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The Impacts of Gold Mining on Vegetation and Mycorrhizal Colonization in Northern Canada

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Abstract

Gold mining is an important part of economic development in Northern Canada. A large portion of the gold that is found in the North is contained within arsenopyrite ores, therefore, arsenic contamination is of special concern. Little is known regarding the impacts of arsenic on plants and mycorrhizae in Northern Ecosystems. Arsenic has been shown to negatively impact plant growth and seed germination in some temperate species, while others are tolerant and can accumulate arsenic concentrations over 1000 mg/kg. In temperate regions, arbuscular mycorrhiza can accelerate the remediation process in gold mines by supporting plant growth in poor soil conditions but they have also been shown to be negatively impacted by arsenic exposure. To assess impact of arsenic on plants and mycorrhizae, field studies were undertaken at Tundra Mine, an inactive gold mine 250 km northeast of Yellowknife. Vegetation surveys were conducted and plant samples were collected for arsenic accumulation and mycorrhizal colonization at 4 sites near a tailings containment area and 4 reference sites further away from the mine. Arsenic concentrations in plants ranged from 0.08 ± 0.01 mg/kg to 870 ± 230 mg/kg. The highest arsenic concentrations were consistently found in plants that grew in the 4 sites adjacent to the tailings containment area. *Carex aquatilis* contained the highest concentration of arsenic at those sites making it a good potential candidate for phytoremediation (roots = 870 ± 230 mg/kg; shoots = 141 ± 49.0 mg/kg). At the sites with the two highest soil arsenic concentrations all plants sampled exceeded arsenic concentrations recommended for plant material designated for human consumption (3.5 mg/kg in fish) and animal feed (8 mg/kg) in some cases by up to three orders of magnitude. Non-metric multidimensional scaling results using vegetation survey data showed that plant communities at the 4 sites adjacent to the mine were distinct from the 4 reference sites. Both arbuscular mycorrhizal and dark septate endophyte

colonization was found at every site, including those with high levels of arsenic contamination. This indicates that mycorrhizae and dark septate endophytes at these sites can tolerate arsenic concentrations reaching 2676 ± 704.6 mg/kg. Hyphal colonization in roots collected from the highest arsenic site (Hambone Lake = 2677 ± 704.6 mg/kg) ranged from 59.83 ± 7.27 % colonization in *Calamagrostis canadensis* to 33.20 ± 10.61% colonization in *Epilobium angustifolium*. No trends of reduced colonization at higher arsenic sites were observed in this study. There were also no clear trends of colonization between plant species.

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Table of Contents

List of Figures

Chapter 1- The impacts of gold mining on vegetation and mycorrhizal colonization in Northern Canada

Chapter 2 - The impacts of gold mining on northern plant communities and arsenic uptake in plants

Chapter 3 – The impacts of gold mining on mycorrhizae

List of Tables

Chapter 2 - The impacts of gold mining on northern plant communities and arsenic uptake in plants

Chapter 3 – The impacts of gold mining on mycorrhizae

Appendix

Chapter 1- Mining impacts and their relation to vegetation and arbuscular mycorrhizal fungi

Mining in Northern Canada

Canada is ranked as one of the top five producers of many metals and minerals (United States Geological Survey 2011). In 2016, the mining and quarrying industry directly employed 71,380 workers in Canada and it is predicted that an additional 17,000 jobs will be provided by 2020 from mining in Northern Canada alone, and as many as 70,000 jobs in fields that support mining output (Natural Resources Canada 2017; Rheaume and Caron-Vuotari 2013). Resource extraction in the Northwest Territories contributes $900 million of the GDP, which is more profitable than any industry in the north by $333 million (Government of Northwest Territories 2016). As the global demand for minerals continues to increase, Northern Canada is in a good position to supply the mineral output needed. The Canadian Shield, occupying Northern Canada, is the major source of minerals and metals (Nature Conservancy Canada 2016). Many of the mines found in Northern Canada are found in the Northwest Territories. Currently most operational mines in the Northwest Territories are diamond mines, but historically gold mines were the most prevalent (Silke 2009). Due to historic instability of gold prices, there are now many abandoned gold mines in the Northwest Territories awaiting proper closure (National Orphaned/Abandoned Mine Initiative 2009).

Today mining companies interested in developing a mine in the Northwest Territories must create a Closure and Reclamation Plan and submit a monetary bond or deposit to the Mackenzie Valley Land and Water Board (MVLWB) and Indigenous and Northern Affairs Canada (INAC). The goal of mine closure in the Northwest Territories is “to return the mine and affected areas to viable and, wherever practicable, self-sustaining ecosystems that are compatible with a healthy environment and with human activities” (MVLWB and AANDC 2013). The most important ecosystem services that are restored during reclamation include maintaining site stability by

reducing soil erosion, nutrient cycling and providing a habitat for wildlife (MVLWB and AANDC 2013). Since these services are provided by plants and the microorganisms associated with them, re-vegetation is a significant part of reclamation (Buscot and Varma 2005).

Mining Impacts

Mining in general has several environmental impacts including the removal of top soil and vegetation to make room for infrastructure (MVLWB and AANDC 2013). The removal of vegetation reduces site stability by increasing soil erosion and the impact of flooding (Meeuwig 1970). By removing vegetation, mining companies are removing food sources as well as a habitat for many animals (Environment and Climate Change Canada 2013). Since heavy equipment is used for many mining activities, soils on site become compacted making it more difficult for plants to naturally establish. Compacted soils can be problematic during revegetation since plants roots are unable to penetrate soil, limiting access to water and nutrients (Densmore et al. 2000). The rate of infiltration of water through soils decreases when soil is compact causing surface water accumulation (Kozlowski 1999). In this case root respiration changes from aerobic to anaerobic respiration which does not release enough energy to maintain root function (Kozlowski and Pallardy 1996).

In addition to the more general impacts, gold mining has some unique effects. Many of the gold deposits in Northern Canada are found within arsenopyrite sulphide ores, which contain naturally-occurring arsenic (Rheaume and Caron-Vuotari 2013). When the arsenopyrite sulphide ores are milled, or roasted to extract gold, arsenic is released as a by-product. Typically, these by-products, also known as tailings, are drained to a nearby lake which is contained by damns (Natural Resources Canada 2016). During the spring months in northern latitudes, large

quantities of snow and ice thaw, causing tailings to flow over the containment dams contaminating adjacent sites with arsenic (MVLWB and AANDC 2013).

Inorganic arsenic is the most common form of arsenic found in soil (Fitz and Wenzel 2002). The two forms of inorganic arsenic that can be readily taken up by plant roots are arsenate (AsO_4^{-3}) and arsenite (AsO_3^{-3}). Arsenite is a trivalent, thiol reactive compound that can bind to multiple cysteine residues or dithiol cofactors in proteins or enzymes (Finnegan and Chen 2012). In the presence of closely spaced cysteine residues in a protein, one arsenite molecule can bind to multiple sites, altering the shape of the protein potentially inhibiting its function (Finnegan and Chen 2012) (Figure 1-1). Because protein and enzyme function is essential in all phases of plant metabolism, arsenite can impair plant performance by altering metabolic processes (Shin et al 2004). Finally, arsenite can generate reactive oxygen species which can damage proteins, lipids and DNA (Liu et al. 2001).

Arsenate is a chemical analogue of inorganic phosphate and can compete for uptake through phosphate transporters (Shin et al 2004) (Figure 1-2). Once inside the cell, arsenate can interfere with any process that utilizes inorganic phosphate. In the presence of arsenate, ATP-synthase (adenosine triphosphate synthase) will form arsenate-ADP (arsenate adenosine diphosphate) instead of ATP (adenosine triphosphate) (Moore et al. 1983). Since arsenate-ADP is unstable, it undergoes hydrolysis, which causes a continuous cycle of the production of arsenate-ADP rather than ATP (Finnegan and Chen 2012). If ADP-arsenate does not dissociate, it can also interfere with ATP production by entering glycolysis. Arsenate from ADP-arsenate enters glycolysis in the 6^{th} step that usually produces 1,3-bisphosphoglycerate from glyceraldehyde 3-phosphate. In the presence of arsenate, 1-arseno-3-phosphoglycerate is formed instead, disrupting the glycolytic chain reaction (Figure 1-3) (Hughes 2002). This causes a

decline in plant performance since energy is not available for metabolic processes (Finnegan and Chen 2012).

Plant species have shown varying degrees of tolerance to arsenic. In an arsenic exposure study using the pea plant (*Pisum sativum*), Garg et al. (2012) found that the shoot growth and root length was reduced by 28% and 23% respectively when exposed to 30 mg/kg arsenic in soil. Reduced shoot growth was also documented in the red clover (*Trifolium pratense*) grown in soil containing 50 mg/kg of arsenic (Mascher et al. 2002). In a similar study using medic (*Medicago trunculata*), Xu et al. (2008) found that 200 mg/kg of arsenic reduced root and shoot dry weight. Another study using maize (*Zea mays*) showed that both root and shoot biomass decreased when plants were grown in 50 mg/kg of arsenic (Yu et al. 2009). Arsenic has also been shown to reduce seed germination. Chun-xi et al. (2007) found that 5 mg/kg of arsenic was enough to reduce seed germination as well as root and shoot growth of wheat seedlings (*Triticum aestivum*).

Understanding the effects of arsenic on vegetation has been skewed by a predominance of studies on vegetation from temperate to southern climates; the aforementioned studies focus on plant species that are not native to Northern Canada. Consequently, it is not clear if effects on northern vegetation are more or less severe than effects on their southern counterparts.

Some plants have been shown to tolerate and even sequester high levels of arsenic in their tissues. Arsenic is naturally occurring in plants, but concentrations rarely exceed 1 mg/kg (Adriano 2001). Arsenic hyperaccumulators are classified as plants that can accumulate 1000 mg/kg of arsenic dry weight (Baker and Brooks 1989). In non-hyperaccumulators, arsenic distribution in tissue generally decreases from root to stem and leaf to fruit, whereas, hyperaccumulators store the greatest amount of arsenic in shoots and leaves (Adriano 2001).

Phytoextraction is a process in which plants are used to extract contaminants from soil (Ghori et al. 2016). After plants have finished growing, they can be harvested and contaminants can be extracted and disposed of in a safe manner (United Nations Environment Programme 2018). Many mine sites in Northern Canada do not have all-season road access, and are only accessible by small propeller planes or winter ice roads. Phytoextraction could be a more cost-effective way of removing arsenic than replacing soil in situations where there is remote access. The Chinese brake fern has been widely studied as an arsenic hyperaccumulator. The brake fern can tolerate soils at concentrations of 1,500 mg/kg of arsenic and can accumulate 22,630 mg/kg of arsenic in above-ground tissue (Ma et al. 2001). The brake fern is native to China, but can be found in the Southern United States, so their use in phytoremediation is limited to that area. Because there is little research on northern vegetation, it is not surprising that arsenic accumulators have not been identified in native northern flora.

If it is not feasible to remove contaminants from the soil because of time or financial constraints, remediation efforts may focus on phytostabilization. Phytostabilization is the process by which vegetation is planted in contaminated areas to reduce mobilization of metals in soil. Plants can help stabilize metals through the sorption of metals onto root surfaces and metal accumulation in root tissue (Mendez and Maier 2008). For example, Fernández et al. (2016) showed that by planting nine plants each of three species of saltbush (*Atriplex atacamensis*, *Atriplex halimus* and *Atriplex nummularia)* they were able to reduce the soil arsenic concentration by 28 mg/kg while maintaining arsenic concentrations below 5 mg/kg in above-ground tissues. Phytostabilization is an important tool in remediation since it reduces the introduction of arsenic into higher trophic levels and, ultimately, the food supply since many

people rely on hunting as a major food source in Northern Canada (Indigenous and Northern Affairs Canada 2017).

Revegetation

Revegetation is a key reclamation strategy outlined in the Guideline for the Closure and Reclamation of Advanced Mineral Exploration and Mine Sites in the Northwest Territories (MVLWB and AANDC 2013). Vegetation provides services that are crucial for the functioning of ecosystems. For example, plants and their associated microorganisms play an important role in biogeochemical cycles including the carbon, nitrogen, phosphorus and water cycles (Schimel 1995; Gruber and Galloway 2008; Oki and Kanae 2006). Plants also convert the sun's energy into biomass acting as the base of the food chain. Vegetation contributes to soil development by supplementing soil with organic matter. Microorganisms subsequently break down the organic matter, mineralizing it and releasing nutrients for new vegetation or microorganisms to grow (Buscot and Varma 2005). Organic matter can also increase the water holding capacity of the soil and contributes to soil structure (Buscot and Varma 2005). In wetlands, wetland plants help provide flood control since they can slow down the movement of water and distribute it slowly over a floodplain (United States Environmental Protection Agency 2002).

Preservation of plant communities in Northern Canada is particularly important because of the interactions between vegetation and permafrost. Vegetation can absorb incoming solar radiation, acting as a protective layer over the ground protecting against permafrost thaw (Rocha and Shaver 2011). Permafrost is important because it has also been shown to be a source of water to arctic plants during drought conditions (Zhang et al. 2011). A decrease in permafrost caused by lack of vegetation could then cause a more hostile growing environment for the future succession of plants.

Plant diversity, the number and distribution of individuals among species, impacts ecosystem services; high diversity is associated with increases in productivity, protective effects against exotic species and the stabilization of the ecosystem process rates in response to disturbances and variation in abiotic conditions (Spehn et al. 2005; Fargione et al. 2007; Hooper 2005). One way higher diversity increases plant productivity is through complementarity effects. Complementarity occurs when niche differences among species, such as differences in resource use, leads to more efficient attainment of limiting resources and, therefore, productivity. In a 10-year biodiversity experiment, Fargione et al. (2007) found that at a higher diversity led to increased plant biomass, plant nitrogen pools and efficiency of nitrogen use. Higher levels of species richness are also believed to decrease the invasibility of a site by decreasing the levels of available nutrients; levels of limiting resources are lower in more diverse ecosystems therefore a lower portion of invaders can become established (Tilman 1997; Tilman et al 1997). Knops et al. (1999) found that the total biomass of external invaders significantly declined with increasing species richness in the invaded community. They also found that species richness played a protective effect against foliar fungal disease (Knops et al. 1999).

In addition to the general ecosystem services provided by vegetation, vegetation also provides ecosystem services that can help mitigate the anthropogenic effects of mining activities. Wetland vegetation acts as a filtration system by allowing sediments to settle out of the water once water movement has been slowed down (United States Environmental Protection Agency 2002). Plants stabilize soils, reducing sediment runoff which is especially important in reclamation efforts in the north, since contaminated sediment can be carried to neighbouring bodies of water during large spring thaws (Grismer and Hogan 2004). Wetland vegetation support communities of sulphate reducing bacteria, which are known to remove metals from

contaminated water by forming metal sulphide precipitates (White and Gadd 1996; White et al. 1995). The organic material in soil, which in part comes from vegetation, can adsorb to metals reducing their mobility and bioavailability (Liu and Gonzalez 1999). The removal of vegetation can therefore cause a dramatic decline in an ecosystem, especially one that has been impacted by mining activities.

Current Approaches to Revegetation

Traditional soil amendment techniques involve increasing organic content, de-compaction and the addition of fertilizers. More recently it has become common for companies to save a stockpile of organic material from the site to use later for re-vegetation. Unfortunately, since the organic layer of soil in Northern Canada can be thin, often the organic material that is available is not enough to properly supplement the large areas that are in need of revegetation (Canadian System of Soil Classification 2013). Currently, a mixture of native and non-native grasses and legumes is used to revegetate areas, but the MVWLB states that the introduction of non-native species should be avoided, as they could contribute to an unnatural ecosystem. Non-native species are not as adapted to site conditions, and to the extreme environment, and they are therefore less likely to be self-sustaining (Densmore and Holmes 1987). In some cases, non-native species can become invasive, quickly spreading and potentially altering natural ecosystems. For example, the European Common Reed (*Phragmites australis*) which was introduced to Canada, can grow in dense monocultures, reducing the biomass, richness and abundance of native plant species (Great Lakes Phragmites Colaborative 2017).

Revegetation in northern climates is limited by lower available nutrients (Densmore et al. 2000). Adding fertilizer can be useful in some situations, but it has been shown that seedlings are often too small to use fertilizer in their first year, risking the chance of fertilizer runoff and

eutrophication (Densmore et al. 2000). Furthermore, it has been shown that for seedlings to successfully establish, there needs to be on-going application of fertilizer over many years (MVLWB and AANDC 2013). Since mines are in such remote areas, it is cost intensive to return to the site the following years to apply fertilizer. One possible way of increasing plant survival and growth rate without the use of nutrient rich fertilizers is using mycorrhiza.

Arbuscular Mycorrhizal Fungi and Dark Septate Endophytes

Studies have shown that arbuscular mycorrhizal symbiosis are present in approximately 80% of vascular plants, and the symbiosis is considered the normal state for most plants under most ecological conditions (Smith and Read 2008). Arbuscular Mycorrhizal Fungi (AMF) are a group of fungi that form mutualistic symbiotic relationships with plants. AMF can only survive within a host plant since they are dependent on plants as a source of essential nutrients. AMF receive an organic source of carbon from plants in exchange for essential nutrients such as nitrogen and phosphorus (Smith and Read 2008). Large amounts of phosphate are required for plant growth and function, but is often not readily available in soil (Smith and Read 2008). Phosphorus is a vital structural component in cells making up the backbone of nucleic acids, as well as the hydrophilic head in phospholipids (Dahm 2005; Singer and Nicolson 1972). Phosphorus also plays a large role in energy metabolism since it is a main component in ATP (Maruyama 1991); it also activates metabolic intermediates, is a component in signal transduction cascades, and regulates enzymes (Ardito et al. 2017). By providing phosphorus to plants, mycorrhizae are vital to numerous biological functions.

AMF are composed of three root inhabiting structures, which includes the arbuscule, hyphae, vesicle, and a separate and external nutrient storage and propagule structure, the spore

(Smith and Read 2008). The arbuscule is a branched system found in cortical cells of the plant root and acts as the site for nutrient exchange and the vesicle acts as a storage unit for nutrients (Smith and Read 2008). The hyphae can be intracellular, acting as a pathway between arbuscules and vesicles, or extracellular, having an absorptive role or acting as a conduit (Smith and Read 2008). It has been shown that arbuscular mycorrhizal plants have two pathways by which phosphate can be taken up (Smith et al. 2003). The direct pathway involves phosphate transporters in the root epidermis and root hairs, which is the same pathway in non-mycorrhizal plant species. The indirect pathway involves hyphae that take up phosphorus and translocate it through the extracellular hyphae to the arbuscule where it is absorbed by the cortical cells of the plant root (Poirier and Bucher 2002; Smith and Read 2008). To retrieve phosphorus from the soil, AMF extend extracellular hyphae past nutrient depletion zones created by the roots and absorb orthophosphate from the soil solution. AMF are more efficient at retrieving phosphorus than plant roots not only because they can pass nutrient depletions zones, but because their hyphae are smaller in diameter than roots, allowing them to access soil pores that are inaccessible to roots, thereby, increasing the volume of soil nutrient solution available for uptake (Smith and Read 2008). It has also been shown that the expenditure of carbon per unit length of hyphae is smaller than that of roots (Tinker et al. 1975).

Arbuscular mycorrhizal spores are thick walled spherical structures containing lipids and some carbohydrates (Smith and Read 2008). They can be found both in the root or soil attached to hyphae or as stand-alone structures (Brundrett 2008). Spores are resistant and overwintering structures that act as a source of inoculum, and the only source that can be dispersed by wind, water and by animals (Koske and Gemma 1990; Friese and Allen 1991). In areas where propagules have been exhausted, such as in areas with no topsoil, spores are the main source of

inoculum for pioneer species since they can be dispersed over long distances (Bellgard 1993; Oehl et al. 2011). Therefore, during revegetation, if mycorrhizae have not been negatively impacted, then spores from neighbouring vegetated areas could help support the succession of plants to include mycorrhizal species (Oehl et al. 2011). If the arbuscular mycorrhizal communities have declined then it is possible to inoculate soil with spores or infected root fragments collected locally since they would be better acclimated to the environment (Orlowska et al 2011).

Because of the nutrients supplied by AMF, plants receive many benefits from being colonized. In numerous studies, AM-colonized plants were larger and contained higher concentrations of phosphorus than uncolonized treatments (Olensniewicz and Thomas 1999; Giri et al. 2003; Bolan 1991). AMF have also been shown to increase plant tolerance to drought. Wu and Xia (2005), found that tangerine plants (*Citrus tangerine*) colonized by AMF accumulated higher levels of soluble sugar, soluble starch, non-structural carbohydrates, K^+, Ca^+ and Mg^+ in roots and leaves compared to non-colonized plants when exposed to drought suggesting greater capacity for osmotic adjustment. Plants accumulate organic and inorganic solutes during drought condition to decrease the plant water potential, driving water into the plant cells. Because of the increased osmotic regulation facilitated by AMF, Wu and Xia also found that under drought conditions, the tangerine plants had higher shoot and root dry weight, plant height, leaf area, leaf number per plants and stem diameter than corresponding non-colonized plants.

Studies have shown that AMF are able to affect plant biodiversity and ecosystem function and increase stress tolerance (Klironomos et al. 2000; van der Heijden et al. 1998; Orlowska et. al 2011; Christopherson et al. 2009; Xia et al. 2007). In a study conducted by van der Heijden et al. (1998), where they compared the effects of a composition of four different

AMF taxa on the species composition and structure of 48 microcosms, they found that eight of eleven plants were almost completely dependent on mycorrhiza to be successful. van der Heijden et al. (1998) also found that the plant biomass of different species varied among treatments with different AMF taxa, showing that different plant species benefit to a different extent depending on the AMF taxa present. They also found that the overall structure of plant communities was impacted by the treatment of different AMF taxa. In the second part of the study where they set up a field experiment with 70 macrocosms inoculated with differing assemblages of mycorrhizal propagules, they found that both plant biodiversity and productivity increased with increasing AMF diversity (van der Heijden et al. 1998).

AMF have been shown to be an important factor in revegetation by increasing plant biomass and seedling survival in mining revegetation efforts (Orlowska et. al 2011). Orlowska et al. (2011) showed that colonized plants growing in contaminated tailings soil had both an increased plant biomass and seedling survival when compared to their uncolonized counterpart. They also found that when native mycorrhiza, extracted from the tailings area was used, the plant biomass was higher than that in plants colonized with non-native commercially available AMF. This shows that native AMF ecotypes may be a better choice for reclamation since they are adapted to the harsh soil conditions and climate (Sylvia and Williams 1992; Weissenhorn et al. 1993; Orlowska et al. 2005).

Because of their ability to increase phosphate intake in plants, mycorrhizae play a protective role against arsenic toxicity. Phosphate and arsenate are chemical analoges; therefore, arsenate is readily taken up into the plant root via phosphate transporters (Shin et al 2004). In a study conducted by Christopherson et al. (2009), they found that upon colonization of AMF, the barley plant (*Hordeum vulgare*) downregulated two genes encoding for phosphate transporters

(*HvPht1;1* and *HvPht1;2)* in the root epidermis and root hairs. This created a bypass through the AM pathway, whose phosphate transporters have a higher affinity for phosphate than for arsenic. Another, and indirect way that AMF colonization could reduce the negative effects of arsenic toxicity is through increased phosphorus nutrition, which causes an increase in plant biomass ultimately diluting the arsenic concentration in the plant tissue (Chen et al. 2007; Ahmed et al. 2006).

In an arsenic exposure study where lentil plants (*Lens culinaris*) were exposed to varying levels of arsenic concentrations in soil, Ahmed et al. (2006) found that plant height, leaf number, pod number, plant biomass and root/shoot concentrations of phosphorus were higher in plants colonized by AMF. They also found that mycorrhizal infection reduced arsenic concentrations in roots and shoots. This shows that AMF can increase nutrition acquisition while excluding arsenic in contaminated soils. Xia et al. (2007) found similar results when experimenting with arsenic uptake in the maize plant. Xia et al. (2007) found that root length and dry weight increased with mycorrhizal colonization and arsenic concentration in shoots decreased compared to non-colonized plants.

Soil disturbance during mining activities has been shown to negatively impact beneficial soil microbiota including arbuscular mycorrhizal fungi (Orlowska et. al 2011). As it relates to mining activities, these disturbances could include the stripping of vegetation, construction of roads and buildings, and soil compaction (Hook and Burke 2000). Physical soil disturbance can break fungal structures, reduce propagules and reduce the number of interaction sites between plant roots and fungi (Rives et al. 1980; Jasper et al. 1987). Arsenic can reduce AMF spore germination as well as colonization of roots. In a germination trial conducted by Wu et al. (2009), it was found that 2.5 mg/kg of arsenic reduced germination in the AMF species *Glomus*

mossae by 40% and *Glomus geosporum, Glomus etunicatum* by 20%. In another experiment, Gonzalez-Chavez et al. (2002) found that 50 mg/kg of arsenic reduced germination in both *Glomus mossae* and *Gigaspora rosea*. Studies have shown that the concentration of arsenic that impacts AMF colonization appears to be dependent on plant host species. In a spiked soil experiment, Yu et al. (2009) found that 25 mg/kg of arsenic reduced colonization of the mycorrhizal species *Glomus etunicatum* and *Glomus contrictum* in maize roots (*Zea mays*). Garg et al. (2012) found that there was a significant decrease in colonization of *Glomus mossae* in pea roots (*Psium sativum*) with 30 mg/kg of arsenic whereas Liu et al. (2005) found a decrease in colonization at 150 mg/kg and in tomato roots (*Solanum lycopersicum)*.

Dark Septate Endophytes (DSE) are another group of root colonizing fungi that are believed to form a symbiotic relationship with plants. They can colonize the root epidermis and the cortex inter/intracellularly but unlike AMF, they are darkly pigmented with septate hyphae (Vergara et al. 2018). DSE can colonize a greater variety of plants than mycorrhizae, and are capable of colonizing roots at the same time as AMF (Jumpponen 2001). DSE have been shown to form a wide range of symbiosis, ranging from mutualistic to parasitic (Mandyam and Jumpponen 2005). In some cases, they have shown that their role is similar to that of AMF (Li et al. 2011; Santos et al. 2017; Vegara et al. 2018). In a meta-analysis compiling 18 independent studies, Newsham (2011) found that DSE colonization raised nitrogen and phosphorus concentrations as well as increased plant biomass by 26-103%. Conversely, in a meta-analysis conducted by Mayerhofer et al. (2013), neutral or even negative impacts on nitrogen concentrations and plant biomass in plants colonized by DSE were noted. Although the influence on DSE on plants is still under debate, the identification of DSE in northern environments is a step towards assessing its use in revegetation in the north. Additionally, studies

assessing the impacts of gold mining and arsenic on DSE are scarce, but it appears that DSE react similarly to AMF, resulting in reduced colonization in high arsenic-contaminated tailings (Orlowska et al. 2011).

Unfortunately, much of the current literature on the impacts of mining on mycorrhizae focuses on temperate or southern mycorrhizal and plant species. Given the benefits provided by AMF, especially with regards to mitigating impacts of metal toxicity and increasing the success of revegetation, it is important to understand how mining can impact mycorrhizae in a northern environment to better guide revegetation efforts.

Northern Environment Considerations

Compared to temperate regions, the north experiences a cooler mean daily temperature. In Yellowknife, NWT the daily average is -26.8°C in January and 16.8°C in June, with a short frost-free period starting in May and ending in late September (Government of Canada 2018). These climate characteristics result in a shorter growing season with lower biodiversity compared to temperate regions (Government of Northwest Territories 2009; Government of Northwest Territories 2012). Since most of Northern Canada was still glaciated until ~10,000 years ago, soils have only been ice-free for a relatively short period of time and are, therefore, shallow and weakly developed. Because of the low average temperature, the rate of decomposition and weathering is slow, resulting in lower available nutrients and decreased productivity (Densmore et al. 2000). As a result, Northern areas may take longer to recover from anthropogenic impacts such as mining and mining closures (Densmore and Holmes 1987). Because of the lack of infrastructure in Northern Canada, many mines are in remote areas where there is limited road access, are accessible only by plane. This limited access can limit

reclamation measures and dramatically elevate costs (MVLWB and AANDC, 2013). For these reasons, it is important to consider using native species found and collected near the mines, as well as soils and material available on site to avoid the need to ship large quantities of soil, fertilizer and seed.

Knowledge Gaps

The MVLWB and INAC, who are responsible for creating the guidelines for the closure and reclamation of mine sites in the Northwest Territories, have expressed the need for more research on northern plants in revegetation efforts. As part of reclamation and remediation, it is important to assess the impacts of mining because it identifies areas that are in need of rehabilitation. In a meta-analysis conducted by Vilmi et al. (2017), authors point out that there are an alarmingly small set of studies that describe the effects of anthropogenic stressors in real-life circumstances in the north. Of the studies Vilmi et al. (2017) did find, they showed that pollution, including contamination of soils, was the most harmful stressor affecting species richness, despite that it was not as commonly studied as land use, such as habitat destruction or fragmentation. Given the lack of information regarding gold mining impacts on plant community structure and arsenic uptake in northern environments; the potential for arsenic, a known contaminant associated with gold mines, to impact plant growth and development and accumulate in plant tissues, and the necessity for this information to guide restoration strategies, my goals were to quantify the level of impact at a northern mine site by quantifying arsenic concentrations in soils, comparing plant community composition and quantifying the arsenic accumulation in plants from sites adjacent to a known arsenic contamination point compared to reference sites further away.

My first objective was to quantify arsenic concentration in soils on and adjacent to a mine site. Quantifying arsenic concentrations at a mine site is important in order to get an understanding of the level of impact potentially caused by the mine (MVLWB and AANDC 2013). Additionally, arsenic concentrations are needed to determine if arsenic is associated with other impacts such as changes in plant community or mycorrhizal colonization. Since the original milling area or tailings containment area were historically the main sources of arsenic contamination, my first hypothesis is that soil arsenic concentrations will be higher adjacent to those areas.

My second objective is to compare arsenic accumulation among species at a given location and within species at differing locations at the mine. Currently there are no studies which show the arsenic accumulation of northern plant species so it is unclear which species will accumulate higher concentrations of arsenic (MVLWB and AANDC 2013). Identifying differences in arsenic accumulation among species has great remediation implications. Species that accumulate and tolerate high arsenic concentrations can be used in the phytoremediation of Northern arsenic contaminated mine sites (Tu and Ma 2003). Conversely plants that accumulate low arsenic concentrations can be used in areas that are expected to experience higher rates of herbivory. Comparing arsenic concentrations in one species across sites is equally as important since it helps determine the levels of impact caused by the mine site (MVLWB and AANDC 2013). My second hypothesis is that arsenic concentration in plants will be greater in plants collected from sites with higher soil arsenic concentrations.

My third objective is to compare plant community composition in areas differing in soil arsenic concentrations. By comparing plant communities growing in high and low arsenic sites, it is possible to determine if soil arsenic concentrations are correlated with changes in plant

communities. Not only will this help determine the impacts caused by mining, but will also be a useful guide for remediation. Un-impacted plant communities can become a long-term target community for revegetation efforts and can be used to assess the recovery of arsenic contaminated mine sites in Northern Canada. Additionally, species that are found in impacted sites can be used in the revegetation of disturbed areas, since they are tolerant to those conditions. Based previous studies which have shown the impacts of arsenic on temperate and southern plant species (Garg et al. 2012; Mascher et al. 2002; Xu et al. 2008; Yu et al. 2009; Chun-xi et al. 2007), my third hypothesis is that plant communities adjacent to the milling and tailings containment area will differ from references sites and those differences will be correlated with soil arsenic concentrations.

Considering the importance of AMF colonization in ameliorating plant performance and survival, alleviating metal toxicity and maintaining biodiversity of plant communities, AMF will be key to revegetation efforts in areas impacted by gold mining. Currently, the Guideline for the Closure and Reclamation of Advanced Mineral Exploration and Mine Sites in the Northwest Territories does not include AMF as part of soil testing or amendment. There is very little research on AMF in northern environments and it is, therefore, unclear whether they are a natural part of that environment. If AMF are naturally-occurring in northern environments they should be considered during revegetation plans not only because they are native to the environment, but because they could increase the success rate of revegetation projects. My goal was to address the lack of research on the impacts of arsenic on mycorrhizae in a northern environment by sampling roots from vegetation in areas close to a known arsenic contamination point and comparing them with roots from reference sites further away.

My fourth objective of this study was to compare mycorrhizal colonization levels among species at a given location and within species at differing locations at the mine. Since there are very few studies on the mycorrhizal colonization of northern plant species it is not possible to predict which species specifically will have higher levels of mycorrhizal colonization. Comparing the mycorrhizal colonization across species can reveal which species may be more highly colonized and therefore a good source of propagules that can be used during revegetation. Comparing the mycorrhizal colonization of each species across several mine sites will shed light on whether the mycorrhizal symbiosis was impacted by elevated arsenic concentrations caused by mining. If colonization is found at high arsenic sites, then the mycorrhiza from those sites could be used as part of the remediation of arsenic impacted areas. Based on several studies which have shown that exposure to arsenic may reduce mycorrhizal colonization (Orlowska et al. 2011; Yu et. al 2009; Garg et al. 2012; Liu et al. 2005), my fourth hypothesis is that mycorrhizal colonization will be will be lower in areas with higher arsenic concentrations.

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Figures

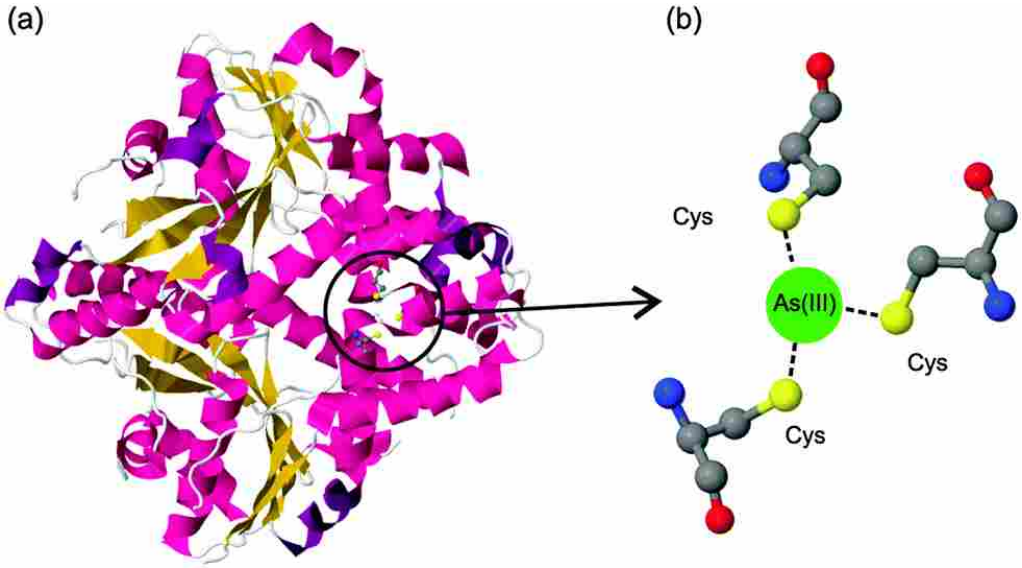

Figure 1- 1: Arsenite bonding with cysteine residues in a protein. These chemical bonds can damage proteins rendering them ineffective (Chen et al. 2014).

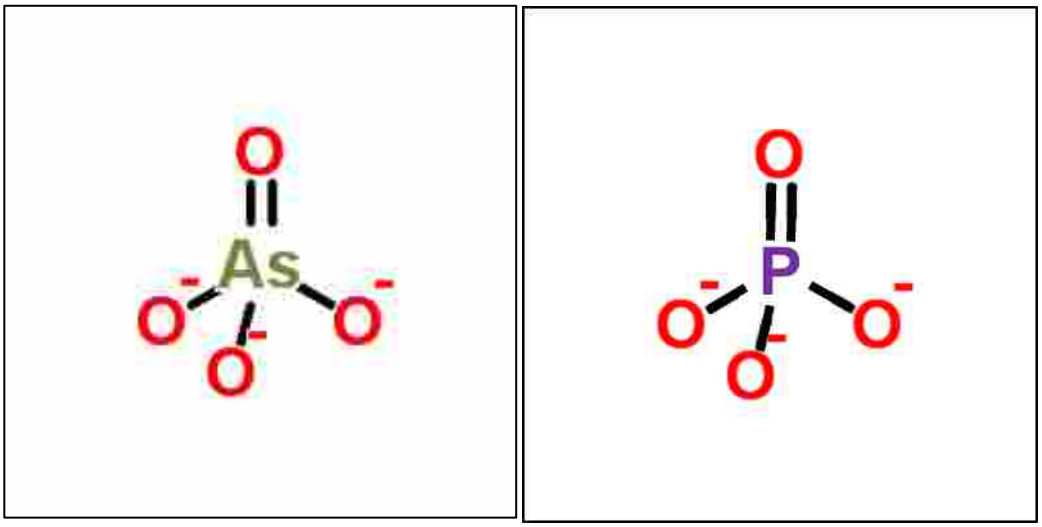

Figure 1- 2: Skeletal chemical model of arsenate and phosphate. Arsenate is a chemical analogue of phosphate, and can interfere with metabolic processes that utilize organic phosphate (Finnegan and Chen 2012). (Pictures obtained from ChemSpider 2015 arsenate:

http://www.chemspider.com/Chemical-Structure.25498.html?rid=11fed839-5d7e-4a10-8677-10c2c111b1ca and phosphate: http://www.chemspider.com/Search.aspx?q=phosphate+ion.

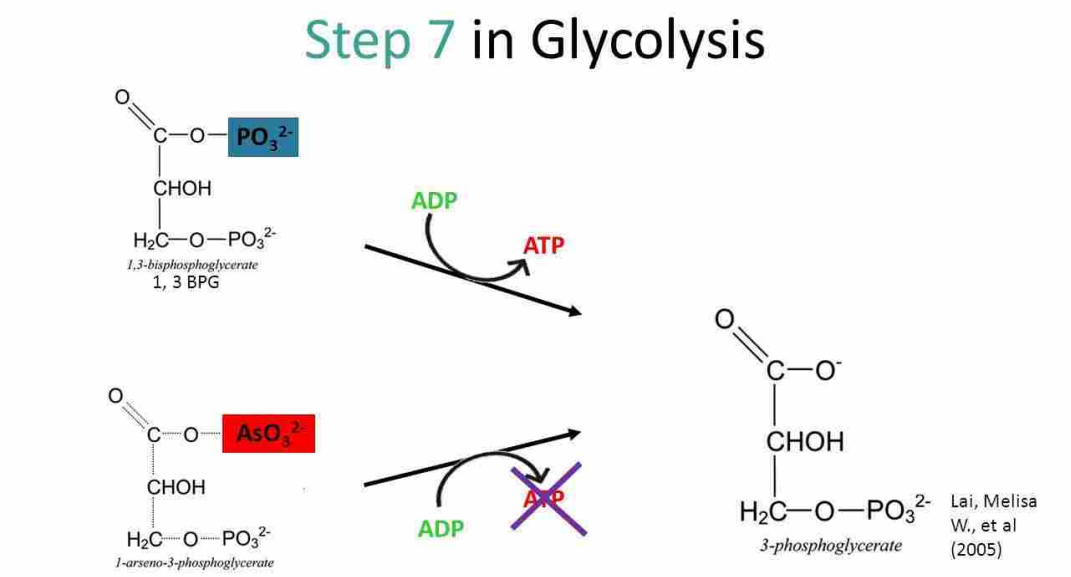

Figure 1- 3: Seventh step of glycolysis in the presence of 1-arseno-3-phosphoglycerate. ADP-arsenate enters glycolysis in the 6th step that usually produces 1,3-bisphosphoglycerate from glyceraldehyde 3-phosphate. In the presence of arsenate, 1-arseno-3-phosphoglycerate is formed instead, disrupting the 7th step of the glycolytic chain, preventing ATP formation (Hughes 2002; Lai et al. 2005).

Chapter 2 - The impacts of gold mining on northern plant communities and arsenic uptake in plants

Abstract

Gold mining is an important part of economic development in Northern Canada. A large portion of the gold that is found in the North is contained within arsenopyrite ores, therefore, arsenic contamination is of special concern. Little is known regarding the impacts of arsenic on northern plant communities or arsenic uptake in northern plant species. Assessing the impacts of mining on plant communities identifies areas that are in need of rehabilitation, and quantifying arsenic concentration in plants shows which species would be best suited for phytoremediation. In temperate regions, arsenic has been shown to negatively impact plant growth and seed germination in some species, while others can be tolerant and even accumulate high concentrations of arsenic. To assess the impact of arsenic on plants, field studies were undertaken at Tundra Mine, an inactive gold mine 250 km northeast of Yellowknife, NWT. Vegetation surveys were conducted and plant samples were collected at 4 sites near a tailings containment area and 4 reference sites further away from the mine. Soil was also collected at these sites and arsenic levels in soils was quantified. Arsenic concentrations in plants ranged from 0.08 ± 0.01 mg/kg to 870 ± 230 mg/kg. As predicted, the highest arsenic concentrations were consistently found in plants that grew in the 4 sites adjacent to the tailings containment area. *Carex aquatilis* contained the highest concentration of arsenic at those sites making it a good potential candidate for phytoremediation (roots = 870 ± 230 mg/kg; shoots = 141 ± 49.0 mg/kg). At the sites with the two highest soil arsenic concentrations all plants sampled exceeded arsenic concentrations recommended for plant material designated for human consumption and animal feed by several orders of magnitude. NMDS results using vegetation survey data showed that plant communities at the 4 sites adjacent to the mine were distinct from the 4 reference sites and correlated with arsenic concentrations in the soil. Species associated with high arsenic sites,

such as *Carex aquatilis* and *Salix athabascensis*, could be used to revegetate high arsenic area. Ericaceous species associated with less impacted sites can represent end target communities during revegetation efforts.

