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Reclamation Of A Limestone Quarry To A Natural Plant Community

by

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Dedicated to my husband, David Galvez, with love.

ABSTRACT

Reclamation of thousands of limestone quarries around the world is challenged by an extremely limiting environment, including steep slopes, high calcium carbonate substrates with low nutrients and low water holding capacity. These issues were addressed at the Exshaw quarry in the Rocky Mountains of southern Alberta. Reintroduction of key components, such as vegetation and ameliorated soil were expected to speed recovery of ecosystem functioning processes. Erosion control blankets and combinations of fertilizer, sulfur and organic amendments at different application rates were evaluated in three limestone substrates in the greenhouse and field. Amendments were hay, straw, wood shavings, pulp mill biosolids, beef manure compost, beef manure mix (6:1:1 manure, waste feed, wood chips), topsoil and clean fill. Revegetation with seeded native grasses (*Poa alpina*, *Agropyron trachycaulum*, *Elymus innovatus*, *Festuca saximontana*, *Trisetum spicatum*), transplanted and seeded woody species (*Picea glauca*, *Pseudotsuga menziesii*, *Populus tremuloides*, *Betula papyrifera*, *Juniperus horizontalis*, *Alnus crispa*, *Arctostaphylos uva-ursi*) and forest floor litter were assessed. Evidence of soil microbial community development, including arbuscular mycorrhizal fungi, was evaluated.

All amended substrates supported plant growth in two greenhouse experiments. Fertilizer with pulp mill biosolids and soil caps significantly increased above and below ground biomass of grasses. *Agropyron*, *Festuca* and *Poa* performed best.

Best treatments from the greenhouse were evaluated for three years in the field. Fall planting and seeding increased plant survival. Erosion control blankets increased seeded plant establishment while reducing unseeded non native species. Manure mix biosolids increased plant establishment, soil nutrients,

microbial biomass and viable fungi and bacteria. *Picea*, *Pseudotsuga* and *Populus* showed revegetation promise. Nursery stock survived better than local transplants. Woody plants did not establish from seed. Arbuscular mycorrhizae infection was found in all 15 species sampled, more in grasses. Site characteristics such as slope, aspect, initial soil nutrient concentrations, surrounding vegetation and browsing by bighorn sheep influenced early plant community development and overall effects of soil treatments.

Reclamation is postulated to be best with erosion control blankets, organic soil amendments such as manure mix, seeded grasses and transplanted woody species. Results from this work can be extrapolated to other limestone quarries or similar disturbances.

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CHAPTER 1. INTRODUCTION

1. INTRODUCTION

Limestone is one of the most important products mined worldwide. It is a sedimentary rock of mainly calcite, a form of calcium carbonate ($CaCO_3$), formed by chemical deposition or by accumulation of shelly remains of marine organisms. Limestone is widely used in the construction industry as aggregates and as the principal component of cement (Kesler 1994). As a source of calcium carbonate, transformed into quick lime (CaO) or slaked lime ($CaOH$), limestone has broad usages in metallurgic, pharmaceutical, food and paper industries, with an increasing production and demand.

Numerous limestone quarries are in use and abandoned in countries around the world. In Ontario alone, there are hundreds of abandoned active quarries (McLellan et al. 1979, Ontario Ministry of Natural Resources 2010), costing the government millions of dollars for reclamation. In other countries where calcium carbonate is mined, the number is also significant (Spalding et al. 1999, Bonifazi et al. 2003, Clemente et al. 2004, Allen et al. 2005).

Loss of vegetation, impacts on surface and ground water, fauna and human health due to dust and noise and loss of interior forest, species habitat and diversity from edge effects created by mining are among the environmental effects of limestone quarries (Martínez-Garza and Howe 2003, Wickham et al. 2007, Darwish et al. 2011). These impacts have a cumulative effect with other human activities in the region of occurrence.

Reclamation of limestone quarries is thus an environmental necessity and is mandatory by law in North America. Despite regulatory requirements, abandoned and operational limestone quarries are not often restored (Bonifazi et al. 2003) or efforts to reclaim them to a wide range of productive land uses, including natural green spaces, meadows, forests, wildlife habitats and wetlands have been mostly unsuccessful.

In many circumstances, quarrying companies are required to establish predisturbance plant communities after mining activities. Natural plant communities result from the interaction among physical, chemical and biological factors, many of which are absent in quarries, such as soil and vegetation. Hypothetically, if key missing biotic and abiotic components are reintroduced to the abandoned quarry, the system should eventually recover functionality and a self sustaining natural community could be established.

In this research program, soil, vegetation and nutrient cycling are considered key components for limestone quarry reclamation. Their introduction and modification in a limestone quarry and plant at Exshaw (Figure 1-1), in the Canadian Rocky Mountains, Southern Alberta, will be expedited through limestone quarry substrate amelioration and assisted plant community establishment.

2. BACKGROUND

2.1 Pedogenesis in the Rocky Mountains

The natural process of soil formation or pedogenesis in the Rocky Mountains has occurred over time through the interaction of climate, soil parent materials, topography, living organisms (particularly vegetation), forest fires and avalanching (Howell and Harris 1978, King and Brewster 1978). Interactions of these factors result in creation of a series of separate but related pedogenic pathways, leading to formation of different soil horizons and profiles (Howell and Harris 1978). The climate, with a short, snow free, growing season retards plant growth and chemical and microbial breakdown of materials; and winds affect soil development by desiccation and transportation. Most of the parent materials in the mountains of Alberta has been transported by wind, gravity or ice; and some in situ development occurred on weathered bedrock (Howell and Harris 1978).

The most recent deglaciation affecting pedogenesis in the Bow Pass area, approximately 30 km northwest of Exshaw, is considered to have occurred 10,000 years ago (Rutter 1972 in King and Brewster 1978). Bedrock is of Middle Cambrian age, mainly mottled dolomitic limestones and sandstones (Price and Mountjoy 1970 in King and Brewster 1978). Glacial deposition resulted in a widespread medium textured calcareous till. Subsequent to deglaciation, initial pedogenesis within the local calcareous till resulted in the formation of Orthic

Regosols. At this stage pedogenesis may have been inhibited by the high base content of the till, resulting in shallow and poorly developed soils. Under conditions of free drainage and on more stable slopes, Orthic Regosols underwent progressive development leading to the formation of Eutric Brunisols. Soils are mainly Brunisols in undisturbed areas surrounding the Exshaw quarry (Alberta Parks and Protected Areas 2002). Common horizon sequence is LFH, Bm, Ck or C and one or more organic surface horizons overlaying a brownish, base saturated B horizon (Soil Classification Working Group 1998). Iron oxidation results in browning (Bm). Elements such as iron, aluminum and carbon can accumulate in illuviated horizons, reflecting movement of organically complexed weathering products from upper to lower horizons (Howitt and Pawluk 1985).

Fire has been a factor influencing soil formation in most forested areas of the Rocky Mountains, increasing avalanche activity and subsequent mass movement after vegetation is burnt (Howell and Harris 1998). Avalanches occur wherever sufficient snow accumulates on slopes steeper than 29^{o} and cause truncation or complete removal of soil profiles on upper slopes, and deposition of this material in the run out zone. Soil denudation and bedrock exposure after avalanches resembles, in a much smaller scale, the condition created by limestone quarrying. Vegetation establishment on the calcareous exposed material is limited by nutrient deficiencies resulting from chemical reactions among the highly concentrated carbonated and high pH, resulting in immobilized iron oxides, causing iron deficiency in plants, phosphorus unavailability due to phosphate adsorption to carbonate minerals or insolubilization, and nitrogen deficiency caused by increased nitrification (Loeppert et al. 1984, Kishchuk 2000).

2.2 Limestone Quarry Disturbances

Limestone is extracted in open pit mines where initial mineral processing takes place. Quarry size varies from a few hundred square meters to hundreds of hectares and their walls can reach dozens of meters in height. To reach the limestone, topsoil, subsoil and overburden are removed with extensive drilling and blasting. Cover soil and subsoil are often salvaged for reclamation purposes, while overburden is salvaged for backfilling and contouring unless otherwise directed. The blasted rock fragments, normally weighing hundreds of kilograms to several tonnes, are removed by large size pit loaders and dumped into large haul trucks that move them from pit to processing plant. There they are crushed and undergo different industrial processes.

Thus the opening, operation and closure of a limestone quarry represents a major perturbation to the natural landscape, creating significant visual and environmental impacts with soil, vegetation, fauna and habitat loss (Sort and Alcañiz 1996, Clemente et al. 2004, Moreno-Peñaranda et al. 2004). Excavation and movement of materials by heavy equipment contribute to the alteration of soil chemical, physical and biological properties, such as dramatically reducing organic matter and nutrients and increasing pH and bulk density. Vegetation on site is completely removed or destroyed.

2.3 Limestone Quarry Reclamation

Reclamation activities on mined areas will be determined by desired and/or mandatory end land uses; so specific revegetation approaches for limestone quarries and different end land uses need to be developed (Muzzi et al. 1997, Ursic et al. 1997). Limestone quarries are difficult to reclaim due to the coarse substrate, mineral deficiencies and often excessive drainage (Bradshaw and Chadwick 1980, Davis et al. 1985). Numerous ecological and geological factors compromise limestone quarry reclamation success (Ruiz-Jaen and Aide 2005, Clemente et al. 2004) especially when the reclamation end land use is reestablishment of predisturbed conditions (Wunderle Jr. 1997, Calow 1998). Without human augmented revegetation, mined areas can remain relatively unvegetated for decades or can be colonized by pioneer and noxious or unwanted species not commonly found in undisturbed conditions (Wheater and Cullen 1997, Cullen et al. 1998, Cooper and MacDonald 2000).

2.3.1 Soil reclamation

Reestablishment of soil and soil dominated processes such as nutrient cycling are necessary for reclamation success (Sort and Alcañiz 1996, Sort and Alcañiz 1999, Allen et al. 2003, Allen et al. 2005). Carbon cycling (Bailey et al. 2006), micro invertebrate abundance (Cullen and Wheater 1993, Wheater and Cullen 1997), appropriate amendments selection (Sopper 1993), soil chemical and physical properties (Vieira et al. 2005) and mycorrhizal interactions (Allen et al. 2003) are among the factors to consider. Improved soil structure on reclaimed sites is critically important to provide soil water and nutrients for plant growth and development (Davis et al. 1985, Clemente et al. 2004, Reid and Naeth 2005a, Reid and Naeth 2005b) and to provide site stability.

After limestone quarrying, soils are mostly characterized by complete loss of topsoil, compaction of the remaining soil, nutrient depletion and high pH that makes natural establishment of vegetation very unlikely. The successful establishment of a plant community could be achieved by soil reconstruction or conditioning through application of amendments to modify soil chemical and physical properties and properties, which adds organic matter, nitrogen, phosphorous and other essential nutrients for plants.

A commonly used technique to enhance degraded soils is application of soil amendments, mainly as cheap organic sources of plant nutrients. Numerous amendments can be used to make reclamation materials a more suitable substrate for revegetation. Amendments can be used to enhance soil conditions depending mainly on desired conditions to be achieved, previous soil properties and conditions, availability of the amendment and regulatory criteria (Walker 2002, Reid and Naeth 2005b).

During the last two decades soil amendments have been used in limestone quarry reclamation. In Spain, sewage sludge provided a source of nutrients and organic matter for vegetation establishment. Plant biomass was significantly higher with sludge (769 g m^{-2}) than without (294 g m^{-2}). Similar results were obtained for plant cover (96% with sludge, 78% without sludge) although the resulting species richness was lower with sludge (31 versus 42). Soil structure was improved with sludge application increasing water retention capacity (Moreno-Peñaranda et al. 2004). In Owl Canyon Quarry, United States of America, organic amendments such as manure and beet residue were tested. The beet residue treatment resulted in the richest, fastest growing vegetation (Mineral Information Institute 2011).

In Clipsham Old Quarry, United Kingdom, plant cover of early seral communities increased 78% with increased nutrient availability, protection against herbivory and sufficient seed input (Davis et al. 1985). Nitrogen, phosphorus, potassium and magnesium (Ceccon et al. 2003) fertilizer was applied at 25% of the minimum recommended rate for initial vegetation establishment (2.1 g m^{-2} nitrogen:phosphorus:potassium:magnesium at 2:2:1:1). In Mexico, survival and recruitment of seedlings of fast growing dominant tree species of tropical dry forest that naturally develop on limestone phosphorous limited soils showed high response to nutrient addition (phosphorus = 75 kg ha^{-1} nitrogen = 220 kg ha^{-1}), in interaction with light availability (Ceccon et al. 2003) during quarry reclamation.

In the Mediterranean, to improve water and nutrient conditions in soil, a water holding polymer (gel), fertilizer and mycorrhizal inoculum were applied in a limestone quarry revegetated with three native evergreen sclerophyllous shrub species (Clemente et al. 2004). Treatment responses were species specific suggesting different water and nutrient use strategies. The researchers recommended use of both gel and fertilizer in revegetation programs but not in combination with each other when revegetating with *Olea europea* L. (olive) and *Pistacia lentiscus* L. (mastic tree) because both species are well adapted to water and nutrient stress deficiencies

Fertilizers are sources of mineral elements essential to plant growth and development. Nitrogen application can be important to enhancing early growth. Although dual application of nitrogen and phosphorus may not increase yield in all soils, positive responses have frequently been observed. Since phosphorus is very immobile in soil, placement near roots is usually advantageous. Potassium fertilizers applied at ≥ 10 lbs K_2O ac^{-1} in direct seed contact commonly causes reduced germination and seedling growth and should be placed below or at the sides (Havlin et al. 2005). Slow release fertilizers have the advantage of releasing nutrients at a slower rate throughout the season. Elemental sulfur is recommended for lowering soil pH. Amount of sulfur to be added will depend on soil type and how much pH needs to be lowered (Jones 1982). To lower pH a value of 0.5 in sandy soils, the recommended rate is 0.5 Mg ha^{-1}. This rate may be appropriate for limestone quarry reclamation considering that the non usable ore of limestone quarries is coarse material with large sized particles. Sulfur as a nutrient for plant uptake occurs mostly as sulfate (SO_4). Small particles degrade much faster than large ones, speeding up the process of soil acidification.

Agricultural materials such as manure and hay or straw can be used as quarry amendments. Addition of manure increases soil organic matter. The carbon:nitrogen (C:N) ratio of the soil is unlikely to be affected by addition of manure and no clear effect on pH has been reported (Land Resources Network Ltd. 1983). In the first year the mineralization rate of manure will be approximately 25% (Moss et al. 2002). Hay and straw are physical improvers of soil. Although the carbon content of straw and wood shavings is very similar (40 to 50%), the organic matter fraction is more easily decomposable in straw (Miller et al. 2000). Application may require addition of fertilizer; otherwise soil available nitrogen will be immobilized. To retain water and minimize seed loss from wind, rain and runoff, hay mulch was successful in an abandoned quarry in Irish Cove Island, Nova Scotia (Hopper and Bonner 2003).

Forestry by products such as wood shavings and sawdust may be appropriate amendments in areas where local forestry operations can provide economical supplies. Wood shavings is an organic waste material with a high C:N ratio and supplemental nitrogen is required to maximize decomposition in most soils (Land Resources Network Ltd. 1983). Its application increases water infiltration and soil water content. Degradation of wood under Alberta climate conditions is accomplished by numerous fungi and bacteria. No agreement exists on appropriate application rates. Higher rates have been associated with better plant response (Land Resources Network Ltd. 1983), although excessive amounts have reduced revegetation significantly (Naeth 2011). To prevent net nitrogen immobilization, addition of 5 to 13 kg nitrogen per tonne of wood waste is usually sufficient. Pulp mill biosolids addition has been recommended to improve physical conditions in soil. Maximum native plant diversity has been achieved at lower rates of pulp mill biosolids application. Adding 5 to 13 kg nitrogen per ton of wood waste added is recommended (Land Resources Network Ltd. 1983).

Topsoil is the uppermost layer of soil (0 to 20 cm), where the majority of biological processes occur. It is rich in organic matter and nutrients. Many plant roots are located in this layer. Although the depth of topsoil varies from site to site, it is a valuable resource in reclamation. Its availability is limited and it is an expensive material for use in large areas. To optimize its benefits in limestone quarries reclamation, hypothetically it should be placed on top of the rocky substrate, preferably in a layer of 5 cm to support early plant germination, emergence and development. Clean fill is a mix of topsoil and subsoil, which may include substances such as clay and partially degraded parent material. It can have some of the benefits of soil such as nutrients, organic matter and good soil structure, but less than topsoil. The resource is often available in limited quantities from housing developments.

2.3.2 Revegetation

The changes in a plant community after perturbation are called succession, and can include changes in biomass, productivity, diversity and plant species composition among others (Odum 1969, Connell and Slatyer 1977, Calow 1998). Primary succession, where no plant propagules remain, will occur after severe perturbation such as quarrying. A major mechanism of successional change in heavily degraded soils is facilitation, where one group of plant species moderates the soil environment and paves the way for later successional species (Connell and Slatyer 1977). Establishment of a plant community through facilitation is more likely to occur on harsh physical conditions (Rousset and Lepart 1999, Bruno 2000, Maestre et al. 2001) such as those found in mined lands. For example, establishment of some species during primary succession, especially in resource limited environments, is highly dependent on previous establishment of environment modifier species (Crocker and Major 1955, Maestre et al. 2001, Gomez-Aparicio 2009). Nitrogen fixation in soil is normally accomplished by plants able to fix atmospheric nitrogen or associated with nitrogen fixing fungi or bacteria. Establishment of these species, mostly legumes which also enhance soil formation, is one of the first steps to be considered during species selection for a restoration project.

The human introduction of some plant species may improve biotic and abiotic conditions of a site, enhancing establishment of other species. Positive effects such as that produced by shrubs on the establishment of trees (Callaway and Davis 1998, Rousset and Lepart 1999), or nitrogen fixing plants on their neighbors (del Moral and Wood 1993, Gomez-Aparicio 2009) are key elements to promote when establishment of a plant community is desired with reclamation (Khater et al. 2003). The presence of undisturbed forest or other plant community fragments close to the degraded site could become a source of seeds, propagules and seed dispersers, enhancing the site's natural regeneration and enrichment of its floristic composition (Wunderle Jr. 1997, Rodrigues et al. 2004).

For restoration planning, analysis of natural regeneration processes represents a valuable starting point for selection of suitable species to be used (Khater et al. 2003). A heterogeneous floristic composition along the regeneration gradient in limestone quarries in Lebanon was found where annual R strategy taxa such as *Inula viscosa* L. (false yellowhead) and *Ainsworthia cordata* (N.J. Jacquin) Boissier (ainsworthia) dominated on very perturbed and degraded sites. Less degraded areas in the quarry were rich in herbaceous perennial or shrub species such as *Geranium dissectum* L. (cut leaf geranium), *Stachys distans* Benth. (distance woundwort), *Salvia triloba* L. (greek sage) and *Ptilostemon chamaepeuce* (L.) Less. (pink). On relatively non degraded areas woody and shrub perennials such as *Pinus brutia* Henry (turkish pine), *Pistacia palaestina* Boiss. (terebinth) and *Quercus calliprinos* Webb. (palestine oak) dominate, along with less stress tolerant taxa such as *Arbutus andrachne* L. (grecian strawberry tree) and *Cistus creticus* L. (pink rock rose) (Khater et al. 2003).

Changes in plant community structure are expected during reclamation. Effects on flora and invertebrate fauna were examined in a *Festuca Helianthemum* community in a limestone quarry near Thrislington Plantation, United Kingdom (Cullen and Wheater 1993). An 8.5 ha area of magnesian limestone grassland was assessed over a period of 8 years. An initial change in some aspects of community flora and invertebrate fauna structure was followed by a recovery period. Bare ground was still evident between relocated turfs early on, but it was successfully colonized by resident species later.

Selection of native species is critical in any restoration project (Davis et al. 1985, Martínez-Garza and Howe 2003, Alvarez-Aquino et al. 2004, de Souza and Batista 2004). Use of native species suitable to the conditions on mined sites will help to retain biodiversity on the reclaimed area (Gerling et al. 1996, Martínez-Garza and Howe 2003). Use of native species is a challenging problem in design and implementation of an optimal reclamation plan (Gerling et al. 1996; Davis et al. 1998, Martínez-Garza and Howe 2003, Riley et al. 2004, Reid and Naeth 2005b, Vieira and Scariot 2006). There is often a lack of information about native species properties and ecology. Seeds and propagules are often low in availability and high in cost. Their germination, emergence and survivability are often unknown. Competition from non native plants and major changes in periodicity and intensity of wet and dry seasons may be an issue. Long term monitoring is needed to determine the speed at which impacted environments achieve the desired community structure (Wheater and Cullen 1997).

For best results, plant species to be used in quarry reclamation should be chosen from among the local vegetation. Selection according to frequency of occurrence during succession will reflect their adaptability to local conditions and their relevance to restoration objectives (Khater et al. 2003).

Provenance of species for revegetation is a long standing debate in reclamation. It is generally considered preferable to introduce local ecotypes and cultivars in reclamation sites. For example, the effect of provenance on establishment of *Lotus corniculatus* L. (birdsfoot trefoil) at a limestone quarry and effect of geographical and ecological distance on plant survival, size and fecundity were addressed (Smith et al. 2005). Local plants had highest survival on treated plots but not on untreated plots where plants from other provenances performed well. Ecological provenance may influence plant fitness when translocated.

The plants chosen to revegetate a limestone quarry should survive and grow under low nutrients, including magnesium, potassium, sodium, iron, copper, zinc and low organic matter availability, high bulk density, low soil water, high pH, and often, steep slopes of various aspects. A variety of species should be used to approximate undisturbed conditions. Some species should be shadow forming trees due to their capability to reduce high air temperature and light irradiance. Use of nitrogen fixing plant species will enhance self sustainability of developing plant communities. Trees and shrubs will enhance revegetation processes.

Many species can be seeded on areas to be reclaimed if seeds are available. Transplanting trees and shrubs is most likely to speed up natural succession reducing the time of secondary forest vegetation establishment. Transplanted trees and shrubs had a high survival rate, particularly when retaining some of the native soil with the transplant to keep the natural occurring mycorrhizae which could play an important role in successful sapling establishment (Allen et al. 2005). Cuttings and stakes are commonly used as vegetative propagules of some local tree species. Stakes are preferred over smaller cuttings (Budowski and Russo 1993). Trees, shrubs, grasses and forbs will protect soil from wind and water erosion but may become a food source and habitat for wild fauna. Grazing and browsing can contribute to plant establishment through seeds dispersion, but may also contribute to stress on the newly established vegetation. Thus grazing and browsing need to be monitored and potentially controlled to facilitate successful revegetation.

Nutrient availability for plants to produce new roots and shoots when out planted has been a concern in severe disturbed sites. Nutrient reserves in the plant and at the site have an effect on successful plant establishment. Fertilization of the planting hole can inhibit root growth, partly associated with release of fertilizer nutrients acting to decrease the need for new root growth to extract soil nutrients (Jacobs et al. 2004). According to Van den Driessche (1985), the improved survival of *Pseudotsuga menziesii* (Douglas fir) seedlings following late season fertilization could have been due to the effect of elevated mineral nutrient reserves on root growth capacity, since plants from the nursery might have higher N concentrations and content than the plants from natural areas. Fertilizer placed directly in the planting hole resulted in greater seedling growth than placement in an adjacent hole for both Douglas fir and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) (Carlson and Preisig 1981, Carlson 1981). The use of slow release fertilizer had greater benefits than regular soluble water fertilizer in coniferous, increasing growth and nutrient concentration for a longer period of time (Haase et al. 2006)

3. RESEARCH PROGRAM OUTLINE AND OBJECTIVES

The series of experiments developed in this research program focused on the suite of processes inherent in primary succession with an interest in speeding up those processes through reclamation techniques directed towards the end plant community establishment. This meant a suitable substrate needed to be developed, plants needed to be introduced and ecological processes such as nutrient cycling and decomposition needed to be re-established or set on a trajectory towards re-establishment. Such endeavours require understanding the predisturbed ecosytem, limitations of the limestone quarry substrate relative to the predisturbed soils and the factors to be balanced in creating a substrate that will support or lead to development of the long term plant community.

Reclamation of limestone quarries has been difficult due to conditions that make sites unsuitable for plant establishment. The predisturbed plant community was the result of a complex array of conditions, strongly supported by the undisturbed soil. Thus an important challenge of limestone quarry reclamation is to develop a substrate with chemical, physical and biological characteristics capable of providing plants with the necessary conditions for establishment and survival.

To develop a suitable limestone quarry substrate, a series of greenhouse and field experiments were conducted. The residual limestone material after quarrying has low water and nutrient holding capacities, high pH, no nutrients, high bulk density and coarse structure. Materials are heterogeneous and ranged from plain crushed limestone to admixed soil and rock fragments of varying sizes. Different substrates and organic amendments available in the area, in combination with fertilizer and sulphur, were used in a greenhouse experiment to develop a suitable substrate for native forb and grasses establishment. Both organic and chemical amendments, including fertilizer, were required to modify those conditions. Adequate amendments and application rates were identified based on response of select native species in a second greenhouse experiment. These greenhouse experiments are described in Chapter 2, and their results were later used and tested in the field, at the Graymont Exhaw quarry.

When active mining concludes at a quarry section, the site is prepared for reclamation. New embankments similar to terraces are built every year to eventually, within the next decade, reach the mountain tree line. These activities had been occurring for decades at the quarry and so there are old and new embankments with diverse topography (steep slopes of varying aspect, flat areas), age, depth of overburden and soil layers and surrounding vegetation, all of which impact reclamation success. Amendment types that plants responded best to in the greenhouse were tested under field conditions at experimental sites at the Graymont Exshaw quarry and lime plant. The objectives were to develop reclamation substrates with locally available soil materials and amendments that would facilitate native plant species establishment and early plant community development on a limestone quarry, and assess to what extent the time of site construction and seeding, the use of amendments and erosion control blankets, the selected species in the seed mix and the heterogeneous characteristics of the sites affect reclamation outcome. This experiment is detailed in Chapter 3.

The end land use plant community at the field experimental site needs to include a diversity of herbaceous and woody species. Vegetation must be established from primary succession. Grasses and forbs with extensive rooting systems are important for their fast establishment and growth, which will increase litter biomass, which when decomposed will facilitate nutrient cycling, enhance soil structure, increase water and nutrient holding capacity, reduce erosion and attract pollinators, dispersers species and other biota. In natural succession, this facilitates establishment of woody species. Natural woody species establishment may be very slow and could be speeded with transplanting. To identify suitable woody species to be used in the field, select native trees and shrubs were transplanted into amended substrates at a quarry site to investigate how species type, season of transplanting and origin of plant material (nursery stock, undisturbed vegetation or propagules from LFH) may affect establishment of woody species at the quarry. This experiment is detailed in Chapter 4.

Quarrying activities alter soil layers and reduce the composition and amount of soil microorganisms. Soil microbial biomass is the primary agent for litter decomposition, nutrient cycling and energy flow in the soil ecosystem (Wardle 1992). About 80 to 90% of the total metabolic activity in the soil is carried out by fungi and bacteria, including actinomycetes (Brady and Weil 2008). Microorganisms respond quickly to anthropogenic disturbances and therefore are increasingly used as ecological indicators of ecosystem health. Biological diversity is an indicator of soil quality because high species diversity may reflect a high degree of functional diversity (Brady and Weil 2008). Re-establishment of soil dominated processes such as nutrient cycling, organic matter build up and decomposition and re-establishment of natural symbiosis such as mycorrhizae, an association between a fungus and plants, are critical to successful limestone quarry reclamation. Effect of amended substrates on microbial carbon and nitrogen and the fungi and bacteria colony forming units were assessed in amended soil of the Exshaw quarry and lime plant. Mycorrhizal colonization of select grass and woody species at the Exshaw quarry and plant disturbed and undisturbed sites was also assessed. Results are detailed in Chapter 5.

Chapter 6 completes the dissertation and includes a research summary of the chapters, management recommendations, limitations of the current research program and some suggested directions and requirements for future research that should be conducted. Documented responses of the early successional community to the newly created substrate provided new knowledge on how limestone quarries can be reclaimed. Management recommendations on the use and application rates of amendments, fertilizer and erosion control blankets; suitable grasses, forbs and woody species and favourable seeding season were summarized, together with highlights on the characteristics identified on the microbial community at the quarry.

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Figure 1-1. View of the Exshaw quarry, Alberta, Canada.

CHAPTER 2. LIMESTONE QUARRY SUBSTRATE AMELIORATION AND EARLY ESTABLISHMENT OF NATIVE GRASSES: GREENHOUSE STUDY

1. INTRODUCTION

Limestone derivates are of global importance to a number of industries including lime for metallurgic and agricultural uses and cement and rock aggregates for use in construction. Production of these derivates requires mining of large amounts of limestone rock involving extensive drilling and blasting. Limestone with the highest purity is selected, screened and crushed to achieve desired sizes for commercialization, or passed through kilns at very high temperatures to produce quick lime.

Some of the blasted rock will not meet the requirements for commercialization and will be left at the quarry or carried back to the quarry after selection and processing. This material, including boulders and rock fragments of varying sizes, will be used as base materials for quarry reconstruction and reclamation. When mountain sides are quarried, rocky materials are used to build terraces up to the mountain top. Materials composed of a mix of soil, smaller rock fragments, organic debris and dust are used on upper layers of the recontoured quarry. These materials have limited suitability for plant establishment and therefore, regulations require that the uppermost layers of recontoured quarries should be of soil. Although specific requirements vary, environmental regulators often recommend covering with 1 m of soil to facilitate vegetation establishment.

Limited soil availability and the poor quality of soil that is available, present considerable problems for reclamation of limestone quarries. Due to the large quantities of soil required to cover the quarried area, any available soil must be used, regardless of its quality. Amelioration of the limestone substrate materials could mean soil may be required in lower quantities or even replaced.

Soil amending is a common method for improving chemical and physical properties of soil. Amendments with high organic matter content can enhance seed germination, provide a microbial community for nutrient cycling and supply minerals which increase plant productivity (Noyd et al. 1996). Although many sources of organic amendments exists, availability at the reclamation site is desired to keep costs manageable. Sewage sludge has been used in limestone quarry reclamation (Hopper and Bonner 2003, Clemente et al. 2004, Moreno-Peñaranda et al. 2004, Almendro-Candel et al. 2007, Jimenez et al. 2007), as has wood bark and topsoil (Richardson and Evans 1986). Chemical amendments such as fertilizer have been used together with organic amendments to increase plant cover (Davis et al. 1985, Richardson and Evans 1986). Amendments with high concentrations of cellulose, such as wood or straw, may require addition of nitrogen fertilizer to improve plant growth (Land Resources Network Ltd. 1983) since nitrogen may be immobilized otherwise. Other organic amendments commonly used to improve soil properties are manure (Moss et al. 2002), municipal biosolids (Wester et al. 2003) and pulp mill biosolids (Eerden 1998).

Use of industrial and municipal by products such as sewage sludge and biosolids is economical and represents an alternative to their disposal in landfills which already have very limited space availability. However, they may oversupply nitrogen, phosphorus or metals if applied at high rates, potentially contaminating soil and water by leaching of nitrates or metals (Almendro-Candel et al. 2007), or changing the composition of soil microorganisms, flora and fauna (Yeates et al. 1997, Bardgett et al. 1999, Bardgett and McAlister 1999). Some guidelines limit nutrient and metal loads to land in Alberta (Land Resources Network Ltd. 1983, Soil Quality Criteria Working Group 1987) indicating upper thresholds for many soil properties. Little research has been conducted to determine whether application rates within the lower range of these thresholds can accomplish desirable outputs such as adequate vegetation establishment, decreased contamination risks and optimized amendments use while reducing costs.

Determining amount and kind of amendments to be used in limestone quarry reclamation requires understanding limiting properties of the substrate and requirements of the desired vegetation. Use of native plant species suitable to quarry site conditions will help retain biodiversity on the reclaimed area (Gerling et al. 1996, Martínez-Garza and Howe 2003) and increase plant establishment due to species adaptations to weather, soil and predators. Native species usually require fewer nutrients and management than non native species and are important for restoring ecosystem function and biological diversity (Lesica and

Allendorf 1999). Plant nutrient uptake depends on availability, species requirements and soil properties (Andrew and Robins 1969). Fertilizer can result in decreased native grass production compared to non fertilized sites (Huffine and Elder 1960), reduced species richness and increased colonization and dominance by invasive species (Green and Galatowitsch 2002, Brooks 2003).

Local amendments are often not available for reclamation in large amounts and thus knowledge on how much amendment will be effective is required. Little detailed research has been conducted for limestone quarry reclamation. We hypothesize some available amendments will mainly modify physical properties of limestone substrates, allowing for better plant root growth and nutrient and water retention. Other amendments will modify chemical properties of limestone substrates, increasing nutrient concentrations or modifying pH, resulting in increased plant establishment and growth. Combinations of amendments may potentiate their individual positive effects.

Greenhouse research can provide a controlled environment in which interactions at a small scale can be studied and evaluated prior to large scale field implementation. The objectives of this greenhouse research were to determine if limestone quarry substrates from the same quarry differ in their effect on plant establishment and growth, to identify readily available amendments that alone or in combination can ameliorate limestone quarry substrates to support native grass establishment, and to determine application rates of these amendments that will support native grass species establishment.

2. MATERIALS AND METHODS

2.1 Limestone Substrate Materials

Substrate materials were procured from the Graymont Exshaw plant and quarry, near Kananaskis, Alberta (51º 07’ N 115º 13’ W. The site occurred at an elevation of 1,350 m, on the north side of the eastern portion of the Bow River Valley, within the Mountain Forest ecoregion (Government of Alberta 2006).

Limestone materials not suitable for commercialization are utilized in quarry backfilling and recontouring as part of quarry reclamation. This material consists of limestone rock mixed with overburden. Three types of limestone substrates that will be referred to as gray, gray brown and crushed limestone were named based on simple visual differences in colour and processing. They were used in this research since they are readily available in large quantities for reclamation at the quarry and are similar to materials from other quarries. Crushed limestone, processed for commercialization, because of its smaller particle size, was expected to be better suited for plant establishment. Gray and gray brown limestone substrates had larger rock fragments compared to crushed limestone. Finer soil particles of gray brown limestone material had brown shades, likely containing more soil from a B horizon.

2.2 Amendment Materials

Eight readily available local amendments alone and in combination with fertilizer and sulfur were used to ameliorate the three limestone substrates. These amendments were expected to ameliorate substrate chemical and physical properties to make them more hospitable for revegetation. Amendments were *Phleum pratense* L (timothy) hay, *Hordeum vulgare* L (barley) straw, wood shavings, pulp mill biosolids, beef manure compost, beef manure mix, topsoil and clean fill. Topsoil, beef manure mix and wood shavings were procured from the University of Alberta research farm. Beef manure mix was a 6:1:1 mix of manure, waste feed and wood chips aged for two years at time of use. Beef manure compost (manure compost) was donated from a Calgary farm. Wood shavings were fine screened from pine and white spruce wood. Alberta Pacific Forest Industries Inc. provided the pulp mill biosolids (biosolids). Clean fill, which is a mix of topsoil with subsoil, was procured from the Exshaw quarry. Fertilizer was slow release Nutricote 14-14-14 Type 100 (14% total nitrogen with 7% ammoniacal nitrogen, 7% nitrate nitrogen, 14% available phosphate phosphorus and 14% soluble potash K_2O, with a polyolefin coating). Elemental sulfur was Garden Sulfur GreenEarth 92%.

Fertilizer was added to the limestone substrate to supply macronutrients (nitrogen, phosphorus, potassium), increasing initial plant growth and development. Sulphur was used to lower substrate pH, increasing initial plant germination, growth and development. The organic amendments hay, straw and wood shavings were used with the purpose of reducing limestone substrate bulk density and thus increasing below ground biomass production. The organic amendments of pulp mill biosolids, beef manure mix and manure compost were used to simultaneously modify chemical and physical properties of the substrates, providing nutrients to plants and reducing substrate bulk density to increase root biomass. Topsoil and clean fill were considered to improve physical and chemical conditions of the substrate, increasing seed soil contact, reducing bulk density and increasing substrate nutrient and water retention capacity.

2.3 Plant Species

Grasses native to the Exshaw area were selected to meet regulatory requirements and for adaptability to site conditions. Seeds of *Poa alpina* L. (alpine blue grass), Agropyron *trachycaulum* (Link) Maltex H.F. Lewis. (slender wheat grass), *Elymus innovatus* Beal. (hairy wild rye), *Festuca saximontana* Rydb. (rocky mountain fescue) and *Trisetum spicatum* (L.) K. Richter (spike trisetum) were obtained from Brett-Young Seeds and used as a seed mix.

2.4 Substrate and Amendment Characterization

Substrates, topsoil and clean fill properties were determined on one composited sample per material. Detailed salinity including sodium adsorption ratio, pH and electrical conductivity were determined in 1:2 soil to water saturated paste (Hendershot et al. 1993). Total carbon was determined by combustion (Nelson and Sommers 1996), total organic carbon by acid digestion then combustion (Skjemstad and Baldock 2007), total inorganic carbon and total inorganic carbon calcium carbonate ($CaCO_3$) equivalent by acid digestion (Loeppert and Suarez 1996). Total nitrogen was determined by combustion (LECO Corporation 2005), total organic nitrogen by kjeldahl procedure (Kalra and Maynard 1991). Available nitrate, phosphate and potassium was determined by modified kelowna extraction (Qiana et al. 1994); available sulfate by extraction with 0.01 M calcium chloride ($CaCl_2$) (Kowalenko 1993). Cation exchange capacity was determined by barium chloride ($BaCl_2$) extraction (Hendershot et al. 1993) and particle size distribution by hydrometer (Sheldric and Wang 1993). Data for manure compost and biosolids were from another project using the materials (Patterson 2008).

2.5 Greenhouse Procedures

Two greenhouse sessions of 12 weeks each were conducted. The first experiment ran from August to October 2006 to evaluate potential of eight amendments, fertilizer and sulfur to ameliorate gray, gray brown and crushed limestone quarry substrates. The second experiment ran from March to June 2007 on gray, the most common quarry substrate, to identify minimum suitable application rates of five amendments selected from the first greenhouse session. The experimental designs were completely randomized. Plastic pots of approximately 15 cm diameter were used. Treatments were seeded with the native grasses mix, five seeds per species, per pot. Pots were randomly located in the greenhouse where temperature was 21 ^oC with a 16 h photoperiod.

In the first experiment, crushed gray and gray brown limestone substrates were assessed with four fertilizer-sulfur treatments: fertilizer with sulfur, sulfur alone, fertilizer alone, no fertilizer and no sulfur. For each substrate and fertilizer-sulfur treatment, eight amendments and an unamended control were assessed on four replicates [3 limestone quarry substrates x 2 fertilizer levels (with fertilizer, without) x 2 sulfur levels (with sulfur, without) x 9 amendments including a control x 4 replicates = 432 pots]. Amendments were incorporated into substrates; topsoil and clean fill were placed as 5 cm thick layers on top substrates, similar to a field scenario. Fertilizer was applied as pellets and covered with 1.5 cm of substrate to avoid direct seed contact. Elemental sulfur was put on the surface.

In the first experiment, amendments were incorporated at field equivalent application rates: hay (3.5 Mg ha^{-1}), straw (3.5 Mg ha^{-1}), wood shavings (45 Mg ha^{-1}), pulp mill biosolids (45 Mg ha^{-1}), beef manure compost (30 Mg ha^{-1}) and beef manure mix (39 Mg ha^{-1}). Layers of topsoil and clean fill were equivalent to 482 Mg ha^{-1}. Fertilizer was applied at 1.1 Mg ha^{-1} and elemental sulfur at 0.5 Mg ha^{-1}. Rates were based on previous research in other harsh reclamation scenarios and on calculations so nitrogen and phosphorus concentrations did not exceed regulatory guidelines (Jones 1982, Land Resources Network Ltd. 1983, Henry et al. 1985, Miller et al. 2000, Reid and Naeth 2005).

In the second experiment, five amendments at three application rates combined with two fertilizer rates were used to amend gray limestone, the most dominant substrate material at Exshaw. Straw, wood shavings, pulp mill biosolids, beef manure compost and clean fill were incorporated at rates considered low, medium and high (Table 2-1). Each amendment treatment was tested with medium and low fertilizer application rates (0.55 and 0.27 Mg ha^{-1}) and replicated five times (5 amendments x 3 levels of amendment application rates x 2 levels of fertilizer application rates x 5 replicates = 150 pots).

2.6 Quantification of Plant Response

Number of plants established and their survival were recorded weekly. Number of plants per species and average species height were monitored on weeks 6 and 12 of each greenhouse session. Mean height was estimated by measuring the tallest and smallest plant per species in each pot. At the end of the experiment plants in each pot were clipped at ground level and sorted into species. Above ground biomass per species from each pot was oven dried to constant weight at 80 ^{o}C for 48 hours. Roots from each pot were collected by hand separating the root materials from the substrates, and oven dried at 80 ^{o}C for 48 hours.

2.7 Statistical Analyses

Data for plant density and above and below ground biomass in experiment 1 were analyzed with four way non parametric analysis of variance (ANOVA) after verifying data did not have normal distribution and equal variances. Fixed factors were substrate, fertilizer, sulfur, amendment. Analyses were with permutational multivariate analysis of variance (PERMANOVA v.1.6) (Anderson 2001, McArdle and Anderson 2001). Permutation tests calculate the probability of getting a value equal to or more extreme than an observed value under a specific null hypothesis by recalculating the test statistic after random data re-ordering (Anderson 2001).

Raw data were neither transformed nor standardized, Analysis was based on the measure of Euclidean distances. A total of 10,000 permutations of raw data were used in all tests (Larson et al. 2007, Chu et al. 2009). A posteriori pair wise comparisons were with PERMANOVA for factors with significant differences.

To identify differences in plant density and above and below ground biomass due solely to the amendment treatments, data were pooled and analyzed with

Kruskal-Wallis one way analysis of variance (ANOVA) on ranks and Tukey test pair wise comparison, using SigmaPlot 12 (Systat Software 2011).

Because of differences in individual species responses to treatments, and to determine effect of amendments within each fertilizer and sulfur combination, above ground biomass, plant density and height of individual species from all three substrates were pooled and analyzed within each fertilizer and sulfur combined treatment using one way ANOVA on ranks and Tukey test. For mean height of individual species, where species did not establish in every replicate pot of a treatment, the value was left blank rather than assumed to be zero, to avoid arithmetically reducing mean height values. Because blank cells result in unequal sample size, pair wise Dunn's test was performed when a significant difference in height was detected due to amendment treatment. Significance for all analyses was accepted at $p<0.05$. Graphics were done using SigmaPlot 12.

Above and below ground biomass data of experiment 2 were analyzed with three way non parametric analyses of variance after verifying the data did not have normal distribution and equal variances. Fixed factors were amendment, amendment rate and fertilizer rate. Raw data were neither transformed nor standardized; analysis was based on the measure of Euclidean distances. A total of 10,000 permutations of raw data were used in all tests (Larson et al. 2007, Chu et al. 2009). A posteriori pair-wise comparisons were done for factors with significant differences. Data was analyzed with PERMANOVA. Plant density data were normal, but were analyzed both with PERMANOVA and three way analyses of variance with SigmaPlot 12. Results were comparable.

3. RESULTS

3.1 Substrate and Amendment Properties

Gray and gray brown substrate materials had similar properties (Table 2-2). They were alkaline at pH 9.0 and 9.1, respectively, and coarse with at least 60% rock fragments ranging from 1 to 7 cm. Crushed limestone particle size was smaller at < 1 cm and pH was 8.8. Amendment properties varied. Total carbon, nitrogen and available nutrients were higher in organic amendments like beef manure compost and biosolids, than in soil amendments such as clean fill. Of the organic amendments, beef manure compost had highest electrical conductivity at 19.7 dS m^{-1} and biosolids had highest cation exchange capacity (Table 2-3).

3.2 Plant Response to Substrate (Experiment 1)

Plant density and above and below ground biomass were similar among gray, gray brown and crushed limestone substrates, with a consistent numerical trend for lower values of all plant response variables in the crushed material (Table 2-4). These differences were sometimes statistically significant for above ground biomass, but pairwise comparison of above ground biomass between substrates were not significant. In the following sections, unless otherwise indicated, analyses of pooled data from all three treated substrates is discussed. Tables and graphs for individual substrates can be found in Appendix A.

3.3 Plant Response to Fertilizer and Sulfur (Experiment 1)

Below and above ground biomass and were significantly higher in treatments with fertilizer whereas plant density decreased with fertilizer (Table 2-5). Above ground biomass was three times higher, below ground biomass was twice as high and there were at least 30% fewer plants with fertilizer than without. Although fertilizer and substrate interactions occurred, further multiple comparisons did not reveal significant differences for any of the plant variables. Use of sulfur did not significantly affect any plant variables but interactions with amendments occurred and will be discussed.

3.4 Plant Response to Amendments (Experiment 1)

Plant density generally increased over the first 6 weeks, then remained relatively constant to the end of week 12, with small drops in a few treatments (Figure 2-1). Averaged across substrates, plant density responded to amendments as follows: topsoil, clean fill, biosolids and wood shavings > straw > hay, manure mix and control > beef manure compost (Figure 2-2).

Above and below ground biomass was generally higher with amendments than without. Highest above ground biomass occurred with topsoil, clean fill and biosolids, followed by manure mix, manure compost and wood shavings (Figure 2-3). Lowest above ground biomass occurred with hay, straw and unamended controls (Figure 2-3). In some cases above ground biomass was lower with straw than in controls. Below ground biomass was highest with topsoil, clean fill and biosolids, followed by manure mix and beef manure compost; it was lowest in control, hay, wood shaving and straw treatments.

3.5 Plant Species Response to Treatments (Experiment 1)

Native grass species responded differently to treatments. Interactions among combinations of fertilizer, sulfur and organic amendments were numerous and plant responses varied within and among individual species. The plant property most affected by treatments was above ground biomass. Overall, biomass for all species was higher with fertilizer and with sulfur in treatments with amendments other than hay and straw or no amendment. Amendments can be divided into those associated with higher (biosolids, beef manure compost, manure mix, clean fill, top soil) and lower (straw, hay, wood shavings, unamended control) biomass. Wood shavings with fertilizer resulted in high above ground biomass.

Agropyron above ground biomass was highest with fertilizer and sulfur (Figure 2-4). It was highest with topsoil, clean fill and biosolids. Plant heights were similar with fertilizer regardless of organic amendment. Plant height was greater with fertilizer and sulfur combined with biosolids and beef manure (Table 2-6). Plant density was similar if sulfur was used, regardless of presence of fertilizer. Plant density was similar with organic amendments but fewer plants established with manure mix, clean fill and no amendments without fertilizer or sulfur. Fertilized wood shavings resulted in higher plant density without sulfur (Figure 2-5).

Elymus above ground biomass was similar across treatments (Figure 2-6). Results should be interpreted cautiously because of low establishment in all treatments. Plant height was greater with biosolids, manure mix, clean fill and beef manure amendments and lower with hay, straw and wood shavings (Table 2-6). Density was similar across treatments (Figure 2-7).

Festuca above ground biomass was similar with and without fertilizer but higher with sulfur amendment. Biosolids and manure mix, with or without fertilizer resulted in more above ground biomass than with fertilized wood shavings. Biomass was lowest in straw and unamended treatments (Figure 2-8). Height was greatest in fertilized treatments and with biosolids and manure mix (Table 2-6). Density was similar across treatments, being highest in fertilized topsoil without sulfur (Figure 2-9).

Poa above ground biomass was greatest with fertilizer and without sulfur. It was greater with biosolids, topsoil and fertilized wood shavings than with hay, straw and no amendment (Figure 2-10). Height was greatest with fertilizer and without sulfur. *Poa* height was greatest with biosolids, beef manure compost, clean fill, hay and topsoil organic amendments and lowest with straw, wood shavings and no amendment (Table 2-6). Density was similar across treatments, but sometimes lower with clean fill, wood shavings and no amendment (Figure 2-11).

Trisetum above ground biomass and height were greatest with fertilizer and sulfur. Above ground biomass was higher with biosolids, clean fill and fertilized wood shavings than with straw, wood shavings and no amendment (Figure 2-12) Height increased with biosolids, clean fill and manure mix and decreased with straw, wood shavings and no amendment (Table 2-6). Overall, density was lowest with fertilizer and similar across organic amendments (Figure 2-13).

3.6 Plant Response to Application Rates of Amendments and Fertilizer in Gray Limestone Substrate (Experiment 2)

As in the first experiment, plant density gradually increased over time during the first six weeks of the second experiment, remaining relatively constant to the end of the experiment at 12 weeks (Figure 2-14). Plant density differed significantly among amendments ($p<0.01$). It was higher with biosolids, clean fill and wood shavings than with beef manure and straw, and was not affected by amendment rates and fertilizer rates.

Above ground biomass was significantly affected by amendment, amendment rate and fertilizer rate ($p<0.01$). It was significantly higher with biosolids and clean fill than with beef manure compost and wood shavings and lowest with straw. Above ground biomass was significantly higher with high than medium amendment ($p<0.01$) (Figure 2-15). Interestingly it was not significantly different with high and low amendment rates. When rates were analyzed within each amendment, the high rate of clean fill was different than medium or low rates. The medium rate of straw was different than the low rate. Medium rate of fertilizer resulted in significantly more above ground biomass than the low rate.

Below ground biomass was significantly affected by amendment, amendment rate and fertilizer rate ($p \leq 0.01$). Medium fertilizer rate resulted in more below ground biomass than low fertilizer rate. Biomass was significantly higher with clean fill and biosolids than with wood shavings and beef manure compost. Below ground biomass was lowest with straw. Among amendments, high application resulted in more below ground biomass except with straw, which resulted in higher biomass at the low rate than medium rate (Figure 2-16).

3.7 Plant Species Response to Application Rates of Amendments and Fertilizer in Gray Limestone Substrate (Experiment 2)

Agropyron above ground biomass and height were greatest with high organic amendment and medium fertilizer rates (Figures 2-17 and 2-18), especially with high rate of clean fill. Heights were similar whenever medium fertilizer rate and high organic amendment rates were used. Density was similar except with beef manure compost medium rate with low fertilizer rate (Table 2-7).

Limited establishment of *Elymus* made it difficult to statistically compare its response to application rates (Table 2-7). The few established plants had overall higher biomass with low amendment and medium fertilizer rates and greater heights at high amendment and medium fertilizer rates (Figures 2-19 and 2-20).

Above ground biomass and height of *Festuca* were greatest with high amendment and medium fertilizer rates, particularly with biosolids (Figures 2-21 and 2-22). Heights were similar with medium fertilizer rate. Density was similar among amendments, except with straw when at high rate with medium fertilizer rate, and with beef manure at low rate with medium fertilizer rate (Table 2-7).

Above ground biomass and height of *Poa* were greatest at high amendment and medium fertilizer rates (Figures 2-23 and 2-24). Above ground biomass was particularly high with clean fill and biosolids, and with wood shavings if applied at high rate with medium fertilizer rate. Heights were greater at high amendment rate with medium fertilizer rate, but no different within the same treatment of amendment and fertilizer rate. Density was similar among amendments, except lower with straw at high rate with medium fertilizer rate, and with beef manure and straw at low rate with medium fertilizer rate (Table 2-7).

Above ground biomass of *Trisetum* was similar with amendments except with medium rate for amendments and low fertilizer rate (Figure 2-25). Overall, above ground biomass was higher with biosolids and beef manure compost at high rate with medium and low fertilizer rates. Lower plant height occurred with high rates of wood shavings, regardless of fertilizer rate (Figure 2-26). Density was similar with all amendments within the same combination of amendment and fertilizer application rates (Table 2-7).

4. DISCUSSION

4.1 Plant Response to Substrate

The slightly better performance of gray and brown substrate materials relative to crushed materials may be due to their higher proportion of particles > 9.55 mm (40 to 47%). These particles result in larger pore spaces where amendments and water could accumulate, creating better microsites for seed germination and plant establishment. Crushed limestone had 53% of particles > 4 mm, which may have created a more compacted surface less favourable for seedling emergence. Nonetheless, all of these substrates supported acceptable plant growth, and their use and further research should be considered particularly because in the majority of quarries mineral soil is in very short supply. By current practices and regulations, the limestone substrates will be covered with a soil layer during reclamation. Results suggest capping substrates with as little as 5 cm of soil could facilitate plant establishment and therefore the soil that is available could be used in thinner layers over larger areas.

4.2 Plant Response to Fertilizer and Sulfur

Chemical improvement of limestone substrates through fertilizer application in this study was similar to results from other studies. Smika et al. (1965) found biomass of native grasses increased linearly with increased rates of 22.5, 45, 90 and 180 kg ha^{-1} of ammonium nitrate (35% nitrogen). Eissenstat and Caldwell (1988) found fertilizer increased root density, 10 to 15 times at the points of liquid fertilizer injection compared to unfertilized sites.

Phosphorus in the slow release fertilizer likely contributed to the increased biomass compared to unfertilized treatments. Limestone substrates are very alkaline and phosphorus is often deficient in alkaline soils (Brady and Weil 2008). The greater shoot:root ratio with fertilizer application was likely directly due to increased nutrient availability (Ingestad and Agren 1991).

Fewer plants in fertilized treatments were reported in other research and may relate to seed sensitivity to components in fertilizer. Urea, one component in 14:14:14 fertilizer, is hydrolyzed in soil into ammonia which may adversely affect seed germination (Bremner and Krogmeier 1989). This response of seeds is species specific. For example, *Avena fatua* L. (wild oats) and invasive species may overcome dormancy with nitrogen fertilizer when soil water is available (Sexsmith and Pittman 1963, Sheppard et al. 2009). Therefore monitoring the plant community is important at the application site to verify unwanted species are not taking advantage of the modified soil nutrient conditions and dominating the plant community. Seed sensitivity for the species used in this study has not been determined, thus impacts on seed germination are speculative.

