COMMUNITY DYNAMICS OF ARBUSCULAR MYCORRHIZAL FUNGI IN A TEMPERATE TREE-BASED INTERCROPPING SYSTEM

A Thesis

Presented to

The Faculty of Graduate Studies

of

The University of Guelph

By

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In partial fulfilment of requirements

For the degree of

Doctor of Philosophy

September, 2011

ABSTRACT

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Arbuscular mycorrhizal (AM) fungi are an important component of agricultural ecosystems, and can directly influence the productivity of these systems. Unfortunately, conventional agricultural practices have been shown to adversely affect AM fungi. The use of more ecologically sustainable agricultural practices such as tree-based intercropping (TBI) may have the potential to reduce the negative impact of agricultural practices on AM fungi. The objectives of this thesis were to determine (1) if trees influence the structuring of AM fungal communities, (2) if TBI systems support a more diverse AM fungal community compared to conventional monocropping (CM) systems, and (3) if differences in AM fungal richness and composition between the two cropping systems have a functional effect on the growth of crops.

Molecular analysis of the AM fungal community in the TBI system revealed 17 phylotypes that all belonged to the family Glomeraceae. Differences in richness and composition among the treatments indicated that trees had an effect on the structuring of AM fungal communities. Intercropping alleys adjacent to white ash and poplar tree rows had a significantly \( P < 0.05 \) richer and different AM fungal community compared to intercropping alleys adjacent to Norway spruce tree rows. When comparing TBI and CM systems, AM fungal abundance was not significantly \( P > 0.05 \) different between the two
cropping systems. However, differences in both richness and community composition of AM fungi were observed between the two cropping systems. The TBI system had a significantly higher AM fungal richness and contained several taxa not found in the CM system. Controlled greenhouse experiments revealed that differences in AM fungal richness and community composition between the TBI and CM systems had no functional effect on the growth of three crops (i.e. barley, canola, and soybean). The similar growth response of crops to AM fungi from the two cropping systems may be due to the lack of functional complementarity among the AM fungi. Overall, the TBI system had a more diverse AM fungal community compared to the CM system and trees appear to be a significant factor in the structuring of these communities.
ACKNOWLEDGEMENTS

Funding for this research was provided by a strategic grant and a graduate scholarship from the Natural Sciences and Engineering Research Council of Canada; by graduate scholarships from the Government of Ontario and the University of Guelph; and a travel award from the A.D. Latornell Foundation.

I would like to thank several people for their help during my time at the University of Guelph including my co-advisors (John Klironomos and Andrew Gordon) and advisory committee (Naresh Thevathasan, and Matthias Rillig) and the Klironomos Lab group including John Klironomos, Alexander Koch, Ben Sikes, Brian Ohsowski, Miranda Hart, Pedro Antunes, and Kevin Courtney. Several people at the University of Guelph provided lab space, equipment, and assistance necessary for the molecular analyses including Brian Husband, Teri Crease, Steve Newmaster, Ben Sikes, and the Genomics Facility Staff (Angela Holliss, Jeff Gross, and Jing Zhang). Mohammed Idris and the University of Guelph Laboratory Services provided assistance with the soil chemical analyses. I would like to thank Alexander Koch for his assistance with the statistical analyses in this thesis and other projects. Many work study, co-op students, and volunteers in the Klironomos Lab assisted with the set-up and harvest of the greenhouse experiments. Finally, a special thank you to Jillian for her help collecting samples in the field, reading manuscripts, and insightful discussions. I have enjoyed our collaborations together both in research and in life.
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CHAPTER ONE:

INTRODUCTION:

ARBUSCULAR MYCORRHIZAL FUNGI IN TREE-BASED INTERCROPPING SYSTEMS: A REVIEW OF THEIR ABUNDANCE AND DIVERSITY

Part of this chapter is published in *Pedobiologia* (2011) 54: 57-61.
Introduction

Environmental concerns associated with conventional agriculture have led to a recent trend toward the adoption of more sustainable and diverse agricultural practices. Over the past century, conventional agriculture has successfully improved the efficiency and productivity of annual crop production. However, this has come at a high cost as it relies heavily on chemical input (e.g. fertilizers and pesticides) and intensive crop production practices. Negative impacts on the environment include soil erosion, contamination of groundwater, release of greenhouse gases, and eutrophication of freshwater and marine ecosystems (Tilman, 1998; Pimentel et al., 2005). Alternative land use strategies such as agroforestry have been suggested as a more sustainable land use practice and a way to reduce the harmful effects of conventional agriculture. Agroforestry systems provide a number of ecological advantages through increased carbon sequestration, biodiversity conservation, and improved water quality (Thevathasan and Gordon, 2004).

Tree-based intercropping

Agroforestry is a term used to describe land-use systems where woody perennials are managed on the same unit of land as agricultural crops, animals or both (ICRAF, 2010). There are various forms of agroforestry systems including windbreaks, silvopastoral, alley cropping, riparian forestry, and forest farming (Williams et al., 1997). This review will primarily focus on the alley cropping form of agroforestry, also referred to as tree-based intercropping (TBI). TBI involves growing agricultural crops in alleys situated between rows of woody perennials (e.g. trees or shrubs) (Jose et al., 2000). This
arrangement of trees and alley crops enables the use of standard farm equipment and practices while gaining the benefits of incorporating trees into the agricultural system (Williams et al., 1997). The success of TBI systems relies on proper design and implementation, in order to optimize positive interactions while minimizing negative interactions between the tree and crop components (Jose et al., 2000; Thevathasan and Gordon, 2004). Similar to most ecosystem processes, the productivity of TBI systems is a net result of the positive and negative interactions among the components (Jose et al., 2004).

There are several key benefits that can result from a properly designed TBI system. The incorporation of tree rows into the agricultural landscape can provide a windbreak, thereby reducing wind speed and subsequently reducing evaporative stress (Jose et al., 2004). They also promote greater earthworm populations compared to conventionally cropped systems, which are important components of maintaining and improving soil structure and stability (Price and Gordon, 1999). Trees not only increase the plant diversity of the site but increase the niche diversity thereby increasing biodiversity conservation (Stamps and Linit, 1998). For example, Williams et al. (1995) found that intercropped areas had a greater number of birds nesting and foraging compared to a monocropped field. In addition, intercropped systems have been shown to have a greater abundance of arthropods and detritivores, and support a higher ratio of parasitoids to herbivores (Thevathasan and Gordon, 2004; Middleton, 2001). This may reduce the need for pest management as trees can provide a habitat that will promote the establishment of natural enemies (Thevathasan and Gordon, 2004; Stamps and Linit, 1998).
In contrast, the addition of trees into an agricultural system can result in negative interactions (e.g. competition) between the trees and annual crops. For example, one of the primary limiting factors in temperate TBI systems is the competition for light (Chirko et al., 1996). Simpson (1999) found that competition for light was the main factor that reduced the crop yield in a temperate agroforestry site compared to a conventional system, and that C4 crops (e.g. *Zea mays* L.) were more vulnerable than C3 crops (e.g. *Glycine max* L. Merr.) to shading.

Within TBI systems there are also inevitable belowground interactions that occur due to the high density of tree roots found within the same region as the crop roots (Jose et al., 2000; Jose et al., 2004). The deep roots of trees can act as a ‘safety net’ by capturing nutrients that leach below the rooting zone of the alley crops and recycle them back into the system (Allen et al., 2004). As a result, inorganic fertilizer rates can be reduced in TBI systems, which in addition to the reduced nutrient leaching can decrease the amount of nitrous oxide emissions compared to conventional agricultural systems (Thevathasan and Gordon, 2004). Another major factor is the below-ground competition for water since the highest concentration of tree roots is situated in the same area as the crop roots (Jose et al., 2004). As a result, competition for belowground resources such as water can become one of the limiting factors in the productivity of temperate TBI systems (Jose et al., 2000). The interaction between tree and crop roots can also have an effect on soil organisms, which play an essential role in the functioning and productivity of agroecosystems. These include arbuscular mycorrhizal (AM) fungi which associate with the roots of most tree and crop species in agroforestry systems.
AM fungi

AM fungi are an important component of natural and agricultural ecosystems, as they form symbiotic associations with most terrestrial plant roots. AM fungi are characterized by fungal structures (arbuscules, vesicles, hyphae) that form in the host root and extra-radical mycelium found in the soil (Smith and Read, 2008). They belong to the phylum Glomeromycota (Schussler et al., 2001) with 214 described species based on spore morphology (Schussler et al., 2009). Fossil and molecular evidence estimate that AM fungi date back to approximately 400 to 600 million years ago and played a critical role in the early establishment of land plants (Remy et al., 1994; Redecker et al., 2000). They are believed to be asexual and colonization of roots can occur via spores, hyphae, or infected root fragments (Klironomos and Hart, 2002).

AM fungi are obligate biotrophs and the symbiosis formed between the host plant and fungal partner is normally mutualistic (Smith and Read, 2008). However, evidence does suggest that the symbiosis can range from parasitic to mutualistic depending on the host plant and AM fungal species involved (Klironomos, 2003). The mycorrhizal association involves the transfer of mineral nutrients from the fungus to the host plant in exchange for carbon (Smith and Read, 2008). In addition to increased access to mineral nutrients, AM fungi provide several other benefits to host plants including tolerance to drought (Auge, 2001) and heavy metals (Joner et al., 2000; Khan et al., 2000), and protection from pathogens (Newsham et al., 1995) and herbivores (Gange and West, 1994). AM fungi have also been shown to influence plant diversity and community structure (Grime et al., 1987; van der Heijden et al., 1998; Bever et al., 2001; Klironomos, 2003; van der Heijden et al., 2006), promote seedling establishment (van der Heijden,
2004) and influence plant invasions (Pringle et al., 2009). As a result, AM fungi can have a significant effect in agricultural systems by improving crop growth and productivity.

**AM fungi in agroecosystems**

From an agroecological perspective, conventional agricultural practices strongly influence the abundance and community composition of AM fungi in temperate regions. In general, arable fields have a low taxonomic diversity of AM fungi (Helgason et al., 1998), and there appears to be an inverse relationship between management intensity and AM fungal species richness (Oehl et al., 2003; Hijri et al., 2006). Common agricultural practices such as tillage, fallow, and fertilization have been shown to alter the community structure of AM fungi (Jansa et al., 2002; Troeh and Lynachan, 2003; Jumpponen et al., 2005). This shift in AM fungal community composition could be due to a number of factors including disturbance of AM fungal hyphal networks, changes in soil nutrient content, altered microbial activity, or changes in weed populations (Jansa et al., 2003). Management strategies that require lower inputs such as organic farming tend to have a greater AM fungal diversity (Oehl et al., 2004). Overall, conventional agricultural practices appear to decrease the abundance of AM fungi and cause a shift in species composition toward species that are inferior mutualists (Douds and Millner, 1999; Johnson, 1993).

Utilizing more sustainable management practices such as TBI has the potential to ameliorate the negative impact agricultural practices have on AM fungi. Increasing the diversity of a field by the incorporation of trees could potentially have a positive influence on AM fungal communities. For example, Burrows and Pfleger (2002) found that
increasing plant diversity had a positive effect on AM fungal sporulation and community composition. In addition, Helgason et al. (1998) found that woodlands had a much higher AM fungal species richness and diversity compared to agricultural fields. AM fungi seem to benefit from increased plant diversity due to the higher number of possible host-fungal pairings, and increased density of plant roots available for colonization (Burrows and Pfleger, 2002). In return, a higher diversity of AM fungi has been shown to increase plant productivity (van der Heijden et al., 1998).

**AM fungi in TBI systems**

The influence of temperate agroforestry systems on AM fungi is not well understood as only two studies to date have investigated AM fungi in these systems. However, due to the higher adoption rate of TBI systems in tropical regions, there have been several studies that have investigated the influence of these systems on the abundance and diversity of AM fungi. The remainder of this review will summarize and synthesize the results from studies that have investigated AM fungi in temperate and tropical TBI systems (Table 1.1). In addition, we will discuss potential paths for future research to further our understanding of AM fungi in TBI systems.

**AM fungi in temperate TBI systems**

The adoption of TBI systems in temperate regions has been minimal, which can likely be attributed to the financial costs associated with incorporating trees into an agricultural system and subsequent loss of arable land. With the limited number of TBI sites in temperate regions there are only two published studies that have investigated the
Table 1.1. Effect of tree-based intercropping (TBI) systems on arbuscular mycorrhizal (AM) fungal abundance and diversity as reported by studies in temperate and tropical regions

<table>
<thead>
<tr>
<th>Reference</th>
<th>Region</th>
<th>Effect</th>
<th>AM fungal measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacombe et al., 2009</td>
<td>Temperate</td>
<td>Positive</td>
<td>PFLA (abundance)</td>
</tr>
<tr>
<td>Chifflot et al., 2009</td>
<td>Temperate</td>
<td>Positive</td>
<td>Molecular diversity (RFLP), spore density, root colonization</td>
</tr>
<tr>
<td>Pande &amp; Tarafdar, 2004</td>
<td>Tropical</td>
<td>Positive</td>
<td>Spore density, root colonization</td>
</tr>
<tr>
<td>Prasad and Mertia, 2005</td>
<td>Tropical</td>
<td>Positive</td>
<td>Spore density, root colonization</td>
</tr>
<tr>
<td>Mutabaruka et al., 2002</td>
<td>Tropical</td>
<td>Positive</td>
<td>Spore density, root colonization</td>
</tr>
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<td>Muleta et al., 2008</td>
<td>Tropical</td>
<td>Positive</td>
<td>Spore density</td>
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<td>Muleta et al., 2007</td>
<td>Tropical</td>
<td>Positive</td>
<td>Spore density</td>
</tr>
<tr>
<td>Boddington &amp; Dodd,</td>
<td>Tropical</td>
<td>Neutral, positive</td>
<td>Spore density and richness, root colonization</td>
</tr>
<tr>
<td>Kumar et al., 2007</td>
<td>Tropical</td>
<td>Neutral, positive</td>
<td>Root colonization</td>
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<td>Cardoso et al., 2003</td>
<td>Tropical</td>
<td>Neutral</td>
<td>Spore density</td>
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<td>Leal et al., 2009</td>
<td>Tropical</td>
<td>Negative</td>
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<td>Jefwa et al., 2006</td>
<td>Tropical</td>
<td>Negative</td>
<td>Spore diversity</td>
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</table>
influence of TBI systems on AM fungi. Both studies found a positive effect of TBI systems on AM fungi. Using phospholipid fatty acids profile analysis, Lacombe et al. (1999) found a higher abundance of AM fungi in two different TBI sites (St. Remi, Quebec and Guelph, Ontario) compared to adjacent conventional monocropping sites. At the St. Remi TBI site, soybean was grown between rows of hybrid poplar (Populus sp.), black walnut (Juglans nigra L.), and white ash (Fraxinus americana L.) trees, and at the Guelph TBI site, crops were grown between rows of black walnut and white ash. In another study at the St. Remi site, Chifflot et al. (2009) found that AM fungal diversity was higher and spatial distribution of spores was significantly different in a TBI site compared to a monoculture forest plantation system. Poplar (Populus nigra L. x P. maximowiczii cv. A. Henry ‘Max 5’) trees, which associate with AM and ectomycorrhizal fungi had a higher diversity of AM fungi associating with their roots in the TBI site compared to the forest plantation system (Chifflot et al., 2009). Although the total spore abundance was similar within both systems, poplar trees had a significant effect on the spatial distribution of spores in the cropping alleys of the TBI system (Chifflot et al., 2009). However, based on these two studies it is not clear how the diversity or composition of AM fungi in TBI systems compares to conventional monocropping systems. Due to the importance of AM fungi in agroecosystems further research is warranted on this topic.

**AM fungi in tropical TBI systems**

There are several studies that have investigated the influence of tropical agroforestry systems on the abundance and diversity of AM fungi. These investigations
have found that TBI can have a positive effect on the AM fungal community, but there are cases where no significant effect or even a negative effect was observed. This may be due to the variety of locations and climates where these studies took place and the diverse combinations of tree and crop species used within these systems. However, these studies provide insight into the effect that TBI systems can have on AM fungi.