Introduction

Gold Mining in the North

Canada is ranked as one of the top five producers of many metals and minerals (United States Geological Survey 2011). As the global demand for minerals and metals continues to increase, Canada is in a good position to supply the output needed. Mineral and metal output is expected to grow by 91% from 2011 to 2020, which is a growth rate four times higher than the Canadian economy (Rheaume and Caron-Vuotari 2013). Mining is especially important in Northern Canada since it is a main contributor to the Northern economy (Government of Northwest Territories 2016). Resource extraction in the Northwest Territories alone contributes $900 million of the GDP, which is more profitable than any industry in the north by $333 million (Government of Northwest Territories 2016).

Many of the mines found in Northern Canada are found in the Northwest Territories. Currently most operational mines in the Northwest Territories are diamond mines, but historically gold mines were the most prevalent (Silke 2009). Commodity prices for gold fluctuate, and when prices plummet, it is no longer economical to continue mining. As a result of unsteady markets, some mines may also be abandoned before reclamation has taken place. Due to historic instability of gold prices, there are now many abandoned gold mines in the Northwest Territories awaiting proper closure (National Orphaned/Abandoned Mine Initiative 2009).

The Mackenzie Valley Land and Water Board (MVLWB) and Indigenous and Northern Affairs Canada (INAC) have created guidelines for the closure and reclamation of mine and mineral sites in the Northwest Territories. Their main goal outlined in the guidelines is “to return the mine and affected areas to viable and, wherever practicable, self-sustaining ecosystems that are compatible with a healthy environment and with human activities” (MVLWB and

AANDC 2013). The most important ecosystem services that are restored during reclamation include maintaining site stability, nutrient cycling and providing a habitat for wildlife (MVLWB and AANDC 2013). Since these services are provided by plants and the microorganisms associated with them, vegetation is a significant part of reclamation (Buscot and Varma 2005).

Ecological Services Provided by Plants

Plant communities provide services that are crucial for the functioning of ecosystems. For example, plants and their associated microorganisms play an important role in biogeochemical cycles including carbon, nitrogen, phosphorus and water cycles (Schimel 1995; Gruber and Galloway 2008; Oki and Kanae 2006). Vegetation contributes to soil development by adding organic matter to the soil. Microorganisms subsequently break down the organic matter, mineralizing it and releasing nutrients for new vegetation or microorganisms to grow (Buscot and Varma 2005). Organic matter, which increases the water holding capacity of the soil and contributes to soil structure (Buscot and Varma 2005). Vegetation is particularly important in Northern Canada because it can absorb incoming solar radiation, acting as a protective layer over the ground protecting against permafrost thaw. It has been shown that lack of vegetation and or an organic layer of soil can cause surface warming, which could lead to permafrost thaw (Rocha and Shaver 2011). Permafrost is important because it has also been shown to be a source of water to arctic plants during drought conditions (Zhang et al. 2011).

Plant diversity, the number and distribution of individuals among species, impacts ecosystem services; high diversity is associated with increases in productivity, protective effects against exotic species and the stabilization of the ecosystem process rates in response to disturbances and variation in abiotic conditions (Spehn et al. 2005; Fargione et al. 2007; Hooper 2005). Higher diversity increases plant productivity through complementarity effects.

Complementarity occurs when niche differences among species, such as differences in resource use, leads to more efficient attainment of limiting resources and therefore productivity. In a 10-year biodiversity experiment, Fargione et al. (2007) found that higher diversity led to increased plant biomass, plant nitrogen pools and efficiency of nitrogen use. Higher levels of species richness are also believed to decrease the invasibility of a site by decreasing the levels of available nutrients; levels of limiting resources are lower in more diverse ecosystems therefore a lower portion of invaders become established (Tilman 1997; Tilman et al. 1997). Knops et al. (1999) found that the total biomass of external invaders significantly declined with increasing species richness in the invaded community. They also found that species richness played a protective effect against foliar fungal disease (Knops et al. 1999).

Impacts of Arsenic on Plants

Many of the gold deposits in Northern Canada are found within arsenopyrite sulphide ores, which releases arsenic when milled or roasted for gold (Rheaume and Caron-Vuotari 2013). Inorganic arsenic is the most common form found in soil (Fitz & Wenzel 2002). The two forms of inorganic arsenic that can be readily taken up by plant roots are arsenate (AsO_4^{-3}) and arsenite (AsO_3^{-}). Arsenite is a trivalent dithiol reactive compound that can bind to multiple cysteine residues or dithiol cofactors in proteins or enzymes (Finnegan and Chen 2012). In the presence of closely spaced cysteine residues in a protein, one arsenite molecule can bind to multiple sites, causing the protein to denature and potentially cease to function (Finnegan and Chen 2012) (Figure 2). Because protein and enzyme function is essential in all phases of metabolism, arsenite can impair metabolic processes (Shin et al 2004). Arsenite has been shown to generate Reactive Oxygen Species which can damage proteins, lipids and DNA and is believed to be the direct cause of carcinogenicity (Liu et al. 2001).

Arsenate is a chemical analog of phosphate and can compete for uptake via phosphate transporters (Shin et al 2004). Once inside the cell, arsenate can interfere any process that utilizes inorganic phosphate. In the presence of arsenate, ATP-synthase will form arsenate-ADP instead of ATP (Moore et al. 1983). Since arsenate-ADP is unstable, it undergoes hydrolysis, which causes a continuous cycle of the production of arsenate-ADP rather than ATP (Finnegan and Chen 2012). If ADP-arsenate does not dissociate, it can also interfere with ATP production by entering glycolysis. If ADP-arsenate is present during the 6^{th} step in glycolysis, 1-arseno-3-phosphoglycerate is formed instead of 1,3-bisphosphoglycerate, disrupting the glycolytic chain reaction and inhibiting the formation of ATP (Hughes 2002). This causes a decline in plant performance since energy is not available for metabolic processes (Finnegan and Chen 2012).

Since arsenic has been shown to affect several biological processes, it is not surprising that it can impact plants. Plant species have shown varying degrees of tolerance to arsenic. In an arsenic exposure study using the pea plant (*Pisum sativum*), Garg et al. (2012) found that the shoot growth and root length was reduced by 28% and 23% respectively when exposed to 30 mg/kg arsenic in soil. Reduced shoot growth was also documented in the red clover (*Trifoiluim pratense*) grown in soil containing 50 mg/kg of arsenic (Mascher et al. 2002). In a similar study using medic (*Medicago trunculata*), Xu et al. (2008) found that 200 mg/kg of arsenic reduced root and shoot dry weight. Another study using maize (*Zea mays*) showed that both root and shoot biomass decreased when plants were grown in 50 mg/kg of arsenic (Yu et al. 2009). Arsenic has also been shown to reduce seed germination. Chun-xi et al. (2007) found that 5 mg/kg of arsenic was enough to reduce seed germination as well as root and shoot growth of wheat seedlings (*Triticum aestivum*).

Understanding the effects of arsenic on vegetation has been skewed by a predominance of studies on vegetation from southern climates; the previously mentioned studies focus on plant species that are not native to Northern Canada. Consequently, it is not clear if effects on northern vegetation are more or less severe than effects on their southern counterparts. One exception is a recent study by Kevin MacColl (2017). MacColl examined the impacts of arsenic on the Reed Canary Grass (*Phalaris arundinaceae*), although not native to the NWT. By growing plants in soils collected from areas in and around Giant Mine, an inactive gold mine in Yellowknife, Northwest Territories MacColl found that the Reed Canary Grass shoot area and root length were negatively correlated with arsenic concentration.

Some plants can tolerate and even sequester high levels of arsenic in their tissues. Arsenic is naturally occurring in plants, but concentrations rarely exceed 1 mg/kg (Adriano 2001). Arsenic hyperaccumulators are classified as plants that can accumulate 1000 mg/kg of arsenic dry weight (Baker and Brooks 1989). In non-hyperaccumulators, arsenic distribution in tissue generally decreases from root to stem and leaf to fruit, whereas hyperaccumulators store the greatest amount of arsenic in shoots and leaves (Adriano 2001). The Chinese brake fern has been widely studied as an arsenic hyperaccumulator. The brake fern can tolerate arsenic concentrations of 1500mg/kg in soils and can accumulate 22,630 mg/kg of arsenic in above ground tissue making is a good candidate for the phytoextraction of arsenic (Ma et al. 2001). Phytoextraction is a process by which plants are used to purify soils through the extraction of contaminants (Ghori et al. 2016). Once hyperaccumulators have established and grown, they are harvested, and contaminants can be extracted and disposed of in a safe manner (United Nations Environment Programme 2018). The brake fern is native to China, and can be found in the Southern United States, so its use in phytoremediation is geographically limited. Many mine

sites in Northern Canada do not have all season road access, and are only accessible by small propeller planes or winter ice roads. Phytoextraction would be a more cost-effective way of removing arsenic than replacing soil because of remote access. Because there is little research on northern vegetation, it is not surprising that arsenic accumulators have not been identified in native northern flora.

If it is not feasible to remove contaminants from the soil because of time or financial constraints, remediation efforts may focus on phytostabilization. Phytostabilization is the process by which vegetation is planted in contaminated areas to reduce mobilization of metals. Plants can help stabilize metals through the sorption of metals onto root surfaces and metal accumulation in root tissue (Mendez and Maier 2008). For example, Fernández et al. (2016) showed that by planting nine plants each of three species of saltbush (*Atriplex atacamensis*, *Atriplex halimus* and *Atriplex nummularia*) they could reduce arsenic concentrations in the soil from 131 mg/kg to 103 mg/kg while maintaining arsenic concentrations below 5 mg/kg in above ground tissues. Phytostabilization is an important tool in remediation since it reduces the introduction of arsenic into higher trophic levels, and ultimately the food supply since many people rely on hunting as a major food source in Northern Canada (Indigenous and Northern Affairs Canada 2017).

Mine Reclamation in the North

Compared to temperate regions, the north experiences a cooler mean daily temperature. For example, in Yellowknife, NWT the daily average is -26.8°C in January and 16.8°C in June, with short frost free period starting in May and ending in late September (Government of Canada 2018). Soil is typically lower in nutrient and water availability (Billings et al. 1984; Shaver et al. 1986). As a result, ecosystem recovery from environmental impacts is much slower in northern climates (Densmore and Holmes 1987). Furthermore, the harsh northern environment would not

be a conducive environment for temperate plant species and reclamation approaches that have been successful in the south may not apply in northern areas. Because of the lack of research available on northern vegetation, the MVLWB and INAC recognize the need to increase vegetation research in Tundra, Subarctic regions to better guide reclamation efforts.

The end goal of revegetation efforts is to have diverse and native plant communities that provide biodiversity and sustainability to the reclaimed sites (MVLWB and AANDC 2013). The use of native species in re-vegetation is especially important since they are well suited to the local environments and consequently, will have a higher chance of survival (Densmore et al. 2000). The use of native species during revegetation is, therefore, self-sustaining because it requires less long-term inputs of resources to support soil formation and nutrient cycling (Brown and Amacher 1999). Native species can support the natural succession of the land eventually restoring functionality of the ecosystem (Brown and Amacher 1999).

The Guidelines for the Closure and Reclamation of Advanced Mineral Exploration and Mine Sites in the Northwest Territories (MVLWB and AANDC 2013) suggest characterizing vegetation in sites that are not impacted and comparing them to sites closer to mining activities to assess the extent of mining impacts and to collected baseline data for potential plant species that could be used for revegetation. A vegetation survey would also identify any species that are lost due to mining, which would need to be reintroduced in as part of the remediation efforts. Since arsenic is a contaminant of concern at gold mine sites the MVLWB and INAC also outline the importance of identifying plant species that are accumulating arsenic so that they can be avoided as part of a re-vegetation plan. Avoidance is recommended due to the potential for arsenic to enter the food chain (Kocar et al. 2004). On the other hand, arsenic accumulators can

be used as a tool in bioremediation of contaminated soils, especially since most mine sites are remote, and removing and replacing contaminated soil is costly.

Given the lack of information regarding gold mining impacts on plant community structure and arsenic uptake in northern environments; the potential for arsenic, a known contaminant associated with gold mines, to impact plant growth and development and accumulate in plant tissues, and the necessity for this information to guide restoration strategies, my goals were to compare plant community composition and arsenic accumulation in plants in sites adjacent to a known arsenic contamination point compared to reference sites further away, and presumably beyond the zone of influence of the mine.

My first objective was to quantify arsenic concentration in soils on and adjacent to a mine site. Quantifying arsenic concentrations at a mine site is important in order to get an understanding of the level of impact potentially caused by the mine. Additionally, arsenic concentrations are needed to determine if arsenic is associated with other impacts such as changes in plant community or mycorrhizal colonization. Since the original milling area or tailings containment area were historically the main sources of arsenic contamination, my first hypothesis is that soil arsenic concentrations will be higher adjacent to those areas.

My second objective is to compare arsenic accumulation among species at a given location and within species at differing locations at the mine. Currently there are no studies which show the arsenic accumulation of northern plant species so it is unclear which species will accumulate higher concentrations of arsenic (MVLWB and AANDC 2013). Identifying differences in arsenic accumulation among species has great remediation implications. Species that accumulate and tolerate high arsenic concentrations can be used in the phytoremediation of Northern arsenic contaminated mine sites (Tu and Ma 2003). Conversely plants that accumulate

low arsenic concentrations can be used in areas that are expected to experience higher rates of herbivory. Comparing arsenic concentrations in one species across sites is equally as important since it helps determine the levels of impact caused by the mine site (MVLWB and AANDC 2013). My second hypothesis is that arsenic concentration in plants will be greater in plants collected from sites with higher soil arsenic concentrations.

My third objective is to compare plant community composition in areas differing in soil arsenic concentrations. By comparing plant communities growing in high and low arsenic sites, it is possible to determine if soil arsenic concentrations are correlated with changes in plant communities. Not only will this help determine the impacts caused by mining, but will also be a useful guide for remediation. Un-impacted plant communities can become a long-term target community for revegetation efforts and can be used to assess the recovery of arsenic contaminated mine sites in Northern Canada. Additionally, species that are found in impacted sites can be used in the revegetation of disturbed areas, since they are tolerant to those conditions. Based previous studies which have shown the impacts of arsenic on temperate and southern plant species (Garg et al. 2012; Mascher et al. 2002; Xu et al. 2008; Yu et al. 2009; Chun-xi et al. 2007), my third hypothesis that plant communities adjacent to the milling and tailings containment area will differ from references sites and those differences will be correlated with soil arsenic concentrations.

Methods

Tundra Gold Mine

Tundra Mine is a gold mine located 250km northeast of Yellowknife, Northwest Territories (64^{o} 03' N, 111^{o} 11' W) (Figure 2-1). The site is part of the Treaty 11 claim, the Akaitcho Territory, Wek'eeshii and Monwhi Gogha De Nittaee areas of the Tlicho Land Claim

Agreement, and the North Slave Métis traditional lands (Golder 2008). The mine is located between two large lakes: Mackay Lake (1060 km^2) can be found five kilometers to the south, and Courageous Lake (250 km^2), nine kilometers to the north. Because the site is remote, it is only accessible using a small chartered aircraft, which can only carry passengers, light weight equipment and food supply. During the winter months, larger heavy equipment or bulk supplies can be brought on site with help of ice roads.

Annual temperature varies from -31°C to 18°C. The shortest day is on December 21 with 3:45 hours of daylight and the longest day is June 20 with 21:45 hours of daylight (Carison et al. 2015). The mean annual precipitation is 345mm and consists approximately of equal parts rain and snow (Carison et al. 2015). It is part of the Taiga Shield and Southern Arctic ecozones, which are characterized by low soil nutrients, a cool and short growing season compared to temperate regions (Government of Northwest Territories 2009, 2012). The topography at Tundra Mine is relatively flat, with gradual slopes and bedrock near the surface. The soils are classified as cryosols, meaning they are frozen 1 meter below the surface and experience waterlogging during spring thaw. The ice formation and thaw causes the soil layers to become disrupted, cracked and to develop hummocks. The hummocks were measured to be up to 30cm in height with a 5cm organic soil containing dark peat with partially decomposed plant matter (Stevens et al. 2017).

Tundra Mine was operational between 1964 and 1968 and produced a total of 104,476 ounces of gold and from 1983 to 1986 processed ore from the nearby Salmita gold mine (MVLWB 2010). The gold extracted at Tundra Mine was from arsenopyrite rock resulting in the release of large amounts of arsenic. Approximately 300,000 tons of tailings produced from both mining operations were placed in Russell Lake (MVLWB 2010). The waste rock produced

contained between 0.2-10 mg arsenic/g of soil which surpassed the CCME guidelines for sediment and soil by several orders of magnitude; (0.0059 and 0.012mg/g repectively). The 1.2 million cubic million liters of water in the tailings pond also contained high levels of arsenic (1.1mg/L-2.4 mg/L) far exceeding the CCME water quality guideline maximum of 0.005mg/L to protect aquatic life (Figure 2-2).

When Russell Lake was turned into a tailings area, it was divided into Upper and Lower Pond (Figure 2-3). To decrease the chances of tailings pond overflow and leaching into surrounding areas, the tailings were moved to a containment area and capped with a geosynthetic liner and rock during remediation. The arsenic from the tailings water was removed with iron co-precipitation and the treated water discharged into Hambone Lake. Since it is almost impossible to remove all arsenic from the tailings, waste rock was excavated and moved to a smaller containment area in lower pond, the bedrock was cleaned and soil was replaced with gravel extracted from the quarry found on site (MVLWB 2010). A discharge channel was placed between the new tailings area and Hambone Lake to facilitate surface drainage (MVLWB 2010).

The areas awaiting revegetation include both dry upland areas as well as wetlands. The soil is a silty sand with some rocks (Stevens et al. 2017). There is some sparse vegetation that has established in the area, but because of the harsh growing conditions, unaided natural seedling establishment and growth could take up to 50 years (Densmore et al. 2000). It is, therefore, crucial that a re-vegetation strategy must be planned and implemented so that the final stage of reclamation can be complete. Currently, the previous tailings pond, the quarry as well as an airstrip is awaiting re-vegetation totaling an area of 73 hectares.

Field Sampling

To assess the impact of mine activities on wetland vegetation, eight vegetated sites were sampled at Tundra mine from June 23rd, 2016 to June 25th, 2016. Four sites were chosen close in proximity to the tailings containment area and original milling area including North Damn (ND), Mill Pond (MP), Hambone Lake (HB) and Trans Saddle Lake (TSL) (Figure 2-4, 2-5, 2-6). The remaining four sites were chosen as far away from the original milling and tailings area as possible given the restricted time frame and road access. The four reference sites, starting with the site furthest away from mining activity and include Reference 1 (R1), Reference 2 (R2), Reference 3 (R3), and Sandy Lake (SL) (Figure 2-5, 2-6). At all sites, three locations for transect establishment were chosen to encompass the variability found at each site. This approach was necessary given limited site access. Each transect was run along a moisture gradient. Three 1x1m quadrats were placed along each transect to include dry, intermediate and wet areas. The moisture gradient is important, as it encompasses the diverse soil types and vegetation that can be found at each site. All vegetation was identified in each quadrat and percent cover of each species of plant was assessed. Plants were identified using "Vascular plants of continental Northwest Territories, Canada" by Cody and Prosild (1980). Not all species were identifiable at the time since the flowers had not yet developed, or had been dried out and were incomplete. In this case plants were identified to the lowest possible taxonomic unit.

To quantify and compare arsenic concentration in plants across sites, the most abundant species at each site were collected. The number of plant samples collected for each species depended on the availability at each site. Plants were collected on a second trip from August 4th to August 7th, 2016, at all sites except for Reference 2 and Reference 3 due to inclement conditions that reduced access to sites. The Bog was also sampled at this time. Plants were collected at each site. Samples were collected as far away from each other as possible to get a

representation of the site conditions. Both woody and non-woody species were collected. Because of time constraints only the above ground portion of the plant was collected for woody species which includes stems, leaves, and fruit when present (Table A-1). Both above and below ground portions were collected for non-woody species which include roots and shoots (Table A-2).

Soil samples were collected from each of the same quadrats used for the vegetation survey using a cylindrical soil corer (20 cm length, 10 cm diameter; Hoskin Scientific Ltd, Ontario Canada). Soil was also collected from the Bog, and from the three areas that are scheduled for revegetation, including Upper Pond (UP), Lower Pond (LP) and the Quarry (QU) (Figure 2-4, 2-7). Soil was collected from the areas that are scheduled for revegetation to help predict the success of the establishment of plants during the revegetation phase. At the Bog three transects containing three quadrats each were randomly chosen, and a soil sample was collected from each quadrat. All soil samples were initially stored at room temperature (approximately 15 ^{o} C) until June 27th, after which they were placed into a freezer at Wilfrid Laurier University until they were analyzed for arsenic and phosphorus in May 2017.

Arsenic and Phosphorus Concentrations in Soil

Soil samples collected from each quadrat at each site were analyzed for total arsenic, total phosphorus and total potassium. Arsenic and phosphorus were extracted from soil using Method 200.2 (Environmental Protection Agency 1994). The soil was first homogenized by placing it in a large container and manually mixing it. The soil was dried in a drying oven for 24 hours at 40 ^{o} C. One gram of soil was then digested in 14mL of 20% hydrochloric acid and 20% nitric followed by a dilution with 85mL of Milli-Q water. The acid solution was then boiled for 30 minutes and then vacuum filtered to remove sediments. The solution was then diluted again by

ten times to ensure element concentrations were within detection limits. Samples were analyzed using a Perkin Elmer Optima 8300 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

Arsenic Concentration in Plant Tissue

Plants were placed in a drying oven at 40^{o} C for three days so that they could be used for arsenic analysis in May 2017. Plants were then sorted into groups based on tissue type (Table A-1). Woody plants were split into stems, leaves and when possible, fruit. Non-woody plants were split into roots and shoots. Dried plant tissue was broken down using a coffee grinder (KitchenAid®) prior to digestion. The coffee grinder was cleaned between the processing of each sample with a moist Kimberly Clark© Kimwipe© to prevent cross contamination. Dried plants were then sent to Guelph University Agriculture and Food Laboratory (Ontario, Canada) for arsenic analysis. There the plants were digested using nitric acid at high temperature and pressure then analyzed by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) using a method developed by the Guelph University Agriculture and Food Laboratory based on Environmental Protection Agency (1998) Method 6020A.

Statistical Analysis

Arsenic and Phosphorus Concentrations in Soil

A one-way analysis of variance (ANOVA) was used to determine if there were any significant differences in mean concentration of arsenic, phosphorus and potassium between sites. Phosphorus and arsenic concentrations were transformed using a natural log transformation to meet the assumptions of homogeneity of variance and normally distributed residuals. If a significant site effect was detected multiple comparisons were conducted using Student's t-tests.

Arsenic Accumulation in Plants

Because not all plants were present at all sites, plants differed in their degree of woodiness and not all plants had fruits, the experimental design was unbalanced and a conventional three-way ANOVA with site, species and tissue type as sources of variation could not be conducted. Consequently, the analysis was conducted in two ways. First, a comparison of the same species at several different sites was conducted followed by a comparison of different species at the same site (within and among site assessments). When the same species was compared across several sites, the tissues assessed included the leaves, stems, fruit, shoots and or fruit. When different species were compared at the same site, the categorical variables were grouped into woody and non-woody tissue type, where woody tissue included stems and non-woody tissue included shoots and leaves. Fruit was analyzed separately. When possible a two-way ANOVA was used where the categorical variables were the tissue type, and either the site or species and the response variable was the arsenic concentration. If it was not possible to use a two-way ANOVA, a one-way ANOVA or t-tests were used to test for differences in arsenic concentration. If the residuals of the model were not normally distributed or there was not homogeneity of variance, the data was transformed and the transformation that most closely met the ANOVA assumptions was used. If significant main and/or interaction effects were detected, multiple comparisons were conducted using student's t-tests. This was the preferred method since there were many multiple comparisons and a Tukey test with a Bonferroni correction would potentially lead to a high rate of false negatives (McDonald 2014).

Characterization of Plant Communities

The Shannon Weiner Index of diversity was calculated using the following equation:

$$= -\Sigma\, pi \ln(pi)$$

where pi is the proportional abundance of each species (i), defined as the percent cover for i species divided by total percent cover of all species at that site.

Non-linear multidimensional scaling was used to assess the differences in plant communities at each site using the software PC-ORD version 7. The percent cover served as the first matrix of the analysis, and a second matrix was added which included arsenic, phosphorus and potassium soil concentrations corresponding to each transect. The number of dimensions were chosen based on a method described by Peck (2010) in *Multivariate Analysis for Community Ecologists* where adding a dimension to the model must reduce the stress by at least 5.0, accompanied by a Monte Carlo randomization test of $p<0.05$.

Results

Arsenic and Phosphorus Concentrations in Soil

Total Arsenic

There was a significant difference in arsenic concentration in the soil among sites (p-value = <2e-16; Table 2-1). The highest arsenic concentration was found at Hambone Lake (HB) (2677 ± 704.6 mg/kg) and the second highest concentration was found at Mill Pond (MP) (1324 ± 398.9 mg/kg) (Figure 2-8). Both sites are vegetated sites that are adjacent to the original tailings pond and milling area. The lowest concentrations were found at Reference 3 (Ref 3) (2.26 ± 1.47 mg/kg) and the Quarry (QU) (0.005 ± 0.00 mg/kg).

Total Phosphorus

There was a significant difference in phosphorus concentration in the soil among sites (p-value = 0.000797; Table 2-1). The highest phosphorus concentration was found in soil collected from Hambone Lake (HB) (587.17 ± 65.76 mg/kg) and Reference 1(Ref 1) (569.83 ± 46.90 mg/kg) (Figure 2-9). Trans Saddle Lake (TSL) (236.7 ± 75.61 mg/kg) had the lowest phosphorus concentration and The Bog had the second lowest concentration (365.56 ± 54.32 mg/kg).

Arsenic Accumulation in Plants

Arsenic concentration in plant species across sites

Overall, the highest levels of arsenic were found in plants collected from Mill Pond, and the lowest levels were found in plants collected from the reference sites. Arsenic concentrations ranged from 0.07 mg/kg in *Empetrum nigrum* fruit collected from Reference 1, the site furthest from mining activity, to 870 mg/kg in *Carex aquatilis* roots collected from Mill Pond, which is the site closest to the original milling area.

Betula glandulosa

There were differences in arsenic concentration that depended upon site and the interaction between site and tissue type (p-value = 0.00253). The highest concentrations of arsenic were found in stems and leaves from Mill Pond (stems = 7.90 ± 3.70 mg/kg; leaves = 1.85 ± 0.78 mg/kg) (Figure 2-10). For both leaves and stems, concentrations tended to decrease with increasing distance from the mine. The lowest arsenic concentration was found in stems (0.08 ± 0.01 mg/kg) and leaves (0.11 ± 0.03 mg/kg) collected from Ref 1, which is the most distal site to mining activity. The second lowest arsenic concentration was found in leaves (0.23 ± 0.00 mg/kg) and stems (0.20 ± 0.03 mg/kg) collected from SL, which is the second most distal

site. At the three sites furthest from the mine, there were no significant differences in arsenic concentrations between leaves and stems. At Mill Pond, the most proximal site, arsenic concentration was higher in stems than leaves. At Hambone Lake, the second most proximal site, there was no significant difference in arsenic concentration between leaves and stems. Samples from North Dam had significantly higher levels of arsenic in leaves than stems (Figure 2-10). Although there were not enough fruit samples to conduct an ANOVA, the samples that were obtained showed a similar pattern as the leaves and stems, with arsenic concentration decreasing with increasing distance from the mine. The highest arsenic concentration was found in fruit collected from Mill Pond (3.20 ± 0.10 mg/kg) while the lowest concentration was found at Reference 1 (0.08 mg/kg).

Salix athabascensis

There was a significant difference in mean arsenic concentration based on site (p-value =2.02E-09) and an interaction between site and tissue type (p-value =0.009; Figure 2-11). The arsenic concentration in the stems and the leaves were not significantly different from each other, other than in samples collected from MP (p-value = 0.0041) and the Bog (p-value = 0.0297). The highest arsenic concentration was found in stems collected from MP (10.9 ± 3.0 mg/kg), followed by leaves from MP (4.5 ± 1.6 mg/kg) and leaves from the Bog (3.75 ± 0.92 mg/kg). The lowest arsenic concentration was found in plants collected further from mining activity including the reference site SL (leaves = 0.37 ± 0.05 mg/kg; stems= 0.22 ± 0.01 mg/kg) and TSL which was next to a quarry (leaves= 0.24 ± 0.05 mg/kg; stems=0.29 ± 0.11 mg/kg).

Empetrum nigrum

There was no significant difference in arsenic concentration between fruit collected from TSL (1.3 ± 1.2 mg/kg) and SL (0.15 ± 0.03 mg/kg) (p-value = 0.1523) (Figure 2-12). There was no significant difference in arsenic concentration in leaves collected from SL (0.59 ± 0.13 mg/kg) versus Ref 1 (0.24 ± 0.03 mg/kg) (p-value = 0.06291). Mean arsenic concentration found in stems significantly differed among sites (p-value = 0.0019). The highest arsenic concentration was found in stems collected from TSL (1.5 ± 0.14 mg/kg) (p-value = 0.004). The two reference sites Ref 1 (0.17 ± 0.09 mg/kg) and SL (0.47 ± 0.05 mg/kg) had the stems with lowest arsenic concentrations and were not significantly different from each other (p-value = 0.117).

Vaccinium uliginosum

There was no significant difference in mean arsenic concentration between leaves collected at MP (6.8 ± 2.3 mg/kg) which is more proximal to the original mining activity than TSL (1.25 ± 0.64 mg/kg; p-value = 0.07917) (Figure 2-13). There was a significant difference between arsenic concentration in stems collected at MP and TSL (p-value = 0.002748). MP being proximal to mining activity, had a significantly higher concentration of arsenic in the stems (14.5 ± 0.71 mg/kg) than TSL, which is found beside a presumably uncontaminated quarry (1.11 ± 0.70 mg/kg).

Carex aquatilis

There were significant differences in means based on site (p-value = 4.31E-06) as well as tissue type (p-value = 0.002), but no significant interaction between site and tissue type (p-value

= 0.227) (Figure 2-14). The highest arsenic concentration was found in roots collected at MP (870 ± 325 mg/kg) and the lowest concentration was found in shoots (0.575 ± 0.08 mg/kg) and roots (1.26 ± 0.92 mg/kg) collected from reference site Ref 1.

Comparing arsenic concentration across species

Hambone Lake

There was a significant difference in arsenic concentration in non-woody tissue among species (p-value = 0.024; Figure 2-15). Although there was only one plant sampled for *Carex aquatilis*, this had the highest arsenic concentration (160 mg/kg). The second highest arsenic concentration was found in *Equisetum arvense* (25.55 ± 24.68 mg/kg) which was significantly greater than *Betula glandulosa* (1.73 ± 0.59 mg/kg) and *Salix athabascensis* leaves (1.5 ± 0.00mg/kg). *Betula glandulosa* had a significantly higher arsenic concentration in the woody tissue (1.2 ± 0.10 mg/kg) than *Salix athabascensis* (0.96 ± 0.04 mg/kg) (p-value = 0.04975).

Mill Pond

Salix athabascensis fruit had a significantly higher arsenic concentration (10.95 ± 1.49 mg/kg) than *Betula glandulosa* fruit (3.2 ± 0.14 mg/kg) (p-value = 0.01802; Figure 2-16). There was a significant difference in mean arsenic concentration among non-woody tissue collected at Mill Pond (p-value = 0.0003). The highest concentration of arsenic was found within *Carex aquatilis* (141 ± 69.30 mg/kg). The arsenic concentration found in *Betula glandulosa* (3.4 ± 0.66 mg/kg), *Salix athabascensis* (4.5 ± 1.56 mg/kg) and *Vaccinium uliginosum* (6.8 ± 2.26 mg/kg) leaves were lower and did not significantly differ from one another. There was no significant

difference in arsenic concentration among *Betula glandulosa, Salix athabascensis* and *Vaccinium uliginosum* stems (p-value = 0.170).

Trans Saddle Lake

There was a significant difference in mean arsenic concentrations in non-woody tissue among species (p-value = 0.00681; Figure 2-17). *Vaccinium uliginosum* had the highest arsenic concentration (1.25 ± 0.64 mg/kg). *Salix athabascensis* (0.24 ± 0.05 mg/kg) and *Betula glandulosa* (0.46 ± 0.02 mg/kg) had the lowest concentration of arsenic and did not significantly differ from one another. One sample of *Empetrum nigrum* leaves could not be included in the ANOVA, but had a concentration of 2.0 mg/kg of arsenic. There was a significant difference in mean arsenic concentration in stems (p-value = 0.0306). In this case, there were enough samples to run an ANOVA with *Empetrum nigrum*, which had the highest concentration of arsenic (1.5 ± 0.14 mg/kg). *Salix athabascensis* stems contained the lowest concentration of arsenic (0.29 ± 0.11 mg/kg). The arsenic concentration in *Vaccinium uliginosum* and *Betula glandulosa* stems was in the middle range and did not significantly differ from *Empetrum nigrum* or *Salix athabascensis*.

The Bog

There was no significant difference in mean arsenic concentration between *Epilobium angustifolium* non-woody tissue (stems and leaves; 7.10 ± 5.51 mg/kg), and *Salix athabascensis* non-woody tissue (leaves; 3.75 ± 0.92 mg/kg; p-value = 0.4861; Figure 2-18).

North Dam

There was a significant difference in mean arsenic concentration in non-woody tissue found at North Dam (p-value = 1.18e-05; Figure 2-19). The highest arsenic concentration was found within *Equisetum arvense* (218 ± 172 mg/kg). The lowest concentrations were found within *Betula glandulosa* (1.85 ± 0.78 mg/kg), *Salix athabascensis* (0.92 ± 0.26), *Oxycoccus microcarpus* (1.60 ± 0.14 mg/kg), *Rubus chamaemorus* (1.10 ± 0.42 mg/kg), which were not significantly different from one another. There was no significant difference in arsenic concentration among the woody tissue in *Betula glandulosa* (0.96 ± 0.06 mg/kg), *Oxycoccus microcarpus* (4.05 ± 1.91 mg/kg), *Rubus chamaemorus* (23.05 ± 31.04 mg/kg) and *Salix athabascensis* (0.68 ± 0.15 mg/kg) (p-value = 0.365) . There was no significant difference in arsenic concentration in roots among *Carex aquatilis* (135 ± 21.21 mg/kg), *Calamagrostis canadensis* (25.9 ± 25.60 mg/kg), *Eriophorum scheuchzeri* (126 ± 160 mg/kg) and *Juncus drummondii* (135 ± 49.5 mg/kg)(p-value = 0.566).

Sandy Lake

There were differences in arsenic concentration that depended upon species but not between species and tissue type (Figure 2-20). *Empetrum nigrum* had the highest concentration of arsenic (non-woody= 0.59 ± 0.13 mg/kg; woody =0.47 ± 0.04 mg/kg). The lowest concentrations of arsenic were found in the woody tissue of *Betula glandulosa* (0.20 ± 0.03 mg/kg) and *Salix athabascensis* (0.22 ± 0.01 mg/kg). Fruit could not be analyzed for differences because of a small sample size, but of the samples collected, both *Betula glandulosa* and *Salix*

athabascensis contained a similar amount of arsenic (*Betula glandulosa* = 0.15 mg/kg; *Salix athabascensis* = 0.15 ± 0.02 mg/kg).

Reference 1

There was a significant difference in mean arsenic concentration in non-woody tissue among the species collected at Reference 1 (p-value = 0.000687; Figure 2-21). The shoots and leaves of *Carex aquatilis* contained the highest concentration of arsenic (0.58 ± 0.08 mg/kg). *Betula glandulosa* (0.11 ± 0.03 mg/kg), *Empetrum nigrum* (0.24 ± 0.03 mg/kg) and *Ledum decumbens* (0.16 ± 0.06 mg/kg) were not significantly different from one another and contained the lowest level of arsenic.

There was no significant difference in mean arsenic concentration in *Betula glandulosa* (0.08 ± 0.01 mg/kg), *Empetrum nigrum* (0.17 ± 0.09 mg/kg) and *Ledum decumbens* (0.16 ± 0.05 mg/kg) woody tissue. Fruit could not be analyzed because of small sample size but *Betula glandulosa* (0.08 mg/kg) and *Empetrum nigrum* (0.07 mg/kg) contained similar arsenic concentrations.

Characterization of Plant Communities

The site with the highest Shannon Weiner Index of diversity was Trans Saddle Lake (H=2.212) and North Dam (H=2.165) and the site with the lowest diversity and evenness was Hambone Lake (H=1.206) (Table 2-8). The site with the highest species richness was Reference 1, which had 20 species, and the site with the lowest species richness was Hambone Lake, which only had 9 species (Table 2-8).

Sixty-eight iterations were used to create the NMDS model (Figure 2-22). Two dimensions were chosen for the model based on the level of stress reduction and Monte Carlo randomization test (p-value = 0.0040). The level of stress on the final iteration was 13.52. 31.9% of the variability is explained by axis 1 and 9.8% of the variability is explained by axis 2. The first axis explains most of the site variability. Arsenic itself loaded 0.79 on axis 1, and -0.30 on axis 2. Potassium loaded less strongly than arsenic with 0.34 on axis 1 and -0.01 on axis 2. Phosphorus had the weakest loading with 0.004 on axis 1 and 0.0007 on axis 2. Sites that have higher arsenic concentrations also loaded most strongly along the first axis; for example, Hambone Lake, the highest arsenic site also had three of the highest scores (l.51, 1.21, 1.25) on axis 1. Low arsenic sites such as Reference 3 (-0.79, -0.45, -1.34), Reference 2 (-1.04, -0.95, -0.96) and Reference 1 (-0.56, -0.51, -0.69) all scored low on the first axis. Little variability was explained by Axis 2. Species are separated along the first axis with typical wetland vegetation, such as sedges (*Carex aquatilis*) and Willows (*Salix athabascensis*, *Salix artica*), scoring highest and woody tundra vegetation such as *Acrctostaphylos alpina, Loiseleuria procumbens, Oxycoccus microcarpus, Rubus chamaemorus* and *Andromeda polifolia* scoring lowest.

Discussion

Currently there are 38 abandoned gold mine sites in the Northwest Territories, not including sites that are inactive and with an identifiable owner (National Orphaned/Abandoned Mine Initiative 2009). Many of those sites are awaiting or in the process of reclamation and remediation (Indian and Northern Affairs Canada 2009). Because arsenic is released as a byproduct of gold extraction from arsenopyrite ore, arsenic is a major contaminant of concern at gold mine sites (Rheaume and Caron-Vuotari 2013). The effects of arsenic on plants and plant communities have been well studied using temperate species in a temperate environment, but

research using northern species in a northern environment is limited. This research is, however, needed to guide reclamation efforts of contaminated sites in Northern Canada (MVLWB and AANDC 2013).

In this study, plant samples were collected from five sites that were close to a contaminated tailings area and original milling area and two reference sites further away and analyzed for arsenic concentration. Vegetation was only collected from two reference sites due to limited access caused by weather. Mean arsenic concentration was compared in one species at a time, across the seven sites to see if there was a higher accumulation of arsenic in plants the closer to the original milling area/main contamination point. Mean arsenic concentration was also compared across species at each site to see if certain species accumulated more arsenic than others. Finally, a vegetation survey was conducted at eight sites, six of the sites overlapped with the sites at which the plants were collected plus two additional reference sites.

Arsenic concentrations were highest in soils collected from Hambone Lake and Mill Pond, sites adjacent to the tailings containment area, which confirms my first hypothesis that arsenic concentrations will be higher in sites adjacent to the original milling area or tailings containment area. A geotechnical survey conducted in 2004 showed that the dams surrounding tailings were degraded, causing a leak into Hambone Lake and Mill Pond (Staples 2005). The survey showed that arsenic was also introduced into those sites through the dams themselves since they were built from waste rock which was contributing to metal leaching. The vegetated sites with the lowest arsenic concentrations was Reference 3, followed by Reference 1 indicating that they are suitable reference sites for this study. The other reference sites, Reference 2 and Sandy Lake had elevated levels of arsenic. It is possible that arsenic was introduced into Sandy Lake through a stream that connects the previous tailings area through Trans Saddle Lake and to

Sandy Lake (Staples 2005). Reference 2 does not have a body of water and, therefore, the arsenic contamination in that case could have been introduced via trucks carrying ore from Salmita Mine. Because of the variance within each site, there was not a significant difference in arsenic concentrations across the rest of the vegetated sites. In the future, more soil samples should be collected to reduce variation making it possible to detect differences across those sites. The Quarry had the lowest arsenic concentration among the non-vegetated sites, which is of no surprise since the Quarry was not part of the previous tailings area and was only used to excavate rock material. The arsenic concentration in soils collected from non-vegetated sites Upper Pond and Lower Pond were higher than in the Quarry indicating that not all the arsenic was removed during remediation efforts. Higher arsenic concentrations at Upper and Lower Pond supports the first hypothesis that arsenic concentrations will be higher in areas adjacent to the original milling or tailings containment area.

The arsenic concentration in plants was highest in plants collected from Mill Pond and Hambone Lake, suggesting that the mine may likely be the cause of arsenic accumulation and confirming my second hypothesis that arsenic accumulation will be greater in plants collected from sites with higher soil arsenic concentrations. The highest arsenic concentrations were consistently found in plants collected at Mill Pond, which has the second highest total arsenic concentration compared to Hambone Lake. Hambone Lake has the highest concentration of phosphorus, which has been shown to decrease arsenic uptake in plants (Tu and Ma 2003).

