Preservation of native biological diversity is one of the

greatest challenges facing ecologists this century

- Carla D'Antonio and Laura Meyerson

Every blade of grass has its Angel that bends

over it and whispers, "Grow, grow."

- The Talmud

Protecting the health of our environment is directly related to our

understanding of the roles of its complex fungal populations

- Paul Stamets

University of Alberta

Non-Native Plant Management And Restoration Of Foothills Fescue Grassland In Waterton Lakes National Park, Alberta

by

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ABSTRACT

Non-native plants are a formidable barrier to native grassland restoration. Foothills fescue prairie restoration was investigated at three southern Alberta sites through reduction of non-native plant cover by steaming, herbicide and mowing; by increasing native plant cover with transplanting, seeding and native cultivar seed; and characterizing arbuscular mycorrhizal fungal (AMF) communities important to grassland plants. Plant responses to restoration treatments were assessed over three growing seasons. AMF in research treatments and undisturbed adjacent native grasslands were compared using 454-pyrosequencing data. Non-native grasses declined with herbicide but did not respond to steaming and mowing. Transplanting was more effective than seeding in establishing native cover. Cultivar seed had higher emergence than wild seed, but equal transplanted seedling survival. AMF were sensitive to soil properties and plant diversity but showed resilience to non-native plant invasion. Long term, prioritized application of researched methods and understanding of species and site specific characteristics will benefit restoration.

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CHAPTER 1. INTRODUCTION

1. ECOLOGICAL RESTORATION OF FOOTHILLS FESCUE PRAIRIE

1.1 Conservation Status And Value Of Foothills Fescue Prairie

Grasslands are among the most endangered ecosystems in North America (Gibson 2009). Foothills fescue grasslands are native to North America and found in southwestern Alberta. Once occupying approximately 3.8 million ha they have been reduced to 17 % of their former range (Adams et al. 2003). They are defined by Orthic Black Chernozem soils and vegetation dominated by *Festuca campestris* Rybd. (Foothills rough fescue), *Danthonia parryi* Scribn. (Parry oatgrass), *Festuca idahoensis* Elmer (Idaho fescue), *Elymus* spp. and *Agropyron* spp. (Wheat grasses). Prairie in the Alberta Foothills Fescue Subregion has been lost due to human impacts such as fire suppression, bison extirpation and non-native species invasion (Widenmaier and Strong 2010).

Fescue grasslands contribute to landscape biodiversity, providing important habitat for several species at risk in Alberta, including threatened *Anthus spragueii* Audubon (Sprague's pipit) and endangered *Charadrius montanus* Townsend (Mountain plover) and *Athene cunicularia* Molina (Burrowing owl) (Committee on the Status of Endangered Wildlife in Canada 2010). Fescue prairies are valuable habitat for ungulates such as *Cervus elaphus* L. (Elk), *Odocoileus virginianus* Zimmermann (White tailed deer) and *Odocoileus hemionus hemionus* Rafinesque (Rocky Mountain mule deer), and are important for livestock production. Native grasses such as rough fescue produce stiff upright culms that are accessible to foraging animals in deep snow, providing important winter food when other grasses are unavailable (Desserud 2006). Research on fescue grassland restoration is essential for conservation of these ecosystems and for Alberta's rare and endangered flora and fauna.

1.2 Waterton Lakes National Park

Waterton Lakes National Park is located in the Rocky Mountains of southwestern Alberta, forming an International Peace Park and United Nations Educational, Scientific and Cultural Organization (UNESCO) World Heritage Site with Glacier National Park. It is approximately 525 km^2 in size and extends southward to the

United States border with Montana and Glacier National Park and westward to the Alberta-British Columbia boundary along the Continental Divide (Achuff et al. 2002). The western boundary borders Akamina-Kishinena Provincial Park in British Columbia. The north and east sides border Alberta crown land and private lands. The northwest corner borders the Blood Indian First Nation Timber Limit in the Belly River area on three sides. Waterton Lakes National Park is the only protected area in Alberta that encompasses four ecoregions: Foothills Parkland, Montane, Subalpine and Alpine. It is the only National Park covering part of the Foothills Parkland Ecoregion.

A total of 971 vascular plant species are found in the park. In 2002, 20 plant species were reported as newly discovered in the Park, including two species new to Alberta and one new to Canada (Achuff et al. 2002). Over 50 % of Alberta's wildflower species are found at Waterton, including 30 rare plant species found nowhere else in Canada.

Most arid areas of the park occur at lowest elevations in the north central region. Grasslands are characterized by Chernozem soils with predominantly calcareous parent materials. Chernozem soils are well drained with dark coloured mineral surface horizons, high in organic matter. The Foothills Parkland Ecoregion is characterized by a warm and dry climate, Aspen Parkland vegetation and a mosaic of grasslands and aspen groves. Other ecosystem types in this ecoregion include sedge fens, wet shrubby meadows and *Populus* spp. (Poplar) forests. Waterton is a safe haven for native species, biodiversity and natural heritage. Although prized for its high biodiversity, land use, historic disturbances and non-native plant species threaten the Park's ecological integrity (Parks Canada 2000). Ecological restoration and non-native plant management are prioritized as a management strategy (Parks Canada 2010).

1.3 Revegetation Practices

Revegetation practices strongly impact restoration success. Revegetation success is highly dependent on seasonal weather. Control of perennial non-natives and precipitation variability are two challenges in grassland restoration. Ideally it is best to plant during wet years and control non-natives during dry years (Bakker et al. 2003). The most optimal planting season depends on

climate, species and logistical limitations. Spring seeding is most advantageous in Aspen Parkland because precipitation in early spring is normally higher than in other months and consistent from May to June. Erichsen-Arychuck (2001) found spring seeding and transplanting resulted in greatest native forb establishment during first and second growing seasons in a grassland restoration study. The ideal season to plant in foothills fescue grassland restoration is species dependent, with an advantage for spring planting (Naeth and Wilkinson 2008).

Revegetation is usually critical for restoring a native plant community. Bakker and Wilson (2004) found only seeded plots had native seedlings in mixed grass prairie restoration in Saskatchewan. Native plant species recruitment was inhibited by competition from non-native species and dispersal limitations that can be overcome by seeding and transplanting with native species. Seeding techniques, timing of seeding and transplanting are known to impact the resulting plant community composition, rate of ground cover and plant community development (Naeth 2000). Although many techniques have been studied, factors affecting native plant species survival are relatively unknown and relationships among species planted and the resulting plant community composition is unclear.

Tyser et al. (1998) found native seed mix design had no significant effect on plant community composition and rapid seeding and establishment of native vegetative cover was necessary after disturbance to prevent invasion and dominance by aggressive non-native plant species. Standard seeding rates were low relative to undisturbed native seed banks and forb seed dormancy characteristics and scarification requirements may have affected germination. Transplanting may improve revegetation success relative to seeding because in the transplant stage, plants have already overcome their most vulnerable stage of growth.

Like other grasslands, foothills fescue grassland restoration is difficult due to a number of formidable challenges that must be overcome through effective reclamation practices and management strategies. *Festuca campestris* and other native perennial grasses take three to five years to establish and the ecology of associated grasses and forbs in these communities is not well known (Johnston and McDonald 1967, Stout et al. 1981, King et al. 1998). Native plant species vary in their ability to establish and survive at a disturbed site. Through

monitoring and research on grassland restoration, native plant species with greater establishment and survival can be identified and targeted for revegetation. These species could then be evaluated for potential to facilitate establishment of more sensitive native species that establish less easily.

Although substantial efforts advanced revegetation practices and establishment of native plant communities, successful ecological restoration of foothills fescue grassland has rarely been documented (Alberta Wilderness Association 2006). Knowledge gaps still exist in timing of planting, whether transplanting or seeding is more successful or what type of seeding or transplanting techniques are most successful. Revegetation practices must be evaluated on a species specific basis (Naeth and Wilkinson 2008). Native plant species with highest rates of establishment must be identified through research and monitoring programs. Revegetation is a critical aspect of any grassland restoration and should not be overlooked in any restoration research program.

The main seed source in National Park restoration is from wild collection, which may limit revegetation success relative to increased competitiveness and survival of native cultivars. Wild collected seed for National Parks restoration must be evaluated for challenges associated with its use and quality and strategies must be developed to improve its success. Little research has been conducted on advantages of using a native cultivar seed mix with wild collected seed to establish a native plant community, prevent monocultures of non-native plants or remove non-native monocultures. In this research, effectiveness of wild collected versus native cultivar seed was investigated to determine if native cultivar seed was advantageous in early stages of restoration to exclude or remove aggressive non-native plant populations from restoration sites and encourage native plant community development in foothills fescue grassland.

2. NON-NATIVE PLANT SPECIES MANAGEMENT

2.1 Overview

Since European colonization in the late 18th Century, over 50,000 non-native species have been introduced to North America; approximately 90 % are benign while about 5,000 have become naturalized or invasive (Morse et al. 1995, Morin

1995). Invasive species are highly variable in traits and ability to be controlled; therefore, guild level research is needed, often on a species basis to design effective control strategies (Kaufmann and Kaufmann 2007). Approximately 1/5 to 1/3 of plant species in North America north of Mexico were introduced from Europe and Asia (Flora of North America Editorial Committee 1993). The non-native species problem is enormous on a global scale and continues to grow. If 10 % of the world's 260,000 vascular plants are good colonizers, then at least 26,000 weedy species may still exist, leaving approximately 20,000 species yet to be introduced. At present, only about 4,000 weedy species have been distributed around the world, leaving the potential for 22,000 additional species to be introduced (Reichard and White 2001).

Preservation of native biological diversity from habitat loss and displacement by invasive plant species is a major challenge of this century (D'Antonio and Meyerson 2002). Invasion by non-native plant species threatens survival of endangered species and ecosystems throughout the world (Vitousek et al. 1997). Plant invasions are expected to increase in some regions due to global climate change based on changes in temperature, precipitation, carbon dioxide, nitrogen deposition and natural and anthropogenic disturbances (Bradley et al. 2010).

Terms to describe non-native plant species are numerous, causing great confusion and debate (Richardson et al. 2000). A non-native plant species and its synonyms (e.g. introduced, non-indigenous, alien, exotic) is considered to be a species not native to its location, which is present outside its natural range due to human mediated dispersal. Naturalized species are considered non-native species that reproduce without human assistance. Invasive species are naturalized species that have spread vastly, developing significant populations outside their natural range that invade undisturbed habitats and displace indigenous flora. Weeds refer to undesired plant species. However, focus should be on classifying rather than labeling non-native species, as populations may behave differently throughout their range (Colautti and MacIssac 2004).

Non-native plants are a major issue in reclamation because of aggressive competition with native plants, ability to proliferate in disturbed environments and plentiful seed sources from urban and agricultural areas (Berger 1993). Human disturbances are a major aid to non-native plant invasions (Bradley et al. 2010).

Many harmful non-native species have persistent seed banks and seeds often live longer in soil stockpiles than native seeds. Abandoned areas often develop persistent, weedy plant communities, especially in enriched former agricultural lands in arid and semi-arid regions (D'Antonio and Meyerson 2002).

Spread of non-native plant species has been facilitated by the reclamation industry because there is greater knowledge of non-native species; they were, and are, cheaper and readily available (Pelech 1997). Knowledge of how to use native plants in reclamation has only become increasingly available through research over the past decade. Vigorous weed growth is a major barrier to restoring native plant communities in North American prairies (Blumenthal et al. 2003). Many native plant species establish slower than aggressive, non-native species, so weed control should assist native species to establish and prevent them being outcompeted by non-natives (Wark et al. 2004). For successful native plant community restoration, non-native perennial species must be controlled (Morgan et al. 1995, Gerling et al. 1996, Wark et al. 2004). The first three to five years of restoration are most critical; once a native prairie community has matured and established, it is less easily invaded (Erichsen-Arychuk 2001). Timing of implementation is critical but for most methods the optimal time for each non-native plant control technique to be implemented is unknown.

Non-native plant species management and management during reclamation, strongly impact reclamation success (Naeth 2000). Several control practices for non-native plant species have been highly effective in reducing their populations (Rice and Toney 1998, Barnes 2004). However, reducing ecological impacts of control measures and effects on endangered plant species recovery is less understood (Tyser et al. 1998). Until the last two decades, most non-native plant control knowledge came from agricultural research (Mars 1984). Although more studies are being conducted, knowledge of how to control non-native plants for conservation and restoration is lacking. Goals of non-native plant management in natural settings, such as species and habitat preservation, maintenance and enhancement of wildlife habitat and plant diversity, are more complex and difficult to obtain than maintenance of monoculture crops in agriculture (Rice et al. 1997).

Three main types of non-native plant management techniques are chemical, mechanical and biological control. Mechanical techniques include hand pulling,

cutting with various types and sizes of equipment and tilling, and is generally labour intensive, time consuming and cheaper than other methods. Hand pulling is ideal for young plants and tap rooted species without extensive root systems (Kaufman and Kaufman 2007). Increasing equipment size such as weed wrenches, root talons and mattocks increases soil disturbance. Current methods to control non-native plant species that were explored in this research include herbicides, prescribed burning, steam and mowing. Burning, grazing, mowing and herbicide application improve fescue grassland restoration and mowing and herbicide application impact plant community composition and decrease the number of non-native invaders in the first few years of reclamation (Naeth 2000). Grazing can be very effective but can disperse seeds and fruit of non-native plant species (Kaufman and Kaufman 2007). Grazing, cutting and burning are human assisted natural disturbances that have maintained grasslands worldwide for centuries and are important for their continued maintenance (Willems 2001).

2.2 Herbicides

Herbicides are typically used as a last resort for species that are very difficult to control or when mechanical control is too damaging (Kaufman and Kaufman 2007). Some species that are repeatedly exposed develop herbicide resistance. Therefore, integrated weed management is necessary for long term applications. Herbicides may be selective or non-selective (broad spectrum), systemic or non-systemic and pre-emergent or post-emergent. Systemic herbicide kills plant tissue by disruption of metabolic processes; non-systemic herbicide inhibits germination and growth. Pre-emergent herbicides kill germinating seed and post-emergent herbicide kills by attacking foliage. Adjuvants, surfactants and fertilizer are commonly added to herbicides to increase uptake.

Glyphosate (N-phosphonomethyl glycine), commonly known as Round Up, is a non-selective, systemic herbicide that controls a large number of annual and perennial non-native plants by inhibiting amino acid synthesis (Tu et al. 2001). It is commonly used in ecological restoration worldwide because it is broad spectrum, low in cost and low in toxicity to most other organisms (Giesy et al. 2000). However, continued or intensive application leaches high concentrations of glyphosate into soil where it readily adsorbs to soil particles. Residual levels in soil may be taken up by plant roots and cause detrimental effects such as tissue

injury, seedling death and decreased root and shoot biomass in plant species used in revegetation after herbicide application, with herbaceous perennial grasses the most sensitive (Cornish and Burgin 2005).

Many herbicides including glyphosate have reduced populations of non-native plant species in revegetation studies in grasslands or grassland understory communities (e.g. Rice et al. 1997, Hitchmough et al. 1994, Wilson and Pärtel 2003, Bakker et al. 2003, Simmons et al. 2007). In tall grass prairie, spring and fall applications of glyphosate, imazapic and clethodim eradicated non-native cool and warm season grasses when combined with revegetation practices (Barnes 2004). For maximum effectiveness, weeds should be eliminated and high quality native seed either drilled into dead or dying sod with a pre-emergent herbicide or the seedbed tilled prior to broadcast seeding, with good seed soil contact. Some native grasses have proven resistant to imazapic and other graminicides (grass specific herbicides) (Hitchmough et al. 1994, Barnes 2004).

Centaurea maculosa L. (Spotted knapweed) was controlled during fescue grassland restoration in Montana with selective systemic herbicides such as clopyralid and picloram which target broadleaf plants. These herbicides reduced *Centaurea maculosa* by over 80 % and increased native grass cover (Rice et al. 1997, Rice and Toney 1998). Control of *Centaurea maculosa* in foothills fescue grassland restoration is more widely known than control of non-native perennial grasses, such as *Elymus repens* (L.) Gould (Quack grass) and *Bromus inermis* Leyss. (Smooth brome). *Agropyron cristatum* L. (Crested wheat grass) in mixed grass prairie in southern Saskatchewan was suppressed (50 %) but not eliminated with glyphosate (Bakker et al. 2003, Wilson and Pärtel 2003). Spraying *Agropyron cristatum* is best in early spring when it has begun growing and C4 native plants are still dormant. Wilson and Gerry (1995) found glyphosate combined with tillage or carbon decreased cover of *Agropyron cristatum* and *Bromus inermis* but increased cover of *Thlaspi arvense* L. (Stinkweed). More information is needed on effectiveness of glyphosate to control annual and perennial non-native forbs and grasses in foothills fescue grassland.

Plant community response to herbicide application during restoration is not well understood as most studies only examine response during the first growing season after treatment. The most effective time to spray is unknown. Some

experimental results showed spring spraying was effective although spraying in late summer or fall may be better when most native plants have completed their life cycles (Rice and Toney 1998). Use of clopyralid late in the season after native forbs enter summer drought induced dormancy decreased impacts on plant community diversity (Rice et al. 1997).

Broad spectrum herbicides such as glyphosate are thought to have negative effects on non-target native plant species and development of the recovering plant community. However, glyphosate application selectively targets and reduces populations of non-native plant species and causes a neutral or positive effect on non-target native species (Brown 1997, Simmons et al. 2007). Release from competition with dominant non-natives by glyphosate application often leads to positive effects on the non-target native plant community such as increased seedling survivorship and native species richness (Bakker et al. 2003). Thus deleterious effects of herbicide are negligible compared to benefits in reduction of the invasive plant population, although effects of glyphosate on the non-target native plant community in invaded foothills fescue grassland are not well known.

2.3 Prescribed Burning

Fire and grazing are natural disturbances that have maintained North American grasslands for the past million years. Fire was used by early Indigenous peoples to increase food production and hunting success and to create travel corridors (Sauer 1950). Fire can be used to restore North American grassland ecosystems by reintroducing the natural disturbance regime, preventing encroachment by woody species and creating habitat for native prairie plants reliant on fire to provide abiotic conditions for growth and to suppress competition by plant species not well adapted to fire. Prescribed burning is defined as a controlled fire used to achieve a management objective and has only been introduced recently as a method for controlling non-native plant species (DiTomaso et al. 2006).

When carefully planned and implemented, burning is an effective tool to control non-native plant species in grasslands. In natural areas, due to the large number of coexisting species, high ecological complexity, patchy variability in fuel structure and unpredictable weather after fire, beneficial effects of fire are often difficult to predict (Pyke et al. 2010). To control undesirable plant species, fires

should be conducted when effects are neutral or beneficial to native plant species and detrimental to non-native plant species. Late spring or early summer burns increase native plant species diversity in grasslands invaded by non-native species (DiTomaso et al. 2006). However, there is a lack of knowledge about effects of burning on non-target native plant populations.

Late spring prescribed burning significantly decreased cover of non-native annual grasses, decreased thatch and increased bare ground, but may increase non-native forbs (Pollak and Kan 1998). Burning is most effective for control of non-native annual species that produce seed late in the growing season (DiTomaso et al. 2006). Effectiveness of burning is dependent on dominant non-native plant species. Annual non-native grasses produce non-dormant seeds with little annual carry over while perennial grasses have more persistent seeds and usually require long term burning for control (Pollak and Kan 1998).

Bromus inermis, *Poa pratensis* L. (Kentucky blue grass) and *Poa compressa* L. (Canada blue grass) have been controlled with repeated burning during the growing season when tiller elongation occurs and native grasses can recolonize. However, limited control has been achieved in northern grasslands with native C3 grasses and in highly infested and disturbed areas where no C4 or C3 native grass is capable of reoccupying the site (Willson and Stubbendieck 1996, DiTomaso et al. 2006). Burning is more successful with heavy thatch, indicating intense burns may be necessary. The best timing for burning non-native perennial *Poa* species is mid to late spring and late spring to early summer for *Bromus inermis*. Some native perennial C3 species are susceptible to burning.

Brown (1997) examined burning, mowing, grazing and glyphosate on *Bromus inermis* and *Poa pratensis* in Alberta foothills fescue grassland. Although burning decreased vigour of *Bromus inermis*, tiller density of *Poa pratensis* significantly increased and *Poa pratensis* replaced *Bromus inermis* in the majority of treatments including burned. It is unclear if burning is useful for foothills fescue grassland or other northern Canadian grasslands with C3 native grasses and forbs and if native species are negatively impacted or benefitted from burning.

Few studies tested burning to control crested wheat grass and quack grass. Fires usually promote non-native perennial forbs, so combining burning with other management techniques is recommended. Fire is ineffective in controlling

Euphorbia esula L. (Leafy spurge), *Linaria dalmatica* L. (Dalmatian toadflax), *Potentilla recta* L. (Sulfur cinquefoil), and *Centaurea maculosa*. *Bromus tectorum* L. (Cheat grass) is difficult to control with prescribed burning because its seed heads shatter and seeds fall to the ground before a sufficient fuel load can build up (DiTomaso et al. 2006). *Bromus tectorum* is fire loving and has increased with increased frequency of wildfires in United States grasslands from about one in twenty years to one in five years (Kaufman and Kaufman 2007).

Non-native perennial grasses are most difficult to control with prescribed burns. Burning must kill perennating structures and suppress resprouting. *Bromus inermis* is extremely difficult to control in natural areas throughout North America. Although harsher and with greater impact on non-target native species, comparative studies suggest herbicides are much more effective in reducing live rhizomes than mowing or burning (Willson and Stubbendieck 1996). However, in some long lived, aggressive stands no technique has proven effective in reducing *Bromus inermis* enough for native grass seedlings to grow. A reduction in live rhizomes of over 80 % is considered necessary to lower *Bromus inermis* competition enough for native grass establishment but this figure has not yet been achieved with any control technique after only one growing season.

Although research has shown dormant season fire to be less successful than herbicide, prescribed burning during the growing season may be more effective than herbicide and other techniques. Simmons et al. (2007) found prescribed burning during the growing season in Texas prairie lead to a greater reduction in canopy cover of non-native Eurasian C4 grass *Bothriochloa ischaemum* (L.) Keng (Yellow bluestem) than repeated glyphosate applications. Fire had a neutral effect on redeveloping a non-target native plant community. Most native species of North American grasslands are likely adapted to growing season fire and may be more adapted than non-native Eurasian species. Further research is needed to determine if growing season fire is more beneficial than dormant season fire at controlling non-native plant species in grasslands.

Fire to control non-native plants in North American grassland has been researched widely. Control of *Bromus inermis* with prescribed burning met with mixed success and needs further research to evaluate effectiveness on this problem species in Canadian fescue prairie. Burning to control *Bromus inermis*,

Elymus repens, *Agropyron cristatum* and *Centaurea maculosa* and other problem species in invaded foothills fescue grassland has never been researched and an assessment is needed on effectiveness of burning to control these species and subsequent native fescue grassland recovery. Few studies investigated the impact of prescribed burning on the non-target native plant community and information is needed on response of fescue prairie plant communities. Prescribed burning investigation was planned for this project, but could not be implemented due to logistical constraints.

2.4 Steam

Steam is a thermal weed control where high pressure, high temperature hot water steam is finely sprayed close to the ground with a pressurized boiler to kill vegetation and seeds (Merfield et al. 2009). Steam can be sprayed to sterilize soil and kill weed seeds or to kill live stands of vegetation. It is typically practiced and almost exclusively researched as an agricultural method of weed control for organic farming (a non-chemical alternative to herbicide). It has proven effective to control annual weeds in agricultural crops (Sirvydas et al. 2004).

Steam exposes vegetation to high temperatures similar to burning but does not remove thatch or litter; therefore, the effects of steaming should be similar to short term effects of burning. Very little research has examined application of steam in weed control of natural areas or in ecological restoration. Effects of steam on vegetation are usually delayed by one growing season but it is effective after an initial lag period (Naeth 2010). The value of this technique for control of non-native plants during ecological restoration needs to be researched.

2.5 Mowing

Mowing or cutting can control non-native plants in natural areas across large or small patches and prevent seed production (Kaufman and Kaufman 2007). Cutting may encourage some woody invasive plants to sucker. If stands that have produced seed are cut, the cut material must be removed or seeds may germinate. Mowing can control annual weeds, which are often the first plants to colonize a disturbed site and usually dominate the first growing seasons (Gerling et al. 1996). Mowing is often recommended when herbicide cannot be used, usually when benefits of herbicide are limited due to growth stages of non-native

and native plant species (Wark et al. 2004). Herbicide delays plant community development more than mowing because it may have residual effects.

On uncompacted sites and at early stages of reclamation, mowing can increase litter to ground contact, improve biocycling and increasing tillering in many grass species (Pelech 1997). Mowing provides a selective advantage to species that can tolerate defoliation and can eliminate some species by breaking their life cycle. Mowing must be timed so annuals are mowed prior to seed maturation and perennials are mowed before they replenish carbohydrate stores (Pelech 1997, Pitchford 2000). Mowing vegetation during reclamation can be expensive and often impractical so timing of mowing to twice per growing season is necessary. However, while mowing may be beneficial, reduction in plant competition by mowing has not led to increased germination and establishment of seeded and transplanted native species (Pelech 1997, Erichsen-Arychuk 2001). Mowing during reclamation has lead to increased bare ground, decreased litter with no effect on tillering in non-native grass species (Pelech 1997).

Pitchford (2000) found seeded native grasses in Alberta Aspen Parkland were most abundant in mowed areas in the first growing season; moss and vegetation cover were significantly greater for two growing seasons but no other differences lasted after the first growing season. In California coastal prairie degraded by dominant non-native annual C3 grasses and nitrogen enrichment by a non-native shrub, mowing doubled forb and slightly increased native grass species richness in the first growing season (Maron and Jeffries 2001). Over the five year study there was no significant decrease in soil nitrogen so benefits of mowing were likely due to improvement of forb germination conditions and removal of the non-native seed source. Simmons et al. (2007) found mowing caused no significant decrease in cover of non-native plants after two growing seasons at two Texas prairie sites heavily dominated by a non-native perennial C4 grass.

Beneficial effects were not apparent for at least two to three years when western Oregon prairie sites were mowed to control non-native perennial grasses; mowing decreased the dominant *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl and C. Presl (Tall oat grass) and increased cover of native grass species (although other non-native perennial grasses increased) (Wilson and Clark 2001). Long term monitoring is needed to assess effectiveness of problem

species control during reclamation. On ecological restoration of species rich, calcareous grasslands in the Netherlands long term (> seven years) reintroduction of mowing and grazing restored pre-disturbance species diversity when accompanied by seed rain from surrounding intact native grasslands (Willems 2001). Mowing was successful because it lowered soil fertility in nitrogen enriched, degraded grassland being restored by removing above ground biomass containing nutrients that would have been returned to the soil. Mowing did not always decrease soil nitrogen pools (Maron et al. 2001).

In tall grass prairie, mowing, grazing and burning enhanced growth and seed production of many native species; however, it is unknown if native plants exhibit this behavior in other Canadian prairies, including fescue prairie (Morgan et al. 1995). Fire and mowing are strongly recommended as follow up to herbicide application to remove above ground vegetation. Techniques are typically performed prior to seeding and considered a type of site preparation in reclamation (Morgan et al. 1995, Gerling et al. 1996). Mowing may benefit native grassland communities by removing excess litter, allowing more sunlight to reach seedlings and encouraging growth (Wark et al. 2004). Mowing can consistently reduce C3 perennial grass cover from year to year relative to herbicide because herbicide applications are often patchier (Wilson and Pärtel 2003).

It is not well understood how long it takes to achieve beneficial effects of mowing, the best time for mowing, frequency of mowing and for what plant communities and target non-native plant species mowing is most effective. No studies have investigated whether mowing can be used to control cool season C3 perennial non-native grasses *Poa compressa*, *Poa pratensis*, *Bromus tectorum*, *Bromus inermis*, *Phleum pratense* L. (Common Timothy), *Agropyron cristatum* and non-native forbs *Centaurea maculosa* and *Thlaspi arvense* in restoration of foothills fescue grassland.

2.6 Other Techniques And Integrated Approaches

Soil impoverishment and substrate removal are potential strategies, but neither has proven successful (Naeth 2000). Soil nitrogen enrichment is often responsible for promoting proliferation of non-native plant species at disturbed sites (Wilson and Gerry 1995). Addition of soil carbon to immobilize nitrogen may

help address this issue (Wark et al. 2004). In tall grass prairie, Blumenthal et al. (2003) found addition of 3346 g of carbon per m^2 decreased nitrogen availability by 86 %, non-native biomass by 54 % and increased prairie species biomass seven fold. However, this method may only be successful in ecosystems being reclaimed if nitrophillic non-natives are present that outcompete native species and the immobilizing effect of carbon can be sustained for a long enough period. Effects of carbon addition were species dependent, with C3 native plants requiring less carbon than C4 for beneficial effects.

Ecological restoration can reduce non-native plants through revegetation with native species. Bakker and Wilson (2004) found seeding native C3 grasses reduced invasion of *Agropyron cristatum* by 1/3 in the first five years of restoration in old fields in Saskatchewan (Grasslands National Park). *Agropyron cristatum* cover decreased with increasing planted native grass cover. At the end of the experiment, 28 % of planted plots were invaded by *Agropyron cristatum* compared to 42 % of unplanted plots, with its cover the same in restored and unrestored plots. Choice of plant species in restoration is important, selection of native plant species with similar functional group to non-native plants will improve competitive ability of the native plant community (Fargione et al. 2003).

Restoration is potentially less damaging and more ecologically sensitive than other techniques for controlling non-native plants. Restoration is effective in grasslands when native perennial grasses are adapted to fire and invaders are intolerant of fire (Berger 1993). Use of short lived sterile hybrids such as *Triticum* L. (Wheat) or commercial grasses in seeding treatments may be beneficial as a cover crop to keep non-native grasses out and promote establishment of native species. Nurse grasses may facilitate soil stabilization on sloped substrates and permit establishment of slower growing native species (Tyser et al. 1998). However, additional study is needed. Restoration of ecological processes, fire regime, hydroperiod, photoperiod, thermoperiod, edaphic conditions or other ecosystem characteristics may also control non-native species (Berger 1993).

Biological control, introduction of organisms in an invaded ecosystem that act as pathogens, parasites or predators in the native range of the invading species, is mainly used in rangelands and agriculture and seldom in ecological restoration (D'Antonio and Meyerson 2002). This approach is relatively inexpensive and

biocontrol agents spread naturally and usually have little environmental impact. Effectiveness is usually limited to reducing or keeping populations in check but biocontrol agents are sometimes able to eliminate entire populations. Beetles of the genus *Chrysolina* are biological control agents for *Hypericum perforatum* L. (St. John's wort) and are capable of eradicating stands of this species in some Canadian ecosystems (Parks Canada 2000).

Integrated approaches combine more than one method for control of non-native plant species in degraded ecosystems. Several integrated approaches have proven more successful than isolated control techniques. Burning or cutting followed by herbicide improved efficacy of herbicides for a number of species and herbicide types including perennials (e.g. *Bromus inermis*), *Bromus tectorum* and pre-emergence herbicides, glyphosate and imazapic (Brown 1997, Washburn et al. 2000, DiTomaso et al. 2006). Burning prior to herbicide application combined with a surfactant increased revegetation success in southern tall grass prairie (Barnes 2004). Many species are better controlled by burning and cutting when pre and post treated with herbicides as this increases fuel load and decreases dependence on herbicides. Single control types often target one non-native species and promote expansion of others. Integrated approaches can address this by being designed to target more than one species.

Wilson and Gerry (1995) showed native seedlings did not establish with carbon addition and tillage unless glyphosate was also applied in a mixed grass prairie restoration in Saskatchewan. Wilson and Pärtel (2003) significantly reduced *Agropyron cristatum* cover from over 80 to 5 % and increased native species richness and cover of *Bouteloua gracilis* (Kunth) Lag. ex Griffiths (Blue grama) in an *Agropyron cristatum* monoculture by combining seeding with native species, glyphosate and cutting. Isolated treatments were significantly less successful. Combined herbicide and grazing or mowing decreased native forb density relative to herbicide alone in Alberta foothills fescue grassland (Brown 1997). If combined use results in intensive disturbance beneficial effects may be reversed.

Various methods for dealing with non-native species and their success is poorly understood relative to the issue of non-native species. The issue has been considered unavoidable because of globalization, which can lead to eventual global homogenization of species across ecosystems (Simberloff 2003). If

management and action is taken, invasive species may be successfully barred from an ecosystem, eradicated from an ecosystem they previously existed in or kept at low insubstantial numbers (D'Antonio and Meyerson 2002). Strategies must be developed to reduce the competitive advantage of these species to make fescue prairie restoration possible (Tyser et al. 1998). Research to date has illustrated the complexity and challenges associated with vegetation management in disturbed sites in protected areas. Restoration of full native fescue prairie is not easily attainable and may not be practically possible. Therefore, goals for restoring fescue prairie should be conservative.

The relative efficacy of various techniques used to control invasive plants is highly variable and depends on ecosystem type and the non-native plant species being controlled. Studies have shown herbicide is more effective than other techniques such as burning or mowing (e.g. Willson and Stubbendieck 1996), but other studies have shown burning and mowing more successful than herbicide (Simmons et al. 2007). In this project, herbicide, steam, mowing and prescribed burning were assessed for effectiveness in controlling target non-native C3 perennial and annual grasses and forbs in disturbed foothills fescue grassland dominated by non-native species.

3. ARBUSCULAR MYCORRHIZAL FUNGI

3.1 Definition Of Arbuscular Mycorrhizal Fungi

Grassland ecosystems are home to many groups of microorganisms that play an important role in shaping population ecology and physiology of grassland plant communities (Gibson 2009). Two main groups of fungi form mutualistic symbioses in grasslands, above ground fungal endophytes and mycorrhizal fungi, which occur in the rhizosphere (soil zone where roots are located). A suite of other microorganisms in the rhizosphere and phyllosphere (total above ground surfaces of a plant as habitat for microorganisms) interact with and may significantly affect grassland flora including non-mycorrhizal fungi, soil bacteria and viruses. A mycorrhiza (plural: mycorrhizae) is the association of a plant root with colonizing soil fungi involving exchange of carbon from the plant with soil nutrients, primarily phosphorous, from the fungi (Kendrick 2000). A broad range of host plant species (majority of the plant kingdom) and fungi from the phyla

Glomeromycota, Basidiomycota and Ascomycota form mycorrhizae (Smith and Read 2008). Mycorrhizal symbiosis is often referred to as one of the most common and ancient symbioses on earth and is thought to have facilitated colonization of land by early terrestrial plants.

Several different forms of mycorrhizae are classified based on host plant species, fungal species involved, structures formed and nature of the symbiotic relationship. Arbuscular mycorrhizal (AM) symbiosis is the most common type of mycorrhizal symbiosis and is formed exclusively with all fungi of the phylum Glomeromycota. Fungi belonging to the Glomeromycota are commonly referred to as arbuscular mycorrhizal fungi (AM fungi or AMF). Like other soil fungi, they produce a vegetative growth structure called a mycelium (plural: mycelia) that forms a vast network throughout the soil that may connect host plant species via roots and facilitate nutrient and metabolite transfer among plants and other soil dwelling organisms (Kendrick 2000). AM fungi are obligate symbionts, meaning they cannot survive without carbon provided by their plant hosts. Thus AM fungi cannot be cultured using traditional methods for other microorganisms and must be grown in pot or root cultures with their plant hosts.

Approximately 80 % of vascular species participate in AM symbiosis, mostly grasses and forbs (Smith and Read 2008). Plant species forming arbuscular mycorrhizae symbiosis are so numerous it is easier to list families that do not (Gerdemann 1968, Smith and Read 2008). Flora involved, representing over 80 % of the plant kingdom, include members of most families of angiosperms and gymnosperms. Glomeromycotan fungi have been identified associating with sporophytes of ferns and lycopods and free living gametophytes of pteridophytes and some hepatics (liverworts), indicating AM symbiosis is present regardless of whether roots or other structures are present or photosynthetic abilities of host species. Although the range of plant hosts is extremely broad, some plant families characteristically do not form mycorrhizae including *Chenopodiaceae*, *Brassicaceae*, *Caryophyllaceae*, *Polygonaceae*, *Juncaceae* and *Proteaceae*, although some fungal colonization has been observed in some species.

An AM fungal mycelium is composed of long (> 1 m), branching, filamentous cells called hyphae (singular: hypha) that colonize roots. Unlike other fungi, AM fungi have broad coenocytic (aseptate) hyphae, meaning their hyphae are wide (> 1

µm) and lack hyphal cell walls (septa) (Kendrick 2000). AM fungal hyphae colonize roots and form structures called vesicles and arbuscles, from which their name is derived. Vesicles are energy storage structures densely filled with lipids produced within and outside the plant root at the end of hyphal tips (Peterson et al. 2004). Arbuscules are very finely branched hyphae (similar to haustoria) in root cortical cells, functioning as carbon and nutrient exchange sites in the root. There are over 200 known species of fungi in Glomeromycota (Schüßler 2010).

In grasslands, AM symbiosis is the most predominant type of mycorrhizal symbiosis as the other types of mycorrhizae typically involve non-herbaceous or woody plant species. Mycorrhizal fungi benefit host plant species by increasing nutrient uptake (primarily phosphorous). AM fungi improve drought tolerance, protect plant hosts from root pathogens and toxic stresses and increase nutrient uptake, resulting in increased productivity (Jeffries et al. 2003). AM fungi are major contributors to plant health and soil fertility and may increase reproduction and offspring survival in some plant hosts at early successional stages (Koide and Dickie 2002). They may negatively impact plant hosts on a parasitism-mutualism continuum, behaving parasitically in less frequent cases when net cost of symbiosis to the plant exceeds net benefit (Johnson et al. 1997).

3.2 Disturbance, Ecological Succession And Arbuscular Mycorrhizal Fungi

AM fungi are sensitive to soil disturbance. Magnitude of disturbance effects varies with ecosystem, being less severe with higher numbers of fungal spores and grass species (Jasper et al. 1989, Jasper et al. 1991). Changes to spore density and mycelia may be less permanent and, therefore, less important than changes in fungal species composition (Richter et al. 2002). The order *Glomerales* may be more susceptible to disturbance than fungi from the family *Gigasporaceae*. Species from *Gigasporaceae* colonize roots primarily by germinating spores whereas *Glomerales* colonize roots primarily from mycelia which are more easily damaged (Hart and Reader 2004). Thus *Glomerales* are more abundant in untilled soil because there are more intact mycelia (Schalamuk and Cabello 2010). Disturbance alters species composition of AM fungi and increases populations of disturbance resistant, abundantly sporulating, fungal species and eliminates species that are more sensitive or sporulate less abundantly (Hamel et al. 1994, Hetrick and Bloom 1983). Disturbance changes

plant and fungal communities which may affect plant-fungal relationships and recovery of disturbed grasslands (Stover 2010, Stover et al. 2012).

Secondary succession occurs in Glomeromycotan fungal communities and is thought to proceed parallel with plant community succession and share several characteristics (Smith and Read 2008). AM fungal communities at disturbed sites move towards original communities over time (Hamel et al. 1994, Li et al. 2007, Sýkorová et al. 2007). At early succession, plant communities are dominated by annual species from *Brassicaceae*, *Chenopodiaceae* and *Polygonaceae* which are mostly non-mycorrhizal. Mycorrhizae may be more beneficial in late successional environments than in early successional disturbed environments dominated by non-mycorrhizal annual plant species (Gibson 2009). As succession proceeds and nutrient availability decreases, competitive advantages should accrue to mycorrhizal dependent plants (Smith and Read 2008).

3.3 Influence Of Arbuscular Mycorrhizal Symbiosis On Plant Communities

Species diversity of arbuscular mycorrhizal fungi is a major factor contributing to plant biodiversity and productivity (van der Heijden et al. 1998, Vogelsang et al. 2006). It differs with plant community composition and vice versa (Stover 2010). AM fungi distribution is dependent on abiotic soil conditions such as pH, organic matter and nutrients and on disturbance and land use (Klironomos et al. 1993, Egerton-Warburton et al. 2007, Su and Guo 2007, Zachow et al. 2009, Dumbrell et al. 2010). Thus fungal species forming AM symbiosis in plant communities varies and may affect host plants differently depending on AM species present.

Mycorrhizal relationships are an important biological factor in healthy prairie plant communities. Knowledge of mycorrhizal symbiosis in recovering grasslands should improve conservation of protected areas (Stover 2010). Important ecological relationships between plants and AM fungi and benefits to individual plants suggests AM fungi play an important role in recovery of disturbed foothills fescue communities. AM fungi are possibly important in explaining slow recovery of native plant communities following disturbance and failed reestablishment of native plant species. Ecological restoration of prairies may be improved by AM fungi inoculum, a source of AM fungal spores and hyphae that can be applied to soils (Smith et al. 1998, White et al. 2008).

A plant-host specificity may exist in AM symbiosis where species of AM fungi are preferentially associated with plant species. Host specificity may affect distribution of plants and AM fungi, explaining differences among ecosystems and disturbance regimes (Bever et al. 1996, Santos-González et al. 2007). Whether plant-host specificity exists in AM symbiosis is critical in determining if AM fungi are important to vulnerable plant species and restoration of native plant communities. AM fungal species vary widely in effects on host plant species (Vogelsang et al. 2006). Diversity and species composition of AM fungi colonizing plant species can vary considerably, even within the same plant families (Vandenkoornhuyse et al. 2003, Santos-González et al. 2007). Plant-host specificity may not exist due to the small number of known fungal species compared to the vast number of plant species (Smith and Read 2008). However, diversity of Glomeromycotan fungi may be highly underestimated based on current species concepts and methods to study fungal diversity (Sanders 2004).

Plant species and functional groups differ in affinity to AM symbiosis. Wilson and Hartnett (1998) found annual grasses had low response to mycorrhizal infection. C3 forbs are considered highly dependent on mycorrhizae and C3 grasses highly independent. Hetrick et al. (1988) found *Liatris aspera* Michx. (Tall blazing star) over 90 % dependent on mycorrhizae and *Bromus inermis* 43 % dependent. C3 grasses may have lower affinity as their roots are better adapted to phosphorous acquisition and they respond less to increased tissue phosphorus (Gibson 2009). In tall grass prairie with low phosphorus, dominant C4 grasses *Andropogon gerardii* Vitman (Big bluestem) and *Sorghastrum nutans* (L.) Nash (Indian grass) had a competitive advantage over dominant C3 grasses *Elymus canadensis* L. (Canada wild rye) and *Koeleria macrantha* (Ledeb.) Schult. (June grass) due to greater affinity and beneficial mycorrhizal associations; mycorrhizae suppression lead to increased species diversity and plant community composition change due to release from competition with highly mycorrhizal species (Hartnett et al. 1994, Hetrick et al. 1994, Hartnett and Wilson 1999, Smith et al. 1999).

Although much knowledge has been gained and considerable research done on arbuscular mycorrhizae, knowledge gaps exist in understanding symbiosis and its implications for ecological restoration. Influence of mycorrhizae on competitive relationships among C4 and C3 plants has been researched, but less is known

about mycorrhizal influences on competition between non-native and native plant species. Through characterization of AM fungi colonizing roots of non-native and native plant species in this study, whether non-native perennial grasses are more dependent on arbuscular mycorrhizae than native species and if specific fungal species are found with native and non-native species was addressed, to determine if plant-host specificity may be important to grassland recovery. The role of arbuscular mycorrhizae and its significance in succession is not well known. Study of arbuscular mycorrhizae was included because a holistic approach is important in ecological restoration and should consider the below ground microbial community. Fungi and other microorganisms are understudied relative to vegetation. Understanding the impact of disturbance on receptors other than the target native plant community may provide insights for restoration.

4. RESEARCH OBJECTIVES AND HYPOTHESES

4.1 Objectives

4.1.1 General

The general research objective was to identify ecologically and economically effective strategies to restore native plant communities in foothills fescue grasslands. Specifically to determine the following.

- Effectiveness of herbicide, steam and mowing at reducing non-native plant species populations and impact on non-target native plant species.
- Effective revegetation methods to improve native plant species survival and competition with non-native plant species.
- How arbuscular mycorrhizal fungi are affected by disturbance and characteristics of mycorrhizal fungal communities in disturbed and undisturbed fescue grassland.

4.1.2 Revegetation

The objective for the revegetation study was to contribute information that can be used to determine the most effective revegetation methods to restore native foothills grassland. Specifically to determine the following.

- What native plant species readily establish in disturbed conditions from broadcast seeding and should be targeted to establish a stable and healthy

early successional plant community to facilitate fescue grassland restoration.

- Whether broadcast seeding or transplanting is most effective for establishing native plant cover and the most effective plant material type for revegetation following non-native plant control.
- If native cultivar seed improves native plant species establishment relative to wild collected native plant seed or transplanted seedlings.

4.1.3 Non-Native Plant Management

The objective for the non-native plant species control study was to determine the most effective method for controlling non-native plant species during restoration of foothills fescue grassland. Specifically to determine the following.

- Relative effectiveness of herbicide, steam and mowing on decreasing cover of non-native plant species.
- Effectiveness of treatments over time and repeated application.
- Impact of treatments on the developing plant community and cover of non-target native plant species.

4.1.4 Arbuscular Mycorrhizal Fungi

The general objective for this research was to provide baseline information about arbuscular mycorrhizal fungi to assist restoration of foothills fescue grasslands. The specific objective was to determine community structure, diversity and species composition of arbuscular mycorrhizal fungi in disturbed and undisturbed foothills fescue grassland.

4.2 Hypotheses

4.2.1 Revegetation

Seasonal weather patterns and other environmental influences will have a major impact on revegetation. The following hypotheses were developed.

- Revegetation success will be highly plant species dependent due to ecological and life history differences among plant species and floral functional groups.
- Transplanting will be more successful than broadcast seeding at increasing native cover due to facilitation of germination and early establishment prior to planting in transplanted species.
- Native cultivar seed will be more successful than wild collected seed at

establishing native plant species due to greater germination rates and survivability of native cultivar seed.

4.2.2 Non-Native Plant Management

Logistics for implementing non-native plant control treatments will strongly influence effectiveness of each specific technique. The following hypotheses were developed.

- Herbicide will be the most effective control treatment by causing the largest decrease in cover of non-native species due to lethality of herbicide and ability to infiltrate below ground root systems.
- Herbicide will be most damaging to non-target native plants as native species are more adapted to mowing and steam causes less damage to roots.
- Steam will stress vegetation and weaken plants but not kill and will be limited in control ability.
- Mowing will be least damaging to the non-target native plant community and more successful at controlling annual species such as *Bromus tectorum*. Mowing will decrease vegetation height and number of non-native and native species in flower due to shoot reduction.

4.2.3 Arbuscular Mycorrhizal Fungi

The following hypotheses were developed.

- Community structure, diversity and species composition of arbuscular mycorrhizal fungi will differ between restoration sites and undisturbed fescue grasslands due to host plant species, soil conditions and disturbance effects.
- Generalist endomycorrhizal fungal species will be present in disturbed and undisturbed environments but species will be absent from disturbed sites and present in undisturbed sites because disturbance and altered plant species composition has led to decline of these fungi in disturbed environments.
- Secondary ecological succession will occur in fungal communities and communities recovering for long periods of time are expected to more closely resemble fungal communities in undisturbed fescue grassland.
- Fungal species richness and diversity will be lower in disturbed sites, than in undisturbed sites, especially where monocultures of invasive non-native plant species are present.

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CHAPTER 2. STEAM, HERBICIDE AND MOWING FOR NON-NATIVE PLANT CONTROL IN FOOTHILLS FESCUE GRASSLAND RESTORATION

1. INTRODUCTION

Native grasslands are globally diverse, highly endangered ecosystems (Gibson 2009). In central Canada, approximately 70 % of native grasslands and 83 % of foothills fescue grasslands have been lost due to agriculture, human disturbance, woody species encroachment, fire suppression, plains bison extirpation and invasion by non-native species (Levesque 2000, Adams et al. 2003, Government of Canada 2010). Thus ecological restoration and maintenance of remaining grasslands is a primary focus. Approximately 20-27 % of the 5,800 vascular plant species found in Canada are non-native (Haber 2002). Non-native plants are a major issue in ecological restoration because of their aggressive competition with native plants, ability to proliferate in disturbed environments, plentiful seed source from urban and agricultural areas and persistent seed banks (Berger 1993, D'Antonio and Meyerson 2002).

Restoration sites are often patchy, with areas of non-native species to eradicate, interspersed with native species to retain (Naeth 2013). Once non-native species are removed, areas need to be repopulated with native species and further invasions of non-native species controlled. Various effective methods to control non-native species are not appropriate in restoration as they can negatively affect the native species to be retained or re-introduced to the site (Naeth 2000). Thus methods like mowing, herbicides and steaming, with low effect on native species, yet high effect for control of non-native species (Naeth 2000), must be assessed.

Mowing can control annual weeds (Wark et al. 2004), increase litter to ground contact, improve biocycling and increase tillering in some grasses (Pelech 1997). It provides a selective advantage to species that tolerate defoliation and can eliminate some species by breaking their life cycle. Mowing can restore nitrogen enriched grasslands invaded by non-native species by removing biomass (Willems 2001) and can more consistently reduce C3 non-native perennial grasses relative to patchier herbicide applications (Wilson and Pärtel 2003). Mowing is often ineffective for perennial species with extensive rhizomes and

root systems and can increase abundance of some non-native species such as *Cirsium arvense* (L.) Scop. (Canada thistle) (Cole et al. 2007, Simmons et al. 2007, Grekul and Bork 2007) and *Chrysanthemum leucanthemum* L. (Ox eye daisy) by removing established vegetation canopies and decreasing competition (Cole et al. 1999). Annuals should be mowed prior to seed maturation and perennials before they replenish carbohydrate stores (Pelech 1997, Pitchford 2000). Mowing can require at least two years of repeated applications to reduce non-native perennials and increase native species (Maron and Jeffries 2001, Willems 2001, Wilson and Clark 2001). Reduction of plant competition by mowing does not always increase germination and establishment of transplanted and seeded native species (Pelech 1997, Erichsen-Arychuk 2001) and effects may be temporary (Pitchford 2000). Mowing is often recommended when herbicide cannot be used, such as when benefits are limited due to growth stages of non-native and native plant species in the managed ecosystem (Wark et al. 2004).