There is evidence that tropical agroforestry systems support a diverse and abundant community of AM fungi. For example, two studies conducted in India found a positive influence of trees on spore abundance and AM fungal colonization of roots collected from the rhizosphere under tree canopies compared to samples collected outside tree canopies (Pande and Tarafdar, 2004, Prasad and Mertia, 2005). A survey of AM fungi in neem (Azadirachta indica L.)-based agroforestry systems revealed a higher density of spores in samples collected from under tree canopies compared to samples collected 25m from trees (Pande and Tarafdar, 2004). Neem trees, which are highly AM-dependent, had a higher level of root colonization compared to the crop and was likely responsible for the increased spore abundance in the rhizosphere under the tree canopies (Pande and Tarafdar, 2004). A similar pattern was observed by Prasad and Mertia (2005) who found higher spore abundance and root colonization under the canopies of three agroforestry tree species that associate with AM fungi (Azadirachta indica, Prosopis cineraria (L.) Druce, and Tecoma undulata (Smith)) and three tree species that associate with AM and ectomycorrhizal fungi (Acacia tortilis (Forsk.) Hayne, Acacia nilotica (L.) Willd. ex Del., and Eucalyptus camaldulensis Dehn.) compared to beyond the tree canopies (12-15m from trees). In addition, there was significant variability in the number of spores and percent root length colonization in the rhizosphere associated with each of the six tree
species, but this did not appear to be correlated with the mycorrhizal status of the agroforestry tree species (Prasad and Mertia, 2005). Similarly, Mutabaruka et al. (2002) found that tree species (*Melia volkensii* Gurke, *Senna spectabilis* (DC.) Irwin & Barneby, *Gliricidia sepium* (Jacq.) Kunth ex Walp, *Leucaena leucocephala* (Lam.) de Wit) in agroforestry systems in Kenya had a variable effect on the abundance of AM fungal spores, with *M. volkensii*, *G. sepium*, and *L. leucocephala* having higher spore populations than monoculture plots. All four tree species were colonized by AM fungi and a significant correlation between levels of tree root colonization and spore abundance was observed (Mutabaruka et al., 2002).

Shade trees in agroforestry coffee (*Coffea arabica* L.) systems also appear to have an effect on AM fungal spore populations compared to monocultural coffee systems. Muleta et al. (2008) investigated the influence of ten shade tree species on the density of AM fungal spores in agroforestry coffee systems compared to monocultural coffee systems. They found significantly higher spore densities in agroforestry coffee systems compared to monocultural coffee systems, and the highest spore populations were found in the top soil layer (0-30cm). In addition, there were variations in the spore abundance among the different shade trees with leguminous tree species (*Albizia* and *Acacia* species) maintaining a higher abundance of spores compared to *Ficus* species, which were not significantly different from monocultural systems. This effect may be attributed to the mycorrhizal associations of the tree species as *Ficus* species are typically less mycorrhizal dependent compared to leguminous tree species, which in turn likely impacts the AM fungal populations in the rhizosphere (Plenchette et al. 2005). This effect was observed by Muleta et al. (2007) as they found higher AM fungal spore populations in the rhizosphere
of coffee plants under leguminous shade trees compared to non-leguminous shade trees. Cardoso et al. (2003) also found that the upper soil layers contained the highest spore densities in agroforestry and monocultural coffee systems. However, monocultural coffee systems had higher densities of spores in the upper soil layers and lower spore densities in the deeper soil layers compared to agroforestry coffee systems. This appeared to be due to the higher abundance of roots in the deeper soil layers in the agroforestry coffee system, which may enable these systems to maintain a higher abundance of AM fungal spores in the deeper soil layers.

In contrast to these previous studies, there is evidence that some TBI systems may have no effect or in some cases a negative effect on the AM fungal community compared to monoculture systems despite utilizing tree species that associate with AM fungi. For example, Boddington and Dodd (2000) found no significant difference between AM fungal spore density and species richness in a *Peltophorum dasyrachis* Kurz ex Baker and maize (*Zea mays* L.) agroforestry system compared to a maize monoculture. However, they did find a higher concentration of extra-radical mycelium in the agroforestry system. A comparison of two agroforestry systems (*Sesbania macrantha* Welw. ex E. Phillips & Hutch and *Sesbania sesban* (L.) Merr.) intercropped with maize and a maize monoculture found that AM fungal species diversity did not differ significantly between the two agroforestry systems, but the *S. macrantha* agroforestry system had a lower species diversity compared to the maize monoculture (Jefwa et al., 2006). Kumar et al. (2007) reported that AM fungal colonization of crops was significantly higher in agroforestry systems compared to local farmer’s fields (monocultures) for twenty-four tree-crop combinations during the rainy season. However, during the winter season, mycorrhizal
colonization was only higher in five tree-crop combinations, and not significantly different in the other fourteen combinations (Kumar et al. 2007). In addition, an evaluation of seven different land use systems found that agroforestry sites and forest sites had the lowest AM fungal spore abundance, considerably lower than samples collected from pastures and cropped land (Leal et al., 2009).

Overall, incorporating trees into agricultural systems can have a range of effects on the AM fungal community. The limited information we have in temperate systems suggests that AM fungal communities are more abundant and diverse in TBI systems compared to monocultures. In tropical regions, TBI systems generally had a positive effect on AM fungi, however, in some cases there was no effect or a negative effect of TBI systems on AM fungi compared to monoculture systems. The variable effect of TBI systems on AM fungi observed in these studies may be a function of the different cultivation techniques, climatic variation, or diverse tree-crop combinations used within these TBI systems. However, it is unclear how this in turn influences the mycorrhizal community that associates with the alley crops and whether this change is functionally beneficial to the crop.

**Objectives**

As AM fungi are important components of agricultural ecosystems and there is limited information about these symbiotic fungi in temperate TBI systems, the goal of this thesis was to increase our understanding of how incorporating trees into temperate agricultural landscapes can influence their composition. In particular, whether TBI systems can help to ameliorate or reverse the negative impact of conventional agricultural
practices on AM fungi. Early indications show that TBI may have a positive effect through increased abundance and diversity on AM fungi (Lacombe et al., 2009; Chifflet et al., 2009). However, there are numerous potential tree-crop combinations that can be employed in temperate TBI systems and their effect on AM fungi may be highly variable. Tree species that are currently planted in or have been proposed for temperate TBI systems have varying mycorrhizal associations. Many of the valuable hardwood trees (e.g. Juglans, Acer, and Fraxinus species) associate with AM fungi, while most coniferous trees (e.g. Picea and Pinus species) associate with ectomycorrhizal fungi, and trees commonly grown as energy crops (e.g. Populus and Salix species) associate with both types of mycorrhizal fungi (Malloch and Malloch, 1981; 1982). Understanding how tree species with varying mycorrhizal associations influence the AM fungal community in TBI systems will enable better system design with regards to choosing appropriate tree-crop combinations that promote beneficial AM fungal communities.

In Chapter 2, a spatially explicit approach was used to assess the influence of three tree species with varying mycorrhizal associations on the AM fungal community composition in a temperate TBI system. The tree species included white ash (Fraxinus americana L.), Norway spruce (Picea abies L. Karst.), and hybrid poplar (Populus deltoides x nigra DN-177). We hypothesized that AM fungal taxa will differ in both richness and composition in cropping alleys adjacent to tree rows occupied by tree species that associate with AM fungi, as compared to cropping alleys adjacent to tree species that do not associate with AM fungi.

In Chapter 3, another field study was conducted in order to compare the effects of TBI and conventional monocropping (CM) systems on AM fungi. We aimed to determine
whether TBI systems promote more abundant and diverse AM fungal communities compared to CM systems. Additionally, sampling occurred throughout the growing season to determine whether AM fungal communities vary temporally during the growing season in these two different cropping systems.

While it is valuable to understand how TBI influences AM fungal communities within these systems, it is unclear how these changes affect crop productivity. In Chapter 4, we used two successive greenhouse experiments to determine whether compositional differences among AM fungal communities in the TBI and CM systems have a functional effect on the growth of crops (i.e. barley, canola, and soybean). As TBI systems harbour a more diverse soil microbial and AM fungal community compared to CM systems (Lacombe et al., 2009; and Chapter 3), we hypothesized that crops would have an increased growth response to soil microbes and specifically to AM fungi from the TBI system.

In Chapter 5, the results from these chapters are synthesized and I present the overall conclusions drawn from these studies along with future areas of research in this field.
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CHAPTER TWO:

INFLUENCE OF TREES ON THE SPATIAL STRUCTURE OF ARBUSCULAR
MYCORHIZAL COMMUNITIES IN A TEMPERATE TREE-BASED
INTERCROPPING SYSTEM

In Press in *Agriculture, Ecosystems & Environment*
Abstract

Tree-based intercropping (TBI) is an ecologically sustainable agricultural practice that may promote a more diverse arbuscular mycorrhizal (AM) fungal community compared to conventional systems, but the influence of the dynamics of these systems on AM fungi has not been established. Soil and root samples were collected in the intercropping alleys along transects perpendicular to tree rows occupied by white ash (*Fraxinus americana*), poplar (*Populus deltoides x nigra*), Norway spruce (*Picea abies*), and rows without trees (control). Molecular analysis of the AM fungal community at the TBI site revealed 17 phylotypes belonging to the Glomeraceae. Overall, the AM fungal community at the TBI site was comparable to other conventional agricultural systems; with the majority of phylotypes belonging to *Glomus* group A. AM fungal phylotype richness and community composition significantly differed among the treatments at the TBI site. AM fungal communities were more diverse in cropping alleys adjacent to trees that associate with AM fungi than trees that do not associate with AM fungi. Norway spruce had a negative influence on the AM fungal community as tree rows and bordering intercropping alleys had a significantly lower phylotype richness and different community composition. These results suggest that to maintain a diverse AM fungal community throughout TBI systems, it may be best to incorporate tree species that associate with AM fungi.
Introduction

Tree-based intercropping (TBI) is an alternative agricultural practice that promotes increased diversity and sustainability compared to conventional farming (Thevathasan and Gordon, 2004). TBI systems involve the incorporation of trees into the same land management unit as agricultural crops. In addition to increased plant diversity, properly designed TBI systems provide a number of other benefits compared to conventional agriculture such as a reduction in wind speed and evaporative stress (Jose et al., 2004), improved soil structure and stability (Price and Gordon, 1999), increased biodiversity conservation (Stamps and Linit, 1998), and both carbon sequestration and reduction in greenhouse gases (Thevathasan and Gordon, 2004). Despite these many ecological benefits, the adoption of TBI systems in North America has been minimal, which is likely attributed to the perceived loss of arable land and financial costs associated with incorporating trees into an agricultural system.

In TBI systems, the deep roots of trees act as a ‘safety net’ by capturing and recycling nutrients that leach below the rooting zone of the alley crops (Jose et al., 2004; Dougherty et al., 2009). However, the majority of tree roots occupy the same region as the crop roots within the top 30 cm of soil, resulting in competition for belowground resources such as water (Jose et al., 2000; Jose et al., 2004). In addition, the presence of trees can modify the microclimate within cropping alleys, which may alter microbial communities (Mungai et al., 2005). Fungi and bacteria within TBI systems are strongly influenced by trees as fungal and bacterial biomass decrease with increasing distance from the tree rows (Seiter et al., 1999). The interaction between tree and crop roots may also have an effect on arbuscular mycorrhizal (AM) fungi, which can play an important role in
the functioning and productivity of agroecosystems (Plenchette et al., 2005). However, little is known about the influence of temperate TBI systems on AM fungal communities, but early evidence suggests that TBI systems promote a higher abundance of AM fungi compared to conventional monocropping systems (Lacombe et al., 2009; Bainard et al., 2011a).

AM fungi are obligate symbiotic fungi that form a mutualistic association with most vascular plants. In return for carbon from the host plant, AM fungi can improve access to nutrients (Smith and Read, 2008), increase drought tolerance (Auge, 2001), and provide protection from pathogens (Newsham et al., 1995). Utilizing management practices that promote AM fungi can improve phosphorus uptake, growth, and grain yield of crops that associate with AM fungi (Arihara and Karasawa, 2000; Karasawa et al., 2002). However, the high use of synthetic chemicals (e.g. fertilizers and pesticides) and intensive land management practices common in conventional agriculture can lead to decreased AM fungal diversity and abundance (Douds and Millner, 1999). Sustainable land use practices such as TBI may be an effective strategy to reduce the negative impact of agricultural practices on AM fungi.

Tree species that are currently planted in or have been proposed for temperate TBI systems have varying mycorrhizal associations. Many of the valuable hardwood trees (e.g. *Juglans*, *Acer*, and *Fraxinus* species) associate with AM fungi, while most coniferous trees (e.g. *Picea* and *Pinus* species) associate almost exclusively with ectomycorrhizal fungi, and trees commonly grown as energy crops (e.g. *Populus* and *Salix* species) associate with both types of mycorrhizal fungi (Malloch and Malloch, 1981; 1982). The objective of this study was to determine if tree species with varying mycorrhizal
associations influence the AM fungal community composition within a temperate TBI system. The tree species included white ash (Fraxinus americana L.), Norway spruce (Picea abies L. Karst.), and hybrid poplar (Populus deltoides x nigra DN-177). We hypothesized that AM fungal taxa will differ in both richness and composition in cropping alleys adjacent to tree rows occupied by tree species that associate with AM fungi, as compared to cropping alleys adjacent to tree species that do not associate with AM fungi.

**Materials and methods**

*Site and experimental design*

The University of Guelph Agroforestry Research Station is a long-term TBI research site established in 1987 on 30 hectares of agricultural land in Guelph, Ontario, Canada. The soil within the site is classified as a Gray Brown Luvisol with a sandy-loam soil texture (65% sand, 25% silt and 10% clay; Order: Alfisols, group: Typic Hapludalf) (Oelbermann and Voroney, 2007). A detailed analysis of the soil physico-chemical properties at the site can be found in Lacombe et al. (2009). Hardwood and coniferous tree species were planted and annually intercropped with corn (Zea mays L.), soybean (Glycine max L. Merr.), and winter wheat (Triticum aestivum L.) using no-till cultivation. Tree density is 111 trees ha⁻¹ and species include silver maple (Acer saccharinum L.), white ash, hazelnut (Corylus avellana L.), black walnut (Juglans nigra L.), Norway spruce, hybrid poplar, red oak (Quercus rubra L.), black locust (Robinia pseudoacacia L.), willow (Salix discolor Muhl.), and white cedar (Thuja occidentalis L.). Tree rows are spaced 12.5 m or 15 m apart, and trees within a row are spaced 3 m or 6 m apart. Tree rows are 2 m wide and occupied by a diverse weed community throughout the TBI site.
The experimental design was a randomized complete block design with four blocks and four TBI treatments. The TBI treatments consisted of rows of at least five successive trees of (i) white ash, (ii) hybrid poplar, or (iii) Norway spruce trees, or (iv) rows without trees (control) within each block. Two random trees or points (control rows) were sampled for each treatment in all blocks. Samples were collected within the tree row and along a transect perpendicular to the tree row at 0.5, 3.0, and 5.5 m into the intercropping alley. Subsamples were collected on both sides of the tree row and on either side of the tree at the edge of the tree canopy within the tree row. This resulted in a total of 256 sampling locations in the TBI site in which soil cores and fine roots samples were collected from. These included 16 sampling locations in the tree rows (4 blocks x 2 replicate trees x 2 directions) and 48 sampling locations in the intercropping alleys (4 blocks x 2 replicate trees x 3 distances x 2 directions) for each TBI treatment. Soybean was planted in the intercropping alleys of the TBI site in May 2007, and sampling took place in August 2007.

**Sampling**

Soil cores were collected to a depth of 20 cm using a 3 cm diameter soil corer and placed into sterile bags. Soil cores collected from the same distance on both sides of the tree row were pooled, homogenized, and stored at -80°C for molecular analysis. Fine root samples were collected from soybean and tree roots to a depth of 20 cm and placed into sterile bags. Tree roots were collected within the tree row at the edge of the tree canopy on either side of the tree. In the control treatment (rows without trees), fine roots were collected from the plants (i.e. weeds) growing in 10 cm² plots located 1.5 m on either side.
of the sampling point in the row. Soybean root samples were collected at 0.5, 3.0, and 5.5 m into the intercropping alley, on both sides of the tree rows. The fine root samples were washed with tap water, lyophilized using a freeze dryer (48 h), and stored at -80°C for molecular analysis.

**DNA extraction and PCR amplification**

The AM fungal community composition in the site was determined by T-RFLP analysis of the FLR3/FLR4 fragments of the LSU rRNA gene (Gollotte et al., 2004). Genomic DNA was extracted from 0.25 g soil samples using a PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, USA). Lyophilized root tissue samples (30 mg) were homogenized by placing them in 2 ml microcentrifuge tubes with a 3 mm ceramic bead and shaking them for 30 s using a Fast-Prep. Genomic DNA was then extracted from the pulverized root tissue samples using a NucleoSpin Plant purification kit (Macherey-Nagel Inc., Bethlehem, USA). Total DNA concentration in each soil and root sample was then quantified spectrophotometrically using a NanoDrop ND-1000 (NanoDrop, Wilmington, USA).

A nested PCR approach was used to amplify AM fungal DNA in the soil and root sample extracts. The first PCR used the primer pair LR1 and FLR2 to amplify the fungal DNA (van Tuinen et al., 1998; Trouvelot et al., 1999). The second PCR used the AM fungal specific 5’-labelled primer pair FLR3-FAM and FLR4-VIC (Gollotte et al., 2004). The 10 µl reaction mixtures for both PCR rounds consisted of 0.5 units Platinum Taq DNA polymerase, 1.5 mM MgCl₂, 0.2 mM deoxynucleoside triphosphate, 0.5 µM of each primer (LR1/FLR2 or FLR3-FAM/FLR4-VIC), 0.5 units BSA, and 100 ng of soil or root
extracted template DNA, or 1 µl of diluted (1:100) PCR product for the second PCR. The cycling parameters for both rounds of amplification included an initial denaturing step for 2 min at 94°C, 35 cycles consisting of 1 min at 93°C, 1 min at 58°C and 1 min at 72°C, followed by a final extension step of 72°C for 10 min.

