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An Assessment of the Plant and Mycorrhizal Community at Steeves Lake Shoreline, Colomac Mine, NWT

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By

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Abstract

This study focused on the environmental impacts of mining and potential techniques to mitigate them. The goals of this study were to (1) Quantify vegetation and fungal colonization at a remediated mine site in northern Canada, and to determine if there were effects from petroleum hydrocarbon contamination, (2) Determine the response of northern plants inoculated with mycorrhizal fungi to diesel contamination. Mycorrhizal colonization was observed in field collected roots and trends in colonization and vegetation cover suggested that these communities were no longer impacted by residual contamination. Mean hyphal colonization of *Elymus trachycaulus* roots collected over two years was 47.3±3.8% and 49.3±2.7% respectively, and *Festuca ovina* and *Hordeum jubatum* collected during one year had mean hyphal colonization of 64.1±2.1% and 56.6. ±2.7% respectively. Two mycorrhizal plants (*E. trachycaulus* and *Erigeron acris*) and one non-mycorrhizal plant (*Carex aquatilis*) were grown with or without mycorrhizal fungi and exposed to four levels of diesel contamination (3,460, 6,500, 10,000 mg/kg and a control). Responses varied between species, though results suggest that PHC concentrations ≥ 6,500 mg/kg significantly affected growth. Mean shoot/root ratio of *C. aquatilis* plants decreased from 0.15 ± 0.01 in the control treatment to 0.10 ± 0.01 and 0.09 ± 0.01in the treatments receiving 6,500 and 10,000 mg/kg diesel respectively. Growth responses between plant species with and without mycorrhizal inoculum suggest that an association with AMF influenced plant growth responses. Significant differences in shoot biomass between inoculum treatments were observed in the mycorrhizal plant species though not in the non-mycorrhizal species. Mean shoot fresh weight of *E. trachycaulus* was significantly higher in plants inoculated with mycorrhizal fungi (0.12 g ± 0.01) compared to non-inoculated plants (0.09 g ± 0.0). Mean shoot fresh weight of *E. acris* plants was 0.10 g ± 0.01 in plants inoculated with mycorrhizal fungi, significantly

greater than non-inoculated plants (0.04 g ± 0.01). Results indicate that mycorrhizal fungi have established at this reclaimed site and are colonizing host species. This research provides insight that will contribute to our understanding of mycorrhizal associations in the north and can be used in future re-vegetation efforts.

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List of Tables

List of Figures

Chapter 1: General introduction

1.1 Mining in Canada

The mining industry is an integral contributor to Canada's economy. As of 2016 a total of 1,192 mining establishments were operating across Canada, with 373,000 persons employed at some level of the mining industry, as well as another 190,000 persons indirectly employed (Marshall, 2015). In 2015, the industry contributed 56 billion dollars to Canada's gross domestic product (GDP), i.e. 3.4% of Canada's GDP, and combined with oil gas extraction is the fourth largest industry in Canada (Marshall, 2016).

Northern Canada has an abundance of natural resources that are actively being mined and many that are currently awaiting extraction. Northern areas have been receiving increased attention; in 2016, 20% of Canadian spending on exploration and deposit appraisal occurred in the three territories (Natural Resources Canada, 2018). The Conference Board of Canada predicts that metal and non-metallic output in the north will grow by 91% between the years 2011 and 2020 (Rhéaume & Caron-Vuotari, 2013). The Northwest Territories and Nunavut Chamber of Mines have identified eleven potential mine sites that are currently under advanced exploration in the NWT in addition to the 3 sites currently producing. Given the abundance of natural resources in the north, it has been suggested that the future of Canada's mining industry lies in remote, northern Canada (Marshall, 2016).

1.2 Environmental impacts of mining

Although mining has had significant, positive impacts on Canada's economy (Marshall, 2016), it also had negative environmental impacts. These impacts include the depletion of non-renewable resources and landscape level disturbance (Azapagic, 2004). Environmental

disturbances, events that are disruptive at all ecological levels, across ecological components and disrupt the biological cycle of organisms affected (Pickett et al., 1989) are common at mine sites. Plans for the responsible closure of mines are recommended to include procedures for remediation of these negative impacts (MVLWB & AANDC). Disturbance due to mining activities can include compacted soils, destruction of vegetation, acidified soils and contamination of soils and water.

Soil compaction at mine sites may arise during mining operations, as well as during the reclamation process. During operation, soil becomes compacted due to vehicle traffic at the site, and the building of mine infrastructure. During the reclamation process, topsoil is transported and re-spread using heavy machinery. This repeated traffic applies high pressure and shear stress, causing soil compaction (McSweeney & Jansen 1984; Chong & Cowsert, 1997). This is referred to as a compressive disturbance: the soil is compressed causing the soil mass to force in on itself resulting in increased soil density and the reduction or elimination of pore space or fissures in the soil (Spoor 2006). Soil compaction can reach deep into the soil profile, up to 0.8 m deep or more (Spoor 2006). Compaction of re-spread topsoil can lead to difficulties re-vegetating a site such as resistance to root penetration and reduced root elongation (Fehrenbacher et al, 1982; Thompson et al., 1987), reduced water infiltration rates and water holding capacity (Indorante et al., 1981; Shukla et al., 2004).

During surface mining, topsoil is removed to access the underlying rock and often stockpiled for later use in reclamation. The action of stripping topsoil and stockpiling it results in the destruction of overlying vegetation and the disruption of unique soil layers, and soil structure (Jansen, 1981), resulting in soils that are not optimal for re-vegetation. Areas where topsoil has been re-spread need to be reclaimed with vegetation quickly to stabilize the soil (Bradshaw,

1997). Vegetation is influenced by water and nutrient availability in the soil and can in turn influence soil characteristics creating a positive feedback (Shukla et al., 2004), with vegetation improving the physical, chemical and biological properties of the re-spread soil (Bradshaw, 1997).

As well as the physical damage to the environment directly caused from excavating open pits there may also be damage due to the formation of acid rock drainage. When exposed rock containing sulphide minerals comes into contact with water and air, sulphuric acid is produced through natural chemical reactions (Bezuidenhout et al., 2009). Soils exposed to acid drainage become acidified, lowering soil fertility and inhibiting plant growth. Haling et al., (2011) found that a decrease in soil pH from 5.2 to 3.0 was enough to inhibit root growth in five plant species including both acid-tolerant and acid-sensitive species.

Mining operations can result in the release of contaminants that persist in the environment effecting surrounding soils and water. Mining has been recognized as a high-risk activity regarding environmental impacts; in the recent decades, some of the worst contamination accidents have been the result of mining activities (Leppänen et al., 2017). Elements commonly found at sites that may reach toxic concentrations include arsenic, cadmium, copper, lead, mercury, nickel, selenium, silver and zinc (Pierzynski et al., 1994). Some of these such as copper, lead, nickel and zinc can restrict the plant growth, as well as negatively affect the diversity and activity of soil organisms (Wong, 2002). Throughout the history of mining in the north there have been significant scars left on the landscape, for example Faro Mine which produced lead and zinc left behind water contaminated with acid and zinc which requires treatment, and Colomac Mine which produced gold and has left behind water contaminated with ammonia, cyanide and metals (Office of the Auditor General of Canada, 2002).

The negative effects of mining activities in the north dictate which closure and remediation options are chosen. The effects of compacted and disrupted soil structure hinder the establishment of vegetation which is necessary to reduce runoff, erosion and loss of soil organic matter (Polyakov & Lal, 2004). Summers in the north are short, and many sites are in remote locations with restricted access allowing only a short amount of time and limited opportunities to re-establish vegetation (MVLWB & AANDC, 2013). Natural attenuation of contaminants, which relies on biological processes to remediate contaminants, may not be effective in the north due to limited nutrients, low temperature, water availability, and possible migration of contaminants during spring freshet (Gore et al. 1999). Consequently, other more intensive methods must be considered such as excavation, soil washing, landfilling and capping, all of which can include high costs, and more disturbance to the environment than what originally occurred through mining operations (Bento et al., 2005).

Although mining can result in the release of numerous contaminants, many of which are specific to a given mining operation, contamination due to petroleum hydrocarbon (PHC) release is a possibility at most remote mine sites. Often PHCs are a secondary contaminant at mine sites; they do not arise from one of the steps in mineral retrieval, but occur through accidental spills. In the Northwest Territories, many remote mine sites do not have access to electric grids. Consequently, they rely on diesel fuel for power generation, leading to a greater likelihood of fuel oil spills (Standing Senate Committee on Energy, the Environment and Natural Resources 2015). In 2014, 39% of spills containing hazardous materials, such as hydrocarbons and metals were fuel oil, and 29% of all reported spills that year were from the mining sector (ENR, 2016). There are many northern mines that have reported fuel spills in the last few decades resulting in contaminated soils, some of which include Colomac, Tundra, Giant and Ekati Mines. These

spills are commonly comprised of diesel fuel which is used to operate machinery and power generators at mine sites (EBA Engineering Consultants, 2001; INAC, 2018; INAC & GNWT, 2010; MVLWB, 2017).

The term petroleum hydrocarbons describes a mixture of organic compounds. Petroleum products can contain varying proportions of hundreds to thousands of different compounds; all are composed of carbon and hydrogen though small amounts of nitrogen, sulfur and oxygen may occur. Many of these compounds are toxic and, depending on their size, they differ in their mobility and the duration of their impacts; larger molecules tend to be less mobile and more persistent than compounds with a smaller molecular weight (Canadian Council of Ministers of the Environment (CCME), 2008). While PHCs are readily broken down by soil microbes, in the north, petroleum spills degrade at a slower rate due to the cold climate. As a result, PHCs persist in the soil for longer periods of time, increasing the chance of exposure to PHCs by plants and wildlife (ENR, 2015). The persistence of contaminants also increases the chances of migration to aquatic habitats which can be devastating to these ecosystems as many water bodies in the north are considered pristine and sensitive to minor changes water chemistry (MVLWB & AANDC, 2013).

1.3 Remediation and reclamation of mine sites

Over the last four decades, improving remediation and reclamation of mine sites in the north has become an increasing priority of the Government of the Northwest Territories (GNWT; MVLWB & AANDC, 2013). As mining has been an integral part of northern development and primary industry for the past half century, and since legislation was lacking to enforce responsible mine closure, many mine sites have been abandoned once mining operations ceased.

Ownership was then transferred to the care of the federal government who have become responsible for site remediation (Bullen & Robb, 2002). In a Report of the Commissioner of the Environment and Sustainable Development (2002), the cleanup costs for Colomac, an abandoned mine in the NWT, were estimated to be $70 million. Only $1.5 million was collected in security deposits from the previous owners, leaving $68.5 million to come from the Federal government (Office of the Auditor General of Canada, 2002). Developing and implementing effective remediation and restoration strategies, therefore, has direct economic benefits to Canadian taxpayers.

The terms remediation, restoration, reclamation and rehabilitation have often been used interchangeably or are unclearly defined in literature and government reports (Lima et al., 2016). As the study site for this project is in the Northwest Territories and is under the jurisdiction of Indigenous and Northern Affairs Canada (INAC), the guidelines referred to and definitions used throughout this thesis are based on what has been published in the INAC, and the Mackenzie Valley Land and Water Board's (MVLWB) Guidelines for the Closure and Reclamation of Advanced Mineral Exploration and Mine Sites in the Northwest Territories (2013), hereafter referred to as the Guidelines. In this document reclamation is defined as, "the process of returning a disturbed site to its natural state or preparing it for other productive uses that prevent or minimize any adverse effects on the environment or threats to human health and safety". Remediation is defined as "the removal, reduction, or neutralization of substances, wastes, or hazardous material from a site in order to prevent or minimize any adverse effects on the environment and public safety now or in the future" (MVLWB & AANDC, 2013).

A primary objective stipulated in the Guidelines is that a community of native vegetation should be established in areas where the natural vegetation has been destroyed by mining

activities, and that this community should be self-sustaining, requiring no future maintenance (MVLWB & AANDC, 2013). Underlying this goal is the recognition that plants form the foundation of most terrestrial ecosystems and that greater plant diversity can lead to greater diversity and abundance at higher trophic levels (Balvanera et al., 2006). It is also recommended in the Guidelines that bioengineering approaches, such as the use of vegetation to reduce erosion rather than erosion control mats, be considered to improve the success of mine closure efforts (MVLWB & AANDC, 2013).

Another important factor in remediation is that project components be measureable and achievable to ensure the project meets the standards of the governing body, whether it is provincial, territorial or federal (MVLWB & AANDC, 2013). Measures of success can be obtained by recording the number of non-native species, identifying the plant species present to calculate plant diversity, assessing the growth of individual plants and recording plant survival (Polster, 2011). Another area of consideration in monitoring is if and how the re-vegetated site is being used by wildlife to determine if a viable wildlife habitat has been created. It is further recommended that the soil physical properties including pH, water and nutrients also be included as indicators of site health (MVLWB & AANDC, 2013). Although these guidelines have monitoring considerations that include above and below ground components, the focus on living organisms has historically been restricted to the above ground communities with little attention to belowground processes (MVLWB & AANDC, 2013).

1.4 Arbuscular mycorrhizal fungi

Mycorrhizal fungi are a group of endophytic fungi that form a symbiotic relationship with vascular plants. Mycorrhizal fungi have been found to form associations with approximately eighty percent of vascular plants surveyed (Jeffries et al., 2003) and are found in

most soils (Mosse, 1981). Plant roots are the point of interaction between mycorrhizal fungi and their plant hosts and although many fungal taxa can form mycorrhizal associations, the most common type of mycorrhizal association found involves arbuscular mycorrhizal fungi (AMF) (Kariya & Toth, 1981). Arbuscular mycorrhizal fungal structures include hyphae, arbuscules and, in some species, vesicles (Figure 1.1). While hyphae and vesicles can be found in other types of mycorrhiza, AMF are distinguished by the presence of arbuscules; highly branched structures that form inside the cortical cells of the host plant roots and are the site where the exchange of resources occurs. Plants primarily supply photosynthates to the fungus in return for phosphorous, and in some cases nitrogen and water (Peterson & Farquhar, 1994). Hyphae may be found inside the root (intraradical hyphae) or outside the root (extraradical), forming networks within the host root as well as extending beyond the roots, expanding the volume of soil from which plants can acquire nutrients. Vesicles are formed within the host root and function in lipid storage and as sources of propagules (Smith & Read 2008)

The association between plants and AMF begins when fungal hyphae are attracted to the surface of the host root. Signalling molecules, such as strigolactones, are released from plant roots and attract fungal hyphae in the soil (López-Ráez et al., 2011). At the point of contact, an hyphopodium forms, and hyphae enter the cortex of the roots, travelling either within or between the cortical cells (Peterson & Farquhar, 1994). Once an association between AMF and a plant host is formed, surrounding plants may be colonized by the extraradical hyphae or through spores produced by the fungus. These spores may lie dormant in the soil and germinate in response to plant signaling molecules (Abbott & Robson, 1991). Mycorrhizal associations are an important component in the successful establishment of new plant communities as this relationship aids in plant nutrient and water uptake, two essential processes in community

establishment (Eom et al., 2000; Jeffries et al., 2003). Given how important mycorrhizal fungi are, both the mycorrhizal and plant community should be considered, together with the interaction between the two when undertaking a reclamation project (Orlowska et al., 2011).

1.5 Effects of mining on plants and mycorrhizal fungi

Often during mining activities, the topsoil is stripped to access the underlying rock resulting in the loss of vegetation and the fertile organic layer (Dudka & Adriano, 1997). Along with the loss of the existing plant community, this removal can disrupt soil microorganisms such as the bacterial and AMF communities (Vergeer et al., 2006). This results in challenges in re-vegetation such as establishing plants on inhospitable substrates and erosion occurring without plant roots to secure the soil. It is well established that soil disturbance can have negative impacts on AMF and the plants they colonize. McGonigle and Miller (1996) observed negative effects of disturbance in *Zea mays* colonized with AMF. Soil disturbance led to a significant decrease in arbuscular and hyphal colonization in host roots, as well as a 50% decrease in shoot mass compared to plants grown in undisturbed soils (McGonigle & Miller, 1996).

As well as impacts on AMF colonization, disturbance can impact spores. Trejo et al. (2016) sampled three sites with increasing amounts of disturbance caused by the removal of forest cover, and transition to agricultural use. Trap plants (*Zea mays*) grown in soil collected from sites with the least forest cover removed (least disturbed site) had significantly higher root colonization than plants grown in soil from the sites with greater disturbance. As well, higher spore viability was observed in the least impacted soils, as determined by vital staining. Though there were no significant differences in spore abundance between sites, there were differences in the viability and ability of AMF to colonize depending on the amount of disturbance. This

indicates that, though spores may still be abundant after soil disturbance, disturbance can negatively affect how AMF function.

In a study by Manoharan et al. (2017), soil was sampled from twenty-five farms representing four different agricultural practices (one organic and three conventionally managed with different crops) and permanent pasture grasslands to determine if there were differences in AMF composition and diversity depending on land use, and if changes in AMF community impacted plant growth. There was a significant effect of land use on AMF diversity; soils sampled from permanent pasture had significantly greater AMF diversity than the three tilled farm soils. AMF community composition was also significantly affected by land use; the AMF community in soils from permanent pasture was significantly different than those of conventional farms. AMF community composition and diversity were significantly correlated with barley (*Hordeum vulgare* L.) growth; AMF composition and diversity of permanent pasture was strongly correlated with high barley grain biomass, shoot phosphorous concentration and nitrogen in the shoots and grains. These authors demonstrated that intensive soil management in agriculture, such as tillage, reduced AMF diversity in the soils and influenced AMF community composition, leading to reduced nutrient uptake in crop plants.

The effects of disturbance on AMF has been noted to show taxon-specific effects. Twenty-one isolates from the AMF suborders Glomineae and Gigasporineae were surveyed by Hart and Reader (2004) to determine the effects of disrupted extra-radical mycelium on host plants including two *Plantago* species and two *Poa* species. These AMF suborders differ in their colonization methods; Gigasporineae colonize through spores whereas Glomineae colonize through living mycelium (Biermann & Lindermann, 1983). Because spores are more resistant to environmental disturbances than hyphae, it was predicted that isolates from the Glomineae

would be more greatly affected by soil disturbance than isolates from Gigasporineae. Although the two isolates differ in their resilience to disturbance, there was a significant decrease in root colonization in both suborders compared to colonization of roots growing in non-disturbed soils. This decrease was, however, greater in the Glomineae isolates. The lower resilience to disturbance in Glomineae isolates also translated into lower biomass in host plants. Plants colonized by Gigasporineae isolates were less severely impacted than plants colonized by Glomineae isolates; only 10% of plants colonized by Gigasporineae isolates exhibited a reduced biomass compared to controls, while 31% of plants colonized by Glomineae isolates showed a reduction in biomass. These results indicate that not only are AMF negatively impacted by soil disturbance, but that these effects also impact vegetation colonized by the AMF. Furthermore, the magnitude of the effect on the fungi differs among fungal taxa.

There is also evidence that a lack of AMF propagules can hinder plant establishment. Cuenca et al. (1998) used outdoor plots with different soil amendments including a control, AMF inoculation, fertilizer and a combination of AMF and fertilizer. Plots were established in a degraded area that had been impacted by transportation infrastructure. Due to a lack of seeds of native vegetation, plots were seeded with the introduced grass, *Bracharia decumbens,* at a rate of 30 kg/ha in each plot. Plants were grown in the field and harvested after five months. Plants growing in plots that received AMF and fertilizer had significantly higher root biomass, root length and shoot percent cover than all the other treatments and plots without any soil amendments had very little grass cover, indicating AMF played an important role in establishing plant cover.

It has been demonstrated that inoculation with AMF improves the success of grassland restoration, and that the use of a mixture of AMF over single species inoculum provides a greater

improvement in plant survival and growth, as well as having benefits relating to plant community composition. To restore a grassland ecosystem, Koziol et al. (2017) seeded plots with native grasses and planted plugs inoculated with AMF, called nurse plants, to introduce AMF into the restoration. These nurse plants were inoculated with an AMF mixture, a single species, or were not inoculated (control). Inoculated nurse plants had significantly higher survival 1 and 2 years after planting, grew larger, and accumulated more leaves than non-inoculated nurse plants. Plots that received nurse plants inoculated with a mixture of AMF recruited desirable, native prairie plants, while plots that received non-inoculated nurse plants or nurse plants inoculated with a single AMF species recruited a higher number of weeds and exotic species. These results indicate that reintroduction of AMF helped shape the resulting plant communities, and a mixture of AMF species may be needed for recruitment of desirable native species.

In addition to physical disturbance to soils, mining activities can also result in contaminated soils. A common contaminant at mine sites are PCHs, specifically diesel fuel due to accidental spills on site (EBA Environmental Engineering, 2001; INAC, 2018; INAC & GNWT, 2010; MVLWB, 2017). Spills are generally not contained at the soil surface but penetrate through the soil profile at rates dependent upon soil physical and chemical properties (CCME, 2008). Diesel- contaminated soils can be directly and indirectly toxic to plants as fuel oil can create hydrophobic and anaerobic conditions around plant roots (Racine, 1994) limiting their access to water and oxygen. Diesel- contaminated soils have been shown to limit or inhibit seed germination and cause decreased plant biomass compared to plants grown in clean soils. Adam and Duncan (1999) surveyed the effect of two diesel fuel concentrations on the germination of 22 plant species, including grasses, herbs, legumes and crop plants. At the lower diesel concentration (25,000 mg/kg), reduced germination was observed in 13 of the species

compared to controls. At the higher concentration (50,000 mg/kg), 19 of the species had reduced germination, and germination was completely inhibited in two species.

Although there are species capable of growing in contaminated soils, their growth is often inhibited compared to that of plants grown in uncontaminated soils. Perennial ryegrass (*Lolium perenne*) growing in soils artificially contaminated with 12,000 mg/kg commercial diesel fuel was observed to have significantly lower shoot mass than plants grown in uncontaminated soil. (Barrutia et al., 2011). Reductions in growth were noted two months after treatment application and were evident five months after diesel exposure (Barrutia et al., 2011). The effects of diesel on a grass community was tested in a study by Palmroth et al. (2002), who grew a grass seed mix in artificially spiked soils (5,000 mg/kg). After growing for 180 days in spiked soil the total mass of the three species taken together (*Festuca rubra, Poa pratensis* and *Lolium perenne*) was 43% of that of control biomass.

PHC contamination has also been shown to have negative effects on mycorrhizal fungi leading to reduced spore germination and infectivity (Franco-Ramírez et al, 2007; Cabello, 1997). In a study by Franco-Ramírez et al. (2007), spore germination of *Glomus ambisporum* was reduced by 30% on a water- agar surface contaminated with light crude oil, and spores that did germinate had inhibited hyphal growth compared to controls. Results from a field study conducted by Cabello (1997) showed that soil collected from contaminated sites contained spores but spore density was lower than that of clean sites. Plants collected from the same PHC contaminated soils also had lower fungal colonization than that of plants from non-contaminated soils. Even though spore density was low in contaminated soils, there was still an average of 226 to 340 spores per 100 g of polluted soils. The presence of spores and low colonization suggests that the fungi were no longer able to colonize roots due to contact with contamination. The lack

of viable AMF propagules can lead to the reduced growth of mycorrhizal- dependent vegetation and limit the number of species that can successfully establish in a re-vegetated site (Wurst et al., 2011). Furthermore, the inhibition of both plant and mycorrhizal fungal growth in the presence of PHC contamination can have negative consequences for re-vegetation projects.

1.6 The role of plants and mycorrhizal fungi in reclamation and remediation

The ultimate goal for the reclamation and remediation of mine sites is to return the site to a state that is as close as possible to its condition prior to the onset of mining operations. A significant step in achieving this goal is establishing a self- sustaining plant community (MVLWB & AANDC, 2013). Though the establishment of a pre-mining plant community can be considered a measure of success, the establishment of such a community may take time and the stages leading up to the establishment of the pre-mining community may be important in site reclamation. Plants provide important ecological functions like nutrient cycling and soil stabilization (Faucon et al., 2017) which help create an hospitable environment for new plants to establish. Plants also contribute to the reclamation of disturbed sites by hosting root- associated organisms like mycorrhizal fungi and rhizospheric bacteria that modify soils and breakdown or immobilize soil contaminants (Umar et al., 2016; Megharaj & Naidu, 2017).

The role of AMF in reclamation efforts has been gaining recognition. AMF have been shown to aid in plant community establishment (Eom et al., 2000; Jeffries et al. 2003) which is important as many impacted sites have been stripped of vegetation and a new community must be established. There is also evidence that establishment of sustainable plant communities during reclamation requires AMF (i.e. Cuenca et al., 1998). Middleton et al. (2015) performed grassland restoration using inoculated plugs to introduce AMF. Experimental plots were planted with

inoculated nurse plants, which included four species, and seeded with a prairie seed mix. The inoculation treatments included native AMF, a commercial inoculum and non-inoculated control. They observed a higher survival rate in three of the four nurse plants inoculated with native AMF than in plants inoculated with commercial inoculum; survival of the fourth species did not significantly differ from those inoculated with commercial inoculum. Similar results were observed in nurse plant growth; both inoculation treatments resulted in larger plants compared to controls, but two of the four nurse plant species grew larger when inoculated with native compared to commercial inoculum. Though commercial inoculum improves plant growth and survival, native inoculum is more beneficial to restoration than commercial. Given the improvements in plant performance in these plots, restoring the AMF community is a necessary component during restoration.

AMF have been shown to contribute to the removal of contaminants from soil and increase plant growth under stressful conditions such as contamination (Joner & Leyval et al., 2003; Tang et al., 2009). Furthermore, they contribute to establishing a rhizosphere environment that supports petroleum degrading microorganisms (Alarcón et al., 2008). Joner and Leyval (2003) found that polycyclic aromatic hydrocarbon (PAH) dissipation was higher around roots with AMF associations than those without, suggesting soil remediation may occur quicker if plants are inoculated with AMF.

A protective effect of AMF on plants growing in contaminated soils has been observed in *Zea mays* grown in diesel- contaminated soils (0, 2,000, 6,000, 10,000, 15,000 mg/kg). Although there were no differences in plant performance due to AMF inoculation at the lowest concentrations of diesel fuel (0 and 2,000 mg/kg), significant differences between AMF treatments were seen at the higher concentrations (6,000, 10,000, 15,000 mg/kg). Total biomass

of *Z. mays* was significantly greater in inoculated plants growing in diesel concentrations of 6,000 mg/kg and higher than that of non-inoculated plants (Tang et al., 2009). *Lolium multiflorum* was grown in crude oil contaminated soils (6,000 mg/kg) and while a protective effect was not observed, there was higher hydrocarbon degradation in treatments inoculated with AMF (Alarcón et al., 2008). Furthermore, treatments with both AMF inoculation of *Rhizophagus irregularis* and microbial addition (*Sphingomonas paucimobilis* and *Cunninghamella echinulata* var *elegans*) showed a 59% reduction in TPH which was significantly higher than degradation with the microbial addition alone (46%; Alarcon et al., 2008).

1.7 Study rationale

Though the mining industry has had a positive impact on Canada's economy, it has also led to the degradation of natural environments. Contamination of soils in, and surrounding mine sites will likely continue as new mines are opened and closed in Canada and effective remediation and reclamation will be needed. Recognizing a need to guide closure and remediation efforts, Indigenous and Northern Development Canada (INAC[1]) and the Mackenzie Valley Land and Water Board published "Guidelines for the Closure and Reclamation of Advanced Mineral Exploration and Mine Sites in the Northwest Territories" (MVLWB & AANDC, 2013). Explicit in the guidelines is the recommendation that reclamation research be conducted to help shape reclamation plans due to a lack of research applicable to northern sites that considers northern vegetation and the use of other living organisms to enhance re-vegetation efforts.

Previous studies have looked at the effect of PHCs on plants and AMF (Figure 1.2) but no studies have looked explicitly at the effects of diesel fuel on northern plants, or the effect on

[1] Previously called Aboriginal Affairs and Northern Development Canada (AANDC)

the association between northern plants and AMF. Of the studies conducted thus far, the reported effects of PHCs on plants and AMF vary greatly. In some cases, AMF were not negatively impacted by contaminated soils. Hernández-Ortega (2012) reported that colonization of clover roots was unaffected when soils were spiked with 7,500 mg/kg diesel fuel. Spore germination, hyphal growth and colonization of transformed carrot roots remained unaffected in diesel concentrations up to 3% (v/w) (Kirk et al., 2005). This contrasts with significant decreases in colonization that have been observed in corn plants grown in 10,000 mg/kg (Tang et al., 2009). Effects on plants are equally inconclusive. Barrutia et al. (2011) observed decreased dry weight in plants grown in 12,000 mg/kg, while Palmroth et al. (2002) observed reduced biomass in plants grown in only 5,000 mg/kg diesel fuel. Together, these results suggest that site and/or species specific approaches are necessary to assess PHC impacts at a given area.

1.8 Objectives and hypotheses

The variation of results in previous research and the need for research with a northern focus led to the research question: how are northern vegetation and mycorrhizal fungi impacted by diesel fuel? To answer this question, my research objectives were to (1) assess whether the current plant and mycorrhizal communities at a northern site with historic contamination show effects of PHC exposure by quantifying plant community structure and mycorrhizal colonization (2) quantify the effects of diesel contamination on the growth of northern vegetation under controlled conditions and (3) assess whether an association with AMF conveys a protective effect on plants grown in soils impacted with diesel fuel. For the first objective, it was hypothesized that if there are any negative effects from residual PHC contamination these would be observed by differences in plant community structure and mycorrhizal colonization that

followed a gradient in PHC exposure. For the second objective, decreases in plant performance were hypothesized between controls (0 mg/kg) and the three levels of diesel contamination. At the highest concentration chosen (10,000 mg/kg) there is strong evidence for this in the literature and it is much higher than both Colomac site specific targets and CCME guideline concentrations. Though the other two concentrations chosen for this study are based on environmental guidelines, results from the studies by Palmroth et al. (2002), Tang et al. (2009) and Driai et al. (2015) suggest these levels may be high enough to cause negative effects on plant growth. For the third objective, it was hypothesized that AMF would have a protective effect on plants grown in contaminated soils. To observe this effect, two species reported to form mycorrhizal associations (*Elymus trachycaulus* and *Erigeron acris*; Zak & Parkinson, 1982; Betekhtina & Veselkin, 2011) and one species reported to be unable to form mycorrhizal associations (*Carex aquatilis*; Thormann et al., 1999; Wang & Qui, 2006) were grown in artificially contaminated soil with and without mycorrhizal inoculum and plant performance was compared between treatments. If there was a protective effect, it would only be observed in the mycorrhizal plant species, as *C. aquatilis* would not benefit from an association with AMF.

