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An Assessment of How Plant and Mycorrhizal Communities Have Been Affected Along a Mine-Impacted Watershed In The Northwest Territories

Kevin MacColl
macc2320@mylaurier.ca

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By

Kevin MacColl

(Honours Bachelor of Science Biology, Wilfrid Laurier University, 2014)

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Abstract

Giant Mine is an inactive gold mine located nine kilometers north of Yellowknife, Northwest Territories. Giant Mine has been the source of arsenic trioxide for the Baker Creek watershed since it opened over 60 years ago. Although arsenic levels in the creek are above the limits considered to be biologically relevant, there is no concrete evidence that plants and mycorrhizae have actually been affected. This study provides an initial assessment of the impacts mining activity at Giant Mine has had on plants and mycorrhizae in the Baker Creek watershed. Nine sites were sampled around Giant Mine: five sites downstream from Giant Mine along Baker Creek, one site upstream, two nearby wetlands and a distant reference site at Yellowknife River. Sites were distinguished by their level of exposure to the mine; downstream sites were considered the most impacted because they received mine water discharges, therefore having the highest arsenic levels. Two species of plants (*Epilobium angustifolium* and *Phalaris arundinacea*) were grown in these soils under laboratory conditions to compare growth responses and mycorrhizal colonization of plants in impacted versus non-impacted soils. A vegetation survey of Baker Creek was also conducted during soil collections in order to determine how patterns in plant species composition differ between sites. Plant roots were also collected to compare levels of colonization among sites to confirm that laboratory results were consistent with those obtained in natural conditions. This study tests the hypothesis that diminished growth and lower mycorrhizal colonization would be observed in plants growing in more impacted soils, and that these results would be reflected in the field through relatively different assemblages of plant species between impacted and non-impacted areas. Results from the growth room study were consistent with this hypothesis.

Mean root length of *P. arundinacea* was 332.99 ± 15.52 cm and 299.75 ± 33.55 cm in soils collected from the upstream site and reference site, respectively, significantly greater than the next highest site downstream from the mine, where mean root length was 155.69 ± 18.01 cm. *E. angustifolium* only grew larger in references soils where mean root length was 44.41 ± 8.74 cm, compared to the next largest, 11.66 ± 2.68 centimeters, from a site downstream from Giant Mine. Mean mycorrhizal colonization was less than 7% in all samples except in plants from reference soils and from those from a reconstructed channel of the creek; mean colonization at these two sites was 13.44 ± 8.94 % and 18.08 ± 3.35 %, respectively, in *P. arundinacea* and 29.0 ± 8.79 % and 16.27 ± 10.54 % in *E. angustifolium*. These two sites were also distinguished from other sites based on different assemblages of plants species, however, opposed to the hypothesis, these were not mycorrhizal plant species that had been excluded from impacted sites. Certain habitat variables exclusive to Reach 4 and Yellowknife River may have favoured the establishment of these plant species, rather than the presence of mycorrhizal fungi. In conclusion, this study has demonstrated disturbed growing conditions along Baker Creek and a reduction in mycorrhizal infectivity; however, it could not relate these trends to patterns in plant species distribution. This study will be the first on the plant and mycorrhizal status of a mine-impacted watershed in Northern Canada, and as such, will contribute to a growing body of work on mine remediation specifically for this region.

Authors Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

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Abbreviations

AANDC: Department of Aboriginal Affairs and Northern Development Canada

As^{V} : Arsenate

As^{III} : Arsenite

CRP: Closure and Reclamation Plan

DMA: dimethylarsinic acid

EIA: Environmental Impact Assessment

GDP: Gross Domestic Product

GNWT: Government of Northwest Territories

GCR: Guidelines for Closure and Reclamation

MMA: monomethylarsonic acid

MVLWB: Mackenzie Valley Land and Water Board

PEL: Probable Effects Limit

Site Abbreviations

PL: Pocket Lake

R0: Reach 0

R2: Reach 2

R4: Reach 4

R5: Reach 5

R6: Reach 6

R7: Reach 7

TL: Trapper Lake

YR: Yellowknife River

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1. Background

1.1 The Canadian Mining Industry

Mining and resource extraction is Canada's fourth largest industry comprising 7.3% of the national Gross Domestic Product (GDP) with a total workforce of over 375,000 full-time employees (Marshall, 2015). The importance of mining is especially apparent in Northern Canada where primary industry is a staple of the economy. Resource extraction in the Northwest Territories comprises $900 million of the territorial GDP, the most profitable industry in this territory by a $333 million margin (GNWT, 2016a). Mineral output in the Canadian North is expected to grow by 91% during this decade, a rate of growth four times greater than the overall Canadian economy (Rheaume & Caron-Vuotari, 2013).

When the first gold mines opened in the Canadian North, there were no regulations over environmental protection, and toxic wastes were released from mines in massive quantities (Bone, 2009; Jardine et al., 2013). Review panels were established in the 1970's to hold mining companies accountable for their imposition on the environment (Bone, 2009). Yet these panels could not enforce law until 1995 with the passing of the Canadian Environmental Assessment Act (Bone, 2009). This act imposed regulations that meant operating on crown land required permits and water licenses issued by the Federal Government (Bone, 2009; AADNC & MVLWB, 2013). Mine operators must now comply with an Environmental Impact Assessment (EIA) and a Closure and Reclamation Plan (CRP) before mining can begin (AADNC & MVLWB, 2013). An overarching guideline of expectations for a CRP is provided by the Guidelines for Closure and Reclamation (GCR) (AADNC, 2002; AANDC & MVLWB 2013). These guidelines

dictate that disturbed areas must be returned to viable and self-sustaining habitats that resemble native nearby ecosystems without the need for long-term monitoring or maintenance (AANDC, 2002; AANDC & MVLWB, 2013). Promoting the establishment of diverse, native plant communities is necessary for revegetation (AANDC & MVLWB, 2013). It has also been recognized that mycorrhizae are important for supporting plants in the revegetation of mine-impacted areas (Gaur & Adholeya, 2004).

1.2 The Role of Plants and Mycorrhizae in Mine Remediation

The ways that mining disturbs plants and mycorrhizae are numerous. The greatest impacts come from mechanical damages as vast areas of habitat and topsoil are stripped (Dudka & Adriano, 1997; Cooke & Johnson, 2002; Wong, 2003). An estimated 1,000 km^2 of viable habitat have been stripped for mining purposes in Canada (Fraser Institute, 2012); much of the topsoil at mines has been buried underground instead of being recycled (Cooke & Johnson, 2002). Once mining is finished, vast areas of compacted soil are left behind due to the presence of buildings and vehicle traffic (King, 1988; Dudka & Adriano, 1997; Cooke & Johnson, 2002). Compacted soils make plant establishment difficult at abandoned mine sites; the high bulk densities associated with construction sites restrict root growth and limit water and nutrient mobility (Cooke & Johnson, 2002).

Soil chemistry is also affected by mining. For example, soil fertility is often low at abandoned mines due to the stripping of topsoil (Dudka & Adriano, 1997; Cooke & Johnson, 2002; Wong, 2003). The remaining mineral substrates then lack organic material; the absence of nitrogen in particular limits plant growth at mine sites (Cooke & Johnson, 2002; Wong, 2003). Soils at mine sites are also usually contaminated with toxic

metals, including Cd, Cu, Pb and Zn (Cooke & Johson, 2002). Metals from mines can persist indefinitely upon their release into the environment; for example, over 4000 km^2 in southwest England have been contaminated with metals from mines that have been closed for over 100 years (Li & Thornton, 1993). Contamination from toxic metals results in areas low in biodiversity where only metal-tolerant species can grow (Gonzalez-Chavez et al., 2002), or in more extreme cases, leaves barren areas where nothing can establish (Cooke & Johnson, 2002). Therefore, soils at mine sites usually require amendments before revegetation can begin; for example, soils are often tilled and fertilized before planting (Cooke & Johnson, 2002).

The primary goal of revegetation, as described by the GCR, is to provide a foundation for self-sustaining habitat through the establishment of diverse native plant communities (AANDC & MVLWB, 2013). But the plants themselves can also be instruments of the remediation process. Plant roots facilitate the precipitation of heavy metals into less available forms, a process known as phytostabilization (Wong, 2003; Mendez & Maier, 2008). Plants can incorporate metals into organic compounds, for example, cadmium and zinc are taken up through root hairs, where the metals are bound to citrate or malate molecules, and either expelled into the soil or sequestered in vacuoles (Steffens, 1990). Phytostabilization has been recognized as a cost-effective method for metal sequestration, and has recently been incorporated into modern remediation techniques (Wong, 2003; Mendez & Maier, 2008).

Root-colonizing bacteria also stabilize metals by incorporating them into organic compounds; however, these bacteria depend on the host they colonize (Mendez & Maier, 2008). In the absence of plant roots, mine spoils are well-known to be dominated by

autotrophic iron- and sulfur-oxidizing bacteria, which themselves have been associated with plant death (Mendez & Maier, 2008). Revegetation is required for introducing roots to these soils, and promoting the activity of heterotrophic bacteria (Mendez & Maier, 2008). Plant roots also reduce erosion by stabilizing arid mine soils, thus preventing the spread of contaminants (Cooke & Johnson, 2002; Wong, 2003; AANDC & MVLWB, 2013). For example, the grass species *Vetiveria zizanioides* has a particularly fine root structure, and for this reason is often chosen for revegetation of mines at more Southern latitudes (Wong, 2003). Once self-sustaining plant communities are established, the canopy they form further reduces the spread of contaminants by preventing wind-erosion (Wong, 2003; Gaur & Adholeya, 2004; Tordoff et al., 2000).

Mobilization of heavy metals is also reduced by the presence of mycorrhizal fungi. Arbuscular mycorrhizal fungi live in symbiosis with plants by colonizing their roots and exchanging nutrients for carbohydrates intracellularly (Smith et al., 2010). Their hyphae are known to bind and sequester metal aggregates, thereby preventing their translocation into plant tissues (Hetrick et al., 1994; Tordoff et al., 2000; Gaur & Adholeya, 2004). For example, Hetrick et al. (1994) found that plants would establish on zinc-contaminated mine tailings only in the presence of both fertilizer and mycorrhizal fungi. Metal-tolerant mycorrhizal fungi isolated from mine sites are believed to support plant growth by sequestering toxic metals (Gonzalez-Chavez et al., 2002); accumulation of Zn in the mycorrhizal species *Glomus mossae* and *G. versiforme* tissues has been observed at levels as high as 1200 mg/kg and 600 mg/kg, respectively, where soil concentrations were only 0.63 mg/kg Zn (Chen et al., 2001).

Mycorrhizae aid in acquisition of nutrients that would otherwise be unavailable to plants due to their low mobility, for example, macronutrients such as phosphorus (Bolan, 1991; Smith et al., 2011). Hyphae can take up phosphorus at rates six times greater than root hairs due to their ability to chemically modulate their environment and release phosphates from sorption sites on soil particles (Bolan, 1991). Mycorrhizal plants can take up phosphorus at rates five times greater than non-mycorrhizal plants (Bolan, 1991; Smith et al., 2011). Increased phosphorus nutrition as a result of mycorrhizal colonization is well-known to ameliorate the effects of metal toxicity in plants (Smith et al., 2010). Increase in biomass as a direct result of additional nutrition dilutes metal-concentrations in plant tissues and reduces the metal's toxic potency, a term described by researchers as 'the dilution effect' (Chen et al., 2007; Cozzolino et al., 2010; Smith et al., 2010). Due to an ability to support plants in disturbed soils, mycorrhizal fungi have become more recognized as important components for remediation strategies in recent years (Gaur & Adholeya, 2004).

1.3 A History of Giant Mine and Arsenic Pollution

Giant Mine is an inactive gold mine located nine kilometers north of the Yellowknife city center in the Northwest Territories. Giant Mine has been a crucial component in the development of the territory; Yellowknife did not exist before mining began at Giant Mine in 1948 (Bullen & Robb, 2002; Watt, 2013). By the early 1950's Giant Mine processed up to 700 tons of ore daily, and by the end of the decade 1,000 tons (AANDC, 2013). When mine operations ended in 1999, there were over 350 employees at the mine, 130 indirectly employed, and an estimated 100 jobs created by servicing

mine workers (Bullen & Robb, 2002). Giant Mine produced seven million ounces of gold, an estimated $2.7 billion revenue over its lifespan, a total contribution of $2 billion to the Territories' GDP, and $360 million in workers' tax dollars (Bullen & Robb, 2002).

Being the most profitable gold mine in Canadian history, Giant Mine has earned national attention for its impacts on the economy and on the environment. Gold in the Canadian Shield is associated with arsenopyrite (FeAsS), which requires a roasting process to separate the gold ore, resulting in the emissions sulfur dioxide (SO_2) and arsenic trioxide (As_2O_3), which is a toxic compound (Houben et al., 2016). Between the years 1948 and 1951, an estimated 2.6 million kg of arsenic trioxide dust was released through the smoke stacks annually, and 25,000 kg of arsenic-laiden tailings were discharged into the Baker Creek watershed every year (de Rosemond et al., 2008; AANDC, 2013). Wildlife was severely affected; reports emerged of Baker Creek becoming barren of aquatic life, herds of livestock perishing, and even the death of an Aboriginal boy in the early 1950's (Jardine et al., 2013). This led to the installation of precipitators to remove arsenic dust from the smoke stacks, preventing its release into the surroundings (AANDC, 2013; Jardine et al., 2013). By the year 1960 aerial emissions of arsenic had dropped to ~250 kg daily for the remainder of mine operations (AANDC, 2013).

Arsenic dust collected from the precipitators was stored underground in chambers sealed by permafrost where today, a total of 237,000 tonnes of arsenic remains (AANDC, 2013; Jardine et al., 2013). However, the permafrost seal on these arsenic chambers has been slowly compromised by ventilation shafts carrying warm air underground (Clark & Raven, 2004; Royle, 2007). The effects of climate change on permafrost have further

degraded this seal so that groundwater can now enter and leave the chambers (Clark & Raven, 2004; Royle, 2007). Arsenic trioxide is soluble, so it easily mixes with groundwater and can escape freely; mine water in the vicinity of these chambers can have arsenic concentrations as high as 4 g/L (Clark & Raven, 2004). Mine water is treated before discharge into Baker Creek, however, complete removal of arsenic is nearly impossible. The water licence granted to the operators of Giant Mine requires that arsenic in mine water discharges remain below 0.5 mg/L, 10 times lower than arsenic's LC50 for algae (AANDC, 2010). Arsenic discharges from Giant Mine tend to be ~0.3 mg/L, and at these concentrations, a total of ~500 kg of arsenic is released into Baker Creek annually (de Rosemond et al., 2008; AANDC, 2013).

While concentrations of arsenic in treated waters are still below toxic levels, arsenic in sediments can remain fairly immobile due a high affinity for sorption sites on oxide surfaces and clay particles (Lombi et al., 2000; Fitz & Wenzel, 2002). There has been a build up of arsenic in Baker Creek sediments far above legally acceptable limits because arsenic molecules have been bound within the sediment matrix (AANDC, 2010). Arsenic concentrations in sediments around the point of discharge can be over 100 times greater than the Probable Effects Limit (PEL). The PEL is a legal limit set by the Canadian Council of Ministers of the Environment (CCME). According to their Canadian Sediment Quality Guidelines, 'adverse biological effects can be expected to occur frequently' when arsenic levels in sediments exceeds 17 μg/g (CCME, 1999).

1.4 Arsenic in Baker Creek and Nearby Wetlands

Baker Creek is a relatively small body of water, only 6.5 kilometers in length, originating 3.5 kilometers northwest of the Giant Mine property, never exceeding 2.3 meters in depth, with a total estimated drainage area of 121 km^2 (Golder Associates, 2013). The creek has been divided into eight channels called 'Reaches': Reach 0, 1 and 2 are downstream from mine property, Reach 3 to 6 run through the mine, and Reach 7 is the furthest upstream in a wilderness area northwest of the mine property (Figure 1.1). Reach 6, at the point of mine water discharge, is the location where previous researchers have found the greatest metal concentrations in sediments: arsenic concentrations can be as high as 3,500 μg/g (AANDC, 2010). However, this same study found arsenic was present along the length of the creek; average arsenic concentrations across sample stations from Reach 0 - 7 was 2,020 μg/g (AANDC, 2010).

There are two exceptional sites where arsenic is low: Reach 4 and Reach 7. Reach 4 contains little to no arsenic because this site had been redirected in 2006, and clean soil for this redirection had been brought in from another location (Hewitt, 2007; Golder Associates, 2013). For example, a study in 2011 found sediment arsenic levels of 23.6 of μg/g at Reach 4, and in contrast a site directly upstream (Reach 5) had arsenic concentrations of 1,370 μg/g (Golder Associates, 2013). Reach 7, upstream from Giant Mine, is the area least exposed to effluents along Baker Creek; this 2011 study found an average of 97 μg/g arsenic in sediments, this site only receiving atmospheric arsenic from the roasters (Golder Associates, 2013).

Although atmospheric emissions have ceased, arsenic persists in the small Northern lakes around Giant Mine because drainage is poor; some of these lakes are

maintained completely by runoff, evaporation and precipitation (Mielko & Woo, 2006). Arsenic is isolated in these lakes due to a solid foundation of either volcanic, sedimentary or granodiorite bedrock (Houben et al., 2016). Palmer et al. (2015) recorded dissolved arsenic concentrations at the 100 - 500 μg/L range in lake waters within a 10 km radius of the mine, and in contrast, the legally acceptable limit of arsenic is 5 μg/L for the protection of aquatic life (Palmer et al., 2015). Sediments in Pocket Lake, an isolated lake to the Northwest of Giant Mine, can have arsenic concentrations as high as 30,000 mg/kg (Thienpont et al., 2016).

1.5 Arsenic Toxicity in Plants and Mycorrhizal Fungi

Arsenic trioxide (As_2O_3) is the form of arsenic that has been released from Giant Mine; however, roughly 25 forms of arsenic have been identified worldwide (Fitz & Wenzel, 2002). Of these, the four most common in soils are arsenate (As^V), arsenite (As^{III}), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Fitz & Wenzel, 2002; Zhao et al., 2009) (Figure 1.2). The inorganic forms, As^{III} and As^V, are the most common at contaminated sites; the organic arsenic molecules DMA and MMA are formed when soil microorganisms take up and methylate inorganic arsenic molecules (Zhao et al., 2009). There is no consensus as to which form is most toxic to plants; however, inorganic arsenic is more easily taken up through the roots, and is the form that plants are the most exposed to (Zhao et al., 2009).

Regardless of which type is taken up, As^{III} is the type that will exert toxicity intracellularly because of the mechanism by which plants process arsenic molecules (Schulz et al., 2008; Zhao et al., 2009). Plants rapidly oxidize all arsenic molecules into

As^{III}, which is then bound to phytochelatin compounds and sequestered in vacuoles (Schulz et al., 2008; Zhao et al., 2009). Abedin et al. (2002) showed that As^{III} is taken up passively in the roots through aquaporin channels; glycerol is also taken up through these channels, and increasing glycerol in a hydroponic system decreased the rate of As^{III} uptake in plants, leading to the conclusion that these molecules are competing for entry. As^{III} exerts toxicity by binding cysteine amino acids and thus altering a protein's primary structure (Figure 1.3) (Kitchin & Wallace, 2006; Finnegan & Chen, 2012). The number of cysteine molecules a protein has will determine its susceptibility to As^{III} toxicity. As an example, based on the sequenced *Arabidopsis* genome, an estimated 23,578 of its 35,386 proteins would be affected (Finnegan & Chen, 2012). Therefore the effects of exposure are numerous; fundamental cellular functioning is inhibited, including a reduction in Rubisco activity and interruption of gene expression, leading ultimately to cell death (Carbonell et al., 1998; Shaibur et al., 2008; Ahsan et al., 2010).

Arsenate (As^{V}) has a nearly identical chemical structure to phosphate (Figure 1.4). This allows As^{V} to enter plant roots by mimicry through phosphate transporters. This has been shown with mutated *Arabidopsis* plants that gained arsenate resistance by lacking phosphate transporters in their roots (Shin et al., 2004). It has been theorized that arsenate's mode of toxicity involves replacing phosphate in key biochemical reactions, particularly in the production of ATP. Moore et al. (1983) provided evidence of this by showing the production of an unstable ADP-arsenate molecule in beef mitochondrial particles, suggesting that arsenate had replaced phosphate in the active site of ATP-synthase. Theoretically, by replacing phosphate in this metabolic reaction, arsenate has

uncoupled oxidative phosphorylation, halting ATP production, and this would eventually exhaust the cell (Gresser, 1981; Moore et al., 1983).

Arsenate's analogy to phosphate is relevant to the role mycorrhizae play in nutrient acquisition. It has been well-established that plants colonized by mycorrhizal fungi are resistant to soils contaminated with arsenic (Ahmed et al., 2006; Chen et al., 2007; Ultra et al., 2007; Xia et. al., 2007). For example, *Zea mays* watered with arsenic-laden water by Xia et al. (2007) produced an above-ground biomass in amounts roughly three times greater than non-inoculated controls. Furthermore, Gonzalez-Chavez et al. (2002) showed a 3-fold decrease in arsenic influx rates in *Holcus lanatus* grass when colonized. The exact mechanisms for resistance are likely a combination of several influences that mycorrhizal fungi have on their hosts. Most simply, it is likely that the additional phosphorus nutrition provided by mycorrhizae would aid in resisting a range of maladies, not specifically arsenic poisoning (Chen et al., 2007). Also, additional phosphorus is believed to give phosphate a competitive advantage during oxidative phosphorylation, enhancing the production of ATP (Zhao et al., 2009). And, there is evidence that plants colonized by mycorrhizal fungi begin to rely on their fungal partners for phosphorus acquisition, resulting in a reduction of phosphate transporters in the roots, reducing the channel for arsenate entry (Smith et al., 2003; Glassop et al., 2005; Chen et al., 2007). Furthermore, mycorrhizal fungi are able to discriminate between arsenate and phosphate. For example, Chen et al. (2007) used a compartmented pot system where arsenic-spiked soil was separated from plant roots by a fine mesh that only hyphae could penetrate, and found nearly an undetectable amount of arsenic was transferred to the plant host via hyphae. It has been theorized that hyphae transfer phosphates from soil to root in

the form of a poly-phosphate molecule; a poly-arsenate molecule would be unstable and should quickly dissociate (Solaiman et al., 1999; Smith et al., 2010).

