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Agaricomycetes of Ontario Tallgrass Prairies

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Graduate Program in Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science
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Abstract

This study provides the first characterization of the Agaricomycetes of Ontario tallgrass prairies, assesses the influence of various environmental factors, and compares results of aboveground mushroom surveys with belowground high-throughput DNA sequencing. Overall, the Mycenaceae, Ceratobasidiaceae and Polyporaceae were the most abundant, and the Clavariaceae, Entolomataceae and Sebacinaceae the richest in species. Position along a transect (geographic region) was the primary factor differentiating Agaricomycete composition of sites whereas tillage history and soil organic carbon content were secondary. The Hygrophoraceae and Clavariaceae were associated with pristine sites, and *Minimedusa* spp. associated with tillage. The belowground method captured most of the minor clades found aboveground and several more unique ones. The aboveground method retrieved 74 species and the belowground method 256 OTUs, with only eight shared between them.

Keywords

Agaricomycetes, tallgrass prairie, grassland, tillage, disturbance, restoration, soil organic carbon, fruiting bodies, mushrooms, soil fungal community, PCR, high-throughput sequencing, conservation, fungal biogeography, biodiversity, ribosomal DNA.

Acknowledgments

Numerous people are acknowledged for their contributions to this project. First, a heartfelt thank-you to my supervisor, Dr. Greg Thorn. I am grateful for Greg's support throughout my thesis: he accepted me as his student, guided me in the formation of this project, helped to provide conference and presentation opportunities, provided technical help in the lab and working with my data, and gave feedback during the final writing process. I also appreciate the guidance from my advisory committee: Dr. Zoë Lindo and Dr. André Lachance, especially Dr. Lindo's assistance with R statistics and Dr. Lachance's answers to questions related to phylogenetics and systematics. As my reader, Dr. Lachance provided the best grammatical and writing style feedback I have ever received. Thanks as well to Dr. Ben Rubin and Dr. Hugh Henry, whom I occasionally consulted for statistical or ecological questions, and Dr. Greg Gloor from biochemistry who provided essential code for my data pipeline and multivariate statistics.

I thank all people who are a part of the Thorn lab for their assistance. I am indebted to Sarah Allan for providing me with step-by-step instruction in wet lab techniques and for the soil she collected in the field. Nimalka Weerasuriya assisted Sarah in soil collection and was indispensable to me during the bioinformatics pipeline stage and later to help with R code. Asma Asemaninejad gave her time to help me with PCR troubleshooting and generously shared her knowledge on appropriate methods of multivariate statistic techniques. Jennifer McDonald was always there when I had questions about anything – from technical details of my project to graduate student life. Catriona Catomeris and Aniruddho Chokroborty-Hoque also collected a share of the soil samples that were used in this project. Special thanks to previous lab members Barbara Bahnmann and Michael Lynch. Hard copies of their theses were always at hand as well-written examples. Thanks to the many work study students and lab volunteers who played a role in sequencing my mushroom specimens, especially my first and longest-lasting volunteer Linxi Xie, who was an exceptional assistant.

I also thank my field site partners, without whom I would have no data: Clint Jacobs and our chaperones of Walpole Island First Nation; Andrea Zolnai, Meaghan

Murphy, and Barbara Macdonell of the Herb-Gray Parkway project, as well as the MTO, MNRF, Parkway SAR team including Parkway Infrastructure Constructors (PIC), AMEC, and AECOM; Jim Wigle, the Ministry of Natural Resources and Forestry representative for Ojibway Prairie Provincial Nature Reserve; Bill Prieksaitis, director of the Elgin Stewardship Council, who permitted me to sample at Dutton-Dunwich prairie and provided directions and site details; Mary Garshore and Peter Carson, who showed me their prairie restoration site and allowed me to collect there; Jill Crosthwaite of the Nature Conservancy of Canada, who permitted me to sample at DeMaere prairie; and Jenna Quinn of the Rare Charitable Research Reserve, who was my contact for sampling at Blair Flats.

Finally, thanks to my family and friends: my parents who helped me in the process of moving to Ontario, my sister and brother-in-law who were always available for conversation, and thanks for the support and fun times to the new friends I've made here – you know who you are.

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List of Site Abbreviations

BF	Blair Flats prairie at the Rare Charitable Research Preserve near Cambridge
DD	Dutton-Dunwich prairie remnant
DM	DeMaere prairie of the Nature Conservancy of Canada in Norfolk County
EL	Eliza's prairie in Walpole Island
HA	FRS #23 of the Herb-Gray Parkway in Windsor
HB	FRS #32 of the Herb-Gray Parkway in Windsor
HC	FRS #27 of the Herb-Gray Parkway in Windsor
HD	FRS #28 of the Herb-Gray Parkway in Windsor
MI	Mike's field in Walpole Island
MP	Mary Gartshore & Peter Carson's prairie restoration in Norfolk County
OA	OPC1 – Area 1 of the Ojibway Prairie Complex in Windsor
OB	OPC2 – Area 2 of the Ojibway Prairie Complex in Windsor
PO	Pottawatomi prairie in Walpole Island
SA	Sandpits in Walpole Island
SI	Silphium prairie in Walpole Island

Chapter 1 - Introduction

1 Introduction

1.1 Agaricomycetes and their role in soils

The Agaricomycetes are a large class of fungi from the phylum Basidomycota, containing about one fifth of all species of fungi (Kirk et al. 2008). Globally, across all ecosystems, as well as within grasslands and shrublands specifically, the Agaricomycetes represent 50% of all fungal soil diversity (Tedersoo et al. 2014). The Agaricomycetes are distinct from the jelly fungi classes Tremellomycetes and Dacrymycetes in the subphylum Agaricomycotina, which are separated from distinct plant-disease fungi in the subphyla Pucciniomycotina (rusts) and Ustilaginomycotina (smuts) (Hibbett et al. 2014). Many species of Agaricomycetes produce conspicuous aboveground fruiting bodies or “mushrooms” in a diversity of forms, but some are more inconspicuous, either creating undistinguished soil crusts or fruiting belowground (Hibbett et al. 2014). The main component of all Agaricomycetes is their vegetative growth – networks of hyphae collectively called a mycelium. Hyphae are thread-like chains of cells that extend through their substrate – in grasslands, from aboveground plant litter, through upper humus layers, and into even deeper strata of soil (Jumpponen et al. 2010).

Soil ecosystems are affected by Agaricomycetes in several ways. Ecologically, the Agaricomycetes span a diversity of guilds: saprotrophs of various substrates, plant pathogens, and partners in symbioses with plants, insects, and algae (Hibbett et al. 2014). These interactions influence nutrient cycling and shape the communities of other organisms. Saprotrophic fungi have major roles in decomposition and nutrient release from plant litter (Baere et al. 1993), although their presence and activities in grasslands are not as well studied as in woodlands (Griffith and Roderick 2008). Texture and stability of soil is improved by fungi. Mycelial nets can hold together the surface of soils (especially sandy ones) to prevent wind erosion, and hyphae release sticky exudates that aggregate soil particles, creating pore spaces that facilitate gas and water exchange and plant root growth (Went and Stark 1968, Caesar-TonThat and Cochran 2000). Certain members of the russuloid clade (Caesar-TonThat et al. 2001) and other Agaricomycetes

such as *Rhizoctonia solani* (Tisdall et al. 1997) are particularly effective at soil particle aggregation, increasing soil stability. Restored and remnant prairie sites have more aggregated soil particles than do agricultural sites(Jastrow 1987), suggesting that fungi and their activities differ between these ecosystems.

1.2 Tallgrass prairies and agriculture

At the centre of North America is a large triangular zone of grasslands known as the prairies. Prairies have minor or no woody cover, and are instead dominated by grasses, and a lesser coverage but high diversity of other herbaceous plants (Sims 1988). A prairie has been defined as having one tree or fewer per acre, while a semi-treed grassland ecosystem (e.g., oak savanna) may have up to 50% canopy cover by trees (Quinlan 2005). North American prairies can be split into three broad, simple groups: shortgrass, mixedgrass, and tallgrass (Sims 1988, Reaume 1993). Tallgrass prairies cover the central to eastern areas, where annual rainfall is higher than prairie regions to the west. In Canada this includes southern Manitoba and Ontario. Prairies in the two provinces are distinct from each other. Ontario tallgrass prairies receive more precipitation than any others in North America, which helps to account for the height of their grasses and high diversity of species (Quinlan 2005). It is likely the particularly wet conditions also encourage proliferation of fungal communities, more so than in drier prairies to the west. Unlike southwestern Manitoba, which is part of the Prairies ecozone, southwestern Ontario is actually classified as Mixedwood Plains, so Ontario prairies (and oak savanna mosaics) are a naturally sporadic but unique component across the Lake Erie Lowlands region (Ecological Stratification Working Group 1995, Barcza and Lebedyk 2014). Sporadic prairies are also present in western Ontario (Quinlan 2005), although they were not assessed in this study.Prairie ecosystems developed hand-in-hand with disturbance events – particularly grazing and fires (Wells 1970, Gibson and Hulbert 1987, Sims 1988). Large ungulates, especially bison, were keystone species in shaping tallgrass prairies by preventing trees from establishing in the Great Plains (Knapp et al. 1999). Fires were ignited by lightning, First Nations peoples, and later to some extent by European farmers. Aboveground vegetation rapidly burns away, but native prairie plants can easily regenerate aboveground growth since they have energy stored in their deep

roots (Bock et al. 1986) while their meristems (growing points) are protected in the ground (Dalgleish and Hartnett 2009). These disturbances are necessary to prevent prairies from succeeding into woody ecosystems (particularly where precipitation is sufficient to encourage tree growth), and to maintain a high diversity of plant species (not allowing any one to become overly dominant over another).

Prairie soils are naturally well supplied with nutrients and organic matter, because they have dense plant cover with fast turnover (herbaceous litter) and dense roots that allow for high microbial activity (Tate 1987). Prairies are ideal for agricultural use, since they are flat, treeless, and have rich soils. Since the 1830s when European homesteading of North America began (Sims 1988), and especially since the invention of the steel plow (Bock and Bock 1995), prairies were steadily converted to agricultural land at a massive scale. In addition to land conversion, Europeans introduced exotic invasive plants, reduced the size and frequency of fires, greatly reduced populations of large mammal grazers, and introduced domestic grazing species, impacting prairies in new ways (Bock and Bock 1995).

The tallgrass prairies of North America are one of the most reduced and imperiled ecosystems in the world, with losses of 85-98% since European settlement (Noss et al. 1995). In southern Ontario, an estimated 3% of fair to good quality tallgrass prairie remains; another 3% exists in poor condition and would require extensive restoration efforts (Barcza and Lebedyk 2014). Remnant patches continue to suffer from serious threats (succession into non-prairie ecosystems, conversion to agricultural land, and replacement of native prairie plants with alien invasives, particularly for smaller patches) and require active management to avoid further declines (Koper et al 2010). As of 2007, there are 21 plant species at risk in Canada that are found in Ontario tallgrass prairies listed in the Species at Risk Act and Endangered Species Act (Tallgrass Ontario 2013). These 21 species include colicroot (*Aletris farinosa*), dense blazing star (*Liatris spicata*), and willowleaf aster (*Symphyotrichum praealtum*), which were found in some of my research sites.

The presence of species at risk in prairies and the realization of the extent of loss of tallgrass prairies has led to increasing efforts to identify and conserve remnants, and regain some of the losses through restoration (e.g., in Ontario; Quinlan 2005). Depending on the condition of the site in question—whether it has been degraded, damaged, or destroyed—restoration may take the form of rehabilitation or complete reconstruction (Society for Ecological Restoration 2004). Rehabilitative restoration is the management of degraded natural areas to improve their quality. For tallgrass prairie sites, a combination of cattle grazing and controlled burns are ideal to create a shifting mosaic of disturbance (Fuhlendorf et al. 2006). Mowing and haying can also be used to create a similar effect, and other more intensive techniques such as herbicides, hand-pulling, and brush-cutting may be required to remove exotic and woody species (Quinlan 2005). Reconstructive restoration is conversion of anthropogenic or severely degraded sites back into a natural state. Agricultural land can be restored to tallgrass prairie through removal of exotic plants (through tillage and/ or herbicide use) and then seeding or planting plugs of native prairie plants. A reconstructed prairie will require ongoing management and rehabilitation for it to establish properly and retain its quality.

1.3 Impacts to and conservation of soil fungi

Impacts from previous agricultural land uses may be carried over in restored tallgrass prairies. Most agricultural systems are disturbed by regular soil tillage, have pesticides applied to them, and have declining soil organic matter and nutrients. Conversion of prairie to agricultural land leads to an initial dramatic drop in the first few years of soil organic matter, air space, aggregation, and water-holding capacity, and then a slow and steady rate of decline of these features, leading to degraded and less productive land (Laws and Evans 1949, Tate 1987). It is expected that reduced soil organic matter (measured experimentally as organic carbon) would have a strong impact on fungi in the soil, since increased carbon in soils is associated with promoted microbial activity (Martyniuk and Wagner 1978, Schnürer et al. 1985, Caesar-TonThat and Cochran 2000, Kjoller and Rosendahl 2014). Between fungi and bacteria in the soil, fungi are greater in biomass and nutrient cycling activity (Anderson and Domsch 1975).

In addition to reduced soil organic carbon, tillage reduces hyphal mass and hyphae lengths of arbuscular mycorrhizal fungi in agricultural soils (Kabir et al. 1998) and tillage would presumably damage non-AMF fungi (e.g., Agaricomyce hyphae) in the same way. Reduced organic carbon and damage to hyphal communities may explain why tillage has been linked with reduced diversity and altered composition of Agaricomycetes in an agricultural context (Lynch and Thorn 2006, Bahnmann 2009, Wong 2012). The importance of a site's tillage history has not been addressed in the context of restored and remnant native prairie ecosystems. Restored prairies are often lower quality in terms of plant diversity and community composition when compared to remnant sites (Sluis 2002, Polley et al. 2005), which may correspond with lower-quality fungal communities as well. Given the negative effects of tillage on organic carbon, hyphae, and plant communities, restored prairies may have very different Agaricomycete communities than pristine remnants.

Our current knowledge about Agaricomyceteson the prairies is very limited, so exploring this group may bring new perspectives to prairie ecology, conservation, and restoration. On a global scale, grassland fungi are not well studied. An exception is Europe, where in recent decades they have been extensively examined (O'Hanlan and Harrington 2011) due to conservation concerns surrounding losses of native grassland to mechanized agriculture (Griffith and Roderick 2008). Fungi are susceptible to threats such as habitat loss, pollution, and climate change, like any other organism, and their conservation requires strong baseline survey and ecology data (Arnolds 1989, Courtecuisse 2001). A plethora of grassland mushroom surveys have been carefully documented from Ireland (Mitchel 2010), south Wales (Rotheroe 2001), Scotland (Newton et al. 2003), the Netherlands (Arnolds 1989), as well as eastern European countries such as Slovakia (Adamčík and Kautmanová 2005). The collection of these baseline scientific datasets has allowed for the development of applied conservation initiatives: systems to classify grassland quality using mushroom indicator taxa (e.g., the waxcap grassland "CHEGD profile" system from Rotheroe et al. 1996), several national species at risk "red lists", as well as a continental red list from the European Council for Conservation of Fungi (which was proposed to the EU Habitat Committee but they voted to delay a decision, Bohlin 2004; and later produced as a reference book for conservation

agencies, Dahlberg and Croneborg 2006). At an international scale, there has been a push for fungi to be more included in biodiversity conservation initiatives, which are usually dominated by plant and animal concerns at the expense of other taxa (Watling 1995, Minter 2011). Consequently, the International Union for the Conservation of Nature developed fungal focus groups, which would include the Agaricomycetes in the "mushroom, bracket, and puffball specialist group" (Vilano et al. 2012)

In North America, data for fungi in grasslands are scarce. Fungi of forests on the west coast have been well-studied in the past from a biodiversity conservation perspective (Castellano et al. 1999) and survey data of mushroom-forming fungi are available for many parks and conservation areas (e.g., Polach 1992, Dewsbury 2006), but often these data exclude grasslands entirely, or else combine them with all other ecosystems in the area as one large, vague, list. The two fungal-related reports produced by the International Biological Program (1964-1974) studied grassland soil and coprophilous (dung) fungi, but focused on moulds and microfungi, not Agaricomycetes (none are mentioned in Christensen and Scarborough 1969, only four are listed in Wicklow and Angel 1974). Surveys specific to grasslands in Canada are rare, and probably mostly exist in smaller nature-group publications, separate from the rigor and accessibility of scientific peer-reviewed journals (e.g., in Saskatchewan mixedgrass prairie; Hay 2013). Checklists and surveys produced by mycological societies from mushroom forays usually take place only in woodlands where mushrooms are large and more common (in my experience, and noted from Europe by Griffith and Roderick 2008). Foray events may or may not include collection of voucher specimens for long-term storage that could later be used for sequencing and confirming identifications, and usually do not attempt to collect abundance data. Recent studies using molecular high-throughput sequencing techniques may offer useful insights into the diversity of Agaricomycetes in tallgrass prairie soils, but are usually focused on addressing other research interests besides characterizing the ecosystem and often cannot provide survey lists at the species level (Penton et al. 2013, Jumpponen and Jones 2014).

1.4 Aboveground and belowground surveys of fungal diversity

Fungal diversity has been estimated to be six times greater than that of plants (Hawksworth 1991), but this estimate has steadily climbed higher (Hawksworth and Rossman 1997, Blackwell 2011) to a plant-fungi ratio of 1:17 equating to 6 million species (Taylor et al. 2014). In contrast, almost all of the world's fungi have not yet been described. Previously, the number of described fungi was estimated to be less than 5% of estimated global diversity (Hawksworth and Rossman 1997). Many more fungi have been described since then, but global diversity estimates have increased dramatically, lowering the percentage of described fungi to only 2% of estimated global diversity (Taylor et al. 2014).

Fungal diversity has been studied via surveys of aboveground fruiting bodies (mushrooms) (detailed methods are described by Rossman et al. 1998 and Lodge et al. 2004), culturing from environmental samples (e.g., Thorn et al. 1996), or else otherwise directly observing features using a microscope (e.g., arbuscular mycorrhizal spores by Stover et al. 2012, or ectomycorrhizal root sheath morphotypes by Matsuda et al. 2013). A major drawback to these methods is the limited diversity they can uncover. Standard dilution plating methods from soil samples overrepresent easily culturable species with high spore production – usually *Penicillium* and *Aspergillus* spp. (e.g., Martyniuk and Wagner 1978). Soil sieving and selective protocols methods were developed to improve soil culturing results (Thorn et al. 1996), but they did not capture as much diversity as cloning and sequencing methods used a decade later on the same soil (Lynch and Thorn 2006). Mushroom surveys face a number of drawbacks. Fruiting body production is variable and sometimes sporadic, meaning committed sampling effort is required over several years to begin to approach a complete survey for an area (Straatsma et al. 2001). Consideration must also be made for differences in longevity of fruiting bodies, fruiting periodicity/ annual fluctuations, and successional changes (Lange 1991, Watling 1995). Mushroom taxonomy is still in transition from traditional morphological species concepts to modern ones utilizing genetic information. Currently defined morpho-species often represent several undefined "cryptic" species (e.g., even in well-known edibles;

Dentinger and Suz 2014). The process of sorting artificial taxa based on morphological characters into phylogenetic groups that represent evolutionary relationships is ongoing (Moncalvo et al. 2002).

The development of high-throughput sequencing (HTS) has revolutionized the study of fungal diversity in environmental samples, since it can produce large numbers of sequences from samples containing genetic material from hundreds of species (Shokralla et al. 2012). Older sequencing methods could produce only one sequence for one genetic specimen at a time (Sanger et al. 1977). Methods of HTS are similar to previous techniques involving DNA extraction from soils and PCR amplification using fungal-specific primers, but instead of labor-intensive cloning and culturing, PCR amplicons with mixed DNA can be sent directly for sequencing, returning hundreds of sequences (Lindahl et al. 2013). A greater diversity of fungi than was previously known has been exposed by HTS, particularly species that are otherwise difficult or impossible to find by culturing or fruiting body surveys. Fungal diversity in soil is increasingly being examined using HTS (e.g., Penton et al. 2013, Jumpponen and Jones 2014).

High-throughput sequencing has also been criticized for a number of reasons. It is not able to distinguish inactive and dormant microbes from active ones, and therefore ecological conclusions from these data are questionable (Klein 2015). Sequences alone are useless for ecological interpretation without sequences from identified reference cultures and (mushroom) specimens to compare with. Despite suggestions to name new species using sequences alone (Kõljalg et al. 2013), tangible samples are still required for naming new species (Blackwell 2011) and Latin binomials remain standard for non-microbial scientists, the public, and legislative bodies (Hibbett 2016). Therefore, mushroom and culture studies (and the collections they contribute to) are still vital and useful to the field of mycology as a whole (Peay 2014). Aboveground fruiting body surveys are also unique in the possibility for amateur mycologists and other interested members of the public (citizen scientists) to contribute, allowing for community involvement and education that is usually not feasible in mycological research.

There has been interest in comparing results of different types of surveys to determine their degree of similarity. Usually there is a large disparity between the results of molecular methods, culture-based approaches, and mushroom surveys (Griffith and Roderick 2008). Results of aboveground mushroom data do not match root tip genotyping of ectomycorrhizal species (Gardes and Bruns 1996, Horton and Bruns 2001). In the same way, molecular methods (cloning) do not match soil culturing (Hunt et al. 2004, Thorn et al. 1996 vs. Lynch and Thorn 2006) or aboveground mushroom surveys (in a hemlock forest; Porter et al. 2008). Depending on the survey type, certain taxa may be missed entirely, such as litter-decomposing fungi missed by soil analysis excluding litter material and inconspicuous fungi missed by fruiting body surveys (Porter et al. 2008). Comparisons of taxa common between two survey types, especially at the species level, often show contrasting relative abundances (high in one and low in the other) (Gardes and Bruns 1996). No published examples can be found in which aboveground and belowground methods were compared in grasslands, or in which mushroom survey results were compared with those of high-throughput sequencing.

1.5 Objectives

1. Characterize Ontario's tallgrass prairies by compiling a list of Agaricomycetes and examining overall abundance and distribution of taxa in this group.
2. Assess how certain factors relate to Agaricomycete composition: geographic region, soil characteristics, vascular plants, and tillage history.
3. Compare two methods of documenting Agaricomycete diversity: aboveground fruiting body collection and belowground soil molecular analysis.

1.6 Hypotheses and predictions

I hypothesized tillage history would be the strongest determining factor for Agaricomycete composition (abundance across Agaricomycete taxa) in tallgrass prairies. My prediction was that my statistical analyses would separate the data first by tillage history (separating pristine and tilled sites from one another). I predicted tillage history would be correlated with organic carbon and plant measures of site quality (native

species richness and adjusted cover-weighted floristic quality index score). I hypothesized that organic carbon and plant diversity would be associated with fungal diversity, so I predicted I would find positive correlations between these variables and richness of Agaricomycetes across my sites.

More specifically in regards to tillage effects, I hypothesized the differences between sites would be driven by taxa believed to be tillage-sensitive: either tillage-associated (higher abundance in tilled sites than pristine ones) or pristine-associated (higher presence in pristine sites than tilled ones). I predicted the Clavariaceae, Hygrophoraceae, Entolomataceae, and Polyporaceae would be pristine-associated and the Cantharellales *incertae sedis* minor clade (*Minimedusa* spp.) and the Lachnellaceae would be tillage-associated (based on Rotheroe et al. 1996 and Bahnmann 2009).

When comparing aboveground and belowground results, I hypothesized there would be relatively little correspondence. The degree of overlap would be particularly low at the taxonomic level of species, but greater at higher taxonomic levels such as family. I predicted that species present in both aboveground and belowground survey types would show contrasting abundances (high in one survey but low in the other). I also predicted that I would find taxa unique to each method, such as litter decomposers unique to the aboveground survey and inconspicuous taxa (such as crusts and non-mushroom-forming soil propagules) unique to the belowground survey. that species with high abundance in one method will be of low abundance in another. There will be certain taxa unique to each method – such as litter decomposers in the aboveground method and hard to find taxa (such as crusts and non-mushroom soil propagules) in the belowground method.

Chapter 2 – Materials and Methods

2 Materials and Methods

2.1 Site descriptions

This project combines data from three studies (this study, Chokroborty-Hoque 2011, and Catomeris 2015), covering a total of fifteen prairie sites across southern Ontario (Figure 2.1). Together, these sites encompass a wide variety of land-use histories, management, and natural landscape features.

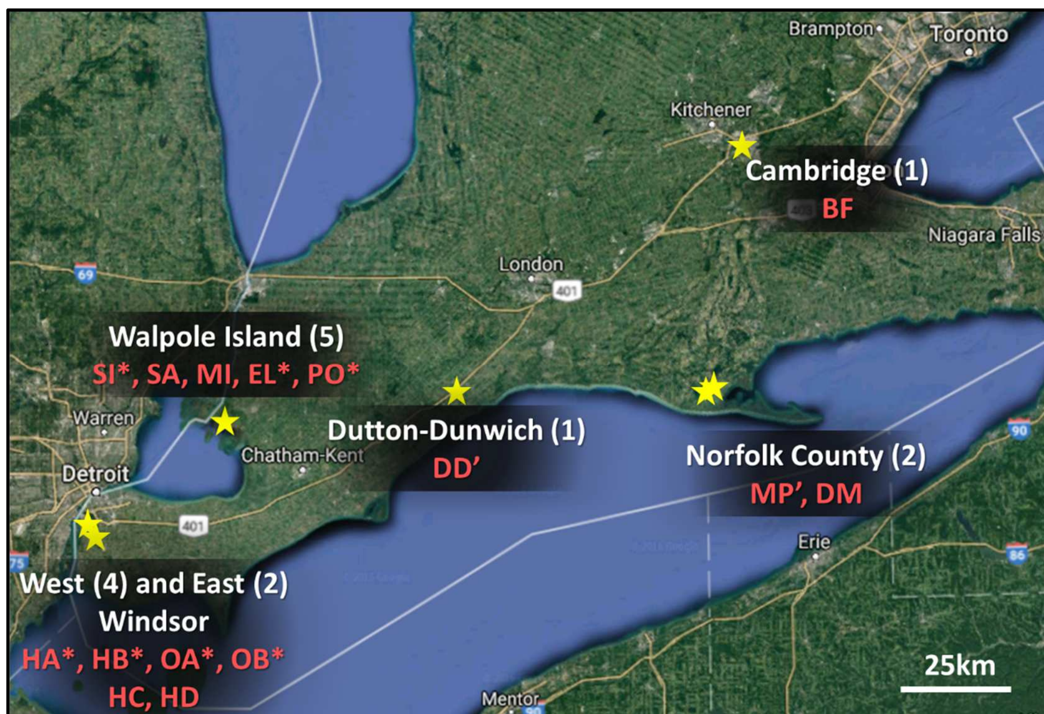

Figure 2.1 Map of 15 tallgrass prairie study sites across five geographic regions of southern Ontario.

Pristine sites are indicated with an asterisk (*) whereas all other sites were recently tilled. An apostrophe (') indicates the site was only surveyed for mushrooms (no soil sampling).

2.1.1 Herb-Gray Parkway

The Herb-Gray Parkway is a major highway construction project started in 2008 to improve traffic flow between Windsor and Detroit (Ontario Ministry of Transportation 2016). Honouring the 2007 Endangered Species Act, the Ontario Ministry of Transportation (MTO) restored tallgrass prairie and oak savannah surrounding the parkway and transplanted to these sites plant species at risk that would have been lost in the construction process (Rt. Hon. Herb Gray Parkway Project Team 2014). Four of these Final Restoration Sites (FRS) located in Windsor and containing tallgrass prairie habitat were chosen for soil sampling and mushroom surveys: FRS #23, FRS #32, FRS #27, and FRS #28. Soils in the Windsor region were developed on thin deposits of sand over the Essex Clay Plain, a flat till plain between Lake Erie and Lake St. Clair, and additional clay was deposited about 13,000 years BP (Chapman and Putnam 1984). Ontario tallgrass prairie is typically found in sandy regions, although the additional clay in the Windsor area meant that pasture fields were the predominant agricultural land use until drainage was later introduced and the land could be tilled for crops (Chapman and Putnam 1984).

