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Disturbance and the Community Composition of Arbuscular Mycorrhizal Fungi in Ontario Tallgrass Prairies

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// Abstract

Arbuscular mycorrhizal fungi (Glomeromycota) form beneficial symbiotic relationships with the majority of land plants, especially in grasslands. Tallgrass prairies in Ontario are an endangered plant community currently being restored on former agricultural land. The objectives of my study were to determine if there were differences in the communities of Glomeromycota between disturbed and undisturbed tallgrass prairies in Ontario and if there were any potential indicator taxa for the ends of the disturbance spectrum. A molecular approach using DNA derived from soil samples was used to compare species composition between disturbed and undisturbed tallgrass prairies. A total of 177 operational taxonomic units (OTUs) in nine genera of Glomeromycota were retrieved. Analyses showed a clear pattern of disturbed and undisturbed prairies clustering separately based on species composition of arbuscular mycorrhizal fungi, and distinguished OTUs that were indicators of disturbed (*Claroideoglomus* and select *Glomus* spp.) or undisturbed (*Ambispora*, *Diversispora*, and *Glomus* spp.) sites.

Keywords

Arbuscular mycorrhizal fungi, Glomeromycota, tallgrass prairie, soil fungal community, disturbance, restoration, Illumina Miseq, ribosomal DNA, PCR, biodiversity.

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List of Abbreviations

ALDEx2	ANOVA-Like Differential Expression tool
AMF	Arbuscular mycorrhizal fungi
BF	Blair Flats *rare* Charitable Research Reserve
DM	De Maere Prairie Nature Conservancy of Canada
EL	Eliza's Prairie in WIFN
FRS 23	Final Restoration site 23 Herb Gray Parkway
FRS 27	Final Restoration site 27 Herb Gray Parkway
FRS 28	Final Restoration site 28 Herb Gray Parkway
FRS 32	Final Restoration site 32 Herb Gray Parkway
MI	Mike's Field in WIFN
OPC1	Ojibway Prairie Complex site 1
OPC2	Ojibway Prairie Complex site 2
OTUs	Operational taxonomic units
PO	Pottawatomi Prairie in WIFN
SA	Sandpits Field in WIFN
SI	*Silphium* Prairie in WIFN
TGPs	Tallgrass prairies
VT	Virtual taxon/taxa
WIFN	Walpole Island First Nation

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Chapter 1

1 Introduction

1.1 Arbuscular mycorrhizal fungi

Certain fungi form a mutualistic relationship with the roots of living plants in a symbiotic association referred to as a mycorrhiza (pl. mycorrhizae). Mycorrhizal fungi exchange soil nutrients (such as phosphorus) and water for the plants' photosynthates. There are several different types of mycorrhizae, of which the most abundant are the arbuscular mycorrhizae (Smith and Read 2008). Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that cannot survive without their plant hosts and colonize a wide variety of land plants with the exception of a few plant families such as Brassicaceae (Smith and Read 2008, Brundrett 2009).

Arbuscular mycorrhizal fungi are part of the monophyletic phylum Glomeromycota (Schüßler et al. 2001). Glomeromycota were once classified under the Zygomycota as the order "Glomales" (a misspelling; Morton and Benny 1990), but molecular analysis suggested that they are a phylum of their own (Schüßler et al. 2001). There are now four orders of Glomeromycota: Glomerales, Paraglomerales, Diversisporales and Achaeosporales (Schüßler and Walker 2010, Redecker et al. 2013). The four orders are divided into 11 families and 25 genera (Schüßler and Walker 2010, Redecker et al. 2013). To date approximately 250 morphospecies have been described with this number increasing regularly due to all of the studies now focusing on AMF (Schüßler and Walker 2010, Redecker et al. 2013). Evidence of AMF spores and hyphae have been discovered in early land plants dating back to the Devonian (Rosendahl 2008). This supports the widely assumed hypothesis that AMF played a major role in the colonization of land by plants (Pirozynski and Malloch 1975, Malloch et al. 1980, Berbee and Taylor 1993, Smith and Read 2008).

A characteristic feature of AMF is the presence of arbuscules, which are structures of finely branched hyphae in the cortical cells of host plant roots (Douds and Millner 1999). An arbuscule forms after the fungus has penetrated a root cortical cell of a plant, and all of its

branches are surrounded by the plant plasmalemma (Smith and Read 2008). The arbuscule is the site of exchange between the fungus and the plant. Some families of Glomeromycota (Archaeosporaceae, Gigasporaceae and Paraglomeraceae) produce vesicles, which are enlarged portions of hyphae used for storage and as potential reproductive structures (Willis et al. 2013). The vast network of extraradical hyphae of AMF goes beyond the plant roots, allowing the fungi to access nutrient pools and water far from the plant root being colonized. The hyphae are coenocytic (multinucleate) and predominantly aseptate. Hyphae of AMF are very broad compared to those of other fungi, being (2-) 10-20 (-50) µm in diameter (http://invam.wvu.edu/the-fungi), but still very fine compared to plant roots. The spores or sporocarps of AMF are thought to be produced asexually (Smith and Read 2008), but there is recent evidence of sexual recombination and mating-type genes in at least some taxa (Halary et al. 2013, Riley and Corradi 2013, Ropars et al. 2016). The spores are produced individually, in the soil or in decaying roots, whereas sporocarps are clusters of thousands of spores, sometimes in a differentiated tissue (Morton 1988). The spores of AMF range from 40 to greater than 800 µm in diameter (Smith and Read 2008).

Host plants provide AMF with photosynthetically derived carbohydrates in exchange for nutrients and water that the plant is unable to obtain. The extensive extraradical mycelial networks of AMF are able to reach and explore more areas than plant roots, protecting plants from nutrient and drought stress. Plant health is aided by AMF by an increase in reproductive success and offspring survival (Koide and Dickie 2002). Plants associated with AMF also are benefited by an increased tolerance of heavy metals, pathogenic fungi and nematodes (Allen et al. 1995, Johnson et al. 1997, van der Heijden et al. 1998, Jeffries et al. 2003, Vandenkoornhuyse et al. 2002). Surrounding soil benefits from AMF as the extraradical hyphae secrete glomalin, a glycoprotein that enhances the stability of soil aggregates, which enhance the resistance of soils to erosion (Wright and Upadhyaya 1996).

1.2 Arbuscular mycorrhizal fungal communities

The importance of AMF for plant success is widely accepted, but studies of the diversity and dynamics of AM fungal communities are only now beginning to take off. Plant community composition and structure are influenced by AM fungal communities (van der Heijden et al. 1998). Understanding AM fungal communities will allow for a better

understanding of how AMF structure plant communities. Communities of AMF consist of many different species and rarely is there only one species of AMF present in a host plant (Morton 1999, Helgason et al. 1999, Bever et al. 2001). Community composition of AMF can be related to host species, season and life stage of host plant (Eom et al. 2000, Bever et al. 2001, Helgason et al. 2002, Husband et al. 2002, Vandenkoornhuyse et al. 2002). More information is needed to determine if there are seasonal differences in AMF community composition, because other studies have not found seasonal differences in the community composition of AMF (Rosendahl and Stukenbrock 2004, Santos-Gonzales et al. 2007). Plant community composition is suggested to influence AM fungal communities (van der Heijdan et al. 1998, Eom et al. 2000, Vogelsang et al. 2006). The addition of nitrogen has been shown to shift AM fungal communities (Egerton-Warburton and Allen 2000, Jumpponen et al. 2005). According to studies conducted on AMF within plant roots, AM fungal communities differ depending on plant community type, such as tropical forests, grasslands and temperate forests (Öpik et al. 2006).

Historically, studies of AMF communities have utilized morphological identification, spore density estimates and greenhouse studies with trap cultures to determine AMF diversity and species composition. Morphological studies require expert knowledge and the ability to determine microscopic variation in spore colour, shape, size, and number and ornamentation of spore wall layers (Willis et al. 2013). The characteristic arbuscules of AMF do not help with identification, because they do not distinguish between different species (Jeffries et al. 2003). The choice of plant species used for trap cultures may influence which AMF are found (Brundrett et al. 1994). Morphological identification is time-consuming and sometimes uncertain or subjective (with identifications varying depending on the observer; Sanders 2004, Willis et al. 2013). Because of these limitations, there has been an increase in molecular identification of AMF, which has permitted better description of AMF communities (Öpik et al. 2006, Öpik et al. 2009, Öpik et al. 2010). Molecular studies allow us to better understand the genetic similarities of AM fungal taxa between AMF communities from different ecosystems.

When comparing past descriptions of AMF communities from morphological and molecular studies we can see that there are differences in the number of AMF species that

are found. Morphological studies find fewer species than molecular studies, usually averaging below 20 species of AMF (Stover et al. 2012), because they may miss taxa that sporulate less abundantly. Early molecular studies from forest ecosystems showed 34 and 47 AM fungal taxa recovered, respectively (Öpik et al. 2008, Öpik et al. 2009). More recent next generation sequencing (NGS) studies have recovered 117 AM fungal OTUs (operational taxonomic units; Bainard et al. 2015), showing that next generation sequencing allows for better characterization of AM fungal communities because of the increased sequencing depth. Morphological studies find the majority of AM fungal taxa to be from Glomerales, while molecular studies can uncover AM fungal taxa from all 4 Glomeromycotan orders.

1.3 Molecular studies and arbuscular mycorrhizal fungi

Morphological identification is difficult and time consuming. Culturing and identifying AMF using morphology is not an exact science as not all AMF produce spores or they can not be cultured (Smith and Read 2008). Studies that focus on culturing AMF, using spore numbers and trap cultures may not be good methods for characterizing AMF communities (Sanders 2004, Jeffries et al. 2003, Willis et al. 2013). Molecular techniques have thus become the typical method for the identification of AMF (Redecker and Raab 2006, Öpik et al. 2006, Rosendahl 2008, Öpik et al. 2014).

Molecular studies involving AMF use PCR primers based on ribosomal DNA (rDNA). Primers target rDNA genes because they have a high copy number, have variable regions and are highly conserved (Redecker et al. 2003). Specifically, most primers target the nuclear ribosomal small subunit (SSU). Numerous AMF-specific primers that target the SSU have been used effectively to characterize AM fungal species from culture collections and environmental samples (Simon et al. 1992, Sato et al. 2005, Lee et al. 2008). Although most primers target the SSU when studying AMF, a few studies have used the internal transcribed spacer (ITS) or large subunit (LSU) regions of the nuclear ribosomal operon (Gollotte et al. 2004, Krüger et al. 2009). The SSU is used because it was the first region used for molecular identification of Glomeromycota, so most information available is based on the SSU region (Öpik et al. 2014). Further studies are required to determine if the SSU is the best target region for AMF identification or if the ITS or LSU regions would be

better for species-level identification (Öpik et al. 2014). Molecular studies on AMF have begun using next generation sequencing to characterize AMF communities (Öpik et al. 2006, Öpik et al. 2009, Lumini et al. 2010, Bainard et al. 2015). Next generation sequencing is a high throughput DNA sequencing technology that builds DNA strands in a massively parallel fashion yielding more sequences than other sequencing methods such as Sanger sequencing. Next generation sequencing allows for more species to be detected from samples. The use of next generation sequencing will provide a depth of information regarding AMF communities that was unattainable with morphological studies.

Molecular studies and interest in AMF is increasing. There is now a curated database of reference sequences dedicated to the DNA-based identification of AMF (MaarjAM; http://maarjam.botany.ut.ee/ ; Öpik et al. 2010). MaarjAM has type sequences called virtual taxa that are helpful in comparing genetic relatedness between sequences. Virtual taxa are phylogenetically defined groups of SSU sequences that have a sequence identity greater or equal to 97 percent; currently there are 348 virtual taxa (Öpik et al. 2014). The MaarjAM database is based on the SSU rDNA gene sequences. The database also collects LSU and ITS sequences of rDNA and sequences of mitochondrial and protein-encoding genes that have been used to identify Glomeromycota (Öpik et al. 2014). This database is focused on streamlining molecular identification and collecting information on ecology and global distribution of AMF, which prior to this database was lacking.

1.4 Arbuscular mycorrhizal fungi and disturbance

Studies on the effects of disturbance on AMF are prevalent in recent research. Determining how AMF are affected by disturbance is important, because it will provide insight into the ecology of AMF, which is not well known. Most studies focus on disturbance related to agriculture, because AMF are associated with crop plants. Unfortunately, most studies focusing on disturbance and AMF utilized morphological and molecular methods that strongly constrained the species of AMF found. Studies using NGS will better characterize how disturbance affects the species composition of AMF.

The effects on AMF of the addition of abiotic factors such as nitrogen and phosphorus also have been studied. The addition of phosphorus-rich fertilizer does not affect spore density

or diversity of AMF (Mathimaran et al. 2005). Plants are often challenged to find available phosphorus in soil and a major benefit of AM symbiosis is the transfer of phosphorus to the plant from the AM fungal partner. A different trend is observed with N addition. Increased nitrogen additions caused a decrease in species richness of AMF and a shift in AMF communities to taxa with small spores, such as *Glomus* (Egerton-Warburton and Allen 2000).

Studies on disturbance related to agricultural practices mainly focus on tillage. Tillage disrupts the hyphal networks of AMF. The extraradical hyphae of AMF play an important role in AM symbiosis, so disruption of the network should have an effect on AMF. Soil disturbance decreases AMF species composition and richness (Boddington and Dodd 2000, Jansa et al. 2002, Lumini et al. 2010). Soil disturbance damages the external mycelial network and causes a decrease in the level of AMF colonization within plant roots (Jansa et al. 2003, Mirás-Avalos et al. 2011). Overall, tilled and no-tilled sites have different communities of AMF (Galvez et al. 2001, Jansa et al. 2002, Li et al. 2007, Lumini et al. 2010, Mirás-Avalos et al. 2011, Stover et al. 2012, Bainard et al. 2015).

Soil disturbance also decreases the overall biomass of AMF in roots and soil (Miller et al. 1995, Schnoor et al. 2011). Both spore density and hyphal length are negatively affected by tillage (Boddington and Dodd 2000, Jansa et al. 2002, Oehl et al. 2003, Galvez et al. 2001, Li et al. 2007). Infectivity of AMF and subsequent nutrient uptake by their host plants is reduced by the disruption of the hyphal network (Jasper et al. 1989, Evans and Miller 1990, Miller et al. 1995, Kabir et al. 1999, Mirás-Avalos et al. 2011). Tillage not only influences AMF, but also affects the soil. Tillage decreases aggregate stability by severing mycelia and causing a decrease in the production of glomalin (Wright and Upadhyaya 1996, Kabir et al. 1999). Thus, in general, agricultural disturbance has a negative influence on AMF, and more information is needed to understand how tillage and land use affect the success of plants and AMF in previously tilled land. This information would be helpful in determining if sites that were used for agriculture can be used in the restoration of endangered ecosystems, such as tallgrass prairies.