The question as to whether mycorrhizal fungi themselves are resistant to arsenic has yielded mixed results; some researchers find no reduction of colonization in arsenic-contaminated soils (Liu et al., 2005; Trotta et al., 2006; Leung et al, 2006; Chen et al., 2007) while roughly an equal amount have seen reductions (Liu et al., 2005; Ahmed et al., 2006; Bona et al., 2010; Garg & Singla, 2012; Ahmed et al., 2012). However, mycorrhizal spore germination is usually impacted by arsenic (Gonzalez-Chavez et al., 2002; Xia et al., 2007). For example, Gonzalez-Chavez et al. (2002) found significant reductions in spore germination in *Glomus mossae* and *Gigaspora rosea* incubated in soils spiked with 50 mg arsenic per kg soil. However, spores isolated from a mine site actually showed an increase in germination (*Glomus caledonium*) or no reduction (*Glomus mossae*), suggesting that strains of mycorrhizal fungi are capable of developing metal resistance (Gonzalez-Chavez et al., 2002).

Figure 1.5 shows a compilation of results from studies that test the effects of arsenic on plants and mycorrhizae. Commonly-used growth variables in plants that are impacted by arsenic include root length, leaf area, chlorophyll production, root biomass and shoot biomass. All of these growth variables are impacted by arsenic levels far lower than those recorded in Baker Creek downstream from Giant Mine. Similarly, with the mycorrhizal fungi, spore germination and root colonization are reduced at arsenic levels below what has been recorded in Baker Creek.

1.6 The Effects of Water Quality on Plant Communities

Water quality is a well-known driver of patterns in plant species diversity: poor water quality tends to be associated with areas of lower diversity as more tolerant species gain a competitive advantage and sensitive species become excluded (Vörösmarty et al., 2010). For example, Del Rio et al. (2002) recorded an intense degradation of plant diversity along the Guadiamar River associated with the Aznalcollar mine in Spain. A pyritic sludge spill in 1998 heavily impacted plant species diversity within the vicinity; only five plant species were collected at the sample site closest to the exposure point, compared to the 76 species collected at a reference site further downstream (Del Rio et al., 2002). In addition to poor water quality, areas surrounding Giant Mine have also been exposed to atmospheric releases of arsenic, which will have an effect on the soil. Metal contaminated soils will affect plant species distributions. For example, a seed bank study on soils from a barium mine by Hernandez & Pastor (2008) found a significant negative correlation between species diversity and metal contamination and recorded a range of species richness from 13.7 to 20.0 between the most and least contaminated soils.

The impacts of mining on mycorrhizal fungi may also translate to the plant communities. Mycorrhizal fungi promote plant diversity by supporting the growth of subordinate species and preventing the spread of more aggressive plants; non-mycorrhizal plant species are given an advantage when mycorrhizal fungi are absent (van der Heijden et al., 1998a). And, mycorrhizal fungi support seedling growth by incorporating young roots into a pre-existing hyphal network, further enhancing plant diversity (van der Heijden, 2004). Diverse mycorrhizal communities have also been related to plant diversity; van der Heijden et al. (1998b) showed plant species richness

was doubled in plots with 14 types of mycorrhizal fungi compared to plots with only one mycorrhizal species. Given the role that mycorrhizal fungi play in supporting plant communities, there is a possibility that plant community structure around Giant Mine has been affected indirectly if mycorrhizal fungi have been impacted by arsenic.

Promoting plant diversity in Baker Creek is one of the goals of the Giant Mine Remediation Plan (AANDC, 2010). Plant diversity has been shown in many studies to enhance primary productivity (Loreau et al., 2001, 2002; Tilman et al., 2001; Balvanera et al., 2006; van Ruijven & Berendse, 2009). For example, in a constructed grassland experiment, Tilman et al. (2001) recorded 50% greater biomass production in plots with 16 plant species than in plots with two species over a four-year period. The consensus is that these researchers are observing a 'complementarity effect': plants each have slightly different mechanism for nutrient acquisition, and will obtain mineral resources from different localities within the same soil volume (Loreau et al., 2002). Therefore, increasing diversity puts into effect a 'niche partitioning' where plants are forced to use their own specialized nutrient-acquisition strategies (Tilman et al., 2001; Loreau et al., 2002; van Ruijven & Berendse, 2009). Researchers have speculated that the 'complementarity effect' should be most pronounced in nutrient-poor environments, such as those in Northern Canada (Loreau et al., 2002). More diverse plant communities are considered more 'stable', i.e. capable of better maintaining a consistent assemblage of plant species while resisting drought, poor water quality, and invasion from alien species (Balvanera et al., 2006). The Giant Mine Remediation Team has also expressed a desire for a diversity of native vegetation along Baker Creek for the functionality this provides (AANDC, 2010).

1.7 Objectives and Hypothesis

The ways that a mine can impact its natural surroundings are numerous. Effective remediation of a mine site must be guided by site-specific research (AADNC & MVLWB, 2013). There are several studies on the plant and mycorrhizal communities around mines at more Southern latitudes, but none on mines in the territorial Canadian North. This region is made distinct by a shorter growing season, lower energy inputs and slower nutrient cycles; the Canadian North is referred to as a 'fragile' ecozone, that is, one that takes particularly long to recover from disturbances (Bone, 2009). Therefore, studies on Southern mines do not necessarily apply to remediation of sub-arctic mines where growing conditions are different. This study on Giant Mine will contribute to a body of work on Northern mine remediation that is currently lacking. The focus is on Baker Creek, an arsenic-impacted watershed, which will now receive remediation. While arsenic in sediments is above the toxic levels where biological effects could be expected, there is at this point no concrete evidence that plants and mycorrhizal fungi have actually been affected. Therefore, the research question of this study is: *how have plant community structure and mycorrhizal activity in the Baker Creek watershed been affected by mining activities at Giant Mine?*

The objective of this study is to show how plant and mycorrhizal fungi communities have been affected by Giant Mine through relative differences between impacted and non-impacted areas. Sites downstream from the mine along Baker Creek are considered impacted areas where previous reports have found greatest amounts of arsenic. These have been compared to reference sites upstream from the mine, at nearby wetlands, and at Yellowknife River (Figure 1.1). Sites were assessed by a two-part study

with lab-based and field-based components. The lab-based study focuses on a bioassay where soils collected from these sites were used to grow plants in a laboratory setting. Growth responses of plants and mycorrhizal colonization in their roots were compared based on sites of soil collection after a six week growth period. Soils were characterized by arsenic and nutrient levels to correlate plant performance with possible abiotic factors that may have been imposed by the mine. The elements carbon, nitrogen, phosphorus and potassium were chosen for this analysis because these are the primary macronutrients required for plant growth. Although other metals exist in Baker Creek at biologically relevant levels (including cadmium, chromium, copper and lead), arsenic was chosen for our soil characterization because arsenic exceeds the levels of any other metal by several orders of magnitude in Baker Creek, and has been largely recognized as the most influential contaminant in this watershed (AADNC, 2010; Golder Associated Ltd. 2013). The field-based component involves contrasting vegetation surveys of sites around the mine to compare species present and their mycorrhizal statuses. The hypothesis of this study is that the bioassays will identify impacted areas through plants growing relatively smaller and with less mycorrhizal colonization than reference sites, and that this will be reflected in the field through different assemblages of plant species between impacted and non-impacted areas. Plants should grow relatively smaller in soils from areas more exposed to the mine and with less mycorrhizal colonization due to pressures from metal toxicity. This should be reflected in the field through more mycorrhizal plant species at reference sites that have been excluded from Baker Creek due to pressures from the mine.

2. Materials and Methods

2.1 Soil Collections and Bioassays

Soils for the bioassays were collected from nine sites in the Giant Mine area: five downstream from Giant Mine along Baker Creek, one upstream Baker Creek site, two nearby wetlands and one distant reference site (Figure 1.1). At each location, three transects were laid out that traversed the site from upland to wetland (with the exception of Reach 7 due to safety concerns with that area). Three 1 x 1 meter quadrats were placed along each transect: one upland quadrat, an intermediate quadrat, and an aquatic quadrat (Figure 2.1). These same quadrats were also used for the vegetation survey described later. A roughly 20 cm deep core was obtained with a spade from the center of each quadrat and stored in a polyethylene bag. Soils were frozen and shipped back to Wilfrid Laurier University.

Two species of plant were selected for our bioassays, *Epilobium angustifolium* and *Phalaris arundinacea*. *E. angustifolium* (common name; fireweed) is a dicotyledon facultative upland species native to the northern regions where we did our sampling (Figure 2.2A). *P. arundinacea* (common name; reed canary grass) is a monocotyledon facultative wetland species that has been introduced to the Northwest Territories (Figure 2.2B). These two species were chosen based on their prevalence along Baker Creek, high rates of germination and mycorrhizal status. Seeds for both species were collected at Reach 4 from Baker Creek. Seeds of these species were germinated in Petri dishes for three days of 25/18 °C, 16/8 hour, day/night cycle, respectively. Three-day-old seedlings were transplanted from Petri dishes directly to the bioassay to ensure a viable plant sample.

Soils for the bioassay consisted of a 4:1 sand : soil mixture to reduce compaction. Three replicates per soil sample were lightly packed into 50 mL centrifuge tubes with drainage holes drilled in the bottom. Soil was filled up to 40 mL mark (roughly 40 grams of soil per tube) to ensure plants were at the same level, and exposed roughly to the same amount of light. Only one seedling of *P. arundinacea* was used per replicate, however, *E. angustifolium* required three transplants per replicate because these seedlings had high mortality and three were required to ensure at least one survived the six-week period. If more than one survived, only the largest seedling was used in the final analysis. During the growth period, plants received a 25/18 °C, 16/8 hour, day/night cycle. Plants were watered every third day with ~2 mL of 1/64 strength Long Ashton nutrient solution, and watered daily with ~10 mL of 'soft' artificial freshwater (WEF & APHA, 2005). This solution most closely resembled water chemistry at the source of Baker Creek. *P. arundinacea* was harvested after four weeks because some roots had grown to the bottom of their tubes, and *E. angustifolium* after six weeks for the same reason.

2.2 Assessing Plant Performance From Bioassays

Growth parameters and biomass of plants were recorded immediately following harvest. Plant growth variables used for this assessment included: root length, root surface area and root fresh weight; shoot surface area, shoot fresh weight and shoot dry weight. Shoots and roots were separated and scanned with an Epson Expression 10000 XL Scanner, and WinRhizo Arabidopsis 2012d software was used to determine surface areas and length. Fresh weights were measured immediately after harvest with a Mettler Toledo NewClassic MF top loading balance. Shoot dry weights were measured after

several weeks in a drying oven at 40 °C. Dry weights of roots could not be obtained because roots went on to be examined for mycorrhizal colonization.

Roots were stained with a 20:1 vinegar : ink mixture by first clearing roots with KOH for 20 minutes at 95°C on a heating block, followed by immersion in ink at the same temperature and for the same time, and 30 minutes of de-staining in 90% diluted vinegar (Vierheilig et al., 1998). Roots were mounted on microscope slides and mycorrhizal colonization quantified by the methods described by McGonigle (1990), where the presence or absence of fungal structures (arbuscules, vesicles and/or hyphae) was determined in 100 fields of view at 200x magnification. This method recognizes that vesicles and arbuscules are originally produced from hyphae, so when scoring vesicles and arbuscules, they are additionally scored as hyphae so that hyphae also represents a measure of total colonization. Slides were examined by light microscopy with a Zeiss Jenaval light microscope, and images captured with Zeiss Zen (blue edition) Imaging Software.

2.3 Soil Characterization

Chemical and nutrient analyses were conducted by different methods depending on the elements being tested. First, all soils were dried for three days at 40 °C in a drying oven. The elements arsenic, phosphorus, and potassium were determined by first digesting one gram of soil in 14 mL of 20% hydrochloric acid and 20% nitric acid followed by dilution with 85 mL of Milli-Q water. The resulting solution was then diluted by ten times to ensure element concentrations were within the detection limit.

Samples were analyzed with a Perkin Elmer Optima 8300 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Method 200.2, described in EPA, 1991).

Available phosphorus was determined by a similar method; however, instead of an acid digestion, a less aggressive extraction was used: 2.5 grams of soil were shaken at 180 oscillations per minute in 25 mL of 1 M sodium bicarbonate solution at room temperature for 15 minutes, followed by analysis with ICP-OES (Jones & Benton, 2000). Soil pH was determined by mixing soil with deionized water in a 50 mL centrifuge tube, with only enough water to saturate the soil before the water level exceeded the height of the soil. This was mixed gently and allowed to sit for 30 minutes before measuring pH with an Accumet pH meter (Kalra, 1995).

Total nitrogen and carbon were measured by first grinding soil in a SPEX SamplePrep ball mill. Two milligrams of pulverized soil were measured with a Sartorius SE-2 ultra microbalance and placed in tin capsules. Tin capsules containing soil were loaded into a Perkin Elmer 2400 Series II Elemental Analyzer for carbon and nitrogen analysis (Braun, 2015).

Soil mycorrhizal spore density was determined with a sucrose extraction method described in Brundrett et al. (1996) where 20 grams of soil were washed through a stack of sieves including 300, 150, 90 and 45 μm meshes. Material from the latter three sizes were suspended separately with deionized water in 50 mL centrifuge tubes. These tubes were spun for five minutes at 2000 RPM in an Allegra X-12R centrifuge in order to remove debris. The pellets were re-suspended in 50% sucrose solution, followed by an additional round of centrifugation for one minute at 2000 RPM. Spores would then be suspended in the supernatant because this 50% sucrose solution has greater density than

mycorrhizal spores, which consist mostly of lipids. Therefore, the spores were easily extractable by decanting the supernatant onto a 0.45 μm filter. This was followed by a rinsing step in water to remove excess sucrose. This filter was then preserved in a moist Petri dish. The number of spores on each Petri dish was counted with a Zeiss Discovery V8 Stereomicroscope to estimate the number of spores per gram of soil.

2.4 Vegetation Survey

A vegetation survey was conducted at the same sites sampled for soil collections used in the bioassays. Nine sites were surveyed: five downstream from Giant Mine along Baker Creek, one upstream Baker Creek site, two nearby wetlands and one distant reference site at Yellowknife River (Figure 1.1). At each location, three transects were laid out that traversed the site from upland to wetland (with the exception of Reach 7, where only two transects were run due to a bear in the area). Plant species were identified within three 1 x 1 meter quadrats along each transect: one quadrat at the most upland location to catalogue grasses and upland species, one at the bottom in the most wetland location to capture emergent and submerged aquatic species, and one quadrat in the middle to capture the intermediate zone where upland transitions into wetland (Figure 2.1). Plant species were identified according to the species key provided by Porsild & Cody (1980). Henceforward, a 'unique' species refers to a plant species that was exclusively present at only one site. This survey also includes the wetland indicator statuses of all species, gathered from the USDA PLANTS database (USDA & NRCS, 2017), and the statuses of plants as Native, Introduced, or Sensitive as determined by the Government of Northwest Territories (GNWT, 2016b). Percent cover was visually

estimated for each species. A Shannon's diversity score for each site was also calculated by the following equation:

$$\text{Diversity} = -\Sigma\, p_i \ln(p_i)$$

where p_i 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.

2.5 Analysis of Mycorrhiza in Field-Collected Roots

To assess the mycorrhizal status of plants around Giant Mine, plant roots were collected during the month of August 2015 at six of the nine sample sites described in the vegetation survey. These sites are Reaches 0, 2, 4, 6, 7 and Yellowknife River. Roots from five individuals of every species present at these sites were collected. Roots were immediately transferred to 50% ethanol for preservation.

To quantify mycorrhizal colonization, roots were stained with a 1% Chlorazol Black solution by first clearing roots with KOH in a Thermo Scientific Lindberg Blue vacuum oven at 95°C for 90-120 minutes set to 25 inches Hg of pressure. A visual inspection step was taken to determine if roots had been adequately cleared because thicker roots required a longer, more rigorous clearing step whereas thinner roots required less time. When roots turned to a pale yellow color, this tended to be long enough of a clearing step for visualizing mycorrhizal structures inside the roots. Roots were then immersed in a 1% Chlorazol Black solution and were returned to the vacuum oven for the same amount of time as that of the clearing step. These staining methods are described by Brundrett et al. (1996); however, they were modified with the use of a vacuum oven rather than a heating block in order to enhance infiltration of KOH and

stain into the roots. Roots were mounted on microscope slides and mycorrhizal colonization quantified by the methods previously described (McGonigle et al., 1990).

2.6 Statistical Analysis

2.6.1 Lab Study

To test the effect of soil collection sites on plant performance from the bioassays, a nested ANOVA design was used following some adjustments to our sample scheme. First, the three replicate plants from each soil sample were nested within the quadrats from which those samples were collected. Aquatic quadrats were eliminated to reduce within-site variability, leaving only three upland and three lowland quadrats per site (in most cases the water was too deep to collect sediment from the aquatic quadrat). This left two lateral transects at each site: an 'upland' and a 'lowland' transect with three quadrats in each (two quadrats in the case of Reach 7 where only two transects were laid originally). Nesting quadrats within these new transects account for a possible effect of moisture. With each ANOVA, an effect test was also run; if nesting quadrats within transect had no significant effect on the model, this level of nesting was removed, leaving six independent soil samples per site (four in the case of Reach 7). However, an effect on the model would imply some effect of moisture, in which case plant performance between sites was compared separately for each transect. A breakdown of this revised sample scheme is provided in Figure 2.3.

Plant performance variables used in this analysis were shoot fresh weight, shoot dry weight, shoot surface area, root fresh weight, root length and root surface area. For *E. angustifolium*, root fresh weight and shoot dry weight were excluded because the

majority of these were below the detection limit of our top-loading balance. To meet the assumptions of an ANOVA, all data were either log or square root transformed to bring them to normality, and Bartlett tests were conducted to ensure equal variance. If a significant effect was found then a Tukey's post hoc was used to compare means between sites. Mycorrhizal colonization data from the bioassays was non-normal by every transformation, so the effect of site was tested with a non-parametric Kruskal-Wallis analysis for hyphal, arbuscular, and vesicular colonization. Comparisons between sites were conducted by a Dunn's test, and supplemented with Wilcoxon pairwise comparisons between all pairs of samples. The possible effect of moisture was tested for by individual pairwise comparisons of colonization data between upland and lowland quadrats for each site. Outputs for Shapiro-Wilks tests, Bartlett tests, ANOVA tables and effects tests are summarized in the Appendix.

To explain trends in plant performance, growth variables were correlated with the nutrient and arsenic levels of the soils that each plant was growing in. In almost all cases, these data violated the assumptions of parametric testing, so a non-parametric Spearman's correlation was used to relate nutrient and arsenic data with plant performance. To eliminate pseudo-replication in plant performance, the mean of the three replicate plants was taken for each data point used in the correlation. Data from quadrat three's were not eliminated from these correlations. Trends in mycorrhizal colonization were analyzed by these same methods. To test the effect of site on arsenic levels and spore density, arsenic data were subjected to an ANOVA followed by Tukey's post-hoc, and spore density analyzed by Kruskal-Wallis and a Dunn's comparison across sites, because these data were non-normal by every transformation.

2.6.2 Field-Study

Non-metric multidimensional scaling (NMDS) was used to distinguish sites based on the average percent coverage of plant species. NMDS produces an ordination of multivariate data based on a specified number of dimensions (or axes). A value of 'stress' is also given for NMDS analyses that is essentially a goodness of fit; adding dimensions reduces stress on the model, but will also make interpretation more difficult by adding more axes. A number of dimensions for NMDS was chosen based on a rule described by Peck (2010) where adding a dimension must reduce stress by at least of value of 5.0, and be accompanied by a Monte Carlo randomization test of $p < 0.05$. Plant species data by site were separated into transects to reduce stress on the model. In addition to NMDS, species diversity and species richness data were also correlated with mean arsenic levels by site using Pearson's product moment correlations.

To compare levels of mycorrhizal colonization in field-collected roots, mean hyphal, arbuscular and vesicular colonization was calculated by site for each mycorrhizal plant species. The effect of site on colonization levels was assessed by either ANOVA for normally distributed data followed by Tukey's post hoc, or Kruskal-Wallis for non-normal data followed by a Dunn's test. Also, mean levels of arbuscular and vesicular colonization in each mycorrhizal species were compared by paired t-tests (for normal data) or Wilcoxon tests (for non-normal data) in colonized roots only. All statistical analyses were performed with JMP Statistical Analysis Software version 11.0, except for NMDS, which was done with PC-ORD version 7 software.

3. Results

3.1 Plant Performance in Bioassays

All growth variables met the assumptions of parametric testing for both species, and there were no effects of nesting quadrats within transects (Tables A1, A2, & A3). With the exception of shoot dry weight, patterns in growth responses were fairly consistent in *P. arundinacea* across sites for all growth variables measured. Seedlings consistently grew larger in soils from Yellowknife River and Reach 7, seedlings from Reaches 0, 2, 4, 5, 6, and Pocket Lake were not significantly different, and Trapper Lake soils produced the smallest seedlings (Figure 3.1). For example, mean shoot surface area of plants grown in soils from Reach 7 and Yellowknife River was 4.19 ± 0.49 and 4.98 ± 1.18 cm^2 respectively, more than double the next highest value at Reach 6, where mean shoot surface area was 1.91 ± 0.26 cm^2. Similarly with mean root length, with soils from Yellowknife River and Reach 7 these values were 299.75 ± 53.95 and 332.99 ± 13.79 cm, respectively, and the next highest value again from Reach 6 was 159.72 ± 21.83 cm.

For *E. angustifolium,* plants consistently grew larger in soils from Yellowknife River than in soils from all other sites. This trend of plants growing largest in soils from Yellowknife River was consistent for all growth parameters across roots and shoot. Mean shoot fresh weight from Yellowknife River soils was 31.88 ± 8.28 mg, and the next highest mean was from Reach 4 at 6.48 ± 1.64 mg. Mean root length was 44.41 ± 11.06 cm in Yellowknife River soils, a value nearly four times greater than the next longest root length, again from Reach 4, at 11.19 ± 3.94 cm (Figure 3.2).