In *Salix athabascensis* and *Betula glandulosa* samples collected from Mill Pond, where arsenic uptake was the highest, the concentration of arsenic was significantly higher in stems than leaves. It is well documented that when plants accumulate arsenic, the concentration is higher in roots than stems, but there is no consensus in the literature on whether it is leaves or

stems that accumulate more arsenic in their tissues. In a study conducted by Mleczek et al. (2017) where they planted six tree species (*Acer platanoides L., Acer pseudoplatanus L., Ulmus laevis Pall., Quercus robur L., Betula pendula Roth,* and *Tilia cordata Miller*.) in mining sludge containing 18,022 mg/kg total arsenic, they found that leaves had a lower concentration of arsenic than stems, which is consistent with this study. Another study using *Salix borealis* and *Salix phylicfolia* planted in mine tailings containing 152 mg/kg of arsenic found no difference in arsenic concentration between leaves and stems (Stoltz and Greger 2002). Finally, in a study conducted by Shi et al. (2008) where they planted tea plants (*Camellia sinensis*) in soil containing 50 mg/kg or 200mg/kg of arsenic, they found that in 50 mg/kg of soil the plant accumulated more arsenic in the stems than in the leaves, whereas, in the 200 mg/kg arsenic contaminated soil, the leaves and stems did not have significantly different concentrations of arsenic. The results in Shi et al. (2008) study show that an increase in soil arsenic concentration does not necessarily translate to a higher arsenic accumulation in stems than leaves.

Carex aquatilis showed the highest potential for use in phytoremediation since it could accumulate and tolerate the highest arsenic concentrations. An arsenic hyperaccumulator is classified as a plant that can accumulate 1000 mg/kg of arsenic in its tissues (Baker and Brooks 1989). The highest concentration of *Carex aquatilis* was 870 ± 230 mg/kg and is within that range of a hyperaccumulator. Although there is not much research on arsenic accumulation and tolerance in *Carex aquatilis,* one study on arsenic in geothermal watersheds of Yellowstone National Park, USA showed that it was present at an arsenic-contaminated site with 170 ± 43 μg/kg bioavailable arsenic (Kocar et al. 2004). The arsenic concentration in *Carex aquatilis* at that site was still much lower than reported in this study, with a mean concentration of 2.5 ± 1.0 mg/kg. *Carex rostrata*, another wetland sedge that is native to Northern Canada, has also been

shown to survive on contaminated tailings containing 151 ± 8.1 mg/kg of arsenic (Stoltz and Greger 2002). Similar to the results in this study, the arsenic concentration was higher in the roots than shoots (26.9 ± 4.2 mg/kg and 5.7 ± 1.1 mg/kg respectively). *Carex aquatilis* also has the potential to be a good candidate for phytoremediation in northern environments because it is a native species that is acclimatized to the harsh northern climate and because it spreads quickly through vegetative rhizomes, which reduces the amount of seeds or propagules needed for revegetation (United States Department of Agriculture and Natural Resources Conservation Service 2011). Since *Carex aquatilis* accumulated higher levels of arsenic in the roots than shoots, it may be more useful in phytostabilization, where the aim it is to reduce mobilization of a contaminant through the accumulation in roots or immobilization in the rhizosphere. *Carex aquatilis* could therefore be used on contaminated tailings if the area was fenced since arsenic concentration exceeds the maximum contaminant level for human consumption (3.5 mg/kg in fish) and animal feed (8 mg/kg) (Health Canada 2018; Canadian Food Inspection Agency 2017). The use of *Carex aquatilis* should, therefore, be avoided in areas where herbivorous animals are expected to frequently visit that may ingest the plant and any accumulated arsenic.

At the high arsenic sites (Mill Pond and Hambone Lake), the above ground portion of all plants collected (*Betula glandulosa, Salix athabascensis, Carex aquatilis, Vaccinium uliginosum* and *Equisetum arvense*) exceeded the maximum contaminant level for human consumption and animal feed as well. As mentioned previously, the goal of reclamation of mine sites in the Northwest Territories is to "to return the mine and affected areas to viable and, wherever practicable, self-sustaining ecosystems that are compatible with a healthy environment and with human activities" (MVLWB and AANDC 2013). To ensure that the sites are compatible with a healthy environment further studies should be conducted to find if these plants are being

regularly consumed and if they are impacting local wildlife (Health Canada 2018; Canadian Food Inspection Agency 2017). *Equisetum arvense* also had the highest arsenic concentration among all species collected from North Dam, and second highest from Hambone Lake. Arsenic accumulation in *Equisetum arvense* has been studied before and studies have shown varying results. Mir et al. (2007) found that *Equisetum arvense* was growing in arsenic contaminated soils at Deloro Gold Mine, ON and contained 241 mg/kg total arsenic. Another study conducted by Chang et al. (2005) at Myoungbong Gold Mine in South Korea, found that *Equesetum arvense* contained 11.3 mg/kg of arsenic, which was the second highest concentration found on site. Although *Equisetum arvense* appears to accumulate high levels of arsenic, it would not be well suited for phytoextraction since it does not have a high biomass or growth rate (Chang et al. 2005; Stevens et al. 2017).

In the non-metric multidimensional scaling, sites separated into two groups along the first axis based on species abundance. The first group, which was positive on the first axis included sites near the original milling area and tailings containment area including Mill Pond (MP), Hambone Lake (HB), Trans Saddle Lake (TSL) and North Dam (ND). The second group, which was negative on the first axis included reference sites Sandy Lake (SL), Reference 1 (Ref 1), Reference 2 (Ref 2), and Reference 3 (Ref 3). Arsenic had the strongest positive correlation with the first axis and, therefore, with species abundance at the sites near the original milling and tailings area which confirms my third hypothesis that plant communities will be different in sites adjacent to known arsenic contamination points when compared to communities further away, and that those differences will be correlated to arsenic concentrations in soils. These results are in agreement with Galbraith et al. (1995), who showed that elevated arsenic concentrations caused by a smelter was correlated with loss of vegetation cover and diversity in plant

communities. A similar study conducted by Espinosa-Reyes et al. (2014) also showed that sites close to a mine had high soil arsenic, lower plant diversity and a change in community composition when compared to unimpacted sites further from the mine.

Carex aquatilis and *Salix athabascensis* are two species that are also associated with high arsenic concentrations. Both species are typically present during the early successional stages of vegetation, such as those caused by recent disturbances (Densmore 2000); therefore, it is possible that the disturbance from arsenic contamination caused the sites near the mine to revert to an early successional stage. These species would be ideal to use in the early stages of revegetation of mine sites since they are capable of growing in disturbed areas.

The reference sites were grouped together lower on axis 1, suggesting that plant species abundance in those areas are negatively associated with high arsenic concentrations. Another noteworthy point is that ericaceous plants that are usually the dominant species found in the subarctic were clustered near the reference sites (Read 1983; Read 1991). These species include *Rubus chamaemorus, Vaccinium uliginosum, Ledum decumbens, Empetrum nigrum, Andromeda polifolia, Oxycoccus microcarpus* and *Acrctostaphylos aplina.* The presence of ericaceous species is indicative of a late successional stage in northern ecosystems, showing that reference sites are likely stable communities in equilibrium with environmental conditions, which have not experienced recent disturbances (Densmore et al. 2000; Sprugel 1991). Identifying plant communities at less disturbed sites, enables land managers to create a goal for the end stages of revegetation. Knowing that the ericaceous plants are more abundant in the reference sites, part of the reclamation plan could be to facilitate the succession of plant to an ericaceous community by transplanting seedlings.

The arsenic concentrations in the soil at Hambone Lake and Mill Pond exceeded those shown to have an impact on plant growth and germination of seeds, but nevertheless there was still vegetation present at these sites (Garg et al. 2012; Mascher et al. 2002; Xu et al. 2008; Yu et al. 2009; Chun-xi et al. 2007). Based on this observation, one can conclude that plant establishment will not be impeded by arsenic concentration in the sites rescheduled for revegetation since arsenic concentration at those sites are significantly lower. Based on the NMDS results, plants that may establish at those sites would include *Carex aquatilis*, *Salix athabascensis*, *Equisetum arvense*, *Salix arctica*, *Potentilla palustre* and bryophytes.

Species richness ranged from 9 species at Hambone Lake to 20 species at Reference 1. Hambone Lake is also the site with the highest arsenic concentration, far exceeding concentrations that have been shown to impact plant performance and seed germination in more temperate species (Mascher et al. 2002; Chun-xi et al. 2007; Yu et al. 2009; Garg et al. 2012; Xu et al. 2008). High metal concentration can decrease biodiversity since only metal-tolerant species can survive and reproduce (Gonzalez-Chavez et al. 2002). Similar results were found in a vegetation survey conducted in an area impacted by gold mining and arsenic in Yellowknife, NWT. MacColl (2017) found 12 species at a site containing 1310 mg/kg of arsenic. This study was the first to assess the plant communities at Tundra Mine, so it is not possible to compare vegetation data across a time scale to determine if species richness was higher prior to mining. However, a meta-analysis of studies has shown that pollution, including arsenic contaminants released by mining, is the number one cause of the decline of species richness in northern environments (Vilmi et al. 2017). Although Reference 1 is the site with the highest species richness, Trans Saddle Lake is the site with the highest Shannon's index of diversity indicating that Trans Saddle Lake had a higher level of species evenness. Trans Saddle Lake is across from

the quarry, which means it would experience some level of disturbance, but not as much as the sites adjacent to the tailings area. At a high level of disturbance, only the tolerant species will grow, whereas, at low disturbance levels, only competitively dominant species and long lived species will be present. At an intermediate level of disturbance, there can be a mix of pioneer species and competitors ultimately increase diversity (Huston 1979). Müller et al.'s (2014) study supported this theory by showing that moderate levels of physical disturbance caused an increase in Shannon's diversity in grasslands. Another study by Biswas and Mallik (2010) found that diversity was highest in riparian areas adjacent to clear-cut or scarified forests when compared to an undisturbed or clear cut forests.

Because this study was field based, it does have some limitations. Species composition differed between sites, so it was not possible to collect the same species at every site, which made it difficult to compare arsenic uptake across sites. It also made it more difficult to compare differences in uptake across species. It was not always possible to collect many replicates for each species, which increased the variability between groups making it harder to detect differences. Finally, it was not possible to control variables in the field, making it more difficult to observe the relationship between arsenic uptake or species abundance and site location. Field research does, however, depict a more realistic image of what occurs in a natural environment. Because there were no previous studies on the vegetation at Tundra Mine, this study serves as a collection of baseline information. In the future, a laboratory study could be conducted to further analyze some of the main findings in this experiment. For example, an arsenic exposure study could be used where seedlings of plants found at Tundra Mine are exposed to differing concentrations of arsenic and survivability, root length, shoot length and arsenic concentration could be measured. This would not only provide information on arsenic uptake in northern

species, but also a better understanding of how these species are impacted by arsenic. Survivability and growth measurements would also help identify species that are more sensitive to arsenic, and that may have been excluded from the sites proximal to mining activity. A seedbank study could also be used to supplement this study by shedding light on all species that were present at sites adjacent to the mining activities. If there are seeds present that belong to species that were more abundant in the reference sites, then we could conclude that these species did not grow due to the mining activities.

This study has shown that high levels of arsenic can accumulate in Northern plant species. This information is useful because it identifies potential hazards to wildlife and nearby communities. All plants collected at the high arsenic sites (Mill Pond and Hambone Lake) contained arsenic concentrations that exceed the maximum contaminant level for human consumption (3.5 mg/kg in fish) and animal feed (8 mg/kg), showing that at high enough concentrations, arsenic is mobilized from the soil and into all plants sampled, thereby, increasing the chances of arsenic consumption by wildlife (Health Canada 2018; Canadian Food Inspection Agency 2017). Identifying high arsenic accumulators can also help guide reclamation efforts. For example, if the main goal is to contain arsenic contamination, and the chances of herbivory are low, then *Carex aquatilis* can be used in the revegetation of arsenic contaminated areas. If consumption by wildlife is a concern, then it could be avoided. The Guidelines for the Closure and Reclamation of Advanced Mineral Exploration and Mine Sites in the Northwest Territories outlines the need for research on northern plant species to help with revegetation efforts, but currently there is no mention of impact of mining on plant communities (MVLWB and AANDC 2013). By showing that mining can impact northern plant communities in areas adjacent to a tailings containment area, original milling area, and even adjacent to an

uncontaminated quarry, it is possible to get a clearer understanding of the level of disturbance associated with mining. Plants provide many ecosystem services such as nutrient and water cycling and protection of permafrost; these services may be altered if plant communities are impacted (Schimel 1995; Gruber and Galloway 2008; Oki and Kanae 2006; Rocha and Shaver 2011). This research can help shed light on the impacts of mining on plants and plant communities, which could ultimately help guide policies governing mine reclamation in Northern Canada.

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Figures and Tables

Figure 2- 1: Location of Tundra Gold Mine. Tundra Mine is located approximately 250km Northeast of Yellowknife, NWT Canada (64º 03' N, 111º 11' W). Map obtained from Google Maps.

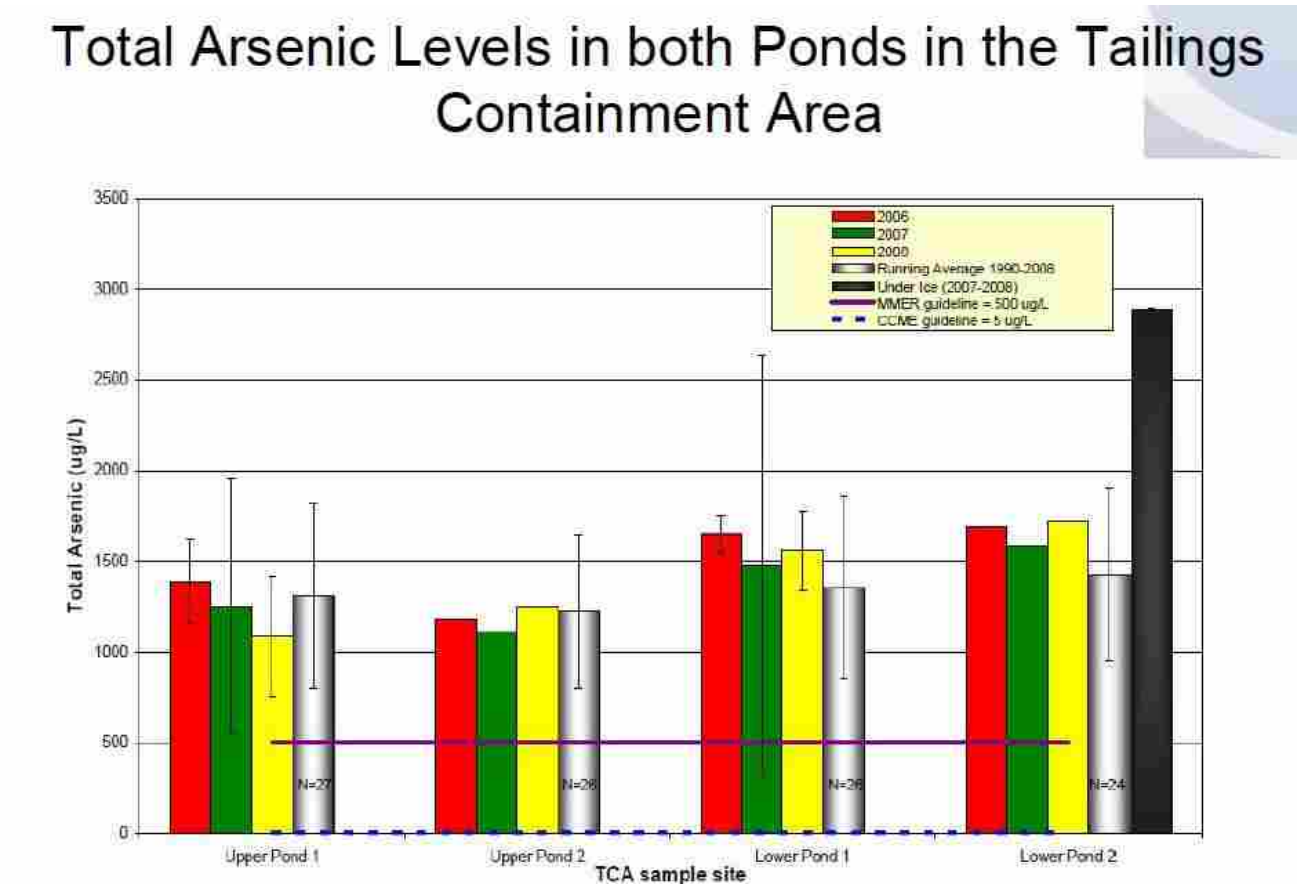

Figure 2- 2: Total arsenic levels in the tailings containment area at Tundra Gold Mine, NWT, Canada (Upper Pond and Lower Pond). CCME water quality guideline for arsenic levels recommends a maximum of 5ug/L to protect aquatic life. Graph obtained from *Report Phase 2 Remedial Action Plan, Tundra Mine Site* (Golder 2008).

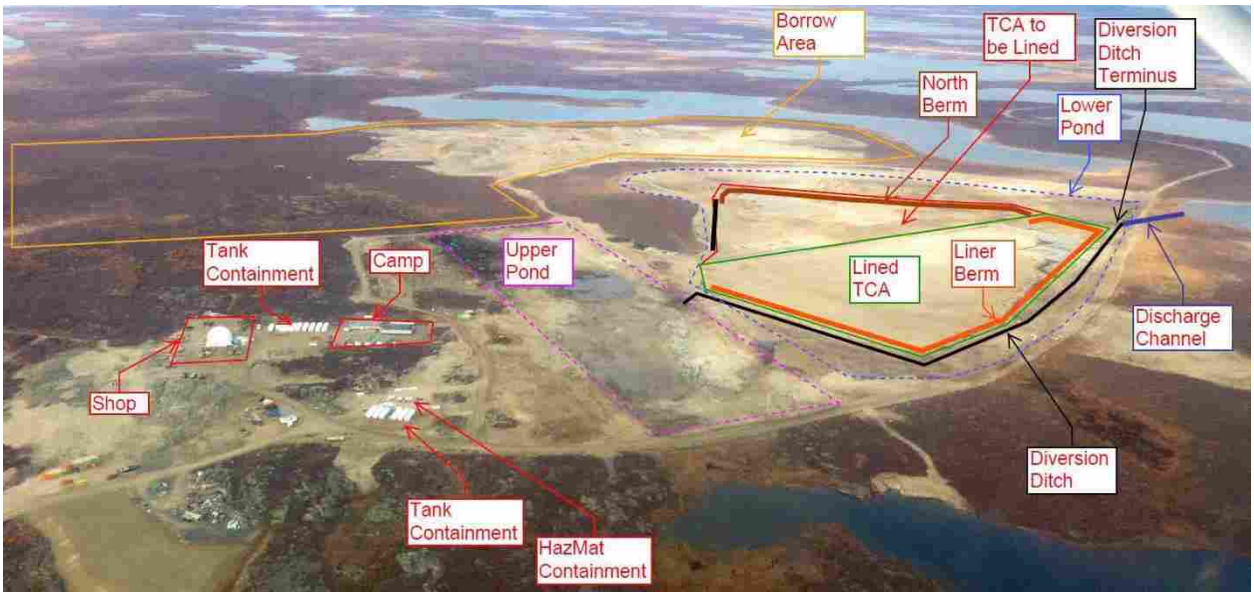

Figure 2- 3: Aerial photo of un-vegetated sites during reclamation phase at Tundra Gold Mine, NWT, Canada. Photo is showing Upper Pond (pink outline) and Lower Pond (blue outline), which were previous tailings area. Lower Pond still contains tailings, but they are now capped and coved with rock material. Borrow Area, is an open quarry that rock material was excavated from to use on the tailings. Photograph obtained from *Report Phase 2 Remedial Action Plan, Tundra Mine Site* (Golder 2008).

Figure 2- 4: Site overview of sites sampled that were near the original milling and tailings containment area. Five vegetated sites labeled in blue, and three non-vegetated sites labeled in red. Photograph obtained from Mary Hewitt (Flat River Consulting) and modified.

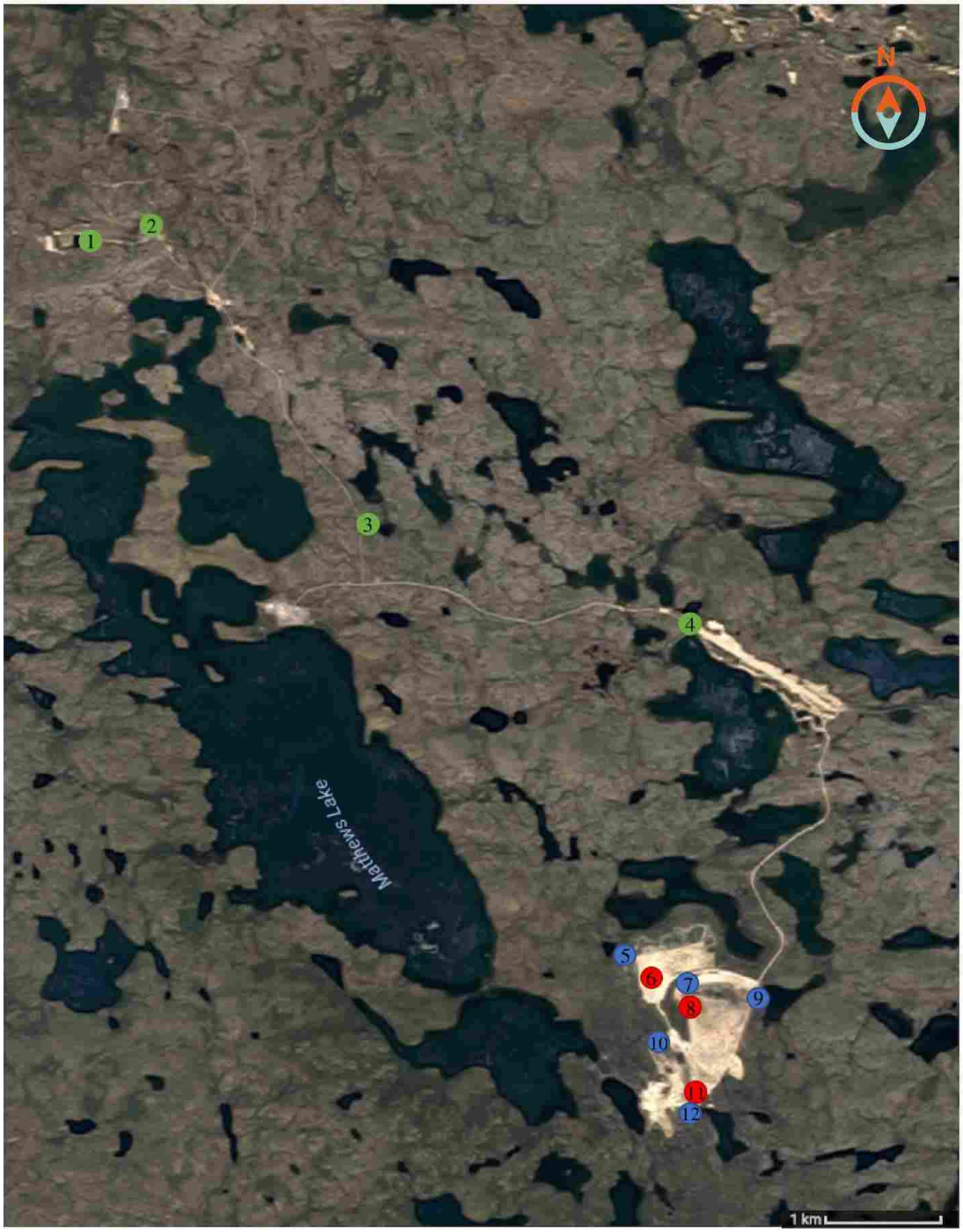

Figure 2- 5: Site overview of sampled sites Tundra Gold Mine. Green sites indicate vegetated reference sites (Reference (1), Reference 2 (2), Reference 3 (3), Sandy Lake (4)) blue sites indicate potentially impacted sites vegetated sites (Hambone Lake (9), Trans Saddle Lake (5), Mill Pond (12), North Dam (7) and the Bog (10)), and red sites indicate potentially impacted non-vegetated sites (Lower Pond (8), Upper Pond (11), Quarry (6)). Map modified from Google Maps.

Figure 2- 6: Overview of vegetated sites including Sandy Lake (top left), Hambone Lake (top right), Reference 1 (bottom left) and Mill Pond (bottom right). Both Hambone Lake and Mill Pond are close to the original milling area, and are presumably impacted. Photographs taken by Sarah Mediouni (top left, bottom left and bottom right) and Kevin Stevens (top right).

Figure 2- 7: Overview of sites awaiting revegetation including Upper Pond (top left), the Quarry (top right, bottom left) and Lower Pond (bottom left). Photographs were taken by Kevin Stevens (top left), Marry Hewitt (top right, bottom left) and Sarah Mediouni (bottom left).

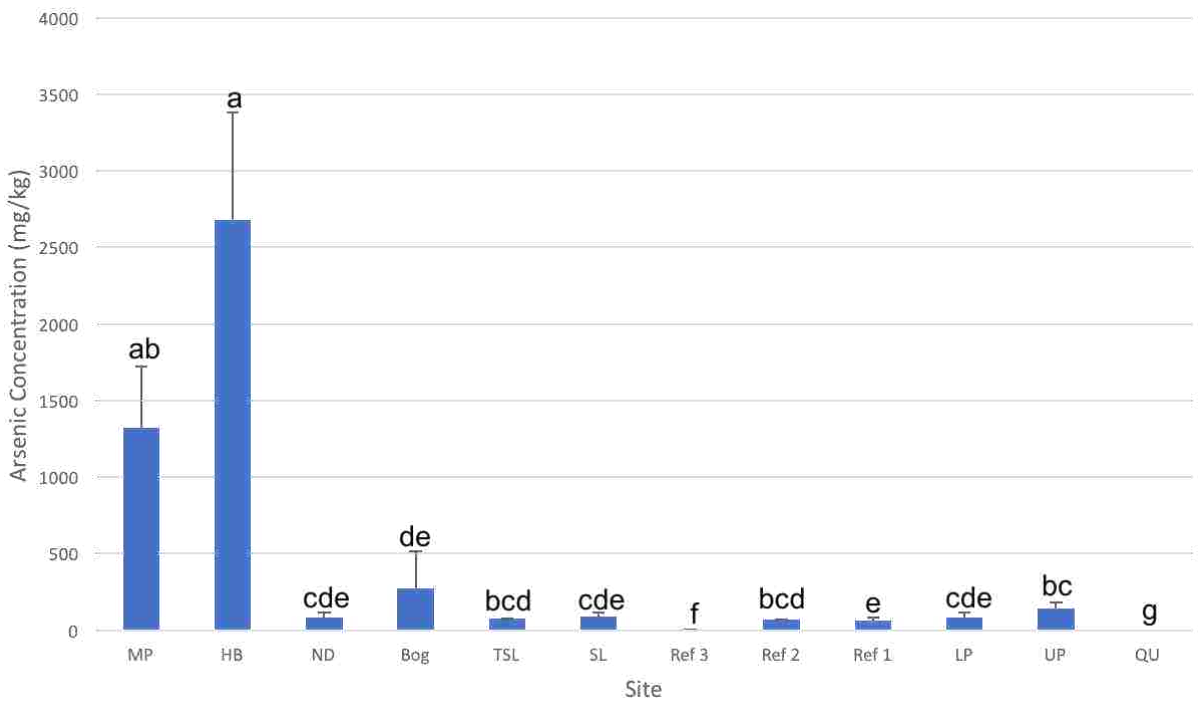

Figure 2- 8: Total arsenic concentration in soil collected from Tundra Gold Mine June 2016. Three transects with three quadrats each were laid out at each site. A soil sample was collected from each quadrat. Arsenic was extracted using EPA Method 200.2 (1994) and then analyzed using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Detection limit was used when the arsenic concentration was too low to detect. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

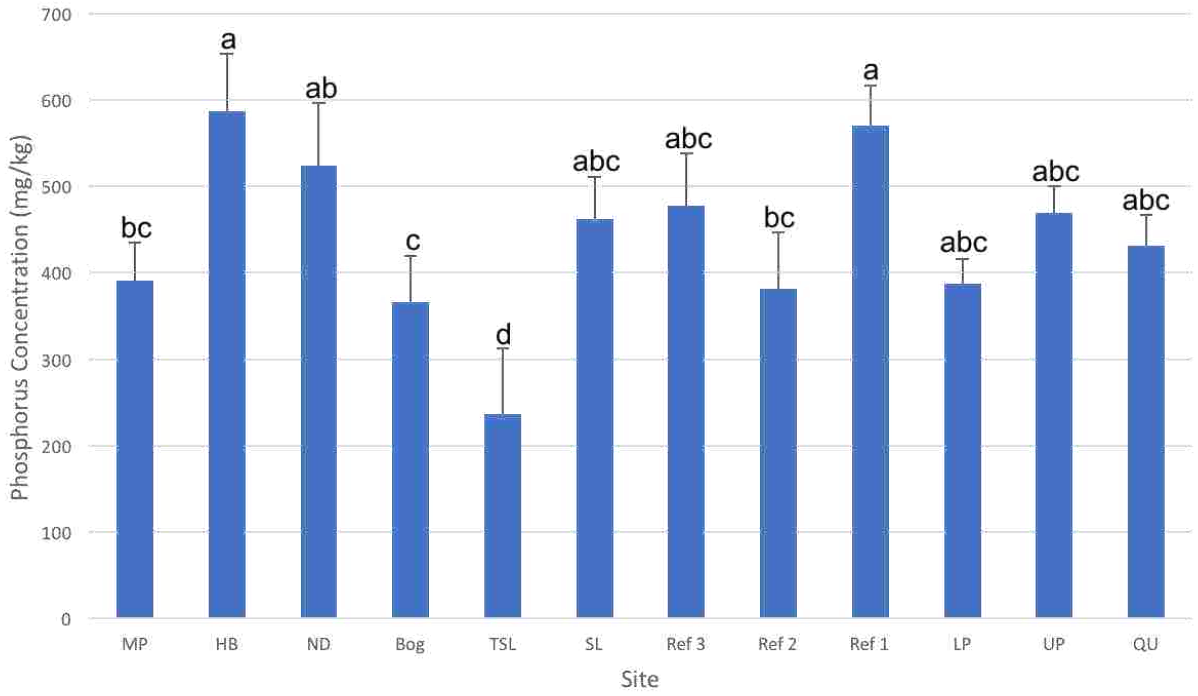

Figure 2- 9: Total phosphorus concentration in soil collected from Tundra Gold Mine June 2016. Three transects with three quadrats each were laid out at each site. A soil sample was collected from each quadrat. Phosphorus was extracted using EPA Method 200.2 (1994) and then analyzed using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

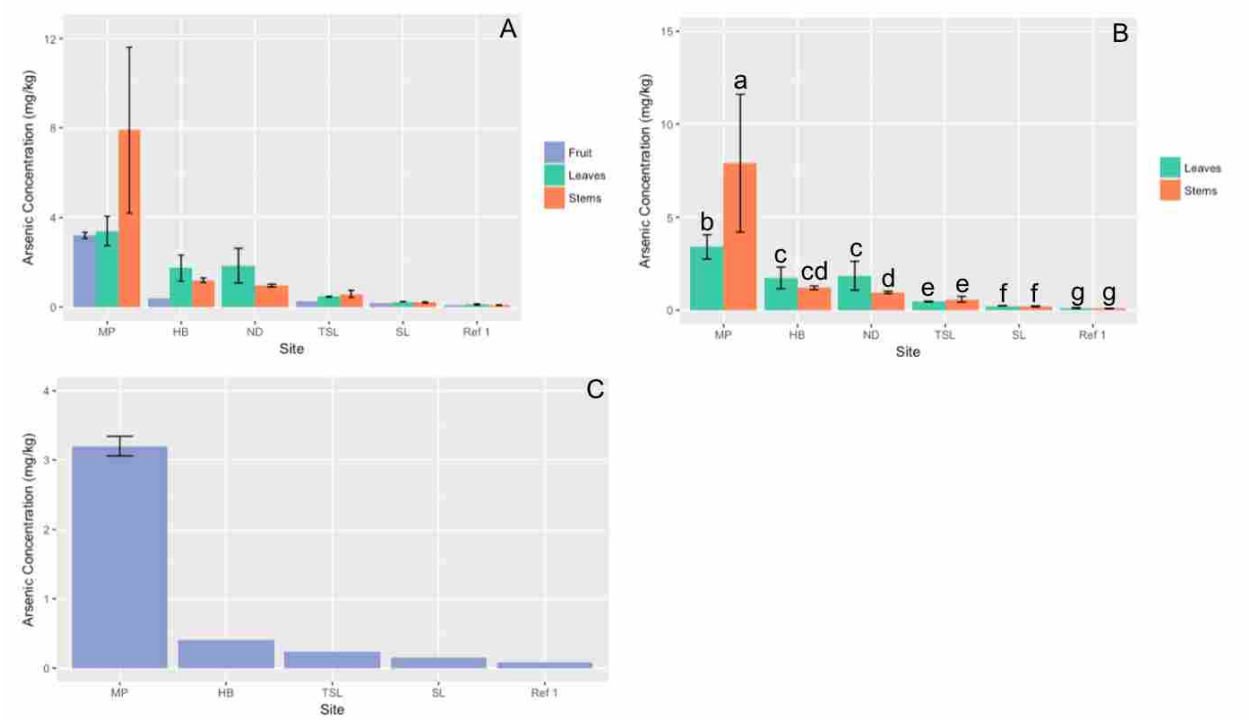

Figure 2- 10: Arsenic concentration in *Betula glandulosa* leaves, stems and fruit collected from Tundra Gold Mine August 2016. Arsenic was extracted using EPA Method 6020A (1998) and analyzed using Inductively Couples Plasma Mass Spectrometry (ICP-MS). A) Overview of *Betula glandulosa* tissue collected at Tundra Gold Mine. B) Arsenic concentration in leaves and stems. C) Arsenic concentration in *Betula glandulosa* fruit. Due to low sample size, it was not possible to compare means of the fruit. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

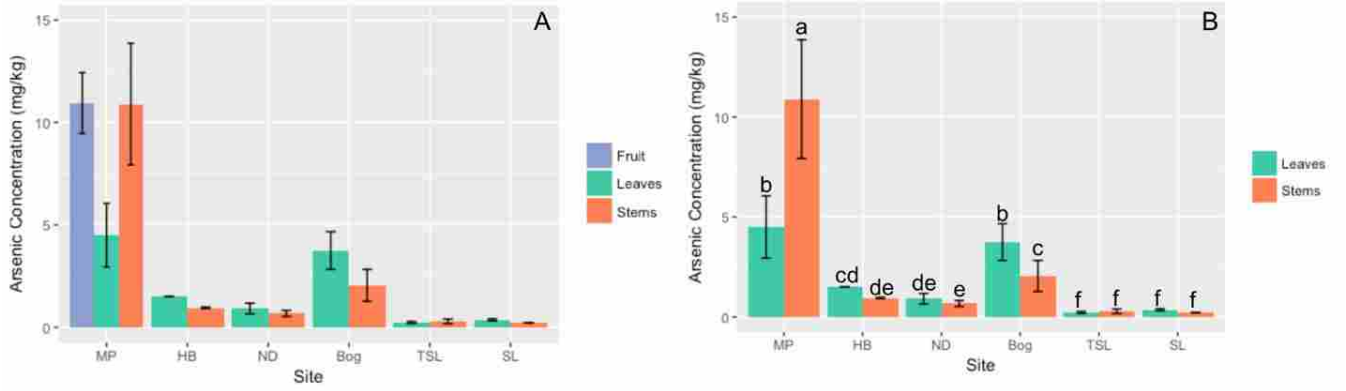

Figure 2- 11: Arsenic concentration in *Salix athabascensis* leaves, stems and fruit collected from Tundra Gold Mine August 2016 Arsenic was extracted using EPA Method 6020A (1998) and analyzed using Inductively Couples Plasma Mass Spectrometry (ICP-MS). A) Overview of all

Salix athabascensis tissue collected at Tundra Gold Mine. B) Arsenic concentration in leaves and stems. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data. Fruit were only found and collected from Mill Pond.

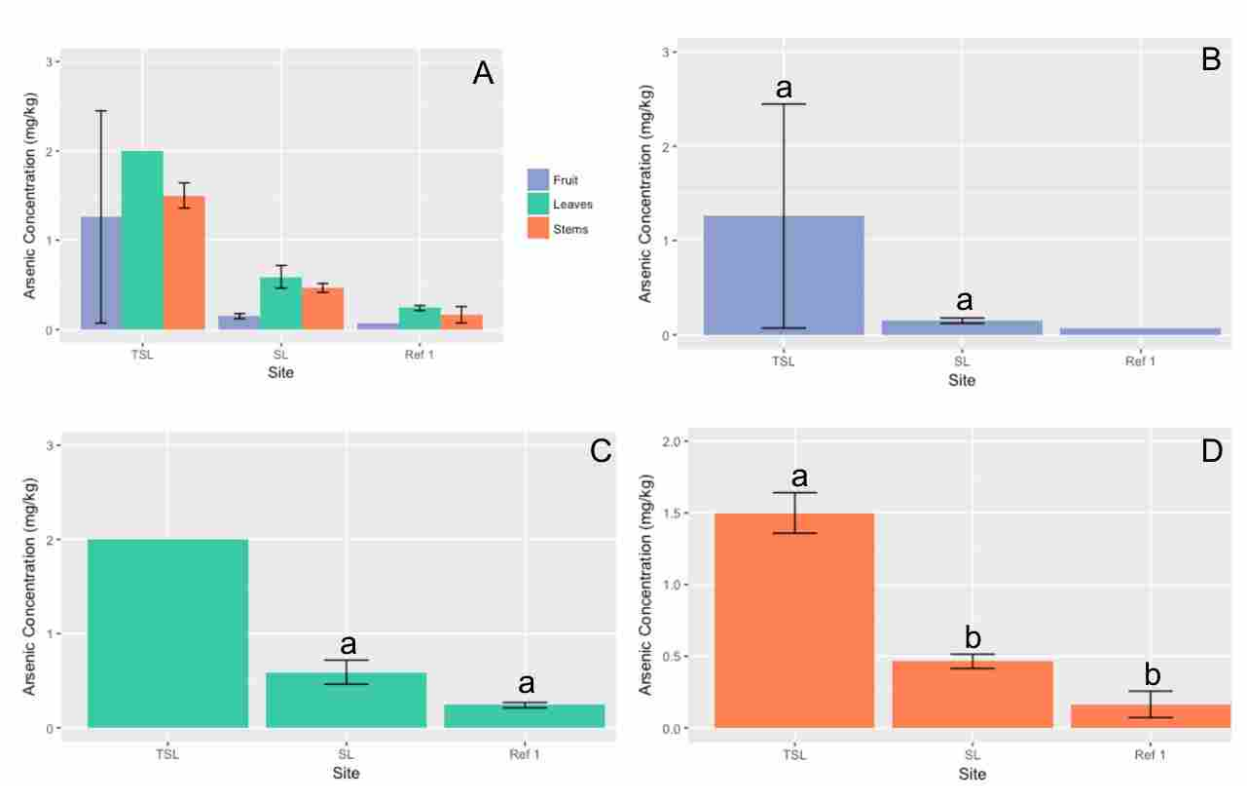

Figure 2- 12: Arsenic concentration in *Empetrum nigrum* leaves, stems and fruit collected from Tundra Gold Mine August 2016. Arsenic was extracted using EPA Method 6020A (1998) and analyzed using Inductively Couples Plasma Mass Spectrometry (ICP-MS). A) Overview of *Empetrum nigrum* tissue collected at Tundra Gold Mine. B) Arsenic concentration in fruit. C) Arsenic concentration in *Empetrum nigrum* leaves. D) Arsenic concentration in *Empetrum nigrum* stems. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data. Bars without error bars indicate that only one sample could be collected for that tissue type at that location.

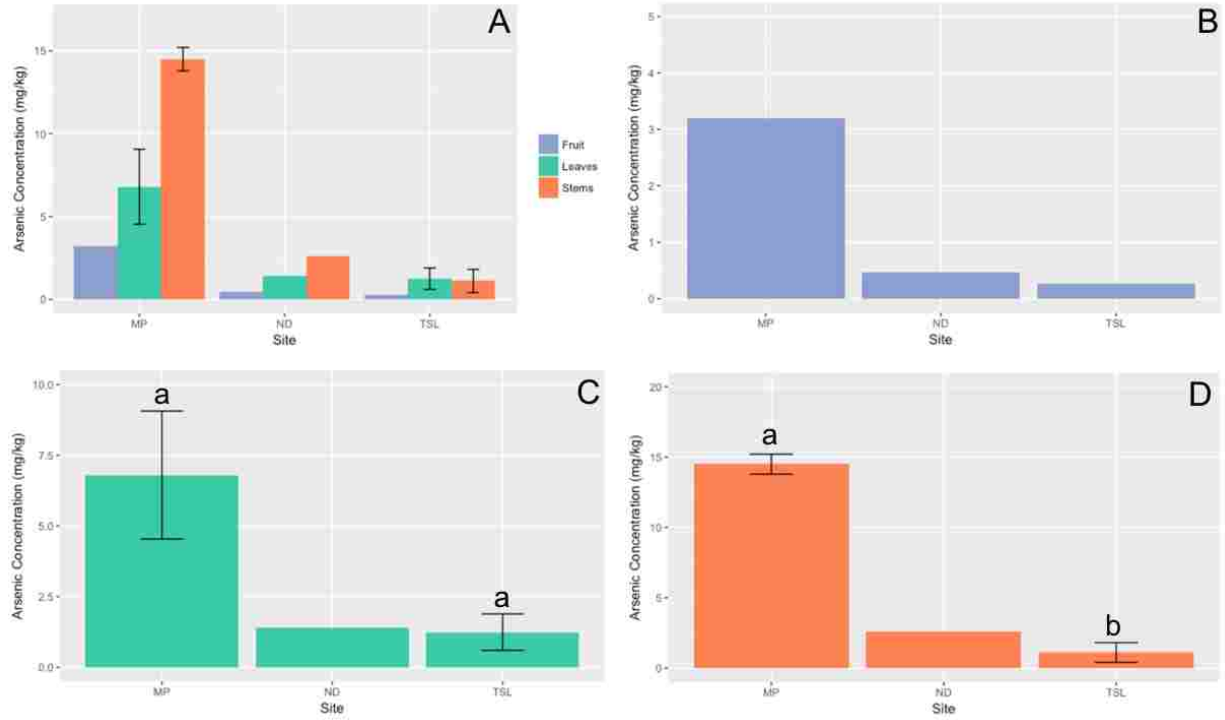

Figure 2- 13: Arsenic concentration in *Vaccinium uliginosum* leaves, stems and fruit collected from Tundra Gold Mine August 2016. Arsenic was extracted using EPA Method 6020A (1998) and analyzed using Inductively Couples Plasma Mass Spectrometry (ICP-MS). A) Overview of *Vaccinium uliginosum* tissue collected at Tundra Gold Mine. B) Arsenic concentration in *Vaccinium uliginosum* fruit. C) Arsenic concentration in *Vaccinium uliginosum* leaves. D) Arsenic concentration in *Vaccinium uliginosum* stems. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data. Bars without error bars indicate that only one sample could be collected for that tissue type at that location.

