PLANT PERFORMANCE ON ANTHROPOSOLS AT HUCKLEBERRY MINE, HOUSTON, BRITISH COLUMBIA

by

.

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B.Sc., University of Northern British Columbia, 2006

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Abstract

Supplies of topsoil are often in limited supply for use in mine reclamation activities; it may be necessary to build soils (Anthroposols) using locally available substrates. Revegetation test plots were established at Huckleberry Mine, Houston, B.C., to investigate plant performance on soils supplemented with (or without) non-acid generating (NAG) sand and fertilizer. The addition of NAG sand reduced some soil properties conducive to plant growth (e.g. cation exchange capacity), yet plant performance was not significantly lower than that observed in soil-only plots. When combined with a fertilizer application, plant performance on NAG sand-supplemented soils significantly increased. Trace element concentrations in supplemented soils were low and should not have any adverse effects on plant growth or the local environment. Plant performance of blue wildrye (mixed genotype variety) was shown to be higher than all other species examined and is suggested as the best candidate for the revegetation at Huckleberry Mine.

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Glossary

Acronyms

	AIC	Akaike Information Criterion
	AMD	Acid Mine Drainage
	ANOVA	Analysis of Variance
	ARD	Acid Rock Drainage
	ATV	All-terrain Vehicle
	BC	British Columbia
	BCMOF	British Columbia Ministry of Forests and Range
	BGC	Biogeoclimatic
	BROMCIL	Bromus ciliatus, fringed brome
	CCME	Canadian Council of Ministers of the Environment
	CEC	Cation Exchange Capacity
	CEQG	Canadian Environmental Quality Guidelines
	EC	Electrical Conductivity
	EFL	Enhanced Forestry Laboratory
	EGC	Environmental Growth Chamber
	ELYMGLA	Elymus glaucus, Blue Wildrye
	EPA	Environmental Protection Agency
	ESSF	Engelmann Spruce-Subalpine Fir biogeoclimatic zone
	ESSFmc	Moist Cold Subzone of the Engelmann Spruce-Subalpine
		Fir Zone
	FESTSAX	Festuca saximontana, Rocky Mountain fescue
	GPS	Global Positioning System
	ICP-AES	Inductively Coupled Plasma Atomic Emission
		Spectrometry
	ICP-MS	Inductively Coupled Plasma Mass Spectrometry
	IFS	Industrial Forestry Service
	LUPIARC	Lupinus arcticus, arctic lupine
	MZE	Main Zone Extension
•	NAG	Non-acid Generating
	NPK	Nitrogen-Phosphorus-Potassium fertilizer
	PAR	Photosynthetically Active Radiation
	PLS	Pure Live Seed
	RBCM	Royal British Columbia Museum
	SBS	Sub-boreal Spruce biogeoclimatic zone
	SBSmc2	Moist Cold Subzone of the Sub-Boreal Spruce Zone
	SE	Standard Error
	SOM	Soil Organic Matter
	TMF	Tailings Management Facility
	UNBC	University of Northern British Columbia
	USA	United States of America

Formulas

CS_2	Carbon Disulphide
CaCO ₃	Calcium Carbonate
Ca(OH) ₂	Calcium Hydroxide
CaO	Calcium Oxide
FeS ₂	Iron Disulphide
NaOH	Sodium Hydroxide
NO ₃ ⁻	Nitrate
NH4 ⁺	Ammonium
PO ₄ ³⁻	Orthophosphate
КОН	Potassium Hydroxide
$ROCS_2^{-}M^+$ (where R = alkyl and	Xanthate
$M^+ = Na^+ \text{ or } K^+)$	

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1.0 Introduction

1.1 Mine substrates

Depending on the type of mine operation and the mineral resources being extracted, a variety of substrates are created as a result of ground disturbance and the production of waste materials from excavated surface and parent material substrates as well as from the processing of mineral concentrated materials. Major mineral resources include heavy metals (e.g., gold [Au], silver [Ag] and copper [Cu]), coal, and industrial minerals (e.g., gypsum, talc and limestone). For each of these resources, the approach to ground extraction and refinement of the minerals differ greatly and this has a significant impact on the amount of disturbed ground, time period of disturbance and the production of waste materials. Overall, there are three major substrates that are commonly produced on mined lands: disturbed and degraded soil and unconsolidated overburden, waste rock materials and processed mine tailings. The conditions associated with each of these substrates differ greatly in terms of their physical, chemical and biological properties, which determine the concentration and mobility of contaminants as well as nutrients, and subsequently the successful establishment and growth of vegetation.

Disturbed and degraded soils are produced when the materials are removed from parent ground and transported to storage locations in the process of site clearing for mine operations. During this process, a considerable amount of soil can be lost as a result of handling and transport. For soil that is successfully salvaged, soil quality, which is defined as the ability of a soil to perform the functions and provide the elements essential for plant and animal growth (Brady and Weil 2002), is often greatly reduced as a result of compaction

from heavy machinery, disruption of soil structure and long-term storage. Compacted soils impede plant growth by reducing the interconnected void space between soil particles, which allow the storage and passage of air and water throughout the soil (Schor and Gray 2007). As a result, oxygen levels within soil are reduced, hydraulic conductivity (the ease of water movement through soil down a gradient) is decreased and the movement of nutrients and ions is restricted (Phelps and Holland 1987). Disruption to soil structure can also reduce the ability of water, air and roots to move through the soil by eliminating pore space between soil surface, it becomes runoff and can result in erosion and a reduction in the availability of water to plant roots (Troeh and Thompson 1993). Long-term storage of soils can result in a significant reduction in microbiological activity in the soil, which can prevent or delay normal nutrient cycling (when compared to undisturbed soils) following respreading during reclamation (Harris et al. 1989).

In mining, overburden is the material overlying a mineral deposit of ore or coal, which is removed in an effort to gain access to the deposit and is either stored for future use or disposed (Aiken and Gunnett 1990). This includes materials such as rock, soil, sands, silts, clays, shale and glacial till. Overburden can be consolidated (i.e., bedrock) or unconsolidated (i.e., loose and unstratified). Overburden materials are often used as alternative media for plant growth when supplies of soil are inadequate (e.g., glacial till at Sullivan Mine, Kimberly, British Columbia [BC]). However, due to the composition and mode of deposition, the physical and chemical properties of overburden can vary widely; hence their ability to support plant growth will depend on how conducive these properties are to plant growth. In a greenhouse experiment conducted by Byrnes and Stockton (1980), the

physical properties, chemical properties and the ability to support plant growth of 18 overburden materials from five surface coal mines in southwestern Indiana, United States of America (USA) were evaluated. The materials examined included unconsolidated A and B soil horizons, lacustrine sediments, glacial till and consolidated, but easily weathered rock strata. From the results of the experiment, the authors concluded that electrical conductivity (EC) and water storage capacity were the most influential properties for plant growth and ranked their suitability as a plant medium as follows: lacustrine sediment \geq A horizon \geq B horizon = glacial till \geq brown shale \geq sandstone \geq grey shale \geq black fissile shale.

Waste rock, produced mostly from open pit mining, is the material excavated from the parent substrate in order to access the mineral concentrated materials and is either stored in waste treatment ponds or in large quarries. Waste rock substrates are potential sources of heavy metal contaminant leachates because exposure to surface weathering conditions can oxidize sulphide from pyritic minerals which then enter surface and ground water, reducing pH (defined as the negative logarithm of the hydrogen ion (H⁺) concentration in a water solution; Troeh and Thompson 1993) and increasing soil salinity (Borden and Black 2005). These substrates are also poor media for plant growth, with little or no organic matter content, reduced nutrient content and harsh physical and chemical conditions due to potentially high metal concentrations (Lottermoser 2010).

Mine tailings are a solid waste product of the milling and mineral concentration process which are commonly produced in base metal and coal mining operations, and are stored in large tailing impoundments (Richmond 2000). During the milling process, the tailings are mixed with water in order to safely transport and store the material in an isolated impoundment. Tailings can have a high metal and sulphate content (if they originate from a

sulphide based ore) and in the presence of water and oxygen, a highly acidic, sulphateenriched slurry, can be produced. Under these conditions, the metals, including iron (Fe), Cu, molybdenum (Mo), aluminum (Al), lead (Pb), zinc (Zn) and cadmium (Cd) are released into solution, resulting in toxic conditions for plants and animals.

1.2 Acid mine drainage

In the presence of oxygen and water, sulphide-based minerals such as Fe pyrite or Fe disulphide (FeS₂) can produce sulphuric acid, also known as acid mine drainage (AMD). This low-pH solution increases the solubility of metals and can result in an effluent with high metal concentrations (Jennings 2008). At a typical metal mining operation which extracts a sulphide-based ore, AMD (also known as acid rock drainage; ARD) can be generated from structures such as waste rock dumps, tailings ponds, open pits and underground workings. Once generated, AMD can have significant adverse effects on downstream aquatic ecosystems (e.g., lakes, rivers).

Eliminating or reducing the risk of AMD generation can be accomplished through a variety of techniques. Overall, by limiting the exposure of sulphide rock to either air or water (or both), the potential for the production of AMD can be greatly reduced (Johnson and Hallberg 2005). Often, a protective cover composed of neutral material (e.g., glacial till, soil, water or synthetic materials) is applied to the surface of tailings impoundments and waste rock dumps to seal and prevent exposure of the sulphide-based minerals to the air and precipitation. With open pits in which the rock walls are exposed to air and water, the most common and most practical method for reducing the potential for AMD is to flood the pit with water.

1.3 Desulphurization of mine tailings

In the early 1990's, treated tailings (known as desulphurized or depyritized tailings) were introduced as a cover material for tailings impoundments (Sjoberg et al. 2001). Desulphurized tailings are essentially a more refined product of the milling process in which the sulphidic fraction of the slurry is removed through an additional flotation stage resulting in a relatively pH-neutral material (Benzaazoua et al. 2000). During the last few years of mine life, treated tailings are produced and then used to cap the sulphuric portion of the tailings impoundment with a layer 1 to 2 m in depth; this layer reduces the oxidation of remaining sulphide minerals within the reactive tailings below by limiting oxygen and water availability (Sjoberg et al. 2003).

Froth flotation (separation of hydrophobic and hydrophilic material) is a treatment stage during the processing of ore-rich rock in which valuable ore is separated from the rock using specialized reagents. These reagents increase the hydrophobicity (the property of being water repellent) of the ore, allowing it to float to the surface of a flotation cell and become concentrated. In addition a secondary flotation stage can be used to remove a significant portion of the remaining sulphidic fraction of the waste material. This results in the production of a final tailings product which has low sulphide content and a low potential to generate acidity (Sjoberg et al. 2003). The sulphidic fraction of the waste material is stored in a separate containment area (e.g., an isolated area of the tailings impoundment) where it can be managed more easily due to the reduced volume (Benzaazoua et al. 2000).

The most common reagent used for non-selective flotation of sulphides are Xanthatebased (Benzaazoua et al. 2000). Xanthate (ROCS₂⁻M⁺ [where R = alkyl and M⁺ = Na⁺ or

 K^+]) is a salt (dithiocarbonate) that is produced by a reaction of alcohol with sodium hydroxide (NaOH), potassium hydroxide (KOH) and carbon disulfide (CS₂). Other common reagents include amine acetate, thiocarbamates and mercaptobenzothiazoles (Benzaazoua et al. 2000).

Regardless of their source, the physical properties of desulphurized tailings are similar to those of untreated tailings, consisting primarily of silt and clay to sand sized particles with a low hydraulic conductivity (Sjoberg et al. 2001). In terms of chemical properties, the tailings maintain a relatively neutral pH (slightly alkaline at Huckleberry Mine) and low cation exchange capacity (CEC) and EC. As it is strictly a product of mined rock, the material is devoid of organic matter and only low concentrations of available plant nutrients are present.

1.4 The use of soil amendments and the concept of Anthroposols

A soil amendment can be defined as a material that is applied to a substrate in an effort to improve its properties by restoring the essential conditions required for adequate plant growth, and/or for reducing the mobility and bioavailability of any soil contaminants. Amendments are often used in the reclamation of soils and unconsolidated overburden, which in almost all cases, lack adequate conditions for plant growth. These substrates can generally be described as having low levels of plant-available nutrients (e.g., nitrogen [N] and phosphorus [P]), high concentrations of heavy metals, low organic matter content, high salt content and lower or higher than normal pH; EPA 2006). By prescribing the appropriate type of amendment(s) and determining the optimal rate, combination and mode of application, organic matter content and nutrient availability can be increased, pH can be

neutralized, microbial soil communities can become re-established and heavy metal contaminants can be immobilized. As a result, the conditions for plant growth are improved and the mobility and incorporation of contaminants into ground water and plant tissues can be reduced.

A multitude of investigations have been completed over the last half-century that have tested many different types of soil amendments in tandem with the operation and decommissioning of mining operations. In some cases, the choice of amendments has been based on addressing specific soil properties (e.g., highly acidic soils, Davis et al. 1999; microbial soil communities, Kubeckova et al. 2003). Amendments tested have been both organic (e.g., sewage sludge; Alvarenga 2009) and inorganic (e.g., bentonite clay; Schuman et al. 2005) in nature. Organic amendments have included municipal wastes (Fuentes et al. 2007), farming wastes (e.g., cow, pig and chicken manure), peat (Burton 2007), soils and wood wastes (Brown and Jackson 1984) and biochar (Lehmann 2007). Inorganic amendments tested have included marble wastes (Murcia et al. 2007), commercial lime products (Stuczynski et al. 2007), commercial fertilizers and coal fly ash (Kumar and Singh 2003). Overall, the types of materials used as soil amendments have been diverse and efforts to test new residual materials with beneficial properties when added to soil from other industrial and commercial processes will likely continue.

Soil amendments can be used to address two main challenges that occur within soils and overburden found on mined landscapes: reducing the mobility and bioavailability of contaminants to plants and animals, and improving substrate conditions to create adequate conditions for plant growth. To address these issues, a full understanding of the physical, chemical and biological conditions of the target substrate must be developed. With this

information, the most appropriate type of amendment, rate of application or amendment combinations and application methods can be chosen.

The type of amendment used to reclaim a minesoil substrate depends on its specific physical, biological and chemical properties. In reclamation efforts, nutrient-poor disturbed and degraded soils are often treated with commercial fertilizers in order to improve plant growth conditions and their use has been shown to increase the production of vegetation cover for a given density of plant seed applied (Burton and Burton 2000). Organic amendments (e.g., municipal solid wastes and farm manure) and inorganic commercial fertilizers have been applied to waste rock substrates in order to promote vegetation establishment by supplying a sufficient source of plant available nutrients. Research by Meikle et al. (1999) tested these amendments with grass seeding treatments on waste rock substrates and found that organic amendments substantially increased vegetation cover compared to mineral fertilizers which was likely the result of enhanced nutrient and water retention ability (i.e., CEC) of organic matter. In the case of acid mine tailings and metal contaminated substrates, a variety of organic and inorganic amendments have been used to reduce acidic conditions and reduce the mobility of metal contaminants and metal uptake by plants. For example, organic residues were added to acidic, metal contaminated soils at a mine in Portugal in order to neutralize acidity, increase organic matter content and reduce the availability to plants (Alvarenga 2009).

Several approaches have been used to determine the most appropriate rate or combination of amendments for application to target substrates. Soil conditions in undisturbed areas adjacent to disturbed sites may be used as a reference for determining what target conditions should be achieved. Previous reclamation and research efforts for similar

sites and soil conditions may also be used to determine target conditions (EPA 2006). However, one of the most effective approaches is to conduct research trials which test the effects of amendment application on plant growth and metal uptake. Amendment applications have been tested both in greenhouse experiments and field trials to determine the most successful concentrations to promote or improve plant growth. In an experiment testing various rates of sewage sludge (wet weight ha⁻¹) to amend minespoil at a coal mine in Utah, USA, it was determined that low application rates (14 Mg ha⁻¹) resulted in foliar N and P concentrations in seeded grasses equivalent to those achieved under the highest application rates (83 Mg ha⁻¹; Topper and Sabey 1986). In a greenhouse experiment conducted by Reid and Naeth (2005a), native grass species were tested on kimberlite tailings amended with combinations of peat moss, paper mill sludge, lake sediment, sewage sludge, Agri-BoostTM, inorganic fertilizer and three calcium (Ca) sources to determine the best approach for reclamation. Results revealed favourable plant growth on sewage sludge, peat moss and paper mill sludge amended tailings and that the combination of peat moss, sewage sludge and fertilizer produced the highest levels of vegetation cover.

Applying amendments to target substrates can sometimes be difficult due to logistical limitations (such as machinery access to target substrates, limited timing windows and transport costs) and soil compaction concerns. Phelps and Holland (1987) compared the effects of bulldozers and small skid-steer loaders on soil compaction using bulk density methods during soil replacement efforts from soil stockpiles at a strip mine in Pennsylvania, USA. It was determined that the spreading of soil by skid steers was more successful at alleviating soil compaction during soil spreading compared to bulldozers. The timing of amendment application can also have a significant impact on its ability to improve soil

conditions. It has been suggested that excessive loss of nitrates in nutrient rich soil amendments may occur in the winter if soil amendments are applied after the growing season; as well, the workability of a land surface may be degraded if amendments are applied during the rainy season (EPA 2006).

With the prescription of the appropriate type and combination of amendment(s), and the rate and mode of application, the substrate conditions for plant growth can be improved and the mobility and incorporation of contaminants within ground water and plant tissues can be reduced. The addition of amendments to these nutrient-limited and contaminated substrates has been shown to increase organic matter content and nutrient availability, neutralize pH, promote increased activity of soil microbes and immobilize heavy contaminants. However, continued research is needed in order to discover new beneficial soil amendments that will provide cost-effective and successful approaches to amending mine soils, with an emphasis on testing the use of industrial, commercial, municipal, agricultural or mining waste products.

Soils which are highly modified or constructed through human activities are defined as Anthroposols (Naeth et al. 2012). These azonal soils are described as soils where one or more of their natural horizons are removed, replaced, added to, or are significantly altered by human activity. Many growth substrates used at mine sites for reclamation activities meet this definition as they contain layers (i.e. "horizons") that are anthropic in origin and contain materials that are significantly altered physically and/or chemically (relative to the original pre-disturbed soil found at the site). In addition to the use of traditional soil amendments (e.g. use of lime to improve conditions for plant growth), a variety of waste materials produced from industrial, commercial and urban development have been added to soils

during land reclamation activities. These materials are most often added to soils as an opportunistic approach to waste disposal (e.g. wood and construction wastes; Murcia et al. 2007), or as a supplement to soils during construction of major infrastructure. The use of waste rock, tailings and other amendments (often at very high application rates) are common inputs into the creation of Anthroposols during mine reclamation. Anthroposols are key to the success of many reclamation projects as they are designed to produce a suitable growth substrate where native soils are lacking, in limited supply, or, are of poor quality.

1.5 Soil properties relevant to plant growth

In order to survive, plants must be provided with a growth medium that can promote root growth, accept, hold, and supply water and mineral nutrients and allow for gas exchange (Schoensholtz et al. 2000). Within any medium, these conditions relevant for plant growth are determined by its physical and chemical properties. A variety of physical and chemical indicators of soil quality have be used in the assessment of agricultural and forest soils as well as in assessing the suitability of waste materials as a media for plant growth in the reclamation of mined lands.

1.5.1 Physical soil properties

Physical soil properties are defined as characteristics, processes or reactions of a soil that relate to its solid particles and how they are aggregated (Brady and Weil 2002). Physical properties used in the assessment of soil quality include soil depth, texture, porosity, color and temperature, bulk density, soil strength, water-holding capacity and hydraulic conductivity (Schoensholtz et al. 2000).

Soil depth influences the availability of resources required for plant growth including nutrients, water and oxygen; hence, greater soil depths often result in a greater availability of resources (Schoensholtz et al. 2000; Bowen et al. 2005). To determine the depth of soil required for maximum production of grass cover at a few mines in Wyoming, Montana and North Dakota, USA, Barth and Martin (1983) tested native and introduced grasses on soil covers over mine spoil ranging in depth and observed a significant increase in grass productivity with increasing soil depth. Similar results were also obtained by Power et al. (1981) where yields of four grass and legume crops planted in various depths of subsoil over sodic mine spoil in North Dakota, USA, were shown to increase with increasing soil depth. Typical target depths for construction of a soil medium on mined areas range from 0.4 to 0.6 m (e.g., North Antellope/Rochelle Mine, Wyoming, USA [Schladweiler et al. 2005]; Huckleberry Mine, Houston, BC [Boxill 2010]) and over the long term, have shown to result in the establishment of productive vegetation cover (Bowen et al. 2005).

Soil texture is described by the relative proportions of sand, silt and clay in a soil and determines the transport, retention and uptake of water, nutrients, and oxygen in a medium (Brady and Weil 2002). For example, sandy soils are often easily permeable to water, air and roots, yet are limited in their ability to store water and nutrients; in comparison, soils with high clay content may have high water-holding capacities but are poorly aerated (Troeh and Thompson 1993).

Soil porosity is the percentage of total soil volume not occupied by solid particles (Coyne and Thompson 2006). Pore size distribution and pore continuity directly influence root growth by determining the amount of soil volume filled with air and water as well as the soil's ability to transport oxygen throughout the rhizosphere. At an air-filled porosity of less than 10 %, the oxygen diffusion rate is inhibited, causing injury to roots and reducing their ability to function (Silva et al. 2004).

Soil color can be attributed to its organic matter content and mineralogy, and has the potential to influence soil temperature because darker surfaces can absorb and release heat more rapidly than lighter colored surfaces (Troeh and Thompson 1993). However, the ability of soil color to influence temperature is dependent on its soil moisture content. Dark colored soils are often high in organic matter content and due to their high water-holding capacity, remain wet and cooler than lighter colored soils (Troeh and Thompson 1993). In contrast, many dark colored waste materials that are often produced on mined lands (e.g., NAG sand, Huckleberry Mine, Houston, BC), have little or no organic matter content and low water holding capacity, allowing for heat to be more readily absorbed.

Soil temperature can influence plant growth by regulating the chemical and biological processes that determine the availability and absorption of water and nutrients (Brady and Weil 2002). In addition to soil color and water content, temperature within a medium is also influence by depth. Overall, soil temperature decreases with depth with the greatest variation in daily temperatures occurring in the first 5 cm below the surface and the greatest annual variation in the first 50 cm (Schaetzl and Anderson 2007).

Bulk density is defined as the mass of a unit volume of dry soil (Brady and Weil 2002) and is a measurement used to assess soil compaction and infer root growth potential and water availability in a soil (Schoensholtz et al. 2000). Average values for bulk density for loamy soils range from approximately 1.3 to 1.5 g cm⁻³ (Coyne and Thompson 2006). At values between 1.4 and 1.9 g cm⁻³ or greater, bulk density can begin to restrict root growth as

a result of high soil strength (Lampurlanes and Cantero-Martinez 2003). Soil compaction often results in high bulk density, which limits the ability for plants to establish on these soils due to high penetration resistance and results in poor root growth due to reduced aeration and water availability within the medium.

Soil strength is the resistance that roots meet when penetrating the soil, either at the surface or within the medium. The most common indicators used to measure soil strength are bulk density and penetration resistance. Penetration resistance measures the pressure required to penetrate a soil surface. As penetration resistance increases, root growth decreases. At values greater than 2 MPa, significant root growth reduction has been reported (Lampurlanes and Cantero-Martinez 2003), leading to decreased plant nutrient uptake and plant stress (Reintam et al. 2009). Generally, penetration resistance varies with soil moisture; as soil moisture decreases, penetration resistance increases. In very dry soils, cementing may occur at the surface, significantly reducing plant establishment from seeds deposited by natural seed rain. However, coarse-textured soils may also exhibit reduced penetration resistance when very dry (Hillel 1998). Soil compaction can also significantly increase penetration resistance (Reintam et al. 2009).

The two indicators that are most often used to describe the availability and movement of water through a soil are the available water holding capacity and hydraulic conductivity, respectively. The available water holding capacity of a soil is defined as the portion of water in a soil that can be readily absorbed by plant roots; hydraulic conductivity is the readiness with which water can pass through a soil (Brady and Weil 2002). Water holding capacity is dependent on various physical soil factors including soil texture, type of clay present, organic matter content, bulk density and soil structure. Sandy soils typically have a lower water

holding capacity than a loam or clay soil; soils with swelling clays (e.g., montmorillonites) also will hold more water than those of non-swelling clays (e.g., kaolinite; Hazelton and Murphy 2007). Soils with low water holding capacity may not maintain an adequate supply of water for plants through periods of drought. Hydraulic conductivity can be a good indicator of how quickly and how efficiently water moves through a soil column, influencing water availability and movement down through the rooting zone.

1.5.2 Chemical soil properties

Organic matter is considered to be one of the key chemical soil properties when assessing soil quality for plant growth. It has a direct influence on aggregate stability, soil porosity, gas exchange and water and nutrient storage, release and availability (Schoensholtz et al. 2000). Other important properties that describe soil quality include levels of available macronutrients and micronutrients, pH, CEC and EC.

Soil organic matter (SOM) consists of fresh residues and humus (organic matter derived from decomposed materials) and is one of the main components of soil to govern both its physical and chemical properties (Troeh and Thompson 1993). With the addition of organic matter, soil porosity is increased, enhancing water infiltration and improving its availability to plant roots. Organic matter also has a high water holding capacity, which helps to hold moisture within the rooting zone. As a major source of nutrients (e.g., N, P, sulphur (S), Ca and magnesium (Mg), micronutrients and trace elements), as well as a high CEC, organic matter contributes significantly to soil quality (Coyne and Thompson 2006). The addition of organic matter also contributes to the soil structure and tilth by promoting soil aggregation and the development of soil structure.

Primary macronutrients (required by plants in large amounts) include N, P and potassium (K). Plant-available forms of N include nitrate (NO_3) and ammonium (NH_4^+); the available form of P is orthophosphate (PO_4^{3-}), while K is released from a variety of mineral sources into the soil solution as K⁺. Secondary macronutrients include S, Ca and Mg. The most important forms of S for plant nutrition within soils are sulphates and other mineralizeable organic compounds (Vanek et al. 2008). Ca and Mg are both found as divalent ions (Ca^{2+} and Mg^{2+}) in the soil solution and on the exchange complex, which can be readily utilized by plants.