Herbicides are typically used in restoration for difficult to control species or when mechanical control is too damaging (Kaufman and Kaufman 2007). Glyphosate (N-phosphonomethyl glycine) is a non-selective, systemic herbicide that controls plants by inhibiting amino acid synthesis (Tu et al. 2001). It is used in ecological restoration worldwide because it is broad spectrum, low in cost and low in toxicity to most other organisms (Giesy et al. 2000). Release from competition with non-native species by glyphosate often leads to positive effects such as increased seedling survival and native species richness (Bakker et al. 2003). Glyphosate reduced non-native plants in grasslands and understories (Rice et al. 1997, Hitchmough et al. 1994, Wilson and Pärtel 2003, Bakker et al. 2003, Simmons et al. 2007). In tall grass prairie, spring and fall applications of glyphosate, imazapic and clethodim combined with revegetation eradicated non-native cool and warm season grasses (Barnes 2004). Herbicide controlled *Centaurea maculosa* L. (Spotted knapweed) in foothills fescue grassland, and in Montana fescue grassland reduced it by over 80 % and increased native grass cover (Rice et al. 1997, Rice and Toney 1998). *Agropyron cristatum* L. (Crested wheat grass) in Saskatchewan mixed grass prairie was reduced by 50 % (Bakker et al. 2003, Wilson and Pärtel 2003). Glyphosate with tillage or carbon addition decreased *Agropyron cristatum* and *Bromus inermis* Leyss. (Smooth brome) cover but increased *Thlaspi arvense* L. (Stinkweed) cover (Wilson and Gerry 1995).

Residual herbicide in soil may cause tissue injury, seedling death and decreased root and shoot biomass in native plants (Cornish and Burgin 2005) and some species can develop resistance with repeated exposure. Herbicides can impact non-target organisms such as native plants and contaminate soil and water.

High pressure, hot water steam can be finely sprayed close to the ground surface with a pressurized steam boiler to kill unwanted vegetation or seeds (Merfield et al. 2009). Steam has been used in organic agriculture (Melander et al. 2005), can control weeds in crops (Sirvydas et al. 2004), horticulture and landscaping (Belker 1990, Labowsky 1990, Randall and Marinelli 1996) and reduce aggressive understory vegetation more than prescribed burning and soil removal in forests (Norberg et al. 1997, Zackrisson et al. 1997). Annual plants can be killed after a few applications; well established perennials require repeated applications, as steaming only damages or kills above ground structures (Kristoffersen et al. 2008, Wei et al. 2010). Deeply rooted plants will survive unless high temperatures reach their roots. Various application methods are used and additives, such as surfactant foam, can improve steam effectiveness by reducing heat loss (Norberg et al. 1997). Plant morphology impacts steam effectiveness; small plants with few or thin leaves and unprotected meristems further from the ground are most vulnerable (Leon and Ferreira 2008). Major limitations to steaming are time, energy and costs, which can be 14 times that of conventional methods (Norberg et al. 1997).

More information is needed on effectiveness of non-native plant management techniques to restore degraded foothills fescue grassland and other native grassland ecosystems. Some research has shown spring herbicide spraying gives good results, but spraying in late summer or fall may be better when most native plants have completed their life cycles (Rice et al. 1997, Rice and Toney 1998). Plant traits and controls are highly variable; thus information on specific non-native plant species or functional groups is required (Kaufmann and Kaufmann 2007). Reducing ecological impacts of control measures and effects on endangered plant species recovery are not well understood (Tyser et al. 1998). Plant community response to herbicide during restoration is unclear as many studies only assess response the first growing season after treatment. In tall grass prairie, mowing enhanced growth and seed production of many native

species; however, it is unknown if native species respond this way in other prairie types, including fescue prairie (Morgan et al. 1995). It is not well understood how long it takes to achieve beneficial effects of mowing, the best time and frequency for mowing and for which plant communities and non-native plant species mowing is effective. Steaming has not been studied in natural ecosystems for non-native plant control during ecological restoration.

2. RESEARCH OBJECTIVES

The objectives of this research were to determine whether steam, herbicide and mowing were effective in reducing or eliminating non-native forbs and grasses. The impacts of these treatments were also assessed on non-target native plant species and the recovering native plant communities.

3. MATERIALS AND METHODS

3.1 Research Location And Study Sites

This research was conducted in Waterton Lakes National Park at three disturbed foothills fescue grassland sites undergoing restoration (Figure 2-1). Waterton Lakes National Park is located in the Rocky Mountains of southwestern Alberta forming an International Peace Park and United Nations Educational, Scientific and Cultural Organization (UNESCO) World Heritage Site with Glacier National Park. It covers approximately 525 km^2, extending south to the Montana border and Glacier National Park and west to the Alberta-British Columbia border along the Continental Divide (Achuff et al. 2002). The western boundary is Akamina-Kishinena Provincial Park in British Columbia. North and east sides border Alberta crown and private lands. In the northwest corner the park borders the Blood Indian First Nation Timber Limit in the Belly River area on three sides. Four Ecoregions occur in the park: Foothills Parkland, Montane, Subalpine and Alpine.

Grasslands are characterized by Chernozem soils with predominantly calcareous soil parent materials, good drainage and dark coloured mineral surface horizons high in organic matter. Climate in the foothills grasslands is characterized by a short growing season (June to August), cool, wet springs (May to June) and hot, dry late summers (August to September). The region is windy, with maximum

daily gusts of 70 to 90 km h^{-1}. Mean annual precipitation is 807.6 mm and minimum and maximum average temperatures were -1.3 ^{o}C and 10.6 ^{o}C, respectively, for 1990 to 2000 (Environment Canada 2012a, 2012b). Daily weather can change rapidly and unpredictably, typical of mountain regions.

A total of 971 vascular plant species are found in the park. In 2002, 20 plant species were discovered, including two new to Alberta and one new to Canada (Achuff et al. 2002). Over 50 % of Alberta wildflower species are found in the park, including 30 rare plant species found nowhere else in Canada.

A former landfill and two borrow pits were selected for study in a 3 km radius with similar topography and soils (Figure 2-1). The sites contain a large and diverse population of non-native annual and perennial species including *Centaurea maculosa*, *Cirsium arvense*, *Bromus inermis*, *Elymus repens* (L.) Gould (Quack grass), *Poa pratensis* L. (Kentucky blue grass), *Poa compressa* L. (Canada blue grass) and *Agropyron cristatum*.

The landfill site (Trade Waste Pit) was used for disposal of a variety of domestic waste, including building materials, fuel, treated wood, scrap metal, batteries and manure between 1952 and 1999 (Naeth and Jobson 2007). Soil and ground water sampled at the site within the past five years had elevated concentrations of aluminum, copper, zinc, strontium, nickel, silver and iron, reflecting natural background concentrations in the region. The site was classified as very low risk with no required remediation (Canadian Council of Ministers of the Environment 2008). In 2006, waste was removed, the site was recontoured and wood chips and topsoil were applied to rebuild the soil. In fall 2006 and spring 2007, native grasses, forbs and shrubs from wild collected seed were planted (seeded and transplanted) and a revegetation study was initiated (Naeth and Wilkinson 2008).

Borrow pit sites were gravel quarries and disturbance details are not well known. Borrow Pit 1 (Potato Patch Pit) is 1.8 ha in size, located near Chief Mountain Highway Junction. Gravel excavation concluded during the 1960s and the site was officially decommissioned in the late 1970s. It became heavily infested with *Centaurea maculosa*, which was sprayed with herbicide and hand pulled in the 1980s, then plowed and revegetated with native plant species. Borrow Pit 2 (Pincher Creek Pit) is 2.2 ha in size, located near the Bison Paddock. In recent years park staff planted native plugs at the sites and tried to control target weeds.

3.2 Experimental Design

In summer 2010, a 0.3 ha block at each site was divided evenly into 12 adjacent plots. A randomized block design included control, mowing, steam and herbicide treatments randomly assigned to three replicate plots in each block (Figures 2-2 to 2-4). Blocks and plots had even topography and uniform vegetation and soils. A 1 m wide buffer zone was located between each plot and plots were marked with painted wooden stakes. Trade Waste Pit plots were rectangular 4 x 54 m except for one 8 x 27 m plot (herbicide). Pincher Creek Pit plots were rectangular 8 x 27 m. Three plots at Potato Patch Pit were 9 x 27 m (mow, herbicide, control), three 15 x 18 m (2 control, 1 mow) and three 12 x 18 m (2 herbicide, 1 mow). Steam was not used at Potato Patch Pit due to limited vehicle access.

Initial treatment applications were performed July 26-30, 2010. Mowing was done with two hand held weed eaters, as low to the ground as possible. Native woody plants were left intact. The herbicide Glyphosate Trans Orb 3 % v/v was applied using backpack sprayers and a constant pressure to maintain a fine spray at an approximate rate of 15 L per plot. Steam was applied by Sunnyside Mobile Wash of Lethbridge, Alberta. A 50 cm long bar was dragged across the vegetation in consecutive swaths and steam was released from narrow openings at the end of the bar at a temperature of 325 ^{o}C and a constant pressure of 800 psi.

In 2011 treatments were again applied. On May 15 through 19, 5 % v/v Glyphosate was applied using the same methods as in 2010. Approximately 20 L of herbicide was applied to each plot. Mowing and steaming were repeated June 8-12 and June 21, respectively, to target non-native species when they were most vulnerable and to allow for revegetation of native species. Mowing methods were the same as in 2010. Steaming was performed by Skywash Services of Calgary, Alberta using a hose held approximately 50 cm from vegetation at a temperature of 225 ^{o}C and pressure of 350 psi.

Revegetation was conducted to provide a source of native propagules and prevent re-colonization by non-native plants. After treatments were applied, during June 13-16, 2011, equal amounts of wild collected native seed was broadcast on all treatment plots (and on June 21 on steam plots after steaming). From May 29 to June 5, 2012, native forbs and grasses grown from wild collected seed were transplanted equally throughout the plots. Details of these seeding

and planting treatments are presented in chapter three as they are lengthy and have no impact on the work reported on in this chapter.

3.3 Meteorological Conditions

Meteorological data were obtained from Environment Canada National Climate Data and Information Archive (Environment Canada 2012a, 2012b). Data were collected from a weather station (Waterton Park Gate Alberta) located at the Waterton Lakes National Park Gate (49°07'52.080" N, 113°48'31.010" W, elevation 1,289 m). Mean daily temperatures were averaged for mean monthly temperature and total monthly precipitation and maximum wind gusts recorded at the weather station were obtained from the online archive. Long term climate normals (1971-2000) were obtained from an inactive weather station (Waterton River Cabin) approximately 0.5 km north of Potato Patch Pit (49°07'00.000" N, 113°50'00.000" W, elevation 1,281 m).

3.4 Vegetation Assessments

For vegetation assessments, transects were run down the length of the plots, and 0.1 m^2 quadrats were systematically placed at equal distance intervals along transects. At Trade Waste Pit (Figure 2-4), a 54 m long transect was run through the middle of each 4 x 54 m plot. In the 8 x 30 m plot, two 30 m long transects were run down the plot, positioned 3 and 6 m along the width of the plot. At Potato Patch Pit (Figure 2-3), three transects were positioned every 3 to 4 m along the width of 15 x 18 m and 12 x 18 m plots and quadrats positioned every 3 to 4 m; 9 x 27 m plots had two transects with quadrats every 5 m. At Pincher Creek Pit (Figure 2-2) plots were 8 x 30 m and two 30 m transects were run 3 and 6 m along the width of each plot, with quadrats positioned every 5 m. Individual plant species and ground (bare ground, moss, vegetation, litter, thatch, rock) cover were determined visually. Nomenclature followed Kuijt (1982), Moss (1983) and Tannas (2003).

Vegetation was assessed June 14-20 and July 26-30, 2010 prior to treatment implementation. Ten quadrats per plot were assessed at Trade Waste Pit and Potato Patch Pit; at Pincher Creek Pit 5 to 10 quadrats were assessed per plot due to heavy rain. Vegetation was assessed September 19-21, 2010 to

determine herbicide and steam effectiveness. Total canopy cover was assessed rather than individual species as plants were difficult to identify at that time.

Post-treatment effectiveness on vascular plants was assessed July 12-17 and August 2-9, 2011 and July 16-28, 2012. In July 2011, canopy cover of non-native and native graminoids and forbs were visually estimated. In July and August 2011, 10 quadrats were used per plot; in August 2011, 3 additional quadrats were randomly placed in each herbicide plot to obtain more data on newly emerging vegetation. In July 2012, 12 quadrats were used per plot to account for increased species richness detected by species area curves.

To assess mowing effectiveness, mean vegetation height in each quadrat was determined in mow and control treatments by measuring average height of vegetation with a measuring tape. Plant health and physiology were assessed for native and non-native vegetation. For health, 1 was assigned to necrotic plants (< 25 % live green), 2 for chlorotic or wilting plants (25 to 75 % live green) and 3 for healthy plants (> 75 % green). For physiology, 1 was assigned to immature plants (e.g. rosette stage), 2 to plants close to flowering or flowering and 3 for plants that had set seed. A single value was assigned per quadrat to each vegetation category (July 2011) or individual species (August 2011). If individual plants in a category had different health and physiology scores, an average was given for the majority in the quadrat.

In August 2011, vegetation was assessed at undisturbed foothills fescue grassland areas surrounding each study site to compare recovering plant communities in treatments with the target ecosystem for restoration. At each site, the closest 8 x 30 m patch of undisturbed grassland without non-native species and with similar topography to control plots was selected. Two transects 30 m long were positioned at 3 and 6 m along the width of the plot, dividing the plot into three parts and 5 quadrats were positioned every 5 m along each. Canopy cover of each vascular plant species and ground cover were visually estimated.

3.5 Soil Sampling And Analyses

Soil was sampled September 19-21, 2011. Transects and quadrat positions from vegetation assessments were used to randomly select soil sampling locations. Soil was sampled to characterize study sites and undisturbed fescue grassland.

A total of 42 samples were collected, one from each control, herbicide, mow and steam plot at each site, giving 12 each for Trade Waste Pit and Pincher Creek Pit and 9 for Potato Patch Pit. Three samples were taken from each undisturbed plot at each site. At each sampling location, a small hole 15 cm deep was dug with trowels and a sharp knife and 500 g of soil was removed, placed in labeled plastic bags and stored at 4 ^{o}C until processing. The hole was filled.

Samples were analyzed by Exova Laboratories in Edmonton, Alberta. Total nitrogen and carbon were determined by dry combustion and Leco combustion (Bremner 1996). Inorganic carbon was determined through carbon dioxide release; total organic carbon was calculated by subtracting inorganic carbon from total carbon (Loeppert and Suarez 1996). C:N ratio was determined by dividing total carbon by total nitrogen. Total phosphorus was determined with strong acid extraction and inductively coupled plasma mass spectrometry (US Environmental Protection Agency 1996). Sand, silt and clay were determined by hydrometer (Kroetsch and Wang 2008). Electrical conductivity, pH, sodium adsorption ratio and available soil calcium, magnesium, sodium and potassium was determined in saturated paste (Miller and Curtin 2008).

3.6 Statistical Analyses

Species cover data by year were grouped into non-native forbs, non-native graminoids, native graminoids and native forbs and shrubs. Cover of non-native species of concern was compared among treatments. Comparisons of response variables were performed using R v. 2.1.3.0 (2010 data) and SAS software v. 9.2 (SAS Institute, Inc. 2003, R Core Development Team 2011). Multivariate analyses were conducted using PC-ORD v. 6 (McCune and Mefford 2011). All data were assessed for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests, respectively. For all statistical tests alpha was 0.05.

To determine if variation among sites would significantly influence outcomes, a two way analysis of variance (ANOVA) was performed for each cover category with proc glm in SAS with site and cover as factors. Site effects were significant (data not shown). Therefore, data from each site were analyzed separately to investigate treatment effects. A second round of analysis was performed where data from each site were combined and analyzed for an overall assessment of treatment effects with statistical models accounting for site variation.

For each year of data, Shannon index of species diversity and species richness were calculated in Microsoft Excel by treatment and by site. 2010 cover data for herbicide and steam treatments at all sites were combined and compared using Student's t-test. Cover data from mow, steam and control treatments at Trade Waste Pit were compared using one way ANOVA with proc mixed in SAS with plot in the random statement to remove inter-plot variation. The herbicide treatment was removed as it was skewed and could not be log transformed.

For 2011 and 2012 data, cover comparisons in all four treatments were made with one way ANOVA using proc mixed in SAS with site specified in the random statement to remove site variation for overall analysis and plot in the random statement for site specific analysis. For significant ANOVAs, Student's t-tests were used to compare treatment means using LSMEANS and pdiff statements in SAS. Resulting p values were adjusted with Bonferroni correction, a multiple comparisons procedure giving strong inference by reducing probability of inaccurate significance (SAS Institute Inc. 2009).

Non-parametric procedures were used for 2011 data with heterogeneous variance and non-normal distributions that could not be log transformed. Wilcoxon Mann-Whitney two sample test was used to compare vegetation height and physiology between mow and control treatments and Friedman's test was used for comparisons of vegetation cover categories in all four treatments; the tables statement was included to remove inter-block (site variation) by stratifying data by site (SAS Institute Inc. 2009). Friedman's test was used for data from all three sites. Kruskal Wallis k-sample test was used for single site data. If significance was detected, proc multest program with perm option was used with contrasts serving as pairwise comparisons to determine significant treatment differences. Proc multtest is a permutation procedure to determine significant probability population distributions (Richter and Higgins 2006).

Impact of non-native plant management treatments on plant community structure and composition was assessed by ordination and group testing analysis. Multivariate analyses were used to determine how site specific soil conditions and plant community composition affected non-native plant control treatments. 2012 vegetation data were grouped with undisturbed fescue grassland and 2011 soil data. Due to the large number of zeros and the heterogeneity, non-metric

multidimensional scaling was used with the Sørensen distance measure, with 500 iterations in the final run, two axes and a random starting configuration. Sørensen is a city block distance measure that does not give importance to zeros (Peck 2010) but does give importance to shared abundances among sampling units. Vegetation and soil data were overlaid to identify plant species and soil properties influencing patterns. Correlations between key variables and ordination axes were tested with Spearman rank correlation in SAS. For multivariate analyses, an accompanying randomization test determined whether a statistically meaningful result was produced relative to that if data were from randomly distributed samples.

Distinct plant community groups associated with treatments and with sites were identified in the ordination and tested for significant differences using multiple response permutation procedure (MRPP) since sample units among groups were unequal. MRPP conducts a statistical permutation test based on similarities within groups (Peck 2010). Indicator species analysis was used to confirm the plant species that were characterizing treatments and sampling locations (Dufrêne and Legendre 1997), based on constancy and distribution of abundance (Peck 2010). Indicator values represent the degree at which a plant species indicates a group and is calculated for each plant species for each group as a product of average abundance of plant species divided by sum of average abundances across groups and number of sample units species is in, then divided by total sample units.

4. RESULTS

4.1 Meteorological Conditions

Mean monthly temperature was similar to average temperature conditions during the study years (Table 2-1). 2010 was wetter than the long term normal with over 100 mm more precipitation during May to August than the long term average. Precipitation in 2011 was similar to average conditions and precipitation in 2012 was drier than normal with 100 mm less precipitation than average. A high frequency of extreme weather occurred in 2012; thunder and hail storms were more intense and more often and there were many hot, dry days followed by periods of heavy rain.

4.2 Pre-Treatment Site Conditions

Soils of the undisturbed fescue grassland surrounding the study sites were Black Chernozems typical of the region. Soils at the study sites were modified by disturbance as indicated by lower organic matter content than undisturbed areas (Table 2-2). All sites had large amounts of rocky, coarse grained sediment typical of the region. Nitrogen and phosphorous were slightly lower in disturbed areas. Pincher Creek Pit had highest sand content and pH and low phosphorous and sodium levels. Overall, soil conditions were similar throughout the study area and did not have a major impact on treatments.

Non-native graminoids dominated sites prior to treatment, followed by native forbs, non-native forbs and native graminoids (Figure 2-5). Non-native species cover was greatest at Trade Waste Pit and least at Pincher Creek Pit, with the opposite trend for native species (Figure 2-5, Table A.1). Species richness and diversity were highest at Pincher Creek Pit (Table 2-3). *Bromus inermis* was most dominant at Potato Patch Pit, *Bromus inermis* and *Elymus repens* at Trade Waste Pit and *Agropyron sibiricum* (Willd.) P. Beauv. (Siberian wheat grass) at Pincher Creek Pit; several non-native species considered invasive in foothills fescue grassland were present (Table 2-4). Non-native species abundance varied among sites, but most were present at all sites (Tables A.2, A.3). More native species were present at Pincher Creek Pit (Tables A.4, A.5).

4.3 First Year Of Treatment Application

Effectiveness of glyphosate, as indicated by decreased vegetation cover, was highly variable within replicates. Glyphosate treatments had significantly lower cover than steam treatments two months after application (Tables 2-5, A.6). Steam and mow treatments did not differ significantly from the control at Trade Waste Pit (Table A.6).

4.4 Second Year Of Treatment Application

Herbicide significantly reduced non-native grasses but was ineffective in decreasing non-native forbs (Figure 2-6, Tables A.7, A.8). Non-native plant species considered invasive in foothills fescue grassland identified in the pre-treatment vegetation assessment had variable responses to herbicide

application. *Bromus inermis*, *Agropyron cristatum* and *Poa pratensis* cover were significantly lower in herbicide plots whereas *Chrysanthemum leucanthemum*, *Centaurea maculosa*, *Melilotus* spp. (Sweet clover), *Medicago lupulina* L. (Black medic) and *Elymus repens* were unaffected by herbicide (Tables 2-4, A.9). *Bromus tectorum* L. (downy brome) and *Cirsium arvense* increased numerically. Herbicide plots were devoid of most original vegetation and generally consisted of bare ground, litter and annual non-native forbs. Herbicide caused a numerical decrease in species diversity and richness (Table 2-3). Native cover was also numerically lower in herbicide than in other treatments, but by August, native forb cover was beginning to increase. Steaming and mowing did not significantly reduce non-native or native cover, species diversity or richness relative to controls (Figure 2-6, Table 2-3). However, mowing had numerically higher native grass cover and lower non-native grass cover than controls (Figure 2-6).

Plant health did not vary among treatments with over 90 % of vegetation having a healthy score. Thus, there was no residual effect of glyphosate. Mowing significantly delayed flowering and seed production in native forbs and grasses and non-native grasses (Tables 2-6, A.9). Mean vegetation height was significantly lower with mowing than in the control (Table 2-6).

Responses to treatments within sites were similar to overall trends (Tables A.10, A.11). However, native forbs were impacted more at Pincher Creek Pit and Potato Patch Pit than at Trade Waste Pit because pre-treatment cover of native forbs at Trade Waste Pit was very low relative to the other sites. At Trade Waste Pit there was a significant increase in non-native forb cover with herbicide.

4.5 Third Monitoring Year With No Application

One year after the second herbicide spraying effectiveness of herbicide waned. Herbicide no longer had significantly less non-native grass cover, which doubled from 2011 to 2012 in the herbicide treatment (Figure 2-7, Table A.12). Herbicide had significantly higher non-native forb cover and numerically higher non-native forb species richness (Figure 2-7, Tables 2-3, A.11). Non-native plant species of concern identified in the pre-treatment vegetation assessment rebounded in the herbicide treatment. Cover of *Bromus inermis*, *Agropyron cristatum* and *Poa pratensis* were no longer significantly lower in herbicide and *Elymus repens*

increased (Tables 2-4, A.13). Cover of non-native forbs *Cirsium arvense*, *Chrysanthemum leucanthemum*, *Melilotus* spp. and *Medicago lupulina* increased but not significantly in the herbicide treatment. There was no change in *Centaurea maculosa*. *Bromus tectorum* cover in herbicide treatment was similar to 2011 and numerically higher than the control (Table 2-4). Native cover was not significantly different among treatments and species diversity and richness were similar (Figure 2-7, Table 2-3). As observed in 2010 and 2011, mowing and steaming had no significant impact on cover, species richness or diversity.

Within site trends were similar to overall results. Native forb and grass cover was restored to previous values at Trade Waste Pit and Potato Patch Pit but was still significantly (forbs) lower at Pincher Creek Pit (Tables A.13, A.14). Native cover was numerically higher in herbicide plots at Trade Waste Pit relative to the control (Table A.14). Native species *Sisyrinchium montanum* Greene (Blue eyed grass) and *Lupinus sericeus* L. (Silky lupine), previously unseen at Trade Waste Pit and Potato Patch Pit, were found colonizing herbicide plots in 2012 (Table A.4). Plant communities demonstrated high resilience and species diversity in response to management. In total, 104 native and 55 non-native species were detected in the study area, occupying about 1 hectare, which represents almost 20 % of the entire Park's vascular plant species (Tables A.2 to A.5). New species were detected that have never been recorded in the Park.

Multivariate analyses showed herbicide dramatically shifted communities dominated by invasive perennial grasses to non-native forb dominated communities (Figure 2-8, Tables A.15, A.16). Mow and steam did not cause any change in plant community structure relative to the control. NMS ordination showed site conditions explained the greater impact of herbicide on native species at Potato Patch Pit and Pincher Creek Pit. A gradient of increasing native plant species and decreasing non-native species was detected from Trade Waste Pit to Potato Patch Pit to Pincher Creek Pit correlated with decreasing soil C:N ratio, clay and silt and increasing sand (Figure 2-8, Tables A.17, A.16). Comparison of treatments with target ecosystem data from undisturbed fescue grasslands showed distribution of individual native and non-native plant species were affected by soil properties contributing to this gradient (Table A.17). For example, *Bromus inermis* was significantly positively correlated with increasing

soil clay content. Undisturbed communities were distinct from disturbed sites, dominated by *Festuca campestris* Rydb. (Rough fescue), *Festuca idahoensis* Elmer (Idaho fescue), *Danthonia parryi* Scribn. (Parry oat grass) and *Selaginella densa* Rydb. (Little club moss) (Figure 2-8, Tables A.15, A.16, A.18). Dominant non-native perennial grasses and *Chrysanthemum leucanthemum* were identified through NMS as strongly influencing Trade Waste Pit and Potato Patch Pit community composition (Figure 2-8, Tables A.15, A.18).

5. DISCUSSION

5.1 Herbicide

This study demonstrated early spring herbicide application is an effective management technique to quickly reduce non-native perennial grasses with little cost and effort. Herbicides such as glyphosate may be key to controlling these challenging species which form dense monocultures and pose a significant threat to conservation of native biodiversity. This finding is consistent with results from other studies (Brown 1997, Rice et al. 1997, Hitchmough et al. 1994, Wilson and Pärtel 2003, Bakker et al. 2003, Simmons et al. 2007). These studies found that non-native grasses targeted early in the growing season, when they are most vulnerable at the three leaf stage, < 15 cm tall and have not reached maturity, often showed the greatest response to herbicide (Martin et al. 1983, Sather 1987, Grilz and Romo 1995).

Broad spectrum glyphosate application is not likely an effective management technique to reduce non-native forbs. Although annual non-native forbs such as *Thlapsi arvense* and *Lepidium densiflorum* Scrad. (Common pepperweed) can remain dominant for 3-4 years during succession, then decrease gradually without any management or control (Gerling et al. 1996), this study showed further control is necessary to reduce both persistent non-native forbs and non-native grass cover. Temporary effects of herbicide and the need for long term control have been reported by other researchers (Rice et al. 1997). Clopyralid, a forb specific herbicide used to control *Centaurea maculosa*, led to major increases in non-native graminoid cover (Tyser et al. 1998). Thus control of forbs by herbicide still requires research to develop beneficial management approaches and balance effects among species groups.

Results showed non-native species respond differently to herbicide application and herbicide can increase undesirable species such as *Bromus tectorum*; therefore, broad spectrum glyphosate application may not be effective for some non-native grass and forb species and may be more effective for other non-native perennial grasses. Variable non-native species response and replacement of dominant species by other non-native species has been documented in other studies (Grilz and Romo 1995, Brown 1997, Cole et al. 1999, DiTomaso 2000, Murphy and Grant 2005, Otfinowski et al. 2007, Simmons et al. 2007).

Our study showed herbicide does not detrimentally affect native plant species abundance and diversity if existing native cover and diversity are low. Herbicide can actually increase native plant abundance and diversity through release from competition with non-native species. Other studies also found impacts of spraying highly disturbed or invaded sites with little existing native cover are neutral or beneficial (Rice et al. 1997, Brown 1997, Simmons et al. 2007). Glyphosate did not appear to negatively impact the health of native seedlings after spraying. This is in contrast to another study which reported tissue damage in plants grown in glyphosate treated soil (Cornish and Burgin 2005).

5.2 Mowing

Mowing was useful in reducing non-native vegetation height and seed production. Other studies had similar findings (Pitchford 2000, Maron and Jeffries 2001, Hansen and Wilson 2006, Cole et al. 2007). With two annual applications mowing is not effective for removal of large and diverse populations of non-native plant species. Short term mowing is likely more effective when only one non-native species to be controlled is present (Rinella et al. 2001). Lack of reduction of non-native cover by short term mowing has been reported in other 1-3 year studies (Pelech 1997, Simmons et al. 2007). Mowing is often more effective when performed repeatedly during the growing season, for longer periods of time and when tiller apices are removed (Sather 1987, Eastin et al. 1964, Paulsen and Smith 1968, Reynolds and Smith 1962, Willson and Stubbendieck 1996, Wilson and Clark 2001, Donkor and Bork 2002). Another disadvantage of mowing is reduced native seed production, which has not been assessed in other studies. However, as with herbicide impact, reduced native seed production is not a major concern if native plants are sparse.

5.3 Steam

Steam is a promising alternative to herbicide but requires more research and development to become a useful non-native plant management technique. Lack of success was likely due to heat loss caused by absence of a steam insulation technique, such as an aluminum box (Norberg et al. 1997) or foam (Belker 1990, Labowsky 1990, Kristoffersen et al. 2007) and insufficient applications and time period (> 2 years) for steam to significantly affect vegetation (Kristoffersen et al. 2007). Perennial non-native grasses dominant in the study are difficult to kill with steam (Wei et al. 2010) and a thermal fingerprint test should be performed to assess effectiveness during the application (Schroeder and Hansson 2006). Innovative strategies like steam are worth investing in to address herbicide resistance and pollution and to search for breakthrough solutions. Very few trials of steaming have been performed in natural areas for non-native plant control. This study represents the first attempt of its use in disturbed grassland.

5.4 Restoration And Management Implications

Based on this research, herbicide is recommended to quickly reduce non-native graminoid cover on disturbed grasslands. The effect is temporary and follow up is needed to control resprouting. Herbicide can control most non-native perennial grasses but may increase other non-native species; thus non-native species need to be considered in management actions. Non-native forbs may increase with broad spectrum herbicides and will require monitoring. Annual non-native forbs usually do not require control as they can provide cover and reduce erosion; perennial non-native forbs will require removal. Techniques like spot spraying, weed wiping (Grekul et al. 2005) or hand pulling could be used at sites with high native cover like Pincher Creek Pit to avoid damaging native plants; more intensive methods can be used at sites with sparse native cover. Glyphosate had greatest impact on dominant vegetation, so vegetation composition should be considered when deciding to use herbicide. Mowing can reduce non-native biomass and seed production but will not remove non-native species when only used for a short duration. Steam requires more research and development and based on this study cannot be recommended for routine use.

Site conditions should play a major role in management decisions. Soil properties correlated with plant distribution can be used in predicting management and

restoration outcomes. Pre-treatment plant community composition plays an important role. Correlations among native and non-native species showed non-native plants suppressed native plants, occur together in non-native communities and can easily replace one another, shifting dominance to sustain a disturbed community. Control of non-native species that thrive after release from competition with dominant competitors is a widely known challenge (Grilz and Romo 1995, Brown 1997, Cole et al. 1999, DiTomaso 2000, Murphy and Grant 2005, Otfinowski et al. 2007, Simmons et al. 2007). In this and other studies *Bromus tectorum* increased after herbicide created bare areas where it was previously suppressed by perennial non-native grasses (Whitson and Koch 1998). Ordination showed *Bromus inermis*, *Poa pratensis*, *Phleum pratense*, *Elymus repens*, *Chrysanthemum leucanthemum* and *Agropyron cristatum* significantly contributed to persistence of non-native monocultures and low biodiversity in plant communities and should be targeted in control efforts.

Multivariate analyses with target ecosystem data identified potentially important native species missing from disturbed habitats and native species tolerant of disturbance that are early successional and perform well on disturbed sites with lower levels of competition from non-native species. *Koeleria macrantha* (Ledeb.) Schult. (Prairie june grass), *Heterotheca villosa* (Pursh) Shinners (Hairy false golden aster), *Festuca campestris*, *Festuca idahoensis*, *Danthonia parryi* and *Selaginella densa* can help facilitate succession to a more biodiverse foothills fescue plant community and suppress weed growth.

Research is needed to determine long term effectiveness of herbicide with follow up control of non-native species and planting of native species to suppress non-native regrowth. Steam could be investigated as a follow up to herbicide when plants are weak and less resilient. Other thermal methods are being developed that can be explored such as hot water and air, proven equally effective as steam (Kristoffersen et al. 2007) and solar tents (Stapleton et al. 2012). Long term, intensive mowing should be further researched; combined with other methods it may provide immediate reduction in biomass to facilitate other control measures.

Restoration of disturbed grasslands dominated by numerous, diverse and aggressive non-native plant species is a complex challenge requiring dedicated long term management. Long standing invasions have been eradicated

throughout the world with continued hard work (Simberloff 2003). If research and management to control spread of non-native species are not vigilantly continued a large proportion of remaining native grassland will be lost (Vaness and Wilson 2007). A strong investment in research and practice of non-native plant control and establishment of a competitive native plant community is critical to circumvent loss of native biological diversity and restore native grasslands.

6. CONCLUSIONS

- Herbicide (glyphosate) was most effective in reducing non-native grasses.
- Herbicide significantly increased non-native forb cover to more than double pre-treatment levels by 2012, shifting plant community structure to an early successional stage dominated by non-native annual forbs.
- Impact of herbicide on native cover and diversity was not significant at sites with sparse native cover.
- Under a two year time frame with low frequency applications, steaming and mowing were ineffective in reducing non-native cover.
- Sites with high pre-treatment native cover, species richness and diversity had significantly lowest native cover and lower richness and diversity after herbicide application. Heavily disturbed and invaded sites had highest non-native cover before and after herbicide. Non-native communities recovered quickly and treatment effectiveness was short lived at these locations. Thus, site conditions must be considered to develop effective control methods.

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Table 2-1. Summer precipitation, temperature, wind gust speed and long term climate normals at Waterton Lakes National Park during the study period.

Year	Month	Total Monthly Precipitation (mm)	Mean Temperature (^{o}C)	Maximum Wind Gust Speed (km/h)
2010	May	119.8	6.0	96
	June	157.8	12.4	96
	July	92.6	15.3	83
	August	68.6	14.4	74
2011	May	126.2	8.0	89
	June	93.2	12.2	76
	July	19.6	16.2	95
	August	72.0	16.7	72
2012	May	37.6	8.9	72
	June	88.8	12.4	87
	July	38.2	17.1	102
	August	42.0	16.9	87
Long Term Normals	May	94.5	8.9 ± 1.3	
	June	80.8	12.5 ± 1.4	
	July	70.8	15.2 ± 1.2	
	August	69	14.5 ± 1.8	

Data from Park Gate and former Waterton River Cabin Weather Stations (Environment Canada 2012a, b).
± = standard deviation.

Table 2-2. Soil chemical and physical properties at research sites in September 2011.

Parameter	Pincher Creek Pit		Potato Patch Pit		Trade Waste Pit	
	Disturbed	Undisturbed	Disturbed	Undisturbed	Disturbed	Undisturbed
C:N Ratio	14.4 ± 3.8	11.5 ± 0.4	23.7 ± 33.6	11.1 ± 0.1	15.1 ± 3.4	13.2 ± 0.7
Total Nitrogen (%)	0.2 ± 0.1	0.7 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.2
Organic Matter (%)	4.5 ± 2.9	16.9 ± 2.5	2.6 ± 1.6	10.0 ± 1.4	6.1 ± 2.6	11.7 ± 4.6
Total Inorganic Carbon (%)	0.6 ± 0.3	0.1 ± 0.0	0.8 ± 0.7	0.1 ± 0.0	0.4 ± 0.3	0.1 ± 0.1
Total Organic Carbon (%)	2.2 ± 1.5	8.5 ± 1.2	1.3 ± 0.8	5.0 ± 0.7	3.1 ± 1.3	5.9 ± 2.3
Total Phosphorus (mg/kg)	606.7 ± 59.2	1013.3 ± 70.4	405.8 ± 65.6	750.0 ± 74.8	828.3 ± 124.9	1093.3 ± 273.5
Sand (%)	82.2 ± 4.5	59.7 ± 4.0	64.4 ± 12.3	59.0 ± 2.7	63.2 ± 4.3	63.2 ± 4.0
Silt (%)	13.8 ± 3.6	36.5 ± 3.3	26.8 ± 9.3	37.7 ± 3.0	29.3 ± 4.0	30.5 ± 3.9
Clay (%)	4.0 ± 1.1	3.8 ± 0.9	8.8 ± 3.6	3.3 ± 0.5	7.42 ± 1.2	6.3 ± 0.9
Hydrogen Ion Concentration (pH)	7.9 ± 0.1	6.8 ± 0.2	5.9 ± 0.2	7.2 ± 0.1	7.5 ± 0.5	6.6 ± 0.6
Electrical Conductivity (dS/m)	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.3	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
Sodium Adsorption Ratio	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Available Calcium (mg/kg)	31.1 ± 11.8	57.6 ± 8.8	40.6 ± 28.0	49.3 ± 11.9	25.3 ± 10.3	17.9 ± 4.8
Available Magnesium (mg/kg)	5.3 ± 2.2	14.4 ± 2.9	8.5 ± 6.0	13.0 ± 2.7	7.1 ± 3.4	5.9 ± 1.0
Available Sodium (mg/kg)	0.3 ± 0.5	3.0 ± 0.8	0.8 ± 0.5	1.7 ± 0.5	1.3 ± 0.4	1.7 ± 0.5
Available Potassium (mg/kg)	5.5 ± 2.8	33.0 ± 12.4	6.2 ± 5.7	16.7 ± 7.4	11.7 ± 7.5	7.0 ± 0.8

Numbers are mean ± standard deviation.

Table 2-3. Effect of treatments on biodiversity and native and non-native species richness.

	Pincher Creek Pit						Potato Patch Pit				Trade Waste Pit				
Species	All	All	C	H	M	S	All	C	H	M	All	C	H	M	S
2010															
Shannon Diversity (H')	0.99	1.71	1.77	1.52	1.8	1.74	0.71	0.66	0.67	0.82	0.72	0.66	0.76	0.56	0.81
Species Richness															
Native Forb	39	33	21	16	23	24	15	8	8	11	3	3	3	3	3
Native Grasses	12	7	3	5	2	3	5	2	3	3	4	2	2	1	0
Non-Native Forb	13	5	4	4	1	2	6	2	4	5	6	2	5	3	3
Non-Native Grasses	7	5	4	5	5	5	7	4	7	5	7	4	7	5	5
2011															
Shannon Diversity (H')	0.86	1.04	1.35	0.3	1.45	1.26	0.58	0.86	0.1	0.86	0.68	0.86	0.34	0.7	0.82
Species Richness															
Native Forb	40	34	21	16	25	24	19	11	6	14	7	2	5	3	2
Native Grasses	10	5	5	3	3	3	5	4	0	3	4	3	0	3	2
Non-Native Forb	14	7	4	4	6	2	9	4	5	4	11	5	7	5	6
Non-Native Grasses	14	11	6	6	8	7	11	6	4	9	12	9	5	8	10
2012															
Shannon Diversity (H')	1.19	1.34	1.53	0.95	1.51	1.39	1.12	1	1.01	1.35	1.09	1.02	1.02	1.22	1.11
Species Richness															
Native Forb	63	43	30	20	31	26	41	26	18	27	15	7	9	6	7
Native Grasses	20	14	6	7	8	7	14	6	9	8	10	8	7	3	4
Non-Native Forb	28	10	6	8	7	6	17	9	16	10	25	13	21	10	12
Non-Native Grasses	16	11	10	8	9	9	12	10	12	10	14	12	13	12	12

C = Control, H = Herbicide, M = Mow, S = Steam.

Table 2-4. Effect of management treatments on cover of specific non-native species considered invasive in fescue grassland.

	Bromus inermis	*Bromus tectorum*	*Agropyron cristatum*	*Agropyron sibiricum*	*Centaurea maculosa*	*Cirsium arvense*	*Poa pratensis*	*Elymus repens*	*Chrysanthemum leucanthemum*	*Melilotus* spp.	*Medicago lupulina*
2010											
Control	8.8 ± 12.5	-	2.2 ± 3.6	3.0 ± 4.5	0.3 ± 2.0		1.2 ± 2.3	3.4 ± 8.8	0.9 ± 3.4	-	0.3 ± 1.3
Herbicide	5.8 ± 8.0	-	3.0 ± 5.2	4.1 ± 4.8	0.3 ± 1.4		1.2 ± 3.0	3.9 ± 9.6	0.6 ± 2.9	0.1 ± 0.5	0.4 ± 1.6
Mow	5.2 ± 6.9	-	2.6 ± 5.4	2.7 ± 6.5	0.3 ± 1.2	0.0 ± 0.3	0.9 ± 2.2	4.0 ± 9.2	0.3 ± 2.0	-	0.6 ± 2.3
Steam	3.9 ± 7.4	-	3.4 ± 7.9	2.1 ± 2.8	0.5 ± 2.2		0.3 ± 1.2	6.6 ± 11.1	1.7 ± 7.2	-	0.6 ± 2.3
2011											
Control	7.1 ± 13.9 a	-	1.8 ± 5.8 a	0.1 ± 0.7	0.2 ± 0.8	-	1.0 ± 2.1 a	1.3 ± 0.6	0.6 ± 0.4	-	0.1 ± 0.4
Herbicide	0.1 ± 0.8 b	0.1 ± 0.3	0.0 ± 0.0 b	0.0 ± 0.1	0.1 ± 0.6	0.2 ± 1.0	0.0 ± 0.2 b	1.4 ± 0.2	0.4 ± 0.3	0.2 ± 0.9	0.3 ± 1.4
Mow	2.0 ± 3.8 a	-	0.9 ± 2.2 a	0.0 ± 0.1	0.2 ± 0.8	-	0.3 ± 0.9 ab	3.9 ± 3.1	0.6 ± 0.3	0.1 ± 0.5	0.1 ± 0.5
Steam	3.6 ± 11.5 a	-	1.5 ± 2.4 ab	0.0 ± 0.0	0.6 ± 3.9	-	0.6 ± 1.6 ab	7.9 ± 1.2	1.2 ± 0.7	-	-
2012											
Control	7.3 ± 16.0	-	1.9 ± 6.4	1.9 ± 4.8	0.7 ± 5.0	0.2 ± 1.2	2.6 ± 6.5	5.0 ± 15.2	0.8 ± 3.7	0.2 ± 0.8	0.6 ± 1.7
Herbicide	1.5 ± 5.5	0.1 ± 1.0	0.6 ± 2.7	0.9 ± 1.2	0.1 ± 0.8	1.8 ± 7.1	0.1 ± 0.4	2.9 ± 9.9	1.1 ± 4.4	3.4 ± 14.7	6.0 ± 12.7
Mow	3.6 ± 18.5	-	1.9 ± 5.5	0.5 ± 3.3	0.4 ± 2.2	-	1.9 ± 5.6	4.7 ± 13.8	0.4 ± 2.1	0.5 ± 3.9	1.0 ± 2.3
Steam	5.8 ± 18.5	-	0.9 ± 2.6	4.1 ± 9.4	0.2 ± 1.0	0.5 ± 3.3	1.9 ± 4.9	6.4 ± 17.7	2.5 ± 8.0	0.0 ± 0.2	0.4 ± 1.7

Numbers are mean ± standard deviation.
Species not detected indicated by -.
Means with different letters are significantly different ($p<0.01$, adjusted for multiple comparisons).

Table 2-5. Effect of treatment on live vegetation cover two months after first treatment application (2010).

Site	Treatment	Canopy Cover (%)
Pincher Creek Pit	Glyphosate	4.9 ± 2.1
	Steam	43.8 ± 12.3
Potato Patch Pit	Glyphosate	3.8 ± 2.8
Trade Waste Pit	Control	64.3 ± 13.5
	Mow	56.8 ± 9.9
	Glyphosate	15.1 ± 12.9
	Steam	59.3 ± 0.9
Overall Mean	Glyphosate	10.0 ± 10.5 a
	Steam	51.6 ± 11.6 b

Numbers are mean ± standard deviation.
Means followed by the same letters are not significantly different ($p<0.01$).
Trade Waste Pit data had no significant difference and herbicide treatment was removed from analysis due to a highly skewed distribution.

Table 2-6. Height and physiology of vegetation with mowing relative to the control one and two months after mowing.

Time	Treatment	Vegetation Height (cm)	Native Forbs and Shrubs	Native Graminoids	Non-Native Forbs	Non-Native Graminoids
July	Control	30.7 ± 3.5 a	0.7 ± 0.8 a	0.6 ± 1.0 a	0.4 ± 0.1 a	0.8 ± 0.5 a
	Mow	12.7 ± 0.6 b	0.7 ± 0.6 b	0.4 ± 0.3 b	0.5 ± 0.1 a	0.4 ± 0.7 a
August	Control	23.9 ± 8.9 a	0.9 ± 1.0 a	1.1 ± 1.3 a	0.8 ± 1.0 a	2.3 ± 0.7 a
	Mow	14.7 ± 4.4 b	0.9 ± 0.9 a	0.7 ± 1.0 b	1.0 ± 1.3 a	1.9 ± 0.9 b

Physiology scores: 1 = immature plant, 2 = flowering, 3 = producing seed.
Mean ± standard deviation presented.
Within months and columns, means with different letters are significantly different ($p<0.01$).

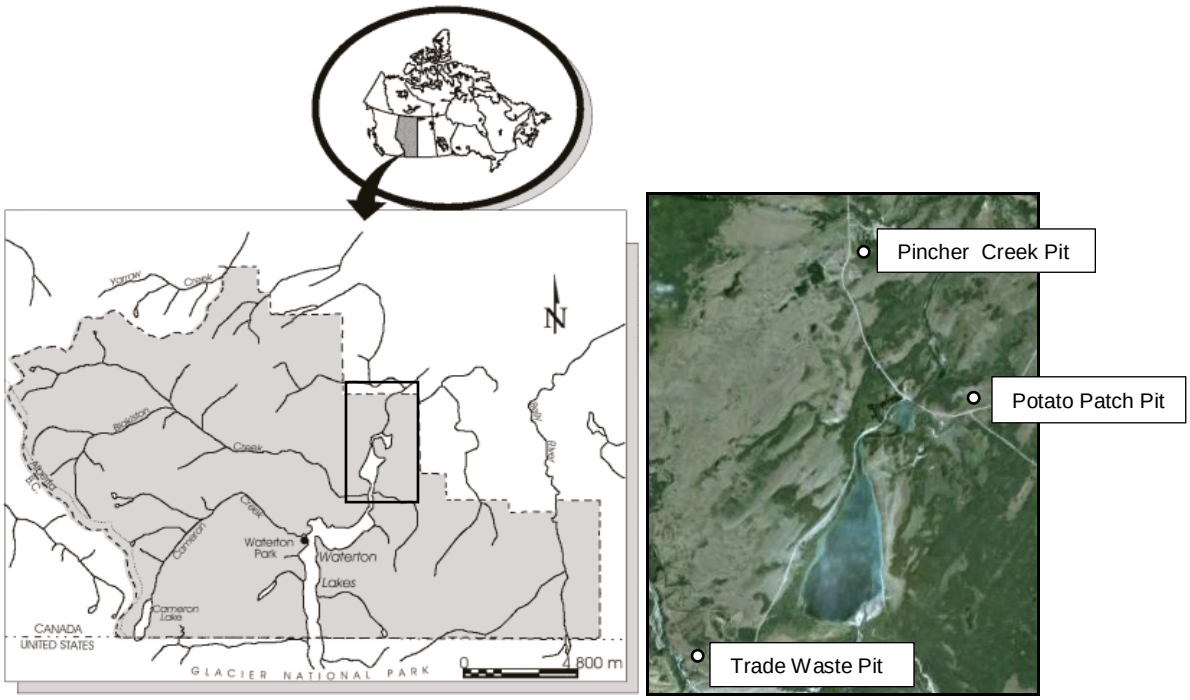

Figure 2-1. Location of Waterton Lakes National Park, Alberta, Canada and research sites (Parks Canada 2009, Google Earth 2012).