FRS #23 is located just south of the E.C. Row Expressway, east of Matchette Road (42.273° N 83.069° W). In 2014 it was classified Fresh-Moist Tallgrass Prairie, with some portions of Gray Dogwood Thicket Swamp and Savannah (Balsdon and Snyder 2015). Its tillage history is uncertain, but it is believed to be a remnant prairie. Aerial photographs show that it has remained flat grassland at least since 1951, with possible mowing for hay and pasture (cattle grazing) preventing surrounding woodland from encroaching (United States Geological Survey 1951).

FRS #32 is just north of Chappus Street and east of Matchette Road (42.272° N 83.070° W), only about 100 m south from FRS #23. In fall 2009, prior to brush cutting and herbicide application to remove unwanted woody vegetation and invasive species, it was classified as Mineral Cultural Thicket (Balsdon and Snyder 2015). In 2014 the site had been altered enough to be re-classified as Forb Meadow Marsh, with some Dry-Moist Old Field Meadow on the eastern edge (Balsdon and Snyder 2015). The site is believed to be another prairie remnant (B. Macdonell, pers. comm., 22 September 2015), and aerial

photography confirms this. It appears that haying, mowing, or pasture use prevented the site from succeeding into a forest community.

FRS #27 is in the south end of Windsor, south of Hwy 3 and west of the Howard Ave Diversion (42.229° N 82.994° W). The site was a fallow agricultural field (tilled and harrowed, but not seeded) until 2011. Since then, prairie species were sod-transplanted and inter-seeded, invasive species were managed with herbicide and manual removal, and the site was allowed to succeed naturally into a Dry-Fresh Old Field Meadow (Balsdon and Snyder 2015). Prior to transplanting and seeding, the site was already in a state of natural recovery, and included a few rare or at-risk prairie plant species. Soil sampling was conducted on areas of land undisturbed by transplanting and seeding, while mushroom surveys were conducted across the entire site.

FRS #28 is adjacent to FRS #27, east off the Howard Avenue Diversion, which separates the two sites (42.228° N 82.993° W). It is split in two by the Howard Ave Connector and a parking lot. Both the north-east and south-west parts were surveyed for mushrooms, but soil could be sampled within the north-east half only, on land undisturbed by transplants. Like FRS #27, this site was tilled agricultural land until 2011, at which point it underwent identical invasive species management and restoration efforts, over the same time period. In 2014 the site was assessed as Dry-Fresh Old Field Meadow vegetation community (the same community type as FRS #27) (Balsdon and Snyder 2015).

2.1.2 Ojibway Prairie Provincial Nature Reserve

The Ojibway Prairie Provincial Nature Reserve (OPPNR) is owned by the Ontario Ministry of Natural Resources and is one of five sites in Windsor collectively referred to as the Ojibway Prairie Complex (Ojibway Nature Centre 2015). It is a large (100 ha), roughly P-shaped block of land located at the south-east corner of Matchette and Titcombe Rd. The OPPNR consists mostly of tallgrass prairie and oak savannah, although micro-landscape variations exist, including shrubby zones and wet fern-dominated areas. Two areas of open grassland 300 m apart were chosen to sample for soil

within this prairie (OPC1: 42.263° N 83.071° W, and OPC2: 42.261° N 83.068° W), and mushroom surveys were conducted in the same general areas.

The Ojibway Prairie Complex has a long and interesting history (Ojibway Nature Centre 2011). It has consistently escaped development: from early French settler farmsteads in the mid-18th century to major industrial proposals that never came to pass due to the depression in the 1930s. In 1961 the City of Windsor set the land aside as a natural park. Since then, appreciation for the ecological aspects of the park increased, neighbouring acquisitions were added, and legal protections of the land were made stronger. The two sampling sites in OPPNR are, as far as can be known, remnant tallgrass prairie and undisturbed from tillage activity.

2.1.3 Walpole Island First Nation

Walpole Island First Nation is located just north of Lake St. Clair and contains five distinct sampling sites. Tallgrass prairies are amenable to the naturally occurring soil conditions in this region - a deltaic sand plain (Chapman and Putnam 1984). The availability of these sites is due to the community's environmental ethic (Beckford et al. 2010), and their allowing the lands to be accessed for research. Unless otherwise stated, tillage history and dominant plants mentioned here are sourced from site descriptions by Stover et al. (2012), who conducted fungal-plant research at the same sites.

Silphium prairie is a high quality prairie remnant located near the northern point of the island (42.628° N 82.502° W). It is dominated by native grasses (Indian grass, big and little bluestem) and herbaceous plants such as prairie dock (*Silphium terebinthinaceum*). Some mature oaks are present, and invasive reed grass encroaching from the south is being actively managed.

Sandpits field is located just southeast of Silphium prairie (42.627° N 82.502° W), and is an old field that was tilled from 2002 to 2006. It is covered with thick, tall vegetation, consisting primarily of goldenrod and sweet and regular clover. This site is representative of a low quality, early successional prairie after agricultural disturbance, although a controlled burn was conducted in 2000 (Turner 2001).

Mike's field (42.580° N 82.494° W) is an old field that was tilled for corn cropping until 1990. Burns were conducted since then, but the site was otherwise left undisturbed and allowed to revegetate. The vegetation is heavily dominated by goldenrod, with some sweet clover and horsetails.

Eliza's prairie is located near the centre of the island (42.580° N 82.489° W). It is a privately owned field that was tilled in 1940 but has since successfully recovered as a quality tallgrass prairie. Panic grass is the dominant plant cover. There is also good cover of small rushes and sedges. There are sporadic woody shrubs, as well as a couple of oaks and some aspen encroachment from the mature forest surrounding the site.

Pottowatomi prairie is located in the south-centre part of the island (42.550° N 82.500° W) with agricultural disturbance noted in some areas from 1943 air photos. It is dominated by native plants such as little bluestem and panic grass, as well as dense blazing star. A few very tall cottonwood trees are present. Except for a human-constructed soil ridge, the site is high quality tallgrass prairie and appears to be undisturbed. Soil organic carbon measurements in this study suggest previous disturbance effects were minimal to none. The site has doubtlessly been burned in the past, including a controlled burn that was conducted in the spring of 2000 (Turner 2001).

2.1.4 Dutton-Dunwich

Located in the township of Dutton-Dunwich (south of Hwy 401 half-way between London and Chatham-Kent), this remnant tallgrass prairie covers two miles of abandoned rail line right-of-way (42.643° N 81.536° W). The prairie is managed in a partnership between the West Elgin Nature Club and Elgin County Stewardship Council. Despite much of the soil being covered with gravel, the site contains many characteristic or rare native prairie plants (such as big bluestem, Indian grass, blazingstar, gray-headed coneflower, compass plant), and is subjected to periodic prescribed burns. These site details are from the Naturally Elgin webpage about the prairie (Naturally Elgin 2012). The site was used for additional mushroom surveys only; no soil samples were collected. A small area east of the road not occupied with ditch, gravel, or aspen forest consists of an apparently undisturbed prairie remnant, and was the most productive part of this site

for prairie mushrooms. The township of Dutton-Dunwich is on the eastern edge of the Bothwell Sand Plain, deposited by the early Thames River during the retreat of the Wisconsin ice sheet (Chapman and Putnam 1984).

2.1.5 Norfolk County

Two sites with different ownership but similar natural features were sampled in Norfolk County. Both are relatively recent restorations from previous tilled cropland and have particularly sandy soils. The sandy soils are typical of the Norfolk Sand Plain, which developed as deltaic deposits in the glacial lakes Whittlesey and Warren (Chapman and Putnam 1984).

DeMaere prairie is located east of the township of Walsingham, south of Highway 24 (42.685° N 80.464° W). The property was used as a tobacco farm until 2003 and then for soy and corn crops until 2010 when the Nature Conservancy of Canada acquired the site and restored it to tallgrass prairie (McPhee *et al.* 2015). The vegetation consists of a mixture of species from the 2010 restoration seed mix and naturally occurring vegetation (both native and exotic), as well as planted sapling pines and oaks. The site is bordered by forest to the east and west, a sand hill separating it from cropland to the south, and by Highway 24 to the north. Soil sampled from this site was used, and subsequent mushroom surveys were conducted.

The other Norfolk County site is a prairie restoration by Mary Gartshore and Peter Carson on their property west of Walsingham, about 10 km west from the DeMaere site on Highway 60/24 (42.641° N 80.572° W). The land was used as a tobacco farm since the 1930s until restoration work began by Gartshore and Carson in 1991/92 (P. Carson, pers. comm. 10 July 2015). It was restored gradually over many years by applying native seed mixes in 1 m strips, totaling 39 rows across the site. Prescribed burns and herbicide were used as needed (about 15 times from 1991 to 2015) to manage the site for woody encroachment and invasive species. A few individuals of staghorn sumac and oaks were retained. There are sandy dune-like areas similar to DeMaere prairie. The site was used for additional mushroom surveys only; no soil samples were taken for this study. The

landowners report finding a large diversity of mushrooms on their restoration after rainy weather.

2.1.6 Blair Flats

The RARE Charitable Research Reserve Cambridge was founded in 2001 and currently includes more than 900 acres of land representing a wide diversity of natural ecosystems (Craig et al. 2014). The organization is based in Cambridge, whereas their properties are located east of there, in the Township of North Dumfries. The Blair Flats (43.384° N 80.373° W) are a part of the reserve with a 60+ year history of corn-soy crop rotations, but in 2009 the eastern half was allowed to naturalize and in 2010 the western half was planted to tallgrass prairie (Germain et al. 2013, Craig et al. 2014). Prior to restoration, the site had been sprayed with glyphosate and plowed, leaving bare soil on which the seed mix of 24 native grasses and forbs were broadcast over the field (Drystek & MacDougall 2014). The site recently underwent a prescribed burn with 80% coverage in April 2015, and future burns are planned on a 5 to 7 year timeline (J. Quinn 2016, pers. comm. 22 February). The vegetative cover includes a strong display of native prairie species (an abundance of goldenrod, big bluestem, and Indian grass, as well as a diversity of native broad-leaved plants). The soil in this region was developed from floodplains formed by the spillway through the till plain when the Wisconsin ice sheet was receding (Chapman and Putnam 1984).

2.2 Field sampling

2.2.1 Soil sampling design

Soil was collected by Sarah Allan from each prairie by arranging six 1 × 1 m plots to capture maximum variety across the landscape. Five soil cores 20 cm deep and 2.5 cm in diameter were taken from each plot and combined into one bag. The top layer of litter was removed from each core. An additional core was taken at each plot for soil composition analysis and combined into one bag across the six plots for the entire site (Figure 2.2). The soil corer was cleaned using a cloth and 70% ethanol to prevent soil from mixing between sampling plots. Bags of soil were kept on ice packs in a cooler

while in the field until they could be transferred into a -20°C freezer for long-term storage.

Sequence data from Walpole sites in June and October 2009 (Silphium, Sandpits, Mike's, Eliza's, and Pottawatomi) were derived from soil collected in the field by Aniruddho Chokroborty-Hoque with a very similar design - two transects with three plots located randomly along each (Chokroborty-Hoque 2011). Sequence data from DeMaere prairie were derived from soil collected by Catriona Catomeris in June and October 2014. The sampling design consisted of a transect of 8 blocks, with three samples taken from each block (Catomeris 2015). All other soil was collected by Sarah Allan in June/July and October 2014 (Walpole Island could be sampled only in October) (Table 2.1).

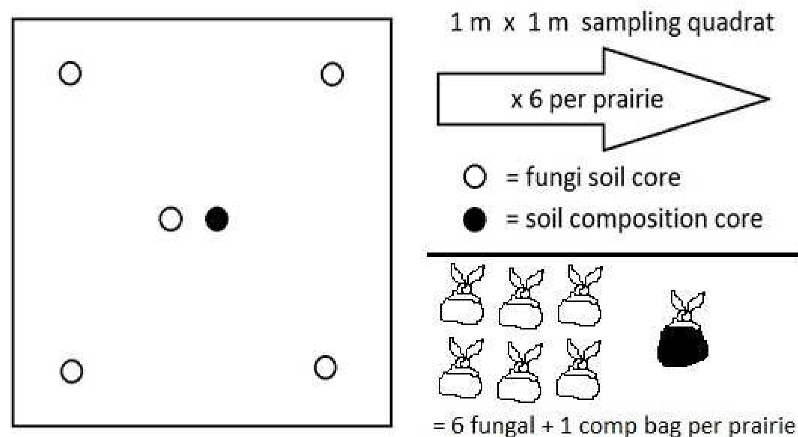

Figure 2.2 Field sampling design and the resulting bags of soil.

Table 2.1 Soil sampling of prairie sites by three researchers from 2009 to 2014. Principal soil sampler: A = Aniruddho Chokroborty-Hoque, C = Catriona Catomeris, and S = Sarah Allan. Dutton-Dunwich and Mary & Peter's prairie were not sampled for soil. Sites are organized by geographic location, from west to east.

	HA	HB	OA	OB	HC	HD	SI	SA	MI	EL	PO	DM	BF
2009-Jun							A	A	A	A	A		
2009-Oct							A	A	A	A	A		
2014-Jun/Jul	S	S	S	S	S	S						S	S
2014-Oct	S	S	S	S	S	S	S	S		S	S	C	S

2.2.2 Mushroom collection

I conducted mushroom surveys at least once in the fall and once in the summer (Table 2.2). All sites with soil sampling were also surveyed for mushrooms, with the exception of Mike's Field, which could not be accessed for logistical reasons. Two additional sites were surveyed for mushrooms that were not sampled for soil: Dutton-Dunwich prairie remnant and Mary & Peter's prairie restoration (Table 2.2). A GPS was used to begin surveys near soil-sampling plots, but the remaining cover of each site was surveyed in a wandering design (as opposed to sampling plots – for maximal coverage) for approximately one hour each visit. Fruiting bodies were counted, genetic individuals estimated (based on proximity and known fruiting patterns for different taxa – e.g. clusters, fairy rings), and a voucher specimen was collected for sequencing of each morpho-species (conservatively estimated in the field). Each voucher was given a specimen code, photographed, and notes were taken on ecology, ephemeral identification features (such as smell, colour, and cap shape), and GPS location. Vouchers were normally small enough to fit into fishing tackle-box cells, otherwise larger containers were used (to avoid cross-contamination, only one cell or container was used per specimen). Mushrooms were preserved using a food dehydrator and stored in labelled paper packets for subsequent lab sequencing and more accurate identification.

Table 2.2 Mushroom sampling over three periods from Oct 2014 through Oct 2015. Collection visits are indicated by "+". Mike's field was not surveyed for mushrooms. Sites are organized by geographic location, from west to east.

	HA	HB	OA	OB	HC	HD	SI	SA	EL	PO	DD	MP	DM	BF
2014-Oct							+	+	+	+				
2015-Jun/Jul	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2015-Oct	+	+	+	+	+	+	+	+	+	+	+	+	+	+

2.3 Soil sieving

Soil from each plot was weighed to 20 g and combined with 100 mL of 0.1 M sodium pyrophosphate in a clean jar. The mixture was shaken vigorously and allowed to sit for 5-10 min to break apart soil colloids before being re-agitated and poured over three

stacked sieves with pore sizes of 1.18 mm, 0.25 mm, and 0.053 mm, then washed with deionized water. The sieving technique allows for the capture of plant debris, fungal hyphae, rhizomorphs, and sclerotia, while removing spores, such as the abundant asexual spores of ascomycetous and zygomycetous molds (Thorn et al. 1996, Lynch and Thorn 2006). Plant roots (including any fungi that may be present on their surfaces) were picked from the top, coarse sieve with forceps and placed in a Falcon tube. Dark organic matter was separated from sand and silt and scooped from the middle sieve and pipetted with a broad tip from the lowest, fine sieve, and added to the Falcon tube until approximately 5 mL was obtained. Sieves were rinsed with deionized water and cleaned using 70% ethanol between each sample to prevent mixing of soil material between samples.

2.4 Molecular protocols

Soil organic matter was lyophilized and ground with liquid nitrogen using a mortar and pestle until a floury texture was reached, whereas mushroom samples were bead-beaten to assist physically in cell wall lysis. Bead beating was carried out in a FastPrepFP120 machine (Bio101, Qbiogene, Inc., Carlsbad, CA, USA) at a setting of 4.0 for 30 sec. Molecular methods from this point forward were similar for both soil and mushroom specimens. Sequencing was attempted on at least one voucher of each mushroom morpho-species. DNA extraction was carried out using a Zymo Research Soil Microbe DNA MicroPrep kit for soil, and a Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit for mushrooms. The concentration of eluted DNA was determined using a Thermo Scientific Nanodrop2000 Spectrophotometer.

PCR reactions were conducted using a total volume of 25 µL; for soil: 1.0 to 3.0 µL template DNA (at ~20 ng/µL), 12.5 µL ToughMix (Quanta Biosciences), 3 µL each for the forward and reverse primers, and 0.5 µL loading dye; for mushrooms: 0.5 to 1 µL template DNA (at ~20 ng/µL), 12.5 µL FroggaMix (FroggaBio), and 1.25 µL each of the forward and reverse primers. For soil samples, a newly developed primer set was used (LSU200-F (AACKGCGAGTGAAGMGGGA)/LSU481-R (TCTTTCCCTCACGGTACTTG)) which targets *ca.* 250 bases at the LSU D1 region of ribosomal DNA which is useful for retrieving and identifying a wide range of fungi, particularly Agaricomycetes (Asemaninejad et al. 2016). For mushrooms, the ITS8F

(AGTCGTAACAAGGTTTCCGTAGGTG) and LR3-mod (GGTCCGTGTTTCAAGACGGG) primer pair was used (Vilgalys and Hester 1990, Dentinger et al. 2010). Gene regions amplified for mushrooms and soil overlapped so that comparisons could be made between aboveground and belowground surveys; different primers were used because Illumina sequencing requires short sequences (here *ca.* 250 bases) whereas mushrooms could be used to obtain longer sequences (here *ca.* 1,250 bases) including the LSU region covered by the soil primers as well as the ITS region standard for mushroom sequencing. The longer mushroom sequences were useful for finer-scale identifications, but were reduced to only the overlapping region with soil sequences for aboveground-belowground shared species comparisons. Soil templates were PCR amplified in a Biometra T1 Thermocycler with a start of 94 °C for 2 min, then 30 cycles of 94 °C for 30 sec, 60 °C for 30 sec, 72 °C for 18 sec, and after cycling, holding at 4 °C. Mushroom templates were PCR amplified in a MWG Biotech Primus96 thermocycler starting with 94 °C for 1 min, then 30 cycles of 94 °C for 30 sec, 58 °C for 30 sec, 72 °C for 1 min 30 sec, and after cycling an extension time of 72 °C for 7 min before holding at 4 °C. PCR products were checked for contamination and successful amplification via gel electrophoresis using agar-agar gels in 1× TAE buffer and a BIO-RAD Power-Pac 3000 to supply electrical charge.

Soil PCR products were pooled for each site (six initial plots pooled to one tube), lyophilized, and rehydrated before being submitted for paired-end Illumina MiSeq high-throughput sequencing at the London Regional Genomics Centre (Robarts Research Institute). Mushroom PCR products were cleaned using a BioBasic EZ-10 Spin Column PCR Products Purification Kit and submitted for Sanger sequencing (Sanger et al. 1977). Because of the length of the mushroom sequences desired, mushroom PCR products needed to be submitted for sequencing four times using different primers to obtain a portion of the total sequence length each time, and were later assembled (using Geneious 8.0.5) to obtain the full sequence. Full mushroom sequences represented a partial sequence of the SSU (18S) rRNA gene, complete sequences for the ITS1, 5.8S, and ITS2 rRNA genes, and a partial sequence of the LSU (28S) rRNA gene. The four primers were: ITS8F, LS1R(-mod) (CTTAAGTTCAGCGGGTAGTCC), LS1-mod (GGACTACCCGCTGAACTTAAG), and LR3-mod (Vilgalys and Hester 1990, Hausner

et al. 1993, Dentinger et al. 2010). All sequencing was carried out at the London Regional Genomics Centre (Robarts Research Institute, London, Ontario, Canada).

2.5 Mushroom identification

Sequences were assembled and checked for errors using Geneious 8.0.5. Assembled sequences were queried through the NCBI GenBank database using their Basic Local Alignment Search Tool for nucleotide sequences (blastn) to check for assembly errors, find nearby matches, and assist in identification.

Mushrooms were identified using macro- and micro-morphological features, as well as occasional chemical tests and ecological information to navigate taxonomic keys. Specimens were kept for long-term storage at the University of Western Ontario's herbarium (UWO), and specimen photos and data were made available online (http://mushroomobserver.org/species_list/show_species_list/652) though MushroomObserver.org (Wilson and Hollinger 2016).

2.6 Vegetation metrics

Custom lists of plant species and percent cover were created for each site using GPS to survey only the areas of soil sampling plots with a buffer of few metres. I performed these surveys in October 2015. After completing surveys, the Universal Floristic Quality Assessment (FQA) Calculator provided data for southern Ontario flora, which were needed to calculate site metrics derived from the plant species lists (Freyman et al. 2015, Oldham et al. 1995). Three metrics were chosen for this study: total and native species richness (TSR, NSR), adjusted cover-weighted Floristic Quality Index (FQI), and mean coefficient of wetness.

Total species richness was calculated as a simple count of the total number of plant species present within each site's survey area. Native species richness includes only native species (excluding alien ones). The decision for defining species as native or alien is sometimes debated, but Oldham et al. (1995) was used as the standard for this study.

Floristic Quality Assessment metrics are based on coefficients of conservatism, which are assigned to each plant species in a region. These scores are based on each plant's sensitivity to degradation and tendency to be present in high quality or pristine ecosystems (Taft et al. 1997). The scores range from 0 to 10, with lower scores belonging to plants well adapted to degradation, middle scores for species common in many communities, and higher scores for plants increasingly limited to natural areas. Several metrics can be derived from C-scores, of which adjusted cover-weighted FQI was chosen for this study. The metric of adjusted cover-weighted FQI does not reduce its score for sites with naturally fewer species that are actually high quality (e.g., bogs), takes percent cover into account so that the effect of missing rare species is not as large, and does not exclude non-native species, which slightly inflates the score (Miller and Waldrop 2006). This is the most fitting metric given that plant surveys were conducted briefly over a small area, rather than being detailed inventories of an entire site. Adjusted cover-weighted FQI (I'_{cw}) is calculated as follows:

$$I'_{cw} = 100\left(\frac{\bar{C}_{\gamma}}{10}\frac{\sqrt{N}}{\sqrt{N+A}}\right)$$

Where $\bar{C}_{\gamma}$ represents mean cover-weighted coefficient of conservatism, N is the native species richness, and A is alien species richness.

Plants can be assigned coefficients of wetness – a similar concept to coefficients of conservatism. Mean coefficient of wetness is the standard measure used to assess hydrology of a site based on its vegetative composition. It is calculated by dividing the sum of wetness coefficients for each plant species present on the site by the total species richness of the site. In the United States, wetness coefficient scores are based on nominal categories (obligate wetland, ±facultative wetland, ±facultative, ±facultative upland, and upland) from national lists of wetland plants (Reed 1988). These can easily be converted into ordinal values from +5 (upland) to 0 (facultative) to -5 (obligate wetland) (Taft et al. 1987). Southern Ontario plants have been assigned scores by the same system (Oldham et al. 1995).

2.7 Soil analyses

Approximately 125 g of soil from each site's bulk composition bag was weighed into tins and dried in a drying oven at 50 °C for 48 hours. The coarse dried soil was used for pH measurements. Remaining soil was gently ground and sieved at 1 mm, re-dried at 100 °C for 24 h, and stored in a desiccator with 454 g Drierite (VWR) before being used for organic carbon and texture measurements.

2.7.1 pH

The procedure for soil pH measurement described by Thomas (1996) was followed, with some minor modifications. A VWR SympHony pH meter model SB20 with a calomel electrode was calibrated using reference solutions (VWR) of pH 7.00, 4.01, and 10.01. Soil and deionized water were combined at a 1:1 ratio (20.0 g soil and 20.0 mL water) in a beaker with a magnetic stirrer. This was repeated three times for each site to account for soil and instrumental variation. The electrode was rinsed with deionized water between readings.

2.7.2 Organic carbon

The loss-on-ignition method from Nelson & Sommers (1996) was used. Crucibles were heated in a muffle furnace at 400 °C for 2 h, cooled in a desiccator with Drierite for 30 min, and dry weights recorded to 0.001 g. Soil was weighed at 2.000 ± 0.001 g and recorded as pre-ignition weight. Soil samples were ignited in the muffle furnace at 400 °C for 16 h to remove organic carbon. They were allowed to cool for 2 h before opening the oven and being placed in the desiccator for 30 min to cool to room temperature. Post-ignition soil plus crucible weights were recorded. Percent organic carbon was calculated for each site as:

$$Organic\ carbon\ \% = \left(\frac{Pre\ ignition\ weight - Post\ ignition\ weight}{Pre\ ignition\ weight}\right)100\%$$

2.7.3 Texture

Texture was assessed using the Finger Assessment of Soil Texture method as described in the Ecological Land Classification for Southern Ontario field guide (Lee et

al. 1998). Sand, silt, and clay were estimated using qualitative tests such as forming a ball or ribbon, as well as feel, taste, and shine tests.

2.8 Geographic region – position on a transect

Broad geographic regions of belowground-sampled sites were translated into one-dimensional values for inclusion in multivariate statistics by creating a diagonal transect across the map (Figure 2.3). Neither latitude nor longitude alone were able to represent location of sites as well as the position on a transect. A preliminary analysis of correlation coefficients on a PCA biplot found position on a transect explained 0.509 of the first axis, whereas latitude and longitude only explained 0.345 and 0.382 percent of the variation respectively. Latitudinal values misrepresent the distance between Walpole and Norfolk sites, whereas longitudinal values misrepresent the distance between the Cambridge and Norfolk sites (Figure 2.3). Other methods of distance measurement were not explored.

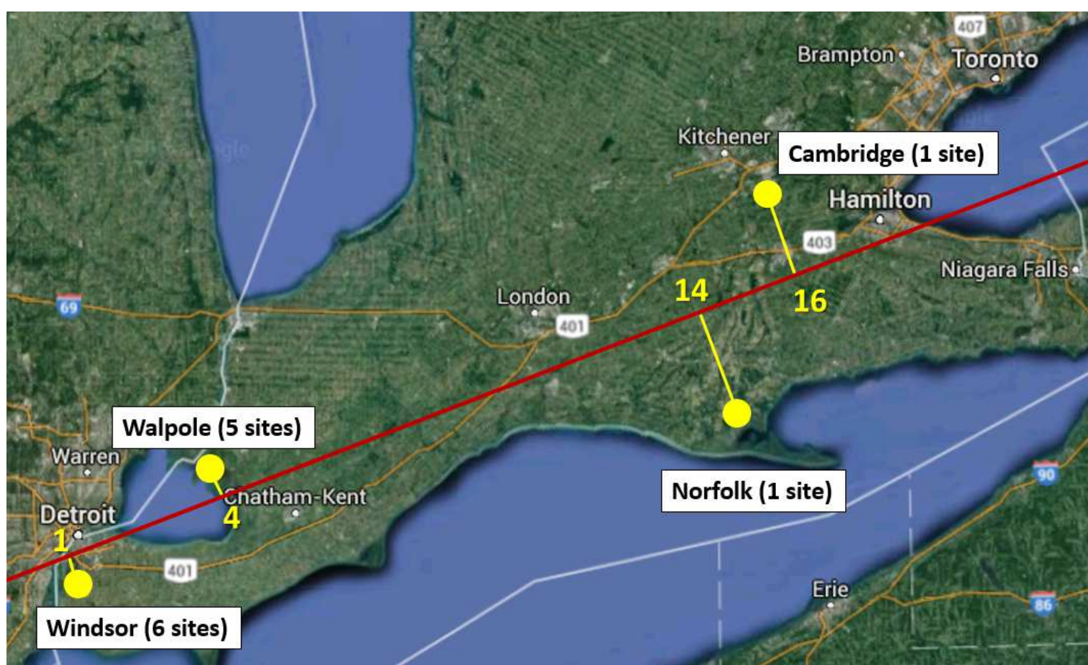

Figure 2.3 Map showing positions of soil-sampled sites on a diagonal transect. The diagonal transect is indicated in red. Yellow circles represent areas where several sites were relatively near to one another. Position on the transect for each group of sites is indicated with a yellow number.

