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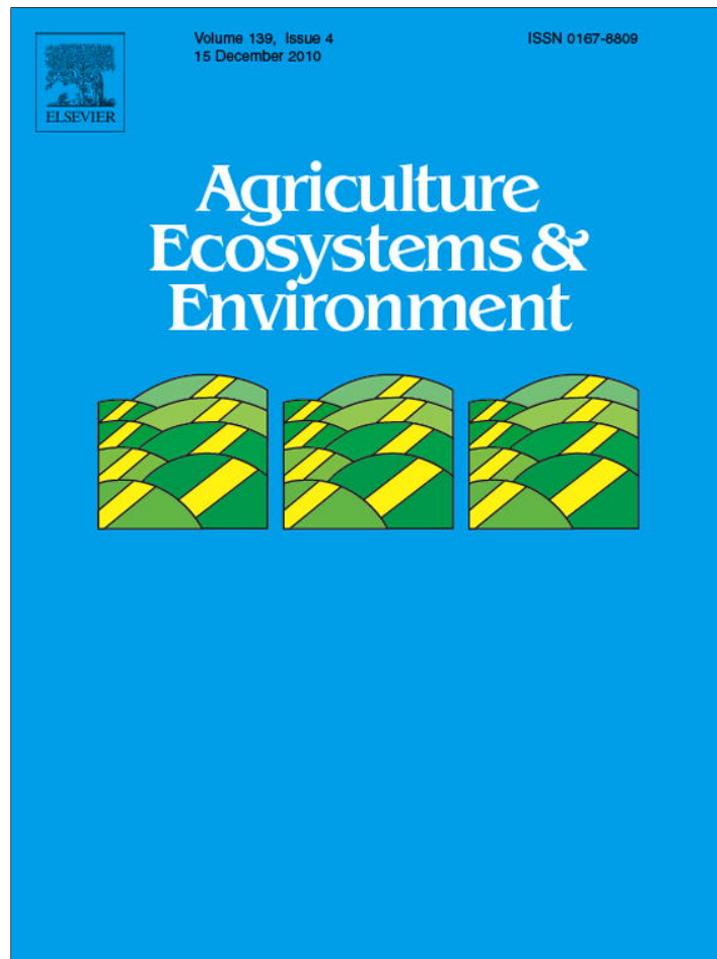
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Influence of trees on the spatial structure of arbuscular mycorrhizal communities in a temperate tree-based intercropping system

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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 deltoids* × *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 in 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 in 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.

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1. 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, 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

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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 site. The tree species included white ash (*Fraxinus americana* L.), Norway spruce (*Picea abies* L. Karst.), and hybrid poplar (*Populus deltoids* × *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.

2. Materials and methods

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. 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) (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 or 15 m apart, and trees within a row are spaced 3 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, 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 × 2 replicate trees × 2 directions) and 48 sampling locations in the intercropping alleys (4 blocks × 2 replicate trees × 3 distances × 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.

2.1. 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 either side 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.

2.2. 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.

2.3. 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 h, 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.

Table 1

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

Factor	Intercropping alleys						Tree rows					
	Phylotype richness			Community			Phylotype richness			Community		
	df	F	P	df	F	P	df	F	P	df	F	P
Block	3	6.7807	0.0081	3	2.8889	0.0011	3	1.7356	0.2097	3	0.9472	0.5640
Treatment	3	5.5031	0.0196	3	1.8906	0.0103	3	35.770	0.0021	3	4.3267	0.0018
Distance	2	1.1798	0.3650	2	1.1868	0.3305						
Sample type	1	283.27	0.0005^a	1	33.456	0.0297^a	1	51.123	0.0058^a	1	14.151	0.0002^a
Replicate (treatment × block)	16	0.8458	0.6294	16	0.8895	0.7477	16	0.5999	0.8245	16	0.8257	0.8947
Block × distance	6	1.6278	0.1773	6	1.1021	0.0004						
Block × sample type	3	0.6743	0.5921	3	2.1648	0.0021	3	0.9521	0.4431	3	0.8316	0.7334
Treatment × distance	6	3.9993	0.0037	6	1.3285	0.0816						
Treatment × sample type	3	0.5176	0.6858	3	1.2624	0.1814	3	15.047	0.0001	3	2.1286	0.0019
Distance × sample type	2	0.0291	0.9715	2	1.2115	0.3150						
Block × distance × sample type	6	1.0059	0.4256	6	0.7503	0.9269						
Treatment × distance × sample type	6	0.3328	0.9189	6	0.7582	0.9225						