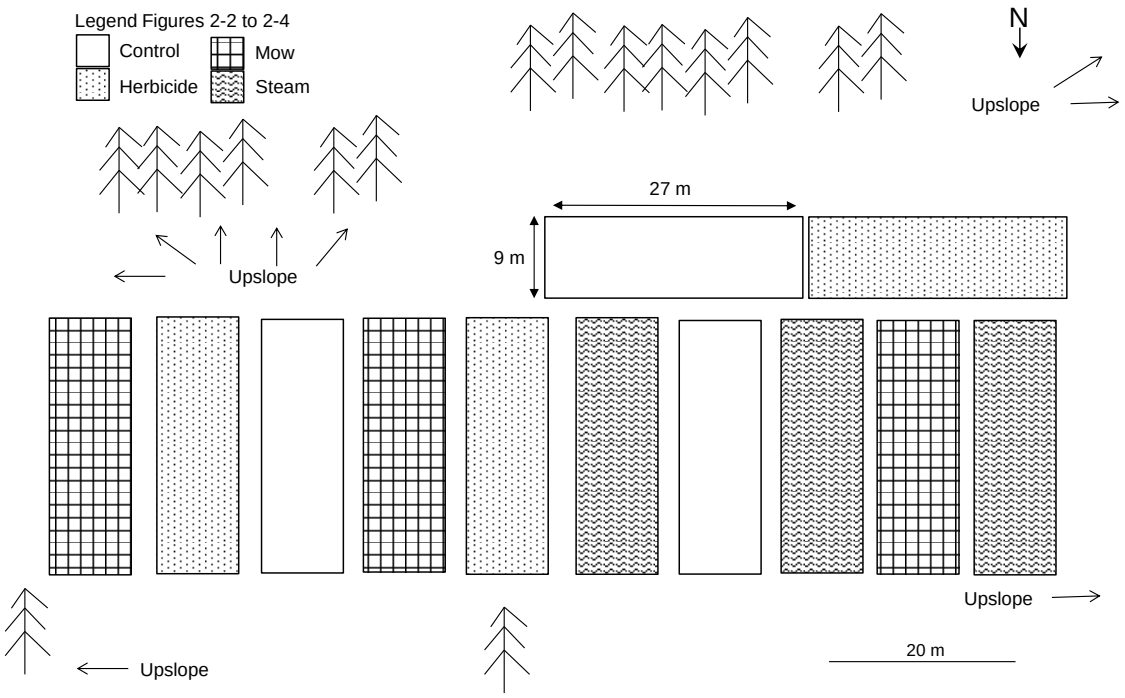

Figure 2-2. Pincher Creek Pit with plot locations.

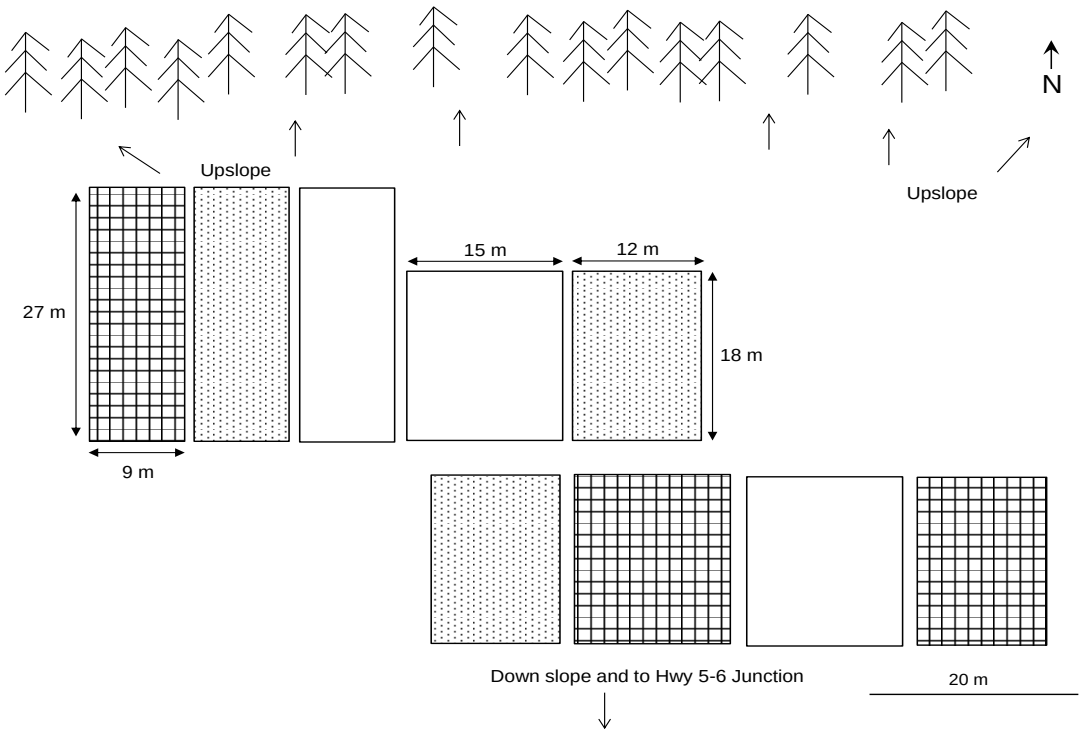

Figure 2-3. Potato Patch Pit with plot locations.

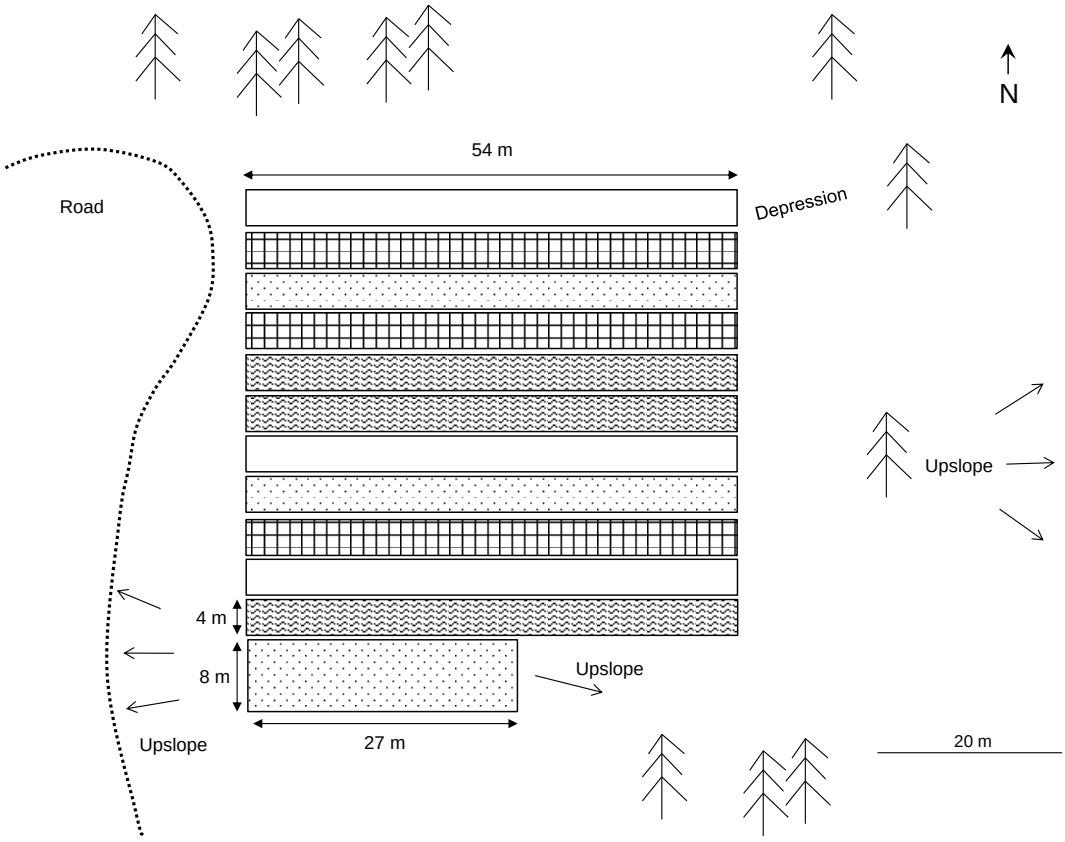

Figure 2-4. Trade Waste Pit with plot locations.

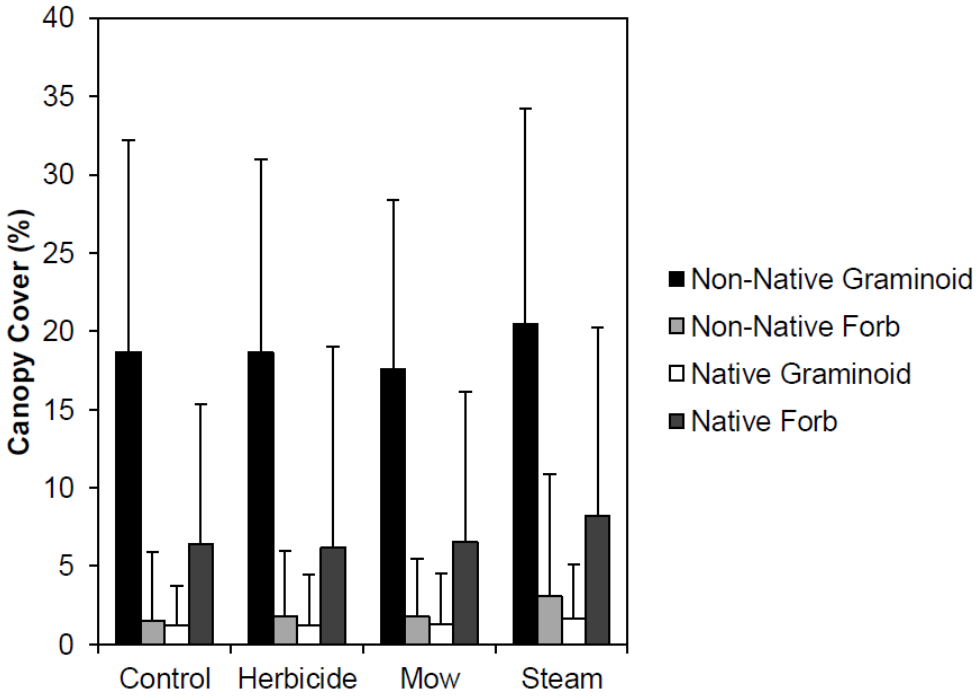

Figure 2-5. Pre-treatment (2010) cover of native and non-native vegetation. Mean and standard deviation are shown.

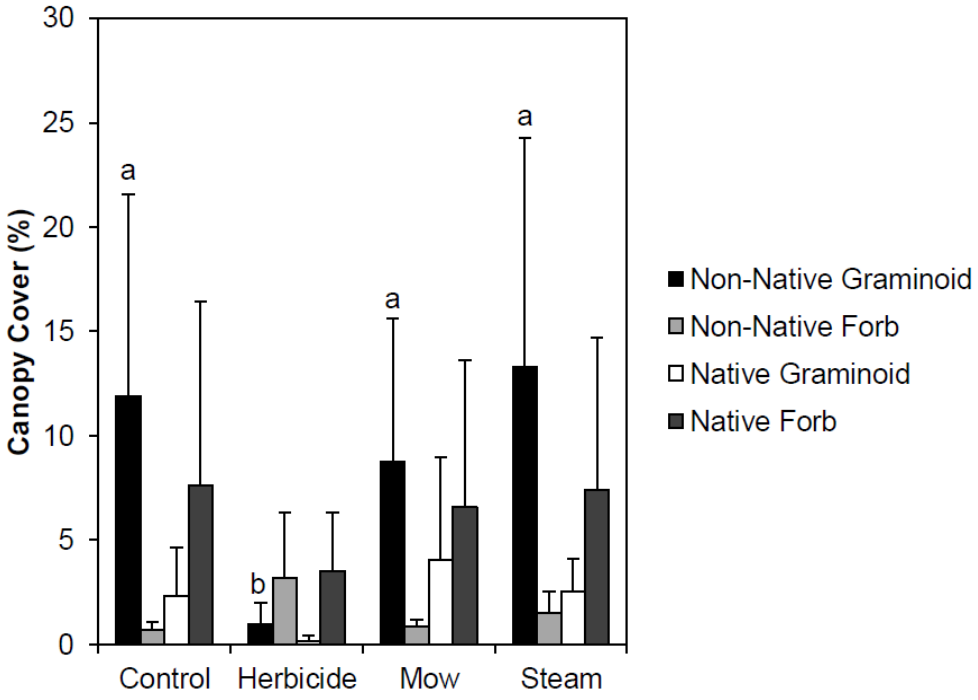

Figure 2-6. Cover two months after the second treatment application (2011). Mean and standard deviation are shown. Treatments with different letters are significantly different for cover group with letter assigned ($p<0.01$).

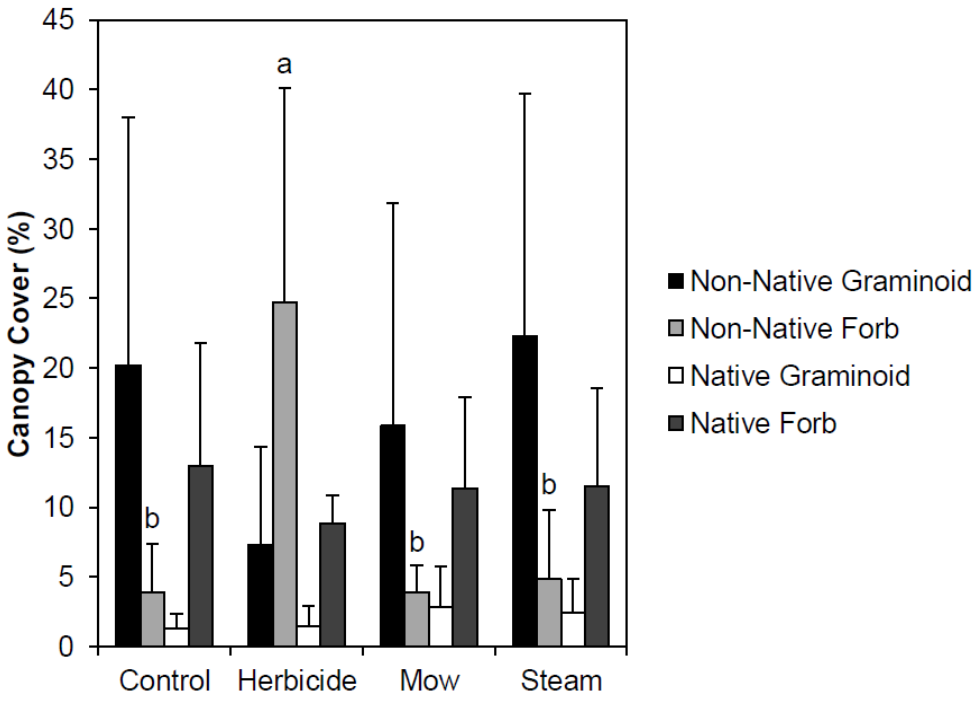

Figure 2-7. Native and non-native cover one year after the second and final treatment application (2012). Mean and standard deviation are shown. Treatments with different letters are significantly different for cover group with letter assigned ($p<0.01$).

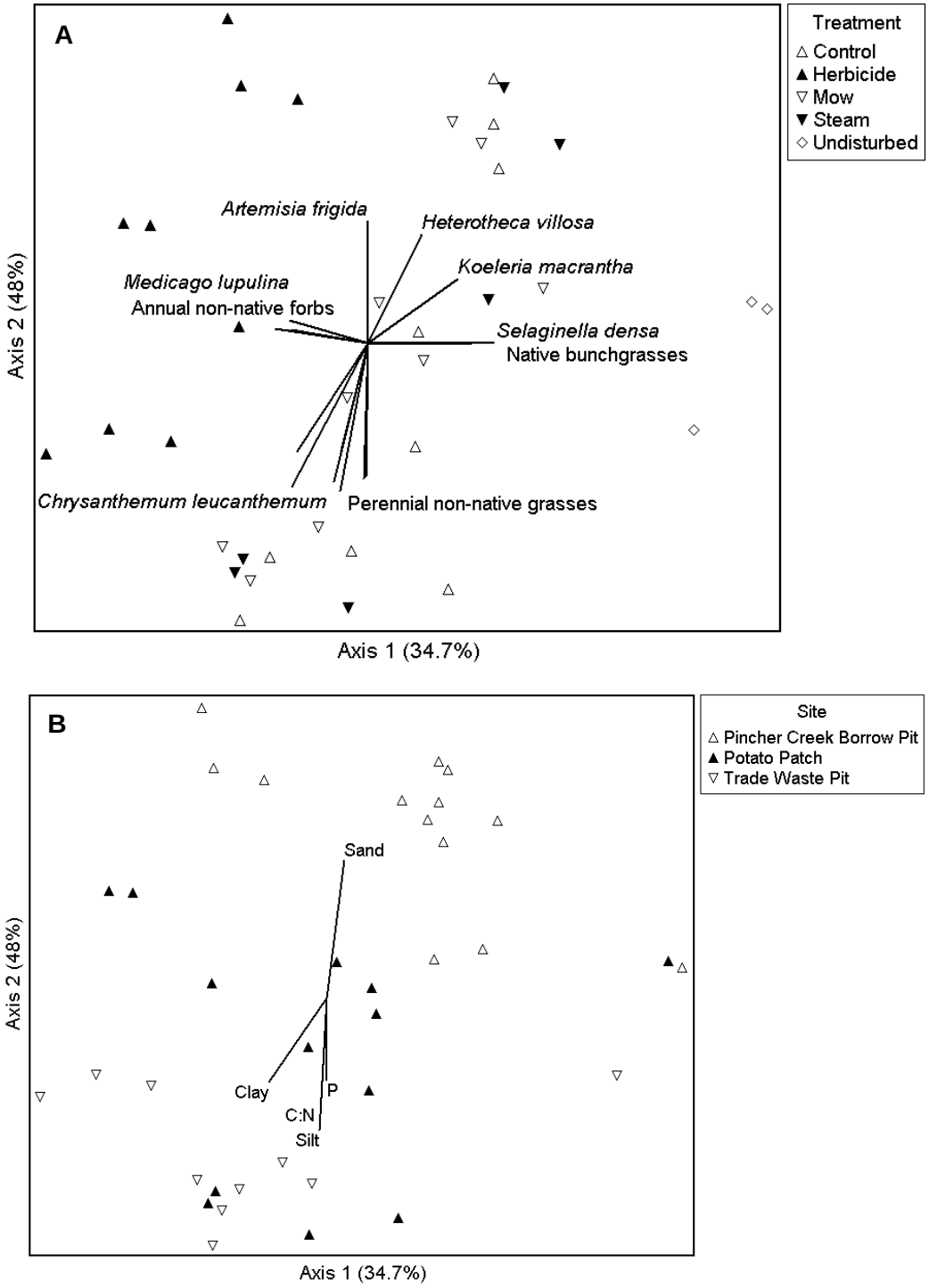

Figure 2-8. NMS ordination of plant communities from different (A) treatments and (B) sites. (A) plant species strongly influencing ordination, Annual non-native forbs = *Lepidium densiflorum, Lepidium ramosissimum* A. Nelson (Manybranched pepperweed), Perennial non-native grasses = *Poa pratensis*, *Elymus repens*, *Phleum pretense*, *Bromus inermis*, *Agropyron cristatum*, Native bunchgrasses = *Festuca idahoensis, F. campestris, Danthonia parryi.* (B) Influential soil properties, C:N = C:N ratio, P = phosphorous.

CHAPTER 3. SEEDING AND TRANSPLANTING FOLLOWING NON-NATIVE PLANT CONTROL IN FOOTHILLS FESCUE GRASSLAND RESTORATION

1. INTRODUCTION

Native grasslands are globally endangered ecosystems with high biodiversity (Gibson 2009). Vast areas of historic native grasslands have been degraded to low biodiversity ecosystems by agriculture and other development activities (Sampson and Knoff 1996). Non-native plant species have proliferated in disturbed areas and invaded large extents of remaining native grassland (Vitousek et al. 1997). Ecological restoration is a necessary solution to this issue but is challenged by lack of revegetation success due to aggressive competition with non-native species and poor establishment of native species (Jordan et al. 1988, Berger 1993, D'Antonio and Meyerson 2002). Foothills fescue grasslands of southwestern Alberta once occupying 1.6 million ha, have been reduced to 17 % of their former range (Adams et al. 2003). These grasslands occur on Orthic Black Chernozem soils and are dominated by *Festuca campestris* Rybd. (Foothills rough fescue), *Danthonia parryi* Scribn. (Parry oatgrass), *Festuca idahoensis* Elmer (Idaho fescue) and forb species. They provide habitat for several species at risk in Alberta, including the threatened *Anthus spragueii* Audubon (Sprague's pipit) and the endangered *Charadrius montanus* Townsend (Mountain plover) and *Athene cunicularia* Molina (Burrowing owl) and provide valuable habitat for grazing ungulates (COSEWIC 2012). Ecological restoration of foothills fescue grassland has been unsuccessful with the exception of sod transfer from a donor source (Revel 1993, Alberta Wilderness Association 2012).

Several abiotic and biotic factors influence introduction of native plant species on a restoration site. The most precarious stages of the plant life cycle are seed dispersal, germination and establishment (Harper 1977). Soil water, topography, slope aspect, nutrients, soil stability, biological crusts and size and number of gaps in vegetation are key factors promoting seedling establishment (Sterling et al. 1984, Hitchmough et al. 1996, Paschke et al. 2000, Elmarsdottir et al. 2003, Morgan 1998, Lulow et al. 2007). Disturbances often disrupt natural ecological succession and plant community sustainability, through a lack of safe sites to

foster establishment and survival of native propagules (Harper et al. 1965), native plant establishment suppression due to aggressive competition with non-native plants (Levine et al. 2003) and unfavourable soil conditions such as compaction and low nutrients and water (Bradshaw 1983, Tsuyuzaki et al. 1997).

Factors affecting native plant establishment are strongly species dependent (Davies et al. 1999, Page and Bork 2005, Naeth and Wilkinson 2008). Readily establishing native species are usually r-strategist, early successional forbs, with late successional graminoids more sensitive to disturbance and more difficult to establish (Moyes et al. 2005). *Festuca campestris* and other native perennial grasses can take three to five years to establish (Johnston and McDonald 1967, Stout et al. 1981, King et al. 1998). Revegetation is highly dependent on seasonal weather, particularly precipitation and on non-native species control (Erichsen-Arychuck 2001, Bakker et al. 2003, Naeth and Wilkinson 2008).

In restoration introducing native propagules after non-native plant control is required to prevent recolonization by non-native species (Tyser et al. 1998, Bakker et al., 2003, Huddleston and Young 2004, Reid et al. 2009). Management treatments such as prescribed burning and herbicide often lead to major changes in vegetation and dominance of non-native plants that may out-compete seeded species (Lulow et al. 2007). Herbicide can increase native plant establishment by creating gaps for seedlings and transplants when non-native plant competition is reduced (Wilson and Gerry 1995, Stromberg and Kephart 1996, Ewing 2002, Huddleston and Young 2005, Page and Bork 2005, Jusaitis and Polomka 2008). For example, prescribed burning prior to planting lead to > 90 % transplant survival by reducing competition with an invasive annual grass (Huddleston and Young 2004); mowing and burning increased native grass seedling density, but the effect was greater with herbicide (Huddleston and Young 2005). Management may not affect plant survival in the short term if it does not influence abiotic or biotic factors affecting transplant or seedling survival (Davies et al. 1999).

Seeding techniques and transplanting can impact resulting plant community composition, ground cover and plant community development (Naeth 2000). In some studies, *Festuca campestris* had greater establishment and survival when transplanted than seeded (Tannas 2011) and in other studies seeding was effective (Sherritt 2012). Standard seeding rates may be low relative to native

seed banks and seed dormancy and scarification requirements may affect germination (Tyser et al. 1998). Transplanting may improve revegetation because native plants have overcome their most vulnerable growth stage (Huddleston and Young 2004, Middleton et al. 2010). Seeding is much less costly and easier to implement, being effective with sufficient seeding rate, competition reduction and site conditions conducive to seedling development (McClaran 1981, Kirt 1990, Cole and Spildie 2000). Seeding with native hay can be highly successful (Desserud and Naeth 2011). Combined techniques may be better than a single technique (Paschke et al. 2000). Replacement of sod following pipeline construction (Desserud et al. 2010) or placement of donor material lead to more complete restoration of plant communities. Research is required to compare transplanting and seeding, particularly for native forbs and shrubs.

Large native perennial grasses and sedge (*Cyperaceae*) transplants had greater survival than small transplants (Steed and DeWald 2003, Page and Bork 2005). Forb transplant size has not been thoroughly investigated but trends appear similar (Davies et al. 1999). In some recovering ecosystems small plants may have higher survival (Bull et al. 2004). Plant size may affect survival below a threshold and for a short time or size may not affect survival in native populations adapted to competition with non-native species (Davies et al. 1999, Fehmi et al. 2004, Thetford et al. 2005). Smaller plants require fewer resources to propagate and can be produced in greater quantities so determining minimum size for successful native plant establishment will help conserve resources. Size may be less important than plant material transplanted (Bull et al. 2004, Burkhart 2006). Using wild collected seed, Naeth and Wilkinson (2007, 2008) found larger forb and shrub transplants had significantly higher survival, seeded native grasses established in the first year but did not surpass less than 1 % cover and forbs took two years to establish from seed. Intense competition with non-native plant species despite control with glyphosate lead to mortality of most species planted.

2. RESEARCH OBJECTIVES

The objective of this research was to determine effectiveness of select revegetation techniques following specific non-native plant control. Broadcast

seeding was compared to transplanting and impact of plant material type and size for transplanting were evaluated.

3. MATERIALS AND METHODS

3.1 Research Location And Study Sites

This research was conducted in Waterton Lakes National Park at three disturbed foothills fescue grassland sites undergoing restoration (Figure 3-1). Waterton Lakes National Park is located in the Rocky Mountains of southwestern Alberta forming an International Peace Park and United Nations Educational, Scientific and Cultural Organization (UNESCO) World Heritage Site with Glacier National Park. It covers 525 km^2, extending south to Montana and Glacier National Park and west to the Alberta-British Columbia border along the Continental Divide (Achuff et al. 2002). The west boundary borders Akamina-Kishinena Provincial Park in British Columbia. North and east sides border Alberta crown and private lands. In the northwest corner it borders the Blood Indian First Nation Timber Limit in the Belly River area on three sides. Four Ecoregions are found in the park: Foothills Parkland, Montane, Subalpine and Alpine.

Grasslands are characterized by Chernozem soils with predominantly calcareous soil parent materials, good drainage and dark coloured mineral surface horizons high in organic matter. Climate in foothills grasslands is characterized by a short growing season (June to August), cool, wet springs (May to June) and hot, dry late summers (August to September). The region is windy, with maximum daily gusts of 70 to 90 km h^{-1}. Mean annual precipitation is 807.6 mm and minimum and maximum average temperatures were -1.3 ^{o}C and 10.6 ^{o}C, respectively, for 1990 to 2000 (Environment Canada 2012a, 2012b). Daily weather can change rapidly and unpredictably, typical of mountain regions.

A total of 971 vascular plant species are found in the park. In 2002, 20 plant species were discovered, including two new to Alberta and one new to Canada (Achuff et al. 2002). Over 50 % of Alberta wildflower species are found in the park, including 30 rare plant species found nowhere else in Canada.

A former landfill and two borrow pits were selected for study in a 3 km radius with similar topography and soils (Figure 3-1). The sites contain a large and diverse

population of non-native annual and perennial species including *Centaurea maculosa* L. (Spotted knapweed), *Cirsium arvense* L. (Canada thistle), *Bromus inermis* Leyss. (Smooth brome), *Elymus repens* (L.) Gould (Quack grass), *Poa pratensis* L. (Kentucky bluegrass), *Poa compressa* L. (Canada bluegrass) and *Agropyron cristatum* L. (Crested wheat grass).

The landfill site (Trade Waste Pit) was used for disposal of a variety of domestic waste, including building materials, fuel, treated wood, scrap metal, batteries and manure between 1952 and 1999 (Naeth and Jobson 2007). Soil and ground water sampled at the site within the past five years had elevated concentrations of aluminum, copper, zinc, strontium, nickel, silver and iron, reflecting natural background concentrations in the region. The site was classified as very low risk with no required remediation (Canadian Council of Ministers of the Environment 2008). In 2006, waste was removed, the site was recontoured and wood chips and topsoil were applied to rebuild the soil. In fall 2006 and spring 2007, native grasses, forbs and shrubs from wild collected seed were planted (seeded and transplanted) and a revegetation study was initiated (Naeth and Wilkinson 2008).

Borrow pit sites were gravel quarries and disturbance details are not well known. Borrow Pit 1 (Potato Patch Pit) is 1.8 ha in size, located near Chief Mountain Highway Junction. Gravel excavation concluded during the 1960s and the site was officially decommissioned in the late 1970s. It became heavily infested with *Centaurea maculosa*, which was sprayed with herbicide and hand pulled in the 1980s, then plowed and revegetated with native plant species. Borrow Pit 2 (Pincher Creek Pit) is 2.2 ha in size, located near the Bison Paddock. In recent years park staff planted native plugs and tried to control target weeds.

3.2 Experimental Design

In summer 2010, a 0.3 ha block at each site was divided evenly into 12 plots. The randomized block design included control, mowing, steam and herbicide treatments randomly assigned to three replicate plots per block (Figures 3-2 to 3-4). Blocks and plots had even topography and uniform vegetation and soils. A 1 m wide buffer was located between plots, marked with painted wooden stakes. Trade Waste Pit plots were rectangular 4 x 54 m with the exception of one 8 x 27 m plot (herbicide). Pincher Creek Pit plots were rectangular 8 x 27 m. Three plots

at Potato Patch Pit were 9 x 27 m (mow, herbicide, control), three 15 x 18 m (2 control, 1 mow) and three 12 x 18 m (2 herbicide, 1 mow). Steam was not used at Potato Patch Pit due to limited vehicle access.

Initial treatment applications were performed July 26-30, 2010. Mowing was done with two hand held weed eaters, as low to the ground as possible. Native woody plants were left intact. The herbicide Glyphosate Trans Orb 3 % v/v was applied using backpack sprayers and a constant pressure to maintain a fine spray at an approximate rate of 15 L per plot. Steam was applied by Sunnyside Mobile Wash of Lethbridge, Alberta. A 50 cm long bar was dragged across the vegetation in consecutive swaths and steam was released from narrow openings at the end of the bar at a temperature of 325 ^{o}C and a constant pressure of 800 psi.

In 2011 treatments were applied May 15-19. A 5 % v/v Glyphosate solution was applied using the same methods as in 2010, with approximately 20 L of herbicide applied to each plot. Mowing and steaming were repeated June 8-12 and June 21, respectively, when non-native species were most vulnerable, at low carbohydrate levels after spring growth. Mowing methods were the same as in 2010. Steaming was performed by Skywash Services of Calgary, Alberta using a hose held approximately 50 cm from vegetation at a temperature of 225 ^{o}C and pressure of 350 psi.

A wild collected seed mix was broadcast on days with calm winds, June 13-16, 2011, after the second round of treatment applications. 15 native forb and 6 grass species were seeded; 376 g of seed was sown per plot (Table 3-1). Steam plots were seeded June 22 after steaming on June 21. Seed was collected from fescue grasslands in Waterton and Glacier National Parks by trained staff. A diversity of native forbs and grasses present in surrounding undisturbed native grassland was chosen. Due to limited availability of wild collected seed, seeding rates were lower than conventional and recommended rates. Total seed for each species was evenly divided among the 33 plots. Paper bags were labeled by plot and site, seed from each species weighed on a portable scale then deposited in each bag. Plots were raked to roughen the surface and encourage seed deposition and anchoring, then seed was evenly scattered on each plot.

In September 2011, germination tests were conducted for each native plant species used. For each species, 12 healthy seeds were positioned evenly on

paper towel in 5 petri dishes moistened with distilled water. Paper towel was wetted every 1 to 2 days and germinating seeds counted daily for 14 days. Total germinating seeds was divided by total number of seeds per dish for percent germination; mean germination was calculated from five replicates per species.

In late May 2012, one year after the second treatment application, revegetation was repeated using a diversity of native forbs and grasses representative of undisturbed fescue grassland. In total 19 forb and 5 grass species, 3,861 cone seedlings, 429 root trainer seedlings and 891 tray seedlings were transplanted (Table 3-2). Transplanting rate was low (1 plant / m^2) due to resources required to propagate and plant. Native plants were grown from January to May 2012 at the Glacier National Park Native Plant Materials Propagation Center in Montana in standard potting soil (peat moss with perlite) in 15.24 cm deep and 3.8 cm wide cones (Figure 3-5). Other plants were grown at the University of Alberta on large trays, then seedlings were transferred into 11.4 cm deep and 2.5 cm wide root trainers (Beaver Plastics Ltd., Acheson, Alberta) after shoots reached approximately 4 cm (Figure 3-5). Some seedlings were transplanted in the field directly from trays. This gave three different types of transplants: cone seedlings, root trainer seedlings and tray seedlings. Effect of different types of transplants on seedling survival was evaluated. Plants were grown in a greenhouse, watered every 3 to 4 days with temperature maintained at 20 ^{o}C. Germination rate of plant species seeded in potting soil was recorded. Plants were monitored daily for 35 days and cumulative seeds germinating was recorded.

Transplanting was conducted May 29 to June 5, 2012; the time of likely maximum precipitation, by 10 University of Alberta assistants and 15 Parks Canada staff. Physical grids with 3 x 3 m cells were made on plots with flagging tape and 40.6 cm metal pigtail stakes; planting grids with 1 x 1 m cells were overlaid on each plot and transplant locations mapped. Planting tools included metal trowels, spades, shovels, post hole diggers and hoedads. Holes were dug carefully to minimize impact to surrounding vegetation, equal to root depth and soil was firmly placed around plants. At each site after transplanting, Plantskydd herbivore deterrent was applied with back pack sprayers at manufacturer recommended rates, evenly throughout each plot targeting transplants. The biodegradable deterrent contains concentrated pig blood powder.

3.3 Vegetation Assessments

Vegetation was assessed June 14-20 and July 26-30, 2010 to document plant communities prior to treatment implementation and revegetation. North facing transects (East for Trade Waste Pit) bisecting each plot were established and 0.1 m^2 quadrats systematically placed at equal distance intervals along the transects. At Trade Waste Pit (Figure 2-4), a 54 m long transect was run through the middle of each 4 x 54 m plot. In the 8 x 30 m plot, two transects 30 m long were positioned at 3 and 6 m along the width of the plot, dividing it into three parts. At Potato Patch Pit (Figure 2-3), three transects were positioned every 3 to 4 m along the width of 15 x 18 m and 12 x 18 m plots and quadrats positioned every 3 to 4 m; 9 x 27 m plots had two transects with quadrats every 5 m. At Pincher Creek Pit (Figure 2-2) all plots were 8 x 30 m and two 30 m transects were positioned at 3 and 6 m along the width of each plot, with quadrats positioned every 5 m along the transect.

Individual plant species and ground (bare ground, moss, vegetation, litter, thatch, rock) cover were ocularly estimated. Botanical nomenclature followed Kuijt (1982), Moss (1983) and Tannas (2003). Ten quadrats per plot were assessed at Trade Waste Pit and Potato Patch Pit; at Pincher Creek Pit between 5 and 10 quadrats were assessed per plot due to heavy rain.

Vegetation was assessed using the same methods as those used in 2010, August 2-9, 2011 after seeding and July 16-28, 2012 after transplanting to determine effectiveness of revegetation. The 10 quadrats per plot in 2011 were increased to 12 per plot in 2012 based on species area curves. Native cover was compared among years to determine if seeding and transplanting increased native species abundance.

Transplants were monitored June 19-27 and July 16-28, 2012. For the assessment, grids were re-established and plants were located using maps. Seedling health was assessed with the following scale. A value of 0 was assigned to plants that could not be located, 1 for dead plants (0 % live material), 2 for necrotic plants (< 25 % live material), 3 for severely chlorotic or wilting plants (25-50 % live material), 4 for chlorotic or wilting plants (51-75 % live material) and 5 for healthy plants (> 75 % live material).

3.4 Meteorological Conditions

Meteorological data were obtained from Environment Canada National Climate Data and Information Archive (Environment Canada 2012a, 2012b). Data were collected from a weather station (Waterton Park Gate Alberta) located at the Waterton Lakes National Park Park Gate (49°07'52.080" N, 113°48'31.010" W, elevation 1,289 m). Mean daily temperatures were averaged for mean monthly temperature and total monthly precipitation and maximum wind gusts recorded at the weather station were obtained from the online archive. Long term climate normals (1971-2000) were obtained from an inactive weather station (Waterton River Cabin) approximately 0.5 km north of Potato Patch Pit (49°07'00.000" N, 113°50'00.000" W, elevation 1,281 m).

3.5 Soil Sampling And Analyses

Soil was sampled in September 2011. Transects and quadrat positions from vegetation assessments were used to randomly select soil sampling locations. Soil was sampled to characterize study sites and undisturbed fescue grassland. A total of 42 samples were collected, one from each control, herbicide, mow and steam plot at each site, giving 12 each for Trade Waste Pit and Pincher Creek Pit and 9 for Potato Patch Pit. Three samples were taken from each undisturbed plot at each site. For each sample, a 15 cm deep hole was dug with trowels and a sharp knife and 500 g of soil removed, placed in labeled plastic bags and stored at 4 ^{o}C until processing. The hole was filled after sampling.

Samples were analyzed by Exova Laboratories in Edmonton, Alberta. Total nitrogen and carbon were determined by Leco combustion (Bremner 1996). Inorganic carbon was determined through carbon dioxide release; total organic carbon was calculated by subtracting inorganic carbon from total carbon (Loeppert and Suarez 1996). C:N ratio was determined by dividing total carbon by total nitrogen. Total phosphorus was determined with strong acid extraction and inductively coupled plasma mass spectrometry (US Environmental Protection Agency 1996). Sand, silt and clay were determined by hydrometer (Kroetsch and Wang 2008). Electrical conductivity, pH, sodium adsorption ratio and available soil calcium, magnesium, sodium and potassium were determined in saturated paste (Miller and Curtin 2008).

3.6 Statistical Analyses

Statistical analyses were performed using SAS software v. 9.2 (SAS Institute Inc. 2003) to test if broadcast seeding and transplanting increased native plant cover in non-native plant management plots and to assess responses of management treatments (mow, steam, herbicide, control) to seeding and transplanting. Mean native plant cover was determined for each plot in 2010 (before revegetation), 2011 (after seeding) and 2012 (after transplanting). Data were assessed for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests, respectively. For all statistical tests alpha was 0.05. For each year of data (2010 to 2012), Shannon index of species diversity and species richness were determined; parameters were calculated in Microsoft Excel.

Mean native plant cover was analyzed using analysis of variance (ANOVA). Two-way ANOVA was performed using proc mixed with site and site x treatment specified in the random statement to remove site variation. The statistical model was native plant cover = management treatment + year + management treatment x year. Following ANOVA, Student's t-tests were performed to compare treatment means using LSMEANS and pdiff statements in SAS. Resulting p values were adjusted using Bonferroni correction. This multiple comparisons procedure gives strong inference by reducing probability of inaccurately finding significance (SAS Institute Inc. 2009). Data were unbalanced as steam was not used at Potato Patch Pit, making data unsuitable to run in SAS using proc mixed for two-way ANOVA. Therefore, ANOVA was run twice, once without steam data with all sites and once with steam data and without data from Potato Patch Pit.

Transplant health data were used to determine survival and performance of transplants and native plant species and how well species performed in each management treatment and site. Analyses were performed twice, once for June data and once for July data to assess changes in transplant health one and two months after planting, respectively. Mean health score (0–5) was determined for each treatment, site and species and for plant material types (cone, tray, root trainer) for each species.

To model influence of species, site, treatment and plant material type on transplant survival, Logit function (log-linear model analysis) and proc catmod in

SAS were used. Logit function models influence of categorical independent variables on probability of obtaining each level of a categorical dependent variable (SAS Institute Inc. 2009). In this case, influence of species, site, management treatment and plant material type on probability of a plant surviving after transplanting. Transplant health data were converted to binary response data indicating survival by grouping scores into two categories: dead (scores 0–1) and alive (scores 2–5). Due to significant interactions and reduced response frequencies (data sparsity), site and species variables were removed from the model and data from each site were analyzed individually. Contrasts were used to perform multiple comparisons for significant effects of independent variables.

4. RESULTS

4.1 Meteorological Conditions

Mean monthly temperature was similar to average conditions during the study years with the exception of 2011 and 2012 being slightly warmer (1-2 ^{o}C) in July and August (Table 3-3). 2010 was wetter than normal with over 100 mm more precipitation during May to August than the long term average. 2011 precipitation was similar to average conditions but July precipitation was lower than normal and 2012 was drier than normal with 100 mm less precipitation than average. The Waterton region has high wind gusts (Table 3-3). A high frequency of extreme weather occurred in 2012; thunder and hail storms were more intense and more often and there were many hot, dry days followed by heavy rain.

4.2 Pre-Treatment Site Conditions

Soils of undisturbed fescue grassland surrounding the study sites were Black Chernozems typical of the region. Soils at the study sites were modified by disturbance as indicated by lower organic matter content relative to undisturbed areas (Table 3-4). All sites had large amounts of rocky, coarse grained sediment typical of the region. Soil was similar throughout the study area and did not have a major impact on revegetation. Non-native graminoids dominated all sites, followed by native forbs, non-native forbs and native graminoids (Table 3-5). Non-native species cover was greatest at Trade Waste Pit and least at Pincher Creek Pit, with the opposite trend for native species.

4.3 Broadcast Seeding

Broadcast seeding did not increase native cover, diversity or richness in any treatment (Figure 3-5, Tables 3.6, B.1). Germination of wild collected seed was low, at < 20 % for 8 species (Table 3-1) and > 50 % for 9 species. Emergence observed while growing transplants in 2012 showed some native forbs, such as *Anemone multifida* Poir (Pacific anemone) did not germinate and emerge for over 20 days, but obtained > 50 % emergence after this (data not shown). Seed of non-native species such as *Phleum pratense* L. (Common timothy) were present in wild collected seed. Although broadcast seeding did not increase native plant cover after seeding, *Penstemon nitidus* Douglas ex Benth (Waxleaf penstemon) was observed at Potato Patch Pit, *Bromus carinatus* Hook. & Arn. (Mountain brome) at Trade Waste Pit and *Penstemon confertus* Douglas ex Lindl. (Yellow penstemon) at all sites (where they were previously absent).

4.4 Transplanting

Native plant species cover, diversity and richness increased numerically after transplanting relative to previous levels in 2010 and 2011 (Figure 3-5, Tables 3-6, B.1). Transplanting re-introduced six native species to the disturbed sites that were previously absent (Table 3-2).

Species with highest health scores were *Festuca* spp., *Heterotheca villosa* (Pursh) Shinners (Hairy false golden aster), *Koeleria macrantha* (Ledeb.) Schult. (Prairie june grass) and *Eriogonum umbellatum* Torr. (Sulphur flower buckwheat) (Tables 3-7, 3-8). Several others performed well and were already present on disturbed sites. Few *Lupinus sericeus* Pursh (Silky lupine) and *Linum lewisii* Pursh (Prairie flax) transplants survived and *Danthonia parryi* transplants had flimsy root systems that became easily exposed. *Elymus trachycaulus* (Link) Gould ex Shinners (Slender wheat grass), *Gaillardia aristata* Pursh (Blanket flower), *Penstemon confertus*, *Heterotheca villosa* and *Erigeron* spp. L. (Fleabane) flowered and produced seed.

Management effect on transplanted seedlings varied. For most sites during both monitoring periods, management did not significantly affect transplant survival (Tables 3-9, 3-10). Canopy cover did not vary greatly among treatments (Table 3-11), although, there were exceptions. Transplant survival was significantly higher

in herbicide plots than the control at Pincher Creek Pit (Tables 3-9, B.2, B.3). Herbicide had lower canopy cover at this site (Table 3-11). Transplant survival at Potato Patch Pit was significantly higher with mowing than herbicide (Tables 3-10, B.3); non-native cover was lower with mowing than herbicide or in the control (Table 3-11). At Trade Waste Pit, management did not significantly affect transplant survival, but a significant interaction occurred between management treatment and plant material (Tables 3-10, B.3). Transplant survival was highest at Pincher Creek Pit and lowest at Trade Waste Pit (Tables 3-9, 3-10, B.4). Native species performed best where they were naturally well established; for example, *Amelanchier alnifolia* at Potato Patch Pit (Tables 3-7, 3-8).

Type of plant material significantly affected survival (Tables 3-12, B.2, B.3, B.4). Tray seedlings had a mean survival rate of 33 %, health score of 1 and significantly lower survival in most sites and treatments than cone and root trainer seedlings (Tables 3-9, 3-10, 3-12). The effect was the same for all species except *Potentilla* spp. (Cinquefoil), which had similar health scores regardless of plant material type (Table 3-12). Mean survival rate of cone and root trainer seedlings was 79 % and mean health scores were 4 and 3, respectively (Tables 3-8, 3-9, 3-12). There was no significant difference in transplant survival between cone and root trainer seedlings in June (Table 3-9). In July, at Pincher Creek Pit cones had significantly higher survival than root trainers (Tables 3-10, B.4). At Trade Waste Pit, plant material performance varied with treatment (Table 3-10).

Mortality increased over time, with species scoring 4 or greater decreasing from 13 out of 24 in June to 9 in July (Tables 3-7, 3-8). *Monarda fistulosa* L. (Wild bergamot), *Artemisia michauxiana* Besser (Michaux's sagebrush) and *Galium boreale* L. (Northern bedstraw) recovered after shoot death with new shoots by July. Some species such as *Penstemon confertus* senesced, with neighbouring naturally occurring plants completing their above ground life cycle by July.

Effectiveness of the herbivore deterrent was difficult to assess. Approximately 25 % of plants showed signs of grazing but had over 75 % live tissue (data not shown). Number of unhealthy or dead plants (score of less than 3) and those heavily grazed was < 5 %. Transplants in a separate experiment studying native cultivar seed planted near the study plots (Chapter four) were not sprayed with deterrent and 1 % died from grazing and 5 % were grazed but scored 5 (healthy).

5. DISCUSSION

5.1 Transplanting

Transplanting increased native cover and species richness and diversity during foothills fescue grassland restoration and facilitated re-establishment of native species on disturbed sites. Transplanting was more effective than seeding in harsher environments with greater competition (Middleton et al. 2010, Tannas 2011). Transplant performance was strongly species dependent, as found in previous research (Davies et al. 1999, Page and Bork 2005, Naeth and Wilkinson 2008). Species producing seedlings with durable, well developed root systems such as *Koeleria macrantha* and *Festuca* bunch grasses or rhizomatous species such as *Heterotheca villosa* had higher health scores and survival than slower growing species such as *Danthonia paryii* and *Lupinus sericeus*. Similar results were found for these species in other studies (Paschke et al. 2000, Ewing 2000).

Management to control non-native plants on a restoration site can enhance transplant seedling survival if canopy cover is reduced, as with herbicide and mowing in this study. Herbicide and mowing increase native transplant survival by reducing resident vegetation (Davies et al. 1999, Erichsen-Arychuck 2001, Wilson and Gerry 1995, Stromberg and Kephart 1996, Ewing 2002, Huddleston and Young 2005, Page and Bork 2005, Jusaitis and Polomka 2008). Mowing may be an environmentally sensitive alternative to herbicide to reduce non-native plant competition. Lack of effect of management on seedling survival was likely due to lack of canopy cover reduction which led to failure in creating hospitable conditions for seedlings (Davies et al. 1999, Middleton et al. 2010).

This research showed transplant survival will depend on specific conditions at a restoration site. Grasslands with higher biomass and lower species diversity like Trade Waste Pit and Potato Patch Pit often had lower seedling and transplant survival (Davies et al. 1999, Middleton et al. 2010).

Plant material type will significantly impact revegetation and larger seedlings with greater root mass are necessary to promote health and survival. Larger size leading to greater transplant survival is consistent with several studies (Davies et al. 1999, Steed and DeWald 2003, Page and Bork 2005), although there are exceptions (Bull et al. 2004). In this study, root depth range of 10 to 15 cm was

necessary for transplant survival. This size is likely at the threshold of becoming too large, as rocky soils do not allow for deeper holes during planting.

Mortality increased with time, consistent with previous research at this location (Naeth and Wilkinson 2008). While short term transplanting was successful, long term effectiveness cannot be assessed from this short term study. Follow up non-native plant control is likely critical to prevent seedling mortality by competition with non-native plants. Mortality from grazing was not substantial and is not often assessed in grassland restoration studies. However, grazing was observed, so use of an animal deterrent is always a worthwhile preventative measure.

5.2 Broadcast Seeding

Broadcast seeding was not a useful technique in establishing native plant cover. Several abiotic and biotic factors affect germination, emergence and survival and may have contributed to lack of native plant establishment from broadcast seeding (Elmarsdottir et al. 2003). Pre-existing vegetation, including non-native plant species, likely created too competitive an environment for seedlings to survive. Precipitation was lower in July 2011, one month after seeding, followed by a dry 2012, which may have negatively impacted seeding results. Wild collected seed can establish cover when directly planted at field sites (Cole and Spildie 2000, Naeth and Wilkinson 2008); thus a higher seeding rate might have been more effective, had seed been available. Although transplanting was more successful than seeding, both broadcast seeding and transplanting introduced native species.

5.3 Restoration And Management Implications

Based on results of this study, broadcast seeding is not recommended for foothills fescue grassland restoration unless higher seeding rates can be used. Transplanting is strongly recommended as a method of quickly establishing native plant cover if sufficient resources are available and transplants can be maintained by controlling competition with non-native plants. Transplanting will lead to quicker and greater increases in native plant populations, species richness and diversity relative to broadcast seeding. Species with high health scores in this study should be used for revegetation. Non-native plant seed being gathered during wild harvesting requires control. Native grasses with strong roots

performed well in this study and should be a focus of revegetation, as native forbs readily colonize disturbed sites from surrounding areas.

Non-native plants should be controlled prior to revegetation; with herbicide and mowing recommended to increase transplant survival. Mowing can be a more environmentally sensitive option than herbicide. Seasonal weather patterns should be considered and revegetation conducted in years with greater precipitation. Site conditions should be carefully evaluated and management and revegetation plans should be based on pre-existing vegetation. Plant material type strongly influences seedling survival and recovery of root stock is important in harsh environments where environmental stresses are high. A plug with sufficient root size is necessary, but roots should not be too large as average minimum depth for planting is 0-15 cm due to the rocky soils. Animal deterrents can be used, although low rates of grazing mortality are expected in foothills fescue grassland.