Although disturbance is known to affect AMF communities, the effect of disturbance on particular taxa of AMF is not well known. Some previous studies have suggested that there are disturbance-sensitive and disturbance-tolerant taxa. According to Hamel et al. (1994), disturbance increases disturbance resistant species which sporulate abundantly and decreases disturbance sensitive species which sporulate less, leading to differences in species composition of AMF. *Scutellospora* have been shown to be reduced in tilled soil and soil with increased nitrogen addition (Jansa et al. 2002, Jansa et al. 2003, Li et al. 2007, Egerton-Warburton and Allen 2000). *Glomus* spp. dominate agricultural soils and are suggested to be tolerant of disturbance (Jansa et al. 2003, Lumini et al. 2010, Mirás-Avalos et al. 2011, Bainard et al. 2015). *Glomus* spp. are also found in abundance in undisturbed soil, suggesting that this genus contains generalist species (Mathimaran et al. 2005, Lumini et al. 2010, Bainard et al. 2015). *Gigaspora* spp. are large-spored and suggested to be disturbance tolerant, because the spores are able to survive tillage and other soil disturbances (Hart and Reader 2004, Soteras et al. 2015). Information on Paraglomerales and Archaeosporales is limited, because these groups are hard to detect with previously utilized primers (e.g., the AM1 primer) that do not match with the SSU portion of these taxa (Redecker 2000, Alguacil et al. 2008). To increase knowledge of AM fungal ecology further, studies of how disturbance structures AM fungal communities and the disturbance threshold of different AM fungal species are necessary. Although most studies have been conducted on agricultural fields, the exploration of AMF community composition in ecosystems undergoing restoration would be beneficial, because habitat restoration and conservation of ecosystems is important for the maintenance of biodiversity.

1.5 Tallgrass prairies

The following information about tallgrass prairies in southern Ontario was based on Bowles (2005) and Rodger (1998). Tallgrass prairies (TGPs) are an endangered plant community type that are characterized by less than 10% tree cover and dominated by bunch-grasses such as Indiangrass (*Sorghastrum nutans*) and Big Bluestem (*Andropogon gerardii*). Tallgrass prairies have a high diversity of vascular plants beyond grasses; many of them rare and only found within remnant prairies. Southern Ontario once contained 100,000 hectares of TGP, but, due to conversion of TGP to agriculture and other land uses

less than one percent of TGP remains. The largest remnants of TGP in Ontario are located in Windsor and Walpole Island First Nation (WIFN).

Tallgrass prairies in southern Ontario are being restored by the Ontario Ministry of Natural Resources and Forestry (MNRF), the Nature Conservancy of Canada (NCC) and by smaller agencies like the *rare* Charitable Research Reserve (http://raresites.org/). Tallgrass prairies in southern Ontario are being restored to ensure that certain endangered species which only grow and live in TGPs are maintained and protected. Remnant Ontario TGPs contain many endangered species, such as Eastern Prairie White Fringed-orchid (*Platanthera leucophaea*) and Small Fringed Lady's-slipper (*Cypripedium candidum*) (Rodger 1998, Bowles 2005). The plant diversity found within southern Ontario remnant TGPs provides shelter and food for a variety of vertebrate and invertebrate animals (Rodger 1998). Some animals supported by TGPs are threatened, such as Butler's garter snakes (*Thamnophis butleri*), or endangered, such as spotted turtles (*Clemmys guttata*) (Rodger 1998, Bowles 2005).

1.6 Tallgrass prairies and arbuscular mycorrhizal fungi

I am surveying AMF communities in TGPs in southern Ontario, because studies in these areas are limited (Chokroborty Hoque 2011, Stover et al. 2012). Previous studies were unable to utilize the improved molecular techniques that are now available for characterizing AMF communities.

Tallgrass prairies are a type of grassland shaped by AMF. The predominant mycorrhizal relationship in TGPs is with AMF. Tallgrass prairies are dominated by warm-season C_4 grasses, such as *Andropogon gerardii* (Big Bluestem), that have high mycorrhizal dependence (Hartnett and Wilson 1999, McCain et al. 2011). Dominant grasses typical of TGPs in southern Ontario require AM fungal associations to receive the soil nutrients that they are unable to take up due to their small root absorptive area.

Due to the importance of AM symbioses in grasslands, many studies of the interaction between AMF and plant communities have been conducted. However, the patterns of AM fungal diversity and composition are still not well known in TGPs. Understanding the

relationship between AM fungal communities and aboveground plant communities is important for the conservation of TGPs. Plant productivity and diversity in TGPs is influenced by the species composition of AMF (van der Heijdan et al. 1998, Eom et al. 2000, Vogelsang et al. 2006). Past studies suggest that AMF shape TGP plant communities due to AMF having some degree of host specificity (van der Heijdan et al. 1998, Hartnett and Wilson 1999, Smith et al. 1999, Vogelsang et al. 2006). Plant community composition in TGPs is altered by the suppression or disruption of the AM fungal community (Hartnett and Wilson 1999, Smith et al. 1999). In studies where AMF were suppressed with fungicides, the abundance of dominant warm-season grasses decreased, while C_3 grasses and forbs, which have lower mycorrhizal dependencies, increased in abundance (Hartnett and Wilson 1999).

The conversion of TGP to agriculture and other land uses has endangered this type of grassland, which is habitat to many endangered plants and animals. Restoration attempts focus on re-establishing the aboveground plant communities, but the belowground microbial communities may also need to be restored. Because many restoration sites were previously agricultural fields, the AMF communities have been degraded by years of tillage, and fertilizer and fungicide application (Jasper et al. 1989, Douds and Milner 1999, Jeffries et al. 2003). Recent studies have investigated the use of commercial AM fungal inoculum versus AM fungal inoculum from native TGPs, and they suggest that inoculum from native TGPs – although more difficult – to obtain may improve restoration attempts (Smith et al. 1998, Paluch et al. 2013, Middleton et al. 2015). More information is needed on AM fungal communities of native and restored TGPs to better understand the influence of AMF on the restoration of TGPs.

1.7 Objectives

The purpose of my research was to understand better the ecology of arbuscular mycorrhizal fungi in Ontario tallgrass prairies, starting with an analysis of their community composition. The objectives of my research were to use next generation sequencing to describe the species composition of arbuscular mycorrhizal fungi in tallgrass prairies with different disturbance histories, and to determine if there are potential indicator taxa for the ends of the disturbance spectrum. I conducted surveys in thirteen tallgrass prairies with

different disturbance histories located in southern Ontario and determined the species composition of arbuscular mycorrhizal fungi in all of the sites. I predicted that recently disturbed sites would have a different species composition of arbuscular mycorrhizal fungi than historically or never disturbed sites.

Chapter 2

2 Materials and Methods

2.1 Sampling locations

Soil samples were collected from never tilled and restored tallgrass prairies in five sampling locations across southern Ontario (Figure 2.1). Thirteen different tallgrass prairies were sampled in June/July and October 2014 to account for AMF with different seasonal patterns. In Walpole Island First Nation there were five sites with different disturbance histories (Figure 2.2); disturbance histories are listed in Table 2. Four sites were converted from natural TGP to agricultural fields at one point in time. *Silphium* Prairie (0.96 ha; 42.628 N, 82.500 W) was the only prairie remnant among these five sites that was not known to have been disturbed by agricultural tillage. Eliza's Prairie (1.9 ha; 42.580 N, 82.489 W) was tilled in the late 1940s. A small part of Pottawatomi Prairie (13 ha; 42.550 N, 82.500 W) was briefly farmed prior to 1943. Mike's Field (0.3 ha; 42.580 N, 82.494 W) was a TGP converted to row crops, then abandoned from corn production in 1990. Since Mike's Field was unavailable for sampling in 2014, all data were from soil collected by Chokroborty Hoque (2011). Sandpits Field (1.67 ha; 42.627 N, 82.502 W) was located beside *Silphium* Prairie and was a TGP converted to agricultural production from 2002 to 2006. Plant communities at these sites were described by Stover et al. (2012). In Norfolk County, Ontario, one tallgrass prairie site (De Maere Home) was sampled; the disturbance history of the site is listed in Table 2. De Maere Home tallgrass prairie (20.9 ha; 42.685 N, 80.464 W) was a tobacco field until 2003, and then alternated between a corn and soybean crop until 2010 (McPhee et al. 2015). In 2010 the site was restored to a tallgrass prairie by the Nature Conservancy of Canada. In June 2010, a nitrogen addition experiment began at this location (McPhee et al. 2015). This location was sampled by Catomeris (2015) in 2014; only data from the control plots were utilized in my analyses. Near Cambridge, Ontario, one tallgrass prairie site was sampled; the disturbance history is listed in Table 2. Blair Flats (17.4 ha; 43.384 N, 80.373 W) was a corn field in 2007 and a soybean field in 2008 and 2009. In 2010 the site was restored to a tallgrass prairie by the *rare* Charitable Research Reserve (Craig et al. 2014). In Windsor, Ontario, two tallgrass prairie sites in the Ojibway Prairie Complex were sampled; the disturbance histories are

listed in Table 2. Ojibway Prairie Provincial Nature Reserve (105 ha) site 1 (42.263 N, 83.071 W) and site 2 (42.261 N, 83.068 W) had never been mechanically tilled for agriculture. The Ojibway Prairie Provincial Nature Reserve was farmsteads from around 1750 to early 1770s and may have been non-mechanically tilled (Lajeunesse 1960; Kenyon 1976). The Ojibway Prairie Provincial Nature Reserve is designated in this thesis as an example of a pristine tallgrass prairie, because it has been burned (earliest burn 2003) and contains species, such as dense blazing star (*Liatris spicata*), indicative of a pristine tallgrass prairie. In Windsor, Ontario, four tallgrass prairie sites part of the Herb Gray Parkway restoration project were sampled; the disturbance histories are listed in Table 2. The current 74 ha tallgrass prairie restoration occurring as part of the Herb Gray Parkway project is one of the largest tallgrass prairie restorations in southern Ontario. The Herb Gray Parkway Restoration project is being coordinated by the Ontario Ministry of Transportation (www.hgparkway.ca). Final restoration site (FRS) 32 (1.59 ha; 42.2717 N, 83.0691 W) was a Mineral Cultural Thicket until 2010 when it was brush cut and prepared for tallgrass prairie restoration (Willowleaf Aster Amendment Plan 2011). Final restoration site 23 (1.745 ha; 42.273 N, 83.069 W) was an old field located beside FRS 32 according to aerial photos. The disturbance histories for FRS 23 and 32 were not certain; both sites may have been mown, or used for pasture, because tree encroachment is not observed in an aerial photo from 1951. These sites were considered to be undisturbed. Final restoration site 27 (4.54 ha; 42.229 N, 82.994 W) and FRS 28 (7.7 ha; 42.2287 N, 82.9920 W) were fallow agricultural fields that were last tilled in 2009 (Willowleaf Aster Amendment Plan 2011).

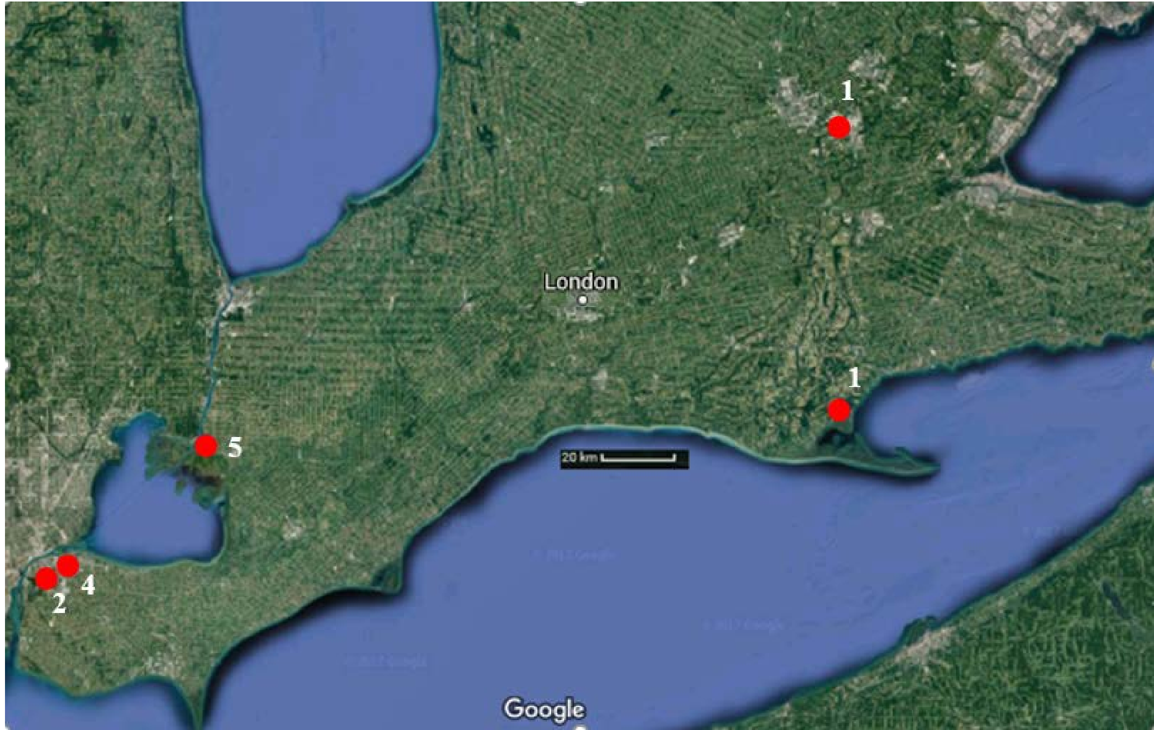

Figure 2.1. Thirteen tallgrass prairies in five sampling locations across southern Ontario. Number beside circle indicates number of tallgrass prairies sampled at each location. Map modified from Google Maps (2017).

Table 2.1. Thirteen tallgrass prairie sites across five sampling locations in southern Ontario and the year the site was last tilled or disturbed.

