

The influence of tillage on the structure of rhizosphere and root-associated arbuscular mycorrhizal fungal communities

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ABSTRACT

Soil environmental factors affect the structure of arbuscular mycorrhizal (AM) fungal communities present in soil. However, it is not understood to which degree management practices such as tillage lead to dissimilarities between intra- and extraradical AM fungal communities. This study aims to assess the influence of two different soil management practices (conventional tillage and no-till) on the diversity of AMF communities, both in rhizosphere soil and inside corn roots. We hypothesized that under no-till, roots are colonized as they grow through the undisturbed fungal mycelia left from the previous crop whereas under conventional tillage they are colonized by those propagules that survived disturbance and can re-establish in their new relocated and mixed environment. We predicted that the degree of similarity of AM fungal communities inside versus outside the roots would be greater under no-till than under tillage. Using terminal restriction fragment length polymorphism (T-RFLP) and denaturing gradient gel electrophoresis we observed a different AM fungal community present in roots under no-till than under conventional tillage. Moreover, the communities present in the rhizosphere soil were different than in the roots of the corn plants. These results suggest that soil management does alter the diversity of AM fungal communities associated with corn roots and that plants influence the structure of the AMF community colonizing their roots. Sequencing results indicated that the majority of AMF species found in this agricultural soil was *Glomus* spp. However, further work is required to determine the extent to which AM fungal genotypic alterations by soil management influences competitive relationships.

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Introduction

Arbuscular mycorrhizal (AM) fungi are integral components of terrestrial ecosystems (Rillig 2004), forming associations with most terrestrial plant families (Wang and Qiu 2006). AM fungi are obligate biotrophs whose hyphae can act as extensions of plant root systems, often resulting in increased phosphorus uptake, and potentially increased plant growth. The AM symbiosis has been shown to impact soil ecosystem function through interacting with plant pathogens, increasing drought tolerance, stabilizing soil aggregates, and stimulating carbon turnover in the soil (Kling and Jakobsen 1998; Finlay 2008). Currently 150–200 species of AMF have been identified, however, DNA based studies suggest that the

diversity is much higher (Finlay 2008). AM fungi typically occur in soil as spatially structured, diverse communities, and multiple taxa can infect a single host plant (Vandenkoornhuysen et al. 2002; Mummey and Rillig 2006; Mummey and Rillig 2008).

In spite of their apparent importance as a determinant of plant community structure (Van der Heijden et al. 1998) and ecosystem function, little is known about how AMF diversity is controlled (Mummey et al. 2005), and why not all fungus-plant combinations are equally beneficial to the host plant (Helgason and Fitter 2009). Several studies have focused on the importance that the host plant may have on the AM fungal mutualism. According to Bever et al. (1996), the host plant may be one of the main factors determining the structure of the AM fungal communities since roots influence each stage of their development. For example, co-occurring plant species can have different AM fungal communities (Helgason et al. 2002; Johnson et al. 2003; Vandenkoornhuysen et al. 2003). However, it has been recently suggested, that soil environmental factors such as drought, temperature, pH, toxins, nutrient availability, grazing by collembolla and parasitism by chytrids are

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important selective pressures that control the structure of AM fungal communities (Helgason and Fitter 2009). These authors suggest, that much of the variation among AM fungal species and isolates is associated with the adaptation of the external mycelium to a complex soil environment rather than the identity of the plant host. In addition, it has recently been shown that destruction of soil spatial structure by tillage, may allow for the proliferation of less-mutualistic microbes (Bever et al. 2009). This may interfere with the interactions among AM fungi or isolates leading to further discrepancies between intra- and extraradical AM fungal communities under tillage managed soils.

Soil environmental conditions in agricultural systems are particularly dynamic, because of the yearly harvest and removal of plant biomass, use of tillage practices and chemical pesticides. Prior work has shown that these variables can affect the composition of the AMF community. Agricultural systems are often dominated by *Glomus* spp. that form spores readily (Daniell et al. 2001), and likely can survive easier in more intensive agricultural systems (Jansa et al. 2003). Soil edaphic factors such as temperature, pH and soil N content have been associated with shifts in the composition of the AMF community in the field (Merryweather and Fitter 1998; Helgason et al. 1999; Santos et al. 2006; Porras-Alfaro et al. 2007; Toljander et al. 2008).

