Molecular analysis of arbuscular mycorrhizal community structure and spores distribution in tree-based intercropping and forest systems

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ABSTRACT

Due to their potential to enhance soil biodiversity and fertility, tree-based intercropping (TBI) systems are recognized as promising agrotechnologies. However, few studies have examined the effects of TBI on the diversity of arbuscular mycorrhizal (AM) fungi. To investigate this aspect, and to compare TBI with a more typical forest plantation (FO) system, a field experiment using soybean (Glycine max (L.) Merr. cv. SO3-W4) and poplar (Populus nigra L. x P. maximowiczii cv. A. Henry ‘Max 5’) was conducted on an arable field site in southwestern Québec. The species of AM fungi colonizing the roots were assessed by PCR-RFLP and DNA sequence analyses of SSU rRNA genes. A total of 13 different phylotypes were identified.

Phylogenetic analyses demonstrated that these belonged to the genera Glomus (11), Acuulospora (1) and Scutellospora (1). The AM fungal diversity, as expressed by the Shannon–Wiener indices, were 0.82 ± 0.08 for the soybean and 0.70 ± 0.11 for poplar under the TBI system, and 0.53 ± 0.08 for poplar under the FO system. The study also investigated the distribution of AM fungal spores in the soil, and this differed in the two systems. Under the TBI system, spore abundance increased with increasing distance from the poplar trunk, while it remained relatively constant under the FO system. Our results also show that neighbouring trees and soybean plants hosted different AM fungal communities, suggesting that TBI systems may enhance AM fungal richness compared to monocultures.

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1. Introduction

Tree-based intercropping (TBI) systems involve the combined culture of trees and arable crops on the same land management unit. The integration of trees into cropping systems increases the soil organic matter content due to the additional deposition of tree leaves and the turnover of fine roots (e.g., Peichl et al., 2006), which may help to improve soil microbial activity and nutrient cycling rates (Seiter et al., 1999; Jose et al., 2000; Mungai et al., 2005). Differences in litter quality between trees and crops, and the additional effect of trees on microclimate, may also result in the development of distinct microbial communities, especially when compared to the rows of trees (Mungai et al., 2005). However, there exists relatively little scientific information concerning the effects of TBI systems on the diversity, distribution and functioning of the soil biota, highlighting the need for further research in this field of agroforestry.

In the past, information concerning the biota of a given soil was limited by the ability to extract and identify the organisms present (Neher, 1999). Recent advances in the use of molecular identification tools have now made it possible to obtain a more complete picture of those organisms present and functionally active in a soil, or a plant root, than via the traditional morphotyping approach. Studies using molecular approaches have provided valuable insights into soil fungal communities such as arbuscular mycorrhizal (AM) fungi, which are symbiotic root colonizing fungi belonging to the phylum Glomeromycota (Schuëller et al., 2001).

The development of polymerase chain reaction (PCR) primers that specifically target species of AM fungi, has allowed the direct characterization of AM fungal communities within both soils and plant roots (Redecker, 2000). Most of these molecular-based studies have involved cloning and sequencing of the fungal ribosomal small subunit (SSU rRNA) genes (e.g., Helgason et al., 1998; Daniell et al., 2001; Vandenkoonhe et al., 2002, 2003). Cloning and sequencing the SSU rRNA genes has the added advantage of offering a measure of the relative abundance of a given fungal taxon. The percentage of a clone with a particular sequence type in a clone library reflects the relative abundance of that particular AM fungal sequence type in an environmental sample. Cloning has been used to demonstrate that co-occurring
2. Materials and methods