Sampling location	Site	Site abbreviation	Disturbance history	Disturbance group	Sampling dates
Walpole Island First Nation	*Silphium* Prairie	SI	Never tilled	Undisturbed	Jun/Oct 2009[a], Oct 2014
	Eliza's Prairie	EL	1940	Undisturbed	Jun/Oct 2009[a], Oct 2014
	Pottawatomi Prairie	PO	≤1943	Undisturbed	Jun/Oct 2009[a], Oct 2014
	Mike's Field	MI	1990	Disturbed	Jun/Oct 2009[a]
	Sandpits Field	SA	2006	Disturbed	Jun/Oct 2009[a], Oct 2014
Ojibway Prairie Provincial Nature Reserve	1	OPC1	1770	Undisturbed	Jul/Oct 2014
	2	OPC2	1770	Undisturbed	Jul/Oct 2014
Herb Gray Parkway	FRS 23	FRS 23	Unknown	Undisturbed	Jul/Oct 2014
	FRS 32	FRS 32	Unknown	Undisturbed	Jul/Oct 2014
	FRS 27	FRS 27	2009	Disturbed	Jul/Oct 2014
	FRS 28	FRS 28	2009	Disturbed	Jul/Oct 2014
rare Charitable Research Reserve	Blair Flats	BF	2009	Disturbed	Jun/Oct 2014
Norfolk County	De Maere Prairie	DM	2010	Disturbed	Jun/Oct 2014[b]

[a] Chokroborty Hoque (2011)

[b] Catomeris (2015)

2.2 Soil Sampling

A total of forty-eight soil samples were collected in June/July 2014 and seventy-two soil samples were collected in October 2014. At each site six 1 m^2 quadrats were selected haphazardly to best characterize the habitat and five soil cores (2.5 cm × 20 cm) were collected from each quadrat (approximately 15 cm on the diagonal from each corner and one from the centre), pooled and homogenized. The soil samples were kept on ice during transportation and frozen at -20 ˚C in the laboratory. All soil samples were wet-sieved using U.S.A. Standard testing sieves (VWR Scientific, West Chester, PA) with different mesh sizes (1.18 mm, 250 μm and 53 μm). A subsample (20 g) of each plot was suspended in a 200 mL glass jar with 200 mL of 0.1 M sodium pyrophosphate ($Na_4P_2O_7$) and shaken for five to ten minutes to break up soil colloids. The subsamples were poured over the three sieves and rinsed with dH_2O for one to two minutes. All organic material (hyphae, spores, roots, etc.) remaining on the three sieves (washed soil) was collected and placed into a falcon tube and frozen at -20 ˚C. The washed soils were lyophilized in a Virtis Bench Top 3.5 L Freeze Dryer (J & M Scientific, Woburn, MA) overnight. All of the freeze-dried washed soils were ground separately with a mortar and pestle using liquid nitrogen.

2.3 DNA extraction with kit

DNA was extracted using a Soil Microbe DNA MicroPrep™ kit (Zymo Research, Irvine, CA). DNA was extracted from all of the June/July and October samples. Subsamples (0.25 g) of the ground washed soils were used as the substrate for DNA extraction. Each sampling site had six separate DNA extracts (one for each sampling quadrat). Each subsample of soil was added to a bead-bashing tube and processed in a FastPrep™ FP210 machine (Bio101, Qbiogene, Inc., Carlsbad, CA, USA) at a speed 4.0 for 30 seconds, causing physical lysis and homogenization of the soil. The lysate was filtered on a Zymo-Spin™ IV Spin Filter and the Soil DNA Binding Buffer was added. The Soil DNA Binding Buffer was filtered on a Zymo-Spin™ IC Column and washed with the Pre-Wash Buffer followed by the Soil DNA Wash Buffer. The DNA was eluted from the Zymo-Spin™ IC Column with 50 μL of DNA Elution Buffer at 60 ˚C into a 1.5 mL microcentrifuge tube for storage. The concentrations of the DNA extracts were determined using a NanoDrop 2000

Spectrophotometer (Thermo Scientific) and diluted to 20 ng/ µL with mH_2O. The diluted DNA extracts were stored in a freezer at -20 ˚C prior to PCR amplification.

2.4 Past soil sampling

Soil samples collected previously by Chokroborty Hoque (2011) from the five sites in Walpole Island First Nation (listed above) had been kept frozen at -20 ˚C and were processed using the same procedure as the 2014 samples. The samples collected by Chokroborty Hoque (2011) were added to this study to better characterize the AM fungal communities present in those sites, because the molecular techniques and primers have improved since the original study. Soil samples from each of the five sample sites were collected in both June and October 2009, whereas only four of the sites were sampled in October 2014.

2.5 Molecular analysis

The extracted DNA was PCR amplified with a Glomeromycota-specific primer pair (AMV4.5N-F(AAACTCGTAGTTGAATTTCG)/AMDG-R (CCCAACTATCCCTATTAATCAT) (Sato et al. 2005). This Glomeromycota-specific primer pair targets the V4 variable region of the small ribosomal subunit (Figure 2.2; Sato et al. 2005). These primers have been shown to detect successfully arbuscular mycorrhizal fungi from all four orders of Glomeromycota (Glomerales, Diversisporales, Archaeosporales and Paraglomerales) from environmental samples (Sato et al. 2005, Lumini et al. 2010, Dai et al. 2013, Bainard et al. 2015). The PCR mix for the Glomeromycota specific primer pair consisted of 9.0 µL of DNA extract, 12.5 µL of AccuStart II PCR ToughMix (Quanta Biosciences, Gaithersburg, MD), 0.5 µL 50x loading dye, and 1.5 µL of each primer with a final reaction volume of 25 µL. Samples were amplified using a T1 plus Thermocycler (Biometra, Montreal) with an initial denaturing step of 2 min at 94 °C, followed by 29 cycles of 30 s of denaturation at 94 °C, 30 s of annealing at 55 °C, and 18 s of elongation at 72 °C. The data obtained from the Glomeromycota-specific primer pair can be found in Appendix A.

The extracted DNA was also PCR amplified with the BG primer pair (LSU200-F(AACKGCGAGTGAAGMGGGA)/LSU481-R(TCTTTCCCTCACGGTACTTG) (Asemaninejad et al. 2016). The BG primer pair targets the D1 variable region of the ribosomal large subunit and is semi-selective for Basidiomycota and Glomeromycota (Figure 2.2; Asemaninejad et al. 2016). The PCR mix for the BG primer pair consisted of 4 µL of DNA extract, 12.5 µL of AccuStart II PCR ToughMix (Quanta Biosciences, Gaithersburg, MD), 0.5 µL 50x loading dye, 3 µL of each primer, and 2 µL mH_2O with a final reaction volume of 25 µL. Samples were amplified with the BG primer pair with an initial denaturation at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 18 s (Asemaninejad et al. 2016). Sequences obtained using the BG primers were not included in statistical analyses because they did not produce a representative sample of Glomeromycota. The data obtained from the BG primers can be found in Appendix B.

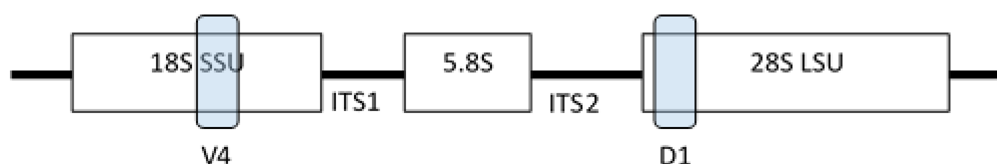

Figure 2.2. Schematic representation of the nuclear ribosomal DNA regions. Vertical boxes represent the two regions amplified by the primer pairs used.

The PCR products were screened for positive amplicons on a 1.5% agar-agar gel containing 0.5 µg/mL ethidium bromide. All PCR products from a site were pooled into the same tube. Multiple PCR amplifications in each site were done to increase the chances of amplifying all AM fungal DNA in the site. Pooled PCR products were then submitted to the London Regional Genomics Centre (Robarts Research Institute, London, ON) for sequencing using the Illumina MiSeq platform using a 2 x 300 v3 kit (Illumina, San Diego, CA). The PCR products were equilibrated using a Qubit fluorometer (Life Technologies) and multiplexed with other samples prior to the Illumina run. In multiplex sequencing individual samples must have unique barcodes to allow for identification following sequencing.

2.6 Bioinformatics

The Illumina MiSeq output was processed through a pipeline provided by Dr. Greg Gloor (Biochemistry Department, The University of Western Ontario). The pipeline can be assessed through the GitHub repository (https://github.com/ggloor/miseq_bin/tree/Jean). To overlap the raw forward and reverse reads, the raw output was processed using PANDAseq (https://github.com/neufeld/pandaseq; Masella et al. 2012). The overlapped output was clustered into identical sequence units (ISUs) and UCHIME (Edgar et al. 2011) was used to remove chimeras. The ISUs were processed using USEARCH v7.0.1090 (Edgar 2010) to cluster the ISUs into operational taxonomic units (OTUs) based on 97% similarity. Rare OTUs (less than 0.1% abundance) were removed from the analyses. A total of three Illumina MiSeq runs were conducted for all of the sites. The data from the three runs were combined into one to be able to compare OTUs from all samples. The OTUs were identified by a nucleotide BLAST search in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and then a final identification comparing with known reference sequences from the MaarjAM database of Glomeromycota sequences (http://maarjam.botany.ut.ee/; Öpik et al. 2010). The sequences were also matched with their respective virtual taxon (Öpik et al. 2010). All OTUs that were non-Glomeromycotan (<90% similarity to known AMF sequences) were removed from further analyses.

2.7 Transformation of sequence data

The transformation of sequence data was conducted in R (RStudio Team 2016). To account for the small proportion of DNA that is extracted from environmental samples and help with the uncertainty of the true number of counts for each OTU the data underwent a centered log-ratio transformation (Fernandes et al. 2013). The count data are transformed to ratios preserving the correspondence to the original sequence data. Transforming the data reduces the number of false positives (Fernandes et al. 2013). The ratios are linearly related allowing for the use of standard statistical analyses. The centered log-ratio transformation is calculated as follows:

$$\log_2\left[\frac{count\ for\ each\ OTU}{geometric\ mean\ of\ all\ OTUs\ in\ sample}\right]$$

2.8 Statistical analyses

To analyze qualitatively the transformed sequence data compositional biplots were constructed using the compositions package in R (van den Boogaart and Tolosana-Delgado 2008). The compositional biplots were conducted using the Phi metric. The Phi metric is a standardized ratio that is good at showing associations (Lovell et al. 2015). Compositional biplots summarize all essential results and are a great tool for describing compositional data. The cluster dendrogram using the Aitchison distance metric and the Ward D2 clustering method was created using the compositions package in R (van den Boogaart and Tolosana-Delgado 2008) to visualize how the sampling locations cluster and the relative abundances of OTUs present at each of the sites.

To determine significant differences in the abundance of individual OTUs between "treatment groups" (disturbed and undisturbed sites as defined in Table 2.1), I used the ANOVA-Like Differential Expression tool (ALDEx2), which uses 1000 Dirichlet Monte Carlo replicates to infer abundance from reads and provides p-values corrected of multiple comparisons (Fernandes et al. 2013). ALDEx2 identifies significant taxa whose differences between groups is strong. ALDEx2 was conducted using the ALDEx2 Bioconductor package in R (Fernandes et al. 2013).
Similarly, a comparison between July and October samples were conducted using ALDEx2 and no differences were found. A comparison between samples collected by Chokroborty Hoque (2011) and my samples was conducted using ALDEx2 and no differences were found. Because these comparisons did not have any significantly different taxa they were combined for all analyses.

Disturbance histories were categorized into two "treatment groups" (disturbed and undisturbed sites as defined in Table 2.1). All disturbed TGP sites were combined as statistical replicates. All undisturbed TGP sites were combined as statistical replicates. The two "treatment groups" were used to determine if there was a significant difference between disturbed and undisturbed sites. The comparison between disturbed and undisturbed sites was conducted using ALDEx2 (Fernandes et al. 2013). The raw ALDEx2 p-values were adjusted using the Benjamini-Hochberg False Discovery Rate to help reduce false positives (Benjamini and Hochberg 1995). Results were used to construct a new

compositional biplot and cluster dendrogram using the significant taxa. A heat map of the significant taxa was created using Heatplus (Ploner 2015) and vegan (Oksanen et al. 2016) packages in R.

2.9 Phylogenetic analysis

The phylogenetic tree was built using MEGA version 7 (Kumar et al. 2016). The type sequences from the matching virtual taxa (http://maarjam.botany.ut.ee/; Öpik et al. 2010) were trimmed and added to the file containing the OTU sequences. These sequences were aligned using MUSCLE (Edgar 2004) in MEGA7. After alignment, a Neighbour-joining phylogenetic reconstruction was used to create a phylogenetic tree with bootstrapping analysis (1000 replicates; Felsenstein 1985, Saitou and Nei 1987), better to confirm the identifications of individual OTUs recovered and to show the evolutionary relationships among them.

Chapter 3

3 Results

3.1 Sequence information

A total of 3 113 434 reads were recovered through the Illumina Miseq platform. After grouping reads into identical sequences (ISUs) and removing those that were flagged as potential chimeras (13 754 ISUs), the remaining ISUs were clustered at 97% similarity to yield a total of 1 175 OTUs (operational taxonomic units). Seven samples were excluded from the statistical analyses, because they had very low read counts (less than 10000).

After classification in BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi), 177 OTUs were matched as Glomeromycota, represented by 870 307 reads, approximately 28% of the total reads recovered. All non-Glomeromycotan OTUs were removed from the following analyses. In my dataset, the Glomeromycotan OTUs were from nine genera in eight families, indicating good coverage of arbuscular mycorrhizal fungi (Figure 3.1a). The Glomeromycotan OTUs were identified by their best match in the MaarjAM database and assigned a virtual taxon identification (Appendix A; http://maarjam.botany.ut.ee/; Öpik et al. 2010). When comparing the proportion of OTUs I recovered to the OTUs accepted by the MaarjAM database for the major genera a similar trend was observed (Figure 3.1b). The 177 Glomeromycotan OTUs matched with 74 virtual taxa (Appendix A).

Sequences obtained using the BG primers were not included in statistical analyses, because they did not produce a representative sample of Glomeromycota, and could not be identified to virtual taxa because those are based on SSU sequences, not LSU as obtained using the BG primers (http://maarjam.botany.ut.ee/; Öpik et al. 2010). The Glomeromycotan OTUs generated from the BG primers were identified by their best match in BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The data obtained from the BG primers can be found in Appendix B. Mike's Field was excluded from Appendix B, because it had no reads.