Shifting agricultural practices are known to impact a variety of soil physical and chemical properties. In a long-term field study at the University of Guelph Elora Research Station, a corn–soybean rotation has been grown under contrasting tillage conditions since 2000. A long-term assessment of this site has indicated that plots differ in soil moisture content, nitrate and ammonium levels, N₂O emissions and diversity of selected bacterial communities (Wagner-Riddle et al. 2007; Smith et al. 2010).

There is evidence that agronomic practices, such as rotation with a non-host crop affect AM fungal colonization in the field (Gavito and Miller 1998). However, these authors did not see an impact of conventional tillage on AM fungal colonization. Some studies suggest that soil disturbance reduced natural soil infectivity of AM fungi (Oliveira and Sanders 1999) and no-till practices increase the colonization of corn roots by AM fungi (Mozafar et al. 2000). The ability of AM fungi to re-establish colonization with the propagules left in the soil, may account for the lack of impact of tillage.

The influence of the tillage practices on the AM fungal communities present within and outside the roots in the rhizosphere soil have not been simultaneously assessed in order to determine if these practices produce a different effect in both communities. Therefore, using molecular tools we characterized the AM fungal community from the roots and rhizosphere soil of corn plants grown under conventional and no-tillage practices. We hypothesized that under no-till the diversity of the AMF community would be different under conventional tillage, because of the disturbance in the fungal mycelium known to be caused by tillage. In addition, we predicted that under no-till, growing roots are mainly colonized by physically undisturbed AM fungal mycelia whereas under conventional tillage they are colonized by AM propagules that survived disturbance and can regrow from their new location within the mixed soil environment. Consequently, we predicted that the AMF community both inside and outside the roots would be different under no-till and conventional tillage.

Materials and methods

Field site and sample collection

Soil and corn root samples were collected at the Elora Research Station, located 20 km north of Guelph (43°39'N, 80°25'W, 376 m above sea level) in Southern Ontario, Canada. Detailed descriptions

of the site can be found in McCoy et al. (2006) and Wagner-Riddle et al. (2007). Soils at the site are classified as Grey-Brown loam-till with imperfect to good drainage (Hoffman et al. 1963). Textural analysis shows that the soil is approximately 29% sand, 52% silt and 19% clay. Total organic C is 2.7%, total organic N is 0.2% and pH (H₂O) is 7.6. The field used in this study is tile drained at a spacing of approximately 16 m and the land generally slopes towards the north at a 3% grade.

The experimental plots are part of a long-term experiment comparing conventional-tillage (CT) agricultural practices against those considered best management practices, including no-tillage (NT). From January 2000 to April 2005, the CT management generally followed recommended guidelines for nitrogen application (OMAFRA 2002), with moldboard tillage to 20 cm after harvest. The NT plots were managed by N fertilizer application according to soil nitrogen tests, no tillage of the soil, and the occasional use of cover crops (Jayasundara et al. 2007). Starting in May 2005, the NT plots were managed similarly to the CT plots, except for tillage which continued to be NT. All plots received the same fertilizer application (urea broadcasted at 150 kg N ha⁻¹) at planting.

Two 100 m × 150 m plots each of both tillage treatments (CT and NT) were located within a larger field (~30 ha). Beginning in 2000, the same crop was grown in all plots in this field with a yearly rotation of corn, soybean and wheat.

Sampling was conducted on 11 July 2007 when corn plants were at physiological maturity. Six soil samples were collected randomly within each plot and additional six samples were collected from surrounding stretches of vegetation outside the plot boundaries in an area containing no corn plants and not managed by agricultural practices. These samples were considered a non-corn rhizosphere control. A total of 30 samples were collected. Root samples were taken by excavation the entire root system of 6 corn plants from each plot. Shoots were removed and the roots with the soil were placed in plastic bags, and transported back to the lab. Samples were stored in a refrigerator (4 °C) overnight prior to analysis.

DNA extractions

Each soil sample was separately homogenised, sieved to 0.5 mm and 5 g of this sieved soil was used for DNA extraction using a PowerMax™ Soil DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA) following the manufacturer's protocol.

Corn roots were thoroughly washed to remove soil particles and surface sterilized using a 1% (v/v) bleach solution. Root samples were freeze-dried for 48 h, ground using a tissuelyser machine (Retsch) and DNA was extracted from 0.2 g of these dry powdered samples using a Dneasy Plant Maxi Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's specifications.