5.4 Future Research

Future research with seeding is necessary to determine minimum rates to establish native plant cover. Combinations of revegetation and management practices could be tested. The impact of site preparation and non-native plant management on canopy characteristics including increases in gap number and width should be researched to better understand how management actions affect survival of planted seedlings. Long term monitoring will provide greater knowledge on effectiveness of revegetation methods used in this study.

Not all methods will have the same outcome in every restoration, even in the same ecosystem type and geographic area. To develop sound knowledge, restoration method effectiveness should be carefully evaluated in the context of restoration location and activities conducted. Control of non-native plants and ecosystem restoration are imperative for conserving biodiversity and represent two of the greatest challenges of this century (D'Antonio and Meyerson 2002). Revegetation should be recognized as an important tool for both restoring and maintaining biodiversity in ecosystems. Although challenges are formidable and progress may be slow, the need for action is great and knowledge from every study can be built upon until effective approaches are developed.

6. CONCLUSIONS

- Broadcast seeding was not effective at increasing native plant cover following non-native plant control.
- Broadcast seeding was not impacted by type of management treatment.
- Transplanting was effective for increasing native plant cover, species diversity and richness following non-native plant control.
- When non-native cover and vegetation height were adequately decreased to reduce competition from non-native plants relative to surrounding conditions, transplant survival was improved.
- Type of plant material significantly affected transplant survival.

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Table 3-1. Native plant species seeding rates and mean percent germination.

Species	Year Collected	Germination (%)	Seeding Rate (g/plot)	Seeding Rate (g/ha)	Seeds/g
Achnatherum nelsonii	2010	8	7.3	291.2	331
Agoseris glauca	n/a	12	0.4	15.6	653
Anemone multifida	2002	0	2.6	103.2	800
Bromus carinatus	2002	10	212.7	8,508.0	151
Danthonia parryi	2005	86	30.3	1,212.0	220
Elymus trachycaulus	n/a	18	21.0	840.0	353
Eriogonum umbellatum	2010	2	5.0	200.0	422
Festuca campestris	n/a	56	11.1	443.6	439-661
Festuca idahoensis	2005	78	35.6	1,424.4	992
Gaillardia aristata	n/a	56	15.9	635.2	291
Geranium viscosissimum	n/a	38	0.3	13.2	121
Geum triflorum	n/a	86	0.2	8.8	991
Hedysarum spp.	n/a	86	1.2	48.4	172
Koeleria macrantha	n/a	84	2.2	87.2	5,104
Linum lewisii	n/a	78	1.4	57.6	319
Lupinus sericius	n/a	22	13.0	520.0	45
Oxytropis campestris	2005	4	0.2	6.0	523
Penstemon confertus	2006, 2008	52	5.4	216.8	10,204
Penstemon nitidis	2010	0	2.6	102.0	3,968
Potentilla gracilis	2006	32	3.0	120.0	2,646
Potentilla spp.	2006	20	4.6	182.0	2,646-3,748
Thalictrum occidentale	2010	n/a	0.03	1.2	388

n/a = not available.

Table 3-2. Species transplanted and their transplanting rate.

Species	Transplanting Rate (plants/plot)		
	Cone	Root Trainer	Tray
Achillea millefolium	0	1	2
*Elymus trachycaulus**	13	0	2
Amelanchier alnifolia	2	0	0
*Anemone multifida**	0	0	2
*Artemisia michauxiana**	8	0	0
Bromus carinatus	0	0	0
*Danthonia parryi**	11	4	2
Erigeron spp.*	3	0	0
Eriogonum flavum	1 per site	0	0
Eriogonum umbellatum	3 per site	0	0
*Festuca campestris**	15	0	0
Festuca idahoensis	11	3	3
Gaillardia aristata	14	0	0
Galium boreale	7	0	0
Geranium viscosissimum	2	0	0
Heterotheca villosa	8	0	0
Koeleria macrantha	10	3	7
Linum lewisii	0	1	1
Lupinus sericeus	1	0	0
Monarda fistulosa	4	1	3
Penstemon confertus	3	0	1
Potentilla spp.	2	1	3
Potentilla arguta	1	0	0
Potentilla gracilis	2	0	1
Rosa woodsii	2	0	0

* Species previously absent from sites until transplanted.

Table 3-3. Summer precipitation, temperature, wind gust speed and long term climate normals at Waterton Lakes National Park during the study period.

Year	Month	Total Monthly Precipitation (mm)	Mean Temperature (^{o}C)	Maximum Wind Gust Speed (km/h)
2010	May	119.8	6.0	96
	June	157.8	12.4	96
	July	92.6	15.3	83
	August	68.6	14.4	74
2011	May	126.2	8.0	89
	June	93.2	12.2	76
	July	19.6	16.2	95
	August	72.0	16.7	72
2012	May	37.6	8.9	72
	June	88.8	12.4	87
	July	38.2	17.1	102
	August	42.0	16.9	87
Long Term Normals	May	94.5	8.9 ± 1.3	
	June	80.8	12.5 ± 1.4	
	July	70.8	15.2 ± 1.2	
	August	69.0	14.5 ± 1.8	

Data from Park Gate and former Waterton River Cabin Weather Stations (Environment Canada 2012a, b).
± = standard deviation.

Table 3-4. Soil chemical and physical properties at research sites in September 2011.

Parameter	Pincher Creek Pit		Potato Patch Pit		Trade Waste Pit	
	Disturbed	Undisturbed	Disturbed	Undisturbed	Disturbed	Undisturbed
C:N Ratio	14.4 ± 3.8	11.5 ± 0.4	23.7 ± 33.6	11.1 ± 0.1	15.1 ± 3.4	13.2 ± 0.7
Total Nitrogen (%)	0.2 ± 0.1	0.7 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.2
Organic Matter (%)	4.5 ± 2.9	16.9 ± 2.5	2.6 ± 1.6	10.0 ± 1.4	6.1 ± 2.6	11.7 ± 4.6
Total Inorganic Carbon (%)	0.6 ± 0.3	0.1 ± 0.0	0.8 ± 0.7	0.1 ± 0.0	0.4 ± 0.3	0.1 ± 0.1
Total Organic Carbon (%)	2.2 ± 1.5	8.5 ± 1.2	1.3 ± 0.8	5.0 ± 0.7	3.1 ± 1.3	5.9 ± 2.3
Total Phosphorus (mg/kg)	606.7 ± 59.2	1013.3 ± 70.4	405.8 ± 65.6	750.0 ± 74.8	828.3 ± 124.9	1093.3 ± 273.5
Sand (%)	82.2 ± 4.5	59.7 ± 4.0	64.4 ± 12.3	59.0 ± 2.7	63.2 ± 4.3	63.2 ± 4.0
Silt (%)	13.8 ± 3.6	36.5 ± 3.3	26.8 ± 9.3	37.7 ± 3.0	29.3 ± 4.0	30.5 ± 3.9
Clay (%)	4.0 ± 1.1	3.8 ± 0.9	8.8 ± 3.6	3.3 ± 0.5	7.42 ± 1.2	6.3 ± 0.9
Hydrogen Ion Concentration (pH)	7.9 ± 0.1	6.8 ± 0.2	5.9 ± 0.2	7.2 ± 0.1	7.5 ± 0.5	6.6 ± 0.6
Electrical Conductivity (dS/m)	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.3	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
Sodium Adsorption Ratio	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Available Calcium (mg/kg)	31.1 ± 11.8	57.6 ± 8.8	40.6 ± 28.0	49.3 ± 11.9	25.3 ± 10.3	17.9 ± 4.8
Available Magnesium (mg/kg)	5.3 ± 2.2	14.4 ± 2.9	8.5 ± 6.0	13.0 ± 2.7	7.1 ± 3.4	5.9 ± 1.0
Available Sodium (mg/kg)	0.3 ± 0.5	3.0 ± 0.8	0.8 ± 0.5	1.7 ± 0.5	1.3 ± 0.4	1.7 ± 0.5
Available Potassium (mg/kg)	5.5 ± 2.8	33.0 ± 12.4	6.2 ± 5.7	16.7 ± 7.4	11.7 ± 7.5	7.0 ± 0.8

Numbers are mean ± standard deviation.

Table 3-5. Pre-treatment canopy cover of native and non-native vegetation.

Site	Treatment	Native Forb	Native Graminoid	Non-Native Graminoid	Non-Native Forb
Pincher Creek Pit	Control	16.0 ± 9.9	2.5 ± 2.9	7.0 ± 4.8	2.2 ± 4.2
	Mow	18.5 ± 9.1	3.8 ± 5.5	8.6 ± 7.1	1.7 ± 4.2
	Herbicide	12.8 ± 9.9	2.7 ± 2.6	7.5 ± 4.5	1.9 ± 2.8
	Steam	18.9 ± 12.4	4.2 ± 4.5	8.4 ± 5.4	1 ± 3.4
Potato Patch Pit	Control	3.9 ± 5.8	1.0 ± 2.4	18.7 ± 15.2	1.0 ± 4.1
	Mow	3.9 ± 7.2	0.6 ± 1.5	13.5 ± 5.2	2.1 ± 3.6
	Herbicide	8.2 ± 18.6	1.3 ± 4.4	16.6 ± 7.6	1.1 ± 2.5
Trade Waste Pit	Control	2.5 ± 5.8	0.5 ± 2.2	27.3 ± 8.8	1.7 ± 5.1
	Mow	0.8 ± 1.3	0.3 ± 1.1	29.4 ± 7.9	1.4 ± 3.7
	Herbicide	1.1 ± 2.4	0.4 ± 1.9	25.9 ± 13.6	2.4 ± 5.7
	Steam	1.1 ± 3.5	0	28.5 ± 11.6	4.5 ± 9.4

Numbers are mean ± standard deviation.
No statistical analyses were performed.

Table 3-6. Effect of treatments on biodiversity and native and non-native species richness.

	Pincher Creek Pit						Potato Patch Pit				Trade Waste Pit				
Species	All	All	C	H	M	S	All	C	H	M	All	C	H	M	S
2010															
Shannon Diversity (H')	0.99	1.71	1.77	1.52	1.8	1.74	0.71	0.66	0.67	0.82	0.72	0.66	0.76	0.56	0.81
Species Richness															
Native Forb	39	33	21	16	23	24	15	8	8	11	3	3	3	3	3
Native Grasses	12	7	3	5	2	3	5	2	3	3	4	2	2	1	0
Non-Native Forb	13	5	4	4	1	2	6	2	4	5	6	2	5	3	3
Non-Native Grasses	7	5	4	5	5	5	7	4	7	5	7	4	7	5	5
2011															
Shannon Diversity (H')	0.86	1.04	1.35	0.3	1.45	1.26	0.58	0.86	0.1	0.86	0.68	0.86	0.34	0.7	0.82
Species Richness															
Native Forb	40	34	21	16	25	24	19	11	6	14	7	2	5	3	2
Native Grasses	10	5	5	3	3	3	5	4	0	3	4	3	0	3	2
Non-Native Forb	14	7	4	4	6	2	9	4	5	4	11	5	7	5	6
Non-Native Grasses	14	11	6	6	8	7	11	6	4	9	12	9	5	8	10
2012															
Shannon Diversity (H')	1.19	1.34	1.53	0.95	1.51	1.39	1.12	1	1.01	1.35	1.09	1.02	1.02	1.22	1.11
Species Richness															
Native Forb	63	43	30	20	31	26	41	26	18	27	15	7	9	6	7
Native Grasses	20	14	6	7	8	7	14	6	9	8	10	8	7	3	4
Non-Native Forb	28	10	6	8	7	6	17	9	16	10	25	13	21	10	12
Non-Native Grasses	16	11	10	8	9	9	12	10	12	10	14	12	13	12	12

C = Control, H = Herbicide, M = Mow, S = Steam.

Table 3-7. Mean transplant health for each species, treatment and site in June 2012.

Species	Overall	Overall				Potato Patch Pit				Pincher Creek Pit					Trade Waste Pit				
		C	H	M	S	Overall	C	H	M	Overall	C	H	M	S	Overall	C	H	M	S
Achillea millefolium	1	2	1	2	2	2	2	1	1	1	1	1	2	1	2	4	1	3	3
Elymus trachycaulus	4	3	4	3	3	4	4	4	4	3	3	4	3	3	4	4	4	3	3
Amelanchier alnifolia	4	4	5	4	4	5	5	5	5	4	4	5	4	4	4	4	4	4	4
Anemone multifida	1	0	1	1	1	1	1	1	0	1	0	1	1	0	1	1	0	1	1
Artemisia michauxiana	4	4	3	4	4	5	5	4	4	3	3	3	3	4	3	4	2	3	4
Danthonia parryi	4	3	4	3	4	4	3	4	3	4	3	4	3	3	4	3	4	4	4
Erigeron spp.	4	3	4	4	3	5	5	5	5	4	3	5	5	4	3	2	4	3	2
Eriogonum flavum	4	5			3	5	5			3				3					
Eriogonum umbellatum	5	5	5	5	4	5	5	5	5	5			5		4				4
Festuca campestris	5	5	5	4	5	5	4	5	5	5	4	5	5	5	4	4	4	3	4
Festuca idahoensis	4	3	4	4	4	3	3	4	3	4	4	4	4	4	4	4	5	4	3
Gaillardia aristata	3	3	3	3	3	4	4	4	4	3	4	4	3	3	3	3	2	2	4
Galium boreale	2	2	2	2	2	3	4	2	3	3	2	2	3	3	2	2	2	2	2
Geranium viscosissimum	3	3	3	4	4	3	5	2	5	4	3	5	4	2	3	3	2	4	4
Heterotheca villosa	4	4	5	4	4	4	4	5	4	5	5	5	5	5	4	3	4	4	4
Koeleria macrantha	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	3	4
Linum lewisii	1	1	0	1	1	1	2	0	1	0	0	0	1	0	1	1	1	1	1
Lupinus sericeus	1	0	2	1	0	0	1	1	0	0	0				1	0	2	1	0
Monarda fistulosa	2	2	2	1	2	2	2	2	2	1	2	2	1	1	2	2	2	1	2
Penstemon confertus	3	3	3	3	3	4	4	3	4	3	3	3	2	2	3	3	3	3	4
Potentilla spp.	2	2	2	2	3	2	2	2	2	2	2	2	2	3	2	2	2	3	3
Potentilla arguta	4	5	4	4	4					4	4	4		4	4	5	4	4	4
Potentilla gracilis	4	4	5	4	4	5	5	5	4	4	4	4	3	3	4	4	4	4	4
Rosa woodsii	4	4	4	4	3	5	5	4	5	4	4	5	3		3	3	2	3	3

C = Control, H = Herbicide, M = Mow, S = Steam.

Table 3-8. Mean transplant health for each species, treatment and site in July 2012.

Species	Overall					Potato Patch Pit				Pincher Creek Pit					Trade Waste Pit				
	Overall	C	H	M	S	Overall	C	H	M	Overall	C	H	M	S	Overall	C	H	M	S
Achillea millefolium	2	3	1	2	2	2	3	2	3	1	2	1	1	0	3	5	0	1	5
Elymus trachycaulus	3	3	4	3	3	4	4	4	4	4	3	4	4	4	3	3	4	2	2
Amelanchier alnifolia	4	4	4	4	4	5	5	5	5	4	3	4	3	4	4	2	4	4	4
Anemone multifida	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	0	1	2
Artemisia michauxiana	4	4	4	4	4	4	5	4	4	4	4	4	4	4	3	3	3	4	4
Danthonia parryi	3	3	4	3	3	4	3	4	3	3	4	4	3	3	3	2	3	3	3
Erigeron spp.	3	3	3	4	4	4	5	3	5	5	4	4	5	5	2	2	3	2	2
Eriogonum flavum	4	5			2	5	5			2				2					
Eriogonum umbellatum	5	5	5	5	2	5	5	5	5	5			5		2				2
Festuca campestris	5	4	4	4	4	4	4	5	4	5	9	9	9	10	2	2	3	2	2
Festuca idahoensis	3	3	4	3	4	3	3	4	3	4	4	4	4	4	3	3	4	3	3
Gaillardia aristata	3	3	3	3	4	4	4	4	4	4	4	4	4	3	3	2	3	2	4
Galium boreale	2	2	2	3	2	4	4	2	5	3	2	2	4	3	2	1	2	2	1
Geranium viscosissimum	2	2	2	3	3	3	4	2	4	3	3	3	4	3	2	1	2	2	3
Heterotheca villosa	4	4	4	4	4	4	4	4	4	5	5	4	5	5	3	2	4	3	3
Koeleria macrantha	3	3	3	3	3	3	3	3	4	3	3	4	3	4	3	2	4	3	3
Linum lewisii	2	3	1	2	1	3	3	3	3	1	2	0	2	0	1	3	0	1	2
Lupinus sericeus	1	1	0	1	0	1	1	1	2	5	5				0	0	0	1	0
Monarda fistulosa	2	2	3	2	2	2	3	2	3	2	2	2	2	2	3	2	3	3	3
Penstemon confertus	2	2	2	2	2	3	4	3	3	2	2	2	2	2	2	1	2	2	3
Potentilla spp.	2	2	2	3	3	2	2	2	3	3	3	2	2	3	2	1	2	3	3
Potentilla arguta	5	5	5	4	5					4	4	4		4	5	5	5	4	5
Potentilla gracilis	5	5	5	4	4	5	5	5	5	4	4	5	4	2	4	4	4	4	4
Rosa woodsii	4	4	4	4	3	5	5	5	5	4	4	4	3		3	2	3	3	3

C = Control, H = Herbicide, M = Mow, S = Steam.

Table 3-9. June transplant survival based on treatment and plant material.

Site	Treatment	Survival (%)	Plant Material	Survival (%)
PCP	Control	62.1 ± 4.6 b	Cone	87.4 ± 1.8 a
			Root trainer	77.4 ± 7.5 a
			Tray	21.7 ± 4.3 b
	Herbicide	72.7 ± 3.7 a	Cone	90.7 ± 1.6 a
			Root trainer	93.3 ± 4.6 a
			Tray	34.0 ± 4.8 b
	Mow	65.9 ± 4.5 ab	Cone	91.7 ± 1.5 a
			Root trainer	80.0 ± 7.3 a
			Tray	25.8 ± 4.5 b
	Steam	70.3 ± 3.5 ab	Cone	92.8 ± 1.5 a
			Root trainer	93.6 ± 4.4 a
			Tray	24.7 ± 4.6 b
PP	Control	67.2 ± 3.8	Cone	87.7 ± 1.8 a
			Root trainer	86.4 ± 5.2 a
			Tray	27.6 ± 4.5 b
	Herbicide	69.5 ± 3.9	Cone	89.5 ± 1.6 a
			Root trainer	87.8 ± 5.1 a
			Tray	31.2 ± 4.8 b
	Mow	69.7 ± 4.0	Cone	86.5 ± 1.8 a
			Root trainer	88.9 ± 5.2 a
			Tray	33.7 ± 4.9 b
TWP	Control	65.4 ± 5.2	Cone	69.7 ± 2.4 a
			Root trainer	75.6 ± 6.7 a
			Tray	50.9 ± 6.6 b
	Herbicide	65.7 ± 4.9	Cone	76.4 ± 2.1 a
			Root trainer	75.0 ± 6.3 a
			Tray	45.8 ± 6.5 b
	Mow	61.9 ± 5.3	Cone	68.5 ± 2.4 a
			Root trainer	72.3 ± 6.5 a
			Tray	44.9 ± 7.1 b
	Steam	69.1 ± 4.9	Cone	73.0 ± 2.3 a
			Root trainer	84.4 ± 5.4 a
			Tray	50.0 ± 6.9 b

PCP = Pincher Creek Pit, PP = Potato Patch Pit, TWP = Trade Waste Pit.
Numbers are mean ± standard error.
Mean survival rates followed by different letters are significantly different within treatment or plant material for a site and monitoring period ($p<0.01$).

Table 3-10. July transplant survival based on treatment and plant material.

Site	Treatment	Survival (%)	Plant Material	Survival (%)
PCP	Control	65.8 ± 4.6	Cone	87.4 ± 1.8 a
			Root trainer	80.7 ± 7.1 b
			Tray	29.3 ± 4.8 c
	Herbicide	66.1 ± 4.3	Cone	89.1 ± 1.7 a
			Root trainer	83.3 ± 6.8 b
			Tray	25.8 ± 4.4 c
	Mow	60.4 ± 4.5	Cone	90.5 ± 1.6 a
			Root trainer	73.3 ± 8.1 b
			Tray	17.2 ± 3.9 c
	Steam	66.2 ± 3.9	Cone	92.5 ± 1.5 a
			Root trainer	87.1 ± 6.0 b
			Tray	19.1 ± 4.2 c
PP	Control	65.9 ± 4.1 ab	Cone	87.4 ± 1.8 a
			Root trainer	81.8 ± 5.8 a
			Tray	28.6 ± 4.6 b
	Herbicide	64.9 ± 3.9 b	Cone	86.6 ± 1.8 a
			Root trainer	85.4 ± 5.5 a
			Tray	22.6 ± 4.3 b
	Mow	74.4 ± 3.5 a	Cone	88.8 ± 1.7 a
			Root trainer	94.4 ± 3.8 a
			Tray	40.0 ± 5.0 b
TWP	Control	48.4 ± 5.5	Cone	46.7 ± 2.6 ab
			Root trainer	63.4 ± 7.5 a
			Tray	35.1 ± 6.3 b
	Herbicide	55.5 ± 5.3	Cone	69.5 ± 2.3 a
			Root trainer	56.3 ± 7.2 ab
			Tray	40.7 ± 6.4 b
	Mow	52.1 ± 5.5	Cone	57.8 ± 2.5 a
			Root trainer	61.7 ± 7.1 a
			Tray	36.7 ± 6.9 b
	Steam	60.6 ± 5.2	Cone	58.0 ± 2.5 b
			Root trainer	77.8 ± 6.2 a
			Tray	46.2 ± 6.9 b

PCP = Pincher Creek Pit, PP = Potato Patch Pit, TWP = Trade Waste Pit.
Numbers are mean ± standard error.
Mean survival rates followed by different letters are significantly different within treatment or plant material for a site and monitoring period ($p<0.01$).

Table 3-11. July 2012 vegetation cover one year after the second treatment application.

Site	Treatment	Native Forbs and Shrubs	Native Graminoids	Non Native Forbs	Non Native Graminoids
Pincher Creek Pit	Control	17.9 ± 3.2	2.5 ± 0.4	0.6 ± 0.1	3.1 ± 1.9
	Mow	15.5 ± 2.6	5.0 ± 3.5	1.6 ± 0.1	2.1 ± 0.6
	Glyphosate	8.5 ± 3.5	0.7 ± 0.3	8.0 ± 1.9	1.3 ± 0.5
	Steam	17.7 ± 2.7	4.4 ± 1.7	0.3 ± 0.1	7.7 ± 10.2
Potato Patch Pit	Control	18.2 ± 7.9	0.8 ± 0.3	4.4 ± 2.2	20.8 ± 15.4
	Mow	14.3 ± 5.6	2.8 ± 2.7	5.3 ± 1.1	9.9 ± 4.8
	Glyphosate	9.4 ± 1.7	1.8 ± 0.7	34.6 ± 14.2	5.6 ± 4.3
Trade Waste Pit	Control	2.8 ± 2.5	0.6 ± 0.2	6.8 ± 3.6	36.7 ± 13.4
	Mow	4.3 ± 4.3	0.8 ± 0.7	4.7 ± 1.5	35.6 ± 8.8
	Glyphosate	8.7 ± 0.4	2.0 ± 2.3	31.6 ± 10.6	15.0 ± 5.6
	Steam	5.4 ± 1.3	0.4 ± 0.3	9.3 ± 1.2	36.9 ± 4.3

Numbers are mean ± standard deviation.
Cover data provided for informational purposes to interpret effect of management on seedling survival (statistical analyses not shown).

Table 3-12. Mean health scores for different plant materials transplanted.

	June			July		
Species	Cone	Root trainer	Tray	Cone	Root trainer	Tray
Achillea millefolium		3	1		3	2
Elymus trachycaulus	4		1	4		1
Amelanchier alnifolia	4		1	4		1
Anemone multifida			1			1
Artemisia michauxiana	4		1	4		1
Danthonia parryi	4	3	1	4	3	1
Erigeron spp.	4			3		
Eriogonum flavum	4			4		
Eriogonum umbellatum	5			5		
Festuca campestris	5			4		
Festuca idahoensis	4	4	2	4	4	2
Gaillardia aristata	3			3		
Galium boreale	2		0	2		0
Geranium viscosissimum	3			2		
Heterotheca villosa	4			4		
Koeleria macrantha	4	4	2	4	4	2
Linum lewisii		1	1		2	2
Lupinus sericeus	1			1		
Monarda fistulosa	2	2	1	3	3	1
Rosa woodsii	4			4		
Penstemon confertus	4	4	1	3	4	1
Potentilla arguta	4		4	5		5
Potentilla gracilis	5	4	2	5	4	4
Potentilla sp.	3	3	1	3	2	2
Overall mean	4	3	1	4	3	1

0 = missing plants that could not be located, 1 = dead plants (0 % live material), 2 = necrotic plants (< 25 % live material), 3 = severely chlorotic or wilting plants (25-50 % live material), 4 = chlorotic or wilting plants (51-75 % live material) and 5 = healthy.

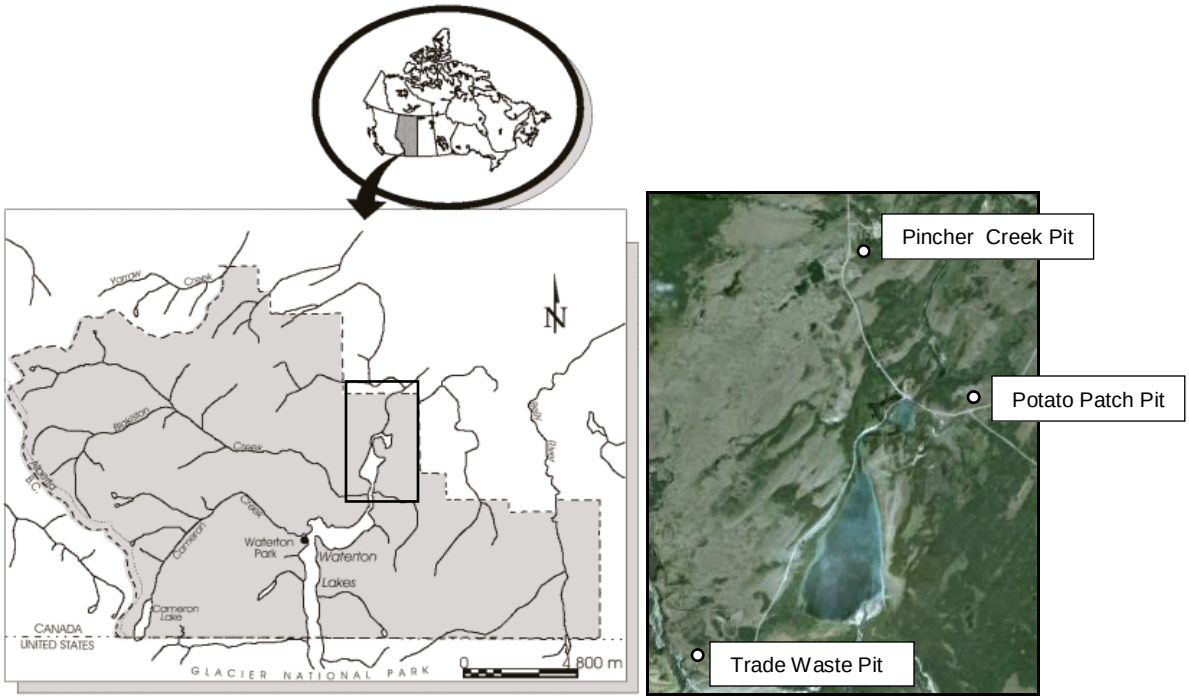

Figure 3-1. Location of Waterton Lakes National Park, Alberta, Canada and research sites (Parks Canada 2009, Google Earth 2012).

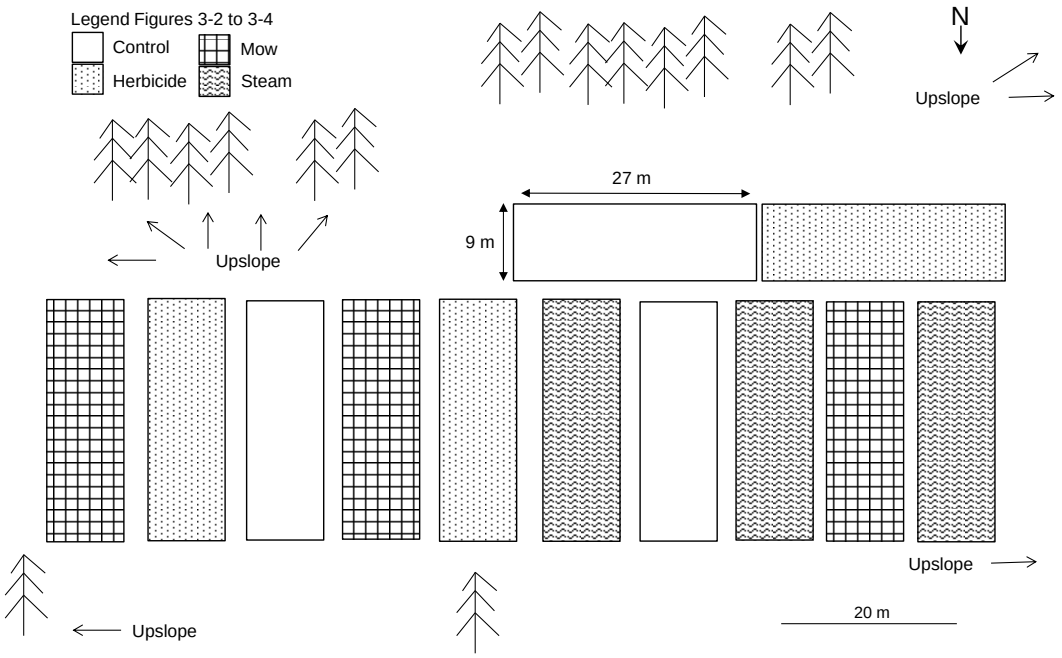

Figure 3-2. Pincher Creek Pit with plot locations.

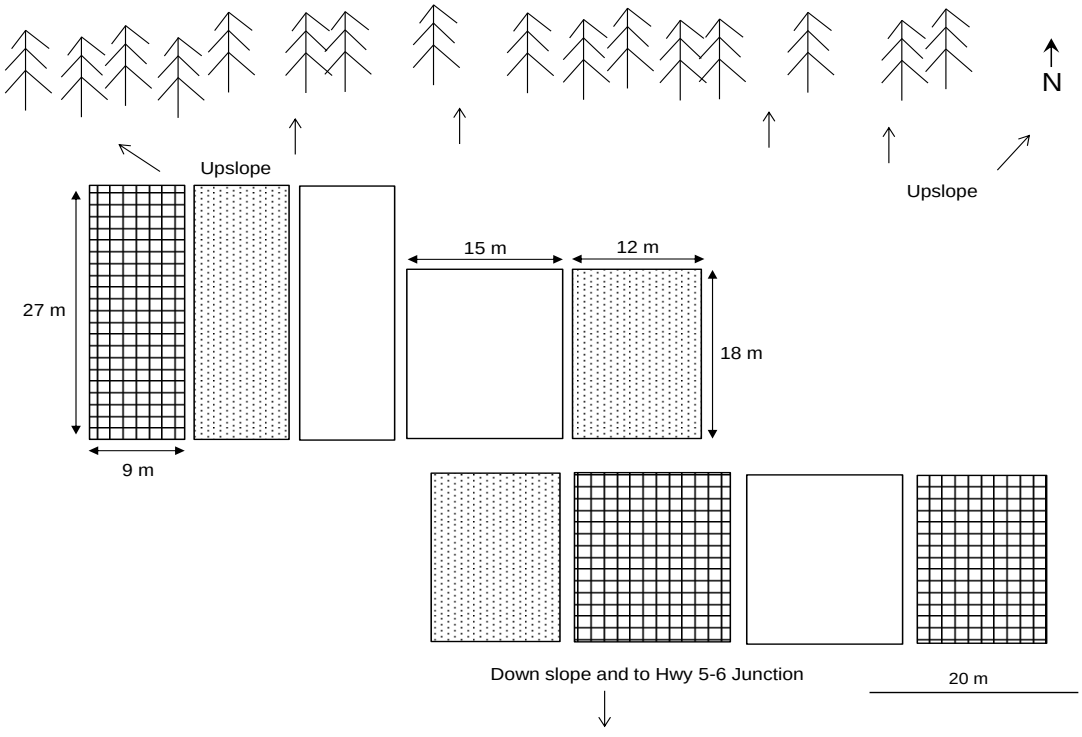

Figure 3-3. Potato Patch Pit with plot locations.

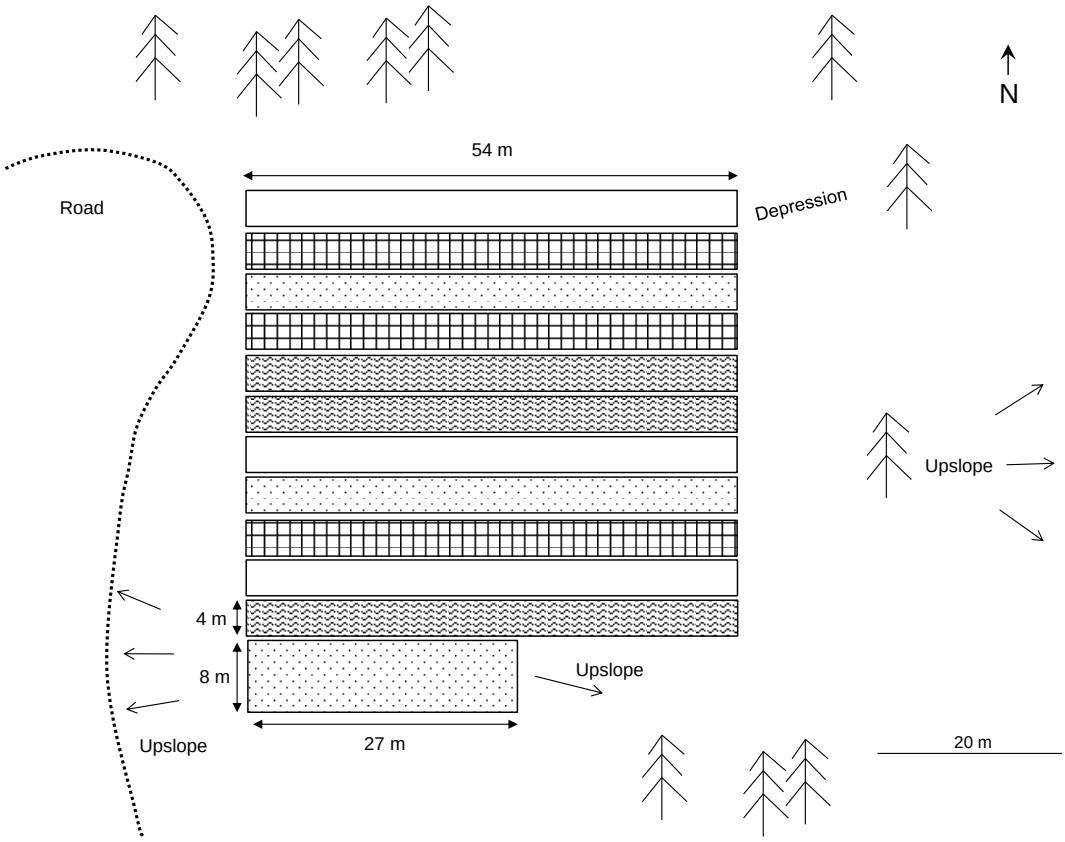

Figure 3-4. Trade Waste Pit with plot locations.

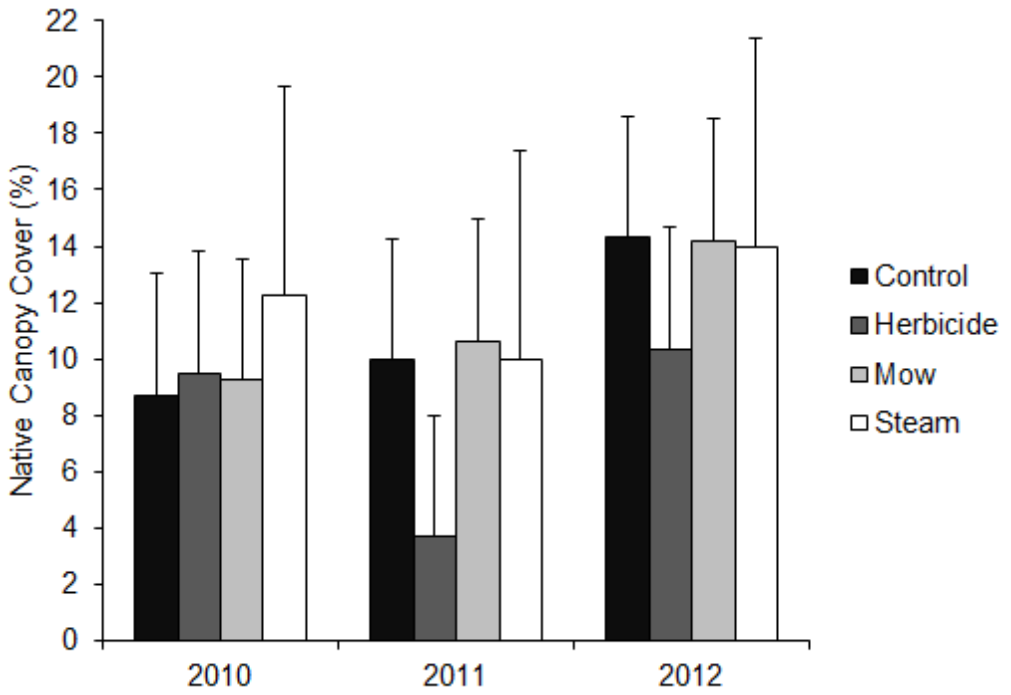

Figure 3-5. Native canopy cover (mean ± standard error) prior to revegetation and non-native plant control (2010), after non-native plant management treatment implementation and broadcast seeding (2011), and after transplanting (2012).

CHAPTER 4. PERFORMANCE OF NATIVE CULTIVAR AND WILD COLLECTED SEED FOR REESTABLISHMENT OF NATIVE GRASSES IN FOOTHILLS FESCUE GRASSLAND RESTORATION

1. INTRODUCTION

Native grasslands are among the most widely distributed, highly biodiverse and threatened ecosystems (Gibson 2009), resulting in global efforts for their restoration. Competition with non-native plant species is considered a formidable barrier to native species reestablishment and restoration of native grassland (Funk et al. 2008). Foothills fescue grasslands in Waterton Lakes National Park, Alberta, Canada are high in biodiversity but have been degraded in many areas due to disturbance and non-native plant invasion. Restoration has been unsuccessful as native plants seeded or planted from wild collected seed are outcompeted and displaced by non-native species (Naeth and Wilkinson 2008).

Many native grass species are commercially grown as cultivars, cultivated varieties with alleged improved performance (Burton and Burton 2002). Use of commercial native cultivar seed bred to have higher fitness and advantageous traits may compete better than native seed, leading to increased native species establishment and succession. Parks Canada has strictly used wild collected seed to protect genetic integrity of native plants. However, cultivars may be a necessary step to avoid heavy losses of native species and circumvent issues of low quantities, availability and germination rates of wild collected seed (Blake 1935, Sorensen and Holden 1974, Voight 1977, Bjugstad and Whitman 1989).

Plant species may have a number of ecotypes, unique populations of plants genetically differentiated in response to specific conditions of an ecosystem such as elevation, precipitation, temperature, soil and growing season (Turesson 1922). Species are expected to have genetic variation exhibited in their natural populations due to life history differences. Outbreeding plants with widely dispersed pollen or seeds that are long lived have high genetic variation among individuals but not populations and early successional species with widespread distributions have low genetic variability among and within populations (Hamrick et al. 1979, Linhart 1995).

Bred cultivated native plant varieties are often adapted to specific environmental conditions and may have less genetic variation than wild collected seed (Burton and Burton 2002). Cultivars sourced from distant locations are not adapted to local growing conditions and may suffer mortality or reduced vigour (Jacobson et al. 1984). Cultivars can degrade genetic diversity of plants in nearby undisturbed areas by interbreeding with native stock and passing on non-adapted genes (Thornburg and Fuchs 1978, Millar and Libby 1989, Linhart 1995, Hufford and Mazer 2003) or may behave invasively due to superior genotypes and outcompete resident species (Conrad and Tischew 2011).

Combined with appropriate breeding strategies and land management practices that promote biodiversity, cultivar ecotypes may be a sustainable way to increase native plant establishment. Even propagation programs sampling wild populations cannot adequately represent the entire range of genetic diversity in donor and target ecosystems, and seed migration and transfer among distant populations is a natural evolutionary process (Burton and Burton 2002). The decision to use local seed or an alternate seed source should be evaluated on a case by case basis using the restoration gene pool concept, a decision making tool for choosing plant material sources for restoration which weighs pros and cons of native provenance and commercial seed sources (Jones 2003). Use of commercial seed mixes including native cultivar and non-native agronomic cultivar grass species from non-local origins has been a common practice in reclamation throughout North America and Europe for many decades (Desserud et al. 2010, Conrad and Tischew 2011, Klopf and Baer 2011). Research on cultivar and local seed sources is necessary to help clarify issues in this debate.

Native plant ecotype selection is often recommended through government policies or required by regulations using collection radius guidelines, seed zones or comprehensive collections. Collection radius guidelines (Thornburg 1982, Romo and Lawrence 1990, Munshower 1994, Wark et al. 2011, Gerling et al. 1996) are based on Cooper (1957), suggesting plants can be moved 150 to 250 km south or 400 to 500 km north of origin to environments with similar soils and climate. Seed zones are recommended for native herbaceous species for more accurate and easier selection of adapted ecotypes (Millar and Libby 1989, Rehfeldt 1991). Seed zones have been established in jurisdictional boundaries

throughout North America, predominantly in forestry, and for native herbaceous species (Johnson et al. 2010). Comprehensive seed collections provide an alternative where native seed collections are prepared with as wide a genetic variability and adaptability as possible (Munda and Smith 1995). Seed sources selected for high genetic diversity improve native seedling establishment at an equal or greater rate than locally collected seed (Bischoff et al. 2010).

There are fewer Canadian native grass cultivars than American due to limited cultivar development and inadequate seed availability (Jefferson et al. 2002). As of 1994, there were 10 Canadian cultivars of five native grass species and 54 American cultivars of 17 species (Joyce 1993, Nykoluk 1994). Advances in Canada's seed industry recently led to an increase in this number, making commercial seed available for many common native species or seed can be obtained from nearby northern United States (Wark et al. 2011). Cultivar research and development is ongoing for a number of species in the Canadian prairie including development of ecovars (Jacobson et al. 1984, Booth and Jones 2001), cultivated native plant breeds selected for genetic breadth and agronomic characteristics, in species such as *Koeleria macrantha* (Ledeb.) Schult. (June grass) and *Bouteloua gracilis* (Kunth) Lag. ex Griffiths (Blue grama) (Friesen 2002). For Waterton, southern cultivars from Montana and nearby areas of the northern United States may be more ecologically suitable than cultivated varieties in Canadian areas further away. Use of cultivars as a more reliable seed source with greater quantities and availability may be an economic reality as long as the species is ecologically adapted to the restoration site (Jones and Johnson 1998). Throughout the prairie provinces, native cultivar cool season grasses from Montana and North Dakota had > 80 % establishment, significantly higher biomass and reduced weed competition than warm season cultivars (Jefferson et al. 2002). Warm season native grass cultivars generally do not survive well in Canadian plantings (Kilcher and Looman 1983) but cool season grasses may be promising in restoration.

In the United States, non-native plant abundance was significantly affected by native grass species seeded with no significant difference in non-native plant abundance between cultivar and local seed planted areas for five C4 native grass species (Wilsey 2010). Weed biomass was lower in local seed grown grass plots

for three out of five species. There was high variation among species in differences between the two seed types; some had higher productivity as cultivars and some as the wild variety; performance was dependent upon species. Cultivar performance can vary depending on cultivated variety, some grow larger than the wild type and some smaller and may be more susceptible to disease and insect herbivory (Gustafson et al. 2001, Gustafson et al. 2004). Cultivars may demonstrate enhanced physiological activity (Lambert et al. 2011) and often have higher cover and biomass with grazing as many are selected for forage value (Chamberlain et al. 2012). While above ground growth differences may be small, seeded cultivars uptake more nutrients and have larger root systems than seeded local plants (Klopf and Baer 2011). Survival and growth over time and through multiple generations needs to be evaluated to determine detrimental genetic effects to restored native plant population (Hufford and Mazer 2003). Species specific and general trends must be studied to provide more information for restoration. Differences in seed type performance for different planting methods should be considered. For example, wild collected seed may be equal or better for transplants and poor for direct seeding.

2. RESEARCH OBJECTIVES

The objective of this research was to evaluate wild collected and native cultivar seed for four species of C3, cool season grasses native to foothills fescue prairie; *Bromus carinatus* Hook. & Arn. (Mountain brome), *Koeleria macrantha*, *Festuca idahoensis* Elmer (Idaho fescue) and *Elymus trachycaulus* (Link) Gould ex Shinners (Slender wheat grass). Wild collected seed and cultivar seed were evaluated to determine differences in survival and growth of transplanted and seeded grasses, to monitor effect of seed type over time, and to determine if differences between the seed types exhibited a general or species specific trend.

3. MATERIALS AND METHODS

3.1 Selected Native Grasses

Bromus carinatus, *Koeleria macrantha* and *Elymus trachycaulus* are early successional species that can colonize and establish on disturbed grasslands and are recommended for restoration (Tannas 2003, USDA 2012). *Elymus*

trachycaulus and *Koeleria macrantha* are widely distributed in North America; *Bromus carinatus* is more commonly found throughout mountains and foothills of the west (USDA 2012). *Festuca idahoensis*, a long lived perennial, is slower establishing, but successful in restorations in western North America (Ewing 2002). *Bromus carinatus* and *Festuca idahoensis* cultivars were developed by the United States Department of Agriculture for high yield, seed viability and germination (Fehr 1987); *Koeleria macrantha* and *Elymus trachycaulus* are Canadian cultivars (Table 4-1). Wild seed was collected from fescue grasslands in Waterton and Glacier National Parks from 2005 to 2010. Cultivar seed was purchased from Eastern Slopes Rangeland Seeds Ltd. (Cremona, Alberta), Pickseed (Edmonton, Alberta) and BrettYoung (Calmar, Alberta); origins of the cultivars was unknown.

3.2 Research Location And Study Sites

Research was conducted in Waterton Lakes National Park at three disturbed foothills fescue grassland sites undergoing restoration (Figure 4-1). Waterton Lakes National Park is located in the Rocky Mountains of southwestern Alberta forming an International Peace Park and United Nations Educational, Scientific and Cultural Organization (UNESCO) World Heritage Site with Glacier National Park. It covers 525 km^2 and extends south to Montana and Glacier National Park and west to the Alberta-British Columbia border along the Continental Divide (Achuff et al. 2002). The western boundary borders Akamina-Kishinena Provincial Park in British Columbia. North and east sides border Alberta crown and private lands. In the northwest corner the park borders the Blood Indian First Nation Timber Limit in the Belly River area on three sides. Four Ecoregions are found in the park: Foothills Parkland, Montane, Subalpine and Alpine.

Grasslands are characterized by Chernozem soils with calcareous soil parent materials, good drainage and mineral surface horizons high in organic matter. Climate in foothills grasslands is characterized by a short growing season (June to August), cool, wet springs and hot, dry late summers. The region is windy, with maximum daily gusts of 70 to 90 km h^{-1}. Mean annual precipitation is 807.6 mm and minimum and maximum average temperatures were -1.3 ^{o}C and 10.6 ^{o}C, respectively, for 1990 to 2000 (Environment Canada 2012a, 2012b). Daily weather can change rapidly and unpredictably, typical of mountain regions.

A total of 971 vascular plant species are found in the park. In 2002, 20 plant species were discovered, including two new to Alberta and one new to Canada (Achuff et al. 2002). Over 50 % of Alberta wildflower species are found in the park, including 30 rare plant species found nowhere else in Canada.

A former landfill and two borrow pits were selected for study within a 3 km radius with similar topography and soils (Figure 4-1). The sites contained a large and diverse population of non-native annual and perennial species including *Centaurea maculosa* L. (Spotted knapweed), *Cirsium arvense* L. (Canada thistle), *Bromus inermis* Leyss. (Smooth brome), *Elymus repens* (L.) Gould (Quack grass), *Poa pratensis* L. (Kentucky bluegrass), *Poa compressa* L. (Canada bluegrass) and *Agropyron cristatum* L. (Crested wheat grass).

The landfill site (Trade Waste Pit) was used for disposal of domestic waste, including building materials, fuel, treated wood, scrap metal, batteries and manure between 1952 and 1999 (Naeth and Jobson 2007). Soil and ground water sampled within the past five years had elevated concentrations of aluminum, copper, zinc, strontium, nickel, silver and iron, reflecting natural background concentrations. The site was classified as very low risk with no required remediation (Canadian Council of Ministers of the Environment 2008). In 2006, waste was removed, the site recontoured and wood chips and topsoil used to build soil. In fall 2006 and spring 2007, native grasses, forbs and shrubs from wild collected seed were seeded and transplanted (Naeth and Wilkinson 2008).