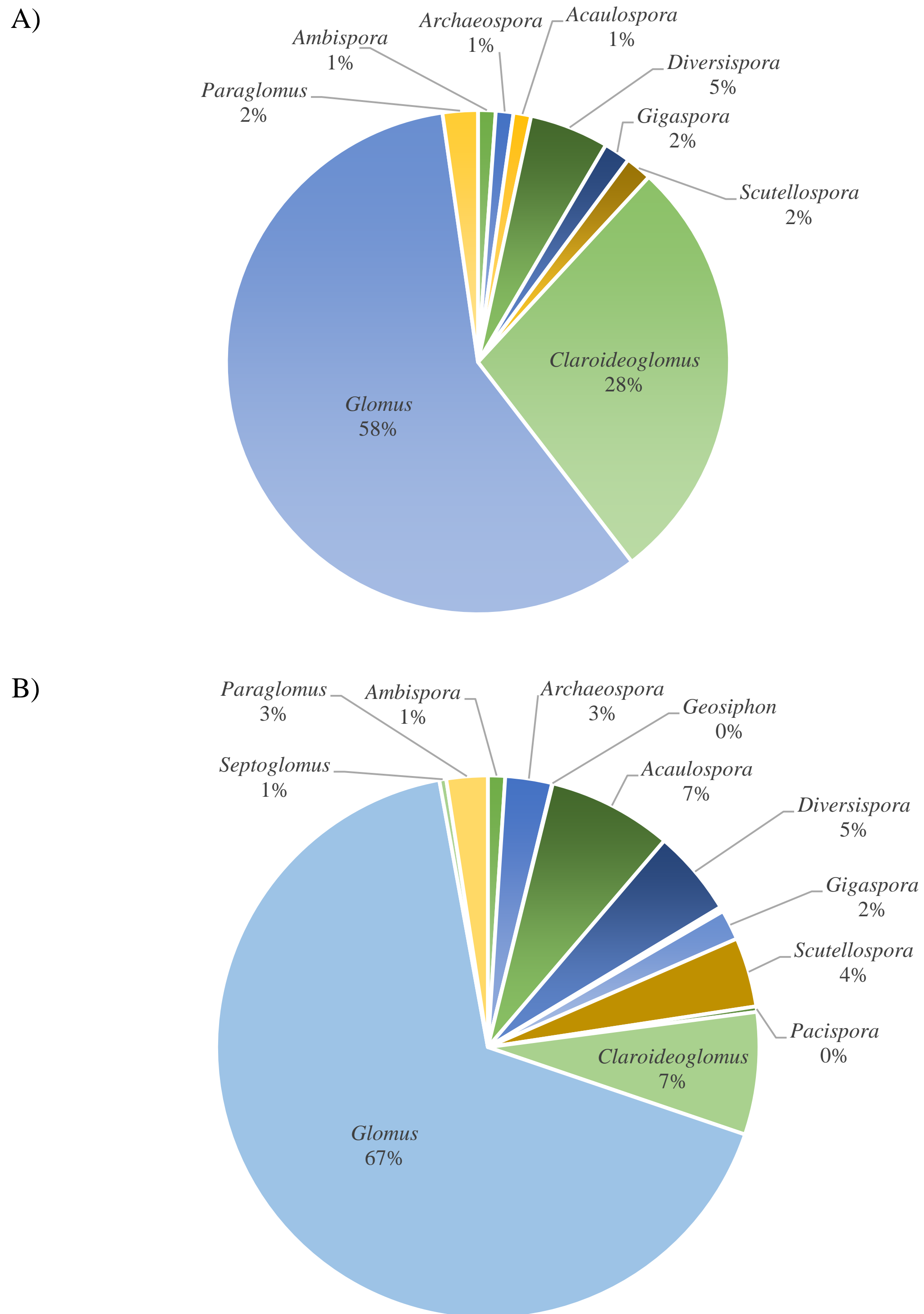

Figure 3.1. A) Genera of 177 Glomeromycotan OTUs obtained from field sampling in 13 tallgrass prairies in Ontario, showing the percentage of OTUs recovered. B) Proportion of OTUs accepted by MaarjAM (http://maarjam.botany.ut.ee/; Öpik et al. 2010) for the major genera.

3.2 Differences between seasons

ALDEx2 and a compositional biplot indicated that there were no differences between samples collected in October 2014 and the samples collected there in June 2009 by Chokroborty-Hoque (2011), and so these samples were combined.

June/July and October samples appear on both sides of the compositional biplot, indicating no split in the data between seasons (Figure 3.2). Principal components 1 and 2 explained 33.9% and 17.7% of the variation, respectively. ALDEx2 did not identify any significantly different OTUs between June/July and October, and so these samples were combined.

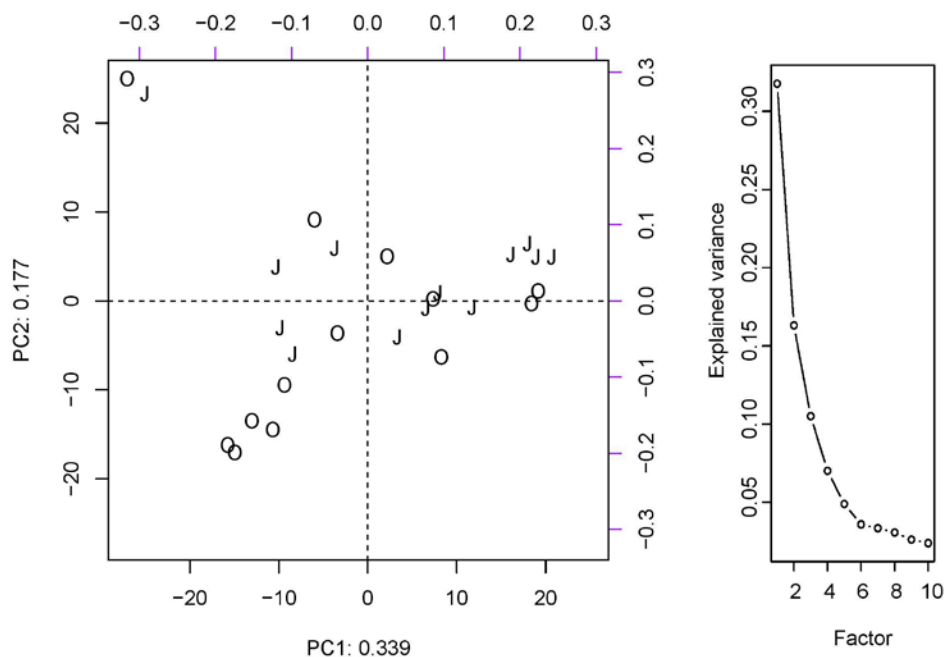

Figure 3.2. Compositional biplot with scree plot for 13 tallgrass prairie sites in Ontario sampled in June/July and October. Compositional data was centre-log transformed. All 177 Glomeromycotan OTUs were used. J= June/July samples and O= October samples.

3.3 Differences between disturbed and undisturbed tallgrass prairies

The initial qualitative comparison of disturbed and undisturbed TGPs, using all 177 OTUs, is shown in Figure 3.3. Principal component 1 and 2 explained 36.2% and 19.7% of the

variation, respectively (Figure 3.3). Principal component 1 appears to be explaining variation based on disturbance history, since disturbed and undisturbed sites appear on separate sides of the compositional biplot. There does appear to be clustering based on geographic location when all 177 OTUs are used (Figure 3.3).

Twenty-five OTUs were identified by ALDEx2 as having significant variation based on disturbance class (Table 3.1). The significant taxa identified by ALDEx2 had P-values less than 0.05 with the exception of OTU 4630, which was included because of its relatively large effect size of -1.02. According to the Benjamini-Hochberg correction only four OTUs had a truly significant difference between disturbed and undisturbed sites (Table 3.1). The four significantly different OTUs were found more abundantly in undisturbed prairies (OTU 222, 377, 611 and 3573). Therefore, there are significant differences between AMF communities in disturbed and undisturbed TGPs in this study. A phylogenetic tree of these significant taxa shows that, among the taxa selected for their significant response to disturbance, 14 OTUs of *Glomus*, 2 of *Ambispora* and 1 of *Diversispora* were found in undisturbed sites and 5 OTUs of *Claroideoglomus* and 2 of *Glomus* (OTUs 4552 and 4630) were found in disturbed sites (Figure 3.4).

The final qualitative comparison between disturbed and undisturbed TGPs used only the 25 significant taxa (Figure 3.5). In it, PC1 explains 67.4% of the variation and appears to be correlated with disturbance history, while PC2 explains 11.2% of the variation. All disturbed sites except Mike's Field are on the left side of the biplot, and the undisturbed sites are on the right (Figure 3.5). How the sites clustered can be observed in the cluster dendrogram (Figure 3.6), in which disturbance history formed the basis of the main division, within which sampling sites clustered by proximity.

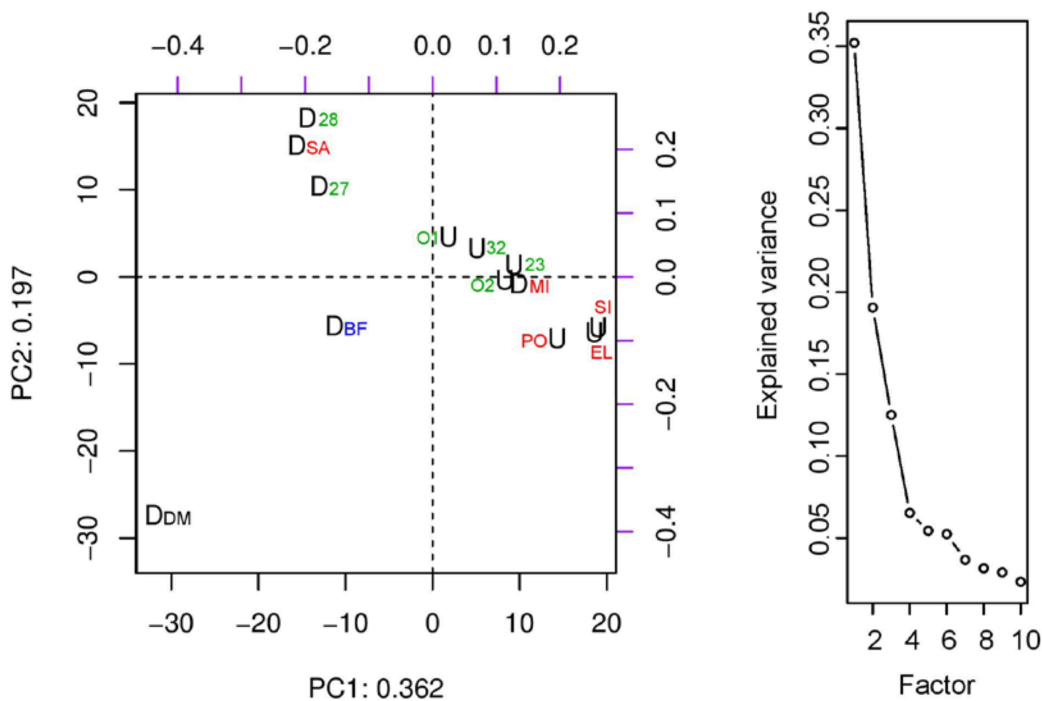

Figure 3.3. Compositional biplot with scree plot for 13 tallgrass prairie sites, combined data from June/July and October. Compositional data was centre-log transformed. All 177 Glomeromycotan OTUs were used. D= Disturbed samples and U= Undisturbed samples. SI= *Silphium* Prairie, EL= Eliza's Prairie, PO= Pottawatomi Prairie, MI= Mike's Field, SA= Sandpits Field, O1= OPC1, O2= OPC2, 23= FRS 23, 32= FRS 32, 27= FRS 27, 28= FRS 28, DM= De Maere Prairie, and BF= Blair Flats. Sites are colour coordinated based on the region the samples were collected (red, black, blue, and green).

Table 3.1. Table of significant taxa derived from ALDEx2 (P<0.05). Benjamini-Hochberg (BH) adjusted P-values included. Positive effect sizes reflect greater proportional abundance in undisturbed sites, while negative effect sizes reflect greater abundance in disturbed sites. * indicates the significant OTUs after the BH correction (P<0.05).

Taxa	diff.btw	diff.win	effect	overlap	P-value	BH P-value
OTU_19	4.740	2.790	1.398	0.122	0.01532	0.1316
OTU_60	7.535	2.298	2.588	0.102	0.00590	0.1020
OTU_75	6.498	3.911	1.484	0.054	0.00718	0.1028
OTU_96	5.548	4.947	1.053	0.133	0.03599	0.1671
OTU_109	5.826	3.435	1.504	0.076	0.01081	0.1168
OTU_124	6.796	5.430	1.181	0.135	0.03125	0.1602
OTU_169	8.450	3.572	1.808	0.105	0.01037	0.1155
OTU_179	-5.139	4.479	-1.035	0.144	0.03740	0.1773
OTU_222	5.188	2.309	2.221	0.000	0.00019	0.0171*
OTU_282	6.159	4.858	1.190	0.082	0.01479	0.1242
OTU_377	6.533	3.324	2.014	0.006	0.00252	0.0472*
OTU_554	-4.633	4.215	-1.015	0.097	0.04092	0.1663
OTU_587	5.240	4.218	1.175	0.085	0.01657	0.1285
OTU_604	5.221	4.119	1.336	0.060	0.01102	0.1093
OTU_611	6.761	3.019	2.170	0.003	0.00258	0.0490*
OTU_846	5.232	4.587	1.066	0.122	0.03443	0.1577
OTU_3028	4.556	3.899	1.099	0.122	0.04900	0.1857
OTU_3338	-3.956	3.190	-1.108	0.134	0.02115	0.1492
OTU_3359	8.575	3.800	1.706	0.146	0.01314	0.1245
OTU_3573	6.633	2.688	2.455	0.000	0.00277	0.0497*
OTU_4431	-4.992	4.831	-1.035	0.096	0.04534	0.1795
OTU_4514	5.379	3.021	1.636	0.018	0.00928	0.1041
OTU_4552	-4.203	3.327	-1.293	0.039	0.00774	0.1015
OTU_4630	-5.213	4.769	-1.016	0.128	0.05599	0.2028
OTU_4646	-4.816	3.845	-1.120	0.120	0.03125	0.1601

