An Investigation of Potential Weed Management Practices and Multivariate Assessment Parameters for Alberta's Oil Sands Reclamation Efforts

by

Leah Amanda deBortoli

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Department of Renewable Resources
University of Alberta

Abstract:

Reclamation efforts that promote the re-establishment of native tree and plant communities subsequent of large-scale oil sands mining land disturbances are crucial in restoring natural ecosystems. It is important that reclamation procedures capable of facilitating the establishment of native species be identified and put into practice. The objective of the first study was to determine plant community development and aspen seedling establishment in response to different combinations of coversoil types and experimental plant establishment treatments on an oil sands overburden waste area. Eighteen field plots, established in 2014, were re-monitored annually to compare plant community development and trembling aspen seedling density on 3 coversoil types (forest floor-mineral mix [FFMM], transitional, peat-mineral mix [PMM]) with 4 plant establishment treatments (seeding native species, weeding undesirable weeds, seeding & weeding, control). Coversoil type was found to be a dominant plant community driver, with FFMM and transitional soils showing higher species richness, diversity, and total vegetation cover than PMM, while PMM supported greater aspen seedling densities. Minimal weed establishment on PMM coversoils resulted in weeding treatments having a lesser effect on plant community development; however, trembling aspen seedling densities were found to have increased. Weeding on FFMM and Transitional did not result in the significant increase of native forb presence. Instead, the decrease in introduced weed species prompted an increase in graminoid cover, particularly *Calamagrostis canadensis* on FFMM.

In addition to the refinement of reclamation procedures, we must work towards developing an effective evaluation framework in order to track ecosystem recovery progress. To date, no official standards, nor suitable criteria and indicators have been established to thoroughly assess and certify reclamation sites. As such, the objective of the second study was to explore the use of multivariate datasets as parameters within a rudimentary ecosystem function assessment framework. Natural reference soil samples and reclamation coversoil samples corresponding to the aforementioned field plots were collected in 2016 following vegetation surveys and in-field bioavailable nutrient profiling. Following a two week laboratory incubation period, soil samples were used to determine microbial function via community level physiological profiling (CLPP). Through the use of non-metric multidimensional scaling ordination analyses, similarities and dissimilarities were determine for bioavailable nutrient profiles, microbial function, and plant community composition parameters between coversoils and natural soils. Ordination analyses were also completed to determine similarities between weeding and control plots on coversoils. As with the first study, coversoils/soils were the dominant drivers of dissimilarities, while weeding treatments did not significantly change bioavailable nutrient profiles or microbial function. Overall, the use of multivariate analyses was able to provide additional insight into the aboveground and belowground recovery on reclamation sites, suggesting that this method of assessment, with further research, holds potential.

Preface:

The following thesis is composed of original data generated and analyzed by Leah Amanda deBortoli, with no data having been published at the time of submission. Data from Chapter 1, “Plant community composition and trembling aspen establishment in response to seeding and weeding treatments on different reclamation coversoils”, was presented at the 2016 Alberta Soil Science Workshop (poster), the 2017 North American Forest Ecology Workshop (oral presentation), the 2017 Ecology Society of America Annual Meeting (poster), and the 2017 Canadian Land Reclamation Association Annual General Meeting (poster). Data from Chapter 2, “Evaluation of oil sands reclamation sites using bioavailable nutrients, microbial function, and plant community multivariate parameters”, was presented at the 2017 Ecological Society of America Annual Meeting. Chapter 1, as presented in this thesis (excluding the conclusion paragraph), has been submitted to the Restoration Ecology journal and is currently waiting for review scores.

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1.1 – Regional Overview:

1.1.1 – The Boreal Zone:

The Canadian boreal zone extends approximately 5.52 million km^2 (Brandt et al. 2013), and is situated between the northern arctic zone (treeless tundra) and the southern temperate zone (NRCan 2017). The boreal zone is comprised of forests, woodlands, naturally treeless alpine regions, heathlands, grasslands, peatlands, as well as rivers and large bodies of water (Brandt 2009; Brandt et al. 2013). Cumulatively, these various ecosystems perform numerous functions, such as primary production, nutrient cycling, and soil formation, as well as ecosystem services, such as food and resource production, climate regulation, water regulation and filtration, erosion control, and recreational opportunities (Hassan et al. 2005). Additionally, the boreal zone provides Canada with a plethora of renewable (timber, pulp, peat, etc.) and non-renewable (base metals, precious metals, oil and gas) ecosystem goods that are crucial in supporting the nation's natural resource-based economy, and social well-being (Bogdanski 2008; Brandt et al. 2013). Despite the many ecosystems found within in the boreal zone, forested regions appropriately referred to as the boreal forest dominate the majority (~ 48%) of the zone's landscape at 2.7 million km^2 (Brandt 2009; Brandt et al. 2013).

1.1.2 – Alberta's Boreal Forest Natural Region:

In Alberta, the Boreal Forest natural region constitutes approximately 58% of the total land area, including the majority of northern Alberta and some southerly extensions as far as Calgary (NRC 2006; Bliss et al. 2015). Due to its extensive size, this natural region is able to support a high level of biodiversity in both plants and wildlife (Bliss et al. 2015; Wiken 2015). The gently undulating landscape allows for the development of upland forests comprised of black and white spruce, jack pine, balsam fir, trembling aspen, balsam poplar and paper birch tree species, as well as wetland ecosystems containing black spruce and tamarack (NRC 2006; Bliss et al. 2015; Wikens 2015). In addition to incredibly diverse plant communities, the boreal forest region is known to support an array of ungulates, other mammals, birds, and fish populations (Wiken 2015).

Certain areas within the Boreal Forest natural region experience discernable differences in topographical, climatic, and vegetative characteristics, which has resulted in the classification of individual subregions (NRC 2006). Given the focus of the subsequent studies discussed in this body of work, the Central Mixedwood natural subregion is of particular interest.

1.1.3 – Alberta's Central Mixedwood Subregion:

The Central Mixedwood is largest of the eight subregions that constitute the Boreal Forest natural region in Alberta (NRC 2006). It experiences short, warm summers with an average frost-free period of 97 days (late May to early September) and long, cold winters (NRC 2006). Mean monthly temperatures range from -17.4ºC in January to 17.1ºC in July, with a mean annual temperature of approximately 1ºC (Government of Canada 2017). Total annual precipitation for the area is estimated to be 418.6mm, with approximately 55 percent occurring as rainfall during May through August (Government of Canada 2017). Aspen-dominated and aspen-white spruce stands are characteristic of natural upland sites, and are typically situated on Luvisolic soils (Beckingham and Archibald 1996; NRC 2006). Lowland sites predominantly support black spruce fens and bogs, and develop thick Organic soils (Beckingham and Archibald 1996; NRC 2006). In addition to land use in the form of forestry operations, the Central Mixedwood subregion of Alberta has been heavily impacted by oil sands mining and exploration disturbances that differ drastically from the region's natural disturbance regime (NRC 2006).

1.2 – Natural Disturbances and Recovery

The natural structure and composition of North American forest ecosystems are often the result of numerous disturbance regimes such as wildfires, wind throw, and insect outbreaks (Chen and Popadiouk 2002; Long 2009; Swanson *et al.* 2011; Kishchuk *et al.* 2015). In the case of the western boreal forests of Alberta, stand-replacing wildfires caused by lightning strikes are the most common, and influential, natural disturbance regime driving forest regeneration dynamics (Chipman and Johnson 2002; Hart and Chen 2008; MacKenzie *et al.* 2014; Kishchuk *et al.* 2015). Environmental characteristics associated with recent burns often include increased light transmission, soil temperature, and nutrient availability (Chen and Popadiouk 2002; Hart and Chen 2006; Swanson *et al.* 2011). As a result, initial colonization often involves intense competition between shade intolerant trees, typically *Populus tremuloides*, *Betula papyrifera*, and *Populus balsamifera*, and shade intolerant vascular plants with high nutrient demands, such as *Chamerion angustifolium, Rubus idaeus*, and *Calamagrostis Canadensis*, *Carex* sp., and *Equisetum* sp. (Chen and Popadiouk 2002; Hart and Chen 2006). The development of a deciduous canopy cover promotes the subsequent establishment of shade tolerant conifers, such as *Picea glauca* and *Picea mariana*, and a more complete canopy closure (Chen and Popadiouk 2002; Hart and Chen 2006). With a decline in light transmission and nutrient availability, total understory biomass decreases, and early stage understory species are out-competed by shade tolerant species, such as *Cornus canadensis*, *Linnea borealis*, and *Aralia nudicaulis*, and *Ribes* spp. (Hart and Chen 2006; De Grandpré *et al.* 2011; Swanson *et al.* 2011).

This repetition of post-fire regeneration throughout the boreal forest contributes to the maintenance of understory plant, and overall forest stand, diversity (Bonan and Shugart 1989).

Ultimately, natural disturbances common to the region are jointly responsible for driving the continuous renewal of biogeochemical cycling, productivity, and landscape variability that gives the boreal forest its dynamic nature (Brandt et al. 2013). However, the establishment of Alberta's oil sands industry has resulted in the introduction of higher severity anthropogenic disturbances within the boreal forest – specifically in the aforemention Central Mixedwood subregion.

1.3 – Alberta Oil Sands, Disturbance, and Reclamation:

1.3.1 – Economic Overview and Forecast:

Canada's landmass contains the third largest crude oil reserve in the world and, with a production rate of approximately 3.85 million barrels per day, is recognized as the sixth largest crude oil producer globally (CAPP 2017b). The majority of Canada's crude oil reserves are situated in the Alberta oil sands region (AOSR) where a reserve of 165 billion barrels was estimated at the conclusion of 2016, of which 133 billion barrels (81%) are to be recovered using in situ steam-assisted gravity drainage (CAPP 2017b). The remaining 32 billion barrels (19%) are located at depths shallow enough for extraction via surface mining operations (NRCan 2013; CAPP 2017b). Both in situ and surface mining production rates are forecasted to increase (CAPP 2017b), resulting in the generation of approximately 3 million jobs nation-wide and a total gross domestic product of approximate 1.7 trillion dollars over the next ten years (Doluweera et al. 2017). This makes the AOSR a crucial source of economic and social security in Canada.

1.3.2 – Mining Operations and Disturbances:

Collectively, the Athabasca, Cold Lake and Peace River oil sands deposits underlie approximately 142,200 km^2 within Alberta's provincial borders, with an estimated 4,800 km^2 situated in the northern portion of the Athabasca deposit deemed suitable for surface mining operations (CAPP 2017). To date, the amount of land that has undergone disturbance from surface mining is approximately 904 km^2 (CAPP 2017). Although the majority of production involves in-situ extraction, surface mining operations generate much greater land disturbances (NRCan 2016). Additionally, unlike natural disturbances, such as wildfires, wind throws, and insects, which mainly result in the temporary loss of vegetation (Chen and Popadiouk 2002; Swanson et al. 2012), oil sands surface mining operations result in the clearances of vegetation, as well as the complete removal of soil (Rowland et al. 2009; Naeth et al. 2012). This results in the need for reclamation procedures to include extensive soil reconstruction prior to revegetation efforts.

1.3.3 – Reclamation Obligations and Procedures:

According to the Environmental Protection and Enhancement Act, reclamation is part of an oil company's legal requirement to restore disturbed areas to an "equivalent land capability" (Province of Alberta 2017). As a means of insurance, mine developers must compile and submit an approved conservation and reclamation plan (revised every five years), as well as provide a security deposit based on estimated reclamation costs, to the Province of Alberta (Fung and Macyk 2000). Once all reclamation activities have been completed in a particular area, mining companies are eligible to apply for a reclamation certification. Should the reclaimed area under assessment meet the necessary legal requirements, the mining company would receive reclamation certification, as well as a refund of their security deposit (Fung and Macyk 2000).

Approved reclamation plans must outline reclamation-based activities throughout the mine's entire lifespan, from initial land clearing to the subsequent reconstruction of soils (Fung and Macyk 2000). Typically, organic material, mineral soil, and overburden overlying oil sand deposits are removed to accommodate oil extraction and processing, and salvaged for subsequent soil reconstruction during land reclamation efforts (Rowland et al. 2009; Audet et al. 2015). Salvaged materials are either used immediately via direct placement reclamation procedures, or stockpiled for later use. Overburden material is used to re-contour disturbed areas, which are then capped with an organic-mineral mix coversoil. Reclamation uses two main coversoils – forest floor-mineral mix (FFMM) comprised of upland forest floor surface organic material and mineral soil, and peat-mineral mix (PMM) comprised of lowland organic material and mineral soil (Pinno and Errington 2015). Due to readily available and abundant supplies, PMM is the more commonly used coversoil (Rowland et al. 2009). However, FFMM is the preferred coversoil because of its abundant native seed bank which has been shown to produce plant communities similar to target upland forests (Mackenzie and Naeth 2010; MacKenzie et al. 2014), and its ability to stimulate microbial activity (McMillan et al. 2007). A third coversoil, referred to as Transitional, is derived from areas of topographical transition between an upland and lowland, and acts as an intermediate organic-mineral mix between FFMM and PMM.

Once soil reconstruction is completed, revegetation efforts can commence. According to the *Guidelines for Reclamation to Forest Vegetation in the Athabasca Oils Sands Region*, it is recommended that either an ecosite or a land-use approach be used to determine revegetation goals (Alberta Environment 2010). The ecosite approach involves determining which ecosite plant community would be most appropriate based on the suspected moisture and nutrient regime of newly reconstructed soils. Alternatively, the land-use approach favours the reconstruction of soils in a way that benefits target species for subsequent land-use (i.e. commercial forestry, wildlife, traditional use, recreation) (Alberta Environment 2010).

To date, nursery-grown white spruce, black spruce, jack pine, and trembling aspen are the most commonly planted tree species. Additional tree species may include tamarack and balsam poplar. In some cases, companies may choose to plant shrub seedlings (low-bush cranberry, blueberry, etc.) and/or seed native understory species to encourage greater vegetation cover of the target plant community. Alternatively, deciduous tree species and understory species may not require planting on site if coversoils contain surviving seeds and propagules, or if strong levels of natural ingress are present.

1.4 – Challenges in Plant Community and Tree Establishment:

Regardless of coversoil type, disturbances to soil during operations often lead to the destruction of viable vegetative propagules and root systems (Mackenzie and Naeth 2010; Pinno and Errington 2015) – a key regeneration strategy used by plant species under natural disturbance regimes (Whittle *et al.* 1997; Roberts 2004). The germination and emergence of native plant species is also reduced as pre-existing seed banks are redistributed to greater depths during soil placement (Mackenzie and Naeth 2010; Lemke *et al.* 2012). As a result, early-stage regeneration of the native plant community on reclamation soils relies more strongly on the limited seed bank and ingress from long-distance seed dispersal (Errington and Pinno 2015).

Long-distance dispersal (LDD) occurs naturally via wind and animal vectors (Taylor *et al.* 2012). However, similar to disturbance regimes, anthropogenic activities are becoming increasingly influential as LDD pathways (Hodkinson and Thompson 1997). Seed dispersal by vehicle transportation (i.e. machinery used to construct reclamation sites) is capable of increasing a species' maximum dispersal distance by several orders of magnitude (Hodkinson and Thompson 1997), and has been identified as an accelerant of non-native plant invasions (Taylor *et al.* 2012).

Invasive weeds possess mechanisms that allow them to establish and reproduce rapidly, to the extent that even dominant native species and tree seedlings are out-competed and displaced (Stinson *et al.* 2006; Cole *et al.* 2007; Hejda *et al.* 2009). The key characteristic of successful weed species is an extensive and rapidly proliferating root network (Drenovsky et al. 2008), such as those found in *Sonchus arvensis*, a common weedy forb species found throughout Alberta (Lemna and Messersmith 1990). Efficient root networks allow species, such as *S.arvensis*, to out-compete slow-growing plants in below-ground resource interception and acquisition, which then allow them to out-compete rival plants aboveground via superior photosynthetic efficiency and biomass production (Drenovsky et al. 2008). These competitive advantages displayed by invasive species are likely amplified on recently disturbed soils, such as oil sands reclamation sites, due to elevated organic matter mineralization rates resulting in increased nitrogen availability (Desserud and Naeth 2013). Weed invasions resulting from these skewed competitive advantages pose a significant threat to biodiversity, which in turn can decrease ecosystem stability, land productivity and habitat quality (Cole et al. 2007; Hejda et al. 2009). In the case of reclamation, weed invasions result in the development of plant communities that deviate from natural post-disturbance regeneration patterns.

1.5 – Challenges in Assessing Reclamation Progress:

As stated in the Environmental Protection and Enhancement Act, reclamation is part of an oil company's legal requirement to restore disturbed areas to an "equivalent land capability" (Province of Alberta 2017). However, no official standards, nor suitable criteria and indicators, have been established in order to thoroughly assess the success of oil sands reclamation efforts. By default, many reclamation sites are evaluated by measuring a collection of isolated chemical or physical indicators such as soil pH, SAR, EC, vegetation cover, tree height and vigour. These indicators meet the requirement of being data that is easy and cost effective to collect (National Research Council 2000); however, they are also susceptible to several common mistakes when criteria and indicators are developed (Failing and Gregory 2003). For example, these univariate indicators can provide a description of reclamation site characteristics, but they cannot be readily translated into a meaningful status report on an area's overall land capability. Additionally, the use of univariate indicators, though helpful during initial determination of baseline conditions, will likely lead to an over-simplified assessment framework given that reclamation targets at set at the ecosystem scale, as well as over a large temporal scale (Failing and Gregory 2003). As a result, current indicator guidelines are not able to meet their full potential in shaping reclamation practice policy, nor in tracking current reclamation recovery progression (Failing and Gregory 2003).