1.9 References

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1.10 Figures

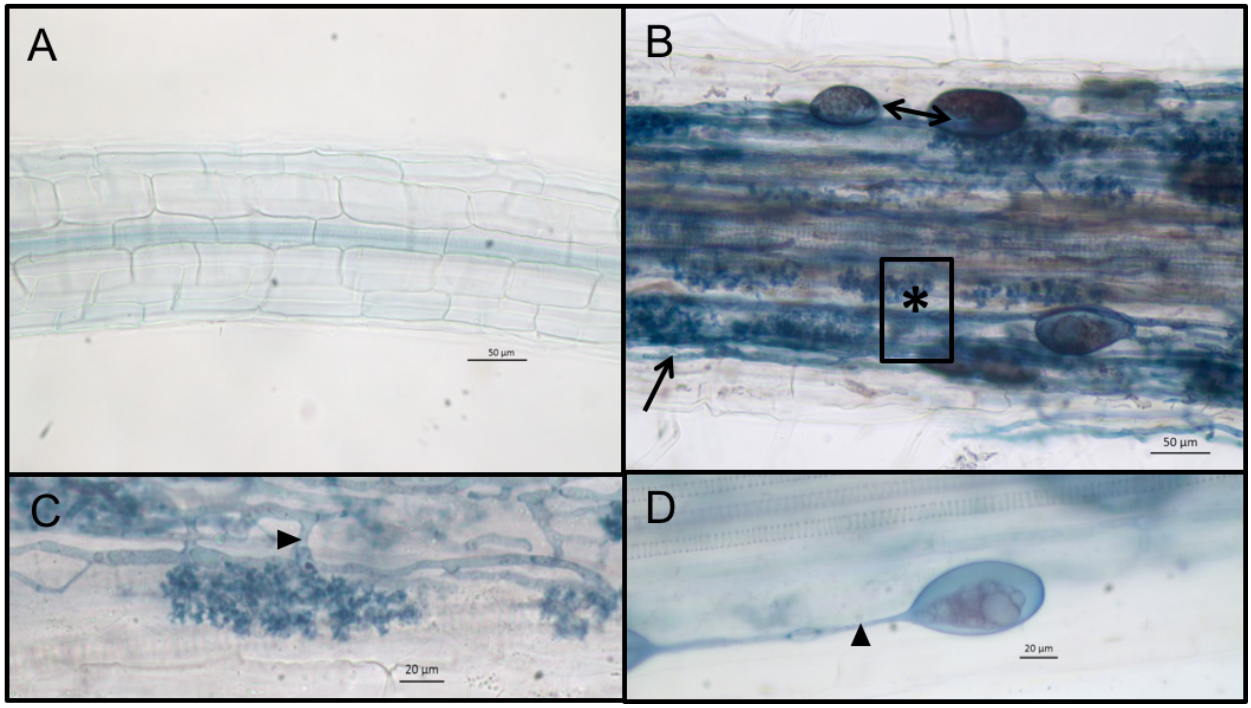
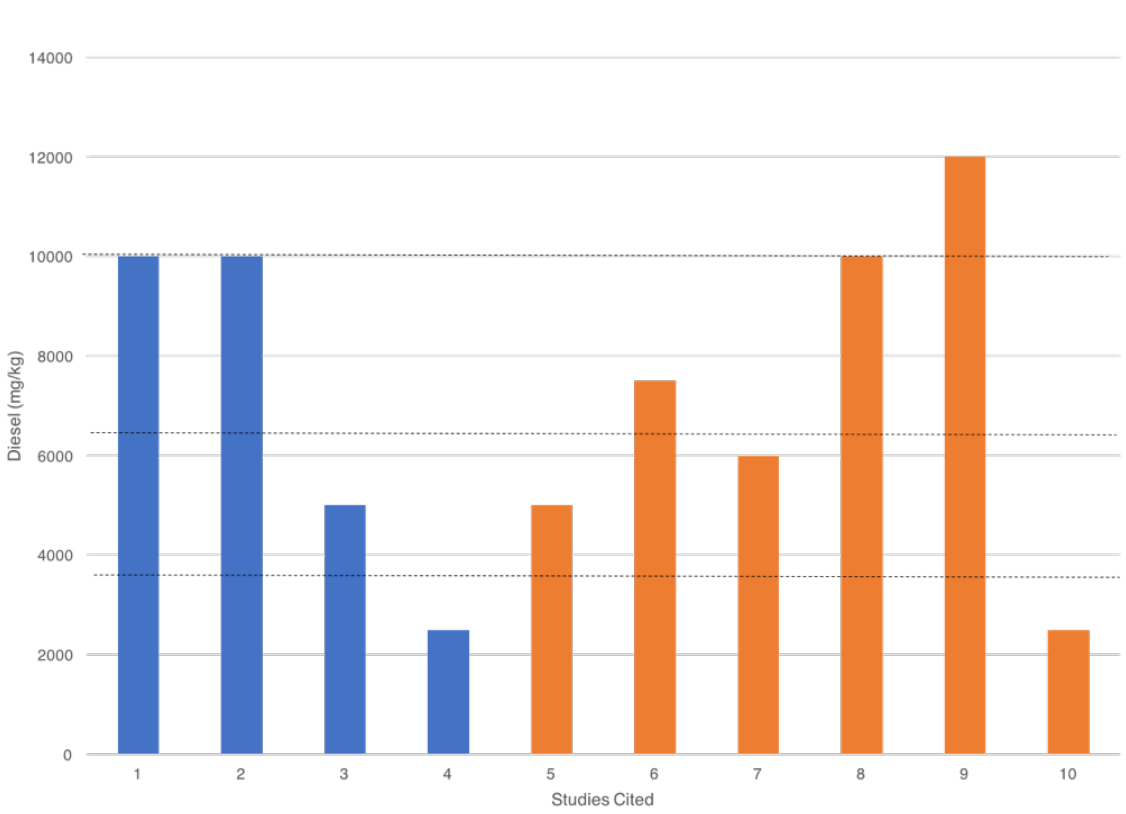


Figure 1.1 Arbuscular mycorrhizal fungi within *Phalaris arundinacea* roots. Roots were cleared in 10% KOH and stained with a 5% ink and vinegar solution (household vinegar containing 5% acetic acid). A is a section of non-colonized root; B-D show arbuscular mycorrhizal fungal structures.
A: An non-colonized section of a *Phalaris arundinacea* root.
B: A *Phalaris arundinacea* root colonized with AMF structures arbuscules (asterisk), vesicles (double arrow) and hyphae (single arrow)
C: An arbuscule with subtending hyphae (arrow head) within a *Phalaris arundinacea* root.
D: A single vesicle with subtending hyphae (arrow head) within a *Phalaris arundinacea* root.



Arbuscular Mycorrhizal Fungi

1) Glomus constrictum; reduced colonization in maize (*Zea mays*) roots (Tang et al., 2009)

2) AMF species unknown; decreased colonization in cordgrass (*Spartina argentinensis)* roots (Redondo-Gomez et al., 2014)

3) Glomus intraradices; delayed hyphal elongation (Kirk et al., 2005)

4) Rhizophagus iregularis; reduced spore germination, hyphal length and colonization in chicory root culture (*Cichorium intybus* L.) (Driai et al., 2015)

Plants

5) Grass seed mix (Red fescue (*Festuca rubra*), Bluegrass (*Poa pratensis*), Perennial ryegrass *(Lolium perenne*)); reduced total biomass (Palmroth et al., 2002)

6) Sweet clover (*Melilotus albus*); reduced total biomass (Hernandez-Ortega et al., 2012)

7) Maize (Zea mays); reduced total biomass (Tang et al., 2009)

8) Cordgrass (*Spartina argentinensis*); reduced total biomass (Redondo-Gomez et al., 2014)

9) White clover (*Trifolium repens*); reduced shoot and root dry weight, Perennial ryegrass (*Lolium perenne*); reduced shoot dry weight (Burrutia et al., 2011)

10) Chicory root culture (Cichorium untybus L.); reduced root length and weight (Driai et al., 2015)

Figure 1.2 An overview of previous studies that have tested the effect of petroleum hydrocarbons on plants and mycorrhizal fungi; the bars indicate the lowest observable effect concentration in each study. The legend on the right lists the plant or fungal species used, the response measured and a citation and the dashed lines on the graph indicate the concentrations used in this study. The lowest dashed line corresponds to a Canadian Council of Ministers of the Environment (CCME) guidelines for PHCs in soil, 3,460 mg/kg. The dashed line in the middle of the graph corresponds to a site-specific guideline (6,500 mg/kg) at the Colomac mine in the Northwest Territories, the site sampled in the field study component of this thesis. The highest line corresponds to the highest concentration (10,000 mg/kg) used in the greenhouse portion of this study; it was chosen as plants have been observed to survive this concentration, but also show effects of PHC exposure at this concentration.

Chapter 2: Field study at Steeves Lake shoreline, Colomac Mine, Northwest Territories

2.1 Abstract

Previous research has indicated that arbuscular mycorrhizal fungi are important in the re-vegetation component of reclamation projects, though little research has focused specifically on northern plants and associated mycorrhizal fungi, and their response to contamination. A field study was performed at a northern site, Steeves Lake shoreline, that has been re-vegetated and has been affected by historic contamination. Vegetation surveys, root collection and soil sampling were conducted to assess (1) the northern vegetation establishing naturally at the site (2) whether arbuscular mycorrhizal fungi had established within the community; and (3) whether trends in vegetation or mycorrhizal colonization indicate negative affects due to historic contamination. It was hypothesized that differences in plant community and mycorrhizal colonization would be observed between samples closest, and furthest away from the area of the shoreline overlaying contaminated sediments. The vegetation surveys revealed the presence of five and six new plant colonizers over two years of sampling respectively, indicating that there are plants colonizing the site naturally. Mycorrhizal colonization was observed in a subsample of species sampled at the site. Hyphal colonization in *Elymus trachycaulus* roots was similar between sampling years, mean hyphal colonization was 47.3±3.8% and 49.3±2.7% in the first and second year respectively. *Festuca ovina* and *Hordeum jubatum* were collected in the second sampling year; mean hyphal colonization of these species was higher than that of *Elymus trachycaulus*; 64.1±2.1% and 56.6. ±2.7% respectively. The results from the vegetation survey did not suggest that there had been strong negative effects on the plant and mycorrhizal community from residual contamination; differences in vegetation cover and mycorrhizal

colonization was not strongly associated with petroleum hydrocarbons concentrations in the soil. The results of this field study suggest that Steeves Lake shoreline is being colonized by native vegetation and that arbuscular mycorrhizal fungi have established at the site and are actively colonizing host plants. Trends in vegetation and mycorrhizal colonization indicate that, though there were hydrocarbons identified in the soil, there were no detectable effects from this contamination.

2.2 Introduction

The mining industry in Canada is expected to grow in the coming decade, and it has been suggested that much of that growth will occur in remote northern areas (Rhéaume & Caron-Vuotari, 2013; Marshall, 2016). Mining activities can cause the destruction of vegetation and animal habitat, and compacted and contaminated soils (Jansen, 1981; McSweeney & Jansen 1984; Leppänen et al. 2017), leading to challenges in the remediation and reclamation of mine sites. Though many environmental disturbances can arise from mining activities, the release of petroleum hydrocarbons (PHCs) is common at many mine sites, especially at remote sites where it is necessary to store large quantities of fuel (Standing Senate Committee on Energy, 2015).

The end goal of a restoration project is the establishment of a native plant community (MVLWB & AANDC, 2013), and while this goal may be hindered due to the impacts of mining, plant establishment can also ameliorate these effects. Restoration often targets physical soil properties such as erosion, and the water holding capacity of soils which have been affected by mining activities (Prach & Tolvanen, 2016). The establishment of a plant community is needed to restore these targeted ecosystem properties, and form the foundation of restored ecosystems (Balvanera et al., 2006). Plant roots stabilize soil, controlling erosion, and cycle nutrients in soils that have been degraded due to mining activities (Bradshaw, 1997; Shukla et al., 2004; Faucon et al., 2017). An obstacle in reaching restoration goals is the presence of contaminated soils at a site. Petroleum hydrocarbon contamination due to accidental releases during mining activities can reduce seed germination (Adam & Duncan, 1999) and limit plant growth (Barrutia et al., 2011) resulting in a decrease in plant biomass at a community level (Palmroth et al., 2002).

There are known impacts of contaminated soils on vegetation, but results from previous studies vary (Palmroth et al., 2002; Tang et al., 2009; Hernández-Ortega et al., 2012). There is

also evidence that plant species exhibit different tolerances or sensitivities to PHC-contaminated soils. Kulakow et al. (2000) grew 29 different plant species in contaminated sediments with a total petroleum hydrocarbon concentration of 18,119 mg/kg collected from an oil refinery. The plant species tested included 26 grass species and 3 legumes. Tolerance was determined by comparing growth parameters such as plant height, biomass, root diameter and root density of plants grown in contaminated soils to non-contaminated controls. There was a large variation in growth parameters within plant species; barley was determined to be the least tolerant to contamination, and three wild rye species were the most tolerant. These results suggest that large variations in tolerance to PHC contamination could exist within a plant community.

Arbuscular mycorrhizal fungi (AMF) are symbiotic fungi that form associations with host plants (Kariya & Toth, 1981) and it has been demonstrated that AMF are an important component in plant community restoration. During surface mining, the nutrient rich organic layer is lost, resulting in nutrient poor soil (Dudka & Adriano, 1997); and when topsoil is re-spread it often becomes compacted, making root penetration in the soil difficult and limiting the water holding capacity of soils (Thompson et al., 1987; Shukla et al., 2004). An association with AMF can help overcome these challenges in re-vegetation; plants associated with AMF have increased access to water and nutrients in the soil due to a hyphal network that extends out from roots into the bulk soil (Smith & Read, 2008). Maltz & Treseder (2015) conducted a meta-analysis of published restoration studies that looked at the effects of mycorrhizal inoculation in restoration projects by examining the percent of root length colonization and plant growth after inoculation under field conditions. The authors surveyed 28 field based studies, 24 of which included inoculation with AMF and 4 that utilized ectomycorrhizal fungi (ECM). These studies were chosen based on the criteria that they included active restoration of a degraded ecosystem, an

inoculated treatment and a non-inoculated control, a measurement of the percent root length colonized and measurements of plant growth. They found that, in general, by inoculating field plots with mycorrhizal fungi, the percent root length colonized in field roots sampled was consistently higher than in non-inoculated plots. They also found that a significant improvement in plant growth was accompanied by an increase in percent root length colonized, demonstrating the strong relationship between AMF inoculation and successful re-vegetation.

Like plant establishment, AMF establishment can aid in the restoration of ecosystem services, but AMF are also negatively affected by PHC contamination in soils. These effects have been noted at several stages in the colonization process. Reduced spore germination and hyphal growth has been observed in water-agar contaminated with crude oil (Franco-Ramírez et al., 2007). Furthermore, reduced colonization has been noted in the roots of plants grown in diesel contaminated soils (Tang et al., 2009; Redondo-Gómez et al., 2014).

Although it has been demonstrated that AMF play an important role in ecosystem restoration, and are also negatively affected by contamination, there is little research applying AMF to restoration projects in the north, and an absence of studies assessing the response of AMF native to northern ecosystems to PHC contamination. In the meta-analysis by Maltz and Treseder (2015) summarized above, none of the studies surveyed were conducted north of 49°; Nunavut and the two territories begin at 60° north. Though the results of these summarized studies are useful in guiding reclamation strategies, results from southern studies don't necessarily apply to northern ecosystems. The lengths of these restoration studies ranged from 4 to 54 months and on average ran for about 15 months. In the north, the growing season, defined as the number of days mean temperature is at or above 5°C (Ecosystems Classifications Group, 2008), is only about 5 months (ENR, 2018). There is limited time for the restoration of

vegetation in northern locations and temperatures may still fall below 0°C in the first month of the growing season (ENR, 2018). Also, because most studies are conducted in more southern locations, the vegetation and AMF are not representative of the native plants and microorganisms in the north, and it is difficult to predict if northern species will respond in the same way. It has been explicitly stated in the Guidelines that research applicable to the north be conducted to fill these gaps in our knowledge of northern site restoration (MVLWB & AANDC, 2013).

To investigate the role of AMF in northern restoration projects, and the effects of residual PHC contamination on northern plants and AMF, a field study was conducted at Steeves Lake shoreline, an area at a northern reclaimed site, Colomac mine. To improve our understanding of restoration in northern ecosystems the following questions were asked: 1) Which plants have naturally colonized a restored northern site? 2) Have AMF established at this site? and 3) Is there an effect of historic contamination on the plant and mycorrhizal community? Two site visits to Colomac Mine were conducted as part of a larger monitoring project by INAC. The vegetation at Steeves Lake shoreline has been monitored yearly since the area was planted in 2010 to determine whether the reclamation of the shoreline could be considered a success. As part of this ongoing monitoring, colonization by arbuscular mycorrhizal fungi was assessed in root samples from the shoreline beginning in the summer of 2015. It is expected that if there are effects from residual contamination, quadrats closest to the original shoreline (quadrats overlying contaminated sediments) may exhibit reduced vegetation cover, larger amounts of bare ground, or species more tolerant to PHC contamination, compared to quadrats furthest from the original shoreline. Mycorrhizal colonization is expected to follow the same trend, root samples collected from quadrats closest to the original shoreline will have lower mycorrhizal colonization compared to samples collected further away.

Understanding more about restoration projects in the north such as native colonizers, prevalence of AMF and impacts from historic contamination will add to the body of reclamation research in the north, and benefit future reclamation projects.

2.3 Methods

2.3.1 Site Description

Colomac mine is an inactive gold mine in the Northwest Territories, 220 km northwest of Yellowknife (Figure 2.1). The mine property is in the Taiga Shield Low Subarctic Ecoregion which is characterized by short cool summers and cold winters with mean annual temperatures ranging from -3.5 ºC to -9.0 ºC. The mean annual precipitation in this region is between 230 and 350 mm, occurring almost equally as snow and rain. The dominant vegetation of this region consists of black spruce forests with lichen and shrub understories, though the area sampled in this study was dominated by native grasses and sedges (Ecosystems Classification Group, 2008). The original owners, Royal Oaks Mines, went bankrupt and abandoned the site, and this resulted in the responsibility for closure being transferred to the Canadian government. The Department of Indigenous and Northern Affairs Canada (INAC) was given the task of land management including ecological restoration (INAC, 2012). Closure and reclamation efforts at the site began in 1999 and were completed in 2010.

The mine property is bordered by two lakes, Baton and Steeves Lake. During operation, there were 23 reported releases of PHC products. These occurred at the main tank farm adjacent to Steeves Lake, as well as other areas of the mine including the mill and camp area. Each release varied in volume and type of product released. Volumes of two major diesel releases were 18,000 L in 1990 and 27,276 L in 1997, both of which were released within area of the tank farm (INAC, 2004). While there were immediate actions taken to contain these spills, near-shore

sediments in Steeves Lake became contaminated. To mitigate persisting PHC contamination, a new shoreline was constructed to contain and filter PHCs in the sediments. Construction and re-vegetation of the shoreline were started in 2010 and completed the same year (Aboriginal Engineering Limited (AEL), 2010; Figure 2.2).

The design of the shoreline included an infill area extending out from the original shoreline contained within an armoured wall. The constructed shoreline is 750 m in length, running north to south, and has an area of 10,000 m^2. Only on-site material such as waste rock and stockpiled soil, was used in construction; coarse-grained waste rock was used to build the shoreline and in the infill, layers of low permeability silt till and peat were used to cap the contaminated sediments. (AEL, 2010). Re-vegetation methods included transplants from the surrounding area as well as seeding with a native plant mix. Alder seeds (*Alnus viridis*), willow cuttings (*Salix* sp.) and water sedge plugs (*Carex aquatilis*) were collected on mine property for transplant; the grass seed mix contained violet wheatgrass (*Elymus violaceus*), Northern fescue (*Festuca saximontana*), annual rye (*Lolium multiflorum*) and glaucous bluegrass (*Poa glauca*) (Flat River Consulting, 2011). The re-vegetation of the shoreline and subsequent monitoring have focused on the above-ground plant community.

2.3.2 2015 Field Season

Sample sizes differed between sampling years due to the amount of time allowed at the site. Colomac Mine is a fly-in site so visits and time permitted at the site were limited by the availability of flights, flight schedules, and the availability of an INAC representative. Sampling in both years was designed to capture an hypothesized gradient in residual contamination, vegetation cover and mycorrhizal colonization (Figure 2.3). Transects began near the original shoreline, which is the area of the shoreline above the contaminated sediments, and ended at the

edge of the shoreline near the lake; the area of the shoreline furthest away from the underlying contaminated sediments.

In August of 2015 the shoreline was sampled over two days. The first objective at the site was a broad assessment of the shoreline to identify areas of possible hydrocarbon contamination. There was an area on the shoreline that appeared to contain surficial PHCs because the soil was darkened in that location and there was an odour suggestive of PHC contamination; low levels of PHC contamination were confirmed in soil samples from this area. Sampling was conducted using 5 transects radiating out from the location of the surficial PHCs, each transect containing 5 equally spaced 1 m x 1 m quadrats (Figure 2.4). In each quadrat, all plant species were identified using the species key by Porsild and Cody (1980). Percent cover of each species was estimated as well and the percent of bare ground in each quadrat. For each plant species, the wetland indicator status was determined through the USDA PLANTS Database (2017) and the mycorrhizal status through published literature. Plant species were categorized as mycorrhizal if there were studies reporting arbuscules, vesicles and hyphae observed in their roots. Plant species were categorized as non-mycorrhizal if studies reported their roots as un-colonized, or given an unknown status if the plant roots had not been observed for colonization in previous studies. Using the Flat River Consulting (2011) report of the shoreline re-vegetation the plants were classified as planted or unplanted, and given a secure or introduced status according to the Working Group on General Status of NWT Species (2016).

The most common species identified within the quadrats were willow (*Salix* sp.) water sedge (*Carex aquatilis*), common horsetail (*Equisetum arvense*), and wild rye (*Elymus trachycaulus*). Though *Salix* sp. was present in most of the quadrats (23 out of 25), there were often only a few individual plants within each quadrat, so to avoid overly disturbing the site *C.*

aquatilis, *E. arvense* and *E. trachycaulus* were chosen for root collection as individuals of these plants were more abundant within quadrats. The roots of two individual plants were collected from each quadrat if present; not all quadrats contained the species chosen for root collection. Entire plants were harvested and an attempt was made to obtain as much of the root system as possible, although pieces of the root system may have been broken off in the process. The roots were separated from the shoots, rinsed with tap water and preserved in 50% ethanol.

Two soil cores (15 cm deep by 8.5 cm wide) were taken from the center of each quadrat using a soil corer (Eijkelkamp Root Auger (Eijkelkamp The Netherlands), and placed in 23 x 11.5 cm Nasco Whirl-Paks ^{TM} (Fisher Scientific Company, Ottawa). These cores were frozen within 24 hours of collection until use in the greenhouse portion of this study where they were used to isolate fungal inoculum.

A third soil sample was taken using a smaller soil corer (10 cm x 35 mm) for a total petroleum hydrocarbon analysis at Taiga Labs in Yellowknife. These samples were placed quickly into glass jars provided by Taiga Labs and packed tightly to reduce head space and avoid the loss of any volatile compounds. Total petroleum hydrocarbons (TPH) were determined through the United States Environmental Protection Agency (US EPA) Method 8260B (1996) for BTEX (benzene, toluene, ethylbenzene and xylene) compounds and CCME Canada- Wide Standard for Petroleum Hydrocarbons in soil (2001) for fractions F1 to F4. In the determination of BTEX compounds, volatile compounds were introduced into a gas chromatograph with a capillary column using a purge and trap method. For the determination of the fractions F1 to F2, 5 g or more of each soil sample underwent extraction with methanol for the F1 (C6 to <C10) fraction, and with 50:50 hexane: acetone in a Soxhlet apparatus for fractions F2, F3 and F4

(>C10 to C50). Analysis was conducted via gas chromatography with a flame ionization detector.

2.3.3 2016 Field Season

In August 2016, the shoreline was sampled over two days. Sampling in the previous year had been limited to a small section of the shoreline, this second sampling was undertaken to include a greater area. Sampling was conducted using 8 transects, each with 4 evenly spaced 1 m x 1 m quadrat. The transects spanned the entire length of the shoreline, from the north to the south end (Figure 2.5). The vegetation survey and classification of plant species was performed as described in the 2015 sampling. Wild rye (*Elymus trachycaulus*), Northern fescue (*Festuca ovina*) and wild barley (*Hordeum jubatum*) were chosen for root collection, and collected and stored as described for the 2015 root samples.

For later use in the greenhouse portion of this study, plants that had gone to seed and were abundant at the site were chosen for seed collection. Seeds of water sedge (*Carex aquatilis*), fleabane (*Erigeron acris*), wild rye (*Elymus trachycaulus*), alder (*Alnus viridis*), wild barley (*Hordeum jubatum*) and dwarf fireweed (*Chamerion latifolium*) were collected from plants growing at the shoreline. Seeds were collected by hand, stored in paper bags, and kept refrigerated at 4 °C until needed.

Soil cores were collected as previously described, though the smaller cores taken for TPH analysis were sent to the Groundwater and Soil Remediation Laboratory at the University of Waterloo. Analysis was performed following a modified version of the CCME Canada –Wide Standard for Petroleum Hydrocarbons in soil (2001). This method used a methylene solvent extraction followed by gas chromatography with a flame ionization detector.

2.3.4 Analyses of collected materials

Quantification of mycorrhizal colonization

To visualize mycorrhizal colonization, roots were first removed from the 50% ethanol and thoroughly rinsed with deionized water. Clearing of the roots followed the method of Brundrett et al. (1996) and staining of the roots followed the method of Vierheilig et al. (1998). Modifications to these methods included the use of a vacuum oven (Thermo Scientific Lindberg Blue M) to heat the samples. To clear the contents of the roots, roots were submerged in a 10% KOH solution and placed in a vacuum oven at 90 °C under 25 inches Hg of pressure for 1 to 2 hours. Roots were then visually inspected under a dissecting microscope, placed back in the oven if they had not been cleared of their contents, and checked at 15 minute intervals. The roots were then rinsed with a 10% vinegar solution, and then submerged in a 5% ink (Sheaffer Black) in vinegar solution to stain the AMF structures within the roots. Roots in the staining solution were placed in a vacuum oven under the same conditions as those described in the clearing step. The roots were then rinsed in 5% vinegar and stored in 50% glycerol.

The root systems were cut into 2 cm length sections, 10 to 15 of which were randomly selected and mounted parallel to the long axis on 75x22 mm microscope slides (Fisherbrand^{TM}) with 50% glycerol and covered with a 50x22 mm cover slip (Fisherbrand^{TM}). Prepared slides were viewed using a Zeiss Jeneval microscope (Carl Zeiss Inc., Germany) under 200 x magnification and images taken with Zeiss Zen (blue edition) Imaging Software. To quantify AMF colonization a modified version of the magnified intersections method (McGonigle et al., 1990) was used. On each slide 100 fields of view were assessed, and percentage of hyphal, arbuscular and vesicular colonization was calculated.

Soil pH and Soil Moisture

To determine soil pH the method by Kalra (1995) was used. Subsamples were taken from each core, homogenized and dried for 48 hours at 40 °C in a drying oven. From these subsamples, 5 g were mixed with 20 mL of deionized water and mixed on a stir plate. This suspension was allowed to sit for 30 minute before taking a pH measurement with a pH meter (Accumet Basic AB 15 pH Meter). Readings were taken again 60 minutes later to ensure the pH had stabilized.

To determine percent soil moisture, approximately 40 g subsamples of the cores were used. After recording the fresh weight, the subsamples were placed in a drying oven (Heratherm^{TM}, ThermoFisher Scientific) at 40 °C and re-weighed daily until the dry weights stabilized and percent moisture was calculated. (Percent moisture = (fresh weight – dry weight)/ fresh weight) x 100).

2.3.5 Data analysis

Non-metric multi-dimensional scaling (NMDS), a multivariate non-parametric technique, was used to identify whether quadrat position (distance from the underlying contaminated sediments) contributed to differences in species composition and cover within quadrats. The NMDS model runs were based on Sorensen distances with no penalty on handling ties and performed using PC-ORD (Version 7, MJM Software Design). Two hundred and fifty runs with real data were made from a random starting point with 10 iterations to evaluate the stability of the model, based on a stability criterion of 0.000001. As the analysis is performed a stress value is assigned that acts as a goodness of fit for the analysis, this stress value is reduced as more axes are added to the model. As described by Peck (2010), the number of axes for the NMDS analysis is chosen when the addition of an axis reduces stress on the model by at least a value of 5 and has an accompanying Monte Carlo randomization test of $p < 0.05$. Percent cover in each quadrat

was used as the main matrix, soil TPH was overlaid as a secondary matrix when analysing the 2015 data, and soil TPH, percent moisture and soil pH overlaid when analysing the 2016 data.

To identify whether there was a relationship between mycorrhizal colonization and quadrat position, Spearman's rank correlations were performed (JMP Statistical Analysis Software version 11.0). Correlations between percent colonization and quadrat position were assessed within individual transects, as well as with the combined data from all transects. Quadrat number increased with increasing distance from contaminated sediments so a significant positive correlation between colonization and quadrat number would indicate agreement with hypothesized trends in colonization.

2.4 Results

2.4.1 2015 Vegetation survey

There were 10 plant species identified within the 25 quadrats and percent cover for each species was estimated within each quadrat (Table A 2.1). Of these species, six were not part of the original re-vegetation of the shoreline, and all are listed as secure by the Working Group on General Status of NWT species (2016). Nine of the 10 species have been reported in previous studies to form an association with mycorrhizal fungi, 8 with AMF and 1 with ectomycorrhizal fungi (ECM); *Carex aquatilis* was the only species identified that has been reported as non-mycorrhizal (Table 2.1), suggesting mycorrhizal fungi are an important component of this community. The determination of wetland indicator status revealed the presence of both wetland and upland plant species; though soil moisture was not determined in this sampling year, this indicates that there is a water gradient present across the shoreline.

2.4.2 Total petroleum hydrocarbon determination in 2015 soil samples

The analysis of the soil samples revealed the presence of benzene, toluene, ethylbenzene, xylenes, fraction 1 (C6 – C10), fraction 2 (C>10 – C16), fraction 3 (C>16 – C34), fraction 4 (C>34 – C50) and total petroleum hydrocarbons. In all the soil samples, total petroleum hydrocarbon concentrations were lower than those specified in the CCME and Colomac site specific- guidelines, ranging from 90 mg/kg to 414 mg/kg (Table A 2.2).

2.4.3 Trends in the 2015 vegetation survey

An initial NMDS model was run on autopilot to find an optimal number of axes that best represented the variation in the data. It was determined from the autopilot run that a two-dimensional NMDS ordination was optimal with final instability of 0.0, stress value (goodness of fit) of 7.9 and a Monte Carlo test result of 0.012. The model included 25 sites (quadrats) and 11 elements (10 plant species and percent bare ground) organized onto two axes that explained 55% and 41% of the variance respectively. Five groups are delineated on the biplot, representing the quadrats 1 through 5 combined from each transect (Figure 2.6). The groups containing quadrats 1 and 2 (quadrats closest to the original shoreline) overlapped with each other on the biplot, as did the groups containing quadrats 4 and 5 (quadrats furthest from the original shoreline). Quadrats 1 and 2 separated out from quadrats 4 and 5 along Axis 2 due to the presence of upland and wetland species. For example, Axis 2 was positively associated with *Carex aquatilis* and *Equisetum arvense* (τ = 0.854, τ = 0.558), species commonly found in hydric soils (soils wet enough in the upper layers to create anaerobic conditions during the growing season; USDA-NRCS, 2010), as denoted by their wetland indicator statuses. Axis 2 was also negatively associated with *Elymus trachycaulus* and *Poa glauca* (τ = -0.455, τ = -0.536), species associated with dry soil as denoted by their facultative upland status and with and bare ground (τ = -0.730) which is also associated with dry soils (Table 2.2).

It was hypothesized that soil contamination would be highest in quadrats 1 and 2 (quadrats closest to the original shoreline) due to underlying contaminated sediments. Correlations between TPH and the two axes did not support this hypothesis, TPH was not correlated with either axes, suggesting that TPH in the soil did not influence the separation of quadrats on the biplot (Table 2.2).

2.4.4 Trends in mycorrhizal colonization in the 2015 root subset

During the 2015 sampling the roots of 3 species were collected (*Carex aquatilis*, *Equisetum arvense* and *Elymus trachycaulus*). Mycorrhizal colonization was observed in all the *E. trachycaulus* root samples, though not in either *C. aquatilis* or *E. arvense* roots (Table A 2.3). Arbuscules, vesicles and hyphae were observed in *E. trachycaulus* roots (Figure 2.7). Among quadrats, mean arbuscular colonization was 3.9±0.7%, mean vesicular 12±1.7% and mean hyphal colonization was 47.3±3.8% (Table 2.3).

It was expected that colonization would be lower in quadrats closer to the original shoreline (quadrats 1 and 2), and higher in quadrats further away from the original shoreline (quadrats 4 and 5) due to a negative influence of underlying contaminated sediments. If percent colonization had followed hypothesized trends, positive correlations between colonization and quadrat position would have been observed. When colonization data from the five transects were combined, there were no significant correlations between quadrat position and arbuscular, vesicular or hyphal colonization (Table 2.4), indicating that underlying contamination has not impacted mycorrhizal colonization of *E. trachycaulus*. This is also supported by the lack of a significant correlation between TPH in the soil and arbuscular, vesicular or hyphal colonization. A significant, negative correlation would have been observed if TPH in the soil had negatively impacted mycorrhizal colonization (Table 2.4).

When *E. trachycaulus* colonization data from individual transects were analyzed, there were significant, positive correlations with quadrat position and arbuscular, vesicular and hyphal colonization (rs (6) = 0.8485, p = 0.0327, rs (6) = 0.9562, p = 0.0028, rs (6) = 0.8367). This does support the hypothesis; in these transects roots collected further from the underlying contaminated sediments were more highly colonized; but this trend was only observed in one transect, transect 5. Analysis of *E. trachycaulus* colonization in the remaining transects revealed no correlation between colonization and quadrat position (transects 3 and 4) and correlations that are contrary to the hypothesis (transects 1 and 2). In these transects, hyphal colonization (transect 1, rs (8) = -0.7363, p = 0.0372,) and vesicular colonization (transect 2, rs (6) = -0.9095, p = 0.0119) were negatively correlated with quadrat positon.

2.4.5 2016 Field season vegetation survey

In the second year of sampling there were 11 plant species identified within the 32 quadrats; percent cover of each species was estimated within each quadrat (Table A 2.4). Seven of the plant species identified were not part of the original re-vegetation, though 2 of these species (*Tussilago farfara* and *Plantago major*) are not native to the Northwest Territories (Table 2.5). The plant community had changed somewhat in species composition from what had been observed in the 2015 sampling; though there were similar proportions of wetland, upland and mycorrhizal species. There were 5 species identified in the 2015 sampling (*Erigeron acris*, *Parnassia palustris*, *Deschampsia cespitosa*, *Alnus viridis* and *Calamagrostis canadensis*), that were not identified in the 2016 quadrats, while (*Tussilago farfara*, *Carex bebbii*, *Plantago major*, *Elymus violaceus*, *Festuca ovina* and *Hordeum jubatum*) were identified in the 2016 but not 2015 quadrats. Similar to what was observed in the 2015 quadrats, 9 of the 11 species have been reported in previous studies to form an association with mycorrhizal fungi, *Carex aquatilis* and

Carex bebbii were the only species identified that have been reported as non-mycorrhizal, suggesting mycorrhizal fungi continue to be an important component of this community. Both wetland and upland plant species were present. Percent soil moisture was determined in this sampling year; associations between percent soil moisture and plant cover within quadrats are discussed along with NMDS results.

2.4.6 Total petroleum hydrocarbon determination in the 2016 soil samples

Petroleum hydrocarbon analysis of the soil included fractions 1 to 4 (C6 – C50). In five of the soil samples no petroleum hydrocarbon presence was detected, and only in one sample were petroleum hydrocarbons in the F2 (>C10 to C16) range detected. In all but 5 of the 32 soil samples, petroleum hydrocarbons in the F3 (C16 to C34) range were detected, though values were lower than both the CCME and Colomac site-specific guidelines (Table A 2.5).