2.9 Sequence and phylogenetic analysis

A pipeline (created by Greg Gloor from Biochemistry, Western University), which incorporated several software programs, was used to process raw sequence data. PANDAseq was used to overlap forward and reverse fastq raw sequence reads, with a minimum overlap of 30 nucleotides (Andre et al. 2012). UCLUST was used to cluster sequences into identical sequence units (ISUs, 100% similarity) then into species-level operational taxonomic units (OTUs) at 97% similarity and to choose a centroid seed OTU sequence (the most common sequence in each OTU cluster) (Edgar 2010). This 97% cutoff is stricter than the 99% level of genetic difference discovered between yeast species in the D1-D2 region of the LSU(25S) rRNA (Peterson and Kurtzman 1991) because our amplicons include only the most variable portion of that region. Only ISUs and OTUs over 1% abundance were kept, and retained ISU and OTU sequence reads were mapped back onto the sites. UCHIME was used to check for and remove chimera sequences (Edgar et al. 2011). Data from three separate Illumina MiSeq runs were processed separately and then combined: A. Chokroborty-Hoque's Walpole site soils from 2009, C. Catomeris' DeMaere prairie soils from 2014, and S. Allan's 2014 soils from other prairies in this study. Subsequently, the OTU_tag_mapped file was checked in Microsoft Excel for low reads within sites and any reads less than 0.1% of the sum of the site were considered absent (0). A cutoff of 0.1% was used instead of 0.01% because when data are combined from multiple sequencing runs the output becomes messier and a more stringent cutoff is required (pers. comm. Greg Gloor, Department of Biochemistry, Western University, May 2015).

Sequences of OTUs were coarsely identified using the sequence classifier from the Ribosomal Database Project (RDP) (Wang et al. 2007) as Agaricomycetes, other fungi (other Basidiomycota, Ascomycota, Glomeromycota, or Zygomycota), Amoebozoa, Animalia, Viridiplantae, or other Eukaryota. A neighbour-joining phylogenetic tree (Saitou and Nei 1987) was produced for all OTUs, and the clade containing mostly Agaricomycetes was extracted. Sequences within this clade that had been classified as non-Agaricomycetes by RDP and sequences classified as Agaricomycetes that were outside of the Agaricomycetes clade in the tree were separately

queried against GenBank sequences to check their matches (http://www.ncbi.nlm.nih.gov/blast/), and only true Agaricomycetes were retained.

OTUs of Agaricomycetes were queried to identify each to genus or family taxonomic levels. Species-level identifications were applied only when query cover and percent identity were each greater than or equal to 98%, without competing species names in this range. Species-identified OTUs should be interpreted with caution; a reference table was produced for these OTUs including the accession numbers of the closest matches (Appendix A). OTUs were placed in major (*ca.* order level) and minor (*ca.* family level) clades based on their query IDs. To visualize the clades, particularly for placing the OTUs with weak matches, neighbour-joining trees with 100 bootstrap replications were produced. Clade-groupings of OTUs, in addition to individual OTUs, were used for subsequent analyses.

2.10 Statistical methods

Agaricomycete OTU (fungal) richness and abundances were determined by combining multiple sampling events (i.e., wells in sequence runs, usually representing seasons) to obtain one value for each OTU per site. OTUs were further combined as needed for minor or major clade analyses. To examine community composition across all sites, belowground high-throughput sequence data was used. Pie charts were produced to display relative abundances of major and minor clades, a bar chart was produced to display richness of OTUs in minor clades, and a ranked list of OTUs by abundance was produced, all using Microsoft Excel. The ranked list of dominant OTUs was produced by combining the 10 most abundant OTUs from each site into a list of 70 different OTUs across all 13 sites with belowground data. To rank the OTUs, read counts were converted into relative abundances and summed across sites for each OTU.

To determine the relative importance of environmental variables in determining Agaricomycete composition, belowground high-throughput data were manipulated using R (RStudio Team 2013). High-throughput soil sequencing data were centre log-ratio (clr) transformed to scale the data and proceed with ratio (abundance) analyses (Gloor 2015). The compositions package in R (van den Boogaart and Tolosana-Delgado 2008) was

used to prepare data to produce the outputs described in this paragraph. A dendrogram grouping sites by abundance in dominant minor clades was produced using the Euclidian distance measure (dist) and ward.D2 hierarchical clustering (hclust), with associated relative abundance bars plotted below each site. A compositional biplot was produced using principal component analysis (PCA) via the prcomp function, including a scree barplot to show eigenvalues of each axis. Environmental variables were treated as metadata for each site and correlated to each axis as correlation coefficients using the cor function and Kendall's tau as the method, which is not dependant on linearity of either dataset (Kendall 1938).

Tillage-sensitive minor clades were examined by comparing relative abundance of reads from tilled sites to pristine sites. These were considered using Walpole sites alone (reduces sample size, but removes geographic autocorelation of sites as a confounding effect) and across all sites (leaves the sample size as large as possible for this study, but the confounding effect of geography remains). Minor clades were selected as tillage-sensitive only if the effect was strong (*ca.* 5 times greater abundance in tilled sites than pristine sites or vice versa) and only if the trend was true when examining the data both ways (if the trend was consistent between all sites and Walpole sites alone). Rare taxa present in only one site were not considered candidates for being included in the list of tillage-sensitive taxa, but absences of taxa from all tilled or all pristine sites did not necessarily disqualify them from being considered tillage-sensitive, since absence in one direction may indicate an extreme effect.

Environmental variables of sites were visualized as bar charts and tables using Microsoft Excel and interpreted with differences between tilled and pristine sites in mind. Sites designated "pristine" were never tilled or tilled for a brief period more than 60 years ago (Pottawatomi and Eliza's prairie) whereas sites designated as "tilled" were used for agriculture much more recently (within four to eight years prior to sampling dates). Relationships between OTU richness of sites and environmental variables were explored by producing regressions (scatter plot trendlines) using Microsoft Excel. These were considered exploratory only, not as inferences of statistical significance, given the lack of independence between sites (sites are autocorrelated by geographic region clusters).

Aboveground and belowground comparisons of abundance and richness in minor clades were visualized using doughnut charts produced using Microsoft Excel. Potential OTU-mushoom matching pairs were found by producing a neighbour joining tree, and then confirmed as 100% identical by alignment using MUSCLE in MEGA6 to check for any dissimilar base pairs (Edgar 2004). To visualize the degree of shared species overlap between the two methods, area-proportional Venn diagrams were produced using the venneuler package in R (Wilkinson 2011). Comparisons of abundance of shared species and their occurrence across sites were visualized through a table and side to side bar charts produced in Microsoft Excel.

Chapter 3 - Results

3 Results

3.1 Sequence recovery

Combined datasets consisted of 529,259 individual sequence units (ISUs), from which the pipeline identified and removed 22,577 as possibly chimeric and 492,352 singletons; the remaining 14,330 were clustered at 97% similarity into 1,275 operational taxonomic units (OTUs). Removing read counts <0.1% of the total reads from each site did not result in the loss of all reads for any OTU (all OTUs were retained despite reducing the dataset). Manual filtering of OTUs using RDP, a neighbor-joining tree, and GenBank querying left 281 OTUs of Agaricomycetes (see Appendix B). Some OTUs from the Cantharellales did not group with other Agaricomycetes in the neighbour-joining tree, but were retained because RDP identified them as belonging to the class. The total of 281 OTUs was further reduced to 256 OTUs after removing nitrogen-treated plot data from DeMaere prairie, leaving only untreated control plots (the full dataset was used for a separate project – Catomeris 2015). The 256 OTUs were used for statistical analyses, but the 281 OTUs are included in Appendix B as a full species list.

A total of 149 collections of fruiting bodies representing 74 morphospecies of Agaricomycetes were collected, and attempts were made to extract, amplify, and sequence rDNA from 92 of the collections. Thirty-six collections were successfully sequenced and assembled for the full *ca.* 1300 bp region (ITS8-F to LR3). Of the remaining 56 collections attempted for sequencing, seven returned mixed product sequences, 18 sequences contained insertions-deletions, and the 31 other collections did not reach the sequencing stage for a variety of reasons (more than half of these collections were small mushrooms where some step failed and there was no material left to try again). Of the 74 morphospecies, 30 were identified to species level (or in five cases, identified to a species group), 39 to genus level (or two genera in one case), and five were considered different unknowns (could not be identified even to family level) (see Appendix C).

3.2 Community composition

3.2.1 Major and minor clade representation

The 256 OTUs were grouped into 19 major clades and are displayed in a phylogenetic context (Figure 3.1). OTU-rich clades included the Tricholomatoid, Agaricoid, and Clavarioid clades, and the Cantharellales, all containing greater than 20 OTUs. Conversely, the Jaapiales, Corticiales, Atheliales, Thelephorales, and Trechisporales each contained fewer than 5 OTUs. By far the Agaricales was the most OTU-rich order in the Agaricomycetes, here containing six major clades and 131 OTUs – just over half of the total OTUs. Finer-scale groupings were produced by splitting the 19 major clades into 55 minor clades (Figure 3.2). The most OTU-rich minor clades were the Clavariaceae, Entolomataceae, Polyporaceae *sensu lato*, Sebacinaceae, and Hygrophoraceae.

When considering major clades by abundance (measured by OTU sequence reads) the Tricholomatoid clade and Cantharellales contain *ca.* 50% of total read abundance. This trend is paralleled by the minor clades that are associated with those major clades containing *ca.* 50% of total abundance: the Mycenaceae (Tricholomatoid), Ceratobasidiaceae (Cantharellales), and Polyporaceae *sensu lato* (Polyporales) (Figure 3.3).

Minor clades with the highest diversity tended to have relatively low read abundance, sitting in the lowest quarter (compare Figure 3.2 with Figure 3.3). The Clavariaceae (Clavarioid major clade) had the highest diversity among minor clades (22 OTUs) but represented only 1.5% of read abundance. Similarly, the Entolomataceae had the second highest diversity (18 OTUs) but only 1.9% read abundance, and the Sebacinaceae with third highest diversity (16 OTUs) had only 2.3% of the read abundance.

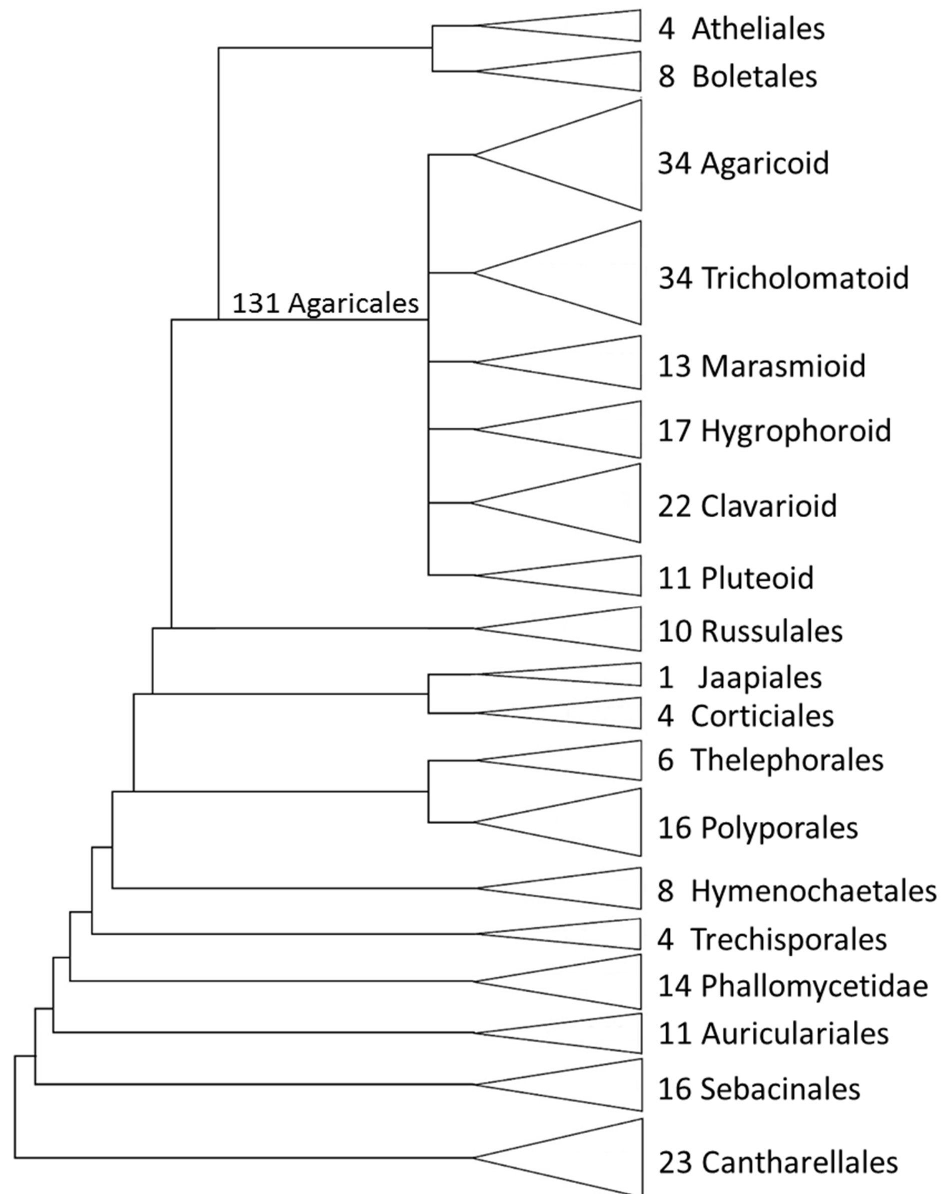

Figure 3.1 Phylogeny of major clades of Agaricomycete OTUs from remnant and restored prairies in southern Ontario.

The tree shows 19 major clades and 256 OTUs. The number of OTUs present in each major clade is shown numerically and by scaled branch tips. The topology is based on a number of sources compiled and arranged by Hibbett et al. (2014), as well as Binder et al. (2010) for approximate Atheliales placement, and Dentinger et al. (2016) for Clavarioid placement.

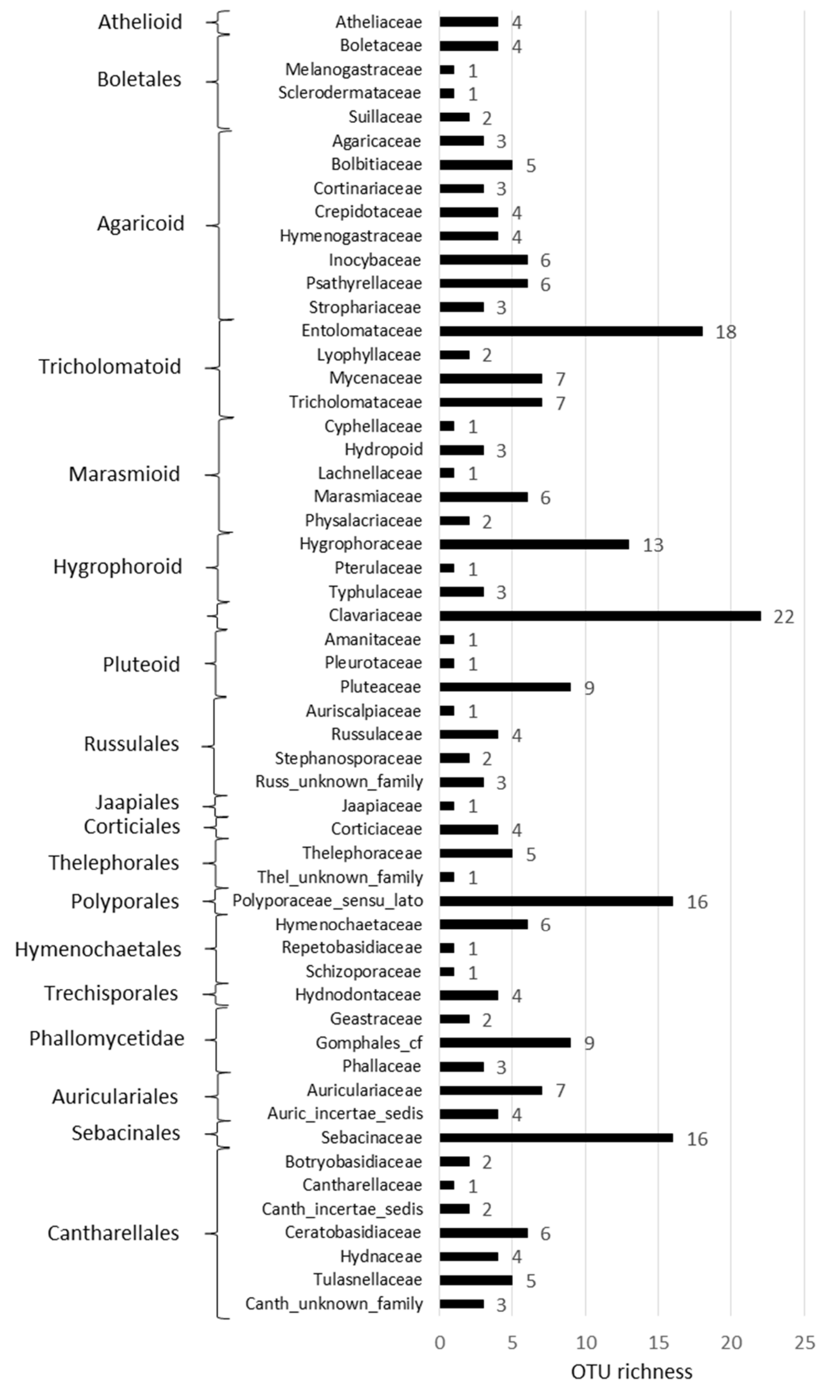

Figure 3.2 Distribution of OTUs among minor clades of Agaricomycetes from restored and remnant tallgrass prairies in southern Ontario.

Corresponding major clade groupings are indicated with brackets to the left of minor clade names.

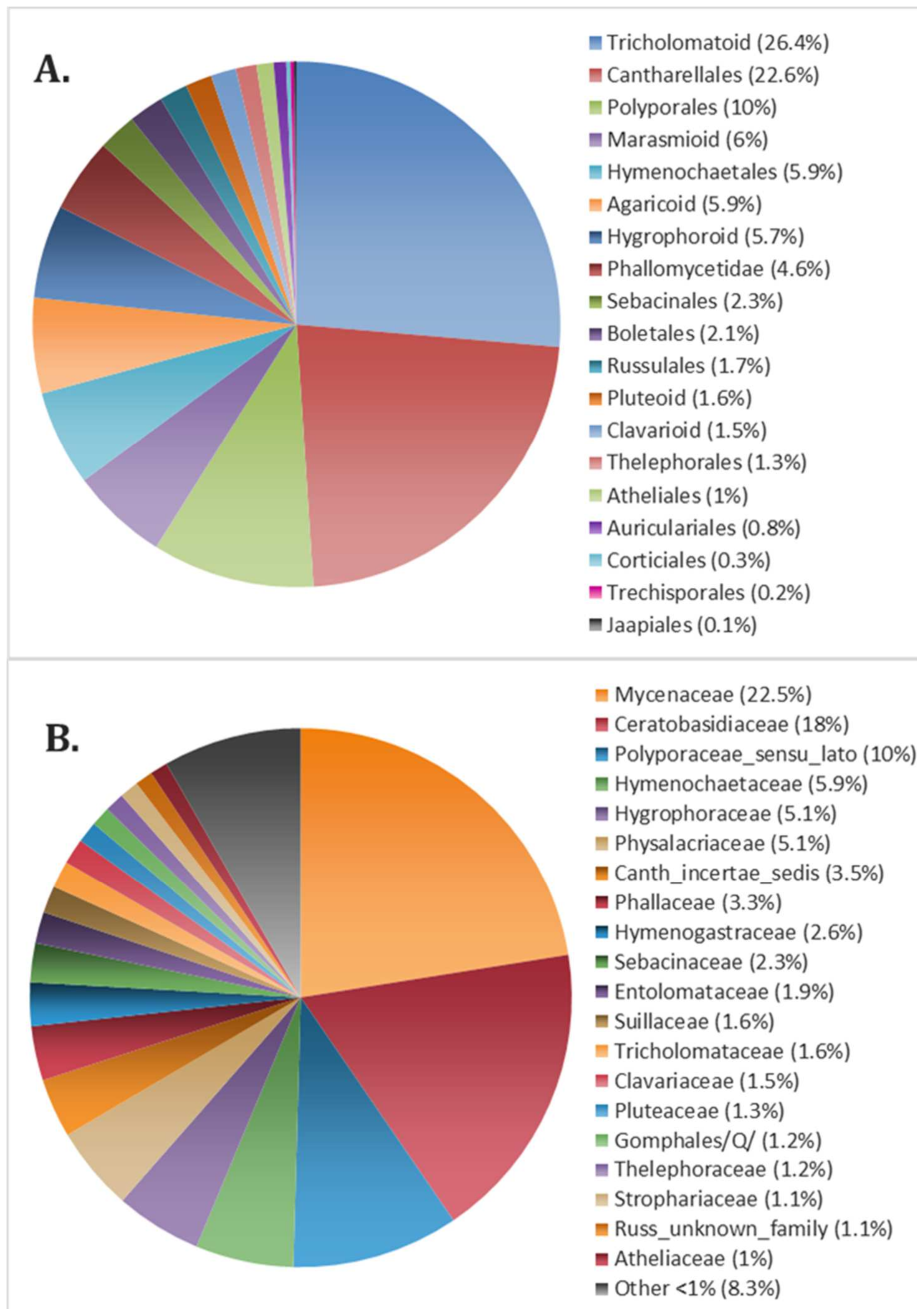

Figure 3.3 Relative abundance of sequence reads in A. major and B. minor clades. The percent relative abundance of each clade is indicated in brackets after the name. All 19 major clades and the most abundant 20 minor clades (≥ 1%) are displayed.

3.2.2 Dominant OTUs

The 10 OTUs with the highest total reads from each site were combined into a list of 70 different OTUs across all 13 sites. The top 10 OTUs from this list contain just over 50% of the total relative abundance from this list (Table 3.1). The most abundant OTU, Ceratobasidiaceae sp. 1, was present in the five Walpole sites only. The second OTU, *Minimedusa polyspora*, was present across all sites to varying degrees, except for Mike's field where it was absent. The third OTU, *Mycena epiptygeria* sp. 1, was highly abundant in all the Walpole sites but was also present with lower abundance in both Ojibway prairie sampling areas. The fourth OTU, *Mutinus elegans,* had the highest abundance of any OTU in any one site (DeMaere prairie) and had low abundance in some others. The fifth OTU, *Hypochicium* sp., was again only present in Walpole sites. Similar trends of local or broad distribution emerge when scanning down the remainder of the list (Table 3.1).

Dominant OTUs tended to be from minor clades of low or moderate richness: OTU_1 Ceratobasidiaceae sp. 1 from the Ceratobasidiaceae (six OTUs), OTU_9 *Minimedusa polyspora* from the Cantharellales *incertae sedis* minor clade (two OTUs, both *Minimedusa* spp., but the minor clades in the Cantharellales ranged from one to only six OTUs), OTU_5 *Mycena epiptygeria* sp. 1 from the Mycenaceae (7 OTUs), and OTU_0 *Mutinus elegans* from the Phallaceae (3 OTUs) (Table 3.1 and Figure 3.2). Similarly, minor clades with the highest diversity tended to have relatively low read abundance, sitting in the lowest quarter (Figure 3.2 and Figure 3.3). This shows the importance of examining both abundance and richness.

Table 3.1 A ranked list of compiled top 10 most abundant OTUs from each of 13 study sites.

Key to abundance: "***" >20%; "**" >5%; "*" >1%; " ' " >0.1%; where percentages represent relative abundance values per site across this list of 70 OTUs. Blanks represent zeroes or near zeroes (≤0.1%) that may or may not actually be present (low read counts are not dependable in high-throughput sequencing data).

Key to sites: HA,B,C,D = FRS #23,32,27,28 Herb-Gray Parkway (Windsor); OA,B = Ojibway Prairie Areas 1 and 2 (Windsor); SI = Silphium, SA = Sandpits, MI = Mike's field, EL = Eliza's prairie, PO = Pottawatomi (Walpole); DM = DeMaere (Norfolk); BF = Blair Flats (Cambridge). Sites are ordered by geographic location, from west to east.

Rank	OTU name	HA	HB	OA	OB	HC	HD	SI	SA	MI	EL	PO	DM	BF
1	OTU_1 Ceratobasidiaceae sp1							***	***	**	***	***		
2	OTU_9 Minimedusa polyspora	**	*	*	*	***	**	'	*		'	'	*	***
3	OTU_5 Mycena epiptygeria sp1			*	'			***	**	***	***	***		
4	OTU_0 Mutinus elegans	'	'	'									***	
5	OTU_12 Hypochnicium sp							**	**	**	**	**		
6	OTU_101 Russulales sp1	***	**	*	'						*			
7	OTU_15 Marasmiaceae sp3							*	**	*	**	*		
8	OTU_21 Hygrocybe conica group sp3	**		*	'			**						
9	OTU_47 Tricholomataceae sp3		*			*	**		'				*	**
10	OTU_19 Mycena sp2							*	**	**	*	*		
11	OTU_22 Gomphales sp3	**	*	*	**				'		*	*	*	*
12	OTU_17 Hyphodontia sp1							**	*	**	*	'		
13	OTU_160 Hygrocybe conica group sp2	*	**	*	*		'	'				*		
14	OTU_35 Sebacinaceae sp2					*	**		'				*	**
15	OTU_376 Fomitopsidaceae sp	*	*	**	*			*	'	'	*	*		*
16	OTU_189 Hymenogastraceae sp	'	'	*				*	**	*	*	*		
17	OTU_200 Tricholomataceae sp2	*		'	**									*

Rank	OTU name	HA	HB	OA	OB	HC	HD	SI	SA	MI	EL	PO	DM	BF
18	OTU_14213 Entoloma sp3	*	*	*	*	*	**	'	'			*		*
19	OTU_80 Lachnellaceae sp		'			*	*						'	**
20	OTU_404 Hygrocybe flavescens/chlorophana			**										
21	OTU_7123 Clitocybe sp	*		'	**									*
22	OTU_242 Pholiota tuberculosa	*	*	*	'		**							*
23	OTU_93 Coprinellus sp2	*	*	*	'	*					'		'	*
24	OTU_137 Typhula phacorrhiza					**	*							
25	OTU_127 Thelephoraceae sp1		'	*	*			'	*	'	*	*		
26	OTU_225 Pluteaceae sp1		'			*	**							
27	OTU_245 Amanita populiphila		**	'										
28	OTU_447 Russula putida				**									
29	OTU_238 Clavaria sp4			**	*	*					*			
30	OTU_480 Lactarius sp		*	**										
31	OTU_274 Pluteaceae sp3						**		'					
32	OTU_8677 Entoloma sp10		'			'	**		'				'	*
33	OTU_6038 Psathyrella sp		*	'	'		*						'	*
34	OTU_60 Suillus cavipes							**	'	'	*	*		
35	OTU_869 Auriculariaceae sp6	*	*			*					'			
36	OTU_36 Hyphodontia sp3		'	*	'							'	*	*
37	OTU_347 Limonomyces roseipellis	*			**									
38	OTU_286 Inocybe squamata		**											
39	OTU_311 Hygrocybe conica group sp4	'	*	*							*	'		
40	OTU_2491 Boletales sp	'		*	*			'						
41	OTU_5877 Hygrophoraceae sp	'		'	**			'			'	'		
42	OTU_247 Gymnopilus sp		**						'					
43	OTU_1087 Cantharellales sp1					'	**						*	
44	OTU_61 Clavariaceae sp3					*	*						*	

Rank	OTU name	HA	HB	OA	OB	HC	HD	SI	SA	MI	EL	PO	DM	BF
45	OTU_1367 Coprinellus sp1												'	**
46	OTU_7077 Cortinarius sp4	'	*	'	*							'		
47	OTU_7800 Hypholoma sp								'	'	*	**		
48	OTU_487 Russula sp			**										
49	OTU_326 Pluteaceae sp4					*	*							
50	OTU_435 Clavaria fuscata	*	*											
51	OTU_1083 Thelephorales sp		*	**										
52	OTU_425 Sclerogaster minor	*			*							'		
53	OTU_488 Vascellum sp	*			*						'	'		
54	OTU_150 Cuphophyllus pratensis	*						*						
55	OTU_1164 Mycena galopus							'	'	'	'	*		
56	OTU_796 Ceratobasidiaceae sp5					*	*					'		
57	OTU_784 Lyophyllaceae sp2	'	'	'	'	'			'	*	'	'		
58	OTU_552 Typhulaceae sp2			**										
59	OTU_4467 Inocybe perlata		**											
60	OTU_236 Russulales sp3				'						*	'		
61	OTU_638 Hodophilus sp			*										
62	OTU_939 Polyporales sp5							'	'	*	'			
63	OTU_174 Serendipita vermifera sp2							'	'	*	'	'		
64	OTU_79 Athelia bombacina												*	
65	OTU_7309 Sistotrema sp3							'	*	'	'	'		
66	OTU_481 Entoloma sp7					*								
67	OTU_7322 Pluteaceae sp6					*								
68	OTU_550 Pluteaceae sp9					*								
69	OTU_49 Mycena adscendens												*	
70	OTU_51 Sistotrema athelioides												*	

3.3 Geographic region and tillage effects

3.3.1 Dendrogram and biplot analyses

In the dendrogram analysis, sites tended to cluster by geographic regions, while the influence of other environmental variables appeared to be weak (Figure 3.4). The five Walpole Island sites clustered together and showed remarkably similar compositions despite varying agricultural histories and aboveground vegetation. Sites geographically near to each other on a similar scale in west Windsor were all pristine and showed similar compositions. The remaining four sites were all tilled, but clustered together despite being far apart geographically and having very different soil textures - the two east Windsor sites and the two remaining individual sites at the far southeast (DeMaere) and northeast (Blair Flats) edges of this study.