1.6 – Study One – Research Objectives:

In the first study of this research project, a field experiment was used to determine the effects of seeding native forbs species and weeding dominant introduced species on plant community composition and aspen establishment on newly constructed reclamation coversoils. The three central questions being asked include:

1) Will broadcast seeding a native forb seed mix promote the establishment of target understory plant communities?
2) Will the removal of dominant weed species improve the natural establishment and growth of trembling aspen seedlings and native understory plants?
3) Does the response to seeding and weeding vary by coversoil type?

1.7 – Study Two – Research Objectives:

The second part of this research project focuses on the use of multivariate parameters pertaining to soil bioavailable nutrients, microbial function, and plant community composition in order to further assess the ecosystem recovery progress of reclamation sites. The objective of this study is to determine whether a multivariate approach is suitable for the evaluation of oil sands reclamation sites. Given that reclamation coversoils and plant communities are the most accessible pathways of influencing ecosystem functioning, this study will apply multivariate assessment tools in order to answer:

1) What level of similarity to natural reference soils, with respect to bioavailable nutrient profiles, microbial function, and plant community composition, will be exhibited based on reclamation coversoil type?
2) Will the removal of dominant weed species increase the level of similarity shared by reclamation coversoils and natural reference soils with respect to bioavailable nutrient profiles and microbial function?
3) After three growing seasons, will reclamation sites have developed a significant plant-microbial interaction?

Chapter 2 – Plant community composition and trembling aspen establishment in response to seeding and weeding treatments on different reclamation coversoils

2.1 – Introduction:

Biodiversity in North American boreal forests is a function of their understory plant communities, which constitute the largest proportion of overall plant diversity (Roberts 2004; Gilliam 2007). Understory plant communities have also been identified as drivers of overstory succession, nutrient cycling, stand productivity, and wildlife communities (Hart and Chen 2006; Gilliam 2007). This is particularly true of early-successional understory communities following disturbances, when a combination of survivor and opportunist plant species elevates overall diversity (Swanson et al. 2011). From an ecosystem management perspective, it is important that we understand the impacts of different disturbances on forests and their understory plant communities.

Natural disturbances such as wildfires, wind throws, and insects are common in boreal forests (Chen and Popadiouk 2002), and play an important role in maintaining plant diversity and spatial heterogeneity throughout the region (Swanson et al. 2011). However, relatively new, and more severe, anthropogenic disturbance regimes have been introduced to Alberta's boreal forests (Mackenzie and Naeth 2010; Errington and Pinno 2015). The severity of these anthropogenic disturbances, in particular oil sands surface mining operations, results not only in the loss of aboveground biomass, but also extensive modification of the soil (Rowland et al. 2009; Naeth et al. 2012). Organic material, mineral soil, and overburden overlying oil sand deposits are removed and salvaged for soil reconstruction during reclamation (Rowland et al. 2009; Audet et al. 2015). Overburden material is used to re-contour disturbed areas, which are then capped with an organic-mineral mix coversoil. Reclamation uses two main coversoils – forest floor-mineral mix (FFMM) comprised of upland forest floor surface organic material and mineral soil, and peat-mineral mix (PMM) comprised of lowland organic material and mineral soil (Pinno and Errington 2015). Due to readily available and abundant supplies, PMM is the more commonly used coversoil (Rowland et al. 2009). However, FFMM contains a larger bank of native seeds and propagules, which has been shown to produce more diverse plant communities similar to those of target upland forest end points (Mackenzie and Naeth 2010; MacKenzie et al. 2014). In contrast, greater establishment of trembling aspen has been observed on PMM due to increased surface roughness and soil moisture and decreased competition, which is favourable for dispersed seed catchment, germination, and growth (Pinno and Errington 2015). A third coversoil, referred to as Transitional, can be derived from areas of topographical transition between an upland and lowland, and acts as an intermediate organic-mineral mix between FFMM and PMM. It has been speculated that Transitional coversoils could produce the ideal medium between native understory and trembling aspen establishment by possessing a similar seed bank to FFMM, as well as similar surface roughness and soil moisture to PMM.

Invasive weeds possess mechanisms that allow for rapid establishment and reproduction, to the extent that even dominant native species and tree seedlings are out-competed and displaced (Stinson et al. 2006; Cole et al. 2007; Hejda et al. 2009). The key characteristic of successful weed species is an extensive and rapidly proliferating root network (Drenovsky et al. 2008), such as those found in *Sonchus arvensis*, a common weedy forb species found throughout Alberta (Lemna and Messersmith 1990). Efficient root networks allow species, such as *S.arvensis*, to out-compete slow-growing plants in below-ground resource interception and acquisition, which then allow them to out-compete rival plants aboveground via superior photosynthetic efficiency and biomass production (Drenovsky et al. 2008). Weed invasions resulting from these skewed competitive advantages pose a significant threat to biodiversity, which in turn can decrease ecosystem stability, land productivity and habitat quality (Cole et al. 2007; Hejda et al. 2009). In the case of reclamation, weed invasions result in the development of plant communities that deviate from natural post-disturbance regeneration patterns. Therefore, determining suitable reclamation management practices that prevent or eliminate weed invasion and encourage the establishment of native plant communities is becoming an increasingly important topic.

Seeding newly reconstructed reclamation coversoils with a native forb seed mix holds potential in promoting the re-establishment of target understory plant communities by alleviating native seed limitations. Literature published by Turnball et al. (2000) and Seabloom et al. (2003) identify limitations in native seed availability as a common obstacle in early successional plant communities. In a field experiment, Seabloom et al. 2003 found that even a single addition of native forb seed to plots dominated by invasive species (over 65% cover) resulted in the establishment of viable native forb stands. Additionally, a study completed by Newman and Redente (2001) identified hand-broadcasting and raking as an effective method of seeding native grasses and forbs during revegetation efforts. Furthermore, hand-broadcasting a native seed mix resulted in greater total plant biomass when compared with seed mixes comprises partially or entirely of introduced species (Newman and Redente 2001).

Alternatively, regular removal of dominant weeds species on newly reconstructed coversoils may reduce the presence of introduced weed species over time, potentially allowing for increased native plant cover and richness, and tree seedling establishment. Studies completed by Biggerstaff and Beck (2007) and Flory and Clay (2009) indicated that hand-pulling target invasive species can result in increased native plant biomass and richness, as well as greater tree regeneration, when native plant communities are being suppressed by invasive plant species.

Both studies suggested that soil disturbances caused by hand-pulling invasive species created patches of bare ground suitable for seed germination (Biggerstaff and Beck 2007; Flory and Clay 2009). Additionally, Sheley et al. (1998) stated that persistent hand-pulling prior to invasive plants species reaching the flowering stage can successfully control invasions given that the entire plant is being removed. This effectively reduces unwanted growth via removal of seed sources, as well as reduction of vegetative propagation (Sheley et al. 1998).

In this study, a field experiment was used to determine the effects of seeding native forbs species and weeding dominant introduced species on plant community composition and aspen establishment on newly constructed reclamation coversoils. The three central questions being asked include:

1) Will broadcast seeding a native forb seed mix promote the establishment of target understory plant communities?
2) Will the removal of dominant weed species improve the natural establishment and growth of trembling aspen seedlings and native understory plants?
3) Does the response to seeding and weeding vary by coversoil type?

2.2 – Methods:

2.2.1 – Site Description:

Field research was completed approximately 70 kilometers north of Fort McMurray, Alberta, at an oil sands mine site situated within the Central Mixedwood subregion of the boreal forest (NRC 2006) which experiences short, warm summers with an average frost-free period of 97 days (late May to early September) and long, cold winters (NRC 2006). Mean monthly temperatures range from -17.4ºC during January to 17.1ºC during July, with a mean annual temperature of approximately 1ºC (Government of Canada 2017a). Total annual precipitation for the area is estimated to be 418.6mm, with approximately 55 percent occurring as rainfall during May through August (Government of Canada 2017a).

The reclamation area used for this study was constructed on an elevated dyke structure during the winter months prior to the initial 2014 growing season. Three organic-mineral mixed coversoils were directly placed at a depth of approximately 30 cm overtop 1 m of suitable overburden. Forest floor-mineral mix (FFMM) was salvaged from cleared upland forest stands, peat-mineral mix (PMM) was salvaged from cleared lowland fens and bogs, and Transitional was salvage from an area of topographic transitional between an upland and lowland. Deciduous tree species and shrubs were not planted on site due to strong levels of natural ingress having been observed on older reclamation areas (CNRL 2015).

2.2.2 – Experimental Design:

Following soil placement, six plots were randomly located within each soil type for a total of 18 plots (Figure 6.1). Each plot was 12m by 12m and divided into 5m by 5m quadrats. A 2m buffer area was established between each quadrat to minimize the disturbance of plant communities during sampling and to thoroughly separate plant establishment treatments. The quadrats within each plot were designated as either control, seeded, weeded, or mixed (seeded and weeded). Quadrats designated as seeded received a one-time seeding treatment during May 2014. A native forb seed mix of *Achillea millefolium*, *Aquilegia brevistyla*, *Aster ciliolatus*, *Aster conspicuous*, *Aster puniceus*, *Cornus canadensis*, *Delphinium glaucum*, *Chamerion angustifolium*, *Galium boreale*, *Solidago canadensis*, *Vicia americana*, and *Viola adunca* seeds mixed with sand (to prevent wind dispersal) was hand-broadcasted over selected quadrats and then gently raked to increase seed contact with the soil (Table 2.1; Figure 6.2). *Sonchus arvensis, Matricaria perforata*, and *Tanaceum vulgare* were identified as the dominant and persistent introduced species on the oil sands mine site (Li et al. 2015), and were therefore selected as targets of the weeding treatment. Hand-pulling on weeded quadrats was completed monthly, starting in June, during the 2014 growing season, which was reduced to twice per growing season during 2015 and 2016. Mixed quadrats received both of the aforementioned treatments, and controls were not manipulated.

2.2.3 – Vegetation Data Collection:

Annual aspen seedling mensuration was completed during the month of August from 2014 to 2016. Seedling mensuration plots were centered within each treatment quadrat and measured 3m by 3m. Stem count, base bole diameter, and seedling height were recorded.

Plant community composition data was collected annually during the months of July and August. Vegetation plots measuring 1m by 1m were nested within the seedling mensuration plots. Surveys were completed by identifying all plant species in each vegetation plot, then estimating their percent cover. Percent cover by plant functional groups was also determined for bryophytes, grasses, forbs, shrubs, and trees measuring less than 1.3m in height. Following plant community composition surveys, a walk-around of the entire 5m by 5m treatment plot was completed to determine species richness on a presence-absence basis (Figure 6.2).

2.2.4 – Soil Data Collection:

Soil nutrient supply rates for the 2014 and 2016 were measured during peak growing season (i.e. July to August) using plant root simulator (PRS) probes (PRS™; Western Ag Innovations, Saskatoon, SK, Canada). Four pairs of anion and cation probes were installed just outside the four boundaries of each 1m by 1m vegetation plot, and collected after 6 weeks. Collected probes were stored in coolers during transport from the field, cleaned thoroughly with deionized water, and sent to Western Ag Innovations where they were extracted with 0.5 M HCl for analysis. Inductively-coupled plasma spectrometry (Optima 8300 ICP-OES; Perkin Elmer Inc., Woodbridge, ON, Canada) was used to measure all nutrient ion contents, except for NO_3-N and NH_4-N ions, which were determined via colorimetry using an automated flow injection analysis system (FIAlab Instruments Inc., Bellevue, WA, USA).

Soil moisture and temperature readings were taken monthly from June to August. Moisture was determined using a TDR moisture probe (Field Scout TDR 300, Spectrum Technologies Inc., Aurora, IL), and temperature by using digital thermometers. Four readings of soil moisture and temperature were collected at a depth of 12 cm at each set of paired PRS probes.

Coversoil samples were collected at a depth of 5 cm to 15 cm in order to determine soil pH, electric conductivity (EC), and total organic carbon (TOC). Soil pH and EC were determined from a soil supernatant (5g soil: 25ml water) using a Mettler Toledo Five Easy pH and EC probe. To determine TOC, air dried soils were ground into a fine powder and weighed into 6mm by 4mm tins for encapsulation. Encapsulated soils were analyzed via a combustion-mode analyzer (Shimadzu TOC-VCSH/CSN, Shimadzu Corporation, Columbia, MD).

2.2.5 – Statistical Analyses:

The following data analyses were performed using R Version 3.2.3 software (R Core Team 2015). Alpha values of 0.10 or smaller were considered significant in order to account for high levels of variability inherent to this reclamation field study. Square root transformations were applied to response variables, as needed, to attenuate deviations from normality and equal variance assumptions. Plant establishment patterns were expected to vary based on coversoil type; therefore, each of the three coversoils were treated as separated datasets in order to effectively assess treatment effects, unless soil was being tested as a main effect.

One-way ANOVA and Tukey's HSD comparisons were used to examine differences in soil pH, EC, TOC, nutrient, and moisture response variables using coversoil as the main effect. Soil properties have been summarized in Table 2.2. The percent cover of each native forb species selected for the seed mix was examined using Kruskal-Wallis ANOVA by mean rank tests in order to assess the effectiveness of the seeding treatment. Plant establishment treatment was selected as the main effect, and research plots as the blocked random effect to account for variation caused by heterogeneity between plot locations. To further assess the effect of the seeding treatment, repeated measures ANOVA tests were used to examine the relative percent cover of native forbs, as well as native forb richness, using seeding and year as the main effects, and plots as the blocked random effect.

Results from the statistical analyses used to examine the effectiveness of seeding indicated minimal change to the plant community composition (refer to results section); therefore, the subsequent statistical analyses used to assess weeding treatments recognized mixed quadrats as additional weeded quadrats, and seeded quadrats as additional control quadrats. Repeated measures ANOVA tests were used to examine aspen density and height, species richness, total vegetation cover, as well as soil moisture and temperature, using coversoil and year as the main effects and plot as the blocked random effect. Additional repeated measures ANOVA tests were completed for aspen density and height, species richness, total vegetation cover, soil moisture and temperature, relative plant functional group cover (i.e. tree, shrub, native forb, introduced forb, graminoid, bryophyte) in order to test weeding and year as the main effects, with plots as the blocked random effect.

Lastly, a series of two sample t-tests (with unequal variances) were run to examine changes in individual grass species in weeded and control plots.

2.2.6 – Research Limitations:

This study used a single reclamation site in its experimental design; therefore, pseudo-replication occurred when analyzing data at the coversoil level given that the research plots established on FFMM, PMM, and Transitional, respectively, were not true replicates (Borcard et al. 2011). As such, this study cannot provide true results, but instead contributes to the findings and speculations of related works.

2.3 – Results:

2.3.1 – Seeding Effect:

Of the twelve native forb species included in the seed mix, *Achillea millefolium* was the only species that significantly increased in cover on seeded quadrats. *Achillea millefolium* cover increased from 1.6% to 7.5% on FFMM (Kruskal-Wallis; $X^2 = 8.91$, $p = 0.031$), from 0% to 4.5% on PMM (Kruskal-Wallis; $X^2 = 16.82$, $p = 0.0008$), and from 0.1% to 3.0% on Transitional (Kruskal-Wallis; $X^2 = 8.34$, $p = 0.039$). Additionally, seeding treatments did not increase the relative cover of native forbs on FFMM (rmANOVA; $F = 2.52$, $p = 0.118$), PMM (rmANOVA; $F = 0.895$, $p = 0.348$), or Transitional (rmANOVA; $F = 0.892$, $p = 0.349$), nor the native forb richness on FFMM (rmANOVA; $F = 0.019$, $p = 0.89$), PMM (rmANOVA; $F = 1.63$, $p = 0.207$), or Transitional (rmANOVA; $F = 2.07$, $p = 0.155$).

2.3.2 – Aspen Establishment:

Aspen seedling density on PMM was significantly greater than on both FFMM and Transitional coversoils (Figure 2.1A). Aspen established on PMM also exhibited a greater average height than both FFMM and Transitional, with average heights of 3.7cm, 25.1cm, and 29.2cm for the 2014, 2015, and 2016 growing seasons, respectively (Figure 2.1B) (soil x year interaction, $p = 0.028$). Weeding increased seedling density on PMM (rmANOVA; $F = 5.47$, $p =$

0.023) from an average of approximately 16,800 stems per hectare on control quadrats to approximately 25,500 stems per hectare on weeded quadrat in the first growing season, but did not significantly affect the seedling density on FFMM (rmANOVA; $F = 0.18$, $p = 0.673$) or Transitional (rmANOVA; $F = 2.50$, $p = 0.119$). Following initial establishment, seedling density did not change with year on any soil type. Weeding did not impact seedling height on FFMM (rmANOVA; $F = 0.006$, $p = 0.941$), PMM (rmANOVA; $F = 0.312$, $p = 0.578$), or Transitional (rmANOVA; $F = 0.32$, $p = 0.574$) coversoils.