2.4.7 Trends in the 2016 vegetation survey

Through an initial autopilot run it was determined that a two-dimensional NMDS ordination was optimal to represent the 2016 vegetation and environmental data. The model had a final instability of 0.0, stress value of 8.7 and a Monte Carlo test result of 0.0040. The model included 32 sites (quadrats) and 12 elements (11 species and percent bare ground) organized onto two axes that explained 74% and 22% of the variation respectively. There are 4 groups delineated on the biplot, representing quadrats 1 through 4 combined from each transect.

Unlike the 2015 quadrats, 2016 quadrats did not separate out into distinct groups, though the axes may again represent a water gradient present at the site (Figure 2.8). The presence of two wetland species were strongly (*Carex aquatilis*, τ= -0.863) and weakly (*Equisetum arvense*, τ -0.184) negatively correlated with Axis 1 while the presence of facultative upland species like

Poa glauca (τ = 0.459) and *Festuca ovina* (τ= 0.404) as well as the presence of bare ground (τ= 0.742) were moderately positively correlated with Axis 1.

The environmental variables measured in the quadrats did not have a strong association with either axes (Table 2.6). Percent soil moisture was weakly associated with the two axes (Axis 1 τ= -0.228, Axis 2 τ= 0.172), indicating that it did not have a strong influence on the separation of quadrats in the biplot. This measurement was determined from one soil sample per quadrat taken on a single day, so the vegetation growing in response to water availability may be a better representation of the water gradient at the site than the percent soil moisture determined from one sample. Soil pH was also weakly associated with the two axes (Axis 1 τ= 0.211, Axis 2 τ= -0.126), suggesting that soil pH did not influence the distribution of quadrats on the biplot.

Correlations between TPH and the two axes did not support the hypothesis that residual contamination has affected the plant community. Soil TPH was not correlated with either axes, indicating that TPH in the soil did not influence the separation of quadrats on the biplot (Table 2.6).

2.4.8. Trends in mycorrhizal colonization in the 2016 root subset

During the 2016 sampling season, the roots of *Festuca ovina, Elymus trachycaulus* and *Hordeum jubatum* were collected. AMF structures were identified in the roots of all three species (Figure 2.9; Tables A 2.6 -2.8). Among quadrats, mean arbuscular colonization in *F. ovina* roots was 7.2±0.8%, mean vesicular was 14.9±1.5% and mean hyphal colonization was 49.3±2.7%. In *E. trachycaulus* roots, mean arbuscular colonization was slightly lower (5.7±0.8 5%) though mean vesicular colonization was highest in this species (17.5±1.4%) as was hyphal colonization (64.4±2.1%). In *H. jubatum* roots the highest mean arbuscular colonization of the three species

was observed (15.5±1.7%) and the lowest mean vesicular colonization (12.6±1.7%). Mean hyphal colonization fell between the other two species (56.6±2.7%) (Table 2.7).

When colonization data for *F. ovina* from all transects were combined, creating a larger sample size, arbuscular colonization was not significantly correlated with quadrat position, ($r_s(42) = -0.1790$, $p = 0.1869$) but vesicular and hyphal colonization were both negatively correlated with quadrat position ($r_s(42) = -0.2823$, $p = 0.0350$; $r_s(42) = -0.3150$, $p = 0.0177$), counter to hypothesized trends (Table 2.8). These results indicate that vesicular and hyphal colonization was higher in quadrats closest to underlying contaminated sediments (quadrats 1 and 2) rather than in quadrats furthest away (quadrats 3 and 4) as hypothesized. Colonization of *F. ovina* roots was not correlated with TPH concentrations in the soil, percent moisture or soil pH, suggesting there are other factors affecting colonization (Table 2.8).

When data from individual transects were analyzed, there were no significant correlations between quadrat positon on the shoreline and percent mycorrhizal colonization in *F. ovina* roots in 5 of the 8 transects, and only arbuscular colonization in transect 8 followed the hypothesized trend ($r_s(8) = 0.7854$, $p = 0.0209$). Vesicular colonization was negatively correlated with quadrat position in transect 5 ($r_s(6) = -0.9562$, $p = 0.0028$) as was arbuscular colonization in transect 7 ($r_s(8) = 0.7854$, $p = 0.0209$), counter to hypothesized trends.

Colonization of *E. trachycaulus* roots was not correlated with quadrat position when data from all transects were combined, within individual transects, or with any of the environmental variables measured (Table 2.9), suggesting that colonization of *E. trachycaulus* roots at the shoreline has not been affected by residual contamination.

When colonization data for *Hordeum jubatum* from all transects were combined, vesicular colonization was positively correlated with quadrat position ($r_s(22) = 0.5409$, $p =$

0.0007), which agrees with hypothesized trends, however there were no correlations between colonization in *H. jubatum* roots and quadrat position in any of the individual transects. Colonization of *H. jubatum* roots was not correlated with TPH or percent soil moisture, though both arbuscular and vesicular colonization had positive correlations with soil pH (Table 2.10), indicating that colonization in *H. jubatum* roots was higher in areas of the shoreline with higher soil pH.

2.5 Discussion

2.5.1 Vegetation survey of Steeves Lake shoreline

The goal of a reclamation project is to return the site, as close as possible, to its original state, or to a state similar to the natural environment (MVLWB & AANDC, 2013). As Steeves Lake shoreline is a man-made site, it could not be returned to an original condition but it could be made compatible with the surrounding environment. There were eight species planted at Steeves Lake shoreline during the re-vegetation, five species through a grass seed mix and three from propagules collected on site. *Carex aquatilis* plugs and *Salix sp*. cuttings were collected on mine property and transplanted to the shoreline, and *Alnus viridis* seeds were collected and used to seed the shoreline as well as the grass seed mix. The results from the 2015 vegetation survey show six species in the quadrats that were not part of the original re-vegetation and seven new species in the 2016 survey. Though there are new plant species colonizing the site, the plant community at the shoreline does not resemble the Taiga vegetation found throughout the surrounding area, suggesting the site is still in transition.

The first plant species to establish at a site act on the ecosystem and influence succession of the community (Burkle & Belote, 2015). It has been suggested that grass pioneer species may outcompete new colonizers as they tend to grow quickly and are highly productive, leading to

lower diversity and biomass of new colonizers (Dickson & Busby, 2007). In the re-vegetation of Steeves Lake shoreline most species used were grasses, though new colonizers have established. In the study by Burkle and Belote (2015), pioneer plant communities of either grasses or forbs were established before the addition of new colonizers. They found that the use of both native and non-native grasses as pioneer species influenced the composition of the new community, with grasses limiting the biomass and diversity of new colonizers compared to pioneer forbs. The community at Steeves Lake shoreline may be a result of similar successional processes. The pioneer community consisted mainly of grasses and the most dominant new colonizers were grasses as well. Though some small forbs were present, they were scarce and only identified in a small percentage of the quadrats sampled. This resulting community could be due to the ability of grasses to better compete with other grasses than with forbs. Most of the species that have come in naturally are native to the Northwest Territories except for *Tussilago farfara* and *Plantago major*. It does not appear as if the site has completed its successional trajectory since the surrounding vegetation is comprised of mostly black spruce (*Picea mariana*), jack pine (*Pinus banksiana*), dwarf birch (*Betula nana*), Labrador tea (*Ledum groenlandicum*), bog cranberry (*Vaccinium oxycoccus*), bog bilberry (*Vaccinium uliginosum*) and black crowberry (*Empetrum nigrum*) (Ecosystem Classification Group, 2008). It is possible that this site may eventually resemble surrounding plant communities, though it may take many years due to the type of surrounding vegetation.

A study by Cray and Pollard (2015) on Tundra vegetation in the Yukon after permafrost disturbance showed that it can take hundreds of years for a Tundra plant community to progress from grass-dominated communities and recover to a forb and dwarf shrub community. They compared the vegetation at five sites that had stabilized after disturbance due to thaw slumps to

the vegetation at two natural, undisturbed sites. Of the disturbed sites, age classes included sites that had been recovering for 0 to 10 years, 10 to 20 years and the oldest age class identified was an area that had been recovering for approximately 250 years. They found that the oldest age class had not recovered the same diversity or species richness as the undisturbed sites, indicating that after a disturbance, succession to a natural community may take over 250 years in a Tundra ecosystem. Although the vegetation at Steeves Lake shoreline does not resemble the surrounding landscape, the shoreline is still in the process of recruiting new colonizers, mostly native species, which does meet the reclamation goal of a diverse native community as set in the Guidelines (MVLWB & AANDC, 2013).

2.5.2 Trends in the vegetation surveys

Trends observed in the vegetation surveys did not indicate that PHCs in the soil were a strong factor in shaping the plant community. It was assumed that, if the shoreline was still being impacted by residual contamination, there would be differences observed in the plant community between quadrats closest to the original shoreline (above underlying contaminated sediments), and quadrats furthest away. Contamination can limit plant growth or cause plant death (Racine 1994, Barrutia et al., 2011) leading to decreased plant density and percent cover, or it can select for species more tolerant to PHC contamination (Sharonova & Breus, 2012). It was hypothesized that plants growing the closest to the original shoreline (quadrats 1 and 2) would be most affected by contamination, leading to observable differences in plant community structure between quadrats. This trend was observed in the 2015 vegetation data, though soil PHC concentrations were all lower than the CCME Tier 1 guideline and Colomac site- specific concentrations, and lower than concentrations that have been shown to cause an effect on plants (Palmroth et al., 2002; Hernández-Ortega et al., 2012; Barrutia et al., 2011) and AMF (Tang et

al., 2009; Redondo-Gómez et al, 2014; Kirk et al., 2005; Driai et al., 2015). As well as the concentrations being low, TPH was not strongly correlated with either axes of the NMDS suggesting that hydrocarbons in the soil are not a driving factor in the separation of these quadrats, even though the grouping of the 2015 quadrats could suggest an effect from PHCs. It was hypothesized that effects from residual contamination would be observed in differences in predominant vegetation, such the presence of plant species more tolerant to PHCs, and a higher percentage of bare ground in quadrats closer to underlying contaminated sediments. Quadrats separated into three groups on the 2015 biplot due to differences in percent cover of species within quadrats, though the species driving the separation were most likely more common in these quadrats because of water availability, and not tolerance to PHCs because there have not been distinct differences in PHC tolerance observed between the species driving the separation of quadrats.

The strongest drivers of quadrat separation along the second axis in the 2015 biplot was a high percent cover of *Carex aquatilis* and *Equisetum arvense* which was common in quadrats 1 and 2 (closest to underlying contaminated sediments), and the presence of *Elymus trachycaulus*, *Poa glauca* and *Erigeron acris* which were common in quadrats 4 and 5 (furthest from the contaminated sediments). There is evidence that growing in contaminated soils does affect the growth of these species, though all are able to tolerate contaminated soils. *Carex aquatilis* and *Equisetum arvense* have been observed growing in wetlands affected by oil sand production (Mollard et al., 2012), and in oil-contaminated soils at a petroleum refinery facility (Hong et al., 2011). Similar effects have been observed in the species associated with quadrats furthest away from underlying contaminated sediments. *Elymus trachycaulus* has been reported to grow in field soil containing 1% TPH from weathered crude oil (Brown and Nadeau, 2002), and in oil

sands processing by-products (Boldt-Burisch et al., 2018). In both studies, though *E. trachycaulus* growth was stunted, plants were still able to grow in contaminated soils. Though there was no available data on the response of *Poa glauca* and *Erigeron acris* to PHC contamination, there have been studies on related species displaying tolerance to contamination. In a study by Robson et al. (2004), plant percent cover was estimated in contaminated and non-contaminated plots and two *Poa* species were identified; *Poa pratensis* L. and *Poa canbyi* (Scribn.) Piper. *Poa canbyi* did not differ in percent cover or frequency between contaminated and un-contaminated sites, though *Poa pratensis* L. percent cover and frequency was significantly lower in contaminated than non-contaminated sites. While there was a decrease in percent cover in one species, both could grow in contaminated soils, suggesting a tolerance to PHCs. There were no available data on the tolerance of *Erigeron acris*, however, *Erigeron annuus* has been shown to tolerate diesel concentrations up to 25,000 mg/kg (Xi et al., 2018). These studies suggest that the plant species driving the separation of the 2015 quadrats may respond in similar ways to contamination, and that the grouping of quadrats is not due to differences in tolerance to PHC contamination.

The 2016 quadrats spanned the entire shoreline; the shoreline is 750 m long so transects were approximately 100 m apart from each other. As a greater area of the shoreline was characterized with by the 2016 sampling data, it is likely a more accurate representation of vegetation trends then data collected in 2015. The 2016 quadrats did not separate out into distinct groups, and again there was not a strong correlation between TPH in the soil and either axes of the NMDS plot. The first axis explained most the variation within the data (74%); like the 2015 data, the axis explaining the most variation was strongly associated with *Carex aquatilis*, *Equisetum arvense* and *Elymus trachycaulus*. As described above, these plant species have all

been observed to tolerate growing in contaminated soils (Brown and Nadeau, 2002; Mollard et al., 2012; Boldt-Burisch et al., 2018 & Hong et al., 2011), so their presence within quadrats is most likely not due to PHC tolerance.

It is possible that the hypothesized trends were not observed because there was no upward migration of contaminants from the sediments to the soil in the shoreline. During construction of the shoreline a peat trench was installed and migrating hydrocarbons may have adsorbed to the peat within the trench (INAC 2012; Kalmykova et al., 2014) halting the movement of hydrocarbons into the infill soil. Contaminants in the sediments may also have migrated or have been degraded. The concentration of PHCs in the sediment before the shoreline was built is unknown, though it must have been environmentally significant to make capping the sediments a priority. Though initial concentrations are unknown, it has been twenty years since the end of mining operations when there were opportunities for diesel spills, possibly long enough for most the contaminants to migrate or degrade. Results from pot experiments have demonstrated an almost 50% decrease in diesel concentrations in unplanted soil after only 9 months and a 35% decrease in 84 days in unplanted soil without any additions to the soil (bacteria or nutrients) (Kuran et al., 2014; Dadrasnia & Agamuthu 2013). It is difficult to characterize the current state of the contaminated sediments without knowing the initial concentrations of the contaminants, their rate of degradation at the low temperatures experienced at the site, and the presence of fuel in fractured bedrock around the shoreline (Iwaken et al., 2008). Regardless of the current state of the contaminated sediments, the current TPH concentrations in the soil, and the current trends in vegetation at the site suggest that the community is not currently being affected by residual contamination.

Water availability is an important element in structuring plant communities (Oddershede et al., 2015) and may be the strongest influence on the plant community at Steeves Lake shoreline. Common to both sampling years was an association between the presence of wetland or upland plants within the quadrats and the two axes. In the 2015 sampling these were *C. aquatilis, E. arvense* and *E. trachycaulus* and C. *aquatilis, E. arvense* and two grasses, *E. trachycaulus* and *P. glauca* in 2016. The USDA classifies *C. aquatilis* as an obligate wetland species, meaning it is commonly found in hydric soils, and *E. arvense* as a facultative species as it is found equally in wetland and upland habitats, whereas *E. trachycaulus* and *P. glauca* are both facultative upland species as they are found almost always in non-wetlands (Lichvar et al., 2012; USDA, NRCS 2017). These species consistently showed opposite responses on the NMDS plots; a negative correlation between wetland species and an axis was accompanied by a positive correlation with upland species. Correlations between percent soil moisture did not support this response, though soil moisture measurements were taken during one sampling season. The species recruited over time are a better reflection of the true water gradient at the site than percent moisture data from one sample as environments that are consistently wet will recruit wetland species such as *C. aquatilis* (Blom & Voesenek 1996) and environments that are consistently dry will recruit species that are to tolerate dry conditions such as facultative upland species (Cornelissen et al., 2003).

2.5.3 Mycorrhizal colonization in field-collected roots

In the past, there have been uncertainties regarding how prevalent AMF are in northern ecosystems (Gardes & Dahlberg, 1996) and their importance in northern plant communities, given that many northern plant species associate with other types of mycorrhizae such as ericoid or ectomycorrhizal fungi. The study site in this thesis is in the low subarctic ecozone, an area

dominated by ericaceous species such as *Ledum groenlandicum*, *Vaccinium oxycoccus*, *Vaccinium uliginosum* and *Empetrum nigrum* (Treu et al., 1996; Massicotte et al., 2005), and species that form ectomycorrhizal associations such as *Pinus banksiana* and *Betula nana* (Deslippe et al., 2011; Onwuchekwa et al., 2014). Though not all the root systems of species that have been reported to associate with AMF could be sampled due to sampling being restricted to two days, five of the new colonizers are reported to be mycorrhizal and many of the root systems sampled were highly colonized by AMF. The high AMF colonization and number of plant species categorized as mycorrhizal though a literature suggests that AMF are important in the plant community at Steeves Lake shoreline and by extension, do play an important role in northern plant communities.

Mycorrhizal colonization was observed in all samples of *Elymus trachycaulus*, *Festuca ovina* and *Hordeum jubatum* roots collected from the shoreline over the two years, though levels of hyphal colonization observed ranged from quite low (8.5±3.75%) to high (78.5±5.25%) in *E. trachycaulus* roots and similar ranges were seen in *F. ovina* and *H. jubatum* roots. Though there was large variation, the colonization in roots collected at Steeves Lake shoreline did tend to be higher than that reported in roots collected from disturbed soils, and more closely resemble what has been observed in undisturbed treatments or natural sites. Gould et al. (1998) sampled roots from reclaimed mine sites in Kentucky multiple years after the reclamation was completed. Roots sampled from soil cores taken two years post reclamation had much lower colonization than those of our study (5 to 11%) as did roots collected eight years post reclamation (26%).

Natural ecosystems in northern Canada have been surveyed for the presence of AMF. As the goal in reclamation is to return a site to its natural condition, colonization in natural ecosystems may be a useful comparison to judge whether the mycorrhizal community has

recovered as well. Root samples have been collected from Banks, Devon, Ellesmere and Ellef Ringnes Islands (Olssen et al., 2004) and again from Devon Island (Peters et al., 2011). In the study by Olssen (2004), colonization ranged greatly between sites. There was no colonization by AMF observed in roots collected from seven species at both Ellesmere and Ellef Ringnes Islands, though colonization of roots from Devon Island ranged from 1.6 to 17% in *Potentilla hookeriana* and *Festuca hyperborean* respectively. In Banks Island samples, colonization ranged from in 37% in *Arnica angustifolia* to as high as 85% *Potentilla hookeriana*. Peters et al. (2011) observed lower levels of colonization in their survey of Devon Island with colonization ranging from 0 to 3% in ten species collected. Results from these surveys indicate that AMF occur naturally in the north, and that there may be a natural heterogeneity in AMF abundance, similar to the heterogeneity observed in colonization at Steeves Lake shoreline. Since colonization in roots collected from the shoreline is equal to or higher than that of natural and reclaimed sites, it can be concluded that by using soil from the surrounding environment, the plants at the site were able to access AMF propagules and become colonized.

Overall, colonization did not follow hypothesized trends. If the mycorrhizal community had been impacted by residual contamination, this would have been observed as a positive correlation between colonization and quadrat position. Although trends in colonization did not follow the expected patterns, there were differences between transects. For example, hyphal colonization in *E. trachycaulus* roots collected in 2015 was positively correlated with quadrat in transect 5, which would support the hypothesis, but had a negative correlation in the first transect, meaning that colonization was higher in quadrats overlying the contaminated sediments. This variation in colonization within transects may have resulted from differences in the plant community across the site. The positive correlation with colonization and quadrat in transect 5

could be because mycorrhizal species were only found in the last three quadrats. In the first transect colonization was negatively correlated with quadrat, but mycorrhizal species were found in a greater number of the quadrats. Other instances of inconsistent trends may be due to differences in water availability or pH between plots, or due to factors such sampling time (August) or the age of plants that roots were collected from.

Studies have shown higher levels of colonization at higher pH (between pH 4 and 7), and that vesicle formation generally increased at a higher pH (Clark & Zeto 1996; Coughlan et al., 2000;) but this response is variable and differs between AMF species (Medeiros et al., 1994; van Aarle et al., 2002). Hyphal and vesicular colonization in *H. jubatum* roots were both positively correlated with soil pH, though not in the roots of any other species collected, possibly due to this variability between AMF species.

Cases where there were opposing trends in colonization may be due to differences in plant age, which was not determined before the roots were sampled. Total percent root colonization and percent colonization by AMF structures may differ depending on the age of the plant. Results from a study conducted by Afek et al. (1990) demonstrated that root colonization can vary within only a 21-day span. They inoculated cotton (*Gossypium hirsutum* L.), onion (*Allium cepa* L.) and pepper (*Capsicum annumm* L.) with three *Glomus* species and harvested plants every 3 days over a 21-day period to determine percent root colonization at each time point. Depending on the plant and AMF species, colonization was detected between 3 and 12 days after inoculation, and in most cases differences in colonization between time points varied. For example, when inoculated with *Glomus deserticola*, colonization of cotton roots increased from 5 to 20% between 15 days and 21 days, but colonization of pepper by *G. deserticola* remained unchanged between these time points (60%).

As well as differences in total percent colonization, differences in AMF structures between time points has also been observed. Rajeshkannan et al. (2009) grew rice (*Oryza sativa* L. var. Ponni) in soil samples collected from a rice field and analyzed root samples every 7 days after planting to determine colonization. They found that hyphal, vesicular and arbuscular colonization varied over a 70-day growth period. Hyphal colonization was observed at the first time point (17% at day 7) and gradually increased throughout the study, and was highest at the last time point (28% at day 70). Vesicular colonization was not observed until 14 days after inoculation (9.8%), increased at day 35 (17.9%) and was lowest at the last time point (day 70, 8.3%), while arbuscular colonization was not observed until 42 days after inoculation (2.9%) and did not change. This observed variation in colonization within a short amount of time may explain some of the trends observed in colonization in the 2015 and 2016 root samples collected. For example, in the *E. trachycaulus* roots collected in 2015 there were no significant correlations with quadrat position and any of the mycorrhizal structures in two of the transects (3 and 4) while in transect 5, the presence of hyphae, vesicles and arbuscules was significantly correlated with quadrat position. These trends may just be due too different ages of the plants collected, or differences in the amount of time since they had become colonized.

From the trends in vegetation and colonization observed in field- collected roots it was concluded that there are most likely no effects from residual contamination on the ability of AMF at the site to colonize host roots. NMDS of the 2015 and 2016 vegetation data showed grouping of the plots due to the presence of plant species that differed more in their wetland indicator status than their tolerance to PHC contamination, indicating that a water gradient is the most likely cause for the separation of quadrats. There were some differences in colonization between transects and quadrats, though none that supported the hypotheses, so there may be

other variables not measured in this study which are causing the observed trends. Because we were unable to conclusively determine whether there had been negative effects on the plant and mycorrhizal community, of if other variable were responsible, a greenhouse study was performed to determine, under controlled conditions, how PHC contamination affects northern vegetation.

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2.7 Tables and Figures

Table 2.1 Plant species identified within the quadrats of the 2015 vegetation survey. Plants were identified using the species key by Porsild and Cody (1980). Wetland indicator status for each species was determined using the USDA PLANTS database and mycorrhizal status through a literature search. Plants were classified as planted if they were included in the re-vegetation of the shoreline; this information was derived from the Flat River Consulting Report (2011): *Colomac Mine, NWT: Habitat Compensation and Re-vegetation for Steeves Lake and Associated Restoration Works, NWT*. Plant species status in the NWT was derived from the Working Group on General Status of NWT Species (2016).

Family	Species	Common Name	Wetland Indicator Status	Mycorrhizal Status	Planted/ unplanted	NWT Status
Asteraceae	*Erigeron acris* L.	bitter fleabane	FAC	AM[9]	Unplanted	Secure
Betulaceae	*Alnus viridis* (Ait.) Pursh	green alder	FAC	ECM[11]	Planted	Secure
Cyperaceae	*Carex aquatilis* Wahlenb var. aquatilis	water sedge	OBL	NM[6]	Planted	Secure
Equisetaceae	*Equisetum arvense* L.	common horsetail	FAC	AM[7] / NM[8]	Unplanted	Secure
Parnassiaceae	*Parnassia palustris* L.	Grass of Parnassuss	OBL	AM[10] / NM[10]	Unplanted	Secure
Poaceae	*Poa glauca* M. Vahl	glaucous bluegrass	FACU	AM[1]	Planted	Secure
Poaceae	*Calamagrostis canadensis* (Michx)	blue joint	FAC	AM[2]	Unplanted	Secure
Poaceae	*Elymus trachycaulus* L.	slender wheatgrass	FACU	AM[3]	Unplanted	Secure
Poaceae	*Deschampsia cespitosa* (L.) Beauv.	tufted hair grass	FAC	AM[4]	Unplanted	Secure
Salicaceae	*Salix sp.*	willow	n/a	ECM[5] / AM[5]	Planted	Secure

Wetland indicator status: (OBL) obligate wetland species, occurs in wetlands ≥ 99 %, (FACW) facultative wetland, occurs in wetlands 67–99 %, (FAC) facultative wetland species, occur equally in wetlands and non-wetlands 34–66 %, FACU facultative upland species, occurs in non-wetlands 67–99 %, UPL obligate upland species, almost always occur in non-wetlands ≥ 99 %, N/A no information, (Lichvar et al., 2012; USDA 2017)

Mycorrhizal status references (AM = arbuscular mycorrhizal, ECM = Ectomycorrhizal, NM = non-mycorrhizal): 1. Betekhtina & Veselkin, 2011, 2. Mejstrik & Benecke, 3. Thorrnann et al., 1999, 4. Turner et al., 2000, 5. Kasowska, 6. Harley & Harley, 7. Akhmetzhanova et al. 2012, 8. Anderson et al., 1984, 9. Zak & Parkinson,10. Gucwa-Przepióra et al., 2013, 11. Wang & Qui, 2006

Table 2.2 Associations between plant species percent cover, total petroleum hydrocarbons, and the two axes of the non-metric multidimensional scaling used to analyse the 2015 vegetation survey of Steeves Lake shoreline. The two axes retained explained 55 and 41% of the variation in the date respectively. Percent cover of each species within each quadrat was used as a main matrix, with total petroleum hydrocarbon concentration within each quadrat as a secondary matrix. Values closet to 1.00 or -1.00 indicate strong positive and negative associations with each axis respectively.

Axis	Axis 1	Axis 2
	Kendall's tau	Kendall's tau
Plant Species		
Salix sp.	-0.374	0.078
Carex aquatilis	-0.498	0.854
Equisetum arvense	-0.551	0.558
Poa glauca	0.374	-0.536
Bare ground	0.280	-0.730
Elymus trachycaulus	0.851	-0.455
Deschampsia cespitosa	-0.047	0189
Erigeron acris	0.299	-0.384
Parnassia palustris	0.071	-0.236
Calamagrostis canadensis	0.165	0.212
Alnus viridis	0.283	-0.024
TPH		
Total petroleum hydrocarbons (TPH) (mg/kg)	0.050	0.097

Table 2.3 Mean arbuscular, vesicular and hyphal colonization in *Elymus trachycaulus* roots collected form Steeves Lake shoreline in August 2015. Root samples were cleared with 10% KOH, stained with a 5% ink in vinegar solution, and mycorrhizal structures were quantified using a modified version of the magnified intersections method (McGonigle et al., 1990). Mean colonization was calculated from thirty-two roots samples collected from the shoreline.

Elymus trachycaulus 2015	Mean Colonization	Standard Error	95% Confidence Interval
% Arbuscular	3.9	0.7	1.4
% Vesicular	12.0	1.7	3.3
% Hyphal	47.3	3.8	7.5

Table 2.4 Spearman's correlation coefficients and sample size ($r_s(n)$) and their statistical significance from Spearman correlations relating colonization in 2015 *Elymus trachycaulus* roots with quadrat position within each transect and all transects combined. Combined data from all transects have also been related to total petroleum hydrocarbon (TPH) concentrations in the soil. Significant, positive correlations between colonization and quadrat position would indicate lower colonization in root samples quadrats closest to underlying contaminated sediments than in those further away. This would agree with hypothesized trends, as would a significant, negative correlation between TPH in the soil and root colonization. Significant correlations are bolded ($p < 0.05$).

% Colonization	By quadrat position						By TPH (mg/kg)
	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Combined Transects	
Arbuscular	r_s (8) = -0.5496, p = 0.1582	r_s (6) = -0.1195, p = 0.8216	r_s (6) = 0.0000, p = 1.000	r_s (6) = -0.3135, p = 0.5452	r_s (6) = 0.8485, p = **0.0327**	r_s (30) = -0.0204, p = 0.9117	r_s (30) = -0.1066, p = 0.6839
Vesicular	r_s (8) = -0.5855, p = 0.1272	r_s (6) = -0.9095, p = **0.0119**	r_s (6) = 0.6063, p = 0.2020	r_s (6) = 0.6179, p = 0.1911	r_s (6) = 0.9562, p = **0.0028**	r_s (30) = 0.0531, p = 0.7730	r_s (30) = -0.2617, p = 0.3193
Hyphal	r_s (8) = -0.7363, p = **0.0372**	r_s (6) = -0.5976, p = 0.2103	r_s (6) = 0.5976, p = 0.2103	r_s (6) = 0.3531, p = 0.4924	r_s (6) = 0.8367, p = **0.0378**	r_s (30) = -0.0452, p = 0.8059	r_s (30) = -0.3569, p = 0.1597

Table 2.5 Plant species identified within the quadrats of the 2016 vegetation survey. Where possible, plants were identified down to the species level using the Porsild and Cody (1980) species key. The wetland indicator statuses were derived from the USDA PLANTS database and mycorrhizal status through a literature search, references are given below. Plants were classified as planted/unplanted according to the Flat River Consulting Report (2011): *Colomac Mine, NWT: Habitat Compensation and Re-vegetation for Steeves Lake and Associated Restoration Works, NWT*, which gives a species list of plants used in the original re-vegetation of the shoreline. The secure/introduced status for each species was determined using the Working Group on General Status of NWT Species (2016); secure species are defined as neither at risk nor sensitive and native to the NWT.

Family	Species	Common Name	Wetland Indicator Status	Mycorrhizal Status	Planted/ Unplanted	Secure/ Introduced
Asteraceae	*Tussilago farfara* L.	coltsfoot	FACU	AM[1]	Unplanted	Introduced
Cyperaceae	*Carex aquatilis* Wahlenb var. aquatilis	water sedge	OBL	NM[2]	Planted	Secure
Cyperaceae	*Carex bebbii* Olney	Bebb's Sedge	OBL	n/a	Unplanted	Sensitive
Equisetaceae	*Equisetum arvense* L.	common horsetail	FAC	AM[3] /NM[4]	Unplanted	Secure
Plantaginaceae	*Plantago major* L.	common plantain	FAC	AM[1]	Unplanted	Introduced
Poaceae	*Elymus violaceus* L.	high wild rye	FACU	AM[5]	Unplanted	Secure
Poaceae	*Festuca ovina* L.	sheep fescue	FACU	AM[6]	Planted	Secure
Poaceae	*Poa glauca* M. Vahl	glaucous bluegrass	FACU	AM[7]	Planted	Secure
Poaceae	*Hordeum jubatum* L.	foxtail barley	FACU	AM[5]	Unplanted	Secure
Poaceae	*Elymus trachycaulus* L.	slender wheatgrass	FACU	AM[8]	Unplanted	Secure
Salicaceae	*Salix sp.*	willow	n/a	ECM[5] + AM[5]	Planted	Secure

Wetland indicator status: (OBL) obligate wetland species, occurs in wetlands ≥ 99 %, (FACW) facultative wetland, occurs in wetlands 67–99 %, (FAC) facultative wetland species, occur equally in wetlands and non-wetlands 34–66 %, FACU facultative upland species, occurs in non-wetlands 67–99 %, UPL obligate upland species, almost always occur in non-wetlands ≥ 99 %, N/A no information, (Lichvar et al., 2012; USDA 2010)

Mycorrhizal Status References (AM = arbuscular mycorrhizal, ECM = Ectomycorrhizal, NM = non-mycorrhizal, n/a = no information reported): 1. Harley & Harley, 1987, 2. Thorrnann et al., 1999, 3. Turner et al., 2000, 4. Kasowska, 1987, 5. Wang & Qui, 2006, 6. Van Der Heijden et al., 1998, 7. Akhmetzhanova et al. 2012, 2, 8. Zak & Parkinson, 1982

Table 2.6 Associations between plant species percent cover, soil pH, percent soil moisture, total petroleum hydrocarbons in the soil, and the two axes of the non-metric multidimensional scaling used to analyse 2016 vegetation survey of Steeves Lake shoreline. The two axes retained explained 74 and 22% of the variation in the data respectively. The percent cover of each species within each quadrat was input as the main matrix in the non-metric multidimensional scaling, and soil pH, percent soil moisture and total petroleum hydrocarbons within each quadrat overlaid as a secondary matrix. Strong negative and positive correlations between each element and axes are indicated with values close to -1.00 and 1.00 respectively.