2.1. Site and experimental design

The experimental site (1.5 ha) is located near St-Rémi (45°14'N, 73°40'W, Quebec, Canada) within the humid temperate continental climatic zone (mean temperature 6.2 °C, mean annual precipitation 978.9 mm of which 22% falls as snow, Environment Canada, 2008). The soil is classified as an orthic melanic brunisol (Agriculture Canada Expert Committee on Soil Survey, 1987) with a loam soil texture (approx. 20% clay, 30% silt and 50% sand) with moderate to imperfect drainage, a cation exchange capacity of 20.6 cmolc kg\(^{-1}\) and a pH\(_{\text{water}}\) of 6.9. The tree stand was established in 2000 and comprises a mixed planting of poplar (Populus nigra L. \(\times\) P. maximowiczii cv. A. Henry 'Max 5'), black walnut (Juglans nigra L.) and white ash (Fraxinus americana L). Prior to 2000, the site was used for corn and soybean production. Poplars, black walnuts and white ashes were planted in alternate rows oriented north–west/south–east and spaced 8 m apart. Poplars were planted at 2-m intervals, black walnuts and white ashes at 3-m intervals, giving a total of nine and six plants per plot, respectively. Strips of black plastic mulch (150-cm wide) were used to suppress weeds close to the trees.

At the beginning of the present project (2005), the poplars were approximately 8-m tall, black walnuts and white ashes approximately 1.8-m tall. The experimental design included two treatments, either a TBI system with soybean (Glycine max (L.) Merr. cv. SO3-W4, 2650 UT) or a forest plantation (FO) system (poplar rows without alley crops). Prior to sowing soybeans, an N–P\(_2\)O\(_5\)–K\(_2\)O fertilizer (5–27–24, 300 kg ha\(^{-1}\)) was applied and the soil was disked to a depth of 10 cm. A conventional herbicide treatment was applied to the soybean at the two to three trifoliolate leaf stage and glyphosate was applied to the mulch–intercropping interface at the end of May. Under the FO system, the alleys were disked twice a year, but did not receive fertilizer.

The experimental design (split block) comprised six blocks (Fig. 1). Each block was divided into two plots representing the two plantation systems (TBI and FO). In each plot unit, the subplot units

![Fig. 1. Experimental design (not to scale) used at the St-Rémi (southwestern Québec) field site. Lines in bold represent poplar rows and dashed lines represent black walnut and white ash rows. The short perpendicular lines represent the randomly chosen transects. The six rectangles represent the six blocks. The grey zone represents the cropped alleys (TBI system) and the white zone represents the disked alleys (FO system).](image-url)
consisted of four distances (0.5, 2.0, 3.5 and 5.0 m) along a transect radiating out from the east side of the trunk of four poplars, at which soil samples were collected. The four trees were chosen at random from the nine trees in each row.

2.2. Sampling

Soil cores (6.3-cm diameter × 20-cm deep) were collected from all plots and subplots on 20 July 2005 when the soybean was flowering (R2 stage). This corresponds to the stage when the mycorrhizal colonization of soybean roots is usually at its highest (Zhang et al., 1995). The 192 samples were stored in plastic bags at 4 °C prior to analysis. Live roots were collected from each core, washed thoroughly in distilled water and separated into a tree fraction (light brown, highly branched with many fine roots) or a crop fraction (white roots with Rhizobium nodules) and frozen at −80 °C prior to DNA extraction.

2.3. Quantification of arbuscular mycorrhizal fungal spores

Arbuscular mycorrhizal fungal spores were isolated from each of the 192 soil samples using wet sieving and sucrose density gradient centrifugation (Jansa et al., 2002). Briefly, a 50-g sample of air-dried soil was passed through a nest of soil sieves (mesh size: 500, 250, 106 and 45 μm). The live spores (containing cytoplasm and lipids) were separated from dead spores under a dissecting microscope at 90-fold magnification and only live spores were counted.

2.4. Quantification of soil parameters

To identify potential factors affecting the spatial variation in AM fungal spore abundance, soil P content and pH were measured in each of the soil samples. Soil pH was determined from a soil:water slurry (1:2.5 by volume). The available P content (mg P kg⁻¹ dry soil) was analyzed using the Mehlich-3 method (Mehlich, 1953).