PCR amplification of a partial *LSU rDNA* region

The primers FLR1 and FLR2 (van Tuinen et al. 1998; Trouvelot et al. 1999) were used for the amplification of the 5' end of *LSU rDNA* sequences in fungi. PCR amplifications were performed in a final volume of 20 µl containing 2 µl 10× PCR buffer (Promega, Madison, WI, USA), 500 nM each primer, 25 mM MgCl₂, 200 nM dNTPs and 1.25 U Go Taq Flexi polymerase (Promega, Madison, WI, USA). An aliquot (1 µl) of diluted soil or corn root DNA extract was added to 19 µl PCR mix. Each reaction was performed in a thermal cycler (Mastercycler®ep, Eppendorf, Hamburg, Germany) programmed as follows: initial denaturation cycle at 95 °C (3 min), annealing at 58 °C (1 min) and extension at 72 °C (1 min) followed by 35 cycles of denaturation at 93 °C (1 min), annealing at 58 °C (1 min), extension at 72 °C (1 min); the last cycle was followed by a final extension at 72 °C for 5 min (van Tuinen et al. 1998).

A nested PCR reaction was used to select for AMF specific LSU rDNA. The first PCR amplification was diluted 1/100, and served as template for a second nested reaction, under the same PCR conditions as described above. For T-RFLP 5' labelled primers FLR3-FAM and FLR4-HEX (Gollotte et al. 2004) were used, for DGGE, no labels were used but a GC clamp on the 3' end of the reverse primer to avoid complete denaturing in the DGGE analysis. All PCR products were confirmed by running on a 1% agarose gel in TAE buffer and visualising under UV light after staining with ethidium bromide. The FLR3/4 primer pair was assessed by Mummey and Rillig (2007) and was found to be highly specific for AMF communities.

Characterization of AMF communities by T-RFLP

PCR products were purified using the UltraClean PCR Clean-Up DNA purification kit (MoBio Laboratories, Solana Beach, CA, USA) and digested with the *AluI* restriction enzyme, according to manufacturer's protocols. In brief, 15 μ l purified PCR product and 5 U *AluI* in the manufacturer's recommended buffer (Invitrogen, Carlsbad, CA, USA), was incubated for 2 h at 37 °C.

Characterization of AMF community by DGGE

DGGE was performed with a D-Code system (BioRad, Hercules, CA, USA), gels containing 8% acrylamide were poured with a gradient of 30–70% denaturant. The gel was left to set for 10 min and then a 0% denaturant stack was poured above this gel to minimize denaturing while in the wells. We defined 100% denaturant as 7 M urea plus formamide (40%). The PCR product was added to the wells in the stack and ran at 20 mV until the temperature reached 65 °C and then the gels were ran at 75 mV for 17 h. The gels were then stained with 0.02 \times SYBR Green for 15 min, UV illuminated and digitally photographed using GeneSnap (Syngene, Cambridge, UK). These digital images were then imported into GeneTools (Syngene, Cambridge, UK), for analysis. Selected DGGE bands were excised for sequencing and placed in 50 μ l of sterile water. The eluted fragments were PCR amplified as described above and PCR products were purified with UltraClean PCR Clean-up DNA Purification Kit (MoBio, Carlsbad, CA, USA). Sequencing was performed by Laboratory Services Department, University of Guelph.

Data analyses

T-RFLP analysis

AM community analysis consisted of determining the profile (sizes, peak heights and areas) of T-RFs in each sample using GeneMapper® software v. 3.5 (Applied Biosystems, Foster City, California, U.S.A.). The Microsoft-Excel macro Treeflap (Rees et al. 2004), available at <http://www.wsc.monash.edu.au/~cwalsh/treeflap.xls>, was used to convert fragment sizes to the nearest integer, aligning them with their respective peak heights side by side in two columns. We then developed an R script (R Development Core Team 2007; available upon request from the authors) that standardizes the amount of total fluorescence among sample profiles (Dunbar et al. 2001) and binary codes T-RFLP fingerprints. Presence/absence matrices for each enzyme/primer combination were combined into a single matrix.