Micronutrients (elements required by plants to complete their life cycle but only in small amounts; Troeh and Thompson 1993) include a variety of trace elements such as boron (B), chlorine (Cl), Cu, Fe, manganese (Mn), Mo and Zn and are only required by plants in trace amounts. Trace element requirements vary somewhat with plant species (Brady and Weil 2002). These elements, released from mineral or organic material in the soil, form one or more ions which then enter the soil solution and become available for plants. Generally, soils with high clay or organic matter content will retain a greater amount of soil nutrients than soils with low clay and organic matter content (Troeh and Thompson 1993).

Soil pH describes the acidity or alkalinity of the soil solution, which strongly influences the availability of nutrients for plant growth (Troeh and Thompson 1993). A plant's ability to survive within a specific pH range depends on its ability to utilize nutrients at the concentrations available. Overall, nutrient availability is greater in neutral to slightly acidic conditions (pH 6-7) than under alkaline conditions or strongly acid conditions (Brady and Weil 2002). pH can also influence the concentration of metal ions in the soil solution

(e.g., Al and Mn) which, at low pH can reach concentrations that are toxic for plants (Troeh and Thompson 1993; Delhaize and Ryan 1995).

Cation exchange capacity is the defined as the sum of the total exchangeable ions that a soil can adsorb per unit mass; the higher the CEC, the greater the capacity of the soil to attract, retain and exchange positively charged ions (Coyne and Thompson 2006). The CEC of a soil is determined by the relative amounts of colloids (organic and inorganic material with a small particle size and large surface area per unit mass) and their individual CEC (Brady and Weil 2002). Generally, colloids in organic matter have the highest CEC compared to colloids of inorganic material (i.e., sand, silt and clays), followed by clays and silts and the lowest CEC for colloids in sand. Therefore, in most soils, a majority of the CEC is contributed by the organic matter content; however, in soils low in SOM, clays contribute the greatest to CEC (Brady and Weil 2002).

Factors which influence the CEC in a soil include organic matter content, texture and pH (Coyne and Thompson 2006). Organic soils generally have a higher CEC compared to mineral soils due to considerably high CEC of organic colloids. In mineral soils, soils classified with high clay content have a higher CEC compared to coarse textured and sandy soils. Clay type can also influence CEC, as the exchange capacities of clay types can differ significantly (e.g., smectites have a much higher CEC than kaolinites; Brady and Weil 2002). pH influences CEC through the constant adsorption and release of H⁺ and Al³⁺ ions between soil particles and the soil solution; as pH increases, the amount of H⁺ and Al³⁺ ions within the soil solution decreases, which allows for a greater availability of adsorption surfaces on soil particles, increasing the CEC (Coyne and Thompson 2006). In addition to this pH-dependent

CEC, many soils exhibit a non-pH dependent (permanent) CEC, based on clay mineralogy (Brady and Weil 2002).

Electrical conductivity measures the ability of a soil to transmit an electric current, and is commonly employed to describe a soil's salinity; the greater the salinity of the soil's solution, the greater the conductivity (Brady and Weil 2002). As a general indicator, soils with an EC value greater than 4 dS m⁻¹ are considered saline (Coyne and Thompson 2006). Soil salinity affects a plant's ability to take up water and nutrients by increasing the soluble ion concentration in the rooting zone, altering osmotic potential in the soil (becoming more negative) and reducing water uptake by plants (Brady and Weil 2002). Under saline conditions, plants may also take up excess amounts of sodium through a pathway that competes with potassium uptake, resulting in nutrient deficiency (Blumwald et al. 2000). Some plant species have adapted over time to tolerate very saline soil conditions while others can only survive on non-saline soils.

1.6 Revegetation practices on mined lands

Prior to and following reclamation and closure of mined lands, much research has investigated the establishment success of native (indigenous to the region) and non-native plant species in amended and non-amended minesoil substrates (e.g., waste rock [Sharon and Smith 2002], metaliferous tailings and soils [Kramer et al. 2000; Macyk 2002; Reid and Naeth 2005b]). The objective of these investigations has been to develop an approach to establish a cover of self-sustaining vegetation which produces a similar level of productivity and land capability as that which existed prior to mining. Species tested in revegetation trials have included a variety of graminoids (e.g., grasses; Burton 2007), forbs, shrubs and trees

(e.g., bigleaf maple [*Acer macrophyllum*]; Kramer et al. 2000). Several methods for the revegetation of minesoils have also been developed and include direct seeding (Tordoff et al. 2000), planting nursery-grown seedlings (e.g., grasses; Sharman and Smith 2002), the use of seed-rich soils (Zhang et al. 2001) and allowing natural colonization of disturbed ground (Borden and Black 2005).

1.6.1 Plant species selection

When designing seeding and planting prescriptions for the revegetation of mined lands, care should be taken to ensure that the plant species utilized are suited to the end land use objective (e.g., agriculture, wildlife habitat, forestry). Depending on the objective, factors such as climate, cost, availability of seed, seedling stock and site conditions (e.g., soil texture, fertility and drainage) may also play a significant role in species selection.

Where the end land use objective is agriculture (e.g., Afton Gold Mine, Kamloops, BC; Schmitt et al. 2008) or the establishment of productive grassland habitat, supplies of appropriate native and non-native agronomic grasses and legumes are typically available from commercial seed houses and can be applied to disturbed sites with relative ease and high success (e.g., Highland Valley Copper, Logan Lake, BC). Areas targeted for these end land use objectives are mostly on plains or in lowland valleys where the growing season is longer (compared to higher elevations) and where the supply and fertility of soils are often more favourable for revegetation.

If the end land use objective is to return wildlife habitat and plant communities to conditions present prior to disturbance, the selection of plant species for revegetation is often considerably more complex. One of the most limiting factors for the establishment of native plant species in reclamation is the availability of plant material. Seed for some native species can be obtained from commercial seed providers or seeds can be collected from the region of the minesite. Both methods are costly (when compared to obtaining agronomic seed stocks) and obtaining enough stock to seed large areas is often not practical. However, seeds obtained through either method can be sent to a seed grower to increase those seed stocks, or to a local nursery for propagation, as seedlings can then be outplanted, thereby maximizing the usage of the costly seed stock in comparison to direct seeding methods.

Often, minesites that designate wildlife habitat as their end land use objective are located on terrain and at elevations which present a variety of challenges for revegetation. These sites are usually at subalpine to alpine locations (e.g., Huckleberry Mine, Kemess South, BC) where growing seasons are short and soils are often shallow (and therefore in limited supply as a medium for reclamation), poorly developed and have low fertility due to the slow rate of nutrient cycling (due to cooler temperatures). In addition, seeds for the native species which naturally inhabit these locations are often difficult to obtain in large quantities, and successful germination for nursery propagation often requires multiple trials to determine appropriate pre-treatments to break dormancy (Kaye 1997).

Where the potential for soil erosion or mass movement has been identified (e.g., steep slopes), the use of quick-establishing agronomic seed mixes has been warranted. However, in areas where the end land use objective aims for the renewal of natural ecosystems and creating or enhancing wildlife habitat, the use of these agronomic species may represent an incompatible option for revegetation. The establishment of non-native vegetation has been shown to preclude or inhibit the natural colonization of native plants species and therefore reduce the land's ability to rebound along natural successional trajectories (Sharman and

Smith 2002; Polster and Howe 2006). Overall, these studies examining the impacts of introduced non-native vegetation have shown that quick establishment of agronomic species may reduce some short-term impacts of mining on soil quality (e.g., soil erosion, plant growth inputs; Forbes and Jefferies 1999). However, the long-term impacts of reduced species diversity and poor prospects for the regeneration of natural plant assemblages (i.e., establishment of successionally stagnant grasslands; Carson et al. 2011) raises questions of their value for the conservation of natural ecosystems (Holl 2002).

In more recent reclamation efforts across BC where the end land use objective is to return wildlife habitat, attempts have been made to initiate natural successional trajectories by establishing a cover of native plant species (e.g., Kemess South mine; Lysay et al. 2010). Once established, studies have shown high success in encouraging the establishment of other native species and initiating successional vegetation development on a site (e.g., red alder [*Alnus rubra*] at Island Copper Mine, Port Hardy, BC; Polster 2001). However, establishing an initial cover of pioneering species may be difficult and costly. Depending on the species, factors such as availability of seed and site conditions may make this method impractical. As a result, incorporation of natural successional trajectories into reclamation plans has been minimal.

1.6.2 Seeding and planting

Technical reclamation and restoration involves the use of seed mixes, seedlings and stem cuttings for revegetating disturbed environments and many studies have demonstrated that these methods provide successful results (Macyk 2002; Gretarsdottir et al. 2004; Petterson et al. 2004; Olfelt et al. 2009). However, on highly disturbed ground and mine soils, the use of

direct seeding has been shown to be more successful and less costly for establishing vegetation than the use of commercially grown or locally collected seedlings and stem cuttings, which often demonstrate poor survival and growth (Macyk 2002). Yet, when applied to areas where soil disturbance is minimal, (e.g., forest cutblocks, roadsides), the use of seedlings (specifically those that are propagated commercially) and stem cuttings can be an effective means of establishing successful tree and shrub cover.

The use of direct seeding for revegetation involves creating a seeding prescription which takes into consideration factors such as species composition, seeding density and method of sowing in an attempt to produce the most successful outcome possible for the target substrate and climate conditions. In terms of species composition, the argument over the use of native versus non-native species has been extensively debated (Jones 2003) and a multitude of studies have be conducted which demonstrate both negative and positive benefits of using non-native species. Forbes and Jefferies (1999) describe how non-native plant species, which are used to provide a quick and temporary establishment of vegetation cover to reduce risks of erosion, will often persist and spread within disturbed environments, inhibiting the establishment of native plant assemblages. In contrast, Antonio and Meyerson (2002) argue that careful investigation of the influences of agronomic species may help to reduce the controversy surrounding their use in restoration and suggests circumstances where their application may be practical.

Seeding density is an important consideration when producing a seeding prescription in order to maximize growth and productivity of vegetation cover for a given area of land. Burton et al. (2006) point out that low seeding densities will not fully occupy the growing space available and high densities may result in intense competition that inhibit growth and
productivity of individual plants. The study by Burton et al. (2006) suggests an optimal density between 750 – 1500 PLS (pure live seed) m⁻² for revegetation efforts on disturbed soils in west-central BC. When considering seed mixes that incorporate species with different life histories (e.g., grasses and shrubs), other factors must be taken into consideration as well. Hild et al. (2006) state that the complex interspecific interactions between shrubs and grasses are not well known and in their study, examined the effect of various grass seeding densities on the growth of big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis*) at a coal mine near Gillette, Wyoming, USA. The results showed that when seeded together, increasing densities of grass lead to a decreased growth and productivity of big sagebrush.

Various methods have been employed in the application of seed mixes on disturbed ground; these include hydroseeding, drill seeding and broadcast seeding. Hydroseeding involves the use of a slurry which contains the seed mix, water and wood fiber (and may also include a tackifier and fertilizer) that is sprayed on the target area using a large tanker with a hose and pressure pump. Drill seeding requires a large piece of machinery known as a seed drill, typically designed for agricultural use or modified from such equipment (e.g., Truax native seed drill [Truax Company Inc.]) which cuts into the soil and distributes seed at an even rate to the freshly disturbed surface. Broadcast seeding, which is often preceded by soil cultivation or harrowing, involves evenly distributing the seed throughout the area, by hand or using a hand-held mechanical device such as a seed slinger (e.g., Truax seed slinger [Truax Company Inc.]) or a mechanical seed spreader mounted on the back of a tractor or an all-terrain vehicle (ATV). Montalvo et al. (2002) compared hydroseeding, imprinting (using machinery which produces troughs that are simultaneously seeded) and drill-seeding

methods for sowing seed. The study found that plant productivity differed significantly among seeding methods and that some methods proved more successful for smaller- or larger-seeded species.

Tree and shrub seedlings, transplants and stem cuttings have been used extensively for reclamation and restoration efforts (Brown and Jackson 1984; Macyk 2002; Huddleston and Young 2004; Petterson 2004; Olfelt et al. 2009). Overall success of commercially grown seedlings has been shown to be high. Olfelt et al. (2009) conducted a study in which seedlings of three-toothed cinquefoil (*Potentilla tridentata*) and poverty grass (*Danthonia spicata*), propagated under greenhouse conditions, were used in the restoration of cliff edges in Tettegouche State Park, Minnesota, USA. Following three years of observation, survival rates were between approximately 60 and 90 % for transplanted seedlings. As well, Huddleston and Young (2004) reported average survival rates of 98 % for planted seedlings of bluebunch wheatgrass (*Pseudoroegneria spicata*) and Idaho fescue (*Festuca idahoensis*) on ground colonized by Lemon's needlegrass (*Achnatherum lemmonii*). However, their research also showed that growth and productivity of planted seedlings can be significantly affected by competition from existing vegetation cover. As well, the cost associated with seedling production can be high (e.g., shrubs) as they must be grown for two seasons or more in order to ensure survival during transplanting (Sharman and Smith 2002).

The use of transplanted seedlings and stem cuttings collected from donor sites has often been less successful, compared to seeding, and survival rates have varied. Macyk (2002) described a study in which coniferous seedlings (e.g., lodgepole pine [*Pinus contorta* var. *latifolia*]) and willow (*Salix* spp.) cuttings were planted in reconstructed soils on coal

spoil dumps, but demonstrated poor growth and survival due to conditions such as frost heaving and difficulty in planting. However, Polster (1997) has shown that stem cuttings can be used successfully to establish vegetation on landslides and unstable slopes through careful stem harvesting, preparation and planting methods.

1.6.3 Natural Regeneration

The process of natural colonization and succession can be utilized either partially or entirely as a suitable and successful method for revegetation of disturbed environments. Natural regeneration of vegetation occurs when an opportunity for colonizing a surface (exposure of bare soil due to surface disturbance or an increase in light availability from canopy removal) occurs in combination with the presence of viable propagules. Seeds, spores and rhizomes can be found in soil, forest floor and peat materials, while seeds and spores can also immigrate from off-site with the aid of dispersal vectors (e.g., wind, wildlife).

Depending on the type of receiving substrate, one source of propagules may dominate (e.g., seed dispersal for non-soil substrates), and in other cases, seed bank and off-site sources may equally contribute. For example, Skrindo and Halvorsen (2008) found a greater frequency of woody and herbaceous perennial species indigenous to adjacent forests along recently constructed forest roads in Norway where soil was applied (with an abundant seed bank) compared to where subsoil (with a sparse or no seed bank) was used. Substrate type can also have a significant effect on the rate of colonization and the abundance and diversity of plant species. Martinez-Ruiz and Fernandez-Santos (2005) compared natural colonization of soil-amended waste rock heaps at a uranium mine in Spain to those amended with fine

non-toxic sediment and found greater species diversity and abundance and reduced time for vegetation establishment on waste heaps amended with soil.

Soils typically have great potential for inducing natural regeneration from an existing seed bank, which was deposited over time during previous generations of vegetation cover. In one study, Iverson and Wali (1981) described seven factors which determine the quality and quantity of seed banks within soils (prior to disturbance), including the reproductive potential of plants, dispersal mechanisms, dormancy patterns, characteristics of soils, biotic influence, weather fluctuations and disturbances to the soil surface. Overall, the study showed decreased seed density with depth within natural grazed and ungrazed prairie grassland soils prior to surface mining and suggested that a large majority of the seed bank occurs within approximately the first 10 cm of soil depth. For activities where ground disturbance is minimal (e.g., forest harvesting), the soil, seed and rhizome bank plays a significant role in the re-establishment of plant cover and greatly influences the subsequent species composition. However, where soil disturbance is high or where developed soils have been completely removed (e.g., mined lands), the seed bank may have less influence or be completely absent. When such impacts can be foreseen, efforts can be made to salvage and conserve the valuable upper soil horizons in which a majority of the seed bank is contained.

Colonization through natural seed dispersal vectors can occur wherever a dispersal vector is in proximity to disturbed ground and propagule sources are found nearby. Success of colonization resulting from dispersal is largely dependent on two main factors which include the distance of seed source (e.g., surrounding forest cover) from the disturbed ground and the receptivity (ability to provide conditions conducive of germination requirements) of its surface to seed. Distance from seed source has a significant effect on the level of seed

contribution by dispersal. Matsumura and Takeda (2010) examined seed dispersal in seminatural grasslands and found that species richness significantly decreased with increasing distance from seed source. The distance from seed source can also have a significant effect on seed rain density. In floodplain forests along the upper Mississippi River, Minnesota, USA, Adams and Sorenson (2009) observed the seed rain density under forest cover and at stratified distances from the forest edge and found a decrease in seed rain density with increasing distance from the forest edge. In terms of seedbed receptivity, some surfaces, such as freshly disturbed soil (Skrindo and Halvorsen 2008), provide ideal conditions for dispersed seeds while others (e.g., waste rock dumps; Borden and Black 2005) provide much more inhospitable conditions for seed germination and plant establishment due to harsh chemical and physical substrate conditions.

Where natural regeneration is incorporated into a revegetation plan, the costs associated with seed stocks and expensive seedlings or transplanting efforts can be reduced or eliminated and successful revegetation attained. Matesanz et al. (2006) found no difference in plant cover, species richness and aboveground biomass between hydroseeded (using a non-native erosion control seed mix) and non-hydroseeded motorways in Malaga, Spain, and concluded that in some cases, seeding was not necessary in order to successfully revegetate a site. Even seeding with site-appropriate native species can, in some cases, prove to be less successful at initiating early seral native plant communities compared to areas that are naturally regenerated. Hodacova and Prach (2003) found a much higher species diversity on areas of coal spoil heaps in the Czech Republic that were naturally revegetated compared to areas that were technically reclaimed with native plant species; these naturally colonized spoil heaps were also found to be further along a successional trajectory.

1.7 Introduction to Huckleberry Mine

Huckleberry mine is an open-pit copper/molybdenum mine located approximately 86 km southwest of Houston in west-central BC. Construction and operation of the mine began in 1997. The minesite occurs within the Ootsa Lake watershed at the base of Huckleberry Mountain at an elevation of approximately 1050 m. The main features of the site include two open pits (the East Zone Pit and the Main Zone Extension [MZE]), a tailings impoundment (TMF-2), three dams (TMF-2 dam, East Dam and East Pit Plug Dam), till borrow pit, millsite, camp and sewage treatment plant (Figure 1).



Figure 1. Overview of the Huckleberry minesite, located approximately 86 kms southwest of Houston, BC, as photographed in the summer of 2007.

At Huckleberry Mine, vegetation and ecosystems in the surrounding area belong to the Sub-Boreal Spruce (SBS) and the Engelmann Spruce-Subalpine Fir (ESSF) biogeoclimatic (BGC) zones; this includes the Babine variant of the moist cold subzone of the Sub-Boreal Spruce (SBSmc2), with some areas of the moist cold subzone of the Engelmann Spruce-Subalpine Fir (ESSFmc) at higher elevations.

The growing season is short, as a deep snowpack forms earlier and stays longer than most other BGC variants in the region. Forest cover is dominated by hybrid white spruce (*Picea glauca x engelmannii*) and subalpine fir (*Abies lasiocarpa*) with lodgepole pine dominating on drier sites and black spruce (*Picea mariana*) on wet sites and poor soils.

Within the perimeter of the minesite (approximately 1911 ha), the landscape is composed of various intensities of disturbed and undisturbed ground with patches of vegetation cover consisting of early colonizing native plant species. Disturbed areas are either subject to continuous disturbance from mining activities or have not been disturbed since the construction of the minesite. These areas initially disturbed during construction, but now inactive (e.g., spur roads, soil stockpiles), have been naturally colonized by local native perennials along with a few agronomic species (e.g., timothy [*Phleum pratense*], alsike clover [*Trifolium hybridum*]). In some areas, a low to moderate cover of native woody plants has also begun to establish (e.g., TMF-2 North Stockpile). The most common species identified on disturbed soils within the minesite include: blue wildrye (*Elymus glaucus*), arctic lupine (*Lupinus arcticus*), bluejoint (*Calamogrostis canadensis*), Merten's sedge

(Carex mertensii), common horsetail (Equisetum arvense), fireweed (Epilobium angustifolium), Sitka alder (Alnus viridus ssp. sinuata) and thimbleberry (Rubus parviflorus).

Soils in the surrounding area of Huckleberry mine are found on a variety of surficial materials forming a thin veneer over glacial and ablation till. The main soil subgroups found include Orthic Humo-Ferric Podzols, Orthic Ferro-Humic Podzols, Sombric Humo-Humic Podzols, Lithic Humo-Ferric Podzols, Orthic Dystric Brunisols and Terric Mesisols. Organic matter is low in these soils except for in wetlands and pond areas. Soil are typically coarse-textured and rapidly draining and the distribution of texture classes is sandy loam (42%), loam (32%), loamy sand (10%), sandy clay loam (6%) and silt loam (3%). N and P levels in the Huckleberry Mine soils are relatively low in all soil types and strata and the pH is mainly acidic, ranging from 4.1 to 6.1 in the B horizon and from 4.5 to 6.3 in the C horizon. In baseline soil studies prior to development, high concentrations of Cu, Zn and arsenic (As) were found (Boxill 2010).

1.7.1 Substrates available for reclamation

The substrates available for reclamation at Huckleberry Mine include stockpiled soil, desulphurized NAG sand (utilized as a cover material for TMF-2), peat and glacial till. Soils stockpiled on the minesite originated during the initial construction of the mine (1996) and during the extension of the MZE in 2006. During construction, a limited amount of peat was salvaged where deposits existed. Glacial till was removed from selected deposits throughout the minesite with one major deposit occurring at the west end of the property. Desulphurized NAG sand currently caps the TMF-2 impoundment and is readily available for use.

During construction, overlying soil was salvaged wherever possible and subsequently stored in separate stockpiles at various locations on the minesite. Soil was salvaged as a mixture of both A and B soil horizons and is therefore classified as an anthroposol. When this thesis research was initiated, stockpiled soil salvaged during initial minesite construction was approximately 10 to 12 years old and newly stockpiled soil, salvaged during the construction of the recently developed MZE pit (Figure 1) was approximately 1 to 2 years old.

Peat salvage occurred during the construction of the mine and this material is stored in a single stockpile at the base of the TMF-2 dam. Attempts were made to salvage as much peat as possible from deposits; however, due to the logistical issues with hauling the water saturated peat, only a limited amount was salvaged for reclamation. Peat which could not be salvaged was buried during the construction of the impoundment. It is estimated that due to the logistical challenges of removing and transporting peat, only a small portion of peat will be salvageable from the current stockpile.

A major deposit of glacial till which was utilized for the construction of TMF-2 is located at the west end of the minesite (till borrow pit; Figure 1) along with a recently established pit near the millsite. This material can also be utilized as a medium for plant growth.

Beginning in 2006, Huckleberry Mine introduced an additional flotation step in processing of ore for the production of desulphurized tailings. The purpose was to provide pH-neutral material that would be used to create a capping layer on TMF-2. To date, the

impoundment is now an abundant source of desulphurized tailings which can be easily accessed for use as a reclamation medium as required.

1.7.2 Overview of mine closure plan

The following is a summary of the current reclamation and mine closure plan for Huckleberry Mine, submitted to the BC Ministry of Mines in 2010 (Boxill 2010). As designated in the closure plan, the targeted end land use has been identified as forested land for wildlife habitat. Upon closure, all open pits and TMF-2 will be flooded. Around the perimeter of the TMF-2 pond and adjacent to all other dam crests (east dam and east pit plug dam), NAG sand beaches will be constructed to act as buffer between the maximum pond elevation and the dam embankment. Existing infrastructure (all buildings, roads, power lines and pipelines) will be deconstructed and removed from the site. Resloping and placement of reclamation media (i.e., stockpiled soil) will be completed on all areas designated to be revegetated and will be applied to site-specific depths (Boxill 2011).

The most abundant media available for reclamation at the mine site will be stockpiled soil. Areas in which reclamation media will be applied include all major dam faces, upper beaches of TMF-2, all road networks, soil stockpiles (once stockpiles have been exhausted), and the site of the sewage treatment, camp and mill. The media will be applied to a depth of at least 30 cm (based on available quantities of soil) on dam faces and approximately 50 to 200 cm depth on NAG beaches; in all other areas, the target depth for media will be approximately 30-50 cm as specified in the current reclamation plan (Boxill 2011).

Revegetation of the minesite will involve the use of a variety of plant species and revegetation techniques. The revegetation prescription is based upon the targeted end land

use objective for the mine, which is to re-establish vegetation cover that provides valuable habitat features for wildlife identified in the area (e.g., pine martin, black bear, grizzly bear and moose [Boxill 2010]).

Plant species will include various grass and legume species (e.g., blue wildrye, fringed brome, alsike clover [*Trifolium hybridum*] and arctic lupine), shrubs (e.g., soopalallie [*Shepherdia canadensis*], Sitka alder and black huckleberry [*Vaccinium membranaceum*] and trees (e.g., lodgepole pine, subalpine fir and trembling aspen [*Populus tremuloides*]). Grass and legume species will be applied in seed mixes either through broadcast seeding or hydroseeding. Shrub and trees seedlings will be propagated using a local nursery and will be planted with products designed to conserve soil water (e.g., anionic polyacrylamide) and supply nutrients (e.g., Nutri-Pak®) where necessary.

On all major dam faces, a grass/legume seed mix (consisting of both native and nonnative species) will be used as an initial cover crop and will be applied by hydroseeding during the fall. After several years when grass cover is presumed to be less vigorous, shrubs and tree seedlings will be planted. Trees will be planted in areas of the dam face where the soil depth is greater than 40 cm and at a density of 1200 to 1400 stems ha⁻¹. The suggested density for shrubs is 400 stems ha⁻¹.

The surface of TMF-2 consists of upper, above water tailings beaches (coarse sand), which make up the perimeter of the impoundment, and a lower central region (with a high concentration of fine sands) that remains flooded throughout the year. On the upper beaches, a grass/legume cover crop will first be established followed by planting both tree and shrub seedlings with species selected based on microsite moisture conditions. In the lower

saturated regions, shrubs adapted to wetlands (e.g., willow and scrub birch [*Betula nana*]) will be planted. In addition, seeding and transplanting of a few wetland herbs (e.g., sedges [*Carex* spp.] and cattail [*Typha* latifolia]) will be experimented with in an effort to establish littoral zone habitat (Boxill 2011).