Terminal restriction fragment length polymorphism (T-RFLP) analysis

PCR products were purified using the Qiaquick PCR Purification kit (Qiagen, Mississauga, Canada) and digested with the restriction enzymes MboI and AluI in separate reactions. The digestion reactions consisted of 5 µl purified PCR product and 1 unit MboI or AluI in the buffer recommended by the manufacturer. The reactions were incubated at 37°C for 6 hours, followed by a heat inactivation step at 65°C for 10 min. The size of the terminal restriction fragments in each sample was determined using an ABI 3730 DNA analyzer (Applied Biosystems, Carlsbad, USA). Fragment data was analyzed using Genemapper software (Applied Biosystems). Only TRFs that ranged from 40 to 400 base pairs in length, had a minimum peak height of 50 relative fluorescent units, and accounted for ≥ 1% of the total peak profile for each sample were considered for analysis.

T-RFLP analysis requires matching unknown T-RFLP profiles to a database of known T-RFLP patterns to identify which species or taxa are in a sample (Dickie et al. 2002). To create a database of known AM fungal sequences from the University of Guelph TBI site, PCR amplicons from DNA extracted from soil and root samples were cloned and sequenced. DNA extracts from soil and root samples were pooled into four composite samples, two for each of the soil and roots samples. In addition, six composite DNA extracts of root samples from a separate study the following year (2008) in the
University of Guelph TBI site were used to create the AM fungal database, for a total of ten composite samples. PCR amplicons were cloned into the pCR4-TOPO vector using the TOPO TA cloning kit (Invitrogen). Positive clones were used as template in DNA sequencing reactions. Clone PCR amplicons were cleaned with Exo-SAP-IT (USB), and sequencing reactions were done in one direction with the M13R primer using BigDye chemistry (Applied Biosystems), and run on an ABI 3730 DNA analyzer.

A total of 454 positive clones were sequenced with only one non-Glomeromycotan sequence detected and subsequently removed from the dataset. Sequences were manually screened for chimeras, which were excluded from further analysis. An alignment of the sequences was performed with Jalview (version 2.5) using the MUSCLE algorithm, and manually edited in the program BIOEDIT (version 7). Phylogenetic analyses were performed using the neighbor-joining (Kimura two-parameter model) and maximum likelihood (GTR + I + G) algorithms implemented in the programs MEGA 5.0 and TOPALi (v. 2.5), respectively. Sequences were conservatively grouped into seventeen phylotypes, which were defined as consistently separated monophyletic groups with high bootstrap support in both phylogenetic trees (Hijri et al., 2006). Names were assigned to sequence phylotypes based on the major AM fungal group to which they belonged.

TRFLP profiles of the database sequences were initially determined in silico by virtually digesting the sequences with AluI and MboI using the program Sequencher 4.9. A representative clone of each unique TRFLP profile was then amplified, digested, and analyzed to determine the real TRFLP profiles of the database sequences. A total of 58 different TRFLP profiles were included in the TRFLP database, with most phylotypes containing multiple TRFLP profiles. The TRAMPR package in the R statistical software
package was used to match known T-RFLP patterns to samples to determine which phylotypes were present in each sample (Dickie and FitzJohn, 2007). To be considered a match, all four terminal restriction fragments within 1.5 base pairs had to be detected for a positive match. This method is considered relatively conservative based on the number of primer/enzyme combinations used in this study (Dickie and FitzJohn, 2007).

A representative sequence for each of the 58 different TRFLP profiles was submitted to GenBank under the accession numbers JN252196 – JN252253. The BLAST function in GenBank was used to retrieve closely related (within 90% similarity) reference Glomeromycotan sequences for each of the representative sequences. An alignment of the representative sequences and reference Glomeromycotan sequences was performed with Jalview (v. 2.5) using the MUSCLE algorithm and manually edited in the program BIOEDIT (v. 7). Phylogenetic analyses were performed using the neighbour-joining and maximum likelihood algorithms implemented in the programs MEGA 5.0 and TOPALi (v. 2.5), respectively.

**Data analyses**

The binary presence-absence data of the detection by T-RFLP of different AM fungal molecular taxa (phylotypes) were analyzed by non-parametric MANOVA using the PERMANOVA add-on package of the PRIMER 6 software (www.primer-e.com). To compare the TRFLP patterns of samples obtained from within the intercropping alley, the five factors “Block” (random, 4 levels), “Treatment” (fixed, 4 levels), “Distance” from the tree row (fixed, 3 levels), “Sample type” (fixed, 2 levels), and “Replicate” transect (random, 2 levels, nested within Treatment and Block) were analyzed. In two separate
analyses the effect of these factors were assessed for the number of different detected phylotypes (i.e. square root transformed “phylotype richness”) and the “community” composition (based on the presence or absence of each of these phylotypes; similarity matrices were calculated based on Euclidian distances in both analyses). For each sampled transect the two root samples of the same distance were pooled, to allow a direct comparison of root and soil samples. To avoid any pseudo-replication separate sub-analyses were performed. First, the factors "Block" and "Tree species" were jointly analyzed by pooling all samples from the same Block and Treatment. The other factors were then analyzed by repeated measures models, by adding one factor at the time, pooling all the respective "pseudo-replicates" and omitting "impossible" interaction terms as recommended by the authors of the software. Analyses of samples taken from within the tree row were performed accordingly, but lacked the factor “Distance”. The reported $P$-values are based on 19,999 permutations (Table 2.1).

Nonmetric multidimensional scaling (NMS; Kruskal, 1964; McCune and Mefford, 2006) was used to visualize compositional differences among the AM fungal communities at the TBI site. Indirect ordination (principle correspondence analysis; ter Braak, 1998) was used to identify the length of the gradients in standard deviations. NMS was used to ordinate the plot data using PC-ORD v5 (MjM Software Design, Glendenen Beach, OR). In NMS, the Bray Curtis distance measure was used because of its robustness for both large and small ecological gradients (Minchin, 1987). NMS was performed using the “slow and thorough” autopilot mode in PC-ORD to determine the optimal ordination solution (i.e. lowest stress and instability) from multiple random starting configurations. This setting performs a maximum of 500 iterations in 250 runs with real data and 250 runs
with randomized data. A three dimensional solution was recommended for each data set, and the significance of these solutions was evaluated using a Monte Carlo test.

**Results**

*Molecular characterization of the AM fungal community*

Molecular analysis of the AM fungal community at the TBI site revealed 17 phylotypes belonging to the Glomeraceae (Fig. 2.1). Rarefaction analysis revealed that cloning and sequencing of the pooled DNA extracts was an effective method for capturing the majority of the targeted AM fungal diversity in soil and root samples collected at the TBI site (Fig. 2.2).

The majority of the phylotypes in the TBI site belonged to *Glomus* group A with 14 phylotypes. The remaining three phylotypes belonged to *Glomus* group B. Reference sequences retrieved from GenBank were closely related to several of the phylotypes, thus providing information on the taxonomic identity of the phylotypes. Three of the more commonly detected phylotypes detected at the TBI site were Glom-A11 (*Glomus constrictum*), Glom-A13 (*Glomus viscosum*), and Glom-A14 (*Glomus intraradices*) (Fig. 1). Glom-A9 (*Glomus mosseae*) and Glom-B3 (*Glomus etunicatum*) were the only other phylotypes for which we found closely related sequences of known AM fungal taxa in this study. The remaining phylotypes had no closely related Glomeromycotan reference sequences available in GenBank including Glom-A1, Glom-A4, Glom-A6, and Glom-A8, which were frequently detected in the TBI site.
Figure 2.1. Neighbor-joining phylogenetic analysis of arbuscular mycorrhizal (AM) fungal LSU rDNA sequences obtained from soil and root samples at the University of Guelph tree-based intercropping (TBI) site. Bootstrap values ≥ 70% for neighbor joining and maximum likelihood analyses are shown above and below the branches, respectively. The brackets indicate the delimitations of the phylotypes.
Figure 2.2. Rarefaction curves of LSU rDNA sequences from soil and root composite samples used to create the University of Guelph tree-based intercropping (TBI) TRFLP database. Soil and root rarefaction curves were separately produced using the Analytical Rarefaction program version 1.3 (http://www.uga.edu/strata/software/index.html).
Effect of sample type on AM fungal community

Sample type had a significant effect on phylotype richness and AM fungal community detected at the TBI site (Table 2.1). Soil samples had a significantly higher phylotype richness compared to root samples in the tree rows ($P = 0.006$) and intercropping alleys ($P < 0.001$). All of the phylotypes except Glom-B2 were detected in both sample types. However, there was significant variation in the distribution of phylotypes among sample types (Fig. 2.3) in both the tree rows ($P < 0.001$) and intercropping alleys ($P = 0.030$). This effect was further supported by the NMS analysis, which showed that AM fungal communities in soil samples were clearly different from root samples (Fig. 2.4). The two sample types showed the most separation along the first axis of the NMS, which explained 54% of the variation in the analysis.

Several phylotypes were more frequently detected in either soil or root samples. For example, *Glomus* group B phylotypes were found in 46% of soil samples, but only in 2% of root samples. Glom-A4 and Glom-A6 were found in 70% and 88% of soil samples, but only 19% and 38% of root samples, respectively. Other phylotypes were more prevalent in root samples, such as Glom-A1 and Glom-A14 (*Glomus intraradices*), which were found in 59% and 84% of root samples, but only 9% and 39% of soil samples, respectively. However, the most frequently detected phylotype, Glom-A13 (*Glomus viscosum*), was common in both soil (93%) and root (82%) samples.

Effect of trees on phylotype richness

AM fungal phylotype richness significantly differed ($P = 0.006$) among the treatments within the tree rows. Roots of white ash, poplar, and the weed community

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Table 2.1. PERMANOVA results for phylotype richness and community composition in the intercropping alleys and tree rows at the University of Guelph tree-based intercropping (TBI) site.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Intercropping alleys</th>
<th></th>
<th>Tree rows</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phylotype richness</td>
<td>Community</td>
<td>Phylotype richness</td>
<td>Community</td>
</tr>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
<td>df</td>
</tr>
<tr>
<td>Block</td>
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<td>6.781</td>
<td><strong>0.008</strong></td>
<td>3</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>5.503</td>
<td><strong>0.020</strong></td>
<td>3</td>
</tr>
<tr>
<td>Distance</td>
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<td>1.180</td>
<td>0.366</td>
<td>2</td>
</tr>
<tr>
<td>Sample type</td>
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<td>283.3</td>
<td>&lt; <strong>0.001</strong></td>
<td>1</td>
</tr>
<tr>
<td>Replicate (treatment x block)</td>
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<td>0.846</td>
<td>0.629</td>
<td>16</td>
</tr>
<tr>
<td>Block x distance</td>
<td>6</td>
<td>1.628</td>
<td>0.177</td>
<td>6</td>
</tr>
<tr>
<td>Block x sample type</td>
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<td>0.674</td>
<td>0.592</td>
<td>3</td>
</tr>
<tr>
<td>Treatment x distance</td>
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<td>3.999</td>
<td><strong>0.004</strong></td>
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<td>0.686</td>
<td>3</td>
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<tr>
<td>Distance x sample type</td>
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<td>Treatment x distance x sample type</td>
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<td>0.333</td>
<td>0.919</td>
<td>6</td>
</tr>
</tbody>
</table>

*P*-value based on a Monte Carlo procedure where the number of unique permutations is less than 500.
Figure 2.3. Distribution of the arbuscular mycorrhizal (AM) fungal phylotypes detected in soil and root samples within the four treatments at the University of Guelph tree-based intercropping (TBI) site.
Figure 2.4. Nonmetric multidimensional scaling (NMS) ordination of arbuscular mycorrhizal (AM) fungal communities in soil (closed symbols) and root samples (open symbols) at the University of Guelph tree-based intercropping (TBI) site. Ordination was based on the AM fungal community detected in each sample. Numbers inside symbols indicate the block that samples were collected from. The total amount of variation in the ordination was 0.885. Final stress value for the three-dimensional solution was 13.53.
(control) had a similar phylotype richness, while AM fungi were rarely detected in Norway spruce roots (Fig. 2.5). A similar trend was observed in soil samples as Norway spruce tree rows had the lowest phylotype richness compared to the other treatments. The phylotype richness was 1.5, 1.6, and 1.4 times higher in white ash, poplar, and control tree rows compared to Norway spruce tree rows.

Trees influenced the phylotype richness in the intercropping alleys as treatment ($P = 0.020$) and the interaction of treatment by distance ($P = 0.004$) had a significant effect. Similar phylotype richness was observed in the intercropping alleys of the white ash, poplar, and control treatments (Fig. 2.6). Phylotype richness did not vary significantly with distance from the tree row for either soil or root samples in each of these treatments. In contrast, Norway spruce trees had an influence on the phylotype richness observed in the intercropping alleys. Samples collected at 5.5 m and 3 m from the tree row had an average of 1.3 (soil) and 1.6 (root) times higher phylotype richness compared to samples collected closest to the Norway spruce tree row. In addition, block had a significant effect on phylotype richness observed in the intercropping alleys ($P = 0.008$), but not in the tree rows ($P = 0.210$) of the TBI site.

Effect of trees on AM fungal community composition

The AM fungal community composition in the tree rows was significantly affected ($P = 0.002$) by treatment. AM fungal communities detected in the roots of white ash, poplar, Norway spruce, and the weed community (control) showed some separation in the NMS analysis (Fig. 2.7). Among the treatments, there was separation between the AM fungal communities in Norway spruce tree rows and the other treatments along axis 2 ($r^2$}
Figure 2.5. Arbuscular mycorrhizal (AM) fungal phylotype richness in soil and root samples from the tree rows of the University of Guelph tree-based intercropping (TBI) site. Bars represent the mean (± standard error) phylotype richness.
Figure 2.6. Arbuscular mycorrhizal (AM) fungal phylotype richness in soil and soybean root samples from the intercropping alleys of the University of Guelph tree-based intercropping (TBI) site. Bars represent the mean (± standard error) phylotype richness.
Figure 2.7. Patterns of arbuscular mycorrhizal (AM) fungal community composition in soybean and tree roots at the University of Guelph tree-based intercropping (TBI) site based on nonmetric multidimensional scaling (NMS) ordination. Ordination was based on the AM fungal community detected in each sample. Symbols are the average (± 1 SE) ordination coordinates of samples in each treatment. Total variance explained by the ordination of all three axes is 0.888. Final stress value for the three-dimensional solution was 13.25.
NMS analysis of the AM fungal communities detected in soil samples revealed a similar trend (Fig. 2.8). AM fungal communities in the Norway spruce tree rows were clearly different from the other treatments with separation along the third NMS axis ($r^2 = 0.398$). Among the other treatments, AM fungal community composition was similar in the soil samples as there was considerable overlap between the communities in white ash, poplar, and control tree rows.

Trees also significantly affected AM fungal community composition in the intercropping alleys (Table 2.1). The AM fungal communities in the intercropping alleys of white ash, poplar, and control treatments were similar as they had considerable overlap in the NMS analyses (Figs. 2.7 and 2.8). Norway spruce trees appear to influence the AM fungal community in the intercropping alley as samples collected 0.5 m from the tree row were strongly separated from other treatments in the soil and root NMS analyses. This effect diminished with increasing distance from the Norway spruce tree row as samples collected 3.0 and 5.5 m into the intercropping alley had similar AM fungal communities as the other treatments. In addition, block had a significant effect ($P = 0.001$) on the AM fungal community composition in the intercropping alleys. Separation among the intercropping alley AM fungal communities were detected within the blocks in the NMS analysis (Fig. 2.4).

Discussion

This study provides the most extensive molecular analysis of AM fungal communities in a TBI system to date. We used a spatially explicit approach to qualitatively assess the influence of three different tree species with varying mycorrhizal
Figure 2.8. Patterns of arbuscular mycorrhizal (AM) fungal community composition in soil at the University of Guelph tree-based intercropping (TBI) site based on nonmetric multidimensional scaling (NMS) ordination. Ordination was based on the AM fungal community detected in each sample. Symbols are the average (± 1 SE) ordination coordinates of samples in each treatment. Total variance explained by the ordination of all three axes is 0.828. Final stress value for the three-dimensional solution was 17.87.
associations on the AM fungal community composition along the tree rows and in the intercropping alleys. Our results show that (1) the AM fungal community at the TBI site is comparable to other conventional agricultural systems, (2) sample type can significantly affect the characterization of AM fungal communities, and (3) trees have a significant effect on the spatial structure of AM fungal communities in TBI systems.