Although sulfur application slightly decreased limestone substrate pH by the desired 0.5 determined during experiment planning, the change had very little effect on plant properties. This may be due to the lower pH not being reached prior to seeding or the plants in the seed mix, being adapted to alkaline substrates. Sulfur application is recommended several weeks prior to seeding for pH decreases (Brady and Weil 2008). The benefits some species showed with sulfur when used with fertilizer and another amendment such as soil, biosolids and manure could be related to sulfur affecting concentrations of labile phosphorus in the soil and amendments which would thus lead to increased biomass. Despite not reducing pH of a calcareous organic soil in South Florida, sulfur applied at 448 kg ha^{-1}, a rate similar to that in our experiment, significantly increased labile phosphorus concentrations 2 months after application, which presumably favoured plant growth (Ye et al. 2011).

4.3 Plant Response to Amendments

Higher above and below ground biomass with topsoil and biosolids application may be associated with their ability to hold water and nutrients. Overall positive effects on grasses of the 5 cm soil layer provides support to the hypothesis that soil can be used in thinner layers than currently mandated. Large, heavily disturbed areas will require an unattainable amount of soil for capping and may hinder reclamation efforts and sustainability of soil at the source. Field research is required to establish an appropriate soil cover depth based on quarry conditions and evaluated to determine soil erosion by wind and water and long term plant performance. Different disturbance types will require specific soil capping depths (Jackson 2011); therefore sustainable capping practices must be scientifically developed and evaluated based on disturbance and site conditions.

The use of biosolids in limestone quarry reclamation may promote a more diverse plant community of native grasses, which is highly desirable for the end land use. Significantly lower amounts of biosolids are required, compared with the amount of soil needed to achieve similar native grass biomass. Other researchers found pulp mill effluent in irrigation increased plant biomass (Patterson et al. 2009). Our results have significant implications for management, transportation and resource sustainability because a 5 cm layer of soil may be equivalent to 482 Mg ha^{-1}, whereas biosolids could be applied at 45 Mg ha^{-1} or even the lower rate of 11 Mg ha^{-1} and achieve similar results for vegetation establishment when used as amendment for limestone substrate.

Amending with manure increased electrical conductivity in the substrate which can inhibit seed germination or plant growth for many species (Singh et al. 1997, Hao et al. 2003, Hao and Chang 2003). This increased electrical conductivity may have led to the reduced above ground biomass and plant density associated with application of manure mix and beef manure compost. Below ground biomass was similar to that with applications of topsoil or biosolids, which is desirable to increase soil organic matter and stabilize slopes at the quarry site.

Above ground biomass of grasses growing on substrates amended with wood shavings was similar to those amended with manure but the lower below ground biomass may be compromised. Roots of grasses on wood shavings treatments were abundant but were very fine and thin in diameter, likely because of the resulting lower bulk density. With low bulk densities, roots will have less anchoring and opportunity for nutrient and water uptake, limiting their growth and development. Roots increased in diameter when growth was limited by mechanical impedance in compacted or rocky soil (Clark et al. 2003).

Hay and straw were not good amendments for the limestone substrates as illustrated by the fewer plants and lower biomass than found in unamended substrates. The very low below ground biomass could be related to phytotoxic damage caused when seedling roots came into close contact with straw (Lynch et al. 1980, Elliott and Lynch 1984). Lower plant biomass may also be due to high carbon concentrations in the hay and straw resulting in nitrogen immobilization. Henriksen and Breland (1999) found that the nitrogen requirement for optimum decomposition of straw was 1.2% of dry matter. Therefore, higher applications of nitrogen as fertilizer or an organic amendment source would be required to improve vegetation growth in straw and hay amended substrates.

Plant species differ in nutrient uptake abilities and growth responses to nutrient availability (Andrew and Robins 1969, Chu et al. 2009). *Agropyron trachycaulum*, *Poa alpina*, *Festuca saximontana and Trisetum spicatum* established better in amended limestone substrates than in the unamended control, but *Festuca* established well in all treatments, even if unfertilized. Research focused on a small number of species likely to be used in limestone quarry reclamation. Thus interpretation of best amendments will need to be interpreted in this regard.

5. CONCLUSIONS AND APPLICATION TO QUARRY RECLAMATION

Gray, gray brown and crushed limestone materials were suitable as reclamation substrates with organic and inorganic amendment. Although gray and gray brown substrate materials were associated with slightly better overall plant performance than crushed materials, all of the substrates supported acceptable plant growth and should be considered for use and research in the field because soil will be in very low supply for quarry reclamation regardless of the global location.

Chemical amendment of limestone substrates through fertilizer application greatly increased above and below ground biomass of grasses despite decreasing the number of established plants. The 14-14-14 nitrogen, phosphorus, potassium fertilizer worked well if applied at 1.1 Mg ha^{-1} and not lower than 0.55 Mg ha^{-1}. Although sulfur was expected to lower substrate pH and thus improve plant response, it did not significantly affect any plant variables.

Topsoil, clean fill and biosolids were consistently the best amendments for plants. Topsoil and clean fill amendments were good as a 5 cm capping layer and other amendments worked when incorporated into the limestone substrate. Biosolids favoured establishment of more plant species and biomass than any other organic amendment even at the low application rate of 11 Mg ha^{-1}. The high rate of 45 Mg ha^{-1} is recommended to provide substrate and plants with more organic matter, nutrients and increased water holding capacity. Beef manure mix should be applied at a minimum of 39 Mg ha^{-1}, wood shavings at 45 Mg ha^{-1} and clean fill at 482 Mg ha^{-1}. Lower rates will result in poor plant performance. Straw, if applied, should be at a low rate of 3.5 Mg ha^{-1} and must be accompanied by fertilizer application to minimize nitrogen immobilization.

Plant species responded differently to amendments and amendment rates. *Agropyron*, *Festuca* and *Poa* performed best of the species tested. *Agropryon* had a significant increase in biomass when fertilizer and sulfur were used together. Soil, manure and biosolids favoured *Agropyron*, and fertilized soil amended substrate was the best treatment. *Elymus* responded best to fertilized soil amended substrate. *Festuca* had similar biomass with or without fertilizer. Biosolids and manure mix, with or without fertilizer were the best treatments. *Poa* biomass increased with fertilizer. Soil, manure and biosolids favoured *Poa*. *Trisetum* had a significant response to fertilizer but less pronounced than other species. Sulfur and fertilizer together increased its biomass. Soil, manure and biosolids favoured the species and fertilized biosolids was the best treatment.

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Table 2-1. Application rates of amendments on gray limestone used in the second greenhouse experiment.

	Application Rate Mg ha^{-1}		
Amendment	High	Medium	Low
Fertilizer		0.55	0.27
Straw	14	7	3.5
Manure Mix	39	19.5	9.75
Pulp Mill Biosolids	45	22.5	11.25
Wood Shavings	45	22.5	11.25
Clean Fill	482	241	120.5

Table 2-2. Particle sizes and pH of limestone substrate materials.

	Crushed	Gray	Gray Brown
Particle Size (%)			
>9.55 mm	0.0	40.0	47.0
Between 4 and 9.55 mm	53.3	31.6	29.3
Sand	27.5	16.3	20.2
Silt and Clay	19.2	12.1	3.5
Hydrogen Ion Concentration (pH)	8.8	9.0	9.1

Table 2-3. Properties of clean fill, beef manure compost and biosolids used in the greenhouse experiments.

Property	Beef Manure	Biosolids	Clean Fill
Total Carbon (%)	13.7	25.0	4.21
Inorganic Carbon (%)	0.3	2.8	2.0
Total Organic Carbon (%)	13.4	21.0	2.2
Calcium Carbonate Equivalent (%)	35.0	34.4	
Total Kjeldahl Nitrogen (%)	1.8	0.8	0.1
Available Nitrate Nitrogen (mg kg^{-1})		15.4	1.1
Available Phosphate (mg kg^{-1})		191.3	41.1
Available Potassium (mg kg^{-1})			205
Available Sulfate (mg kg^{-1})			14.4
Soluble Chloride (mg L^{-1})	6360		14
Soluble Calcium (mg L^{-1})	1480		252
Soluble Potassium (mg L^{-1})	8560		9
Soluble Magnesium (mg L^{-1})	1180		22
Soluble Sodium (mg L^{-1})	2820		8
Sodium Adsorption Ratio	13.3		0.13
Sulphate (mg L^{-1})	5060		69
Saturation (%)	120.0	411.4	82.2
Hydrogen Ion Concentration (pH)	6.7	8.0	7.6
Electrical Conductivity (dS m^{-1})	19.7	0.6	1.5
Cation Exchange Capacity (meq 100 g^{-1})	6.0	20.2	8.4
Sand (%)			50
Silt (%)			32
Clay (%)			18
Texture	Loam		Loam

Blank spaces indicate the parameters were not measured.

Table 2-4. Mean plant density and above and below ground biomass per pot of native grasses grown in limestone substrates.

Substrate	Density (plant pot^{-1})	Above Ground Biomass (g)	Below Ground Biomass (g)
Crushed	8.7 ± 0.4	1.0 ± 0.1	0.4 ± 0.1
Gray	9.5 ± 0.4	1.3 ± 0.1	0.5 ± 0.1
Gray Brown	9.2 ± 0.4	1.2 ± 0.1	0.5 ± 0.1

Density and above ground biomass n = 432 pots (4 replicates).

Below ground biomass n = 324 pots (3 replicates).

Values are mean ± standard error.

Values within a column were not significantly different at $p \leq 0.05$.

Table 2-5. Fertilizer effects on native grasses density and biomass in limestone substrate.

Treatment	Density (plant pot^{-1})	Above Ground Biomass (g pot^{-1})	Below Ground Biomass (g pot^{-1})
Fertilized	7.3 ± 0.3 b	1.7 ± 0.1 a	0.7 ± 0.0 a
Unfertilized	10.8 ± 0.2 a	0.5 ± 0.0 b	0.3 ± 0.0 b

Error bars are ± standard errors.

Density and above ground biomass n = 216, below ground biomass n = 162.

Different letters within columns indicate significant differences at $p<0.05$).

Table 2-6. Height (cm) of native grasses at each treatment averaged across substrates at the end of Experiment 1.

Treatment	*Agropyron*	*Elymus*	*Festuca*	*Poa*	*Trisetum*
Fertilizer - Sulfur					
Control	24.5 ± 4.8	30.6 ± 1.7	2.6 ± 0.1	12.5 ± 1.0	4.0 ± 0
Beef Manure Compost	35.7 ± 3.3	24.6 ± 3.0	6.2 ± 0.8	16.2 ± 1.2	12.4 ± 1.5
Biosolids	37.6 ± 3.5	32.6 ± 1.85	6.5 ± 0.4	13.8 ± 1.5	13.5 ± 1.7
Clean Fill	34.7 ± 2.2	29.4 ± 3.9	4.3 ± 0.3	11.2 ± 0.7	14.5 ± 2.2
Hay	26.8 ± 3.1	26.3 ± 2.3	4.6 ± 0.7	12.0 ± 1.3	10 ± 1.7
Manure Mix	33.3 ± 2.1	26.5 ± 3.1	6.1 ± 0.8	11.5 ± 1.7	11.5 ± 1.8
Straw	30.58 ± 2.4	26.8 ± 2.4	5.6 ± 0.6	10.9 ± 2.0	9.6 ± 1.3
Topsoil	33.4 ± 1.7	33.5 ± 2.4	5.0 ± 0.4	14.0 ± 1.5	9.7 ± 0.7
Wood Shavings	28.4 ± 2.5	22.2 ± 2.9	5.7 ± 0.7	11.6 ± 1.5	12.0 ± 1.2
Fertilizer – No Sulfur					
Control	26.5 ± 2.5	21.5 ± 3.0	2.0 ± 0	10.6 ± 2.0	13.3 ± 3.3
Beef Manure Compost	25.9 ± 4.8	24.0 ± 2.9	7.2 ± 0.3	15.1 ± 0.8	12.7 ± 2.6
Biosolids	30.6 ± 2.5	37.5 ± 1.0	6.3 ± 0.5	15.3 ± 1.1	9.5 ± 0.7
Clean Fill	31.4 ± 2.2	29.7 ± 1.2	5.4 ± 0.5	15.3 ± 2.7	11.1 ± 1.8
Hay	27.7 ± 2.4	33.0 ± 4.2	5.6 ± 0.5	12.0 ± 0.7	9.3 ± 0.7
Manure Mix	22.7 ± 4.0	34.1 ± 4.6	11.2 ± 3.9	13.5 ± 2.0	11.0 ± 1.1
Straw	24.8 ± 2.7	22.5 ± 3.4	4.7 ± 0.4	11.8 ± 0.6	7.0 ± 0.8
Topsoil	34.1 ± 1.3	25.0 ± 0	5.5 ± 0.2	13.7 ± 1.1	9.4 ± 0.7
Wood Shavings	29.7 ± 2.4	23.8 ± 3.4	5.1 ± 0.4	12.4 ± 1.5	10.4 ± 1.1

Mean ± standard errors.

Letters indicate significant differences among amendment treatments within same fertilizer and sulfur combination treatment.

Table 2-6. Height of native grasses at each treatment averaged across substrates at the end of Experiment 1 (cont'd).

Treatment	*Agropyron*	*Elymus*	*Festuca*	*Poa*	*Trisetum*
No Fertilizer - Sulfur					
Control	9.7 ± 1.0 b	10.0 ± 1.4 bc	2.3 ± 0.3 b	2.1 ± .2 b	2.8 ± 0.3 bcd
Beef Manure Compost	26.5 ± 2.3 a	21.0 ± 4.0 abc	5.0 ± 0.5 ab	9.3 ± 1.0 a	7.3 ± 1.3 ac
Biosolids	28.1 ± 1.3 a	34.6 ± 3.2 a	5.9 ± 0.4 a	10.3 ± .7 a	10.2 ± 0.9 a
Clean Fill	22.6 ± 1.6 a	16.8 ± 1.6 abc	3.9 ± 0.4 ab	5.5 ± 0.5 ab	7.7 ± 1.1 ab
Hay	17.0 ± 1.6 ab	19.0 ± 3.7 bc	5.0 ± 0.5 ab	7.2 ± 0.6 a	6.5 ± 0.6 ac
Manure Mix	25.8 ± 2.3 a	25.2 ± 2.3 ab	6.0 ± 0.5 a	9.9 ± 1.5 a	8.7 ± 0.7 ab
Straw	8.25 ± 0.4 b	7.2 ± 0.5 c	1.9 ± 0.1 b	2.2 ± 0.2 b	2.0 ± 0.2 cd
Topsoil	23.7 ± 1.6 a	19.5 ± 2.2 bc	4.3 ± 0.1 ab	8.3 ± 0.8 a	5.7 ± 0.5 acd
Wood Shavings	7.5 ± 0.4 b	7.5 ± 0.5 c	4.6 ± 2.0 b	1.5 ± 0.2 b	1.3 ± 0.1 d
No Fertilizer - No Sulfur					
Control	11.8 ± 1.2 bc	8.5 ± 0.4 b	2.2 ± 0.2 b	3.0 ± 0.4 b	3.1 ± 0.4 bc
Beef Manure Compost	24.9 ± 2.4 ab	29.5 ± 2.4 a	5.1 ± 0.3 ab	9.1 ± 0.7 ab	13.8 ± 1.5 a
Biosolids	25.5 ± 2.5 ab	24.8 ± 1.9 ab	6.3 ± 0.4 a	10.9 ± 1.3 a	10.4 ± 0.6 a
Clean Fill	21.8 ± 1.7 ab	17.1 ± 1.2 ab	2.9 ± 0.2 b	6.8 ± 0.7 ab	5.6 ± 0.5 abc
Hay	19.8 ± 2.9 ab	24.0 ± 3.6 ab	4.6 ± 0.5 ab	7.7 ± 1.0 ab	7.2 ± 0.9 ab
Manure Mix	24.5 ± 2.0 ab	23.5 ± 4.3 ab	6.1 ± 0.4 a	9.5 ± 0.9 ab	10.1 ± 0.7 a
Straw	8.7 ± 0.4 bc	6.1 ± 0.1 b	2.6 ± 0.3 b	3.5 ± 0.5 b	3.3 ± 0.4 bc
Topsoil	26.2 ± 1.9 a	18.7 ± 1.0 ab	4.6 ± 0.2 ab	9.0 ± 0.5 ab	6.5 ± 0.6 abc
Wood Shavings	6.3 ± 0.5 c	6.6 ± 0.9 b	1.6 ± 0.1 b	1.8 ± 0.3 b	1.5 ± 0.1c

Mean ± standard errors.

Letters indicate significant differences among amendment treatments within same fertilizer and sulfur combination treatment.

Table 2-7. Density (plants pot^{-1}) of native grasses species in each treatment at the end of Experiment 2.

	Amendment	*Agropyron*	*Elymus*	*Festuca*	*Poa*	*Trisetum*
Amendment High Fertilizer Low	Beef manure compost	0.8 ± 0.3	0.4 ± 0.2	2.2 ± 0.7	0.4 ± 0.2	0.2 ± 0.2
	Biosolids	1.4 ± 0 .4	0.6 ± 0.4	3 ± 0.3	1.6 ± 0.2	1.4 ± 0.5
	Clean fill	2 ± 0.4	0.8 ± 0.2	2.4 ± 0.4	3 ± 0.4	1.6 ± 0.6
	Wood shavings	1.6 ± 0.4	0 ± 0	3.4 ± 0.6	2.2 ± 0.5	1 ± 0.3
	Straw	0.8 ± 0 .4	0.2 ± 0.2	1.2 ± 0.8	0.2 ± 0.2	0 ± 0
Amendment High Fertilizer Medium	Beef manure compost	1.4 ± 0.2	0.2 ± 0.2	2.6 ± 0.5 ab	0.8 ± 0.2 ab	1.2 ± 0.3
	Biosolids	2 ± 0.7	0 ± 0	2.8 ± 0.5 ab	1 ± 0.3 ab	2 ± 0.3
	Clean fill	1.6 ± 0.5	0.4 ± 0.2	2.4 ± 0.7 ab	1.4 ± 0.4 ab	1.4 ± 0.6
	Wood shavings	2.8 ± 0.5	0 ± 0	4.4 ± 0.2 a	2 ± 0.4 a	2.4 ± 0.2
	Straw	0.6 ± 0.2	0.2 ± 0.2	1.4 ± 0.6 b	0 ± 0 b	0.8 ± 0.3
Amendment Medium Fertilizer Low	Beef manure compost	0.6 ± 0.4	0.2 ± 0.2	3.2 ± 0.9	1 ± 0.4	0.8 ± 0.3
	Biosolids	1.2 ± 0.3	0.6 ± 0.2	2.2 ± 0.3	2.4 ± 0.5	1.4 ± 0.2
	Clean fill	1 ± 0.3	0.4 ± 0.2	3 ± 0.7	3 ± 0.4	1.8 ± 0.5
	Wood shavings	1.4 ± 0.2	0.2 ± 0.2	2.6 ± 0.6	3.8 ± 0.7	1 ± 0.7
	Straw	0.4 ± 0.4	0 ± 0	1.2 ± 0.4	1.6 ± 0.9	0.2 ± 0.2
Amendment Medium Fertilizer Medium	Beef manure compost	0 ± 0 b	0 ± 0	2 ± 0.5	1 ± 0.3 b	2 ± 0.3
	Biosolids	0.6 ± 0.2 ab	0.2 ± 0.2	2.4 ± 0.5	1 ± 0.3 ab	1.4 ± 0.4
	Clean fill	1.8 ± 0.5 ab	0.2 ± 0.2	2.8 ± 0.3	2.4 ± 0.2 a	0.8 ± 0.3
	Wood shavings	2 ± 0.5 a	0.4 ± 0.2	4 ± 0.6	1.4 ± 0.6 a	1.4 ± 0.2
	Straw	0.6 ± 0.4 ab	0 ± 0	2 ± 0.8	0.8 ± 0.3 b	0.6 ± 0.4
Amendment Low Fertilizer Low	Beef manure compost	0.8 ± 0.3	0 ± 0	2 ± 0.4	1.8 ± 0.3	1.4 ± 0.4
	Biosolids	1.4 ± 0.5	0.2 ± 0.2	3 ± 0.7	1.6 ± 0.2	1.8 ± 0.8
	Clean fill	1.6 ± 0.4	0.2 ± 0.2	3.2 ± 0.8	2 ± 0.5	1.8 ± 0.3
	Wood shavings	1.6 ± 0.8	0 ± 0	3.2 ± 0.8	2.2 ± 0.5	1.2 ± 0.4
	Straw	1.6 ± 0.5	0.4 ± 0.2	3 ± 0.4	2 ± 0.4	0.6 ± 0.4
Amendment Low Fertilizer Medium	Beef manure compost	0.6 ± 0.4	0 ± 0	0.6 ± 0.4 b	0.6 ± 0.2	1 ± 0.6
	Biosolids	2.4 ± 0.5	0.4 ± 0.4	2.8 ± 0.3 a	2 ± 0	1.8 ± 0.3
	Clean fill	2 ± 0.5	0.2 ± 0.2	1.6 ± 0.4 ab	2.6 ± 0.5	1.6 ± 0.5
	Wood shavings	1.6 ± 0.9	0.4 ± 0.2	2.4 ± 0.4 ab	2.4 ± 0.5	1.4 ± 0.4
	Straw	0.6 ± 0.2	0 ± 0	1.6 ± 0.5 ab	0.6 ±0.4	0.8 ± 0.5

Mean ± standard errors.
Letters indicate significant differences among amendment treatments within same amendment and fertilizer rate treatment.

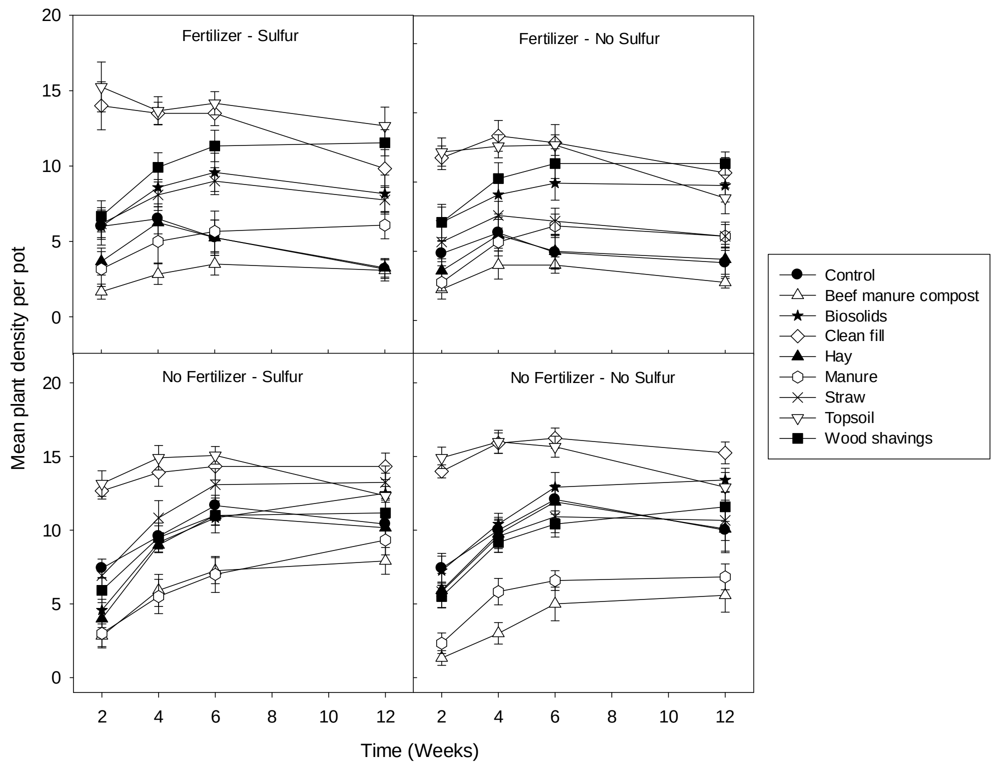

Figure 2-1. Mean plant density over 12 weeks averaged across limestone substrates with and without fertilizer and sulfur and nine organic and soil amendments. Error bars are ± standard error. N = 12.

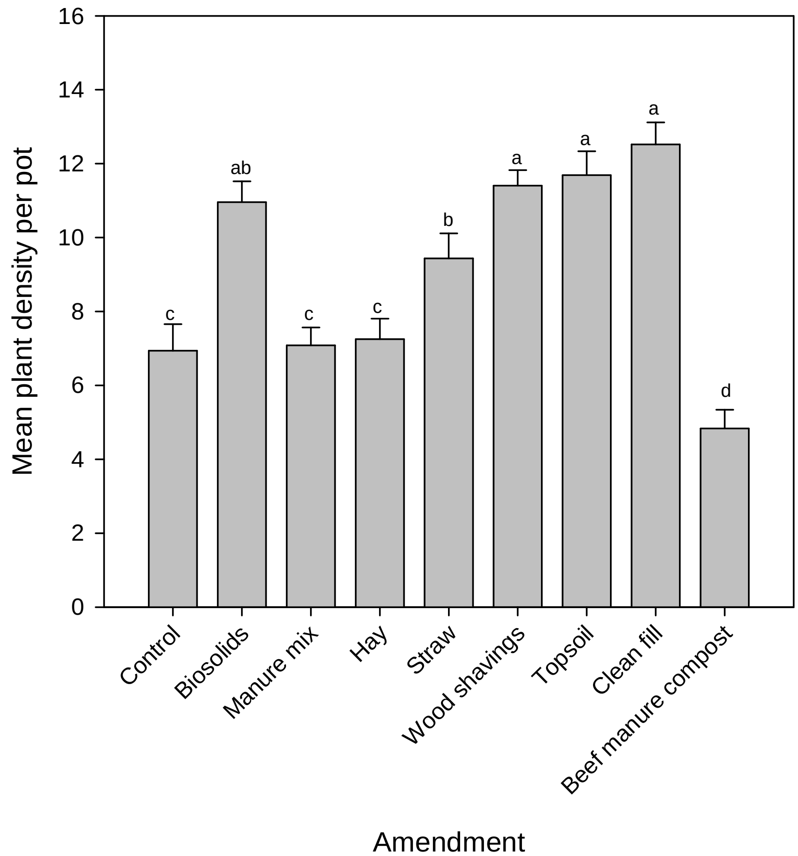

Figure 2-2. Plant density response to amendments averaged across substrates at the end of the experiment in week 12. Error bars are ± standard errors. Different letters indicate significant differences. N = 48.

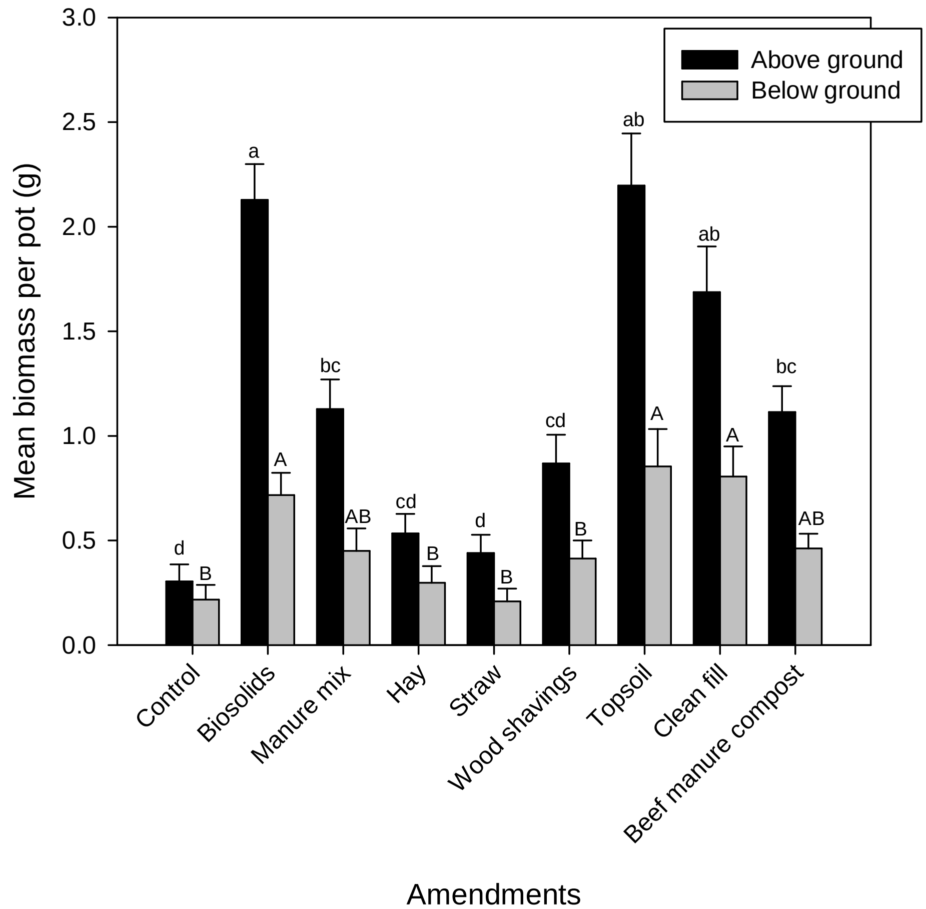

Figure 2-3. Above and below ground biomass response to amendments at the end of week 12. Error bars are ± standard errors. Letters indicate significant differences among treatments; lower case letters for above ground biomass (n = 48) and upper case letters for below ground biomass (n = 36).

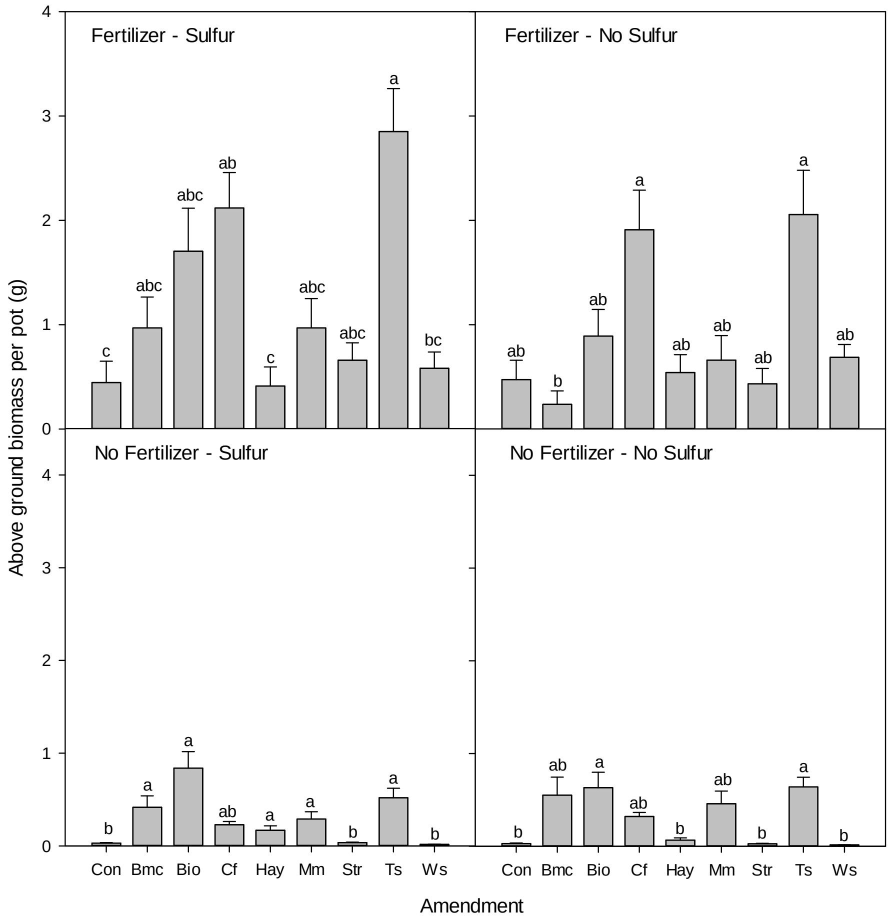

Figure 2-4. Above ground biomass of *Agropyron trachycaulum* at each amendment averaged across substrates at the end of the experiment in week 12. Error bars are ± standard errors. N = 12. Different letters indicate significant differences among amendment treatments within a fertilizer and sulfur combination treatment. Amendments are control (Con), beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), Hay, straw (Str), topsoil (Ts), wood shavings (Ws).

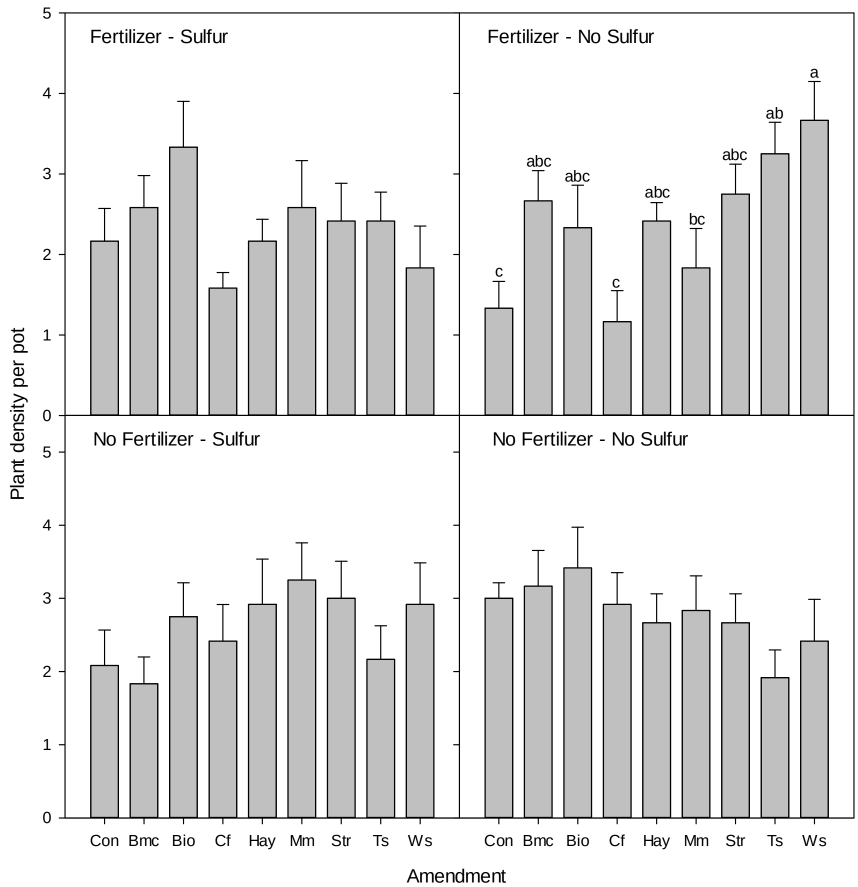

Figure 2-5. Plant density per pot of *Agropyron trachycaulum* at each amendment averaged across substrates at the end of the experiment in week 12. Error bars are ± standard errors. N = 12. Different letters indicate significant differences among amendment treatments within a fertilizer and sulfur combination treatment. Absence of letters indicates no statistical differences. Amendments are control (Con), beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), Hay, straw (Str), topsoil (Ts), wood shavings (Ws).

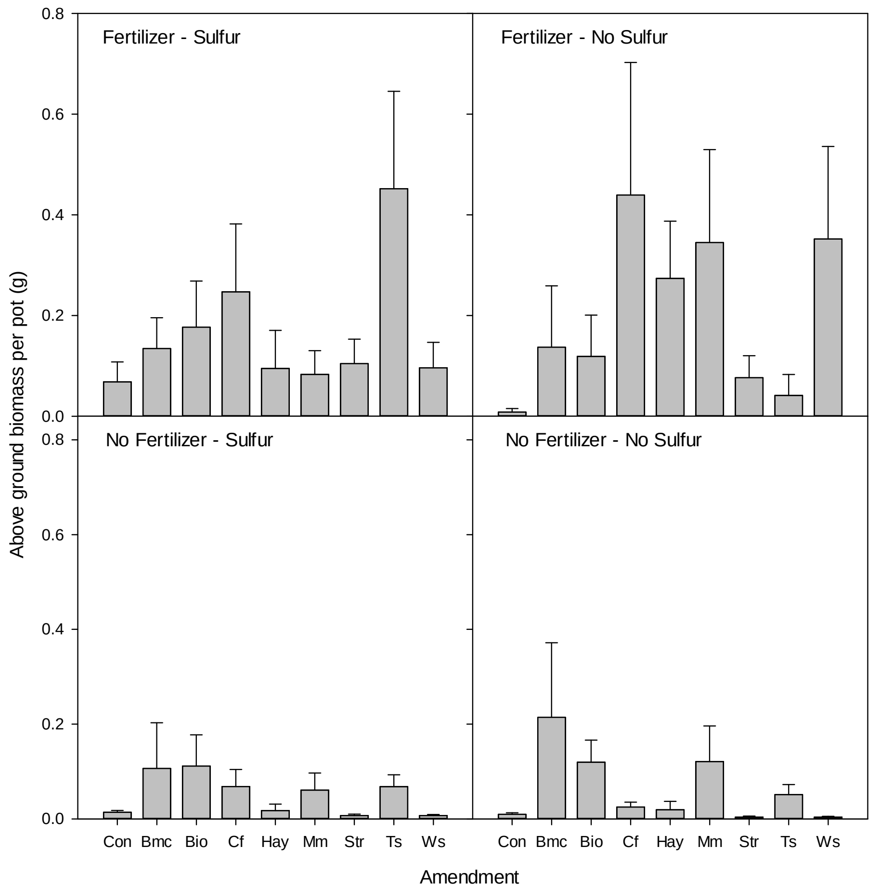

Figure 2-6. Above ground biomass of *Elymus innovatus* at each amendment averaged across substrates at the end of the experiment in week 12. Error bars are ± standard errors. N = 12. There were no statistical differences. Amendments are control (Con), beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), Hay, straw (Str), topsoil (Ts), wood shavings (Ws).

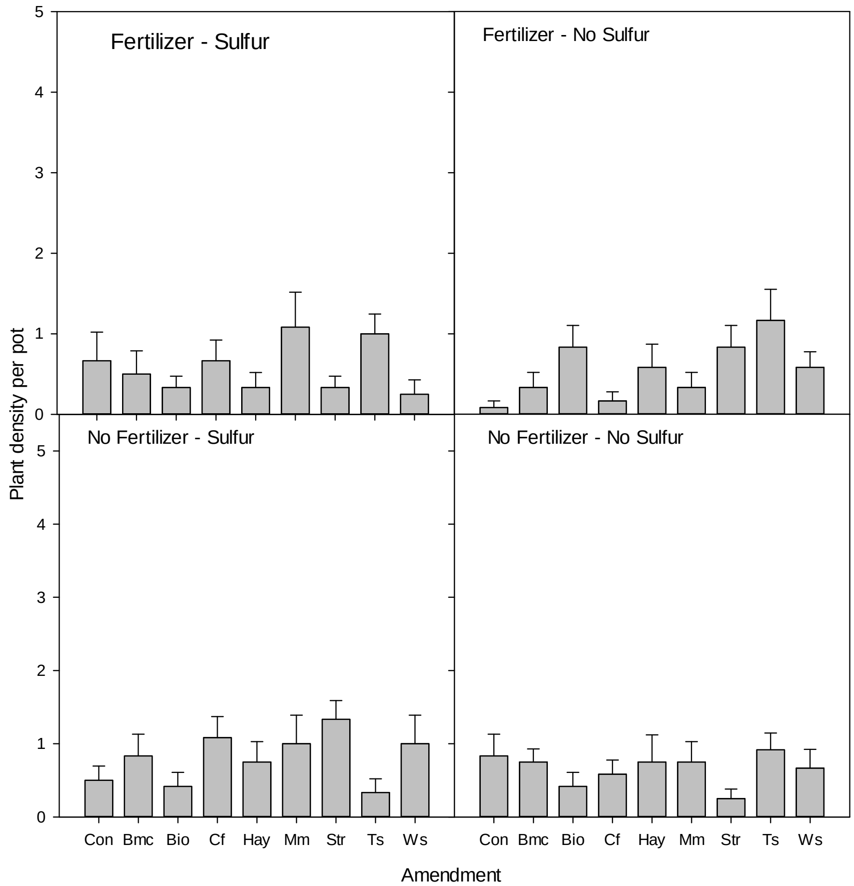

Figure 2-7. Plant density per pot of *Elymus innovatus* at each amendment averaged across substrates at the end of the experiment in week 12. Error bars are ± standard errors. N = 12. There were no statistical differences. Amendments are control (Con), beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), Hay, straw (Str), topsoil (Ts), wood shavings (Ws).

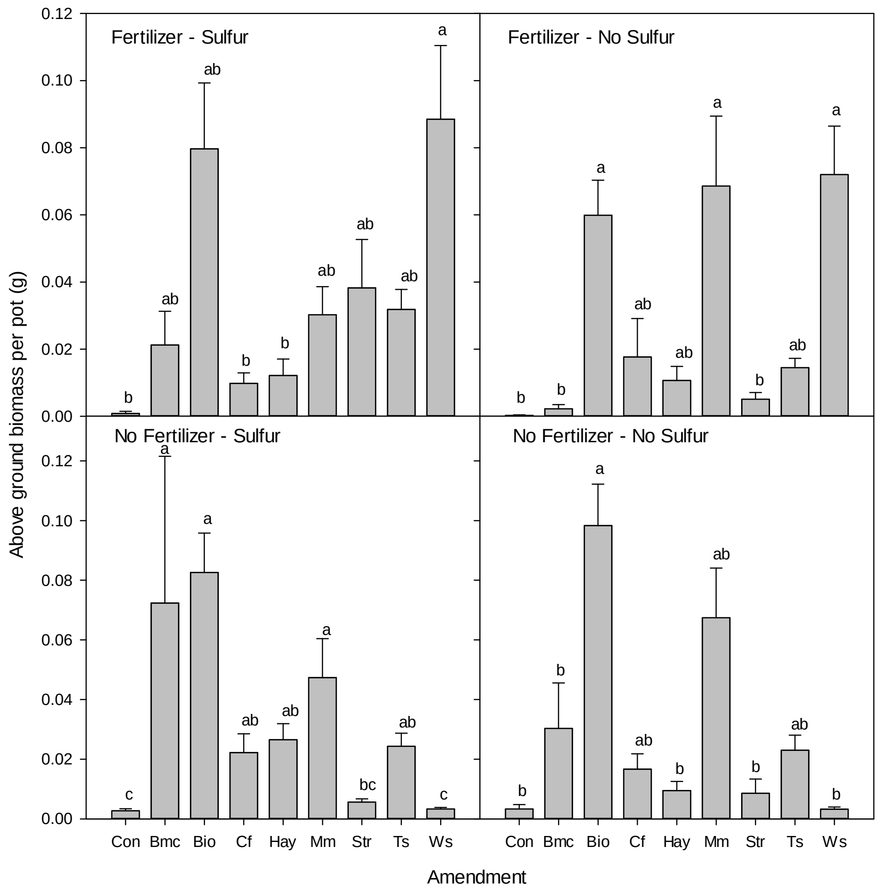

Figure 2-8. Above ground biomass of *Festuca saximontana* at each amendment averaged across substrates at the end of the experiment in week 12. Error bars are ± standard errors. N = 12. Different letters indicate significant differences among amendment treatments within a fertilizer and sulfur combination treatment. Amendments are control (Con), beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), Hay, straw (Str), topsoil (Ts), wood shavings (Ws).

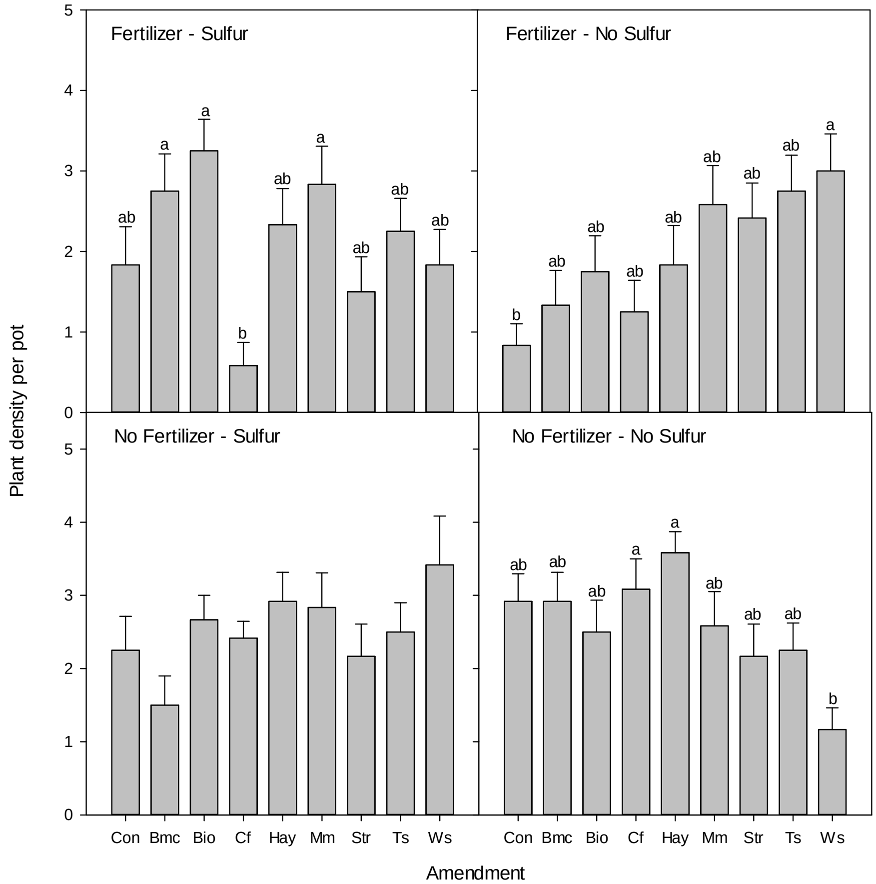

Figure 2-9. Plant density per pot of *Festuca saximontana* at each treatment averaged across substrates at the end of the experiment in week 12. Error bars are ± standard errors. N = 12. Letters indicate significant differences among amendment treatments within a fertilizer and sulfur combination treatment. Absence of letters indicates no differences. Amendments are control (Con), beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), Hay, straw (Str), topsoil (Ts), wood shavings (Ws).

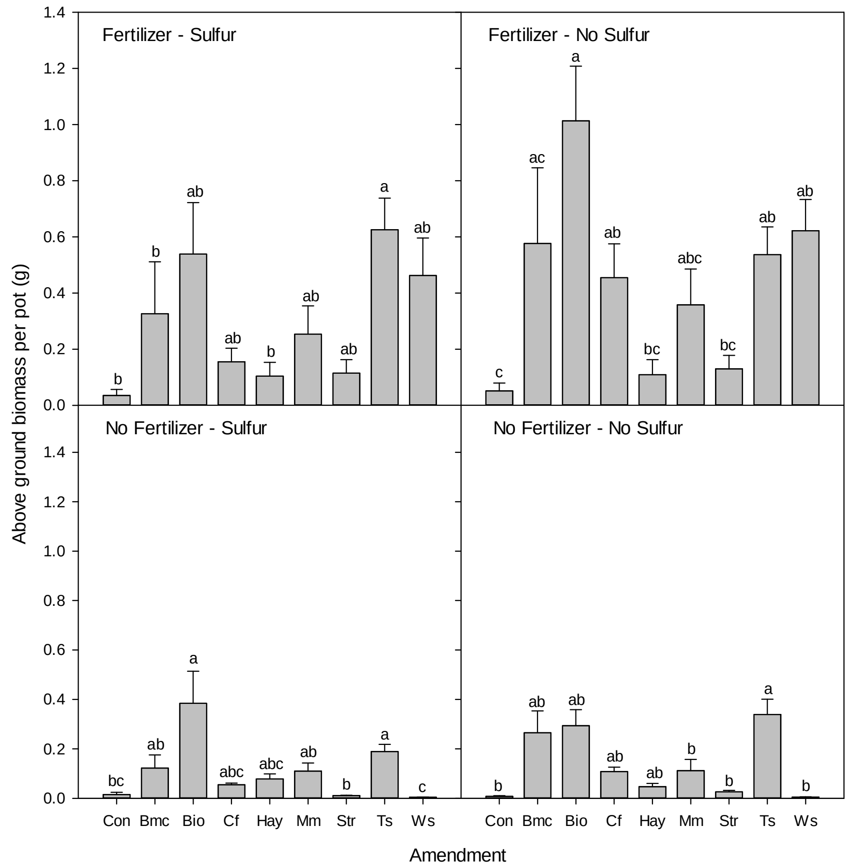

Figure 2-10. Above ground biomass of *Poa alpina* at each amendment averaged across substrates at the end of the experiment in week 12. Error bars are ± standard errors. N = 12. Different letters indicate significant differences among amendment treatments within a fertilizer and sulfur combination treatment. Amendments are control (Con), beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), Hay, straw (Str), topsoil (Ts), wood shavings (Ws).

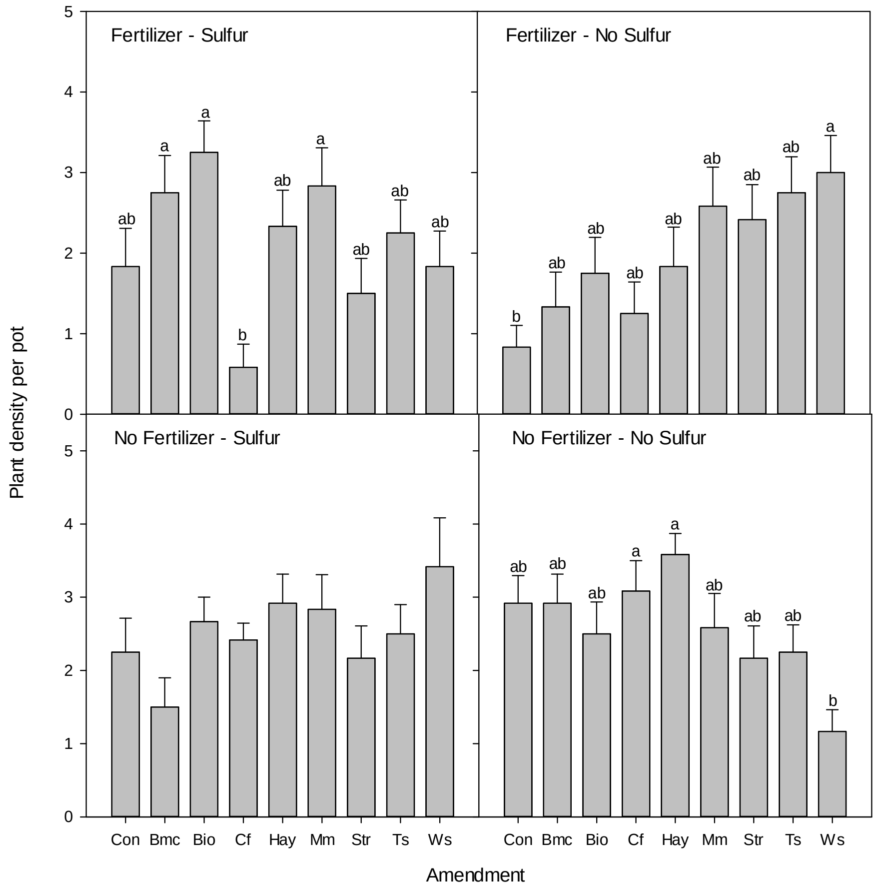

Figure 2-11. Plant density per pot of *Poa alpina* at each amendment averaged across substrates at the end of the experiment in week 12. Error bars are ± standard errors. N = 12. Different letters indicate significant differences among amendment treatments within a fertilizer and sulfur combination treatment. Absence of letters indicates no statistical differences. Amendments are control (Con), beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), Hay, straw (Str), topsoil (Ts), wood shavings (Ws).

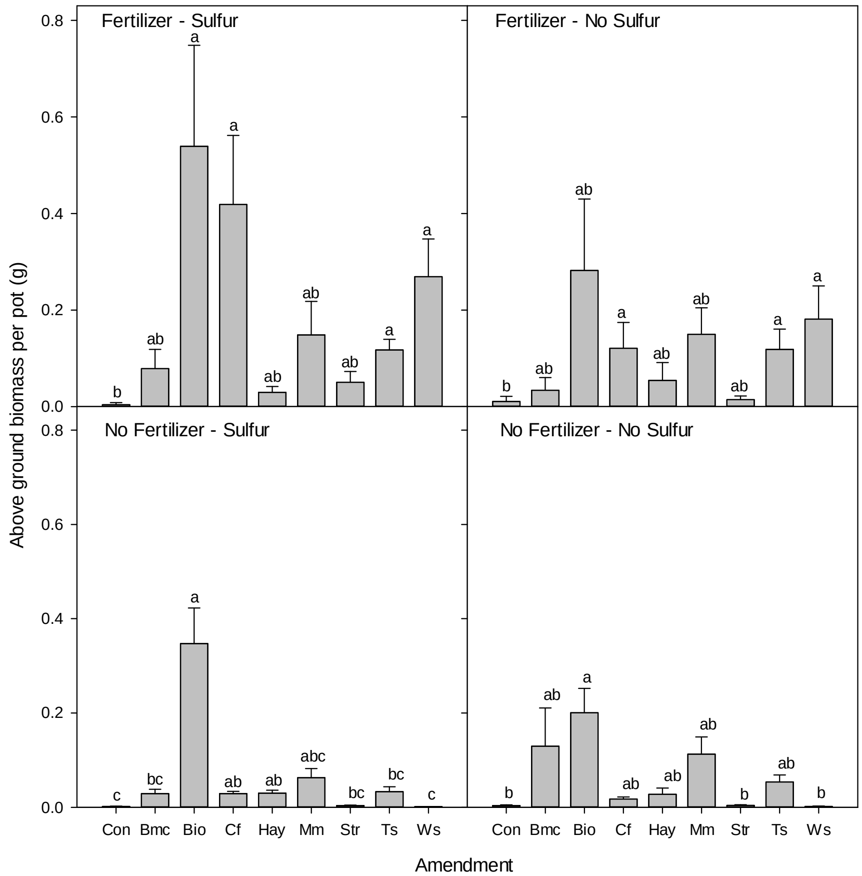

Figure 2-12. Above ground biomass of *Trisetum spicatum* at each amendment averaged across substrates at the end of the experiment in week 12. Error bars are ± standard errors. N = 12. Different letters indicate significant differences among amendment treatments within a fertilizer and sulfur combination treatment. Amendments are control (Con), beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), Hay, straw (Str), topsoil (Ts), wood shavings (Ws).

