Exploring the Feasibility of Enhancing Indigenous Arbuscular Mycorrhizal Fungi Communities for Sustainable Soybean Production in Northern Ontario

by

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Author’s Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Arbuscular mycorrhizal (AM) fungi have been widely recognized as a promising approach to attain sustainability in the agricultural industry. These fungi have been known to offer numerous benefits to crops and have been commercially employed as inoculants due to their capacity to enhance yields and protect crops from biotic and abiotic stressors. However, it is widely believed that certain conventional agricultural practices, including high fertilization, monocultures, and non-mycorrhizal crops, can cause a decline in the abundance and diversity of AM fungal communities. This, in turn, can lead to a decrease in the transfer of benefits from AM fungi to crops, thereby compromising crop productivity and sustainability. This thesis aimed to investigate the feasibility of enhancing the preexisting indigenous AM fungal communities in the soil to potentially confer additional benefits to soybeans (*Glycine max* (L.) Merr.) in rotation after canola (*Brassica napus*). Since indigenous AM fungi are already adapted to local conditions and possess a more diverse community than the typical commercial inoculants, it was hypothesized that augmenting indigenous AM fungi may serve as a viable substitute for chemical fertilizers. The objective was to evaluate the capacity of indigenous AM fungi to confer additional benefits to crops under varying levels of fertilizer. It was hypothesized the indigenous AM fungi would maintain or improve soybean growth metrics with decreasing levels of fertilizer use to a higher degree when compared to commercial inoculant. This study took place at three field sites in northern Ontario, Canada where it is common to see a canola-soybean crop rotation. Canola, a non-mycorrhizal crop, was first planted in the 2021 field season. Based on the literature, it was assumed that a non-

mycorrhizal crop would have a detrimental effect on AM fungi. Concurrently, during this season, sorghum-sudangrass (*Sorghum* × *drummondii*), a highly mycotrophic plant, was planted in a small adjacent plot with the objective of increasing the abundance of indigenous AM fungi in the soil. Rhizosphere soil from the sorghum-sudangrass plot was subsequently collected and used as indigenous inoculant in the main experiment the following year. In 2022, soybeans were planted where canola had grown previously. This study used a randomized split plot experimental design with fertilizer as the main treatment (at 0, 50, and 100% the recommended rate according to provincial guidelines) and AM fungal inoculant (commercial, indigenous, and no additions) as the subplot treatment with 4 replicates for a total of 36 experimental plots at each site. Root colonization and biomass were assessed at V2 and R2 while apparent harvest index, yield, protein and oil content, and spore counts were assessed after the soybeans reached physiological maturity. Overall, no evidence was found that the treatments (fertilizer and AM fungal inoculant) had a statistically significant effect on AM fungal root colonization, biomass, apparent harvest index, yield, protein content, or oil content of soybeans and the hypothesis was rejected. The findings suggest that the benefits of AM fungi are context dependent. In soils with relatively moderate nutrient content, there may not be a significant advantage to introducing additional AM fungal inputs. Additionally, these results indicate that conventional agricultural practices may not be consistently detrimental to AM fungal inoculum potential. Furthermore, canola and soybean farmers in northern Ontario can direct their resources towards promoting sustainable

practices and maintaining soil health, rather than relying on AM fungal inoculants, to overall facilitate a healthy microbial community and increase the robustness of the agroecosystem.

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1.0 GENERAL INTRODUCTION

The agricultural industry must transition to a sustainable state to continue to maintain food production without contributing further to climate change and environmental degradation. It is thought that arbuscular mycorrhizal (AM) fungi represent an opportunity for increased sustainability in agriculture (van der Heijden, 2010). AM fungi are of vital importance to the pedosphere. AM fungi are ubiquitous soil dwelling microbes that form symbiotic relationships with most vascular plants (Brundrett & Tedersoo, 2018). This relationship between the plants and fungi is mutually beneficial; the plants gain enhanced access to nutrients and improved stress tolerance while the fungi are provided with carbon (Kiers et al., 2011; Pepe et al., 2018). AM fungi provide many benefits to crops, such as improved yield and greater abiotic and biotic stress resistance (Pepe et al., 2018). Although various studies have produced conflicting results, the prevailing view is that the success of AM fungi in agriculture is dependent on factors such as soil type, environmental conditions, and crop type (Frew, 2021; Islam et al., 2021; Sosa-Hernández et al., 2019).

Commercial AM fungal inoculants have been available for use in agriculture since the late 1990s (Agnihotri et al., 2022; Antoine et al., 2021). However, their limited fungal diversity and inconsistent results (Hart et al., 2018), raise questions about their suitability as compared to using indigenous mycorrhizal alternatives. Indigenous fungi are better adapted to the local context and have more diverse communities than the exotic commercial inoculants (Bencherif et al., 2021). The objective of this thesis was to investigate whether

augmenting indigenous AM fungi could improve crop productivity and provide a viable alternative to chemical fertilization in a canola-soybean (*Brassica napus – Glycine max* (L.) Merr.) crop rotation. It was hypothesized the indigenous AM fungi would provide more benefits to the soybeans with decreasing levels of fertilizer use when compared to commercial inoculant after canola cultivation. To date, studies showing that augmenting indigenous AM fungi on-farm would provide these benefits to crops after the cultivation of a non-mycorrhizal crop under field conditions are limited. Using a split plot experiment in northern Ontario this project aimed to address this research gap. The goal of this study was to establish the groundwork for the agricultural industry to utilize the mutually beneficial relationship between AM fungi and crops through the development of a novel management technique. It was anticipated that this approach could reduce the use of fertilizers, which in turn could conserve resources and minimize eutrophication in the surrounding environment. Considering the context specific nature of AM fungi, this project aimed to evaluate the efficiency of indigenous AM fungi in northern Ontario to determine whether local farmers could incorporate this method into their management practices in a canola-soybean crop rotation. This rotation was chosen as it is typical in northern Ontario and canola has been demonstrated to reduce the AM fungal community, thus reducing the benefits AM fungi can confer onto the subsequent crops (Isobe et al., 2014). Soybeans were selected as the focal crop due to their prevalence in northern Ontario, where canola-soybean rotations are commonly practiced. Additionally, research has revealed increased crop growth through co-inoculation with rhizobia and AM fungi in soybeans (Wang et al., 2011), thus demonstrating

the capacity for a strong mutualistic relationship forming between AM fungi and soybeans. Although AM fungi are ubiquitous in the soil, current farming practices are not focused on encouraging their growth. Thus, this study explored whether indigenous AM fungi could be optimized to reap their benefits in agroecosystems.

2.0 LITERATURE REVIEW

This project aimed to distinguish the effects that management of indigenous AM fungi compared with using commercial AM fungi inoculants have on cultivating soybeans in northern Ontario after the cultivation of canola. The following studies demonstrate the current knowledge of how AM fungi function within soils and how they can be used to benefit the agricultural system. The relationship between canola and AM fungi, the use of fungi as inoculants, their potential in restoring degraded soils, and the benefits of AM fungi will be explored herein. Additionally, the ambiguity that still exists within the literature will be discussed with the conclusion that more research needs to be completed on the use of AM fungi and their impact on the agroecosystem.

2.1 **Sustainability in the Agricultural Industry**

This thesis aimed to test a novel agronomic management practice aimed at enhancing the presence of AM fungi in agricultural soils, providing long-lasting benefits to crops. Even though this research is focused on specific crops in a local agroecosystem, the findings can have important global implications. To support the survival and growth of fungi in the soil, changes need to be implemented within the agricultural industry, promoting more sustainable methods. These may include crop rotation which uses a variety of nutrients in the soil and reduces pest pressures (Manoharan et al., 2017), reduced tilling to mitigate erosion (Säle et al., 2015), and reduced fertilizer use (Williams et al., 2017) to decrease eutrophication and costs to farmers. However, reduced fertilizer use may also result in lower yields, and less tilling may increase herbicide usage. To counterbalance these issues, planting cover crops

can reduce erosion, control weeds, and enhance soil quality. Cover cropping with mycotrophic plants, such as sorghum-sudangrass, has also been shown to increase the AM fungal potential in soils which can then increase the benefit that AM fungi provide to the subsequent crop (Kaur et al., 2020; Panja & Chaudhuri, 2004). The utilization of cover crops has multiple benefits that aid in the transition towards sustainable farming practices.

It is vital to provide farmers with information on sustainable methods and strategies that work well in their geographical area, as well as to create knowledge-sharing events where they can exchange knowledge of best practices. Although there may be some uncertainty with these changes towards sustainability, the benefits of improved soil health, including enhanced soil aggregates, water retention, and nutrient cycling (Rillig, 2004; Thirkell et al., 2017), are long-term commodities that should not be ignored in favor of immediate yield gains.

Overall, the importance of soil health and microbial communities, including AM fungi, in sustainable agriculture cannot be understated. By implementing more sustainable methods, farmers can enhance the health of their soil, reduce fertilizer use and eutrophication, increase long-term crop productivity, and ultimately contribute to a more sustainable agricultural industry.

The benefits of fungi are context-specific and influenced by various factors, such as location, crop type, land history, soil microbial community, and weather. Therefore, it may not be feasible to implement the same approach universally without adaptation.

Consequently, continued research is crucial to determine the longevity and context-specific benefits of fungi. The present thesis represents an initial step in the research process to assess the feasibility of on-farm AM fungi augmentation. This study may provide a framework for transforming the agricultural system into a sustainable one by establishing a method that can be applied across the farming industry.

2.2 **Agriculture in northern Ontario**

There are both challenges and advantages surrounding agriculture in northern Ontario. Currently, agriculture in northern Ontario faces obstacles such as shorter growing seasons, climate change, extremes in weather, and youth migration (Carrillo et al., 2022; Chapagain, 2017). However, projected temperature increases in the coming decades can allow for more crops to be produced in northern areas, thereby facilitating further expansion of northern agriculture (Glaros et al., 2022). Glaros et al. (2022) also state that northern agriculture has the capacity to enhance local food security and generate additional economic benefits. Northern Ontario, with its abundant fertile lands covering approximately 6.5 million hectares along with the affordability of land (Chapagain 2017), represents an ideal region for the expansion of agriculture. Additionally, the government of Ontario has made investments into northern agriculture and in 2011 formulated a framework to promote growth in the workforce, economy, and communities (OMAFRA, 2016). These factors uniquely position northern Ontario to further expand its agricultural sector in the coming years; however, some consideration must be taken. It is important to recognize the potential impact of land conversion on ecosystems and take measures to mitigate these effects through sustainable

methods and the application of traditional knowledge (Glaros et al., 2022). The continuation of research in this field is vital to understand the present and future impacts on ecosystems while developing strategies and crops that will benefit the future of agriculture in the north. This thesis took place at three sites in northern Ontario to further expand on the understanding of sustainable northern farming practices, specifically analysing a potential sustainable method using indigenous AM fungi that could be put into practice by local farmers. While there is significant potential for agriculture in the north, the continuation of research into northern Ontario agriculture is vital to ensure sustainability within communities and ecosystems in the coming decades of northern agricultural expansion.

2.3 **Background on AM fungi**

AM fungi form associations with plants all over the world and are ubiquitous in soil with over 200 known species (Brundrett & Tedersoo, 2018; Hajiboland et al., 2020; Hazard et al., 2013). AM fungi form obligate mutualistic associations with plants, this relationship has coevolved with the plants and fungi for over 400 million years (Gianinazzi-Pearson, 1996). This symbiotic relationship is mutually beneficial with the plant receiving enhanced access to nutrients such as phosphorous, while the fungi are provided with carbon (Pepe et al., 2018). This exchange of resources allows for the plants to have more efficient access to these nutrients (Pepe et al., 2018). This occurs via the arbuscules located within the plant root cells. These highly branched structures serve as the sites of nutrient exchange between the plant and fungi (Pepe et al., 2018). In addition to arbuscules AM fungi consist of spores, hyphae (both inside the root and in the soil), and vesicles (which act as storage structures)

(Müller et al., 2017). Overall, understanding the symbiosis between AM fungi and plants provides a critical foundation for exploring their potential applications in sustainable agriculture.

2.4 **AM Fungi Propagation**

AM fungi are able to propagate in several ways. Depending on the taxa, propagules consist of spores, hyphae, or colonized root fragments that can grow and colonize future plant roots (Schalamuk & Cabello, 2010). The different propagules have different infectivity, and these differing life strategies vary at the family level (Schalamuk & Cabello, 2010). AM fungal spores are considered to be an enduring source of inoculum that can persist in the soil over extended periods of time (Schalamuk et al., 2013), as demonstrated by Kytöviita and Vestberg (2020) spores persisted for up to six years. However, fungivores in the soil can consume spores thereby reducing AM abundance and species richness (Thomsen & Hart, 2018). Numerous vectors, including organisms such as earthworms, birds, ants, and gophers, as well as abiotic factors like wind and water, can disperse AM fungal spores throughout the environment (Thomsen & Hart, 2018). In the presence of host crops, AM fungi can also spread up to 3.2 m per year via hyphae (Powell, 1979). The movement of fungal mycelia throughout the soil can also be influenced by soil chemistry (Bala Chaudhary et al., 2022). These diverse dissemination strategies have enabled AM fungi to achieve a ubiquitous presence in soils across the globe.

AM fungi experience seasonality in their lifecycle. There is seasonal variation in the hyphal density (Kabir et al., 1997) and in sporulation, with some genus' sporulating in early

spring while others sporulate in the late fall (Oehl et al., 2009). Additionally, different AM fungi produce spores at different times and rates (Oehl et al., 2009). Further, some AM fungi experience longer spore dormancy periods while other AM fungi use hyphae as propagules more often than they use spores (Oehl et al., 2009). It is crucial to understand the diversity in AM fungi life strategies when considering agricultural applications and when evaluating and comparing literature.

2.5 **Canola & AM Fungi**

Canola is an important crop in northern Ontario with 27,222 acres harvested in 2022 and is responsible for almost half of Ontario's total canola production (OMAFRA, 2023). Canola is a member of the Brassicaceae family and is considered to be a non-host to AM fungi (Cosme et al., 2018; Thompson & Wildermuth, 1989). There are numerous sources that state that canola is a non-host to AM fungi (Cosme et al., 2018; Koide & Peoples, 2012; Thomsen & Hart, 2018; Valetti et al., 2016). This has implications in agriculture as there is evidence from different studies showing that after canola is grown there is a reduction in the AM fungal community. This is thought to be partially due to a reduction in propagules as root and AM fungal hyphae fragments typically survive for only about six months (Gosling et al., 2006). This reduction in the AM fungal community was found in different studies that used molecular methods and spore identification to observe a reduction in species diversity as well as propagule numbers (de Souza & Santos, 2018; Gosling et al., 2006; Sommermann et al., 2018), with some observing a decrease in subsequent crop growth (Arihara & Karasawa, 2000; C. Castillo et al., 2017; Isobe et al., 2014). For instance, a field study that

took place in Andosol soils in Kanagawa Prefecture, Japan from 2007 to 2011 and found that a 50% reduction in soybean yield was observed after 3 years of a winter canola - soybean rotation (Isobe et al., 2014). Based on these findings and other supporting literature (Arihara & Karasawa, 2000; C. Castillo et al., 2017; Gosling et al., 2006; Sommermann et al., 2018), there is a general acceptance that canola is detrimental for the AM fungal symbiosis of subsequent crops.

Further exploration of the relationship between AM fungi and canola reveals additional complexities. A study conducted by Floc'h et al. (2022) took place from 2008 to 2018 with two study sites in Alberta in the sub-humid brown soil zone and one site in Saskatchewan in the semi-arid brown soil zone. Floc'h et al.'s study used molecular methods and found that after ten years in a canola monoculture there was still presence of AM fungi, albeit a less diverse community. While canola may have an effect on the AM fungal community, the relationship between canola and AM fungi is complex, and subsequent crops might not be as negatively affected as previously shown. This may depend on the original level of inoculum potential, soil type, presence of other plants (e.g., weeds), and other geographic and climatic factors. While some studies suggest that canola is harmful to AM fungal communities and future crops (Arihara & Karasawa, 2000; Castillo et al., 2017), other research has shown that the relationship between canola and AM fungi is not clear-cut, and that prior cropping of canola does not necessarily have a significant effect on the yield of future crops (Ryan & Angus, 2003). Therefore, further research is necessary to fully understand the dynamics of this relationship.

2.6 **AM Fungi as Inoculants**

AM fungi have potential applications in agriculture as they can be used as an alternative or supplement to chemical fertilizers to increase crop yields. Currently, commercial AM fungal inoculants are being used by farmers to achieve this goal (Agnihotri et al., 2022). In 2019, the global demand for AM fungal inoculum was $268.8 million and is estimated to increase to over $600 million by 2025 (Srivastava et al., 2021). However, it is unclear whether farmers can enhance the indigenous AM fungal communities that are already present in the soil to reap the benefits of AM fungi without adding commercial products. Several studies have demonstrated that indigenous AM fungi are more efficient than commercial inoculants (Bencherif et al., 2021; Herrera-Parra et al., 2021; Kouadio et al., 2017). Despite this, there is minimal research on developing a method for on-farm augmentation of indigenous AM fungi after a non-mycorrhizal crop in a conventional agricultural system. To address this gap, this project aimed to investigate the feasibility of increasing indigenous AM fungi in agricultural soil to achieve improved crop yields in a canola-soybean rotation. This research sought to determine whether farmers could use techniques to enhance the abundance and effectiveness of existing AM fungi in the soil. By doing so, farmers might be able to use an alternative, more effective and sustainable practice than resorting to commercial AM fungal inoculants.

This thesis focused on the use of indigenous AM fungi, which refers to genotypes native to the region, which have been shown to improve crops' nutrient access and stress tolerance (Begum et al., 2019; Kiers et al., 2011; Pepe et al., 2018). In contrast, exotic AM

fungi are isolated from different locations and artificially cultivated in mass to be introduced into foreign soil. Additionally, indigenous AM fungal communities consist of a multitude of diverse species adapted to the local context while the commercial AM fungal inoculants typically contain only one, exotic species (Faye et al., 2013). Although the potential benefits of mycorrhizal fungi have been recognized for some time, there has been a recent increase in research examining the establishment success of AM fungi (Köhl et al., 2016; Kokkoris et al., 2019; Pellegrino et al., 2012; Sýkorová et al., 2012; Verbruggen et al., 2013). However, there are few studies that have compared the use of commercial inoculants versus managing indigenous mycorrhizal fungi on conventional farms. Thus, this study aimed to address this knowledge gap by investigating the feasibility of on-farm augmentation of indigenous AM fungi and the potential for the use of AM fungi in the transition to a sustainable agricultural industry. The importance of the context dependency in studies on AM fungi deserves consideration. The research discussed next will encompass a wide range of crops, diverse geographical locations, varying weather patterns, historical land use, different strains of AM fungi, and various study lengths and endpoints, including both field and glasshouse studies. Many factors, such as tilling, water availability, and fertilizers, have been identified as significant influences on AM fungi, leading to context-specific results in many cases (Kokkoris et al., 2019; Manoharan et al., 2017; Säle et al., 2015). These studies serve to provide valuable context for ongoing research in this field. However, it is worth noting that the research has produced a variety of results and conclusions (Hart et al., 2018), which

emphasizes the importance of assessing each paper's transferability to the context of this project.