**AM fungal community at the TBI site**

Temperate TBI systems have been promoted as sustainable low-input agricultural systems as the incorporation of trees reduces the need for inorganic fertilizer and synthetic pesticides (Thevathasan and Gordon, 2004). In addition, TBI systems have greater plant diversity compared to conventionally managed systems due to the intercropping of trees and crops, and diverse weed community that occupies tree rows. Increasing plant diversity has been shown to have a positive influence on AM fungal diversity (Helgason et al., 1998; Burrows and Pfleger, 2002). As a result, we predicted that the University of Guelph TBI site would harbour a rich AM fungal community, including taxa from outside the Glomeraceae. However, all AM fungal sequences obtained in this study belonged to *Glomus* group A (14 phylotypes) or *Glomus* group B (3 phylotypes). These results are comparable to other studies that have investigated the molecular diversity of AM fungal communities in conventional agricultural systems (Hijri et al., 2006; Cesaro et al., 2008; Toljander et al., 2008; Galvan et al., 2009; Sasvari and Posta, 2010; Sasvari et al., 2011). In general, the most frequently detected species or phylotypes in conventional agricultural systems belong to *Glomus* group A and B, including many of the rapidly sporulating AM fungal species, such as *Glomus mosseae*, *Glomus geosporum*, and *Glomus etunicatum*.
(Oehl et al., 2003). However, these species were infrequently detected in this study. AM fungi from the other major Glomeromycotan groups have been detected in arable fields, but are associated with agricultural systems with lower inputs (Hijri et al., 2006).

Comparing our results to previous studies that have investigated AM fungal communities in TBI systems becomes difficult, as most studies were completed in tropical regions and used classical techniques (Bainard et al., 2011a). Assessment of AM fungal communities based on spore morphology can be difficult as Glomeromycotan sequence data suggests that the described species only represent a small fraction of the AM fungal diversity (Helgason and Fitter, 2009). A more accurate comparison can be made with the study by Chifflet et al. (2009), who found that the majority of sequences in a young TBI system in Quebec, Canada also belonged to *Glomus* group A. In addition, they detected sequences from the Gigasporaceae and Acaulosporaceae in poplar and soybean roots (Chifflet et al., 2009). Based on the limited number of studies, it appears that TBI systems harbour similar AM fungal taxa as conventional agricultural systems. However, we have recently completed a study that shows that although the broad taxonomic groups are similar between sites, the TBI site contains a significantly higher number of AM fungal taxa within *Glomus* group A compared to an adjacent conventional agricultural site (Chapter 3).

The lack of AM fungi detected outside of the *Glomeraceae* in the TBI site could in part be due to the methodology used in this study. Samples were collected at a single point in the season, which may have affected the AM fungal community that was detected in the TBI site. Oehl et al. (2009) found that there are successional sporulation dynamics that can occur within agricultural systems. They found strong seasonal variation in the
sporulation of different AM fungal species. In addition, Hijri et al. (2006) found that certain AM fungal species are more readily detected at different times; for example, *Acaulospora* species were detected more frequently during the early part of the growing season. In addition, the molecular methods used in this study may have influenced which AM fungal taxa were detected. The primers (FLR3 and FLR4) used in this study are AM fungal specific, but they may not amplify sequences of all AM fungal groups equally (Mummey and Rillig, 2007; Gamper et al., 2009). However, these primers have successfully amplified AM fungi from each of the major Glomeromycotan groups including Glomeraceae, Acaulosporaceae, Gigasporaceae, Paraglomeraceae, and Diversisporaceae (Gollotte et al., 2004; Mummey and Rillig, 2007; Schnoor et al., 2011).

**Effect of sample type on AM fungal community**

Several studies have shown that molecular analysis of soil and root samples from the same sampling location can produce different AM fungal communities (Clapp et al., 1995; Hempel et al., 2007; Cesaro et al., 2008; Wilde et al., 2009). In this study we found some overlap in the phylotypes detected in each sample type, but overall, there was significant variation in the AM fungal communities detected in soil and root samples. The most frequently detected phylotype, Glom-A13 (*G. viscosum*), was common in both soil and root samples. In contrast, several phylotypes were more prominently detected in either soil or root samples. The *Glomus* group B phylotypes were rarely detected in root samples, but found in nearly 50% of the soil samples. Glom-A14 (*G. intraradices*) was the most frequently detected (84%) phylotype in the roots, but only detected in 39% of the soil samples. Cesaro et al. (2008) observed a similar effect, as they had a high detection
rate of *G. intraradices* sequences in potato roots, but rarely detected any sequences in the soil. The results from this study provide additional support for the necessity to use both soil and root samples in order to provide an accurate assessment of the AM fungal community.

**Effect of trees on AM fungal community**

There were differences in the mycorrhizal status and associated AM fungal communities among the tree species in the TBI site. White ash is colonized by AM fungi, Norway spruce is colonized by ectomycorrhizal fungi, and poplar is colonized by both AM fungi and ectomycorrhizal fungi. White ash and poplar had the highest AM fungal richness and similar communities in their roots and in soil collected from respective tree rows. These results were not expected considering that tree species that form a tripartite association with AM fungi and ectomycorrhizal fungi generally have low levels of AM fungal colonization as they mature (Bainard et al., 2011b). The TBI site may lack or contain low levels of ectomycorrhizal inoculum, which could promote greater AM fungal colonization in the poplar trees. Assessment of the mycorrhizal colonization levels in roots could provide insight into this effect.

Another unexpected result was the detection of AM fungi in Norway spruce roots. Norway spruce is a member of the Pinaceae which are considered obligate ectomycorrhizal hosts that do not associate with AMF (Horton et al., 1998). However, there have been several reports indicating that some Pinaceae species can be colonized by AM fungi (Horton et al., 1998; Smith et al., 1998; Wagg et al., 2008). More specifically, *G. intraradices* has been observed to colonize Pinaceae roots (Smith et al., 1998), which
is the primary phylotype that was detected in Norway spruce roots in this study. Regardless, soil collected within Norway spruce tree rows had significantly lower AM fungal richness compared to rows occupied by white ash and poplar trees, and rows without trees (control).

The AM fungal community composition in the intercropping alleys was also affected by the trees, but to varying degrees. White ash and poplar had no effect on the community composition in the intercropping alleys, while the intercropping alleys closest to the Norway spruce tree row (0.5m) showed decreased AM fungal richness and a different AM fungal community. As the distance increased away from the Norway spruce tree row (3.0 m and 5.5 m from the tree row), the AM fungal community composition and richness became the same as the rest of the TBI site. Additionally, AM fungal richness and composition in intercropping alleys adjacent to white ash and poplar tree rows were similar to those in intercropping alleys adjacent to control rows (tree rows where trees were not growing). These results suggest that Norway spruce negatively impacts the AM fungal community in the TBI system.

Conclusion

Spatial analysis of the AMF community at the University of Guelph TBI site revealed significant variation in the AMF community composition. The results from this study support our original hypothesis that AMF communities are more diverse in cropping alleys adjacent to trees that associate with AMF than trees that do not host AMF. We did not find evidence to indicate that white ash and poplar increase the AMF diversity in the tree rows or intercropping alleys, as similar AMF communities were observed in rows
without trees (control) and adjacent intercropping alleys. However, Norway spruce clearly has a negative influence on the AMF community in tree rows and bordering intercropping alleys.
Literature Cited


CHAPTER THREE:

TEMPORAL PATTERNS OF ARBUSCULAR MYCORRHIZAL FUNGI IN
CONVENTIONAL MONOCROPPING AND TREE-BASED INTERCROPPING
SYSTEMS
Abstract

Previous research has found that conventional agricultural systems adversely affect arbuscular mycorrhizal (AM) fungi. However, there is little information on how more ecologically sustainable agricultural practices such as tree-based intercropping (TBI) influence AM fungal communities. In this study, we investigated whether TBI promotes a more abundant and diverse AM fungal community compared to conventional monocropping (CM). Molecular analysis of the AM fungal community was assessed using T-RFLP analysis of LSU rRNA genes amplified from roots in the two cropping systems. Overall, AM fungal abundance was similar in both systems as corn roots from the CM and TBI system were heavily colonized by AM fungi throughout the growing season. Additionally, soil samples from the CM and TBI systems contained similar spore densities and hyphal length. Molecular analysis of AM fungal communities in the CM and TBI systems revealed significant temporal and compositional differences between the two cropping systems. The TBI system had a higher AM fungal richness and contained several taxa not found in the CM system. In addition, tree species differentially influenced the AM fungal community composition within the TBI system.
Introduction

Arbuscular mycorrhizal (AM) fungi form a symbiotic association with a majority of crop species (Smith and Read, 2008), making them an important component of the soil microbial community in agroecosystems. AM fungi are abundant in field soils of most terrestrial ecosystems and have been estimated to account for 5-10% of soil microbial biomass (Fitter et al., 2011). The AM symbiosis can be beneficial to crop plants through increased access to nutrients (Smith and Read, 2008), protection from pathogens and herbivores (Newsham, 1995; Gange and West, 1994), and improved water relations (Auge, 2001; Auge, 2004). In addition, AM fungi can provide a number of beneficial ecosystem services such as improving soil structure (Caravaca et al., 2006; Rillig and Mummey, 2006; Bedini et al., 2009), influencing plant and microbial community structure (van der Heijden et al., 1998; Bever et al., 2001; Klironomos, 2003; van der Heijden, 2006; Marschner et al., 2001; Vestergard et al., 2008), suppressing weed populations (Rinaudo et al., 2010), and influencing major element cycles (e.g. carbon, phosphorus, nitrogen) (Fitter et al., 2011). Based on the ubiquitous nature and ecological services provided by these symbiotic organisms, AM fungi have the ability to alter crop productivity and support ecosystem sustainability in agricultural systems (Gianinazzi et al., 2010).

Over the past half century, the green revolution has led to the development of highly intensive agricultural practices that are heavily mechanized and rely on the application of synthetic fertilizers and pesticides. Although these practices have dramatically increased the global food supply, recent evidence suggests there are a number of environmental concerns associated with these practices that may not make
them ecologically sustainable over time (Tilman et al., 2002). These concerns include contamination of groundwater, eutrophication of aquatic ecosystems, release of greenhouse gases, loss of crop genetic diversity, loss of soil fertility, and soil erosion (Tilman, 1998). In addition, AM fungi can be strongly impacted by conventional agricultural practices. Studies have shown that practices such as tillage and fallow (Jansa et al., 2002; Troeh and Loynachan, 2003), monoculture cropping (Douds and Millner, 1999), and fertilization (Jumpponen et al., 2005; Oehl et al., 2004) can have a negative impact on the abundance and diversity of AM fungi (Oehl et al., 2005). As a result, arable systems generally have lower taxonomic diversity of AM fungi compared to natural systems (Helgason et al., 1998), and there appears to be an inverse relationship between management intensity and AM fungal richness (Oehl et al., 2003; Hijri et al., 2006). This shift in AM fungal community composition and diversity may be due to a number of factors including disturbance of AM fungal hyphal networks, changes in soil nutrient content, altered microbial activity, or changes in weed populations (Jansa et al., 2003).

Environmental concerns associated with conventional agricultural practices have led to the development and implementation of more sustainable agricultural practices. Several examples include reducing tillage for soil and water conservation, eliminating synthetic chemical inputs in organic agriculture, and increasing diversity by incorporating multiple crops (e.g. intercropping) or trees (e.g. agroforestry) into an agricultural system. In this study we focused on tree-based intercropping (TBI) or alley cropping, which is a form of agroforestry that involves planting annual crops in alleys between rows of trees (Jose et al., 2000). TBI systems enable the use of standard or conventional agricultural practices. However, properly designed TBI systems can provide a number of ecological
advantages compared to conventional agriculture such as reduced wind speed and evaporative stress (Jose et al., 2004), improved soil structure and stability (Price and Gordon, 1999), increased biodiversity conservation (Stamps and Linit, 1998), enhanced carbon sequestration (Thevathasan and Gordon, 2004), and reduced greenhouse gas emissions (Thevathasan and Gordon, 2004). However, little is known about how temperate TBI systems influence AM fungal communities, although early evidence suggests that temperate TBI systems promote a higher abundance of AM fungi compared to conventional agricultural systems (Lacombe et al., 2009; Bainard et al., 2011a).

In this study, we investigated the influence of intercropping on the abundance and diversity of AM fungi. We aimed to determine (1) if TBI systems promote a more abundant and diverse AM fungal community compared to conventional monocropping (CM) systems, and (2) whether AM fungal communities vary temporally during the growing season in these agricultural systems. Abundance was evaluated by measuring spore density and hyphal length in soil, and AM fungal colonization of crop roots. We hypothesized that the higher plant diversity within the TBI system supports a more abundant and rich AM fungal community compared to the CM system.

Materials & Methods

Study site

The University of Guelph Agroforestry Research Station is a long-term TBI research site established in 1987 on 30 ha of agricultural land in Guelph, Ontario, Canada. Trees were planted and annually intercropped with corn (Zea mays L.), soybean (Glycine max L. Merr.), winter wheat (Triticum aestivum L.) or barley (Hordeum vulgare L.) using
no-till cultivation. Trees species include silver maple (*Acer saccharinum* L.), white ash (*Fraxinus americana* L.), hazelnut (*Corylus avellana* L.), black walnut (*Juglans nigra* L.), Norway spruce (*Picea abies* L. Karst.), hybrid poplar (*Populus deltoides* x *nigra* DN-177), red oak (*Quercus rubra* L.), black locust (*Robinia pseudoacacia* L.), willow (*Salix discolor* Muhl.), and white cedar (*Thuja occidentalis* L.). Tree rows are spaced 12.5 or 15 m apart, and the trees within a row are spaced at either 3 or 6 m. The tree rows are 2 m wide and currently reduce by approximately 16% the total crop production area (Peichl et al., 2006). The CM system is located directly adjacent to the TBI system. Crops at the CM system are managed in a similar manner as the TBI system using no-till cultivation and the same crop rotation. Prior to planting trees, the CM and TBI fields had a similar cultivation history over the past half century. The fields were under hay until the mid 1970s and then cultivated with corn, soybean, and winter barley. The soil at the University of Guelph Agroforestry Research Station is classified as a Gray Brown Luvisol with a sandy-loam soil texture (65% sand, 25% silt and 10% clay; Order: Alfisols, Group: Typic Hapludalf) (Oelbermann and Voroney, 2007). A detailed analysis of the soil physico-chemical properties at the TBI and CM site can been found in Lacombe et al. (2009).

**Experimental design and sampling**

The experimental design in the TBI system was a randomized complete block design with six blocks and three TBI treatments. The TBI treatments consisted of rows of at least five successive trees of i) white ash, ii) silver maple, or iii) Norway spruce trees within each block. Samples were collected on either direction of the tree rows within 1 m² quadrats situated in the intercropped alleys (2.5 – 3.5 m from the tree row). In the CM
system, samples were collected from six randomly selected, but pseudo-replicated locations. At each location, samples were collected from within two 1 m² quadrats that were set up in a similar spatial pattern as the TBI system. In order to assess the temporal variation of AM fungal communities, sampling took place at four time periods throughout the 2008 growing season: i) early spring (May 6th, prior to emergence of crop seedlings), ii) late spring (June 11-13th, four weeks of crop growth), iii) early summer (July 9-12th, eight weeks of crop growth), and iv) late summer (August 14-17th, thirteen weeks of crop growth). Corn (*Zea mays*) was planted in the TBI and CM systems on April 24th-25th and seedlings emerged from the soil on May 15th.

At each sampling period, soil cores were collected to a depth of 20 cm using a 3 cm diameter soil corer and placed in sterile plastic bags. Soil cores were thoroughly homogenized and stored at 4°C prior to assessment of spore density and extraradical hyphal length. Fine root samples were collected at the three sampling periods following crop emergence to assess the level of root length colonization and molecular analysis of AM fungi. Tree and crop roots were collected within 1 m² quadrats to a depth of 20 cm and placed in sterile plastic bags. Root samples were rinsed with tap water and divided into two subsamples. The first set of subsamples was stored in 50% ethanol to assess the proportion of AM fungal root length colonization. The second set of subsamples was freeze dried and stored at -80°C for molecular analysis.

*Spore density*

AM fungal spores were extracted from a 100 g soil subsample by wet sieving and sucrose density gradient centrifugation (Daniels and Skipper, 1982). Soil subsamples were air-dried and passed through 1000 μm, 500 μm, and 38 μm sieves. The contents on the
500 µm and 38 µm sieves were collected and separated from soil and organic debris using sucrose density gradient centrifugation. This was achieved by adding the sieve contents to a 50% water-sucrose solution (50% wt/vol) gradient and centrifugation at 2000 rpm for 2 min. The supernatant was decanted onto a 38 µm sieve, washed with distilled water, and transferred to a petri dish for enumeration.

Hyphal length

Hyphal length was measured to provide another measure of the abundance of AM fungi in the soil. Hyphae were extracted from a 4 g soil subsample by an aqueous extraction and membrane filter technique (Miller et al., 1995). Hyphal length was quantified using the magnified gridline-intersects method (McGonigle et al., 1990).

Root colonization

Corn fine roots were rinsed with distilled water and cleared with 10% potassium hydroxide. Cleared root samples were thoroughly rinsed with distilled water and stained with 0.05% Chlorazol Black E (Brundrett, 1994). A random subset of roots from each sample was mounted onto microscope slides and the proportion of root length colonized by arbuscules, vesicles, and hyphae (total colonization) was determined using the magnified gridline-intersects method (McGonigle et al., 1990).

