# SOIL TILLAGE AFFECTS THE COMMUNITY STRUCTURE OF MYCORRHIZAL FUNGI IN MAIZE ROOTS

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*Abstract.*    In this study we tested whether communities of arbuscular mycorrhizal fungi (AMF) colonizing the roots of maize (*Zea mays* L.) were affected by soil tillage practices (plowing, chiseling, and no-till) in a long-term field experiment carried out in Tänikon (Switzerland). AMF were identified in the roots using specific polymerase chain reaction (PCR) markers that had been developed for the AMF previously isolated from the soils of the studied site. A nested PCR procedure with primers of increased specificity (eukaryotic, then fungal, then AMF species or species-group specific) was used. Sequencing of amplified DNA confirmed that the DNA obtained from the maize roots was of AMF origin. Presence of particular AMF species or species-group was scored as a presence of a DNA product after PCR with specific primers. We also used single-strand conformation polymorphism analysis (SSCP) of amplified DNA samples to check if the amplification of the DNA from maize roots matched the expected profile for a particular AMF isolate with a given specific primer pair.

Presence of the genus *Scutellospora* in maize roots was strongly reduced in plowed and chiseled soils. Fungi from the suborder *Glomineae* were more prevalent colonizers of maize roots growing in plowed soils, but were also present in the roots from other tillage treatments. These changes in community of AMF colonizing maize roots might be due to (1) the differences in tolerance to the tillage-induced disruption of the hyphae among the different AMF species, (2) changes in nutrient content of the soil, (3) changes in microbial activity, or (4) changes in weed populations in response to soil tillage. This is the first report on community composition of AMF in the roots of a field-grown crop plant (maize) as affected by soil tillage.

*Key words:    arbuscular mycorrhiza; community structure; field experiment; maize; molecular identification; root colonization; soil tillage; specific primers.*

## INTRODUCTION

The practice of sowing crop into no-till soil has been developed to reduce soil erosion and costs of agricultural production (Lal 1991, Tebrügge and During 1999). In the USA $15 \times 10^6$ ha and in Brazil $14 \times 10^6$ ha are annually planted without tillage (Six et al. 2002). Increases in topsoil bulk density (Kaspar et al. 1991), soil aggregation (Wright et al. 1999) and penetration resistance (Unger and Jones 1998), increased water retaining capacity (Lal 1995), and lower soil temperature (Arshad and Azooz 1996) have been described in no-till soils as compared to tilled soils. Higher concentrations of soil organic matter and inorganic nutrients such as P and K have been observed in the topsoil of no-till compared to tilled soils (Doran 1987, Robbins and Voss 1991, Angers et al. 1995). Fungal biomass is also enhanced in the topsoil under no-till (Frey et al. 1999). Streit et al. (2000) observed an increase in the pro-

portion of perennial weeds, and O'Donovan and McAndrew (2000) observed a decrease in the proportion of annual weeds in no-till soils compared to the tilled soils. However, the extent of the soil tillage effect on various soil parameters strongly depends on soil type and climate (Blevins and Frye 1993).

Arbuscular mycorrhizal fungi (AMF) colonize roots of the majority of terrestrial plants (Smith and Read 1997), including most crop species. These fungi affect nutrient uptake, growth, and reproduction of the host plants (Subramanian and Charest 1997, Miller 2000). Van der Heijden et al. (1998) showed that an increase in AMF species richness resulted in an increased dry matter production in a model grassland ecosystem. Jastrow et al. (1998) showed that AMF also improve aggregate stability, as their extraradical hyphae can bind soil particles together both mechanically (Miller and Jastrow 1990) and chemically through the exudation of glomalin (Wright and Upadhyaya 1998).

Tillage and high-input management have a negative impact on AMF spore numbers (Galvez et al. 2001) and on the density of AMF hyphae in soil (Kabir et al. 1998). Higher AMF infection potential as well as faster

development of AMF colonization have been shown in soils under reduced tillage conditions (McGonigle and Miller 1996). Spores of *Glomus etunicatum* were found to be more abundant in tilled soils, while spores of *Glomus occultum* were more frequent under no-till (Douds et al. 1995). Jansa et al. (2002) observed an increased abundance of large spores of *Glomus mosseae* in plowed soils compared to no-till soils, where diverse communities consisting of several small-spored *Glomus* species and non-*Glomus* AMF were present. They also found that the community structure of AMF spore communities was different under the different tillage treatments.

Morphological, enzymatic, and immunological approaches are less powerful for assessing the diversity of AMF in the roots (Merryweather and Fitter 1998) than molecular approaches (van Tuinen et al. 1998*a*). The development of the latter has, however, been slow for two reasons. First, the purity and amount of the fungal material is severely limited, as AMF cannot currently be cultured without host plant roots (Smith and Read 1997). Second, relatively large variability of sequences within an AMF individual has been described (Sanders et al. 1995). This is probably due to genetic differences among nuclei within each individual and evolutionary mechanisms that allow the maintenance of these differences (Kuhn et al. 2001). Recent studies are based on a nested PCR procedure, targeted to the genes coding for 18S (Clapp et al. 1995, Redecker 2000) or the 28S ribosomal subunit (van Tuinen et al. 1998*a, b*). Most recent studies have been carried out under greenhouse conditions (van Tuinen et al. 1998*a*, Kjøller and Rosendahl 2000) or in polluted soils with low diversity of AMF communities (Jacquot et al. 2000, Turnau et al. 2001). Less work has been done in complex natural communities such as agricultural fields or woodlands (Clapp et al. 1995, Helgason et al. 1998, 1999, Daniell et al. 2001). These last studies indicated a high diversity of mycorrhizal symbionts in undisturbed environments as compared to arable soils. However, the absence of an intensive sampling design hampered an in-depth statistical evaluation of the results. Some studies also used molecular markers, which were either not exclusively AMF-specific or too specific, preventing the observation of a number of AMF phylogenetic groups (Redecker 2000). At that time the necessity of tuning the identification tools for local AMF communities (including extensive isolation of AMF from the studied site) had not yet been recognized (Turnau et al. 2001).

The objective of this work was to further develop and use some molecular techniques to test whether AMF community structure in roots of field-grown maize was affected by soil tillage. In this study, soil tillage is defined as proposed by SSSA (1997) as any kind of soil manipulation performed by application of mechanical forces to the soil. Three tillage treatments (plowing, chiseling, and no-till) were tested, representing a gradient in tillage intensity (Mozafar et al. 2000, Streit et al. 2000).

