Native forb cover	-0.22	0.496	-0.15	0.638	-0.19	0.564	-0.04	0.901
Volunteer cover	**0.72**	**0.008**	**0.75**	**0.005**	**0.76**	**0.004**	**0.74**	**0.006**
P. spicata cover	-0.52	0.082	-0.46	0.132	-0.54	0.070	-0.36	0.247
Poa spp. cover	-0.34	0.275	-0.32	0.314	-0.31	0.321	-0.25	0.437
E. trachycaulus cover	0.27	0.394	0.39	0.208	0.37	0.232	0.44	0.149
Plant community diversity								
Species richness (*S*)	0.11	0.725	0.11	0.729	0.17	0.591	0.10	0.746
Shannon index (*H*)	0.21	0.506	0.27	0.402	0.20	0.532	0.27	0.404
Simpson index (*D*)	0.24	0.445	0.28	0.373	0.21	0.503	0.29	0.353

Bolded values are significant at the 5% probability level. *Excludes *Artemisia tridentata* nurse plant. [a]sagebrush canopy elliptical volume; [b]sagebrush aboveground biomass; [c]sagebrush horizontal canopy area; [d]sagebrush canopy cover
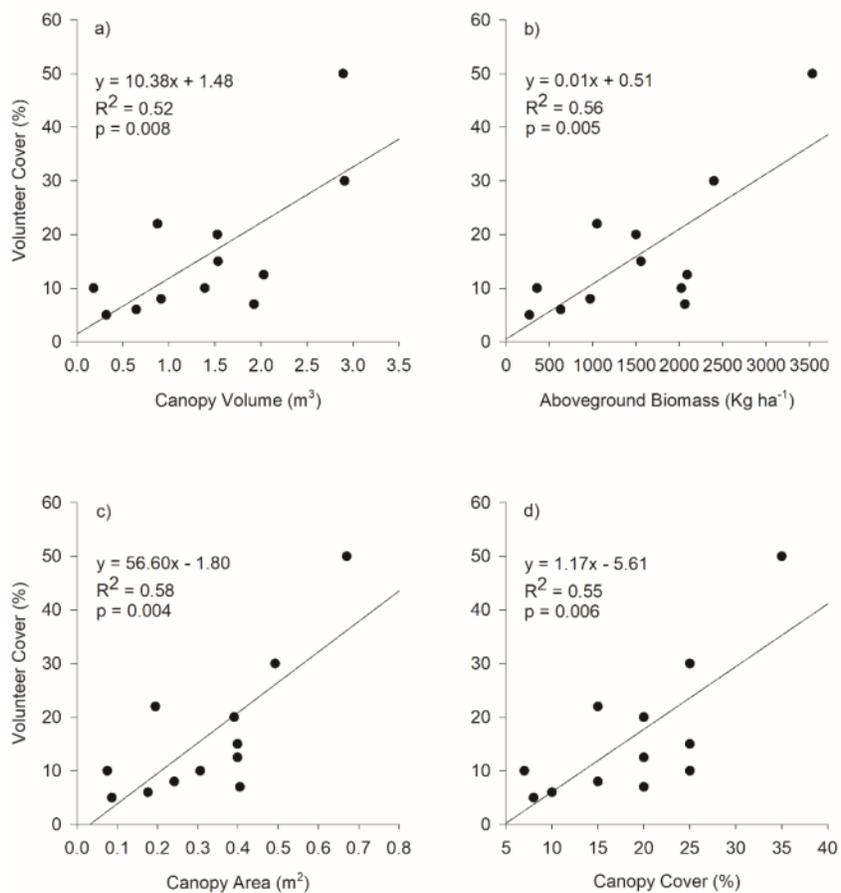
Figure 3.15. Relationship between volunteer species cover and sagebrush abundance parameters a) canopy elliptical volume, b) aboveground biomass, c) horizontal canopy area and d) canopy cover.
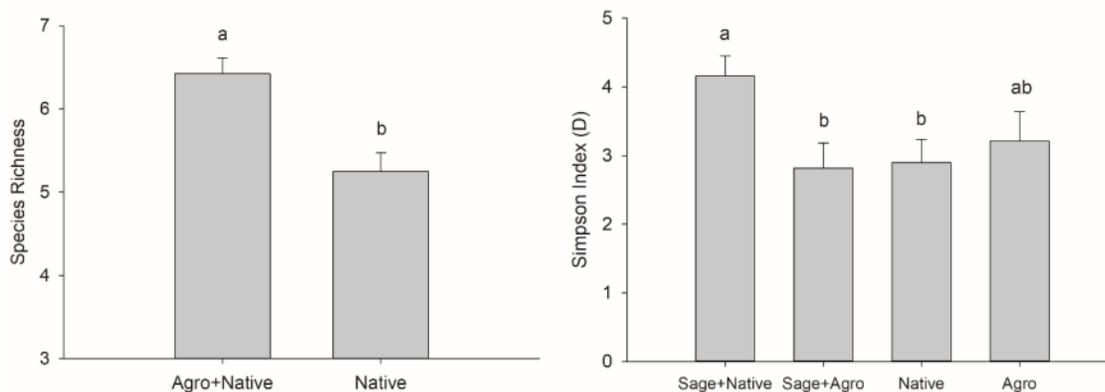
Figure 3.16. Effect of cover crops on species richness (left) and effect of nurse plant/cover crops on Simpson's diversity index (right). Error bars represent standard error of the mean. Treatments with different letters are statistically different at the 5% probability level.

DISCUSSION

Effect of Nurse Plants on Abiotic and Biotic Parameters

Artemisia nurse plants lowered mean daily soil temperature of the study plots by only a small margin (0.3 °C) during the early months of the growing season (April – May). When investigating a time course over the hottest day in May, it was found that soil temperatures under nurse plants were up to 2 °C cooler than in the exposed location. The lower soil temperatures under nurse plants can attributed to shading by shrub canopies, which limited the amount of solar radiation reaching the soil surface during daylight hours, and may be a critical process for seedling survival and establishment (Franco and Nobel, 1989; Padilla and Pugnaire, 2009). Since the nurse plants used in this study were only small, one-year-old seedlings (15-35 cm in height), the degree of shading was minimal compared to what has been reported in similar environments under large, adult nurse plants. For example, Franco & Nobel (1989) examined the nurse effects of various arid shrub species in the Sonoran Desert, USA and reported soil surface temperatures of up to 13.5 °C lower under adult *Hilaria rigida* plants compared to an exposed location (where soil surface temperatures reached 71 °C). Another study by Padilla & Pugnaire (2009) in a Mediterranean semiarid region of Spain found that air temperature averaged about 9 °C lower underneath the canopy of mature *Retama sphaerocarpa* shrubs compared to in gaps over a 6-day period during the summer. It is likely that the effect of shading on the understory soil surface temperature will become more prominent as the *Artemisia* canopies grow larger and are able to provide more shade.