	Axis 1	Axis 2
	Kendall's tau	Kendall's tau
Plant Species		
Carex aquatilis	-0.863	0.388
Salix sp.	-0.032	0.230
Elymus trachycaulus	0.277	-0.231
Poa glauca	0.459	-0.286
Bare ground (BG)	0.724	-0.682
Equisetum arvense	-0.184	0.608
Festuca ovina	0.404	-0.052
Hordeum jubatum	-0.058	-0.042
Elymus violaceus	0.159	-0.063
Carex bebbii	0.120	0.024
Tussilago farfara	0.220	0.058
Plantago major	0.250	-0.121
Environmental Variables		
Soil pH	0.211	-0.126
Soil Moisture (%)	-0.228	0.172
Total petroleum hydrocarbons (mg/kg)	0.088	0.052

Table. 2.7 Mean arbuscular, vesicular and hyphal colonization in the roots of three species, *Festuca ovina*, *Elymus trachycaulus* and *Hordeum jubatum*, collected from Steeves Lake shoreline in August 2016. Briefly, the collected roots were cleared with 10% KOH, stained with a 5% ink in vinegar solution, and mycorrhizal structures were quantified using a modified version of the magnified intersections method (McGonigle et al., 1990). Sample sizes varied between plant species; *Festuca ovina* (n = 42), *Elymus trachycaulus* (n = 34) and *Hordeum jubatum* (n = 22).

	Mean Colonization	Standard Error	95% Confidence Interval
Festuca ovina			
% Arbuscular	7.2	0.8	1.5
% Vesicular	14.9	1.5	3.0
% Hyphal	49.3	2.7	5.2
Elymus trachycaulus			
% Arbuscular	5.7	0.8	1.5
% Vesicular	17.5	1.4	2.6
% Hyphal	64.1	2.1	4.0
Hordeum jubatum			
% Arbuscular	15.5	1.7	3.3
% Vesicular	12.6	1.7	3.3
% Hyphal	56.6	2.7	5.2

Table 2.8 Spearman's correlation coefficients, sample size ($r_s(n)$) and their statistical significance, from Spearman correlations relating colonization in *Festuca ovina* roots to quadrat position and environmental variables. Colonization was determined from root samples collected in August 2016 from Steeves Lake shoreline, Colomac mine. Correlations between colonization and quadrat position in separate transects were performed, as well as within all transects combined, each transect contained 4 quadrat positions. Colonization was also related to total petroleum hydrocarbons in the soil (TPH), soil percent moisture, and soil pH. Colonization was hypothesized to be lowest in quadrat positon 1, and highest in position 4 due to TPH in the soil. Significant, positive correlations between colonization and position would agree with hypotheses; as would significant negative correlations between colonization and soil TPH. Percent soil moisture and soil pH were included as possible explanatory variables for trend that do not follow hypotheses. Significant correlations are bolded ($p < 0.05$).

% Colonization	By quadrat position									By environmental variables		
	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 7	Transect 8	Combined Transects	TPH (mg/kg)	% Moisture	Soil pH
Arbuscular	r_s (8) = 0.3436, p = 0.4046	r_s (4) = -0.8944, p = 0.1056	r_s (6) = -0.5809, p = 0.2266	r_s (8) = 0.1482, p = 0.7262	r_s (6) = -0.2390, p = 0.6483	r_s (8) = -0.5186, p = 0.1879	r_s (8) = -0.8396, p = **0.0091**	r_s (8) = 0.7854, p = **0.0209**	r_s (42) = -0.1790, p = 0.1869	r_s (42) = -0.1215, p = 0.5630	r_s (42) = 0.2308, p = 0.2670	r_s (42) = 0.0079, p = 0.9701
Vesicular	r_s (8) = -0.4880, p = 0.2199	r_s (4) = -0.2357, p = 0.7643	r_s (6) = -0.4851, p = 0.3295	r_s (8) = -0.1952, p = 0.6432	r_s (6) = -0.9562, p = **0.0028**	r_s (8) = -0.0245, p = 0.9540	r_s (8) = 0.4880, p = 0.2199	r_s (8) = -0.3664, p = 0.3720	r_s (42) = -0.2823, p = **0.0350**	r_s (42) = -0.1476, p = 4815	r_s (42) = -0.0485, p = 0.8179	r_s (42) = -0.0886, p = 0.6737
Hyphal	r_s (8) = -0.1952, p = 0.6432	r_s (4) = 0.000, p = 1.000	r_s (6) = -0.5457, p = 0.2627	r_s (8) = -0.1718, p = 0.6841	r_s (6) = -0.7276, p = 0.1012	r_s (8) = -0.3416, p = 0.4076	r_s (8) = 0.0491, p = 0.9081	r_s (8) = -0.0976, p = 0.8182	r_s (42) = -0.3159, p = **0.0177**	r_s (42) = 0.1082, p = 0.6067	r_s (42) = -0.0473, p = 0.8223	r_s (42) = -0.1540, p = 0.4624

Table 2.9 Spearman's correlation coefficients, sample size ($r_s(n)$) and statistical significance, from Spearman correlations relating colonization in *Elymus trachycaulus* roots to quadrat position. Colonization was quantified in roots collected in August 2016 from Steeves Lake shoreline at Colomac mine, NWT. Correlations between colonization and quadrat position in separate transects were performed, as well as within all transects combined, each transect contained 4 quadrat positions. Colonization was also related to total petroleum hydrocarbons in the soil (TPH), soil percent moisture, and soil pH. Colonization was hypothesized to be lowest in quadrat positon 1, and highest in position 4 due to TPH in the soil. Significant, positive correlations between colonization and position would agree with hypotheses; as would significant negative correlations between colonization and soil TPH. Percent soil moisture and soil pH were included as possible explanatory variables for trend that do not follow hypotheses. Significant correlations are bolded ($p < 0.05$).

% Colonization	By quadrat position									By environmental variable		
	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 7	Transect 8	Combined Transects	TPH (mg/kg)	% Moisture	Soil pH
Arbuscular	r_s (4) = -0.2357, p = 0.7643	r_s (3) = 0.000, p = 1.000	r_s (6) = -0.3032, p = 0.5592	r_s (8) = -0.0252, p = 0.9528	r_s (3) = -0.8660, p =0.3333	r_s (8) = 0.0000, p = 1.000	r_s (8) = 0.1500, p = 0.7229	r_s (8) = -0.0988, p = 0.8160	r_s (34) = 0.0298, p = 0.8409	r_s (34) = -0.1214, p = 0.5630	r_s (34) = 0.2308, p = 0.2670	r_s (34) = -0.0079, p = 0.9701
Vesicular	r_s (4) = 0.000, p = 1.000	r_s (3) = 0.8660, p = 0.3333	r_s (6) = 0.0000 p = 1.0000	r_s (8) = -0.4939 p = 0.2136	r_s (3) = 0.8660 p = 0.3333	r_s (8) = 0.3682 p = 0.3695	r_s (8) = -0.2440, p = 0.5604	r_s (8) = -0.4692, p = 0.2409	r r_s (34) = -0.0253, p = 0.8647	r_s (34) = -0.1476, p = 0.4815	r_s (34) = -0.0485, p = 0.8179	r_s (34) = -0.0886, p = 0.6737
Hyphal	r_s (4) = -0.8944, p = 0.1056	r_s (3) = 0.8660, p = 0.3333	r_s (6) = -0.2390, p = 0.6483	r_s (8) = -0.6872, p = 0.0597	r_s (3) = -0.8660, p = 0.3333	r_s (8) = 0.5891, p = 0.1244	r_s (8) = -0.0488, p = 0.9087	r_s (8) = 0.2700, p = 0.5178	r_s (34) = -0.0807 p = 0.5858	r_s (34) = 0.1082, p = 0.6067	r_s (34) = -0.0473, p = 0.8223	r_s (34) = -0.1540, p = 0.4624

Table 2.10 Spearman's correlation coefficients, sample size ($r_s(n)$) and statistical significance, from Spearman correlations relating colonization in *Hordeum jubatum* roots to quadrat position. Root samples were collected in August 2016, from Steeves Lake shoreline, Colomac mine, NWT. Correlations between colonization and quadrat position in separate transects were performed, as well as within all transects combined, each transect contained 4 quadrat positions. Colonization was also related to total petroleum hydrocarbons in the soil (TPH), soil percent moisture, and soil pH. Colonization was hypothesized to be lowest in quadrat positon 1, and highest in position 4 due to TPH in the soil. Significant, positive correlations between colonization and position would agree with hypotheses; as would significant negative correlations between colonization and soil TPH. Percent soil moisture and soil pH were included as possible explanatory variables for trend that do not follow hypotheses. Significant correlations are bolded ($p < 0.05$).

% Colonization	By quadrat position									By environmental variable		
	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 7	Transect 8	Combined Transects	TPH (mg/kg)	% Moisture	Soil pH
Arbuscular	r_s (4) = -0.8944, p = 0.1056	r_s (3) = -0.8660, p = 0.3333	r_s (2) = N/A	r_s (3) = 0.8660, p = 0.3333	r_s (6) = 0.0000 p = 1.0000	r_s (6) = -0.7171, p = 0.1087	r_s (8) = 0.5645, p = 0.1449	r_s (4) = -0.8889 p = 0.1111	r_s (22) = -0.0800, p = 0.6427	r_s (22) = -0.0460, p = 0.8473	r_s (22) = 0.1483, p = 0.5327	r_s (22) = 0.3552, p = 0.1244
Vesicular	r_s (4) = 0.8944, p = 0.1056	r_s (3) = 0.8660, p = 0.3333	r_s (2) = N/A	r_s (3) = 0.8660, p = 0.3333	r_s (6) = 0.3810, p = 0.4562	r_s (6) = 0.1195, p = 0.8216	r_s (8) = 0.5645 p = 0.1449	r_s (4) = -0.7379 p = 0.2621	r_s (22) = 0.5409, p = **0.0007**	r_s (22) = -0.4287, p = 0.0593	r_s (22) = -0.4431, p = 0.0504	r_s (22) = 0.5558, p = **0.0109**
Hyphal	r_s (4) = -0.4472, p = 0.5528	r_s (3) = 0.8660, p = 0.3333	r_s (2) = N/A	r_s (3) = 0.8660, p = 0.3333	r_s (6) = 0.3586, p = 0.4852	r_s (6) = -0.1195 p = 0.8216	r_s (8) = 0.1952, p = 0.6432	r_s (4) = -0.7379 p = 0.2621	r_s (22) = 0.2605, p = 0.1249	r_s (22) = -0.1928, p = 0.4153	r_s (22) = -0.1926, p = 0.4160	r_s (22) = 0.5183, p = **0.0192**

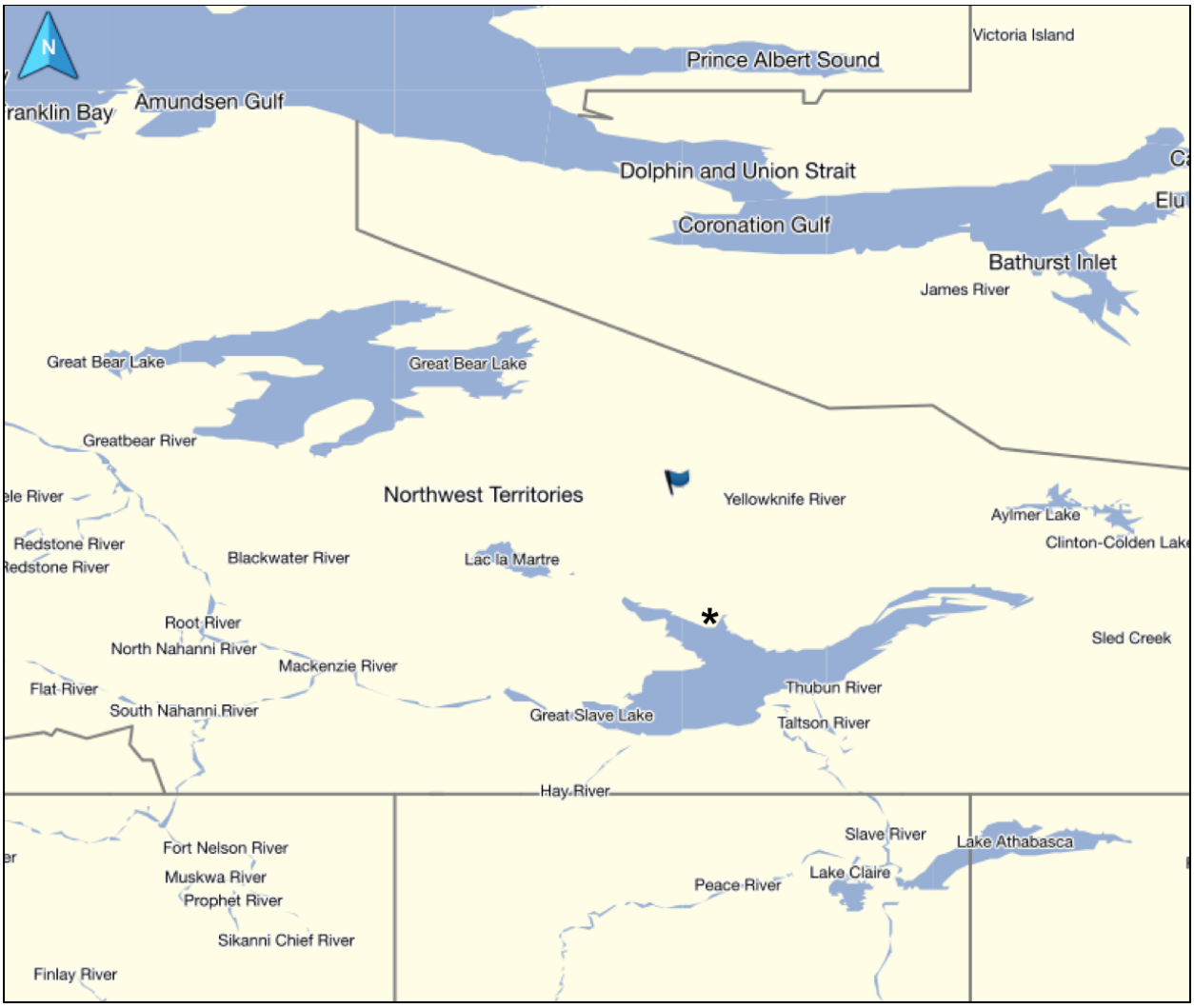

Figure 2.1 The location of Colomac Mine (flag) and Yellowknife (asterisk) in the Northwest Territories.

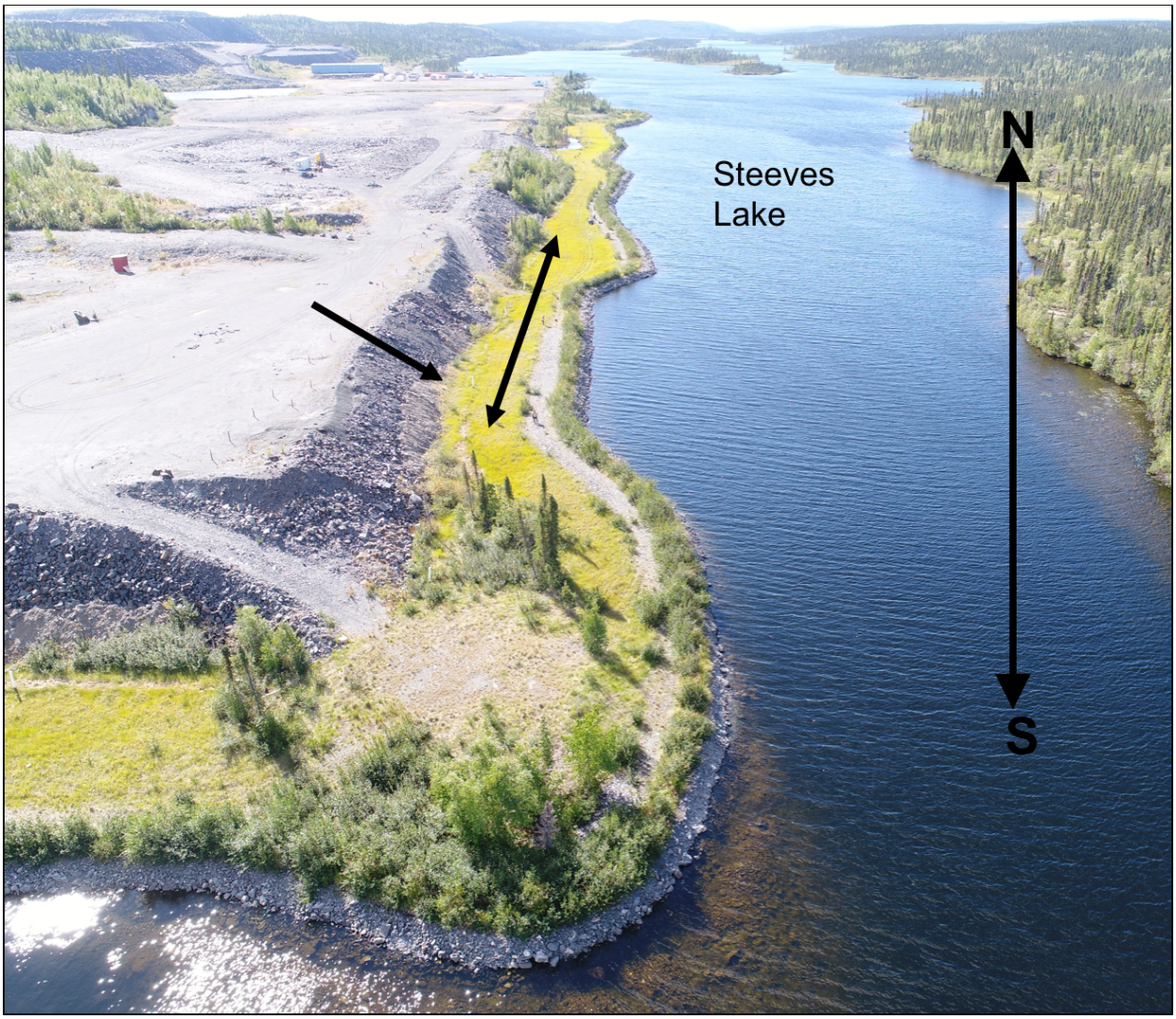

Figure 2.2 Overview of the Steeves Lake shoreline. The shoreline runs north to south, and is bordered by Steeves Lake on the right. The single head arrow shows the location of the original shoreline, and where the new man-made shoreline begins. The double headed arrow is overlaying the new shoreline that has been re-vegetated and is the area chosen for sampling in this field study.

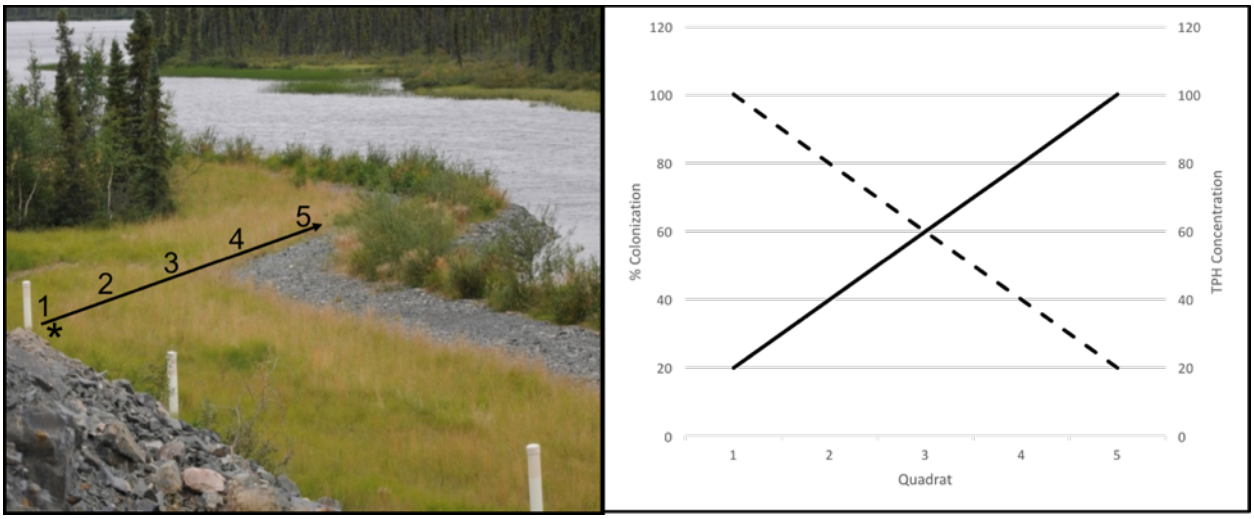

Figure 2.3 Overview of Steeves Lake shoreline (left) overlaid with the sampling design. The numbers show the position of the quadrats across the shoreline, and the asterisk shows the hypothesized location of underlying contaminated sediments. Hypothesized trends (right) in mycorrhizal colonization (solid line) and the hypothesized PHC gradient (dashed line**)** are shown**.** It was hypothesized that colonization would be lower in quadrats closest to the underlying contaminated sediments, and hydrocarbon concentration would be highest in these quadrats. Plants and mycorrhizal fungi in the quadrats closest to the underlying contaminated sediments are hypothesized to have been negatively affected by these contaminated sediments.

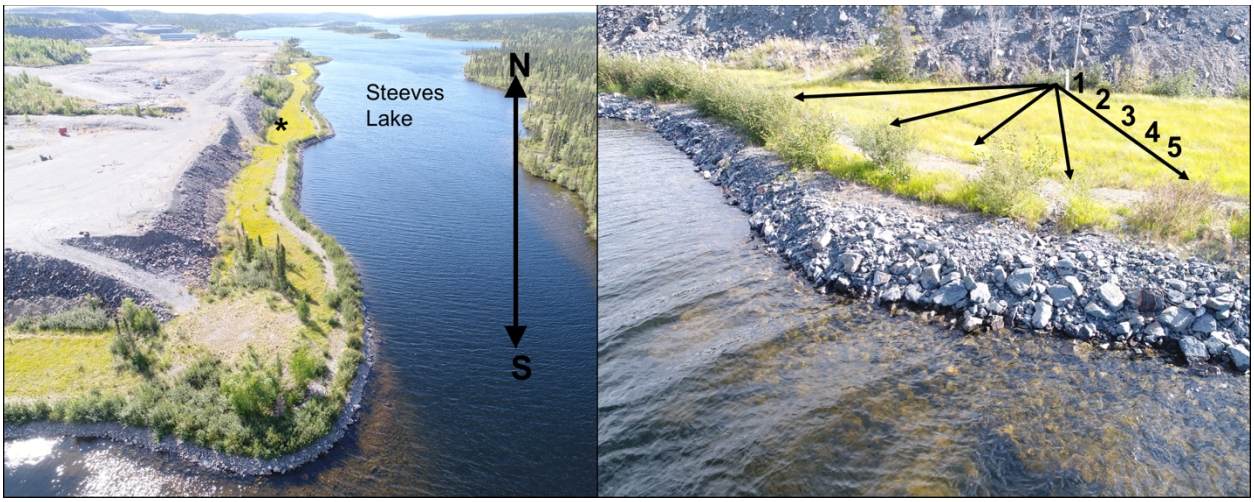

Figure 2.4 Overview of the Steeves Lake shoreline showing the 2015 sampling design. The image on the left shows the entire shoreline, and the location of the area sampled, denoted with an asterisk. The image on the right shows the radiating pattern of transects used to sample a localized area of the shoreline. The numbers show the approximate location of five quadrats within each of the five transects.

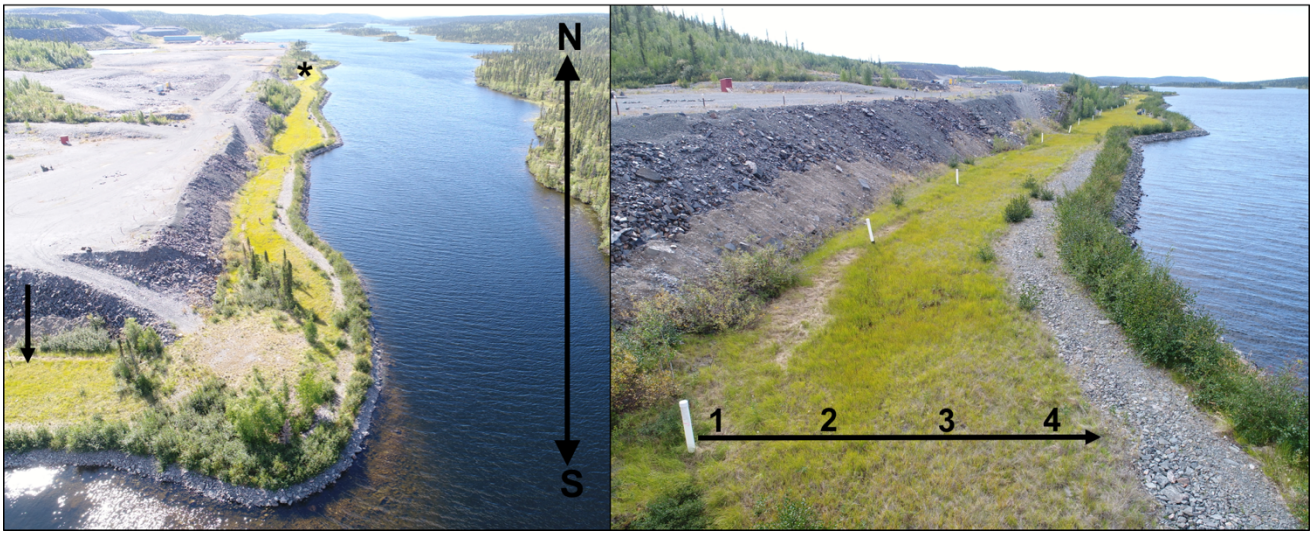

Figure 2.5 Overview of the Steeves Lake shoreline line showing the 2016 sampling design. The image on the left shows the entire shoreline; the asterisk represents the location of the first of eight transects, and the arrow represents the location of the eighth transect, the remaining six transects were spread out evenly between these points along the shoreline. The image on the right shows the approximate location of the four quadrats within each of the eight transects.

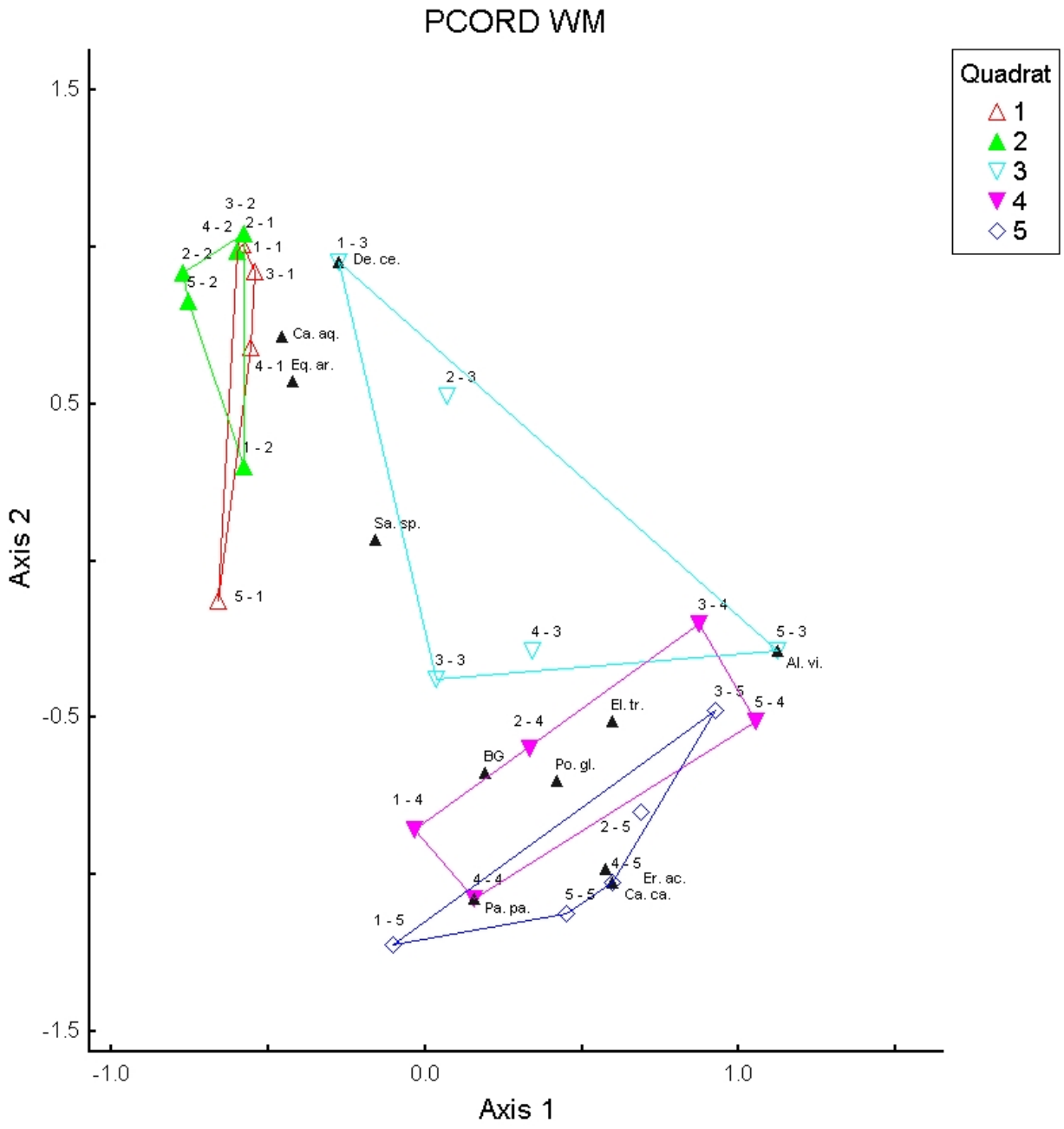

Figure 2.6 Non-metric multidimensional scaling of the 2015 vegetation survey of Steeves Lake shoreline based on Sorensen distances with no penalty on handling ties (stress = 7.9, p = 0.0012). The symbols represent quadrats one through five from the five transects sampled at the shoreline. The quadrats closest to the original shoreline have grouped together (quadrats 1 and 2) as well as the quadrats furthest from the original shoreline (quadrats 4 and 5). The percent cover of plant species plotted include *Salix* sp. (Sa.sp.) *Carex aquatilis* (Ca.aq.), *Equisetum arvense* (Eq.ar.), *Elymus trachycaulus* (El.tr.), *Deschampsia cespitosa* (De.ce.), *Erigeron acris* (Er.ac.), *Parnassia palustris* (Pa.pa), *Calamagrostis canadensis* (Ca.ca), *Alnus viridis* (Al.vi.), and bare ground (BG).

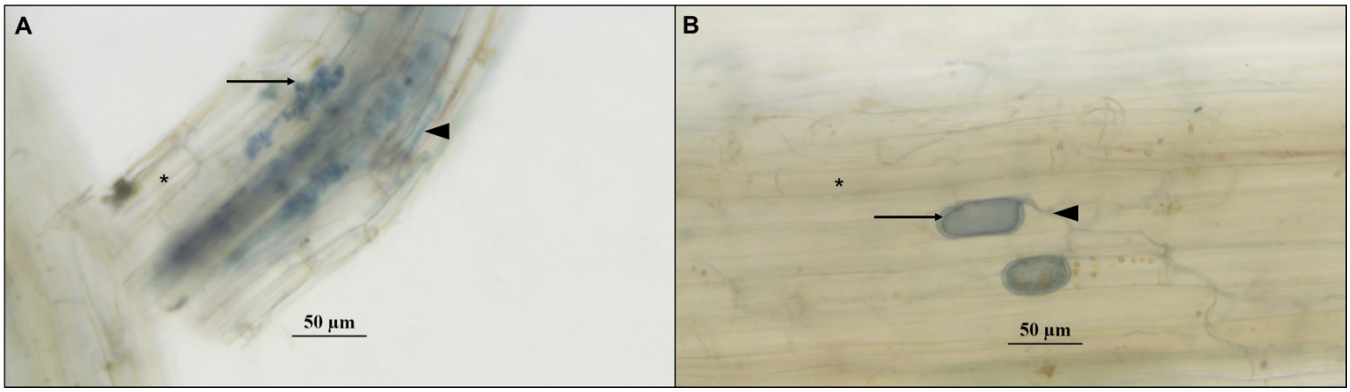

Figure 2.7 Cleared and stained roots of *Elymus trachycaulus* collected from Steeves Lake shoreline, Colomac Mine NWT, in August 2015. Root samples were cleared in a 10% KOH solution, stained with a 5% ink in vinegar solution and mycorrhizal structures were quantified using a modified version of the magnified intersections method (McGonigle et al., 1990).
A: A colonized area of an *E. trachycaulus* root with visible arbuscules (arrow), and intercellular hyphae (arrow head). Non-colonized cortical cells appear translucent (asterisk)
B: A vesicle (arrow), and subtending hyphae (arrow head) within an *E. trachycaulus* root. Surrounding non-colonized cortical cells appear translucent (asterisk).