Molecular analysis

AM fungal communities in the TBI and CM systems were investigated by terminal restriction fragment length polymorphisms (T-RFLP) analyses of the FLR3/FLR4 fragments of the LSU rRNA gene (Gollotte et al., 2004). Lyophilized root tissue samples (30 mg) were homogenized by placing them in 2 ml microcentrifuge tubes with a 3 mm ceramic bead and shaking them for 30 s using a Fast-Prep. Genomic DNA was then
extracted from pulverized root tissue samples using a DNeasy Plant Mini kit (Qiagen, Mississauga, Canada). Total DNA concentration in each root sample was quantified spectrophotometrically using a NanoDrop ND-8000 (NanoDrop, Wilmington, USA).

A nested PCR approach was used to amplify AM fungal DNA in the root sample extracts. The first PCR used the primer pair LR1 and FLR2 to amplify the fungal DNA (van Tuinen et al., 1998; Trouvelot et al., 1999). The second PCR used the AM fungal specific 5’-labelled primer pair FLR3-FAM and FLR4-VIC (Gollotte et al., 2004). The 25 µl reaction mixtures for both PCR rounds consisted of 1 unit of AmpliTaq Gold DNA polymerase, 1.5 mM MgCl₂, 0.2 mM deoxynucleoside triphosphate, 0.5 µM of each primer pair, and 100 ng of root extracted template DNA (round one), or 2 µl of diluted (1:10) PCR product (round two). The cycling parameters included an initial denaturing step for 2 min at 94°C, 25 cycles (round one) or 30 cycles (round two) consisting of 1 min at 94°C, 1 min at 58°C and 1 min at 65°C, followed by a final extension step of 65°C for 10 min.

PCR products were digested with the restriction enzymes MboI and AluI in separate reactions. The digestion reactions consisted of 5 µl of diluted (1:5) PCR product and 1 unit MboI or AluI in the manufacturers recommended buffer. The reactions were incubated at 37°C for 6 hours, followed by a heat inactivation step at 65°C for 10 min. Samples were purified with Exo-SAP-IT (USB), followed by a Sephadex column clean-up step. The size of the terminal restriction fragments (TRFs) in each sample was determined using an ABI 3730 DNA analyzer (Applied Biosystems, Carlsbad, USA). Fragment data was analyzed using Genemapper software (Applied Biosystems). Only TRFs ranging from 40 to 400 base pairs in length, had a minimum peak height of 50
relative fluorescent units, and accounted for ≥ 1% of the total peak profile for each sample were considered for analysis.

T-RFLP requires matching unknown T-RFLP profiles to a database of known T-RFLP patterns to identify which species or taxa are in a sample (Dickie et al., 2002). A database of AM fungal sequences at the University of Guelph TBI and CM systems was previously created for an earlier study (Chapter 2). The database consists of 17 AM fungal phylotypes and a total of 59 different T-RFLP profiles, with most phylotypes containing multiple T-RFLP profiles. The TRAMPR package in the R statistical software package was used to match known T-RFLP patterns to samples to determine which phylotypes were present in each sample (Dickie and FitzJohn, 2007). To be considered a match, all four TRFs within 1.5 base pairs had to be detected for a positive match. This method is considered relatively conservative based on the number of primer/enzyme combinations used in this study (Dickie and FitzJohn, 2007).

Data analyses

Nonmetric multidimensional scaling (NMS; Kruskal, 1964; McCune and Mefford, 2006) was used to visualize compositional differences among the AM fungal communities in the TBI and CM systems and treatments in the TBI system. NMS was used to ordinate the plot data using PC-ORD v5 (MjM Software Design, Glenden Beach, OR). In NMS, the Bray Curtis distance measure was used because of its robustness for both large and small ecological gradients (Minchin, 1987). NMS was performed using the “slow and thorough” autopilot mode in PC-ORD to determine the optimal ordination solution (i.e. lowest stress and instability) from multiple random starting configurations. This setting performs a maximum of 500 iterations in 250 runs with real data and 250 runs with
randomized data. A three dimensional solution was recommended for each data set, and the significance of these solutions was evaluated using a Monte Carlo test.

Both morphological and molecular AM fungal data were analyzed by non-parametric MANOVA using the PERMANOVA add-on package of the PRIMER 6 software (www.primer-e.com). We analyzed data in two complementary statistical models. The first model (Table 1, “CM vs TBI systems”) was a three-factor mixed (M)ANOVA model that included the crossed factors “Site” (fixed, two levels) and “Sampling period” (fixed, 4 levels for AM fungal morphological traits, and 3 levels for molecular data), and “Block” (random, nested within site). For samples from the TBI system we disregarded the different TBI treatments, which were analyzed in the second model. Separate ANOVA models were calculated for AM fungal spore abundance and hyphal lengths, and the three different AM fungal colonization measures were analyzed in one MANOVA. For the molecular data, we analyzed the binary phylotype T-RFLP data, and performed ANOVA and MANOVA on AM fungal phylotype richness and phylotype community (presence/absence of the different phylotypes), respectively. In a second model we disregarded all samples from the CM site (Table 1, “TBI system alone”) and analyzed the crossed factors “Block” (random, 6 levels), TBI treatment (fixed, 3 levels), “Sampling period” (same as before), and “Direction” (fixed, 2 levels: samples were either recovered north east or south west of the tree rows), in an analogous way as the first model. To avoid any pseudo-replication separate sub-analyses were performed. First, the factors "Block" and "Tree species" were jointly analyzed by pooling all samples from the same Block and Treatment. The other factors were subsequently analysed by repeated measures models, by adding one factor at a time, pooling all the respective "pseudo-
replicates" and omitting "impossible" interaction terms as recommended by the authors of
the software. If necessary variables were transformed prior to all analyses and reported $P$-
values are based on 19,999 permutations (Table 1).

**Results**

*Abundance of AM fungi*

*Spore density*

There was no significant difference in spore density ($P = 0.640$) between the TBI
and CM systems (Table 3.1). However, spore density was influenced by tree species ($P =
0.038$) in the TBI system. The highest spore densities were found in soil from the white
ash treatment, and lowest in soil from the Norway spruce treatment (Fig. 3.1). There was
no significant temporal (sampling period) effect on spore density in the two cropping
systems ($P = 0.393$). However, temporal trends in spore density were evident in the TBI
and CM systems. The highest spore densities were found in late spring (June 11-13th) for
all treatments in the TBI system, and declined during the summer sampling periods (July
9-12th and August 14-17th) to early spring (May 6th) levels. A more delayed response was
observed in the CM system, as spore densities peaked during the early summer and then
returned to spring levels at the final sampling period. In addition, no significant effect of
sampling direction ($P = 0.330$), block ($P = 0.236$), or interaction among factors (see Table
1) were detected for spore density in the TBI system.

*Hyphal length*

Hyphal length in the soil was not significantly different between the TBI and CM
systems ($P = 0.102$), or among the treatments in the TBI system ($P = 0.261$) (Fig. 3.2).
Table 3.1. PERMANOVA results for AM fungal abundance (spore density, hyphal length, and root colonization),
phylotype richness and community composition in conventional monocropping (CM) and tree-based intercropping
(TBI) systems at the University of Guelph Agroforestry Research Station.

<table>
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<tr>
<th>Factor</th>
<th>Spore abundance</th>
<th>Hyphal length</th>
<th>AMF colonization</th>
<th>Phylotype richness</th>
<th>Phylotype community</th>
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<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
<td>df</td>
<td>F</td>
</tr>
<tr>
<td><strong>CM vs. TBI systems</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site</td>
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<td>0.640</td>
<td>1</td>
<td>2.99*</td>
</tr>
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<td>3</td>
<td>0.66</td>
</tr>
<tr>
<td>Block (site)</td>
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<td>0.236</td>
<td>10</td>
<td>1.09</td>
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<tr>
<td>Site x sampling period</td>
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<td>0.202</td>
<td>3</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.236</td>
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<td>0.12</td>
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<td>2</td>
<td>1.51</td>
</tr>
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<td>0.13</td>
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<tr>
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<td>1</td>
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</tr>
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<td>0.607</td>
<td>15</td>
<td>0.70</td>
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<td>6</td>
<td>1.37</td>
</tr>
<tr>
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<td>0.507</td>
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<td>6</td>
<td>0.72</td>
</tr>
</tbody>
</table>

*P-value based on a Monte Carlo procedure where the number of unique permutations is less than 100.
Figure 3.1. Spore density in soil collected from (a) conventional monocropping (CM) and tree-based intercropping (TBI) systems at the University of Guelph Agroforestry Research Station, and (b) treatments in the TBI system across four sampling periods. Symbols represent the mean (± standard error) spore density.
Figure 3.2. Hyphal length in soil collected from (a) conventional monocropping (CM) and tree-based intercropping (TBI) systems at the University of Guelph Agroforestry Research Station, and (b) treatments in the TBI system across four sampling periods. Symbols represent the mean (± standard error) hyphal length.
We also did not observe any significant variation in hyphal length among the sampling periods in the two cropping systems ($P = 0.568$). Hyphal length ranged between 3-4 m g$^{-1}$ soil in both systems throughout the growing season. In addition, no significant effect of sampling direction ($P = 0.939$), block ($P = 0.982$), or interaction among factors (see Table 1) were observed for hyphal length in the TBI system.

**Root colonization**

AM fungal colonization of corn roots was not significantly different between the TBI and CM systems ($P = 0.801$), or among the treatments in the TBI system ($P = 0.836$) (Fig. 3.3). Corn roots were heavily colonized by AM fungi after only four weeks of growth and exhibited no significant temporal variation in both cropping systems ($P = 0.590$). In addition, no significant effect of sampling direction ($P = 0.939$), block ($P = 0.982$), or interaction among factors (see Table 3.1) were observed for AM fungal colonization in the TBI system. Total (hyphal) AM fungal colonization of corn roots ranged between 54 – 67% in both cropping systems throughout the growing season. The proportion of root length colonized by arbuscules and vesicles were also quantified and we did not observe any significant variation between the two cropping systems, the treatments within the TBI system, or throughout the growing season.

**Molecular analysis of AM fungi**

We successfully amplified AM fungal DNA from all corn, white ash, and silver maple root samples despite freeze drying the samples (Bainard et al., 2010). We detected fifteen AM fungal phylotypes in the TBI and CM systems. Fourteen of the phylotypes belonged to *Glomus* group A, and one phylotype belonged to *Glomus* group B. A detailed description of the phylotypes detected in the two cropping systems can be found in
Figure 3.3. Arbuscular mycorrhizal (AM) fungal colonization of corn roots from (a-c) conventional monocropping (CM) and tree-based intercropping (TBI) systems at the University of Guelph Agroforestry Research Station, and (d-f) treatments in the TBI system across three sampling periods. Symbols represent the mean (± standard error) arbuscular, vesicular, and hyphal colonization of corn roots.
Chapter 2.

Phylotype richness

AM fungal phylotype richness significantly differed \( (P = 0.034) \) between the TBI and CM systems (Table 3.1). Corn roots from the CM system had the lowest phylotype richness at each sampling period (Fig. 3.4), and lowest total number of phylotypes compared to corn growing in the TBI system. Only eight phylotypes were detected in the CM system, whereas thirteen phylotypes were found in corn roots growing in the white ash, silver maple, and Norway spruce treatments in the TBI system (Fig. 3.5). Several AM fungal phylotypes colonizing corn roots were only detected in the TBI system including Glom-A3, Glom-A4, Glom-A5, Glom-A7, Glom-A8, and Glom-B1. Within the TBI system, tree species had no effect on phylotype richness of corn as there was no significant difference \( (P = 0.240) \) among the treatments. However, when tree roots were included in a separate analysis there was a significant difference \( (P < 0.001) \) in phylotype richness among the treatments, which can be attributed to the low detection of AM fungi in Norway spruce roots. In a pair-wise test, Norway spruce roots had a significantly different \( (P < 0.05) \) phylotype richness compared to both silver maple and white ash roots, which had similar phylotype richness.

Sampling period had a significant effect \( (P = 0.008) \) on AM fungal phylotype richness of corn in the TBI and CM systems (Table 3.1). Phylotype richness of corn roots were generally higher in early summer and subsequently declined over the summer in both the TBI and CM systems, but remained relatively constant in tree roots (Fig. 3.4). In addition, no significant effect of sampling direction, block, or interaction among factors except ‘block x sampling period’ (see Table 3.1) were observed for AM fungal phylotype
Figure 3.4. Phylotype richness of AM fungi in (a) corn roots from conventional monocropping (CM) and tree-based intercropping (TBI) systems, (b) corn roots from treatments in the TBI system, and (c) trees roots from the TBI system across three sampling periods. Symbols represent the mean (± standard error) phylotype richness.
Figure 3.5. Distribution of AM fungal phylotypes detected in (a) corn roots from the conventional monocropping (CM) system, (b) corn roots from the white ash TBI treatment, (c) corn roots from the silver maple TBI treatment, (d) corn roots from the Norway spruce TBI treatment, (e) white ash tree roots, and (f) silver maple tree roots across three sampling periods.
richness in the TBI system.

Community composition

AM fungal community composition significantly differed \((P < 0.001)\) between the TBI and CM systems (Table 3.1). Several of the AM fungal phylotypes detected in the TBI system (Glom-A4, Glom-A5, Glom-A7, Glom-A8, Glom-A11, and Glom-A12) were either lacking or detected at a much lower frequency in the CM system (Fig. 3.5). In addition, Glom-A9 and Glom-A10 were detected in 92% of corn roots in the CM system, but only 33% of corn roots in the TBI system. Differences among AM fungal communities in the two cropping systems were also evident in the NMS ordination (Fig. 6). AM fungal communities in the CM system were clearly different from the TBI system with separation occurring along the second NMS axis \((r^2 = 0.56)\).

Trees also had a significant effect \((P = 0.020)\) on the AM fungal community composition of corn roots in the TBI system (Table 3.1). In particular, a pair-wise test within the ‘treatment’ factor found that corn roots growing in the Norway spruce treatment had a distinct AM fungal community compared to silver maple treatments. Among the treatments, the majority of the AM fungal community variation was observed along the first axis \((r^2 = 0.21)\) of the NMS ordination (Fig. 3.6). The majority of the AM fungal community variation between the treatments in the TBI system occurred during the late spring and early summer. Interestingly, temporal changes lead to more similar AM fungal communities in corn roots among the TBI treatments at the final sampling period in late summer than earlier in the season (Figs. 3.5 and 3.6).

Sampling period also had a significant effect \((P < 0.001)\) on AM fungal community composition in the TBI and CM systems (Table 3.1). A pair-wise test within
Figure 3.6. Nonmetric multidimensional scaling (NMS) ordinations of AM fungal communities in (a) corn roots from CM and TBI systems at the University of Guelph Agroforestry Research Station, and (b) corn and tree roots from TBI treatments across three sampling periods. Ordinations were based on the AM fungal community detected in each sample. Symbols are the average (± 1 SE) ordination coordinates of samples in each (a) cropping system and (b) treatment in the TBI system. Numbers inside symbols indicate the time of sampling: (1) late spring (June 11-13th), (2) early summer (July 9-12th), and (3) late summer (August 14-17th). Total variance explained by the ordination of all three axes was (a) 0.90 and (b) 0.88. Final stress values for the three-dimensional solutions were (a) 11.68 and (b) 14.31.
the ‘sampling period’ factor found a significant difference ($P < 0.05$) between late summer AM fungal communities and those found in early spring and early summer. These results indicate a more gradual shift in AM fungal community composition earlier in the growing season and more pronounced later in the season. In the NMS ordinations, the AM fungal communities in the corn roots showed separation between the sampling periods along the second ($r^2 = 0.56$) and third ($r^2 = 0.18$) axes in the CM and TBI systems (Fig. 3.6a), and first ($r^2 = 0.21$) and second ($r^2 = 0.41$) axes in the TBI treatments (Fig. 3.6b). In contrast, tree roots exhibited very little temporal variation in AM fungal community composition. Seasonal patterns of a few AM fungal phylotypes (Fig. 3.5) explain some of the temporal variation. For example, Glom-A14 (Glomus intraradices) was detected at a high frequency in late spring and early summer in corn roots and declined in late summer, but detected a high frequency throughout the growing season in tree roots. Glom-A9 (Glomus mosseae) and Glom-A10 showed different temporal patterns in the two cropping systems. They were detected at a high frequency at every sampling period in the CM system and declined considerably through the growing season in the TBI system.