2.7 **Potential of AM fungi to Restore Degraded Soils**

Several studies have explored the potential of AM fungi in restoring degraded soils and increasing benefits to agroecosystems. Considering the recognized soil degradation linked to intensive agricultural practices (Kopittke et al., 2019), the utilization of AM fungi to aid in the restoration of these compromised soils presents a potential avenue to enhance sustainability and increase soil health within the agricultural industry. One long-term field study conducted by Johnson (1993) that lasted for eight years in Minnesota, United States evaluated the effects of fertilization on the AM fungal community. The study found that annual fertilization with: 50 g/m^2 ammonium nitrate; 20 g/m^2 phosphorus pentoxide; 20 g/m^2 potassium oxide; 40 g/m^2 calcium carbonate; 30 g/m^2 magnesium sulfate; 18 ug/m^2 cupric sulfate; 37.7 ug/m^2 zinc sulfate; 15.3 ug/m^2 cobalt chloride; 322 ug/m^2 manganese (II) chloride; and 15.1 ug/m^2 sodium permanganate led to a decrease in the relative abundance of most species within the community. The results have implications for the wider agricultural industry, which relies on chemical fertilizers (Johnson, 1993). Fertilization decisions that do not take AM fungi into account could potentially result in detrimental outcomes for the AM fungal community. After fertilization occurs the plant can obtain sufficient phosphorus from the soil, it is believed that the plant decreases the carbon allocated to the AM fungi which adversely affects the AM fungi, leading to a decline in the AM fungal community (Liu et al., 2012). These results also help to explain the well-known phenomenon of sub-optimal AM

fungal communities in agricultural soils (Pellegrino & Bedini, 2014). A meta-analysis investigated the role of AM fungi in restoring a variety of degraded ecosystems (such as lands impacted by logging, agriculture, and grazing) (Maltz & Treseder, 2015). The analysis found that local fungi can be more effective than exotic or commercial fungi at restoring ecosystems, and that the benefits that AM fungi provide (such as increased plant biomass) can last for several years (Maltz & Treseder, 2015). This finding has applications in the agricultural industry, as some agricultural fields can be classified as degraded ecosystems and could benefit from remediation aimed at increasing soil health. Furthermore, the results suggest that using commercial AM fungal inoculants might be less effective than using local fungi, as found in these studies. Together, these studies demonstrate that there is potential to use local AM fungi to increase the benefits conferred upon the agroecosystem.

2.8 **Benefits of AM Fungi**

This thesis aimed to investigate on-farm augmentation of indigenous AM fungi, building on recent research demonstrating their potential in enhancing agricultural productivity. This potential has been demonstrated through a field study conducted on yam (*Dioscorea rotundata* Poir.) that took place at two study sites (one with neutral sandy loam soil and the other with acidic sand soil) in Yamoussoukro, Côte d'Ivoire (Kouadio et al., 2017). The ecological context in Côte d'Ivoire is distinct from that in this thesis, with different soil context and varying climate conditions. Nonetheless, Kouadio et al. (2017) showed that indigenous fungal inoculants observed higher crop growth parameters than the exotic commercial inoculant. Numerous studies have revealed that indigenous AM fungal

inoculants are superior to commercial/exotic inoculants regarding fungal abundance, crop growth, and production (Bencherif et al., 2021; Herrera-Parra et al., 2021; Kouadio et al., 2017). Pellegrino and Bedini (2014) conducted a study in the Mediterranean at a field site with sandy loam soil that looked at the agronomic effects of local and exotic inoculum on chickpeas (*Cicer arietinum* L). This experiment had varied sowing time and utilized AM fungal inoculum in pots rather than in the field. Nevertheless, their findings revealed that the local AM fungal inoculum resulted in a significant (75%) increase in chickpea yield compared to the exotic AM fungal inoculum. Moreover, the local AM fungal inoculant was associated with higher nutrient uptake and enhanced nutritional value (Pellegrino & Bedini, 2014). These results support the validity of using AM fungi as inoculants. This thesis aimed to expand on this line of work to determine if fungi can be augmented on-farm and if this approach could be implemented in northern Ontario.

AM fungi not only have impacts on the plants but also contribute to overall soil health. Studies have shown that AM fungi contribute to improved soil structure (Thirkell et al., 2017); this is due to the hyphae of the AM fungi acting as a structure to stabilize the soil (Rillig, 2004). Moreover, AM fungi produce a protein, glomalin, that helps improve soil quality by contributing to the soil organic carbon and aiding in carbon and nitrogen storage, as well as enhancing soil aggregate stability (Rillig, 2004). This allows for enhanced water retention and decreases erosion (Thirkell et al., 2017). Overall, these findings highlight the importance of considering AM fungi in sustainable soil management practices.

2.9 **Ambiguity**

Studies examining the effects of AM fungi in the agroecosystem have yielded diverse and sometimes contradictory outcomes. For example, Antunes et al. (2009) analyzed the effects of commercial inoculants using a fine sandy loam soil for this experiment that took place in a greenhouse in Guelph, Ontario, Canada. They found that commercial inoculants did not impact the indigenous community of AM fungi. However, the study acknowledged that longer research periods may reveal other effects. Conversely, Berruti et al. (2017) conducted a study near Carmagnola, Italy in sandy loam soil and found that commercial inoculants altered the indigenous AM fungal community by increasing evenness and diversity. The researchers concluded that the success of commercial AM fungal inoculants is context dependent as the symbiotic relationship between the plant and the fungi can be influenced by variation in genotypes and the soil conditions. Furthermore, the survivability of inoculants varies between studies. For instance, Pellegrino et al. (2011) carried out a study at a field site in Pisa, Italy with sandy loam soil in a clover (*Trifolium alexandrinum)* and corn (*Zea mays*) crop rotation that looked at the effectiveness of indigenous and exotic AM fungi. They found that AM fungal inoculum, both indigenous and exotic, remained present and effective after two years. This is contrasted by the findings of Islam et al. (2021) who conducted an experiment that looked at the survival of commercial AM fungal inoculant in 128 aluminum cores at four field sites in Saskatchewan, Canada: Swift Current (Brown soil), Scott (Dark Brown soil), Melfort (Black soil), and Outlook (Dark Brown soil). They found that commercial inoculants persisted no longer than three months in one field site, but up to

three years in another site. The study concluded that the commercial inoculant's persistence in the environment and its impact on the indigenous AM fungi depended on different edaphic conditions. These findings highlight the variability and context-specific nature of studying AM fungi, which contributes to the conflicting views surrounding the benefits of AM fungal inoculants. Further research is necessary to deepen the understanding of the complex and context-dependent relationship between AM fungi and the agroecosystem. Longer research periods and a thorough analysis of local soil and environmental conditions can help develop more definitive and context-specific recommendations for farmers on the use of AM fungal inoculants. Accurate information for farmers is crucial, particularly in light of the limited diversity, quality, and effectiveness of commercial inoculants being sold, as the exploration of local, indigenous inoculants may offer a promising alternative that can potentially provide greater benefits to crops. More research is needed on methods that consider the health of the pedosphere and overall agroecosystem in order to provide accurate, context specific information to farmers to aid in transforming the industry towards sustainability.

3.0 MATERIALS AND METHODS

3.1. Study Site Descriptions

Field trials were conducted during the 2021 and 2022 growing seasons at three locations across northern Ontario, Canada: New Liskeard (47.5183553N, 79.6700641W), Plummer (46.392963N, 83.790729W), and Mt. Zion (46.4007936N, 83.7159118W) (Figure 3.1). The selection of research locations for this study was driven by the practical considerations of land availability within the scope of the research group. The climate is humid continental (New Liskeard) and temperate continental (Plummer and Mt Zion) (Laporte et al., 2002; Rabearison et al., 2023). The Plummer and Mt Zion sites are commercial farms while the trial at New Liskeard was performed at the Ontario Crop Research Centre – New Liskeard, a research station. The previous crop grown in 2020 at each site was oats (New Liskeard), hay (Mount Zion), and corn (Plummer). Soil physical and chemical properties were determined via a soil test prior to the experiment. At all sites, there is presence of clay and sand due to glacier deposits during the Pleistocene Epoch after the recession of a continental icesheet (Engineering and Terrain Geology Section Staff, 1988). The soil composition at Mt Zion and Plummer was fine sandy loam, Orthic Gray Luvisol, while at New Liskeard the soil was clay, Humic Gleysol (Table 3.1) (Agriculture & Canada, 2013; Bedard-Haughn, 2011). Initial soil fertility levels were also measured, focused on determining P (using the Olsen method) and K (using the ammonium acetate method) availability at each site (Olsen, 1954; Schollenberger & Simon, 1945).

Table 3.1 Soil characteristics at the New Liskeard, Plummer, and Mt Zion sites, northern Ontario, Canada in spring of 2021.

Location	**pH**	**Soil Organic Matter (%)**	**Soil Texture**
New Liskeard	6.7	5.6	clay
Plummer	5.6	3.9	fine sandy loam
Mt Zion	5	3.9	fine sandy loam

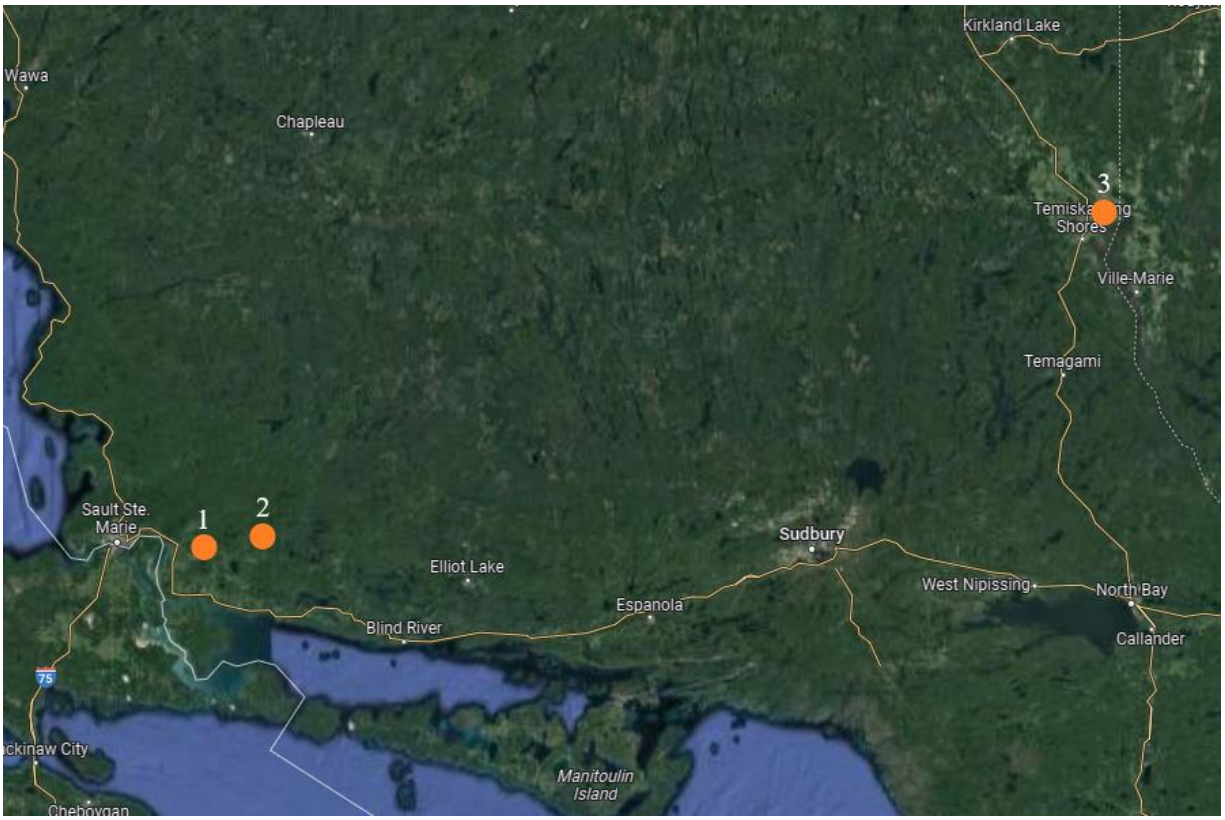

Figure 3.1 Map of three study sites Plummer (1), Mt Zion (2), and New Liskeard (3) in northern Ontario, Canada. Retrieved from Google Maps.

3.2. Canola and Sorghum-Sudangass Cultivation

At all sites in 2021, canola, a non-mycorrhizal crop, was planted in the field that was used in the 2022 experiment. Directly adjacent to the canola, sorghum-sudangrass (*Sorghum* × *drummondii*) was planted in a small area at the New Liskeard and Mt. Zion sites. Sorghum-sudangrass forms strong associations with AM fungi and was grown to promote the growth of indigenous AM fungi (Figure 3.2). The canola and sorghum-sudangrass were harvested at physiological maturity. New Liskeard was tilled in the fall of 2021 to incorporate the fertilizer for the following growing season while Plummer and Mt Zion were not tilled in the fall of 2021 nor the spring of 2022. In 2022, soybeans were grown in the canola field.

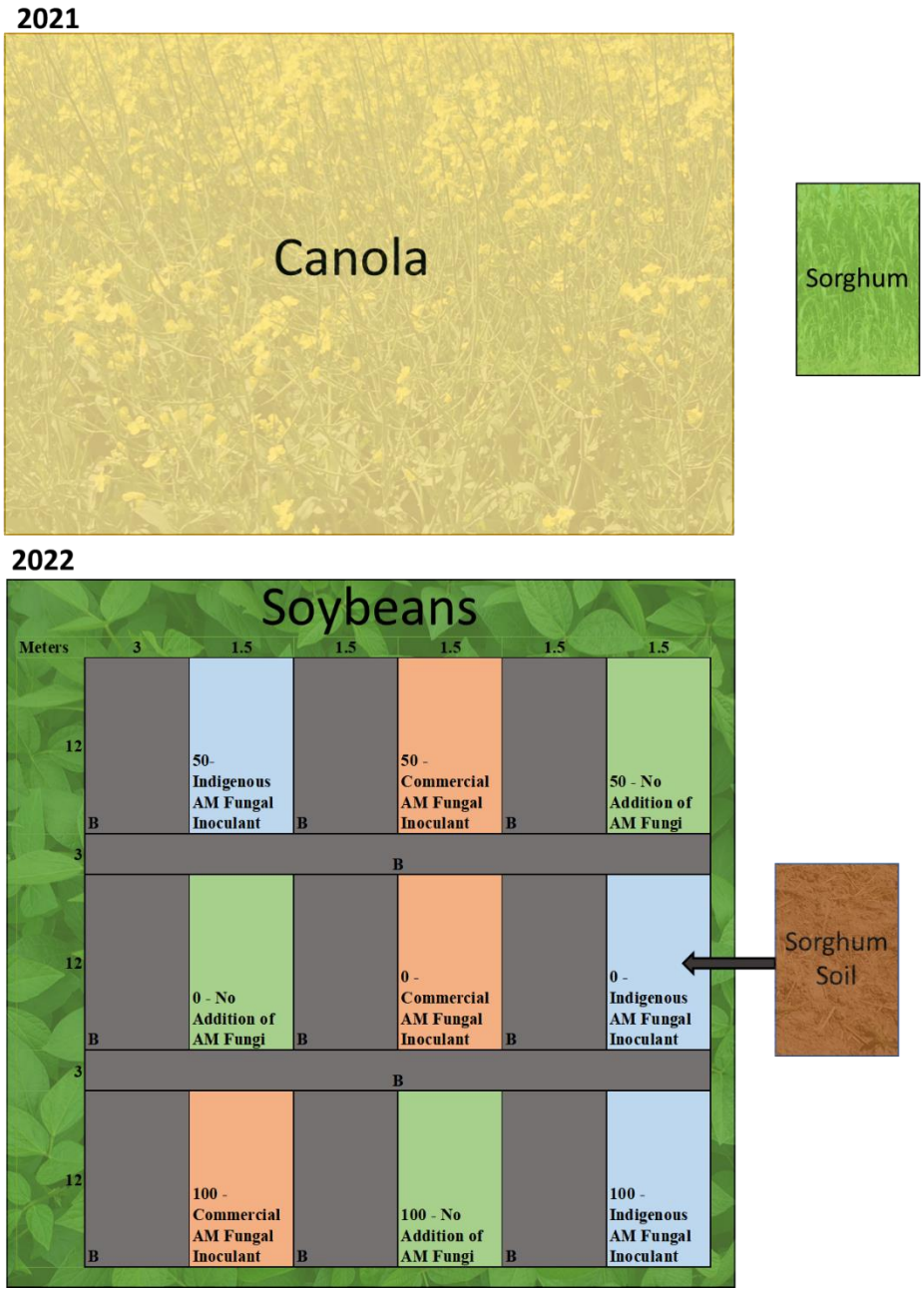

Figure 3.2 Field layout for the 2021 and 2022 growing season in northern Ontario, Canada. In 2021, canola was cultivated in the main field, while sorghum-sudangrass was planted adjacently in a small plot. In 2022, soybeans were planted in the main field. One block of the soybean plot plan is shown depicting the main treatment of varied fertilizer levels (0 = No fertilizer, 50 = 50% recommended P and K rate, 100 = 100% recommended P and K rate according to the provincial guidelines) along with three AM fungal treatments (indigenous AM fungal inoculant, commercial AM fungal inoculant, and no additions of AM fungi) Buffers indicated with "B". Partially adapted from the plot plan by Joshua Nasielski (personal communication, March 22, 2022). Photographs by Melinda Drummond, 2021.

3.3. Experimental and Treatment Design

A split-plot experiment was conducted in 2022 with fertilizer P and K application rate as the main-plot factor and AM fungal inoculation as the split-plot factor. There were three main-plot factor levels, with P and K each applied at either the recommended rate, half the recommended rate, or unfertilized, denoted as 100%, 50% and 0% respectively. The recommended rate was site-specific and based on soil test P and K levels determined in 2021. Ontario's Ministry of Agriculture, Food and Rural Affairs (OMAFRA) maintains a P and K rate recommendation tool for soybean that is based on soil test P and K levels and this tool was used to determine the recommended rate of P and K for each site (Table 3.2). P was always applied as mono-ammonium phosphate (MAP; 11-52-0) and K was always applied as potash (0-0-62). At New Liskeard only, no K was applied in the 100% treatment because no K was recommended based on the soil test. P and K were broadcast at New Liskeard on November 1, 2021 and May 31, 2022 at Mt Zion and Plummer. At New Liskeard, plots were tilled after broadcasting the fertilizer while Mt Zion and Plummer were not tilled after fertilization. The split-plot factor was AM fungal addition. The commercial inoculant, AgTiv field crops granular (Premier Tech, Québec, Canada), was applied according to the manufacturer's instructions, in-furrow with the seed at a rate of 0.57 g /m^2. At Mt Zion plots assigned to the commercial inoculant were seeded last to avoid cross-contamination. In contrast, at Plummer plots receiving the commercial AM fungal inoculum were seeded first as the inoculum was already in the seeder. The seeder was then carefully vacuumed to

remove all the inoculant and remaining seed before adding non-AM fungi inoculated seed. The control plots did not contain any mycorrhizal inoculant.

The indigenous mycorrhizal inoculant was sourced from the field where sorghum was planted in 2021. The soil was rototilled to ensure that the soil was churned as well as to break up the remaining rootballs and sorghum residues to aid in the harvesting of the soil. The soil was harvested from the top 8 cm from random areas around the field to collect a representative quantity. The gravimetric moisture content of the soil was taken according to Routledge and Sabey (1976). Accordingly, the equivalent of 10 kg of dry soil was placed on the corresponding plots (for instance, at Mt Zion the soil was found to have a moisture content of 21.66% so 12.75 kg of wet soil was added per plot). Due to proximity of the sites and land available, Mt Zion and Plummer used the same source of indigenous inoculum. Before planting, the indigenous AM fungal inoculant was sprinkled over the plots and then raked in the top 8 cm of the field by hand. There was a comparable number of spores per gram of source material between the commercial and the indigenous inoculum (Table 3.3). However, the rate of application of the indigenous inoculum was much higher; this was to test an extreme amount of indigenous inoculum to increase the likelihood of observing a response while also ensuring proper mixing throughout the plots.

