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THE IMPACT OF TILLAGE PRACTICES ON ARBUSCULAR MYCORRHIZAL FUNGAL DIVERSITY IN SUBTROPICAL CROPS

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Abstract. Arbuscular mycorrhizal fungi (AMF) are a main component of soil microbiota in most agrosystems. As obligately mutualistic symbionts, they colonize the roots of the majority of plants, including crop plants. We used molecular techniques to investigate how different tillage systems (moldboard, shred-bedding, subsoil-bedding, and no tillage) can influence the AM fungal community colonizing maize, bean, and sorghum roots in an experimental site located in northern Tamaulipas, Mexico. Roots from 36 plants were analyzed using AM fungal-specific primers to partially amplify the small subunit (SSU) of the ribosomal DNA genes. More than 880 clones were screened for restriction fragment length polymorphism (RFLP) variation, and 173 of these were sequenced. Ten AM fungal types were identified and clustered into three AM fungal families: Gigasporaceae, Glomaceae, and Paraglomaceae. *Glomus* was the dominating taxon in all the samples. Four of the 10 identified types were distinct from any previously published sequences and could correspond to either known unsequenced species or unknown species. The fungal diversity was low in the four agriculture management systems, but the multidimensional scaling (MDS) analysis and log-linear-saturated model indicated that the composition of the AMF community was significantly affected by the tillage system. In conclusion, since some fungal types were treatment specific, agricultural practices could directly or indirectly influence AM biodiversity.

Key words: arbuscular mycorrhizal fungi; fungal biodiversity; Gigasporaceae; Glomaceae; Paraglomaceae; SSU rDNA; soil tillage practices; subtropical conditions.

Introduction

The practice of conservation tillage is based on the use and management of crop residues that cover at least 30% of the soil surface, thus preventing or minimizing problems connected to soil erosion and degradation. No tillage and reduced/minimum tillage are two of these practices. In Mexico, ~650 000 hectares are planted under conservation tillage (Claveran-Alonso et al. 2001), and the use of this system is increasing because of heightened interest in sustainable agriculture (Roldán et al. 2003).

Conservation tillage systems, in particular no tillage, have several positive effects such as reduction in erosion (Tiscareño et al. 1999), weed problems, and chemical-fertilizer use, and restoration of soil fertility (Salinas-Garcia et al. 2002), which are fundamentally due to crop residue. An increase in soil organic matter, lower soil temperature (Arshad and Azooz 1996), higher water retaining capacity (Lal 1995), and penetration resistance (Unger and Jones 1998) have been described in no-tillage soils compared to till soils (Angers et al. 1995, Roldán et al. 2005*a*, *b*), together with higher values of water-soluble C, enzyme activities, and aggregate stability (Roldán et al. 2005*a*, *b*). However, the extent of the soil-tillage effect on various soil parameters closely depends on the soil crop type and climate (Blevins and Frye 1993).

Arbuscular mycorrhizal fungi (AMF) represent one of the main components of soil microbiota in most agroecosystems. These obligately mutualistic symbionts belonging to the phylum Glomeromycota (Schüßler et al. 2001) form intimate associations with the majority of plants (~80% of families), including most crop plants. AM fungi improve soil structure and plant nutrition, particularly phosphorous uptake, and protect plants from pathogens and abiotic stresses (Smith and Read 1997). Extraradical hyphae play a main role in soil stable aggregate formation (Kabir and Koide 2002) through the exudation of glomalin, a glycoprotein that acts as a glue-like agent for soil particles (Wright and Anderson 2000). It is evident that all these beneficial effects contribute to make AM fungi a main component for the functioning of terrestrial ecosystems. Plant diversity and productivity in ecosystems are significantly influenced by AM fungal diversity in the soil (van der Heijden et al.

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1998). Therefore, information regarding the active AMF in roots is crucial in ecological field studies, not only in natural systems, but also in sites where agricultural practices may have an effect on the diversity and functionality of AM fungi (Hijri et al. 2006).

Since the persistence of AM fungi in soil depends on the survival of their active propagules (e.g., spores, hyphae, and colonized roots), tillage can negatively affect AM fungi. Roldán et al. (2007) observed the highest levels of mycorrhizal propagules in maize and bean crop soil under no-tillage compared to tillage soils. Soil tillage alters the ability of AMF to colonize roots, breaking up their hyphal network. As a consequence, there is a significant reduction in root colonization and, in turn, in the nutrient absorption from the soil (McGonigle and Miller 1996).

A number of studies exist on the effect of soil tillage on the diversity and structure of AMF communities in temperate (Jansa et al. 2002, 2003) and tropical (Boddington and Dodd 2000) soils. However, no information is available about the effect of soil management strategies, such as tillage, on the biodiversity of AMF in subtropical agroecosystems. This is an important requisite for the effective management and preservation of AMF diversity in sustainable agricultural systems. The objective of this study was therefore to test whether the AMF community in roots of cultivated maize, bean, and sorghum were affected by soil tillage in a subtropical system.