The facilitative effect of nurse plants on soil surface temperature diminished (shifting from positive to neutral) during the mid to later summer months (June – July). This observation is consistent with reports of interactions shifting with temporal variations in the environment (Bruno et al., 2003; Maestre et al., 2009), and could be partially due to the effect of shading by nurse plant canopies becoming less significant as the understory grasses and other vegetation developed. Similar to the nurse plants, the established vegetation would have also been able to modify soil surface temperature by casting shade on the understory, resulting in similar conditions to the habitat under nurse plants. The observed nurse effect on soil surface temperature will likely persist further into the growing season in subsequent years as the *Artemisia* shrubs mature.

Whether or not the lower temperatures under nurse plants were beneficial for seedling survival is arguable because the conditions early in the growing season (when the nurse effect was observed) were not that extreme. Models of plant-plant interactions suggest that the magnitude of facilitation increases with abiotic stress (Bertness and Callaway, 1994; Callaway and Walker, 1997; Maestre et al., 2009). In extreme arid conditions, seedlings can benefit from the shade under nurse plants because it reduces heat stress and water loss (Franco and Nobel, 1989). However, when conditions are less severe, the positive benefits of shade might be outweighed by the negative aspects of growing under a shrub canopy such as increased competition for light, nutrients and water (Padilla and Pugnaire, 2009; Walker et al., 2001). More detailed measurements of other physical parameters associated with microclimatic amelioration (e.g. solar radiation, wind speed, air temperature, relative humidity) might have helped to further explain the facilitative processes under *Artemisia* shrubs.

It is important to note that shade can have a negative effect if light becomes limiting (Franco and Nobel, 1989; Padilla and Pugnaire, 2009). However, past field experiments have shown a large variation in seedling response to shade (Maestre et al., 2003; Padilla and Pugnaire, 2009; Walker et al., 2001). For example, a study by Huber-Sannwald & Pyke (2005) assessed *P. spicata* seedling survival under artificial shade and reported higher mortality rates for seedlings growing under strong shade conditions as opposed to full exposure or moderate shade. This could also be true for the other grassland species assessed in the current study. Whether or not shade results in a net facilitative effect on the plant community depends on the degree to which other interacting variables or combinations of variables are influenced by nurse plants (Walker et al., 2001). The effect of canopy protection by *Artemisia* seedlings on the understory microclimate warrants further investigation.

Contrary to reports of improved water availability near *Artemisia* shrubs, there was no indication of nursing success under canopies with regard to soil moisture levels during any of the months investigated. In fact, there was slight evidence of shrubs having a negative effect on soil moisture in May and June, as indicated by the weak negative correlation between shrub abundance and soil moisture levels. The decline in soil water with shrub abundance could likely be a result of high spring water demand (Drivas and Everett, 1988; Lysne, 2005) coupled with increased transpiration from the larger canopies. Larger plants would typically have greater leaf area which would increase transpiration rates during the day (when stomata are open) and lead to

a loss of water from the system (Cardon et al., 2013; Evans and Black, 1993). It is known that *Artemisia* shrubs take advantage of spring moisture (mostly from snowmelt) by allocating resources to aboveground vegetative growth early in the season, and that growth rates subsequently diminish in the summer and fall when moisture is more limiting and the plants are in their reproductive growth phase (Lysne, 2005; McArthur et al., 1998). In a greenhouse study investigating *Artemisia tridentata* seedling growth, Booth et al., (1990) reported substantial early aboveground growth rates which diminished after about 12 weeks into the experiment. The rapid early growth is believed to be an evolutionary adaptation to drier habitats where summer drought is common (Booth et al., 1990; Mcarthur and Welch, 1982). The field data suggests that *Artemisia* seedlings may exert a competitive effect on neighbors with regard to soil moisture uptake during the spring, and that this effect increases with canopy size and shrub biomass. This is consistent with ideas in the literature that increased facilitator size can result in an increase in the relative strength of competition, especially when ecological conditions are not severe (Callaway and Walker, 1997; Maestre et al., 2009).

The lack of evidence supporting a positive nurse effect on soil moisture can be attributed to several factors. Firstly, due to the small size of the nurse plants, the effect of shading was minimal; therefore, it is possible that evaporation rates at the soil surface were not much different under shrubs compared to in the open (during early months) or under the established grasses and other vegetation (during later months). Secondly, the shrubs may not have had adequate time to develop their root systems because they were only transplanted during the fall prior the study year; therefore, the contribution of water from hydraulic lifting from deeper soil layers was probably minimal to negligible. In a field study, Richards & Caldwell (1987) documented enhanced water availability at the subsurface soils (35-80 cm) of a mature *Artemisia tridentata* stand (where rooting depths reached over 2 m) as a result of hydraulic lifting from deeper soil layers. In the current study, the rooting depth of the transplanted seedlings was limited by the initial pot size (15 cm) as well as the depth of the study plots (61 cm), and so the soil moisture near and beyond the bottom of the containers would not have been accessible for root uptake. Finally, since the study year was a particularly wet year, the facilitative effects of nurse shrubs may have been less obvious because soil moisture was not as limiting as during a normal precipitation year. The effect of nurse plants on soil moisture would likely be more prominent during a drier year (Maestre et al., 2003; Padilla and Pugnaire, 2009) and under mature shrubs

(Cardon et al., 2013; Richards and Caldwell, 1987). As the plant root systems mature and are able to access water from deeper soil layers, hydraulic lifting could become a more significant factor influencing soil moisture and other important soil resources within the vicinity of *Artemisia* shrubs (Cardon et al., 2013; Richards and Caldwell, 1987).

The results suggest that *Artemisia* seedlings competed for water during the early months of the growing season but had no effect later in the season when conditions were drier. Contrarily, nurse canopies had a positive effect on soil temperature during the early months, but no effect during the later months when temperatures were warmer. This is consistent with reports of plant-plant interactions (i.e. facilitation vs. competition) shifting over a temporal scale (in this case, from negative to neutral for soil moisture, and from positive to neutral for soil temperature) in response to changes in nurse plant physiology and growth stage (Maestre et al., 2003; Pugnaire et al., 1996) and across an abiotic stress gradient (Maestre et al., 2009; Tewksbury and Lloyd, 2001). In semiarid environments, water is often more limiting than light or nutrients (Casper and Jackson, 1997), therefore it is possible that competition for water early in the season may be a more important factor determining survivability than the microclimatic amelioration provided by the nurse canopy.

Several studies have reported facilitation of perennial grasses and other plant species by shrubs in semiarid ecosystems (Padilla and Pugnaire, 2006). In these areas, the facilitative mechanisms within the vicinity of shrubs are often associated with resource enhancement (e.g. improved soil fertility and water retention) (Moro et al., 1997), microclimatic amelioration (e.g. reduced temperature and solar radiation levels, increased humidity) (Franco and Nobel, 1989) and protection from herbivores (Bruno et al., 2003; Ren et al., 2008). Cavieres & Badano (2009) demonstrated that these positive plant-plant interactions can improve the fitness of individuals of certain species and lead to an overall increase in species diversity at the community level. The results of the current study are in agreement with their work, and support the hypothesis that facilitation by *Artemisia* nurse plants leads to an increase in species diversity.