Borrow pit sites were gravel quarries and disturbance details are not well known. Borrow Pit 1 (Potato Patch Pit) is 1.8 ha in size, located near Chief Mountain Highway Junction. Gravel excavation ended in the 1960s and the site was decommissioned in the late 1970s. It became heavily infested with *Centaurea maculosa*, which was sprayed with herbicide and hand pulled in the 1980s, then plowed and revegetated with native plant species. Borrow Pit 2 (Pincher Creek Pit) is 2.2 ha in size, located near the Bison Paddock. In recent years Park staff planted native plugs at these sites and tried to control target weeds.

3.3 Experimental Design

During May 15-19, 2011, two 2 x 2 m plots were located at each site to assess wild collected and native cultivar seed. Locations were randomly chosen near

existing research plots on even terrain with similar soils and vegetation. Plots were measured and marked with wire flags, vegetation was removed with glyphosate and soil was lightly tilled with shovels. A split plot design was used to increase statistical power, with native cultivar and wild collected seed as the main effect and the four grass species randomly assigned to locations within the main plot as the subplot effect (Figures 4-2 to 4-4). Each species and seed type was planted in one location in the plot in a grid 0.5 to 1.0 m apart. On June 13-16, 2011, at each planting location, two holes, 2-5 cm deep, were dug beside each other and 20 healthy seeds were deposited and lightly covered with soil.

No seed treatment was used as little is known about germination requirements of the native plant species in this study. Insufficient data were available on germination, emergence and survivability. Therefore, to determine how seed viability may have affected seed performance, in September 2011, germination tests were conducted for each native grass species used. For each species, 12 healthy seeds were positioned evenly on paper towel in 5 replicate petri dishes moistened with distilled water. Paper towel was wetted every 1 to 2 days and germinating seeds counted daily for 14 days. Total germinating seeds was divided by total seeds per dish for percent germination and mean percent germination was calculated from five replicates for each species.

Performance of native cultivar and wild collected transplants was assessed in 2012. *Koeleria macrantha*, *Elymus trachycaulus*, *Bromus carinatus* and *Festuca idahoensis* were grown from the cultivar and wild collected seed used in 2011 in seeding experiments at the University of Alberta. Plants were grown on large trays and seedlings transferred to 11.4 cm deep and 2.5 cm wide root trainers after reaching approximately 4 cm. Growing viable seed in trays germinated viable seed before preparing transplant containers, saving time and resources. Plants were grown in a greenhouse, watered every 3 to 4 days with temperature maintained at 20 ^{o}C. Emergence of plants seeded in potting soil was recorded. Plants were monitored daily for 35 days and cumulative emergence recorded. *Bromus carinatus* seed had poor germination so was not used in the experiment.

May 29 to June 5, 2012, native grasses were transplanted in 2 x 2 m plots. In the same region species and seed type (e.g. *Festuca idahoensis* cultivar) were seeded in 2011, 16 plants were planted. Transplanting was timed for late May

and early June for maximum precipitation, late enough to avoid spring snowfall. University of Alberta Research Assistants and Parks Canada Staff assisted with planting. Planting tools included metal trowels, spades and shovels. Large and more robust tools such as post hole diggers and hoedads provided by Parks Canada were needed in rocky areas with many boulders, especially at Trade Waste Pit. Holes were dug carefully to minimize impact to surrounding vegetation, equal to root depth and soil was firmly replaced around the plant with no air gaps to prevent erosion and root exposure.

3.4 Vegetation Assessments

Vegetation was assessed June 14-20 and July 26-30, 2010. North facing transects (East for Trade Waste Pit) were established and 0.1 m^2 quadrats systematically placed at equal distance intervals along the transects. Individual plant species and ground (bare ground, moss, vegetation, litter, thatch, rock) cover were ocularly determined. Botanical nomenclature followed Kuijt (1982), Moss (1983) and Tannas (2003). Twelve transects and 99 quadrats were assessed at Trade Waste Pit, 9 and 74 at Potato Patch Pit and 12 and 64 at Pincher Creek Pit, respectively.

Seedlings were monitored six times; July 12-17, August 2-9 and August 26, 2011 and May 29-June 5, June 19-27 and July 16-28, 2012. Transplants were monitored June 19-27 and July 16-28, 2012. Plant emergence and growth were assessed in each planting location. In each plot, number of seedlings and height was determined for each species and seed type. Health was assessed by assigning a score for each plant. A 0 was assigned to plants that could not be located, 1 for dead plants (0 % live material), 2 for necrotic plants (< 25 % live material), 3 for severely chlorotic or wilting plants (25-50 % live material), 4 for chlorotic or wilting plants (51-75 % live material) and 5 for healthy plants (> 75 % live material). Other significant observations were recorded.

3.5 Meteorological Conditions

Meteorological data were obtained from Environment Canada National Climate Data and Information Archive (Environment Canada 2012a, 2012b). Data were collected from a weather station (Waterton Park Gate Alberta) located at the Waterton Lakes National Park Park Gate (49°07'52.080" N, 113°48'31.010" W,

elevation 1,289 m). Mean daily temperatures were averaged for mean monthly temperature and total monthly precipitation and maximum wind gusts recorded at the weather station were obtained from the online archive. Long term climate normals (1971-2000) were obtained from an inactive weather station (Waterton River Cabin) approximately 0.5 km north of Potato Patch Pit (49°07'00.000" N, 113°50'00.000" W, elevation 1,281 m).

3.6 Soil Sampling And Analyses

Soil was sampled to characterize study sites and undisturbed fescue grassland September 19-21, 2011. Transects from the 2010 vegetation assessment were used to randomly select soil sampling locations. A total of 42 samples were collected, 12 from Trade Waste Pit, 12 from Pincher Creek Pit and 9 from Potato Patch Pit. Three samples were taken from undisturbed grassland neighbouring each of the three sites. At each sampling location, a 15 cm deep hole was dug with trowels and a sharp knife and 500 g of soil was removed, placed in labeled plastic bags, and stored at 4 ^{o}C until processing. The hole was filled with soil following sampling.

Samples were analyzed by Exova Laboratories in Edmonton, Alberta. Total nitrogen and carbon were determined by dry combustion and Leco combustion (Bremner 1996). Inorganic carbon was determined through carbon dioxide release; total organic carbon was calculated by subtracting inorganic carbon from total carbon (Loeppert and Suarez 1996). C:N ratio was determined by dividing total carbon by total nitrogen. Total phosphorus was determined with strong acid extraction and inductively coupled plasma mass spectrometry (US Environmental Protection Agency 1996). Sand, silt and clay were determined by hydrometer (Kroetsch and Wang 2008). Electrical conductivity, pH, sodium adsorption ratio and available soil calcium, magnesium, sodium and potassium were determined in saturated paste (Miller and Curtin 2008).

3.7 Statistical Analyses

Statistical analyses were completed in SAS software v. 9.2 (SAS Institute, Inc. 2003) using an alpha of 0.05. Wild collected and native cultivar growth data were analyzed to assess performance of each seed source for each species. Germination data were assessed to determine viability of seed types. Number of

seedlings and height were analyzed for 2011 and 2012 data using two-way analysis of variance (ANOVA) with species and seed type as the two factors. Two-way ANOVA was performed using proc mixed addressing the split plot design with plot and plot x seed type in the random statement to remove plot variation from the model. The statistical model was mean number of seedlings/height = species + seed type + species x seed type. For significant ANOVAs, Student's t-tests were used to compare treatments using LSMEANS and pdiff statements. Resulting p values were adjusted using Bonferroni correction. Health scores and height were analyzed using two-way ANOVA for transplant data with the same statistical model. Factors such as seedling and transplant germination and mortality, grazing and physiological development were used to assess overall effectiveness of cultivar and wild collected seed.

4. RESULTS

4.1 Meteorological Conditions

Mean monthly temperature was similar to average conditions during the study years with the exception of 2011 and 2012 being slightly warmer (1-2 ^{o}C) in July and August (Table 4-2). 2010 was wetter than normal with over 100 mm more precipitation during May to August than the long term average. 2011 had precipitation similar to average conditions but July precipitation was lower than normal and 2012 was drier than normal with 100 mm less precipitation than average. The Waterton region experiences heavy wind gusts. A high frequency of extreme weather occurred in 2012; thunder and hail storms were more intense and more often and there were many hot, dry days followed by heavy rain.

4.2 Site Conditions

Soils of undisturbed fescue grassland surrounding the study sites were Black Chernozems typical of the region. Study site soils were modified by disturbance as indicated by lower organic matter content relative to undisturbed areas (Table 4-3). All sites had large amounts of rocky, coarse grained sediment typical of the region. Soils were similar throughout the study area and did not have a major impact on revegetation. Non-native graminoids dominated all sites, followed by native forbs, non-native forbs and native graminoids (Table 4-4). Non-native

species cover was greatest at Trade Waste Pit and least at Pincher Creek Pit, with the opposite trend for native species.

4.3 Seedling Performance

Germination did not differ with seed type but showed a species specific trend. *Bromus carinatus* and *Koeleria macrantha* wild seed had higher germination than cultivars and *Festuca idahoensis* and *Elymus trachycaulus* cultivars had higher germination than wild seed (Table 4-1). *Bromus carinatus* had very low germination, < 15 %. Number of seedlings emerging over time was similar between cultivar and wild, with cultivar seed having numerically higher emergence (Table 4-5). Emergence patterns were similar to germination except emergence was higher in *Bromus carinatus* cultivar seed than wild seed.

Number of seedlings and seedling height did not differ significantly between native cultivar and wild collected seed (Tables 4-6, B.5). Native cultivar seed produced more seedlings and greater height than wild seed in three of four species studied (Figures 4-5, 4-6). *Koeleria macrantha* had more seedlings with greater height from the wild collected than cultivar seed type. Cultivar and wild seedlings of *Elymus trachycaulus* produced seed; cultivar plants produced slightly more (data not shown).

Overall patterns were similar over time in both study years from 2011 to 2012. Number of cultivar seedlings increased in 2012 and very few *Bromus carinatus* seedlings were found in either seed type so emergence drastically decreased over time in this species (Tables 4-1, 4-6). Out of 1,920 seeds planted or 240 seeds per species and seed type, 530 seedlings emerged. Thus approximately 28 % of total seeds planted germinated and emerged. Differences among species were detected that would be expected based on species specific genetic traits. For example, *Elymus trachycaulus* seedlings were significantly taller than *Koeleria macrantha* seedlings (Tables 4-6, B.5).

4.4 Transplant Performance

Transplant performance was similar for cultivar and wild seed with no significant differences in health or height (Tables 4-7, B.6). Transplants of *Elymus trachycaulus* grown from cultivar seed produced slightly more seed than wild

collected transplants (data not shown). Phenotypic differences were observed between transplants of the two seed types. For example, *Festuca idahoensis* cultivar transplants had lime green, soft leaves instead of the typical bluish green, stiff leaves of local populations. Mortality and grazing increased 50 days after planting and was higher in cultivars than wild plants (Table 4-7). *Elymus trachycaulus* and *Festuca idahoensis* cultivar seedlings had higher mortality than wild seedlings; *Koeleria macrantha* mortality between the two seed types were similar. Differences among species were also observed in transplanted seedlings. *Elymus trachycaulus* was significantly taller than *Koeleria macrantha* and *Festuca idahoensis* and *Festuca idahoensis* had significantly greater health than *Elymus trachycaulus* (Tables 4-7, B.6). Transplant health was significantly lower in *Elymus trachycaulus* than *Festuca idahoensis* and *Koeleria macrantha* (Tables 4-7, B.6).

5. DISCUSSION

5.1 Seedling Performance

This study suggests establishment of native grasses, measured by germination, emergence, seedling number and height, is equally successful using wild collected or native cultivar seed. Thus the choice of seed type may be more strongly influenced by cost and availability than performance (Jones and Johnson 1998). Equal performance of wild collected seed highlights the importance of adaptation to local environmental conditions (Jacobson et al. 1984, Bischoff et al. 2010), which may change as more cultivars are developed and improved. This is one of few investigations of C3 native grass cultivars for ecological restoration and one of the first for foothills fescue grassland restoration.

Species specific responses indicate the most effective seed source will differ with plant species. Variation among species in seed type performance and overall equal performance was found in previous research (Jacobson et al. 1984, Bugg et al. 1997, Bischoff et al. 2010). Wilsey (2010) outlined three working hypotheses based on ecological and evolutionary theory (Lesica and Allendorf 1999) to explain the outcome of a comparison of a cultivar and local seed source under different grassland restoration scenarios, the cultivar vigour hypothesis,

local adaptation hypothesis and a null hypothesis where growth is equal. This study suggests for cool season grasses that any of the above three hypotheses may be operating to explain cultivar and local seed performance because responses are strongly species or ecotype specific (observed by Wisley 2010 and Gustafson et al. 2004 for warm season grasses). Species vary greatly in intraspecific genetic variation due to life history (Hamrick et al. 1979), which is likely a factor driving high variability in species responses to seed types.

As predicted by the strong establishment of C3 American cultivars by Jefferson et al. (2002) the *Festuca idahoensis* cultivar had high establishment. *Bromus carinatus*, from American wild collected and cultivar sources, had poor growth with both seed types and appeared to act as an annual. Changes in lifespan have been observed in different provenances of a species when moved to a new location (Bischoff et al. 2010). Attempts to cultivate *Bromus carinatus* in Canada have been highly unsuccessful due to the same observations in seed crops along with a limited seed shelf life of < a year (Weir 2012). As evidenced with *Bromus carinatus* being a good reclamation species in the United States (Bugg et al. 1997, USDA 2012) not all native C3 grasses will establish well when moved north in latitude. This species is sensitive to translocation due to changes in climate (Johnson et al. 2010). *Bromus carinatus* should be seeded, if used at all in Canadian restoration projects due to its short lifespan and its performance using both revegetation methods in this study. *Koeleria macrantha* populations in Waterton National Park are adapted to disturbance and can colonize a variety of harsh substrates and compete with non-native plants in dry, rocky areas, which may explain better performance of wild collected seed in this study.

Seedlings of all species were small and slow growing, reaching heights of 10 cm compared to resident perennial non-native grasses which quickly reached over 20 cm height in surrounding cleared areas and with extensive abundance and cover in the study area after one growing season. There was poor establishment with seeding compared to transplanting; therefore, high seeding rates are needed to establish native plant cover and transplanting may be necessary to overcome competition with non-native plants. Low establishment from seeding may be partially due to drier conditions in late summer 2011 and in the entire 2012 growing season.

5.2 Transplant Performance

Wild collected and cultivar transplants had very similar health scores and height for each species; therefore, wild collected transplants are likely sufficient for re-establishment of native grasses, as long as an adequate amount of seed is available. Another study found variation in seed type performance among species in transplanted seedlings similar to results of direct seeding in this study (Wilsey 2010), while another observed greater below ground growth in cultivar seedlings (Klopf and Baer 2011). Limited research has been done on seed type performance comparisons with transplanted seedlings.

Species was a significant factor affecting health and height of transplanted seedlings. *Festuca idahoensis* is difficult to establish from seed (Bugg et al. 1997) but had significantly higher transplant health than *Elymus trachycaulus*, suggesting it is a good restoration species for transplanting. Phenotypic differences between *Festuca idahoensis* seed types were only observed in transplanted seedlings. These morphological differences were possibly due to a varied response to environmental conditions or differing characteristics during immaturity at the seedling stage. *Elymus trachycaulus* was significantly larger and taller than the other species and produced good canopy cover (also found by Bugg et al. 1997) and should be used in future restorations.

This and other studies (Jacobson et al. 1984) found transplanted cultivar seedlings may suffer mortality from grazing or other causes. Native cultivars are often bred for greater forage value (Chamberlain et al. 2012) and have larger root systems and uptake more nutrients (Klopf and Baer 2011); therefore, they may provide a good food source for wildlife but have a higher mortality risk.

5.3 Restoration And Management Implications

This research indicates native cultivar grass species are no more successful than grasses grown from wild collected seed and establishment from seeding is very low. Therefore, transplanting is strongly recommended for revegetation and wild collected seed is an effective seed source for growing transplants. *Koeleria macrantha*, *Elymus trachycaulus* and *Festuca idahoensis* all had high health scores in this study and are highly suitable for foothills grassland restoration. *Bromus carinatus* is not recommended, but if seed is available in large quantities,

it could be seeded for an annual cover crop. Seed source selection must be made on a species specific basis as some perform better as cultivars and some from wild sources. Long term research and further study is needed to determine whether native cultivars are advantageous for foothills fescue grassland restoration. Non-local seed sources should be used carefully in full consideration of all of potential negative ecological consequences. Significant differences in health and height among species indicates *Elymus trachycaulus* may compete better with non-native grasses and *Festuca idahoensis* may be more tolerant of environmental stresses.

5.4 Future Directions

This was a small scale, pilot study so future research should implement treatments on a larger scale more practical to restoration. Long term data are lacking and studies should be conducted over longer periods to fully evaluate seed type differences. Competition characteristics between cultivars and non-native plant species and local native plant populations need to be evaluated. More native plant species and cultivar types should be studied. Research is needed in development of new and improved cultivars for Canadian restoration.

Near-natural restoration, novel ecosystems and plant provenance are all important current topics in restoration (Andel and Aronson 2012). Small scale restoration studies that address these broad concepts will aid in development of restoration strategies. Invasive perennial grasses challenging current restoration projects escaped from cultivation and were once agronomic crops bred for high forage value and stand production. Globally, restorations often employ commercially cultivated seed from non-local origin, which will likely have major implications for restored ecosystems (Conrad and Tischew 2011). Negative outcomes from using commercial seed mixes have been widely postulated (Burton and Burton 2002) but not yet thoroughly researched. More ecological studies are needed assessing outcomes of commercial seed in restored ecosystems to determine negative impacts and potential mitigation and management measures and opportunities and methods for sustainable uses of commercial seed or strategies for improving the sustainability and ecological sensitivity of the seed industry.

6. CONCLUSIONS

- There was no significant difference between native cultivar and wild collected seed types in establishment of cool season native grasses in disturbed foothills fescue prairie.
- Species specific responses occurred, highlighting the importance of species characteristics as a significant factor affecting native grass re-establishment and the need for making seed source decisions on a species specific basis.
- A greater number of seedlings with greater height established from *Festuca idahoensis* and *Elymus trachycaulus* cultivars from Oregon and Saskatchewan, respectively, compared to wild collected seed.
- More *Bromus carinatus* seedlings established from cultivar seed (sourced from Montana) than wild collected seed but this species had very low overall germination and establishment and is not recommended for restoration.
- *Koeleria macrantha* had had greater establishment from wild collected seed than cultivar seed.
- Transplanted seedlings performed similarly for both seed types but grazing was more frequent and led to greater mortality in cultivar plants.

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Table 4-1. Native plant species seed source information and germination.

Species	Seed source information	Year Collected	Germination (%)
Bromus carinatus	Wild, Glacier National Park, Montana	2002	10
	Cultivar “Bromar”, Montana: ‘disease resistant, taller, more leaves, later maturing’	2010	2
Elymus trachycaulus	Wild, Waterton National Park, Alberta	unknown	18
	Cultivar “Adanac Slender Wheatgrass”, Saskatchewan: ‘salinity tolerance, produces rhizomes, taller’	2010	74
Festuca idahoensis	Wild, Waterton National Park, Alberta	2005	78
	Cultivar “Joseph’s Idaho Fescue”, Oregon: ‘higher seed production, larger seeds, increased germination’	2010	86
Koeleria macrantha	Wild, Waterton National Park, Alberta	n/a	84
	Cultivar “Common”, Alberta	2009	64

Cultivated variety of *Koeleria macrantha* is unregistered so labeled as “Common” for legislative purposes.

Table 4-2. Summer precipitation, temperature, wind gust speed and long term climate normals at Waterton Lakes National Park during the study period.

Year	Month	Total Monthly Precipitation (mm)	Mean Temperature (^{o}C)	Maximum Wind Gust Speed (km/h)
2010	May	119.8	6.0	96
	June	157.8	12.4	96
	July	92.6	15.3	83
	August	68.6	14.4	74
2011	May	126.2	8.0	89
	June	93.2	12.2	76
	July	19.6	16.2	95
	August	72.0	16.7	72
2012	May	37.6	8.9	72
	June	88.8	12.4	87
	July	38.2	17.1	102
	August	42.0	16.9	87
Long Term Normals	May	94.5	8.9 ± 1.3	
	June	80.8	12.5 ± 1.4	
	July	70.8	15.2 ± 1.2	
	August	69	14.5 ± 1.8	

Data from Park Gate and former Waterton River Cabin Weather Stations (Environment Canada 2012a, b).
± = standard deviation.

Table 4-3. Soil chemical and physical properties at research sites.

Parameter	Pincher Creek Pit		Potato Patch Pit		Trade Waste Pit	
	Disturbed	Undisturbed	Disturbed	Undisturbed	Disturbed	Undisturbed
C:N Ratio	14.4 ± 3.8	11.5 ± 0.4	23.7 ± 33.6	11.1 ± 0.1	15.1 ± 3.4	13.2 ± 0.7
Total Nitrogen (%)	0.2 ± 0.1	0.7 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.2
Organic Matter (%)	4.5 ± 2.9	16.9 ± 2.5	2.6 ± 1.6	10.0 ± 1.4	6.1 ± 2.6	11.7 ± 4.6
Total Inorganic Carbon (%)	0.6 ± 0.3	0.1 ± 0.0	0.8 ± 0.7	0.1 ± 0.0	0.4 ± 0.3	0.1 ± 0.1
Total Organic Carbon (%)	2.2 ± 1.5	8.5 ± 1.2	1.3 ± 0.8	5.0 ± 0.7	3.1 ± 1.3	5.9 ± 2.3
Total Phosphorus (mg/kg)	606.7 ± 59.2	1013.3 ± 70.4	405.8 ± 65.6	750.0 ± 74.8	828.3 ± 124.9	1093.3 ± 273.5
Sand (%)	82.2 ± 4.5	59.7 ± 4.0	64.4 ± 12.3	59.0 ± 2.7	63.2 ± 4.3	63.2 ± 4.0
Silt (%)	13.8 ± 3.6	36.5 ± 3.3	26.8 ± 9.3	37.7 ± 3.0	29.3 ± 4.0	30.5 ± 3.9
Clay (%)	4.0 ± 1.1	3.8 ± 0.9	8.8 ± 3.6	3.3 ± 0.5	7.42 ± 1.2	6.3 ± 0.9
Hydrogen Ion Concentration (pH)	7.9 ± 0.1	6.8 ± 0.2	5.9 ± 0.2	7.2 ± 0.1	7.5 ± 0.5	6.6 ± 0.6
Electrical Conductivity (dS/m)	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.3	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
Sodium Adsorption Ratio	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Available Calcium (mg/kg)	31.1 ± 11.8	57.6 ± 8.8	40.6 ± 28.0	49.3 ± 11.9	25.3 ± 10.3	17.9 ± 4.8
Available Magnesium (mg/kg)	5.3 ± 2.2	14.4 ± 2.9	8.5 ± 6.0	13.0 ± 2.7	7.1 ± 3.4	5.9 ± 1.0
Available Sodium (mg/kg)	0.3 ± 0.5	3.0 ± 0.8	0.8 ± 0.5	1.7 ± 0.5	1.3 ± 0.4	1.7 ± 0.5
Available Potassium (mg/kg)	5.5 ± 2.8	33.0 ± 12.4	6.2 ± 5.7	16.7 ± 7.4	11.7 ± 7.5	7.0 ± 0.8

Numbers are mean ± standard deviation.

Table 4-4. Vegetation composition and canopy cover (%) at each research site.

Site	Native Forbs	Native Graminoids	Non-Native Forbs	Non-Native Graminoids
Pincher Creek Pit	18.0 ± 6.0	2.4 ± 1.5	0.7 ± 0.3	3.6 ± 1.0
Potato Patch Pit	4.7 ± 4.2	0.7 ± 1.0	0.6 ± 0.1	12.0 ± 8.6
Trade Waste Pit	0.2 ± 0.3	3.9 ± 3.3	0.8 ± 0.7	20.1 ± 9.7

Numbers are mean ± standard deviation.

Table 4-5. Cumulative number of seedlings emerging over time since seeded (June 16, 2011) for four native grass species from native cultivar and wild collected seed sources.

Species	Seed Type	July 14, 2011	August 8, 2011	August 26, 2011	June 5, 2012	July 20, 2012
Bromus carinatus	Cultivar	7	0	20	0	0
	Wild	2	1	0	4	0
Elymus trachycaulus	Cultivar	4	25	45	59	9
	Wild	5	4	28	49	8
Festuca idahoensis	Cultivar	2	12	7	26	15
	Wild	14	1	7	9	12
Koeleria macrantha	Cultivar	2	2	3	28	11
	Wild	3	14	20	40	32
Overall	Cultivar	15	39	75	113	35
	Wild	24	20	55	102	52

Table 4-6. Performance of native cultivar and wild collected seed establishing from direct seeding.

Species	Seed Type	Mean number of seedlings	Mean seedling height (cm)
2011			
Bromus carinatus	Cultivar	4.5 ± 3.2	2.8 ± 1.3 ab
	Wild	0.5 ± 3.2	1.5 ± 1.3 ab
Elymus trachycaulus	Cultivar	10.7 ± 3.1	4.8 ± 1.2 a
	Wild	6.2 ± 3.2	3.8 ± 1.3 a
Festuca idahoensis	Cultivar	3.3 ± 3.2	1.3 ± 1.3 ab
	Wild	3.5 ± 3.1	2.3 ± 1.2 ab
Koeleria macrantha	Cultivar	1.2 ± 3.2	1.2 ± 1.3 b
	Wild	6.2 ± 3.2	1.0 ± 1.3 b
Overall	Cultivar	5.0 ± 3.2	2.5 ± 1.3
	Wild	4.1 ± 3.1	2.2 ± 1.3
2012			
Bromus carinatus	Cultivar	0	0
	Wild	0	0
Elymus trachycaulus	Cultivar	11.3 ± 4.6	9.7 ± 2.5 a
	Wild	9.5 ± 4.6	9.3 ± 2.5 a
Festuca idahoensis	Cultivar	6.8 ± 4.6	4.4 ± 2.5 ab
	Wild	3.5 ± 4.6	2.5 ± 2.5 ab
Koeleria macrantha	Cultivar	6.5 ± 4.6	1.3 ± 2.5 b
	Wild	11.7 ± 4.6	3.6 ± 2.5 b
Overall	Cultivar	6.2 ± 4.6	3.9 ± 2.5
	Wild	6.2 ± 4.6	3.9 ± 2.5

Mean ± standard error is given.
Means within columns followed by different letters are significantly different ($p<0.02$).
No *Bromus carinatus* seedlings survived after 2011.

Table 4-7. Performance of transplants grown from native cultivar and wild collected seed.

Time Since Planting	Species	Seed Type	Mortality (%)	Grazing (%)	Mortality Due To Grazing (%)	Health	Height (cm)
20 days	*Elymus trachycaulus*	Cultivar	1.0 ± 2.6	1.0 ± 2.6	1.0 ± 2.6	4.2 ± 1 b	14.7 ± 3.8 a
		Wild	0	0	0	3.8 ± 1 b	13.8 ± 4.6 a
	Festuca idahoensis	Cultivar	0	3.1 ± 7.7	0	4.8 ± 0.4 a	10.5 ± 3.2 b
		Wild	0	0	0	4.5 ± 0.5 a	10.2 ± 2.4 b
	Koeleria macrantha	Cultivar	0	0	0	4.8 ± 0.4 ab	9.2 ± 1 b
		Wild	0	0	0	4.8 ± 0.4 ab	7.2 ± 1.3 b
50 days	*Elymus trachycaulus*	Cultivar	6.3 ± 10.5	6.3 ± 10.5	6.3 ± 10.5	4.2 ± 0.8 b	14.7 ± 3.8 a
		Wild	0	14.6 ± 35.7	0	4.3 ± 0.8 b	13.8 ± 4.6 a
	Festuca idahoensis	Cultivar	3.1 ± 5.2	15.6 ± 30.0	0	4.8 ± 0.4 a	10.5 ± 3.2 b
		Wild	2.1 ± 5.1	0	0	4.7 ± 0.5 a	10.2 ± 2.4 b
	Koeleria macrantha	Cultivar	1.0 ± 2.6	0	0	4.5 ± 0.5 ab	8.7 ± 1.6 b
		Wild	2.5 ± 5.6	0	0	4.2 ± 0.8 ab	9.3 ± 5.4 b

Numbers are mean ± standard deviation.
Means within columns followed by different letters are significantly different ($p<0.02$).
Health scores: 0 = missing, 1 = dead (0 % live material), 2 = necrotic (< 25 % live material), 3 = severely chlorotic or wilting (25-50 % live material), 4 = chlorotic or wilting plants (51-75 % live material) and 5 = healthy (> 75 % live material).

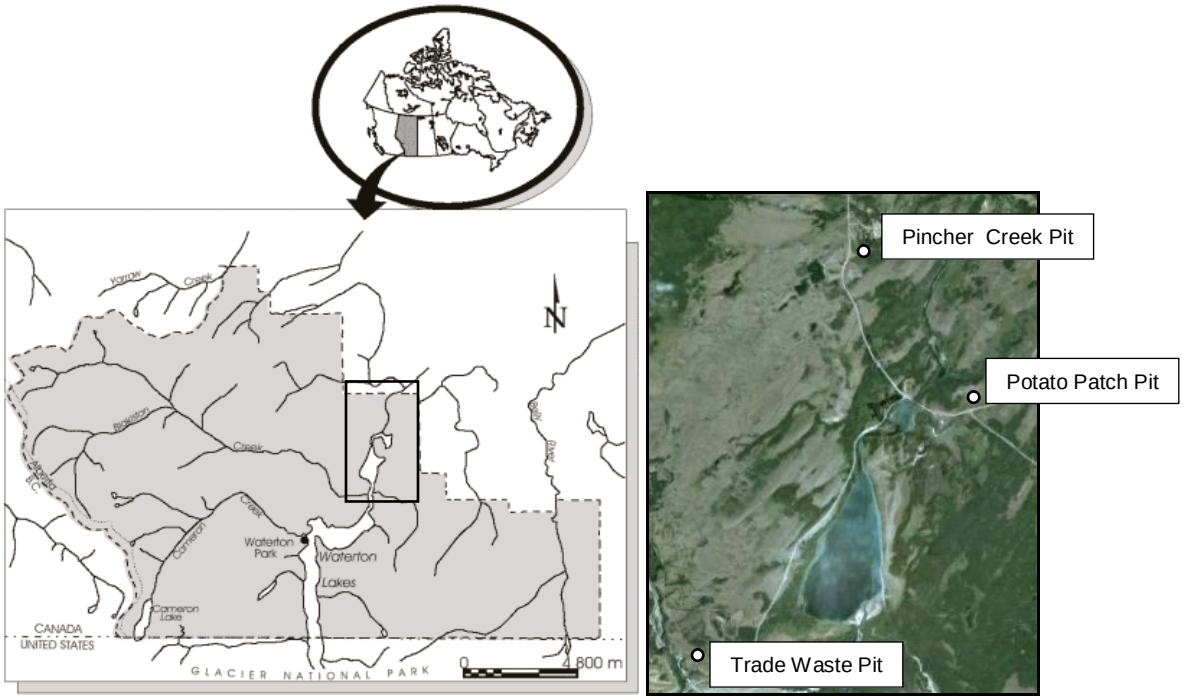

Figure 4-1. Location of Waterton Lakes National Park, Alberta, Canada and research sites (Parks Canada 2009, Google Earth 2012).

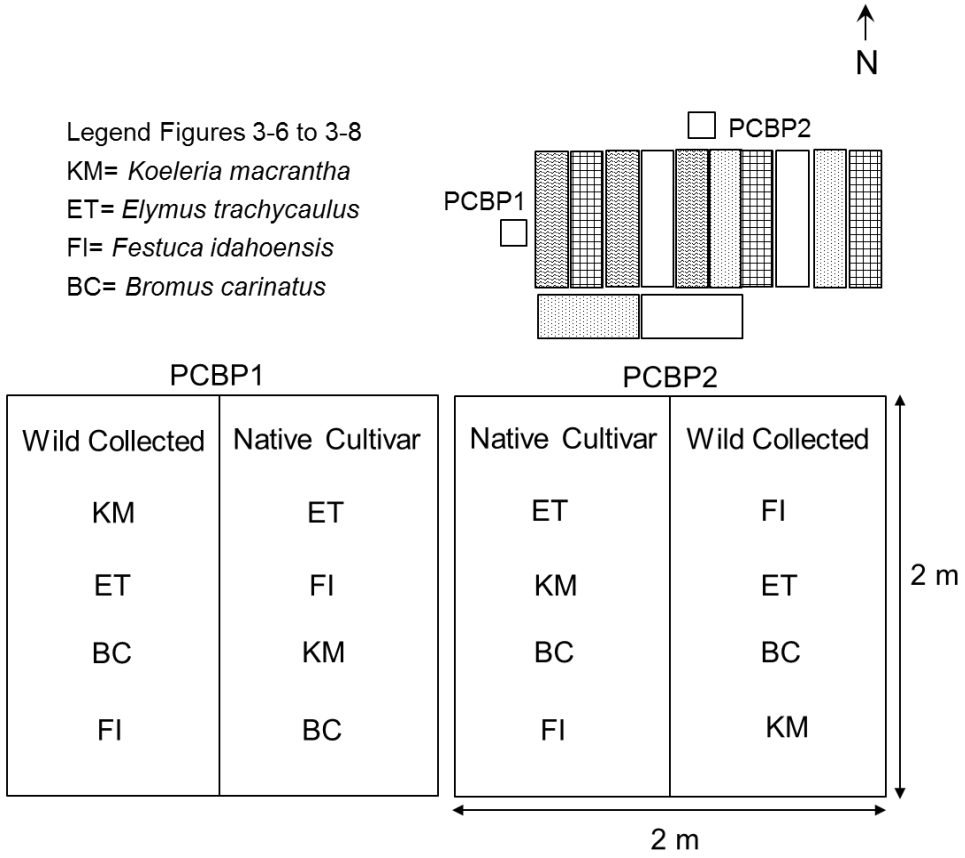

Figure 4-2. Planting and plot locations at Pincher Creek Pit for seed type experiment. Diagram in upper right indicates where plots are located relative to research plots used in a different experiment (Chapters two and three).

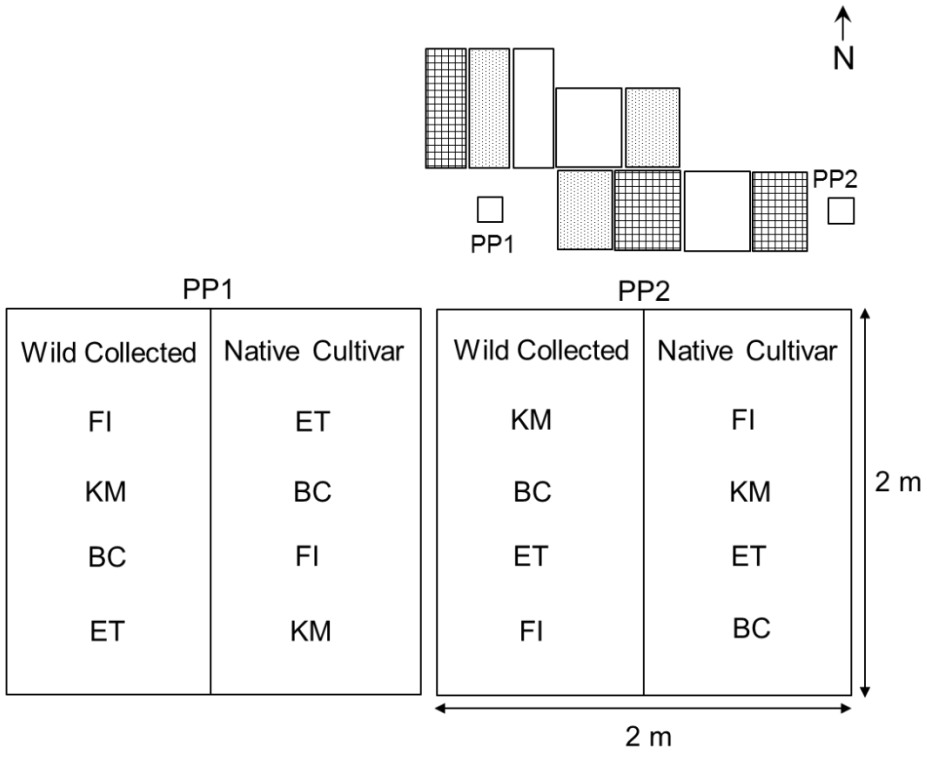

Figure 4-3. Planting locations at Potato Patch Pit for seed type experiment.

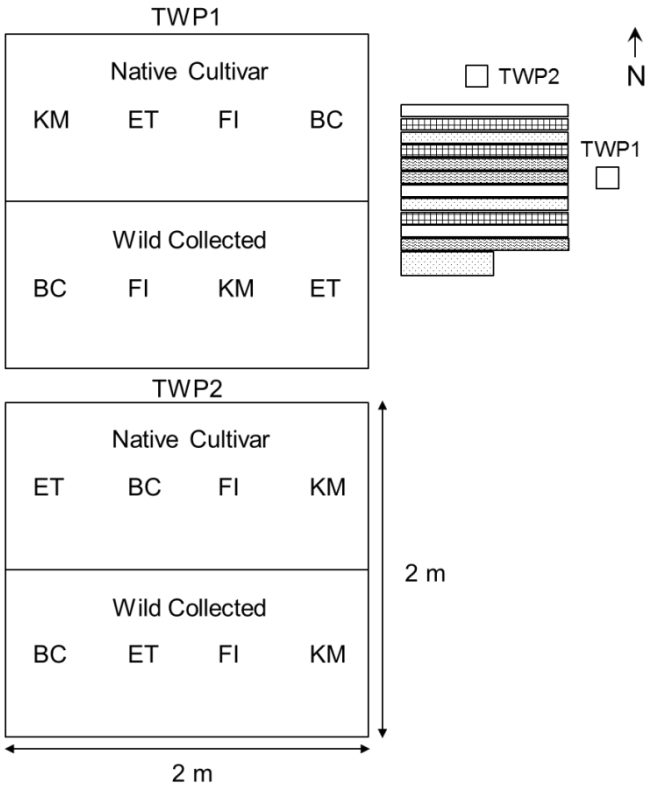

Figure 4-4. Planting locations at Trade Waste Pit for seed type experiment.

CHAPTER 5. ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES OF FOOTHILLS FESCUE GRASSLAND: ASSESSMENT OF DISTURBANCE AND INVASION IMPACTS

1. INTRODUCTION

Native grasslands are distributed worldwide, are high in biodiversity and perform important ecosystem functions (Tilman et al. 1996, Gibson 2009). Therefore, their conservation and restoration are of increasing global concern (Mlot 1990). Two major challenges in native grassland restoration are poor native plant species reestablishment and competition with non-native plant species (D'Antonio and Meyerson 2002). Foothills fescue grasslands have been reduced to 17 % of their former extent (Adams et al. 2003), with important remaining remnants protected in Waterton Lakes National Park, a United Nations Educational, Scientific and Cultural Organization (UNESCO) world heritage site. Large populations of non-native plant species have permanently established in disturbed grasslands, including several non-native species which form dense monocultures and spread into native grasslands.

Symbionts in the soil microbial community, especially arbuscular mycorrhizal fungi (AMF), may be an important biological factor altered by disturbance and non-native plant invasion (Allen 1991, Hartnett and Wilson 2002, Van der Heijden 2004). Alterations in AMF communities could affect native plant reestablishment and competition with non-native species (Van der Heijden et al. 1998, Klironomos 2002, 2003, Hart et al. 2003). However, little is understood about AMF community characteristics in disturbed and invaded relative to pristine habitats.

AMF are obligate symbionts and cannot survive without carbon provided by host plants in exchange for nutrients. Many AMF cannot be cultured using traditional methods and must be grown in pot cultures or root organ culture; they are difficult to identify physically due to conserved morphological features (Schenck and Pérez 1990). AMF are exclusive members of the phylum *Glomeromycota* (Schüßler et al. 2001), an ancient fungal lineage originating over 460 million years ago and thought to have acted as primordial root structures for plants, facilitating their colonization of land (Redecker 2000). A large number of plants (approximately 80 % of vascular species) participate in AM symbiosis, the

majority of which are grasses and forbs (Smith and Read 2008). AMF hyphae colonize plant roots and form structures called vesicles and arbuscules, from which their name is derived. Vesicles are energy storage structures densely filled with lipids produced within and outside the plant root at the end of hyphal tips; arbuscules are very finely branched hyphae located in root cortical cells, where carbon and nutrient exchange takes place (Peterson et al. 2004). There are 249 known AMF species (Schüßler and Walker 2010, Schüßler 2013), with a much larger number expected (Helgason et al. 2002, Redecker 2002, Fitter 2005). Molecular studies reported new isolates and data showing AMF diversity is similar to plant diversity (Öpik et al. 2006, 2009, 2010).

In grasslands, AMF are the dominant type of mycorrhiza. AMF improve drought tolerance, protect plant hosts from root pathogens and toxic stresses and increase nutrient uptake, resulting in increased productivity (Jeffries et al. 2003). AMF contribute to plant health and soil fertility and may increase reproduction and offspring survival in some plant hosts at early successional stages (Koide and Dickie 2002). AMF species diversity is a major factor contributing to plant biodiversity and productivity (Van der Heijden et al. 1998, Vogelsang et al. 2006) but AMF can negatively affect plant hosts on a parasitism-mutualism continuum (Johnson et al. 1997). Disturbance causes changes in species composition of plant and fungal communities (Hart and Reader 2004, Stover 2010, Stover et al. 2012) and can select for generalist AMF species (Hetrick and Bloom 1983, Hamel et al. 1994, Schalamuk and Cabello 2010, Öpik et al. 2009). Secondary succession occurs in Glomeromycotan fungal communities and succeeds towards the pre-disturbance community (Hamel et al. 1994, Li et al. 2007, Sýkorová et al. 2007). AMF distribution is dependent on abiotic soil conditions such as pH, organic matter and nutrient concentrations, and on disturbance and land use (Klironomos et al. 1993, Egerton-Warburton et al. 2007, Su and Guo 2007, Zachow et al. 2009, Dumbrell et al. 2010).

Whether plant-host specificity exists in AM symbiosis is critical in determining if AMF are important to native plant communities. AMF species vary widely in effects on host plant species (Klironomos 2003, Vogelsang et al. 2006). Diversity and species composition of AMF colonizing different plant species can vary considerably, even within the same plant families (Bever et al. 1996,

Vandenkoornhuyse et al. 2003, Santos-González et al. 2007). Plant-host specificity may be low due to the small number of known fungal species compared to the vast number of plant species (Smith and Read 2008). However, diversity of Glomeromycotan fungi may be highly underestimated based on current available data, species concepts and methods used to study fungal diversity (Sanders 2004). Experimental evidence is increasing in support of plant-host specificity (Hartnett and Wilson 1999, Helgason et al. 2002, Vandenkoornhuyse et al. 2002, 2003, Johnson et al. 2003, Klironomos 2003).

There is compelling evidence non-native plant species invasion affects AMF communities with implications for native host plants (Pringle et al. 2009). Non-native plants host generalist AMF taxa in their place of origin and their new introduced range in contrast to neighboring native plants which host a more diverse assemblage of local AMF species (Moora et al. 2011). Non-native plants can reject AMF colonization (Allen et al. 1989) and reduce AMF diversity and abundance via production of allelochemicals (Roberts and Anderson 2001, Mummey and Rillig 2006, Vogelsang and Bever 2010). Native plant species are often more dependent on mycorrhizal fungi than non-native plants. For example, Hetrick et al. (1988) found native *Liatris aspera* Michx. (Tall blazing star) was over 90 % dependent on mycorrhizal colonization whereas invasive *Bromus inermis* ssp. *inermis* Leyss. (Smooth brome) was 43 % dependent. AMF can be used as a competitive advantage by dominant species in plant communities (Goodwin 1992, Hartnett et al. 1994, Hetrick et al. 1994, Hartnett and Wilson 1999, Smith et al. 1999) and aid non-native plant invasions through disruption of mycorrhizal networks which native plants depend on (e.g. *Centaurea maculosa* L. (Spotted knapweed) Marler et al. 1999, Callaway et al. 2001, 2003, Zabinski et al. 2002, Walling and Zabinski 2004, Carey et al. 2004).

It is difficult and time consuming to culture and identify AMF using morphological characteristics; not all species can be cultured or produce spores for identification and culturing data may not adequately reflect true communities (Merryweather and Fitter 1998, Sanders 2004). Thus, molecular techniques have become central to AMF identification (Redecker 2002, Redecker and Raab 2006, Rosendhal 2008, Sharmah et al. 2010, Gorzelak et al. 2012). Most molecular identification systems for AMF are based on ribosomal DNA sequences (rDNA)

(Figure 5-1). Taxa can be distinguished with high resolution as rDNA genes are high in copy number, highly conserved and have variable sectors (Redecker et al. 2003). Next generation sequencing technology has made large datasets more easily and quickly available and sequencing of different samples and isolates possible simultaneously (Parameswaran et al. 2007, Hamady et al. 2008). Research on AMF employing next generation sequencing technology has begun (Öpik et al. 2009, Lumini et al. 2010, Moora et al. 2011, Dumbrell et al. 2011, Lekberg et al. 2012), which will provide great insights into the ecology of AMF.

Critical knowledge gaps exist in understanding AMF symbiosis and its implications for restoring native plant communities (Koide and Dickie 2004). Little is known about the influence of mycorrhizae on dynamics of competition between non-native and native plant species. *Festuca* grasses of the western United States and Canada host two to five AMF (Molina et al. 1978, Dalpé and Aiken 1998). *Festuca* grasses may use mycorrhizal hyphae to feed nutrients to offspring seedlings from adult plants, while mycorrhizae alone in the presence of seedlings may behave parasitically (Desserud 2011). Otherwise, there is very little knowledge about AMF in fescue grasslands and this is the first investigation of AMF communities in foothills fescue grassland.

2. RESEARCH OBJECTIVES

The objectives of this research were to provide baseline information about arbuscular mycorrhizal fungi by determining diversity and species composition of fungal communities in disturbed and undisturbed foothills fescue grassland and to assess the impact of disturbance modifications of soil properties and plant communities and non-native plant invasion on AMF to assist with ecological management and restoration of foothills fescue prairie.

3. MATERIALS AND METHODS

3.1 Research Sites

This research was conducted in Waterton Lakes National Park at three disturbed foothills fescue grassland sites undergoing restoration (Figure 5-2). Waterton Lakes National Park is located in the Rocky Mountains of southwestern Alberta

forming an International Peace Park and United Nations Educational, Scientific and Cultural Organization (UNESCO) World Heritage Site with Glacier National Park. It covers 525 km^2 and extends south to Montana and Glacier National Park and west to the Alberta-British Columbia border along the Continental Divide (Achuff et al. 2002). The western boundary borders Akamina-Kishinena Provincial Park in British Columbia. North and east sides border Alberta crown and private lands. In the northwest corner the park borders the Blood Indian First Nation Timber Limit in the Belly River area on three sides. Four Ecoregions are found in the park: Foothills Parkland, Montane, Subalpine and Alpine.

Grasslands are characterized by Chernozem soils with predominantly calcareous soil parent materials, good drainage and dark coloured mineral surface horizons high in organic matter. Climate in the foothills grasslands is characterized by a short growing season (June to August), cool, wet springs and hot, dry late summers. The region is windy, with maximum daily gusts of 70 to 90 km h^{-1}. Mean annual precipitation is 807.6 mm and minimum and maximum average temperatures were -1.3 ^{o}C and 10.6 ^{o}C, respectively, for 1990 to 2000 (Environment Canada 2012a, 2012b). Daily weather can change rapidly and unpredictably, typical of mountain regions.

A total of 971 vascular plant species are found in the park. In 2002, 20 plant species were discovered, including two new to Alberta and one new to Canada (Achuff et al. 2002). Over 50 % of Alberta wildflower species are found in the park, including 30 rare plant species found nowhere else in Canada.

A former landfill and two borrow pits were selected for study in a 3 km radius with similar topography and soils (Figure 5-2). The sites contain a large and diverse population of non-native annual and perennial species including *Centaurea maculosa*, *Cirsium arvense* L. (Canada thistle), *Bromus inermis* Leyss. (Smooth brome), *Elymus repens* (L.) Gould (Quack grass), *Poa pratensis* L. (Kentucky bluegrass), *Poa compressa* L. (Canada bluegrass) and *Agropyron cristatum* L. (Crested wheat grass).

The landfill site (Trade Waste Pit) was used for disposal of a variety of domestic waste, including building materials, fuel, treated wood, scrap metal, batteries and manure between 1952 and 1999 (Naeth and Jobson 2007). Soil and ground water sampled at the site within the past five years had elevated concentrations

of aluminum, copper, zinc, strontium, nickel, silver and iron, reflecting natural background concentrations in the region. The site was classified as very low risk with no required remediation (Canadian Council of Ministers of the Environment 2008). In 2006, waste was removed, the site was recontoured and wood chips and topsoil were applied to rebuild the soil. In fall 2006 and spring 2007, native grasses, forbs and shrubs from wild collected seed were planted (seeded and transplanted) and a revegetation study was initiated (Naeth and Wilkinson 2008).

Borrow pit sites were gravel quarries and disturbance details are not well known. Borrow Pit 1 (Potato Patch Pit) is 1.8 ha in size, located near Chief Mountain Highway Junction. Gravel excavation concluded during the 1960s and the site was officially decommissioned in the late 1970s. It became heavily infested with *Centaurea maculosa*, which was sprayed with herbicide and hand pulled in the 1980s, then plowed and revegetated with native plant species. Borrow Pit 2 (Pincher Creek Pit) is 2.2 ha in size, located near the Bison Paddock. In recent years park staff planted native plugs and tried to control target weeds.