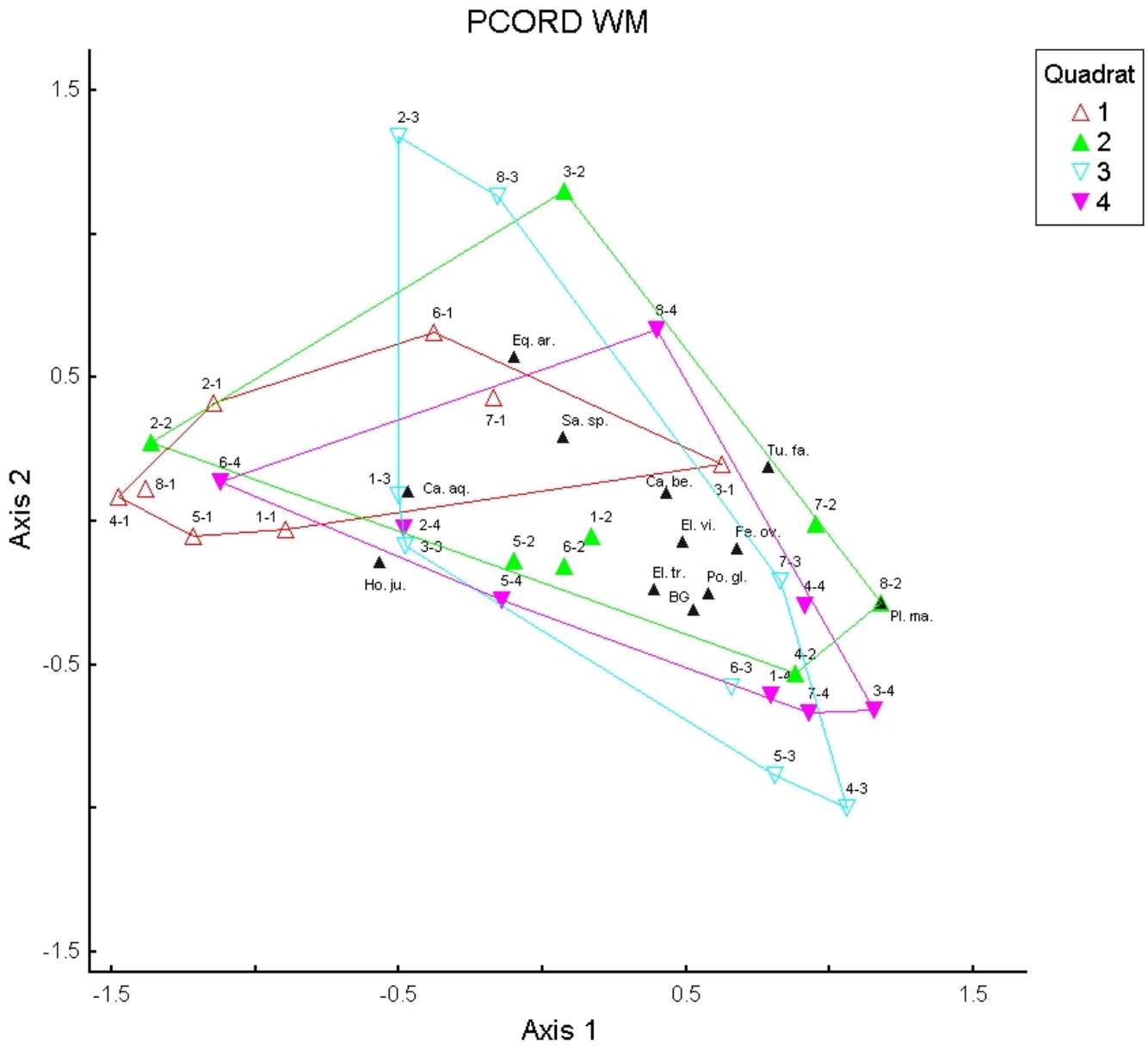

Figure 2.8 Non-metric multidimensional scaling of the 2016 vegetation survey of Steeves Lake shoreline based on Sorensen distances with no penalty on handling ties (stress = 8.7, p = 0.0040). The symbols represent quadrats one through four from the eight transects sampled at the shoreline. The ordination was based on the percent cover of plant species within quadrats, there was no clear separation of quadrats within the biplot. The percent cover of plant species plotted include *Salix* sp. (Sa.sp.) *Carex aquatilis* (Ca.aq.), *Equisetum arvense* (Eq.ar.), *Elymus trachycaulus* (El.tr.), *Deschampsia cespitosa* (De.ce.), *Erigeron acris* (Er.ac.), *Parnassia palustris* (Pa.pa), *Calamagrostis canadensis* (Ca.ca), *Alnus viridis* (Al.vi.), and bare ground (BG).

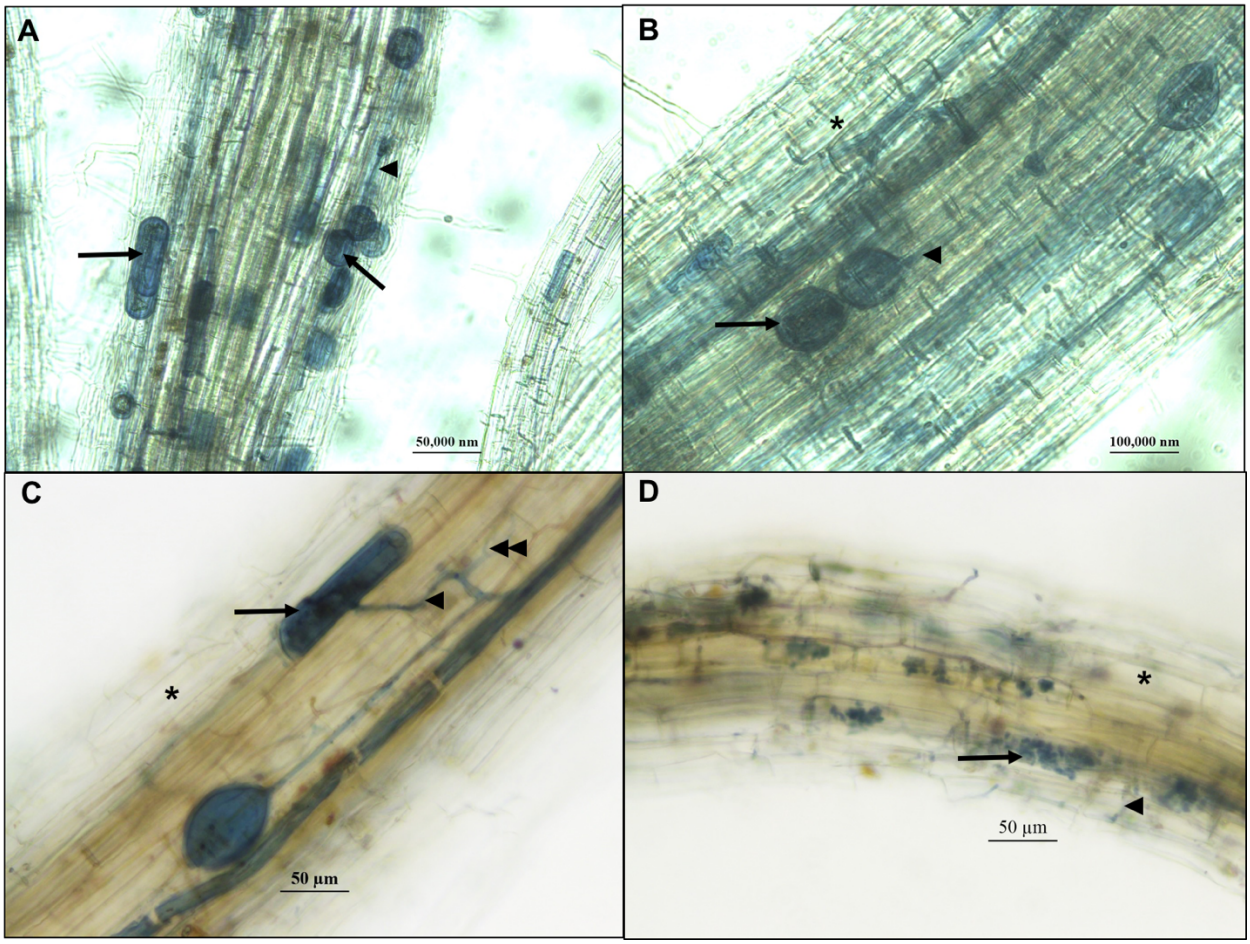

Figure 2.9 Cleared and stained roots of *Festuca ovina*, *Elymus trachycaulus* and *Hordeum jubatum* collected from Steeves Lake shoreline, Colomac Mine NWT, in August 2016. Root samples were cleared in a 10% KOH solution, stained with a 5% ink in vinegar solution and mycorrhizal structures were quantified using a modified version of the magnified intersections method (McGonigle et al., 1990).
A: A colonized area of an *F. ovina* root with visible vesicles (arrows) and intraradical hyphae (arrow head).
B: An area of an *H. jubatum* colonized root with visible vesicles (arrow) and subtending hyphae (arrow head). Surrounding, non-colonized cortical cells appear translucent (*)
C: A colonized section of *E. trachycaulus* root with vesicles (arrow), subtending hyphae (arrow head) and intercellular hyphae (double arrow), and non-colonized cortical cells (*)
D: A colonized area of an *E. trachycaulus* root with arbuscules (arrow) and intercellular hyphae (arrow head). Surrounding non-colonized cortical cells appear translucent (*).

Chapter 3: Greenhouse study examining the effects of commercial diesel fuel on northern plants, with and without an association with arbuscular mycorrhizal fungi

3.1 Abstract

The accidental release of petroleum hydrocarbons into the environment is a common problem across Canada, and especially harmful in northern locations were site access and cold temperatures make remediation difficult. This study was conducted to determine the effects of these spills in northern ecosystems as there is little research on how northern plants respond to contamination, or specifically how northern plants associated with arbuscular mycorrhizal fungi respond to diesel fuel, a common type of fuel spilled in remote northern locations. It was hypothesized that plants would be negatively affected by diesel fuel, and that these affects would increase with increasing concentrations of diesel fuel. It was also predicted that an association with arbuscular mycorrhizal fungi may convey a protective effect on plants affected by diesel fuel. One non-mycorrhizal plant species, *Carex aquatilis*, and two mycorrhizal species *Elymus trachycaulus* and *Erigeron acris* were collected from a northern site and grow in soils contaminated with three concentrations of commercial diesel fuel (3,460, 6,500 and 10,000 mg/kg) and un-contaminated soil. Plants were inoculated with a mixture of mycorrhizal fungi and bacteria isolated from soil samples taken from the same northern site, or with bacteria only. The response to diesel contamination varied between the three species, though results suggest that the two highest concentrations, 6,500 mg/kg and 10,000 mg/kg have significant, negative effects on plant growth. *Carex aquatilis* shoot/root ratios were significantly reduced in plants growing in 6,500 mg/kg diesel, as were *Erigeron acris* shoot/root ratios, and root and total fresh weight, and shoot surface area in *Elymus trachycaulus*. A comparison of plant growth between mycorrhizal and non-mycorrhizal plants indicated that there may be a protective effect conveyed to plants associating with mycorrhizal fungi. *Erigeron acris*, one of the mycorrhizal species,

inoculated with both fungi and the bacterial wash consistently had greater shoot and root fresh weight and surface area than plants that received a bacterial wash only, whereas in most of the *Carex aquatilis* growth responses there were no differences between inoculum treatments. This study has demonstrated that different plant species respond differently to diesel contamination, and concentrations of diesel fuel 6,500 mg/kg and greater can significantly negatively affect northern plants.

3.2 Introduction

In the north, there are many remote areas that rely on diesel fuel for power generation as they lack access to electric grids; consequently, there is a greater likelihood of fuel oil spills (Standing Senate Committee on Energy, the Environment and Natural Resources 2015). The accidental release of fuel into the environment is a common occurrence in the Northwest Territories, and the number and size of spills has been growing. Between 2015 and 2016, fuel oil spills increased by 2% which corresponded to an increased volume of 62,599 L of fuel oil spilled (ENR, 2016). The same levels of contamination in warmer areas have a more detrimental impact in the north as low temperature ecosystems are adapted to harsh conditions that make them more sensitive to contamination. Biodegradation of contaminants is slower in the north due to limitations caused by cold temperatures and low nutrient levels. Low temperatures can increase the viscosity of spilled fuel, which limits the bioavailability of contaminants by slowing diffusion of degradable hydrocarbons to the fuel-water interface where they come into contact with microbes and are broken down (Atlas, 1981, Atlas & Bragg, 2009), and the limited availability of nutrients such as nitrogen and phosphorous in northern ecosystems may also delay the biodegradation of contaminants (Margesin & Schinner, 1999; Snape et al., 2003).

A reduction in growth of plants exposed to PHCs is common and may be due to oxidative stress and negative effects on plant physiology. Liu et al. (2009) examined the effect of polycyclic aromatic hydrocarbons (PAHs) on *Arabidopsis thaliana* using phenanthrene contaminated medium and found that, as well as reduction in germination and root length, *A. thaliana* plants growing in phenanthrene contamination had significantly lower leaf chlorophyll concentrations, and significantly higher concentrations of two antioxidant enzymes, superoxide dismutase and peroxidase, and hydrogen peroxide. Petroleum hydrocarbon contamination can

trigger oxidative stress in plants and induce the formation of reactive oxygen species such as superoxide, hydrogen peroxide, and hydroxyl groups, as well as increased levels of antioxidant enzymes to mitigate the effects of these free radicals such as superoxide dismutase, catalase and peroxidase (Parida et al., 2004). These antioxidant enzymes relieve oxidative stress by converting reactive oxygen species to innocuous substances; superoxide dismutase interacts with superoxide anions, creating an intermediate, hydrogen peroxide, which interacts with catalase and peroxidase to produce water and oxygen (Matés, 2000). PHC exposure may also affect plant protein content, and photosynthetic activity. Tran et al. (2018) observed reduced chlorophyll and protein content in *Acacia raddiana* seedlings growing in soil contaminated by oil spills in 1997 and 2014. Significant reductions in chlorophyll and protein content were observed in leaves, and reduced protein content in the roots of plants growing in contaminated compared to non-contaminated soils (Tran et al., 2018). Kummerová et al. (2010) observed a significant decrease in net photosynthetic rate in *Pisum sativum* plants grown in fluoranthene contaminated medium after 21 days compared to control plants. These observations suggest that the reductions in plant growth commonly observed in plants affected by PHC contamination are due to a variety of plant physiological responses to PHC induced stress.

Petroleum hydrocarbon contamination has been shown to reduce AMF spore germination (Franco-Ramírez et al., 2007), reduce hyphal infectivity (Cabello, 1997) and there is evidence that some PAHs can affect phosphorous transport between AMF and host roots. Colonne et al. (2014) looked at the effect of two PAHs, anthracene and benzo[a]pyrene, on the mycorrhizal fungus *Rhizophagus irregularis* and transformed chicory roots growing in contaminated medium. Phosphorous (P) uptake from the medium by mycorrhizal mycelium, P translocation and P transport into chicory root was monitored through radiolabelled ^{33}P to determine if there

was an impact of PAHs on one or more of these steps. They found that when extraradical mycelium was grown in benzo[a]pyrene or the combination of both PAHs, there was a decrease in phosphorous quantity in chicory roots compared to un-contaminated controls. By monitoring the ^{33}P, they concluded that AMF mycelium could take up phosphorous, but PAHs affected a step in P transport to plant roots. These results suggest the that, even if plant can form AMF associations in contaminated soils, the relationship may be altered, and be less beneficial to the plant host.

It has been shown that plants and their associated mycorrhizal fungi can contribute to the dissipation of PHCs in soil, Joner & Leyval et al. (2003) observed significant reductions in PAH concentrations around clover and ryegrass roots associated with AMF compared to planted treatments without AMF. It has also been noted that AMF convey a protective effect on plants growing in contaminated soils. *Lolium multiflorum* grown in 6,000 mg/kg crude oil had significantly higher total dry mass and leaf area when inoculated with *Glomus intraradices* than plants grown without AMF inoculation (Alarcón et al., 2008). Tang et al. (2009) demonstrated that inoculation with *Glomus constrictum* increased the height and basal diameter, as well as chlorophyll and protein content in *Zea mays* plants grown in diesel contaminated soils compared to non-inoculated plants. These results indicate that plants can establish a relationship with AMF under PHC stress, though many of these studies have been conducted in southern sites that do not face the same limitations as northern ones. It is important to understand the effects of PHCs on northern plants, and which species can survive long enough in contaminated soils to establish a relationship with AMF, and contribute to the remediation of contaminated soils.

The Guidelines for the Closure and Reclamation of Advanced Mineral Exploration and Mine Sites in the Northwest Territories (MVLWB & AANDC, 2013) and the Canada – Wide

Standard for Petroleum Hydrocarbons (PHC) in Soil (PHC CWS) (CCME 2008) have both made recommendations for reclamation and remediation research that form the foundation for this chapter. The MVLWB & AANDC guidelines have recommended that reclamation research be conducted to help shape reclamation plans, especially since there is a lack of research applicable to northern sites that considers northern vegetation and the use of other living organisms to enhance natural re-vegetation. Consequently, this study will add to current knowledge about northern plants and their associated mycorrhizal fungi by testing the effects of diesel fuel on northern plant species growing with, or without an association with mycorrhizal fungi isolated from a northern site.

The PHC CWS (2008) identifies a need for research on the effects of specific PHC mixtures and studies on a broader range of soil organisms as the current knowledge is limited. The PHC CWS currently uses a three-tiered standard which was developed for the protection of environmental and human health. These are applied across four generic land use categories including industrial, commercial, residential/parkland and agriculture. In this approach, soil quality benchmarks have been developed for individual PHC fractions (F1, F2, F3, F4) which correspond roughly to whole products. For example, kerosene corresponds to the F2 fraction (nC9 to nC17) and diesel corresponds roughly to 50% F2 and 50% F3 fractions (nC9 to nC20). The plant species used to develop these benchmarks were alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), northern wheatgrass (*Agropyron dasystachyum*). Since these assessments utilize specific individual fractions, and diesel is comprised of both F2 and F3 fractions, knowledge of the effects of diesel as a whole product on plants is limited. The PHC CWS Scientific Rationale cites only one study looking at the effects of diesel fuel as a whole; a study by Marwood et al. (1998) is cited where the effects of fresh diesel were tested on lettuce seed

germination. This reductionist approach has consequences for setting guideline concentrations. Data derived from studies using single fractions may not be a useful predictor of the effects from whole product, and it is the whole product that is often a contaminant being spilled at mine sites (ENR, 2015). Though the CCME guidelines include toxicity data for many soil dwelling invertebrates and for individual PHC fractions, it is recognized that the range of organisms should be expanded to include northern vegetation and AMF, and on the toxicity of PHC mixtures, especially mixtures like diesel fuel, a common contaminant (ENR, 2015).

To address the lack of data on northern plant and mycorrhizal response to diesel contamination, this study was being performed to determine how northern plants will respond to diesel stress, and how an association with AMF will affect this response. From results reported in previous literature it was expected that plants would respond negatively to diesel contamination with effects manifested as reduced biomass, or changes in biomass allocation. It was also predicted that an association with AMF may protect plants from stress experienced in contaminated soils. To test these hypotheses this study was performed to 1) assess the effects of diesel contamination on northern vegetation under controlled conditions and 2) assess whether an association with AMF conveys a beneficial effect on plants grown in soils impacted with diesel fuel. It was predicted that plants may be able to survive all diesel concentrations, but there would be significant, negative effects on plant growth response with increasing diesel concentrations. To determine if plants stress is alleviated when associated with AMF, plant performance between plants growing with and without AMF inoculum can be compared, as well as performance between mycorrhizal and non-mycorrhizal plant species. It was expected that plants inoculated with AMF would less negatively affected by contamination compared to those that did not

receive AMF, and that in non-mycorrhizal plants there would be no difference in performance, regardless of inoculation.

The diesel concentrations used in this study were based on site-specific guidelines for PHCs in soil at Colomac mine (6,500 mg/kg), guidelines obtained from the CCME for PHCs in coarse agricultural soils (3,460 mg/kg), and a high-level concentration (10,000 mg/kg) previously shown to elicit effects on plants and mycorrhizal fungi (Kirk et al., 2005; Tang et al., 2009; Redondo-Gómez et al., 2014).

3.3 Methods and Materials

3.3.1 Plant species chosen for this study

Three plant species, two mycorrhizal (*Elymus trachycaulus* and *Erigeron acris)* and one non-mycorrhizal (*Carex aquatilis*), were chosen for this experiment (Figure 3.1). All three grow along Steeves Lake shoreline and were found within the quadrats sampled. *E. trachycaulus*, commonly known as slender wheatgrass, was planted at the site as part of the native grass seed mix used in the re-vegetation. *E. trachycaulus* is a perennial grass native to the NWT. It is a fast-growing grass with a life span of about 4 years and is commonly used in reclamation (USDA, 2002). *E. acris*, commonly called bitter fleabane, was not planted as part of the original re-vegetation, though is also native to the NWT (USDA, 2002). *C. aquatilis,* or water sedge, is native to the NWT and was planted during the re-vegetation as plugs which were collected from the surrounding area (Flat River Consulting, 2011).

3.3.2 Inoculum treatments

The inoculum for this study was obtained from the soil cores taken during the 2015 sampling. The cores were thawed, homogenized, and mixed with sterile peat (Sun Gro ® Peat Moss Grower Grade Green, Sungro Horticulture) and sand (Nepheline syenite, Unimin Canada

Ltd.) to obtain a substrate for the trap plants, *Elymus trachycaulus* and *Erigeron acris*. Seeds of these plants were germinated in petri dishes on filter paper for 2-3 days; germination was considered to have occurred as soon as the radicle emerged. Once seeds had germinated the seedlings were transplanted onto the substrate which was contained within one 1x1x 0.1 m container. The trap plants were grown in this container under greenhouse conditions (16/8 light/dark cycle and temperature 24-28 °C) for 3 months, receiving moderately hard artificial fresh water (Water Environmental Federation & American Public Health Association, 2005) every other day until the soil was saturated. Plants also received weekly 1/50 strength Long Ashtons nutrient solution (Table A 3.1) which was added to the artificial fresh water. After three months, all the trap plants within the container were harvested and the shoots were separated from the roots. The roots of all the trap plants were combined; this included both *E. trachycaulus* and *E. acris* roots. During the harvest, some of the trap plant roots were set aside to check that the roots had been successfully colonized by AMF using the same methods as those described previously. Five root subsamples were analyzed and colonization by AMF was confirmed in all subsamples. Mean hyphal colonization of the subsamples was 83.2 ± 5.6%, vesicular colonization 20.8 ± 2.2% and arbuscular colonization was 20.6 ± 5.9% (Table A 3.2)

The remaining trap plant roots were rinsed with deionized water to remove large soil particles and cut into 0.5 cm segments. These root segments were suspended in moderately hard artificial fresh water and the suspension was shaken by hand. The suspension containing root segments was divided into two equal parts, corresponding to the two inoculum treatments used in this study. The suspended root segments for the treatment receiving mycorrhizal propagules did not require further processing and was placed in a refrigerator at 4°C until needed. This treatment

included mycorrhizal propagules within or attached to the root segments, as well as the bacterial community.

The other half of the root segment suspension was prepared for the treatment not receiving mycorrhizal propagules. The roots were separated from the liquid in the suspension and placed in a separate flask which was autoclaved to kill any viable AMF propagules within, or attached to the roots. The liquid from this suspension was filtered through a 3μm filter paper to remove mycorrhizal propagules suspended within the liquid. The filtered liquid was then added back to the autoclaved root segments and stored at 4°C until needed. This treatment included sterilized roots and a filtrate containing the bacterial community (Figure 3.2).

3.3.3 Experimental design

Pots used in this study were sterilized by soaking in 10% bleach for 24 hours. The soil used was a 4:1 (w: w) mixture of sterile peat: sand; the peat and sand were autoclaved separately before being measured out and mixed. Each pot received 200 g of the sterile soil mixture and 5 g of the root segment suspension. To prepare the different inoculum treatments, each pot was filled with 140 g of the soil mixture, 5 g of inoculum was layered on top and covered with 60 g of the soil mixture. All the pots were filled on a top-loading balance to ensure equal soil mass.

The *Elymus trachycaulus*, *Erigeron acris*, and *Carex aquatilis* seeds used in this study were collected from Steeves Lake shoreline and refrigerated at 4 °C until needed. The seeds were sterilized for 1 minute in 0.1% bleach and germinated in petri dishes on filter paper in a growth chamber (Bio Chambers, Model TPC-19) under a 25/18 °C, 16/8 hour, day/night cycle. Germinated seedlings were transplanted to the experimental pots as soon as the radicle emerged. After transplanting, the pots were watered daily with 10 ml of moderately hard artificial fresh water with 1/50 strength Long Ashton nutrient solution. Plants were grown in a greenhouse with

a 16/8-hour day/night cycle and average day/night temperature of 26/19 °C for 5 weeks, and then another 6 weeks after the soil was spiked with diesel fuel.

Diesel Concentrations and Preparation

The diesel used for this study was bought at a Canadian Tire gas station. The fuel was placed under a fume hood and stirred with a magnetic stir bar for 3 days to allow for evaporation of the most volatile compounds. Samples were taken from the fuel at this point and sent to the University of Waterloo for analyses of PHC Fractions 1 to 4 (Table A 3.3). The analysis was performed using a modified version of the Canadian Council of Ministers of the Environment (CCME) method "Reference Method for the Canada-Wide Standard for Petroleum Hydrocarbons (CWS-PHC) in Soil – Tier 1 Method" (2008). This method used a methylene chloride solvent extraction followed by gas chromatography-flame ionization detection (GC-FID).

The experimental pots were spiked with diesel fuel using glass syringes to simulate a fuel spill within a plant community. The diesel treatment consisted of three diesel concentrations (3,460, 6,500, and 10,000 mg/kg soil as well as a control (0 mg/kg). These corresponded to CCME guidelines, Colomac site specific guidelines, and a concentration that has been shown to have significant negative effects on plants and mycorrhizal fungi (Tang et la. 2009).

To achieve the first concentration (3,460 mg/kg), 0.2 ml of diesel fuel was injected into each pot at one location. For consistency, each pot receiving this concentration of fuel was injected at the same distance from the plant in the pot. Fuel was injected into the soil 1 cm to the right of the plant, and 3 cm below the top of the pot. The syringes were marked on the needle to ensure the injection was made at the same depth in each pot. To achieve the second concentration (6,500 mg/kg), 0.7 ml of fuel was injected into each treatment pot at two locations. These injections were made 1 cm to the left and right of the plant, and at 3 and 5 cm below the

top of the pot. The third concentration (10,000 mg/kg), was achieved by injecting 2.5 ml of fuel into each pot at four locations. These injections were made 1 cm from each side of the plant, two at 3 cm and two at 5 cm below the top of the pot. The control pots (0 mg/kg) also received four 2.5 ml injections using water to control for any physical disturbance caused by the injections. Treatments included plants inoculated with AMF and a bacterial wash, and plants inoculated with bacterial wash only, growing in four diesel concentration (0, 3,460, 6,500 and 10,000 mg/kg). The experiment was laid out in a completely randomized design with eight replicates per treatment.

3.3.4 Analysis of plant growth response

Plant performance was assessed using shoot and root fresh weight, shoot surface area and shoot to root ratio. Fresh weights were determined immediately after the plants were harvested using a Mettler Toledo NewClassic MS analytical balance. Shoots were scanned with an Epson Expression 10000 XL Scanner and surface area determined using WinRhizo Arabidopsis 2012d software (Regent Instruments, Quebec, Canada).

3.3.5 Data analysis

There were 3 different species used in this experiment; due to differences between plant species, all the performance data were analyzed separately for each species. Plant growth responses were analyzed using a two-way analysis of variance (ANOVA) in JMP (Statistical Analysis Software version 11.0.) with diesel and inoculum as main effects and diesel x inoculum as an interaction effect. Log and square root transformations were used when data did not meet the assumptions of normality (Table A 3.4) and equal variance (Tables A 3.5 - 7). Data that could not be transformed were analyzed using a non-parametric Kruskal-Wallis test. ANOVA testing yielding significant effects was followed by a Student's t test to determine which means

were significantly different. To answer the research questions, plant performance at the three diesel concentrations was compared to control values, and performance of inoculated plants compared to non-inoculated.

3.4 Results

Over the course of the experiment there were a few cases of mortality, of the 192 plants used in this study, three *Erigeron acris* plants died.

3.4.1 Carex aquatilis growth response

Carex aquatilis plants growing in the two highest diesel concentrations (6,500 mg/kg and 10,000 mg/kg) had significantly reduced shoot/root ratio than plants grown in the uncontaminated control. The ratio of shoot to root fresh weight was 0.09 ± 0.01 and 0.10 ± 0.01 at the highest diesel concentration (10000 mg/kg) and at the Colomac guideline concentration (6,500 mg/kg), respectively, both of which were significantly lower than the control, 0.15 ± 0.01 (Figure 3.3) There was also a significant reduction in the shoot surface area of *C. aquatilis* plants grown in 10,000 mg/kg diesel, though this reduction was only observed in the plants that were inoculated with bacterial wash; plants inoculated with AMF and the bacterial wash did not have reduced shoot surface area in any of the diesel concentrations compared to controls (Figure 3.4). There were no significant effects of diesel, type of inoculum or the interaction of diesel and inoculum type on the shoot fresh weight, root fresh weight, or total fresh weight of *C. aquatilis*. This indicates that, though diesel fuel significantly affected biomass allocation in *C. aquatilis* plants, it did not affect overall all fresh biomass (Table 3.1).

3.4.2 Elymus trachycaulus growth response

Elymus trachycaulus shoot, root and total fresh weight and shoot surface area were all significantly affected by inoculum, or the interaction between inoculum and diesel (Table 3.2).

There was a significant effect of inoculum treatment on *E. trachycaulus* shoot fresh weight; shoot fresh weight was significantly greater in plants that received AMF inoculum and the bacterial wash compared to those that received the bacterial wash only (Figure 3.5).

Root fresh weight and total fresh weight of *E. trachycaulus* plants were significantly affected by the interaction between diesel and inoculum. Root fresh weight (Figure 3.6) and total fresh weight (Figure 3.7) showed the same response to the interaction between diesel concentration and inoculum treatment. Plants growing in the control treatment (0 mg/kg diesel), the lowest concentration (3,460 mg/kg) and the highest concentration (10,000 mg/kg) that received fungal inoculum had a significantly greater root and total fresh weight than plants that received the bacterial wash only, though significantly lower root and total fresh weight at 6,500 mg/kg. Within the treatment receiving both AMF and bacterial wash the responses of plants growing in the two highest concentrations were not as predicted; plants growing in 6,500 mg/kg diesel had significantly lower root and total fresh weight than control plants but *E. trachycaulus* plants growing in the highest diesel concentration (10,000 mg/kg) had significantly higher root and total fresh weight than control plants. Within the treatment that received only the bacterial wash there were no significant differences in root or total fresh weight between diesel concentrations.

E. trachycaulus shoot surface area was also significantly affected by the interaction between diesel and inoculum. Shoot surface areas was significantly greater in plants inoculated with both AMF and a bacterial wash than plants that received the microbial wash only in the uncontaminated control, and in two of the diesel treatments (3,460 mg/kg and 10,000 mg/kg), but in the plants growing in 6,500 mg/kg there was no differences in shoot surface area between inoculum treatments (Figure 3.8). Within plants that received only the bacterial wash, there were

no significant differences in shoot surface area between diesel concentrations, though in plants that received AMF and a bacterial wash, shoot surface area was significantly lower in plants grown in 6,500 mg/kg than all other diesel treatments including the control. There were no significant differences in *Elymus trachycaulus* shoot/ratio growing in any diesel concentrations compared to controls, and there were no significant differences between inoculum treatments (Table 3.3).

3.4.3 Erigeron acris growth response

All the performance variables measured in *Erigeron acris* plants were significantly affected by diesel, inoculum treatment, or the interaction of the two (Table 3.4). There were significant effects of diesel fuel and inoculum treatment on *E. acris* shoot fresh weight and shoot surface area; the same response to diesel fuel and inoculum treatment was observed for both growth responses. Shoot fresh weight (Figure 3.9) and surface area (Figure 3.10) were significantly lower in plants grown in the two highest concentrations (6,500 and 10,000 mg/kg) compared to plants growing in 3,460 mg/kg, though there were no differences compared to plants grown in uncontaminated soil. Between inoculum treatments, plants inoculated with AMF and the bacterial wash had significantly greater shoot fresh weight (Figure 3.11) and surface area (Figure 3.12) than plants that received the bacterial wash only.

The shoot/root ratio of *E. acris* plants tended to decrease with increasing diesel concentrations, though the only significant reduction in the shoot/root ratio from control plants was observed in plants grown in 6,500 mg/kg (Figure 3.13).

The root fresh weight and total fresh weight of *E. acris* plants responded the same way to diesel fuel and inoculum treatment. Plants that received both the AMF inoculum and the bacterial wash had significantly greater root and total fresh weight than plants inoculated only

with the microbial wash in the control, and in the two highest concentrations (6,500 mg/kg and 10,000 mg/kg), though in plants grown in 3,460 mg/kg diesel there was no difference between inoculum treatments (Figure 3.14; Figure 3.15).Within inoculum treatments there were no significant decreases in root or total fresh weight in any of the contaminated treatments compared to controls.

3.5 Discussion

The hypothesized trends in plant performance were based on plant responses to diesel fuel that have been reported previously in the literature (Palmroth et al., 2002; Tang et al., 2009; Hernández-Ortega., 2012) and a predicted protective effect of AMF (Joner and Leyval et al., 2003; Tang and Chen, 2009). It was expected that plants would respond to diesel fuel with reduced biomass or mortality in a linear way; growth decreasing with increasing diesel concentrations. There were three cases of mortality in *E. acris*, and some cases of decreased biomass among species, though these decreases did not follow hypothesized trends.