Hyphae, arbuscules and vesicles were observed in the roots of both species (Figure 3.3). Mean mycorrhizal colonization was less than 7% at all sites except for

Reach 4 and Yellowknife River for both species (Table 3.1 & 3.2). Few plants grown in soils other than from these sites were colonized, 15 plants for *P. arundinacea* roots and five for *E. angustifolium.* In a Kruskal-Wallis ranking of sites, Reach 4 and Yellowknife River ranked higher than all other sites for hyphal, arbuscular and vesicular colonization in both species. But within-site variability was high. For example, mean hyphal colonization in *P. arundinacea* at Yellowknife River was 13.44 ± 8.94%; however, the upper and lower 95% confidence interval around the mean ranged from 36.41 to -9.52%. This made detecting differences between sites difficult; a Dunn's test could not detect differences between Yellowknife River and sites where colonization was 0% (Figure 3.4). However, pairwise comparisons between Yellowknife River and all the other sites showed significantly greater hyphal colonization than the three lowest sites, Pocket Lake, Trapper Lake and Reach 0. This is also true for arbuscular colonization; however, vesicular colonization was low across all sites and there were no significant differences. In *E. angustifolium* a Dunn's test was only sensitive enough to detect differences among sites for hyphal colonization, where Reach 4 plants were higher than plants growing in soils from all other sites except Yellowknife River and Reach 6 (Figure 3.5). In terms of pairwise comparisons, only hyphal colonization was high enough to detect differences between sites, where Reach 4 plants were significantly more colonized than plants growing in soils obtained from the other sites except from that of Yellowknife River. There were no differences among sites for arbuscular and vesicular colonization (Table 3.2).

Spearman's correlations showed that arsenic, carbon and nitrogen had a significant relationship with root length for both species (Table 3.3 A,B). Arsenic had the

strongest correlation with root growth for both species, yielding Spearman's **ρ**-values of -0.609 and -0.530 for *E. angustifolium* and *P. arundinacea*, respectively. Low levels of mycorrhizal colonization made correlations difficult because the vast majority of roots were uncolonized. In *P. arundinacea*, a significant negative correlation was only observed with nitrogen, but with *E. angustifolium*, arsenic, nitrogen and carbon were negatively correlated with colonization, and potassium and total phosphorus were positively correlated (Table 3.3 C,D).

Spearman's correlations between all combinations of soil-nutrient and arsenic data revealed some strong relationships (Table 3.4). Of these, carbon and nitrogen had the strongest because these elements coincide in organic soils. The next strongest was between arsenic and carbon, yielding a **ρ**-value of 0.640, indicating that organic soils contained high levels of arsenic. Arsenic was not correlated with pH levels. Site of soil collection was significantly affected by arsenic levels; Reach 4 and Yellowknife River had significantly less arsenic than all other sites (Figure 3.6).

Although no efforts were made to identify mycorrhizal species, a brown and a yellow morphotype for mycorrhizal spores were observed the most frequently (Figure 3.7). Within-site variability in spore density made detecting an effect of site difficult; although spore density at Reach 4 could be as high as 70 spores per gram, a Dunn's test only found that spore density at Reach 4 was greater than Reach 0 and Reach 2 (Figure 3.8). Overall, spore density was low at all sites; with the exception of Pocket Lake, mean spore density was less than six spores per gram in all other soils.

3.2 Vegetation Survey

A complete list of all plant species identified is shown in Table 3.5. Thirty-two plant species were identified in total across 19 families. Twenty-two species, comprising the majority of plants, were obligate wetland plants, although some facultative species were identified in upland quadrats. Two species designated as 'Sensitive' by the GNWT were identified: *Alisma triviale* at Reach 4 and *Potamogeton pectinatus* at Reach 2. Most other species were native plants, except *Melilotus alba*, *P. arundinacea*, *Sonchus arvensis* and *Trifolium repens*; each is designated as 'Introduced' and all four found only at Reach 4. The three most common species overall were *Carex aquatilis, Equisetum fluviatile* and *Typha latifolia*. Table 3.6 lists Shannon's species diversity scores and species richness of all sites sampled; Yellowknife River had the greatest diversity score although not the highest species richness, the most species-rich site was Reach 4. However, no significant correlation was found between Shannon's diversity or species richness against mean arsenic levels (Figure 3.9).

Figure 3.10 and Table 3.7 show the results of the non-metric multidimensional scaling of our vegetation survey. The first three dimensions were retained for analysis. Axes 1, 2 and 3 explained 33.4, 34.7 and 13.6% of the variance respectively; only the first two are represented in the bi-plot. Four groups are delineated in the bi-plot: Baker Creek sites, nearby sites, Reach 4 and Yellowknife River. Reach 4 and Yellowknife River separated along the second axis because of the species exclusively found at these sites. For example, the species only found at Yellowknife River, *Carex rostrata, Sagittaria cuneata, Sium suave* and *Sparganium hyperborium* all were negatively associated with the first and second axis, and for this reason transects from Yellowknife River can be

found in the bottom left quadrant of the bi-plot. Similarly with Reach 4, the exclusive presence of *A. triviale*, *Juncus alpinus*, *M. alba*, *P. arundinacea*, *S. arvensis* and *T. repens* positively associated with the two axes is the reason why Reach 4 transects can be found in the upper right quadrant. The remaining sites are grouped together along the second axis because of similar species distributions: dry areas typically consisted of the grasses *Calamagrostis canadensis* and *Agrostis scabra* where wetter areas were dominated by *C. aquatilis*, *E. fluviatile* and *T. latifolia*. Separation of these sites along the first axis is related to some unique species at these sites. For example, *Calla palustris* and *Potamogeton richardsonii* at Reach 7 and Reach 0, respectively, gave these sites a negative association with the first axis; *Nuphar variegatum* found exclusively at Pocket Lake gave this site a strong positive score on the first axis. Arsenic was associated with many of the Baker Creek transects by sharing a similar neutral association with the second axis and negative relationship with the first axis. Potassium was associated with Reach 4 because this is where the highest potassium levels were recorded.

3.3 Analysis of Mycorrhiza in Field-Collected Roots

Table 3.8 contains a list of all plant species collected for mycorrhizal analysis and the sites where colonization was observed. Only 5 of our 17 species were colonized: *C. canadensis*, *Equisetum arvense*, *E. angustifolium*, *P. arundinacea* and *Potentilla fruticosa*. *C. canadensis* was the only mycorrhizal plant present at all sites, and the only species colonized at Reach 0. Although hyphal colonization was fairly even for this species, site did have an effect on percent colonization, which was significantly higher at Reach 0 and Reach 6 than at Reach 2 (Table 3.9). Site also had an effect on hyphal

colonization for *E. arvense*, which was significantly higher at Reach 4 than at Reach 0 and Reach 2. Patterns of colonization for *E. angustifolium* did not match those obtained with the bioassays, this species had greater colonization at Reach 6 than at Reach 4. *P. arundinacea* was only present at Reach 4, so across site comparisons could not be made with this species. *P. fruticosa* had greater colonization at Reach 7 than at Yellowknife River (Table 3.9). In general, vesicular colonization was low to detect differences between sites; site only had an effect on vesicular colonization in two species (Table 3.10). *E. arvense* had greater colonization at Reach 4 than at Reach 0, where colonization was absent; this was consistent with the results obtained with hyphal colonization. *P. fruticosa* had greater vesicular colonization at Reach 7 than at Yellowknife River (Table 3.10). In most species, arbuscular colonization was too low to detect differences between sites. However, *E. arvense* had more arbuscular colonization at Reach 4 than at all other sites, and *P. fruticosa* had greater colonization at Reach 7 than at Yellowknife River (Table 3.11).

Hyphae, vesicles and arbuscules were observed in field-collected roots (Figure 3.11). Table 3.12 shows vesicular and arbuscular colonization in both bioassay and field-collected roots. Arbuscular colonization was generally higher in bioassay roots. Mean arbuscular colonization in *P. arundinacea* was 10.42 ± 1.51%, over ten times greater than vesicular colonization, which was 0.95 ± 0.37%. In field-collected roots vesicular colonization tended to be greater than arbuscular, with four of the five species having significantly more vesicles than arbuscules. For example, mean vesicular colonization in *E. angustifolium* was 19.10 ± 3.26% yet no arbuscules were observed.

4. Discussion

4.1 Plant Performance in Bioassays

Root length and shoot surface area of *P. arundinacea* was significantly greater in soils from Yellowknife River and Reach 7, our reference site and upstream Baker Creek site, respectively. *E. angustifolium* seedlings only grew larger at Yellowknife River; the vast majority of the other *E. angustifolium* seedlings barely survived the 6-week growing period and probably would have died if harvested at a later date. *E. angustifolium* is evidently a more sensitive species than *P. arundinacea*, which is an aggressive invasive grass species well-known for withstanding disturbed conditions and dominating entire wetlands (Lavergne & Molofsky, 2004). Overall, results from the bioassays were consistent with the hypothesis that plants would grow larger in soils from Yellowknife River and upstream of the mine, indicating some sort of stressor at other sites likely imposed by Giant Mine.

Arsenic is likely the strongest stressor in our soil samples. This is indicated by the negative correlations with root length in both species; arsenic had the strongest correlation with plant performance of all the soil variables measured. Furthermore, Yellowknife River soils had one of the lowest arsenic levels and mean root length was highest at this site. At the other low-arsenic site, Reach 4, plants did not grow in a manner significantly different from that of plants growing in soils from other Baker Creek sites. However, there are other signs of disturbance apart from arsenic at Reach 4 that are discussed later.

Our two nearby wetland sites, Trapper and Pocket Lake, had more arsenic than expected; these were supposed to act as reference sites but plants grew roughly the same

size as those growing in soils from the Baker Creek sites. Evidently arsenic from the mine is wide-spread rather than restricted to Baker Creek. For example, Trapper Lake is directly adjacent to the Northwest Tailings Pond, the single most contaminated tailings pond on Giant Mine property. Arsenic seepage from the pond has been recorded in the past, and is likely the major source of arsenic found at Trapper Lake (AANDC, 2010). Pocket Lake, located roughly one kilometer Northwest of the mine site, is in the direction of prevailing winds carrying arsenic dust from the mine. For this reason, many of the small lakes to the northwest of Giant Mine have elevated levels of dissolved arsenic; a 2016 study showed dissolved arsenic levels exceeding 100 µg/L in the small lakes northwest of Giant Mine, and lakes an equivalent distance to the Southeast were all below 50 µg/L (Palmer et al., 2015). Arsenic is especially concentrated in these small lakes isolated within the Canadian Shield due to poor drainage; with the exception of a brief period during winter melt off, Pocket Lake is only maintained by runoff, evaporation and precipitation (Mielko & Woo, 2006). Thienpont et al. (2016) recorded arsenic levels that exceeded 30,000 mg/kg in sediment cores from Pocket Lake.

Arsenic tends to accumulate in fine-textured soil due to a high number of sorption sites and the negative charge of clay (Fitz & Wenzel, 2002). But in this study, arsenic was most present in our organic soils. This is apparent because the single strongest correlation of this entire study is that between carbon and arsenic. Northern peatlands are ideal for the accumulation of arsenic because ecosystems at these latitudes are largely considered to be 'carbon sinks' where rates of organic carbon accumulation exceed rates of decomposition, and consequently, atmospheric arsenic that settles in organic soils becomes immobilized (Rothwell et al., 2009). Inorganic forms of arsenic are taken up by

plants and fungi and incorporated into organic compounds such as monomethylarsonic acid, dimethylarsinic acid, or more complex organic molecules such as arsenobetaine (Koch et al., 2000a, 2000b; Rothwell et al., 2009). At Northern latitudes where rates of decomposition and nutrient cycles are especially slow, organic soils will accumulate arsenic to a greater extent than mineral soils (Rothwell et al., 2009). This is why strong negative correlations were also observed between root lengths and carbon.

That arsenic would reduce root length is consistent with the findings of Singh et al. (2007) who observed that the root length of *Phaseolus aureus* was reduced by half when arsenic in a hydroponic system was increased by a factor of five (from 10 μM to 50 μM). These researchers also recorded evidence of lipid peroxidation in root cells and concluded that arsenic was exerting toxicity by disrupting cellular membranes, because of increased cellular malondialdehyde concentrations and electrolyte leakage (Singh et al., 2007). Malondialdehyde is an indicator of oxidative stress because these molecules result from the peroxidation of the unsaturated fatty acids that compose lipid membranes (Singh et al., 2007). Mascher et al. (2002) obtained similar findings when studying arsenic's effect on *Trifolium pratense*, where increased oxidative stress with increasing arsenic led them to conclude that arsenic exposure results in lipid bilayer degradation.

Similar to our root data, shoot surface area was significantly greater in plants growing in reference soils. Arsenic is sequestered in root cells before its translocation to the shoots, therefore reductions in shoot surface area are likely an indirect result of arsenic exerting toxicity on the roots (Zhao et al., 2009). A reduced root system would be less capable of supporting above-ground biomass production. For example, Carbonell et al. (1998) watered *Spartina alterniflora* plants with 2.0 mg/L arsenic solution and

measured arsenic concentrations of over 500 mg/g dry weight in the roots and less than 12 mg/g in the shoots. Similarly, Quaghebeur & Rengel (2004) when studying *Arabidopsis thaliana* found significant reductions in shoot biomass production at soil arsenic concentrations of 100 mg/g, yet less than 3% of this arsenic had been translocated to the shoots. These findings relate to plants' defense against metal toxicity; regardless of what form of arsenic is taken from the soil, these molecules are reduced to arsenite (AsO_3^{3-}) and sequestered by phytochelatin compounds in root-cell vacuoles; thus they are prevented to translocate to the shoots (Zhao et al., 2009).

Mycorrhizal colonization was lower than expected; the original hypothesis was that colonization would be depressed in Baker Creek sites, yet in many cases colonization was completely absent. However there were two distinct sites: Yellowknife River, our reference site, and Reach 4, a reconstructed channel of the creek where greater mycorrhizal colonization is probably related to the lower levels of arsenic; however, this could not be said definitively without further experimentation. For example, spore density in Yellowknife River soil is not significantly different from that at sites where mean colonization was less than 2%, suggesting that this amount of spores is sufficient to cause colonization. And spore germination can be interrupted at arsenic levels comparable to those found in Baker Creek; Gonzalez-Chavez et al. (2002) found that 0.05 mg/g of total arsenic per gram of soil was sufficient to reduce rate of hyphal emergence.

Not knowing exactly to how much arsenic plants and mycorrhizal fungi are exposed is one of the limitations of our bioassays. Soil was mixed with four parts sand, which dilutes the amount of arsenic exposure by a factor of five. If soils had been left

undiluted, we could have expected trends in the bioassay to be more pronounced. And we only have a measure of total arsenic determined by acid digestion, but determining bioavailable arsenic is difficult because arsenic's mobility is variable between different types of soils, and different species of plants can chemically modulate their rhizosphere to different extents (Anawar et al., 2008). Gonzalez-Chavez et al. (2002) approached this problem by using a clay substrate in their mycorrhizal spore germination assay. Arsenic's mobility is reduced in fine-textured clay due to the negative charge and high number of sorption sites; this imitates field conditions where arsenic is not completely available. Under these conditions, arsenic levels of 0.05 mg/g was sufficient to impact spore germination, a level lower than all sites except those of Yellowknife River and Reach 4. So consistent with the original hypothesis, sites with lower arsenic levels were the ones with the greatest colonization. However, directly relating arsenic levels to mycorrhizal colonization cannot be done without further experimentation on bioavailable arsenic.

4.2 Vegetation Survey

The bioassays distinguished Reach 4 and Yellowknife River for colonized roots and low arsenic levels. These sites are distinguished again in our vegetation survey. In non-metric multidimensional scaling Reach 4 and Yellowknife River have been separated from other sites along the second axis. This separation is due to the presence of unique species at these sites.

Six species were identified exclusively at Reach 4 and at no other sites: *A. triviale*, *J. alpinus*, *M. alba*, *P. arundinacea*, *S. arvensis*, and *T. repens*. Several of these species may have established at Reach 4 because this is a reconstructed channel of the

creek. For example, *M. alba*, *S. arvensis*, and *T. repens* are all species classified as 'Introduced'. None of these species were included in the seed mix used for the revegetation of Reach 4 following construction (Hewitt, 2007), and all three are synonymous with moist, recently disturbed areas such as roadside ditches and construction sites (Reznicek et al., 2011). These species may themselves be indicators of disturbance related to construction even though Reach 4 had the lowest arsenic levels of any sites. Another indicator of disturbance is potassium which is strongly associated with Reach 4, potassium likely indicates the influence of the road and may be related to road maintenance. Reach 4 is directly adjacent to the Ingraham Trail; salt from the road is the likely source of potassium. However, the 'road effect' is a well-documented source of contamination in ways that extend beyond road salts, including the introduction of asbestos, cadmium, copper, hydrocarbons and lead, especially for roads that run directly through mine sites (Coffin, 2007).

Yellowknife River also had several unique species: *C. rostrata*, *S. cuneata*, *S. suave*, and *S. hyperborium*. Whether these are arsenic-sensitive species that have been excluded from Baker Creek is hard to say without experimentation. It is well-known that habitat is the strongest driver of species distributions (Guisan & Zimmermann, 2000). Ecological models will use 'direct' and 'indirect' habitat variables weighted based on their importance to predict species distributions. 'Direct' habitat variables, and arguably the most influential, are those that directly determine the suitability of an area for certain plant species to live in; these include nutrient availability, soil moisture, temperature and Photosynthetically Active Radiation. 'Indirect' habitat variables are those that influence

the previously listed variables; examples are wind, cloudiness/canopy cover, precipitation and soil texture (Guisan & Zimmermann, 2000).

Many of these habitat variables become redundant when comparing Yellowknife River to Baker Creek because these two sites are only four kilometers apart. Still, there were some differences in soil characteristics that may have influenced species establishment; for instance, carbon and nitrogen were generally lower at Yellowknife River than at Baker Creek, indicating lesser amounts of organic material in this substrate. There was also a substantial canopy cover at Yellowknife River that may have favoured species specialized for understory living. And Yellowknife River is part of a much larger watershed than Baker Creek; there is simply more potential for different species upstream to have dispersed seeds that could have established at our sample site. Nonetheless, nearly undetectable arsenic levels were the defining difference between Yellowknife River and most of our other sites. Whether this alone is the reason that four of the nine species here were unique to Yellowknife River cannot be said definitively without further study. Comparing the arsenic tolerance in seed germination trials between seeds collected at Yellowknife River and at Baker Creek, and subsequently the seedling performance, would help answer the question as to whether Baker Creek plants are simply arsenic-resistant. Plants are capable of developing arsenic resistance through the suppression of genes that encode high-affinity phosphate transporters in the roots (Gonzalez-Chavez et al., 2002). However, to my knowledge this has never been reported in any of the species we found along Baker Creek. To conclude, I believe the assemblage of different species at Yellowknife River is the product of both habitat variables and lower amounts of arsenic working together to create a distinct environment from Baker Creek.

The remaining sites are grouped together because of their similar plant species. Within these sites dry areas were mostly dominated by the grasses *A. scabra* and *C. canadensis*, whereas wetter areas were dominated by *C. aquatilis*, *E. fluviatile* and *T. latifolia*. These were the most common species in our vegetation survey in terms of abundance and presence at most sites. This is consistent with the vegetation survey of Baker Creek performed by Jacques Whitford Ltd. (2003), whose three most common species were *C. aquatilis*, *E. fluviatile* and *T. latifolia*. All three are common wetland species across the entire continent. The vegetation survey from Jacques Whitford Ltd. (2003) contains fewer species than ours does, only 18 species as opposed to our 32; however, theirs was done three years before the realignment at Reach 4, at which time 11 new grass species were introduced. Additionally, Jacques Whitford Ltd. (2003) did not survey Yellowknife River like we did, which itself included several unique species.

Typical of Northern ecosystems, the Baker Creek watershed has apparently lower species diversity than similar sites at more southern latitudes. In a seed bank study of a mine impacted area in Spain, Hernandez & Pastor (2008) recorded species richness values of at least 13.7 at all of their sites, with their highest being 27.0; our species richness values ranged from 6 - 18. In a similar study on a mine-impacted watershed in Spain, Del Rio et al. (2002) recorded a range in species richness of 5 - 73 between their most and least contaminated sites. The objective of our vegetation survey was to relate signs of disturbance with reduced species diversity, however, the inherently lower species diversity of Northern climates reduces the sensitivity of our assessment. This made drawing correlations between diversity and arsenic levels difficult. Detecting a

relationship with arsenic levels would have been more likely if sites had been more species-rich.

However, the distributions of some individual species were consistent with this original objective. One such species, *A. triviale* is classified as 'Sensitive', and was only found at Reach 4, a low-arsenic site. Its classification as 'Sensitive' does not imply the species is at risk; however, it designates it as a species that could become endangered if not given special attention (GNWT, 2016b). Jacques Whitford Ltd. (2003) also recorded trace amounts of *A. triviale* (less than 5%) in an isolated area upstream of Reach 6, the point of mine water discharge. Jacques Whitford Ltd. (2003) conducted their survey three years before the redirection at Reach 4. They likely found the source of seeds for *A. triviale* that was recorded in our survey. Being a sensitive species, *A. triviale* may not be able to establish at sites downstream from Reach 6; however, since Reach 4 had been redirected, there is now a low-arsenic area where seeds travelling downstream could establish. In a similar case, *C. palustris* is a species recorded by both ourselves and Jacques Whitford Ltd. (2003) upstream from Giant Mine. This could be another species incapable of establishing at downstream Baker Creek sites because of metal levels. In terms of future studies, seedlings survivorship trials under arsenic exposure would clarify whether *A. triviale* and *C. palustris* are sensitive species being excluded from sites downstream of the mine.

4.3 Mycorrhizal Status of Plants in Baker Creek

One objective of this study was to relate results from the bioassays to patterns in plant species distributions along Baker Creek. We tested the hypothesis that soils where

mycorrhizal fungi have been impacted would produce plants with lower levels of colonization in our bioassays, and that this pressure would select for non-mycorrhizal plant species in our vegetation survey. Our findings were not consistent with this hypothesis; the vast majority of plants, even at our reference sites, were non-mycorrhizal plant species. For example, soils from Yellowknife River produced some of our most colonized plants, however, this site was dominated by the non-mycorrhizal sedges *C. aquatilis* and *C. rostrata* (Muthukumar et al., 2000). Some unique mycorrhizal plant species were found at Reach 4 and may have been the source of propagules that colonized plants in the bioassays: *M. alba*, *P. arundinacea*, *S. arvensis* and *T. repens*. However, rather than pressures from arsenic, their establishment at Reach 4 is more likely related to the reconstruction of this channel in 2006; *P. arundinacea* was included in the seed mix used for revegetation, and as previously discussed, the remaining three species are synonymous with recently disturbed areas such as construction sites and road-side ditches (Reznicek et al., 2011).