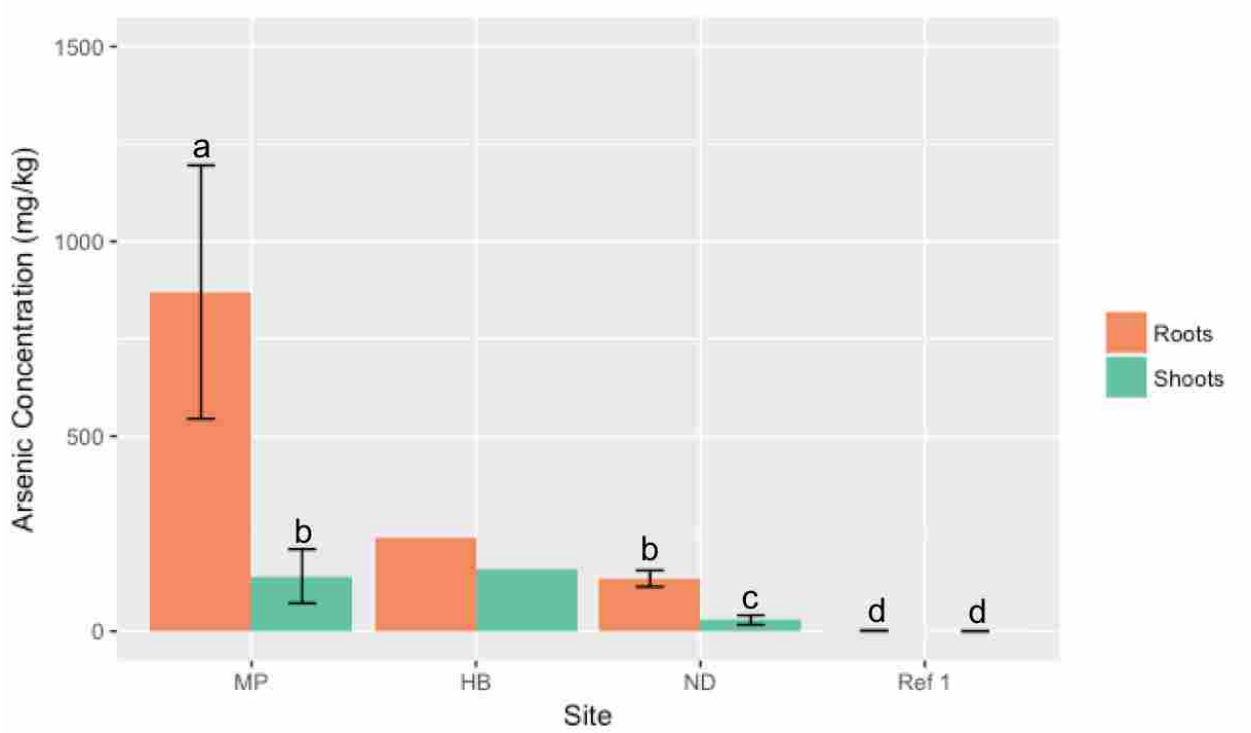

Figure 2- 14: Arsenic concentration in *Carex aquatilis* shoots and roots collected from Tundra Gold Mine August 2016. Arsenic was extracted using EPA Method 6020A (1998) and analyzed using Inductively Couples Plasma Mass Spectrometry (ICP-MS). Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data. Bars without error bars indicate that only one sample could be collected for that tissue type at that location.

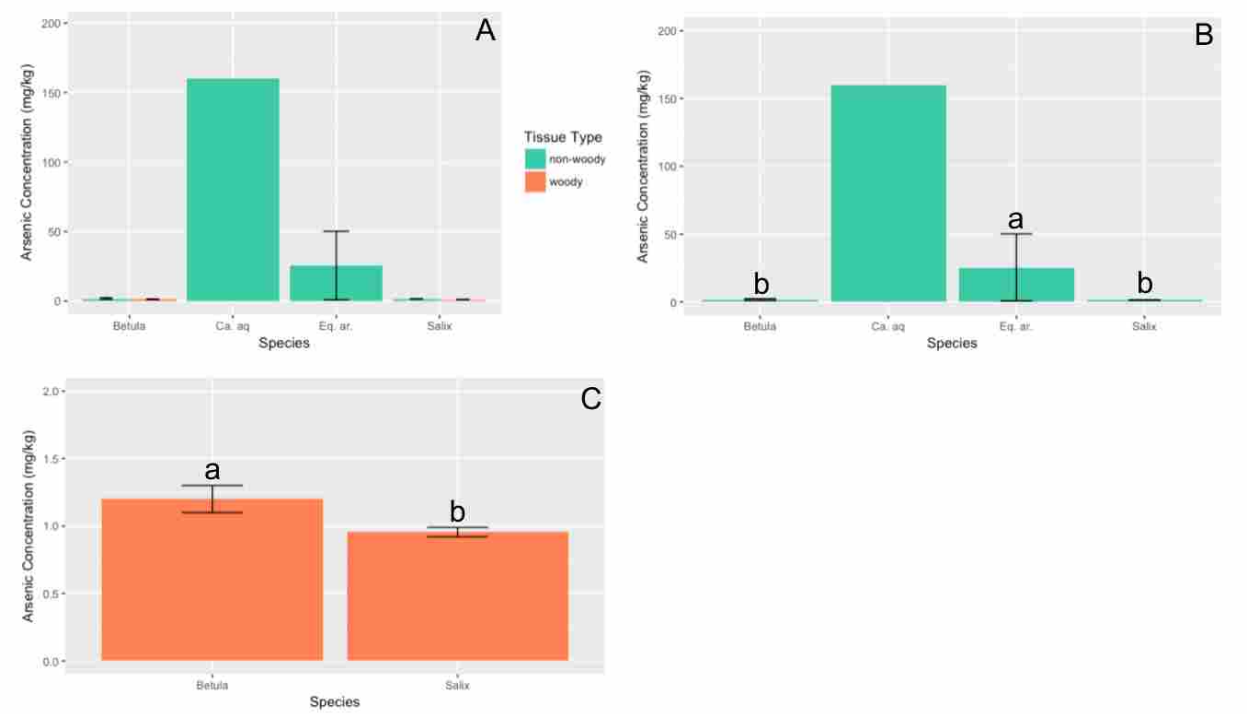

Figure 2- 15: Arsenic concentration found in plants collected from Hambone Lake at Tundra Gold Mine August 2016. Plants include *Betula glandulosa* (Betula), *Carex aquatilis* (Ca. aq.), *Equisetum arvense* (Eq. ar.) and *Salix athabascensis*. Arsenic was extracted using EPA Method 6020A (1998) and analyzed using Inductively Couples Plasma Mass Spectrometry (ICP-MS). A) Overview of all non-woody and woody tissue of plants collected at Hambone Lake. B) Arsenic concentration in non-woody tissue C) Arsenic concentration in woody tissue. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data. Error bars are not provided for *Carex aquatilis* (non-woody) since only one sample could be collected at Hambone Lake.

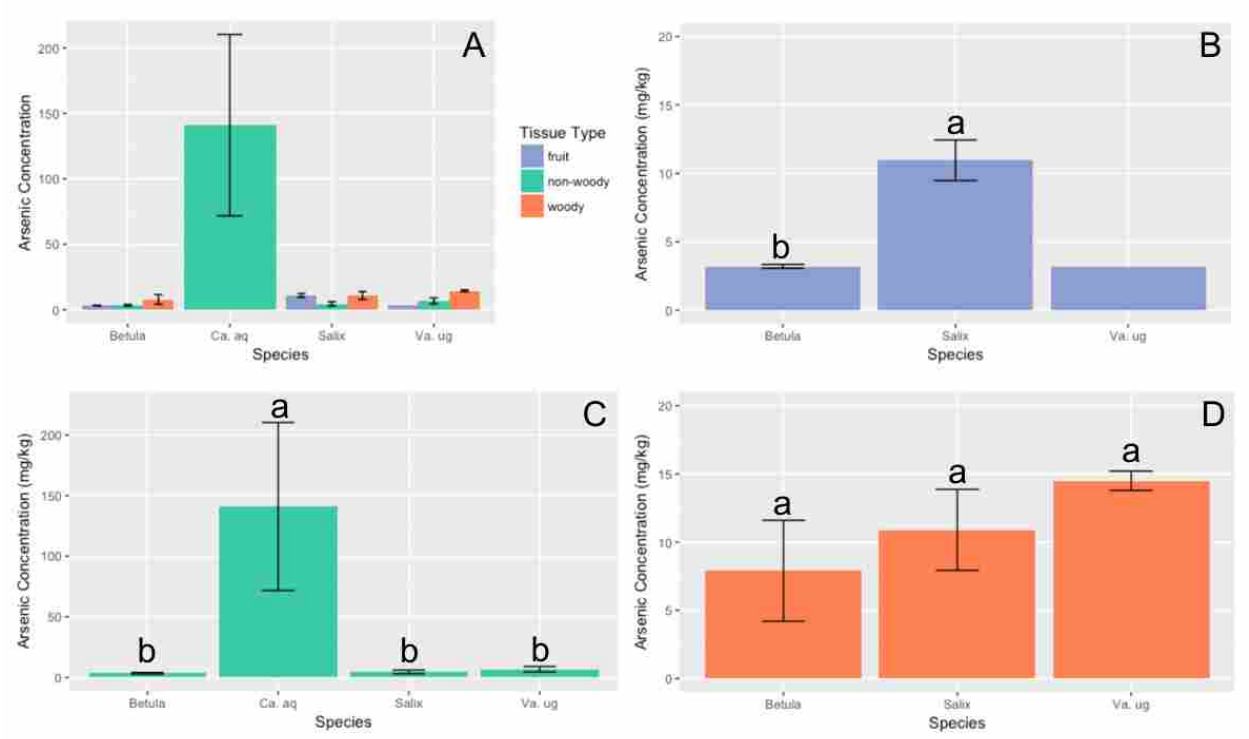

Figure 2- 16: Arsenic concentration in plants collected from Mill Pond at Tundra Gold Mine August 2016. Species include *Betula glandulosa* (Betula), *Carex aquatilis* (Ca. aq.), *Salix athabascensis* (Salix) and *Vaccinium uliginosum* (Va. ug.). Arsenic was extracted using EPA Method 6020A (1998) and analyzed using Inductively Couples Plasma Mass Spectrometry (ICP-MS). A) Overview of all non-woody, woody and fruit tissue collected at Mill Pond. B) Arsenic concentration in fruit. C) Arsenic concentration non-woody. D) Arsenic concentration in woody tissue. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data. Error bars are not provided for *Vaccinium uliginosum* fruit since only one sample could be collected at Mill Pond.

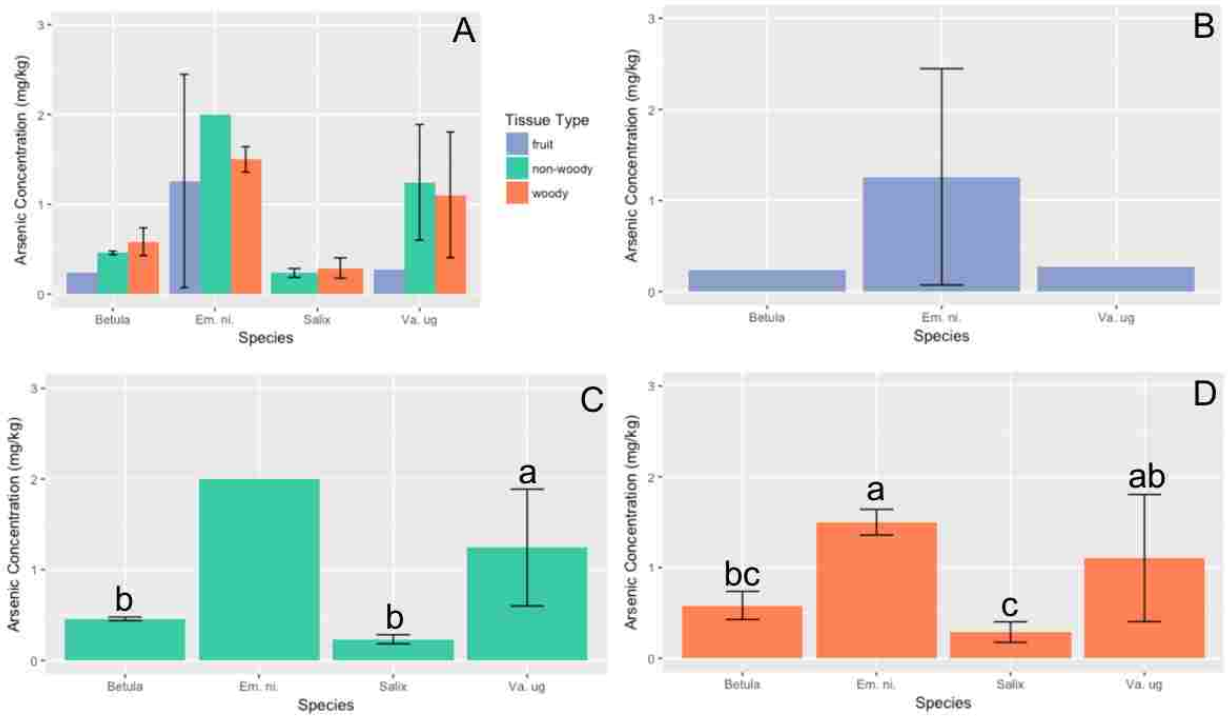

Figure 2- 17: Arsenic concentration in plants collected from Trans Saddle Lake at Tundra Gold Mine August 2016. Species include *Betula glandulosa* (Betula), *Empetrum nigrum* (Em. ni.), *Salix athabascensis* (Salix) and *Vaccinium uliginosum* (Va. ug.). Arsenic was extracted using EPA Method 6020A (1998) and analyzed using Inductively Couples Plasma Mass Spectrometry (ICP-MS). A) Overview including all non-woody, woody and fruit tissue. B) Arsenic concentration in fruit. C) Arsenic concentration non-woody tissue. D) Arsenic concentration in woody tissue. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data. Bars without error bars indicate that only one sample could be collected for that species.

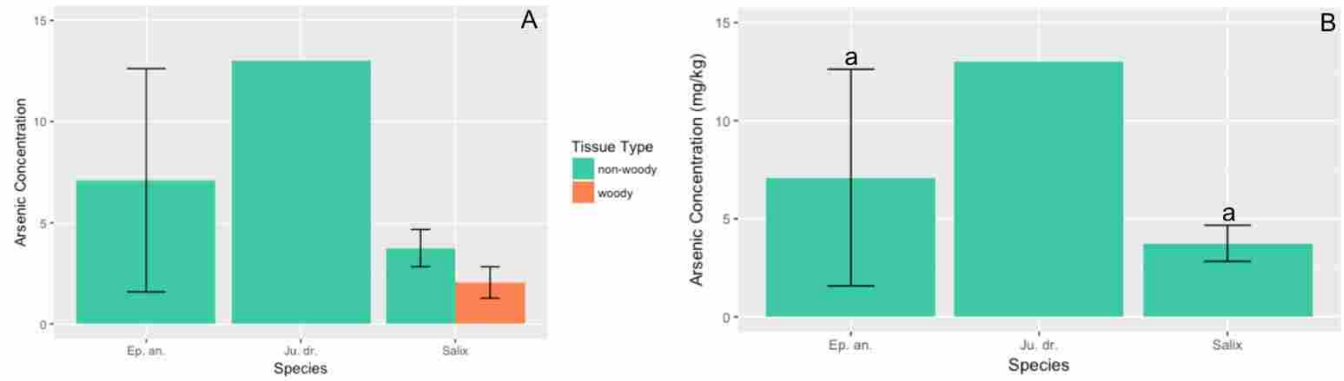

Figure 2- 18: Arsenic concentration in plants collected from the Bog at Tundra Gold Mine August 2016. Species include *Epilobium angustifolium* (Ep. an.), *Juncus drummondii* (Ju. dr.) and *Salix athabascensis* (Salix). Arsenic was extracted using EPA Method 6020A (1998) and analyzed using Inductively Couples Plasma Mass Spectrometry (ICP-MS). A) Overview

including all non-woody and woody tissue. B) Arsenic concentration in non-woody tissue. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data. Error bar missing from *Juncus drummodii* since only one sample could be collected at the Bog.

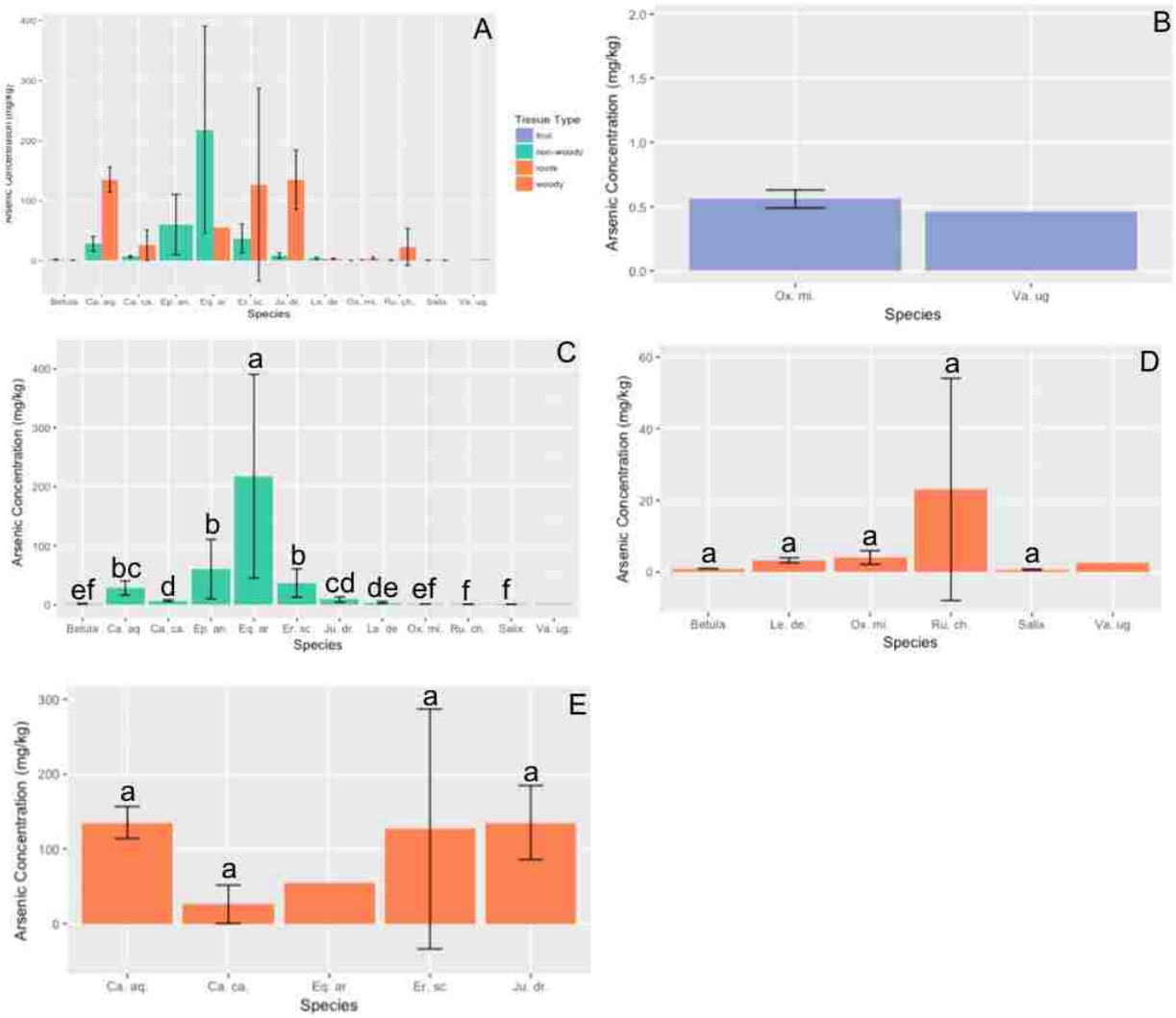

Figure 2- 19: Arsenic concentration in plants collected from North Dam at Tundra Gold Mine August 2016. Plants include *Betula glandulosa* (Betula), *Carex aquatilis* (Ca. aq.), *Calamagrostis canadensis* (Ca. ca.), *Epilobium angustifolium* (Ep. an.), *Equisetum arsense* (Eq. ar.), *Eriophorum scheuchzeri* (Er. sc.), *Juncus drummondii* (Ju. dr.), *Ledum decumbens* (Le. de.), *Oxycoccus microcarpus* (Ox. mi.), *Rubus chamaemorus* (Ru. ch.), *Empetrum nigrum* (Em. ni.), *Salix athabascensis* (Salix) and *Vaccinium uliginosum* (Va. ug.). Arsenic was extracted using EPA Method 6020A (1998) and analyzed using Inductively Couples Plasma Mass Spectrometry (ICP-MS). A) Overview including all fruit, non-woody, woody and root tissue. B) Arsenic concentration in fruit. C) Arsenic concentration non-woody tissue. D) Arsenic concentration in woody tissue. E) Arsenic concentration in root tissue. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from

non-transformed data. Bars without error bars indicate that only one sample could be collected for that species.

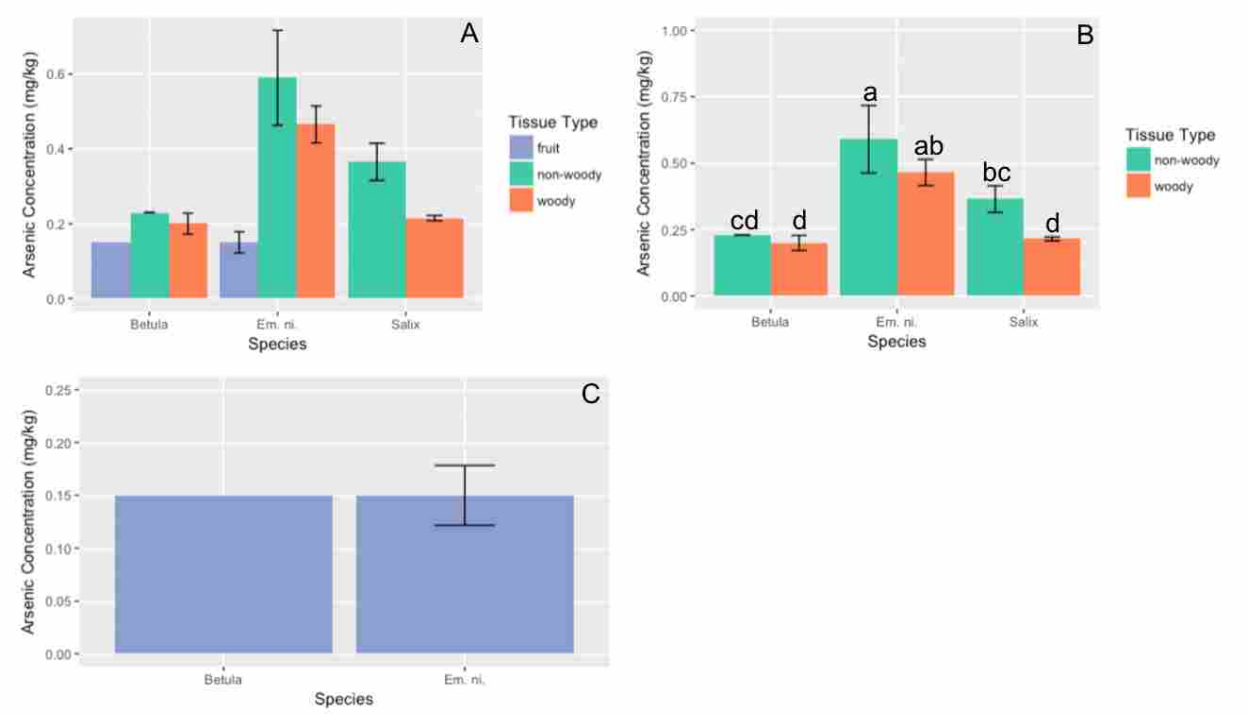

Figure 2- 20: Arsenic concentration in plants collected from Sandy Lake at Tundra Gold Mine August 2016. Plants include *Betula glandulosa* (Betula), *Empetrum nigrum* (Em. ni.) and *Salix athabascensis* (Salix). Arsenic was extracted using EPA Method 6020A (1998) and analyzed using Inductively Couples Plasma Mass Spectrometry (ICP-MS). A) Overview including all non-woody, woody and fruit tissue. B) Arsenic concentration non-woody and woody tissue. C) Arsenic concentration in fruit. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data. Bars without error bars indicate that only one sample could be collected for that species.

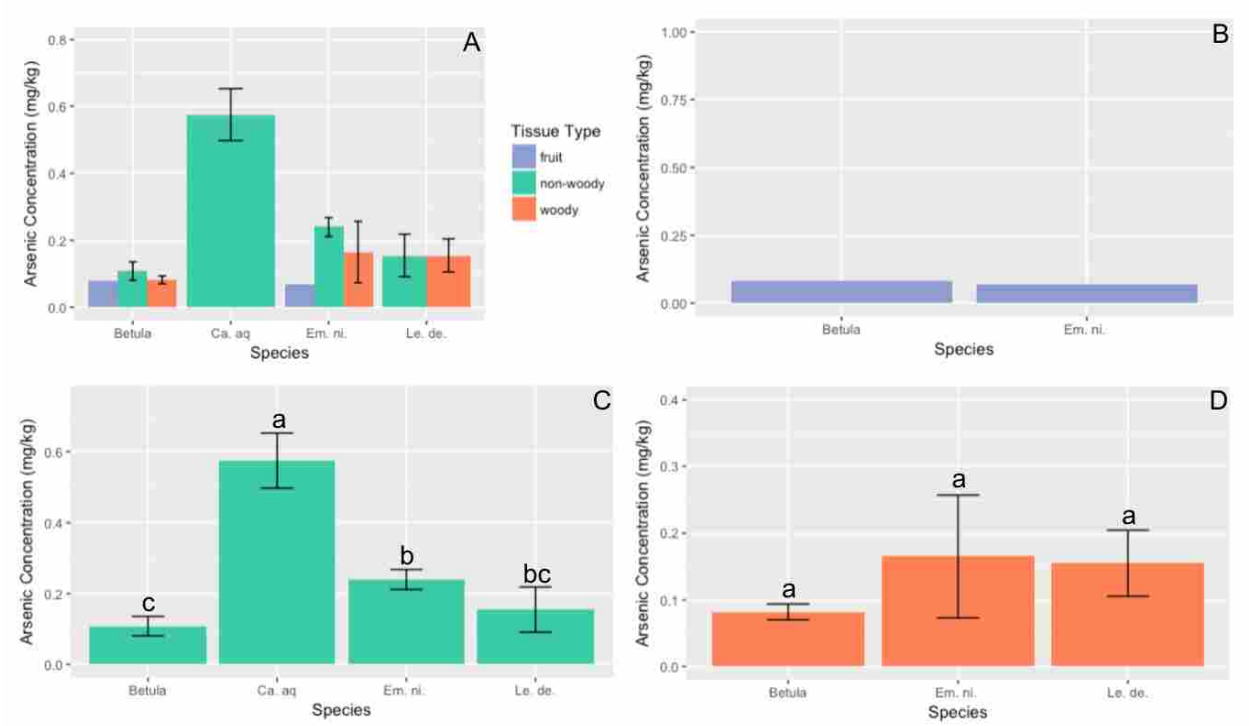

Figure 2- 21: Arsenic concentration in plants collected from Reference 1 at Tundra Gold Mine August 2016. Plants include *Betula glandulosa* (Betula), *Carex aquatilis* (Ca. aq.), *Empetrum nigrum* (Em. ni.) and *Ledum decumbens* (Le. de.). Arsenic was extracted using EPA Method 6020A (1998) and analyzed using Inductively Couples Plasma Mass Spectrometry (ICP-MS). A) Overview of arsenic concentration in non-woody, woody and fruit tissue. B) Arsenic concentration in fruit (Error bars missing because only one sample could be collected for each species). C) Arsenic concentration non-woody tissue. D) Arsenic concentration in woody tissue. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

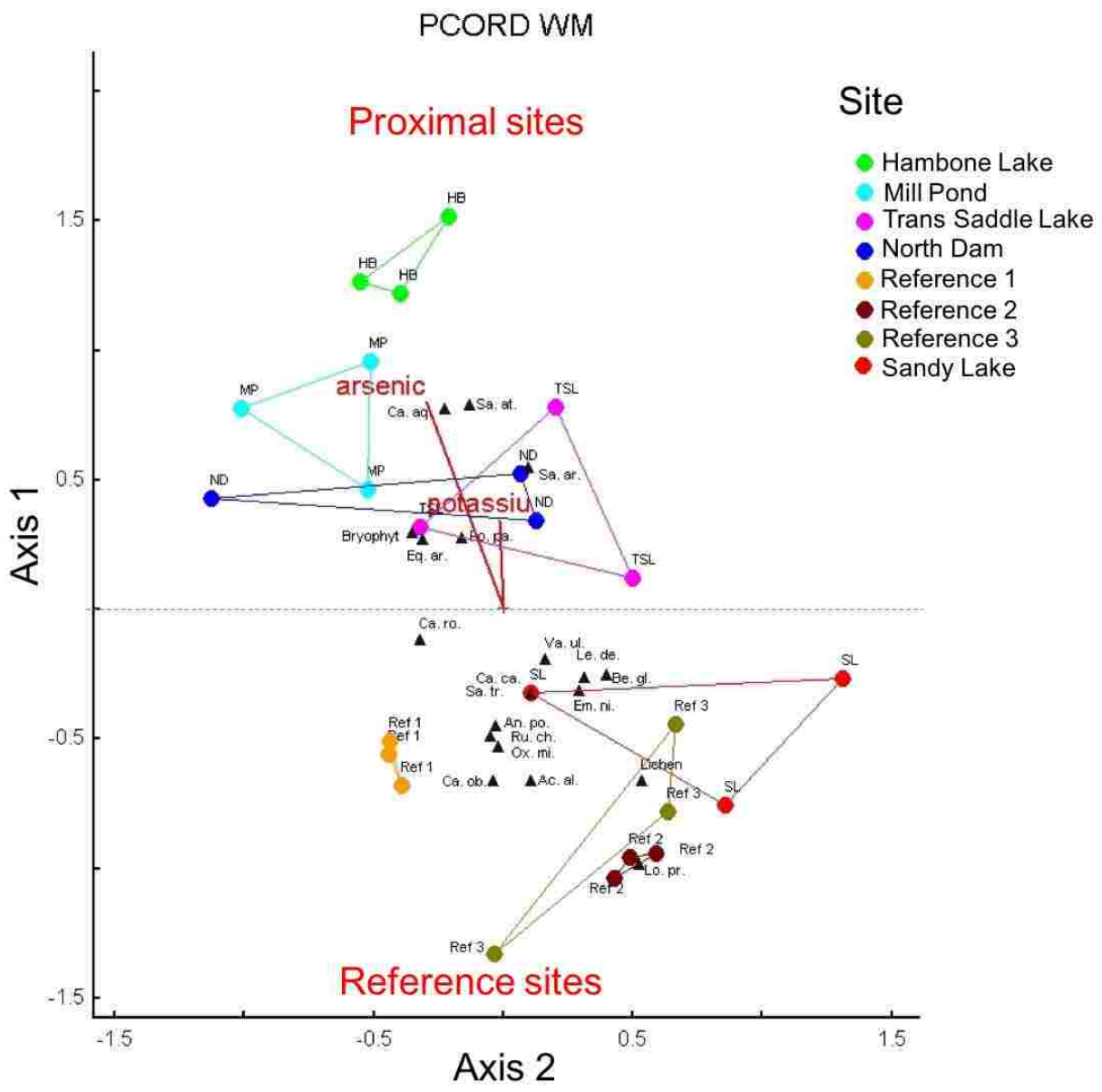

Figure 2- 22: Ordination of the two dimensions for non-metric multidimensional scaling of sample sites in the vegetation survey at Tundra Gold Mine (stress = 13.52; p-value from randomization test = 0.0040). Ordination based on average percent cover of plant species in three transects for each site, with arsenic, phosphorus and potassium concentration overlaid on the ordination space. Species include *Carex aquatilis* (Ca. aq.), *Carex rotundata* (Ca. ro.), *Carex obtusata* (Ca. ob.), *Calamagrostis canadensis* (Ca. ca.), *Betula glandulosa* (Be. gl.), *Acrctostaphylos alpine* (Ac. al.), *Andromeda polifolia* (An. po.), *Empetrum nigrum* (Em. ni.), *Ledum decumbens* now classified as *Rhododendron subarcticum* (Le. de.), *Loiseleuria procumbens* (Lo. pr.), *Oxycoccus microcarpus* (Ox. mi.), *Vaccinium uliginosum* (Va. ul.), *Potentilla palustre* (Po. pa.), *Rubus chamaemorus* (Ru. ch.), *Salix athabascensis* (Sa. at.), *Salix arctica* (Sa. ar.) and *Saxifrage tricuspidat* (Sa. tr.). Bryophytes and lichen were also included in the survey.

Table 2- 1: Output from one-way ANOVAs conducted on the mean arsenic and phosphorus concentration in soils collected at Tundra Gold Mine. ANOVA is comparing mean arsenic concentration across sites. Table also includes tests for the assumptions of a one-way ANOVA including a Shapiro test, to test for normality of residuals and a Levene's test, to test for homogeneity of variance.

Element	Transformation	Shapiro p-value	Levene p-value	Site ndf/ddf	Site F	Site Pr(>F)
Total Arsenic	Natural log	1.764e-08	0.03498	11/100	21.9	**<2e-16**
Total Phosphorus	Natural log	0.07242	0.6921	11/100	3.249	**0.000797**

Table 2- 2: Output from two-way ANOVAs conducted on the mean arsenic concentration in plants collected at Tundra Gold Mine. ANOVA is comparing mean arsenic concentration in species across sites. Table also includes tests for the assumptions of a two-way ANOVA including a Shapiro test, to test for normality of residuals and a Levene's test, to test for homogeneity of variance.

Species	Tissues included	Transformation	Shapiro p-value	Levene p-value	Site ndf/ddf	Site F	Site Pr(>F)	Tisue Type ndf/ddf	Tissue Type F	Tissue Type Pr(>F)	Site*Tissue ndf/ddf	Site*Tissue F	Site*Tissue Type Pr(>F)
Betula glandulosa	Leaves and Stems	Natural log	0.4764	0.4611	5/20	190.89	**3.66E-16**	1/20	0.053	0.82016	5/20	5.449	**0.00253**
Salix athabascensis	Leaves and Stems	Natural log	0.1596	< 2.2e-16	5/12	102.72	**2.02E-09**	1/12	1.739	0.21183	5/12	5.229	**0.00888**
Carex aquatilis	Shoots and roots	Natural log	0.9487	< 2.2e-16	2/6	181.38	**4.31E-06**	1/6	25.80	**0.00227**	2/6	1.92	0.22676

Table 2- 3: Output from Student's t-tests conducted on the mean arsenic concentration in plants collected at Tundra Gold Mine. The Student's t-tests is comparing mean arsenic concentration in species across sites. Table also includes tests for the assumptions of a one-way ANOVA including a Shapiro test, to test for normally distributed data and a Bartlett test, to test for equal variance.

Species	Tissues included	Transformation	Shapiro p-value	Bartlett p-value	df	t	Site Pr(>F)

Empetrum nigrum	Leaves	none	NA	0.2845	2	-3.7963	0.06291
Empetrum nigrum	Fruit	Natural log	NA	0.2205	2	2.2595	0.1523
Vaccinium uliginosum	Leaves	none	NA	0.3548	2	3.3395	0.07917
Vaccinium uliginosum	Stems	none	NA	0.9935	2	19.038	**0.002748**

Table 2- 4: Output from one-way ANOVAs conducted on the mean arsenic concentration in plants collected at Tundra Gold Mine. ANOVA is comparing mean arsenic concentration in *Empetrum nigrum* across sites. Table also includes tests for the assumptions of a one-way ANOVA including a Shapiro test, to test for normality of residuals and a Levene test, to test for homogeneity of variance.

Species	Tissues included	Transformation	Shapiro p-value	Levene p-value	Site ndf/ddf	Site F	Site Pr(>F)
Empetrum nigrum	Stems	none	0.7341	< 2.2e-16	2/3	95.26	**0.00193**

Table 2- 5: Output from one-way ANOVAs conducted on the mean arsenic concentration in plants collected at Tundra Gold Mine. ANOVA is comparing mean arsenic concentration across species at each site. Table also includes tests for the assumptions of a one-way ANOVA including a Shapiro test, to test for normality of residuals and a Levene test, to test for homogeneity of variance.

Site	Tissues included	Transformation	Shapiro p-value	Levene p-value	Species ndf/ddf	Species F	Species Pr(>F)
Hambone Lake	Non-woody	Reciprocal	0.5755	0.4197	2/4	12.91	**0.018**
Mill Pond	Non-woody	Natural log	0.2557	0.07187	3/5	53.8	**0.000315**
Mill Pond	Woody	none	0.9008	0.5465	2/4	2.856	0.17
Trans Saddle Lake	Non-woody	Natural log	0.111	9.85E-07	2/4	5.533	0.0705
Trans Saddle Lake	Woody	Natural log	0.9315	0.07459	3/5	7.008	**0.0306**
North Dam	Non-woody	Natural log	0.3723	< 2.2e-16	10/11	20.09	**1.18E-05**
North Dam	Woody	Natural log	0.04566	< 2.2e-16	4/5	1.36	0.365
North Dam	Roots	none	0.7828	< 2.2e-16	3/5	0.775	0.566
Reference 1	Non-woody	none	0.5436	0.1173	3/5	38.88	**0.000687**

Reference 2	Woody	Reciprocal	0.5921	0.1516	2/4	3.747	0.121

Table 2- 6: Output from a Student's t-test conducted on the mean arsenic concentration in plants collected at Tundra Gold Mine. Student's t-test is comparing mean arsenic concentration across species across sites. Table also includes tests for the assumptions of a one-way ANOVA including a Shapiro test, to test for normally distributed data and a Bartlett test, to test for equal of variance.

Site	Tissues included	Transformation	Shapiro p-value	Bartlett p-value	df	t	Site Pr(>F)
Hambone Lake	Woody	none	Betula = 1, Salix = NA	0.3857	3	3.1889	**0.04975**
Mill Pond	Fruit	none	NA	0.136	2	-7.3477	**0.01802**
Bog	Non-woody	none	NA	0.2205	2	0.84729	0.486

Table 2- 7: Output from two-way ANOVA's conducted on the mean arsenic concentration in plants collected at Tundra Gold Mine. ANOVA is comparing mean arsenic concentration across species at Sandy Lake. Table also includes tests for the assumptions of a two-way ANOVA including a Shapiro test, to test for normality of residuals and a Levene test, to test for homogeneity of variance.

Site	Tissues included	Transfor-mation	Shapiro p-value	Levene p-value	Species ndf/ddf	Species F	Species Pr(>F)	Tissue Type nds/ddf	Tissue Type F	Tissue Type Pr(>F)	Species* Tissue Type ndf/ddf	Species* Tissue Type F	Species* Tissue Type Pr(>F)
Sandy Lake	Non-woody and woody	none	0.8479	< 2.2e-16	2/6	29.100	**0.000816**	1/6	8.476	**0.026932**	2/6	1.096	0.393996

Table 2- 8: Shannon Weiner Index of diversity and evenness. Species richness is shown in brackets. Vegetation survey was conducted at Tundra Gold Mine, June 2016. At all vegetated sites, three transects were chosen to encompass the variability within the site. Each transect contained three 1x1m quadrats that were placed in each soil moisture gradient ranging from dry to wet. The vegetation survey was conducted in each quadrat where percent cover of each species of plant was categorized into 5% increments. Plants were identified using "Vascular plants of continental Northwest Territories, Canada" by Cody and Prosild (1980).