Shrubs had a positive effect on the Simpson's diversity index (*D*) (although this effect also depended on whether cover crops were planted with nurse plants). The combination of nurse plants without cover crops yielded the highest *D* value, and the treatment with nurse plants and cover crops yielded the lowest. This indicates that, in the absence of competition from cover crops, *Artemisia* shrubs facilitated the establishment of a more diverse ecological community.

One explanation for the increased diversity under nurse plants could be due to the increase in volunteer species cover. Relative cover of *Puccinellia nuttalliana*, *Danthonia intermedia, and Hordeum jubatum* were all higher on plots with nurse plants and total cover of these three grass species (grouped together as "volunteer cover") increased with all of the four measured sage abundance parameters. It is possible that *Artemisia* canopies intercepted the wind-dispersed seed of these colonizer species and facilitated their establishment through climatic amelioration and other facilitative processes. A study by Moro, Pugnaire, Haase, & Puigdefabregas (1997) suspected the same mechanism was responsible for increased species richness under *Retama sphaerocarpa* canopies in a semiarid region of Spain. The suspected ability of *Artemisia* shrubs to intercept wind-dispersed seed of colonizer species and facilitate their establishment could lead to the formation of fertile shrub islands (i.e. islands of fertility) over time (Moro et al., 1997; Walker et al., 2001) which can aid in the natural recovery of disturbed grassland ecosystems. The increased species diversity under nurse plants is an important finding because plant communities with high diversity generally have greater ecosystem functionality and so they are more resilient to environmental perturbations (Callaway and Walker, 1997; Cavieres and Badano, 2009).

Both plant biomass and total plant cover were lower under shrub canopies compared to in the open. This could be a direct result of belowground competition (for water and nutrients) with the shallow, fibrous roots of the *Artemisia* plants limiting the growth of the understory plant species. In a field study, Huber-Sannwald & Pyke (2005) reported an overall increase in *Pseudoroegneria spicata* biomass under *Artemisia* canopies when seedlings were planted in root-exclusion tubes, which suggested that root interactions between nurse plant and associated species can limit seedling growth. Competition for water increases when both the nurse plant and the target species have shallow rooting systems and when there are differences in water potential between nurse and target species (Franco and Nobel, 1989). The belowground competition between *Artemisia* and neighbors is likely to be greater when the shrubs are in their seedling phase because the rooting zone is co-occupied by both species (Maestre et al., 2003). The species that are most able to benefit from the interaction with a nurse plant are those that can withstand the negative effects of root interference (Liancourt et al., 2005). As the shrubs develop deeper root systems, belowground root competition with associated species is expected to decrease because the respective rooting systems will each occupy their own niche within the soil profile.

Evidence of such a phenomenon was observed in a study by Moro et al., (1997) who reported that plant biomass was higher under mature shrubs compared to younger, immature shrubs.

When analyzing by plant functional group, native gramnoid cover was highest when nurse plants and cover crops were excluded and lowest when cover crops were included. When looking at individual species, *Poa spp.* cover followed a similar trend. *Poa spp.* were also the most dominant of the native grass species on all plots. This could be because the *Poa* species planted are known to be tolerant of alkaline conditions (Dobb and Burton, 2013). Despite *Poa spp.* performing well on the tailings, the results suggest that belowground competition from nurse plants and cover crops can limit their establishment on the amended tailings.

Effect of Agronomic Cover Crops on Abiotic and Biotic Parameters

Despite high germination in the greenhouse (see Appendix A), the establishment success of *Medicago sativa* in the field was limiting; therefore, the other agronomic species, *Lolium multiflorum*, can be considered the main driver of the observed changes in abiotic and biotic parameters with cover crops. The agronomic cover crop had a minor effect on soil temperatures in June. Increased shade under the vigorous cover of *L. multiflorum* can explain why soil temperatures were lower compared to plots planted with slower-establishing native grasses. Choi & Mohan, (1995) suspected that improved microclimatic conditions under a similar cover crop, *Panicum virgatum*, facilitated the establishment of later successional native species on an iron mine tailings site in New York, USA.

Soil moisture levels during the months of June and July were lower on plots seeded with cover crops compared to those without. This finding suggests that *Lolium multiflorum* competed for soil moisture during the drier months of the growing season. The competitive abilities of *L. multiflorum* in terms of its rapid growth and resource acquisition rates have been demonstrated (Tribouillois et al., 2014). In June, soil moisture depended on an interactive effect involving cover crops and soil amendments, with the compost-cover crop treatment having the lowest moisture levels. This could be because improved nutrients on the compost-amended plots (see Chapter 2) led to enhanced belowground growth and increased plant water uptake.

Cover crops interacted with nurse plants to influence the Simpson diversity (*D*) index. Species diversity values on plots with cover crops and no nurse plants was statistically similar to the values for plots with nurse plants and no cover crops. This finding indicates that cover crops

may also be an important facilitative tool for long-term grassland recovery. A study by Choi & Mohan, (1995) determined that the annual grass *Panicum virgatum* facilitated natural colonization of an iron mine tailings site in eastern USA by capturing wind-blown seed of woody species from surrounding areas and acting as a "nurse crop" during establishment. In their study, *P. virgatum* cover decreased substantially after several years, giving way for the colonized native species to dominate the plant community. It is possible that the same mechanism (capturing of volunteer seed) is responsible for the improved species diversity with *Lolium multiflorum*. A longer term study is needed to investigate how *L. multiflorum* influences grassland plant community dynamics over time.

The agronomic cover crop (dominated by *Lolium multiflorum*) inhibited the growth of native gramnoid species *Pseudoroegneria spicata, Elymus trachycaulus* and *Poa spp*. Previous field studies of grassland restoration have shown that non-native annual grasses can exert "priority effects" (competitive advantages of early-growing species) that reduce the growth of native perennial grasses both in the short- and long-term, and that can play a major role in determining future plant community composition (Fukami et al., 2005; Grman and Suding, 2010; Plückers et al., 2013). In the short-term, these effects are primarily a result of agronomics outcompeting native species by germinating and growing more rapidly, but in the long term, soil legacies (alteration of soil physiochemical conditions such that growth of other species is inhibited) can play a major role (Grman and Suding, 2010; Viall et al., 2014). The greenhouse results (Appendix A) coupled with the field data suggest that strong priority effects exhibited by *L. multiflorum* are driving the observed reduction in native species cover on plots seeded with agronomics.