^a P-value based on a Monte Carlo procedure where the number of unique permutations is less than 500.

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 (Hijiri 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 TRAMP 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 deposited in 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 neighbor-joining and maximum likelihood algorithms implemented in the programs MEGA 5.0 and TOPALi (v. 2.5), respectively.

2.4. 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.primers-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 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.

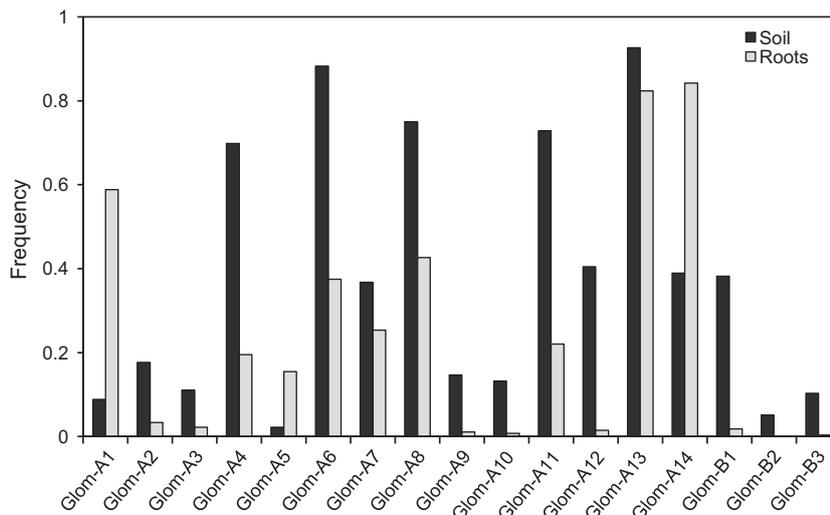


Fig. 1. Distribution of arbuscular mycorrhizal (AM) fungal phylotypes detected in soil and root samples within the four treatments at the University of Guelph tree-based intercropping (TBI) site.

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, Gleneden 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.

3. Results

3.1. 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. S1). 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. S2).

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 (*G. viscosum*), and Glom-A14 (*G. intraradices*) (Fig. 1). Glom-A9 (*G. mosseae*) and Glom-B3 (*G. 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.

3.2. 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 1). Soil sam-

ples had a significantly higher phylotype richness compared to root samples in tree rows ($P=0.0058$) and intercropping alleys ($P=0.0005$). 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. 1) in both the tree rows ($P=0.0002$) and intercropping alleys ($P=0.0297$). 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). 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 (*G. 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 (*G. viscosum*), was common in both soil (93%) and root (82%) samples.

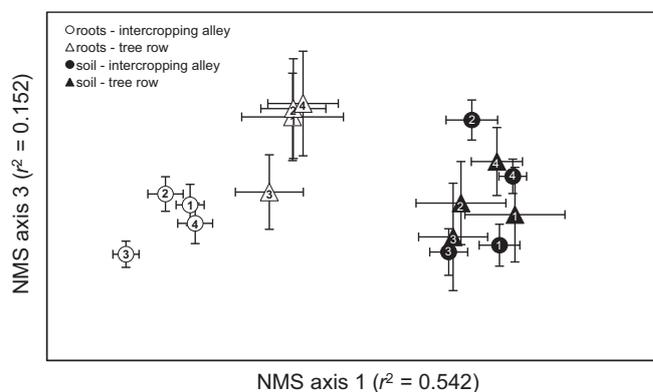


Fig. 2. Nonmetric multidimensional scaling (NMS) ordination of arbuscular mycorrhizal (AM) fungal communities in soil (closed symbols) and root samples (open symbols) at the University of Guelph tree-based intercropping (TBI) site. Ordination was based on the AM fungal community detected in each sample. Symbols are the average (± 1 SE) ordination coordinates of samples in each block. 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.