2.5. Molecular identification of arbuscular mycorrhizal fungal phylotypes

For the molecular identification of AM fungal phylotypes, 72 soil cores containing roots samples were used (24 for poplar in the TBI system, 24 for soybean in the TBI system and 24 for poplar in the FO system). Roots were ground in liquid nitrogen. Total DNA was extracted from powdered roots using a DNeasy Plant Mini Kit (Qiagen, Canada) according to the manufacturer’s instructions, and frozen at −20 °C until PCR amplification. A partial sequence of the SSU rRNA gene was amplified (550 bp) using the universal eukaryotic primer pair 5'-TTG GAG GGC TCT GGT GCC-3' and 5'-GTT CCC ATG CCT ACG CCA AA-3' (Helgason et al., 1998), in order to exclude plant DNA sequences. The reaction mix comprised 0.5 mmol MgSO₄, 10 mmol dNTPs, 10 mmol of each primer and 1 unit of the proofreading Platinum Pfx DNA polymerase (Invitrogen, Canada) suspended in the manufacturer’s reaction buffer to give a final volume of 20 μL. The cycling regime (94 °C for 2 min, 35 cycles at 94 °C for 15 s, 60 °C for 30 s and 68 °C for 30 s, and 68 °C for 5 min) was done using a PTC-225 (MJ Research, Boston, MA, USA). Amplified SSU rRNA fragments were identified in 1% agarose gels. The resulting blunt-ended products were cloned into pCR-Blunt II-TOPO and transformed into One Shot TOP10 Chemically Competent Escherichia coli (Zero Blunt TOPO PCR cloning Kit, Invitrogen, Canada). Putative positive transformants were screened using standard M13R/M13F amplification. To identify restriction length polymorphism (RFLP) types, up to 10 positives from each individual were digested with the restriction enzymes Alul and Hinf1, according to the manufacturer’s instructions. The Kodak 1D software was used to analyze the RFLP patterns. One clone of each RFLP type was sequenced and the sequences obtained were submitted to GenBank. Sequences relating to other fungi were occasionally amplified, but were excluded on the basis of their species-specific RFLP pattern.

2.6. Data analyses

Forward and reverse sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999) and reference sequences of the SSU region were obtained from GenBank. Multiple alignments of sequences were processed using CLUSTALW software (Thompson et al., 1994). Phylogenetic relationships were inferred with the help of MEGA 4 using the neighbor-joining (NJ) method (Saitou and Nei, 1987). Bootstrap support values for branches were estimated from 1000 replicates for the NJ tree. The putative choanozoan Corallochytrium limacisporum, a close relative of fungi (Cavallier-Smith and Allsopp, 1996), was chosen as the out-group.

A two-way analysis of variance (ANOVA) followed by the Tukey multiple means test, was done to compare AM fungal spore abundance (number of spores per gram of soil) and soil parameters (soil P content and pH) among distances and plantation systems. To simplify the data presentation, block effects and interactions containing them are not shown. Subsequently, interactions were used as whole plot and subplot error terms. The number of spores was log₁₀(N + 1) transformed to satisfy normal distribution parameters and homogeneity of variance assumptions (St. John and Koske, 1988). Estimates of soil P content and pH were correlated with spore counts. All statistical analyses were performed using the statistical analysis system (SAS) software version 9.1 (SAS Institute Inc, 2002).

Arbuscular mycorrhizal fungal diversity in poplar and soybean roots was evaluated using the Shannon–Wiener index, which combines two components of diversity: richness and evenness of individuals among the species. The Shannon–Wiener index was estimated by calculating $H = \sum p_j \ln p_j$, where $p_j$ is the relative abundance of each phylotype, calculated as the proportion of individuals of a given phylotype in the total number of individuals in each root sample. Sequence phylotypes were defined as separated monophyletic groups in the phylogenetic trees (see Hijri et al., 2006 for the complete definition of sequence phylotypes). Two-way ANOVA and Tukey multiple means test were also used to compare the AM fungal diversity among hosts and distances.

Univariate ANOVA tests following multivariate analyses of variance (MANOVA) were performed to assess: (1) the effect of the soybean intercrop on the relative abundance of phylotypes associated with poplar roots under the TBI system, and (2) the AM fungal host specificity with co-occurring poplar and soybean roots under the TBI system. In the latter analysis, only co-occurring plant roots sampled at 2.0, 3.5 and 5.0 m were considered. In MANOVA, the $F$-value was derived from an $F$-ratio calculated from Hotelling–Lawley trace statistics as previously described by Jansa et al. (2002). Only phylotypes common to both hosts were added to the model statement for each MANOVA.