The species that had moderate germination success in the greenhouse did not perform well in the field. While germination rates of *Festuca campestris*, *Koeleria macrantha*, and *Gaillardia aristata* ranged from 60 to 80% in the greenhouse (see Appendix A, Figure A.2.), none of these species were observed on any of the study plots in the field. This suggests that species establishment on the amended tailings may have been limited by competition from the faster-growing species such as *Lolium multiflorum* and *Pseudoroegneria spicata*, and that some of the native grasses are also capable of exerting priority effects that limit the establishment of other native species.

The physical data suggests that competition for water (from both cover crops and nurse plants) may be one mechanism explaining the reduction in native species productivity on the treatment plots. Studies have shown that native bunchgrasses are sensitive to below-ground competition from other grasses (Herron et al., 2001) and shrubs (Huber-Sannwald and Pyke, 2005) until they can establish a mature root system (Cline et al., 1977). A longer term study is needed to determine whether the annual cover crop will persist, or whether native perennials will eventually take over. A study by Skousen & Venable (2008) assessing native species establishment on highway roadsides determined that competition from agronomic cover crops was most intense during the first year of reclamation, but that co-seeded native grasses were able to increase in subsequent years. Contrarily, another study assessing priority effects during dry acidic grassland restoration observed that plant community composition four years after seedling was similar to that of the initial seed mixtures (Plückers et al., 2013).

The results obtained in the current study contradict those reported by Espeland & Perkins, (2013) who found that the agronomic cover crop *Avena sativa* did not inhibit early growth of perennial grasses including *Elymus trachycaulus* (which was also used in this study). In the current study, it is possible that the improved nutrients on the compost-amended tailings led to enhanced growth and subsequent competition by *Lolium multiflorum*, which in turn reduced *E. trachycaulus* cover. Grman & Suding (2010) suggested that seeding native species prior to the establishment of agronomics could increase restoration success. Doing so would allow the practitioner to benefit from the short-term practical uses of cover crops (e.g. erosion control, aesthetics, nutrient retention, invasion resistance) while the perennial grass community develops over time.

CONCLUSION

The results of the field study provide several important considerations for mine restoration practices in B.C.'s interior semiarid grasslands. The presence of *Artemisia* nurse plant seedlings improved microclimatic conditions by reducing soil surface temperature early in the growing season, but there was some evidence of a competitive effect with regard to soil water usage in the spring. This study did not assess other environmental parameters, such as air temperature, solar radiation, relative humidity and wind speed under nurse canopies, and so it is difficult to ascertain whether the young *Artemisia* plants had a net facilitative effect on the

understory abiotic environment. It is suspected that belowground competition with shrubs led to an overall decrease in grassland species cover and biomass. This effect is expected to diminish as nurse plant roots develop and become capable of extracting water from deeper soil layers. Furthermore, species diversity responded positively to nurse plants indicating that some facilitative mechanisms are at work. These mechanisms may promote the formation of "islands of fertility" around *Artemisia* shrubs over time due to enhanced productivity and subsequent buildup of organic matter. Given the short duration of this study, the influence of nurse plants on soil physiochemical parameters was not assessed. A longer term study would reveal more insight into the facilitative processes taking place within the vicinity of *Artemisia* shrubs. The influence of nurse plants on the parameters investigated should increase as the shrubs grow and mature.

The hypothesis that cover crops would impede native species diversity was not supported. The plots planted with nurse plants had the highest diversity, but those seeded with cover crops were statistically similar. Despite high germination success in the greenhouse, *Medicago sativa* establishment was not successful in the field, and therefore this species was only a minor component of the agronomic cover crop. Competition from *Lolium multiflorum* with the native perennials at the onset of reclamation resulted in a strong priority effect after the first year. A longer term study is needed to assess whether the competitive effects of *L. multiflorum* will persist or whether native perennial grass cover will improve over time.

Despite a negative response to the wood ash amendment in the greenhouse (see Chapter 2), plant performance did not appear to be affected in the field. The two soil amendment treatments were statistically similar to one another with regard to the abiotic parameters investigated in the field. The addition of soil amendments reduced the bulk density of the tailings by almost twofold, which shows that these materials can be useful for addressing some of the physical limitations of mine tailings. An interactive effect between soil amendments and cover crops resulted in lower cover of *Elymus trachycaulus*, likely because the improved soil conditions with the compost-amended tailings led to increased competition from the fast-growing agronomic annual, *Lolium multiflorum*.

The results of the study suggest that *Artemisia* nurse plants are a promising tool for native grassland recovery on mine tailings under the circumstances tested. Although the agronomic cover crop treatment increased total biomass, the emergence of key native grass species was inhibited which indicates that cover crops are less useful for grassland recovery on mine tailings.

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CHAPTER 4 – RESEARCH CONCLUSIONS, MANAGEMENT IMPLICATIONS AND FUTURE RESEARCH

RESEARCH CONCLUSIONS

The natural habitats and ecosystems within British Columbia's interior semiarid grasslands have been altered by human activities such as urban development, mining, and agriculture. Conservation and restoration of these grasslands is important because they offer a wide array of ecological and economic benefits including biodiversity, wildlife habitat, water filtration, and carbon storage (Wilson, 2009). Traditional restoration practices involved seeding degraded land with fast-growing agronomic species, but more recently, the importance of re-establishing pre-existing native plant communities and biodiversity is increasingly being recognized (Burton and Burton, 2002; Skousen and Venable, 2008). Mine tailings, however, are not an ideal growing medium for native plants because they tend to lack the physiochemical and biological attributes of a native soil (Gardner et al., 2010; Pepper et al., 2013). Restoration in arid and semiarid environments can be especially challenging because extreme temperatures and desiccation threaten seedling survival (Padilla and Pugnaire, 2006). Successful development of native grassland communities on such sites depends on the ability of practitioners to manipulate the abiotic environment such that it is more favourable for plant growth (Pueyo et al., 2009) and reconstruct natural ecological processes such as soil and plant succession (Bradshaw, 1997). The aim of this thesis was to investigate the efficacy of a variety of restoration practices including 1) the use of locally available soil amendments for improving tailings physiochemical conditions, 2) remediation of metals and other contaminants through phytostabilization, and 3) facilitation of native grassland species with nurse plants and cover crops. These methods were assessed though a two-part study involving a one-year field study at the Historic Afton Tailings Storage Facility (TSF) and a three-month greenhouse experiment at the Thompson Rivers University Research Greenhouse in Kamloops, B.C.

Greenhouse Study

The objectives of the greenhouse experiment were to 1) investigate the suitability of two native, semiarid bunchgrasses (*Pseudoroegneria spicata* and *Festuca campestris*) for

phytostabilization of the historic Afton tailings, and 2) to assess the effect of soil amendments on soil physiochemical attributes and plant productivity.