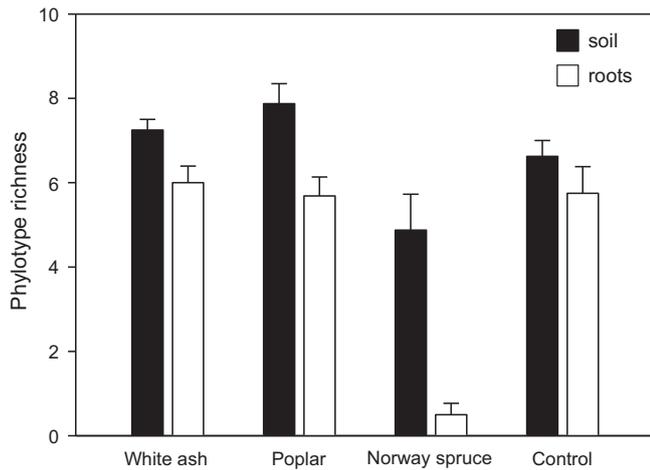


Fig. 3. Arbuscular mycorrhizal (AM) fungal phylotype richness in tree rows at the University of Guelph tree-based intercropping (TBI) site.

3.3. Effect of trees on AM fungal community

AM fungal phylotype richness significantly differed ($P=0.0058$) among the treatments within the tree rows. Roots of white ash, poplar, and the weed community (control) had a similar phylotype richness, while AM fungi were rarely detected in Norway spruce roots (Fig. 3). 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 ($P=0.0196$) and the interaction of treatment by distance ($P=0.0037$) had a significant effect. Similar phylotype richness was observed in the intercropping alleys of white ash, poplar, and control treatments (Fig. 4). 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 phylotype richness observed in the intercropping alleys. Samples collected at 3 and 5.5 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

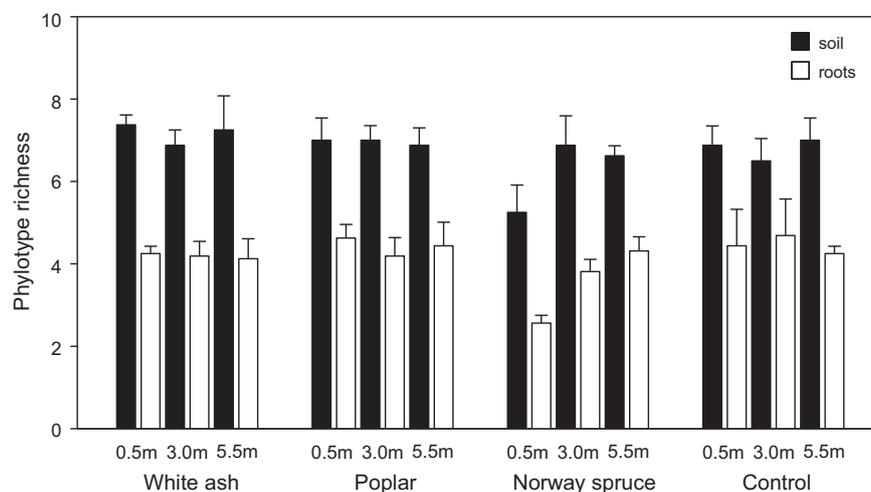


Fig. 4. Arbuscular mycorrhizal (AM) fungal phylotype richness in intercropping alleys at the University of Guelph tree-based intercropping (TBI) site.