Plant roots from Baker Creek were analyzed with the objective of comparing levels of colonization among sites. But comparing sites became difficult because so few plants were actually colonized: only five of the 17 species collected showed colonization. Of these five, only two species were colonized across more than two sites: *C. canadensis* and *E. arvense*. Roots of *C. canadensis* were less colonized at Reach 2; however, this is inconsistent with plants from the bioassays, where colonization of plants grown in soils from Reach 2 was not significantly lower than many of the other sites.

E. arvense roots had significantly greater colonization at Reach 4 than at Reach 0 and Reach 2, consistent with the findings of the bioassay. Again inconsistent with the

bioassays, *E. angustifolium* were more colonized at Reach 6 than at Reach 4. However, *E. angustifolium* is a perennial species; we did not discriminate between ages of plants when collecting roots, so Reach 6 plants may have been older and had more time for colonization to develop. Similarly with *P. fruticosa*, inconsistencies between the bioassays may be the product of different ages of plants and not necessarily the sites they were collected.

Precaution must be taken when comparing mycorrhizal colonization from the bioassay to that of field-collected roots. There is an issue of host-specificity when comparing levels of mycorrhizal colonization across different plant species. Several researchers have demonstrated mycorrhizal host-specificity through significant differences in biomass production and nutrient acquisition when single species of plants are inoculated with different types of mycorrhizal fungi (van der Heijden et al., 1998a; Klironomos, 2000). However, these researchers routinely show that at least some interaction occurs between plant and mycorrhizal fungi. For example, Klironomos (2000) crossed ten species of plant with ten mycorrhizal fungi and found only four of these one hundred combinations were incompatible. So in light of these findings, we would have expected at least some colonization in roots of the bioassays in soils from Reach 0, 5 and 7, given that field-collected *C. canadensis* was colonized at these sites. But field-collected roots overall had more potential to be colonized because these were collected at the end of the growing season and had several more weeks to interact with mycorrhizal fungi. So discrepancies between bioassays and field-collected roots may be a combination of differences in amount of time to interact with mycorrhizal fungi, and a lack of compatibility between host plant species. The Giant Mine Remediation Team

should also consider the diversity of belowground fauna to enhance the diversity of plant species during revegetation efforts.

Of all the five mycorrhizal plants collected, *P. arundinacea* was one of our most heavily colonized species. This species is known to adopt growth forms that maximize resource capture, making it an exploiter of productive environments; its association with mycorrhizal fungi is one of the reasons why this invasive species is such an effective competitor (Lavergne & Molofsky 2004). *C. canadensis* and *E. angustifolium* are two northern species whose association with mycorrhizal fungi has been recorded by other researchers in habitats similar to that of Baker Creek (Malloch & Malloch, 1982). The mycorrhizal status of the *Equisetum* genus has been a topic of debate, with some researchers in the past classifying this genus as non-mycorrhizal, while others argued lack of colonization was only the result of this species' preference for moist areas (Dhillion, 1993). However, *E. arvense* from our field-collected roots were consistently colonized. Our finding is similar to the findings of Dhillion (1993) whose field collected roots ranged in colonization from 32 - 42%. Dhillion (1993) also recorded traces of colonization in *E. fluviatile*, although no higher than 5%. Dhillion (1993) discusses this finding as unusual because this is a strictly aquatic species, and suggests that colonization occurred when water levels were lower; he proposes that what he observed are actually non-functional remnants of mycorrhizal structures. In contrast, our samples of *E. fluviatile* were collected from the water channel and there was no colonization, although there seems to have been a potential for colonization if roots were collected at a time when water levels were lower.

Another mycorrhizal species with potential for colonization was *T. latifolia*, although no traces of mycorrhizal structures were observed in its roots. Again, root samples may have been collected from areas where moisture was too high to support mycorrhizal development. This would be consistent with the findings of Ray & Inouye (2006) who found a direct negative correlation between flooding and mycorrhizal colonization in *T. latifolia*. *J. alpinus* is another species with potential for colonization because this was found at Reach 4, a site where other plants showed mycorrhizal colonization. However, roots were collected from an area possibly too wet to support mycorrhizal growth, as this plant is know to be capable of forming associations with mycorrhizal fungi (Turner et al., 2000).

P. arundinacea from the bioassays had a significantly greater number of arbuscules than vesicles, where *E. angustifolium* had similar levels of both. Production of vesicles and arbuscules is under the control of the host plant, so differences in types of colonization may be the result of a different symbiotic physiology for the two species (Strack et al., 2003). For example, arbuscules are only produced during times of nutrient exchange between fungus and host because of the energy demanding process required for their formation; arbuscules only exist for a maximum of 10 days before they are digested (Strack et al., 2003). In the bioassays, roots may have been harvested during a time that favoured arbuscular colonization in *P. arundinacea* but not in *E. angustifolium*.

Field-collected roots seemed to favour vesicular colonization where plants from the bioassays had more arbuscules. For example, four of our five mycorrhizal plant species from the field had significantly greater number of vesicles than of arbuscules. This is likely an effect of seasonality. Our bioassays essentially represent the beginning

of the growing season because seedlings were only grown for six weeks. Field-collected roots represent the end of the growing season because plants were collected in late August. Seasonality can affect the types of mycorrhizal structures produced (Smith & Smith, 1997; Sigüenza et al., 1996; Lutgen et al., 2003; Lingfei et al., 2005). Other researchers have found decreased arbuscular colonization later in the season (Sigüenza et al, 1996; Lutgen et al., 2003). Arbuscules are the primary location of nutrient exchange, where vesicles are lipid-rich structures used for energy storage (Harrison, 1999; Strack et al., 2003). Plants may favour arbuscule production during the rapid growth period of their seedling stage, and then favour the production of vesicles towards the end as plant and fungus prepare for dormancy. Whether this is the case in Baker Creek could be tested in the future through multiple root collections at different dates in the growing season.

4.4 Summary and Future Directions

Baker Creek is a mine-impacted watershed well-known for arsenic contamination. Previous reports have shown arsenic in sediments far above the levels that are considered biologically relevant, but this study provides the first piece of evidence that plants and mycorrhizae have actually been affected. This was done through contrasting sampling of heavily impacted downstream sites, less impacted upstream sites, nearby wetlands and a reference site further away. Soil samples were collected to grow plants in a laboratory setting to compare their responses. Vegetation surveys were conducted at these sites to assess plant species distributions relative to the mine, and roots were collected to assess the mycorrhizal status of plants. The objective was to relate patterns in plant and mycorrhizal biology to the abiotic factors that may have been imposed by the mine. The

overarching hypothesis was that impacts from the mine would be apparent in our bioassays through plants growing relatively smaller and with less mycorrhizal colonization in soils collected downstream from the mine due to pressures from arsenic, and this would be consistent in the field through lower plant species diversity at these sites, tending towards non-mycorrhizal plant species.

Results from the bioassay were consistent with our hypothesis. Plants grew significantly larger in soils collected upstream from Giant Mine and our reference site. A strong negative correlation between total arsenic and root length was also observed, indicating that arsenic levels may have been impacting plant growth. However, knowing exactly how much arsenic the plants were exposed to would not be possible without further analysis on arsenic bioavailability. This also applies to our assessment of mycorrhizal colonization; the two sites with the lowest total arsenic levels also supported the most colonized plants. But determining if arsenic levels were sufficient to interrupt spore germination is not possible without tests on bioavailability. These results are still largely consistent with the original hypothesis; reference soils from Yellowknife River had the least amount of arsenic, supported the largest seedlings, and high levels of colonization. The highest colonization was found at Reach 4, another low-arsenic site, but other signs of disturbance are present here related to construction.

Our original prediction was to find greater species richness in sites distinguished by the bioassays; soils producing the largest and most colonized plants should also support unique species that have been excluded from Baker Creek either because of low arsenic tolerance or because of mycorrhizal dependency. But the inherently low species diversity of this Northern ecosystem reduced the sensitivity of the assessment. There

were unique species at Yellowknife River, but contrary to the hypothesis, these were non-mycorrhizal plant species. Unique species were also identified at Reach 4, but these were invasive plants that probably established here following reconstruction. Too few mycorrhizal plant species were present, even at our reference sites, to draw comparisons of mycorrhizal colonization between sites under field conditions.

To conclude, this study provides evidence of disturbed growing conditions along Baker Creek and an absence of mycorrhizal activity. However, the hypothesis that mycorrhizal plant species are being excluded from these areas was not supported by our vegetation survey. Evidence would suggest that arsenic is underlying the patterns observed in our bioassays, but this cannot be said definitively without further experimentation. For example, one could conduct an experiment whereby mycorrhizal spores collected from Baker Creek along with an assessment of bioavailable arsenic levels. Such an experiment could be used to determine if arsenic levels found along the creek are sufficient to impact mycorrhizal spore germination. A similar study could be done on the plants, such as another bioassay of Baker Creek soils side-by-side with an arsenic exposure to plants growing in sterile sand. Similar results between these two experiments would imply arsenic contamination in Baker Creek is driving the patterns observed in this study.

5. Significance of this Study

5.1 Northern Research

Mineral extraction projects have only existed in the Northwest Territories since the 1950's. Mining is a relatively young industry in this region compared to other places

where mining has been practiced for hundreds of years. This makes Giant Mine one of several mines that have only closed down recently and for which reclamation must now be considered. However, ecological studies on mines in the Canadian North are very few. Other studies have recorded arsenic levels in the environment around Yellowknife, including a series of papers by Koch et al. (2000a,b; 2005) and more recently an assessment of arsenic in lake waters and sediments by Thienpont et al. (2016) and Houben et al. (2016). However, my study is the first to directly investigate the influence Giant Mine has had over vegetation communities as a result of arsenic emissions.

Our vegetation survey of Baker Creek has been the first to relate plant species distributions to the location of Giant Mine, unlike the study by Jacques Whitford Ltd. (2003) who only catalogued species. Furthermore, ours is different because it also incorporates a full soil characterization which relates arsenic levels to plant species at each sample location. And our incorporation of a bioassay is a technique seldom used to assess soil conditions around disturbed areas, and is certainly the first to have been used to assess a mine in Northern Canada. Evaluating the infectivity of mycorrhizal fungi in our soil samples was one of the goals of this bioassay. Our consideration of mycorrhizae is another unique aspect of this project. Mycorrhizae have increasingly been recognized as an integral part of the remediation of mine-disturbed areas because of their ability to support plant growth in adverse conditions. Mycorrhizal fungi have been studied extensively in mines at Southern latitudes, for example, the frequently cited papers by Gonzalez-Chavez et al. (2002) and Wu et al. (2009); however, there have been none in places as far north as Giant Mine.

Northern climates are distinct from those where most other ecological studies of mine sites have taken place. The lower energy inputs at Northern latitudes translates to shorter growing seasons, diminished plant growth, and slower nutrient cycles (Kankaanpää & Huntington, 2001; Bone, 2009). As a result plant species diversity is lower, and species present are specialized for surviving these adverse conditions. Mycorrhizal fungi too are recognized as being in lower abundance at Northern latitudes; levels of colonization are highly variable in Northern peatlands and vast areas can be dominated by non-mycorrhizal plant species (Gardes & Dahlberg, 1996). The Canadian North is therefore a region that requires a greater body of work than currently exists. And given a well-recognized requirement for site-specific research to support mine site remediation, this study may contribute to the reclamation of Baker Creek, and to the remediation of Northern mines overall.

This study being an initial assessment of Baker Creek, we answered some original questions. For example, we initially predicted an arsenic gradient along the creek with areas closest to the point of discharge being most impacted. However we disproved this prediction by showing fairly even arsenic levels along the length of the creek, and relatively homogenous plant species distributions. Also, we found that impacts from mining are more wide-spread than previously anticipated. Sites we had initially planned as reference sites that were disconnected from Baker Creek, for example Pocket Lake, had as much arsenic as Baker Creek sites and plants in our bioassays growing in soils from Pocket Lake did not behave any differently from plants growing in other soils. And we identified sensitive plant species in Baker Creek, for example, *P. pectinatus* and *A.*

triviale. These species should receive special attention, given that preserving plant diversity is cited as one of the goals for the Giant Mine Remediation Plan.

5.2 Integrative Biology

Ecology itself is inherently an integrative study of biology. From my experience, because the primary goal of ecologists is to analyze the interactions of organisms with each other and their environments, research questions in ecology span across several streams of biology. Conservation ecology is the research of threatened populations in the interest of preserving biodiversity and it prevents the reduction of biotic interactions due to anthropogenic pressures. An integrative approach is necessary for the study of conservation ecology. Unlike 'interdisciplinary' biology, which is a combination of different streams to enhance information gathering, conservation ecology is 'integrative' because these streams are inextricably linked in the answering of single questions.

The study of Baker Creek is an example of truly integrative biology. While our vegetation survey had a singular objective, there are aspects of plant biology, microbiology and chemistry that are inseparable in answering our questions. For example, we set out to determine the mycorrhizal status of plants living along the creek. This required an ability to identify plant species through general knowledge of plant biology and floral structures. Analyzing the distributions of these species required knowledge of habitat, for instance, explaining why certain species were only found at certain sites and not others. Finally, assessing the roots for colonization required

knowledge of soil microbial communities. All of these streams are inseparable for answering a single question: Has mining at Giant Mine impacted mycorrhizal communities along Baker creek?

Our bioassays were a similar example of integrative biology. A singular question about the quality of soil around Giant Mine required the integration of several streams of biology. Soil chemistry was also incorporated into this process by assessing nutrient and arsenic levels before the bioassays began. Then an analysis of plant performance acted as an indicator of soil quality from the different sites. This analysis required knowledge of plant responses to metal-contaminated soils. And knowledge of soil microbial communities was also required in the assessment of mycorrhizal infectivity. Again, several streams of biology and chemistry were inseparable in answering the single question: Has mining at Giant Mine impacted soils to the point of disturbing plant growth and mycorrhizal infectivity?

An integrative approach was necessary for answering these questions. Our vegetation survey revealed that sites ranged in species richness depending on their exposure to the mine. These findings were consistent with chemical analyses of soil, showing that arsenic tended to be lower in these areas. An assessment of plant roots revealed only a single area along the creek where mycorrhizae existed in abundance. A compilation of these results is necessary in answering whether mining activity has influenced plant and mycorrhizal communities along Baker Creek. Our bioassays revealed similar patterns to our plant survey. Significant differences were observed in plant performance between sites. Plants generated greater biomass in areas less exposed to the mine. This is an indication of lower soil disturbance. A chemical analysis

confirmed that these areas tended to have less arsenic than other sites. Mycorrhizal colonization too was higher in areas low-arsenic areas, consistent with our hypothesis. An integrative assessment of soils was necessary for confirming that mining activity has been affecting the habitats around Giant Mine.

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7. Tables

Table 3.1 Summary table of mycorrhizal colonization data in *P. arundinacea* from the bioassays for hyphal, arbuscular and vesicular colonization. Sites ranked by Kruskal-Wallis analysis in order from highest to lowest. Included are means ± 1 SE, upper and lower 95% confidence limits. Two rows sharing a letter are not significantly different as determined by Wilcoxon pairwise comparisons ($p < 0.05$). Site abbreviations: R0 - 7, Reach 0 - 7; PL, Pocket Lake; TL, Trapper Lake; YR, Yellowknife River.

Table 3.1

	Site	Mean ± 1 SE	Upper CL	Lower CL	Wilcoxon Pairwise Comparisons
Hyphal Colonization	R4	18.08 ± 3.34	26.68	9.48	a
	YR	13.44 ± 8.94	36.41	-9.52	ab
	R7	0.67 ± 0.41	1.97	-0.63	bc
	R6	3.25 ± 3.05	11.10	-4.60	bc
	R2	2.17 ± 2.17	7.74	-3.40	bc
	R5	0.39 ± 0.39	1.39	-0.61	bc
	PL	0.27 ± 0.27	0.99	-0.44	c
	TL	0.11 ± 0.11	0.40	-0.17	c
	R0	0	0	0	c
	Chi-Square			26.45	
	Prob>ChiSq			0.001*	
Arbuscular Colonization	R4	11.53 ± 2.53	18.04	5.02	a
	YR	8.83 ± 5.42	22.78	-5.11	ab
	R6	2.5 ± 2.31	8.43	-3.43	bc
	R7	0.58 ± 0.34	1.68	-0.51	bc
	R2	2.00 ± 2.00	7.14	-3.14	bc
	PL	0.39 ± 0.25	1.03	-0.25	bc
	R5	0.22 ± 022	0.79	-0.35	c
	TL	0.06 ± 0.06	0.20	-0.09	c
	R0	0	0	0	c
	Chi-Square			26.40	
	Prob>ChiSq			0.001*	
Vesicular Colonization	YR	1.72 ± 1.40	5.33	-1.89	a
	R4	0.44 ± 0.21	0.97	-0.08	a
	R6	0.08 ± 0.08	0.30	-0.13	a
	PL	0.06 ± 0.06	0.20	-0.09	a
	R5	0.06 ± 0.06	0.20	-0.09	a
	R7	0	0	0	a
	R0	0	0	0	a
	R2	0	0	0	a
	TL	0	0	0	a
	Chi-Square			14.45	
	Prob>ChiSq			0.071	

Table 3.2 Summary table of mycorrhizal colonization data in *E. angustifolium* from the bioassays for hyphal, arbuscular and vesicular colonization. Sites ranked by Kruskal-Wallis analysis in order from highest to lowest. Included means ± 1 SE, upper and lower 95% confidence limits. Two rows sharing a letter are not significantly different as determined by Wilcoxon pairwise comparisons ($p < 0.05$). Site abbreviations: R0 - 7, Reach 0 - 7; PL, Pocket Lake; TL, Trapper Lake; YR, Yellowknife River.

Table 3.2

	Site	Mean ± 1 SE	Upper CL	Lower CL	Wilcoxon Pairwise Comparisons
Hyphal Colonization	R4	29.00 ± 8.97	51.60	6.40	a
	YR	16.27 ± 10.54	43.48	-10.83	ab
	R6	6.56 ± 5.37	20.37	-7.26	b
	R2	2.51 ± 2.51	8.97	-3.95	b
	PL	0.41 ± 0.41	1.47	-0.65	b
	R7	0	0	0	b
	R0	0	0	0	b
	R5	0	0	0	b
	TL	0	0	0	b
	Chi-Square		29.19		
	Prob>ChiSq		<0.001*		
Arbuscular Colonization	YR	6.75 ± 4.25	17.66	-4.17	a
	R4	2.51 ± 1.84	7.24	-2.23	a
	R6	1.98 ± 1.76	6.50	-2.54	a
	PL	0.41 ± 0.41	1.47	-0.65	a
	R2	0.38 ± 0.38	1.37	-0.60	a
	R7	0	0	0	a
	R0	0	0	0	a
	R5	0	0	0	a
	TL	0	0	0	a
	Chi-Square		13.46		
	Prob>ChiSq		0.097		
Vesicular Colonization	R4	2.21 ± 1.31	5.57	-1.14	a
	YR	2.08 ± 1.79	6.68	-2.52	a
	R2	1.28 ± 1.28	4.59	-2.02	a
	R6	0.39 ± 0.39	1.41	-0.62	a
	R7	0	0	0	a
	PL	0	0	0	a
	R0	0	0	0	a
	R5	0	0	0	a
	TL	0	0	0	a
	Chi-Square		12.72		
	Prob>ChiSq		0.121		

Table 3.3 Spearman's rho values (**ρ**) and significance levels from all Spearman correlations relating plant performance (root length) and mycorrhizal colonization with all soil nutrient, pH, and arsenic data for **A)** Root length in *E. angustifolium* **B)** Root length in *P. arundinacea* **C)** Mycorrhizal colonization in *E. angustifolium* **D)** Mycorrhizal colonization in *P. arundinacea*. Significant correlations are highlighted in red ($p < 0.05$).

A

Root Length (*E. angustifolium*)

Variable	ρ	Prob > ρ
Available Phosphorus	**-0.263**	**0.033**
Arsenic	**-0.609**	**<.0001**
Carbon	**-0.574**	**<.0001**
Nitrogen	**-0.494**	**<.0001**
pH	-0.041	0.771
Potassium	0.206	0.098
Total Phosphorus	0.065	0.605

B

Root Length (*P. arundinacea*)

Variable	ρ	Prob > ρ
Available Phosphorus	-0.134	0.285
Arsenic	**-0.530**	**<.0001**
Carbon	**-0.389**	**0.001**
Nitrogen	**-0.253**	**0.041**
pH	-0.079	0.593
Potassium	0.090	0.472
Total Phosphorus	0.163	0.191

C

Mycorrhizal Colonization (*E. angustifolium*)

Variable	ρ	Prob > ρ
Available Phosphorus	-0.001	0.997
Arsenic	**-0.443**	**0.0002**
Carbon	**-0.342**	**0.005**
Nitrogen	**-0.371**	**0.002**
pH	0.144	0.774
Potassium	**0.309**	**0.012**
Total Phosphorus	**0.247**	**0.046**

D

Mycorrhizal Colonization (*P. arundinacea*)

Variable	ρ	Prob > ρ
Available Phosphorus	0.242	0.277
Arsenic	-0.363	0.097
Carbon	-0.355	0.105
Nitrogen	**-0.441**	**0.040**
pH	0.058	0.682
Potassium	0.263	0.237
Total Phosphorus	0.201	0.370

Table 3.4 Spearman's rho values (**ρ**) and significance levels from Spearman's correlations between all combinations of soil nutrient, pH, and arsenic data. Significant correlations are highlighted in red ($p < 0.05$).