**Discussion**

In this study, we investigated whether an ecologically sustainable agricultural system such as TBI promotes a more abundant and diverse AM fungal community compared to CM. Our results show that (1) AM fungal abundance (i.e. spore density, hyphal length, and root colonization) does not significantly differ between the two cropping systems, but that (2) TBI supports a richer AM fungal community compared to
AM fungal abundance

Similar levels of spore density, hyphal length, and mycorrhizal colonization was observed in the TBI and CM systems suggesting the incorporation of trees into TBI systems has little effect on the abundance of AM fungi. Chifflot et al. (2009) observed similar results in a TBI (poplar-soybean combination) and poplar forest plantation system in eastern Canada, as no difference in spore abundance was observed between the two systems. However, TBI systems had a higher concentration of AM fungal fatty acids compared to CM systems at two different sites in eastern Canada, including the University of Guelph TBI research station (Lacombe et al., 2009). Differences observed in AM fungal abundance at the University of Guelph TBI and CM systems between the present study and Lacombe et al. (2009) may be due to methodology (e.g. morphological measurements vs. phospholipid fatty acid profiles), sampling time (e.g. May – August 2008 vs. September 2006), or sampling location in the TBI site (white ash, silver maple, and Norway spruce rows vs. silver maple and black walnut rows). Clearly more research is required to understand the effect of TBI systems on AM fungal abundance as there are several potential tree-crop combinations and climatic variables that can influence AM fungi in temperate agricultural systems.

Previous studies in tropical regions have shown that TBI systems can harbour more abundant AM fungal communities compared to CM systems (see Bainard et al., 2011a for a review). Crops growing in close proximity to trees had a higher level of root colonization and greater spore densities in the rhizosphere relative to crops growing
beyond the tree canopies and monoculture plots (Mutabaruka et al., 2002; Pande and Tarafdar, 2004; Prasad and Mertia, 2005). In addition, studies in agroforestry coffee (Coffea arabica L.) systems observed higher spore densities in the rhizosphere of coffee plants under shade trees compared to monocultural coffee systems (Muleta et al., 2007; Muleta et al., 2008). In contrast, other studies have also shown no effect or in some cases a negative effect of TBI systems on AM fungi (Boddington and Dodd, 2000; Leal et al., 2009). The variable effect of TBI systems on AM fungi, as also shown in temperate regions, could be a function of different cultivation techniques, crop types, climatic variation, or diverse tree-crop combinations in these studies (Bainard et al., 2011a; Chapter 2).

**Temporal dynamics of AM fungal abundance**

Although we did not observe significant temporal variation in spore density, seasonal trends were evident at the TBI and CM sites. Spore density peaked during the late spring and declined throughout the summer in the TBI site. A more delayed response was observed in the CM site, as spore density peaked during the early summer and declined at the final sampling period. The delayed response in the CM site is in agreement with results from previous studies on corn (Sasvari et al., 2011; Tian et al., 2011). These studies found that spore density increased through the growing season and peaked during the reproductive stages of corn and subsequently declined at the end of the growing season (Sasvari et al., 2011; Tian et al., 2011). The earlier increase in spore density in the TBI system may be a function of the different AM fungal community compared to the CM system. There is evidence of contrasting seasonal sporulation dynamics among AM
fungal species (Pringle and Bever, 2002; Oehl et al., 2009), which may account for the different temporal trends observed in the TBI and CM systems.

The abundance of hyphae in the soil did not exhibit any temporal variation. These results are in contrast to previous studies that measured hyphal length in agroecosystems planted with corn (Kabir et al., 1997; Tian et al., 2011). These studies found the lowest hyphal abundance in the early growth stages of corn, peaked at the reproductive stages, and then declined toward the end of the growing season (Kabir et al., 1997; Tian et al., 2011). Similarly, in a tallgrass prairie and ungrazed pasture, hyphal lengths increased throughout the growing season (Miller et al., 1995).

Corn roots were rapidly colonized by AM fungi and showed no temporal variation in mycorrhizal colonization throughout the growing season. The rapid colonization of corn roots was not unexpected considering that corn is a mycorrhizal dependent species and easily colonized by AM fungi (Hamel and Smith, 1991). Previous studies have also reported high levels of AM fungal colonization of corn roots at early growth stages (Hamel and Smith, 1991; Galvez et al., 2001; Tian et al., 2011). In fact, Galvez et al. (2001) observed the highest levels of AM fungal colonization of corn roots 23 days after planting in a no-till conventional field and low-input chisel-disk field. Similar to the present study, Tian et al. (2011) found no significant temporal variability in total AM fungal colonization of corn roots between four growth stages. However, temporal variation of arbuscule and vesicle colonization levels was observed, with colonization levels peaking at the reproductive stages of corn growth (Tian et al., 2011). This study provides support for the reported rapid colonization of this highly mycorrhizal dependent crop species.
**AM fungal community**

Molecular analysis of AM fungal communities revealed a higher richness in the TBI system. Fourteen AM fungal phylotypes belonging to *Glomus* group A and B were found in corn roots in the TBI system, and eight phylotypes belonging to *Glomus* group A in the CM system. A similar AM fungal community was found in the TBI system the previous year, as these fourteen AM fungal phylotypes plus an additional two phylotypes were detected in soybean roots (Chapter 2). However, corn had a considerably higher (1.5 times) AM fungal richness compared to soybean. The higher AM fungal richness in the TBI system may be linked to the greater plant diversity in the site compared to the CM system as stated in our hypothesis. Previous studies have shown that host plant composition can influence the diversity and composition of AM fungal communities especially in soils with a small range of soil chemical factors (Helgason et al., 2007; Dumbrell et al., 2010). Comparisons of the soil physico-chemical properties at the Guelph TBI and CM systems has revealed very little variation in nutrient content and pH of the soils in both systems (Lacombe et al., 2009; Chapter 4). These findings suggest that host plant composition rather than soil chemistry is likely a stronger factor in structuring AM fungal communities in these cropping systems.

Beyond the University of Guelph Agroforestry Station, molecular analysis of AM fungal communities has only been conducted in one other TBI system. In a young TBI system in eastern Canada, Chifflot et al. (2009) found that the majority of the twelve phylotypes detected in poplar and soybean roots belonged to *Glomus* group A. In addition, they detected a higher AM fungal diversity in the TBI system compared to a poplar forest plantation system (Chifflot et al., 2009). Based on the limited number of studies to date, it
appears that temperate TBI systems harbour a more diverse AM fungal community compared to CM systems, with the majority of taxa found within \textit{Glomus} group A.

The dominance of \textit{Glomus} group A in agricultural systems has been frequently reported (Daniell et al., 2001; Oehl et al., 2003; Hijri et al., 2006; Cesaro et al., 2008, Toljander et al., 2008; Galvan et al., 2009; Sasvari and Posta, 2010, Sasvari et al., 2011). AM fungal taxa from other Glomeromycotan families are generally rare in agroecosystems and are associated with agriculture systems with lower inputs or reduced disturbance (Daniell et al., 2001; Hijri et al., 2006). Despite the lack of diversity among other AM fungal families, agricultural systems can have substantial diversity within \textit{Glomus} group A. For example, Sasvari et al. (2011) detected twenty-two AM fungal operational taxonomic units (OTUs) in corn roots from a long-term monoculture experiment, with eighteen OTUs belonging to \textit{Glomus} group A. In the studies at the University of Guelph TBI system we detected fourteen phylotypes belonging to \textit{Glomus} group A, several of which no closely related sequences were available in GenBank (Chapter 2). The prevalence of \textit{Glomus} group A taxa in agroecosystems is thought to be associated with their ability to tolerate disruption of the mycelia network via soil cultivation (Daniell et al., 2001; Jansa et al., 2003). Further research into the functional characteristics of the diverse taxa within Glomus group A will provide valuable information on the role of AM fungi in agroecosystems.

\textit{Temporal dynamics of AM fungal communities}

The results from this study revealed significant temporal variation across a growing season in the richness and community composition of AM fungi in the TBI and CM systems. Only a few studies to date have investigated the temporal dynamics of AM
fungal communities colonizing plant roots. In separate studies, Santos-Gonzalez et al. (2007) and Rosendahl and Stukenbrock (2004) found no seasonal variation in the community composition of AM fungi colonizing grassland plant species. However, Dumbrell et al. (2011) suggests that the lack of seasonal variation was due to the exclusion of sampling during winter months. They found significantly different AM fungal assemblages and diversity during the cooler and warmer periods of the year in temperate grassland and suggested that variation in community composition during the growing season is likely a response to local soil chemistry (e.g. pH, phosphorus, C/N ratio) (Dumbrell et al., 2010; 2011). The results from our study along with Daniell et al. (2001) reveal that significant variation in AM fungal richness and community composition can occur within a growing season in agricultural systems.

Strong seasonal trends were also evident among some of the dominant AM fungal phylotypes. For example, Glom-A14 (*Glomus intraradices*) was detected at a high frequency in corn roots during late spring and early summer, but at a low frequency later in the summer. Santos-Gonzalez et al. (2007) observed a similar effect in grassland plant species, as a *G. intraradices* sequence type was common among spring and early summer root samples, but rare in September and October. The opposite trend was observed in white ash tree roots in the present study, as Glom-A14 was detected at a higher frequency as the growing season progressed, and silver maple tree roots remained highly colonized throughout the growing season. Different trends in the frequency of Glom-A9 (*Glomus mosseae*) and closely related Glom-A10 phylotypes were observed in the two cropping systems in this study. These phylotypes were detected at a high frequency throughout the growing season in the CM system, but decreased substantially as the season progressed in
the TBI system. These seasonal trends may be associated with the differences among the two cropping systems. Crops growing in the CM system may rely more on sporulating species due to the lack of living roots in the soil following harvest until the next spring to maintain active AM fungal communities. Some of the most frequently detected taxa in CM systems are rapidly sporulating AM fungal species including *G. mosseae* (Oehl et al., 2003).

**Conclusion**

Molecular analysis of AM fungal communities in the TBI and CM systems revealed significant temporal and compositional differences between the cropping systems. The TBI system had a significantly higher AM fungal richness, with the majority of taxa found within *Glomus* group A. Despite differences in richness and composition, AM fungal abundance was similar in both systems. In addition, we provide evidence of changing AM fungal communities in agricultural systems over a growing season. Future research should focus on determining whether compositional differences among AM fungal communities in TBI and CM systems have a functional effect on crop growth and productivity.
Literature Cited


mycorrhiza, but not to reductions in microbial activity induced by foliar cutting.

FEMS Microbiol. Ecol. 64, 78-89.
CHAPTER FOUR:

GROWTH RESPONSE OF CROPS TO SOIL MICROBES AND ARBUSCULAR MYCORRHIZAL FUNGI FROM CONVENTIONAL MONOCROPPING AND TREE-BASED INTERCROPPING SYSTEMS
Abstract

Soil microbes play an important role in agricultural ecosystem processes and can directly affect the productivity of these systems. Intensive management strategies employed in conventional agricultural systems have been shown to strongly impact or influence microbial communities. Alternative agricultural practices such as tree-based intercropping (TBI) have been shown to promote a more diverse microbial community compared to conventional monocropping (CM) practices. In this study, we use a series of greenhouse experiments to test the growth response of three crop species to soil microbes and specifically to AM fungi from these two cropping systems. Barley (*Hordeum vulgare*) and canola (*Brassica napus*) had a negative growth response (63% and 12% reduction in biomass, respectively) to soil microbes, while soybean (*Glycine max*) was not affected. However, overall the crops had a similar growth response to the soil microbial communities from both cropping systems. During the AM fungal establishment phase of the second experiment, nurse plants (*Trifolium pratense, Daucus carota, Allium porrum*) had a strong positive growth response (1.9 fold increase in biomass over non-mycorrhizal control) to AM fungal inoculation. When comparing the two cropping systems, nurse plants had a significantly higher biomass when inoculated with AM fungi from the CM system. Soybean was the only crop species to exhibit a significant positive growth response (1.3 fold increase in biomass over non-mycorrhizal control) to AM fungal inoculation. Similar to soil microbes, AM fungi from the two cropping systems did not differ in their effect on crop growth. Overall, AM fungi from both cropping systems had a positive effect on the growth of plants that formed a functional symbiosis.
Introduction

Soil microbes play a key role in the development of sustainable agriculture. Soil microbes are a diverse group of microorganisms that are multifunctional and involved in many important ecosystem processes including nutrient acquisition (Smith and Read, 2008), biogeochemical cycling (Beare et al., 1995), and soil aggregation (Rillig and Mummey, 2006). Soil microbes can have a strong influence on plant productivity by way of direct or indirect effects (van der Heijden et al., 2008). Direct effects on plant productivity occur through mutualistic or pathogenic symbiotic relationships formed between soil microbes and plants. In agricultural systems, mutualistic microbes include arbuscular mycorrhizal (AM) fungi and nitrogen-fixing bacteria that can enhance productivity of crop plants by increasing the availability and supply of limiting nutrients. Indirect effects occur via free-living soil microbes that effect nutrient and resource availability through nutrient mineralization, and competition for resources and nutrients (van der Heijden et al., 2008).

While conventional agricultural practices have drastically increased crop yields due to high mechanization and reliance on synthetic chemical inputs this has come at a high cost. These practices have significantly reduced the microbial diversity (Matson et al., 1997) and prevented the introduction and establishment of beneficial microbial populations in agricultural systems (Hart and Trevors, 2005). Diversification of cropping systems or use of ecologically sustainable agricultural practices has been suggested as a method to improve conditions for soil microbes, promoting more sustainable and resilient agricultural systems. Tree-based intercropping (TBI) or alley cropping has been promoted as an ecologically sustainable agricultural system that enables the use of standard or
conventional agricultural practices. TBI involves planting annual crops in alleys between widely spaced rows of trees.

The incorporation of trees in agricultural systems results in a higher abundance and patchier distribution of soil organic matter and roots compared to conventional monocropping (CM) systems (Thevathasan and Gordon, 1997; Lacombe et al., 2009). For example, higher soil mineral N and total C pools were observed in soils within 2.5 m distance from poplar tree rows compared to the middle of the cropping alley (Thevathasan and Gordon, 1997). In addition, the presence of trees can modify the microclimate within the cropping alleys of TBI systems. These factors combined can alter the composition of microbial communities in TBI systems (Mungai et al., 2005) as they have been strongly linked to the physical-chemical properties of soil (Franklin and Mills, 2009). Within TBI systems, fungi and bacteria are strongly influenced by trees as fungal and bacterial biomass have been shown to decrease with increasing distance from tree rows (Seiter et al., 1999). When comparing TBI and CM systems, higher microbial beta-diversity has been observed in TBI systems (Lacombe et al., 2009).

The interaction between tree and crop roots may also have an effect on AM fungi, which can play an important role in the functioning and productivity of agricultural ecosystems (Plenchette et al., 2005). Lacombe et al. (2009) found a higher abundance of AM fungi in TBI systems compared to CM systems. In addition, we found a more diverse AM fungal community in a temperate TBI system compared to a CM system, but no significant difference in abundance between the two cropping systems (Chapter 3). These studies have shown that the incorporation of trees can influence and modify the
composition of soil microbes in agricultural systems, but it is not clear how these changes functionally affect the growth and productivity of crops.

In the current study, we aimed to determine whether crops respond differently to soil microbes and more specifically to AM fungi from CM and TBI systems. To address this question we collected soil samples from CM and TBI systems at the University of Guelph Agroforestry Research Station (Guelph, Ontario, Canada), which were used for two successive greenhouse experiments. In addition, we aimed to determine whether crops responded differently to soil microbes and AM fungi from the rhizosphere of four tree species in the TBI system as trees have been shown to differentially influence AM fungal community composition in the TBI system (Chapter 2). The tree species included white ash (*Fraxinus americana* L.), silver maple (*Acer saccharinum* L.), Norway spruce (*Picea abies* L. Karst.), hybrid poplar (*Populus deltoides* x *nigra* DN-177), and control tree rows that lacked trees. As TBI systems have been found to harbour a more diverse soil microbial and AM fungal community compared to CM systems (Lacombe et al., 2009; Chapter 3), we hypothesized that crops would have a higher growth response to soil microbes and AM fungi from the TBI system.

**Materials & Methods**

*Site description*

The University of Guelph Agroforestry Research Station is a long-term TBI research site established on 30 hectares of agricultural land in Guelph, Ontario, Canada. See Chapters 2 and 3 for a detailed description of the tree-based intercropping system. The conventional monocropping (CM) fields are located directly adjacent to the TBI
system. CM fields are managed in a similar manner as the TBI system using no-till cultivation and the same crop rotation. Prior to planting trees, the CM and TBI fields had a similar cultivation history over the past half century. The fields were under hay until the mid 1970s and then cultivated with corn, soybean, and winter barley. The soil at the University of Guelph Agroforestry Research Station is classified as a Gray Brown Luvisol with a sandy-loam soil texture (65% sand, 25% silt and 10% clay; Order: Alfisols, Group: Typic Hapludalf) (Oelbermann and Voroney, 2007).

Soil sampling and analyses

Soil samples were collected from the CM and TBI systems to examine the growth response of crops to soil microbial and AM fungal communities from these two different cropping systems. The experimental design in the TBI system was a randomized complete block design with five blocks and five TBI treatments. The TBI treatments consisted of rows of at least five successive trees of i) white ash, ii) silver maple, iii) hybrid poplar, iv) Norway spruce, or v) rows without trees (control) within each block. Soils were collected systematically from each of 25 sampling locations by pooling soil cores taken along transects on either side of the tree rows at 0.5, 3.0, and 5.5 m into the cropping alleys for a total of 50 soil samples (5 TBI treatments x 5 blocks x 2 directions). Soils were also collected from five randomly selected locations in each of three different CM fields that were located adjacently to the TBI system. At each location, soil samples were collected along transects in a similar spatial pattern as the TBI system for a total of 30 soil samples (3 fields x 5 sampling locations x 2 directions). All soil samples were collected to a depth of 20 cm using a 3 cm diameter soil corer and placed into sterile bags. At each location,
soil samples collected from either half of the transect were pooled, homogenized, and stored at 4°C. Sampling took place on April 29-30, 2009 before the crop was planted in either system.