The influence of tillage regime on community composition of roots and rhizosphere soil was determined by distance-based redundancy analysis (db-RDA) (Legendre and Anderson 1999). Bray Curtis' coefficients of similarity were first calculated between samples and used to compute principal coordinates in PrCoord 1.0 (part of Canoco version 4.51, Biometris, Wageningen, The Netherlands). All the principal coordinate axes (PCAs) were then exported to Canoco and treated as "species" data. Factors (tillage and sample

type) were entered as dummy binary variables (one level per column) and their effects tested by using the combined levels of a factor as the explanatory variable in the model while removing the variance explained by the other factors by entering their levels as covariates. Where appropriate we used forward selection of environmental variables and conducted Monte-Carlo permutation tests based on 999 permutations. The results of the ordination of the AM fungal community composition, as assessed by PCR-T-RFLP, were displayed as PCA ordination diagrams.

The population diversity of the microbial community was examined by the Shannon's index of general diversity ($H' = -\sum (ni/N) \log(ni/N)$), Margalef's richness index ($d = (S - 1) / \log(N)$) and Pielou's evenness index ($J = H' / \log(S)$). H' , d and J were calculated on the basis of banding patterns as described in (Costa et al. 2006). Variance analysis was performed using the aov procedure of R software (R Development Core Team 2007) to determine variation due to origin (root or soil), tillage treatment and tillage treatment within each origin. Means comparison were done using Tukey's adjustment. A significance level of 0.05 was used throughout the study.

DGGE analysis

The images of the DGGE gels were analyzed with GeneTools software (Syngene, Cambridge, UK) and corrected visually to identify true peaks. The corrected DGGE profiles from GeneTools (Syngene, Cambridge, UK) were imported into GeneDirectory (Syngene, Cambridge, UK) and compared to each other based on clustering via the unweighted pair group method with mathematical averages (UPGMA: Dice coefficient of similarity). A similarity matrix of the gels were analyzed with non-metric multidimensional scaling (MDS) and analysis of similarity (ANOSIM) in Primer v.6 (Primer-E Ltd., Plymouth, UK).

DGGE sequences were subjected to analysis by Genbank's BLAST utility to determine similarity to known AMF LSU sequences. DNA sequences were aligned with LSU sequences from known AMF by using ClustalX software version 2.0, with manual adjustments as necessary. Phylogenetic analysis of the sequences was performed by using a neighbour-joining algorithm with 1000 bootstrap replicates implemented with Geneious Pro v. 4.0.4 (Biomatters Ltd.). An LSU sequence from *Scutellospora* sp. was used to root the tree.

Nucleotide sequence accession numbers

The sequences of the DGGE bands identified in this study have been deposited in GenBank under accession numbers GU049660–GU049665.

Results

Characterization of AMF communities by T-RFLP

Overall, using the tree-flap methodology, a total of 66 different T-RF sizes were detected (Fig. 1). An average of 32.33 (± 1.22), 28.75 (± 1.27), 31.75 (± 1.24) and 25.5 (± 1.98) different T-RF sizes were detected within all T-RFLP profiles derived from CT soils, CT roots, NT soils and NT roots, respectively. There were 17 unique T-RFs associated with soil, that were not found in plant root AMF communities and 11 T-RFs were unique to plant root associated AMF communities, suggesting that there are some differences in the AMF community structure between each of these treatments (Fig. 1). This was supported by canonical correspondence analysis (CCA) indicating that the AMF community structure associated within roots differed from that associated with rhizosphere soil (Fig. 2). Using CT and NT as covariables roots and rhizosphere explain a significant portion (11%) of the total variance (F -ratio = 1.869; $P = 0.001$). Ecological indices also indicated that the diversity of the AMF population was lower in the roots ($H' = 1.46$)