Variable	ρ	Prob > ρ
Nitrogen x Carbon	**0.891**	**<.0001**
Carbon x Arsenic	**0.640**	**<.0001**
Nitrogen x Arsenic	**0.534**	**<.0001**
Carbon x Available Phosphorus	**0.476**	**<.0001**
Available Phosphorus x Arsenic	**0.380**	**0.002**
Potassium x Total Phosphorus	**0.372**	**0.002**
Nitrogen x Available Phosphorus	**0.333**	**0.006**
Potassium x Available Phosphorus	0.229	0.064
Nitrogen x pH	-0.185	0.189
Carbon x pH	-0.169	0.232
Potassium x Arsenic	-0.136	0.275
Potassium x pH	0.147	0.300
Available Phosphorus x Total Phosphorus	0.104	0.405
Total Phosphorus x Arsenic	0.100	0.425
Nitrogen x Total Phosphorus	0.087	0.488
Available Phosphorus x pH	-0.093	0.512
Carbon x Total Phosphorus	0.081	0.517
Arsenic x pH	-0.081	0.569
Total Phosphorus x pH	-0.077	0.588
Carbon x Potassium	0.055	0.664
Nitrogen x Potassium	0	0.999

Table 3.5 Complete species list of all plants identified in the vegetation survey of Baker Creek and surrounding area, and the sites where they were identified

Monocotyledon	Alismataceae	*Alisma triviale*	Al. tr.	Northern Water Plantain	OBL	S			+						
		Sagittaria cuneata	Sa. cu.	Northern Arrowhead	OBL	N									+
	Araceae	*Calla palustris*	Ca. pa.	Water Dragon	OBL	N						+			
	Cyperaceae	*Carex aquatilis*	Ca. aq.	Water Sedge	OBL	N	+	+	+	+	+		+	+	+
		Carex rostrata	Ca. ro.	Swollen Beaked Sedge	OBL	U									+
		Scirpus validus	Sc. va.	Soft-stem Bulrush	OBL	U				+					
	Juncaceae	*Juncus alpinus*	Ju. al.	Northern Green Rush	OBL	N			+						
	Onagraceae	*Epilobium angustifolium*	Ep. an.	Fireweed	FACU	N	+		+		+		+		
	Poaceae	*Agrostis scabra*	Ag. sc.	Rough Bentgrass	FAC	N	+		+	+	+		+	+	
		Calamagrostis canadensis	Ca. ca.	Blue-jointed Reed Grass	OBL	N	+	+			+		+		+
		Hordeum jubatum	Ho. ju.	Fox-Tail Barley	FACU	N			+		+				
		Phalaris arundinacea	Ph. ar.	Reed Canary Grass	OBL	U			+						
	Potamogetonaceae	*Potamogeton filiformis*	Po. fi.	Slender Pondweed	OBL	N			+				+		
		Potamogeton gramineus	Po. gr.	Grassy Pondweed	OBL	N				+		+	+		+
		Potamogeton pectinatus	Po. pe.	Sago Pondweed	OBL	S		+							
		Potamogeton richardsonii	Po. ri.	Richardson's Pondweed	OBL	N	+								
	Sparganiaceae	*Sparganium angustifolium*	Sp. an.	Northern Bur-reed	OBL	N			+	+	+	+			
		Sparganium hyperborium	Sp. hy.	Narrow-leaf Bur-reed	OBL	N									+
	Typhaceae	*Typha latifolia*	Ty. la.	Broad-leaf Cattail	OBL	N	+	+	+	+	+		+		

Table 3.5 *(continued)*

Group	Family	Species	Abbrv.	Common Name	Wetland Indicator Status	Native/ Introduced/ Sensitive/ Undetermined (N/I/S/U)	R 0	R 2	R 4	R 5	R 6	R 7	T L	P L	Y R
Dicotyledon	Apiaceae	*Sium suave*	Si. su.	Water Parsnip	OBL	N									+
	Asteraceae	*Asteraceae sp.*	Aster.	n/a	n/a	n/a	+		+	+				+	
		Sonchus arvensis	So. ar.	Field Sow Thistle	FACU	I			+						
	Fabaceae	*Melilotus alba*	Me. al.	White Sweet-Clover	FACU	I			+						
		Trifolium repens	Tr. re.	White Clover	FACU	I			+						
	Halorgaceae	*Myriophyllum exalbuscens*	My. ex.	Spiked Water Milfoil	OBL	N	+					+	+		
	Nymphaceae	*Nuphar variegatum*	Nu. ve.	Variegated Pond Lily	OBL	N								+	
	Rosaceae	*Potentilla fruticosa*	Po sp.	n/a	n/a	n/a						+			+
	Rubiaceae	*Galium triflorum*	Ga. tr.	Fragrant Bedstraw	FAC	N					+	+			
	Salicaceae	*Salix sp*	Sa. sp.	Willow sp.	n/a	n/a	+	+	+	+					
	Saxifragaceae	*Parnassia palustris*	Pa. pa.	Marsh Grass-of-parnassus	FACW	N	+		+				+	+	
Fern	Equisetaceae	*Equisetum arvense*	Eq. ar.	Field Horsetail	FAC	N	+	+	+		+			+	
		Equisetum fluviatale	Eq. fl.	Water Horsetail	OBL	N	+	+	+	+	+	+			+

Table 3.6 Species richness, evenness, and Shannon's diversity scores for all sites sampled in our vegetation survey of Baker Creek

	Reach 0	Reach 2	Reach 4	Reach 5	Reach 6	Reach 7	Trapper Lake	Pocket Lake	Yellowknife River
Diversity	0.51	0.54	1.24	1.04	1.19	0.21	0.91	0.98	1.37
Evenness	0.22	0.30	0.44	0.50	0.51	0.19	0.51	0.55	0.77
Richness	12	7	18	9	10	7	9	6	9

Table 3.7 Summary of the associations plant species had with the first three ordination axes of the non-metric multidimensional scaling used for analyzing the vegetation survey of Baker Creek

Variable	**Axis1**	**Axis2**	**Axis3**
Ag. sc.	0.793	-0.272	0.115
Al. tr.	0.687	1.064	0.255
Aster.	0.677	0.579	-0.170
Ca. aq.	0.485	-0.261	0.123
Ca. ca.	-0.166	-0.527	-0.120
Ca. pa.	-0.848	0.093	-0.312
Ca. ro.	-0.596	-0.875	0.410
Ep. an.	-0.282	0.674	-0.151
Eq. ar.	0.544	1.098	-0.033
Eq. fl.	-0.590	0.127	0.078
Ga. tr.	-0.470	0.431	-0.377
Ho. ju.	0.542	0.500	0.124
Ju. al.	0.616	1.347	-0.072
Me. al.	0.616	1.347	-0.072
My. ex.	-0.468	0.022	-0.148
Nu. ve.	1.065	-0.442	0.454
Pa. pa.	0.745	-0.263	0.233
Ph. ar.	1.008	0.486	-0.458
Po. sp.	-0.158	-0.889	-0.092
Po. fi.	0.739	0.172	-0.542
Po. gr.	-0.271	-0.225	-0.647
Po. pe.	-0.405	0.387	0.523
Po. ri.	-0.974	0.481	0.048
Sa. sp.	0.040	0.670	0.166
Sag. sp.	-0.398	-1.271	-0.462
Sc. va.	-0.104	0.202	0.125
Si. su.	-0.398	-1.271	-0.462
So. ar.	0.628	1.300	-0.017
Sp. an.	-0.880	-0.867	0.389
Sp. sp.	0.049	0.185	0.204
Tr. re.	1.008	0.486	-0.458
Ty. la.	0.131	0.232	-0.214

Table 3.8 A list of all plant species where roots were collected for mycorrhizal analysis, and the sites where they were colonized (**C**) or uncolonized (**U**); blank squares imply species was not present at that site. Site abbreviations: R0 - 6, Reach 0 - 6; YR, Yellowknife River.

Species	Site					
	R0	**R2**	**R4**	**R6**	**R7**	**YR**
Ca. aq.	U	U	U	U		U
Ca. ca.	**C**	**C**	**C**	**C**	**C**	**C**
Ca. pl.					U	
Ca. ro.					U	U
Ep. an.			**C**	**C**		
Eq. ar.	U	**C**	**C**	**C**		
Eq. fl.	U			U	U	U
Hi. vu.			U		U	
Ju. al.			U			
Ph. ar.			**C**			
Po. sp.					**C**	**C**
Ro. pa.					U	
Sa. sp.			U			U
Sc. sp.			U	U		
Si. su.	U					U
Sp. sp.			U	U	U	U
Ty. la.	U	U	U	U	U	

Table 3.9 Means (± 1 SE) of hyphal colonization in field-collected roots from five naturally found plant species from five sites along Baker Creek and one site at Yellowknife River. Means sharing letters are from data that are not significantly different (Ca. ca., ANOVA $p < 0.05$; Ep. an., Eq. ar. & Po. sp. Wilcoxon $p < 0.05$). Site abbreviations: R0 - 6, Reach 0 - 6; YR, Yellowknife River.

Species	**Site**					
	R0	**R2**	**R4**	**R6**	**R7**	**YR**
Ca. ca.	22.20 ± 5.17 a	4.40 ± 1.50 b	18.00 ± 2.43 ab	22.00 ± 3.08 a	15.20 ± 3.61 ab	13.40 ± 2.91 ab
Eq. ar.	0 ± 0 b	1.60 ± 1.17 b	23.80 ± 5.49 a	7.20 ± 2.40 ab		
Ep. an.			57.80 ± 9.87 b	96.20 ± 2.33 a		
Ph. ar.			47.00 ± 9.47			
Po. sp.					9.80 ± 2.56 a	1.00 ± 1.00 b

Table 3.10 Means (± 1 SE) of vesicular colonization in field-collected roots from five naturally found plant species from five sites along Baker Creek and one site at Yellowknife River. Rows without letters implies no significant effect of site on colonization, and means sharing letters are from data that are not significantly different (Ca. ca. & Ep. an., ANOVA $p < 0.05$; Eq. ar. & Po. sp. Kruskal-Wallis $p < 0.05$). Site abbreviations: R0 - 6, Reach 0 - 6; YR, Yellowknife River.

Species	**Site**					
	R0	**R2**	**R4**	**R6**	**R7**	**YR**
Ca. ca.	6.80 ± 0.97	2.00 ± 0.89	5.80 ± 1.39	7.40 ± 2.06	3.80 ± 0.58	4.80 ± 1.80
Eq. ar.	0 ± 0 b	0.60 ± 0.60 ab	10.20 ± 2.63 a	2.80 ± 1.36 ab		
Ep. an.			15.80 ± 1.96	22.40 ± 6.20		
Ph. ar.			17.20 ± 5.19			
Po. sp.					4.40 ± 1.36 a	0 ± 0 b

Table 3.11 Means (± 1 SE) of arbuscular colonization in field-collected roots from five naturally found plant species from five sites along Baker Creek and one site at Yellowknife River. Rows without letters implies no significant effect of site on colonization, and means sharing letters are from data that are not significantly different (Kruskal-Wallis $p < 0.05$). Site abbreviations: R0 - 6, Reach 0 - 6; YR, Yellowknife River.

Species	**Site**					
	R0	**R2**	**R4**	**R6**	**R7**	**YR**
Ca. ca.	1.60 ± 0.81	0 ± 0	1.60 ± 0.75	0.60 ± 0.60	2.40 ± 1.50	0.40 ± 0.40
Eq. ar.	0 ± 0 b	0 ± 0 b	3.80 ± 1.91 a	0 ± 0 b		
Ep. an.			0 ± 0	0 ± 0		
Ph. ar.			9.40 ± 3.98			
Po. sp.					4.40 ± 1.36 a	0 ± 0 b

Table 3.12 Mean levels of mycorrhizal colonization (± 1 SE) in colonized roots only, summarizing hyphal, vesicular and arbuscular colonization in roots from bioassay and field-collected roots. Letters are from within-species comparisons of arbuscular versus vesicular colonization, means accompanied by the same letters are from data that are not significantly different as determined by Wilcoxon matched pairs comparisons ($p < 0.05$).

Sample Source	Species	Hyphal Colonization (%)		Vesicles (%)	Arbuscules (%)
Bioassay	Ep. an.	35.86± 4.32		4.09± 1.29 a	7.89± 2.07 a
	Ph. ar.	15.53± 2.30		0.95± 0.37 a	10.42± 1.51 b
Field-Collected Roots	Ca. ca.	16.41± 1.63		5.28± 0.61 a	1.14± 0.35 b
	Ep. an.	77.00± 7.99		19.10± 3.26 a	0 ± 0 b
	Eq. ar.	15.90± 3.81		6.80± 1.77 a	1.90± 1.10 b
	Ph. ar.	47.00± 9.47		18.00± 4.72 a	7.20± 2.85 b
	Po. sp.	10.80± 1.62		4.40± 1.36 a	0.60± 0.40 a

8. Figures

Figure 1.1 An aerial image of Baker Creek and all sites sampled. Downstream sites include Reaches 0, 2, 4, 5, 6 (R0, R2, R4, R5, R6). Our upstream site was Reach 7 (R7). Nearby wetland sites include Pocket Lake (PL) and Trapper Lake (TL). Yellowknife River (YR) was our reference site.

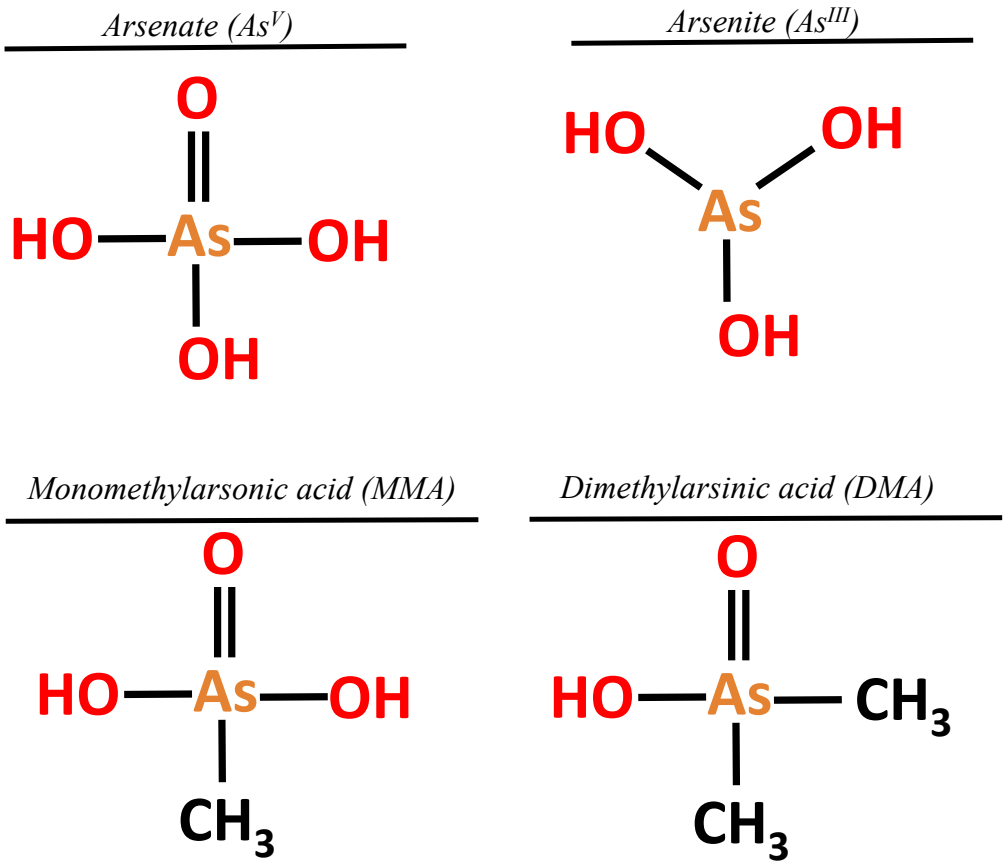


Figure 1.2 Molecular structures of the four most common forms of arsenic: arsenate (As^V), arsenite (As^{III}), monomethylarsonic acid (MMA), and dimethylarsinic acid. These are 2-dimensional depictions of these molecules; in reality As^V, DMA and MMA would take tetrahedral shapes and As^{III} would be planar. These molecules are depicted as if under basic conditions; as pH lowers the hydroxyl groups are deprotonated leaving negatively charged oxygen atoms.

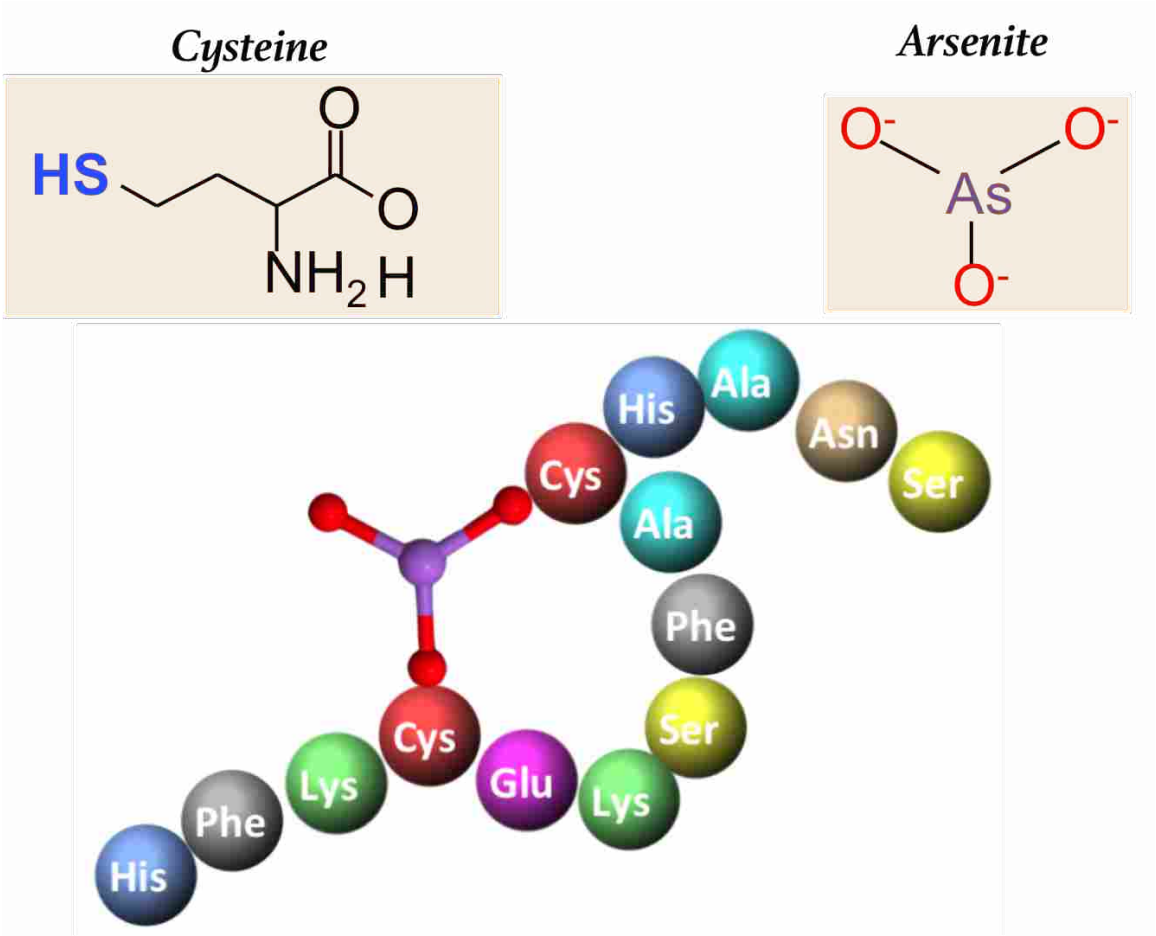


Figure 1.3 Arsenite damages proteins by disrupting primary structure. A schematic diagram of how arsenite disrupts protein primary structure; negatively charged oxygens can bind ionically to the sulfhydryl groups of multiple cysteine amino acids simultaneously. This causes bends in the protein's primary structure and will alter its biological function.