	Hambone Lake	Sandy Lake	Mill Pond	Trans Saddle Lake	North Dam	Reference 1	Reference 2	Reference 3
Dry	1.170 (4)	1.696 (9)	1.219 (8)	2.102 (9)	2.175 (11)	1.982 (10)	1.747 (7)	1.827 (9)
Intermediate	1.187(5)	1.273 (8)	1.522 (6)	1.886 (8)	1.599 (8)	1.759 (9)	1.744 (8)	1.762 (10)
Wet	0.632(3)	1.961 (11)	0.349(2)	0.974 (3)	0.555 (3)	0.530 (3)	1.678 (7)	1.885 (7)
Entire site	1.206 (9)	2.026 (19)	1.668 (17)	2.212 (16)	2.165 (18)	2.080 (20)	1.958(16)	2.043 (13)

Chapter 3 – The impacts of gold mining on mycorrhizae

Abstract

Gold mining continues to be an important part of economic development in Northern Canada. A large portion of the gold that is found in the Northern territories is contained within arsenopyrite ores, therefore, arsenic byproduct is of special concern. Currently, not much research has been done on the impacts of arsenic on arbuscular mycorrhizae in Northern Ecosystems, and it is unknown whether they are a natural part of the ecosystem. Previous research done in temperate regions has shown that the symbiosis formed between plants and arbuscular mycorrhizae can accelerate the remediation process in gold mines by supporting plant growth in poor soil conditions. However, it has also been shown that at high enough concentration arsenic can reduce mycorrhizal colonization and germination. The mine studied in this project is Tundra Mine, an inactive gold mine 250 km northeast of Yellowknife. Eight vegetated sites were chosen at varying distances from the original milling and tailings containment area to include a range of arsenic contamination levels. Mycorrhizal plant species were collected from each site in June and August 2016 for assessment of arbuscular mycorrhizal and dark septate endophyte colonization. Colonization was compared in one species at a time across several sites or several species were compared across one site at a time. Both arbuscular mycorrhizal and dark septate endophyte colonization was found at every site, including those with high levels of arsenic contamination reaching up to 2677 ± 704.6 mg/kg of arsenic in soil. This indicates that mycorrhizae and dark septate endophytes at these sites can tolerate high arsenic concentrations. Mycorrhizal hyphal colonization in roots collected from the site with the highest arsenic (Hambone Lake = 2677 ± 704.6 mg/kg) ranged from 59.83 ± 7.27 % colonization in *Calamagrostis canadensis* to 33.20 ± 10.61% colonization in *Epilobium angustifolium*. At the site with the lowest arsenic concentration (Reference 1 = 64.82 ± 19.32 mg/kg) arbuscular

hyphal colonizations ranged from 66.47 ± 9.32 % in *Agrostis scabra* to 37.05 ± 9.31% in *Calamagrostis deschampsioides.* No trends of reduced colonization at higher arsenic sites were observed in this study, indicating a tolerance to arsenic. There were also no clear trends of colonization between plant species. This study has shown that arbuscular mycorrhizae are part of the Northern ecosystem and can be used as a tool in revegetation strategies. If high arsenic soils are scheduled for revegetation, then native mycorrhiza, extracted from high arsenic sites could potentially be used. Further research in a lab setting could shed light to the arsenic tolerance levels of the mycorrhizae living in the contaminated sites, as well as to determine if there are differences in colonization based on species.

Introduction

Gold Mining in the North

Canada is ranked as one of the top five producers of many metals and minerals (United States Geological Survey 2011). As the global demand for minerals and metals continues to increase, Canada is in a good position to supply the output needed. Mineral and metal output is also expected to grow by 91% from 2011 to 2020, which is a growth rate four times higher than the Canadian economy (Rheaume and Caron-Vuotari 2013). Mining is especially important in Northern Canada since it is a main contributor to the Northern economy (Government of Northwest Territories 2016). Resource extraction in the Northwest Territories alone contributes $900 million of the GDP, which is more profitable than any industry by $333 million (Government of Northwest Territories 2016). Many of the mines found in Northern Canada are found in the Northwest Territories.

Currently most operational mines in the Northwest Territories are diamond mines, but historically gold mines were the most prevalent (Silke 2009). Commodity prices for gold fluctuate, and when prices plummet, it is no longer economical to continue mining causing companies to file for bankruptcy before appropriate closure procedures have been followed. Currently there are 38 abandoned gold mine sites in the Northwest Territories, not including sites that are inactive and with an identifiable owner (National Abandoned/Orphaned Mines Initiative, 2004). The most important ecosystem services that are restored during reclamation include erosion control, nutrient cycling and providing a habitat for wildlife; Since these services are provided by plants and the microorganisms associated with them, revegetation is a significant part of reclamation (MVLWB and AANDC 2013; Buscot and Varma 2005).

Mining Impacts

Many of the gold deposits in Northern Canada are found within arsenopyrite sulphide ores, which contain naturally-occurring arsenic (Rheaume and Caron-Vuotari 2013). When arsenopyrite sulphide ores are milled, or roasted to extract gold, arsenic is released as a by-product. Typically, these by-products, also known as tailings, are drained to a nearby lake which is contained by dams (Natural Resources Canada 2016). Arsenic is found in four elemental states including arsenate As(V), arsenite As(III), elemental arsenic As (0) and arsenide As (-III). Arsenate and arsenite are the most common forms of arsenic found in contaminated soil (Fitz & Wenzel 2002). Arsenite is a trivalent, thiol reactive compound that can bind to multiple cysteine residues or dithiol cofactors in proteins or enzymes (Finnegan and Chen 2012). In the presence of closely spaced cysteine residues in a protein, one arsenite molecule can bind to multiple sites, causing the protein to denature and potentially cease to function (Finnegan and Chen 2012). Because protein and enzyme function is essential in all phases of metabolism, arsenite can impair metabolic processes (Shin et al 2004). Arsenite has also been shown to generate Reactive Oxygen Species which can damage proteins, lipids and DNA and is believed to be the direct cause of carcinogenicity (Liu et al. 2001).

Arsenate is a chemical analog of phosphate and can compete for uptake via phosphate transporters (Shin et al 2004). Once inside the cell, arsenate can interfere with any process that utilizes inorganic phosphate. In the presence of arsenate, ATP-synthase will form arsenate-ADP instead of ATP (Moore et al. 1983). Since arsenate-ADP is unstable, it undergoes hydrolysis, which causes a continuous cycle of the production of arsenate-ADP rather than ATP (Finnegan and Chen 2012). If ADP-arsenate does not dissociate, it can also interfere with ATP production by entering glycolysis. If ADP-arsenate is present during the 6^{th} step in glycolysis, 1-arseno-3-

phosphoglycerate is formed instead of 1,3-bisphosphoglycerate, disrupting the glycolytic chain reaction and inhibiting the formation of ATP (Hughes 2002). Since arsenic has been shown to affect several biological processes, it is not surprising that it can impact revegetation efforts.

Arbuscular Mycorrhizal Fungi and Dark Septate Endophytes

Traditional soil amendment techniques involve increasing organic content, de-compacting and the addition of fertilizers. Adding fertilizer can be useful in some situations, but it has been shown that seedlings are often too small to use fertilizer in their first year, risking the chance of fertilizer runoff (Densmore et al. 2000). It has also been shown that for seedlings to successfully establish, there needs to be on-going application of fertilizer over many years (MVLWB and AANDC 2013). Since mines are in such remote areas, it is cost intensive to return to the site the following years to apply fertilizer. Also, since the plants will already be establishing, applying fertilizer would have to be done carefully and manually, to avoid damaging the seedlings. One possible way of increasing plant survival and growth rate without the use of nutrient rich fertilizers is using mycorrhiza.

Studies have shown that mycorrhizal symbiosis are present in approximately 80% of vascular plants that have been surveyed so far, and the symbiosis is considered the normal state for most plants under most ecological conditions (Smith and Read 2008). Arbuscular Mycorrhizal Fungi (AMF) are a group of fungi that form a mutualistic symbiotic relationship with plants. AMF can only survive within a host plant since they are dependent on plants as a source essential nutrients. In the symbiotic relationship, AMF receive an organic source of carbon from plants in exchange for essential nutrients such as phosphorus (Smith and Read 2008). AMF are composed of three main parts: the arbuscule, hyphae and vesicle (Smith and Read 2008). The arbuscule is a branched system found in cortical cells of the plant root and acts

as the site for nutrient exchange and the vesicle acts as a storage unit for nutrients (Smith and Read 2008). The hyphae can be intracellular, acting as a pathway between arbuscules and vesicles, or extracellular, having an absorptive role or acting as a conduit (Smith and Read, 2008). It has been shown that arbuscular mycorrhizal plants have two pathways by which phosphate can be taken up (Smith et al. 2003). The direct pathway involves phosphate transporters in the root epidermis and root hairs, which is the same pathway in non-mycorrhizal plant species, and the indirect pathway by which hyphae take up phosphorus and translocate it through the extracellular hyphae leading to the arbuscule and ultimately being absorbed by the cortical cells of the plant root (Poirier and Bucher 2002; Smith and Read 2008). To retrieve nutrients from the soil, AMF extend extracellular hyphae past nutrient depletion zones created by the roots and absorb orthophosphate from the soil solution. AMF are more efficient at retrieving phosphorus than plant roots not only because they can pass nutrient depletions zones, but because their hyphae is smaller in diameter than roots, allowing them to access soil pores that are inaccessible to roots, thereby, increasing the volume of soil nutrient solution available for uptake (Smith and Read 2008). It has also been shown that the expenditure of carbon per unit length of hyphae is smaller than that of roots (Tinker et al. 1975).

Spores are resistant and function as long-term survival structures that can be dispersed by wind, water or animals (Koske and Gemma 1990; Friese and Allen 1991). AMF colonization can occur when there are spores, hyphae or colonized root fragments in the soil. Soil can be amended with AMF by using commercially available spores, or ideally through spores or colonized root fragments collected on site since they would be acclimatized to the environment (Orlowska et al. 2011).

Because of the nutrients supplied by AMF, plants receive many benefits from being colonized. In numerous studies, AM colonized plants were larger and contained higher concentration of phosphorus than the un-colonized treatments (Olensniewicz and Thomas 1999; Giri et al. 2003; Bolan 1991). AMF have also been shown to increase plant tolerance to drought conditions. In a study conducted by Wu and Xia (2005), where they compared osmotic adjustment to drought conditions in tangerine plants (*Citrus tangerine*) they found that plants colonized by AMF accumulated higher levels of soluble sugar, soluble starch, non-structural carbohydrates, K^+, Ca^+ and Mg^+ in roots and leaves compared to non-colonized plants. Plants accumulate organic and inorganic solutes during drought conditions to decrease plant water potential, driving water into the plant cells. Because of the increased osmotic regulation facilitated by AMF Wu and Xia (2005) also found that under drought conditions, the tangerine plants had higher shoot and root dry weight, plant height, leaf area, leaf number per plants and stem diameter than corresponding non-colonized plants.

Studies have shown that AMF are able to affect plant biodiversity and ecosystem function and increase stress tolerance (Klironomos et al. 2000; van der Heijden et al. 1998; Orlowska et. al 2011; Christopherson et al. 2009; Xia et al. 2007). In a study conducted by van der Heijden et al. (1998), where they compared the effects of a composition of four different AMF taxa on the community structure and composition of 48 microcosms, they found that eight of eleven plants were almost completely dependent on mycorrhiza to be successful. van der Heijden et al. (1998) also found that the plant biomass of different species varied among treatments with different AMF taxa, showing that different plant species benefit to a different extent based on the AMF taxa present. They also found that the overall structure of plant communities was impacted by the treatment of different AMF taxa. In the second part of the

study where they set up a field experiment with 70 macrocosms with the number of AMF species randomly selected from a pool, they found that both plant biodiversity and productivity increased with increasing AMF diversity.

AMF have been shown to be an important factor in revegetation. Orlowska et al. (2011) showed that colonized seedlings growing in contaminated tailings soil had both an increased plant biomass and seedling survival when compared to their un-colonized counterpart. They also found that when native mycorrhiza, extracted from the tailings area, was used, the plant biomass was higher than that in plants colonized with non-native commercially available AMF. This shows that native AMF ecotypes may be a better choice for reclamation since they are adapted to the harsh soil conditions and climate (Sylvia and Williams 1992; Weissenhorn et al. 1993; Orlowska et al. 2005).

Because of their ability to increase phosphate intake in plants, AMF play a protective role against arsenic (Xu et al. 2008). Because phosphate and arsenate are chemical analogs, arsenate is readily taken up into the plant root via phosphate transporters (Shin et al 2004). Christopherson et al. (2009) found that colonization of AMF downregulated two genes encoding for phosphate transporters (*HvPht1;1* and *HvPht1;2)* in the root epidermis and root hairs of barley (*Hordeum vulgare*). The barley plants received phosphate through AMF, whose phosphate transporters have a higher affinity for phosphate than for arsenic. Another, indirect way that AMF colonization could reduce the negative effects of arsenic toxicity is through increased phosphorus nutrition. The increase in nutrition causes an increase in plant biomass ultimately diluting the metal concentration in the plant tissue (Chen et al. 2007; Ahmed et al. 2006).

In an arsenic exposure study where lentil plants (*Lens culinaris*) were exposed to varying levels of arsenic concentrations in soil, Ahmed et al. (2006) found that plant height, leaf number, pod number, plant biomass and root/shoot concentrations of phosphorus were higher in plants colonized by AMF. They also found that mycorrhizal colonization reduced arsenic concentrations in roots and shoots. This shows that AMF can increase nutrition acquisition while excluding arsenic in contaminated soils. Xia et al. (2007) found similar results when experimenting with arsenic uptake in the maize plant. Xia et al. (2007) found that root length and dry weight increased with mycorrhizal colonization and arsenic concentration in shoots decreased compared to non-colonized plants.

Dark Septate Endophytes (DSE) are another group of root colonizing fungi that form a symbiotic relationship with plants ranging from parasitic to mutualistic (Mandyam and Jumpponen 2005). They colonize the root epidermis and the cortex inter/intracellularly, but unlike AMF they are melanized with septate hyphae (Vegara et al. 2018). DSE lack vesicles and arbuscules, but have microsclerotia, which are inflated hyphae that reside inside of cortical cells. Microsclerotia are believed to serve as a storage structure for nutrients (Yu et al. 2001) or as propagules (Currah et al. 1993). DSE have regularly been found co-occurring with arbuscular mycorrhizae, but are capable of colonizing a greater variety of plants (Jumpponen 2001). Compared to arbuscular mycorrhizae, knowledge on the role of DSE is limited, but some studies have shown that they can assist with nutrient acquisition, as well as mitigate stress on plants (Mandyam and Jumpponen 2005). Studies on the tomato (*Solanum lycopersicum*) and rice plant (*Oryza sativa*) have shown that DSE colonization increased nitrogen, phosphorus, potassium and magnesium concentrations and increased above ground biomass when compared to the non-colonized treatment (Vergara et al. 2017; Vergara et al. 2018). A meta-analysis compiling 18

independent studies, Newsham (2011) reported that DSE colonization raised nitrogen and phosphorus concentrations as well as increased plant biomass by 26-103%. Although their symbiosis with northern plant species is not well understood (Newsham et al. 2009), DSE appear to have the potential to assist with mine revegetation in the same way as AMF.

DSE have been found in extreme environments such as arctic and alpine zones (Read and Haselwandter 1981; Väre et al. 1992), high salinity (Sonjak et al. 2009) and metal contaminated soils (Likar and Regvar 2013; Li et al. 2011; Orlowska et al. 2011), which led researchers to investigate their ability to confer stress tolerance in plants. Likar and Regvar (2013) conducted a study on metal uptake and DSE and found that DSE colonization decreased cadmium and zinc uptake in the goat willow (*Salix caprea*) and increased chlorophyll concentration when compared to the non-colonized treatment. Another study by Li et al. (2011) showed that DSE colonization improved the tolerance of maize in lead, zinc and cadmium contaminated soil by increasing the root and shoot biomass. Because DSE have been shown to increase nutrient acquisition, as well as reduce metal uptake, and increase biomass of plants in metal contaminated soils, they should not be ignored during mine revegetation efforts.

Impacts of Mining on Arbuscular Mycorrhizae and DSE

Mining activity can cause severe soil disturbance, sometimes resulting in the complete loss of topsoil. Physical soil disturbance can break AMF hyphal networks leading to a decrease in infectivity and propagules (Jasper et al. 1987; Rives et al. 1980). The overflow of contaminated tailings can introduce metals into surrounding soils. Arsenic, a known byproduct of gold mining, can reduce AMF spore germination as well as colonization of roots. In a germination trial conducted by Wu et al. (2009), they found that 2.5 mg/kg of arsenic reduced germination in *Glomus mossae* by 40% and *Glomus geosporum, Glomus etunicatum* by 20%. In

another experiment, Gonzalez-Chavez et al. (2002) found that 50 mg/kg of arsenic reduced germination in both *Glomus mossae* and *Gigaspora rosea*. The concentration of arsenic which has been shown to impact mycorrhizal colonization appears to be dependent on plant host species. In a spiked soil experiment, Yu et al. (2009) found that 25 mg/kg of arsenic reduced colonization of the mycorrhizal species *Glomus etunicatum* and *Glomus contrictum* in maize roots (*Zea mays*). Garg et al. (2012) found that there was a significant decrease in colonization of *Glomus mossae* in pea roots (*Psium sativum*) with 30 mg/kg of arsenic whereas Liu et al. (2005) found a decrease in colonization at 150 mg/kg and in tomato roots (*Solanum lycopersicum)*. Studies assessing the impacts of gold mining and arsenic on DSE are scarce, but it appears that DSE react similarly to AMF, resulting in reduced colonization in high arsenic contaminated tailings (Orlowska et al. 2011).

Northern knowledge gaps

Given the importance of AMF colonization in ameliorating plant performance and survival, alleviating metal toxicity and maintaining biodiversity of plant communities, AMF has the potential to aid in revegetation efforts in areas impacted by gold mining (Klironomos et al. 2000; van der Heijden et al. 1998; Orlowska et. al 2011; Christopherson et al. 2009; Xia et al. 2007). Currently the Guideline for the Closure and Reclamation of Advanced Mineral Exploration and Mine Sites in the Northwest Territories does not include AMF as part of soil testing or amendment; although it does highlight the need for further research on revegetation to ensure that the closure plans will be successful. If AMF are naturally occurring in northern environments they should be considered during revegetation plans not only because they are native to the environment, but because they could increase the success rate of revegetation projects.

Given the lack of research on the impacts of gold mining on mycorrhiza in a northern environment; the potential for arsenic, a contaminant associated with gold mine, to impact AMF germination and colonization, and the necessity for quantifying impacts on AMF to help guide restoration strategies in the north, my goal was to sample roots from vegetation in mine impacted areas and adjacent reference sites and to examine relationships mycorrhizal colonization and environmental conditions in and adjacent to a northern mine site.

The objective of this study was to compare mycorrhizal colonization levels among species at a given location and within species at differing locations at the mine. Since there are very few studies on the mycorrhizal colonization of northern plant species it is not possible to predict which species specifically will have higher levels of mycorrhizal colonization. Comparing the mycorrhizal colonization across species can reveal which species may be more highly colonized and therefore a good source of propagules that can be used during revegetation. Comparing the mycorrhizal colonization of each species across several mine sites will shed light on whether the mycorrhizal symbiosis was impacted by elevated arsenic concentrations caused by mining. If colonization is found at high arsenic sites, then the mycorrhiza from those sites could be used as part of the remediation of arsenic impacted areas. Based on several studies which have shown that exposure to arsenic may reduce mycorrhizal colonization (Orlowska et al. 2011; Yu et. al 2009; Garg et al. 2012; Liu et al. 2005), I hypothesize that mycorrhizal colonization will be will be lower in areas with higher arsenic concentrations.

Methods

Tundra Gold Mine

Tundra Mine is a gold mine located 250km northeast of Yellowknife, Northwest Territories (64º 03' N, 111º 11' W) (Figure 3-1). The site is part of the Treaty 11 claim, the Akaitcho Territory, Wek'eeshii and Monwhi Gogha De Nittaee areas of the Tlicho Land Claim Agreement, and the North Slave Métis traditional lands (Golder 2008). The mine is located between two large lakes: Mackay Lake (1060 km^2) can be found five kilometers to the south, and Courageous Lake (250 km^2), nine kilometers to the north. Because the site is remote, it is only accessible using a small chartered aircraft, which can only carry passengers, light weight equipment and food supply. During the winter months, larger heavy equipment or bulk supplies can be brought on site with help of ice roads.

Annual temperature varies from -31°C to 18°C. The shortest day is on December 21 with 3:45 hours of daylight and the longest day is June 20 with 21:45 hours of daylight (Carison et. al 2015). The mean annual precipitation is 345 mm and consists approximately of equal parts rain and snow (Carison et. al 2015). It is part of the Taiga Shield and Southern Arctic, which are characterized by low soil nutrients, a cool and short growing season compared to temperate regions (Government of Northwest Territories 2009, 2012).

The topography at Tundra Mine is relatively flat, with gradual slopes and bedrock near the surface. The soils are classified as cryosols, meaning they are frozen 1 meter below the surface and experience waterlogging during spring thaw. The ice formation and thaw causes the soil layers to become disrupted, cracked and to develop hummocks. The hummocks were measured to be up to 30 cm tall with a 5cm organic soil containing dark peat with partially decomposed plant matter (Stevens et al. 2017).

Tundra Mine was operational between 1964 and 1968 and produced a total of 104,476 ounces of gold but was reopened from 1983 to 1986 to process ore from Salmita mine

(MVLWB, 2010). The gold extracted at Tundra Mine was from arsenopyrite rock resulting in the release of large amounts of arsenic. Approximately 300,000 tons of tailings produced from both mining operations were placed in Russell Lake (MVLWB, 2010). The waste rock produced contained between 0.2 -10 mg arsenic/g of soil which surpassed the CCME guidelines for sediment (0.0059 mg/g) and soil (0.012 mg/g). The tailings, which were 1.2 million cubic million liters, also contained high levels of arsenic (1.1 mg/L – 2.8 mg/L) compared to the CCME water quality guideline which recommends a maximum of 0.005 mg/L to protect aquatic life (Figure 3-2). When Russell Lake was turned into a tailings area, it was divided into Upper and Lower Pond (Figure 3-3). To decrease the chances of tailing overflow and leaching into surrounding areas, the tailings were moved to a containment area and capped with a geosynthetic liner and rock. The arsenic from the tailings water was removed with iron co-precipitation and the treated water discharged into Hambone Lake. Since it is almost impossible to remove all arsenic from the tailings, waste rock was excavated and moved to a smaller containment area in lower pond, the bedrock was cleaned and soil was replaced with gravel extracted from the on-site quarry (MVLWB 2010). A discharge channel was placed between the new tailings area and Hambone Lake to facilitate surface drainage (MVLWB 2010).

In the previous chapter "Chapter 2- Mining impacts and their relation to vegetation and arbuscular mycorrhizal fungi" total arsenic and total phosphorus was quantified at all sites sampled in this part of the study. Hambone Lake (HB) (2677 ± 704.6 mg/kg) and at Mill Pond (MP) (1324 ± 398.9 mg/kg) contain the highest arsenic concentrations in the soil. The lowest arsenic concentrations were found at Reference 3 (Ref 3) (2.26 ± 1.47 mg/kg) followed by the Quarry (QU) (0.005 ± 0.00 mg/kg) (Figure 3-4). The differences between total phosphorus were less dramatic with the highest phosphorus concentration in soils collected from Hambone Lake

(HB) (587.17 ± 65.76 mg/kg) and Reference 1 (Ref 1) (569.83 ± 46.90 mg/kg) while the lowest concentration was at Trans Saddle Lake (TSL) (236.7 ± 75.61 mg/kg)(Figure 3-5).

The areas awaiting revegetation include both dry upland areas as well as wetlands. The soil appears to be silty with some rocks (Stevens et al. 2017). There is some sparse vegetation growing in the area, but because of the harsh growing conditions, unaided natural seedling establishment and growth could take up to 50 years (Densmore et al. 2000). It is therefore crucial that a revegetation strategy must be planned and implemented so that the final stage of reclamation can be complete. Currently, the previous tailings pond, the quarry as well as an airstrip is awaiting revegetation totaling an area of 73 hectares (Figure 3-6).

Field Sampling

Nine vegetated sites were sampled at Tundra mine from June 23rd, 2016 to June 25th, 2016. Five sites were chosen close in proximity to the tailings containment area and original milling area. These five sites are sites that experienced mining disturbances, as evidenced by proximity to the tailings and active quarry, and were compared to reference sites further away from mining activity. The five possibly impacted vegetated sites include North Damn (ND), Mill Pond (MP), Hambone Lake (HB), Trans Saddle Lake (TSL) and the Bog (Figure 3-7 to 3-9). The remaining four sites were chosen as far away from the original milling and tailings area as possible given the restricted time frame and road access. The four reference sites, starting with the site furthest away from mining activity and include Reference 1 (R1), Reference 2 (R2), Reference 3 (R3), and Sandy Lake (SL) (Figure 3-8 to 3-9). All vegetated sites except Reference 2 and Reference 3 were visited again from August 4th to August 7th, 2016, since there was no

access to Reference 2 and Reference 3 due to lack of access during wet conditions. Again, five plant samples were collected for each AM species at every vegetated site.

Five individual plant samples were collected for each plant species that are anticipated to harbour AMF at every vegetated site (Harley and Harley 1987) (Table A-16 to A-17). Each plant sample was carefully uprooted and placed in a sterile Whirl-Pak (eNasco, Fort Atkinson, Wisconsin, USA) with soil from the site. The soil was added to prevent the root system from drying. Plants were identified to the lowest possible taxon using the identification key "Vascular plants of continental Northwest Territories, Canada" (Cody and Prosild 1980). Due to incomplete samples not all species were identifiable to the species level.

Quantification of Arbuscular Mycorrhizal Fungi Colonization

To quantify levels of AMF colonization, roots were rinsed with deionized water, then cleared in 10% KOH at 90˚C in a Thermo Scientific Lindber Blue vacuum oven at 25 inches Hg of pressure (Brundrett et al. 1996). Roots were removed from the KOH solution every 10-15 minutes to be visually inspected under Zeiss Jenalumar light microscope in order to assess the clearing. Total clearing time ultimately depended on thickness of the roots, with thicker roots needing more time to clear. Roots were then rinsed with deionized water and placed in a solution of 10% blue ink in acetic acid (Vierheilig et al. 1998) for the same amount of time that was needed to clear them. Roots were then rinsed with deionized tap water and place in slightly acidified water overnight to allow roots to de-stain. After roots were stained, they were mounted on a slide with 50% glycerol. If the entire root system was too large to fit on one slide, random samples were chosen using a grid and a random number generator and placed on a microscope slide until it was full. Colonization was quantified using the magnified intersections method at 250x magnification with a Zeiss Jenalumar light microscope (Mcgonigle et al. 1990). One

hundred fields of view were assessed and the presence of hyphae, arbuscules, vesicles and DSE was recorded.

Statistical Analysis

Presence of Arbuscular Mycorrhizal Fungi and Dark Septate Endophytes

Mycorrhizal colonization was assessed in two ways. The first way was comparing the colonization in one plant species across several sites and the second was comparing the colonization of different species at the same site. One-way ANOVAs were used on the hyphal, arbuscular, vesicular and DSE colonization separately. If the residuals of the model were not normally distributed or the variance was not equal, then the data was transformed (Table 3-1 to 3-16). When the ANOVA showed significant differences, a Student's t-test was used to obtain letters of significance. If only two species or sites were compared, then a Student's t-test was used to test significant differences.

Results

Arbuscular Mycorrhizal and DSE Colonization Across Sites

Calamagrostis canadensis June

Calamagrostis canadensis was collected from Sandy Lake, North Dam, Trans Saddle Lake and Reference 2 in June 2016. Hyphal, arbuscular and DSE colonization were present in roots collected from all sites. Vesicular colonization was found in all roots except those collected from North Dam. There was a significant difference in hyphal colonization in roots collected in June (Figure 3-10A; p-value = 0.000386). The highest colonization was found in roots collected from Trans Saddle Lake (97.50 ± 1.50 %). Roots collected from Sandy Lake (24.50 ± 3.94),

North Dam (30.93 ± 9.96 %) and Reference 2 (43.53 ± 10.24 %) were lower in colonization and not significantly different from one another. The same trend was observed with arbuscular and vesicular colonization (Figure 3-10B, 3-10C). The highest arbuscular colonization was 20.50 ± 12.50 % and vesicular colonization was 25.00 ± 13.00% and was found in roots collected from Trans Saddle Lake. There was no significant difference in DSE colonization among sites (Figure 3-10D).

Calamagrostis canadensis August

Calamagrostis canadensis was collected at Hambone Lake, North Dam and Trans Saddle Lake in August 2016. Hyphal, arbuscular, vesicular and DSE colonization were found in roots collected from all sites. There was a significant difference in hyphal colonization among sites (Figure 3-11A; p-value = 0.0316). The highest hyphal colonization was found in roots collected from North Dam (81.25 ± 8.75%) while the lowest colonization was found in roots collected form Hambone Lake (33.20 ± 10.61%). There was a significant difference in arbuscular colonization based on site (p-value = 0.0395) and the highest colonization was found within roots collected from North Dam (28.75 ± 19.11%) (Figure 3-11B). There were no significant differences in vesicular colonization based on site (Figure 3-11C; p-value = 0.682), however, there was a significant difference in DSE colonization (Figure 2-11D; p-value = 0.0293). The roots collected from Trans Saddle Lake had the highest DSE colonization (50.96 ± 11.99%), while roots collected from Hambone Lake (18.40 ± 9.04%) and North Dam (9.75 ± 5.80%) were lower and did not significantly differ from one another.

Calamagrostis deschampsioides June

Calamagrostis deschampsioides was collected at Reference 1 and Reference 2 in June 2016. Hyphal, arbuscular, vesicular and DSE colonization was present at all sites. There was no significant difference in hyphal, arbuscular or DSE colonization between roots collected at Reference 1 versus Reference 2 (hyphal p-value = 0.341; arbuscular p-value = 0.7072; DSE p-value = 0.1617) (Figure 3-12). Reference 2 (4.31 ± 2.01%) had significantly higher vesicular colonization than Reference 1 (0.20 ± 0.20%) (Figure 3-12C; p-value = 0.03813).

Epilobium angustifolium August

Epilobium angustifolium was collected at Hambone Lake, the Bog and Reference 1. Hyphal, arbuscular, vesicular and DSE colonization was found at all sites except the Bog, where roots lacked arbuscular and DSE colonization and Hambone Lake, where roots lacked arbuscular colonization. There was a significant difference in hyphal colonization among sites (Figure 3-13A; p-value = 0.00623). Roots collected from Hambone Lake and Reference 1 had the highest hyphal colonization and were not significantly different from one another (Hambone Lake = 59.83 ± 7.27%; Reference 1 = 44.75 ± 13.99%) (p-value = 0.535). The Bog had the lowest hyphal colonization (6.45 ± 1.91%). There were no significant differences in arbuscular or vesicular colonization among sites (Figure 3-13B, 3-13C; arbuscular p-value = 0.441; vesicular p-value = 0.211). Reference 1 (41.23 ± 11.29%) and Hambone Lake (37.28 ± 14.70%) had the highest DSE colonization and did not significantly differ from one another while the Bog had the lowest DSE colonization (0.00 ± 0.00%) (Figure 3-13D; p-value = 0.0233).

Epilobium palustre August

Epilobium palustre was collected at Mill Pond, the Bog and North Dam. Hyphal, arbuscular, vesicular and DSE colonization was found at all sites except the Bog, which did not have arbuscular, vesicular or DSE colonization and Mill Pond which did not have vesicular colonization. There was no significant difference in hyphal or vesicular colonization among sites (Figure 3-14A. 3-14C; hyphal p-value = 0.101; vesicular p-value = 0.141), but there was a significant difference in arbuscular colonization (Figure 3-14B; p-value = 0.0144). Mill Pond had the highest arbuscular colonization (33.64 ± 15.65%) compared to the Bog and North Dam which were significantly different from one another (the Bog = 0.00 ± 0.00%; North Dam = 0.60 ± 0.60%). There was also a significant difference in DSE colonization among sites (Figure 3-14D; p-value = 0.0384). The roots collected from North Dam had the highest DSE colonization (12.00 ± 4.57%) and the roots from the Bog had the lowest colonization (0.00 ± 0.00%). The DSE colonization in roots from Mill Pond were not significantly different than those collected at North Dam or the Bog (8.30 ± 4.93%).

Mycorrhizal Colonization Across Species

North Dam June

Calamagrostis canadensis (Ca. ca.) and *Epilobium palustre* (Ep. pa.) were collected at North Dam in June 2016. Hyphal, arbuscular and DSE colonization was found in both plant species, whereas, vesicular colonization was absent. There were no significant differences in hyphal, arbuscular, vesicular or DSE colonization between plant species (Figure 3-15; hyphal p-value = 0.4379; arbuscular p-value = 0.3498; DSE p-value = 0.2154).

Reference 2 June

Calamagrostis canadensis (Ca. ca.) and *Calamagrostis deschampsioides* (Ca. de.) were collected at Reference 2 in June 2016. Hyphal, arbuscular, vesicular and DSE colonization was present in both species. Hyphal colonization was significantly higher in *Calamagrostis canadensis* than *Calamagrostis deschampsioides* (Figure 3-16A)(*Calamagrostits canadensis* = 43.53 ± 10.24%; *Calamagrostis deschampsioides* = 15.25 ± 6.69%). There were no significant differences in arbuscular, vesicular or DSE colonization between species (Figure 3-16B to 3-16D; arbuscular p-value = 0.9995; vesicular p-value = 0.5523; DSE p-value = 0.1578).

Sandy Lake June

Calamagrostis canadensis (Ca. ca.), *Stellaria longipes* (St. lo.) and *Trisetum spicatum* (Tr. Sp.) were collected at Sandy Lake in June 2016. Hyphal, arbuscular, vesicular and DSE colonization was found in all plant species except for *Stellaria longipes*, which did not have arbuscular or vesicular colonization. There was a significant difference in hyphal, arbuscular and vesicular colonization among species (Figure 3-17; hyphal p-value = 0.00721; arbuscular p-value = 00275; vesicular p-value = 0.0213). *Trisetum spicatum* had the highest hyphal colonization (57.52 ± 8.84%). Hyphal colonization in *Calamagrostis canadensis* and *Stellaria longipes* was lower and did not significantly differ from one another (*Calamagrostis canadensis* = 24.51 ± 3.17%; *Stellaria longipes* = 20.01 ± 8.20%) (p-value = 0.8848). The highest arbuscular colonization was found in *Calamagrostis canadensis* roots (5.27 ± 1.86%). *Stellaria longipes* and *Trisetum spicatum* colonization was lower and not significantly different from each other (*Stellaia longipes* = 0.00 ± 0.00%; *Trisetum spicatum* = 1.00 ± 1.42%) (p-value = 0.3871). The highest vesicular colonization was found in *Trisetum spicatum* (5.00 ± 1.78%). Colonization in

Stellaria longipes (0.00 ± 0.00%) was lower and was not significantly different from *Trisetum spicatum* (0.97 ± 0.85%). There was no significant difference in DSE colonization among species (Figure 3-17D; p-value = 0.0662).

The Bog August

Epilobium angustifolium (Ep. an.), *Epilobium palustre* (Ep. pa.), *Poa sp. 1* and *Poa sp.. 3* were collected at the Bog in August 2016. Hyphal colonization was found in all plant species. Arbuscular colonization was not detected in any collected roots. Vesicular colonization was found in *Epilobium angustifolium*, *Poa sp. 1* and *Poa sp. 3*, but not in *Epilobium palustre*. DSE colonization was only found in *Poa sp. 1*. There was a significant difference in hyphal and DSE colonization based on plant species (hyphal p-value = 0.0106; DSE p-value = 1.13e-14). *Poa sp. 3* had the highest hyphal colonization (45.40 ± 14.19%). *Epilobium angustifolium* (6.45 ± 1.91%) and *Epilobium palustre* (13.69 ± 4.07%) had the lowest colonization and did not significantly differ from one another (Figure 3-18A). The highest DSE colonization was found in *Poa sp. 1* roots (79.12 ± 7.57%) and there was no colonization found in all other species (Figure 3-18D). Finally, there were no significant differences in vesicular colonization based on species (Figure 3-18C; p-value = 0.124).

Reference 1 August

Agrostis scabra (Ag. Sc.), *Epilobium angustifolium* (Ep. an.) and *Calamagrostis deschampsioides* (Ca. de.) were collected at Reference 1 in August 2016. Although hyphal, arbuscular, vesicular and DSE colonization was present in all species collected, there were no significant differences among species (Figure 3-19; hyphal p-value = 0.169; arbuscular p-value = 0.691; vesicular p-value = 0.861; DSE p-value = 0.140).

Hambone Lake August

Epilobium angustifoilum (Ep. an.) and *Calamagrostis canadensis* (Ca. ca.) were collected at Hambone Lake in August 2016. Hyphal, vesicular, and DSE colonization was found in both species collected. Arbuscules were only found in *Calamagrostis canadensis.* There were no significant differences found in colonization between species (Figure 3-20; hyphal p-value = 0.09139; arbuscular p-value = 0.4071; vesicular p-value = 0.9846; DSE p-value = 0.2899).

North Dam August

Calamagrostis canadensis (Ca. ca.) and *Epilobium angustifolium* (Ep. an.) were collected at North Dam in August 2016. Hyphal, arbuscular, vesicular and DSE colonization was found in both species collected. There were no significant differences found in colonization between the two species (Figure 3-21; hyphal p-value = 0.154; arbuscular p-value = 0.2372; vesicular p-value = 0.1639; DSE p-value = 0.766).

Discussion

Mycorrhizal colonization has been shown to alleviate arsenic toxicity and increase the biomass of seedlings, making it an important component that can be used in revegetation at gold mines (Ahmed et al. 2006). However, it has also been shown that at high concentrations, arsenic can decrease mycorrhizal colonization and germination of spores (Wu et al. 2009; Garg et al. 2012; Liu et al. 2005). Currently, there is not much research on mycorrhiza in northern environments. It is unclear whether they are a part of the natural ecosystem and whether the impacts of arsenic on mycorrhiza are different in a northern ecosystem. The discovery of

mycorrhiza in northern ecosystems would help facilitate gold mine revegetation effort by supporting plant communities (van der Heijden et al. 1998; Orlowska et al. 2005; Orlowska et al. 2011). During revegetation soils are usually amended with fertilizer, which can be expensive since many mine sites are remote (Densmore and Holmes 1987; Densmore et al 2000). Also, by adding fertilizer there is a risk of runoff into pristine waters ultimately leading to eutrophication (Huang et al 2017). An alternative would be inoculating soils with native mycorrhizae that are acclimatized to the harsh northern environment.

In this study, AM plants were collected at varying distances from a known arsenic contaminated area, and roots were assessed for mycorrhizal colonization. The mycorrhizal colonization was then compared across sites to see if arsenic had an impact on colonization. Mycorrhizal and DSE colonization were found at all sites sampled, indicating that mycorrhizae are part of the ecosystem at Tundra Mine. In a study conducted 200km south of Tundra Mine, MacColl (2017) also found colonization in plants collected along Baker Creek. Either arbuscular and or vesicular colonization was found in *Epilobium angustifolium, Epilobium palustre* (which has not yet been documented), *Calamagrostis canadensis, Calamagrostis deschampsiodes, Trisetum spicatum, Agrostis scabra* and two unknown species of grass. *Stellaria longipes* was the only species sampled that did not have arbuscules or vesicles, but hyphae were present and appeared morphologically similar to hyphae in plants colonized with arbuscules and vesicles. Hyphae are the first fungal structures to form, therefore, it is possible that when roots were collected, arbuscules and vesicles had not yet developed (Brundrett 2008). Nevertheless, the hyphal colonization cannot be conclusively identified as arbuscular mycorrhizal.

Reductions in spore germination and root colonization caused by arsenic have been observed at concentrations ranging from 2.5 mg/kg to 50 mg/kg and 25 mg/kg to 150 mg/kg

respectively (Wu et al. 2009; Gonzalez-Chavez et al. 2002; Yu et al. 2009; Garg et al. 2012; Liu et al. 2005). Although research on impacts of arsenic on DSE is limited, Orlowska et al. (2011) showed that colonization is reduced at 83 mg/kg of arsenic in the hopbush (*Dodonaea viscosa*). Based on the aforementioned studies, I would have expected a decrease in mycorrhizal and DSE colonization in plants collected from Hambone Lake and Mill Pond since those sites had arsenic concentrations exceeding those shown to decrease germination or colonization. When comparing percent colonization across sites, there were no clear trends of mycorrhizal colonization based on proximity to the original tailings area or arsenic concentration in the soil leading to a rejection of the hypothesis that mycorrhizal colonization will be lower in areas more proximal to the gold mining activities and that the mycorrhizal colonization will be related to arsenic. It is possible that mycorrhizae in the contaminated areas can tolerate arsenic. Several studies have shown that mycorrhizae can acquire a tolerance to mining contaminants. Wu et al. (2009) compared the germination of a metal-contaminated isolate versus uncontaminated isolate of *Glomus mossae* and found that the metal-contaminated isolate had a significantly higher germination rate than all uncontaminated isolates in 2.5mg/kg of arsenic in soil. In a similar study done by Gonzalez-Chavez et al. (2002), where they extracted spores of *Glomus caledonium* and *Glomus mossae* from mine soil contaminated with arsenic, they found that spores of *Glomus caledonium* exhibited an increased germination and *Glomus mossae* showed no difference in germination when exposed to 250 mg/kg of arsenic. In the study, the germination rates of spores that were non-mine isolates were significantly lower than mine isolates when exposed to the same 250 mg/kg arsenic concentration. It is, therefore, possible that the mycorrhiza at Tundra Mine can tolerate high concentrations of arsenic, although further studies must be conducted to confirm this.