Our experiment was carried out to identify AM fungi taxa in roots on the basis of the small subunit (SSU) rDNA sequences subjected to restriction fragment length polymorphism and phylogenetic analyses. We demonstrate that agricultural practices do in fact influence AM biodiversity, since some fungal types were treatment specific.

Materials and Methods

Site description

This research was conducted at the Río Bravo experimental site in northern Tamaulipas, Mexico (25°57′ N, 98°01′ W). The dominant soil type is Vertisol (FAO 1988), which has developed from alluvial sediments and has a clay texture (28% sand, 41% clay, and 31% silt), 1.2% organic matter, and a pH of 7.8 (1:2 soil:water). The climate of the region is classified as warm subtropical, with hot, wet summers and dry winters. The mean annual temperature and rainfall are 23°C and 635 mm, respectively. The topography of the area is mainly flat with slopes that do not exceed 3%. The climax vegetation of this area, which has almost disappeared due to agriculture, is currently represented by shrub species, such as *Prosopis juliflora* and *Acacia farnesiana*, and halophytic pasture.

Experimental design and layout

The experiment was conducted using a factorial design with two factors and triple-fold replication. The first factor included four tillage treatments: moldboard plow (disking stalks after harvest followed by moldboard plow and disking, then building the rows), subsoil-bedding (shredding stalks after harvest followed by subsoiling on row centers and forming beds in the same operation), shred-bedding (shredding stalks after harvest followed by bedding on the old rows) and no tillage (shredding stalks after harvest and spraying glyphosate [1.5 L/ha] and 2-4 D amine [1.5 L/ha] as necessary for weed control). The second factor involved three crop types: maize (*Zea mays* L.), bean (*Phaseolus vulgaris* L.), and sorghum (*Sorghum bicolor* L.) Moench. The 12 plots measured 22.4 × 52 m. All the cropping systems were established in 2000, as Roldán et al. (2005*a*) described. The maize, bean, and sorghum were planted in late January and harvested in the first half of June each year from 2000 to 2005.

Sampling

All samples were collected in the second half of May 2005. Three plants per treatment from each crop type, each plant taken from a different plot, were sampled and taken to the laboratory (a total of 36 plants). Root fragments of each plant were washed free of soil, air-dried at room temperature, and stored at 4°C. The roots were used both for morphological and molecular analysis.

Surface crop residues were collected after primary tillage from two midrow to midrow 1-m^2 areas that were considered representative of each tillage treatment, dried (60°C for 48 h), weighed and then converted to kg/ha (Steiner et al. 1994).

The percentage of surface cover was determined using a line-transect measurement. This involved stretching a 10-m string with 100 marks (10 cm apart), across the field at a 45° angle to the rows, walking along the line, looking straight down at the ground, and counting the number of times a piece of residue was under a mark. The number of "hits" indicated the percentage of cover (Steiner et al. 1994).

Mycorrhizal determinations

The percentage of root length colonized by AM fungi was calculated using the gridline intersect method (Giovannetti and Mosse 1980) after staining with trypan blue (Phillips and Hayman 1970).

Statistical analysis

Treatment effects on measured variables (surface crop residues and degree of AM fungal colonization) were tested using analysis of variance, and comparisons among treatment means were made using a least significant difference (LSD) test calculated at $P < 0.05$. Statistical procedures were carried out with the Statgraphics for Windows 7.0 software package (StatPoint 2005).

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Molecular analysis

Total DNA was extracted from four pieces (1–2 cm each) of the stored root samples for each plant according to the protocol described in Perotto et al. (1996). The DNA was re-suspended in 20 μl of water. Several dilutions of extracted DNA (1/10, 1/50, 1/100, 1/250) were prepared. Partial ribosomal small subunit (SSU) DNA fragments were amplified using 1.0 unit of Red*Taq* DNA polymerase (Sigma-Aldrich, St. Louis, Missouri, USA) and two different sets of primers. One set consisted of the NS31 universal eukaryotic primer (Simon et al. 1992) and the AM1 primer, designed to amplify AM fungal SSU sequences but not plant sequences (Helgason et al. 1998).

The second set of amplification was with the ARCH1311 primer (Redecker 2000) in combination with NS8 (White et al. 1990). Polymerase chain reactions (PCR) were performed using 0.1 μg/μL BSA (Bovine Serum Albumine), 0.2 mmol/L dNTPs and 0.5 μmol/L of each primer, and the supplied reaction buffer to a final volume of 25 μL (PCR conditions: 95°C for 3 min, then 35 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 2 min, then 72°C for 10 min). All the PCR reactions were run on a Cetus DNA thermal cycler (Perkin Elmer, Norwalk, Connecticut, USA). The samples that did not show any PCR product were further purified with an additional chloroform extraction to remove any low molecular mass inhibitory compounds.