Key Findings of Greenhouse Study:

- **Compost improved native bunchgrass productivity on the amended tailings**

A study by Piorkowski et al., (2015) emphasized the economic and ecological importance of using locally available soil amendments for soil reclamation. I was interested in determining the most effective amendment mixture – in terms of plant growth response – of the locally available materials at hand. The results of the greenhouse study determined that, of the three soil amendments investigated, the City of Kamloops municipal yard waste compost was the most effective at increasing aboveground and belowground biomass and shoot heights of native bunchgrasses *Pseudoroegneria spicata* and *Festuca campestris*. Contrarily, the Domtar wood ash appeared to have a negative effect on productivity, as I found that increasing the relative amount of ash in the amendment mixture produced shorter seedlings with lower overall biomass.

- *Pseudoroegneria spicata* **(bluebunch wheatgrass) and** *Festuca campestris* **(rough fescue) are not suitable candidates for phytostabilization of the historic Afton Tailings**

The objective of phytostabilization is to promote the growth of plant species that can stabilize metals in the rhizosphere while limiting shoot uptake (Mendez and Maier, 2008, 2007). The translocation factor (ratio of shoot elemental concentration-to-root elemental concentration) is a useful metric for determining species suitability. Both grass species assessed had TF values of >1 for molybdenum and zinc which indicates that these species are "hyper-accumulators" of these metals and therefore are not suitable candidates for phytostabilization of the historic Afton tailings because excess amounts of these metals can be toxic to wildlife and domestic animals.

Field Study

      The objectives of the field study were to assess 1) whether *Artemisia tridentata* seedlings can be used as nurse plants to facilitate native grassland species establishment, 2) if agronomic cover crops benefit or impede native plant community development, and 3) the effect of soil amendments on revegetation success.

Key Findings of Field Study:

- ***Artemisia tridentata* nurse plants facilitated the establishment of a more diverse grassland plant community**

      Studies have shown that, under adverse ecological conditions, certain shrubs called nurse plants can facilitate the establishment of neighbouring plants through a variety of mechanisms including abiotic amelioration, resource enhancement, and protection from herbivory (Bruno et al., 2003; Padilla and Pugnaire, 2006), and that this process can lead to an overall improvement in plant community diversity (Cavieres and Badano, 2009). I was interested in determining whether *Artemisia tridentata* seedlings were capable of facilitating the establishment of a more diverse grassland plant community on the mine tailings site. The results of the field study indicated greater plant community diversity under nurse plants which was possibly a result of the improved microhabitat (e.g. lower temperatures, protection from wind, solar radiation) under *A. tridentata* canopies.

- **Agronomic cover crops had a negative effect on native grassland species establishment**

      In agricultural settings, cover crops are useful tools for reducing nitrogen leaching, preventing erosion, and improving soil organic matter during the fallow period (Espeland and Perkins, 2013). These fast-growing, annual grasses can also be planted during restoration to provide quick benefits and improve the abiotic conditions for the slower-growing native species to establish (Choi and Mohan, 1995; Skousen and Venable, 2008). But in some instances, agronomics can out-compete native species and sterilize the process of native plant community

development and succession (Davis et al., 2005; Grman and Suding, 2010). I was interested in finding out if the agronomic grass species *Lolium multiflorum* and legume *Medicago sativa* facilitate or interfere with native grassland community establishment during early mine reclamation. The results of the one-year field study indicate that cover crops had a negative effect on native grassland species productivity and suggest that competition for moisture during the hot and dry summer months may have been a limitation to native species establishment.

MANAGEMENT IMPLICATIONS & FUTURE RESEARCH
Soil Amendments

Surface mining requires the removal of pre-existing soil and vegetation which often results in long-term impairment of ecological processes (Bradshaw, 1997; Shrestha and Lal, 2011). The degraded material (e.g. tailings, waste rock) left behind cannot facilitate plant life or soil processes because it is low in organic matter and nutrients, and lacks the physical attributes of well-functioning soils (Gardner et al., 2010; Sheoran et al., 2010). Land application of organic waste by-products such as compost, wood ash, and biosolids is an effective method for restoring the soil's physiochemical attributes and its ability to promote primary production (Larney and Angers, 2012; Zebarth et al., 1999). These materials are often high in organic matter and include nutrients such as nitrogen and phosphorous which makes them a good soil amendment for mine reclamation (Park et al., 2011; Shrestha et al., 2009). Vast quantities of amendments are needed in order to cover the disturbed surface, and so sourcing locally available industrial waste materials can reduce transport costs and be more efficient (Piorkowski et al., 2015).

My study assessed three locally sourced organic materials in terms of their potential for use as soil amendments at the TSF. Of the materials investigated, the City of Kamloops municipal yard waste compost seemed to have the best qualities and the most noticeable effect on plant growth, although, further research assessing higher application rates is necessary to determine the full potential of this amendment. The wood ash – created from "hog fuel" incineration at the Domtar pulp mill in Kamloops, B.C. – was a less promising product for amending the alkaline tailings because of its high pH and total aluminum content, which most likely caused the observed negative effect on plant growth. Because of its high pH, the ash may be a more suitable amendment for reclamation of acidic mine tailings where it can act as a soil conditioner to buffer pH levels and limit heavy metal bioavailability (Solís-Dominguez et al., 2012). There were no

clear effects of wood chips on plant growth, but there were improvements in soil organic matter and carbon which indicates that this material could be mixed with other amendments as a slow-release carbon source that can be beneficial in the long term.

Phytostabilization

Phytostabilization is an emerging remediation technology that utilizes a vegetative cap to prevent the dispersion of toxic metals and other harmful contaminants from tailings and other mine waste sites (Mendez and Maier, 2008, 2007). A recent study by Solís-Dominguez et al., (2012) emphasized that there is currently a lack of information regarding species-specific performance and suitability for phytostabilization. Information regarding plant tissue metal accumulation is especially important because revegetated mine sites are often grazed by livestock and/or wildlife (Schuman et al., 2010; Solís-Dominguez et al., 2012). Suitable species are those which can tolerate the adverse physiochemical conditions of mine soils while avoiding shoot uptake of metals.

My study investigated growth responses and plant tissue metal accumulation of two bunchgrass species native to B.C.'s semiarid grasslands and found that neither species were promising candidates for phytostabilization of the historic Afton tailings because both species accumulated high amounts of Mo and Zn in their shoots. In terms of growth and productivity on the mine tailings, *Pseudoroegneria spicata* is the better candidate and may still have a use as for other remediation techniques such as phytoextraction (Mendez and Maier, 2008). With this technology, shoot uptake is encouraged and plants containing the sequestrated metals are mowed and subsequently removed from the site, resulting in a reduction in tailings metals concentrations over time. Some of the other native grassland species that were successful in the field, such as *Poa spp.*, *Elymus trachycaulus* and *Puccinellia nuttalliana*, should also be considered, as they appeared to perform well on the alkaline tailings. The suitability of other native species that can tolerate alkaline soils, such as *Distichlis spicata* and *Hordeum jubatum* (Porensky et al., 2014; Robson et al., 2004) should also be investigated.