Plot size varied across sites. At New Liskeard and Plummer plot sizes were 1.5 m wide by 12 m long, and 1.8 m by 12 m at Mt Zion. Buffer plots (3 m wide) were used between main plots used to avoid any movement of fertilizer across main plot treatments

during tillage operations (Figure 3.2). Buffer plots (1.5 m wide) were used between all split-plot treatments to avoid cross-contamination of split-plots. The front half of every plot (6 m) was reserved for in-season sampling while the back half of every plot was reserved for harvest after physiological maturity.

3.4. Soybean Management

Soybean cultivar Bourke (Secan; Ottawa, Ontario, Canada) was sown at all three locations. Bourke is an early-season soybean with a relative maturity (RM) of 00.4 and was chosen because it is widely grown in northern Ontario (Secan, 2023). Seeds were treated with a fungicide (EverGol Energy; Bayer, Missouri, United States) and Rhizobium japonium inoculum (Cell-Tech peat for soybeans; Novozymes, Bagsværd, Denmark) based on manufacturer label instructions.

The Mt Zion site was planted on May 24, 2022, while the Plummer and New Liskeard sites were planted on May 25, 2022. At Plummer and Mt Zion, the soybeans were planted with a no-till drill with row spacing of 19.05 cm at a rate of 34 kg of seed per 0.4045 ha as is typical for farmers in the area. At New Liskeard the soybeans were planted with 35.56 cm row spacing with the same seed rate as the other locations (Figure 3). Thus, each experimental unit (plot) comprised 4 rows (New Liskeard), 7 rows (Plummer), and 9 rows (Mt Zion). Round-up was used as a herbicide for weed management as the soybeans were a Roundup Ready variety. New Liskeard was sprayed with Round-up early June, Plummer was sprayed mid-June and Mt Zion was sprayed mid-July of 2022. Soybean harvest occurred on October 5, 2022 at New Liskeard and October 11, 2022 at Mt Zion and Plummer.

3.5. Fertilization

Due to the observed negative impact of fertilizers on the AM fungal community (Johnson, 1993), the effect of reducing fertilizer levels on the benefits conferred by AM fungi to soybeans was investigated. One of the objectives of this study was to determine whether AM fungi, which can provide additional nutrients to plants they form mutualistic associations with (Aka-Kacar et al., 2010), would be more effective under reduced fertilizer conditions. This study involved treatments with 0, 50 and 100 % the recommended fertilizer levels according to the OMAFRA guidelines. New Liskeard was fertilized November 1, 2021, 11-52-0 (N-P-K) at 11.1 kg and 20.5 kg of P/ha, in the 50% and 100% treatments, respectively. No K was necessary. Mt. Zion and Plummer were fertilized May 31, 2022, with 11-52-0 and 0-0-62. At Mt. Zion the rates were 39.23 kg/ha of P and 79.58 kg/ha of K at the 100% and 19.06 kg/ha of P and 39.23 kg/ha of K for the 50% plots. While at Plummer 19.06 kg/ha of P and 39.23 kg/ha of K for the 100% plots and 9.98 kg/ha of P and 19.06 kg/ha of K for the 50% plots. No nitrogen application was needed since nitrogen fertilization is not typically required for soybeans as the majority of the nitrogen the soybeans require will come from nitrogen fixation by rhizobia bacteria (Bagg et al., 2017).

Table 3.2 Initial soil nutrient levels and nutrient additions required to reach the Ontario Ministry of Agriculture, Food and Rural Affairs recommended levels at three sites in northern Ontario, Canada. Standard error of the mean is presented in brackets (n=6).

Location	**Before Planting**		**Additions to Reach 100% Recommended Rate**	
	Average P (ppm)	Average K (ppm)	P Addition (kg/ha of P_2O_5)	K Addition (kg/ha of K_2O)
New Liskeard	15 (1.8)	234 (5.1)	20.5	0
Plummer	19 (2.1)	97 (8.4)	19.1	39.2
Mt Zion	9 (0.7)	54 (5.2)	39.2	79.6

Note: Table 3.2 only presents the fertilizer amounts in the 100% treatment, there were also 0% and 50% treatments in this study.

Figure 3.3 Planting of Bourke R2X soybeans in New Liskeard, Ontario, Canada in spring of 2022. Photograph by Melinda Drummond, 2022.

Table 3.3 Comparison of AM fungal inoculant spore counts between commercial and sorghum soil sources. Standard error of the mean is presented in brackets (n=6).

Inoculant	**Spores/g of Source Material**	**Rate of Application (**g/m^2)
Indigenous Inoculum from Sorghum Soil	139 (28.5)	555
Commercial Inoculum	142*	0.57

*according to manufacturer's label

3.6. Crop Sampling

Throughout the season the health of the plants at each site was noted, if there were any issues the issues were scored and recorded (Appendix D.2). Soybean stand counts (plants/m^2) taken in every plot at the V2 growth stage by counting the number of emerged plants in 1 m length of two inner rows. Weed density in each plot was calculated (weed plants/m^2) at the V2 growth stage by counting the number of weed plants in two 0.25 m^2 quadrats placed randomly in the plot. Due to heavy weed pressure At Mt. Zion, a second weed density measurement was performed using the same procedure (Appendix D.3).

Soybean biomass sampling occurred at the V2 and R2 growth stages, occurring approximately at the end of June and mid-July respectively. Exact sampling dates varied across locations (Appendix E.1). At each sampling date, all aboveground biomass in an area of 1.5 m^2 (at New Liskeard and Plummer) or 1.8 m^2 (at Mt. Zion) was harvested. In-season sampling areas were separated by at least 2 m to avoid border effects. Biomass was immediately placed in a dryer after harvest (45˚C in New Liskeard and 60˚C at Mt. Zion and Plummer) until constant moisture achieved. After drying, the weight of each sample was

recorded. At the same time as aboveground biomass sampling, roots of five soybean plants were excavated. Roots were then sampled for AM fungal root colonization (details below) and frozen for future molecular work.

At the V2 and R2 stages of soybean growth, Normalized difference vegetation index (NDVI) was measured using a handheld GreenSeeker (Trimble, Colorado, United States). This was used as a metric to aid in assessing crop health. Additionally at the same time, fractional green canopy cover was measured with the Canopeo mobile application (Canopeo, Oklahoma, United States). This allowed for a measure of the land cover of the soybeans.

At the R8 growth stage (physiological maturity), apparent harvest index was calculated at all sites by hand harvesting 1 m^2 area. Apparent harvest index was calculated as (grain mass) / (total plant tissue mass). The harvested biomass was dried (45˚C) until constant moisture was achieved and then threshed to separate the grain for remaining soybean biomass. The beans were weighed to obtain seed weight. Additionally, at this time, the roots from three plants per treatment plot were combined and frozen for later molecular work.

Grain yield and grain quality was determined by harvesting the 6 m length of each plot reserved for final harvest. Soybean harvest occurred on October 5, 2022 at New Liskeard and October 11, 2022 at Mt. Zion and Plummer. A combine was used at New Liskeard (SPC40; ALMACO, Nevada, USA) while soybeans were hand harvested at the other sites and later threshed using a combine (SPC40; ALMACO, Nevada, USA). The area harvested was recorded for all plots at all sites. After the grain was weighed, a subsample of grain (1 L)

was used to determine grain moisture content, grain protein content and grain oil content using a near-infrared analyzer (FOSS Infratec 1241; FOSS, Hillerød, Denmark). Grain yield was adjusted to 13.5% for reporting. At Mt Zion, due to very low yields, an insufficient amount of seed was available to confidently use the near-infrared analyzer. Thus, grain yield from Mt Zion was adjusted to 13.5% moisture based on reported grain moisture at harvest from the Plummer site.

3.7. AM fungal Spore Extraction

Spores were extracted from 50 g of soil according to Daniel and Skipper 1982 method, using a 50% sugar solution. Spores were counted using the INVAM (2023) method for spore enumeration with the following alterations. Instead of using a grid to count spores, spores present in the ocular field were tallied and averaged across 15 observations. From there, calculations were performed to obtain an estimate of the spores per unit area, expressed as spores per square meter.

3.8. Root Colonization

AM fungal root colonization was determined using the method by Vierheilig et al. (1998). First, the tissue cassettes (Epredia – Thermo Fisher Scientific, Massachusetts, United States) that held the roots were placed in a 10% KOH solution for 30 minutes at 80˚C. The roots were subsequently rinsed under deionized water (DI) three times and then placed in vinegar for 15 minutes. Then the roots were placed in an 80˚C ink solution for 15 minutes and rinsed 3 times in DI water. The roots were stored in de-staining solution (1:1:1 glycerol : DI water : vinegar) for long-term storage and were not mounted for at least 24 hours. The

roots were mounted on slides and examined for colonization using the method from (McGonigle et al., 1990). Percent root colonization was obtained by dividing the total number of intersections by the number of intersections with an AM fungal structure (i.e., hyphae, arbuscule, or vesicle) present and multiplying by 100 adapted from Hart and Reader (2002).

3.9. Statistical Analysis

R Studio (2022.07.2+554) was used to analyze the data in this thesis. Data was first visualized via boxplots to gain insight to the distribution of the data. The effects of the treatments on the soybeans were analyzed with a linear mixed model (LMM) with fertilizer as the main plot and AM fungal treatment as the subplot factor. The 'lmerTest' library was used for the LMM in this thesis. A LMM was chosen due to needing to elucidate the interactions between fertilizer and AM fungi, as anticipated by the hypothesis. Additionally, due to the nested design of this thesis, with the AM fungal treatment nested within the fertilizer blocks as well as the need to account for both fixed and random effects a LMM was chosen to address these aspects. The site and replicates were considered random effects. The following formula was used in the LMM: response ~ fertilizer * AM fungal treatment + (1|rep) + (1|rep/site). This allowed for observance of the main effects of fertilizer and AM fungal treatment as well as their interaction. The response variables assessed herein were AM fungal root colonization at V2 and R2, soybean biomass at V2 and R2, apparent harvest index, grain yield, oil and protein content, and AM fungal spore counts. At New Liskeard, Mt Zion, and Plummer the sample size was n = 35, n = 35, n = 36, respectively. The assumptions

of homoscedasticity and normality of the residuals of the LMM were assessed using residual and Q-Q plots. The linearity assumption was assessed visually by plotting the model residuals vs. fitted and the assumption of independence was considered with the experimental and model design. The assumptions were found to be adequately met by the data and therefore the data did not need to be transformed. Tukey's honestly significant difference (HSD) correction was used with the "emmeans()" function to correct for multiple comparisons and minimize the chance of making a type I error, using the Kenward-Roger degrees-of-freedom method and a 95% confidence level. For all analysis herein significance level of $\alpha = 0.05$ was used. Due to lack of significance detected in the response variables observed, the mean of the metric assessed will be presented herein.

4.0 RESULTS

4.1. Site Conditions

In general, the New Liskeard site experienced slightly higher temperatures and a lower amount of precipitation when compared to the Plummer and Mt Zion sites (Figure 4.1, 4.2). A five-year average for the full months of the growing season indicates that at New Liskeard, Plummer and Mt Zion, the temperatures that occurred in 2022 were similar to the historic data. The 2022 season experienced a lower than the historical average amount of precipitation in September at Plummer and Mt Zion and in July, August, and September at New Liskeard (Table 4.1, 4.2).

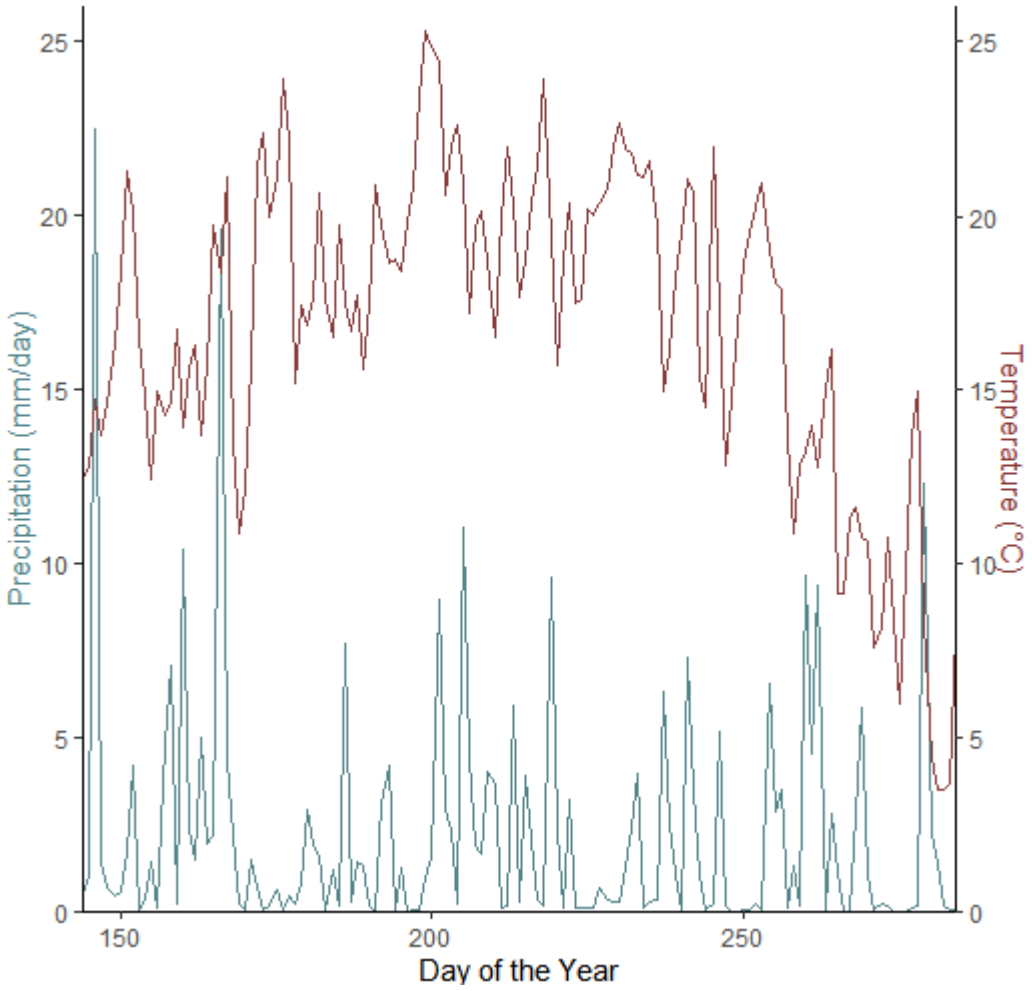

Figure 4.1 Daily average temperature and precipitation at New Liskeard, Ontario, Canada during the 2022 growing season. Data sourced from the NASA Langley Research Center (LaRC) POWER Project funded through the NASA Earth Science/Applied Science Program (NASA, 2023). The data was obtained from the POWER Project's Hourly 2.0.0 version on 2023/05/28.

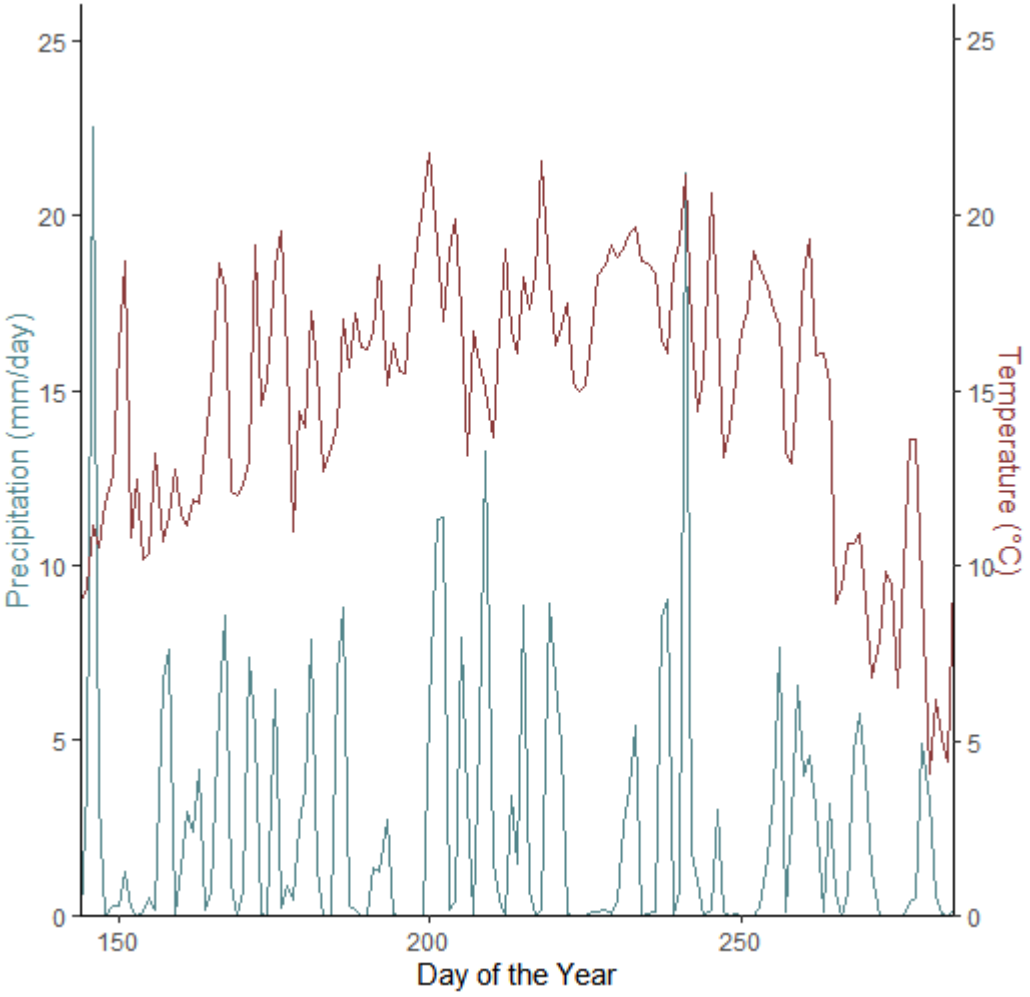

Figure 4.2 Daily average temperature and precipitation at the Plummer and Mt Zion sites in northern Ontario, Canada during the 2022 growing season. Due to the proximity of the sites (7.1 km apart), the temperature and precipitation data available for both locations were the same. Data sourced from the NASA Langley Research Center (LaRC) POWER Project funded through the NASA Earth Science/Applied Science Program (NASA, 2023). The data was obtained from the POWER Project's Hourly 2.0.0 version on 2023/05/28.

Table 4.1 Comparison of historical monthly precipitation (mm/day) and daily temperature (C˚) data at Plummer and Mt Zion sites located in Ontario, Canada with 5-year (2015-2020) averages and 2022 growing season data for average daily temperature and monthly precipitation. Standard deviation is presented in brackets.

	Jun	**Jul**	**Aug**	**Sep**
Historical Temperature	14.1 (3.2)	17.9 (2.4)	17.6 (2.5)	14.6 (3.8)
Historical Monthly Precipitation	77 (38)	72 (29)	80 (24)	83 (27)
2022 Temperature	13.7	16.7	17.7	14.3
2022 Monthly Precipitation	78.2	83.9	90.2	58.0

Data sourced from the NASA Langley Research Center (LaRC) POWER Project funded through the NASA Earth Science/Applied Science Program (NASA, 2023).