While total biomass of *C. aquatilis* did not differ between diesel concentrations, plants grown in 6,500 and 10,000mg/kg allocated more resources to root biomass than to shoot biomass; at both concentrations, the ratio of shoot to root mass decreased by about one and a half times. There was a similar trend in biomass allocation in *E. acris*. Generally, the shoot/root ratio decreased at each increasing level of diesel contamination, though only plants grown in 6,500mg/kg had a significantly different biomass allocation to roots from that of the control. It has been demonstrated in previous studies that plants often increase biomass allocation to roots in response to stress caused by hydrocarbon pollution (Adam and Duncan, 1999; Nie et al., 2010; Hernández-Ortega et al.; 2012, Redondo-Gómez.; 2014). This allocation may help alleviate stress from contamination such as reduced ability to take up water and compensate for root death

caused by direct toxic effects of PHCs (Racine, 1994). It has also been posited that this allocation is accompanied by increased carbon release from root systems to stimulate the microbial community. Using labelled $^{13}CO_2$, Nie et al. (2010) observed increased carbon allocation to the soil and to soil microbial biomass around the root systems of *Phragmites australis* grown in crude oil polluted soils, indicating that the carbon released belowground was being quickly turned over by the microbial community. Allocation of biomass to roots may depend on the diesel concentration; in a study by Adam and Duncan (1999), shoot/root ratio of *Lolium multiflorum* decreased by ten times in plants grown in 5,000 mg/kg diesel compared to controls, though only by four times in plants grown in 10,000 mg/kg, similar to results of *E. acris* shoot/root ratio. Changes in biomass allocation to the roots in response to PHC exposure was not observed in *E. trachycaulus* plants, possibly due to the fibrous root system of *E. trachycaulus*, common to species found in grass (Poaceae) family. Compared to those of other plant types, roots systems found in grasses have the largest root surface area per square metre of soil (Aprill and Sim, 1990) so these plants may not have needed to alter biomass allocation.

Plant growth may also have differed from predictions due to the medium used in this study. Plants were grown in 4:1 mixture of commercial soil made up of sphagnum peat moss (80%) and sterile sand (20%) (soil: sand, 4:1). Deiss et al (2004) conducted a study on the movement of diesel range organics (DRO) through peat soil columns and found that DROs have greater movement horizontally through peat than vertically; and in a field-scale study conducted in peatlands it was observed that both free-phase and dissolved hydrocarbons have limited mobility in peat soils compared to mineral soils (Gharedaghloo & Price, 2018). In our study, fuel was injected at different points into the soil rather than mixed into the soil. Due to the high proportion of peat used in the experimental pots, fuel movement may have been limited; the

diesel may have remained close to the injection point, therefore limiting the interaction between plant roots and contaminants. Barrutia et al. (2011) and Tang et al. (2009) observed decreases in biomass of grasses grown in 12,000mg/kg diesel and 10,000mg/kg diesel whereas this was not consistently observed in this study. It is possible that the differences in results between these studies and this current study could be the use of substrates less limiting to fuel movement. In the study conducted by Barrutia et al. (2011) a clay loam soil was used, and Tang et al. (2009) conducted their study using a field collected soil that had been ground and mixed with fine sand before use in the experiment, possibly allowing for greater interaction between plants and contaminants.

The interaction between plant growth, diesel and inoculum in *E. trachycaulus* and *E. acris* compared to *C. aquatilis* indicate that there was a protective effect conveyed by an association with AMF. There were consistent significant inoculum effects observed in *E. trachycaulus* and *E. acris* performance, but not in *C. aquatilis*, indicating that these effects were due to an association with AMF. The only significant difference in *C. aquatilis* growth between inoculum treatments was observed in shoot surface area at the highest diesel concentration (10,000 mg/kg). The interaction effect on shoot surface area was unexpected as *C. aquatilis* has been reported to be non-mycorrhizal (Thormann et al., 1999, Wang and Qiu, 2006) so there should not have been differences between inoculum treatments. As this difference was only observed in one performance variable and at one diesel concentration, it was concluded that AMF did not have a protective effect on *C. aquatilis* growth.

It is possible the AMF associated with *E. trachycaulus* did not survive 10,000 mg/kg diesel, this would explain the trends observed in *E. trachycaulus* root and total fresh weight in inoculated plants, both were significantly lower at 6,500 mg/kg than at other diesel

concentrations. There were no differences between diesel concentrations in plants inoculated with the microbial wash only, indicating that the trend observed in plants inoculated with both AMF and the bacterial wash is due to their association with AMF. Reductions in colonization and hyphal growth have been observed in concentrations lower than 6,500 mg/kg in roots grown in spiked media (Kirk et al., 2005; Driai et al., 2015), though not in soil. In soil, reductions in colonization have been observed at 10000 mg/kg diesel and higher (Tang et al., 2009; Redondo-Gómez et al., 2014). It may be that AMF associated with *E. trachycaulus* were able to survive 6,500 mg/kg diesel, but the association was strongly reduced at 10,000 mg/kg, causing plants to allocate more resources to root their root system to compensate.

The effect of diesel contamination on different plant species is difficult to predict; responses of other grasses growing in contaminated soil provide evidence for this (Palmroth et al., 2002; Tang et al., 2009 and Barrutia et al., 2011). Decreased biomass was observed in grass species when grown alone, and when multiple grass species were grown together at PHC concentrations below or near 10,000mg/kg, the highest concentration used in the current study. This indicates that while grasses have been shown to be affected by PHC contamination*, E. trachycaulus* may not be, as there was no significant decrease in plant growth in plants grown in 10,000 mg/kg diesel compared to control plants grown in clean soil. This has implications for remediation efforts, it has been suggested that grasses are useful in remediation efforts due to their extensive roots systems (Aprill and Sims 1990), though results from previous studies and this current study show that the choice of grass species may strongly effect the outcome of a remediation project.

Species- specific responses to diesel fuel have been previously reported (Adam and Duncan, 2002; Palmroth et al., 2002; and Shahsavari et al., 2013). Adam and Duncan (2002)

found that germination of seeds in diesel contaminated soils was highly dependent on species. Palmroth et al. (2002) found differences in plant biomass between legume and grass mixtures; the biomass of grasses growing in contaminated soils was only 43% of that of control biomass, whereas legumes biomass reached 64% of that of controls growing on the same concentration of diesel fuel. There is an effect of plant species on response to contamination as well as an effect of contaminant on response. Shahsavari et al. (2013) observed species- dependent effects as well as contaminant- dependent effects. Shortened root length was observed in two different species of clover grown in diesel and crude oil. *Medicago sativa* root growth was negatively affected by diesel but not by crude oil, while *Trifolium michelianum* was impacted by crude oil and not diesel. These species- dependent and contaminant- dependent effects may be the reason for the differences in performance between species in the current study. Our results demonstrate the importance of using specific products, like diesel fuel, when testing the effects of PHCs on plant performance, and using multiples plant species. There were effects on plant growth observed at the Colomac site-specific guidelines in the form of reduced shoot/root ratios in *C. aquatilis* and *E. acris* plants, and reduced fresh biomass and leaf surface area in *E. trachycaulus* plants. The Colomac guideline concentration is for total petroleum hydrocarbons in soil, though results suggest that when this guideline concentration is applied as a specific product (i.e. diesel), this guideline may recommend concentrations that are above levels found in this study to cause a change in plant growth Further studies are needed to determine if these changes in plant growth have impacts at a community level.

3.6 References

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3.7 Tables and Figures

Table 3.1 Summary of ANOVA showing the effects of diesel fuel, inoculum treatment, and their interaction on shoot, root and total fresh weight, shoot surface area and shoot/root fresh weight ratio of *Carex aquatilis*. Significant effects are bolded ($p < 0.05$).

Carex	Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Shoot Fresh Weight	Diesel	3	3	0.8830649	2.1803	0.1005
	Inoculum	1	1	0.1327115	0.9830	0.3257
	Inoculum*Diesel	3	3	1.0385466	2.5642	0.0637
Root Fresh Weight	Diesel	3	3	0.7091872	1.0996	0.3570
	Inoculum	1	1	0.0948881	0.4414	0.5092
	Inoculum*Diesel	3	3	1.1700822	1.8143	0.1550
Total Fresh Weight	Diesel	3	3	0.4726908	0.8058	0.4959
	Inoculum	1	1	0.1021477	0.5224	0.5728
	Inoculum*Diesel	3	3	1.0931223	1.8635	0.1463
Shoot/Root Ratio	Diesel	3	3	0.03487000	**6.6687**	**0.0006**
	Inoculum	1	1	0.00020146	0.1156	0.7351
	Inoculum*Diesel	3	3	0.00556967	1.0652	0.3713
Shoot Surface Area	Diesel	3	3	0.8863343	2.3202	0.0851
	Inoculum	1	1	0.1282321	1.0070	0.3199
	Inoculum*Diesel	3	3	1.1970193	**3.1335**	**0.0326**

Table 3.2 Summary of ANOVA showing the effects of diesel fuel, inoculum treatment, and their interaction on shoot, root and total fresh weight, shoot surface area and shoot/root fresh weight ratio of *Elymus trachycaulus*. Significant effects are bolded ($p < 0.05$).

Elymus	Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Shoot Fresh Weight	Diesel	3	3	0.00173371	0.6334	0.5966
	Inoculum	1	1	0.01175056	**12.8790**	**0.0007**
	Inoculum*Diesel	3	3	0.00705513	2.5776	0.0627
Root Fresh Weight	Diesel	3	3	0.8517440	**3.2199**	**0.0294**
	Inoculum	1	1	0.6023700	**6.8316**	**0.0115**
	Inoculum*Diesel	3	3	1.9446535	**7.3516**	**0.0003**
Total Fresh Weight	Diesel	3	3	0.9239635	**3.2822**	**0.0274**
	Inoculum	1	1	0.7823845	**8.3379**	**0.0055**
	Inoculum*Diesel	3	3	2.1766028	**7.7320**	**0.0002**
Shoot Surface Area	Diesel	3	3	11.843565	**3.4282**	**0.0231**
	Inoculum	1	1	18.357047	**15.9405**	**0.0002**
	Inoculum*Diesel	3	3	11.659710	**3.3749**	**0.0246**

Table 3.3 A summary of Kruskal-Wallis comparisons between *Elymus trachycaulus* root/shoot fresh weight ratio between inoculum treatments and diesel concentrations.

Shoot/Root Ratio by Diesel	Diesel Concentration (mg/kg)	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Elymus	0	16	616.000	520.000	38.5000	1.481
	3460	16	487.000	520.000	30.4375	-0.504
	6500	16	499.000	520.000	31.1875	-0.318
	10000	16	478.000	520.000	29.8750	-0.634
Chi Square (2.2554)	DF (3)	Prob>ChiSq (0.5211)				
Shoot/Root Ratio by Inoculum	Inoculum	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Elymus	M+AM	32	1127.00	1040.00	35.2188	1.161
	Mic	32	953.000	1040.00	29.7813	-1.161
Chi Square (1.3636)	DF (1)	Prob>ChiSq (0.2427)				

Table 3.4 Summary of ANOVA showing the effects of diesel fuel, inoculum treatment, and their interaction on shoot, root and total fresh weight, shoot surface area and shoot/root fresh weight ratio of *Erigeron acris*. Significant effects are bolded ($p < 0.05$).

Erigeron	Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Shoot Fresh Weight	Diesel	3	3	0.06979110	3.4995	**0.0216**
	Inoculum	1	1	0.21333121	32.0910	**<0.0001**
	Inoculum*Diesel	3	3	0.01753292	0.8791	0.4579
Root Fresh Weight	Diesel	3	3	0.12805349	1.8486	0.1496
	Inoculum	1	1	0.75449772	32.6762	**<0.0001**
	Inoculum*Diesel	3	3	0.23347300	3.3705	**0.0251**
Total Fresh Weight	Diesel	3	3	3.002908	1.7200	0.1740
	Inoculum	1	1	23.446838	40.2887	**<0.0001**
	Inoculum*Diesel	3	3	5.980708	3.4255	**0.0236**
Shoot/Root Ratio	Diesel	3	3	5.1066130	3.2387	**0.0292**
	Inoculum	1	1	0.5663317	0.8873	0.3505
	Inoculum*Diesel	3	3	1.9743489	1.2522	0.3002
Shoot Surface Area	Diesel	3	3	1.5840768	2.8178	**0.0478**
	Inoculum	1	1	7.5976068	40.5446	**<0.0001**
	Inoculum*Diesel	3	3	0.9170512	1.6313	0.1931

Figure 3.1 The plant species used in the greenhouse study, from left to right are Carex aquatilis, *Elymus trachycaulus* and *Erigeron acris*. These images were taken five weeks after seedlings were transplanted into the experimental pots.

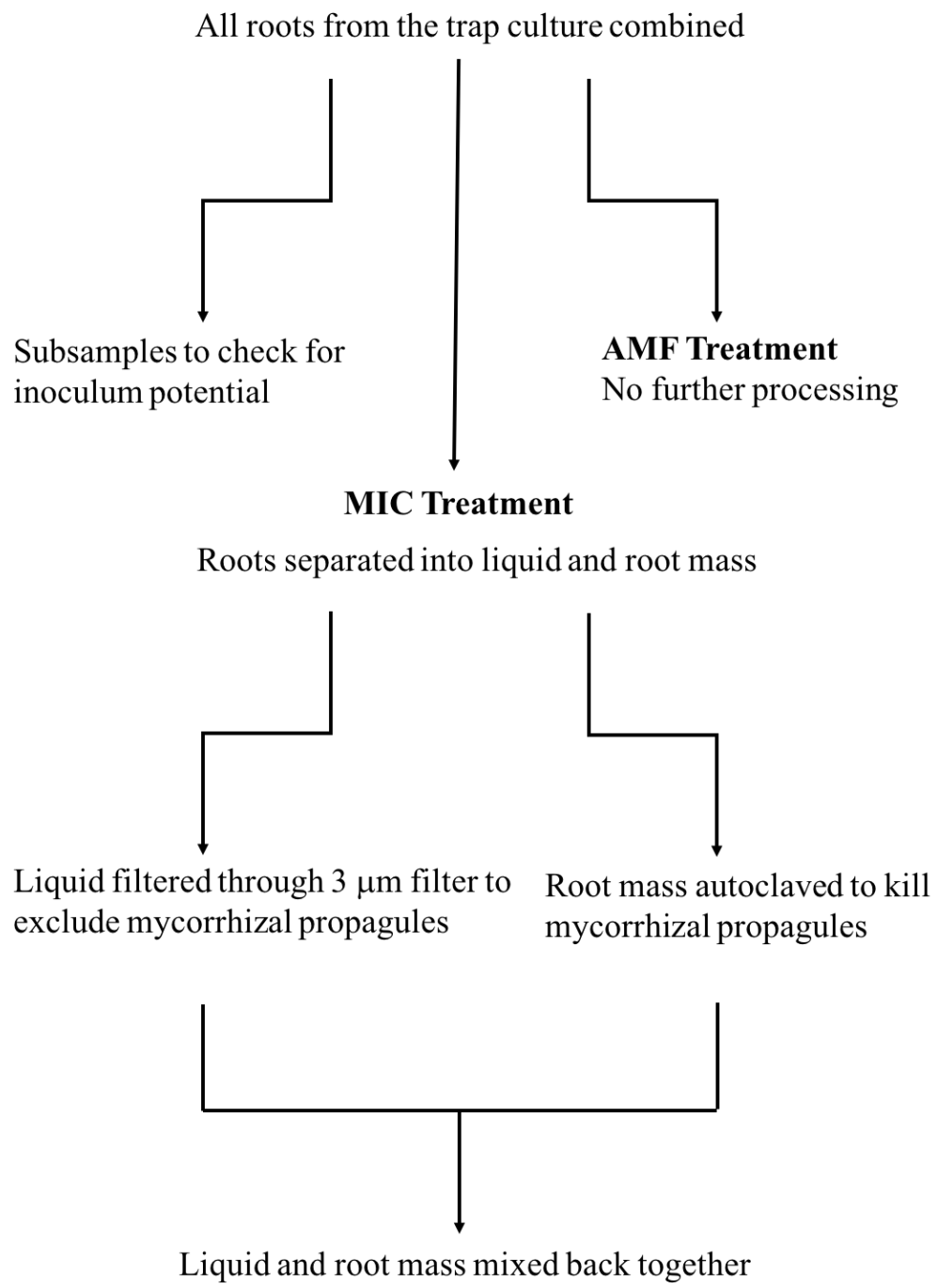

Figure 3.2 A flow chart summarizing how the inoculum for the two inoculum treatments (AM and MIC) were derived from the roots of trap culture plants. The AMF treatment included both root fragments and the bacterial wash. The MIC treatment included autoclaved root fragments mixed with bacterial wash to exclude fungal propagules but still provide a bacterial community to each experimental pot.

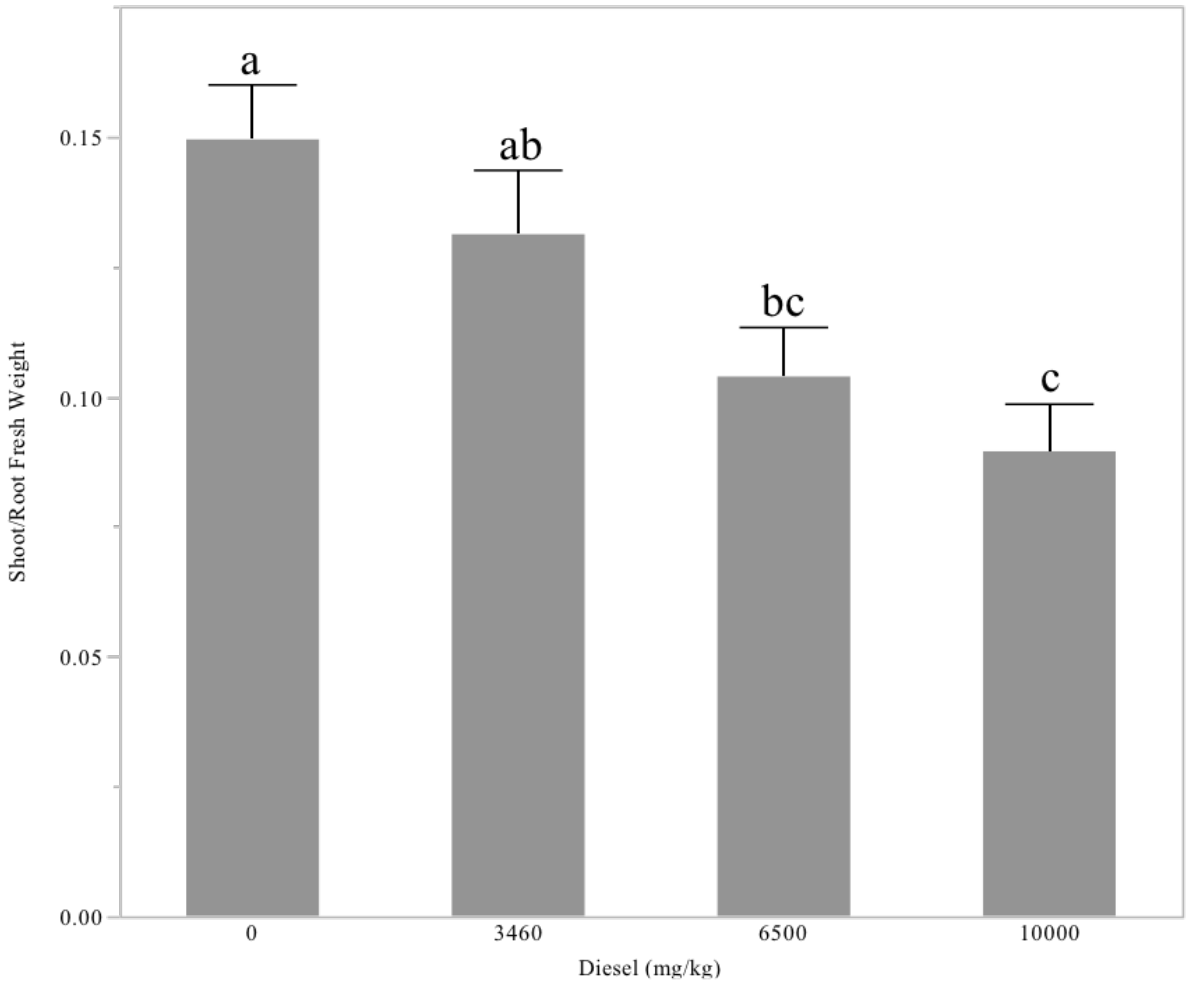

Figure 3.3 *Carex aquatilis* shoot/root fresh weight ratio in response to diesel contamination. Significant effects of diesel fuel on *C. aquatilis* shoot/root fresh weight were determined through a two-way analysis of variance (ANOVA), and differences between diesel concentrations were determined using Student's t multiple comparisons. Bars represent means ± 1 standard error and bars with the same letter are not significantly different.

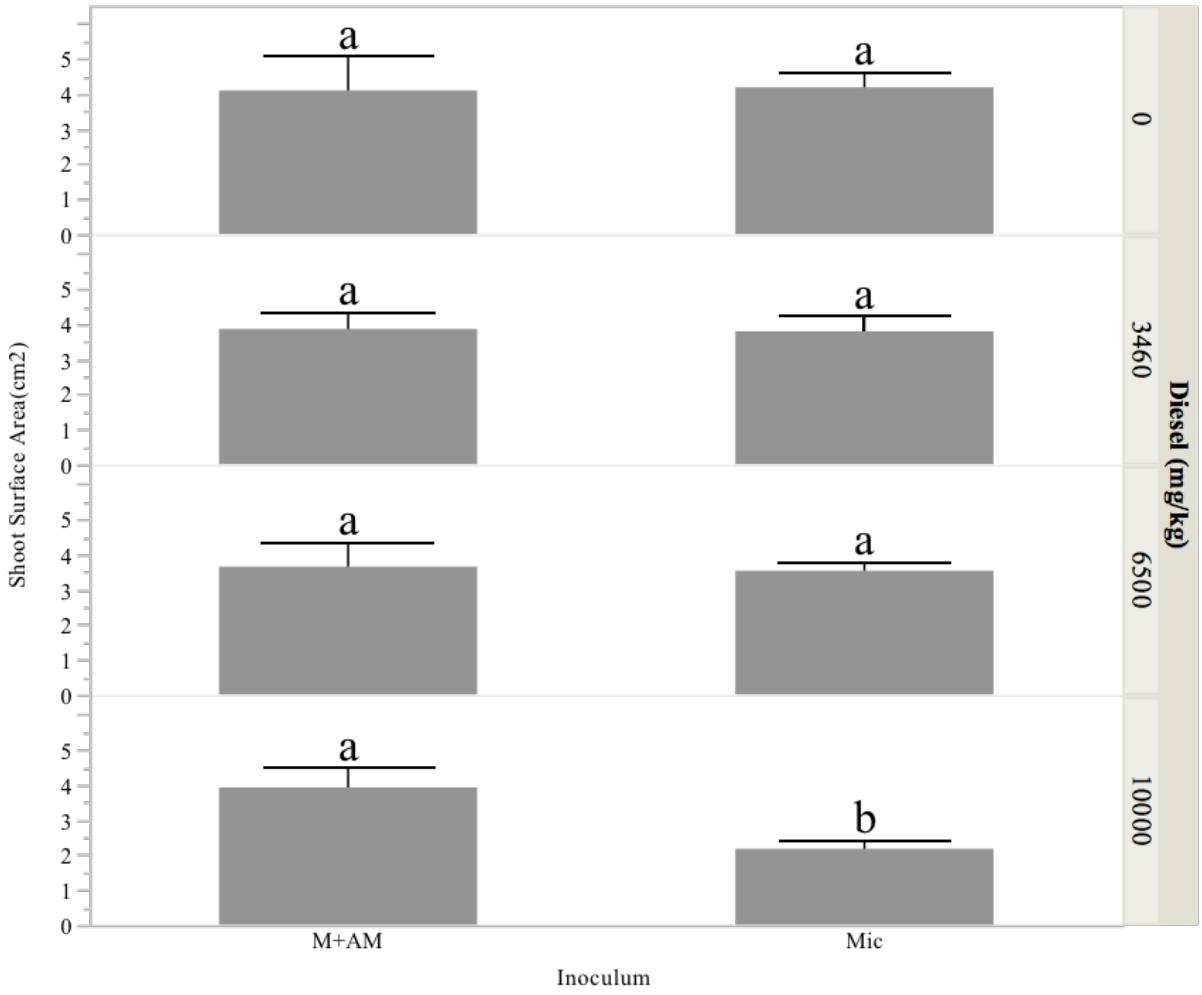

Figure 3.4 A comparison of *Carex aquatilis* shoot surface area (cm^2) between plants grown with mycorrhizal inoculum and a bacterial wash (M+AM) or grown with the bacterial wash only (Mic) at each concentration of diesel fuel (0, 3,460, 6,500, and 10,000 mg/kg). The effects of diesel fuel and inoculum treatment on *C. aquatilis* shoot surface area were determined through a two-way analysis of variance (ANOVA), and differences between diesel concentrations and inoculum treatments were determined using Student's t tests. Bars represent means ± 1 standard error and bars with the same letter are not significantly different.

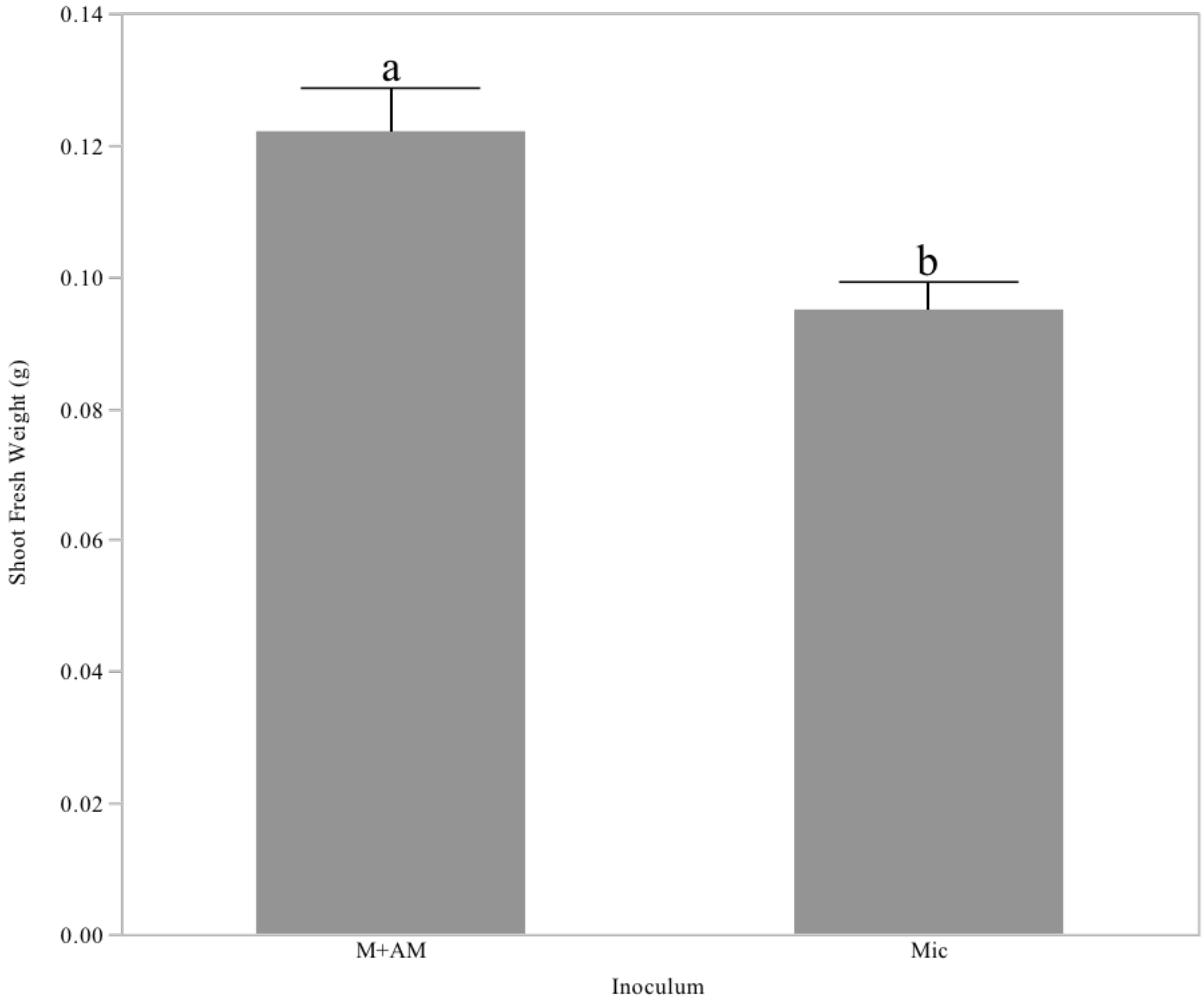

Figure 3.5 *Elymus trachycaulus* shoot fresh weight (g) in response to inoculum treatment. The effects of two inoculum treatments were assessed, plants that received both mycorrhizal inoculum and bacterial wash (M+AM) or plants that received the bacterial wash only (Mic). Significant effects of inoculum treatment on *E. trachycaulus* shoot fresh weight were determined through a two-way analysis of variance (ANOVA), and differences between diesel concentrations were determined using Student's t tests. Bars represent means ±1 standard error and bars with the same letter are not significantly different.

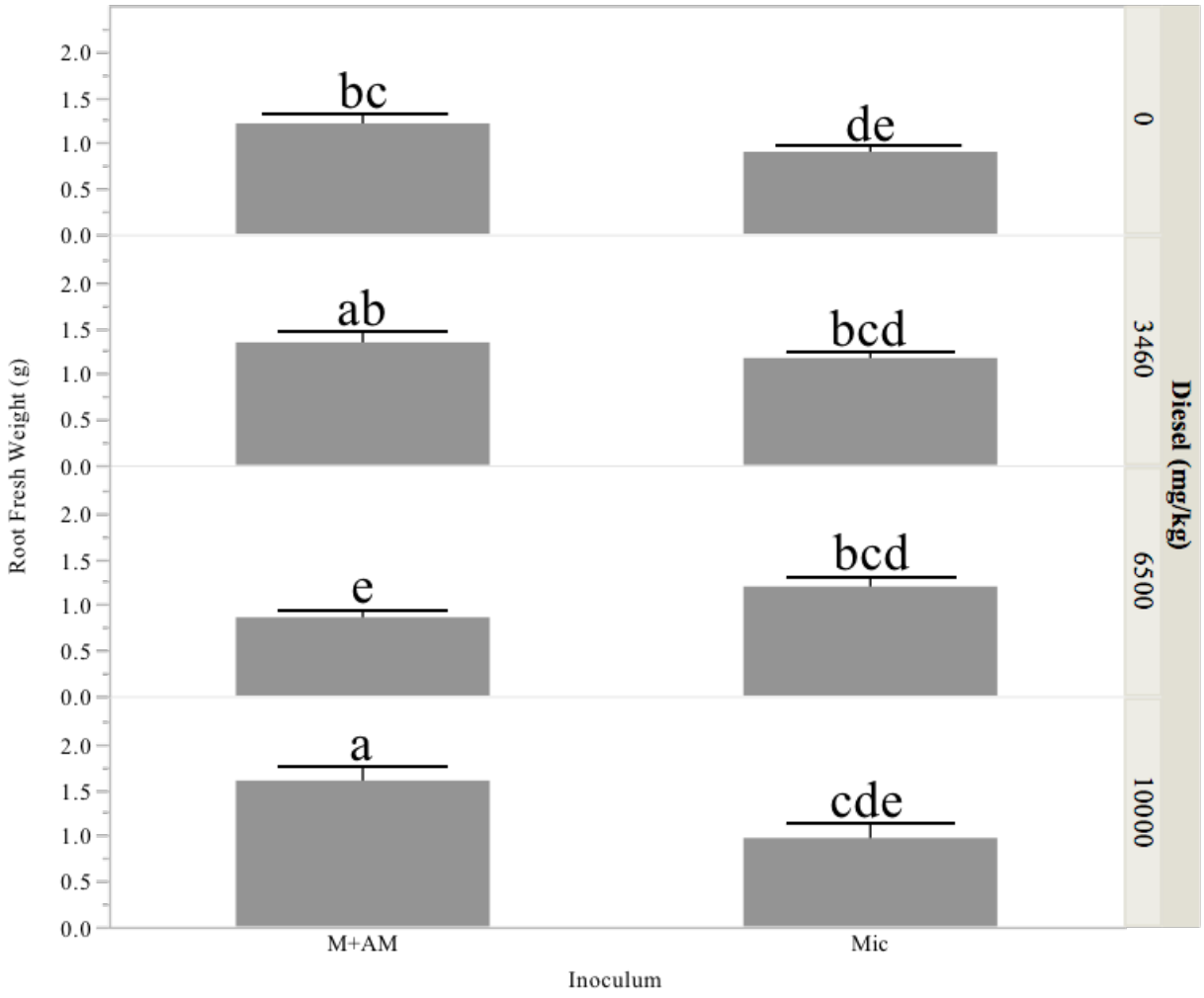

Figure 3.6 A comparison of *Elymus trachycaulus* root fresh weight (g) between plants grown with mycorrhizal inoculum and a bacterial wash (M+AM) or grown with the bacterial wash only (Mic) at each concentration of diesel fuel (0, 3,460, 6,500, and 10,000 mg/kg). The effects of diesel fuel and inoculum treatment on *E. trachycaulus* root fresh weight were determined through a two-way analysis of variance (ANOVA), and differences between diesel concentrations and inoculum treatments were determined using Student's t tests. Bars represent means ±1 standard error and bars with the same letter are not significantly different.