Nurse Plants

Under extreme ecological conditions, such as in arid and semiarid environments, facilitation by nurse plants can enhance target species establishment (Franco and Nobel, 1989;

Padilla and Pugnaire, 2006). Facilitation between nurse plant and associated species typically includes some form of habitat amelioration, resource enhancement and/or protection from grazing that results in a net positive effect on plant establishment (Bruno et al., 2003; Padilla and Pugnaire, 2006). Recently, facilitation with nurse plants and has been deemed as an appropriate technique for the restoration of degraded semiarid environments (Maestre et al., 2003; Pueyo et al., 2009). However, since competition and facilitation occur simultaneously, in some instances, competitive effects may be stronger than facilitation, resulting in a net interaction that is negative (Bertness et al., 1999; Padilla and Pugnaire, 2006). Because of this, there is a need to investigate facilitative mechanisms across a wide range of environments and plant communities (Cavieres and Badano, 2009; Padilla and Pugnaire, 2009; Pueyo et al., 2009), and to determine which species (both the facilitator and beneficiary) are appropriate for facilitation in a given system (Padilla and Pugnaire, 2006).

My study assessed the suitability of a semiarid shrub *Artemisia tridentata* (big sagebrush) as a nurse plant for grassland restoration at the TSF. The study revealed that nurse plant canopies were effective at reducing soil surface temperatures early in the growing season but that competition for soil moisture with neighbors can potentially outweigh this positive effect. Nonetheless, nurse plants appeared to successfully promote a more diverse ecological community which may be a result of canopies intercepting wind dispersed seed from surrounding plant communities and facilitating their establishment. Soil temperature and soil moisture were the only abiotic variables assessed, and so further research looking at other parameters such as wind speed, air temperature and solar radiation, under nurse plants (Padilla and Pugnaire, 2009) is recommended. It would also be beneficial to plant nurse shrubs with only one species (rather than an entire community) to determine the specific interactions taking place between *A. tridentata* and target grassland species (Huber-Sannwald and Pyke, 2005). Comparing the environment and biotic responses under nurse plants to artificial shade structures would also be interesting (Pueyo et al., 2009).

Cover Crops

Because of their wide use in agricultural systems (Espeland and Perkins, 2013; Tribouillois et al., 2014), there is potential for agronomic cover crops to be used as a tool for restoration of mine tailings sites. Cover crops (fast-growing agronomic annual grasses and/or

legumes) are typically used to provide quick ecological and economic benefits, such as erosion control, prevention of N leaching, and addition of green manure (i.e. organic matter) which can make the environment more favourable for the target plant community to establish (Espeland and Perkins, 2013). However, in some instances, these fast-growing introduced species can outcompete slower-growing native species and halt the process of ecological succession (Davis et al., 2005) due to the "priority effects" of being the first to establish (Grman and Suding, 2010; Plückers et al., 2013). Other studies, however have shown that, given adequate time, native species can, in fact, establish on sites that have been seeded with agronomics (Espeland and Perkins, 2013; Skousen and Venable, 2008).

My study investigated the use of *Lolium multiflorum* and *Medicago Sativa* as cover crops for facilitating native grassland species establishment at the TSF. The data suggests that cover crops (primarily *L. multiflorum*, as *M. sativa* establishment was poor) impeded native species establishment and resulted in the formation of less diverse plant communities after one growing season. This was likely because the agronomic species exhibited rapid germination and growth and competition for soil moisture. A longer-term study would be beneficial to determine if these "priority effects" persist, or whether native species cover will increase with time. As with the nurse plants, it would also be interesting to assess the interactions between *L. multiflorum* and a single target species growing within close proximity to one another.

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APPENDIX A – SEED GERMINATION TRIAL

INTRODUCTION

Seed dormancy and viability of seed stock are barriers to successful germination of native seed during restoration projects. Pre-treatments such as mechanical scarification, cold stratification and application of hormones can break dormancy and improve germination success in some species (Dobb and Burton, 2013). Gibberellic acid (GA), or Gibberellin is a naturally occurring plant hormone that stimulates growth of germinating seeds. The use of GA as a seed treatment has had variable success, depending on the species tested (Çetinbaş and Koyuncu, 2006; Gonzalez-Melero et al., 1997).

During winter 2015, a seed germination trial was conducted at the Thompson Rivers University Research Greenhouse (Kamloops, B.C.) to test the effects of GA on seed germination of the native species being used in this study. The objectives of the trial were threefold: 1) to determine the viability of the native seed stock acquired for the field experiment, 2) to assess the effects of GA on seed germination of the study species and 3) to compare seed germination between agronomic and native species.

MATERIALS & METHODS

Experimental Design

Germination rates of the thirteen study species (see Chapter 3, Table 2) were assessed for two treatments: 'GA' and 'control'. Each treatment was replicated three times (13 species × 2 treatments × 3 replicates = 78). The germination trial was conducted over a 30-day period under controlled conditions (temperature 21 °C, natural and artificial light, day/night 16 hrs/8 hrs). Seventy-eight petri dishes (35 mm diameter × 18 mm deep) were lined with filter paper and labelled by species and treatment. Each dish received 20 seeds of a single species. Petri dishes were randomly assigned to a single block using "The Random Number Generator" iPhone iOS application (Nicholas Dean, 2013) (Figure A1). Petri dishes were placed at the centre of the greenhouse pod (where temperature and lighting were most stable). The filter paper was kept saturated with either a) 1000 ppm Gibberellic acid solution (GA) (Çetinbaş and Koyuncu, 2006) or b) deionized water (control), depending on the assigned treatment. The greenhouse pod was monitored daily and the number of germinated seeds was recorded every second day. Seeds were

considered germinated when the radicle length reached twice the radicle width. The germinated seeds were removed from the petri dishes once they were counted.

Figure A.1. View of randomized block layout (top left), *Festuca campestris* seed prior to germinating (top right), emerging *Balsamorhiza sagittata* seed (bottom left) and *Allium cernuum* seed (bottom right). Treatments were solutions containing either a) 1000 ppm Gibberellic acid or b) deionized water (top left).

Statistical Analysis

Mean germination rates were calculated for each 2-day interval in order to show cumulative germination over the 30-day trial period. The time to first germination (T_0) (Ranal and Santana, 2006) was recorded for each replicate. Time to 50% germination (T_{50}) was calculated by using the equation from Çalişkan et al. (2012):

$$T_{50} = ti \left[\frac{\frac{(N+1)}{2} - ni}{nj - ni} \right] (tj - ti) \qquad [4]$$

where *N* is the final number of seeds germinated and *ni* and *nj* are the total number of seeds germinated at time *ti* and *tj* (where $ni < (N+1)/2 < nj$). Two sample t-tests were used to compare the final germination rates and T_{50} between treatments for each species. Significances were accepted at the 5% probability level.