Other areas designated for revegetation such as the millsite, till borrow pits and haul roads will also be initially seeded with a grass/legume cover crop, followed by planting of tree and shrub seedlings. Lodgepole pine and aspen are suggested where sites are more south-facing, with subalpine fir and spruce limited to cooler, wetter sites. Stocking rates for trees will be between 1200 and 1600 stems ha⁻¹ and for shrubs between 400 and 600 stems ha⁻¹ (Boxill 2011).

Of the total 1911 ha of property, approximately 404 ha has been designated as disturbed areas to be reclaimed. Areas such as the open pits (East Zone Pit and MZE) and the central portion of the TMF-2 are not included as they will be permanently flooded.

Following the completion of all major closure objectives, post-closure monitoring will include periodic assessments of the performance of revegetation efforts. Assessments will determine whether vegetation succession is occurring on reclaimed sites and evaluate whether further planting, seeding and/or fertilization efforts will be required.

1.7.3 Past reclamation research at Huckleberry Mine

In 2006, revegetation test trials were conducted on the Huckleberry minesite which investigated the success of 10 perennial plant species grown from seed, sown within disturbed soil during the fall and then monitored at the end of the second growing season (Burton 2007). Seed treatments compared locally collected native seeds to genetically diverse native seeds from plants grown in cultivation (Burton and Burton 2002). Overall, the growth and survival of plants grown from locally collected seeds were superior to those from seeds collected from cultivated plants of the same species, exhibiting significantly higher cover, shoot and root biomass and overall plant biomass (Burton 2007).

1.8 Thesis Research Objectives

The availability of NAG sand, obtained from desulphurized tailings at Huckleberry Mine, offered an opportunity to assess a possible low-cost solution to the challenge of limited soil availability, a common problem on mined lands in BC. It was hypothesized that by supplementing stockpiled soil with NAG sand, the amount of suitable growth medium available for reclamation could be increased. As a Masters of Science thesis project, the objective was to evaluate the use of NAG sand and fertilizer as a supplement to stockpiled soil in an effort to increase the quantity of suitable plant growth medium available at the site. The suitability of the medium was assessed by comparing a) physical (e.g., texture) and chemical soil properties (e.g., trace elements and plant nutrients) and b) plant performance (establishment and productivity) between soils supplemented with or without NAG sand. To clarify measures of plant performance, establishment here is defined as the ability of a seed to successfully establish on a soil (e.g., % emergence, seedling density) and productivity is the defined as the size, form and mass of a plant (e.g., plant height, % cover and above- and belowground biomass). In addition, the specific performance of blue wildrye established from locally collected and genetically diverse seed stocks was compared to evaluate the effects of seed origin on plant establishment and productivity.

1.8.1 Objective 1: NAG Sand as a Supplement to Stockpiled Soil

The use of stockpiled soil for reclamation is an effective approach; however, due to poor salvaging and storage techniques, supplies of soil are often limited. As an alternative to locating additional sources of soil, this experiment sought to determine whether the addition of NAG sand could act as a volume-enhancing supplement to stockpiled soil, increasing the amount of suitable medium available for reclamation. In order to measure medium suitability, plant performance in soil supplemented with or without NAG sand was compared. To proclaim soil supplemented with NAG sand as a suitable medium for plant growth, it was decided that plant performance would have to be relatively equal to or greater than the performance observed on non-supplemented soil.

To further test the suitability of soil supplemented with NAG sand as a growth medium, two stockpiled soil sources, differing in age since salvage (2 and 10 years) were examined. The age and composition of stockpiled soil at Huckleberry Mine varies considerably and therefore, it was important to ensure that the criterion of a suitable medium was met for a variety of soils supplemented with NAG sand. In addition, to ensure that comparison of plant performance between soils supplemented with or without NAG sand was not limited to a single plant species, a variety of plant species with different growth forms were tested (i.e., grasses and legumes).

1.8.2 Objective 2: Comparing the Performance of Native Grasses and Legumes

When establishing vegetation cover on disturbed soils, where conditions for plant growth are often less than optimal, the most appropriate species for revegetation are those that can successfully establish in poor growing conditions, provide dense and long-term vegetation cover and require little or no maintenance. To identify candidate species for revegetation at Huckleberry Mine, the performance of five native species (three grass, one sedge and one legume) sown in supplemented and non-supplemented soils was compared. The evaluation of these species was considered important as grasses, sedges and legumes all have different regenerative strategies and a variety of these species types are currently colonizing the minesite. The candidate species selected were native to the region of the minesite and were chosen based on the availability of seed and ease of seed collection.

1.8.3 Objective 3: Influence of Genetic Origin on Performance of Blue Wildrye

Collecting native seed from areas surrounding the minesite is costly compared to obtaining similar seed stocks from local suppliers. To assess whether the higher costs of obtaining local seed stocks are justified, the performance of locally collected and cultivated varieties of blue wildrye was examined in this experiment. In effect, the objective was to compare the performance of blue wildrye established from locally collected (local genotype) and genetically diverse (mixed genotype) seed stocks to evaluate the effects of seed origin on plant establishment and productivity.

2.0 Methods

2.1 Seed Collection

During the minesite reconnaissance in late June 2008, a few local native perennials were selected as candidates for incorporation into revegetation test plots. The search for these candidates was conducted in areas of disturbed ground on the minesite property that had been colonized by native vegetation, most notably along the western boundaries. The most common species identified within these areas included blue wildrye, arctic lupine, bluejoint, reedgrass, Merten's sedge, common horsetail, fireweed and Sitka alder. Candidate species were evaluated for their potential ease of seed collection and overall success of propagation. Three species were chosen for incorporation into test plots; these were blue wildrye, arctic lupine and Merten's sedge.

In August and September, 2008, seeds from candidate species were collected from the local area (10 km radius) at appropriate dates to their expected ripening times. Seeds for arctic lupine were collected in the first week of August and seed for blue wildrye and Merten's sedge were collected in early September. Geographic coordinates (from a global positioning system [GPS] receiver) and dates were recorded for each area in which seeds were collected (Appendix 1).

Once seed materials had been collected, recommended methods for each species were utilized to clean and prepare the seeds for sowing the test plots. The cleaned seeds were stored in low light conditions at room temperature between the time of collection and seeding efforts. Seed cleaning and preparation methods followed procedures recommended in a local

manual that included species-specific guidelines for the three species collected (Burton and Burton 2003).

2.2 Seed Preparation

2.2.1 Arctic Lupine

Lupine seeds were collected on August, 2008 at four locations within the local area of the minesite. Sites included the two locations around the TMF-2 north stockpile, a location along the Morice Forest Service Road at km 114 and an inactive airfield at km 111. At each location, lupine plants were assessed for pod ripeness (see Figure 2a for an example of ripe pods). Where ripe pods were found, the entire inflorescence was harvested using clippers. Harvested inflorescence were collected in small brown paper bags and then transferred to larger paper bags for transport and storage.

Following field collection efforts, the resulting pod stock was stored in large paper bags (sealed closed) for approximately two weeks to allow pods to fully ripen and dehisce. Seeds from dehisced pods were then collected from the bottoms of each bag. Remaining unopened pods were crushed by hand and then crushed material was filtered through two different screens (4 mm and 1 mm mesh; Figure 2b and 2c, respectively) in order to extract seeds. A final hand screening process was completed to eliminate insect larvae from the seed stock; this insect was probably the lupine aphid, *Macrosiphum alibifrons* (Cohen and Mackauer 1986; Figure 2d).



Figure 2. Seed collection and preparation of arctic lupine with a) example of ripe pods (black and dry) during collection, b) screening of crushed pod material for initial and c) final screening; d) insect larvae were separated by hand from final seed stock (close up of larvae top left).

After seed cleaning, an estimated 830 g of pure seed were collected. An analysis of seed purity by weight determined that the cleaned seed lot was approximately 99 % pure. The total number of seeds available was then calculated based on an average number of seeds per gram (determined by taking an average of the total number of seeds in three random one gram samples, as per Burton and Burton 2003). With an average of 108 seeds g⁻¹, it was estimated that approximately 90,000 lupine seeds were collected. Samples of lupine seeds from each collection location were then sent to a commercial seed testing laboratory (20/20 Seed Labs Inc., Nisku, Alberta) for viability testing ("Between Paper" method at 20°C for 10 days; light and dark periods, 8 and 16 respectively) to determine the percent germination capacity of each seed lot.

2.2.2 Blue Wildrye and Merten's Sedge

Seeds from blue wildrye and Merten's sedge were collected from ten locations around the minesite (six within the current minesite boundaries and four outside the boundaries) between September 3rd and 16th, 2008. Pure seed from blue wildrye was collected by running hands through the inflorescence and accumulating loosened seeds, or the inflorescence was clipped from the stalk for processing later. Seed material from Merten's sedge was collected by clipping the inflorescence from stalks. The seed material for both species was collected in brown paper bags.

Seed material collected from blue wildrye was cleaned using soil screening equipment available in the metallurgical laboratory at Huckleberry Mine. Before screening, detritus material and chaff was removed by hand. Using a series of six soil screens, decreasing in mesh size from top to bottom, the hand cleaned material was placed in the top screen, with the largest mesh size. Screens were then placed in the sieve shaker and processed (vibrated or shaken) for ten minutes. The shaking separated most of the seeds from the seed heads, with seeds retained in one or two of the middle sieves. After the sieving process, the seed stock of blue wildrye was determined to be approximately 100 % purity by weight.

For Merten's sedge, the attempt to purify seed stock through the series of sieves proved unsuccessful, as floral bracts were very similar to the seeds in size and shape. Therefore, seeds (with bracts) were separated from the inflorescence by hand and a seed purity analysis was conducted in order to estimate the number (proportion) of seeds within the total seed stock material. Purity was determined to be 85.5 % by weight.

2.3 Test Plot Construction

A total of twelve revegetation test plots were constructed at four different locations (three test plots per location) at the Huckleberry minesite between September 22nd and October 6th, 2008 (Figure 3). Test plot locations were chosen based on criteria that included accessibility to heavy machinery for ground preparation and substrate delivery (stockpiled soil and NAG sand) and low risk of disturbance from mine operation over the lifetime of the project.

Before test plots were constructed, the ground at each location was leveled to create similar surface conditions for each site. Following site preparation, stockpiled soil and desulphurized NAG sand were delivered to each site and piled for use in the construction of the test plots; approximately 9 m³ of desulphurized NAG sand and 27 m³ of soil from stockpiles was delivered to each site.

At each of the four locations, two supplemented soil test plots were constructed using soil from either a 2-year soil stockpile (sourced from the stockpiles south of the MZE pit; Figure 3) or 10-year old soil stockpile (sourced from the TMF-2 North Stockpile) and desulphurized NAG sand (sourced from a single location within the TMF-2 impoundment). Test plots at two locations (TMF-2 North Stockpile and Upper TMF-2 test plots) were constructed using 10-year old stockpiled soil and the remaining two locations (Lower East Dam and Millsite test plots) were constructed using 2-year old stockpiled soil. In addition, a single NAG sand test plot was constructed at each of the four test plot locations.



Figure 3. An aerial view of Huckleberry mine (2011), located 86 kms southwest of Houston, BC, indicating the four locations of the revegetation test plots (TMF-2 North Stockpile, Upper TMF-2, Lower East Dam and Millsite) and the MZE stockpile.

Supplemented soil test plots were composed of 32 subplots, each measuring 10.4 m by 5.2 m, arranged in a split-strip-plot design (Little and Hills 1978) and NAG sand test plots were composed of eight subplots (Figure 4). The supplemented soil test plots were divided into two equal sections, each consisting of 16 subplots. In one section, the subplots were composed of a stockpiled soil and the other section, a 50:50 mix (by volume) of stockpiled soil and NAG sand. Within each of these treatment substrates, each of the eight subplots was fertilized with 57.69 g (equivalent to 576.9 kg ha⁻¹) of 13-16-10 NPK (74.9 kg ha⁻¹ N, 92.3 kg ha⁻¹ P₂O₅ and 57.7 kg ha⁻¹ K₂O; EvergroTM, Kelowna, BC) fertilizer and the remaining eight were left unfertilized. The rate of fertilizer applied to subplots was based on a similar

rate used in revegetation research plots established at Huckleberry Mine in 2005 (Burton 2007). A list of test plot treatment substrates is provided in Table 1.



Figure 4. Overview diagram of soil treatments (each square representing a subplot) for NAG sand test plots (left) and the supplemented soil test plots (right).

Substrate composition and fertilization treatments were prepared using two 0.085m³ gas powered cement mixers and mixed material was transported between substrate source piles and subplots by shovel and wheelbarrow (Figure 5a, b). In order to create the 50:50 mix of soil and NAG sand, equal amounts of each material were shovelled into cement mixers and allowed to mix at a medium rotation speed for approximately two minutes (Figure 5c). Fertilization followed the same procedure in which the material for the top 10 cm of each fertilized subplot was mixed in a cement mixer with the prescribed fertilizer for two minutes (Figure 5d).

Substrates	Stockpile Age	50:50 NAG	Fertilizer
Substrates		Sand:soil	
Stockpiled soil	2	No	No
Stockpiled soil	2	No	Yes
Stockpiled soil	2	Yes	No
Stockpiled soil	2	Yes	Yes
Stockpiled soil	10	No	No
Stockpiled soil	10	No	Yes
Stockpiled soil	10	Yes	No
Stockpiled soil	10	Yes	Yes
NAG sand	N/A	No	No

Table 1. A list of the nine treatment substrates for revegetation test plots established in September 2008.

The target surface area for each subplot was 1.0 m^2 . To achieve this, a 2.6 m² ground surface area, designated for each subplot was covered with the treatment substrate, resulting in a substrate depth of approximately 0.2-0.25 m and 0.3 m buffer around the subplot perimeters (Figure 5e). Following the completion of subplots, buffer spaces were backfilled using neutral parent soil from the area surrounding the test plots (Figure 5f). For photographs of each of the four test plot locations upon completion of construction, see Appendix 2.



e) f) Figure 5. Photographs illustrating the construction of the test plots, showing a) mixing of soil and NAG material, b) transporting mixed material to subplots, c) procedure for mixing substrates, d) mixing fertilizer with the top layer of fertilization subplots, e) standardized dimensions of subplots and f) backfilling buffer spaces between subplots.

2.4 Test Plot Seeding

In total, seven seeding treatments were applied to all 12 test plots initiated on the minesite (Table 2) on October 5th and 6th, 2008. Treatments consisted of sowing either a single plant species or a mix of species. Seeds stocks included: 1) native seeds collected from the local area of the mine (locally specialized genotypes) and 2) seeds obtained from seed increase plots where seeds from a variety of locations were sown to propagate genetically diverse seed crops (mixed genotype or ecovars) grown at the Industrial Forest Service Ltd. (IFS) Ness Lake Nusery, located northwest of Prince George, BC.

Treatments were applied to all subplots at a standardized sowing density of 750 PLS m⁻² which was chosen based on optimal sowing densities recommended as the result of a previous study (Burton et al. 2006). The amount of seed to weigh out in order to obtain the desired PLS was calculated by adjusting the bulk seed lot weights on the basis of their germination and purity values (Table 2). For locally collected seeds, percent germination was determined from suggested values reported by Burton and Burton (2003) and percent purity and seeds g⁻¹ were determined by weight from samples of each seed lot. Percent germination and purity values and seeds g⁻¹ for genetically diverse seeds were obtained from previous seed germination tests for seed from the same increase plots (20/20 Seed Labs Inc., Nisku, Alberta). Prior to seeding treatments, seed quantities for each subplot were weighed using a scale with a 0.01 g resolution (ProScale; LC-50; Fletcher, NC, USA) and then packaged in paper envelopes (Figure 6a).

Test plots were sown during the period of October 5th-6th, 2008. At each location subplots for each treatment were individually hand sown (sowing was conducted by only one

person to prevent bias (Figure 6b). Immediately after sowing each subplot, the soil surface was lightly raked to promote good seed/soil contact and to prevent seed loss from wind (Figure 6c). An example of seed distribution across the surface of the subplot is shown in Figure 6d. A diagram displaying the arrangement of seeding treatments within test plots is shown in Figure 7.



Figure 6. Images illustrating procedures associated with test plot seeding, including a) weighing and packaging of seed quantities for subplots prior to sowing b) hand seeding procedure c) raking after sowing to promote seed germination and d) representative seed coverage across the 1.0 m^2 surface of a subplot (before raking).

Seed Treatment	Seed Stock	Composition by weight (%)	Seeds g ⁻¹	Germination (%)	Purity (%)	Bulk Seed Application (g m ⁻²) ^a
Single-Species	an a					
fringed brome	ecovar	100	420	80.5	79.0	2.81
blue wildrye	ecovar	100	219	86.5	85.0	4.66
blue wildrye	local	100	219	80.0	99.0	4.32
Rocky Mountain fescue	ecovar	100	1500	95.0	96.0	0.55
arctic lupine	local	100	108	44.3	97.0	16.16
Mixed-Species						
blue wildrye: fringed brome:	ecovar	33 3. 33 3. 33 3	219: 420:	86 5. 80 5. 95 0	85.0: 79.0:	1 55 0 94 0 18
Rocky Mountain fescue	ccovar	<i></i>	1500	00.5. 00.5. 95.0	96.0	1.55. 0.74. 0.10
blue wildrye: arctic lupine:	local	33 3. 33 3. 33 3	219: 108:	80 0. 44 0. 37 2	99.0: 97.0:	1.44: 5.39: 0.58
Merten's sedge	IUCAI		1555	00.0. 44 .0. J7.2	86.4	

Table 2. Seed treatment types and derived bulk seed application weights for 1.0 m² subplots based on estimated percent germination and percent purity values for each seed lot.

^aAmount of bulk seed stock sown on each 1m² subplot surface. The prescribed application is based on the desired seeding density of 750 PLS m⁻², where bulk seed application = $\left(\frac{750 \text{ PLS m}^{-2} \times \% \text{ composition}}{\text{Seeds g}^{-1} \times \% \text{ germination} \times \% \text{ purity}}\right)$.

In addition to the seven seeding treatments mentioned above, a non-native domestic seed mix treatment was also applied to the test plots during the seeding dates. However, due to a miscalculation, the sowing rate of the domestic seed mix treatment (estimated at >4000 PLS m⁻²) was considerably higher than the target seeding rate (750 PLS m⁻²) and thus, was not comparable with the other seeding treatments. To compensate, the domestic seed mix treatment was removed from the subplots in the following spring (top 5cm of subplots removed by shovel) and the subplots were designated as non-vegetated subplots (Figure 7).



NAG Sand Test Plot

Supplemented Soil Test Plot

Figure 7. Overview diagram of seeding treatments (each square representing a subplot) for NAG test plots (left) and supplemented soil test plots (right). Subplots were either seeded with one of the seven seeding treatments or designated as non-vegetated.

2.4.1 Prior to Test Plot Construction and Seeding

Due to the short time period between seed collection efforts and test plot seeding, germination testing of locally collected seeds was only conducted for arctic lupine. Four samples (100 seeds per sample) were sent to 20/20 Seed Labs Inc. where germination testing was conducted within a temperature controlled environment (daytime period of 8 hours at 20°C) for a 20 day period.

2.4.2 Following Test Plot Construction and Seeding

Germination testing of the seed lots used in test plot seeding treatments was initiated at the University of Northern British Columbia (UNBC) Enhanced Forestry Laboratory (EFL) on April 8, 2009. These tests were completed in an effort to acquire actual germination rates in comparison to the estimated rates used in calculating bulk seed application rates. The actual germination rates could then be used to more accurately report sowing rate (PLS m⁻²) as implemented, and percent emergence. In total, four replicates of each of the seven seeding treatments were tested within an Environmental Growth Chamber (EGC; Model GCW 30, Chagrin Falls, Ohio, USA). For each replicate, 100 seeds of a seed lot were counted and placed in a clear plastic germination test box (Tristate Plastics, Dixon, Kentucky). Additionally, a pre-treatment was applied to the locally collected arctic lupine seeds (knicking seed coat with a razor blade) to hasten germination. Seeds were applied to a thick, pre-moistened pad of cellulose wadding (Kimpack®) using distilled water in germination test boxes and then placed in the EGC for incubation. Replicates for each seeding treatment were evenly distributed throughout the chamber (4 groups of replicates, non-randomly placed in the EGC) to account for any variability in light and temperature in the EGC.

The controlled conditions within the EGC were as follows: photosynthetically active radiation (PAR) at 600 W m⁻² for a daytime period of 14 hours and a day/night temperature regime of 20/10 °C. During incubation, germination test boxes were monitored every two to three days to ensure that paper cloth substrates remained moist. As a preventative measure, a fungicide (No-DampTM) was diluted into water and used to keep cloth substrates moist (1:100 No-Damp:Water dilution). During monitoring, the number of seeds that had germinated was recorded for each germination test box. Seed germination (defined as penetration of the seed coat by the radicle; Bewley 1997) was monitored until no new seed germination was observed for a consecutive four-day period; observations were completed over a 60-day period. Germinated seeds were not removed from the germination boxes until observations were completed.

2.5 Test Plot Monitoring

2.5.1 Year One (2009)

2.5.1.1 Vegetation Sampling

Sampling of vegetation was conducted for 252 out of the 288 1.0 m² subplots in the twelve established revegetation test plots during late June and August, 2009 (36 subplots were designated as non-vegetated, Figure 7). Each subplot was divided into a grid consisting of 16 sampling grid locations (625 cm² area [25 cm x 25 cm]). Using a random number generator in Excel (Microsoft Office 2007), a single grid location for each subplot was chosen for sampling (Figure 8a). In subplots sown with a single-species seeding treatment, sampling included a count of seedlings (seedlings m⁻²; Figure 8b), percent cover (Figure 8c) in both June and August, and plant height (Figure 8d) in August (no seedling counts were completed

for Rocky Mountain fescue (Festuca saximontana) due to the difficulty in distinguishing individual seedlings). For subplots sown with mixed-species seeding treatments, cover was evaluated and measurements of height were taken for each species. Sampled grid locations were marked using four pins and flagging tape (blue) at plot corners for future reference.



c)

Figure 8. Images from Year One vegetation sampling, completed in June and August 2009 showing a) a 25 cm x 25 cm sampling grid location (corners marked by blue flagging) with a 1 m x 1 m wood frame to delineate the16 possible sampling grid locations, b) an example of seedling density observed in subplots of blue wildrye, c) measurements of percent cover for sampling plots, and d) measuring plant height.

2.5.1.2 Substrate Treatment Sampling

Soil samples representing each of the nine treatment substrates (Table 1) were collected from the 252 of the 288 1.0 m² subplots within the 12 test plots in August 2009 (non-vegetated subplots were not sampled as a subplots were significantly disturbed during the removal of

domestic seed mix). Soil samples were collected from subplot sampling groups (each sampling group representing a single treatment substrate; Figure 9). The sampling design was chosen in an effort to obtain a sample of each treatment substrate that was representative across all subplots. In each test plot, one sample was collected for each subplot sampling group (two from each of the four NAG test plots and eight from each of the eight supplemented soil test plots) for a total of 72 samples. Each sample was a composite, composed of core samples collected from all subplots designated to a specific sampling group. Within each subplot, cores were collected from three random locations within a 625 cm² sampling grid using a regular 7.6 cm diameter soil auger to the depth of approximately 15 cm (using a 16.5 cm long auger bucket). Remaining auger holes were filled with soil from the surrounding area and marked using a pin and flag to avoid soil sampling there in the future. Any damage to subplot vegetation was noted. All cores collected for each composite sample were collected into a 20 L bucket and mixed prior to packaging. Samples were packaged in ziplock bags, appropriately labelled and stored in coolers and were transported from the minesite to UNBC for sample preparation.

Initial sample preparation was completed at UNBC and then sent to the Ministry of Forests and Range (BCMOF) Research Branch Laboratory in Victoria, BC, and ALS Canada Ltd., Vancouver, BC, for analysis. Sample preparation included air drying of samples (for at least a seven day period) followed by sieving using a 2 mm sieve. Coarse fragment (material >2 mm) content (percent by mass) for each sample was recorded after sieving. The fine soil fraction material was then packaged in small cardboard sampling boxes and shipped to the BCMOF laboratory for analysis. Table 3 lists the chemical and physical properties tested; further references for analyses conducted are listed in Appendix 3. Following return of

samples from the BCMOF laboratory, a subset of the samples was sent to ALS Canada Ltd. laboratory (Vancouver, BC) for total elemental analysis (Table 3).



NAG Sand Test Plot

Soil Supplemented Test Plot

Figure 9. A diagram displaying subplot sampling groups from NAG sand test plots (left) and supplemented soil test plots (right). Each subplot is designated to a specific sampling group. Each sampling group (two in each NAG sand test plot and eight in each supplemented soil test plot) represents a single substrate treatment. Nonvegetated subplots were not sampled.

Laboratory/Analysis	Preparation Method	Analytical Method	Year of Analysis
BCMOF Laboratory			
Particle Size Analysis	Sedimentation Rate	Gravimetric	2009
Total Carbon and Nitrogen	Combustion Elemental Analysis	Elemental Analyzer	2009, 2010
Total Inorganic Carbon	Combustion Elemental Analysis	Elemental Analyzer	2009, 2010
Total Sulphur	Combustion Elemental Analysis	Elemental Analyzer	2009, 2010
Available Phosphorus (PO ₄ -P)	Olsen's Extraction Method	UV/visible	2010
Minerizeable Nitrogen	Anaerobic incubation, 1N KCl extraction	Colorimetric · Auto - Analyzer	2010
Available Ammonium (NH4-N) and Nitrate (NO3-N)	Extraction with 2N KCl	Colorimetric - Auto - Analyzer	2010
Carbonate (CaCO ₃) equivalent	Empirical – Acid Neutralization	pH meter	2010
pH (Calcium Chloride)	1:1 for Mineral Soil	pH/lon Meter	2009, 2010
pH (water)	1:1 for Mineral Soil	pH/Ion Meter	2009, 2010
Electrical Conductivity	Saturated Paste	Conductivity Meter	2009, 2010
Extractable Elements (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn	Mehlich III Extraction	ICP-AES ^a	2009, 2010
Exchangeable Cations (Ca, K, Mg, Na) and CEC	Neutral Ammonium Acetate	ICP-AES	2009, 2010
Trace Elements (As, Ba, Bi, Cd, Co, Cr, Hg, Mo, Ni, Pb, Sb, Sn, Ti and V)	Mehlich III Extraction	ICP-AES	2009
ALS Canada Ltd. Laboratory			
Total Elemental Analysis for Rare Earth and Trace Elements	Lithium Borate Fusion followed by Acid Dissolution	ICP-MS [♭]	2009
Base Metals	Aqua Regia Digestion	ICP-AES	2009
Major Oxides	Lithium Metaborate/Lithium Tetraborate Fusion followed by Acid Dissolution	ICP-AES	2009

Table 3. Chemical analysis conducted on soil samples (completed by BCMOF and ALS Canada Ltd.) collected from test plot treatment substrates in August 2009 and 2010. Further descriptions and references are listed in Appendix 3.