## MATERIALS AND METHODS

### Field experiment

Fungal isolates were obtained from a long-term tillage experiment in Tänikon (Switzerland, 47°29′ 10.0″ N, 8°55′10.1″ E, altitude 540 m). The field experiment was established in 1987 on a well-drained stony Orthic Luvisol with the following characteristics (0–20 cm depth): 51% sand, 31% silt, 16% clay, pH 6.2 (Anken et al. 1997). Six different management practices were under study in combination with two plant-residue managements. The $6 \times 12$ m plots were organized in a completely randomized block design with four replicates. Crop rotation was the same for all soil treatments and consisted of a sequence of winter wheat (*Triticum aestivum* L.)—maize (*Zea mays* L.)—winter wheat—canola (*Brassica napus* L.), with one harvest every year. We studied three treatments: (1) plowing (soil plowed annually to the depth of 25 cm with a moldboard plow); (2) chisel treatment (soil loosened annually with a wing share chisel, which cuts the soil to the depth of 25 cm, but does not turn it over); and (3) no-till. We only studied soils from treatments where organic residues had been left in the fields. In the no-till treatment, 3L/ha of the herbicide glyphosate was sprayed yearly to control weeds. Average fertilization of 112 kg N, 33 kg P, and 101 kg K per hectare was applied yearly in a water-soluble form as standard in all treatments. No insecticides or fungicides were used so as to minimize the effects on soil microflora. Under no-till, pellets containing 6% metaldehyde were routinely applied in canola and maize cultivation against slugs. In the other tillage methods, those pellets were applied according to the amount of infestation. Total P and N, available P and K, and organic carbon contents were measured in 1999 down to 40 cm in three or four (in case of total P) field replicates of the three tillage treatments (Table 1). The AMF were isolated and identified in soil sampled in the three tillage treatments in January 1999, after the canola season, using trap cultures and single-spore cultures of *Glomus* spp. (Jansa et al. 2002).

### Development of molecular markers

For the development of molecular markers we used AMF material from 12 single-spore cultures belonging to four species of *Glomus* sp., and spores isolated from trap cultures for Gigasporaceae (Jansa et al. 2002). The single-spore cultures of AMF were deposited in the International Culture Collection of Arbuscular and Vesicular–Arbuscular Mycorrhizal Fungi (INVAM)[5] under the accession numbers SW201–SW212, and in The International Bank for the Glomales (BEG)[6] under the

[5] URL: ⟨http://invam.caf.wvu.edu⟩
[6] URL: ⟨http://www.ukc.ac.uk/bio/beg⟩

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TABLE 1. Soil properties under different tillage treatments (Till, Chisel, No-till) and across depths in the long-term field experiment in Tänikon (Switzerland).

| | Depth | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Till | | | | Chisel | | | |
| | 0–10 | 10–20 | 20–30 | 30–40 | 0–10 | 10–20 | 20–30 | 30–40 |
| $P_{total}$ (mg/kg)‡ | 964 | 980 | 659 | 616 | 1093 | 992 | 659 | 620 |
| $P_{available}$ (mg/kg)§ | 1.70 | 1.75 | 1.20 | 0.41 | 2.32 | 1.44 | 0.56 | 0.25 |
| $K_{available}$ (mg/kg)§ | 24.3 | 18.5 | 20.8 | 16.6 | 47.9 | 25.5 | 12.2 | 8.3 |
| Organic C%‖ | 1.54 | 1.52 | 1.24 | 0.82 | 1.91 | 1.49 | 0.95 | 0.69 |
| Total N%¶ | 0.22 | 0.21 | 0.15 | 0.12 | 0.23 | 0.23 | 0.16 | 0.13 |

*Note:* Means and *F* ratios for the two-way ANOVA showing the soil treatment (T) and depth effects (D), as well as ANOVA comparing correlation coefficients between soil properties and soil depth among different soil treatments are given.

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant.

‡ P extracted by 2 mol/L HCl after incineration at 550°C for 6 h (Saunders and Williams 1955).

§ P and K extracted by $CO_2$-saturated $H_2O$ (Dirks and Scheffer 1930).

‖ Estimated by the dichromate-oxidation method (Sims and Haby 1971).

¶ Measured on the Nitrogen Analyzer 1500 (Carlo Erba Strumentazione, Schlieren, Switzerland).

accession numbers BEG155–BEG162. Both substrate-borne spores and roots of maize or wheat colonized by the single-spore cultures were used for developing molecular markers. Multispore cultures of *Gigaspora margarita* and *Scutellospora pellucida* were established only recently from the studied field site and deposited in BEG under the accession numbers BEG152 and BEG153.

The DNA from single spores was extracted according to Sanders et al. (1995). Single spores were crushed in 5 μL of PCR-grade water by freshly flamed Pasteur pipette. Five microliters of Chelex-100 (20%, Bio-Rad Laboratories, Hercules, California, USA) were added and samples were placed onto a hot plate (95°C) for three minutes, then transferred to ice, and incubated at 0°C for 5 minutes. Five microliters of the liquid phase were taken as template for PCR amplification of the large ribosomal subunit gene, 28S. Root pieces of maize and wheat colonized by single-spore cultures of *Glomus* sp. (5-cm fragments of fine roots, two months old) were carefully cleaned from adjacent spores and mycelium, frozen with liquid $N_2$ in a ceramic mortar, and powdered immediately. DNA was extracted by

DNeasy Plant Mini Kit (Qiagen Sciences, Germantown, Maryland, USA) following manufacturer's recommendations and a 5-μL aliquot was used for PCR amplification of the 28S ribosomal subunit gene. Root pieces of nonmycorrhizal maize and wheat were used as a negative control.

A nested PCR procedure employing, first, eukaryotic-specific primers ITS3 + NDL22 (White et al. 1990) and, second, fungal-specific primers LR1 + FLR2 (van Tuinen et al. 1998*b*, Turnau et al. 2001) was performed on both spore and root DNA samples. There were 30 cycles with each primer pair. The product of the first PCR was diluted 1000 times, and 5 μL of the diluted mixture was used for the second reaction. The PCR conditions were the same as those described by van Tuinen et al. (1998*b*), the annealing temperature being 60°C in both PCR steps.