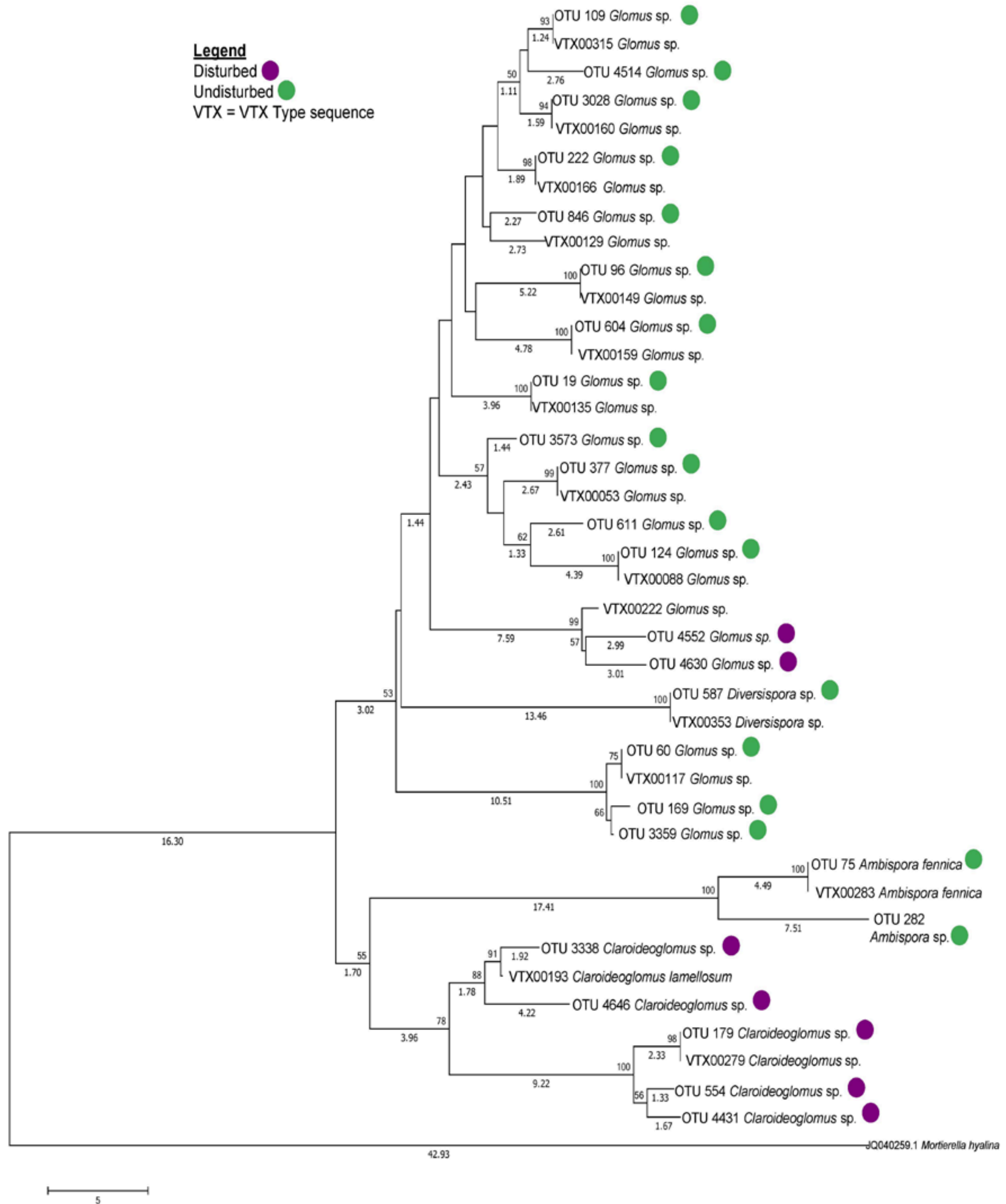

Figure 3.4. Evolutionary relationships of 25 taxa differing significantly in their occurrence between disturbed and undisturbed TGPs in Ontario. Type sequences for the matching virtual taxa were included. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016).

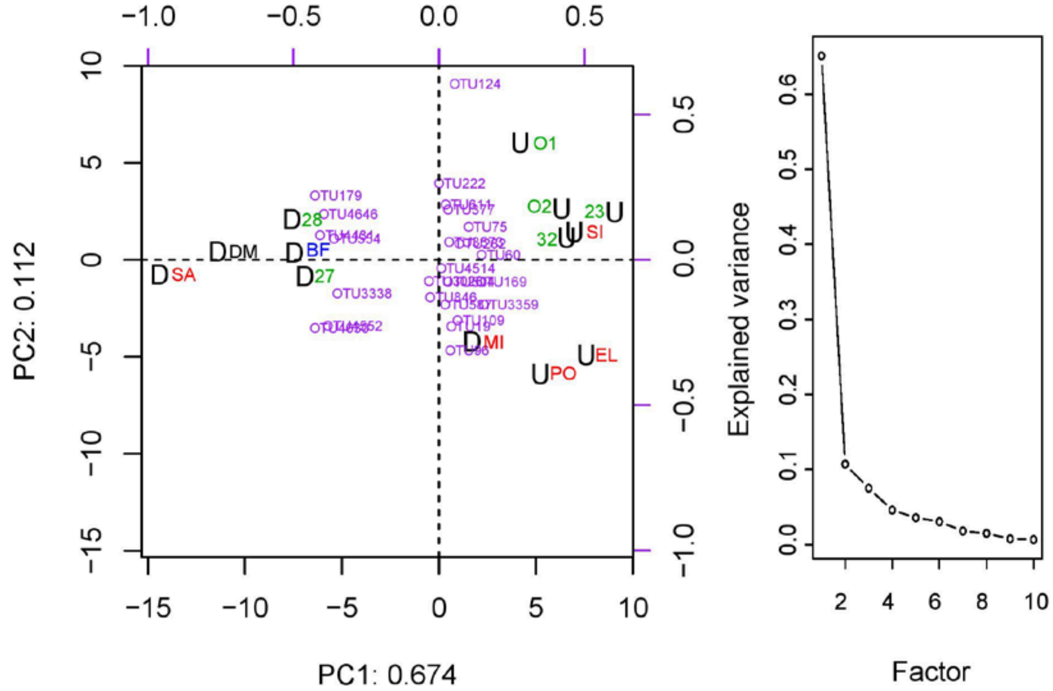

Figure 3.5. Compositional biplot with scree plot for 13 tallgrass prairie sites, combined data from June/July and October. Compositional data was centre-log transformed. Twenty-five important OTUs determined using ALDEx2 were used. D= Disturbed samples and U= Undisturbed samples. SI= *Silphium* Prairie, EL= Eliza's Prairie, PO= Pottawatomi Prairie, MI= Mike's Field, SA= Sandpits Field, O1= OPC1, O2= OPC2, 23= FRS 23, 32= FRS 32, 27= FRS 27, 28= FRS 28, DM= De Maere Prairie, and BF= Blair Flats. Sites are colour coordinated based on region samples were collected (red, black, blue, and green).

3.4 Arbuscular mycorrhizal communities and potential indicator taxa

In total, nine genera of Glomeromycota were found (Figure 3.1). The majority of the taxa were *Glomus* (103 OTUs) and *Claroideoglomus* (49 OTUs) species. This study also found representative of *Diversispora* (9 OTUs), *Gigaspora* (3 OTUs), *Scutellospora* (3 OTUs), *Acaulospora* (2 OTUs), *Ambispora* (2 OTUs), *Paraglomus* (4 OTUs) and *Archaeospora* (2 OTUs), which shows the success of the AMF primers used.

The differing AMF communities of disturbed and undisturbed TGPs can be observed visually in the relative abundance bar plots below the cluster dendrogram (Figure 3.6) as well as the phylogenetic tree (Figure 3.4). The disturbed TGPs were characterized by 7 OTUs whereas the undisturbed TGPs were characterized by 18 OTUs (Figure 3.4; Figure 3.6).

The dendrogram supports the clustering of sites based on disturbance history (Figure 3.6), with the exception that Mike's Field clustered with the undisturbed sites. The pattern of OTUs differs between disturbed and undisturbed sites. The dendrogram shows that undisturbed sites are dominated by OTU 19 and OTU 60 (both *Glomus* sp.). Disturbed sites have less of a distinctive pattern when it comes to the relative abundance of OTUs present across the different sites within the treatment group (Figure 3.6). Disturbed sites have a larger abundance of OTU 3338, OTU 4630, and OTU 179 (Figure 3.6; Figure 3.7). A heatmap of relative abundances shows that the OTUs may occur in both disturbed and undisturbed TGPs but at a significantly lower abundance in one than the other (Figure 3.7). For a full list of all 177 OTUs and which sites they were found see Appendix A.

The significant taxa may be indicator taxa of disturbed and undisturbed TGPs. The disturbed TGPs are dominated by *Glomus* and *Claroideoglomus* (Figure 3.4; Figure 3.6). The undisturbed TGPs have *Glomus* and *Claroideoglomus*, but they also contain *Ambispora* and *Diversispora* (Figure 3.4; Figure 3.6). *Ambispora* and *Diversispora* may be indicators of undisturbed TGPs. *Glomus* and *Claroideoglomus* may be disturbance-tolerant taxa or generalists.

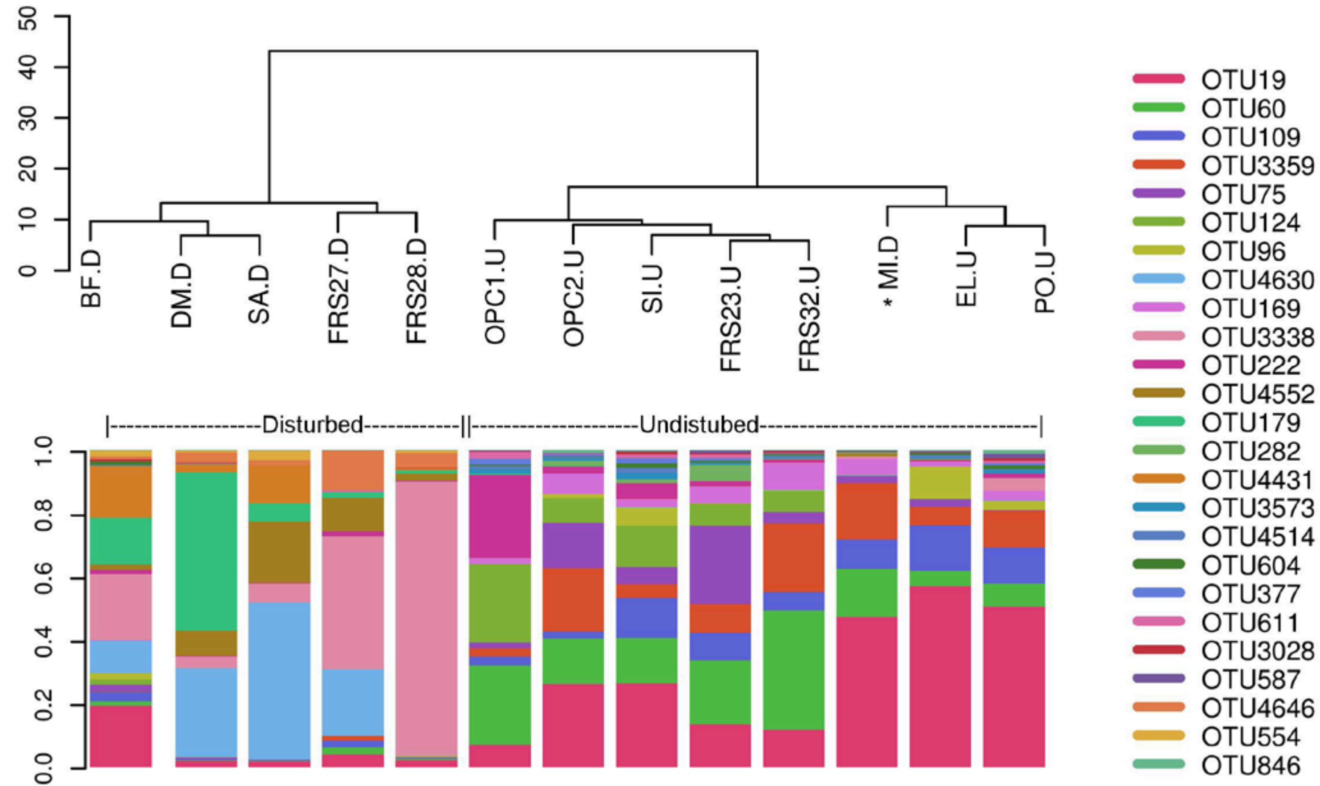

Figure 3.6. Cluster dendrogram and relative abundance bar plots for 13 tallgrass prairies with different disturbance histories in Ontario. Clustering based on the Aitchison distance metric and the Ward D2 clustering method. Compositional data was centre-log transformed. Twenty-five significant OTUs were used. D= Disturbed samples and U= Undisturbed samples. * indicates disturbed site that clustered with undisturbed sites. For sampling site abbreviations see Table 2.1. For OTU identification see Appendix A.

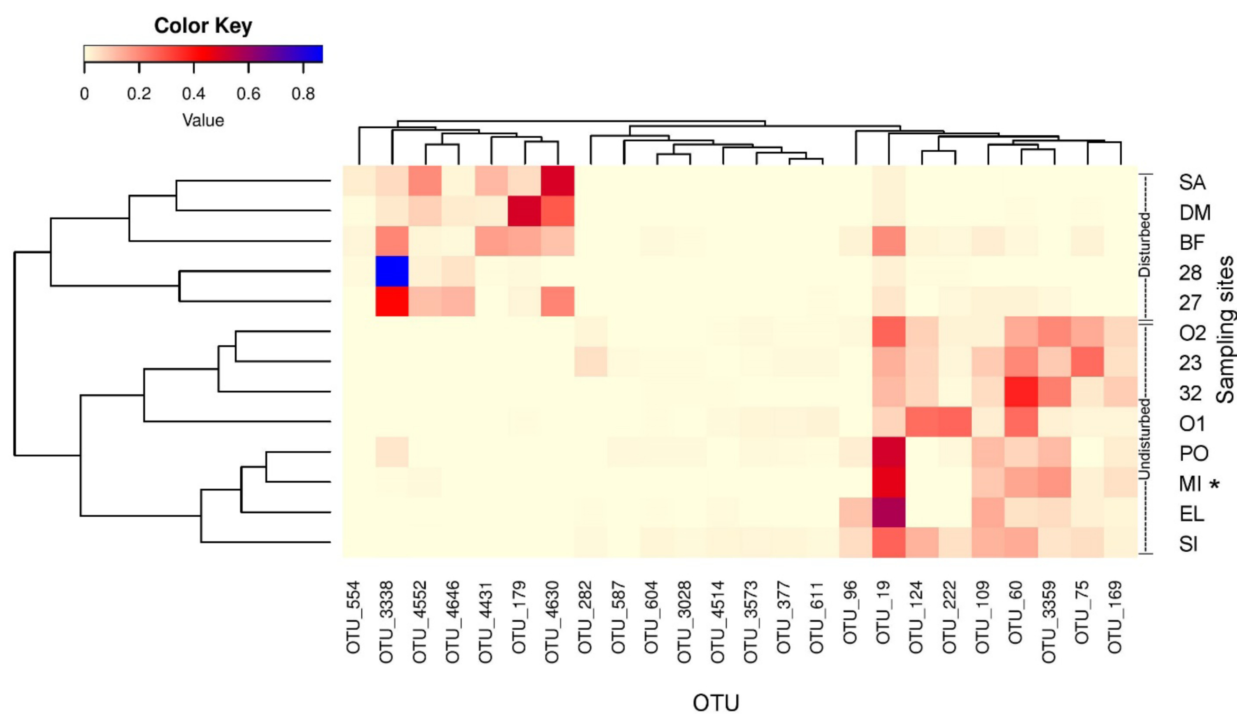

Figure 3.7. Heatmap of 25 significant OTUs with different relative abundances between disturbed and undisturbed tallgrass prairies. For OTU identification see Appendix 1. SA= Sandpits, DM= De Maere, BF= Blair Flats, 28= FRS 28, 27= FRS 27, O2= OPC2, 23= FRS 23, 32= FRS 32, O1= OPC1, PO= Pottawatomi, MI= Mike's Field, EL= Eliza's Prairie, and SI= Silphium Prairie. * indicates disturbed site that clustered with undisturbed sites. Heatmap created using Heatplus (Ploner 2015) and vegan (Oksanen et al. 2016) packages in R. Clustering of sampling sites is shown in the dendrogram (left) and phylogenetic clustering of OTUs in the dendrogram across the top.