2.3.3 – Plant Community Composition:

FFMM supported the greatest species richness throughout the study, while PMM consistently supported the lowest species richness (Figure 2.2A). The species richness found on Transitional and PMM coversoil had similar total vegetation cover during the initial growing season, but cover on Transitional increased by the final growing season to the point of resembling FFMM species richness (soil x year interaction, $p < 0.0001$). Weeding did not result in significant changes to species richness on FFMM (rmANOVA; $F = 1.89$, $p = 0.175$), PMM (rmANOVA; $F = 0.27$; $p = 0.607$), or Transitional (rmANOVA; $F = 0.32$, $p = 0.574$).

Total vegetation cover on FFMM was significantly greater than the other coversoils each year, while total vegetation cover on Transitional became significantly greater than PMM after the initial growing season (Figure 2.2B) (soil x year interaction, $p = 0.018$). FFMM and Transitional coversoils experienced similar patterns of increasing total cover; however, initial plant establishment on FFMM was significantly greater than on Transitional. Both the initial plant establishment and rate of total vegetation cover increase were significantly lower on PMM. Weeding did not result in significant changes to the total vegetation cover on FFMM (rmANOVA; F = 2.02, p = 0.16), PMM (rmANOVA; F = 0.001; p = 0.972), or Transitional (rmANOVA; F = 0.36, p = 0.552).

Although total vegetation cover did not change significantly as a result of weeding, significant changes to the relative cover of different plant functional groups were observed on both FFMM and Transitional coversoils. Weeding on FFMM significantly lowered relative cover of introduced forbs (rmANOVA; F = 12.08, p = 0.0009), and significantly increased relative cover of graminoids (rmANOVA; F = 4.18, p = 0.045) (Figure 2.3A). By the third growing season, FFMM weeded and control plots had an average relative introduced forb cover of 3 and 18 percent, respectively, and an average relative graminoid cover of 45 percent and 30 percent, respectively. These differences were due to an increase in a single grass species, specifically, *Calamagrostis canadensis*. Weeded quadrats on Transitional coversoil also had significantly lower relative cover of introduced forbs (rmANOVA; F = 17.32, p = 0.0001) and significantly higher relative cover of graminoids (rmANOVA; F = 7.15, p = 0.01) (Figure 2.3B). The average relative introduced forb cover for Transitional weeded and control plots were 10 and 18 percent, respectively, and the average relative graminoid cover 38 percent and 22 percent, respectively. Overall, by comparing the relative cover composition of control and weeded plots on FFMM (2014 to 2016) and Transitional (2015 to 2016), we can see that weeding accelerated the rate at which introduced species cover declined and grass cover increased.

The relative cover of introduced forbs (rmANOVA; F = 12.45, p = 0.0008) and graminoids (rmANOVA; F = 5.01, p = 0.029) on PMM were significantly different over the years; however, there was no consistent trend with respect to the weeding treatment (Figure 2.3C).

2.4 – Discussion:

Seeding had minimal impact on plant community composition. With the exception of *Achillea millefolium,* hand broadcasting and raking the native forb seed mix did not result in increased native forb cover, regardless of coversoil type. The lack of native forb seed establishment could be attributed to the limited seed supply available at the time of this study. In their study, Newman and Redente (2001) successfully established significantly greater forb biomass by broadcasting a forb seed mix of 3 species at a rate of 3.4 kilograms per hectare. In this study, the native forb seed mix, consisting of 12 species, was broadcasted at a rate of 1.5 kilograms per hectare. Furthermore, *Achillea millefolium,* the only species to significantly increase in cover, comprised just over 23% of the total seed mix. This suggests that using a greater quantity of seeds for each species may have resulted in significant increases in native forb cover. The composition of this study's seed mix was greatly influenced by the availability and attainability of native seeds from local producers and therefore, highlights a logistical challenge associated with incorporating seeding into reclamation practices.

The success of broadcast seeding may have also been dependent on small-scale variations in precipitation experienced during the seeding year, as suggested by Bakker et al. (2003). In their study, Bakker et al. (2003) found that increased rainfall during June (following May seeding) resulted in greater germination and establishment of seeded species; however, it also led to a decrease in survivorship due to elevated competition-induced mortality. Total precipitation for June 2014, following broadcast seeding of our native forb mix, was 34.2mm (Government of Canada 2017b), which was well below the climate normal of 73.3mm for the region (Government of Canada 2017a). This suggests that inadequate precipitation decreased the success of seed germination, with the exception of *Achillea millefolium*, which has been shown to experience increased germination under patterns of rapidly drying and re-moistening of soils (Robocker 1977).

A mature aspen tree is capable of producing approximately 1.6 million seeds each year (Hellum 1976). These seeds are small in size and have a tuft of fine hair that aid in facilitating long-distance wind dispersal (Peterson and Peterson 1992), with some seeds having been recorded traveling up to 18 km from their source tree (Landhäusser et al. 2010). As a result, natural ingress of aspen seedlings was able to occur on the study site via seed rain from the surrounding forest. However, aspen seeds require very specific environmental conditions to successfully germinate, in particular continuous availability of moisture and minimal competition for light (Peterson and Peterson 1992). These requirements provide rationale as to why PMM supported a significantly greater seedling density in comparison to FFMM and Transitional coversoils. Moisture readings from June to August in 2014 indicate that PMM had the greatest volumetric moisture content, at approximately 27.5% (Table 2.2). More importantly, this moisture content appears to have been maintained throughout the growing season. PMM also had the lowest total vegetation cover, indicating that there was little competition for light. This combination of continuous moisture and increased access to light provides a probable explanation to why PMM has been able to support a much larger density of aspen seedlings. Furthermore, PMM had the highest rates of bioavailable NH_4 and NO_3 amongst the three coversoil types which may explain the significantly greater height of trembling aspen on PMM (Pinno et al. 2012).

The significantly greater density of aspen seedlings on weeded PMM plots may be better explained by microsite availability. Schott et al. (2014) found that an increased presence of microsites, particularly those capable of retaining greater soil moisture contents, enhanced aspen establishment from seed. Alternatively, Bullied et al. (2012) linked sufficient soil water as a driver of weed seed germination and recruitment. This suggests that the greater density of aspen seedlings established on weeded PMM plots was due to an increase in suitable microsites once competing weed seedlings were removed.

Greater species richness and total vegetation cover found on FFMM coversoil is in agreement with previous oil sands reclamation studies (Mackenzie and Naeth 2010; Brown and Naeth 2014). This can be attributed to a large abundance in vegetative propagules and seeds situated in the LFH horizon of upland forest systems from which FFMM is derived (Mackenzie and Naeth 2010). Additionally, it would be expected that FFMM propagules have more successful plant emergence rates given that the upland derived coversoil was placed onto an upland reclamation site. After a delay in the first growing season, species richness and vegetation cover on Transitional coversoil have followed a similar pattern as FFMM. This delay in cover establishment is likely attributed to Transitional coversoils containing fewer seeds and propagules of plant species adapted to upland mixedwood forests.

Alternatively, PMM supported the lowest species richness and vegetation cover. This too is in agreement with the previous studies completed by Mackenzie and Naeth (2010) and Brown and Naeth (2014). Vegetative propagules found in bog and fen derived PMM likely consist of species that prefer to grow in subhydric to hydric conditions (Beckingham and Archibald 1996). PMM placed on upland sites during reclamation tends to become dryer due to increased drainage, likely making it difficult for these propagules to establish and produce any vigorous aboveground cover. The minimal availability of viable propagules and aboveground vegetation cover is likely why weeding had little effect on PMM plant community composition. There were simply not enough plants established to generate a competition limited plant community.

Total vegetation cover of weeded plots on FFMM and Transitional coversoils were not significantly different from their associated control plots, which suggests habitat vacancies created by weeding facilitated the establishment or expansion of competing plant species. The vacant habitat led to a significant increase in graminoid relative cover, which agrees with a previous study completed by Flory and Clay (2009), where graminoid cover increased significantly after two years of hand-pulling a dominant invasive species.

The increased relative cover of graminoids on FFMM coversoil was mainly attributed to an average increase of 3.54% in the cover of *Calamagrostis canadensis*. Its ability to rapidly colonize rhizomatously (Winder and Macey 2001), as well as increase seed production and dispersal when stressed, makes *C. canadensis* highly invasive in recently disturbed areas (Lieffers et al. 1993). In their study, Lieffers et al. (1993) observed that *C. canadensis* rhizomes are capable of spreading up to 50cm annually, and often times concentrate in relatively unoccupied soils. This behaviour suggests that the augmented *C. canadensis* cover in FFMM weeded plots resulted from the rhizomatous expansion of pre-existing plants into microsite vacancies.

Although a native grass species to boreal forests in the region (Beckingham and Archibald 1996), increased *C. canadensis* is considered a negative effect of the weeding treatment. An overabundance of *C. canadensis* can negatively impact both white spruce (Lieffers et al. 1993) and aspen (Landhäusser and Lieffers 1998) seedlings – the two main tree species desired for restoring upland boreal forests. Dense mats of *C. canadensis* can delay soil warming, and maintain cooler temperatures throughout the growing season, stunting growth and increasing mortality in both white spruce (Lieffers et al. 1993) and aspen (Landhäusser and Lieffers 1998) seedlings. Alternatively, white spruce and aspen grown alongside forbs, shrubs, or other trees rather than grasses, such as *C. canadensis,* experience better survival and growth (Lieffers et al. 1993). This suggests that, without the successful stimulation of native forb establishment by broadcast seeding, the removal of dominant weed species on reclamation sites may only accelerate the colonization of a more problematic grass species.

2.5 – Conclusion:

This field study has provided us with useful insight related to the impacts of weeding treatments completed without successful seeding of target understory species. The unintended increase of more problematic grass species suggests that, in some cases, allowing the presence of weed species may be the better alternative for plant community development. It is recommended that a more effective seeding procedure be implemented in future field studies. Coupled with weeding treatments, a successful seeding method could insure that vacant microsites created by weed removal be inhabited by target understory plants, and not dominated by grasses. Alterations to the broadcasting seeding method in this study could include increasing the number of seeds broadcasted, increasing the frequency of seeding treatment to coincide with the weeding treatment (i.e. immediately after weeding to ensure seeds collect in microsites), and scheduling seeding treatments in accordance to predicted precipitation patterns to ensure favourable germination conditions. Overall, this study has provided us with knowledge relating to plant community management on reclamation sites, allowing for further progression toward the development of suitable best management practice guidelines for oil sand reclamation.

2.6 – Tables and Figures:

Table 2.1 – Listing the forb species selected for the seeding mix, the seed collection year, approximate grams broadcasted per quadrat, approximate number of seeds broadcasted per quadrat, and the seed mix percentage represented by each forb.

Species	Grams Per 25m^2 Quadrat	Seeds Per 25m^2 Quadrat	Seed Mix Composition (by Number of Seeds)
Achillea millefolium	0.080	684	23.35%
Aquilegia brevistyla	0.375	334	11.42%
Aster ciliolatus	0.003	16	0.53%
Aster conspicuous	0.045	94	3.21%
Aster puniceus	0.120	411	14.04%
Chamerion angustifolium	2.50	275	9.40%
Cornus canadensis	0.188	463	15.81%
Delphinium glaucum	0.113	128	4.39%
Galium boreale	0.113	275	9.40%
Solidago canadensis	0.023	210	7.18%
Vicia americana	0.158	11	0.37%
Viola adunca	0.025	27	0.91%

Table 2.2 – Mean basic soil characteristics for reclamation coversoils during the 2014 growing season when the one-time broadcast seeding procedure took place (late May), with the exception of TOC which was determined using 2016 soil samples. Table reports means with standard error in brackets. Coversoil characteristics sharing the same letter are not significantly different from one another. TOC (%) = Total Organic Carbon; VWC (%) = Volumetric Water Content. Nutrient rates are measured as µg/10cm^2/6 weeks.

Coversoil	**pH**	**EC**	**TOC**	**NO_3**	**NH_4**	**P**	**K**	**VWC (June)**	**VWC (August)**
FFMM	5.9 **a** (0.2)	865.17 **b** (192.30)	5.47 **a** (1.08)	4.91 **b** (1.93)	3.92 **b** (0.45)	1.23 **a** (0.17)	80.89 **a** (20.77)	25.48 **a** (2.12)	17.13 **b** (2.15)
PMM	5.2 **a** (0.4)	1526.33 **ab** (277.52)	14.73 **ab** (3.29)	50.10 **a** (23.21)	8.70 **a** (1.87)	0.72 **a** (0.10)	32.09 **a** (6.49)	27.51 **a** (3.53)	28.13 **a** (2.45)
Transitional	6.2 **a** (0.3)	2101.83 **a** (374.09)	20.57 **b** (4.01)	4.48 **b** (1.92)	4.83 **ab** (0.47)	1.00 **a** (0.15)	68.62 **a** (8.94)	24.39 **a** (2.38)	20.62 **ab** (1.46)

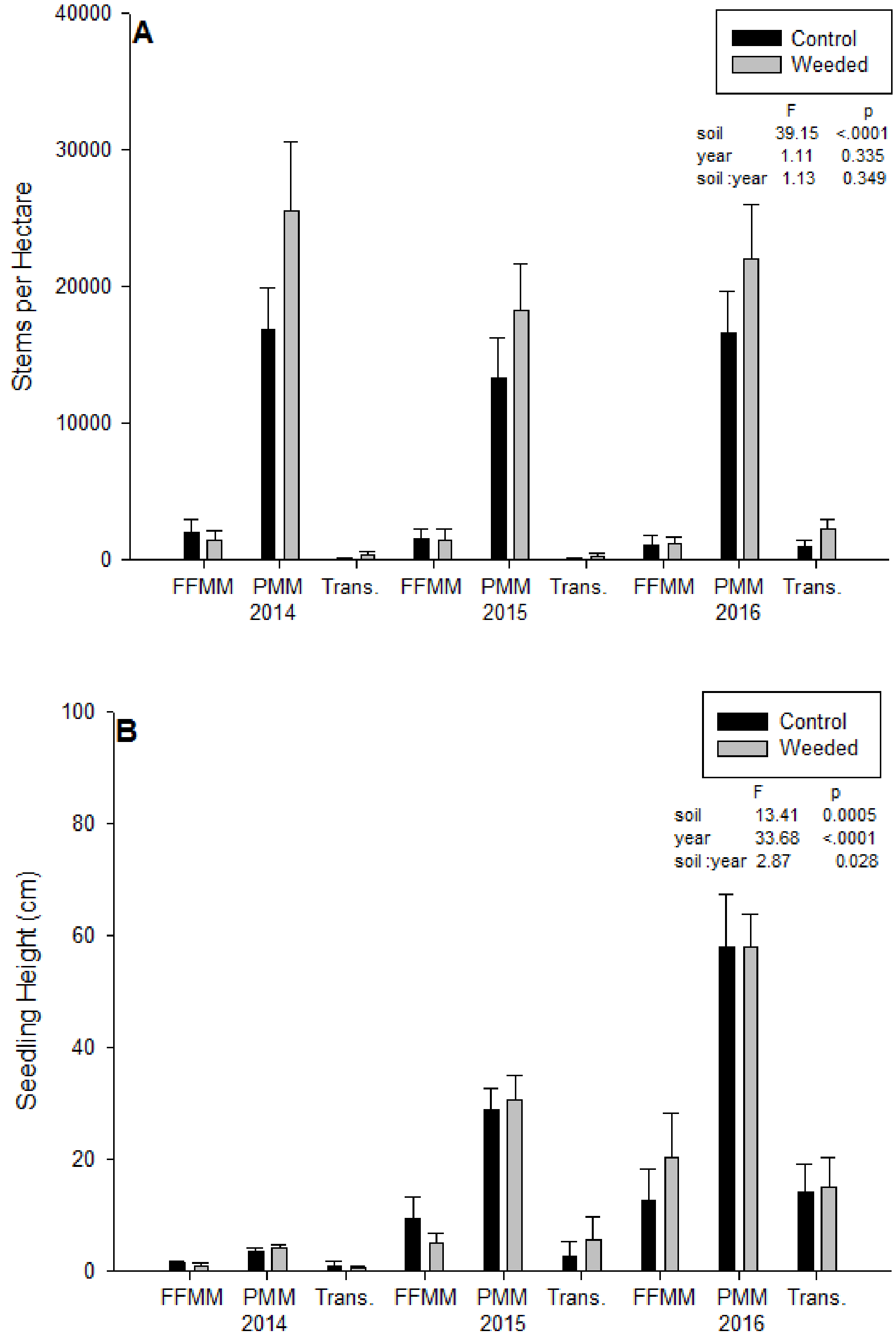

Figure 2.1 – Repeated measures ANOVA of (A) aspen seedling density and (B) aspen seedling height on FFMM, PMM, and Transitional coversoils in response to weeding treatments over three growing seasons. Paired columns with the same letter are not significantly different from one another. P-values ≤ 0.1 are considered significant.