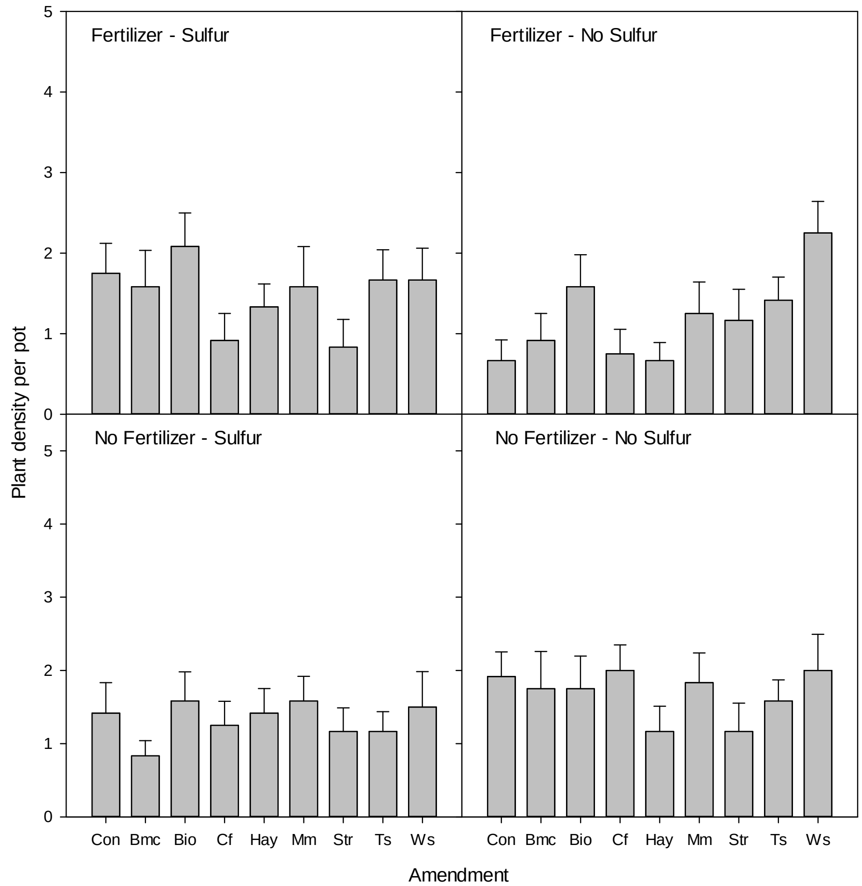

Figure 2-13. Plant density per pot of *Trisetum spicatum* at each amendment averaged across substrates at the end of the experiment in week 12. Error bars are ± standard errors. N = 12. There were no statistical differences. Amendments are control (Con), beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), Hay, straw (Str), topsoil (Ts), wood shavings (Ws).

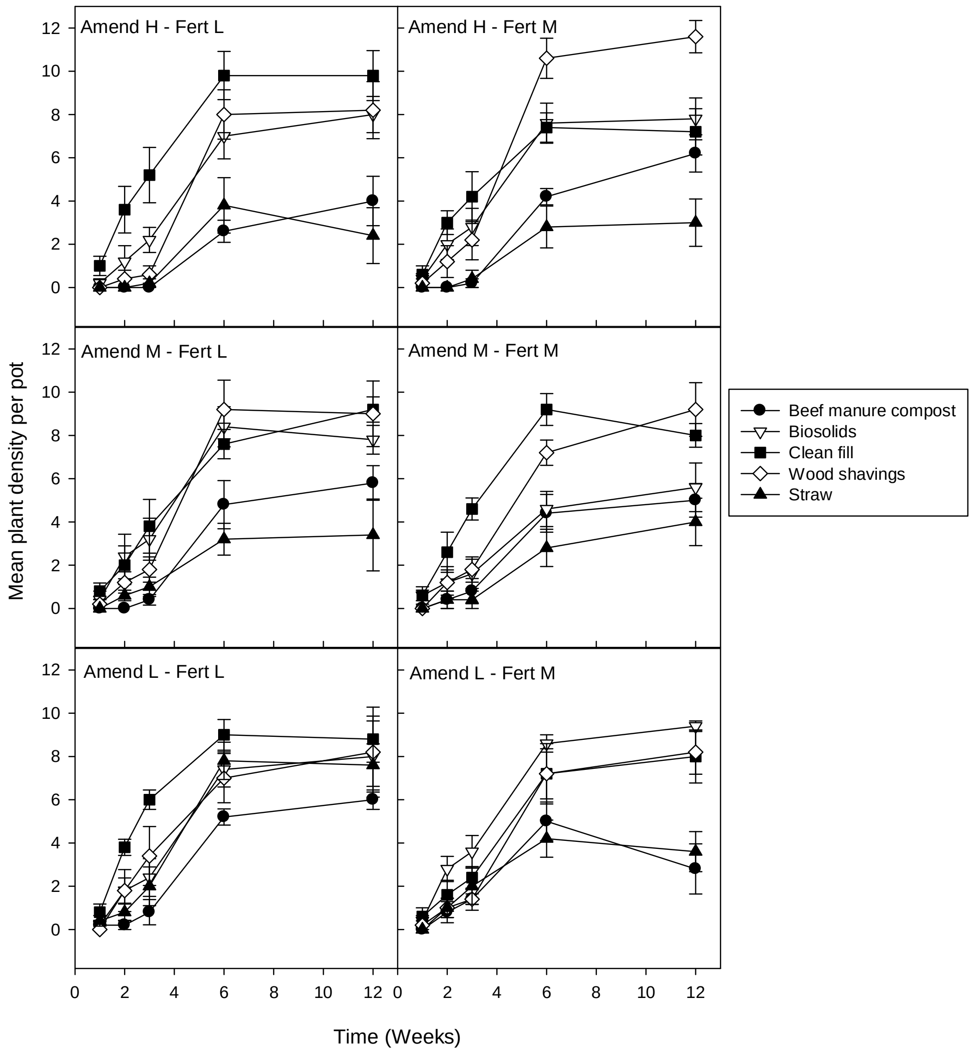

Figure 2-14. Plant density per pot over 12 weeks. Gray substrate was treated with high (top row), medium (center row) and low (bottom row) amendment rates and low (left column) and medium (right column) fertilizer rates. Error bars are ± standard errors. N = 5.

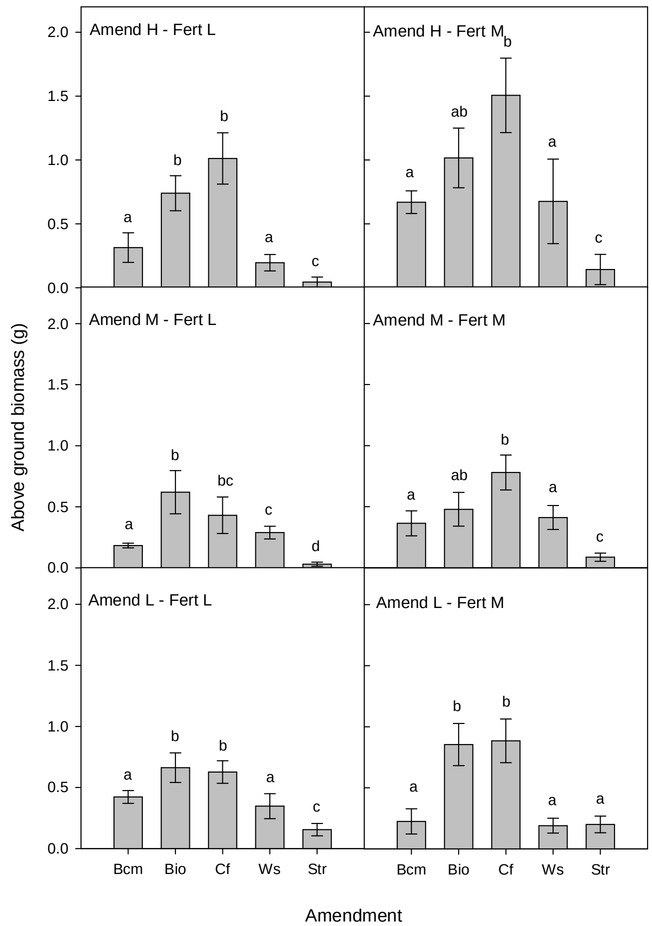

Figure 2-15. Native grass above ground biomass per pot in gray limestone substrate with high (top row), medium (center row) and low (bottom row) amendment application rates, and low (left column) and medium (right column) fertilizer application rates. Error bars are ± standard errors. N = 5. Different letters indicate significant differences among amendments within fertilizer and amendment rates combination treatments. Amendments are beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), wood shavings (Ws) and straw (Str).

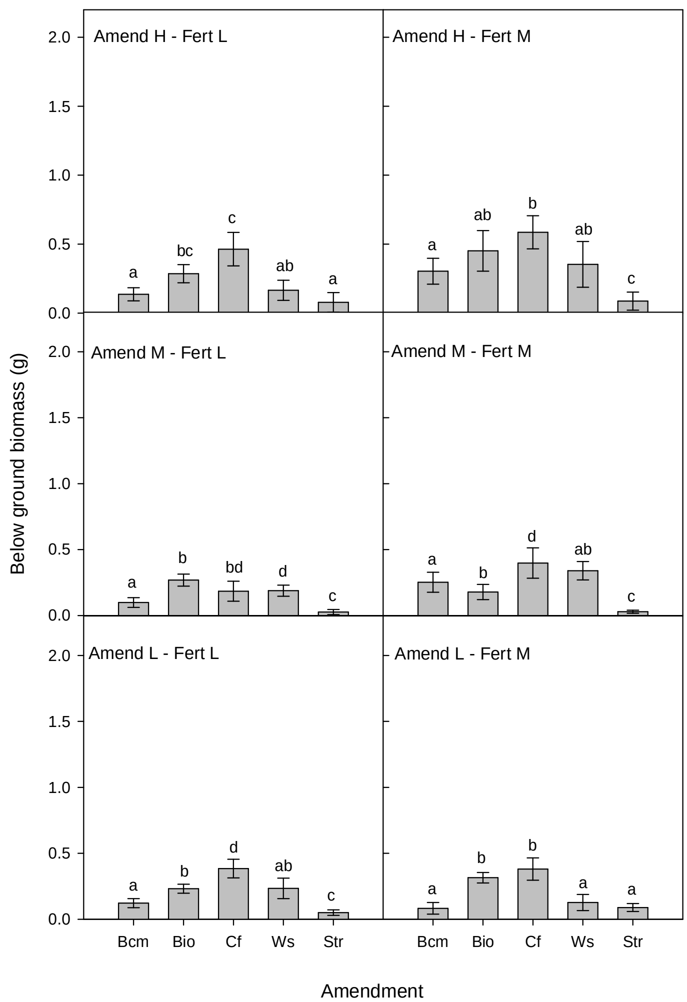

Figure 2-16. Native grass below ground biomass per pot in gray limestone substrate with high (top row), medium (center row) and low (bottom row) amendment application rates, and low (left column) and medium (right column) fertilizer application rates. Error bars are ± standard errors. N = 5. Different letters indicate significant differences among amendments within fertilizer and amendment rates combination treatments. Amendments are beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), wood shavings (Ws) and straw (Str).

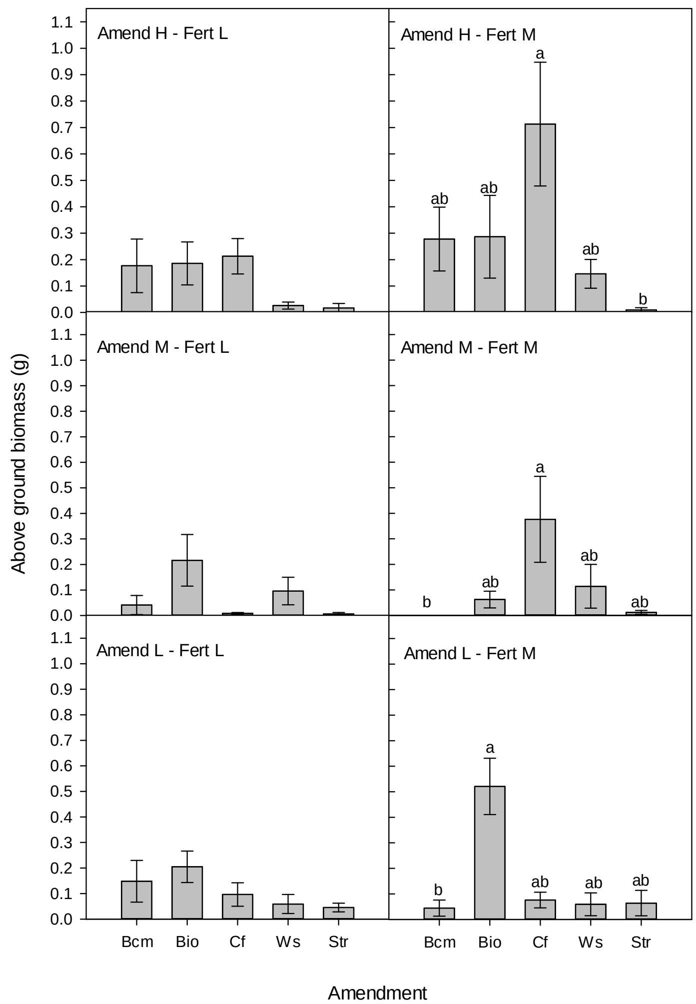

Figure 2-17. Mean above ground biomass of *Agropyron trachycaulum* in gray limestone substrate with high (top row), medium (center row) and low (bottom row) amendment application rates, and low (left column) and medium (right column) fertilizer application rates. Error bars are ± standard errors. N = 5. Different letters indicate significant differences among amendments within fertilizer and amendment rates combination treatments. Absence of letters indicates no statistical differences. Amendments are beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), wood shavings (Ws) and straw (Str).

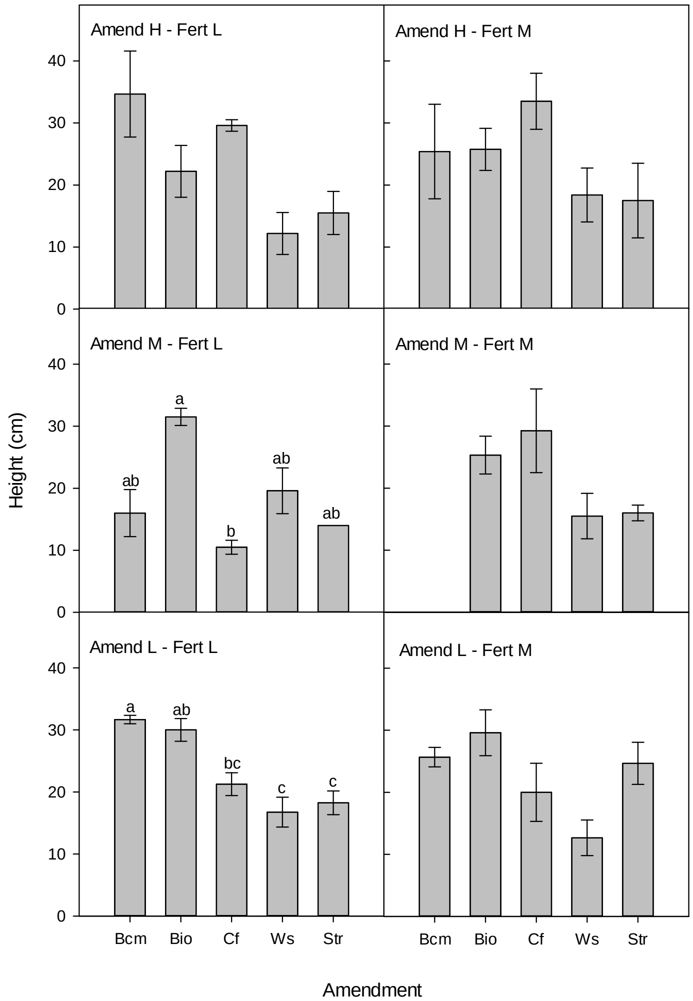

Figure 2-18. Mean height of *Agropyron trachycaulum* in gray limestone substrate with high (top row), medium (center row) and low (bottom row) amendment application rates, and low (left column) and medium (right column) fertilizer application rates. Error bars are ± standard errors. N = 5. Different letters indicate significant differences among amendments within fertilizer and amendment rates combination treatments. Absence of letters indicates no statistical differences. Amendments are beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), wood shavings (Ws) and straw (Str).

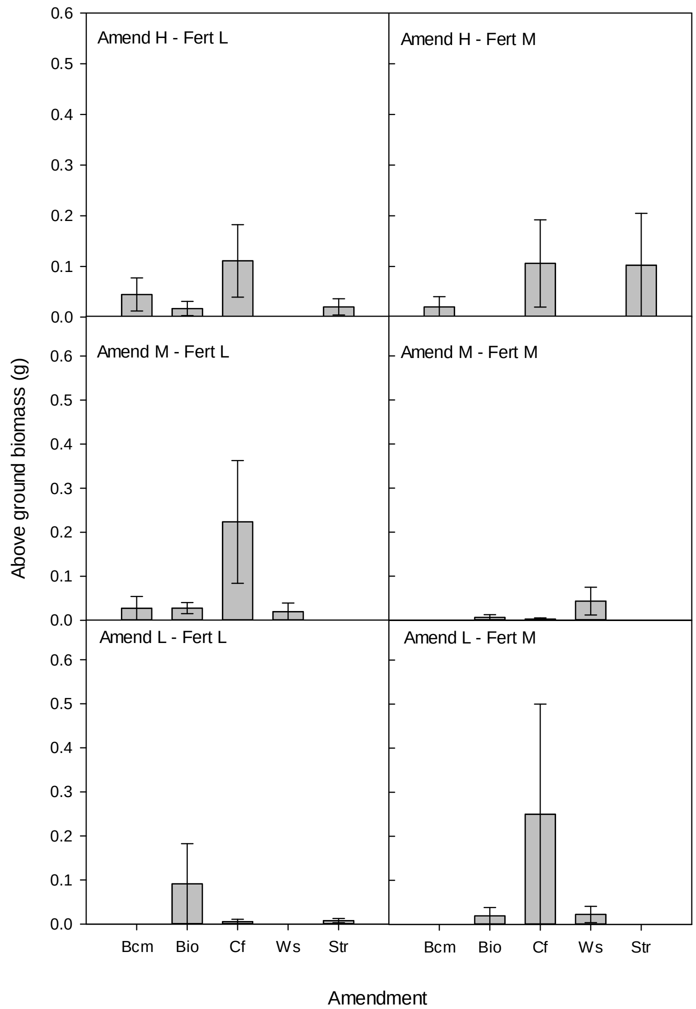

Figure 2-19. Mean above ground biomass of *Elymus innovatus* in gray limestone substrate with high (top row), medium (center row) and low (bottom row) amendment application rates, and low (left column) and medium (right column) fertilizer application rates. Error bars are ± standard errors. N = 5. There were no statistical differences. Amendments are beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), wood shavings (Ws) and straw (Str).

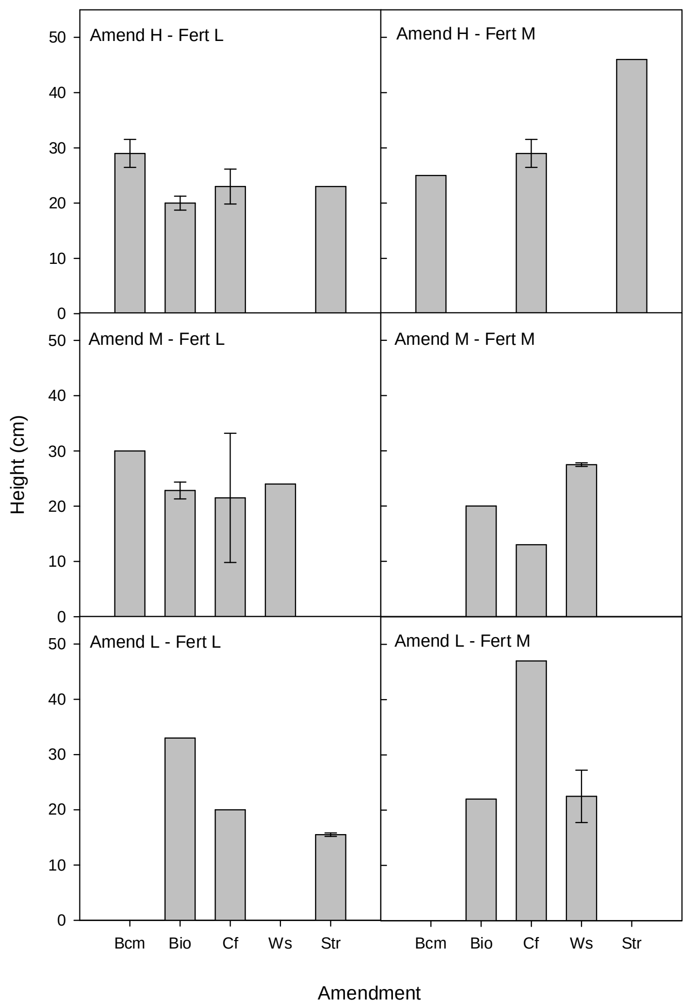

Figure 2-20. Mean height of *Elymus innovatus* in gray limestone substrate with high (top row), medium (center row) and low (bottom row) amendment application rates, and low (left column) and medium (right column) fertilizer application rates. Error bars are ± standard errors. N = 5. There were no statistical differences. Amendments are beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), wood shavings (Ws) and straw (Str). Lack of establishment appears as absent columns and or error bars.

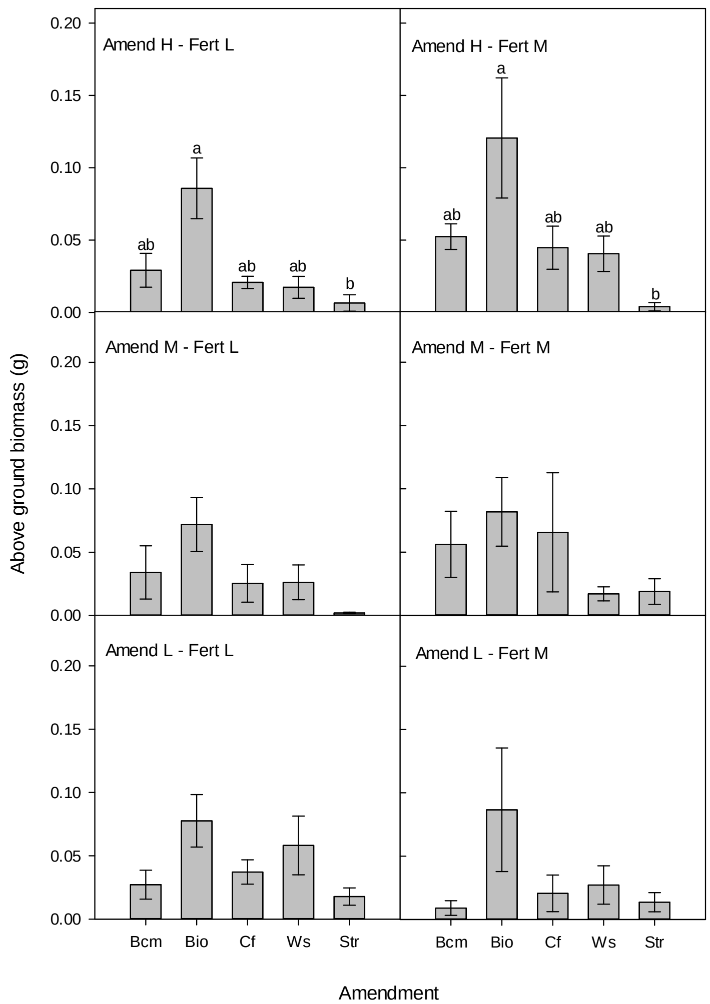

Figure 2-21. Mean above ground biomass of *Festuca saximontana* in gray limestone substrate with high (top row), medium (center row) and low (bottom row) amendment application rates, and low (left column) and medium (right column) fertilizer application rates. Error bars are ± standard errors. N = 5. Different letters indicate significant differences among amendments within fertilizer and amendment rates combination treatments. Absence of letters indicates no statistical differences. Amendments are beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), wood shavings (Ws) and straw (Str).

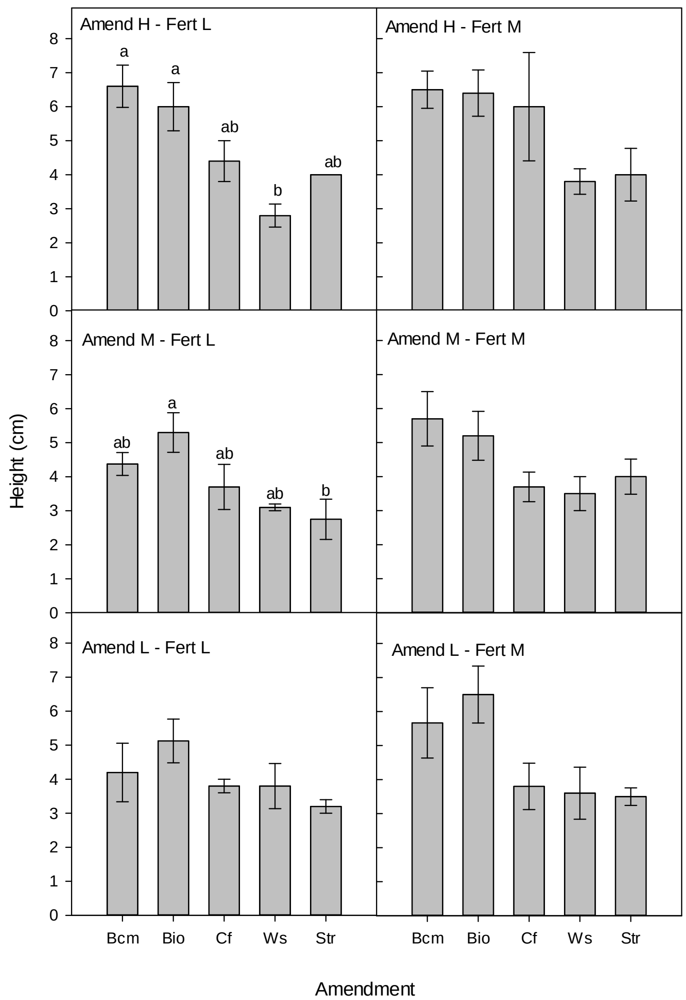

Figure 2-22. Mean height of *Festuca saximontana* in gray limestone substrate with high (top row), medium (center row) and low (bottom row) amendment application rates, and low (left column) and medium (right column) fertilizer application rates. Error bars are ± standard errors. N = 5. Different letters indicate significant differences among amendments within fertilizer and amendment rates combination treatments. Absence of letters indicates no statistical differences. Amendments are beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), wood shavings (Ws) and straw (Str).

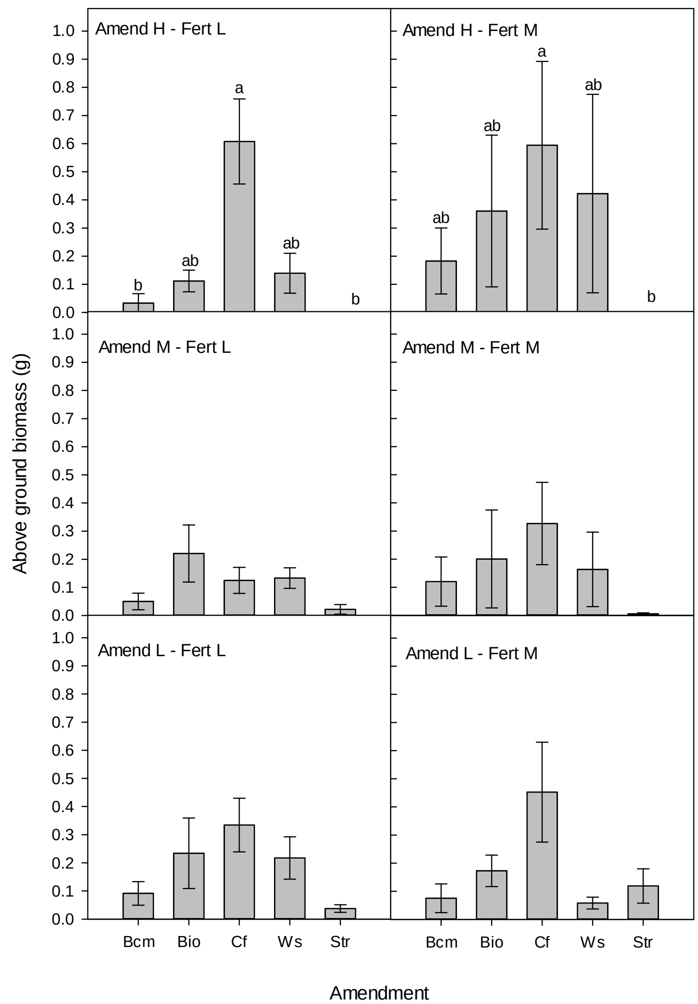

Figure 2-23. Mean above ground biomass of *Poa alpina* in gray limestone substrate with high (top row), medium (center row) and low (bottom row) amendment application rates, and low (left column) and medium (right column) fertilizer application rates. Error bars are ± standard errors. N = 5. Different letters indicate significant differences among amendments within fertilizer and amendment rates combination treatments. Absence of letters indicates no statistical differences. Amendments are beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), wood shavings (Ws) and straw (Str).

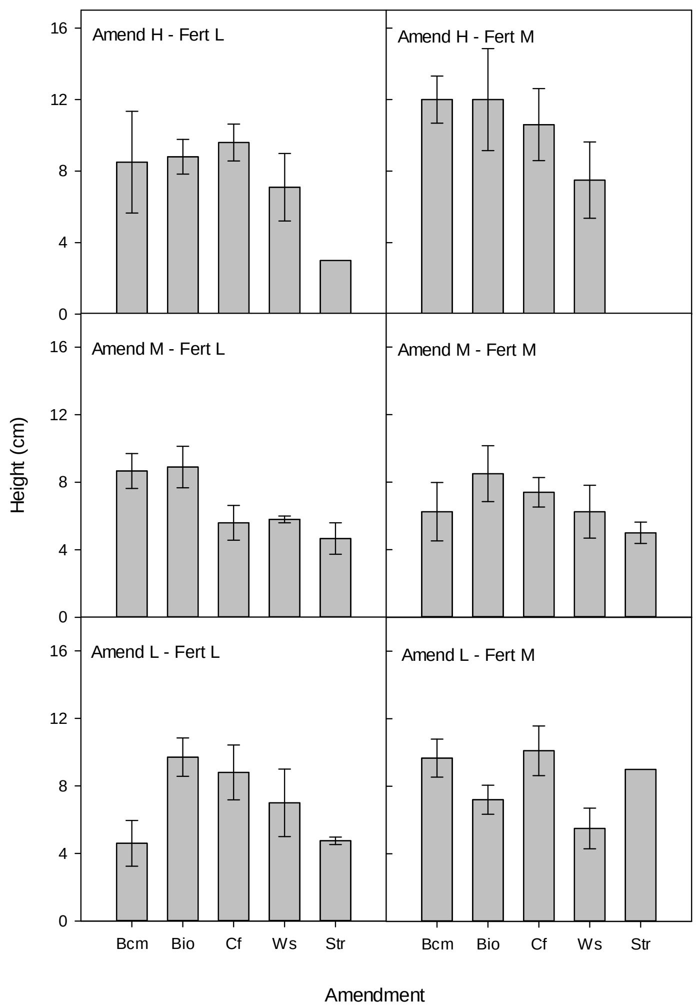

Figure 2-24. Mean height of *Poa alpina* in gray limestone substrate with high (top row), medium (center row) and low (bottom row) amendment application rates, and low (left column) and medium (right column) fertilizer application rates. Error bars are ± standard errors. N = 5. There were no statistical differences. Amendments are beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), wood shavings (Ws) and straw (Str).

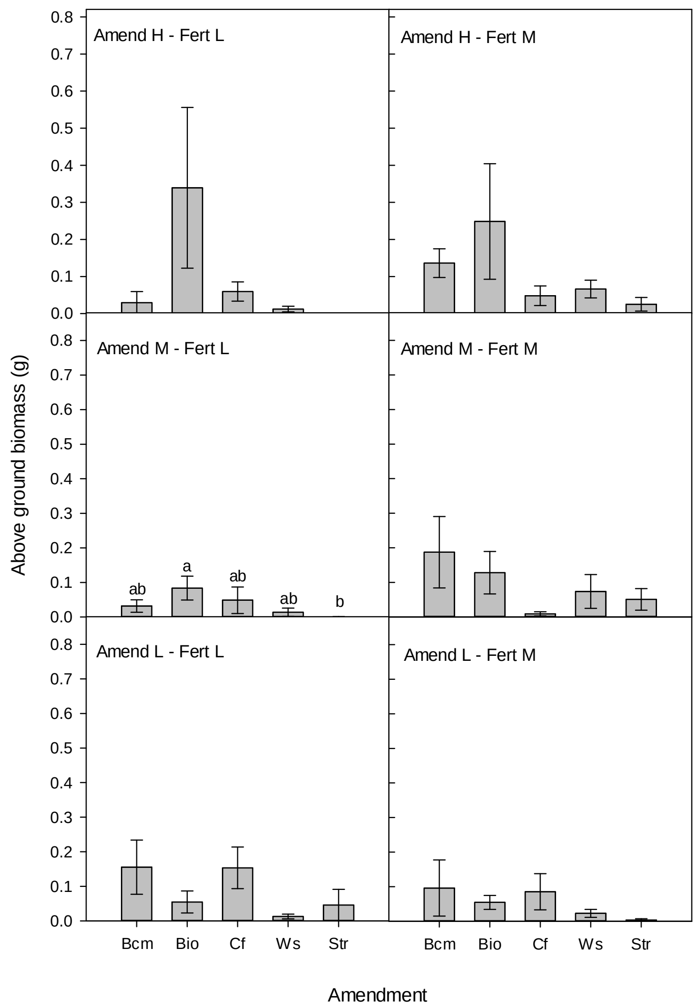

Figure 2-25. Mean above ground biomass of *Trisetum spicatum* in gray limestone substrate with high (top row), medium (center row) and low (bottom row) amendment application rates, and low (left column) and medium (right column) fertilizer application rates. Error bars are ± standard errors. N = 5. Different letters indicate significant differences among amendments within fertilizer and amendment rates combination treatments. Absence of letters indicates no statistical differences. Amendments are beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), wood shavings (Ws) and straw (Str).

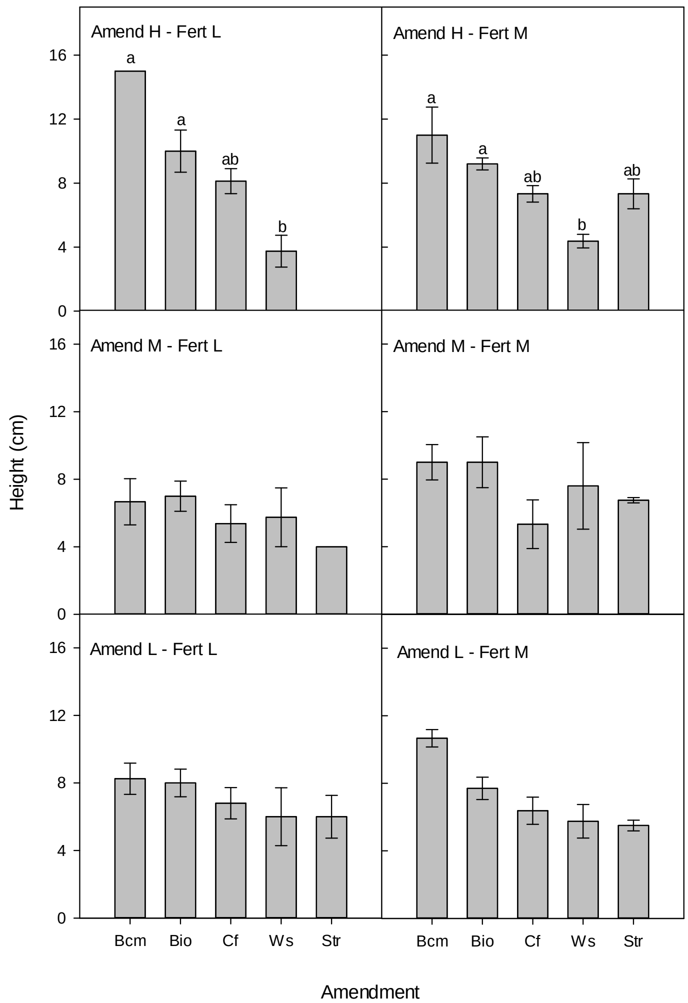

Figure 2-26. Mean height of *Trisetum spicatum* in gray limestone substrate with high (top row), medium (center row) and low (bottom row) amendment application rates, and low (left column) and medium (right column) fertilizer application rates. Error bars are ± standard errors. N = 5. Different letters indicate significant differences among amendments within fertilizer and amendment rates combination treatments. Absence of letters indicates no statistical differences. Amendments are beef manure compost (Bmc), biosolids (Bio), clean fill (Cf), wood shavings (Ws) and straw (Str).

CHAPTER 3. LIMESTONE QUARRY SUBSTRATE AMELIORATION AND EARLY ESTABLISHMENT OF VEGETATION: FIELD STUDY

1. INTRODUCTION

Limestone, a sedimentary rock of mainly calcium carbonate, is widely used in a variety of industries including construction, metallurgic, pharmaceutics, food and agriculture, with an increasing worldwide production and demand (Kesler 1994). Limestone is mainly extracted in open pit mines by drilling and blasting, resulting in disturbances that can be dozens of hectares in size and dozens of meters in height (Davis et al. 1985, Ursic et al. 1997). This can result in significant impacts to soil, vegetation and fauna and to habitat loss (Sort and Alcañiz 1996, Clemente et al. 2004, Moreno-Peñaranda et al. 2004). Although increasing environmental awareness and regulatory requirements make limestone quarry reclamation mandatory in North America, reclamation success has been very limited (Bonifazi et al. 2003, Allen et al. 2005), particularly when the reclaimed land must resemble predisturbance conditions (Wunderle Jr. 1997, Calow 1998).

Soil is a complex system formed by interacting chemical, physical and biological components. Topsoil, the uppermost layer of soil where the majority of soil biological processes occur, is rich in organic matter and nutrients. Although it is a valuable resource in reclamation, its availability is limited and expensive for use in large areas.

Soil available for limestone quarry reclamation is usually scarce, coarse and nutrient deficient (Bradshaw and Chadwick 1980, Davis et al. 1985), consequently, vegetation establishment is very difficult. To modify such conditions, improvement of soil physical, chemical and biological properties and reestablishment of processes, such as nutrient cycling and interaction among microinvertebrate, microorganism and mycorrhizae, are necessary (Sort and Alcañiz 1999, Allen et al. 2003, Allen et al. 2005, Bailey et al. 2006). A commonly used technique to enhance degraded soils is application of soil amendments, mainly of organic sources (Sopper 1993, Calderón et al. 2005), because they increase available nutrients, water content, serve as buffers for pH, and improve texture. Amendments can enhance soil depending mainly on desired conditions to be achieved, previous soil nutrient status, availability of the amendment and regulatory requirements (Reid and Naeth 2005).

During the past three decades soil amendments have been used in limestone quarry reclamation with limited success. In Spain, sewage sludge was used as a source of nutrients and organic matter for vegetation establishment, increasing plant biomass and cover, although the resulting species richness was lower than without sludge (Moreno-Peñaranda et al. 2004). In Clipsham Old Quarry, United Kingdom, plant cover of early seral communities increased 78% with increased nutrient availability, protection against herbivory and sufficient seed input (Davis et al. 1985). Hay mulch has been used to retain water and minimize seed loss from wind, rain and runoff in an abandoned quarry in Irish Cove Island, Nova Scotia (Hopper and Bonner 2003).

In a series of greenhouse experiments (Chapter 2), we developed suitable substrates for plant establishment using limestone quarry materials and agricultural waste materials such as beef manure compost (Land Resources Network Ltd. 1983), forestry by products such as pulp mill biosolids and wood shavings, and soil as amendment; all with the objectives of modifying physical and chemical conditions of the substrates increasing soil organic matter, water infiltration, water holding capacity and nutrients and to reduce soil bulk density. The substrates made with these amendments in combination with fertilizer were suitable for native grasses establishment. Their suitability for large scale reclamation at a limestone quarry, where uncontrolled conditions and site heterogeneity may affect reclamation results require evaluation. The diverse topography (steep slopes of varying aspect, flat areas), quarry reconstruction characteristics (age, overburden depth, soil layers, soil type) and seed inputs from surrounding vegetation, may affect the overall ability of the amended substrates to facilitate early vegetation establishment.

Establishment of a plant community through facilitation (modification of the environment via successive plant species or communities) is likely to occur under limiting physical conditions (Rousset and Lepart 1999, Bruno 2000, Maestre et al. 2001), such as those found in mined lands. Ameliorating soil conditions at a limestone quarry should facilitate establishment of seeded vegetation non seeded species that will colonize the newly created sites. Establishment of some target species during primary succession, especially in resource limited environments, is highly dependent on previous establishment of environment modifying plant species (Maestre et al. 2001). Mined areas can remain relatively unvegetated for decades or can be colonized by pioneer and unwanted plant species not commonly found in undisturbed conditions without human augmented revegetation (Wheater and Cullen 1997, Cooper and MacDonald 2000). The characteristics of these early plant communities may alter the trajectories of mid and late successional communities in the long term.

Introduction of native grasses on quarry steep slopes can control soil erosion and accelerate natural successional processes for establishment of a native plant community. The use of erosion control blankets can provide additional physical protection against wind and water erosion. Selection of native species is critical in any restoration project (Davis et al. 1985, Wunderle Jr. 1997, Alvarez-Aquino et al. 2004, Alvarez-Aquino et al. 2004, de Souza and Batista 2004);

Identification of species able to thrive in the particular conditions of a reclamation site are important. However, the use of native species for reclamation is challenging (Gerling et al. 1996, Davis et al. 1998, Riley et al. 2004, Reid and Naeth 2005, Vieira and Scariot 2006). Seeds and propagules are often scarcely available and economically expensive. There is usually limited information about native species characteristics and ecology, including their responses to season of seeding (Skousen and Fortney 2003), nutrient addition or to competing non-native vegetation. At northern latitudes, amending and seeding in the fall, when seeds will not immediately germinate, could result in a substrate with fewer nutrients by the following spring when plants start to emerge but plants will have a longer growing season and water available from early spring snow melt. If substrates are prepared and seeded in the spring, the emerging plants may have a shorter growing season to establish.

We hypothesize seeding season will affect establishment of vegetation and that physical and chemical modification of the substrate through addition of organic amendments and fertilizer and physical protection against wind and water erosion will favour establishment of seeded vegetation at the quarry. To address these issues, this research focused on the following objectives: to determine if limestone quarry fall and spring soil preparation and seeding differ in their effect on soil properties and seeded native grasses; to determine the effect of soil amendments and erosion blankets on soil properties; to determine if ameliorated quarry substrate and erosion blankets differ in their ability to increase plant establishment; to identify characteristics of the early established plant community of seeded and non seeded species due to amendments and quarry locations. The results from this work will contribute to generating the knowledge and practices for aiding development of a self sustaining ecosystem. It will contribute to meeting regulatory approval requirements for reclamation and can be extrapolated to other limestone quarries or similar disturbances. Knowledge of best performing amendments may be incorporated into reclamation planning and reconstruction of the quarry. Performance of plant species used in the seed mix provides information on plant species suitability for limestone quarry reclamation in the area, seeding rates and long term monitoring and management.

2. MATERIALS AND METHODS

2.1 Study Area

Research was conducted at the Graymont Exshaw limestone quarry near Kananaskis, Alberta, Canada (51º 07' N 115º 13' W). The quarry was located on a south facing slope of the Rocky Mountains, at an elevation of approximately 1,350 m, below the tree line that occurs from 2,000 to 2,300 m. The climate is montane-subalpine (Alberta Parks and Protected Areas 2002). Long term mean annual precipitation for the area is 296.2 mm as rain and 234.1 cm as snow. Mean daily temperature is 3 ^{o}C; mean maximum is 21.9 ^{o}C in July and mean minimum is -14.1 ^{o}C in January (Environment Canada 2011a).

In the undisturbed surrounding areas, soils are mainly Brunisols with underlying calcareous parent material (Alberta Parks and Protected Areas 2002). Vegetation is coniferous and deciduous mixed wood forests. Primary canopy species are *Pinus contorta* Douglas ex Louden (lodgepole pine), *Picea glauca* (Moench) Voss (white spruce), *Abies lasiocarpa* (Hook.) Nutt. (alpine fir), *Populus tremuloides* Michx. (trembling aspen), *Pseudotsuga menziesii* (Mirb.) Franco (douglas fir) and *Populus balsamifera* L. (balsam poplar) (Alberta Parks and Protected Areas 2002). Main understory species include *Shepherdia canadensis* (buffalo berry), *Juniperus horizontalis* Moench (creeping juniper), *Arctostaphylos uva-ursi* (L.) Spreng. (bear berry), *Salix* spp. (willow), grasses such as *Elymus innovatus* Beal. (hairy wild rye) and various forb species. Various species of wild life use the site. Conspicuous fauna at the quarry area include big horn sheep (*Ovis canadensis* Shaw 1804) that like to climb the rocky quarry faces and slopes.

The reclamation research treatments were established at three locations in the quarry within a km of each other and one at the lime plant 7 km away. Sites were named based on site age and characteristics. Sites New and New HM were located in one newly built south facing embankment, on the east side of the quarry road. Embankments are engineered piles of waste rock from limestone mining, covered with substrate (topsoil or clean fill, which is admixed topsoil with subsoil). New embankments are built every year resembling terraces that will eventually reach the tree line. The embankment where sites New and New HM were located was approximately 188 m long and 7 to 15 m wide, with a 30 degree slope, and covered with clean fill, to a 30 cm depth. A 65 m long segment at the east half of the embankment was covered from top to bottom with a thin layer (1-2 cm) of horse manure so it was considered a separate site (site HM). Site Old was located in a south facing, older embankment, on the west side of the quarry road. It had 30 degree slopes of varying length, covered with clean fill to a depth of 60 cm. At the Exshaw lime plant, site Plant was situated at a northwest facing berm, which had 15 to 20 degree slopes of 10 to 30 m lengths covered with at least 30 cm of clean fill. These sites collectively represent the heterogeneity of site conditions found on a typical limestone quarry requiring reclamation around the world.

2.2 Experimental Design

The experimental design addressed the need of testing several treatments for substrate amelioration in the quarry. Embankment characteristics (slope and aspect, age of construction, surrounding vegetation) may affect vegetation establishment on the treated substrate, and therefore all ameliorated substrates were tested and replicated in each embankment (Site). Season of amending and seeding also had to be replicated at each site. Therefore each site formed an experimental block in which all treatments were replicated (sites New, New HM, Old, Plant). In one of the embankments (site New HM) not all the treatments were tested because of the smaller area and different substrate characteristics.

In August 2007, 140 research plots were established, 40 in each of sites New, Old and Plant and 20 in site New HM. Each plot had an area of 2.25 m^2 (1.5 x 1.5 m). Plots were in a horizontal line on each site separated by 1 m between plots. To test effect of season, half of the plots in each site were randomly selected for fall and half for spring soil preparation and seeding (referred to as spring and fall treatments hereafter). Within spring and fall treatments, soil treatments were randomly assigned plots. Soil treatments included incorporated manure mix and wood shavings and placement of erosion control blankets on the plot surface. Every treatment, including a control, was replicated five times. Blocks New, Old and Plant had the following experimental design: 2 seeding seasons x 4 soil treatments including control x 5 replicates = 40 plots. Block New HM was not amended because it was already covered with horse manure, therefore the only treatments were the erosion control blanket and a control with the following experimental design: 2 seeding seasons x 2 soil treatment including control x 5 replicates = 20 plots.

2.3 Soil Treatments

Amendments that improved the physical and chemical properties of the limestone substrate in the greenhouse experiments described in Chapter 2, were tested in the field. Beef manure mix (manure) and wood shavings (wood) were selected because they resulted in adequate plant establishment and because these organic amendments are locally available and could be used without a special environmental application permit. This was not the case of pulp mill biosolids, one of the best performing amendments in the greenhouse, that could not be used in the field due to time constraints to obtain the application permit. Erosion control blankets (blanket) were used to provide physical protection to soil and seeds. The soil treatments including a control (control) were used in combination with fertilizer, which improved plant establishment in the greenhouse and is a regular practice in other types of quarry reclamation.

The beef manure mix was a 2 year old aged 6:1:1 mix of manure, waste feed and wood shavings. The wood shavings were fine screened from pine and white spruce wood. The manure mix and the wood shavings were procured from the University of Alberta farm. The blanket was Nilex SC150BN made with coconut and straw. The beef manure mix was applied at a rate of 30 Mg ha^{-1} and the wood shavings at a rate of 11.25 Mg ha^{-1}, then incorporated into the soil by hand with rakes and shovels to a depth of 5-10 cm. Slow release fertilizer (Type 100 Nutricote, 14-14-14 nitrogen, phosphorus, potassium) was used as a source of macronutrients and applied to the treatments at a rate of 1.1 Mg ha^{-1}. Fall plots were amended in August 2007 and spring plots in May 2008. Fertilizer was applied at time of seeding. Blankets were cut to individual plot size and installed to cover plots immediately after seeding.

Due to the extensive bighorn sheep grazing at the research plots, Plantskydd, a biodegradable chemical deterrent made of dry pig blood was applied in June 2008 and April 2009. The product was applied using a solution of 1 kg product per 6 gallons (22.7 L) of water applied according to the manufacturer's recommended rate. The product was sprayed by hand over the complete area of each of the research blocks at each application time.

2.4 Plant Species

A seed mix consisting of 5 grasses and one forb was designed for relatively rapid establishment. The grass species were *Poa alpina* L. (alpine blue grass) 20%, *Agropyron trachycaulum* (Link) Maltex H.F. Lewis. (slender wheat grass) 15%, *Elymus innovatus* Beal. (hairy wild rye) 15%, *Festuca saximontana* Rydb. (rocky mountain fescue) 15%, *Trisetum spicatum* (L.) K. Richter variety ARC sentinel (spike trisetum) 15%, *Bromus carinatus* Hook. & Arn. 10% (mountain brome grass). The forb was *Vicia americana* Muhl. ex Willd. (american vetch) 10%. The native plant species were chosen for their potential adaptability to quarry site conditions due to their tolerance to dry slopes, rocky or gravelly sites, alkaline soils and grazing. A nitrogen fixing forb was included to increase available soil nitrogen. The species, except *Bromus* and *Vicia*, had been tested for limestone quarry material in the greenhouse. Fall plots were seeded October 8-10, 2007 and spring plots May 13-15, 2008.

2.5 Quantification of Soil Properties

To determine soil properties prior to reclamation treatment (August 2007), soil was sampled in three locations at each site. A 0 to 10 cm soil core was collected at each sampling locations, then composited to one sample per site. To determine soil properties after reclamation treatment, when seeds of both spring and fall treatments were beginning to establish, samples were taken in May 2008 and at the end of the second growing season in August 2009. One 0 to 10 cm core from each of the 140 research plots was taken. This depth was expected to be the root growing portion of the soil for the native species seeded.

Soil samples were analyzed by a commercial laboratory. Detailed salinity including sodium adsorption ratio, pH and electrical conductivity were determined in a 1:2 soil to water saturated paste (Hendershot et al. 1993). Total carbon was determined by combustion (Nelson and Sommers 1996), total organic carbon by acid digestion then combustion (Skjemstad and Baldock 2007) and total inorganic carbon and total inorganic carbon calcium carbonate ($CaCO_3$) equivalent by acid digestion (Loeppert and Suarez 1996). Total nitrogen was determined by combustion (LECO Corporation 2005), total organic nitrogen by kjeldahl procedure (Kalra and Maynard 1991). Available nitrate, phosphate and potassium were determined by modified Kelowna extraction (Carter 1993, Qiana et al. 1994) and available sulphate by extraction with 0.01 M calcium chloride ($CaCl_2$) (Kowalenko 1993). Cation exchange capacity was determined by barium chloride ($BaCl_2$) extraction (Hendershot et al. 1993) and particle size distribution by hydrometer (Sheldric and Wang 1993)

2.6 Vegetation Assessment

Vegetation was assessed at each site August 28 to 30, 2007, prior to plot set up and soil treatment, since some remnant vegetation from a previous failed seeding at site Old and Plant and naturally invading weeds were present in very small numbers. The assessment was done by visually determining % total canopy cover and % individual species cover within a 0.1 m^2 quadrat (20 x 50 cm) placed at 10 random locations in each site. Data were used to estimate an average % canopy cover per site and a baseline data of species richness.