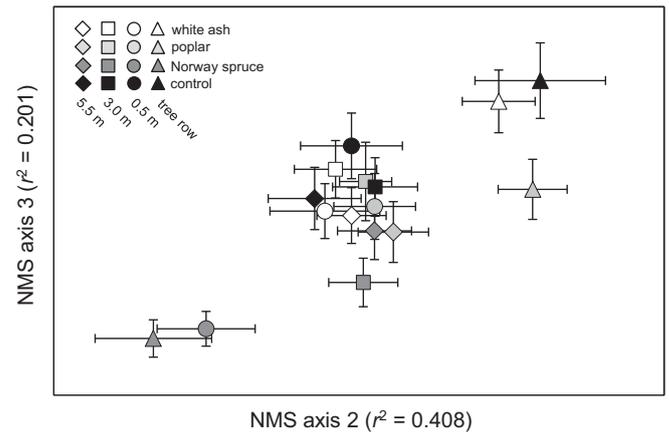


Fig. 5. Patterns of arbuscular mycorrhizal (AM) fungal community composition in roots at the University of Guelph tree-based intercropping (TBI) site based on non-metric multidimensional scaling (NMS) ordination. Ordination was based on the AM fungal community detected in each sample. Symbols are the 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.

richness observed in the intercropping alleys ($P=0.0081$), but not in the tree rows ($P=0.2097$) of the TBI site.

The AM fungal community composition in the tree rows was significantly affected ($P=0.0018$) by treatment. AM fungal communities detected in roots of white ash, poplar, Norway spruce, and the weed community (control) showed some separation in the NMS analysis (Fig. 5). Among the treatments, there was separation between AM fungal communities in Norway spruce tree rows and the other treatments along axis 2 ($r^2=0.408$) and 3 ($r^2=0.201$). NMS analysis of AM fungal communities detected in soil samples revealed a similar trend (Fig. 6). AM fungal communities in 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 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 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. 5 and 6). Norway spruce trees appear to influence the AM fungal community in the intercropping alley

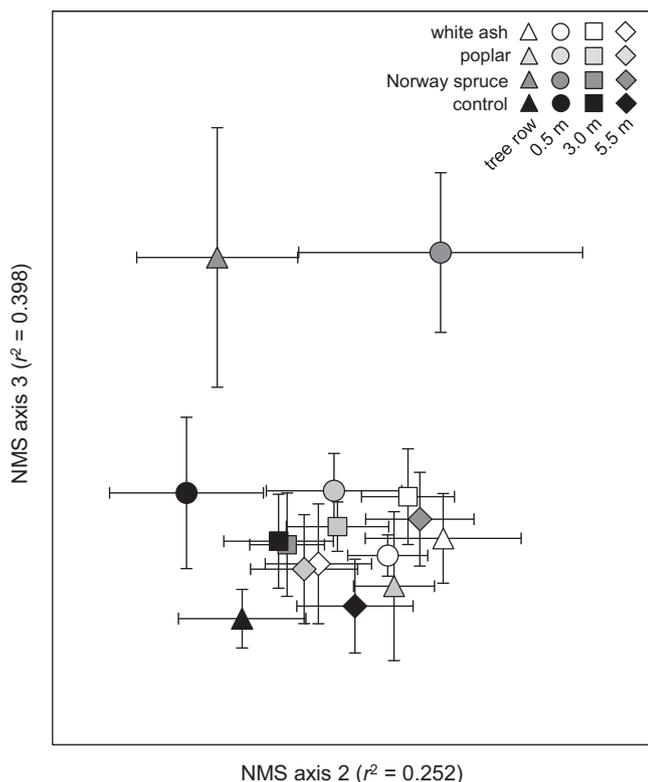


Fig. 6. Patterns of arbuscular mycorrhizal (AM) fungal community composition in soil at the University of Guelph tree-based intercropping (TBI) site based on non-metric multidimensional scaling (NMS) ordination. Ordination was based on the AM fungal community detected in each sample. Symbols are the 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.