Chapter 4

4 Discussion

In this study, 13 tallgrass prairies (TGPs) in Ontario were sampled to characterize arbuscular mycorrhizal fungal communities in disturbed and undisturbed TGPs. Understanding the effect that disturbance has on the species composition of arbuscular mycorrhizal fungi (AMF) is beneficial, because information on this aspect of the ecology of AMF is lacking. This is the first study to use next generation sequencing to explore AM fungal communities in Ontario TGPs. The study shows that Ontario TGPs host a variety of AMF. A total of 177 operational taxonomic units (OTUs), representing nine genera and all four orders of Glomeromycota, were recovered from the Ontario TGPs surveyed. Molecular studies of AMF using next generation sequencing are increasing, because they allow for discovery of a greater diversity than previous methods based on culturing or spore isolation and micromorphology, or cloning and sequencing of PCR products from soil-extracted DNA (Chokroborty Hoque 2011, Stover et al. 2012). For example, this study detected 160 OTUs of AMF from five prairie sites in WIFN, compared to 16 species found in the same sites using a trap culture and spore micromorphology approach (Stover et al. 2012) or 19/14 OTUs found using a PCR, cloning and sequencing approach with two different primers (Chokroborty Hoque 2011). These preliminary studies were able to detect some effect of agricultural disturbance on AMF community composition (Chokroborty Hoque 2011, Stover et al. 2012). The methods used in this study will be a good reference for future studies focusing on the community composition of AMF.

The MaarjAM database of curated AMF reference sequences is a great resource for AMF studies and aided in the identification of the OTUs recovered (http://maarjam.botany.ut.ee/ ; Öpik et al. 2010). As this database grows the uncertainty of the identification of AMF will decrease improving the detail and precision of future studies on AMF diversity.

The AMV4.5N-F/AMDG-R primer pair (Sato et al. 2005) with the Illumina Miseq high-throughput sequencing platform was a great combination for sequence recovery of AMF. Other studies using this primer pair found similar success in recovering AMF. Two studies focusing on AMF communities in Canadian prairies using the AMV4.5N-F/AMDG-R

primer pair found 122 Glomeromycotan OTUs (Dai et al. 2013) and 117 Glomeromycotan OTUs (Bainard et al. 2015). A study by Lumini et al. (2010) used two AMF specific primer pairs, of which AMV4.5NF/AMDG-R (Sato et al. 2005) recovered 117 OTUs of Glomeromycota and NS31/AML (Simon et al. 1992, Lee et al. 2008) recovered 28 OTUs. The NS31/AML primer pair in another study recovered OTUs matching with 133 AM fungal VTs (García de León et al. 2016), whereas in this study my 177 OTUs matched with 74 VTs. When comparing the OTUs recovered in this study with the OTUs/taxa accepted by the MaarjAM database, similar proportions of genera were recovered (Figure 3.1).

4.1 Arbuscular mycorrhizal fungal communities in Ontario tallgrass prairies

Some studies have shown there to be seasonal differences in community composition of AMF (Husband et al. 2002, Vandenkoornhuyse et al. 2002), while others state that there are no seasonal differences in the community composition of AMF (Rosendahl and Stukenbrock 2004, Santos-Gonzales et al. 2007). My study did not show seasonal differences in the species composition of AMF in the TGPs studied, but lacked sufficient information to make definite conclusions. When examining the compositional biplot (Figure 3.2) there is no decisive trend observed of the samples from the two different seasons (June/July and October) grouping together. In this study, each site had one replication per season, so for statistical analyses the sites were combined into statistical replicates for determining seasonal differences. The combination of all sites into statistical replicates could have decreased the chances of observing seasonal differences, because many factors could be influencing how the sites clustered together. Multiple replications per site within the same year would allow for June and October AMF communities for each site to be compared. If comparisons between different seasons within the same site are used it would be a better method for determining seasonal differences in AMF communities. The growth stages of AMF have been shown to differ throughout the seasons, with hyphal biomass peaking in spring and autumn and spore density – for some species – peaking in autumn (Klironomos et al. 1993, Galvez et al. 2001). Understanding seasonal differences in AMF can help us to determine the best time to collect soils to ensure a representative

sample of the AMF community. More studies are needed to determine if there are seasonal differences in AM fungal communities of TGPs.

This study provides the first baseline for the diversity of AMF in Ontario TGPs, and improved depth and resolution over previous studies of AMF in TGPs of Walpole Island First Nation (Chokroborty Hoque 2011, Stover et al. 2012). Nonetheless, it should still be considered a preliminary assessment, for various reasons. The primer pair used amplified a section of the SSU approximately 250 bp long, and when looking at species differences between AMF that may not be a sufficient length. Thiéry et al. (2012) sequenced a 4960 bp segment of SSU, ITS and part of the LSU, and suggested there is low intra- and interspecific variation of the small subunit regions typically studied. This suggests that a larger section of the ribosomal operon may be needed when studying AM fungal communities. Secondly, this study did not consider the plant populations or soil characteristics of the TGPs, which have been shown to influence AMF communities (Egerton-Warburton and Allen 2000, Eom et al. 2000, Bever et al. 2001, Helgason et al. 2002, Husband et al. 2002, Vandenkoornhuyse et al. 2002). The study was small-scale with only one replication for each season, which decreases the information that could have been obtained. Ideally, multiple replications over multiple years would have provided a more thorough characterization of the AMF communities of Ontario TGPs. However, in this preliminary assessment, the number of OTUs of Glomeromycota obtained was greater than what was found in most studies that used the AMV4.5NF/AMDG-R with 454 pyrosequencing (Lumini et al. 2010, Dai et al. 2013, Bainard et al. 2015), and much greater than earlier studies of the same sites using culture-based or cloning and sequencing methods (Chokroborty Hoque 2011, Stover et al. 2012).

The majority of OTUs recovered (103 of 177) best matched sequences in MaarjAM that were identified as *Glomus* spp. Unfortunately, *Glomus* is a vaguely defined genus that includes many unnamed species, and such OTUs should be considered Glomerales incertae sedis (Redecker et al. 2013). This finding is similar to those of other studies focusing on the species composition of AMF. Lumini et al. (2010) recovered 117 Glomeromycotan OTUs, 76.5% of which matched closest to Glomerales. Other studies found similar proportions of Glomeraceae with 76 OTUs (Bainard et al. 2015) and 78 OTUs (Dai et al.

2013) out of approximately 100 Glomeromycotan OTUs. *Claroideoglomus* had the second highest proportion of OTUs, also following with past studies (Oehl et al. 2003, Lumini et al. 2010, Dai et al. 2013, Bainard et al. 2015). Glomeraceae are the most prevalent taxa found in both molecular and past morphological studies. The detection of genera outside the Glomeraceae and the Claroideoglomeraceae (i.e. members of Archaeosporales and Paraglomerales) is a good indicator that in this study a good range of Glomeromycota were recovered (Appendix A). Some past studies have been unsuccessful at detecting Archaeosporales and Paraglomerales (Eom et al. 2000, Li et al. 2007, Alguacil et al. 2008).

4.2 Arbuscular mycorrhizal fungi and disturbance

The hyphal networks of AMF are extensive, ranging across multiple plant hosts throughout an ecosystem (Smith and Read 2008). Agricultural tillage disrupts the hyphal networks of AMF, not only for the interaction with one host plant, but for all host plants connected in the vast web of hyphae produced by AMF (Jasper et al. 1989, Smith and Read 2008). The disruption of the hyphal network has negative effects on AMF and on the associated plants. When tillage destroys the hyphal network AMF species richness and density decreases (Boddington and Dodd 2000, Jansa et al. 2002). Soil disturbance negatively affects the infectivity of AMF and nutrient uptake by their host plants, causing a decrease in the mutualistic symbiosis AMF have with their host plants (Jasper et al. 1989, Evans and Miller 1990, Miller et al. 1995, Kabir et al. 1999, Mirás-Avalos et al. 2011). Infectivity is negatively affected by disturbance because the hyphal networks need to recolonize the host plant roots, and tillage reduces the number of active hyphae and damages the AMF associated with the plants, which are also disturbed (Kabir et al. 1999, Galvez et al. 2011, Mirás-Avalos et al. 2011). In the AM symbiosis, the hyphal network is essential, because the exchange and transport of nutrients depends on the mycelial network (Miller et al. 1995). Since tillage destroys the mycelial network, it would be expected that disturbed land has a different community composition of AMF than undisturbed land. Knowing the distinction between AMF communities in disturbed versus undisturbed sites is beneficial in understanding AMF ecology and the importance of AMF communities in ecosystem restorations.

My study supported my prediction (based on past research – Boddington and Dodd 2000, Jansa et al. 2002, Lumini et al. 2010, Chokroborty Hoque 2011, Stover et al. 2012) that disturbed TGPs would have a different species composition of AMF than undisturbed TGPs. A similar study conducted on prairies in western Canada also determined that land use history influences AMF community composition (Bainard et al. 2015). With this information, we can conclude that agricultural tillage disrupts the mycelial network of AMF and affects the species composition of AMF.

The negative effect of agricultural tillage on AMF filters up to the host plants, which also may be affected negatively. The negative effects on AMF are informative, because agricultural land is typically used for the restoration of TGPs. Agricultural lands have altered AM fungal communities; therefore, more research is needed regarding the influence of AM fungal communities on restoration projects. It has been stated that restoration should also focus on belowground rehabilitation, not just the aboveground plant community (Smith et al. 1998, Li et al. 2007, Paluch et al. 2013, Middleton et al. 2015). Recent studies have suggested that AM fungal inoculum should be transplanted from native ecosystems to restoration sites to have better success at a complete ecosystem restoration (Smith et al. 1998, Paluch et al. 2013, Middleton et al. 2015). Native AM fungal inocula have been isolated from native prairies and identified using morphology; the inocula were then pot-cultured using *Sorghum bicolor* and incorporated into prairie restorations (Middleton et al. 2015). The Herb Gray Parkway Restoration utilized soil block transplants from remnant TGPs in Windsor that needed to be relocated, but sampling was prohibited from these specific areas. Soil block transplants have been shown to be beneficial for plant establishment in restorations (Conlin and Ebersole 2001, Bay and Ebersole 2006), but their effect on AM fungal communities has not been studied. Using native inoculum is more time demanding and expensive, but this study supports the idea that using native inoculum may improve ecosystem restorations. If healthy and infective AMF propagules (spores and hyphae) are readily available, they will be able to better aid host plants, because there will be less damage to the AM fungal community. Host plants would benefit from healthy AMF in a restoration, because the plants would be able to gain the benefits of the AM symbiosis quicker. This may cause the restoration site to establish the important characteristics of the desired ecosystem sooner than a site with a damaged AM fungal community. A healthy

AM fungal community is important to the restoration of TGPs, because the characteristic plants of TGPs (such as *Andropogon gerardii*) are heavily dependent on mycorrhizal associations (Hartnett and Wilson 1999, McCain et al. 2011). The suppression of mycorrhizae in TGPs has been shown to alter the plant community to favour C3 grasses and forbs, which have lower dependence on mycorrhizae, and are not the dominant plants in established TGPs (Hartnett and Wilson 1999).

When examining the clustering of TGPs in this study there was an exception to the overall trend. Mike's Field, although classified as a disturbed TGP, clustered with the undisturbed TGPs. This pattern of clustering suggests that Mike's Field, which was released to prairie in 1990, has an AM fungal community closer to the undisturbed TGPs of WIFN. This could mean that the AMF communities of undisturbed TGPs are present after approximately 19 years (Table 2.1) of not being agriculturally tilled. The clustering of Mike's Field with undisturbed TGPs is supported by past studies suggesting that AM fungal communities can transition back to native communities over time (Hamel et al. 1994, Li et al. 2007). The grouping of Mike's Field with the undisturbed TGPs also was observed in a previous study of AMF communities in WIFN (Chokroborty Hoque 2011). Therefore, a better classification of Mike's Field would be with the undisturbed TGPs in this study. Within WIFN, the TGP locations were very close in proximity. Mike's Field and Eliza's Prairie and Sandpits Field and *Silphium* Prairie were adjacent to one another and were most likely the same native TGPs before agricultural conversions. If geographic location was a primary factor, causing the clustering of Mike's Field with Eliza's Prairie and Pottawatomi Prairie, then there should have been similar clustering between Sandpits Field and *Silphium* Prairie. However, there were differences between the AM fungal communities of Sandpits Field and *Silphium* Prairie, which suggests that disturbance history had a greater influence on AM fungal communities than geographic location. However, when examining the cluster dendrogram (Figure 3.6), there was some secondary clustering of sites based on geographic location, because FRS 27 and 28, FRS 32 and 23 and OPC1 and OPC2 clustered together.

Arbuscular mycorrhizal fungi have different colonization strategies, which could explain the differences between disturbed and undisturbed AM fungal communities. Certain Glomeromycota (i.e. Glomeraceae) that colonize host plants using hyphae would be

negatively affected by agricultural tillage, because the mycelial networks lose viability when broken up by tillage (Hamel et al. 1994, Hart and Reader 2004, Mathimaran et al. 2005). Glomeromycota that use spores to colonize host plants (i.e. Gigasporaceae) would be less affected by tillage, because spores have a better chance of survival (Hamel et al. 1994, Hart and Reader 2004, Soteras et al. 2015). The colonization strategies of AMF present in the community would determine which species are viable/present following agricultural tillage. Hart and Reader (2004) also suggest that some Glomeraceae may be able to survive disturbance if the hyphae are well established within plant roots that are not damaged, because this would allow the hyphae to grow out of the roots and colonize other host plants.