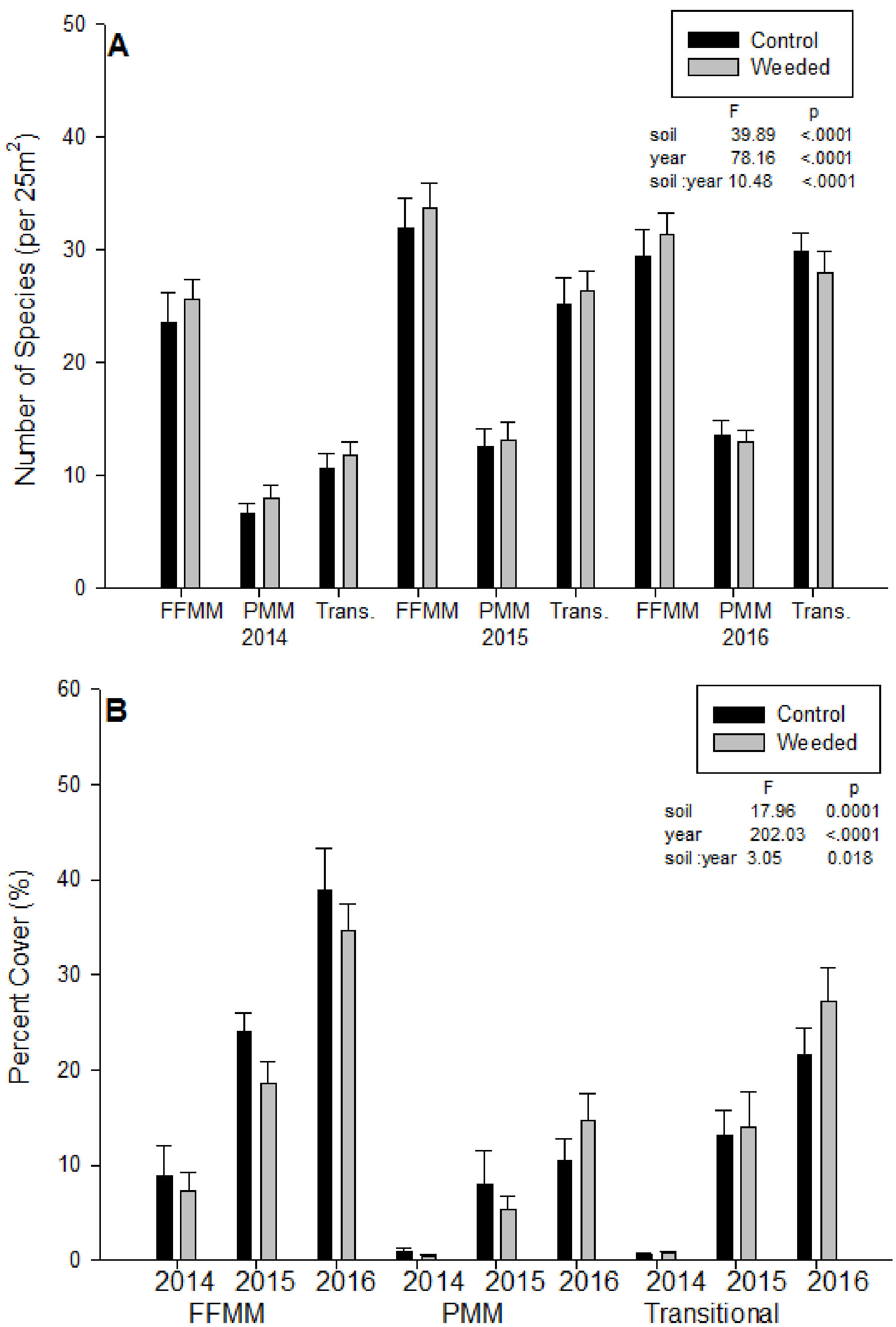

Figure 2.2 – Repeated measures ANOVA of (A) species richness and (B) total vegetation cover on FFMM, PMM, and Transitional coversoils in response to weeding treatments over three growing seasons. Paired columns with the same letter are not significantly different from one another. P-values ≤ 0.1 are considered significant.

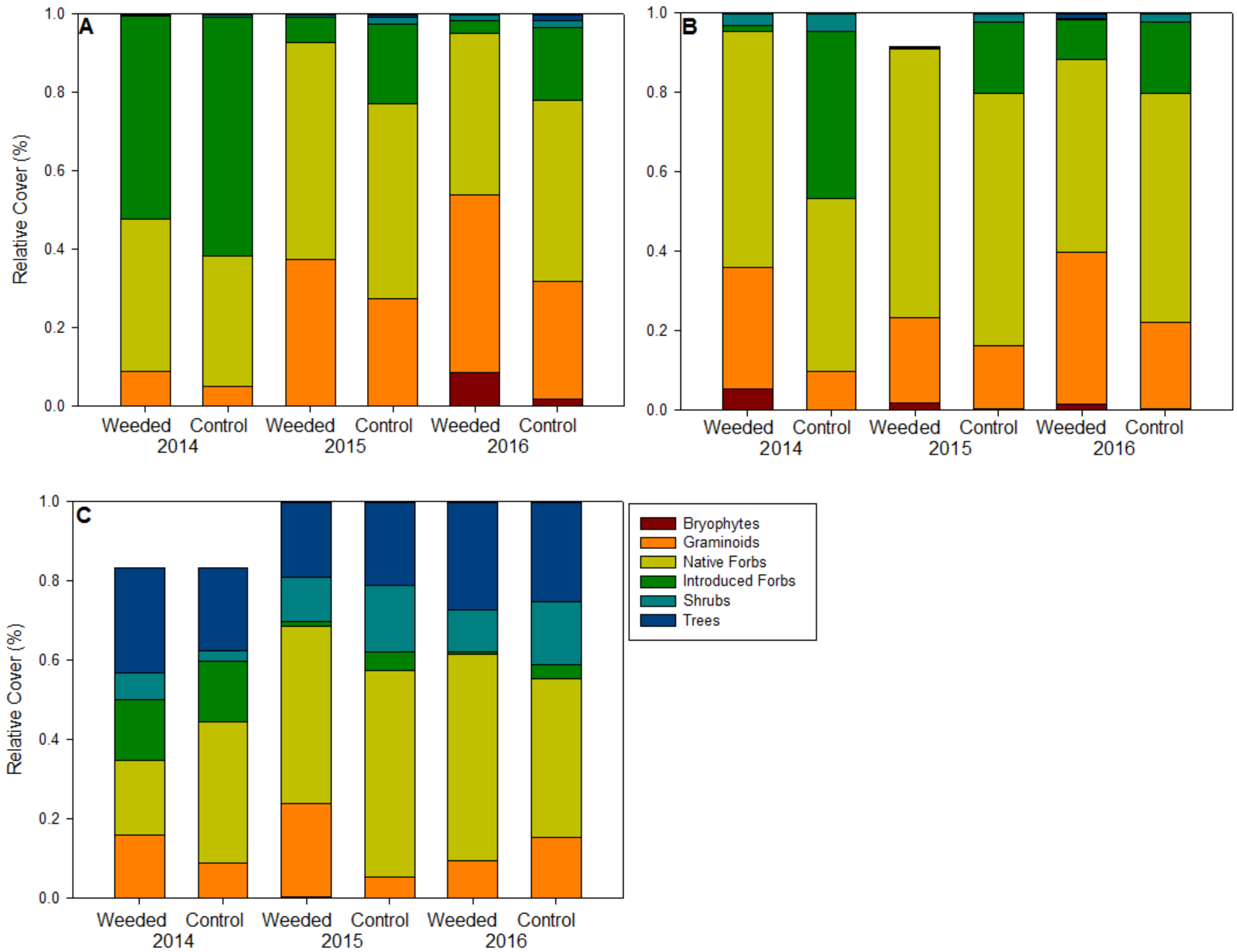

Figure 2.3 – Relative covers of plant functional groups in on (A) FFMM, (B) Transitional, and (C) PMM over three growing seasons, in response to weeding treatments. Columns that do not reach 1.0 were a result of some research plots lacking vegetation during initial growing seasons.

Chapter 3 – Evaluation of oil sands reclamation coversoil using bioavailable nutrients, microbial function, and plant community composition multivariate parameters

3.1 – Introduction:

Collectively, the Athabasca, Cold Lake and Peace River oil sands deposits underlie approximately 142,200 km^2 within Alberta's provincial borders, with an estimated 4,800 km^2 situated in the northern portion of the Athabasca deposit deemed suitable for surface mining operations (CAPP 2017). To date, the amount of land that has undergone disturbance from surface mining is approximately 904 km^2 (CAPP 2017). Unlike natural disturbances, such as wildfires, wind throws, and insects, which mainly result in the temporary loss of vegetation (Chen and Popadiouk 2002; Swanson et al. 2012), oil sands surface mining operations result in the complete removal of vegetation and extensive soil modification (Rowland et al. 2009; Naeth et al. 2012). Organic material, mineral soil, and overburden overlying oil sand deposits are removed to accommodate oil extraction and processing, and salvaged for subsequent soil reconstruction during land reclamation efforts (Rowland et al. 2009; Audet et al. 2015). Overburden material is used to re-contour disturbed areas, which are then capped with an organic-mineral mix coversoil. Reclamation uses two main coversoils – forest floor-mineral mix (FFMM) comprised of upland forest floor surface organic material and mineral soil, and peat-mineral mix (PMM) comprised of lowland organic material and mineral soil (Pinno and Errington 2015). Due to readily available and abundant supplies, PMM is the more commonly used coversoil (Rowland et al. 2009). However, FFMM is the preferred coversoil because of its abundant native seed bank which has been shown to produce plant communities similar to target upland forests (Mackenzie and Naeth 2010; MacKenzie et al. 2014), and its ability to stimulate microbial activity (McMillan et al. 2007). A third coversoil, referred to as Transitional, can be derived from areas of topographical transition between an upland and lowland, and acts as an intermediate organic-mineral mix between FFMM and PMM.

As stated in the Environmental Protection and Enhancement Act, reclamation is part of an oil company's legal requirement to restore disturbed areas to an "equivalent land capability" (Province of Alberta 2017). However, no official standards, nor suitable criteria and indicators, have been established in order to thoroughly assess the success of oil sands reclamation efforts. By default, many reclamation sites are evaluated by measuring a collection of isolated chemical or physical indicators such as soil pH, SAR, EC, vegetation cover, tree height and vigour. However, evaluating reclamation through the use of multivariate bioavailable nutrient profiles, microbial function patterns, and plant community composition parameters may have the potential to better describe an area's level of recovery and progress toward a functioning ecosystem.

Ecosystem function is a product of biological (i.e. plant and microbial) activities performed in conjunction with abiotic environmental characteristics such as climate, topography, and soil properties (Hooper et al. 2005), and provides society with an abundance of essential ecosystem services such as food production, air and water quality control, organic matter decomposition, nutrient cycling, soil development, and climate control (Hooper et al. 2005; Balvanera et al. 2006). Due to their sheer abundance and diversity, soil microbial communities play a tremendous role in an ecosystem's functional capability, with soil microbes being responsible for the vast majority of organic matter decomposition, as well as the catalysis of nitrogen, sulphur, and phosphorus nutrient cycling (Allison and Martiny 2008). This enhanced nutrient cycling associated with more robust microbial communities has been found to support more productive plant communities via improved nutrient acquisition (van der Heijden et al. 2008). Additionally, microbial communities actively contribute to the biogeochemical processes that promote pedogenesis (Poncelet et al. 2014). As such, microbial communities can be regarded as highly influential regulators of both soil properties and plant communities (Laureto et al. 2015). In return, plant community composition has been identified as a driver of soil microbial communities (Wardle et al. 2004), particularly within the rhizosphere where microbes are heavily influenced by plant-specific root exudates (Berg and Smalla 2009). Plant litter composition has also been found to have an effect on the overall structure and function of microbial communities (Elgersma et al. 2012), with diverse litter often generating a greater variety in microhabitats and available substrates capable of supporting larger and more diverse microbial communities (Hansen and Coleman 1998; Chapman and Newman 2010; Chapman et al. 2013). Given the amount of influence they have on one another, plants and soil microbes are recognized as highly interconnected mutual drivers, as well as major contributors to overall ecosystem functioning (Wardle et al. 2004).

This mutual symbiosis between plants and soil microbes within the rhizosphere serves as a mitigation strategy to cope with limitation in abiotic resources such as energy and mineral nutrients (Morgan et al. 2005; Hartmann et al. 2009). Plants supply belowground microbes with photosynthetically fixed carbon compounds via root exudates and litter, and in return soil microbes provide plants with bioavailable mineral nutrients via the metabolism of organic compounds (Reynolds et al. 2003; Jacoby et al. 2017). Given that nutrient cycling is one of the central forces driving microbial-plant interactions, an assessment of the bioavailable nutrient profiles of coversoils could provide insight into initial functional aspects of reclamation ecosystems.

To successfully incorporate bioavailable nutrient profiles, microbial function, and plant community composition parameters into an oil sands reclamation assessment framework, we must continue to expand our knowledge by studying current reclamation and suitable natural reference sites. This will allow for the evaluation of post-oil sands disturbance recovery of ecosystem function, as well as the development of reasonable certification targets. Additionally, the identification of differences in drivers of microbial functional diversity between reclamation and natural sites would aid in the inception and refinement of best management practices for the re-establishment of ecosystem function.

The objective of this study is to determine whether a multivariate approach is suitable for the evaluation of oil sands reclamation sites. Given that reclamation coversoils and plant communities are the most accessible pathways of influencing ecosystem functioning, this study will apply multivariate assessment tools in order to answer:

1) What level of similarity to natural reference soils, with respect to bioavailable nutrient profiles, microbial function, and plant community composition, will be exhibited based on reclamation coversoil type?
2) Will the removal of dominant weed species increase the level of similarity shared by reclamation coversoils and natural reference soils with respect to bioavailable nutrient profiles and microbial function?
3) After three growing seasons, will reclamation sites have developed a significant plant-microbial interaction?

3.2 – Methods:

3.2.1 – Site Description:

Field research was completed approximately 70 kilometers north of Fort McMurray, Alberta, at an oil sands mine site. The area falls within the Central Mixedwood sub-region of the boreal forest (NRC 2006) which experiences short, warm summers with an average frost-free period of 97 days (late May to early September) and long, cold winters (NRC 2006). Mean monthly temperatures range from -17.4ºC in January to 17.1ºC in July, with a mean annual temperature of approximately 1ºC (Government of Canada 2017). Total annual precipitation for the area is estimated to be 418.6mm, with approximately 55 percent occurring as rainfall during May through August (Government of Canada 2017). Aspen-dominated and aspen-white spruce stands are characteristic of natural upland sites, and are typically situated on Luvisolic soils (Beckingham and Archibald 1996; NRC 2006). Lowland sites predominantly support black spruce fens and bogs, and develop thick Organic soils (Beckingham and Archibald 1996; NRC 2006).

The reclamation area used for this study was constructed on an elevated dyke structure during the winter months prior to the 2014 growing season. Three organic-mineral mixed coversoils were directly placed at a depth of approximately 30 cm overtop 1 m of suitable overburden. Forest floor-mineral mix (FFMM) was derived from Luvisolic soils established under an aspen dominated upland mixedwood forest containing white spruce. A 60:40 (organic:mineral) peat-mineral mix (PMM) was derived from Organic soils under black spruce dominated bogs and fens (CNRL 2013; CNRL 2014). A third coversoil, referred to as Transitional, was derived from an area of topographical transition between upland and lowland salvage sites, and acted as an intermediate organic-mineral mix between FFMM and PMM. Deciduous tree species and shrubs were not planted on site due to strong levels of natural ingress having been observed on older reclamation areas (CNRL 2015).

Several natural reference sites within a 25km radius of the reclamation study site, including both mature (aged 58 to 70 years) and post-fire (2011 Richardson Fire) forest stands, were selected for comparison purposes. A total of 5 mature and 5 post-fire plots were established. All natural references sites were characterized by a dominant trembling aspen overstory accompanied by white spruce as a secondary tree species in either the main canopy (mature stands) or the intermediate canopy (post-fire) (Errington and Pinno 2015).

3.2.2 – Experimental Design:

Following soil placement, six plots were randomly located within each coversoil type for a total of 18 plots (Figure 6.1). Each plot was 12m by 12m and divided into 5m by 5m quadrats. A 2m buffer area was established between each quadrat to minimize the disturbance of plant communities during sampling and to thoroughly separate treatments. The quadrats within each plot were either weeded or left as a control, with each plot containing two weeded and two control quadrats. Weeding was completed in order to test the impact of plant community manipulation on bioavailable nutrients, microbial function, and the plant community itself (refer to Chapter 2), on different coversoils. *Sonchus arvensis, Matricaria perforata*, and *Tanaceum vulgare* were identified as the dominant and persistent introduced species on the oil sands mine site (Li *et al.* 2015), and were therefore selected as targets of the weeding treatment. Hand-pulling on weeded quadrats was completed monthly, starting in June, during the 2014 growing season, which was reduced to twice per growing season during 2015 and 2016.