Table 4.2 Comparison of historical monthly precipitation (mm/day) and daily temperature (C˚) data from New Liskeard, Ontario, Canada with 5-year (2015-2020) averages and 2022 growing season data for average daily temperature and monthly precipitation. Standard deviation is presented in brackets.

	Jun	**Jul**	**Aug**	**Sep**
Historical Temperature	15.7 (3.8)	20.1 (3.1)	18.8 (3.0)	15 (4.4)
Historical Monthly Precipitation	78.9 (32.1)	80.2 (31.0)	109.4 (29.4)	75.7 (21.9)
2022 Temperature	17.0	19.8	19.7	14.3
2022 Monthly Precipitation	77.5	66.6	60.1	58.5

Data sourced from the NASA Langley Research Center (LaRC) POWER Project funded through the NASA Earth Science/Applied Science Program (NASA, 2023).

4.2. Metrics of AM Fungi

4.2.1.AM Fungal Root Colonization

At the V2 stage of soybean growth, root colonization was assessed at all sites (Figure 4.3) and the mean percent of root colonization was determined (Table 4.3). The mean percent colonization at all sites in all treatments ranged from 10.04 to 40.86 % at the V2 stage. The linear mixed effects model revealed a significant intercept term ($\beta = 29.47$, t-value = 6.86, p-value = 0.00013). This indicates that the mean value of the percent root colonization is 29.47% when all other predictors (fertilizer and AM fungal inoculant) in the model are equal to zero. There were no significant differences between the fertilizer treatments or among the AM fungal treatments (Appendix A.1). Overall, there was no apparent effect of the treatments on soybean root colonization at V2 in any of the three field locations.

Table 4.3 Mean of percent am fungal root colonization at the V2 growth stage of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions (none). Standard error of the mean is presented in brackets (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were found to be not statistically significant at $p>0.05$.

Percent of Fertilizer Added	**AM Fungi Treatment**	**New Liskeard**	**Plummer**	**Mt Zion**
0	indigenous	26.36 (7.25)	16.86 (7.81)	20.22 (7.35)
	commercial	23.20 (11.36)	14.22 (1.85)	35.69 (6.64)
	none	34.08 (5.63)	28.15 (6.80)	14.94 (8.07)
50	indigenous	22.56 (13.46)	26.09 (3.67)	26.99 (4.41)
	commercial	30.51 (9.89)	18.20 (3.19)	25.59 (10.38)
	none	31.58 (8.55)	21.47 (4.63)	40.86 (8.83)
100	indigenous	28.52 (9.89)	13.34 (1.65)	22.49 (8.17)
	commercial	25.34 (6.93)	10.04 (2.10)	17.83 (4.35)
	none	27.79 (4.48)	21.56 (4.81)	31.82 (8.80)

Note: Each treatment had 4 replicates

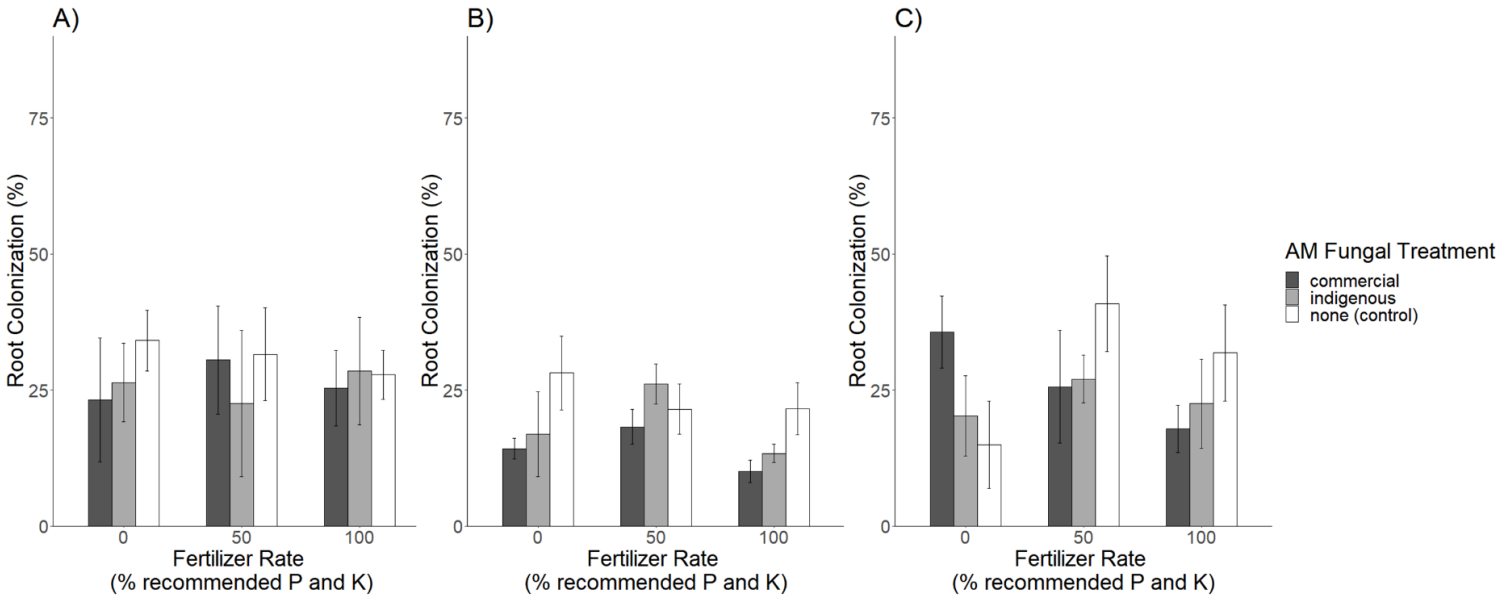

Figure 4.3 Percent AM fungal root colonization of soybeans at the V2 growth stage at (A) New Liskeard (A) (n = 35), Plummer (B) (n = 36), and Mt. Zion (C) (n = 35) located in northern Ontario, Canada in 2022. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no addition. Vertical bars represent standard error. There were no significant differences between the fertilizer treatments or among the AM fungal treatments. Statistical tests were conducted in R using a Linear Mixed Model.

At the R2 growth stage the soybeans were again assessed for root colonization of AM fungi and the mean of percent root colonization was determined (Table 4.4). Mean root colonization ranged from 31.86 to 79.55 % across all treatments and sites. Overall, there was an increase in percent root colonization from the V2 stage in all the treatments and at all the sites (Figure 4.4). The site with the highest colonization was New Liskeard. Again, there was a significant intercept term in the linear mixed model ($\beta = 63.62$, t-value = 6.51, p-value = 0.013) which illustrates that the mean value of the percent root colonization is 63.62% when the fertilizer and AM fungal inoculant variables in the model are equal to zero (see Appendix

A.2). Additionally, there were significant differences in the indigenous AM fungi addition (β = -10.54, t-value = 1.09, p-value = 0.028) and in the interaction between the fertilizer and indigenous AM fungal treatment (β = 0.17, t-value = 2.31, p-value = 0.023). The "emmeans()" function was used in R to compute estimated marginal means and pairwise comparisons to examine pairwise differences between the fertilizer treatment groups and the AM fungal treatment groups. It was found that none of the pairwise comparisons between the groups were statistically significant after adjusting for multiple comparisons using the Tukey method (all adjusted p-values > 0.05) (see Appendix B.1). Therefore, there was no significant effect of the treatments on root colonization at R2.

Table 4.4 Mean of Percent Root Colonization at the R2 growth stage of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions (none). Standard error of the mean is presented in brackets (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were found to be not statistically significant at $p>0.05$.

Percent of Fertilizer Added	**AM Fungi Treatment**	**New Liskeard**	**Plummer**	**Mt Zion**
0	indigenous	74.72 (6.30)	38.69 (4.50)	38.10 (4.22)
	commercial	71.58 (5.29)	44.26 (6.92)	43.10 (3.16)
	none	74.50 (5.47)	56.22 (2.12)	43.40 (14.98)
50	indigenous	64.16 (21.60)	43.24 (3.34)	64.83 (6.32)
	commercial	79.55 (3.04)	51.54 (8.67)	49.34 (7.55)
	none	78.09 (1.17)	41.27 (7.71)	67.11 (6.19)
100	indigenous	78.49 (5.11)	42.17 (6.63)	64.22 (6.19)
	commercial	67.26 (8.89)	44.40 (10.87)	56.15 (4.62)
	none	72.72 (8.18)	31.86 (5.47)	65.54 (2.58)

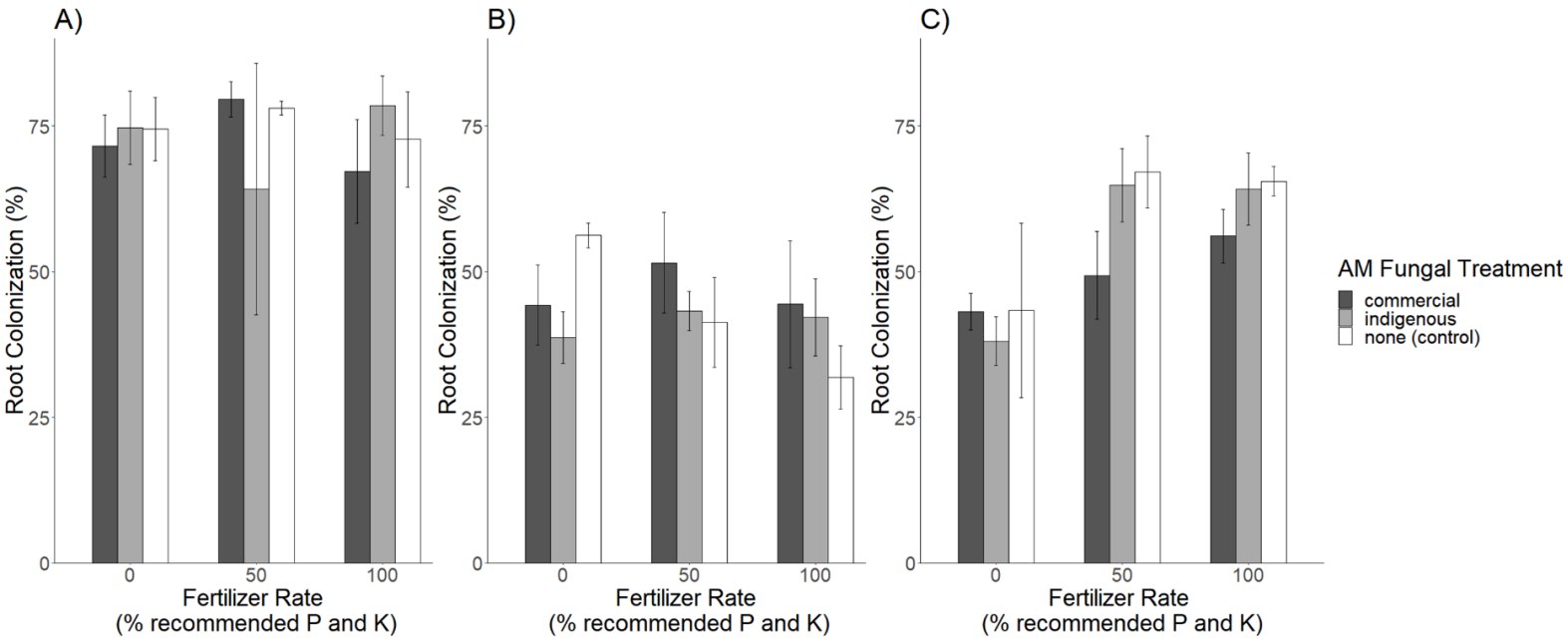

Figure 4.4 Percent AM fungal root colonization of soybeans at R2 growth stage at New Liskeard (A) (n = 35), Plummer (B) (n = 36), and Mt. Zion (C) (n = 35) located in northern Ontario, Canada in 2022. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no addition. Vertical bars represent standard error. Statistical tests were conducted in R using a Linear Mixed Model and Tukey's honestly significant difference correction was used to correct for multiple comparisons. There were no significant differences between the fertilizer treatments or within the AM fungal treatments.

4.2.2.Spore Counts

In the spring of 2022, spore counts were obtained at Plummer and Mt Zion (Table 4.5). The amount of spores present in the soil was 14.2 and 18.9 million per square meter at Plummer and Mt Zion, respectively. New Liskeard was not assessed due to a sample not being available at the time. The plots in the study received an application of commercial and indigenous inoculants, with spore concentrations of 80.9 and 77100 per square meter, respectively (Table 4.5).

Table 4.5 Comparing initial AM fungal spore abundance and number of spores added via inoculant at Mt Zion and Plummer (n=6) in northern Ontario, Canada in the spring of 2022.

Location	**Initial Spores in Soil per m^2**	**Spores Added per m^2**		**Total Spores per m^2 After Planting**	
		Commercial Inoculant	Indigenous Inoculant	Commercial Treatment Plots	Indigenous Treatment Plots
Mt Zion	18.9 million	0.0000809 million	0.0771 million	18.9000809 million	18.9771 million
Plummer	14.2 million	0.0000809 million	0.0771 million	14.2000809 million	14.2771 million

Subsequent spore counts were completed on soil that was sampled at soybean maturity. Spore counts ranged from 14.63 to 56.27 million spores/m^2 with the highest spore counts observed at New Liskeard (Table 4.6, Figure 4.5). After conducting a LMM, the intercept term was found to be significant ($\beta = 31.11$, t-value = 3.53, p-value = 0.063). That is, with fertilizer and AM fungal inoculation at zero, the mean spore count is expected to be 31.11 million spores/m^2. There was no significance observed between the fertilizer treatments or within the AM Fungi treatments (Appendix A.9)**.**

Table 4.6 Mean spore counts in millions of spores per m^2 across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions (none). Standard error of the mean is presented in brackets (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were not statistically significant at $p>0.05$.

Percent of Fertilizer Added	**AM Fungi Treatment**	**New Liskeard**	**Plummer**	**Mt Zion**
0	indigenous	50.60 (5.14)	19.53 (2.83)	30.95 (1.74)
	commercial	43.40 (8.73)	16.60 (2.67)	25.36 (2.81)
	none	47.02 (4.50)	14.63 (2.30)	27.05 (2.24)
50	indigenous	53.73 (3.40)	22.42 (1.40)	29.47*
	commercial	43.20 (1.30)	17.11 (2.51)	29.04 (9.64)
	none	47.22 (8.54)	16.24 (2.09)	26.67 (2.68)
100	indigenous	43.67 (5.56)	18.89 (1.66)	23.87 (5.93)
	commercial	56.27 (3.61)	20.52 (3.15)	27.96 (2.12)
	none	46.16 (5.65)	20.67 (1.43)	23.18 (2.72)

*Only one sample was available from this treatment so standard error was not able to be calculated

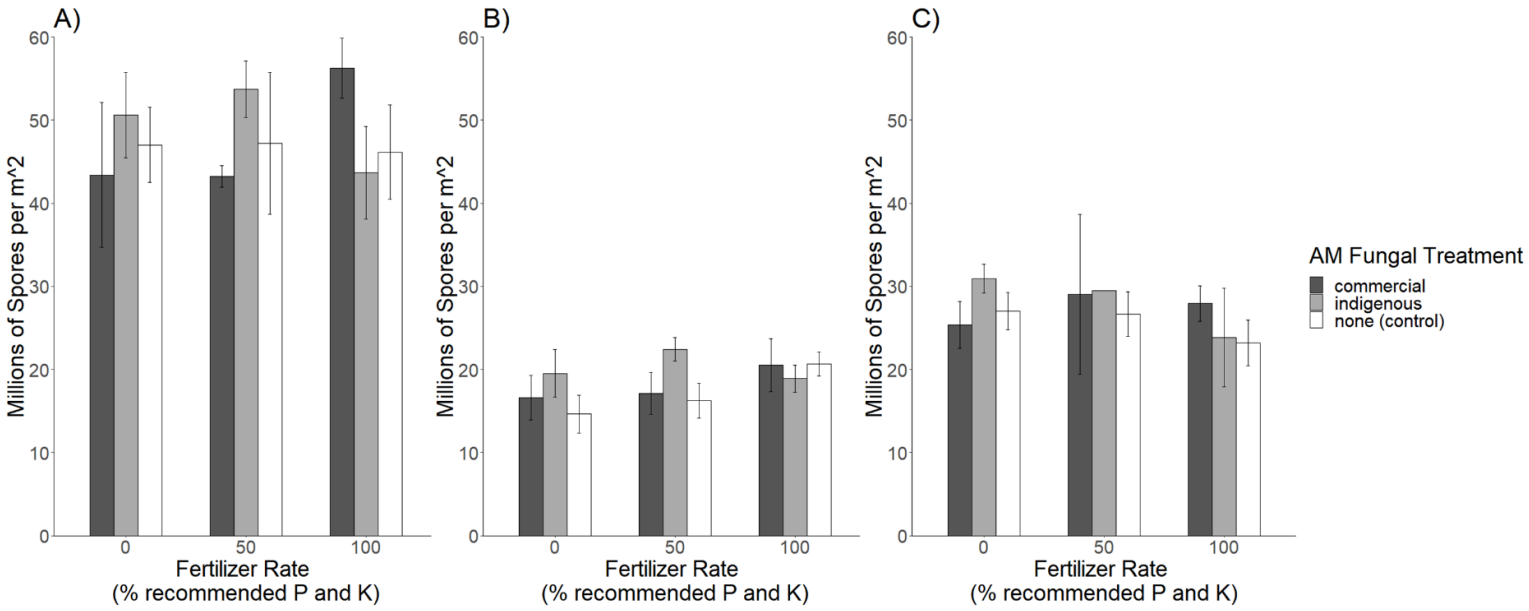

Figure 4.5 End of season spore counts in millions of spores per m^2 at New Liskeard (A) (n = 35), Plummer (B) (n = 36), and Mt. Zion (C) (n = 35) located in northern Ontario, Canada in 2022. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no addition. Vertical bars represent standard error. There were no significant differences between the fertilizer treatments or within the AM fungal treatments. Statistical tests were conducted in R using a Linear Mixed Model.

4.3. Soybean Crop Metrics

4.3.1. Soybean Biomass

The soybeans were evaluated for dry biomass at the V2 growth stage. Mt Zion had the lowest biomass at this stage (Figure 4.6). There was high weed pressure present at Mt Zion (Appendix D.1). At all sites, in all treatments the biomass ranged from 6.01 to 18.60 g/m^2 (Table 4.7). A LMM was conducted, and it was found that the intercept term was significant ($\beta = 11.789$, t-value = 5.300, p-value = 0.019). Additionally, the interaction between the fertilizer and commercial AM fungal inoculant was significant ($\beta = 0.037$, t-value = 2.058, p-value = 0.042) (Appendix A.4). Accordingly, pairwise comparisons were

used to examine pairwise differences between the fertilizer treatment groups and the AM fungal treatment groups. It was found that none of the pairwise comparisons between the groups were statistically significant after adjusting for multiple comparisons using the Tukey method (all adjusted p-values > 0.05) (Appendix B.2). Therefore, there was no significant effect of the treatments on biomass at V2.

Table 4.7 Mean biomass (g/m^2) at the V2 growth stage of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions (none). Standard error of the mean is presented in brackets (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were not statistically significant at p>0.05.