Ten microliters of reaction mixture was used to check amplification on 1.2% agarose gel (SeaKem LE, Flowgen, Leicestershire, UK). The remaining product was then purified using QIAquick PCR Purification Kit (Qiagen Sciences), cloned into a blue script vector (pGEM-T Easy, Promega-Catalys AG, Wallisellen,

TABLE 2. Specificity and length of the expected fragment with primers developed for identification of AM fungi in plant roots.

| Primer name | DNA sequence in 5′-end to 3′-end orientation | Targeted specificity | Product length (base pairs) |
|---|---|---|---|
| Clar1A | CGA TCG AAG TCA GTC GTG CTG GCG | *Glomus claroideum* group | 265 |
| Clar1B | CGA TTG AAG TCA GTC GTG CTG GCG | | |
| Clar2A | AAG AGA AGC CAG GTG GAA CAG CCC | | |
| Clar2B | AAG AGA GGC CAG GTG GAA CAG CCC | | |
| Intra1 | GGT GCG ATT CTG TGG AGT GTG AGG | *Glomus intraradices* | 254 |
| Intra2 | CAA GCT TTC GGC ACC AGA GCA ACG | | |
| Cal1 | CCT CTT GAG TTT GGT CTC GTG GG | *Glomus caledonium–Glomus mosseae* group | 248 |
| Cal2 | AGG CAA CGT TTC AGA GAT CAG ACG | | |
| Gig1 | GGT ATC ATA GAG GGT GAG AAT CCC | *Gigaspora* sp. (genus) | 308 |
| Gig2 | AAA TCG ACG CTA ACC TGC CAA ACG | | |
| Scut1 | AGG TAT CAT GGA GGG TGA GAA TCC C | *Scutellospora* sp. (genus) | 434 |
| Scut2 | CGT ATT AGA GAC CAG GCG GTT AAC C | | |

TABLE 1. Extended.

| Depth | | | | | | | | $F$ ratios | | | |
| No-till | | | | | | | | | | | |
| 0–10 | 10–20 | 20–30 | 30–40 | $df_T$ | $df_D$ | $df_{T\times D}$ | $df_{residual}$ | Tillage | Depth | Interaction T × D | Comparison of correction coefficients |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1076 | 950 | 690 | 617 | 2 | 3 | 6 | 36 | 0.50 NS | 47.4*** | 0.48 NS | 0.08 NS |
| 4.01 | 1.47 | 0.62 | 0.19 | 2 | 3 | 6 | 24 | 0.65 NS | 10.8*** | 1.54 NS | 2.75† |
| 47.6 | 23.5 | 12.7 | 7.5 | 2 | 3 | 6 | 24 | 0.67 NS | 25.3*** | 3.94** | 9.31*** |
| 1.95 | 1.57 | 0.99 | 0.68 | 2 | 3 | 6 | 24 | 0.13 NS | 64.0*** | 2.62* | 5.49** |
| 0.24 | 0.24 | 0.16 | 0.13 | 2 | 3 | 6 | 24 | 2.65† | 67.4*** | 0.13 NS | 0.25 NS |

Switzerland), and transformed into bacterial strain *E. coli* JM109 by the heat-shock method. The size of the insert in growing bacterial colonies was checked after PCR amplification using M13+ and M13− primers that were targeted to the cloning site of the vector. Plasmid DNA was isolated from transformed bacteria following standard miniprep procedure (Sambrook et al. 1989), and used as a template for cycle sequencing using BigDye Terminator (Applied Biosystems, Faster City, California, USA). Sequencing analysis was performed on ABI-310 Capillary Sequencer (PerkinElmer, Wellesley Massachusetts, USA). All the sequence data were computer edited and reedited manually. Sequences obtained from this screening were deposited in the GenBank (National Center for Biotechnology Information, Bethesda, Maryland, USA)[7] under accession numbers AF396782-AF396799.

Fungal 28S sequences obtained from both the above-described study and from the Genbank (28S sequences from *Gigaspora rosea*, *Scutellospora castanea*, and several *Glomus* sp. sequences) were aligned by ClustalW software (Thompson et al. 1994). Phylogenetic relationships were inferred using Phylip Program Package (Felsenstein 1993), employing a neighbor joining with the Kimura distance model setting the transition/transversion ratio at 2. The identity of outgroup species used to root the dendrograms is indicated for each analysis. Sequences obtained by amplification of nonmycorrhizal roots showed similarity to basidiomycetous and ascomycetous fungi.

Using the sequence information we designed the primer pairs to specifically amplify five disjunctive groups of AMF (Table 2, Fig. 1). DNA extracts from AMF spores were used to cross check that the primers only amplified the targeted fungal group. The primers were used (separately for each fungal group) in a third nested PCR. The second PCR product was diluted 1:1000, and 5 μL of the solution was used as a template for the third reaction. This was run under stringent conditions, denaturation at 95°C for three minutes, 32 cycles of 93°C (60 seconds)–68°C (60 seconds)–72°C (60 seconds), and final extension at 72°C for five minutes. The composition and concentration of PCR mix-

ture was identical to that referred to above, except for the primers. Amplification product was analyzed on 2% agarose gel. Subsamples of these products were cloned and sequenced and compared with the 28S sequences obtained previously. This comparison showed that the sequences were very similar and that using many PCR cycles had not introduced too much sequencing and/or Taq polymerase reading error.

### Root sampling

From each plot (12 plots in three soil treatments), three 90-d-old maize plants (cultivar LG 22.65, planted at a density of 11 plants/m²) were randomly sampled in mid-July 2000 and their roots taken from the 0–15 cm soil depth. Maize roots were carefully cleaned with tap water and washed several times with deionized water. Roots were cut into 5-cm pieces, and four fine root pieces (<1 mm in diameter) were selected per plant. Each root piece was further washed in PCR-grade sterile water and then the DNA was extracted using the protocol described above. A total of 144 root pieces were used for the molecular analysis.

### Molecular identification of AMF in the roots

DNA samples from root pieces were used in the same nested PCR protocol as previously described. Five separate PCR reactions were performed with five different primer pairs on each DNA sample (specific primers for the detection of *Glomus claroideum* group were partially degenerated at one base each, Table 2). Presence/absence of a reaction product was scored after an electrophoresis of a 10-μL aliquot on 2% agarose gel. The molecular identification of AMF in the roots with PCR allowed us to calculate two parameters: species richness and colonization frequency. Species or species-group richness is defined as the number of species or species groups found in a plot. The frequency of colonization is calculated as the percentage of root fragments in a plot colonized by a specific species or a species group.