3.2.3 – Plant Community Data Collection:

Plant community composition data was collected during the months of July and August. Vegetation plots measuring 1m by 1m were nested at the center of each quadrat. Surveys were completed by identifying all plant species in each vegetation plot, then estimating their percent cover. Percent cover by plant functional groups was also determined for bryophytes, grasses, forbs, shrubs, and trees measuring less than 1.3m in height. Following plant community composition surveys, a walk-around of the entire 5m by 5m quadrat was completed to determine species richness on a presence-absence basis (Figure 6.2).

3.2.4 – Soil Data Collection:

Soil nutrient supply rates for 2016 were measured during peak growing season (i.e. July to August) using plant root simulator (PRS) probes (PRS™; Western Ag Innovations, Saskatoon, SK, Canada). Four pairs of anion and cation probes were installed just outside the four boundaries of each 1m by 1m vegetation plot, and collected after 6 weeks (Figure 6.2). Collected probes were stored in coolers during transport from the field, cleaned thoroughly with deionized water, and sent to Western Ag Innovations where they were extracted with 0.5 M HCl for analysis. Inductively-coupled plasma spectrometry (Optima 8300 ICP-OES; Perkin Elmer Inc., Woodbridge, ON, Canada) was used to measure all nutrient ion contents, except for NO_3-N and NH_4-N ions, which were determined via colorimetry using an automated flow injection analysis system (FIAlab Instruments Inc., Bellevue, WA, USA).

3.2.5 – Soil Sampling:

Following vegetation surveys, approximately 400 grams of coversoil was collected from each 1m by 1m vegetation plot (Figure 6.2) at a depth of 5 cm to 15 cm in order to determine soil pH, electrical conductivity (EC), total organic carbon (TOC), and total nitrogen (TN), and to setup soil incubation chambers for community level physiological profiling (CLPP), microbial biomass carbon and nitrogen (MBC/N), and soil respiration. The 5 cm to 15 cm collection depth was chosen in order to avoid surficial crusting of peat and organic matter that had developed due to a lack of rain. Furthermore, Howell and MacKenzie (2017) determined that on reclamation coversoils, properties such as bioavailable nutrients, microbial biomass carbon, and microbial function did not change significantly due to coversoil depth. Samples were transported from the field in coolers and stored at 4˚C prior to laboratory analyses. Soil samples were also collected from a mature aspen-spruce mixedwood forest and a recently burned forest stand.

3.2.6 – Soil Properties:

Soil pH and EC were determined from a soil supernatant (5g soil: 25ml water) that was shaken four 4 hours, allowed to settle for 30 minutes, and then vacuum filtered. A Mettler Toledo Five Easy pH and EC probe was used to determine pH and EC. To determine both TOC and TN, oven dried soils (24hrs at 105˚C) were ground into a fine powder and encapsulated for elemental analysis by dry combustion (Flash 2000 Organic Elemental Analyzer, ThermoFisher Scientific).

3.2.7 – Soil Incubation:

Approximately 100 grams of coversoil was weighed into 500 mL glass jars and brought to 60% field water capacity using deionized water. Oxoid anaerobic indicator strips were placed in eight incubation jars – two per coversoil type (FFMM, PMM, Transitional) and one per reference soil type (Mature, Fire), to ensure aerobic conditions were maintained. Sealed incubation jars were kept in an incubator set at 30˚C for 14 days. Each incubation jar was aerated for 8 minutes and brought back to 60% field water capacity every second day. Soil respiration, MBC/N, and CLPP data was collect from incubated soils at the conclusion of the 14 day period.

3.2.8 – Soil Respiration:

A LI-COR LI-8100A Automated Soil CO_2 Flux system was used to measure soil respiration. Chamber dimensions, pre-purge (30 sec), observation length (90 sec), post-purge (45 sec), and data logging output options were programmed in advance to allow for immediate readings once soils were removed from the incubator. Once incubation chambers were attached to the LI-COR LI-8100A, CO_2 gas fluxes were measured by tracking the changes concentration due to microbial respiration within the closed chamber over time.

3.2.9 – Microbial Biomass C and N:

MBC/N was determined using the direct chloroform extraction method (Gregorich et al. 1990). For each sample, two 5 grams subsamples of soil were weighed into a plastic centrifuge tube and a glass tube, respectively. Samples in centrifuge tubes were controls, receiving only 40ml of 0.5M K_2SO_4. Samples in glass tubes received both 40ml of 0.5M K_2SO_4 and 0.5ml EtOH-free chloroform in order to extract organic carbon and nitrogen. All samples were shaken for 4 hours, and then given 10 minutes to settle. Once settled, approximately 30ml of extract was decanted and vacuum filtered, prior to being bubbled vigorously for 30 minutes to remove chloroform. Both a control and a chloroform-exposed blank (containing no soil) were made in the same manner (needed for subsequent calculations). Non-purgeable organic C and total dissolved N data was used to determine MB-C/N using the calculations outlined by Voroney *et al.* (2008).

3.2.10 – Community Level Physiological Profiling:

CLPP was completed using a microplate based respiration system and multi-substrate induced respiration experiment, as described by Campbell et al. (2003). Each soil was uniformly loaded into a 48-well deepwell microplate containing 15 different carbon substrates (Table 3.1) and one blank (deionized water), in triplicate. Loaded deepwells were immediately fitted with a sealing gasket and detection microplate, and then incubated for 6 hours at 30˚C. The detection microplates contained a 1:2 (agar:indicator) gel comprised of purified agar, cresol red, potassium chloride, and sodium bicarbonate. This allowed substrate-induced catabolic activity of the microbial community to be determined via colorimetric detection. During the incubation, carbonic acid would form as mineralized CO_2 produce from microbial activity was absorbed by the indicator gel, prompting a decrease in pH. Differing amounts of CO_2 respiration led to individual wells in the detection microplates to experience different levels of pH decrease which was expressed by color changes in the indicator gel. CO_2 absorbance of each detection microplate was determined immediately after the 6 hour incubation using a spectrophotometer (Biotek Instruments Inc., Winooski, VT, USA) at a 570nm wavelength. The initial (taken at time zero) post-incubation absorbance values were used to model CO_2 production rates (µg CO_2-C/g/hr) using equations provided by MicroResp™ (Cameron 2015), thus allowing for comparisons of the microbial activity amongst the different soil types.

3.2.11 – Statistical Analysis:

Non-metric multidimensional scaling (NMS) was used to plot each research quadrat into ordination space based on bioavailable nutrient profile, microbial function (i.e. CLPP), and plant community composition. Plant community data matrices were modified in order to alleviate sparsity (many zero values) and to standardize prior to analysis. Species columns containing less than 1 non-zero value were eliminated. Data was subsequently standardized using a general relativization (by column total) in order to reduce any skewness of influence between common and rare species occurrences (Peck 2016). The microbial and soil data matrices did not require modification for sparsity, but was standardized using the same general relativization as the plant community data. Multiple response permutations procedures (MRPP) with a Bray-Curtis distance measure were used to statistically determine significant differences amongst each of the aforementioned functional parameters between reclamation coversoils and natural reference soils. NMS and MRPP were also used to assess the impact of plant community manipulation on each functional parameter within each coversoil type. Additionally, Mantel correlation tests were completed using a Bray-Curtis distance measure in order to identify any significant correlations between plant community composition and belowground microbial function (Peck 2016). Multivariate data analyses were performed using PC-ORD Version 6 software (Peck 2016).

Statistical comparison of CO_2 production rates derived by carbohydrates, carboxylic acids, and amino acids during the CLPP procedure were completed at the coversoil/soil level (Table 3.3). Significant differences in univariate soil properties including pH, EC, TOC, TN, soil respiration, MB-C/N, and metabolic quotient (qCO_2) (Table 3.4), as well as individual nutrient response variables (Table 3.2), were also examined using coversoil/soil as the main effect. An assessment of ANOVA assumptions, normality of residuals and homogeneity of variances, was complete for each univariate (Borcard et al. 2011). If assumptions were met, a one-way ANOVA followed by a Tukey HSD analysis was performed. Alternatively, if assumptions were not met, a non-parametric Kruskal-Wallis ranked means test was performed (Borcard et al. 2011). One-way ANOVAs with Tukey HSD or Kruskal-Wallis ranked means tests were completed using R Version 3.2.3 software (R Core Team 2015). Alpha values of 0.10 or smaller were considered significant in order to account for high levels of variability inherent to this reclamation field study.

3.2.12 – Research Limitations:

This study used a single reclamation site in its experimental design; therefore, pseudo-replication occurred when analyzing data at the coversoil level given that the research plots established on FFMM, PMM, and Transitional, respectively, were not true replicates (Borcard et al. 2011). As such, this study cannot provide true results, but instead contributes to the findings and speculations of related works.

3.3 – Results:

NMS and MRPP analyses revealed that each coversoil type (FFMM, PMM, and Transitional) conveyed significantly different bioavailable nutrient profiles, microbial function, and plant community composition from one another. When compared to Fire and Mature reference soils, only FFMM microbial function was found to share similarities. Additionally, with the exception of the plant community composition parameter, coversoil ordinations displayed much larger ellipses suggesting that the heterogeneous placement of reclamation coversoils has caused considerable variation in the composition of functional parameters.

3.3.1 – *Bioavailable Nutrient Profiles:*

The bioavailable nutrient profiles between all reclamation coversoils and natural reference soils were significantly different ($p < 0.0005$), with the exception of Fire and Mature soils that exhibited similarities (MRPP; $T = -0.86$, $A = 0.03$, $p = 0.168$) (Figure 3.1). Although, significantly different from each reference soil, FFMM, PMM, and Transitional coversoils shared a similar trend in which the level of separation from Mature soil ($T = -16.40$, -11.43, and -16.24, respectively) was greater than the separation from Fire soil ($T = -11.67$, -9.73, and -12.43, respectively). The dissimilarities between coversoil and reference soil bioavailable nutrient profiles is predominantly attributed to coversoils having significantly greater available calcium (ANOVA; $F = 38.61$, $p < 0.001$), magnesium (ANOVA; $F = 25.07$, $p < 0.001$), and sulphur (ANOVA; $F = 34.88$, $p < 0.001$) while reference soils had significantly greater rates of available potassium (Kruskal-Wallis; $X^2 = 37.46$, $p < 0.001$) and phosphorus (ANOVA; $F = 44.02$, $p < 0.001$) (Table 3.2). The smaller level of separation observed between PMM and the references soils is likely due to similar rates of available manganese that exceed those found in FFMM and Transitional (ANOVA; $F = 13.72$, $p < 0.001$) (Table 3.2).

At the coversoil level, weeding treatments did not result in significant changes to the bioavailable nutrient profiles of FFMM (MRPP; T = 0.47, A = -0.006, p = 0.63), PMM (MRPP; T = 0.20, A = -0.004, p = 0.51), or Transitional (MRPP; T = 0.84, A = -0.01, p = 0.80) (Figure 6.3).

3.3.2 – Microbial Function:

Similar to the bioavailable nutrient profiles, the microbial function of FFMM, PMM, and Transitional coversoils exhibited greater separation from Mature soil (T = -0.83, -2.07, and -4.87, respectively) than from Fire soil (T = -0.18, -1.75, and -4.44, respectively). However, the overall separation between coversoils and reference soils was much lower than what was observed in bioavailable nutrient profiles. As expected, Fire and Mature soils shared similarities in microbial function (MRPP; T = -0.78, A = 0.03, p = 0.19). Alternatively, FFMM microbial function shared a stronger similarity to Fire (MRPP; T = -0.18, A = 0.001, p = 0.34) than the similarity between Mature and Fire soils (Figure 3.2). FFMM microbial function also exhibited similarities with Mature soil (MRPP; T = -0.83, A = 0.006, p = 0.17). Microbial function trends in PMM were similar to FFMM (MRPP; T = -1.06, A = 0.01, p = 0.14) and Transitional (MRPP; T = - 1.24, A = 0.009, p = 0.11) coversoils, but differed from Fire (MRPP; T = -1.74, A = 0.03, p = 0.06) and Mature (MRPP; T = -2.07, A = 0.04, p = 0.04) reference soils. In contrast, the microbial function of Transitional coversoil was found to be significantly different from all other coversoils and soils, with the exception of PMM. The similarities and differences observed in microbial function are due to significantly different catabolic rates pertaining to amino acid (ANOVA; F = 13.58, p <0.001) and carbohydrate (ANOVA; F = 3.88, p = 0.006) substrates, as well as marginal differences associated with carboxylic acid (ANOVA; F = 1.82, p = 0.13) substrates. The microbial communities in Transitional and PMM coversoils demonstrated greater rates of catabolic activity than FFMM and the reference soils when induced with amino acids. When induced with carbohydrates or carboxylic acids, Transitional still exhibits the greatest rate of catabolic activity, but PMM becomes similar (or marginally greater than) to the reference soils and FFMM (Table 3.3).

At the coversoil level, weeding treatments did not result in significant changes to the microbial function of FFMM (MRPP; $T = 0.47$, $A = -0.004$, $p = 0.63$), PMM (MRPP; $T = 1.15$, $A = -0.02$, $p = 0.88$), or Transitional (MRPP; $T = 0.58$, $A = -0.008$, $p = 0.67$) (Figure 6.4).

3.3.3 – Plant Community Composition:

The plant community composition between all reclamation coversoils (control plots) and natural reference soils were significantly different (Figure 3.3), including the plant communities established on Fire and Mature soils (MRPP; $T = -2.57$, $A = 0.07$, $p = 0.016$), which have up until now demonstrated similarities in functional parameters.

Both the Fire and Mature plant communities differed from those supported by reclamation coversoils due to a higher cover of native forbs and shrubs, in particular, *Rosa acicularis*, *Cornus canadensis*, *Maianthemum canadense*, and *Viburnum edule*. However, the natural reference soils differ, with the Fire plant community consisting of more forbs (including some introduced species) and graminoids such as *Petasites palmatus*, *Galium boreale*, *Polygonum arenastrum*, *Poa palustris*, *Linnaea boreale*, *Plantago major*, and *Carex* sp. Alternatively, Mature plant communities supported a greater shrub cover consisting of predominantly *Lonicera dioica*, *Amelanchier alnifolia*, *Alnus viridis*, and *Ribes triste*.

The plant communities observed on reclamation coversoils had fewer distinguishing plant species than the reference soils, with FFMM plant communities being associated with mainly *Sonchus arvensis*. Neither PMM nor Transitional plant communities displayed a strong association with a particular group of species; however, plant community comparisons made by deBortoli (2017 – Ch. 2) indicated that after a delay in the first growing season, species richness and vegetation cover on Transitional coversoil began to follow a similar pattern as FFMM. This delay in cover establishment is likely attributed to Transitional coversoils containing fewer seeds and propagules of plant species adapted to upland mixedwood forests. Alternatively, deBortoli (2017 – Ch. 2) found that PMM plant communities remained poorly established, supporting under 20 plant species with a vegetation cover less than 20 percent, which could explain the lack of strongly associated plant species.

3.3.4 – Cumulative Functional Parameters:

When all three multivariate matrices of the bioavailable nutrient profile, microbial function, and plant community functional parameter were combined, each coversoil and natural reference soil was significantly different from one another, with Fire and Mature sharing the greatest similarity in overall ecosystem characteristics (MRPP; $T = -2.43$, $A = 0.05$, $p = 0.017$) (Figure 3.4). Overall, this cumulative ordination bi-plot indicates that dissimilarities between the reclamation and natural ecosystems are predominant due coversoils lacking bioavailable potassium and phosphate, as well as *Rosa acicularis* and *Cornus canadensis* plant species, while containing a surplus of bioavailable calcium.

3.3.5 – Plant-Microbial Interaction:

The removal of dominant weed species resulted in FFMM and Transitional plant communities containing a larger amount of grass (*Calamagrostis canadensis* and *Agrostis scabra*, respectively) and fewer introduced species, and PMM supporting a greater density of *Populus tremuloides* seedlings (deBortoli 2017 – Ch. 2). However, these alterations to the plant community did not affect the microbial function in FFMM (MRPP; $T = 0.47$, $A = -0.004$, $p =$

0.62), PMM (MRPP; T = 1.15, A = -0.02, p = 0.88), or Transitional (MRPP; T = 0.58, A = -0.008, p = 0.67) coversoils.

Additionally, no significant correlations were identified between plant community composition and microbial function on FFMM (Mantel; r = 0.072; p = 0.252), PMM (Mantel; r = 0.090; p = 0.258), or Transitional (Mantel; r = -0.094, p = 0.133) coversoils. This suggests that three growing seasons after initial soil reconstruction reclamation sites have not developed a significant plant-microbial interaction.