There were differences in mycorrhizal colonization between species in two of the sites sampled. One reason for the lack of detectable difference in the remaining five sites is partially due to the high variance in colonization in the sample replicates. To get a better understanding of the differences in colonization that are attributed to host species, a lab experiment could be used where more replicates are included to reduce variance.

Calamagrostis canadensis, *Epilobium angustifolium*, and *Epilobium palustre* collected from the high arsenic sites were all colonized with AMF. If site managers wish to revegetate the tailings containment area as part of the final stages of reclamation, or are concerned that the tailings could leach into the unvegetated areas, they could isolate AMF from those species and inoculate the soil to facilitate the symbiosis of AMF and newly developing plants. Since the AMF from those sites originated from soils that contained high levels of arsenic, they may perform better non-native AMF because they are acclimated to those conditions. One experiment comparing plant growth in tailings showed that when mycorrhiza extracted from the tailings was used to inoculate plants that were native to the mine site, plants grew larger than non-native plants inoculated with commercially available mycorrhiza (Orlowska et al. 2011). The use of native AMF in this case may be a better choice for reclamation since they are adapted to the harsh soil conditions and climate (Sylvia and Williams 1992; Weissenhorn et al. 1993; Orlowska et al. 2005).

Mycorrhizal colonization in *Calamagrostis canadensis* collected in June had the highest hyphal, arbuscular and vesicular colonization when collected from Trans Saddle Lake, which is the site with the lowest total phosphorus. In soils where phosphorus is readily available to plants, the cost of carbon needed to form a symbiotic relationship with mycorrhiza can outweigh the supply of nutrients provided to the plant (Bethlenfalvay et al 1983; Peng et al. 1993). However,

if phosphorus supply is limited then the cost of carbon to form the symbiotic relationship is little compared to the benefit because mycorrhizal hyphae can access phosphorus that roots cannot access alone (Smith and Read 2008); therefore, it is not uncommon for higher colonization to occur at lower phosphorus concentrations (Propster and Johnson 2015; Bethlenfalvay et al 1983; Stevens et al. 2002). For example, in an agroforestry bioassay experiment , Shukla et al. (2012) found that a decrease in phosphorus supply resulted in higher arbuscular and vesicular colonization in wheat *(Triticum aestivum*) and red gum *(Eucalyptus tereticornis*). Kahiluoto et al. (2000) had similar results, showing that the cumulative addition of phosphorus caused a decrease in AMF colonization.

There were no clear trends of DSE colonization based on site. DSE and AMF were almost always found in the same root system, showing their ability to coexist. In a study comparing DSE and AMF colonization in plants collected from disturbed and undisturbed sites, Chaudhry et al. (2009) found that the majority of plant roots sampled had both AMF and DSE colonization together regardless of disturbance levels. Another study by Lugo et al. (2018), examining AMF and DSE colonization in 20 native grasses in the Argentine Puna found that grasses were co-colonized by AMF and DSE. In this study, DSE colonization was even found at the highest arsenic site confirming that DSE can withstand extreme environments (Read and Haselwandter 1981; Väre et al. 1992; Sonjak et al. 2009; Likar and Regvar 2013; Li et al. 2011). DSE are found in many terrestrial ecosystems, ranging from the tropics to arctic zones (Jumpponen et al. 1998), but it has been shown that they are more prominent in cold, nutrient poor and arid environments (Frenot et al. 2005; Olsson et al. 2004). Although the symbiosis between DSE and plants is not as clearly understood as the AMF-plant symbiosis, there have been some studies showing that DSE have the potential to assist with revegetation efforts.

Studies have shown that DSE colonization have increased macronutrient concentrations and biomass in plants (Vergara et al. 2017; Vergara et al. 2018; Newsham et al. 2009). DSE have been shown to alleviate metal toxicity by reducing metal uptake and increasing biomass in plants grown in metal contaminated soils (Likar and Regvar 2013; Li et al. 2011). Because DSE colonization was present at all sites, DSE are likely to be a natural part of the ecosystem and, therefore, play an ecological role and should not be ignored during revegetation efforts.

Because this experiment was a field study, it does have some limitations. Since not all sites sampled had the same species present, it was difficult to compare colonization across all sites. Also, due to the nature of a field study, there were variables that were not controlled that could have affected results, although a field study does reflect a real-world situation. This experiment showed not only that mycorrhiza and DSE are present in a northern environment, but that some may also be arsenic tolerant. In the future, a lab experiment could be used to assess arsenic tolerance by comparing mycorrhizal and DSE colonization from isolates obtained from arsenic contaminated areas such as Hambone Lake and Mill Pond compared to non-contaminated isolates from Reference 2. Bioassays with varying soil arsenic concentrations, could be used to grow a native plant host inoculated with mycorrhizal and DSE isolates and colonization could be quantified to determine if isolates from the arsenic-contaminated areas can withstand higher concentrations of arsenic. In a lab experiment where the plant host and soil is consistent across all samples, and where only arsenic concentration and AMF or DSE isolate vary, it is possible get a clearer understanding of the impacts of arsenic.

This research can help guide remediation and reclamation efforts at Tundra Mine and arsenic-contaminated mine sites across Northern Canada. Northern climate is characterized by low soil nutrients, a cool and short growing season and high winds, making revegetation efforts

challenging (Government of Northwest Territories 2009; Government of Northwest Territories 2012). This study has shown that arbuscular mycorrhizae and DSE are part of the Northern ecosystem and can be used as a tool in revegetation strategies. If high arsenic soils are scheduled for revegetation, then native mycorrhiza and DSE, extracted from high arsenic sites could potentially be used. AMF can also help decrease arsenic uptake in plants, which is beneficial since colonization would prevent arsenic from entering higher trophic levels (Ahmed et al. 2006). Because AMF provides plants with nutrients, it would also decrease the need for fertilizer, which can be costly and harmful to the environment. It is especially important to reduce environmental impact because northern ecosystems recover from impacts more slowly (Densmore 2000).

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Figures and Tables

Figure 3- 1: Location of Tundra Gold Mine. Tundra Mine is found approximately 250km Northeast of Yellowknife, NWT Canada (64º 03' N, 111º 11' W). Map obtained from Google Maps.

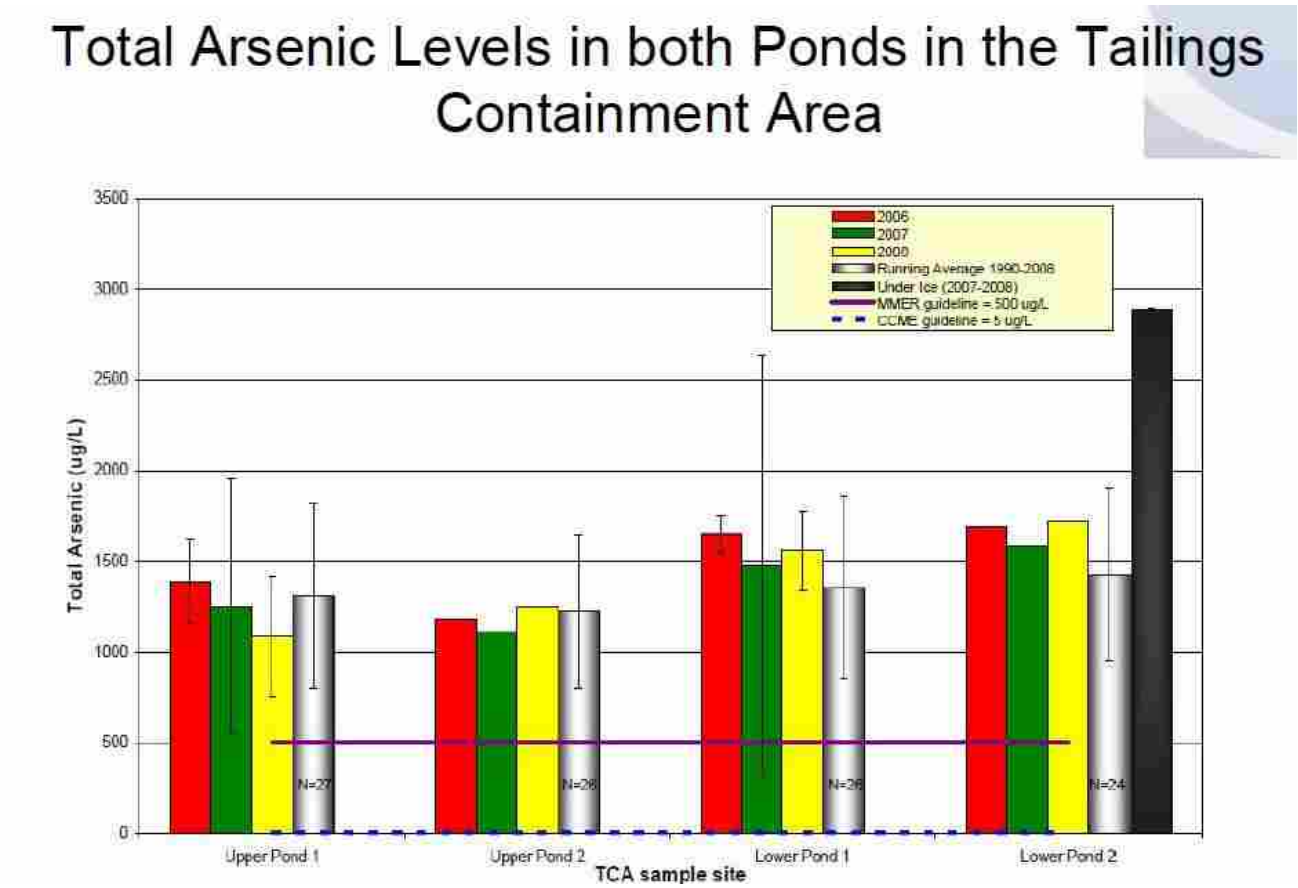

Figure 3- 2: Total arsenic levels in the Tailings Containment Area at Tundra Gold Mine, NWT, Canada (Upper Pond and Lower Pond). CCME water quality guideline for arsenic levels recommends a maximum of 5ug/L to protect aquatic life. Graph obtained from *Report Phase 2 Remedial Action Plan, Tundra Mine Site* (Golder 2008).

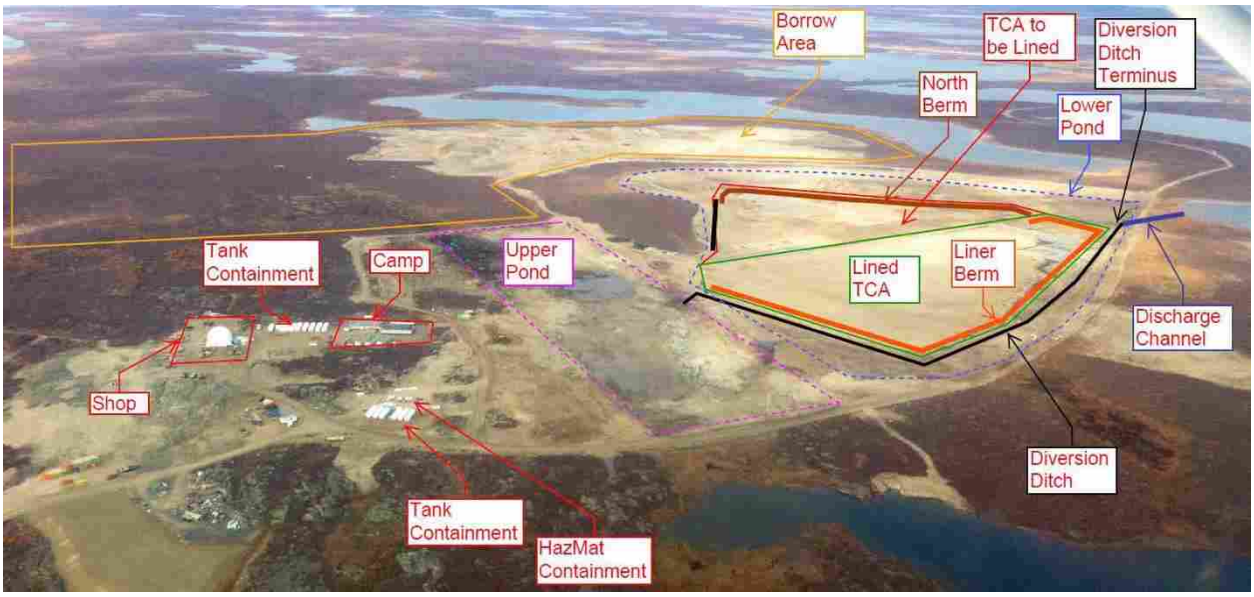

Figure 3- 3: Aerial photo of un-vegetated sites during remediation phase at Tundra Gold Mine, NWT, Canada. Photo is showing Upper Pond (pink outline) and Lower Pond (blue outline), which were previous tailings area. Lower Pond still contains tailings, but they are now capped and coved with rock material. Borrow Area, is an open quarry that rock material was excavated from to use on the tailings. Photograph obtained from *Report Phase 2 Remedial Action Plan, Tundra Mine Site* (Golder 2008).

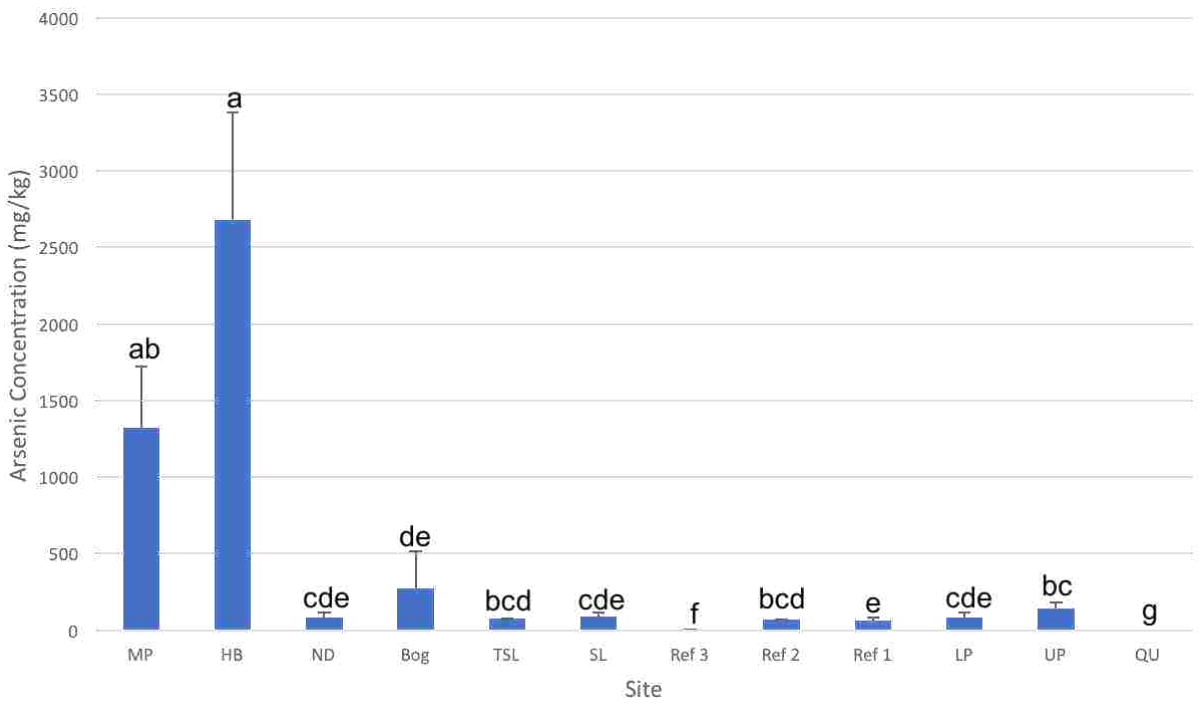

Figure 3- 4: Mean total arsenic concentration in soil collected from Tundra Gold Mine June 2016. Plots include raw data and error bars showing standard error. Bars sharing letters are from data that are not significantly different. Graph was obtained from the previous chapter in this study "The impacts of gold mining on northern plant communities and arsenic uptake in plants."

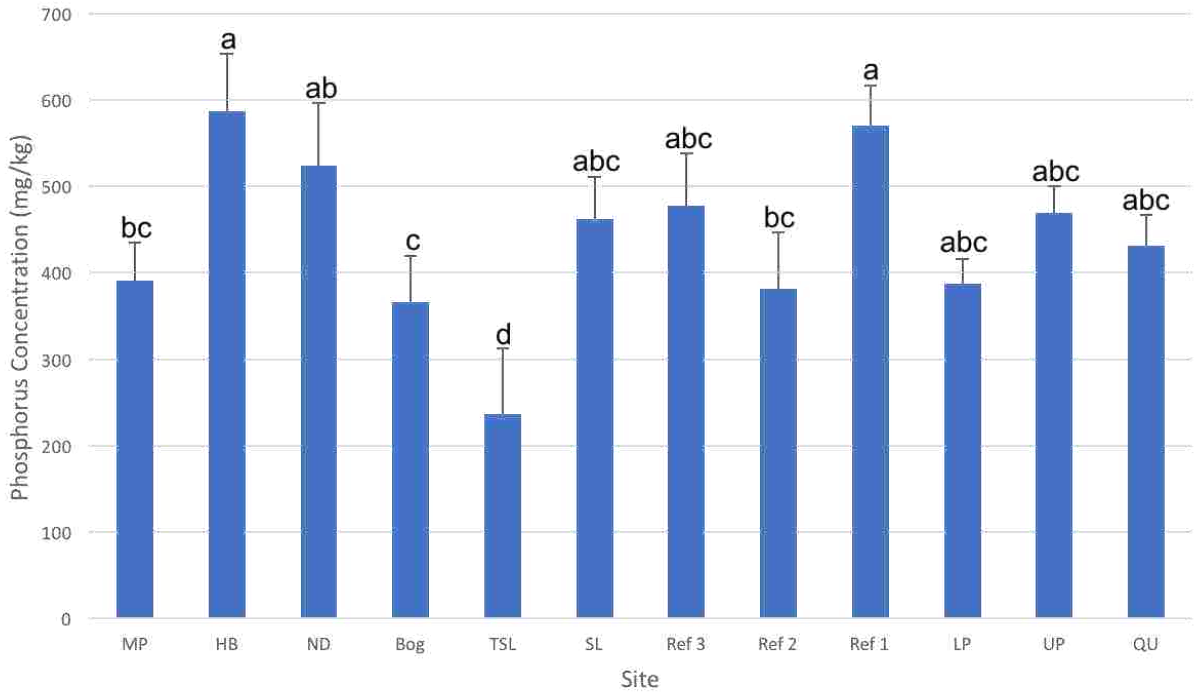

Figure 3- 5 : Mean total phosphorus concentration in soil collected from Tundra Gold Mine June 2016. Plots include raw data and error bars showing standard error. Bars sharing letters are from data that are not significantly different. Graph was obtained from the previous chapter in this study "The impacts of gold mining on northern plant communities and arsenic uptake in plants."

Figure 3- 6: Overview of sites awaiting revegetation including Upper Pond (top left), the Quarry (top right, bottom left) and Lower Pond (bottom Left). Photographs taken by Kevin Stevens (top left), Marry Hewitt (top right, bottom left) and Sarah Mediouni (bottom left).

Figure 3- 7: Site overview of sites sampled that were near the original milling and tailings containment area. Five vegetated sites labeled in blue, and three non-vegetated sites labeled in red. Photograph obtained from Mary Hewitt (Flat River Consulting) and modified.

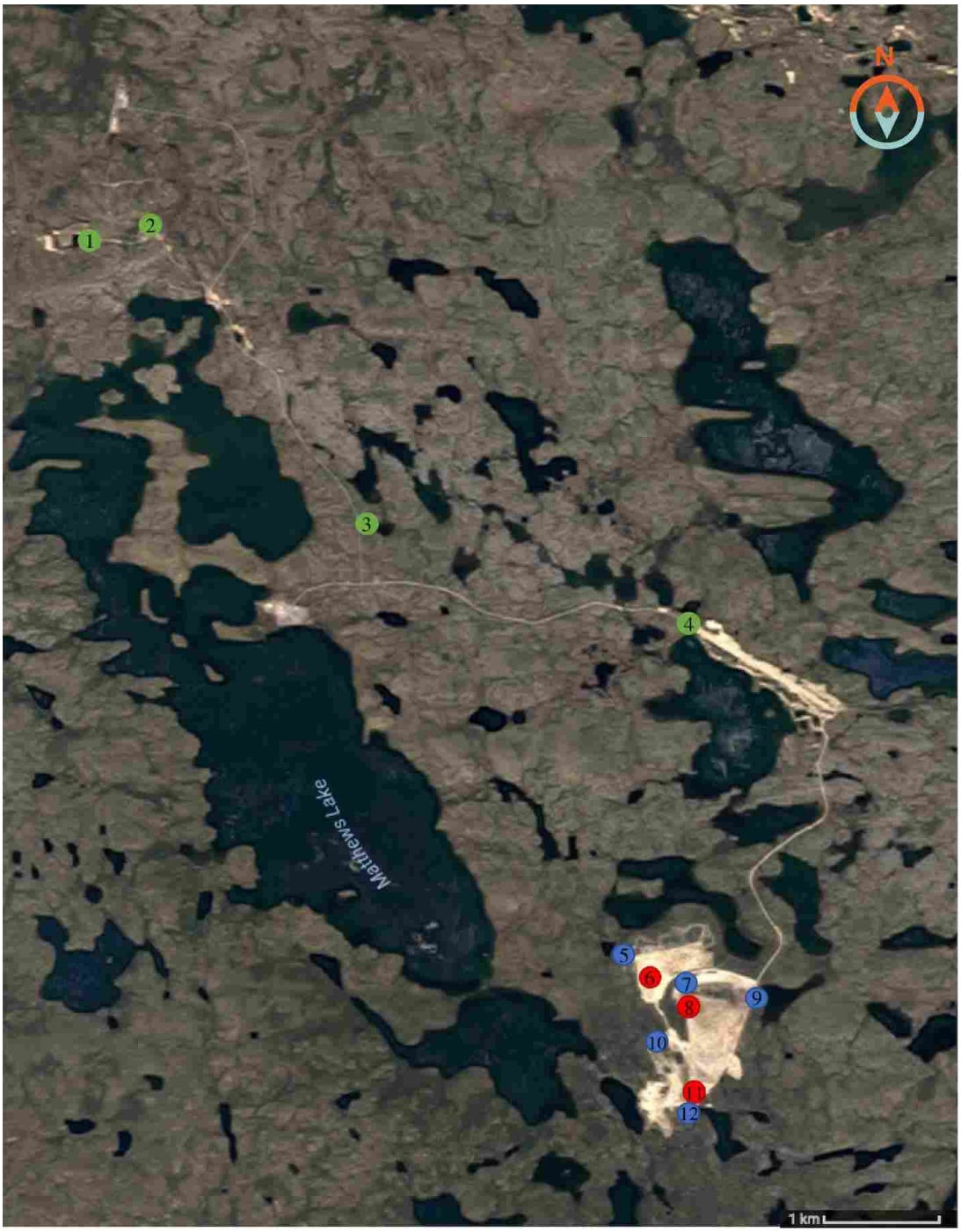

Figure 3- 8: Site overview of sampled sites Tundra Gold Mine. Green sites indicate vegetated reference sites (Reference (1), Reference 2 (2), Reference 3 (3), Sandy Lake (4)) blue sites indicate potentially impacted sites vegetated sites (Hambone Lake (9), Trans Saddle Lake (5), Mill Pond (12), North Dam (7) and the Bog (10)), and red sites indicate potentially impacted un-vegetated sites (Lower Pond (8), Upper Pond (11), Quarry (6)). Map modified from Google Maps.

Figure 3- 9: Overview of vegetated sites including Sandy Lake (top left), Hambone Lake (top right), Reference 1 (bottom left) and Mill Pond (bottom right). Both Hambone Lake and Mill Pond are close to the original milling area, and are presumably impacted. Photographs taken by Sarah Mediouni (top left, bottom left and bottom right) and Kevin Stevens (top right).

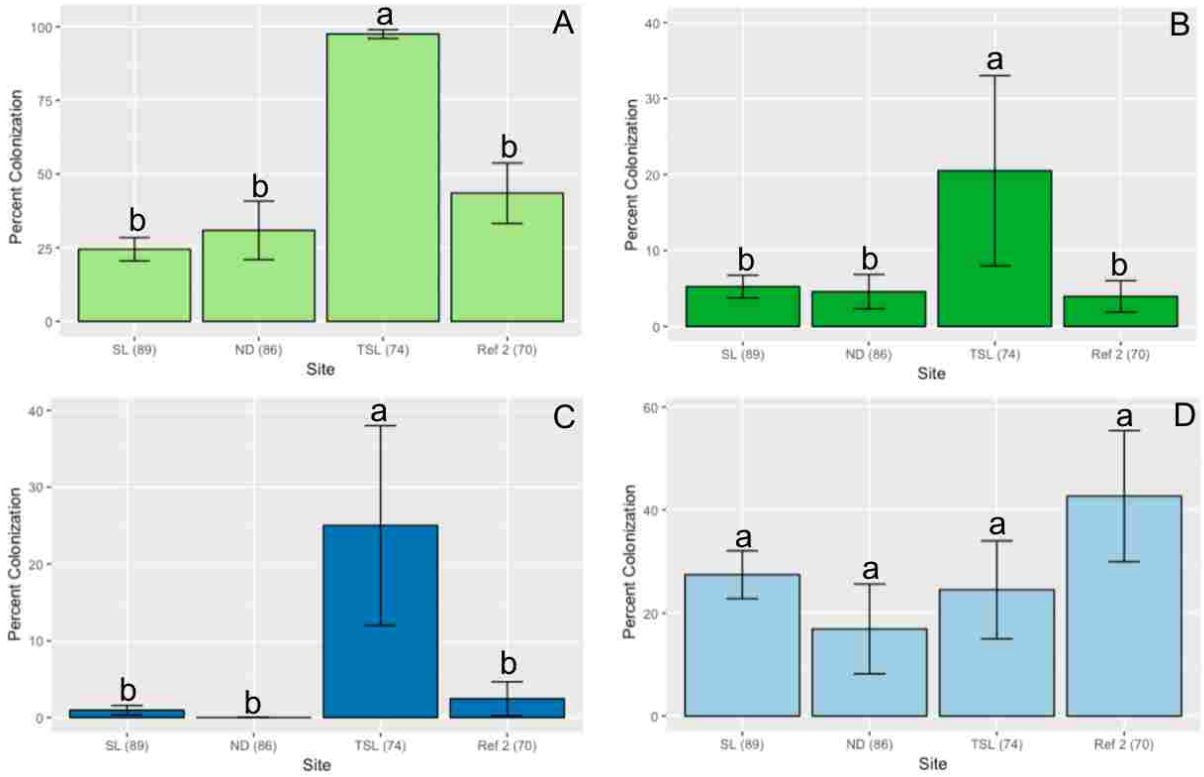

Figure 3- 10: Mycorrhizal colonization of *Calamagrostis canadensis* roots collected from Tundra Gold Mine June 2016. Roots were collected at Sandy Lake (SL), North Dam (ND), Trans Saddle Lake (TSL) and Reference 2 (Ref 2). In brackets beside the x-axis site labels is the arsenic concentration found at each site in mg/kg. Roots were cleared in 10% KOH and stained with 10% ink in acetic acid (Vierheilig 1998). Colonization was measured using the magnified intersections method at 250x magnification (Mcgonigle et al. 1990). A) Mean hyphal colonization. B) Mean arbuscular colonization. C) Mean vesicular colonization. D) Mean DSE colonization. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

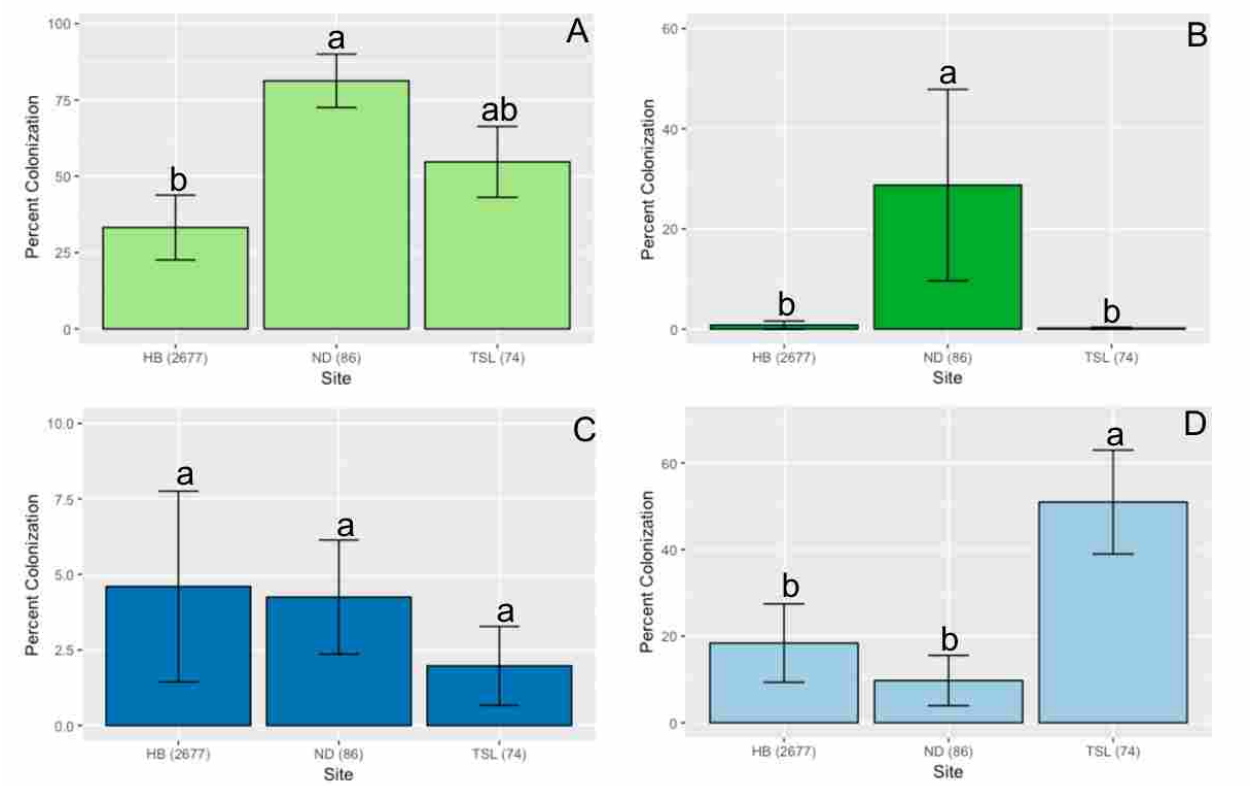

Figure 3- 11: Mycorrhizal colonization of *Calamagrostis canadensis* roots collected from Tundra Gold Mine August 2016. Roots were collected at Hambone Lake (HB), North Dam (ND), Trans Saddle Lake (TSL). In brackets beside the x-axis site labels is the arsenic concentration found at each site in mg/kg Roots were cleared in 10% KOH and stained with 10% ink in acetic acid (Vierheilig 1998). Colonization was measured using the magnified intersections method at 250x magnification (Mcgonigle et al. 1990). A) Mean hyphal colonization. B) Mean arbuscular colonization. C) Mean vesicular colonization. D) Mean DSE colonization Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

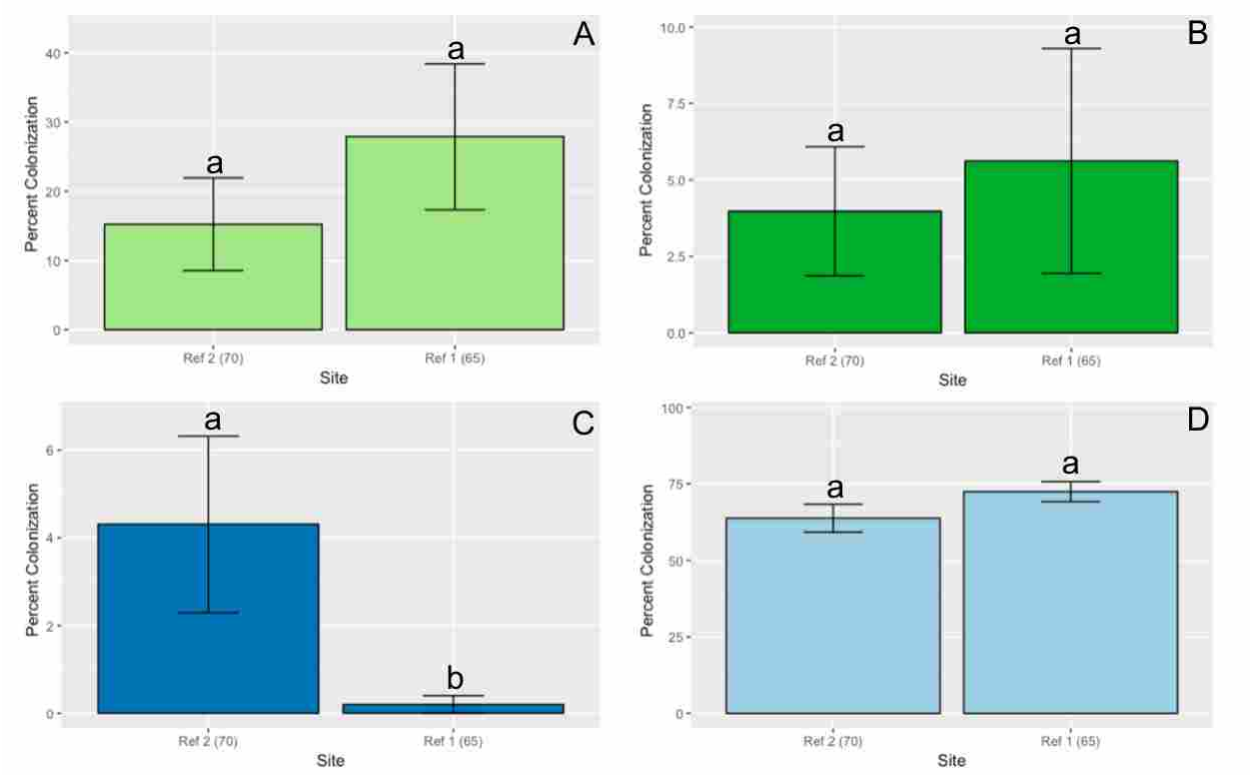

Figure 3- 12: Mycorrhizal colonization of *Calamagrostis deschampsioides* roots collected from Tundra Gold Mine June 2016. Roots were collected at Reference 2 (Ref 2) and Reference 1 (Ref 1). In brackets beside the x-axis site labels is the arsenic concentration found at each site in mg/kg. Roots were cleared in 10% KOH and stained with 10% ink in acetic acid (Vierheilig 1998). Colonization was measured using the magnified intersections method at 250x magnification (Mcgonigle et al. 1990). A) Mean hyphal colonization. B) Mean arbuscular colonization. C) Mean vesicular colonization. D) Mean DSE colonization. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

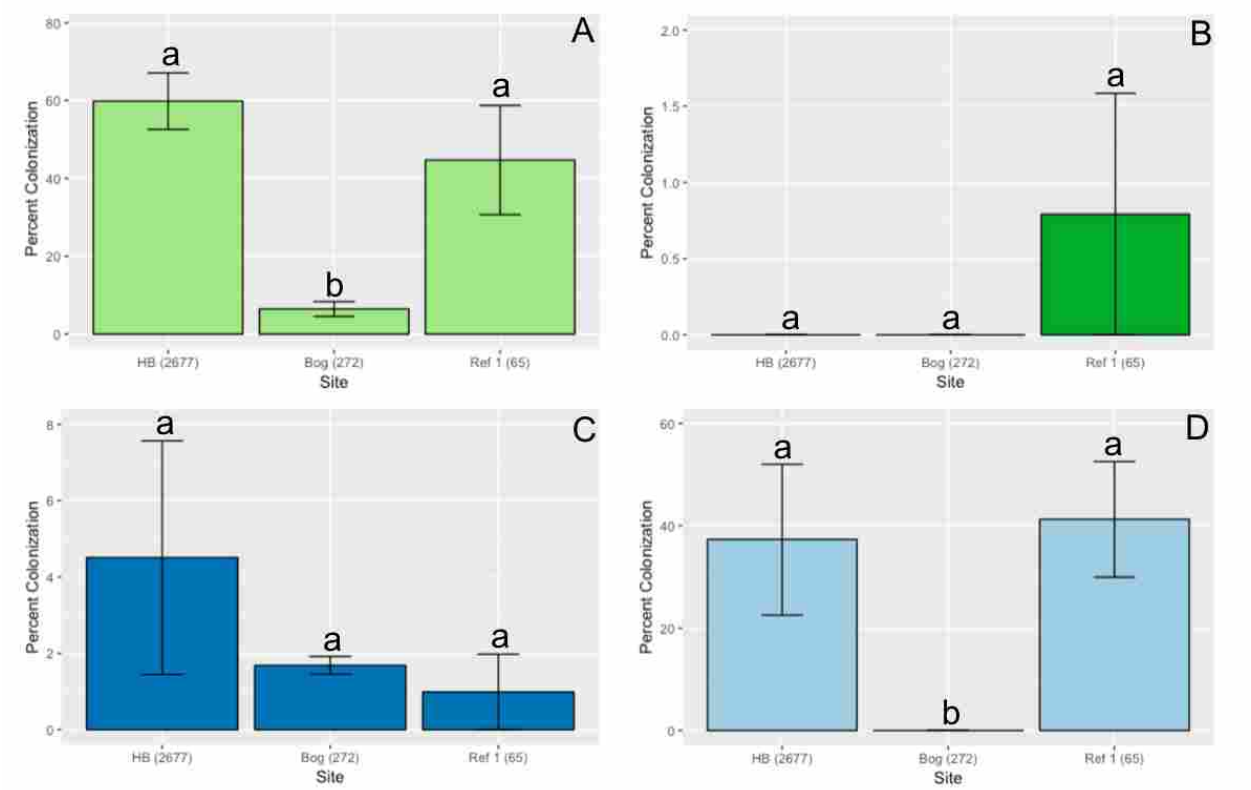

Figure 3- 13: Mycorrhizal colonization of *Epilobium angustifolium* roots collected from Tundra Gold Mine August 2016. Roots were collected at Hambone Lake (HB), the Bog and Reference 1 (Ref 1). In brackets beside the x-axis site labels is the arsenic concentration found at each site in mg/kg. Roots were cleared in 10% KOH and stained with 10% ink in acetic acid (Vierheilig 1998). Colonization was measured using the magnified intersections method at 250x magnification (Mcgonigle et al. 1990). A) Mean hyphal colonization. B) Mean arbuscular colonization. C) Mean vesicular colonization. D) Mean DSE colonization Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

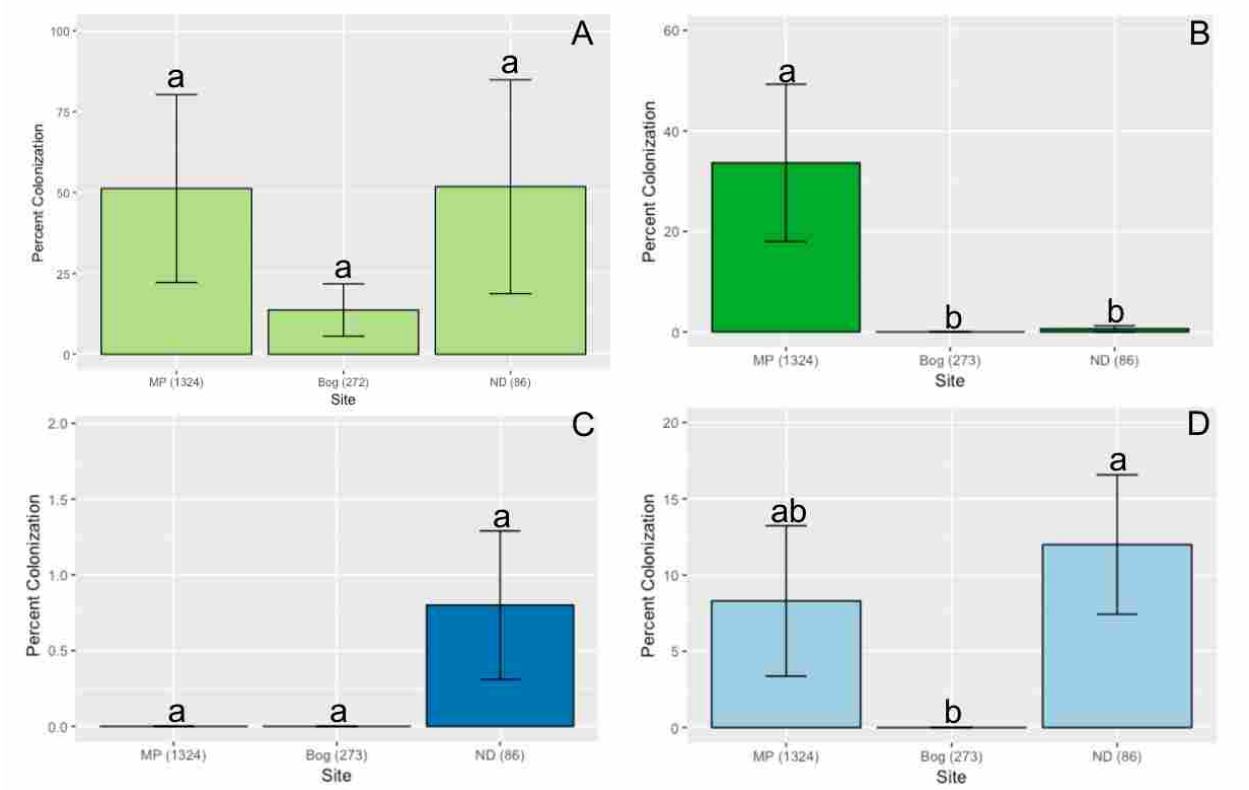

Figure 3- 14: Mycorrhizal colonization of *Epilobium palustre* roots collected from Tundra Gold Mine August 2016. Roots were collected at Mill Pond (MP), the Bog and North Dam (ND). In brackets beside the x-axis site labels is the arsenic concentration found at each site in mg/kg. Roots were cleared in 10% KOH and stained with 10% ink in acetic acid (Vierheilig 1998). Colonization was measured using the magnified intersections method at 250x magnification (Mcgonigle et al. 1990). A) Mean hyphal colonization. B) Mean arbuscular colonization. C) Mean vesicular colonization. D) Mean DSE colonization. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