After treatment application, vegetation monitoring was performed twice a year, early (May 13 to 15, 2008 and May 19 to 24, 2009) and late in the growing season (August 11 to 15, 2008 and August 17 to 24 2009), although only data collected at the end of each growing season was statistically analyzed. All plants in each plot were identified and counted (seeded and non-seeded species) to determine plant density per species. Percent total canopy cover and litter were determined within a 0.5 x 0.5 m (0.25 m^2) quadrat located at the center of each of the 140 plots. Height of two tall and two short plants of each seeded species were measured to obtain average height within the quadrat. Phenological stage (presence of flowers, seed heads and senescence) and overall health and vigour, noted as healthy, stunted, necrotic and chlorotic of seeded species, were determined. In August 2009, additional parameters were evaluated. Cover of individual seeded species was assessed and seed heads counted. A partial assessment to verify plant survival was carried out in August 2010 only in spring plots by counting plant density per seeded species within the 0.25 m^2 quadrat.

2.7 Statistical Analyses

Two way analysis of variance (ANOVA) was performed on soil data with SigmaPlot 12 (Systat Software Inc. 2011). To isolate which groups differed, all pair wise multiple comparison procedures (Holm Sidack method) were performed. Plant density and electrical conductivity data did not comply with assumptions of normality and equality of variance, thus permutational analysis of variance was performed with PERMANOVA v.1.6 (Anderson 2001, McArdle and Anderson 2001). Permutation tests calculate the probability of getting a value equal to or more extreme than an observed value under a specified null hypothesis by recalculating the test statistic after random re-ordering of data (Anderson 2001). Permutational analysis of variance was performed with the fixed factors season and amendment. Two levels of season were spring and fall and four levels of amendments were wood, blanket, manure and control. Raw data were neither transformed nor standardized, and analysis was based on the measure of Bray-Curtis dissimilarities distance (Bray and Curtis 1957). A total of 10,000 permutations of raw data were used in all tests (Larson et al. 2007, Chu et al. 2009). A posteriori pair-wise comparisons with PERMANOVA were done for treatments with significant differences. Differences in density of individual seeded species due to seeding season were analyzed with Mann-Whitney rank sum test for most of the data and with T test when data were normal.

To identify differences in total seeded plant density and individual seeded species density due solely to amendment and blanket treatments at each site, fall and spring treatment data were pooled and one way non parametric analysis of variance used with the fixed factor amendment and four levels (wood, blanket, manure, control). Raw data were neither transformed nor standardized, and the analysis was based on the measure of Bray-Curtis dissimilarities distance. A total of 10,000 permutations of raw data were used and posteriori pair-wise comparisons were done for treatments with significant differences.

Differences in total plant cover, total litter cover, heights and seed heads density of individual seeded species and plant density of individual non seeded species due to amendment and blanket treatments were analyzed with one way ANOVA if data had normal distribution and equal variance and ANOVA on ranks (Kruskal-Wallis for sites New, Old and Plant and Mann-Whitney rank sum test for site New HM) otherwise, using SigmaPlot 12 (Systat Software 2011). Plant cover of individual species was analyzed with ANOVA on ranks (Kruskal-Wallis).

Comparison of plant cover of seeded and non seeded species within the same treatment at each site was performed with Mann-Whitney U rank sum test using SigmaPlot 12 software. Mann-Whitney U rank sum is a nonparametric test to assess whether one of two samples of independent observations tends to have larger values than the other and Kruskal-Wallis is an extension of the Mann-Whitney test that assesses differences among three or more independently sampled groups on a single, non normally distributed continuous variable (Kruskal and Wallis 1952, McKight and Najab 2010). Differences in plant density of individual species due to amendment and blanket treatments were analyzed with one way Holm-Sidak ANOVA if data had normal distribution and equal variance and ANOVA on ranks otherwise, using Sigmaplot 12.

To investigate the response of vegetation (density of seeded and non seeded species) to site and soil properties (total nitrogen, total carbon, total organic carbon, pH, electrical conductivity), redundancy analyses (RDA) on Hellinger-transformed data were performed. Hellinger tranformations (Legendre and Gallagher 2001) prevent distortion of redundancy analysis results by species with low abundance. Soil data were standardized. Analyses were performed with statistical package R (version 2.13.0) (R Development Core Team 2011) and Vegan library (version 1.17-10) (Oksanen et al. 2011).

3. RESULTS

3.1 Unamended Soil Properties

Prior to amendment, the proportion without rocks of the soil in the upper 10 cm of the profile was loam in texture, with similar proportions of sand, silt and clay at all sites (Table 3-1). Electrical conductivity, pH and sodium adsorption ratio were well within soil criteria guidelines; organic carbon was low, ranging from 0.5 to 1.5%. Soil properties were very similar among sites with a few exceptions in site New HM, which generally had highest nutrient concentrations, sodium adsorption ratio and electrical conductivity and lowest organic carbon.

3.2 Fall and Spring Soil Preparation and Seeding Effects on Soils and Vegetation

Overall, soil chemical properties and plant density were not significantly different between spring and fall treatments in either study year (Tables 3-2 and 3-3). Only site Plant had significantly more total plants in fall than spring 2008, but this difference did not persist in 2009. Mean plant density was generally numerically higher in fall treatments than spring treatments. Largest differences occurred in sites New HM and Plant; in 2008 there were 16 and 33 more plants per m^2 in fall than spring and in 2009 there were 40 and 18 more plants per m^2, respectively.

The growing season of 2008 was colder than that of 2009. Mean temperatures ranged from 4.4 ^{o}C in June to 0.1 ^{o}C in October 2008 whereas in 2009 temperature ranged from 11.2 to 0.7 ^{o}C. Precipitation was higher in 2008, with total precipitation between June to October being 355.1 mm, whereas in 2009 it was 265.7 mm (Environment Canada 2011b). Despite 2008 being colder and with more precipitation than 2009, seeded plants had an overall increase in density from 2008 to 2009.

When analyzing the effect of season on individual species density, no consistent pattern across sites was found. Fall treatments resulted in higher densities for some species at sites New HM and Plant. *Poa* and *Bromus* had significantly higher density in fall treatments at site New HM in 2008. These differences did not persist to the following year. *Trisetum* density was significantly higher in fall treatments in 2009 in site New HM. *Agropyron* density was significantly higher in fall treatments in 2008 in site Plant and *Poa* and *Vicia* in 2009, in site Plant (Table 3-4 and 3-5). *Festuca* and *Elymus* densities were not significantly different due to seeding season.

3.3 Soil Amendments and Erosion Blanket Effects on Soils

Total nitrogen, total carbon and total organic carbon was higher in manure amended treatments than in controls. These increases were often statistically significant but numerically small. Total nitrogen and organic carbon were almost double that of controls in 2008 (Table 3-6) and remained higher in 2009 (Table 3-7). Total nitrogen, carbon and organic carbon did not follow a discernible trend over time after amendment with wood shavings, although total organic carbon was marginally higher at all sites than the control. Erosion control blankets had very little effect on nitrogen and carbon concentrations relative to the control but concentrations were often significantly lower than with manure or wood shavings.

Soil pH averaged 8 and varied little with time and treatment. Amendment with manure increased electrical conductivity in 2008 at the three sites where it was used (Table 3-6). Electrical conductivity in manure treatments at sites New, Old and Plant were over twice as high as in the control. A year later these differences had almost disappeared (Table 3-7).

3.4 Soil Amendments and Erosion Control Blanket Effects on Seeded Vegetation

Seeded plant density was generally higher with amendments than in controls (Figure 3-1). In 2008 and 2009 significant differences in density occurred at site New with manure; densities were twice as high as in the control. Wood shavings had less effect on plant density than manure and were often similar to the control but lower at site Old in both years. Blankets had a significant effect across sites and over time. In 2008 plant density was almost twice as high with blankets than the control at sites New and New HM; by 2009 values were over twice as high. Plant density increased in most treatments and sites from 2008 to 2009, except at site New HM.

Seeded plant density, cover and height varied with species seeded, treatment and site. *Agropyron trachycaulum*, *Festuca saximontana*, *Poa alpina* and *Trisetum spicatum* were the best performing species in the seed mix. Density of *Festuca saximontana*, *Poa alpina* and *Trisetum spicatum* increased from 2008 to 2009, whereas density of other species generally decreased (Table 3-8). *Agropyron trachycaulum* had the highest plant density at all sites in both years except at site Old in 2009 where *Festuca saximontana* density was highest. *Vicia americana*, *Bromus carinatus* and *Elymus innovatus* had limited establishment and were often absent in most plots regardless of year.

Agropyron trachycaulum had higher density in blanket treatments. Although in 2008 its density was fairly similar among treatments (Table 3-9), by 2009 densities in blankets were significantly higher (Table 3-10). Densities remained similar in 2010 (Table 3-11). *Agropyron* height increased from 2008 to 2009 and was higher at sites New HM and Plant (Table 3-13 and 3-14). Height was often higher with manure, and significantly different than controls at site New in 2008 (Table 3-13) and at site New and Plant in 2009 (Table 3-14). In 2010, height was similar among treatments within site (Table 3-15). Seed head density was highest in blanket and manure treatments. Seed heads in controls were significantly fewer at all sites compared to other treatments (Table 3-16). Fall and spring treatment cover of *Agropyron* is presented in Tables 3-17, and 3-18, respectively. Differences in cover due to soil treatments within fall or spring treatments were not detected. When spring and fall data were analyzed together, cover was often significantly higher in blanket and manure treatments. At site New, cover in manure treatments was significantly higher than controls. At site New HM cover was significantly higher with blankets than in controls. At site Old, cover was significantly higher with blankets than wood shavings, and at site Plant, blankets and manure had significantly more cover than wood and control treatments. No significant differences were detected in *Agropyron* cover in spring plots in 2010 (Table 3-19).

Festuca saximontana density varied among treatments. In 2008, its density was similar among treatments within a site (Table 3-9), but in 2009 densities with blankets were significantly higher than controls at site New, wood at sites Old and Plant and manure at site Plant (Table 3-10). Densities were similar in 2010 in spring treatments (Table 3-11). Height increased from 2008 to 2009 and was greater at sites Old and Plant although similar across treatments (Table 3-13 and 3-14). In 2010, height was similar among treatments within site and taller plants were found at site Plant (Table 3-15). Seed head density was similar within treatments at each site and higher at sites Old and Plant (Table 3-16). *Festuca* cover in fall and spring is depicted in Tables 3-17 and 3-18, respectively. Differences in cover due to soil treatments within fall or spring treatments were not detected, even when season data were analyzed together. No significant differences were detected for cover in spring plots in 2010 (Table 3-19).

Poa alpina density varied with treatment. In 2008, densities were similar across treatments (Table 3-9), but lower with manure at site New. In 2009, densities were significantly higher with blankets except at site Plant (Table 3-10). Densities remained similar in 2010 in spring treatments (Table 3-12).Height increased from 2008 to 2009 and was similar across treatments although significantly higher with blankets at site Plant than in controls in 2008 (Table 3-13) and to wood in 2009 (Table 3-14). Height was greater at site Plant in 2009. In 2010, heights were similar among treatments in spring plots (Table 3-15). Seed head density was similar within treatments at each site although overall higher with blankets (Table 3-16). *Poa* cover in fall and spring treatments are presented in Tables 3-17 and 3-18, respectively. No differences in cover due to soil treatments within fall or spring treatments were detected. When spring and fall treatments were analyzed together, blankets resulted in significantly more cover than controls at site New and controls and wood shavings at site Old. No significant differences were detected in cover in spring plots in 2010 (Table 3-19).

Trisetum spicatum density varied with treatment. In 2008, plant density was low at sites New and Old (Table 3-9); in 2009 plants occurred at all sites, and densities were higher with erosion control blanket and manure treatments (Table 3-10). More plants established at site Plant than at any of the other sites. Plant densities remained similar in 2010 in the spring treatments (Table 3-11). Height was similar among treatments of the few plants that established in 2008 (Table 3-13) and in 2009 (Table 3-14). Taller plants established at sites New HM and Plant in 2009. A broad range of heights of plants was detected in spring treatments in 2010 (Table 3-15). The seed head density on the plants was low and similar within treatments at each site (Table 3-16). *Trisetum spicatum* cover in fall and spring treatments is presented in Tables 3-17 and 3-18, respectively. No differences in cover due to soil treatments within fall or spring treatments were detected. No differences were detected when the spring and fall data were pooled for statistical analyses. Overall, greater plant cover occurred in sites New HM and Plant than any of the other sites. No significant differences in any plant measurements were detected in the spring treatments when measurements were taken in 2010 (Table 3-19).

Extremely low establishment of *Elymus innovatus* limited the ability to conclude any effect of amendments on plant density (Tables 3-8 to 3-10) or plant height (Tables 3-13 to 3-15) of the species. No seed heads were present at any of the treatments or sites (Table 3-16), nor was it possible to determine effect of treatments on cover (Tables 3-17 to 3-19).

Low establishment of *Vicia americana* limited the ability to conclude any effect of amendments on density (Tables 3-8 to 3-10, 3-12) or height (Tables 3-13 to 3-15) of the species. Seed heads were present only with the manure treatment at site Plant (Table 3-16). No significant differences were detected but higher cover occurred in the fall treatments (Table 3-17) than in the spring treatments (Table 3-18) at site Plant. Data were insufficient to detect differences in cover at spring plots in 2010 (Table 3-19).

Low establishment of *Bromus carinatus* limited the ability to conclude any effect of treatments on plant density (Tables 3-8 to 3-11) or height (Table 3-13 to 3-15) of the species. Seed heads were only present at site Plant, and the density was numerically higher with erosion control blankets (Table 3-16). Data were insufficient to determine effect of treatments on cover (Tables 3-17 to 3-19), although in site Plant the cover was numerically higher with erosion control blankets (Table 3-17).

3.5. Amendments and Quarry Location Effects on the Established Early Plant Community of Seeded and Non Seeded Species

Before research plot establishment the ground in the research area was mostly bare. Although a previous revegetation attempt was made, it had been highly unsuccessful. Average vegetation cover was 4.9, 2.2, 3.3% and 6% at sites New, New HM, Old and Plant, respectively. This low cover of vegetation was relatively homogeneously distributed across the site, so there were no areas of any higher vegetation cover.

Site New had mainly *Erucastrum gallicum* with traces of *Agropyron trachycaulum*, *Melilotus officinalis* (L.) Lam. (yellow sweetclover) and *Bromus inermis* Leyss (smooth brome). At site New HM, plant cover was mainly *Erucastrum gallicum,* with individual plants of *Bromus inermis*, *Avena fatua* L. (wild oats*)*, *Polygonum aviculare* L. (postrate knot weed), *Thlaspi arvense* L. (stinkweed), *Cirsium arvense* (L.) Scop. (canada thistle), *Trifolium hybridum* L (alsike clover), *Hordeum jubatum* L. (foxtail barley), *Lathyrus nevadensis* S. Watson (purple peavine) and *Sonchus arvensis* L. (sow thistle). Site Old cover was almost half *Erucastrum gallicum* (1.2%) with 1% *Agropyron trachycaulum*, 0.3% *Festuca* spp, 0.3% *Poa* spp, 0.3% *Lolium perenne* L. (perennial rye grass) and traces of *Hordeum jubatum* L. (foxtail barley) and *Cerastium arvense* L. (prairie chickweed). Site Plant had 1.8% *Agropyron trachycaulum*, 0.4% *Erucastrum gallicum, 3.7% Festuca rubra* L. (red fescue), 0.2% *Polygonum* spp. (knotweed) and 0.1% *Chenopodium album* L. (lamb's quarters).

Total plant cover was similar among treatments, except in 2008 when it was significantly higher with manure at site New and blankets at site New HM (Table

3-20). Litter cover increased after treatment and decreased from 2008 to 2009 (Table 3-20). Manure and wood treatments had significantly more litter than the control. Litter was not assessed under blankets as it could not be differentiated from straw and coconut mesh. Plant cover remained similar in 2010 in spring plots and litter cover increased slightly (Table 3-21). Figure 3-2 depicts differences in plant cover of seeded and non seeded species in 2009. Cover of non seeded species was significantly lower than seeded species at sites New HM and Plant. There were few significant differences in plant cover between seeded and non seeded species at sites New or Old. Overall, blankets resulted in highest seeded plant cover and lowest non seeded plant cover at sites New HM, Old and Plant, significantly increasing desirable species cover. Seeded species cover increased with manure amendment, being higher than any other treatment at site New. Wood shavings resulted in more non seeded than seeded plant cover at sites New and Old. Controls had more seeded plant cover at sites New HM and Plant and more non seeded plant cover at sites New and Old.

Plant densities of five non seeded species at all sites and three species listed as noxious in Alberta, Canada, were analyzed. Densities varied with site and reclamation treatment but differences were only significant for *Erucastrum gallicum* (Willd.) O.E. Schulz. (dog mustard) (Table 3-22). This species was often more dense in controls and had fewer plants with blankets. *Erucastrum gallicum* generally occurred at higher densities than most other non seeded species at all sites, except at site Plant where *Melilotus* had higher density (Table 3-22). The noxious species *Cirsium arvense* and *Avena fatua* had extremely low densities.

Constrained ordination on plant density of seeded and non seeded species across sites indicated higher effect of site than soil properties or reclamation treatments on plant community composition (Table 3-23). This explains low overlap among sites (Figure 3-3). Total nitrogen and total carbon emerged as soil properties exerting the strongest influence on vegetation. Total nitrogen and electrical conductivity were a stronger influence at site New HM where *Agropyron trachycaulum* and *Chenopodium album* were more abundant. Total carbon was the soil property exerting greater influence at site Plant, where conditions favoured *Trisetum spicatum and Poa alpina* more than at any other site. *Melilotus* spp was the dominant non seeded species. *Festuca saximontana* was most favoured by conditions at sites Old and Plant. Among seeded species, *Agropyron trachycaulum* benefited most from total nitrogen, whereas *Festuca saximontana and Poa alpina* benefited least from total nitrogen. Among non seeded species *Melilotus* species benefited the most from total carbon and *Erucastrum gallicum* benefitted the least. *Chenopodium* species benefitted from total nitrogen.

4. DISCUSSION

4.1 Fall and Spring Soil Preparation and Seeding

Although soil properties were similar between fall or spring prepared sites, fall preparation may create better conditions for germination in spring, such as over winter decomposition and mineralization of organic matter increased available nutrients and water content from snow melt. From a management perspective, either season of site preparation and seeding will work; although fall seeding could be most beneficial, particularly if site conditions are less harsh. Seeded species could then potentially take advantage of soil water content from snow melt to establish and growth roots, before temperatures rise and the soil surface dries out in summer. Fall seeding benefits numerous species (Kilcher 1961), although others like *Brasica napus* L. (canola) (Clayton et al. 2004) or *Medicago sativa* L. (alfalfa) (Kilcher 1961) are favoured by spring seeding. In the seed mix used at the Exshaw quarry, *Agropyron*, *Bromus* and *Poa* were favoured by fall seeding during the first growing season, but only at sites with better conditions (higher soil nutrients or northwest facing slope) and differences did not persist to the second growing season. *Festuca* density, on the other hand, was similar between fall and spring treatments. Overall, the limited differences in plant densities between fall and spring soil preparation and seeding in the current study suggests the species in the grass mix can be used at any seeding season.

4.2 Amendments and Erosion Control Blanket Effects on Soil and Seeded Vegetation

Soil modification by amendments and erosion control blankets generally yielded favourable plant responses. Adding manure increased total nitrogen and organic carbon. From this and other studies, it is evident manure will provide an overall improvement to low quality reclamation soils with little organic matter and low nutrient concentrations (Saviozzi et al. 1997, Hao et al. 2003, Larney et al. 2006, Yan et al. 2007) and nutrient concentrations will remain higher a few years after application. In the current study, even though manure was purposely added at a moderate rate, it increased nitrogen concentrations considerably after application and this effect remained high at the end of the second year. Plant density increased with treatments in relation to the control, particularly with manure and blankets. Manure also raised native grass plant density at a greenhouse study with amended limestone materials (Chapter 2).

Tolerance of electrical conductivity is plant species specific with higher values inhibiting germination or plant growth for many species (Singh et al. 1997). In our study, all values remained well below the 2 dS m^{-1} known threshold for adequate plant establishment (Soil Quality Criteria Working Group 1987), even though manure is known to increase electrical conductivity (Hao and Chang 2003).

Plant performance with wood shavings was lower than with manure, similar to results in the greenhouse experiments. Wood shavings with fertilizer was favourable for some species like *Poa* in the greenhouse. Fertilized wood shavings in the field were expected to enhance plant response but often plants were stunted. Some granules of fertilizer did not degrade suggesting a different kind of fertilizer should be used in the future. At higher rates wood products such as saw dust decreased nitrogen mineralization and reduced competitive ability of species with higher nitrogen requirements (Averett et al. 2004).

Erosion control blankets provided mechanical anchorage for seeds and plants, helping seeded vegetation to better establish. Nutrient uptake by the significantly higher number of established plants may explain the lower concentrations of total carbon, total organic carbon and total nitrogen with blankets. The relatively high cover of seeded species and fewer non seeded species with blankets has been found in other studies (Faucette et al. 2006). Reasons for this are not known, although speculatively the favourable conditions may reduce competitive ability of non native weedy species that normally do well under adverse conditions.

Blankets increased the plant density, plant cover, height and seed heads density of the native grasses seeded.

4.3 Species for Revegetation

The plant community of seeded species began development during the first growing season (2008), but continued to grow in numbers, cover and height in the following year (2009) and stabilized by 2010. *Agropyron* consistently established at high densities across soil treatments and sites, confirming its ability to establish in highly disturbed sites. Due to this ability, it has often been labelled as an aggressive species, potentially warranting recommendations to limit its use to <10% pure live seed in the seed mix (Native Plant Working Group 2000). Although *Agropyron* established well in the current study, plants of other seeded species would often intermingle with it and grow healthier and in more numerous clumps close to it. Whether the robust *Agropyron* plants provided physical shelter for other species, or modified their environment in some other way is worth further study. In view of the harsh environmental conditions at the quarry that limits establishment of most species, and the fact that *Agropyron* appears to facilitate development of other species, the recommendation to limit it to 10% of the mix appears unwarranted in limiting quarry environments. Despite being the species with greater seed head density, its overall density and cover stabilized and decreased slightly in the three years of the study, suggesting it was not going to dominate and restrict other species.

Poa cover in many cases was equivalent to that of *Agropyron* and *Festuca* even with fewer plants, highlighting the value of a diverse plant mix that includes tufted species. *Poa alpina* is not currently widely used in native grass mixes for Subalpine and Montane areas in the Bow Valley and results from this study suggest it should be more widely used. *Poa* was favoured by higher nutrients of site New HM and manure treatments as in the greenhouse (Chapter 2).

Elymus did not establish in the field, similar to the poor greenhouse results (Chapter 2). Because seed was obtained from the same commercial source, it is highly possible that seed viability was low and the site conditions impeded viable seeds from establishing. *Elymus* plants naturally grow in the surrounding areas of the quarry (see Chapter 4) and therefore the species should be further evaluated. Germination and viability tests prior to seeding are recommended.

Festuca could adequately grow in all treatments in the field just as it did in the greenhouse, where it achieved similar biomass with or without fertilizer. This makes it a good candidate to include in the quarry seed mix because it can establish at various management regimes.

Trisetum density increased greatly one year after seeded and it did much better when site conditions were less harsh, such as site New HM and Plant. *Vicia* and *Bromus* did not establish well although the few plants that established looked healthy. Increasing seeding rates is an option but *Vicia* is an expensive seed so this approach may not be economical. Fortunately, natural colonization of *Vicia* could occur from plants in the surrounding areas.

Plant species selection is always an issue to be addressed in land reclamation scenarios. Seeding mid to late successional species is desirable to develop a stable community as soon as possible, however, soil properties have often not been modified sufficiently (facilitation) to favour the mid to late successional species seeded. In the current study only four out of seven species in the seed mix, established at desired amounts. The lack of establishment of three species that were 35% of the seed mix limited plant cover. Thus more understanding of native species to be seeded, their site requirements, and seed viability tests are needed for successful early plant community development. Another potential factor reducing plant density was likely fertilizer, as this effect was also found in the greenhouse (Chapter 2). However, plants were healthier and with more above and below ground biomass in the greenhouse where plant density was 30% less than in unfertilized treatments but biomass was at least two times higher. Small drops in establishment are normal, and were also observed in the greenhouse after 12 weeks.

Selectively reseeding the quarry could be considered after the third year of reclamation if desired plant cover has not been achieved. Species that did well in the first three years after reclamation could be included in the seed mix, although introducing other species would potentially increase biodiversity. Reseeding could occur even if the ground was covered with blankets because, after 2 years, holes in the mesh will allow seeding the exposed soil surface. Seeding rate could be increased to achieve greater cover. Transplanting of woody species could be included. Woody species survival is achievable (Chapter 4) and could set the plant community on a later successional stage in a more expedient way.

4.4 Site and Soil Treatment Effects on the Early Plant Community

Site characteristics such as slope and aspect, soil chemical properties, availability of limiting resources such as nutrients (Tilman 1987) and early colonizers that attain dominance (García-Palacios et al. 2011), are among the factors that influence structure and dynamics of plant communities. Control treatments often had greater cover of non seeded species than seeded species confirming the need for amelioration of the quarry substrate to achieve a desired early plant community with a high percent of seeded species. More favourable sites, such as the northwest facing berm at the plant (site Plant), and the site with horse manure (site New HM), provided for higher seeded plant density, cover, taller plants and more seed heads. The northwest facing site with smoother slopes promoted establishment of more plants from seeded species possibly due to more soil water and available nutrients, which is common on north facing slopes. For example, soils at north facing slopes on dryland in Southeast Nebraska had 20% more water available throughout the year than south facing slopes (Hanna et al. 1982). In a sandy grassland restoration project in Hungary, influence of carbon addition on nitrogen immobilization was more pronounced at sites where soil organic matter and water were lowest (Török et al. 2000).

On south facing slopes, sites New and New HM present an interesting point of comparison, because although located in the same embankment, the horse manure cover at site New HM yielded a denser vegetation of seeded species. Harsher sites, because of lower nutrient content and higher exposure to wind (New and Old) had a higher proportion of non seeded plant cover and overall lower establishment of seeded species. Unless the non seeded species are listed as noxious, their presence can provide many benefits to the early plant community, reducing soil erosion, adding organic matter to the soil and buffering temperature for the seeded species. Establishment of certain species in early stages of succession may modify biotic and abiotic conditions of a site, facilitating establishment of more species in the community (facilitation model), or arresting incorporation of new individuals (inhibition model) (Connell and Slatyer 1977).

Site properties and their modification will in turn affect the plant community. Our results of constrained ordination indicate that there is a stronger effect of site on plant density, than of amendment or blanket treatments. But modification of the site by addition of nutrients will affect species differently. Some species, like *Erucastrum gallicum*, even though it was the most dominant of the non seeded species at every site, was drastically reduced with organic amendments addition and blankets. *Chenopodium* benefited from the increase of total nitrogen. The seeded species *Trisetum* and *Poa* benefited more at site Plant with higher total carbon. These species were also favoured by biosolids in the greenhouse (Chapter 2) which has high amounts of total carbon.

Many inhibiting species are non native to a site and have been identified and listed as noxious. These species are often early successional species that are regulated (Government of Alberta 2008), and require monitoring and control or they may inhibit establishment of desired species. In early successional stages of herbaceous communities, inhibition will usually dominate and characteristics of the pre-existing vegetation will have a large influence on success of species establishment in degraded systems (Gomez-Aparicio 2009). *Sonchus arvensis* and *Cirsium arvense*, two of the most frequently listed noxious broad leaved weeds in North America (Skinner et al. 2000), and the grassy weeds *Hordeum jubatum* and *Avena fatua,* were at Exshaw but not in sufficient quantities to create a significant reduction in seeded species density and cover.

Certain management practices such as use of fertilizer, although desirable during initial revegetation activities to favour seeded species establishment (Cohen-Fernández and Naeth unpublished), can also increase unwanted species. Fertilizer nitrates can break dormancy of some weedy species like *Avena fatua* (Sexsmith and Pittman 1963) which can then compete with seeded crops (Davis and Liebman 2001) and native grasses (Huffine and Elder 1960), taking advantage of added nutrients. The potential problems associated with fertilizer may thus be prevented or modified, for example, with the use of blankets. This in turn can move a community, from inhibition stages to facilitation stages much sooner than without human augmentation. Soil nutrient management over the long term in the form of fertilizer, amending and incorporation of legumes in the seed mix, will contribute nitrogen and other nutrients to the soil. This is desirable to achieve increases of soil organic matter from established vegetation over time, which otherwise may be limited with soil nutrient deficits.

5. CONCLUSIONS AND APPLICATIONS TO QUARRY RECLAMATION

Season of soil preparation and seeding did not significantly affect soil properties or vegetation although fall preparation and seeding gave an advantage to *Agropyron*, *Poa* and *Trisetum* at sites with better conditions provided by more nutrients and northwest facing slopes.

Among measured soil properties, total nitrogen and carbon significantly improved establishment of seeded grasses and non seeded species, increasing plant density. Increased nitrogen and carbon in constructed soils were best achieved through manure at 39 Mg ha^{-1}. Electrical conductivity at this rate remained below threshold values. Manure mix addition increased plant density and cover.

Erosion control blankets facilitated a denser native grass establishment despite not significantly changing chemical properties of the constructed soils. They affected composition of the plant community by facilitating better establishment of seeded species and hindering non seeded species. Its use increased plant density, cover, height and seed head density of seeded species.

Wood shavings are not recommended as an amendment due to their unfavourable effect on plant health (stunted plants) and thus lower plant cover.

Seeded species began development during the first growing season (2008), but continued to grow in number, cover and height the following year (2009) and stabilized by 2010. Best establishing seeded species were *Agropyron trachycaulum*, *Festuca saximontana*, *Poa alpina* and *Trisetum spicatum*.

In view of the environmental conditions at the quarry that limits establishment of most plant species, and the fact that *Agropyron trachycaulum* appears to facilitate development of other species, the general reclamation practice of limiting it to 10% of the mix appears unwarranted.

Poa alpina should be included in the seed mix. This species is not currently widely used in native grass mixes for Subalpine and Montane areas in the Bow Valley. Its success in this study suggests it should be more widely used.

Assisted revegetation increased plant cover from < 6% to 50% and reduced cover of non seeded species. Non seeded species density varied with site and reclamation treatment. *Erucastrum gallicum* was more prevalent in controls while other species were favoured by reclamation treatments. *Erucastrum gallicum* generally occurred at higher densities than most other non seeded species. Site characteristics such as slope, aspect, initial soil nutrient concentrations and the surrounding plant community influenced early plant community development at the quarry and plant and overall effects of soil treatments.

Soil nutrient management over the long term in the form of fertilizer, amending and incorporation of legumes in the seed mix, will contribute nitrogen and other nutrients to the soil. A combined approach of soil nutrient enrichment through manure application and protection with erosion control blankets could further increase revegetation success at the limestone quarry and plant.

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Table 3-1. Chemical and physical properties of the soil at Exshaw quarry research sites in August 2007 prior to treatments application.

Parameter	Site			
	New	New HM	Old	Plant
Total Carbon (%)	6.9	6.5	6.9	7.6
Inorganic Carbon (%)	5.8	5.9	6.3	6.1
Total Organic Carbon (%)	1.0	0.5	0.6	1.5
Inorganic Carbon/$CaCO_3$ Equivalent (%)	5.8	5.9	6.2	6.1
$CaCO_3$ Equivalent (%)	48.8	49.8	52.7	51.1
Total Kjeldahl Nitrogen (%)	0.1	0.1	0.1	0.1
Available Nitrate Nitrogen (mg kg^{-1})	10.2	14.0	5.4	3.6
Available Phosphate Phosphorus (mg kg^{-1})	2.0	2.0	2.0	2.0
Available Potassium (mg kg^{-1})	67.0	304	77	50
Available Sulfate Sulfur (mg kg^{-1})	123	257	120	16
Soluble Chloride (mg L^{-1})	20	370	20	40
Soluble Calcium (mg L^{-1})	89	149	87	100
Soluble Potassium (mg L^{-1})	6	169	5	5
Soluble Magnesium (mg/L)	25	59	21	31
Soluble Sodium (mg L^{-1})	22	82	13	29
Sodium Adsorption Ratio	0.5	1.4	0.3	0.7
Sulphate (mg L^{-1})	90	123	49	90
Saturation (%)	28.1	28.6	26.0	27.9
Hydrogen Ion Activity (pH)	7.6	7.5	7.6	7.7
Electrical Conductivity (dS m^{-1})	0.6	1.7	0.5	0.6
Cation Exchange Capacity (meq 100 g^{-1})	8.4	6.0	11.4	8.2
Sand (%)	50	43	50	51
Silt (%)	32	39	32	35
Clay (%)	18	18	18	14
Texture	Loam	Loam	Loam	Loam

Table 3-2. Mean measured soil properties and seeded plant density in fall and spring treatments in 2008.

		Site			
Parameter	Season	New	New HM	Old	Plant
Total Carbon (%)	Fall	6.96 ± 0.05	7.47 ± 0.18	6.88 ± 0.07 a	7.69 ± 0.16
	Spring	6.99 ± 0.08	7.48 ± 0.15	6.99 ± 0.08 b	7.86 ± 0.13
Total Organic Carbon (%)	Fall	0.84 ± 0.06	1.18 ± 0.19	0.89 ± 0.06	1.76 ± 0.01
	Spring	0.90 ± 0.07	1.11 ± 0.17	0.96 ± 0.08	1.75 ± 0.01
Total Nitrogen (%)	Fall	0.06 ± 0.00	0.14 ± 0.02	0.07 ± 0.00	0.10 ± 0.01
	Spring	0.07 ± 0.00	0.13 ± 0.02	0.09 ± 0.00	0.12 ± 0.01
Electrical Conductivity (dS m^{-1})	Fall	0.20 ± 0.01	0.44 ± 0.03	0.23 ± 0.01	0.29 ± 0.01 a
	Spring	0.33 ± 0.06	0.42 ± 0.05	0.37 ± 0.07	0.48 ± 0.08 b
Hydrogen Ion Activity (pH)	Fall	8.14 ± 0.02	8.25 ± 0.03	8.2 ± 0.01	8.28 ± 0.01
	Spring	8.15 ± 0.01	8.22 ± 0.02	8.19 ± 0.02	8.29 ± 0.01
Density (plants m^{-2})	Fall	26.75 ± 4.79	76.64 ± 11.0	25.77 ± 3.2	85.15 ± 10.60 a
	Spring	21.02 ± 5.17	60.96 ± 4.99	17.51 ± 2.39	52.55 ± 4.80 b

Values are mean ± standard error.

Values of ± 0.00 in the table were <0.006.

Letters indicate statistically significant differences between fall and spring treatments of the same site.

Absence of letters indicates no statistical differences.

Table 3-3. Mean measured soil properties and seeded plant density in fall and spring treatments in 2009.

		Site			
Parameter	Season	New	New HM	Old	Plant
Total Carbon (%)	Fall	7.25 ± 0.06	7.40 ± 0.12	7.20 ± 0.11	8.87 ± 0.24
	Spring	7.37 ± 0.08	7.49 ± 0.25	7.22 ± 0.08	9.23 ± 0.26
Total Organic Carbon (%)	Fall	1.02 ± 0.03	1.19 ± 0.08	0.99 ± 0.03	1.32 ± 0.05
	Spring	1.03 ± 0.05	1.21 ± 0.13	1.06 ± 06	1.31 ± 0.06
Total Nitrogen (%)	Fall	0.08 ± 0.00	0.11 ± 0.01	0.08 ± 0.003	0.09 ± 0.00
	Spring	0.09 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	0.08 ± 0.00
Electrical Conductivity (dS m^{-1})	Fall	0.32 ± 0.03	0.33 ± 0.02	0.34 ± 0.04	0.33 ± 0.03
	Spring	0.40 ± 0.03	0.35 ± 0.03	0.32 ± 0.02	0.37 ± 0.03
Hydrogen Ion Activity (pH)	Fall	8.18 ± 0.04	8.06 ± 0.06	8.12 ± 0.03	8.09 ± 0.05
	Spring	8.05 ± 0.03	8.1 ± 0.07	8.12 ± 0.03	8.06 ± 0.05
Density (plants m^{-2})	Fall	25.93 ± 2.68	79.50 ± 16.34	42.06 ± 4.41	82.84 ± 7.91
	Spring	24.37 ± 3.01	39.73 ± 4.65	43.13 ± 5.24	65.6 ± 4.85

Values are mean ± standard error.

Values of ± 0.00 in the table were <0.006.

Letters indicate statistically significant differences among treatments in a site.

Absence of letters indicates no statistical differences.

Table 3-4. Mean density of individual species (plants m^{-2}) in fall and spring treatments in 2008.

		Site			
Species	Season	New	New HM	Old	Plant
Agropyron	Fall	22.1 ± 3.8	47.5 ± 7	16 ± 2.3	48.3 ± 5.4 a
	Spring	18.4 ± 4.6	53.3 ± 4.2	11.1 ± 1.7	33.8 ± 3.6 b
Bromus	Fall	0.4 ± 0.1	1.3 ± 0.4 a	0.1 ± 0.1	
	Spring		0.2 ± 0.1 b		
Elymus	Fall				
	Spring			0.1 ± 0.1	
Festuca	Fall	1.3 ± 0.6	12.8 ± 3.1	8.8 ± 1.7	19.2 ± 4.3
	Spring	0.5 ± 0.3	2.6 ± 1.1	5.5 ± 1.2	12.1 ± 2.1
Poa	Fall	2.8 ± 1.1	12.2 ± 3.1 a	0.6 ± 0.2	15.7 ± 4.6
	Spring	1.5 ± 0.7	3.3 ± 1.5 b	0.6 ± 0.2	5.9 ± 1.4
Trisetum	Fall		1.4 ± 0.9		0.2 ± 0.2
	Spring		0.2 ± 0.1		
Vicia	Fall	0.3 ± 0.1	1.4 ± 0.6	0.3 ± 0.1	1.5 ± 0.3
	Spring	0.6 ± 0.1	1.3 ± 0.3	0.2 ± 0.2	0.6 ± 0.2

Values are mean ± standard error.

Letters indicate statistically significant differences between season treatments within a site.

Absence of letters indicates no statistical differences.

Blank cells indicate no plants established at that site and treatment.

Table 3-5. Mean density of individual species (plants m^{-2}) in fall and spring treatments in 2009.

		Site			
Species	Season	New	New HM	Old	Plant
Agropyron	Fall	18.7 ± 1.4	46.5 ± 9.1	13.9 ± 1.7	27 ± 4.3
	Spring	19 ± 2.1	27.2 ± 2.2	14.8 ± 1.5	28.7 ± 3.5
Bromus	Fall		0.6 ± 0.5		
	Spring		0.2 ± 0.1		
Elymus	Fall			0.2 ± 0.2	
	Spring				
Festuca	Fall	1.7 ± 0.6	6.4 ± 2.2	20.7 ± 3.3	27.7 ± 4.7
	Spring	1.1 ± 0.2	2.9 ± 1.2	19.3 ± 3.3	22.6 ± 3.4
Poa	Fall	4.5 ± 1.1	11.8 ± 3.5	6.3 ± 1.1	20.6 ± 4.2 a
	Spring	3.8 ± 1.1	6.9 ± 2.9	7.4 ± 1.5	10.1 ± 2.1 b
Trisetum	Fall	0.9 ± 0.3	10.1 ± 2.9 a	0.8 ± 0.2	5.5 ± 1.0
	Spring	0.3 ± 0.1	2.0 ± 1.0 b	1.5 ± 0.2	3.8 ± 0.6
Vicia	Fall	0.1 ± 0.1	0.4 ± 0.1		1.3 ± 0.3 a
	Spring	0.2 ± 0.2	0.6 ± 0.2	0.2 ± 0.2	0.3 ± 0.1 b

Values are mean ± standard error.

Letters indicate statistically significant differences between season treatments within a site.

Absence of letters indicates no statistical differences.

Blank cells indicate no plants established at that site and treatment.

Table 3-6. Mean measured soil properties in soil treatments in 2008.

Site	Treatment	Total Carbon (%)	Total Organic Carbon (%)	Total Nitrogen (%)	Hydrogen Ion Activity (pH)	Electrical Conductivity (dS m^{-1})
New	Control	6.81 ± 0.05 b	0.68 ± 0.02 b	0.06 ± 0.00 b	8.17 ± 0.04	0.18 ± 0.01 b
	Blanket	6.74 ± 0.03 b	0.61 ± 0.04 b	0.05 ± 0.00 b	8.11 ± 0.02	0.22 ± 0.02 b
	Manure	7.25 ± 0.09 a	1.18 ± 0.09 a	0.10 ± 0.01 a	8.17 ± 0.02	0.50 ± 0.11 a
	Wood	7.10 ± 0.10 a	1.01 ± 0.08 a	0.06 ± 0.00 b	8.14 ± 0.01	0.17 ± 0.01 b
New HM	Control	7.73 ± 0.15	1.45 ± 0.17 a	0.18 ± 0.02 a	8.25 ± 0.03	0.48 ± 0.03
	Blanket	7.23 ± 0.13	0.83 ± 0.11 b	0.10 ± 0.01 b	8.22 ± 0.02	0.38 ± 0.05
Old	Control	6.73 ± 0.10 b	0.74 ± 0.09 b	0.06 ± 0.00 b	8.16 ± 0.02	0.18 ± 0.00 c
	Blanket	6.81 ± 0.09 ab	0.70 ± 0.09 b	0.07 ± 0.00 b	8.16 ± 0.03	0.25 ± 0.02 b
	Manure	7.13 ± 0.09 a	1.16 ± 0.08 a	0.11 ± 0.01 a	8.21 ± 0.03	0.56 ± 0.11 a
	Wood	7.05 ± 0.10 ab	1.09 ± 0.08 a	0.07 ± 0.00 b	8.24 ± 0.02	0.19 ± 0.00 c
Plant	Control	7.69 ± 0.21	1.46 ± 0.13 b	0.10 ± 0.01 b	8.29 ± 0.01	0.30 ± 0.02 b
	Blanket	7.68 ± 0.19	1.41 ± 0.12 b	0.08 ± 0.01 b	8.27 ± 0.01	0.30 ± 0.03 b
	Manure	8.06 ± 0.25	2.59 ± 0.46 a	0.16 ± 0.01 a	8.27 ± 0.01	0.70 ± 0.14 a
	Wood	7.67 ± 0.18	1.57 ± 0.14 b	0.10 ± 0.01 b	8.31 ± 0.01	0.26 ± 0.02 b

Values are mean ± standard error.

Values of ± 0.00 in the table were <0.006.

Letters indicate statistically significant differences among treatments in a site.

Absence of letters indicates no statistical differences.

Table 3-7. Mean measured soil properties in soil treatments in 2009.

Site	Treatment	Total Carbon (%)	Total Organic Carbon (%)	Total Nitrogen (%)	Hydrogen Ion Activity (pH)	Electrical Conductivity (dS m^{-1})
New	Control	7.15 ± 0.07 b	0.93 ± 0.03 b	0.07 ± 0.00 b	8.18 ± 0.07	0.33 ± 0.04
	Blanket	7.10 ± 0.07 b	0.90 ± 0.02 b	0.08 ± 0.01 b	8.19 ± 0.05	0.33 ± 0.05
	Manure	7.53 ± 0.11 a	1.22 ± 0.07 a	0.11 ± 0.01 a	8.07 ± 0.05	0.38 ± 0.04
	Wood	7.45 ± 0.10 a	1.05 ± 0.04 ab	0.09 ± 0.01 b	8.02 ± 0.05	0.39 ± 0.04
New HM	Control	7.49 ± 0.18	1.25 ± 0.10	0.11 ± 0.01	8.1 ± 0.08	0.34 ± 0.03
	Blanket	7.39 ± 0.22	1.14 ± 0.11	0.12 ± 0.01	8.06 ± 0.06	0.34 ± 0.02
Old	Control	6.96 ± 0.05	0.86 ± 0.03 b	0.07 ± 0.00	8.2 ± 0.06	0.32 ± 0.03
	Blanket	7.31 ± 0.21	0.99 ± 0.04 b	0.07 ± 0.01	8.03 ± 0.06	0.36 ± 0.06
	Manure	7.28 ± 0.17	1.16 ± 0.10 a	0.10 ± 0.01	8.15 ± 0.03	0.32 ± 0.04
	Wood	7.28 ± 0.05	1.08 ± 0.03 ab	0.08 ± 0.01	8.1 ± 0.04	0.31 ± 0.04
Plant	Control	8.56 ± 0.22	1.26 ± 0.07	0.07 ± 0.00 b	8.18 ± 0.07	0.30 ± 0.02
	Blanket	9.08 ± 0.33	1.26 ± 0.05	0.08 ± 0.00 b	8.16 ± 0.04	0.32 ± 0.04
	Manure	9.60 ± 0.42	1.41 ± 0.09	0.11 ± 0.01 a	7.97 ± 0.09	0.39 ± 0.05
	Wood	8.96 ± 0.40	1.33 ± 0.11	0.08 ± 0.00 b	7.99 ± 0.08	0.40 ± 0.07

Values are mean ± standard error.

Values of ± 0.00 in the table were <0.006.

Letters indicate statistically significant differences among treatments in a site.

Absence of letters indicates no statistical differences.

Table 3-8. Mean plant density (plants m^{-2}) of individual seeded species at each site in 2008 and 2009.

		Site			
Species	Year	New	New HM	Old	Plant
Agropyron trachycaulum	2008	20.2 ± 2.9	50.5 ± 3.9	13.5 ± 1.4	41.0 ± 3.4
	2009	18.8 ± 1.2	36.6 ± 5.2	14.3 ± 1.1	27.8 ± 2.7
Bromus carinatus	2008	0.2 ± 0.1	0.7 ± 0.2	0.1 ± 0.0	0.1 ± 0.0
	2009		0.4 ± 0.2		0.4 ± 0.1
Elymus innovatus	2008			0.1 ± 0.0	
	2009			0.1 ± 0.1	
Festuca saximontana	2008	0.9 ± 0.3	7.4 ± 1.9	7.1 ± 1.0	15.6 ± 2.4
	2009	1.3 ± 0.3	4.8 ± 1.3	20.0 ± 2.3	25.1 ± 2.9
Poa alpina	2008	2.1 ± 0.6	7.5 ± 1.9	0.6 ± 0.1	10.8 ± 2.5
	2009	4.1 ± 0.7	9.7 ± 2.3	6.8 ± 0.9	15.3 ± 2.4
Trisetum spicatum	2008		0.7 ± 0.4		0.1 ± 0.1
	2009	0.6 ± 0.1	6.3 ± 1.7	1.1 ± 0.1	4.6 ± 0.5
Vicia americana	2008	0.4 ± 0.1	1.3 ± 0.3	0.2 ± 0.1	1.0 ± 0.1
	2009	0.1 ± 0.1	0.4 ± 0.1	0.1 ± 0.1	0.8 ± 0.1

Values are mean ± standard error.

Values of ± 0.0 in the table were <0.05.

Blank cells indicate no plants established at that site and year.

Table 3-9. Mean density (plants m^{-2}) of seeded species at the end of the first growing season in August 2008.

Site/Treatment	*Agropyron*	*Bromus*	*Elymus*	*Festuca*	*Poa*	*Trisetum*	*Vicia*
New							
Control	20.4 ± 4.7	0.5 ± 0.2		2.3 ± 1.1	2.6 ± 1.6 a		0.1 ± 0.1 ab
Blanket	23.7 ± 6.2	0.2 ± 0.1		0.3 ± 0.2	2.9 ± 1.4 ab		0.4 ± 0.1 b
Manure	11.1 ± 2.8			0.1 ± 0.1	0.4 ± 0.3 b		0.7 ± 0.2 a
Wood	25.6 ± 8.5	0.1 ± 0.1		0.9 ± 0.6	2.6 ± 1.4 a		0.4 ± 0.1 ab
New HM							
Control	53.1 ± 4.4 a	0.2 ± 0.1		2.7 ± 1.2	3.6 ± 1.5	0.2 ± 0.1	1.4 ± 0.3
Blanket	47.5 ± 7 b	1.3 ± 0.4		12.8 ± 3.1	12.2 ± 3.1	1.4 ± 0.9	1.4 ± 0.6
Old							
Control	12.4 ± 2.5			5.5 ± 1.2	0.6 ± 0.2		0.1 ± 0.1
Blanket	19.6 ± 3.5	0.2 ± 0.1		12.1 ± 2.9	0.7 ± 0.2		0.4 ± 0.2
Manure	11 ± 2.5			6 ± 2	0.8 ± 0.4		0.3 ± 0.3
Wood	11.2 ± 2.3		0.2 ± 0.1	5.1 ± 1.5	0.4 ± 0.2		0.1 ± 0.1
Plant							
Control	44.6 ± 4.7	0.3 ± 0.2		9.5 ± 2	12.6 ± 3.5		1.6 ± 0.5
Blanket	52 ± 9.9	0.2 ± 0.1		28.9 ± 7.4	18.9 ± 8.7	0.5 ± 0.5	1.4 ± 0.3
Manure	27.8 ± 5.9			11.2 ± 3.3	5.2 ± 2		0.4 ± 0.2
Wood	39.9 ± 3.5			13 ± 2.8	6.7 ± 2.1		0.8 ± 0.3

Values are mean ± standard error.

Different letters indicate significant differences among treatments within a site.

Absence of letters indicates no statistical differences.

Blank cells indicate no plants established at that site and treatment.

Table 3-10. Mean density of seeded species at the end of the second growing season in August 2009.

Site/Treatment	*Agropyron*	*Bromus*	*Elymus*	*Festuca*	*Poa*	*Trisetum*	*Vicia*
New							
Control	15.3 ± 2 b			0.1 ± 0.1 b	1.1 ± 0.3 b	0.1 ± 0.1 b	
Blanket	24.5 ± 2 a			2.4 ± 1.1 a	8.3 ± 1.7 a	1.2 ± 0.6 ab	0.2 ± 0.1
Manure	21.2 ± 2.6 ab			2.2 ± 0.3 a	6.0 ± 1.5 c	0.8 ± 0.2 a	0.4 ± 0.3
Wood	14.3 ± 2 b			0.9 ± 0.3 c	1.2 ± 0.4 b	0.3 ± 0.1 ab	
New HM							
Control	22.7 ± 2.3 b	0.2 ± 0.1		2.6 ± 0.6	2.8 ± 0.6 b	1.7 ± 0.5	0.6 ± 0.2
Blanket	49.2 ± 8.2 a	0.6 ± 0.5		6.9 ± 2.4	16 ± 3.4 a	10.6 ± 2.9	0.4 ± 0.1
Old							
Control	10.5 ± 1.6 bc			25.4 ± 6.4 ab	3.9 ± 1.0 b	0.6 ± 0.3 b	
Blanket	23 ± 1.9 a		0.4 ± 0.3	24.9 ± 4.4 a	14.6 ± 1.8 a	2.0 ± 0.4 a	0.1 ± 0.1
Manure	14.4 ± 1.5 b			18.5 ± 3.4 ab	5.1 ± 1.1 b	1.0 ± 0.2 a	0.4 ± 0.4
Wood	9.4 ± 1.3 c			11.2 ± 2.1 b	3.9 ± 1.1 b	1.1 ± 0.3 ab	
Plant							
Control	20.3 ± 3.6 c	0.4 ± 0.2		26.2 ± 5.2 ab	8.3 ± 2.4	3.5 ± 0.9	1.1 ± 0.2
Blanket	33.8 ± 6.6 c	1 ± 0.5		31.3 ± 5.2 a	24.6 ± 7.1	5.8 ± 1	1.5 ± 0.6
Manure	35.6 ± 6 ab			15.7 ± 4.7 b	15.7 ± 4.3	6.0 ± 1.7	0.3 ± 0.1
Wood	21.9 ± 4.3 bc	0.1 ± 0.1		27.4 ± 7.4 ab	12.8 ± 4	3.4 ± 0.9	0.3 ± 0.2

Values are mean ± standard error.
Different letters indicate significant differences among treatments within a site.
Absence of letters indicates no statistical differences.
Blank cells indicate no plants established at that site and year.

Table 3-11. Mean density of *Agropyron*, *Bromus* and *Festuca* in 0.25 m^2 quadrat at spring treatment plots from 2008 to 2010.

Treatment	*Agropyron*			*Bromus*			*Festuca*		
	2008	2009	2010	2008	2009	2010	2008	2009	2010
New									
Control	8.2 ± 2	6.2 ± 1.4	26.8 ± 6.5				0.2 ± 0.2	0.2 ± 0.2	
Blanket	5.2 ± 3.5	20.2 ± 2	15.8 ± 2.1					1 ± 0.4	2.4 ± 1.3
Manure	22 ± 8.4	14.8 ± 2.5	23.6 ± 8.4				1 ± 0.8	1.2 ± 0.7	0.2 ± 0.2
Wood	9.6 ± 5.2	7.8 ± 1.5	10.4 ± 1.6				0.4 ± 0.4	0.4 ± 0.2	4.6 ± 2.8
New HM									
Control	30.4 ± 4.3	20 ± 3.4	22.8 ± 8.4				1.6 ± 0.7	0.8 ± 0.6	1.4 ± 1.4
Blanket	51 ± 10.1	16.6 ± 2.3	18.4 ± 3.9			0.2 ± 0.2	0.8 ± 0.6	1 ± 0.4	0.8 ± 0.8
Old									
Control	3.4 ± 2.4	7.4 ± 2.6	5.2 ± 2.2				5.2 ± 2	4.8 ± 2.4	2.8 ± 1.2
Blanket	7.4 ± 2.3	14.6 ± 2.9	10.2 ± 2.5	0.2 ± 0.2			2.8 ± 2.8	15.6 ± 5	17.6 ± 6.9
Manure	8.8 ± 2.3	9 ± 2.2	10 ± 4.3				1.6 ± 0.9	7.4 ± 2.1	6.2 ± 2.5
Wood	2.6 ± 1	3.6 ± 0.9	4.4 ± 1.3				0.4 ± 0.4	3 ± 1.3	2.4 ± 0.9
Plant									
Control	14.4 ± 5.4	18.6 ± 6.1	17.6 ± 8.4				3.8 ± 2.2	8 ± 2.1	24 ± 10.7
Blanket	18.4 ± 2.5	16.2 ± 1.5	8.6 ± 4.4	0.2 ± 0.2	0.2 ± 0.2		5.8 ± 2.3	24.8 ± 9	15.6 ± 7.6
Manure	21.6 ± 4.1	24.2 ± 7.8	35.4 ± 4.8				5.4 ± 1.6	5 ± 2.7	13 ± 8
Wood	25.4 ± 6.7	16 ± 6.6	12 ± 5.6				4.8 ± 1.4	13.2 ± 3.9	11.8 ± 4.8

Elymus establishment was negligible and therefore is not included in the table.