The PCR products were then purified using a QIAquick or gel extraction kit (Qiagen, Hilden, Germany) cloned into pGEM-T Easy (Promega, Madison, Wisconsin, USA) and transformed into *Escherichia coli* (Xl1 blue). Putative positive transformants were screened in each resulting SSU rRNA gene library, using a second NS31/AM1 or ARCH1311/NS8 amplification following the same conditions described in the previous paragraph. Positive clones from each sample were tested for restriction fragment length polymorphism (RFLP) by independent digestion with *Hinf*I and *Hsp*92II for the NS31/AM1 sequences or with *Hinf*I and *Alu*I for the ARCH1311/NS8 ones, according to the manufacturer's instructions (Promega). Finally, they were analyzed on 2.5% agarose (in tris-borate-EDTA; TBE) gel electrophoresis. Examples of each RFLP type were chosen for sequencing, which was performed by GeneLab (Rome, Italy) using the SP6 and T7 universal primers.

Sequence editing was conducted using the Sequencher version 4.1.4 program (Gene Codes 2000). The sequences were deposited at the National Centre for Biotechnology Information (NCBI) GenBank with accession numbers AM412060–AM412115 (*available online*).[5]

Phylogenetic analysis

Sequence similarities were determined using the BLASTn sequence similarity search tool (Altschul et al. 1997) provided by GenBank. The sequences were aligned to previously published sequences using the ClustalX program (Thompson et al. 1997), and the alignment was adjusted manually in GeneDoc (Nicholas et al. 1997). Neighbor-joining (NJ) and maximum parsimony (MP) phylogenetic analyses (Saitou and Nei 1987) were performed with the program PAUP4.08b (Swofford 2002) and using the default parameters. The putative choanozoan *Corallochytrium limacisporum*, a close relative of fungi (Cavalier-Smith and Allsopp 1996), was chosen as outgroup and used to root the trees on the basis of the widely accepted phylogenetic hypothesis that the fungal radiation is contemporary with the choanozoan radiation (Vandenkoornhuyse et al. 2002).

Patterns of AM fungal community composition

The experimental design allowed us to compare changes in the composition of an AM fungal community among plants of a given species, plant species, and tillage systems. The data were analyzed using multidimensional scaling (MDS) implemented in SPSS 14.0 for Windows (Ferrán Aranaz 1996). MDS was designed to analyze distance-like data, in this case, representing the degree of dissimilarity of three AM fungal communities. Euclidean distances were computed to model dissimilarities. A log-linear saturated model that contained all the independent variables as well as combinations of the variables was constructed to examine the relationships among the variables. The saturated model reproduced the observed data exactly. The relative importance of a given interaction term was estimated after having removed this term from the saturated model. The overall goodness-of-fit statistics were then calculated in SPSS 14.0 using likelihood ratio χ^2 to determine the significance levels and deduce whether this new unsaturated model fit the data. Here, the host plant, the tillage system, and the AM fungal species (i.e., RFLP pattern) counts were included in the model. The five most frequent AM fungal types were used in the model.

The Shannon-Weaver (H') index was calculated as an additional measure of AMF diversity, as it combines two diversity components, i.e., species richness and evenness. It was calculated from the equation $H' = -\Sigma\, p_i(\ln p_i)$, where p_i is the proportion of individuals found in the ith species (in a sample, the true value of pi is unknown, but is estimated as n_i/N, [here and throughout, n_i is the number of individuals in the ith species]).

Results

Surface crop residue

The surface crop residue was significantly affected by tillage practices and crop type (Table 1). No tillage produced the highest residue amount (~4.5, 5.0, and 9.5 times more residue than moldboard plowing for maize, bean, and sorghum crops, respectively) and the greatest residue cover (19 times more cover than the soil under moldboard plow for all crops) on the soil surface. The

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TABLE 1. Surface residues of maize, bean, and sorghum crops affected by different management tillage systems ($n = 3$ enumerated replicates).

Crop species and tillage system	Residue cover (%)	Residue amount (kg/ha)
Maize		
Moldboard	5.0[a]	1322[a]
Shred-bedding	17.0[b]	2808[b]
Subsoil-bedding	17.3[b]	2826[b]
No tillage	94.7[c]	6054[c]
Bean		
Moldboard	1.7[a]	122[a]
Shred-bedding	6.3[b]	369[b]
Subsoil-bedding	6.0[b]	449[c]
No tillage	33.7[c]	635[d]
Sorghum		
Moldboard	5.0[a]	980[a]
Shred-bedding	20.3[b]	6253[b]
Subsoil-bedding	21.3[b]	7263[c]
No tillage	98.3[c]	9303[d]

Note: For each crop, values in columns followed by the same letter do not differ significantly ($P < 0.05$) as determined by the LSD test.

soils subject to moldboard plowing showed the lowest amount and cover of residue for all the crops. There were no significant differences between subsoil-bedding and shred-bedding with respect to increasing surface crop residue.