4.3 Potential indicator taxa

My study identified some taxa as potential indicators for the ends of the disturbance spectrum. This information could be used in restoration attempts, because knowing which AM fungal species are indicative of undisturbed soils could determine which AM fungal inoculum would be best for restoration. If restorations are seeded with AM fungal taxa that are present in healthy TGPs, it could aid in the success and speed of establishment of dominant C4 grasses that are dependent on mycorrhizae (Hartnett and Wilson 1999, McCain et al. 2011). A summary of what is known about disturbance sensitive and disturbance tolerant AMF can be found in Table 4.1.

The significant taxa segregated by ALDEx2 could be indicator taxa of different disturbance histories, because these taxa occurred in higher abundances in either disturbed or undisturbed TGPs. In this study, disturbed TGPs were dominated by *Claroideoglomus* and *Glomus* spp. The undisturbed TGPs had OTUs of *Ambispora* (OTU 75 and OTU 282) and *Diversispora* (OTU 587) in higher abundance than disturbed TGPs. Undisturbed TGPs also contained various OTUs of *Glomus* (e.g. OTUs 19, 60, 96, 109 and 11 others) that were found in lower abundance in disturbed sites. Although certain OTUs of *Claroideoglomus* were not more abundant in undisturbed TGPs, there were OTUs of *Claroideoglomus* present across both disturbed and undisturbed TGPs. The high abundance of *Claroideoglomus* and certain *Glomus* found across all of the TGPs in this study indicates that *Claroideoglomus* and these *Glomus* spp. may be generalists. Members of *Glomus* have

been shown to dominate both agricultural and untilled soils (Jansa et al. 2003, Mathimaran et al. 2005, Lumini et al. 2010, Mirás-Avalos et al. 2011, Bainard et al. 2015). Unfortunately, information on *Ambispora* and *Diversispora* in the literature does not indicate whether these taxa are disturbance sensitive. With the information obtained from this study I propose that *Ambispora* and *Diversispora* are sensitive to disturbance by agricultural tillage (Table 4.1). *Diversispora* are in the same Order as *Gigaspora*, which is considered disturbance tolerant (Hamel et al. 1994, Hart and Reader 2004, Soteras et al. 2015). Further research should be done in other disturbed and undisturbed locations to support these findings.

Another important genus of Glomeromycota to mention from this study is *Gigaspora*. The number of *Gigaspora* spp. recovered in this study was low (3 OTUs), but the location where these OTUs were found is interesting. *Gigaspora* spp. were found only in De Maere Prairie (Norfolk County) and Sandpits Field (WIFN; Appendix A). These sites are two of the most recently disturbed sites in this study. This supports past literature that Gigasporaceae are disturbance tolerant taxa due to their large spore size and colonization of roots from spores (Hart and Reader 2004, Soteras et al. 2015). The presence of Gigasporaceae in Sandpits Field also was discovered by Stover et al. (2012). In the latter study, Gigasporaceae were found in highest abundance in Sandpits Field, and our studies shared the same five sampling locations in WIFN. Thus, this study supports that *Gigaspora* spp. are tolerant of disturbance by agricultural tillage.

Table 4.1. Potential indicator taxa for soil disturbance from literature and findings from this study. Asterisk*s* indicate information was obtained from this study.

	Genus	Colonization strategy	References
Disturbance sensitive	*Scutellospora*	colonization by spores	Egerton-Warburton and Allen (2000), Jansa et al. (2002), Jansa et al. (2003), Li et al. (2007)
	Acaulospora	colonization by spores	Li et al. (2007)
	Ambispora	unknown	* (select taxa; OTU 75, OTU 282)
	Diversispora	unknown	* (OTU 587)
	Glomus	unknown	* (select taxa; OTU 19, OTU 60, OTU 96, OTU 109, OTU 124, OTU 169, OTU 222, OTU 377, OTU 604, OTU 611, OTU 846, OTU 3028, OTU 3359, OTU 3573, OTU 4514)
Disturbance tolerant	*Gigaspora*	colonization by spores	* (select taxa; OTU 244, OTU 514, OTU 3339), Hart and Reader (2004), Stover et al. (2012), Soteras et al. (2015)
	Glomus	colonization by hyphae	* (select taxa; OTU 4552, OTU 4630)
	Claroideoglomus	colonization by hyphae	* (OTU 179, OTU 554, OTU 3338, OTU 4431, OTU 4646)
Generalists	*Glomus*	colonization by hyphae	Egerton-Warburton and Allen (2000), Jansa et al. (2002), Jansa et al. (2003), Mathimaran et al. (2005), Lumini et al. (2010), Mirás-Avalos et al. (2011), Bainard et al. (2015)
	Claroideoglomus	colonization by hyphae	Lumini et al. (2010), Bainard et al. (2015)

4.4 Future research

Because of the lack of replications and the observational nature of this study, there were limitations regarding the statistical power of the results. Unfortunately, gaining high statistical power with such a study would not be easy, because locating multiple sampling locations with the same properties and disturbance histories was difficult. Collecting and processing soil from a large number of sampling locations is a big undertaking. In this study, information about the plant community and soil properties were not collected. Future studies that focus on the influence of other factors, such as geographic location, plant community, and soil properties (such as pH, nitrogen and phosphorus content) would be beneficial and add much needed information about what else could be affecting AM fungal communities.

Future studies conducted on AM fungal communities that are being manipulated by disturbance, nutrient additions, and plant community alterations would aid in furthering our knowledge of AMF communities. Establishing more information on AMF and disturbance in a controlled experimental design would be beneficial, because there is potential for greater statistical power and more definitive conclusions about what is influencing AM fungal communities. This kind of analysis could be conducted at the Michigan Kellogg Biological Station (KBS), because they have many established experimental plots with varying levels of disturbance histories (http://lter.kbs.msu.edu/). The Kellogg Biological Station has a replicated design of common soils, and soils with different agricultural disturbance histories. The experimental plots at KBS would allow for a manipulative microplot experiment imposed on never tilled soil, which would not be possible in restoration areas.

The next steps in AMF research should focus on refining the phylum Glomeromycota. The information available on the Glomeromycota has improved dramatically over the past few years, but more information is needed. Better understanding identification of AM fungal taxa and their characteristics should be a focus of future studies on AMF. These studies will allow for improvements to the identification of DNA-based sequences, which is the easiest way to study AMF community composition. Studies that focus on increasing the

sections of genome used for identifying Glomeromycota will not only help with identification of Glomeromycota, but also help us understand the evolution and relatedness between species. Advancement in this area has begun with single molecule real time (SMRT) sequencing, which was capable of sequencing a large portion of the ribosomal DNA region of Glomeromycota (Schlaeppi et al. 2016).

4.5 Conclusions

This is the first soil survey of the community composition of AMF in Ontario tallgrass prairies utilizing next generation sequencing. The AMF-specific primer pair used recovered a good range of genera from Glomeromycota, although predominantly *Glomus* were found across all sites. This study added substantially to what was previously known about AMF in Ontario TGPs. My prediction that the AMF communities of disturbed and undisturbed TGPs would be different was supported in this study, except for the AMF community of a single disturbed site (Mike's Field) that grouped with those of undisturbed TGPs. The presence of differences in community composition of AMF based on disturbance supports the idea that native inoculum from established TGPs would be beneficial in any future TGP restoration attempts. Some OTUs were potential indicators of undisturbed TGPs: *Ambispora*, *Diversispora*, and some *Glomus* spp. may be disturbance-sensitive taxa. This study supported the literature suggesting that certain *Glomus* spp., *Claroideoglomus* and *Gigaspora* spp. are tolerant of disturbance by agricultural tillage. Overall, this study was successful in studying how disturbance is affecting the community composition of AMF in Ontario TGPs and will be a useful resource for future studies on AMF community composition and ecosystem restorations.

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Appendices

Appendix A. A list of all 177 OTUs of Glomeromycota recovered from 13 tallgrass prairies in Ontario using AMF primers. Classification of Glomeromycotan OTUs based on the best match to BLAST against the MaarjAM database (http://maarjam.botany.ut.ee/; Öpik et al. 2010). The percent in brackets represents the % identity of the match. “x” indicates presence of OTU at a site (June/July and October data combined). PO= Pottawatomi Prairie, EL= Eliza’s Prairie, SI= *Silphium* Prairie, MI= Mike’s Field, SA= Sandpits Field, O1= OPC1, O2= OPC2, BF= Blair Flats, DM= De Maere Prairie, 23= FRS 23, 27= FRS 27, 28= FRS 28, 32= FRS 32. Asterisks indicate 25 significant taxa found using ALDEx2.

OTU ID	Best match in MaarjAM	Virtual taxon	PO	EL	SI	MI	SA	O1	O2	BF	DM	23	27	28	32
1170	*Acaulospora* sp. (97.7%)	VTX00231							x						
1744	*Acaulospora* sp. (99.5%)	VTX00026								x					
75*	*Ambispora fennica* (99.1%)	VTX00283	x	x	x	x		x	x	x		x			x
282*	*Ambispora* sp. (97.2%)	VTX00283	x	x	x	x			x			x			x
94	*Archaeospora* sp. (91.1%)	VTX00005									x				
1180	*Archaeospora* sp. (99.5%)	VTX00338									x				
5	*Claroideoglomus* sp. (100.0%)	VTX00056	x		x	x	x		x		x		x	x	x
11	*Claroideoglomus* sp. (100.0%)	VTX00278	x		x	x	x	x	x		x	x	x	x	x
24	*Claroideoglomus* sp. (100.0%)	VTX00193	x	x	x	x	x	x	x	x	x	x	x	x	x
53	*Claroideoglomus* sp. (100.0%)	VTX00055	x	x	x	x	x	x	x			x	x	x	x
67	*Claroideoglomus* sp. (100.0%)	VTX00057	x	x	x	x	x	x	x	x	x	x	x	x	x
334	*Claroideoglomus* sp. (100.0%)	VTX00225					x					x	x	x	x
507	*Claroideoglomus* sp. (100.0%)	VTX00225					x					x	x		x
524	*Claroideoglomus* sp. (100.0%)	VTX00225											x		
576	*Claroideoglomus* sp. (100.0%)	VTX00193					x			x	x				
1564	*Claroideoglomus* sp. (100.0%)	VTX00055	x		x	x	x	x	x			x		x	x
3338*	*Claroideoglomus* sp. (100.0%)	VTX00193	x		x	x	x	x	x	x	x		x	x	x
46	*Claroideoglomus* sp. (90.3%)	VTX00056									x				
4528	*Claroideoglomus* sp. (92.8%)	VTX00056									x				
1289	*Claroideoglomus* sp. (93.2%)	VTX00056		x				x					x	x	x
769	*Claroideoglomus* sp. (94.6%)	VTX00278									x		x		

OTU ID	Best match in MaarjAM	Virtual taxon	PO	EL	SI	MI	SA	O1	O2	BF	DM	23	27	28	32
448	*Claroideoglomus* sp. (95.0%)	VTX00402					x				x		x		
2902	*Claroideoglomus* sp. (95.0%)	VTX00278									x				
3095	*Claroideoglomus* sp. (95.0%)	VTX00056					x								
1267	*Claroideoglomus* sp. (95.4%)	VTX00279	x		x	x						x	x	x	
4951	*Claroideoglomus* sp. (95.5%)	VTX00056					x								
850	*Claroideoglomus* sp. (95.8%)	VTX00056									x				
3641	*Claroideoglomus* sp. (95.8%)	VTX00056	x			x	x	x	x	x					x
4431*	*Claroideoglomus* sp. (95.9%)	VTX00279				x	x			x	x			x	
1353	*Claroideoglomus* sp. (96.0%)	VTX00340	x	x	x	x	x								x
3331	*Claroideoglomus* sp. (96.1%)	VTX00056									x				
179*	*Claroideoglomus* sp. (96.3%)	VTX00279					x	x		x	x		x	x	
554*	*Claroideoglomus* sp. (96.3%)	VTX00279					x			x				x	
73	*Claroideoglomus* sp. (96.4%)	VTX00055									x				x
200	*Claroideoglomus* sp. (96.4%)	VTX00402	x	x	x	x	x	x	x	x	x	x	x	x	x
517	*Claroideoglomus* sp. (96.4%)	VTX00278					x		x		x		x		
2615	*Claroideoglomus* sp. (96.4%)	VTX00056					x		x		x		x		
2978	*Claroideoglomus* sp. (96.7%)	VTX00056									x				
1586	*Claroideoglomus* sp. (96.8%)	VTX00279					x							x	
7	*Claroideoglomus* sp. (96.9%)	VTX00276		x	x	x	x	x	x	x		x	x	x	x
4910	*Claroideoglomus* sp. (96.9%)	VTX00056					x		x		x		x	x	
3797	*Claroideoglomus* sp. (97.2%)	VTX00056						x	x						x
404	*Claroideoglomus* sp. (97.8%)	VTX00340	x		x	x	x		x	x	x				x
545	*Claroideoglomus* sp. (98.1%)	VTX00278							x		x				
4472	*Claroideoglomus* sp. (98.1%)	VTX00276						x	x				x		x
819	*Claroideoglomus* sp. (98.2%)	VTX00056		x		x	x	x	x	x		x	x	x	x
2593	*Claroideoglomus* sp. (98.2%)	VTX00276									x		x	x	
3551	*Claroideoglomus* sp. (98.6%)	VTX00056		x		x	x	x	x	x		x		x	x
4340	*Claroideoglomus* sp. (98.6%)	VTX00056					x				x				
4646*	*Claroideoglomus* sp. (98.6%)	VTX00193					x				x		x	x	
2454	*Claroideoglomus* sp. (98.7%)	VTX00056									x				
3822	*Claroideoglomus* sp. (99.1%)	VTX00276	x	x	x	x	x	x	x	x	x	x	x	x	
18	*Claroideoglomus* sp. (99.5%)	VTX00278		x	x	x	x	x	x	x		x	x	x	x
3344	*Claroideoglomus* sp. (99.5%)	VTX00225					x		x	x			x		
16	*Claroideoglomus* sp. (99.6%)	VTX00056	x	x	x	x	x	x	x	x	x	x	x	x	x
22	*Diversispora* sp. (100.0%)	VTX00062	x	x	x	x	x		x	x	x	x			x