Redundancy analysis was computed using CANOCO software version 4.0 (TerBraak, 1988). This analysis was employed to assess the contribution of the identity of the host plant to the AM fungal community structure, as previously described by Jansa et al. (2002). The $F$-values shown represent the significance of results obtained using the Monte-Carlo permutation test.
3. Results

3.1. Spatial variation of arbuscular mycorrhizal fungal spore abundance

With regards to spore abundance, there was a strong interaction between plantation systems and distance from the poplar trunk ($F = 12.59, P < 0.0001$). Spore abundance increased with increasing distance from the poplar trunk under the TBI system, while it remained relatively constant under the FO system (Fig. 2). Total spore counts were similar in both plantation systems but varied significantly with distances from the poplar trunk ($F = 60.28, P < 0.0001$). Total AM fungal spore abundances were $22.70 \pm 1.08$ spores $g^{-1}$ soil under the TBI system and $22.96 \pm 0.94$ spores $g^{-1}$ soil under the FO system. Under both plantation systems, the spore count was lowest at 0.5 m from the poplar trunk (i.e., under the black plastic mulch).

There was also a significant interaction between the plantation systems and distance from the poplar trunk for the soil P content ($F = 4.58, P < 0.0080$). As for the spore count, soil P increased with increasing distance from the poplar trunk under the TBI system, while it remained relatively constant and low under the FO system (Fig. 3). There were strongly significant differences between the plantation systems ($F = 41.89, P < 0.0001$) and the distances from the poplar trunk ($F = 6.64, P = 0.0011$). Available P under the TBI system, where fertilizer was applied, was $26.11 \pm 2.52 \text{ mg P kg}^{-1}$ dry soil. By contrast, available P under the FO system, where no fertilizer was applied, was $8.03 \pm 0.95 \text{ mg P kg}^{-1}$ dry soil.

Soil pH was $6.44 \pm 0.04$ under the TBI system and $6.48 \pm 0.05$ under the FO system and there were no statistical differences between the plantations or between distances from the poplar trunk.

There was a strong correlation between AM fungal spore abundance and soil P content ($r^2 = 0.78, P = 0.0100$) under the TBI system, while no correlation was found under the FO system. Spore abundance and pH were not correlated.

3.2. Phylotypes detected in the field

Detailed analysis of AM fungal communities done for 72 root samples yielded almost 800 PCR product clones. After RFLP screening, approximately 120 clones were sequenced and analyzed phylogenetically. Representative sequences of each phylotype were submitted to the NCBI database and included in the phylogenetic trees shown in Fig. 4. The phylogenetic tree topology is largely in agreement with previously published phylogenies. The AM fungi colonizing poplar and soybean roots were from the Glomeraceae, Acaulosporaceae and Gigasporaceae. By far, the most diverse clade at the field site was *Glomus* group A (G1GrA; following the phylogeny of Schüßler et al., 2001, see also Schwarzott et al., 2001), with 11 sequence types, which comprised three subclades (G1GrAa, b and c). One phylotype belonging to the Acaulosporaceae and a Gigasporaceae type related to *Scutellospora gilmorei* were also detected.

3.3. Arbuscular mycorrhizal fungal diversity

The highest AM fungal diversity occurred under the soybean intercrop of the TBI system (Fig. 5) and the lowest under the poplars of the FO system. Differences between the hosts, and between distances from the poplar trunk, were not statistically significant. The mean Shannon–Wiener index for poplars and soybean roots under the TBI system was 0.78 ± 0.07, compared to 0.53 ± 0.08 for poplars grown alone under the FO system.

3.4. AM fungal community structure

Fig. 6 shows details of the AM fungal community structure for soybean and poplars under the TBI system and for poplars under...
the FO system. Under the TBI system, nine phylotypes were associated with soybean roots and ten with poplar roots. Seven phylotypes were common to co-occurring trees and crops. The AM fungal colonization of poplar roots under the TBI system was dominated by GIGrAb phylotypes (which contains species such as *Glomus intraradices* and *Glomus fasciculatum*) and one sequence phylotype (No. 4) accounted for 53.4\% of the colonization. The colonization of soybean roots was more uniform, but was slightly dominated by one GIGrAa phylotype (which contains the species *Glomus mosseae*) and two GIGrAb phylotypes. Eight phylotypes were found on poplar roots under the FO system. Colonization of poplars under the FO system was also dominated by phylotype No. 4 (76.1\%).