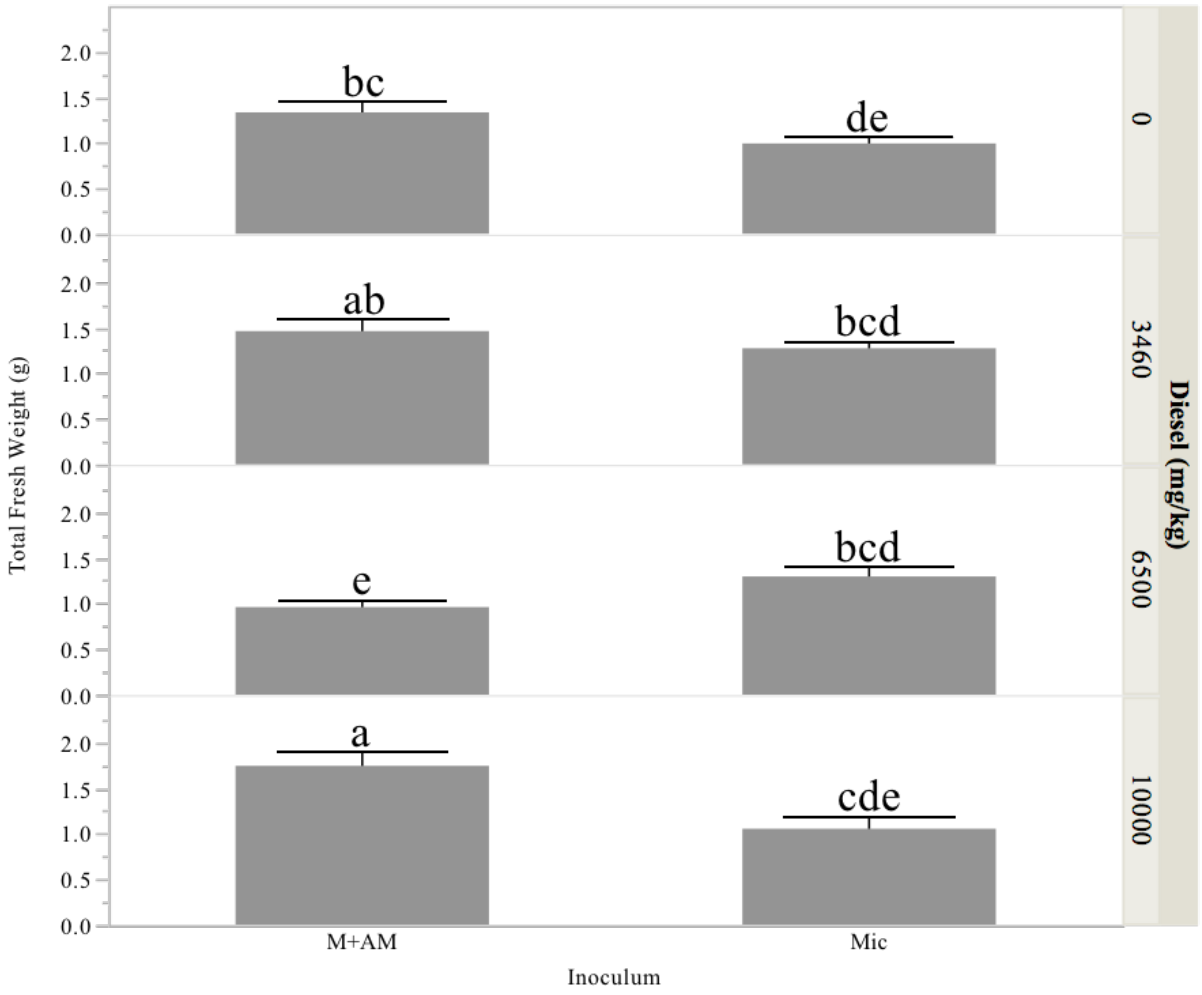

Figure 3.7 A comparison of *Elymus trachycaulus* total fresh weight (g) between plants grown with mycorrhizal inoculum and a bacterial wash (M+AM) or grown with the bacterial wash only (Mic) at each concentration of diesel fuel (0, 3,460, 6,500, and 10,000 mg/kg). The effects of diesel fuel and inoculum treatment on *E. trachycaulus* total fresh weight were determined through a two-way analysis of variance (ANOVA), and differences between diesel concentrations and inoculum treatments were determined using Student's t tests. Bars represent means ± 1 standard error and bars with the same letter are not significantly different.

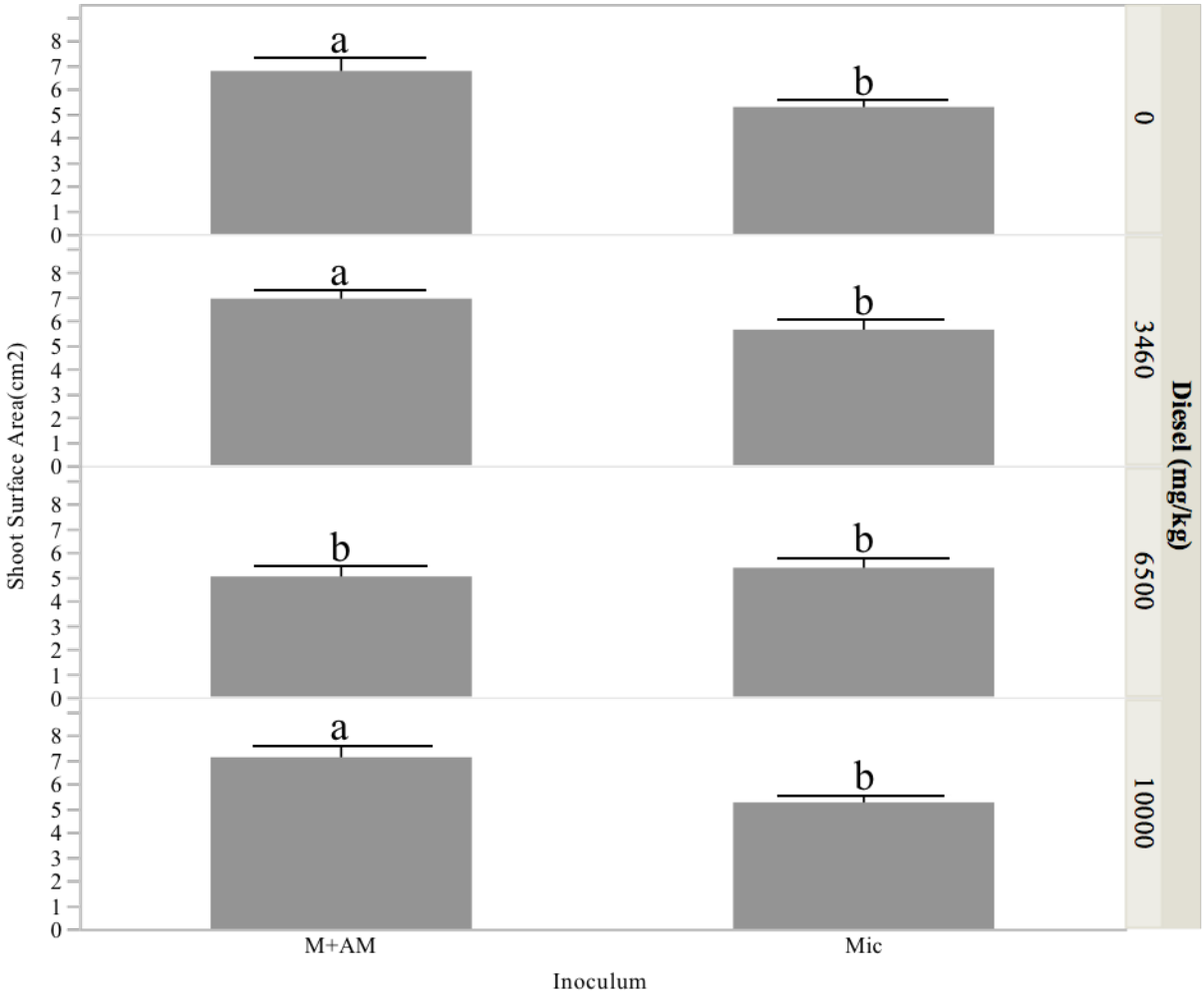

Figure 3.8 A comparison of *Elymus trachycaulus* shoot surface area (cm^2) between plants grown with mycorrhizal inoculum and a bacterial wash (M+AM) or grown with the bacterial wash only (Mic) at each concentration of diesel fuel (0, 3,460, 6,500, and 10,000 mg/kg). The effects of diesel fuel and inoculum treatment on *E. trachycaulus* shoot surface area were determined through a two-way analysis of variance (ANOVA), and differences between diesel concentrations and inoculum treatments were determined using Student's t tests. Bars represent means ± 1 standard error and bars with the same letter are not significantly different.

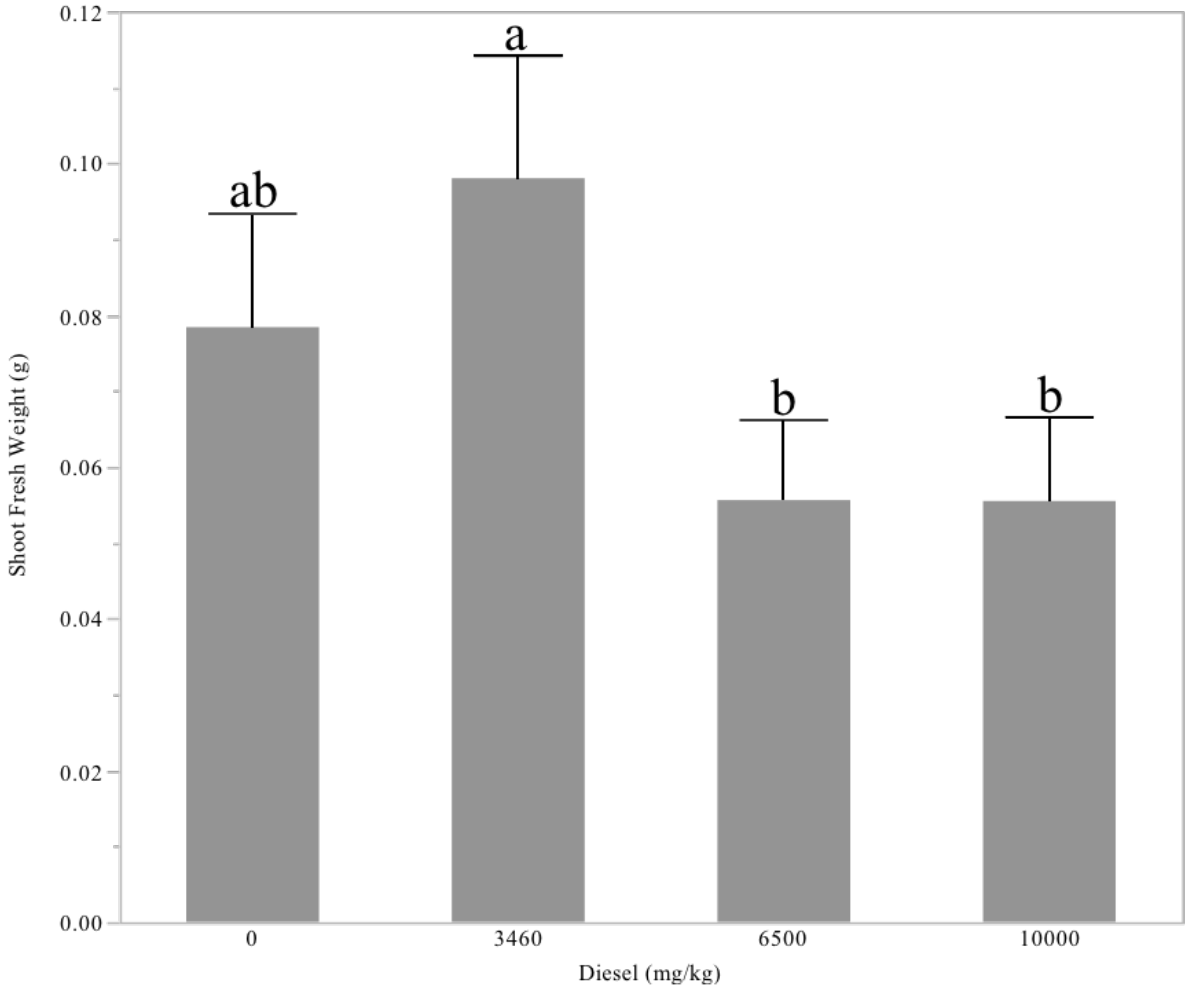

Figure 3.9 The response of *Erigeron acris* shoot fresh weight (g) to four concentrations of diesel contamination (0, 3,460, 6,500, and 10,000 mg/kg). Significant effects of diesel fuel on *E. acris* shoot fresh weight were determined through a two-way analysis of variance (ANOVA), and differences between diesel concentrations and inoculum treatments were determined using Student's t tests. Bars represent means ± 1 standard error and bars with the same letter are not significantly different.

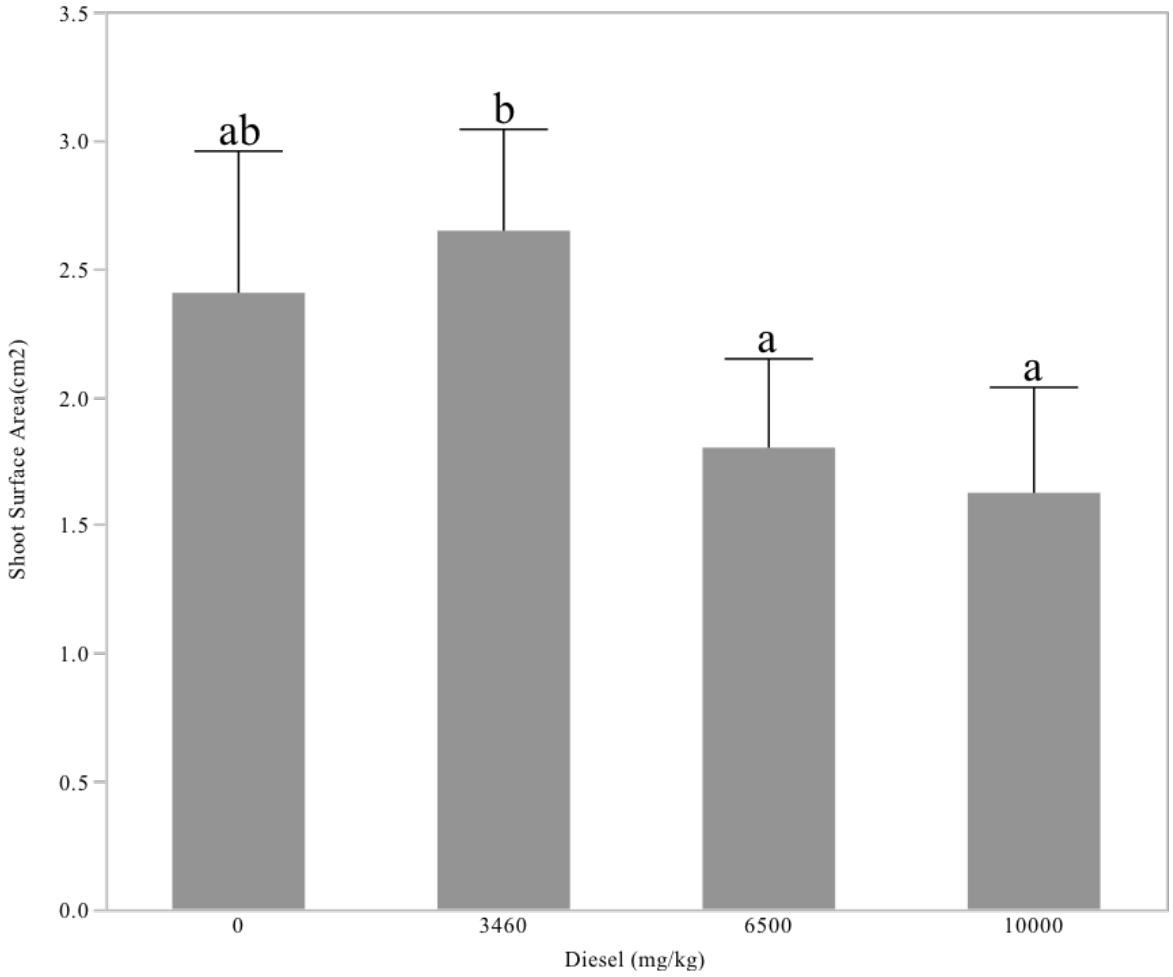

Figure 3.10 The response of *Erigeron acris* shoot surface area (cm^2) to four concentrations of diesel contamination (0, 3,460, 6,500, and 10,000 mg/kg). Significant effects of diesel fuel on *E. acris* shoot surface area were determined through a two-way analysis of variance (ANOVA), and differences between diesel concentrations and inoculum treatments were determined using Student's t tests. Bars represent means ± 1 standard error and bars with the same letter are not significantly different.

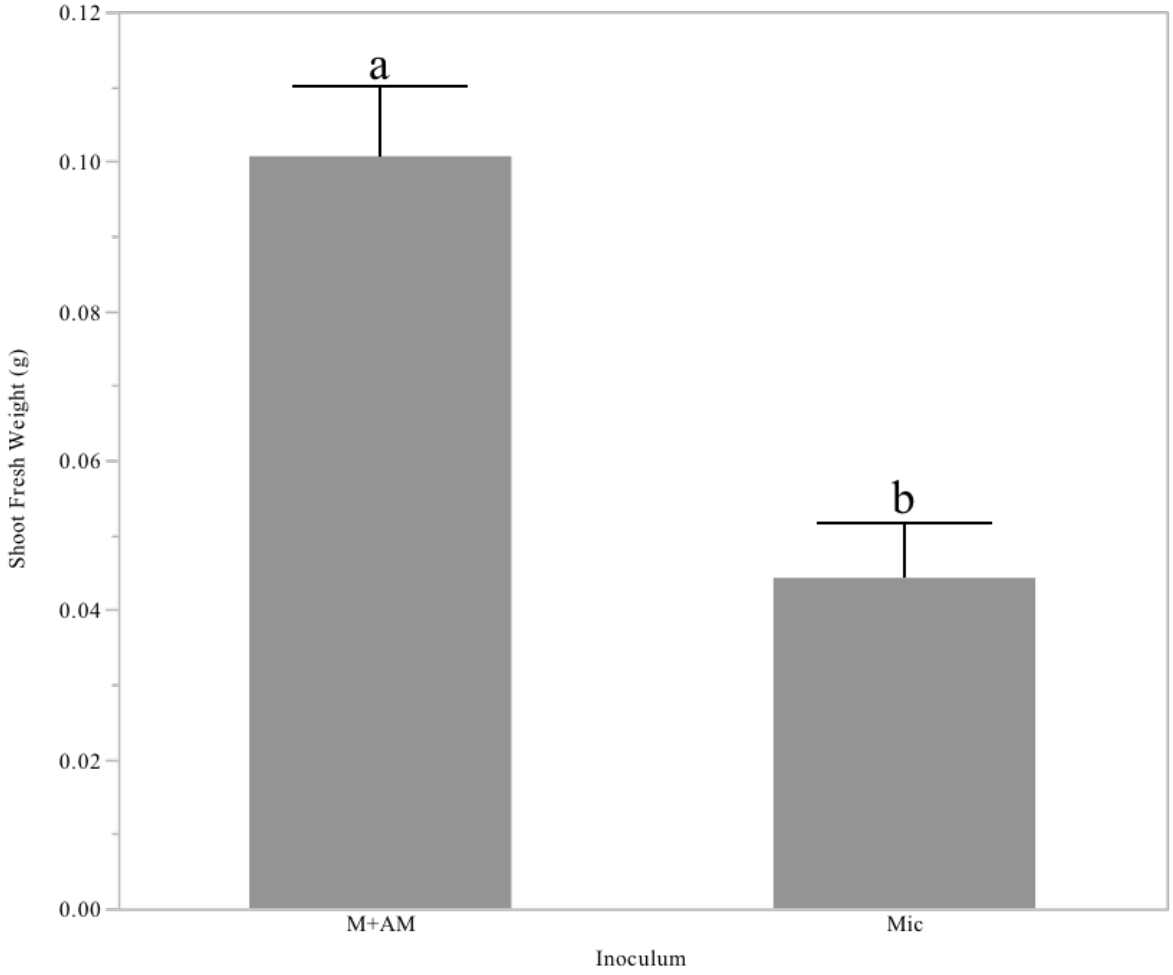

Figure 3.11 The effect of inoculum treatment on *Erigeron acris* shoot fresh weight (g). The effect of addition of mycorrhizal inoculum and a bacterial wash (M+AM) or bacterial wash only (Mic) was determined through a two-way analysis of variance (ANOVA), and differences between inoculum treatments were determined using Student's t tests. Bars represent means ±1 standard error and bars with the same letter are not significantly different.

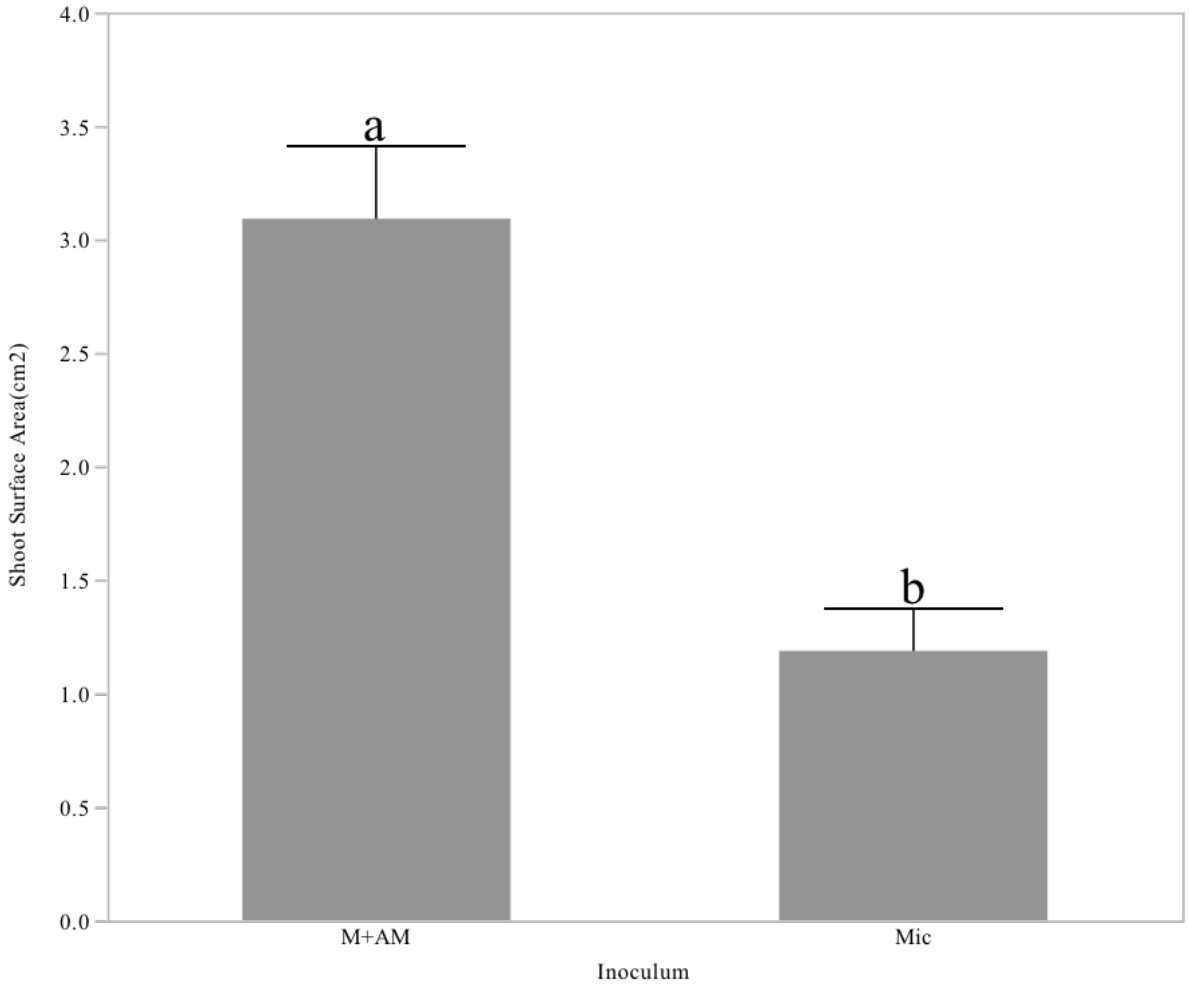

Figure 3.12 The effect of inoculum treatment on *Erigeron acris* shoot surface area (cm^2). The effect of addition of mycorrhizal inoculum and a bacterial wash (M+AM) or bacterial wash only (Mic) was determined through a two-way analysis of variance (ANOVA), and differences between inoculum treatments were determined using Student's t tests. Bars represent means +/- 1 standard error and bars with the same letter are not significantly different.

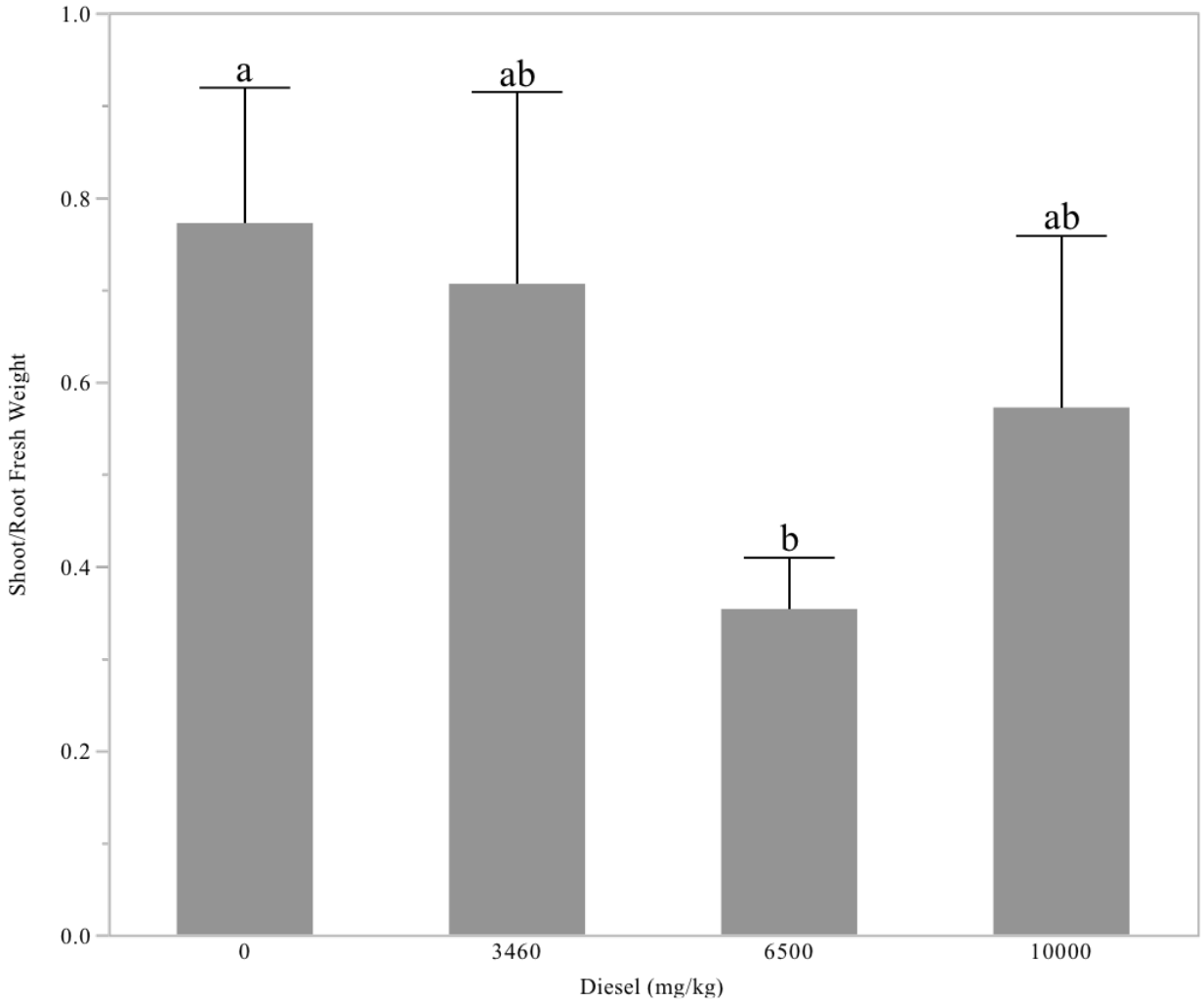

Figure 3.13 The response of *Erigeron acris* shoot/root fresh weight ratio to four diesel concentrations (0, 3,460, 6,500 and 10,000 mg/kg). A significant effect of diesel fuel on shoot/rot ratio was determined through a two-way analysis of variance (ANOVA) and differences between inoculum treatments were determined using Student's t tests. Bars represent means +/- 1 standard error and bars with the same letter are not significantly different.

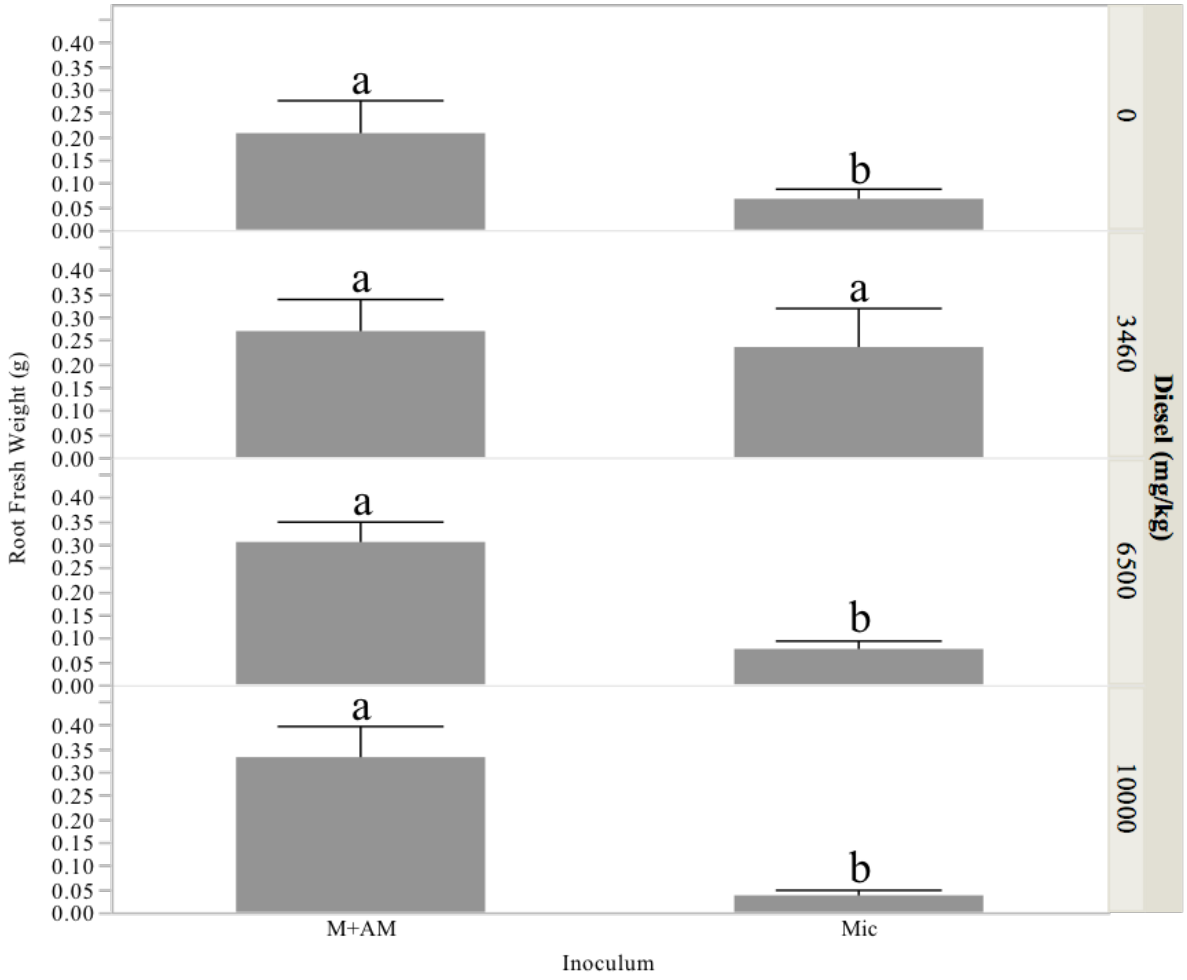

Figure 3.14 A comparison of *Erigeron acris* root fresh weight between plants grown with mycorrhizal inoculum and a bacterial wash (M+AM) or grown with the bacterial wash only (Mic) at each concentration of diesel fuel (0, 3,460, 6,500, and 10,000 mg/kg). The effects of diesel fuel and inoculum treatment on *E. acris* root fresh were determined through a two-way analysis of variance (ANOVA), and differences between diesel concentrations and inoculum treatments were determined using Student's t tests. Bars represent means ± 1 standard error and bars with the same letter are not significantly different.