^aInductively Coupled Plasma Atomic Emission Spectrometry

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^bInductively Coupled Plasma Mass Spectrometry

"The term base metals is used by the mining industry to identify metals used in non-alloyed forms (e.g., Cu, Pb, Zn, Sn; Lottermoser 2010).

2.5.2 Year Two (2010)

2.5.2.1 Vegetation Sampling

In late August, 2010, vegetation was sampled from 252 of the 288 1.0 m^2 subplots of the 12 revegetation test plots (36 subplots were designated as non-vegetated, Figure 7). In each subplot, destructive vegetation sampling was completed in and outside the four central sampling grid locations and non-destructive sampling was completed in the four central sampling grid locations. The outside corners of the four central sampling grid locations were marked using four pins and pink flagging tape and a 1 m x 1 m wood frame was used to mark the subplot boundary.

Non-destructive sampling was first completed in the four central sampling grid locations and included percent cover and plant height determinations. Destructive sampling from both inside and outside the four central sampling grid locations followed, and consisted of harvesting above- and belowground biomass.

Inside the four central sampling grid locations, aboveground biomass (live shoot biomass above the soil surface) was harvested by clipping the stems at the soil surface; an example of a subplot where the aboveground biomass was harvested is shown in Figure 10a. The stems were then placed upright in a 20 L bucket and the number of seedlings was determined by counting the number of stems (Figure 10b); for grasses, care was taken to distinguish primary and secondary shoots and only primary shoots were counted. No seedling counts were completed for Rocky Mountain fescue due to difficulties in distinguishing between individual seedlings. Following seedling counts, the samples were packaged in labelled brown paper bags.

Outside the four central sampling grid locations representative seedlings were harvested from each of the subplots (Figure 10c). For each of the subplots sown with singlespecies seeding treatments, four seedlings were harvested and for those with mixed-species seeding treatments, between one and four seedlings of each species present was harvested. Using a garden trowel and shovel, seedlings were harvested from the subplots by digging out the earth surrounding the root system to the base of the subplot (~25 cm) and then carefully removing the soil from the roots by hand. To further reduce the amount of soil around the roots, the roots of each plant sample were soaked in water for a one minute period (Figure 10d). The seedlings were kept intact (shoot and root biomass not separated) and packaged in labelled brown paper bags. Once harvesting was complete, aboveground biomass and seedling samples were packaged in cardboard boxes and shipped to UNBC; samples arrived within 10 days of collection with no signs of mold or deterioration.

In the EFL at UNBC, aboveground biomass and seedling samples were washed, dried and weighed over a four month period (September to December 2010). Upon arrival from the minesite, the samples were placed in a sunny, glassed-in open area and air-dried for a period of approximately two weeks in an effort to stabilize the samples (i.e., prevent any decomposition or molding). During sample preparation and drying, all samples were kept in their original labelled brown paper bags.

Aboveground biomass samples were oven-dried in a laboratory oven (Isotemp® Standard Incubators, 600 series; Fisher Scientific; model: 650d; Toronto, Ontario) for 48 hours at 70 °C. After drying, biomass samples were sieved (Figure 11a) to eliminate any residual sand or soil particles and then samples were weighed using a top-loading digital balance with a 0.01 g resolution (Sartorius, model ISO 9001; Edgewood, NY, USA).


Figure 10. Photographs showing the harvesting of biomass from revegetation test plots: a) a subplot after aboveground biomass has been harvested from the four central sampling grid locations; b) seedling counts and packaging of biomass samples; c) individual seedlings with shoot and root biomass collected from outside the four central sampling grid locations; and d) seedlings bathed in water to clean residual soil on roots.

Before oven-drying, seedling samples with both root and shoot biomass were soaked for a 12 hour period (to loosen soil particles still attached to the roots; Figure 11b) and then washed with a pressurized hose over a 1 mm sieve to remove remaining soil particles (Figure 11c). Seedling samples were then oven-dried in a laboratory ovens referred to above for 48 hours at 70 °C.

Once drying was completed, seedling samples were left to equilibrate to room temperature for approximately 20 minutes before further preparation and weighing. The above- and belowground biomass of seedling samples was then separated by clipping the samples at approximately 1cm above the shoot:root transition zone (Figure 11d), simulating the approximate location in which aboveground biomass was clipped when harvested from the test plots. Once clipped, the root and shoot mass were measured separately using a digital balance with a 0.0001 g resolution (Sartorius, model MC4105; Edgewood, NY, USA).



Figure 11. Photographs illustrating preparation of aboveground biomass and seedling samples prior to weighing. Images show a) aboveground biomass samples being sieved prior to weighing; b) soaking the roots of seedling samples for a 12 hour period; c) washing seedling samples over a 1 mm sieve, d) separating the above- and belowground biomass on seedling samples.

2.5.2.2 Substrate Treatment Sampling

In August 2010, soil samples were collected from subplots sown with local blue wildrye, ecovar blue wildrye and arctic lupine (total = 108 samples) single seeding treatments. A composite sample composed of three core samples was collected from each subplot containing one of the three seeding treatments to the depth of approximately 15 cm (using a 16.5 cm long auger bucket). The composite sample was mixed thoroughly in a bucket and then a smaller random sample was collected from the mix. Samples were collected into ziplock bags, labelled, packaged in coolers and then sent to the BCMOF laboratory for analysis; Table 3 lists the physical and chemical properties that were tested.

Samples for bulk density measurements were collected on site using a volume excavation method (15.24 cm diameter ring, board, hammer and trowel). Four samples were collected for each of the nine substrate treatments; sampled subplots were randomly selected using a random number generator in Excel (Microsoft Office 2007) to select four subplots for each substrate treatment. Each sample was collected by hammering the ring to a depth of approximately 4.5 cm and then extracting the core using a trowel. Bulk density samples were then processed (i.e., dried and weighed) at UNBC. Soil samples were dried within a laboratory oven (Isotemp® Standard Incubators, 600 series; Fisher Scientific; model: 650d; Toronto, Ontario) for 48 hours at 70 °C and then weighed using a scale with 0.01 g accuracy (Sartorius, model ISO 9001; Edgewood, NY, USA).

In addition, the compressive strength (kg cm⁻¹) of the soil surface was measured during soil sampling in all subplots (except non-vegetated subplots; Figure 8) using a pocket penetrometer (Humboldt Manufacturing, model H-4200; Schiller Park, IL, USA). For each subplot, measurements were taken in four randomly selected sampling grid locations (one measurement per grid location, selected using a random number generator in Excel (Microsoft Office 2007).

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2.6 Statistical Analyses

Statistical analysis of vegetation data was conducted using R (version 2.11.1; R Development Core Team 2010). A linear mixed effects model was used to describe the response of dependent variables to independent treatment variables (Table 4 and 5) using the R nlme package (Pinheiro et al. 2009). Analysis of 2- and 10-year old stockpiled soil data was completed separately. Fixed effects for models included NAG sand, fertilizer and seeding treatment; blocking (in terms of the four separate test site locations) was incorporated into the model as a random effect. The fixed-effects structure of the maximal model fit by maximum likelihood was: [y ~ NAG sand * fertilizer * seeding treatment] where * indicates interactions.

Analytical assumptions, namely homogeneity of variances and normal distribution of residuals, were examined by inspecting residual plots. If required, a square root transformation was used to satisfy model assumptions. Maximal models (a model that includes all fixed and random effects and interactions) were simplified by sequentially deleting non-significant terms (starting with highest-order interactions) and comparing each model using the Akaike information criterion (AIC, Burnham and Anderson 1998) and likelihood ratio tests, until minimal adequate models were retrieved. A likelihood ratio test was also used to determine whether the lowest level of nesting should be removed. The minimal model was compared to a null model : $[y \sim 1]$ using a likelihood ratio test to assess the validity of the mixed effects analyses.

The response of dependent variables to independent treatment variables were examined using analysis of variance (ANOVA) on transformed or non-transformed data.

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Where no significant interactions were detected, Tukey's pairwise comparisons (R package multcomp; Hothorn et al. 2008) were utilized to investigate differences between treatment levels. Calculated p-values ≤ 0.05 were considered statistically significant. Where significant interactions were detected, mean values of treatment effects were obtained (biology package; Logan 2008) and plotted on a bar graph.

For soil data, mean values (± standard error [SE]) have been calculated from raw data in an effort to characterize physical and chemical properties of substrate treatments. Soil variables were not tested for significant differences among the experimental treatments.

Variable	Effects	Number of Treatments	Description
Block	random	4	Test plots, two at each location
NAG sand	Fixed	2	50:50 mix of soil:NAG sand – yes or no
Fertilizer	Fixed	2	Granular fertilizer, 577 kg ha ⁻¹ (13-16-10 NPK) – yes or no
Seeding Treatment	Fixed	7	Seeding treatments, either single-species or mixed-species; sown at 750PLS m ⁻²

Table 4. Independent variables for native vegetation test plots constructed with 2- or 10-year old stockpiled soil.

Table 5. Dependent variables for native vegetation test plots constructed with 2 or 10 year old stockpiled soil. Vegetation

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percent emergence (% seedling density PLS density<sup>-1</sup>)<sup>a</sup>
seedling density (seedlings m^{-2})
percent cover (%)
plant height (cm)
cover per seedling (% seedling<sup>-1</sup>)<sup>b</sup>
above ground biomass (g m^{-2})
estimated belowground biomass (g m<sup>-2</sup>)<sup>c</sup>
shoot:root ratio<sup>d</sup>
aboveground biomass per seedling (g seedling^{-1})<sup>e</sup>
estimated belowground biomass per seedling (g seedling<sup>-1</sup>)<sup>f</sup>
Soil
coarse fragments (%)
fine fragments (sand, silt and clay; %) bulk density (g \text{ cm}^{-3})
penetration resistance (Mg m^{-2})
total carbon (organic and inorganic; %)
total nitrogen (%)
total sulphur (%)
ammonium-N (mg kg<sup>-1</sup>)
phosphorus (Mehlich III and Olsen's; mg kg<sup>-1</sup>)
nitrate-N (mg kg<sup>-1</sup>)
mineralizeable nitrogen (mg kg^{-1})
pH (water)
electrical conductivity (saturated paste; mS cm<sup>-1</sup>)
cation exchange capacity and exchangeable cations (cmol(+) kg^{-1})
extractable elements (Mehlich III; mg kg^{-1})
major elements reported as oxides (%)
rare earth and trace elements (Mehlich III; mg kg<sup>-1</sup>)
base metals (mg kg<sup>-1</sup>)
calcium carbonate equivalent (%)
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^aPercent emergence was calculated by dividing seedling density by actual PLS (refer to Appendix 8 for actual PLS values).

^bCover per seedling was calculated by dividing percent cover by seedling density.

"Estimated belowground biomass was calculated by dividing aboveground biomass by the shoot:root ratio.

^dThe shoot:root ratio was calculated by dividing shoot biomass by root biomass of seedling samples.

^eAboveground biomass per seedling was calculated by dividing aboveground biomass by seedling density. ^fEstimated belowground biomass per seedling was calculated by dividing the estimated belowground biomass by seedling density.

3.0 Results

3.1 Soil Characterization

Physical properties of 2- and 10-year old stockpiled soil supplemented with NAG sand differed considerably from non-supplemented stockpiled soil (Table 6). Percent coarse fragments, percent silt and percent clay content were lower and percent sand was higher in supplemented compared to non-supplemented soils. Bulk density (g cm⁻³) and soil strength (MPa) were higher and organic matter content (SOM; %) was lower in soil supplemented with NAG sand compared to non-supplemented soils.

Chemical properties differed significantly between 2- and 10-year old stockpiled soil supplemented with and without NAG sand (Table 6). Soil pH was higher in supplemented compared to non-supplemented soils. Total carbon (C; %) and N (%) were lower in supplemented 2- and 10-year old soil and total S (%) was lower in supplemented 2-year old soil, but higher in supplemented 10-year old soil compared to non-supplemented soils. The amount of organic C (%) was lower in supplemented soils and the C/N ratio was higher in supplemented 2-year old soil and lower in supplemented 10-year old soils compared to non-supplemented 2-year old soil and lower in supplemented 10-year old soils compared to non-supplemented soils. Electrical conductivity (mS cm⁻¹) was higher and calcium carbonate equivalent (CaCO₃) concentration was lower in supplemented compared to non-supplemented soils. Concentrations of exchangeable cations (Ca²⁺, Na⁺, Mg²⁺ and K⁺) in supplemented soils were relatively equal to or lower than concentrations in non-supplemented soils.

	2-Year Soil		10-3		
	Soil	Soil + NAG sand	Soil	Soil + NAG sand	NAG sand
Physical Properties					
Coarse Fragments (%)	56.1 ± 6.3	32.4 ± 5.8	43.2 ± 4.3	22.3 ± 3.4	1.4 ± 0.3
Sand (% of fine fraction)	51.3 ± 0.3	80.5 ± 0.7	46.3 ± 0.4	77.7 ± 0.5	89.5 ± 0.3
Silt (% of fine fraction)	29.4 ± 0.4	12.6 ± 0.4	32.5 ± 0.3	13.5 ± 0.3	6.3 ± 0.5
Clay (% of fine fraction	19.3 ± 0.4	6.8 ± 0.3	21.2 ± 0.3	9.1 ± 0.4	4.2 ± 0.3
Bulk Density (g cm ⁻³)	0.76 ± 0.12	1.17 ± 0.09	0.80 ± 0.04	1.18 ± 0.02	1.23 ± 0.02
Soil Strength (MPa)	$0.04 \pm < 0.01$	$0.01 \pm < 0.01$	$0.06 \pm < 0.01$	0.03 ± <0.01	0.01 ± <0.01
Soil Organic Matter (%) ^b	2.75 ± 0.16	1.09 ± 0.28	3.13 ± 0.16	0.58 ±0.32	<0.01
Chemical Properties					
pH (water)	7.08 ± 0.10	7.31 ± 0.04	5.61 ± 0.03	7.16 ± 0.03	8.25 ± 0.03
Total Carbon (%)	1.25 ± 0.08	0.45 ± 0.03	1.51 ± 0.05	0.36 ± 0.02	0.09 ± 0.02
Total Nitrogen (%)	0.06 ± <0.01	$0.07 \pm < 0.01$	0.09 ± <0.01	0.02 ± <0.01	<0.01
Total Sulphur (%)	0.42 ± 0.02	0.23 ± 0.01	0.04 ± <0.01	0.16 ± 0.003	0.19 ± 0.01
Total Organic Carbon (%) ^a	1.10 ± 0.06	0.43 ± 0.11	1.25 ± 0.07	0.23 ± 0.13	<0.01
C/N Ratio ^c	20.96 ± 0.11	22.13 ± 0.59	16.52 ± 0.13	14.63 ± 0.27	33.13 ± 6.53
Electrical Conductivity (mS cm ⁻¹)	1.59 ± 0.19	1.66 ± 0.20	0.23 ± 0.01	0.83 ± 0.07	1.00 ± 0.14
Calcium Carbonate (%)	0.89 ± 0.02	0.76 ± 0.03	0.54 ± 0.01	0.50 ± 0.01	0.65 ± 0.04
CEC (cmol(+) kg ⁻¹) ^d	13.98 ± 0.94	5.02 ± 0.28	10.47 ± 0.15	4.39 ± 0.14	2.11 ± 0.09
Exchangeable Ca (cmol(+) kg ⁻¹) ^d	19.92 ± 0.29	8.75 ± 0.67	6.33 ± 0.13	5.63 ± 0.23	4.74 ± 0.28
Exchangeable Na (cmol(+) kg ⁻¹) ^d	0.20 ± 0.02	0.09 ± 0.03	0.07 ± 0.01	0.05 ± 0.02	0.05 ± 0.02
Exchangeable Mg $(cmol(+) kg^{-1})^d$	0.68 ± 0.03	0.17 ± 0.01	0.61 ± 0.02	0.14 ± 0.01	0.08 ± 0.01
Exchangeable K (cmol(+) kg ⁻¹) ^d	0.50 ± 0.13	0.29 ± 0.02	0.32 ± 0.03	0.32 ± 0.03	0.17 ± 0.01

Table 6. Mean values (± SE) of physical and chemical soil properties in 2- and 10-year old stockpiled soil supplemented with or without NAG sand (n=12) and NAG sand (n=12).

Samples were collected from test plots during the first growing season (August 2009) with the exception of bulk density (n=4), penetration resistance (n=32) and calcium carbonate equivalent (n=16), which were sampled in August 2010. ^aTotal Organic C = Total organic C – Total inorganic C; ^bSoil Organic Matter = Total Organic C x 2.5 for B horizon (Troeh and Thompson 1993); ^cC/N ratio

= Total Carbon/Total Nitrogen; ^dSamples prepared using Neutral Ammonium Acetate.

Mean concentrations of Mehlich III extractable elements (mg kg⁻¹) differed between 2- and 10-year old stockpiled soil supplemented with or without NAG sand (Table 7). For 2year old stockpiled soil, concentrations of extractable elements were lower in supplemented soil compared to non-supplemented soils with the exception of chromium (Cr), Cu, Fe, P, titanium (Ti) and vanadium (V). For 10-year old stockpiled soil, concentrations of elements were lower in NAG sand supplemented soils, with the exception of cobalt (Co), Cr, Cu, Mn, S, Ti, V and Zn.

Total concentrations of rare earth and trace elements (mg kg⁻¹) differed between 2and 10-year old stockpiled soil supplemented with or without NAG sand (Table 8). In general, total concentrations of rare earth and trace elements were similar to or lower in supplemented 2- and 10-year old soil with the exception of V, erbium (Er), rubidium (Rb) and yttrium (Y). Cesium (Cs), Gallium (Ga) and ytterbium (Yb) were also higher in 10-year old supplemented soil.

In addition, total concentrations of rare earth and trace elements (mg kg⁻¹) within all treatment substrates were found to be lower than concentrations outlined in the Canadian Council of Ministers of the Environment (CCME) Canadian Environmental Quality Guidelines (CEQG) for agricultural land (CCME 2004), with the exception of Cr, Mo, V and Cu (Table 8). When comparing concentrations of these four elements between 2 and 10-year old stockpiled soils supplemented with or without NAG sand, only V was found to be higher in supplemented soils.

Element	2-Ye	ar Soil			
	Soil	Soil + NAG sand	Soil	Soil + NAG sand	NAG sand
Al	1616.46 ± 19.96	836.02 ± 22.90	1955.77 ± 8.88	1041.39 ± 16.82	320.48 ± 7.07
В	0.69 ± 0.01	0.56 ± 0.02	0.45 ± 0.01	0.46 ± 0.01	0.52 ± 0.03
Ba	31.99 ± 2.32	8.46 ± 0.28	36.65 ± 0.51	11.72 ± 0.41	1.61 ± 0.11
Ca	4202.56 ± 130.36	2343.55 ± 125.28	1161.95 ± 18.69	1316.54 ± 21.17	1586.08 ± 82.67
Cd	$0.17 \pm < 0.01$	0.13 ± <0.01	$0.13 \pm < 0.01$	$0.11 \pm < 0.01$	$0.09 \pm < 0.01$
Co	1.07 ± 0.06	0.70 ± 0.01	0.41 ± 0.01	0.63 ± 0.01	$0.36 \pm < 0.01$
Cr	0.19 ± 0.02	0.38 ± 0.01	0.21 ± <0.01	0.38 ± 0.01	0.92 ± 0.02
Cu	32.89 ± 2.18	38.40 ± 1.02	18.41 ± 0.31	39.20 ± 0.85	46.27 ± 1.66
Fe	222.40 ± 2.94	421.59 ± 5.66	162.34 ± 2.22	333.39 ± 5.97	474.56 ± 7.27
K	99.64 ± 2.32	65.04 ± 2.45	89.24 ± 2.39	76.96 ± 1.60	51.99 ± 1.00
Mg	92.73 ± 3.71	50.01 ± 1.54	76.71 ± 2.24	45.62 ± 0.69	64.04 ± 1.47
Mn	104.98 ± 1.34	74.35 ± 2.10	24.99 ± 1.07	38.81 ±0.79	28.76 ± 1.20
Na	32.22 ± 1.42	20.22 ± 1.30	14.75 ± 0.44	15.59 ± 0.64	22.87 ± 0.79
Ni	0.31 ± 0.01	0.34 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.01
P	16.83 ± 1.17	17.16 ± 1.08	13.85 ± 0.82	15.73 ± 0.86	5.86 ± 0.34
Pb	2.68 ± 0.13	1.51 ± 0.05	1.05 ± 0.01	1.02 ± 0.03	0.67 ± 0.02
S	215.88 ± 30.24	159.61 ± 21.59	22.41 ± 1.45	47.44 ± 4.47	59.35 ± 9.37
Ti	1.87 ± 0.19	4.56 ± 0.15	1.75 ± 0.10	5.99 ± 0.07	4.56 ± 0.01
V	0.58 ± 0.01	0.67 ± 0.01	$0.39 \pm < 0.01$	0.64 ± <0.01	0.55 ± 0.01
Zn	2.04 ± 0.07	1.92 ± 0.08	0.87 ± 0.02	1.29 ± 0.04	1.84 ± 0.05

Table 7. Mean values (\pm SE) for the concentration of extractable elements (mg kg⁻¹) in 2- and 10-year old stockpiled soil supplemented with or without NAG sand (n=12), prepared using Mehlich III extraction method and analysed by ICP-MS.

Samples were collected from test plots during the first growing season (August 2009).

Element	Element 2-Year Soil		10-Y	ear Soil		
	Soil	Soil + NAG sand	Soil	Soil + NAG sand	NAG sand	CEQG Standard
Ag	<1 -1	<1 -1	<1 -1	<1 -1	<1 -1	20
Ba	494.00 ± 9.80	300.00 ± 11.10	540.50 ± 7.50	370.30 ± 10.00	255.80 ± 14.60	750
Ce	32.10 ± 1.00	19.70 ± 0.80	36.00 ± 0.60	23.20 ± 0.20	15.70 ± 0.50	
Co	27.20 ± 0.70	14.90 ± 1.10	16.90 ± 0.20	15.20 ± 0.80	12.30 ± 0.40	40
Cr	65.00 ± 5.00	45.00 ± 6.50	67.50 ± 2.50	45.00 ± 2.90	25.00 ± 2.90	64
Cs	8.10 ± 0.10	7.20 ± 0.10	3.80 ± 0.10	6.00 ± 0.10	7.10 ± 0.10	
Cu	529.00 ± 67.51	365.75 ± 44.09	252.63 ± 2.55	270.00 ± 3.12	257.75 ± 16.13	63
Dy	4.16 ± 0.04	4.43 ± 0.04	3.84 ± 0.03	4.44 ± 0.10	4.54 ± 0.20	
Er	2.45 ± 0.03	2.77 ± 0.06	2.31 ± 0.01	2.71 ± 0.04	2.95 ± 0.10	
Eu	1.22 ± 0.02	1.16 ± 0.02	1.18 ± 0.02	1.16 ± 0.02	1.13 ± 0.04	
Ga	15.70 ± 0.30	16.20 ± 0.30	14.30 ± 0.1	15.90 ± 0.10	16.40 ± 0.30	
Gd	4.10 ± 0.10	3.80 ± 0.02	4.10 ± 0.10	3.80 ± 0.10	3.80 ± 0.10	
Hf	2.88 ± 0.08	2.18 ± 0.05	3.35 ± 0.06	2.35 ± 0.03	2.03 ± 0.06	
Но	0.88 ± 0.01	0.95 ± 0.02	0.81 ± 0.002	0.95 ± 0.02	1.00 ± 0.02	
La	15.30 ± 0.50	8.90 ± 0.40	17.00 ± 0.30	10.6 ± 0.20	7.00 ± 0.30	
Lu	0.38 ± 0.01	0.45 ± 0.01	0.35 ± <0.01	0.44 ± <0.01	0.47 ± 0.01	
Мо	28.5 ± 7.60	8.50 ± 1.30	6.30 ± 0.30	6.30 ± 0.30	9.50 ± 3.40	5
Nb	4.63 ± 0.03	2.63 ± 0.18	5.40 ± 0.04	3.15 ± 0.03	1.98 ± 0.05	
Nd	16.90 ± 0.60	11.80 ± 0.20	17.9 ± 0.40	13.30 ± 0.20	10.80 ± 0.30	

Table 8. Mean values (\pm SE) for the total concentration of rare earth and trace elements (mg kg⁻¹) in 2- and 10-year old stockpiled soil supplemented with or without NAG sand (n=4) and NAG sand (n=4) and maximum recommended concentrations in the Canadian Environmental Quality Guidelines (CEQG) outlined by the Canadian Council for the Ministers of Environment for agricultural land (CCME 2004).

Samples were collected from test plots during the first growing season (August 2009).

Samples were prepared using lithium borate fusion followed by acid dissolution and analysed using ICP-MS.