OTU ID	Best match in MaarjAM	Virtual taxon	PO	EL	SI	MI	SA	O1	O2	BF	DM	23	27	28	32
313	*Diversispora* sp. (100.0%)	VTX00356	x	x	x	x				x	x				x
443	*Diversispora* sp. (100.0%)	VTX00061	x	x		x	x	x	x	x	x	x	x	x	x
1830	*Diversispora* sp. (100.0%)	VTX00054	x					x	x	x	x				x
5490	*Diversispora* sp. (96.9%)	VTX00356				x					x				
125	*Diversispora* sp. (98.6%)	VTX00054								x	x				
587*	*Diversispora* sp. (98.6%)	VTX00353	x	x	x	x						x			x
540	*Diversispora* sp. (99.1%)	VTX00060	x		x	x			x	x	x		x	x	x
1720	*Diversispora* sp. (99.5%)	VTX00061	x	x	x	x	x	x	x	x	x	x	x	x	x
514	*Gigaspora* sp. (93.9%)	VTX00039									x				
244	*Gigaspora* sp. (99.1%)	VTX00039					x				x				
3339	*Gigaspora* sp. (99.5%)	VTX00039					x				x				
8	*Glomus* sp. (100.0%)	VTX00130	x	x	x	x	x	x	x	x	x	x	x	x	x
19*	*Glomus* sp. (100.0%)	VTX00135	x	x	x	x	x	x	x	x	x	x	x	x	x
23	*Glomus* sp. (100.0%)	VTX00212	x	x	x	x	x	x	x	x	x	x	x	x	x
33	*Glomus* sp. (100.0%)	VTX00067	x	x	x	x	x	x	x	x	x	x	x	x	x
57	*Glomus* sp. (100.0%)	VTX00222	x	x	x	x	x	x	x	x	x	x	x	x	x
60*	*Glomus* sp. (100.0%)	VTX00117	x	x	x	x		x	x	x		x	x		x
61	*Glomus* sp. (100.0%)	VTX00113	x	x	x	x	x	x	x	x	x	x	x	x	x
66	*Glomus* sp. (100.0%)	VTX00419	x	x			x		x	x	x			x	
68	*Glomus* sp. (100.0%)	VTX00143	x								x				
107	*Glomus* sp. (100.0%)	VTX00214	x	x	x	x	x	x	x	x		x	x	x	x
122	*Glomus* sp. (100.0%)	VTX00177	x	x	x	x	x	x	x	x		x		x	x
124*	*Glomus* sp. (100.0%)	VTX00088		x	x	x		x	x			x		x	x
208	*Glomus* sp. (100.0%)	VTX00084	x		x			x	x	x			x	x	x
210	*Glomus* sp. (100.0%)	VTX00151		x		x	x				x				
215	*Glomus* sp. (100.0%)	VTX00219			x			x	x	x	x		x		x
220	*Glomus* sp. (100.0%)	VTX00084						x	x				x		
222*	*Glomus* sp. (100.0%)	VTX00166	x	x	x		x	x	x			x	x	x	x
242	*Glomus* sp. (100.0%)	VTX00156	x		x	x	x			x	x			x	
243	*Glomus* sp. (100.0%)	VTX00222	x	x	x	x	x	x	x	x	x	x	x	x	x
265	*Glomus* sp. (100.0%)	VTX00172	x		x						x		x		
300	*Glomus* sp. (100.0%)	VTX00064	x	x	x	x	x	x	x	x		x	x	x	x
310	*Glomus* sp. (100.0%)	VTX00086	x	x	x	x	x	x	x			x	x		x
315	*Glomus* sp. (100.0%)	VTX00063	x		x	x	x		x		x		x	x	
360	*Glomus* sp. (100.0%)	VTX00143									x				

OTU ID	Best match in MaarjAM	Virtual taxon	PO	EL	SI	MI	SA	O1	O2	BF	DM	23	27	28	32
368	*Glomus* sp. (100.0%)	VTX00345	x		x										x
458	*Glomus* sp. (100.0%)	VTX00063	x			x	x						x	x	
515	*Glomus* sp. (100.0%)	VTX00409					x							x	
601	*Glomus* sp. (100.0%)	VTX00175				x							x	x	
611*	*Glomus* sp. (100.0%)	VTX00053	x	x	x			x	x			x			x
678	*Glomus* sp. (100.0%)	VTX00165	x	x	x	x						x			
684	*Glomus* sp. (100.0%)	VTX00063					x							x	
688	*Glomus* sp. (100.0%)	VTX00223						x	x						
699	*Glomus* sp. (100.0%)	VTX00093			x			x	x						
711	*Glomus* sp. (100.0%)	VTX00222	x	x	x	x	x			x	x		x	x	
846*	*Glomus* sp. (100.0%)	VTX00129	x	x	x				x						
951	*Glomus* sp. (100.0%)	VTX00172			x			x							x
1302	*Glomus* sp. (100.0%)	VTX00165			x	x									
1312	*Glomus* sp. (100.0%)	VTX00155											x		
1646	*Glomus* sp. (100.0%)	VTX00070						x							
703	*Glomus* sp. (93.0%)	VTX00149			x										
271	*Glomus* sp. (94.4%)	VTX00103	x	x	x	x			x						
3675	*Glomus* sp. (94.5%)	VTX00202	x	x	x	x						x			
1654	*Glomus* sp. (94.8%)	VTX00130	x						x	x	x				
720	*Glomus* sp. (95.3%)	VTX00149	x	x	x				x						
694	*Glomus* sp. (95.5%)	VTX00122		x	x	x									
314	*Glomus* sp. (95.8%)	VTX00149			x		x		x			x			x
1762	*Glomus* sp. (95.8%)	VTX00212	x	x	x	x			x		x				
2854	*Glomus* sp. (95.8%)	VTX00212	x								x				
29	*Glomus* sp. (96.3%)	VTX00129	x	x	x	x	x		x	x		x			x
3677	*Glomus* sp. (96.3%)	VTX00202	x	x	x	x			x			x			
2647	*Glomus* sp. (96.7%)	VTX00130													
2892	*Glomus* sp. (96.7%)	VTX00129	x	x	x	x			x						x
2903	*Glomus* sp. (96.7%)	VTX00130								x	x				
389	*Glomus* sp. (96.8%)	VTX00202	x	x	x	x						x			
492	*Glomus* sp. (97.0%)	VTX00130	x						x	x	x				
96*	*Glomus* sp. (97.2%)	VTX00149	x	x	x	x			x	x					x
1995	*Glomus* sp. (97.2%)	VTX00143									x				
3278	*Glomus* sp. (97.2%)	VTX00063					x								
4523	*Glomus* sp. (97.2%)	VTX00222			x		x		x		x				

OTU ID	Best match in MaarjAM	Virtual taxon	PO	EL	SI	MI	SA	O1	O2	BF	DM	23	27	28	32
603	*Glomus* sp. (97.7%)	VTX00419							x	x	x				
698	*Glomus* sp. (97.7%)	VTX00143									x				
871	*Glomus* sp. (97.7%)	VTX00418					x						x	x	
1958	*Glomus* sp. (98.1%)	VTX00093	x		x	x	x						x	x	
2942	*Glomus* sp. (98.1%)	VTX00143									x				
3531	*Glomus* sp. (98.1%)	VTX00214			x	x	x			x				x	
4514*	*Glomus* sp. (98.1%)	VTX00315	x	x	x	x		x	x			x			x
4686	*Glomus* sp. (98.1%)	VTX00177	x	x	x	x			x						
5138	*Glomus* sp. (98.1%)	VTX00129	x	x	x	x			x						
5369	*Glomus* sp. (98.1%)	VTX00130	x		x	x	x			x	x				
437	*Glomus* sp. (98.6%)	VTX00103			x				x						
478	*Glomus* sp. (98.6%)	VTX00129	x	x	x	x			x						x
604*	*Glomus* sp. (98.6%)	VTX00159	x	x	x	x		x	x			x			x
1626	*Glomus* sp. (98.6%)	VTX00130			x		x		x	x	x				
2601	*Glomus* sp. (98.6%)	VTX00130	x		x	x	x		x	x	x				x
3573*	*Glomus* sp. (98.6%)	VTX00053	x	x	x			x	x			x			x
3935	*Glomus* sp. (98.6%)	VTX00219		x	x			x	x				x		
3975	*Glomus* sp. (98.6%)	VTX00143									x				
4552*	*Glomus* sp. (98.6%)	VTX00222	x	x	x	x	x				x		x	x	x
4720	*Glomus* sp. (98.6%)	VTX00222	x	x	x	x	x	x	x		x		x		x
169*	*Glomus* sp. (99.1%)	VTX00117	x	x	x	x		x	x			x			x
254	*Glomus* sp. (99.1%)	VTX00202	x	x	x	x	x					x			x
1054	*Glomus* sp. (99.1%)	VTX00177	x	x	x	x	x								
1065	*Glomus* sp. (99.1%)	VTX00222		x		x						x			
4447	*Glomus* sp. (99.1%)	VTX00214					x							x	
109*	*Glomus* sp. (99.5%)	VTX00315	x	x	x	x	x	x	x	x		x	x		x
184	*Glomus* sp. (99.5%)	VTX00125	x	x	x	x	x	x	x	x	x	x	x	x	x
260	*Glomus* sp. (99.5%)	VTX00122			x	x									
373	*Glomus* sp. (99.5%)	VTX00287	x	x		x						x			
377*	*Glomus* sp. (99.5%)	VTX00053	x		x			x	x			x			x
430	*Glomus* sp. (99.5%)	VTX00129	x	x	x	x	x		x						
450	*Glomus* sp. (99.5%)	VTX00151	x	x	x	x	x							x	x
548	*Glomus* sp. (99.5%)	VTX00146		x		x							x	x	
599	*Glomus* sp. (99.5%)	VTX00214	x			x	x			x			x	x	
1045	*Glomus* sp. (99.5%)	VTX00069					x							x	x

OTU ID	Best match in MaarjAM	Virtual taxon	PO	EL	SI	MI	SA	O1	O2	BF	DM	23	27	28	32
2739	*Glomus* sp. (99.5%)	VTX00197									x				
3028*	*Glomus* sp. (99.5%)	VTX00160	x	x	x	x						x			x
3219	*Glomus* sp. (99.5%)	VTX00063					x							x	
3359*	*Glomus* sp. (99.5%)	VTX00117	x	x	x	x		x	x			x	x		x
3439	*Glomus* sp. (99.5%)	VTX00247	x	x	x	x	x	x			x	x			x
3813	*Glomus* sp. (99.5%)	VTX00064				x	x		x	x		x			x
4630*	*Glomus* sp. (99.5%)	VTX00222	x		x	x	x			x	x		x		
4952	*Glomus* sp. (99.5%)	VTX00199	x	x	x	x				x	x		x		
5175	*Glomus* sp. (99.5%)	VTX00222	x	x	x	x	x	x	x		x	x	x		x
15	*Paraglomus* sp. (100.0%)	VTX00281	x	x	x	x			x	x	x	x	x	x	x
1627	*Paraglomus* sp. (96.0%)	VTX00351													x
617	*Paraglomus* sp. (96.2%)	VTX00281									x				
3511	*Paraglomus* sp. (99.1%)	VTX00239							x	x	x		x	x	
35	*Scutellospora gilmorei* (100.0%)	VTX00041	x							x	x				
477	*Scutellospora* sp. (100.0%)	VTX00052	x								x				
710	*Scutellospora* sp. (100.0%)	VTX00049						x	x			x			

Appendix B. A list of all 43 OTUs of Glomeromycota recovered from 13 tallgrass prairies in Ontario using BG primers. Classification of Glomeromycotan OTUs based on the best match to BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The percent in brackets represents the % identity of the match. "x" indicates presence of OTU at a site (June/July and October data combined). PO= Pottawatomi Prairie, EL= Eliza's Prairie, SI= *Silphium* Prairie, SA= Sandpits Field, O1= OPC1, O2= OPC2, BF= Blair Flats, DM= De Maere Prairie, 23= FRS 23, 27= FRS 27, 28= FRS 28, 32= FRS 32.

OTU ID	Best match in BLASTn	PO	EL	SI	SA	O1	O2	BF	DM	23	27	28	32
392	*Claroideoglomus claroideum* (100%)		x	x	x			x	x	x	x	x	x
66	*Claroideoglomus claroideum* (87%)		x		x	x			x		x		
513	*Claroideoglomus claroideum* (88%)								x				
11593	*Claroideoglomus walkeri* (88%)				x			x				x	x
309	*Claroideoglomus walkeri* (89%)				x	x	x	x	x			x	x
1186	*Dominikia difficilevidera* (100%)	x	x		x		x				x		x
701	*Funneliformis mosseae* (100%)		x		x	x	x	x	x	x	x	x	x
88	Glomeromycota sp. (100%)				x	x		x	x		x	x	
131	Glomeromycota sp. (100%)								x		x		
838	Glomeromycota sp. (93%)										x	x	
5644	Glomeromycota sp. (93%)				x						x	x	
403	Glomeromycota sp. (95%)										x	x	x
5969	Glomeromycota sp. (95%)						x						
98	Glomeromycota sp. (96%)								x		x		
222	Glomeromycota sp. (96%)				x				x		x		
1406	Glomeromycota sp. (97%)				x								
1644	Glomeromycota sp. (99%)										x		
803	*Glomus aggregatum* (100%)								x				
846	*Glomus constrictum* (100%)				x			x	x		x	x	
782	*Glomus coronatum* (92%)	x	x	x		x	x			x			x
187	*Glomus drummondi* (89%)				x				x		x	x	x
92	*Glomus drummondi* (90%)		x								x	x	x
936	*Glomus drummondi* (90%)							x			x		
1589	*Glomus drummondi* (91%)				x								
2381	*Glomus invermaium* (95%)			x		x							
507	*Glomus microaggregatum* (93%)				x						x	x	

OTU ID	Best match in BLASTn	PO	EL	SI	SA	O1	O2	BF	DM	23	27	28	32
1047	*Glomus sinuosum* (100%)											x	x
958	*Glomus* sp. (100%)							x				x	
279	*Glomus* sp. (79%)	x	x	x	x	x	x	x	x	x		x	x
994	*Glomus* sp. (88%)									x			x
252	*Glomus* sp. (93%)	x			x		x	x	x			x	x
14068	*Glomus* sp. (93%)											x	x
331	*Glomus* sp. (95%)	x			x		x	x	x		x	x	x
765	*Glomus* sp. (95%)				x							x	x
675	*Glomus* sp. (98%)	x			x	x		x	x		x	x	x
1535	*Glomus* sp. (98%)				x						x	x	
1728	*Glomus* sp. (99%)							x				x	
75	*Paraglomus brasilianum* (96%)							x	x				
181	*Paraglomus brasilianum* (99%)				x	x		x	x		x		x
991	*Paraglomus occultum* (98%)												
6176	*Paraglomus occultum* (99%)												
459	*Scutellospora gilmorei* (99%)							x	x				
1130	*Septoglomus viscosum* (100%)				x						x	x	

Curriculum Vitae

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