Degree of AM fungal colonization

The lowest degree of root colonization was found in bean plants (Table 2). The colonization percentage of bean and sorghum roots under subsoil-bedding and no tillage were significantly higher than under moldboard plowing. The maize roots under no tillage showed significantly higher colonization rates than both under shred-bedding and moldboard plowing. The sorghum species had the highest colonization.

Sequences amplified using NS31/AM1 and RFLP type analysis

All the PCR experiments were run using DNA preparations from pooled roots of individual plants. The occurrence of AMF was monitored in 36 root samples (three root samples for each crop species and tillage system). Template DNA was amplified with the AM1/NS31 primer pair. Thirty-four samples (94% of all roots) generated PCR products of the expected band of 550 bps that were used to create a clone library. Overall, 1085 clones were screened by PCR; out of these, 691 contained the SSU rRNA gene fragment. The clones were grouped according to RFLP typing by two enzymes, *Hin*fI and *Hsp*92II. According to Vallino et al. (2006), 15–20 clones/sample were demonstrated to be sufficient to cover the AM fungal biodiversity present in our study. When the number of analyzed clones increased, no new RFLP types were found.

Two clones of each sample for each RFLP type were sequenced for a total of 149 clones, while the remaining 542 clones were classified by RFLP typing. After preliminary BLASTn searches, a total of 213 clones resulted to correspond to AM fungi sequences, while the remaining clones were identified as non-AMF. Our analysis was mostly focused on the AMF sequences, which were grouped into 16 AM fungal types according to their RFLP patterns (Table 3). The RFLP patterns identified as RFLP1, RFLP2, and RFLP4 were present in all the crops, and represented 68%, 11%, and 6% of the analyzed clones, respectively. The RFLP1 pattern was present in maize roots in all the tillage systems, as well as in bean and sorghum roots under moldboard, shred-bedding, and no tillage. The highest number of clones showing the RFLP2 type was found in maize and sorghum crops in the no-tillage system. The RFLP4 pattern was found in all the crops under no tillage, while the RFLP3 pattern was instead exclusively found in sorghum crop under moldboard and subsoil-bedding systems.

The other RFLP types contributed to >3% of the clones and were only recovered once.

Identification of AM fungal groups

Although primer specificity has been confirmed in many investigations (Helgason et al. 1998, Redecker 2000), a high number of non-AM clones were identified. Apart from sequences that showed high similarity (98–99% identity) to AM fungi that belong to members of the Glomeromycota phylum, there were also sequences corresponding to Ascomycetes as recently reported by Douhan et al. 2005.

Alignment of the sequences obtained in this study with those corresponding to the closest matches from GenBank produced a tree that revealed that the sequences belong to the Glomerales and Diversisporales

TABLE 2. Degree of arbuscular mycorrhizal (AM) fungal colonization of the roots of maize, bean, and sorghum under different management tillage systems ($n = 3$ enumerated replicates).

Crop species and tillage system	Colonization (%)
Maize	
Moldboard	39.5[a]
Shred-bedding	40.3[a]
Subsoil-bedding	41.4[ab]
No tillage	45.5[b]
Bean	
Moldboard	35.6[a]
Shred-bedding	39.2[ab]
Subsoil-bedding	40.5[b]
No tillage	41.8[b]
Sorghum	
Moldboard	41.0[a]
Shred-bedding	43.4[a]
Subsoil-bedding	46.7[b]
No tillage	51.3[b]

Note: For each crop, values in columns followed by the same letter do not differ significantly ($P < 0.05$) as determined by the LSD test.

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TABLE 3. Number of clones of each restriction fragment length polymorphism (RFLP) type obtained for each crop species under different management tillage systems ($n = 3$ enumerated replicates).