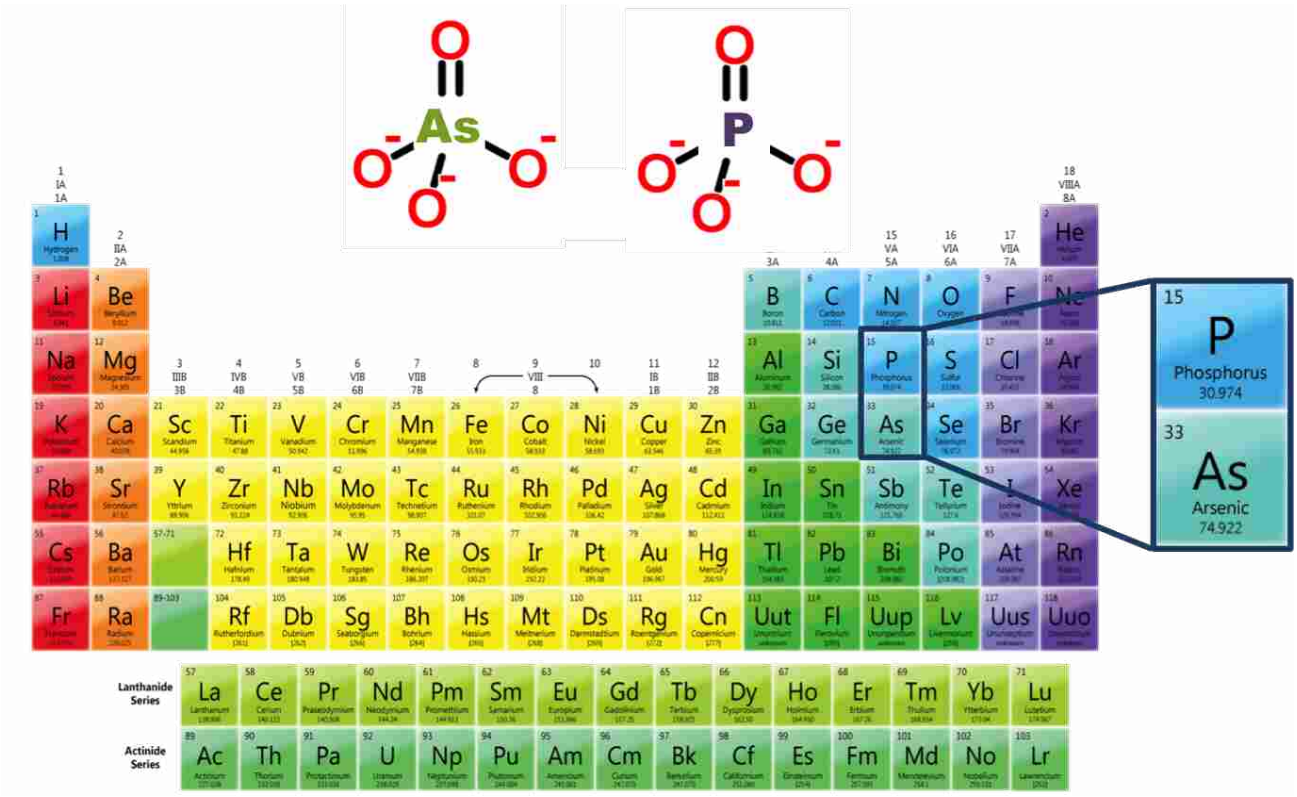
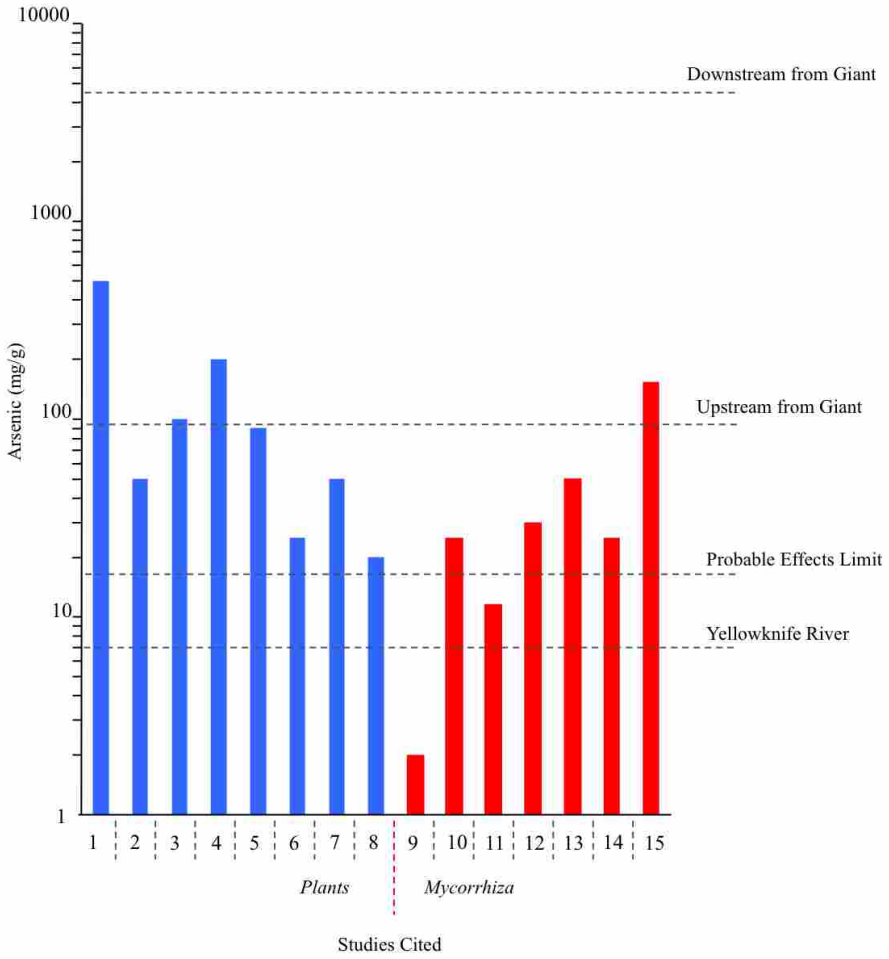


Figure 1.4 Arsenate exerts toxicity by mimicking phosphate**.** A periodic table showing arsenic and phosphorus in the same chemical family and therefore having the same number of valence electrons. When bound to four oxygens arsenate and phosphate have a nearly identical molecular structure.



1) American mountain mint, Upright sedge, Prairie cordgrass (*Pycnanthemum virginianum*, *Carex stricta, Spartina pectinata*); reduced leaf area, root/shoot biomass (Rofkar & Dwyer, 2013).

2) Clover (*Trifolium pratense*); reduced shoot biomass and chlorophyll production (Masher et al., 2002)

3) Maize (*Zea mays*); Reduced root Length (Yu et al., 2009)

4) Medick (*Medicago trunculata*); Reduced root length (Xu et al., 2008)

5) Pea (*Pisum sativum*); Reduced root length (Garg & Singla, 2012)

6) Tomato (*Solanum lycopersicum*); Reduced shoot/root dry weight (Liu et al., 2005)

7) Tomato (*Solanum lycopersicum*); Reduced stem height (Miteva, 2002)

8) Wheat (*Triticum aestivum*); Root/shoot length and biomass (Li et al., 2007)

9) *Glomus etunicatum*, *G. geosporum*, *G. mossae*; Reduced germination (Wu et al., 2009)

10) *Glomus etunicatum*, *G. constrictum, G. mossae*; Reduced colonization in corn (*Zea mays*) roots (Yu et al., 2010)

11) *Glomus mossae*; Reduced colonization in lentil (*Lens culinaris*) roots (Ahmed et al., 2006)

12) *Glomus mossae*; Reduced colonization in pea (*Pisum sativum*) roots (Garg et al., 2012)

13) *Gigaspora rosea*, *Glomus mossae*; Reduced germination (Gonzalez-Chavez et al., 2002)

14) *Glomus mossae*; Reduced colonization of Chines brake fern (*Pteris vittata*) roots (Bona et al., 2010)

15) *Glomus mossae*; Reduced colonization in tomato (*Solanum lycopersicum*) roots (Liu et al., 2005)

Figure 1.5 A review of studies that tested the effects of arsenic on plants and mycorrhizae. Bars indicate the levels of arsenic in soils that elicited a negative growth response. Listed on the right are the species of plants or mycorrhizae being tested, the growth response measured, and a citation for that study. Dashed lines indicate mean levels of arsenic from a 2011 study on sediments in Baker Creek and Yellowknife River, and the CCME Probable Effects Limit.

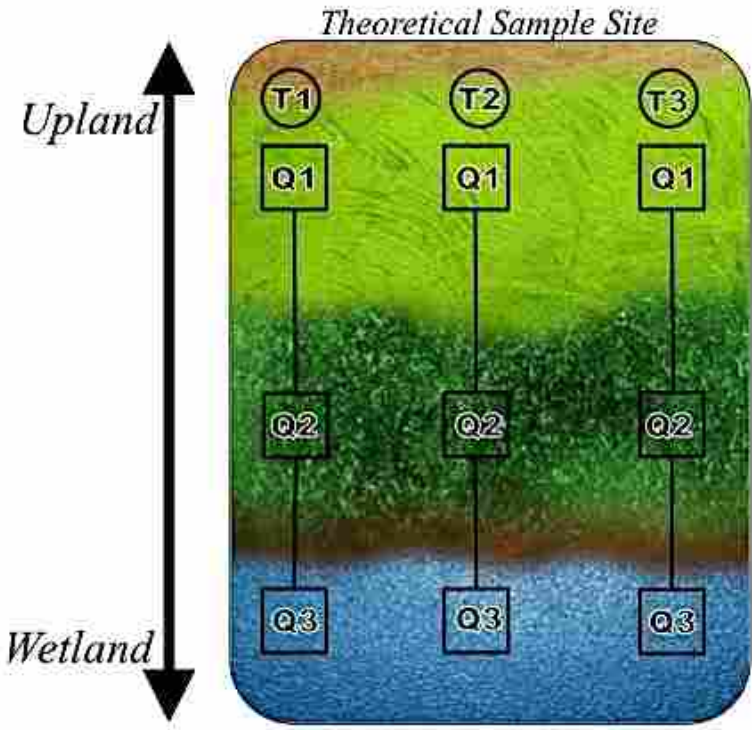


Figure 2.1 Overhead view of a theoretical sample site. Soil collections and vegetation surveys at each site were conducted by the scheme showed above. Three transects were used at each site (T1, T2, T3) and each transect had an upland, intermediate and wetland quadrat (Q1, Q2, Q3 respectively). This diagram represents a transition from upland to wetland, with the light green area representing strictly upland vegetation, dark green representing an intermediate zone with facultative wetland species, and the blue representing the water column where strictly aquatic species were sampled.

Figure 2.2 Images of plant species used in the bioassays A) *E. angustifolium* B) *P. arundinacea*. Both are images of full grown plants growing in natural conditions

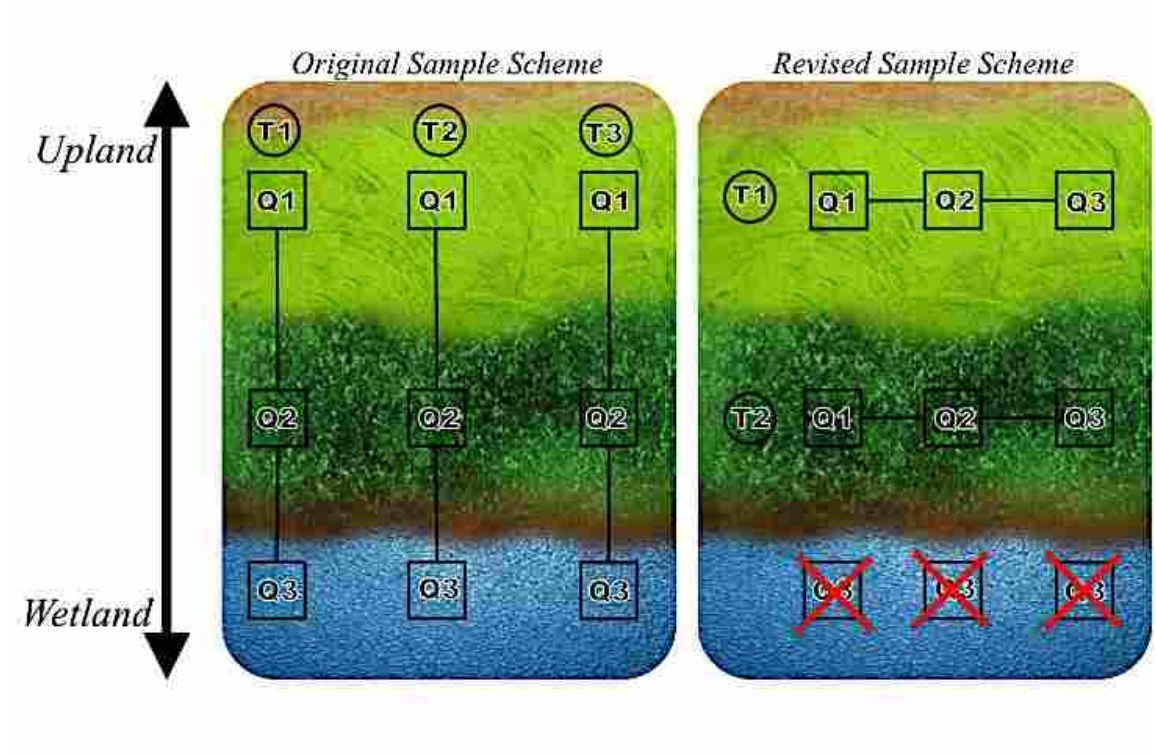


Figure 2.3 Side-by-side comparison of the original sampling scheme used for soil collections (left) and a revised version used for statistical analysis (right) to account for a possible moisture effect. For example, because arsenic trioxide is soluble, one might expect wetter areas to have higher arsenic levels.

Figure 3.1 Growth responses in *P. arundinacea* to soils collected at sites in Baker Creek, nearby wetlands and a reference site for **A)** Shoot dry weight **B)** Shoot fresh weight **C)** Shoot surface area **D)** Root fresh weight **E)** Root length **F)** Root surface area. Bars represent means ± 1 SE. Bars arranged in descending order according to the original hypothesis; plants were expected to grow largest in soils from Yellowknife River, followed by Reach 7, then our nearby sites, followed by Baker Creek sites in ascending order from the point of discharge at Reach 6. Connected letters generated from a Tukey's post-hoc analysis following log transformations for all shoot data, and all root data was square root transformed. Bars sharing letters represent means that are not significantly different (ANOVA, $p < 0.05$). Site abbreviations: R0 - 7, Reach 0 - 7; PL, Pocket Lake; TL, Trapper Lake; YR, Yellowknife River.

Figure 3.1

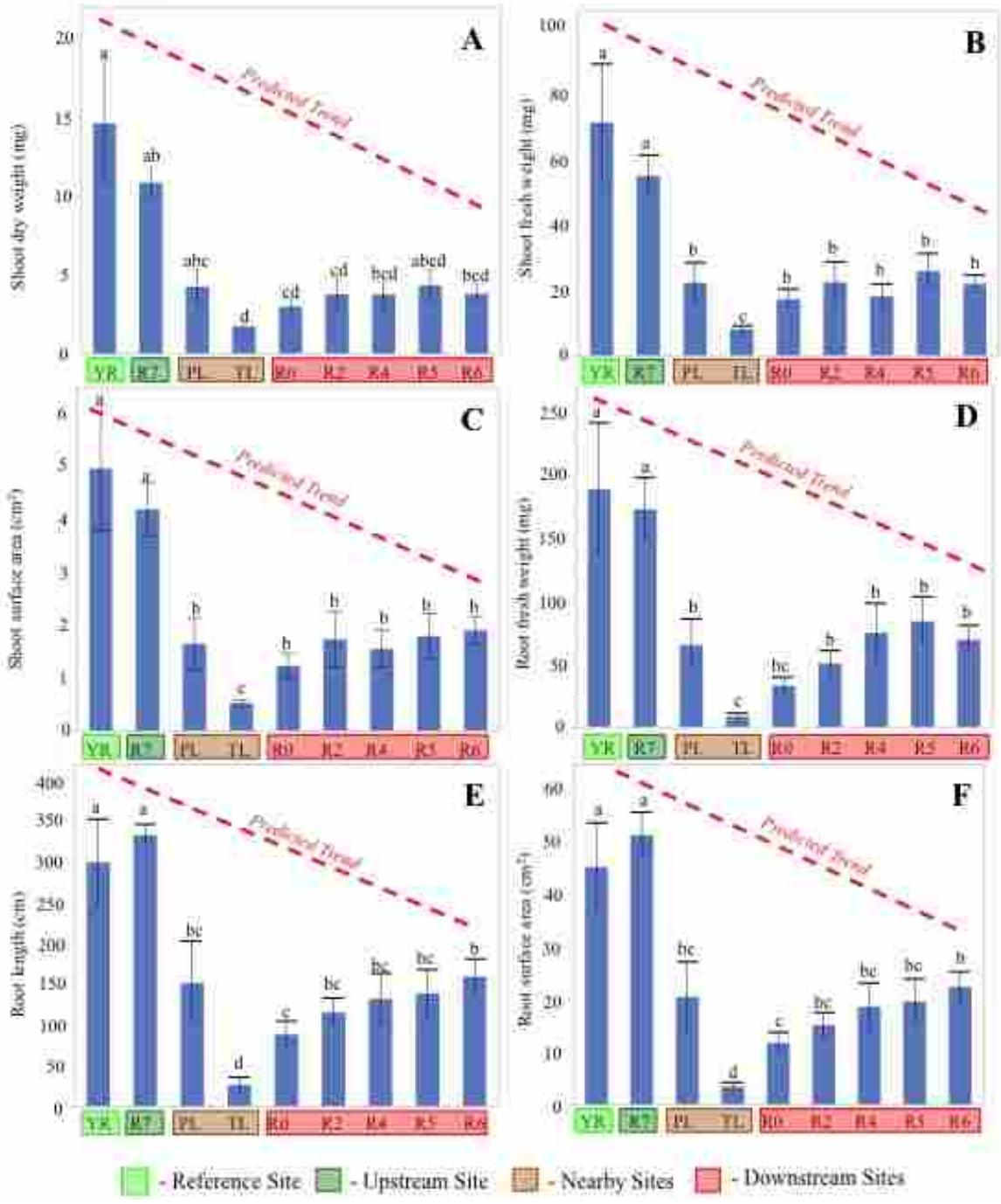
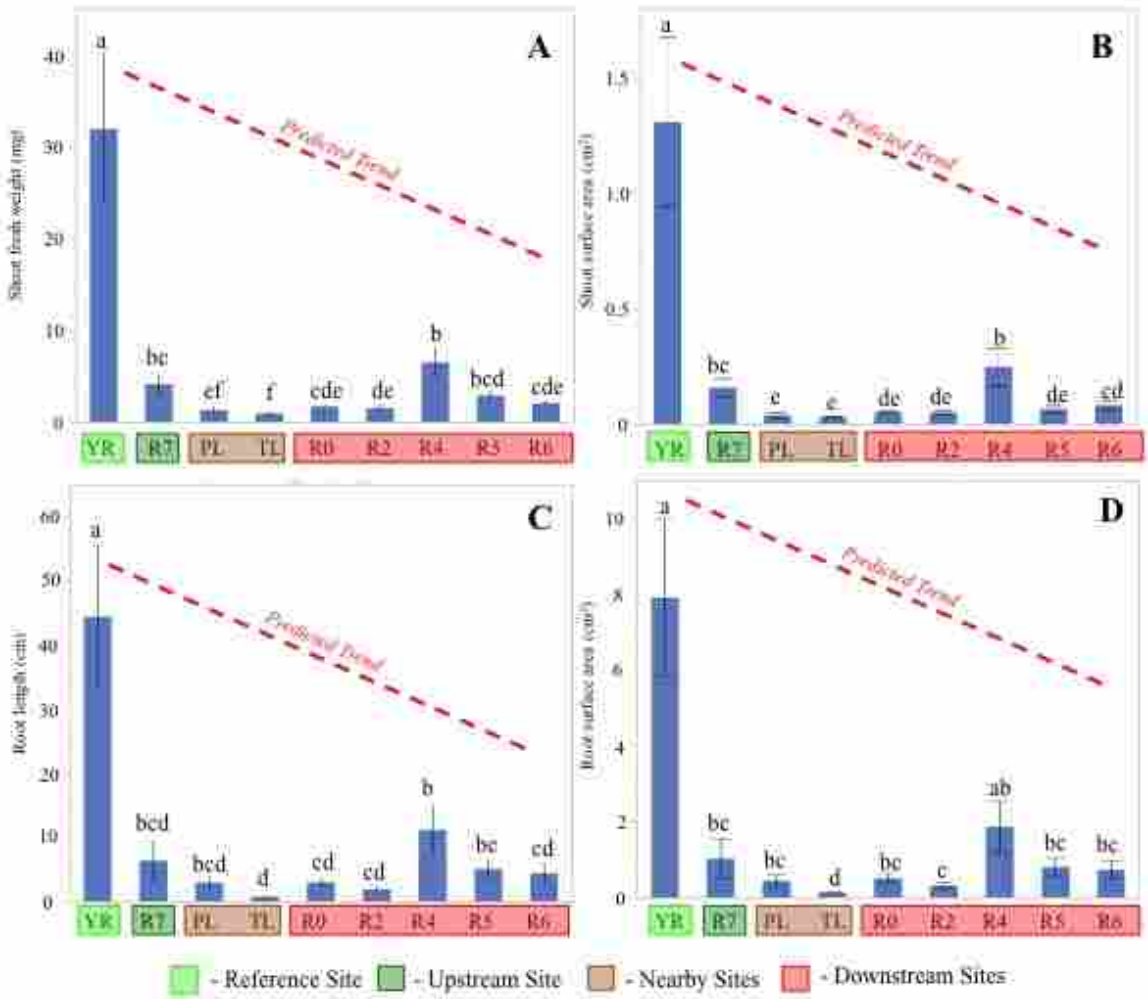

A
B
C
D
E
F
Predicted Trend
Shoot dry weight (mg)
Shoot fresh weight (mg)
Shoot surface area (cm²)
Root fresh weight (mg)
Root length (cm)
Root surface area (cm²)
YR
R7
PL
TL
R0
R2
R4
R5
R6
- Reference Site
- Upstream Site
- Nearby Sites
- Downstream Sites

Figure 3.2 Growth responses in *E. angustifolium* to soils collected at sites in Baker Creek, nearby wetlands and our reference site for **A)** Shoot fresh weight **B)** Shoot surface area **C)** Root length **D)** Root surface area. Bars represent means ± 1 SE. Bars arranged in descending order according to the original hypothesis; plants were expected to grow largest in soils from Yellowknife River, followed by Reach 7, then our nearby sites, followed by Baker Creek sites in ascending order from the point of discharge at Reach 6. Connected letters generated from a Tukey's post-hoc analysis following log-transformations. Bars sharing letters represent means that are not significantly different (ANOVA, $p < 0.05$). Site abbreviations: R0 - 7, Reach 0 - 7; PL, Pocket Lake; TL, Trapper Lake; YR, Yellowknife River.

Figure 3.2


A
B
C
D
Predicted Trend
YR
R7
PL
TL
R0
R2
R4
R5
R6
- Reference Site
- Upstream Site
- Nearby Sites
- Downstream Sites

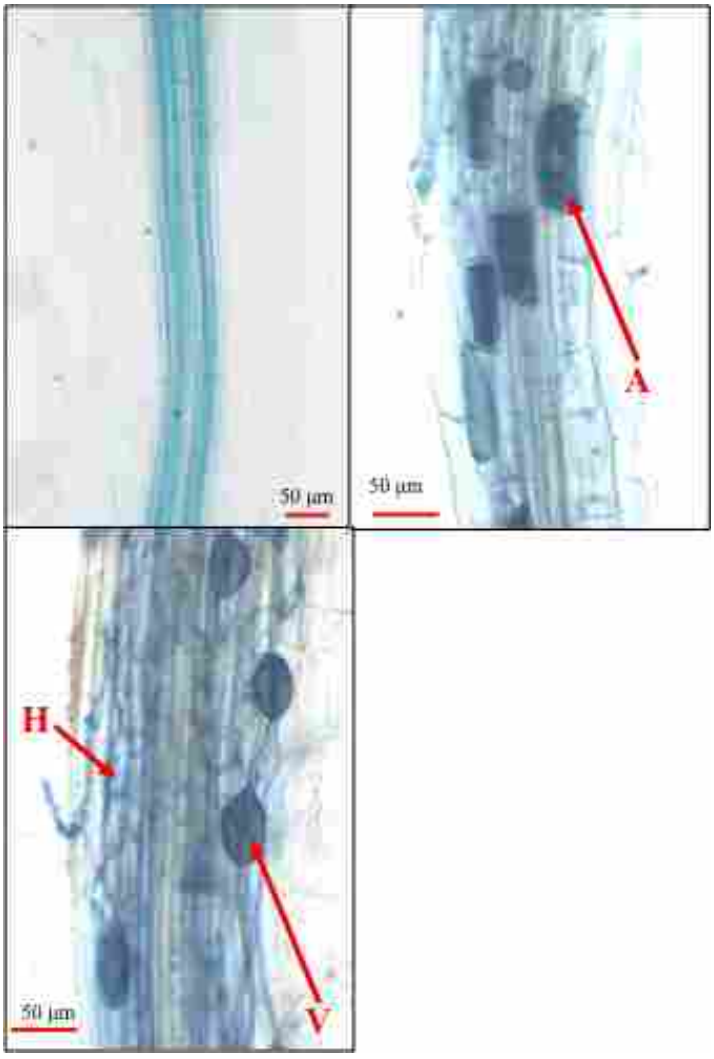


Figure 3.3 Micrographs of mycorrhizal colonization in plant roots from the bioassays. **A)** A cleared root of *P. arundinacea* with no structures after four weeks of growth in the bioassay **B)** Arbuscules (A) in roots of *P. arundinacea* stained blue with ink & vinegar **C)** Vesicles (V) and hyphae (H) in *E. angustifolium* roots after six weeks of growth in the bioassays, stained blue with ink & vinegar stain

Figure 3.4 Effects of site of soil collection on percent mycorrhizal colonization in roots of *P. arundinacea* from the bioassays for **A)** Hyphal colonization **B)** Arbuscular colonization **C)** Vesicular colonization. Boxes represent the middle fifty percent of the distributions, medians are represented by middle lines, values greater than 1.5 times the interquartile range are depicted as circles above the box, and whiskers denote the maximum and minimum values that did not qualify as outliers. Connected letters from Dunn's non-parametric comparisons of data distributions by site; boxes sharing letters represent data distributions that are not significantly different (Kruskal-Wallis, $p < 0.05$). Site abbreviations: R0 - 7, Reach 0 - 7; PL, Pocket Lake; TL, Trapper Lake; YR, Yellowknife River.