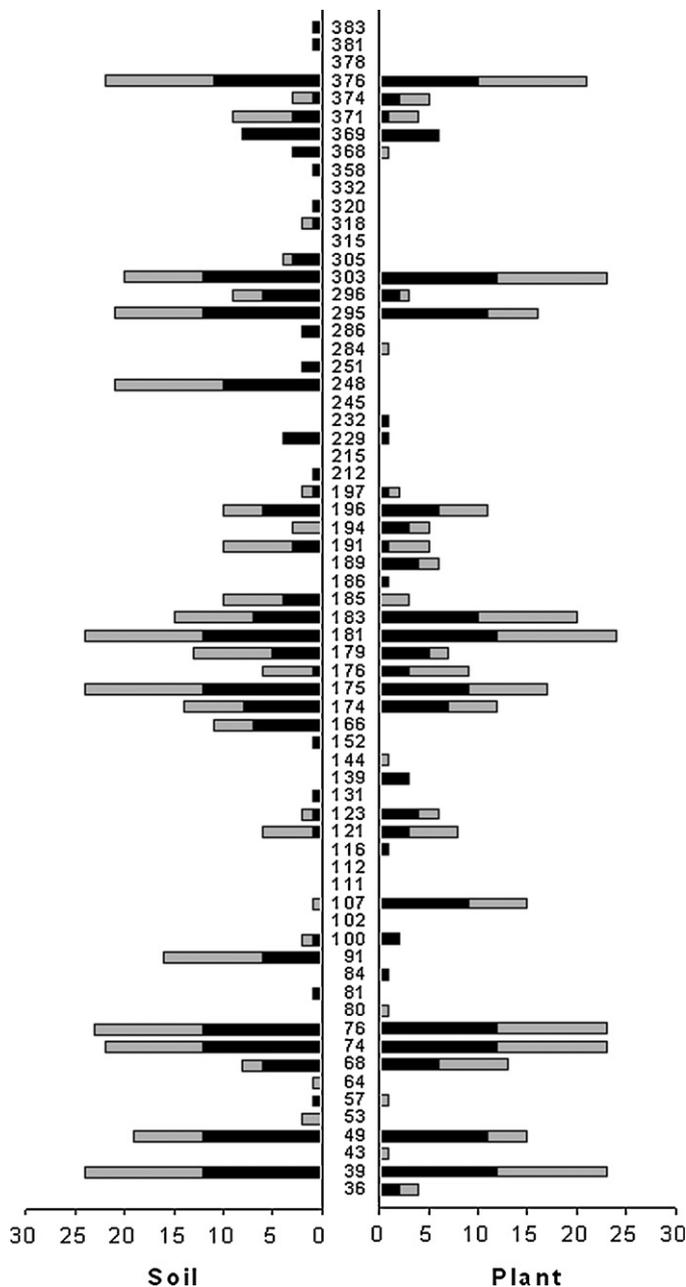


Fig. 1. Graphical presentation of the number of samples in which T-RF sizes were found in plant and soil under no tillage (grey) and conventional tillage (black) conditions.

compared to the rhizosphere ($H' = 1.63$), although only marginally significant ($P = 0.054$). The AMF community within the roots had lower richness in the no-till plots ($d = 2.72$) compared to the rhizosphere ($d = 4.26$) ($P = 0.042$), but no significant difference in richness between roots ($d = 3.8$) and rhizosphere ($d = 3.28$) was seen in the conventional tillage plots ($P = 0.25$). There were no differences in Pielou's evenness index (J) between AMF communities within the roots ($J = 0.52$) and rhizosphere ($J = 0.56$) ($P = 0.13$).

There was also some separation between tillage systems within the root-associated and soil-associated AMF communities (Fig. 2). A significant portion of the variance in the community (11.7%) could be attributed to tillage differences regardless of soil and root origin (F -ratio = 1.504; $P = 0.001$). Ecological indices also indicated that there was a difference in the composition of the community due to tillage system. Shannon's diversity indices were higher in con-

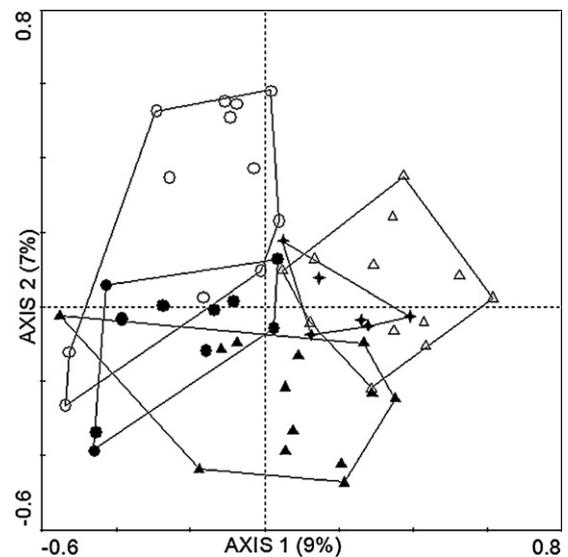


Fig. 2. Ordination diagram of principal components 1 and 2 (Axis 1 \times Axis 2). The distance between the symbols (each representing an individual sample) in the diagram approximates the dissimilarity of their T-RF composition, measured by their Euclidean distance. Circles and triangles are plant and soil samples, respectively and open and solid represent no till and conventional till, respectively. The stars represent the samples collected from the side lanes (non-corn rhizosphere control samples).