Analysis of the composition of the AM fungal phylotypes by MANOVA, showed a significant effect of the plantation system (TBI or FO system) on the AM fungal community of poplar roots ($F = 4.81, P = 0.0276$). The plantation system significantly affected the relative abundance of phylotypes No. 4 ($F = 5.19, P = 0.0403$) and No. 12 ($F = 6.37, P = 0.0254$). A subsequent MANOVA was conducted to test the AM fungal host specificity in co-occurring tree and crop roots under the TBI system at 2.0, 3.5 and 5.0 m from the poplar trunk (i.e., in the cropped alley). The MANOVA revealed a large host effect on the composition of the AM fungal community under the TBI system ($F = 4.91, P = 0.0018$). The relative abundances of the phylotypes No. 4 ($F = 11.08, P = 0.0025$) and No. 10 ($F = 12.69, P = 0.0013$) were significantly affected by the host.

A redundancy analysis allowed the effect of host identity (soybean and poplar under the TBI system and poplars under the FO system) on the AM fungal community of poplar roots ($F = 6.19, P = 0.0034$). The plantation system significantly affected the relative abundance of phylotypes No. 4 ($F = 5.19, P = 0.0403$) and No. 12 ($F = 6.37, P = 0.0254$). A subsequent MANOVA was conducted to test the AM fungal host specificity in co-occurring tree and crop roots under the TBI system at 2.0, 3.5 and 5.0 m from the poplar trunk (i.e., in the cropped alley). The MANOVA revealed a large host effect on the composition of the AM fungal community under the TBI system ($F = 4.91, P = 0.0018$). The relative abundances of the phylotypes No. 4 ($F = 11.08, P = 0.0025$) and No. 10 ($F = 12.69, P = 0.0013$) were significantly affected by the host.

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Fig. 7. Effect of host species on the composition of arbuscular mycorrhizal fungal communities under tree-based intercropping (TBI) or traditional tree plantation (FO) systems. Results of redundancy analysis are shown using the relative abundances of each AM fungal phylotype as affected by host plant. Vectors representing factors (host plant) are shown as broken lines; vectors representing AM fungal phylotypes are shown as solid lines. Size and orientation of the vectors represent correlation among them and with the axis. The smaller the angle between the vectors (or a vector and an axis) and the longer the vectors, the more the variables represented by the vectors are correlated.

FO system) on AM fungal community composition (Fig. 7) to be quantified. A Monte-Carlo permutation test confirmed that the AM fungal community structure was significantly affected by the host. This effect accounted for 20.8% of the variability and was highly significant ($F = 9.17, P = 0.0020$). This analysis confirmed significant differences in AM fungal community composition between poplars under the TBI system and poplars under the FO system, as well as the AM fungal host preference hypothesis for co-occurring trees and crop.

4. Discussion

4.1. Distance dependent variation in spore density under tree-based intercropping

Arbuscular mycorrhizal fungal spore abundance found in this study was similar to that observed by Jansa et al. (2002) and Oehl et al. (2003, 2004, 2005) in agricultural soils with essentially similar clay contents to those of the present study site. Total spore abundances were similar under both plantation systems. Under the FO system, mechanical weed control did not suppress all weeds. A certain number of these may have formed arbuscular mycorrhiza, which, in turn, may have maintained the number of AM fungal propagules in the soil. The two plantation systems differed only in the distribution of AM fungal spores. Under the TBI system, AM fungal spore abundance increased with increasing distance from the poplar trunk. In a recent companion study by Rivest et al. (2009) at the St-Rémi site, the authors observed that overhead shading by poplars reduced light transmittance levels close to the poplar rows, resulting in a significant reduction in soybean yield. Shading by associated trees has been repeatedly shown to reduce net assimilation and yield in temperate TBI systems (see reviews by Jose et al., 2004; Reynolds et al., 2007). As AM fungi obtain their carbohydrate from their host plants, any decrease in the host's photosynthetic rate may indirectly affect root infection, ERM elongation and spore production (Heinemeyer et al., 2003).