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 m and 5.5 m into the intercropping alley had similar AM fungal communities as the other treatments. In addition, block had a significant effect ($P=0.0011$) on 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 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.

4.1. 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 Pflieger, 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 *G. mosseae*, *G. geosporum*, and *G. 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 Chiffot 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 (Chiffot 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 (Unpublished data).

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).

4.2. 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 soil samples. Glom-A14 (*G. intraradices*) was the most frequently detected (84%) phylotype in roots, but only detected in 39% of 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.

4.3. Effect of trees on AM fungal community

There were differences in the mycorrhizal status and associated AM fungal communities among 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 poplar trees.

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).

AM fungal community composition in the intercropping alleys was also affected by trees, but to varying degrees. White ash and poplar had no effect on community composition in the intercropping alleys, while intercropping alleys closest to the Norway spruce tree row (0.5 m) showed decreased AM fungal richness and a different AM fungal community. As the distance increased away from the Norway spruce tree row (3.0 and 5.5 m from the tree row), the AM fungal community composition and richness was similar to the rest of the TBI site. Additionally, AM fungal richness and composition in intercropping alleys adjacent to white ash and poplar tree rows was 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. This study provides evidence to suggest that utilizing tree species that form an association with AM fungi will support a diverse AM fungal community throughout TBI systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.agee.2011.07.014.