RESULTS & DISCUSSION

Viability of Native Seed

Germination rates of native grasses ranged from 62 to 97%, with *Festuca campestris* having the lowest germination and *Elymus trachycaulus* having the highest after 30 days (Figure A1). Native forb germination was more variable and ranged from 0 to 87% (*Allium cernuum* and *Achillea millifollium*, respectively) (Figure A2). The native forbs *Balsamorhiza sagittata* and *A. cernuum* were the slowest to germinate (Tables A1 and A2) and final germination rates were low compared to the other forbs (Figure A2).
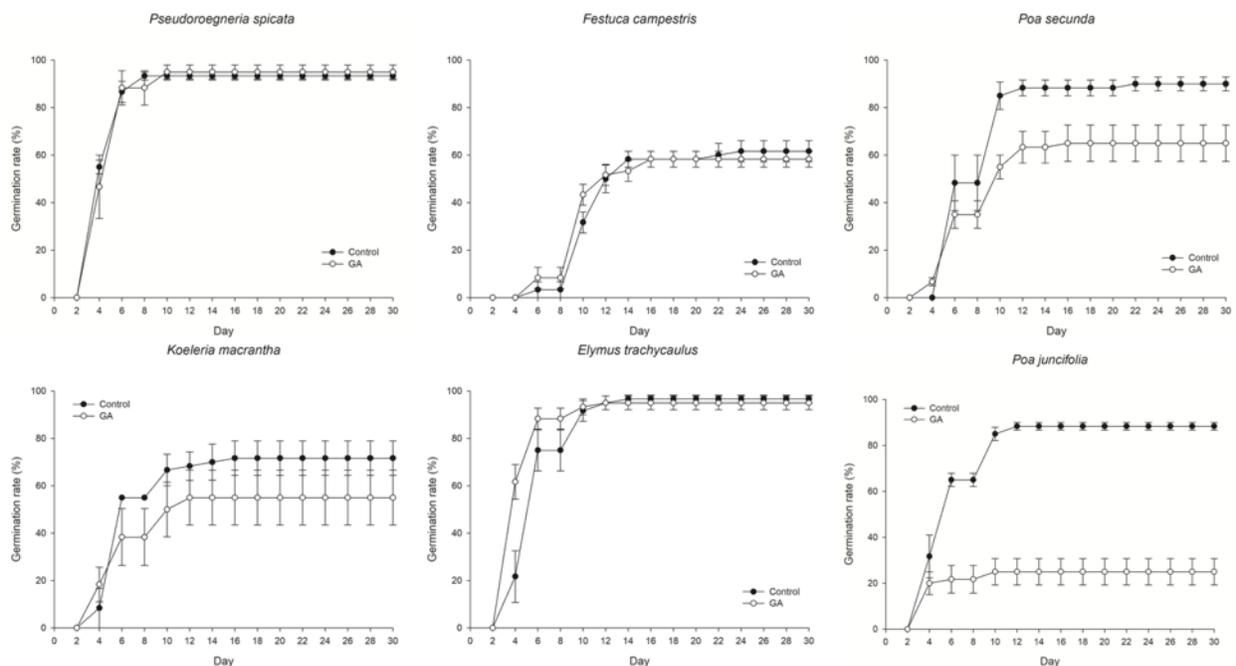
Figure A.2 Cumulative germination rates of native grasses over a 30-day greenhouse trial. Error bars are standard error of the mean. Treatments were a 1000 ppm Gibberellic acid solution (GA) and deionized water (control).
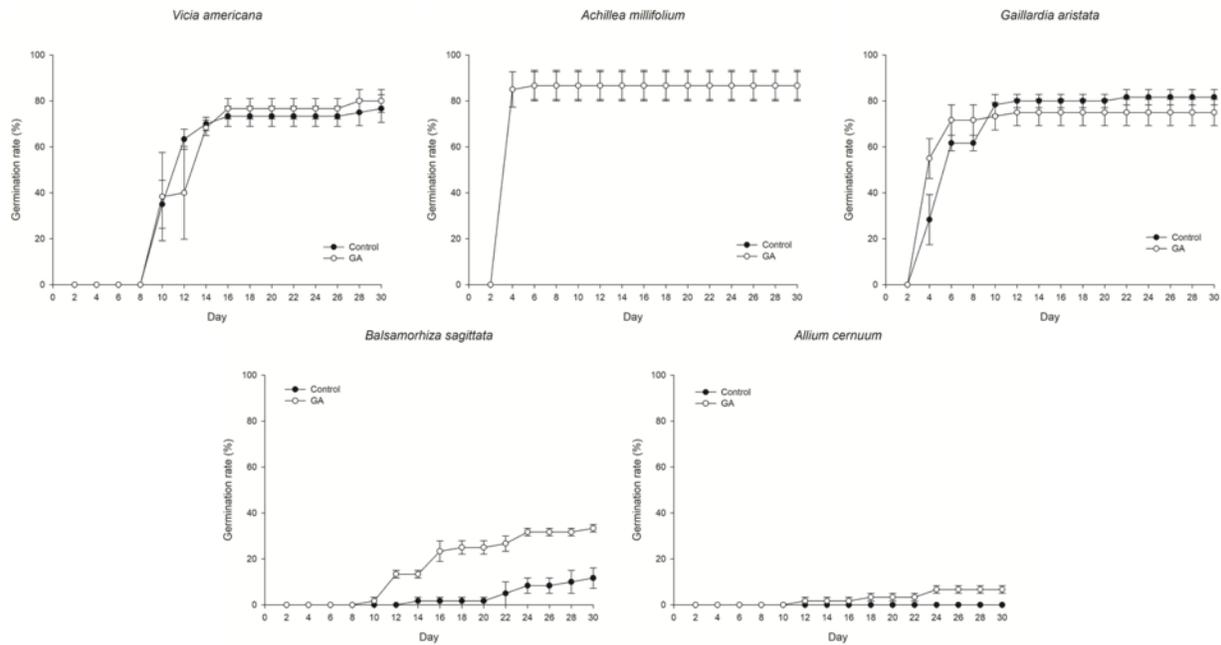
Figure A.3. Cumulative germination rates of native forbs over a 30-day greenhouse trial. Error bars are standard error of the mean. Treatments were a 1000 ppm Gibberellic acid solution (GA) and deionized water (control).
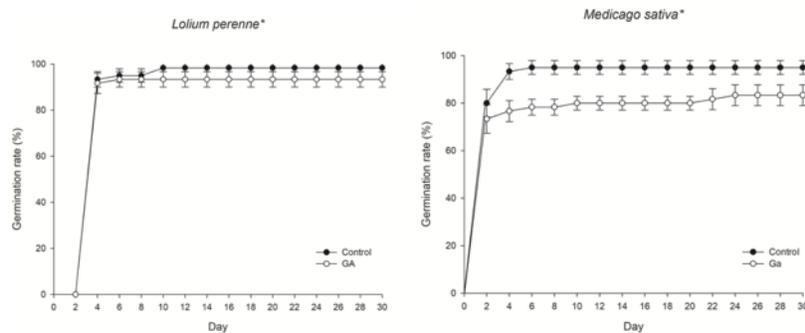
Figure A.4. Cumulative germination rates of agronomic species during a 30-day greenhouse trial. Error bars are standard error of the mean. Treatments were a 1000 ppm Gibberellic acid solution (GA) and deionized water (control).