A sequencing control was performed on the samples amplified from the field roots after the three PCR steps. Those sequence data were used to check the identity of the samples from roots, and they were later deposited in the GenBank under accession numbers AF396800–
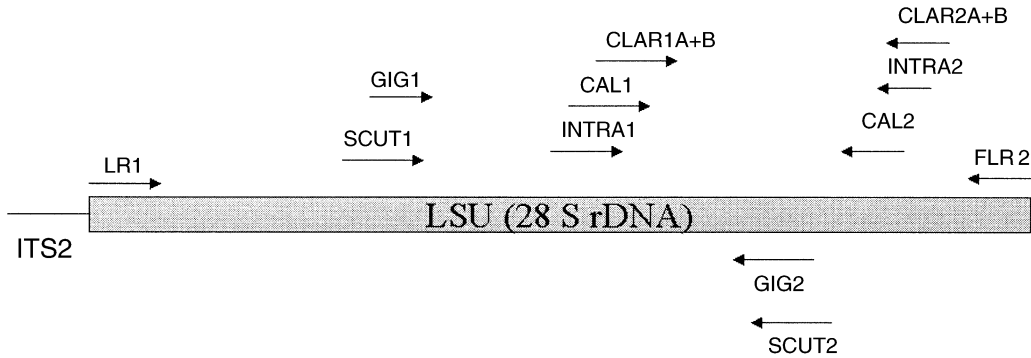
Fig. 1. Position of primer binding sites for the primers used for detection of AMF within host plant roots for the third PCR of the nested PCR procedure. Binding sites of fungal-specific primers LR1 and FLR2 (van Tuinen et al. 1998*b*) are also shown.

AF396826. Phylogenetic analysis based on sequence data was performed by comparing sequences obtained from the field samples with sequences obtained by screening pure isolates of AMF, and with the sequences obtained from the GenBank.

### Single-strand conformation polymorphism analysis of amplified DNA samples

Where positive amplification with specific markers occurred, the amplified DNA samples were further analyzed by single-strand conformation polymorphism analysis (SSCP). This was done in order to distinguish lower taxonomic units within the species or species-groups of AMF targeted by specific primers. The SSCP analysis allows identification of specific sequence types in a mixed sample by examining the mobility of single DNA strands folded according to their sequence composition. Considering the fact that the genes coding for ribosomal RNA are variable within both AMF species and individuals (Kuhn et al. 2001), the different molecular types represented by different sequences (Sheffield et al. 1993) must not necessarily represent different individuals and/or species, but different genotypes within the same individual and/or species. However, for some AMF species, identification of species-specific SSCP patterns could still have been achieved due to extensive calibration with pure cultures. For others, we prefer to keep the label ''molecular type'' until more information is available.

AMF species or species-group specific primer pairs were designed so that the size of the DNA fragments obtained after the third PCR would fit the optimal range of resolution for SSCP. DNA was denatured by mixing 4 μL of PCR sample with 8 μL of formamide (HCONH$_2$, containing 10 mmol/L NaOH), heating at 95°C for five minutes, and placing on ice for five minutes. Samples were loaded on precooled (5°C) SSCP precast gels (GMA Wide Mini S-26; Elchrom Scientific, Cham, Switzerland) and resolved on an Elchrom SEA-2000 electrophoresis apparatus at 5 V/cm for 16 hours. Gels were stained with SYBR Gold II dye (Mo-

lecular Probes, Eugene, Oregon, USA) and photographed on a shortwave (254 nm) transilluminator (Elchrom).

DNA samples from spores of the single-spore isolates of AMF were also subjected to the same SSCP. The profiles obtained were used as a reference to identify the profiles produced from root-extracted DNA, and as a further check that we had successfully amplified the targeted AMF. Presence or absence of a specific SSCP profile in field samples amplified with respective primers was scored, and root colonization by a given banding profile (molecular type) of AMF was calculated per plot. In the case where pure isolates were not available (e.g., *Gigaspora* and *Scutellospora*) or if a completely distinct profile was identified among field samples, which could not be related to any of the pure isolate samples studied (e.g., *G. caledonium*), separate SSCP profiles were described and evaluated independently.

### Statistical analysis

The hypothesis that soil tillage affects the distribution of nutrients and organic carbon in different soil depths was tested by two approaches. This was done, first, by using two-way ANOVA with tillage treatment and soil depth as factors and examining the significance of interaction between the factors among different tillage treatments. Second, the analysis was done by comparing slopes of regression lines between soil properties and soil depth among different tillage treatments using comparison of correlation coefficients (Kleinbaum et al. 1988).

Colonization frequencies of each fungus in each experimental plot were used in a one-way ANOVA with soil treatment as a factor with three levels and four replicate plots for each treatment, following a MANOVA analysis. Statistical analyses were carried out with SAS software (SAS 1987) and Statgraphics software version 3.1 (Manugistics 1997). The acceptable level of significance for this study was set at $P < 0.05$. Results with $0.05 \le P < 0.1$ were considered to be
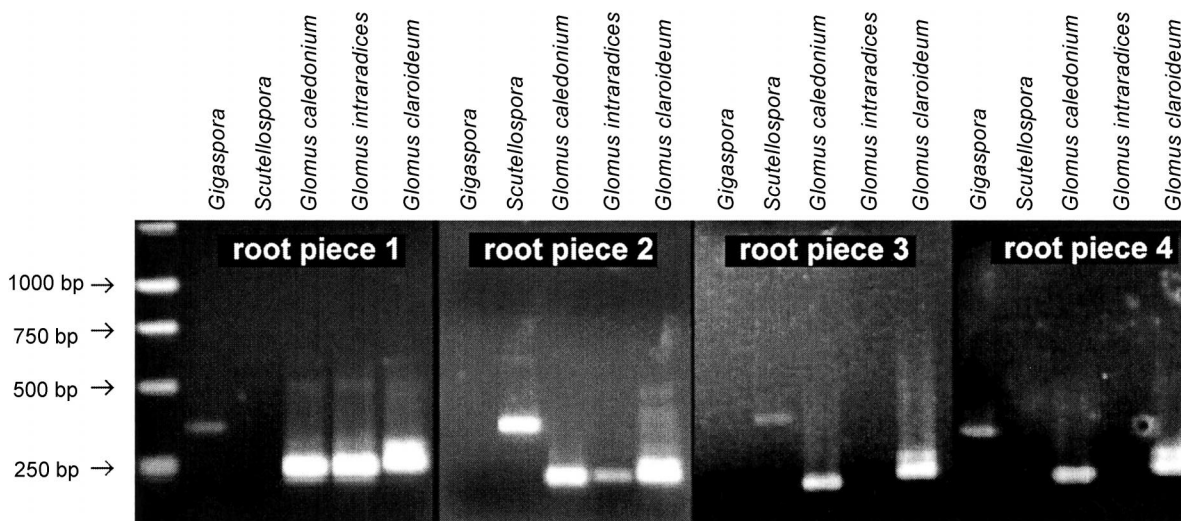
FIG. 2. Presence and absence of different AMF in four root pieces from a single plant, as visualized by staining of PCR products after the third step of nested PCR using five primer pair combinations in the final PCR. Variability of fungal species colonizing different root pieces from a single root system is shown. The specificity of the primers is indicated above each lane; bp = base pairs.

marginally significant, with regard to the fact that the study was carried out under field conditions. LSD multiple range test was performed to test significant differences among the means, provided that the previous ANOVA analysis gave a significant result. Both Hotelling-Lawley Trace statistics and Wilks' lambda statistics were used to estimate the $F$ ratio of the MANOVA analysis (Jongman et al. 1987).