Blank cells indicate no plants established at that treatment and year.

Table 3-12. Mean density of *Poa*, *Trisetum* and *Vicia* in 0.25 m^2 quadrat at spring treatment plots from 2008 to 2010.

Treatment	*Poa*			*Trisetum*			*Vicia*		
	2008	2009	2010	2008	2009	2010	2008	2009	2010
New									
Control		0.6 ± 0.4							
Blanket	0.2 ± 0.2	4 ± 2.3	5.4 ± 2.5		0.2 ± 0.2	0.8 ± 0.8	0.6 ± 0.4	0.8 ± 0.8	0.8 ± 0.8
Manure	4 ± 2.8	2.8 ± 1.9	2.6 ± 1.9		0.2 ± 0.2	2 ± 2	0.6 ± 0.4		
Wood	0.2 ± 0.2	0.6 ± 0.4	0.2 ± 0.2			0.2 ± 0.2	0.2 ± 0.2		
New HM									
Control	2 ± 0.6	5.6 ± 3.3	7.6 ± 7.6		0.4 ± 0.4	1 ± 1	0.8 ± 0.4		
Blanket	4.2 ± 4	1 ± 0.4		0.6 ± 0.6	1.2 ± 1.2	0.8 ± 0.8	0.8 ± 0.4	0.2 ± 0.2	
Old									
Control		2 ± 1.5	0.8 ± 0.6		0.8 ± 0.6	0.4 ± 0.2			
Blanket	1.2 ± 1	10.6 ± 2.4	14.8 ± 2.6		1.2 ± 0.5	2.2 ± 0.9	0.2 ± 0.2		0.2 ± 0.2
Manure		2.4 ± 1.2	1.8 ± 1.1		0.2 ± 0.2	0.6 ± 0.2			
Wood	0.2 ± 0.2	2.4 ± 1	1.8 ± 1.1		0.2 ± 0.2	0.2 ± 0.2			
Plant									
Control	1.2 ± 0.6	5.4 ± 2.1	4.6 ± 2.4		1.8 ± 1.1	1.2 ± 1.2			
Blanket	8.8 ± 2.9	14.4 ± 6.6	9.6 ± 4.5	0.2 ± 0.2	3.4 ± 1.8	3.4 ± 1.2	0.2 ± 0.2	0.2 ± 0.2	
Manure	8.2 ± 3.5	4.4 ± 1.6	1.4 ± 0.7		0.8 ± 0.4	0.2 ± 0.2		0.2 ± 0.2	0.6 ± 0.4
Wood	5.8 ± 2.2	6.2 ± 2.4	6.8 ± 3.9		1.6 ± 1.1	1.6 ± 0.8			

Blank cells indicate no plants established at that treatment and year.

Table 3-13. Mean height (cm) of seeded species measured in August 2008

Site/Treatment	*Agropyron*	*Bromus* *	*Elymus**	*Festuca*	*Poa*	*Trisetum* *	*Vicia*
New							
Control	4.2 ± 0.3 b			1 ± 0.0			1 ± 0.0
Blanket	15.8 ± 5.6 ab				2.8 ± 0.5		4.3 ± 0.7
Manure	16.2 ± 1.0 a	11 ± 3.3		2.0 ± 0.2	2.4 ± 0.2		9.5 ± 2.25
Wood	6.5 ± 0.9 ab	13 ± 0.0		3.2 ± 0.2	2 ± 0.0		2.9 ± 0.0
New HM							
Control	25.0 ± 1.5	40.9 ± 0.0		3.0 ± 0.4	4.0 ± 0.4		5.5 ± 0.3
Blanket	30.2 ± 2.9	37.8 ± 8.5		3.8 ± 0.1	5.0 ± 0.5	6.7 ± 1.7	6.5 ± 1.1
Old							
Control	13.6 ± 2.7			12.5 ± 2.8	4.3 ± 1.3		
Blanket	10.1 ± 1.7	5.4 ± 0.8		11.8 ± 2.7	2.3 ± 0.4		2 ± 0.1
Manure	14.8 ± 3.8			8.1 ± 1.86	2 ± 0.0		13 ± 0.0
Wood	6.5 ± 0.6			2.5 ± 0.6	3.3 ± 0.0		7 ± 0.0
Plant							
Control	14.5 ± 2.8			13.4 ± 2.8	1.3 ± 0.9 b		4.3 ± 0.3
Blanket	17.5 ± 2.6	16 ± 1.9		7.4 ± 1.2	5.5 ± 1.4 a	5 ± 0.8	5.2 ± 0.3
Manure	21.4 ± 1.4	32.5 ± 0.0		5.2 ± 1.3	2.2 ± 0.1 ab		6.4 ± 0.0
Wood	22.7 ± 4.2			6.0 ± 2.1	2.6 ± 0.6 ab		5.6 ± 0.9

* Not enough height values to analyze variance at all sites.

Values are mean ± standard error.

Different letters indicate significant differences among heights within treatments of same site.

Blank cells indicate no plants established at that treatment and year.

Table 3-14. Mean height of seeded species in August 2009

Site/Treatment	*Agropyron*	*Bromus* *	*Elymus* *	*Festuca*	*Poa*	*Trisetum*	*Vicia* *
New							
Control	23.8 ± 3.2 b				5.0 ± 0.6	4.3 ± 0.1	
Blanket	29.9 ± 3.3 ab			6.6 ± 1.6	7.3 ± 0.7	4.3 ± 0.7	9.2 ± 2
Manure	38.3 ± 1.8 a			4.4 ± 0.2	7.3 ± 0.6	6.8 ± 0.6	
Wood	30.0 ± 2.4 ab			7 ± 2.1	4.7 ± 0.4	5.7 ± 0.9	
New HM							
Control	50.6 ± 2.4	40.5 ± 9		5.9 ± 0.8	9.1 ± 1.13	7.7 ± 1.1	20.7 ± 2.8
Blanket	52.1± 3.9			8.5 ± 1.1	8.8 ± 0.8	8.3 ± 0.6	14 ± 1
Old							
Control	19.9 ± 2.4		14 ± 0.0	11.2 ± 2	5.8 ± 1.6	2 ± 0.3	
Blanket	25.1 ± 2.2		16.1 ± 0.0	11.9 ± 2.3	5.2 ± 0.7	3.4 ± 0.3	
Manure	30.2 ± 3.1			10 ± 1.8	4.7 ± 0.7	4.2 ± 1.1	
Wood	22.8 ± 3.5			4.9 ± 1.2	4.1 ± 0.7	3.4 ± 0.7	
Plant							
Control	36.5 ± 3.6 b	40.3 ± 6.9		14.9 ± 2.6	7.3 ± 1.5 ab	4.4 ± 0.5	10.2 ± 1.3
Blanket	48.9 ± 4 ab	57.8 ± 3.8		21.9 ± 4.1	11.7 ± 1.6 a	7.5 ± 1.3	9 ± 2.4
Manure	51.1 ± 2.6 a	65.8 ± 5.9		15.7 ± 2.6	8.2 ± 1.3 ab	7.1 ± 1.1	23.3 ± 4.4
Wood	41.7 ± 3.5 ab	74 ± 0.4		14.7 ± 2.3	5.6 ± 0.8 b	5.7 ± 1.4	16.8 ± 3.9

* Not enough height values to analyze variance at all sites.

Values are mean ± standard error.

Different letters indicate significant differences among heights within treatments of same site.

Blank cells indicate no plants established at that treatment and year.

Table 3-15. Mean height (cm) of seeded species at spring treatments in August 2010. Only species in 0.25 m^2 monitoring quadrat were measured.

Site	Treatment	*Agropyron*	*Bromus* *	*Festuca*	*Poa*	*Trisetum*	*Vicia* *
New	Control	26.1 ± 3.5					
	Blanket	40.3 ± 2.6		6.9 ± 1.6	9.7 ± 2.3	7.3	14.3
	Manure	30.6 ± 6.9		5	6.3 ± 0.5	19.3	
	Wood	40.2 ± 4		4.3 ± 0.3	3.5	2	
New HM	Control	38.5 ± 4.2	28	9.5		5.8	
	Blanket	47.9 ± 4.3		5.3	7.5	7.3	
Old	Control	22.1 ± 6.4		14.4 ± 6	5.1 ± 1.2	3 ± 0	
	Blanket	27.7 ± 5.1		10 ± 2.5	7.7 ± 1.8	4.5 ± 0.8	8
	Manure	38.2 ± 3.9		11.7 ± 2.6	3.5 ± 0.2	10.8 ± 4.1	
	Wood	32.5 ± 9.2		10.1 ± 3.4	5.6 ± 0.3	20	
Plant	Control	40.8 ± 13.2		18.4 ± 4.1	4.5 ± 0.4	4.4	
	Blanket	50.3 ± 3.8		30 ± 1.3	7.3 ± 0.7	9.4 ± 2.1	
	Manure	47.3 ± 3.3		11.9 ± 2.6	7 ± 1.9	7	13 ± 1.3
	Wood	49.7 ± 2.6		20.6 ± 3	6.3 ± 1.1	8.9 ± 1	

* Not enough height values to analyze variance at all sites.

Values are mean ± standard error.

Different letters indicate significant differences among heights within treatments of same site.

No *Elymus* plants survived to 2010 and species is not included in the table.

Blank cells indicate no plants established at that site and treatment.

Table 3-16. Mean density of seed heads (seed heads m^{-2}) in August 2009.

Site/Treatment	*Agropyron*	*Bromus*	*Festuca*	*Poa*	*Trisetum*	*Vicia*
New						
Control	3.6 ± 0.9 b					
Blanket	25.2 ± 6.8 ab		0.4 ± 0.4	2.4 ± 1.4		
Manure	36 ± 8.1 a			1.2 ± 0.9		
Wood	18 ± 2.8 ab		0.4 ± 0.4			
New HM						
Control	46 ± 7.4 b			4 ± 3.2	0.4 ± 0.4	
Blanket	98 ± 19.5 a		3.2 ± 1.9	4 ± 2.5	2 ± 1.4	
Old						
Control	6.4 ± 1.5 b		11.2 ± 5.4	0.8 ± 0.8		
Blanket	24.4 ± 5.3 a		13.6 ± 6.2	2 ± 1.6		
Manure	16.4 ± 5.1 ab		4.8 ± 1.9		0.4 ± 0.4	
Wood	5.6 ± 1.4 b		0.8 ± 0.5			
Plant						
Control	25.6 ± 9.2 b	0.8 ± 0.5	24 ± 11.9	0.8 ± 0.8	0.4 ± 0.4	
Blanket	59.2 ± 9.9 a	2.8 ± 1.2	15.6 ± 3.3	10 ± 4	0.8 ± 0.5	
Manure	55.6 ± 10.8 ab	0.4 ± 0.4	7.6 ± 3.2	2.8 ± 1	1.2 ± 0.9	0.8 ± 0.5
Wood	33.2 ± 11.6 ab	0.4 ± 0.4	10 ± 3.2		0.8 ± 0.8	

Values are mean ± standard error.

Different letters indicate significant differences among heights within treatments of same site.

No *Elymus* plants had seed heads therefore the species is not included in the table.

Blank cells indicate no plants established at that site and treatment.

Table 3-17. Mean plant cover (%) of seeded species in fall treatments in August 2009.

Site/Treatment	*Agropyron*	*Bromus*	*Elymus*	*Festuca*	*Poa*	*Trisetum*	*Vicia*
New							
Control	1.6 ± 0.7						
Blanket	4.3 ± 2			0.6 ± 0.4	2.8 ± 1.8	0.5 ± 0.3	
Manure	12.6 ± 2.7			0.8 ± 0.4	3.1 ± 1	0.9 ± 0.3	
Wood	5.8 ± 1.8			0.4 ± 0.2	0.9 ± 0.6		
New HM							
Control	12 ± 3.4			0.6 ± 0.3	0.5 ± 0.5	1 ± 1	
Blanket	21.4 ± 5			2.6 ± 0.9	6 ± 0.8	5 ± 0.8	
Old							
Control	1.2 ± 0.4			6.7 ± 2.9	1.2 ± 0.7	0.1 ± 0.1	
Blanket	2.4 ± 0.9		0.4 ± 0.4	6.4 ± 3.2	2.7 ± 0.5	0.5 ± 0.2	
Manure	1.3 ± 0.4			2 ± 0.8	1.8 ± 0.5	0.7 ± 0.2	
Wood	1 ± 0.3			0.7 ± 0.4	0.6 ± 0.4	0.1 ± 0.1	
Plant							
Control	3.4 ± 1.9	0.2 ± 0.1		26.4 ± 16.9	3.8 ± 2.6	1 ± 0.4	0.5 ± 0.4
Blanket	17 ± 2.1	0.7 ± 0.2		6 ± 1.9	10.9 ± 3.7	1.3 ± 0.7	1.2 ± 1.2
Manure	14.4 ± 4.7	0.2 ± 0.2		3.9 ± 1.5	7.2 ± 1.5	2.4 ± 0.7	0.4 ± 0.4
Wood	3.7 ± 0.8	0.4 ± 0.3		6.7 ± 3.6	2.9 ± 1.1	0.3 ± 0.2	1.2 ± 0.5

Values are mean ± standard error.

No statistical differences were detected among soil treatments within a site.

Blank cells indicate no plants established at that site and treatment.

Table 3-18. Mean plant cover (%) of seeded species in spring treatments in August 2009.

Site/Treatment	*Agropyron*	*Bromus*	*Elymus*	*Festuca*	*Trisetum*	*Vicia*
New						
Control	3.4 ± 1.7			0.3 ± 0.2		
Blanket	9.6 ± 5.5			2.8 ± 1.4	0.1 ± 0.1	0.6 ± 0.6
Manure	23.4 ± 11.5			2.6 ± 1.5	0.2 ± 0.2	
Wood	3.8 ± 0.9			0.2 ± 0.1		
New HM						
Control	26.8 ± 9.9			2.1 ± 1.2	0.8 ± 0.8	0.4 ± 0.4
Blanket	43.8 ± 10.3			1.6 ± 0.9	0.1 ± 0.1	
Old						
Control	1.4 ± 0.7			1 ± 0.8	0.1 ± 0.1	
Blanket	3.2 ± 0.6			6.8 ± 2.8	0.5 ± 0.2	
Manure	4 ± 0.8			1.9 ± 1.1	0.3 ± 0.2	
Wood	0.9 ± 0.4			1.2 ± 0.6	0.1 ± 0.1	
Plant						
Control	5 ± 2.1			2.5 ± 1.4	0.7 ± 0.4	
Blanket	13.6 ± 2.3	0.2 ± 0.2		13 ± 6.6	0.8 ± 0.4	0.1 ± 0.1
Manure	19.4 ± 4.4			1.8 ± 0.6	0.3 ± 0.2	0.2 ± 0.2
Wood	9.6 ± 2.7			3.2 ± 1.6	0.7 ± 0.6	

Values are mean ± standard error.
No statistical differences were detected among soil treatments within a site.
Blank cells indicate no plants established at that site and treatment.

Table 3-19. Mean plant cover (%) of seeded species in spring treatments in August 2010.

Site/Treatment	*Agropyron*	*Bromus*	*Elymus*	*Festuca*	*Poa*	*Vicia*
New						
Control	2.7 ± 0.8					
Blanket	7.6 ± 2			0.5 ± 0.2	0.1 ± 0.1	0.4 ± 0.4
Manure	7.6 ± 2.4			0.3 ± 0.2	1.4 ± 1.4	
Wood	6.6 ± 1.2			0.6 ± 0.2	0.2 ± 0.2	
New HM						
Control	14 ± 2.5	0.2 ± 0.2		0.2 ± 0.2	0.4 ± 0.4	
Blanket	20.4 ± 2.3			0.2 ± 0.2	0.2 ± 0.2	
Old						
Control	1.5 ± 0.6			2.2 ± 0.7	0.3 ± 0.2	
Blanket	1.8 ± 0.8			4.6 ± 1.3	1.2 ± 0.4	0.2 ± 0.2
Manure	2.4 ± 0.2			3.8 ± 1.2	0.6 ± 0.2	
Wood	1.8 ± 0.8			1.9 ± 1.1	0.1 ± 0.1	
Plant						
Control	4 ± 1.3			2.4 ± 0.9	3*	
Blanket	3.8 ± 0.6			9.3 ± 1.4	0.6 ± 0.2	
Manure	10.2 ± 2.5			4 ± 2.2	0.25*	0.3 ± 0.2
Wood	3.3 ± 1.5			14 ± 1.6	0.7 ± 0.2	

Values are mean ± standard error.

No statistical differences were detected among soil treatments within a site.

*Value from a single plot.

Blank cells indicate no plants established at that site and treatment.

Table 3-20. Mean plant and litter cover at each site and treatment in August 2008 and 2009.

Site	Treatment	Plant Cover (%)		Litter Cover (%)*	
		2008	2009	2008	2009
New	Control	1.9 ± 0.4 b	15.2 ± 3.3	1.1 ± 0.3 b	0.6 ± 0.2 b
	Blanket	2.6 ± 1.2 b	26.7 ± 4.5		
	Manure	12.9 ± 5.3 a	24.6 ± 5.3	3.75 ± 1.0 ab	2.7 ± 0.5 a
	Wood	3.7 ± 2.3 b	26.2 ± 4.2	15.8 ± 4.0 a	3.6 ± 0.6 a
New HM	Control	15.5 ± 2.7 b	35.2 ± 5.6	4.9 ± 1.3	1.3 ± 0.2
	Blanket	29.0 ± 3.8 a	45.5 ± 5.5		
Old	Control	4.7± 1.4	18 ± 3.6	1.1 ± 0.2 b	0.6 ± 0.1 b
	Blanket	2.7 ± 0.8	16.9 ± 2.7		
	Manure	7.8 ± 2.8	20 ± 4.2	4.2 ± 1.2 a	2.1 ± 0.5 b
	Wood	2.5 ± 0.5	7.6 ± 1.8	18.5 ± 4.8 a	12.8 ± 3.2 a
Plant	Control	9.4 ± 4.4	27.4 ± 7.9	0.7 ± 0.1 b	1.1 ± 0.2
	Blanket	16.2 ± 4.4	41.5 ± 4.9		
	Manure	18.3 ± 4.1	37.9 ± 6.8	3.7 ± 0.8 a	1.25 ± 0.3
	Wood	10.9 ± 2.9	31.1 ± 7.0	8.7 ± 3.4 a	2.9 ± 1.2

* Litter was not quantifiable at blanket treatments and cells were left blank.

Values are mean ± standard error.

Letters indicate statistically significant differences between values for treatments at the same site and year. Absence of letters indicates no statistical differences.

Table 3-21. Mean plant and litter cover in spring treatments in August 2010.

Site	Treatment	Plant Cover (%)	Litter Cover (%)*
New	Control	13.7 ± 3.5	3.2 ± 0.7
	Blanket	20.8 ± 3.9	
	Manure	15.2 ± 3.1	5.2 ± 1.0
	Wood	22.4 ± 5.2	3.2 ± 0.7
New HM	Control	24 ± 4.5	5.8 ± 1.2
	Blanket	29.8 ± 3.7	
Old	Control	12.1 ± 4.1	2.6 ± 0.7
	Blanket	19 ± 2.2	
	Manure	28.4 ± 9.0	3.2 ± 0.8
	Wood	11.8 ± 1.5	7.8 ± 4.6
Plant	Control	7.6 ± 2.0	2.6 ± 0.9
	Blanket	12.9 ± 3.5	
	Manure	18.6 ± 3.1	7.8 ± 3.1
	Wood	16.8 ± 6.4	3.75 ± 0.9

* Litter cover was not quantifiable at blanket treatments.

Values are mean ± standard error.

Letters indicate statistically significant differences between values for treatments at the same site.

Absence of letters indicates no statistical differences.

Table 3-22. Mean density (plants m^{-2}) of non-seeded species at each site and treatment in 2009.

Site Treatment	*Erucastrum gallicum*	*Chenopodium album*	*Melilotus* spp	*Sonchus arvensis*	*Polygonum* spp	*Hordeum jubatum*	*Avena fatua*	*Cirsium arvense*
New								
Control	22.2 ± 3.2 a	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0 ± 0	0.7 ± 0.2	0 ± 0	0 ± 0
Blanket	16.6 ± 2.7 ab	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.2	0.3 ± 0.3	0.1 ± 0.1	0 ± 0	0 ± 0
Manure	7.9 ± 1.4 b	1.1 ± 0.9	0.6 ± 0.1	0.6 ± 0.6	0 ± 0	0.9 ± 0.5	0 ± 0	0 ± 0
Wood	22.4 ± 4.7 a	0.1 ± 0.0	0.4 ± 0.2	1.6 ± 1.4	0 ± 0	0.1 ± 0.0	0 ± 0	0 ± 0
New HM								
Control	5.7 ± 1.7	11.6 ± 3.1	0.1 ± 0.1	0.7 ± 0.6	1.0 ± 0.5	0 ± 0	0 ± 0	0 ± 0
Blanket	3.5 ± 0.8	4.4 ± 1.6	0.0 ± 0.0	0.2 ± 0.2	1.9 ± 0.5	0.2 ± 0.1	0 ± 0	0.7 ± 0.7
Old								
Control	19.9 ± 4.2 a	0.0 ± 0.0	0.3 ± 0.2	0 ± 0	0 ± 0	0.1 ± 0.0	0 ± 0	0 ± 0
Blanket	7.9 ± 2.1 b	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Manure	17.0 ± 2.9 ab	1.0 ± 1.0	0.2 ± 0.1	2.8 ± 2.1	0 ± 0	0.1 ± 0.0	0 ± 0	0 ± 0
Wood	12.3 ± 2.3 ab	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 1.12	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Plant								
Control	7.9 ± 2.4	0.2 ± 0.2	12.6 ± 2.8	0.1 ± 0.1	2.4 ± 1.2	0.1 ± 0.0	0 ± 0	0 ± 0
Blanket	1.3 ± 0.6	0.1 ± 0.1	17.9 ± 6.1	0.3 ± 0.1	1.1 ± 0.4	0 ± 0	0 ± 0	0 ± 0
Manure	2.6 ± 1.3	0.4 ± 0.2	16.7 ± 4.6	0.2 ± 0.1	2.7 ± 0.9	0 ± 0	0 ± 0	0 ± 0
Wood	4.7 ± 1.5	0.4 ± 0.1	26.7 ± 5.2	0.9 ± 0.5	2.5 ± 1.0	0.1 ± 0.1	0.1 ± 0.1	0 ± 0

Values are mean ± standard error.

Letters indicate statistically significant differences between values for treatments at the same site.

Absence of letters indicates no statistical differences.

Sonchus arvensis, Cirsium arvense and Avena fatua are considered noxious weeds in Alberta, Canada.

Table 3-23. Variation partitioning using redundancy analyses (RDA) on Hellinger transformed density of seeded and non seeded species in 2009.

				Proportion Explained			Significant p Values for Individual Variables		
Explanatory Variables	df	F	p	RDA1 (p)	RDA2 (p)	RDA3 (p)	Total Nitrogen	Total Carbon	Soil Treatment
Sites	3	37.88	0.005	0.267 (0.005)	0.127 (0.005)	0.061 (0.005)			
Soil Properties	5	6.06	0.005	0.137 (0.005)	0.032 (0.005)	0.011	-0.01	-0.01	
Soil Treatments	3	3.591	0.005	0.059 (0.005)	0.012	0.001			-0.01

Variation partitioning was based on RDAs against three sets of explanatory variables: site (New, New HM Old and Plant), soil properties (standardized values of total nitrogen, carbon, organic carbon, electrical conductivity and hydrogen ion activity) and soil treatments (control, blanket, manure, wood).

Only significant p values are indicated for RDA axes (p).

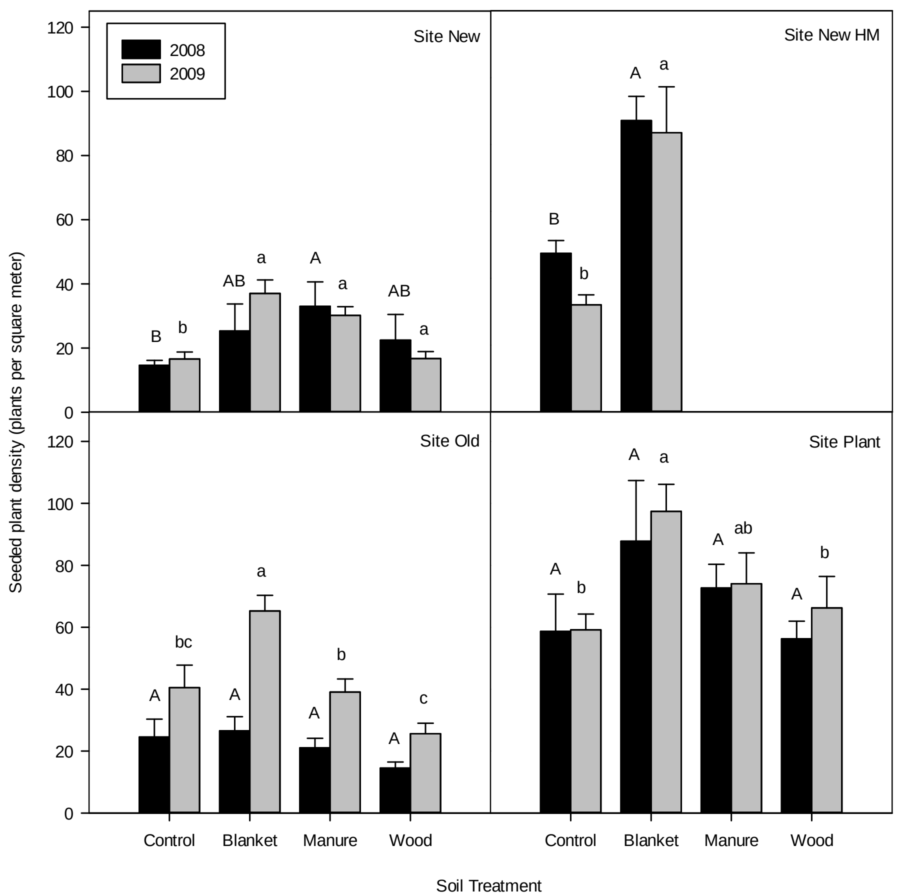

Figure 3-1. Mean seeded plant density at each soil treatment and site in 2008 and 2009. Soil treatments, particularly blanket and manure, increased plant density in relation to the controls at all sites. Letters indicate significant differences among soil treatments in the same year; upper case letters for 2008 and lower case letters for 2009. Bars indicate standard error.

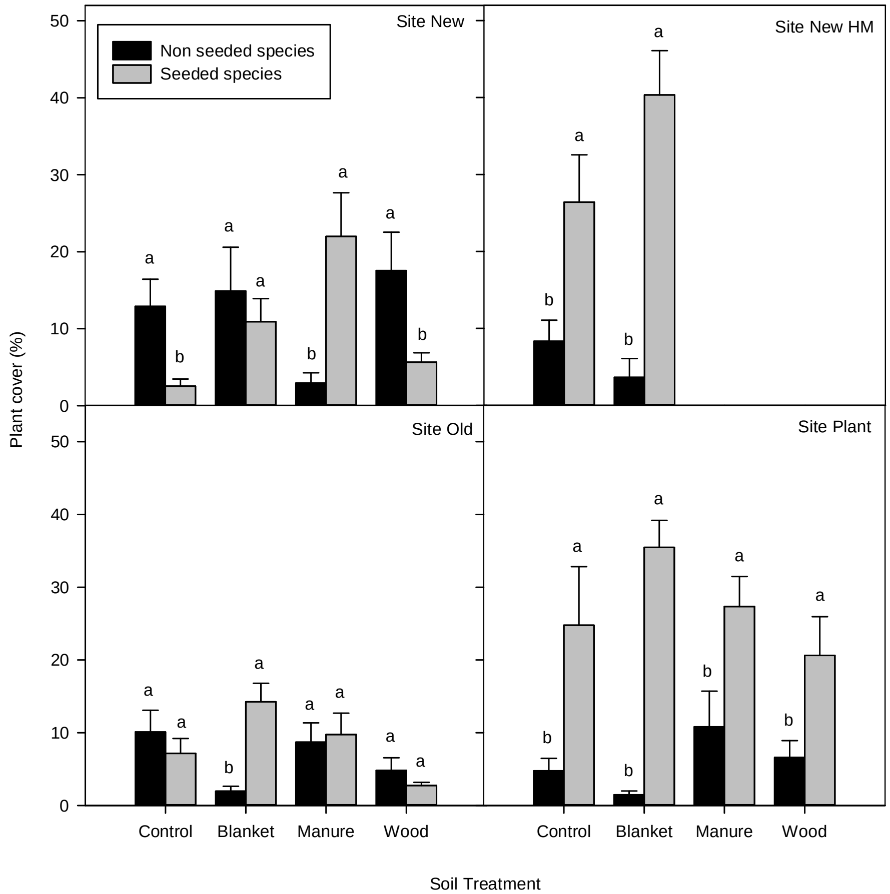

Figure 3-2. Mean percent plant cover of seeded and non seeded species in 2009. Plant cover of desirable species varied with site and amendment type, being higher at sites New HM and Plant and in the erosion control blanket treatment. Letters indicate significant differences between plant cover of seeded and non seeded species of the same treatment within a site. Bars indicate standard error.

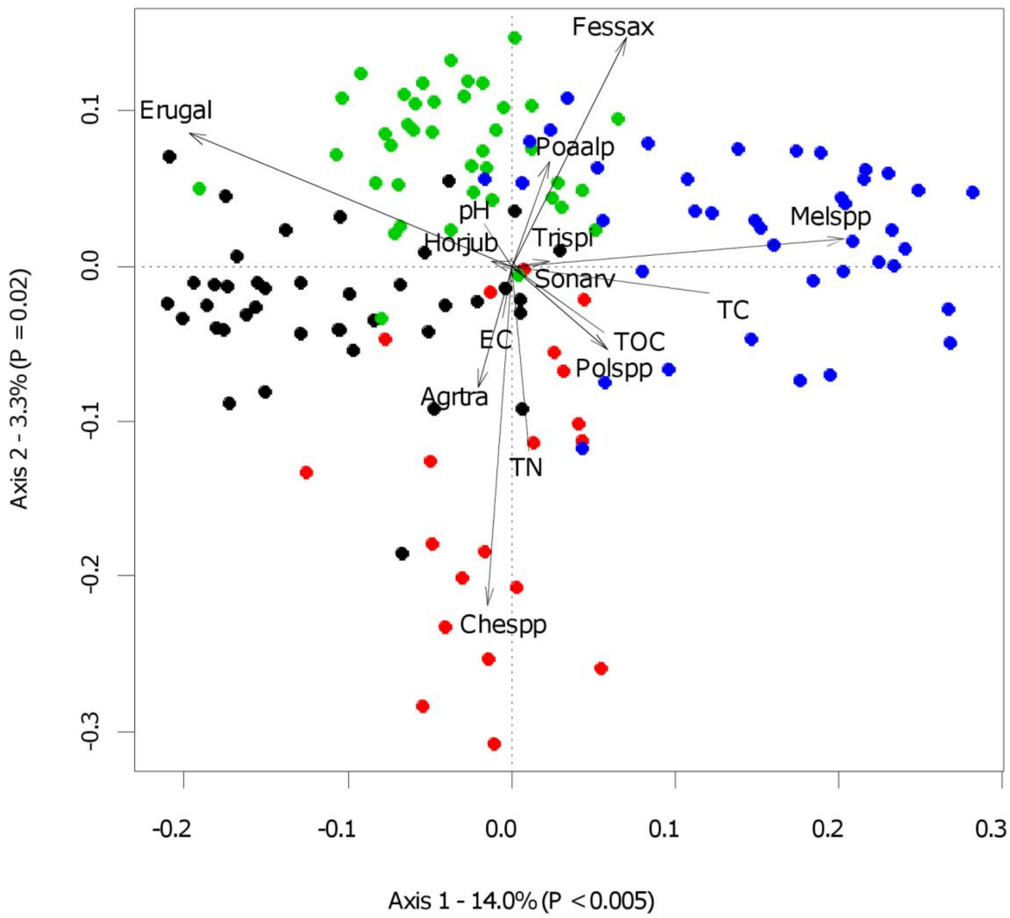

Figure 3-3. Triplot of effect of standardized soil properties (lines) on Hellinger transformed species plant density (arrows). Plant and soil conditions at each site differed, resulting in little overlap among sites. Total nitrogen (TN) and total carbon (TC) were soil properties exerting greater influence in the plant community with larger effects at sites New HM and Plant, respectively. Species codes are the first three letters of genus and species names. Plots at each site are in different dot colours: site New black, site New HM red, site Old green and site Plant blue. Other soil parameters in the figure are hydrogen ion concentration (pH), total organic carbon (TOC) and electrical conductivity (EC).

CHAPTER 4. ESTABLISHMENT OF WOODY SPECIES FROM SEEDS AND SEEDLINGS AT A LIMESTONE QUARRY

1. INTRODUCTION

Limestone quarries are often located in ecologically sensitive areas where their large disturbances contribute to habitat fragmentation, loss of interior forest, species habitat and diversity from edge effects (Martínez-Garza and Howe 2003, Wickham et al. 2007). These impacts often have a cumulative effect with other human activities in the region of occurrence, increasing their impact. To fulfill regulatory requirements and contribute to a wide range of ecological services reclamation of many quarries must be to a natural plant community, often including woody species. The ability of a community to maintain these ecological services has been linked to high biodiversity and species richness (Petchey and Gaston 2002, Menninger and Palmer 2006). For example, a mixed stand forest will have higher resilience to disease than a single species stand (Cole et al. 1999) and will provide a better habitat for wildlife (Densmore and Page 1992). Planting of disturbed land is increasingly viewed as an exchangeable commodity for carbon sequestration (Dewar and Cannell 1992, Newell and Stavins 2000) and even energy production in the form of biofuel crops (Evans et al. 2010).

Establishment of woody vegetation on highly disturbed land is challenging. Natural establishment may be very slow. It may take years for individual plants to reach their reproductive stage and seedlings commonly have low survival rates. Although natural forest regeneration and enrichment of floristic composition could occur if undisturbed forest or other plant community fragments near the disturbed area become a source for seeds, propagules and seed dispersers (Wunderle Jr. 1997, Rodrigues et al. 2004), human assisted revegetation is required for more rapid development of a diverse community. Reclamation practices can incorporate facilitation, a natural mechanism of succession by which the establishment of some species modifies the environment to make it more suitable for other species to establish (Crocker and Major 1955, Maestre et al. 2001, Gomez-Aparicio 2009). One example is purposely introducing fast growing shade trees to accelerate forest regeneration and nurse plants to assist recovering vegetation (Menninger and Palmer 2006). Other interactions will act simultaneously to shape the structure and composition of the plant community, such as predation (grazing, browsing) and competition. For example, tree seedlings may be outcompeted by certain grasses (Lieffers et al. 1993).

Ideally, the woody species to be introduced during assisted revegetation should be chosen from local vegetation. The use of native species helps to retain biodiversity on a reclaimed area (Gerling et al. 1996, Martínez-Garza and Howe 2003); preferably, local ecotypes and cultivars should be chosen to increase survival and fitness (Campbell and Sorensen 1978, Smith et al. 2005). A variety of deciduous and evergreen trees and shrubs should be used to approximate undisturbed conditions and build the functional diversity of the community. For example, shrubs have positive effects on tree establishment (Callaway and Davis 1998, Rousset and Lepart 1999); nitrogen fixing plants have positive effects on their neighbors (del Moral and Wood 1993, Gomez-Aparicio 2009); shadow forming trees can reduce high air temperature and light irradiance.

Several techniques can be used to introduce woody plants to a large disturbed site. Seeding and transplanting are the most common techniques, but other approaches have had positive results. Transplanting trees and shrubs is most likely to speed up natural succession, reducing the time for secondary forest vegetation establishment. Transplanted trees from local sources may provide additional benefits if root systems are transplanted with native soil containing natural occurring mycorrhizae, which could improve successful establishment of the seedling (Allen et al. 2005). Although plant survival may increase if saplings and young or adult trees are transplanted, costs may increase. Planting is labour intensive and more costly where mechanization is not feasible due to steep and irregular rocky landscape. Seeding can occur when seeds are available by purchasing or collecting. Seeding may be a less expensive way to introduce woody species but establishment from seeds is often low, requiring the use of much seed to account for non viable seeds and low survival rates. Donor soils have been used as a source of native plant propagules for revegetation of mine sites (Iverson and Wali 1981, Holmes 2001, Mackenzie and Naeth 2009). The uppermost layer containing litter (L), fermented and fragmented litter (F) and humus (H), the LFH horizon, has potentially higher concentrations and viability of propagules (Mackenzie and Naeth 2009). It may contain propagules from a variety of species for which seed can not be collected or purchased.

Seedlings may be purchased or obtained from donor sites. In the nursery, seedlings can be produced as plugs, bare root and containerized plants; stock type may have an effect on tree survival once out planted. Stock type was the most important factor affecting spruce (*Picea*) growth during the first few years after planting in two separate experiments (Vyse 1981, Burdett et al. 1984). Stock that produced higher root mass positively affected tree growth, likely due to increased water uptake by the roots. Size of plants to be introduced is an important factor in revegetation. If soils are shallow, small seedlings may be more successful than large seedlings (Dobbs1976, Thomson and McMinn 1989). Plants taken from donor sites will often successfully transplant, if the root mass is not too disturbed, but donor sites must be chosen carefully (Land Owner Resource Centre 2000) and the amount of material removed must be small to minimize damage to the donor site.

Transplanting and seeding season may affect survival. Plants introduced in spring may establish quickly, taking advantage of an increasingly longer photoperiod over the summer, but they are at greater risk of overheating and desiccation. Plants introduced in the fall will likely go dormant and delay growing until the next spring when they will rely on their root reserves to start growing. Tolerance to environmental conditions related to season of planting may vary with species. Within species, individual plant characteristics such as greater root mass or height may provide an advantage to survive low soil water content, high soil temperature or frost (Eis 1965, Ehleringer and Sandquist 2006).

Plant species chosen to revegetate a limestone quarry should survive and grow under conditions of low nutrients, rocky substrate, low soil water, high pH and often, steep slopes of various aspects. Scarcity of topsoil forces use of subsoil and overburden as substrates. These materials are characteristically low in organic matter and nutrients. Slope position and aspect will often further constrain vegetation establishment. Modification of these conditions through soil amending may improve plant establishment. Trees like *Betula* can regenerate naturally, but germination is limited by vegetative competition and lack of suitable substrate for germination (Densmore and Page 1992).

Nutrient reserves in the plant and at the site had an effect on successful plant establishment. Nutrients must be available for plants to produce new roots and shoots when planted. Seedling growth and survival may increase through fertilizer application (Carlson and Preisig 1981, Carlson 1981, Van Den Driessche 1985, Haase et al. 2006), although abundance of nutrients in some cases may inhibit root growth (Jacobs et al. 2004). Modification of conditions at the planting hole can increase establishment success. For example, better quality soil can decrease bulk density and wood shavings can increase water content.

Understanding and modification of the main factors that limit establishment of a diverse plant community with woody species at a limestone quarry is necessary to devise effective reclamation strategies for limestone quarries around the world. The study quarry requires reclamation to a natural plant community with woody species, to fulfill regulatory requirements and contribute to a wide range of ecological services. This research was designed to assess limestone quarry reclamation potential of select species of native trees and shrubs; specifically to determine whether plant material source (nursery, local transplants, local LFH, seeds), planting and seeding season and soil amendments would affect woody species survival and establishment.

2. MATERIALS AND METHODS

2.1 Study Area

Research was conducted at the Graymont Exshaw limestone quarry near Kananaskis, Alberta, Canada (51º 07’ N 115º 13’ W). The quarry was located on a south facing slope of the Rocky Mountains, at an elevation of approximately 1,350 m, below the tree line that occurs from 2,000 to 2,300 m. The climate is montane-subalpine (Alberta Parks and Protected Areas 2002). Long term mean annual precipitation for the area is 296.2 mm as rain and 234.1 cm as snow. Mean daily temperature is 3 ^{o}C, mean maximum is 21.9 ^{o}C in July and mean minimum is -14.1 ^{o}C in January (Environment Canada 2011).

In the undisturbed surrounding areas, soils are mainly brunisols with underlying calcareous parent material (Alberta Parks and Protected Areas 2002). Vegetation is mixed wood forest. Dominant woody species are *Pinus contorta* Douglas ex Louden (lodgepole pine), *Picea glauca* (Moench) Voss (white spruce), *Abies lasiocarpa* (Hook.) Nutt. (alpine fir), *Populus tremuloides* Michx. (trembling aspen), *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir) and *Populus balsamifera* L. (balsam poplar) (Alberta Parks and Protected areas 2002). Main understory woody species includes *Shepherdia canadensis* (L.) Nutt. (buffaloberry), creeping juniper (*Juniperus horizontalis* Moench), *Arctostaphylos uva-ursi* (L.) Spreng. (bear berry), *Salix spp.* (willow). Wildlife includes noticeable mammals such as rocky mountain goat (*Oreamos americanus* de Blainville), moose (*Alces alces* L.), grizzly bear (*Ursos arctos horribilis* Ord.), black bear (*Ursus americanus* Pallas) and bighorn sheep (*Ovis canadensis* Shaw) (UNEP 1998). Big horn sheep like to climb the rocky quarry faces and recontoured slopes and can be a great detriment to revegetation.

This reclamation research was conducted at the northwest end of the Exshaw quarry at an elevation of 1,525 m. The site is an embankment with a steep, long south facing slope approximately 75 m long, 150 m wide and with 30 degree inclination. Embankments are engineered piles of boulders (waste rock from limestone mining), covered with substrate. At Exshaw quarry, the majority of subsbtrate is clean fill, which is admixed topsoil with subsoil. The embankment where the woody species experiment was established was covered with a < 10 cm layer of clean fill. The surface was very rocky, about 70%. Size of rocks varied from 5 cm pebbles to large rocks of 30 cm or more. The embankment was seeded with a grass mix in 2000. In 2007, when the woody species experiment being described was set up, grass cover was less than 9% across the site and it was not possible to distinguish differences in vegetation due to prior experimental trial with grasses.

2.2 Treatments and Experimental Design

2.2.1 Soil amendments and fertilizer

Two amendments and fertilizer were based on best results from two greenhouse experiments to ameliorate limestone substrate (Chapter 2). Wood shavings were fine screened from pine and white spruce wood; procured from the University of Alberta dairy farm. Clean fill, consisting of subsoil excavation material was procured from the Exshaw quarry stock piles, originally obtained from the town of Canmore. Fertilizer was slow release Nutricote 14-14-14 (nitrogen, phosphorus, potassium) type 100.

Amendment treatments were prepared by hand mixing the soil excavated at each hole for seeding and transplanting with a proportional amount of amendment. Rocks were removed from the excavated soil which left enough room for the added amendments. Wood shavings were applied at a rate of 11.25 Mg ha^{-1}, approximately 35 g and 70 g to seeding and planting holes, respectively. Clean fill was applied at a rate of 482 Mg ha^{-1}, approximately 1,514 g and 3,028 g to seeding and planting holes, respectively.

Fertilizer was applied by hand at a rate of 1.1 Mg ha^{-1}, that is 3.5 g per transplant or seed holes. Both spring and fall season, all soil amendments and all plant source materials were fertilized, except LFH.

2.2.2 Plant species and sources, transplanting and seeding

Transplants and seeds of seven plant species and locally collected LFH were used for a total of 13 plant sources. Plant species were initially selected based on being native to the study area and ultimately on availability. Species included *Picea glauca* (Moench) Voss (white spruce), *Pseudotsuga menziesii* (Mirbel) Franco (douglas fir), *Populus tremoloides* Michx. (trembling aspen), *Betula papyrifera* Marshall (paper birch), *Juniperus horizontalis* Moench (creeping juniper), *Alnus crispa* (Aiton.) Pursh (green alder) and *Arctostaphylos uva-ursi* (L.) Spreng. (bear berry).

Seedlings and wild collected seeds were purchased from Bow Point Nursery which is located approximately 50 km East from the study site. Species that were introduced both as plug transplants and seeds from the nursery were *Pseudotsuga*, *Populus, Betula* and *Picea*. Seeded only species were *Alnus* and *Arctostaphylos.* Plugs were one year old stock and average height was 30 cm.

Local seedlings were procured from the quarry site and forested adjacent areas. *Juniperus* was collected from adjacent forested sites, and only used if after lifting the plant from the soil it still had a large portion of roots in good condition. Local *Picea* transplants were collected from the gravelly quarry compound where they established from naturally dispersed seeds. Age of local transplants is unknown, but likely between one to few years old, and average height was 20 cm.

LFH was collected from a forested area adjacent to the experimental site with shovels. To avoid disturbing larger areas, LFH was collected with a shovel from three scattered sites of 1 x 1 m to a depth of 5 cm. LFH from the three sites was composited and homogenized in large plastic bags prior to its placement on the allocated holes as a 5 cm thick layer. Additional LFH samples were taken from the same area in May 2008 and August 2009 and frozen until used to characterize the seed bank. On October 2009 LFH was thawed and placed in trays as a layer of 3 cm on top of a 3 cm layer of potting soil. The trays were watered regularly. After one month in the greenhouse, all emerged propagules were identified and removed, the LFH was hand tilled to allow for potential propagules in the deeper portion of the layer to emerge and continued to be watered regularly until May 2010, when vegetation was assessed again, including cover of individual species.

All plants were transplanted within 48 hours of being carried from the nursery or lifted from the local sites. One individual was transplanted per planting hole. To avoid over crowding the seeding holes, only 10 seeds of each woody species were sown. Because of their smaller size, 20 *Populus* seeds were sown. *Betula*, *Alnus* and *Populus* seeds were placed on substrate surface and slightly pressed into the substrate; *Arctostaphylos*, *Pseudotsuga* and *Picea* seeds were placed and covered with a very thin layer of the substrate from the hole, only as thick to be the equivalent to 1 or 2 times the diameter of the species seed. Seeding and planting of woody species occurred on two different dates in 2007; the spring treatments from June 14 to 18 and the fall treatments from August 31 to September 3.

During the four days of the spring planting, a total of 91 mm of rain registered in the Bow Valley weather station, followed by nine days without any additional rain. Maximum temperatures were 11 to 17 ^{o}C and minimum temperatures were 2.5 to 6 ^{o}C. On the first two days of the fall planting a total of 8 mm of rain was registered in the Bow Valley station, followed by four days without any rain. The maximum temperature ranged from 17 to 22 ^{o}C and the minimum ranged between 2.5-7 ^{o}C.

2.2.3 Experimental design

The experimental design was completely randomized to test effect of season of transplanting and seeding, plant source and soil amendment on plant survival and establishment. A 1,040 m^2 (52 x 20 m) area was divided into four 10 x 20 m sections, each separated by a 4 m buffer. Two of these sections were allocated to spring season and two to fall season, alternated across the area, to decrease effect of location within the research area. A grid of holes 1 m apart were excavated with a shovel to a standard size for transplanting and seeding; transplant holes were 20 cm diameter x 20 cm depth; seeding and LFH holes were 15 cm diameter x 10 cm depth. Individuals of each plant source were planted or seeded into the three soil treatments, which were randomly assigned a location within the grid of holes. Every combination of soil treatment and plant source was replicated ten times. The experimental design is therefore: 2 transplanting and seeding seasons x 3 soil treatments including control x 13 plant sources x 10 replicates = 780 planting and seeding holes.

2.2.4 Fauna management

Due to intense bighorn sheep grazing at the research plots, Plantskydd, a biodegradable chemical deterrent made of dry pig blood was applied to the entire study area in June 2008 and April 2009. The product was applied using a solution of 1 kg product per 6 gallons (22.7 L) of water applied at the manufacturer recommended rate.

2.3 Vegetation Assessment

In June 2007, prior to research site establishment, 40 random sites (10 in each spring and fall allocated section) were selected to assess plant cover and species composition using 0.1 m^2 quadrats. Assessments took place twice per year.

The first assessment of plant survival and emergence occurred on October 2007. Regular assessments followed in spring (May 14 to 15, 2008 and May 23 to 24, 2009) and late in the growing season (August 14 to 15, 2008, August 23 to 24 2009 and August 25, 2010). Assessments included survival of transplants, emergence and survival of seeded species and LFH seed and propagule emergence and survival. Plants were evaluated for evidence of browsing or pulling out by bighorn sheep (*Ovis canadensis canadensis* Shaw) and for presence of buds. In August 2008 height of all transplants was measured, after which, height was measured at every assessment date to estimate increase of each transplant between monitoring dates.

In August 2010, the surrounding forested area close to the study site was assessed to determine general characteristics of the soil and plant communities. Two quadrats, 5 x 5 m in size, were located in two distinct plant communities. Within each quadrat, a smaller 20 x 50 cm quadrat was placed in a discrete location to identify grasses. Beside each quadrat a hole 20 to 30 cm in diameter was dug to characterize soil type and depth of topsoil. Vegetation surrounding the garage compound where the *Picea* local trees were collected was characterized in a 5 x 5 m quadrat.

2.4 Statistical Analyses

Plant survival data were analyzed using a logistic regression. The variation explained by transplanting season, soil amendments and species to plant survival during four growing seasons was assessed by examining the change in residual deviance resulting from removing factors from the model. Model fitting for survival was done using a logit link (Agresti 2002, Jones et al. 2005). The response variable was survival at each assessment date from October 2007 to August 2010 (0 = died; 1 = survived), and the explanatory variables were transplanting season (fall and spring), soil treatments (clean fill, wood shavings and unamended control) and transplanted species. Graphics were done using SigmaPlot 12 (Systat Software 2011).

Analysis of deviance of the residuals was used to determine differences in survival due to species and amendments within fall and spring treatments. Akaike information criterion (AIC) values were used to compare how good the logit model explained survival of fall and spring treatments. AIC judges a model by how close fitted values tend to be to the true expected values, as summarized by an expected distance between the two (Agresti 2007). Smaller AIC values indicate fitted values are closer to true expected values. Effect of planting season on survival of individual species was conducted by analyses of deviance and Chi-square statistics were used for the calculation of the significance of the factor.

The chi-square test is the most appropriate for a model with known dispersion, binomial in the case of survival data. R statistical language (R Development Core Team 2011) was used for the analyses.

Mortality of plants of every species was determined as time intervals among planting and assessment dates. For example, the first estimation was calculated as: % mortality = ((live transplants $_{\text{(planting date)}}$ - live transplants $_{\text{(assessment date 1)}}$) / live transplants $_{\text{(planting date)}}$)* 100. The height increments of each transplant in a growing season (from May to August) and between years (August to August the following year) were calculated as the difference from a measured height minus the previous measured height of each transplant between assessment dates. Mortality and height data were summarized but not statistically analyzed.

Plant data from seeds or LFH were very limited and did not allow for statistical analyses. Established seedlings at each assessment date and treatment were counted and data were summarized.

3. RESULTS

3.1 In Situ and Surrounding Plant Communities Prior to Treatments and LFH Seed Bank

Prior to this woody species experiment in June 2007, mean vegetation cover on the research site was 9% and mean litter cover was 5.5% (data not shown). Dominant species were *Festuca ovina* L. (sheep fescue) (6.22%) followed by *Agropyron dasystachyum* (Hook.) Scribn. (northern wheat grass) (2.53%). *Stipa viridula* Trin. (green needle grass), *Chrysopsis villosa* (Pursh) Nutt. (hairy golden aster) and *Bromus tectorum* L. (downy brome) together had 0.05% cover. The vegetation was sparsely distributed fairly homogeneously patchy.

Two vegetation communities in proximity to the research site (300 m to the North) were identified (data not shown). The bearberry-logepole pine community had *Pinus contorta* Douglas ex Louden (lodgepole pine), *Pseudotsuga menziesii*, *Picea galuca*, *Juniperus horizontalis*, *Arctostaphylos uva-ursi*, *Galium boreale* L. (northern bedstraw) and *Potentilla fruticosa* L. (shrubby cinquefoil). LFH depth was 3 cm. The *Pinus contorta* and *Elymus* community had *Pinus contorta*, *Pseudotsuga menziesii*, *Populus tremuloides*, *Juniperus horizontalis*, *Elymus innovatus* Beal. (hairy wild rye), *Galium boreale*, *Pyrola asarifolia* Michx. (liver leaf wintergreen), *Lathyrus ochroleucus* Hook. (cream pea) and moss. Soil was an Orthic Utric Brunisol, with a 30 cm depth.

The garage compound where *Picea* local trees were collected for the experiment is situated at the bottom of a hill on the edge of the quarry. The vegetation uphill consisted mainly of *Populus*, *Pseudotsuga*, *Juniperus*, *Arctostaphylos*, *Rosa acicularis* Lindl. (prickly rose), *Festuca saximontana* Rydb. (rocky mountain fescue), *Medicago sativa* L. (alfalfa), *Galium*, *Cirsium arvense* (L.) Scop. (canada thistle) and *Solidago* spp. (golden rod) (data not shown).