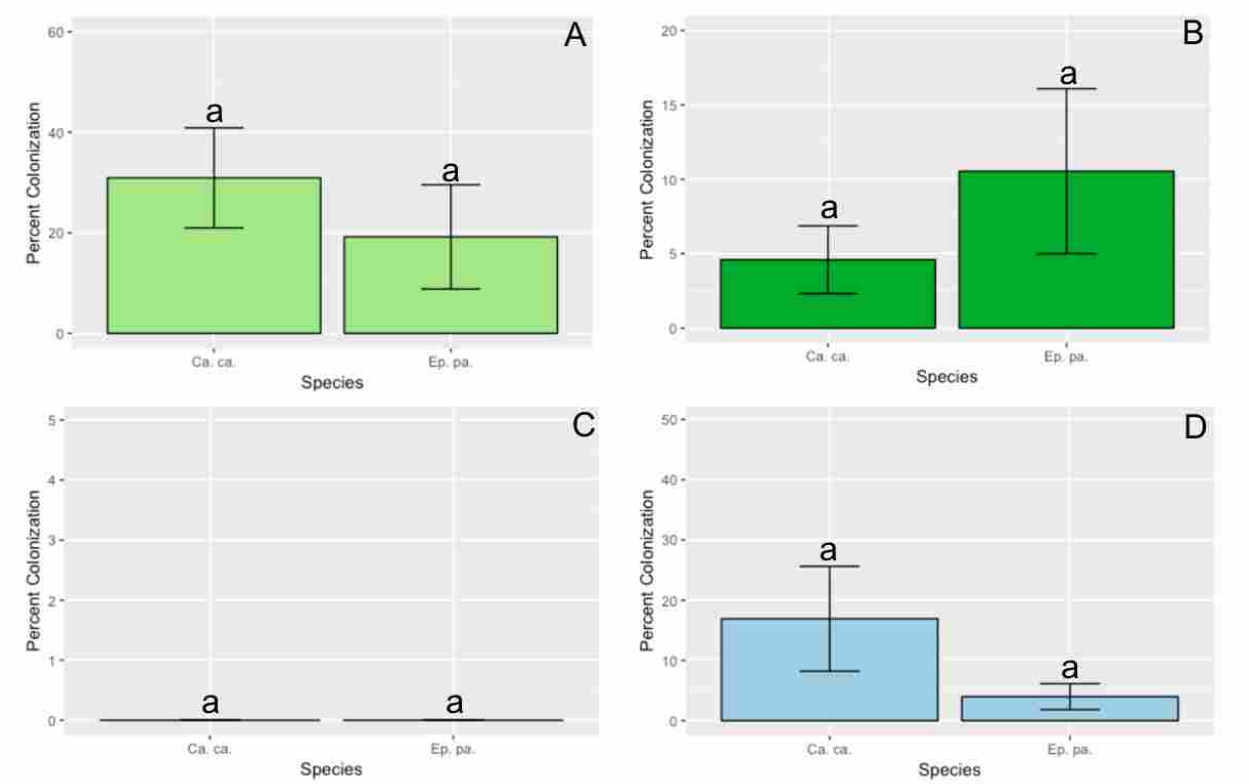

Figure 3- 15: Mycorrhizal colonization of roots collected from North Dam at Tundra Gold Mine June 2016. Species collected include *Calamagrostis canadensis* (Ca. ca) and *Epilobium palustre* (Ep. pa.). Roots were cleared in 10% KOH and stained with 10% ink in acetic acid (Vierheilig 1998). Colonization was measured using the magnified intersections method at 250x magnification (Mcgonigle et al. 1990). A) Mean hyphal colonization. B) Mean arbuscular colonization. C) Mean vesicular colonization. D) Mean DSE colonization. Plots include raw data and error bars showing standard deviation. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

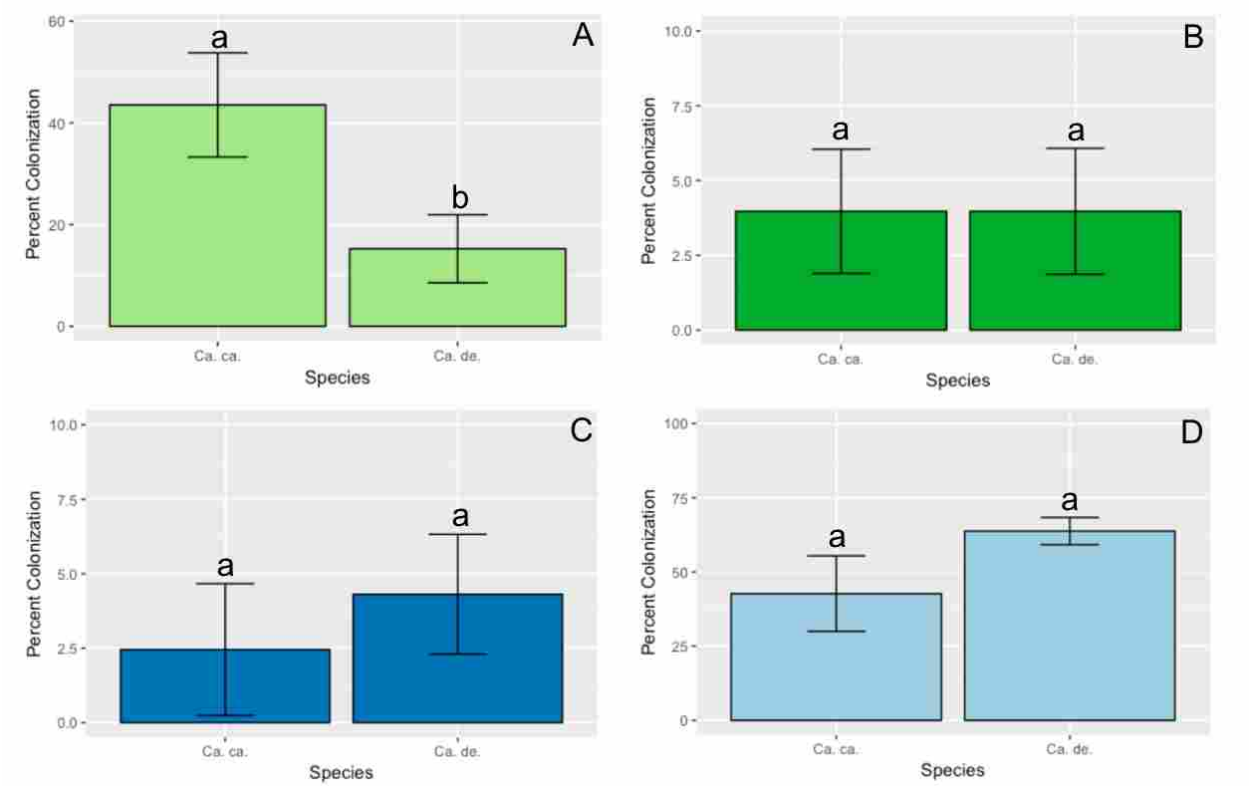

Figure 3- 16: Mycorrhizal colonization of roots collected from Reference 2 at Tundra Gold Mine June, 2016. Species collected include *Calamagrostis canadensis* (Ca. ca) and *Calamagrostis deschampsioide* (Ca. de.). Roots were cleared in 10% KOH and stained with 10% ink in acetic acid (Vierheilig 1998). Colonization was measured using the magnified intersections method at 250x magnification (Mcgonigle et al. 1990). A) Mean hyphal colonization. B) Mean arbuscular colonization. C) Mean vesicular colonization. D) Mean DSE colonization. Plots include raw data and error bars showing standard deviation. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

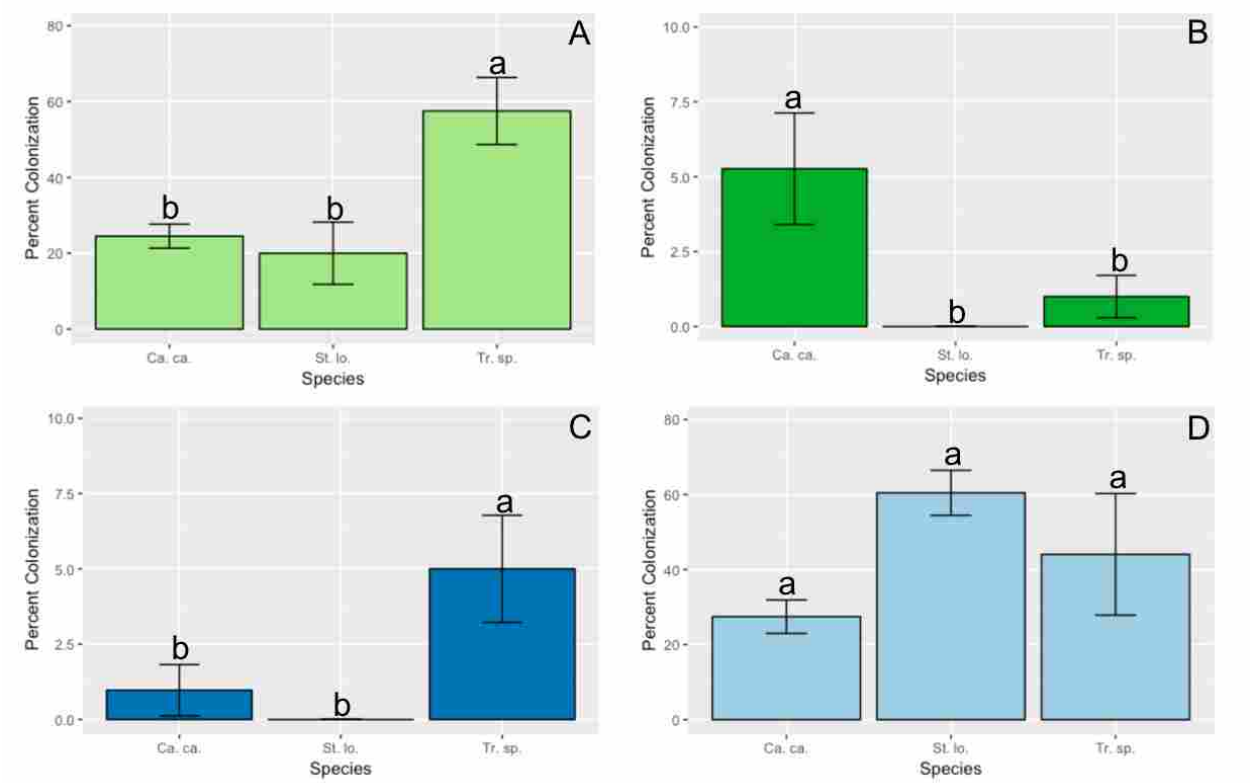

Figure 3- 17: Mycorrhizal colonization of roots collected from Sandy Lake at Tundra Gold Mine June 2016. Species collected include *Calamagrostis canadensis* (Ca. ca), *Stellaria longipes* (St. lo.) and *Trisetum spicatum* (Tr. sp.). Roots were cleared in 10% KOH and stained with 10% ink in acetic acid (Vierheilig 1998). Colonization was measured using the magnified intersections method at 250x magnification (Mcgonigle et al. 1990). A) Mean hyphal colonization. B) Mean arbuscular colonization. C) Mean vesicular colonization. D) Mean DSE colonization. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

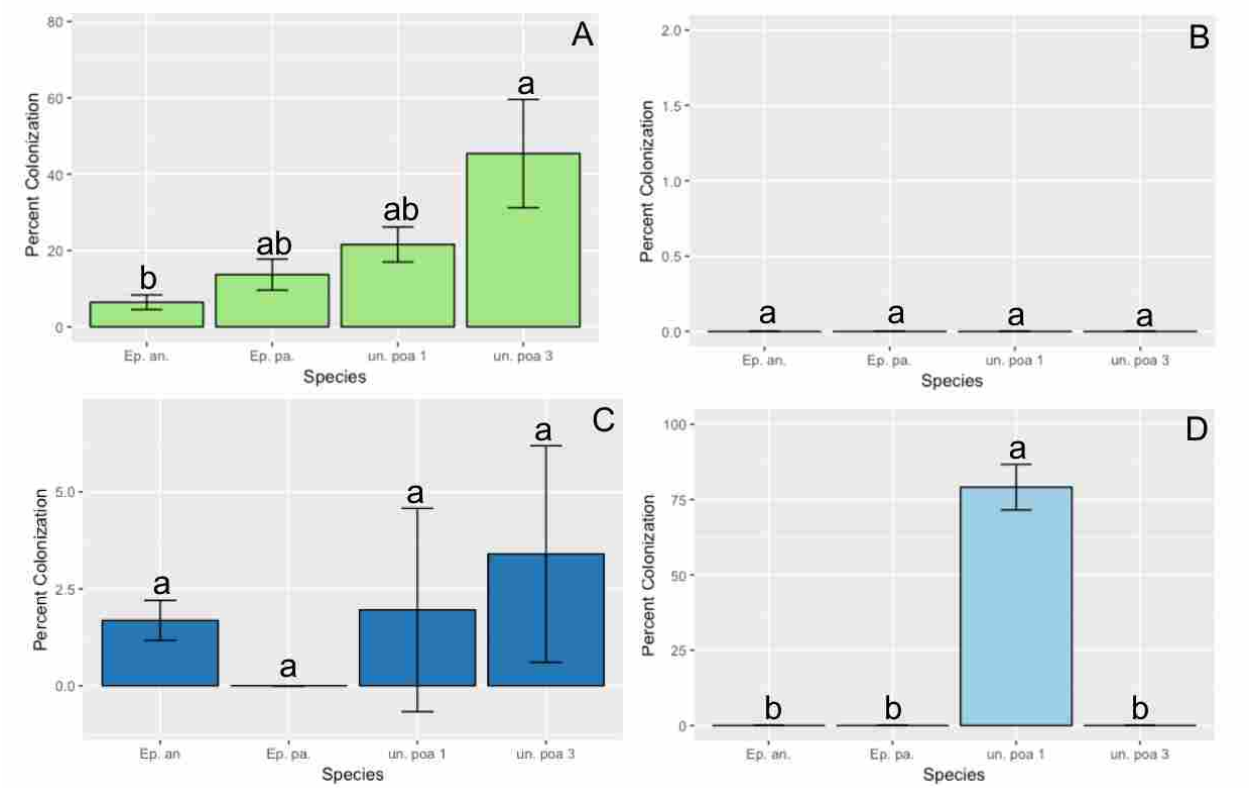

Figure 3- 18: Mycorrhizal colonization of roots collected from the Bog at Tundra Gold Mine August 2016. Species collected include *Epilobium angustifolium* (Ep. an.), *Epilobium palustre* (Ep. pa.), *Poa sp. 1* and *Poa sp. 3.* Roots were cleared in 10% KOH and stained with 10% ink in acetic acid (Vierheilig 1998). Colonization was measured using the magnified intersections method at 250x magnification (Mcgonigle et al. 1990). A) Mean hyphal colonization. B) Mean arbuscular colonization. C) Mean vesicular colonization. D) Mean DSE colonization Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

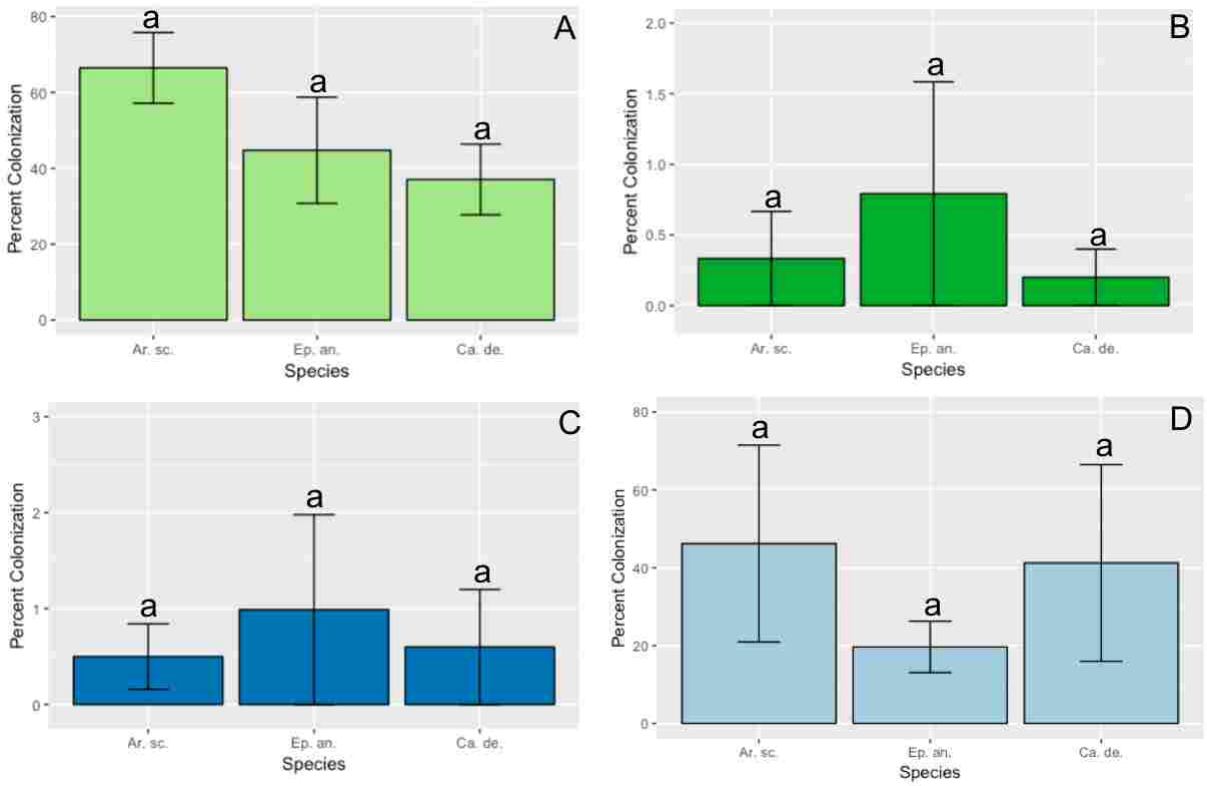

Figure 3- 19: Mycorrhizal colonization of roots collected from Reference 1 at Tundra Gold Mine August 2016. Species collected include *Agrostis scabra* (Ag. sc.), *Epilobium angustifolium* (Ep. an.) and *Calamagrostis deschampsioides.* Roots were cleared in 10% KOH and stained with 10% ink in acetic acid (Vierheilig 1998). Colonization was measured using the magnified intersections method at 250x magnification (Mcgonigle et al. 1990). A) Mean hyphal colonization. B) Mean arbuscular colonization. C) Mean vesicular colonization. D) Mean DSE colonization. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

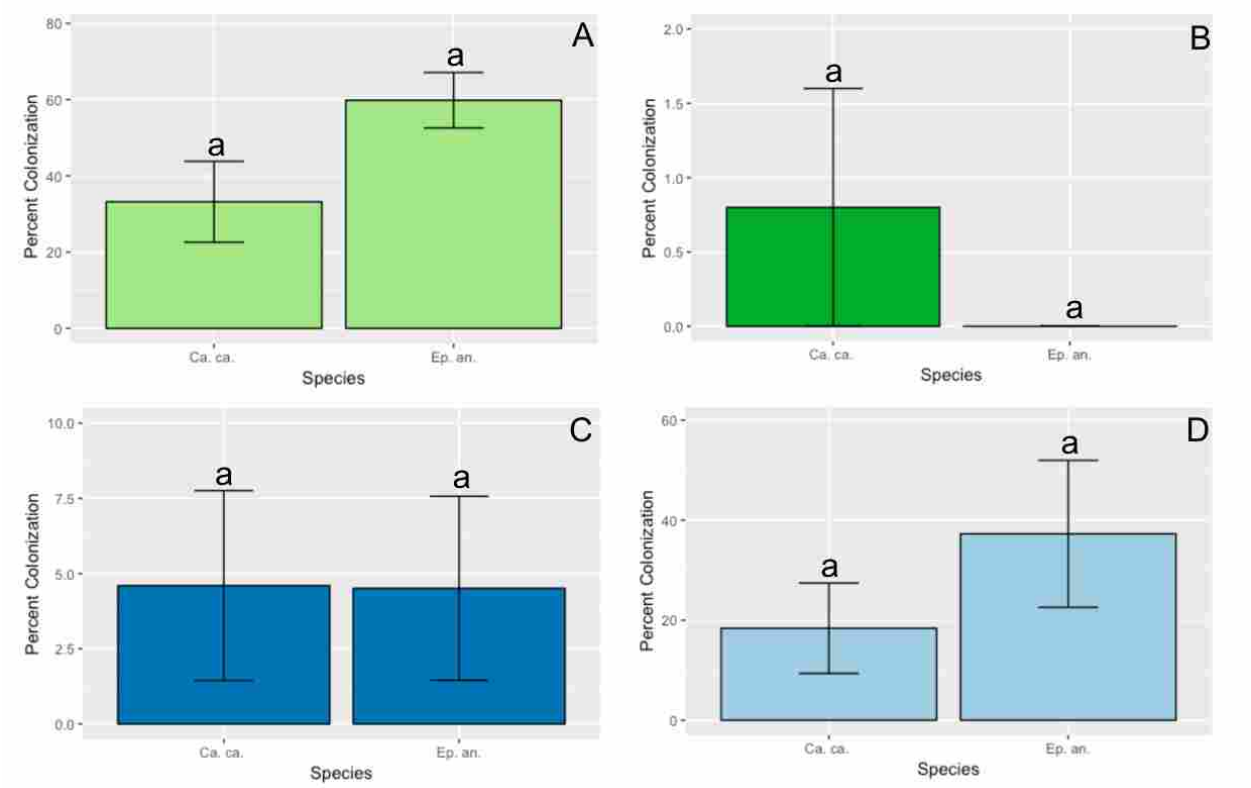

Figure 3- 20: Mycorrhizal colonization of roots collected from Hambone Lake at Tundra Gold Mine August 2016. Species collected include *Calamagrostis canadensis* (Ca. ca) and *Epilobium angustifolium* (Ep. an.). Roots were cleared in 10% KOH and stained with 10% ink in acetic acid (Vierheilig 1998). Colonization was measured using the magnified intersections method at 250x magnification (Mcgonigle et al. 1990). A) Mean hyphal colonization. B) Mean arbuscular colonization. C) Mean vesicular colonization. D) Mean DSE colonization. Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

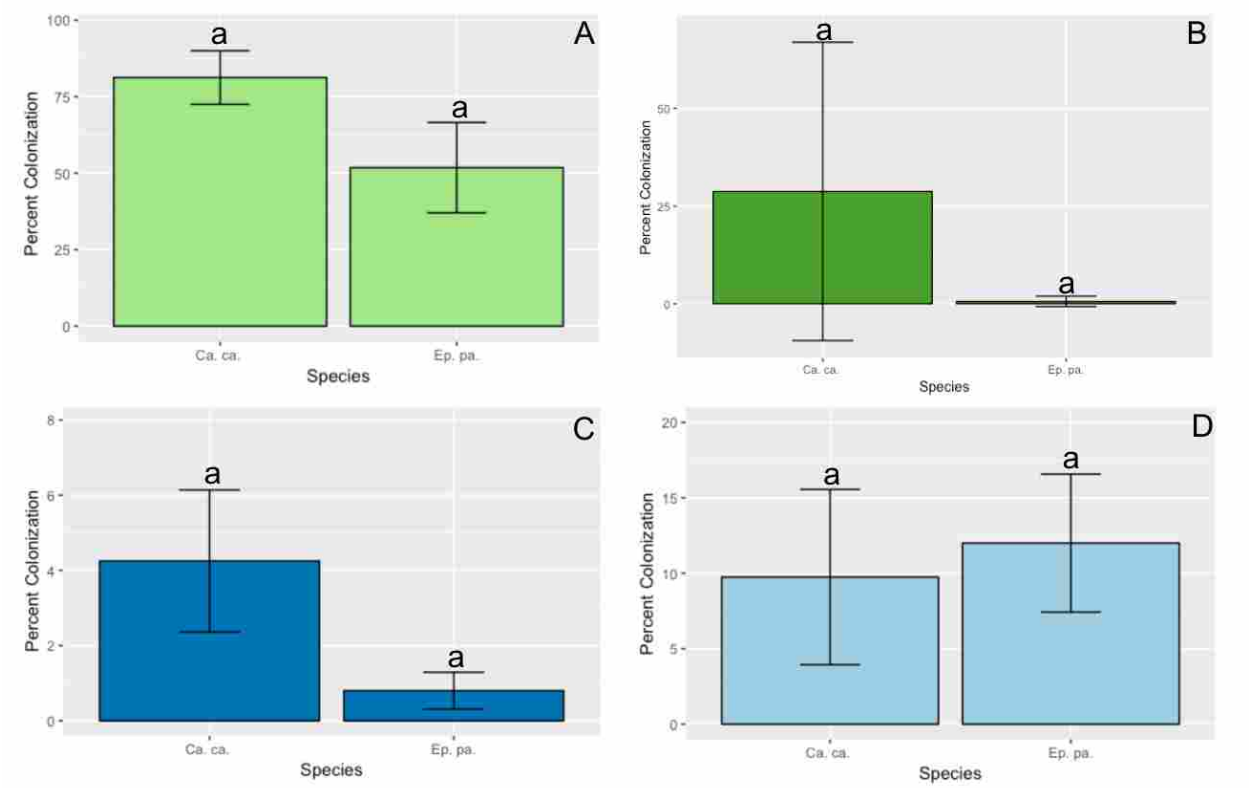

Figure 3- 21: Mycorrhizal colonization of roots collected from North Dam at Tundra Gold Mine August 2016. Species collected include *Calamagrostis canadensis* (Ca. ca) and *Epilobium palustre* (Ep. pa.). Roots were cleared in 10% KOH and stained with 10% ink in acetic acid (Vierheilig 1998). Colonization was measured using the magnified intersections method at 250x magnification (Mcgonigle et al. 1990). A) Mean hyphal colonization. B) Mean arbuscular colonization. C) Mean vesicular colonization. D) Mean DSE colonization Bars sharing same letters are from data that are not significantly different ($p < 0.05$). Means and standard error were calculated from non-transformed data.

Table 3- 1: Output from one-way ANOVA's comparing the mean hyphal colonization across sites collected at Tundra Gold Mine, 2016. Table also includes tests for the assumptions of a one-way ANOVA including a Shapiro test, to test for normality of residuals and a Levene test, to test for homogeneity of variance.

Species	Month of Collection	Transformation	Shapiro p-value	Levene p-value	Site ndf/ddf	Site F	Site Pr(>F)
Calamagrostis canadensis	June	None	0.3974	0.2787	3/18	10.16	**0.000386**
Calamagrostis canadensis	August	None	0.06737	0.8696	2/11	4.809	**0.0316**
Epilobium angustifolium	August	None	0.6902	0.0944	2/11	8.346	**0.00623**
Epilobium palustre	August	None	0.7214	0.3009	2/11	2.837	0.101

Table 3- 2: Output from one-way ANOVA's comparing the mean arbuscular colonization across sites collected at Tundra Gold Mine, 2016. Table also includes tests for the assumptions of a one-way ANOVA including a Shapiro test, to test for normality of residuals and a Levene test, to test for homogeneity of variance.

Species	Month of Collection	Transformation	Shapiro p-value	Levene p-value	Site ndf/ddf	Site F	Pr(>F)
Calamagrostis canadensis	June	None	0.3371	0.001507	3/18	3.886	**0.0265**
Calamagrostis canadensis	August	Square root	0.02747	0.005285	2/11	4.397	**0.0395**
Epilobium angustifolium	August	None	2.32E-05	0.4406	2/11	0.884	0.441
Epilobium palustre	August	Square root	0.06788	0.0104	2/11	6.387	**0.0144**

Table 3- 3: Output from one-way ANOVA's comparing the mean vesicular colonization across sites collected at Tundra Gold Mine, 2016. Table also includes tests for the assumptions of a one-way ANOVA including a Shapiro test, to test for normality of residuals and a Levene test, to test for homogeneity of variance.

Species	Month of Collection	Transformation	Shapiro p-value	Levene p-value	Site ndf/ddf	Site F	Pr(>F)
Calamagrostis canadensis	June	Square root	0.00624	0.3109	3/18	11.3	**0.000212**
Calamagrostis canadensis	August	None	0.05576	0.6311	2/11	0.396	0.682
Epilobium angustifolium	August	Square root	0.01043	0.371	2/11	1.801	0.211
Epilobium palustre	August	None	0.001684	0.1406	2/11	2.357	0.141

Table 3- 4: Output from one-way ANOVA's comparing the mean DSE colonization across sites collected at Tundra Gold Mine, 2016. Table also includes tests for the assumptions of a one-way ANOVA including a Shapiro test, to test for normality of residuals and a Levene test, to test for homogeneity of variance.

Species	Month of Collection	Transformation	Shapiro p-value	Levene p-value	Site ndf/ddf	Site F	Pr(>F)
Calamagrostis canadensis	June	None	0.8768	0.5174	3/18	1.5	0.248
Calamagrostis canadensis	August	None	0.04576	0.6312	2/11	4.951	**0.0293**
Epilobium angustifolium	August	None	0.01637	0.1384	2/11	5.392	**0.0233**
Epilobium palustre	August	Square root	0.1636	0.124	2/11	4.45	0.0384

Table 3- 5: Output from a t-test comparing the mean hyphal colonization in *Calamagrostis deschampsioides* across sites at Tundra Gold Mine, 2016. Table also includes tests for the assumptions of a t-test including a Shapiro test, to test for normality of residuals and a Bartlett test, to test variance.

Species	Month of Collection	Transformation	Shapiro p-value	Bartlett p-value	df	t	Pr(>F)
Calamagrostis deschampsioide	June	None	Ref 1= 0.4704 Ref 2= 0.576	0.401	8	1.0122	0.3411

s							

Table 3- 6: Output from a t-test comparing the mean arbuscular colonization in *Calamagrostis deschampsioides* across sites at Tundra Gold Mine, 2016. Table also includes tests for the assumptions of a t-test including a Shapiro test, to test for normality of residuals and a Bartlett test, to test variance.

Species	Month of Collection	Transformation	Shapiro p-value	Bartlett p-value	df	t	Pr(>F)
Calamagrostis deschampsioides	June	None	Ref 1= 0.0653 Ref 2= 0.1414	0.3054	8	0.38933	0.7072

Table 3- 7: Output from a t-test comparing the mean vesicular colonization in *Calamagrostis deschampsioides* across sites at Tundra Gold Mine, 2016. Table also includes tests for the assumptions of a t-test including a Shapiro test, to test for normality of residuals and a Bartlett test, to test variance.

Species	Month of Collection	Transformation	Shapiro p-value	Bartlett p-value	df	t	Pr(>F)
Calamagrostis deschampsioides	June	None	Ref 1= 0.000131 Ref 2= 0.9784	0.06359	8	-2.4796	**0.03813**

Table 3- 8: Output from a t-test comparing the mean DSE colonization in *Calamagrostis deschampsioides* across sites at Tundra Gold Mine, 2016. Table also includes tests for the assumptions of a t-test including a Shapiro test, to test for normality of residuals and a Bartlett test, to test variance.

Species	Month of Collection	Transformation	Shapiro p-value	Bartlett p-value	df	t	Pr(>F)
Calamagrostis deschampsioides	June	None	Ref 1= 0.6745 Ref 2= 0.4055	0.5318	8	1.5417	0.1617

Table 3- 9: Output from a t-test comparing the mean hyphal colonization across sites at Tundra Gold Mine, 2016. Species include *Calamagrostis canadensis* (Ca. ca.), *Epilobium palustre* (Ep. pa.) and *Epilobium angustifolium* (Ep. an.). Table also includes tests for the assumptions of a t-test including a Shapiro test, to test for normality of residuals and a Bartlett test, to test variance.

Site	Month of Collection	Transformation	Shapiro p-value	Bartlett p-value	df	t	Pr(>F)
North Dam	June	None	Ca. ca. = 0.546 Ep. pa. =0.1654	0.9378	8	0.81634	0.4379
Reference 2	June	None	Ca. ca. = 0.6447 Ca. de. = 0.576	0.4287	8	2.3117	**0.04956**
Hambone Lake	August	None	Ca. ca. = 0.2714 Ep. an. = 0.8034	0.4162	7	-1.9558	0.09139
North Dam	August	None	Ca. ca. = 0.8941 Ep. pa. = 0.1344	0.3002	7	-1.5983	0.154

Table 3- 10: Output from a t-test comparing the mean arbuscular colonization across sites at Tundra Gold Mine, 2016. Species include *Calamagrostis canadensis* (Ca. ca.), *Epilobium palustre* (Ep. pa.) and *Epilobium angustifolium* (Ep. an.). Table also includes tests for the assumptions of a t-test including a Shapiro test, to test for normality of residuals and a Bartlett test, to test variance.

Site	Month of Collection	Transformation	Shapiro p-value	Bartlett p-value	df	t	Pr(>F)
North Dam	June	None	Ca. ca. = 0.4368 Ep. pa. =0.1089	0.1126	8	-0.99293	0.3498
Reference 2	June	None	Ca. ca. = 0.1483 Ca. de. = 0.1414	0.981	8	-0.0006767	0.9995
Hambone Lake	August	None	Ca. ca. = 0.0001 Ep. an. = NA	< 2.2e-16	7	0.88192	0.4071

North Dam	August	None	Ca. ca. = 0.2261 Ep. pa. = 0.0001	1.985e-05	3.0059	1.4723	0.2372

Table 3- 11: Output from a t-test comparing the mean vesicular colonization across sites at Tundra Gold Mine, 2016. Species include *Calamagrostis canadensis* (Ca. ca.), *Epilobium palustre* (Ep. pa.) and *Epilobium angustifolium* (Ep. an.). Table also includes tests for the assumptions of a t-test including a Shapiro test, to test for normality of residuals and a Bartlett test, to test variance. NA indicates that colonization was not found.

Site	Month of Collection	Transformation	Shapiro p-value	Bartlett p-value	df	t	Pr(>F)
North Dam	June	NA	NA	NA	NA	NA	NA
Reference 2	June	None	Ca. ca. = 0.0009 Ca. de. = 0.5534	0.8585	8	-0.62029	0.5523
Hambone Lake	August	None	Ca. ca. =0.03863 Ep. an. =0.0786	0.8064	7	0.02002	0.9846
North Dam	August	None	Ca. ca. = 0.976 Ep. pa. = 0.0065	0.00647	3.4062	1.7692	0.1639

Table 3- 12: Output from a t-test comparing the mean DSE colonization across sites at Tundra Gold Mine, 2016. Species include *Calamagrostis canadensis* (Ca. ca.), *Epilobium palustre* (Ep. pa.) and *Epilobium angustifolium* (Ep. an.). Table also includes tests for the assumptions of a t-test including a Shapiro test, to test for normality of residuals and a Bartlett test, to test variance.

Site	Month of Collection	Transformation	Shapiro p-value	Bartlett p-value	df	t	Pr(>F)
North Dam	June	None	Ca. ca. =0.1466 Ep. pa =0.1751	0.01966	4.4854	1.4417	0.2154

Reference 2	June	None	Ca. ca. = 0.7974 Ca. de. = 0.0406	0.07351	8	-1.5583	0.1578
Hambone Lake	August	None	Ca. ca. =0.08944 Ep. an. = 0.6864	0.5153	7	-1.1448	0.2899
North Dam	August	None	Ca. ca. = 0.4068 Ep. pa. = 0.123	0.8244	7	-0.30938	0.766

Table 3- 13: Output from one-way ANOVA's comparing the mean hyphal colonization across species collected at Tundra Gold Mine, 2016. Table also includes tests for the assumptions of a one-way ANOVA including a Shapiro test, to test for normality of residuals and a Levene test, to test for homogeneity of variance.

Species	Month of Collection	Transformation	Shapiro p-value	Levene p-value	Species ndf/ddf	Species F	Pr(>F)
Sandy Lake	June	None	0.9087	0.6004	2/11	7.983	**0.00721**
Bog	August	Square Root	0.9551	0.1062	3/14	5.475	**0.0106**
Reference 1	August	Square Root	0.7871	0.6327	3/13	2.046	0.169

Table 3- 14: Output from one-way ANOVA's comparing the mean arbuscular colonization across species collected at Tundra Gold Mine, 2016. Table also includes tests for the assumptions of a one-way ANOVA including a Shapiro test, to test for normality of residuals and a Levene test, to test for homogeneity of variance. NA indicates that colonization was not found.

Species	Month of Collection	Transformation	Shapiro p-value	Levene p-value	Species ndf/ddf	Species F	Pr(>F)
Sandy Lake	June	Square Root	0.315	0.00976	2/11	5.807	**0.00275**
Bog	August	NA	NA	NA	NA	NA	NA
Reference 1	August	Square Root	0.0001026	0.6911	2/13	0.38	0.691

Table 3- 15: Output from one-way ANOVA's comparing the mean vesicular colonization across species collected at Tundra Gold Mine, 2016. Table also includes tests for the assumptions of a one-way ANOVA including a Shapiro test, to test for normality of residuals and a Levene test, to test for homogeneity of variance.

Species	Month of Collection	Transformation	Shapiro p-value	Levene p-value	Species ndf/ddf	Species F	Pr(>F)
Sandy Lake	August	Square Root	0.2302	0.3193	2/11	5.571	**0.0213**
Bog	August	None	0.3351	0.09589	3/14	2.283	0.124
Reference 1	August	Square Root	0.0001258	0.8608	2/13	0.152	0.861

Table 3- 16: Output from one-way ANOVA's comparing the mean DSE colonization across species collected at Tundra Gold Mine, 2016. Table also includes tests for the assumptions of a one-way ANOVA including a Shapiro test, to test for normality of residuals and a Levene test, to test for homogeneity of variance.

Species	Month of Collection	Transformation	Shapiro p-value	Levene p-value	Species ndf/ddf	Species F	Pr(>F)
Sandy Lake	June	None	0.7743	0.1068	2/11	3.51	0.0662
Bog	August	Square Root	2.778e-05	2.456e-05	3/14	535.1	**1.13e-14**
Reference 1	August	Square Root	0.6816	0.144	2/13	2.298	0.14

Chapter 4 – General Discussion

Discussion

The purpose of this study was to get a better understanding of how gold mining, and more specifically how arsenic, a contaminant released as a by-product of gold mining. can impact plant communities and mycorrhizal colonization in northern plant species. Since there is a concern that vegetation around mines is being consumed by wildlife, this study also assesses arsenic accumulation in northern plant species. Currently, there are 38 abandoned gold mine sites in the Northwest Territories, not including sites that are inactive and with an identifiable owner (National Orphaned/Abandoned Mine Initiative 2004). Many of those sites are awaiting or in the process of reclamation and remediation (Indian and Northern Affairs Canada 2009). Because arsenic is released as a byproduct of gold extraction from arsenopyrite ore, arsenic is a major contaminant of concern at gold mine sites (Rheaume and Caron-Vuotari 2013). The effects of arsenic on mycorrhizal colonization and plant communities have been well studied using temperate species in a temperate environment, but research using northern species in a northern environment is limited.

In this study plant samples were collected from five sites that were close to a contaminated tailings area and the original milling area and two reference sites further away, and analyzed for arsenic concentration. Mean arsenic concentration was compared in one species at a time, across the seven sites to see if there was a higher accumulation of arsenic in plants the closer to the original milling area/main contamination point. Mean arsenic concentration was also compared across species at each site to see if certain species accumulated more arsenic than others. Finally, a vegetation survey was conducted at eight sites, six of the sites overlapped with the sites at which the plants were collected plus two additional reference sites. The two additional reference sites were not accessible during the time of plant collection.

Arsenic concentrations were highest in soils collected from Hambone Lake and Mill Pond, sites adjacent to the tailings containment area, which confirms the first hypothesis that arsenic concentrations will be higher in sites adjacent to the original milling area or tailings containment area since those were the main sources of arsenic contamination. A geotechnical survey conducted in 2004 showed that the dams surrounding tailings were degraded, causing a leak into Hambone Lake and Mill Pond (Staples 2005). The survey showed that arsenic was also introduced into those sites through the dams themselves since they were built from waste rock which was contributing to metal leaching. The vegetated sites with the lowest arsenic concentrations were Reference 3, followed by Reference 1 indicating that they are suitable reference sites for this study. The Quarry had the lowest arsenic concentration among the non-vegetated sites, which is of no surprise since the Quarry was not part of the previous tailings area and was only used to excavate rock material. The arsenic concentration in soils collected from non-vegetated sites Upper Pond and Lower Pond were higher than in the Quarry indicating that not all the arsenic was removed during remediation efforts.

The arsenic concentration was higher in plants collected from Mill Pond and Hambone Lake, suggesting that the mine may likely be the cause of arsenic accumulation and confirming my second hypothesis that arsenic accumulation will be greater in plants collected from sites with higher soil arsenic concentrations. The highest arsenic concentrations were consistently found in plants collected at Mill Pond, which has the second highest total arsenic concentration compared to Hambone Lake. The decreased arsenic concentration in Hambone Lake compared to Mill Pond could have been caused by site-specific conditions that decreased arsenic uptake in plants. For example, an increase in phosphorus concentration has been shown to inhibit arsenic

uptake in the Chinese brake fern (Tu and Ma 2003). Hambone Lake also has the highest concentration of phosphorus, which could be decreasing the arsenic uptake in plants.