## RESULTS

### PCR identification of fungi in the roots

Some plants were colonized by fungi of all five of the AMF species or species groups that were tested (Fig. 2). The richness of AMF species or species groups in the roots of maize was significantly lower (ANOVA $F_{(2,9)} = 9.0$, $P < 0.007$) in the plowed than in the no-till soils. This difference was due to higher abundance of *Scutellospora* sp. in the roots from the no-till soil.

A subsample of the DNA that had gone through the three-step PCR reaction was cloned and sequenced to provide proof regarding the identity of the sequences amplified from the field roots. This was performed because there was a possibility that the primers could amplify DNA from fungi, which had not previously been isolated from the field or which the primers had not been designed for. Phylogenetic analysis of a selection of those sequences together with previously published respective GenBank accessions is shown in Fig. 3. Sequences obtained from roots by primers targeted for three *Glomus* groups fitted into the expected clusters (Fig. 3A). Sequences obtained using specific primers targeted to *Scutellospora* and *Gigaspora* clustered also within these AMF genera. However, the diversity of fungi from the genus *Gigaspora* in our ex-

perimental field was substantially higher than that expected from previous analysis of spores formed in trap pots. This conclusion is drawn from the deeply branched tree structure of *Gigaspora* sequences from the roots sampled in the fields (Fig. 3B). This heterogeneity of sequences from the field root samples is contrasting with the uniformity of sequences from the *Gigaspora* sp. spores recovered from the trap cultures that are clustering together with the reference sequence of *Gigaspora rosea* (Fig. 3B).

The frequencies of colonization of different AMF in maize roots from the fields confirmed differences in community structure of AMF due to soil tillage treatments (Hotelling-Lawley Trace statistic $F_{10,56} = 39.5$, $P < 0.0001$; Wilks' lambda $F_{26,42} = 20.1$, $P < 0.0001$; Fig. 4). *Scutellospora* sp. colonization in roots was significantly reduced in both plowed and chiseled soils compared to the no-till treatment. Colonization of roots by *Glomus intraradices* was significantly increased with increasing tillage intensity, i.e., a higher colonization was observed in plowed and chiseled treatments than in the no-till treatment. This trend was also true for colonization by the *G. claroideum* group, although this was only marginally significant. Root colonization by the *G. mosseae–G. caledonium* species group as well as by *Gigaspora* sp. was not affected by the soil tillage treatment (Fig. 4).

### SSCP analysis of amplified DNA samples

Using SSCP analysis, closely related AMF species such as *Glomus mosseae* and *G. caledonium* could be distinguished within samples where they were co-amplified by species group specific primers. Identification of distinct molecular types within other AMF groups
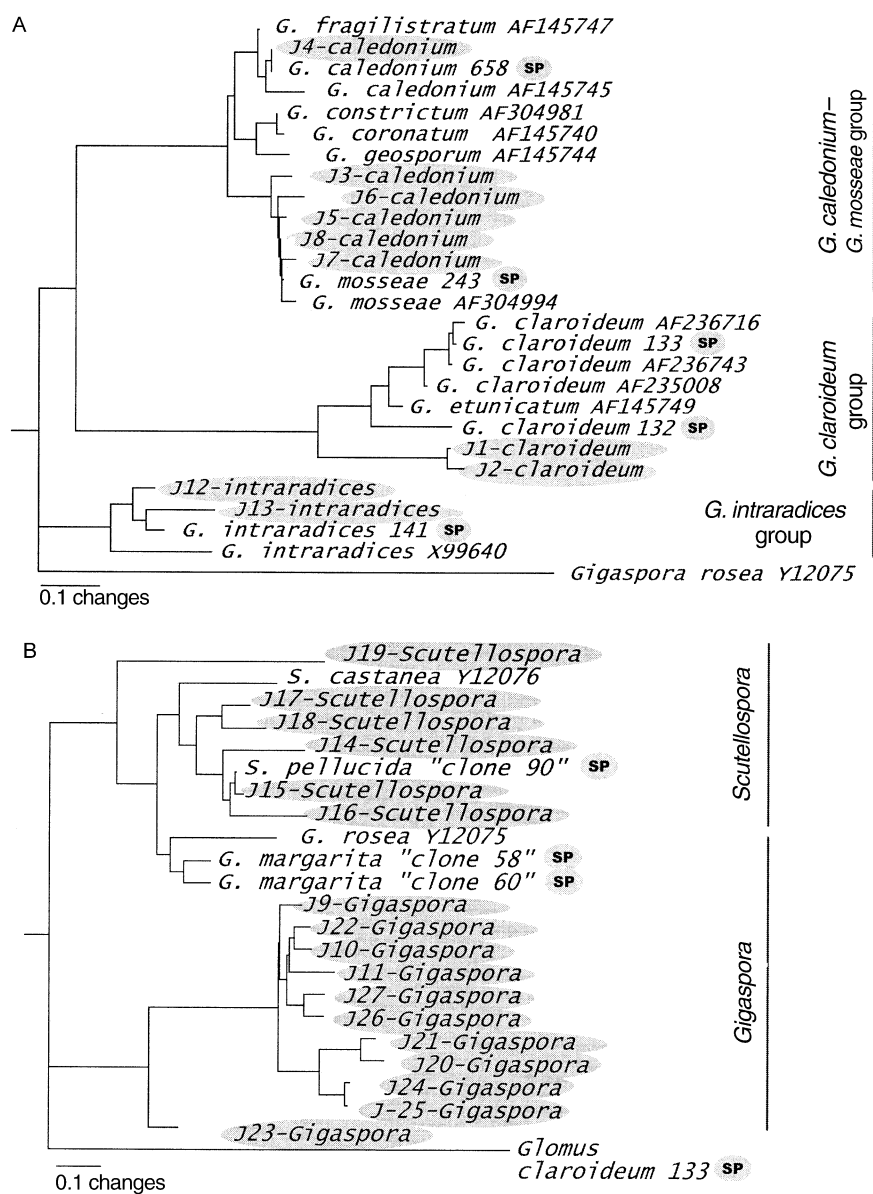
FIG. 3. Phylogenetic analysis to check the origin of the sequences obtained from maize roots from the field, using the five group-specific primer pairs. Sequences obtained from the roots are contrasted by gray background, and they are labeled with J and the name of the fungal group that the primers were targeted for (shown in boldface). Sequences obtained from GenBank are labeled with their respective entry numbers (AF, X, Y). Sequences labeled with three-digit numbers originate from single-spore cultures isolated from the same field site. The sequences of *Gigaspora* and *Scutellospora* labeled with the clone number were obtained from spores in trap cultures. All the sequences obtained from AMF spores from the studied field site are additionally labeled with a suffix **SP** highlighted by gray background. (A) Phylogeny of sequences amplified from maize roots using group-specific primer pairs targeted for different *Glomus* sp. groups. A *Gigaspora rosea* sequence from GenBank was used as the outgroup. (B) Phylogeny of sequences amplified from maize roots using group-specific primer pairs targeted for *Gigaspora* sp. and *Scutellospora* sp. A sequence from a single-spore isolate of *Glomus claroideum* was used as the outgroup. The position of the branch containing the only 28S sequence of *Gigaspora* currently present in the GenBank (*Gigaspora rosea* Y12075), clustering in the dendrogram close to *Scutellospora* sp., could not be justified by parsimony analysis.