Species in the LFH seed bank, after one month of growing in the greenhouse, included one *Picea* seedling and plants from three forb species, *Crepis tectorum* L. (annual hawksbeard)*, Epilobium anagallidifolium* Lam. (willow herb) and *Erucastrum gallicum (Willd.) Britt,* (dog mustard). In May 2010, the LFH collected in 2008 resulted in 25% plant cover, 10% moss cover and three species *Crepis tectorum, Epilobium anagallidifolium* and *Cirsium arvense* (L.) Scoop. (Canada thistle). The LFH collected in 2009 had 45% plant cover of *Crepis tectorum* and *Epilobium anagallidifolium*.

3.2 Survival and Growth of Out Planted Trees

Total transplant survival of all species responded significantly to transplanting season ($p<0.001$). After four growing seasons, trees out planted in fall had higher survival than those out planted in spring (Table 4-1). These differences were evident since the first assessment date and persisted to the end of the experiment. Differences in survival due to planting season were statistically analyzed for the first and last assessment dates. Planting season affected *Picea* local ($p<0.001$), *Picea* nursery ($p<0.001$), *Populus* ($p<0.003$) and *Pseudotusga* ($p<0.003$) the year of planting. At the end of the experiment, the significant effect only persisted for *Picea* local. At this time, survival of *Betula* showed significant differences related to planting season (Tables 4-1 and 4-2). Survival was not significantly affected by soil treatments in either spring or fall planting (Table 4-3). Survival of *Populus*, *Pseudotsuga* and *Picea* (nursery) although lower when spring planted, were higher than the other species (Figure 4-1 and Table 4-1).

Twice as many plants of *Betula* and *Picea* (local) survived fall planting. *Juniperus* survival was low regardless of planting season.

Highest mortality for most species occurred during the first months after planting, especially for spring plantings for most species (Table 4-4). *Juniperus* had the highest mortality. Although *Betula* mortality was lower than the other species after planting, it had higher consistent mortality over the years and the second highest mortality at the end of the experiment. *Picea* local had higher mortality than *Picea* nursery particularly if planted in spring. *Populus* had the lowest mortality throughout the experiment. *Pseudotsuga* had the least deaths after the first winter. Mortality with soil treatments had no discernible pattern (Table 4-5).

Evidence of browsing was observed on every assessment date, contributing to or causing plant mortality (Table 4-6). During the first month after planting 44% of *Betula* and *Populus* deaths in spring plantings and 100% of deaths in fall planting of *Betula* and *Pseudotsuga* were attributed to bighorn sheep trampling and/or browsing. Large amounts of deaths of *Betula*, *Picea* (local), *Populus* and *Pseudotsuga*, were attributed to bighorn sheep the following year.

Trees that survived to the end of the experiment had a similar 2 to 6 cm height increase over the course of the experiment (Table 4-7). Overall, height increased more for fall planted than spring planted trees. Within fall planted trees, *Betula*, *Picea* (local) and *Picea* (nursery) achieved greatest heights. Within spring planted trees, *Picea* (local), *Picea* (nursery) and *Populus* achieved greater heights. Depending on species, 38 to 60% of the planted trees increased in height during the four years of the experiment. Only a few plants consistently increased in height over two consecutive assessment dates and appeared to do so by escaping browsing.

3.3 Emergence and Survival of Seedlings

The extremely low emergence of all seeded species did not allow for statistical testing of treatment effects. The harsh site conditions and frequent trampling by bighorn sheep caused pebbles to fall and partly or completely cover most of the seeded holes. Of the 9 seedlings that emerged, 6 were seeded in fall and 3 in spring (Table 4-8); *Pseudotsuga* (n = 4, 3 fall 1 spring), *Betula* (n = 1 fall), *Arctostaphylos* (n = 1 fall), *Alnus* (n = 1 spring), propagule from LFH (n = 2, 1 fall 1 spring). In October 2007, 5 and 3 months after spring and fall seeding respectively, a seedling from *Betula* and an LFH propagule emerged but did not survive to the following spring. Most seedlings emerged in 2008 (*Pseudotsuga* 4 seedlings). One of these seedlings survived to the end of 2009, but died by 2010. In 2008 an *Alnus* seedling emerged. An *Arctostaphylos* seedling and a propagule from LFH emerged in May 2009 but died during the summer. No plants emerged in wood shavings amended soil and no emergence occurred in 2010.

4. DISCUSSION

4.1 Survival and Growth of Out Planted Trees

The lower survival of spring transplanted seedlings may be associated with a higher planting water stress shock. Seedlings spend more energy for respiration under warmer temperatures (Kramer and Kozlowski 1979) and therefore may have not being able to grow or withstand the shock of out planting. Increasing temperatures over summer can reduce soil water content which will also affect seedling survival (Harrington 1991).

Although mean air temperatures were similar during spring and fall planting in 2007 (Table 4-9), water gradients may have imposed different challenges to plant establishment and survival. Spring plants were introduced in June, coinciding with days when precipitation occurred (about 90 mm in 4 days). After seeding no precipitation occurred until end of the month (12 mm). In July, total precipitation was 6 mm. In August, when fall planting occurred, total precipitation was 74 cm of snow. In spring, higher temperatures may have increased plant respiration costs and water stress. This together with browsing during summer, may explain the higher mortality rates with spring planting. Nonetheless, it was a particularly dry summer, judging by precipitation in 2006 (32 cm) (Environment Canada 2011) and 2008 (70 cm) (Table 4-10). Fall lower air temperatures and shorter photoperiods could have reduced tree photosynthesis and reserve accumulation, both key physiological processes to plant survival over winter. These stresses may have a cumulative effect over time on plant survival. In one study in New

Mexico, survival dropped from 80% in the first 3 years to 43% by year 12 although the majority happened within the first 9 years (Young et al. 2009).

Wood shavings were used because they improved substrate properties, including water retention, in a greenhouse experiment (Chapter 2). Treatments preventing soil water loss were considered desirable. *Pseudotsuga* was planted with a blanket type mulch, structurally similar to the wood shavings used in our study (Flint and Childs 1987). Wood shavings did not negatively affect out planted trees, and were not beneficial for grass establishment at other sites of the quarry (Chapter 3). Clean fill and wood shavings failed to produce a substrate functionally and structurally different from unamended controls, which were physically improved during soil preparation prior to transplanting. While preparing the planting hole, larger rocks and pebbles were removed. Once transplants were in place, holes were filled with substrate which was very similar to clean fill, except for the initial amount of rocks. Other amendments, such as pulp mill biosolids may increase soil nutrients and water content and reduce bulk density, and should be studied with woody transplanting.

The higher survival of *Picea* nursery stock than local sourced material, may be related to the larger plants with greater root mass. *Picea* from the nursery and *Pseudotsuga* had well developed root systems better than local *Picea* and *Juniperus*. This may have reduced water stress compared to plants with smaller root volumes. *Picea* trees grown from bare roots had reduced height relative to container stock in British Columbia (Burdett et al. 1984). Container plants used slow release fertilizer (nitrogen, phosphorus, potassium) applied at planting to improve shoot growth the first and second growing season compared to bare root stock. When planted in summer, container seedlings survived better than bare root stock (Lapage and Pollack 1886 in Cole), similar to our higher survival of *Picea* (nursery) than *Picea* (local).

The high mortality of *Juniperus* was likely because root systems were damaged during extraction from natural areas. Extraction of *Juniperus* plants with relatively intact root systems was difficult due to substrate characteristics and the extensive root system, sometimes shared with a parental plant. The low mortality of *Pseudotsuga* from the nursery was likely the result of better initial growth conditions resulting in better root systems and hence lower transplant shock.

Success in planting should consider not only survival but growth (Johnson and Rogers 1985). Continuous tree growth over time was limited due to site conditions (limited soil water, high sun exposure, extreme temperatures, slope, rocky substrate) and browsing. These site conditions and browsing limits plant growth by reducing photosynthesis, which in return limits reserve accumulation. Water stress decreases bud production, which may affect following year growth (Haase and Rose 1993).

According to Burdett et al. (1984), an increase in height can be expected in the third growing season, but this didn't happen in our experiment. Poor height growth for all stock types could be associated with dry sites resulting from southern aspects and coarse parent material. Vyse (1981) found that plantations passed through two seasons of slow height growth before beginning accelerated growth sometimes lasting three years. In this period annual growth rarely exceeded 8 cm for *Picea* and decreased in the second year for some stock types. In our experiment, *Picea* had an increment of 3 to 4 cm, similar to other studies. For example, in western Alberta, *Picea* grew 30 cm in 5 years (Johnston 1976 in Cole 1991).

The quarry does not have ideal conditions for *Pseudotusga, Populus or Betula* (King 1981, Cole and Newton 1987, Wang, Hawkins and Letchford 1998, Woodruff et al. 2001). Low height increase has been observed in other species in overburden. A four to seven year establishment period was needed before *Pinus ponderosa* P. & C. Lawson (ponderosa pine) trees planted on overburden reached appreciable height growth. It may take many years (19 at the ponderosa pine plantation) for trees on overburden to achieve growing rates similar to those of the same species at undisturbed sites (Harrington and Loveall 2006).

Herbivores have a huge impact on seedling survival and may confound experimental results (Cole et al. 1999). Browsing and trampling caused more mortality to *Populus* and *Betula* than to *Picea* or *Pseudotsuga*. *Pseudotsuga* had very low mortality, but in fact, the single dead tree in the fall treatment in 2007 was due to sheep. Higher mortality due to sheep browsing occurred in summer 2007, when nearly half of *Populus* and *Betula* transplants were killed by sheep. Numerous sheep and lambs were observed on site in June. The roots of the newly planted seedlings in spring treatments may not have been established well enough to endure browsing and numerous seedlings were uprooted, particularly *Populus* and *Betula*. Seasonal migration patterns of bighorn sheep has been observed in the south eastern slopes of the Rocky Mountains, and the use of slopes increases as the animals move to alpine summer grounds (higher elevations in midsummer)(McCann 1956, Shannon et al. 1975). Less grazing thus likely occurs at the study site in fall as sheep may migrate during rut season and to winter grounds (McCann 1956, Shannon et al. 1975).

Diverse communities are more resistant and resilient to perturbation, and efforts to restore entire ecological communities or ecosystems are becoming more common (Cattelino et al. 1979). A mix stand of woody species will be preferable to a single stand and therefore, all the species tested in this research could be considered for planting, except *Juniperus* unless a different plant source other than local transplants is obtained. Animal use and browsing is a part of the system and may require incorporating species that will be browsed. Management could be effective to improve plant survival and growth. For example, overall increment in plant height was higher in the 2009 growing season, the last season when chemical deterrent was applied, than in the 2010 growing season, when no deterrent was used. Lower herbivore damage occurred when chemical deterrent was applied but this observation requires further research.

4.2 Plant Establishment from Seeds

The lack of woody plant establishment form seed is likely due to extreme temperatures and low water availability at the site and trampling by bighorn sheep. Most seeding holes were partially covered with rock at every assessment date. Of the nine seedlings established from seed four were *Pseudotsuga*, making it the most successful species to establish from seed at the site. Three of the established *Pseudotsuga* seedlings were seeded in fall 2007 and emerged in 2008, after winter. Seeds from many woody species including *Pseudotsuga*, *Betula* and *Picea* benefit from cold stratification to germinate (Campbell and Sorensen 1978).

Germination is the most limiting stage for seeded species (Harper 1977 in Bevington 1986). Seeds must be viable and have an adequate seed bed to germinate. The seeding hole and surrounding substrate at the quarry had considerable bare ground. Exposed mineral soil is regarded as adequate substrate for *Picea* seedling establishment, because it provides seeds with light and allows for root development if water is stable (Steven 1991). At the quarry, rock and pebble abundance may impede seedling root growth and limit available water. Small seedlings at bare ground sites are prone to thermal stress. Seedlings a bit above the ground, for example, on a seedbed log, gain more heat than those in positions near the ground (DeLong et al. 1997). Leaf and bud tissues elevated 5 to 10 cm above the soil surface can often avoid freezing stress (DeLong et al. 1997). Soil temperature can also be much higher than air temperature when exposed to the sun, especially with limited available water. Slavoj (1965) found greater mortality of *Picea* seedlings at fully exposed dry sites in British Columbia, because the soil temperature was at least 6 ^{o}C higher than the air temperature in summer (Ehleringer and Sandquist 2006).

Site conditions and species characteristics affect germination and seedling survival differently. In a study in the boreal forest, low temperatures limited establishment of *Picea* and only 6.8% of viable seeds produced established seedlings. In contrast, 53% of planted seedlings established (Eis 1965). *Picea* seedling mortality was related to summer drought. Water content of the upper 20 to 40 mm of mineral soil was reduced below wilting point probably causing the 45% seedling mortality. Seedlings that survived had longer roots (35 mm) than those that died (18 mm) (DeLong et al. 1997). *Populus* seeds are known to have low viability (Eis 1965). Lower survival of *Populus* than *Picea* was found in other studies (Moss 1938). In Alaska, *Betula* seeds did not germinate in B horizon soil, and required A horizon soil (Johnstone and Chapin 2006).

Although viability tests of seeds were not performed, some germination reinforces the hypothesis that lack of establishment from seeds was due to harsh site conditions which included trampling. This was the case for LFH. Despite having propagules for woody and forb species which emerged in the greenhouse, propagules did not successfully emerge in the field. If more germination from LFH occurred, radicles may not have been able to reach compacted horizons as occurred in a British Columbia study when at the depth of root penetration, raw humus was at wilting point a few days after rain (Densmore and Page 1992).

Therefore it will be interesting to test LFH at quarry sites with different conditions, such as the north facing slope where more soil water is available.

The undisturbed surrounding vegetation at the quarry may provide a source of plant propagules, for species such as *Picea* and *Pseudotsuga*. *Populus* may be intolerant to competition from other plant species (Cattelino et al. 1979), and should be introduced to the site during the reclamation process. Although *Populus* will likely appear naturally during ecological succession, human introduction will facilitate its arrival since there are few seed sources in the mature surrounding vegetation. This was confirmed as several *Picea* seedlings were observed establishing on the site naturally from dispersed seeds from the surrounding areas.

5. CONCLUSIONS AND IMPLICATIONS FOR QUARRY RECLAMATION

Fall transplants of the woody species used in this experiment had higher survival than spring transplants. *Pseudotsuga* and *Populus* had highest survival. These species together with *Picea* (nursery) can be planted in either spring or fall but *Betula* and *Picea* (local) could have twice as many surviving plants if planted in fall. *Juniperus* plants dug from the adjacent forest had low survival with either spring or fall planting and different sources should be considered. *Picea* local transplants had lower survival than *Picea* from the nursery. *Picea*, *Pseudotsuga* and *Populus* could all be considered for limestone quarry reclamation.

Higher mortality occurred in the first months after planting. Browsing was more intense in summer affecting recent spring transplants the most. Browsing and trampling caused greater mortality to *Betula* and *Populus* than to *Picea* or *Pseudotsuga*. Height increase of transplanted species was limited particularly for broad leaved plants (*Populus* and *Betula*), which suffered more browsing. Only a few plants increased height over two consecutive growing seasons.

Soil treatments were no different than controls in improving plant establishment and survival. However, amendments used were similar to unamended substrate. Thus testing amendments which will more dramatically modify microsites to reduce soil temperature and increase soil water content could be useful.

Overall, plants from seed did not establish. *Pseudotsuga* seedlings emerged in slightly higher numbers than the other species. Site conditions caused heat and water stress, preventing germination or killing emerged seedlings.

Bighorn sheep are part of the quarry ecosystem and animal use and browsing must be considered when planting woody species at the quarry. Use of a variety of species including a few favourites of sheep such as *Betula*, *Amelanchier alnifolia* Nutt., *Salix* spp. and rose (McCann 1956, Shannon et al. 1975), could be considered. Diverse communities are more resistant and resilient to perturbation. Attraction of sheep to certain plant species, may reduce pressure to others such as *Picea* and *Pseudotsuga*. These species were subject to browsing but to a much lesser extent than all others. If not being browsed, individuals of *Picea* and *Pseudotsuga* will survive and establish. In turn they will be aesthetically pleasing pillars of the woody community, which is one of the reclamation goals for public approval. Planting density should be planned to avoid potential domination and thus exclusion of others. More intense management of herbivores should be investigated. Plantskyd was successful in reducing herbivore use and could be applied on a more frequent basis over the growing season.

Soil amending and fertilizing, protection against hervibores and high density planting of nursery woody plants and local woody plants could be more integrated. Large plants could be planted sparsely to help build the landscape and to create or improve micro sites (deeper, amended planting soil). Coniferous species, particularly *Pseudotsuga*, could be used to survive the bighorn sheep browsing. Small areas could be planted with *Betula* far away from the taller trees to attract bighorn sheep and if heavily browsed, replanting *Betula* in these areas could be considered. Appropriate timing of fertilizer and even irrigation water could be included. Other woody species including shrubs for grazing and as nitrogen fixers could be included to facilitate establishment of the plant community at these highly disturbed sites.

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Table 4-1. Surviving plants in spring (S) and fall (F) planting treatments over four growing seasons.

	2007		2008				2009				2010	
	October		May		August		May		August		August	
Species	S	F	S	F	S	F	S	F	S	F	S	F
Betula	21	26	18	25	18	24	12	24	10	23	10 b	18 a
Juniperus	3	6	2	2	1	2	1	2	1	2	1	2
Picea (local)	13 b	27 a	12	26	12	24	12	23	11	23	11 b	22 a
Picea (nursery)	17 b	30 a	17	27	17	24	17	23	17	23	17	23
Populus	21 b	29 a	20	29	19	26	19	23	19	22	19	22
Pseudotsuga	16 b	29 a	16	25	16	25	16	25	16	25	15	25

There were 30 initial transplants per treatment.

Different letters indicate significant differences in survival between spring and fall planting of the same species.

Data were statistically analyzed for 2007 and 2010.

Table 4-2. Variation of residuals and Akaike information criterion values (AIC) obtained from logistic regression of survival data of trees planted in spring and fall.

Monitoring Dates	Explanatory Variable	Spring		Fall	
		Residual Deviance	AIC	Residual Deviance	AIC
October 2007	Species	206.7	218.0	90.6	104.6
May 2008		214.4	226.4	120.4	134.4
August 2008		209.8	221.8	152.0	166.0
May 2009		206.7	218.7	166.5	180.5
August 2009		206.6	218.6	171.3	185.3
August 2010		206.7	218.7	181.3	195.3
October 2007	Soil treatment	248.0	254.0	170.8	176.8
May 2008		247.6	253.6	204.5	210.5
August 2008		247.1	253.1	220.2	226.2
May 2009		244.3	250.3	226.7	232.7
August 2009		242.3	248.3	229.9	235.9
August 2010		241.8	247.8	236.1	242.1

Table 4-3. Surviving plants in soil treatment and spring (S) and fall (F) planting treatments.

		2007		2008				2009				2010	
		October		May		August		May		August		August	
Species	Soil Treatment	S	F	S	F	S	F	S	F	S	F	S	F
Betula	Control	6	10	5	10	5	9	3	9	3	8	3	5
	Clean fill	6	6	5	5	5	5	4	5	3	5	3	4
	Wood	9	10	8	10	8	10	5	10	4	10	4	9
Juniperus	Control	1	1	1	0	1	0	1	0	1	0	1	0
	Clean fill	1	2	0	2	0	2	0	2	0	2	0	2
	Wood	1	3	1	0	0	0	0	0	0	0	0	0
Picea (local)	Control	4	9	4	8	4	7	4	7	4	7	4	7
	Clean fill	5	10	5	10	5	10	5	10	4	10	4	10
	Wood	4	8	3	8	3	7	3	6	3	6	3	5
Picea (nursery)	Control	5	10	5	9	5	8	5	8	5	8	5	8
	Clean fill	6	10	6	9	6	9	6	8	6	8	6	8
	Wood	6	10	6	9	6	7	6	7	6	7	6	7
Populus	Control	7	10	7	10	7	8	7	8	7	8	7	8
	Clean fill	6	10	5	10	5	10	5	10	5	9	5	9
	Wood	8	9	8	9	7	8	7	5	7	5	7	5
Pseudotsuga	Control	6	10	6	8	6	8	6	8	6	8	5	8
	Clean fill	7	9	7	9	7	9	7	9	7	9	7	9
	Wood	3	10	3	8	3	8	3	8	3	8	3	8

There were 10 initial transplants per treatment.

Soil treatments did not significantly affect plant survival.

Table 4-4. Mortality (%) of trees in spring (S) and fall (F) planting treatments in relation to the previous assessment date.

	2007		2008				2009				2010		Final	
	October		May		August		May		August		August			
Species	S	F	S	F	S	F	S	F	S	F	S	F	S	F
Betula	30	13	10	3	0	3	20	0	7	3	0	17	67	40
Juniperus	90	80	3	13	3	0	0	0	0	0	0	0	97	93
Picea (local)	57	10	3	3	0	7	0	3	3	0	0	3	63	27
Picea (nursery)	43	0	0	10	0	10	0	3	0	0	0	0	43	23
Populus	30	3	3	0	3	10	0	10	0	3	0	0	37	27
Pseudotsuga	47	3	0	13	0	0	0	0	0	0	3	0	50	17

Table 4-5. Mortality (%) of trees in spring (S) and fall (F) planting treatments in relation to the previous assessment date.

Species	Soil Treatment	2007 October S	2007 October F	2008 May S	2008 May F	2008 August S	2008 August F	2009 May S	2009 May F	2009 August S	2009 August F	2010 August S	2010 August F	Total Mortality S	Total Mortality F
Betula	Control	40	0	10	0	0	10	20	0	0	10	0	30	70	50
	Clean fill	40	40	10	10	0	0	10	0	10	0	0	10	70	60
	Wood	10	0	10	0	0	0	30	0	10	0	0	10	60	10
Juniperus	Control	90	90	0	10	0	0	0	0	0	0	0	0	90	100
	Clean fill	90	80	10	0	0	0	0	0	0	0	0	0	100	80
	Wood	90	70	0	30	10	0	0	0	0	0	0	0	100	100
Picea (local)	Control	60	10	0	10	0	10	0	0	0	0	0	0	60	30
	Clean fill	50	0	0	0	0	0	0	0	10	0	0	0	60	0
	Wood	60	20	10	0	0	10	0	10	0	0	0	10	70	50
Picea (nursery)	Control	50	0	0	10	0	10	0	0	0	0	0	0	50	20
	Clean fill	40	0	0	10	0	0	0	10	0	0	0	0	40	20
	Wood	40	0	0	10	0	20	0	0	0	0	0	0	40	30
Populus	Control	30	0	0	0	0	20	0	0	0	0	0	0	30	20
	Clean fill	40	0	10	0	0	0	0	0	0	10	0	0	50	10
	Wood	20	10	0	0	10	10	0	30	0	0	0	0	30	50
Pseudotsuga	Control	40	0	0	20	0	0	0	0	0	0	10	0	50	20
	Clean fill	30	10	0	0	0	0	0	0	0	0	0	0	30	10
	Wood	70	0	0	20	0	0	0	0	0	0	0	0	70	20

Table 4-6. Number of deaths attributed to bighorn sheep trampling and/or browsing and corresponding mortality (%) in relation to total deaths on October 2007 and May 2008, when high mortalities occurred.

	October 2007				May 2008			
	Spring		Fall		Spring		Fall	
Species	Deaths	%	Deaths	%	Deaths	%	Deaths	%
Betula	4	44	4	100	3	25	0	0
Juniperus	2	7	0	0	0	0	1	4
Picea (local)	1	6	0	0	0	0	1	25
Picea (nursery)	0	0	0	0	0	0	0	0
Populus	4	44	0	0	3	30	0	0
Pseudotsuga	0	0	1	100	3	21	0	0

Table 4-7. Numbers of trees from total live plants in 2010, that had increased height at any given time interval between assessment dates (2007-2010).

Planting Season	Species	Number of Survivors	Number of Trees with Height Increase	% Trees with Increased Height	Mean Height Increase (cm)	Range of Increase from Individual Plants(cm)
Spring	*Betula*	10	4	40	2.9	1-5
	Juniperus	1	1	100	2.2	1.5-3
	Picea (local)	11	10	91	4.2	1-9
	Picea (nursery)	17	14	82	4.4	1-17
	Populus	19	9	47	4.8	1-10.5
	Pseudotsuga	15	11	73	2.1	1-6
Fall	*Betula*	18	6	33	6.5	1-16
	Juniperus	2	2	100	5.3	4-6
	Picea (local)	22	20	91	4.5	1-10
	Picea (nursery)	23	14	61	3.1	1-13
	Populus	22	9	44	2.8	1-8
	Pseudotsuga	25	15	60	2.5	1-9

Table 4-8. Emerged seedlings from seeds at each assessment date.

Species / Soil Treatment	2007 October		2008 May		2008 August		2009 May		2009 August		2010 August		Total	
	S	F	S	F	S	F	S	F	S	F	S	F	S	F
Alnus														
Control					1								1	
Arctostaphylos														
Clean Fill								1						1
Betula														
Control		1												1
LFH														
Clean Fill								1					1	1
Control	1													
Pseudotsuga														
Control				1*	1	1*		1*		1*			1	3
Clean Fill				2										

*Plant emerged and survived in 2008 and 2009, but died before the 2010 assessment.

Table 4-9. Climate data at Bow Valley Provincial Park, Alberta for 2007.

Month	Mean Maximum Temperature (°C)	Mean Temperature (°C)	Mean Minimum Temperature (°C)	Total Precipitation (mm)
January	0.1	-4.9	-9.8	7.3
February	-1.4	-6.3	-11.2	20.5
March	8.1	2.5	-3.0	31.5
April	7.7	2.5	-2.8	46.9
May	M	M	M	79.9
June	19.5	12.9	6.3	150.4
July	27.4	18.8	10.0	6.6
August	20.3	12.6	5.0	74.6
September	16.3	9.4	2.5	102.1
October	11.3	6.1	0.8	28.4
November	3.1	-1.8	-6.6	28.8

Latitude: 51°05'00.000" N, Longitude: 115°04'00.000" W, Elevation: 1,318.00 m, Climate: 3050779. Environment Canada http://www.climate.weatheroffice.gc.ca.

M = data missing at the source.

Table 4-10. Climate data at Bow Valley Provincial Park, Alberta for 2008.

Month	Mean Maximum Temperature (^{o}C)	Mean Temperature (^{o}C)	Mean Minimum Temperature (^{o}C)	Total Precipitation (mm)
January	-2.2	12.9	7.6	43.2
February	3.2	-9.8	-3.3	42.1
March	4.9	-5.2	-0.1	40.7
April	7.1	-5.1	1.0	33.9
May	14.5	2.1	8.3	78.6
June	19.0	4.4	11.7	76.6
July	22.0	7.7	14.8	70.8
August	22.5	7.4	15.0	103.3
September	17.6	2.4	16.0	84.0
October	11.8	0.1	6.0	20.5
November	6.9	-1.8	2.6	25.1
December	-6.0	-17.8	-11.9	48.7

Latitude: 51°05'00.000" N, Longitude: 115°04'00.000" W, Elevation: 1,318.00 m, Climate: 3050779. Environment Canada http://www.climate.weatheroffice.gc.ca.

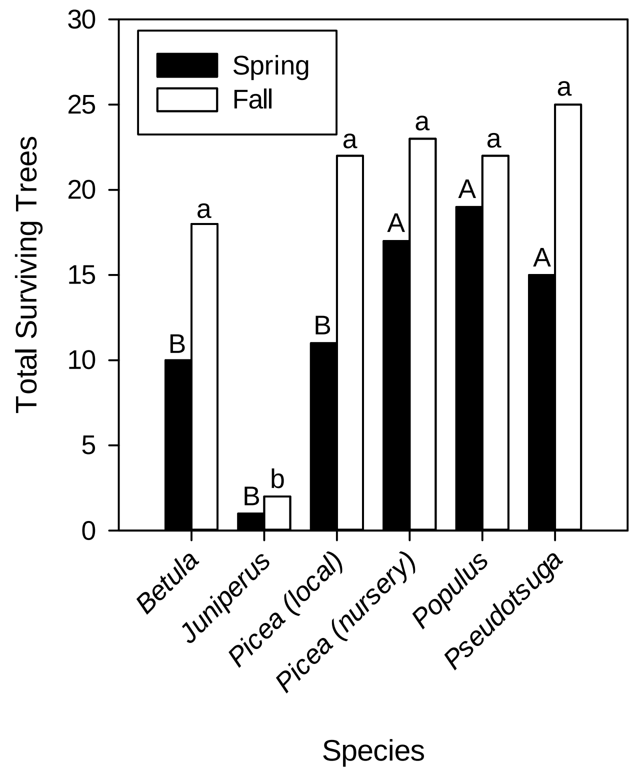

Figure 4-1. Total number of surviving tree in 2010 surviving four years after transplanting. Different letters indicate significant differences in species survival in spring season treatment (upper case) and fall season treatment (lower case). N = 30.

CHAPTER 5. MICROBIAL BIOMASS, VIABLE FUNGI AND BACTERIA AND ARBUSCULAR MYCORRHIZAE AT A LIMESTONE QUARRY

1. INTRODUCTION

Limestone quarry soils are commonly nutrient and organic matter deficient. Quarrying activities alter soil and remove vegetation, which is the primary source of soil organic matter. Soil microbial biomass is the primary agent for litter decomposition, nutrient cycling and energy flow in the soil ecosystem (Dalal and Mayer 1987, Wardle 1992), with 80 to 90% of total soil metabolic activity carried out by fungi, bacteria and actinomycetes (Brady and Weil 2008).

Numerous factors affect composition and abundance of microorganisms in the soil. Among the most important are organic matter quantity and quality, concentrations of organic carbon and nitrogen, pH (Wardle 1992) and physical soil disruption like quarrying. Re-establishment of soil dominated processes such as nutrient cycling and organic matter build up and decomposition, are critical to limestone quarry reclamation success. Reclamation practices can alter microbial populations. Addition of organic amendments can increase soil organic matter in degraded soils (Sopper 1993, Calderón et al. 2005). These amendments vary widely in their composition, affecting microbial community composition. For example in limestone quarries, addition of sewage sludge increased biochemical and microbiological processes in the soils (Jimenez et al. 2007).

Bacteria rapidly consume easily digestible components of organic matter such as sugars and starch. Fungi degrade more persistent components such as cellulose and lignin (Brady and Weil 2008). Actinomycetes degrade resistant substances such as cellulose, chitin and phospholipids. *Frankia* can fix atmospheric nitrogen into ammonium nitrogen.

Arbuscular mycorrhizal fungal hyphae can penetrate cortical root cell walls and form structures (arbuscules) and an association (symbiosis) from which fungi obtain sugars directly from plant roots and plants increase root absorption surface and uptake of water and nutrients. Soil disruption destroys the hyphae network, reducing mycorrhizae effectiveness. Their recovery after disturbance depends on spore count, soil nutrients, host plant genotype, plant cover and time since reclamation (Allen and Allen 1980).

Microorganisms respond quickly to anthropogenic disturbances and therefore are increasingly used as ecological indicators of ecosystem health (Harris 2003). Biological diversity is used by soil scientists as an indicator of soil quality because high species diversity may reflect high functional diversity (Brady and Weil 2008). Soil quality is defined as capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, to maintain or enhance water and air quality and to support human health and habitation and must be determined relative to natural variability in site properties, such as soil type, soil use and climate (Beck et al. 2005).

To evaluate the effect of organic amendments on soil microbiological properties, determination of soil microbial biomass has been recommended (Goyal et al. 1999, Garcí a-Gil et al. 2000). Measurements of the microbial community in amended treatments at the quarry (Chapter 3) can be used as indicators for selection of soil treatments that increase soil microbial community, nutrient availability and plant establishment. Re-establishment of a hyphal network of mycorrhizae may increase plant establishment and contribute to soil formation. This could naturally occur if there are host plants.

The objectives of this research were to determine if reclamation soil treatments for substrate amelioration at a limestone quarry affected soil microbial biomass, number of viable fungi and bacteria colony forming units and arbuscular mycorrhizae infection in key plant species. These data could be used to determine whether ecosystem development was occurring in the reclaimed sites.

2. MATERIALS AND METHODS

2.1 Study Site

Research was conducted at the Graymont Exshaw limestone quarry near Kananaskis, Alberta, Canada (51º 07’ N 115º 13’ W). The quarry was located on a south facing slope of the Rocky Mountains, at an elevation of approximately 1,350 m, below the tree line that occurs from 2,000 to 2,300 m. The climate is montane-subalpine (Alberta Parks and Protected Areas 2002). Long term mean annual precipitation for the area is 296.2 mm as rain and 234.1 cm as snow. Mean daily temperature is 3 ^{o}C; mean maximum is 21.9 ^{o}C in July; mean minimum is -14.1 ^{o}C in January (Environment Canada 2011).

In the surrounding areas, soils are mainly Brunisols with underlying calcareous parent material (Alberta Parks and Protected Areas 2002). Vegetation is coniferous and deciduous mixed wood forests. Primary canopy species are *Pinus contorta* Douglas ex Louden (lodgepole pine), *Picea glauca* (Moench) Voss (white spruce), *Abies lasiocarpa* (Hook.) Nutt. (alpine fir), *Populus tremuloides* Michx. (trembling aspen), *Pseudotsuga menziesii* (Mirb.) Franco (douglas fir) and *Populus balsamifera* L. (balsam poplar). Main understory species include *Shepherdia canadensis* (buffalo berry), *Juniperus horizontalis* Moench (creeping juniper), *Arctostaphylos uva-ursi* (L.) Spreng. (bear berry), *Salix* spp. (willow), grasses such as *Elymus innovatus* Beal. (hairy wild rye) and various forbs.

2.2 Experimental Design

Soil microorganisms were assessed in research plots for a substrate amelioration experiment (Chapter 3). Microbial biomass, fungi and bacteria assessments were conducted on spring treatment plots, established May 13 to 15, 2008.

Sites New and New HM were located in a newly built south facing embankment, on the east side of the quarry road. The embankment was approximately 188 m long and 7 to 15 m wide, with a 30 degree slope, and covered with 30 cm of clean fill. A 65 m long segment at the east half was covered previously from top to bottom with 1 to 2 cm of horse manure (site New HM). Site Old was located in a south facing, older embankment, on the west side of the quarry road. It had 30 degree slopes of varying length, covered with 60 cm of clean fill material. At the Exshaw lime plant, site Plant was situated on a northwest facing berm, with 15 to 20 degree slopes of 10 to 30 m lengths covered with at least 30 cm of clean fill material. These sites represent the heterogeneity of the overall site conditions on a typical limestone quarry requiring reclamation around the world.

The experimental design built on an experiment to address substrate amelioration and native grass establishment (Chapter 3). In August 2007, 140 research plots were established, 40 in each of sites New, Old and Plant and 20 in site New HM. Each plot was 2.25 m^2 (1.5 x 1.5 m). Half of the plots in each site were randomly selected for fall and half for spring soil preparation and seeding (spring and fall treatments hereafter). Within spring and fall treatments, soil treatments were randomly assigned plots. Soil treatments included incorporated manure mix and wood shavings and placement of erosion control blankets on the plot surface (see Chapter 3 for further details). Every treatment, including a control, was replicated five times. Blocks New, Old and Plant had the following experimental design: 2 seeding seasons x 4 soil treatments including control x 5 replicates = 40 plots. Block New HM was not amended because it was already covered with horse manure, therefore the only treatments were the erosion control blanket and a control with the following experimental design: 2 seeding seasons x 2 soil treatment including control x 5 replicates = 20 plots.

Selected amendments were placed on randomly assigned plots and incorporated to 5 to 10 cm depth. Amendments were wood shavings (wood), beef manure mix (manure) and erosion control blankets (blankets) and an unamended control (control). All were fertilized with slow release fertilizer 14-14-14 nitrogen, phosphorus, potassium at time of seeding. Plots were seeded with a native grass seed mix of alpine blue grass (*Poa alpina* L.) 20%, slender wheatgrass (*Agropyron trachycaulum* (Link) Maltex H.F. Lewis.) 15%, hairy wild rye (*Elymus innovatus* Beal.) 15%, rocky mountain fescue (*Festuca saximontana* Rydb.) 15%, spike trisetum (*Trisetum spicatum* (L.) K. Richter) variety ARC sentinel 15%, mountain brome grass (*Bromus carinatus* Hook. & Arn.) 10% and american vetch (*Vicia americana* Muhl. ex Willd.) 10%.

Discrete sampling was undertaken for arbuscular mycorrhizal fungi by choosing plants from dominant species at various locations within the quarry and the plant facilities. Plant samples were collected within 100 m down the slope of site Old, in stockpiled soil with a vegetation patch; at an old reclaimed site near the quarry entrance; and at an old landfill 300 m west of site Old which has been covered with topsoil and historically seeded with *Agropyron repens* (L.) Gould (quack grass). A few uprooted (by grazing sheep) tree transplants from the woody species reclamation experiment (Chapter 4) were collected. Plant samples were collected within 20 m of site Plant, in a vegetation patch at the Exshaw plant site.

2.3 Estimation of Microbial Biomass

One soil sample from each of the 80 spring plots was collected for microbial biomass carbon and nitrogen assessments. Samples were collected with a shovel from 0-5 cm depth in August 2009. Samples were doubled bagged, stored in coolers with ice with final storage in a freezer (-20 ^{o}C). Microbial biomass carbon and nitrogen were estimated by chloroform fumigation-extraction (Vance et al. 1987) from December 2009 to May 2010. Extraction was done with 0.5 M K_2SO_4 solution. Analyses were performed using a Total Organic Carbon / Total Organic Nitrogen analyzer (Shimadzu). Microbial biomass carbon and nitrogen were calculated using equations 1 and 2 and then transformed to ug of organic carbon and organic nitrogen per g of soil.

Equation 1**.** Microbial biomass carbon (% dry wt) = EC / kEC * (100 + M) %

Equation 2. Microbial biomass nitrogen (% dry wt) = EN / kEN * (100 + M) %

EC = ECf – ECnf = Total carbon extractable to 0.5M K_2SO_4 by fumigation
ECf = Average of the duplicate [Cf] * V / W
ECnf = Average of the duplicate [Cnf] * V / W
[Cf] = Total carbon concentration in the extract of fumigated soil (g mL^{-1})
[Cnf] = Total carbon concentration in the extract of non-fumigated soil (g mL^{-1})
kEC = Extraction coefficient † = 0.45
EN = ENf – ENnf = Total nitrogen extractable to 0.5M K_2SO_4 by fumigation
ENf = Average of the duplicate [Nf] * V / W
ENnf = Average of the duplicate [Nnf] * V / W
[Nf] = Total nitrogen concentration in the extract of fumigated soil (ug mL^{-1})
[Nnf] = Total nitrogen concentration in the extract of non-fumigated soil (ug mL^{-1})
kEN = Extraction coefficient = 0.45
V = Volume of K_2SO_4 extractant used = 100 mL
W = Weight of wet soil subsample (g)
M = Water content of soil subsample (% dry wt)

2.4 Viable Fungi and Bacteria

In August 2010, one soil sample for each spring plot was collected with a shovel from 0 to 3 cm, double bagged, stored in coolers with ice while transporting, then at -20 ^oC until plating in September 2010. Serial dilution plate counts were used to determine total heterotrophic aerobic bacteria on plate count agar and fungi and actinomycetes on rose bengal – malt extract agar (Ottow and Glathe 1968).

Plate count agar was prepared by dissolving 23.5 g of plate count agar in 1 L of deionized water in a 2 L round bottom flask. Rose bengal – malt extract agar was prepared by dissolving 20 g of agar, 20 g of malt extract and 0.5 g of K_2HPO_4 in 1 L of tap water and adding 3 ml of micronutrients solution (100 ppm of each of ferric iron, molybdenum, copper and cobalt) and 8 ml of rose bengal solution. Rose bengal solution was prepared by dissolving 3 g of rose bengal in 500 ml deionized water, equivalent to 0.006 g ml^{-1} rose bengal. Plate count agar and Rose bengal – malt extract agar were sterilized in an autoclave at 121 ^oC for 30 minutes before dispersing into petri dishes to solidify.

Soil inocula were prepared by adding 10 g of each soil sample, previously sieved in a flame sterilized sieve, into a 90 ml phosphate buffer solution blank (10^{-1} dilution) and shaken 40 minutes in a laboratory shaker. Shaking time was based on a previous test with soil samples being shaken for increasing 10 minutes periods up to an hour, after which serial dilutions were completed and 0.1 ml samples were plated and colony forming units of bacteria were counted to verify that numbers were no longer increasing or decreasing due to the shaking times. After this step, 10 ml of the soil solution were added to another 90 ml phosphate buffer blank (10^{-2} dilution) and then shaken by hand for 15 seconds. These 10^{-2} diluted aliquots were then in turn serially diluted to 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}.

Four plates for each dilution of a soil sample were prepared. A 100 µl volume of a given dilution was inoculated into each plate. Plated dilutions for plate count agar were 10^{-5}, 10^{-6} and 10^{-7}. Plated dilutions for rose bengal – malt extract agar were 10^{-3},10^{-4} and 10^{-5}. Plates were placed in a plastic bag to avoid water evaporation of the medium and incubated in the dark, at room temperature (21 ^oC), for 2 weeks. Colony forming units were cumulatively counted each week.

A subsample from each soil sample was used to measure soil water content. The soil subsamples were weighed, oven dried at 80 °C for 24 hours and weighed again. A water factor was calculated as weight of wet soil / weight of dry soil. The factor was used to estimate number of colony forming units per g of dry soil.

Mean number of bacteria and fungi colony forming units obtained from each soil sample was multiplied by the water factor for that sample. Morphotypes of fungi, bacteria and actinomycetes on each plate were described based on shape (regular, irregular, conical, round), colour and texture (glossy, opaque, velvety).

2.5 Estimation of Arbuscular Mycorrhizae

Arbuscular mycorrhizae were estimated indirectly by quantifying glucosamine in the roots of selected plants. In May 2008, 25 plants of 15 different species growing at the limestone quarry were dug out with their roots with a shovel. The plants were collected from the reclamation sites (disturbed) and semi disturbed areas in close proximity to the reclamation sites at the quarry and lime plant. All plant samples were bagged, placed in coolers with ice, transported and stored in a freezer at -20 ^{o}C until processing.

Species sampled were the grasses *Agropyron repens* (L.) Gould (quack grass), *Agropyron subsecundum* (Link) Hitchc (bearded wheat grass), *Agropyron trachycaulum* (Link) Maltex H.F. Lewis. (slender wheat grass), *Bromus inermis* Leyss. (smooth brome), *Festuca campestris Rydb.* (rough fescue), *Festuca ovina* L. (sheep fescue), *Poa pratensis* L. (Kentucky blue grass); one forb *Medicago sativa* L. (alfalfa) and the woody species *Populus tremoloides* Michx (tembling aspen), *Picea glauca* (Moench) Voss (white spruce), *Betula papyrifera* Marsh. (paper birch), *Elaeagnus commutata* Bernh. ex Rydb (silver berry), *Arctostaphylos uva-ursi* (L.) Spreng (bear berry) and *Juniperus horizontalis* Moench (creeping juniper).

Arbuscular mycorrhizae infection was estimated through quantification of glucosamine ($C_6H_{13}NO_5$) (Braid and Line 1981, Ekblad and Näsholm 1996, Nilsson and Bjurman 1998) the repeating sugar amine chitin in the cell walls of arbuscular mycorrhizal fungi (Bartnicki-Garcia 1968). In January 2009, roots were separated from each plant and washed thoroughly with distilled water to remove soil particles and external glucosamine sources, such as non-mycorrhizal fungi, bacteria, arthropods and soil invertebrates (Ekblad and Näsholm 1996) and a modified glucosamine assay technique followed (Nilsson and Bjurman 1998). Roots were dried at 80 °C for 24 hours and ground to very fine powder then placed in clean threaded glass test tubes. Samples weighing 100 to 300 mg (large sample) were suspended in 5 ml of 6 N hydrochloric acid (Braid and Line 1981). Samples weighing less than 100 mg (small sample) were suspended in 2 ml of 6 N hydrochloric acid. This step is the first dilution in the final glucosamine calculation. Test tubes were tightly capped and samples were hydrolyzed at 96 °C for 48 hours, then cooled to ambient temperature. Five ml of de-ionized water were added to large samples and 2 ml to small samples (second dilution).

A 2 ml volume of diluted hydrolysate was taken from large samples and 1 ml from small samples and placed in clean test tubes. These samples were evaporated in a 50 °C water bath, assisted by air injection. Compressed air was gently introduced into each test tube with a pasteur pipette attached with plastic tubing to a compressed air tank regulator. Each evaporated precipitate was resuspended with 5 ml de-ionized water (third dilution), then 1 ml was extracted and placed in Teflon wrapped threaded test tubes. A glucosamine standard was prepared with 50 µg/ml glucosamine (Table 5-1). To the 1 ml samples and glucosamine standard, 0.25 ml of acetylacetone solution (4% by volume acetylacetone in 1.25 N sodium carbonate) were added. Test tubes were tightly capped and bathed in a 100 °C water bath 1 hour, then cooled to ambient temperature in a cool water bath. 2 ml ethanol were added to each sample then mixed with a vortex mixer for 5 seconds to dissolve the precipitate, then 0.25 ml ehrlich reagent (1.6 g of N-N-dimethyl-P-aminobenzaldehyde in 60 ml 1:1 mixture of ethanol and concentrated HCl) were added and again vortexed for 5 seconds.

Absorbance was measured at 530 nanometers with a spectrophotometer (Spectronic 20), using glucosamine S_0 standard to zero the spectrophotometer. Readings were compared to a standard curve from glucosamine and used to calculate amount of glucosamine per dry gram of root by equations 3 and 4.

Equation 3. total µg = µg glucosamine per 1 ml sample x 3rd dilution factor x 2nd dilution factor x 1st dilution factor / fraction of hydrolysate used for the assay

Equation 4. µg glucosamine per g root = total µg in sample / g root (dry material)

2.6 Statistical Analyses

The differences in microbial biomass, number of colony forming units and in the morphotypes of fungi and bacteria due to amendment and erosion control blanket treatments were analyzed with a one way analysis of variance (ANOVA) if the data had normal distribution and equal variance and they were analyzed with ANOVA on ranks otherwise. Normality was verified with the Shapiro-Wilk test. Posteriori pair wise multiple comparison procedures (Holm Sidack method) were performed when statistically significant differences among groups were detected. All the analyses and the graphic were performed with SigmaPlot 12 (Systat Software 2011).

3. RESULTS

3.1 Soil Microbial Biomass

Microbial biomass was higher in amended soil than in unamended control, 15 months after amendment applications. Microbial biomass nitrogen in amended soil was numerically higher in amended treatments at the quarry (sites New, New HM and Old) but not at the plant (site Plant) (Figure 1). However, differences were not statistically significant. Values were similar across treatments and sites but extreme values were found at Site Old, ranging from 17.01 ug g^{-1} soil in the control to 47.24 ug g^{-1} soil at a manure treatment (Table 5-2). Manure treatment often contained the higher values of microbial biomass nitrogen (Figure 1).

Estimation of microbial biomass carbon in limestone soil by the total organic carbon analyzer was not reliable. Many values were eliminated because they were negative. The chloroform used in the procedure may have reacted with the calcium carbonate of the soil liberating carbon in the form of carbon dioxide and creating an overall reduction of carbon. Abiotic evolution of carbon dioxide by decomposition of bicarbonate has been suggested to explain the drop in the soil biomass when the soil is coming into equilibrium with an atmosphere containing less carbon dioxide for soils containing high proportions of carbonate (Ayanaba et al. 1976, Powlson and Jenkinson 1976).

3.2 Fungi and Bacteria Colony Forming Units

Twenty eight months after soil amendment, soil treatments had numerically higher numbers of viable fungi colony forming units at all sites compared to the unamended control (Table 5-3); values were not statistically different. In sites New and Old, more viable fungal colony forming units were present in soil amended with wood; whereas in site New HM, more fungal colony forming units were present in the erosion control blanket treatment. Number of viable fungal colony forming units was similar among treatments at site Plant; however, the control counts were lower.

Numbers of viable bacterial colony forming units was similar across soil treatments within the same site, but the range of values differed among sites (Table 5-3). Site New HM had the higher numbers regardless of soil treatment. Erosion control blankets and the control had similar mean values, around 81 x 10^6 colony forming units g $soil^{-1}$. Sites New, Old and Plant ranged from 7 x 10^6 colony forming units g $soil^{-1}$ to 55 x 10^6 g $soil^{-1}$.

A total of 12 morphotypes of bacteria, 5 of actinomycetes (included with bacteria in table) and 15 of fungi were observed in the plate counts. Morphotype differences were not statistically significant among soil treatments. Mean number of morphotypes ranged from 6 to 12 for bacteria and 6 to 15 for fungi (Table 5-4). Higher numbers of bacteria morphotypes were in soil from the erosion control blanket treatment at site New HM and lower numbers from the site Plant control.

3.3 Arbuscular Mycorrhizae

Glucosamine was found in the roots of all sampled plants. Values ranged from 277 to 1,449 ug of glucosamine per g of roots. Glucosamine was higher in roots from grasses than from woody species (Table 5-5). Within woody species, shrubs had higher glucosamine than trees. The highest value was in the forb *Medicago sativa* (1,449 ug g^{-1} of dry root). The second highest was the grass *Poa pratensis* (1,380 ug g^{-1} of dry root), which was growing at an undisturbed site. The lowest value was obtained from root samples of *Poa pratensis* growing in a disturbed, reclaimed old site.

The highest value for a woody species was found in roots of *Elaeagnus commutata* (1,308 ug g^{-1} of dry root) at a disturbed site. The second highest value was from *Populus tremuloides* (1,094 ug g^{-1} of dry root), grown in a nursery but transplanted to the quarry and uprooted by bighorn sheep. The lowest glucosamine was from the tree *Betula papyrifera* (527 ug g^{-1} of dry root).

4. DISCUSSION

4.1 Soil Microbial Biomass

The high microbial biomass in soil amended with manure, wood shavings and erosion control blankets after 15 months of soil being treated was likely even higher, particularly with manure, soon after application. The organic matter in manure is easier to decompose than wood. Whereas the carbon:nitrogen ratio in wood could be 600, in manure compost it ranges from 7 to 25 (Tang et al. 2006). Likely the stimulation within the microbial community caused by manure addition and continued decomposition of organic matter and dead microorganisms resulted in mineralization and an increase of available nitrogen that was then utilized by seeded and non seeded plants. Manure treatment resulted in higher plant density and more vigorous plants (Chapter 3). Even after 15 months, higher microbial biomass nitrogen and significantly higher total nitrogen and total organic carbon was found in manure treatments.

High microbial biomass nitrogen at site Plant in wood shavings treatments was probably associated with high decomposition rates due to more soil water because of its north facing position. North facing slopes commonly have more water (Hanna et al. 1982) which is important for microbial decomposition of organic matter and thus increases microbial population and biomass (Kieft et al. 1987). Microbial activity at the time of measurements had likely already decomposed the more readily decomposable fraction of the amendments and mineralized some nutrients which were utilized by vegetation. This is supported by the more lush and dense vegetation observed at site Plant (Chapter 3). Microorganisms feeding on the wood were then increasing and yielding higher microbial biomass nitrogen in the wood treatment compared to the soil in the other treatments.

The overall slightly higher microbial biomass nitrogen at site Old was associated with the lowest density of seeded and non seeded plants (Chapter 3). Site Old was a south facing slope, more exposed to wind. Higher nitrogen immobilization occurs at sites with lower soil water and organic matter (Török et al. 2000). In contrast, site New HM had higher nutrient concentrations in soil to begin with and higher electrical conductivity.

Multiple factors affect activity and stability of microbial biomass in soil (Dalal and Mayer 1987). Other researchers have found that composition of roots exudates differed among plant species and result in differences in microbial soil community feeding from exudates (Grayston et al. 1998, Bardgett et al. 1999).

4.2 Fungi and Bacteria Colony Forming Units

The number of organisms in the quarry soil, despite being highly disturbed, corresponds to a range described by other researchers (10^4 to 10^5) (Beck et al. 2005). Although only a small number of bacteria in soil can be cultured by standard isolation techniques (Wawrik et al. 2005) it gives an indication of differences in detectable microorganisms caused by treatment. High pH and calcium carbonate of a limestone quarry site are more favourable for bacteria than fungi. This partially explains the higher bacterial (colony forming units) counts compared to fungal counts at all locations within the overall site.

Wood shavings in the soil favoured the fungal community at south facing, exposed sites. The cultured organisms were predominantly molds, because wood shavings have lignocellulose as one component and various fungi are able to degrade lignocellulose (Rodriguez et al. 1996). The readily available food supply may have resulted in more fungi than in other treatments where bacteria may have competed for resources. Fungi continue to decompose complex organic material after bacteria and actinomycetes have ceased to function (Brady and Weil 2008). Soil in the wood shavings treatment had less water and fewer plants. Erosion control blankets favoured fungi likely because of straw and shade. Manure treatments did not cause a significant increase in colony forming units and morphotypes.

Site New HM covered with horse manure had conditions for more bacteria colony forming units and more morphotypes. This could result from bacteria inherent in the horse manure or by more organic matter and better chemical and physical conditions for local bacteria to establish.

Variability in number of viable colony forming units and morphotypes of bacteria and fungi increased under more exposed conditions. More favourable north facing sites resulted in more stable (similar numbers) fungi and bacteria colony forming units across amended treatments and controls. More than a third of the morphotypes were actinomycetes. They may become more dominant during later stages of decay when easily metabolized substrate has been used (Brady and Weil 2008). Soil at site Plant had higher water content regardless of soil treatment and there was a small wetland at the bottom of the berm. Site Plant had fewer morphotypes of bacteria and fungi. Amendments may have depleted more quickly. Wetter conditions at the site and addition of amendments may have resulted in an increase of bacteria. Bacteria live for hours or days and adequate conditions increase population, decay and decomposition. More rapid mineralization of nutrients in amendments and dead microorganisms were used by plants and therefore removed from the soil.