3.2 Experimental Design

The study utilized 3 rectangular plots, approximately 200 m^2, at each of the disturbed sites designated as controls for comparison with restoration treatments. At each site, a fourth plot was established in the closest patch of undisturbed grassland without non-native species and with similar topography to disturbed plots. All undisturbed plots were within 400 m of the disturbed plots and neighboring disturbed area. All plots had even topography and uniform vegetation and soils. A 1 m wide buffer zone was located around each plot and plots were marked with painted wooden stakes. Trade Waste Pit plots were 4 x 54 m, Pincher Creek Pit plots 8 x 27 m and at Potato Patch Pit one plot was 9 x 27 m and two 15 x 18 m. Undisturbed plots were 8 x 30 m.

3.3 Vegetation Assessment

Vegetation was assessed August 2-9, 2011 to characterize host plant communities at mycorrhizal sampling locations. This time was chosen to capture the majority of flowering vascular plant species near the end of their peak growing season. North facing transects (East for Trade Waste Pit) bisecting each plot were established, transects were run down the length of the plots, and 10,

0.1 m^2 quadrats were systematically placed at equal distance intervals along these transects. At Trade Waste Pit, a 54 m long transect was run through the middle of each 4 x 54 m plot. At Potato Patch Pit, three transects were positioned every 3 to 4 m along the width of 15 x 18 m plots and quadrats positioned every 3 to 4 m; 9 x 27 m plots had two transects with quadrats every 5 m. At Pincher Creek Pit and all undisturbed plots two 30 m transects were positioned at 3 and 6 m along the width of each plot, with quadrats positioned every 5 m. Individual plant species and ground (bare ground, moss, vegetation, litter, thatch, rock) cover were determined by visual estimation. Botanical nomenclature followed Kuijt (1982), Moss (1983) and Tannas (2003).

3.4 Soil And Root Sampling And Analyses

Soil was sampled for AMF spores in early November 2010. Spore isolation by wet sieving and decanting (Gerdemann and Nicolson 1963) showed spore densities of arbuscular mycorrhizal fungi were extremely low in foothills fescue grasslands. Therefore, a molecular approach was chosen using field sampled roots from host plant communities. Soil sampling accompanied root sampling to characterize soil chemical and physical properties at sampling locations.

Soil and root sampling was conducted September 19-21, 2011. Transects and quadrat positions from vegetation assessments were used to randomly select soil and root sampling locations. Eighteen soil samples were collected; one from each control plot and three from each undisturbed plot, giving 6 per site. A total of 36 root samples were collected; 18 disturbed and 18 undisturbed, 2 from each disturbed plot and 6 from each undisturbed plot. At each sampling location, a 15 cm deep hole was dug with trowels and a sharp knife, with 500 g of soil and 100 g of roots removed as individual samples. Samples were placed in labeled plastic bags and stored at 4 ^{o}C until processing. After sampling, the hole was filled and covered with turf to minimize disturbance.

Soil samples were analyzed by Exova Laboratories in Edmonton, Alberta. Total nitrogen and carbon were determined by dry combustion and Leco combustion (Bremner 1996). Inorganic carbon was determined through carbon dioxide release; total organic carbon was calculated by subtracting inorganic carbon from total carbon (Loeppert and Suarez 1996). C:N ratio was determined by dividing

total carbon by total nitrogen. Total phosphorus was determined with strong acid extraction and inductively coupled plasma mass spectrometry (US Environmental Protection Agency 1996). Sand, silt and clay were determined by hydrometer (Kroetsch and Wang 2008). Electrical conductivity, pH, sodium adsorption ratio and available soil calcium, magnesium, sodium and potassium was determined in saturated paste (Miller and Curtin 2008).

Mycorrhizal root samples were kept at 4 ^{o}C for 2 weeks while being processed. Soil was removed by shaking and gently pulling aggregates from the roots. Roots were dry sieved using a 1 mm sieve to further remove soil, then gently washed in tap water until most soil was removed, patted dry with paper towel and air dried at room temperature (20 ^{o}C) for two hours. After drying, root samples were stored in paper envelopes and kept in glass jars with Drierite (Drierite Co. Ltd., USA) at room temperature. Roots were checked weekly for excess water and moulding. A subsample of roots from each plot was placed in a labeled glass vial containing 50 % ethanol for assessment of mycorrhizal colonization (Brundrett et al. 1994).

In November 2011, root samples were assessed to confirm mycorrhizal colonization prior to molecular analysis by staining with a simple and non-toxic ink and vinegar method (Vierheilig et al. 1998 with modifications based on Brundrett et al. 1994). Root samples stored in 50 % ethanol were removed from vials with micro forceps and washed with distilled water on a 250 µm sieve to remove ethanol and debris. Subsamples of 1 g of root tissue were placed in glass vials one-third filled with potassium hydroxide (10 % m/v KOH in dH_2O), covered with loosely sealed caps, placed in a tube rack and autoclaved 15 minutes at 121 ^{o}C to clear roots of cellular contents. Samples were rinsed thoroughly on a 250 µm sieve to remove solution, either under a gentle stream of cold tap water or using a 500 ml squeeze bottle. Samples were placed in one-third full vials of 5 % v/v ink vinegar solution. Black Shaeffer ink was used and diluted to 5 % in household vinegar. Samples were autoclaved 3 minutes at 121 ^{o}C to stain roots. After staining, roots were rinsed several times for 5 to 10 minutes in cold tap water with a 500 ml squeeze bottle acidified with a few drops of vinegar. Roots were stained an opaque black; therefore, destaining was necessary so mycorrhizal structures could be seen. Root samples were placed in vials containing 50 % v/v glycerol (in dH_2O), sealed and left for 3 to 5 days. Pieces of

root tissue were mounted on glass slides in distilled water and polyvinyl lacto glycerol, a temporary preservative and mounting medium. Roots were examined under a compound confluorescence microscope at 100 to 400 magnification.

3.5 Molecular Procedures

Root samples were immersed in liquid nitrogen and ground to a fine powder with mortar and pestle. Grinding was performed as bead beating was insufficient to fully homogenize root tissue, a necessary step for obtaining DNA of intraradical AMF. DNA was isolated from 50 mg of each sample using a MoBio Power Plant DNA isolation kit according to manufacturer's instructions (MoBio Laboratories Inc., USA) and stored at -20 ^{o}C in aliquots. 2 µl of DNA extract was run on a 0.7 % agarose gel in 1X TAE buffer to confirm DNA isolation was successful.

The universal eukaryotic primer NS31 and AMF specific primer AML2 were used to amplify a central fragment of the V3-V4 region of the 18S rRNA gene (Figure 5-1) (Simon et al. 1992, Lee et al. 2008). AML2 was used instead of the common NS31 and AM1 pair for better coverage of basal AMF families *Archaeosporaceae* and *Paraglomeraceae* (Helgason et al. 1998, Davison et al. 2012). The 18S gene was chosen because it has a large amount of data from previous studies (Öpik et al. 2010). Polymerase chain reaction (PCR) was performed with NS31 and AML2 attached to 454 sequencing adapters A and B. Adapter A, CCATCTCATCCCTGCGTGTCTCCGACTCAG, was inserted before NS31 and B, CCTATCCCCTGTGTGCCTTGGCAGTCTCAG, before AML2. Barcodes were used to identify sequences belonging to each sample. A barcode sequence 10 nucleotides long was inserted in between adapter A and NS31. 36 different barcodes were used in the forward composite primer to identify sequences from each sample. A barcode was inserted between adapter B and AML2. Barcodes and adapters were designed by Roche 454 Life Sciences Corporation.

HotStarTaq Master Mix Kit was used for PCR (Qiagen Inc., Canada). For each reaction the following was added: 10 µl HotStarTaq Master Mix, 3 µl template DNA (2 ng/µl), 1 µl of each composite primer (10 µM), 0.6 µl bovine serum albumin (Fermentas Canada Inc.) (20 mg/ml) and 4.4 µl nuclease free water. PCRs were run on a S1000 Thermocycler (Bio-Rad Laboratories Canada Ltd.) with initial denaturation and HotStarTaq polymerase activation step of 95 ^{o}C for

15 min, followed by 30 cycles of denaturation at 94 ^{o}C for 45 sec, annealing at 60 ^{o}C for 45 sec, polymerization at 72 ^{o}C for 1 min and a final elongation step of 72 ^{o}C for 7 min. Following PCR, replicate reactions per sample were pooled to 100 µl and purified using Qiagen QiaQuick PCR Purification Kit (Qiagen Inc. Canada) and stored at -20 ^{o}C. 2 µl of PCR product from each sample was run on a 1.5 % agarose gel to confirm the targeted gene product was amplified. PCR samples were measured using a Qubit® 1.0 Fluorometer (Invitrogen Ltd. and Molecular Probes Inc.), diluted to 20 ng/µl minimum concentration and sent for sequencing. Sequencing services were provided by the Génome Québec Innovation Centre in Montréal, Québec. Samples were further purified by the sequencing provider using Agencourt AMPure XP PCR purification kit (Beckman Coulter Canada Inc.). After purification, samples were screened for quality control, measured and pooled in equal concentrations. Sequencing was performed using a half-plate of a Roche GS-FLX Titanium Sequencer.

3.6 Bioinformatics

A detailed description of the 454 data analysis pipeline used is provided in Appendix C. A total of 770,812 raw reads were obtained in the half-plate sequencing run (mean per sample = 21,411 ± 5,646 standard deviation). Number of raw reads per sample was consistent except for a few samples (Figure C.1). Roche-454 technology was developed for genome sequencing which is more robust to sequencing error and is not greatly affected by errors in individual sequencing reads. However, using 454 reads for interpretation of sequences from microbial communities requires confidence in correctness of individual reads (Schloss et al. 2011). PCR errors can introduce chimeras, which can increase chances of inaccurately identifying novel taxa (Haas et al. 2011). Therefore, strict quality control was performed on sequencing reads prior to taxonomic analysis using procedures to decrease sequencing error rate from ~0.6 to 0.02 % (Schloss et al. 2011). Mothur SOP pipeline was applied to the data (Schloss 2013) using Mothur v. 1.28.0 (Schloss et al. 2009) and adapted for analysis of the partial 18S rRNA gene and targeted sequences belonging to AMF.

Issues were encountered in the original 454 dataset where a common insertion error in the forward primer occurred in over 50 % of reads. Re-processing of run data with up to date software from Roche resolved the issue. Sequencing noise

was reduced with PyroNoise, a component of AmpliconNoise (Quince et al. 2009, Quince et al. 2011). Sequences shorter than 200 bp and with homopolymers longer than 8 bp were removed. Most raw reads were approximately 500 bp in length (mean = 487) and reduced to approximately 280 bp after denoising (mean = 274). Multiple sequence alignment was performed in Mothur using a reference alignment for the Glomeromycota 18S rRNA gene (Krüger et al. 2012). Aligned sequences were screened to check that they overlapped in the same alignment space and a pre-clustering step merged sequence counts for reads within two base pairs of more abundant sequences (Huse et al. 2010). Potentially chimeric sequences were removed using chimera.uchime (Edgar et al. 2011); 169 chimeric sequences were detected and removed. A reference database of Glomeromycota 18S rDNA sequences was retrieved from MaarjAM database in fasta format (Öpik et al. 2010). Sequences were classified in Mothur using sequences and taxonomy metadata from MaarjAM and Silva Eukarya (Pruesse et al. 2007); sequences not identified as AMF were removed. Mothur's default classification settings were used which implement Ribosomal Database Project's Bayesian Classifier (Wang et al. 2007), with the exception that a cutoff of 95 % was used instead of 80 % to more accurately classify sequences to species.

Operational Taxonomic Units (OTUs) were used to characterize AMF communities. They are sequence clusters grouped at a specific level of sequence similarity and taxonomic hierarchy for taxonomic comparison and assignment (Schloss and Handelsman 2005, Schloss et al. 2009, Sun et al. 2009, Davison et al. 2012, Bik et al. 2012). The default OTU construction method in Mothur was employed, which utilizes the furthest neighbor clustering algorithm with a distance matrix cutoff of 0.15 (Schloss and Westcott 2011). OTUs were clustered at 97 % similarity and further refined and classified by performing a BLAST search against MaarjAM database. For taxonomic assignment, MaarjAM contains published Glomeromycota 18S rRNA gene sequences amplified by NS31/AM1 and AML2 primers grouped at ≥ 97 % similarity referred to as virtual taxa. OTUs were classified as MaarjAM virtual taxa and published named Glomeromycota sequences if the similarity score was ≥ 97 %, alignment length covered the full 454 read and was no more than 10 bp shorter than the query length and a BLAST e-value of <1e-50 was obtained; for multiple hits, the match with the highest BLAST score was selected (Öpik et al. 2009, Davison et al. 2012). OTUs

represented by two or more sequences that met criteria but received a similarity score of 96 % were classified to genus and retained in the data set. Single OTUs that did not classify to a MaarjAM taxon were removed.

3.7 Statistical Analyses

For unbiased comparison of diversity, samples were standardized by randomly subsampling to the minimum number of reads in the smallest sample (7,124) (Brazelton et al. 2010, Gihring et al. 2012). Subsampling to the minimum was chosen as the risk of making an inaccurate conclusion due to unequal sample representation was more important than including a larger data set. This approach eliminated one sample not included in the statistical analyses. The effect of 454 read processing on read length and number of sequences per sample was assessed by comparing read lengths and numbers before and after processing. Adequacy of sequencing and sampling depth was assessed in Mothur with rarefaction curves, resampling without replacement which gives the number of OTUs observed per sample and number of sequences based on the 1,000 iterations.

For all statistical tests, $\alpha = 0.05$, and was corrected using the Bonferroni adjustment for multiple comparisons. Multivariate analyses were conducted using PC-ORD v. 6.08 (Peck 2010, McCune and Mefford 2011). Due to numerous zeros and heterogeneity in the data, non-metric multidimensional scaling (NMS) was chosen with the Sørensen distance measure with 500 iterations in the final run, two axes and a random starting configuration. Taxa only occurring once in were removed to reduce sparsity. Vegetation and soil data were overlaid on the ordination to identify key plant species and soil properties influencing AMF community structure.

Correlations between key variables and ordination axes were tested for significance using Spearman Rank Correlation test in SAS v. 9.2 (SAS Institute Inc. 2003). NMS ordination was performed on host plant data. Ordinations were rotated 90 degrees manually to more clearly illustrate ordination patterns. AMF communities from each site and treatment were tested to determine if they were significantly different using multiple response permutation procedure (MRPP). MRPP and NMS were each accompanied by a randomization test. Two-way

clustering using the Sørensen distance measure and average group linkage method was used to identify potential AMF generalist and specialist taxa and further elucidate community distribution patterns. AMF species richness, evenness and Shannon and Simpson diversity indices were all compared between disturbed and undisturbed fescue grassland using Student's t-test (n = 6). Species richness was included to have an abundance-independent diversity estimate as sequence number is not necessarily a true reflection of abundance. Correlation between AMF diversity measures and plant species diversity with the same calculated parameters was tested using Spearman Rank Correlation and correlation between AMF diversity and non-native, native and *Bromus inermis* cover. To test for potential confounding spatial autocorrelation in the variation in AMF and plant communities among sites, the Mantel test was performed using Euclidean distance matrix on the latitude and longitude of sampling locations and a Sørensen distance matrix on species abundances.

4. RESULTS

4.1 Soil Chemical And Physical Properties

Soils of the undisturbed fescue grassland surrounding the study sites were Black Chernozems typical of the region. Soils in disturbed areas were modified by disturbance as indicated by lower organic matter and total organic carbon content relative to undisturbed areas (Table 5-1). Nitrogen and phosphorous were slightly lower in disturbed areas. Sites had rocky sediment typical of the region. Soil conditions were similar throughout the study area. Pincher Creek Pit had highest sand content and pH and low phosphorous and sodium.

4.2 Vegetation

Plant communities in disturbed areas were dominated by non-native vegetation with lower biodiversity (Table 5-2). Non-native perennial grass species *Bromus inermis* was abundant at Trade Waste Pit and Potato Patch Pit but only traces occurred at Pincher Creek Pit and this species was not present in undisturbed areas. Non-native cover was greatest at Trade Waste Pit and least at Pincher Creek Pit, with the opposite trend for native species. Undisturbed fescue grassland had higher biodiversity and only traces of non-native vegetation.

4.3 Arbuscular Mycorrhizal Fungi

Results from mycorrhizal colonization (root staining) showed plots where roots were sampled contained plant species colonized by arbuscular mycorrhizal fungi (Figures C.2 to C.4). Since all sample areas contained roots colonized by AM fungi, molecular identification of AMF from root samples was validated and unbiased by sampling location. Results from PCR showed pyrosequencing primers were able to amplify genomic DNA in one round of PCR, reducing PCR reactions necessary to obtain PCR amplicons for sequencing (Figure C.5).

A total of 8,283 sequences were identified as other taxa (e.g. Metazoa, other fungi) and were removed, representing 1.76 % of total processed reads. Number of reads per sample was reduced after sequence processing, but number of reads among samples remained relatively even with a few exceptions (Figure C.1). After processing, mean read length was reduced from 487 to 260 bp. Total number of processed sequences obtained was 461,307 and generated 491 operational taxonomic units clusters in Mothur at 97 % similarity. After classification in MaarjAM using BLAST, 190 OTUs representing 349 sequences were removed that did not meet classification criteria, considered spurious AMF sequences (e.g. chimeras, sequencing artifacts). 202 OTUs were redundant, classifying to the same virtual taxon as other OTU groupings. Rarefaction analysis showed sampling depth adequately covered the observed AMF diversity (Figure C.6). For sequencing coverage, number of OTUs detected in the majority of samples began to plateau at < 2,000 sequences, confirming sequencing depth was adequate to characterize diversity in each sample and subsampling to the minimum depth of 7,124 sequences for statistical analysis would not result in a major loss of information (Figure C.7).

In total, 92 arbuscular mycorrhizal fungal taxa were found including 15 OTUs that may be newly discovered taxa which met classification criteria but did not match at 97 % with any published sequence records (Table C.1). *Glomus* comprised the majority of species, while only small numbers of other genera were observed. Ten most abundant taxa represented 92 % of the data set and the most abundant taxon *Glomus* WOTU1 represented 34 %. AMF taxon abundance varied distinctly between disturbed and undisturbed locations or randomly with no pattern. For example, *Glomus* WOTU1 was more abundant in undisturbed than

disturbed locations and *Glomus* sp. VTX177 was more abundant in disturbed. Most taxa were less abundant, restricted to one or two locations. Several AMF were identified to taxa detected from field sites in Europe, North America and elsewhere or were known globally distributed species. Species richness of AMF was significantly higher in undisturbed native grassland than disturbed sites but no significant differences between disturbed and undisturbed habitats were detected for other diversity parameters (Tables 5-3, C.2).

AMF communities were highly variable compared to their host plant communities and showed much greater similarity among sampling locations. An AMF community was found at Pincher Creek Pit disturbed grassland characterized by *Glomus* spp. VTX177, VTX165 and MO.G8 VTX130 that was significantly different from all other areas (Figure 5-3, Table C.2). A highly variable community was found at Trade Waste Pit disturbed grassland that was significantly different from its neighbouring undisturbed grassland and was characterized by *Glomus* spp. VTX143, WOTU20 and WOTU58 (Figure 5-3, Table C.2). Undisturbed AMF communities were similar, forming a broad cluster characterized by *Glomus* spp. MO.G27 VTX160, WOTU1 and QU.Glo7 VTX187, including Potato Patch Pit disturbed. The AMF community at Potato Patch Pit disturbed grassland showed no uniqueness relative to other sites. Dominant species characteristic of each community group were significantly correlated with NMS ordination axes (Tables 5-4, C.3). When sites were combined as statistical replicates undisturbed and disturbed communities were significantly different (Table C.2).

4.4 Relationship Between Arbuscular Mycorrhizal Fungi, Host Plant Species And Soil Properties

NMS ordination of host plant communities showed a distinct native plant grassland community at undisturbed sites, non-native communities at Trade Waste Pit and Potato Patch Pit and a distinct community at Pincher Creek Pit, obtained a final stress value of 11.83 and produced a significant pattern according to the randomization test (Figure 5-4). NMS ordination of AMF communities showed high variation within sampling locations and similarity among undisturbed sites and Potato Patch Pit disturbed grassland (Figure 5-5). Pincher Creek Pit grouped separately as a distinct AMF community. Trade Waste Pit disturbed grassland diverged from undisturbed communities forming a highly

variable plant community type. AMF ordination was significant and produced a stress value of 14.6.

A soil gradient was detected in the ordination that was also observed for plant communities. Plant and AMF communities shifted along a gradient of decreasing silt, organic matter, total organic carbon, sodium, nitrogen and phosphorous and increasing sand and pH from undisturbed community grouping towards Pincher Creek Pit disturbed grassland. This gradient produced significant correlations between soil factors and NMS axes (Figures 5-4, 5-5; Tables 5-4, C.3). AMF taxa were significantly correlated with soil factors along the gradient (Tables 5-5, C.4).

Several plant species, both native and non-native, were significantly correlated with NMS axes, reflecting potential importance for specific AMF communities (Tables 5-4, C.3). AMF taxa were significantly correlated with some host plant species (Tables 5-5, C.4). For example, *Glomus* spp. WOTU20 and WOTU58 were significantly positively correlated with non-native perennial grasses *Agropyron cristatum*, *Poa compressa* and *Bromus inermis*. Species richness and diversity was higher for AMF communities than their host plant communities, as the ratio of AMF to host plant species was 92:62 in total and 23:8 on average in each sampling location. Total number of species detected at sampling sites, 159, would bring the overall ratio to 92:159 (Tables A.2 to A.5). AMF species richness was significantly positively correlated with host plant species richness and Shannon diversity index (Tables 5-6, C.5). There was no significant relationship between AMF diversity and non-native, native or *Bromus inermis* cover although large Spearman correlation coefficients were obtained. There was no significant spatial autocorrelation detected in AMF or plant communities (Table C.2).

5. DISCUSSION

5.1 Arbuscular Mycorrhizal Fungi Of Foothills Fescue Grassland

Foothills grasslands in Waterton Lakes National Park contain a high proportion (37 %) of known arbuscular mycorrhizal fungal taxa including several which are globally distributed and found in a broad range of ecosystems (grassland, coniferous forest, anthropogenic vineyard) (Öpik et al. 2010). These fungi were sampled in a surface area of 3.6 m^2 across 17 km^2. There were 15 novel

sequences identified that could represent newly discovered species which may be unique regionally or globally. The large number of taxa found were expected due to the variety of habitats sampled. Richness of arbuscular mycorrhizal fungi significantly decreases due to disturbance and non-native plant invasion, even after >20 years. Previous investigations also found high diversity of arbuscular mycorrhizal fungi from the 18S rRNA gene (Haug et al. 2010, Lumini et al. 2010) and reduced diversity in recovering disturbed areas with non-native vegetation (Li et al. 2007, Li et al. 2010). The average ratio of AMF to host plant species in each sampling location (23:8) was similar to 3-5 AMF taxa associated with western North America *Festuca* species (Molina et al. 1978, Dalpé and Aiken 1998) and the 8 AMF per plant species found globally in grassland ecosystems (Öpik et al. 2006). Number of colonizing AMF per plant species is thought to increase with seedling survivorship (Veresoglou and Halley 2012).

Potential AMF generalists and specialists identified were not restricted to undisturbed native plant communities, in contrast to previous studies and current belief that disturbance and non-native plant invasion promote generalists and specialists are restricted to pristine environments (Öpik et al. 2009). AMF communities in disturbed and undisturbed locations were much more similar to each other than vegetation was, suggesting resilience in AMF communities to disturbance and non-native plant invasion; however, within each disturbed sampling location there was high variability suggesting community instability. Lekberg et al. (2012) found high within site variability of AMF communities and high similarity among AMF communities overall between disturbed and undisturbed sampling locations.

Results of this study suggest AMF communities of foothills fescue grassland are low sporulating but abundant intraradically. This was observed in the only other report of AMF in Canadian fescue grassland (Molina et al. 1978) and may be an important characteristic of these ecosystems. This is the first detailed description of mycorrhizal communities in foothills fescue grassland.

5.2 Impact Of Disturbance Modified Soil Properties

Changes in soil properties from disturbance significantly impact AMF community composition, much greater than changes in host plant community, and soil

factors affect distribution of host plants. Soil conditions in the study area were similar, highlighting AMF sensitivity to soil properties. Abiotic soil conditions were previously established as a strong control of AMF distribution (Johnson et al. 1992, Klironomos et al. 1993, Allen et al. 1995, Egerton-Warburton et al. 2007, Su and Guo 2007, Zachow et al. 2009, Dumbrell et al. 2010, Oehl et al. 2010, Zarei et al. 2010), although disturbance, land use, plant species richness and functional diversity have potentially greater direct influences on AMF communities (König et al. 2010, Lumini et al. 2010).

Further research is needed to characterize important relationships among AMF and these soil factors. Theories such as neutral models for community assembly are thought to apply to AMF due to dispersal limitation; application of such theories to microbial communities is lacking and can provide insight for understanding community dynamics (Caruso et al. 2012). This study demonstrated AMF communities are strongly affected by niche differentiation in soil abiotic factors, confirming results from previous studies (Li et al. 2007, Dumbrell et al. 2010, Li et al. 2010).

5.3 Impact Of Host Plant Community

Soil conditions were a much better predictor of plant and AMF community structure than either symbiotic partner. Other studies found weaker relationships with plant hosts such as AMF productivity and plant species diversity (Koch et al. 2012). However, increasing plant diversity and richness was strongly and significantly correlated with increasing AMF richness and observed widely in previous research (Van der Heijden et al. 1998, Vogelsang et al. 2006, König et al. 2010). According to this study, plant host specificity may occur with select groups of plant species and AM fungi within communities. This was previously hypothesized; host plant species may affect AMF communities due to their influence on a specific subset of AMF taxa, as AMF taxa differ in response to changes in plant species composition (Johnson et al. 1992) and show plant host specificity (Hartnett and Wilson 1999, Helgason et al. 2002, Vandenkoornhuyse et al. 2002, 2003, Johnson et al. 2003, Klironomos 2003, Gollotte et al. 2004).

High AMF diversity and ecological group specificity has been observed in forest AMF communities (Öpik et al. 2009, Davison et al. 2011). While identified AMF

taxa may be present in floristically different locations, their behaviour and ecological function in the presence of different plant species is unknown, and in previous studies varied greatly depending on plant host species (Klironomos 2003, Vogelsang et al. 2006). There may be high functional diversity in AM fungi; therefore, if one AMF taxon is altered, there may be a change in resource acquisition and growth by plant hosts (Allen et al. 1995).

5.4 Non-Native Plant Invasion And Arbuscular Mycorrhizal Fungi

Understanding effects of non-native plant invasion on AMF communities is critical to ecological management, as it may provide useful information for mitigating loss of native biological diversity (Pringle et al. 2009). A diverse and large population of non-native plants were present in the study system (55 species), providing an opportunity to evaluate invasion impacts from a broad range of species. Both negative and neutral impacts of non-native plants on AMF were observed and evidence was generated supporting two alternative hypotheses surrounding non-native plants and mycorrhizal symbiosis. The long standing hypothesis that invasive species detrimentally affect AMF, posing problems for management and native plant restoration (Hawkes et al. 2005, Koch et al. 2011, Moora et al. 2011) or alternatively that there may be little effect of invasions on AMF communities; therefore, damage to AMF may not be an additional challenge in addressing invasion and reestablishment of native plants (Lekberg et al. 2011). Conflicting results illustrate the complexity of this issue and the need for further research.

5.5 Study Considerations And Use Of 454 Sequencing

Several approaches have been used to analyze 454 sequencing data in AMF studies (Öpik et al. 2009, Lumini et al. 2010, Moora et al. 2011, Dumbrell et al. 2011, Lekberg et al. 2012, Davison et al. 2012). Mothur standard operating procedure was chosen as it significantly reduces sequencing error (Schloss et al. 2011), a major issue with 454 pyrosequencing technology. Subsampling to minimum number of reads can result in significant information loss, for which alternative techniques are strongly supported (Aguirre de Càrcer et al. 2011). Due to the large number of reads and importance of accurate comparison of diversity, subsampling was to the minimum. Sequence processing steps and single round of PCR may have increased amplification specificity which could

have led to lower percentage of observed non-AMF reads. Taxon abundances derived from molecular datasets are often questioned due to systematic biases inherent in processing, PCR and sequencing. 454 pyrosequencing can cause biases in sequence number (Amend et al. 2010, Berry et al. 2011). Abundance-independent assessments used (e.g. species richness) help address this issue.

Spatial autocorrelation and sampling and sequencing adequacy are important to assess in microbial ecology studies (Mummey and Rillig 2008, Unterseher et al. 2011). Research on design of an optimal sampling scheme to characterize AMF communities has only begun (Öpik and Moora 2012), but can be assessed using rarefaction and spatial autocorrelation analysis, as in this study, and through these analyses were not a significant issue. Studies on AMF communities may be biased by temporal variation (Husband et al. 2002), but this was negligible (Davison et al. 2012), to vary the greatest between summer and winter (Dumbrell et al. 2011) and communities are known to develop increased distinctiveness later in the growing season (Douds and Millner 1999, Davison et al. 2011). Therefore, since sampling was conducted in late summer, timing of sampling did not likely negatively affect observation of full diversity of AMF communities.

5.6 Restoration And Management Implications

High AMF biodiversity observed in Waterton foothills fescue grasslands supports their conservation and restoration. Maintenance of plant diversity will likely benefit diversity of arbuscular mycorrhizae. Strong resilience of AMF communities was observed at Potato Patch Pit, indicating, in some cases, AMF communities are not greatly damaged by disturbance and non-native plant invasion. This provides optimism for restoring native plant communities as symbiotic fungi are present to re-establish mutualistic networks. Restoration and conservation practitioners can expect greatest changes in AMF communities when soil properties have been significantly altered and invasions are more extensive and long term (Trade Waste Pit). Competition with non-native plants will remain a challenge for management, as results also indicate invasive plants are capable of forming fully-functional mycorrhizae with native AMF.

This investigation emphasizes the importance of soil science in restoration ecology and soil drivers of above and below ground community composition.

Results clearly illustrate the importance of soil chemical and physical properties in shaping AMF communities. The contrasting results highlight the challenge of understanding complex ecosystems and symbiotic relationships. Interesting relationships between AMF and host plant richness and between AMF and host plant species were revealed. Identification of multiple working hypotheses to explain behaviour of different functional groups within symbiotic communities such as arbuscular mycorrhizae is likely a better approach than attempting to draw universal conclusions that can be applied to all species participating in a symbiosis. AMF are important symbionts contributing towards maintenance of healthy ecosystems and plant communities. Knowledge from this study can be used to further understand characteristics of AMF communities in ecosystems and develop well informed approaches to ecosystem management and conservation of native plant species.

6. CONCLUSIONS

- Waterton Lakes National Park grasslands contain at least 92 different AMF taxa and 15 putatively novel AMF species.
- AMF are highly sensitive to soil chemical and physical properties and vary in species composition based on changes in soil properties.
- Disturbed grasslands in Waterton National Park invaded by non-native plant species have similar AMF communities to undisturbed native grasslands when soil conditions are similar but disturbed AMF communities are highly variable and unstable and have lower species richness.
- AMF species richness may be negatively affected by decreasing plant species richness, diversity and native cover and increasing non-native and *Bromus inermis* cover.
- Abundance of specific native and non-native species is significantly related to abundance of specific AMF.

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Table 5-1. Soil chemical and physical properties at research sites in September 2011.

Parameter	Pincher Creek Pit		Potato Patch Pit		Trade Waste Pit	
	Disturbed	Undisturbed	Disturbed	Undisturbed	Disturbed	Undisturbed
C:N Ratio	14.4 ± 3.8	11.5 ± 0.4	23.7 ± 33.6	11.1 ± 0.1	15.1 ± 3.4	13.2 ± 0.7
Total Nitrogen (%)	0.2 ± 0.1	0.7 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.2
Organic Matter (%)	4.5 ± 2.9	16.9 ± 2.5	2.6 ± 1.6	10.0 ± 1.4	6.1 ± 2.6	11.7 ± 4.6
Total Inorganic Carbon (%)	0.6 ± 0.3	0.1 ± 0.0	0.8 ± 0.7	0.1 ± 0.0	0.4 ± 0.3	0.1 ± 0.1
Total Organic Carbon (%)	2.2 ± 1.5	8.5 ± 1.2	1.3 ± 0.8	5.0 ± 0.7	3.1 ± 1.3	5.9 ± 2.3
Total Phosphorus (mg/kg)	606.7 ± 59.2	1013.3 ± 70.4	405.8 ± 65.6	750.0 ± 74.8	828.3 ± 124.9	1093.3 ± 273.5
Sand (%)	82.2 ± 4.5	59.7 ± 4.0	64.4 ± 12.3	59.0 ± 2.7	63.2 ± 4.3	63.2 ± 4.0
Silt (%)	13.8 ± 3.6	36.5 ± 3.3	26.8 ± 9.3	37.7 ± 3.0	29.3 ± 4.0	30.5 ± 3.9
Clay (%)	4.0 ± 1.1	3.8 ± 0.9	8.8 ± 3.6	3.3 ± 0.5	7.42 ± 1.2	6.3 ± 0.9
Hydrogen Ion Concentration (pH)	7.9 ± 0.1	6.8 ± 0.2	5.9 ± 0.2	7.2 ± 0.1	7.5 ± 0.5	6.6 ± 0.6
Electrical Conductivity (dS/m)	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.3	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
Sodium Adsorption Ratio	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Available Calcium (mg/kg)	31.1 ± 11.8	57.6 ± 8.8	40.6 ± 28.0	49.3 ± 11.9	25.3 ± 10.3	17.9 ± 4.8
Available Magnesium (mg/kg)	5.3 ± 2.2	14.4 ± 2.9	8.5 ± 6.0	13.0 ± 2.7	7.1 ± 3.4	5.9 ± 1.0
Available Sodium (mg/kg)	0.3 ± 0.5	3.0 ± 0.8	0.8 ± 0.5	1.7 ± 0.5	1.3 ± 0.4	1.7 ± 0.5
Available Potassium (mg/kg)	5.5 ± 2.8	33.0 ± 12.4	6.2 ± 5.7	16.7 ± 7.4	11.7 ± 7.5	7.0 ± 0.8

Numbers are mean ± standard deviation.

Table 5-2. Plant community characteristics at sampling locations.

Parameter	Pincher Creek Pit		Potato Patch Pit		Trade Waste Pit	
	Disturbed	Undisturbed	Disturbed	Undisturbed	Disturbed	Undisturbed
Native Cover (%)	25.1 ± 13.2	109.4 ± 27.7	14.5 ± 21.3	73.1 ± 18.6	3.7 ± 5.4	54.4 ± 8.3
Non-Native Cover	3.9 ± 2.6	0.0 ± 0.0	20 ± 9.7	1.1 ± 1.39	51.1 ± 20.1	1.5 ± 1.9
Bromus inermis Cover	0.0 ± 0.0	0.0 ± 0.0	9.5 ± 2.7	0.0 ± 0.0	14.33 ± 10	0.0 ± 0.0
Shannon Diversity	1.5	2.0	1.2	1.5	1.2	1.7
Species richness	7	13	4	9	6	7
Evenness	0.8	0.8	0.8	0.7	0.7	0.7
Simpson Diversity	0.7	0.8	0.6	0.7	0.6	0.7

Numbers are mean ± standard deviation.

Table 5-3. AMF diversity in disturbed and native grassland.

	Pincher Creek Pit		Potato Patch Pit		Trade Waste Pit	
	Disturbed	Undisturbed	Disturbed	Undisturbed	Disturbed	Undisturbed
Species Richness	22 ± 2 a	25 ± 3 b	19 ± 3 a	23 ± 4 b	20 ± 2 a	26 ± 1 b
Evenness	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Shannon Diversity	1.7 ± 0.3	1.5 ± 0.3	1.5 ± 0.2	1.4 ± 0.2	1.5 ± 0.2	1.7 ± 0.2
Simpson Diversity	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1

Numbers are mean ± standard deviation.
Means with different letters are significantly different ($p<0.05$).

Table 5-4. Correlations of key soil and plant variables and AMF taxa with NMS ordination axes.

Variable	Axis 1	Axis 2
Organic Matter	0.334	-0.67*
Total Organic Carbon	0.334	-0.67*
Nitrogen	0.356	-0.618*
Phosphorous	0.208	-0.756*
Sand	-0.449	0.344
Silt	0.44	-0.45
pH	-0.315	0.757*
Na	0.284	-0.587*
Non-Native Plant Species		
Bromus inermis	-0.295	0.219
Poa compressa	-0.349	0.547*
Agropyron sibiricum	-0.037	0.416
Native Plant Species		
Festuca idahoensis	0.39	-0.67*
Galium boreale	0.21	-0.5
Heterotheca villosa	-0.227	0.534*
Koeleria macrantha	-0.06	0.48
Oxytropis sericea	-0.222	0.408
Dantonia parryi	0.123	-0.544*
Glomus WOTU1	0.648*	-0.73*
Glomus VTX177	0.05	0.825*
Glomus VTX143	-0.706*	0.183
Glomus VTX165	-0.495	0.551*
Glomus MO.G8 VTX130	-0.379	0.526*
Glomus QU.Glo7 VTX187	0.509*	-0.565*
Glomus VTX140	-0.123	0.573*

Spearman Correlation Coefficient is listed.
For all correlations marked with *, $p<0.001$.

Table 5-5. Correlations of soil and plant variables with AMF taxa.

Variable	AMF Taxa	r_s
Organic Matter	WOTU1, VTX165, VTX187, VTX140	0.64*, -0.44, 0.60*, -0.43
Total Organic Carbon	WOTU1, VTX165, VTX187, VTX140	0.64*, -0.44, 0.60*, -0.43
Nitrogen	WOTU1, VTX165, VTX187	0.66*, -0.46, 0.64*
Phosphorous	WOTU1, VTX177, VTX187, VTX140, Wirsel VTX137, acnaGlo1 VTX137	0.51, -0.64*, 0.48, -0.59*, -0.49, -0.43
Sand	WOTU1, VTX165, VTX130, VTX187	-0.58*, 0.47, 0.53, -0.55*
Silt	WOTU1, VTX165, VTX130, VTX187	0.65*, -0.51, -0.49, 0.64*
pH	WOTU1, VTX177, VTX165, VTX187, VTX140	-0.64*, 0.56*, 0.47, -0.59*, 0.53*
Na	WOTU1, VTX187, VTX140	0.54*, 0.61*, -0.45
Non-Native Species		
Agropyron sibiricum	WOTU1, VTX165, VTX130, VTX137	-0.42, 0.39, 0.48, 0.46
Agropyron cristatum	WOTU20	0.54*
Poa compressa	WOTU1, VTX165, VTX187, WOTU20	-0.63*, 0.63*, 0.62*, 0.51
Bromus inermis	MO.G20 VTX143, WOTU58, WOTU20	0.42, 0.54*, 0.59*
Native Species		
Festuca idahoensis	WOTU1, VTX177, VTX165, VTX187	0.68*, 0.40, 0.49, 0.54*
Heterotheca villosa	WOTU1, VTX165, VTX130, VTX140, Wirsel VTX137, acnaGlo1 VTX137	-0.52, 0.52, 0.67*, 0.41, 0.51, 0.64*
Koeleria macrantha	VTX177, VTX130	0.53, 0.44
Dantonia parryi	WOTU1, VTX177, VTX165, VTX187, VTX140	0.42, -0.41, -0.43, 0.41, -0.49

Spearman Correlation Coefficient is listed.
For all correlations marked with *, $p<0.001$.

Table 5-6. Correlations between AMF diversity and plant community properties.

	Arbuscular Mycorrhizal Fungi			
	Species Richness	Evenness	Shannon Diversity	Simpson Diversity
Host Plant Species				
Species Richness	0.83*****	-0.71	-0.43	-0.37
Evenness	-0.48	0.26	0.14	-0.02
Shannon Diversity	0.83*****	-0.71	-0.43	-0.37
Simpson Diversity	0.66	-0.14	0.09	0.03
Native Cover (%)	0.77	-0.6	-0.37	-0.43
Non-Native Cover	-0.77	0.6	0.37	0.43
Bromus inermis Cover	-0.78	0.13	-0.03	0.03

Spearman Correlation Coefficient is listed.
For all correlations marked with *, $p<0.05$.

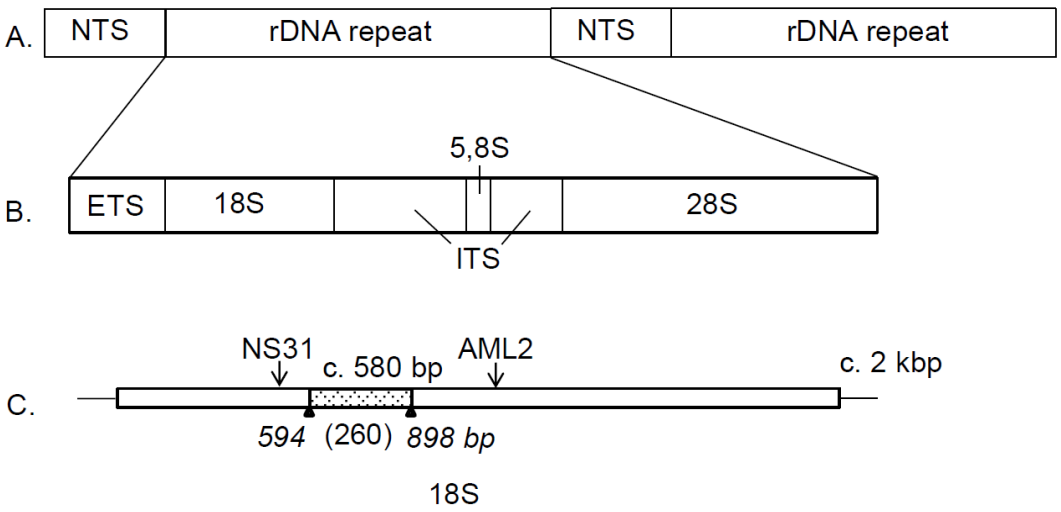

Figure 5-1. (A) Structure of eukaryotic ribosomal DNA, which has numerous copies in eukaryotic genomes occurring in tandem, repetitive clusters with a non-transcribed spacer (NTS) segment occurring between each repeat (Hillis and Dixon 1991). (B) structure of the ribosomal DNA repeat (Redecker 2000) bordered by an external transcribed spacer (ETS) segment, and (C) schematic of the 18S rRNA gene showing primer binding sites, PCR amplicon length (580 bp) and mean 454 read length and start and end position after quality processing and sequence alignment.

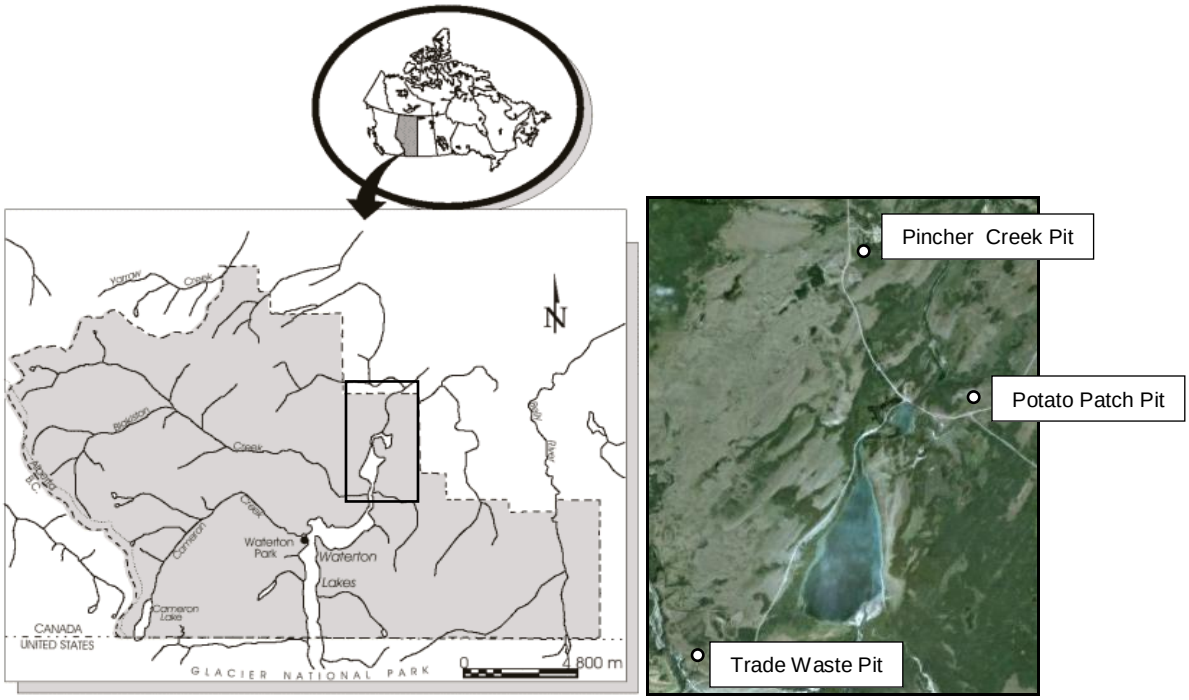

Figure 5-2. Location of Waterton Lakes National Park, Alberta, Canada and research sites (Parks Canada 2009, Google Earth 2012).

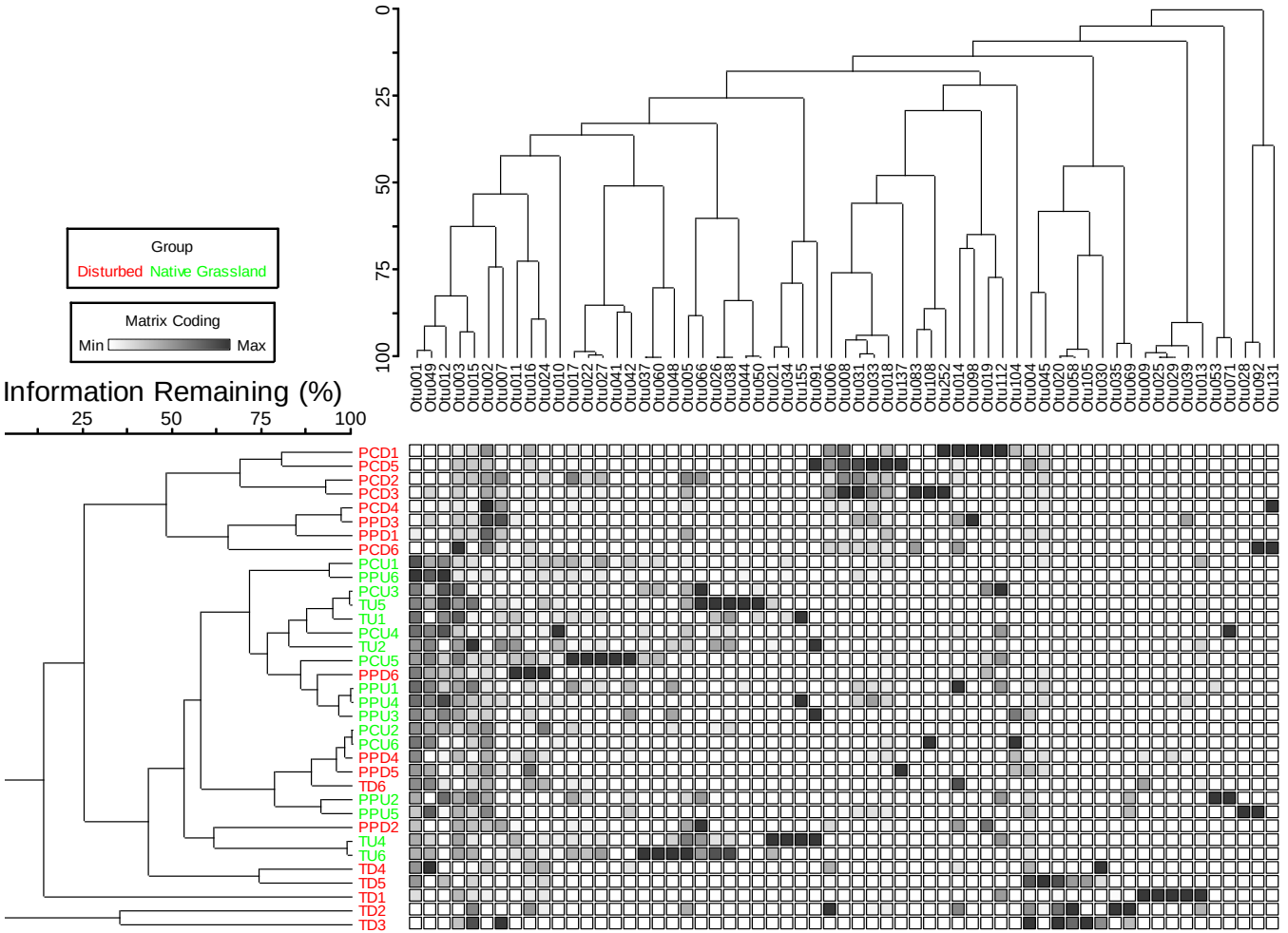

Figure 5-3. Two-way clustering of AMF communities. PC = Pincher Creek Pit, T = Trade Waste Pit, PP = Potato Patch Pit, D = disturbed, U = undisturbed. For OTU taxon name, refer to Table C.1.