In addition, the soil cores revealed that fine root density increased with increasing distance from the poplar trunk, at least in the superficial (0–20 cm) layer considered in this study. Poplar roots were very scarce compared to soybean roots. While this is probably due to soil disking before sowing, inter-plant competition may also have encouraged poplar roots to proliferate deeper within the soil profile (Mulia and Dupraz, 2006). If this is the case, soybean root distribution may explain the observed spore distribution in the shallower soil layer. However, to confirm this, further studies are needed to investigate AM fungal spore abundance and fine root distribution lower in the soil profile.

A significant positive correlation between the abundance of AM fungal propagules and available soil P was observed under the TBI system. This is in contrast to many other studies that report lower AM fungal spore numbers following P fertilization (e.g., Ezawa et al., 2000; Kahliluoto et al., 2001). Although fertilizer was applied in 2005, the available P content of the soil was much lower than that usually recommended for soybean (CRAAQ, 2003). Therefore, it is possible that the correlation observed in the present study was due to the low overall P content of the soil, which may have stimulated spore production. The increase of soil P content with the distance from the poplar trunk was probably due to the fact that the fertilizers were applied with a cyclone spreader. The strip closest to the poplar row may have received less P than the middle of the alley. This effect is often underestimated in studies dealing with tree-crop interactions.

The lower spore abundance found at 0.5 m from the poplar trunk under FO and TBI systems suggests that the AM fungal sporulation might be inhibited by the black plastic mulch, despite an apparently higher poplar root density (V. Chifflot, personal observation). Plastic mulch is usually used to eliminate weeds, but it has also been shown to control pathogenic soil borne fungi through solar heating (see review by Nicot et al., 2000). This effect may have led to the unintentional inhibition of symbiotic fungi.

4.2. Species richness

Although spore abundance is likely to be a good indicator of a soil’s mycorrhizal inoculum potential (Johnson et al., 1991), the composition of the AM fungal spore population does not necessarily reflect the AM fungal community that is actually colonizing the plant roots (Clapp et al., 1995). In the present study, the AM fungal community associated with the roots of soybean and poplar was obtained using molecular identification tools. Only 13 AM fungal phylotypes were recorded at the study site. Most of these belonged to Glomus group A. In other studies dealing with AM fungal communities in conventionally managed fields, the number of AM fungal species ranged from 11–26 (Jansa et al., 2002; Oehl et al., 2003, 2004; Mathimaran et al., 2005). On arable sites, the AM fungal community may be dominated by a single species (e.g., G. intraradices, Mathimaran et al., 2005 or G. mosseae, Helgason et al., 1998). At such sites, the loss of diversity is characterized by the virtual elimination of certain genera such as Acaulospora and Gigaspora, which are usually abundant in undisturbed communities (Daniell et al., 2001). The dominance of a given community by a single species (Leake et al., 2004; Oehl et al., 2004) might be related to the contrasting inter-specific differences in the developmental patterns of the ERM. Species of Glomus (but not Gigaspora and Scutellospora) readily form anastomoses between hyphae and might therefore have the ability to re-establish an interconnected network after limited mechanical disruption (Voets et al., 2006). Furthermore, conventional farming probably tends to allow only those AM fungal species capable of tolerating high nutrient levels. These may be less beneficial, or perhaps even detrimental, to crops (Johnson et al., 1992; Johnson, 1993). However, other AM fungal species may have found refuge in the deeper soil layers under the ploughing depth (Oehl et al., 2005). As pointed out by Daniell et al. (2001), it is also
possible that some additional species were present in the roots that we examined, but they were not detected by the methods we used. No sequences were detected within the Archaeasporaceae or Paraglomaceae. Indeed, AM1 and NS31 primers are known to exclude these newly defined AM fungal families (Redecker et al., 2000). Therefore, the true diversity may be higher than recorded here.