ventional plots ($H' = 1.63$) compared to no-tillage plots ($H' = 1.47$), although this was only marginally significant ($P = 0.075$). In addition, the AMF community within the plants grown under the conventional tillage system had higher richness ($d = 3.80$) compared to communities from plants grown under no-tillage ($d = 2.72$) ($P = 0.015$), but there were no differences in the richness of the AMF communities from the rhizosphere soil of the no-till plots, compared to the conventional tillage plots. There were also no differences in Pielou's evenness index (J) between AMF communities in conventional tillage ($J = 0.56$) and no-tillage plots ($J = 0.52$).

Characterization of AMF communities by DGGE

DGGE analysis also indicates that the community of AMF associated with corn roots is different than that associated with corn rhizosphere soil, and that tillage regime affected these communities (Figs. 3 and 4). DGGE was used to visualize the AMF community and identify AMF sequences from the field site (Fig. 3a). DGGE banding patterns suggest that certain detected AMF taxa were only detected in soils from conventional tillage plots, and not in no-tillage plots (Fig. 3a). Select bands were sequenced from the DGGE gels, all were identified as *Glomus* spp. through NCBI Blast. A phylogenetic analysis indicated that 3 of the bands clustered with known *Glomus intraradices* isolates, 2 of the bands clustered with an uncultured glomeromycotan fungus, and one clustered most closely with a group of *Glomus claroideum* sequences (Fig. 3b).

Multidimensional scaling analysis indicates that the community structure of these communities are separated on an MDS plot, ANOSIM indicates that there are significant differences in the community structure associated with plant roots compared to rhizosphere soil ($R = 0.7$, $P = 0.033$), and also differences between conventional and no-tillage plots ($R = 0.96$, $P = 0.04$) (Fig. 4). Note that an ANOSIM value usually lies between 0 and 1. A value of $R = 0$ indicates that there are no differences in community structure between sample intervals, and $R = 1$ indicates complete difference in community structure.