3.4 – Discussion:

3.4.1 – Bioavailable Nutrient Profiles:

The rates of certain bioavailable nutrients in coversoils differed significantly in comparison to rates found in the natural reference soils. These alterations can be attributed, as previously observed by Lavkulich and Arocena (2011) and Howell et al. (2017), to the admixing of surface organic materials with underlying mineral horizons during soil salvage and reconstruction. Both phosphorus and potassium availability were significantly lower in coversoils. However, extensive literature identifying phosphorus as a key determinant of ecosystem dynamics, predominantly net primary productivity (Elser et al. 2007; Vitousek et al. 2010; Menge et al. 2012), suggests that reduced phosphorus availability could be more problematic than reduced potassium on reclamation sites. This is particularly true of Transitional and PMM coversoils where available nitrogen, the more common limiting nutrient (Schachtman et al. 1998), is found to be 2.85 times and 3.66 times greater than available phosphorus, respectively. The lowered phosphorus availability found in coversoils could be attributed to significantly elevated levels of available calcium. Available calcium, which was found to be approximately 2.5 times on coversoils, is capable of diminishing the amount of phosphorus available for plant uptake via increased precipitation of calcium phosphates (Hopkins and Ellsworth 2005; Cao and Harris 2008).

The limitation posed by low bioavailable phosphorus rates could explain the differences in vegetation cover establishment on different coversoils. PMM had the least amount of bioavailable phosphorus, followed by Transitional, then FFMM. Correspondingly, PMM was also observed to have the lowest percentage of vegetation cover (~12.6%), followed by Transitional (~24.4%), than FFMM (~36.8%). Given this information, the incorporation of a fertilizer of mainly phosphorus may alleviate plant growth limitations and prompt and more rapid re-establishment of vegetation cover on reclamation sites.

3.4.2 – Microbial Function:

Of the three coversoils, FFMM was the only one found to have similar microbial function to Fire and Mature reference soils, while PMM and Transitional differed. Greater catabolic rates were observed in Transitional, followed closely by PMM, when induced with carbohydrates, carboxylic acids, and amino acids alike. This is likely attributed to the significantly greater TOC percentages found within these two coversoils supporting significantly greater microbial biomass carbon – a trend also observed by Howell and MacKenzie (2017). Literature by Fenner and Freeman (2011) on drought-induced carbon loss in peatlands further explains the elevated microbial function observed in Transitional and PMM coversoils. Peatlands and water-saturated environments are known to accumulate large amounts of carbon as a result of inhibited microbial activity. Once these areas are exposed to drought conditions, a series of previously inhibited biogeochemical activities can promote rapid microbial (mainly bacterial) growth and elevated catabolic activity as vast carbon stocks start to decompose (Fenner and Freeman 2011). Transitional and PMM soils derived from previously water-saturated areas likely experienced the same stimulation of microbial growth and carbon decomposition when placed, and allowed to dry, on the upland reclamation site.

Lastly, Transitional and PMM coversoils displayed significantly higher metabolic quotients than FFMM and the reference soils (Table 3.4). This is likely an indication of these two coversoils experiencing greater levels of disturbance and more persistent levels of environmental stress (Alvarez et al 1995; Wardle and Ghani 1995). This is in agreement with PMM and Transitional coversoils experiencing greater disturbance given that they were moved from areas of low-lying topography and placed on an upland slope (Errington and Pinno 2015; deBortoli 2017 – Ch. 2). Alternatively, the metabolic quotient of FFMM, an upland soil transferred to an upland reclamation site, are much lower. From this, we can speculate that PMM and Transitional coversoils are likely to experience longer periods of environmental stress during upland reclamation processes.

3.4.3 – Plant Community Composition:

The plant community composition differed significantly between all coversoils and reference soils. As expected, Fire and Mature plant communities were the most similar as a result surviving root systems and seed banks favouring the regeneration of native species (Roberts 2004; Errington and Pinno 2015). What was unexpected was the greater dissimilarity between FFMM plant communities in comparison to natural reference sites, than the plant communities established on PMM and Transitional coversoils. According to deBortoli (2017 – Ch. 2), FFMM had the highest percentage of vegetation cover, as well as the greatest number of plant species, of the three coversoils. Despite these favourable cover and richness characteristics, FFMM remains strongly associated with a large proportion of introduced weed species (predominantly *S. arvensis*) and grasses. Alternatively, plant communities supported by Transitional and PMM coversoils, though lower in total vegetation cover, were found to have greater proportions of native forbs and shrubs, and in the case of PMM, trees. This illustrates the potential importance of developing effective plant establishment treatments such as the seeding of native plant species and the control of weed species.

3.4.4 – Plant-Microbial Interaction:

Lastly, comparisons of microbial function between weeded and control vegetation plots on each coversoil, as well as insignificant correlation tests, indicated that no substantial plant-microbial interactions had developed at the functional level after three growing seasons. In their study, Tscherko et al. (2005) observed and described the influence of plant community development on belowground rhizosphere microbial communities following a recent deglaciation event. Although this study was completed in an alpine environment, the primary successional timeline observed could serve as a rudimentary analogy and forecast for rhizosphere development on reconstructed reclamation sites. According to Tscherko et al. (2005), microbial biomass and activity was not affected by early successional plant communities that developed approximately 45 years post-glaciation, with rhizosphere microbial communities exhibiting no differences from communities observed in the bulk soil. In fact, microbial biomass accumulation in the rhizosphere, as well as the development of plant species specific influences on microorganisms, was not observed until between 75 and 135 years post-glaciation (Tscherko et al. 2005). The placement of FFMM and Transitional coversoils allowed for the establishment of plant communities with similar vegetation cover and richness as those observed by Tscherko et al. (2005) at 45 years post-glaciation after only three growing seasons. As such, the development of plant-microbial interactions and the development of a distinct rhizosphere may require at least another 30 of plant community development.

Additionally, Bohlen et al. (2001) found that under certain situations it is possible for the effects of plants on the microbial community to be overridden by other factors such as soil characteristics and topography. The increased microbial biomass and activity associated with rhizospheres develop as a result of organic carbon root exudates attracting microorganisms through chemotaxis (Mitter et al. 2017). Chemotaxis in soil is defined as the attraction of mobile microorganisms (i.e. bacteria) in response to chemical gradients – in this case, carbon compounds (Haichar et al. 2014). By migrating toward carbon-rich rhizosphere soils, previously carbon-limited microorganisms can experience increased growth (Grayston et al 2006). However, given the higher TOC percentages found in reclamation coversoils used in this study, it is suspected that a suitable carbon gradient has not yet developed. As such, it is possible that the development of the rhizosphere and its plant-microbial interactions will be delayed due to a lack of chemo-tactical attraction of microorganisms toward developing root networks.

3.5 – Conclusion:

The use of multivariate analyses to compare bioavailable nutrients, microbial function, and plant community composition parameters at a community-level scale allowed for a more holistic assessment of reclamation sites in comparison to mature and post-fire natural reference ecosystems. Further data collection pertaining to target natural ecosystems holds potential for aiding in the development of reclamation certification standards, while continued monitoring of ecosystem functional parameters on reclamation sites could provide companies with a quantitative trajectory of recovery. The identification of key soil, microbial, and vegetation characteristics that cause dissimilarities between reclamation sites and their target endpoint ecosystems can provide insight on how we can continue to refine best management practices, and ultimately encourage a more rapid re-establishment of crucial ecosystem functioning drivers such as plant-microbial interactions.

3.6 – Tables and Figures:

Table 3.1 – Carbon substrates selected to determine microbial function of FFMM, PMM Transitional coversoils, as well as Fire and Mature reference soils, using the community level physiological profiling method.

Carbohydrates	**Carboxylic Acids**	**Amino Acids**
L-(+)-arabinose	N-acetyl glucosamine	Citric acid
D-(-)-fructose	L-alanine	α-ketoglutaric acid
D-(+)-galactose	ϒ-amino butyric acid	L-malic acid
D-(+)-glucose	L-arginine	Oxalic acid
D-(+)-trehalose	L-cysteine-HCL	
	L-lysine-HCL	

Table 3.2 – Mean bioavailable nutrient rates present in reclamation coversoils and natural references soils during 2016 measurements using PRS™ probes. Table reports means with standard errors in brackets. Coversoil and soil nutrients sharing the same letter (per column) are not significantly different from one another. Nutrient rates are measured as μg/10cm^2/6 weeks.

	NO_3	NH_4	P	K	Ca	Mg	S	Fe	Mn	Cu
FFMM	2.22 **b** (0.24)	0.33 **c** (0.06)	2.05 **b** (0.27)	66.40 **b** (9.87)	1214.30 **b** (78.57)	275.95 **b** (16.62)	232.56 **b** (37.17)	21.63 **b** (3.93)	2.79 **b** (0.37)	0.15 **c** (0.02)
PMM	3.55 **b** (0.82)	0.69 **b** (0.10)	1.16 **b** (0.15)	21.94 **c** (2.14)	1947.77 **a** (63.86)	438.21 **a** (17.32)	700.33 **a** (51.56)	57.35 **a** (7.24)	7.51 **a** (1.62)	0.55 **a** (0.10)
Transitional	4.47 **a** (0.32)	0.47 **b** (0.03)	1.75 **b** (0.20)	32.81 **c** (4.36)	1934.49 **a** (83.03)	415.19 **a** (22.34)	235.25 **b** (35.30)	11.77 **c** (1.32)	1.01 **c** (0.08)	0.28 **b** (0.06)
Mature	2.26 **b** (0.90)	4.10 **a** (0.75)	9.52 **a** (1.30)	308.42 **a** (41.79)	570.94 **c** (39.61)	174.36 **bc** (30.16)	20.36 **c** (4.98)	5.66 **d** (0.26)	6.90 **a** (2.43)	0.04 **d** (0.02)
Fire	1.64 **b** (0.10)	2.38 **a** (0.79)	11.76 **a** (2.62)	246.36 **a** (47.12)	834.64 **c** (74.54)	120.20 **c** (21.98)	19.18 **c** (3.96)	5.38 **d** (0.63)	4.44 **ab** (1.03)	0.02 **d** (0.02)

Table 3.3 – Mean CO_2 production rates (µg CO_2-C/g/hr) of reclamation coversoils and nature reference soils following carbon-substrate induced respiration. Table reports means with standard errors in brackets. Coversoil and soil CO_2 production rates sharing the same letter (per column) are not significantly different from one another.

	Carbohydrates	**Carboxylic Acids**	**Amino Acids**
FFMM	1.69 **b** (0.20)	6.18 **b** (0.96)	1.26 **b** (0.15)
PMM	2.42 **ab** (0.32)	9.28 **ab** (1.79)	2.33 **a** (0.20)
Transitional	2.90 **a** (0.27)	11.61 **a** (1.83)	2.80 **a** (0.25)
Mature	1.80 **ab** (0.27)	6.96 **ab** (1.12)	0.47 **b** (0.15)
Fire	1.27 **b** (0.36)	6.29 **ab** (1.78)	0.77 **b** (0.21)

Table 3.4 – Mean soil characteristics for reclamation coversoils and natural reference soils during 2016. Table reports means with standard errors in brackets. Characteristics sharing the same letter (per column) are not significantly different from one another. EC = electrical conductivity; TOC = total organic carbon; TN = total nitrogen; MB-C = microbial biomass carbon; MB-N = microbial biomass nitrogen; qCO_2 = metabolic quotient.

	pH	**EC (µS/cm)**	**TOC (%)**	**TN (%)**	**MB-C (mg/L)**	**MB-N (mg/L)**	**Respiration (pmm/sec)**	**qCO_2**
FFMM	5.6 **b** (0.1)	942.71 **b** (122.12)	6.87 **b** (0.98)	0.32 **bc** (0.05)	1.31 **b** (0.20)	0.15 **b** (0.01)	0.78 **b** (0.22)	1.05 **b** (0.23)
PMM	5.0 **c** (0.3)	1508.63 **a** (140.44)	14.51 **a** (1.81)	0.47 **ab** (0.07)	1.52 **ab** (0.27)	0.07 **c** (0.01)	2.17 **a** (0.43)	3.62 **a** (1.00)
Transitional	6.2 **a** (0.1)	1908.25 **a** (171.63)	17.14 **a** (1.67)	0.65 **a** (0.07)	2.40 **a** (0.33)	0.27 **a** (0.03)	3.92 **a** (1.16)	4.22 **a** (1.32)
Mature	4.9 **c** (0.2)	91.02 **c** (7.52)	2.12 **b** (0.16)	0.08 **d** (0.01)	1.08 **b** (0.23)	0.07 **bc** (0.01)	0.52 **b** (0.15)	0.50 **b** (0.12)
Fire	6.0 **ab** (0.2)	95.38 **c** (8.66)	3.43 **b** (1.54)	0.12 **cd** (0.04)	0.65 **b** (0.08)	0.03 **c** (0.01)	0.49 **b** (0.30)	0.63 **b** (0.33)

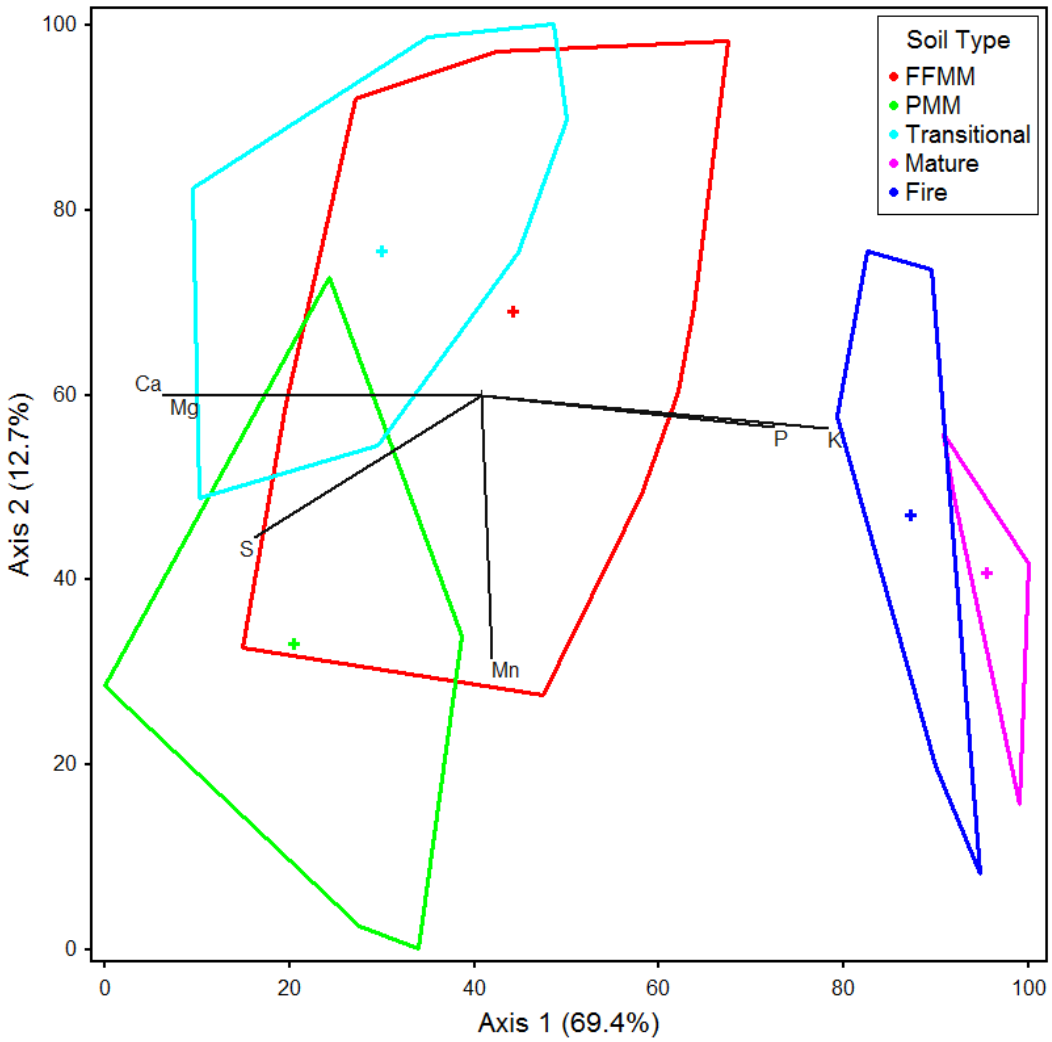

Figure 3.1 – Non-metric multidimensional scaling ordination bi-plot of reclamation coversoil and natural reference soil bioavailable nutrient profiles using a Bray-Curtis distance measure. Vector associations ($r^2 = 0.568$) are weighted by length and include proportionate responses to bioavailable rates of NO_3, NH_4, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, and Al.