Table 8 Continued						
Element	2-Ye	ear Soil	10-Y	ear Soil		
	Soil	Soil + NAG sand	Soil	Soil + NAG sand	NAG sand	CEQG Standard
Ni	31.30 ± 0.30	27.50 ± 2.90	31.30 ± 0.50	23.00 ± 4.30	35.00 ± 11.60	50
Pb	24.50 ± 0.50	11.50 ± 0.60	13.50 ± 0.30	9.00	7.00 ± 0.40	70
Pr	4.10 ± 0.10	2.70 ± 0.10	4.40 ± 0.10	3.10 ± 0.10	2.20 ± 0.10	
Rb	59.30 ± 1.40	94.80 ± 1.20	44.60 ± 0.20	84.10 ± 0.60	108.50 ± 2.90	
Se	<2	<2	<2	<2	<2	1
Sm	4.00 ± 0.02	3.20 ± 0.10	4.10 ± 0.10	3.50 ± 0.04	3.20 ± 0.02	
Sn	1.00	1.30 ± 0.30	1.00	1.30 ± 0.03	1.30 ± 0.30	5
Sr	236.00 ± 2.20	215.25 ± 2.53	259.25 ± 0.62	237.13 ± 1.78	212.80 ± 6.50	
Ta	0.30	0.20 ± 0.03	0.40	0.20	0.10	
Тb	0.68 ± 0.01	0.66 ± 0.01	0.65 ± 0.001	0.67 ± 0.001	0.68 ± 0.02	
Th	2.88 ± 0.08	1.64 ± 0.06	3.25 ± 0.02	1.94 ± 0.04	1.24 ± 0.02	
Tl	<0.5 - 0.8	<0.5 - 0.8	<0.5 - 0.8	<0.5 - 0.8	<0.5 - 0.8	1
Tm	0.38 ± 0.01	0.42 ± 0.01	0.35 ± <0.01	0.43 ± 0.01	0.44 ± 0.02	
U	1.40 ± 0.05	1.00 ± 0.04	1.69 ± 0.02	1.13 ± 0.01	0.88 ± 0.02	23
v	148.3 ± 2.20	173.0 ± 2.30	124.8 ±0.9	166.0 ± 2.30	190.5 ± 7.10	130
W	6.50 ± 0.50	15.30 ± 3.30	3.00	7.50 ± 0.60	28.00 ± 8.50	
Y	21.90 ± 0.30	24.00 ± 0.20	20.00 ± 0.20	23.40 ± 0.20	24.50 ± 0.80	
Yb	2.47 ± 0.06	2.95 ± 0.02	2.24 ± 0.02	2.81 ± 0.07	2.93 ± 0.11	
Zn	112.38 ± 2.07	75.00 ± 3.60	84.75 ± 0.67	72.75 ± 5.11	67.80 ± 11.30	
Zr	111.50 ± 1.87	75.13 ± 1.06	132.50 ± 6.05	88.38 ± 3.35	65.50 ± 1.80	200

Samples were collected from test plots during the first growing season (August 2009). Samples were prepared using lithium borate fusion followed by acid dissolution and analysed using ICP-MS.

Mean concentrations of base metals differed between 2- and 10-year old stockpiled soil supplemented with or without NAG sand (Table 9). Total concentrations of base metals were lower in supplemented compared to in non-supplemented soils with the exception of Cu; within 10-year old soil, concentrations of Cu were higher in NAG sand supplemented soil. Base metal concentrations were also found to be lower than concentrations outlined in the CEQG for agricultural land (CCME 2004) with the exception of Cu and Mo.

Mean percent composition of major elements reported as oxides for 2- and 10-year old stockpiled soil supplemented with or without NAG sand are shown in Table 10. Percent composition of these oxides were higher in supplemented compared to in non-supplemented soils.

During the second growing season (2010), differences in the level of plant available nutrients between fertilized and unfertilized substrates for 2- and 10-year old stockpiled soil were detected (Table 11). For 10-year old soil, mineralizeable N (mg kg⁻¹) was lower in fertilized compared to in unfertilized soils. Concentrations of K (prepared using Mehlich III and Neutral Ammonium Acetate) and P were higher in fertilized compared to unfertilized soils. Concentrations of B and Mn were higher in fertilized 2-year old soil and B was lower in fertilized 10-year old soil compared to unfertilized soils. Concentrations of Mn in fertilized and unfertilized 10-year old soils did not differ considerably.

Table 9. Mean values (\pm SE) for base metal concentrations (mg kg ⁻¹) in 2- and 10-year old stockpiled soil supplemented with or without NAG sand (n=4) and
NAG sand (n=4) and maximum recommended concentrations in the Canadian Environmental Quality Guidelines (CEQG) outlined by the Canadian Council
for the Ministers of Environment for agricultural land (CCME 2004).

	2-Ye	ear Soil	10-Y	ear Soil		
	Soil	Soil + NAG sand	Soil	Soil + NAG sand	NAG sand	CEQG Standard
Ag	<0.5-0.6	<0.5-0.6	<0.5-0.6	<0.5-0.6	<0.5-0.6	20
As	<5.0	<5.0	<5.0	<5.0	<5.0	12
Со	23.75 ± 0.48	13.75 ± 0.75	14.25 ± 0.25	12.50 ± 0.29	12.30 ± 0.44	40
Cd	<0.5	<0.5	<0.5	<0.5	<0.5	1.4
Cu	560.50 ± 110.99	373.75 ± 78.11	256.75 ± 1.80	275.25 ± 1.03	257.75 ± 16.13	63
Hg	<0.1-1	<0.1-1	< 0.1-1	<0.1-1	<0.1-1	6.6
Mo	15.00 ± 2.58	6.75 ± 1.54	5.00	4.25 ± 0.25	5.00 ± 0.41	5
Ni	22.75 ± 0.25	11.25 ± 0.63	20.50 ± 0.50	12.75 ± 0.25	7.75 ± 0.25	50
Pb	21.75 ± 0.25	8.00 ± 0.41	10.50 ± 0.65	7.25 ± 0.48	5.00 ± 0.41	70
Zn	105.25 ± 4.80	66.00 ± 3.94	72.50 ± 1.55	54.25 ± 1.75	<u>5</u> 7.75 ± 7.65	200

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Samples were collected from test plots during the first growing season (August 2009). Samples were prepared using lithium borate fusion followed by acid dissolution and analysed using ICP-MS.

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	2-Y	ear Soil	10-Y		
	Soil	Soil + NAG sand	Soil	Soil + NAG sand	NAG sand
BaO	<0.01 - 0.06	<0.01 - 0.06	< 0.01 - 0.06	<0.01 - 0.06	< 0.01 - 0.06
SiO ₂	57.55 ± 0.57	59.48 ± 0.28	61.50 ± 0.27	61.53 ± 0.09	60.8 ± 0.50
Al ₂ O ₃	14.58 ± 0.14	15.50 ± 0.15	14.05 ± 0.06	15.13 ± 0.03	15.45 ± 0.20
Fe ₂ O ₃	8.18 ± 0.04	8.69 ± 0.06	6.30 ± 0.05	8.00 ± 0.05	9.00 ± 0.20
CaO	2.58 ± 0.07	3.96 ± 0.10	2.05 ± 0.03	3.60 ± 0.04	4.48 ± 0.03
Cr ₂ O ₃	<0.1	<0.1	<0.1	<0.1	<0.1
MgO	2.16 ± 0.03	2.24 ± 0.01	1.70 ± 0.01	2.15 ± 0.01	2.32 ± 0.02
Na ₂ O	2.09 ± 0.05	2.81 ± 0.05	2.60 ± 0.02	2.93 ± 0.01	3.05 ± 0.06
K ₂ O	1.42 ± 0.02	1.57 ± 0.01	1.40 ± 0.01	1.56 ± 0.01	1.68 ± 0.01
TiO ₂	0.71 ± <0.01	0.85 ± 0.01	0.71 ± 0.01	$0.84 \pm < 0.01$	0.92 ± 0.01
MnO	0.14	0.13	0.10 ± <0.01	$0.12 \pm < 0.01$	$0.13 \pm < 0.01$
P_2O_5	0.18 ± 0.01	0.24 ± 0.02	0.18 ± 0.01	0.21 ± 0.01	0.28 ± 0.03
Loi ^a	9.94 ± 0.43	4.13 ± 0.41	9.37 ± 0.29	3.94 ± 0.26	1.77 ± 0.21

Table 10. Mean values (\pm SE) for the percent composition of major elements reported as oxides in 2- and 10-year old stockpiled soil supplemented with or without NAG sand (n=4) and NAG sand (n=4). Oxide concentrations were calculated from the determined elemental concentration. _

Samples were collected from test plots during the first growing season (August 2009). Samples were prepared using lithium borate fusion followed by acid dissolution and analysed using ICP-AES. ^aLoss on ignition at 1000°C.

	2-Ye	ear Soil	10-Year Soil		
	Soil	Soil + Fertilizer	Soil	Soil + Fertilizer	
pH (water)	7.08 ± 0.05	7.03 ± 0.07	6.35 ± 0.16	6.31 ± 0.16	
Nitrate-N (mg kg ⁻¹)	1.37 ± 0.25	1.48 ± 0.40	1.64 ± 0.43	1.06 ± 0.31	
Ammonium-N (mg kg ⁻¹)	0.68 ± 0.11	0.90 ± 0.16	1.20 ± 0.21	1.20 ± 0.20	
Mineralizeable Nitrogen (mg kg ⁻¹)	7.83 ± 0.49	8.60 ± 0.85	9.02 ± 0.53	7.91 ± 0.51	
Total Carbon (%)	0.85 ± 08	0.94 ± 0.10	1.00 ± 0.11	1.00 ± 0.10	
Total Nitrogen (%)	$0.04 \pm < 0.01$	0.04 ± <0.01	$0.06 \pm < 0.01$	0.06 ± <0.01	
Total Sulphur (%)	0.33 ± 0.02	0.33 ± 0.02	0.09 ± 0.01	0.09 ± 0.01	
CEC (cmol(+)/kg) ^c	9.29 ± 1.04	8.73 ± 0.85	5.64 ± 0.44	5.69 ± 0.44	
Exchangeable Ca (cmol(+)/kg) ^c	14.16 ± 1.21	14.77 ± 1.34	6.55 ± 0.16	6.45 ± 0.14	
Exchangeable Na (cmol(+)/kg) ^c	0.12 ± 0.01	0.10 ± 0.01	$0.04 \pm < 0.01$	0.04 ± <0.01	
Exchangeable Mg (cmol(+)/kg) ^c	0.17 ± 0.04	0.18 ± 0.04	0.27 ± 0.06	0.25 ± <0.01	
Exchangeable K (cmol(+)/kg) ^c	0.28 ± 0.01	0.29 + 0.01	$0.23 \pm < 0.01$	0.26 ± <0.01	
Boron (mg kg ⁻¹) ^b	$0.02 \pm < 0.01$	$0.04 \pm < 0.01$	$0.05 \pm < 0.01$	0.03 ± <0.01	
Calcium (mg kg ⁻¹) ^b	4374.56 ± 149.05	4030.57 ± 208.49	1188.06 ± 24.56	1135.85 ± 27.09	
Copper (mg kg ⁻¹) ^b	35.73 ± 1.24	33.19 ± 1.54	26.42 ± 1.98	25.26 ± 1.65	
Iron (mg kg ⁻¹) ^b	214.20 ± 3.20	230.60 ± 3.70	163.91 ± 2.71	160.77 ± 3.58	
Phosphorus (mg kg ⁻¹) ^a	4.58 ± 0.32	7.98 ± 0.56	3.21 ± 0.13	5.39 ± 0.27	
Potassium (mg kg ⁻¹) ^b	86.44 ± 2.86	90.84 ± 2.83	80.05 ± 1.57	84.68 ± 1.56	
Magnesium (mg kg ⁻¹) ^b	59.11 ± 4.36	55.03 ± 4.37	52.07 ± 3.96	52.35 ± 3.89	
Manganese (mg kg ⁻¹) ^b	75.29 ± 3.36	66.63 ± 2.75	26.75 ± 1.45	26.19 ± 1.35	
Sulphur (mg kg ⁻¹) ^b	215.48 ± 45.59	216.27 ± 41.79	22.41 ± 1.45	19.80 ± 0.80	
Zinc $(mg kg^{-1})^{b}$	1.82 ± 0.09	1.86 ± 0.07	1.20 ± 0.10	1.19 ± 0.13	

Table 11. Mean values (± SE) of chemical soil properties of 2- and 10-year old stockpiled soil with or without the addition of fertilizer. Samples were collected from test plots during the second growing season (August 2010).

^aOlsen's extraction method; ^bMehlich III extraction; ^cNeutral Ammonium Acetate.

3.2 Plant Growth

3.2.1 Non-destructive Measurements

The addition of NAG sand had no significant effect on seeding density (seedlings m⁻²) on 2year old stockpiled soil test plots (Table 12); however, significant effects were observed with fertilizer and seeding treatment. The addition of fertilizer significantly increased seedling density from 226 ± 37 seedling m⁻² to 333 ± 37 seedlings m⁻² (*p*=0.0007). Seedling density differed significantly among single-species seeding treatments (*p*<0.0001) with the highest value for genetically diverse blue wildrye and lowest for arctic lupine; seedling density of genetically diverse blue wildrye was also significantly higher than that of locally collected blue wildrye seed (Figure 12). No seedlings of Merten's sedge were detected on 2-year old soil.

The addition of NAG sand had no significant effect on seeding density on 10-year old stockpiled soil test plots (Table 12). Significant effects were observed with fertilizer and seeding treatment. The addition of fertilizer significantly increased seedling density from 243 ± 38 seedling m⁻² to 322 ± 41 seedlings m⁻² (p<0.0104). Seedling density differed significantly among single-species seeding treatments (p<0.0001) with the highest value for genetically diverse blue wildrye and lowest for arctic lupine (Figure 12). No seedlings of Merten's sedge were detected on 10-year old soil.

Table 12. Summary of linear mixed-effects model results for percent emergence, seedling density, percent cover and plant height (single-species treatments) collected from 2- and 10-year old stockpiled soil test plots in September 2010. For each of the four response variables (columns), the details of the minimal adequate models are listed in the rows, with explanatory variables and their interactions (first column) retained in the models, their corresponding F- and p-values, plus the number of observations and the minimum model AIC values. Bold font indicates significant p-values.

Predictor Variable	Seedling	Density ^a	Emerg	ence ^a	Percent (Cover	Plant H	eight ^a
2-year Soil								
NAG Sand	$F_{1,55}=0.0193$	p=0.8901	$F_{1,55}=0.2163$	<i>p</i> =0.6437	$F_{1,100}=0.1426$	<i>p</i> =0.7065	$F_{1,56}=0.3860$	p=0.5369
Fertilizer	$F_{1,55}=13.06$	<i>p=</i> 0.0007	$F_{1,55}=13.22$	<i>p=</i> 0.0006	$F_{1,100}=34.26$	<i>p</i> <0.0001	$F_{1.56}=30.65$	<i>p</i> <0.0001
Seeding Treatment	$F_{3,55}=60.62$	<i>p</i> <0.0001	$F_{3,55}=79.16$	<i>p</i> <0.0001	$F_{6,100}=9.980$	<i>p</i> <0.0001	$F_{4,56}=10.18$	<i>p<</i> 0.0001
NAG sand X Fertilizer	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
NAG sand X Seeding Treatment	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
Fertilizer X Seeding Treatment	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	$F_{4,56}=2.884$	P=0.0303
NAG sand X Fertilizer X Seeding	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
Treatment								
Number of Observations	64	ļ	64	ļ	112		71	
AIC of maximum model	807	.3	275	.6	891.3	2	280	.9
AIC of minimum model	805	.5	266	.5	870.1	7	263	.8
10-Year Soil								
NAG sand	$F_{1,ss}=0.6869$	p=0.4108	$F_{1,0}=1.176$	p=0.2831	$F_{1,99}=0.0066$	p=0.9356	$F_{1,64}=0.7399$	p=0.3929
Fertilizer	$F_{1.55} = 7.035$	p=0.0104	$F_{1,51} = 10.79$	p=0.0018	$F_{1.99}=23.22$	p<0.0001	$F_{1.64} = 19.86$	p<0.0001
Seeding Treatment	$F_{3.55}=77.23$	p<0.0001	$F_{1,51}=88.70$	p<0.0001	$F_{6.00} = 18.06$	p<0.0001	$F_{4.64}=24.82$	p<0.0001
NAG sand X Fertilizer	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
NAG sand X Seeding Treatment	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
Fertilizer X Seeding Treatment	Excluded	Excluded	$F_{1,s1}=3.829$	p=0.0149	Excluded	Excluded	Excluded	Excluded
NAG sand X Fertilizer X Seeding	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
Treatment								
Number of Observations	64	ļ	64	•	111		74	
AIC of maximum model	372.3	317	269.1	764	900.30	47	266.5	264
AIC of minimum model	367.7	658	262.5	318	892.6	85	249.2	311

^asquare root; AIC, Akaike information criterion; 'excluded' indicates terms excluded during model simplification (stepwise approach).





Figure 12. Overall seeding treatment effects on mean values (\pm SE) of seedling density (seedlings m⁻²) across all substrate treatments in 2-year (above) and 10-year (below) old stockpiled soil. Single-species seeding treatments include seeds obtained from genetically diverse seed increase plots (ecovars) of fringed brome (BROMCIL), blue wildrye (ELYMGLA) and seeds collected from the local area of the minesite (local) of blue wildrye and arctic lupine (LUPIARC). Bars which share a common letter are not significantly different (p<0.05).

In both 2- and 10-year old stockpiled soil test plots, the highest seedling densities for single-species seeding treatments were observed in soils where fertilizer and fertilizer + NAG sand treatments were applied. In 2-year old soil test plots, seedling density was similar between fertilizer and fertilizer + NAG sand treated soil, however, in 10-year old soil, the mean value of seedling density in the fertilizer + NAG sand treated soils was slightly higher compared to seedling density in soil treated with just fertilizer.

The addition of NAG sand had no significant effect on percent emergence (%) on the 2-year old stockpiled soil test plots; however, significant effects on percent emergence were observed with fertilizer and seeding treatment (Table 12). The addition of fertilizer significantly increased percent emergence from 43 ± 8 % to 63 ± 8 % (*p*=0.0006). Percent emergence differed significantly among single-species seeding treatments (*p*<0.0001) with the highest value for genetically diverse blue wildrye and lowest for arctic lupine. Percent emergence of genetically diverse blue wildrye was significantly higher than that of locally collected blue wildrye seed (Figure 13).

The addition of NAG sand had no significant effect on percent emergence on the 10year old stockpiled soil test plots; however, there was a significant interaction between seeding treatment and fertilizer for percent emergence (p=0.0149; Table 12). Percent emergence for all seeding treatments increased in response to the addition of fertilizer, most notably for the genetically diverse fringed brome and blue wildrye seeding treatments (Figure 13).

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Figure 13. Overall seeding treatment effects on mean values (\pm SE) of emergence (%) across all substrate treatments in 2-year (above) and 10-year (below) old stockpiled soil. Single-species seeding treatments include seeds obtained from genetically diverse seed increase plots (ecovars) of fringed brome (BROMCIL), blue wildrye (ELYMGLA) and seeds collected from the local area of the minesite (local) of blue wildrye and arctic lupine (LUPIARC). For 2-year old soil (above), bars which share a common letter are not significantly different (p<0.05).

In both 2- and 10-year old stockpiled soil test plots, the highest mean percent emergence for single-species seeding treatments were observed in soils treated with fertilizer and fertilizer + NAG sand, with the exception of arctic lupine in 10-year old soil. In both 2and 10-year old soil test plots, mean percent emergence in the fertilizer + NAG sand treated soils was slightly higher compared to percent emergence in soil trated with just fertilizer for most seeding treatments.

The addition of NAG sand had no significant effect on percent cover (%) on the 2year old stockpiled soil test plots; however, significant effects were observed with fertilizer and seeding treatment (Table 12). The addition of fertilizer significantly increased percent cover of seeding treatments from 12.6 ± 1.3 % to 24.8 ± 1.3 % (p<0.0001). Percent cover significantly differed among seeding treatments (p<0.0001) with the highest value for genetically diverse blue wildrye and lowest for fringed brome (Figure 14).

The addition of NAG sand had no significant effect on percent cover (%) on the 10year old stockpiled soil test plots; however, significant effects were observed with fertilizer and seeding treatment (Table 12). The addition of fertilizer significantly increased percent cover of seeding treatments from 24.4 ± 1.8 % to 33.2 ± 1.8 % (p<0.0001). Percent cover differed significantly among single-species seeding treatments (p<0.0001) with the highest value for genetically diverse blue wildrye and lowest for arctic lupine (Figure 14). Blue wildrye (mixed genotype) had significantly higher mean percent cover compared to the locally collected blue wildrye (Figure 14).

In both 2- and 10-year old stockpiled soil test plots, the highest percent cover values for seeding treatments were observed in soils treated with fertilizer and fertilizer + NAG

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sand. There was no considerable difference in mean values between the two substrate treatments in either soil.

In the 2-year old stockpiled soil test plots, the addition of NAG sand had no significant effect on plant height (cm) for single-species seeding treatments; however, there was a significant interaction between seeding treatments and fertilizer (p=0.0303; Table 12). Mean plant height for all seeding treatments increased in response to the addition of fertilizer, most notably for fringed brome and Rocky Mountain fescue (Figure 15).

The addition of NAG sand had no significant effect plant height for single-species seeding treatments on the 10-year old stockpiled soil test plots; however, significant effects were observed with fertilizer and seeding treatment (Table 12). The addition of fertilizer significantly increased mean plant height from 26.6 ± 2.4 cm to 39.2 ± 2.6 cm (p<0.0001). Plant height significantly differed among seeding treatments (p<0.0001) with the highest values for genetically diverse blue wildrye and the lowest for Rocky Mountain fescue and arctic lupine. Genetically diverse blue wildrye had significantly higher mean plant height compared to the locally collected blue wildrye (Figure 15).

In both 2- and 10-year old stockpiled soil test plots, the highest mean plant height for single-species seeding treatments were observed in soils treated with fertilizer and fertilizer + NAG sand. In 2-year old soil, the mean value of plant height in the fertilizer + NAG sand treated soils was slightly higher than values observed in plots with just the fertilizer treatment. No considerable difference in mean plant height of seeding treatments was visible between fertilizer and fertilizer + NAG sand substrate treatments in 10-year old soil.

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Figure 14. Overall seeding treatment effects on mean values (\pm SE) of plant cover (%) across all substrate treatments in 2-year (above) and 10-year (below) old stockpiled soil. Single-species seeding treatments include seeds obtained from genetically diverse seed increase plots (ecovars) of fringed brome (BROMCIL), blue wildrye (ELYMGLA) and Rocky Mountain fescue (FESTSAX) or seeds collected from the local area of the minesite (local) of blue wildrye and arctic lupine (LUPIARC). Mixed seeding treatments include an ecovar mix (blue wildrye, fringed brome and Rocky Mountain fescue) and a local mix (blue wildrye, arctic lupine and Merten's sedge (*Carex mertensii*). No comparisons between single and mixed-species seed treatments were made. Bars which share a common letter are not significantly different (p<0.05).

2-Year Soil







Figure 15. Overall species effects on mean values (±SE) of plant height (cm) for single-species seeding treatments across all substrate treatments in 2-year (above) and 10-year (below) old stockpiled soil. Singlespecies seeding treatments include seeds obtained from genetically diverse seed increase plots (ecovars) of fringed brome (BROMCIL), blue wildrye (ELYMGLA) and Rocky Mountain fescue (FESTSAX) and seeds collected from the local area of the minesite (local) of blue wildrye and arctic lupine (LUPIARC). For 10 year old soil (below), bars which share a common letter are not significantly different (p < 0.05).

For mixed-species seeding treatments in 2-year old stockpiled soil test plots, the addition of NAG sand had no significant effect on plant height; however, significant effects were observed with fertilizer and seeding treatment (Table 13). Fertilizer significantly increased mean plant height from 23.8 ± 4.0 cm to 43.2 ± 4.3 cm (p<0.0001). Mean height for plant species differed significantly with the highest values for genetically diverse and locally collected blue wildrye and the lowest for arctic lupine (p=0.0091; Figure 16).

The addition of NAG sand had no significant effect on plant height for mixed-species seeding treatments on the 10-year old stockpiled soil test plots; however, significant effects were observed with fertilizer and seeding treatment (Table 13). Fertilizer significantly increased mean plant height from 41.8 ± 4.2 cm to 53.1 ± 4.3 cm (p=0.0001). Mean plant height among species within mixed-species seeding treatments differed significantly (p<0.0001); the highest value was for genetically diverse blue wildrye and the lowest was for arctic lupine (Figure 16). Genetically diverse blue wildrye had significantly higher mean plant height compared to the locally collected blue wildrye (Figure 16).

Table 13. Summary of linear mixed-effects model results for plant height for mixed-species treatments collected from 2- and 10-year old stockpiled soil test plots in September 2010. Details of the minimal adequate models are listed in the rows, with explanatory variables and their interactions (first column) retained in the models, their corresponding F- and p-values, plus the number of observations and the minimum and maximum model AIC values (bottom rows). Bold font indicates significant p-values.

Predictor Variable	Plant height ^a		
2-Year Soil			
NAG Sand	$F_{1,37}=2.201$	p=0.1460	
Fertilizer	$F_{1,37}=42.75$	<i>p</i> <0.0001	
Species	F3.37=3.966	<i>p</i> =0.0091	
NAG sand X Fertilizer	Excluded	Excluded	
NAG sand X Seeding Treatment	Excluded	Excluded	
Fertilizer X Seeding Treatment	Excluded	Excluded	
NAG sand X Fertilizer X Seeding Treatment	Excluded	Excluded	
Number of Observations	4	6	
AIC of maximum model	166	5.57	
AIC of minimum model	162	2.57	
10-Year Soil			
NAG sand	$F_{1,45}=2.082$	<i>p</i> =0.1561	
Fertilizer	$F_{1,45}=18.66$	<i>p</i> =0.0001	
Species	$F_{3,45}=36.70$	<i>p</i> <0.0001	
NAG sand X Fertilizer	Excluded	Excluded	
NAG sand X Seeding Treatment	Excluded	Excluded	
Fertilizer X Seeding Treatment	Excluded	Excluded	
NAG sand X Fertilizer X Seeding Treatment	Excluded	Excluded	
Number of Observations	5	4	
AIC of maximum model	442	2.60	
AIC of minimum model	438	3.60	

^asquare root transformed; AIC, Akaike information criterion; 'excluded' indicates terms excluded during model simplification (stepwise approach).

In both 2- and 10-year old stockpiled soil test plots, the highest mean plant heights for species in mixed seeding treatments were observed in soils treated with fertilizer and fertilizer + NAG sand. In 2-year old soil test plots, mean plant height for species was higher in soil treated with fertilizer compared to soil treated with fertilizer + NAG sand (with the exception of fringed brome). However, in 10-year old soil, the mean value of plant height for species seeded in the fertilizer + NAG sand treated soils was slightly higher compared to mean plant height in soil treated with just fertilizer.



Figure 16. Overall species effects on mean values (\pm SE) of plant height (cm) for mixed-species seeding treatments across all substrate treatments in 2-year (above) and 10-year (below) old stockpiled soil. Species in mixed-species seeding treatments include seeds obtained from genetically diverse seed increase plots (ecovars) of fringed brome (BROMCIL), blue wildrye (ELYMGLA) and Rocky Mountain fescue (FESTSAX) and seeds collected from the local area of the minesite (local) of blue wildrye and arctic lupine (LUPIARC). Bars which share a common letter are not significantly different (p<0.05).