was also possible (Fig. 5A). Multivariate ANOVA confirmed significant differences in abundance of different molecular types of AMF with respect to different soil tillage treatments (Hotelling-Lawley trace statistic $F_{26,40} = 10.8$, $P < 0.088$; Wilks' lambda $F_{26,42} = 7.1$, $P < 0.009$). Root colonization by both molecular types of *Scutellospora* sp. in the roots was significantly reduced in plowed and chiseled soils compared with the
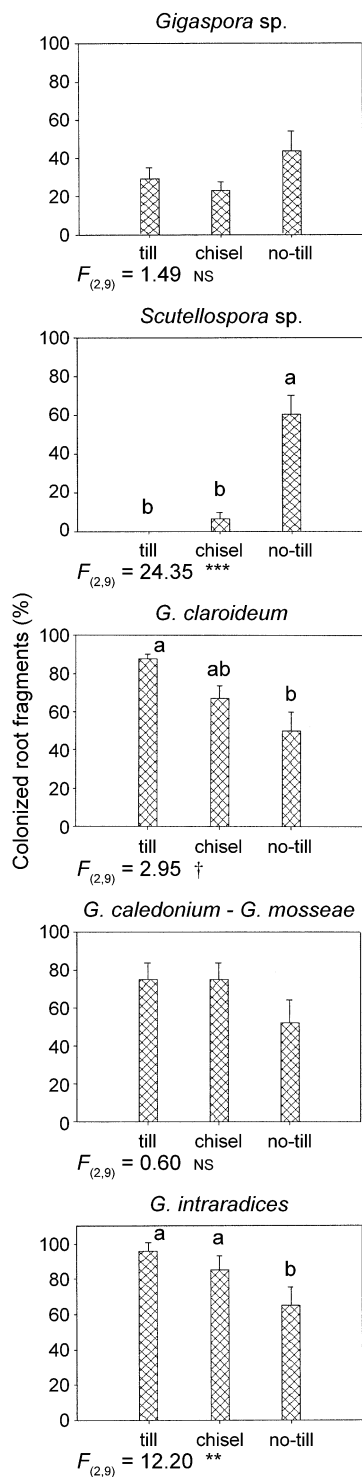
FIG. 4. Differences in percentage of colonization of maize root fragments by five different AMF groups as measured by amplification with AMF-group-specific primers and as affected by soil tillage treatment. Different letters above bars indicate a significant difference according to LSD tests (at the $P < 0.05$ level of significance). The $F$ ratio for the ANOVA showing the soil treatment effect is given below each graph. Bars represent one standard error of the mean.

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant.

no-till soils. One of the molecular types of *Scutellospora* was detected only in the no-till soils, and it was absent from any other soil tillage treatment (molecular type A, Fig. 5B). None of the three molecular types of *Gigaspora* was affected by soil tillage. No significant differences ($P < 0.05$) in abundance of *Glomus mosseae* and *Glomus caledonium* could be observed with respect to the different soil tillage treatment. However, increase in root colonization by *Glomus caledonium* (type B) and *Glomus claroideum* (type A) with increased tillage intensity was observed at a 90% probability level (Fig. 5B). Colonization of all three molecular types of *Glomus intraradices* in the roots of field-grown maize have increased as a result of increased soil tillage intensity (Fig. 5B).

## DISCUSSION

This study has shown for the first time that an agricultural management practice, namely soil tillage, affects the AMF community structure in maize roots. This was possible because of the combination of intensive sampling within an established experimental design, and also because reference AMF cultures had previously been isolated from the same field site (Jansa et al. 2002). High diversity of AMF, including several genera, was observed in the field under intensive agricultural use: 17 species from five genera in the no-till soil, 13 species from three genera in the chiseled soil, and 12 species from two genera in the plowed soil (Jansa et al. 2002). This contradicts previous molecular studies, which have reported only low diversity of mycorrhizal symbionts in the roots of plants from arable soils (Helgason et al. 1998, Daniell et al. 2001). However, the statistical analysis in those referred studies was limited due to absence of a suitable sampling design. Thus, the within-treatment variation could not be compared to among-site variation in presence or absence of different AMF taxa.

The changes we observed in AMF community in response to different soil tillage practices might be due to: (1) disturbance of AMF hyphae integrity by the tillage, (2) changes in nutrient content of the soil, (3) changes in microbial activity, or (4) changes in weed populations.