In this study *Carex aquatilis* showed the highest potential for use in phytoremediation since it could accumulate and tolerate the highest arsenic concentrations. An arsenic hyperaccumulator is classified as a plant that can accumulate 1000 mg/kg of arsenic (Baker and Brooks 1989). The highest concentration of arsenic was found in *Carex aquatilis* and was 870 ± 230 mg/kg and is within that range. Although there is not much research on arsenic accumulation and tolerance in *Carex aquatilis*, one study on arsenic in geothermal watersheds of Yellowstone National Park, USA showed that it was present at an arsenic contaminated site with 170 ± 43 μg/kg bioavailable arsenic (Kocar et al. 2004). The arsenic concentration in *Carex aquatilis* at that site was still much lower than reported in this study, with a mean concentration of 2.5 ± 1.0 mg/kg. *Carex rostrata*, another wetland sedge that is native to northern Canada, has also been shown to survive on contaminated tailings containing 151 ± 8.1 mg/kg of arsenic (Stoltz and Greger 2002). Similar to the results in this study, the arsenic concentration was higher in the roots than shoots (26.9 ± 4.2 mg/kg and 5.7 ± 1.1 mg/kg respectively). Further studies should be conducted where more samples are analyzed to determine if *Carex aquatilis* is a hyperaccumulator. *Carex aquatilis* also has the potential to be a good candidate for phytoremediation in northern environments because it is a native species that is acclimatized to the harsh northern climate and because it spreads quickly through vegetative rhizomes, which reduces the amount of seeds or propagules needed for revegetation (United States Department of Agriculture and Natural Resources Conservation Service 2011). Since *Carex aquatilis* accumulated higher levels of arsenic in the roots than shoots, it may be more useful in phytostabilization, to reduce mobilization of a contaminant through the accumulation in roots or

immobilization in the rhizosphere. *Carex aquatilis* could, therefore, be used on contaminated tailings if the area was fenced since arsenic concentration exceeds the maximum contaminant level for human consumption (3.5 mg/kg in fish) and animal feed (8 mg/kg) (Health Canada 2018; Canadian Food Inspection Agency 2017). The use of *Carex aquatilis* should therefore be avoided in areas where herbivorous animals are expected to frequently visit.

At the high arsenic sites (Mill Pond and Hambone Lake), the above ground portion of all plants collected (*Betula glandulosa, Salix athabascensis, Carex aquatilis, Vaccinium uliginosum* and *Equisetum arvense*) exceeded the maximum contaminant level for human consumption and animal feed as well. As mentioned previously, the goal of reclamation of mine sites in the Northwest Territories is to "to return the mine and affected areas to viable and, wherever practicable, self-sustaining ecosystems that are compatible with a healthy environment and with human activities" (MVLWB and AANDC 2013). To ensure that the sites are compatible with a healthy environment further studies should be conducted to find if these plants are being regularly consumed and if they are impacting local wildlife.

Based on plant species abundance in the non-metric multidimensional scaling, sites separated into two groups along the first axis. Soil arsenic concentrations had the strongest association with the first axis indicating that arsenic was correlated with the species abundance of sites found higher on the first axis, which include sites that were near the original milling area and tailings containment area (Mill Pond (MP), Hambone Lake (HB), North Dam (ND) and Trans Saddle Lake (TSL). This confirms the third hypothesis that plant communities will differ in sites adjacent to the tailings area when compared to reference sites further away, and that those differences would be correlated to arsenic concentration in soils. These results are in agreement with Galbraith et al. (1995), who showed that elevated arsenic concentrations caused

by a smelter was correlated with loss of vegetation cover and diversity in plant communities. A similar study conducted by Espinosa-Reyes et al. (2014) also showed that sites close to a mine had high soil arsenic, lower plant diversity and a change in community composition when compared to un-impacted sites further from the mine.

Carex aquatilis and *Salix athabascensis* are two species that are also associated with high arsenic concentrations. Both species are typically present during the early successional stages of vegetation, such as those caused by recent disturbances (Densmore 2000); therefore, it is possible that the disturbance from arsenic contamination caused the sites near the mine to revert to an early successional stage. These species would be ideal to use in the early stages of revegetation of mine sites since they are capable of growing in disturbed areas.

The second group, which was negative on the first axis and, therefore, negatively correlated with arsenic, included reference sites Sandy Lake (SL), Reference 1 (Ref 1), Reference 2 (Ref 2), and Reference 3 (Ref 3). Another noteworthy point is that ericaceous plants that are usually the dominant species found in the subarctic were clustered near the reference sites (Read 1983; Read 1991). These species include *Rubus chamaemorus*, *Vaccinium uliginosum*, *Ledum decumbens*, *Empetrum nigrum*, *Andromeda polifolia*, *Oxycoccus microcarpus* and *Acrtostaphylos aplina.* The presence of ericaceous species is indicative of a late successional stage in northern ecosystems, showing that reference sites are likely stable communities in equilibrium with environmental conditions, which have not experienced recent disturbances (Densmore et al. 2000; Sprugel 1991). Identifying plant communities at less disturbed sites, enables land managers to create a goal for the end stages of revegetation. Knowing that the ericaceous plants are more abundant in the reference sites, part of the

reclamation plan could be to facilitate the succession of plant to an ericaceous community by transplanting seedlings.

To assess impacts of gold mining on mycorrhizae, AM plants were collected in June at four sites that were adjacent to the contaminated tailings and original milling area and at three reference sites further away, and collected again in August at five sites adjacent to the contaminated tailings and original milling area and two reference sites further away (Table A-16 and A-17). Mycorrhizal and DSE colonization was found at all sites sampled, indicating that mycorrhizae are part of the ecosystem at Tundra Mine. In a study conducted 200km south of Tundra Mine, MacColl (2017) also found colonization in plants collected along Baker Creek. In this study, mycorrhizal colonization was found in *Epilobium palustre* and based on a literature search, has not been documented before.

Reductions in spore germination and root colonization caused by arsenic have been observed at concentrations ranging from 2.5 mg/kg to 50 mg/kg and 25 mg/kg to 150 mg/kg respectively (Wu et al. 2009; Gonzalez-Chavez et al. 2002; Yu et al. 2009; Garg et al. 2012; Liu et al. 2005). Although research on impacts of arsenic on DSE is limited, one study showed that colonization is reduced at 83 mg/kg of arsenic (Orlowska et al. 2011). Based on the aforementioned studies, I would have expected a decrease in mycorrhizal and DSE colonization in plants collected from Hambone Lake and Mill Pond since those sites had arsenic concentrations exceeding those shown to decrease germination or colonization. When comparing percent colonization across sites, there were no clear trends of mycorrhizal colonization based on proximity to the original tailings area or arsenic concentration in the soil leading to the rejection of the fourth hypothesis that mycorrhizal colonization will be lower in areas more proximal to the gold mining activities and that the mycorrhizal colonization will be related to arsenic. Several

studies have shown that mycorrhizae can acquire a tolerance to mining contaminants. Wu et al. (2009) compared the germination of metal-contaminated versus uncontaminated isolate of *Glomus mossae* and found that the metal-contaminated isolate had a significantly higher germination rate than all uncontaminated isolates in 2.5mg/kg of arsenic in soil. In a similar study done by Gonzalez-Chavez et al. (2002), where they extracted spores of *Glomus caledonium* and *Glomus mossae* from mine soil contaminated with arsenic, they found that spores of *Glomus caledonium* exhibited an increased germination and *Glomus mossae* showed no difference in germination when exposed to 250 mg/kg of arsenic. In the study, the germination rates of spores that were nonmine isolates were significantly lower than mine isolates when exposed to the same 250 mg/kg arsenic concentration.

There were differences in mycorrhizal colonization between species in two of the sites sampled. One reason for the lack of detectable difference in the remaining five sites is partially due to the high variance in colonization in the sample replicates. To get a better understanding of the differences in colonization that are attributed to host species, a lab experiment could be used where more replicates are included to reduce variance.

Calamagrostis canadensis, *Epilobium angustifolium*, and *Epilobium palustre* were all colonized with AMF when collected from the high arsenic sites. If the site managers wish to revegetate the tailings containment area as part of the final stages of reclamation, or are concerned that the tailings could leach into the non-vegetated areas, they could isolate the AMF from those plants and inoculate the soil to facilitate the symbiosis of AMF and newly developing plants. Since the AMF from those sites originated from soils that contained high levels of arsenic, they may perform better non-native AMF because they are acclimated to those conditions. It has been shown that a combination of native mycorrhiza extracted from tailings

combined with native plants had a higher plant biomass when planted in contaminated soil than non-native mycorrhiza and non-native plant combination (Orlowska et al. 2011). The use of native AMF in this case may be a better choice for reclamation since they are adapted to the harsh soil conditions and climate (Sylvia and Williams 1992; Weissenhorn et al. 1993; Orlowska et al. 2005).

This study has shown that high levels of arsenic can accumulate in Northern plant species. This information is useful because it identifies potential hazards to wildlife and nearby communities. All plants collected at the high arsenic sites contained arsenic concentrations that exceed the maximum contaminant level for human consumption (3.5 mg/kg in fish) and animal feed (8 mg/kg), showing that at high enough concentrations, arsenic is mobilized from the soil and into all plants sampled, thereby, increasing the chances of arsenic consumption by wildlife (Health Canada 2018; Canadian Food Inspection Agency 2017). Identifying arsenic accumulators can also help guide reclamation efforts. For example, if the main goal is to contain arsenic contamination, and the chances of herbivory are low, then *Carex aquatilis* can be used in the revegetation of arsenic-contaminated areas. If consumption by wildlife is a concern, then it could be avoided. By showing that mining can impact northern plant communities in areas adjacent to a tailings containment area, original milling area, and even adjacent to an uncontaminated quarry, it is possible to get a clearer understanding of the level of disturbance associated with mining. Plants provide many ecosystem services such as nutrient and water cycling and protection of permafrost; these services may be altered if plant communities are impacted (Schimel 1995; Gruber and Galloway 2008; Oki and Kanae 2006; Rocha and Shaver 2011). By identifying disturbed and undisturbed plant communities it also sheds light on what type of vegetation may be able to grow in the disturbed unvegetated areas, as well as provides an

end goal type as to the type of vegetation that should be growing once the reclamation process is considered over. Several studies have shown the benefits of AMF in revegetation (van der Heijden et al. 1998; Orlowska et al. 2011; Ahmed et al. 2006; Xia et al. 2007).

Northern climate is characterized by low soil nutrients, a cool and short growing season and high winds, making it a hostile environment for plants (Government of Northwest Territories 2009; Government of Northwest Territories 2012). This study has shown that arbuscular mycorrhizae are part of the Northern ecosystem and can be used as a tool in revegetation strategies. If high arsenic soils are scheduled for revegetation, then native mycorrhiza, extracted from high arsenic sites could potentially be used. AMF can also help decrease arsenic uptake in plants, which is beneficial since colonization would prevent arsenic from entering higher trophic levels (Ahmed et al. 2006). Because AMF provides plants with nutrients, it would also decrease the need for fertilizer, which can be expensive and harmful to the environment. It is especially important to reduce environmental impact because northern ecosystems recover from impacts more slowly.

Integrative Biology

This work is integrative in nature because it is the field of ecology, which studies the relations of organisms to one another and their physical environment. In this study, I examined the relationship between plant distribution and arsenic uptake in plants and their relation to soil chemistry. These fields require knowledge and application of plant toxicology, and physiology as well as the field of chemistry. To assess the distribution of plants themselves it required a knowledge of plant anatomy and floral structures. In the second part of this study I assessed the impacts of mining on mycorrhizal colonization, which is considered work in mycology. In order to analyze the data collected in this study, statistical analysis was used which is in the field of

mathematics. Finally, to apply the information gathered in this study to help guide mine reclamation efforts in Northern Canada, requires background knowledge on the mining industry, as well as current reclamation and revegetation techniques used in environmental engineering.

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Appendix

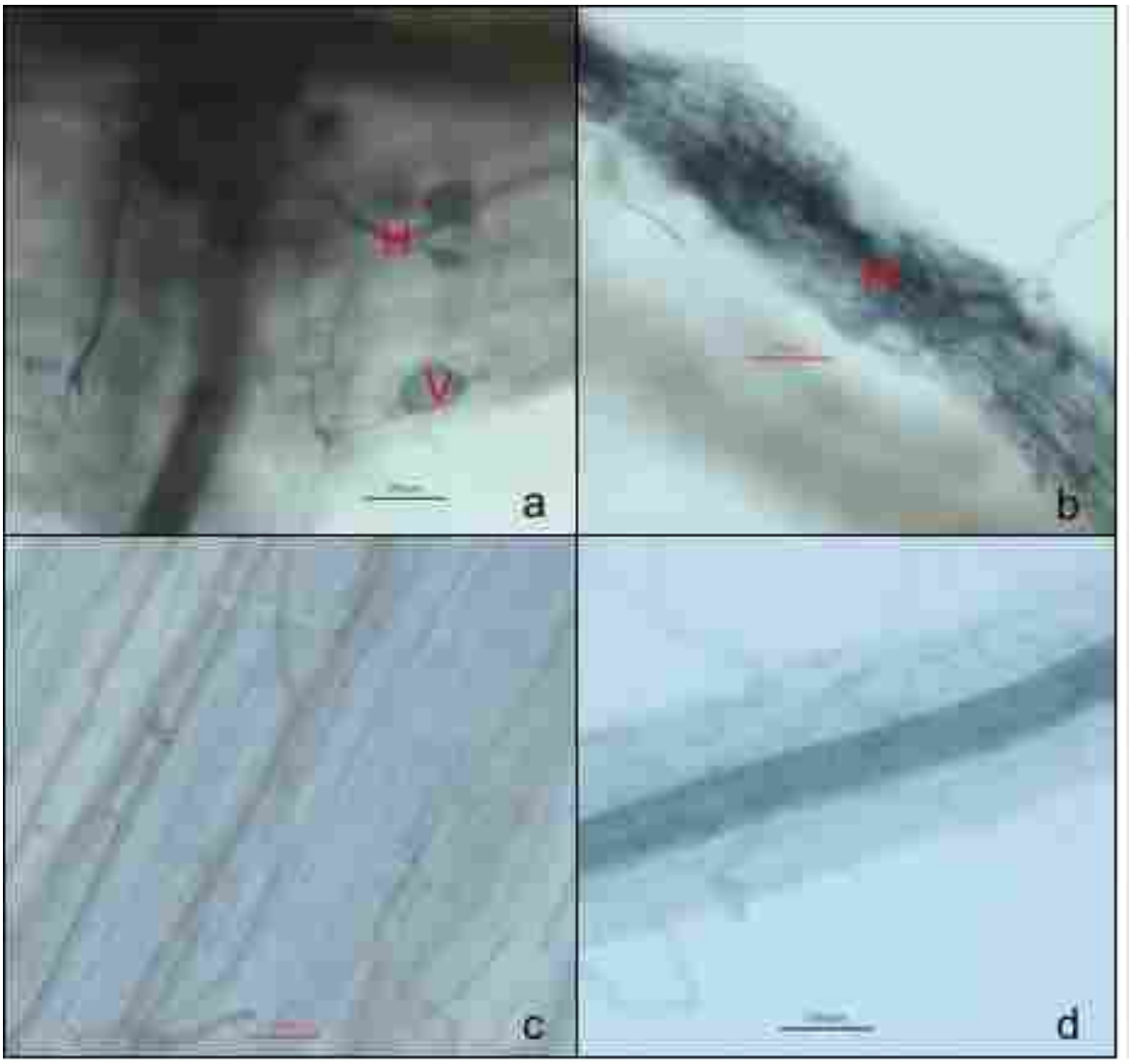

Figure A-1: Arbusuclar mycorrhizal fungal and dark septate endophyte colonization in vegetation from sites in and around the Tundra Mine site. (a) hyphal and vesicular colonization in *Epilobium angustifolium* collected from Hambone Lake (b) hyphal and arbuscular colonization in *Epilobium angustifolium* collected from Hambone Lake (c) Dark septate endophyte hyphae. (d) non-colonized root.

Table A- 1: Mean arsenic concentration in woody plants collected at Tundra Gold Mine, NWT August 2016. Sites include the Bog, Hambone Lake (HB), Mill Pond (MP), North Dam (ND), Trans Saddle Lake (TSL), which are sites located proximal to the tailings area or original milling area, and Reference 1 (Ref 1) and Sandy Lake (SL), which are further away. Plant material was dried, digested and analyzed for arsenic using ICP-MS.

	Leaves	Stems	Fruit

Site	Species	Mean ± Standard Error	Number of Samples	Mean ± Standard Error	Number of Samples	Mean ± Standard Error	Number of Samples
HB	*Betula glandulosa*	1.73 ± 0.34	3	1.20 ± 0.06	3	0.41	1
MP	*Betula glandulosa*	3.40 ± 0.38	3	7.90 ± 2.14	3	3.20 ± 0.10	2
ND	*Betula glandulosa*	1.85 ± 0.55	2	0.96 ± 0.05	2		
TSL	*Betula glandulosa*	0.46 ± 0.01	3	0.58 ± 0.09	3	0.24	1
Ref 1	*Betula glandulosa*	0.11 ± 0.02	3	0.08 ± 0.01	3	0.08	1
SL	*Betula glandulosa*	0.23 ± 0.00	2	0.20 ± 0.02	2	0.15	1
Bog	*Salix athabascensis*	3.75 ± 0.65	2	2.05 ± 0.55	2		
HB	*Salix athabascensis*	1.50 ± 0.00	2	0.96 ± 0.03	2		
MP	*Salix athabascensis*	4.50 ± 1.10	2	10.90 ± 2.10	2	10.95 ± 1.05	2
ND	*Salix athabascensis*	0.92 ± 0.19	2	0.68 ± 0.11	2		
TSL	*Salix athabascensis*	0.24 ± 0.04	2	0.29 ± 0.08	2		
SL	*Salix athabascensis*	0.37 ± 0.04	2	0.22 ± 0.01	2		
TSL	*Empetrum nigrum*	2	1	1.50 ± 0.10	2	1.26 ± 0.84	2
Ref 1	*Empetrum nigrum*	0.24 ± 0.02	2	0.17 ± 0.07	2	0.07	1
SL	*Empetrum nigrum*	0.59 ± 0.09	2	0.47 ± 0.04	2	0.15 ± 0.02	2
MP	*Vaccinium uliginosum*	6.80 ± 1.60	2	14.50 ± 0.50	2	3.2	1
ND	*Vaccinium uliginosum*	1.4	1	2.6	1	0.46	1
TSL	*Vaccinium uliginosum*	1.25 ± 0.46	2	1.11 ± 0.50	2	0.27	1
ND	*Oxycoccus microcarpus*	1.60 ± 0.10	2	4.05 ± 1.35	2	0.56 ± 0.05	2
ND	*Ledum decumbens*	3.95 ± 1.25	2	3.30 ± 0.50	2		
Ref 1	*Ledum decumbens*	0.16 ± 0.05	2	0.16 ± 0.04	2		

Table A- 2: Mean arsenic concentration in non-woody plants collected at Tundra Gold Mine, NWT August 2016. Sites include the Bog, Hambone Lake (HB), Mill Pond (MP), North Dam (ND), Trans Saddle Lake (TSL), which are sites located proximal to the tailings area or original milling area, and Reference 1 (Ref 1) and Sandy Lake (SL), which are further away. Plant material was dried, digested and analyzed for arsenic using ICP-MS.

		Shoots		Roots	
Site	Species	Mean ± Standard Error	Number of Samples	Mean ± Standard Error	Number of Samples
HB	*Carex aquatilis*	160	1	160	1
MP	*Carex aquatilis*	141.00 ± 49.00	2	141.00 ± 49.00	2
ND	*Carex aquatilis*	28.50 ± 8.50	2	28.50 ± 8.50	2
Ref 1	*Carex aquatilis*	0.58 ± 0.06	2	0.58 ± 0.06	2
Bog	*Juncus drummondii*	13	1	110	1
ND	*Juncus drummondii*	8.85 ± 3.15	2	135.00 ± 35.00	2
HB	*Equisetum arvense*	25.55 ± 17.45	2		
ND	*Equisetum arvense*	218.00 ± 122.00	2	55	1
ND	*Eriophorum scheuchzeri*	37.00 ± 17.00	2	126.50 ± 113.50	2
ND	*Calamagrostis canadensis*	7.10 ± 1.20	2	7.10 ± 1.20	2

Table A- 3: Mean total arsenic and total phosphorus concentrations in soils collected from Tundra Gold Mine, 2016. Sites include the Bog, Hambone Lake (HB), Mill Pond (MP), North Dam (ND), Trans Saddle Lake (TSL), which are vegetated sites located proximal to the tailings area or original milling area, and Reference 1 (Ref 1) and Sandy Lake (SL), which are further away. Lower Pond (LP), Upper Pond (UP) and the Quarry (QU) are sites awaiting revegetation. Soil was acid digested and arsenic and phosphorus concentrations were quantified using ICP-OES using EPA Method 200.2.

Site	Total Phosphorus (mg/kg)	Total Arsenic (mg/kg)	Number of Samples
MP	390.83 ± 43.73	1,324.47 ± 398.93	9
HB	587.17 ± 65.76	2,676.94 ± 704.57	9
ND	523.62 ± 72.09	85.85 ± 32.12	9
Bog	365.56 ± 54.32	272.59 ± 244.08	9
TSL	236.73 ± 75.61	73.74 ± 1.49	9

SL	461.74 ± 49.59	89.41 ± 28.85	9
Ref 3	477.31 ± 60.46	2.26 ± 1.48	9
Ref 2	381.73 ± 65.39	70.07 ± 1.38	9
Ref 1	569.83 ± 46.90	64.82 ± 19.32	9
LP	386.99 ± 28.49	84.74 ± 29.48	9
UP	468.84 ± 30.85	144.11 ± 34.87	12
QU	431.69 ± 35.00	0.01 ± 0.00	9

Table A- 4: Vegetation survey conducted June 2016 at Sandy Lake, Tundra Gold Mine. Other species that were found on site, but were not in the vegetation survey include *Pedicularis labradorica*, *Eriphorym scheuchzeri*, *Stelleria longipes* and *Trisetum spicatum*.

Sandy Lake	T1			T2			T3		
Species	Dry	Interm ediate	Wet	Dry	Interm ediate	Wet	Dry	Interm ediate	Wet
Carex rotundata	2.5		2.5						
Bryophyte	60					5			15
Saxifrage tricuspidat	5								
Calamagrostis canadensis	10	5							
Rhododendron subarcticum (*Ledum decumbens)*		10	5		20	15	5	10	10
Rubus chamaemorus		2.5				2.5			
Vaccinium uliginosum		20							
Oxycoccus microcarpus		2.5	5		20		5		
Lichen		80		30	30	10	40	90	
Empetrum nigrum			15	50			30		
Andromeda polifolia			2.5			2.5			2.5
Salix athabascensis			10					15	
Betula glandulosa			5		10	15	40		60
Carex obtusata									20
Salix arctica									2.5

Table A- 5: Vegetation survey conducted June 2016 at Hambone Lake, Tundra Gold Mine. Other species that were found on site, but were not in the vegetation survey include *Equisetum arvense, Epilobium angustifolium, Rubus chamaemorus, Calamagrostis canadensis*.

Hambone Lake	T1			T2			T3		
Species	Dry	Intermediate	Wet	Dry	Intermediate	Wet	Dry	Intermediate	Wet
Salix athabascensis	30	2.5	5	2.5	5	5	10	5	10
Carex aquatilis	15	5	30	20	5	40	10	10	20
Bryophyte	5	20		40	2.5			40	5
Vaccinium uliginosum		2.5							
Betula glandulosa					10		2.5		

Table A- 6: Vegetation survey conducted June 2016 at Mill Pond, Tundra Gold Mine. Other species that were found on site, but were not in the vegetation survey include *Arctostaphylos alpine. Kalmia polifolia, Epilobium palustre, Juncus stygius, Juncus drummondii.*

Mill Pond	T1			T2			T3		
Species	Dry	Intermediate	Wet	Dry	Intermediate	Wet	Dry	Intermediate	Wet
Betula glandulosa	5	5					5		
Empetrum nigrum	25								
Bryophytes	40	25		60			50		
Rhododendron subarcticum (Ledum decumbens)	15			5			10		
Oxycoccus microcarpus	5								
Carex aquatilis		5	15		5	5		10	20
Salix athabascensis		2.5			5			15	5
Rubus chamaemorus		2.5		5					
Andromeda polifolia				2.5					
Vaccinium							5		

uliginosum									
Salix arctica								5	

Table A- 7: Vegetation survey conducted June 2016 at Trans Saddle Lake, Tundra Gold Mine. Other species that were found on site, but were not in the vegetation survey include *Rubus chamaemorus, Kalmia polifolia, Calamagrostis canadensis* and *Equisetum sylvaticum*.

Trans Saddle Lake	T1			T2			T3		
Species	Dry	Intermediate	Wet	Dry	Intermediate	Wet	Dry	Intermediate	Wet
Equisetum arvense	5								
Salix athabascensis	10			5	15		5		
Rhododendron subarcticum (*Ledum decumbens)*	35	5			5		5	5	
Lychen	10								
Vaccinium uliginosum	15	20		15	5		5	10	
Empetrum nigrum	15	15		10			10		
Oxycoccus microcarpus	15	10		20	5		2.5		
Carex aquatilis		5	5	5		15	5	15	30
Potentilla palustre			2.5			10			
Bryophyte			20		50	15		20	
Betula glandulosa		20		15	20		10	10	
Rhododendron groelandicum (*Ledum groelandicum)*				5					

Table A- 8: Vegetation survey conducted June 2016 at North Dam Tundra Gold Mine. Other species that were found on site, but were not in the vegetation survey include *Salix athabascensis, Eriophorym scheuchzeri, Epilobium angustifolium, Calamagrostis canadensis, Epilobium palustre* and *Juncus drummondii*.

North Dam	T1			T2			T3		
Species	Dry	Intermediate	Wet	Dry	Intermediate	Wet	Dry	Intermediate	Wet
Rhododendron subarcticum (Ledum decumbens)	15	2.5		15					
Betula glandulosa	10	5		20			5		
Empetrum nigrum	10								
Oxycoccus microcarpus	15	5		10			5		
Acrctostaphylos alpina	10								
Vaccinium uliginosum	5			10					
Lychen	5							5	
Rubus chamaemorus		2.5		2.5			2.5		
Carex aquatilis		2.5	50		5	25		5	20
Carex rotundata		15			20		15	30	
Bryophytes		15	5	25			20		15
Andromeda polifolia		5					10		
Equisetum arvense								2.5	2.5

Table A- 9: Vegetation survey conducted June 2016 at Reference 1 Tundra Gold Mine. Other species that were found on site, but were not in the vegetation survey include *Salix arctica, Kalmia polifolia, Rumex occidentalis, Carex rotundata, Calamagrostis deschampsioides, Epilobium angustifolium and Agrostis scabra.*

Reference 1	T1			T2			T3		
Species	Dry	Intermediate	Wet	Dry	Intermediate	Wet	Dry	Intermediate	Wet
Betula	10	15		5	15			15	

glandulosa									
Vaccinium uliginosum	15	5			5		2.5	5	
Rubus chamaemorus	5	5		2.5	5			5	
Rhododendron subarcticum (Ledum decumbens)	5	10		2.5	10		5	10	
Bryophytes	50	30		25	30	15		30	
Empetrum nigrum	5	5			5			5	
Andromeda polifolia	5	5			5			5	
Oxycoccus microcarpus	2.5	60			60		35	60	
Lichen	10	10		10	10		15	10	
Carex obtusata			50			20			35
Acrctostaphylos alpina				25			5		
Carex aquatilis									5

Table A- 10: Vegetation survey conducted June 2016 at Reference 2 Tundra Gold Mine. Other species that were found on site, but were not in the vegetation survey include *Salix athabascensis, Epilobium angustifolium, Calamagrostis canadensis, Calamagrostis deschampsioides* and *Eriophorum scheuchzeri.*

Reference 2	T1			T2			T3		
Species	Dry	Intermediate	Wet	Dry	Intermediate	Wet	Dry	Intermediate	Wet
Acrctostaphylos alpina	15	2.5			5		5	15	
Lichen	40	40		40	25		40	20	
Oxycoccus microcarpus	20	20		5	20		15	20	
Rhododendron subarcticum (Ledum decumbens)	5	5		2.55	5	2.5	15	5	
Empetrum nigrum	5	5		15	2.5	5	5		
Vaccinium uliginosum	2.5	10		2.5				15	

Betula glandulosa	2.5	15		5	15		10	5	5
Andromeda polifolia						5			2.5
Carex rotundata		2.5	2.5			5			5
Carex obtusata			25						
Rubus chamaemorus						5			

Table A- 11: Vegetation survey conducted June 2016 at Reference 3 Tundra Gold Mine. Other species that were found on site, but were not in the vegetation survey include *Bryophytes* and *Eriophorum scheuchzeri.*

Reference 3	T1			T2			T3		
Species	Dry	Intermediate	Wet	Dry	Intermediate	Wet	Dry	Intermediate	Wet
Rubus chamaemorus	5	5		5			10		
Rhododendron subarcticum (Ledum decumbens)	20	5		20	0			2.5	
Betula glandulosa	15	5	5	15	20	2.5	5	5	
Oxycoccus microcarpus	10	10	5	5			10	5	
Lichen	25	60		20	30		30	10	
Andromeda polifolia		2.5		2.5	10		5	5	
Salix athabascensis	2.5								
Empetrum nigrum		2.5	5						
Vaccinium uliginosum		5	5	5	15		5		
Carex aquatilis			2.5	5	5	5			
Carex obtusata						2.5			10
Carex rotundata								15	10

Table A- 12: Loading scores based on site from the non-linear multidimensional scaling using the software PC-ORD version 7. Vegetation survey was conducted on three quadrats on three transects at each site, for a total of nine quadrats. Percent cover for each plant species was

averaged out for each transect at each site. The percent cover served as the first matrix of the analysis, and a second matrix was added which included arsenic, phosphorus and potassium soil concentrations corresponding to each transect.

Sites	Axis 1	Axis 2
SL	-0.32601	0.10546
SL	-0.76205	0.85792
SL	-0.27381	1.30737
HB	1.51156	-0.21027
HB	1.21122	-0.39959
HB	1.2578	-0.55323
MP	0.45676	-0.52281
MP	0.77149	-1.01226
MP	0.95309	-0.5113
TSL	0.11343	0.49686
TSL	0.31381	-0.32342
TSL	0.7732	0.19916
ND	0.33784	0.12791
ND	0.51785	0.06549
ND	0.42218	-1.12585
Ref 1	-0.56167	-0.4438
Ref 1	-0.5127	-0.43835
Ref 1	-0.68518	-0.3938
Ref 2	-1.04203	0.43049
Ref 2	-0.94638	0.58924
Ref 2	-0.96008	0.48985
Ref 3	-0.78636	0.63273
Ref 3	-0.44851	0.6671
Ref 3	-1.33544	-0.03488

Table A- 13: Loading scores based on plant species from the non-linear multidimensional scaling using the software PC-ORD version 7. Vegetation survey was conducted on three quadrats on three transects at each site, for a total of nine quadrats. Percent cover for each plant species was averaged out for each transect at each site. The percent cover served as the first matrix of the analysis, and a second matrix was added which included arsenic, phosphorus and potassium soil concentrations corresponding to each transect. Species include *Carex aquatilis* (Ca. aq.), *Carex rotundata* (Ca. ro.), *Carex obtusata* (Ca. ob.), *Calamagrostis canadensis* (Ca. ca.), *Betula glandulosa* (Be. gl.), *Acrctostaphylos alpine* (Ac. al.), *Andromeda polifolia* (An. po.), *Empetrum nigrum* (Em. ni.), *Ledum decumbens* now classified as *Rhododendron subarcticum* (Le. de.), *Loiseleuria procumbens* (Lo. pr.), *Oxycoccus microcarpus* (Ox. mi.), *Vaccinium uliginosum* (Va.

ul.), *Potentilla palustre* (Po. pa.), *Rubus chamaemorus* (Ru. ch.), *Salix athabascensis* (Sa. at.), *Salix arctica* (Sa. ar.) and *Saxifrage tricuspidat* (Sa. tr.). Bryophytes and lichen were also included in the survey.

species	Axis 1	Axis 2
Bryophyte	0.29113	-0.35154
Lichen	-0.66291	0.53532
Eq. ar.	0.26781	-0.31449
Ca. aq.	0.77139	-0.2257
Ca. ro.	-0.12269	-0.32185
Ca. ob.	-0.66659	-0.04007
Ca. ca.	-0.32601	0.10546
Be. gl.	-0.25512	0.39693
Ac. al.	-0.66708	0.10458
An. po.	-0.45458	-0.03005
Em. ni.	-0.316	0.29074
Le. de.	-0.2693	0.31251
Lo. pr.	-0.98464	0.52574
Ox. mi.	-0.53263	-0.0202
Va. ul.	-0.19763	0.16146
Po. pa.	0.27373	-0.15936
Ru. ch.	-0.49112	-0.05139
Sa. at.	0.78347	-0.13068
Sa. ar.	0.54412	0.09492
Sa. tr.	-0.32601	0.10546

Table A- 14: Loading scores based on soil elements from the non-linear multidimensional scaling using the software PC-ORD version 7. Vegetation survey was conducted on three quadrats on three transects at each site, for a total of nine quadrats. Percent cover for each plant species was averaged out for each transect at each site. The percent cover served as the first matrix of the analysis, and a second matrix was added which included arsenic, phosphorus and potassium soil concentrations corresponding to each transect.

	Axis 1	Axis 2
arsenic	0.7943	-0.29775
phosphorus	0.00385	0.00071
potassium	0.3374	-0.0145

Table A- 15: Complete species list of all plants identified in the vegetation survey of Tundra Gold Mine and the sites where they were identified in.

Family	Species	Wetland Indicator Status (Alaska)	HB	MP	ND	TSL	Bog	Ref 1	Ref 2	Ref 3	SL
Bryophyte	Bryophyte		o	o	o	o	o	o		o	o
Lichen	Lichen				o	o		o	o	o	o
Equisetaceae	*Equisetum arvense*	FAC FACU FACW	o		o	o					
Equisetaceae	*Equisetum sylvaticum*	FAC				o					
Monocots											
Cyperaceae	*Carex aquatilis*	OBL	o	o	o	o		o		o	
Cyperaceae	*Carex rotundata*	OBL		o	o			o	o	o	o
Cyperaceae	*Carex obtusata*	OBL						o	o	o	o
Cyperaceae	*Eriophorum scheuchzeri*	OBL			o				o	o	o
Juncaceae	*Juncus drummondii*	FACW		o			o				
Juncaceae	*Juncus stygius*	OBL		o							
Poaceae	*Calamagrostis canadensis*	FAC OBL	o		o	o			o		o
Poaceae	*Calamagrostis deschampsioides*	FACW						o	o		
	Agrostis scabra	FAC						o			
	Trisetum spicatum	FAC									o

Dicots											
Betulaceae	*Betula glandulosa*	FAC OBL	o	o	o	o		o	o	o	o
Caryophyllaceae	*Stellaria longipes*	FAC FACU OB									o
Ericaceae	*Arctostaphylos alpina*	FAC		o	o			o	o		
Ericaceae	*Andromeda polifolia*	OBL		o	o			o	o	o	o
Ericaceae	*Empetrum nigrum*	FAC FACU FACW		o	o	o		o	o	o	o
Ericaceae	*Kalmia polifolia*	OBL		o		o		o			
Ericaceae	*Rhododendron subarcticum (Ledum decumbens)*	FACW		o	o	o		o	o	o	o
Ericaceae	*Rhododendron groelandicum(Ledum decumbens groelandicum)*	FACW				o					
Ericaceae	*Oxycoccus microcarpus*	OBL		o	o	o		o	o	o	o
Ericaceae	*Vaccinium uliginosum*	FAC FACU FACW	o	o	o	o		o	o	o	o
Onagraceae	*Epilobium angustifolium*	FACU	o		o		o	o	o		
Onagraceae	*Epilobium palustre*	OBL		o	o		o				
Orobanchaceae	*Pedicularis labradorica*	FACW									o
Plantaginaceae	*Hippuris vulgaris*	OBL					o				

Polygonaceae	*Rumex occidentalis*	OBL						o			
Rosaceae	*Potentilla palustre*	OBL				o					
Rosaceae	*Rubus chamaemorus*	FACW	o	o	o	o		o	o	o	o
Salicaceae	*Salix athabascensis*	OBL	o	o	o	o	o	o	o	o	o
Salicaceae	*Salix arctica*	FACU		o				o			o
Saxifragaceae	*Saxifrage tricuspidata*	FACU									o
Diversity index			1.206	1.668	2.165	2.212	NA	2.080	1.958	2.043	2.026
Species richness			9	17	18	16	6	20	16	14	19

Table A- 16: Mean mycorrhizal and DSE colonization in plants collected at Tundra Gold Mine June 2016. Roots were cleared in 10% KOH at 90˚C and stained with 10% ink in acetic acid. Colonization was measured using the magnified intersections method at 250x magnification. 100 fields of view were used and the presence of hyphae, arbuscules, vesicles were recorded.

Site	Species	Hyphal colonization (Mean ± SEM)	Arbuscular colonization (Mean ± SEM)	Vesicular colonization (Mean ± SEM)	DSE colonization (Mean ± SEM)	Number of samples
Bog	*Epilobium palustre*	0	0	0	0	1
LP	*Epilobium angustifolium*	2.5	0	0	5	1
ND	*Calamagrostis canadensis*	30.93 ± 9.96	4.59 ± 2.27	0.00 ± 0.00	16.91 ± 8.71	5
ND	*Epilobium palustre*	19.19 ± 10.38	10.54 ± 5.54	0.00 ± 0.00	3.99 ± 2.15	5
Ref 1	*Calamagrostis deschampsioides*	27.87 ± 10.52	5.62 ± 3.67	0.20 ± 0.20	72.43 ± 3.28	5
Ref 2	*Calamagrostis canadensis*	43.53 ± 10.24	3.97 ± 2.08	2.45 ± 2.21	42.69 ± 12.72	5
Ref 2	*Calamagrostis deschampsioides* .	15.25 ± 6.69	3.97 ± 2.10	4.31 ± 2.01	63.75 ± 4.58	5
SL	*Calamagrostis canadensis*	24.51 ± 3.17	5.27 ± 1.86	0.97 ± 0.85	27.45 ± 4.45	5
SL	*Stellaria longipes*	20.01 ± 8.20	0.00 ± 0.00	0.00 ± 0.00	60.44 ± 6.00	5
SL	*Trisetum spicatum*	57.52 ± 8.84	1.00 ± 0.71	5.00 ± 1.78	44.05 ± 16.20	4
TSL	*Calamagrostis canadensis*	97.50 ± 1.50	20.50 ± 12.50	25.00 ± 13.00	24.50 ± 9.50	2

Table A- 17: Mycorrhizal and DSE colonization in plants collected at Tundra Gold Mine August 2016. Roots were cleared in 10% KOH at 90˚C and stained with 10% ink in acetic acid. Colonization was measured using the magnified intersections method at 250x magnification. 100 fields of view were used and the presence of hyphae, arbuscules, vesicles were recorded.

Site	Species	Hyphal colonization (Mean ± SEM)	Arbuscular colonization (Mean ± SEM)	Vesicular colonization (Mean ± SEM)	DSE colonization (Mean ± SEM)	Number of samples

Bog	*Epilobium angustifolium*	6.45 ± 1.91	0.00 ± 0.00	1.69 ± 0.23	0.00 ± 0.00	5
Bog	*Epilobium palustre*	13.69 ± 4.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4
Bog	Poa 1	21.57 ± 4.59	0.00 ± 0.00	1.96 ± 1.31	79.12 ± 7.57	4
Bog	Poa 3	45.40 ± 14.19	0.00 ± 0.00	3.40 ± 1.25	0.00 ± 0.00	5
HB	*Calamagrostis canadensis*	33.20 ± 10.61	0.80 ± 0.80	4.60 ± 3.16	18.40 ± 9.05	5
HB	*Epilobium angustifolium*	59.83 ± 7.27	0.00 ± 0.00	4.51 ± 3.06	37.28 ± 14.70	4
MP	*Epilobium palustre*	51.26 ± 12.98	33.64 ± 15.65	0.00 ± 0.00	8.30 ± 4.93	5
ND	*Calamagrostis canadensis*	81.25 ± 8.75	28.75 ± 19.11	4.25 ± 1.89	9.75 ± 5.81	4
ND	*Epilobium palustre*	51.80 ± 14.77	0.60 ± 0.60	0.80 ± 0.49	12.00 ± 4.57	5
Ref 1	*Agrostis scabra*	66.47 ± 9.32	0.33 ± 0.33	0.50 ± 0.34	46.21 ± 10.32	6
Ref 1	*Calamagrostis deschampsioides*	37.05 ± 9.31	0.20 ± 0.20	0.60 ± 0.60	19.67 ± 2.95	5
Ref 1	*Epilobium angustifolium*	44.75 ± 13.99	0.79 ± 0.79	0.99 ± 0.99	41.23 ± 11.29	5
SL	*Trisetum spicatum*	57.52 ± 8.84	1.00 ± 0.71	5.00 ± 1.78	44.05 ± 16.20	4
TSL	*Calamagrostis canadensis*	54.70 ± 11.62	0.20 ± 0.20	1.97 ± 1.30	50.97 ± 11.99	5