References

- Arihara, J., Karasawa, T., 2000. Effect of previous crops on arbuscular mycorrhizal formation and growth of succeeding maize. *Soil Sci. Plant Nutr.* 46, 43–51.
- Auge, R.M., 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11, 3–42.
- Bainard, L.D., Klironomos, J.N., Gordon, A.M., 2011a. Arbuscular mycorrhizal fungi in tree-based intercropping systems: a review of their abundance and diversity. *Pedobiologia* 54, 57–61.
- Bainard, L.D., Klironomos, J.N., Gordon, A.M., 2011b. The mycorrhizal status and colonization of 26 tree species growing in urban and rural environments. *Mycorrhiza* 21, 91–96.
- Burrows, R.L., Pfeiler, F.L., 2002. Arbuscular mycorrhizal fungi respond to increasing plant diversity. *Can. J. Bot.* 80, 120–130.
- Cesaro, P., van Tuinen, D., Copetta, A., Chatagnier, O., Berta, G., Gianinazzi, S., Lingua, G., 2008. Preferential colonization of *Solanum tuberosum* L. roots by the fungus *Glomus intraradices* in arable soil of a potato farming area. *Appl. Environ. Microb.* 74, 5776–5783.
- Chiffot, V., Rives, D., Olivier, A., Cogliastro, A., Khasa, D., 2009. Molecular analysis of arbuscular mycorrhizal community structure and spores distribution in tree-based intercropping and forest systems. *Agr. Ecosyst. Environ.* 131, 32–39.
- Clapp, J.P., Young, J.P.W., Merryweather, J.W., Fitter, A.H., 1995. Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. *New Phytol.* 130, 259–265.
- Dickie, I.A., Xu, B., Koide, R.T., 2002. Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytol.* 156, 527–535.
- Dickie, I.A., FitzJohn, R.G., 2007. Using terminal restriction fragment length polymorphism (T-RFLP) to identify mycorrhizal fungi: a methods review. *Mycorrhiza* 17, 259–270.
- Douds, D.D., Millner, P.D., 1999. Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. *Agr. Ecosyst. Environ.* 74, 77–93.
- Dougherty, M.C., Thevathasan, N.V., Gordon, A.M., Lee, H., Kort, J., 2009. Nitrate and *Escherichia coli* NAR analysis in tile drain effluent from a mixed tree intercrop and monocrop system. *Agr. Ecosyst. Environ.* 131, 77–84.
- Galvan, G.A., Paradi, L., Burger, K., Baar, J., Kuyper, T.W., Scholten, O.E., Kik, C., 2009. Molecular diversity of arbuscular mycorrhizal fungi in onion roots from organic and conventional farming systems in the Netherlands. *Mycorrhiza* 19, 317–328.
- Gamper, H.A., Walker, C., Schussler, A., 2009. *Diversispora celata* sp. nov.: molecular ecology and phylotaxonomy of an inconspicuous arbuscular mycorrhizal fungus. *New Phytol.* 182, 495–506.
- Gollotte, A., van Tuinen, D., Atkinson, D., 2004. Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaries* and *Lolium perenne* in a field experiment. *Mycorrhiza* 14, 111–117.
- Helgason, T., Daniell, T.J., Husband, R., Fitter, A.H., Young, J.P.W., 1998. Ploughing up the wood-wide web? *Nature* 394, 431.
- Helgason, T., Fitter, A.H., 2009. Natural selection and the evolutionary ecology of the arbuscular mycorrhizal fungi (Phylum Glomeromycota). *J. Exp. Bot.* 60, 2465–2480.
- Hempel, S., Renker, C., Buscot, F., 2007. Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem. *Environ. Microbiol.* 9, 1930–1938.
- Hijri, I., Sykora, Z., Oehl, F., Ineichen, K., Mader, P., Wiemken, A., Redecker, D., 2006. Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. *Mol. Ecol.* 15, 2277–2289.
- Horton, T.R., Cazares, E., Bruns, T.D., 1998. Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire. *Mycorrhiza* 8, 11–18.
- Jose, S., Gillespie, A.R., Seifert, J.R., Biehle, D.J., 2000. Defining competition vectors in a temperate alley cropping system in the Midwestern USA. 2. Competition for water. *Agroforestry Syst.* 48, 41–59.
- Jose, S., Gillespie, A.R., Pallardy, S.G., 2004. Interspecific interactions in temperate agroforestry. *Agroforestry Syst.* 61, 237–255.
- Karasawa, T., Kasahara, Y., Takebe, A., 2002. Differences in growth responses of maize to preceding cropping caused by fluctuation in the population of indigenous arbuscular mycorrhizal fungi. *Soil Biol. Biochem.* 34, 851–857.
- Kruskal, J.B., 1964. Non-metric multidimensional scaling: a numerical method. *Psychometrika* 29, 115–129.
- Lacombe, S., Bradley, R.L., Hamel, C., Beaulieu, C., 2009. Do tree-based intercropping systems increase the diversity and stability of soil microbial communities? *Agr. Ecosyst. Environ.* 131, 25–31.
- Malloch, D., Malloch, B., 1981. The mycorrhizal status of boreal plants: species from northeastern Ontario. *Can. J. Bot.* 59, 2167–2172.
- Malloch, D., Malloch, B., 1982. The mycorrhizal status of boreal plants: additional species from northeastern Ontario. *Can. J. Bot.* 60, 1035–1040.