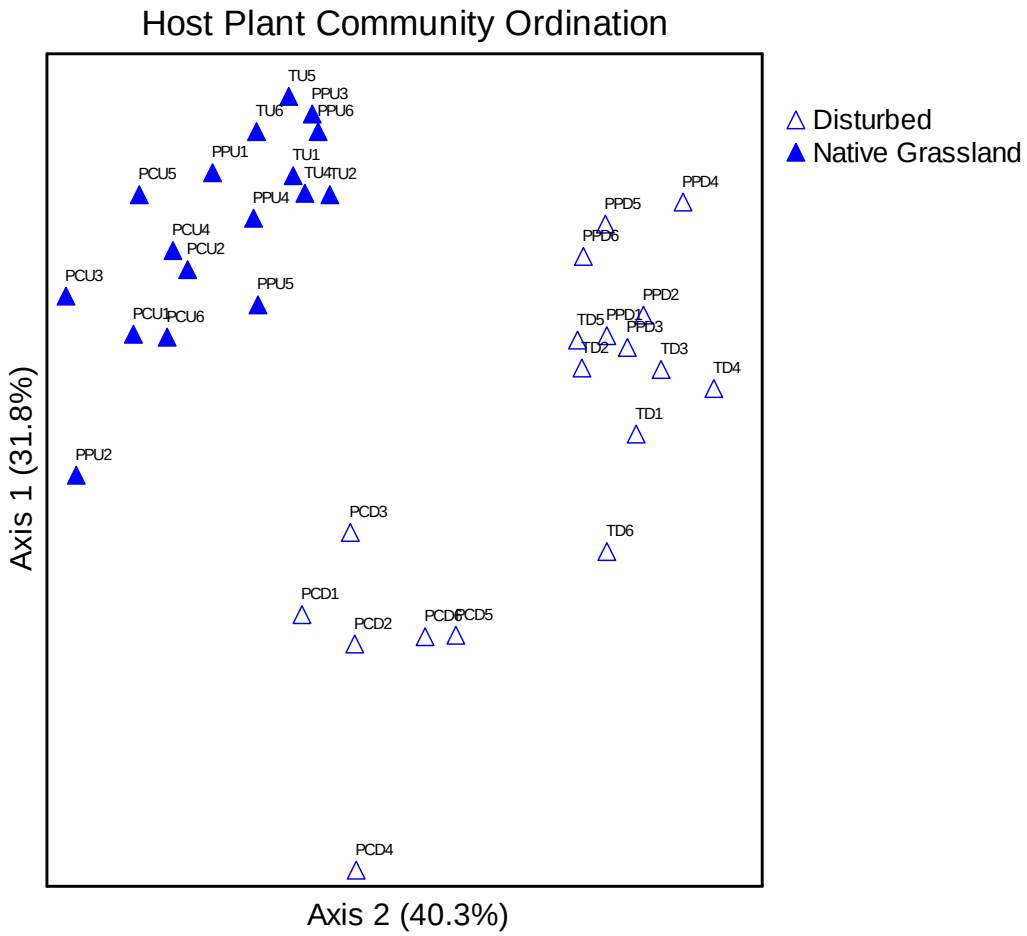

Figure 5-4. NMS ordination of plant communities from disturbed and native foothills fescue grassland and the three sampling sites (PC = Pincher Creek Pit, T = Trade Waste Pit, PP = Potato Patch Pit, D = disturbed, U = undisturbed).

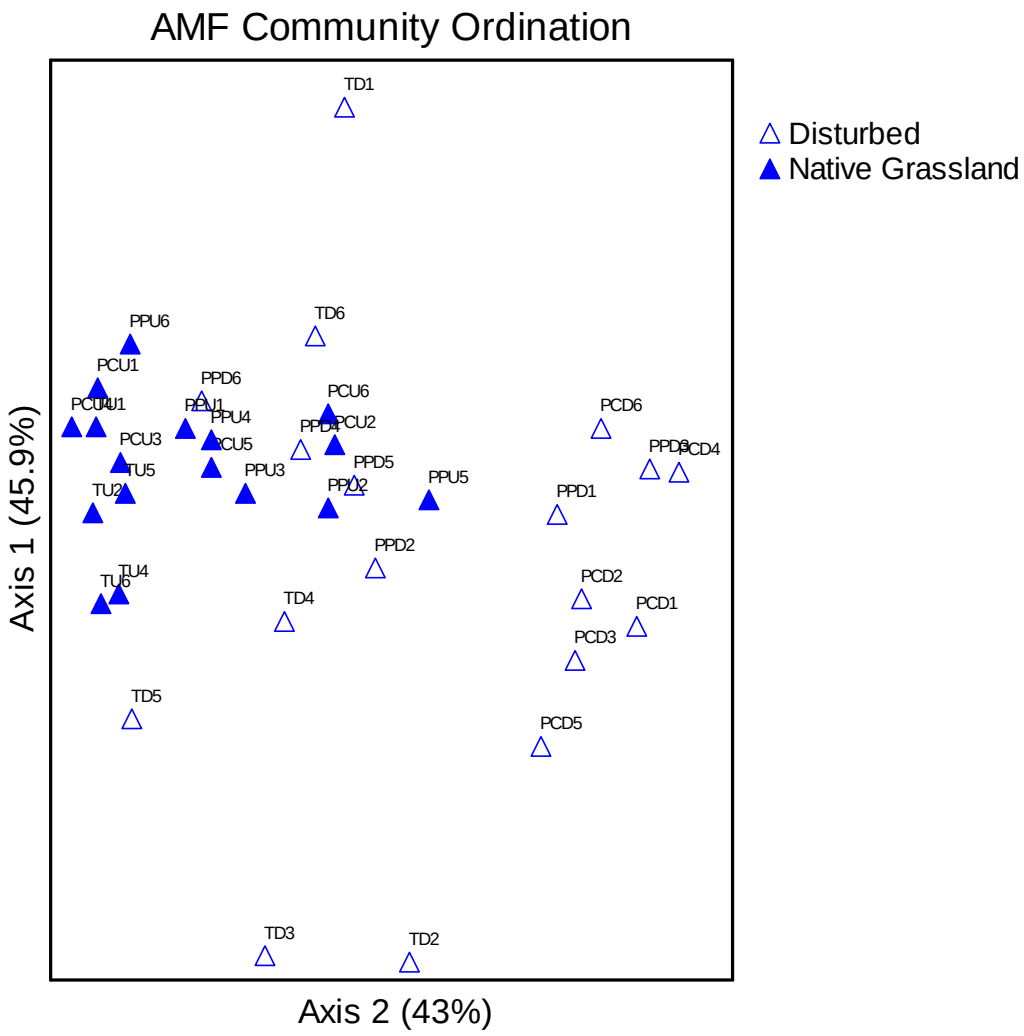

Figure 5-5. NMS ordination of AMF communities from disturbed and native foothills fescue grassland and the three sampling sites (PC = Pincher Creek Pit, T = Trade Waste Pit, PP = Potato Patch Pit, D = disturbed, U = undisturbed).

CHAPTER 6. SYNTHESIS AND FUTURE RESEARCH

1. RESEARCH SUMMARY

Objectives of this study were to investigate non-native plant control methods, revegetation techniques and arbuscular mycorrhizal fungi and how they affect grassland restoration. Reduction in non-native plant cover by steaming, glyphosate (herbicide) application and mowing were compared to a control at three disturbed sites heavily invaded by non-native species. Revegetation success was compared for wild collected and native cultivar seed and broadcast seeding was compared with transplanting. Arbuscular mycorrhizal fungi (AMF) communities at disturbed sites were compared with those in undisturbed grassland using 454 pyrosequencing and molecular identification of partial 18S ribosomal gene sequences.

Glyphosate was most effective in reducing non-native species but most detrimental to native species and steaming and mowing were ineffective. Native cultivar seed had greater establishment than wild collected seed but effectiveness depended on species and plant material type. Transplanting is recommended over broadcast seeding as a follow up to non-native plant control. AMF communities between disturbed and undisturbed sampling locations were similar, suggesting AMF may be resilient to non-native plant invasion, and were very sensitive to changes in soil properties. Knowledge from this research can be used to develop restoration strategies for degraded grasslands.

2. IMPLICATIONS FOR RESTORATION AND MANAGEMENT

Prioritization of management areas and actions is important to conserve limited resources. Disturbed borders of biodiverse fescue grasslands are suggested as priority areas for restoration. Non-native grasses should be incorporated into non-native management plans along with increased revegetation measures and monitoring. Successful restoration will require several years of management with non-native plant control, monitoring and revegetation with native species known to establish on disturbed sites. Herbicide is likely the best control method for non-native plants but long term and repeated use of mowing and steaming may be successful. Use of transplanted native seedlings and suppression of competing

non-native plants is required. Native wild collected seed from populations close to restoration areas, when grown as seedlings and planted, is likely the most ecologically effective seed source for restoration. Reestablishment of native plant populations at disturbed sites with soil conditions similar to the target ecosystem will likely be supported by native AM fungi present but challenged by the ability of non-native plants to form competitively functional mycorrhizae with resident AMF.

3. RESEARCH LIMITATIONS

High environmental variability was observed in plant communities and arbuscular mycorrhizal fungal communities, potentially confounding statistical significance in some cases. Use of more homogeneous field plots or greenhouse study may have improved this issue, but was beyond the scope of this research project.

The time scale for complete ecosystem restoration may require multiple decades (Dobson et al. 1997). This research was conducted over a relatively short period, making it difficult to fully assess all experimental objectives.

Many of the plant species studied are late successional, slow growing and require several years for establishment. The study location was also characterized by a short growing season. These factors limited the ability to fully observe the effects of restoration treatments.

To study effects of disturbance and non-native plant invasion, it was necessary to substitute space for time, so inferences about effects on disturbed ecosystems are limited by comparisons with available target ecosystem sites rather than a comparison with their true, previous undisturbed state.

Knowledge gaps in arbuscular mycorrhizal fungal research limit the certainty of research findings until a universal primer set and accompanying reference database is developed that can be realistically used in robust metagenomic studies and methods used to obtain and analyze data are further refined.

4. FUTURE RESEARCH

This research provided information on short term use of non-native plant control techniques and future research should build upon these data and test the long

term effectiveness of control techniques with greater intensity of applications. Strategies to reduce non-native forbs should be researched as broad spectrum herbicide application was ineffective in controlling this group. More research is needed on steam and mowing to develop methods of using these techniques that are more successful in reducing non-native cover. Further seeding research is needed to determine effective seeding rates and techniques for foothills fescue grassland restoration. The impact of site preparation and non-native plant management on revegetation success and competition from non-native plant species should be researched to develop more effective restoration strategies. Long term monitoring is needed to provide greater knowledge on the effectiveness of non-native plant management and revegetation methods.

Research on seed sources in grassland restoration should continue to evaluate the performance of cultivar seed types compared to local wild seed with additional plant species and expand beyond pilot testing to larger scale restoration experiments. Long term monitoring data are greatly lacking in seed source studies and are strongly needed for more informed management and restoration decisions. The competitive ability of cultivar seed types with non-native plant species should be directly tested. Development of newer and improved cultivars for Canadian restoration projects is needed.

This project provided information on characteristics of AMF communities in disturbed and undisturbed foothills fescue grassland. Further research is needed to clarify the importance of host plant species relationships, functional diversity and the impact of non-native plant invasion on AMF. This observational research work was necessary to build a foundation of baseline knowledge and should be expanded upon with experimental studies to gain more information on the ecology of arbuscular mycorrhizal fungi and their role in ecological restoration. The use of mock community assessments to refine 454 pyrosequencing data analysis in AMF studies for 18S and other eukaryotic genetic markers is also required (Schloss et al. 2011).

5. REFLECTIONS

The grasslands in Waterton Lakes National Park have very high biodiversity and contain a unique assemblage of mountain and prairie species. In an area of only

about 1 ha, almost 20 % of the Park's vascular plant species were found. Non-native plant species add to the Park's species richness (55 species) but have the tendency to exclude other species, form dense monocultures, displace native species and prevent native grassland recovery in disturbed areas. Funding and resources available for restoration and management are limited. Due to the extent of non-native plant invasion and limited resources, prioritization of management areas is strongly needed. Areas of intact native foothills fescue grassland need to be identified and management prioritized to degraded zones surrounding the perimeters of these areas. Undisturbed grasslands need to be protected from disturbance and monitored carefully for non-native plant invaders.

Non-native plants moving in should be removed immediately before they spread out of control and habitat is lost. In addition to non-native forb species being controlled by Park staff, non-native grasses need to be controlled. All control efforts where large patches of vegetation are affected must be accompanied by revegetation and monitoring. Research should examine potential of less aggressive invaders to become invasive. Populations of non-native plants known to be invasive that are growing should be monitored to stop their spread. For example, *Agropyron elongatum* (Host) P. Beauv. (Tall wheat grass) is considered invasive but has not currently spread at Waterton. It may be spreading at Pincher Creek Pit where it was observed in large patches in 2012. *Bromus tectorum* is spreading but if managed rigorously now, could be kept under control. It appears to be currently suppressed by the dominant non-native perennial grasses at disturbed sites.

In cases of disturbed sites with longstanding invasions, the seed bank of native species has likely been depleted and dispersal of native species is limited. Management will require extensive revegetation with control efforts to build competitive native plant communities. The seed bank and population of non-native species in these areas are large and will require long term control for many years before non-native species are eradicated. Impact to native species and biodiversity is low in these areas; therefore, intensive management can be taken. In areas with higher biodiversity and native species (e.g. Pincher Creek Pit) spot applications (e.g. herbicide wicking) or other selective control techniques should be performed to avoid harming the non-target plant community.

Waterton Lakes National Park has unique challenges for ecosystem restoration including extreme winds, a short growing season and window for spring planting, a harsh growing environment with sites that are often difficult to access with personnel and equipment and intense competition from a large number of non-native plant species. Limited seed and plant material and financial and personnel resources also reduce the amount of restoration work that can be conducted. Long term monitoring and data from each year of restoration should be collected and used to build a strong knowledge base to conduct effective restoration.

At Trade Waste Pit, despite an aggressive, dense and large population of non-native grasses and forbs, traces of *Koeleria macrantha* (Ledeb.) Schult. (June grass), *Lupinus sericeus* L. (Silky lupine), *Sisyrinchium montanum* Greene (Blue eyed grass) and other native species can be found. The significance of reasons why these species prevail in small populations is unknown. Native species growing on disturbed sites (Tables A.4, A.5) are a very important resource; they should be conserved and used for revegetation (seed collection) when possible as they are genetically adapted to the disturbed environment and can help form the foundation of the recovering native plant community. For example, *Carex siccata* Dewey (Dryspike sedge) grows abundantly at Trade Waste Pit in wetter areas, forms large (> 15 cm wide) tussocks and competes well with non-native grasses. Native species are needed to help control non-native species (Hobbs and Humphries 1995). Perennial native grasses such as *Elymus trachycaulus* (Link) Gould ex Shinners (Slender wheat grass) can help suppress weedy annuals such as *Bromus tectorum* while restoring the native plant community.

The unique biodiversity and ecology of the Waterton Lakes grassland should be given more attention in the scientific community and deserves more research. For example, *Festuca campestris* roots can reach 120 cm (Budd 1987), yet this project only sampled the top 15 cm of roots to avoid severe disturbance to native plant communities. If 92 different mycorrhizal fungi were found in the top 15 cm of roots, it would be fascinating and valuable to know the composition of the entire fungal community along the entire root length. Phenotypes observed of *Agoseris glauca* (Pursh) Raf. (Pale agoseris), *Cryptantha celosioides* (Eastw.) Payson (Butte candle) and *Cryptantha nubigena* (Greene) Payson (Sierra cryptantha) were dissimilar to descriptions in floras, which may be novel varieties or indicate

updates are needed to species descriptions. The phenotype of *Elymus repens* (L.) Gould (Quackgrass) showed characters of *Agropyron smithii* Rydb. (Western wheat grass), *Agropyron violaceum* (Hornem.) Lange (Broad glumed wheat grass) and *Agropyron intermedium* (Host) Beauv. (Intermediate wheat grass) at Trade Waste Pit in dense *Elymus repens* monocultures. The meaning behind this should be determined and could be hybridization, with implications for conservation genetics of native grasses. *Orobanche fasciculata* Nutt. (Clustered broomrape) was detected once, in late June 2012. This species was exclusively located in herbicide plots with *Artemisia frigida* Willd. (Prairie sagewort), its parasitic host. Perhaps herbicide application, a form of disturbance, stimulated the growth of this species on *Artemisa frigida* plants developing during secondary succession. At Pincher Creek Pit, the dry, rocky soil creates a thin vegetative cover and forbs and grasses often appear growing directly together in tight clusters with shoots overlapping and sprouting from the same locations. The ecology of these observations remains unknown.

6. REFERENCES

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APPENDIX A. CHAPTER 2 SUPPLEMENTARY DATA

Table A.1. Pre-treatment canopy cover of native and non-native vegetation.

Site	Treatment	Native Forb and Shrub	Native Graminoid	Non-Native Graminoid	Non-Native Forb
Pincher Creek Pit	Control	16.0 ± 9.9	2.5 ± 2.9	7.0 ± 4.8	2.2 ± 4.2
	Mow	18.5 ± 9.1	3.8 ± 5.5	8.6 ± 7.1	1.7 ± 4.2
	Herbicide	12.8 ± 9.9	2.7 ± 2.6	7.5 ± 4.5	1.9 ± 2.8
	Steam	18.9 ± 12.4	4.2 ± 4.5	8.4 ± 5.4	1 ± 3.4
Potato Patch Pit	Control	3.9 ± 5.8	1.0 ± 2.4	18.7 ± 15.2	1.0 ± 4.1
	Mow	3.9 ± 7.2	0.6 ± 1.5	13.5 ± 5.2	2.1 ± 3.6
	Herbicide	8.2 ± 18.6	1.3 ± 4.4	16.6 ± 7.6	1.1 ± 2.5
Trade Waste Pit	Control	2.5 ± 5.8	0.5 ± 2.2	27.3 ± 8.8	1.7 ± 5.1
	Mow	0.8 ± 1.3	0.3 ± 1.1	29.4 ± 7.9	1.4 ± 3.7
	Herbicide	1.1 ± 2.4	0.4 ± 1.9	25.9 ± 13.6	2.4 ± 5.7
	Steam	1.1 ± 3.5	0	28.5 ± 11.6	4.5 ± 9.4

Numbers are mean ± standard deviation.
No statistical analyses were performed on these data.

Table A.2. Mean percent cover (measured in July 2012) of non-native forb and shrub species found at research sites. Species with 'x' have mean cover of less than 0.1% and with * are newly reported to the Park.

Species	Fescue Grassland	Pincher Creek Pit	Potato Patch Pit	Trade Waste Pit
Alyssum alyssoides	0.0	0.0	0.0	x
Arenaria serpyllifolia	0.0	x	0.2	0.7
Artemisia absinthium	0.0	0.0	0.1	0.0
Artemisia dracunculus	0.0	0.0	1.6	0.0
Arctium minus	0.0	0.0	0.1	x
Carduus nutans	0.0	0.0	0.0	0.5
Centaurea maculosa	0.0	x	0.5	0.7
Chenopodium alba	0.0	x	0.2	x
Chrysanthemum leucanthemum	0.0	0.0	0.0	3.0
Cirsium arvense	0.0	0.0	1.7	0.4
Descurania sophia	0.0	0.0	0.0	0.2
Dianthus armeria	0.0	0.0	0.0	x
Erysimum chieranthoides	0.0	0.0	x	0.0
Erysimum inconspicuum	0.0	0.0	0.0	x
Hypericum perforatum	0.0	0.0	0.0	0.4
Lappula squarrosa	0.0	0.0	0.0	x
Linaria vulgaris	0.0	0.0	0.0	x
Medicago lupulina	0.0	1.4	4.0	1.4
Medicago sativa	0.0	x	0.0	0.0
Melilotus alba	0.0	0.1	0.1	0.5
Melilotus officinale	0.0	0.0	0.3	1.3
Melilotus sp.	0.0	x	0.1	0.1
Plantago major	0.0	0.0	0.0	0.3
Rumex crispus	0.0	0.0	0.0	0.4
Salsola kali	0.0	0.0	x	x
Sisymbrium altissimum	0.0	0.0	x	x
Sonchus sp.	0.0	0.0	0.0	x
Taraxacum officinale	0.0	x	0.8	x
Thlapsi arvense	0.0	0.0	0.9	0.3
Tragopogon dubius	0.0	x	0.5	0.2
Trifolium pratense	0.0	0.0	0.0	x
*Trifolium pratense x repens**	0.0	0.0	0.0	0.1
Unknown *Fabaceae*	0.0	0.0	x	x
Verbascum thapsus	0.0	0.0	0.0	1.7

Unknown *Fabaceae* did not resemble any native *Fabaceae*.

Table A.3. Mean percent cover (measured in July 2012) of non-native graminoid species found at research sites. Species with 'x' have mean cover of less than 0.1% and with * are newly reported to the Park.

Species	Fescue Grassland	Pincher Creek Pit	Potato Patch Pit	Trade Waste Pit
Agropyron cristatum	0.0	0.0	1.7	2.4
*Agropyron elongatum**	0.0	0.1	0.2	0.0
*Agropyron intermedium**	0.0	x	0.0	0.0
*Agropyron sibiricum**	0.0	1.9	0.0	0.0
*Agropyron trichophorum**	0.0	x	0.0	0.0
*Agrostis gigantea**	0.0	0.0	0.0	0.1
Agrostis stolonifera	0.0	0.0	0.0	0.3
Bromus inermis	0.0	0.5	7.0	6.4
Bromus inermis ssp. *inermis* x ssp. *pumpellianus*	0.0	0.0	0.2	0.1
Bromus tectorum	0.0	0.0	0.1	x
Dactylis glomerata	0.0	x	0.0	x
Elymus repens	0.0	0.0	0.0	12.6
Festuca ovina	0.0	x	x	x
Festuca rubra	0.0	0.3	0.1	1.1
Hordeum jubatum	0.0	0.0	0.0	x
Phleum pratense	0.0	0.0	0.1	3.3
Poa compressa	0.0	0.7	0.5	2.1
Poa pratensis	0.9	0.0	2.4	2.6
*Schedonnardus paniculatus**	0.0	0.0	0.0	x

Table A.4. Mean percent cover (measured in July 2012) of native forb and shrub species found at research sites. Species with 'x' have mean cover of less than 0.1% and with * are newly reported to the Park.

Species	Fescue Grassland	Pincher Creek Pit	Potato Patch Pit	Trade Waste Pit
Achillea millefolium	1.4	0.7	0.9	2.4
Agoseris glauca	0.7	0.4	0.2	0.0
Allium cernum	x	x	0.0	0.0
Amelanchier alnifolia	0.0	0.0	0.1	0.1
Androsace septentrionalis	x	0.5	x	x
Anemone multifida	0.1	0.0	0.0	0.0
Anemone patens	0.3	0.0	0.0	0.0
Antennaria parvifolia	0.2	x	0.0	0.0
Antennaria pucherrima	0.3	0.0	0.0	0.0
Antennaria umbrinella	0.6	0.0	0.0	0.0
Arabis divaricarpa	0.0	x	0.0	0.0
Arabis glabra	0.0	0.0	0.1	0.0
Arabis holboellii	0.0	0.0	0.1	0.0
Arenaria capillaria var. americana	1.2	0.2	0.1	0.0
Artemisia campestris	0.0	0.4	0.3	0.0
Artemisia frigida	0.1	1.7	1.9	0.0
Artemisia ludoviciana	0.0	0.1	0.3	0.0
Artemisia michauxiana	0.0	0.0	0.1	0.1
Aster falcatus	0.0	x	0.1	0.0
Aster laevis	0.6	x	0.1	0.0
Bupleurum americanum	0.0	x	0.0	0.0
Campanula rotundifolia	0.3	0.1	0.4	0.0
Castilleja flava	0.0	x	0.0	0.0
Comandra umbellata	0.8	0.2	0.0	0.0
Cryptantha celosioides	0.0	x	0.0	0.0
Cryptantha nubigena	0.0	x	0.0	0.0
Dalea purpurea	0.0	x	0.0	0.0
Draba cana	0.0	x	0.0	0.0
Epilobium angustifolium	0.0	0.0	x	x
*Epilobium glandulosum**	0.0	0.0	0.0	x
Equisetum arvense	0.0	0.0	0.5	0.0
Erigeron caespitosus	1.7	0.8	x	0.0
Erigeron compositus	0.0	0.4	0.0	0.0
Erigeron sp.	0.0	x	x	x

Table A.4, con'd. Mean percent cover (measured in July 2012) of native forb and shrub species found at research sites. Species with 'x' have mean cover of less than 0.1%.

Species	Fescue Grassland	Pincher Creek Pit	Potato Patch Pit	Trade Waste Pit
Eriogonum flavum	0.0	0.3	0.0	0.0
Fragaria virginiana	0.0	0.0	x	x
Gaillardia aristata	0.5	0.3	0.6	0.2
Galium boreale	3.0	x	0.8	x
Gentiana amarella	0.1	0.0	0.0	0.0
Geranium viscosissimum	0.0	0.0	0.1	0.4
Hedysarum alpinum	1.3	0.0	0.0	0.0
Hedysarum boreale	0.0	x	0.0	0.0
Helianthus nuttalii	0.3	0.0	0.0	x
Heterotheca villosa	x	3.1	1.3	0.0
Juniperus horizontalis	1.0	0.7	0.0	0.0
Juniperus virginiana	0.0	x	0.0	0.0
Lepidium densiflorum	x	0.2	1.4	0.3
*Lepidium ramosissimum**	0.0	0.7	2.6	0.5
Liatris punctata	0.0	0.6	0.0	0.0
Linum lewisii	0.0	x	0.0	0.0
Lithospermum ruderale	0.4	x	0.1	0.0
Lupinus sericeus	3.7	1.3	1.0	x
Maianthemum racemosum	0.0	0.0	x	0.0
Monarda fistulosa	1.0	0.2	1.0	0.1
Oenothera biennis	0.0	x	0.0	0.8
Orobanche fasiculata	0.0	x	x	0.0
Orthocarpus luteus	0.0	x	0.0	0.0
Oxytropis deflexa	0.0	0.9	0.0	0.0
Oxytropis sericea	0.1	0.6	0.1	0.0
Oxytropis splendens	0.0	x	0.0	0.0
Penstemon confertus	0.0	0.0	0.1	0.0
Penstemon nitidus	0.0	0.2	x	0.0
Potentilla arguta	0.0	x	0.0	0.0
Potentilla fruticosa	0.8	0.0	0.0	0.0
Potentilla gracilis	0.0	0.0	0.1	0.0
Potentilla hippiana	0.0	x	0.0	0.0
Potentilla pensylvanica	0.2	0.0	0.0	0.0
Rosa arkansana	1.8	0.0	0.0	0.0
Rosa woodsii	0.2	0.0	0.5	x

Table A.4, con'd. Mean percent cover (measured in July 2012) of native forb and shrub species found at research sites. Species with 'x' have mean cover of less than 0.1%.

Species	Fescue Grassland	Pincher Creek Pit	Potato Patch Pit	Trade Waste Pit
Rubus idaeus	0.0	0.0	0.0	x
Sedum lanceolatum	0.0	0.1	0.0	0.0
Selaginella densa	10.2	x	0.1	0.0
Senecio canus	0.0	x	x	0.0
Silene menziesii	0.0	0.0	x	0.0
Sisyrinchium montanum	0.4	x	x	x
Smilacina stellata	0.0	0.0	x	0.0
Solidago canadensis	0.0	0.0	1.8	0.0
Solidago missouriensis	0.2	0.7	0.3	0.0
Symphoricarpos albus	0.0	x	0.5	0.0
Verbena bracteata	0.0	0.0	x	1.2
Vicia americana	0.0	0.0	0.3	x
Viola sp.	x	0.0	0.0	0.0

Table A.5. Mean percent cover (measured in July 2012) of native graminoid species found at research sites. Species with 'x' have mean cover of less than 0.1% and with * are newly reported to the Park.

Species	Fescue Grassland	Pincher Creek Pit	Potato Patch Pit	Trade Waste Pit
Agropyron albicans var. *griffithsii**	0.2	0.0	0.1	0.0
Agropyron dasystachum	0.5	0.2	0.3	0.0
Agropyron dasystachum var. *albicans**	0.0	x	0.0	0.0
Agropyron smithii	0.0	0.2	x	0.1
Agropyron trachycaulum	0.0	x	0.1	0.0
Agropyron trachycaulum var. *trachycaulum*	0.0	0.0	0.1	0.1
Agropyron trachycaulum var. *unilaterale*	x	x	0.0	x
Agropyron violaceum	0.0	0.0	0.0	0.1
Bromus carinatus	x	0.0	0.0	0.0
Calamagrostis montanensis	0.0	0.0	0.0	x
Carex filifolia	0.0	0.0	x	
Carex siccata	0.0	0.0	0.0	x
Carex stenophylla ssp. *eleocharis**	0.0	1.9	x	0.1
Festuca campestris	6.2	x	0.2	x
Danthonia parryi	0.0	10.7	0.0	x
Festuca idahoensis	9.7	x	0.3	0.1
Festuca saximontana	0.0	0.0	x	0.0
Juncus balticus	0.0	0.0	0.0	x
Koeleria macrantha	0.0	0.8	1.9	0.1
Poa alpina	0.0	0.0	0.1	0.0
Stipa columbiana	0.4	x	x	x
Stipa comata	0.3	x	0.0	0.0
Stipa curtiseta	0.0	0.1	0.1	0.0
Stipa richardsonii	1.5	0.4	0.5	0.0

Table A.6. Effectiveness of herbicide, steam and mowing compared to control (2010-July 2011).

Location	Parameter	Comparison	p value
2010			
All sites	Canopy cover	Glyphosate, steam	0.0001537
Trade Waste Pit	Canopy cover	Mow, steam, control	0.7749
July 2011			
All sites	Native forb and shrub	All	0.1072
	Native graminoid		0.2664
	Non-native forb		0.2361
	Non-native graminoid		0.0718
Pincher Creek Pit	Native forb and shrub		0.0943
	Native graminoid		0.0827
	Non-native forb		0.4285
	Non-native graminoid		0.0594
Potato Patch Pit	Native forb and shrub		0.0379
		Control, mow	0.0887
		Control, glyphosate	0.0392
		Glyphosate, mow	0.9142
	Native graminoid		0.2246
	Non-native forb		0.0349
		Control, mow	0.1265
		Control, glyphosate	0.2535
		Glyphosate, mow	0.0070
	Non-native graminoid		0.0340
		Control, mow	0.1191
		Control, glyphosate	0.0139
		Glyphosate, mow	1.0000
Trade Waste Pit	Native forb and shrub	All	0.3625
	Non-native forb		0.2411
	Non-native graminoid		0.0439
		Control, mow	0.3313
		Control, glyphosate	0.0383
		Glyphosate, mow	0.0383
All sites	Native forb and shrub physiological stage	Control, mow	0.0009
	Native graminoid physiological stage		0.0003
	Non-native forb physiological stage		0.2776
	Non-native graminoid physiological stage		0.3718
	Mean height		<0.0001

Table A.7. Overall performance for July 2011 vegetation cover one month after the second treatment application.

Treatment	Native Forbs and Shrubs	Native Graminoids	Non Native Forbs	Non Native Graminoids
Control	4.4 ± 3.7	0.2 ± 0.3	0.7 ± 0.6	3.6 ± 5.3
Mow	3.3 ± 4.4	1.0 ± 1.4	0.7 ± 0.0	0.7 ± 1.1
Glyphosate	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
Steam	4.2 ± 5.6	0.3 ± 0.4	0.7 ± 0.8	2.8 ± 3.4

Numbers are mean and standard deviation.

Table A.8. Effectiveness of treatments compared to control (August 2011).

Location	Parameter	Comparison	p value
All sites	Native forb and shrub	All	0.5289
	Native graminoid		0.1116
	Non-native forb		0.0606
	Non-native graminoid		0.0129
		Control, glyphosate	0.0046
		Control, mow	0.4822
		Control, steam	0.8248
		Glyphosate, mow	0.0093
		Glyphosate, steam	0.0066
		Mow, steam	0.4128
Pincher Creek Pit	Native forb and shrub	All	0.0434
		Control, glyphosate	0.0244
		Control, mow	0.2784
		Control, steam	0.5526
		Glyphosate, mow	0.4088
		Glyphosate, steam	0.1947
		Mow, steam	0.9407
	Native graminoid	All	0.0752
	Non-native forb		0.9324
	Non-native graminoid		0.0329
		Control, glyphosate	0.0149
		Control, mow	0.3888
		Control, steam	0.9237
		Glyphosate, mow	0.1721
		Glyphosate, steam	0.0056
		Mow, steam	0.1868
Potato Patch Pit	Native forb and shrub	All	0.9565
	Native graminoid		0.1199
	Non-native forb		0.1479
	Non-native graminoid		0.0665
Trade Waste Pit	Native forb and shrub		0.0823
	Native graminoid		0.0567
	Non-native forb		0.0476
		Control, glyphosate	0.0344
		Control, mow	1.0000
		Control, steam	0.9024
		Glyphosate, mow	0.0405
		Glyphosate, steam	0.2856
		Mow, steam	0.9433
	Non-native graminoid		0.0534

Table A.9. Mowing effects and effect of treatments on non-native species August 2011.

Location	Parameter	Comparison	p value
All sites	Native forb and shrub physiological stage	Control, mow	0.0693
	Native graminoid physiological stage		0.0018
	Non-native forb physiological stage		0.3344
	Non-native graminoid physiological stage		0.0009
	Mean height		0.0411
	Bromus inermis canopy cover	All	0.0005
		Control, mow	0.0474
		Control, glyphosate	0.0040
		Control, steam	0.2114
		Steam, glyphosate	0.0063
		Steam, mow	0.4296
		Mow, glyphosate	0.0041
	Agropyron cristatum canopy cover	All	0.0073
		Control, mow	0.2125
		Control, glyphosate	0.0005
		Control, steam	0.1423
		Steam, glyphosate	0.0410
		Steam, mow	0.2944
		Mow, glyphosate	0.0018
Pincher Creek Pit	*Agropyron sibiricum* canopy cover	All	0.2240
All sites	*Centaurea maculosa* canopy cover	All	0.6958
	Poa pratensis canopy cover	All	0.0409
		Control, mow	0.0529
		Control, glyphosate	0.0080
		Control, steam	0.4286
		Steam, glyphosate	0.0103
		Steam, mow	0.1757
		Mow, glyphosate	0.0588
Trade Waste Pit	*Elymus repens* canopy cover	All	0.0762
	Chrysanthemum leucanthemum canopy cover		0.6595

Table A.10. Site specific results for July 2011 vegetation cover one month after the second treatment application.

Site	Treatment	Native Forbs and Shrubs	Native Graminoids	Non Native Forbs	Non Native Graminoids
Pincher Creek Pit	Control	6.7 ± 3.5	0.5 ± 0.0	0.3 ± 0.2	0.2 ± 0.1
	Mow	8.4 ± 1.2	0.4 ± 0.2	0.7 ± 0.9	0.0 ± 0.0
	Glyphosate	0.3 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0
	Steam	8.1 ± 6.7	0.6 ± 0.4	0.1 ± 0.1	0.4 ± 0.5
Potato Patch Pit	Control	6.4 ± 4.1 a	0.0 ± 0.1	0.3 ± 0.1 ab	1.0 ± 1.3 a
	Mow	1.3 ± 0.9 ab	0.1 ± 0.1	0.7 ± 0.3 a	0.1 ± 0.1 ab
	Glyphosate	0.0 ± 0.1 b	0.0 ± 0.0	0.0 ± 0.0 b	0.0 ± 0.0 b
Trade Waste Pit	Control	0.1 ± 0.1	0.0 ± 0.0	1.4 ± 1.2	9.7 ± 12.5 a
	Mow	0.3 ± 0.4	2.7 ± 4.6	0.7 ± 0.2	2.0 ± 1.4 a
	Glyphosate	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.1 b
	Steam	0.2 ± 0.2	0.0 ± 0.0	1.2 ± 1.6	5.2 ± 1.4 a

Numbers are mean ± standard deviation.
Means with different letters are significantly different ($p<0.01$).

Table A.11. Vegetation cover two months after the second treatment application (August 2011).

Site	Treatment	Native Forbs and Shrubs	Native Graminoids	Non Native Forbs	Non Native Graminoids
Pincher Creek Pit	Control	18.0 ± 6.0 a	2.4 ± 1.5	0.7 ± 0.3	3.6 ± 1.0 a
	Mow	11.1 ± 1.0 ab	3.4 ± 1.1	0.8 ± 0.3	2.3 ± 0.6 ab
	Glyphosate	5.4 ± 2.4 b	0.5 ± 0.2	0.8 ± 0.4	0.5 ± 0.4 b
	Steam	13.2 ± 5.7 ab	3.1 ± 1.0	0.7 ± 0.3	4.0 ± 1.4 a
Potato Patch Pit	Control	4.7 ± 4.2	0.7 ± 1.0	0.6 ± 0.0	12.0 ± 8.6
	Mow	8.3 ± 10.0	0.2 ± 0.1	0.7 ± 0.3	10.1 ± 4.0
	Glyphosate	3.8 ± 3.7	0.0 ± 0.0	3.6 ± 3.4	0.3 ± 0.3
Trade Waste Pit	Control	0.2 ± 0.3	3.9 ± 3.3	0.8 ± 0.7 a	20.1 ± 9.7
	Mow	0.3 ± 0.3	8.5 ± 6.5	1.0 ± 0.5 ab	14.0 ± 8.0
	Glyphosate	1.3 ± 0.0	0.0 ± 0.0	5.2 ± 3.6 b	2.1 ± 0.8
	Steam	1.6 ± 1.2	2.0 ± 2.0	2.3 ± 0.8 a	22.5 ± 6.4

Numbers are mean ± standard deviation.
Means with different letters are significantly different ($p<0.01$).

Table A.12. Effectiveness of treatments compared to control (July 2012).

Location	Parameter	Comparison	p value
All sites	Native forb and shrub	All	0.6124
	Native graminoid		0.3499
	Non-native graminoid		0.2121
	Non-native forb		<0.0001
		Control, glyphosate	<0.0001
		Control, mow	0.9939
		Control, steam	0.8477
		Glyphosate, mow	<0.0001
		Glyphosate, steam	0.0001
		Mow, steam	0.8423
Pincher Creek Pit	Native forb and shrub	All	0.0164
		Control, glyphosate	0.0051
		Control, mow	0.3616
		Control, steam	0.9205
		Glyphosate, mow	0.0215
		Glyphosate, steam	0.0059
		Mow, steam	0.4125
	Native graminoid	All	0.0969
	Non-native graminoid		0.0500
	Non-native forb		<0.0001
		Control, glyphosate	<0.0001
		Control, mow	0.2129
		Control, steam	0.6951
		Glyphosate, mow	<0.0001
		Glyphosate, steam	<0.0001
		Mow, steam	0.1165
Potato Patch Pit	Native forb and shrub	All	0.2417
	Native graminoid		0.3800
	Non-native forb		<0.0001
		Control, glyphosate	0.0043
		Control, mow	0.8880
		Glyphosate, mow	0.0050
	Non-native graminoid		0.2161
Trade Waste Pit	Native forb and shrub		0.1076
	Native graminoid		0.4086
	Non-native forb		0.0014
		Control, glyphosate	0.0007
		Control, mow	0.6588
		Control, steam	0.6028
		Glyphosate, mow	0.0004
		Glyphosate, steam	0.0014
		Mow, steam	0.3465

Table A.13. Effect of treatments on specific non-native species (July 2012).

Location	Parameter	Comparison	p value
All sites	*Bromus inermis* canopy cover	All	0.2177
	Agropyron cristatum canopy cover		0.4499
	Agropyron sibiricum canopy cover		0.4671
	Poa pratensis canopy cover		0.2652
	Elymus repens canopy cover		0.8682
	Mean height		0.4069
	Non-native graminoid	All	0.0405
		Control, glyphosate	0.0166
		Control, mow	0.8852
		Control, steam	0.9760
		Glyphosate, mow	0.0208
		Glyphosate, steam	0.0158
		Mow, steam	0.8616

Table A.14. Site specific results for July 2012 vegetation cover one year after the second treatment application.

Site	Treatment	Native Forbs and Shrubs	Native Graminoids	Non Native Forbs	Non Native Graminoids
Pincher Creek Pit	Control	17.9 ± 3.2 a	2.5 ± 0.4	0.6 ± 0.1 a	3.1 ± 1.9
	Mow	15.5 ± 2.6 ab	5.0 ± 3.5	1.6 ± 0.1 a	2.1 ± 0.6
	Glyphosate	8.5 ± 3.5 b	0.7 ± 0.3	8.0 ± 1.9 b	1.3 ± 0.5
	Steam	17.7 ± 2.7 a	4.4 ± 1.7	0.3 ± 0.1 a	7.7 ± 10.2
Potato Patch Pit	Control	18.2 ± 7.9	0.8 ± 0.3	4.4 ± 2.2 a	20.8 ± 15.4
	Mow	14.3 ± 5.6	2.8 ± 2.7	5.3 ± 1.1 a	9.9 ± 4.8
	Glyphosate	9.4 ± 1.7	1.8 ± 0.7	34.6 ± 14.2 b	5.6 ± 4.3
Trade Waste Pit	Control	2.8 ± 2.5	0.6 ± 0.2	6.8 ± 3.6 a	36.7 ± 13.4
	Mow	4.3 ± 4.3	0.8 ± 0.7	4.7 ± 1.5 a	35.6 ± 8.8
	Glyphosate	8.7 ± 0.4	2.0 ± 2.3	31.6 ± 10.6 b	15.0 ± 5.6
	Steam	5.4 ± 1.3	0.4 ± 0.3	9.3 ± 1.2 a	36.9 ± 4.3

Numbers are mean ± standard deviation.
Means with different letters are significantly different ($p<0.01$).

Table A.15. Spearman Correlation Coefficients (r) for explanatory variables and NMS axes formed from ordination of plant communities in non-native plant management treatments and sites.

Variable	r Axis 1	p	r Axis 2	p
C:N Ratio	-0.26657	0.1160	-0.54730	0.0006
Sand	0.31582	0.0606	0.55955	0.0004
Silt	-0.27300	0.1072	-0.54704	0.0006
Koeleria macrantha	0.67668	<0.0001	0.41243	0.0124
Chrysanthemum leucanthemum	-0.50280	0.0018	-0.58902	0.0002
Heterotheca villosa	0.60422	<0.0001	0.59267	0.0001
Agropyron cristatum	-0.35153	0.0355	-0.69517	<0.0001
Agropyron repens	-0.56478	0.0003	-0.73826	<0.0001
Bromus inermis	-0.29820	0.0773	-0.68917	<0.0001
Poa pratensis	-0.16283	0.3427	-0.88872	<0.0001
Phleum pratense	-0.52375	0.0010	-0.80316	<0.0001
Poa compressa	-0.24282	0.1536	-0.47778	0.0032
Agropyron sibiricum	0.40407	0.0145	0.77653	<0.0001
Danthonia parryi	0.30176	0.0737	-0.07362	0.6696
Festuca idahoensis	0.20985	0.2193	-0.05105	0.7675
Selaginella densa	0.54028	0.0007	0.299902	0.0765
Festuca campestris	0.13162	0.4442	0.03528	0.8381
Artemisia frigida	0.19373	0.2576	0.74311	<0.0001
Lepidium ramosissimum	-0.57265	0.0003	0.20298	0.2351
Lepidium densiflorum	-0.48495	0.0027	0.37825	0.0229
Medicago lupulina	-0.57996	0.0002	0.02968	0.8636

Variables with higher r and lower p values are more strongly correlated with the ordination axes and influential in forming the patterns observed in the ordination.

Table A.16. Multivariate analysis comparing plant communities in treatments and undisturbed grassland (July 2012).

Location	Test	Comparison	p value
All sites	Randomization test for NMS Axis 1 and 2	All	0.004
	MRPP randomization test for significantly different groups		0.00047569
		Control, glyphosate	0.00047569
		Control, undisturbed	0.00172538
		Mow, glyphosate	0.00074488
		Mow, undisturbed	0.00081489
		Steam, undisturbed	0.00640862
		Glyphosate, steam	0.00662828
	Randomization test for Indicator Species Analysis	All	0.0002
		Koeleria macrantha	0.0002
		Heterotheca villosa	0.0002
		Solidago missouriensis	0.004
		Artemisia frigida	0.081
		Agropyron sibiricum	0.0002
		Thlapsi arvense	0.0136
		Chenopodium alba	0.0038
		Medicago lupulina	0.006
		Lepidium ramosissimum	0.0002
		L. densiflorum	0.0002
		Artemisia dracunculus	0.0012
		Symphoricarpos albus	0.0686
		Equisetum arvense	0.0306
		Bromus inermis	0.036
		Poa pratensis	0.0956
		Chrysanthemum leucanthemum	0.009
		Achillea millefolium	0.0836
		Phleum pratense	0.0002
		Poa compressa	0.0002
		Agropyron cristatum	0.0082
		Agropyron repens	0.0002
		Danthonia parryi	0.0002
		Carex stenophylla	0.0002
		Festuca idahoensis	0.0002
		Festuca campestris	0.0002
		Stipa richardsonii	0.0038
		Rosa arkansana	0.0002
		Comandra umbellata	0.002
		Erigeron caespitosus	0.0032
		Arenaria capillaris	0.0002
		Selaginella densa	0.0096
		Anemone patens	0.0046
		Lupinus sericeus	0.0086
		Galium boreale	0.0028
		Antennaria spp.	0.007

NMS = Non-Metric Multidimensional Scaling, MRPP = Multiple Response Permutation Procedure.

Table A.17. Significant relationships among key explanatory variables identified in NMS ordination. The rank abundances between each of the two variables compared were significantly correlated, indicating increasing (positive correlation) or decreasing (negative correlation) abundance of one variable is related to increases in the other variable. Spearman Correlation Coefficients (r) for explanatory variables and p values for the correlation are given above and below, respectively.

	Sand	Silt	Clay	*Agropyron repens*
Heterotheca villosa	0.5538 0.0005	-0.5709 0.0003	-0.6375 <0.0001
Bromus inermis	0.6847 <0.0001	0.8193 <0.0001
Chrysanthemum leucanthemum	
Agropyron cristatum	
Agropyron sibiricum	0.7173 <0.0001	-0.7047 <0.0001	-0.6601 <0.0001
Poa pratensis	0.5482 0.0005	0.8759 <0.0001
Phleum pratense	0.5501 0.0005	0.6722 <0.0001
Koeleria macrantha	
Agropyron cristatum	
Poa compressa	

Table A.18. Indicator Species Analysis results, species listed are characteristic of groups identified by NMS ordination.

Group	Species	IV
Pincher Creek Pit disturbed	*Koeleria macrantha*	69.4
	Heterotheca villosa	67.2
	Solidago missouriensis	56
	Artemisia frigida	38.6
	Agropyron sibiricum	88.7
Herbicide	*Thlapsi arvense*	55.6
	Chenopodium alba	66.7
	Medicago lupulina	65.4
	Lepidium ramosissimum	92.9
	Lepidium densiflorum	84
Potato Patch Pit disturbed	*Artemisia dracunculus*	76
	Symphoricarpos albus	29.7
	Equisetum arvense	33.3
Trade Waste Pit disturbed	*Bromus inermis*	47.1
	Poa pratensis	44.5
	Chrysanthemum leucanthemum	56.2
	Achillea millefolium	41
	Phleum pratense	91.4
	Poa compressa	60
	Agropyron cristatum	55.2
	Agropyron repens	83.1
Foothills fescue	*Danthonia parryi*	99.7
	Carex stenophylla	94.2
	Festuca idahoensis	95.5
	Festuca campestris	95.4
	Stipa richardsonii	55.8
	Rosa arkansana	100
	Comandra umbellata	78.1
	Erigeron caespitosus	59.9
	Arenaria capillaris	76.5
	Selaginella densa	65.5
	Anemone patens	66.7
	Lupinus sericeus	54.1
	Galium boreale	73.1
	Antennaria sp.	66.7

IV = Indicator Value (%), degree at which species characterizes a group.
Disturbed includes mow, control and steam treatments, foothills fescue is undisturbed native grassland target ecosystem.

APPENDIX B. CHAPTERS 3 AND 4 SUPPLEMENTARY DATA

Table B.1. Results for two-way ANOVAs performed to assess the effects of revegetation on native cover.

Effect	Num DF	Den DF	F	p
ANOVA without steam				
Treatment	2	4	1.23	0.3827
Year	2	12	2.85	0.0974
Treatment*Year	4	12	0.63	0.6486
Differences of least squares means				
Control, herbicide				0.2713
Control, mow				0.8835
Herbicide, mow				0.2256
2010, 2011				0.6289
2010, 2012				0.1016
2011, 2012				0.0425
ANOVA without Potato Patch Pit				
Treatment	3	3	0.72	0.6029
Year	2	8	0.86	0.4594
Treatment*Year	6	8	0.32	0.9080
Differences of least squares means				
Control, herbicide				0.3455
Control, mow				0.9151
Control, steam				0.9119
Herbicide, mow				0.3055
Herbicide, steam				0.3041
Mow, steam				0.9967
2010, 2011				0.5068
2010, 2012				0.5558
2011, 2012				0.2267

Table B.2. Results for log-linear analysis of effect of treatment and material on transplant survival.

Site, Time	Source	DF	Chi-Square	p
Pincher Creek Pit, June	Intercept	1	104.76	<0.0001
	Treatment	3	9.27	0.0259
	Material	2	477.07	<0.0001
Pincher Creek Pit, July	Intercept	1	76.24	<0.0001
	Material	2	489.33	<0.0001
Potato Patch Pit, June	Intercept	1	96.28	<0.0001
	Material	2	318.52	<0.0001
Potato Patch Pit, July	Intercept	1	94.19	<0.0001
	Treatment	2	6.5	0.0389
	Material	2	315.59	<0.0001
Trade Waste Pit, June	Intercept	1	80.38	<0.0001
	Material	2	53.52	<0.0001
Trade Waste Pit, July	Intercept	1	6.16	0.0131
	Material	2	31.13	<0.0001
	Treatment	3	7.02	0.0714
	Material*Treatment	6	14.87	0.0213

Table B.3. Contrasts performed for significant independent variables from log-linear analysis on transplant survival.