Walpole sites were dominated by the Mycenaceae and Ceratobasidiaceae, with moderate representation of Polyporaceae *sensu lato*. West Windsor sites were dominated by the Hygrophoraceae, again with moderate representation of Polyporaceae *sensu lato*. East Windsor sites and the other two sites much farther east (DeMaere and Blair Flats) were dominated by the Sebacinaceae. The two east Windsor sites also had strong representation from the Ceratobasidiaceae.

The dendrogram topology was fairly stable, since the dataset reduced to the top 15 minor clades (Fig. 3.8) remained nearly identical to dendrograms produced using all 55 minor clades or all 256 OTUs individually (data not shown). Some otherwise not-apparent patterns of dominance at the OTU level can be seen in Table 3.1. Blair Flats and east Windsor sites had a strong component of OTU_9 *Minimedusa polyspora* (Cantharellales *incertae sedis*) and DeMaere prairie was dominated (with over 50% of total reads) by OTU_0 *Mutinus elegans* (Phallaceae). The minor clades containing these OTUs would have dramatically shifted relative abundances in those specific sites were the minor clades not removed in the process of reducing this dataset from 55 to 15 minor clades.

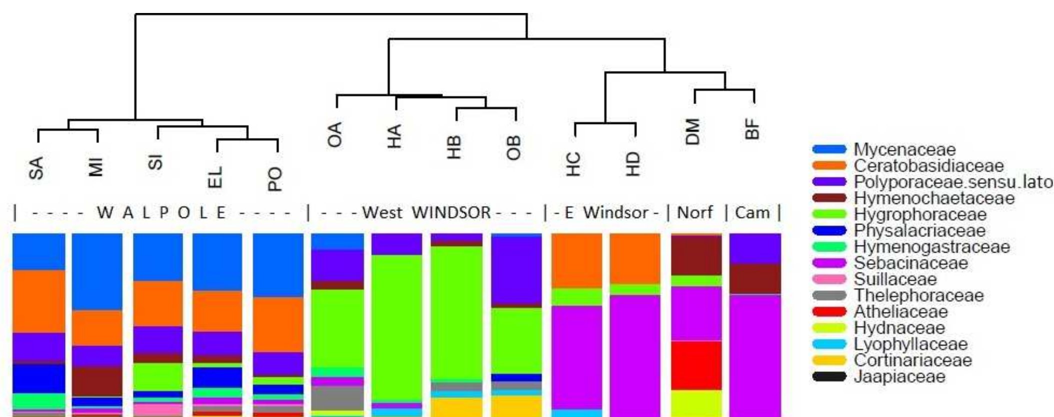

Figure 3.4 Cluster dendrogram and relative abundance bar plots for 13 prairie sites in southwestern Ontario.

Sites are organized by transformed compositional data for the 15 most abundant minor clades. Sites tended to cluster by geographic regions, indicated above relative abundance bars (Norf = Norfolk County; Cam = Cambridge). Walpole sites were dominated by the Mycenaceae and Ceratobasidiaceae, west Windsor sites were dominated by the Hygrophoraceae, and east Windsor sites and other eastern sites were dominated by the Sebacinaceae.

The PCA biplot analysis complemented the dendrogram and relative abundance bar plot results by showing position on a transect (geographic region) as a primary factor, but revealed tillage (and organic carbon) as secondary factors and other variables as having much less influence (Figure 3.5). Principal component 1 explained 40.5% of the variation in the dataset and was most strongly correlated with position on a transect (60.1%; Table 3.2). Walpole sites are tightly clustered in the left half of the biplot, while Windsor and the two easternmost sites are spread out over the right half. Principal component 2 explained 27.1% of the variation and is most strongly correlated with tillage and organic carbon (71.4% and 52.7% for tillage and organic carbon respectively; Table 3.2). There is a clean division between pristine and tilled sites on the biplot. Mike’s field, Sandpits, and all the other tilled sites were placed in the bottom half of the plot, whereas other pristine Walpole sites and the pristine Windsor ones were placed in the top half.

Principal components beyond the second were not examined, as the explained variance rapidly dropped (as seen in the scree plot in Figure 3.5). There were no other strong (>0.5) correlation coefficients in the environmental variables analysis (Table 3.2).

Minor clades associated with pristine sites (through higher read abundance values) were the Mycenaceae and Physalacriaceae (Walpole); Thelephoraceae and Polyporaceaceae *sensu lato* (generally); and Cortinariaceae, Lyophyllaceae, and Hygrophoraceae (west Windsor). Minor clades associated with tilled sites are the Ceratobasidiaceae (also associated with Walpole) and especially the Sebacinaceae (which was more associated with east Windsor and farther east sites – DeMaere and Blair Flats).

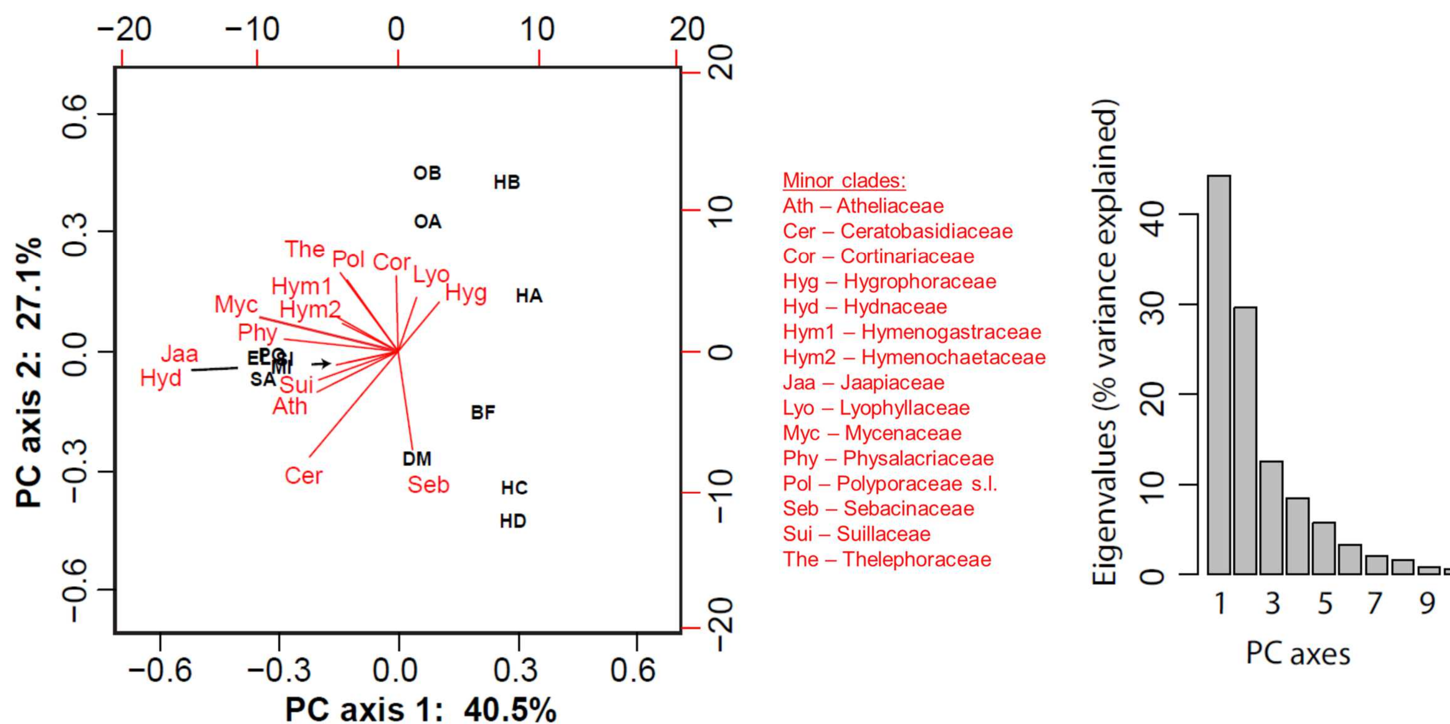

Figure 3.5 PCA biplot and associated scree plot for 13 prairie sites in southwestern Ontario based on transformed compositional data for the top 15 most abundant minor clades.

PC1 (40.5%) was associated with position on a transect (geographic region) (60.1%; Table 3.2) and PC2 (27.1%) was associated with tillage and organic carbon (71.4%, 52.7%; Table 3.2). Minor clades are abbreviated to their first three letters. Explained variance rapidly dropped after the first two component axes, as is evident in the scree plot (histogram).

Table 3.2 Environmental variable correlation coefficients associated with the principal components (PC) in the PCA biplot analysis.

Correlation coefficients >0.5 in PC1 and PC2 were highlighted. Position on a transect (geographic region) explained most of the variance compared with other variables for PC1, whereas tillage and correlated organic carbon explained the most variance for PC2. Dominant influences are not as easily discerned for later PCs. Tillage: 0 = tilled, 1 = pristine; TSR = total species richness (plants); NSR = native species richness (plants); adj-cw-FQI = adjusted cover-weighted Floristic Quality Index; Wetness = wetness score; OrgC = percent organic carbon; PosTrans = position on a transect (geographic region).

	Tillage	TSR	NSR	adj-cw-FQI	Wetness	OrgC	pH	PosTrans
PC1	-0.204	-0.038	-0.229	-0.127	0.273	-0.200	-0.309	-0.509
PC2	0.714	0.076	0.229	0.091	0.200	0.527	-0.091	-0.324
PC3	-0.204	-0.114	-0.267	0.127	0.018	0.055	0.236	-0.370
PC4	-0.510	-0.343	-0.381	-0.091	-0.055	-0.164	0.164	0.277
PC5	0.357	-0.038	0.114	0.164	0.127	0.382	0.345	0.092

3.3.2 Tillage-sensitive minor clades

Minor clades predicted or found to be sensitive to tillage in one direction or another (with much higher relative abundance in either tilled or pristine sites) are presented in Figure 3.6. Nine minor clades were pristine-associated: Boletaceae, Russulales unknown family, Agaricaceae, Hygrophoraceae, Clavariaceae, Suillaceae, Corticiaceae, and (to some degree) Entolomataceae and Polyporaceae *sensu lato*. Four minor clades were tillage-associated: Cantharellales *incertae sedis* (*Minimedusa* spp.), Tulasnellaceae, Hydnodontaceae, and Lachnellaceae. Minor clades that were present across many sites were more amenable to supporting conclusions; these were the Hygrophoraceae, Clavariaceae, Entolomataceae, Polyporaceae *sensu lato*, and Cantharellales *incertae sedis*. The Cantharellales *incertae sedis* clade (*Minimedusa* spp.) was present in many pristine sites, but despite this it was much more abundant in tilled sites.

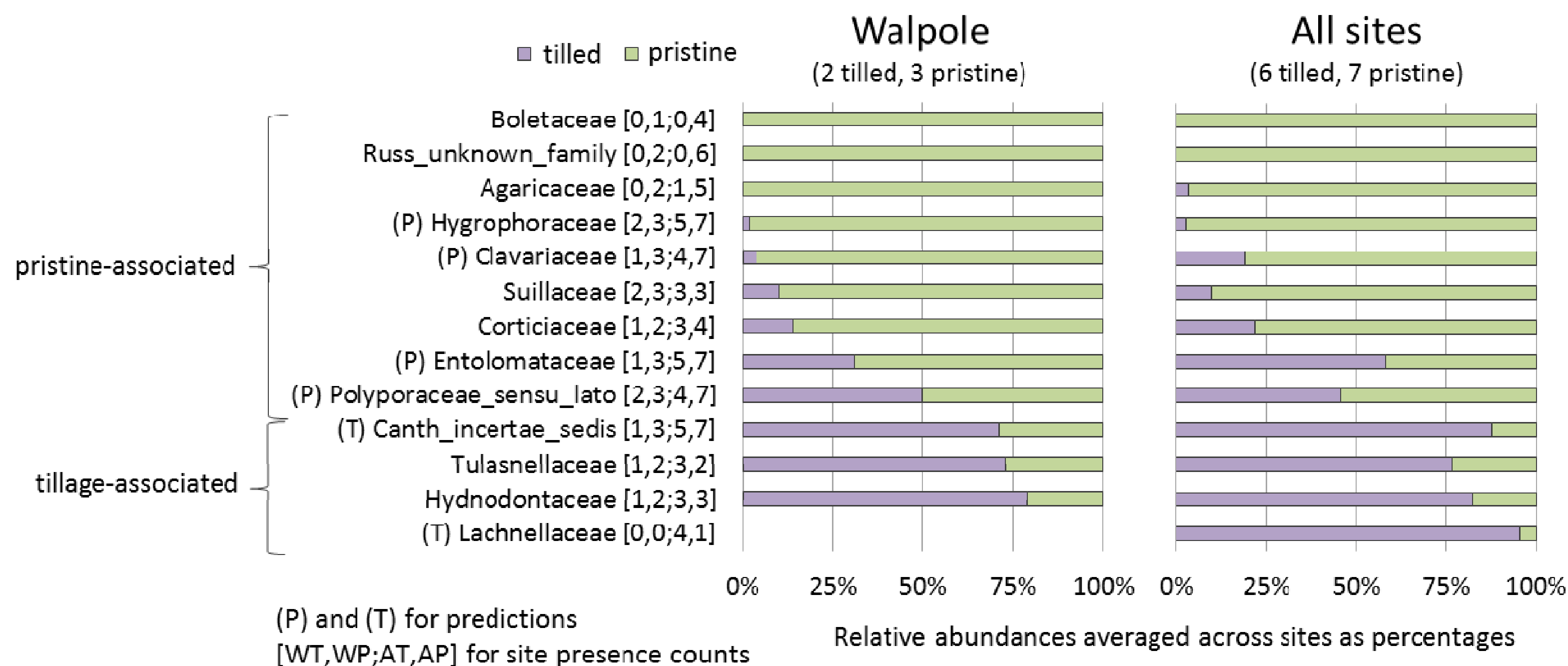

Figure 3.6 Stacked bar charts showing tillage sensitive minor clades in Walpole sites alone and across all sites. P = predicted to be pristine-associated, T = predicted to be tillage-associated. WT,WP;AT,AP = number of sites the minor clade was present in across: WT = tilled sites in Walpole, WP = pristine sites in Walpole, AT = all tilled sites, AP = all pristine sites.

3.4 Exloration of environmental variables

3.4.1 Tillage history association with soil and vegetation metrics

Soil organic carbon tended to be lower at tilled sites than pristine ones (Figure 3.7). Soil pH did not vary meaningfully between sites; most sites were close to the overall average of pH 6.55 with the exception of OA, which was much more acidic than OB, sampled from just 300 m away at the same site (Figure 3.8). Soil texture did not show any trends by geographic region or between tilled and pristine sites (Table 3.3).

Generally, tilled sites had lower native than total species richness, but native richness values were not necessarily lower for tilled sites than pristine ones. DeMaere was recently tilled but had the highest native richness of any site at 15, while the pristine FRS23 (HA) had a relatively low native richness of eight. Adjusted cover-weighted floristic quality index scores helped to separate pristine and tilled sites better, but not for every site. FRS27 and 28 (HC and HD) were recently tilled, but their scores became inflated above other pristine Windsor sites by their low richness values. Mean wetness coefficient scores showed a range of 2.44 (wettest -1.51; dryest 0.93). Wetness was apparently not associated with geographic region or agricultural history, meaning it would be useful as an independent environmental factor. Raw data from plant surveys of sites is displayed in Appendix D.

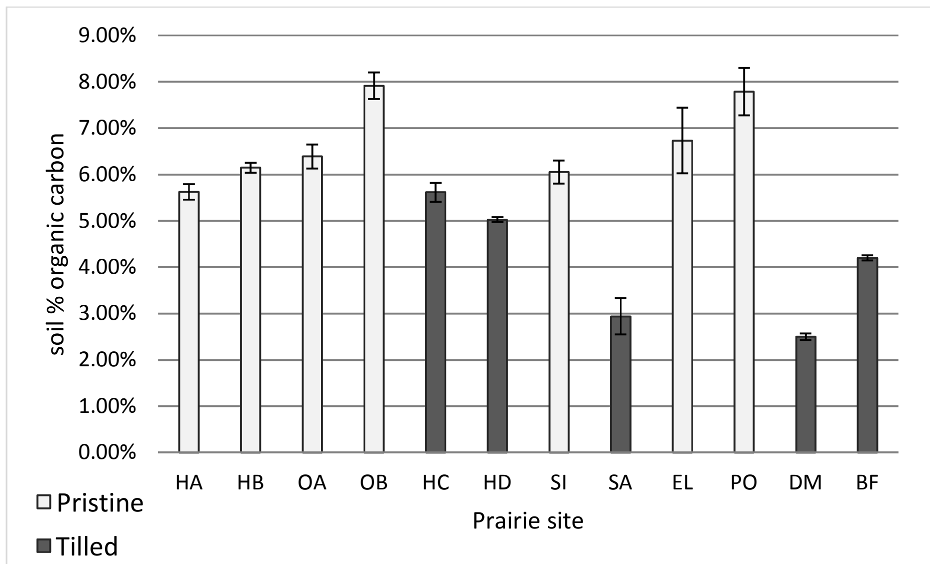

Figure 3.7 Soil percent organic carbon for 12 prairie sites in southwestern Ontario. Error bars represent standard deviation across four lab measurements from one composite bag of soil per site. Sites are arranged geographically, from west to east. Mike's field was excluded. Pristine sites had higher organic carbon than tilled ones.

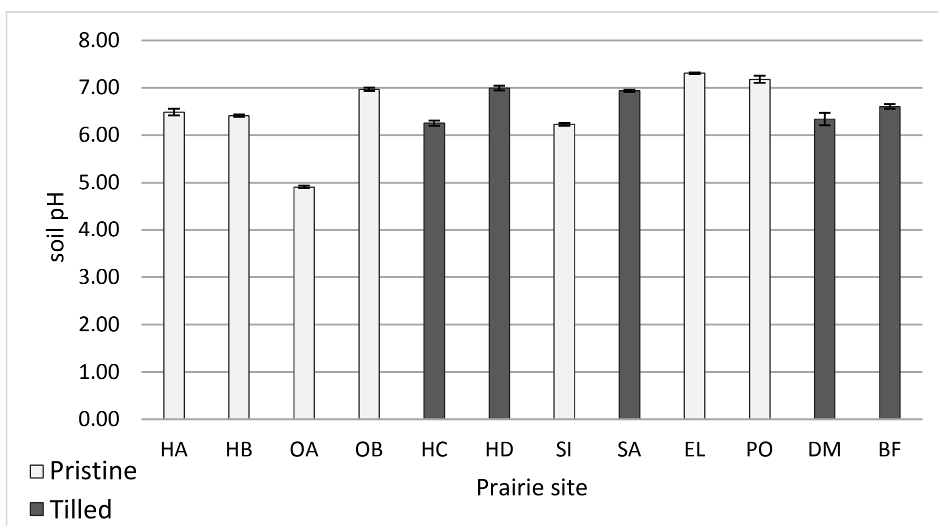

Figure 3.8 Soil pH measured at 12 prairie sites in southwestern Ontario. Error bars represent standard deviation across three lab measurements from one composite bag of soil per site. Sites are arranged geographically, from west to east.

Table 3.3 Soil texture measured at 12 prairie sites in southwestern Ontario

An asterisk marks pristine (as opposed to recently tilled) sites. Sites are listed in geographic order from west to east. Mike's field was excluded. There were no apparent trends between soil texture and geographic region or tillage history.

Region	Site	Soil Texture
West Windsor	HA*	Loam
West Windsor	HB*	Loamy Sand
West Windsor	OA*	Sand
West Windsor	OB*	Silty Sand
East Windsor	HC	Silty Clay
East Windsor	HD	Silty Clay
Walpole	SI*	Loam
Walpole	SA	Silty Sand
Walpole	EL*	Loam
Walpole	PO*	Clay Loam
Norfolk	DM	Loamy Sand
Cambridge	BF	Silty Clay Loam

Table 3.4 Vegetation metrics for 12 prairie sites in southwestern Ontario.

An asterisk marks pristine (as opposed to recently tilled) sites. Sites are listed in geographic order from west to east. Mike's field was excluded. See 2.6 Vegetation metrics for detailed descriptions and Appendix D for plant survey raw data.

	HA*	HB*	OA*	OB*	HC	HD	SI*	SA	EL*	PO*	DM	BF
total species richness	8	11	14	8	12	8	13	6	12	11	16	7
native species richness	8	11	12	8	8	5	13	2	12	11	15	6
adjusted cover-weighted FQI	27.8	19.6	53.5	58.6	45.6	36.9	81.0	9.5	30.5	49.9	35.9	29.5
mean Wetness coefficient	0.80	0.24	-0.57	0.93	0.20	-0.79	0.87	-0.45	-0.97	-1.51	0.81	0.06

3.4.2 Regressions of environmental variables and OTU richness

OTU richness values were calculated per-sample as average counts from each sampling event (different seasons), which were then averaged for each site. Walpole data from 2009 were excluded because plant surveys conducted in 2009 did not use the same methods as in this study (therefore the data are not comparable) and additional soil collected for pH and organic carbon measurements were no longer available. The first six of eight plots were used to determine OTU richness at DeMaere prairie (to be even with all other sites, which had only six plots). Richness values ranged from 16.5 to 38.0 across the 12 sites considered here, allowing for a window of comparison against environmental variables (Figure 3.9).

Regressions between OTU richness (of Agaricomycete OTUs only, but here also called "fungal richness") and the six measured environmental variables (plant total and native species richness, adjusted cover-weighted FQI, wetness score, soil organic carbon, and soil pH) are displayed with trendlines in Figure 3.10. Soil organic carbon had the strongest relationship with fungal richness of any environmental variable ($R^2 = 0.28$) (Figure 3.10 e). Plant measures had the next strongest relationships with fungal richness ($R^2 = 0.13$ to 0.19) (Figure 3.10 a,b,c).

Mean wetness coefficient had little relation to fungal richness based on the high degree of scatter and low R^2 value (0.02) (Figure 3.10 d). Similarly to mean wetness coefficient, soil pH was poorly related to fungal richness ($R^2 = 0.01$) (Figure 3.10 f). The OA site was removed since it was an outlier, with unusually high richness (38 OTUs) and an unusually acidic pH value (4.9) (perhaps due to the presence of woody vegetation nearby, including a large oak, hosting additional mycorrhizal species).

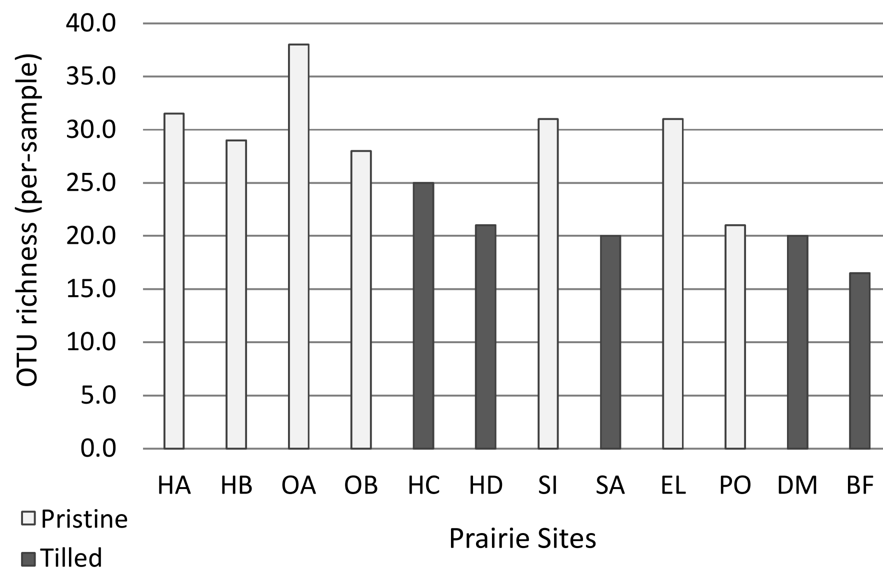

Figure 3.9 OTU richness (per-sample) for 12 prairie sites.

Pristine sites tended to have higher richness values than tilled ones. Only data from the first six of eight plots at DeMaere prairie (DM) were used, since all other sites had only six plots. Sites are organized by geographic location, from west to east.

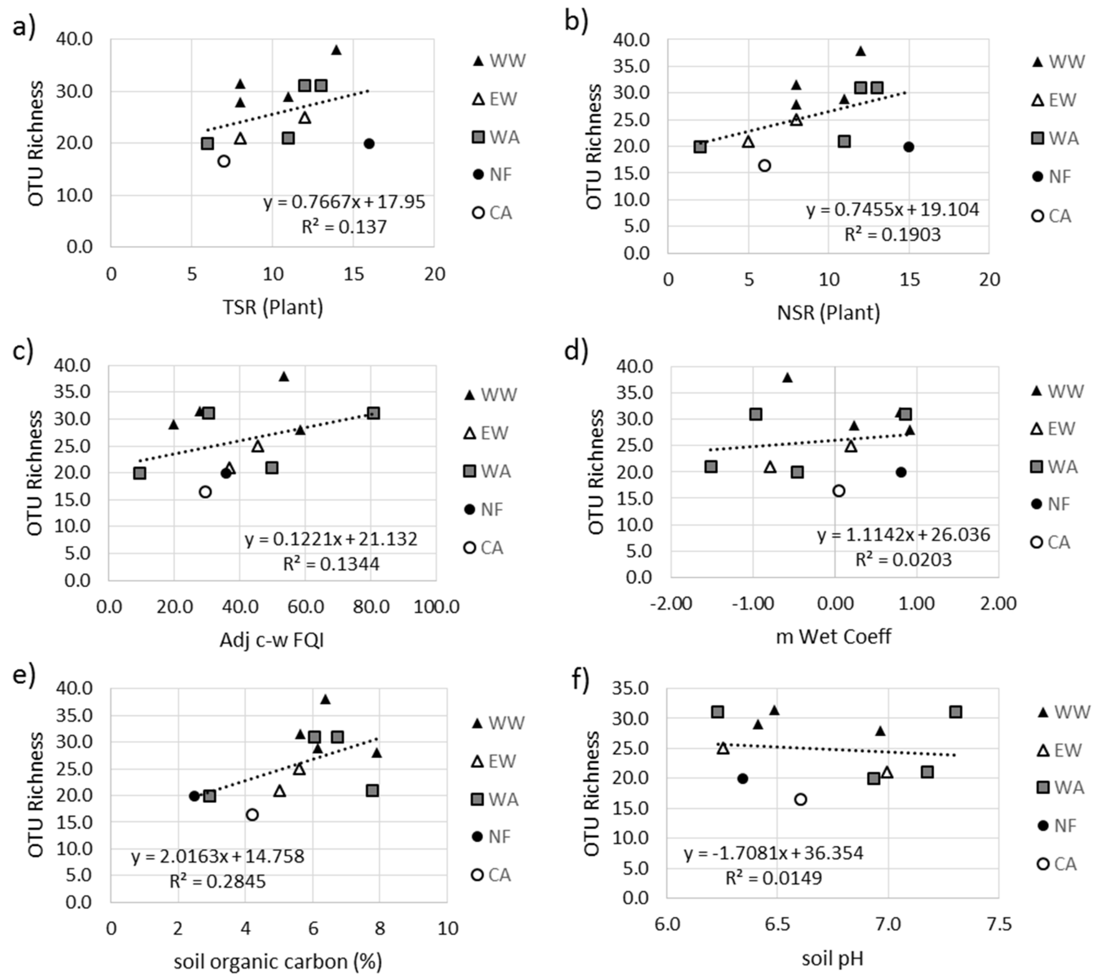

Figure 3.10 Regressions of OTU richness against environmental variables for 12 prairie sites in southwestern Ontario, grouped by geographic region.