Two representative soil subsamples from each direction of the 40 sampling locations in the CM and TBI systems were pooled and homogenized before nutrient analyses of soils. Each subsample was air-dried at room temperature, sieved (< 2mm), and analyzed for soil pH, total soil carbon (Leco-combustion method), phosphorus (sodium bicarbonate extraction), calcium and magnesium (ammonium acetate extraction), and soil ammonium and nitrate (KCl extraction). We conducted the soil ammonium and nitrate analyses, while all other analyses were conducted by University of Guelph Laboratory Services.

Experiment 1: Growth response of crops to soil microbes

All soil samples were divided into two subsamples, one of which was sterilized using gamma-irradiation to 3.2 MRad, while the other half remained unsterilized. Gamma-irradiation was used to sterilize the field soil as it effectively eliminates nearly all microbes in soil, while having minimal impact on the edaphic conditions (McNamara et al., 2003). Each replicate pot (D40L Deepot, Stuewe & Sons) in the experiment consisted of a 25 ml base layer of sterile sunshine potting mix (Sun Gro Horticulture), followed by 500 ml field soil or sterile field soil/silica sand mix (1:1), and topped with a thin layer (25 ml) of Turface (Turface Athletics). Silica sand, sunshine potting mix, and Turface were sterilized by autoclaving (45 min at 121°C) twice. For each of the 160 soil types (40
sampling locations x 2 directions x 2 soil treatments) three replicate pots were prepared, one for each crop species.

Barley (*Hordeum vulgare* L.), canola (*Brassica napus* L.), and soybean (*Glycine max* L.) seeds were surface sterilized by incubating them in a 5% sodium hypochlorite solution for 1 min, 70% ethanol for 1 min, and thoroughly rinsing in sterile distilled water. Four surface sterilized seeds were placed below the Turface layer in each pot. Following emergence, seedlings were thinned to one plant per pot. Experiment 1 consisted of 480 total pots, with 160 pots of each crop species. Pots were arranged in a completely randomized design on benches in a greenhouse, with temperatures maintained at 22-24°C during the day and 16-18°C at night, with a 16 h photoperiod. Plants were watered regularly and fertilized once per week with a low phosphorus nutrient solution.

Plants were harvested after ten weeks of growth. Aboveground plant material was harvested, dried at 60°C, and weighed to determine biomass. A subsample of roots was taken from each pot, thoroughly washed to remove soil particles, chopped into small segments (approx. 2 cm), and stored in 50% ethanol. Root subsamples were then cleared in 10% potassium hydroxide and stained with Chlorazol Black E (Brundrett, 1994) to assess the level of arbuscular, vesicular, and hyphal colonization using the magnified intersects method (McGonigle et al., 1990). The remainder of the soil and roots from each pot were stored at 4°C for one week prior to being used in Experiment 2.

*Experiment 2: Growth response of crops to AM fungi*

The AM fungal component of the unsterilized field soil from the first experiment was used for a second greenhouse experiment to determine the growth response of crops
to AM fungi from the CM and TBI systems. AM fungal inoculum (i.e. spores and hyphae) was isolated from the field soil component of the pots from Experiment 1 using wet sieving and sucrose gradient centrifugation (Daniels and Skipper, 1982) for each of the 80 soil samples (40 sampling locations x 2 directions), after pooling 400 ml of substrate from each of the three replicate pots (one per crop species). Field soil samples were passed through 1000 µm, 500 µm, and 38 µm sieves, and contents of the 38 µm sieves were collected and separated from soil and organic debris using sucrose density gradient centrifugation. The supernatant containing the AM fungal spores and hyphae was decanted onto a 38 µm sieve, washed with sterile distilled water, and stored at 4°C for one week prior to inoculation. In addition, a common microbial filtrate (<38 µm) was prepared from a composite soil sample from all of the replicates to account for differences in microbial contaminants introduced with the AM fungal inoculum, 10 ml of which were added to each replicate pot.

Soil was collected from the CM and TBI systems and gamma-irradiated (to 3.2 MRad) to establish a common sterile field soil used to set-up pots as in Experiment 1. For each of the 80 AM fungal inoculums, three replicate pots were prepared, one for each crop species. In addition, 10 replicate pots for each crop species were prepared as non-mycorrhizal controls for a total of 270 pots in Experiment 2. In order to allow the AM fungal communities to establish in the pots, ‘nurse’ plants were initially planted into the pots by placing surface sterilized Trifolium pratense L., Daucus carota L., and Allium porrum L. seeds below the Turface layer in each pot. Following emergence, seedlings were thinned to one seedling of each plant species per pot, for a total of three seedlings per pot. Pots were arranged in a completely randomized design on benches in a
greenhouse, with temperatures maintained at 22-24°C during the day and 16-18°C at night, with a 16 h photoperiod. Plants were watered regularly and fertilized once every three weeks (see Experiment 1). After twelve weeks of growth, the aboveground plant material was harvested, dried at 60°C, and weighed to determine biomass.

Following the AM fungal establishment phase, four surface sterilized seeds were placed below the Turface layer in each pot to establish seedlings for each crop species (barley, canola, and soybean). Following emergence, seedlings were thinned to one plant per pot. Plants were watered regularly and fertilized once per week, and harvested after ten weeks of growth. Aboveground plant material was dried at 60°C and weighed to determine shoot biomass for each replicate. A subsample of roots was taken from each pot, thoroughly washed to remove soil particles, chopped into small segments (approx. 2 cm), and stored in 50% ethanol. Root subsamples were then cleared and stained (see Experiment 1) to assess the level of arbuscular, vesicular, and hyphal colonization using the magnified intersects method (McGonigle et al., 1990).

Data analysis

Soil nutrient content, Experiment 1, and Experiment 2 data were separately analyzed by non-parametric (Multivariate) Analysis of Variance, (M)ANOVA, using the PERMANOVA add-on package of PRIMER 6 software (www.primer-e.com). Analyses were focused on assessing the effects of soil microbes and AM fungi from a) the two cropping systems (CM and TBI), and b) the different treatments within the TBI system on plant growth and AM fungal root colonization. To test if the cropping systems had a significant effect, we used mixed models that included the random factor Plot nested
within the fixed factor Cropping System. In these analyses, the different Blocks within the TBI system, and each of the three fields in the CM system were considered as replicate plots.

For Experiment 1, the factor Cropping System was crossed with the fixed factors Soil (non-sterilized or sterilized field soil) and Crop (plant species identity) to analyze the biomass of crop shoots, and proportion of root length colonized by AM fungi (arbuscules, vesicles, and hyphae). Because soil sterilization effectively killed all AM fungal propagules, and canola is non-mycorrhizal, we also ran the same model that excluded all canola and sterilized soil samples and AM fungal colonization as response variables.

For Experiment 2, to analyze the overall effect of AM fungal inoculation on total shoot biomass of nurse plants, we analyzed a one-way fixed model to compare samples from the TBI system, CM systems, and sterilized (non-mycorrhizal) controls. To analyze the effect of AM fungal inoculation on total shoot biomass of the different crops we analyzed a similar two-way fixed model that included Crop as an additional (crossed) factor. Our main interest for these analyses was to compare the contrasts between controls and either TBI and CM, which were assessed by pairwise t-tests. To compare whether AM fungi from the TBI and CM systems had different effects on crop biomass and AM fungal root colonization, the same models as in Experiment 1 were used, omitting all control samples and nesting Plot within Cropping System, which was crossed with Crop (see above).

To analyze treatment effects within the TBI system, all samples from the CM system were excluded. For Experiment 1, data (crop biomass and AM fungal root colonization) were analyzed using a mixed model that included the random factor
“Block”, and the fixed factors TBI-treatment (5 levels: 4 tree species or no-tree controls), Soil (sterilized or not), Crop, and Direction (2 levels: the soil sample location within each block and TBI-treatment was on either side of the tree row). For Experiment 2, plant data (total nurse plant, and crop biomass) and fungal root colonization data were analyzed using the same model, but without the factor “Soil”. Because the different TBI treatments were not replicated within each Block, all interactions terms that included TBI-treatment and Block were excluded from the models. Similarly, analyses of nurse plant data omitted the factor Crop and all interactions that included the factor Crop.

If necessary variables were box-cox transformed prior to all analyses and reported $P$-values are based on 19,999 and 1,111 permutations for soil nutrient content and all other analyses, respectively. For MANOVA, where several response variables were jointly analyzed (i.e. soil nutrients, or fungal root colonization data consisting of arbuscular, vesicular, and hyphal colonization) all variables were standardized to equal variance, to give each of them equal “weight”.

**Results**

*Soil nutrient content*

To characterize the nutrient content of the soil used in the current study nine chemical properties were measured and the mean values are shown in Table 4.1. Soil from the CM and TBI systems had similar properties as there was no significant difference ($P = 0.13$) in soil nutrient content between the cropping systems. In addition, there was no significant variation ($P = 0.40$) in nutrient content of the soil among the treatments in the TBI system.
Table 4.1 Nutrient content of field soil from conventional monocropping (CM) and tree-based intercropping (TBI) systems at the University of Guelph Agroforestry Research Station and treatments within the TBI system

<table>
<thead>
<tr>
<th>Cropping system</th>
<th>Carbon (% Inorganic)</th>
<th>Carbon (% Organic)</th>
<th>Carbon (% Total)</th>
<th>pH</th>
<th>µg g soil(^1) P</th>
<th>µg g soil(^1) Mg</th>
<th>µg g soil(^1) K</th>
<th>µg g soil(^1) NO(_3^-)</th>
<th>µg g soil(^1) NH(_4^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM</td>
<td>0.76±0.15</td>
<td>1.45±0.07</td>
<td>2.21±0.11</td>
<td>7.65±0.04</td>
<td>12.9±1.2</td>
<td>408.3±13.8</td>
<td>63.3±4.5</td>
<td>3.48±0.17</td>
<td>0.76±0.23</td>
</tr>
<tr>
<td>TBI</td>
<td>1.21±0.15</td>
<td>1.36±0.05</td>
<td>2.57±0.12</td>
<td>7.70±0.02</td>
<td>12.6±1.8</td>
<td>344.4±14.6</td>
<td>71.0±7.1</td>
<td>4.03±0.16</td>
<td>0.65±0.17</td>
</tr>
<tr>
<td>TBI treatments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White ash</td>
<td>1.66±0.41</td>
<td>1.08±0.12</td>
<td>2.75±0.35</td>
<td>7.76±0.02</td>
<td>9.7±2.1</td>
<td>330.0±20.8</td>
<td>67.3±5.4</td>
<td>3.97±0.45</td>
<td>0.56±0.46</td>
</tr>
<tr>
<td>Silver maple</td>
<td>0.90±0.16</td>
<td>1.60±0.04</td>
<td>2.50±0.14</td>
<td>7.66±0.02</td>
<td>12.1±4.2</td>
<td>395.0±20.0</td>
<td>66.0±7.3</td>
<td>4.52±0.28</td>
<td>0.99±0.39</td>
</tr>
<tr>
<td>Poplar</td>
<td>1.30±0.30</td>
<td>1.42±0.08</td>
<td>2.73±0.24</td>
<td>7.68±0.04</td>
<td>15.2±6.8</td>
<td>342.5±29.7</td>
<td>71.8±8.7</td>
<td>4.37±0.29</td>
<td>0.55±0.53</td>
</tr>
<tr>
<td>Norway spruce</td>
<td>0.85±0.26</td>
<td>1.36±0.13</td>
<td>2.21±0.16</td>
<td>7.66±0.04</td>
<td>13.5±4.2</td>
<td>345.0±29.2</td>
<td>92.8±33</td>
<td>3.75±0.38</td>
<td>0.62±0.32</td>
</tr>
<tr>
<td>Control</td>
<td>1.35±0.49</td>
<td>1.33±0.11</td>
<td>2.68±0.40</td>
<td>7.74±0.05</td>
<td>12.2±2.9</td>
<td>309.3±53.0</td>
<td>57.3±6.8</td>
<td>3.53±0.31</td>
<td>0.51±0.33</td>
</tr>
</tbody>
</table>
**Crop growth response to soil microbes**

The three crop species responded significantly differently ($P < 0.001$) to the soil treatments (with and without soil microbes) (Fig. 4.1). Both barley and canola had a negative growth response to soil microbes from the CM and TBI systems. Barley had a 63% and canola a 12% mean reduction in biomass when grown in unsterilized field soil (with microbes) compared to sterilized field soil (without microbes). In contrast, soybean biomass was similar when grown in unsterilized and sterilized field soil. Cropping system (CM vs. TBI) and cropping system x soil treatment interaction had no significant effect ($P > 0.05$) on crop growth. This indicated that any variation in soil microbial communities (unsterilized soil) or abiotic soil characteristics (sterilized soil) between the CM and TBI systems had no effect on the growth of crops. Within the TBI system, TBI treatment did not have a significant effect ($P = 0.32$) on crop growth, but the TBI treatment x soil treatment x crop ($P = 0.033$) interaction term was significant (Fig. 4.2). This can be explained by the variable response of crops grown in unsterilized and sterile soil from the five TBI treatments. For example, soybean had a higher biomass when grown in unsterilized soil compared to sterile soil from the Norway spruce and poplar treatments and similar or lower biomass when grown in unsterilized soil for all other TBI treatments. In contrast, barley had a considerably lower biomass when grown in unsterilized soil compared to sterile soil from all TBI treatments.

**AM fungal colonization**

Gamma-irradiation effectively eliminated the majority of AM fungi in the sterile soil treatment. Only trace levels of arbuscules or vesicles ($< 1\%$) were observed in the
Figure 4.1. Growth response of crops to unsterilized (+ soil microbes) and sterilized (- soil microbes) field soil from conventional monocropping (CM) and tree-based intercropping (TBI) systems at the University of Guelph Agroforestry Research Station. Bars represent the mean (± standard error) shoot biomass.
Figure 4.2. Growth response of crops to unsterilized (+ soil microbes) and sterilized (- soil microbes) field soil from treatments in the TBI system at the University of Guelph Agroforestry Research Station. Bars represent the mean (± standard error) shoot biomass.
roots of crops growing in the sterile soil. Barley and soybean were well colonized by all AM fungal structures in the unsterilized soil, while only hyphal colonization was detected in canola roots (Fig. 4.3). Crop species (barley and soybean) significantly differed ($P = 0.002$) in the proportion of root length colonized by AM fungi (i.e. arbuscules, vesicles, and hyphae). However, cropping system (CM vs. TBI) had no significant effect ($P = 0.89$) on the proportion of root length colonized by AM fungi. Within the TBI system, no significant difference ($P = 0.60$) in AM fungal colonization was observed among the five treatments in the TBI site (Fig.4.4).

**Crop growth response to AM fungi**

The ‘nurse’ plants (*Trifolium pratense*, *Daucus carota*, and *Allium porrum*) grown during the establishment phase of the second experiment had a significant ($P < 0.001$) positive growth response to AM fungal inoculum from the CM and TBI systems compared to non-mycorrhizal (NM) controls (Fig. 4.5). In a pair-wise comparison, ‘nurse’ plants had a significantly ($P = 0.003$) higher biomass when inoculated with AM fungi from the CM system compared to the TBI system. In addition, ‘nurse’ plants significantly ($P < 0.001$) varied in their response to AM fungal inoculum from the TBI treatments. When inoculated with AM fungi from control (rows without trees) and Norway spruce treatments, ‘nurse’ plants had a greater biomass compared to those inoculated with AM fungi from white ash, silver maple, and poplar treatments.

Soybean was the only crop species that showed a significant growth response when inoculated with AM fungi from the CM ($P = 0.002$) and TBI ($P = 0.002$) systems
Figure 4.3. Arbuscular mycorrhizal (AM) fungal colonization of crop roots grown in unsterilized (+ soil microbes) field soil from conventional monocropping (CM) and tree-based intercropping (TBI) systems at the University of Guelph Agroforestry Research Station. Bars represent the mean (± standard error) arbuscular, vesicular, and hyphal colonization of crop roots.
Figure 4.4. Arbuscular mycorrhizal (AM) fungal colonization of crops grown in unsterilized (+ soil microbes) field soil from treatments in the TBI system at the University of Guelph Agroforestry Research Station. Bars represent the mean (± standard error) arbuscular, vesicular, and hyphal colonization of crop roots.
Figure 4.5. Growth response of ‘nurse’ plants to non-mycorrhizal controls (NM) and AM fungal inoculum from (a) conventional monocropping (CM) and tree-based intercropping (TBI) systems at the University of Guelph Agroforestry Research Station, and (b) treatments in the TBI system. Bars represent the mean (± standard error) shoot biomass.
Barley exhibited a small but not significant positive growth response when inoculated with AM fungi from the CM ($P = 0.12$) and TBI ($P = 0.46$) systems. Whereas, canola did not respond to AM fungal inoculum from either the CM ($P = 0.77$) or TBI ($P = 0.75$) system. Cropping system AM fungal inoculum (CM vs. TBI) did not have a significant effect ($P = 0.88$) on crop growth. Within the TBI system, crops showed a significantly different ($P < 0.001$) growth response to AM fungal inoculum from the TBI treatments (Fig. 4.7). Similar to the ‘nurse’ plants, crops inoculated with AM fungi from the control and Norway spruce treatments had a greater biomass compared to the other treatments.