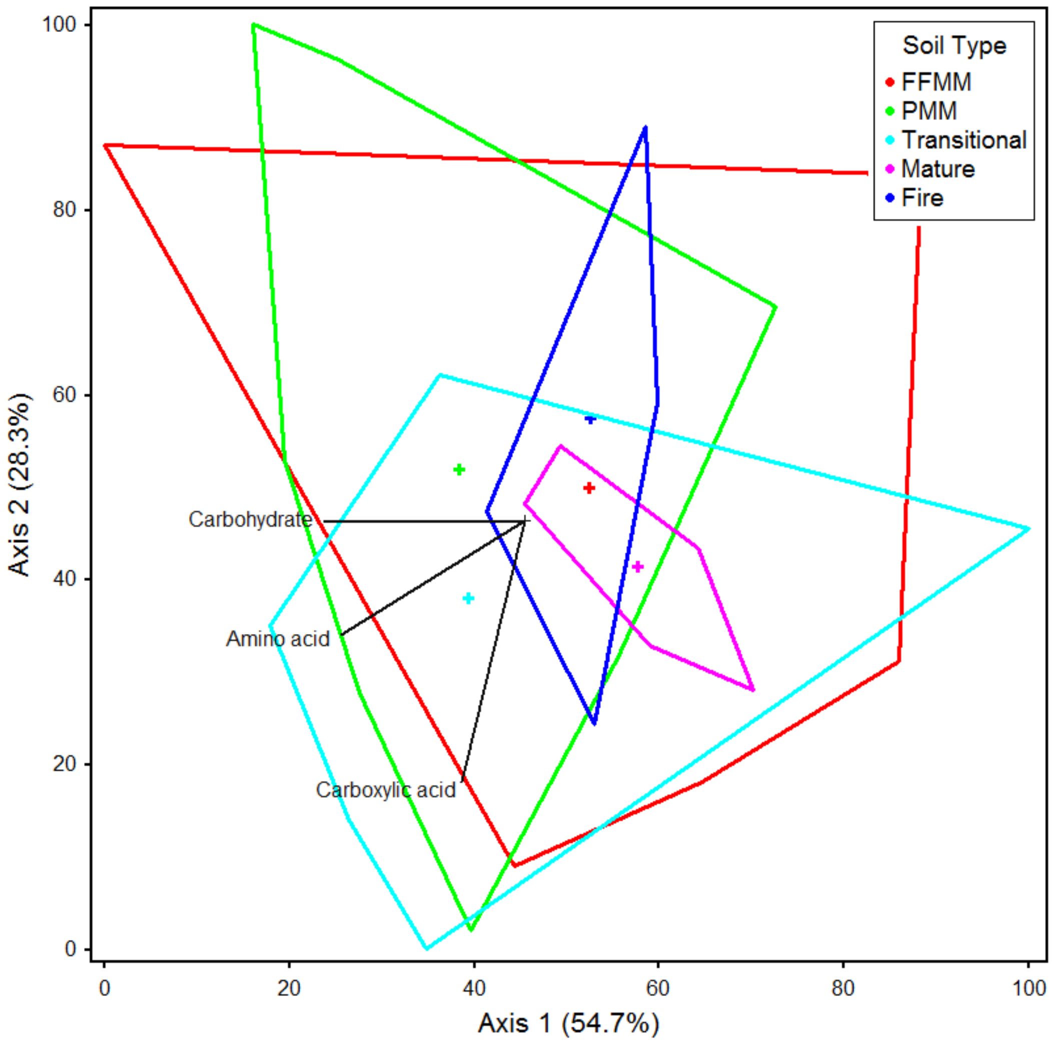

Figure 3.2 – Non-metric multidimensional scaling ordination bi-plot of reclamation coversoil and natural reference soil microbial function (as measured by CO_2 production) using a Bray-Curtis distance measure. Vector associations (r^2 = 0.434) are weighted by length and include proportionate responses to carbohydrates (L-(+)-arabinose, D-(-)-fructose, D-(+)-galactose, D-(+)-glucose, D-(+)-trehalose), carboxylic acids (N-acetyl glucosamine, L-alanine, ϒ-amino butyric acid, L-arginine, L-cysteine-HCL, L-lysine-HCL), and amino acids (Citric acid, α-ketoglutaric acid, L-malic acid, Oxalic acid).

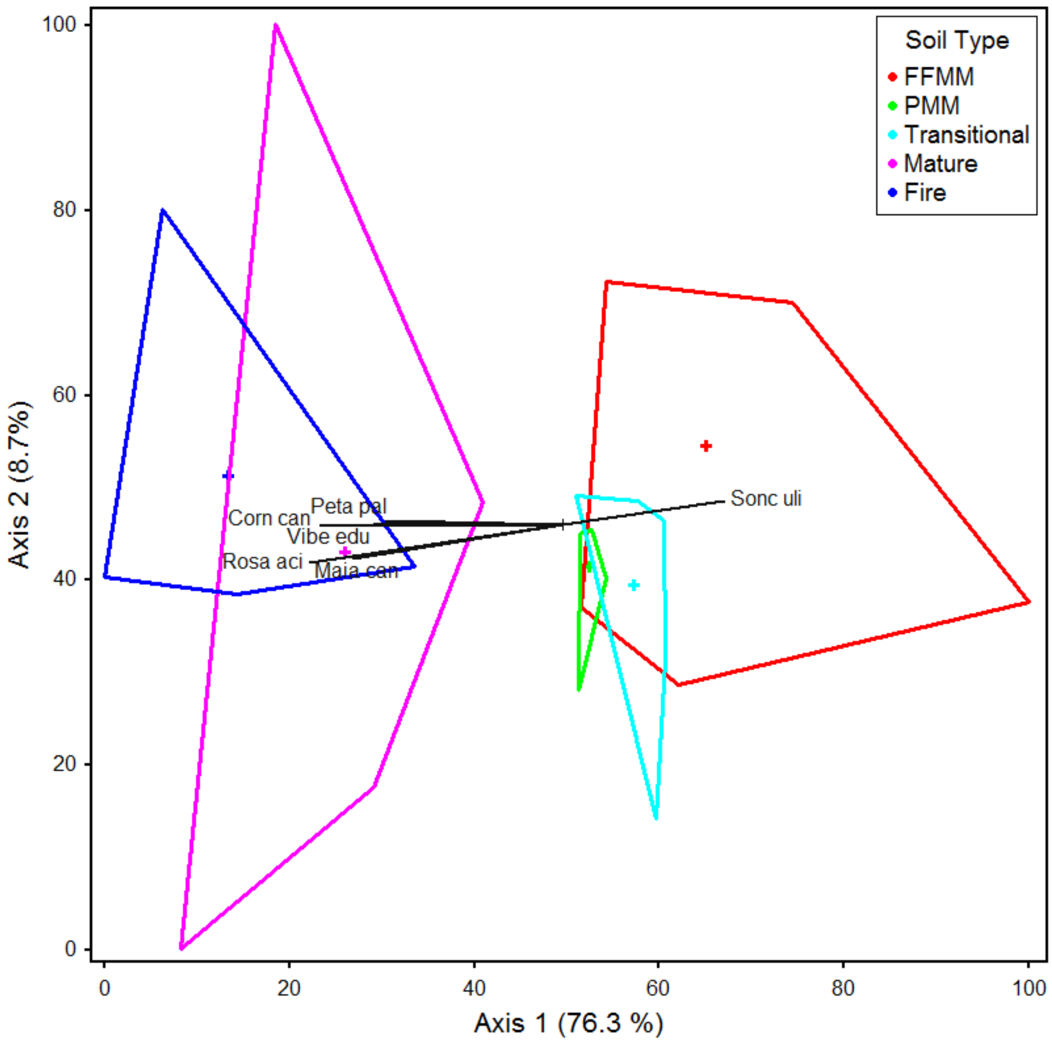

Figure 3.3 – Non-metric multidimensional scaling ordination bi-plot of reclamation coversoil and natural reference soil plant community composition using a Bray-Curtis distance measure. Vector associations ($r^2 = 0.354$) are weighted by length and include proportionate responses to 77 understory plant species.

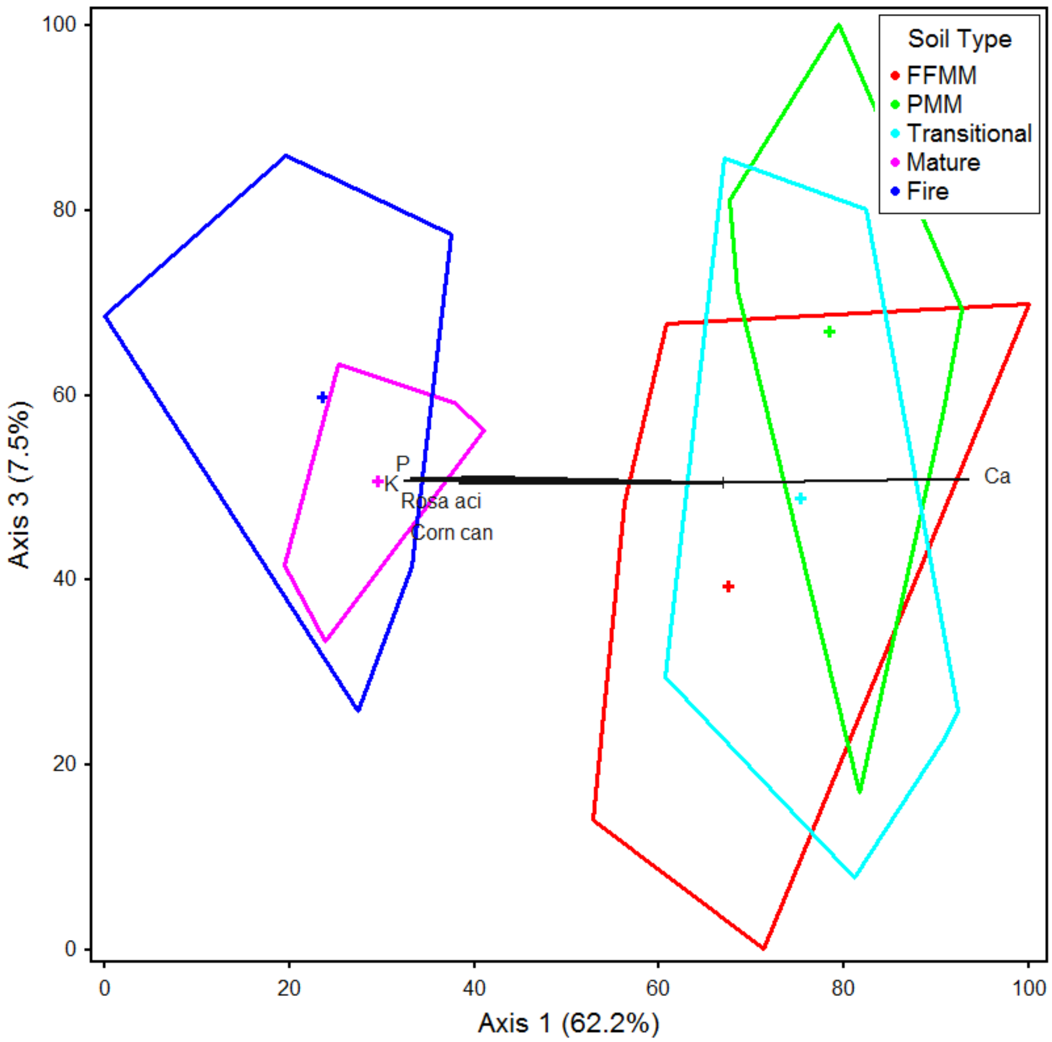

Figure 3.4 – Non-metric multidimensional scaling ordination bi-plot of reclamation coversoil and natural reference soil's bioavailable nutrient profiles, microbial function, and plant community composition multivariate parameters using a Bray-Curtis distance measure. Vector associations ($r^2 = 0.5$) are weighted by length.

Chapter 4 – Technical Transfer

4.1 – Study One – Implications:

Although the weeding treatment did result in a beneficial increase in aspen seedling density on PMM, the removal of dominant introduced species did not necessarily align understory plant community composition to a trajectory similar to that of a post-disturbance (i.e. fire) boreal mixedwood forest. Instead, it appears that weeding without successful facilitation of native forb establishment via broadcast seeding acted only as an accelerant to the pre-existing trend of a declining introduced forb cover in favour of an increasingly dominant graminoid cover. This accelerated trend is particularly problematic on FFMM given that the species benefiting the most from weeding is *Calamagrostis canadensis*, a native grass that has been shown to inhibit growth and elevated mortality of desired aspen and white spruce seedlings when overabundant.

It is therefore recommended that a more effective seeding procedure be determined in future field studies. If coupled with weeding treatments, a successful seeding method should be able to insure that the vacant microsites created by weed removal be inhabited by target understory plants, not dominated by grasses. Some alterations to the broadcasting seeding method in this study could include (1) increasing the amount of seed mix being broadcasted, (2) increasing the frequency of seeding treatment to coincide with weeding treatments (i.e. immediately after weeding to ensure seeds collect in microsites), and/or (3) scheduling weeding and seeding treatments in accordance to predicted precipitation patterns to ensure favourable establishment conditions.

From a managerial perspective, hand-pulling dominant weed species would become cumbersome as the collective area of reclamation sites increased over the years. Therefore, it is recommended that weed management efforts be allocated specifically to areas experiencing high levels of introduced plant invasion. Of the two plant establishment treatments explored during this study, a successful seeding practice would likely be a more operationally applicable plant community management strategy for large scale reclamation efforts.

An alternative to reactively removing dominant weed species would be to incorporate various methods of proactive weed prevention into reclamation plans. This could include the placement of coarse woody debris (CWD) amendments, as well as the planting of fast-growing deciduous tree species such as trembling aspen and balsam poplar. The use of CWD, which includes large branches, logs, standing snags, and coarse roots (Kwak et al. 2015), has been shown to regulate soil temperature and water content by providing coversoils with protection from immense levels of sunlight (Brown and Naeth 2014; Kwak et al. 2015). Decreased light exposure and soil temperatures caused by CWD holds the potential to reduce weed invasion by diminishing the germination of introduced seeds (Lemna and Messersmith 1990; Woo et al. 1991). For example, the germination rate of the most prevalent weed species in this study, *S. arvensis*, experiences a notable decline in response to decreased light exposure coupled with temperatures below 20˚C (Lemna and Messersmith 1990). Additionally, the placement of CWD was observed, by Brown and Naeth (2014), to promote the diversification of reclamation habitats via the creation of microsites. Individual plant species have their own unique set of establishment and survival requirements (Grubb 1977); therefore, microsites generated by CWD can provide reclamation sites with a greater diversity of plant species via cohabitation, and prevent a single [weed or grass] species from dominating the area.

In addition to CWD, it is recommended that trembling aspen and balsam poplar seedlings be planted on FFMM and Transitional coversoils. Apart from contributing to the establishment early-successional tree species on site, planting fast-growing deciduous seedlings would also be able to influence understory development via increased shading from leaf area (Pinno and Errington 2015). Much like the CWD, development of even a partial canopy cover from deciduous seedlings would likely translate into lower light transmission to the understory level and cooler soil temperatures; thereby diminishing weed germination. Furthermore, Lieffers et al. (1993) found that in a deciduous dominated stand with 40 percent canopy closure, the biomass of this study's potentially problematic grass species, *C. canadensis,* was halved compared to in clearings. In which case, it is suspected that the early development of a deciduous canopy cover on reclamation sites would prevent the extensive establishment of both invasive weed species and highly competitive grasses.

4.2 – Study Two – Implications:

From the of multivariate dataset analyses used in this second study, we are able to suggest additional reclamation strategies, as well as refine some of the aforementioned recommendations derived from the first study. The evaluation of bioavailable nutrients indicated a lack of phosphorus and potassium accompanied by a surplus of calcium and magnesium on reclamation coversoils in comparison to natural reference soils. Although fertilizers high in nitrogen have been discouraged by past studies due to the possibility of encouraging the growth of grass and weeds (Heady and Child 1999; Sloan and Jacobs 2013; Howell et al. 2017), a fertilizer low in nitrogen, but high in phosphorus and potassium, may be able to alleviate nutrient limitations and promote increased plant growth.

Analysis of plant community composition revealed that *S. arvensis* is strongly associated with coversoils (particularly FFMM), and is likely the cause of increased dissimilarity between reclamation sites and nature reference areas. This further supports the need for an effective weed management plan during reclamation efforts. Alternatively, both Fire and Mature plant communities held strong associations with various shrub species – most notably, *Rosa acicularis, Rubus idaeus, Viburnum edule,* and *Shepherdia canadensis*. Therefore, it is recommended that future seeding treatments utilize a seed mix that contains both shrub and forb species. To maximize the potential effectiveness of seeding, it is suggested that the majority of the seed mix be comprised of species that are known to thrive in areas with elevated light transmission – for example, *Achillea millefolium, Chamerion angustifolium, Rubus idaeas,* and *Rosa acicularis* (Chen and Popadiouk 2002; Tannis 2004; Hart and Chen 2006).

Lastly, the increased response to carbon substrates, as well as the elevated metabolic quotients, observed for PMM and Transitional during microbial function analyses suggests that these coversoils are experiencing higher levels of environmental stress than FFMM and natural reference sites. In order to reduce environmental stress experienced by PMM and Transitional coversoils, it is recommended that, when possible, reclamation sites be re-contoured into areas with gently undulating topography in order to accommodate the ratio of upland:lowland derived coversoil available. In addition to reducing environmental stress by encouraging increased water saturation in PMM or Transitional capped depressions, the proposed reconstructed topography would resemble that of a pre-disturbance boreal forest natural region (NRC 2006; Bliss et al. 2015; Wikens 2015), and reduce the amount of CO_2 emissions from drought-induced organic material (Fenner and Freeman 2011).