The environmental variables are: plant a) total and b) native species richness, c) adjusted cover-weighted floristic quality index, d) mean wetness coefficient, d) soil organic carbon, and e) soil pH. Trend lines with their associated equation and R^2 values are included. OTU richness values were calculated per-sample (season) and then averaged for each site. Soil organic carbon had the strongest relationship with fungal richness (R^2 = 0.28). Geographic regions: WW = West Windsor (HA, HB, OA, OB); EW = East Windsor (HC, HD); WA = Walpole (SI, SA, EL, PO); NF = Norfolk (DM); CA = Cambridge (BF).

3.5 Comparisons of aboveground and belowground survey results

3.5.1 Minor clade comparisons by OTU richness and abundance

Certain minor clades showed high richness and abundance by either the aboveground or belowground surveys, others were present in both surveys but had lower richness and abundance, and a few minor clades were unique only to the aboveground survey whereas many more minor clades were unique to the belowground survey (Figure 3.11). The five minor clades with the highest OTU richness were the same as the five minor clades with the highest abundance. These minor clades were the Entolomataceae, Hygrophoraceae, Mycenaceae, Clavariaceae, and Polyporales *sensu lato*. The richest minor clades unique to the belowground method were the Sebacinaceae, Gomphales cf., and Pluteaceae, and the most abundant were by far the Ceratobasidiaceae followed by the Hymenochaetaceae, Physalacriaceae, and Cantharellales *incertae sedis* (*Minimedusa* spp.). The richest and most abundant minor clades unique to the aboveground survey were the Tubariaceae and Nidulariaceae.

The belowground survey captured most of the minor clades found aboveground and many more unique minor clades. Of the 55 minor clades found in the belowground survey, 38 (over half) were unique. The minor clades unique to the belowground survey represent 52.7% of the richness and 47.7% of the abundance found by that survey. In contrast, only 22 minor clades were found aboveground and only five (less than a quarter) were unique. The minor clades unique to the aboveground survey represent only 10.8% of the richness and 8.3% of the abundance. Both above and belowground surveys captured representatives from a wide diversity of Agaricomycete taxa across 60 collective minor clades.

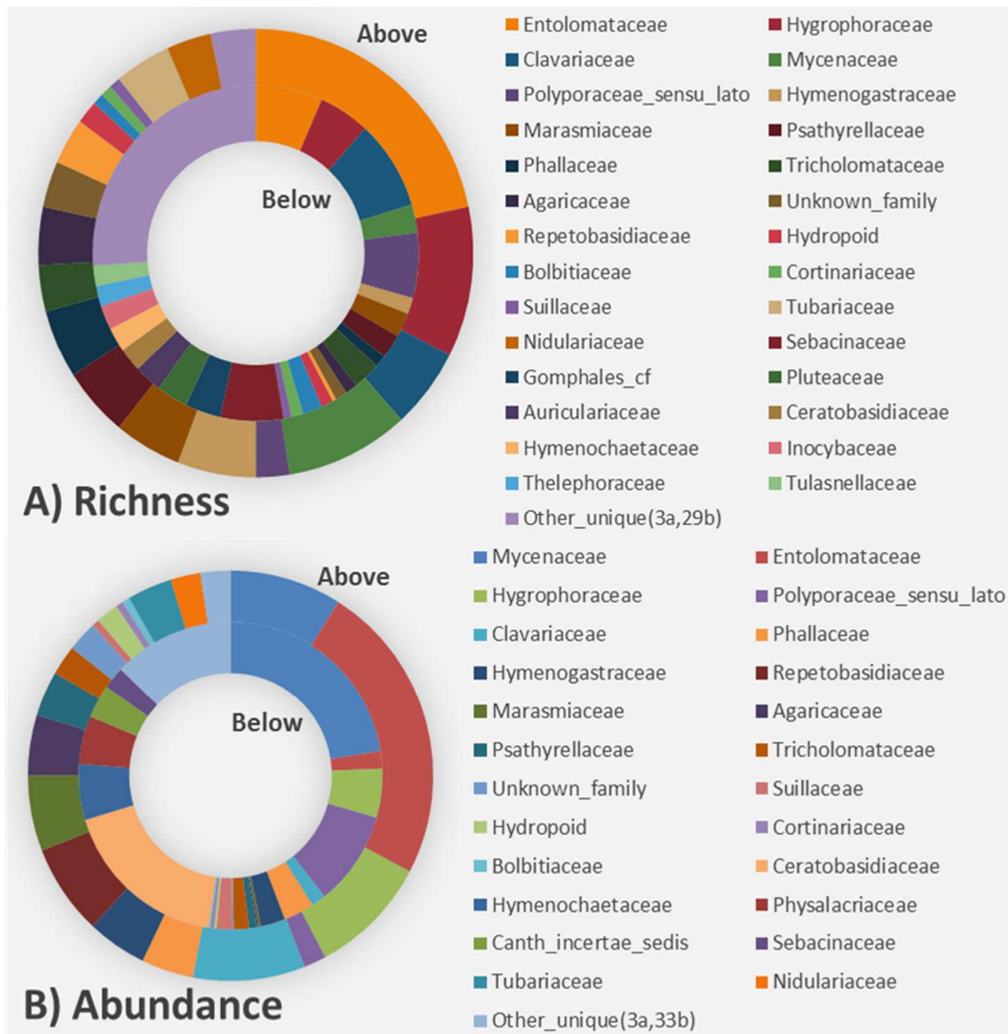

Figure 3.11 Doughnut charts comparing A) richness of species or OTUs and B) abundance of individuals or reads, in minor clades between aboveground and belowground surveys.

Aboveground and belowground minor clade proportions are represented by outer and inner doughnuts respectively. Red lines denote the split from shared to unique minor clades (only found in one survey type). The group “other unique” contains minor clades unique to one survey or the other with <20% richness or abundance values, and the number of families contained therein are indicated (“a” for above, “b” for below). The belowground survey retrieved many more unique minor clades than the aboveground survey.

3.5.2 Species-level overlap between the two survey types

Eight pairs of identical sequences (each pair consisting of one derived from a mushroom fruiting body collected during this study and the other from a soil-derived OTU sequence) were detected (Figure 3.12). These eight shared species represent 3.1% of the OTU richness and 10.8% of mushroom species richness.

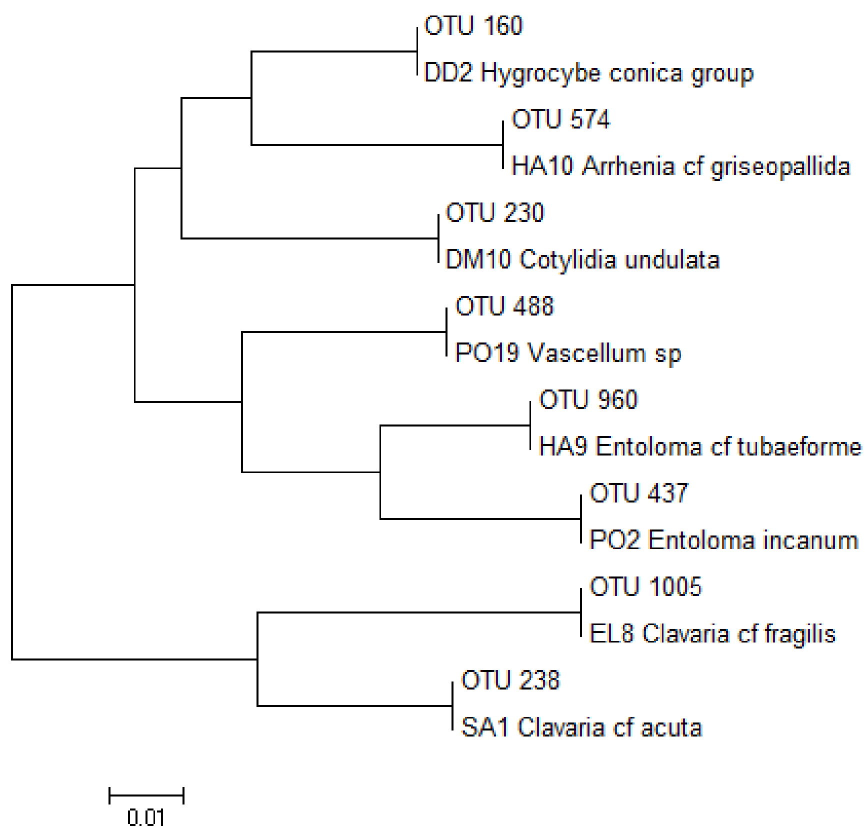

Figure 3.12 Neighbour joining tree of shared mushroom-OTU sequences.

Given the limited mushroom sequencing success rate, these numbers can be extrapolated to find the expected degree of overlap by the following equation:

$$\text{expected shared species} = \frac{\text{mushroom morphospecies } \times \text{shared species}}{\text{unique mushroom sequences obtained}}$$

The expected number of shared species is 16, representing 6.3% of OTU richness and 21.6% of mushroom species richness. These statistics were visualized as area-proportional Venn diagrams (Figure 3.13).

Figure 3.13 Area-proportional Venn diagrams of a) observed and b) expected shared species richness between aboveground and belowground surveys.

3.5.3 Shared species abundance and occurrence

Species found in high abundance by one survey type may not exhibit a similar abundance in the other (Figure 3.14). In three of the eight shared species, there was a pronounced disparity between aboveground and belowground abundances. *Cotylidia undulata* and *Entoloma* cf. *tubaeforme* were found in the highest abundance (of the eight shared species) aboveground but among the lowest belowground, and *Clavaria* cf. *acuta* had the highest relative abundance of the eight shared species belowground but the lowest of the eight aboveground.

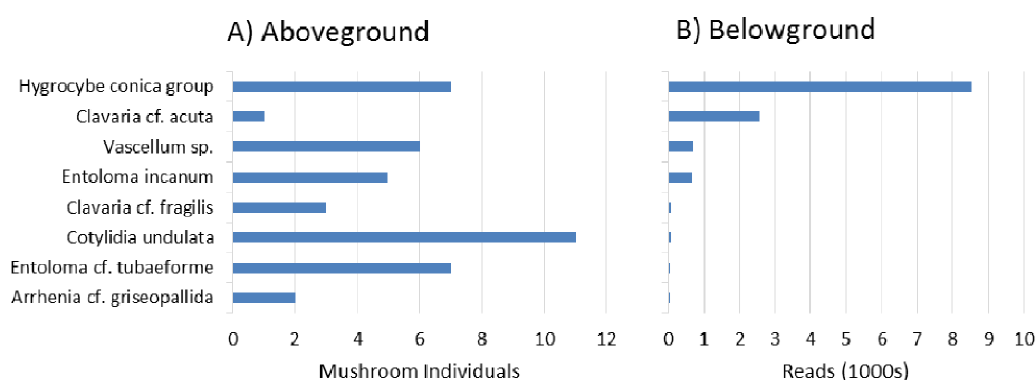

Figure 3.14 Correspondences between number of aboveground mushroom individuals and belowground reads across the eight shared species.
Often high or low abundance by one survey type does not positively correlate with similar abundance by the other survey type.

There is no apparent pattern as to whether species will be found by one survey type or another at any one site – sometimes species were found by both survey types at a site, but more often they were only found by one survey type or the other (Table 3.5). *Arrhenia* cf. *griseopallida* and *Clavaria* cf. *acuta* were never found at the same site using both survey types. *Cotylidia undulata* was found by both survey types, but only at one site. The *Vascellum* sp. was found at two sites by both survey types as well as four other sites by one survey type or the other.

Table 3.5 Occurrence of species in both the aboveground and belowground survey types across thirteen tallgrass prairie sites.
"A" represents an aboveground and "B" represents a belowground occurrence at a site.

	HA	HB	OA	OB	HC	HD	SI	SA	MI	EL	PO	DD	MP	DM	BF
Arrhenia cf. *griseopallida*	A										A			B	
Clavaria cf. *acuta*			B	B	B			A		B					
Clavaria cf. *fragilis*		B								A					
Cotylidia undulata														AB	
Entoloma incanum		B	B	B						AB	A				
Entoloma cf. *tubaeforme*	AB	A									A				
Hygrocybe conica group	B	B	B	B		B	B	A			AB	A			
Vascellum sp.	B			B			A			AB	AB		A		

4 Discussion

4.1 Significance of environmental variables

4.1.1 Geographic region

Contrary to my hypothesis, geographic region was the most important factor (not tillage history) for determining Agaricomycete composition. Sites did not spread across the biplot in order of positions along the diagonal transect (which would be correlated with latitudinal or longitudinal order), suggesting that no there is no broad gradient effect connecting my geographic regions. Between the most southern and most northern sites in this study, the latitudinal difference is *ca.* 125 kilometres – not enough for latitudinal climate effects to be a factor. At this broad scale, all sites in this study are fairly close (southern Ontario; mixedwood plains ecozone, Ecological Stratification Working Group 1995). Instead, the geographic region effect in this study probably represents local underlying edaphic (patchy soil type distribution) or climate variables (e.g., lake effects). Peay et al. (2016) argue against the "everything is everywhere and the environment selects" hypothesis, suggesting that patterns of fungal community distributions may be related to spatial dispersal of fungal spores being more limited than previously believed. This may have also been an important factor in community compositions being distinctly different between the geographic regions in my study.

Global biogeography of fungi is mainly determined by mean annual precipitation and distance from the equator (latitude), but different fungal groups prefer specific soil conditions – especially in regards to pH, calcium, and phosphorus (Tedersoo et al. 2014). Global drivers may not necessarily be relevant at a regional scale, such as my study where latitude and pH are not important. A regional study of British grasslands showed numerous edaphic factors played a role in soil microbial composition, including pH, nitrogen, phosphorus, and carbon, especially carbon-nitrogen ratios (deVries et al. 2012). A study of fungal biogeography at a regional scale in alpine grasslands found moisture to be important to other fungal classes, but not the Agaricomycetes (Pellissier et al. 2014). Soil texture may play a role, but does not always explain differences between regions (e.g., Sandpits with sandy soil and Pottowatomi with clayey soil, both in Walpole, still

had very similar community composition). It is possible the driving force behind differences in community composition between regions in my study has to do with underlying parent material and soil minerals that were not measured (such as calcium, phosphorus, and nitrogen).

4.1.2 Tillage and soil organic carbon

I had hypothesized that tillage would the the most important factor in determining Agaricomycete composition. Tillage was actually second most important, as it was overshadowed by position on a transect (geographic region). Tillage has long been known to decrease soil organic carbon (Laws and Evans 1949) and so it is not surprising that I found the two to be correlated. Others have found that increased soil organic carbon is related to increased fungal activity (Martyniuk and Wagner 1978, Schnürer et al. 1985, Caesar-TonThat and Cochran 2000, Kjoller and Rosendahl 2014). My findings suggested greater soil organic carbon is also associated with greater fungal diversity.

Although tillage and organic carbon were correlated, tillage had a stronger influence than organic carbon. In a similar study in an agroecosystem context, Bahnmann (2009) also found tillage to be a stronger driver of community composition than soil organic matter. The two variables have different biological relevance, since tillage is a form of intense, acute disturbance whereas soil organic carbon depends on multiple factors that are continually in action (plant growth, death, and incorporation into the soil). Tillage breaks apart hyphae and reduces colonization ability (Wardle 1995) whereas soil organic matter is an important energy source for most fungi (even mutualists feed on it – Griffith and Roderick 2008).

Since my study was observational, not experimental, it is important to consider that there may be underlying reasons why my pristine sites are pristine and tilled sites are tilled. For example, Liang and colleagues (2012) acknowledge that their prairie sites had sandy soils while their crop sites had heavy clay soils. Reviewing soil textures from my site descriptions (Section 2.1 – pg 11), pristine sites and tilled sites both contain a range of clayey to loamy to sandy soils. Within Walpole alone, pristine sites were more loam to clay-loam textured than tilled sites. Pristine sites may have been too wet for agriculture

(due to location and clayey soils) and sandier soils may have been preferred for tillage. This is not the case when comparing wetness values across all sites (summing wetness scores separately for pristine and tilled sites produces almost equal values). Pristine Walpole sites were somewhat wetter in total (especially Pottowatomi), but between Sandpits and Silphium (which were adjacent to one another) it is actually the pristine Silphium that has a higher positive wetness score (indicating dryer conditions). A previous study using the same Walpole island sites and including more rigorous vegetational surveys produced similar wetness scores (Stover et al. 2012). Soil pH was not very different between tilled sites and pristine ones and neither was soil texture; only soil organic carbon was correlated with tillage, as discussed earlier. There is no evidence for any factors considered in my study other than tillage (and correlated organic carbon) accounting for fungal community differences between tilled and pristine sites.

4.1.3 Tillage-sensitive taxa

As predicted, the Hygrophoraceae and Clavariaceae were pristine-associated. The two families are particularly well represented as they were present across many sites – both tilled and pristine. Half of my Hygrophoraceae minor clade consists of *Hygrocybe* OTUs, so it is comparable to Bahnmann’s (2009) *Hygrocybe* minor clade and the many *Hygrocybe* spp. mentioned in studies of the British Isles (e.g., Rotheroe 2001, Newton et al. 2003, Mitchel 2010). The Clavariaceae family in my study is treated as a distinct phylogenetic clade the same way as the clavarioid clade from Bahnmann (2009), so the two are directly comparable. European fruiting body surveys probably exclude crust-like and agaric Clavariaceae (since these members are difficult to identify as Clavariaceae without sequencing), and it is possible that clavarioid members of the Clavulinaceae (Cantharellales) may be incorrectly included, but the Clavariaceae probably coincide well between European studies and mine. Along with *Hygrocybe* spp., the Clavariaceae are considered sensitive to fertilizer and agricultural disturbance in European studies (Rotheroe et al. 1996, Arnolds 1989). In Bahnmann (2009), the *Hygrocybe* minor clade was absent in agriculturally active (conventional and no till) sites but present in historically and never-tilled sites. Similarly, the clavarioid clade was highly abundant in

historically and never-tilled sites and of relatively low abundance in agriculturally active ones.

Newton and colleagues (2003), in mushroom surveys of 511 grassland sites in Scotland, found richness of *Hygrocybe* taxa to be correlated with Clavariaceae taxa (but not with other CHEGD taxa such as *Entoloma* spp.). Birkebak and colleagues (2013) note in their overview of the Clavariaceae that Clavariaceae and Hygrophoraceae members are often found together in the same habitat and often near each other – grasslands in Europe and forests in North America (incidentally, according to my study, they are also found together in North American grasslands, not just North American forests) – supporting their belief that these two groups both have the same unknown nutritional mode. It makes sense that both of these families would prefer pristine sites over recently tilled ones.

Contrary to my prediction, the Entolomataceae showed mixed results and did not appear to be pristine-associated. Results for the *Entoloma* minor clade from Bahmann (2009) were also mixed – its highest abundance was in never-tilled sites, but it was also moderately abundant in agriculturally active sites (conventional and no till), and absent in historically tilled sites. Newton and colleagues (2003) examined occurrence of CHEGD taxa across sites and found *Entoloma* taxa tended to cluster with themselves and not be correlated with richness of any other families. The Entolomataceae remain a highly diverse but ecologically mysterious (saprotrophic, or at least believed to be) family of grassland mushrooms.

The Polyporaceae also did not show trends as being pristine-associated, despite my prediction. Although Bahnmann (2009) did not find any of the Polyporales in agriculturally active (conventional and no till) sites, the Polyporales were found in both historically and never tilled sites. To some extent, the historically tilled sites in Bahnmann (2009) are probably similar to the tilled sites in my study, and never tilled sites in Bahnmann (2009) are similar to the pristine ones in my study. All of these types of sites where the Polyporaceae were found have perennial vegetation cover and lots of litter, which is probably the most important factor for determining presence of the Polyporaceae (not soil disturbance).

As predicted, the Cantharellales *incertae sedis* minor clade (*Minimedusa* spp.) was tillage-associated. The genus *Pneumatospora* was present in all of the KBS site types in Michigan, but its abundance was by far the highest in actively conventionally tilled sites (Bahnmann 2009). The most abundant OTU in that study was noted as matching well with sequences of *Pneumatospora obcoronata*, which was also highly abundant from a previous study in the same site (Lynch and Thorn 2006). The current preferred synonym is *Minimedusa obcoronata* which may be OTU_9652 *Minimedusa* sp. but is also very similar to the second most abundant OTU in my study, OTU_9 *Minimedusa polyspora*, since both produce bulbil propagules. Bahnmann (2009) suggests these propagules may be easily spread across a site by tillage, assisting the fungus to colonize a large area, and it may be a saprotroph preferring agricultural residues of wheat, corn, and soy. Remnants of these agricultural influences were apparently strong enough in my recently tilled sites for the fungus to reach higher abundance than pristine sites.

The Lachnellaceae minor clade was also tillage-associated, as predicted. My prediction was based on the *Lachnella/Calathella* (*Nia*) clade having the second-highest abundance in conventionally tilled sites but being absent from historically and never tilled sites in Bahnmann's study (2009). No *Calathella* spp. were identified in my study; my Lachnellaceae minor clade consisted of one unknown Lachnellaceae species. *Lachnella alboviolascens* had an unresolved phylogenetic placement in Moncalvo (2002), but the Lachnellaceae family was later placed in the Marasmioid major clade and considered synonymous with the *Nia* minor clade by Matheny et al. (2006). *Lachnella* spp. are culturable saprotrophs of plant litter with a cyphelloid (cup-shaped) fruiting body form (Agerer 1983).

A number of additional taxa not predicted to be tillage-sensitive showed strong association with pristine or tilled sites. The Boletales major clade includes the Boletaceae and Suillaceae, both of which were pristine-associated. The Boletales are mostly believed to be ectomycorrhizal based on field observations of mushrooms, but the order actually includes many brown-rot and white-rot saprotrophs of wood as well (Binder and Hibbett 2006). The Boletales were completely absent from Michigan agroecosystem soils, and this has been attributed to lack of suitable ectomycorrhizal plant hosts (especially wood

species) at most of the sites (Wong 2012). I found four Boletaceae OTUs, all matching with ectomycorrhizal genera and found only in pristine sites where trees were nearby – especially oak (*Quercus* spp.). There were only two Suillaceae OTUs: OTU_874 *Suillus luteus* found only in DeMaere (where many tree seedlings were present, and sapling pines with fruiting bodies of *Suillus americanus* nearby) and OTU_60 *Suillus cavipes*, which was found in Walpole only, especially the pristine sites. For both the Boletaceae and Suillaceae, the trend towards pristine sites is probably a byproduct of ectomycorrhizal plant hosts (woody species) tending to be lacking at recently tilled sites where they have not been able to establish.

The Russulales unknown family was found in pristine sites only and represents three OTUs lacking strong GenBank sequence matches. Russulaceae OTUs found matches for *Lactarius* and *Russula* spp., so the unknown family represents other taxa. The Russulales order comes in every fruiting body form: resupinate (crust-like), discoid, clavarioid, pileate (typical umbrella mushrooms), and gasteroid (stomach-like), and the order consists of mostly saprotrophs but also ectomycorrhizals (e.g., *Russula* spp), root parasites, and insect symbionts (Miller et al. 2006). It is unknown what the three OTUs from this group may represent and why they were found in pristine sites only. An important soil aggregating species from the Russulales with an identity near the genus *Peniophora* was found in short grass prairie (Caesar-TonThat et al. 2001). No species of *Peniophora* were otherwise matched with my OTUs, so it is possible these OTUs may belong there.

The Agaricaceae represents three OTUs from *Agaricus* (button mushrooms), *Lepiota* (parasol mushrooms), and *Vascellum* (small puffball) genera. They were found almost exclusively in pristine sites, except for the *Lepiota* sp. which was also found in DeMaere. Fruiting bodies of these genera are known to occur in grasslands, especially pastures (e.g., Hay 2013) where *Agaricus* spp. can form large “fairy rings” in the grass (Griffith and Roderick 2008). Mushrooms of *Agaricus campestris* and a *Vascellum* sp. were found fruiting in several sites in my study. Many *Lepiota* spp. are nitrophilic, and some prefer dune habitats (Bon 1993), perhaps explaining presence in the sandy soils of DeMaere. The preference of this family for pristine sites may be explained by higher

levels of organic carbon found in pristine sites than tilled ones, given that members in this family are all saprotrophic. The Agaricaceae are apparently less abundant in sites where soil molecular analysis has been conducted (with very low abundance in historically tilled sites in Bahnmann 2009 and not mentioned at all in Jumpponen et al. 2010 or Penton et al. 2013). Agaricaceae members that form fairy rings have active hyphae and often fruiting bodies at the ring's edge, but apparently die back inside the ring and are presumably not present outside the ring (Dowson et al. 1989, Griffith and Roderick 2008). It is possible studies sampling with soil cores are better suited to finding taxa with smaller and more evenly dispersed individuals.

The Corticiaceae had a moderate preference for pristine sites over tilled ones. There was a split between the four OTUs of the Corticiaceae. Two OTUs were found in only tilled sites and are plant pathogens (*Waitea circinata* and *Laetisaria arvalis*) and two OTUs were found in only pristine sites and are saprotrophic crusts that grow on wood (best matches to *Vuilleminia macrospora* and *Limonomyces roseipellis*). Apparently the two pristine-associated crusts were abundant enough to influence the association of this family, and they can grow on grassland litter or organic matter in the soil as well as woody material.

In addition to the two predicted tillage-associated families, two more were found in my study. The Tulasnellaceae form patchy crust-like fruiting bodies and have been reported as saprotrophs of wood (pine) or mycorrhizal with orchids (Roche et al. 2010). Five OTUs, mostly *Tulasnella* spp. (of uncertain species identity), were each found at only one tilled site except for one found at two pristine Walpole sites (see Appendix B). Perhaps they are saprotrophs of remnant agricultural residues (preferring corn, soy, and wheat over natural plant litter).

The second tillage-associated family, Hydnodontaceae, consisted of four OTUs: Hydnodontaceae sp., *Subulicystidium* sp., and *Trechispora* sp. 1 and 2. These members form white crusts and are believed to be mycorrhizal with a side variety of plants. In total they were present in about as many pristine sites as tilled ones, but had much higher abundance in the tilled sites. It is unclear why this preference may exist.

4.1.4 Vegetation metrics

There is growing research interest about how well a site's plant diversity and soil microbial (bacterial and fungal) composition correspond. Some studies have found plant diversity to be strongly associated with microbial composition (Carney and Matson 2006, Grueter et al. 2006), whereas others have found this not to be the case (Liang et al. 2012). This question of the similarity or dissimilarity of plant and fungal characteristics of sites has implications for fungal conservation. Nature reserve organizations preferentially preserve sites that rank highly using vegetative assessments, and so mycologically valuable sites that rank lower using those methods are overlooked as a result (Rotheroe 2001). I did find some support for this notion, since at least fungal richness was poorly associated with conservatism value of sites. As well, sites of close proximity (in which geographic region was controlled) with very different plant diversity and management histories still had similar fungal communities. For example, Silphium and Sandpits were adjacent to each other and had vastly different aboveground vegetation and management histories, but their fungal composition was very similar. Liang and colleagues (2012) note similar observations. Even without tillage as a confounding factor, there was no difference in microbial composition between old switchgrass monoculture fields and diverse mixedgrass prairie sites. More research is needed to determine what, if not aboveground vegetation, may indicate a site of fungal importance.