RFLP types	Maize				Bean				Sorghum				Total	
	MB	Shred	Sub	NT	MB	Shred	Sub	NT	MB	Shred	Sub	NT	*n*	%
1	2	21	7	1	2	22	0	32	6	28	0	24	145	68.1
2	0	0	1	5	2	1	0	0	0	0	0	14	23	10.8
3	0	0	0	0	0	0	0	0	4	0	9	0	13	6.1
4	0	2	0	8	0	0	0	1	0	0	0	1	12	5.6
5	0	0	0	0	0	0	0	0	0	0	7	0	7	3.3
6	0	0	1	0	0	0	0	0	0	0	1	0	2	0.9
7	0	0	0	0	0	0	0	0	0	1	0	1	2	0.9
8	0	0	0	1	0	0	0	0	0	0	0	0	1	0.5
9	0	0	0	0	0	1	0	0	0	0	0	0	1	0.5
10	0	0	0	0	0	0	0	1	0	0	0	0	1	0.5
11	0	0	0	0	0	0	0	1	0	0	0	0	1	0.5
12	0	0	0	0	0	0	0	0	1	0	0	0	1	0.5
13	0	0	0	0	0	0	0	0	0	0	1	0	1	0.5
14	0	0	0	0	0	0	0	0	1	0	0	0	1	0.5
15	0	1	0	0	0	0	0	0	0	0	0	0	1	0.5
16	0	1	0	0	0	0	0	0	0	0	0	0	1	0.5
Total	2	25	9	15	4	24	0	35	12	29	18	40	213	100

Notes: The first column lists 16 RFLP types that were detected by restriction analysis of PCR fragments with the *Hinf*I and *Hsp92*II enzymes. Abbreviations are: MB, moldboard; Shred, shred-bedding; Sub, subsoil-bedding; NT, no tillage.

groups (Fig. 1). As expected, no AM fungal types from the genera *Archaeospora* or *Paraglomus* were detected because the AM1 primer does not match the SSU sequences of these taxa (Redecker 2000). The phylogenetic relationships among the sequences clearly revealed discrete sequence groups and identified nine potential taxonomic units or fungal types: eight belong to Glomeraceae and one to Gigasporaceae. All sequence clusters were supported by a bootstrap >75%. Two clusters (Glo1 and Glo8) were not well supported by the bootstrap value, but they were identified as distinct fungal types, since they were distinguished by the *Hinf*I and *Hsp*92II RFLP patterns.

The majority of the AM fungal types did not cluster closely with any sequences of AM fungi maintained as reference AMF isolates in international culture collections and they might be unique to this environment. Clade Glo5 included sequences with over 99% identity to the sequences for *G. sinuosum* (AJ133706), while clade Gig1 included sequences with 99% identity to the *Gigaspora decipiens* (U96146), which appeared to be specific for maize and sorghum crops under the subsoil-bedding system. In many cases, sequences from different crops under different tillage systems were identical. In other groups the sequences were more variable.

Sequences amplified using ARCH1311/NS8

The same DNA samples used for the AM1/NS31 amplification were used in PCR reactions with ARCH1311/NS8 to analyze whether the AMF belonging to Paraglomeraceae and Archaeosporaceae were present inside the roots of the investigated crops. Only 7 out of the 36 analyzed samples gave a positive result. All the products amplified by ARCH1311/NS8 were sufficient for cloning. A total of 208 clones were screened for the presence of the insert and 197 clones resulted positive; these were then analyzed with RFLP using the *Hin*fI and *Rsa*I enzymes: 8 patterns were identified. Two clones were sequenced for each RFLP type for a total of 24 sequences.

BLASTn searches in the GenBank database showed that only one of the analyzed sequences belonged to Paraglomerales. The sequence obtained gave high homology (95% identity) with *Paraglomus brasilianum*, and it was present in maize roots under subsoil-bedding. All the other sequences gave homology with *Ascomycota* and *Basidiomycota* (data not shown).

AM fungal distribution

As the properties of each AM fungal type varied according to the tillage system and crop type (Table 4), log-linear modeling was used to assess the relative importance of these factors. The relationship between the explanatory (tillage system and host) and response (AM fungi) variables was explored with this analysis. The results showed highly significant differences among the AM fungal populations for the different management tillage systems (Table 4, model 1a). Along with the log-linear analysis, the Euclidean-based MDS analysis highlighted differences in community composition for the different tillage systems (Fig. 2). The AM fungal communities in the roots of the plants under subsoil-bedding and moldboard system were very similar. The AM communities in plants under shred-bedding also grouped together, but differed from those in plants under subsoil-bedding and moldboard systems. Conversely, the AM fungal communities in the roots of the sampled plants under the no-tillage system were different both from each another and from the other treatments (Fig. 2). The no-tillage system had in fact a higher number of fungal types colonizing bean and sorghum crops than the other management systems