4.3 – Future Research:

As is common with research, the completion of these two reclamation studies has to the generation of several more related research questions. Some subsequent studies that could branch from this research include:

1. **The investigation of alternative seeding methods.** A number of seeding enhancements, such as seed priming, pelleting, and/or agglomerating, have been studied and utilized in the agricultural and rangeland industries (Madsen et al. 2012; Schoonmaker et al. 2014). Given the first study's speculation of dry weather conditions inhibiting seeding success, it would be helpful to identify methods of overcoming this obstacle during future seeding treatments. This could include testing the effectiveness of hydropriming to enhance drought resistance (Jisha et al. 2013), pelletizing to improve seeding success of miniscule seed (i.e. fireweed and common yarrow) (Schoonmaker et al. 2014), or agglomerating a mixture of native seeds to enhance emergence and growth in unideal soil conditions (Madsen et al. 2012).
2. **The construction of reclamation trajectories.** The continuous collection and analysis of bioavailable nutrient profiles, microbial function, and plant community composition would allow for the generation of reclamation trajectories over the years. As time progresses, we would be able to determine whether certain coversoil, plant establishment, or other managerial practices prompted reclamation sites to successful converge with natural reference sites, or alternatively, created a novel ecosystem.
3. **The collection of multivariate parameter datasets for reclamation and reference wetland systems.** Although much more challenging than upland forest reclamation, the reclamation of wetlands has been recognized as a crucial step toward achieving equivalent land capability in guideline manuals (CEMA 2014). As such, it is important to start critically developing a set a criteria and indicators for the effective tracking and evaluation of future wetland reclamation efforts. This could be done through the

monitoring of current wetland reclamation projects such as Syncrude’s Sandhill Fen and Suncor’s Nikanotee Fen.

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Appendix:

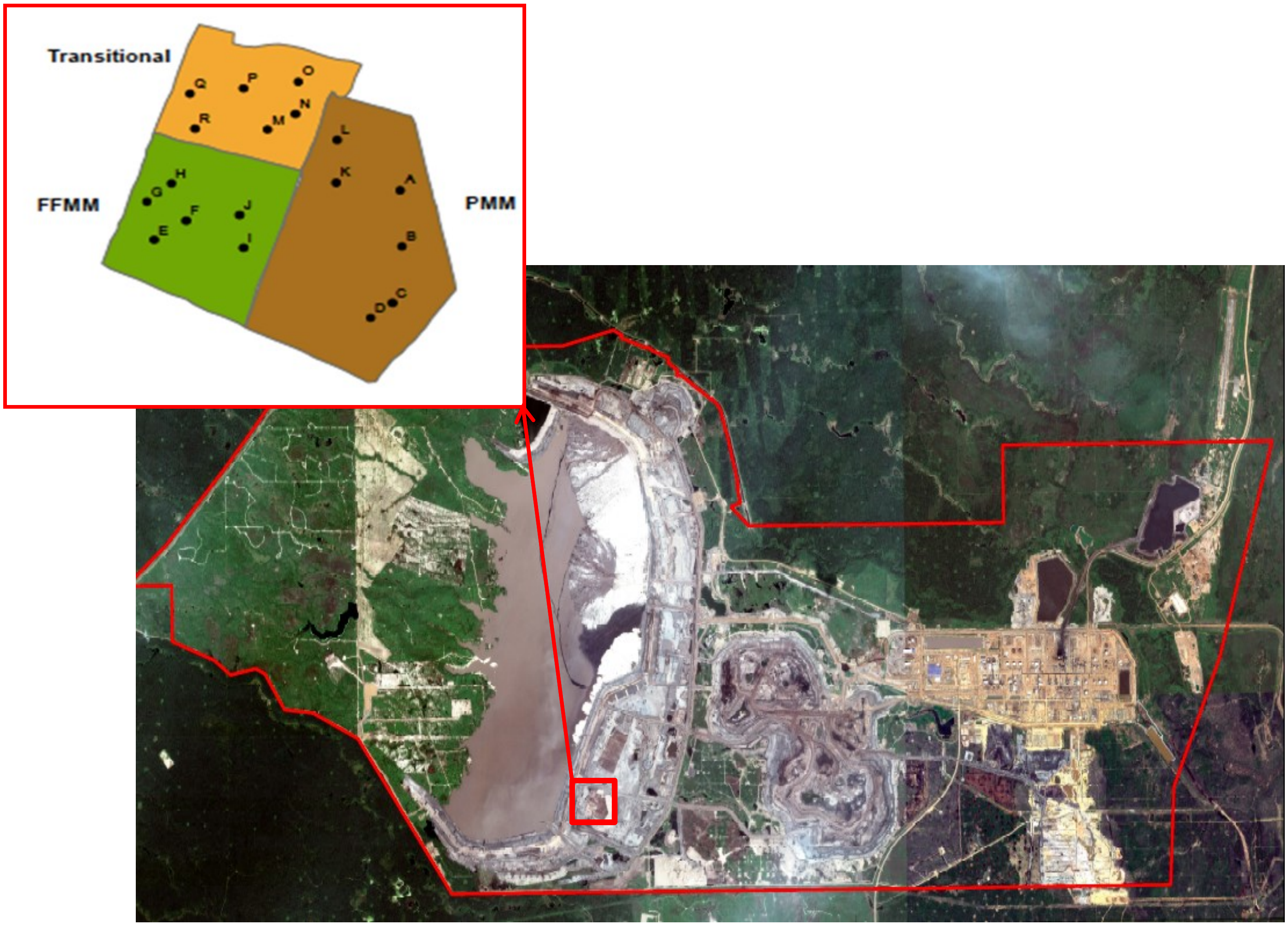

Figure 6.1 – A satellite image of the oil sands site and reclamation study area. Research plot placement for FFMM, PMM, and Transitional coversoils is shown via magnification of the reclamation study area.

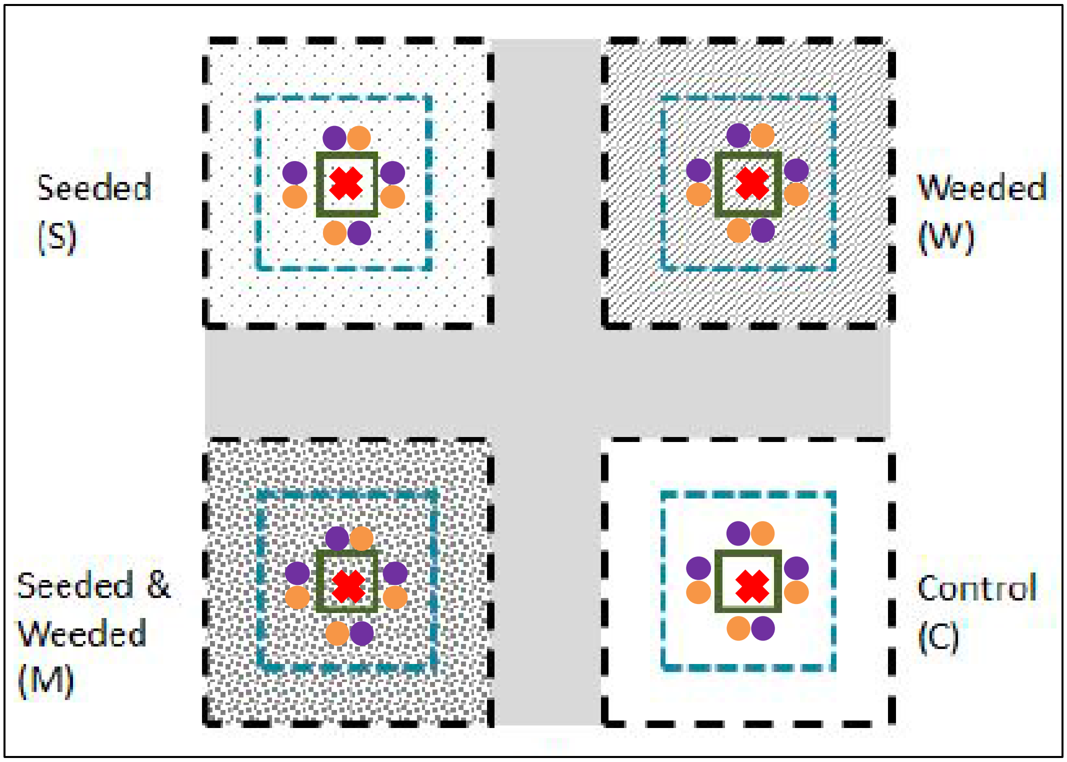

Figure 6.2 – A schematic of individual research plot breakdowns. Seeded, weeded, seeded & weeded, and control treatment quadrats (5m by 5m) are outlined by black dashed lines. Seedling mensuration plots (3m by 3m) are outlined by blue dashed lines. Vegetation survey plots (1m by 1m) are outline by green lines. Plant root simulator probe installments are depicted by paired purple (cation) and orange (anion) circles. Soil sampling locations marked with a red X.

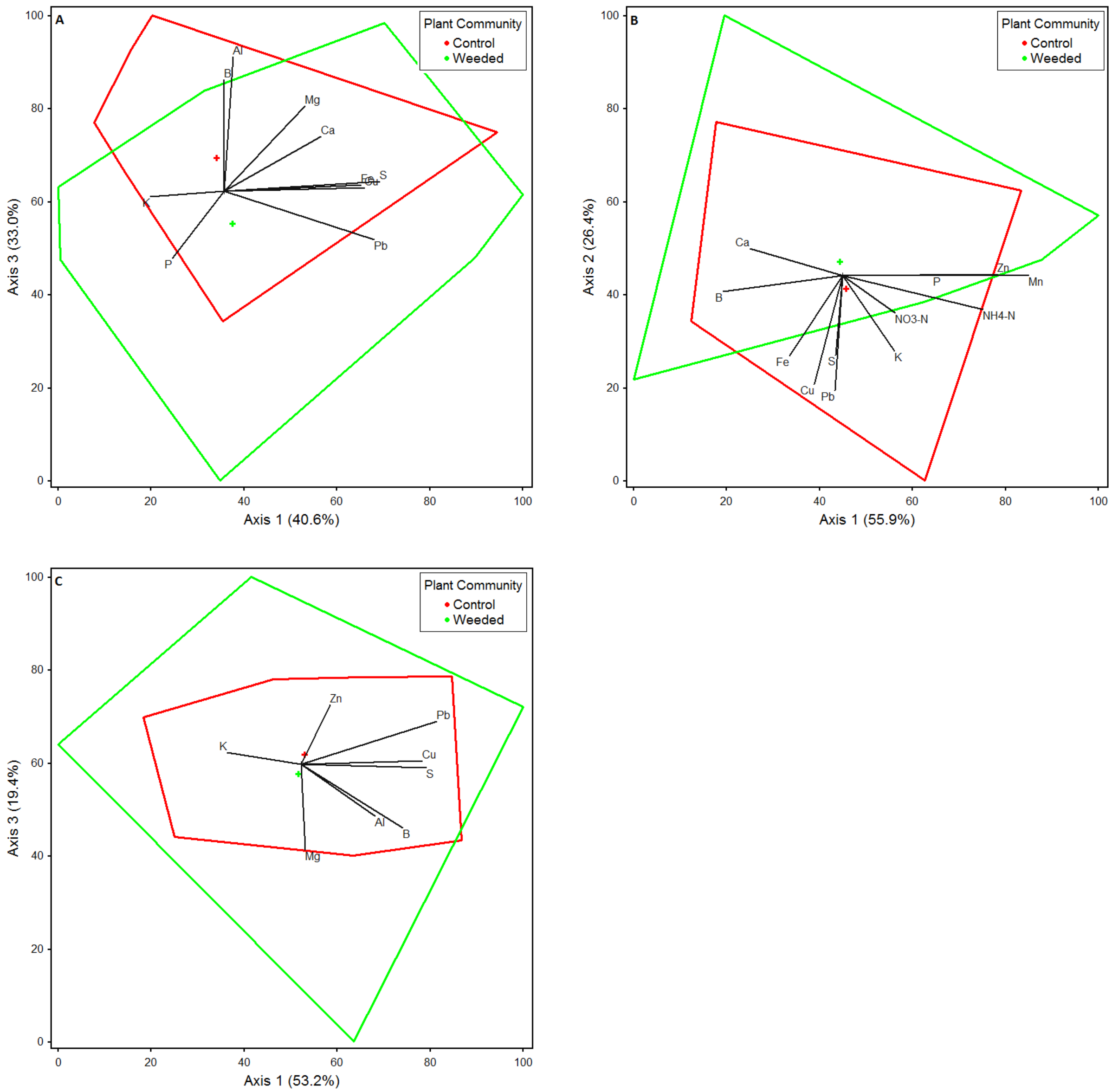

Figure 6.3 – Non-metric multidimensional scaling ordination bi-plots of soil bioavailable nutrient profiles between weeded and control plots on A) FFMM, B) PMM, and C) Transitional coversoils. A Bray-Curtis distance measure was used. Vector associations are weighted by length and include proportionate responses to bioavailable rates of NO_3, NH_4, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, and Al.

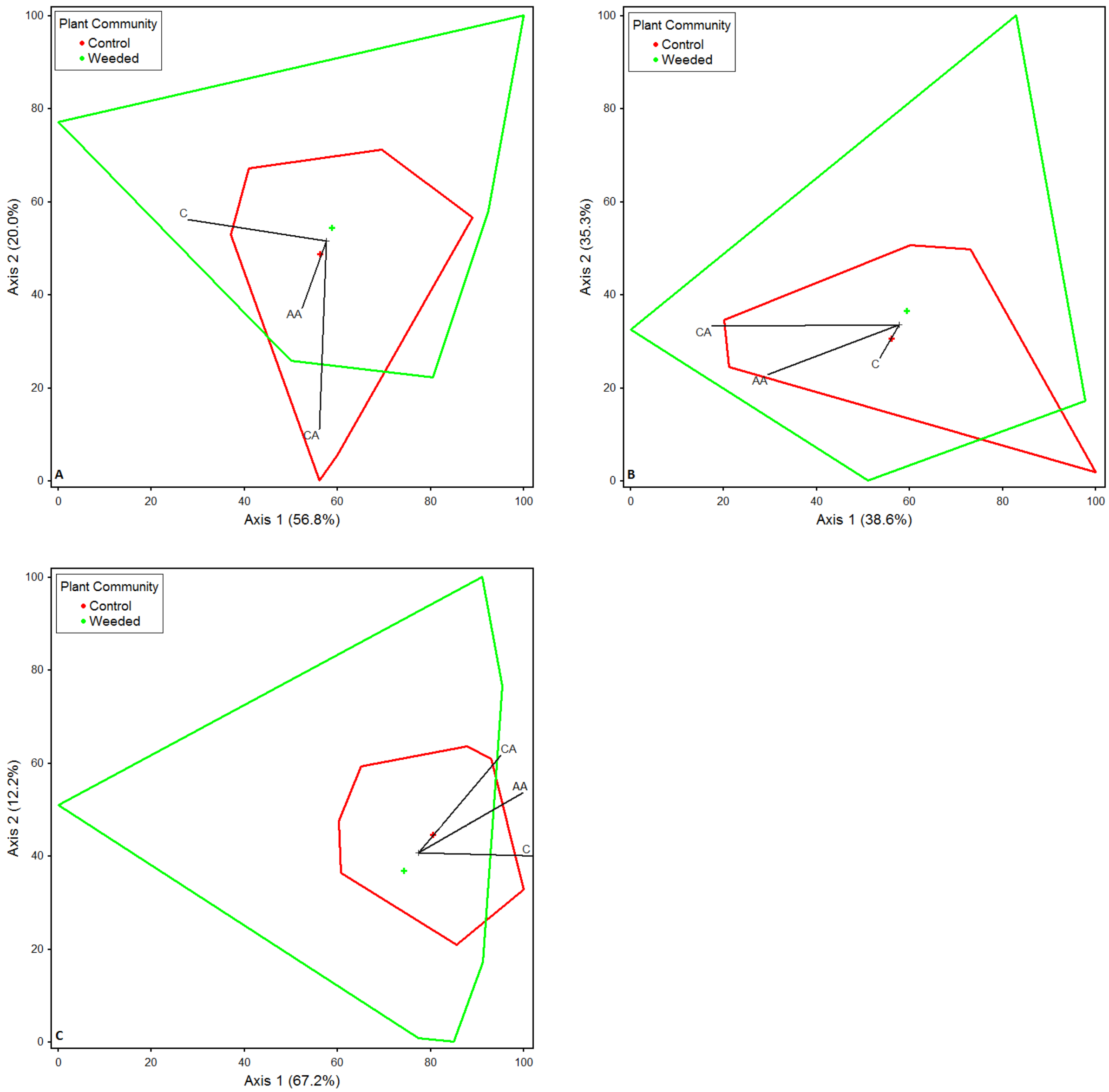

Figure 6.4 – Non-metric multidimensional scaling ordination bi-plots of soil microbial function (as measured by CO_2 production) between weeded and control plots on A) FFMM, B) PMM, and C) Transitional coversoils. A Bray-Curtis distance measure was used. Vector associations are weighted by length and include proportionate responses to carbohydrates (L-(+)-arabinose, D-(-)-fructose, D-(+)-galactose, D-(+)-glucose, D-(+)-trehalose), carboxylic acids (N-acetyl glucosamine, L-alanine, ϒ-amino butyric acid, L-arginine, L-cysteine-HCL, L-lysine-HCL), and amino acids (Citric acid, α-ketoglutaric acid, L-malic acid, Oxalic acid).