4.2 Aboveground and belowground comparisons

In some studies, aboveground mushroom survey results have been compared with results obtained by other survey types on the same site, but none so far have made comparisons with high-throughput sequencing. Aboveground mushroom survey data was compared with belowground root tip genotyping of ectomycorrhizal fungi by Gardes and Bruns (1996). Correspondence between the two methods was limited – some species were commonly encountered using either method, but others were rare aboveground and common belowground or common aboveground and rare belowground. My results support this to some degree, but it is difficult to draw confident conclusions about whether this applies when the belowground survey includes high-throughput sequencing, given that I only found eight species shared between the two survey types.

Horton and Bruns (2001) reviewed studies of ectomycorrhizal fungi comparing aboveground fruiting body surveys to belowground root tip genotyping, and found that species common by one method are rare by the other, as a rule. Such trends are accounted for partly by differences in sampling between the two methods, but it is believed the dissimilarity largely exists for a biological reason: different investment strategies for different species (Horton and Bruns 2001). Some species invest more energy into belowground vegetative growth and competition than aboveground reproduction (fruiting body structures to spread spores) whereas others take the opposite approach (Horton and Bruns 2001). My results, then, suggest that *Clavaria* cf. a*cuta* invests more energy in vegetative growth and competition (belowground activities) than reproduction (aboveground fruiting body production) whereas *Cotylidia undulata* uses the opposite investment strategy.

Porter et al. (2008) used aboveground mushroom surveys and belowground soil rDNA cloning to compare the degree of overlap and ability to capture fungal diversity of a site. Similarly to the root tip genotyping studies, and the results of my study, there was little overlap between the two survey types. Unlike the root tip studies, the study by Porter and colleagues assessed degrees of overlap at different taxonomic scales (species, but also genus-order level), and using richness, abundance, and phylogenetic diversity as measures. Naturally, the degree of overlap between the two survey types increases at coarser taxonomic resolutions – most orders were present in either survey to some degree, and the Agaricales were the largest component of both survey types. The Agaricales were dominant at the level of order in my study as well, but already similarities began to break down at the finer scale of minor clades. At the species level, Porter et al. (2008) found only 13 OTUs in common, representing 10% of aboveground mushroom diversity (132 total) and 20% of belowground soil diversity (66 total). In my study, the expected overlap was 16 species, representing 22% of aboveground diversity (74 total) and 6% of belowground soil diversity (256 total). The differences between the study of Porter and colleagues (2008) and mine are associated with the total number of species that were found by either method.

It is important to consider sampling effort when comparing results of two survey types (Horton and Bruns 2001). Porter and colleagues (2008) found about twice as many species aboveground than belowground, whereas I found about four times as many species belowground than above. Their belowground method was different (cloning is more labour intensive than high-throughput sequencing) and they put more effort into aboveground mushroom collection and sequencing than I did (they visited their site several times per week in peak season). Their study was also in a forested ecosystem, where mushrooms are more abundant. Unlike my study, they found about as many orders unique to the aboveground survey as belowground (5:6). In my study the aboveground survey captured far fewer unique clades than the belowground surey (5:38). Despite these differences, her study and mine found similar degrees of total overlap between both survey types – about one-third (6/18 shared orders in their study and 17/60 shared minor clades in mine). This suggests that despite some differences in aboveground sampling effort and belowground methods, only about a third of orders-families are found using either survey type.

There are many reasons why some taxa are unique to one survey type or the other. Obviously the aboveground survey missed minor clades that lack conspicuous fruiting bodies: Ceratobasidiaceae, Hymenochaetaceae, Cantharellales *incertae sedis* (*Minimedusa* spp.), and Tulasnellaceae, to name those with higher abundance or richness. Similarly in Porter et al. (2008), orders lacking conspicuous fruiting bodies such as the Atheliales, Sebacinales, and Trechisporales were unique to the belowground survey (missed by the aboveground survey). Most of the other minor clades missed in my study by the aboveground survey produce conspicuous fruiting bodies but they are (surprisingly, in a grassland environment) saprotrophic on wood or else mycorrhizal with woody plants: Auriculariaceae, Gomphales cf., Inocybaceae, Pluteaceae (except for *Volvariella*, which produces mushrooms in grasslands), and Thelephoraceae.

Five minor clades were detected by the aboveground survey and not by the belowground survey: Tubariaceae, Nidulariaceae, Paxillaceae, Hydnangiaceae, and Peniophoraceae. I propose three explanations. 1) Some of these families were only found growing directly on aboveground litter that was not attached to the soil, and so they

would naturally be excluded when removing the litter layer from the top of soil cores when sampling. This would apply to the Nidulariaceae (*Cyathus stercoreus* and *Nidula candida*) and Peniophoraceae (*Peniophora versiformis*). 2) The aboveground survey type can cover a much larger area of land, and so it is possible that fruiting bodies were found at some distance from the point of soil sampling, too far away to capture belowground hyphae. I believe that this was the case with the Paxillaceae. In the one site where this family was found, a few fruiting bodies of a *Paxillus* were found at a considerable distance from the small area where soil sampling was permitted. The Hydnangiaceae were represented by one *Laccaria* sp. that was found fruiting throughout DeMaere prairie, but never within several meters of where soil cores were taken along one edge of the site. This may have also been the case for the Tubariaceae. Two *Tubaria* species were found throughout DeMaere, but they were also found in Silphium prairie and Sandpits on Walpole Island, which were smaller sites where soil sampling was more widely dispersed. The sampling distance effect is possible, but less likely in those cases. This brings me to the third possibility. 3) The belowground survey type misses some taxa due to its short sequence requirement negatively impacting OTU identifications. If this is the case, the Tubariaceae sequences were retrieved by the belowground survey but could not be confidently identified as such. For example, OTU_197 Bolbitiaceae_sp1 could not be identified with confidence despite matching well with a *Tubaria furfuracea* sequence from aboveground collections in this study (3/246 base pair mismatches). The OTU retrieved sequences from a variety of taxa in Genbank (e.g., *Deconica xeroderma* (KC669340), *Psilocybe* cf. *subviscida/crobula* (KC176337), and *Tubaria serrulata* (DQ987906) - all with 100% coverage, 2e-122 E value, and 99% identity), and grouped with the Bolbitiaceae in my phylogenetic tree. Therefore it is possible the belowground survey misses some taxa due to its short sequence read requirement negatively impacting OTU identifications. The use of additional primer sets, including ones targeting the ITS1 or ITS2 regions, may provide better identifications for some taxa that lack diagnostic sequences in the D1 region of LSU used in this study (Asemaninejad et al. 2016).

4.3 Ecology and conservation of prairie Agaricomycetes

4.3.1 Ecological roles of predominant taxa

4.3.1.1 Clavariaceae

Based on soil DNA sequence data, the Clavariaceae were the most OTU-rich minor clade (22 OTUs) but only 14th most abundant (1.5%). Previous studies in the agricultural and old-field context of the Kellogg Biological Station (KBS) in Michigan consistently found the Clavariaceae to be the most OTU-rich basidiomycete taxon in the soil (Lynch 2004, Bahnmann 2009, Wong 2012). Fruting bodies of white clavate (club-shaped with a wider tip than base) *Clavaria* spp. found during mushroom surveys in my study appeared macroscopically identical but sequencing of two collections revealed two different species at the genetic level (*Clavaria* cf. *acuta* and *Clavaria* cf. *fragilis*). Two other Clavariaceae mushrooms were found: *Clavulinopsis laeticolor* that was similar to the white *Clavaria* spp. except it was yellow, and a *Ramariopsis* that was very small and highly branched. A moderately high richness of Clavariaceae fruiting bodies are found in European grassland mushroom surveys, usually second to the Hygrophoraceae and Entolomataceae (e.g., Arnolds 1989). Given these findings, the diversity of the Clavariaceae is probably underestimated in surveys that do not involve sequencing, particularly as some members of the family are agaricoid or crust-like (Birkebak et al. 2013) and not otherwise recognizable as Clavariaceae.

Besides lignicolous (wood-decaying) species, the Clavariaceae are believed to be mostly biotrophic (a broad term for any symbiosis with a living partner – in opposition to necrotrophic where the symbiosis leads to the death of the partner) (Birkebak et al. 2013). Some of the Clavariaceae are mycorrhizal with ericoid plants (Seviour et al. 1973, Petersen and Litten 1989) and others are algal-associates. All Clavariaceae fruiting bodies collected in this study were attached to bare soil. It could be that they have a biotrophic relationship with the roots of grassland plants or they may be lignicolous on buried non-woody but lignin-containing plant debris.

4.3.1.2 Entolomataceae

The Entolomataceae were the second most OTU-rich minor clade (18 OTUs) but only the 11th most abundant (1.9%). They constituted the third most OTU-rich minor clade in KBS soils from Michigan (Lynch and Thorn 2006, Bahnmann 2009). Unlike the Clavariaceae, the diversity of the Entolomataceae is more readily apparent in mushroom surveys without requiring sequencing. Eleven different mushroom morphospecies were encountered in this study, making it the most species-rich family from my mushroom surveys. In grasslands of The Netherlands it was by far the richest—55 species were listed from grasslands and sandy sites in Arnolds (1989). Most of the Entolomataceae (including those identified to species in this study) are regarded as saprotrophs (obtaining energy from decomposing organic matter in the soil). Noordeloos (1981) notes *Entoloma* as a genus of terrestrial saprophytic mushrooms of humus. The designation of this family as saprotrophic is questionable and needs further study – most Entolomataceae do not grow in culture and many seem to be associated with mosses or algal crusts (Greg Thorn pers. comm.). The ecological category of "saprotroph" has been considered a dumping ground for leftover fungal taxa with unknown, poorly examined, or semi-saprotrophic but truly more complex ecological roles (Griffith and Roderick 2008) and groups considered saprotrophic in the past have been discovered to have other roles (see my discussion of *Hygrocybe* spp. in 4.3.1.4 Hygrophoraceae). Some Entolomataceae species are mycorrhizal with shrubby plants from the rose family (Kobayashi and Yamada 2003), but none of the known mycorrhizal Entolomataceae or their potential Rosaceae host plants were identified in any of the field sites in this study.

4.3.1.3 Sebacinaceae

The Sebacinaceae were the third/fourth most OTU-rich minor clade (16 OTUs – tied with the Polyporaceae *sensu lato*) but only 10th most abundant (2.3%). In Michigan soils, this group was found to have much lower richness compared to richness of other families (Wong 2012) and the group was apparently absent or of too low abundance to mention in other grassland molecular studies (Jumpponen et al. 2010, Penton et al. 2013). It is possible Sebacinaceae were present in these soils but were not detected because the primers used could not detect their sequences; Sebacinaceae sequences are unusual, being

basal in the Agaricomycetes next to the Cantharellales and this group has long been overlooked (Weiss 2011).

No fruiting bodies from this family were encountered in mushroom surveys from my study, although conspicuous white tremelloid crust-like masses are known to be formed by some members of this family. The Sebacinaceae are known for their variety of symbioses with plants: ectomycorrhizas (where a fungal layer is formed on roots of tree species), orchid mycorrhizas, ericoid mycorrhizas, jungermannioid (associated with the Jungermanniales – an order of liverworts) mycorrhizas, and probably a diversity of other mycorrhizas that are yet to be uncovered (Weiss et al. 2004). Tedersoo et al. (2010) warn against considering all *Sebacina* spp. as mycorrhizal, since the Sebacinaceae includes other ecological groups (e.g., OTU_981 *Efibulobasidium* sp., which they consider a saprotroph) and many species are still crypic with unresolved ecologies. Many Sebacinaceae species are endophytes (apparently symptomless symbionts in plants that are now often believed to improve the plant's growth and resilience) that are found around the world in seemingly all angiosperm families, as well as in ferns, mosses, and liverworts (Weiss et al. 2011). Three OTUs in my study matched closely with *Piriformospora indica*, a root endophyte found in a wide range of host plants, including monocots, dicots, and legumes (Varma et al. 2012). The two most abundant OTUs from this group (OTU_35 Sebacinaceae sp. 2 and OTU_174 *Serendipita vermifera* sp. 2) were found almost exclusively in recently tilled sites, and many of the Sebacinaceae OTUs were present only in tilled sites (see Appendix B). *Serendipita vermifera* has been confirmed as an ectomycorrhizal species with a variety of hosts (Warcup 1988), quite possibly including plants present in restored tilled sites. The hosts and activities of the majority of the Sebacinaceae encountered in this study remain unknown.

4.3.1.4 Hygrophoraceae

The Hygrophoraceae were the fifth most OTU-rich minor clade (13 OTUs) and also the fifth most abundant (5.1%). Other soil molecular studies did not find Hygrophoraceae OTU richness to be as relatively high as in my study (Jumpponen et al. 2010, Wong 2012, Penton et al. 2013) but this group is famously rich and abundant from mushroom surveys in waxcap grasslands of Europe (Rotheroe et al. 1996). My study

encountered only four morphospecies of *Hygrocybe*, but it is likely more would have been encountered with greater survey effort (my study has low sampling effort compared to other mushroom studies; e.g., Porter et al. 2008) and sequencing within morphospecies to discover cryptic species that are known to occur in the group (e.g., Ainsworth et al. 2013). There were 49 *Hygrocybe* spp. recorded from grasslands in Scotland (Newton et al. 2003), and 26 from grasslands in The Netherlands (Arnolds 1989) – relatively high richness for this genus in both studies, but still lower than the number of *Entoloma* spp.

The ecology of this group has been elusive for some time despite being studied extensively. *Hygrophorus* spp. are ectomycorrhizal with trees, so none were found. *Cuphophyllus* spp. are probably biotrophic; from this genus only one OTU (Appendix B) and two aboveground mushroom species were found (Appendix C). *Hygrocybe* spp. were first believed to be saprotrophs (Arnolds 1982, as cited in Griffith et al 2002) until *Hygrocybe* and *Arrhenia* spp. were discovered to be biotrophic (Seitzman et al. 2011). *Hygrocybe* spp. are probably associated with grasses (Griffith et al. 2014) and *Arrhenia* spp. with mosses or algae. The mushroom *Omphalina rivulicola/pyxidata* (identified to one of those two species) is closely related but separate from the lichen-forming species of *Lichenomphalia* (Redhead et al. 2002). There was one instance of a basidiolichen found belowground (OTU_2 *Acantholichen/Dictyonema* sp.) but despite the top two GenBank matches both having 100% coverage and 96% identity for *Acantholichen pannarioides* (KT429807) and *Dictyonema aeruginosulum* (EU825954), this identity is questionable since these genera are known to be only tropical in distribution (Ertz et al. 2008, Dal-Forno et al. 2016).

In my study, the Hygrophoraceae minor clade was found to prefer pristine sites over tilled ones. In Europe, *Hygrocybe* spp. are considered sensitive to fertilizer application and human disturbance, and are being lost as low-productivity natural grasslands are converted into agriculturally improved nutrient-rich, high-productivity sites (Arnolds 1989). The importance of *Hygrocybe* spp. in my study and others is further discussed in sections 4.1.2 and 4.2.2.

4.3.1.5 Mycenaceae

The Mycenaceae were the most abundant minor clade (22.5%). Most of the abundance comes from two OTUs that were dominant in Walpole sites – *Mycena epiptygeria* sp. 1 and *Mycena* sp. 2. There were only seven OTUs, but four (or maybe five – one collection was identified to either *Marasmius* or *Mycena*) mushroom morphospecies were encountered. The Mycenaceae are saprotrophic primary colonizers on plant debris or rarely humus (Moncalvo et al. 2002). Often Mycenaceae fruiting bodies are attached directly to their substrate; I observed mushrooms on leaves (e.g., from New Jersey Tea – *Mycena* sp. (longstem)), grass litter (*Mycena* cf. *stylobates*), and some on bare soil (*Mycena* sp. (white)). The number of mushroom morphospecies encountered and the omission of litter from belowground sequencing suggests the Mycenaceae may be even more prominent in tallgrass prairies than shown in this study.

4.3.1.6 Ceratobasidiaceae

The Ceratobasidiaceae were the second most abundant minor clade (22.5%), with most of this abundance coming from one species – the most abundant OTU (OTU_1 *Ceratobasidiaceae* sp. 1). No other OTUs in this minor clade could be identified to a higher resolution than family except for one with a strong query match for *Thanatephorus cucumeris* – a plant pathogen (see Appendix B). The Ceratobasidiaceae were less abundant and received little attention in studies of Michigan soils (Wong 2012) and tallgrass prairies in Kansas (Jumpponen et al. 2010) and Oklahoma (Penton et al. 2013), although they have been found in abundance in soils of an Australian agricultural site (Midgley et al. 2007).

A variety of ecological roles occur across the species in this family. Many species are able to switch between different roles depending on conditions or may sit somewhere on a spectrum ofmany roles (Veldre et al. 2013). This makes it difficult to ascertain what ecological activities they were carrying out in the context of this tallgrass prairie study. The Ceratobasidiaceae include crop pathogens (necrotrophs, that kill the host and feed on dead tissue), orchid mycorrhizae, saprotrophs, and endophytes (which live in plant tissues but cause no symptoms) (Veldre et al. 2013). OTUs found in recently tilled sites may be

crop pathogens that are still harbored in the soil from the recent agricultural activity. Midgley et al. (2007) found their one agriculturally active site to be dominated by the Ceratobasidiales. In my study, many OTUs in the Ceratobasidiaceae were found in both tilled and pristine sites, and so they may be saprotrophs or else endophytes or pathogens of non-agricultural prairie plants. It is unlikely any represent orchid mycorrhizae because orchid species were not present in the sampling plots of any site. This family does not produce macroscopic fruiting bodies, except for inconspicuous anamorphic sclerotia (Veldre et al. 2013), so they were not observed at all in aboveground surveys. It is possible that OTU_1 *Ceratobasidiaceae* sp. 1 had such high abundance in this experiment from its sclerotia being captured during soil sampling and retained in the soil sieving procedure.

4.3.1.7 Polyporaceae *sensu lato*

The Polyporaceae *sensu lato* were the third most abundant (10%) and third/fourth richest minor clade (16 OTUs – tied with the Sebacinaceae). The majority of the abundance arises from OTU_5 *Hypochnicium* sp. and OTU_376 Fomitopsidaceae sp. The OTUs found in this group were taxonomically diverse, covering at least 10 genera (see Appendix B). Similarly to the Mycenaceae, it is possible that diversity was undersampled in the belowground method, since members were observed fruiting on aboveground litter (such as *Trametes* spp. on incidental woody debris or decaying vines of *Vitis riparia*).

It is perhaps surprising that this group was so rich and abundant in grasslands. The Polyporaceae are known for being abundant in forests, decaying lignin and/or cellulose in standing wood and fallen wood as brown rots and white rots (Hibbett et al. 2014). Some of the Polyporaceae form agaricoid fruiting bodies (with pores instead of gills) whereas others are corticioid (crust-like). *Hypochnicium* spp. are corticioid on fallen wood (Telleria et al. 2010). Fomitopsidaceae members are usually saprotrophic brown rots of wood (decomposing cellulose and hemicellulose). Apparently these “wood-rotting” fungi are also found in tallgrass prairies, presumably decomposing non-woody plant material in the soil. It has been suggested that lignin-rich rough-textured herbaceous plant material such as *Solidago* spp. (goldenrod) stems are an abundant and likely substrate in grasslands (Bahnmann 2009). There were no apparent tilled-pristine trends in

OTUs from the Polyporaceae *sensu lato* (see Appendix B), so ecological roles could not be further determined using site history.

4.3.2 Comparisons of composition with other grassland studies

Only a few studies have applied high-throughput sequencing to soil Agaricomycetes in tallgrass prairies. Penton and colleagues (2013) compared fungal composition of Oklahoma tallgrass prairies to Alaskan permafrost under warming conditions. Dominant Agaricomycete taxa were the Tricholomataceae and Marasmiaceae at the family level, and *Moniliophthora* (Marasmiaceae), *Leucopaxillus* (Tricholomataceae), *Camarophyllopsis* (Clavariaceae), and *Camarophyllus* (syn. *Cuphophyllus*; Hygrophoraceae) at the genus level. In my study of Ontario tallgrass prairies, the Tricholomatoid and Marasmioid major clades were first and fourth most abundant, so there is some correlation with Oklahoma. However, Ontario contains a stronger component of the Ceratobasidiaceae (Cantharellales) and Polyporaceae *sensu lato* (=Polyporales). In both Ontario and Oklahoma, members of the Clavariaceae and Hygrophoraceae are prominent. Their dominant *Moniliophthora* sp. (4.5% of all their sequences) was not found in my study, perhaps because the genus name applied to their OTU is doubtful (some other Marasmiaceae species is more likely). A search of all seven species of *Moniliophthora* from Index Fungorum shows no indication of this genus occurring in the United States, but only being described from tropical and Eastern regions (associated with *Theobroma cacao*, Phillips-Mora et al. 2007, Meinhardt et al. 2008; Southeast Asia, Kerekes and Desjardin 2009; littoral forests of Polynesia, Kropp and Albee-Scott 2012; and Korea, Antonin et al. 2014). Naming of OTUs depends on closest matches available, which may represent only a closely related taxon (e.g. my OTU_62 *Acantholichen/Dictyonema* sp. which are also only known from the tropics (Ertz et al. 2008, Dal-Forno et al. 2016)).

Jumpponen and colleagues (2010) applied high-throughput sequencing to study soils at Konza tallgrass prairie in Kansas. Ontario prairies were similar to Kansas in having strong representation of the Cantharellales and Polyporales (in the Kansas study, second and fourth most abundant respectively), which was lacking in the Oklahoma prairies from Penton (2013). However the Kansas prairie had much stronger

representation from the Atheliales (third most abundant) than was found in Ontario or Oklahoma. The most abundant order from Kansas was the Agaricales, which would include both the Tricholomataceae and Marasmiaceae from Penton's study (Oklahoma) or Tricholomatoid and Marasmioid major clades from my study (Ontario). Jumpponen et al. (2010) list genera that were encountered and ones that had significant correlations with certain soil strata, which was the focus of their study. All of the Agaricomycete genera from this list were also found in my study, usually in both the aboveground and belowground surveys and with high abundance of their families: *Omphalina* and *Hygrocybe* (Hygrophoraceae), *Marasmiellus* (Marasmiaceae), *Mycena* (Mycenaceae), *Clitopilus* (Entolomataceae), and *Ceratobasidium* (Ceratobasidiaceae – Cantharellales).

To summarize, high-throughput sequencing of tallgrass prairies in Ontario, Oklahoma, and Kansas shows that composition tends to consist mostly of the following broad taxa by abundance: Tricholomatoid clade (Agaricales), Marasmioid clade (Agaricales), Cantharellales (especially Ceratobasidiaceae), and Polyporales. Certain other families tend to have high richness: Hygrophoraceae, Entolomataceae, Clavariaceae, and Mycenaceae.

Aboveground mushroom surveys of grasslands in North America are rare. Hay (2013) surveyed mixedgrass prairie from Grasslands National Park in southern Saskatchewan. There were many species in common with this study: *Hygrocybe* spp., a *Clitopilus* sp. (misidentified as *Arrhenia* sp.), *Phallus hadriani*, and puffballs from the Agaricaceae (*Lycoperdon/ Bovista/ Vascellum*). Some mushrooms from the mixedgrass prairie were found only in Mary & Peter's prairie in this study of Ontario: *Agaricus* spp. and *Calvatia cyathiformis* (both Agaricaceae). Other Saskatchewan mixedgrass prairie mushroom species were not encountered at all in Ontario tallgrass prairies: *Marasmius oreades* (Marasmiaceae) and an unknown *Clitocybe* sp. (Tricholomataceae) that were both highly abundant, *Volvariella* sp. and *Volvopluteus gloiocephalus* (Pluteaceae), various coprophilous species (from genera *Coprinopsis*, *Panaeolus*, and *Protostropharia*) probably only lacking in the Ontario sites due to absence of cattle dung, and the desert species *Battarea phalloides*.

In Europe, mushroom surveys have been used to recognize and designate high quality grasslands of conservation concern (e.g., Scotland by Newton et al. 2003, Netherlands by Arnolds 1989, South Wales by Rotheroe 2001, and many other European regions not listed here). Grasslands of high conservation quality in Europe have been termed "waxcap grasslands" and are characterized by high diversity of waxcap (Hygrophoraceae – particularly *Hygrocybe* spp.) mushrooms (Griffith et al. 2002).

Rotheroe and colleagues (1996) proposed a method of assessing conservation quality of waxcap grasslands using mushroom taxa common in this ecosystem: *Hygrocybe* spp. *sensu lato*, clavarioid fungi (=Clavariaceae), Geoglossaceae, *Entoloma* spp. *sensu lato*, and *Dermoloma* spp (Tricholomataceae) – counting the number of species found at a site for each group, noting them by their first letters: C, H, E, G, and D. It should be noted that the *Dermoloma* spp. group ("D") traditionally also includes *Camarophyllopsis* spp. which are now placed in the Clavariaceae (Griffith et al. 2013). Using equal sampling effort across multiple sites, the sites can be compared by their counts (for examples, see Rotheroe 2001). Generally, the Hygrophoraceae, Clavariaceae, and Entolomataceae were found to have high richness and moderately high abundance using belowground and aboveground surveys in my study. The Geoglossaceae are members of the Ascomycota and therefore were not considered in my study, although it is worth noting none were found incidentally in aboveground surveys. *Dermoloma* spp. were encountered the least in Rotheroe's examples, and only one *Camarophyllopsis* sp. OTU (syn. *Hodophilus* sp. – Appendix B) was encountered in Ontario tallgrass prairies by my study (although it is possible any of the Clavariaceae sp. 1-4 may represent *Camarophyllopsis* species).

I applied the CHEGD method to my aboveground and belowground data. West Windsor (pristine) sites tended to score the highest, with pristine (except for Sandpits) Walpole sites in second place. The highest scoring site by the aboveground survey data was Pottawatomi prairie (a pristine site in Walpole Island) at **C**0, **H**2, **E**6, **G**0, **D**0. In second place was HA (FRS #23 – a pristine site in Windsor) with more even diversity at **C**2, **H**2, **E**3, **G**0, **D**0. The highest scoring site by the belowground method was Ojiway prairie area A (pristine site in Windsor) at **C**6, **H**5, **E**3, **G-**, **D**1. Despite this, no

mushrooms were encountered at the site in aboveground surveys (despite searching below thick vegetation and litter). It is difficult to compare my CHEGD scores with other studies, since, for the aboveground method, my study had relatively low sampling effort (only two or three visits per site) and the CHEGD method has not been applied to belowground soil high-throughput sequencing studies. However, there seems to be a much higher diversity of *Hygrocybe* spp. (H) in European grasslands (e.g., **C**3, **H**28, **E**0, **G**5 in Rotheroe 2001).

I compared my aboveground mushroom survey results of Ontario tallgrass prairies with Netherlands grasslands and similar sandy ecosystems surveyed by Arnolds (1989) as one European example. The CHEGD taxa were highly diverse in the Netherlands surveys, especially species of *Entoloma* (55) and from the Hygrophoraceae (33), with fewer from the Clavariaceae (only 17 – or 10 if the seven *Camarophyllopsis* spp. are traditionally placed in the "D" group) and again fewer species from the Geoglossaceae and *Dermoloma*. Similar proportions of species of *Entoloma*, *Hygrocybe*, and from the Clavariaceae were found in aboveground surveys in this study, but in smaller numbers (11, 4, and 5 respectively). Increased sampling visits may have uncovered richness similar to that in the Netherlands. Ontario sites were visited only two or three times, which probably uncovered only about a third to a half of the true species diversity according to species accumulation curves of these taxa from Newton et al. (2003). From a belowground perspective, the Clavariaceae are by far the most species-rich of the CHEGD group (24 OTUs, vs. only 11 for the Entolomataceae and eight for the Hygrophoraceae), but this is not apparent in aboveground surveys. Several species were shared between the Netherlands and Ontario: *Entoloma incanum*, *E. excentricum* (perhaps; the uncertain identity in my study was *E. excentricum/sericellum*), *E. mougeotii*, *Hygrocybe conica* (group), *H. glutinipes*, *H. flavescens*, *Phallus hadriani*, and *Cyathus stercoreus*. The genera *Tubaria* and *Hebeloma* were present in Ontario tallgrass prairies but absent in the Netherlands grasslands. Species of *Hebeloma* have been recorded in forests and roadsides in the Netherlands, but not in any type of grassland there, while *Tubaria* wasn't recorded from any ecosystems in that study at all (Arnolds 1989). Conversely, Ontario tallgrass prairies lacked members of *Dermoloma* and *Camarophyllopsis* – at least aboveground (from CHEGD), as well as other genera:

Conocybe, *Lepiota*, *Lepista*, *Psathyrella*, *Psilocybe*, and *Volvariella*. It is possible these seemingly unique taxa may be uncovered with higher sampling effort (in the Netherlands or Ontario).