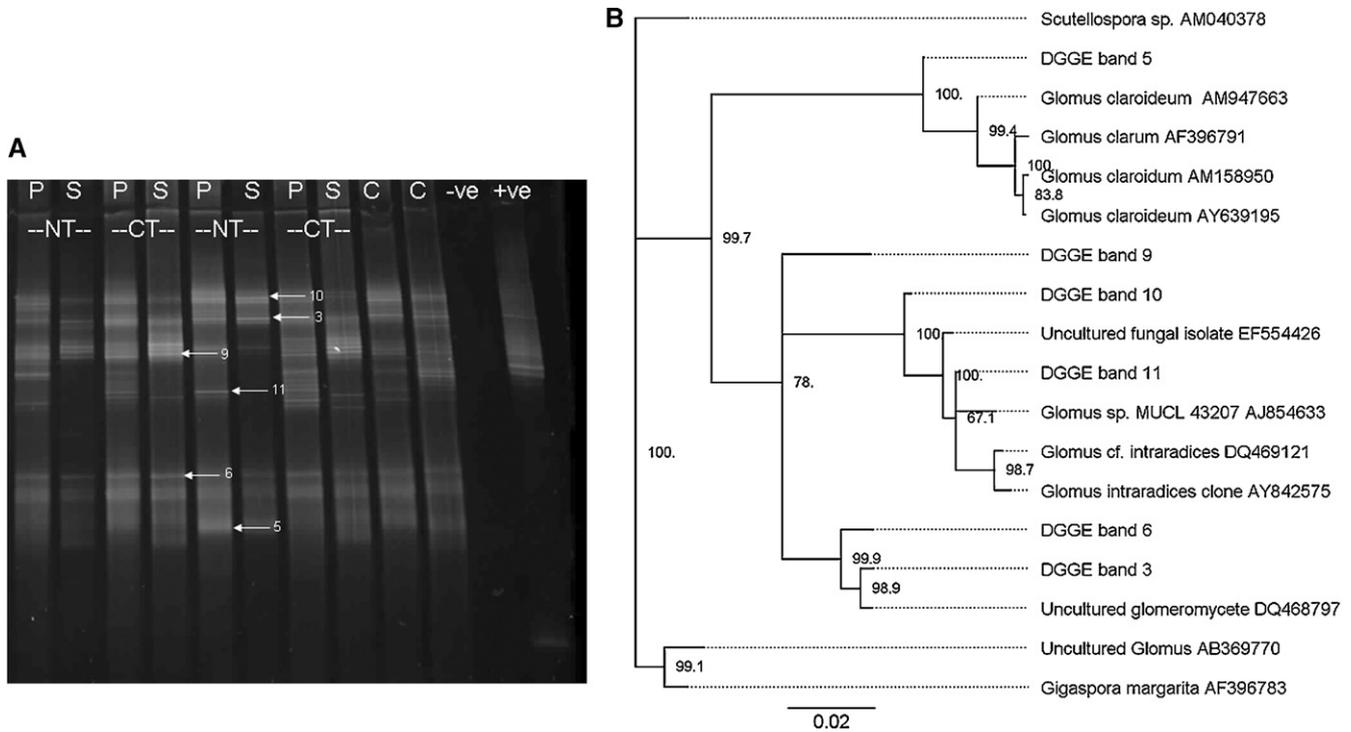


Fig. 3. (a) Sample DGGE gel indicating the AMF community associated with plant roots (P), or rhizosphere soil (S), from corn plants grown under no-tillage (NT) or conventional tillage (CT) regimes. AMF profile from an adjacent field soil with no corn (C) was added for comparison. A negative control (-ve), and 100 bp ladder (+ve) were also included. Numbers by the arrows identify DGGE bands that were sequenced and included in phylogenetic analysis (b). (b) Neighbour-joining analysis (1000 bootstrap replicates) of large subunit ribosomal DNA from DGGE band sequences and known *Glomerales* sequences found in Genbank, accession numbers for DGGE band 3 GU049660; band 5, GU049661; band 6 GU049662; band 9 GU049663; band 10 GU049664; band 11 GU049665. Bootstrap values <70% are not shown. *Scutellospora* sp. was defined as the outgroup.

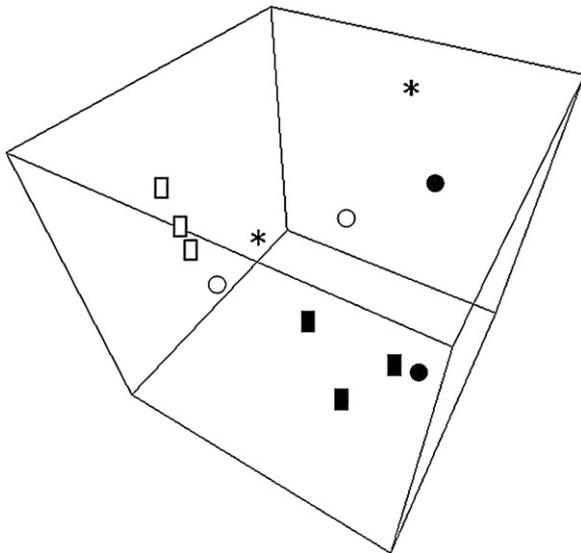


Fig. 4. Multidimensional scaling plot of DGGE analysis. Each point on the plot represents the AMF communities associated with plant roots (solid symbols), or rhizosphere soil (open symbols), from corn plants grown under no-tillage (circles) or conventional tillage (squares) regimes. The stars represent the samples collected from the side lanes (non-corn rhizosphere control samples). The 3D stress value is 0.08, the ANOSIM values (*R*) indicates significant differences between plant and soil (0.396, $P < 0.04$), and between tillage treatments (0.7, $P < 0.033$).

Discussion

In the current study, application of T-RFLP and DGGE approaches demonstrate that root-associated AMF communities have a different structure than rhizosphere-soil associated AMF communities.