**AM fungal colonization**

Arbuscule and vesicle colonization was absent in the NM control treatment for each crop species. Some hyphal colonization was evident in the roots from the NM control treatment but was likely from non-AM fungal propagules introduced by the microbial wash. Barley and soybean roots were well colonized by all AM fungal structures in each treatment, while only hyphal colonization was observed in canola roots (Fig. 4.8). Crop species (barley and soybean) significantly differed ($P < 0.001$) in the proportion of root length colonized by AM fungi (i.e. arbuscules, vesicles, and hyphae). Cropping system (CM vs. TBI) had a significant effect ($P = 0.014$) on the proportion of root length colonized by AM fungi. In addition, there was significant variation ($P < 0.001$) in AM fungal colonization of crop roots inoculated with AM fungi from the TBI treatments (Fig. 4.9). Barley and soybean inoculated with AM fungi from the Norway
Figure 4.6. Growth response of crops to non-mycorrhizal controls (NM) and AM fungal inoculum from conventional monocropping (CM) and tree-based intercropping (TBI) systems at the University of Guelph Agroforestry Research Station. Bars represent the mean (± standard error) shoot biomass.
Figure 4.7. Growth response of crops to non-mycorrhizal controls (NM) and AM fungal inoculum from treatments in the TBI system at the University of Guelph Agroforestry Research Station. Bars represent the mean (± standard error) shoot biomass.
Figure 4.8. Arbuscular mycorrhizal (AM) fungal colonization of crop roots inoculated with AM fungi from conventional monocropping (CM) and tree-based intercropping (TBI) systems at the University of Guelph Agroforestry Research Station. Bars represent the mean (± standard error) arbuscular, vesicular, and hyphal colonization of crop roots.
Figure 4.9. Arbuscular mycorrhizal (AM) fungal colonization of crops inoculated with AM fungi from treatments in the TBI system at the University of Guelph Agroforestry Research Station. Bars represent the mean (± standard error) arbuscular, vesicular, and hyphal colonization of crop roots.
The spruce treatment had the lowest proportion of root length colonized by arbuscules and vesicles among the TBI treatments.

**Discussion**

Previous studies have found that TBI systems support more diverse microbial and AM fungal communities compared to CM systems (Lacombe et al. 2009; Chapter 3). In this study, we aimed to determine whether differences in soil microbial and AM fungal communities from these contrasting cropping systems have a functional effect on the growth of crops. We found that (1) crop species responded differently to soil microbes and AM fungi, (2) cropping system (CM vs. TBI) had no effect on the growth response of crops to soil microbes and AM fungi, and (3) AM fungi from both cropping systems had a positive effect on crop growth.

The crop species exhibited a range of growth responses to soil microbes from the cropping systems. Soil microbes from both cropping systems strongly reduced the growth of barley by an average of 63%, slightly reduced the growth of canola by 12%, and had no effect on soybean. The negative response of barley suggests that pathogenic microbes in the soil had a stronger effect on the growth of barley than mutualistic microbes, even though roots were well colonized by AM fungi. Although the AM fungal symbiosis can be beneficial to many plant or crop species, barley does not appear to benefit greatly from this relationship. When inoculated with AM fungi from the two cropping systems in the second experiment, we found that barley was unresponsive despite the positive growth response of soybean and ‘nurse’ plants to AM fungal inoculation. Similarly, previous studies have found that AM fungal inoculation has no effect (Jensen, 1984) or causes a
growth reduction in barley (Khaliq and Sanders, 2000; Grace et al., 2009). However, other studies have found that when available soil phosphorus contents are below 10-20 mg kg\(^{-1}\), barley can respond positively to AM fungal colonization (Clarke and Moss, 1981; Jakobsen and Jensen, 1981; Powell, 1981; Jensen, 1982). The phosphorus content of the soil used for our experiments was within or below this critical range for mycorrhizal responsiveness, however, we failed to see any effect on barley. This study further supports the observation that barley is nonresponsive to AM fungi.

The slightly negative response of canola may also have been due to a direct effect of pathogenic microbes. Canola does not form a symbiotic relationship with AM fungi or nitrogen-fixing bacteria. However, a small proportion (>10%) of canola roots were colonized by AM fungal hyphae, but arbuscules and vesicles were absent. Other studies have also found low levels of hyphal colonization of canola roots (Marschner and Timonen, 2005; Solaiman et al., 2007). In fact, Marschner and Timonen (2005) found that low levels of hyphal colonization by *Glomus intraradices* increased biomass of canola compared to uncolonized plants, while *Glomus versiforme* colonization decreased canola biomass. In contrast, we found that canola was nonresponsive to AM fungal colonization in the second experiment supporting the observation that canola does not form a functional symbiosis with AM fungi (Solaiman et al., 2007).

Soybean was nonresponsive to soil microbes as similar biomass was found in both unsterilized and sterilized field soil from both cropping systems. Unlike the other crops, soybean roots benefit from being able to form a symbiotic association with both AM fungi and nitrogen-fixing bacteria. In this study we observed the presence of both AM fungal colonization and nodules on soybean roots. This tripartite symbiosis generally has a
positive effect on plant growth through a synergistic effect on nutrient supply (Meghvansi et al., 2008). AM fungi from both cropping systems had a positive effect on soybean as evidenced by the second experiment in this study, and typically dual inoculation with nitrogen-fixing bacteria and AM fungi increases plant growth to a greater extent than inoculation with only one (Chalk et al., 2006). However, any positive benefits that soybean gained from the tripartite symbiosis in the first experiment may have been negated by pathogenic microbes.

The original hypothesis of this study was rejected as there were no discernable differences in crop growth response to soil microbes and AM fungi from the CM and TBI systems. This is interesting given that previous studies have observed significant differences in microbial and AM fungal communities in these contrasting cropping systems, particularly at the University of Guelph Agroforestry Research Station (Lacombe et al., 2009; Chapter 3). Soil microbes play key roles in ecosystem processes and directly influence the productivity of plant communities; yet only a few studies have directly examined the effect of microbial diversity on plant productivity (van der Heijden et al., 2008). Two separate studies found that increasing AM fungal diversity had a positive effect on plant productivity (van der Heijden et al., 1998; Maherali and Klironomos, 2007), while another study found no effect (van der Heijden et al., 2006). A possible explanation for the lack of effect is that van der Heijden et al. (2006) used AM fungal taxa that belonged to one family (Glomeraceae). Maherali and Klironomos (2007) found that plant productivity was not stimulated (similar to nonmycorrhizal controls) when AM fungal taxa belonged to a single family, but increased when taxa from two complementary families were present.
The lack of functional complementarity among the AM fungal taxa may explain why no significant differences in crop growth response to AM fungal communities from the two cropping systems were observed in this study. In an earlier study, we found that the TBI system had a higher AM fungal richness compared to the CM system (Chapter 3). We hypothesized that the higher AM fungal richness in the TBI system would have a positive effect on crop productivity compared to the CM system. However, we rejected this hypothesis as crops exhibited similar growth responses to the AM fungal communities from both systems. This is likely due to the lack of functional complementarity among the AM fungi in both systems as all taxa belonged to the Glomeraceae. This may also explain the lack of differences in crop growth responses to AM fungal communities from the five TBI treatments. Variation in AM fungal community composition among treatments in the TBI system have been observed (Chapter 2; Chapter 3), but all taxa belonged to the Glomeraceae. Any differences in community composition or richness may be redundant as all taxa within this family generally have similar functional traits, which include high levels of root colonization that aid in pathogen protection (Maherali and Klironomos, 2007).

While no significant variation in the response of crops to AM fungi from the CM and TBI systems were observed, the ‘nurse’ plants did respond differently. Unexpectedly, the ‘nurse’ plants had a higher biomass when inoculated with AM fungi from the CM system compared to the TBI system. In addition, AM fungi from the Norway spruce treatment stimulated the greatest growth response in the ‘nurse’ plants compared to the other TBI treatments. The CM system and Norway spruce treatment from the TBI system both had a lower AM fungal richness and more similar AM fungal community
compositions compared to the other treatments in the TBI system (Chapter 3). It is unclear why lower AM fungal richness would stimulate higher productivity unless the TBI system harboured taxa that were parasitic or less beneficial.

Overall, the AM fungi within both cropping systems had a positive effect on plant productivity. The ‘nurse’ plant biomass increased by 101% and 77% over NM controls when inoculated with AM fungi from the CM and TBI systems, respectively. In addition, crops that formed a functional symbiosis with AM fungi showed a positive growth response to AM fungi from both cropping systems. These are encouraging results as agricultural systems are generally thought to harbour AM fungi that are inferior mutualists (Johnson, 1993; Oehl et al., 2003). Future research should focus on further investigating and increasing the functional diversity of microbial communities to promote more resilient and sustainable agricultural systems.
Literature Cited


colonization, fungal phosphorus uptake or effects on expression of plant phosphate transporter genes. New Phytol. 181:938-949.


CHAPTER FIVE:

SYNTHESIS AND CONCLUSIONS
AM fungi play an important functional role in agricultural ecosystems, yet little is known about the community dynamics of AM fungi in tree-based intercropping (TBI) systems. This thesis sought to illuminate several aspects of AM fungi in temperate TBI systems, including community composition, spatial and temporal variation, and the functional response of crops to AM fungi. Through a thorough review of the literature, Chapter 1 reveals that TBI systems generally have a positive effect on AM fungi as compared to conventional monoculture (CM) systems, by having a more abundant and diverse AM fungal community. However, the majority of these studies were in tropical regions, which have distinct tree and crop combinations, climatic conditions, and cultivation methods. In addition, these studies used classical techniques to assess the AM fungal communities within agroforestry systems, which cannot accurately represent the AM fungal community in a site. Identification of AM fungal species using classical methods is based solely on AM fungal spores, and Glomeromycotan sequence data suggests that the described species (based on spore morphology) only represent a small fraction of the diversity of AM fungi (Helgason and Fitter, 2009). These factors, along with the limited number of studies (two) on AM fungi in temperate TBI systems, indicate a need for research focusing on AM fungi in temperate TBI systems.

The University of Guelph Agroforestry research station provides a good location to conduct research and compare TBI and CM systems. The two systems are directly adjacent to each other, allowing for similar soil types and climatic variables such as precipitation and temperature. Prior to the establishment of the TBI system in 1987, the land in both agricultural systems had a similar cultivation history for the previous 30
years. In addition, both systems have been managed in a similar manner, including the implementation of tillage, fertilizer, pest management, and cropping regimes.

To begin exploring the role of AM fungi in temperate TBI systems, Chapter 2 focuses on the influence of different tree species on the community structure of AM fungi. Molecular (i.e. cloning and sequencing, and terminal restriction fragment length polymorphisms) tools were utilized to characterize AM fungal communities found in soil and roots in close proximity to three different tree species in the TBI system. We found that there are compositional differences in AM fungal communities based on the tree species. White ash and poplar appeared to have no effect on AM fungal communities, as the communities were similar to the control treatment (where no trees were growing in the TBI site). However, Norway spruce had a negative influence on AM fungal communities, as evidenced by reduced AM fungal richness in areas of the intercropping alley adjacent to Norway spruce trees.

Once we established that AM fungal communities differed within the TBI system, we also wanted to determine how AM fungi in the TBI system differ from CM systems. In Chapter 3 we investigated the effect of TBI systems on both the abundance and diversity of AM fungi compared to the CM system. Previous studies have found that increasing plant diversity has a positive effect on AM fungal communities, suggested to be due to the higher number of possible host-fungal pairings and increased density of plant roots available for colonization (Burrows and Pfleger, 2002). Alternatively, soil chemistry has been suggested to have the strongest effect on AM fungal diversity or compositional variation (Dumbrell et al. 2010; 2011). Both of these factors could have a role in TBI systems. We found different AM fungal communities and richness between the TBI and
CM systems, but all measures of AM fungal abundance (i.e. spore density, hyphal length, and root colonization) were similar in both systems throughout the growing season. Due to the similar cultivation history and soil chemistry (Chapter 4) in the CM and TBI systems, our results suggest that incorporation of tree rows into the cropping system supports a more diverse AM fungal community. In addition, the initial planting of trees into the TBI system may have introduced “new” AM fungal taxa and accounted for differences in richness and composition between the two cropping systems. However, it may not be trees alone that influence AM fungal communities, as there is a diverse weed community throughout the tree rows of the TBI system. In general, the diversity of weed communities in TBI systems is comparable to CM systems (Kotey 1996), but the tree rows appear to harbour a more permanent weed community (personal observation). The contribution of weeds to the AM fungal community in TBI systems warrants further investigation to understand the AM fungal-weed dynamics in these systems.

The significance of variation in AM fungal community composition and richness to crop productivity is unclear. In Chapter 4, we aimed to determine if compositional differences between the CM and TBI systems had a functional effect on the growth of crops. Overall, there were no discernable differences in crop growth response to AM fungi from the two cropping systems. The similar growth response of crops to AM fungal communities from the CM and TBI systems may be due to the lack of functional complementarity among the AM fungi. All AM fungal taxa from the CM and TBI systems belonged to the Glomeraceae, which generally provide a similar function to the host plant (i.e. pathogen protection) (Maherali and Klironomos, 2007). These results further confirm that increased diversity within an AM fungal functional group has little effect on plant
productivity. Despite the lack of differences between the CM and TBI systems, we found that AM fungi from both cropping systems had a positive effect on plant growth. These results are in contrast to previous studies that suggest agricultural systems harbour AM fungal taxa that are inferior mutualists (Johnson, 1993; Oehl et al., 2003).

A number of different limitations were encountered during the research of this thesis. Due to the original design of the Guelph TBI system, the CM system could not be properly replicated into the experimental design. Samples collected within the CM system were not independent and therefore pseudo-replicated. A proper experimental design would incorporate CM treatments into each block of the TBI system, and sampling would occur at multiple TBI systems or geographic locations. Additionally, the CM system was classified as the control system to determine the effect of trees on AM fungi in agricultural systems. However, an alternative control could have included a monoculture forest plantation system, to determine the effect of intercropping trees and crops together on AM fungi. Another limitation included the primers (FLR3 and FLR4) used for the molecular analysis of AM fungal communities in this thesis. These primers are AM fungal specific, but they may not amplify sequences of all AM fungal groups equally (Mummey and Rillig, 2007; Gamper et al., 2009). However, these primers have successfully amplified AM fungi from each of the major Glomeromycotan groups (Gollotte et al., 2004; Mummey and Rillig, 2007; Schnoor et al., 2011). Despite these limitations, the research from this thesis provides valuable insight into the effect of TBI systems on AM fungi.

The results from this thesis support the assertion that AM fungi play a beneficial role in TBI systems. This research also provides valuable insights into possible
management strategies regarding the development and maintenance of a thriving AM fungal community in TBI systems. It appears that utilizing tree species that associate with AM fungi will at least maintain or have no effect on AM fungal communities, as opposed to having a negative effect as shown by Norway spruce trees. Future research should focus on the effect of trees that form a tripartite symbiosis with N-fixing bacteria and AM fungi in temperate TBI systems. These are common tree species planted in tropical regions and generally have a positive effect on AM fungal communities (Bainard et al. 2011). Additionally, many of the tree species planted in temperate TBI systems form an association with ectomycorrhizal fungi (e.g. Picea, Pinus, Populus, Salix species). Future research should determine whether TBI systems have the ability to maintain healthy ectomycorrhizal communities to ensure maximum productivity of these trees species.

From a broader perspective, more research should be pursued that addresses the fundamental relationship between AM fungi and plant species in agricultural systems. More specifically, do TBI systems maintain AM fungal diversity while CM systems reduce AM fungal diversity? Future research could investigate whether adding new AM fungal taxa, including those from other functional groups, be better maintained in TBI systems as compared to CM systems. Another over-arching question revolves around the broad impact of increased AM fungal richness and diversity on agricultural systems. From an ecological perspective, higher species diversity is often considered more desirable, but within agricultural systems, does increased AM fungal diversity actually benefit crops or the system as a whole in terms of productivity? While previous studies have indicated that increasing AM fungal diversity generally has a positive effect on plant productivity, this still remains to be explored in TBI systems.
Overall, the research from this thesis provides the most detailed molecular analysis of AM fungal communities in a TBI system, and provides a strong foundation upon which further research can be built to improve the productivity and sustainability of agricultural systems.
Literature cited