4.3.3 Notable species and conservation significance

Cotylidia undulata is apparently rare in Ontario tallgrass prairies, as it was found only at the DeMaere prairie (by both aboveground and belowground methods), where it is apparently associated with moss on the sandy soil. The species is found around the world (for example, is listed as occurring in degraded or dessicated *Sphagnum* peat bogs in the Netherlands by Arnolds 1989), but has been listed as vulnerable on a red list of species at risk by the British Mycological Society (Evans et al. 2006). Once deposited, the sequence obtained from a collection of fruiting bodies in this study will be only the second sequence available in GenBank for this species.

Psathyrella ammophila (dune brittlestem mushroom) was found only in the two Norfolk prairies due to their sandy soils. This species requires sand dune habitat and has a relationship with plant roots – particularly with *Ammophila* spp. (beachgrass) (Watling and Rotheroe 1989, First Nature 2015). Conservation managers should keep variation within ecosystems in mind, since (for example) sandy tallgrass prairies are habitat to unique fungi not found in tallgrass prairies with other soil types.

Arrhenia cf. *griseopallida* was found at two sites, but is easy to overlook given its tiny size and gray-brown colour similar to soil. Whether it is actually rare or only overlooked would require survey efforts focused on finding more occurrences of this mushroom specifically. Investigations are under way to clarify species of *Arrhenia* and name undescribed ones, of which a sequence from one of the collections in this study is a part (Andrus Voitk pers. comm.). It is possible genetic studies may discover that cryptic taxa within this morphospecies are unique to grasslands in North America (different from European specimens).

Minimedusa polyspora (OTU_9) produces raspberry-like bulbils (0.1-0.2 mm) (Weresub and LeClair 1971) and members of the Ceratobasidiaceae (perhaps including

OTU_1 Ceratobasidiaceae sp. 1) produce sclerotia (0.250 mm - 0.500 mm) (Kumar et al. 2002). These unusual bulbils and sclerotia propagules may explain the high abundance of the two species in this study. Since the propagules are larger than the openings of the finest sieve used in the soil sieving procedure (0.053 mm) there would have been much higher proportion of fungal matter for these species than others. *Pneumatospora obcoronata* (syn. *Minimedusa obcoronata*) also produces bulbils and was by far the most abundant OTU in studies of Michigan soils (Bahnmann 2009) that used the same sieveing method (Thorn et al. 1996).

4.4 Limitations

The belowground survey type is highly limited by small sampling size. In each field site only a small fraction of the land is sampled by thin vertical soil cores (cores are 2.5 cm diameter with 5×6 taken per site, so that's 4.9×10^{-6} % of a one hectare site). This is further reduced in the lab when only a few milligrams of each sample are added to an Eppendorf tube for DNA extraction, and then only a few microliters of DNA extract are used for PCR. The sequencing process itself also subsamples by using only some of the PCR products to create a library and then sequencing only part of that library. The limitations of such repeated subsampling are described by Gloor (2015). The consequence is that rare species are easily lost in the process. Fortunately a few grams of soil can contain tens to hundreds of fungal species (Lynch and Thorn 2006, Peay et al. 2016). I found one Agaricomycete OTU per gram of raw soil, or seven OTUs per gram before any taxonomic filtering (Agaricomycetes, other fungi, and representatives from other kingdoms).

The aboveground survey type does not face the limitations of repeated subsampling. Sampling area is less of a limitation, as a large area can be searched for fruiting bodies, but it is still impossible to cover the entire site, and so it is possible that some mushrooms were present but not found. This is particularly likely for inconspicuous (small and/or drab-coloured) fruiting bodies that are hidden under grass and litter. Another major limiting factor in aboveground surveys is the seasonal and sporadic nature of fruiting body production. Sites should ideally be visited more than once each season and over several years (Rossman et al. 1998, Lodge et al. 2004). This is not a limiting

factor in belowground sampling because hyphal materials are found in the soil even when no mushrooms are being produced.

The belowground survey type applied to soil microbes has also been criticized for being too sensitive, capturing DNA from unwanted biological materials. The soil sieving technique I used washes away the spores of almost all fungi (most importantly, spores deposited hundreds of years ago and those from other ecosystems), retaining only plant debris, fungal hyphae, rhizomorphs, and sclerotia (ecologically active material belonging to the sampled ecosystem) (Parkinson and Williams 1960, Thorn et al. 1996, Lynch and Thorn 2006). Without sieving, inactive and active fungal materials would both be present in samples and it would be impossible to distinguish them after sequencing, leading to incorrect ecological inferences (Klein 2015). Another way of targeting only active organisms is to use rRNA (ribosomal RNA), which has a faster turnover rate than rDNA, or mRNA (messenger RNA for genes actively expressed in the environment). My study used rDNA because it is more stable across time and more appropriate for capturing a wide range of diversity with limited field sampling (Porter et al. 2008).

Sequence-based identification is particularly difficult when: 1) the query OTU retrieves a wide variety of taxa that are all equally well-matched or 2) the best matches are highly dissimilar to the query OTU (noted in Penton et al. 2013). Various underlying limitations come into play. GenBank is limited by frequent incorrectly annotated sequences (i.e. wrong identifications) (Nilsson et al. 2012) and still holding too few sequences. Of the world's estimated eight million species of fungi (Taylor et al. 2014), GenBank currently holds sequences for only 125,865 (see NCBI taxonomy statistics: http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter=statistics&?&m=0). The data gap comes from both described fungi with herbarium specimens that have not yet been sequenced (Brock et al. 2009) and undescribed fungi, which include those without conspicuous fruiting bodies or which cannot be cultured.

I addressed the limitation of undescribed, unsequenced fungi by constructing of a large phylogram combining OTU and mushroom sequences from this study with quality GenBank reference sequences (not shown). OTUs with weak matches could at least be

placed in a broader taxonomic group (such as order or family). Of note are the following minor clades which probably represent undescribed soil fungi: Cantharellales unknown family, Russulales unknown family, Thelephorales unknown family, most of the Pluteaceae, Pleurotaceae, and all of the Gomphales cf. group.

All OTUs from the Gomphales clade grouped together strongly in my phylogenetic tree, but returned diverse and highly dissimilar matches with a maximum identity of *ca.* 90%, usually with representatives from the Gomphales, but sometimes also from the Cantharellales, Russulales, Strophariaceae, Hymenogastraceae, Pluteaceae, Auriculariaceae, and Phallaceae. The Gomphales OTUs were placed between reference sequences from the Gomphales and Phallaceae in my phylogenetic tree. In a study of Michigan soils, three OTUs from no till and historically tilled plots were placed in the gomphoid/phalloid clade and were on their own branch on a phylogram, next to a branch with reference sequences of *Phallus hadriani* and *Mutinus elegans* (Wong 2012). The 10 Gomphales cf. clade OTUs from my study and the three gomphoid/phalloid OTUs from Michigan apparently represent a region of undescribed fungal species.

Studies in Michigan soils repeatedly returned a “sister clade to *Volvariella*” or “Pluteoid clade” that was very OTU rich (Lynch 2004, Bahnmann 2009, and Wong 2012). This seems to correspond to my 11 Pluteaceae spp., which did not return strong GenBank matches and created their own branch on my phylogram. Again, these are apparently a group of closely related undescribed fungi.

4.5 Future studies

This study is exploratory, and future studies can test the trends observed here in controlled experimental systems. The impact of tillage on communities of Agaricomycetes ought to be examined in a design where edaphic and climate factors are the same between tilled and pristine plots. This could be accomplished by finding pairs of adjacent sites that are identical except for tillage history, or by tilling portions of a pristine prairie as a long-term experiment (e.g., tillage microplots in never-tilled “T8” plots at the Kellogg Biological Station in Michigan).

Much of the ecology and basic biology of fungal families in this study remains unknown. The Sebacinaceae, Ceratobasidiaceae, and Polyporaceae, three families whose ecological roles in grassland soils are not clear, could be isolated and cultured from soils where they are abundant to test for pathogenicity (Thorn et al. 1996, Midgley et al. 2007) and to obtain longer sequences. Until we know more about the ecological roles of soil fungi it remains difficult to infer ecological significance of the taxa recovered in exploratory studies.

Aboveground litter is often omitted in high-throughput sequencing studies of soil biota (such as this one, and noted in Porter et al. 2008). Inclusion of litter would add another dimension to compare with belowground soil and aboveground mushroom surveys and may uncover overlooked diversity in the Mycenaceae and the Polyporaceae, and the otherwise absent Tubariaceae and Nidulariaceae, helping to bring a more complete picture to the mycological communities of grasslands.

Combining detailed vegetative data of sites with fungal high-throughput sequencing would allow for in-depth comparisons between fungal richness and plant composition. Sites suspected of being vegetationally uninteresting but important mycologically could be included to test whether conservation agencies have a gap in their land acquisition priorities. This has been conducted using aboveground mushroom surveys in South Wales (Rotheroe 2001), but not yet using high-throughput sequencing technology.

4.6 Conclusion

This has been the first study in North America to characterize grassland fungal communities using high-throughput sequencing of soil samples. Although observational, my study provides important baseline data for future studies in tallgrass prairies and examining the composition of Agaricomycetes in soils. The discovery that North American grasslands contain many of the same dominant fungal families as European ones facilitates moving on with the next steps of applying European methods of study and fungal conservation assessment here as well.

Despite the influence of unknown factors related to geographic regions of sites, restored tallgrass prairies with a history of tillage were found to have compositional differences from pristine remnant sites. Examination of tillage-sensitive taxa confirmed their sensitivity to tillage disturbance (such as the Hygrophoraceae and Clavariaceae) and several taxa showing tillage-sensitive trends were proposed for further examination. These findings further support the need for stringent conservation of remnant ecosystems (such as the tallgrass prairies), and that restoration is not a replacement for conserving lands in the first place.

Comparisons of the aboveground mushroom survey with belowground high-throughput sequencing showed that the belowground survey successfully captures most of the taxa found aboveground and many more. Degrees of overlap between the two survey types at different taxonomic scales supports findings from Porter et al. (2008) that overlap decreases at finer scales. I showed that shared species can be determined (identical sequences from aboveground and belowground can be matched together), but they are only a small percentage of total species richness in either survey type. Comparisons of abundance in species shared between the two survey types brings new perspectives to aboveground versus belowground energy investment (especially for *Clavaria* cf. *acuta* and *Cotylidia undulata*).

This study clearly demonstrates the usefulness of applying high-throughput sequencing to belowground fungal surveys. Hopefully new technologies such as these will continue to illuminate the diversity and importance of fungi and facilitate our understanding and conservation of the world's imperiled ecosystems.

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Appendix A Accession numbers of best matched sequences from GenBank for soil OTUs and mushroom specimens with species-level names.

OTU/Specimen	Name (query ID)	Accession
HA10	Arrhenia cf. griseopallida	U66436
SA1	Clavaria cf. acuta	HQ877680
EL8	Clavaria cf. fragilis	HQ877687
HA5	Clavulinopsis laeticolor	EU118618
MP13	Clitocybe dealbata	AF042589
SI11	Clitopilus scyphoides	KC176282
DM10	Cotylidia undulata	JN649335
HA9	Entoloma cf. tubaeforme	KJ845724
PO4, PO7	Entoloma excentricum/sericellum	KF771047
DM8	Hebeloma cf. incarnatulum	AF430291
DM12	Omphalina rivulicola/pyxidata	U66450
DM12	Omphalina rivulicola/pyxidata	U66451
BF4	Pholiotina sulcata	JX968153
MP15	Psathyrella ammophila	KC992871
DM6, DM9	Tubaria cf. furfuracea (T. hiemalis)	FJ717494
OTU_245	Amanita_populiphila	KP224345
OTU_2278	Amauroderma_intermedium	KU315209
OTU_2392	Athelia_arachnoidea	GU187557
OTU_79	Athelia_bombacina	LN714523
OTU_213	Athelia_epiphylla	AY586633
OTU_1602	Boletinellus/Gyrodon_merulioides	AY612807
OTU_1398	Boletellus_chrysenteroides	KP327645
OTU_878	Vuilleminia_macrospora	JX892941
OTU_545	Burgoa_anomala	AB972757
OTU_83	Calyptella_capula	AY570994
OTU_3713	Clavaria_acuta	GU299506
OTU_1005	Clavaria_fragilis	HQ877687
OTU_435	Clavaria_fuscata	HQ877691
OTU_1168	Clavaria_incarnata_sp1	KP257245
OTU_1327	Clavaria_incarnata_sp2	JQ415937
OTU_1027	Clavulinopsis_helvola	GU299510
OTU_230	Cotylidia_undulata	JN649335
OTU_656	Craterellus_tubaeformis	DQ898741
OTU_150	Cuphophyllus_pratensis	AF261457

OTU/Specimen	Name (query ID)	Accession
OTU_210	Endoperplexa_enodulosa	AY505543
OTU_404	Hygrocybe_flavescens/chlorophana	KF291121
OTU_1673	Hygrocybe_lepida/cantharellus	KF306334
OTU_1051	Hymenochaete_tenuis	JQ279641
OTU_1336	Inocybe_cookei	AY702014
OTU_129	Inocybe_curvipes	JN035294
OTU_4467	Inocybe_perlata	JN975013
OTU_744	Inocybe_splendens	KJ399959
OTU_286	Inocybe_squamata	FJ904136
OTU_578	Jaapia_ochroleuca	GU187670
OTU_540	Laetisaria_arvalis	EU622842
OTU_820	Lepista_saeva	KJ417193
OTU_347	Limonomyces_roseipellis	KF824722
OTU_148	Merulicium_fusisporum	EU118647
OTU_9	Minimedusa_polyspora	AB972779
OTU_0	Mutinus_elegans	AY574643
OTU_49	Mycena_adscendens	KT900143
OTU_5	Mycena_epiptygeria_sp1	HQ604772
OTU_9841	Mycena_epiptygeria_sp2	KP454034
OTU_1164	Mycena_galopus	HM240534
OTU_4223	Mycena_purpureofusca	HQ604765
OTU_587	Omphalina_grisella	U66443
OTU_984	Omphalina_velutipes	U66455
OTU_506	Paulisebacina_allantoidea	KF061266
OTU_242	Pholiota_tuberculosa	AY207276
OTU_1599	Piriformospora_indica_sp2	KT762618
OTU_90	Piriformospora_indica_sp3	KF061284
OTU_557	Polyozellus_multiplex	EF561637
OTU_180	Ramariopsis_corniculata	GU299495
OTU_940	Ramariopsis_pulchella_sp1	GU299497
OTU_7506	Ramariopsis_pulchella_sp2	KP012919
OTU_198	Rogersella_griseliniae	DQ873651
OTU_1162	Russula_cremeirosea	KT933844
OTU_447	Russula_putida	HG798526
OTU_425	Sclerogaster_minor	FJ435976
OTU_12273	Serendipita_vermifera_sp1	KT762620
OTU_174	Serendipita_vermifera_sp2	EU625994

OTU/Specimen	Name (query ID)	Accession
OTU_407	Serendipita_vermifera_sp3	EU626002
OTU_879	Serendipita_vermifera_sp4	EU625994
OTU_9073	Serendipita_vermifera_sp5	AY505555
OTU_51	Sistotrema_athelioides	DQ898700
OTU_78	Sphaerobolus_ingoldii	AF139975
OTU_69	Sphaerobolus_stellatus	HQ604795
OTU_192	Thanatephorus_cucumeris	KP171644
OTU_191	Tylospora_fibrillosa	JN938845
OTU_137	Typhula_phacorrhiza	AF261374
OTU_515	Waitea_circinata	KC176341

Appendix B All 281 Agaricomycete OTUs from 13 tallgrass prairie sites where soil sampling was conducted.

Key to sites: HA,B,C,D = FRS #23,32,27,28 Herb-Gray Parkway (Windsor); OA,B = Ojibway Prairie Areas 1 and 2 (Windsor); SI = Silphium, SA = Sandpits, MI = Mike's field, EL = Eliza's prairie, PO = Pottawatomi (Walpole); DM* = DeMaere (Norfolk) (* = dataset includes all 24 sample plots including nitrogen treatments, not just control plots; presence marked with "(x)"); BF = Blair Flats (Cambridge). Sites are ordered by geographic location, from west to east.

OTU	Name (based on query ID)	Minor Clade	Major Clade	HA	HB	OA	OB	HC	HD	SA	SI	MI	EL	PO	DM*	BF
OTU_1084	Agaricus_sp	Agaricaceae	Agaricoid	x		x									x	
OTU_9976	Lepiota_sp	Agaricaceae	Agaricoid												x	
OTU_488	Vascellum_sp	Agaricaceae	Agaricoid	x			x						x	x		
OTU_197	Bolbitiaceae_sp1	Bolbitiaceae	Agaricoid												x	
OTU_4574	Bolbitius_sp	Bolbitiaceae	Agaricoid						x	x					x	
OTU_1302	Conocybe_sp	Bolbitiaceae	Agaricoid				x									
OTU_12334	Conocybe_sp	Bolbitiaceae	Agaricoid										x		x	
OTU_14293	Conocybe_sp	Bolbitiaceae	Agaricoid			x							x			
OTU_255	Pholiotina_sp	Bolbitiaceae	Agaricoid												(x)	
OTU_11219	Cortinarius_sp1	Cortinariaceae	Agaricoid							x	x	x	x	x	x	
OTU_297	Cortinarius_sp2	Cortinariaceae	Agaricoid												(x)	
OTU_5283	Cortinarius_sp3	Cortinariaceae	Agaricoid		x		x						x	x	x	
OTU_7077	Cortinarius_sp4	Cortinariaceae	Agaricoid	x	x	x	x							x	x	
OTU_1194	Crepidotus_sp1	Crepidotaceae	Agaricoid			x										
OTU_2994	Crepidotus_sp2	Crepidotaceae	Agaricoid												x	x
OTU_14025	Simocybe_sp1	Crepidotaceae	Agaricoid												x	
OTU_899	Simocybe_sp2	Crepidotaceae	Agaricoid					x								
OTU_3849	Galerina_sp1	Hymenogastraceae	Agaricoid			x					x					
OTU_445	Galerina_sp2	Hymenogastraceae	Agaricoid							x	x		x	x		
OTU_4878	Galerina_sp3	Hymenogastraceae	Agaricoid								x					

OTU	Name (based on query ID)	Minor Clade	Major Clade	HA	HB	OA	OB	HC	HD	SA	SI	MI	EL	PO	DM*	BF
OTU_189	Hymenogastraceae_sp	Hymenogastraceae	Agaricoid	x	x	x				x	x	x	x	x	x	
OTU_1336	Inocybe_cookei	Inocybaceae	Agaricoid			x										
OTU_129	Inocybe_curvipes	Inocybaceae	Agaricoid												x	
OTU_4467	Inocybe_perlata	Inocybaceae	Agaricoid		x											
OTU_1769	Inocybe_sp	Inocybaceae	Agaricoid				x									
OTU_744	Inocybe_splendens	Inocybaceae	Agaricoid		x											
OTU_286	Inocybe_squamata	Inocybaceae	Agaricoid		x											
OTU_1367	Coprinellus_sp1	Psathyrellaceae	Agaricoid												x	x
OTU_93	Coprinellus_sp2	Psathyrellaceae	Agaricoid	x	x	x	x	x					x		x	x
OTU_13279	Coprinopsis_sp1	Psathyrellaceae	Agaricoid			x	x		x				x			
OTU_7346	Coprinopsis_sp2	Psathyrellaceae	Agaricoid					x							x	
OTU_8222	Coprinopsis_sp3	Psathyrellaceae	Agaricoid										x			
OTU_6796	Cyathus_sp	Psathyrellaceae	Agaricoid												(x)	
OTU_6038	Psathyrella_sp	Psathyrellaceae	Agaricoid		x	x	x		x						x	x
OTU_247	Gymnopilus_sp	Strophariaceae	Agaricoid	x	x					x					x	
OTU_7800	Hypholoma_sp	Strophariaceae	Agaricoid							x		x	x	x		
OTU_242	Pholiota_tuberculosa	Strophariaceae	Agaricoid	x	x	x	x		x						x	x
OTU_2392	Athelia_arachnoidea	Atheliaceae	Atheliales												x	
OTU_79	Athelia_bombacina	Atheliaceae	Atheliales												x	
OTU_213	Athelia_epiphylla	Atheliaceae	Atheliales							x	x	x	x	x		
OTU_191	Tylospora_fibrillosa	Atheliaceae	Atheliales							x	x	x	x	x		
OTU_870	Auriculariales_sp	Auric_incertae_sedis	Auriculariales							x				x		
OTU_1006	Basidiodendron_sp	Auric_incertae_sedis	Auriculariales								x		x			
OTU_816	Elmerina_sp	Auric_incertae_sedis	Auriculariales												x	
OTU_210	Endoperplexa_enodulosa	Auric_incertae_sedis	Auriculariales												x	
OTU_14200	Auricularia_sp	Auriculariaceae	Auriculariales												x	
OTU_12515	Auriculariaceae_sp1	Auriculariaceae	Auriculariales								x					

OTU	Name (based on query ID)	Minor Clade	Major Clade	HA	HB	OA	OB	HC	HD	SA	SI	MI	EL	PO	DM*	BF
OTU_183	Auriculariaceae_sp2	Auriculariaceae	Auriculariales	x							x				x	
OTU_185	Auriculariaceae_sp3	Auriculariaceae	Auriculariales						x				x		x	x
OTU_833	Auriculariaceae_sp4	Auriculariaceae	Auriculariales												x	
OTU_86	Auriculariaceae_sp5	Auriculariaceae	Auriculariales					x							x	
OTU_869	Auriculariaceae_sp6	Auriculariaceae	Auriculariales	x	x			x			x		x		x	
OTU_1602	Boletellus/Gyrodon_merulioides	Boletaceae	Boletales		x											
OTU_1398	Boletellus_chrysenteroides	Boletaceae	Boletales			x										
OTU_179	Boletus_sp	Boletaceae	Boletales	x							x				x	
OTU_1614	Tylopilus_sp	Boletaceae	Boletales			x										
OTU_2491	Boletales_sp	Melanogastraceae	Boletales	x		x	x				x					
OTU_281	Scleroderma_sp	Sclerodermataceae	Boletales												x	
OTU_60	Suillus_cavipes	Suillaceae	Boletales							x	x	x	x	x		
OTU_874	Suillus_luteus	Suillaceae	Boletales												x	
OTU_401	Botyrobasidiaceae_sp	Botryobasidiaceae	Cantharellales												x	
OTU_545	Burgoa_anomala	Botryobasidiaceae	Cantharellales												x	x
OTU_9	Minimedusa_polyspora	Canth_incertae_sedis	Cantharellales	x	x	x	x	x	x	x	x		x	x	x	x
OTU_9652	Minimedusa_sp	Canth_incertae_sedis	Cantharellales				x								x	
OTU_1087	Cantharellales_sp1	Canth_unknown_family	Cantharellales					x	x						x	
OTU_1394	Cantharellales_sp2	Canth_unknown_family	Cantharellales			x										
OTU_7444	Cantharellales_sp3	Canth_unknown_family	Cantharellales										x			
OTU_656	Craterellus_tubaeformis	Cantharellaceae	Cantharellales							x						
OTU_1	Ceratobasidiaceae_sp1	Ceratobasidiaceae	Cantharellales							x	x	x	x	x	x	
OTU_12552	Ceratobasidiaceae_sp2	Ceratobasidiaceae	Cantharellales							x	x		x			
OTU_5231	Ceratobasidiaceae_sp3	Ceratobasidiaceae	Cantharellales					x							x	
OTU_6779	Ceratobasidiaceae_sp4	Ceratobasidiaceae	Cantharellales												x	
OTU_796	Ceratobasidiaceae_sp5	Ceratobasidiaceae	Cantharellales					x	x					x	x	
OTU_192	Thanatephorus_cucumeris	Ceratobasidiaceae	Cantharellales												x	

OTU	Name (based on query ID)	Minor Clade	Major Clade	HA	HB	OA	OB	HC	HD	SA	SI	MI	EL	PO	DM*	BF
OTU_305	Burgella_flavoparmeliae	Clavulinaceae	Cantharellales												(x)	
OTU_51	Sistotrema_athelioides	Hydnaceae	Cantharellales												x	
OTU_27	Sistotrema_sp1	Hydnaceae	Cantharellales			x							x		x	
OTU_2019	Sistotrema_sp2	Hydnaceae	Cantharellales												x	
OTU_7309	Sistotrema_sp3	Hydnaceae	Cantharellales							x	x	x	x	x		
OTU_234	Tulasnella_sp1	Tulasnellaceae	Cantharellales												x	
OTU_426	Tulasnella_sp2	Tulasnellaceae	Cantharellales												x	
OTU_611	Tulasnella_sp3	Tulasnellaceae	Cantharellales									x				
OTU_797	Tulasnella_sp4	Tulasnellaceae	Cantharellales										x	x		
OTU_1361	Tulasnellaceae_sp	Tulasnellaceae	Cantharellales					x								
OTU_3713	Clavaria_acuta	Clavariaceae	Clavarioid			x										
OTU_1005	Clavaria_fragilis	Clavariaceae	Clavarioid		x											
OTU_435	Clavaria_fuscata	Clavariaceae	Clavarioid	x	x						x					
OTU_1168	Clavaria_incarnata_sp1	Clavariaceae	Clavarioid								x					
OTU_1327	Clavaria_incarnata_sp2	Clavariaceae	Clavarioid	x												
OTU_1071	Clavaria_sp1	Clavariaceae	Clavarioid	x		x										
OTU_674	Clavaria_sp10	Clavariaceae	Clavarioid	x	x											
OTU_1256	Clavaria_sp2	Clavariaceae	Clavarioid								x					
OTU_13081	Clavaria_sp3	Clavariaceae	Clavarioid												(x)	
OTU_238	Clavaria_sp4	Clavariaceae	Clavarioid			x	x	x					x		x	
OTU_4000	Clavaria_sp5	Clavariaceae	Clavarioid		x											
OTU_516	Clavaria_sp6	Clavariaceae	Clavarioid												(x)	
OTU_520	Clavaria_sp7	Clavariaceae	Clavarioid								x	x	x	x	x	
OTU_613	Clavaria_sp8	Clavariaceae	Clavarioid											x		
OTU_6560	Clavaria_sp9	Clavariaceae	Clavarioid		x											
OTU_3841	Clavariaceae_sp1	Clavariaceae	Clavarioid				x									
OTU_592	Clavariaceae_sp2	Clavariaceae	Clavarioid			x					x					