Percent of Fertilizer Added	**AM Fungi Treatment**	**New Liskeard**	**Plummer**	**Mt Zion**
0	indigenous	14.56 (1.06)	11.52 (0.60)	7.72 (1.48)
	commercial	14.38 (0.82)	14.88 (1.91)	6.38 (0.33)
	none	14.23 (0.91)	12.47 (1.91)	6.57 (2.58)
50	indigenous	18.60 (5.75)	11.57 (1.36)	7.68 (1.29)
	commercial	14.20 (0.63)	12.42 (1.73)	7.83 (0.38)
	none	13.44 (1.33)	12.95 (1.49)	8.11 (1.30)
100	indigenous	16.34 (0.95)	13.17 (1.01)	7.79 (0.68)
	commercial	15.13 (1.02)	17.77 (3.72)	11.75 (1.21)
	none	15.13 (2.15)	10.25 (1.11)	6.01 (0.64)

Note: Each treatment had 4 replicates

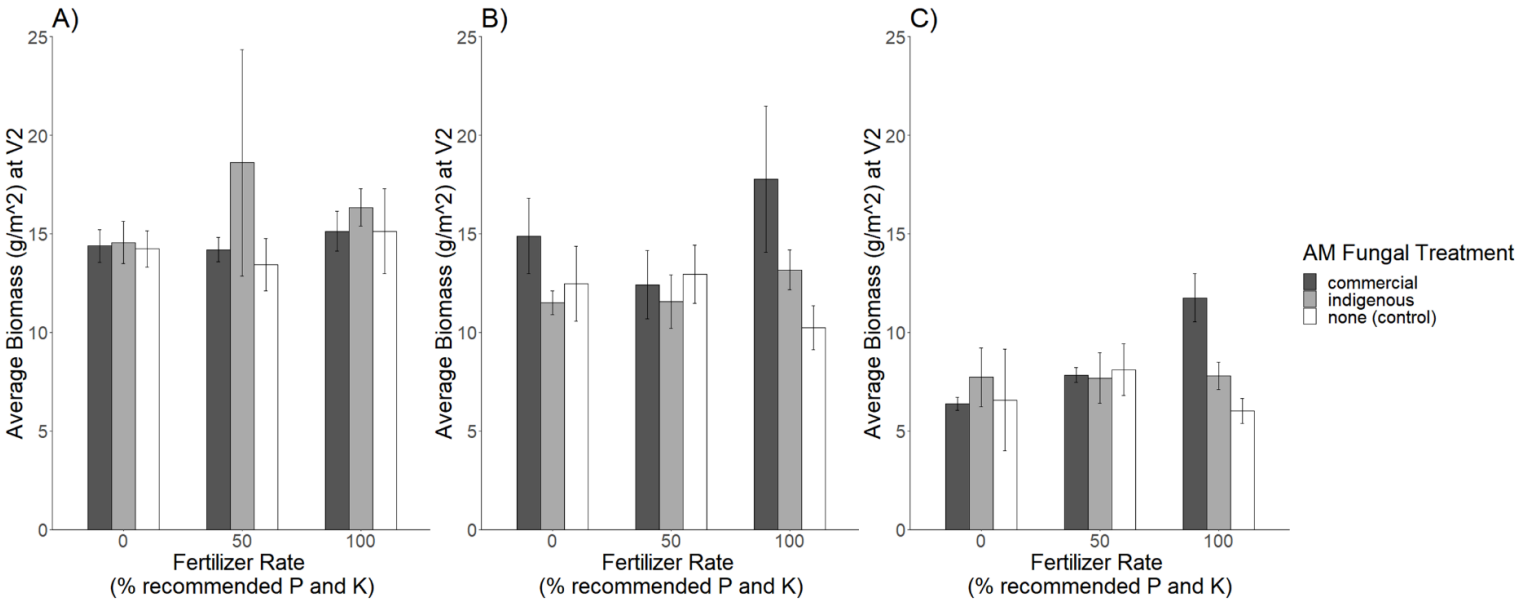

Figure 4.6 Dry aboveground soybean biomass in grams per m^2 obtained at the V2 growth stage at New Liskeard (A) (n = 35), Plummer (B) (n = 36), and Mt. Zion (C) (n = 35) located in northern Ontario, Canada in 2022. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no addition. Vertical bars represent standard error. There were no significant differences between the fertilizer treatments or within the AM fungal treatments. Statistical tests were conducted in R using a Linear Mixed Model.

At the R2 growth stage the soybeans were assessed for dry biomass. At all sites the biomass increased from the V2 assessment with the highest biomass being observed at New Liskeard (Figures 4.6 & 4.7). The biomass ranged over all treatments, at all sites from 15.63 to 142.92 g/m^2 (Table 4.8). The Mt Zion site was experiencing both weed pressure and herbivory at this time which likely affected the amount of biomass present. There was no effect of the treatments on biomass at R2 (all p-values > 0.05) (Appendix A.4).

Table 4.8 Mean Biomass (g/m^2) at the R2 growth stage of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions (none). Standard error of the mean is presented in brackets (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were not statistically significant at $p>0.05$.

Percent of Fertilizer Added	**AM Fungi Treatment**	**New Liskeard**	**Plummer**	**Mt Zion**
0	indigenous	128.90 (7.28)	47.32 (6.90)	20.22 (7.35)
	commercial	134.42 (12.61)	61.80 (11.07)	35.69 (6.64)
	none	142.92 (8.73)	54.97 (8.00)	14.94 (8.07)
50	indigenous	131.58 (11.72)	48.93 (4.85)	26.99 (4.41)
	commercial	127.67 (2.65)	59.72 (6.84)	25.59 (10.38)
	none	130.12 (5.35)	44.72 (5.48)	40.86 (8.83)
100	indigenous	138.77 (13.93)	52.98 (8.43)	22.49 (8.17)
	commercial	128.15 (15.19)	39.90 (3.64)	17.83 (4.35)
	none	147.28 (11.55)	43.98 (6.84)	31.82 (8.80)

Note: Each treatment had 4 replicates

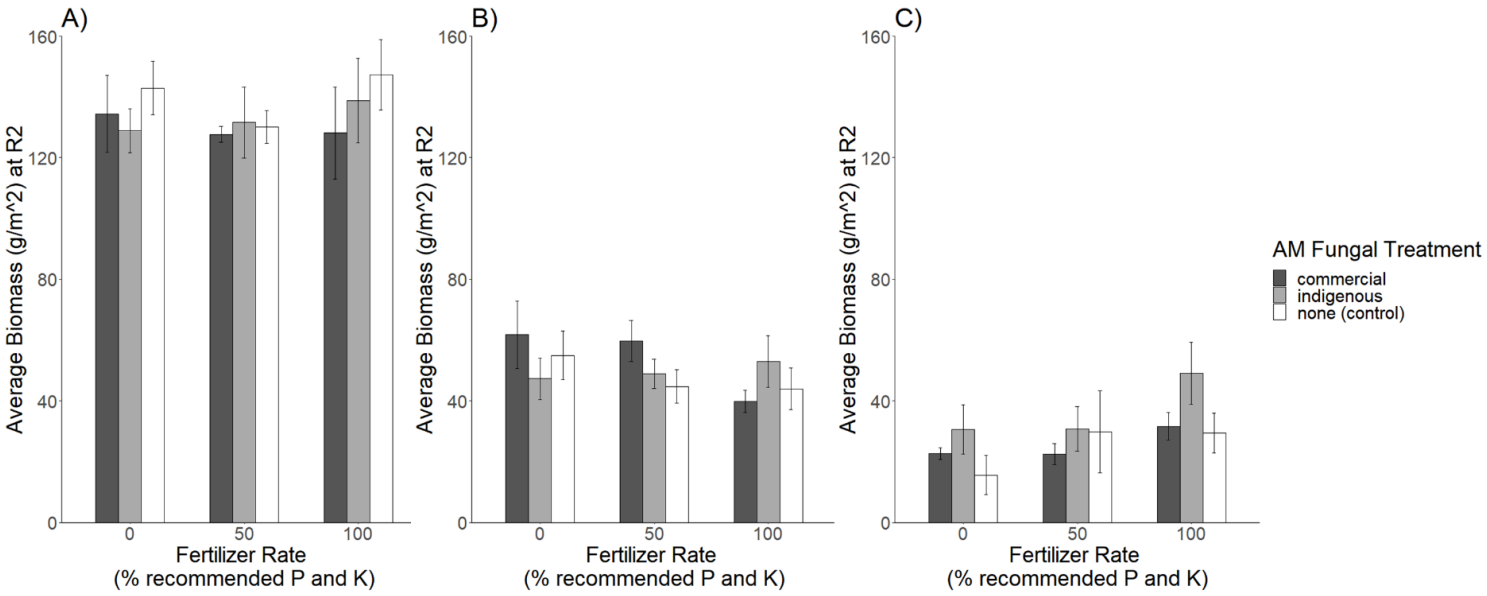

Figure 4.7 Dry aboveground soybean biomass in grams per m^2 obtained at the R2 growth stage at New Liskeard (A) (n = 35), Plummer (B) (n = 36), and Mt. Zion (C) (n = 35) located in northern Ontario, Canada in 2022. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no addition. Vertical bars represent standard error. There were no significant differences between the fertilizer treatments or within the AM fungal treatments. Statistical tests were conducted in R using a Linear Mixed Model.

4.3.2. Apparent Harvest Index

Apparent harvest index was assessed after the soybeans reached maturity. The apparent harvest index ranged from 0.37 to 0.50 (Table 4.9). The highest variability was seen at Mt Zion (Figure 4.8), again this site experienced high herbivory and weed pressure. The intercept term of the linear mixed model was significant ($\beta = 0.47$, t-value = 22.74, p-value = 0.000011) (Appendix A.5). This indicates that the mean value of apparent harvest index is 0.47 when all the fertilizer and AM fungal inoculant variables in the model are equal to zero. There were no significant differences between the fertilizer treatments or within the AM Fungi treatments (Appendix A.5).

Table 4.9 Mean Apparent Harvest Index of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions (none). Standard error of the mean is presented in brackets (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were not statistically significant at $p>0.05$.

Percent of Fertilizer Added	**AM Fungi Treatment**	**New Liskeard**	**Plummer**	**Mt Zion**
0	indigenous	0.47 (0.00)	0.47 (0.02)	0.37 (0.06)
	commercial	0.48 (0.01)	0.50 (0.01)	0.42 (0.04)
	none	0.48 (0.00)	0.49 (0.01)	0.49 (0.04)
50	indigenous	0.48 (0.01)	0.50 (0.01)	0.41 (0.03)
	commercial	0.48 (0.01)	0.49 (0.02)	0.38 (0.03)
	none	0.48 (0.01)	0.49 (0.02)	0.41 (0.04)
100	indigenous	0.48 (0.01)	0.49 (0.01)	0.48 (0.02)
	commercial	0.46 (0.01)	0.50 (0.01)	0.48 (0.02)
	none	0.49 (0.01)	0.49 (0.01)	0.47 (0.05)

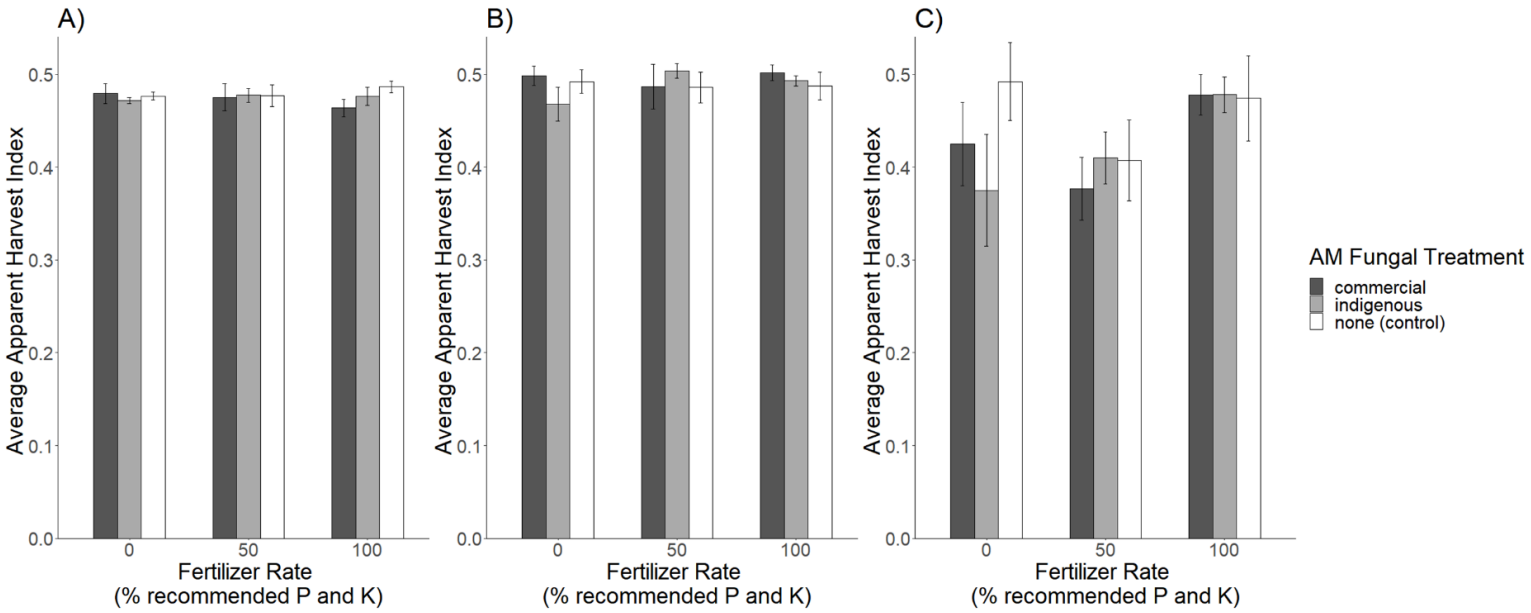

Figure 4.8 Apparent harvest index of soybeans at New Liskeard (A) (n = 35), Plummer (B) (n = 36), and Mt. Zion (C) (n = 35) located in northern Ontario, Canada in 2022. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no addition. Vertical bars represent standard error. There were no significant differences between the fertilizer treatments or within the AM fungal treatments. Statistical tests were conducted in R using a Linear Mixed Model. Note that Mt. Zion was affected by weeds and herbivory.

4.3.3. Crop Grain Yield

The soybean yield was obtained at harvest. Across all sites and treatments, grain yield ranged from 73.0 to 3406.7 kg/ha (Table 4.10). Mt Zion had much lower yields than the other sites (Figure 4.9), again, this site was observed to have high weed pressure and experienced severe herbivory (Appendix D.1). There were no significant results observed in the LMM (Appendix A.6). Overall, there was no observable effect of the treatments on yield.

Table 4.10 Mean yield of soybeans at 13.5% (kg/ha) across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions (none). Standard error of the mean is presented in brackets (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were not statistically significant at $p>0.05$.

Percent of Fertilizer Added	**AM Fungi Treatment**	**New Liskeard**	**Plummer**	**Mt Zion**
0	indigenous	3406.7 (156.2)	2458.1 (66.5)	73.0 (22.9)
	commercial	3217.8 (142.3)	2182.8 (240.7)	104.3 (31.7)
	none	3160.0 (82.9)	1351.2 (463.7)	124.2 (16.5)
50	indigenous	3404.5 (139.7)	1947.5 (408.7)	181.9 (73.4)
	commercial	3099.4 (109.0)	2054.8 (194.0)	102.6 (16.8)
	none	3113.4 (74.7)	2360.1 (165.6)	139.1 (39.6)
100	indigenous	3173.7 (73.8)	2362.3 (222.5)	112.0 (31.2)
	commercial	2979.8 (202.6)	2140.1 (32.2)	117.2 (23.0)
	none	3248.4 (188.0)	2111.1 (62.7)	100.2 (38.5)

Note: Each treatment had 4 replicates

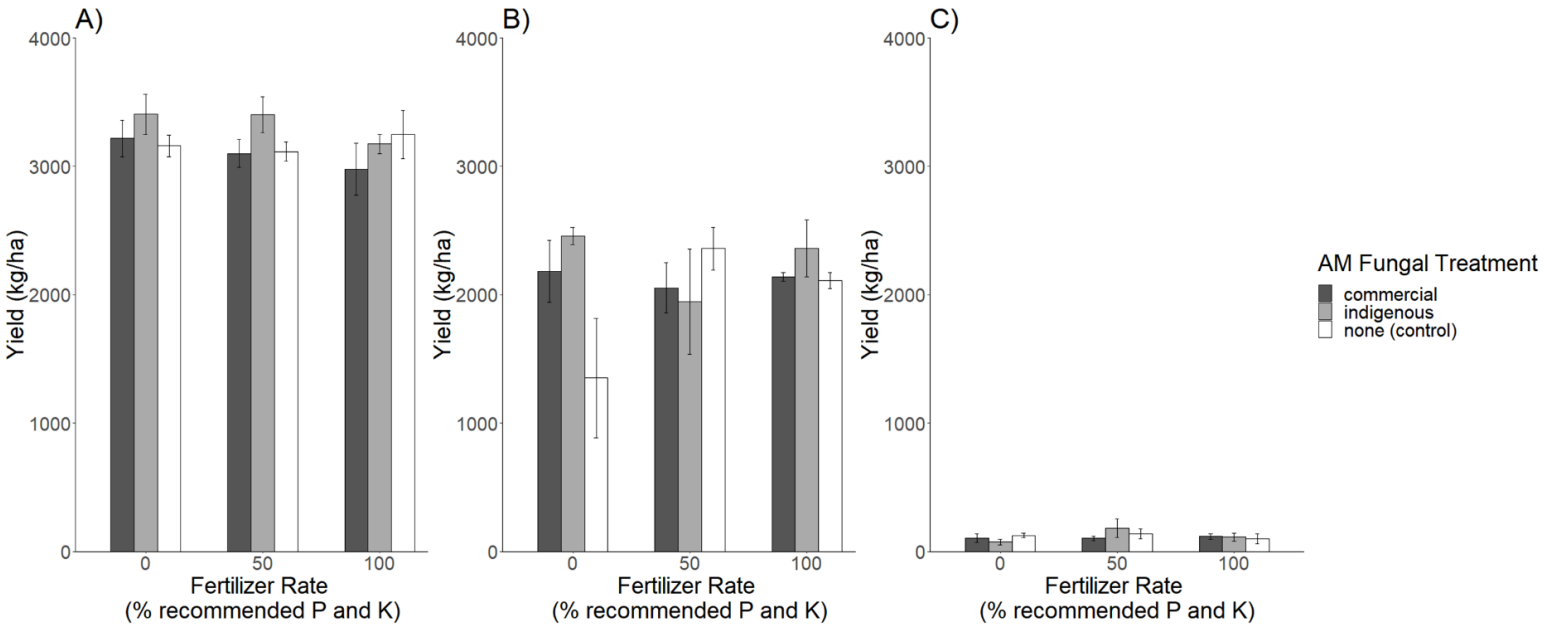

Figure 4.9 Yield of soybeans at 13.5% moisture kg/ha adjusted for plot size at New Liskeard (A) (n = 35), Plummer (B) (n = 36), and Mt. Zion (C) (n = 35) located in northern Ontario, Canada in 2022. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no addition. Vertical bars represent standard error. There were no significant differences between the fertilizer treatments or within the AM fungal treatments. Statistical tests were conducted in R using a Linear Mixed Model. The yield at Mt Zion was affected by herbivory and weed pressure.