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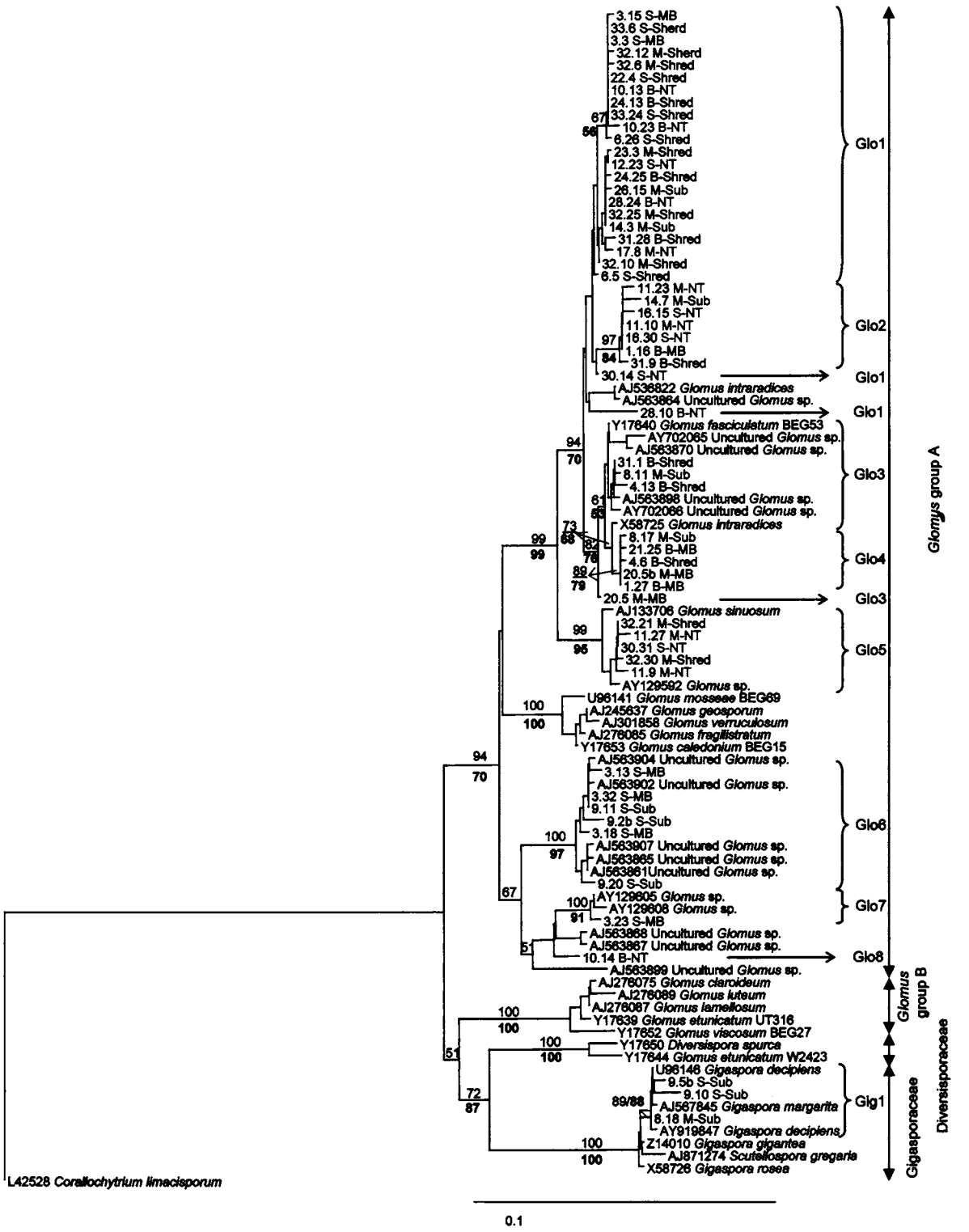

FIG. 1. Neighbor-joining (NJ) phylogenetic tree showing arbuscular mycorrhizal (AM) fungal sequences isolated from roots of maize, bean, and sorghum crop roots under different tillage systems and reference sequences from GeneBank. Numbers above the branches indicate the bootstrap values (above 50%, 100 replicates) of the NJ analysis; numbers below the branches indicate the bootstrap values of the maximum parsimony analysis. Clone identifiers relate to the tillage system and crop species; abbreviations are: MB, moldboard; Shred, shred-bedding; Sub, subsoil-bedding; NT, no tillage (see the Appendix for a color-coded version of this figure showing the tillage system groups); S, sorghum; M, maize; and B, bean. Group identifiers (for example, Glo1) are the AM fungal sequences types found in our study. *Corallochytrium limacisporum* (Cavalier-Smith and Allsopp 1996) was used as the outgroup.

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TABLE 4. Likelihood ratios (LR) produced by general log-linear analysis.

Model	LR	df	*P*
1a) Tillage system main effect	92	3	<0.001
1b) Host main effect	12	2	<0.001
1c) Tillage system × host interaction	42	6	<0.001

Notes: The table shows the LR produced by the model when the specified term is removed. Higher LR values indicate greater deviation from the saturated model.