OTU	Name (based on query ID)	Minor Clade	Major Clade	HA	HB	OA	OB	HC	HD	SA	SI	MI	EL	PO	DM*	BF
OTU_61	Clavariaceae_sp3	Clavariaceae	Clavarioid					x	x						x	
OTU_802	Clavariaceae_sp4	Clavariaceae	Clavarioid		x											
OTU_1027	Clavulinopsis_helvola	Clavariaceae	Clavarioid			x	x				x					
OTU_638	Hodophilus_sp	Clavariaceae	Clavarioid			x										
OTU_180	Ramariopsis_corniculata	Clavariaceae	Clavarioid	x							x				x	
OTU_940	Ramariopsis_pulchella_sp1	Clavariaceae	Clavarioid	x												
OTU_7506	Ramariopsis_pulchella_sp2	Clavariaceae	Clavarioid			x										
OTU_878	Vuilleminia_macrospora	Corticiaceae	Corticiales							x	x			x		
OTU_540	Laetisaria_arvalis	Corticiaceae	Corticiales						x						x	
OTU_1622	Laetisaria_fuciformis	Corticiaceae	Corticiales												(x)	
OTU_347	Limonomyces_roseipellis	Corticiaceae	Corticiales	x			x								x	
OTU_515	Waitea_circinata	Corticiaceae	Corticiales						x						x	
OTU_62	Acantholichen/Dictyonema_sp	Hygrophoraceae	Hygrophoroid					x							x	
OTU_574	Arrhenia_sp	Hygrophoraceae	Hygrophoroid												x	
OTU_150	Cuphophyllus_pratensis	Hygrophoraceae	Hygrophoroid	x							x				x	
OTU_11255	Hygrocybe_concia_group_sp1	Hygrophoraceae	Hygrophoroid											x		
OTU_160	Hygrocybe_concia_group_sp2	Hygrophoraceae	Hygrophoroid	x	x	x	x		x		x			x	x	
OTU_21	Hygrocybe_concia_group_sp3	Hygrophoraceae	Hygrophoroid	x		x	x				x				x	
OTU_311	Hygrocybe_concia_group_sp4	Hygrophoraceae	Hygrophoroid	x	x	x							x	x		
OTU_404	Hygrocybe_flavescens/chlorophana	Hygrophoraceae	Hygrophoroid			x										
OTU_1673	Hygrocybe_lepida/cantharellus	Hygrophoraceae	Hygrophoroid			x										
OTU_5877	Hygrophoraceae_sp	Hygrophoraceae	Hygrophoroid	x		x	x			x	x	x	x	x	x	
OTU_1182	Omphalina_ericetorum	Hygrophoraceae	Hygrophoroid												x	
OTU_587	Omphalina_grisella	Hygrophoraceae	Hygrophoroid												x	
OTU_984	Omphalina_velutipes	Hygrophoraceae	Hygrophoroid									x			x	
OTU_148	Merulicium_fusisporum	Pterulaceae	Hygrophoroid												x	
OTU_137	Typhula_phacorrhiza	Typhulaceae	Hygrophoroid					x	x						x	

OTU	Name (based on query ID)	Minor Clade	Major Clade	HA	HB	OA	OB	HC	HD	SA	SI	MI	EL	PO	DM*	BF
OTU_1931	Typhula_sp1	Typhulaceae	Hygrophoroid												(x)	
OTU_7090	Typhula_sp2	Typhulaceae	Hygrophoroid						x							
OTU_752	Typhula_sp3	Typhulaceae	Hygrophoroid												(x)	
OTU_2969	Typhulaceae_sp1	Typhulaceae	Hygrophoroid												(x)	
OTU_552	Typhulaceae_sp2	Typhulaceae	Hygrophoroid			x										
OTU_6859	Hymenochaetaceae_sp	Hymenochaetaceae	Hymenochaetales			x										
OTU_1051	Hymenochaete_tenuis	Hymenochaetaceae	Hymenochaetales								x					
OTU_644	Hyphodontia_sambuci	Hymenochaetaceae	Hymenochaetales												(x)	
OTU_17	Hyphodontia_sp1	Hymenochaetaceae	Hymenochaetales							x	x	x	x	x		
OTU_301	Hyphodontia_sp2	Hymenochaetaceae	Hymenochaetales							x		x	x			
OTU_36	Hyphodontia_sp3	Hymenochaetaceae	Hymenochaetales		x	x	x							x	x	x
OTU_9489	Hyphodontia_sp4	Hymenochaetaceae	Hymenochaetales										x			
OTU_230	Cotylidia_undulata	Repetobasidiaceae	Hymenochaetales												x	
OTU_198	Rogersella_griseliniae	Schizoporaceae	Hymenochaetales												x	x
OTU_578	Jaapia_ochroleuca	Jaapiaceae	Jaapiales							x	x	x	x	x		
OTU_617	Marasmiaceae_sp5	Cyphellaceae	Marasmioid								x					
OTU_268	Hydropoid_sp	Hydropoid	Marasmioid			x					x				x	
OTU_1848	Hydropus_sp	Hydropoid	Marasmioid			x										
OTU_49	Mycena_adscendens	Hydropoid	Marasmioid												x	
OTU_860	Flagelloscypha_sp1	Lachnellaceae	Marasmioid												(x)	
OTU_970	Flagelloscypha_sp2	Lachnellaceae	Marasmioid												(x)	
OTU_80	Lachnellaceae_sp	Lachnellaceae	Marasmioid		x			x	x						x	x
OTU_83	Calyptella_capula	Marasmiaceae	Marasmioid						x						x	
OTU_262	Crinipellis_sp	Marasmiaceae	Marasmioid							x					x	
OTU_1115	Marasmiaceae_sp1	Marasmiaceae	Marasmioid			x										
OTU_11566	Marasmiaceae_sp2	Marasmiaceae	Marasmioid		x		x				x					
OTU_177	Marasmius_sp	Marasmiaceae	Marasmioid		x	x									x	

OTU	Name (based on query ID)	Minor Clade	Major Clade	HA	HB	OA	OB	HC	HD	SA	SI	MI	EL	PO	DM*	BF
OTU_9613	Tetrapyrgos_sp	Marasmiaceae	Marasmioid								x	x	x			
OTU_15	Marasmiaceae_sp3	Physalacriaceae	Marasmioid							x	x	x	x	x		
OTU_519	Marasmiaceae_sp4	Physalacriaceae	Marasmioid				x						x			
OTU_425	Sclerogaster_minor	Geastraceae	Phallomycetidae	x			x							x		
OTU_78	Sphaerobolus_ingoldii	Geastraceae	Phallomycetidae												x	
OTU_69	Sphaerobolus_stellatus	Geastraceae	Phallomycetidae												(x)	
OTU_1118	Gomphales_sp1	Gomphales cf	Phallomycetidae								x					
OTU_8876	Gomphales_sp10	Gomphales cf	Phallomycetidae												x	
OTU_12188	Gomphales_sp2	Gomphales cf	Phallomycetidae			x	x									
OTU_22	Gomphales_sp3	Gomphales cf	Phallomycetidae	x	x	x	x			x	x		x	x	x	x
OTU_383	Gomphales_sp4	Gomphales cf	Phallomycetidae						x	x					x	
OTU_474	Gomphales_sp5	Gomphales cf	Phallomycetidae												(x)	
OTU_503	Gomphales_sp6	Gomphales cf	Phallomycetidae							x						x
OTU_5543	Gomphales_sp7	Gomphales cf	Phallomycetidae												x	
OTU_6412	Gomphales_sp8	Gomphales cf	Phallomycetidae							x					x	
OTU_843	Gomphales_sp9	Gomphales cf	Phallomycetidae			x										
OTU_121	Aseroe_sp	Phallaceae	Phallomycetidae												(x)	
OTU_0	Mutinus_elegans	Phallaceae	Phallomycetidae	x	x	x									x	
OTU_1261	Phallaceae_sp1	Phallaceae	Phallomycetidae												x	
OTU_161	Phallaceae_sp2	Phallaceae	Phallomycetidae												x	
OTU_245	Amanita_populiphila	Amanitaceae	Pluteoid		x	x										
OTU_9509	Pleurotaceae_sp	Pleurotaceae	Pluteoid	x	x											
OTU_225	Pluteaceae_sp1	Pluteaceae	Pluteoid		x			x	x							
OTU_755	Pluteaceae_sp10	Pluteaceae	Pluteoid												(x)	
OTU_773	Pluteaceae_sp11	Pluteaceae	Pluteoid												(x)	
OTU_2360	Pluteaceae_sp2	Pluteaceae	Pluteoid										x	x		
OTU_274	Pluteaceae_sp3	Pluteaceae	Pluteoid						x	x						

OTU	Name (based on query ID)	Minor Clade	Major Clade	HA	HB	OA	OB	HC	HD	SA	SI	MI	EL	PO	DM*	BF
OTU_326	Pluteaceae_sp4	Pluteaceae	Pluteoid					x	x	x					x	
OTU_684	Pluteaceae_sp5	Pluteaceae	Pluteoid					x								
OTU_7322	Pluteaceae_sp6	Pluteaceae	Pluteoid					x		x					x	
OTU_9206	Pluteaceae_sp7	Pluteaceae	Pluteoid					x	x							
OTU_388	Pluteaceae_sp8	Pluteaceae	Pluteoid												(x)	
OTU_550	Pluteaceae_sp9	Pluteaceae	Pluteoid					x								
OTU_343	Pluteus_sp	Pluteaceae	Pluteoid												x	
OTU_2278	Amauroderma_intermedium	Polyporaceae_sensu_lato	Polyporales	x		x	x			x	x		x		x	
OTU_376	Fomitopsidaceae_sp	Polyporaceae_sensu_lato	Polyporales	x	x	x	x			x	x	x	x	x	x	x
OTU_12	Hypochnicium_sp	Polyporaceae_sensu_lato	Polyporales							x	x	x	x	x	x	
OTU_13068	Irpex_sp	Polyporaceae_sensu_lato	Polyporales	x												
OTU_2262	Ischnoderma_sp1	Polyporaceae_sensu_lato	Polyporales										x			
OTU_6694	Ischnoderma_sp2	Polyporaceae_sensu_lato	Polyporales							x	x	x	x	x		
OTU_1568	Neofavolus_sp1	Polyporaceae_sensu_lato	Polyporales												x	
OTU_535	Neofavolus_sp2	Polyporaceae_sensu_lato	Polyporales	x		x	x									
OTU_2708	Phanerochaete_sp	Polyporaceae_sensu_lato	Polyporales												(x)	
OTU_2532	Phlebia_sp	Polyporaceae_sensu_lato	Polyporales				x			x	x	x	x	x	x	
OTU_1122	Polyporales_sp1	Polyporaceae_sensu_lato	Polyporales							x						
OTU_1318	Polyporales_sp2	Polyporaceae_sensu_lato	Polyporales												x	
OTU_212	Polyporales_sp3	Polyporaceae_sensu_lato	Polyporales							x	x	x	x	x		
OTU_378	Polyporales_sp4	Polyporaceae_sensu_lato	Polyporales							x	x	x	x	x		
OTU_939	Polyporales_sp5	Polyporaceae_sensu_lato	Polyporales							x	x	x	x			
OTU_294	Scytinostromella_sp	Polyporaceae_sensu_lato	Polyporales							x					x	
OTU_1460	Xenasmatella_sp	Polyporaceae_sensu_lato	Polyporales									x				
OTU_4919	Auriscalpium_sp	Auriscalpiaceae	Russulales												x	
OTU_101	Russulales_sp1	Russ_unknown_family	Russulales	x	x	x	x						x		x	
OTU_1140	Russulales_sp2	Russ_unknown_family	Russulales				x									

OTU	Name (based on query ID)	Minor Clade	Major Clade	HA	HB	OA	OB	HC	HD	SA	SI	MI	EL	PO	DM*	BF
OTU_236	Russulales_sp3	Russ_unknown_family	Russulales				x						x	x		
OTU_836	Russulales_sp4	Russ_unknown_family	Russulales												(x)	
OTU_480	Lactarius_sp	Russulaceae	Russulales		x	x										
OTU_1162	Russula_cremeirosea	Russulaceae	Russulales		x	x										
OTU_447	Russula_putida	Russulaceae	Russulales				x								x	
OTU_487	Russula_sp	Russulaceae	Russulales			x										
OTU_30	Stephanosporaceae_sp1	Stephanosporaceae	Russulales												x	
OTU_470	Stephanosporaceae_sp2	Stephanosporaceae	Russulales	x	x	x	x							x		
OTU_981	Efibulobasidium_sp	Sebacinaceae	Sebacinales												x	
OTU_1013	Helvellosebacina_concrescens	Sebacinaceae	Sebacinales												x	
OTU_506	Paulisebacina_allantoidea	Sebacinaceae	Sebacinales			x							x			
OTU_13646	Piriformospora_indica_sp1	Sebacinaceae	Sebacinales												x	
OTU_1599	Piriformospora_indica_sp2	Sebacinaceae	Sebacinales												x	
OTU_90	Piriformospora_indica_sp3	Sebacinaceae	Sebacinales			x			x						x	
OTU_1470	Sebacinaceae_sp1	Sebacinaceae	Sebacinales	x												
OTU_35	Sebacinaceae_sp2	Sebacinaceae	Sebacinales					x	x	x					x	x
OTU_558	Sebacinaceae_sp3	Sebacinaceae	Sebacinales									x	x			
OTU_607	Sebacinaceae_sp4	Sebacinaceae	Sebacinales					x	x							
OTU_8735	Sebacinaceae_sp5	Sebacinaceae	Sebacinales					x	x	x					x	
OTU_886	Sebacinaceae_sp6	Sebacinaceae	Sebacinales												(x)	
OTU_12273	Serendipita_vermifera_sp1	Sebacinaceae	Sebacinales					x	x				x		x	
OTU_174	Serendipita_vermifera_sp2	Sebacinaceae	Sebacinales							x	x	x	x	x	x	
OTU_407	Serendipita_vermifera_sp3	Sebacinaceae	Sebacinales							x	x	x	x	x	x	
OTU_879	Serendipita_vermifera_sp4	Sebacinaceae	Sebacinales							x	x	x	x	x		
OTU_9073	Serendipita_vermifera_sp5	Sebacinaceae	Sebacinales							x				x		
OTU_1083	Thelephorales_sp	Thel_unknown_family	Thelephorales		x	x									x	
OTU_557	Polyozellus_multiplex	Thelephoraceae	Thelephorales							x	x	x	x	x		

OTU	Name (based on query ID)	Minor Clade	Major Clade	HA	HB	OA	OB	HC	HD	SA	SI	MI	EL	PO	DM*	BF
OTU_127	Thelephoraceae sp1	Thelephoraceae	Thelephorales		x	x	x			x	x	x	x	x		
OTU_2226	Thelephoraceae sp2	Thelephoraceae	Thelephorales												(x)	
OTU_6583	Thelephoraceae sp3	Thelephoraceae	Thelephorales		x											
OTU_7654	Thelephoraceae sp4	Thelephoraceae	Thelephorales										x			
OTU_817	Thelephoraceae sp5	Thelephoraceae	Thelephorales			x										
OTU_1166	Tomentella_sp	Thelephoraceae	Thelephorales												(x)	
OTU_733	Hydnodontaceae_sp	Hydnodontaceae	Trechisporales					x								
OTU_821	Subulicystidium_sp	Hydnodontaceae	Trechisporales				x								x	
OTU_775	Trechispora_alnicola	Hydnodontaceae	Trechisporales												(x)	
OTU_186	Trechispora_sp1	Hydnodontaceae	Trechisporales												x	
OTU_390	Trechispora_sp2	Hydnodontaceae	Trechisporales							x	x		x			
OTU_321	Clitopilus_sp	Entolomataceae	Tricholomatoid		x	x									x	
OTU_10003	Entoloma_sp1	Entolomataceae	Tricholomatoid			x							x			
OTU_8677	Entoloma_sp10	Entolomataceae	Tricholomatoid		x			x	x	x					x	x
OTU_9891	Entoloma_sp11	Entolomataceae	Tricholomatoid					x								
OTU_10659	Entoloma_sp2	Entolomataceae	Tricholomatoid	x										x		
OTU_14213	Entoloma_sp3	Entolomataceae	Tricholomatoid	x	x	x	x	x	x	x	x			x	x	x
OTU_1467	Entoloma_sp4	Entolomataceae	Tricholomatoid	x							x				x	
OTU_1536	Entoloma_sp5	Entolomataceae	Tricholomatoid	x												
OTU_437	Entoloma_sp6	Entolomataceae	Tricholomatoid		x	x	x						x			
OTU_481	Entoloma_sp7	Entolomataceae	Tricholomatoid					x							x	
OTU_5724	Entoloma_sp8	Entolomataceae	Tricholomatoid					x								
OTU_84	Entoloma_sp9	Entolomataceae	Tricholomatoid												x	
OTU_64	Entolomataceae_sp1	Entolomataceae	Tricholomatoid					x	x	x					x	x
OTU_1093	Entolomataceae_sp2	Entolomataceae	Tricholomatoid		x								x	x		
OTU_1752	Pouzarella_sp1	Entolomataceae	Tricholomatoid								x					
OTU_683	Pouzarella_sp2	Entolomataceae	Tricholomatoid					x	x							

OTU	Name (based on query ID)	Minor Clade	Major Clade	HA	HB	OA	OB	HC	HD	SA	SI	MI	EL	PO	DM*	BF
OTU_8315	Richoniella_sp1	Entolomataceae	Tricholomatoid	x		x	x						x	x		
OTU_960	Richoniella_sp2	Entolomataceae	Tricholomatoid	x												
OTU_1315	Lyophyllaceae_sp1	Lyophyllaceae	Tricholomatoid	x												
OTU_784	Lyophyllaceae_sp2	Lyophyllaceae	Tricholomatoid	x	x	x	x	x		x	x	x	x	x		
OTU_5	Mycena_epiptygeria_sp1	Mycenaceae	Tricholomatoid			x	x			x	x	x	x	x	x	
OTU_9841	Mycena_epiptygeria_sp2	Mycenaceae	Tricholomatoid							x	x	x	x	x		
OTU_1164	Mycena_galopus	Mycenaceae	Tricholomatoid							x	x	x	x	x		
OTU_4223	Mycena_purpureofusca	Mycenaceae	Tricholomatoid								x	x	x	x		
OTU_14227	Mycena_sp1	Mycenaceae	Tricholomatoid										x			
OTU_19	Mycena_sp2	Mycenaceae	Tricholomatoid							x	x	x	x	x		
OTU_9988	Mycenaceae_sp	Mycenaceae	Tricholomatoid								x	x	x			
OTU_7123	Clitocybe_sp	Tricholomataceae	Tricholomatoid	x		x	x								x	x
OTU_820	Lepista_saeva	Tricholomataceae	Tricholomatoid			x										
OTU_7752	Resupinatus_sp1	Tricholomataceae	Tricholomatoid				x								x	
OTU_9999	Resupinatus_sp2	Tricholomataceae	Tricholomatoid		x										x	
OTU_1385	Tricholomataceae_sp1	Tricholomataceae	Tricholomatoid											x		
OTU_200	Tricholomataceae_sp2	Tricholomataceae	Tricholomatoid	x		x	x								x	x
OTU_47	Tricholomataceae_sp3	Tricholomataceae	Tricholomatoid		x			x	x	x					x	x

Appendix C Morphospecies of the 74 mushrooms found across 12 tallgrass prairie sites.

Key to sites: HA,B,C,D = FRS #23,32,27,28 Herb-Gray Parkway (Windsor); OA,B = Ojibway Prairie Area 1 and 2 (Windsor); SI = Silphium, SA = Sandpits, MI = Mike's field, EL = Eliza's prairie, PO = Pottawatomi (Walpole); DD = Dutton-Dunwich; MP = Mary & Peter's prairie, DM = DeMaere (Norfolk); BF = Blair Flats (Cambridge). Sites are ordered by geographic location, from west to east. Mushroomobserver numbers and “ref” are listed respectively to specimen code order. “ref” indicates the specimen was not posted but other specimens for this same species can be viewed for reference. “?” following species names indicates uncertain identifications, and following “ref” indicates uncertainty whether the specimen is truly the same species as the others.

Species	Spec Code(s)	Mushroom Observer.org/	HA	HB	OA	OB	HC	HD	SI	SA	MI	EL	PO	DD	MP	DM	BF
Agaricus campestris	MP7, PO15	231253, ref											x		x		
Agrocybe sp	MP1	215566													x		
Amanita sp	MP14	231251													x		
Arrhenia cf griseopallida	HA10, PO21	222131, 222132	x										x				
Astraeus hygrometricus	MP4	215730													x		
Atheniella cf flavoalba	PO24	222137											x				
Bovista cf pusilla	MP2	215568													x		
Bovista sp	PO13	215272											x				
Calvatia cyathiformis	MP12	231250													x		
Clavaria cf acuta	SA1	182133								x							
Clavaria cf fragilis	EL8	215241										x					
Clavaria sp (white)	BF1, BF2, SA7	182133 ref?								x							x
Clavulinopsis laeticolor	HA5	215748	x														
Clitocybe cf dealbata	MP13	236059													x		
Clitopilus cf scyphoides	SI11	215274							x								

Species	Spec Code(s)	Mushroom Observer.org/	H A	H B	O A	O B	H C	H D	S I	S A	M I	E L	P O	D D	M P	D M	B F
Coprinellus sp	SI4, BF5, BF6	215396, 215560, ref							x								x
Coprinellus sp (large)	PO9	215267											x				
Cortinarius sp	PO16, MP16	222130, ref											x		x		
Cotylidia cf diaphana	SI5	not posted							x								
Cotylidia undulata	DM3, DM10, DM11	215734,ref,ref														x	
Cuphophyllus pratensis group	EL12	222044										x					
Cuphophyllus virgineus	HA12, SI18	222046, 222045	x						x								
Cyathus stercoreus	DM4, SA2, SA6	215735, 182134, ref								x						x	
Entoloma excentricum/sericellum	PO4, PO7	182145, 215264											x				
Entoloma incanum	EL2, EL5, El6, PO2, PO3	182137, 140, 141, 143, 144											x	x			
Entoloma cf mougeotii	DD3, SI12, SI6	215744, ref, ref							x					x			
Entoloma sericeum	BF8, BF9, MP6, SI13	221924, ref...							x						x		x
Entoloma sp A (subgenus Alboleptonia)	EL1, HA6, PO1	182136, ref, 182142	x									x	x				
Entoloma cf tubaeforme	HA9, PO14, HB1	215751, 215255, ref	x	x									x				
Entoloma sp C (colours)	PO6	215259											x				
Entoloma sp D (dimpled)	SA5, HA4	215273, ref	x							x							
Entoloma sp E (Eccilia)	PO8	215265											x				
Entoloma sp F (subgenus Pouzarella)	SA3	182135								x							
Entoloma subserrulatum	EL9, EL10	215251, ref										x					
Galerina marginata	DD6	221929												x			
Hebeloma cf eburneum	SI14	182139 ref?							x								
Hebeloma cf incarnatulum	DM8, DM15, DM17	215740, ref, ref														x	
Hebeloma sp	EL4, MP10, SI19, PO17	182139, 231249, 235647, ref								x			x	x		x	
Hygrocybe conica group	DD2, PO12, PO20, SA9	215741, ref...								x			x	x			
Hygrocybe flavescens	HA8	215750	x														

Species	Spec Code(s)	Mushroom Observer.org/	HA	HB	OA	OB	HC	HD	SI	SA	MI	EL	PO	DD	MP	DM	BF
Hygrocybe glutinipes var rubra	HA7	222157	x														
Hygrocybe cf miniata	PO10, SI20	215269, 222161							x				x				
Inocybe sp	MP11	235645													x		
Laccaria sp	DM19	not posted														x	
Leccinum sp	DD5	221926												x			
Lycoperdon sp	MP5	215731													x		
Marasmiellus sp	PO11, BF3, HA1, HA3, HB3	215271, ref...	x	x									x				x
Mutinus caninus/elegans	DM16, DM2, DM1, EL13, PO22	221931, 215732, ref...										x	x			x	
Mycena sp (longstem)	HA13, SI16	222047, 222048	x						x								
Mycena sp (midpt)	HB2, SA10, SI15	215752, 231257, ref		x					x	x							
Mycena sp (white)	SI1, SI3, SI10, EL7, EL11, MP3	182131, 215394, ref...							x			x			x		
Mycena cf stylobates	DM5	222138														x	
Nidula candida	SA12	222166								x							
Omphalina rivulicola/pyxidata	DM7, DM12, DM14	215739, 221937, ref														x	
Parasola sp	HD1	215789						x									
Paxillus sp	HB8, HB5	222050, ref		x													
Peniophora versiformis	SA13	231260								x							
Phallus hadriani	PO23	221970											x				
Pholiotina sulcata	BF4	215557															x
Psathyrella ammophila	DM13, MP15	231256, 231252													x	x	
Ramariopsis/Clavulinopsis sp (branched)	HA11	222049	x														
Stropharia coronilla	MP8	231255													x		
Suillus americanus	DM20	221940														x	
Trametes sp	EL15, HB9	222035, 231248		x								x					
Trametopsis cervina	HB7	231247		x													

Species	Spec Code(s)	Mushroom Observer.org/	HA	HB	OA	OB	HC	HD	SI	SA	MI	EL	PO	DD	MP	DM	BF
Tricholoma sp	EL14	221963										x					
Tubaria cf furfuracea	DM6, DM9, SA4	215736, 215738, 182311								x						x	
Tubaria sp	SA11, SI17	221959, 231261							x	x							
Unknown sp (buttons)	SA14	not posted								x							
Unknown sp (fluffs)	DM18	221938														x	
Unknown sp (Marasmius/Mycena sp grass)	DD4, HB6, SI7	221925, ref...		x					x					x			
Unknown sp (soil crust polypore)	HB4	215753		x													
Unknown sp (tiny)	BF7	not posted															x
Vascellum sp	EL3, SI2, PO19, MP9	182138, 182132, 222038, ref								x			x	x		x	

Appendix D Estimated percent cover values for plants in areas surrounding sampling plots of 12 tallgrass prairie sites.

Plant names are presented as seven letter codes derived from their scientific names: the first four letters of the genus immediately followed by the last three letters of the epithet. Key to sites: HA,B,C,D = FRS #23,32,27,28 Herb-Gray Parkway (Windsor); OA,B = Ojibway Prairie Area 1 and 2 (Windsor); SI = Silphium, SA = Sandpits, MI = Mike's field, EL = Eliza's prairie, PO = Pottawatomi (Walpole); DM = DeMaere (Norfolk); BF = Blair Flats (Cambridge). Sites are ordered by geographic location, from west to east.

	HA	HB	OA	OB	HC	HD	SI	SA	EL	PO	DM	BF
AGRIPAR		2										
AGROGIG					1							
AGROSTO									2			
AMPHBRA								8				
ANDRGER			45		1		18.5			4.5	5	
ANTENEG							8		1			
BROMSPU			1									
CAREGRA	3	2										
CARESPP	10		2						3			
CAREVUL		2										
CHAMANG						1						
CIRSMUT									1			
CORNDRU	6											
CORNFOE									6			
CORYAME			1	3							1	
CYPERAC									6			
DAUCCAR			2		3.5	3						
DESMCAN					10	4					1	
DICHIMP		2										
ELYMCAN							1					
EQUIHYE										3.5	1	
FRAGVIR	2.5								2	6		
JUNCSPU		2										
LESPCAP					3		6				1	
LIATSPI										3		
MELIALB								7				
MONAFIS				2							5	5
MUHLMEX				6								
PANISPU			2		1							
PANIVIR									28	30.5		

	HA	HB	OA	OB	HC	HD	SI	SA	EL	PO	DM	BF
PHRAAUS		2					3					
PINUSTR											5	
POA ANN								4				
POPUDEL		2									1	
PTERAQU				2								
PYCNVIR			2	2					2	3.5		3
QUERMAC							4				1	
QUERRUB		2	2								1	
RUBUFLA	25		5									
RUBUIDA				7								
RUDBHIR			5				2.5				5	5
SALIBEB			2				1.5		1			
SALISPP		2										
SCHISCO										21	25	3
SETAVIR					1	1						
SILPTER							37.5					
SOLISPP	25	12	20	15	1	6	6	32	3	3.5	5	40
SORGNUT			14	60	35	45	16		6	4.5	5	12.5
SYMPERI					13.5					2		
SYMPNOV	1											
SYMPPRA		12			18	6						
SYMPSPP			2				9				5	
TANAVUL												3
TARAOFF											1	
TRIFHYB					15.5							
TRIFPRA						3		6				
TRIFREP								15				
ULMURUB	2.5											
VIOLCUC										1.5		
ZIZIAUR							3.5					

Curriculum Vitae

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	Telfer, A. C., M. R. Young, J. Quinn, […], C. Hay, […], and J. R. deWaard. 2015. Biodiversity inventories in high gear: DNA barcoding facilitates a rapid biotic survey of a temperate nature reserve. Biodiversity Data Journal (3): e6313. doi: 10.3897/BDJ.3.e6313
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