The absence or lower abundance of *Scutellospora* sp. in plowed and chiseled plots, respectively, might be attributed to the yearly disruption of the extraradical hyphae of these fungi induced by soil tillage. The presence of *Gigaspora* sp. in the roots from all the plots (tilled, chiseled, and no-tilled), on the other hand, suggests that these fungi were more tolerant to this stress. Our results confirm the hypothesis that the undisturbed hyphal network plays an important role in the survival and in root infection of *Scutellospora* sp., as proposed by Jasper et al. (1993). However, we show that this hypothesis is not valid for all *Gigaspora* sp., as proposed earlier by Boddington and Dodd (2000), who performed a study based on the capacity to form my-
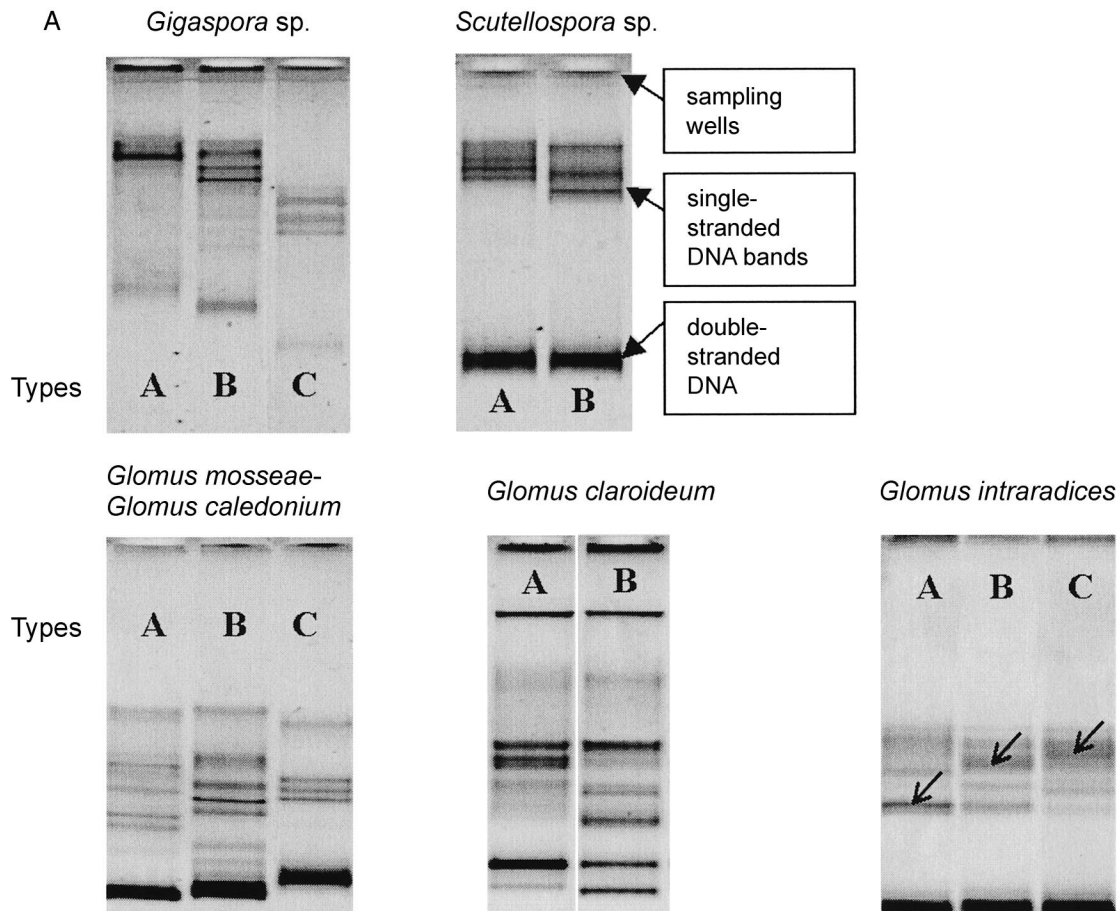
FIG. 5. (A) Definition of molecular types of AMF, based on SSCP banding pattern of DNA samples of isolated AMF cultures, amplified in three-step PCR procedure. SSCP types were identified in the groups of *Gigaspora* and *Scutellospora* based on field samples. SSCP types in the *Glomus mosseae–Glomus caledonium* group were defined based on profiles both from single-spore isolates (type A and B, corresponding to spores of *Glomus mosseae* and *Glomus caledonium*, respectively) and from field samples (type C). SSCP types in the *Glomus claroideum* group were defined based on profiles of single-spore isolates. Within *Glomus intraradices*, only presence/absence of particular bands could be detected. Band composition was very similar among the samples; samples differed mostly in intensity of the bands. Three particular bands (positions shown with arrows) could be distinguished and separately evaluated. Colors of SSCP images were inverted (black to white and vice versa) to obtain better contrast. (B) (facing page) Colonization of roots of maize grown in the field soil subjected to different tillage, by different molecular types of AMF. Different letters above the bars denote significant differences between treatments according to LSD multiple range test ($P < 0.05$), which was only performed where the ANOVA showed significant differences among tillage treatments. $F$ ratio for the ANOVA showing the soil treatment effect is given below each graph. Bars represent one standard error of the mean.

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; NS, not significant.

corrhizas by the extraradical mycelium of different AMF subjected to different levels of soil disturbance. The increased incidence of *Glomus* sp. in tilled soils can be explained by a stimulatory effect of tillage on hyphae growth as suggested by Boddington and Dodd (2000) and/or by the lower competition for root colonization due to the absence of other AMF species. We are, however, aware that other AMF (*Acaulospora* and *Entrophospora*) might have been missed in this study, because we could not develop appropriate molecular detection tools for them.

Nutrient availability affects the composition of AMF communities in the soil (Johnson 1993, Ezawa et al.

2000, Titus and Lepš 2000). Water-soluble fertilizer inputs, especially N inputs, have a negative effect on Gigasporaceae populations (Egerton-Warburton and Allen 2000). In the studied field, total N and P contents were similar in the different horizons of the three tillage treatments, while available P and K contents were higher in the upper horizon of the no-till soil compared to the chiseled and plowed soil treatments (Table 1). The increased occurrence of *Scutellospora* sp. in the roots from the no-till soil showed that these fungi were tolerant to this higher P and K availability.

Both total microbial activity (Palma et al. 2000) and the structure of microbial communities (Lupwayi et al.