Table 14. Summary of linear mixed-effects model results for percent cover per seedling (% seedling⁻¹) calculated using cover and seedling density collected from 2- and 10-year old stockpiled soil test plots in September 2010. Details of the minimal adequate models are listed in the rows, with explanatory variables and their interactions (first column) retained in the models, their corresponding F- and p-values, plus the number of observations and the minimum and maximum model AIC values. Bold font indicates significant p-values.

Predictor variable	Percent Cover per Seeding			
2-Year Soil				
NAG Sand	$F_{1.55}=0.0571$	<i>p</i> =0.8121		
Fertilizer	$F_{1,55}=1.901$	<i>p</i> =0.1735		
Seeding Treatment	$F_{3,55}=1.101$	<i>p</i> =0.3567		
NAG sand X Fertilizer	Excluded	Excluded		
NAG sand X Seeding Treatment	Excluded	Excluded		
Fertilizer X Seeding Treatment	Excluded	Excluded		
NAG sand X Fertilizer X Seeding	Excluded	Excluded		
Treatment				
Number of Observations		64		
AIC of maximum model	-4	.868		
AIC of minimum model	-19	9.13		
10-Year Soil				
NAG sand	$F_{1,54} = 6.640$	<i>p</i> =0.0129		
Fertilizer	$F_{1,54}=0.0501$	p=0.8238		
Seeding Treatment	$F_{3.54} = 5.713$	<i>p</i> <0.0019		
NAG sand X Fertilizer	Excluded	Excluded		
NAG sand X Seeding Treatment	$F_{3,53}=5.141$	<i>p</i> =0.0035		
Fertilizer X Seeding Treatment	Excluded	Excluded		
NAG sand X Fertilizer X Seeding	Excluded	Excluded		
Treatment				
Number of Observations		63		
AIC of maximum model	-12	35.3		
AIC of minimum model	-14	49.8		

^asquare root; AIC, Akaike information criterion; 'excluded' indicates terms excluded during model simplification (stepwise approach).

NAG sand, fertilizer and seeding treatment had no significant effects on average plant size (expressed as mean cover per seedling) in 2-year old stockpiled soil test plots. However, in the 10-year old stockpiled soil test plots, there was a significant interaction between NAG sand and seeding treatment for cover per seedling (p=0.0035; Table 14). Mean cover per seedling decreased in response to the addition of NAG sand for arctic lupine (Figure 17).



Figure 17. Overall species effects on mean values (\pm SE) of cover per seedling (% seedling⁻¹) for single-species seeding treatments across all substrate treatments in 10-year old stockpiled soil. Single-species seeding treatments include seeds obtained from genetically diverse seed increase plots (ecovars) of fringed brome (BROMCIL), blue wildrye (ELYMGLA) and seeds collected from the local area of the minesite (local) of blue wildrye and arctic lupine (LUPIARC).

In 2-year old stockpiled soil test plots, mean values of cover per seedling for singlespecies seeding treatments was the highest in fertilizer + NAG sand treated soils. However, in 10-year old soil test plots, mean values of cover per seedling for seeding treatments did not differ considerably between substrate treatments, with the exception of arctic lupine where cover per seedling considerably decreased with the addition of NAG sand.

In NAG sand test plots (i.e., with no fertilizer or soil), seeding treatments had a significant effect on percent emergence (Table 15; p=0.0202) and seedling density (p=0.0344). The highest percent emergence and seedling densities on NAG sand test plots were found for genetically diverse blue wildrye and the lowest were for fringed brome (Figure 18). Seeding treatments had no significant effect on percent cover or plant height (for both single and mixed seeding treatments) on NAG sand substrates (Tables 15, 16).

Seeding treatment in NAG sand test plots had no significant effect on cover per seedling (% seedling⁻¹; Table 16).

Nodules of *Rhizobium* were detected on the roots of arctic lupine seedlings when they were first collected from the test plots. However, due to difficulties identifying nodulation on dried samples, the number of inoculated seedlings and the number of nodules per seedling was not recorded.

Table 15. Summary of linear mixed-effects model results for emergence, seedling density, percent cover and plant height (single-species treatments) collected from NAG sand test plots in September 2010. Details of the minimal adequate models are listed in the rows, with explanatory variables and their interactions (first column) retained in the models, their corresponding F- and p-values, plus the number of observations (bottom row). Bold font indicates significant p-values.

Predictor Variable	Eme	rgence ^a	Seedling Density ^a		
NAG Sand Seeding Treatment Number of Observations	F _{6,8} =5.489	р=0.0202 16	F _{6.8} =4.495	<i>p=</i> 0.0344	
Predictor Variable	Percent Cover		Plant Height		
NAG Sand					
Seeding Treatment	$F_{6,18} = 1.678$	<i>p</i> =0.1744	F _{2,2} =0.9697	p=0.3757	
Number of Observations	26		66		

^asquare root transformed.

Table 16. Summary of linear mixed-effects model results for plant height (mixed-species treatments) and percent cover per seedling (% seedling⁻¹), calculated using cover and seedling density, collected from NAG sand testplots in September 2010. Details of the minimal adequate model are listed in the rows, with explanatory variables and their interactions (first column) retained in the models, their corresponding F- and P-values, plus the number of observations (bottom rows).

Predictor Variable	Plant	Height	Percent Cover per Seedling		
NAG Sand Seeding Treatment	F _{1.2} =7.861	p=0.2181	F _{3,11} =0.6809	p=0.5856	-
Number of Observations		5		0	



Figure 18. Overall seeding treatment effects on mean values (\pm SE) of emergence (%; above) and seedling density (seedlings m⁻²; below) in NAG sand. Single-species seeding treatments include seeds obtained from genetically diverse seed increase plots (ecovars) of blue wildrye (ELYMGLA) and seeds collected from the local area of the minesite (local) of blue wildrye and arctic lupine (LUPIARC). Bars which share a common letter are not significantly different (p<0.05).

3.2.2 Shoot and Root Biomass

The addition of NAG sand to 2-year old stockpiled soil had no significant effect on aboveground biomass (g m⁻²); however, significant effects were observed with fertilizer and seeding treatment (Table 17). The addition of fertilizer significantly increased aboveground biomass after two growing seasons from 16.59 ± 4.72 g m⁻² to 67.61 ± 4.45 g m⁻² (p<0.0001). Aboveground biomass differed significantly among single-species seeding treatments (p=0.0001) with the highest value for genetically diverse blue wildrye and lowest for fringed brome; a comparion of mixed-species seeding treatments showed no significant difference (Figure 19). Genetically diverse blue wildrye had significantly higher aboveground biomass compared to locally collected blue wildrye (Figure 19).

Within 10-year old stockpiled soil test plots, there were significant interactions between seeding treatment and NAG sand (p=0.0072) and seeding treatment and fertilizer (p=0.0003) for aboveground biomass (Table 17). In response to the NAG sand supplement, all seeding treatments increased in aboveground biomass compared to their performance on non-supplemented soils (except for arctic lupine and the local native seed mix); Rocky Mountain fescue increased considerably more than the other seeding treatments. The interaction between seeding treatment and fertilizer showed increases in aboveground biomass for all seeding treatments in response to fertililzer with the exception of arctic lupine (Figure 19). Table 17. Summary of linear mixed-effects model results for aboveground biomass, estimated belowground biomass and shoot:root ratio collected from 2and 10-year old stockpiled soil test plots in September 2010. For each of the three response variables (columns), the details of the minimal adequate models are listed in the rows, with explanatory variables and their interactions (first column) retained in the models, their corresponding F- and p-values, plus the number of observations and the minimum and maximum model AIC values (bottom rows). Bold font indicates significant p-values.

Predictor Variable	Aboveground Biomass ^a		Shoot:Root Ratio ^a		Estimated Belowground Biomass ^a	
2-Year Soil						
NAG Sand	$F_{1,100}=2.893$	<i>p</i> =0.0921	$F_{1,62}=4.046$	p=0.0486	$F_{1.64} = 7.408$	<i>p</i> =0.0083
Fertilizer	$F_{1,100} = 52.24$	p<0.0001	$F_{1,62}=13.84$	<i>p</i> =0.0004	$F_{1,64} = 9.000$	p<0.0001
Seeding Treatment	$F_{6,100}$ =4.665	p=0.0003	$F_{4,62}=29.71$	<i>p<</i> 0.0001	F _{4.64} =7.852	<i>p</i> =0.0038
NAG sand X Fertilizer	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
NAG sand X Seeding Treatment	Excluded	Excluded	Excluded	Excluded	$F_{4,64}=2.595$	p=0.0444
Fertilizer X Sceding Treatment	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
NAG sand X Fertilizer X Seeding Treatment	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
Number of Observations	112		72		79	
AIC of maximum model	616.3		110.5		421.6	
AIC of minimum model	588.9		88.07		403.7	
10-Year Soil						
NAG sand	$F_{1,86}=1.132$	p=0.2903	$F_{1.65} = 11.04$	p=0.0015	$F_{1.64} = 1.432$	p=0.2358
Fertilizer	$F_{1.86} = 87.95$	p=0.0001	$F_{1.65} = 3.182$	p=0.0791	$F_{1.64} = 19.53$	p<0.0001
Seeding Treatment	$F_{6.86}=22.51$	p < 0.0001	$F_{4.65} = 48.71$	p<0.0001	$F_{4.64} = 5.217$	p=0.0010
NAG sand X Fertilizer	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
NAG sand X Seeding Treatment	$F_{6.86}=3.176$	p = 0.0072	Excluded	Excluded	F _{4.64} =7.282	p=0.0001
Fertilizer X Seeding Treatment	$F_{6.86}$ =4.703	p=0.0003	Excluded	Excluded	Excluded	Excluded
NAG sand X Fertilizer X Seeding Treatment	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
Number of Observations	112		75		79	
AIC of maximum model	532.5		85.57		306.2	
AIC of minimum model	525.4		66.14		307.1	

^asquare root transformed; AIC, Akaike information criterion; 'excluded' indicates terms excluded during model simplification (stepwise approach).





Figure 19. Overall seeding treatment effects on mean values (\pm SE) of aboveground biomass (g m⁻²) across all substrate treatments in 2-year (above) and 10-year (below) old stockpiled soil. Single-species seeding treatments include seeds obtained from genetically diverse seed increase plots (ecovars) of fringed brome (BROMCIL), blue wildrye (ELYMGLA) and Rocky Mountain fescue (FESTSAX) or seeds collected from the local area of the minesite (local) of blue wildrye and arctic lupine (LUPIARC). Mixed seeding treatments include an ecovar mix (blue wildrye, fringed brome and Rocky Mountain fescue) and a local mix (blue wildrye, arctic lupine and Merten's sedge (*Carex mertensii*)). No comparisons between single and mixed-species seed treatments were made. For 2-year old soil (above), bars which share a common letter are not significantly different (p<0.05).

In both 2- and 10-year old stockpiled soil test plots, the highest mean aboveground biomass for seeding treatments was observed in soils treated with fertilizer and fertilizer + NAG sand. When comparing the two treatments, the mean aboveground biomass for seeding treatments in both soils was considerably higher in soils treated with fertilizer + NAG sand (with the exception of arctic lupine and the local native mix seeding treatments in 10-year old soil).

Within 2-year old stockpiled soil test plots, fertilizer had a significant effect on estimated belowground biomass and there was a significant interaction between seeding treatment and NAG sand substrate treatment (Table 17). Fertilizer significantly increased estimated belowground biomass from 4.10 ± 3.55 g m⁻² to 14.89 ± 3.22 g m⁻² (p<0.0001; Figure 20). The interaction between seeding treatment and NAG sand substrate treatment showed an increase in estimated belowground biomass in response to the addition of NAG sand for all seeding treatments with a significant increase for arctic lupine (p=0.0001; Figure 20).

Fertilizer had a significant effect on estimated belowground biomass on 10-year old stockpiled soil test plots and there was a significant interaction between seeding treatment and NAG sand (Table 17). Fertilizer significantly increased estimated belowground biomass from 11.56 ± 2.18 g m⁻² to 22.77 ± 3.07 g m⁻² (p<0.0001). The interaction between the NAG sand and seeding treatments showed an increase in estimated belowground biomass for all seeding treatments on soil supplemented with NAG sand, compared to those of non-supplemented soil, with the exception of arctic lupine, which showed the opposite trend (p<0.0001; Figure 20).

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Figure 20. Overall seeding treatment effects on mean values (\pm SE) of estimated belowground biomass (g m⁻²) across all substrate treatments in 2-year (above) and 10-year (below) old stockpiled soil. Single-species seeding treatments include seeds obtained from genetically diverse seed increase plots (ecovars) of fringed brome (BROMCIL), blue wildrye (ELYMGLA) and Rocky Mountain fescue (FESTSAX) or seeds collected from the local area of the minesite (local) of blue wildrye and arctic lupine (LUPIARC).

In both 2- and 10-year old stockpiled soil test plots, the highest mean estimated belowground biomass for single-species seeding treatments was observed in soils treated with fertilizer and fertilizer + NAG sand. When comparing the two treatments, the mean estimated belowground biomass for most seeding treatments in both soils was considerably higher in soils treated with fertilizer + NAG sand (with the exception of locally collected blue wildrye and arctic lupine seeding treatments in 10-year old soil).

For 2-year old stockpiled soil test plots, NAG sand, fertilizer and seeding treatments had significant effects on the shoot:root ratio (Table 17). The addition of NAG sand significantly decreased the shoot:root ratio from 4.25 ± 0.34 to 3.53 ± 0.35 (p=0.0486). Fertilizer significantly increased the shoot:root ratio from 4.25 ± 0.34 to 5.69 ± 0.37 (p=0.0001). Significant differences in the shoot:root ratio were detected among single-species seeding treatments (p<0.0001) with the highest values for fringed brome and locally collected blue wildrye and the lowest for arctic lupine (Figure 21). Genetically diverse blue wildrye (Figure 21).

NAG sand supplement and seeding treatments had significant effects on the shoot:root ratio in 10-year old stockpiled soil test plots (Table 17). The addition of NAG sand significantly increased the shoot root ratio from 4.89 ± 0.31 to 5.63 ± 0.32 (p=0.0015). Significant differences for the shoot root ratio were detected among single-species seeding treatments (p<0.0001) with arctic lupine values significantly lower than all other single-species seeding treatments (Figure 21).





Figure 21. Overall seeding treatment effects on mean values (\pm SE) of the shoot:root ratio across all substrate treatments in 2-year (above) and 10-year (below) old stockpiled soil. Single-species seeding treatments include seeds obtained from genetically diverse seed increase plots (ecovars) of fringed brome (BROMCIL), blue wildrye (ELYMGLA) and Rocky Mountain fescue (FESTSAX) or seeds collected from the local area of the minesite (local) of blue wildrye and arctic lupine (LUPIARC). No comparisons between single and mixed-species seed treatments were made. Bars which share a common letter are not significantly different (p<0.05).

In both 2- and 10-year old stockpiled soil test plots, the highest mean shoot:root ratio for single-species seeding treatments was observed in soils treated with fertilizer and fertilizer + NAG sand. In 2-year old soil, the mean shoot:root ratio was higher in fertilized soil compared to soils treated with both fertilizer + NAG sand; however, the opposite trend was observed in 10-year old soil.

For 2-year old stockpiled soil test plots, the addition of NAG sand had no significant effect on the average aboveground biomass per seedling (g seedling⁻¹); however, a significant effect was observed for fertilizer (Table 18). Fertilizer significantly increased the aboveground biomass per seedling from 0.12 ± 0.02 to 0.29 ± 0.02 (*p*=0.0056).

In the 10-year old stockpiled soil test plots, fertilizer had significant effect on the aboveground biomass per seedling and there was a significant interaction between NAG sand and seeding treatment (Table 18). Fertilizer significantly increased the aboveground biomass per seedling from 0.16 ± 0.02 to 0.27 ± 0.02 (p=0.0132). The interaction between seeding treatment and NAG sand substrate treatment showed a species dependent increase or decrease in aboveground biomass per seedling in response to the addition of NAG sand, the most notable effect being an considerable decrease for arctic lupine (p=0.0039; Figure 22).

Table 18. Summary of linear mixed-effects model results for above- and estimated belowground biomass per seedling calculated using above- and estimated belowground biomass and seedling density collected from 2- and 10-year old stockpiled soil test plots in September 2010. For each of the two response variables (columns), the details of the minimal adequate models are listed in the rows, with explanatory variables and their interactions (first column) retained in the models, their corresponding F- and p-values, plus the number of observations and the minimum and maximum model AIC values (bottom rows). Bold font indicates significant p-values.

Predictor Variable	Aboveground Biomass per Seedling ^a		Estimated Belowground Biomass per Seedling ^a		
2-Year Soil					
NAG Sand	$F_{1,55}=1.908$	p=0.1728	$F_{1,54}=3.716$	p=0.0592	
Fertilizer	$F_{1.55} = 8.307$	<i>p</i> =0.0056	$F_{1,54}=2.450$	<i>p</i> =0.1234	
Seeding Treatment	$F_{3,55}=1.290$	p=0.2871	$F_{3.54}=10.98$	<i>p</i> <0.0001	
NAG sand X Fertilizer	Excluded	Excluded	Excluded	Excluded	
NAG sand X Seeding Treatment	Excluded	Excluded	Excluded	Excluded	
Fertilizer X Seeding Treatment	Excluded	Excluded	Excluded	Excluded	
NAG sand X Fertilizer X Seeding Treatment	Excluded	Excluded	Excluded	Excluded	
Number of Observations	64		63		
AIC of maximum model	48.94		54.06		
AIC of minimum model	30.61		44.19		
10-Year Soil					
NAG sand	$F_{1.51}=2.258$	p=0.1390	$F_{1,40}=13.75$	<i>p</i> =0.0006	
Fertilizer	$F_{1,51}=6.582$	p=0.0132	$F_{1,40}=2.245$	p=0.1412	
Seeding Treatment	$F_{3.51} = 1.292$	p=0.2869	$F_{3,40}=26.96$	p<0.0001	
NAG sand X Fertilizer	Excluded	Excluded	$F_{1,40}=3.420$	p=0.0712	
NAG sand X Seeding Treatment	$F_{3,51}=5.026$	p=0.0039	$F_{3,40}=13.57$	<i>p</i> <0.0001	
Fertilizer X Seeding Treatment	Excluded	Excluded	$F_{3,40}=0.2529$	<i>p</i> =0.8589	
NAG sand X Fertilizer X Seeding Treatment	Excluded	Excluded	F _{3,40} =4.485	<i>p</i> =0.0078	
Number of Observations	64		63		
AIC of maximum model	-13.02		-29.42		
AIC of minimum model	-19.44		-33.42		

^asquare root transformed; AIC, Akaike information criterion; 'excluded' indicates terms excluded during model simplification (stepwise approach).

In both 2- and 10-year old stockpiled soil test plots, the highest mean aboveground biomass per seedling for seeding treatments was observed in soils treated with fertilizer and fertilizer + NAG sand. When comparing the two treatments, the mean aboveground biomass per seedling for seeding treatments in both soils was considerably higher in soils treated with fertilizer + NAG sand (with the exception of locally blue wildrye and arctic lupine seeding treatments in 10-year old soil).

For 2-year old stockpiled soil test plots, the addition of NAG sand had no significant effect on the estimated belowground biomass per seedling (g seedling⁻¹); however, a significant effect was observed for seeding treatment (p<0.0001; Table 18). Estimated belowground biomass per seedling for arctic lupine was significantly higher than all other seeding treatments (Figure 23).

In the 10-year old stockpiled soil test plots, there was a significant interaction for estimated belowground biomass per seedling between NAG sand and seeding treatment and between NAG sand, fertilizer and seeding treatment (Table 18). Estimated belowground biomass per seedling significantly decreased in response to the addition of NAG sand for arctic lupine (p<0.0001). In addition, fertilizer plus NAG sand further reduced estimated belowground biomass per seedling for arctic lupine (p<0.0078; Figure 23).



Figure 22. Overall seeding treatment effects on mean values (\pm SE) of the aboveground biomass per seedling (g seedling⁻¹) across all substrate treatments in 2-year (above) and 10-year (below) old stockpiled soil. Single-species seeding treatments include seeds obtained from genetically diverse seed increase plots (ecovars) of fringed brome (BROMCIL), blue wildrye (ELYMGLA) or seeds collected from the local area of the minesite (local) of blue wildrye and arctic lupine (LUPIARC).

In both 2- and 10-year old stockpiled soil test plots, the highest mean estimated belowground biomass per seedling for seeding treatments was observed in soils treated with fertilizer and fertilizer + NAG sand (with the exception of locally blue wildrye and arctic lupine seeding treatments in 10-year old soil).

In NAG sand test plots, seeding treatments had no significant effect on aboveground and estimated belowground biomass and shoot:root ratio (Table 19). Seeding treatment also had no significant effect on aboveground and estimated belowground biomass per seedling (Table 20).



10-Year Soil



Figure 23. Overall seeding treatment effects on mean values (\pm SE) of the estimated belowground biomass per seedling (g seedling⁻¹) across all substrate treatments in 2-year (above) and 10-year (below) old stockpiled soil. Single-species seeding treatments include seeds obtained from genetically diverse seed increase plots (ecovars) of fringed brome (BROMCIL), blue wildrye (ELYMGLA) or seeds collected from the local area of the minesite (local) of blue wildrye and arctic lupine (LUPIARC). For 2 year old soils (above), bars which share a common letter are not significantly different (p<0.05).

Table 19. Summary of linear mixed-effects model results for aboveground biomass, estimated belowground biomass and shoot:root ratio collected from NAG sand testplots in September 2010. Details of the minimal adequate models are listed in the rows, with explanatory variables and their corresponding F- and p-values, plus the number of observations (bottom rows).

Predictor Variable	Aboveground Biomass		Shoot:Root Ratio	
NAG Sand Seeding Treatment Number of Observations	<i>F</i> _{6.20} ≃0.8447 2	<i>P</i> =0.5522	F _{6,18} =0.7983	<i>P</i> =0.5851
Predictor Variable	Estimated Belowground Biomass		······	Noting to the second
NAG Sand Seeding Treatment Number of Observations	F _{4.5} =1.402	<i>P=</i> 0.3757	_	

Table 20. Summary of linear mixed-effects model results for above- and estimated belowground biomass per seedling calculated using aboveground biomass and seedling density collected from NAG sand testplots in September 2010. Details of the minimal adequate models are listed in the rows, with explanatory variables and their corresponding F- and p-values, plus the number of observations (bottom rows).

	Aboveground Diomass per Seconing		Estimated Delowground Diomass per Seeding		
NAG Sand					
Seeding Treatment	F _{3.11} =0.7473	P=0.5507	F _{3.11} =0.8753	P=0.4932	
Number of Observations	16		16		

4.0 Discussion

The main objective of this experiment was to determine whether NAG sand could be utilized as a supplement to stockpiled soil to effectively increase the quantity of adequate plant growth medium available for use in reclamation. The experiment set out to evaluate this option by determining how soil properties (physical and chemical) respond to the addition of NAG sand. A number of plant performance parameters were also used to evaluate the response of plants to the addition of NAG sand to soil. Plant-available nutrients (and trace elements) were estimated using a variety of methods: exchangeable cations; Mehlich III extractable elements; Olson P, extractable P and mineral N (nitrate and ammonium).

Overall concentrations of plant-available nutrients were reduced as a result of supplementing soil with NAG sand; however, physical conditions in supplemented soils (e.g., temperature, aeration) may have been more conducive for plant growth. To provide context for trace element concentrations, values were compared to CCME soil quality guidelines agricultural soil. Total concentrations of trace elements (including base metals) in soils supplemented with NAG sand were found to be below concentrations outlined in the CEQG for agricultural land, with the exception of Cr, Mo, V and Cu (CCME 2004). However, when comparing soils supplemented with NAG sand to background levels found in non-supplemented soils, only V was found to be significantly higher in the supplemented soils. Vanadium is an element that has been shown to have low mobility and phytoavailability (Martin and Kaplan 1998).

Plant performance on soils supplemented with NAG sand was shown to be equal to or higher than their performance in non-supplemented soils. With the addition of fertilizer,

plant performance significantly increased in soils supplemented with or without NAG sand. When combined, the effects of both NAG sand and fertilizer on some measures of plant performance appeared to be additive and resulted in the highest levels of plant performance.

Further to the main objective, the plant performance of three grass species (two varieties of blue wildrye, a single variety of fringed brome and a single variety of Rocky Mountain fescue) and a single legume (arctic lupine) were compared. Overall, the performance was highest for the two varieties of blue wildrye compared to the other two grasses and legume. In addition, a significant difference in performance was also observed between two varieties of blue wildrye (locally specialized versus mixed genotype), with the highest performance observed for the mixed genotype variety.

4.1 Soil Characterization

4.1.1 Physical Soil Properties

Differences in the physical properties between 2- and 10- year old stockpiled soils supplemented with or without NAG sand were apparent. The addition of NAG sand to the soils resulted in an increase in the sand content and a proportional reduction in the clay, silt and organic matter. This was expected, as the 50:50 mixtures of loam soil and NAG sand would necessarily have an intermediate soil texture. Bulk density of the soils increased and soil porosity (which was not directly measured) decreased in response to the addition of NAG sand. The change in bulk density and soil porosity also accompanied with a decrease in soil strength. As an incidental observation, the surfaces of soils supplemented with NAG sand were visibly darker when compared to the non-supplemented soils, which may have influenced surface temperatures. Furthermore, visual assessments of the surface of nonsupplemented soils suggest that there may have been some surface sealing (i.e., crusting), which was not observed on soils supplemented with NAG sand.

With the addition of NAG sand, the percentage of sand in the fine fraction of the stockpiled soils increased. Non-supplemented soils were classified as a loam and soils supplemented with NAG sand as a sandy loam (Troeh and Thompson 1993). Sandy soils are usually well aerated as most pore spaces within this soil type are large enough to allow water to enter and drain through quickly (Troeh and Thompson 1993). As a result of the higher percentage of sand, air and water infiltration in the soils likely increased with the addition of NAG sand.

The percentage of clay and silt in the fine fraction of the stockpiled soils decreased with the addition of NAG sand. Clay content contributes significantly to a soil's ability to store water and nutrients (Brady and Weil 2002). Therefore, a reduction in clay content in soils supplemented with NAG sand likely resulted in a reduction in their water and nutrient storage capacity.