Figure 3.4

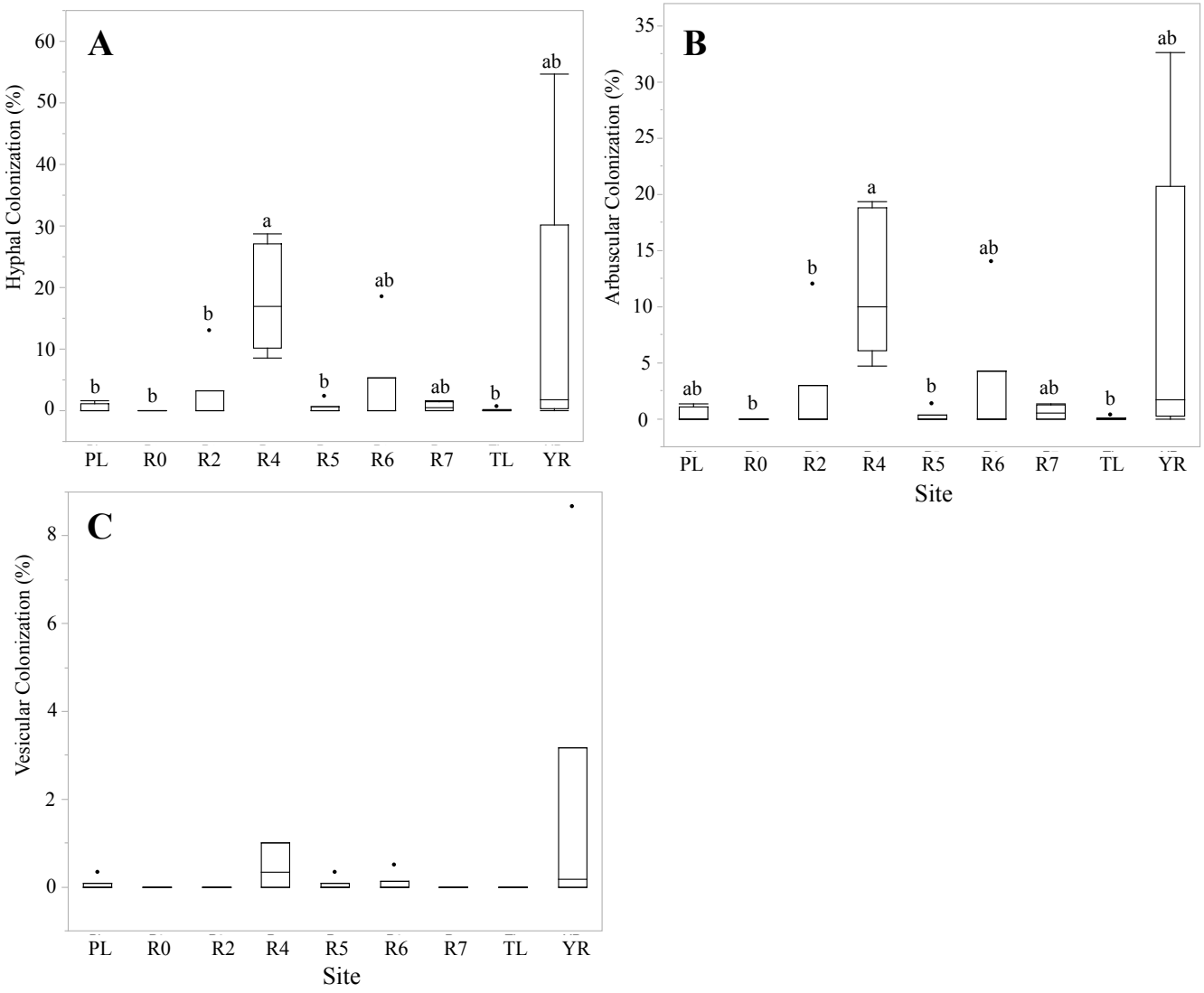
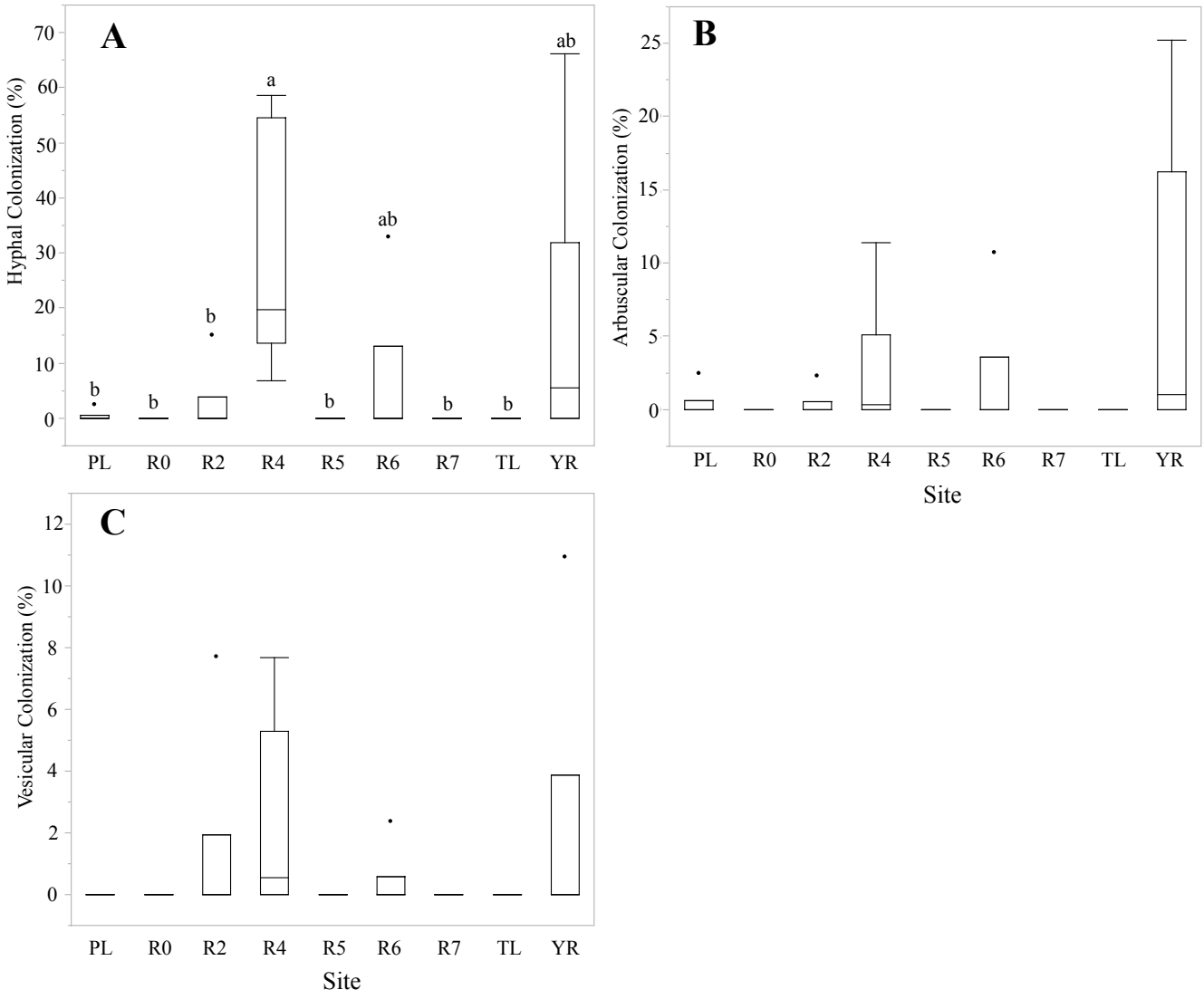

A
Hyphal Colonization (%)
60
50
40
30
20
10
0
b
b
b
a
b
ab
ab
b
ab
PL
R0
R2
R4
R5
R6
R7
TL
YR
B
Arbuscular Colonization (%)
35
30
25
20
15
10
5
0
ab
b
b
a
b
ab
ab
b
ab
PL
R0
R2
R4
R5
R6
R7
TL
YR
Site
C
Vesicular Colonization (%)
8
6
4
2
0
PL
R0
R2
R4
R5
R6
R7
TL
YR
Site

Figure 3.4 Effects of site of soil collection on percent mycorrhizal colonization in roots of *E. angustifolium* from the bioassays for **A)** Hyphal colonization **B)** Arbuscular colonization **C)** Vesicular colonization. Boxes represent the middle fifty percent of the distributions, medians are represented by middle lines, values greater than 1.5 times the interquartile range are depicted as circles above the box, and whiskers denote the maximum and minimum values that did not qualify as outliers. Connected letters from Dunn's non-parametric comparisons of data distributions by site; boxes sharing letters represent data distributions that are not significantly different (Kruskal-Wallis, $p < 0.05$). Site abbreviations: R0 - 7, Reach 0 - 7; PL, Pocket Lake; TL, Trapper Lake; YR, Yellowknife River.

Figure 3.5


A
Hyphal Colonization (%)
70
60
50
40
30
20
10
0
b
b
b
a
b
ab
b
b
ab
PL
R0
R2
R4
R5
R6
R7
TL
YR
B
Arbuscular Colonization (%)
25
20
15
10
5
0
PL
R0
R2
R4
R5
R6
R7
TL
YR
Site
C
Vesicular Colonization (%)
12
10
8
6
4
2
0
PL
R0
R2
R4
R5
R6
R7
TL
YR
Site

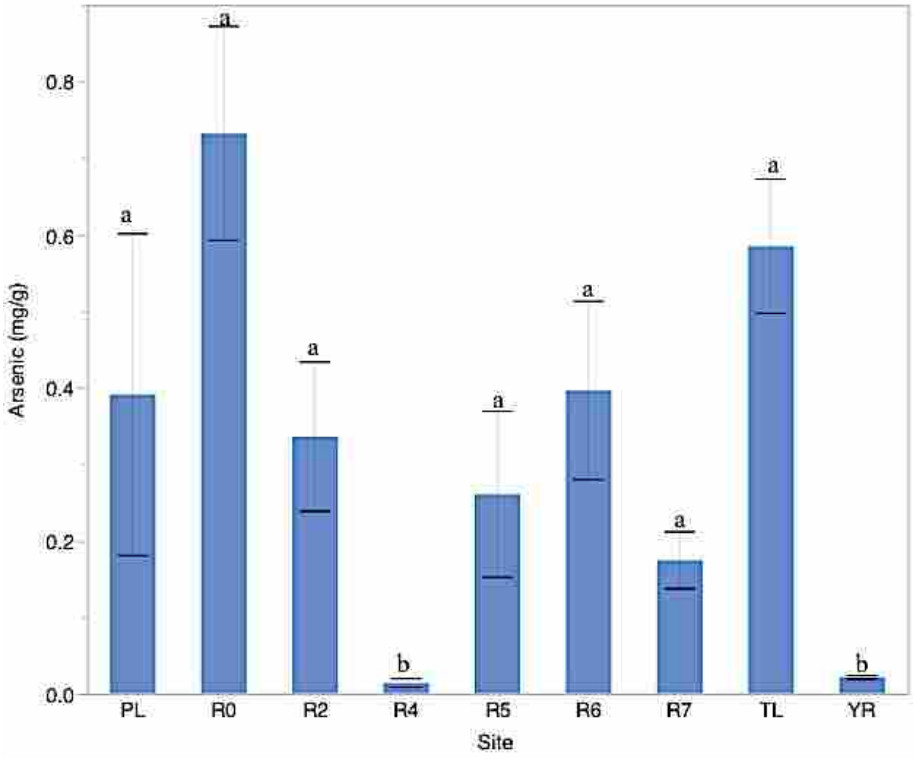


Figure 3.6 Arsenic levels by site from soils used in bioassays. Bars represent means ± 1 SE; connected letters from a Tukey's HSD post hoc comparison of means from log-transformed data. Bars sharing letters represent means that are not significantly different (ANOVA, $p < 0.05$). Site abbreviations: R0 - 7, Reach 0 - 7; PL, Pocket Lake; TL, Trapper Lake; YR, Yellowknife River.

Figure 3.7 Images of mycorrhizal spores, a brown spore (left) and a yellow spore (right) with vestigial hyphae, indicated by the red arrow

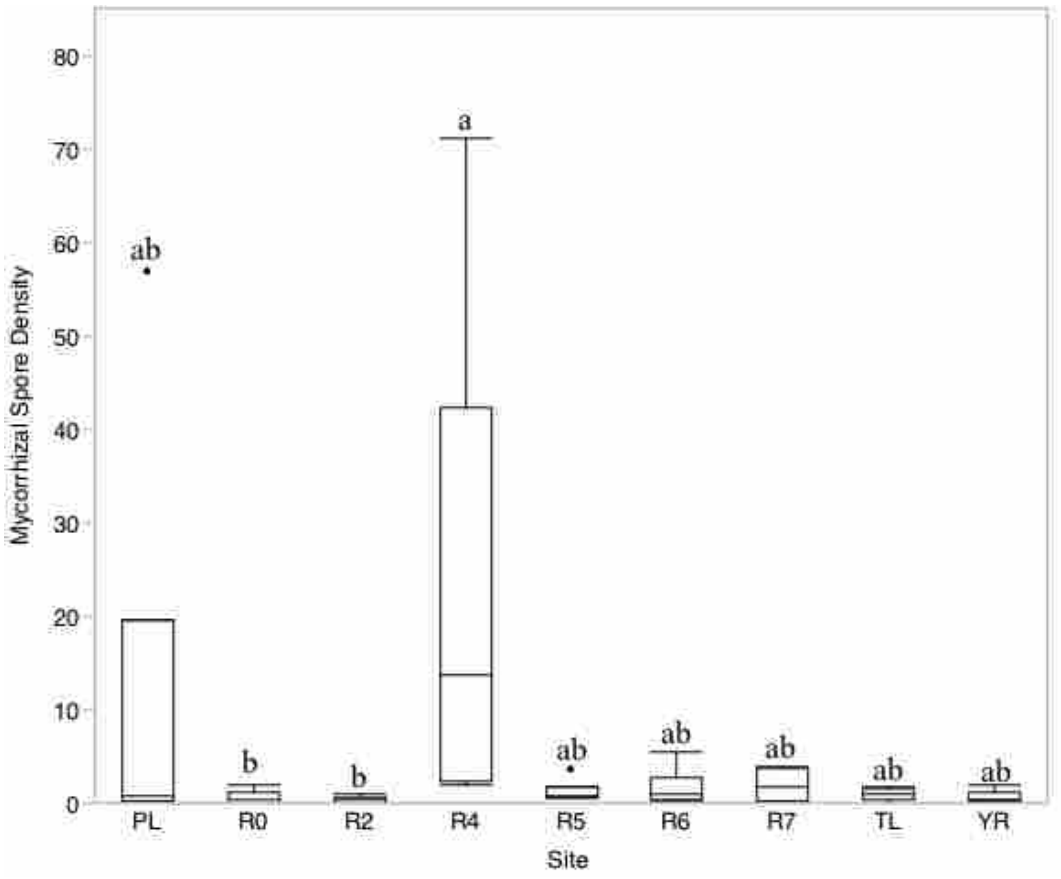


Figure 3.8 Mycorrhizal spore density per gram of soil by site in soils used for the bioassays. Boxes represent the middle fifty percent of the distributions, medians are represented by middle lines, values greater than 1.5 times the interquartile range are depicted as circles above the box, and whiskers denote the maximum and minimum values that did not qualify as outliers. Connected letters from Dunn's non-parametric comparisons of data distributions by site; boxes sharing letters represent data distributions that are not significantly different (Kruskal-Wallis, $p < 0.05$). Site abbreviations: R0 - 7, Reach 0 - 7; PL, Pocket Lake; TL, Trapper Lake; YR, Yellowknife River.

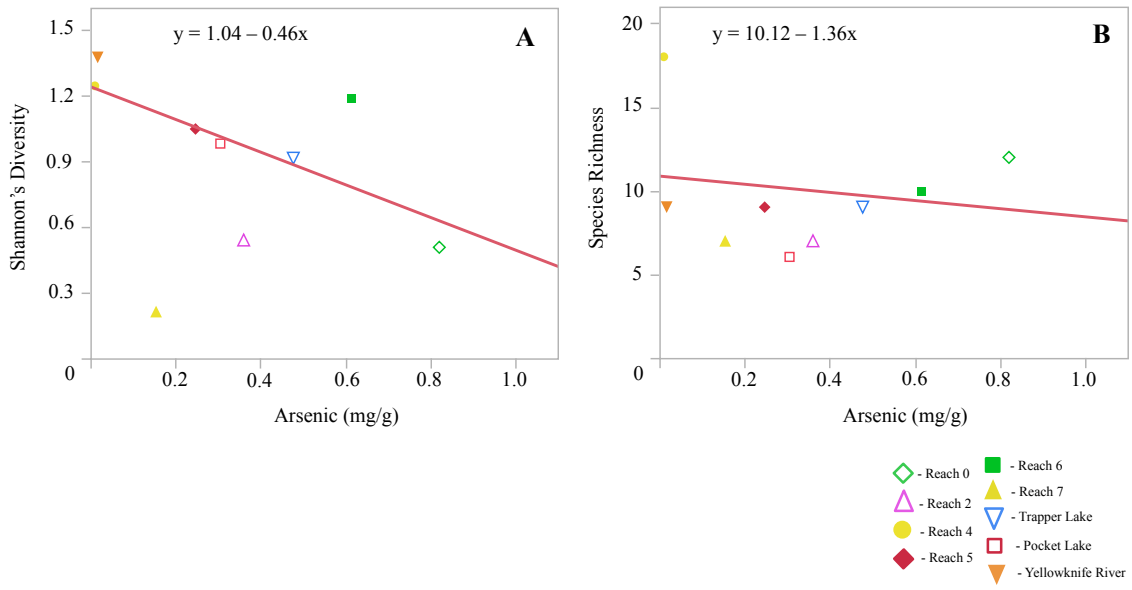


Figure 3.9 A) Correlation of Shannon's species diversity against mean arsenic levels by site in our vegetation survey of Baker Creek and surrounding wetlands. Fitted with line generated from Pearson's correlation (Correlation = -0.321 p = 0.399)

B) Correlation of species richness against mean arsenic levels by site in our vegetation survey of Baker Creek and surrounding wetlands. Fitted with line generated from Pearson's correlation (Correlation = -0.101 p = 0.795)

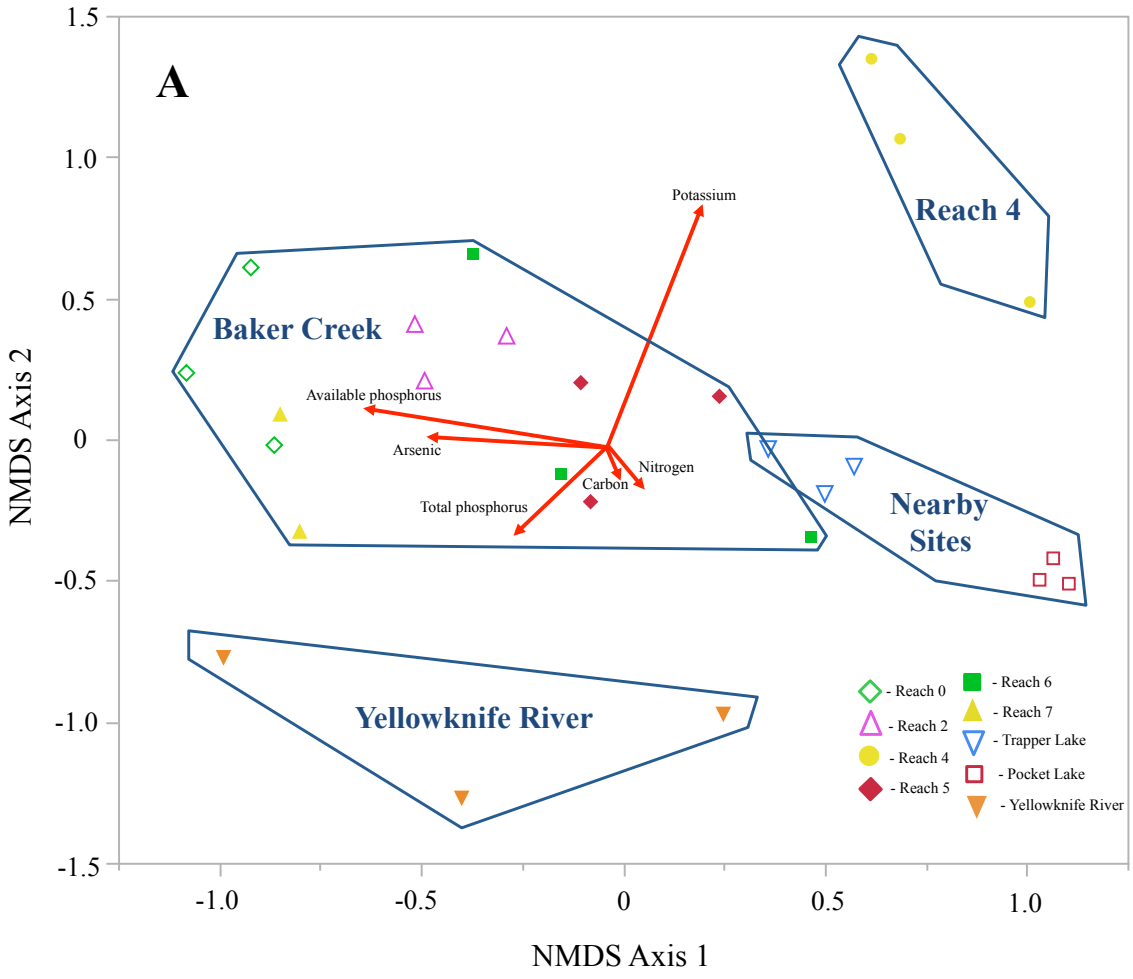


Figure 3.10 Ordination of the first two dimensions for non-metric multidimensional scaling of sample sites in the vegetation survey of Baker Creek (stress = 11.439; p-value from randomization test = 0.016). Ordination based on average percent cover of plant species in three transects for each site, with environmental variables overlaid on the ordination space. **A)** A biplot of the first two ordination axes **B)** Vectors for each species used in the analysis. Site abbreviations: R0 - 7, Reach 0 - 7; PL, Pocket Lake; TL, Trapper Lake; YR, Yellowknife River.