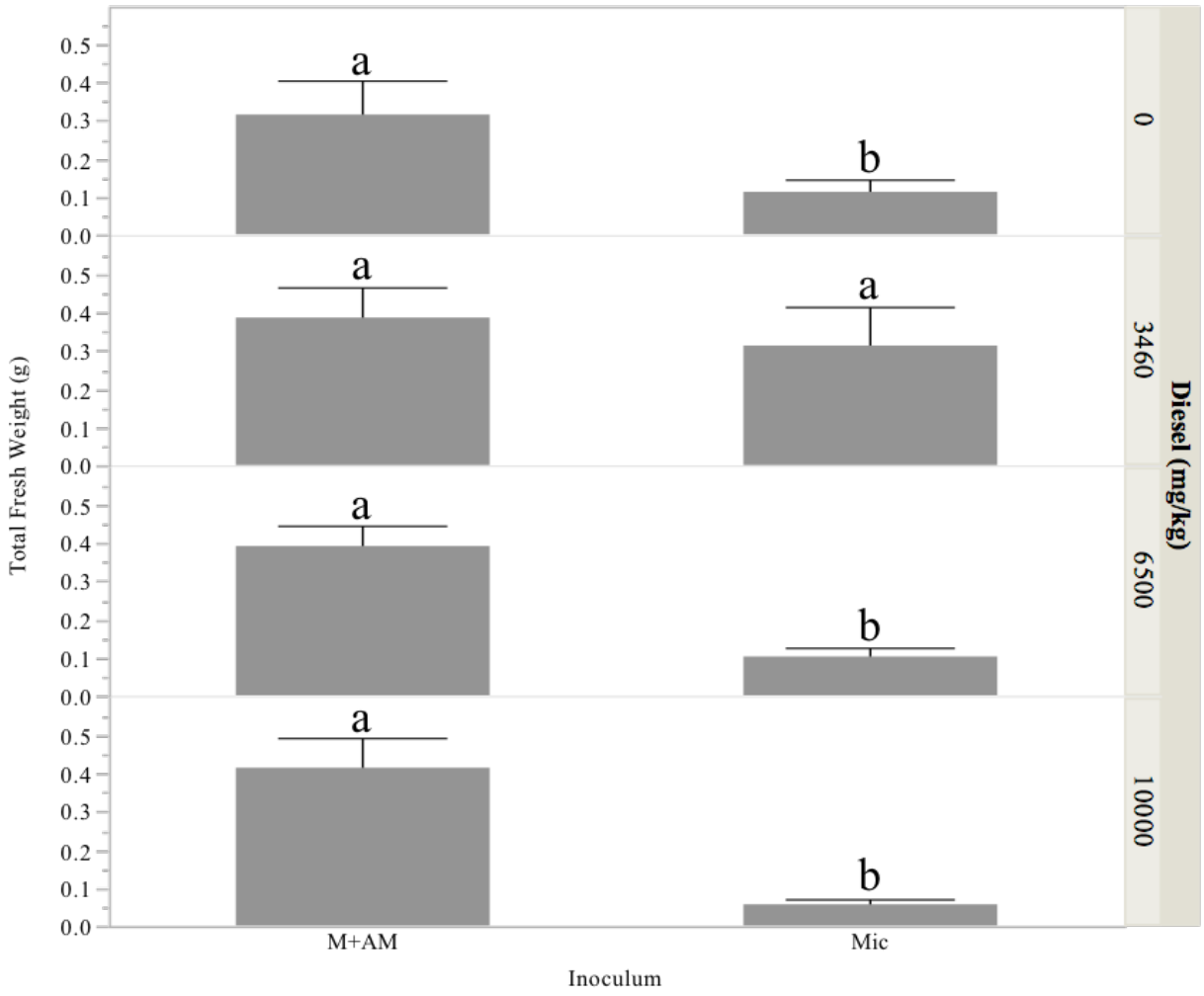

Figure 3.15 A comparison of *Erigeron acris* total fresh weight between plants grown with mycorrhizal inoculum and a bacterial wash (M+AM) or grown with the bacterial wash only (Mic) at each concentration of diesel fuel (0, 3,460, 6,500, and 10,000 mg/kg). The effects of diesel fuel and inoculum treatment on *E. acris* total fresh were determined through a two-way analysis of variance (ANOVA), and differences between diesel concentrations and inoculum treatments were determined using Student's t tests. Bars represent means ± 1 standard error and bars with the same letter are not significantly different.

Chapter 4: Summary of results and integrative biology

4.1 Summary and future directions

This field portion of this study was performed to assess the plant and mycorrhizal community at a northern site, Steeves Lake shoreline, previously affected by PHC contamination. The goals were to determine which plant species had naturally colonized the site, and to quantify vegetation cover and mycorrhizal colonization. Furthermore, we wanted to determine if there were trends in plant cover and colonization that indicated negative affects due to historic contamination. By comparing the species identified in the vegetation survey to the list of species planted at the site, we determined that Steeves Lake shoreline is recruiting new colonizers, and most of the new colonizers are species native to the area. Trends in vegetation cover did not support the hypothesis that there had been negative effects from residual contamination**;** differences observed in plant composition between plots was most likely due to water availability as the axis assigned by the NMDS were strongly influenced by the presence of wetland species and facultative upland species.

Though there have been questions regarding the abundance of AMF in northern ecosystems, results from this study show that AMF were present at a northern site. Colonization in roots collected from the Steeves Lake shoreline was similar and in some cases higher than what has been found in natural areas or in control treatments in previous studies. The hypothesis that residual contamination may still be affecting the site was not supported; there were no trends in colonization to indicate effects from contamination.

The greenhouse portion of this study was conducted to determine how northern plants with or without an association with mycorrhizal fungi respond to diesel fuel contaminated soils. Results from the greenhouse experiment were not consistent with hypothesized effects; a linear decrease

in plant biomass due to diesel contamination was expected but was not consistently observed in any of the three plant species. This may have been due to the plant species used in this study, or the influence of the substrate and how it impacted the mobility of diesel in the experimental pots, and how the substrate impacted access of plant roots to the contaminant. This study would need to be repeated using different soil types and soil mixtures to determine if there was an effect caused by the substrate, to apply the results to a greater range of ecosystems.

It was clear from our results that an association with AMF influenced plant growth responses. Significant differences in shoot biomass were consistently observed in the mycorrhizal plant species, though not in the non-mycorrhizal species *C. aquatilis*, indicating that the association with AMF was responsible for increased biomass. Some of the trends in *E. trachycaulus* responses were difficult to explain, and may have been caused by PHC toxicity to AMF in the highest diesel treatment. Future quantification of AMF colonization in these roots will help further explain trends in plant responses to diesel fuel. For example, if differences in *E. trachycaulus* root fresh weight were caused by PHC toxicity to the AMF, we would expect to see high colonization in roots from the 6,500 mg/kg treatment, and significantly lower colonization in 10,000 mg/kg, causing the plants to compensate and allocate more resources belowground. It was shown that AMF have a positive effect on plants growing in contaminated soils, and it has been posited that AMF also increase PHC dissipation in soils. Analysis of the end of experiment TPH concentrations in the experimental pots will be able to provide more insight into the benefits provided by an association with AMF.

One of the recommendations in the guidelines put out by MVLWB and AANDC (2013) for mine site closure and reclamation is the use of living organisms to help meet goals at the site such as the re-vegetation of a site or the remediation of soils. While it has been shown that AMF

are useful in these projects, there have also been questions about their abundance in northern ecosystems. Before AMF can be incorporated into remediation and reclamation projects in the north, more information is need about their presence in northern ecosystems. This study has shown that AMF are present at a northern site, and actively colonizing plant roots. The AMF propagules at Steeves Lake shoreline came from soil near the shoreline, this indicates that AMF are found naturally at a northern site, and can therefore could be incorporated into future projects easily by transplanting soil from reference areas.

It was stated the PHC CWS (2008) that there is a need for more data on the toxic effects of specific PHC mixtures and data on a larger range of organisms. This study utilized diesel fuel and northern plants and found effects on plant performance at the Colomac site-specific guideline concentration. In both *C. aquatilis* and *E. acris* shoot/root fresh weight ratios were significantly lower than controls and in *E. trachycaulus* plants inoculated with AMF, root fresh weight, total fresh weight and shoot surface area were all significantly reduced. Results from this study indicate that the Colomac guideline concentration may not be appropriate for northern plants, or for diesel fuel.

4.2 Integrative biology

The field of ecology requires an integrative approach, this is clearly shown in the current study. This study was conducted out of a wetland ecology lab though it encompassed aspects of plant biology, microbiology, chemistry and toxicology. Part of the field of ecology is the study of interactions between organisms, in our research we try to understand whole communities, including plants and the microorganisms associated with them and the abiotic factors affecting the community. For example, in the current study it was hypothesized that if the plant community at the study site had been influenced by residual contamination, this might be

observed through predicted trends in the vegetation, such as the type plant species and percent cover between plots. This relationship may not be evident when just looking at vegetation alone. Biotic and abiotic factors need were included in the site survey as well as an analysis of contamination in the soil. Factors such as soil characteristics like pH and percent moisture were measured, and the relationships between plants and their associations with soil microorganisms like mycorrhizal fungi, were considered. Though the results from our first year of sampling may have indicated an effect of residual effects from contamination, when other variables were included it was determined that trends in vegetation were more likely due to a water gradient than contamination.

One of the goals of this research was to add to our current understanding of how mycorrhizal fungi could be utilised in the field of bioremediation. Bioremediation projects need to consider not only the type of vegetation planted, but how the different species will interact with each other, and the microbial community. The field of bioremediation is inherently integrative, often specific plants and microorganisms are chosen to remediate a contaminant, for example, with PHC contamination plant species need to be able to survive in contaminated soils to provide habitat for bacteria, and that there is population of PHC degrading bacteria. Abiotic factors such as the length of growing season, type of soil and hydrology at the site need to be considered to determine how long it will take for the contaminant to degrade, or movement of the contaminant, and concentrations of contaminants in the soil need to be measured to ensure remediation targets are being met.

Appendix

Table A 2.1 A summary of the vegetation survey of Steeves Lake shoreline, Colomac Mine, NWT in August 2015. Each species within a quadrat was identified using the key by Porsild and Cody (1980) and the percent cover for each species was estimated as well as the percent bare ground in each quadrat.

		% Cover									
Transect	Quadrat	*Salix* sp.	*Carex aquatilis*	*Equisetum arvense*	*Poa glauca*	*Elymus trachycaulus*	*Deschampsia cespitosa*	*Erigeron acris*	*Calamagrostis canadensis*	*Alnus viridis*	Bare ground
1	1	10	70	5	0	0	0	0	0	0	0
1	2	20	60	5	5	0	0	0	0	0	30
1	3	5	70	5	0	10	5	0	0	0	0
1	4	5	10	0	10	15	0	0	0	0	45
1	5	10	0	0	10	15	0	0	0	0	60
2	1	10	70	15	0	0	0	0	0	0	0
2	2	5	60	20	0	0	0	0	0	0	0
2	3	10	40	20	0	30	0	0	0	0	0
2	4	10	5	5	5	30	0	0	0	0	30
2	5	15	0	0	10	40	0	5	0	0	20
3	1	10	70	10	0	0	0	0	0	0	5
3	2	5	70	10	0	0	0	0	0	0	0
3	3	5	15	5	5	15	0	0	0	0	20
3	4	5	5	5	0	50	0	0	0	0	10
3	5	5	5	0	5	50	0	0	0	0	10
4	1	10	60	15	0	0	0	0	0	0	10
4	2	10	70	5	0	0	0	0	0	0	0
4	3	5	15	0	0	20	0	0	0	0	15
4	4	5	0	0	5	15	0	0	0	0	20
4	5	15	0	0	15	40	0	5	5	0	50
5	1	15	20	20	0	0	0	0	0	0	25
5	2	15	60	5	0	0	0	0	0	0	0
5	3	0	10	0	15	40	0	0	0	10	10
5	4	0	5	0	0	50	0	0	0	0	15

5	5	5	0	0	0	30	0	5	0	0	50

Table A 2.2 Total petroleum hydrocarbon determination in soil samples collected from Steeves Lake shoreline, Colomac Mine, NWT, in August 2015. Total petroleum hydrocarbons (TPH) was determined through EPA Methods 8260B (1996) for BTEX (benzene, toluene, ethylbenzene and xylene) compounds and CCME Canada- Wide Standard for Petroleum Hydrocarbons in soil (2001) for fractions F1 to F4. (ND means not detected)

Transect	Quadrat	Benzene (mg/kg)	Toluene (mg/kg)	Ethylbenzene (mg/kg)	Xylenes (mg/kg)	F1Fraction (mg/kg)	F2 Fraction (mg/kg)	F3 Fraction (mg/kg)	F4 Fraction (mg/kg)	F4 Fraction Gravimetric (mg/kg)	Total Hydrocarbons (mg/kg)
1	1	<0.01	<0.1	<0.03	<0.14	<20	<30	181	75	<500	256
1	2	<0.005	<0.005	<0.015	<0.1	<10	<20	92	46	ND	137
1	3	<0.005	<0.005	<0.015	<0.1	<10	<20	49	139	ND	188
1	4	<0.01	<0.1	<0.03	<0.14	<20	<20	94	43	ND	137
1	5	<0.005	<0.005	<0.015	<0.1	<10	<20	83	33	ND	116
2	1	<0.005	<0.05	<0.015	<0.1	<10	<30	170	72	<500	242
2	2	<0.005	<0.05	<0.015	<0.1	<10	<20	79	28	ND	107
2	3	<0.005	<0.05	<0.015	<0.1	<10	<20	111	48	ND	159
2	4	<0.005	<0.05	<0.015	<0.1	<10	<20	97	49	ND	146
2	5	<0.005	<0.05	<0.015	<0.1	<10	<20	97	33	ND	130
3	1	<0.005	<0.05	<0.015	<0.1	<10	166	208	40	ND	414
3	2	<0.005	<0.05	<0.015	<0.1	<10	<20	145	63	<500	208
3	3	<0.005	<0.05	<0.015	<0.1	<10	<20	95	38	ND	133
3	4	<0.01	<0.1	<0.03	<0.14	<20	<20	130	65	550	195
3	5	<0.005	<0.05	<0.015	<0.1	<10	<20	117	47	ND	164
4	1	<0.005	<0.05	<0.015	<0.1	<10	24	66	<20	ND	90
4	2	<0.005	<0.05	<0.015	<0.1	<10	<20	48	<20	ND	48
4	3	<0.005	<0.05	<0.015	<0.1	<10	<20	98	48	ND	146
4	4	<0.005	<0.05	<0.015	<0.1	<10	21	190	62	ND	273
4	5	<0.005	<0.05	<0.015	<0.1	<10	<20	138	67	ND	205
5	1	<0.01	<0.1	<0.03	<0.14	<20	<20	190	82	<500	272
5	2	<0.005	<0.05	<0.015	<0.1	<10	<20	179	78	<500	257

5	3	<0.01	<0.1	<0.03	<0.14	<20	<20	172	81	<500	253
5	4	<0.01	<0.1	<0.03	<0.14	<20	<20	164	78	<500	242
5	5	<0.005	<0.05	<0.015	<0.1	<10	<20	122	52	ND	174

Table A 2.3 Mean (±1 SE) arbuscular, vesicular and hyphal colonization in the roots of *Elymus trachycaulus* collected from Steeves Lake shoreline, Colomac Mine, NWT in August 2015. the collected roots were cleared with 10% KOH, stained with a 5% ink in vinegar solution, and mycorrhizal structures were quantified using a modified version of the magnified intersections method (McGonigle et al., 1990). (n=2, * Indicates a samples size of 1, and empty cells indicates that the *E. trachycaulus* was not found within the quadrat).

Transect	Quadrat	% Arbuscular Colonization	% Vesicular Colonization	% Hyphal Colonization
1	1			
1	2	5.5±1.75	15±1.5	66 ±2.5
1	3	5.5±1.75	15.5±0.25	55.5±1.25
1	4	3.5±0.75	13.5±4.25	50.5±10.25
1	5	1±0.5	1.5±0.25	8.5±3.75
2	1			
2	2			
2	3	8±3.5	35±1	78.5±5.25
2	4	7±1.5	14.5±0.75	60.5±7.75
2	5	5±1	11.5±0.75	59±2.5
3	1			
3	2			
3	3	7±2	6±0	37.5±1.25
3	4	6.5±0.75	8.5±4.25	38±9.5
3	5	6.5±2.75	18.5±4.25	58±3
4	1			
4	2	2*	3*	38*
4	3	5±1.5	10.5±2.75	49.5±6.75
4	4	1*	2*	27*
4	5	2±1	19.5±0.75	59±3
5	1			
5	2			
5	3	0	1±0.5	18±3.5
5	4	0	8.5±2.75	33±10
5	5	1.5±0.25	19.5±1.25	67.5±0.75

Table A 2.4 A summary of the vegetation survey of Steeves Lake shoreline, Colomac Mine, NWT in August 2016. Each species within a quadrat was identified using the key by Porsild and Cody (1980) and the percent cover for each species was estimated as well as the percent bare ground in each quadrat.

Transect	Quadrat	*Carex aquatilis*	*Salix* sp.	*Elymus trachycaulus*	*Equisetum arvense*	*Festuca ovina*	*Poa glauca*	*Hordeum jubatum*	*Elymus violaceous*	*Carex bebbii*	*Tussilago farfara*	*Plantago major*	Bare ground
1	1	80	0	0	0	0	0	0	0	0	0	0	20
1	2	20	5	0	0	5	10	0	0	0	0	0	40
1	3	60	5	5	5	5	5	0	0	0	0	0	20
1	4	20	5	10	0	0	5	0	0	0	0	0	60
2	1	65	5	0	5	0	0	0	0	0	0	0	5
2	2	90	5	0	10	0	0	0	0	0	0	0	5
2	3	30	5	0	5	5	0	0	0	0	0	0	5
2	4	60	5	5	5	0	0	0	0	0	0	0	25
3	1	30	15	0	10	0	0	0	0	0	0	0	50
3	2	30	10	0	15	0	5	0	0	0	0	0	10
3	3	65	5	5	5	0	5	0	0	0	0	0	30
3	4	10	5	5	0	15	5	0	0	0	0	0	65
4	1	95	5	0	0	0	0	0	0	0	0	0	0
4	2	20	0	5	0	5	10	0	0	0	0	0	60
4	3	10	0	10	0	0	10	0	0	0	0	0	75
4	4	20	5	5	0	15	0	0	0	0	0	0	50
5	1	85	0	0	4	0	0	10	0	0	0	0	10
5	2	50	5	0	5	5	0	0	0	0	0	0	40
5	3	15	5	0	5	0	0	0	0	0	0	0	70
5	4	50	5	0	0	0	0	10	0	0	0	0	40
6	1	50	0	0	30	0	0	0	5	0	0	0	20
6	2	50	5	0	0	5	5	0	0	5	0	0	45
6	3	25	5	0	0	0	5	5	5	0	0	0	65
6	4	80	5	10	5	0	5	0	0	0	0	0	5
7	1	50	5	0	20	10	0	0	0	0	0	0	25

7	2	20	5	5	15	15	10	0	0	0	0	0	50
7	3	25	5	5	5	10	20	0	0	5	0	0	50
7	4	15	5	5	0	0	10	0	0	0	0	0	60
8	1	90	5	0	5	0	5	0	0	0	0	0	0
8	2	15	0	0	5	0	10	0	5	0	5	5	50
8	3	40	10	5	25	5	5	0	0	0	0	0	5
8	4	30	5	10	10	5	5	5	0	5	5	0	20

Table A 2.5 Total petroleum hydrocarbon concentration in soil samples collected from Steeves Lake shoreline, Colomac Mine, NWT, in August 2016. Total petroleum hydrocarbons (TPH) was determined through a modified version of the CCME Canada –Wide Standard for Petroleum Hydrocarbons in soil (2001). Soil pH was determined using the method by Kalra (1995) and percent moisture gravimetrically. (ND = not detected)

Transect	Quadrat	pH	% Moisture	F1 Fraction (mg/kg)	F2 Faction (mg/kg)	F3 Fraction (mg/kg)	F4 Fraction (mg/kg)
1	1	7.31	49.46	ND	ND	12.5	ND
1	2	7.68	30.41	ND	ND	18.4	ND
1	3	7.64	33.86	ND	ND	14.8	ND
1	4	7.63	35.54	ND	ND	10.7	ND
2	1	7.39	45.91	ND	ND	ND	ND
2	2	7.29	46.12	ND	ND	ND	ND
2	3	7.74	37.35	ND	ND	6.0	ND
2	4	7.46	34.29	ND	ND	3.2	ND
3	1	7.24	50.09	ND	58.0	39.0	ND
3	2	7.68	41.50	ND	ND	8.7	ND
3	3	8.14	45.14	ND	ND	4.7	ND
3	4	7.85	37.50	ND	ND	8.9	ND
4	1	7.65	35.44	ND	ND	20.3	ND
4	2	7.66	33.02	ND	ND	12.8	ND
4	3	7.49	24.56	ND	ND	4.2	ND
4	4	8.1	33.22	ND	ND	11.1	ND
5	1	7.3	50.67	ND	ND	12.5	ND
5	2	7.71	47.02	ND	ND	29.1	ND
5	3	7.16	38.48	ND	ND	16.0	ND
5	4	8.26	25.41	ND	ND	ND	ND
6	1	7.39	43.24	ND	ND	20.3	ND
6	2	7.58	31.15	ND	ND	28.1	ND
6	3	7.52	37.14	ND	ND	48.7	ND
6	4	7.61	25.90	ND	ND	ND	ND
7	1	7.37	39.72	ND	ND	22.5	ND
7	2	7.53	37.35	ND	ND	20.2	ND
7	3	7.85	23.84	ND	ND	13.2	ND
7	4	7.75	22.74	ND	ND	ND	ND
8	1	7.54	35.33	ND	ND	22.6	ND
8	2	7.66	40.09	ND	ND	24.3	ND
8	3	7.58	33.79	ND	ND	36.7	ND
8	4	7.71	29.21	ND	ND	9.7	ND

Table A 2.6 Mean (±1 SE) arbuscular, vesicular and hyphal colonization in the roots of *Festuca ovina* collected from Steeves Lake shoreline, Colomac Mine, NWT in August 2016. the collected roots were cleared with 10% KOH, stained with a 5% ink in vinegar solution, and mycorrhizal structures were quantified using a modified version of the magnified intersections method (McGonigle et al., 1990). (n=2, * Indicates a samples size of 1, and empty cells indicates that *F. ovina* was not found within the quadrat).

Transect	Quadrat	% Arbuscular Colonization	% Vesicular Colonization	% Hyphal Colonization
1	1	5±3	18.5±2.5	54.5±5.5
1	2	5±1	8.5±3.5	38±2
1	3	5±1	23±8	66±2
1	4	14.5±8.5	3.5±2.5	33±13
2	1			
2	2			
2	3	3±1	1.5±1.5	14.5±2.5
2	4	0.5±0.5	0.5±0.5	19.5±12.5
3	1			
3	2	17±9	28±3	75±12
3	3	5±3	31.5±19.5	66±21
3	4	5±3	16.5±8.5	49.5±3.5
4	1	8.5±1.5	19±7	55±3
4	2	3±1	17.5±0.5	49.5±13.5
4	3	15±5	18.5±5.5	40.5±4.5
4	4	9.5±7.5	14.5±5.5	50.5±10.5
5	1			
5	2	7±4	23±16	50±17
5	3	10±5	5.5±0.5	41±3
5	4	4.5±2.5	0.5±0.5	17±0
6	1	10.5±9.5	11±1	54±10
6	2	12.5±2.5	17±4	79.5±3.5
6	3	3.5±2.5	8±7	42.5±28.5
6	4	3±3	9±6	38.5±10.5
7	1	8.5±2.5	5.5±5.5	45.5±0.5
7	2	11±5	23.5±2.5	79.5±10.5
7	3	4.5±0.5	3.5±0.5	35.5±9.5
7	4	3±1	28.5±5.5	60.5±5.5
8	1	5.5±0.5	20.5±9.5	54.5±11.5
8	2	5±2	33±3	75±3
8	3	7±1	11±0	40.5±5.5
8	4	10.5±1.5	16±5	56.5±11.5

Table A 2.7 Mean (±1 SE) arbuscular, vesicular and hyphal colonization in the roots of *Elymus trachycaulus* collected from Steeves Lake shoreline, Colomac Mine, NWT in August 2016. the collected roots were cleared with 10% KOH, stained with a 5% ink in vinegar solution, and mycorrhizal structures were quantified using a modified version of the magnified intersections method (McGonigle et al., 1990). (n=2, * Indicates a samples size of 1, and empty cells indicates that *E. trachycaulus* was not found within the quadrat).

Transect	Quadrat	% Arbuscular Colonization	% Vesicular Colonization	% Hyphal Colonization
1	1			
1	2			
1	3	4±3	23±10	78±2
1	4	2±1	25.5±5.5	53±12
2	1			
2	2			
2	3	5.5*	14.5*	47*
2	4	6±5	33±1	86.5±1.5
3	1			
3	2	18.5±9.5	14±0	69.5±7.5
3	3	3±1	15.5±5.5	65±2
3	4	7±3	13.5±10.5	54±16
4	1	3±0	23.5±5.5	75±8
4	2	2.5±0.5	16.5±0.5	57.5±8.5
4	3	3±1	24±5	55.5±7.5
4	4	5±4	9±7	54.5±11.5
5	1			
5	2			
5	3	13*	4*	62*
5	4	6±2	9±4	46±10
6	1	8.5±4.5	12±5	51±2
6	2	1±0	7±4	58±4
6	3	1±1	13±10	43±17
6	4	10.5±1.5	20.5±5.5	68±6
7	1	0	27±8	76±6
7	2	11.0±6.0	13.5±0.5	72.5±6.5
7	3	6.5±0.4	25±3.0	74.5±3.5
7	4	1.5±1.5	15.5±4.5	72±18
8	1	5±0	16.5±0.5	62±3
8	2	7±3	28±15	72±9
8	3	9±6	12.5±2.5	72.5±3.5
8	4	5.5±4.5	14.5±1.5	68.5±7.5

Table A 2.8 Mean (±1 SE) arbuscular, vesicular and hyphal colonization in the roots of *Hordeum jubatum* collected from Steeves Lake shoreline, Colomac Mine, NWT in August 2016. the collected roots were cleared with 10% KOH, stained with a 5% ink in vinegar solution, and mycorrhizal structures were quantified using a modified version of the magnified intersections method (McGonigle et al., 1990). (n=2, * Indicates a samples size of 1, and empty cells indicates that *H. jubatum* was not found within the quadrat).

Transect	Quadrat	% Arbuscular Colonization	% Vesicular Colonization	% Hyphal Colonization
1	1			
1	2			
1	3	21.5±6.5	10.5±2.5	58±3
1	4	7.5±4.5	21.5±6.5	52.5±7.5
2	1			
2	2			
2	3	35±3	3±2	46.5±6.5
2	4	6*	22*	68*
3	1			
3	2			
3	3			
3	4	19.5±1.5	15.5±1.5	63.5±4.5
4	1			
4	2			
4	3	14*	5*	43*
4	4	28±2	34.5±3.5	88.5±4.5
5	1			
5	2	14±2	5±2	42.5±6.5
5	3	32±2	3±0	50.5±5.5
5	4	15±5	17.5±12.5	58.5±15.5
6	1	19.5±1.5	6.5±2.5	45.5±10.5
6	2			
6	3	2±1	7±6	36.5±3.5
6	4	2.5±2.5	13.5±11.5	43.5±14.5
7	1	4±2	10±9	66.5±12.5
7	2	10.5±4.5	3.5±3.5	42.5±2.5
7	3	19±10	13±2	70±8
7	4	11±3	24.5±5.5	74±2
8	1			
8	2	18*	14*	71*
8	3	18*	15*	76*
8	4	10.5±4.5	10.5±1.5	50±2

Table A 3.1 The micronutrient and macronutrient ingredients in Long Ashtons nutrient stock solution.

Macronutrient	M.W. (g)	mMconc	g/L	1L stock for 100L (g)
KNO_3	101.106	4	0.4044	40.4424
$Ca(NO_3)_2$	164.088	4	0.6564	65.635
$MgSO_4$-$7H_2O$	246.477	1.5	0.3697	36.972
NaH_2PO_4-$2H_2O$	156.007	1.33	0.2075	20.749
Micronutrients	M.W. (g)	mMconc	g/L	1L stock for 100L (g)
$MnSO_4$-$4H_2O$	223.058	0.01	$2.231x10^{-3}$	0.223
$CuSO_4$-$5H_2O$	249.675	0.001	$2.497x10^{-4}$	0.0250
$ZnSO_4$-$7H_2O$	287.535	0.001	$2.875x10^{-4}$	0.0575
H_3BO_3	61.832	0.05	$3.0916x10^{-3}$	0.3092
NaCl	58.444	0.1	$5.844x10^{-3}$	0.584
$(NH_4)_6Mo_7O_{24}$-$4H_2O$	1235.85	$7x10^{-5}$	$8.65x10^{-5}$	$8.65x10^{-3}$
Fe EDTA	367.05	0.05	$1.835x10^{-2}$	1.835

Table A 3.2 Mycorrhizal colonization in roots collected from trap plants grown to create the inoculum treatments in the greenhouse study. The roots were cleared with 10% KOH, stained with a 5% ink in vinegar solution, and mycorrhizal structures were quantified using a modified version of the magnified intersections method.

Replicate	% Hyphal Colonization	% Vesicular Colonization	% Arbuscular Colonization
1	89	21	11
2	84	20	23
3	83	21	27
4	74	18	22
5	86	24	20

Table A 3.3 Total petroleum hydrocarbon determination of commercial diesel fuel purchased to contaminate experimental pots. Total petroleum hydrocarbons (TPH) were determined through a modified version of the CCME Canada –Wide Standard for Petroleum Hydrocarbons in soil (2001). (ND means not detected)

Subsample	F1 Fraction (mg/L)	F2 Fraction (mg/L)	F3 Fraction (mg/L)	F4 Fraction (mg/L)
1	46581.5	444534.6	295583.4	ND
2	52354.5	513285.0	288092.3	ND
3	41491.7	448404.8	282406.5	ND

Table A 3.4 Shapiro-Wilks for testing the normality of data distributions for *Carex aquatilis*, *Elymus trachycaulus*, and *Erigeron acris* growth variables, and transformation used when data did not fit a normal distribution.

		Shoot Fresh Weight	Root Fresh Weight	Total Fresh Weight	Shoot Surface Area	Shoot/Root Ratio
Carex aquatilis	Transformation	Log	Log	Log	Log	Normal
	W	0.980748	0.985182	0.985879	0.977501	0.980337
	Prob<W	0.4167	0.6390	0.6773	0.2920	0.3989
Elymus trachycaulus	Transformation	Normal	Normal	Normal	Normal	Non-normal
	W	0.969685	0.969486	0.968426	0.967490	N/A
	Prob<W	0.1165	0.1137	0.1001	0.0895	N/A
Erigeron acris	Transformation	Square Root	Square Root	Log	Square Root	Log
	W	0.989818	0.959468	0.961527	0.909168	0.978824
	Prob<W	0.8939	0.0416	0.0527	0.8674	0.3699

Table A 3.5 Results from tests assessing variance for all comparisons of means between *Carex aquatilis* growth variables.

		O'Brien	Brown-Forsythe	Levene	Bartlett
Shoot Fresh Weight	DF	7	7	7	7
	F-Ratio	2.0528	2.6640	3.3184	2.2180
	Prob > F	0.0642	0.0188	0.0050	0.0298
Root Fresh Weight	DF	7	7	7	7
	F-Ratio	0.5908	0.3472	0.6286	0.8162
	Prob > F	0.7606	0.9283	0.7302	0.5736
Total Fresh Weight	DF	7	7	7	7
	F-Ratio	0.5227	0.2898	0.4312	0.4506
	Prob > F	0.8136	0.9553	0.8786	0.8704
Shoot Surface Area	DF	7	7	7	7
	F-Ratio	1.4924	2.2795	2.7897	1.8865
	Prob > F	0.1889	0.0408	0.0146	0.0673
Shoot/Root Ratio	DF	7	7	7	7
	F-Ratio	0.6691	0.9007	0.9972	0.5707
	Prob > F	0.6971	0.5125	0.4430	0.7803

Table A 3.6 Results from tests assessing variance for all comparisons of means between *Elymus trachycaulus* growth variables.

		O'Brien	Brown-Forsythe	Levene	Bartlett
Shoot Fresh Weight	DF	7	7	7	7
	F-Ratio	1.3625	1.3727	1.9437	2.6063
	Prob > F	0.2393	0.2349	0.0796	0.0109
Root Fresh Weight	DF	7	7	7	7
	F-Ratio	0.9899	0.9864	1.2339	1.9062
	Prob > F	0.4480	0.4505	0.3001	0.0642
Total Fresh Weight	DF	7	7	7	7
	F-Ratio	0.9455	0.9082	1.1146	1.7276
	Prob > F	0.4795	0.5069	0.3670	0.0975
Shoot Surface Area	DF	7	7	7	7
	F-Ratio	1.1458	0.9482	1.2749	0.4922
	Prob > F	0.3485	0.4776	0.2794	0.8410
Shoot/Root Ratio	DF	N/A			
	F-Ratio	N/A			
	Prob > F	N/A			

Table A 3.7 Results from tests assessing variance for all comparisons of means between *Erigeron acris* growth variables.

		O'Brien	Brown-Forsythe	Levene	Bartlett
Shoot Fresh Weight	DF	7	7	7	7
	F-Ratio	0.9562	0.9549	1.1414	0.8837
	Prob > F	0.4724	0.4733	0.3520	0.5182
Root Fresh Weight	DF	7	7	7	7
	F-Ratio	1.1574	1.1622	1.7137	2.0528
	Prob > F	0.3428	0.3400	0.1257	0.0450
Total Fresh Weight	DF	7	7	7	7
	F-Ratio	0.8142	0.9407	1.3873	1.5347
	Prob > F	0.5796	0.4835	0.2301	0.1502
Shoot Surface Area	DF	7	7	7	7
	F-Ratio	0.5846	0.8218	0.8547	1.0918
	Prob > F	0.7654	0.5736	0.5479	0.3651
Shoot/Root Ratio	DF	7	7	7	7
	F-Ratio	1.8983	2.2162	3.3751	2.2983
	Prob > F	0.0882	0.0473	0.0058	0.0016