The percentage of SOM in the stockpiled soils also decreased with the addition of NAG sand. No appreciable amounts of SOM were present in NAG sand; thus, the decrease in percent SOM of stockpiled soils in response to the addition of NAG sand was expected. Soil organic matter is another important contributor to the storage and retention of water and nutrients (Gregorich et al. 1994). Therefore, the decreased concentration in the SOM of soils with the addition of NAG sand may have also contributed to a reduction in their water and nutrient storage capacity. For soils supplemented with or without NAG sand, percent SOM (0.58 - 3.13 %) was lower than the average percent SOM in grassland soils (approximately 4

%) reported by Troeh and Thompson (1993). Consequently, it can be inferred that the contribution of SOM to nutrient and water storage in the stockpiled soils was substandard, regardless of the NAG supplement.

Bulk density in the stockpiled soils increased with the addition of NAG sand. Models examining soil porosity by Stolf et al. (2011) showed that as bulk density increases, soil porosity (total porosity and macro- and microporosity) decreases. Based on these models, which estimate soil porosity using bulk density and percent sand content, the addition of NAG sand to stockpiled soils likely resulted in a decrease in total, macro- and microporosity. This seems valid as finer textured soils are associated with higher porosities and lower bulk densities (Brady and Weil 2002). However, the amount of air-filled porosity of stockpiled soils may have increased in response to the NAG sand supplement. In comparison to clay soils, sandy soils have higher water infiltration rates and thus, the capacity to retain water within the medium is reduced, resulting in increased air-filled porosity (Baker et al. 1999). Therefore, the addition of NAG sand to stockpiled soils likely resulted in an increase to water infiltration and air-filled porosity.

Soil strength at the surface of soils supplemented with or without NAG sand was measured using a small handheld penetrometer. In responses to the addition of NAG sand, soil strength decreased, and in both supplemented and non-supplemented soils, values were lower than 2 MPa, a level at which, root growth starts to be impacted (Lampurlanes and Cantero-Martinez 2003). It should be noted that soil strength varies with soil moisture. We did not measure soil moisture at the time of penetrometer measurements but all samples were obtained over a 2 hour period. We expected soil moisture conditions to be relatively constant over the sample period.

A considerable difference in soil color was observed between stockpiled soils supplemented with or without NAG sand (Appendix 4). Soils supplemented with NAG sand were darker than non-supplemented soils and this difference in color may have resulted in a considerable difference in soil temperature during the growing season, especially before plant cover became well established. The darker the surface, the greater the amount of solar radiation absorbed, which is then converted into heat. Darker soils have a low albedo, resulting in less reflection of radiation than light-coloured soils. Thus the darker color of soil supplemented with NAG sand may have equated to higher temperatures as compared to the non-supplemented soils. Dark soils do not always equate to warmer soils as the dark color is often due to higher organic matter content; soils with high organic matter have a very high water-holding capacity that when saturated, keeps the soil cool (Troeh and Thompson 1993). However, the dark color of soil supplemented with NAG sand is due to the addition of sand, a mineral material that has no organic matter content and low water-holding capacity; therefore, it is unlikely that the increased heat production from the absorbance of solar radiation in soils supplemented with NAG sand would be impeded by wet soil conditions.

In addition to soil color, soil texture may have also significantly influenced soil temperature. Soils with higher percent sand content are likely to have a lower heat capacity and greater thermal conductivity, thereby allowing for greater absorption and transfer of heat energy throughout the soil (Shaw 1952). Therefore, soils supplemented with NAG sand may have experienced higher daily surface and sub-surface temperatures in comparison to the non-supplemented soils throughout the spring, summer and fall when snow cover did not interfere with solar irradiance.

Following periods of precipitation, what appeared to be the development of a surface seal (a thin layer of structureless material on the soil surface) was observed on soils that were not supplemented with NAG sand. In addition, other areas of the minesite reclaimed with the same stockpiled soil exhibited water pooling on the surface too (Appendix 5). During periods of heavy precipitation, aggregates on the soil surface can be broken down from the force of raindrops into smaller particles (called slaking). These particles, along with dispersed clay particles, can then clog soil pores; as the soil dries, it forms a crust. Surface seals formed in this manner can significantly reduce water infiltration, limiting the availability of water for plant growth (Brady and Weil 2002). The development of a surface seal was not observed on soils supplemented with NAG sand.

4.1.2 Chemical Soil Properties

The addition of NAG sand to 2- and 10-year old stockpiled soils resulted in considerable changes to their chemical properties. Cation exchange capacity was shown to decrease in stockpiled soils with the addition of NAG sand. This was expected as the addition of NAG sand would dilute the soil, lowering the organic matter and clay content, thereby reducing nutrient retention. As a result of the lower capacity for nutrient retention, total and available macro- and micronutrient concentrations in soils supplemented with NAG sand were lower in comparison with non-supplemented soils (with a few exceptions) and concentrations of some nutrients were considered deficient for plant growth. The pH of stockpiled soils increased with the addition of NAG sand, most notably in 10-year old soil. Electrical conductivity increased and calcium carbonate equivalence slightly decreased with the addition of NAG sand.

Cation exchange capacity in stockpiled soils decreased with the addition of NAG sand, most likely in response to a reduction in SOM and clay content. The majority of negatively charged particles (micelles) that make up the exchange complex are composed of clay and organic matter particles (Troeh and Thompson 1993). Consequently, the reduction in SOM and clay content observed in the stockpiled soils in response to the NAG sand supplement was likely responsible for the decrease in cation exchange capacity.

In 2- and 10-year old stockpiled soil, several nutrient deficiencies were observed. Available concentrations (Mehlich III extraction) for P and K (10-year old soil), and total concentrations of N were found to be at levels deficient for plant growth (Marx et al. 1999). In addition, concentrations of the micronutrients B and Zn were also found be deficient (Brady and Weil 2002). In response to the NAG sand supplement, concentrations of macronutrients (with the exception of P) and micronutrients (with the exception of Cu, Fe and Ni) in the stockpiled soils decreased and further nutrient deficiencies for Mg, K (for 2year old soil) and the micronutrient Mn were observed. Concentrations of macro- and micronutrients in soils supplemented with or without NAG sand were all below concentrations which would be considered toxic to plants (Brady and Weil 2002).

With the addition of NAG sand, pH in the stockpiled soils increased, most notably in 10-year old soil. It is hypothesized that the increase in pH was due to higher concentrations of calcium oxide (CaO) in NAG sand compared to stockpiled soils. With the addition of NAG sand, concentrations of CaO in the supplemented soils may have increased significantly. However, values of oxide concentrations were calculated from the determined elemental concentrations and therefore it is unknown whether Ca existed in the form of CaO in the NAG sand or soil materials. In addition, the percent of SOM in soils supplemented

with NAG sand was low and soils with a low SOM have been shown to have a lower buffering capacity as the complex structure of SOM acts as an efficient reservoir for H⁺ ions, (Brady and Weil 2002).

Soil pH has numerous effects on plant growth but its most important influence is on nutrient availability. Nutrient release through weathering, solubility in soil solution and storage associated with CEC is governed by soil pH. For optimum nutrient availability, a range in pH of 6.0-7.5 has been suggested (Troeh and Thompson 1993). In response to the addition of NAG sand, both stockpiled soils where shown to have a pH at the high end of this optimum range.

Electrical conductivity increased in stockpiled soils with the addition of NAG sand. Values of EC in soils supplemented with or without NAG sand were not considered saline (<4 mS cm⁻²; Brady and Weil 2002). Soils which become saline can adversely affect plant growth by reducing their ability to absorb water and nutrients from the soil. However, from the changes in EC observed in the stockpiled soils with the addition of NAG sand, it is unlikely that soil salinity had any appreciable influence on plant growth.

The percentage of calcium carbonate equivalence decreased in the stockpiled soils in response to the addition of NAG sand. As a source of Ca^{2+} ions, $CaCO_3$ (or Mg^{2+}) can increase a soil's pH by exchanging Ca^{2+} (or Mg^{2+}) for H⁺ ions in the soil's cation exchange complex. Carbonate (CO_3^{2-}) ions may also form OH- through the hydrolysis of water. Calcium carbonate equivalence in NAG sand was not considerably higher than the concentrations observed in stockpiled soils and overall, concentrations in both mediums are considered low. Thus, it is unlikely that $CaCO_3$ (or other carbonate) concentrations in NAG

sand played a significant role in the changes to pH observed in stockpiled soils with the addition of NAG sand.

With the addition of fertilizer, total and plant-available concentrations of macro- and micronutrients in 2- and 10-year stockpiled soils were either similar to or higher than concentrations in unfertilized soils. Concentrations of available mineral N (nitrate and ammonium) increased in 2-year old soil but in 10-year old soil remained relatively equal to concentrations observed in unfertilized soils. Available concentrations of P (Olsen extractable) and exchangeable K (for 2-year old soil) were shown to increase in response to the addition of fertilizer. No difference in the total concentrations of N and S, available Ca, Na, Mg, K, S or Zn was observed between fertilized and unfertilized soils. In addition, no significant change in CEC and pH was observed with the addition of fertilizer. CEC is not expected to change unless significant changes in pH are observed.

Despite the increase in the availability of N, P and K as a result of fertilizer application, concentrations of macronutrients in fertilized and unfertilized soils may have been at levels considered to be deficient for plant growth (Marx et al. 1999). Soils with a low CEC have low capacity for nutrient retention and this is likely a factor that has contributed to deficiency levels of macronutrients in the stockpiled soils.

Concentrations of total trace elements in soils supplemented with or without NAG sand were found to be below concentrations outlined in the CEQG for agricultural land with the exception of Cr, Mo, V and Cu (CCME 2004). However, when comparing total concentrations between soils supplemented with or without NAG sand, only V was shown to be higher in the supplemented soils. Total concentrations of trace elements were expected to

be lower in soil supplemented with NAG sand compared to non-supplemented soils as it was hypothesized that NAG sand (originating from desulphurization tailings) would have relatively low total trace element content, and when combined with soil would not lead to in an increased trace element concentrations.

Concentrations of V in 2-year old soil were above CEQG concentrations and just below CEQG concentrations in 10-year old soil. With the addition of NAG sand, concentrations of V increased. This suggests that although natural levels of V in the stockpiled soils are high, NAG sand contributed to even higher concentrations when mixed with soil. Vanadium is a transition metal that has been shown to have low mobility and phytoavailabilty in soils with low clay and organic matter content (Martin and Kaplan 1998).

The term base metals refers to trace elements that are relevant to the mining industry; these are elements are usually used in pure form rather than as alloys (Lottermoser 2010). Concentrations of base metals in soils supplemented with or without NAG sand were found to be below concentrations outlined in the CEQG for agricultural land with the exception of Cu and Mo (for 2 year old soil). Concentrations of base metals in soils supplemented with NAG sand were lower in comparison to non-supplemented soils with the exception of Cu in 10-year old soil for which higher concentrations were found in NAG sand supplemented soils. Since NAG sand has relatively low base metal concentrations compared to nonsupplemented stockpiled soils, the lower base metal concentrations soils supplemented with NAG sand were expected.

4.2 Plant performance

In response to the addition of NAG sand to 2- and 10-year old stockpiled soil, plant performance was either similar to the performance observed in non-supplemented soils or showed a significant increase. When comparing measures of plant performance between soils supplemented with or without NAG sand, no significant difference in percent emergence, seedling density, percent cover or plant height was found. In addition, no significant difference in percent cover per seedling was observed between 2-year soil supplemented with or without NAG sand; however in 10-year old soil, a significant interaction was observed between seeding treatment and NAG sand. Estimated belowground biomass for seeding treatments was significantly higher in soils supplemented with NAG sand compared to non-supplemented soils. Furthermore, the shoot:root ratio was shown to be significantly higher 2-year old soil supplemented with NAG sand compared to the nonsupplemented soil; the opposite trend was found in 10-year old soil. This evidence suggests that the addition of NAG sand to stockpiled soils altered the physical properties of soil in such that conditions for plant growth may have been slightly improved. It is hypothesized that surface temperature, water infiltration and soil aeration (i.e., air-filled porosity) were more favorable for plant establishment and growth in soils supplemented with NAG sand than those that were non-supplemented.

Although soil temperatures were not measured in this experiment, the darker soil color and higher sand content of soils supplemented with NAG sand suggests that they may have experienced higher soil temperatures throughout the growing season. In cold climates such as the sub-boreal/subalpine transition zone of the study area, soil temperature can significantly influence plant establishment and growth (specifically in the spring) by increasing seed germination at the surface and improving nutrient and water uptake by roots (Shaw 1952). To infer germination and emergence rates, seeding densities and percent emergence were compared between soils supplemented with or without NAG sand. There was no significant increase in percent emergence or seedling density with the addition of NAG sand. However, in 10-year old soil, slightly higher mean seedling densities were found in soils supplemented with NAG sand compared to non-supplemented soil. This finding may indicate the possibility that higher surface temperatures resulting from the darker color of soils supplemented with NAG sand could positively influence germination rates.

Soil temperature may have also influenced the production of belowground biomass in soils supplemented with NAG sand. There was a significant interaction between the NAG sand supplement and seeding treatment in affecting the estimated belowground biomass for both 2- and 10-year old stockpiled soils. However, despite the interaction, all seeding treatments established in soils supplemented with NAG sand showed higher mean values of estimated belowground biomass compared to seeding treatments in non-supplemented soils. The production of root mass for barley seedlings (*Hordeum vulgare*) grown in a growth chamber at uniform soil temperatures of 10, 15 and 20°C as well as a vertical gradient of 20-10 °C (top to bottom) was observed by Fullner et al. (2011). The authors found that seedlings grown at the higher soil temperatures had significantly higher root mass and the highest root mass was found for seedlings grown in the vertical gradient. These findings support the hypothesis that potentially higher soil temperatures in soils supplemented with NAG sand compared to non-supplemented soils, may have contributed to a higher production of belowground biomass for seeding treatments.

Although it has been suggested here that soil temperatures in soils supplemented with NAG sand may have contributed to higher belowground biomass production for seedling treatments, the increase in air-filled porosity with the addition of NAG sand may have also contributed. The production of belowground biomass for creeping bentgrass (*Agrostis stolonifera*) in response to changes in soil aeration was demonstrated by Huang et al. (1999) when seedlings grown in a saturated medium were compared to seedlings grown in a well aerated medium. The authors showed that grass seedlings grown in a saturated medium had significantly lower dry root mass compared to seedlings from the well aerated medium. Although there is no indication that stockpiled soils in this experiment were frequently saturated, the addition of sand to the soils likely increased water infiltration, reducing field capacity and increasing air-filled porosity, resulting in an increase in the productivity of belowground biomass.

When resources required by plants for growth are limited in one area, plants respond to the imbalance by allocating new biomass to organs in which the resources are most limiting. If plants are short of C (due to shading or herbivory), they respond by allocating growth to shoots and if nutrients in the soil are limiting, plants respond by producing more root mass (Chapin et al. 1987). In this experiment, the shoot:root ratio for seedlings established in soils supplemented with or without NAG sand was compared. It was found that the shoot:root ratio was significantly lower in 2-year old soil supplemented with NAG sand compared to non-supplemented soil; the exact opposite relationship was observed for seedlings grown in 10-year old soil supplemented with or without NAG sand. In accordance with the growth allocation response explained by Chapin et al. (1987), these results suggest that 2-year old soil was more nutrient limiting in comparison to 10-year old soil. However,

despite this difference, seedlings compensated to the change in nutrient availability without a significant reduction in plant performance as root and shoot biomass was not adversely affected by the addition of NAG sand.

Fertilizers are often added to soil to improve plant performance by supplementing a soil's natural fertility (Troeh and Thompson 1993). Various experiments examining plant performance in degraded soils have shown a significant increase in plant performance in response to the addition of fertilizer (Greipsson and Davy 1997; Burton and Burton 2000; Gardner et al. 2012). In this experiment, the addition of fertilizer to 2- and 10-year old stockpiled soil significantly improved plant performance for the seeding treatments tested. Measures of plant performance, including percent emergence, seedling density, percent cover, plant height, aboveground and estimated belowground biomass, aboveground biomass per seedling and shoot:root ratio were significantly higher in fertilized soils, with the exception of the shoot:root ratio for 10-year old soil. The increase in plant performance in fertilized soils is likely the result of an increased availability of primary macronutrients, including N (nitrate and ammonium), P and K, which were found to be at levels of deficiency in the unfertilized soils. Fertilizer (13-16-10 expressed as % N, P₂O₅, K₂O) was added to the treatment substrates at a rate of 576.9 kg ha⁻¹. Slight increases in the availability of these macronutrients were shown in fertilized soils during the second growing season, most notably for P.

In this experiment, percent emergence for seeding treatments sown in 2- and 10-year old soils significantly increased in response to the addition of fertilizer. Research testing the effects of fertilizer and sowing rate on seed emergence and seedling densities for similar grass and legume species was completed by Burton et al. (2006) at degraded sites in northern BC. For all sowing densities tested, percent emergence was shown to be significantly higher on fertilized compared to non-fertilized soils. Additionally, in a glasshouse potting experiment by Agenbag and Villiers (1989), seedling emergence of wild oat (*Avena fatua*) seeds sown in sandy and loamy soils treated with various concentrations of limestone, ammonium nitrate (prills) and liquid ammonium nitrate were compared to unfertilized controls. Seedling emergence was shown to significantly increase in response to increasing concentrations of nitrate application, all of which had significantly higher percent seedling emergence compared to the unfertilized control treatment. The results from these two examples support the results of our own experiment showing an increase in seedling emergence in response to the addition of fertilizer, most notably when applied to degraded (i.e., nutrient poor) substrates.

In a few experiments, the success of seedling emergence has been shown to significantly increase in response to the addition of fertilizers. In the experiment by Agenbag and Villiers (1989), the number of wild oat seedlings significantly increased with increasing concentrations of nitrate applied. Similar results were obtained by Greipsson and Davy (1997) in which the number of dune-building grass (*Leymus arenarius*) seedlings, established on a sandy plain in southern Iceland, increased five-fold in response to the addition of a slow-release fertilizer. Such findings agree with the results of this experiment in which seedling densities in the 2- and 10 year old soil treatments significantly increased in response to the addition of fertilizer. Rather than positively influencing germination rates (thereby increasing seedling survival during the first and second growing season, and hence higher seedling densities compared to unfertilized treatments.

The addition of fertilizer to 2- and 10-year old soil was shown to significantly increase the percent cover of plants established from seeding treatments. Similar results were observed by Burton and Burton (2000) during seed trials in northern British Columbia in which a specific seed mix was sown at a variety of seeding densities on fertilized and unfertilized compacted forest soils. Regardless of seeding density, Burton and Burton (2000) observed a significant increase in percent cover of seedlings in response to the addition of fertilizer.

Plant height is a simple, non-destructive measurement often used in agricultural research for observing the effects of fertilizer on plant performance. Bolton et al. (1982) measured the effects of drainage, crop rotation and fertilizer on yield, plant height and leaf nutrient composition of corn (*Zea mays*); the results showed a significant increase in the height of corn plants in response to fertilizer. Similar responses of plant height to fertilizer application have also been shown in many other agricultural experiments (Pertuit et al. 2001; Law-ogbomo and Law-ogbomo 2009). In the experiment reported here, the response of plant height to fertilizer application was similar, with significantly greater plant heights observed for seedlings established in fertilized soils compared to seedlings growing in unfertilized soils.

Biomass measurements also showed a significant increase in aboveground and estimated belowground biomass in response to fertilizer application (with the exception of estimated belowground biomass per seedling) which was consistent with the results of our productivity proxies. Similar results were found in agricultural experiments in which fertilizer applications significantly increased crops yields for corn (Bolton et al. 1982; Lawogbomo and Law-ogbomo 2009). In addition, many reclamation research trials with cover crops (i.e., grasses and legumes) have also shown the same productivity response to fertilizer applications (Redente and Richards 1997; Gardner et al. 2012).

In this experiment, there was a significant increase in the allocation of growth to aboveground biomass for seedlings in fertilized compared to unfertilized soils. Increased allocation to aboveground biomass in fertilized plots suggests that soil nutrient limitations were alleviated by the fertilization treatment, as suggested by Chapin et al.'s (1987) interpretation of limiting resources. It also suggests that available nutrients in fertilized soils were present at concentrations which may have no longer been deficient. In a greenhouse experiment by Bonifas et al. (2005), the response of root and shoot biomass of corn and velvetleaf (*Abutilon theophrasti*) was measured under a variety of N applications. As the concentration of N applied to pots was increased, the shoot:root ratio for plants increased; plants in the pots with no N added had the lowest shoot:root ratios. Therefore, the plants in this experiment allocated more growth to shoots in response to increasing N supply.

Treating stockpiled soil with NAG sand or fertilizer resulted in similar or increased plant performance when compared to non-treated soils. When combined, the effect of these treatments on some measures of plant performance appeared to be additive and resulted in the highest levels of plant performance. The production of aboveground and estimated belowground biomass and the shoot:root ratio (10 year old soil) for most seeding treatments was shown to be highest in soils treated with both NAG sand and fertilizer, most notably for aboveground and estimated belowground biomass per seedling in 2-year old soil. No interaction between NAG sand and fertilizer was detected for any of these measures of plant performance. Therefore, it is likely that, when combined, the effects of NAG sand and fertilizer on soil do not significantly influence one another, but instead result in a cumulative

improvement to soil conditions for plant growth. An example of the additive effects of multiple soil treatments on plant growth was likewise demonstrated by Bolten et al. (1982) who showed that the maximum yield for corn crops resulted from the combined effects of drainage spacing, crop rotation and fertilizer.

4.3 Species-specific plant performance

The way in which plants respond to differences in their environment varies considerably among species. Although most plant species require a similar balance of energy, water and mineral nutrients (Chapin et al. 1987), their strategies for responding to stress and disturbance (Grime 1977) can differ. These different strategies can confer significant advantages or disadvantages for a species under any given set of environmental conditions (e.g., local adaptation to climate or soil conditions; Macel et al. 2007). In this experiment, significant differences were observed in the performance of four grasses and a legume sown on stockpiled soils treated with or without NAG sand and/or fertilizer. Interspecific differences in plant performance can be interpreted as resulting from different primary strategies for which they have evolved to respond to environmental stress and disturbance. Grime (1977) describes three primary plant strategies of plant species in which species are adapted to thrive in specific permutations of high and low levels of stress and disturbance; these include competitors (adapted to low stress and disturbance), stress tolerators (adapted to high stress, low disturbance) and ruderals (adapted to low stress, but high disturbance).

In an experiment where germination trials for seeds from 403 plant species were examined, Grime et al. (1981) ranked initial germinability of freshly collected seed by family as follows: Poaceae >Asteraceae > Fabaceae and Cyperaceae > Apiaceae. In response to

storage (simulating overwintering conditions), germinability significantly increased for Poaceae and Asteraceae, but not Apiaceae and Fabaceae. The number of seedlings which emerge each spring is dependent on the number of seeds that germinate, and so it is logical to assume that given an equal number of seeds, the number of grass seedlings emerging from a soil would be greater than the number of legume seedlings due to their higher germinability. For this experiment, percent emergence and seedling density for blue wildrye, fringed brome and arctic lupine seeds sown at a similar rate (\sim 750 PLS m⁻²) and under the same conditions (treated and non-treated stockpiled soils) during the second growing season were compared. Blue wildrye and fringed brome had significantly higher percent emergence compared to arctic lupine. The lower percent emergence of arctic lupine in comparison to the blue wildrye and fringed brome agrees with the germinability ranking proposed by Grime et al. (1981) and in their findings which showed that seeds which were dark colored, had a thick seed coat and were dispersed through an explosive mechanism (dehiscence), had low germinability (often due to coat-enhanced dormancy; Bewley 1997). Such is the case for arctic lupine; where as seeds of grass species such as blue wildrye and fringed brome, which are characterized as having features that are beneficial for germination on the soil surface (cylinder shaped with hygroscopic appendages), exhibited significantly higher germinability.

In terms of its relationship to ranking of germinability for plant families, it can only be speculated as to why a significantly lower seedling density and percent emergence was observed for fringed brome in comparison to the blue wildrye varieties as both are a member of the gramineae family. As suggested by Grime et al. (1981), species and even individuals of the same species can differ in germination requirements (e.g., temperature and light conditions). Based on environmentally controlled germination tests for the two grass species

by Burton and Burton (2003), fringed brome was shown to have lower mean germination rates at cooler temperatures (15°/25 °C night/day; 57.7 %) compared to blue wildrye (79.2 %) for untreated seeds. In addition, stratification was suggested to slightly increase germination rates for blue wildrye. This evidence suggests that the lower seedling density of fringed brome compared to blue wildrye (which were sown in our test plots located at a subalpine elevation) may be a reflection of warmer temperature requirements for germination of fringed brome.

Percent cover of vegetation is often used as a proxy for measures of plant productivity. Grass cover is often perceived to be highly productive due to its dense and consolidated aboveground growth. In contrast, legume cover is often seen to be less productive because cover is patchy, shoots are usually highly branched and branch morphology can vary widely (e.g, sicklepod [*Senna obtusifolia*]; Smith and Jordan 1994). In this experiment, both varieties of blue wildrye had significantly higher percent cover compared to all other single-species seeding treatments on both 2- and 10-year old soil. On 2-year old soil, percent cover for the arctic lupine seeding treatment was significantly higher than the percent cover of Rocky Mountain fescue and fringed brome; the trend was reversed in 10-year old soil. However, when comparing percent cover per seedling grown in 2-year old soil, arctic lupine had significantly higher percent cover compared to fringed brome and both varieties of blue wildrye. Overall, the high aboveground productivity of the three grass species in comparison to the single legume species suggests an ability of the grasses to more efficiently utilize resources from the environment, mostly in the first and second year.

Plant height is another proxy of plant productivity. Taller plants have a competitive advantage for light, giving them greater access to the resource, which often allows them to be

more productive (Falster and Westoby 2003). The mean height for the three grass species compared in this experiment was significantly greater than that of the single legume, with the greatest heights observed for the two varieties of blue wildrye. Therefore, when in competition, the taller stature of the grass species would allow for greater access to the light resource in comparison to the legume. In response to fertilizer, plant height significantly increased for the grass species but did not for the legume. This further suggests that the grass species are able to utilize the increased availability of nutrients at greater rates in comparison to the legume.