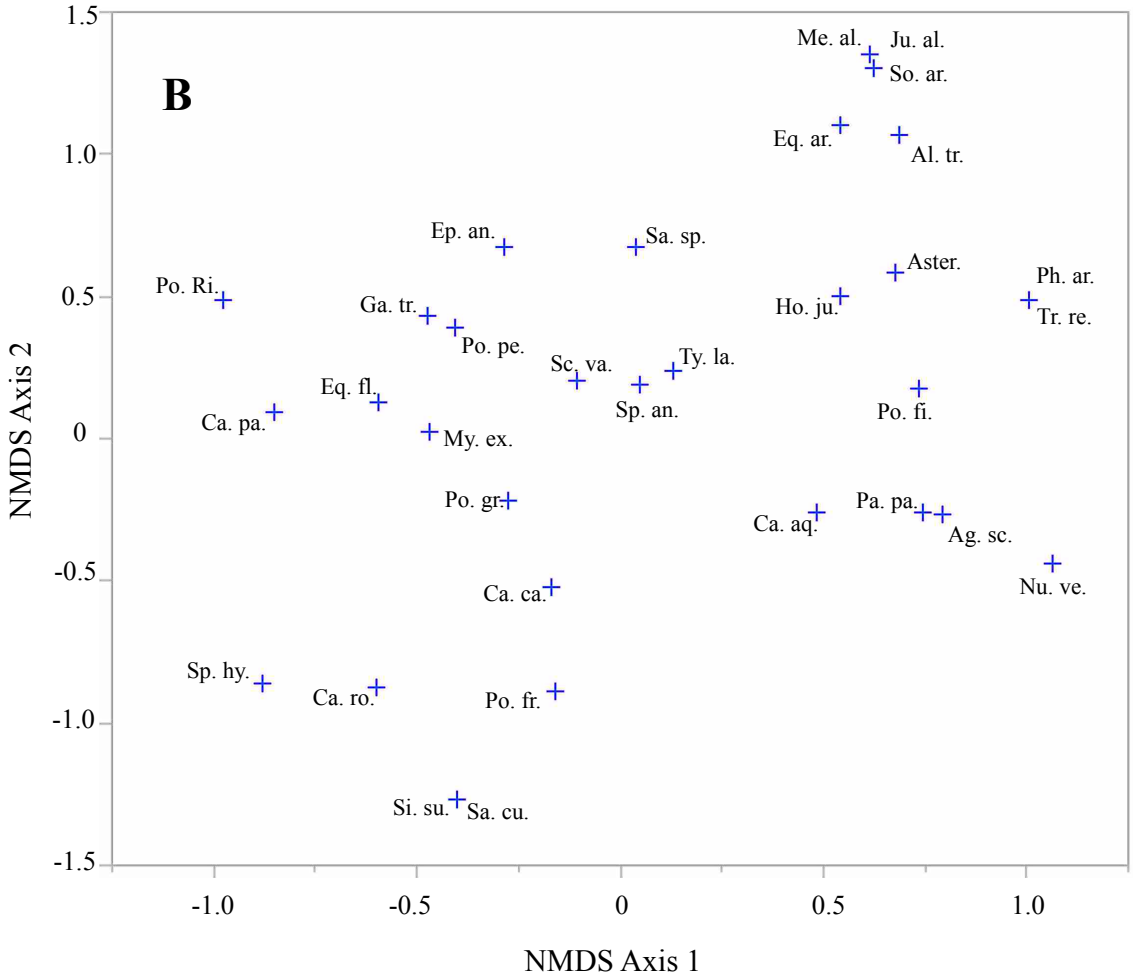


Figure 3.10 *(continued)*.

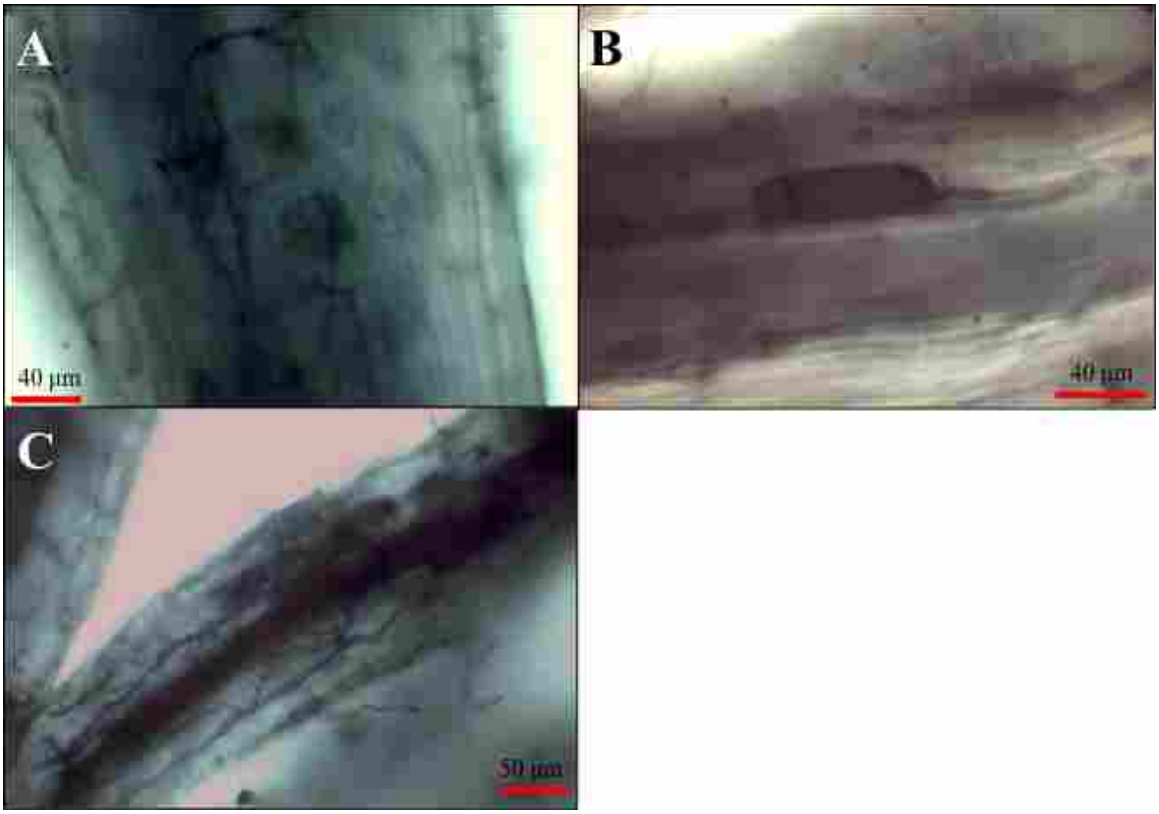


Figure 3.11 Images of mycorrhizal structures from field-collected roots **A)** Arbuscules in *C. canadensis* **B)** A Vesicle in *E. arvense* **C)** Hyphae in *P. arundinacea*

9. Appendix

Table A1. Shapiro-Wilks scores for testing the normality of data distributions by site for **A)** Shoot dry weight in *P. arundinacea* **B**) Shoot fresh weight in *P. arundinacea* **C)** Shoot surface area in *P. arundinacea* **D)** Root fresh weight in *P. arundinacea* **E)** Root length in *P. arundinacea* **F)** Root surface area in *P. arundinacea* **G)** Shoot fresh weight for *E. angustifolium* **H)** Shoot surface area for *E.* **I)** Root length in *E. angustifolium* **J)** Root surface area in *E. angustifolium* **K)** Arsenic data by site. All data was log transformed except root data in *P. arundinacea*, which was square root transformed. Due to the large amount of variables being tested, and the inherently noisy nature of biological field data, a significance level of $p < 0.01$ was used. Site abbreviations: R0 - 7, Reach 0 - 7; PL, Pocket Lake; TL, Trapper Lake; YR, Yellowknife River.

		A	B	C	D	E	F	G	H	I	J	K
PL	**W**	0.93	0.95	0.95	0.94	0.94	0.93	0.94	0.97	0.96	0.99	0.83
	Prob<W	0.59	0.74	0.72	0.70	0.66	0.61	0.70	0.92	0.83	0.99	0.10
R0	**W**	0.60	0.24	0.93	0.55	0.94	0.62	0.04	0.96	0.95	0.24	0.78
	Prob<W	0.87	0.96	0.59	0.89	0.67	0.94	0.94	0.84	0.72	0.93	0.04
R2	**W**	0.98	0.97	0.94	0.96	0.91	0.96	0.90	0.92	0.89	0.86	0.91
	Prob<W	0.95	0.88	0.63	0.85	0.41	0.82	0.40	0.50	0.33	0.19	0.44
R4	**W**	0.81	0.91	0.98	0.39	0.96	0.64	0.19	0.93	0.85	0.45	0.98
	Prob<W	0.99	1.00	0.96	0.96	0.85	0.93	0.94	0.60	0.17	0.86	0.92
R5	**W**	0.82	0.97	0.94	0.93	0.95	0.85	0.91	0.98	0.93	0.99	0.87
	Prob<W	0.14	0.82	0.65	0.57	0.72	0.21	0.48	0.93	0.61	0.97	0.24
R6	**W**	0.06	0.04	0.99	0.49	0.89	0.75	0.58	0.90	0.88	0.77	0.87
	Prob<W	0.94	0.95	0.98	0.90	0.33	0.86	0.95	0.39	0.27	0.91	0.24
R7	**W**	0.93	0.95	0.99	0.94	0.90	0.93	0.94	0.90	0.98	0.99	0.82
	Prob<W	0.59	0.74	0.96	0.70	0.41	0.61	0.70	0.43	0.91	0.99	0.14
TL	**W**	0.60	0.24	0.90	0.55	0.79	0.62	0.04	0.95	0.96	0.24	0.94
	Prob<W	0.87	0.96	0.39	0.89	0.05	0.94	0.94	0.77	0.84	0.93	0.70
YR	**W**	0.98	0.97	0.92	0.96	0.92	0.96	0.90	0.96	0.90	0.86	0.97
	Prob<W	0.95	0.88	0.54	0.85	0.48	0.82	0.40	0.78	0.35	0.19	0.90

Table A2 Results from Bartlett's tests assessing variance for all comparisons of means between sites **A)** Shoot dry weight in *P. arundinacea* **B**) Shoot fresh weight in *P. arundinacea* **C)** Shoot surface area in *P. arundinacea* **D)** Root fresh weight in *P. arundinacea* **E)** Root length in *P. arundinacea* **F)** Root surface area in *P. arundinacea* **G)** Shoot fresh weight for *E. angustifolium* **H)** Shoot surface area for *E.* **I)** Root length in *E. angustifolium* **J)** Root surface area in *E. angustifolium* **K)** Arsenic data by site. All data was log transformed except root data in *P. arundinacea*, which was square root transformed. Due to the large amount of variables being tested, and the inherently noisy nature of biological field data, a significance level of $p < 0.01$ was used.

	DF	F - ratio	Prob > F
A	8	1.70	0.09
B	8	1.32	0.23
C	8	1.19	0.30
D	8	2.05	0.04
E	8	1.82	0.07
F	8	1.74	0.08
G	8	0.95	0.47
H	8	1.81	0.07
I	8	0.61	0.77
J	8	0.68	0.71
K	8	2.09	0.04

Table A3 Effects tests evaluating the influence of nesting quadrats within transects for all comparisons of means between sites for **A)** Shoot dry weight in *P. arundinacea* **B**) Shoot fresh weight in *P. arundinacea* **C)** Shoot surface area in *P. arundinacea* **D)** Root fresh weight in *P. arundinacea* **E)** Root length in *P. arundinacea* **F)** Root surface area in *P. arundinacea* **G)** Shoot fresh weight for *E.* **H)** Shoot surface area for *E. angustifolium* **I)** Root length in *E. angustifolium* **J)** Root surface area in *E. angustifolium* **K)** Arsenic data by site. All data was log transformed except root data in *P. arundinacea*, which was square root transformed. Due to the large amount of variables being tested, and the inherently noisy nature of biological field data, a significance level of $p < 0.01$ was used.

		Nparm	DF	Sum of Squares	F Ratio	Prob > F
A	Site	8	8	50.55	6.46	<.0001
	Transect[Site]	9	9	10.83	1.23	0.284
	Quadrat[Transect, Site]	34	34	76.78	2.31	0.0001
B	Site	8	8	60.70	24.03	<.0001
	Transect[Site]	9	9	5.32	1.87	0.065
	Quadrat[Transect, Site]	34	34	39.76	3.70	<.0001
C	Site	8	8	57.18	25.41	<.0001
	Transect[Site]	9	9	4.10	1.62	0.120
	Quadrat[Transect, Site]	34	34	46.29	4.84	<.0001
D	Site	8	8	1331.89	24.30	<.0001
	Transect[Site]	9	9	56.63	0.92	0.513
	Quadrat[Transect, Site]	34	34	941.22	4.04	<.0001
E	Site	8	8	1907.29	36.95	<.0001
	Transect[Site]	9	9	76.08	1.31	0.241
	Quadrat[Transect, Site]	34	34	1181.96	5.39	<.0001
F	Site	8	8	314.38	42.18	<.0001
	Transect[Site]	9	9	8.67	1.03	0.419
	Quadrat[Transect, Site]	34	34	167.39	5.28	<.0001
G	Site	8	8	182.53	33.65	<.0001
	Transect[Site]	9	9	13.42	2.20	0.029
	Quadrat[Transect, Site]	34	34	47.45	2.06	0.003
H	Site	8	8	164.36	64.73	<.0001
	Transect[Site]	9	9	6.17	2.16	0.032
	Quadrat[Transect, Site]	34	34	32.51	3.01	<.0001
I	Site	8	8	139.36	25.19	<.0001
	Transect[Site]	9	9	8.72	1.40	0.203
	Quadrat[Transect, Site]	34	34	51.62	2.20	0.002
J	Site	8	8	233.63	12.08	<.0001
	Transect[Site]	9	9	23.67	1.09	0.381
	Quadrat[Transect, Site]	34	34	129.63	1.58	0.049
K	Site	8	8	80.91	16.11	<0.001
	Transect[Site]	9	9	11.41	02.02	0.070

Table A4 Results from ANOVA's testing the effect of site of soil collection on growth responses in *P. arundinacea* from the bioassays. **A)** Shoot dry weight **B)** Shoot fresh weight **C)** Shoot surface area **D)** Root fresh weight **E)** Root length **F)** Root surface area. All shoot data received log-transformations and root data was square root transformed. * Indicates a significant effect of site

A	**df**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob > F**
Model	51	136.41	2.67	2.73	<.0001*
Error	98	95.80	0.98		
C. Total	149				
B	**df**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob > F**
Model	51	106.12	2.08	6.59	<.0001*
Error	98	30.94	0.32		
C. Total	149	137.07			
C	**df**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob > F**
Model	51	106.31	2.08	7.41	<.0001*
Error	98	27.57	0.28		
C. Total	149	133.87			
D	**df**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob > F**
Model	51	2338.10	45.85	6.69	<.0001*
Error	98	671.40	6.85		
C. Total	149	3009.50			
E	**df**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob > F**
Model	51	3155.57	61.87	9.59	<.0001*
Error	98	632.26	6.45		
C. Total	149	3787.83			
F	**df**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob > F**
Model	51	490.78	9.62	10.33	<.0001*
Error	98	91.31	0.93		
C. Total	149	582.10			

Table A5 Results from ANOVA's testing the effect of site of soil collection on growth responses in *E. angustifolium* from the bioassays **A)** Shoot fresh weight **B)** Shoot surface **C)** Root length **D)** Root surface area. All data was log-transformed before analysis. *Indicate a significant effect of site

A	**df**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob > F**
Model	51	236.54	4.64	6.84	<0.0001*
Error	95	64.41	0.68		
C. Total	146	300.95			
B	**df**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob > F**
Model	51	203.29	3.99	12.56	<0.0001*
Error	95	30.15	0.32		
C. Total	146	233.44			
C	**df**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob > F**
Model	51	200.53	3.93	5.69	<0.0001*
Error	77	53.24	0.69		
C. Total	128	253.77			
D	**df**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob > F**
Model	51	437.22	8.57	3.55	<0.0001*
Error	83	200.70	2.42		
C. Total	134	637.92			

Table A6. Results from Wilcoxon pairwise comparisons testing the effect of transect on mycorrhizal colonization by site in roots from the bioassays. Site abbreviations: R0 - 7, Reach 0 - 7; PL, Pocket Lake; TL, Trapper Lake; YR, Yellowknife River.

Species	**Site**	*Hyphal Colonization*		*Arbuscular Colonization*		*Vesicular Colonization*	
		ChiSqu	Prob > ChiSqu	ChiSqu	Prob > ChiSqu	ChiSqu	Prob > ChiSqu
E. angustifolium	**PL**	1.00	0.318	2.40	0.121	1.00	0.318
	R0	0.00	1.00	0.00	1.000	0.00	1.000
	R2	1.00	3.18	1.00	0.318	0.00	1.000
	R4	1.19	0.275	1.19	0.275	0.22	0.637
	R5	1.00	3.180	1.00	0.318	1.00	0.318
	R6	2.40	0.121	2.40	0.121	1.00	0.318
	R7	2.67	0.103	2.67	0.103	0.00	1.00
	TL	1.00	0.318	1.00	0.318	0.00	1.00
	YR	3.86	0.050	3.86	0.050	4.35	0.04
P. arundinacea	**PL**	1.00	0.318	1.00	0.318	0.00	1.000
	R0	0.00	1.000	0.00	1.000	0.00	1.000
	R2	1.00	3.18	1.00	0.318	1.00	0.318
	R4	0.43	0.513	0.48	0.487	0.48	0.487
	R5	0.00	1.000	0.00	1.000	0.00	1.000
	R6	0.07	0.796	0.07	0.796	1.00	0.318
	R7	0.00	1.000	0.00	1.000	0.00	1.000
	TL	0.00	1.000	0.00	1.000	0.00	1.000
	YR	1.34	0.246	1.34	0.246	1.40	0.121

Table A7. Results from ANOVA testing the effects of site on arsenic levels in soils used for the bioassays; data was log-transformed before analysis. * Indicates a significant effect of site

	df	Sum of Squares	Mean Square	F-Ratio	Prob > F
Model	8	95.50	11.94	16.30	<0.0001*
Error	43	31.50	20.73		
C. Total	51	125.99			

Table A8. Results from ANOVA's testing the effect of site on levels of mycorrhizal colonization in field-collected roots for **A)** Hyphal colonization in *C. canadensis* **B)** Vesicular colonization in *C. canadensis* **C)** Vesicular colonization in *E. angustifolium*

A	**df**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob > F**
Model	5	1101.47	220.29	4.01	0.009*
Error	24	1318.00	54.92		
C. Total	29	2419.47			
B	**df**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob > F**
Model	5	100.30	20.06	2.09	0.102
Error	24	230.40	9.60		
C. Total	29	330.70			
C	**df**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Prob > F**
Model	1	108.90	108.90	1.03	0.340
Error	8	846.00	105.75		
C. Total	9	954.90			

Table A9. Results from Kruskal-Wallis analyses testing the effect of site on levels of mycorrhizal colonization in field collected roots for **A)** Hyphal colonization in *E. arvense* **B)** Hyphal colonization in *E. angustifolium* **C)** Hyphal colonization in *P. fruticosa* **D)** Vesicular colonization in *E. arvense* **E)** Vesicular colonization in *P. fruticosa* **F)** Arbuscular colonization in *C. canadensis* **G)** Arbuscular colonization in *E. arvense* **H)** Arbuscular colonization in *P. fruticosa*

	df	**Chi-Square**	**Prob<ChiSq**
A	3	13.88	0.003
B	1	6.99	0.008
C	1	4.51	0.034
D	1	0.10	0.751
E	1	5.54	0.019
F	5	6.04	0.302
G	2	9.88	0.007
H	1	2.22	0.136

Table A10. Test scores from Wilcoxon matched pairs (non-normal) or t-test (normal) comparisons between levels of arbuscular and vesicular colonization in colonized roots only for **A)** *E. angustifolium* from the bioassays **B)** *P. arundinacea* from the bioassays **C)** Field-collected *C. canadensis* **D)** Field-collected *E. angustifolium* **E)** Field-collected *E. arvense* **F)** Field-collected *P. fruticosa*

G) Field-collected *P. arundinacea.* (**A-G)** Wilcoxon matched pairs, (**F)** Paired t-test.

Variable	n	Test Statistic S	Prob > S
A	27	39.50	0.898
B	43	-473.00	<.0001
C	30	-172.50	<.0001
D	10	-27.50	0.002
E	10	-22.50	0.004
G	5	-5.00	0.125
Variable	**n**	**t-ratio**	**Prob > t**
F	5	-5.74	0.005