4.3 Arbuscular Mycorrhizae

Glucosamine was found in all root samples supporting the hypothesis that mycorrhizae associations are a rule more than an exception in roots of higher plants (Brady and Weil 2008). Annual plants that do not form mycorrhizal associations such as those in *Cruciferae* and *Chenopodiaceae* families (Brady and Weil 2008) are dominant non seeded species at the quarry and plant sites (e.g., *Erucastrum gallicum* and *Chenopodium album*). Despite this, all plants sampled presented mycorrhizal infections. The grass *Poa pratensis* had a high amount of glucosamine in roots from a plant growing under a deciduous tree in a forested site adjacent to quarry stock piled soil, but had the lowest value in a plant near the driveway at the entrance of the Exshaw quarry. This is the same site where *Medicago sativa* had high glucosamine, and could be evidence of the differences between two species to respond to mycorrhizal colonization under similar site conditions. There is high variability of glucosamine in roots within plant families. Appuhn and Joergensen (2006) found glucosamine in roots of *Poa pratensis* of 850 ug g^{-1}. The highest they found in their greenhouse experiment was in *Papaver* (poppy) corresponding to 2,950 ug g^{-1}. In our study we found glucosamine in roots of *Poa pratensis* of 270 and 1,340 ug g^{-1}.

Indications of the effect of site such as management history, soil condition and neighboring plants were found. *Festuca ovina* had similar values at both collection sites, even though the landfill site had better soil quality (top soil brought to cover it). Molina et al (1978) found no differences in mycorrhizal development between *Festuca* species. Many species may have an infection range that will not change greatly under similar conditions at the quarry as may be the case for *Festuca* ovina, although *Festuca campestris* presented the lowest values of all the grasses. *Agropyron repens* in the landfill had less glucosamine than in site Plant at the lime plant. Soil in the landfill had a much better appearance and supported a dense and vigorous plant cover. Glucosamine in roots of *Agropyron subsecundum* and *Agropyron sp*. were in the range of values above 1,000 ug g^{-1} root whereas *Festuca campestris* glucosamine was below.

More glucosamine was found in roots from the grasses than the woody species. Glucosamine from the trees was similar to that of the shrub species growing in close proximity to the trees. This indicates values could indicate the range of infection that could be expected for species even though roots from tree species came from transplants grown in a nursery, transplanted and uprooted by bighorn sheep, possibly affecting infection from onsite arbuscular mycorrhizae.

When studying wheat cultivars, infections of roots were related to the amount of sugars exuded by roots more than by sugars present in roots (Azcon and Ocampo 1981). Soil fertility may play a role in response of species to arbuscular mycorrhizal fungi. Further research will be valuable to detect if differences in plant responses to mycorrhizae change with soil fertility from amendments.

5. CONCLUSIONS AND APPLICATIONS TO QUARRY RECLAMATION

Amending with manure, wood shavings and erosion control blankets increased microbial biomass in quarry soils in relation to the controls, even after 15 months time. Soil treatments increased the numbers of microorganisms at the quarry. The increased nutrients, microbial biomass and fungi and bacteria counts, particularly in soil amended with manure, was still present 24 months after amendment.

The manure treatment was the best for soil microorganisms. It resulted in more microbial biomass nitrogen which means more nutrients for plant uptake.

Wood shavings treatments increased organic matter and resulted in more fungi.

Erosion control blankets did not modify the soil community compared to wood shavings or manure, but were better than the control. Increasing plant density and cover is expected to lead to decaying root biomass and exudates which will sustain a larger microorganism community, compared to unamended controls.

Site differences including soil properties and established plant species affected number of bacteria and fungi colony forming units, 27 months after amendment. Wood shavings and erosion control blankets favoured fungi whereas bacteria were slightly higher with manure. Actinomycetes could be increasing soil nutrients by nitrogen fixation.

If vegetation thrives at a reclamation site, the soil community will also likely thrive. Thus vegetation monitoring may provide information on the soil microbial community.

All sampled species of grass, trees and forbs at the quarry, plant, disturbed sites and semi disturbed sites had mycorrhizae infections. Disturbance and management practices may have an effect on arbuscular mycorrhizal infection within the same species. Further research on infection and effect (beneficial or detrimental) due to amendment treatments, management practices and effect of target seeded and non seeded species can be investigated. Quantification of glucosamine may be a useful method to indirectly assess arbuscular mycorrhizal fungi infection. Soil has been severely disrupted yet plants have arbuscular mycorrhizal fungi. Therefore, hyphae networks maybe naturally restore over time and artificial inoculation may not be required.

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Table 5-1. Preparation of glucosamine 50 µg ml^{-1} standard.

Standard	50 µg/ml Glucosamine Solution Added (µl)	Total Glucosamine (ug)	Distilled Water (µl)	Total Solution (ml)
S_0	0	0	1000	1
S_5	100	5	900	1
S_{10}	200	10	800	1
S_{20}	400	20	600	1
S_{30}	600	30	400	1
S_{40}	800	40	200	1
S_{50}	1000	50	0	1

Table 5-2. Microbial biomass nitrogen, soil properties and plant density at spring plots at the Exshaw quarry and lime plant in August 2009.

Site/Soil Treatment	Microbial Biomass Nitrogen (ug g^{-1} soil)	Total Carbon (%)	Total Organic Carbon (%)	Total Nitrogen (%)	Electrical Conductivity (dS m^{-1})	Hydrogen Ion Concentration (pH)	Density (plants m^{-2})
New							
Control	17.00 ± 6.04	7.21±0.11 b	0.94±0.02 b	0.07±0.00 b	0.44±0.05	8.00±0.03	14.67±2.39 b
Blanket	25.45 ± 6.73	7.15±0.11 b	0.89±0.04 b	0.07±0.01 b	0.30±0.04	8.20±0.05	36.09±6.64 a
Manure	23.60 ± 1.45	7.75±0.14 a	1.31±0.13 a	0.14±0.02 a	0.43±0.08	8.02±0.10	34.04±2.11 a
Wood	26.93 ± 9.41	7.38±0.20 a	0.98±0.06 ab	0.10±0.00 b	0.44±0.08	7.98±0.09	12.71±1.88 b
New HM							
Control	26.47± 10.26	7.53±0.36	1.22±0.19	0.12±0.02	0.36±0.05	8.02±0.14	32.62±4.14 b
Blanket	28.81 ± 5.60	7.45±0.41	1.20±0.21	0.12±0.03	0.35±0.03	8.18±0.08	46.84±7.41 a
Old							
Control	19.14 ± 10.08	6.97±0.08	0.81±0.04 b	0.07±0.01	0.37±0.07	8.16±0.12	32.44±10.98 bc
Blanket	29.23 ± 5.22	7.21±0.10	1.05±0.08 b	0.08±0.01	0.31±0.04	8.06±0.07	69.42±8.61 a
Manure	47.24 ± 17.70	7.43±0.30	1.28±0.19 a	0.11±0.02	0.30±0.03	8.16±0.02	43.82±5.58 b
Wood	24.44 ± 7.34	7.28±0.10	1.10±0.05 ab	0.09±0.01	0.31±0.07	8.10±0.07	26.84±5.34 c
Plant							
Control	24.58 ± 9.12	8.54±0.17	1.39±0.09	0.08±0.00 b	0.31±0.04	8.22±0.11	52.44±5.52 b
Blanket	24.57 ± 3.41	8.73±0.38	1.30±0.08	0.07±0.01 b	0.37±0.07	8.10±0.07	89.96±8.74 a
Manure	25.33 ± 5.70	10.26±0.57	1.38±0.14	0.11±0.01 a	0.44±0.06	7.92±0.11	59.91±10.61b
Wood	22.13 ± 10.65	9.40±0.64	1.18±0.20	0.09±0.01 b	0.39±0.11	8.00±0.15	60.09±4.61b

Values are mean ± standard error.
Letters indicate statistically significant differences among soil treatments of the same site. Absence of letters indicates no differences.

Table 5-3. Colony forming units (CFU) of fungi and bacteria in amended soil in August 2010.

Soil Treatment	Site A	Site New HM	Site Old	Site Plant
	$\times 10^{4}$ Fungi CFU g^{-1} soil			
Control	14.61 ± 5.73	12.73 ± 5.77	4.66 ± 1.63	14.34 ± 3.65
Blanket	15.45 ± 14.54	23.7 ± 1.6	9.82 ± 5.84	17.4 ± 4.43
Manure	19.16 ± 3.01		13.94 ± 3.29	20.22 ± 8.7
Wood	44.49 ± 16.69		40.07 ± 15.29	18.79 ± 14.91
	$\times 10^{6}$ Bacteria CFU g^{-1} soil			
Control	32.81 ± 31.06	81.47 ± 41.53	10.9 ± 0.83	39.91 ± 18.07
Blanket	11.52 ± 10.01	82.88 ± 9.11	25.7 ± 11.77	49.26 ± 10.99
Manure	7.34 ± 7.01		41.76 ± 6.83	55.18 ± 15.49
Wood	16.79 ± 13.87		45.9 ± 31.68	39.17 ± 26.53

Values are mean ± standard error.

Different letters indicate statistically significant differences among soil treatments of the same site.

Absence of letters indicates no statistical differences.

Table 5-4. Number of morphotypes of aerobic bacteria grown in plate count agar medium and fungi in Rose benagal malt extract medium, from samples of treated soil in August 2010.

Site	Treatment			
	Control	Blanket	Manure	Wood
			Bacteria	
New	7.0 ± 2.83	9.0 ± 0	6.67 ± 1.53	8.00 ± 1.00
New HM	11.0 ± 2.0	12.33 ± 1.53		
Old	8.50 ± 0.71	8.67 ± 1.15	8.67 ± 4.51	10.33 ± 0.58
Plant	6.33 ± 1.53	7.0 ± 1.0	10.33 ± 3.22	
			Fungi	
New	15.0 ± 0	9.50 ± 2.12	11 ± 3.46	8.5 ± 2.12
New HM	7.33 ± 3.05	7.33 ± 1.15		
Old	8.50 ± 0.71	9.67 ± 3.79	12 ± 2.65	7.33 ± 1.53
Plant	6.00 ± 1.00	6.00 ± 2.65	7.33 ± 1.53	5.67± 1.53

Values are mean ± standard error.

Different letters indicate statistically significant differences among soil treatments of the same site.

Absence of letters indicates no statistical differences.

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Table 5-5. Estimation of glucosamine from arbuscular mycorrhizae fungi in roots of plant species in May 2008.

Species	Common Name	Location	Disturbance	Glucosamine (µg g root^{-1})
Trees				
Populus tremuloides	Aspen (nursery)	Woody species experiment	Disturbed	1094.67
Picea glauca	White spruce (nursery)	Woody species experiment	Disturbed	841.73
Populus tremuloides	Aspen (nursery)	Woody species experiment	Disturbed	734.58
Populus tremuloides	Aspen (nursery)	Woody species experiment	Disturbed	689.38
Picea glauca	White spruce (nursery)	Woody species experiment	Disturbed	668.70
Betula papyrifera	Paper birch (nursery)	Woody species experiment	Disturbed	527.64
Shrubs				
Elaeagnus commutata	Silver berry	Lime plant Site Plant	Undisturbed	1308.64
Arctostaphylos uva-ursi	Bear berry	Woody species experiment	Undisturbed	960.36
Juniperus horizontalis	Creeping juniper	Woody species experiment	Undisturbed	886.60
Grasses				
Festuca ovina	Sheep fescue	Woody species experiment	Disturbed	1216.63
Festuca ovina	Sheep fescue	Reclaimed Landfill	Disturbed	1192.49
Agropyron subsecundum	Bearded wheat grass	Quarry reclaimed	Disturbed	1180.03
Agropyron subsecundum	Bearded wheat grass	Quarry reclaimed	Disturbed	1077.34
Agropyron sp	Wheat grass	Woody species experiment	Disturbed	1064.28
Agropyron repens	Quack grass	Lime plant Site Plant	Disturbed	900.17
Agropyron trachycaulum	Slender wheat grass	Quarry soil stockpile	Disturbed	540.80
Agropyron repens	Quack grass	Landfill	Disturbed	376.60
Poa pratensis	Kentucky blue grass	Quarry under deciduous tree	Undisturbed	1380.16
Poa pratensis	Kentucky blue grass	Quarry reclaimed	Disturbed	277.08
Bromus inermis	Smooth brome	Lime plant Site Plant	Undisturbed	1132.13
Bromus inermis	Smooth brome	Lime plant Site Plant	Undisturbed	1105.09
Agropyron repens	Quack grass	Lime plant Site Plant	Undisturbed	997.31
Festuca campestris	Foothills rough fescue	Quarry under conifers	Undisturbed	892.95
Festuca campestris	Foothills rough fescue	Lime plant Site Plant	Undisturbed	348.21
Forbs				
Medicago sativa	Alfalfa	Quarry reclaimed	Disturbed	1449.75

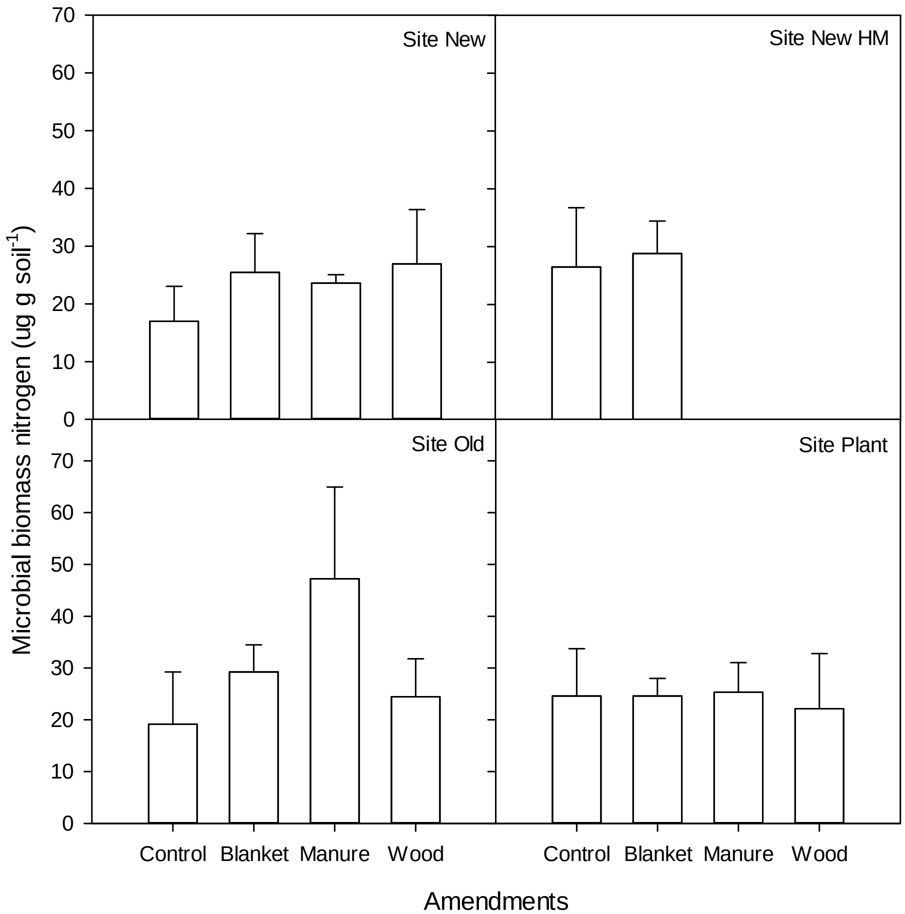

Figure 5-1. Mean microbial biomass nitrogen in soil samples from spring plots at the quarry (sites New, New HM and Old) and lime plant (site Plant) in samples collected in August 2009. Bars are standard errors. There were no significant differences in microbial biomass within same site.

CHAPTER 6. SYNTHESIS AND FUTURE RESEARCH

1. DISSERTATION OVERVIEW

This research program focused on two main issues of significance in global limestone quarry reclamation, which were addressed at the Exshaw limestone quarry in the Rocky Mountains of southern Alberta. The first issue was modification of the inherent reclamation substrate materials to increase the plant required nutrients, increase the organic matter content and reduce the alkalinity. The second issue was to assist revegetation to meet the regulatory reclamation requirements.

The limiting conditions of the research site, like those of thousands of limestone quarries around the world, have resulted in limited reclamation success of this type of disturbance. We hypothesized that if key components were re-introduced into the reclaimed system, it would eventually recover its functioning processes. In this research, soil, vegetation and nutrient cycling were considered to be the key components for limestone quarry reclamation. Their introduction and modification were expedited through substrate amelioration and assisted plant community establishment in a series of greenhouse and field experiments.

The results will contribute to development and/or modification of best reclamation practices, meeting approval requirements for reclamation and can be extrapolated to other limestone quarries or similar disturbances around the world. In the following sections each phase of the research, as presented in the preceding chapters, is summarized.

1.1 Greenhouse Amelioration of Limestone Quarry Substrate Material

Two consecutive greenhouse experiments, detailed in Chapter 2, were designed to investigate suitability of amended limestone substrates and application rates of amendments for establishment of a native grass mix. Each experiment lasted three months. The first experiment tested three limestone substrates from the quarry treated with readily available amendments, used alone and in combination with fertilizer to enhance available nutrients and with sulphur to reduce pH. The amendments were pulp mill biosolids, two types of manure compost, hay, straw, wood shavings and capping with two types of soils. Minimum application rates of best performing amendments were tested in a second greenhouse experiment. Plant density, above and below ground biomass and height data were used to assess substrate suitability.

Three limestone substrates, gray, gray brown and crushed, supported grass establishment when amended. Gray limestone, the most abundant of the three substrates in the Exshaw quarry, produced overall more biomass and supported higher plant density than the other two.

Addition of slow release 14:14:14 nitrogen, phosphorus and potassium fertilizer yielded three times more above ground biomass and two times more root biomass, increased plant heights and reduced plant density by half. Addition of sulfur reduced substrate pH but the treatment only increased biomass when combined with biosolids, soil and wood shavings in the presence of fertilizer. Capped top soil and clean fill and incorporated biosolids consistently produced higher above and below ground biomass and plant density. Biosolids favoured establishment of more species than any of the other organic amendments. Hay and straw did not favour establishment of vegetation.

Grass species that established best from the seed mix were *Agropyron trachycaulum* (Link) Maltex H.F. Lewis. (slender wheat grass), *Festuca saximontana* Rydb. (rocky mountain fescue) and *Poa alpina* L. (alpine blue grass). Higher application rates of fertilizer and amendments were always better than medium and low application rates for all amendments except biosolids, where the low application rate resulted in plant performance which was almost as good as the high rate.

1.2 Field Amelioration of Limestone Quarry Substrate Material

Some of the best performing amendments and application rates from the greenhouse research were used under field conditions at the Graymont Exshaw quarry and lime processing plant (Chapter 3). Manure mix, wood shavings and erosion control blankets were evaluated to determine their effect on soil properties and native grasses establishment. Season of soil preparation and seeding were also evaluated. Plots were established in blocks situated in three old and newly built embankments of the quarry and one berm at the plant. Soil and vegetation were monitored over two growing seasons. Effect of amendment treatment on soil properties and plant species composition and density of seeded and non seeded species were analyzed.

Season (fall, spring) of soil amending and seeding did not significantly affect revegetation or soil propertiess. Site characteristics such as slope, aspect, initial soil nutrients and the surrounding plant communities influenced early plant community development and overall effects of soil treatments.

The use of erosion control blankets resulted in the highest seeded plant cover and the lowest non seeded plant cover. Among the measured soil properties, total nitrogen and carbon significantly improved establishment of seeded grasses and non seeded species, increasing plant density. Increased nitrogen and carbon in the constructed soils were best achieved through addition of manure. Electrical conductivity, known to increase with manure amendment, remained below threshold values of concern and decreased by the second year of the research. Erosion control blankets facilitated a denser native grass establishment despite not significantly changing chemical properties of the constructed soils. Wood shavings did not favour establishment of vegetation and resulted in similar, and in some cases less, vegetation than controls.

Assisted revegetation increased plant cover from < 6 to 50 % and reduced cover of non seeded species. Best establishing seeded species were the grasses *Agropyron trachycaulum*, *Festuca saximontana*, *Poa alpina* and *Trisetum spicatum (L.) K. Richter variety ARC sentinel (spike trisetum)*. Non seeded species density varied with site and reclamation treatment. *Erucastrum gallicum* (Willd.) O.E. Schulz. (dog mustard) was more prevalent in controls while other species were favoured by the reclamation treatments. *Erucastrum gallicum* generally occurred at higher densities than most other non seeded species.

1.3 Field Establishment of Woody Species

Native trees and shrubs were transplanted into amended substrates at an Exshaw quarry site to investigate how species type, season of transplanting and seeding and origin of plant material (nursery stock, undisturbed vegetation, propagules from LFH) may affect establishment of woody species at the quarry (Chapter 4). Planted and seeded species included *Picea glauca* (Moench) Voss (white spruce), *Pseudotsuga menziesii* (Mirbel) Franco (douglas fir), *Populus tremuloides* Michx. (trembling aspen), *Betula papyrifera* Marshall (paper birch), *Juniperus horizontalis* Moench (creeping juniper), *Alnus crispa* (Aiton.) Pursh (mountain alder) and *Arctostaphylos uva-ursi* (L.) Spreng. (bear berry). Transplants, seeds and LFH together totaled 13 plant sources. Individuals of each plant source were planted or seeded in spring and fall, into three soil treatments consisting of wood shavings, clean fill and an unamended control. During three growing seasons plant emergence, survival and height and signs of browsing were monitored.

Transplanting season affected survival rate of the woody species used in this experiment. Seedlings transplanted in fall had higher survival than those transplanted in spring. Soil treatments were no different than the control in improving establishment of plants. Browsing and trampling caused greater mortality to *Populus tremuloides* and *Betula papyrifera* than to *Picea glauca* or *Pseudotsuga menziesii*. Browsing was more intense in summer affecting recent spring transplanted plants the most.

Plant survival varied depending on species and source type. Local transplants had lower survival than plants from the nursery. Height increase of transplanted species was limited particularly for broad leaved plants (*Populus tremuloides* and *Betula papyrifera*) which suffered more browsing. Only a few plants were able to increase height over two consecutive growing seasons. For those plants with detectable height increases, the mean increase within a growing season was 3 to 4 cm. Overall, plants from seed did not establish. *Pseudotsuga menziesii*. seedlings emerged in slightly higher numbers than the other species. Bare soil and the rocky substrate of the exposed south facing slope may have caused heat and water stress, preventing germination or killing of the emerged seedlings.

1.4 Field Microbial Evaluations

In Chapter 5 an assessment of representative microbial characteristics was done to serve as an indication of re-establishing ecological functions in a control and in amended substrates of the Exshaw quarry and lime plant after the soil was treated with manure compost, wood shavings and erosion control blankets. Microbial biomass nitrogen and carbon was quantified 15 months after soil treatment; number of fungi and bacteria colony forming units after 2 years; and arbuscular mycorrhizae fungi infection in key plant species at disturbed and semi disturbed sites at the Exshaw quarry and plant. Soil samples were collected from spring seeded plots described in Chapter 3. Arbuscular mycorrhizae fungi infection was determined in roots of 25 plants of 15 different key species; the grasses *Agropyron repens* (L.) Gould (quack grass), *Agropyron subsecundum* (Link) Hitchc (bearded wheat grass), *Agropyron* species, *Agropyron trachycaulum* (Link) Maltex H.F. Lewis. (slender wheat grass), *Bromus inermis* Leyss. (smooth brome), *Festuca campestris* Rydb. (rough fescue), *Festuca ovina* L. (sheep fescue), *Poa pratensis* L. (kentucky blue grass); one forb *Medicago sativa* L. (alfalfa) and the woody species *Populus tremuloides*, *Picea glauca*, *Betula papyrifera*, *Elaeagnus commutata* Bernh. ex Rydb (silver willow), *Arctostaphylos uva-ursi* and *Juniperus horizontalis*.

Amendment treatments, particularly manure, were associated with more microbial biomass nitrogen. The estimation of microbial biomass carbon was highly variable due to properties of the calcareous substrate. This is not unexpected due to natural variability. Amended soil had numerically more fungi colony forming units than the controls but did not result in significantly more bacteria colony forming units. Site differences affected the number of bacteria (10^6) and fungi (10^4) colony forming units.

All sampled species of grass, trees and forbs at the quarry and plant, and at disturbed and semi disturbed sites had mycorrhizal infections. Disturbance and management practices may have had an effect on this infection within the same species. Overall, higher values of glucosamine were found in roots from the grasses than from the woody species sampled. The forb *Medicago sativa* had the highest glucosamine value from the sampled species (1,449.75 µg g root^{-1}) and *Poa pratensis* had the lowest glucosamine values even though they were collected at the same disturbed site (277.08 µg g root^{-1}). A much higher value of glucosamine in roots of *Poa pratensis* was detected at a semi disturbed site (1,380.16 µg g root^{-1}).

2. LIMESTONE QUARRY RECLAMATION APPLICATIONS

2.1 Limestone Quarry Substrate Material Use

All three of the limestone substrates tested could be used to reclaim the Exshaw quarry if they are amended. However, better results will likely be achieved using the gray and gray brown substrates. Crushed limestone is produced at the plant to be sold but it can be used to make a substrate suitable for plants.

2.2 Limestone Quarry Substrate Material Amendment

Amendment of any of the limestone substrate materials will be required for reclamation. Current recommendations can be based on the rates tested in this field and greenhouse research.

Fertilizer addition greatly increased above and below ground biomass of grasses. We used 14-14-14 nitrogen, phosphorus, potassium fertilizer applied at 1.1 Mg ha^{-1} and not less than 0.55 Mg ha^{-1}.

Soil amendments can be used even as a 5 cm capping layer or incorporated to the limestone substrate (482 Mg ha^{-1}). Clean fill should be applied at 482 Mg ha^{-1}. Lower rates will result in poor plant performance.

Pulp mill biosolids favoured biomass production even at a low rate of 11 Mg ha^{-1} but a higher rate of 45 Mg ha^{-1} is recommended to provide substrate and plants with more organic matter, nutrients and increased water holding capacity.

Increased nitrogen and carbon in constructed soils were best achieved through addition of beef manure mix at 39 Mg ha^{-1}. Adding manure should provide an overall improvement to low quality reclamation soils with low organic matter and nutrient concentrations because of its high organic carbon and nitrogen which can last a few years after application. In the current study, even though manure was purposely added at a moderate rate, it increased nitrogen concentrations considerably and this effect remained high at the end of the second year. Electrical conductivity at this rate remained below threshold values of concern. Manure mix addition increased plant density and cover.

Wood shavings applied at a rate of 45 Mg ha^{-1} were favourable in the greenhouse but not in the field. Unless water can be supplied to transplants, wood shavings will not be a good soil amendment. Wood shavings are not recommended as an amendment due to their unfavourable effect on plant health (stunted plants) and thus lower plant cover in the field.

The use of hay and straw is not recommended because of the low plant density and biomass produced, even lower than the unamended substrate. If used it should be applied at a low rate of 3.5 Mg ha^{-1}.

Sulfur if used with fertilizer and other amendments such as soil, biosolids or manure should increase biomass of *Agropyron*, *Trisetum* and *Poa*.

Use of erosion control blankets is recommended to increase establishment of seeded native grasses and reduce density and cover of non seeded species. Its use increased plant density, cover, height and seed head density of seeded species. A combined approach of soil nutrient enrichment through manure application and protection with erosion control blankets could further increase revegetation success.

Use of organic forest floor material (LFH) at exposed south facing, bare slopes is a consideration. LFH should be used only on protected microsites or if water is not limiting and in small rather than large areas to optimize the resource.

Transplanting holes can be prepared to have more and better soil than the remaining area, for example 30 cm soil depth.

2.3 Timing of Substrate Development, Amendment and Revegetation

From a management perspective, site preparation and seeding can be done in spring or fall. However, fall species seeded could potentially have the advantage of using early spring soil water from snow melt, before the often drying conditions of summer, and therefore establish in higher numbers. Fall preparation and seeding gave an advantage to *Agropyron*, *Poa* and *Trisetum* at sites with better conditions provided by more nutrients and northwest facing slopes.

Transplanting woody species in fall is recommended. Fall transplanting may improve survival because less grazing occurred in fall when sheep migrated to rut and winter grounds (McCann 1956, Shannon et al. 1975). Although not assessed quantitatively in this research, spring transplanted plants may have had a high water stress shock at the time of transplanting, particularly if planting coincides with a dry summer.

Assisted revegetation increased plant cover from < 6% to 50% and reduced cover of non seeded species. *Erucastrum gallicum,* the most abundant of the non seeded species, was not favoured by soil amending and its presence can be reduced with soil amendments.

Site characteristics such as slope, aspect, initial soil nutrient concentrations and the surrounding plant community influenced early plant community development at the quarry and plant and overall effects of soil treatments. Thus different slopes and locations on a typical quarry may require slightly different reclamation practices.

Soil nutrient management over the long term in the form of fertilizer, amending and incorporation of legumes in the seed mix, will contribute nitrogen and other nutrients to the soil. A combined approach of soil nutrient enrichment through manure application and protection with erosion control blankets could further increase revegetation success at the limestone quarry and plant.

2.4 Plant Species for Revegetation

In view of the environmental conditions at the quarry that limits establishment of most plant species, and the fact that *Agropyron trachycaulum* appears to facilitate development of other species, the general reclamation practice of limiting this species to 10% of the mix appears unwarranted.

Poa alpina should be incorporated in the seed mix. Its cover in many cases was equivalent to that of *Agropyron trachycaulum* and *Festuca saximontana* even with fewer plants. This highlights the value of using a diverse plant mix with tufted species. *Poa alpina* is not currently widely used in native grass mixes for Subalpine and Montane areas in the Bow Valley and results from this study suggest it should be more widely used.

Pseudotsuga and *Populus* had highest survival. *Juniperus* plants dug from the adjacent forest had low survival and different sources should be considered, for example, purchasing at a local nursery. Quarry conditions are favourable for this species and it has been observed establishing naturally in disturbed areas of the quarry. This rate of establishment is very slow and likely not conducive to rapid reclamation, however, it could augment longer term plant community development. *Picea* local transplants had lower survival than *Picea* from the nursery. Nonetheless, *Picea* local plants could be collected and transplanted in fall, when chances of survival will be higher, to supplement purchases from nurseries, *Picea*, *Pseudotsuga* and *Populus* could be considered for limestone quarry reclamation with likelihood of high survival.

Populus could be planted to increase primary production, as it is unlikely to regenerate naturally from the surrounding areas in large enough numbers. *Populus* is more commonly established by suckers than seeds, and there are few aspen trees that could reproduce vegetatively and spread to other areas of the quarry in the short term.

Planting nitrogen fixing plant species such as *Alnus crispa* may contribute to increased soil nutrients and may provide a balance of palatable and unpalatable species for wildlife that occupy the area. For example, *Alnus* has low palatability and can tolerate browsing and low soil nutrients (Tannas 2003), similar to *Pseudotsuga* or Picea; whereas *Betula* and *Populus* are more prone to browsing.

For any transplanted species, a mortality rate of 10 to 50% should be expected during the first year after planting and therefore it may be necessary to consider over planting to compensate for tree mortality from browsing and harsh site conditions. Further increased mortality can be expected over the next decade after planting. *Populus* and *Betula*, both deciduous broad leaved species, more frequently presented signs of browsing compared to evergreen trees. Low rates of height increase should be expected, between 2 to 6 cm per year, particularly for species that will likely be browsed. These low rates of height increase may last over a decade (Young et al. 2009).

The limiting site conditions and frequent trampling of bighorn sheep makes seeding inadvisable for woody species to establish a plant community. If seeding will be done, germination or viability tests will provide some indication of seeding rates to use. In particular, seeding *Betula* or other species that could be browsed is not advised, because even if established the seedlings may be subjected to a high pressure from browsing. Non palatable species are preferable for seeding, such as *Pseudotsuga*, *Picea* and *Juniperus*.

2.5 Management of Bighorn Sheep

Bighorn sheep are part of the quarry ecosystem and animal use and browsing must be considered when planting woody species at the quarry. Use of a variety of species including a few favourites of sheep such as *Betula* and *Amelanchier alnifolia* Nutt. (saskatoon), *Salix* (willow) spp., could be considered. Diverse communities are more resistant and resilient to perturbation. Attraction of sheep to certain plant species, may reduce pressure to others such as *Picea* and *Pseudotsuga*. These species were subject to browsing but to a much lesser extent than all others. If not being browsed, individuals of *Picea* and *Pseudotsuga* will be more likely to survive and establish. In turn they will be aesthetically pleasing pillars of the woody community, which is one of the reclamation goals for public approval. Planting density, however, should be planned to avoid potential domination and thus exclusion of others.

More intense management of herbivores should be investigated. Plantskyd was successful in reducing herbivore use of the site and could be applied on a more frequent basis over the growing season.

2.6 Specific Revegetation Strategies

Soil amending and fertilizing, protection against herbivores and high density planting of nursery woody plants and local woody plants could be more integrated. Large plants could be planted sparsely to help build the landscape and to create or improve micro sites (deeper, amended planting soil). Coniferous species, particularly *Pseudotsuga*, could be used to survive the bighorn sheep browsing. Small areas could be planted with *Betula* far away from the taller trees to attract bighorn sheep and if heavily browsed, replanting *Betula* in these areas could be considered. Appropriate timing of fertilizer and even irrigation water could be included. Other woody species including shrubs for grazing and as nitrogen fixers could be included to facilitate establishment of the plant community at these highly disturbed sites.

2.7 Field Microbial Evaluations

Amending with manure, wood shavings and erosion control blankets should assist in increasing microbial biomass in quarry soils in relation to controls and result in higher viable fungi and bacteria which is a sign of soil health. Manure treated soil will increase the microbial population the most, whereas wood shavings will favour fungi. While erosion control blankets did not affect the microbial community as much as organic amendments incorporated into the soil, their effect at increasing plant density and cover is expected to lead to decaying root biomass and exudates in the soil which will sustain a larger community of microorganisms than untreated soil.

Mycorrhizae were found in roots of all tested plants, indicating that management practices such as artificially inoculating mycorrhizae is not necessary, particularly if the initial establishment of vegetation is paired with soil amending and addition of phosphorus.

3. RESEARCH LIMITATIONS AND FUTURE RESEARCH DIRECTIONS

3.1 Limitations of the Current Research

The variety of characteristics of sites within the quarry and the different results they produced for vegetation establishment, illustrates the variability that could be expected at various locations. Therefore, it would have been ideal to develop this research in more than one limestone quarry. That was not possible due to budget constraints in this research program. However, the research could be applied to other sites at other points in time to better extrapolate the data beyond the current study site.

The plant communities established in the various treatments, including woody species, grasses and forbs, were assessed for a few years only. Effects of soil treatments on the plant community over the long term were also not assessed.

The study was confined to the time of a PhD program. However, longer term follow up is possible if the sites can be retained for further assessment over time.

A broader range of parameters for characterization of the developed substrates were not assessed such as substrate water holding capacity, field water potential, runoff and chemical parameters such as available nutrients and soluble ions. Funding and time were not available in this research program, but should be included in further research or longer term assessments if possible.

3.2 Main Questions Raised from the Current Research

Are moderate rates of organic amendments sufficient for establishment of a sustainable native plant community or will more organic matter be needed as a first or subsequent applications?

Could sulfur added several months prior to seeding increase plant establishment by increasing phosphorus availability for plants?

What other organic amendments will improve the physical, chemical and biological characteristics of the substrate?

What other woody, grass and forb native species can adequately establish at the quarry and thus be used to increase plant diversity?

What characteristics of microsites could be used to increase vegetation establishment of woody, grass and forb native species?

What is the extent of use of bighorn sheep at the quarry and what strategies can be implemented for their management and inclusion for quarry revegetation?

What is the composition and how is the activity of the microbial community at different reclamation treatments compared to the undisturbed microbial community? Is the microbial community including mycorrhizae hyphae network developing at the reclaimed site?

3.3 Research Directions for Substrate Amelioration

Due to limited water availability at the reclamation quarry sites, which is critical for early revegetation stages, further greenhouse and field research with more amendments and combinations of amendments that will retain water is warranted. There are several water retaining synthetic materials that should be tested. Lack of water on the exposed slopes is a critical factor limiting reclamation of limestone quarries.

Measurement of physical properties of the amended substrates is required to better quantify the soil response to amendments. Previous research provides a lot of information on why many specific amendments alter soil physical properties; however, this has often not been quantified in the field and will have a large impact of determining loading rates of amendments.

Although amendments ameliorated site conditions to some degree, the harsh environment of the quarry requires even more enhancement. Research is needed to address further site modification, including soil nutrient conditions, slope aspect and microsites. Creation of microsites has been a major factor of importance in arctic environments (Drozdowski et al. 2011, Naeth and Wilkinson in preparation). Small microsites developed during recontouring could accumulate organic matter and water and have an angle protected from full sun exposure, emulating north facing slopes. Introduction of other forms of microsites such as woody debris would be valuable to study based on their reclamation use in oil sands reclamation (Brown and Naeth in preparation). Woody debris may also assist with erosion control.

The erosion control blankets favourably modified soil physical conditions for the seeded vegetation. Research is needed on erosion control blankets in combination with manure or other organic amendments that improve the chemical conditions. Since seeded species had a positive response to increased nitrogen and carbon, higher rates of manure application than the one used in this study could be investigated to develop optimum application rates. Follow up research testing sulfur in the field, application time prior to seeding and use of fertilizer with sulfur to characterize its effect as nutrients will be useful.

More soil treatments should be tested to further improve the soil nutrient content and the soil depth for a more hospitable plant growing environment. Research needs to identify ways or treatments to prevent soil water loss, for example, using pulp mill biosolids in the field with the woody species.

Long term studies are needed to evaluate if and when to re-apply manure or other organic amendments to prevent regression of the plant community due to poor soil nutrients.

3.4 Research Directions for Plant Species and Revegetation

Long term studies are needed to evaluate succession of the plant community, effect of non seeded species on the plant community and required management to prevent regression of the plant community due to poor soil physical and / or chemical properties.

Whether the robust *Agropyron trachycaulum* plants provided physical shelter for other species, or modified their environment in some other way is worth further study. This will provide an ideal opportunity to further test the hypothesis of facilitation in a field setting.

Use of other native grass and forb species will need to be tested to provide an appropriate level of biodiversity for reclaimed sites. Whether this could be addressed with later stage seedings or transplantings is worth pursuing. Use of other woody species such as *Alnus* and *Salix* spp as cuttings and containerized stock is needed.

Research on microsites to identify ways to improve woody establishment from seeds would be useful. For example, to determine is seeds should be placed in close proximity to the forest edge or near transplants or logs that will provide protection from temperature stress. How to modify microsites to reduce soil temperature and to increase soil water content requires further research.

Research is needed on planting densities and proportion of each species to be planted. Several planting densities could be studied. LFH in several smaller areas within a larger site could be tested and appropriate size of LFH covered area could be determined.

Long term monitoring is required to study growing rates of different species used to reforest the quarry and to compare to natural areas. Effect of browsing needs to be further addressed. Establishment needs to be better verified, for example, using root growth and basal growth as indicators of establishment.

Planting taller plants, out of the reach of sheep, could be tested. More species of trees, shrubs and grasses could be planted to increase diversity of the plant community. If areas not visited by bighorn sheep are identified within the quarry and/or an exclusion method is devised to isolate specific areas, the effect of amendments and reclamation treatments on tree growth can be determined with and without the impact of browsing.

Growing rates of different wood species could be assessed at various reclamation prescriptions. Ideally, these treatments should be long term monitored (more than 10 years), to capture trends in tree survival and growth. Assessment of root development as result of reclamation prescriptions could be undertaken. Better prescriptions will lead to healthier root systems and increase tree survival and stability and may be particularly important to keep the trees at the steep and wind exposed quarry slopes.

Because LFH is a potential good source of native propagules (Mackenzie and Naeth 2009), identification of better sites or microsites for its use in the quarry could be investigated. These sites may have different conditions, such as the north facing slope at the plant, where more soil water is available, sheltered areas by logs or close to planted trees, placement on top of better substrate. Potential of LFH from different undisturbed sites could be assessed prior to its use to identify better characteristics for an LFH donor site.

Treatments that can prevent the need for weed management by limiting establishment of non desired species should be developed. By testing several combinations of soil treatments including a diversity of organic amendments and application rates, prescriptions with lower establishment of non desirable species could be identified, just as it was detected with this research that erosion control blankets decrease the establishment of non seeded species and that *Erucastrum* density decreases with soil amending. This will favour the establishment of the target plant community and potentially reduce management costs.

3.5 Research Direction for Herbivore Management

To develop strategies for bighorn sheep management, the extent of use of the quarry site by bighorn sheep, along with patterns of seasonal use and frequented paths could be investigated. Establishment of vegetation at frequented and not frequented areas could be contrasted and the pressure of grazing and browsing could be quantified.

Seeding and planting prescriptions could be investigated to both attract and detract bighorn sheep consumption. For example, it could be determined if bighorn sheep are attracted to areas specifically planted with their preferred food sources such as *Betula* or *Salix*, and thus browsing pressure to other planted areas may decrease.

Effectiveness of chemical deterrents such as Plantskyd could be further assessed. Use of these chemical deterrents several times over the full growing season may provide better control. These are cost effective alternatives to the physical barriers such as fences, which are not feasible for use at the quarry because of the rocky substrate that prevents installation. The size and strength of the bighorn sheep could likely break the fence easily if it were not well installed. Smaller physical deterrents, such as individual tree wire protection and grazing cages could be assessed for use with woody vegetation.

3.6 Research Directions for Microorganisms

It will be useful to compare fungi and bacteria numbers and characterize functional groups at undisturbed and semi disturbed sites in the surroundings in addition to in the amended substrate, to have a better idea of the characteristics of the soil community that occur naturally and therefore aim for it in the reclaimed soils. Because of seasonal changes in the soil community, this assessment could be done over distinct seasons (spring, summer, fall, winter). For the amended soil community, the assessment could target an evolution over time (1 to several years). This research would help identify soil treatments that provide conditions for a better soil community and better vegetation in the medium and long term.

Further research is needed to detect if differences in plant responses to arbuscular mycorrhizae infection change with levels of soil fertility caused by amending. A wider range of species (the seeded ones for example) could be tested for arbuscular mycorrhizae infection. Currently, a high proportion of the non seeded plant cover at the quarry is composed of annual plant species (non native) that do not form mycorrhizae associations. Low phosphorus in the soil makes it desirable to have mycorrhizae to facilitate establishment of desired native plants. This makes assessment of mycorrhizae infection in key species important to evaluate reclamation success. Quantification of glucosamine seems to be a useful method to indirectly assess arbuscular mycorrhizae infection and could be tested against other method to further evaluate the method.

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APPENDIX A

Table A-1. Fertilizer effects on native grasses density and biomass in limestone substrates.

Substrate	Treatment	Density (plant pot^{-1})	Above Ground Biomass (g pot^{-1})	Below Ground Biomass (g pot^{-1})
Crushed	Fertilized	6.3 ± 0.5	1.5 ± 0.1	0.60 ± 0.1
	Unfertilized	11.0 ± 0.5	0.47 ± 0.0	0.27 ± 0.0
Gray	Fertilized	7.7 ± 0.5	1.87 ± 0.1	0.75 ± 0.1
	Unfertilized	11.2 ± 0.5	0.67 ± 0.0	0.29 ± 0.0
Gray Brown	Fertilized	8.0 ± 0.4	1.85 ± 0.1	0.68 ± 0.0
	Unfertilized	10.3 ± 0.5	0.54 ± 0.0	0.27 ± 0.0

Error bars are ± standard errors.

Density and above ground biomass n=72, below ground biomass n=54

Table A-2. Permutational analyses of variance values for plant density and above and below ground biomass of native grasses in treated limestone substrates in experiment 1.

	Plant Density			Above Ground Biomass		Below Ground Biomass		
	df	F	p	F	p	df	F	p
Substrate	2	2.18	0.11	5.58	0.004*	2	0.68	0.51
Fertilizer	1	122.33	<0.001*	274.4	<0.001*	1	44.54	<0.001*
Sulfur	1	3.31	0.06	1.38	0.23	1	0.72	0.39
Amendment	8	31.6	<0.001*	43.51	<0.001*	8	7.93	<0.001*
Substrate x Fertilizer	2	4.44	0.01 *	1.27	0.28	2	0.43	0.66
Substrate x Sulfur	2	0.15	0.85	0.09	0.90	2	0.12	0.88
Substrate x Amendment	16	1.58	0.07	2.07	0.008*	16	1.39	0.14
Fertilizer x Sulfur	1	0.79	0.38	3.95	0.04*	1	0.12	0.73
Fertilizer x Amendment	8	4.12	<0.001*	14.92	<0.001*	8	5.21	<0.001*
Sulfur x Amendment	8	1.67	0.10	1.68	0.10	8	1.37	0.20
Substrate x Fertilizer x Sulfur	2	0.64	0.53	0.53	0.59	2	0.16	0.85
Substrate x Fertilizer x Amendment	16	2.07	0.01*	1.50	0.09	16	1.42	0.12
Substrate x Sulfur x Amendment	16	2.06	0.01*	1.93	0.01*	16	1.15	0.30
Fertilizer x Sulfur x Amendment	8	1.25	0.26	2.29	0.02*	8	2.26	0.02*
Substrate x Fertilizer x Sulfur x Amendment	16	0.84	0.63	1.24	0.22	16	0.85	0.62
Residual	324					216		
Total	431					323		

Table A-3. Permutational analyses of variance of plant density and above and below ground biomass at week 12 in gray substrate with 5 amendments applied at three rates and two fertilizer rates in experiment 2.

		Density		Above Ground Biomass		x Below Ground Biomass	
Source	df	F	p	F	p	F	p
Amendment (Amend)	4	24.60	<0.001 *	29.42	<0.001 *	16.65	<0.001 *
Amendment Rate (Amend Rate)	2	0.27	0.75	9.23	<0.001 *	4.81	0.007 *
Fertilizer Rate (Fert Rate)	1	1.18	0.27	9.91	0.001 *	6.32	0.01 *
Amend x Amend Rate	8	1.63	0.12	2.00	0.05	0.99	0.45
Amend x Fert Rate	4	1.27	0.28	1.08	0.36	0.34	0.84
Amend Rate x Fert Rate	2	2.12	0.12	3.33	0.03	2.05	0.12
Amend x Amend Rate x Fert Rate	8	1.72	0.09	0.73	0.66	0.94	0.48
Residual	120						
Total	149						

Amendments are biosolids, wood shavings, clean fill, beef manure compost, straw.

Amendment rates are low, medium, high.

Fertilizer rates are low, medium.

* Indicates significance at $p \leq 0.05$.

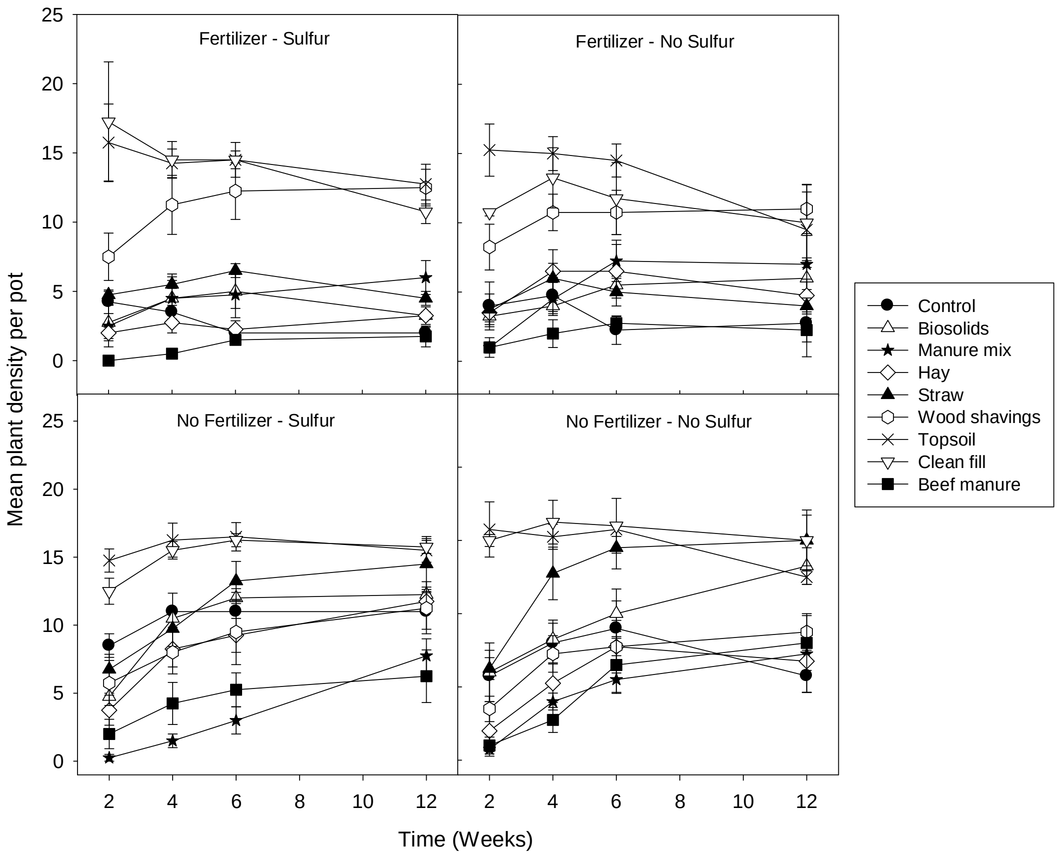

Figure A-1. Mean plant density in crushed limestone with nine treatments with and without fertilizer and sulfur over 12 weeks. Error bars are ± standard errors. N = 4.

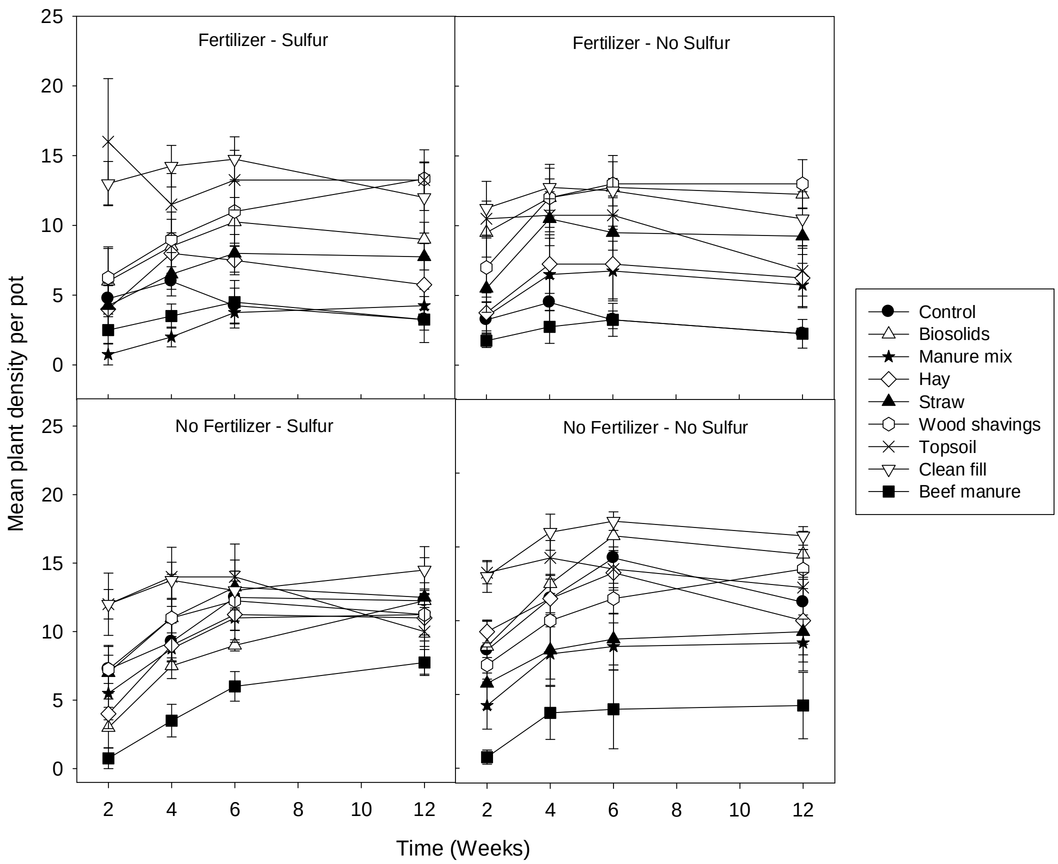

Figure A-2. Mean plant density in gray limestone with nine treatments with and without fertilizer and sulfur over 12 weeks. Error bars are ± standard errors. N = 4.