Competition between grass species and between grass and legume was also examined in this experiment and was shown to influence plant height. When sown together in the locally native seed mix (consisting of blue wildrye, arctic lupine and Merten's sedge), mean height values for blue wildrye and arctic lupine increased when compared to their mean heights in single-species seeding treatments, most notably in fertilized soil (no emergence of Merten's sedge was detected in the second growing season). These results suggest that when nutrient resources are not limiting (as assumed from the application of fertilizer), blue wildrye and arctic lupine respond to competition with each other by increasing in height. However, caution should be taken when interpreting these results, as the N-fixing capacity of *Rhizobium* nodules (which were detected on the roots of lupines collected from the test plots) may have contributed to the N pool in the locally native mixed seeding treatment plots, influencing the growth response of all species. For the ecovar native mix seeding treatment (consisting of blue wildrye, Rocky Mountain fescue and fringed brome) sown in the stockpiled soils, the mean height of fringed brome and Rocky Mountain fescue either decreased or showed no significant change in comparison to their mean heights in the single-

species seeding treatments (most notably in 10-year old soil), and the mean height of blue wildrye increased significantly. This suggests that the competitive ability of blue wildrye may be greater than that of fringed brome and Rocky Mountain fescue in that its ability to access light resources is enhanced in the presence of competition with other species.

The most direct measure of net productivity for a plant species is the amount of aboveground and estimated belowground biomass produced over a fixed given period of time. In this experiment, the production of aboveground biomass in response to the NAG sand supplement or fertilizer was species dependent and overall, the most productive species were the two varieties of blue wildrye. This result further supports the hypothesis that blue wildrye retains a greater competitive advantage in the capture and utilization of nutrients in comparison to Rocky Mountain fescue, fringed brome and arctic lupine in the stockpiled soils.

With a reduction in the availability of nutrients (as seen with the addition of NAG sand), the four grass species tested were able to capture and utilize enough resources to maintain productivity compared to the single legume for which productivity decreased (i.e., aboveground biomass per seedling). These findings further support the interpretation that blue wildrye has a competitive advantage in the capture and utilization of nutrients compared to arctic lupine.

In contrast, the high production of belowground biomass for arctic lupine suggests a greater investment in the development of biomass for the utilization of soil resources (e.g., water and mineral nutrients) in comparison to the four grass species tested. Arctic lupines are associated with nitrogen-fixing *Rhizobium*. Other than the production of plant-available

N through atmospheric N fixation, rhizobacteria have been shown to provide a variety of other direct and indirect benefits to plants including protection from phytopathogens, sequestration of plant-available forms of Fe, solubilisation of minerals including P and the production of plant growth regulators (Noel et al. 1996). These additional benefits to arctic lupine may provide this species with a greater tolerance to stress in comparison to the grass species, which do not have these symbiotic relationships.

Chapin et al. (1987) explains a large allocation of growth to roots is usually in response to low nutrient availability in the soil. This may explain the significant increase in root biomass for arctic lupine in response to the dilution of soil (and therefore the dilution of mineral nutrients) through the addition of NAG sand. Similar results were obtained by Nicholas and McGinnies (1982) when the growth of two legumes, alfalfa (*Medicago sativa*) and cicer milkvetch (*Astragalus cicer*), established in topsoil or coal mine spoil (a mixture of shale and sandstone that was shown to have lower nutrient availability than the topsoil) was compared. Both legumes produced significantly greater root biomass when established in mine spoil compared to topsoil. All other grass species in this experiment displayed this same trend with either similar or slightly increased levels of estimated belowground biomass in response to the NAG sand supplement. On the other hand, the decrease in estimated belowground biomass for arctic lupine in response to the addition of NAG sand was unexpected and remains unexplained.

The grass species contributed a significantly higher proportion of their growth to aboveground biomass in comparison to the arctic lupine. Root architecture differs greatly between three grasses and the single legume species examined here. The grass species utilize a fibrous root system, whereas the legume, arctic lupine, utilizes a taproot. This difference in architecture is likely what determined the difference in shoot:root ratios between the two lifeforms. Taproots have the advantage of reaching deeper into the soil to access water, which suggests arctic lupine may have a significant advantage in dealing with drought stress compared to the fibrous rooted grass species. However, fibrous roots have the advantage of absorbing available nutrients at greater rates than that of taproots, suggesting a competitive advantage under nutrient-poor conditions for the grass species.

Growth allocation responses to fertilizer also differed significantly between the grass and legume species. With the addition of fertilizer, there was a significant increase in allocation to aboveground biomass for all grass species; however, allocation of growth for arctic lupine showed no significant change. This significant shift in allocation in response to fertilizer for the grass species suggests a greater morphological plasticity in comparison to the arctic lupine which showed no significant allocation response. Species with a greater morphological plasticity are likely to have a competitive advantage (Grime et al. 1988) in that their ability to direct growth towards limited resources (as inferred by Chapin et al. 1987) is greater than species with limited morphological plasticity.

The differences in plant performance among species in this experiment reflect different plant growth strategies that have evolved to respond to environmental stress and disturbance (competition, abiotic stress or frequent disturbance; Grime et al. 1988). The growth characteristics displayed by the three grass species (blue wildrye, Rocky Mountain fescue and fringed brome) suggests a primary competitive strategy. These characteristics included high seed germinability, tall stature and dense canopies (which was less apparent with fringed brome and Rocky Mountain fescue) and an ability to quickly utilize and improve access to resources in response to competition with other species (e.g., light and

nutrients), leading to high morphological plasticity. In comparison to the grass species, the growth characteristics displayed by arctic lupine suggest a stress-tolerator primary strategy. Characteristics displayed by arctic lupine included low seed germinability, low stature (in comparison to the grass species), no significant response to competition with other species (i.e., blue wildrye), greater ability to store resources through the use of thick rhizomes and association with nitrogen-fixing *Rhizobium* and low morphological plasticity.

In addition to the differences in plant performance between the grass and legume species, significant differences in plant performance were also observed between two varieties of blue wildrye. The two varieties consisted of seeds collected from the local area around the minesite (locally specialized genotype) and from seed increase plots (Industrial Forest Service Ltd.) in which seeds from a variety of locations were sown to create genetically diverse seed crops (mixed genotype or ecovar). Overall, the blue wildrye mixed genotype had higher mean values for all measures of plant performance compared to the locally specialized genotype (in most cases the differences were significant) with a few exceptions. It is therefore concluded that the benefits associated with establishment from seeds sourced from a broader geographic area, may be greater than the benefits associated with a narrower genotype supposedly adapted to local soil and climate conditions. However, let it be noted that stockpiled soils were highly disturbed (classified as Anthroposols) and therefore, not representative of the undisturbed soil conditions for the area. Thus, the higher performance observed by the blue wildrye mixed genotype in comparison to the local genotype may also be a reflection of its ability to establish in poor soil conditions.

Although adaptations to local climate and soil conditions for a specific plant species can provide benefits to plant fitness (Macel et al. 2007), the benefits may not always result in

superior productivity when compared to more genetically diverse varieties of the same species. In an experiment by Bischoff et al. (2010), performance was assessed for four species of wildflowers, established from seeds that were collected from a variety of individuals (mother plants) from the local area of the experimental site and from four distant provenances (distance ranged from 120 to 900 km from the experimental site). Significant differences in fitness-related traits were observed between plants established from seeds collected from four distant provenances (locations) and the local area. However, individuals established from seeds collected from the local area showed no superior fitness (significantly higher values for fitness-related traits) compared to the four provenances. Furthermore, the productivity of genetically diverse seed plots (consisting of plants established from seeds of 12 mother plants) was greater than that of low diversity plots (established from seeds of 2 mother plants). These results are similar to the results of this experiment in which fitnessrelated traits (e.g., height, seedling density and biomass) were shown to be greater for blue wildrye plants established from a mixed genotype seeding treatment. Both experiments thus support the theory that benefits of local adaptation may not always results in superior productivity when compared to genetically diverse populations of the same species.

Theory on local adaptation, as explained by Bischoff et al. (2010), predicts that the performance of local genotypes should be greater than genotypes of distant provenances when grown at the local site. However, this theory assumes that conditions at the local site have not been altered from the conditions in which the local genotype has adapted to over time. In this experiment, the seeds of the local and mixed genotypes of blue wildrye were sown in stockpiled soil in which soil conditions did not resemble those prior to disturbance.

In this case, a mixed genotype exhibits greater benefits when sown in such altered soil conditions to which neither genotype is specifically adapted to.

In contrast to the results of this experiment, Burton (2007) observed greater plant performance (i.e., percent emergence, percent cover and total biomass) following two growing seasons (2006 and 2007) for the blue wildrye seedlings established from the local genotype in comparison to the mixed genotype (ecovar) when sown in disturbed soil at the same minesite. Weather conditions (i.e., mean monthy total precipitation and minimum and maximum temperatures; Appendix 6 and 7 respectively) during the growing seasons for this experiment and the experiment conducted by Burton (2007) were compared. Overall, no considerable differences in total precipitation and temperatures were visible between the periods of these two experiments. Therefore, it is likely that the contrasting results may be due to a considerable difference in soil conditions between the stockpiled soils and those of the soil covered area in which the revegetation trial by Burton (2007) was conducted (although soil conditions for this experiment were not tested and therefore cannot be compared to the conditions of the stockpiled soils). It may therefore be hypothesized that when comparing the local to mixed genotype of blue wildrye, superior plant performance of one genotype over the other may be strongly influenced by site specific soil conditions. This hypothesis is further supported by our findings that mean height of blue wildrye seedlings established from local seeds was less than the mean height for the mixed genotype in 2-year old soil, but in 10-year old soil, the opposite trend was observed.
4.4 Limitations of Experimental Design

A few limitations became apparent in this experiment, mostly related to the design of the revegetation test plots. During the initial construction and seeding of the text plots, the pattern in which soil and seeding treatments were applied to subplots was not different between test plots; in effect, the treatments were not randomized. It is therefore possible that the pattern in which treatments were applied may have had confounding effects in that one subplot with a specific combination of treatments may have had unforeseen influences on its neighboring subplot and this influence may have then been expressed across all test plots.

During the construction of the test plots, the test plots constructed with 2-year old soil were all located at the east end of the minesite and those constructed of 10-year old soil were all located at the west end of the minesite. These areas differed slightly in elevation and exposure, which may have resulted in a slight difference in the influence of climate and weather between the two soils. In addition, a considerable difference in the amount of original vegetation cover was noted between 2- and 10-year old soils, including the locations in which these soils were excavated for construction of the test plots. The 2-year old stockpiled soil had almost no vegetation cover; only a few incidents of volunteer colonization were detected. However, along most of the surface of 10-year old stockpiled soil, a well-established herbaceous cover was present. This difference may have resulted in a considerable difference in soil characteristics, most notably in terms of the amount of biological activity (which was not measured in this experiment). Therefore, in order to avoid the influence of potential differences in climate and biological soil activity, the effects of soil and seeding treatments on plant performance in 2- and 10-year old soil were analyzed

separately and only minor comparisons for plant performance and soil characteristics between the two soils were made.

In order to calculate the amount of seed required for sowing seeding treatments at a standardized density, the pure live seed (PLS) count for each seed stock had to be calculated. PLS is calculated using the percent germination and percent purity of a seed stock to obtain an accurate count of the number of germinating seeds within a given weight of seed stock (PLS g^{-1}). At a targeted sowing rate of 750 PLS m^{-2} , the PLS g^{-1} was used to calculate the weight of seed stock required for seeding treatments applied to the 1m² subplots. For the seed stocks supplied by IFS Ltd. (ecovars; blue wildrye, Rocky Mountain fescue and fringed brome), values of percent germination and percent purity from a past analysis were used to calculated PLS. For the remaining seed stocks (locally collected blue wildrye, arctic lupine and Merten's sedge), percent purity was assessed following seed cleaning, however, due to time constraints, percent germination was estimated. Percent germination of blue wildrye seeds was estimated at 80 %, arctic lupine at 44 % and Merten's sedge at 32.7 % using the lowest values reported by Burton and Burton (2003). To assess the accuracy of our estimated germination rates, a variety of germination tests were conducted. In the fall of 2008, germination tests of arctic lupine seeds were conducted by 20/20 seed labs and in 2009 and 2010, seed germination trials were also conducted at UNBC for seeding treatments sown in the test plots. A comparison of germination rates used for calculating sowing rates and actual germination rates determined from germination testing of seeding treatments is shown in Appendix 8. Due to these differences in germination rates, actual sowing rates were either higher or lower than the targeted sowing rate. The actual sowing rates are as follows: ecovar blue wildrye (416 PLS m⁻²), ecovar fringed brome (314 PLS m⁻²), ecovar Rocky Mountain

fescue (642 PLS m⁻²), local blue wildrye (703 PLS m⁻²) and local arctic lupine (847 PLS m⁻²), local native seed mix (1105 PLS m⁻²) and ecovar native seed mix (482 PLS m⁻²). Assessment of the accuracy of estimated germination rates for Merten's sedge is not necessary as no seedlings were detected in the test plots for this species during the second growing season.

During the literature review for this experiment, an important factor in the assessment of NAG sand as a supplement to soil, which was not examined in this experiment, was recognized. One of the major constituents in the desulphurization process for Cu tailings (and therefore in the production of NAG sand) is the compound known as xanthate. In a laboratory experiment conducted by Xu et al. (1988), the effects of various concentrations of xanthate on duckweed (*Lemna minor*) and a species of daphnid (*Daphnia magna*) was examined. The results showed that concentrations of xanthate >2 mg L⁻¹ were toxic to both species and concluded that xanthate should be considered a micropollutant to aquatic systems. However, the experiment also revealed a rapid rate of degradation for xanthate (2.5 to 4 day half-life) which suggests a short-lived presence in aquatic environments. In this experiment, we did not test for xanthate concentrations in NAG sand and so the potential effects of supplementing soil with NAG sand to aquatic environments downstream from which this treatment would be applied are unknown, as are its effects on terrestrial plants and microbes.

5.0 Conclusions and Recommendations

This study confirms that the addition of NAG sand to stockpiled soils can increase the quantity of growth medium available (i.e. Anthroposols) for reclamation while maintaining plant performance observed in non-supplemented soils. When combined with a fertilizer application, plant performance on soils supplemented with NAG sand can be significantly increased over non-supplemented soils. Concentrations of total and extractable trace elements (including base metals) in soils supplemented with NAG sand are not likely to result in any adverse effects to plants or the local environment. However, it is recommended that use of NAG sand as a soil supplement be limited to hydrologically isolated areas of the minesite (e.g. tailings impoundments and tailings-filled pits) until further studies examine the residual concentrations of xanthate in NAG sand, and the potential mobility of xanthate into surrounding aquatic ecosystems.

This study has shown that blue wildrye is an excellent candidate for the revegetation of tailings and other minesoils at Huckleberry Mine. In terms of seed source, plant performance of blue wildrye established from a mixed genotype seed source (ecovar) performed better than that of the local genotype. However, previous research comparing these two seed sources has shown conflicting results. Thus, in order to achieve an optimal cover of blue wildrye, the best result may come from the use of seed stocks which are composed of both genotypes.

Plant establishment and productivity on 10-year old soil appears to be greater than establishment and productivity on the 2-year old soil (however, this was not statistically tested). This result is most apparent when comparing percent cover and plant height of

plants established in the two different-aged soils. Assuming the effects of elevation and climate differences between the locations of 2- and 10-year old stockpiled soil test plots are negligible, physical and chemical conditions in the 10-year old stockpiled soil are likely to be more conducive to plant growth. It is hypothesized that the considerably higher amount of vegetation cover established on the 10-year old in comparison to the 2-year old stockpiled soil may have resulted in a greater amount of biological contributions. The amount of SOM and ammonium, mineralizeable N and total N was higher in 10-year old compared to 2-year old soil and this may be an indication a greater amount of biological contributions resulting from the well-established vegetation cover.

Huckleberry Mine may want to re-evaluate their 2010 reclamation plan, given the results of this study. During the Huckleberry Mine Closure Meeting workshop in March 2009, the use of a soil cover in place of the currently proposed water cover for TMF-2 was investigated. It was determined that although the use of a soil cover would provide an adequate cover for PAG (potentially acid generating) materials, the efforts required to obtain the necessary soil materials needed to completely cover the impoundment were deemed impractical (Boxill 2010). However, if the conservative approach to the use of soil supplies examined in this experiment is applied to the surface of TMF-2, successful revegetation of the entire impoundment may be possible.

In order to investigate the feasibility of this alternative, further revegetation trials utilizing this technique are recommended. Large-scale revegetation plots (e.g. 0.25 ha), in which soil is mixed into the NAG sand surface, should be constructed directly on the tailings impoundment and sown using a standard native seed mix. In order to assess the potential financial costs of soil application, various methods and rates of application should be tested. In addition, the use of fertilizer and peat moss as amendments to NAG sand should also be tested as an alternative method for improving plant performance on the surface of the impoundment.

Currently, analysis of metal uptake for plant samples collected from stockpiled soils supplemented with or without NAG sand is being conducted. This information will allow us to identify whether concentrations of metals within plant tissues possess a potential risk to browsing animals (e.g., ungulates) which feed on native grasses in the area of the minesite. However, if further tests of using NAG sand in reclamation are conducted, they should be accompanied by further analysis of metal uptake. We expect metal uptake to be low, based on the low total and Mehlich III extractable metal concentrations in soils supplemented with NAG sand.

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7.0 Appendices

the local area of the Huckleberry Mille (10 kin radius).							
Species	Collectors	Date ^a	UTM	Easting	Northing	Elevation (m)	
arctic lupine	Burton, Carson	Aug 1 st	09U	625287	5957133	1010	
arctic lupine	Burton, Carson, Sparks	Aug 1 st	09U	618402	5949205	1002	
arctic lupine	Burton, Carson, Sparks	Aug 1 st	09U	618603	5949900	1099	
arctic lupine	Burton, Carson, Sparks	Aug 2 nd	09U	625032	5952888	975	
arctic lupine	Burton, Carson, Sparks	Aug 2 nd	09U	624729	5953458	973	
Merten's sedge	Burton, Carson	Aug 1 st	09U	618402	5949205	1002	
Merten's sedge	Burton, Carson, Sparks	Aug 2 nd	09U	624729	5953458	973	
blue wildrye	Davis	Sept 3 rd	09U	618728	5949241	1049	
blue wildrye	Robichaud	Sept 4 th					
blue wildrye	Burton, Davis	Sept 15 th	09U	621505	5348764	1067	
blue wildrye	Burton, Davis	Sept 15 th	09U	618728	5949241	1049	
blue wildrye	Burton, Davis	Sept 15 th	09U	618765	5949165	1045	
blue wildrye	Burton, Davis	Sept 15 th	09U	618485	5948939	1009	
blue wildrye	Burton, Davis	Sept 16 th	09U	625358	5956214	968	
blue wildrye	Burton, Davis	Sept 16 th	09U	625307	5956416	979	

Appendix 1. Details of the dates, locations and participants for the collection of locally native seeds of blue wildrye, arctic lupine and Merten's sedge within the local area of the Huckleberry Mine (10 km radius).

^aDates of seed collection in 2008; ^bcollectors include Ron Robichaud, Mike Davis, Sara Sparks, Allan Carson and Carla Burton.



Appendix 2. Photographs of each of the test plot locations, including a) millsite, b) lower east dam, c) upper <u>TMF-2 and d</u>) TMF-2 north stockpile.

Laborator y/Anarysis	
BCMOF Laboratory	
Particle Size Analysis	Carter, M. J. (Ed), 1993, Soil sampling and methods of analysis. Florida, USA: Lewis publishing, pp. 503, 507-509.
Total Carbon, Nitrogen and Sulphur and	Carter, M. R., and E. G. Gregorich (Eds). 2008. Soil Sampling and Methods of Analysis (2nd edition). Florida, USA: CRC Press, pp. 226.
Inorganic Carbon	Leco Corporation Instruction and Reference Documents for Truspec NC Analyzer
	Leco Corporation Instruction and Reference Documents for Truspec Add-on S Analyzer
	Thermo Instruments Ltd. Instruction and Reference Documents for NA-1500 Analyzer
Available Phosphorus (PO ₄ -P)	Missouri Agricultural Experiment Station SB 1001. 1998. Recommended chemical soil test procedures for the north central region. North Central Regional Research Publication NO. 221 (Revised).
Minerizeable Nitrogen	Bremner, J.M. 1965. Nitrogen Availability Indexes. <i>In</i> Methods of soil analysis: part 2 - chemical and biological properties, C.A. Black (Ed). Wisconsin, USA: American Society of America, pp. 1324-1345.
	Waring, S.A., and J.M. Bremner. 1964. Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. Nature 201: 951-952.
Available Ammonium (NH ₄ -N) and	Carter, M. J. (Ed). 1993. Soil sampling and methods of analysis. Florida, USA: Lewis publishing. pp 25-37.
Nitrate (NO ₃ -N)	Bremner, J.M. 1965. Inorganic forms of nitrogen. In Methods of soil analysis: part 2 - chemical and biological properties, C.A. Black (Ed). Wisconsin, USA: American Society of America, pp. 1179-1237.
Carbonate (CaCO ₃) equivalent	Carter, M. J. (Ed). 1993. Soil sampling and methods of analysis. Florida, USA: Lewis publishing, pp. 177-179.
pH (Calcium Chloride and Water)	Kalra, Y.P., and Maynard, D.G. 1991. Methods Manual for Forest Soil and Plant Analysis, Forestry Canada. Forestry Canada, Northwest Region, Northern Forestry Centre. Edmonton, Alberta, Information Report NOR-X-319E, 116pp.
	Atkinson, H. J., G. R. Giles, A. J. MacLean, and J. R. Wright. 1958. Chemical Methods of Soil Analysis - Contribution No. 169 (Revised).
	Chemistry Division - Science Service Canada Department of Agriculture, Ottawa. 90pp.
Electrical Conductivity	Carter, M. R., and E. G. Gregorich (Eds). 2008. Soil Sampling and Methods of Analysis (2^{nu} ed.). Florida, USA: CRC Press. 1264pp. United States Salinity Laboratory Staff. 1954. Diagnosis and improvement of saline and alkali soils", United States Department of Agriculture
	Handbook No: 60. United States Government Printing Office, Washingtion, D.C. 160pp. Kalra, Y.P., and Maynard, D.G. 1991. Methods Manual for Forest Soil and Plant Analysis, Forestry Canada. Forestry Canada, Northwest Region, Northern Forestry, Control Admonton, Alberta, Information Papert NOR X 3105.
Extractable Elements (Al, B, Ca, Cu, Fe, K Mg Mn Na P S and Zn	Carter, M. J. (Ed). 1993. Soil sampling and methods of analysis. Florida, USA: Lewis publishing, pp. 43-49.
Exchangeable Cations (Ca, K, Mg, Na) and CEC	Carter, M. R., and E. G. Gregorich (Eds). 2008. Soil Sampling and Methods of Analysis (2nd edition). Florida, USA: CRC Press, pp. 203.
Trace Elements (As, Ba, Bi, Cd, Co, Cr, Ha, Mo, Ni, Ph, Sh, Sh, Ti and V)	Carter, M. J. (Ed). 1993. Soil sampling and methods of analysis. Florida, USA: Lewis publishing, pp. 43-49. (Modified in-house to include
rig, wo, w, ro, so, sii, ar and v)	
ALS Canada Ltd. Laboratory	
Total Elemental Analysis for Rare Earth	A prepared sample (0.200 g) is added to lithium borate flux (0.90 g), mixed well and fused in a furnace at 1000°C. The resulting melt is then applied and discound in 100 mL of 4% (10.02 / 2 % MCl solution. This solution is then applying the product of MS.
and trace Elements	coorde and unsolved in 100 mL of 4 % mNO 7 2 % mO solution. This solution is then analyzed by ICP-MS.
Dase metals	A prepared sample (0.25 g) is digested with perchloric, nitric, nydronuoric and nydrochloric acids. The residue is topped up with dilute hydrochloric acid and the resulting solution is analyzed by ICP-AES. Results are corrected for spectral inter-element interferences.
Major Oxides	A prepared sample (0.200 g) is added to lithium metaborate/lithium tetraborate flux (0.90 g), mixed well and fused in a furnace at 1000°C. The resulting melt is then cooled and dissolved in 100 mL of 4 % nitric acid/2 % hydrochloric acid. This solution is then analyzed by ICP-AES and the results are corrected for spectral inter-element interferences. Oxide concentration is calculated from the determined elemental concentration and the result is reported in that format.

Appendix 3. Physical and chemical analyses conducted on soil samples collected from the 12 revegetation test plots in 2009 and 2010. Laboratory/Analysis Descriptions and References of Analyses

Appendix 4. Photographs showing the difference in soil color (i.e., darkness) for a) soils supplemented with NAG sand and b) without NAG sand using the test plot located near the millsite as an example. Soil from the area surrounding the test plot was placed in between subplots to act as a buffer and can be seen here.



b)

Appendix 5. Photographs displaying what appears to be a) surface sealing on stockpiled soils compared to b) the surface NAG sand supplemented soils where surface sealing is not visible. Where the same stockpiled soil was used to reclaim another area of the minesite, surface pooling following a rainfall event was observed (c).



c)

Appendix 6. Monthly total precipitation (mm) at Huckleberry Mine for each month in 2006, 2007, 2009 and 2010. Rainfall and snowfall measurements were collected from the mine weather station located on the roof of the administration building at the camp and mill site (refer to Figure 1 for location). Total precipitation was calculated using a snow:rainfall conversion ratio of 10:1 (Environment Canada 2012).



Appendix 7. Monthly daily minimum (above) and maximum (below) temperatures (°C) at Huckleberry Mine for each month in 2006, 2007, 2009 and 2010. Temperature measurements were collected from the mine weather station located on the roof of the administration building at the camp and mill site (refer to Figure 1 for location).



Seeding Treatment	Seed Stock	Estimated (%)	Actual (%)
Single-species			
fringed brome	ecovar	80.5	41.5
blue wildrye	ecovar	86.5	48.0
blue wildrye	local	80.0	67.8
Rocky Mountain fescue	ecovar	95.0	81.3
arctic lupine	local	44.0	50.0
Mixed-Species			
blue wildrye: fringed	local	87.3	73.25
brome: Rocky Mountain			
fescue			
blue wildrye: arctic	local	52.3	64.5
lupine: Merten's sedge			

Appendix 8. A comparison of estimated and actual percent germination (%) for 2 and 10-year old stockpiled soil test plot seeding treatments.