(Table 3). In addition to modification of the AM fungal community under different tillage systems, our data clearly demonstrated a host-plant significant effect (Table 4, model 1b), despite the observation that communities on the three host species were not clearly separated by the first two dimensions of the MDS analysis (Fig. 2). This effect could be related to the higher fungal diversity found in sorghum compared to the others plants. The diversity of AMF measured using the Shannon-Weiner diversity index was low in general ($H' = 0.6$); however, this index was higher in sorghum ($H' = 0.8$) than it was in bean and maize ($H' = 0.4$ and $H' = 0.6$, respectively). On the other hand, our results showed an interaction between the tillage system and host (Table 4, model 1c), suggesting that, under a determined tillage system, the hosts could be colonized by different AM fungal populations.

DISCUSSION

The success of AMF colonization is crop and treatment dependent

This study has shown, for the first time, how different tillage systems (moldboard, subsoil-bedding, shred-bedding, and no tillage) affect the AMF community structure in maize, bean, and sorghum roots under warm subtropical conditions. The quantification of AMF colonization confirms that these crops show a good degree of AMF colonization (~40%). This was expected, because maize, bean, and sorghum are considered obligatory mycorrhizal species, being readily colonized by many AM fungi (Khalil et al. 1994). However, significant crop and treatment-related differences were revealed. Sorghum never showed the highest root colonization percentage under any tillage system. Grasses, like sorghum, are generally considered to be better host plants for inoculum production, because they produce a greater length of mycorrhizal roots, which should result in more massive sporulation than for other hosts (Simpson and Daft 1990, Douds 1994). The lowest degree of AMF colonization was under the moldboard system for all three crops. Tillage of soil breaks up the AM fungi hyphal network, leading to a significant reduction in the mycorrhizal colonization of roots and in P absorption from soil (McGonigle and Miller 1996).

Molecular analyses reveal that Glomerales are the most represented taxa

In spite of the high number of non-AMF sequences, the primer combinations that were used allowed us to reliably detect and identify all currently known AM fungal taxa. The primer combination NS31-AM1 in fact amplified sequences (31%) related to fungi belonging to the Glomales and Diversisporales orders (i.e., Glomeraceae, Acaulosporaceae, Diversisporaceae, and Gigasporaceae) while the ARCH1311-NS8 primer combination amplified one sequence of Paraglomerales.

The high number of unrelated AMF sequences is surprising; however, problems of non-glomeralean DNA amplification with the NS31-AM1 primer pair have already been reported (Helgason et al. 1999). However, only recently, Douhan et al. (2005) clearly demonstrated the reason for the problem that is related to the AM1 primer for in planta AMF identification. They showed very different results that depend on the host species and the amount of host mycorrhizal colonization and, interestingly, they found that members

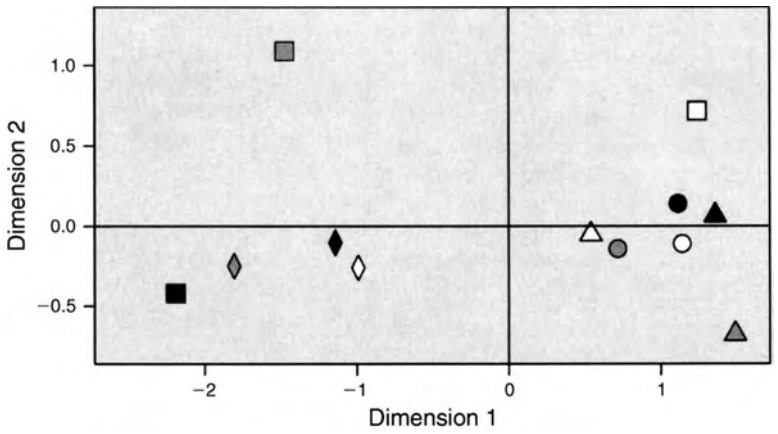

FIG. 2. Euclidean-based multidimensional scaling (MDS) analysis emphasizing changes in the patterns of AM fungal community composition. Each point represents an AM fungal root community, with communities of similar composition being located close together. Key: black symbols, bean; gray symbols, sorghum; open symbols, maize; triangles, subsoil-bedding; circles, moldboard plow; squares, no tillage; rhombuses, shred-bedding.

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of Ascomycetes, like *Nectria,* can be detected, which is in agreement with our results.