In a review, Helgason and Fitter (2009) suggested that much of the variation among AMF species and isolates in soil is associated with adaptation of external mycelium to a complex soil environment rather than to the identity of the plant host. Our data suggest that the soil environment interacts with the host plant in determining the structure of the AMF community colonizing plant roots, since the community of AMF differs significantly between rhizosphere soil and root communities, within a tillage treatment. This suggests that the plant does have an influence on the structure of the AMF community, colonizing its roots.

The impact of tillage on AMF communities presented in the literature is not clear. In general, tillage is considered to 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). Several authors reported that reduced soil tillage increases the mycorrhizal colonization of plant roots (McGonigle and Miller 1996; Mozafar et al. 2000; Borie et al. 2006). While an early study examining the impact of tillage on AMF associated with corn grown in Ontario soils, showed relatively little impact of the tillage system on colonization (Gavito and Miller 1998), Jansa et al. (2003) determined that soil tillage alters the composition of AMF communities colonizing corn roots under field conditions. Under conventional tillage, more members of the genus *Glomus* were detected in the roots. They suggested that changes could be due to disturbance of AMF hyphae by tillage, or due to differences in biological and chemical soil conditions.

In the current study, we assessed AMF communities associated with corn plants growing in four adjacent plots as part of a long-term field study where a corn-soybean rotation has been grown under contrasting tillage conditions since 2000. Our prior work from this long-term study has shown that plots differ in soil moisture content, nitrate and ammonium levels, N₂O emissions and diversity of selected nitrifying and denitrifying bacterial communi-

ties (Wagner-Riddle et al. 2007; Smith et al. 2010). In combination, these studies suggest, that at this site, shifting agricultural practices have impacted a variety of soil physical, chemical and biological properties. Our current data also demonstrates significantly different AMF community structure in the CT compared to NT plots. Due to the large size of the established plots (1500 m²) required for the ag-meteorological instrumentation available on-site, only two field replications were possible. However, multivariate analysis (Figs. 2 and 4) clearly shows that while there is some variability in the community structure of AMF communities within plots, there are clear groupings of communities according to tillage practice.

Results from this study suggest that NT soils are dominated by few species, whereas tillage allows for more species to coexist. The intermediate disturbance theory (Grime 1973), suggests that ecological communities seldom reach an equilibrium state, in which competitively superior species exclude others, because disturbances that kill or damage individuals will continually set back the process of competitive elimination by opening space for colonization by less competitive individuals. It is possible that AMF observed under conditions of less disturbance (NT) are more competitive and disturbance (tillage) practices may promote less-competitive species. Therefore, possibly explaining why our results show that the number of co-existing species is lower in CT than in NT. Furthermore, tillage may affect the rate of infective propagule production for different AMF species producing the alteration of AMF communities infecting corn roots.

Recently, molecular biological tools have significantly increased the ability to characterize microbial communities *in situ*. These molecular studies involve cloning and sequencing of rRNA genes. In addition, T-RFLP analysis (Liu et al. 1997) has been widely used for discrimination of AMF communities within plant roots (Vandenkoornhuysse et al. 2003; Mummey et al. 2005; Mummey and Rillig 2007).

Dickie and FitzJohn (2007) reviewed the use of T-RFLP in mycorrhizal fungi identification studies, pointing out some limitations and several sources of error in these techniques. These included three major potential errors in T-RFLP: (1) erroneous combinations of either real peaks or “noise” in electropherograms, (2) shared T-RFLP profiles between two species, and (3) multiple T-RFLP profiles within a single species. They suggested combining T-RFLP methodologies with DGGE analysis in order to strengthen the conclusions supported by the former. According to this, a combination of both methods was employed in the current study. Results from DGGE confirmed the findings obtained by the T-RFLP analysis, and further allowed a portion of the bands to be sequenced. Interestingly, we also found that the sequences of selected DGGE bands, lead to identical T-RFLP profiles, despite, our ability to differentiate the organisms phylogenetically.

Our data suggest that soil management does alter the diversity of AM fungal communities in soil and roots. Corn takes some of the AMF present in soil thereby serving as another filter. These results highlight the need for further study to determine to which extent AM fungi genotypic alterations by soil management influences AMF competitive relationships. It also calls for the potential benefit that intercropping may have to help sustain AMF diversity in soils under high disturbance regimes.

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