4.3.4. Oil Content

The oil content of the soybeans was analyzed at harvest. Results were not obtained at Mt Zion as the analysis could not be conducted due to the insufficient quantity of sample. There was very low variation between the treatments and sites (Figure 4.10). The oil content ranged from 18.58 to 19.08% across both sites and all treatments (Table 4.11). After conducting a LMM, the intercept term was found to be significant ($\beta = 18.89$, t-value = 110.08, p-value = 0.000023) (Appendix A.7). This signifies that the mean value for oil content would be 18.89 % with the other factors (fertilizer and AM fungal inoculant) equal to

zero. There was no significance observed between the fertilizer treatments or within the AM Fungi treatments (Appendix A.7)**.**

Table 4.11 Mean oil content (%) of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions (none). Standard error of the mean is presented in brackets (At Plummer n=36 and at New Liskeard). All treatments were not statistically significant at $p>0.05$.

Percent of Fertilizer Added	**AM Fungi Treatment**	**New Liskeard**	**Plummer**
0	indigenous	19.00 (0.07)	18.60 (0.30)
	commercial	18.88 (0.16)	18.68 (0.18)
	none	18.98 (0.09)	18.77 (0.23)
50	indigenous	18.93 (0.12)	18.58 (0.23)
	commercial	18.95 (0.06)	18.60 (0.25)
	none	19.03 (0.06)	18.78 (0.11)
100	indigenous	18.93 (0.11)	18.80 (0.25)
	commercial	19.08 (0.08)	18.65 (0.26)
	none	18.98 (0.10)	18.78 (0.23)

Note: Each treatment had 4 replicates

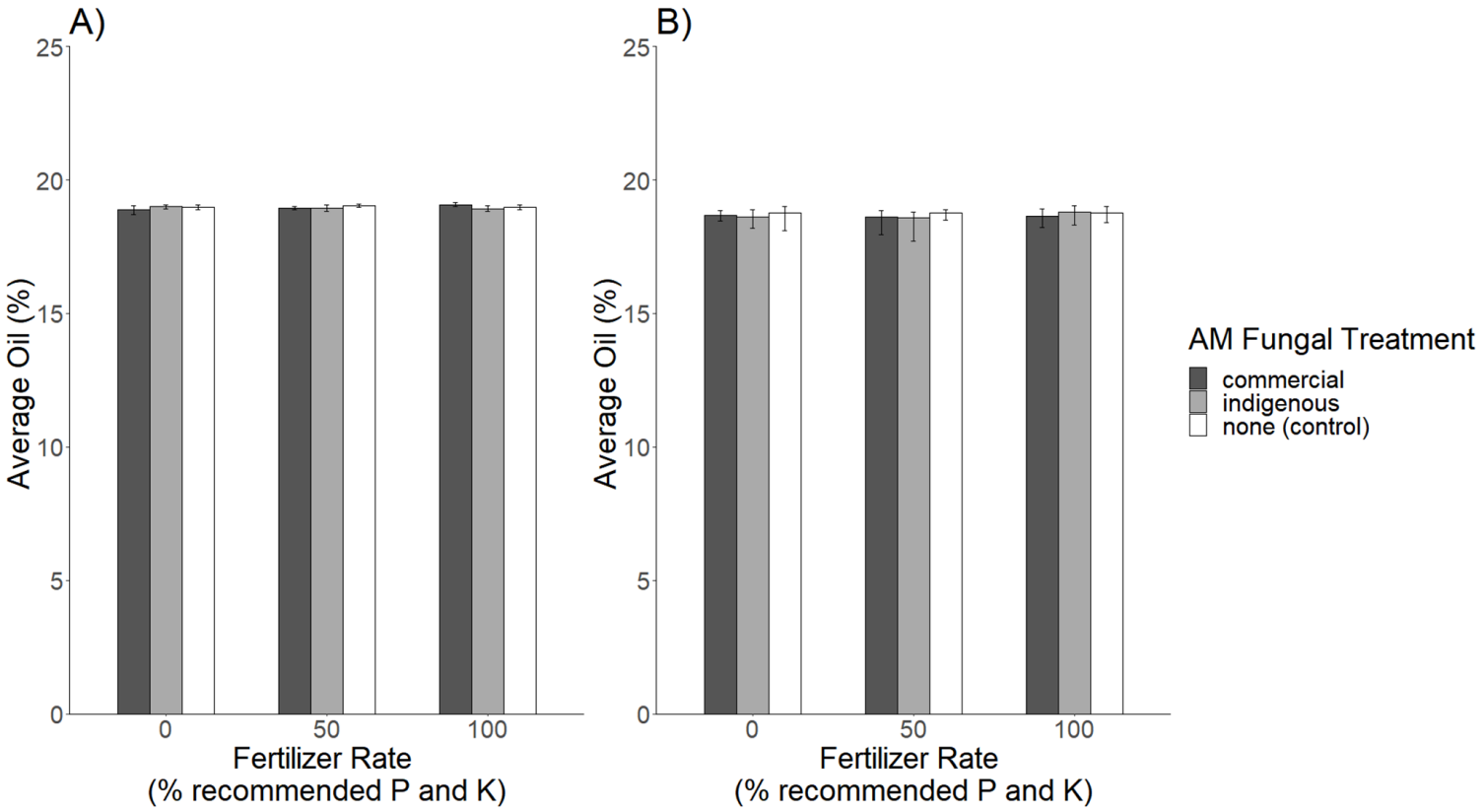

Figure 4.10 Percent oil content in soybeans at New Liskeard (A) (n = 35), Plummer (B) (n = 36), and Mt. Zion (C) (n = 35) located in northern Ontario, Canada in 2022. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no addition. Vertical bars represent standard error. There were no significant differences between the fertilizer treatments or within the AM fungal treatments. Statistical tests were conducted in R using a Linear Mixed Model. The Mt Zion site was omitted due to insufficient quantity of sample for analysis.

4.3.5.Protein Content

Protein content of the soybeans was assessed at harvest. Again, the Mt Zion site is omitted from these results due to insufficient quantity of sample. The protein content ranged from 30.20 to 32.45 % across all treatments at both sites (Table 4.12). There was low variability between treatments and sites, with slightly lower protein content being observed at

New Liskeard (Figure 4.11). After performing a LMM, the intercept term was found to be significant ($\beta = 30.96$, t-value = 42.26, p-value = 0.0064) (Appendix A.8). This suggests that if all other variables (fertilizer and AM fungal inoculant) were set at zero the mean protein content would be 30.96%. All other factors and interactions were observed to not be significant (Appendix A.8).

Table 4.12 Mean protein content of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions (none). Standard error of the mean is presented in brackets (At Plummer n=36 and at New Liskeard n=35) All treatments were not statistically significant at $p>0.05$.

Percent of Fertilizer Added	**AM Fungi Treatment**	**New Liskeard**	**Plummer**
0	indigenous	30.48 (0.21)	31.78 (0.40)
	commercial	30.45 (0.24)	32.45 (0.22)
	none	30.28 (0.13)	31.93 (0.66)
50	indigenous	30.30 (0.26)	32.23 (0.86)
	commercial	30.20 (0.21)	31.78 (0.64)
	none	30.35 (0.32)	31.05 (0.30)
100	indigenous	30.48 (0.17)	31.53 (0.47)
	commercial	30.30 (0.26)	31.88 (0.43)
	none	30.48 (0.21)	31.33 (0.36)

Note: Each treatment had 4 replicates

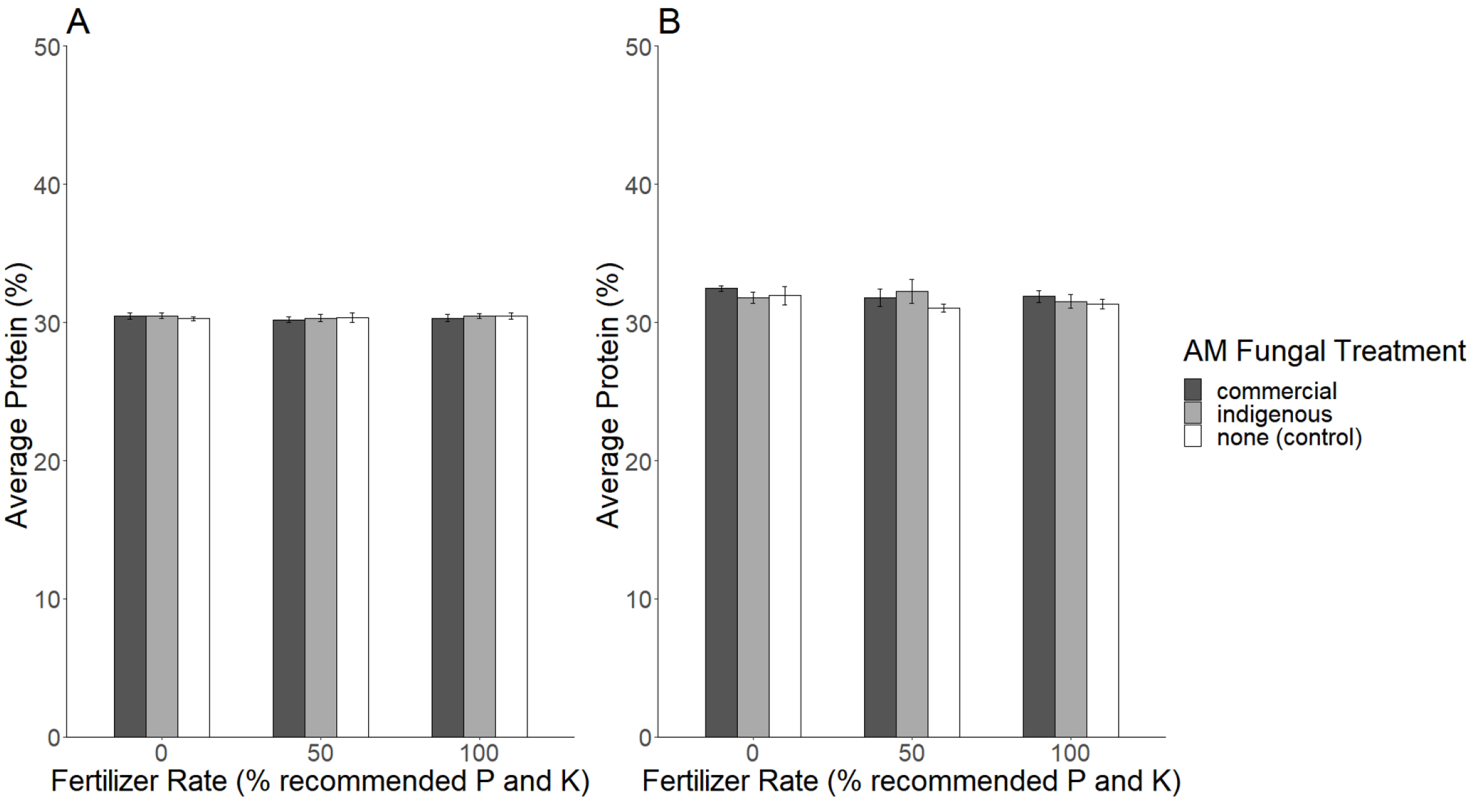

Figure 4.11 Percent protein content in soybeans at New Liskeard (A) (n = 35), Plummer (B) (n = 36), and Mt. Zion (C) (n = 35) located in northern Ontario, Canada in 2022. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no addition. Vertical bars represent standard error. There were no significant differences between the fertilizer treatments or within the AM fungal treatments. Statistical tests were conducted in R using a Linear Mixed Model. The Mt Zion site was omitted due to insufficient amount of sample for the analysis.

4.3.6. Health Status of the Soybeans

At V2, New Liskeard was observed to have the lowest number of weeds while Plummer had the highest number of weeds (Appendix D.1). As each site was sprayed at different points in the season, the weeds at each location grew and died back at different rates. Throughout the season it was observed that there was a very low presence of weeds at New Liskeard. At Plummer there was noted to be a reduction in weed presence after V2 with

a low weed presence for the remainder of the season. However, after V2, Mt Zion continued to experience moderate to severe weed pressure for the entirety of the growing season (Appendix D.2). At Mt Zion the stand counts were typical, however the plants were observed to have variable development (i.e., plants were at multiple growth stages) due to the weed pressure (Appendix D.3). Additionally, Mt Zion also experienced herbivory which impacted the endpoints that were assessed herein.

5.0 DISCUSSION

5.1. Overview

The objective of this research was to determine whether using a novel approach for the augmentation of indigenous AM fungal communities naturally present in the soil could provide equal or greater benefits to crops than inoculating with a commercial AM fungal inoculum in northern Ontario. This research holds particular significance in crop rotations with a non-mycorrhizal crop which is thought to reduce the AM fungal community and the benefits conferred to subsequent crops. In addition, this thesis aimed to explore the potential to use AM fungi in place of or with a reduction in fertilizer use to transition the agricultural industry towards sustainability. To investigate the impact of fertilization on AM fungal communities, treatments with 0, 50, and 100% of the recommended rate of fertilizer were implemented according to the OMAFRA guidelines, simultaneously at three field sites in northern Ontario to better ascertain the reproducibility of the results to draw more robust conclusions. It was hypothesized that a reduction in fertilizer would allow the AM fungal communities to be more effective mutualists, ultimately promoting crop growth and yield. However, as will be discussed herein, no evidence was found to support this hypothesis as there was no difference among treatments based on the agronomic metrics that were observed, and the hypothesis was rejected.

Analysis encompassing several indicators were carried out, including for AM fungi, root colonization and spore abundance in the soil, and, for soybeans, biomass, apparent harvest index, yield and protein and oil content. These response variables are consistent with

those measured in studies evaluating crop responses to AM fungi and they were also meant to inform northern Ontario farmers about the potential economic benefits of on-farm augmentation of AM fungi. It was hypothesized that the treatments receiving 100% fertilizer would have greater crop productivity indicators. Additionally, it was hypothesized that there would be an interactive effect between fertilizer treatments and AM fungal inoculant treatments resulting in a higher root colonization and spore abundance, and the maintenance of crop productivity indicators as fertilizer application declined (Johnson, 1993). As will be discussed in the following sections, these hypotheses were generally not supported by the results and were rejected.

Subsequent sections of this thesis draw on studies sourced from across the globe. The distinctive context of northern Ontario and the potential contrasts in factors such as soil composition, climate conditions, and agricultural practices in this thesis can affect the local AM fungal community and the AM fungi-soybean relationship in ways different from those observed in other parts of the world (Pedroso et al., 2022; Soudzilovskaia et al., 2015). Nonetheless, the selection of AM fungi studies from various global contexts represents the most suitable avenue for comparison, enabling a robust evaluation of this thesis' findings. Situating this thesis within the global context of AM fungal studies allows for additional insights while acknowledging the unique conditions specific to northern Ontario. However, it is crucial to recognize that the results of each study should be considered in their local context.

5.2. Metrics of AM Fungi

It was hypothesized that there would be higher AM fungal indicators as the fertilizer application rate declined. Overall, there was no treatment effect on AM fungal root colonization nor spore counts, and the hypothesis was rejected. AM fungal root colonization was measured to determine the presence and degree of the symbiotic relationship between the fungi and soybeans. This allowed for observation of any differences in the amount of colonization in the different fertilizer and AM fungal inoculant treatments. Again, there was no response of the treatments on AM fungal root colonization. However, as expected, at all sites, and in all treatments AM fungal colonization increased from V2 to R2.

Across all sites and treatments AM fungal root colonization was approximately 10.04 to 40.86 % and 31.86 to 79.55 % at V2 and R2, respectively. These averages were consistent with the results of McGonigle et al. (1999)'s field study that took place in Orthic Humic Gleysol soil at Ridgetown, Ontario, Canada. They reported root colonization of 30 to 40% at 29 days after planting (V2 stage) and 50 to 60% after 50 days (R2 stage) (McGonigle et al., 1999). These results may be similar to the results in this thesis due to the location (Ontario) and similar soil type to the New Liskeard site herein (Humic Gleysol). As Oehl et al. (2010) found that soil type can influence AM fungal diversity and the community composition. There are generalist AM fungi, that are found across soil types, and specialist, that are typically exclusive to certain soil types, which can contribute to potential similarities in the community and subsequent rate of colonization observed herein (Oehl et al., 2010).

Another study, that took place in a greenhouse with soil of consisting of equal parts vermiculite, sand, clay, and field soil found slightly higher colonization of soybeans; ranging from 37 to 51% 38 days after planting (about V4), with the amount of colonization varying across different soybean varieties (Cameron et al., 2017). This increase in colonization compared to the results herein is likely due to the optimal conditions in a greenhouse in comparison to the variable conditions in the field (Ryan & Graham, 2018). A field study that took place in Andhra Pradesh, India in sandy clay and clay soils, found that AM fungal colonization ranged from 23.58 to 57.55 % and 53.08 to 76.92% at 30 days after planting (about V2) and just before harvest in soybeans (about R8), respectively (Hindumathi & Reddy, 2011). These results are again similar to those found herein, this could be due to the similar soil texture (of sandy clay and clay in their study compared to sandy loam and clay present in this thesis). The texture of the soil can greatly affect AM fungal communities (Jansa et al., 2014). Different species of AM fungi have varying colonization strategies with some species colonizing early or more extensively than others (Hart & Reader, 2005). Therefore, AM fungal communities with different species compositions of can have differing amounts of root colonization. This might explain why similar colonization results are observed with studies with similar soil characteristics (i.e., texture) as similar species will exist and can have analogous colonization strategies, allowing for a comparable amount of root colonization. These studies were all done on soybeans around the same stages of this thesis which could also account for these similarities between the results of the studies and this thesis.

In contrast, the experiment by Higo et al. (2018) in Kanagawa, Japan in volcanic ash soil observed AM fungal root colonization ranging between 5 and 10% for all treatments at R2 which is lower than the findings in this thesis. This difference could be due to the location and the soil texture being very different from that in the present thesis, perhaps allowing for a different AM fungal community with differing colonization strategies. Additionally, climate and soil characteristics (pH, and C and N availability) also have an effect on root colonization; for example, higher colonization is often present in moderately warm temperatures compared to hot and cold temperatures (Soudzilovskaia et al., 2015). The climate could also have an impact on the colonization and the differences between studies and this thesis. Overall, there are many factors that can influence the AM fungal community and alter the rate of colonization. Again, there was no effect of the different AM inoculants or fertilization treatments on root colonization of soybeans in northern Ontario, Canada (see discussion below).

Spores were counted to estimate inoculum potential after canola production, after sorghum-sudangrass production, and after soybean cultivation. AM fungal spore counts allowed for a preliminary observation of the presence of AM fungi and, when combined with root colonization analysis, provided a more comprehensive understanding of the AM fungal community in the soil. In this thesis, the end of season spore counts ranged from 14.63 to 56.27 million of spores/m^2 (75.0 to 288.6 spores/g) across all treatments at all sites. The spore abundance between Plummer and Mt Zion was more similar compared to that found in the New Liskeard site, possibly due to their closer geographical proximity.

The abundance of spores reported in the literature is highly varied. This large variation in spore abundance is likely due to several factors. The estimated number of AM fungal taxa worldwide is between 300 to 1600 (van der Heijden et al., 2015) and with this diversity comes varying life strategies that influence their spore production (Oehl et al., 2009). Different AM fungal species produce spores at different times (Oehl et al., 2009), so the timing of collection can greatly alter the observed spore abundance. Additionally, soil disturbance can affect the AM fungi and their spores, with tillage spreading the spores out more in the soil (Schalamuk et al., 2013). As previously mentioned, there are many vectors (both biotic and abiotic) that can influence the distribution and thus the concentration of spores in the soil (Thomsen & Hart, 2018).