BLAST searches with closely related AM fungal sequences in GenBank and through phylogenetic analysis of the obtained sequences assigned the fungal sequences to 10 glomeralean groups. Eight groups belong to the genus *Glomus* group A, one to *Gigaspora,* and one to *Paraglomus* (Fig. 1). Predominance of the Glomeraceae has been reported for tropical forests (Onguene and Kuyper 2001, Husband et al. 2002*a, b*, Wubet et al. 2003), agricultural sites (Daniell et al. 2001, Hijri et al. 2006), and wetland soils (Wirsel 2004). According to Daniell et al. (2001), *Glomus* types dominate arable crops, because they seem to be better adapted to disturbed environments, since their high sporulation rates can enable them to recover more readily (Oehl et al. 2003). In addition, Glomeraceae colonize via fragments of mycelium or mycorrhizal root pieces, readily form hyphal anastomoses (Giovannetti et al. 1999), and therefore have the ability to reestablish a network after mechanical disruption. Gigasporaceae instead propagate via sporal dispersal or infection from an intact mycelium (Biermann and Linderman 1983, Daniell et al. 2001). Such differences clearly explain the dominance of Glomeraceae over Gigasporaceae in environments characterized by repeated severe physical disturbances, such as tillage.

Out of the 10 taxonomic units (TUs) found in the roots, two (Glo6 and Glo7) were exclusively found in sorghum, one (Glo8) in bean, and one (*Paraglomus*) in maize roots. Furthermore, Glo7 was only found under the moldboard system, Glo8 under the no-tillage system, and Gig1 and *Paraglomus* under the subsoil-bedding system. More extensive sampling would be advisable to ascertain whether these findings indicate a host or system specificity as already described in previous studies (Vandenkoornhuyse et al. 2002, Stukenbrock and Rosendahl 2005).

Some sequences were closely related to fungal types observed in previous studies (Husband et al. 2002*a*, Wirsel 2004). However, the majority of clones, grouped into Glo1, Glo2, and Glo4 fungal types, were distinct from any previously published sequence and may be either known, yet unsequenced, or still unknown species. These dominant new fungal types could possess some functional traits, giving them the capability to proliferate under subtropical agricultural conditions. In more general terms, this work supports the concept that more AMF taxa than previously thought probably exist in nature (Husband et al. 2002*a*, Wirsel 2004).

Biodiversity of AMFs in subtropical fields is low

The AM fungal diversity colonizing roots of maize, bean, and sorghum subject to different agricultural practices under subtropical conditions was found to be low ($H' = 0.6$) compared to other ecosystems, such as dry forest ($H' = 2.58$ from one host species; Wubet et al. 2003), tropical forest ($H' = 2.33$ from two host species; Husband et al. 2002*a*), wetland grass ($H' = 2.4$ from one host species; Wirsel 2004), temperate grassland ($H' = 1.71$ from two host species; Vandenkoornhuyse et al. 2002), or seminatural woodland ($H' = 1.44$ from five host species; Helgason et al. 1999). In general, our findings are in agreement with previous studies (Helgason et al. 1998, Daniell et al. 2001, and Oehl et al. 2003) indicating that intensification of agriculture practices negatively affects AMF abundance and diversity, even though a recent work by Hijri et al. (2006) showed that the diversity of AMF is not always low in arable soils. Another important point is that AMF fungal richness might have been underestimated because of the low specificity shown by the primers in our conditions. For instance, many non-AM fungal sequences found in our work correspond to different fungal taxa that colonized decayed roots of conifer seedlings, as reported by Menkis et al. (2006). We suggest that other fungal symbionts (for instance, endophytes) might functionally replace AM fungi or coexist with them as competitors within the same ecological niche when carbon compounds from a host plant are available. Other studies illustrate how different fungal species have a cosmopolitan distribution in cultivated soils and are mostly associated with organic matter and plant debris (Burgess 1981, Rheeder and Marasas 1998). In general, tillage promotes soil organic matter decomposition through crop residue incorporation in a greater volume of soil and the physical breakdown of residues (Salinas-García et al. 2002) by increasing contact between soil microorganisms and crop residues (Henriksen and Breland 2002). Thus, the fact that the highest number of clones of non-AM fungi occurred in the roots of bean and maize under the moldboard tillage system could be due to the increase in fungal and bacterial microorganisms that degraded the cellulose and hemicellulose. These results suggest that nutritional competition with other fungal symbionts may influence the composition of AMF consortia in subtropical habitats.

Conclusions

Mycorrhizal symbiosis and their propagules have been recognized as being fundamental for ecosystem stability and sustainability (Jeffries et al. 2003). Our study confirms that fungal diversity in agricultural ecosystems is quite low and demonstrates that agricultural practices (applied in the experimental fields of Rio Bravo, Mexico) can influence AM specificity, selecting fungal types that are treatment specific. From the perspective of sustainable production, it is very important to ascertain tillage practices that can promote mycorrhizal diversity and, therefore, contribute to the long-term sustainability of agroecosystems under subtropical conditions.

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APPENDIX

Color-coded version of Fig. 1 showing the tillage system groups (*Ecological Archives* A018-014-A1).

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