Comparison of Native Species vs. Agronomic Species

The agronomic grass *Lolium multiflorum* had the highest germination rate (98%) of all the species examined (Figure A3). The native bunchgrasses *Poa secunda*, *Pseudoroegneria spicata* and *Elymus trachycaulus* were comparable at 90, 93 and 97%, respectively (Figure A1). Although, *L. multiflorum* emergence was much quicker compared to the native grasses (Tables

A1 and A2). The fastest species to germinate was the agronomic legume *Medicago sativa*. The native legume *Vicia americana* was comparably slow (Figure A2). Germination speed of the pioneer species, *Achillea millefolium* was similar to the agronomic species.

Effect of Gibberellic Acid on Seed Germination

Depending on the species, treatment with GA solution either hindered, had no effect, or improved seed germination. The GA treatment had a significant positive effect on emergence of the native forbs *Balsamorhiza sagittata* (P=0.010) and *Allium cernuum* (P=0.016) (Figure A2), but had a negative effect on both *Poa* species (P=0.037 and P=0.0005 for *Poa secunda* and *Poa juncifolia*, respectively) (Figure A1). Interestingly, without the GA treatment, *A. cernuum* seeds could not break dormancy within the 30-day trial period (Figure A2; Table A2). With regards to germination speed, GA solution improved the performance of *Elymus trachycaulus* and *B. sagittata* and had no effect on any of the other species (Tables A1 and A2).

Table A.1. Days to first germination (T_0) by treatment for each of the study species.

Species	Control	GA
Pseudoroegneria. spicata	<4	<4
Festuca campestris	<6	<6
Poa secunda	<6	<4
Koeleria macrantha	<4	<6
Elymus trachycaulus	<4	<4
Poa juncifolia	<4	<4
Vicia americana	<10	<10
Achillea millefolium	<4	<4
Balsamorhiza sagittata	<14	<10
Gaillardia aristata	<4	<4
Allium cernuum	-	<12
*Lolium perenne**	<4	<4
*Medicago sativa**	<2	<2

GA: 1000 ppm Gibberellic acid solution; Control: deionized water.
*Agronomic species

Table A.2. Mean days to 50% germination (T_{50}) ± standard error for each of the study species and two-sample t-test results comparing means between treatments.

Species	Control	GA	P-value (t-test)
Pseudoroegneria spicata	3.8 ± 0.07	4.0 ± 0.34	0.583
Festuca campestris	10.3 ± 0.36	9.3 ± 0.33	0.121
Poa secunda	6.7 ± 0.99	6.6 ± 1.04	0.976
Koeleria macrantha	5.2 ± 0.23	5.5 ± 0.73	0.702
Elymus trachycaulus	5.1 ± 0.30	3.7 ± 0.17	**0.016**
Poa juncifolia	4.7 ± 0.41	3.6 ± 0.23	0.078
Vicia americana	10.3 ± 0.59	10.8 ± 1.24	0.761
Achillea millefolium	3.1 ± 0.00	3.1 ± 0.03	0.444
Balsamorhiza sagittata	24.8 ± 2.20	15.7 ± 0.69	**0.017**
Gaillardia aristata	4.7 ± 0.58	3.5 ± 0.24	0.121
Allium cernuum	-	17.8 ± 3.32	-
*Lolium perenne**	3.1 ± 0.03	3.1 ± 0.02	0.433
*Medicago sativa**	1.6 ± 0.03	1.6 ± 0.02	0.564

GA: 1000 ppm Gibberellic acid solution; Control: deionized water. Bolded values are statistically significant ($P < 0.05$).
*Agronomic species

SUMMARY/CONCLUSIONS

- The agronomic species were quicker to germinate compared to the native species.

- Germination rates of the native bunchgrasses *Poa spp., Pseudoroegneria spicata* and *Elymus trachycaulus* were similar to the agronomic grass *Lolium multiflorum*.

- Treatment with Gibberellic acid improved germination success of *Balsamorhiza sagittata, Allium cernuum* and *Elymus trachycaulus*, but hindered *Poa spp.* seed emergence.

- The effects of Gibberellic acid on seed germination were variable and appeared to be species dependent; seed size and seed coat thickness may be a factor.

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APPENDIX B – LABORATORY ANALYTICAL RESULTS

Table B.1. Elemental concentrations of experimental materials and federal soil quality guidelines.

Element	Ash	Substrate Compost	Tailings	Soil Quality Guideline* CCME AL[a]	CCME IL[b]
Main Elements (mg kg^{-1})					
Al	1967	828	75	-	-
Ag	< 2	< 2	< 2	**20**	**40**
As	< 3.0	< 3.0	< 3.0	**12**	**12**
B	27.2	8.9	< 1.0	**2**	-
Ba	1225	475	492	-	-
Ca	35594	9793	4770	-	-
Cd	< 1.0	< 1.0	< 1.0	**1.4**	**22**
Co	25.7	16.9	30.8	**40**	**300**
Cr	51.8	52.2	**138**	**64**	**87**
Cu	**70.7**	**77.9**	**600**	**63**	**91**
Fe	486	545	525	-	-
Hg	< 3	< 3	< 3	**6.6**	**60**
Li	10.7	10.7	8.4	-	-
Mg	3888	1775	706	-	-
Mn	680.1	83.8	23.7	-	-
Mo	3.15	3.81	**10.5**	**5**	**40**
Na	3897	597	712	-	-
Ni	30.9	26.1	**90.7**	**45**	**89**
Pb	< 1.0	6.6	< 1.0	**70**	**600**
S	918	452	977	-	-
Sb	6.0	< 3.0	6.1	-	-
Se	**9.74**	**8.49**	**11.2**	**1**	**2.9**
Si	43	177	213	-	-
Sn	< 2	< 2	< 2	-	-
Sr	509	225	365	-	-
Ti	1864	2096	2993	-	-
Tl	< 6	< 6	< 6	-	-
V	53.8	58.0	180	-	-
Y	8.00	8.17	14.0	-	-
Zn	**216.0**	105.9	19.6	**200**	**360**
Zr	84.9	81.2	55.4	-	-
Total Plant Nutrients (%)					
N	0.05	1.18	0.01	-	-
P	0.47	0.30	0.11	-	-
K	2.49	1.32	1.09	-	-
S	0.15	0.21	0.26	-	-
C	22.5	24.3	1.12	-	-
Available Nutrients (mg/kg)					
NO$_3$-N	19.7	35.1	1.22	-	-
NH$_4$-N	< 0.01	6.31	0.78	-	-

*Canadian Council of Ministers of the Environment Soil Quality Guidelines for [a] Agricultural Land Use and [b] Industrial Land Use (Canadian Council of Ministers of the Environment, 2014).