There are numerous factors contributing to AM fungal spore abundance in soil that can contribute to the variation in spore abundance observed throughout literature. For instance, a study in the Rhine River valley in France, Germany, and Switzerland with Calcaric Regosols, Haplic Luvisols, and Rendzic Leptosol soils, respectively, was looking at the effects of land use on species' diversity of AM fungi (Oehl et al., 2003). They found a range of 35 to 65, 9.7 to 12.5, 2.5 to 8 spores/g in grassland, crop rotation and monoculture, respectively (Oehl et al., 2003), which is much lower than spore abundances reported herein. This difference could be due to Oehl et al. (2003) presenting spore counts from the spring, as they encountered difficulties in identifying species in fall spore samples due to the higher proportion of young spores. Additionally, the differing soil characteristics and climate of these locations may have altered the AM fungal community, potentially favoring fungi with

different life strategies (Oehl et al., 2009) than those present in northern Ontario soils (i.e., species that produce fewer spores). Consequently, the differing timing of collection, soil conditions, and climate may all account for the contrasting results observed when comparing the findings herein to those of Oehl et al. (2003).

Another study that focused on sodic and saline environments reported a range of 0 to 199 spores/g, and one of the locations in the study Szabadszállás, Hungry (a similar latitude to Sault Ste Marie, Ontario) had a count of 25 to 199 spores/g which is more similar to the spore abundance found herein (Landwehr et al., 2002). AM fungal spore abundance was not found to have a relationship with latitude in a study conducted by Álvarez-Sánchez and Johnson (2011). However, this study was limited in scope as it only examined a total of eight sites located in the United States and Mexico. Therefore, it is possible that a larger sample size and a wider geographic area may yield different results and provide a more comprehensive and detailed understanding of the abundance of mycorrhizal fungi across different latitudes. A study conducted by Egan et al. (2014) found an average of approximately 25 to 300 spores/g across eighteen ecoregions in six North American biomes, which is consistent with data in this thesis. These counts may algin more with those in this thesis due to the sample locations being in relatively similar geographical regions (i.e., across North America). Additionally, Egan et al. (2014) collected four samples throughout the year which allows for a more comprehensive understanding of spore abundance due to accounting for seasonal variation (Ohel et al., 2009).

However, many studies found lower spore counts than those reported herein (Burrows, 2014; Mathimaran et al., 2005; Zubek et al., 2022), especially in agricultural studies (Bedini et al., 2007; Boddington & Dodd, 2000; Nongkling & Kayang, 2017; Oehl et al., 2017; Priyadharsini et al., 2012; Schalamuk et al., 2013; Singh et al., 2022). This difference in spore counts observed herein compared to that of literature may be due to the cold winters in northern Ontario. Soudzilovskaia et al. (2015) described five-to-ten-fold increases in spore germination and reduction of the mortality of AM fungal spores after long periods of cold stratification. This highlights the significance of considering regional climatic conditions and its impact on the dynamics of AM fungal spores, further emphasizing the need for localized studies to gain a full understanding of northern AM fungal communities and their effects on the agroecosystem.

In this thesis there were no observable effects of fertilization on spore count. The effects of no P and an excess of P fertilization were tested in a 31-year field study in Gleyic Cambisol soil in Canton Vaud, Switzerland by Mathimaran et al. (2005) where they observed AM fungal spore abundance. They found no evidence that P fertilization had an effect on spore density, composition, and diversity of AM fungal communities (Mathimaran et al., 2005). However, they did find that the crop had an effect on AM fungal spore density and diversity with canola having the lowest (around 1 spore/g) spore abundance with wheat (*Triticum aestivum*) and *Phacelia tanacetifolia* having spore abundance of 12 to 15 and 20 to 25 spores/g, respectively (Mathimaran et al., 2005). This study is consistent with the results herein considering the absence of a fertilization effect on spore abundance. This thesis used

OMAFRA's guidelines which follow a sufficiency approach to fertilization (OMAFRA, 2023). This approach typically recommends lower fertilization rates compared to the approach used by Mathimaren et al. (2005), which bases fertilization on the amount of nutrients removed by the crop. It is important to note that some treatments in the study by Mathimaren et al. (2005) exceeded the recommended fertilization rates based on the crop removal approach. To sum up, even with the application of excess fertilizer the study by Mathimaren et al. (2005), they did not observe an effect on spore density, similar to this thesis. Whether canola reduced the spore numbers in the soil in this thesis is unknown. However, the initial spore levels in this thesis were higher than 1 spore/g as observed by Mathimaran et al. (2005).

The lack of difference in spore abundance between fertilizer treatments was unexpected as previous research found fertilization impacts the AM fungal communities and reduces the spore abundance (Johnson, 1993; Liu et al., 2012). Although fertilization can alter the AM fungal community, it is possible that in this thesis, the soils in northern Ontario had a robust community of AM fungi that was able to shift composition resulting in another species of AM fungi being able to adapt to the higher fertility conditions (Treseder & Allen, 2002). This would allow for a species to take over that niche and maintain spore abundance in the soil (Treseder & Allen, 2002). More studies analyzing the composition of the AM fungal community and the effects of fertilization in the northern Ontario context are needed to further understand dynamics of the AM fungal communities present in the local agroecosystem.

Variation in spore abundance across six sites was observed in a study by Deveautour et al. (2020) with different soil present across the sites: brown calcareous sand, red soil, clay with gravel, grey clay, red clay and red sandy soil. The average spore abundance ranged from about 50 to 225 spores/g at different sites, in both arid and grasslands in Australia (Deveautour et al., 2020). This range in spore abundance is very comparable to the abundance of spores found herein. The findings by Deveautour et al. (2020) regarding variation in AM fungal spore abundance across sites sheds light on the large variation in spore counts reported in the literature. This variation in AM fungal spore abundance could be due to the multiple factors that influence AM fungi (e.g., tilling, crop rotation, fertilization, plant species present (Burrows, 2014; Pellegrino et al., 2019; Säle et al., 2015)). This does confirm the findings herein and that of literature that the benefits of AM fungi are context dependent, likely due to high variation within local AM fungal communities. Overall, these results show that the spore counts in this thesis were consistent with some studies in the literature, while differing from others, indicating a high degree of variability of AM fungal communities in different environmental conditions. Despite the lack of response to these treatments, these findings provide valuable insights into the complex dynamics of AM fungal communities and highlight the need for further research to fully understand the factors that influence their composition and functioning.

Spore counts were conducted at two different times in this thesis, and it should be noted that the data is seasonal in nature and cannot be compared to the prior spore count (Vieira Junior et al., 2020). The initial spore counts were taken in spring, while the end-of-

season counts were taken in fall. In the Cerrado, a study found that AM fungi activity is higher in dry, winter versus rainy, summer seasons indicating the seasonality of AM fungi (Vieira Junior et al., 2020). Another study observing the seasonality of AM fungi was carried out in the Opole Silesia region of Poland by Zubek et al. (2022) that looked at spore abundance in spring, summer, and fall. They observed the highest number of spores in summer with the lowest in autumn (Zubek et al., 2022). Another factor to consider is the effects of pesticides on spores. A study near Partido de Azul, Argentina with Natraquoll soil looked at the effect of glyphosate on AM fungi found a reduction in spore viability as well as a decrease in arbuscular presence in plant roots (Druille et al., 2013). In this thesis root colonization was observed and therefore the presence of AM fungi was confirmed, however the effects of glyphosate could have had an impact on the results herein as all plots were sprayed with Roundup. There was not a separate control left not sprayed by glyphosate to elucidate any effects of glyphosate in the context of this thesis. Another consideration is that spores can have little to do with the propagation of an AM fungal species as there are other ways that AM fungi can propagate (e.g., mycelium or colonized roots) (Martinez & Johnson, 2010; Schalamuk & Cabello, 2010a). Further, some AM fungal species exhibit infrequent spore production (Clapp et al., 1995). It is also important to note that some spores may be dormant, which means that spore counts may not always reflect the active infectibility status of AM fungi (Oehl et al., 2009). These factors contribute to the complexity of observing spore abundance as an indicator of the AM fungal community. In order to mitigate the potential uncertainties associated with spore viability, Oehl et al. (2009) recommended an

approach that involves assessing the root colonization of AM fungi in the soil. In addition to the spore counts, this thesis implemented this approach of also observing root colonization to identify the active AM fungi that were present. The initial spore counts and results (no significant findings between AM fungal inoculum treatments), raise questions about the underlying hypothesis, that canola decreases the AM fungal community resulting in fewer benefits being conferred to the crops from the AM fungi.

The prevailing view in the literature is that canola, a non-mycorrhizal crop, reduces the AM fungal community in soils and diminishes their potential benefits for subsequent crops (Castillo et al., 2017; de Souza & Santos, 2018; Hansen et al., 2019; Koide & Peoples, 2012; Owen et al., 2010). However, the sites in this study had high levels of spores even after canola cultivation. Again, Floc'h et al. (2022) observed the persistence of AM fungi after 10 years in a canola monoculture. There are a few hypotheses for this persistence of AM fungi in the soil despite lacking a main host; for example, a bacteria biofilm may interact with the AM fungi in the soil providing the necessary nutrients for survival in an unfavorable environment like canola rhizosphere soil (Floc'h et al., 2022). Additionally, there is evidence that there can be limited colonization of *Brassicaceae* by AM fungi (i.e., low colonization, rarely the presence of arbuscules) (Anthony et al., 2020). A study by Poveda et al. (2019) that took place in a greenhouse in Haarlem, The Netherlands with three parts peat to one part vermiculite growth medium demonstrated that co-inoculation of AM fungi and *Trichoderma harzianum* resulted in a presence of AM fungi in the roots of canola (Poveda et al., 2019). This study demonstrated that there are certain circumstances that allow for AM fungal

colonization of canola roots which could be another possible avenue for persistence of AM fungi in soil where canola had been grown. A recent study by Valetti et al. (2016) that took place in argiudol soils in Provincia de Buenos Aires, Argentina found no significant impact on soybean biomass with prior cropping of canola but did decrease soybean root colonization by AM fungi. These studies along with the findings herein suggest that canola's impact on the AM fungal community may not be as uniformly deleterious to subsequent crops as previously assumed.

There were no treatment effects on any of the endpoints measured herein, which was not expected. A cause for the lack of responses to the AM fungal inoculants could be due to the initial levels of inoculum potential in the soil measured in terms of spore abundance. Again, spore counts allow for preliminary observation of the presence of AM fungi. The initial number of spores present in the soil was 18.9 million and 14 million spores per m^2 at Mt Zion and Plummer, respectively. The AM fungal treatments added approximately 80.9 and 77100 spores per m^2 with the commercial and indigenous inoculants, respectively. Clearly, these numbers are small compared to those already present in the soil which may be the cause of the lack of observable response on the metrics studied with the AM fungal inoculum treatments. Perhaps, different results would be observed with lower initial spore levels, future research should be conducted at sites that have highly varied spore abundance to further explore the effectiveness of AM fungal inoculants.

It is thought that conventional agriculture with high inputs is not conducive for the AM fungal community, but some studies have challenged this notion. A study by Dai et al.

(2013) that sampled over 130 sites across the Canadian prairies observed no negative effect of crop production on diversity of AM fungi, they did note a shift on community structure when compared to natural areas. Overall, this study found that the richness of AM fungal communities in croplands remains, and the AM fungal communities are able to withstand disturbance even though there is a shift in community structure that occurs due to agricultural land use (Dai et al., 2013). This indicates that agricultural practices may not be as detrimental to the AM fungal community as previously believed. This thesis observed high spore levels at the study sites which aligns with the findings by Dai et al. (2013). In addition, a review by Ryan and Graham (2018) found that most mycorrhizal research is very optimistic regarding the benefits AM fungi can provide for crops, however agronomic research does not hold the same sentiments. They also noted that the AM fungi community appears more resilient to agricultural practices than previously assumed (Ryan & Graham, 2018). Ryan and Graham (2018) advocate for a holistic approach to agriculture that considers the entire agroecosystem, rather than focusing solely on the AM fungal community. This approach includes sustainable farming practices such as crop rotation, intercropping, and efficient use of inputs, which can naturally coexist with the AM fungal community and promote overall soil and crop health (Ryan & Graham, 2018). Moreover, these findings suggest that while AM fungi may play a role in crop health and soil fertility, their responsiveness to agricultural practices may have been overstated, or may be context specific. The results of this thesis support this hypothesis by Ryan and Graham (2018), that the effects of AM fungi on crops are very context dependent. These findings present a contradiction to the foundational

assumptions of the hypothesis herein, which based on previous literature assumed that agricultural practices, such as canola cultivation and fertilization, would significantly negatively impact the AM fungal community (Arihara & Karasawa, 2000; C. Castillo et al., 2017; Isobe et al., 2014; Johnson, 1993; Liu et al., 2012). However, the results obtained herein do not provide evidence to support the hypothesis. Specifically, this thesis' hypothesis suggested that indigenous AM fungi would offer greater benefits to soybeans with decreasing levels of fertilizer use in comparison to commercial inoculants following canola cultivation. The aim of this thesis was to assess the potential of integrating the use of indigenous AM fungi in northern Ontario into the management practices of local farmers within a canola-soybean crop rotation. However, in the context of this study in northern Ontario, no evidence was found to show that using AM fungi as inoculants benefited soybeans in an observable manner, leading to this hypothesis being rejected. As such, farmers should consider other factors as a priority in their management practices, while still keeping in mind the potential benefits of maintaining healthy AM fungal communities in their soils.

5.3. Crop Metrics

It was hypothesized that the treatments receiving 100 % the recommend rate according to OMAFRA would have greater crop productivity indicators and that there would be an interactive effect between the fertilizer and AM fungi treatments that would result in the maintenance of crop productivity metrics with decreasing fertilizer treatments. There were no observable effects of the fertilizer nor the AM fungal inoculant on crop productivity indicators and the hypotheses were rejected. Biomass at V2 and R2 was observed to gather

an early insight into the possible impact of the treatments on the plants, specifically any effect that might translate into yield. The average biomass at all sites in all treatments ranged from 6.01 to 18.60 g/m^2 and 15.63 to 142.92 g/m^2 at V2 and R2, respectively. These results are consistent with those of Yusuf et al. (1999) who carried out a field study in Illinois, United States in silt loam soil, that reported an average biomass of approximately 12.18 g/m^2 at the V2 stage and 141.17 g/m^2 at the R2 stage. The biomass results were also similar to those reported by Higo et al. (2018) whose study took place in Kanagawa, Japan in volcanic ash soil. They observed that when canola was used as a cover crop preceding soybean cultivation, the soybean biomass during the R2 stage ranged from 50 to 100 g/m^2 without the addition of P fertilizer, and 100 to 200 g/m^2 with the addition of P fertilizer (Higo et al., 2018). The findings by Higo et al. (2018) are within range of the biomass found in this thesis. However, in contrast to the observations herein, they found a difference in biomass in treatments with and without P fertilizer additions (see discussion below in section 5.6). The apparent harvest index observed in this study (ranged from 0.37 to 0.50) is comparable to the values reported in other experiments. For instance, Umburanas et al. (2022) found a harvest index 0.35 and 0.42 (using 2017/2018 cultivars in clayey Oxisol soils in Paraná State, Brazil) while Krisnawati and Adie (2015) found an average harvest index of 0.38 over 29 different soybean genotypes in Indonesia (Krisnawati & Adie, 2015). This indicates that the apparent harvest index in this thesis falls within the range reported in the literature. Soybeans have been selected for stable agronomic traits across environments and varying climates (Cucolotto et al., 2007; Rao et al., 2002) which could be attributed to the similarity of the

agronomic results found in this thesis, however environmental conditions (soil type, weather precipitation, solar radiation) can still have an effect on these metrics.

The yield in this study ranged from 1351.2 to 3406.7 kg/ha across all treatments at Plummer and New Liskeard while at Mt Zion, since all the treatments were affected by weeds and herbivory, the yield ranged from 73.0 to 181.9 kg/ha. The findings from Plummer and New Liskeard are consistent with the soybean yields reported in other studies. Specifically, in Manitoba, Canada during the 2022 season, the average yield of Bourke soybeans was 44 bushels per acre (equivalent to 2959 kg/ha) according to Manitoba Agricultural Services Corporation (2023). The Ontario Soybean And Canola Committee (OSACC) reported the five yield average yield of Bourke R2X soybeans as 3380 kg/ha. These findings align with the results obtained in this thesis; the similarity is due to both reports being conducted within the Canadian context and observing the same soybean variety as this thesis.

The oil content in this thesis ranged from 18.58 to 19.08 % across both sites (Plummer and New Liskeard) and in all treatments, which is consistent with previously reported findings. For instance, a study in Brazil in clayey Oxisol soils analyzing different soybean cultivars documented an average oil content of approximately 190 to 215 mg/g (equivalent to 19 to 21.5 %) (Umburanas et al., 2022). This study analyzed a wide range of 26 soybean cultivars, which likely contributed to the consistent findings reflected in this thesis. Furthermore, a greenhouse experiment in Kurukshetra, India, investigated the possible effects of co-inoculation with two indigenous AM fungi and two other microbes

(*Trichoderma viride* and *Bradyrhizobium japonicum*) on soybeans reported that the average oil content of their control group was 17.88%, whereas the treatments involving indigenous fungi and the other bioinoculants resulted in a range of average oil content between 19.08 to 24.88% (Yadav et al. 2013). This is slightly higher than the oil content observed within the treatments herein. This difference could be due to enhanced nutrient uptake by the plants due to the additional bioinoculants, an interaction between the AM fungi and the bioinoculants, or due to the differences of variable conditions experienced by soybeans in an agricultural field compared to the optimized conditions of a greenhouse experiment. In this thesis the protein content ranged from 30.20 to 32.45 % across all treatments at both sites, this is slightly lower than what can be observed in literature. For instance, the study by Yadav et al. (2013) found an average of 34.02 to 42.88 protein content for their treatments. The discrepancy between this thesis and the results by Yadav et al. (2013) could be attributed again to the additional bioinoculants present in the study, the soybean variety, or environmental differences (greenhouse versus field). A study investigating the agronomic responses of different soybean cultivars in Brazil found an average protein content of about 340 to 375 mg/g, which is equivalent to 34 to 37.5% (Umburanas et al., 2022). Bellaloui et al. (2020) found that seeding rate, row spacing, herbicide treatment, and nitrogen fertilization all influenced protein content which could account for differences between this thesis and other studies in literature. Additionally, OSACC reported the five-year average of Bourke R2X oil and protein content to be 20.4 % and 36.8 %, respectively. However, they also reported that for the years 2021 and 2022 Bourke R2X experienced a below average protein

and oil content (OSACC, 2023). This matches the findings herein as this thesis observed a lower protein and slightly lower oil content than typical. This is likely due to environmental conditions such as year-to-year variations in temperature and precipitation altering seed-filling processes (i.e., the assimilate transport pathway) (Sehgal et al., 2018).