B



*Scutellospora* type A  
$F_{(2, 9)}$ = 7.38 *

*Scutellospora* type B  
$F_{(2, 9)}$ = 9.10 **

*Gigaspora* type A  
$F_{(2, 9)}$ = 0.26 NS

*Gigaspora* type B  
$F_{(2, 9)}$ = 1.95 NS

*Gigaspora* type C  
$F_{(2, 9)}$ = 1.50 NS

*Glomus mosseae* (type A)  
$F_{(2, 9)}$ = 0.74 NS

*Glomus caledonium* I (type B)  
$F_{(2, 9)}$ = 3.10 †

*Glomus caledonium* II (type C)  
$F_{(2, 9)}$ = 2.55 NS

*Glomus claroideum* (type A)  
$F_{(2, 9)}$ = 3.22 †

*Glomus claroideum* (type B)  
$F_{(2, 9)}$ = 0.73 NS

*Glomus intraradices* type A  
$F_{(2, 9)}$ = 3.13 †

*Glomus intraradices* type B  
$F_{(2, 9)}$ = 6.28 *

*Glomus intraradices* type C  
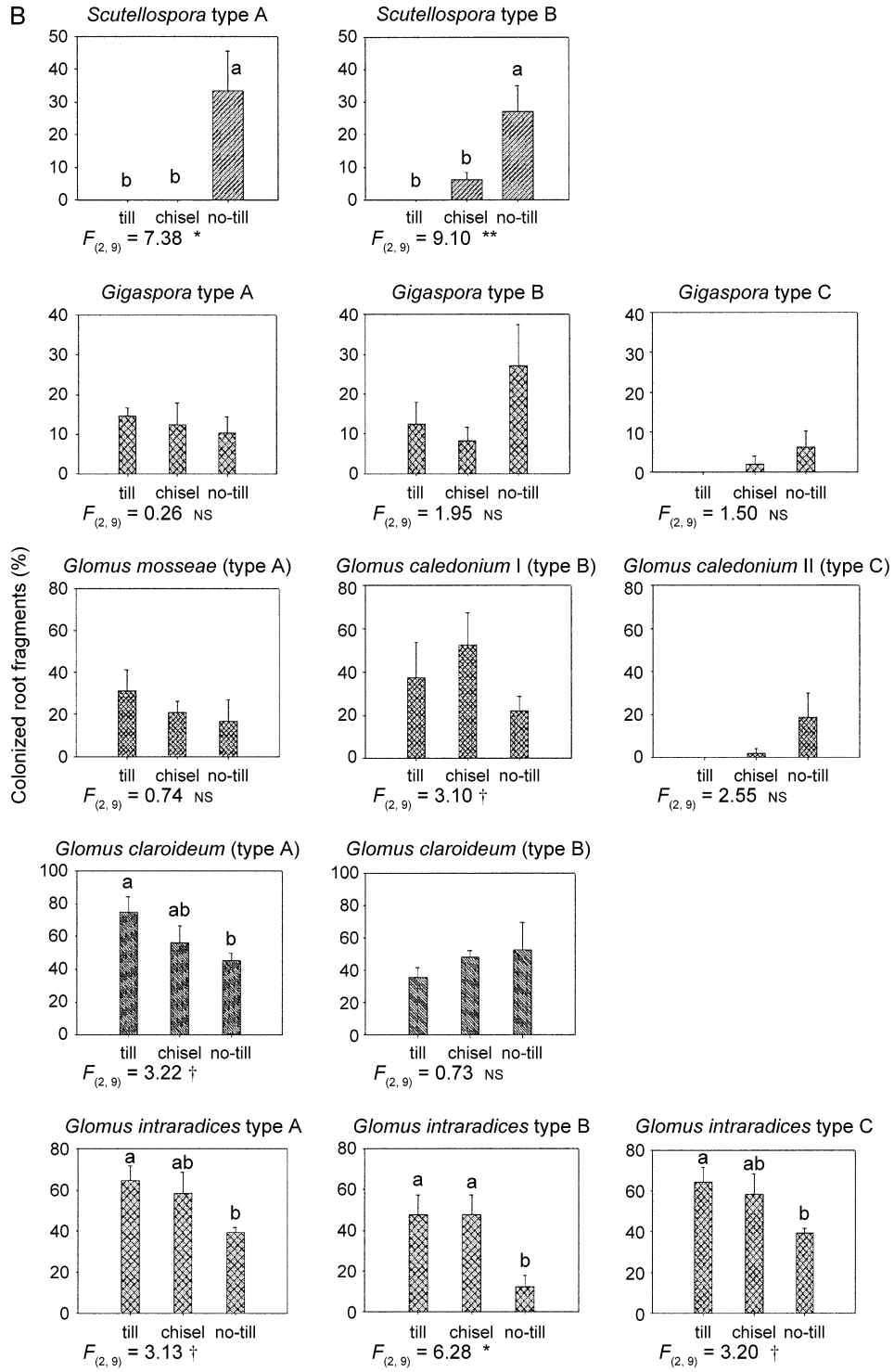$F_{(2, 9)}$ = 3.20 †

Colonized root fragments (%)

FIG. 5. Continued.

1998, Guggenberger et al. 1999) are affected by soil tillage. Thus, it is possible that the effects of soil tillage on AMF might have been mediated by changes in the microbial communities. However, it is necessary to continue studying this issue, because currently little information is available about the interaction of AMF with other soil microorganisms (Germida and Walley 1996).

Another possible reason explaining our results showing altered AMF community composition in the roots of no-till soils as compared with the plowed soils might be the changes in composition in weed plant community in response to different tillage practices (O'Donovan and McAndrew 2000, Streit et al. 2000). With the increased abundance of long-living weeds in the no-till plots (Streit et al. 2000), *Scutellospora* sp. might have established a symbiosis with a more suitable plant host than cereals or maize (Bever et al. 1996), which would have facilitated the production of new spores. These spores would then provide sufficient infectious potential to colonize roots of a less suitable host such as the maize sown in those fields the next season.

The molecular identification methods used in this study have only been developed recently (van Tuinen et al. 1998*b*, Kjøller and Rosendahl 2000), and thus are prone to irregularities. This approach also requires a lot of time and money due to extensive sequencing necessary for development of markers and for the feedback controls. The cross-specificity tests and the sequencing controls performed with the DNA samples amplified from field samples showed that the AMF species or species-groups, which were targeted by the specific PCR markers, are genetically disjunctive and do not share ribosomal gene sequences among them. This confirmed the use of the PCR technique for the identification of AMF, and allowed study of the AMF community within the roots. It must, however, be mentioned that the markers developed in this study might co-amplify some other AMF from other ecosystems and that much more information is needed for the development of universal AMF-specific markers useful in any ecosystem worldwide. This study also showed that coupling of different molecular approaches improved the characterization of AMF diversity in their ecological niche.

## CONCLUSIONS

We have shown that soil tillage alters composition of AMF communities colonizing maize roots under field conditions. Colonization of maize roots by AMF from the genus *Scutellospora* was depressed by intensive tillage, while the opposite could be shown for some members of genus *Glomus*. This might be due to (1) disturbance of AMF hyphae integrity by the tillage, (2) changes in nutrient content of the soil, (3) changes in microbial activity, or (4) changes in weed populations. The future challenge is to investigate influence of the

functional aspects of the AMF community change on the performance of field-grown plants. We know that different AMF species differ in their symbiotic efficiency (Jakobsen et al. 1992, Smith et al. 2000), and thus any induced changes in AMF community structure could lead to changes in crop nutrient uptake and/or productivity.

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