Site, Time	Contrast	Chi-Square	p
Pincher Creek Pit, June	Control, herbicide	7.26	0.0071
	Control, mow	3.39	0.0655
	Control, steam	6.17	0.0130
	Herbicide, mow	0.75	0.3874
	Herbicide, steam	0.04	0.8505
	Mow, steam	0.45	0.5040
	Cone, root trainer	2.61	0.1063
	Cone, tray	467.46	<0.0001
	Root trainer, tray	98.65	<0.0001
Pincher Creek Pit, July	Cone, root trainer	8.45	0.0037
	Cone, tray	484.65	<0.0001
	Root trainer, tray	103.81	<0.0001
Potato Patch Pit, June	Cone, root trainer	0.01	0.9317
	Cone, tray	307.04	<0.0001
	Root trainer, tray	82.74	<0.0001
Potato Patch Pit, July	Cone, root trainer	0.05	0.8275
	Cone, tray	305.42	<0.0001
	Root trainer, tray	84.13	<0.0001
	Control, herbicide	0.35	0.5546
	Control, mow	3.59	0.0582
	Herbicide, mow	6.06	0.0138
Trade Waste Pit, June	Cone, root trainer	1.86	0.1721
	Cone, tray	48.65	<0.0001
	Root trainer, tray	33.11	<0.0001
Trade Waste Pit, July	Cone, root trainer	4.04	0.0444
Control	Cone, tray	2.63	0.1046
	Root trainer, tray	7.45	0.0063
Herbicide	Cone, root trainer	3.42	0.0644
	Cone, tray	17.67	<0.0001
	Root trainer, tray	2.55	0.1102
Mow	Cone, root trainer	0.26	0.6115
	Cone, tray	7.48	0.0062
	Root trainer, tray	5.86	0.0155
Steam	Cone, root trainer	6.20	0.0127
	Cone, tray	2.58	0.1081
	Root trainer, tray	9.61	0.0019

Table B.4. Logit function for each site and time period predicting transplant survival based on material type and treatment.

Site, Time	Parameter		Estimate	Standard Error	Chi-Square	P
Pincher Creek Pit, June	Intercept		1.0355	0.1012	104.76	<0.0001
	Treatment	Control	-0.3512	0.1202	8.54	0.0035
		Herbicide	0.1893	0.1264	2.24	0.1343
		Mow	0.0119	0.1241	0.01	0.9234
	Material	Cone	1.2546	0.1154	118.27	<0.0001
		Root trainer	0.8037	0.1821	19.48	<0.0001
Pincher Creek Pit, July	Intercept		0.8094	0.0927	76.24	<0.0001
	Material	Cone	1.3725	0.1069	164.88	<0.0001
		Root trainer	0.6503	0.1626	15.98	<0.0001
Potato Patch Pit, June	Intercept		1.0419	0.1062	96.28	<0.0001
	Material	Cone	0.9394	0.1194	61.87	<0.0001
		Root trainer	0.9135	0.1914	22.77	<0.0001
Potato Patch Pit, July	Intercept		1.0136	0.1044	94.19	<0.0001
	Treatment	Control	-0.0813	0.1025	0.63	0.4278
		Herbicide	-0.1843	0.1022	3.25	0.0714
	Material	Cone	0.9553	0.1176	66.04	<0.0001
		Root trainer	0.8932	0.1871	22.78	<0.0001
Trade Waste Pit, June	Intercept		0.6860	0.0765	80.38	<0.0001
	Material	Cone	0.2582	0.0832	9.62	0.0019
		Root trainer	0.5108	0.1272	16.12	<0.0001
Trade Waste Pit, July	Intercept		0.1809	0.0729	6.16	0.0131
	Material	Cone	0.1515	0.0789	3.69	0.0549
		Root trainer	0.4519	0.1176	14.78	0.0001
	Treatment	Control	-0.2473	0.1266	3.82	0.0508
		Herbicide	0.0522	0.1207	0.19	0.6653
		Mow	-0.0979	0.1257	0.61	0.4361
	Material X Treatment	Cone, control	-0.2193	0.1369	2.57	0.1092
		Cone, herbicide	0.4406	0.1320	11.15	0.0008
		Cone, mow	0.0811	0.1361	0.36	0.5512
		Root trainer, control	0.1646	0.2051	0.64	0.4222
		Root trainer, herbicide	-0.4337	0.1928	5.06	0.0245
		Root trainer, mow	-0.0579	0.1983	0.09	0.7701

Table B.5. P values for two-way ANOVAs comparing native cultivar and wild collected seed performance in 2011.

Response variable	Variable(s) tested or compared	p
Mean number of seedlings	Seed type	0.6144
	Species	0.0468
	Seed type x species	0.1382
	Bromus carinatus, Elymus trachycaulus	0.0114
	Bromus carinatus, Festuca idahoensis	0.6760
	Bromus carinatus, Koeleria macrantha	0.6087
	Elymus trachycaulus, Festuca idahoensis	0.0281
	Elymus trachycaulus, Koeleria macrantha	0.0386
	Festuca idahoensis, Koeleria macrantha	0.9178
	Koeleria macrantha wild collected vs. cultivar seed	0.1270
	Elymus trachycaulus wild collected vs. cultivar seed	0.1477
Mean height (cm)	Seed type	0.7217
	Species	0.0279
	Seed type x species	0.7156
	Bromus carinatus, Elymus trachycaulus	0.0519
	Bromus carinatus, Festuca idahoensis	0.7391
	Bromus carinatus, Koeleria macrantha	0.3275
	Elymus trachycaulus, Festuca idahoensis	0.0226
	Elymus trachycaulus, Koeleria macrantha	0.0048
	Festuca idahoensis, Koeleria macrantha	0.5045
	Elymus trachycaulus wild collected vs. cultivar seed	0.5494
	Festuca idahoensis wild collected vs. cultivar seed	0.5685

Table B.6. P values for two-way ANOVAs comparing native cultivar and wild collected seed performance in 2012.

Response variable	Variable(s) tested or compared	p
Mean number of seedlings	Seed type	1
	Species	0.4042
	Seed type x species	0.5302
	Koeleria macrantha wild collected vs. cultivar seed	0.4369
	Festuca idahoensis wild collected vs. cultivar seed	0.6144
	Elymus trachycaulus wild collected vs. cultivar seed	0.7812
Mean height (cm)	Seed type	0.9904
	Species	0.0130
	Seed type x species	0.6612
	Elymus trachycaulus, Festuca idahoensis	0.0167
	Elymus trachycaulus, Koeleria macrantha	0.0063
	Festuca idahoensis, Koeleria macrantha	0.6679
	Elymus trachycaulus wild collected vs. cultivar seed	0.9049
	Festuca idahoensis wild collected vs. cultivar seed	0.5840
	Koeleria macrantha wild collected vs. cultivar seed	0.5209
Mean transplant health score	Seed type	0.1975
	Species	0.0049
	Seed type x species	0.8365
	Elymus trachycaulus, Festuca idahoensis	0.0016
	Elymus trachycaulus, Koeleria macrantha	0.0172
	Festuca idahoensis, Koeleria macrantha	0.3109
Mean transplant height (cm)	Seed type	0.8227
	Species	0.0021
	Seed type x species	0.8896
	Elymus trachycaulus, Festuca idahoensis	0.0068
	Elymus trachycaulus, Koeleria macrantha	0.0008
	Festuca idahoensis, Koeleria macrantha	0.3625

APPENDIX C. CHAPTER 5 SUPPLEMENTARY DATA

454 Data Analysis Pipeline

Raw 454 sequencing data were obtained from the sequencing provider as 36 individual standard flowgram format files (sff files) representing 36 unique DNA libraries for each root sample. sff files are binary format files containing the fasta sequences, flowgram and quality scores for each sample. A total of 770,812 raw reads were obtained in the half-plate sequencing run (mean per sample=21,411 ± 5,646 S.D.). Number of raw reads per sample was consistent with the exception of a few samples (Figure C.1). Typical Titanium 454 half runs from Génome Québec are known to have between 360,000 to 520,000 reads. Errors occurring during next generation sequencing can have major implications for downstream analysis. Roche-454 technology was developed for genome sequencing which is more robust to sequencing error and is not greatly affected by errors in individual sequencing reads. However, using 454 reads for interpretation of sequences from microbial communities requires confidence in the correctness of each individual read (Schloss et al. 2011). In addition, PCR errors can introduce chimeras, which can increase the chances of inaccurately of identifying novel taxa (Haas et al. 2011). Therefore, strict quality control was performed on the sequencing reads prior to taxonomic analysis using procedures that are known to decrease sequencing error rate from ~0.6% to 0.02 % (Schloss et al. 2011). The Mothur SOP pipeline was applied to the data (Schloss 2013) using Mothur v. 1.28.0 (Schloss et al. 2009) and adapted for analysis of the partial 18S rRNA gene and targeted sequences belonging to arbuscular mycorrhizal fungi.

The Mothur implementation of sffmultiple and sffinfo were first used to extract the fasta, flowgram and quality files from each sff file. The flow data was then used to denoise the sequences by removing reads with less than 450 flows and trim longer reads to 450 flows along with removal of sequences containing barcode sequences with more than one error and primer sequences with more than two errors. Read quality is known to significantly decrease after 450 flows and in reads shorter than 450 flows (Schloss et al. 2011). Errors in the barcode and forward sequencing primer are correlated with increased errors throughout the read. Sequencing noise was further reduced through implementation of PyroNoise, a component of the AmpliconNoise suite of programs (Quince et al.

2009, Quince et al. 2011). Sequences shorter than 200 bp and with homopolymers longer than 8 bp were then removed from the data. The majority of raw reads were approximately 500 bp in length (mean = 487) and were reduced to approximately 280 bp after denoising (mean = 274).

Multiple sequence alignment was performed in Mothur using a reference alignment database for the Glomeromycota 18S rRNA gene (Krüger et al. 2012) and the Mothur implementation of NAST (DeSantis et al. 2006, Schloss 2009). Aligned sequences were screened to check that all sequences overlapped in the same alignment space, outlying sequences were removed and overhanging sequences were trimmed so that each sequence had the same start and end position along the 18S gene segment. A pre-clustering step was then used to further reduce error on the aligned sequences by merging sequence counts for reads within two base pairs of more abundant sequences (Huse et al. 2010). Potentially chimeric sequences were removed using chimera.uchime, a version of the original chimera removal program UCHIME created by Edgar et al. (2011). Chimera.uchime was run in database independent mode where the most abundant sequences in the dataset were used as parent references for chimera checking. A total of 169 chimeric sequences were detected and removed. A reference database of Glomeromycota 18S rDNA sequences was retrieved from the MaarjAM database in fasta format (Öpik et al. 2010). Sequences were classified in Mothur using sequences and taxonomy metadata from the MaarjAM database and the Silva Eukarya database (Pruesse et al. 2007) and 8,283 sequences that did not identify as arbuscular mycorrhizal fungi were removed from the data. Mothur's default classification settings were used which implement the Ribosomal Database Project's Bayesian Classifier (Wang et al. 2007), with the exception that a cutoff of 95% was used instead of 80% to more accurately classify sequences at the species level of taxonomic hierarchy.

Operational Taxonomic Units (OTUs) were used to characterize AMF communities. OTUs are sequence clusters grouped at a specific level of sequence similarity and taxonomic hierarchy for taxonomic comparison and assignment (Schloss and Handelsman 2005, Schloss et al. 2009, Sun et al. 2009, Davison et al. 2012, Bik et al. 2012). The default OTU construction method in Mothur was employed, which utilizes the average neighbor clustering algorithm

with a distance matrix cutoff of 0.15 (Schloss and Westcott 2011). OTUs were clustered at 97% similarity. To reduce the number of spurious OTUs, OTU clusters represented by four or fewer sequences were removed that did not identify to the species or virtual taxon level for *Glomus* or genus level for other genera. A strong relationship has been established between increasing sequence number and increasing spurious OTUs generated from sequencing error known as “sequencing artifacts” (Schloss et al. 2011). For this reason and for an unbiased comparison of diversity in the samples, all samples were standardized by randomly subsampling to the minimum number of reads in the smallest sample, which was 7,124 (Brazelton et al. 2010, Gihring et al. 2012). This eliminated one sample that was not included in statistical analyses. The majority consensus taxonomy was determined for each OTU in Mothur using the MaarjAM and Silva databases and the same taxonomic classification described for sequence classification during non-AMF sequence removal.

Table C.1. Arbuscular mycorrhizal fungal taxa detected at research sites.

OTU	Taxon	PCP		PP		TWP	
		D	U	D	U	D	U
1	*Glomus* WOTU1	31	8215	4146	6549	2434	4740
2	*Glomus* sp. VTX177	5372	2242	4803	2949	842	4
3	*Glomus* MO.G3 VTX113	1800	1819	1342	1604	353	2183
4	*Glomus* sp. VTX143	1002	355	721	929	2771	465
5	*Glomus* sp. VTX166	700	607	645	137	223	1665
6	*Glomus* sp. VTX165	1495	2	211	1	849	2
7	*Glomus* MO.G15 VTX135	607	124	815	97	392	516
8	*Glomus* MO.G8 VTX130	1387	1	13	13	x	1
9	*Glomus* ORVIN.GLO6 VTX212	36	33	139	18	899	14
10	*Glomus* Glo.D VTX103	x	686	0	88	0	256
11	*Glomus* MO.G21 VTX129	1	170	425	1	1	434
12	*Glomus* QU.Glo7 VTX187	2	280	31	298	37	123
13	*Glomus* Glom.1B.10 VTX108	1	201	x	2	339	146
14	*Glomus* PF14 VTX083	212	10	130	114	171	0
15	*Glomus* Glo.G8 VTX193	41	58	54	92	80	115
16	*Glomus* NES06 VTX199	63	54	142	35	79	28
17	*Glomus indicum* VTX222	64	144	10	86	8	56
18	*Glomus* sp. VTX140	181	33	52	43	12	x
19	*Glomus* MO.G19 VTX140	91	66	136	3	0	x
20	*Glomus* WOTU20	x	0	0	0	266	x
21	*Glomus* Winther07.E VTX142	0	0	x	x	0	247
22	Glom.1B.2 VTX125	18	84	3	22	3	34
24	*Glomus* Glo.G8 VTX057	6	47	41	8	30	13
25	*Glomus* NES14 VTX151	x	0	0	0	100	0
26	*Glomus* VDGlo10 VTX117	0	6	0	1	0	82
27	*Glomus* LES30 VTX222	9	43	1	6	1	21
28	*Glomus* NF10 VTX155	0	0	0	37	1	0
29	*Paraglomus* Pa1 VTX335	0	0	6	0	31	0
30	*Glomus* Glomus3 VTX143	0	0	x	0	60	0
31	*Glomus* Wirsel.OTU14 VTX137	21	1	5	7	0	0
33	*Glomus* acnaGlo1 VTX137	16	x	4	6	x	0
34	*Glomus* ORVIN.GLO3A VTX072	0	4	0	1	0	22
35	*Glomus* Glo.G4 VTX166	x	0	0	0	25	0
36	*Glomus* NF17 VTX159	22	0	0	0	0	0
37	*Scutellospora aurigloba* VTX052	0	10	0	0	0	10
38	*Glomus* QU.Glo10 VTX117	0	2	0	x	0	14
39	*Glomus* sp. VTX064	1	0	5	3	6	x
41	*Glomus* sp. VTX325	0	15	0	0	0	0
42	*Diversispora* NF29 VTX062	x	6	x	6	x	x
43	*Glomus* MO.G11 VTX067	0	0	0	11	0	0

PCP = Pincher Creek Pit, PP = Potato Patch Pit, TWP = Trade Waste Pit, U = Undisturbed, D = Disturbed.

Table C.1. con'd. Arbuscular mycorrhizal fungal taxa detected at research sites.

OTU	Taxon	PCP		PP		TWP	
		D	U	D	U	D	U
44	*Acaulospora* PSAM.Aca.1 VTX023	0	x	0	1	0	6
45	*Glomus* Ligrone07.sp VTX143	5	1	3	2	5	0
48	*Archaeospora trappei* VTX245	0	x	0	2	0	3
49	*Glomus* MO.G27 VTX160	x	4	3	4	2	4
50	*Paraglomus* Paraglomus1 VTX281	0	1	0	0	0	3
53	*Glomus* WOTU53	0	0	0	3	0	0
55	*Glomus* VDGlo11 VTX064	0	0	2	0	0	0
58	*Glomus* WOTU58	0	0	0	0	2	0
60	*Scutellospora* Schechter08.Scut1 VTX052	0	1	0	0	0	1
64	*Glomus* WOTU64	0	0	2	0	0	0
65	*Scutellospora* sp. VTX049	0	0	0	0	0	1
66	*Glomus* VDGlo4 VTX166	x	x	1	x	0	2
69	*Glomus* Glo4 VTX143	0	0	0	1	1	0
71	*Glomus* Glo60 VTX315	0	x	0	x	0	x
76	*Glomus* NF09 VTX143	0	0	0	0	1	0
77	*Glomus* WOTU77	0	0	x	1	0	0
80	*Ambispora gerdemannii* VTX283	0	1	0	0	0	0
83	*Glomus perpusillum* VTX287	1	0	0	0	0	0
87	*Pacispora* Schechter08.Paci1	0	x	0	x	0	0
90	*Glomus* WOTU90	0	1	0	0	0	0
91	*Glomus* sp. VTX113	x	0	0	x	0	x
92	*Glomus* 6 VTX113	x	0	0	x	0	0
96	*Glomus* WOTU96	0	0	0	x	0	x
98	*Glomus* Glom.1B.8 VTX135	x	0	x	0	x	0
104	*Glomus* MO.G20 VTX143	x	1	1	1	0	x
105	*Glomus* Glom.1B.5 VTX143	0	0	0	x	1	0
107	*Glomus* WOTU107	0	0	0	0	0	x
108	*Glomus* WOTU108	x	x	0	0	0	0
110	*Glomus* QU.Glo5 VTX166	0	0	0	x	0	x
112	*Glomus* MO.G13 VTX115	1	1	x	1	x	1
118	*Glomus* VeGlo18 VTX166	0	0	0	0	x	0
125	*Diversispora* WOTU125	0	x	0	0	0	0
129	*Glomus* WOTU129	0	0	x	x	0	0
131	*Glomus* VeGlo8 VTX114	x	0	0	0	0	0
132	*Glomus* WOTU132	0	x	0	x	0	0
135	*Glomus* WOTU135	0	0	x	0	0	0
137	*Glomus* Schechter08.Glo7 VTX125	x	0	x	0	0	0
153	*Glomus* Porras.Alfaro03.OTU2 VTX177	x	0	0	0	0	0
155	*Glomus* QU.Glo8 VTX129	0	0	x	x	0	x
174	*Glomus* Alguacil09c.Glo2 VTX113	x	0	0	0	0	0

PCP = Pincher Creek Pit, PP = Potato Patch Pit, TWP = Trade Waste Pit, U = Undisturbed, D = Disturbed.

Table C.1. con'd. Arbuscular mycorrhizal fungal taxa detected at research sites.

OTU	Taxon	PCBP		PP		TWP	
		D	U	D	U	D	U
192	*Glomus* VDGlo3 VTX142	0	0	0	x	0	x
236	*Glomus* sp. VTX193	x	0	0	0	0	0
242	*Glomus* BV.WUB.2 VTX113	0	0	0	x	0	0
246	*Glomus* Glo.G1 VTX105	0	0	0	0	x	0
252	*Glomus* sp. VTX130	x	0	0	0	0	0
282	*Scutellospora* MO.S2 VTX052	0	x	0	0	0	0
314	*Paraglomus majewskii* VTX335	0	0	x	0	0	0
336	*Ambispora* WOTU336	0	0	0	0	0	x
404	*Glomus* Glo8 VTX113	0	0	x	0	0	0
414	*Glomus irregulare* VTX115	0	x	0	0	0	0
437	*Glomus* MO.G7 VTX199	0	0	0	x	0	0
439	*Glomus* Glomus 14 VTX222	x	0	0	0	0	0

PCP = Pincher Creek Pit, PP = Potato Patch Pit, TWP = Trade Waste Pit, U = Undisturbed, D = Disturbed.

Table C.2. P values for Student's t-test for AMF diversity and MRPP.

Test	P
T-test Disturbed vs. Undisturbed	
Species richness	0.02615
Evenness	0.3583
Shannon Diversity	0.8164
Simpson's Diversity	0.4994
MRPP $\alpha_{adjusted}$=0.0033333	0.00000005
PCD vs. PCU	0.00055133
PCD vs. PPD	0.01074235
PCD vs. PPU	0.00088949
PCD vs. TD	0.00061996
PCD vs. TU	0.00081256
PCU vs. PPD	0.04556931
PCU vs. PPU	0.32076941
PCU vs. TD	0.00389190
PCU vs. TU	0.02135788
PPD vs. PPU	0.12243720
PPD vs. TD	0.02831253
PPD vs. TU	0.00223696
PPU vs. TD	0.00716545
PPU vs. TU	0.00305341
TD vs. TU	0.00085584
Disturbed vs. Undisturbed	0.00000634
Mantel Test	
AMF	0.665897
Vegetation	0.333311

D = Disturbed, U = Undisturbed, PC = Pincher Creek Pit, PP = Potato Patch Pit, T = Trade Waste Pit.

Table C.3. P values for correlations of key soil and plant variables and AMF taxa with AMF ordination axes.

Variable	p Axis 1	p Axis 2
Organic Matter	0.0497	<0.0001
Total Organic Carbon	0.0497	<0.0001
Nitrogen	0.0360	<0.0001
Phosphorous	0.2311	<0.0001
Sand	0.0069	0.0432
Silt	0.0081	0.0067
pH	0.0656	<0.0001
Na	0.0989	0.0002
Bromus inermis	0.0858	0.2088
Poa compressa	0.0396	0.0007
Agropyron sibiricum	0.8345	0.0129
Festuca idahoensis	0.0207	<0.0001
Galium boreale	0.2356	0.0024
Heterotheca villosa	0.1904	0.001
Koeleria macrantha	0.7528	0.0036
Oxytropis sericea	0.1999	0.0150
Dantonia paryii	0.4828	0.0007
Glomus WOTU1	<0.0001	<0.0001
Glomus VTX177	0.7620	<0.0001
Glomus VTX143	<0.0001	0.2936
Glomus VTX165	0.0025	0.0006
Glomus MO.G8 VTX130	0.0248	0.0012
Glomus QU.Glo7 VTX187	0.0018	0.0004
Glomus VTX140	0.4856	0.0003

Table C.4. P values for correlations between AMF taxa and soil and plant variables.

Variable	AMF Taxa	p
Organic Matter	WOTU1, VTX165, VTX187, VTX140	<0.0001, 0.0076, 0.0001, 0.0096
Total Organic Carbon	WOTU1, VTX165, VTX187, VTX140	<0.0001, 0.0076, 0.0001, 0.0096
Nitrogen	WOTU1, VTX165, VTX187	<0.0001, 0.0051, <0.0001
Phosphorous	WOTU1, VTX177, VTX187, VTX140, Wirsel VTX137, acnaGlo1 VTX137	0.0018, <0.0001, 0.0036, 0.0002, 0.0027, 0.0095
Sand	WOTU1, VTX165, VTX130, VTX187	0.0003, 0.0044, 0.0011, 0.0006
Silt	WOTU1, VTX165, VTX130, VTX187	<0.0001, 0.0019, 0.0026, <0.0001
pH	WOTU1, VTX177, VTX165, VTX187, VTX140	<0.0001, 0.0004, 0.0044, 0.0002, 0.0010
Na	WOTU1, VTX187, VTX140	0.0008, <0.0001, 0.0074
Agropyron sibiricum	WOTU1, VTX165, VTX187, WOTU20	0.0121, 0.0190, 0.0039, 0.0052
Agropyron cristatum	WOTU20	0.0008
Poa compressa	WOTU1, VTX165, VTX187, WOTU20	0.349
Bromus inermis	MO.G20 VTX143, WOTU58, WOTU20	0.011, 0.0008, 0.0002
Festuca idahoensis	WOTU1, VTX177, VTX165, VTX187	<0.0001, 0.0176, 0.0030, 0.0009
Heterotheca villosa	WOTU1, VTX165, VTX130, VTX140, Wirsel VTX137, acnaGlo1 VTX137	0.0013, 0.0013, <0.0001, 0.0144, 0.0019, <0.0001
Koeleria macrantha	VTX177, VTX130	0.0011, 0.0087
Dantonia paryii	WOTU1, VTX177, VTX165, VTX187, VTX140	0.0113, 0.0155, 0.0109, 0.0144, 0.0026

Table C.5. P values for correlations between AMF diversity and plant community properties.

	Arbuscular Mycorrhizal Fungi			
	Species Richness	Evenness	Shannon Diversity	Simpson Diversity
Host Plant Species				
Species Richness	0.04	0.1	0.4	0.5
Evenness	0.3	0.6	0.14	0.9
Shannon Diversity	0.04	0.1	0.4	0.5
Simpson Diversity	0.2	0.8	0.9	0.9
Native Cover (%)	0.07	0.2	0.5	0.4
Non-Native Cover	0.07	0.2	0.5	0.4
Bromus inermis Cover	0.07	0.8	0.9	0.9

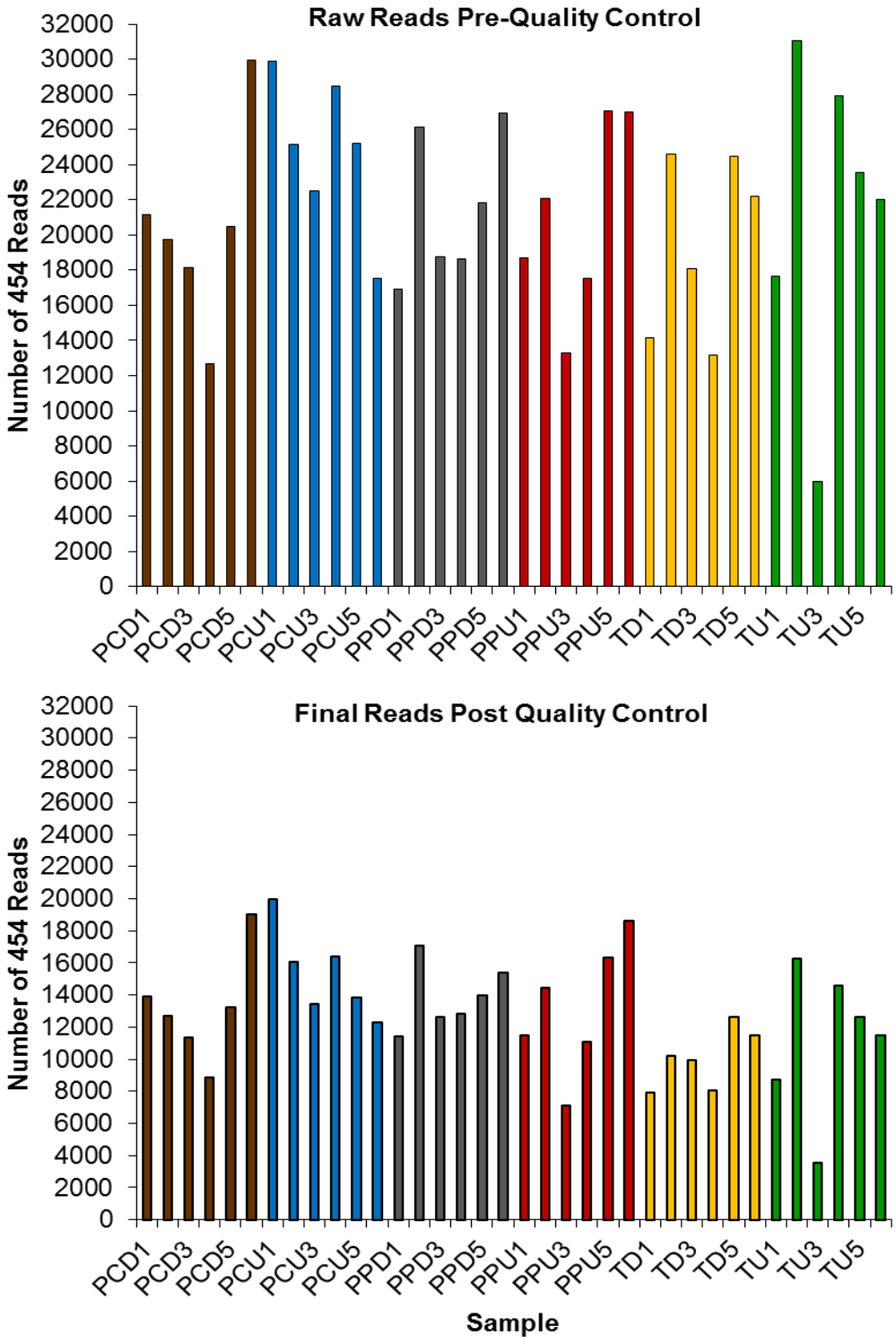

Figure C.1. Number of 454 sequencing reads obtained for the study. Above, the total number of raw reads are shown per sample; below, the number of reads after quality filtering. (PC = Pincher Creek Pit, T = Trade Waste Pit, PP = Potato Patch Pit, D = disturbed, U = undisturbed).

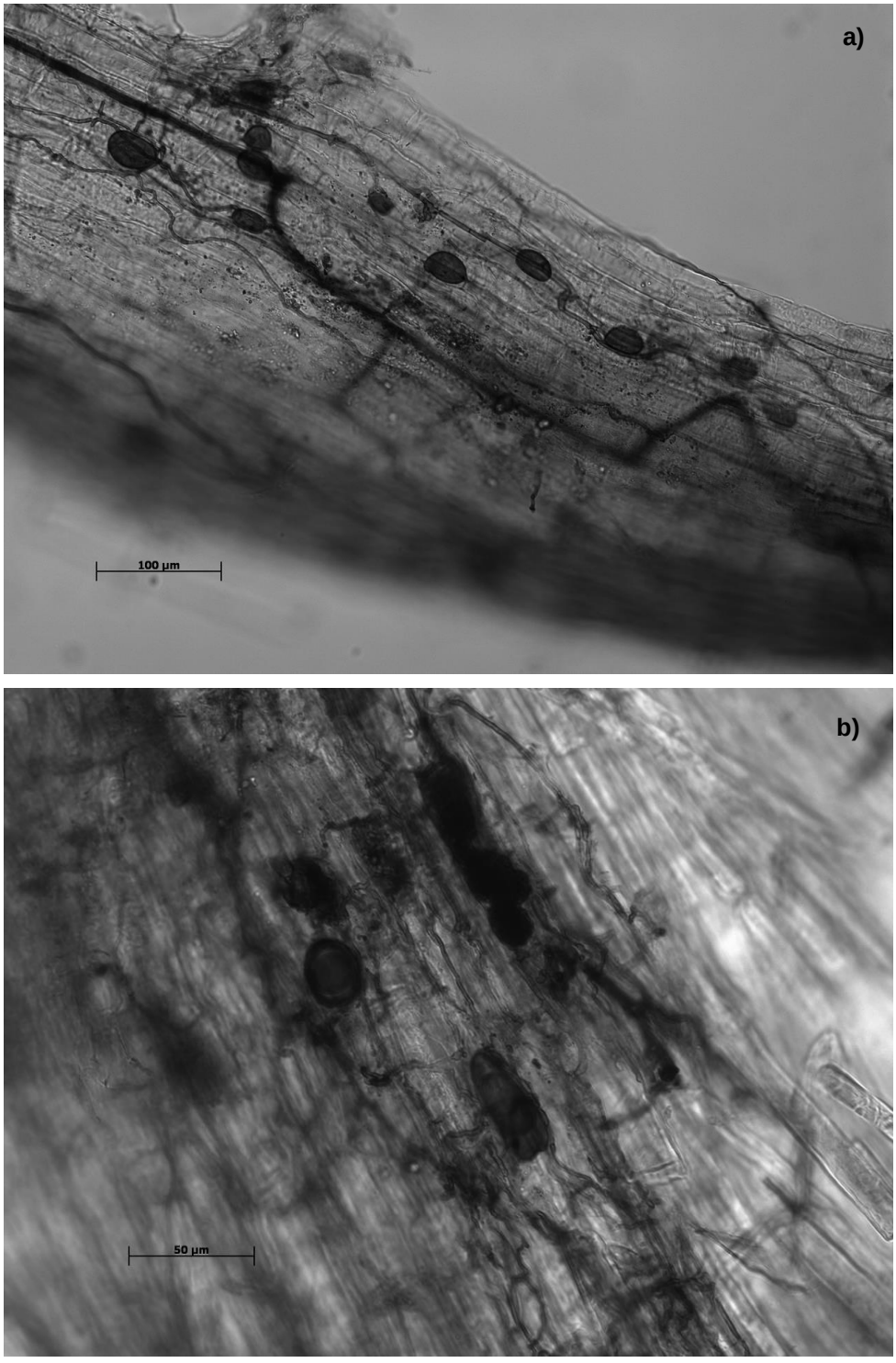

Figure C.2. Images of roots cleared and stained for assessment of mycorrhizal colonization. a) and b) Trade Waste Pit, a) disturbed, b) undisturbed. 200-400X.

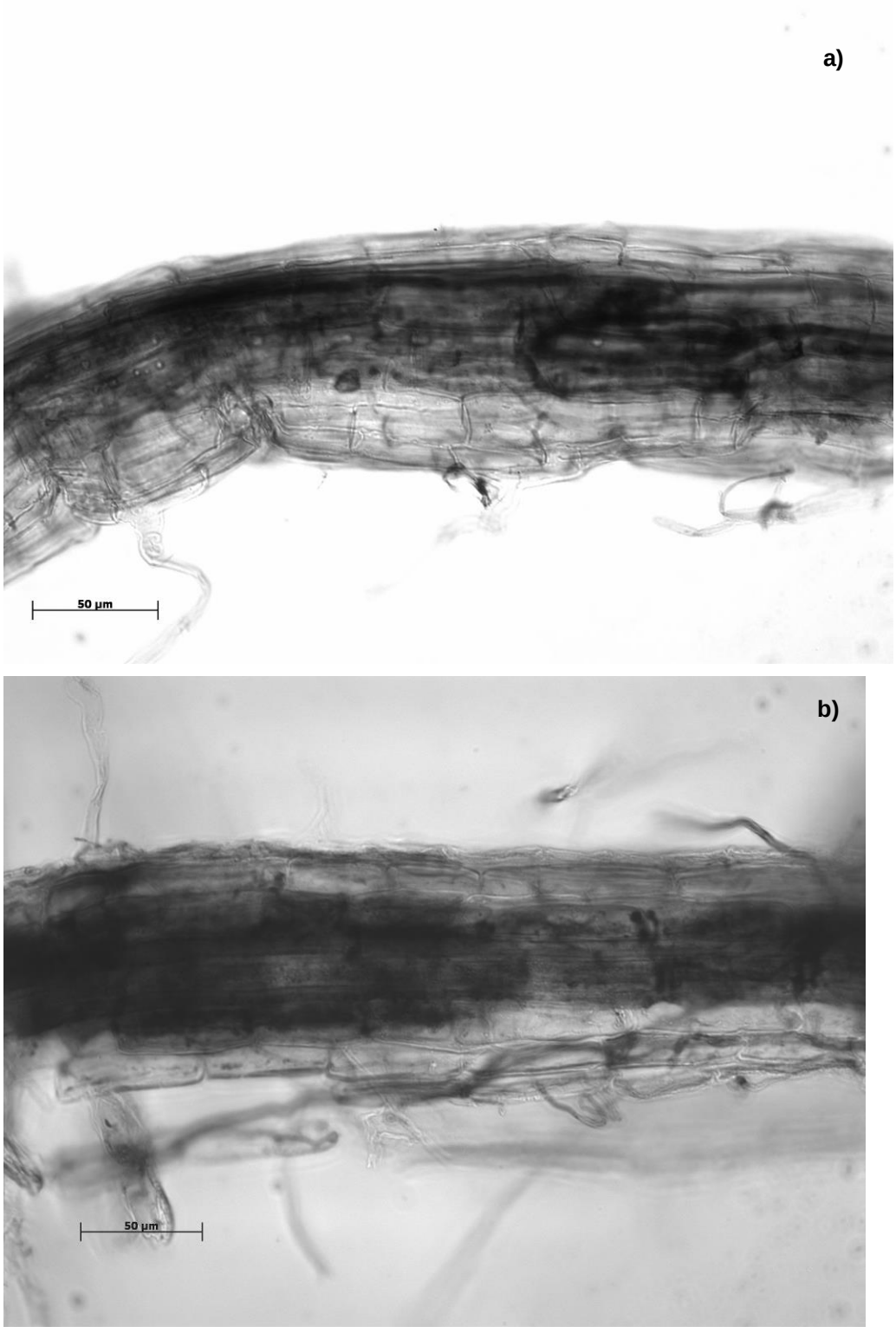

Figure C.3. Images of roots cleared and stained for assessment of mycorrhizal colonization. a) and b) Pincher Creek Pit, a) disturbed, b) undisturbed. 200-400X.

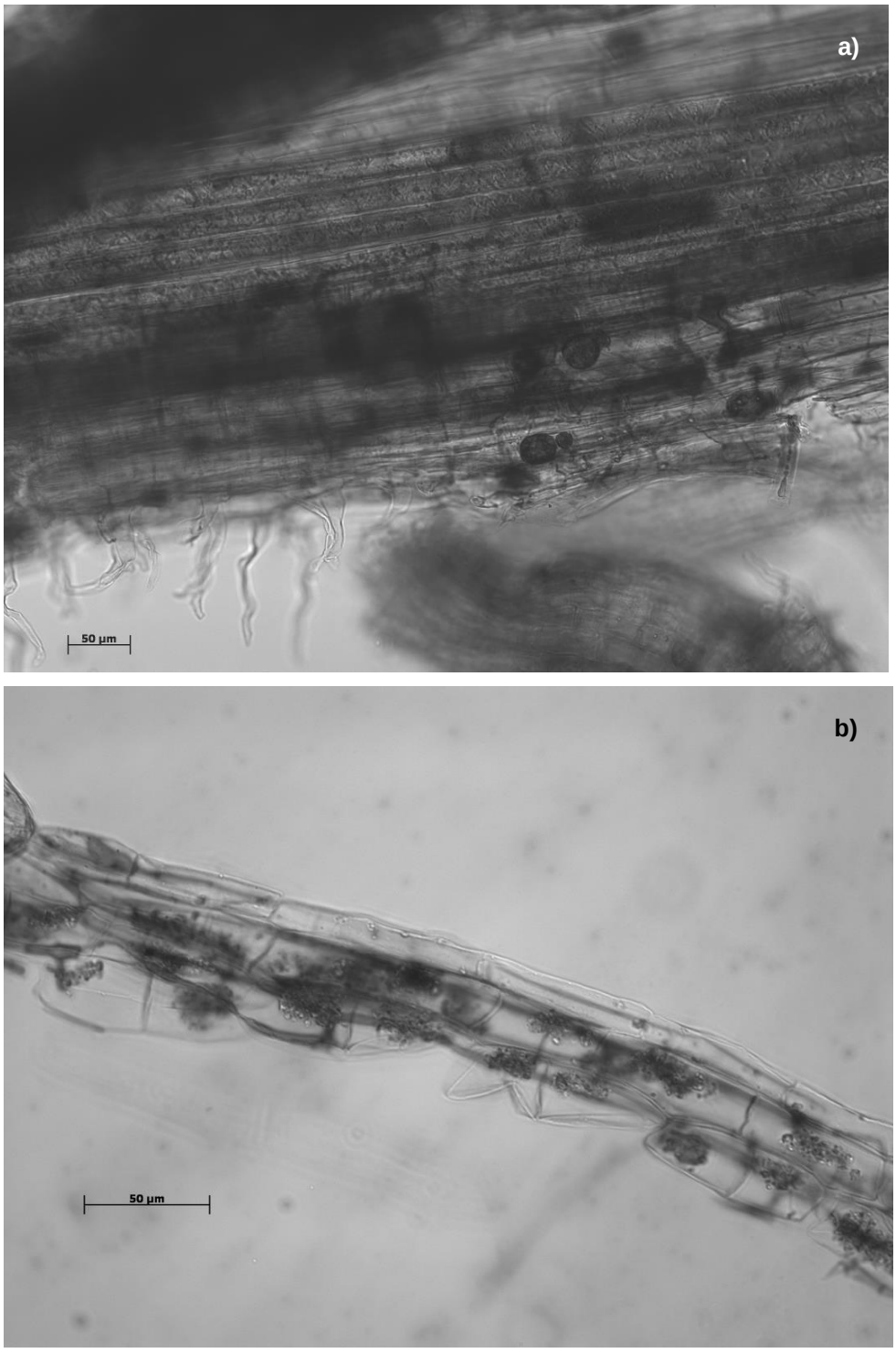

Figure C.4. Images of roots cleared and stained for assessment of mycorrhizal colonization. a) and b) Potato Patch Pit, a) disturbed, b) undisturbed. 200-400X.

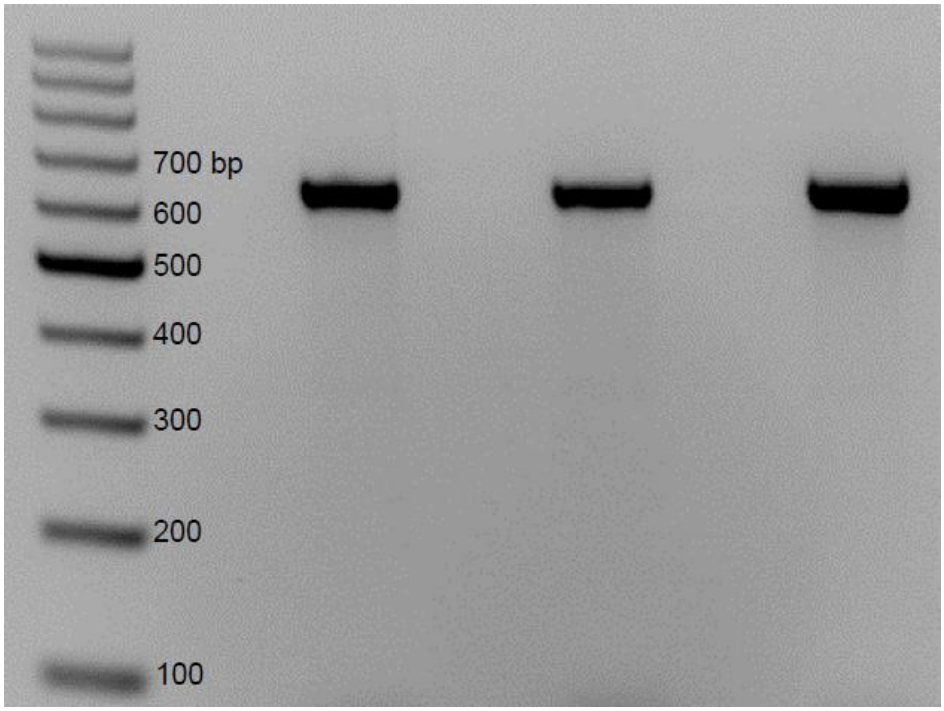

Figure C.5. Image of 1.5 % agarose gel and PCR products amplified from pyrosequencing primers and isolated genomic DNA from three experimental samples. Previous investigations with these primers required two PCR reactions to obtain amplification with pyrosequencing primers, one with template primers only then one with template primer attached to pyrosequencing adapter and barcode, using amplicons from the previous reaction as the template. Here it is demonstrated that the reaction can be performed once with pyrosequencing primers and genomic DNA as the template.

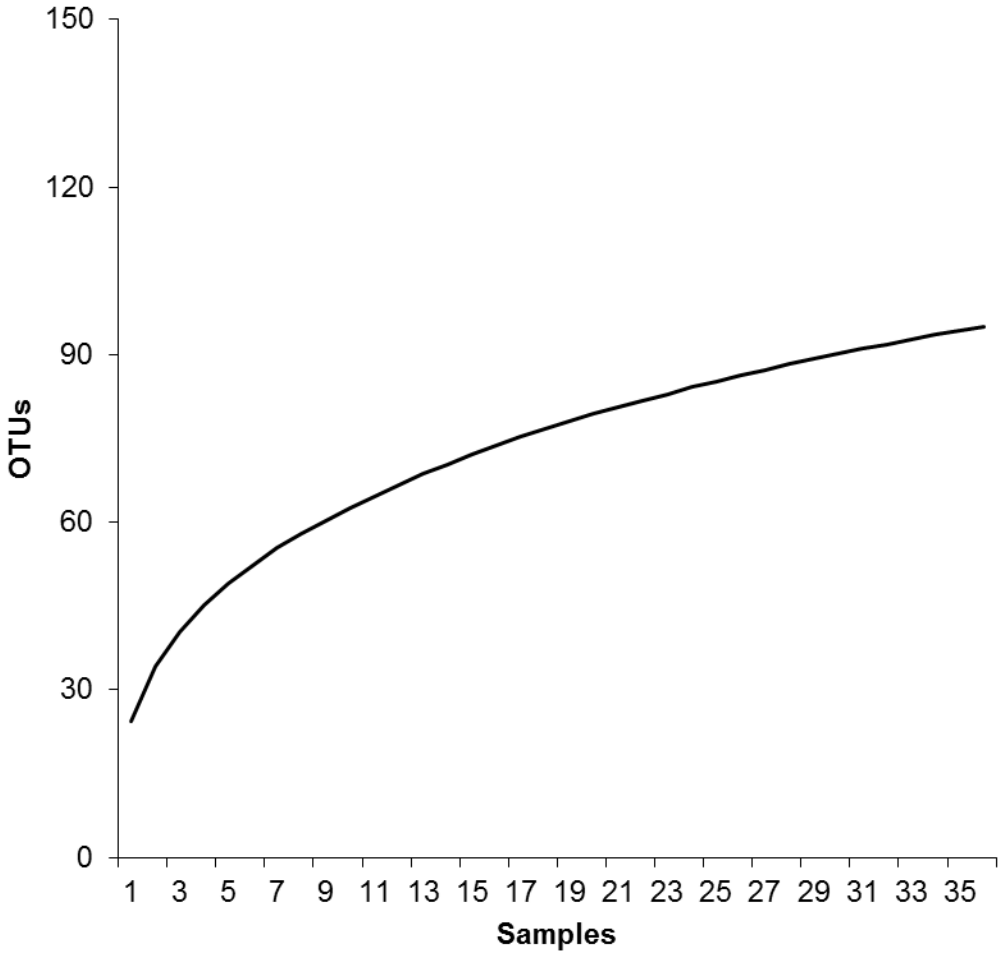

Figure C.6. Rarefaction curve showing number of Operational Taxonomic Units detected per number of samples used.

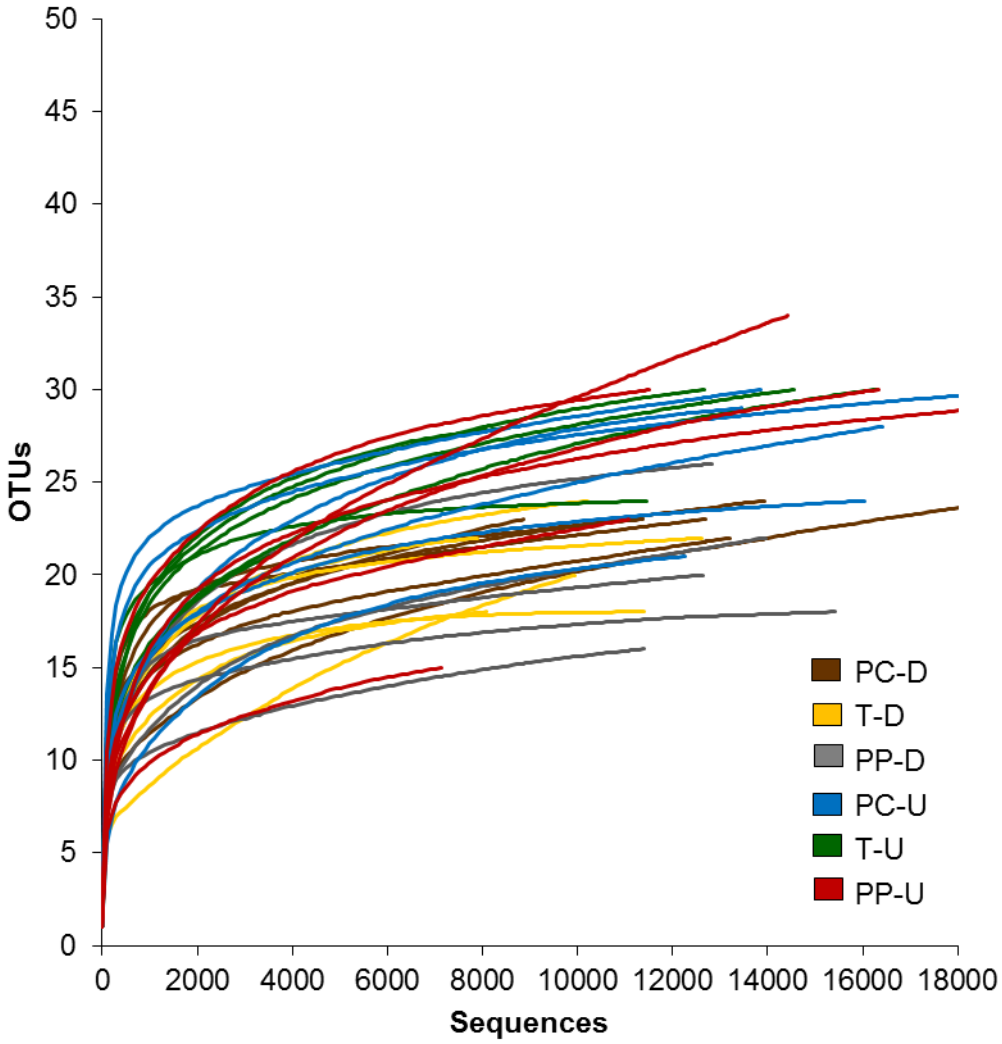

Figure C.7. Rarefaction curve showing number of Operational Taxonomic Units detected as the number of sequences obtained increases in each sample. (PC = Pincher Creek Pit, T = Trade Waste Pit, PP = Potato Patch Pit, D = disturbed, U = undisturbed).