5.4. AM Fungal Inoculant and Fertilizer Response

There was likely no response on the endpoints assessed to the fertilizer treatments as according to OMAFRA the initial levels of P (Appendix C.1) indicated a low, medium, and high response at Plummer, New Liskeard, and Mt Zion, respectively (Appendix C.2) (Bagg et al., 2017). Thus, a low and moderate response to fertilizer at Plummer and New Liskeard was expected due to the initial levels of soil P. Since the initial levels of P in the soil were not significantly low, it is possible that the difference between the controls (without P additions) and the treatments (with P additions) may not have been substantial enough to observe an effect of fertilizer. The site where the highest response to fertilization would have likely been observed, Mt Zion, was affected by weeds and herbivory which greatly affected the data that was gathered at that site. Moreover, a lack of yield response to fertilizer in soybeans has been observed previously by Lauzon and Miller (1997) who attributed this finding to P not limiting the growth of the soybeans. This indicates that there is a precedent for soybeans not responding to fertilization, which could offer further explanation for the null results found herein. A study that took place in New Mexico, United States in Shiprock fine sandy loam soils showed that AM fungi were more beneficial to crops (i.e., had a much larger effect in increasing crop biomass) in nutrient poor soil than in nutrient rich soil (Martinez & Johnson,

2010). Although the soil at the sites in this thesis was not nutrient rich, Plummer and New Liskeard did not have nutrient poor soils, which could also contribute to the lack of observed response for the AM fungal inoculants. Overall, the initial environmental conditions (nutrient and high spore levels in the soil) likely contributed to lack of response observed in this study. This further shows that the effectiveness of AM fungi inoculants is context specific especially in the environmental and soil conditions of northern Ontario. Future research efforts should aim to investigate the impact of specific environmental factors on AM fungal inoculants impact on specific crops to better inform agricultural practices in northern Ontario.

Overall, the benefits of using AM fungi as inoculants appear quite context specific with some research showing benefits conferred to crops while other studies do not. AM fungi do play an important part in the agroecosystem as a whole, in C storage and improving soil health (Rillig, 2004). This thesis focused on the agronomic endpoints (yield, protein and oil content) that are of economic interest for farmers. There was no evidence found that commercial or indigenous AM fungal inoculants were effective in the scope of this research. The hypothesis was rejected as, the indigenous AM fungi treatment and the commercial AM fungal inoculant did not confer any observable benefits to the soybeans in the context of northern Ontario at the sites studied herein. This has significant practical implications, as it enables farmers in northern Ontario to make informed decisions about the efficacy of additional amendments, based on their specific environmental context and financial considerations. This is of great importance due to the warming climate and the expected

expansion of agriculture into the north in the coming decades (Altdorff et al., 2021). Facilitating the dissemination of knowledge among farmers, enabling them to effectively optimize their available resources, is imperative for cultivating sustainable agriculture in the northern region. This ensures the preservation of food production while simultaneously protecting the integrity of local ecosystems. A research centric approach with emphasis on knowledge dissemination which is crucial to determine a sustainable path forward in the expansion of agriculture as land conversion can exacerbate climate change by triggering the release of stored carbon (Unc et al., 2021). The results of this thesis demonstrate that sustainable farming methods must be studied in northern Ontario before implementation to ensure efficacy in the local context.

5.5. Limitations of this Study

A potential limitation of this study is that the fertilizer was applied at different times, the New Liskeard site was fertilized in the fall of 2021 while the Plummer and Mt Zion sites were fertilized in the spring of 2022, since fertilizer takes time to become bioavailable this could have impacted this study and contributed to the increased biomass and yield observed at New Liskeard. Additionally, at the Plummer and Mt Zion sites the fertilizer was broadcast on top of the soil and not worked in, which could also have impacted the effectiveness of the fertilizer. While at New Liskeard the site was tilled which could have impacted the AM fungal communities present. Further, while this thesis attempted to follow the guidelines set out by OMAFRA, the Plummer site was fertilized when the OMAFRA recommendations indicated that it did not need to be. Another limitation of this study was that there was not a

replicated experiment on canola cultivation that assessed the effects of canola on the AM fungal community. This would have been able to determine if canola reduced the AM fungal community as this was an underlying assumption of this work. Additionally, the effects of glyphosate could have been studied to provide a more comprehensive understanding of the AM fungal community in an agricultural context. The lack of spore abundance data prior to canola growth and the initial spore levels from spring of 2023 in New Liskeard is another limitation of this study. A further limitation could have been the decision to rototill the sorghum-sudangrass soil, the source of indigenous AM fungi, as tilling has been known to disrupt the community (Säle et al., 2015) and could have impacted the effectiveness of the indigenous inoculum. Additionally, Mt Zion being so strongly affected by weeds and herbivory greatly limited the data that was able to be obtained at that site. Further, molecular methods of analyzing the AM fungal community were not used but could have provided greater insight to the community.

5.6. Recommendations for Future Study

Future research should focus on quantifying the number of spores in agricultural fields in northern Ontario and identifying community compositions as this work would enhance the understanding of the indigenous fungi communities present in this area. To further investigate the effect of canola on the AM fungal community, future studies could consider including an additional control group that did not experience canola cultivation in the previous year as well as a control that was not sprayed with glyphosate to ensure there are no effects on glyphosate on the AM fungal community. In addition, including soils under

canola cultivation over several seasons, and consistently conducting spore counts and molecular analyses to assess the impact of canola on the AM fungal community, could provide further insight into the potential consequences for subsequent mycorrhizal crops. Additionally, this study could be repeated in locations with more degraded soils (reduced fertility), with a lower AM fungal community abundance (i.e., lower spore counts then the initial numbers in this study), and with a different mycorrhizal crop to investigate potential crop-specific responses to indigenous AM fungi and to further the elucidate the effects of AM fungi in the agroecosystem.

6.0 CONCLUSIONS AND FUTURE RESEARCH

In summary, there was not found to be any advantage to producing indigenous AM fungi on-farm or in using a commercial inoculant in the context of this thesis. The treatments were not found to be effective at the study sites. Thus, the hypothesis that the indigenous fungal inoculant would result in the maintenance or improvement of crop productivity indicators as fertilizer application declined, was rejected. There was likely no response in the metrics observed to the AM fungal inoculant treatments due to the initial high levels of indigenous spores already present in the soil. Further, it is concluded that in addition to soybeans known occasional predisposition to a low response to fertilizer, the initial levels of P and K indicated an expected low to moderate response of fertilizer on soybeans at Plummer and New Liskeard, which lead to no fertilizer response being observed. At Mt Zion, weeds and herbivory overrode any potential treatment effects.

Further research in degraded soils may provide more insight into the effects of fertilizer relative to those of different AM fungal inoculants. Other studies have demonstrated the success of inoculants is context specific (Frew, 2021; Islam et al., 2021). Such context dependence is associated with soil factors and genotypic variation in plants and fungi that affect the symbiotic relationship (Berruti et al., 2017). More research should be done in northern Ontario to examine the effectiveness of AM fungi in the local context. However, based on the results from this thesis, overall, it is recommended that farmers in northern Ontario prioritize other factors in their management practices, while acknowledging the potential advantages of preserving a robust AM fungal community in their soils.

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Appendix A - Linear Mixed Model Results

Table A.1 Results of a linear mixed model conducted in R of AM fungal root colonization at the V2 growth stage of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions. (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were not statistically significant at $p > 0.05$.

Treatment	Estimate	Std. Error	Df	T Value	Pr(>|T|)
(Intercept)	29.47	4.29	7.98	6.86	0.00013
Fertilizer	-0.011	0.050	90.36	-0.21	0.83
Commercial	-3.73	4.57	90.58	-0.82	0.42
Indigenous	-6.34	4.53	89.60	-1.40	0.17
Fertilizer: Commercial	-0.058	0.071	91.67	-0.82	0.41
Fertilizer: Indigenous	0.015	0.070	89.95	0.21	0.83

Table A.2. Results of a linear mixed model conducted in R of AM fungal root colonization at the R2 growth stage of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions. (At Plummer n=36, at New Liskeard and Mt Zion n=35).

Treatment	**Estimate**	**Std. Error**	**Df**	**T Value**	**Pr(>|T|)**
(Intercept)	63.62	9.77	2.50	6.51	0.012
Fertilizer	-0.058	0.052	92.31	-1.11	0.27
Commercial	-8.37	4.73	92.77	-1.77	0.08
Indigenous	-10.54	4.72	90.28	-2.23	0.028*
Fertilizer:Commercial	0.080	0.0731	95.18	1.09	0.28
Fertilizer:Indigenous	0.17	0.073	91.26	2.312	0.023*

Significant ($p<0.05$) results indicated with *
See Appendix B1 for Tukey honestly significant difference (HSD) correction.

Table A.3 Results of a linear mixed model conducted in R of biomass at the V2 growth stage of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions. (At Plummer n=36, at New Liskeard and Mt Zion n=35).

Treatment	Estimate	Std. Error	Df	T Value	Pr(>|T|)
(Intercept)	11.79	2.22	2.58	5.30	0.019
Fertilizer	-0.011	0.013	91.28	-0.85	0.40
Commercial	-0.34	1.15	91.63	-0.30	0.77
Indigenous	-0.32	1.15	89.92	-0.28	0.78
Fertilizer:Commercial	0.037	0.018	93.43	2.06	0.042*
Fertilizer:Indigenous	0.021	0.018	90.57	1.21	0.23

Significant ($p<0.05$) results indicated with *
See Appendix B2 for Tukey honestly significant difference (HSD) correction.

Table A.4 Results of a linear mixed model conducted in R of biomass at the R2 growth stage of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions. (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were not statistically significant at $p > 0.05$.

Treatment	Estimate	Std. Error	Df	T Value	Pr(>|T|)
(Intercept)	71.15	32.28	2.07	2.20	0.15
Fertilizer	0.014	0.067	91.79	0.21	0.84
Commercial	1.83	6.10	92.20	0.30	0.77
Indigenous	-3.11	6.07	90.11	-0.51	0.61
Fertilizer:Commercial	-0.077	0.095	94.32	-0.81	0.42
Fertilizer:Indigenous	0.094	0.093	90.92	1.01	0.32

Table A.5 Results of a linear mixed model conducted in R of apparent harvest index of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions. (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were not statistically significant at $p > 0.05$.

Treatment	Estimate	Std. Error	Df	T Value	Pr(>|T|)
(Intercept)	0.47	0.021	4.33	22.74	0.000011
Fertilizer	0.000072	0.00019	90.83	0.37	0.71
Commercial	-0.0071	0.018	91.15	-0.41	0.69
Indigenous	-0.030	0.017	89.66	-1.70	0.092
Fertilizer:Commercial	-0.000042	0.00027	92.75	-0.15	0.88
Fertilizer:Indigenous	0.00034	0.00027	90.21	1.26	0.21

Table A.6 Results of a linear mixed model conducted in R for yield of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions. (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were not statistically significant at $p > 0.05$.

Treatment	Estimate2	Std. Error	Df	T Value	Pr(>|T|)
(Intercept)	175.1	90.87	2.03	1.93	0.19
Fertilizer	0.10	0.12	94.01	0.84	0.40
Commercial	7.33	10.94	93.21	0.67	0.50
Indigenous	19.54	10.95	91.14	1.79	0.078
Fertilizer:Commercial	-0.20	0.17	95.57	-1.16	0.25
Fertilizer:Indigenous	-0.20	0.17	92.07	-1.19	0.24

Table A.7 Results of a linear mixed model conducted in R for oil content (%) of soybeans at the Plummer and New Liskeard sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions. (At Plummer n=36, at New Liskeard n=35). All treatments were not statistically significant at $p > 0.05$.

Treatment	Estimate	Std. Error	Df	T Value	Pr(>|T|)
(Intercept)	18.89	0.17	2.32	110.08	0.000023
Fertilizer	-0.000074	0.0016	59.30	-0.045	0.96
Commercial	-0.12	0.15	58.37	-0.80	0.43
Indigenous	-0.12	0.15	58.32	-0.78	0.44
Fertilizer:Commercial	0.00075	0.0023	58.82	0.33	0.74
Fertilizer:Indigenous	0.0007	0.0023	58.30	0.31	0.76

Table A.8 Results of a linear mixed model conducted in R for Protein Content (%) of soybeans at the Plummer and New Liskeard sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions. (At Plummer n=36, at New Liskeard n=35). All treatments were not statistically significant at $p > 0.05$.

Treatment	Estimate	Std. Error	Df	T Value	Pr(>|T|)
(Intercept)	30.96	0.73	1.25	42.26	0.0064
Fertilizer	-0.0015	0.0038	59.51	-0.39	0.70
Commercial	0.36	0.35	58.49	1.05	0.30
Indigenous	0.24	0.35	58.40	0.70	0.49
Fertilizer:Commercial	-0.0015	0.0053	59.01	-0.27	0.78
Fertilizer:Indigenous	0.00023	0.0053	58.38	0.042	0.97

Table A.9 Results of a linear mixed model conducted in R for AM fungal spore counts in millions of spores per square meter across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions. (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were not statistically significant at $p > 0.05$.

Treatment	Estimate	Std. Error	Df	T Value	Pr(>|T|)
(Intercept)	31.11	8.82	2.19	3.53	0.063
Fertilizer	-0.006	0.031	68.24	-0.19	0.85
Commercial	-0.98	2.88	69.51	-0.34	0.73
Indigenous	2.91	2.91	65.23	1.00	0.32
Fertilizer:Commercial	0.03	0.045	69.99	0.67	0.51
Fertilizer:Indigenous	-0.050	0.045	65.79	-1.10	0.27

Appendix B – Tukey HSD Results

Table B.1 Results of pairwise comparison on AM fungal root colonization of soybeans at the R2 growth stage using Tukey adjustment conducted in R across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions. (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were not statistically significant at $p > 0.05$.

1.000000	**Estimate**	**Se**	**Df**	**T.Ratio**	**P.Value**
Fertilizer50.4716981132075 None- Fert50.4716981132075 Commercial	4.34	2.94	89.2	1.48	0.31
Fertilizer50.4716981132075 None - Fertilizer50.4716981132075 Indigenous	2.08	2.96	89.3	0.70	0.76
Fertilizer50.4716981132075 Commercial - Fertilizer50.4716981132075 Indigenous	-2.26	2.94	89.2	-0.77	0.72

Table B.2 Results of pairwise comparison on biomass of soybeans at theV2 growth stage using Tukey adjustment conducted in R across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions. (At Plummer n=36, at New Liskeard and Mt Zion n=35). All treatments were not statistically significant at $p > 0.05$.

1.000000	**Estimate**	**Se**	**Df**	**T.Ratio**	**P.Value**
Fertilizer50.4716981132075 None - Fertilizer50.4716981132075 Commercial	-1.52	0.71	89.1	-2.13	0.09
Fertilizer50.4716981132075 None - Fertilizer50.4716981132075 Indigenous	-0.76	0.72	89.2	-1.06	0.54
Fertilizer50.4716981132075 Commercial - Fertilizer50.4716981132075 Indigenous	0.76	0.71	89.1	1.06	0.54

Appendix C – Soil Nutrient Levels

Table C.1 Initial phosphorus levels at obtained in June of 2021 at three sites in northern Ontario, Canada. Standard error of the mean is presented in brackets (n = 6).

Location	**Average P (ppm)**
New Liskeard	15 (1.8)
Plummer	19 (2.1)
Mt Zion	9 (0.7)

Table C.2 Phosphorus soil test, recommended amount of phosphate required and expected response to the phosphate for soybean cultivation per Ontario's Ministry of Agriculture, Food and Rural Affairs Agronomy Guide adapted from (Bagg et al., 2017).

Phosphorus Soil Test (ppm)	**Phosphate Required (kg/ha)**
8-9	40 (High Response)
13-15	20 (Moderate Response)
16-30	0 (Low Response)

Appendix D – Health of Soybeans

Table D.1 Average results of weed census (average number of weeds per m^2) at the V2 growth stage of soybeans across all three sites in northern Ontario, Canada. Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions (none). (At Plummer n=36, at New Liskeard and Mt Zion n=35).

Am Fungi Treatment	Fertilizer Rate	New Liskeard	Plummer	Mt Zion
Indigenous	0	14.50 (2.60)	54.50 (14.71)	37.50 (4.57)
Commercial	0	13.00 (2.68)	46.00 (12.49)	38.00 (2.45)
None	0	15.75 (1.38)	49.50 (3.59)	36.00 (6.48)
Indigenous	50	11.25 (1.38)	61.50 (17.35)	26.50 (4.57)
Commercial	50	15.00 (2.74)	52.00 (9.20)	34.00 (3.74)
None	50	13.00 (1.68)	39.50 (7.54)	31.50 (2.22)
Indigenous	100	12.75 (1.80)	56.50 (10.31)	35.50 (2.36)
Commercial	100	11.50 (2.06)	58.00 (14.07)	29.50 (4.03)
None	100	13.00 (2.42)	48.50 (10.59)	32.50 (3.30)

Table D.2 Average area and severity affected by weeds and herbivory of soybean plots at the Mt Zion site located in northern Ontario, Canada in August 2022 (n=4). Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions. The severity of these effects was rated on a scale of 1 (low severity) to 5 (high severity).

Am Fungi Treatment	Fertilizer Rate	Average Area Affected By Weeds (%)	Average Area Affected By Herbivory (%)	Average Severity Of Weeds In Plots (1- 5)	Average Severity Of Herbivory In Plots (1 - 5)
Indigenous	0	8.75 (4.27)	80.00 (16.71)	3.75 (1.25)	2.88 (0.66)
Commercial	0	18.75 (8.98)	81.25 (13.75)	5.00 (0.00)	3.00 (0.58)
None	0	11.67 (4.41)	33.33 (18.56)	5.00 (0.00)	1.67 (0.33)
Indigenous	50	18.75 (9.66)	50.00 (16.71)	3.75 (1.25)	2.25 (0.63)
Commercial	50	31.25 (9.44)	41.25 (18.19)	5.00 (0.00)	3.25 (0.75)
None	50	18.75 (9.66)	50.00 (16.71)	3.75 (1.25)	2.25 (0.63)
Indigenous	100	12.50 (5.95)	41.25 (18.53)	3.75 (1.25)	2.25 (0.63)
Commercial	100	8.75 (2.39)	37.50 (8.54)	5.00 (0.00)	1.75 (0.25)
None	100	8.75 (3.75)	67.50 (14.51)	5.00 (0.00)	3.13 (0.13)

Note: It was observed that the plots that were more severely affected was due to placement on the field (i.e., close to the wetland and tree line).

Table D.3 Weed density and stand counts of soybean plots observed at the R2 growth stage of soybeans at the Mt Zion site located in northern Ontario, Canada in August 2022 (n=4). Main treatment of fertilizer varied at 0, 50, and 100 % the recommended rate according to the provincial guidelines, and subplot treatment of indigenous AM fungal inoculum, commercial AM fungal inoculum or no inoculum additions.

Am Fungi Treatment	**Fertilizer Rate**	**Weed Density (weeds /m^2)**	**Stand Count (soybean plants/m^2)**
Indigenous	0	123.50 (9.67)	6.75 (1.89)
Commercial	0	122.00 (7.57)	4.75 (0.95)
None	0	124.50 (7.80)	4.50 (2.72)
Indigenous	50	99.50 (12.76)	4.25 (0.25)
Commercial	50	117.50 (12.89)	6.75 (0.48)
None	50	138.50 (25.61)	8.00 (0.41)
Indigenous	100	147.00 (17.99)	5.75 (0.75)
Commercial	100	123.50 (15.56)	6.25 (2.29)
None	100	116.50 (3.20)	7.00 (0.58)

Appendix E – Sampling Dates

Table E.1 Sampling dates for the 2022 growing season at the New Liskeard, Plummer, and Mt Zion sites located in northern Ontario, Canada

Stage	**New Liskeard**	**Plummer**	**Mt Zion**
Planting	May 25, 2022	May 25, 2022	May 24, 2022
V2	June 20, 2022	June 29, 2022	June 27, 2022
R2	July 15, 2022	July 19, 2022	July 18, 2022
Scoring of Weeds and Herbivory	n/a	n/a	August 22, 2022.
Harvest	October 5, 2022	October 11, 2022	October 11, 2022