- McCune, B., Mefford, M.J., 2006. PC-ORD. Multivariate Analysis of Ecological Data (Version 5). MjM Software Design, Gleneden Beach, OR.
- Minchin, P.R., 1987. An evaluation of the relative robustness of techniques for ecological ordination. *Vegetatio* 69, 89–107.
- Mumme, D.L., Rillig, M.C., 2007. Evaluation of LSU rRNA-gene PCR primers for analysis of arbuscular mycorrhizal fungal communities via terminal restriction fragment length polymorphism analysis. *J. Microbiol. Methods* 70, 200–2004.
- Mungai, N.W., Motavalli, P.P., Kremer, R.J., Nelson, K.A., 2005. Spatial variation of soil enzyme activities and microbial diversity in temperate alley cropping systems. *Biol. Fert. Soils* 42, 129–136.
- Newsham, K.K., Fitter, A.H., Watkinson, A.R., 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *J. Ecol.* 83, 991–1000.
- Oehl, F., Sieverding, E., Ineichen, K., Mader, P., Boller, T., Wiemken, A., 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of central Europe. *Appl. Environ. Microbiol.* 69, 2816–2824.
- Oehl, F., Sieverding, E., Ineichen, K., Mader, P., Wiemken, A., Boller, T., 2009. Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long-term microcosms. *Agr. Ecosyst. Environ.* 134, 257–268.
- Oelbermann, M., Voroney, R.P., 2007. Carbon and nitrogen in a temperate agroforestry system: using stable isotopes as a tool to understand soil dynamics. *Ecol. Eng.* 29, 342–349.
- Plenchette, C., Clermont-Dauphin, C., Meynard, J.M., Fortin, J.A., 2005. Managing arbuscular mycorrhizal fungi in cropping systems. *Can. J. Plant Sci.* 85, 31–40.
- Price, G.W., Gordon, A.M., 1999. Spatial and temporal distribution of earthworms in a temperate intercropping system in southern Ontario, Canada. *Agroforest. Syst.* 44, 141–149.
- Sasvari, Z., Posta, K., 2010. Effect of different plant densities on the diversity of arbuscular mycorrhizal fungi community in a long-term maize monocrop system. *Span. J. Agric. Res.* 8, S123–S130.
- Sasvari, Z., Hornok, L., Posta, K., 2011. The community structure of arbuscular mycorrhizal fungi in roots of maize grown in a 50-year monoculture. *Biol. Fert. Soils* 47, 167–176.
- Schnoor, T.K., Lekberg, Y., Rosendahl, S., Olsson, P.A., 2011. Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland. *Mycorrhiza* 21, 211–220.
- Seiter, S., Ingham, E.R., William, R.D., 1999. Dynamics of soil fungal and bacterial biomass in a temperate climate alley cropping system. *Appl. Soil Ecol.* 12, 139–147.
- Smith, J.E., Johnson, K.A., Cazares, E., 1998. Vesicular mycorrhizal colonization of seedlings of Pinaceae and Betulaceae after spore inoculation with *Glomus intradices*. *Mycorrhiza* 7, 279–285.
- Smith, S.E., Read, D.J., 2008. *Mycorrhizal Symbiosis*, 3rd ed. Academic Press, New York, NY.
- Stamps, W.T., Linit, M.J., 1998. Plant diversity and arthropod communities: implications for temperate agroforestry. *Agroforest. Syst.* 39, 73–89.
- ter Braak, C.J.F., 1998. *CANOCO Reference Manual and User's Guide to CANOCO for Windows*. Center for Biometry, Wageningen, The Netherlands.
- Thevathasan, N.V., Gordon, A.M., 2004. Ecology of tree intercropping systems in the North temperate region: experiences from southern Ontario, Canada. *Agroforest. Syst.* 61, 257–268.
- Toljander, J.F., Santos-Gonzalez, J.C., Tehler, A., Finlay, R.D., 2008. Community analysis of arbuscular mycorrhizal fungi and bacteria in the maize mycorrhizosphere in a long-term fertilization trial. *FEMS Microbiol. Ecol.* 65, 323–338.
- Trouvelot, S., van Tuinen, D., Hijri, M., Gianinazzi-Pearson, V., 1999. Visualization of ribosomal DNA loci in spore interphasic nuclei of glomalean fungi by fluorescence in situ hybridization. *Mycorrhiza* 8, 203–206.
- van Tuinen, D.E., Jacquot, E., Zhao, B., Gollotte, A., Gianinazzi-Pearson, V., 1998. Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. *Mol. Ecol.* 7, 879–887.
- Wagg, C., Pautler, M., Massicotte, H.B., Peterson, R.L., 2008. The co-occurrence of ectomycorrhizal, arbuscular mycorrhizal, and dark septate fungi in seedlings of four members of the Pinaceae. *Mycorrhiza* 18, 103–110.
- Wilde, P., Manal, A., Stodden, M., Sieverding, E., Hildebrandt, U., Bothe, H., 2009. Biodiversity of arbuscular mycorrhizal fungi in roots and soils of two salt marshes. *Environ. Microbiol.* 11, 1548–1561.