4.3. Variation of arbuscular mycorrhizal fungal community composition with host

Although species diversity was similar, co-occurring trees and crops showed differences in their respective AM fungal communities. As pointed by Jansa et al. (2002), the Shannon–Wiener index may not properly reflect the community structure, especially if the differences in abundance between different species are too large, as was the case in the present study. Nevertheless, the diversity of AM fungal phylotypes in soybean roots tended to be higher than in poplar roots. Host preference by AM fungi has been previously described for co-occurring plants in grassland ecosystems (e.g., Vandenkornhuyse et al., 2002, 2003) as well as in crop plants in adjacent fields (Mathimaran et al., 2005). Jansa et al. (2002) showed the potential for species of host plant to influence species-specific fungal development. The results of the present study support the idea that AM fungi can show host preference when in the presence of co-occurring plant roots (Figs. 6 and 7). However, distance from the poplar trunk did not affect diversity indices and phylotype abundance in poplar or soybean roots. One reason for this could be that the number of clones analyzed from each sample was too low to observe any distance effect. Furthermore, a number of soil cores lacked poplar roots, especially at 3.5 and 5.0 m from the poplar trunk. The root samples that did not yield any PCR products were probably not colonized by AM fungi.

4.4. Effect of soybean on arbuscular mycorrhizal fungal community structure of poplar

The present study suggests that intercropping affects the community structure of AM fungi colonizing poplar roots. Although not statistically significant, the diversity of AM fungal phylotypes found on poplar roots under the TBI system was higher than that associated with poplar roots under the FO system. The differences in community composition of the AM fungi associated with poplar roots may be due to soil properties, such as soil P content, that differed between the two plantation systems. Furthermore, composition of the AM fungal community of co-occurring roots of soybean could have played an important role, facilitating AM fungal colonization of trees via ERM links (Enkhtuya and Vosatka, 2005). This effect may increase the functional diversity AM fungi associated with trees under the TBI systems. Functional diversity studies in TBI systems would help shed light in this aspect. The AM fungal community structures given in Fig. 6, show that phylotypes No. 12 (Acaulosporaceae) and No. 13 (Gigasporaceae) were more common in soybean and poplar root under the TBI system, than in poplar roots under the FO system. This suggests a possible colonization by AM fungi from one root system to the other when trees and crops coexist. Although the effect of black walnuts and white ashes was not studied, it is possible that these species could influence the AM fungal community of soybean.

5. Conclusion and future directions

Arbuscular mycorrhizal fungal spore counts indicated that the heterogeneity of spore distribution was higher under the TBI system than under the FO system. Under the TBI system, spore distribution appeared to be influenced by soybean yield and soil properties. Our study also showed an effect of the soybean intercrop on the AM fungal community structure of poplar. The neighbouring trees and soybean plants hosted different AM fungal communities, suggesting that TBI systems may enhance AM fungal richness compared to monocultures. In the present study, mechanical weed control under the FO system did not suppress all weed species. According to the host preference hypothesis, some of these hosts may potentially harbour different AM fungal phylotypes. An AM fungal community analysis that considers all potential hosts present in both plantation systems, including weeds, would help to obtain a more complete picture of AM fungal diversity and the factors controlling it.

Symbiotic relations with AM fungi and N2-fixing bacteria (rhizobia) have been much studied in tropical agroforestry systems because these associations enable trees and crops to sustain growth in the P- and N-deficient soils typically encountered in these regions (e.g., Haselwandter and Bowen, 1996; Ingleby et al., 2007). The benefits of the AM symbiosis in temperate agroforestry systems remain uncertain as the agronomic regime is intensive and soil fertility, in most cases, is high. However, rising concerns about the increasing loss of biodiversity and its potential to affect agriculture has increased the interest in TBI systems. Further work is required to confirm the hypothesis that trees used in TBI systems, or as windbreaks or buffer strips, may represent a perennial source of additional AM fungal diversity that may benefit adjacent crops. Trees may also improve the structural stability of the soil by promoting the development of extensive ERM networks within the soil matrix and supplying different sources of organic matter. Studying the potential role of AM fungi in mediating tree-crop interactions under low fertility, or low external input, may also provide important information. Yield reduction close to trees may be attenuated by the presence of an intact extraradical mycelium in the tree row at the start of AM fungal colonization of crop roots. Increased AM fungal diversity under TBI systems may increase hyphal length in the soil, and enhance P extraction for the benefit of the whole cropping system.

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References