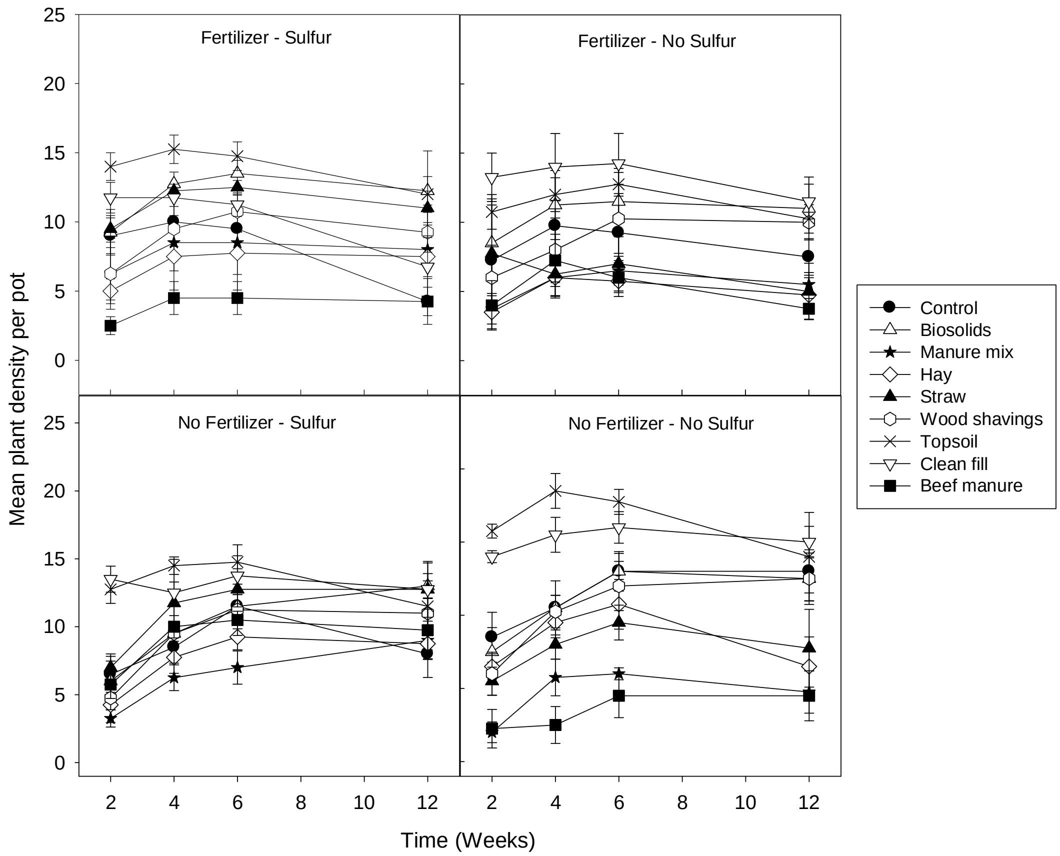

Figure A-3. Mean plant density in gray brown limestone with nine treatments with and without fertilizer and sulfur over 12 weeks. Error bars are ± standard errors. N = 4.

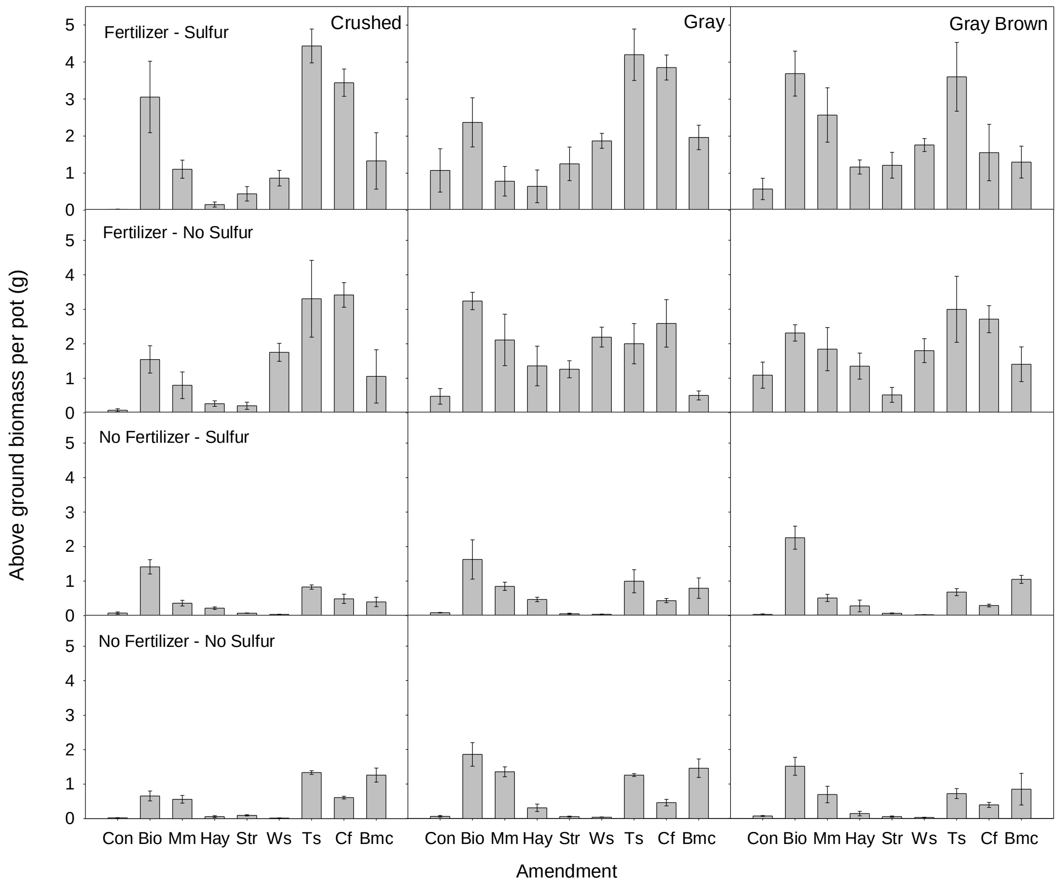

Figure A-4. Above ground biomass in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from the graph top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars are ± standard errors. N = 4.

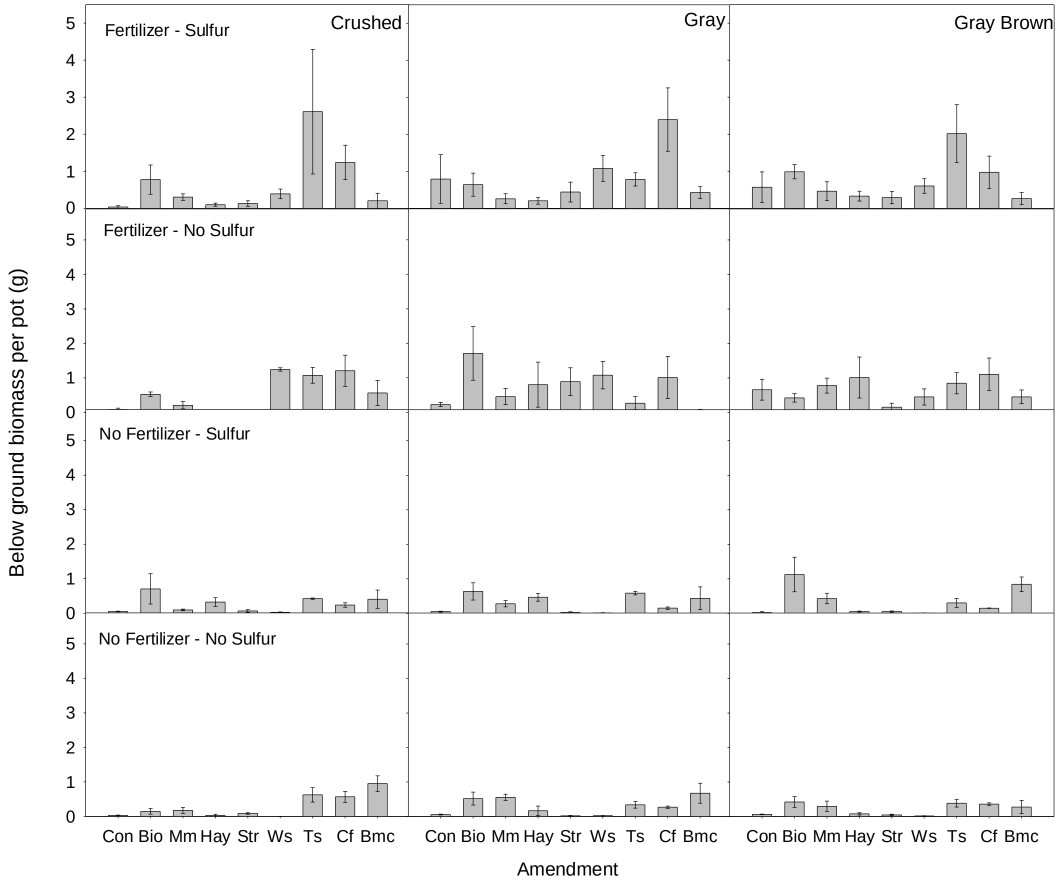

Figure A-5. Below ground biomass in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from the graph top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars are ± standard errors. N = 3.

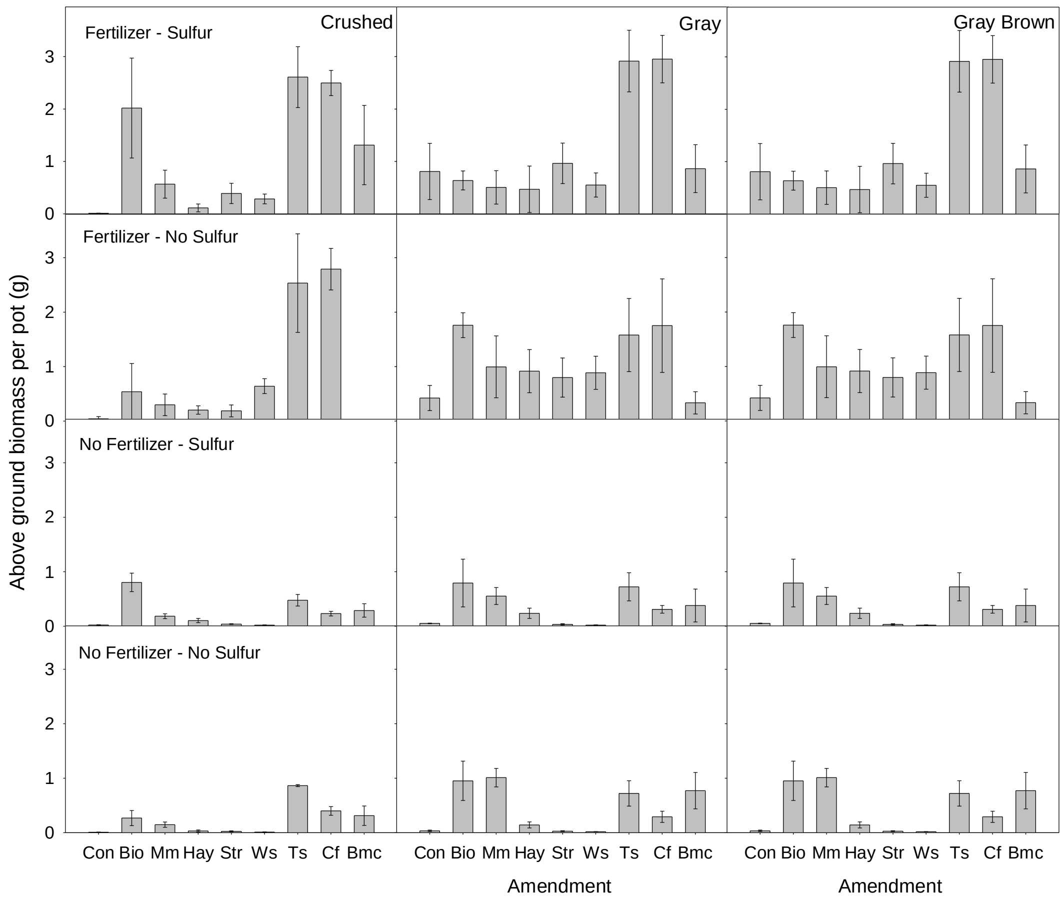

Figure A-6. Above ground biomass of *Agropyron trachycaulum* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars are ± standard errors. N = 4.

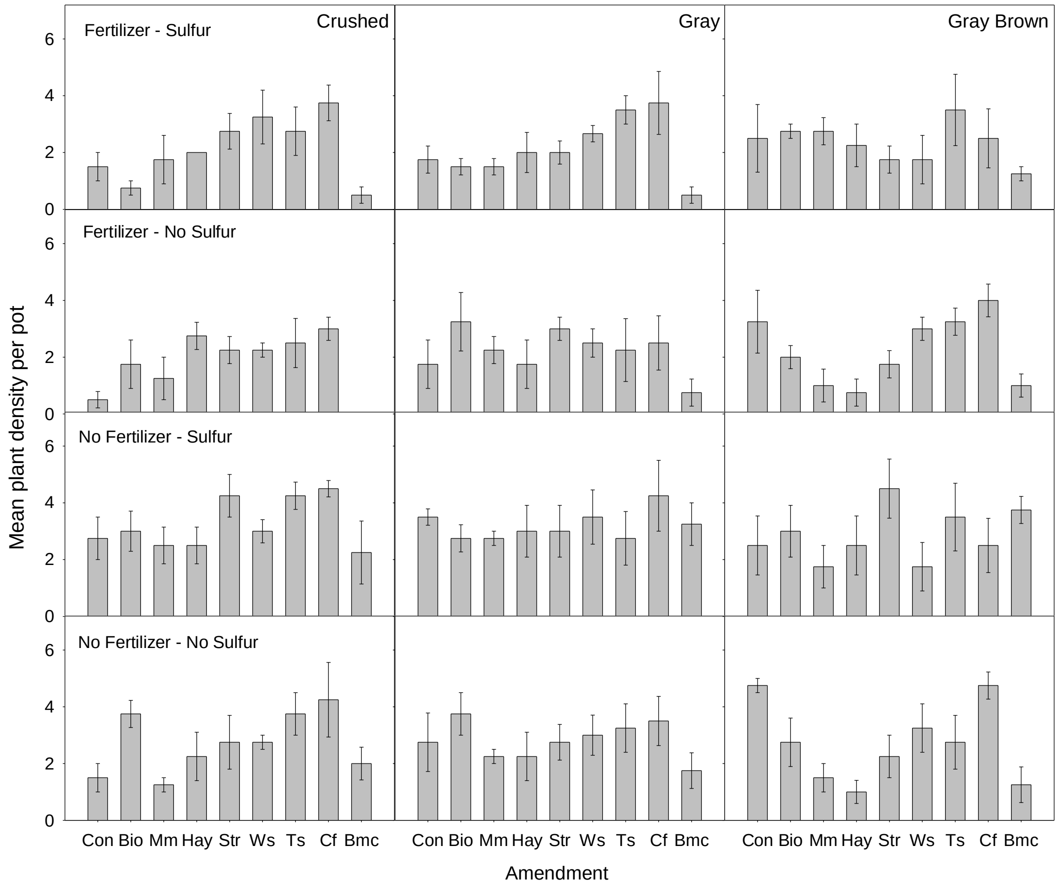

Figure A-7. Plant density of *Agropyron trachycaulum* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars are ± standard errors. N = 4.

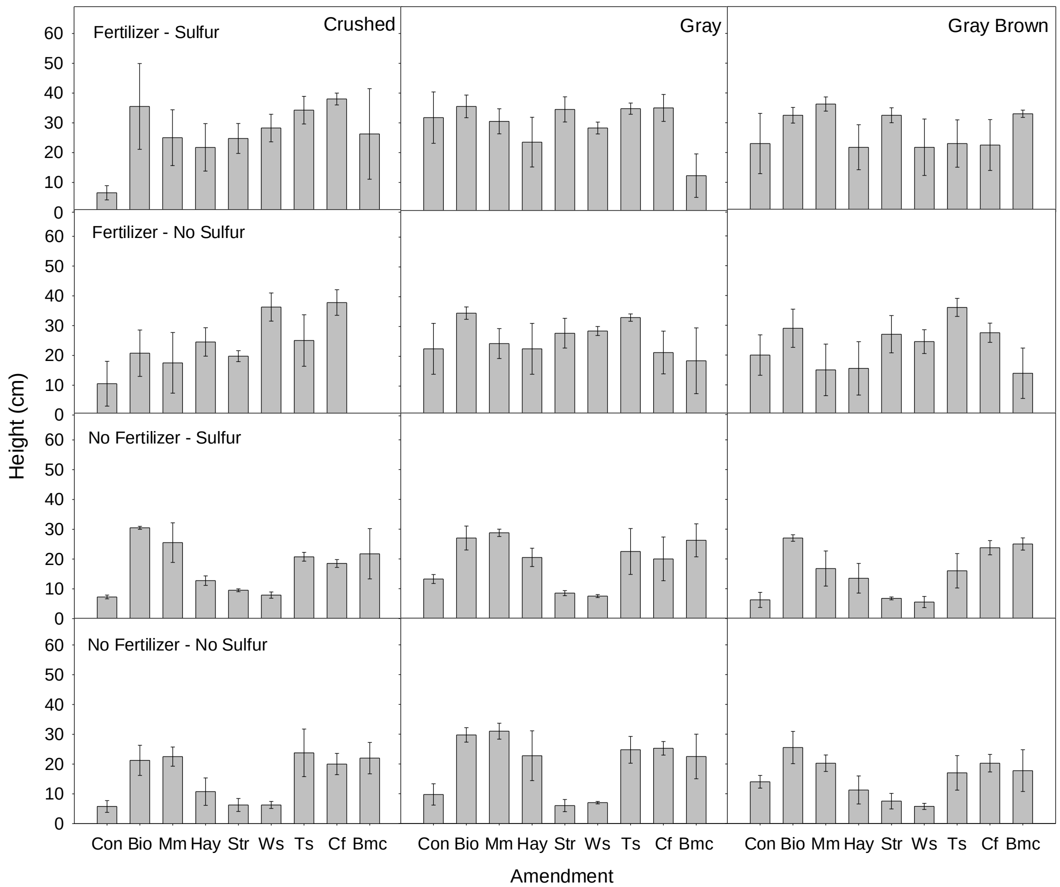

Figure A-8. Height of *Agropyron trachycaulum* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars ± standard errors. N = 4.

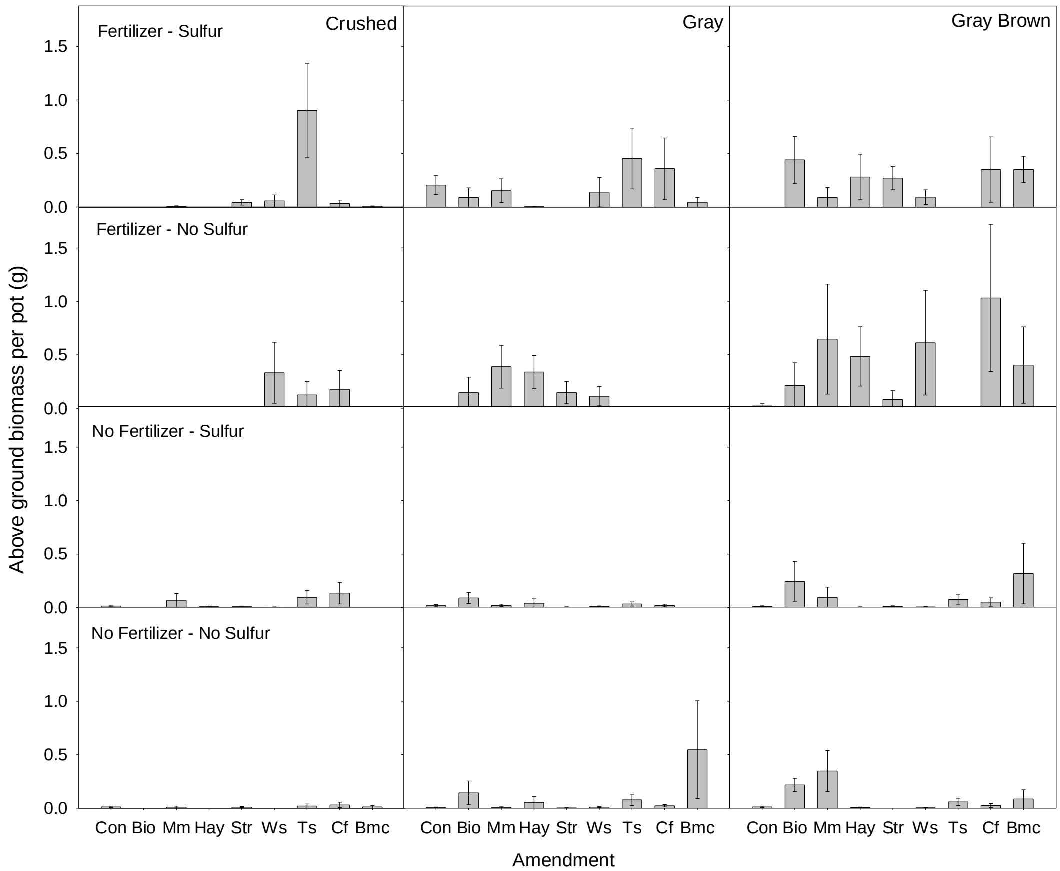

Figure A-9. Above ground biomass of *Elymus innovatus* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars are ± standard errors. N = 4.

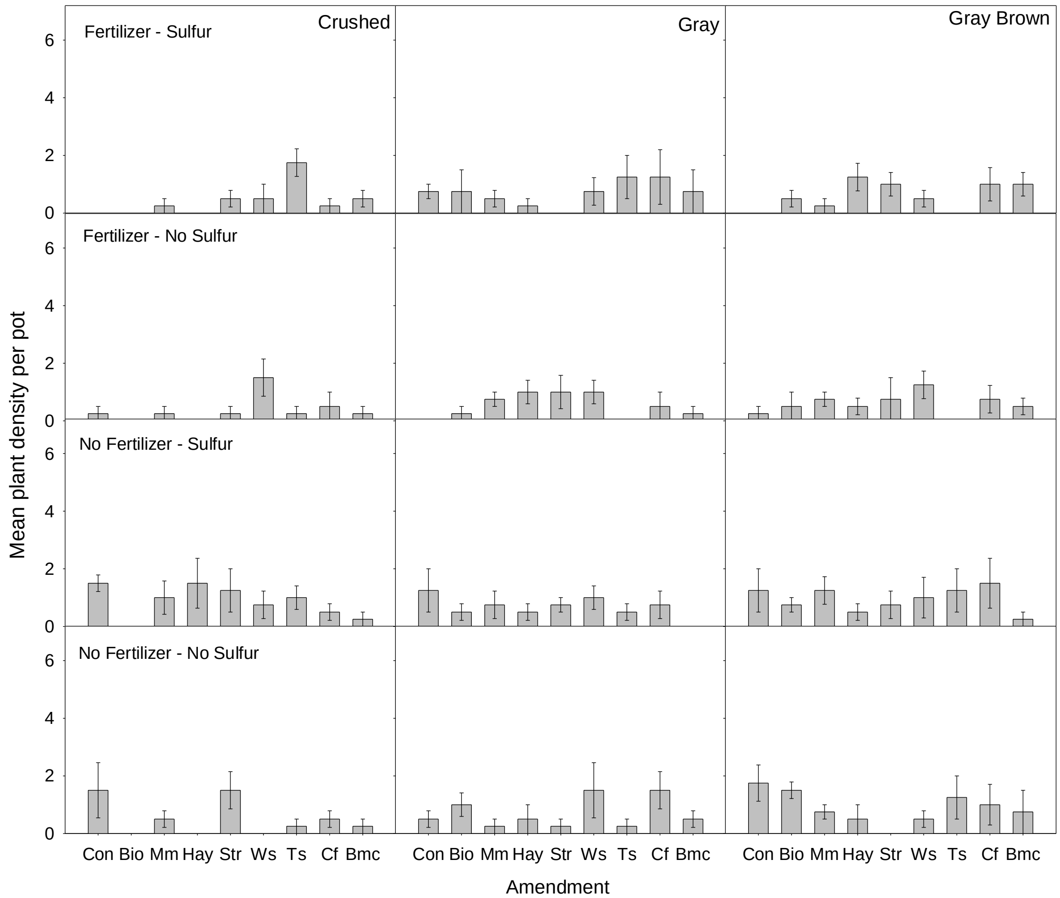

Figure A-10. Plant density of *Elymus innovatus* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars ± standard errors. N = 4.

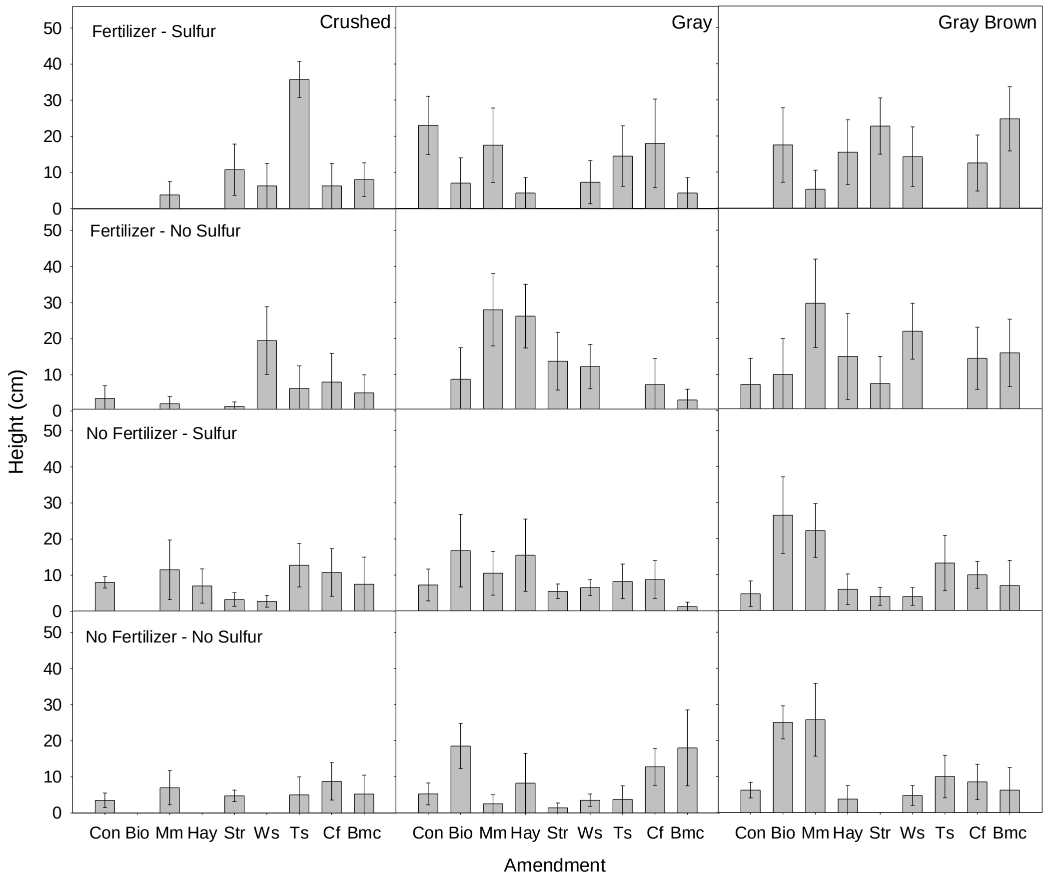

Figure A-11. Height of *Elymus innovatus* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars ± standard errors. N = 4.

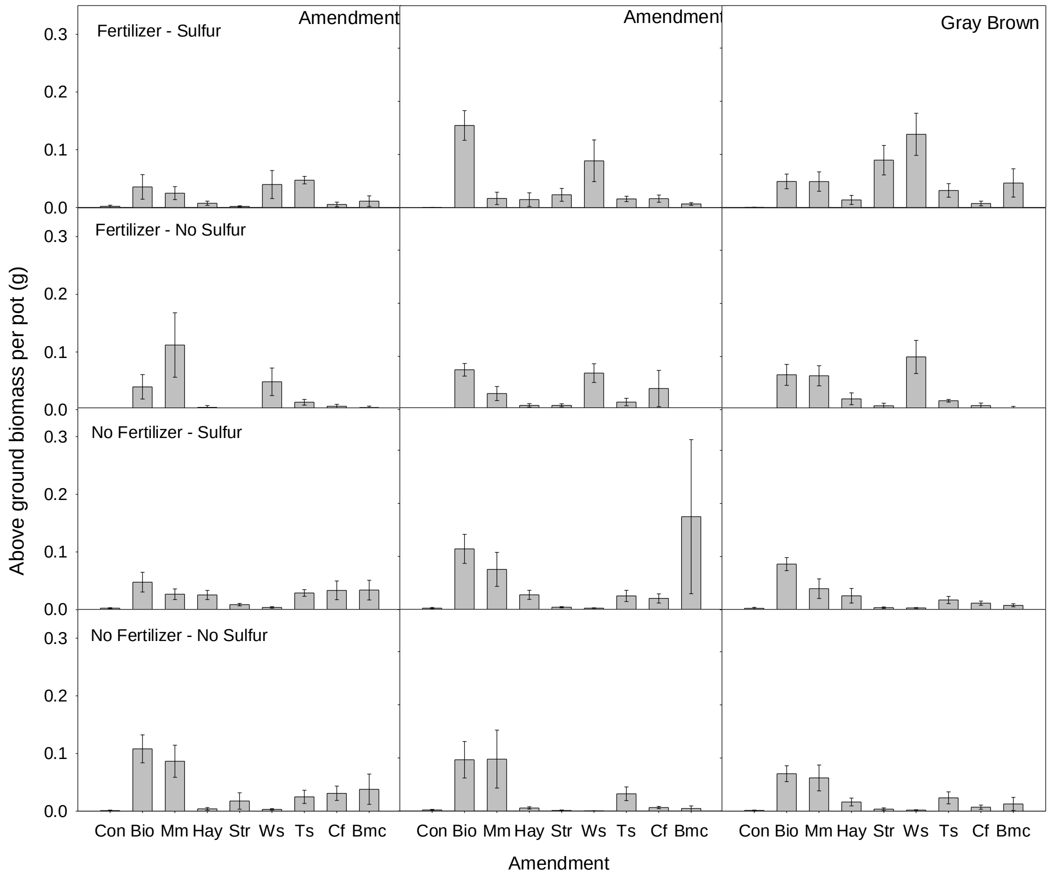

Figure A-12. Above ground biomass of *Festuca saximontana* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars are ± standard errors. N = 4.

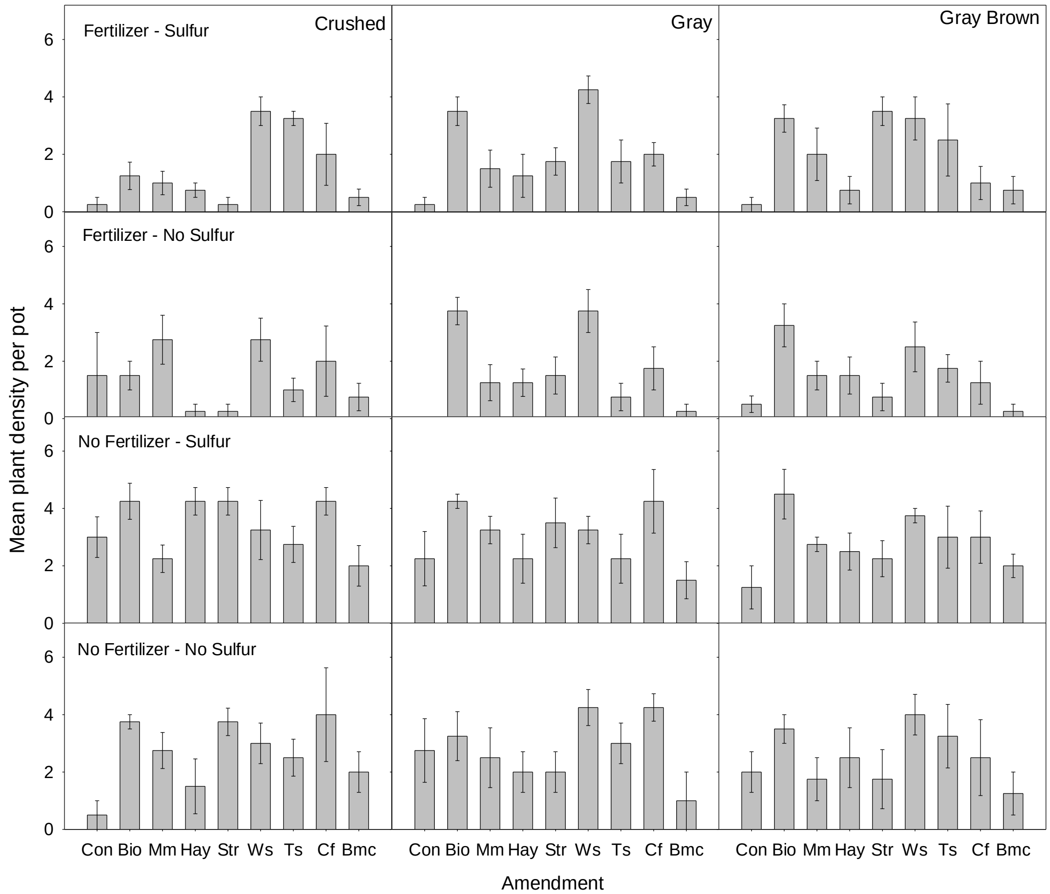

Figure A-13. Plant density of *Festuca saximontana* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars ± standard errors. N = 4.

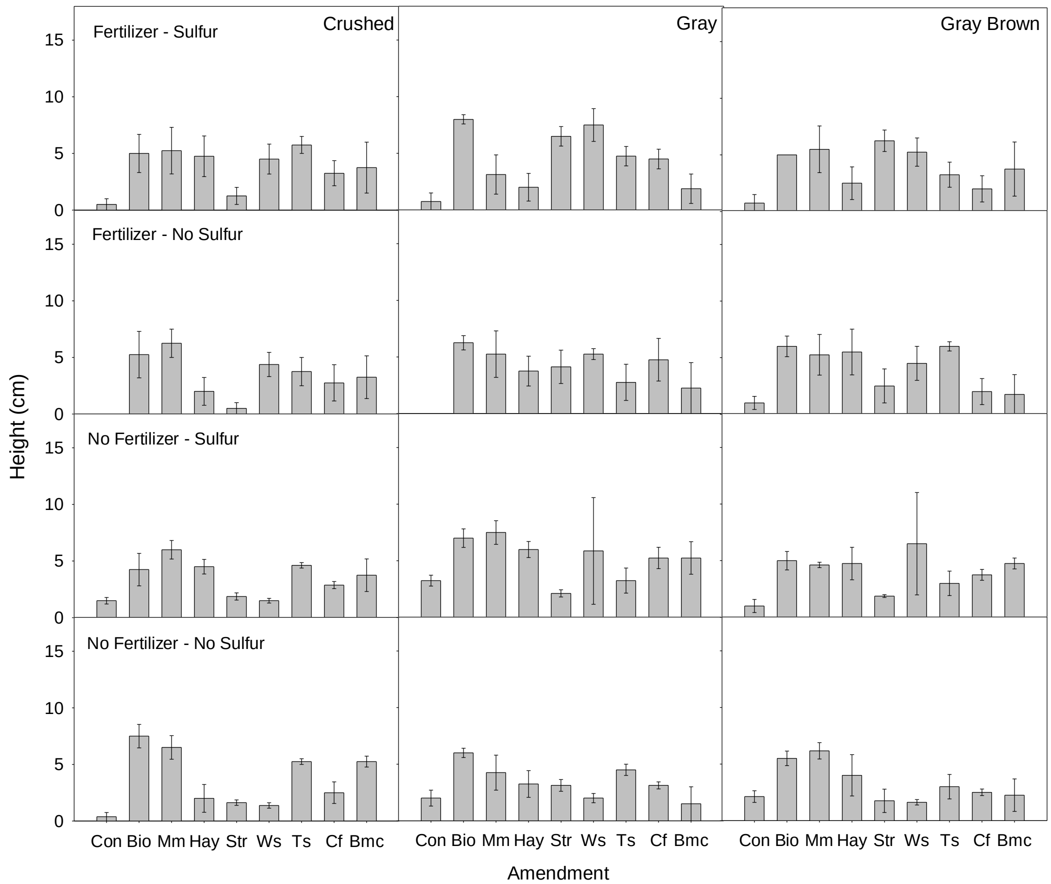

Figure A-14. Height of *Festuca saximontana* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars ± standard errors. N = 4.

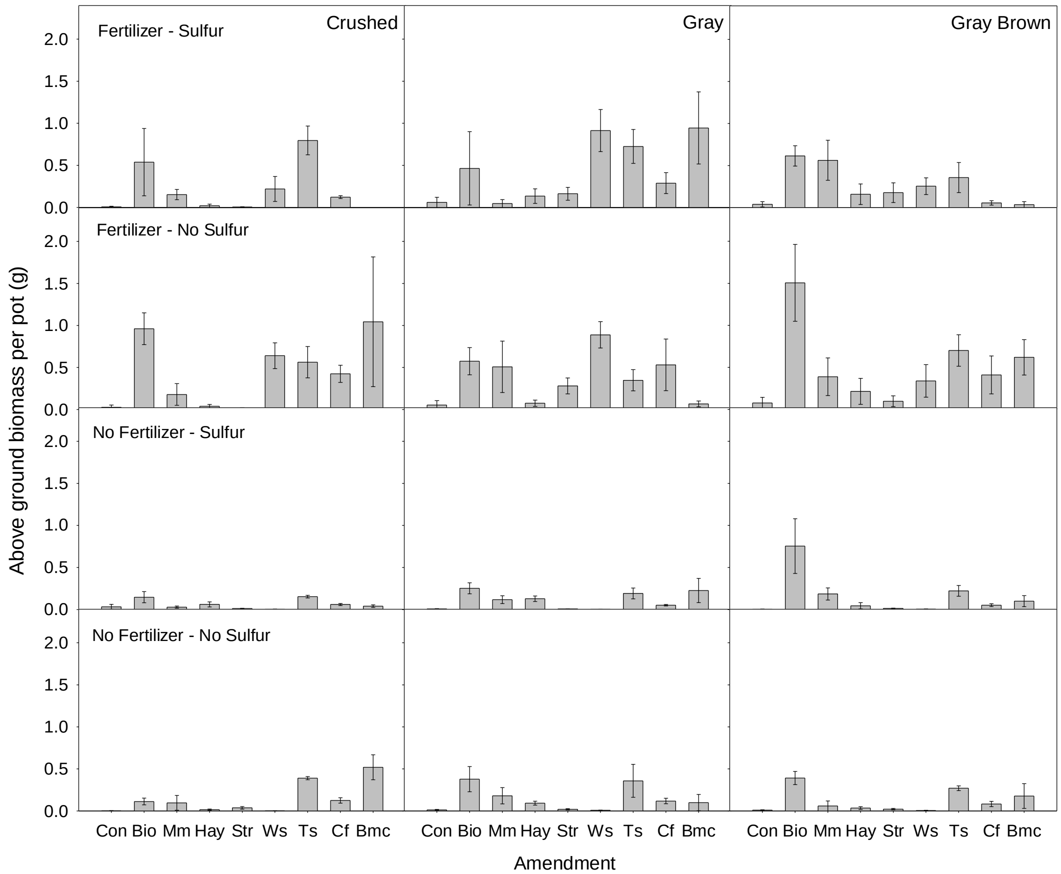

Figure A-15. Above ground biomass of *Poa alpina* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars ± standard errors. N = 4.

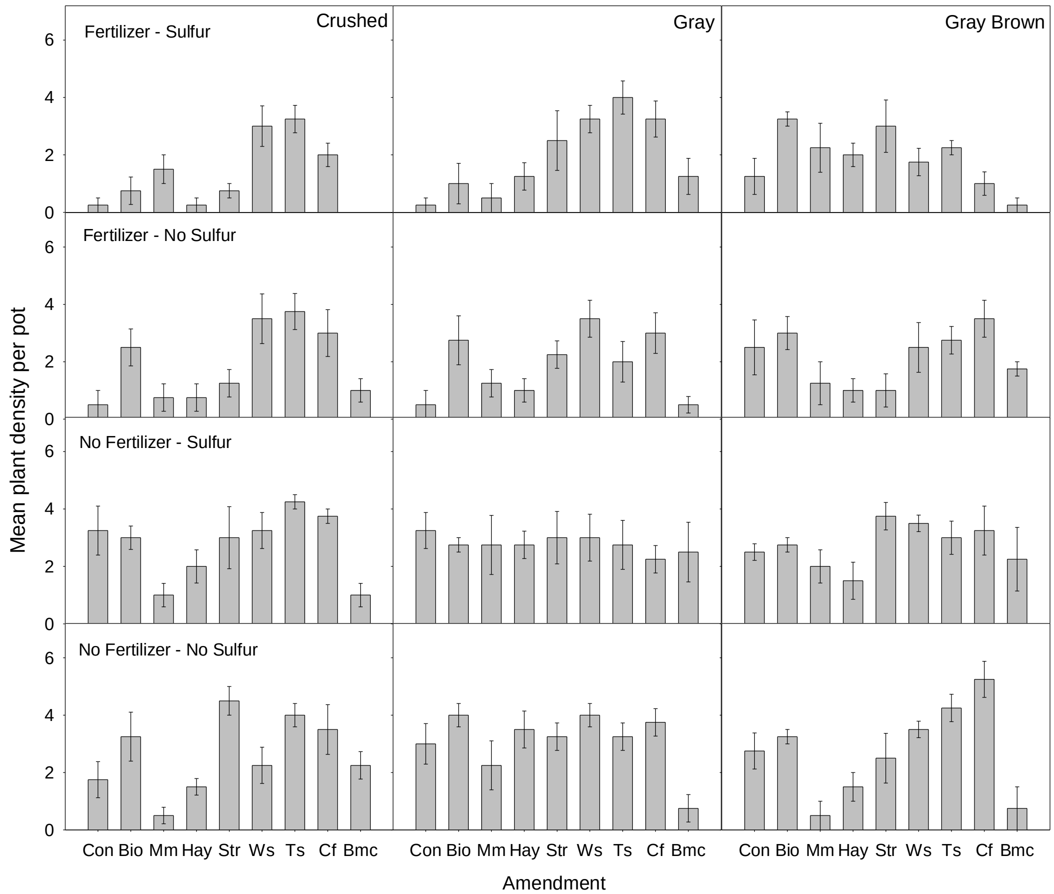

Figure A-16. Plant density of *Poa alpina* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from the graph top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars are ± standard errors. N = 4.

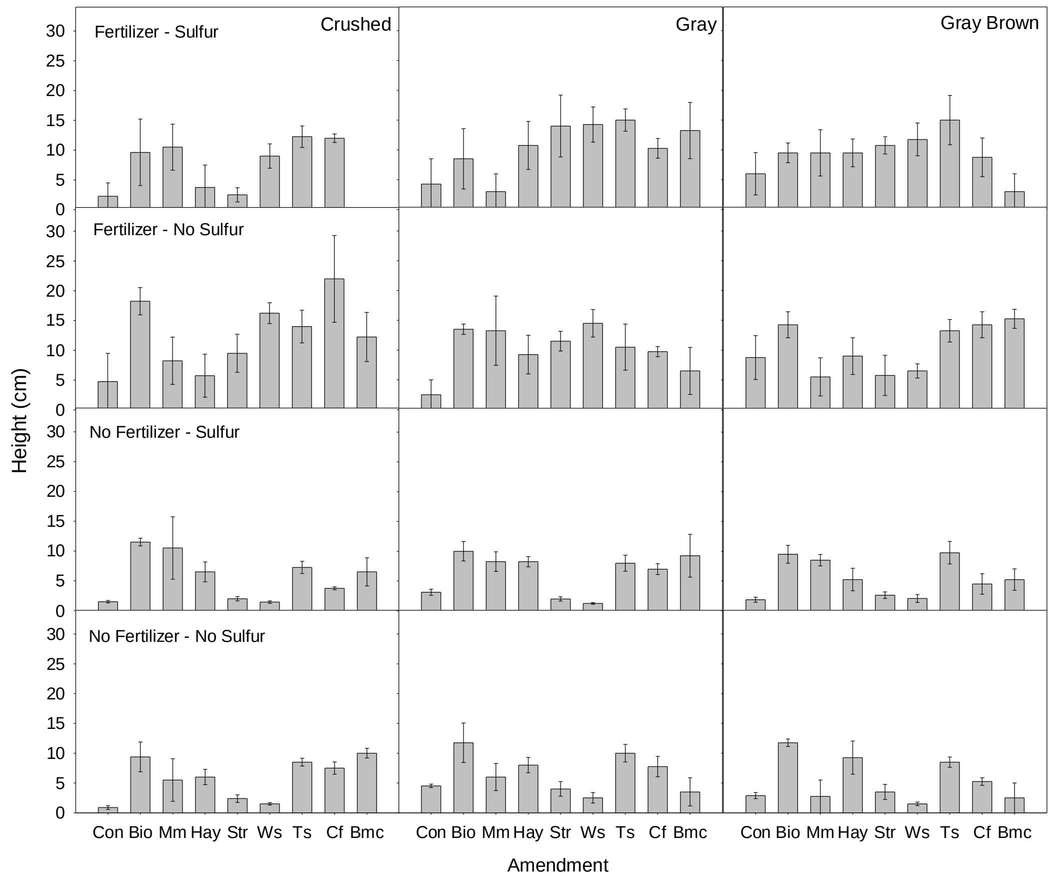

Figure A-17. Height of *Poa alpina* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars are ± standard errors. N = 4.

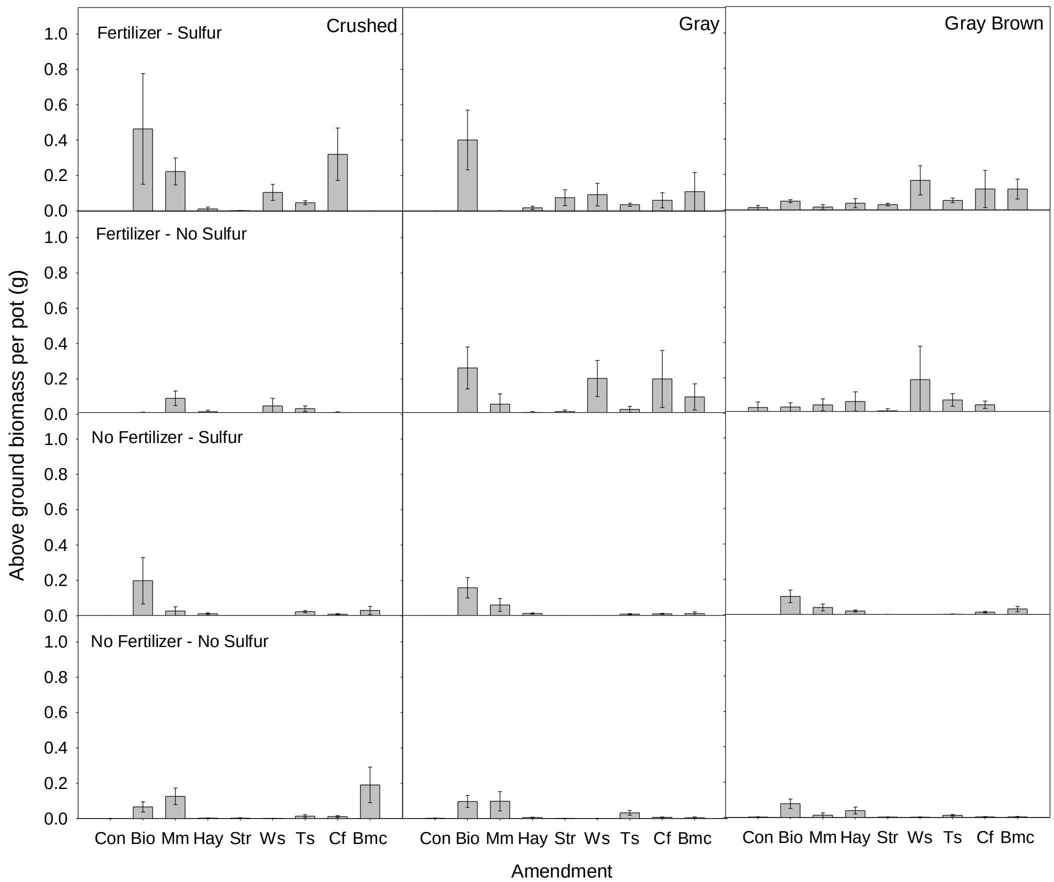

Figure A-18. Above ground biomass of *Trisetum spicatum* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars are ± standard errors. N = 4.

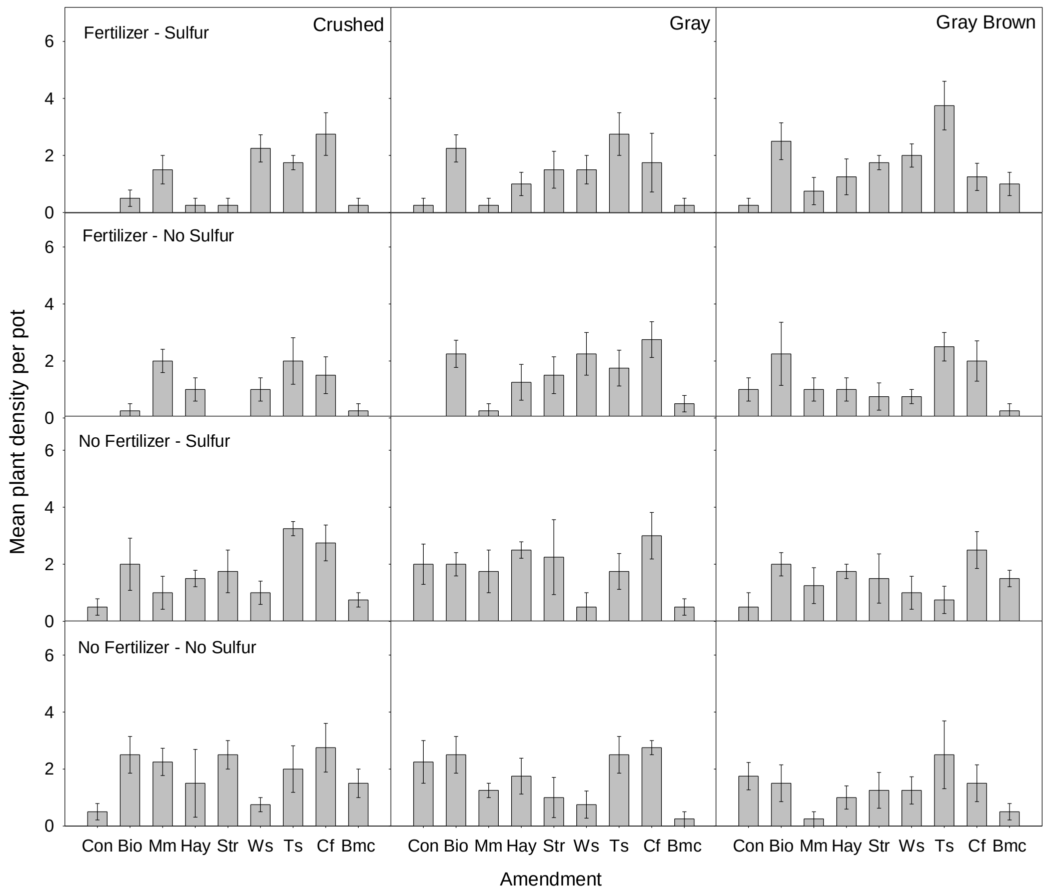

Figure A-19. Plant density of *Trisetum spicatum* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars ± standard errors. N = 4.

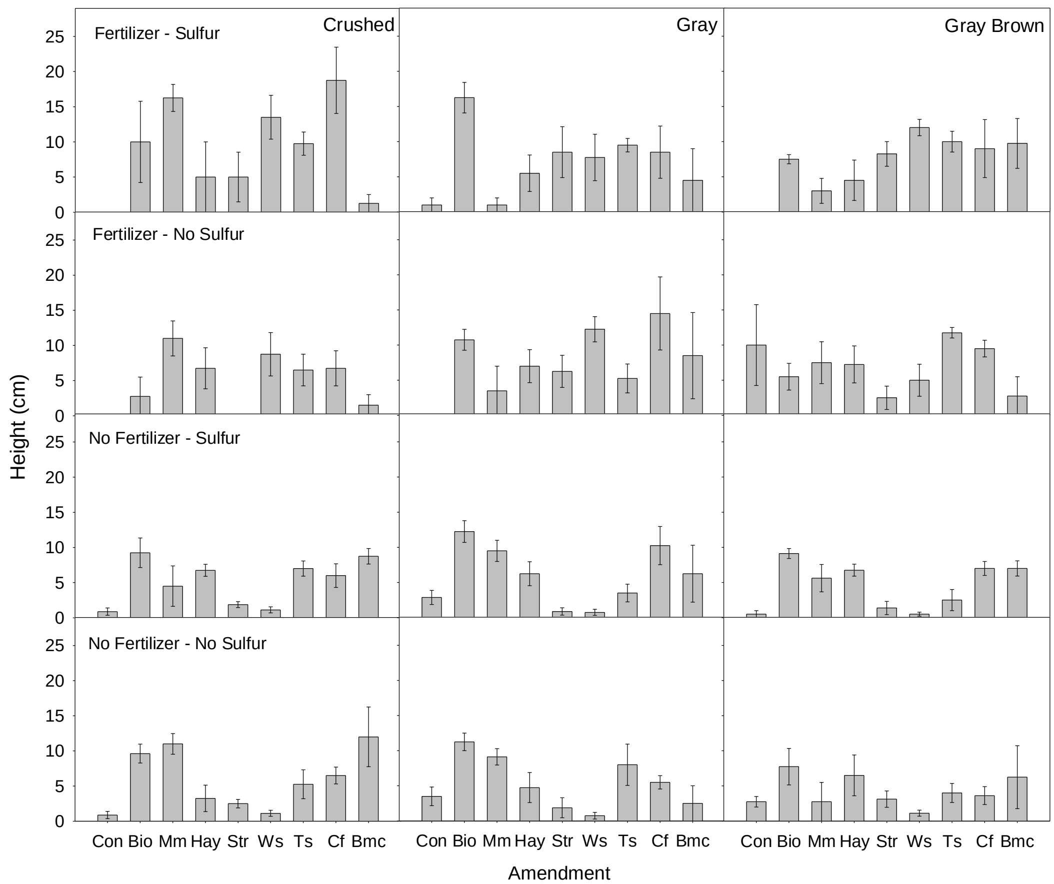

Figure A-20. Height of *Trisetum spicatum* in crushed (left column), gray (center column) and gray brown (right column) limestone substrates. Fertilizer and sulfur treatments are in rows from the graph top to bottom. Amendments are control (Con), biosolids (Bio), manure mix (Mm), Hay, straw (Str), wood shavings (Ws), topsoil (Ts), clean fill (Cf) and beef manure compost (Bmc). Error bars are ± standard errors. N = 4.